Exploration of space to achieve scientific breakthroughs

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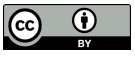

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Exploration of Space to Achieve Scientific Breakthroughs

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ABSTRACT

Living organisms adapt to changing environments using their amazing flexibility to remodel themselves by a process called evolution. Environmental stress causes selective pressure and is associated with genetic and phenotypic shifts for better modifications, maintenance, and functioning of organismal systems. The natural evolution process can be used in complement to rational strain engineering for the development of desired traits or phenotypes as well as for the production of novel biomaterials through the imposition of one or more selective pressures. Space provides a unique environment of stressors (e.g., weightlessness and high radiation) that organisms have never experienced on Earth. Cells in the outer space reorganize and develop or activate a range of molecular responses that lead to changes in cellular properties. Exposure of cells to the outer space will lead to the development of novel variants more efficiently than on Earth. For instance, natural crop varieties can be generated with higher nutrition value, yield, and improved features, such as resistance against high and low temperatures, salt stress, and microbial and pest attacks. The review summarizes the literature on the parameters of outer space that affect the growth and behavior of cells and organisms as well as complex colloidal systems. We illustrate an understanding of gravity-related basic biological mechanisms and enlighten the possibility to explore the outer space environment for application-oriented aspects. This will stimulate biological research in the pursuit of innovative approaches for the future of agriculture and health on Earth.

Keywords: Space, Evolution, Microgravity, Stress response, Mutation, Horizontal gene transfer, Epigenetics, Tissue engineering, Cancer research, Colloids

1. Introduction

The scientific approach lends itself to the creation of definitions or rules that box life into specific lanes. However, upon further investigations, these lanes require repeated adjustments due to life being an ever-evolving continuous spectrum of possibilities that are based on a few absolute natural laws. The result is an ambiguous division between what is living and what is not. Life can be further thought of as a magnification of physical and chemical laws driven by local energy and order states overlaid by selection history and constraints. In recent years, and with the advent of molecular biology, the classical definition of species and life has come to be questioned. While Darwin's inheritance, variation, and selection still play a key role in the process of life's evolution, the source of the inheritance, variation, and process of selection has expanded. This expansion can result in a selection of inherited variability that is not associated with perceived optimal fitness at the time of selection. Key processes that can lead to such results include genetic hitchhiking, genetic drift via bottleneck or founder event, horizontal gene transfer (HGT) and, to a lesser extent, gene flow and epigenetics. Besides, some changes in the DNA do not seem to have any effect on amino acid translation or fitness and maybe deemed silent or neutral mutations, respectively. When gauging fitness and speciation we must consider the spatial and time aspect that is the distribution over time. Speciation occurs in either a gradual consistent pace (gradualism) or abrupt pulses (punctuated equilibria) with the main difference being the time scale of the speciation events. Punctuated events can be seen in places that have undergone rapid environmental changes (e.g., the Hawaiian Islands). The space environment with its gravitational and radiation changes affords such a novel abrupt environmental change for terrestrial life. This environment can be used to decipher underlying pathways that are not apparent on Earth, test countermeasures for observed changes, and develop new organisms with novel attributes.

Since the late 1950s space technology has developed spacecraft for transporting terrestrial life into low Earth orbit (LEO) to study, in situ, its responses to selected conditions of space (Brinckmann, 2007; Clément and Slenzka, 2006; Horneck et al., 2010; Nickerson et al., 2004). The space shuttle (Baglioni et al., 2007; Horneck et al., 1984b; Horneck et al., 1984a) and the space stations, e.g., the Russian MIR (Rettberg et al., 2002) and the International Space Station (ISS) (Baglioni et al., 2007) can truly remove the influence of gravity in an experimental setting in the LEO. The orbital microgravity environment provided by these spaceships has allowed us to perform unique experiments to study the influence of microgravity on living systems that could not be carried out on Earth. Changed environmental factors onboard a spacecraft include weightlessness (technically called "microgravity"), ionizing and non-ionizing solar or cosmic radiations, temperature extremes, and high vacuum. Among these, microgravity and cosmic radiations are of particular interest for scientific research. Regardless of the various primary experimental objectives and subsequent, sometimes-disparate conclusions drawn from the data, the majority of investigations to date have indicated that microgravity has a significant influence on cell growth and behavior. With technological advancement in molecular science and analytics, the focus of attention in modern space and gravitational biology is switching to explore the influence of microgravity on living systems at the cellular and molecular levels. Emphasis is on the investigation of genetic stability, growth, development, reproduction, aging, life span, behavior, and orientation of plants and animals in space environmental conditions. In recent years, studies under simulated or real microgravity resulted in significant outcomes in cancer research and cell and tissue engineering among others.

1.1. Scope of the review

This review covers why we need to think out of the box and explore space environmental conditions to achieve breakthroughs for a sustainable future. The review explains the possibility of exploration of the space environment to accelerate the development of new varieties of crops or other species and novel substances using the natural evolution process. We discuss the molecular machinery and mechanisms that may lead to the evolution of organisms in space's unique environment. We consider the effects of the environment, constrained selection process, unrestricted and extensive gene expression, mutation, gene exchange, and epigenetics on the biology, adaptation, and evolution of cells or organisms in space. Technically, natural evolutionary mechanisms can be employed to obtain strains with desired traits (self-guided evolution) by setting controlled environmental and selection factors. A review of literature relevant to the effects of microgravity on growth, behavior, or survival of microbes,

plants, and cell and tissue cultures is provided. We also cover the need to study colloids in real microgravity conditions to unveil some key physicochemical aspects, like the natural movement of colloids that experiments performed on Earth, in the presence of gravity may mask, alter, or simply hinder. Taken altogether, this review encompasses the different aspects of space biology that have been studied to date. By providing a comprehensive review of this currently unfolding space research discipline, we intend to illustrate organisms' responses to the space environment, enlighten a new understanding of biology, and recognize the potential of the space environment for boosting biological research in the pursuit of new solutions for the future of agriculture and health on Earth. The review not only explains gravity-related basic biological mechanisms at the cellular level but also gives a picture to explore space for application-oriented aspects, such as to produce and develop an array of products for food, pharmaceutics, cosmetics, home-care with longer shelf lives and new functionalities.

1.2. Effects of human activities on the global ecosystem

Earth has immense resources and opportunities for the development of different technologies and products to meet the current needs of the global population. However, human activities, in particular, the overuse of fossil fuels, uncontrolled deforestation, intensive land use for agriculture, and enormous animal production, are dramatically affecting the global climate and causing considerable stress to land resources, including water, soil, nutrients, plants, and animals (Dupuy et al., 1976; Grossi et al., 2019; Masson-Delmotte et al., 2018). Global temperature rise (~0.85ºC in the last 130 years) continues to result in shrinking of ice sheets, glacial retreat, sea-level rise, extreme weather events, ocean acidification, soil salination, desertification, and reduced availability of drinking water (Masson-Delmotte et al., 2018). These changes are affecting agricultural productivity and food availability, safety, and quality at global, regional, and local levels (Brown et al., 2015). While the human population is continually growing, there is no unlimited availability of natural resources such as arable land and freshwater, which is further amplifying the threats of climate change on food security. According to the World Economic Forum, our food supplies will be under far greater pressure with the global population expected to reach 9.8 billion by 2050 (Godfray et al., 2010). The demand for food will be 60% higher than it is today. With the current state-of-the-art agricultural practices and productivity, it will be impossible to meet the demands. Furthermore, in the last several decades, there is a significant decrease in the number of cultivated crop species as well as in the diversity within major crops (Cardinale et al., 2012; Nordin and Nordin, 2017). Plant domestication and subsequent selection in agriculture have limited the genetic diversity of modern crops and increased their susceptibility to multiple diseases and extreme environmental stresses (weather and soil conditions) (Huang et al., 2016). Furthermore, the stress due to the changing environment is weakening plants' defense mechanisms, making them more vulnerable to several pathogens, such as viruses, bacteria, fungi, oomycetes ("egg fungi"), nematodes, and parasitic plants (Dean et al., 2012; Savary et al., 2019; Vurro et al., 2010). These pathogens' range and distribution are increasing with climate change (Anderson et al., 2004; Backlund et al., 2008), causing extensive crop damage. For instance, a study on climate change-related spreading of downy mildew (*Plasmopara viticola*) concluded that the production costs in vineyards are increasing in a range of 20-50% due to higher costs in disease management (Francesca et al., 2006). Increasing the genetic diversity within cropping systems by the use of variety or species mixtures offer several potential advantages, such as limiting disease development, increasing yield stability, and improving resilience to abiotic factors (Newton, 2016; Savary et al., 2019; Schöb et al., 2015). Furthermore, it delivers other ecosystem services including greater biodiversity. Unpredictable weather events, poor crop yields, augmented pathogenesis, serious consequences of uncontrolled agrochemical utilization, and increasing food insecurity have set off alarms that we cannot afford to ignore. It is important to focus on the development of stress-resistant crops that are well adapted to thrive in a changing environment and have stable yields under stress conditions to ensure future food security. The need is to deploy the rich biodiversity by generating a suite of new varieties of crops, not just one.

Climate change is also intensifying health problems that already pose a major burden to susceptible populations and will bring new and emerging health issues soon. According to an assessment by World Health Organization (WHO), between 2030 and 2050, climate change is expected to cause approximately 250,000 additional deaths per year from malnutrition, malaria, diarrhea, and heat stress. The socioeconomic costs of health problems are considerable and will increase over time. Besides, misuse and overuse of conventional antibiotics in humans, animals, and plants are accelerating the

development and spread of antimicrobial resistance. Drug-resistant diseases already cause at least 700,000 deaths globally per year, a figure that could increase to 10 million per year by 2050 if no action is taken (Interagency Coordination Group on Antimicrobial Resistance, 2019). Antimicrobial resistance could cause the death of 2.4 million people in high-income countries between 2015 and 2050 if no sustainable efforts are made to control it. The discovery and development of novel antibiotic compounds have been slow and our arsenal of effective antibiotics is dwindling. The WHO has recommended prioritizing research on the discovery and development of new antibiotics to deal with the serious problem of drug-resistant infections (World Health Organization, 2017). There is an urgent need for the development of new methods and novel classes of potent drugs to combat antimicrobial-resistant pathogens.

1.3. Approaches to tackle problems of the fast-changing world

Researchers from all disciplines have constantly attempted developing novel strains or bio-products by both conventional approaches and emerging technologies, but the results are not as envisioned. Conventional screening programs have increasingly resulted in the rediscovery of already known species, strains, and metabolites (Wohlleben et al., 2016). It is assumed that only a few new metabolites are left to be isolated from nature's repository but needs massive screening programs (Baltz, 2007; Monciardini et al., 2014). Furthermore, the discovery and process development of natural chemicals for pharmaceutical, agricultural, and related applications are labor- and resource-intensive as they are produced in low yields in native organisms, and sometimes even in multiple forms, thus complicating their identification, isolation, and structural elucidation (Pickens et al., 2011). Besides, many of the natural chemicals are structurally complex, which makes their chemical synthesis difficult and therefore restricting their commercial utilization. These limitations within the natural product discovery led to the development and expansion of innovative methods, such as genetic or metabolic engineering, for producing novel biomaterials. Traditional breeding methods rely on hybridization and recombination to stack favorable alleles together. This process is usually time-consuming and labor-intensive, especially when the crop is not fully or newly domesticated.

The modern techniques, like genetic or metabolic engineering that include the manipulation of genetic material or targeted modification of metabolic pathways of an organism using recombinant DNA technology (Courchesne et al., 2009; King et al., 2016; Pickens et al., 2011; Yang et al., 2007) have created a foothold in scientific discovery and development. In the recent years, several genetic toolbox and methods have been developed and implemented for strain improvements such as cloning and expression, shuttle vectors, transformation, reporter genes, selection markers, RNA interference, and DNA microarrays (Amiri and Karimi, 2019; Doron et al., 2016; Prasad et al., 2014; Yadav et al., 2018; Yao et al., 2015; Zhang et al., 2018). Genetic engineering strategy has enabled the improvement of species and natural product titers, production of a new characteristic in an organism, and generation of a wide variety of novel compounds for pharmaceutical and agricultural sectors, but has several limitations. There is still the need for optimization to achieve titers, yields, and productivities that are sufficient for industrial applications. Successful transformation is the main hurdle that prevents the genetic modification of many species. Unfortunately, standard transformation methods successfully used in model organisms often fail when applied to other organisms. Many of the strains developed by genetic or metabolic engineering are unstable and lose engineered properties over time, thus hampering the commercial application (Prasad et al., 2014; Prasad et al., 2019a; Prasad et al., 2019b). It is a general observation that a successful transgene integration does not always result in the efficient expression of foreign genes. This is generally attributed to inadequate recognition of the promoter that drives gene expression, transgene silencing, post-transcriptional gene silencing, and/or bias in codon usage (Prasad et al., 2019a). Furthermore, for genetic or metabolic engineering, it is important to consider every biochemical network in the cell as well as the cellular responses to genetic changes and environmental stimuli (Yang et al., 2007). For this, we need to have complete knowledge of the cell and it's complex metabolic and regulatory networks, which is impossible in the near future. Thus, our partial and flawed knowledge of organism metabolism, physiology, and regulatory networks limits the development of competent strains with a high titer, yield, and/or productivity through genetic or metabolic engineering. Rapid progress in genetic engineering shows the development of clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (Cas)9 system, which allows robust and flexible genome editing and gene regulation in a multitude of model organisms and cell types, but has

some technical limitations, like the efficiency of Cas9 activity, target site selection, and off-target effects (Vasiliou et al., 2016). Although many of the advances in the genome-engineering territory are viewed as huge scientific achievements, their use is vigorously debated and restricted in most countries, which is a major obstacle for their widespread adoption.

Evolutionary engineering is a complementary approach to conventional techniques and imitates the natural evolution process to increase the tolerance of organisms to stress (biotic or abiotic), improve cell performance, improve utilization of a target substrate, improve the production of compounds, or produce new natural chemicals (Jin et al., 2016; Lee et al., 2015; Zhang et al., 2016). In this approach, the natural evolutionary process is rapid, directed, rational, and efficient for the improvement of strains or development of desired traits through the imposition of one or more selective pressures and is associated with genetic, physiological, and morphological changes (Sharma et al., 2019). This approach takes a broad view of the complex cellular network and is the most effective tool for developing multiple or complex traits that are a result of the interaction of several genes or for which genes are unknown. In this review, we term this process as 'self-guided evolution'. In fact, evolutionary engineering is the most ancient tool for the development of novel phenotypes. It has long been used in agriculture, animal farming, and in recent times for the production of antibiotics and other industrially important biomaterials. For example, penicillin from *Penicillium chrysogenum* and the yeast *Saccharomyces cerevisiae* for brewing, winemaking, and ethanol fermentation at an industrial scale. Space can be explored as a platform to induce the self-guided evolution of organisms (Fig. 1). Exposure of an organism to the unique space environment (discussed in Section 3) will increase evolutionary rate and processes and result in genetically diverse phenotypes, which would not form under a load of gravity. This is because organisms never experienced the absence of gravity during their whole evolution. Subjecting organisms in the space environment could produce new valuable strains of plants, yeasts, bacteria, and others and products of pharmaceutical and agricultural relevance (Horneck et al., 2010; Huang et al., 2015). Also, the observed differentiation effects on cancer cells or the induction of 3D-organoids from cell cultures under microgravity conditions are very promising applications for commercial use of space-born technology with high return values. Before we discuss the potential of space and relevant experiments, we provide a brief overview of how evolution works and the different processes involved in bringing evolutionary changes to organisms.

2. Evolution and mechanisms of evolution

The term 'evolution' stands for patterns of change in living beings and the process of natural selection responsible for these patterns. Living things reproduce and pass on their characteristics to their offspring. In a population, individuals that are poorly adapted are less likely to reproduce. However, over time, the characteristics that help the survival of an individual become more common, and a species gradually changes. Given enough time, these small changes can add up to the extent that a new species altogether can evolve. Thus, organisms adapt to their habitats by evolving or developing special features that help them to survive. Taken as a whole, this is evolution and it occurs only when there is a change in gene frequency within a population over time. These genetic differences are heritable and passed on to the next generations. According to Gingerich, (2001), three-time scales are of interest in evolution: (i) the generational time scale, the change from one generation to the next, (ii) the micro-evolutionary time scale regarding changes within 2 – 100 generations, and (iii) the macro-evolutionary time scale dealing with changes of patterns in a scale of hundred and more generations.

Evolution is not always a slow process, but also may happen more quickly. The evolution of a species occurs due to gradualism, punctuated equilibrium, or a combination of both (Rhodes, 1983; Saylo et al., 2011; Scoville, 2019). Gradualism is a more gradual selection and variation, which is hard to notice over a short period. In punctuated equilibrium, changes are sudden and huge. In the latter case, there is a period of little change, and then one or a few huge changes occur, often through mutations in the genes of a few individuals. Mutations that result in punctuated equilibrium are helpful to the individuals in their environments because these mutations allow the survival of those that have them. Evolution by punctuated equilibrium occurs because of huge or sudden changes in the environmental conditions, which causes strong selective pressure. This results in more rapid changes, leading to genetic and phenotypic shifts in the organisms in a matter of generations.

2.1. What happens to an organism if a constant important stimulus is no longer impinging?

The dramatic effect of an absence of a constant environmental parameter on the evolution of organisms can be seen in creatures that move from light-exposed habitats into dark environments, such as caves, deep sea, or temporally shift their activity into the night. Rétaux and Casane, (2013) analyzed all available publications about eye loss in cave inhabiting animals and concluded that genetic drift, as well as the selection of eyeless phenotypes, is the reason for eye degeneration. Interesting is the adaptation of many deep-sea fishes to their reduced light habitat: many of them did not reduce their eyes but increased the capabilities of their visual systems. Some deep-sea fish developed the ability to view color. In *Diretmus argenteus*, 38 genes for synthesis of rhodopsin were found making this fish the vertebrate bearing the highest number of genes for visual pigment synthesis (Musilova et al., 2019). In addition, many deep-sea organisms have developed strategies to replace the absence of light by producing own light utilizing bioluminescence (Haddock et al., 2010), which plays a role in communication, camouflage, or attraction of prey. Loss or reduction of light as an environmental stimulus has also led to the evolution of completely new features in animals, such as echolocation in bats and whales, or sideline organs in fish and some amphibians. An impression of the potential of evolution in the case of the absence of gravity can be seen in aquatic organisms. Although water as habitat cannot be compared to microgravity, it shares the reduced mechanical load acting onto a submerged body. Living permanently in water under reduced mechanical load, living organisms often strongly reduced stabilizing elements in their body, for e.g., in whales, which originated from dog-like land animals about 50 million years ago (Thewissen et al., 2009). However, various other factors, such as respiratory organs, increased thermal conduction, a necessity to develop new organs for locomotion (e.g., fins) affect the shape of aquatic organisms. These examples indicate the mighty potential that the space environment may have on the evolution of an exposed organism.

2.2. How does evolution work?

In living cells, genes encode proteins, and proteins guide cell function and structure. Phenotypic characters are formed during the development process by the complicated interaction of a large number of different genes, which is coordinated by molecular mechanisms. Signal molecules activate signaling pathways and complex gene interactions occur as a form of gene regulatory networks to develop phenotypic characters (Carroll et al., 2009; Davidson, 2006). Usually, many signaling pathways are involved in the development of a phenotypic character. As the complexity of the phenotypic character increases, the proportion of genes involved in signaling pathways or genetic networks increases (Nei and Rooney, 2005; Vogel and Chothia, 2006). The development of phenotypic characters depends on the function of various physiological processes or characters. Similarly, the function of physiological characters is based on the development of morphological characters. Both characters cannot be separated easily.

Changes in nucleotide sequences and coordination of genes expression at different times and dimensions in the development process play important roles in evolution. To understand the evolution of physiological or morphological characters, it is important to know the mechanism of gene regulation in the developmental process. In general, two essential cellular processes - transcription (DNA to RNA) and translation (RNA to protein) are involved in the production of proteins. The expression of thousands of genes and their coordinated regulation determine what a cell can do. The control of gene expression is important to all organisms. Activation of specific genes and the amount of protein or RNA product production are regulated by the complex interactions between genes, RNA molecules, regulatory proteins, and other components of the gene expression system. A gene is composed of protein-coding sequences and the regulatory region. During gene expression, the regulatory region that consists of the cis-regulatory elements and the transcription promoter (TATA box) initiates the transcription of the coding region of messenger RNAs (mRNAs). The basic components of gene expression regulation are the transcription factors and co-regulators, and the cis-regulatory elements, which serve as a docking site for transcription factors (Yilmaz and Grotewold, 2010). The transcription factors generated by different pathways interact with one another in the development process. Regulatory proteins that affect RNA polymerase activity bind to DNA sequences in the control regions of the genes that they regulate through specific protein-protein interactions, transfer signals to the transcriptional machinery, and results in specific rates of gene expression. In eukaryotes, chromatin structure also plays a significant

role in modulating gene expression. Recent studies have shown non-coding RNAs (ncRNAs) (e.g., microRNAs (miRNAs), short interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs)) as key components in the regulation of gene expression as well (Gomes et al., 2013; Nei, 2013; Patil et al., 2014; Yilmaz and Grotewold, 2010).

Evolution of genes and ultimately characters occur through changes (nucleotide changes or gene duplication) in the protein-coding or regulatory regions of DNAs or both. Mutation and selection play an important role in the evolution of characters. However, new functional genes may also evolve by employing or co-opting other genes for a new function (gene co-option) or by adopting foreign genes that come from outside the individual (i.e., HGT). Changes in the protein-coding region result in the alternation of the amino acid sequence of the encoded protein. This alternation sometimes changes protein function, and this change may affect physiological or phenotypic characters. Amino acid substitutions vary extremely among different proteins. A strong relationship exists between the functional constraints of gene products and the rates of amino acid or nucleotide substitutions (Nei, 2005). For instance, the amino acid substitution rate is much lower in significant proteins, such as hemoglobin and cytochrome c than in less important protein, like fibrinopeptides. In addition, the evolution rate is much lower in the active site of the protein than other regions to maintain the same function. A study on the interspecific comparison of the evolution of protein molecules (e.g., cytochrome c, hemoglobin, and insulin) involved in various physiological functions showed that the amino acid substitutions occurring in the active sites primarily lead to the functional change of these proteins (King and Jukes, 1969; Nei, 2005) and most amino acid substitutions in structural proteins are relatively neutral. This study supports the basic principle of the evolution of proteins controlling physiological characters (Nei, 1987; Nei, 2005). Pseudogenes, for example, globin gene families, which do not have any function, evolve at the fastest rate because of the non-existence of selective constraints.

Many developmental biologists emphasized that morphological evolution is a result of mutations of regulatory genes and not the structural genes (Carroll et al., 2009; Davidson, 2006; King and Wilson, 1975). In contrast, Nei, (1987) proposed the major gene effect hypothesis, which states that morphological evolution occurs by small mutations of major effects in the structural or regulatory regions of the DNA. Studies on the evolutionary changes of the protein-coding regions of genes involved in morphological evolution show that the pattern of amino acid substitution is similar to the genes involved in physiological characters (Hoekstra et al., 2006; Liao et al., 2010; Nei, 2013). However, most amino acid substitutions do not affect physiological characters noticeably, while in the case of morphological characters, a few amino acid substitutions can cause dramatic alternations. Nucleotide deletion/insertion, exon deletion, exon shuffling, and transposon insertion may also cause functional or structural changes in the case of morphogenes (Hoekstra and Coyne, 2007; Nei, 2013; Reid and Ross, 2011; Xu et al., 2012). Hoekstra et al., (2006) reported that a single amino acid substitution in the *MC1R* gene contributes to the adaptive beach color pattern in the beach mouse, *Peromyscus polionatus*. Mutation in the gene *SBE1* that is responsible for causing wrinkled seeds in pea (Bhattacharyya et al., 1993) is due to the insertion of a transposon. Albinism in cavefish is caused by the loss of function mutations (exon deletion) in the *OCA2* gene, which operates during the first step of the melanin synthesis pathway (Bilandžija et al., 2013). These examples suggest that new mutations are important for adaptation of organisms to the challenging or new environment and the innovative changes are incorporated into the genome or spread through the population by natural selection (elimination of previous genotypes) or by genetic and genomic drift. It appears that natural selection plays a more important role in phenotypic evolution than in physiological or molecular evolution, but is secondary to mutation.

Evolution is associated with both direct and indirect selection. Strong selection pressure for resistance imposed by the use of antibiotics led to the propagation of antibiotic resistance in bacteria. This points to direct selection as an underlying evolutionary mechanism (Barlow, 2009). Indirect selection can be seen for example in animals living in caves. The selection pressure induced by the dark environment in caves reduced eye size and pigmentation but promoted the development of other sensory and neuromodulatory systems in the cavefish *Astyanax* for the orientation in a dark environment (Rétaux and Casane, 2013). One may also speculate that the useless neuronal capacity for the procession of visual signals is reduced in favor of other sensory elements, such as olfactory.

2.3. Processes modulating evolution of species

2.3.1. Mutation — the major force of the evolution of species

Genetic variation is vital to evolutionary change. Without it, no subsequent change can be accomplished (Hershberg, 2015). Genetic variation in an organism is primarily generated by mutation (Nei, 2013; Wayne and Miyamoto, 2006), which, in the simplest form, is a change of DNA sequence that could not be repaired by the cellular repair system. A mutation can result from a single base-pair substitution (point mutation), an insertion of a transposable element in a regulatory sequence, a gene deletion, or a duplication of a particular gene, chromosome, or the entire genome. Point mutations, whereby a single nucleotide base is changed, inserted, or deleted from a DNA sequence, perhaps change a single protein and thus seem unimportant (Nei, 2013). However, many tiny point mutations over time affect the history of species. On the other hand, mutations that alter specific segments of genes or whole chromosomes are commonly associated with species differences or genetic diseases. In most cases, mutations have little or neutral effects, while harmful mutations reduce the fitness of an organism. Sometimes, the change in the DNA sequences is useful (advantageous mutations) and has a positive effect (Eyre-Walker, 2006). Although rare, this can lead to significant evolutionary change. The genotype generated by this mutation has higher fitness than the pre-existing ones and may form an innovative phenotype, allowing organisms to adapt better to their environment. The new genetic variant (mutant) which feeds, grows, or reproduces more effectively becomes more abundant within the population over time. Thus, mutations lead to changes in an organism, making it biologically different and unique from the original population.

Numerous studies acknowledge that organisms evolve systems to respond to environmental cues by controlling mutation rates. Mutation rate data is vital for understanding patterns of molecular diversity within species and divergence among species (Lynch, 2010a). High-throughput genome sequencing and mutation accumulation experiments, coupled with comparative approaches and constant theoretical progress are rapidly expanding our knowledge on natural mutation variation both within and among species. There is substantial evidence that mutation rates vary widely among different forms of life (Baer et al., 2007; Sung et al., 2012). In a recent study, 36 whole-genome single nucleotide polymorphism datasets from 16 species of bacteria sampled over periods extending over 2000 years were compared to estimate and compare their evolutionary rates (Duchêne et al., 2016). The evolutionary rates in the most rapidly evolving bacteria, such as *Enterobacterium faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii* were noted to be between 10^{-5} and 10^{-6} substitutions per site year^{-1}. These rates were found to be within the range estimated for other rapidly evolving bacterial species (Holt et al., 2012; Kennemann et al., 2011; Pérez-Losada et al., 2007; Ward et al., 2014) and similar to the rates estimated for slowly evolving DNA viruses (Duchêne et al., 2014; Firth et al., 2010). The pathogenic bacteria *Mycobacterium leprae* and *Yersinia pestis* show very low change of base pairs with estimates of ~10^{-8} substitutions per site year^{-1} and are considered as slow evolving bacteria (Duchêne et al., 2016; Kay et al., 2015). However, the unicellular eukaryote *Chlamydomonas reinhardtii* (large genome) exhibits an extremely low per-base mutation rate of 6.76 x 10^{-11} per cell division (Ness et al., 2012). The analysis shows somatic mutation rate is higher than germline mutation rate in humans (Lynch, 2010a, 2010b), in a wide range of tissue types in mouse and rat, in the medaka fish (Winn et al., 2000), and in *Drosophila melanogaster* (Garcia et al., 2007). The average mutation rate in human somatic cells (1.02×10^{-9} per nucleotide site per cell division) is higher than *S. cerevisiae* and *Escherichia coli* (Lynch, 2006; Lynch et al., 2008). The mutation rates in the unicellular microbes *S. cerevisiae* and *E. coli* are not much different from the germline mutation rates of multicellular species, such as *D. melanogaster* and *Arabidopsis thaliana* (Lynch, 2010a). In addition, the mutation rate differs across the nuclear genome within a species and mainly depends on the base composition, DNA repair domains, recombination rate, and gene expression and density.

The proportion of advantageous or beneficial, neutral, and deleterious mutations vary among species (Baer et al., 2007; Eyre-Walker and Keightley, 2007). Analysis suggests that about 30, 16, and 2.8% of the amino-acid-changing (non-synonymous) mutations in hominids, *Drosophila* spp., and enteric bacteria, respectively, are effectively neutral (Charlesworth and Eyre-Walker, 2006; Eyre-Walker et al., 2002), and the remaining amino-acid mutations are deleterious (Eyre-Walker and Keightley, 2007). The rate of effectively neutral mutations occurring outside protein-coding sequences (in non-coding DNA) is estimated to be at least 95% in humans and around 50% in *D. melanogaster* (Eyre-Walker and Keightley, 2007). The percentage of advantageous mutations are rare and estimated to be 0% in *E. coli* (Elena et al., 1998) and in the RNA bacteriophage φ6 (Burch et al., 2007), 4% in vesicular stomatitis virus (Sanjuán et al., 2004), and 6% in *S. cerevisiae* (Thatcher et al., 1998). Studies

indicate that deleterious mutations are far more common than advantageous ones (Drake, 2006). Also, the majority of genotypic mutations that affect phenotype are harmful (Eyre-Walker and Keightley, 2007). Therefore, an important question arises: what keeps a check on the mutation rate? An array of data shows that natural selection and genetic drift plays a significant role in determining genomic mutation rates across a wide range of taxa (Baer et al., 2007; Eyre-Walker and Keightley, 2007; Nei, 2013; Vos et al., 2015). Selection functions mostly in large populations, while drift prevails during periods of small population size (Ohta, 1992). For instance, because of its large population size, natural selection is more effective in reducing genomic mutation rates in the unicellular eukaryote *C. reinhardtii* than in the multicellular eukaryote *D. melanogaster* (Sniegowski and Raynes, 2013). Mutations and selection are dependent on each other. When the effective population size is large, selection tends to remove deleterious mutations that have negative fitness effects on populations and brings the mutation rate down to zero. However, the reduction is counterbalanced by an increase in mutation rates to facilitate ongoing adaptation. Thus, natural selection adjusts mutation rates to an optimum level by maintaining a balance between deleterious mutations and mutations that act as a source of genetic variation required for adaptation to a changing environment (Lynch, 2010a; Sung et al., 2012; Vos et al., 2015). This suggests that strongly selected advantageous mutations are exponentially distributed. There exists a variation in natural selection among individuals or species, which is associated to the differential regulation of DNA polymerases that have varying fidelities in replication, differences in mutagen exposure, and abilities of species to neutralize or block mutagens, recognize and repair DNA damage, and modulate or buffer mutational effects (Baer et al., 2007).

The speed and mode of evolution are dependent on the rate at which genomes accumulate random mutations. Sometimes evolution works much more quickly. Treatments of cells by high-energy sources such as radiation (X-rays, and nuclear radiation), chemicals, etc., induce deleterious genetic changes, but also may cause advantageous ones. Exposure of bacteria to mutagens or stress (mutation-inducing chemicals, UV-radiation, or antibiotics) increases bacterial mutation rates to evolve and adapt to the challenging environmental conditions (Cox and Battista, 2005; MacLean et al., 2013; MacLean and Buckling, 2009).

2.3.2. Role of HGT in the evolution of species

HGT, also known as lateral gene transfer, is the transfer of genetic material between organisms independent of sexual reproduction. HGT events can result in uptake of whole genes, 'genomic islands', operons, or plasmids and an instant acquisition of new functions that could be beneficial for adaptation to novel niches. Thus, HGT enables organisms to increase their genetic diversity and evolutionary fitness probably due to the gain of new genes, the gain of paralogs of existing genes, and the gain of phylogenetically distant orthologous gene (Koonin et al., 2001; Kuo and Ochman, 2009; Rocha, 2008; Sieber et al., 2017).

Horizontal gene exchange is more common in closely related organisms but also occurs between distantly related organisms, contributing to evolution (Fig. 2) (Andersson, 2005; Boto, 2010; Zhaxybayeva et al., 2006; Zhaxybayeva et al., 2007). Gene exchange is reported between bacteria and archaea (Gophna et al., 2004; Koonin et al., 1997; Nelson et al., 1999; Rest and Mindell, 2003), between bacteria and eukaryote (Anderson and Seifert, 2011; Brown et al., 1998; Dröge et al., 1998; Guljamow et al., 2007; Watkins and Gray, 2006), from archaea to eukaryote (Andersson et al., 2003), and within bacteria (Felmingham and Washington, 1999), archaea, and eukaryote (Nedelcu et al., 2008; Richards et al., 2006). For instance, the development of antibiotic-resistant and pathogenic strains due to HGT between bacterial species (Davies and Davies, 2010; Lerminiaux and Cameron, 2019). Another example is the formation of crown galls (woody protrusions) on the trunks of fruit trees because of the transfer of bacterial DNA (tumor-inducing plasmid) of the *Agrobacterium* genus to plant somatic cells and the expression of this DNA (Gelvin, 2003; Kondo et al., 2002; Pitzschke and Hirt, 2010).

It is often difficult to conclude the exact mechanisms of gene transfer between organisms as they are rare evolutionary events and identified by their consequences and not at the time of happening. The molecular basis of inter-domain HGT has been largely understood by studies of genes in model species or cell lines. The fundamental steps involved in a successful gene transfer between two lineages include the transfer of the foreign genetic material into the host cell as naked DNA, incorporation of the transferred gene in the recipient nucleus or existence as an extrachromosomal DNA, and expression into a protein that must provide a function for selection in a population. In prokaryotes, HGT is generally mediated by three potential mechanisms – transformation, conjugation, and transduction (Jain et al.,

2002). Transformation is the ability of cells to obtain foreign DNA from the extracellular environment and incorporate it into the genome of a cell. Transduction is the process of movement of genes from one species to another via viruses. Conjugation involves cell-to-cell contact for the transfer of genetic material to a recipient cell, which is facilitated by a tube-like structure known as the pilus. The process of conjugation was thought to exist in closely related bacteria because of the compatibility, similar codon usage, and higher success rate for homologous recombination (Beiko et al., 2005; Ochman et al., 2000). However, there is considerable evidence that distantly related bacteria can potentially conjugate with one another and exchange DNA (Cordero and Hogeweg, 2009; Lerat et al., 2005). These processes are less dominant in eukaryotes. Physical contact of symbiotic or host-parasite relationships, as well as transfection of a virus, have been implicated for the DNA transfer of unrelated species into eukaryotic cells (Gogarten, 2003).

HGT includes the successful transfer of genetic material as well as the existence of the transferred genetic material throughout generations (Boto, 2010). After genes are transferred, natural selection determines which of them spread throughout populations (Gogarten et al., 2002; Pál et al., 2005). Genes that have useful functions are preserved, whereas useless ones are generally removed (positive selection) (Hao and Golding, 2008; Nakamura et al., 2004). Thus, the impact of HGT in the evolution of an organism is dependent on the number of transferred genes, their successful maintenance in the recipient genomes over generations, and the extent of the phenomena concerning the evolutionary time framework and phylogenetic distance between organisms (Boto, 2010). The long-term persistence of horizontally transferred genes confirms that they confer a selective advantage to recipient organisms. There is substantial evidence that the transfer of even a single or few genes gives the recipient new functions, allowing them to exploit and adapt to the new environment (Fournier and Gogarten, 2008). For example, several anaerobes have acquired many metabolic genes from bacteria for their adaptation to anaerobic environments (Keeling and Palmer, 2008). The acquisition of genes involved in the catabolism of complex carbohydrates by rumen ciliates (Ricard et al., 2006) and the rumen fungus *Orpinomyces joyonii* (Garcia-Vallvé et al., 2000) from bacteria have facilitated their colonization and adaptation to the anaerobic environment of the ruminant gut. The pathogenesis of certain intracellular parasitic bacteria, such as *Chlamydia* and *Rickettsia* is anticipated to be due to the HGT of the chloroplast-type ATP/ADP translocases from plants, which allows them to scavenge ATP from the host (Koonin et al., 2001).

Comparative analyses of data from whole-genome sequencing projects suggest that HGT has significantly influenced the evolutionary mechanism throughout the history of life and emergence of the three domains of life: Archaea, Bacteria, and Eukarya (Andersson et al., 2003; Boto, 2010; Boucher et al., 2003; Brown, 2003; Daubin et al., 2003; Richards et al., 2006; Soucy et al., 2015). With advances in sequencing technologies, the information on the importance of HGT in microbial evolution continues to expand (Choi and Kim, 2007; Dagan et al., 2008; Koonin and Wolf, 2008; Pál et al., 2005; Treangen and Rocha, 2011). In many prokaryotes, gene gain due to HGT is considered to be a more significant process than gene duplication (Dagan et al., 2008; Treangen and Rocha, 2011; Vos et al., 2015), while the relevance of HGT in gene loss is not adequately understood (Kurland et al., 2003; Polz et al., 2013). The last few years of attained results show that HGT plays a far superior role in the evolution of archaea and bacteria than formerly thought (Kloesges et al., 2011; Sieber et al., 2017; Smillie et al., 2011; Vos et al., 2015; Wiedenbeck and Cohan, 2011). The genes and species that are associated with HGT between the archaea and the bacteria are highly diverse (Brown, 2003). Koonin et al., (2001) reported that between 1.6 and 32.6% of the genes of each microbial genome are acquired by HGT indicating that a high level of gene transfer exists for most bacterial and archaeal genomes. Almost 24% of the genes in the bacterium *Thermotoga maritima* that grow well at 80°C are more similar to hyperthermophilic archaeal genes than other bacterial genes (Nelson et al., 1999). *T. maritima* acquired genes from archaea through extensive HGT. Both cultures share reverse gyrase gene, which perhaps provides them adaptation to live at very high temperatures (Forterre et al., 2000). Significant differences in gene content (over 30%) among strains of *E. coli* demonstrate that HGT has a considerable effect on genome evolution even at the species level (Hayashi et al., 2001). Dagan et al., (2008) analyzed evolutionary history among 539,723 genes distributed across 181 sequenced prokaryotic genomes and found that at least 81 ± 15% of genes in each genome were involved in HGT. They indicated that HGT has very low barriers and can substantially affect all gene families among prokaryotes over long evolutionary time scales.

The impact of HGT in eukaryotic evolution remains controversial, in particular when multicellular eukaryotes are concerned (Huang, 2013; Ku et al., 2015; Salzberg et al., 2001; Stanhope et al., 2001). It is anticipated that HGT plays a less prominent role in the evolution of eukaryotes than in prokaryotes. A group of researchers supports that gene inheritance in eukaryotes is vertical (Ku et al., 2015) and that eukaryotic genomes show no evidence for either continuous or recent gene acquisitions from prokaryotes (Ku and Martin, 2016). However, the last decade has seen an increasing number of reports on the HGT of genes into eukaryotic genomes from both endosymbionts and free-living organisms (Alsmark et al., 2013; Andersson, 2005; Dunning Hotopp et al., 2007; Hall and Dietrich, 2007; Kishore et al., 2013; Mackiewicz et al., 2013; Marin et al., 2007; Soanes and Richards, 2014). These studies indicate that HGT had a strong impact in modulating both unicellular and multicellular eukaryote evolution. Significant numbers of HGT events contributing to genome evolution have been reported in rumen ciliates (Ricard et al., 2006), the parasitic protozoan *Entamoeba histolytica* (Loftus et al., 2005) and *Trichomonas vaginalis* (Carlton et al., 2007), the anaerobic parasite *Blastocystis* (Eme et al., 2017), the diatom *Phaeodactylum tricornutum* (Bowler et al., 2008), and the multicellular *Hydra magnipapillata* (Chapman et al., 2010). The phylogenomic analysis revealed that the metabolic repertoire of *E. histolytica* and *T. vaginalis* is shaped by the HGT of bacterial genes into their genome (Carlton et al., 2007; Loftus et al., 2005). Extensive gene transfer events have provided the diatom *P. tricornutum* with novel possibilities for metabolite management and the perception of environmental signals to thrive in oceans (Bowler et al., 2008). In a recent study, Eme et al., (2017) identified that *Blastocystis* has horizontally transferred genes both from prokaryote and eukaryote donors and the genes are involved in diverse metabolic pathways facilitating adaptation of the parasite to the gut environment, infection, and circumvention of host defenses. The gain of genes from bacteria and other eukaryotes has also resulted in the evolution of bdelloid rotifers (Gladyshev et al., 2008), fungi (Marcet-Houben and Gabaldón, 2010; Richards et al., 2006), and even plants (Huang and Gogarten, 2008). Analysis of prokaryotic-derived HGTs in 60 fully sequenced genomes showed that inter-domain transferred genes in fungi mostly included bacterial arsenite reductase, catalase, different racemases, and peptidoglycan metabolism enzymes (Marcet-Houben and Gabaldón, 2010). The coffee berry borer beetle, *Hypothenemus hampei*, which is a devastating pest of coffee, gained the bacterial *HhMAN1* gene through HGT to hydrolyze coffee berry galactomannana and colonize the coffee berries (Acuña et al., 2012). Genes that are horizontally transferred from eukaryotes to bacteria or archaea are functionally diverse and do not belong to just one or a few categories (Koonin et al., 2001; Koonin and Wolf, 2008). An example of gene transfer from eukaryote to bacteria, mainly proteobacteria, is the glutaminyl-tRNA synthetase gene, which is not present in other bacteria, the archaea (archaebacteria), and organelles (Brown and Doolittle, 1999).

Overall, there is abundant evidence to demonstrate that extensive genetic exchange has occurred in both prokaryotic and eukaryotic organisms and HGT has played a significant role in shaping our complex and remarkable biological world. HGT could potentially change genomes more rapidly than point mutation and has important implications in the evolution of organisms, such as adaptation to novel niches. Genes acquired by HGT have clear functional or environmental significance for the recipient. The acquisition of foreign genes via HGT allows organisms to thrive in new and competitive environments. As a globally important evolutionary mechanism, HGT could be used to generate novel strains that would allow them to potentially exploit and adapt to new and challenging environments.

2.3.3. Epigenetic gene regulation in the evolution of species

Epigenetics represents the changes in gene expression and function that occur without alteration in the DNA sequence (Casadesús and Low, 2006; Crisp et al., 2016; Eichten et al., 2014; Ito, 2014). Phenotype variations due to epigenetic processes are stable and inherited to the offspring. Epigenetic processes involve DNA-methylation, histone modification, and non-coding RNA (ncRNA) activity. DNA methylation includes the addition of a methyl group to specific nucleotides catalyzed by DNA methyltransferases (Casadesús and Low, 2006; Law and Jacobsen, 2009). Histone modification is a post-translational modification of the histone proteins via acetylation, methylation, phosphorylation, and ubiquitylation (Gendrel and Colot, 2005; Ito, 2014), which can influence gene expression by modifying chromatin structure or employing histone modifiers (Bannister and Kouzarides, 2011). Histone modifications act in processes such as chromosome packaging, transcriptional activity, and DNA damage or repair. The ncRNAs, such as short ncRNAs (<30 bp; miRNAs, siRNAs, and piRNAs) and the long ncRNAs (>200 bp) are also shown to regulate gene expression at the transcriptional and post-

transcriptional level by playing a role in histone modification, DNA methylation targeting, heterochromatin formation, and gene silencing (Crisp et al., 2016; Ito, 2014; Palazzo and Lee, 2015). DNA methylation change, histone modification, and chromatin and small interfering RNA (siRNA) abundance are all perturbed (induced or blocked) by both biotic and abiotic environmental conditions, resulting in the alternation of gene expression (Boyko et al., 2010; Ding et al., 2012; Khraiwesh et al., 2012; Sani et al., 2013; Zong et al., 2013).

Epigenetic effects are not only common but they can also underlie and influence many aspects of evolution (Lind and Spagopoulou, 2018) in several organisms including plants, animals, and microbes. This is true both for epigenetic effects that are visible only in a single generation as well as for transgenerational epigenetic inheritance (Fig. 3). Epigenetic regulation enables a rapid increase in variation within a species in times of environmental stresses or cues, providing an opportunity for adaptation to the arising selection pressures. The passing on of these variations to the offspring establishes epigenetic inheritance (Holliday, 2006), which is important for adaptation, above all in organisms with limited genetic variation. Plants have developed innovative approaches to survive harsh environmental stresses (Khraiwesh et al., 2012; Zhu et al., 2008; Zong et al., 2013). Among others, the efficient strategy is the epigenetic regulation of gene activity throughout their lifespan. In plants, DNA methylation and histone modification affect various biological processes in the current as well as in subsequent generations, such as growth and development, flowering time, fertility, and morphology. They regulate the silencing of transgenes and transposons as well as control genomic imprinting (Bai and Settles, 2014; Crisp et al., 2016; Ito, 2014). Stress-induced epigenetic changes can be inherited through cell division (meiotic and mitosis) (Calarco et al., 2012; Eichten et al., 2014; Feng et al., 2010). Earlier investigation showed that the DNA methylation pattern is not passed to the next generation (Monk, 1995) in mammalian cells but later studies indicated that epigenetic modifications of some genes can be transmitted through the germline (Anway et al., 2005; Cropley et al., 2006; Rakyan et al., 2003). Like in many eukaryotes, epigenetic regulation by DNA methylation (adenine instead of cytosine) also influences various cellular functions in bacteria (Casadesús and Low, 2006). It governs virulence pattern in a wide range of pathogens, controls cell cycle, provides signal for DNA replication, repair of DNA, chromosome segregation, exchange of plasmids, and bacteriophage genome packaging, and regulates activities of transposase and certain promoters as well as expression of genes (Bhagwat and Lieb, 2002; Casadesús and Low, 2006; Løbner-Olesen et al., 2005; Roberts, 1985; Wion and Casadesús, 2006).

Collectively, epigenetic modifications enable (i) survival of organism in changing environments without the need for genetic alterations (Burggren, 2016), (ii) adaptation to the new environment by the development of rapid variation due to higher rate of methylation or demethylation (i.e., the epimutation rate) of DNA and histones compared to genetic mutations (van der Graaf et al., 2015), and (iii) adaptation of small populations with a limited genetic variation or of asexual organisms to new environments. Thus, epigenetics provides a means for the development of variations within a species that can adapt at times of stress or deprivation.

2.4. Stress accelerates evolution

The interaction between an organism and the environment is central in evolution (Fig. 4). Stress is the major force driving the evolution of living organisms (Nevo, 2011; Parsons, 2005). Parsons defined stress as "an environmental factor causing potential changes to biological systems with the potential for impacts on evolutionary processes" (Parsons, 2005). Stress plays a significant role in facilitating adaptation by allowing better modifications, maintenance, and functioning of organismal systems to the ever-changing environment (Badyaev, 2005; Rutherford, 2003). Organisms become extinct when they fail to change and evolve adaptive mechanisms to diminish the detrimental effects of environmental changes.

Living cells are constantly subject to a myriad of external and internal stresses that affect their performance and well-being. External stresses are naturally occurring or man-made changes in abiotic factors, such as temperature, light, other climatic factors, and chemical components, and biotic aspects including competition, predation, and parasitism (Bijlsma and Loeschcke 2005). Internal stresses consist of genetic architecture changes in organisms brought naturally or artificially by numerous mutagenic agents, such as oxygen-free radical metabolic by-products (Baer et al., 2007; Bijlsma and Loeschcke, 1997). Both external and internal stresses result in a wide array of biological responses ranging from genotypic to physiological to phenotypic. Based on the intensity, frequency, and uniformity of stress, these responses are either associated with the development of stress tolerance, stress resistance, and

novel traits, or the reduction, migration, and extinction of a population. Evolutionary change is not always associated with phenotypic changes. Under a normal range of environments, stress result only in the expression and accumulation of novel genetic variation and physiological adaptations and no changes in morphology (Badyaev, 2005). Moderate stress is vital for normal growth and differentiation of cells and organisms (Badyaev, 2005; Clark and Fucito, 1998; Müller, 2003). For example, gravity influences the development of cells and organisms profoundly and subtly in terms of shape, function, and behavior (Bizzarri et al., 2015). Extreme environmental changes disrupt normal development, function, and may cause mass extinction events but are also associated with evolutionary changes and the origin of new species adapted to new environments (Nevo, 2011; West-Eberhard, 2003). Harsh environments occasionally lead to novel phenotypic alternations and simultaneously initiate strong phenotypic selection (directional selection) for survival and succession in changing and new environments (Badyaev, 2005; Rutherford, 2003; Wright, 2004). Examples of such shifts in populations include responses to selection arising from human activities, such as the evolution of antibiotic resistance in bacteria, pesticide resistance in insects, and heavy metals resistance in plants and invertebrates (Davies and Davies, 2010; Hoffmann and Hercus, 2000; Lerminiaux and Cameron, 2019; McKenzie and Batterham, 1994). Nonetheless, the mechanisms behind the relationship between extreme environments and phenotypic evolution are rarely understood.

Organisms' ability to recognize, assess, and react to stressors, in particular to those that are extreme, occasional, and shorter than the generation time of an organism and associated with phenotypic evolution is quite remarkable. It is intriguing to see how an organism survives stress, accommodate stress-induced modifications, and reproduce without reducing its functionality. With the increase in evolutionary studies of stress, it is widely recognized that adaptation of an organism to ever-changing environments is generally associated with an increase of recombination and mutation rates (genetic variance) and amplification of phenotypic variation (Badyaev, 2005; Hoffmann and Merilä, 1999; Horne et al., 2014; MacLean et al., 2013; Wright, 2004). Stress increases mutations rates in prokaryotes as well as in eukaryotes, which is probably the reason for discontinuous (punctuated) evolution (Velkov, 2002). The mutation rate in bacteria under stress is up to 100 to 200-fold higher compared to non-stressed cells (Foster, 2000). This increase is due to (i) activation of an SOS-regulon, which lead to the formation of a DNA polymerase IV that produces many errors during DNA-replication, (ii) activation of the homologous recombination system that is associated with single- and double-strand breaks in DNA by recombination enzymes, (iii) activation of double-strand break repair system, which restores replication of a stalled or damaged replication fork, and (iv) transient switch-off of the mismatch repair system (Bruijn, 2016; Kuzminov, 1999; McKenzie et al., 2000; Motamedi et al., 1999). Furthermore, stress increases the efficiency of interspecific gene transfer. Plasmids are considered as evolutionary tools as they can accelerate evolution by increasing mutations of their own and chromosomal genes as well (Velkov, 2002). The adaptive mutation rate is higher in plasmid than chromosomal genes (Rosenberg, 2001). In stress, bacterial cells also suppress their own restriction-modification system transiently to improve their chances of gaining new foreign genes, thereby facilitating evolutionary changes (Kelleher and Raleigh, 1995; Velkov, 2002). In eukaryotes, the heat shock protein Hsp90 enables the native folding of proteins (e.g., signal transduction and morphogenetic proteins) under normal conditions. However, under stress, this protein is involved in other important use that leads to increased accumulation of multiple mutations in vital genes (Rutherford, 2000).

3. Space environment as a toolbox for application-oriented biological research

Space is a unique environment in many aspects (Horneck et al., 2010). It provides a combination of stressors that organisms have never experienced on Earth (Fig. 5). Certain changes are noticeable only in space. Studies conducted so far show that space environment leads to alternations in genetics and variations in morphology and physiology, producing negative or positive effects on the organisms. For example, space conditions increases virulence, reduces the susceptibility of pathogenic microorganisms against antimicrobial agents, and enhances bacterial growth (reduced lag phase and enhanced exponential phase) (Horneck et al., 2010; Klaus et al., 1997; Mermel, 2012). Space offers the extraordinary environmental conditions of high radiation of galactic and solar origin, high vacuum, extreme temperatures, and microgravity (Horneck et al., 2010). The radiation in LEO is strong enough to increase the mutation rate (about 100-fold compared with that on Earth), and microgravity forces

biological systems to reorganize, adapt, and evolve. Microgravity is the absence of gravity and leads to pronounced physical changes in a sample or system environment.

3.1. Gravity and microgravity

Gravity is one of the four basic forces in physics and has two effects on an object as a function of its mass: displacement (motion) and/or deformation (weight). It is the reason for the mutual mass-dependent attraction of objects. The magnitude of the attractive force between two bodies of mass is determined from Newton's law of gravitation: $F_{12} = (G\, m_1\, m_2)\, r^{-2}$, where G is the gravitational constant (6.672×10^{-11} Nm^2kg^{-2}), F_{12} is the force resulting from the attraction by a particle of mass (m_1) on another mass particle (m_2) a distance (r) away. Force accelerate bodies. The acceleration effect of the small body (e.g., human) on a huge mass (e.g., Earth) is so negligible that the high-mass object attracts the body. The gravity field of Earth accelerates masses at the Earth's radius with about 9.81 m/s^2 (differs concerning geographic position). At Earth's surface, the path of an object to the Earth center is inhibited and gravity becomes noticeable as weight force ($F = m\, a$, where, m is mass and a is gravitational acceleration). The outcome of this relationship is so ubiquitous in our daily lives that weight is usually not considered as a variable to be experimentally manipulated. The fact that the Earth's gravity gives weight to attract matter leads to various phenomena, such as the formation of water bodies and atmosphere, hydrostatic pressure, sedimentation, convection, and increased shear forces. In addition, the physical solubility of gases such as oxygen in water depends on acceleration. Since the origin of life, all organisms are constantly exposed to the vector of gravity. Organisms, above all terrestrial ones, developed structures to counteract the impact of gravitational forces.

To annul gravity on Earth is not easy. Only conditions of free fall enable real microgravity conditions. Microgravity means acceleration conditions close to weightlessness (Horneck et al., 2010; Klaus, 2004). Weightlessness leads to pronounced physical changes in the sample environment, such as no sedimentation, no hydrostatic pressure difference within the samples or the surrounding medium in open containers, no buoyancy-driven convection: transport of metabolites reduced to the range of diffusion, and low shear forces. Acceleration directly influences the properties of membranes, as has been determined in experiments with defined vesicles and living cells (Kohn et al., 2017; Sieber et al., 2016). In cells as well as in vesicles, membrane fluidity increases significantly in microgravity, which, for example, influences the uptake and effects of drugs.

3.1.1. Sedimentation

Under microgravity, no sedimentation takes place. Various organisms use intracellular heavy particles (e.g., amyloplasts or otoliths) or the whole cell body as statoliths to sense the vector of gravity. This phenomenon is based on the higher density of those statoliths which sediment within the less dense cytoplasm. Without gravity or external acceleration this movement stops.

3.1.2. Diffusion and convection

Diffusion is essentially based on the Brownian motion and results finally in an even mixing of all types of molecules in a given limited volume. Diffusion is fast only over small distances (scale of a cell). In contrast, convection depends on specific density differences in a given volume. It allows a very effective mass transport over large distances. Diffusion is not gravity-dependent, while convection rate is. The diffusion rate of a particle depends on its diffusion coefficient D [$D = (k\, T)/(4\, \pi\, \eta\, a)$], where k is the Boltzmann constant ($1.3806488 \times 10^{-23}$ J/K) divided by NA (Avogadro number: 6.022×10^{23}), T is the temperature in Kelvin (K), η is the viscosity of the medium, and a is the radius of the particle. Typical values for 'D' are about 5×10^{-6} cm^2s^{-1} for small molecules, such as sucrose, 6×10^{-7} cm^2s^{-1} for large molecules and values below 10^{-10} cm^2s^{-1} for organelles. The square distance of the migration of a particle can be calculated by $d^2 = 2\, D\, t$. The time (t) for a particle to be translocated depends on the square of the distance (d). This means that diffusion processes are rather slow. For example, the time of a protein needed to travel 3 mm from an effector to a receptor cell would last about 2 h.

Convection is a gravity-dependent buoyancy-driven effect. The specific weight of air or fluids (except water at its point of an anomaly) becomes lower with increasing temperature. With uneven temperature distribution (e.g., presence of a heating source), heated water or air begins to move upward, while it is replaced by colder air and water. Convection results in a circulation, which also mixes the air or water and leads to a better distribution of oxygen or metabolites. No convection occurs when gravity

is absent. This may lead to severe effects on the macroscopic and eventually on the microscopic scale. The availability of oxygen is impaired when particle translocation is solely based on diffusion. Stout et al., (2001) clearly showed that hypoxia occurs in the root zone of *Brassica rapa* under microgravity. Among others, the activity of ADH (alcohol dehydrogenase) was found to be increased several-fold in microgravity indicating anaerobiosis compared to 1*g*. Not only oxygen supply but also nutrient uptake as well as dissipation of metabolic waste products are most likely hampered in microgravity. As mentioned before, many studies under real and simulated microgravity reported that metabolic enzymes were affected. The lack of convection is an indirect effect of microgravity. To overcome these problems convection needs to be replaced by air and fluid streams (which is, of course, difficult when roots are embedded in agar and also introduces a force in a force-free environment). Most likely non-motile organisms or cells are more sensitive against depleting-effects in their direct environment. Motile organisms can constantly change their position from depleted to non-depleted sites. In this respect, it would be interesting to compare e.g., the development of motile and non-motile bacteria in not-stirred containers under microgravity.

The high viscosity of the cytoplasm reduces the diffusion speed so that the Peclet number (ratio between convection and diffusion) is >1 (Todd, 1989). This means that convection contributes considerably to movement within the cytoplasm (assuming the cells do not show plasma streaming). Convection and diffusion are not only important for mass transport but also for heat transport. In cells with a high-energy conversion ratio, it would be theoretically possible that focal heat-producing cellular components, such as mitochondria, the nucleus during DNA-replication, or centrosomes may become overheated under microgravity, because of a misbalance between convectional and diffusional heat transport. Overheating of mitochondria may account for intrinsic-induced apoptosis due to cytochrome c release from damaged mitochondria [space-related apoptosis and other effects are reviewed among others in Majima et al., (2009)]. Indeed, in a variety of publications effects on abnormal distribution or ultrastructure of mitochondria were reported (Bell et al., 1992; Klymchuk, 2007; Mao et al., 2013; Nikawa et al., 2004; Philpott et al., 1990; Popova, 2003). To prove this, we propose a simple experiment in which temperature inside a cell will be measured spatially in microgravity and under 1 *g*. Recently different methods for temperature detection have been developed, based on fluorescence lifetime-imaging of thermosensitive fluorophores (Okabe et al., 2012) or sub-micrometer thermometry using nanodiamonds (Kucsko et al., 2013).

3.1.3. Shear forces

Shear forces are strongly reduced under microgravity. The random positioning machine (RPM) leads to increased shear forces and stress on biological systems because the samples are constantly moved in the field of gravity. Clinorotation, in contrast, provides an environment with very low shear forces (Hauslage et al., 2017). The luminescent dinoflagellate *Pyrocystis noctiluca*, which produces bioluminescence when experiencing shear forces, was subjected to clinorotation. The authors constructed hardware, which can be fixed on different "simulated-gravity"-devices and recorded shear force-induced light signals from the dinoflagellates. Random-positioning was found to induce strong luminescence indicating the significant presence of shear forces. In contrast, no luminescence was induced upon clinorotation. This indicates that clinorotation is probably the closest match to real microgravity which can be achieved on Earth in terms of shear forces.

3.2. Space radiation

The main fraction of space radiation reaching LEO consists of protons from solar particle events and high-energy galactic cosmic rays (mainly hydrogen ions and helium ions and some high-atomic-number nuclei and high-energy nuclei), as well as other high-energy solar particle events (Häder et al., 2009a; Horneck et al., 2010; Suparta and Zulkeple, 2014). The ionizing radiation (γ-ray, x-ray, β-ray, α-radiation, and neutron radiation) in LEO is considerably increased compared to Earth, with the perceived radiation strongly dependent on the orbit of the spacecraft. We calculated the yearly dose in millisieverts during different LEO space missions according to the data provided in a corresponding NASA report (space shuttle, Skylab, and ISS) (Moreno-Villanueva et al., 2017). The perceived level of ionizing radiation is between 85 times (space shuttle mission 41-C: about 0.69 mSv/d) and 250 times (Skylab 4: about 2.04 mSv/d) that at ground level (about 0.0082 mSv/d). Polar orbits or frequent passages through the so-called South Atlantic Anomaly, where the Earth inner Van Allen belt is thin (only about 190-200 km), will exceed the values of the above-mentioned missions multiple times. The

investigation on the influence of space ionizing radiation on different organisms is an important field of study. Ionizing radiation interacts with DNA and may lead to stable and inheritable genetic alternations, changes in gene function, metabolism, physiology, growth, shape, and structure. The effects of radiation on organisms depend on the quality and quantity of radiation, and cell age and characteristics (Horneck et al. 2010; Matallana-Surget and Wattiez 2013). Space radiation cannot be simulated on Earth to its full complexity.

4. Simulated and real microgravity platforms

Based on free fall, different measures are available to achieve real microgravity. These include drop towers (microgravity time about 5 s), aircraft parabolic flights (microgravity time about 20 – 30 s), sounding rockets (microgravity time 5 – 13 min), and orbital platforms such as satellites or space stations (microgravity time weeks to years) (Fig. 6). The "microgravity carriers" differ widely in terms of microgravity-time and microgravity-quality, as well as cost and accessibility (Pletser, 2004; Ruyters and Friedrich, 2006). Drop tower experiments and aircraft parabolic flight experiments are relatively reasonably priced and accessible in a foreseeable time scale (weeks or months). Residual accelerations are very low during drops, while they are higher in parabolic flights. Additionally, the typical acceleration profile of a parabola includes two phases of 1.8 *g* before and after the µg-phase. Sounding rockets or orbital carriers demand a long time of experiment preparation and are quite cost-intensive. Due to the high costs of those technical microgravity opportunities, extensive ground-based data sets are required for the in-depth analysis of the microgravity experiments.

To inhibit the orientation of organisms to the vector of gravity, different devices have been developed by which the gravity vector is randomized. The principle of randomizing the acceleration vector is the rotation of the samples around one or two axes. These methods are often referred to as "simulated microgravity". In any case, it is important to emphasize that "simulated" or "functional" microgravity is different from real microgravity because (omnidirectional) acceleration forces are still acting on the samples. In contrast to real microgravity, no gravitational unloading is achieved. Next to averaging the vector of gravity, these devices inhibit the sedimentation of the samples. Most common devices for averaging the vector of gravity are:

- 2D-clinostat and 3D-clinostat, where the samples are rotated around one and two axes, respectively (Hemmersbach et al., 2006). A clinostat reproduces the quiescent, unstirred fluid conditions that are achievable on orbit.
- Rotating wall vessel (RWV) bioreactors are special 2D-clinostat for liquid cultures. RWV is a suspension culture system that allows cells to grow under physiologically relevant low fluid shear conditions normally experienced by cells cultured in a spaceflight environment. A tube with a narrow diameter is rotated around its length axis (Hammond and Hammond, 2001; Klaus, 2001). The RWV bioreactor enables perfusion of nutrients to and waste from the culture.
- The random positioning machine (RPM), which in principle works similar to a 3D-clinostat, but arbitrarily changes the movement direction (Patel et al., 2007; van Loon, 2007).

Recently, diamagnetic levitation was thoroughly tested as a measure to produce microgravity-like conditions. In principle, all molecules in a sample should be lifted using an extremely strong magnetic field (10 - 16.5 T) in a way that the samples in the center of the solenoid are in the condition of weightlessness. The solenoid produces the magnetic field. Different magnetic fields result in different effective gravity levels. Manzano et al., (2012) constructed sample holders with different sample locations in the solenoid. With this method, simultaneously microgravity, Moon-, Martian- and hyper-gravity can be achieved. A "1 *g*-position" serves as an additional control. Magnetic levitation is presently the only facility which enables microgravity and low gravity-like effects in a stable environment (Manzano et al., 2012). Prerequisite is that all components of a sample have the same magnetic susceptibility and therefore this method is not suited for a variety of organisms. For example, since barium sulfate statoliths in *Chara* rhizoids have a far different magnetic susceptibility than the rest of the cell, investigations of gravitropism in this organism using diamagnetic levitation are not possible (Herranz et al., 2013). Also, a magnetic field alone can exert effects on living organisms. Paul et al., (2006) reported changes in gene expression patterns in *Arabidopsis* induced by strong magnetic fields. Significant effects of magnetic levitation on the orientation of *Euglena gracilis* as well as *Paramecium* cells, are also described (Hemmersbach et al., 2014). Under normal conditions, both organisms show a precise orientation behavior, termed as negative gravitaxis. However, upon exposure to a strong

magnetic field, both cell types aligned to the magnetic field lines. While *Paramecium*-cells were aligned parallel to the field lines, chloroplast-containing *E. gracilis* cells showed a perpendicular alignment towards the field lines. In addition, even upon application of very strong magnetic fields, it was not possible to levitate immobilized cells. This clearly shows that magnetic levitation is, at least for various biological systems, no substitute for real microgravity. Herranz et al., (2013) compared literature data of experiments performed under simulated microgravity with data from real microgravity. The survey revealed that the optimal simulated microgravity facility to achieve comparable results to real microgravity is specific for the investigated organism and the investigated phenomenon. However, after profound literature research, Kiss et al., (2019) strongly promote the application of ground-based gravity simulation but also recommend a very stringent and careful standardization of the experiments to allow better comparison between the data. Taken together, due to the fundamental physical differences between real and simulated microgravity, it is important to consider the applied experiments' environmental needs.

5. Microbiological studies in space environment or simulated microgravity conditions

To study the influence of various space conditions, microbes have been sent into space for over 60 years (Clément and Slenzka, 2006; Crabbé et al., 2011; Crabbé et al., 2013; Horneck et al., 2010; Huang et al., 2015; Juergensmeyer et al., 1999; Kacena et al., 1999; Kim et al., 2013a; Kim et al., 2013b; Nickerson et al., 2004; Rosenzweig and Chopra, 2012; Taylor, 1974; Wilson et al., 2007; Wilson et al., 2008). The two main physical factors that microbes experience in space are microgravity and cosmic radiation, while other factors, like space vacuum, thermal extremes, and solar UV radiation are mitigated by spacecraft design. Besides, several studies have been performed utilizing a low-shear modeled microgravity (LSMMG) environment to understand the influence of the space environment on a range of microorganisms (Altenburg et al., 2008; Arunasri et al., 2013; Baker et al., 2004; Chopra et al., 2006; Gao et al., 2011; Huang et al., 2015; Kim et al., 2016; Lawal et al., 2013; Nickerson et al., 2000; Pacello et al., 2012; Rosenzweig et al., 2014; Searles et al., 2011; Soni et al., 2014; Tucker et al., 2007; Xu et al., 2015). These microbes include among others *Salmonella enterica* serovar Typhimurium, *Yersinia pestis*, *E. coli*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Streptococcus mutans*, *Mycobacterium marinum*, *Bacillus subtilis*, *Streptomyces coelicolor*, *S. clavuligerus*, *S. hygroscopicus*, *Serratia marcescens*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Rhodospirillum rubrum*, and the yeasts *Candida albicans* and *Saccharomyces cerevisiae* (Horneck et al., 2010; Senatore et al., 2018; Tirumalai et al., 2019). Microbial characters were found to be changed in real and simulated microgravity conditions including metabolism, genetic transfer, growth, morphology, stress sensitivity, and pathogenicity.

A majority of microbiological studies in space have reported similar responses in space across several bacterial species, including reduced lag phase and increased cell population (Benoit and Klaus, 2007; Horneck et al., 2010). Unexplained inconsistencies deviating from these typical findings were occasionally reported over the years. A literature survey substantially proposes that cell motility is the key variable responsible for the seemingly incongruent results (Benoit and Klaus, 2007; Horneck et al., 2010). Studies of microgravity effects on motile and non-motile cells have shown that non-motile cells exhibit a difference in growth kinetics in space, while growth kinetics of motile strains is not affected under microgravity and static conditions. This is perhaps due to the lack of convection, which limits the mass transfer between cells and the fluid environment (Klaus et al., 2004; Klaus, 2004). That means the transport of nutrients from the medium to non-motile cells as well as the convection-driven distribution of waste products from cells is reduced (the only diffusion occurs), which in turn leads to the accumulation of waste products and reduction of oxygen and nutrients around the cells. This causes specific biological responses in non-motile cells, altering their growth and behavior relative to 1 *g* controls. However, these do not account for motile cells as they are constantly changing their position in the medium. A strong correlation exists between motility and cell number of culture in spaceflight (Benoit and Klaus, 2007; Horneck et al., 2010). This suggests that bacteria lack gravity-dependent physiological mechanisms but are indirectly influenced by the lack of convection in microgravity conditions. Also, it is proposed that due to their small size, it is unlikely that bacteria respond to gravity using physiological mechanisms (Horneck et al., 2010; Klaus et al., 1997). Above all, the Brownian motion superimposes the gravitational force in small objects (>10 μm) (Klaus et al., 1997). Convection is present but minimized under sub-1 *g* accelerations and thus it is important to determine the degree of

convection required for optimal cell growth and development in reduced gravity conditions (Benoit et al., 2008; Janosi et al., 2002).

Several groups of bacteria (e.g., Proteobacteria, Firmicutes, Staphylococcaceae, and Enterobacteriaceae) are present onboard the ISS (Ichijo et al., 2016), which are mostly transferred from the bodies of the astronauts. It is important to study the relationship between humans and microbes in space habitats, as they are crucial for long-term manned space missions. Space influences microbes but microbes also influence spaceflight and may become a potential threat to the crewmembers. Microorganisms play an important role in several biological processes, e.g., in wastewater treatment, but may become problematic through the buildup of biofilms that can negatively affect life-support systems. During long-term missions, they also pose risks of increased pathogenicity and development of antibiotic resistance due to unpredictable genomic changes (Tirumalai et al., 2017), which was concluded based on a growth study of Lac+ *E. coli* cells for over 1000 generations in lysogeny broth under LSMMG. Previous and recent studies have shown that space conditions increase virulence and reduce the susceptibility of pathogenic microorganisms against antimicrobial agents (increase in minimum inhibitory concentrations) (Horneck et al., 2010; Ilyin, 2005; Kalpana et al., 2016; Lapchine et al., 1986; Mermel, 2012; Nickerson et al., 2000; Senatore et al., 2018; Tirumalai et al., 2019). *S. enterica* serovar Typhimurium exhibited increased pathogenicity in mice infected with cells exposed to simulated microgravity compared to 1 *g* conditions (Nickerson et al., 2000). The cells subjected to simulated microgravity were also found to be more acid-resistant, suggesting the possibility of enhanced survival in the gastrointestinal tract, as well as showed altered protein expression patterns. Low fluid shear forces as present in microgravity increase virulence, gene expression, and stress response. This was concluded based on experiments conducted in RWV bioreactor using a *Salmonella* liquid culture (Yang et al., 2016). The increase in microbial virulence and resistance against antimicrobials in the space environment could be attributed to reduced lag phase, increased population growth, and enhanced secondary metabolite production (Horneck et al., 2010). But, conflicting reports also exist showing reduced resistance and virulence in some bacteria in microgravity conditions (Hammond et al., 2013; Juergensmeyer et al., 1999). Hammond et al., (2013) assessed the effects of spaceflight, clinorotation in a 2-D clinorotation device, and static ground controls on virulence by determining the ability of pathogens *Listeria monocytogenes*, methicillin-resistant *S. aureus* (MRSA), *Enterococcus faecalis*, and *Candida albicans* to kill *Caenorhabditis elegans*. They found all four pathogens less virulent in microgravity.

Moreover, the biofilm formation in a microgravity environment is different compared to normal gravity controls (Kim et al., 2013b; Mauclaire and Egli, 2010), which could be associated with increased pathogenesis and tolerance to antibiotics. Increased viable cells and thick and higher biomass biofilms with a column-and-canopy structure is observed in spaceflight compared to 1 *g* controls, indicating that the community-level behaviors of bacteria are highly influenced in microgravity (Kim et al., 2013b). Due to the differences in extracellular polymeric substances responsible for biofilm formation, differences in cell aggregates and clumping have been observed in *S. typhimurium* (Wilson et al., 2007) and *P. aeruginosa* (Kim et al., 2013a) cultured in space. Crabbé et al., (2011) noted differential expression of genes involved in biofilm formation and cell aggregation as well as validated microscopically the enhanced self-aggregation behavior of space flight grown *C. albicans* compared to synchronous 1 *g* controls. Increased biofilm formation has also been reported in *C. albicans* grown under LSMMG determined by microscopy and morphology-specific gene expression profiling (Searles et al., 2011). Enhanced biofilm formation ability has been noted in *Klebsiella pneumonia* in simulated microgravity conditions (Wang et al., 2016). A thick biofilm showing high tolerance to antibiotics, salt, and ethanol treatment has also been reported in LSMMG grown *E.coli* cells compared to controls (Lynch et al., 2006; Tucker et al., 2007). Recently, Cheng et al., (2014) reported that simulated microgravity alters the biofilm structure of *S. mutans* as well as increases its proportion within a dual-species biofilm (co-cultured with *S. sanguinis*). Taken together, the changes in microbial virulence, antibiotic susceptibility, and biofilm formation in combination with suppression of the immune system in astronauts because of various spaceflight stressors increase health-related concerns during a long-term manned space mission.

Significant changes in membrane fluidity have been reported in microbial cells under reduced gravity conditions as well. Vukanti et al., (2012) reported that variations in membrane potential and integrity under modeled microgravity conditions make *E. coli* and *S. aureus* cells physiologically more active and viable compared to 1 *g* samples. Kim and Rhee, (2016) also showed that the simulated space

environment alter physiological characteristics of a foodborne pathogen *E. coli* O157:H7. The study indicated that heat resistance of *E. coli* decreased under LSMMG conditions most likely due to the increases in membrane fluidity and downregulation of relevant heat stress genes.

Gravity-dependent physical alterations influence metabolic pathways, gene expression, and genetics, affecting physiology and phenotype of microbes. In the last two decades, research groups are focusing on the gene expression changes (up- or down-regulation) in response to real or simulated microgravity. Microarray analysis revealed differential expression of about 100 genes mostly involved in transcriptional regulation, virulence, lipopolysaccharide synthesis, and iron-utilization in *Salmonella* under simulated microgravity (Wilson et al., 2002). Global microarray and proteomic analyses of *Salmonella* grown aboard space shuttle mission (STS-115) showed that 167 transcripts and 73 proteins changed expression with the RNA-binding protein Hfq as a global transcriptional regulator involved in the microbial response to spaceflight conditions (Wilson et al., 2007). Crabbé et al., (2011) also found Hfq as a global transcriptional regulator, while investigating the global transcriptional and proteomic responses of spaceflight-induced *P. aeruginosa*. They reported differential regulation of 167 genes and 28 proteins and found transcriptional response data similar to cells grown in microgravity analog conditions using the RWV bioreactor. Among others, genes for oxygen metabolism, virulence-related (the *lecA* and *lecB* lectin) genes, and the gene for rhamnosyltransferase (*rhlA*) involved in rhamnolipid production were induced in spaceflight. Recently, Orsini et al., (2017) investigated the effects of simulated microgravity on *S. mutans* and reported up-regulation of 153 genes and down-regulation of 94 genes (both ≥2-fold). Genes involved in carbohydrate metabolism, translation, and stress responses, as well as genes of extrachromosomal elements, were affected. When a waterborne pathogen *Mycobacterium marinum* was grown under LSMMG in high aspect ratio vessels (HARVs) using a rotary cell culture system or the control orientation (normal gravity), it transitioned from exponential phase growth earlier than the normal gravity culture. LSMMG significantly altered transcript levels and induced a reduction in translation, a downregulation of metabolism, an increase in lipid degradation, and increased chaperone and mycobactin expression. In summary, these results suggest that LSMMG may simulate a nutrient-deprived environment (Abshire et al., 2016) as would be expected in a diffusion-limited environment as previously discussed. To understand how the microbial transcriptome responds to the environment of spaceflight, the model Gram-positive bacterium *B. subtilis* was flown on two separate missions to the ISS (Morrison et al., 2019). Cells were grown to late-exponential/early stationary phase, frozen, and then returned to Earth for RNA-seq analysis in parallel with matched ground control samples. The results indicated differences in oxygen availability between flight and ground control samples, likely due to diffusion limitation in microgravity, and to differences in cell sedimentation.

The global transcriptional profiling of the human opportunistic fungal pathogen *C. albicans* revealed that cells subjected to short-term spaceflight differentially regulated 452 genes compared to synchronous ground controls (Crabbé et al., 2013). Among others, genes involved in cell aggregation (similar to flocculation), antifungal agent resistance, stress tolerance, ABC transportation, oxidative stress resistance, transcriptional regulation, actin cytoskeleton, and ergosterol-encoding genes were observed to be differentially expressed. Enhanced random budding was observed in spaceflight-cultured cells compared to bipolar budding for ground samples. Although microgravity induced a virulence-related phenotype, no increased virulence in a murine intraperitoneal infection model was observed. This study supported the finding that physical forces in the human body, such as low fluid-shear, play a role in the infection process, advancing our understanding of infectious diseases on Earth to develop novel treatment strategies. A study on the metabolic and phenotypic changes of the yeast *S. cerevisiae* strains under microgravity revealed the upregulation of proteins related to anaerobic conditions and alternation in budding pattern with a switch towards more random budding (van Mulders et al., 2011).

Spaceflight and ground-based studies have shown that the stress environment of space (microgravity) can also affect the transfer of gene fragments or even the whole chromosome between cells. Boever et al., (2007) reported the efficient transfer of genes via plasmid-mediated conjugation in Gram-positive bacterial strain (*B. thuringiensis*) in spaceflight compared to ground control. In contrast, no differences in gene transfer were noticed between spaceflight and ground control Gram-negative species *E. coli* and *Cupriavidus metallidurans*. Another study also showed HGT of small or large plasmids in members of *Bacillus* genera (Timmery et al., 2011), suggesting that such a process could contribute to the spread of virulence potential of *Bacillus* in confined environments. However, Beuls et al., (2009) reported no significant difference in the conjugative and mobilizable transfer frequencies in

B. thuringiensis strains between 1 *g* and the three different simulated microgravity conditions established using the RWV, the RPM, and a superconducting magnet. The spread and increase of antibiotic resistance and bacterial virulence in spaceflight could be linked to efficient gene transfer events (Senatore et al., 2018; Timmery et al., 2011; Tirumalai et al., 2019). Horneck et al., (2010) suggested the need to investigate microbial adaptation and evolution via transferable genetic elements, like phages, extrachromosomal DNA, and transposons in the space environment. On the other hand, it is shown that space environmental conditions can influence the frequency of mutation in microbial cells. Short-term spaceflights experiments have shown no effect on the mutation rate or repair activity in *E. coli* (Harada et al., 1997) and S. cerevisiae (Pross and Kiefer, 1999) but a long-term experiment has shown a pronounced effect on mutation frequency (Fukuda et al., 2000). After 40 days aboard the Russian space station MIR, the mutation rate for a cloned bacterial gene was found to be 2- to 3-fold higher than the ground control (Fukuda et al., 2000). Alternation in frequency and spectrum of spontaneous mutation has also been reported in *Staphylococcus epidermidis* and *B. subtilis* cells flown aboard the ISS. Compared to ground controls the mutation rate of the *rpoB* gene leading to rifampicin resistance was noted to be significantly higher in the spaceflight samples (Fajardo-Cavazos et al., 2018; Fajardo-Cavazos and Nicholson, 2016). These findings suggest that space environment induces stresses on bacterial cells causing changes in mutagenic potential and gene transfer events.

The exploration of the unique environment of outer space (microgravity and radiation) to improve microbial strains for the production of biotechnology-relevant secondary metabolites gained interest because space environment favored microbial growth (shortened lag phase, faster growth rate, prolonged exponential phase, higher cell density, and proliferation in presence of antibiotic concentrations), promoted efficient DNA exchange, and elicited a wide spectrum of metabolic responses. Due to the high potential of the space environment to mold organisms with desired traits, Horneck et al., (2010) proposed the commercial application of space for the development of new pharmaceuticals, which would enhance our understanding of how bioprocesses occur under the absence of the ever-present gravity to design schemes and approaches to develop novel compounds. Improvement of the production of secondary metabolites, such as actinomycin D (Lam et al., 2002), monorden (Lam et al., 1998), and kanglemycin C (an immunosuppressant) (Zhou et al., 2006) by space cultured *Streptomyces plicatus*, *Humicola fuscoatra* WC5157, and *Nocardia mediterranei* var. *kanglensis*, respectively, have been reported. Recently, Huang et al., (2015) investigated the effects of microgravity on antibiotic production by *Streptomyces coelicolor* A3 and found that microgravity conditions altered secondary metabolism and resulted in the production of more bioactive substances highly active against *B. subtilis.* Several groups have also studied the effect of simulated microgravity using LSMMG. However, ground-based facilities do not produce results as observed in spaceflight experiments. Experiments to test the production of antibiotics using the RWV, which partially simulate the weightless environment, resulted in inhibition of antibiotics, such as the microcin B17 by *E. coli* (Fang et al., 1997a), β-lactam by *Streptomyces clavuligerus* (Fang et al., 1997c), and rapamycin by *S. hygroscopicus* (Fang et al., 2000). In addition, no effect of simulated gravity was noticed in the case of gramicidin-S production by *B. brevis* (Fang et al., 1997b). The response of these organisms to actual spaceflight experiments to produce antibiotics are not performed to draw conclusive results on actual microgravity effects. In addition, pilot experiments have been conducted to develop new vaccines against diarrhea-causing strains of *Salmonella* aboard the ISS (Horneck et al., 2010). Taken together, these studies suggest that space microgravity environment can alter secondary metabolism in microbes, suggesting potential production, development, and commercial application of compounds, such as antibiotics.

5.1. Conclusions from observed effects in microbes

Majority of the studies conducted till date indicate that microgravity has a strong influence on microbial growth and behavior and could lead to the evolution of properties, which does not occur on Earth, such as a column-and-canopy structure of bacterial biofilms and attainment of a growth stage that facilitates circumvention of the immune system. The mechanisms responsible for the observed biological responses, however, are not yet fully understood. We are, however, just beginning to scratch the surface in unraveling the molecular and genetic mechanisms by which gravity influences microbial growth and behavior. The major influence of a lack of gravity is a direct effect of the environment surrounding the cell simply because there is no convection only diffusion. This suggests that in

microgravity the observed changes in microbial growth and behavior are primarily indirect. Future studies focused on studying microbial behavior (e.g., biofilm production, antibiotic resistance, etc.) in conjunction with genetic studies on the regulation of the genes known to be involved with a particular behavior will lead to a clear understanding of the role gravity plays in microbial growth, development, and behavior. Studies on the interactions of both harmful and beneficial microbes with humans under altered gravity conditions need to be emphasized. Experiments with recent molecular tools are likely going to advance our knowledge and understanding and provide an intriguing foundation for the exploration of the space environment to develop novel products.

6. Microgravity and cancer research

Spaceflight affects human physiology in both a positive and a negative direction. In addition to the variety of the observed negative biological effects on the human body, caused by a long-term spaceflight (reduction in bone density, loss of muscle mass, diminished cardiac function, distorted balance, impaired vision and others (White and Averner, 2001), several studies indicate that microgravity may have an anticancer effect (Krüger et al., 2019c). The low-gravity environment significantly affects key cellular processes, including gene transcription and intercellular contacts (Hughes-Fulford et al., 2015; Lewis et al., 1998; Martinez et al., 2015) and thus influences cell growth, cell cycle, self-renewal (Arun et al., 2019) and differentiation of cancer cells without the need for drugs (Chen et al., 2019; Jessup et al., 2000; Vidyasekar et al., 2015). Microgravity slows proliferation and elevates apoptosis of different cancer cell types derived from lymphoma (Kim et al., 2017), glioma (Zhao et al., 2017), osteosarcoma (Wei et al., 2013), and different carcinomas (Chang et al., 2013; Grimm et al., 2002; Kopp et al., 2018; Kossmehl et al., 2002; Kossmehl et al., 2003; Masiello et al., 2014; Qian et al., 2008). Cytoskeletal alterations occur very early in cancer cells (Corydon et al., 2016a; Nassef et al., 2019) and are often suggested to be an effect of gravitational unloading (Vorselen et al., 2014). The impaired cytoskeleton of cancer cells in microgravity is assumed to be attributed to cell death (Arun et al., 2017; Vidyasekar et al., 2015). Qian et al., (2008) reported that simulated-microgravity affects cell migration and adhesion of breast cancer cells. Furthermore, microgravity has been reported to cause stress signaling due to mechanical forces of tensegrity (Ingber, 1999; Ingber, 2014), cellular oxidative and endoplasmic reticulum stresses (Morabito et al., 2017). Hodgkin's lymphoma cells exposed to microgravity showed dysfunctional mitochondria leading to reduced ATP levels and autophagy (Jeong et al., 2018), which was found elevated in various cell types after annulling gravity (Sambandam et al., 2014). Autophagy is involved in stemness regulation of human stem cells and contributes to the pluripotency of cancer stem cells (Kenific et al., 2016; Sharif et al., 2017). Experiments using simulated microgravity produced by an RPM (Kopp et al., 2015) as well as space missions, such as SimBox/Shenzhou-8 (Ma et al., 2013) or CellBox-2 (Melnik et al., 2018), indicated that thyroid carcinoma cells are considerably less aggressive in microgravity than on Earth. Prostate cancer cells were also found to be less aggressive and showed changes in lipid second messengers and signal transduction after exposure to simulated microgravity using the NASA-developed High Aspect Ratio Vessel (HARV) (Clejan et al., 1996; Clejan et al., 2001). This finding will now be evaluated in real microgravity. In addition, breast, prostate, and thyroid carcinoma cells form unique morphological features when exposed to simulated microgravity (Fig. 7A) - a further sign of differentiation (Corydon et al., 2016b; Kopp et al., 2015; Lelkes et al., 1998).

However, most mechanisms by which microgravity induces these cellular responses are still poorly understood and will be a subject of future research in space medicine. In this regard, microgravity-based research provides a very special culture environment to detect changes in proteins representing new targets for drug development in cancer. Many of these proteins and available drugs were found by analyzing multiple pilot studies with the help of semantic analyses (Bauer et al., 2017; Bauer et al., 2018). Using these results, scientists will be able to target or silence individual genes in cancer cells with pharmacological substances to inhibit selected signaling pathways. This way, they may be able to elucidate cellular changes in cancer development. The first success was achieved with MCF-7 breast cancer cells: metastasis-like spheroid formation was prevented by c-Src inhibition but promoted by blocking E-cadherin activity (Sahana et al., 2018). After annulling gravity, cells can arrange themselves scaffold-free into large three-dimensional (3D) aggregates without any signs of necrosis (Fig. 7B) (Ingram et al., 1997; Kopp et al., 2015).

The extracellular matrix of these multicellular tumor spheroids comprises a complex 3D network of cell-cell and cell-matrix interactions that differs in amount and composition from corresponding monolayer cultures (Ulbrich et al., 2011). It affects the distribution and function of hormones and growth factors and thus fundamentally determines the regulation of cell growth, differentiation, and apoptosis (Kunz-Schughart, 1999). The model system 'Multicellular Spheroid' (MCS) mimics metastasis of solid tumors and delivers valuable information about cancer progression *in vivo* and is often used for pharmacological drug testing (Braddock, 2019; Zanoni et al., 2019). The special 3D architecture of tumor spheroids might facilitate the penetration and action of drugs more closely mimicking the situation of a tumor in the human body. In addition, the tumor spheroids and the more complex cancer organoids that were grown from breast tumor (Kaur et al., 2011; Kunz-Schughart et al., 2001) or prostate tumor cells (Zhau et al., 1997) together with fibroblasts have become important *ex vivo* models to study cancer key processes like neoangiogenesis, tumor cell invasion, and to investigate tumor-microenvironment and cell-cell-interactions (Hamilton, 1998; Kunz-Schughart, 1999; Kunz-Schughart et al., 2004).

Microgravity, in space or produced by special devices on Earth, provides a unique environment for cancer research that has allowed insights in cancer biology that would not have been possible under normal cell culture conditions. The results already influence conventional cancer research and contribute to the understanding of cancer progression and the development of new drugs and cancer treatment strategies.

Upcoming projects will examine the roles of both microgravity and cosmic radiation in tumor growth and development onboard the ISS and the Chinese Space Station (CSS). The studies of cosmic radiation, the main concern for space exploration by humans, will also help with understanding the cancer risk for astronauts on long-duration stays on space stations, or longer journeys, such as to Mars.

7. Tissue engineering in space

Microgravity-based scaffold-free tissue engineering is a novel technique in translational regenerative medicine. For this purpose, the cells are exposed to microgravity in space or to devices simulating microgravity conditions on Earth, so-called ground-based facilities (GBF), acknowledged among others by ESA (European Space Agency) and NASA. A large number of studies performed in real and simulated microgravity revealed that one part of the cultured cells of various cell types detaches from the bottom of the culture dish and starts forming 3D aggregates or MCS (Grimm et al., 2018), while another part remains growing as a 2D monolayer on the bottom of the culture flask. These MCS resemble the organs or original tissues from which the cells originally derived. In this section, we review the current knowledge about tissue engineering of cartilage, bone, and tube formation by endothelial cells in space and on ESA-GBF.

7.1. Cartilage tissue engineering

Diseases of the cartilage comprise osteoarthritis, a frequent cause of chronic pain, and a disorder with increased prevalence worldwide due to the aging population. Unfortunately, no causal treatment is available, and the major objective is to treat the pain and to attenuate the disease progression. Therefore, new research focusing on the chondrocytes is mandatory and new ideas are welcome. Microgravity-based approaches of cartilage tissue engineering have been performed in space on the MIR space station. Bovine chondrocytes were grown in 3D polyglycolic acid matrices at 1 *g* and cultured in rotating bioreactors for three months. One of these reactors was subsequently sent to space (MIR) where the cell culture continued to grow for another four months. In parallel, the remaining samples were kept as ground controls. Both culture conditions resulted in functional cartilage tissues, but the cartilage sample cultured in space revealed a lower size and mechanical strength (Freed et al., 1997; Freed et al., 1999; Freed and Vunjak-Novakovic, 1997).

Other researchers performed a scaffold-free experiment with porcine chondrocytes, which were cultured in special cylindrical culture chambers. The cells were exposed to real microgravity on the ISS, to simulated microgravity on an RPM and 1 *g* on Earth. The ISS samples revealed a reduced amount of extracellular matrix proteins together with a reduced cell density compared to the 1 *g* controls (Stamenković et al., 2010). Various experiments with chondrocytes have been performed to engineer cartilage constructs using ESA GBF (Aleshcheva et al., 2016). Ohyabu et al., (2006) demonstrated that

rabbit bone marrow mesenchymal stem cells cultured in an RWV bioreactor formed large cylindrical cartilaginous pieces without scaffolds. Another interesting approach was the production of hyaline cartilage using a RWV (Sakai et al., 2009). Human mesenchymal stem cells (MSC) exposed to simulated microgravity revealed an increased proliferation, and among others, a higher expression of collagen II compared to 1 *g*-samples (Yuge et al., 2006). Only the simulated-microgravity samples were able to form hyaline cartilage after transplantation in cartilage defective mice (Yuge et al., 2006).

Taken together, cartilage tissue engineering with and without scaffolds is an elegant technique to produce viable, functional cartilage constructs (Fig. 7C) (Grimm et al., 2018). Tissue engineering of comparable constructs is not possible with conventional static 1*g*-culture conditions. Furthermore, future research should increase scaffold-free approaches in space accompanied by GBF studies, avoiding potential pitfalls associated with non-autologous biomaterials. A more personalized approach should be introduced by studying patient's chondrocytes after, for example, an injury to engineer autologous cartilage.

7.2. Tissue engineering of bone

Microgravity-induced osteopenia and bone loss is a serious health problem for long-term space missions (Grimm et al., 2016). Astronauts lose between 1 and 2% of weight-bearing bone mass during long-term missions (Cappellesso et al., 2015). These changes in bone structure and metabolism are still an important health concern of humans in space today (Chatani et al., 2015; Grimm et al., 2016; Vico et al., 2000). Despite this knowledge, it is of interest that simulated microgravity also induces bone formation. Bone tissue engineering using microgravity-based methods is currently a hot topic in regenerative medicine. The incidence of bone diseases is increasing because of aging populations, obesity, and reduced physical activity (Amini et al., 2012). The destruction of bone tissue is often a result of trauma or disease. The use of stem cells, specialized cells, scaffolds, and growth factors to improve bone formation are discussed as possible therapeutic strategies. Tissue-engineered bone fragments from human osteoblasts might serve as a potential alternative to the conventional use of bone grafts.

Various research teams used the NASA-developed RWV in bone tissue engineering studies. These RWV-derived bone constructs from osteoblasts and stem cells were comparable to natural bone and had demonstrated an improved *in vivo* effectiveness in bone repair in animal studies (Cerwinka et al., 2012; Cui et al., 2018; Han and Dai, 2016; Jin et al., 2010; Sikavitsas et al., 2002; Ulbrich et al., 2014). Clarke et al., (2013) had developed a new method for tissue engineering of bone. The authors used successfully a low shear stress rotating bioreactor, such as a HARV culture system. Mann et al., (2019) exposed human fetal osteoblast (hFOB 1.19) cells to an RPM for 7 and 14 days. This new study was the first that demonstrated a scaffold-free approach to engineer 3D bone constructs on the RPM (Fig. 7D). The next step will be to investigate these constructs in an *in vivo* animal model.

Taken together, new discoveries have been published in the field of microgravity-based bone tissue engineering. Due to the huge impact of musculoskeletal medical conditions on Earth and for future space exploration, further studies on the influence of microgravity on osteoblasts and stem cells are important. This novel technology first can be used to study the mechanisms behind spaceflight-related bone loss and second, the engineered bone constructs might be suitable for the repair of bone diseases such as osteonecrosis or bone injuries.

7.3. Growing blood vessels in space

Tissue engineering of scaffold-free small biocompatible blood vessels, which are applicable for vascular reconstruction purposes in transplantation, plastic, aesthetic, and hand surgery (Fig. 7E), is an important medical research topic. A key benefit of this technique is the scaffold-free engineering of 3D tubes without any foreign materials. Under normal static gravity (1*g*) conditions in Earth-based laboratories, a 2D monolayer of endothelial cells (ECs) is comparable to the inner surface of blood vessels (intima) but does not completely show the vascular characteristics found *in vivo*.

Sanford et al., (2002) cultured bovine aortic ECs in an RWV for up to 30 days. They found large tissue-like aggregates (containing 20 or more beads) after 30 days. This was the first report of a unique growth pattern for ECs, resulting in enhanced NO production and barrier properties. Another group used a perfusion system and developed a cell culture model for a small-diameter vessel engineered from

adipose tissue stromal cells together with human umbilical vein endothelial cells using a tube-like gelatin scaffold (Frerich et al., 2006; Frerich et al., 2008). Human umbilical cord blood stem cells (CBSC) were exposed to an RWV for 14 days (Chiu et al., 2005). CD34+ mononuclear cells were cultured in the bioreactor with or without microcarrier beads (MCB) and vascular endothelial growth factor. RWV cultures showed increased proliferation and 3D tissue-like aggregates. At day 4, CD34+ cells without MCB developed vascular tubular assemblies and exhibited endothelial phenotypic markers. The CD34+ CBSC is capable to differentiate in a vascular endothelial cell phenotype and to form 3D tissue structures when exposed to the RWV (Chiu et al., 2005). Another study focused on MSCs exposed to simulated microgravity for 72 h. The authors demonstrated that MSC was able to differentiate into endothelial-like cells, express endothelial-specific molecules (Flk-1 and vWF), and form a capillary network (Zhang et al., 2013). Using a clinostat, (Shi et al., 2016) showed that clinorotation induced cell migration and tube formation in ECs *in vitro* depending on caveolin-1. A large number of studies of our team investigating ECs exposed to simulated microgravity conditions had demonstrated that ECs can form 3D tube-like structures (intima constructs) (Dittrich et al., 2018; Grimm et al., 2009; Grimm et al., 2010; Infanger et al., 2006; Ma et al., 2013; Ma et al., 2014; Ulbrich et al., 2008). In microgravity, the *de novo* construction of scaffold-free, functional, organo-typic tissues has a great advantage, because ECs grow as spheroids and as 3D tubular structures without requiring any artificial matrix or scaffolds (Grimm et al., 2018). These tubes can be cultured for more than 35 days. Interestingly, they do not develop necrosis or stop growing (Dittrich et al., 2018). Human endothelial cells of the EAhy926 cell line had been sent to the ISS (SpaceX CRS-8 mission) in 2016 to determine the impact of real microgravity on 3D growth (Pietsch et al., 2017). The ESA-SPHEROIDS project confirmed the formation of 3D vessel constructs (Krüger et al., 2019b; Pietsch et al., 2017). Cell culture in space was possible with a specifically designed automatic hardware, which allowed programmed cell feeding and fixation (Pietsch et al., 2017). A recent study reported the impact of clinorotation on endothelial progenitor cells (EPC) (Hagiwara et al., 2018). The gene expression analysis revealed an increase in angiogenic factors, including vascular endothelial growth factor. Moreover, the initial cultivation on a 3D clinostat followed by cultivation under 1*g* remarkably improved the EPC expansion rates and the angiogenic potential (Hagiwara et al., 2018). This interesting approach should be investigated in real microgravity in future space experiments.

In summary, microgravity-based research contributes to new developments in regenerative medicine using stem cells or ECs. Co-culture experiments of ECs with vascular smooth muscle cells and fibroblasts on an RPM will be a further step to engineer a complete, functional vessel. These constructed vessels can be co-cultured with cancer MCS and might be suitable for the use in pharmacological drug testing to reduce the number of animal experiments, or for radiation experiments.

8. Plants and algae in real or simulated microgravity

8.1. Plant-based studies

Same as in animals, genetic programs and networks control plant growth and development. Plants use a variety of stimuli, such as light, temperature, gravity, and water to orient themselves in their natural habitat. Mechano-perception is crucial for the regulation of plant growth and development as well as for its life. Mechanical touch perception enables adaptation to environmental conditions, such as wind. In trees, the weight of a branch induces the increased formation of wood at its onset to overcome the notch stress. Responses to stimuli such as gravity and light are important for the orientation of root and stem growth in plants to anchor and absorb water and minerals as well as to orient their leaves towards sunlight (Kordyum, 2014; Kordyum and Chapman, 2017). These factors are the most stable and reliable signal utilized by plants for their survival throughout evolution. Physiological orientations towards light and gravity are termed as phototropism and gravitropism, respectively. Microgravity experiments allow us to elucidate the mechanisms of gravitropism in plants. A notable number of spaceflight experiments with plants (monocots and eudicots) *in vivo* and *in vitro* (organ, tissue, cell, and protoplast cultures) have been performed on board satellites, space shuttles, and orbital stations. These studies show that gravity and other spaceflight factors influence plants at the organismal, cellular, subcellular, and molecular levels, leading to changes in orientation, response to stress, development, metabolism, and growth. These experiments have contributed to the understanding of gravity perception, signal transduction, intrinsic cell-division processes, adaptation to microgravity, and gravity-induced distribution of organelles in plants and accompanying morphometric traits (Correll et al., 2013; Ferl et

al., 2002; Kiss, 2000; Kordyum and Chapman, 2017; Paul et al., 2012a; Paul et al., 2012b; Stutte et al., 2006).

In earlier investigations of gravity and microgravity, solely the measurement of physiological or anatomical parameters was possible. Currently, an arsenal of molecular biological methods is available to investigate transcription levels, protein expression and modification, or metabolic profiles. Kimbrough et al., (2004) carried out whole-genome microarray analysis of *Arabidopsis* root apices after gravity stimulation (reorientation) and mechanical stimulation to investigate the effect of gravity and mechanical forces on plants. Out of 22,744 monitored genes, 1730 (7.6%) and 1691 (7.5%) genes were found to be altered in transcript abundance in response to gravity or mechanical stimulation, respectively. An increase in transcription factors, genes encoding cell wall modifying enzymes (xyloglucan endotransglycosylases, and pectinesterases), transporters (ions, sugar, purines), and genes involved in the response to other environmental stresses (dehydration, cold, and pathogen response) were observed after gravity stimulation. The severe effect of just reversing the position of a polar plant organ towards the gravity vector was demonstrated with the gynophore (a stalk supporting the female part of a flower) of peanut (Li et al., 2013). Some of the gynophores were positioned upside-down, while the others were kept in their normal position and changes in transcriptome and proteins were detected. After 6 and 12 h, more than 170 and 420 genes were found to display altered expression levels, respectively. The proteomic analysis performed via 2D-gel electrophoresis and subsequent MALDI-TOF analysis revealed that especially proteins involved in stress or plant defense as well as proteins of the carbohydrate metabolism were influenced due to reversing the position of gynophores. Several studies have reported the effects of gravity on the transcriptome and proteome of *Arabidopsis* (Hausmann et al., 2014; Martzivanou et al., 2006; Martzivanou and Hampp, 2003). Hyper-gravity of 7 x *g* for 1 h resulted in up- or down-regulation of around 200 genes among 4100 analyzed genes of *A. thaliana* (Martzivanou and Hampp, 2003). Mainly genes of major pathways, genes of proteins involved in cellular organization and cell wall formation, phosphorylation events, and signaling were altered. The fraction of stress response as well as genes involved in gravity-sensing was considerably lower. Significant changes in the expression of various genes in *A. thaliana* due to the effect of subsequent short-term microgravity phases (each about 20 s) achieved during parabolic flight maneuvers have also been reported (Paul et al., 2011). Transcriptome profiling revealed that parabolic flight caused changes in gene expression by prominence of genes associated with signal transduction, stress, and biotic and abiotic stimuli.

Various experiments with *A. thaliana* callus cultures as a test system have been performed under different accelerations and microgravity conditions as well (Babbick et al., 2007; Barjaktarovic et al., 2009; Barjaktarović et al., 2009; Hausmann et al., 2014; Martzivanou and Hampp, 2003). It seems likely that also in plant cell cultures, acceleration or microgravity induces a variety of unspecific effects ranging from changes in transcription level to protein translation and modification as well as changes in the metabolome. Proteome analysis (2D-gel electrophoresis and subsequent ESI-MS/MS) of *Arabidopsis* callus cells following simulated microgravity (RPM) as well as hypergravity revealed that a variety of proteins changed. Largely, proteins involved in radical scavenging and detoxification of reactive oxygen species (ROS), primary metabolism, and signaling were found to be different from controls (Barjaktarović et al., 2009). ROS generation is likely due to the activity of calcium-dependent NADPH oxidases (Hausmann et al., 2014) or linked to auxin signaling (Tan et al., 2011). Transcription levels of ROS-related enzymes, like ascorbate- and glutathione peroxidases, which are involved in ROS homeostasis of cells altered during microgravity (Barjaktarovic et al., 2009; Barjaktarović et al., 2009). Significant changes in protein phosphorylation, mainly in stress proteins are also reported (Barjaktarovic et al., 2009). Calcium-dependent genes also changed in short and long-term microgravity exposure (Hausmann et al., 2014; Paul et al., 2012b; Salmi et al., 2011; Salmi and Roux, 2008).

Wang, (2006) investigated the effect of vertical clinorotation on the proteome of *Arabidopsis* calli (horizontally rotated calli served as a control). Among others, stress response proteins, enzymes of the carbohydrate metabolism (e.g., glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate-isomerase), signaling proteins, and transcription factors were found to be altered. Determination of clinostat effects on the proteome of intact plants (wild type and a *pin2* mutant, which exhibits a gravity-insensitive root growth phenotype) came to a similar result (Tan et al., 2011). Altered gravity conditions significantly elevated the expression of certain classes of proteins, like those involved in detoxification (GST 6, NAD^+-ADH, GS-FDH), in chaperone functions (HSP70) and in mediating resistance to stress factors correlated with energy metabolism (mitochondrial ATP synthase, putative inosine-5'-

monophosphate dehydrogenase, and isocitrate dehydrogenase) in both wild type and *pin2* mutant (Tan et al., 2011).

During a parabolic flight different molecular and physiological parameters were investigated (Neef et al., 2015). During microgravity, ROS molecules and cytosolic calcium increased in *A. thaliana* cell cultures (Ca^{2+}-sensor YC3.6-cells). Phosphorylation analysis revealed that in hyper-*g*, superoxide dismutase, ascorbate peroxidase, glycerol kinase, phosphoglyceromutase, annexin, a microtubule-associated protein and ATP-dependent NAD(P)H hydrate hydratase, serine/threonine phosphatase (protein regulation), UDP glucose dehydrogenase, V-Type proton ATPase, Hsp70, and malate-dehydrogenase genes were influenced. Under microgravity, phosphorylation of triose-P isomerase, inorganic pyrophosphatase, pyruvate dehydrogenase, citrate synthase, Hsp70, CML14 (Ca^{2+}-binding protein), phosphoenolpyruvate carboxykinase, fructokinase, and dehydrogenase ferritin-2 were found to be altered compared to the control. While only a few proteins changed by varying gravitational conditions, the effect on gene expression (measured with Affymetrix GeneChip expression analysis) was much more pronounced. Gene expression in hyper-*g* and microgravity showed a strong overlap in 1378 up- and 1769 down-regulated genes. In addition, many genes were exclusively altered in microgravity (396 up and 485 down) or hyper-*g* (413 up and 637 down directly at the onset of 1.8*g*). Genes involved included calcium-binding, signaling (calcium Ins(1,4,5,)P3), stress response, and detoxification (e.g., catalase). Microarray analysis of *A. thaliana* calli cultures subjected to magnetic levitation, which "simulates" different gravity levels (microgravity, 0.1 *g*, 1 *g*, and hyper-*g*) has revealed changes in stress and metabolic genes among others in high gradient magnetic fields (Manzano et al., 2012). Also, Paul et al., (2013) found significant stress responses (effects on heat shock proteins and chaperons) of *Arabidopsis* tissue cultures in space.

Correll et al., (2013) investigated the transcriptome of *A. thaliana* seedlings in space (TROPI-2 experiment on the ISS) and found that the transcription of 982 genes differed from ground controls. Among these genes, 135 and 143 were noted to be up- and down-regulated at least two-fold, respectively. Next to many undefined genes, a large number of genes involved in salt stress, oxidative stress, detoxification, biotic-induced stress, water stress, and defense response (and other stress responses) were among the differentially expressed ones. In addition, changes in the actin cytoskeleton organization that among other functions is involved in the transport of cell wall components to the cell periphery on exposure to microgravity were noted. In a spaceflight experiment (BRIC-16 on STS-131, 2010), Paul et al., (2012b) analyzed and compared the transcriptome of *A. thaliana* seedlings and cell cultures. While the seedlings reacted moderately over a variety of functions, the cell cultures reacted mainly with the transcription of stress response and heat shock proteins. For instance, the expression of the heat shock transcription factor HSFA2 or the heat shock protein Hsp70 was noted to be 20-fold higher in cell cultures. This shows that even undifferentiated plant cells perceive the absence of gravity as stress but do so in a more general sense than plant seedlings, which have specialized organs to sense their environment and react. In seedlings, among others, genes involved in pathogen response and wounding, stress response (cold and drought stress), auxin-mediated root development, and calcium signaling were also found to be different compared to the ground control. During a long-term experiment onboard the ISS, Paul et al., (2013) investigated the seedlings of *A. thaliana* and found 1480 genes to be differentially expressed from 1*g* controls (centrifuge). Also, gene expression was found to be organ-specific. About 181 genes were found to be exclusively altered in roots, only 114 in the hypocotyl, and only 113 in the leaves. Leaves, hypocotyl, and roots shared only a few genes, which were altered in all organs (26 of 408). Differentially expressed genes codes for proteins of touch-wounding response, cell wall remodeling, growth hormone, and signal transduction. Perhaps altered hormone-signaling affect the cell wall remodeling genes, influencing the growth and cell expansion (Schüler et al., 2015).

Changes in transcription in plants after transfer to microgravity are not surprising considering the report of Millar and Kiss, (2013), where, transcription and metabolomics during tropistic responses were investigated. In this study, seedlings were irradiated unidirectional with red or blue light to measure phototropic responses and tilted towards the gravity vector to induce gravitropic responses. In all tropisms, genes for carbohydrate and secondary metabolism were found to be downregulated, while different genes involved in amino acid metabolism were found to be upregulated. Changes in gene expression were highest in gravitropism compared to different phototropisms. Especially genes coding for proteins of subcellular localization, proteins with binding function, defense genes, and metabolic genes were more profoundly altered during gravitropism. It was found that various metabolites, which are typical for biotic and abiotic stress, such as asparagine, phenylalanine (interaction between ethylene

and auxin), threonine, sucrose (correlation to osmosis), etc. were increased upon all tropistic reactions. In a very recent study, Vandenbrink et al., (2019) reported down-regulation of light-associated pathways (e.g., photosynthesis-antenna proteins, photosynthesis, porphyrin, and chlorophyll metabolism) in *A. thaliana* grown in a microgravity environment and irradiated with a blue light onboard the ISS compared to 1 *g* control. In contrast, pathways involved in ribosome biosynthesis, which has been associated with elements controlling cell growth and proliferation (Kamal et al., 2018), and oxidative phosphorylation were upregulated. The authors suggest a connection between gravity and phototropism in *Arabidopsis*.

In other investigated plants, acceleration changes led to changes in protein patterns or transcription profiles as well. The transcription profiles of spores of the fern *Ceratopteris richardii* germinating in microgravity and 1 *g* analyzed during the NASA space shuttle mission STS-93 revealed changes in the mRNA expression of roughly 5% of genes analyzed (Salmi and Roux, 2008). Various genes were noted to be altered, e.g., transferases, hydrolases, and heat shock proteins. Bushart et al., (2013) also identified potential modulators (calcium pumps and apyrases) for gravity response in *Ceratopteris*. Jin et al., (2015) analyzed rice calli grown on the Chinese satellite Shenzhou-8. In comparison to 1 *g* ground and 1 *g* inflight controls, they found 627 microgravity responsive transcripts, mostly involved in the cell wall structure, primary metabolism, transcription, calcium regulation, and others. Stutte et al., (2006) investigated the effect of microgravity on dwarf wheat (leaf ultrastructure, biochemical, physiological, and transcription levels) grown in real microgravity (located in a Biomass Production System (BPS)-device) during a space shuttle flight and subsequent installation on the ISS. Interestingly only marginal changes in leaf ultrastructure (e.g., thinner cross section and higher cell density) and chloroplast (more ovoid shape, slightly different thylakoid structure-no effect of photosynthetic performance) compared to a 1 *g*-ground control were detected (Stutte et al., 2006). Transcription was not significantly impaired. Xu and Guo (Xu and Guo, 2013) investigated RPM-effects on six conserved miRNAs in *Solanum lycopersicum*. After 30 days of RPM, the expression level in six investigated miRNAs was found to be increased after transient reduced expression before. Putative targeted proteins of this miRNA are involved in physiological and metabolic processes (protein kinases, oxidoreductase), transcription factors, and others. Real microgravity resulted in the detection of 19 differentially expressed proteins in *Oryza sativa* investigated using 2D-gel electrophoresis and mass spectrometry (Ma et al., 2007). Four of these proteins could be identified: hydrogen peroxide oxidase, ascorbate peroxidase, a manganese-stabilizing protein, and a ribosomal protein.

Recent studies have indicated that epigenetic mechanisms (DNA methylation and histone modification) control gene expression in plants in gravitational alterations indicating that gravitational changes are sensed as stress by plants (Kamal et al., 2018; Ou et al., 2010; Xu et al., 2018; Zhou et al., 2019). The regulation of genes expression via epigenetic modification affects plant responses to environmental stresses as discussed before. Kamal et al., (2018) performed experiments with cell cultures of *Arabidopsis* under simulated micro- and Martian-gravity (RPM-treatment) as well as under hypergravity (centrifugation) and observed that samples exposed to artificial microgravity and a lower extend cells exposed to Martian-gravity showed an increased DNA methylation level compared to 1 g and hypergravity conditions. Also, this study revealed effects of simulated microgravity or simulated Martian gravity on the cell cycle (lower fraction of cells in G1-phase), nucleolus structure (decrease of cells showing compact nucleolus structure and increase of fibrillary structure) as well as changes in levels of proteins involved in cell cycle regulation. Recently, Xu et al., (2018) investigated the methylation pattern changes in *Arabidopsis* seedlings exposed to real microgravity conditions onboard the Chinese recoverable scientific satellite SJ-10. They found widespread methylation of genes involved in metabolic pathways, biosynthesis of secondary metabolites, ribosome pathway, starch and sucrose metabolism, phenylalanine biosynthesis, purine, glutathione, amino sugars, and nucleotide sugar metabolism), proteasomes, nucleotide excision repair, and ribosomes among others. Ou et al., (2010) found significant changes in genetics, as well as DNA methylation frequencies (hyper- and hypo-methylation), in rice plants (*O. sativa* L.) germinated from seeds kept in space for 18 days. Among the assessed 460 genomic loci, the genetic change frequencies ranged from 0.7% to 6.7%, while hypermethylation frequencies were about 1.95% for CG and 1.44% for CNG. The hypomethylation frequencies were noted to be 0.76% (CG) and 0.80% (CNG). In a very recent whole-genome survey of DNA methylation, Zhou et al., (2019) found differentially methylated genes in microgravity-exposed *Arabidopsis* (ISS).

Chinese space researchers combined space, biological, and plant-breeding technologies for screening and identifying diverse new varieties of plants through a space mutation breeding program

carried out from 1987 to 2005 (Chengzhi, 2011; He et al., 2006; Xianfang et al., 2004). They pointed out that the space environment may result in genetic mutagenesis of seeds or seedlings and thus open the route to develop new varieties of plants. He et al., (2006) summarized the progress of this space mutation breeding program and reported that Chinese scientists and agricultural biologists sent more than 1000 varieties of different plant seeds (crops, floricultural plants, vegetables, fruits, medicinal plants, and forest trees) into space. The space-exposed seeds grown on Earth showed positive physiological and genetic variations. Through this 'space breeding' program, Chinese research institutes (70 involved) identified more than 200 promising new varieties of plants exhibiting high quality, high yield, and disease resistance properties. Many of the space-breed plant varieties are cultivated in large scales in some provinces in China (extended up to 270000 hectares) and are bringing social-economic benefits (Xianfang et al., 2004).

Based on several years of studies on plant systems under different acceleration and microgravity conditions it is evident that gravity affects several mechanisms in plants. Plant systems develop or activate a range of molecular responses to raise their defenses upon exposure to gravity-related conditions. These conditions change directly or indirectly the information in the genome, which influence the transcription process and eventually result in the production of changed proteins. Gene activity is affected by non-genetic mechanisms, and the level of stress-induced transcripts alters. In addition, certain repressive RNA transcripts activate and signaling molecules vital for cell survival and adaptation accumulate. The combination of all these responses changes the global systemic organization of plants for the potential recovery, development, and growth under gravity-related conditions. For instance, *A. thaliana* germinated in space but plants grew smaller and roots showed different growth patterns (Millar et al., 2011; Paul et al., 2012a; Paul et al., 2012b). Altered acceleration leads to changes in gene expression which is associated with specific responses in whole plants, seedlings, and plant cell cultures, and differs between different plant organs (Paul et al., 2012a; Paul et al., 2012b; Paul et al., 2013) but share some common gene changes. For example, genes involved in root hair development and generation, cell wall remodeling, cell wall modifying enzymes, actin cytoskeleton association, metabolism and response to ROS, calcium signaling, and general stress responses (Correll et al., 2013; Fengler et al., 2015; Fengler et al., 2016; Hausmann et al., 2014; Mazars et al., 2014a; Mazars et al., 2014b; Paul et al., 2005; Paul et al., 2011; Paul et al., 2013; Schüler et al., 2015; Schüler et al., 2016; Zhang et al., 2015; Zhang and Zheng, 2015). The involvement of the second messengers ROS, Ca^{2+}, and heat shock proteins, as well as distinct changes in the transcriptome, indicates that microgravity is a significant stressor for plants. In summary, whole plants seem to be less affected by microgravity-induced physical phenomena than cell cultures because (i) the cells possess a high internal pressure (turgor) caused by the osmotic pressure of the huge internal vacuole which pushes the cytoplasm against a rigid cell wall and (ii) because of intracellular cytoplasma-streaming, which mixes the cytoplasm also in the absence of microgravity.

8.2. Algae-based studies

Intensive gravitational biology work over many years has made green macroalgae *Chara* a well-established model system for studies on polarized growth and gravity-sensing in plants (Braun et al., 1999; Braun et al., 2004; Braun and Limbach, 2006; Braun and Richter, 1999; Braun and Sievers, 1994). Microgravity experiments during a parabolic flight campaign revealed that the physical contact to a hypothetical receptor protein is responsible for signal transduction and not the force applied by the statoliths on the lower membrane (Limbach et al., 2005). Many studies have been conducted with unicellular photosynthetic organisms. The single cellular green algae *C. reinhardii* shows precise gravitactic behavior. Although non-gravitactic mutants exist (Yoshimura et al., 2003), it is still controversially discussed, whether this phenomenon is based on active physiological mechanisms or solely on physical effects such as buoyancy or bottom-heaviness (Häder et al., 2006b; Häder et al., 2006a). Mathematic models and calculations support physical mechanisms (Hagen et al., 2014). In recent years, the unicellular flagellate *Euglena gracilis* has become a model organism for gravitational research. Its behaviors (phototaxis and gravitaxis, i.e., response to the direction of light and gravity as stimuli) have been extensively investigated during various space experiments (Nasir et al., 2014; Strauch et al., 2010) because *E. gracilis* use physiological mechanisms to sense gravity and light for orientation in its habitat. Several experiments in microgravity helped to develop a working hypothesis of the mechanisms involved in gravitaxis. According to it, mechano-sensitive channels are periodically opened

when the cells deviate from a vertical path upwards (Lebert and Häder, 1996, 1997a, 1997b). This allows for the influx of calcium (Richter et al., 2001; Richter et al., 2002), which in turn activates a calmodulin (Daiker et al., 2010; Häder et al., 2009b). Subsequently, an adenylate cyclase increases the intracellular cAMP levels, thereby activating a specific protein kinase A (PKA) (Daiker et al., 2011; Streb et al., 2002; Tahedl et al., 1998). Curiously, the phototactical signal transduction chain also leads to that PKA (Häder et al., 2005; Häder et al., 2017; Ntefidou et al., 2003). A molecule interacting with PKA was found in the flagellum (Nasir et al., 2018) (Fig. 8).

After a spaceflight aboard the Chinese Satellite Shenzhou-8, *Euglena* cells were analyzed for possible adaptations to microgravity (Nasir et al., 2014). Genes related to oxidative stress or heat shock proteins were upregulated, as well as genes suspected to play a role in gravitaxis, like calmodulin, calcium-binding and other signal proteins, and proteins involved in cell cycle control. Of the tested 32 genes, 18 were up-regulated and one down-regulated, while the rest remained unchanged. Recent advances in the sequencing of the nuclear and mitochondrial genome of *Euglena* (Ebenezer et al., 2019) allowed microarray construction and investigate the transcription pattern in the course of a parabolic flight campaign (Krüger et al., 2019a). The study supported that significant changes in gene expression can be achieved within a very short time. Different signal transduction elements, such as mitogen-activated kinase kinase kinase NPK1, hybrid sensor histidine kinase/response regulator, adenylate guanylate cyclase domain-containing protein, etc., were found to be regulated as well as transport proteins, phosphorylases, primary metabolism, and DNA and protein modification genes. Microgravity might also affect the photosynthesis and perception of light: genes involved in these processes were differently expressed, even though the experiment was performed in darkness (Daiker et al., 2011; Nasir et al., 2018). Some of the altered transcripts showed similarities to those observed in *A. thaliana* (Hausmann et al., 2014; Martzivanou et al., 2006). Taken together, the study contributed to the understanding of the short-term effects of microgravity and different accelerations on gene expression, which can be used as a reference data set for further on-ground and spaceflight-related studies.

8.3. Conclusions from observed effects on plants and algae

In plants and algae, like in animal cells, the current knowledge about gravity perception and the connected signal transduction chains is very limited. However, photosynthetic organisms (plants, plant cell cultures, and algae) and human or animal systems share similar patterns of "disturbed-gravity-induced" changes in certain classes of proteins or gene expression. For instance, signal transduction chains involving calcium are affected in plants and animals alike (Hashemi et al., 1999; Kordyum, 2003; Paul et al., 2012b). Six orthologue genes between *Drosophila melanogaster* and *Caenorhabditis elegans* were found to be differentially expressed (Leandro et al., 2007). Therefore, there appears to be something like a common response to spaceflight. Unfortunately, differences in the experimental setups (duration, hardware, limited numbers of experiments and replicates, quality of microgravity, etc.) make it very difficult to compare results of space experiments and draw a definite interpretation and conclusion. Future space experiments need to be performed under standardized conditions to attain reproducible results and conclusions (Ruyters and Braun, 2014; Wolff et al., 2014). A general database should be developed comprising of genomics, proteomics, metabolomics, and transcriptomics datasets obtained from space experiments and corresponding ground studies of different organisms.

9. Colloids in microgravity

Grape juice and its fermented form, wine, are complex multi-component fluids containing sugars, different alcohols, hundreds of different tannins, or polyphenols prone to various attractive and repulsive interactions (hydrophobic, van der Waals, electrostatic, hydrogen bonding). The tannins are likely to aggregate (haze formation) with time (ageing), thus making the red wine a meta-stable colloidal dispersion: a system of fine particles suspended in a fluid (Zanchi et al., 2008). Other common examples of colloidal dispersion than wine are paints, inks, mayonnaise, ice cream, milk, and biological fluids such as blood. Although these systems are routinely produced, used, and studied, we still have much to learn about the underlying properties of colloidal systems. Hence, understanding and controlling them is a central issue in many technological and scientific fields as it will foster academic and industrial research to fine-tune their physico-chemical structures to put forward new functionalities and products.

The force of gravity may in certain cases impact the colloidal phase behavior as well as dynamical processes. It can, for example, destabilize a dispersion through either the rising toward the liquid/fluid interface of some of the components lighter than the dispersing fluid as in oil-in-water emulsions (creaming) or the sedimentation of the denser ones as silica particles in water. Indeed, at the colloidal level, the so-called gravitational length $l_g = \frac{k_B T}{G}$, where $k_B T$ is the thermal energy source of the Brownian motion and $G = \frac{\pi D^3 \Delta\rho g}{6}$ the buoyant weight (with D the colloidal diameter, $\Delta\rho$ the density mismatch between the colloid and the solvent, and g the gravitational acceleration. It characterizes at what height gravity will start to play an important role in the equilibrium phase behavior (Royall et al., 2005). In a gravitational field, the balance between diffusive forces, pushing the particles up, and gravity, pulling them down, results in hydrostatic equilibrium, the sedimentation profile, where the volume fraction of non-interacting colloids (dilute regime) $\phi = \frac{\pi\rho}{6D^3}$ decays exponentially with height as $\phi(z) = \phi_0 \exp(-z/l_g)$, with a decay length, l_g, historically determined by Perrin in his 1916 seminal work on the measurement of the Boltzman's constant k_B (Perrin, 1916). It follows that if the size of a colloid in the dispersion is somehow below 1µm (with small $\Delta\rho$), its motion will be mainly diffusive with a negligible impact of gravity-driven convection (sedimentation or creaming). A useful (dimensionless) quantity to weigh the influence of gravity in colloidal dynamics is the Péclet number (Pe) that measures the ratio between convective and diffusive transport in a gravitational field (El Masri et al., 2012; Okubo et al., 2000):

$$P_e = \frac{D}{2l_g} = \frac{\pi D^4 \Delta\rho g}{12 k_B T} = \frac{\tau_B}{\tau_S}$$

Where, τ_B and τ_S are the time scales for Brownian diffusion and sedimentation (creaming). When P_e <<1, gravity may be neglected, when P_e >>1 the colloidal dispersion will be strongly affected by gravity. Indeed, for 1µm silica particles in water (~$\Delta\rho$ = 1 g cm^{-3}) the sedimentation rate due to gravity is 100 times larger than Brownian diffusion. By selecting the right particle size and matching the particle-solvent density one can significantly reduce the influence of gravity in laboratory experiments. It is however very difficult to reduce $\Delta\rho$ significantly below 10^{-3} g.cm^{-3} without extreme temperature control (better than 0.01°C) required to reach the condition of microgravity. Furthermore, for optical studies, the fluid and the disperse component should be index-matched which practically excludes that they are density-matched at the same time, something impossible to achieve for all the different colloidal systems used and studied every day all over the world. Colloidal stability is a key issue in various domains ranging from soft-matter, physical chemistry, colloidal and macromolecular science to material science and biotechnology through micro and synthetic biology as it relates to end-user products, drugs.

These systems are often structured by attractive forces between building blocks such as micelles, macromolecules, proteins, globules, nanoparticles in the form of weak, flowable gels. These structures in turn age through coasering where they diffuse to encounter and then compact or merge. When they can no longer support the gravitational stresses, they collapse, sediment, or sink, often abruptly without warning (delayed collapse) with a dynamic much faster than Brownian motion. Consequently, all the motions are biased by hydrodynamics as in the separation of emulsions into layers of different density for example. This pinpoints the difficulties to fully capture and understand the fundamentals of coasering but also crystallization (Okubo et al., 2000; Zhu et al., 1997), aggregation (Veen et al., 2012), gelation (Manley et al., 2004), crystal nucleation and growth, late stage of spinodal decomposition (Bailey et al., 2007), the collapse of tenuous structures, the spatial distribution of aerosols, interfaces, bubbles, and droplets shapes.

Another interesting aspect is the possibility to construct synthetic cells with life-like properties to tackle key societal challenges, such as environmental cleanup, energy production, and drug delivery but also to bring new insights concerning the possible pathways responsible for the origin of life. This bottom-approach aim at producing artificial compartments drawing on a combination of natural biomolecules and synthons to mimic cell structure and functions by incorporating i) cellular extracts containing proteins and nucleic acids required for DNA replication and ii) enzymes to develop biochemical reactions and reactors. To date, two types of compartments have been used to assemble synthetic cell models, namely membrane-bound vesicles and membrane-free micron-sized droplets formed by all-in-water emulsions (ATPS, complex coacervates) (Douliez et al., 2018; Dzieciol and Mann, 2012). The former ensure long-term solute encapsulation and confinement of biomolecules but

the lipid bilayer limits molecular exchange, while the latter allows spontaneous solute uptake but the lack of membrane leads to low confinement times and then support facile solute exchange. Bioreactors capable of synthesizing proteins have been developed using vesicles (Ciancaglini et al., 2012; Noireaux and Libchaber, 2004), while all-in-water emulsions have been suggested as a step toward the production of protocell models (Dzieciol and Mann, 2012). The design of synthetic cells holds great promise but their studies and development are facing the same problems as encountered with a more classical dispersion. The coarsening of the compartments leading to fast sedimentation or macroscopic phase separation if not correctly stabilized, large scale convection taking over the diffusion of small biomolecules, nutriment, and actives.

Real microgravity conditions are then needed to unveil some key physicochemical aspects like the natural movement of colloids that experiments performed on Earth, in the presence of gravity may mask, alter, or simply hinder. Studies in microgravity will open the routes for the pharmaceutical, food, agricultural, and chemical companies to develop a wide range new products (DiFrancesco and Olson, 2015). Since more than 30 years, numerous scientific experiments on colloids in microgravity have been carried out on aircraft (and Space Shuttles) (Lee and Israelsson, 2001; Okubo et al., 1999; Okubo et al., 2000; Sprenger et al., 1993; Tomita et al., 2018; Zhu et al., 1997). If the characteristic time scale of the given experiment is longer (an aging process for example that needs to unfold slowly and evenly) then the unique capabilities of the ISS is an ideal location to study more deeply the various physical, physico-chemical aspects and mechanism(s) at play (*vide supra*) in colloid science in microgravity. In particular, ISS has developed the so-called Light Microscopy Module (LMM), a modified commercial and highly flexible, state-of-the-art light imaging microscope facility that provides researchers with powerful diagnostic hardware and software with the capability of remotely acquiring and downloading digital images and videos across many levels of magnification. This provides a unique environment to observe colloidal self-assembly in 3-D and microgravity for a variety of shapes, sizes, and particle interactions to build understanding and drive colloidal engineering (Bailey et al., 2007; Manley et al., 2004; McPherson and DeLucas, 1999; Safari et al., 2017; Veen et al., 2012).

10. Future perspectives of space research

Life on Earth has always developed within the boundaries of gravity. In space, organisms encounter completely novel conditions, such as weightlessness and radiation. The majority of space experiments until date have revealed that microgravity has a significant influence on organisms' growth and behavior. Future space and gravitational biology research programs should focus on the exploration of the influence of microgravity on living systems at the cellular and molecular levels, as well as on genetic stability, growth, and development of animal and plant systems utilizing advanced scientific methods and tools. The space environment can be explored to develop new products for the benefit of humankind. For instance, we can generate natural crop varieties with higher nutrition and yields and improved features, such as resistance against high and low temperatures, salt stress, microbial and pest attacks, drought, and other stresses. Introduction of plants to artificial stressors in microgravity will activate the expression of dormant genes, change the global systemic organization of plants, and improve their recovery processes in subsequent stresses, like the ability to adapt rapidly to a disease-causing organism. Strains improved in space environmental conditions can be a valuable partner for conventional engineering strategies. Characterization of evolved strains and identification of the important mutations or molecular basis that enabled evolved phenotype or behavior can increase our understanding of organisms' physiology and metabolism as well as uncover the significance of molecular mechanisms contributing to strain fitness and performance. Application of emerging genome and transcriptome sequencing and analysis tools, and advanced metabolic identification and characterization methods are required to interpret the story behind the evolution-based strain improvement approach. These investigations can reveal novel strategies for improving the tolerance of strains to biotic or abiotic factors, increasing utilization of a target substrate for growth and yield, or improve the production of target compounds.

Several infrastructures and space operation capacities are currently present to execute various biological experiments in space. In addition, more advanced technologies are being developed. The current Dragon spacecraft by SpaceX, the Cygnus spacecraft by Orbital ATK, and the new Shepard spacecraft by Blue Origin, and the robust space platforms, such as the ISS and the Russian Earth-orbiting satellites BION and FOTON are available as well as space platforms and spacecrafts from China and

India will be accessible in the near future. Most platforms are currently used for publicly driven space programs, serving and servicing the ISS or far more ambitious space exploration programs such as to the Moon or Mars. ISS offers the most sophisticated research facility in the world. Only highly elaborated ground-based research facilities, such as the Large Hadron Collider at CERN, can compete with the ISS in terms of complexity and operating expense. Differences in experimental setups (viz. duration, hardware, limited numbers of experiments and replicates, and microgravity quality) have limited our understanding of space research data to achieve breakthroughs in biological research. It is therefore required to carry out space experiments under defined and standardized conditions. Factors that need to be emphasized in space research are numbers, time, and on-site monitoring of relevant factors. The required changes cannot be expected to happen often, but by increasing the number of exposed subjects, and by increasing mutational pressure, one can increase the probability that the changes will occur. The longer the subjects are exposed, the higher the chance of change. However, there should be a threshold of exposure time to allow survivability of the biological system. Recent advances in online monitoring of genetic and metabolic changes will allow close tracking of occurring changes. The development of a general database consisting of datasets from different experimental analyses is highly recommended.

In conclusion, space offers unique types of stressors or environmental conditions (i.e., weightlessness and high radiation) that can be used to accelerate evolutionary processes in an organism for the development of a new strain adapted to the challenging environments. Microgravity as an environmental condition never experienced before by any organism on Earth most likely increases mutations rates and influences metabolic and signal transduction pathways in a way that phenotypes may occur, which would not develop under 1*g*. We anticipate that the evolutionary changes in space will be considered fast. Evolution will be based on random processes with positive selection guiding the development of phenotypes with desired properties. Microgravity potentially constitutes a platform to develop valuable strains and bio-products as well as to study more deeply the various physical, physicochemical aspects, and mechanism(s) at play in colloid science. We propose to craft different varieties of organisms in space to meet Earth's demand in the age of ultimate planetary- and human-impacting threats. Something that would definitely help academia and industry to formulate a wide range of new products for food, medicine, pharmaceutics, cosmetics, home-care with longer shelf lives and new functionalities.

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References

Abshire, C.F., Prasai, K., Soto, I., Shi, R., Concha, M., Baddoo, M. et al., 2016. Exposure of *Mycobacterium marinum* to low-shear modeled microgravity: effect on growth, the transcriptome and survival under stress. npj Microgravity 2, 16038. 10.1038/npjmgrav.2016.38.

Acuña, R., Padilla, B.E., Flórez-Ramos, C.P., Rubio, J.D., Herrera, J.C., Benavides, P. et al., 2012. Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee. Proc. Natl. Acad. Sci. U. S. A. 109, 4197–4202. 10.1073/pnas.1121190109.

Aleshcheva, G., Bauer, J., Hemmersbach, R., Egli, M., Wehland, M., Grimm, D., 2016. Tissue Engineering of Cartilage on Ground-Based Facilities. Microgravity Sci. Technol. 28, 237–245. 10.1007/s12217-015-9479-0.

Alsmark, C., Foster, P.G., Sicheritz-Ponten, T., Nakjang, S., Martin Embley, T., Hirt, R.P., 2013. Patterns of prokaryotic lateral gene transfers affecting parasitic microbial eukaryotes. Genome Biol. 14, R19. 10.1186/gb-2013-14-2-r19.

Altenburg, S.D., Nielsen-Preiss, S.M., Hyman, L.E., 2008. Increased Filamentous Growth of *Candida albicans* in Simulated Microgravity. Genomics, Proteomics Bioinformatics 6, 42–50. 10.1016/S1672-0229(08)60019-4.

Amini, A.R., Laurencin, C.T., Nukavarapu, S.P., 2012. Bone tissue engineering: recent advances and challenges. Crit. Rev. Biomed. Eng. 40, 363–408. 10.1615/CritRevBiomedEng.v40.i5.10.

Amiri, H., Karimi, K., 2019. Biobutanol Production. In: Advanced Bioprocessing for Alternative Fuels, Biobased Chemicals, and Bioproducts: Elsevier. p. 109–133.
Anderson, M.T., Seifert, H.S., 2011. Opportunity and means: horizontal gene transfer from the human host to a bacterial pathogen. mBio 2, e00005-11. 10.1128/mBio.00005-11.
Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R., Daszak, P., 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends Ecol. Evol. 19, 535–544. 10.1016/j.tree.2004.07.021.
Andersson, J.O., 2005. Lateral gene transfer in eukaryotes. Cell Mol. Life Sci. 62, 1182–1197. 10.1007/s00018-005-4539-z.
Andersson, J.O., Sjögren, Å.M., Davis, L.A.M., Embley, T.M., Roger, A.J., 2003. Phylogenetic Analyses of Diplomonad Genes Reveal Frequent Lateral Gene Transfers Affecting Eukaryotes. Current Biol. 13, 94–104. 10.1016/s0960-9822(03)00003-4.
Anway, M.D., Cupp, A.S., Uzumcu, M., Skinner, M.K., 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308, 1466–1469. 10.1126/science.1108190.
Arun, R.P., Sivanesan, D., Patra, B., Varadaraj, S., Verma, R.S., 2019. Simulated microgravity increases polyploid giant cancer cells and nuclear localization of YAP. Sci. Rep. 9, 10684. 10.1038/s41598-019-47116-5.
Arun, R.P., Sivanesan, D., Vidyasekar, P., Verma, R.S., 2017. PTEN/FOXO3/AKT pathway regulates cell death and mediates morphogenetic differentiation of Colorectal Cancer Cells under Simulated Microgravity Sci. Rep. 7, 5952. 10.1038/s41598-017-06416-4.
Arunasri, K., Adil, M., Venu Charan, K., Suvro, C., Himabindu Reddy, S., Shivaji, S., 2013. Effect of simulated microgravity on *E. coli* K12 MG1655 growth and gene expression. PLoS ONE 8, e57860. 10.1371/journal.pone.0057860.
Babbick, M., Dijkstra, C., Larkin, O.J., Anthony, P., Davey, M.R., Power, J.B. et al., 2007. Expression of transcription factors after short-term exposure of *Arabidopsis thaliana* cell cultures to hypergravity and simulated microgravity (2-D/3-D clinorotation, magnetic levitation). Adv. Space Res. 39, 1182–1189. 10.1016/j.asr.2007.01.001.
Backlund, P., Janetos, A., Schimel, D. [Internet]. The Effects of Climate Change on Agriculture, Land Resources, Water Resources, and Biodiversity. Report, 2008. Available from: https://www.fs.fed.us/rm/pubs_other/rmrs_2008_backlund_p003.pdf.
Badyaev, A.V., 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. Proceedings. Biol. Sci. 272, 877–886. 10.1098/rspb.2004.3045.
Baer, C.F., Miyamoto, M.M., Denver, D.R., 2007. Mutation rate variation in multicellular eukaryotes: causes and consequences. Nat. Rev. Genet. 8, 619–631. 10.1038/nrg2158.
Baglioni, P., Sabbatini, M., Horneck, G., 2007. Astrobiology Experiments in Low Earth Orbit: Facilities, Instrumentation, and Results. In: Horneck G, Rettberg P, editors. Complete Course in Astrobiology: Wiley-VCH Verlag GmbH & Co. KGaA. Weinheim, Germany. p. 273–319.
Bai, F., Settles, A.M., 2014. Imprinting in plants as a mechanism to generate seed phenotypic diversity. Frontiers in plant science 5, 780. 10.3389/fpls.2014.00780.
Bailey, A.E., Poon, W.C.K., Christianson, R.J., Schofield, A.B., Gasser, U., Prasad, V. et al., 2007. Spinodal decomposition in a model colloid-polymer mixture in microgravity. Phys Rev Lett 99, 205701. 10.1103/PhysRevLett.99.205701.
Baker, P.W., Meyer, M.L., Leff, L.G., 2004. *Escherichia coli* growth under modeled reduced gravity. Microgravity Sci. Technol. 15, 39–44.
Baltz, R., 2007. Antimicrobials from Actinomycetes: Back to the Future. Microbe 2, 125–131.
Bannister, A.J., Kouzarides, T., 2011. Regulation of chromatin by histone modifications. Cell Res. 21, 381–395. 10.1038/cr.2011.22.
Barjaktarovic, Z., Schütz, W., Madlung, J., Fladerer, C., Nordheim, A., Hampp, R., 2009. Changes in the effective gravitational field strength affect the state of phosphorylation of stress-related proteins in callus cultures of *Arabidopsis thaliana*. J. Exp. Bot. 60, 779–789. 10.1093/jxb/ern324.
Barjaktarović, Ž., Babbick, M., Nordheim, A., Lamkemeyer, T., Magel, E., Hampp, R., 2009. Alterations in Protein Expression of *Arabidopsis thaliana* Cell Cultures During Hyper- and Simulated Micro-Gravity. Microgravity Sci. Technol. 21, 191–196. 10.1007/s12217-008-9058-8.
Barlow, M., 2009. What antimicrobial resistance has taught us about horizontal gene transfer. Methods Mol. Biol. 532, 397–411. 10.1007/978-1-60327-853-9_23.

Bauer, J., Grimm, D., Gombocz, E., 2017. Semantic analysis of thyroid cancer cell proteins obtained from rare research opportunities. J. Biomed. Inform. 76, 138–153. 10.1016/j.jbi.2017.10.011.
Bauer, J., Wehland, M., Infanger, M., Grimm, D., Gombocz, E., 2018. Semantic Analysis of Posttranslational Modification of Proteins Accumulated in Thyroid Cancer Cells Exposed to Simulated Microgravity. Int. J. Mol. Sci. 19. 10.3390/ijms19082257.
Beiko, R.G., Harlow, T.J., Ragan, M.A., 2005. Highways of gene sharing in prokaryotes. Proc. Natl. Acad. Sci. U. S. A. 102, 14332–14337. 10.1073/pnas.0504068102.
Bell, G.J., Martin, T.P., Ilyina-Kakueva, E.I., Oganov, V.S., Edgerton, V.R., 1992. Altered distribution of mitochondria in rat soleus muscle fibers after spaceflight. J. Appl. Physiol. 73, 493–497.
Benoit, M.R., Brown, R.B., Todd, P., Nelson, E.S., Klaus, D.M., 2008. Buoyant plumes from solute gradients generated by non-motile *Escherichia coli*. Phys. Biol. 5, 46007. 10.1088/1478-3975/5/4/046007.
Benoit, M.R., Klaus, D.M., 2007. Microgravity, bacteria, and the influence of motility. Adv. Space Res. 39, 1225–1232. 10.1016/j.asr.2006.10.009.
Beuls, E., van Houdt, R., Leys, N., Dijkstra, C., Larkin, O., Mahillon, J., 2009. *Bacillus thuringiensis* conjugation in simulated microgravity. Astrobiology 9, 797–805. 10.1089/ast.2009.0383.
Bhagwat, A.S., Lieb, M., 2002. Cooperation and competition in mismatch repair: very short-patch repair and methyl-directed mismatch repair in *Escherichia coli*. Mol. Microbiol. 44, 1421–1428. 10.1046/j.1365-2958.2002.02989.x.
Bhattacharyya, M., Martin, C., Smith, A., 1993. The importance of starch biosynthesis in the wrinkled seed shape character of peas studied by Mendel. Plant Mol. Biol. 22, 525–531. 10.1007/BF00015981.
Bijlsma R, Loeschcke V, editors, 1997. Environmental Stress, Adaptation and Evolution Birkhäuser Verl, Basel/Switzerland.
Bilandžija, H., Ma, L., Parkhurst, A., Jeffery, W.R., 2013. A potential benefit of albinism in Astyanax cavefish: downregulation of the oca2 gene increases tyrosine and catecholamine levels as an alternative to melanin synthesis. PLoS ONE 8, e80823. 10.1371/journal.pone.0080823.
Bizzarri, M., Monici, M., van Loon, J.J.W.A., 2015. How microgravity affects the biology of living systems. Biomed Res. Int. 2015, 863075. 10.1155/2015/863075.
Boever, P. de, Mergeay, M., Ilyin, V., Forget-Hanus, D., Van der Auwera, G., Mahillon, J., 2007. Conjugation-mediated plasmid exchange between bacteria grown under space flight conditions. Microgravity Sci. Technol. 19, 138–144. 10.1007/BF02919469.
Boto, L., 2010. Horizontal gene transfer in evolution: facts and challenges. Proc. Biol. Sci. 277, 819–827. 10.1098/rspb.2009.1679.
Boucher, Y., Douady, C.J., Papke, R.T., Walsh, D.A., Boudreau, M.E.R., Nesbø, C.L. et al., 2003. Lateral gene transfer and the origins of prokaryotic groups. Annu. Rev. Genet. 37, 283–328. 10.1146/annurev.genet.37.050503.084247.
Bowler, C., Allen, A.E., Badger, J.H., Grimwood, J., Jabbari, K., Kuo, A. et al., 2008. The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. Nature 456, 239–244. 10.1038/nature07410.
Boyko, A., Blevins, T., Yao, Y., Golubov, A., Bilichak, A., Ilnytskyy, Y. et al., 2010. Transgenerational Adaptation of *Arabidopsis* to Stress Requires DNA Methylation and the Function of Dicer-Like Proteins. PLoS ONE 5, e9514. 10.1371/journal.pone.0009514.
Braddock, M., 2019. From Target identification to Drug Development in Space: Using the Microgravity Assist. Curr. Drug Discov. Technol. 10.2174/1570163816666190112150014.
Braun, M., Buchen, B., Sievers, A., 1999. Electron microscopic analysis of gravisensing *Chara* rhizoids developed under microgravity conditions. FASEB J. 13, S113-S120.
Braun, M., Hauslage, J., Czogalla, A., Limbach, C., 2004. Tip-localized actin polymerization and remodeling, reflected by the localization of ADF, profilin and villin, are fundamental for gravity-sensing and polar growth in characean rhizoids. Planta 219, 379–388.
Braun, M., Limbach, C., 2006. Rhizoids and protonemata of characean algae: model cells for research on polarized growth and plant gravity sensing. Protoplasma 229, 133–142.
Braun, M., Richter, P., 1999. Relocalization of the calcium gradient and a dihydropyridine receptor is involved in upward bending by bulging of *Chara* protonemata, but not in downward bending by bowing of *Chara* rhizoids. Planta 209, 414–423.

Braun, M., Sievers, A., 1994. Role of the microtubule cytoskeleton in gravisensing *Chara* rhizoids. Eur. J. Cell Biol. 63, 289–298.
Brinckmann, E., 2007. Biology in Space and Life on Earth Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
Brown, J.R., 2003. Ancient horizontal gene transfer. Nat. Rev. Genet. 4, 121–132. 10.1038/nrg1000.
Brown, J.R., Doolittle, W.F., 1999. Gene descent, duplication, and horizontal transfer in the evolution of glutamyl- and glutaminyl-tRNA synthetases. J. Mol. Evol. 49, 485–495. 10.1007/PL00006571.
Brown, J.R., Zhang, J., Hodgson, J.E., 1998. A bacterial antibiotic resistance gene with eukaryotic origins. Curr. Biol. 8, R365-S12. 10.1016/s0960-9822(98)70238-6.
Brown, M.E., Antle, J.M., Backlund, P., Carr, E.R., Easterling, W.E., Walsh, M.K. et al. [Internet]. Climate Change, Global Food Security, and the U.S. Food System., 2015. Available from: http://www.usda.gov/oce/climate_change/FoodSecurity2015Assessment/FullAssessment.pdf.
Bruijn, F.J. de, 2016. Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria. John Wiley & Sons, Inc, Hoboken, NJ, USA.
Burch, C.L., Guyader, S., Samarov, D., Shen, H., 2007. Experimental estimate of the abundance and effects of nearly neutral mutations in the RNA virus phi 6. Genetics 176, 467–476. 10.1534/genetics.106.067199.
Burggren, W., 2016. Epigenetic Inheritance and Its Role in Evolutionary Biology: Re-Evaluation and New Perspectives. Biol. 5. 10.3390/biology5020024.
Bushart, T.J., Cannon, A.E., Ul Haque, A., San Miguel, P., Mostajeran, K., Clark, G.B. et al., 2013. RNA-seq analysis identifies potential modulators of gravity response in spores of *Ceratopteris* (Parkeriaceae): Evidence for modulation by calcium pumps and apyrase activity. Am. J. Bot. 100, 161–174. 10.3732/ajb.1200292.
Calarco, J.P., Borges, F., Donoghue, M.T.A., van Ex, F., Jullien, P.E., Lopes, T. et al., 2012. Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. Cell 151, 194–205. 10.1016/j.cell.2012.09.001.
Cappellesso, R., Nicole, L., Guido, A., Pizzol, D., 2015. Spaceflight osteoporosis: current state and future perspective. Endocrine regulations 49, 231–239. 10.4149/endo_2015_04_231.
Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P. et al., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67. 10.1038/nature11148.
Carlton, J.M., Hirt, R.P., Silva, J.C., Delcher, A.L., Schatz, M., Zhao, Q. et al., 2007. Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. Sci. 315, 207–212. 10.1126/science.1132894.
Carroll, S.B., Grenier, J., Weatherbee, S., 2009. From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design. 2nd ed. John Wiley & Sons Ltd, Hoboken.
Casadesús, J., Low, D., 2006. Epigenetic gene regulation in the bacterial world. Microbiol. Mol. Biol. Rev. 70, 830–856. 10.1128/MMBR.00016-06.
Cerwinka, W.H., Sharp, S.M., Boyan, B.D., Zhau, H.E., Chung, L.W.K., Yates, C., 2012. Differentiation of human mesenchymal stem cell spheroids under microgravity conditions. Cell Regen. 1, 2. 10.1186/2045-9769-1-2.
Chang, D., Xu, H., Guo, Y., Jiang, X., Liu, Y., Li, K. et al., 2013. Simulated microgravity alters the metastatic potential of a human lung adenocarcinoma cell line. In Vitro Cell. Dev. Biol. -Animal 49, 170–177. 10.1007/s11626-013-9581-9.
Chapman, J.A., Kirkness, E.F., Simakov, O., Hampson, S.E., Mitros, T., Weinmaier, T. et al., 2010. The dynamic genome of *Hydra*. Nature 464, 592–596. 10.1038/nature08830.
Charlesworth, J., Eyre-Walker, A., 2006. The rate of adaptive evolution in enteric bacteria. Mol. Biol. Evol. 23, 1348–1356. 10.1093/molbev/msk025.
Chatani, M., Mantoku, A., Takeyama, K., Abduweli, D., Sugamori, Y., Aoki, K. et al., 2015. Microgravity promotes osteoclast activity in medaka fish reared at the international space station. Sci. Rep. 5, 14172. 10.1038/srep14172.
Chen, Z.-Y., Guo, S., Li, B.-B., Jiang, N., Li, A., Yan, H.-F. et al., 2019. Effect of Weightlessness on the 3D Structure Formation and Physiologic Function of Human Cancer Cells. Biomed Res. Int. 2019, 4894083. 10.1155/2019/4894083.
Cheng, X., Xu, X., Chen, J., Zhou, X., Cheng, L., Li, M. et al., 2014. Effects of simulated microgravity on *Streptococcus mutans* physiology and biofilm structure. FEMS Microbiol. Lett. 359, 94–101. 10.1111/1574-6968.12573.

Chengzhi, L., 2011. Agronomy in space–China's crop breeding program. Space Policy, 27(3), 157-164. https://doi.org/10.1016/j.spacepol.2011.06.001

Chiu, B., Wan, J.Z.-M., Abley, D., Akabutu, J., 2005. Induction of vascular endothelial phenotype and cellular proliferation from human cord blood stem cells cultured in simulated microgravity. Acta Astronaut. 56, 918–922. 10.1016/j.actaastro.2005.01.018.

Choi, I.-G., Kim, S.-H., 2007. Global extent of horizontal gene transfer. Proc. Natl. Acad. Sci. U. S. A. 104, 4489–4494. 10.1073/pnas.0611557104.

Chopra, V., Fadl, A.A., Sha, J., Chopra, S., Galindo, C.L., Chopra, A.K., 2006. Alterations in the Virulence Potential of Enteric Pathogens and Bacterial–Host Cell Interactions Under Simulated Microgravity Conditions. J. Toxicol. Env. Heal. A 69, 1345–1370. 10.1080/15287390500361792.

Ciancaglini, P., Simão, A.M.S., Bolean, M., Millán, J.L., Rigos, C.F., Yoneda, J.S. et al., 2012. Proteoliposomes in nanobiotechnology. Biophys. Rev. 4, 67–81. 10.1007/s12551-011-0065-4.

Clark, A.G., Fucito, C.D., 1998. Stress tolerance and metabolic response to stress in Drosophila melanogaster. Heredity 81 (Pt 5), 514–527. 10.1046/j.1365-2540.1998.00414.x.

Clarke, M.S.F., Sundaresan, A., Vanderburg, C.R., Banigan, M.G., Pellis, N.R., 2013. A three-dimensional tissue culture model of bone formation utilizing rotational co-culture of human adult osteoblasts and osteoclasts. Acta biomater. 9, 7908–7916. 10.1016/j.actbio.2013.04.051.

Clejan, S., O'Connor, K., Rosensweig, N., 2001. Tri-dimensional prostate cell cultures in simulated microgravity and induced changes in lipid second messengers and signal transduction. J. Cell Mol. Med. 5, 60–73. 10.1111/j.1582-4934.2001.tb00138.x.

Clejan, S., O'Connor, K.C., Cowger, N.L., Cheles, M.K., Haque, S., Primavera, A.C., 1996. Effects of simulated microgravity on DU 145 human prostate carcinoma cells. Biotechnol. Bioeng. 50, 587–597. 10.1002/(SICI)1097-0290(19960605)50:5<587:AID-BIT14>3.0.CO;2-G.

Clément, G., Slenzka, K., (eds.) 2006. Fundamentals of space biology, research on cells, animals and plants in space. Microcosm Press, El Secondo, CA.

Cordero, O.X., Hogeweg, P., 2009. The impact of long-distance horizontal gene transfer on prokaryotic genome size. Proc. Natl. Acad. Sci. U. S. A. 106, 21748–21753. 10.1073/pnas.0907584106.

Correll, M.J., Pyle, T.P., Millar, Katherine D. L., Sun, Y., Yao, J., Edelmann, R.E. et al., 2013. Transcriptome analyses of *Arabidopsis thaliana* seedlings grown in space: implications for gravity-responsive genes. Planta 238, 519–533. 10.1007/s00425-013-1909-x.

Corydon, T.J., Kopp, S., Wehland, M., Braun, M., Schütte, A., Mayer, T. et al., 2016a. Alterations of the cytoskeleton in human cells in space proved by life-cell imaging. Sci. Rep. 6, 20043. 10.1038/srep20043.

Corydon, T.J., Mann, V., Slumstrup, L., Kopp, S., Sahana, J., Askou, A.L. et al., 2016b. Reduced Expression of Cytoskeletal and Extracellular Matrix Genes in Human Adult Retinal Pigment Epithelium Cells Exposed to Simulated Microgravity. Cell Physiol. Biochem. 40(1-2), 1–17. 10.1159/000452520.

Courchesne, N.M.D., Parisien, A., Wang, B., Lan, C.Q., 2009. Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. J. Biotechnol. 141, 31–41. 10.1016/j.jbiotec.2009.02.018.

Cox, M.M., Battista, J.R., 2005. *Deinococcus radiodurans* - the consummate survivor. Nat. Rev. Micro. 3, 882–892. 10.1038/nrmicro1264.

Crabbé, A., Nielsen-Preiss, S.M., Woolley, C.M., Barrila, J., Buchanan, K., McCracken, J. et al., 2013. Spaceflight Enhances Cell Aggregation and Random Budding in *Candida albicans*. PLoS ONE 8, e80677. 10.1371/journal.pone.0080677.

Crabbé, A., Schurr, M.J., Monsieurs, P., Morici, L., Schurr, J., Wilson, J.W. et al., 2011. Transcriptional and proteomic responses of *Pseudomonas aeruginosa* PAO1 to spaceflight conditions involve Hfq regulation and reveal a role for oxygen. Appl. Environ. Microbiol. 77, 1221–1230. 10.1128/AEM.01582-10.

Crisp, P.A., Ganguly, D., Eichten, S.R., Borevitz, J.O., Pogson, B.J., 2016. Reconsidering plant memory: Intersections between stress recovery, RNA turnover, and epigenetics. Sci. Adv. 2, e1501340. 10.1126/sciadv.1501340.

Cropley, J.E., Suter, C.M., Beckman, K.B., Martin, D.I.K., 2006. Germ-line epigenetic modification of the murine A vy allele by nutritional supplementation. Proc. Natl. Acad. Sci. U. S. A. 103, 17308–17312. 10.1073/pnas.0607090103.

Cui, Y., Lu, C., Chen, B., Han, J., Zhao, Y., Xiao, Z. et al., 2018. Restoration of mandibular bone defects with demineralized bone matrix combined with three-dimensional cultured bone marrow-derived mesenchymal stem cells in minipig models. Journal of materials science. J. Mater. Sci.: Mater. Med 29, 147. 10.1007/s10856-018-6152-3.

Dagan, T., Artzy-Randrup, Y., Martin, W., 2008. Modular networks and cumulative impact of lateral transfer in prokaryote genome evolution. Proc. Natl. Acad. Sci. U. S. A. 105, 10039–10044. 10.1073/pnas.0800679105.

Daiker, V., Häder, D.-P., Richter, P.R., Lebert, M., 2011. The involvement of a protein kinase in phototaxis and gravitaxis of *Euglena gracilis*. Planta 233, 1055–1062. 10.1007/s00425-011-1364-5.

Daiker, V., Lebert, M., Richter, P., Häder, D.-P., 2010. Molecular characterization of a calmodulin involved in the signal transduction chain of gravitaxis in *Euglena gracilis*. Planta 231, 1229–1236. 10.1007/s00425-010-1126-9.

Daubin, V., Moran, N.A., Ochman, H., 2003. Phylogenetics and the cohesion of bacterial genomes. Sci. 301, 829–832. 10.1126/science.1086568.

Davidson, E.H., 2006. The regulatory genome: Gene regulatory networks in development and evolution Academic, Burlington, MA, San Diego.

Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev. 74, 417–433. 10.1128/MMBR.00016-10.

Dean, R., van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D. et al., 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13, 414–430. 10.1111/j.1364-3703.2011.00783.x.

DiFrancesco, J.M., Olson, J.M., 2015. The economics of microgravity research. npj Microgravity 1, 15001. 10.1038/npjmgrav.2015.1.

Ding, Y., Fromm, M., Avramova, Z., 2012. Multiple exposures to drought 'train' transcriptional responses in *Arabidopsis*. Nat. Commun. 3, 740. 10.1038/ncomms1732.

Dittrich, A., Grimm, D., Sahana, J., Bauer, J., Krüger, M., Infanger, M. et al., 2018. Key Proteins Involved in Spheroid Formation and Angiogenesis in Endothelial Cells After Long-Term Exposure to Simulated Microgravity. Cell. Physiol. Biochem. 45, 429–445. 10.1159/000486920.

Doron, L., Segal, N.'a., Shapira, M., 2016. Transgene Expression in Microalgae-From Tools to Applications. Front. Plant Sci. 7, 505. 10.3389/fpls.2016.00505.

Douliez, J.-P., Martin, N., Beneyton, T., Eloi, J.-C., Chapel, J.-P., Navailles, L. et al., 2018. Preparation of Swellable Hydrogel-Containing Colloidosomes from Aqueous Two-Phase Pickering Emulsion Droplets. Angew. Chem. 130, 7906–7910. 10.1002/ange.201802929.

Drake, J.W., 2006. Chaos and Order in Spontaneous Mutation. Genetics 173, 1–8.

Dröge, M., Pühler, A., Selbitschka, W., 1998. Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern. J. Biotechnol. 64, 75–90. 10.1016/s0168-1656(98)00105-9.

Duchêne, S., Holmes, E.C., Ho, S.Y.W., 2014. Analyses of evolutionary dynamics in viruses are hindered by a time-dependent bias in rate estimates. Proc. Biol. Sci. 281. 10.1098/rspb.2014.0732.

Duchêne, S., Holt, K.E., Weill, F.-X., Le Hello, S., Hawkey, J., Edwards, D.J. et al., 2016. Genome-scale rates of evolutionary change in bacteria. Microb. Genom. 2, e000094. 10.1099/mgen.0.000094.

Dunning Hotopp, J.C., Clark, M.E., Oliveira, D.C.S.G., Foster, J.M., Fischer, P., Muñoz Torres, M.C. et al., 2007. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Sci. 317, 1753–1756. 10.1126/science.1142490.

Dupuy, P., Grossin, F., Trotet, G., 1976. Methode de culture des Cyanophycees sur support mineral solide. C.R. Acad. Sci. 170, 44.

Dzieciol, A.J., Mann, S., 2012. Designs for life: protocell models in the laboratory. Chem. Soc. Rev. 41, 79–85. 10.1039/C1CS15211D.

Ebenezer, T.E., Zoltner, M., Burrell, A., Nenarokova, A., Novák Vanclová, A.M.G., Prasad, B. et al., 2019. Transcriptome, proteome and draft genome of *Euglena gracilis*. BMC Biol. 17, 11. 10.1186/s12915-019-0626-8.

Eichten, S.R., Schmitz, R.J., Springer, N.M., 2014. Epigenetics: Beyond Chromatin Modifications and Complex Genetic Regulation. Plant Physiol. 165, 933–947. 10.1104/pp.113.234211.

El Masri, D., Vissers, T., Badaire, S., Stiefelhagen, J.C.P., Vutukuri, H.R., Helfferich, P. et al., 2012. A qualitative confocal microscopy study on a range of colloidal processes by simulating microgravity conditions through slow rotations. Soft Matter 8, 6979. 10.1039/C2SM07217C.

Elena, S.F., Ekunwe, L., Hajela, N., Oden, S.A., Lenski, R.E., 1998. Distribution of fitness effects caused by random insertion mutations in *Escherichia coli*. Genetica 102-103, 349–358.
Eme, L., Gentekaki, E., Curtis, B., Archibald, J.M., Roger, A.J., 2017. Lateral Gene Transfer in the Adaptation of the Anaerobic Parasite *Blastocystis* to the Gut. Curr. Biol. 27, 807–820. 10.1016/j.cub.2017.02.003.
Eyre-Walker, A., 2006. The genomic rate of adaptive evolution. Trends Ecol. Evol. 21, 569–575. 10.1016/j.tree.2006.06.015.
Eyre-Walker, A., Keightley, P.D., 2007. The distribution of fitness effects of new mutations. Nat. Rev. Genet. 8, 610–618. 10.1038/nrg2146.
Eyre-Walker, A., Keightley, P.D., Smith, N.G.C., Gaffney, D., 2002. Quantifying the slightly deleterious mutation model of molecular evolution. Mol. Biol. Evol. 19, 2142–2149. 10.1093/oxfordjournals.molbev.a004039.
Fajardo-Cavazos, P., Leehan, J.D., Nicholson, W.L., 2018. Alterations in the Spectrum of Spontaneous Rifampicin-Resistance Mutations in the *Bacillus subtilis rpoB* Gene after Cultivation in the Human Spaceflight Environment. Front. Microbiol. 9, 192. 10.3389/fmicb.2018.00192.
Fajardo-Cavazos, P., Nicholson, W.L., 2016. Cultivation of Staphylococcus epidermidis in the Human Spaceflight Environment Leads to Alterations in the Frequency and Spectrum of Spontaneous Rifampicin-Resistance Mutations in the rpoB Gene. Front. Microbiol. 7, 999. 10.3389/fmicb.2016.00999.
Fang, A., Pierson, D.L., Koenig, D.W., Mishra, S.K., Demain, A.L., 1997a. Effect of simulated microgravity and shear stress on microcin B17 production by *Escherichia coli* and on its excretion into the medium. Appl. Environ. Microbiol. 63, 4090–4092.
Fang, A., Pierson, D.L., Mishra, S.K., Demain, A.L., 2000. Growth of *Steptomyces hygroscopicus* in rotating-wall bioreactor under simulated microgravity inhibits rapamycin production. Appl. Microbiol. Biotechnol. 54, 33–36.
Fang, A., Pierson, D.L., Mishra, S.K., Koenig, D.W., Demain, A.L., 1997b. Gramicidin S production by *Bacillus brevis* in simulated microgravity. Curr Microbiol 34, 199–204.
Fang, A., Pierson, D.L., Mishra, S.K., Koenig, D.W., Demain, A.L., 1997c. Secondary metabolism in simulated microgravity: beta-lactam production by *Streptomyces clavuligerus*. J. Ind. Microbiol. Biotechnol. 18, 22–25. 10.1038/sj.jim.2900345.
Felmingham, D., Washington, J., 1999. Trends in the antimicrobial susceptibility of bacterial respiratory tract pathogens--findings of the Alexander Project 1992-1996. J. Chemother. 11 Suppl 1, 5–21. 10.1179/joc.1999.11.Supplement-2.5.
Feng, S., Jacobsen, S.E., Reik, W., 2010. Epigenetic reprogramming in plant and animal development. Sci. 330, 622–627. 10.1126/science.1190614.
Fengler, S., Spirer, I., Neef, M., Ecke, M., Hauslage, J., Hampp, R., 2016. Changes in Gene Expression of *Arabidopsis thaliana* Cell Cultures Upon Exposure to Real and Simulated Partial-g Forces. Microgravity Sci. Technol. 28, 319–329. 10.1007/s12217-015-9452-y.
Fengler, S., Spirer, I., Neef, M., Ecke, M., Nieselt, K., Hampp, R., 2015. A whole-genome microarray study of *Arabidopsis thaliana* semisolid callus cultures exposed to microgravity and nonmicrogravity related spaceflight conditions for 5 days on board of Shenzhou 8. Biomed. Res. Int. 2015, 547495. 10.1155/2015/547495.
Ferl, R., Wheeler, R., Levine, H.G., Paul, A.-L., 2002. Plants in space. Curr Opin Plant Biol 5, 258–263.
Firth, C., Kitchen, A., Shapiro, B., Suchard, M.A., Holmes, E.C., Rambaut, A., 2010. Using time-structured data to estimate evolutionary rates of double-stranded DNA viruses. Mol. Biol. Evol. 27, 2038–2051. 10.1093/molbev/msq088.
Forterre, P., La Bouthier de Tour, C., Philippe, H., Duguet, M., 2000. Reverse gyrase from hyperthermophiles: probable transfer of a thermoadaptation trait from Archaea to Bacteria. Trends Genet. 16, 152–154. 10.1016/S0168-9525(00)01980-6.
Foster, P.L., 2000. Adaptive mutation: implications for evolution. Bioessays 22, 1067–1074. 10.1002/1521-1878(200012)22:12<1067:AID-BIES4>3.0.CO;2-Q.
Fournier, G.P., Gogarten, J.P., 2008. Evolution of acetoclastic methanogenesis in *Methanosarcina* via horizontal gene transfer from cellulolytic *Clostridia*. J. Bacteriol. 190, 1124–1127. 10.1128/JB.01382-07.

Francesca, S., Simona, G., Francesco, N.T., Andrea, R., Vittorio, R., Federico, S. et al., 2006. Downy mildew (*Plasmopara viticola*) epidemics on grapevine under climate change. Global Change Biol. 12, 1299–1307. 10.1111/j.1365-2486.2006.01175.x.

Freed, L.E., Langer, R., Martin, I., Pellis, N.R., Vunjak-Novakovic, G., 1997. Tissue engineering of cartilage in space. Proc. Natl. Acad. Sci. U. S. A. 94, 13885–13890. 10.1073/pnas.94.25.13885.

Freed, L.E., Vunjak-Novakovic, G., 1997. Microgravity tissue engineering. In Vitro Cell. Dev. Biol. - Animal 33, 381–385. 10.1007/s11626-997-0009-2.

Freed, L.F., Pellis, N.R., Searby, N., Luis, J. de, Preda, C., Bordonaro, J. et al., 1999. Microgravity cultivation of cells and tissues. Gravit. Space Biol., 12, 57–67.

Frerich, B., Zückmantel, K., Hemprich, A., 2006. Microvascular engineering in perfusion culture: immunohistochemistry and CLSM findings. Head Face Med. 2, 26. 10.1186/1746-160X-2-26.

Frerich, B., Zückmantel, K., Winter, K., Müller-Dürwald, S., Hemprich, A., 2008. Maturation of capillary-like structures in a tube-like construct in perfusion and rotation culture. Int. J. Oral Maxillofac. Surg. 37, 459–466. 10.1016/j.ijom.2008.01.014.

Fukuda, T., Fukuda, K., Takahashi, A., Ohnishi, T., Nakano, T., Sato, M. et al., 2000. Analysis of deletion mutations of the rpsL gene in the yeast *Saccharomyces cerevisiae* detected after long-term flight on the Russian space station Mir. Mutat. Res. Genet. Toxicol. Environ. Mutagen 470, 125–132. 10.1016/S1383-5742(00)00054-5.

Gao, H., Liu, Z., Zhang, L., 2011. Secondary metabolism in simulated microgravity and space flight. Protein Cell 2, 858–861. 10.1007/s13238-011-1125-z.

Garcia, A.M., Derventzi, A., Busuttil, R., Calder, R.B., Perez, E., Chadwell, L. et al., 2007. A model system for analyzing somatic mutations in *Drosophila melanogaster*. Nat. Meth. 4, 401–403. 10.1038/nmeth1027.

Garcia-Vallvé, S., Romeu, A., Palau, J., 2000. Horizontal gene transfer of glycosyl hydrolases of the rumen fungi. Mol. Biol. Evol. 17, 352–361. 10.1093/oxfordjournals.molbev.a026315.

Gelvin, S.B., 2003. Agrobacterium-mediated plant transformation: the biology behind the "gene-jockeying" tool. Microbiol. Mol. Biol. Rev. 67, 16-37, 10.1128/MMBR.67.1.16-37.2003.

Gendrel, A.-V., Colot, V., 2005. *Arabidopsis* epigenetics: when RNA meets chromatin. Curr. Opin. Plant Biol. 8, 142–147. 10.1016/j.pbi.2005.01.007.

Gingerich, P.D., 2001. Rates of evolution on the time scale of the evolutionary process. Genetica 112-113, 127–144.

Gladyshev, E.A., Meselson, M., Arkhipova, I.R., 2008. Massive horizontal gene transfer in bdelloid rotifers. Sci. 320, 1210–1213. 10.1126/science.1156407.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F. et al., 2010. Food security: the challenge of feeding 9 billion people. Sci. 327, 812–818. 10.1126/science.1185383.

Gogarten, J.P., 2003. Gene Transfer: Gene Swapping Craze Reaches Eukaryotes. Curr. Biol. 13, R53-R54. 10.1016/s0960-9822(02)01426-4.

Gogarten, J.P., Doolittle, W.F., Lawrence, J.G., 2002. Prokaryotic evolution in light of gene transfer. Mol. Biol. Evol. 19, 2226–2238. 10.1093/oxfordjournals.molbev.a004046.

Gomes, A.Q., Nolasco, S., Soares, H., 2013. Non-coding RNAs: multi-tasking molecules in the cell. Int. J. Mol. Sci. 14, 16010–16039. 10.3390/ijms140816010.

Gophna, U., Charlebois, R.L., Doolittle, W.F., 2004. Have archaeal genes contributed to bacterial virulence? Trends Microbiol. 12, 213–219. 10.1016/j.tim.2004.03.002.

Grimm, D., Bauer, J., Kossmehl, P., Shakibaei, M., Schöberger, J., Pickenhahn, H. et al., 2002. Simulated microgravity alters differentiation and increases apoptosis in human follicular thyroid carcinoma cells. FASEB J. 16, 604–606.

Grimm, D., Bauer, J., Ulbrich, C., Westphal, K., Wehland, M., Infanger, M. et al., 2010. Different responsiveness of endothelial cells to vascular endothelial growth factor and basic fibroblast growth factor added to culture media under gravity and simulated microgravity. Tissue Eng. Part A 16, 1559–1573. 10.1089/ten.tea.2009.0524.

Grimm, D., Egli, M., Krüger, M., Riwaldt, S., Corydon, T.J., Kopp, S. et al., 2018. Tissue Engineering Under Microgravity Conditions-Use of Stem Cells and Specialized Cells. Stem Cells Dev. 27, 787–804. 10.1089/scd.2017.0242.

Grimm, D., Grosse, J., Wehland, M., Mann, V., Reseland, J.E., Sundaresan, A. et al., 2016. The impact of microgravity on bone in humans. Bone 87, 44–56. 10.1016/j.bone.2015.12.057.

Grimm, D., Infanger, M., Westphal, K., Ulbrich, C., Pietsch, J., Kossmehl, P. et al., 2009. A delayed type of three-dimensional growth of human endothelial cells under simulated weightlessness. Tissue Eng. Part A 15, 2267–2275. 10.1089/ten.tea.2008.0576.

Grossi, G., Goglio, P., Vitali, A., Williams, A.G., 2019. Livestock and climate change: impact of livestock on climate and mitigation strategies. Animal Front. 9, 69–76. 10.1093/af/vfy034.

Guljamow, A., Jenke-Kodama, H., Saumweber, H., Quillardet, P., Frangeul, L., Castets, A.M. et al., 2007. Horizontal gene transfer of two cytoskeletal elements from a eukaryote to a cyanobacterium. Curr. Biol. 17, R757-9. 10.1016/j.cub.2007.06.063.

Haddock, S.H.D., Moline, M.A., Case, J.F., 2010. Bioluminescence in the sea. Annu. Rev. Mar. Sci. 2, 443–493. 10.1146/annurev-marine-120308-081028.

Häder, D.-P., Braun, M., Grimm, D., Hemmersbach, R., 2017. Gravireceptors in eukaryotes-a comparison of case studies on the cellular level. npj Microgravity 3, 13. 10.1038/s41526-017-0018-8.

Häder, D.-P., Richter, P., Lebert, M., 2006a. Signal transduction in gravisensing of flagellates. Signal Transduct. 6, 422–431. 10.1002/sita.200600104.

Häder, D.-P., Richter, P., Ntefidou, M., Lebert, M., 2005. Gravitational sensory transduction chain in flagellates. Adv. Space Res. 36, 1182–1188.

Häder, D.-P., Richter, P., Schuster, M., Dachev, T., Tomov, B., Georgiev, P. et al., 2009a. R3D-B2-Measurement of ionizing and solar radiation in open space in the BIOPAN 5 facility outside the FOTON M2 satellite. Adv. Space Res. 43, 1200–1211.

Häder, D.-P., Richter, P.R., Schuster, M., Daiker, V., Lebert, M., 2009b. Molecular analysis of the graviperception signal transduction in the flagellate *Euglena gracilis*: Involvement of a transient receptor potential-like channel and a calmodulin. Adv. Space Res. 43, 1179–1184. 10.1016/j.asr.2009.01.029.

Häder, D.-P., Richter, P.R., Strauch, S.M., Schuster, M., 2006b. Aquacells - flagellates under long-term microgravity and potential usage for life support systems. Micrograv. Sci. Technol. 18, 210–214.

Hagen, B. ten, Kümmel, F., Wittkowski, R., Takagi, D., Löwen, H., Bechinger, C., 2014. Gravitaxis of asymmetric self-propelled colloidal particles. Nat. Commun. 5, 4829. 10.1038/ncomms5829.

Hagiwara, H., Higashibata, A., Ogawa, S., Kanazawa, S., Mizuno, H., Tanaka, R., 2018. Effectiveness of endothelial progenitor cell culture under microgravity for improved angiogenic potential. Sci. Rep. 8, 14239. 10.1038/s41598-018-32073-2.

Hall, C., Dietrich, F.S., 2007. The reacquisition of biotin prototrophy in Saccharomyces cerevisiae involved horizontal gene transfer, gene duplication and gene clustering. Genetics 177, 2293–2307. 10.1534/genetics.107.074963.

Hamilton, G., 1998. Multicellular spheroids as an in vitro tumor model. Cancer Lett. 131, 29–34. 10.1016/S0304-3835(98)00198-0.

Hammond, T.G., Hammond, J.M., 2001. Optimized suspension culture: the rotating-wall vessel. Am. J. Physiol. Renal Physiol. 28, 12–25.

Hammond, T.G., Stodieck, L., Birdsall, H.H., Becker, J.L., Koenig, P., Hammond, J.S. et al., 2013. Effects of microgravity on the virulence of *Listeria monocytogenes*, *Enterococcus faecalis*, *Candida albicans*, and methicillin-resistant *Staphylococcus aureus*. Astrobiology 13, 1081–1090. 10.1089/ast.2013.0986.

Han, J., Dai, J., 2016. Microgravity may help future organ/tissue manufacture. Science China. Life Sci. 59, 850–853. 10.1007/s11427-016-5079-5.

Hao, W., Golding, G.B., 2008. Uncovering rate variation of lateral gene transfer during bacterial genome evolution. BMC Genomics 9, 235. 10.1186/1471-2164-9-235.

Harada, K., Obiya, Y., Nakano, T., Kawashima, M., Miki, T., Kobayashi, Y. et al., 1997. Cancer risk in space due to radiation assessed by determining cell lethality and mutation frequencies of prokaryotes and a plasmid during the Second International Microgravity Laboratory (IML-2) Space Shuttle experiment. Oncol. Rep. 4, 691–695. 10.3892/or.4.4.691.

Hashemi, B.B., Penkala, J.E., Vens, C., Huls, H., Cubbage, M., Sams, C.F., 1999. T cell activation responses are differentially regulated during clinorotation and in spaceflight. FASEB J. 13, 2071–2082. 10.1096/fasebj.13.14.2071.

Hauslage, J., Cevik, V., Hemmersbach, R., 2017. Pyrocystis noctiluca represents an excellent bioassay for shear forces induced in ground-based microgravity simulators (clinostat and random positioning machine). npj Microgravity 3, 1. 10.1038/s41526-017-0016-x.

Hausmann, N., Fengler, S., Hennig, A., Franz-Wachtel, M., Hampp, R., Neef, M. et al., 2014. Cytosolic calcium, hydrogen peroxide and related gene expression and protein modulation in *Arabidopsis thaliana* cell cultures respond immediately to altered gravitation: parabolic flight data. Plant Biol. J. 16, 120–128. 10.1111/plb.12051.

Hayashi, T., Makino, K., Ohnishi, M., Kurokawa, K., Ishii, K., Yokoyama, K. et al., 2001. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res. 8, 11–22. 10.1093/dnares/8.1.11.

He, X., Liu, M., Lu, J., Xue, H., Pan, Y. Space Mutation Breeding: A Brief Introduction of, UK Global Science Books, UK, 2006.

Hemmersbach, R., Simon, A., Waßer, K., Hauslage, J., Christianen, P.C.M., Albers, P.W. et al., 2014. Impact of a high magnetic field on the orientation of gravitactic unicellular organisms--a critical consideration about the application of magnetic fields to mimic functional weightlessness. Astrobiology 14, 205–215. 10.1089/ast.2013.1085.

Hemmersbach, R., Strauch, S.M., Seibt, D., Schuber, M., Häder, D.-P., 2006. Comparative studies on gravisensitive protists on ground (2D and 3D clinostats) and in microgravity. Microgravity Sci. Technol. 18, 257–259.

Herranz, R., Anken, R., Boonstra, J., Braun, M., Christianen, P.C.M., Geest, M. de et al., 2013. Ground-Based Facilities for Simulation of Microgravity: Organism-Specific Recommendations for Their Use, and Recommended Terminology. Astrobiology 13, 1–17. 10.1089/ast.2012.0876.

Hershberg, R., 2015. Mutation--The Engine of Evolution: Studying Mutation and Its Role in the Evolution of Bacteria. Cold Spring Harb. Perspect. Biol. 7, a018077. 10.1101/cshperspect.a018077.

Hoekstra, H.E., Coyne, J.A., 2007. The locus of evolution: evo devo and the genetics of adaptation. Evol. 61, 995–1016. 10.1111/j.1558-5646.2007.00105.x.

Hoekstra, H.E., Hirschmann, R.J., Bundey, R.A., Insel, P.A., Crossland, J.P., 2006. A single amino acid mutation contributes to adaptive beach mouse color pattern. Sci. 313, 101–104. 10.1126/science.1126121.

Hoffmann, A.A., HERCUS, M.J., 2000. Environmental Stress as an Evolutionary Force. BioSci. 50, 217.

Hoffmann, A.A., Merilä, J., 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol. Evol. 14, 96–101. 10.1016/S0169-5347(99)01595-5.

Holliday, R., 2006. Epigenetics: a historical overview. Epigenetics 1, 76–80. 10.4161/epi.1.2.2762.

Holt, K.E., Baker, S., Weill, F.-X., Holmes, E.C., Kitchen, A., Yu, J. et al., 2012. *Shigella sonnei* genome sequencing and phylogenetic analysis indicate recent global dissemination from Europe. Nat. Genet. 44, 1056–1059. 10.1038/ng.2369.

Horne, S.D., Chowdhury, S.K., Heng, H.H.Q., 2014. Stress, genomic adaptation, and the evolutionary trade-off. Front. Genet. 5, 92. 10.3389/fgene.2014.00092.

Horneck, G., Bucker, H., Dose, K., Martens, K.D., Bieger, A., Mennigmann, H.D. et al., 1984a. Microorganisms and biomolecules in space environment experiment ES 029 on Spacelab-1. Adv. Space Res. 4, 19–27.

Horneck, G., Bucker, H., Reitz, G., Requardt, H., Dose, K., Martens, K.D. et al., 1984b. Life Sci.: Microorganisms in the Space Environment. Sci. 225, 226–228. 10.1126/science.225.4658.226.

Horneck, G., Klaus, D.M., Mancinelli, R.L., 2010. Space Microbiology. Microbiol. Mol. Biol. Rev. 74, 121–156. 10.1128/MMBR.00016-09.

Huang, B., Liu, N., Rong, X., Ruan, J., Huang, Y., 2015. Effects of simulated microgravity and spaceflight on morphological differentiation and secondary metabolism of *Streptomyces coelicolor* A3(2). Appl. Microbiol. Biotechnol. 99, 4409–4422. 10.1007/s00253-015-6386-7.

Huang, J., 2013. Horizontal gene transfer in eukaryotes: the weak-link model. BioEssays 35, 868–875. 10.1002/bies.201300007.

Huang, J., Gogarten, J.P., 2008. Concerted gene recruitment in early plant evolution. Genome Biol. 9, R109. 10.1186/gb-2008-9-7-r109.

Huang, L., Raats, D., Sela, H., Klymiuk, V., Lidzbarsky, G., Feng, L. et al., 2016. Evolution and Adaptation of Wild Emmer Wheat Populations to Biotic and Abiotic Stresses. Annual Rev. Phytopathol. 54, 279–301. 10.1146/annurev-phyto-080614-120254.

Hughes-Fulford, M., Chang, T.T., Martinez, E.M., Li, C.-F., 2015. Spaceflight alters expression of microRNA during T-cell activation. FASEB J. 29, 4893–4900. 10.1096/fj.15-277392.

Ichijo, T., Yamaguchi, N., Tanigaki, F., Shirakawa, M., Nasu, M., 2016. Four-year bacterial monitoring in the International Space Station-Japanese Experiment Module "Kibo" with culture-independent approach. npj Microgravity 2, 16007. 10.1038/npjmgrav.2016.7.

Ilyin, V.K., 2005. Microbiological status of cosmonauts during orbital spaceflights on Salyut and Mir orbital stations. Acta Astronaut. 56, 839–850.

Infanger, M., Kossmehl, P., Shakibaei, M., Baatout, S., Witzing, A., Grosse, J. et al., 2006. Induction of three-dimensional assembly and increase in apoptosis of human endothelial cells by simulated microgravity: Impact of vascular endothelial growth factor. Apoptosis 11, 749–764. 10.1007/s10495-006-5697-7.

Ingber, D., 1999. How cells (might) sense microgravity. FASEB J. 13 Suppl, S3-15. 10.1096/fasebj.13.9001.s3.

Ingber, D.E., 2014. Mechanobiology, Tissue Development and Organ Engineering. In: Principles of Tissue Engineering: Elsevier. p. 309–322.

Ingram, M., Techy, G.B., Saroufeem, R., Yazan, O., Narayan, K.S., Goodwin, T.J. et al., 1997. Three-dimensional growth patterns of various human tumor cell lines in simulated microgravity of a NASA bioreactor. In Vitro Cell. Dev. Biol. -Animal 33, 459–466. 10.1007/s11626-997-0064-8.

Interagency Coordination Group on Antimicrobial Resistance [Internet]. No time to Wait: Securing the future from drug-resistant Infections. A report to the Secretary-General of the United Nations, 2019. Available from: http://oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/IACG2019/IACG_final_report_EN.pdf.

Ito, H., 2014. Plant Models of Transgenerational Epigenetic Inheritance. In: Transgenerational Epigenetics: Elsevier. p. 147–161.

J. Majima, H., P. Indo, H., Tomita, K., Iwashita, Y., Suzuki, H., Masuda, D. et al., 2009. Bio-Assessment of RISK in Long-Term Manned Space Exploration-Cell Death Factors in Space Radiation and/or Microgravity: A Review. Biol. Sci. Space 23, 43–53. 10.2187/bss.23.43.

Jain, R., Rivera, M.C., Moore, J.E., Lake, J.A., 2002. Horizontal gene transfer in microbial genome evolution. Theor. Popul. Biol. 61, 489–495.

Janosi, I.M., Czirok, A., Silhavy, D., Holczinger, A., 2002. Is bioconvection enhancing bacterial growth in quiescent environments? Environ..Microbiol. 4, 525–531.

Jeong, A.J., Kim, Y.J., Lim, M.H., Lee, H., Noh, K., Kim, B.-H. et al., 2018. Microgravity induces autophagy via mitochondrial dysfunction in human Hodgkin's lymphoma cells. Sci. Rep. 8, 14646. 10.1038/s41598-018-32965-3.

Jessup, J.M., Frantz, M., Sonmez-Alpan, E., Locker, J., Skena, K., Waller, H. et al., 2000. Microgravity culture reduces apoptosis and increases the differentiation of a human colorectal carcinoma cell line. Nat. Med. 36, 367. 10.1290/1071-2690(2000)036<0367:MCRAAI>2.0.CO;2.

Jin, F., Zhang, Y., Xuan, K., He, D., Deng, T., Tang, L. et al., 2010. Establishment of three-dimensional tissue-engineered bone constructs under microgravity-simulated conditions. Artif. Organs 34, 118–125. 10.1111/j.1525-1594.2009.00761.x.

Jin, J., Chen, H., Cai, W., 2015. Transcriptome Analysis of *Oryza sativa* Calli Under Microgravity. Microgravity Sci. Technol. 27, 437–453. 10.1007/s12217-015-9432-2.

Jin, T., Chen, Y., Jarboe, L.R., 2016. Evolutionary Methods for Improving the Production of Biorenewable Fuels and Chemicals. In: Biotechnology for Biofuel Production and Optimization: Elsevier. p. 265–290.

Juergensmeyer, M.A., Juergensmeyer, E.A., Guikema, J.A., 1999. Long-term exposure to spaceflight conditions affects bacterial response to antibiotics. Microgravity Sci. Technol. 12, 41–47.

Kacena, M.A., Manfredi, B., Todd, P., 1999. Effects of space flight and mixing on bacterial growth in low volume cultures. Microgravity Sci. Technol. 12, 74–77.

Kalpana, D., Im, C., Lee, Y.S., 2016. Comparative growth, cross stress resistance, transcriptomics of Streptococcus pyogenes cultured under low shear modeled microgravity and normal gravity. Saudi J. Biol. Sci. 23, 24–33. 10.1016/j.sjbs.2015.02.004.

Kamal, K.Y., Herranz, R., van Loon, J.J.W.A., Medina, F.J., 2018. Simulated microgravity, Mars gravity, and 2g hypergravity affect cell cycle regulation, ribosome biogenesis, and epigenetics in *Arabidopsis* cell cultures. Sci. Rep. 8, 6424. 10.1038/s41598-018-24942-7.

Kaur, P., Ward, B., Saha, B., Young, L., Groshen, S., Techy, G. et al., 2011. Human breast cancer histoid: an in vitro 3-dimensional co-culture model that mimics breast cancer tissue. J. Histochem. Cytochem. 59, 1087–1100. 10.1369/0022155411423680.

Kay, G.L., Sergeant, M.J., Zhou, Z., Chan, J.Z.-M., Millard, A., Quick, J. et al., 2015. Eighteenth-century genomes show that mixed infections were common at time of peak tuberculosis in Europe. Nat. Commun. 6, 6717. 10.1038/ncomms7717.

Keeling, P.J., Palmer, J.D., 2008. Horizontal gene transfer in eukaryotic evolution. Nat. Rev. Genet. 9, 605–618. 10.1038/nrg2386.

Kelleher, J.E., Raleigh, E.A., 1995. On the regulation and diversity of restriction in *Escherichia coli*. Gene 157, 229–230. 10.1016/0378-1119(94)00678-L.

Kenific, C.M., Wittmann, T., Debnath, J., 2016. Autophagy in adhesion and migration. J. Cell Sci. 129, 3685–3693. 10.1242/jcs.188490.

Kennemann, L., Didelot, X., Aebischer, T., Kuhn, S., Drescher, B., Droege, M. et al., 2011. *Helicobacter pylori* genome evolution during human infection. Proc. Natl. Acad. Sci. U. S. A. 108, 5033–5038. 10.1073/pnas.1018444108.

Khraiwesh, B., Zhu, J.-K., Zhu, J., 2012. Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta 1819, 137–148. 10.1016/j.bbagrm.2011.05.001.

Kim, H.J., Lee, J., Choi, J.-H., Bahinski, A., Ingber, D.E., 2016. Co-culture of Living Microbiome with Microengineered Human Intestinal Villi in a Gut-on-a-Chip Microfluidic Device. J. Vis. Exp (114): 54344. 10.3791/54344.

Kim, H.W., Rhee, M.S., 2016. Influence of Low-Shear Modeled Microgravity on Heat Resistance, Membrane Fatty Acid Composition, and Heat Stress-Related Gene Expression in *Escherichia coli* O157:H7 ATCC 35150, ATCC 43889, ATCC 43890, and ATCC 43895. Appl. Environ. Microbiol. 82, 2893–2901. 10.1128/AEM.00050-16.

Kim, W., Tengra, F.K., Shong, J., Marchand, N., Chan, H.K., Young, Z. et al., 2013a. Effect of spaceflight on Pseudomonas aeruginosa final cell density is modulated by nutrient and oxygen availability. BMC Microbiol. 13, 241. 10.1186/1471-2180-13-241.

Kim, W., Tengra, F.K., Young, Z., Shong, J., Marchand, N., Chan, H.K. et al., 2013b. Spaceflight promotes biofilm formation by Pseudomonas aeruginosa. PLoS ONE 8, e62437. 10.1371/journal.pone.0062437.

Kim, Y.J., Jeong, A.J., Kim, M., Lee, C., Ye, S.-K., Kim, S., 2017. Time-averaged simulated microgravity (taSMG) inhibits proliferation of lymphoma cells, L-540 and HDLM-2, using a 3D clinostat. Biomedical Eng. Online 16, 48. 10.1186/s12938-017-0337-8.

Kimbrough, J.M., Salinas-Mondragon, R., Boss, W.F., Brown, C.S., Sederoff, H.W., 2004. The fast and transient transcriptional network of gravity and mechanical stimulation in the *Arabidopsis* root apex. Plant Physiol 136, 2790–2805.

King, J.L., Jukes, T.H., 1969. Non-Darwinian evolution. Sci. 164, 788–798. 10.1126/science.164.3881.788.

King, J.R., Edgar, S., Qiao, K., Stephanopoulos, G., 2016. Accessing Nature's diversity through metabolic engineering and synthetic biology. F1000Res. 5. 10.12688/f1000research.7311.1.

King, M.C., Wilson, A.C., 1975. Evolution at two levels in humans and chimpanzees. Sci. 188, 107–116. 10.1126/science.1090005.

Kishore, S.P., Stiller, J.W., Deitsch, K.W., 2013. Horizontal gene transfer of epigenetic machinery and evolution of parasitism in the malaria parasite *Plasmodium falciparum* and other apicomplexans. BMC Evol. Biol. 13, 37. 10.1186/1471-2148-13-37.

Kiss, J.Z., 2000. Mechanisms of the early phases of plant gravitropism. Crit. Rev. Plant Sci. 19, 551–573.

Kiss, J.Z., Wolverton, C., Wyatt, S.E., Hasenstein, K.H., van Loon, J.J.W.A., 2019. Comparison of Microgravity Analogs to Spaceflight in Studies of Plant Growth and Development. Front. Plant Sci. 10, 1577. 10.3389/fpls.2019.01577.

Klaus, D., Simske, S., Todd, P., Stodieck, L., 1997. Investigation of space flight effects on *Escherichia coli* and a proposed model of underlying physical mechanisms. Microbiol. 143 (Pt 2), 449–455. 10.1099/00221287-143-2-449.

Klaus, D.M., 2001. Clinostats and bioreactors. Gravit. Space Biol. Bull.14(2):55-64.

Klaus, D.M., 2004. Gravitational influence on biomolecular engineering processes. Gravit. Space Biol. Bull. 17, 51–65. 10.1016/S1389-0344(04)00014-0.

Klaus, D.M., Benoit, M.R., Nelson, E.S., Hammond, T.G., 2004. Extracellular mass transport considerations for space flight research concerning suspended and adherent in vitro cell cultures. J Gravit. Physiol. 11, 17–27.

Kloesges, T., Popa, O., Martin, W., Dagan, T., 2011. Networks of gene sharing among 329 proteobacterial genomes reveal differences in lateral gene transfer frequency at different phylogenetic depths. Mol. Biol. Evol. 28, 1057–1074. 10.1093/molbev/msq297.

Klymchuk, D.O., 2007. Structural and functional organization of mitochondria in soybean root statocytes under microgravitation. Tsitol. Genet. 41, 30–35.

Kohn, F., Hauslage, J., Hanke, W., 2017. Membrane Fluidity Changes, A Basic Mechanism of Interaction of Gravity with Cells? Microgravity Sci. Technol. 29, 337–342. 10.1007/s12217-017-9552-y.

Kondo, N., Nikoh, N., Ijichi, N., Shimada, M., Fukatsu, T., 2002. Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. Proc. Natl. Acad. Sci. U. S. A. 99, 14280–14285. 10.1073/pnas.222228199.

Koonin, E.V., Makarova, K.S., Aravind, L., 2001. Horizontal gene transfer in prokaryotes: quantification and classification. Annu. Rev. Microbiol. 55, 709–742. 10.1146/annurev.micro.55.1.709.

Koonin, E.V., Mushegian, A.R., Galperin, M.Y., Walker, D.R., 1997. Comparison of archaeal and bacterial genomes: computer analysis of protein sequences predicts novel functions and suggests a chimeric origin for the archaea. Mol. Microbiol. 25, 619–637. 10.1046/j.1365-2958.1997.4821861.x.

Koonin, E.V., Wolf, Y.I., 2008. Genomics of bacteria and archaea: the emerging dynamic view of the prokaryotic world. Nucleic Acids Res. 36, 6688–6719. 10.1093/nar/gkn668.

Kopp, S., Krüger, M., Bauer, J., Wehland, M., Corydon, T.J., Sahana, J. et al., 2018. Microgravity Affects Thyroid Cancer Cells during the TEXUS-53 Mission Stronger than Hypergravity. Int. J. Mol. Sci. 19. 10.3390/ijms19124001-.

Kopp, S., Warnke, E., Wehland, M., Aleshcheva, G., Magnusson, N.E., Hemmersbach, R. et al., 2015. Mechanisms of three-dimensional growth of thyroid cells during long-term simulated microgravity. Sci. Rep. 5, 16691. 10.1038/srep16691.

Kordyum, E., 2003. A role for the cytoskeleton in plant cell gravisensitivity and Ca2+-signaling in microgravity. Cell Biol. Internat. 27, 219–221. 10.1016/S1065-6995(02)00315-3.

Kordyum, E.L., 2014. Plant cell gravisensitivity and adaptation to microgravity. Plant Biol. 16 Suppl 1, 79–90. 10.1111/plb.12047.

Kordyum, E.L., Chapman, D.K., 2017. Plants and microgravity: Patterns of microgravity effects at the cellular and molecular levels. Cytol. Genet. 51, 108–116. 10.3103/S0095452717020049.

Kossmehl, P., Shakibaei, M., Cogoli, A., Infanger, M., Curcio, F., Schönberger, J. et al., 2003. Weightlessness induced apoptosis in normal thyroid cells and papillary thyroid carcinoma cells via extrinsic and intrinsic pathways. Endocrinol. 144, 4172–4179. 10.1210/en.2002-0171.

Kossmehl, P., Shakibaei, M., Cogoli, A., Pickenhahn, H., Paul, M., Grimm, D., 2002. Simulated microgravity induces programmed cell death in human thyroid carcinoma cells. J. Gravit. Physiol. 9, 295-306.

Krüger, J., Richter, P., Stoltze, J., Strauch, S.M., Krüger, M., Daiker, V. et al., 2019a. Changes of Gene Expression in *Euglena gracilis* Obtained During the 29th DLR Parabolic Flight Campaign. Sci. Rep. 9, 14260. 10.1038/s41598-019-50611-4.

Krüger, M., Kopp, S., Wehland, M., Bauer, J., Baatout, S., Moreels, M. et al., 2019b. Growing blood vessels in space: Preparation studies of the SPHEROIDS project using related ground-based studies. Acta Astronaut. 159, 267–272. 10.1016/j.actaastro.2019.03.074.

Krüger, M., Melnik, D., Kopp, S., Buken, C., Sahana, J., Bauer, J. et al., 2019c. Fighting Thyroid Cancer with Microgravity Research. Int. J. Mol. Sci. 20. 10.3390/ijms20102553.

Ku, C., Martin, W.F., 2016. A natural barrier to lateral gene transfer from prokaryotes to eukaryotes revealed from genomes: the 70 % rule. BMC Biol. 14, 89. 10.1186/s12915-016-0315-9.

Ku, C., Nelson-Sathi, S., Roettger, M., Sousa, F.L., Lockhart, P.J., Bryant, D. et al., 2015. Endosymbiotic origin and differential loss of eukaryotic genes. Nature 524, 427–432. 10.1038/nature14963.

Kucsko, G., Maurer, P.C., Yao, N.Y., Kubo, M., Noh, H.J., Lo, P.K. et al., 2013. Nanometre-scale thermometry in a living cell. Nature 500, 54–58. 10.1038/nature12373.

Kunz-Schughart, L.A., 1999. Multicellular tumor spheroids: intermediates between monolayer culture and in vivo tumor. Cell Biol. Internat. 23, 157–161. 10.1006/cbir.1999.0384.

Kunz-Schughart, L.A., Freyer, J.P., Hofstaedter, F., Ebner, R., 2004. The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. J. Biomol. Screen. 9, 273–285. 10.1177/1087057104265040.

Kunz-Schughart, L.A., Heyder, P., Schroeder, J., Knuechel, R., 2001. A heterologous 3-D coculture model of breast tumor cells and fibroblasts to study tumor-associated fibroblast differentiation. Exp. Cell Res. 266, 74–86. 10.1006/excr.2001.5210.

Kuo, C.-H., Ochman, H., 2009. The fate of new bacterial genes. FEMS Microbiol. Rev. 33, 38–43. 10.1111/j.1574-6976.2008.00140.x.

Kurland, C.G., Canback, B., Berg, O.G., 2003. Horizontal gene transfer: a critical view. Proc. Natl. Acad. Sci. U. S. A. 100, 9658–9662. 10.1073/pnas.1632870100.

Kuzminov, A., 1999. Recombinational Repair of DNA Damage in *Escherichia coli* and Bacteriophage λ. Microbiol. Mol. Biol. Rev. 63, 751–813.

Lam, K.S., Gustavson, D.R., Pirnik, D.L., Pack, E., Bulanhagui, C., Mamber, S.W. et al., 2002. The effect of space flight on the production of actinomycin D by *Streptomyces plicatus*. J. Ind. Microbiol. Biotechnol. 29, 299–302. 10.1038/sj.jim.7000312.

Lam, K.S., Mamber, S.W., Pack, E.J., Forenza, S., Fernandes, P.B., Klaus, D.M., 1998. The effects of space flight on the production of monorden by *Humicola fuscoatra* WC5157 in solid-state fermentation. Appl. Microbiol. Biotechnol. 49, 579–583.

Lapchine, L., Moatti, N., Gasset, G., Richoilley, G., Templier, J., Tixador, R., 1986. Antibiotic activity in space. Drugs Exp. Clin. Res. 12, 933–938.

Law, J.A., Jacobsen, S.E., 2009. Molecular biology. Dynamic DNA methylation. Sci. 323, 1568–1569. 10.1126/science.1172782.

Lawal, A., Kirtley, M.L., van Lier, C.J., Erova, T.E., Kozlova, E.V., Sha, J. et al., 2013. The effects of modeled microgravity on growth kinetics, antibiotic susceptibility, cold growth, and the virulence potential of a *Yersinia pestis* ymoA-deficient mutant and its isogenic parental strain. Astrobiology 13, 821–832. 10.1089/ast.2013.0968.

Leandro, L.J., Szewczyk, N.J., Benguría, A., Herranz, R., Laván, D., Medina, F.J. et al., 2007. Comparative analysis of *Drosophila melanogaster* and *Caenorhabditis elegans* gene expression experiments in the European Soyuz flights to the International Space Station. Adv. Space Res. 40, 506–512. 10.1016/j.asr.2007.05.070.

Lebert, M., Häder, D.-P., 1996. How *Euglena* tells up from down. Nature 379, 590.

Lebert, M., Häder, D.-P., 1997a. Behavioral mutants of *Euglena gracilis*: functional and spectroscopic characterization. J Plant Physiol 151, 188–195.

Lebert, M., Häder, D.-P., 1997b. Effects of hypergravity on the photosynthetic flagellate, *Euglena gracilis*. J Plant Physiol 150, 153–159.

Lee, M.C., Israelsson, U.E., 2001. New microgravity fundamental physics research areas in the ISS era. Acta Astronaut. 48, 597–602. 10.1016/S0094-5765(01)00029-7.

Lee, S.-M., Young, E.M., Alper, H.S., 2015. Remaining Challenges in the Metabolic Engineering of Yeasts for Biofuels. In: Direct Microbial Conversion of Biomass to Advanced Biofuels: Elsevier. p. 209–237.

Lelkes, P.I., Galvan, D.L., Hayman, G.T., Goodwin, T.J., Chatman, D.Y., Cherian, S. et al., 1998. Simulated microgravity conditions enhance differentiation of cultured PC12 cells towards the neuroendocrine phenotype. In Vitro Cell. Dev. Biol. -Animal 34, 316–325. 10.1007/s11626-998-0008-y.

Lerat, E., Daubin, V., Ochman, H., Moran, N.A., 2005. Evolutionary origins of genomic repertoires in bacteria. PLoS Biol. 3, e130. 10.1371/journal.pbio.0030130.

Lerminiaux, N.A., Cameron, A.D.S., 2019. Horizontal transfer of antibiotic resistance genes in clinical environments. Can. J. Microbiol. 65, 34–44. 10.1139/cjm-2018-0275.

Lewis, M.L., Reynolds, J.L., Cubano, L.A., Hatton, J.P., Lawless, B.D., Piepmeier, E.H., 1998. Spaceflight alters microtubules and increases apoptosis in human lymphocytes (Jurkat). FASEB J. 12, 1007–1018.

Li, H.-F., Chen, X.-P., Zhu, F.-H., Liu, H.-Y., Hong, Y.-B., Liang, X.-Q., 2013. Transcriptome profiling of peanut (*Arachis hypogaea*) gynophores in gravitropic response. Funct. Plant Biol. 40, 1249. 10.1071/FP13075.

Liao, B.-Y., Weng, M.-P., Zhang, J., 2010. Contrasting genetic paths to morphological and physiological evolution. Proc. Natl. Acad. Sci. U. S. A. 107, 7353–7358. 10.1073/pnas.0910339107.

Limbach, C., Hauslage, J., Schäfer, C., Braun, M., 2005. How to activate a plant gravireceptor. Early mechanisms of gravity sensing studied in characean rhizoids during parabolic flights. Plant Physiol. 139, 1030–1040. 10.1104/pp.105.068106.
Lind, M.I., Spagopoulou, F., 2018. Evolutionary consequences of epigenetic inheritance. Heredity 121, 205–209. 10.1038/s41437-018-0113-y.
Løbner-Olesen, A., Skovgaard, O., Marinus, M.G., 2005. Dam methylation: coordinating cellular processes. Curr. Opin. Microbiol. 8, 154–160. 10.1016/j.mib.2005.02.009.
Loftus, B., Anderson, I., Davies, R., Alsmark, U.C.M., Samuelson, J., Amedeo, P. et al., 2005. The genome of the protist parasite *Entamoeba histolytica*. Nature 433, 865–868. 10.1038/nature03291.
Lynch, M., 2006. The origins of eukaryotic gene structure. Mol. Biol. Evol. 23, 450–468. 10.1093/molbev/msj050.
Lynch, M., 2010a. Evolution of the mutation rate. Trends Genet. 26, 345–352. 10.1016/j.tig.2010.05.003.
Lynch, M., 2010b. Rate, molecular spectrum, and consequences of human mutation. Proc. Natl. Acad. Sci. U. S. A. 107, 961–968. 10.1073/pnas.0912629107.
Lynch, M., Sung, W., Morris, K., Coffey, N., Landry, C.R., Dopman, E.B. et al., 2008. A genome-wide view of the spectrum of spontaneous mutations in yeast. Proc. Natl. Acad. Sci. U. S. A. 105, 9272–9277. 10.1073/pnas.0803466105.
Lynch, S.V., Mukundakrishnan, K., Benoit, M.R., Ayyaswamy, P.S., Matin, A., 2006. *Escherichia coli* Biofilms Formed under Low-Shear Modeled Microgravity in a Ground-Based System. Appl. Environ. Microbiol. 72, 7701–7710. 10.1128/AEM.01294-06.
Ma, X., Pietsch, J., Wehland, M., Schulz, H., Saar, K., Hübner, N. et al., 2014. Differential gene expression profile and altered cytokine secretion of thyroid cancer cells in space. FASEB J. 28, 813–835. 10.1096/fj.13-243287.
Ma, X., Wehland, M., Schulz, H., Saar, K., Hübner, N., Infanger, M. et al., 2013. Genomic approach to identify factors that drive the formation of three-dimensional structures by EA.hy926 endothelial cells. PLoS ONE 8, e64402. 10.1371/journal.pone.0064402.
Ma, Y., Cheng, Z., Wang, W., Sun, Y., 2007. Proteomic analysis of high yield rice variety mutated from spaceflight. Adv. Space Res. 40, 535–539. 10.1016/j.asr.2007.05.028.
Mackiewicz, P., Bodył, A., Moszczyński, K., 2013. The case of horizontal gene transfer from bacteria to the peculiar dinoflagellate plastid genome. Mob. Genet. Elements 3, e25845. 10.4161/mge.25845.
MacLean, R.C., Buckling, A., 2009. The distribution of fitness effects of beneficial mutations in *Pseudomonas aeruginosa*. PLoS Genet. 5, e1000406. 10.1371/journal.pgen.1000406.
MacLean, R.C., Torres-Barceló, C., Moxon, R., 2013. Evaluating evolutionary models of stress-induced mutagenesis in bacteria. Nat. Rev. Genet. 14, 221–227. 10.1038/nrg3415.
Manley, S., Cipelletti, L., Trappe, V., Bailey, A.E., Christianson, R.J., Gasser, U. et al., 2004. Limits to gelation in colloidal aggregation. Phys. Rev. Lett. 93, 108302. 10.1103/PhysRevLett.93.108302.
Mann, V., Grimm, D., Corydon, T.J., Krüger, M., Wehland, M., Riwaldt, S. et al., 2019. Changes in Human Foetal Osteoblasts Exposed to the Random Positioning Machine and Bone Construct Tissue Engineering. Int. J. Mol. Sci. 20. 10.3390/ijms20061357.
Manzano, A.I., van Loon, Jack JWA, Christianen, P.C.M., Gonzalez-Rubio, J.M., Medina, F.J., Herranz, R., 2012. Gravitational and magnetic field variations synergize to cause subtle variations in the global transcriptional state of *Arabidopsis* in vitro callus cultures. BMC Genomics 13, 105. 10.1186/1471-2164-13-105.
Mao, X.W., Pecaut, M.J., Stodieck, L.S., Ferguson, V.L., Bateman, T.A., Bouxsein, M. et al., 2013. Spaceflight Environment Induces Mitochondrial Oxidative Damage in Ocular Tissue. Radiat. Res. 180, 340–350. 10.1667/RR3309.1.
Marcet-Houben, M., Gabaldón, T., 2010. Acquisition of prokaryotic genes by fungal genomes. Trends Genet. 26, 5–8. 10.1016/j.tig.2009.11.007.
Marin, B., Nowack, E.C.M., Glöckner, G., Melkonian, M., 2007. The ancestor of the *Paulinella* chromatophore obtained a carboxysomal operon by horizontal gene transfer from a *Nitrococcus*-like gamma-proteobacterium. BMC Evol Biol 7, 85. 10.1186/1471-2148-7-85.
Martinez, E.M., Yoshida, M.C., Candelario, T.L.T., Hughes-Fulford, M., 2015. Spaceflight and simulated microgravity cause a significant reduction of key gene expression in early T-cell activation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 308, R480-8. 10.1152/ajpregu.00449.2014.

Martzivanou, M., Babbick, M., Cogoli-Greuter, M., Hampp, R., 2006. Microgravity-related changes in gene expression after short-term exposure of *Arabidopsis thaliana* cell cultures. Protoplasma 229, 155–162. 10.1007/s00709-006-0203-1.

Martzivanou, M., Hampp, R., 2003. Hyper-gravity effects on the *Arabidopsis* transcriptome. Physiol. Plant 118, 221–231.

Masiello, M.G., Cucina, A., Proietti, S., Palombo, A., Coluccia, P., D'Anselmi, F. et al., 2014. Phenotypic switch induced by simulated microgravity on MDA-MB-231 breast cancer cells. Biomed. Res. Int. 2014, 652434. 10.1155/2014/652434.

Masson-Delmotte, V., Zhai, P., Pörtner, H.O., Roberts, D., Skea, J., Shukla, P.R. et al. 1. IPCC, 2018: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty, 2018.

Mauclaire, L., Egli, M., 2010. Effect of simulated microgravity on growth and production of exopolymeric substances of *Micrococcus luteus* space and earth isolates. FEMS Immunol. Med. Microbiol. 59, 350–356. 10.1111/j.1574-695X.2010.00683.x.

Mazars, C., Brière, C., Grat, S., Pichereaux, C., Rossignol, M., Pereda-Loth, V. et al., 2014a. Microgravity induces changes in microsome-associated proteins of *Arabidopsis* seedlings grown on board the international space station. PLoS ONE 9, e91814. 10.1371/journal.pone.0091814.

Mazars, C., Brière, C., Grat, S., Pichereaux, C., Rossignol, M., Pereda-Loth, V. et al., 2014b. Microsome-associated proteome modifications of *Arabidopsis* seedlings grown on board the International Space Station reveal the possible effect on plants of space stresses other than microgravity. Plant Signal. Behav. 9, e29637. 10.4161/psb.29637.

McKenzie, G.J., Harris, R.S., Lee, P.L., Rosenberg, S.M., 2000. The SOS response regulates adaptive mutation. Proc. Natl. Acad. Sci. U. S. A. 97, 6646–6651. 10.1073/pnas.120161797.

McKenzie, J.A., Batterham, P., 1994. The genetic, molecular and phenotypic consequences of selection for insecticide resistance. Trends Ecol. Evol. 9, 166–169. 10.1016/0169-5347(94)90079-5.

McPherson, A., DeLucas, L., 1999. Crystal-growing in space. Science 283, 1459.

Melnik, D., Krüger, M., Kopp, S., Wehland, M., Bauer, J., Infanger, M. et al., 2018. Microgravity-based Modulation of VEGF Expression in Human Thyroid Carcinoma Cells. Front. Physiol. 9. 10.3389/conf.fphys.2018.26.00002.

Mermel, L.A., 2012. Infection Prevention and Control During Prolonged Human Space Travel. Clin. Infect. Dis. 56, 123–130. 10.1093/cid/cis861.

Millar, K.D., Johnson, C.M., Edelmann, R.E., Kiss, J.Z., 2011. An endogenous growth pattern of roots is revealed in seedlings grown in microgravity. Astrobiology 11, 787–797. 10.1089/ast.2011.0699.

Millar, K.D., Kiss, J.Z., 2013. Analyses of tropistic responses using metabolomics. Am. J. Bot. 100, 79–90. 10.3732/ajb.1200316.

Monciardini, P., Iorio, M., Maffioli, S., Sosio, M., Donadio, S., 2014. Discovering new bioactive molecules from microbial sources. Microbial biotechnology 7, 209–220. 10.1111/1751-7915.12123.

Monk, M., 1995. Epigenetic programming of differential gene expression in development and evolution. Develop. Genet. 17, 188–197. 10.1002/dvg.1020170303.

Morabito, C., Guarnieri, S., Catizone, A., Schiraldi, C., Ricci, G., Mariggiò, M.A., 2017. Transient increases in intracellular calcium and reactive oxygen species levels in TCam-2 cells exposed to microgravity. Sci. Rep. 7, 15648. 10.1038/s41598-017-15935-z.

Moreno-Villanueva, M., Wong, M., Lu, T., Zhang, Y., Wu, H., 2017. Interplay of space radiation and microgravity in DNA damage and DNA damage response. npj Microgravity 3, 14. 10.1038/s41526-017-0019-7.

Morrison, M.D., Fajardo-Cavazos, P., Nicholson, W.L., 2019. Comparison of *Bacillus subtilis* transcriptome profiles from two separate missions to the International Space Station. npj Microgravity 5, 1. 10.1038/s41526-018-0061-0.

Motamedi, M.R., Szigety, S.K., Rosenberg, S.M., 1999. Double-strand-break repair recombination in *Escherichia coli*: physical evidence for a DNA replication mechanism in vivo. Genes Dev. 13, 2889–2903.

Müller, G.B., 2003. Embryonic motility: environmental influences and evolutionary innovation. Evol. Dev. 5, 56–60. 10.1046/j.1525-142X.2003.03009.x.

Musilova, Z., Cortesi, F., Matschiner, M., Davies, W.I.L., Patel, J.S., Stieb, S.M. et al., 2019. Vision using multiple distinct rod opsins in deep-sea fishes. Sci. 364, 588–592. 10.1126/science.aav4632.
Nakamura, Y., Itoh, T., Matsuda, H., Gojobori, T., 2004. Biased biological functions of horizontally transferred genes in prokaryotic genomes. Nat. Genet. 36, 760–766. 10.1038/ng1381.
Nasir, A., Le Bail, A., Daiker, V., Klima, J., Richter, P., Lebert, M., 2018. Identification of a flagellar protein implicated in the gravitaxis in the flagellate *Euglena gracilis*. Sci. Rep. 8, 7605. 10.1038/s41598-018-26046-8.
Nasir, A., Strauch, S.M., Becker, I., Sperling, A., Schuster, M., Richter, P.R. et al., 2014. The influence of microgravity on *Euglena gracilis* as studied on Shenzhou 8. Plant Biol. J. 16, 113–119. 10.1111/plb.12067.
Nassef, M.Z., Kopp, S., Wehland, M., Melnik, D., Sahana, J., Krüger, M. et al., 2019. Real Microgravity Influences the Cytoskeleton and Focal Adhesions in Human Breast Cancer Cells. Int. J. Mol. Sci. 20. 10.3390/ijms20133156.
Nedelcu, A.M., Miles, I.H., Fagir, A.M., Karol, K., 2008. Adaptive eukaryote-to-eukaryote lateral gene transfer: stress-related genes of algal origin in the closest unicellular relatives of animals. J. Evol. Biol. 21, 1852–1860. 10.1111/j.1420-9101.2008.01605.x.
Neef, M., Ecke, M., Hampp, R., 2015. Real-time Recording of Cytosolic Calcium Levels in *Arabidopsis* thaliana Cell Cultures during Parabolic Flights. Microgravity Sci. Technol. 27, 305–312. 10.1007/s12217-015-9461-x.
Nei, M., 1987. Molecular evolutionary genetics Columbia University Press, New York.
Nei, M., 2005. Selectionism and neutralism in molecular evolution. Mol. Biol. Evol. 22, 2318–2342. 10.1093/molbev/msi242.
Nei, M., 2013. Mutation-driven evolution Oxford University Press, Oxford.
Nei, M., Rooney, A.P., 2005. Concerted and birth-and-death evolution of multigene families. Annu. Rev. Genet. 39, 121–152. 10.1146/annurev.genet.39.073003.112240.
Nelson, K.E., Clayton, R.A., Gill, S.R., Gwinn, M.L., Dodson, R.J., Haft, D.H. et al., 1999. Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of *Thermotoga maritima*. Nature 399, 323–329. 10.1038/20601.
Ness, R.W., Morgan, A.D., Colegrave, N., Keightley, P.D., 2012. Estimate of the spontaneous mutation rate in *Chlamydomonas reinhardtii*. Genetics 192, 1447–1454. 10.1534/genetics.112.145078.
Nevo, E., 2011. Evolution under environmental stress at macro- and microscales. Genome Biol. Evol. 3, 1039–1052. 10.1093/gbe/evr052.
Newton, A.C., 2016. Exploitation of Diversity within Crops-the Key to Disease Tolerance? Front. Plant Sci. 7, 665. 10.3389/fpls.2016.00665.
Nickerson, C.A., Ott, C.M., Mister, S.J., Morrow, B.J., Burns-Keliher, L., Pierson, D.L., 2000. Microgravity as a novel environmental signal affecting *Salmonella enterica* serovar Typhimurium virulence. Infect. Immun. 68, 3147–3152.
Nickerson, C.A., Ott, C.M., Wilson, J.W., Ramamurthy, R., Pierson, D.L., 2004. Microbial responses to microgravity and other low-shear environments. Microbiol. Mol. Biol. Rev. 68, 345–361.
Nikawa, T., Ishidoh, K., Hirasaka, K., Ishihara, I., Ikemoto, M., Kano, M. et al., 2004. Skeletal muscle gene expression in space-flown rats. FASEB J. 18, 522–524. 10.1096/fj.03-0419fje.
Noireaux, V., Libchaber, A., 2004. A vesicle bioreactor as a step toward an artificial cell assembly. Proc. Natl. Acad. Sci. U. S. A. 101, 17669–17674. 10.1073/pnas.0408236101.
Nordin, S.M., Nordin, K.J., 2017. Food, the source of Nutrition. WN 8, 87. 10.26596/wn.20178187-94.
Ntefidou, M., Iseki, M., Watanabe, M., Lebert, M., Häder, D.-P., 2003. Photoactivated adenylyl cyclase controls phototaxis in the flagellate *Euglena gracilis*. Plant Physiol. 133, 1517–1521.
Ochman, H., Lawrence, J.G., Groisman, E.A., 2000. Lateral gene transfer and the nature of bacterial innovation. Nature 405, 299–304. 10.1038/35012500.
Ohta, T., 1992. The Nearly Neutral Theory of Molecular Evolution. Annu. Rev. Ecol. Syst. 23, 263–286. 10.1146/annurev.es.23.110192.001403.
Ohyabu, Y., Kida, N., Kojima, H., Taguchi, T., Tanaka, J., Uemura, T., 2006. Cartilaginous tissue formation from bone marrow cells using rotating wall vessel (RWV) bioreactor. Biotechnol. Bioeng. 95, 1003–1008. 10.1002/bit.20892.
Okabe, K., Inada, N., Gota, C., Harada, Y., Funatsu, T., Uchiyama, S., 2012. Intracellular temperature mapping with a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy. Nat. Commun. 3, 705. 10.1038/ncomms1714.

Okubo, T., Tsuchida, A., Okuda, T., Fujitsuna, K., Ishikawa, M., Morita, T. et al., 1999. Kinetic analyses of colloidal crystallization in microgravity — aircraft experiments. Colloids **Surf**. A Physicochem. Eng. Asp. 153, 515–524. 10.1016/S0927-7757(98)00474-9.

Okubo, T., Tsuchida, A., Takahashi, S., Taguchi, K., Ishikawa, M., 2000. Kinetics of colloidal alloy crystallization of binary mixtures of monodispersed polystyrene and/or colloidal silica spheres having different sizes and densities in microgravity using aircraft. Coll. Polymer Sci. 278, 202–210. 10.1007/s003960050033.

Orsini, S.S., Lewis, A.M., Rice, K.C., 2017. Investigation of simulated microgravity effects on Streptococcus mutans physiology and global gene expression. npj Microgravity 3, 4. 10.1038/s41526-016-0006-4.

Ou, X., Long, L., Wu, Y., Yu, Y., Lin, X., Qi, X. et al., 2010. Spaceflight-induced genetic and epigenetic changes in the rice (*Oryza sativa* L.) genome are independent of each other. Genome 53, 524–532. 10.1139/G10-030.

Pacello, F., Rotilio, G., Battistoni, A., 2012. Low-Shear Modeled Microgravity Enhances Salmonella Enterica Resistance to Hydrogen Peroxide Through a Mechanism Involving KatG and KatN. Open Microbiol. J. 6, 53–64. 10.2174/1874285801206010053.

Pál, C., Papp, B., Lercher, M.J., 2005. Adaptive evolution of bacterial metabolic networks by horizontal gene transfer. Nat. Genet. 37, 1372–1375. 10.1038/ng1686.

Palazzo, A.F., Lee, E.S., 2015. Non-coding RNA: what is functional and what is junk? Frontiers in genetics 6, 2. 10.3389/fgene.2015.00002.

Parsons, P.A., 2005. Environments and evolution: interactions between stress, resource inadequacy and energetic efficiency. Biol. Rev. Camb. Philos. Soc. 80, 589–610. 10.1017/S1464793105006822.

Patel, M.J., Liu, W., Sykes, M.C., Ward, N.E., Risin, S.A., Risin, D. et al., 2007. Identification of mechanosensitive genes in osteoblasts by comparative microarray studies using the rotating wall vessel and the random positioning machine. J. Cell. Biochem. 101, 587–599. 10.1002/jcb.21218.

Patil, V.S., Zhou, R., Rana, T.M., 2014. Gene regulation by non-coding RNAs. Crit. Rev. Biochem. Mol. Biol. 49, 16–32. 10.3109/10409238.2013.844092.

Paul, A.-L., Amalfitano, C.E., Ferl, R.J., 2012a. Plant growth strategies are remodeled by spaceflight. BMC Plant Biol. 12, 232. 10.1186/1471-2229-12-232.

Paul, A.-L., Ferl, R.J., Meisel, M.W., 2006. High magnetic field induced changes of gene expression in *Arabidopsis*. Biomagn. Res. Technol. 4, 7. 10.1186/1477-044X-4-7.

Paul, A.-L., Manak, M.S., Mayfield, J.D., Reyes, M.F., Gurley, W.B., Ferl, R.J., 2011. Parabolic flight induces changes in gene expression patterns in *Arabidopsis thaliana*. Astrobiology 11, 743–758. 10.1089/ast.2011.0659.

Paul, A.-L., Popp, M.P., Gurley, W.B., Guy, C., Norwood, K.L., Ferl, R.J., 2005. *Arabidopsis* gene expression patterns are altered during spaceflight. Adv. Space Res. 36, 1175–1181. 10.1016/j.asr.2005.03.066.

Paul, A.-L., Zupanska, A.K., Ostrow, D.T., Zhang, Y., Sun, Y., Li, J.-L. et al., 2012b. Spaceflight transcriptomes: Unique responses to a novel environment. Astrobiology 12, 40–56. 10.1089/ast.2011.0696.

Paul, A.-L., Zupanska, A.K., Schultz, E.R., Ferl, R.J., 2013. Organ-specific remodeling of the *Arabidopsis* transcriptome in response to spaceflight. BMC Plant Biol. 13, 112. 10.1186/1471-2229-13-112.

Pérez-Losada, M., Porter, M.L., Tazi, L., Crandall, K.A., 2007. New methods for inferring population dynamics from microbial sequences. Infect. Genet. Evol. 7, 24–43. 10.1016/j.meegid.2006.03.004.

Perrin, J., 1916. Atoms Constable, London & Co.

Philpott, D.E., Popova, I.A., Kato, K., Stevenson, J., Miquel, J., Sapp, W., 1990. Morphological and biochemical examination of Cosmos 1887 rat heart tissue: Part I--Ultrastructure. FASEB J. 4, 73–78.

Pickens, L.B., Tang, Y., Chooi, Y.-H., 2011. Metabolic engineering for the production of natural products. Annu. Rev. Chem. Biomol. Eng. 2, 211–236. 10.1146/annurev-chembioeng-061010-114209.

Pietsch, J., Gass, S., Nebuloni, S., Echegoyen, D., Riwaldt, S., Baake, C. et al., 2017. Three-dimensional growth of human endothelial cells in an automated cell culture experiment container during the SpaceX CRS-8 ISS space mission - The SPHEROIDS project. Biomaterials 124, 126–156. 10.1016/j.biomaterials.2017.02.005.

Pitzschke, A., Hirt, H., 2010. New insights into an old story: *Agrobacterium*-induced tumour formation in plants by plant transformation. EMBO J 29, 1021–1032. 10.1038/emboj.2010.8.
Pletser, V., 2004. Short duration microgravity experiments in physical and life sciences during parabolic flights: the first 30 ESA campaigns. Acta Astronaut. 55, 829–854. 10.1016/j.actaastro.2004.04.006.
Polz, M.F., Alm, E.J., Hanage, W.P., 2013. Horizontal gene transfer and the evolution of bacterial and archaeal population structure. Trends Genet. 29, 170–175. 10.1016/j.tig.2012.12.006.
Popova, A.F., 2003. Comparative characteristic of mitochondria ultrastructural organization in *Chlorella* cells under altered gravity conditions. Adv. Space Res. 31, 2253–2259.
Prasad, B., Lein, W., Lindenberger, C.P., Buchholz, R., Vadakedath, N., 2019a. An optimized method and a dominant selectable marker for genetic engineering of an industrially promising microalga—*Pavlova lutheri*. J. Appl. Phycol. 31, 1163–1174. 10.1007/s10811-018-1617-9.
Prasad, B., Lein, W., Thiyam, G., Lindenberger, C.P., Buchholz, R., Vadakedath, N., 2019b. Stable nuclear transformation of rhodophyte species *Porphyridium purpureum*: advanced molecular tools and an optimized method. Photosynth. Res. 140, 173–188. 10.1007/s11120-018-0587-8.
Prasad, B., Vadakedath, N., Jeong, H.-J., General, T., Cho, M.-G., Lein, W., 2014. *Agrobacterium tumefaciens*-mediated genetic transformation of haptophytes (*Isochrysis* species). Appl. Microbiol. Biotechnol. 98, 8629–8639. 10.1007/s00253-014-5900-7.
Pross, H.D., Kiefer, J., 1999. Repair of cellular radiation damage in space under microgravity conditions. Radiat. Environ. Biophys. 38, 133–138. 10.1007/s004110050149.
Qian, A., Zhang, W., Xie, L., Weng, Y., Yang, P., Wang, Z. et al., 2008. Simulated weightlessness alters biological characteristics of human breast cancer cell line MCF-7. Acta Astronaut. 63, 947–958. 10.1016/j.actaastro.2008.01.024.
Rakyan, V.K., Chong, S., Champ, M.E., Cuthbert, P.C., Morgan, H.D., Luu, K.V.K. et al., 2003. Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. Proc. Natl. Acad. Sci. U. S. A. 100, 2538–2543. 10.1073/pnas.0436776100.
Reid, J.B., Ross, J.J., 2011. Mendel's genes: toward a full molecular characterization. Genetics 189, 3–10. 10.1534/genetics.111.132118.
Rest, J.S., Mindell, D.P., 2003. SARS associated coronavirus has a recombinant polymerase and coronaviruses have a history of host-shifting. Infect. Genet. Evol. 3, 219–225. 10.1016/j.meegid.2003.08.001.
Rétaux, S., Casane, D., 2013. Evolution of eye development in the darkness of caves: adaptation, drift, or both? EvoDevo 4, 26. 10.1186/2041-9139-4-26.
Rettberg, P., Eschweiler, U., Strauch, K., Reitz, G., Horneck, G., Wanke, H. et al., 2002. Survival of microorganisms in space protected by meteorite material: results of the experiment 'EXOBIOLOGIE' of the PERSEUS mission. Adv. Space Res. 30, 1539–1545.
Rhodes, F.H., 1983. Gradualism, punctuated equilibrium and the Origin of Species. Nature 305, 269–272. 10.1038/305269a0.
Ricard, G., McEwan, N.R., Dutilh, B.E., Jouany, J.-P., Macheboeuf, D., Mitsumori, M. et al., 2006. Horizontal gene transfer from Bacteria to rumen Ciliates indicates adaptation to their anaerobic, carbohydrates-rich environment. BMC Genomics 7, 22. 10.1186/1471-2164-7-22.
Richards, T.A., Dacks, J.B., Campbell, S.A., Blanchard, J.L., Foster, P.G., McLeod, R. et al., 2006. Evolutionary origins of the eukaryotic shikimate pathway: gene fusions, horizontal gene transfer, and endosymbiotic replacements. Eukaryot. Cell 5, 1517–1531. 10.1128/EC.00106-06.
Richter, P., Lebert, M., Tahedl, H., Häder, D.-P., 2001. Calcium is involved in the gravitactic orientation in colorless flagellates. J. Plant Physiol. 158, 689–697.
Richter, P., Ntefidou, M., Streb, C., Lebert, M., Häder, D.-P., 2002. Cellular perception and transduction mechanisms of gravity in unicellular organisms. Curr. Top. Plant Biol. 3, 143–154.
Roberts, D., 1985. IS10 transposition is regulated by DNA adenine methylation. Cell 43, 117–130. 10.1016/0092-8674(85)90017-0.
Rocha, E.P., 2008. Evolutionary patterns in prokaryotic genomes. Curr. Opin. Microbiol. 11, 454–460. 10.1016/j.mib.2008.09.007.
Rosenberg, S.M., 2001. Evolving responsively: adaptive mutation. Nat. Rev. Genet. 2, 504–515. 10.1038/35080556.

Rosenzweig, J.A., Ahmed, S., Eunson, J., Chopra, A.K., 2014. Low-shear force associated with modeled microgravity and spaceflight does not similarly impact the virulence of notable bacterial pathogens. Appl. Microbiol. Biotechnol. 98, 8797–8807. 10.1007/s00253-014-6025-8.

Rosenzweig, J.A., Chopra, A.K., 2012. The effect of low shear force on the virulence potential of *Yersinia pestis*: new aspects that space-like growth conditions and the final frontier can teach us about a formidable pathogen. Front. Cell. Infect. Microbiol. 2, 107. 10.3389/fcimb.2012.00107.

Royall, C.P., van Roij, R., van Blaaderen, A., 2005. Extended sedimentation profiles in charged colloids: the gravitational length, entropy, and electrostatics. J. Phys.: Condens. Matter 17, 2315–2326. 10.1088/0953-8984/17/15/005.

Rutherford, S.L., 2000. From genotype to phenotype: buffering mechanisms and the storage of genetic information. Bioessays 22, 1095–1105. 10.1002/1521-1878(200012)22:12<1095:AID-BIES7>3.0.CO;2-A.

Rutherford, S.L., 2003. Between genotype and phenotype: protein chaperones and evolvability. Nat. Rev. Genet. 4, 263–274. 10.1038/nrg1041.

Ruyters, G., Braun, M., 2014. Plant biology in space: Recent accomplishments and recommendations for future research. Plant Biol. 16 Suppl 1, 4–11. 10.1111/plb.12127.

Ruyters, G., Friedrich, U., 2006. From the Bremen drop tower to the international space station ISS - ways to weightlessness in the German Space Life Sciences Program. Signal Transduct. 6, 397–405.

Safari, M.S., Poling-Skutvik, R., Vekilov, P.G., Conrad, J.C., 2017. Differential dynamic microscopy of bidisperse colloidal suspensions. npj Microgravity 3, 21. 10.1038/s41526-017-0027-7.

Sahana, J., Nassef, M.Z., Wehland, M., Kopp, S., Krüger, M., Corydon, T.J. et al., 2018. Decreased E-Cadherin in MCF7 Human Breast Cancer Cells Forming Multicellular Spheroids Exposed to Simulated Microgravity. Proteomics 18, e1800015. 10.1002/pmic.201800015.

Sakai, S., Mishima, H., Ishii, T., Akaogi, H., Yoshioka, T., Ohyabu, Y. et al., 2009. Rotating three-dimensional dynamic culture of adult human bone marrow-derived cells for tissue engineering of hyaline cartilage. J. Orthop. Res. 27, 517–521. 10.1002/jor.20566.

Salmi, M.L., Roux, S.J., 2008. Gene expression changes induced by space flight in single-cells of the fern Ceratopteris richardii. Planta 229, 151–159. 10.1007/s00425-008-0817-y.

Salmi, M.L., Ul Haque, A., Bushart, T.J., Stout, S.C., Roux, S.J., Porterfield, D.M., 2011. Changes in gravity rapidly alter the magnitude and direction of a cellular calcium current. Planta 233, 911–920. 10.1007/s00425-010-1343-2.

Salzberg, S.L., White, O., Peterson, J., Eisen, J.A., 2001. Microbial genes in the human genome: lateral transfer or gene loss? Sci. 292, 1903–1906. 10.1126/science.1061036.

Sambandam, Y., Townsend, M.T., Pierce, J.J., Lipman, C.M., Haque, A., Bateman, T.A. et al., 2014. Microgravity control of autophagy modulates osteoclastogenesis. Bone 61, 125–131. 10.1016/j.bone.2014.01.004.

Sanford, G.L., Ellerson, D., Melhado-Gardner, C., Sroufe, A.E., Harris-Hooker, S., 2002. Three-dimensional growth of endothelial cells in the microgravity-based rotating wall vessel bioreactor. In Vitro Cell Dev. Biol. Animal 38, 493–504.

Sani, E., Herzyk, P., Perrella, G., Colot, V., Amtmann, A., 2013. Hyperosmotic priming of *Arabidopsis* seedlings establishes a long-term somatic memory accompanied by specific changes of the epigenome. Genome Biol. 14, R59. 10.1186/gb-2013-14-6-r59.

Sanjuán, R., Moya, A., Elena, S.F., 2004. The distribution of fitness effects caused by single-nucleotide substitutions in an RNA virus. Proc. Natl. Acad. Sci. U. S. A. 101, 8396–8401. 10.1073/pnas.0400146101.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., Nelson, A., 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3, 430–439. 10.1038/s41559-018-0793-y.

Saylo, M.C., Escoton, C.C., Saylo, M.M., 2011. Punctuated equilibrium vs. phyletic gradualism. Int. J. BioSci. Biotechnol. 3, 27–42.

Schöb, C., Kerle, S., Karley, A.J., Morcillo, L., Pakeman, R.J., Newton, A.C. et al., 2015. Intraspecific genetic diversity and composition modify species-level diversity-productivity relationships. New Phytol. 205, 720–730. 10.1111/nph.13043.

Schüler, O., Hemmersbach, R., Böhmer, M., 2015. A Bird's-Eye View of Molecular Changes in Plant Gravitropism Using Omics Techniques. Front. Plant Sci. 6, 1176. 10.3389/fpls.2015.01176.

Schüler, O., Krause, L., Görög, M., Hauslage, J., Kesseler, L., Böhmer, M. et al., 2016. ARADISH - Development of a Standardized Plant Growth Chamber for Experiments in Gravitational Biology Using Ground Based Facilities. Microgravity sci. Technol. 28, 297–305. 10.1007/s12217-015-9454-9.

Scoville, H., 2019. Gradualism vs. Punctuated Equilibrium. Available from: https://www.thoughtco.com/gradualism-vs-punctuated-equilibrium-1224811.

Searles, S.C., Woolley, C.M., Petersen, R.A., Hyman, L.E., Nielsen-Preiss, S.M., 2011. Modeled microgravity increases filamentation, biofilm formation, phenotypic switching, and antimicrobial resistance in *Candida albicans*. Astrobiology 11, 825–836. 10.1089/ast.2011.0664.

Senatore, G., Mastroleo, F., Leys, N., Mauriello, G., 2018. Effect of microgravity & space radiation on microbes. Future Microbiol. 13, 831–847. 10.2217/fmb-2017-0251.

Sharif, T., Martell, E., Dai, C., Kennedy, B.E., Murphy, P., Clements, D.R. et al., 2017. Autophagic homeostasis is required for the pluripotency of cancer stem cells. Autophagy 13, 264–284. 10.1080/15548627.2016.1260808.

Sharma, A., Singh, R.S., Gupta, G., Ahmad, T., Kaur, B., 2019. Metabolic Engineering of Enzyme-Regulated Bioprocesses. In: Advances in Enzyme Technology: Elsevier. p. 293–323.

Shi, F., Zhao, T.-Z., Wang, Y.-C., Cao, X.-S., Yang, C.-B., Gao, Y. et al., 2016. The Impact of Simulated Weightlessness on Endothelium-Dependent Angiogenesis and the Role of Caveolae/Caveolin-1. Cell. Physiol. Biochem. 38, 502–513. 10.1159/000438646.

Sieber, K.B., Bromley, R.E., Dunning Hotopp, J.C., 2017. Lateral gene transfer between prokaryotes and eukaryotes. Exp. Cell. Res. 358, 421–426. 10.1016/j.yexcr.2017.02.009.

Sieber, M., Kaltenbach, S., Hanke, W., Kohn, F.P.M., 2016. Conductance and Capacity of Plain Lipid Membranes under Conditions of Variable Gravity. J. Biomed. Sci. Eng. 09, 361–366. 10.4236/jbise.2016.98031.

Sikavitsas, V.I., Bancroft, G.N., Mikos, A.G., 2002. Formation of three-dimensional cell/polymer constructs for bone tissue engineering in a spinner flask and a rotating wall vessel bioreactor. J. Biomed. Mater. Res. A 62, 136–148. 10.1002/jbm.10150.

Smillie, C.S., Smith, M.B., Friedman, J., Cordero, O.X., David, L.A., Alm, E.J., 2011. Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480, 241–244. 10.1038/nature10571.

Sniegowski, P., Raynes, Y., 2013. Mutation rates: how low can you go? Curr. Biol. 23, R147-9. 10.1016/j.cub.2013.01.018.

Soanes, D., Richards, T.A., 2014. Horizontal gene transfer in eukaryotic plant pathogens. Annu. Rev. Phytopathol. 52, 583–614. 10.1146/annurev-phyto-102313-050127.

Soni, A., O'Sullivan, L., Quick, L.N., Ott, C.M., Nickerson, C.A., Wilson, J.W., 2014. Conservation of the Low-shear Modeled Microgravity Response in Enterobacteriaceae and Analysis of the trp Genes in this Response. Open Microbiol. J. 8, 51–58. 10.2174/1874285801408010051.

Soucy, S.M., Huang, J., Gogarten, J.P., 2015. Horizontal gene transfer: building the web of life. Nat. Rev. Genet. 16, 472–482. 10.1038/nrg3962.

Sprenger, H.J., Marquardt, P., Tadros, T.F., 1993. The scope of microgravity experiments in colloid science. Adv. Colloid Interface Sci. 46, 343–347. 10.1016/0001-8686(93)80048-G.

Stamenković, V., Keller, G., Nesic, D., Cogoli, A., Grogan, S.P., 2010. Neocartilage formation in 1 g, simulated, and microgravity environments: implications for tissue engineering. Tissue Eng. Part A 16, 1729–1736. 10.1089/ten.tea.2008.0624.

Stanhope, M.J., Lupas, A., Italia, M.J., Koretke, K.K., Volker, C., Brown, J.R., 2001. Phylogenetic analyses do not support horizontal gene transfers from bacteria to vertebrates. Nature 411, 940–944. 10.1038/35082058.

Stout, S.C., Porterfield, D.M., Briarty, L.G., Kuang, A., Musgrave, M.E., 2001. Evidence of Root Zone Hypoxia in *Brassica rapa* L. Grown in Microgravity. Int. J. Plant Sci. 162, 249–255. 10.1086/319585.

Strauch, S.M., Richter, P., Schuster, M., Häder, D.-P., 2010. The beating pattern of the flagellum of *Euglena gracilis* under altered gravity during parabolic flights. J. Plant Physiol. 167, 41–46. 10.1016/j.jplph.2009.07.009.

Streb, C., Richter, P., Ntefidou, M., Lebert, M., Häder, D.-P., 2002. Sensory transduction of gravitaxis in *Euglena gracilis*. J. Plant Physiol. 159, 855–862.

Stutte, G.W., Monje, O., Hatfield, R.D., Paul, A.-L., Ferl, R.J., Simone, C.G., 2006. Microgravity effects on leaf morphology, cell structure, carbon metabolism and mRNA expression of dwarf wheat. Planta 224, 1038–1049. 10.1007/s00425-006-0290-4.

Sung, W., Ackerman, M.S., Miller, S.F., Doak, T.G., Lynch, M., 2012. Drift-barrier hypothesis and mutation-rate evolution. Proc. Natl. Acad. Sci. U. S. A. 109, 18488–18492. 10.1073/pnas.1216223109.

Suparta, W., Zulkeple, S.K., 2014. Spatial Analysis of Galactic Cosmic Ray Particles in Low Earth Orbit/Near Equator Orbit Using SPENVIS. J. Phys.: Conf. Ser. 495, 12040. 10.1088/1742-6596/495/1/012040.

Tahedl, H., Richter, P., Lebert, M., Häder, D.-P., 1998. cAMP is involved in gravitaxis signal transduction of *Euglena gracilis*. Microgravity Sci. Technol. 11, 173–178.

Tan, C., Wang, H., Zhang, Y., Qi, B., Xu, G., Zheng, H., 2011. A proteomic approach to analyzing responses of *Arabidopsis thaliana* root cells to different gravitational conditions using an agravitropic mutant, pin2 and its wild type. Proteome Sci. 9, 72. 10.1186/1477-5956-9-72.

Taylor, G.R., 1974. Space Microbiology. Annu. Rev. Microbiol. 28, 121–137. 10.1146/annurev.mi.28.100174.001005.

Thatcher, J.W., Shaw, J.M., Dickinson, W.J., 1998. Marginal fitness contributions of nonessential genes in yeast. Proc. Natl. Acad. Sci. U. S. A. 95, 253–257. 10.1073/pnas.95.1.253.

Thewissen, J.G.M., Cooper, L.N., George, J.C., Bajpai, S., 2009. From Land to Water: the Origin of Whales, Dolphins, and Porpoises. Evo. Edu. Outreach 2, 272–288. 10.1007/s12052-009-0135-2.

Timmery, S., Hu, X., Mahillon, J., 2011. Characterization of Bacilli isolated from the confined environments of the Antarctic Concordia station and the International Space Station. Astrobiology 11, 323–334. 10.1089/ast.2010.0573.

Tirumalai, M.R., Karouia, F., Tran, Q., Stepanov, V.G., Bruce, R.J., Ott, C.M. et al., 2017. The adaptation of *Escherichia coli* cells grown in simulated microgravity for an extended period is both phenotypic and genomic. npj Microgravity 3, 15. 10.1038/s41526-017-0020-1.

Tirumalai, M.R., Karouia, F., Tran, Q., Stepanov, V.G., Bruce, R.J., Ott, C.M. et al., 2019. Evaluation of Acquired Antibiotic Resistance in *Escherichia coli* Exposed to Long-Term Low-Shear Modeled Microgravity and Background Antibiotic Exposure. mBio. 10. 10.1128/mBio.02637-18.

Todd, P., 1989. Gravity-dependent phenomena at the scale of the single cell. ASGSB Bull. 2, 95–113.

Tomita, Y., Seki, T., Fukaya, N., Nishikawa, S., Sato, N., Imai, M. et al., 2018. Crystallization of Charged Colloids under Microgravity during Aircraft Parabolic Flights. Int. J. Microgravity Sci. Appl. 35, 1–6.

Treangen, T.J., Rocha, E.P.C., 2011. Horizontal transfer, not duplication, drives the expansion of protein families in prokaryotes. PLoS Genet 7, e1001284. 10.1371/journal.pgen.1001284.

Tucker, D.L., Ott, C.M., Huff, S., Fofanov, Y., Pierson, D.L., Willson, R.C. et al., 2007. Characterization of *Escherichia coli* MG1655 grown in a low-shear modeled microgravity environment. BMC Microbiol. 7, 15. 10.1186/1471-2180-7-15.

Ulbrich, C., Pietsch, J., Grosse, J., Wehland, M., Schulz, H., Saar, K. et al., 2011. Differential gene regulation under altered gravity conditions in follicular thyroid cancer cells: relationship between the extracellular matrix and the cytoskeleton. Cell. Physiol. Biochem. 28, 185–198. 10.1159/000331730.

Ulbrich, C., Wehland, M., Pietsch, J., Aleshcheva, G., Wise, P., van Loon, J.J.W.A. et al., 2014. The impact of simulated and real microgravity on bone cells and mesenchymal stem cells. Biomed Res. Int. 2014, 928507. 10.1155/2014/928507.

Ulbrich, C., Westphal, K., Baatout, S., Wehland, M., Bauer, J., Flick, B. et al., 2008. Effects of basic fibroblast growth factor on endothelial cells under conditions of simulated microgravity. J. Cell Biochem. 104, 1324–1341. 10.1002/jcb.21710.

van der Graaf, A., Wardenaar, R., Neumann, D.A., Taudt, A., Shaw, R.G., Jansen, R.C. et al., 2015. Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. Proc. Natl. Acad. Sci. U. S. A. 112, 6676–6681. 10.1073/pnas.1424254112.

van Loon, J.J.W.A., 2007. Some history and use of the random positioning machine, RPM, in gravity related research. Adv. Space Res. 39, 1161–1165. 10.1016/j.asr.2007.02.016.

van Mulders, S.E., Stassen, C., Daenen, L., Devreese, B., Siewers, V., van Eijsden, R.G.E. et al., 2011. The influence of microgravity on invasive growth in *Saccharomyces cerevisiae*. Astrobiology 11, 45–55. 10.1089/ast.2010.0518.

Vandenbrink, J.P., Herranz, R., Poehlman, W.L., Alex Feltus, F., Villacampa, A., Ciska, M. et al., 2019. RNA-seq analyses of *Arabidopsis thaliana* seedlings after exposure to blue-light phototropic stimuli in microgravity. Am. J. Bot. 106, 1466–1476. 10.1002/ajb2.1384.

Vasiliou, S.K., Diamandis, E.P., Church, G.M., Greely, H.T., Baylis, F., Thompson, C. et al., 2016. CRISPR-Cas9 System: Opportunities and Concerns. Clin. Chem. 62, 1304–1311. 10.1373/clinchem.2016.263186.

Veen, S.J., Antoniuk, O., Weber, B., Potenza, M.A.C., Mazzoni, S., Schall, P. et al., 2012. Colloidal aggregation in microgravity by critical Casimir forces. Phys. Rev. Lett. 109, 248302. 10.1103/PhysRevLett.109.248302.

Velkov, V.V., 2002. New Insights into the Molecular Mechanisms of Evolution: Stress Increases Genetic Diversity. Mol. Biol. 36, 209–215. 10.1023/A:1015365805383.

Vico, L., Collet, P., Guignandon, A., Lafage-Proust, M.-H., Thomas, T., Rehailia, M. et al., 2000. Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. Lancet 355, 1607–1611. 10.1016/S0140-6736(00)02217-0.

Vidyasekar, P., Shyamsunder, P., Arun, R., Santhakumar, R., Kapadia, N.K., Kumar, R. et al., 2015. Genome Wide Expression Profiling of Cancer Cell Lines Cultured in Microgravity Reveals Significant Dysregulation of Cell Cycle and MicroRNA Gene Networks. PLoS ONE 10, e0135958. 10.1371/journal.pone.0135958.

Vogel, C., Chothia, C., 2006. Protein family expansions and biological complexity. PLoS Comput. Biol. 2, e48. 10.1371/journal.pcbi.0020048.

Vorselen, D., Roos, W.H., MacKintosh, F.C., Wuite, G. J. L., van Loon, J.J.W.A., 2014. The role of the cytoskeleton in sensing changes in gravity by nonspecialized cells. FASEB J. 28, 536–547. 10.1096/fj.13-236356.

Vos, M., Hesselman, M.C., te Beek, T.A., van Passel, M.W.J., Eyre-Walker, A., 2015. Rates of Lateral Gene Transfer in Prokaryotes: High but Why? Trends Microbiol. 23, 598–605. 10.1016/j.tim.2015.07.006.

Vukanti, R., Model, M.A., Leff, L.G., 2012. Effect of modeled reduced gravity conditions on bacterial morphology and physiology. BMC Microbiol. 12, 4. 10.1186/1471-2180-12-4.

Vurro, M., Bonciani, B., Vannacci, G., 2010. Emerging infectious diseases of crop plants in developing countries: impact on agriculture and socio-economic consequences. Food Sec. 2, 113–132. 10.1007/s12571-010-0062-7.

Wang, H., 2006. A proteomic approach to analysing responses of *Arabidopsis thaliana* callus cells to clinostat rotation. J. Exp. Bot. 57, 827–835. 10.1093/jxb/erj066.

Wang, H., Yan, Y., Rong, D., Wang, J., Wang, H., Liu, Z. et al., 2016. Increased biofilm formation ability in *Klebsiella pneumoniae* after short-term exposure to a simulated microgravity environment. MicrobiologyOpen 5, 793–801. 10.1002/mbo3.370.

Ward, M.J., Gibbons, C.L., McAdam, P.R., van Bunnik, B.A.D., Girvan, E.K., Edwards, G.F. et al., 2014. Time-Scaled Evolutionary Analysis of the Transmission and Antibiotic Resistance Dynamics of *Staphylococcus aureus* Clonal Complex 398. Appl. Environ. Microbiol. 80, 7275–7282. 10.1128/AEM.01777-14.

Watkins, R.F., Gray, M.W., 2006. The frequency of eubacterium-to-eukaryote lateral gene transfers shows significant cross-taxa variation within amoebozoa. J. Mol. Evol. 63, 801–814. 10.1007/s00239-006-0031-0.

Wayne, M., Miyamoto, M.M., 2006. In Evolutionary Genetics: Concepts and Case Studies Oxford Univ. Press, New York.

Wei, L., Diao, Y., Qi, J., Khokhlov, A., Feng, H., Yan, X. et al., 2013. Effect of change in spindle structure on proliferation inhibition of osteosarcoma cells and osteoblast under simulated microgravity during incubation in rotating bioreactor. PLoS ONE 8, e76710. 10.1371/journal.pone.0076710.

West-Eberhard, M.J., 2003. Developmental plasticity and evolution Oxford University Press, New York.

White, R.J., Averner, M., 2001. Humans in space. Nature 409, 1115–1118. 10.1038/35059243.

Wiedenbeck, J., Cohan, F.M., 2011. Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiol. Rev. 35, 957–976. 10.1111/j.1574-6976.2011.00292.x.

Wilson, J.W., Ott, C.M., Quick, L., Davis, R., zu Bentrup, Kerstin Höner, Crabbé, A. et al., 2008. Media Ion Composition Controls Regulatory and Virulence Response of *Salmonella* in Spaceflight. PLoS ONE 3, e3923. 10.1371/journal.pone.0003923.

Wilson, J.W., Ott, C.M., Ramamurthy, R., Porwollik, S., McClelland, M., Pierson, D.L. et al., 2002. Low-Shear modeled microgravity alters the Salmonella enterica serovar typhimurium stress response in an RpoS-independent manner. Appl. Environ. Microbiol. 68, 5408–5416. 10.1128/AEM.68.11.5408-5416.2002.

Wilson, J.W., Ott, C.M., zu Bentrup, K. H., Ramamurthy, R., Quick, L., Porwollik, S. et al., 2007. Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. Proc. Natl. Acad. Sci. U. S. A. 104, 16299–16304. 10.1073/pnas.0707155104.

Winn, R.N., Norris, M.B., Brayer, K.J., Torres, C., Muller, S.L., 2000. Detection of mutations in transgenic fish carrying a bacteriophage lambda cII transgene target. Proc. Natl. Acad. Sci. U. S. A. 97, 12655–12660. 10.1073/pnas.220428097.

Wion, D., Casadesús, J., 2006. N6-methyl-adenine: an epigenetic signal for DNA-protein interactions. Nat. Rev. Micro. 4, 183–192. 10.1038/nrmicro1350.

Wohlleben, W., Mast, Y., Stegmann, E., Ziemert, N., 2016. Antibiotic drug discovery. Microb. Biotechnol. 9, 541–548. 10.1111/1751-7915.12388.

Wolff, S.A., Coelho, L.H., Karoliussen, I., Jost, A.-I.K., 2014. Effects of the Extraterrestrial Environment on Plants: Recommendations for Future Space Experiments for the MELiSSA Higher Plant Compartment. Life 4, 189–204. 10.3390/life4020189.

World Health Organization [Internet]. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis, 2017. Available from: http://www.who.int/medicines/areas/rational_use/PPLreport_2017_09_19.pdf?ua=1.

Wright, B.E., 2004. Stress-directed adaptive mutations and evolution. Mol. Microbiol. 52, 643–650. 10.1111/j.1365-2958.2004.04012.x.

Xianfang, W., Long, Z., Weixu, D., Chunhua, L., 2004. Study of space mutation breeding in China. Appl. Life Sci. 18, 241–246.

Xu, B., Li, C., Zheng, Y., Si, S., Shi, Y., Huang, Y. et al., 2015. Simulated microgravity affects ciprofloxacin susceptibility and expression of acrAB-tolC genes in *E. coli* ATCC25922. Int. J. Clin. Exp. Pathol. 8, 7945–7952.

Xu, D., Guo, S., 2013. Identification of Conserved miRNAs in *Solanum Lycopersicum* Response to Long-term RPM-treatment. JOLST, 136–141. 10.12720/jolst.1.2.136-141.

Xu, G., Guo, C., Shan, H., Kong, H., 2012. Divergence of duplicate genes in exon-intron structure. Proc. Natl. Acad. Sci. U. S. A. 109, 1187–1192. 10.1073/pnas.1109047109.

Xu, P., Chen, H., Jin, J., Cai, W., 2018. Single-base resolution methylome analysis shows epigenetic changes in *Arabidopsis* seedlings exposed to microgravity spaceflight conditions on board the SJ-10 recoverable satellite. npj Microgravity 4, 12. 10.1038/s41526-018-0046-z.

Yadav, D., Tanveer, A., Malviya, N., Yadav, S., 2018. Overview and Principles of Bioengineering. In: Omics Technologies and Bio-Engineering: Elsevier. p. 3–23.

Yang, J., Barrila, J., Roland, K.L., Ott, C.M., Nickerson, C.A., 2016. Physiological fluid shear alters the virulence potential of invasive multidrug-resistant non-typhoidal *Salmonella Typhimurium* D23580. npj Microgravity 2, 16021. 10.1038/npjmgrav.2016.21.

Yang, S.-T., Liu, X., Zhang, Y., 2007. Metabolic Engineering – Applications, Methods, and Challenges. In: Bioprocessing for Value-Added Products from Renewable Resources: Elsevier. p. 73–118.

Yao, J., Weng, Y., Dickey, A., Wang, K.Y., 2015. Plants as Factories for Human Pharmaceuticals: Applications and Challenges. Int. J. Mol. Sci. 16, 28549–28565. 10.3390/ijms161226122.

Yilmaz, A., Grotewold, E., 2010. Components and mechanisms of regulation of gene expression. Methods Mol. Biol. 674, 23–32. 10.1007/978-1-60761-854-6_2.

Yoshimura, K., Matsuo, Y., Kamiya, R., 2003. Gravitaxis in *Chlamydomonas reinhardtii* studied with novel mutants. Plant Cell Physiol. 44, 1112–1118.

Yuge, L., Kajiume, T., Tahara, H., Kawahara, Y., Umeda, C., Yoshimoto, R. et al., 2006. Microgravity potentiates stem cell proliferation while sustaining the capability of differentiation. Stem Cells Dev. 15, 921–929. 10.1089/scd.2006.15.921.

Zanchi, D., Poulain, C., Konarev, P., Tribet, C., Svergun, D.I., 2008. Colloidal stability of tannins: astringency, wine tasting and beyond. J. Phys.: Condens. Matter 20, 494224. 10.1088/0953-8984/20/49/494224.

Zanoni, M., Pignatta, S., Arienti, C., Bonafè, M., Tesei, A., 2019. Anticancer drug discovery using multicellular tumor spheroid models. Expert Opin. Drug Discov. 14, 289–301. 10.1080/17460441.2019.1570129.

Zhang, H., Li, Y., Zhu, J.-K., 2018. Developing naturally stress-resistant crops for a sustainable agriculture. Nat. Plants 4, 989–996. 10.1038/s41477-018-0309-4.

Zhang, J., Wang, S., Wang, Y., 2016. Biobutanol Production From Renewable Resources. In: Elsevier. p. 1–68.

Zhang, X., Nan, Y., Wang, H., Chen, J., Wang, N., Xie, J. et al., 2013. Model microgravity enhances endothelium differentiation of mesenchymal stem cells. Die Naturwissenschaften 100, 125–133. 10.1007/s00114-012-1002-5.

Zhang, Y., Wang, L., Xie, J., Zheng, H., 2015. Differential protein expression profiling of *Arabidopsis* thaliana callus under microgravity on board the Chinese SZ-8 spacecraft. Planta 241, 475–488. 10.1007/s00425-014-2196-x.

Zhang, Y., Zheng, H.Q., 2015. Changes in Plastid and Mitochondria Protein Expression in *Arabidopsis* Thaliana Callus on Board Chinese Spacecraft SZ-8. Microgravity Sci. Technol. 27, 387–401. 10.1007/s12217-015-9431-3.

Zhao, J., Ma, H., Wu, L., Cao, L., Yang, Q., Dong, H. et al., 2017. The influence of simulated microgravity on proliferation and apoptosis in U251 glioma cells. In Vitro Cell. Dev. Biol. -Animal 53, 744–751. 10.1007/s11626-017-0178-6.

Zhau, H.E., Goodwin, T.J., Chang, S.M., Baker, T.L., Chung, L.W., 1997. Establishment of a three-dimensional human prostate organoid coculture under microgravity-simulated conditions: evaluation of androgen-induced growth and PSA expression. In Vitro Cell. Dev. Biol. -Animal 33, 375–380. 10.1007/s11626-997-0008-3.

Zhaxybayeva, O., Gogarten, J.P., Charlebois, R.L., Doolittle, W.F., Papke, R.T., 2006. Phylogenetic analyses of cyanobacterial genomes: quantification of horizontal gene transfer events. Genome Res. 16, 1099–1108. 10.1101/gr.5322306.

Zhaxybayeva, O., Nesbø, C.L., Doolittle, W.F., 2007. Systematic overestimation of gene gain through false diagnosis of gene absence. Genome Biol. 8, 402. 10.1186/gb-2007-8-2-402.

Zhou, J., Sun, C., Wang, N., Gao, R., Bai, S., Zheng, H. et al., 2006. Preliminary report on the biological effects of space flight on the producing strain of a new immunosuppressant, Kanglemycin C. J. Ind. Microbiol. Biotechnol. 33, 707–712. 10.1007/s10295-006-0118-z.

Zhou, M., Sng, N.J., LeFrois, C.E., Paul, A.-L., Ferl, R.J., 2019. Epigenomics in an extraterrestrial environment: organ-specific alteration of DNA methylation and gene expression elicited by spaceflight in *Arabidopsis thaliana*. BMC Genomics 20, 205. 10.1186/s12864-019-5554-z.

Zhu, J., Jeong, J.C., Zhu, Y., Sokolchik, I., Miyazaki, S., Zhu, J.-K. et al., 2008. Involvement of *Arabidopsis* HOS15 in histone deacetylation and cold tolerance. Proc. Natl. Acad. Sci. U. S. A. 105, 4945–4950. 10.1073/pnas.0801029105.

Zhu, J., Li, M., Rogers, R., Meyer, W., Ottewill, R.H., Russel, W.B. et al., 1997. Crystallization of hard-sphere colloids in microgravity. Nature 387, 883–885. 10.1038/43141.

Zong, W., Zhong, X., You, J., Xiong, L., 2013. Genome-wide profiling of histone H3K4-tri-methylation and gene expression in rice under drought stress. Plant Mol. Biol. 81, 175–188. 10.1007/s11103-012-9990-2.

Figure Legends

Fig. 1. A simplified scheme of self-guided evolution. Diverse genetic variants of strains can be achieved by subjecting cells in microgravity and radiation in combination with other biotic and abiotic stressors. Suitable mutants selected according to defined parameters and isolated subsequently. Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Fig. 2. Scheme of horizontal gene transfer between non-related organisms. Horizontal gene exchange is common in closely related organisms but also occurs between distantly related organisms. Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Fig. 3. Stress-induced epigenetics in plants. Epigenetic bring about new traits without altering the genetic code. Epigenetic regulation increases variation within a species in times of environmental stresses or signals (biotic and abiotic factors), thereby provide ways for adaptation to the arising selection pressures.

Fig. 4. The central dogma of evolution. In biology and ecology, environmental factors or stressors (abiotic or biotic components) affect living organisms, leading to adaptive changes over time employing evolution, which includes mutation, gene flow (horizontal gene transfer), genetic drift, epigenetic regulation, and natural selection as mechanisms of change.

Fig. 5. Simplified representation of parameters cells or organisms encounters in outer space. Lack of gravity on board of a spacecraft considerably changes the environment in which thermal convection, mechanical load as well as hydrostatic does not exist. This has significant impacts on cells in terms of gas, nutrient, and heat transfer. Space unique environment provides a combination of stressors (e.g., high radiation and weightlessness/microgravity) that organisms have never experienced on Earth. Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Fig. 6. Different facilities for the investigation of altered gravitational conditions Platforms include aircraft-based parabolic flights, 3D and 2D clinostats, random-positioning machine (RPM), sounding rocket, and drop tower. Clinostat and RPM constantly change the position of the test object to the vector of gravity. With drop tower, aircraft-based parabolic flights, and sounding rockets, microgravity conditions can be achieved ranging from few seconds to several minutes.

Fig. 7. Applications of microgravity-based research on human cells. (A) MCF-7 breast cancer cells form unique ring-like structures when grown for 14 days in s-µ*g* produced by an RPM. **(B)** Schematics of cancer research using microgravity. In µ*g*, cancer cells detach from the growth surface and form 3D MCS composed of less aggressive, more differentiated cells. Using Omics research and bioinformatics, scientists try to elucidate the mechanisms behind the alteration of the phenotype to mimic these effects with drugs. The results may be used in the development of novel cancer therapies. Furthermore, µ*g* provides a suitable culture environment for scaffold- and necrosis-free tissue engineering of **(C)** cartilage, **(D)** bone, and **(E)** blood vessels. Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Fig. 8. Simplified scheme of the physiological mechanism involved in the gravitaxis of *Euglena gracilis*. If a vertically upward swimming cell (A) deviates from its vertical movement path (B and C), mechano-sensitive membrane patches are activated due to forces generated by the weight of the cell body (C). This triggers a physiological signal cascade (orange insert) in which calcium channels, a particular calmodulin (CAM 2, activated by increasing concentrations of calcium ions), a not yet

identified adenylate cyclase are involved. Increased levels of cAMP, synthesized from activated adenylate cyclase activates a protein kinase A, which most likely phosphorylates flagellar proteins. This results in reorientation strokes of the flagella, aligning the cell back to the vertical position (D). In addition, a recently identified flagellar protein EgPCDUF4201 is involved in gravitaxis, but its role in the signal transduction chain is yet to be determined. The Figure is adopted and modified according to (Nasir et al., 2018). Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

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Exploration of Space to Achieve Scientific Breakthroughs

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Running title: Space for Revolutionizing Biological Research

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ABSTRACT

Living organisms adapt to changing environments using their amazing flexibility to remodel themselves by a process called evolution. Environmental stress causes selective pressure and is associated with genetic and phenotypic shifts for better modifications, maintenance, and functioning of organismal systems. The natural evolution process can be used in complement to rational strain engineering for the development of desired traits or phenotypes as well as for the production of novel biomaterials through the imposition of one or more selective pressures. Space provides a unique environment of stressors (e.g., weightlessness and high radiation) that organisms have never experienced on Earth. Cells in the outer space reorganize and develop or activate a range of molecular responses that lead to changes in cellular properties. Exposure of cells to the outer space will lead to the development of novel variants more efficiently than on Earth. For instance, natural crop varieties can be generated with higher nutrition value, yield, and improved features, such as resistance against high and low temperatures, salt stress, and microbial and pest attacks. The review summarizes the literature on the parameters of outer space that affect the growth and behavior of cells and organisms as well as complex colloidal systems. We illustrate an understanding of gravity-related basic biological mechanisms and enlighten the possibility to explore the outer space environment for application-oriented aspects. This will stimulate biological research in the pursuit of innovative approaches for the future of agriculture and health on Earth.

Keywords: Space, Evolution, Microgravity, Stress response, Mutation, Horizontal gene transfer, Epigenetics, Tissue engineering, Cancer research, Colloids

1. Introduction

The scientific approach lends itself to the creation of definitions or rules that box life into specific lanes. However, upon further investigations, these lanes require repeated adjustments due to life being an ever-evolving continuous spectrum of possibilities that are based on a few absolute natural laws. The result is an ambiguous division between what is living and what is not. Life can be further thought of as a magnification of physical and chemical laws driven by local energy and order states overlaid by selection history and constraints. In recent years, and with the advent of molecular biology, the classical definition of species and life has come to be questioned. While Darwin's inheritance, variation, and selection still play a key role in the process of life's evolution, the source of the inheritance, variation, and process of selection has expanded. This expansion can result in a selection of inherited variability that is not associated with perceived optimal fitness at the time of selection. Key processes that can lead to such results include genetic hitchhiking, genetic drift via bottleneck or founder event, horizontal gene transfer (HGT) and, to a lesser extent, gene flow and epigenetics. Besides, some changes in the DNA do not seem to have any effect on amino acid translation or fitness and maybe deemed silent or neutral mutations, respectively. When gauging fitness and speciation we must consider the spatial and time aspect that is the distribution over time. Speciation occurs in either a gradual consistent pace (gradualism) or abrupt pulses (punctuated equilibria) with the main difference being the time scale of the speciation events. Punctuated events can be seen in places that have undergone rapid environmental changes (e.g., the Hawaiian Islands). The space environment with its gravitational and radiation changes affords such a novel abrupt environmental change for terrestrial life. This environment can be used to decipher underlying pathways that are not apparent on Earth, test countermeasures for observed changes, and develop new organisms with novel attributes.

Since the late 1950s space technology has developed spacecraft for transporting terrestrial life into low Earth orbit (LEO) to study, in situ, its responses to selected conditions of space (Brinckmann, 2007; Clément and Slenzka, 2006; Horneck et al., 2010; Nickerson et al., 2004). The space shuttle (Baglioni et al., 2007; Horneck et al., 1984b; Horneck et al., 1984a) and the space stations, e.g., the Russian MIR (Rettberg et al., 2002) and the International Space Station (ISS) (Baglioni et al., 2007) can truly remove the influence of gravity in an experimental setting in the LEO. The orbital microgravity environment provided by these spaceships has allowed us to perform unique experiments to study the influence of microgravity on living systems that could not be carried out on Earth. Changed environmental factors onboard a spacecraft include weightlessness (technically called "microgravity"), ionizing and non-ionizing solar or cosmic radiations, temperature extremes, and high vacuum. Among these, microgravity and cosmic radiations are of particular interest for scientific research. Regardless of the various primary experimental objectives and subsequent, sometimes-disparate conclusions drawn from the data, the majority of investigations to date have indicated that microgravity has a significant influence on cell growth and behavior. With technological advancement in molecular science and analytics, the focus of attention in modern space and gravitational biology is switching to explore the influence of microgravity on living systems at the cellular and molecular levels. Emphasis is on the investigation of genetic stability, growth, development, reproduction, aging, life span, behavior, and orientation of plants and animals in space environmental conditions. In recent years, studies under simulated or real microgravity resulted in significant outcomes in cancer research and cell and tissue engineering among others.

1.1. Scope of the review

This review covers why we need to think out of the box and explore space environmental conditions to achieve breakthroughs for a sustainable future. The review explains the possibility of exploration of the space environment to accelerate the development of new varieties of crops or other species and novel substances using the natural evolution process. We discuss the molecular machinery and mechanisms that may lead to the evolution of organisms in space's unique environment. We consider the effects of the environment, constrained selection process, unrestricted and extensive gene expression, mutation, gene exchange, and epigenetics on the biology, adaptation, and evolution of cells or organisms in space. Technically, natural evolutionary mechanisms can be employed to obtain strains with desired traits (self-guided evolution) by setting controlled environmental and selection factors. A review of literature relevant to the effects of microgravity on growth, behavior, or survival of microbes,

plants, and cell and tissue cultures is provided. We also cover the need to study colloids in real microgravity conditions to unveil some key physicochemical aspects, like the natural movement of colloids that experiments performed on Earth, in the presence of gravity may mask, alter, or simply hinder. Taken altogether, this review encompasses the different aspects of space biology that have been studied to date. By providing a comprehensive review of this currently unfolding space research discipline, we intend to illustrate organisms' responses to the space environment, enlighten a new understanding of biology, and recognize the potential of the space environment for boosting biological research in the pursuit of new solutions for the future of agriculture and health on Earth. The review not only explains gravity-related basic biological mechanisms at the cellular level but also gives a picture to explore space for application-oriented aspects, such as to produce and develop an array of products for food, pharmaceutics, cosmetics, home-care with longer shelf lives and new functionalities.

1.2. Effects of human activities on the global ecosystem

Earth has immense resources and opportunities for the development of different technologies and products to meet the current needs of the global population. However, human activities, in particular, the overuse of fossil fuels, uncontrolled deforestation, intensive land use for agriculture, and enormous animal production, are dramatically affecting the global climate and causing considerable stress to land resources, including water, soil, nutrients, plants, and animals (Dupuy et al., 1976; Grossi et al., 2019; Masson-Delmotte et al., 2018). Global temperature rise (~0.85ºC in the last 130 years) continues to result in shrinking of ice sheets, glacial retreat, sea-level rise, extreme weather events, ocean acidification, soil salination, desertification, and reduced availability of drinking water (Masson-Delmotte et al., 2018). These changes are affecting agricultural productivity and food availability, safety, and quality at global, regional, and local levels (Brown et al., 2015). While the human population is continually growing, there is no unlimited availability of natural resources such as arable land and freshwater, which is further amplifying the threats of climate change on food security. According to the World Economic Forum, our food supplies will be under far greater pressure with the global population expected to reach 9.8 billion by 2050 (Godfray et al., 2010). The demand for food will be 60% higher than it is today. With the current state-of-the-art agricultural practices and productivity, it will be impossible to meet the demands. Furthermore, in the last several decades, there is a significant decrease in the number of cultivated crop species as well as in the diversity within major crops (Cardinale et al., 2012; Nordin and Nordin, 2017). Plant domestication and subsequent selection in agriculture have limited the genetic diversity of modern crops and increased their susceptibility to multiple diseases and extreme environmental stresses (weather and soil conditions) (Huang et al., 2016). Furthermore, the stress due to the changing environment is weakening plants' defense mechanisms, making them more vulnerable to several pathogens, such as viruses, bacteria, fungi, oomycetes ("egg fungi"), nematodes, and parasitic plants (Dean et al., 2012; Savary et al., 2019; Vurro et al., 2010). These pathogens' range and distribution are increasing with climate change (Anderson et al., 2004; Backlund et al., 2008), causing extensive crop damage. For instance, a study on climate change-related spreading of downy mildew (*Plasmopara viticola*) concluded that the production costs in vineyards are increasing in a range of 20-50% due to higher costs in disease management (Francesca et al., 2006). Increasing the genetic diversity within cropping systems by the use of variety or species mixtures offer several potential advantages, such as limiting disease development, increasing yield stability, and improving resilience to abiotic factors (Newton, 2016; Savary et al., 2019; Schöb et al., 2015). Furthermore, it delivers other ecosystem services including greater biodiversity. Unpredictable weather events, poor crop yields, augmented pathogenesis, serious consequences of uncontrolled agrochemical utilization, and increasing food insecurity have set off alarms that we cannot afford to ignore. It is important to focus on the development of stress-resistant crops that are well adapted to thrive in a changing environment and have stable yields under stress conditions to ensure future food security. The need is to deploy the rich biodiversity by generating a suite of new varieties of crops, not just one.

Climate change is also intensifying health problems that already pose a major burden to susceptible populations and will bring new and emerging health issues soon. According to an assessment by World Health Organization (WHO), between 2030 and 2050, climate change is expected to cause approximately 250,000 additional deaths per year from malnutrition, malaria, diarrhea, and heat stress. The socioeconomic costs of health problems are considerable and will increase over time. Besides, misuse and overuse of conventional antibiotics in humans, animals, and plants are accelerating the

development and spread of antimicrobial resistance. Drug-resistant diseases already cause at least 700,000 deaths globally per year, a figure that could increase to 10 million per year by 2050 if no action is taken (Interagency Coordination Group on Antimicrobial Resistance, 2019). Antimicrobial resistance could cause the death of 2.4 million people in high-income countries between 2015 and 2050 if no sustainable efforts are made to control it. The discovery and development of novel antibiotic compounds have been slow and our arsenal of effective antibiotics is dwindling. The WHO has recommended prioritizing research on the discovery and development of new antibiotics to deal with the serious problem of drug-resistant infections (World Health Organization, 2017). There is an urgent need for the development of new methods and novel classes of potent drugs to combat antimicrobial-resistant pathogens.

1.3. Approaches to tackle problems of the fast-changing world

Researchers from all disciplines have constantly attempted developing novel strains or bio-products by both conventional approaches and emerging technologies, but the results are not as envisioned. Conventional screening programs have increasingly resulted in the rediscovery of already known species, strains, and metabolites (Wohlleben et al., 2016). It is assumed that only a few new metabolites are left to be isolated from nature's repository but needs massive screening programs (Baltz, 2007; Monciardini et al., 2014). Furthermore, the discovery and process development of natural chemicals for pharmaceutical, agricultural, and related applications are labor- and resource-intensive as they are produced in low yields in native organisms, and sometimes even in multiple forms, thus complicating their identification, isolation, and structural elucidation (Pickens et al., 2011). Besides, many of the natural chemicals are structurally complex, which makes their chemical synthesis difficult and therefore restricting their commercial utilization. These limitations within the natural product discovery led to the development and expansion of innovative methods, such as genetic or metabolic engineering, for producing novel biomaterials. Traditional breeding methods rely on hybridization and recombination to stack favorable alleles together. This process is usually time-consuming and labor-intensive, especially when the crop is not fully or newly domesticated.

The modern techniques, like genetic or metabolic engineering that include the manipulation of genetic material or targeted modification of metabolic pathways of an organism using recombinant DNA technology (Courchesne et al., 2009; King et al., 2016; Pickens et al., 2011; Yang et al., 2007) have created a foothold in scientific discovery and development. In the recent years, several genetic toolbox and methods have been developed and implemented for strain improvements such as cloning and expression, shuttle vectors, transformation, reporter genes, selection markers, RNA interference, and DNA microarrays (Amiri and Karimi, 2019; Doron et al., 2016; Prasad et al., 2014; Yadav et al., 2018; Yao et al., 2015; Zhang et al., 2018). Genetic engineering strategy has enabled the improvement of species and natural product titers, production of a new characteristic in an organism, and generation of a wide variety of novel compounds for pharmaceutical and agricultural sectors, but has several limitations. There is still the need for optimization to achieve titers, yields, and productivities that are sufficient for industrial applications. Successful transformation is the main hurdle that prevents the genetic modification of many species. Unfortunately, standard transformation methods successfully used in model organisms often fail when applied to other organisms. Many of the strains developed by genetic or metabolic engineering are unstable and lose engineered properties over time, thus hampering the commercial application (Prasad et al., 2014; Prasad et al., 2019a; Prasad et al., 2019b). It is a general observation that a successful transgene integration does not always result in the efficient expression of foreign genes. This is generally attributed to inadequate recognition of the promoter that drives gene expression, transgene silencing, post-transcriptional gene silencing, and/or bias in codon usage (Prasad et al., 2019a). Furthermore, for genetic or metabolic engineering, it is important to consider every biochemical network in the cell as well as the cellular responses to genetic changes and environmental stimuli (Yang et al., 2007). For this, we need to have complete knowledge of the cell and it's complex metabolic and regulatory networks, which is impossible in the near future. Thus, our partial and flawed knowledge of organism metabolism, physiology, and regulatory networks limits the development of competent strains with a high titer, yield, and/or productivity through genetic or metabolic engineering. Rapid progress in genetic engineering shows the development of clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (Cas)9 system, which allows robust and flexible genome editing and gene regulation in a multitude of model organisms and cell types, but has

some technical limitations, like the efficiency of Cas9 activity, target site selection, and off-target effects (Vasiliou et al., 2016). Although many of the advances in the genome-engineering territory are viewed as huge scientific achievements, their use is vigorously debated and restricted in most countries, which is a major obstacle for their widespread adoption.

Evolutionary engineering is a complementary approach to conventional techniques and imitates the natural evolution process to increase the tolerance of organisms to stress (biotic or abiotic), improve cell performance, improve utilization of a target substrate, improve the production of compounds, or produce new natural chemicals (Jin et al., 2016; Lee et al., 2015; Zhang et al., 2016). In this approach, the natural evolutionary process is rapid, directed, rational, and efficient for the improvement of strains or development of desired traits through the imposition of one or more selective pressures and is associated with genetic, physiological, and morphological changes (Sharma et al., 2019). This approach takes a broad view of the complex cellular network and is the most effective tool for developing multiple or complex traits that are a result of the interaction of several genes or for which genes are unknown. In this review, we term this process as 'self-guided evolution'. In fact, evolutionary engineering is the most ancient tool for the development of novel phenotypes. It has long been used in agriculture, animal farming, and in recent times for the production of antibiotics and other industrially important biomaterials. For example, penicillin from *Penicillium chrysogenum* and the yeast *Saccharomyces cerevisiae* for brewing, winemaking, and ethanol fermentation at an industrial scale. Space can be explored as a platform to induce the self-guided evolution of organisms (Fig. 1). Exposure of an organism to the unique space environment (discussed in Section 3) will increase evolutionary rate and processes and result in genetically diverse phenotypes, which would not form under a load of gravity. This is because organisms never experienced the absence of gravity during their whole evolution. Subjecting organisms in the space environment could produce new valuable strains of plants, yeasts, bacteria, and others and products of pharmaceutical and agricultural relevance (Horneck et al., 2010; Huang et al., 2015). Also, the observed differentiation effects on cancer cells or the induction of 3D-organoids from cell cultures under microgravity conditions are very promising applications for commercial use of space-born technology with high return values. Before we discuss the potential of space and relevant experiments, we provide a brief overview of how evolution works and the different processes involved in bringing evolutionary changes to organisms.

2. Evolution and mechanisms of evolution

The term 'evolution' stands for patterns of change in living beings and the process of natural selection responsible for these patterns. Living things reproduce and pass on their characteristics to their offspring. In a population, individuals that are poorly adapted are less likely to reproduce. However, over time, the characteristics that help the survival of an individual become more common, and a species gradually changes. Given enough time, these small changes can add up to the extent that a new species altogether can evolve. Thus, organisms adapt to their habitats by evolving or developing special features that help them to survive. Taken as a whole, this is evolution and it occurs only when there is a change in gene frequency within a population over time. These genetic differences are heritable and passed on to the next generations. According to Gingerich, (2001), three-time scales are of interest in evolution: (i) the generational time scale, the change from one generation to the next, (ii) the micro-evolutionary time scale regarding changes within 2 – 100 generations, and (iii) the macro-evolutionary time scale dealing with changes of patterns in a scale of hundred and more generations.

Evolution is not always a slow process, but also may happen more quickly. The evolution of a species occurs due to gradualism, punctuated equilibrium, or a combination of both (Rhodes, 1983; Saylo et al., 2011; Scoville, 2019). Gradualism is a more gradual selection and variation, which is hard to notice over a short period. In punctuated equilibrium, changes are sudden and huge. In the latter case, there is a period of little change, and then one or a few huge changes occur, often through mutations in the genes of a few individuals. Mutations that result in punctuated equilibrium are helpful to the individuals in their environments because these mutations allow the survival of those that have them. Evolution by punctuated equilibrium occurs because of huge or sudden changes in the environmental conditions, which causes strong selective pressure. This results in more rapid changes, leading to genetic and phenotypic shifts in the organisms in a matter of generations.

2.1. What happens to an organism if a constant important stimulus is no longer impinging?

The dramatic effect of an absence of a constant environmental parameter on the evolution of organisms can be seen in creatures that move from light-exposed habitats into dark environments, such as caves, deep sea, or temporally shift their activity into the night. Rétaux and Casane, (2013) analyzed all available publications about eye loss in cave inhabiting animals and concluded that genetic drift, as well as the selection of eyeless phenotypes, is the reason for eye degeneration. Interesting is the adaptation of many deep-sea fishes to their reduced light habitat: many of them did not reduce their eyes but increased the capabilities of their visual systems. Some deep-sea fish developed the ability to view color. In *Diretmus argenteus*, 38 genes for synthesis of rhodopsin were found making this fish the vertebrate bearing the highest number of genes for visual pigment synthesis (Musilova et al., 2019). In addition, many deep-sea organisms have developed strategies to replace the absence of light by producing own light utilizing bioluminescence (Haddock et al., 2010), which plays a role in communication, camouflage, or attraction of prey. Loss or reduction of light as an environmental stimulus has also led to the evolution of completely new features in animals, such as echolocation in bats and whales, or sideline organs in fish and some amphibians. An impression of the potential of evolution in the case of the absence of gravity can be seen in aquatic organisms. Although water as habitat cannot be compared to microgravity, it shares the reduced mechanical load acting onto a submerged body. Living permanently in water under reduced mechanical load, living organisms often strongly reduced stabilizing elements in their body, for e.g., in whales, which originated from dog-like land animals about 50 million years ago (Thewissen et al., 2009). However, various other factors, such as respiratory organs, increased thermal conduction, a necessity to develop new organs for locomotion (e.g., fins) affect the shape of aquatic organisms. These examples indicate the mighty potential that the space environment may have on the evolution of an exposed organism.

2.2. How does evolution work?

In living cells, genes encode proteins, and proteins guide cell function and structure. Phenotypic characters are formed during the development process by the complicated interaction of a large number of different genes, which is coordinated by molecular mechanisms. Signal molecules activate signaling pathways and complex gene interactions occur as a form of gene regulatory networks to develop phenotypic characters (Carroll et al., 2009; Davidson, 2006). Usually, many signaling pathways are involved in the development of a phenotypic character. As the complexity of the phenotypic character increases, the proportion of genes involved in signaling pathways or genetic networks increases (Nei and Rooney, 2005; Vogel and Chothia, 2006). The development of phenotypic characters depends on the function of various physiological processes or characters. Similarly, the function of physiological characters is based on the development of morphological characters. Both characters cannot be separated easily.

Changes in nucleotide sequences and coordination of genes expression at different times and dimensions in the development process play important roles in evolution. To understand the evolution of physiological or morphological characters, it is important to know the mechanism of gene regulation in the developmental process. In general, two essential cellular processes - transcription (DNA to RNA) and translation (RNA to protein) are involved in the production of proteins. The expression of thousands of genes and their coordinated regulation determine what a cell can do. The control of gene expression is important to all organisms. Activation of specific genes and the amount of protein or RNA product production are regulated by the complex interactions between genes, RNA molecules, regulatory proteins, and other components of the gene expression system. A gene is composed of protein-coding sequences and the regulatory region. During gene expression, the regulatory region that consists of the cis-regulatory elements and the transcription promoter (TATA box) initiates the transcription of the coding region of messenger RNAs (mRNAs). The basic components of gene expression regulation are the transcription factors and co-regulators, and the cis-regulatory elements, which serve as a docking site for transcription factors (Yilmaz and Grotewold, 2010). The transcription factors generated by different pathways interact with one another in the development process. Regulatory proteins that affect RNA polymerase activity bind to DNA sequences in the control regions of the genes that they regulate through specific protein-protein interactions, transfer signals to the transcriptional machinery, and results in specific rates of gene expression. In eukaryotes, chromatin structure also plays a significant

role in modulating gene expression. Recent studies have shown non-coding RNAs (ncRNAs) (e.g., microRNAs (miRNAs), short interfering RNAs (siRNAs), and piwi-interacting RNAs (piRNAs)) as key components in the regulation of gene expression as well (Gomes et al., 2013; Nei, 2013; Patil et al., 2014; Yilmaz and Grotewold, 2010).

Evolution of genes and ultimately characters occur through changes (nucleotide changes or gene duplication) in the protein-coding or regulatory regions of DNAs or both. Mutation and selection play an important role in the evolution of characters. However, new functional genes may also evolve by employing or co-opting other genes for a new function (gene co-option) or by adopting foreign genes that come from outside the individual (i.e., HGT). Changes in the protein-coding region result in the alternation of the amino acid sequence of the encoded protein. This alternation sometimes changes protein function, and this change may affect physiological or phenotypic characters. Amino acid substitutions vary extremely among different proteins. A strong relationship exists between the functional constraints of gene products and the rates of amino acid or nucleotide substitutions (Nei, 2005). For instance, the amino acid substitution rate is much lower in significant proteins, such as hemoglobin and cytochrome c than in less important protein, like fibrinopeptides. In addition, the evolution rate is much lower in the active site of the protein than other regions to maintain the same function. A study on the interspecific comparison of the evolution of protein molecules (e.g., cytochrome c, hemoglobin, and insulin) involved in various physiological functions showed that the amino acid substitutions occurring in the active sites primarily lead to the functional change of these proteins (King and Jukes, 1969; Nei, 2005) and most amino acid substitutions in structural proteins are relatively neutral. This study supports the basic principle of the evolution of proteins controlling physiological characters (Nei, 1987; Nei, 2005). Pseudogenes, for example, globin gene families, which do not have any function, evolve at the fastest rate because of the non-existence of selective constraints.

Many developmental biologists emphasized that morphological evolution is a result of mutations of regulatory genes and not the structural genes (Carroll et al., 2009; Davidson, 2006; King and Wilson, 1975). In contrast, Nei, (1987) proposed the major gene effect hypothesis, which states that morphological evolution occurs by small mutations of major effects in the structural or regulatory regions of the DNA. Studies on the evolutionary changes of the protein-coding regions of genes involved in morphological evolution show that the pattern of amino acid substitution is similar to the genes involved in physiological characters (Hoekstra et al., 2006; Liao et al., 2010; Nei, 2013). However, most amino acid substitutions do not affect physiological characters noticeably, while in the case of morphological characters, a few amino acid substitutions can cause dramatic alternations. Nucleotide deletion/insertion, exon deletion, exon shuffling, and transposon insertion may also cause functional or structural changes in the case of morphogenes (Hoekstra and Coyne, 2007; Nei, 2013; Reid and Ross, 2011; Xu et al., 2012). Hoekstra et al., (2006) reported that a single amino acid substitution in the *MC1R* gene contributes to the adaptive beach color pattern in the beach mouse, *Peromyscus polionatus*. Mutation in the gene *SBE1* that is responsible for causing wrinkled seeds in pea (Bhattacharyya et al., 1993) is due to the insertion of a transposon. Albinism in cavefish is caused by the loss of function mutations (exon deletion) in the *OCA2* gene, which operates during the first step of the melanin synthesis pathway (Bilandžija et al., 2013). These examples suggest that new mutations are important for adaptation of organisms to the challenging or new environment and the innovative changes are incorporated into the genome or spread through the population by natural selection (elimination of previous genotypes) or by genetic and genomic drift. It appears that natural selection plays a more important role in phenotypic evolution than in physiological or molecular evolution, but is secondary to mutation.

Evolution is associated with both direct and indirect selection. Strong selection pressure for resistance imposed by the use of antibiotics led to the propagation of antibiotic resistance in bacteria. This points to direct selection as an underlying evolutionary mechanism (Barlow, 2009). Indirect selection can be seen for example in animals living in caves. The selection pressure induced by the dark environment in caves reduced eye size and pigmentation but promoted the development of other sensory and neuromodulatory systems in the cavefish *Astyanax* for the orientation in a dark environment (Rétaux and Casane, 2013). One may also speculate that the useless neuronal capacity for the procession of visual signals is reduced in favor of other sensory elements, such as olfactory.

2.3. Processes modulating evolution of species

2.3.1. Mutation — the major force of the evolution of species

Genetic variation is vital to evolutionary change. Without it, no subsequent change can be accomplished (Hershberg, 2015). Genetic variation in an organism is primarily generated by mutation (Nei, 2013; Wayne and Miyamoto, 2006), which, in the simplest form, is a change of DNA sequence that could not be repaired by the cellular repair system. A mutation can result from a single base-pair substitution (point mutation), an insertion of a transposable element in a regulatory sequence, a gene deletion, or a duplication of a particular gene, chromosome, or the entire genome. Point mutations, whereby a single nucleotide base is changed, inserted, or deleted from a DNA sequence, perhaps change a single protein and thus seem unimportant (Nei, 2013). However, many tiny point mutations over time affect the history of species. On the other hand, mutations that alter specific segments of genes or whole chromosomes are commonly associated with species differences or genetic diseases. In most cases, mutations have little or neutral effects, while harmful mutations reduce the fitness of an organism. Sometimes, the change in the DNA sequences is useful (advantageous mutations) and has a positive effect (Eyre-Walker, 2006). Although rare, this can lead to significant evolutionary change. The genotype generated by this mutation has higher fitness than the pre-existing ones and may form an innovative phenotype, allowing organisms to adapt better to their environment. The new genetic variant (mutant) which feeds, grows, or reproduces more effectively becomes more abundant within the population over time. Thus, mutations lead to changes in an organism, making it biologically different and unique from the original population.

Numerous studies acknowledge that organisms evolve systems to respond to environmental cues by controlling mutation rates. Mutation rate data is vital for understanding patterns of molecular diversity within species and divergence among species (Lynch, 2010a). High-throughput genome sequencing and mutation accumulation experiments, coupled with comparative approaches and constant theoretical progress are rapidly expanding our knowledge on natural mutation variation both within and among species. There is substantial evidence that mutation rates vary widely among different forms of life (Baer et al., 2007; Sung et al., 2012). In a recent study, 36 whole-genome single nucleotide polymorphism datasets from 16 species of bacteria sampled over periods extending over 2000 years were compared to estimate and compare their evolutionary rates (Duchêne et al., 2016). The evolutionary rates in the most rapidly evolving bacteria, such as *Enterobacterium faecium*, *Staphylococcus aureus*, and *Acinetobacter baumannii* were noted to be between 10^{-5} and 10^{-6} substitutions per site year^{-1}. These rates were found to be within the range estimated for other rapidly evolving bacterial species (Holt et al., 2012; Kennemann et al., 2011; Pérez-Losada et al., 2007; Ward et al., 2014) and similar to the rates estimated for slowly evolving DNA viruses (Duchêne et al., 2014; Firth et al., 2010). The pathogenic bacteria *Mycobacterium leprae* and *Yersinia pestis* show very low change of base pairs with estimates of ~10^{-8} substitutions per site year^{-1} and are considered as slow evolving bacteria (Duchêne et al., 2016; Kay et al., 2015). However, the unicellular eukaryote *Chlamydomonas reinhardtii* (large genome) exhibits an extremely low per-base mutation rate of 6.76 x 10^{-11} per cell division (Ness et al., 2012). The analysis shows somatic mutation rate is higher than germline mutation rate in humans (Lynch, 2010a, 2010b), in a wide range of tissue types in mouse and rat, in the medaka fish (Winn et al., 2000), and in *Drosophila melanogaster* (Garcia et al., 2007). The average mutation rate in human somatic cells (1.02×10^{-9} per nucleotide site per cell division) is higher than *S. cerevisiae* and *Escherichia coli* (Lynch, 2006; Lynch et al., 2008). The mutation rates in the unicellular microbes *S. cerevisiae* and *E. coli* are not much different from the germline mutation rates of multicellular species, such as *D. melanogaster* and *Arabidopsis thaliana* (Lynch, 2010a). In addition, the mutation rate differs across the nuclear genome within a species and mainly depends on the base composition, DNA repair domains, recombination rate, and gene expression and density.

The proportion of advantageous or beneficial, neutral, and deleterious mutations vary among species (Baer et al., 2007; Eyre-Walker and Keightley, 2007). Analysis suggests that about 30, 16, and 2.8% of the amino-acid-changing (non-synonymous) mutations in hominids, *Drosophila* spp., and enteric bacteria, respectively, are effectively neutral (Charlesworth and Eyre-Walker, 2006; Eyre-Walker et al., 2002), and the remaining amino-acid mutations are deleterious (Eyre-Walker and Keightley, 2007). The rate of effectively neutral mutations occurring outside protein-coding sequences (in non-coding DNA) is estimated to be at least 95% in humans and around 50% in *D. melanogaster* (Eyre-Walker and Keightley, 2007). The percentage of advantageous mutations are rare and estimated to be 0% in *E. coli* (Elena et al., 1998) and in the RNA bacteriophage φ6 (Burch et al., 2007), 4% in vesicular stomatitis virus (Sanjuán et al., 2004), and 6% in *S. cerevisiae* (Thatcher et al., 1998). Studies

indicate that deleterious mutations are far more common than advantageous ones (Drake, 2006). Also, the majority of genotypic mutations that affect phenotype are harmful (Eyre-Walker and Keightley, 2007). Therefore, an important question arises: what keeps a check on the mutation rate? An array of data shows that natural selection and genetic drift plays a significant role in determining genomic mutation rates across a wide range of taxa (Baer et al., 2007; Eyre-Walker and Keightley, 2007; Nei, 2013; Vos et al., 2015). Selection functions mostly in large populations, while drift prevails during periods of small population size (Ohta, 1992). For instance, because of its large population size, natural selection is more effective in reducing genomic mutation rates in the unicellular eukaryote *C. reinhardtii* than in the multicellular eukaryote *D. melanogaster* (Sniegowski and Raynes, 2013). Mutations and selection are dependent on each other. When the effective population size is large, selection tends to remove deleterious mutations that have negative fitness effects on populations and brings the mutation rate down to zero. However, the reduction is counterbalanced by an increase in mutation rates to facilitate ongoing adaptation. Thus, natural selection adjusts mutation rates to an optimum level by maintaining a balance between deleterious mutations and mutations that act as a source of genetic variation required for adaptation to a changing environment (Lynch, 2010a; Sung et al., 2012; Vos et al., 2015). This suggests that strongly selected advantageous mutations are exponentially distributed. There exists a variation in natural selection among individuals or species, which is associated to the differential regulation of DNA polymerases that have varying fidelities in replication, differences in mutagen exposure, and abilities of species to neutralize or block mutagens, recognize and repair DNA damage, and modulate or buffer mutational effects (Baer et al., 2007).

The speed and mode of evolution are dependent on the rate at which genomes accumulate random mutations. Sometimes evolution works much more quickly. Treatments of cells by high-energy sources such as radiation (X-rays, and nuclear radiation), chemicals, etc., induce deleterious genetic changes, but also may cause advantageous ones. Exposure of bacteria to mutagens or stress (mutation-inducing chemicals, UV-radiation, or antibiotics) increases bacterial mutation rates to evolve and adapt to the challenging environmental conditions (Cox and Battista, 2005; MacLean et al., 2013; MacLean and Buckling, 2009).

2.3.2. Role of HGT in the evolution of species

HGT, also known as lateral gene transfer, is the transfer of genetic material between organisms independent of sexual reproduction. HGT events can result in uptake of whole genes, 'genomic islands', operons, or plasmids and an instant acquisition of new functions that could be beneficial for adaptation to novel niches. Thus, HGT enables organisms to increase their genetic diversity and evolutionary fitness probably due to the gain of new genes, the gain of paralogs of existing genes, and the gain of phylogenetically distant orthologous gene (Koonin et al., 2001; Kuo and Ochman, 2009; Rocha, 2008; Sieber et al., 2017).

Horizontal gene exchange is more common in closely related organisms but also occurs between distantly related organisms, contributing to evolution (Fig. 2) (Andersson, 2005; Boto, 2010; Zhaxybayeva et al., 2006; Zhaxybayeva et al., 2007). Gene exchange is reported between bacteria and archaea (Gophna et al., 2004; Koonin et al., 1997; Nelson et al., 1999; Rest and Mindell, 2003), between bacteria and eukaryote (Anderson and Seifert, 2011; Brown et al., 1998; Dröge et al., 1998; Guljamow et al., 2007; Watkins and Gray, 2006), from archaea to eukaryote (Andersson et al., 2003), and within bacteria (Felmingham and Washington, 1999), archaea, and eukaryote (Nedelcu et al., 2008; Richards et al., 2006). For instance, the development of antibiotic-resistant and pathogenic strains due to HGT between bacterial species (Davies and Davies, 2010; Lerminiaux and Cameron, 2019). Another example is the formation of crown galls (woody protrusions) on the trunks of fruit trees because of the transfer of bacterial DNA (tumor-inducing plasmid) of the *Agrobacterium* genus to plant somatic cells and the expression of this DNA (Gelvin, 2003; Kondo et al., 2002; Pitzschke and Hirt, 2010).

It is often difficult to conclude the exact mechanisms of gene transfer between organisms as they are rare evolutionary events and identified by their consequences and not at the time of happening. The molecular basis of inter-domain HGT has been largely understood by studies of genes in model species or cell lines. The fundamental steps involved in a successful gene transfer between two lineages include the transfer of the foreign genetic material into the host cell as naked DNA, incorporation of the transferred gene in the recipient nucleus or existence as an extrachromosomal DNA, and expression into a protein that must provide a function for selection in a population. In prokaryotes, HGT is generally mediated by three potential mechanisms – transformation, conjugation, and transduction (Jain et al.,

2002). Transformation is the ability of cells to obtain foreign DNA from the extracellular environment and incorporate it into the genome of a cell. Transduction is the process of movement of genes from one species to another via viruses. Conjugation involves cell-to-cell contact for the transfer of genetic material to a recipient cell, which is facilitated by a tube-like structure known as the pilus. The process of conjugation was thought to exist in closely related bacteria because of the compatibility, similar codon usage, and higher success rate for homologous recombination (Beiko et al., 2005; Ochman et al., 2000). However, there is considerable evidence that distantly related bacteria can potentially conjugate with one another and exchange DNA (Cordero and Hogeweg, 2009; Lerat et al., 2005). These processes are less dominant in eukaryotes. Physical contact of symbiotic or host-parasite relationships, as well as transfection of a virus, have been implicated for the DNA transfer of unrelated species into eukaryotic cells (Gogarten, 2003).

HGT includes the successful transfer of genetic material as well as the existence of the transferred genetic material throughout generations (Boto, 2010). After genes are transferred, natural selection determines which of them spread throughout populations (Gogarten et al., 2002; Pál et al., 2005). Genes that have useful functions are preserved, whereas useless ones are generally removed (positive selection) (Hao and Golding, 2008; Nakamura et al., 2004). Thus, the impact of HGT in the evolution of an organism is dependent on the number of transferred genes, their successful maintenance in the recipient genomes over generations, and the extent of the phenomena concerning the evolutionary time framework and phylogenetic distance between organisms (Boto, 2010). The long-term persistence of horizontally transferred genes confirms that they confer a selective advantage to recipient organisms. There is substantial evidence that the transfer of even a single or few genes gives the recipient new functions, allowing them to exploit and adapt to the new environment (Fournier and Gogarten, 2008). For example, several anaerobes have acquired many metabolic genes from bacteria for their adaptation to anaerobic environments (Keeling and Palmer, 2008). The acquisition of genes involved in the catabolism of complex carbohydrates by rumen ciliates (Ricard et al., 2006) and the rumen fungus *Orpinomyces joyonii* (Garcia-Vallvé et al., 2000) from bacteria have facilitated their colonization and adaptation to the anaerobic environment of the ruminant gut. The pathogenesis of certain intracellular parasitic bacteria, such as *Chlamydia* and *Rickettsia* is anticipated to be due to the HGT of the chloroplast-type ATP/ADP translocases from plants, which allows them to scavenge ATP from the host (Koonin et al., 2001).

Comparative analyses of data from whole-genome sequencing projects suggest that HGT has significantly influenced the evolutionary mechanism throughout the history of life and emergence of the three domains of life: Archaea, Bacteria, and Eukarya (Andersson et al., 2003; Boto, 2010; Boucher et al., 2003; Brown, 2003; Daubin et al., 2003; Richards et al., 2006; Soucy et al., 2015). With advances in sequencing technologies, the information on the importance of HGT in microbial evolution continues to expand (Choi and Kim, 2007; Dagan et al., 2008; Koonin and Wolf, 2008; Pál et al., 2005; Treangen and Rocha, 2011). In many prokaryotes, gene gain due to HGT is considered to be a more significant process than gene duplication (Dagan et al., 2008; Treangen and Rocha, 2011; Vos et al., 2015), while the relevance of HGT in gene loss is not adequately understood (Kurland et al., 2003; Polz et al., 2013). The last few years of attained results show that HGT plays a far superior role in the evolution of archaea and bacteria than formerly thought (Kloesges et al., 2011; Sieber et al., 2017; Smillie et al., 2011; Vos et al., 2015; Wiedenbeck and Cohan, 2011). The genes and species that are associated with HGT between the archaea and the bacteria are highly diverse (Brown, 2003). Koonin et al., (2001) reported that between 1.6 and 32.6% of the genes of each microbial genome are acquired by HGT indicating that a high level of gene transfer exists for most bacterial and archaeal genomes. Almost 24% of the genes in the bacterium *Thermotoga maritima* that grow well at 80°C are more similar to hyperthermophilic archaeal genes than other bacterial genes (Nelson et al., 1999). *T. maritima* acquired genes from archaea through extensive HGT. Both cultures share reverse gyrase gene, which perhaps provides them adaptation to live at very high temperatures (Forterre et al., 2000). Significant differences in gene content (over 30%) among strains of *E. coli* demonstrate that HGT has a considerable effect on genome evolution even at the species level (Hayashi et al., 2001). Dagan et al., (2008) analyzed evolutionary history among 539,723 genes distributed across 181 sequenced prokaryotic genomes and found that at least 81 ± 15% of genes in each genome were involved in HGT. They indicated that HGT has very low barriers and can substantially affect all gene families among prokaryotes over long evolutionary time scales.

The impact of HGT in eukaryotic evolution remains controversial, in particular when multicellular eukaryotes are concerned (Huang, 2013; Ku et al., 2015; Salzberg et al., 2001; Stanhope et al., 2001). It is anticipated that HGT plays a less prominent role in the evolution of eukaryotes than in prokaryotes. A group of researchers supports that gene inheritance in eukaryotes is vertical (Ku et al., 2015) and that eukaryotic genomes show no evidence for either continuous or recent gene acquisitions from prokaryotes (Ku and Martin, 2016). However, the last decade has seen an increasing number of reports on the HGT of genes into eukaryotic genomes from both endosymbionts and free-living organisms (Alsmark et al., 2013; Andersson, 2005; Dunning Hotopp et al., 2007; Hall and Dietrich, 2007; Kishore et al., 2013; Mackiewicz et al., 2013; Marin et al., 2007; Soanes and Richards, 2014). These studies indicate that HGT had a strong impact in modulating both unicellular and multicellular eukaryote evolution. Significant numbers of HGT events contributing to genome evolution have been reported in rumen ciliates (Ricard et al., 2006), the parasitic protozoan *Entamoeba histolytica* (Loftus et al., 2005) and *Trichomonas vaginalis* (Carlton et al., 2007), the anaerobic parasite *Blastocystis* (Eme et al., 2017), the diatom *Phaeodactylum tricornutum* (Bowler et al., 2008), and the multicellular *Hydra magnipapillata* (Chapman et al., 2010). The phylogenomic analysis revealed that the metabolic repertoire of *E. histolytica* and *T. vaginalis* is shaped by the HGT of bacterial genes into their genome (Carlton et al., 2007; Loftus et al., 2005). Extensive gene transfer events have provided the diatom *P. tricornutum* with novel possibilities for metabolite management and the perception of environmental signals to thrive in oceans (Bowler et al., 2008). In a recent study, Eme et al., (2017) identified that *Blastocystis* has horizontally transferred genes both from prokaryote and eukaryote donors and the genes are involved in diverse metabolic pathways facilitating adaptation of the parasite to the gut environment, infection, and circumvention of host defenses. The gain of genes from bacteria and other eukaryotes has also resulted in the evolution of bdelloid rotifers (Gladyshev et al., 2008), fungi (Marcet-Houben and Gabaldón, 2010; Richards et al., 2006), and even plants (Huang and Gogarten, 2008). Analysis of prokaryotic-derived HGTs in 60 fully sequenced genomes showed that inter-domain transferred genes in fungi mostly included bacterial arsenite reductase, catalase, different racemases, and peptidoglycan metabolism enzymes (Marcet-Houben and Gabaldón, 2010). The coffee berry borer beetle, *Hypothenemus hampei*, which is a devastating pest of coffee, gained the bacterial *HhMAN1* gene through HGT to hydrolyze coffee berry galactomannana and colonize the coffee berries (Acuña et al., 2012). Genes that are horizontally transferred from eukaryotes to bacteria or archaea are functionally diverse and do not belong to just one or a few categories (Koonin et al., 2001; Koonin and Wolf, 2008). An example of gene transfer from eukaryote to bacteria, mainly proteobacteria, is the glutaminyl-tRNA synthetase gene, which is not present in other bacteria, the archaea (archaebacteria), and organelles (Brown and Doolittle, 1999).

Overall, there is abundant evidence to demonstrate that extensive genetic exchange has occurred in both prokaryotic and eukaryotic organisms and HGT has played a significant role in shaping our complex and remarkable biological world. HGT could potentially change genomes more rapidly than point mutation and has important implications in the evolution of organisms, such as adaptation to novel niches. Genes acquired by HGT have clear functional or environmental significance for the recipient. The acquisition of foreign genes via HGT allows organisms to thrive in new and competitive environments. As a globally important evolutionary mechanism, HGT could be used to generate novel strains that would allow them to potentially exploit and adapt to new and challenging environments.

2.3.3. Epigenetic gene regulation in the evolution of species

Epigenetics represents the changes in gene expression and function that occur without alteration in the DNA sequence (Casadesús and Low, 2006; Crisp et al., 2016; Eichten et al., 2014; Ito, 2014). Phenotype variations due to epigenetic processes are stable and inherited to the offspring. Epigenetic processes involve DNA-methylation, histone modification, and non-coding RNA (ncRNA) activity. DNA methylation includes the addition of a methyl group to specific nucleotides catalyzed by DNA methyltransferases (Casadesús and Low, 2006; Law and Jacobsen, 2009). Histone modification is a post-translational modification of the histone proteins via acetylation, methylation, phosphorylation, and ubiquitylation (Gendrel and Colot, 2005; Ito, 2014), which can influence gene expression by modifying chromatin structure or employing histone modifiers (Bannister and Kouzarides, 2011). Histone modifications act in processes such as chromosome packaging, transcriptional activity, and DNA damage or repair. The ncRNAs, such as short ncRNAs (<30 bp; miRNAs, siRNAs, and piRNAs) and the long ncRNAs (>200 bp) are also shown to regulate gene expression at the transcriptional and post-

transcriptional level by playing a role in histone modification, DNA methylation targeting, heterochromatin formation, and gene silencing (Crisp et al., 2016; Ito, 2014; Palazzo and Lee, 2015). DNA methylation change, histone modification, and chromatin and small interfering RNA (siRNA) abundance are all perturbed (induced or blocked) by both biotic and abiotic environmental conditions, resulting in the alternation of gene expression (Boyko et al., 2010; Ding et al., 2012; Khraiwesh et al., 2012; Sani et al., 2013; Zong et al., 2013).

Epigenetic effects are not only common but they can also underlie and influence many aspects of evolution (Lind and Spagopoulou, 2018) in several organisms including plants, animals, and microbes. This is true both for epigenetic effects that are visible only in a single generation as well as for transgenerational epigenetic inheritance (Fig. 3). Epigenetic regulation enables a rapid increase in variation within a species in times of environmental stresses or cues, providing an opportunity for adaptation to the arising selection pressures. The passing on of these variations to the offspring establishes epigenetic inheritance (Holliday, 2006), which is important for adaptation, above all in organisms with limited genetic variation. Plants have developed innovative approaches to survive harsh environmental stresses (Khraiwesh et al., 2012; Zhu et al., 2008; Zong et al., 2013). Among others, the efficient strategy is the epigenetic regulation of gene activity throughout their lifespan. In plants, DNA methylation and histone modification affect various biological processes in the current as well as in subsequent generations, such as growth and development, flowering time, fertility, and morphology. They regulate the silencing of transgenes and transposons as well as control genomic imprinting (Bai and Settles, 2014; Crisp et al., 2016; Ito, 2014). Stress-induced epigenetic changes can be inherited through cell division (meiotic and mitosis) (Calarco et al., 2012; Eichten et al., 2014; Feng et al., 2010). Earlier investigation showed that the DNA methylation pattern is not passed to the next generation (Monk, 1995) in mammalian cells but later studies indicated that epigenetic modifications of some genes can be transmitted through the germline (Anway et al., 2005; Cropley et al., 2006; Rakyan et al., 2003). Like in many eukaryotes, epigenetic regulation by DNA methylation (adenine instead of cytosine) also influences various cellular functions in bacteria (Casadesús and Low, 2006). It governs virulence pattern in a wide range of pathogens, controls cell cycle, provides signal for DNA replication, repair of DNA, chromosome segregation, exchange of plasmids, and bacteriophage genome packaging, and regulates activities of transposase and certain promoters as well as expression of genes (Bhagwat and Lieb, 2002; Casadesús and Low, 2006; Løbner-Olesen et al., 2005; Roberts, 1985; Wion and Casadesús, 2006).

Collectively, epigenetic modifications enable (i) survival of organism in changing environments without the need for genetic alterations (Burggren, 2016), (ii) adaptation to the new environment by the development of rapid variation due to higher rate of methylation or demethylation (i.e., the epimutation rate) of DNA and histones compared to genetic mutations (van der Graaf et al., 2015), and (iii) adaptation of small populations with a limited genetic variation or of asexual organisms to new environments. Thus, epigenetics provides a means for the development of variations within a species that can adapt at times of stress or deprivation.

2.4. Stress accelerates evolution

The interaction between an organism and the environment is central in evolution (Fig. 4). Stress is the major force driving the evolution of living organisms (Nevo, 2011; Parsons, 2005). Parsons defined stress as "an environmental factor causing potential changes to biological systems with the potential for impacts on evolutionary processes" (Parsons, 2005). Stress plays a significant role in facilitating adaptation by allowing better modifications, maintenance, and functioning of organismal systems to the ever-changing environment (Badyaev, 2005; Rutherford, 2003). Organisms become extinct when they fail to change and evolve adaptive mechanisms to diminish the detrimental effects of environmental changes.

Living cells are constantly subject to a myriad of external and internal stresses that affect their performance and well-being. External stresses are naturally occurring or man-made changes in abiotic factors, such as temperature, light, other climatic factors, and chemical components, and biotic aspects including competition, predation, and parasitism (Bijlsma and Loeschcke 2005). Internal stresses consist of genetic architecture changes in organisms brought naturally or artificially by numerous mutagenic agents, such as oxygen-free radical metabolic by-products (Baer et al., 2007; Bijlsma and Loeschcke, 1997). Both external and internal stresses result in a wide array of biological responses ranging from genotypic to physiological to phenotypic. Based on the intensity, frequency, and uniformity of stress, these responses are either associated with the development of stress tolerance, stress resistance, and

novel traits, or the reduction, migration, and extinction of a population. Evolutionary change is not always associated with phenotypic changes. Under a normal range of environments, stress result only in the expression and accumulation of novel genetic variation and physiological adaptations and no changes in morphology (Badyaev, 2005). Moderate stress is vital for normal growth and differentiation of cells and organisms (Badyaev, 2005; Clark and Fucito, 1998; Müller, 2003). For example, gravity influences the development of cells and organisms profoundly and subtly in terms of shape, function, and behavior (Bizzarri et al., 2015). Extreme environmental changes disrupt normal development, function, and may cause mass extinction events but are also associated with evolutionary changes and the origin of new species adapted to new environments (Nevo, 2011; West-Eberhard, 2003). Harsh environments occasionally lead to novel phenotypic alternations and simultaneously initiate strong phenotypic selection (directional selection) for survival and succession in changing and new environments (Badyaev, 2005; Rutherford, 2003; Wright, 2004). Examples of such shifts in populations include responses to selection arising from human activities, such as the evolution of antibiotic resistance in bacteria, pesticide resistance in insects, and heavy metals resistance in plants and invertebrates (Davies and Davies, 2010; Hoffmann and Hercus, 2000; Lerminiaux and Cameron, 2019; McKenzie and Batterham, 1994). Nonetheless, the mechanisms behind the relationship between extreme environments and phenotypic evolution are rarely understood.

Organisms' ability to recognize, assess, and react to stressors, in particular to those that are extreme, occasional, and shorter than the generation time of an organism and associated with phenotypic evolution is quite remarkable. It is intriguing to see how an organism survives stress, accommodate stress-induced modifications, and reproduce without reducing its functionality. With the increase in evolutionary studies of stress, it is widely recognized that adaptation of an organism to ever-changing environments is generally associated with an increase of recombination and mutation rates (genetic variance) and amplification of phenotypic variation (Badyaev, 2005; Hoffmann and Merilä, 1999; Horne et al., 2014; MacLean et al., 2013; Wright, 2004). Stress increases mutations rates in prokaryotes as well as in eukaryotes, which is probably the reason for discontinuous (punctuated) evolution (Velkov, 2002). The mutation rate in bacteria under stress is up to 100 to 200-fold higher compared to non-stressed cells (Foster, 2000). This increase is due to (i) activation of an SOS-regulon, which lead to the formation of a DNA polymerase IV that produces many errors during DNA-replication, (ii) activation of the homologous recombination system that is associated with single- and double-strand breaks in DNA by recombination enzymes, (iii) activation of double-strand break repair system, which restores replication of a stalled or damaged replication fork, and (iv) transient switch-off of the mismatch repair system (Bruijn, 2016; Kuzminov, 1999; McKenzie et al., 2000; Motamedi et al., 1999). Furthermore, stress increases the efficiency of interspecific gene transfer. Plasmids are considered as evolutionary tools as they can accelerate evolution by increasing mutations of their own and chromosomal genes as well (Velkov, 2002). The adaptive mutation rate is higher in plasmid than chromosomal genes (Rosenberg, 2001). In stress, bacterial cells also suppress their own restriction-modification system transiently to improve their chances of gaining new foreign genes, thereby facilitating evolutionary changes (Kelleher and Raleigh, 1995; Velkov, 2002). In eukaryotes, the heat shock protein Hsp90 enables the native folding of proteins (e.g., signal transduction and morphogenetic proteins) under normal conditions. However, under stress, this protein is involved in other important use that leads to increased accumulation of multiple mutations in vital genes (Rutherford, 2000).

3. Space environment as a toolbox for application-oriented biological research

Space is a unique environment in many aspects (Horneck et al., 2010). It provides a combination of stressors that organisms have never experienced on Earth (Fig. 5). Certain changes are noticeable only in space. Studies conducted so far show that space environment leads to alternations in genetics and variations in morphology and physiology, producing negative or positive effects on the organisms. For example, space conditions increases virulence, reduces the susceptibility of pathogenic microorganisms against antimicrobial agents, and enhances bacterial growth (reduced lag phase and enhanced exponential phase) (Horneck et al., 2010; Klaus et al., 1997; Mermel, 2012). Space offers the extraordinary environmental conditions of high radiation of galactic and solar origin, high vacuum, extreme temperatures, and microgravity (Horneck et al., 2010). The radiation in LEO is strong enough to increase the mutation rate (about 100-fold compared with that on Earth), and microgravity forces

biological systems to reorganize, adapt, and evolve. Microgravity is the absence of gravity and leads to pronounced physical changes in a sample or system environment.

3.1. Gravity and microgravity

Gravity is one of the four basic forces in physics and has two effects on an object as a function of its mass: displacement (motion) and/or deformation (weight). It is the reason for the mutual mass-dependent attraction of objects. The magnitude of the attractive force between two bodies of mass is determined from Newton's law of gravitation: $F_{12} = (G\, m_1\, m_2)\, r^{-2}$, where G is the gravitational constant (6.672×10^{-11} Nm^2kg^{-2}), F_{12} is the force resulting from the attraction by a particle of mass (m_1) on another mass particle (m_2) a distance (r) away. Force accelerate bodies. The acceleration effect of the small body (e.g., human) on a huge mass (e.g., Earth) is so negligible that the high-mass object attracts the body. The gravity field of Earth accelerates masses at the Earth's radius with about 9.81 m/s^2 (differs concerning geographic position). At Earth's surface, the path of an object to the Earth center is inhibited and gravity becomes noticeable as weight force ($F = m\, a$, where, m is mass and a is gravitational acceleration). The outcome of this relationship is so ubiquitous in our daily lives that weight is usually not considered as a variable to be experimentally manipulated. The fact that the Earth's gravity gives weight to attract matter leads to various phenomena, such as the formation of water bodies and atmosphere, hydrostatic pressure, sedimentation, convection, and increased shear forces. In addition, the physical solubility of gases such as oxygen in water depends on acceleration. Since the origin of life, all organisms are constantly exposed to the vector of gravity. Organisms, above all terrestrial ones, developed structures to counteract the impact of gravitational forces.

To annul gravity on Earth is not easy. Only conditions of free fall enable real microgravity conditions. Microgravity means acceleration conditions close to weightlessness (Horneck et al., 2010; Klaus, 2004). Weightlessness leads to pronounced physical changes in the sample environment, such as no sedimentation, no hydrostatic pressure difference within the samples or the surrounding medium in open containers, no buoyancy-driven convection: transport of metabolites reduced to the range of diffusion, and low shear forces. Acceleration directly influences the properties of membranes, as has been determined in experiments with defined vesicles and living cells (Kohn et al., 2017; Sieber et al., 2016). In cells as well as in vesicles, membrane fluidity increases significantly in microgravity, which, for example, influences the uptake and effects of drugs.

3.1.1. Sedimentation

Under microgravity, no sedimentation takes place. Various organisms use intracellular heavy particles (e.g., amyloplasts or otoliths) or the whole cell body as statoliths to sense the vector of gravity. This phenomenon is based on the higher density of those statoliths which sediment within the less dense cytoplasm. Without gravity or external acceleration this movement stops.

3.1.2. Diffusion and convection

Diffusion is essentially based on the Brownian motion and results finally in an even mixing of all types of molecules in a given limited volume. Diffusion is fast only over small distances (scale of a cell). In contrast, convection depends on specific density differences in a given volume. It allows a very effective mass transport over large distances. Diffusion is not gravity-dependent, while convection rate is. The diffusion rate of a particle depends on its diffusion coefficient D [$D = (k\, T)/(4\, \pi\, \eta\, a)$], where k is the Boltzmann constant ($1.3806488 \times 10^{-23}$ J/K) divided by NA (Avogadro number: 6.022×10^{23}), T is the temperature in Kelvin (K), η is the viscosity of the medium, and a is the radius of the particle. Typical values for 'D' are about 5×10^{-6} cm^2s^{-1} for small molecules, such as sucrose, 6×10^{-7} cm^2s^{-1} for large molecules and values below 10^{-10} cm^2s^{-1} for organelles. The square distance of the migration of a particle can be calculated by $d^2 = 2\, D\, t$. The time (t) for a particle to be translocated depends on the square of the distance (d). This means that diffusion processes are rather slow. For example, the time of a protein needed to travel 3 mm from an effector to a receptor cell would last about 2 h.

Convection is a gravity-dependent buoyancy-driven effect. The specific weight of air or fluids (except water at its point of an anomaly) becomes lower with increasing temperature. With uneven temperature distribution (e.g., presence of a heating source), heated water or air begins to move upward, while it is replaced by colder air and water. Convection results in a circulation, which also mixes the air or water and leads to a better distribution of oxygen or metabolites. No convection occurs when gravity

is absent. This may lead to severe effects on the macroscopic and eventually on the microscopic scale. The availability of oxygen is impaired when particle translocation is solely based on diffusion. Stout et al., (2001) clearly showed that hypoxia occurs in the root zone of *Brassica rapa* under microgravity. Among others, the activity of ADH (alcohol dehydrogenase) was found to be increased several-fold in microgravity indicating anaerobiosis compared to 1*g*. Not only oxygen supply but also nutrient uptake as well as dissipation of metabolic waste products are most likely hampered in microgravity. As mentioned before, many studies under real and simulated microgravity reported that metabolic enzymes were affected. The lack of convection is an indirect effect of microgravity. To overcome these problems convection needs to be replaced by air and fluid streams (which is, of course, difficult when roots are embedded in agar and also introduces a force in a force-free environment). Most likely non-motile organisms or cells are more sensitive against depleting-effects in their direct environment. Motile organisms can constantly change their position from depleted to non-depleted sites. In this respect, it would be interesting to compare e.g., the development of motile and non-motile bacteria in not-stirred containers under microgravity.

The high viscosity of the cytoplasm reduces the diffusion speed so that the Peclet number (ratio between convection and diffusion) is >1 (Todd, 1989). This means that convection contributes considerably to movement within the cytoplasm (assuming the cells do not show plasma streaming). Convection and diffusion are not only important for mass transport but also for heat transport. In cells with a high-energy conversion ratio, it would be theoretically possible that focal heat-producing cellular components, such as mitochondria, the nucleus during DNA-replication, or centrosomes may become overheated under microgravity, because of a misbalance between convectional and diffusional heat transport. Overheating of mitochondria may account for intrinsic-induced apoptosis due to cytochrome c release from damaged mitochondria [space-related apoptosis and other effects are reviewed among others in Majima et al., (2009)]. Indeed, in a variety of publications effects on abnormal distribution or ultrastructure of mitochondria were reported (Bell et al., 1992; Klymchuk, 2007; Mao et al., 2013; Nikawa et al., 2004; Philpott et al., 1990; Popova, 2003). To prove this, we propose a simple experiment in which temperature inside a cell will be measured spatially in microgravity and under 1 *g*. Recently different methods for temperature detection have been developed, based on fluorescence lifetime-imaging of thermosensitive fluorophores (Okabe et al., 2012) or sub-micrometer thermometry using nanodiamonds (Kucsko et al., 2013).

3.1.3. Shear forces

Shear forces are strongly reduced under microgravity. The random positioning machine (RPM) leads to increased shear forces and stress on biological systems because the samples are constantly moved in the field of gravity. Clinorotation, in contrast, provides an environment with very low shear forces (Hauslage et al., 2017). The luminescent dinoflagellate *Pyrocystis noctiluca*, which produces bioluminescence when experiencing shear forces, was subjected to clinorotation. The authors constructed hardware, which can be fixed on different "simulated-gravity"-devices and recorded shear force-induced light signals from the dinoflagellates. Random-positioning was found to induce strong luminescence indicating the significant presence of shear forces. In contrast, no luminescence was induced upon clinorotation. This indicates that clinorotation is probably the closest match to real microgravity which can be achieved on Earth in terms of shear forces.

3.2. Space radiation

The main fraction of space radiation reaching LEO consists of protons from solar particle events and high-energy galactic cosmic rays (mainly hydrogen ions and helium ions and some high-atomic-number nuclei and high-energy nuclei), as well as other high-energy solar particle events (Häder et al., 2009a; Horneck et al., 2010; Suparta and Zulkeple, 2014). The ionizing radiation (γ-ray, x-ray, β-ray, α-radiation, and neutron radiation) in LEO is considerably increased compared to Earth, with the perceived radiation strongly dependent on the orbit of the spacecraft. We calculated the yearly dose in millisieverts during different LEO space missions according to the data provided in a corresponding NASA report (space shuttle, Skylab, and ISS) (Moreno-Villanueva et al., 2017). The perceived level of ionizing radiation is between 85 times (space shuttle mission 41-C: about 0.69 mSv/d) and 250 times (Skylab 4: about 2.04 mSv/d) that at ground level (about 0.0082 mSv/d). Polar orbits or frequent passages through the so-called South Atlantic Anomaly, where the Earth inner Van Allen belt is thin (only about 190-200 km), will exceed the values of the above-mentioned missions multiple times. The

investigation on the influence of space ionizing radiation on different organisms is an important field of study. Ionizing radiation interacts with DNA and may lead to stable and inheritable genetic alternations, changes in gene function, metabolism, physiology, growth, shape, and structure. The effects of radiation on organisms depend on the quality and quantity of radiation, and cell age and characteristics (Horneck et al. 2010; Matallana-Surget and Wattiez 2013). Space radiation cannot be simulated on Earth to its full complexity.

4. Simulated and real microgravity platforms

Based on free fall, different measures are available to achieve real microgravity. These include drop towers (microgravity time about 5 s), aircraft parabolic flights (microgravity time about 20 – 30 s), sounding rockets (microgravity time 5 – 13 min), and orbital platforms such as satellites or space stations (microgravity time weeks to years) (Fig. 6). The "microgravity carriers" differ widely in terms of microgravity-time and microgravity-quality, as well as cost and accessibility (Pletser, 2004; Ruyters and Friedrich, 2006). Drop tower experiments and aircraft parabolic flight experiments are relatively reasonably priced and accessible in a foreseeable time scale (weeks or months). Residual accelerations are very low during drops, while they are higher in parabolic flights. Additionally, the typical acceleration profile of a parabola includes two phases of 1.8 *g* before and after the µg-phase. Sounding rockets or orbital carriers demand a long time of experiment preparation and are quite cost-intensive. Due to the high costs of those technical microgravity opportunities, extensive ground-based data sets are required for the in-depth analysis of the microgravity experiments.

To inhibit the orientation of organisms to the vector of gravity, different devices have been developed by which the gravity vector is randomized. The principle of randomizing the acceleration vector is the rotation of the samples around one or two axes. These methods are often referred to as "simulated microgravity". In any case, it is important to emphasize that "simulated" or "functional" microgravity is different from real microgravity because (omnidirectional) acceleration forces are still acting on the samples. In contrast to real microgravity, no gravitational unloading is achieved. Next to averaging the vector of gravity, these devices inhibit the sedimentation of the samples. Most common devices for averaging the vector of gravity are:

- 2D-clinostat and 3D-clinostat, where the samples are rotated around one and two axes, respectively (Hemmersbach et al., 2006). A clinostat reproduces the quiescent, unstirred fluid conditions that are achievable on orbit.
- Rotating wall vessel (RWV) bioreactors are special 2D-clinostat for liquid cultures. RWV is a suspension culture system that allows cells to grow under physiologically relevant low fluid shear conditions normally experienced by cells cultured in a spaceflight environment. A tube with a narrow diameter is rotated around its length axis (Hammond and Hammond, 2001; Klaus, 2001). The RWV bioreactor enables perfusion of nutrients to and waste from the culture.
- The random positioning machine (RPM), which in principle works similar to a 3D-clinostat, but arbitrarily changes the movement direction (Patel et al., 2007; van Loon, 2007).

Recently, diamagnetic levitation was thoroughly tested as a measure to produce microgravity-like conditions. In principle, all molecules in a sample should be lifted using an extremely strong magnetic field (10 - 16.5 T) in a way that the samples in the center of the solenoid are in the condition of weightlessness. The solenoid produces the magnetic field. Different magnetic fields result in different effective gravity levels. Manzano et al., (2012) constructed sample holders with different sample locations in the solenoid. With this method, simultaneously microgravity, Moon-, Martian- and hyper-gravity can be achieved. A "1 *g*-position" serves as an additional control. Magnetic levitation is presently the only facility which enables microgravity and low gravity-like effects in a stable environment (Manzano et al., 2012). Prerequisite is that all components of a sample have the same magnetic susceptibility and therefore this method is not suited for a variety of organisms. For example, since barium sulfate statoliths in *Chara* rhizoids have a far different magnetic susceptibility than the rest of the cell, investigations of gravitropism in this organism using diamagnetic levitation are not possible (Herranz et al., 2013). Also, a magnetic field alone can exert effects on living organisms. Paul et al., (2006) reported changes in gene expression patterns in *Arabidopsis* induced by strong magnetic fields. Significant effects of magnetic levitation on the orientation of *Euglena gracilis* as well as *Paramecium* cells, are also described (Hemmersbach et al., 2014). Under normal conditions, both organisms show a precise orientation behavior, termed as negative gravitaxis. However, upon exposure to a strong

magnetic field, both cell types aligned to the magnetic field lines. While *Paramecium*-cells were aligned parallel to the field lines, chloroplast-containing *E. gracilis* cells showed a perpendicular alignment towards the field lines. In addition, even upon application of very strong magnetic fields, it was not possible to levitate immobilized cells. This clearly shows that magnetic levitation is, at least for various biological systems, no substitute for real microgravity. Herranz et al., (2013) compared literature data of experiments performed under simulated microgravity with data from real microgravity. The survey revealed that the optimal simulated microgravity facility to achieve comparable results to real microgravity is specific for the investigated organism and the investigated phenomenon. However, after profound literature research, Kiss et al., (2019) strongly promote the application of ground-based gravity simulation but also recommend a very stringent and careful standardization of the experiments to allow better comparison between the data. Taken together, due to the fundamental physical differences between real and simulated microgravity, it is important to consider the applied experiments' environmental needs.

5. Microbiological studies in space environment or simulated microgravity conditions

To study the influence of various space conditions, microbes have been sent into space for over 60 years (Clément and Slenzka, 2006; Crabbé et al., 2011; Crabbé et al., 2013; Horneck et al., 2010; Huang et al., 2015; Juergensmeyer et al., 1999; Kacena et al., 1999; Kim et al., 2013a; Kim et al., 2013b; Nickerson et al., 2004; Rosenzweig and Chopra, 2012; Taylor, 1974; Wilson et al., 2007; Wilson et al., 2008). The two main physical factors that microbes experience in space are microgravity and cosmic radiation, while other factors, like space vacuum, thermal extremes, and solar UV radiation are mitigated by spacecraft design. Besides, several studies have been performed utilizing a low-shear modeled microgravity (LSMMG) environment to understand the influence of the space environment on a range of microorganisms (Altenburg et al., 2008; Arunasri et al., 2013; Baker et al., 2004; Chopra et al., 2006; Gao et al., 2011; Huang et al., 2015; Kim et al., 2016; Lawal et al., 2013; Nickerson et al., 2000; Pacello et al., 2012; Rosenzweig et al., 2014; Searles et al., 2011; Soni et al., 2014; Tucker et al., 2007; Xu et al., 2015). These microbes include among others *Salmonella enterica* serovar Typhimurium, *Yersinia pestis*, *E. coli*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Streptococcus mutans*, *Mycobacterium marinum*, *Bacillus subtilis*, *Streptomyces coelicolor*, *S. clavuligerus*, *S. hygroscopicus*, *Serratia marcescens*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Rhodospirillum rubrum*, and the yeasts *Candida albicans* and *Saccharomyces cerevisiae* (Horneck et al., 2010; Senatore et al., 2018; Tirumalai et al., 2019). Microbial characters were found to be changed in real and simulated microgravity conditions including metabolism, genetic transfer, growth, morphology, stress sensitivity, and pathogenicity.

A majority of microbiological studies in space have reported similar responses in space across several bacterial species, including reduced lag phase and increased cell population (Benoit and Klaus, 2007; Horneck et al., 2010). Unexplained inconsistencies deviating from these typical findings were occasionally reported over the years. A literature survey substantially proposes that cell motility is the key variable responsible for the seemingly incongruent results (Benoit and Klaus, 2007; Horneck et al., 2010). Studies of microgravity effects on motile and non-motile cells have shown that non-motile cells exhibit a difference in growth kinetics in space, while growth kinetics of motile strains is not affected under microgravity and static conditions. This is perhaps due to the lack of convection, which limits the mass transfer between cells and the fluid environment (Klaus et al., 2004; Klaus, 2004). That means the transport of nutrients from the medium to non-motile cells as well as the convection-driven distribution of waste products from cells is reduced (the only diffusion occurs), which in turn leads to the accumulation of waste products and reduction of oxygen and nutrients around the cells. This causes specific biological responses in non-motile cells, altering their growth and behavior relative to 1 *g* controls. However, these do not account for motile cells as they are constantly changing their position in the medium. A strong correlation exists between motility and cell number of culture in spaceflight (Benoit and Klaus, 2007; Horneck et al., 2010). This suggests that bacteria lack gravity-dependent physiological mechanisms but are indirectly influenced by the lack of convection in microgravity conditions. Also, it is proposed that due to their small size, it is unlikely that bacteria respond to gravity using physiological mechanisms (Horneck et al., 2010; Klaus et al., 1997). Above all, the Brownian motion superimposes the gravitational force in small objects (>10 µm) (Klaus et al., 1997). Convection is present but minimized under sub-1 *g* accelerations and thus it is important to determine the degree of

convection required for optimal cell growth and development in reduced gravity conditions (Benoit et al., 2008; Janosi et al., 2002).

Several groups of bacteria (e.g., Proteobacteria, Firmicutes, Staphylococcaceae, and Enterobacteriaceae) are present onboard the ISS (Ichijo et al., 2016), which are mostly transferred from the bodies of the astronauts. It is important to study the relationship between humans and microbes in space habitats, as they are crucial for long-term manned space missions. Space influences microbes but microbes also influence spaceflight and may become a potential threat to the crewmembers. Microorganisms play an important role in several biological processes, e.g., in wastewater treatment, but may become problematic through the buildup of biofilms that can negatively affect life-support systems. During long-term missions, they also pose risks of increased pathogenicity and development of antibiotic resistance due to unpredictable genomic changes (Tirumalai et al., 2017), which was concluded based on a growth study of Lac+ *E. coli* cells for over 1000 generations in lysogeny broth under LSMMG. Previous and recent studies have shown that space conditions increase virulence and reduce the susceptibility of pathogenic microorganisms against antimicrobial agents (increase in minimum inhibitory concentrations) (Horneck et al., 2010; Ilyin, 2005; Kalpana et al., 2016; Lapchine et al., 1986; Mermel, 2012; Nickerson et al., 2000; Senatore et al., 2018; Tirumalai et al., 2019). *S. enterica* serovar Typhimurium exhibited increased pathogenicity in mice infected with cells exposed to simulated microgravity compared to 1 *g* conditions (Nickerson et al., 2000). The cells subjected to simulated microgravity were also found to be more acid-resistant, suggesting the possibility of enhanced survival in the gastrointestinal tract, as well as showed altered protein expression patterns. Low fluid shear forces as present in microgravity increase virulence, gene expression, and stress response. This was concluded based on experiments conducted in RWV bioreactor using a *Salmonella* liquid culture (Yang et al., 2016). The increase in microbial virulence and resistance against antimicrobials in the space environment could be attributed to reduced lag phase, increased population growth, and enhanced secondary metabolite production (Horneck et al., 2010). But, conflicting reports also exist showing reduced resistance and virulence in some bacteria in microgravity conditions (Hammond et al., 2013; Juergensmeyer et al., 1999). Hammond et al., (2013) assessed the effects of spaceflight, clinorotation in a 2-D clinorotation device, and static ground controls on virulence by determining the ability of pathogens *Listeria monocytogenes*, methicillin-resistant *S. aureus* (MRSA), *Enterococcus faecalis*, and *Candida albicans* to kill *Caenorhabditis elegans*. They found all four pathogens less virulent in microgravity.

Moreover, the biofilm formation in a microgravity environment is different compared to normal gravity controls (Kim et al., 2013b; Mauclaire and Egli, 2010), which could be associated with increased pathogenesis and tolerance to antibiotics. Increased viable cells and thick and higher biomass biofilms with a column-and-canopy structure is observed in spaceflight compared to 1 *g* controls, indicating that the community-level behaviors of bacteria are highly influenced in microgravity (Kim et al., 2013b). Due to the differences in extracellular polymeric substances responsible for biofilm formation, differences in cell aggregates and clumping have been observed in *S. typhimurium* (Wilson et al., 2007) and *P. aeruginosa* (Kim et al., 2013a) cultured in space. Crabbé et al., (2011) noted differential expression of genes involved in biofilm formation and cell aggregation as well as validated microscopically the enhanced self-aggregation behavior of space flight grown *C. albicans* compared to synchronous 1 *g* controls. Increased biofilm formation has also been reported in *C. albicans* grown under LSMMG determined by microscopy and morphology-specific gene expression profiling (Searles et al., 2011). Enhanced biofilm formation ability has been noted in *Klebsiella pneumonia* in simulated microgravity conditions (Wang et al., 2016). A thick biofilm showing high tolerance to antibiotics, salt, and ethanol treatment has also been reported in LSMMG grown *E.coli* cells compared to controls (Lynch et al., 2006; Tucker et al., 2007). Recently, Cheng et al., (2014) reported that simulated microgravity alters the biofilm structure of *S. mutans* as well as increases its proportion within a dual-species biofilm (co-cultured with *S. sanguinis*). Taken together, the changes in microbial virulence, antibiotic susceptibility, and biofilm formation in combination with suppression of the immune system in astronauts because of various spaceflight stressors increase health-related concerns during a long-term manned space mission.

Significant changes in membrane fluidity have been reported in microbial cells under reduced gravity conditions as well. Vukanti et al., (2012) reported that variations in membrane potential and integrity under modeled microgravity conditions make *E. coli* and *S. aureus* cells physiologically more active and viable compared to 1 *g* samples. Kim and Rhee, (2016) also showed that the simulated space

environment alter physiological characteristics of a foodborne pathogen *E. coli* O157:H7. The study indicated that heat resistance of *E. coli* decreased under LSMMG conditions most likely due to the increases in membrane fluidity and downregulation of relevant heat stress genes.

Gravity-dependent physical alterations influence metabolic pathways, gene expression, and genetics, affecting physiology and phenotype of microbes. In the last two decades, research groups are focusing on the gene expression changes (up- or down-regulation) in response to real or simulated microgravity. Microarray analysis revealed differential expression of about 100 genes mostly involved in transcriptional regulation, virulence, lipopolysaccharide synthesis, and iron-utilization in *Salmonella* under simulated microgravity (Wilson et al., 2002). Global microarray and proteomic analyses of *Salmonella* grown aboard space shuttle mission (STS-115) showed that 167 transcripts and 73 proteins changed expression with the RNA-binding protein Hfq as a global transcriptional regulator involved in the microbial response to spaceflight conditions (Wilson et al., 2007). Crabbé et al., (2011) also found Hfq as a global transcriptional regulator, while investigating the global transcriptional and proteomic responses of spaceflight-induced *P. aeruginosa*. They reported differential regulation of 167 genes and 28 proteins and found transcriptional response data similar to cells grown in microgravity analog conditions using the RWV bioreactor. Among others, genes for oxygen metabolism, virulence-related (the *lecA* and *lecB* lectin) genes, and the gene for rhamnosyltransferase (*rhlA*) involved in rhamnolipid production were induced in spaceflight. Recently, Orsini et al., (2017) investigated the effects of simulated microgravity on *S. mutans* and reported up-regulation of 153 genes and down-regulation of 94 genes (both ≥2-fold). Genes involved in carbohydrate metabolism, translation, and stress responses, as well as genes of extrachromosomal elements, were affected. When a waterborne pathogen *Mycobacterium marinum* was grown under LSMMG in high aspect ratio vessels (HARVs) using a rotary cell culture system or the control orientation (normal gravity), it transitioned from exponential phase growth earlier than the normal gravity culture. LSMMG significantly altered transcript levels and induced a reduction in translation, a downregulation of metabolism, an increase in lipid degradation, and increased chaperone and mycobactin expression. In summary, these results suggest that LSMMG may simulate a nutrient-deprived environment (Abshire et al., 2016) as would be expected in a diffusion-limited environment as previously discussed. To understand how the microbial transcriptome responds to the environment of spaceflight, the model Gram-positive bacterium *B. subtilis* was flown on two separate missions to the ISS (Morrison et al., 2019). Cells were grown to late-exponential/early stationary phase, frozen, and then returned to Earth for RNA-seq analysis in parallel with matched ground control samples. The results indicated differences in oxygen availability between flight and ground control samples, likely due to diffusion limitation in microgravity, and to differences in cell sedimentation.

The global transcriptional profiling of the human opportunistic fungal pathogen *C. albicans* revealed that cells subjected to short-term spaceflight differentially regulated 452 genes compared to synchronous ground controls (Crabbé et al., 2013). Among others, genes involved in cell aggregation (similar to flocculation), antifungal agent resistance, stress tolerance, ABC transportation, oxidative stress resistance, transcriptional regulation, actin cytoskeleton, and ergosterol-encoding genes were observed to be differentially expressed. Enhanced random budding was observed in spaceflight-cultured cells compared to bipolar budding for ground samples. Although microgravity induced a virulence-related phenotype, no increased virulence in a murine intraperitoneal infection model was observed. This study supported the finding that physical forces in the human body, such as low fluid-shear, play a role in the infection process, advancing our understanding of infectious diseases on Earth to develop novel treatment strategies. A study on the metabolic and phenotypic changes of the yeast *S. cerevisiae* strains under microgravity revealed the upregulation of proteins related to anaerobic conditions and alternation in budding pattern with a switch towards more random budding (van Mulders et al., 2011).

Spaceflight and ground-based studies have shown that the stress environment of space (microgravity) can also affect the transfer of gene fragments or even the whole chromosome between cells. Boever et al., (2007) reported the efficient transfer of genes via plasmid-mediated conjugation in Gram-positive bacterial strain (*B. thuringiensis*) in spaceflight compared to ground control. In contrast, no differences in gene transfer were noticed between spaceflight and ground control Gram-negative species *E. coli* and *Cupriavidus metallidurans*. Another study also showed HGT of small or large plasmids in members of *Bacillus* genera (Timmery et al., 2011), suggesting that such a process could contribute to the spread of virulence potential of *Bacillus* in confined environments. However, Beuls et al., (2009) reported no significant difference in the conjugative and mobilizable transfer frequencies in

B. thuringiensis strains between 1 *g* and the three different simulated microgravity conditions established using the RWV, the RPM, and a superconducting magnet. The spread and increase of antibiotic resistance and bacterial virulence in spaceflight could be linked to efficient gene transfer events (Senatore et al., 2018; Timmery et al., 2011; Tirumalai et al., 2019). Horneck et al., (2010) suggested the need to investigate microbial adaptation and evolution via transferable genetic elements, like phages, extrachromosomal DNA, and transposons in the space environment. On the other hand, it is shown that space environmental conditions can influence the frequency of mutation in microbial cells. Short-term spaceflights experiments have shown no effect on the mutation rate or repair activity in *E. coli* (Harada et al., 1997) and S. cerevisiae (Pross and Kiefer, 1999) but a long-term experiment has shown a pronounced effect on mutation frequency (Fukuda et al., 2000). After 40 days aboard the Russian space station MIR, the mutation rate for a cloned bacterial gene was found to be 2- to 3-fold higher than the ground control (Fukuda et al., 2000). Alternation in frequency and spectrum of spontaneous mutation has also been reported in *Staphylococcus epidermidis* and *B. subtilis* cells flown aboard the ISS. Compared to ground controls the mutation rate of the *rpoB* gene leading to rifampicin resistance was noted to be significantly higher in the spaceflight samples (Fajardo-Cavazos et al., 2018; Fajardo-Cavazos and Nicholson, 2016). These findings suggest that space environment induces stresses on bacterial cells causing changes in mutagenic potential and gene transfer events.

The exploration of the unique environment of outer space (microgravity and radiation) to improve microbial strains for the production of biotechnology-relevant secondary metabolites gained interest because space environment favored microbial growth (shortened lag phase, faster growth rate, prolonged exponential phase, higher cell density, and proliferation in presence of antibiotic concentrations), promoted efficient DNA exchange, and elicited a wide spectrum of metabolic responses. Due to the high potential of the space environment to mold organisms with desired traits, Horneck et al., (2010) proposed the commercial application of space for the development of new pharmaceuticals, which would enhance our understanding of how bioprocesses occur under the absence of the ever-present gravity to design schemes and approaches to develop novel compounds. Improvement of the production of secondary metabolites, such as actinomycin D (Lam et al., 2002), monorden (Lam et al., 1998), and kanglemycin C (an immunosuppressant) (Zhou et al., 2006) by space cultured *Streptomyces plicatus*, *Humicola fuscoatra* WC5157, and *Nocardia mediterranei* var. *kanglensis*, respectively, have been reported. Recently, Huang et al., (2015) investigated the effects of microgravity on antibiotic production by *Streptomyces coelicolor* A3 and found that microgravity conditions altered secondary metabolism and resulted in the production of more bioactive substances highly active against *B. subtilis.* Several groups have also studied the effect of simulated microgravity using LSMMG. However, ground-based facilities do not produce results as observed in spaceflight experiments. Experiments to test the production of antibiotics using the RWV, which partially simulate the weightless environment, resulted in inhibition of antibiotics, such as the microcin B17 by *E. coli* (Fang et al., 1997a), β-lactam by *Streptomyces clavuligerus* (Fang et al., 1997c), and rapamycin by *S. hygroscopicus* (Fang et al., 2000). In addition, no effect of simulated gravity was noticed in the case of gramicidin-S production by *B. brevis* (Fang et al., 1997b). The response of these organisms to actual spaceflight experiments to produce antibiotics are not performed to draw conclusive results on actual microgravity effects. In addition, pilot experiments have been conducted to develop new vaccines against diarrhea-causing strains of *Salmonella* aboard the ISS (Horneck et al., 2010). Taken together, these studies suggest that space microgravity environment can alter secondary metabolism in microbes, suggesting potential production, development, and commercial application of compounds, such as antibiotics.

5.1. Conclusions from observed effects in microbes

Majority of the studies conducted till date indicate that microgravity has a strong influence on microbial growth and behavior and could lead to the evolution of properties, which does not occur on Earth, such as a column-and-canopy structure of bacterial biofilms and attainment of a growth stage that facilitates circumvention of the immune system. The mechanisms responsible for the observed biological responses, however, are not yet fully understood. We are, however, just beginning to scratch the surface in unraveling the molecular and genetic mechanisms by which gravity influences microbial growth and behavior. The major influence of a lack of gravity is a direct effect of the environment surrounding the cell simply because there is no convection only diffusion. This suggests that in

microgravity the observed changes in microbial growth and behavior are primarily indirect. Future studies focused on studying microbial behavior (e.g., biofilm production, antibiotic resistance, etc.) in conjunction with genetic studies on the regulation of the genes known to be involved with a particular behavior will lead to a clear understanding of the role gravity plays in microbial growth, development, and behavior. Studies on the interactions of both harmful and beneficial microbes with humans under altered gravity conditions need to be emphasized. Experiments with recent molecular tools are likely going to advance our knowledge and understanding and provide an intriguing foundation for the exploration of the space environment to develop novel products.

6. Microgravity and cancer research

Spaceflight affects human physiology in both a positive and a negative direction. In addition to the variety of the observed negative biological effects on the human body, caused by a long-term spaceflight (reduction in bone density, loss of muscle mass, diminished cardiac function, distorted balance, impaired vision and others (White and Averner, 2001), several studies indicate that microgravity may have an anticancer effect (Krüger et al., 2019c). The low-gravity environment significantly affects key cellular processes, including gene transcription and intercellular contacts (Hughes-Fulford et al., 2015; Lewis et al., 1998; Martinez et al., 2015) and thus influences cell growth, cell cycle, self-renewal (Arun et al., 2019) and differentiation of cancer cells without the need for drugs (Chen et al., 2019; Jessup et al., 2000; Vidyasekar et al., 2015). Microgravity slows proliferation and elevates apoptosis of different cancer cell types derived from lymphoma (Kim et al., 2017), glioma (Zhao et al., 2017), osteosarcoma (Wei et al., 2013), and different carcinomas (Chang et al., 2013; Grimm et al., 2002; Kopp et al., 2018; Kossmehl et al., 2002; Kossmehl et al., 2003; Masiello et al., 2014; Qian et al., 2008). Cytoskeletal alterations occur very early in cancer cells (Corydon et al., 2016a; Nassef et al., 2019) and are often suggested to be an effect of gravitational unloading (Vorselen et al., 2014). The impaired cytoskeleton of cancer cells in microgravity is assumed to be attributed to cell death (Arun et al., 2017; Vidyasekar et al., 2015). Qian et al., (2008) reported that simulated-microgravity affects cell migration and adhesion of breast cancer cells. Furthermore, microgravity has been reported to cause stress signaling due to mechanical forces of tensegrity (Ingber, 1999; Ingber, 2014), cellular oxidative and endoplasmic reticulum stresses (Morabito et al., 2017). Hodgkin's lymphoma cells exposed to microgravity showed dysfunctional mitochondria leading to reduced ATP levels and autophagy (Jeong et al., 2018), which was found elevated in various cell types after annulling gravity (Sambandam et al., 2014). Autophagy is involved in stemness regulation of human stem cells and contributes to the pluripotency of cancer stem cells (Kenific et al., 2016; Sharif et al., 2017). Experiments using simulated microgravity produced by an RPM (Kopp et al., 2015) as well as space missions, such as SimBox/Shenzhou-8 (Ma et al., 2013) or CellBox-2 (Melnik et al., 2018), indicated that thyroid carcinoma cells are considerably less aggressive in microgravity than on Earth. Prostate cancer cells were also found to be less aggressive and showed changes in lipid second messengers and signal transduction after exposure to simulated microgravity using the NASA-developed High Aspect Ratio Vessel (HARV) (Clejan et al., 1996; Clejan et al., 2001). This finding will now be evaluated in real microgravity. In addition, breast, prostate, and thyroid carcinoma cells form unique morphological features when exposed to simulated microgravity (Fig. 7A) - a further sign of differentiation (Corydon et al., 2016b; Kopp et al., 2015; Lelkes et al., 1998).

However, most mechanisms by which microgravity induces these cellular responses are still poorly understood and will be a subject of future research in space medicine. In this regard, microgravity-based research provides a very special culture environment to detect changes in proteins representing new targets for drug development in cancer. Many of these proteins and available drugs were found by analyzing multiple pilot studies with the help of semantic analyses (Bauer et al., 2017; Bauer et al., 2018). Using these results, scientists will be able to target or silence individual genes in cancer cells with pharmacological substances to inhibit selected signaling pathways. This way, they may be able to elucidate cellular changes in cancer development. The first success was achieved with MCF-7 breast cancer cells: metastasis-like spheroid formation was prevented by c-Src inhibition but promoted by blocking E-cadherin activity (Sahana et al., 2018). After annulling gravity, cells can arrange themselves scaffold-free into large three-dimensional (3D) aggregates without any signs of necrosis (Fig. 7B) (Ingram et al., 1997; Kopp et al., 2015).

The extracellular matrix of these multicellular tumor spheroids comprises a complex 3D network of cell-cell and cell-matrix interactions that differs in amount and composition from corresponding monolayer cultures (Ulbrich et al., 2011). It affects the distribution and function of hormones and growth factors and thus fundamentally determines the regulation of cell growth, differentiation, and apoptosis (Kunz-Schughart, 1999). The model system 'Multicellular Spheroid' (MCS) mimics metastasis of solid tumors and delivers valuable information about cancer progression *in vivo* and is often used for pharmacological drug testing (Braddock, 2019; Zanoni et al., 2019). The special 3D architecture of tumor spheroids might facilitate the penetration and action of drugs more closely mimicking the situation of a tumor in the human body. In addition, the tumor spheroids and the more complex cancer organoids that were grown from breast tumor (Kaur et al., 2011; Kunz-Schughart et al., 2001) or prostate tumor cells (Zhau et al., 1997) together with fibroblasts have become important *ex vivo* models to study cancer key processes like neoangiogenesis, tumor cell invasion, and to investigate tumor-microenvironment and cell-cell-interactions (Hamilton, 1998; Kunz-Schughart, 1999; Kunz-Schughart et al., 2004).

Microgravity, in space or produced by special devices on Earth, provides a unique environment for cancer research that has allowed insights in cancer biology that would not have been possible under normal cell culture conditions. The results already influence conventional cancer research and contribute to the understanding of cancer progression and the development of new drugs and cancer treatment strategies.

Upcoming projects will examine the roles of both microgravity and cosmic radiation in tumor growth and development onboard the ISS and the Chinese Space Station (CSS). The studies of cosmic radiation, the main concern for space exploration by humans, will also help with understanding the cancer risk for astronauts on long-duration stays on space stations, or longer journeys, such as to Mars.

7. Tissue engineering in space

Microgravity-based scaffold-free tissue engineering is a novel technique in translational regenerative medicine. For this purpose, the cells are exposed to microgravity in space or to devices simulating microgravity conditions on Earth, so-called ground-based facilities (GBF), acknowledged among others by ESA (European Space Agency) and NASA. A large number of studies performed in real and simulated microgravity revealed that one part of the cultured cells of various cell types detaches from the bottom of the culture dish and starts forming 3D aggregates or MCS (Grimm et al., 2018), while another part remains growing as a 2D monolayer on the bottom of the culture flask. These MCS resemble the organs or original tissues from which the cells originally derived. In this section, we review the current knowledge about tissue engineering of cartilage, bone, and tube formation by endothelial cells in space and on ESA-GBF.

7.1. Cartilage tissue engineering

Diseases of the cartilage comprise osteoarthritis, a frequent cause of chronic pain, and a disorder with increased prevalence worldwide due to the aging population. Unfortunately, no causal treatment is available, and the major objective is to treat the pain and to attenuate the disease progression. Therefore, new research focusing on the chondrocytes is mandatory and new ideas are welcome. Microgravity-based approaches of cartilage tissue engineering have been performed in space on the MIR space station. Bovine chondrocytes were grown in 3D polyglycolic acid matrices at 1 *g* and cultured in rotating bioreactors for three months. One of these reactors was subsequently sent to space (MIR) where the cell culture continued to grow for another four months. In parallel, the remaining samples were kept as ground controls. Both culture conditions resulted in functional cartilage tissues, but the cartilage sample cultured in space revealed a lower size and mechanical strength (Freed et al., 1997; Freed et al., 1999; Freed and Vunjak-Novakovic, 1997).

Other researchers performed a scaffold-free experiment with porcine chondrocytes, which were cultured in special cylindrical culture chambers. The cells were exposed to real microgravity on the ISS, to simulated microgravity on an RPM and 1 *g* on Earth. The ISS samples revealed a reduced amount of extracellular matrix proteins together with a reduced cell density compared to the 1 *g* controls (Stamenković et al., 2010). Various experiments with chondrocytes have been performed to engineer cartilage constructs using ESA GBF (Aleshcheva et al., 2016). Ohyabu et al., (2006) demonstrated that

rabbit bone marrow mesenchymal stem cells cultured in an RWV bioreactor formed large cylindrical cartilaginous pieces without scaffolds. Another interesting approach was the production of hyaline cartilage using a RWV (Sakai et al., 2009). Human mesenchymal stem cells (MSC) exposed to simulated microgravity revealed an increased proliferation, and among others, a higher expression of collagen II compared to 1 *g*-samples (Yuge et al., 2006). Only the simulated-microgravity samples were able to form hyaline cartilage after transplantation in cartilage defective mice (Yuge et al., 2006).

Taken together, cartilage tissue engineering with and without scaffolds is an elegant technique to produce viable, functional cartilage constructs (Fig. 7C) (Grimm et al., 2018). Tissue engineering of comparable constructs is not possible with conventional static 1*g*-culture conditions. Furthermore, future research should increase scaffold-free approaches in space accompanied by GBF studies, avoiding potential pitfalls associated with non-autologous biomaterials. A more personalized approach should be introduced by studying patient's chondrocytes after, for example, an injury to engineer autologous cartilage.

7.2. Tissue engineering of bone

Microgravity-induced osteopenia and bone loss is a serious health problem for long-term space missions (Grimm et al., 2016). Astronauts lose between 1 and 2% of weight-bearing bone mass during long-term missions (Cappellesso et al., 2015). These changes in bone structure and metabolism are still an important health concern of humans in space today (Chatani et al., 2015; Grimm et al., 2016; Vico et al., 2000). Despite this knowledge, it is of interest that simulated microgravity also induces bone formation. Bone tissue engineering using microgravity-based methods is currently a hot topic in regenerative medicine. The incidence of bone diseases is increasing because of aging populations, obesity, and reduced physical activity (Amini et al., 2012). The destruction of bone tissue is often a result of trauma or disease. The use of stem cells, specialized cells, scaffolds, and growth factors to improve bone formation are discussed as possible therapeutic strategies. Tissue-engineered bone fragments from human osteoblasts might serve as a potential alternative to the conventional use of bone grafts.

Various research teams used the NASA-developed RWV in bone tissue engineering studies. These RWV-derived bone constructs from osteoblasts and stem cells were comparable to natural bone and had demonstrated an improved *in vivo* effectiveness in bone repair in animal studies (Cerwinka et al., 2012; Cui et al., 2018; Han and Dai, 2016; Jin et al., 2010; Sikavitsas et al., 2002; Ulbrich et al., 2014). Clarke et al., (2013) had developed a new method for tissue engineering of bone. The authors used successfully a low shear stress rotating bioreactor, such as a HARV culture system. Mann et al., (2019) exposed human fetal osteoblast (hFOB 1.19) cells to an RPM for 7 and 14 days. This new study was the first that demonstrated a scaffold-free approach to engineer 3D bone constructs on the RPM (Fig. 7D). The next step will be to investigate these constructs in an *in vivo* animal model.

Taken together, new discoveries have been published in the field of microgravity-based bone tissue engineering. Due to the huge impact of musculoskeletal medical conditions on Earth and for future space exploration, further studies on the influence of microgravity on osteoblasts and stem cells are important. This novel technology first can be used to study the mechanisms behind spaceflight-related bone loss and second, the engineered bone constructs might be suitable for the repair of bone diseases such as osteonecrosis or bone injuries.

7.3. Growing blood vessels in space

Tissue engineering of scaffold-free small biocompatible blood vessels, which are applicable for vascular reconstruction purposes in transplantation, plastic, aesthetic, and hand surgery (Fig. 7E), is an important medical research topic. A key benefit of this technique is the scaffold-free engineering of 3D tubes without any foreign materials. Under normal static gravity (1*g*) conditions in Earth-based laboratories, a 2D monolayer of endothelial cells (ECs) is comparable to the inner surface of blood vessels (intima) but does not completely show the vascular characteristics found *in vivo*.

Sanford et al., (2002) cultured bovine aortic ECs in an RWV for up to 30 days. They found large tissue-like aggregates (containing 20 or more beads) after 30 days. This was the first report of a unique growth pattern for ECs, resulting in enhanced NO production and barrier properties. Another group used a perfusion system and developed a cell culture model for a small-diameter vessel engineered from

adipose tissue stromal cells together with human umbilical vein endothelial cells using a tube-like gelatin scaffold (Frerich et al., 2006; Frerich et al., 2008). Human umbilical cord blood stem cells (CBSC) were exposed to an RWV for 14 days (Chiu et al., 2005). CD34+ mononuclear cells were cultured in the bioreactor with or without microcarrier beads (MCB) and vascular endothelial growth factor. RWV cultures showed increased proliferation and 3D tissue-like aggregates. At day 4, CD34+ cells without MCB developed vascular tubular assemblies and exhibited endothelial phenotypic markers. The CD34+ CBSC is capable to differentiate in a vascular endothelial cell phenotype and to form 3D tissue structures when exposed to the RWV (Chiu et al., 2005). Another study focused on MSCs exposed to simulated microgravity for 72 h. The authors demonstrated that MSC was able to differentiate into endothelial-like cells, express endothelial-specific molecules (Flk-1 and vWF), and form a capillary network (Zhang et al., 2013). Using a clinostat, (Shi et al., 2016) showed that clinorotation induced cell migration and tube formation in ECs *in vitro* depending on caveolin-1. A large number of studies of our team investigating ECs exposed to simulated microgravity conditions had demonstrated that ECs can form 3D tube-like structures (intima constructs) (Dittrich et al., 2018; Grimm et al., 2009; Grimm et al., 2010; Infanger et al., 2006; Ma et al., 2013; Ma et al., 2014; Ulbrich et al., 2008). In microgravity, the *de novo* construction of scaffold-free, functional, organo-typic tissues has a great advantage, because ECs grow as spheroids and as 3D tubular structures without requiring any artificial matrix or scaffolds (Grimm et al., 2018). These tubes can be cultured for more than 35 days. Interestingly, they do not develop necrosis or stop growing (Dittrich et al., 2018). Human endothelial cells of the EAhy926 cell line had been sent to the ISS (SpaceX CRS-8 mission) in 2016 to determine the impact of real microgravity on 3D growth (Pietsch et al., 2017). The ESA-SPHEROIDS project confirmed the formation of 3D vessel constructs (Krüger et al., 2019b; Pietsch et al., 2017). Cell culture in space was possible with a specifically designed automatic hardware, which allowed programmed cell feeding and fixation (Pietsch et al., 2017). A recent study reported the impact of clinorotation on endothelial progenitor cells (EPC) (Hagiwara et al., 2018). The gene expression analysis revealed an increase in angiogenic factors, including vascular endothelial growth factor. Moreover, the initial cultivation on a 3D clinostat followed by cultivation under 1*g* remarkably improved the EPC expansion rates and the angiogenic potential (Hagiwara et al., 2018). This interesting approach should be investigated in real microgravity in future space experiments.

In summary, microgravity-based research contributes to new developments in regenerative medicine using stem cells or ECs. Co-culture experiments of ECs with vascular smooth muscle cells and fibroblasts on an RPM will be a further step to engineer a complete, functional vessel. These constructed vessels can be co-cultured with cancer MCS and might be suitable for the use in pharmacological drug testing to reduce the number of animal experiments, or for radiation experiments.

8. Plants and algae in real or simulated microgravity

8.1. Plant-based studies

Same as in animals, genetic programs and networks control plant growth and development. Plants use a variety of stimuli, such as light, temperature, gravity, and water to orient themselves in their natural habitat. Mechano-perception is crucial for the regulation of plant growth and development as well as for its life. Mechanical touch perception enables adaptation to environmental conditions, such as wind. In trees, the weight of a branch induces the increased formation of wood at its onset to overcome the notch stress. Responses to stimuli such as gravity and light are important for the orientation of root and stem growth in plants to anchor and absorb water and minerals as well as to orient their leaves towards sunlight (Kordyum, 2014; Kordyum and Chapman, 2017). These factors are the most stable and reliable signal utilized by plants for their survival throughout evolution. Physiological orientations towards light and gravity are termed as phototropism and gravitropism, respectively. Microgravity experiments allow us to elucidate the mechanisms of gravitropism in plants. A notable number of spaceflight experiments with plants (monocots and eudicots) *in vivo* and *in vitro* (organ, tissue, cell, and protoplast cultures) have been performed on board satellites, space shuttles, and orbital stations. These studies show that gravity and other spaceflight factors influence plants at the organismal, cellular, subcellular, and molecular levels, leading to changes in orientation, response to stress, development, metabolism, and growth. These experiments have contributed to the understanding of gravity perception, signal transduction, intrinsic cell-division processes, adaptation to microgravity, and gravity-induced distribution of organelles in plants and accompanying morphometric traits (Correll et al., 2013; Ferl et

al., 2002; Kiss, 2000; Kordyum and Chapman, 2017; Paul et al., 2012a; Paul et al., 2012b; Stutte et al., 2006).

In earlier investigations of gravity and microgravity, solely the measurement of physiological or anatomical parameters was possible. Currently, an arsenal of molecular biological methods is available to investigate transcription levels, protein expression and modification, or metabolic profiles. Kimbrough et al., (2004) carried out whole-genome microarray analysis of *Arabidopsis* root apices after gravity stimulation (reorientation) and mechanical stimulation to investigate the effect of gravity and mechanical forces on plants. Out of 22,744 monitored genes, 1730 (7.6%) and 1691 (7.5%) genes were found to be altered in transcript abundance in response to gravity or mechanical stimulation, respectively. An increase in transcription factors, genes encoding cell wall modifying enzymes (xyloglucan endotransglycosylases, and pectinesterases), transporters (ions, sugar, purines), and genes involved in the response to other environmental stresses (dehydration, cold, and pathogen response) were observed after gravity stimulation. The severe effect of just reversing the position of a polar plant organ towards the gravity vector was demonstrated with the gynophore (a stalk supporting the female part of a flower) of peanut (Li et al., 2013). Some of the gynophores were positioned upside-down, while the others were kept in their normal position and changes in transcriptome and proteins were detected. After 6 and 12 h, more than 170 and 420 genes were found to display altered expression levels, respectively. The proteomic analysis performed via 2D-gel electrophoresis and subsequent MALDI-TOF analysis revealed that especially proteins involved in stress or plant defense as well as proteins of the carbohydrate metabolism were influenced due to reversing the position of gynophores. Several studies have reported the effects of gravity on the transcriptome and proteome of *Arabidopsis* (Hausmann et al., 2014; Martzivanou et al., 2006; Martzivanou and Hampp, 2003). Hyper-gravity of 7 x *g* for 1 h resulted in up- or down-regulation of around 200 genes among 4100 analyzed genes of *A. thaliana* (Martzivanou and Hampp, 2003). Mainly genes of major pathways, genes of proteins involved in cellular organization and cell wall formation, phosphorylation events, and signaling were altered. The fraction of stress response as well as genes involved in gravity-sensing was considerably lower. Significant changes in the expression of various genes in *A. thaliana* due to the effect of subsequent short-term microgravity phases (each about 20 s) achieved during parabolic flight maneuvers have also been reported (Paul et al., 2011). Transcriptome profiling revealed that parabolic flight caused changes in gene expression by prominence of genes associated with signal transduction, stress, and biotic and abiotic stimuli.

Various experiments with *A. thaliana* callus cultures as a test system have been performed under different accelerations and microgravity conditions as well (Babbick et al., 2007; Barjaktarovic et al., 2009; Barjaktarović et al., 2009; Hausmann et al., 2014; Martzivanou and Hampp, 2003). It seems likely that also in plant cell cultures, acceleration or microgravity induces a variety of unspecific effects ranging from changes in transcription level to protein translation and modification as well as changes in the metabolome. Proteome analysis (2D-gel electrophoresis and subsequent ESI-MS/MS) of *Arabidopsis* callus cells following simulated microgravity (RPM) as well as hypergravity revealed that a variety of proteins changed. Largely, proteins involved in radical scavenging and detoxification of reactive oxygen species (ROS), primary metabolism, and signaling were found to be different from controls (Barjaktarović et al., 2009). ROS generation is likely due to the activity of calcium-dependent NADPH oxidases (Hausmann et al., 2014) or linked to auxin signaling (Tan et al., 2011). Transcription levels of ROS-related enzymes, like ascorbate- and glutathione peroxidases, which are involved in ROS homeostasis of cells altered during microgravity (Barjaktarovic et al., 2009; Barjaktarović et al., 2009). Significant changes in protein phosphorylation, mainly in stress proteins are also reported (Barjaktarovic et al., 2009). Calcium-dependent genes also changed in short and long-term microgravity exposure (Hausmann et al., 2014; Paul et al., 2012b; Salmi et al., 2011; Salmi and Roux, 2008).

Wang, (2006) investigated the effect of vertical clinorotation on the proteome of *Arabidopsis* calli (horizontally rotated calli served as a control). Among others, stress response proteins, enzymes of the carbohydrate metabolism (e.g., glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate-isomerase), signaling proteins, and transcription factors were found to be altered. Determination of clinostat effects on the proteome of intact plants (wild type and a *pin2* mutant, which exhibits a gravity-insensitive root growth phenotype) came to a similar result (Tan et al., 2011). Altered gravity conditions significantly elevated the expression of certain classes of proteins, like those involved in detoxification (GST 6, NAD^+-ADH, GS-FDH), in chaperone functions (HSP70) and in mediating resistance to stress factors correlated with energy metabolism (mitochondrial ATP synthase, putative inosine-5'-

monophosphate dehydrogenase, and isocitrate dehydrogenase) in both wild type and *pin2* mutant (Tan et al., 2011).

During a parabolic flight different molecular and physiological parameters were investigated (Neef et al., 2015). During microgravity, ROS molecules and cytosolic calcium increased in *A. thaliana* cell cultures (Ca^{2+}-sensor YC3.6-cells). Phosphorylation analysis revealed that in hyper-*g*, superoxide dismutase, ascorbate peroxidase, glycerol kinase, phosphoglyceromutase, annexin, a microtubule-associated protein and ATP-dependent NAD(P)H hydrate hydratase, serine/threonine phosphatase (protein regulation), UDP glucose dehydrogenase, V-Type proton ATPase, Hsp70, and malate-dehydrogenase genes were influenced. Under microgravity, phosphorylation of triose-P isomerase, inorganic pyrophosphatase, pyruvate dehydrogenase, citrate synthase, Hsp70, CML14 (Ca^{2+}-binding protein), phosphoenolpyruvate carboxykinase, fructokinase, and dehydrogenase ferritin-2 were found to be altered compared to the control. While only a few proteins changed by varying gravitational conditions, the effect on gene expression (measured with Affymetrix GeneChip expression analysis) was much more pronounced. Gene expression in hyper-*g* and microgravity showed a strong overlap in 1378 up- and 1769 down-regulated genes. In addition, many genes were exclusively altered in microgravity (396 up and 485 down) or hyper-*g* (413 up and 637 down directly at the onset of 1.8*g*). Genes involved included calcium-binding, signaling (calcium Ins(1,4,5,)P3), stress response, and detoxification (e.g., catalase). Microarray analysis of *A. thaliana* calli cultures subjected to magnetic levitation, which "simulates" different gravity levels (microgravity, 0.1 *g*, 1 *g*, and hyper-*g*) has revealed changes in stress and metabolic genes among others in high gradient magnetic fields (Manzano et al., 2012). Also, Paul et al., (2013) found significant stress responses (effects on heat shock proteins and chaperons) of *Arabidopsis* tissue cultures in space.

Correll et al., (2013) investigated the transcriptome of *A. thaliana* seedlings in space (TROPI-2 experiment on the ISS) and found that the transcription of 982 genes differed from ground controls. Among these genes, 135 and 143 were noted to be up- and down-regulated at least two-fold, respectively. Next to many undefined genes, a large number of genes involved in salt stress, oxidative stress, detoxification, biotic-induced stress, water stress, and defense response (and other stress responses) were among the differentially expressed ones. In addition, changes in the actin cytoskeleton organization that among other functions is involved in the transport of cell wall components to the cell periphery on exposure to microgravity were noted. In a spaceflight experiment (BRIC-16 on STS-131, 2010), Paul et al., (2012b) analyzed and compared the transcriptome of *A. thaliana* seedlings and cell cultures. While the seedlings reacted moderately over a variety of functions, the cell cultures reacted mainly with the transcription of stress response and heat shock proteins. For instance, the expression of the heat shock transcription factor HSFA2 or the heat shock protein Hsp70 was noted to be 20-fold higher in cell cultures. This shows that even undifferentiated plant cells perceive the absence of gravity as stress but do so in a more general sense than plant seedlings, which have specialized organs to sense their environment and react. In seedlings, among others, genes involved in pathogen response and wounding, stress response (cold and drought stress), auxin-mediated root development, and calcium signaling were also found to be different compared to the ground control. During a long-term experiment onboard the ISS, Paul et al., (2013) investigated the seedlings of *A. thaliana* and found 1480 genes to be differentially expressed from 1*g* controls (centrifuge). Also, gene expression was found to be organ-specific. About 181 genes were found to be exclusively altered in roots, only 114 in the hypocotyl, and only 113 in the leaves. Leaves, hypocotyl, and roots shared only a few genes, which were altered in all organs (26 of 408). Differentially expressed genes codes for proteins of touch-wounding response, cell wall remodeling, growth hormone, and signal transduction. Perhaps altered hormone-signaling affect the cell wall remodeling genes, influencing the growth and cell expansion (Schüler et al., 2015).

Changes in transcription in plants after transfer to microgravity are not surprising considering the report of Millar and Kiss, (2013), where, transcription and metabolomics during tropistic responses were investigated. In this study, seedlings were irradiated unidirectional with red or blue light to measure phototropic responses and tilted towards the gravity vector to induce gravitropic responses. In all tropisms, genes for carbohydrate and secondary metabolism were found to be downregulated, while different genes involved in amino acid metabolism were found to be upregulated. Changes in gene expression were highest in gravitropism compared to different phototropisms. Especially genes coding for proteins of subcellular localization, proteins with binding function, defense genes, and metabolic genes were more profoundly altered during gravitropism. It was found that various metabolites, which are typical for biotic and abiotic stress, such as asparagine, phenylalanine (interaction between ethylene

and auxin), threonine, sucrose (correlation to osmosis), etc. were increased upon all tropistic reactions. In a very recent study, Vandenbrink et al., (2019) reported down-regulation of light-associated pathways (e.g., photosynthesis-antenna proteins, photosynthesis, porphyrin, and chlorophyll metabolism) in *A. thaliana* grown in a microgravity environment and irradiated with a blue light onboard the ISS compared to 1 *g* control. In contrast, pathways involved in ribosome biosynthesis, which has been associated with elements controlling cell growth and proliferation (Kamal et al., 2018), and oxidative phosphorylation were upregulated. The authors suggest a connection between gravity and phototropism in *Arabidopsis*.

In other investigated plants, acceleration changes led to changes in protein patterns or transcription profiles as well. The transcription profiles of spores of the fern *Ceratopteris richardii* germinating in microgravity and 1 *g* analyzed during the NASA space shuttle mission STS-93 revealed changes in the mRNA expression of roughly 5% of genes analyzed (Salmi and Roux, 2008). Various genes were noted to be altered, e.g., transferases, hydrolases, and heat shock proteins. Bushart et al., (2013) also identified potential modulators (calcium pumps and apyrases) for gravity response in *Ceratopteris*. Jin et al., (2015) analyzed rice calli grown on the Chinese satellite Shenzhou-8. In comparison to 1 *g* ground and 1 *g* inflight controls, they found 627 microgravity responsive transcripts, mostly involved in the cell wall structure, primary metabolism, transcription, calcium regulation, and others. Stutte et al., (2006) investigated the effect of microgravity on dwarf wheat (leaf ultrastructure, biochemical, physiological, and transcription levels) grown in real microgravity (located in a Biomass Production System (BPS)-device) during a space shuttle flight and subsequent installation on the ISS. Interestingly only marginal changes in leaf ultrastructure (e.g., thinner cross section and higher cell density) and chloroplast (more ovoid shape, slightly different thylakoid structure-no effect of photosynthetic performance) compared to a 1 *g*-ground control were detected (Stutte et al., 2006). Transcription was not significantly impaired. Xu and Guo (Xu and Guo, 2013) investigated RPM-effects on six conserved miRNAs in *Solanum lycopersicum*. After 30 days of RPM, the expression level in six investigated miRNAs was found to be increased after transient reduced expression before. Putative targeted proteins of this miRNA are involved in physiological and metabolic processes (protein kinases, oxidoreductase), transcription factors, and others. Real microgravity resulted in the detection of 19 differentially expressed proteins in *Oryza sativa* investigated using 2D-gel electrophoresis and mass spectrometry (Ma et al., 2007). Four of these proteins could be identified: hydrogen peroxide oxidase, ascorbate peroxidase, a manganese-stabilizing protein, and a ribosomal protein.

Recent studies have indicated that epigenetic mechanisms (DNA methylation and histone modification) control gene expression in plants in gravitational alterations indicating that gravitational changes are sensed as stress by plants (Kamal et al., 2018; Ou et al., 2010; Xu et al., 2018; Zhou et al., 2019). The regulation of genes expression via epigenetic modification affects plant responses to environmental stresses as discussed before. Kamal et al., (2018) performed experiments with cell cultures of *Arabidopsis* under simulated micro- and Martian-gravity (RPM-treatment) as well as under hypergravity (centrifugation) and observed that samples exposed to artificial microgravity and a lower extend cells exposed to Martian-gravity showed an increased DNA methylation level compared to 1 g and hypergravity conditions. Also, this study revealed effects of simulated microgravity or simulated Martian gravity on the cell cycle (lower fraction of cells in G1-phase), nucleolus structure (decrease of cells showing compact nucleolus structure and increase of fibrillary structure) as well as changes in levels of proteins involved in cell cycle regulation. Recently, Xu et al., (2018) investigated the methylation pattern changes in *Arabidopsis* seedlings exposed to real microgravity conditions onboard the Chinese recoverable scientific satellite SJ-10. They found widespread methylation of genes involved in metabolic pathways, biosynthesis of secondary metabolites, ribosome pathway, starch and sucrose metabolism, phenylalanine biosynthesis, purine, glutathione, amino sugars, and nucleotide sugar metabolism), proteasomes, nucleotide excision repair, and ribosomes among others. Ou et al., (2010) found significant changes in genetics, as well as DNA methylation frequencies (hyper- and hypo-methylation), in rice plants (*O. sativa* L.) germinated from seeds kept in space for 18 days. Among the assessed 460 genomic loci, the genetic change frequencies ranged from 0.7% to 6.7%, while hypermethylation frequencies were about 1.95% for CG and 1.44% for CNG. The hypomethylation frequencies were noted to be 0.76% (CG) and 0.80% (CNG). In a very recent whole-genome survey of DNA methylation, Zhou et al., (2019) found differentially methylated genes in microgravity-exposed *Arabidopsis* (ISS).

Chinese space researchers combined space, biological, and plant-breeding technologies for screening and identifying diverse new varieties of plants through a space mutation breeding program

carried out from 1987 to 2005 (Chengzhi, 2011; He et al., 2006; Xianfang et al., 2004). They pointed out that the space environment may result in genetic mutagenesis of seeds or seedlings and thus open the route to develop new varieties of plants. He et al., (2006) summarized the progress of this space mutation breeding program and reported that Chinese scientists and agricultural biologists sent more than 1000 varieties of different plant seeds (crops, floricultural plants, vegetables, fruits, medicinal plants, and forest trees) into space. The space-exposed seeds grown on Earth showed positive physiological and genetic variations. Through this 'space breeding' program, Chinese research institutes (70 involved) identified more than 200 promising new varieties of plants exhibiting high quality, high yield, and disease resistance properties. Many of the space-breed plant varieties are cultivated in large scales in some provinces in China (extended up to 270000 hectares) and are bringing social-economic benefits (Xianfang et al., 2004).

Based on several years of studies on plant systems under different acceleration and microgravity conditions it is evident that gravity affects several mechanisms in plants. Plant systems develop or activate a range of molecular responses to raise their defenses upon exposure to gravity-related conditions. These conditions change directly or indirectly the information in the genome, which influence the transcription process and eventually result in the production of changed proteins. Gene activity is affected by non-genetic mechanisms, and the level of stress-induced transcripts alters. In addition, certain repressive RNA transcripts activate and signaling molecules vital for cell survival and adaptation accumulate. The combination of all these responses changes the global systemic organization of plants for the potential recovery, development, and growth under gravity-related conditions. For instance, *A. thaliana* germinated in space but plants grew smaller and roots showed different growth patterns (Millar et al., 2011; Paul et al., 2012a; Paul et al., 2012b). Altered acceleration leads to changes in gene expression which is associated with specific responses in whole plants, seedlings, and plant cell cultures, and differs between different plant organs (Paul et al., 2012a; Paul et al., 2012b; Paul et al., 2013) but share some common gene changes. For example, genes involved in root hair development and generation, cell wall remodeling, cell wall modifying enzymes, actin cytoskeleton association, metabolism and response to ROS, calcium signaling, and general stress responses (Correll et al., 2013; Fengler et al., 2015; Fengler et al., 2016; Hausmann et al., 2014; Mazars et al., 2014a; Mazars et al., 2014b; Paul et al., 2005; Paul et al., 2011; Paul et al., 2013; Schüler et al., 2015; Schüler et al., 2016; Zhang et al., 2015; Zhang and Zheng, 2015). The involvement of the second messengers ROS, Ca^{2+}, and heat shock proteins, as well as distinct changes in the transcriptome, indicates that microgravity is a significant stressor for plants. In summary, whole plants seem to be less affected by microgravity-induced physical phenomena than cell cultures because (i) the cells possess a high internal pressure (turgor) caused by the osmotic pressure of the huge internal vacuole which pushes the cytoplasm against a rigid cell wall and (ii) because of intracellular cytoplasma-streaming, which mixes the cytoplasm also in the absence of microgravity.

8.2. Algae-based studies

Intensive gravitational biology work over many years has made green macroalgae *Chara* a well-established model system for studies on polarized growth and gravity-sensing in plants (Braun et al., 1999; Braun et al., 2004; Braun and Limbach, 2006; Braun and Richter, 1999; Braun and Sievers, 1994). Microgravity experiments during a parabolic flight campaign revealed that the physical contact to a hypothetical receptor protein is responsible for signal transduction and not the force applied by the statoliths on the lower membrane (Limbach et al., 2005). Many studies have been conducted with unicellular photosynthetic organisms. The single cellular green algae *C. reinhardii* shows precise gravitactic behavior. Although non-gravitactic mutants exist (Yoshimura et al., 2003), it is still controversially discussed, whether this phenomenon is based on active physiological mechanisms or solely on physical effects such as buoyancy or bottom-heaviness (Häder et al., 2006b; Häder et al., 2006a). Mathematic models and calculations support physical mechanisms (Hagen et al., 2014). In recent years, the unicellular flagellate *Euglena gracilis* has become a model organism for gravitational research. Its behaviors (phototaxis and gravitaxis, i.e., response to the direction of light and gravity as stimuli) have been extensively investigated during various space experiments (Nasir et al., 2014; Strauch et al., 2010) because *E. gracilis* use physiological mechanisms to sense gravity and light for orientation in its habitat. Several experiments in microgravity helped to develop a working hypothesis of the mechanisms involved in gravitaxis. According to it, mechano-sensitive channels are periodically opened

when the cells deviate from a vertical path upwards (Lebert and Häder, 1996, 1997a, 1997b). This allows for the influx of calcium (Richter et al., 2001; Richter et al., 2002), which in turn activates a calmodulin (Daiker et al., 2010; Häder et al., 2009b). Subsequently, an adenylate cyclase increases the intracellular cAMP levels, thereby activating a specific protein kinase A (PKA) (Daiker et al., 2011; Streb et al., 2002; Tahedl et al., 1998). Curiously, the phototactical signal transduction chain also leads to that PKA (Häder et al., 2005; Häder et al., 2017; Ntefidou et al., 2003). A molecule interacting with PKA was found in the flagellum (Nasir et al., 2018) (Fig. 8).

After a spaceflight aboard the Chinese Satellite Shenzhou-8, *Euglena* cells were analyzed for possible adaptations to microgravity (Nasir et al., 2014). Genes related to oxidative stress or heat shock proteins were upregulated, as well as genes suspected to play a role in gravitaxis, like calmodulin, calcium-binding and other signal proteins, and proteins involved in cell cycle control. Of the tested 32 genes, 18 were up-regulated and one down-regulated, while the rest remained unchanged. Recent advances in the sequencing of the nuclear and mitochondrial genome of *Euglena* (Ebenezer et al., 2019) allowed microarray construction and investigate the transcription pattern in the course of a parabolic flight campaign (Krüger et al., 2019a). The study supported that significant changes in gene expression can be achieved within a very short time. Different signal transduction elements, such as mitogen-activated kinase kinase kinase NPK1, hybrid sensor histidine kinase/response regulator, adenylate guanylate cyclase domain-containing protein, etc., were found to be regulated as well as transport proteins, phosphorylases, primary metabolism, and DNA and protein modification genes. Microgravity might also affect the photosynthesis and perception of light: genes involved in these processes were differently expressed, even though the experiment was performed in darkness (Daiker et al., 2011; Nasir et al., 2018). Some of the altered transcripts showed similarities to those observed in *A. thaliana* (Hausmann et al., 2014; Martzivanou et al., 2006). Taken together, the study contributed to the understanding of the short-term effects of microgravity and different accelerations on gene expression, which can be used as a reference data set for further on-ground and spaceflight-related studies.

8.3. Conclusions from observed effects on plants and algae

In plants and algae, like in animal cells, the current knowledge about gravity perception and the connected signal transduction chains is very limited. However, photosynthetic organisms (plants, plant cell cultures, and algae) and human or animal systems share similar patterns of "disturbed-gravity-induced" changes in certain classes of proteins or gene expression. For instance, signal transduction chains involving calcium are affected in plants and animals alike (Hashemi et al., 1999; Kordyum, 2003; Paul et al., 2012b). Six orthologue genes between *Drosophila melanogaster* and *Caenorhabditis elegans* were found to be differentially expressed (Leandro et al., 2007). Therefore, there appears to be something like a common response to spaceflight. Unfortunately, differences in the experimental setups (duration, hardware, limited numbers of experiments and replicates, quality of microgravity, etc.) make it very difficult to compare results of space experiments and draw a definite interpretation and conclusion. Future space experiments need to be performed under standardized conditions to attain reproducible results and conclusions (Ruyters and Braun, 2014; Wolff et al., 2014). A general database should be developed comprising of genomics, proteomics, metabolomics, and transcriptomics datasets obtained from space experiments and corresponding ground studies of different organisms.

9. Colloids in microgravity

Grape juice and its fermented form, wine, are complex multi-component fluids containing sugars, different alcohols, hundreds of different tannins, or polyphenols prone to various attractive and repulsive interactions (hydrophobic, van der Waals, electrostatic, hydrogen bonding). The tannins are likely to aggregate (haze formation) with time (ageing), thus making the red wine a meta-stable colloidal dispersion: a system of fine particles suspended in a fluid (Zanchi et al., 2008). Other common examples of colloidal dispersion than wine are paints, inks, mayonnaise, ice cream, milk, and biological fluids such as blood. Although these systems are routinely produced, used, and studied, we still have much to learn about the underlying properties of colloidal systems. Hence, understanding and controlling them is a central issue in many technological and scientific fields as it will foster academic and industrial research to fine-tune their physico-chemical structures to put forward new functionalities and products.

The force of gravity may in certain cases impact the colloidal phase behavior as well as dynamical processes. It can, for example, destabilize a dispersion through either the rising toward the liquid/fluid interface of some of the components lighter than the dispersing fluid as in oil-in-water emulsions (creaming) or the sedimentation of the denser ones as silica particles in water. Indeed, at the colloidal level, the so-called gravitational length $l_g = \frac{k_B T}{G}$, where $k_B T$ is the thermal energy source of the Brownian motion and $G = \frac{\pi D^3 \Delta\rho g}{6}$ the buoyant weight (with D the colloidal diameter, $\Delta\rho$ the density mismatch between the colloid and the solvent, and g the gravitational acceleration. It characterizes at what height gravity will start to play an important role in the equilibrium phase behavior (Royall et al., 2005). In a gravitational field, the balance between diffusive forces, pushing the particles up, and gravity, pulling them down, results in hydrostatic equilibrium, the sedimentation profile, where the volume fraction of non-interacting colloids (dilute regime) $\phi = \frac{\pi\rho}{6D^3}$ decays exponentially with height as $\phi(z) = \phi_0 \exp(-z/l_g)$, with a decay length, l_g, historically determined by Perrin in his 1916 seminal work on the measurement of the Boltzman's constant k_B (Perrin, 1916). It follows that if the size of a colloid in the dispersion is somehow below 1µm (with small $\Delta\rho$), its motion will be mainly diffusive with a negligible impact of gravity-driven convection (sedimentation or creaming). A useful (dimensionless) quantity to weigh the influence of gravity in colloidal dynamics is the Péclet number (Pe) that measures the ratio between convective and diffusive transport in a gravitational field (El Masri et al., 2012; Okubo et al., 2000):

$$P_e = \frac{D}{2l_g} = \frac{\pi D^4 \Delta\rho g}{12 k_B T} = \frac{\tau_B}{\tau_S}$$

Where, τ_B and τ_S are the time scales for Brownian diffusion and sedimentation (creaming). When P_e <<1, gravity may be neglected, when P_e >>1 the colloidal dispersion will be strongly affected by gravity. Indeed, for 1µm silica particles in water (~$\Delta\rho$ = 1 g cm^{-3}) the sedimentation rate due to gravity is 100 times larger than Brownian diffusion. By selecting the right particle size and matching the particle-solvent density one can significantly reduce the influence of gravity in laboratory experiments. It is however very difficult to reduce $\Delta\rho$ significantly below 10^{-3} g.cm^{-3} without extreme temperature control (better than 0.01°C) required to reach the condition of microgravity. Furthermore, for optical studies, the fluid and the disperse component should be index-matched which practically excludes that they are density-matched at the same time, something impossible to achieve for all the different colloidal systems used and studied every day all over the world. Colloidal stability is a key issue in various domains ranging from soft-matter, physical chemistry, colloidal and macromolecular science to material science and biotechnology through micro and synthetic biology as it relates to end-user products, drugs.

These systems are often structured by attractive forces between building blocks such as micelles, macromolecules, proteins, globules, nanoparticles in the form of weak, flowable gels. These structures in turn age through coasering where they diffuse to encounter and then compact or merge. When they can no longer support the gravitational stresses, they collapse, sediment, or sink, often abruptly without warning (delayed collapse) with a dynamic much faster than Brownian motion. Consequently, all the motions are biased by hydrodynamics as in the separation of emulsions into layers of different density for example. This pinpoints the difficulties to fully capture and understand the fundamentals of coasering but also crystallization (Okubo et al., 2000; Zhu et al., 1997), aggregation (Veen et al., 2012), gelation (Manley et al., 2004), crystal nucleation and growth, late stage of spinodal decomposition (Bailey et al., 2007), the collapse of tenuous structures, the spatial distribution of aerosols, interfaces, bubbles, and droplets shapes.

Another interesting aspect is the possibility to construct synthetic cells with life-like properties to tackle key societal challenges, such as environmental cleanup, energy production, and drug delivery but also to bring new insights concerning the possible pathways responsible for the origin of life. This bottom-approach aim at producing artificial compartments drawing on a combination of natural biomolecules and synthons to mimic cell structure and functions by incorporating i) cellular extracts containing proteins and nucleic acids required for DNA replication and ii) enzymes to develop biochemical reactions and reactors. To date, two types of compartments have been used to assemble synthetic cell models, namely membrane-bound vesicles and membrane-free micron-sized droplets formed by all-in-water emulsions (ATPS, complex coacervates) (Douliez et al., 2018; Dzieciol and Mann, 2012). The former ensure long-term solute encapsulation and confinement of biomolecules but

the lipid bilayer limits molecular exchange, while the latter allows spontaneous solute uptake but the lack of membrane leads to low confinement times and then support facile solute exchange. Bioreactors capable of synthesizing proteins have been developed using vesicles (Ciancaglini et al., 2012; Noireaux and Libchaber, 2004), while all-in-water emulsions have been suggested as a step toward the production of protocell models (Dzieciol and Mann, 2012). The design of synthetic cells holds great promise but their studies and development are facing the same problems as encountered with a more classical dispersion. The coarsening of the compartments leading to fast sedimentation or macroscopic phase separation if not correctly stabilized, large scale convection taking over the diffusion of small biomolecules, nutriment, and actives.

Real microgravity conditions are then needed to unveil some key physicochemical aspects like the natural movement of colloids that experiments performed on Earth, in the presence of gravity may mask, alter, or simply hinder. Studies in microgravity will open the routes for the pharmaceutical, food, agricultural, and chemical companies to develop a wide range new products (DiFrancesco and Olson, 2015). Since more than 30 years, numerous scientific experiments on colloids in microgravity have been carried out on aircraft (and Space Shuttles) (Lee and Israelsson, 2001; Okubo et al., 1999; Okubo et al., 2000; Sprenger et al., 1993; Tomita et al., 2018; Zhu et al., 1997). If the characteristic time scale of the given experiment is longer (an aging process for example that needs to unfold slowly and evenly) then the unique capabilities of the ISS is an ideal location to study more deeply the various physical, physico-chemical aspects and mechanism(s) at play (*vide supra*) in colloid science in microgravity. In particular, ISS has developed the so-called Light Microscopy Module (LMM), a modified commercial and highly flexible, state-of-the-art light imaging microscope facility that provides researchers with powerful diagnostic hardware and software with the capability of remotely acquiring and downloading digital images and videos across many levels of magnification. This provides a unique environment to observe colloidal self-assembly in 3-D and microgravity for a variety of shapes, sizes, and particle interactions to build understanding and drive colloidal engineering (Bailey et al., 2007; Manley et al., 2004; McPherson and DeLucas, 1999; Safari et al., 2017; Veen et al., 2012).

10. Future perspectives of space research

Life on Earth has always developed within the boundaries of gravity. In space, organisms encounter completely novel conditions, such as weightlessness and radiation. The majority of space experiments until date have revealed that microgravity has a significant influence on organisms' growth and behavior. Future space and gravitational biology research programs should focus on the exploration of the influence of microgravity on living systems at the cellular and molecular levels, as well as on genetic stability, growth, and development of animal and plant systems utilizing advanced scientific methods and tools. The space environment can be explored to develop new products for the benefit of humankind. For instance, we can generate natural crop varieties with higher nutrition and yields and improved features, such as resistance against high and low temperatures, salt stress, microbial and pest attacks, drought, and other stresses. Introduction of plants to artificial stressors in microgravity will activate the expression of dormant genes, change the global systemic organization of plants, and improve their recovery processes in subsequent stresses, like the ability to adapt rapidly to a disease-causing organism. Strains improved in space environmental conditions can be a valuable partner for conventional engineering strategies. Characterization of evolved strains and identification of the important mutations or molecular basis that enabled evolved phenotype or behavior can increase our understanding of organisms' physiology and metabolism as well as uncover the significance of molecular mechanisms contributing to strain fitness and performance. Application of emerging genome and transcriptome sequencing and analysis tools, and advanced metabolic identification and characterization methods are required to interpret the story behind the evolution-based strain improvement approach. These investigations can reveal novel strategies for improving the tolerance of strains to biotic or abiotic factors, increasing utilization of a target substrate for growth and yield, or improve the production of target compounds.

Several infrastructures and space operation capacities are currently present to execute various biological experiments in space. In addition, more advanced technologies are being developed. The current Dragon spacecraft by SpaceX, the Cygnus spacecraft by Orbital ATK, and the new Shepard spacecraft by Blue Origin, and the robust space platforms, such as the ISS and the Russian Earth-orbiting satellites BION and FOTON are available as well as space platforms and spacecrafts from China and

India will be accessible in the near future. Most platforms are currently used for publicly driven space programs, serving and servicing the ISS or far more ambitious space exploration programs such as to the Moon or Mars. ISS offers the most sophisticated research facility in the world. Only highly elaborated ground-based research facilities, such as the Large Hadron Collider at CERN, can compete with the ISS in terms of complexity and operating expense. Differences in experimental setups (viz. duration, hardware, limited numbers of experiments and replicates, and microgravity quality) have limited our understanding of space research data to achieve breakthroughs in biological research. It is therefore required to carry out space experiments under defined and standardized conditions. Factors that need to be emphasized in space research are numbers, time, and on-site monitoring of relevant factors. The required changes cannot be expected to happen often, but by increasing the number of exposed subjects, and by increasing mutational pressure, one can increase the probability that the changes will occur. The longer the subjects are exposed, the higher the chance of change. However, there should be a threshold of exposure time to allow survivability of the biological system. Recent advances in online monitoring of genetic and metabolic changes will allow close tracking of occurring changes. The development of a general database consisting of datasets from different experimental analyses is highly recommended.

In conclusion, space offers unique types of stressors or environmental conditions (i.e., weightlessness and high radiation) that can be used to accelerate evolutionary processes in an organism for the development of a new strain adapted to the challenging environments. Microgravity as an environmental condition never experienced before by any organism on Earth most likely increases mutations rates and influences metabolic and signal transduction pathways in a way that phenotypes may occur, which would not develop under 1*g*. We anticipate that the evolutionary changes in space will be considered fast. Evolution will be based on random processes with positive selection guiding the development of phenotypes with desired properties. Microgravity potentially constitutes a platform to develop valuable strains and bio-products as well as to study more deeply the various physical, physicochemical aspects, and mechanism(s) at play in colloid science. We propose to craft different varieties of organisms in space to meet Earth's demand in the age of ultimate planetary- and human-impacting threats. Something that would definitely help academia and industry to formulate a wide range of new products for food, medicine, pharmaceutics, cosmetics, home-care with longer shelf lives and new functionalities.

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References

Abshire, C.F., Prasai, K., Soto, I., Shi, R., Concha, M., Baddoo, M. et al., 2016. Exposure of *Mycobacterium marinum* to low-shear modeled microgravity: effect on growth, the transcriptome and survival under stress. npj Microgravity 2, 16038. 10.1038/npjmgrav.2016.38.

Acuña, R., Padilla, B.E., Flórez-Ramos, C.P., Rubio, J.D., Herrera, J.C., Benavides, P. et al., 2012. Adaptive horizontal transfer of a bacterial gene to an invasive insect pest of coffee. Proc. Natl. Acad. Sci. U. S. A. 109, 4197–4202. 10.1073/pnas.1121190109.

Aleshcheva, G., Bauer, J., Hemmersbach, R., Egli, M., Wehland, M., Grimm, D., 2016. Tissue Engineering of Cartilage on Ground-Based Facilities. Microgravity Sci. Technol. 28, 237–245. 10.1007/s12217-015-9479-0.

Alsmark, C., Foster, P.G., Sicheritz-Ponten, T., Nakjang, S., Martin Embley, T., Hirt, R.P., 2013. Patterns of prokaryotic lateral gene transfers affecting parasitic microbial eukaryotes. Genome Biol. 14, R19. 10.1186/gb-2013-14-2-r19.

Altenburg, S.D., Nielsen-Preiss, S.M., Hyman, L.E., 2008. Increased Filamentous Growth of *Candida albicans* in Simulated Microgravity. Genomics, Proteomics Bioinformatics 6, 42–50. 10.1016/S1672-0229(08)60019-4.

Amini, A.R., Laurencin, C.T., Nukavarapu, S.P., 2012. Bone tissue engineering: recent advances and challenges. Crit. Rev. Biomed. Eng. 40, 363–408. 10.1615/CritRevBiomedEng.v40.i5.10.

Amiri, H., Karimi, K., 2019. Biobutanol Production. In: Advanced Bioprocessing for Alternative Fuels, Biobased Chemicals, and Bioproducts: Elsevier. p. 109–133.
Anderson, M.T., Seifert, H.S., 2011. Opportunity and means: horizontal gene transfer from the human host to a bacterial pathogen. mBio 2, e00005-11. 10.1128/mBio.00005-11.
Anderson, P.K., Cunningham, A.A., Patel, N.G., Morales, F.J., Epstein, P.R., Daszak, P., 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. Trends Ecol. Evol. 19, 535–544. 10.1016/j.tree.2004.07.021.
Andersson, J.O., 2005. Lateral gene transfer in eukaryotes. Cell Mol. Life Sci. 62, 1182–1197. 10.1007/s00018-005-4539-z.
Andersson, J.O., Sjögren, Å.M., Davis, L.A.M., Embley, T.M., Roger, A.J., 2003. Phylogenetic Analyses of Diplomonad Genes Reveal Frequent Lateral Gene Transfers Affecting Eukaryotes. Current Biol. 13, 94–104. 10.1016/s0960-9822(03)00003-4.
Anway, M.D., Cupp, A.S., Uzumcu, M., Skinner, M.K., 2005. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308, 1466–1469. 10.1126/science.1108190.
Arun, R.P., Sivanesan, D., Patra, B., Varadaraj, S., Verma, R.S., 2019. Simulated microgravity increases polyploid giant cancer cells and nuclear localization of YAP. Sci. Rep. 9, 10684. 10.1038/s41598-019-47116-5.
Arun, R.P., Sivanesan, D., Vidyasekar, P., Verma, R.S., 2017. PTEN/FOXO3/AKT pathway regulates cell death and mediates morphogenetic differentiation of Colorectal Cancer Cells under Simulated Microgravity Sci. Rep. 7, 5952. 10.1038/s41598-017-06416-4.
Arunasri, K., Adil, M., Venu Charan, K., Suvro, C., Himabindu Reddy, S., Shivaji, S., 2013. Effect of simulated microgravity on *E. coli* K12 MG1655 growth and gene expression. PLoS ONE 8, e57860. 10.1371/journal.pone.0057860.
Babbick, M., Dijkstra, C., Larkin, O.J., Anthony, P., Davey, M.R., Power, J.B. et al., 2007. Expression of transcription factors after short-term exposure of *Arabidopsis thaliana* cell cultures to hypergravity and simulated microgravity (2-D/3-D clinorotation, magnetic levitation). Adv. Space Res. 39, 1182–1189. 10.1016/j.asr.2007.01.001.
Backlund, P., Janetos, A., Schimel, D. [Internet]. The Effects of Climate Change on Agriculture, Land Resources, Water Resources, and Biodiversity. Report, 2008. Available from: https://www.fs.fed.us/rm/pubs_other/rmrs_2008_backlund_p003.pdf.
Badyaev, A.V., 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. Proceedings. Biol. Sci. 272, 877–886. 10.1098/rspb.2004.3045.
Baer, C.F., Miyamoto, M.M., Denver, D.R., 2007. Mutation rate variation in multicellular eukaryotes: causes and consequences. Nat. Rev. Genet. 8, 619–631. 10.1038/nrg2158.
Baglioni, P., Sabbatini, M., Horneck, G., 2007. Astrobiology Experiments in Low Earth Orbit: Facilities, Instrumentation, and Results. In: Horneck G, Rettberg P, editors. Complete Course in Astrobiology: Wiley-VCH Verlag GmbH & Co. KGaA. Weinheim, Germany. p. 273–319.
Bai, F., Settles, A.M., 2014. Imprinting in plants as a mechanism to generate seed phenotypic diversity. Frontiers in plant science 5, 780. 10.3389/fpls.2014.00780.
Bailey, A.E., Poon, W.C.K., Christianson, R.J., Schofield, A.B., Gasser, U., Prasad, V. et al., 2007. Spinodal decomposition in a model colloid-polymer mixture in microgravity. Phys Rev Lett 99, 205701. 10.1103/PhysRevLett.99.205701.
Baker, P.W., Meyer, M.L., Leff, L.G., 2004. *Escherichia coli* growth under modeled reduced gravity. Microgravity Sci. Technol. 15, 39–44.
Baltz, R., 2007. Antimicrobials from Actinomycetes: Back to the Future. Microbe 2, 125–131.
Bannister, A.J., Kouzarides, T., 2011. Regulation of chromatin by histone modifications. Cell Res. 21, 381–395. 10.1038/cr.2011.22.
Barjaktarovic, Z., Schütz, W., Madlung, J., Fladerer, C., Nordheim, A., Hampp, R., 2009. Changes in the effective gravitational field strength affect the state of phosphorylation of stress-related proteins in callus cultures of *Arabidopsis thaliana*. J. Exp. Bot. 60, 779–789. 10.1093/jxb/ern324.
Barjaktarović, Ž., Babbick, M., Nordheim, A., Lamkemeyer, T., Magel, E., Hampp, R., 2009. Alterations in Protein Expression of *Arabidopsis thaliana* Cell Cultures During Hyper- and Simulated Micro-Gravity. Microgravity Sci. Technol. 21, 191–196. 10.1007/s12217-008-9058-8.
Barlow, M., 2009. What antimicrobial resistance has taught us about horizontal gene transfer. Methods Mol. Biol. 532, 397–411. 10.1007/978-1-60327-853-9_23.

Bauer, J., Grimm, D., Gombocz, E., 2017. Semantic analysis of thyroid cancer cell proteins obtained from rare research opportunities. J. Biomed. Inform. 76, 138–153. 10.1016/j.jbi.2017.10.011.
Bauer, J., Wehland, M., Infanger, M., Grimm, D., Gombocz, E., 2018. Semantic Analysis of Posttranslational Modification of Proteins Accumulated in Thyroid Cancer Cells Exposed to Simulated Microgravity. Int. J. Mol. Sci. 19. 10.3390/ijms19082257.
Beiko, R.G., Harlow, T.J., Ragan, M.A., 2005. Highways of gene sharing in prokaryotes. Proc. Natl. Acad. Sci. U. S. A. 102, 14332–14337. 10.1073/pnas.0504068102.
Bell, G.J., Martin, T.P., Ilyina-Kakueva, E.I., Oganov, V.S., Edgerton, V.R., 1992. Altered distribution of mitochondria in rat soleus muscle fibers after spaceflight. J. Appl. Physiol. 73, 493–497.
Benoit, M.R., Brown, R.B., Todd, P., Nelson, E.S., Klaus, D.M., 2008. Buoyant plumes from solute gradients generated by non-motile *Escherichia coli*. Phys. Biol. 5, 46007. 10.1088/1478-3975/5/4/046007.
Benoit, M.R., Klaus, D.M., 2007. Microgravity, bacteria, and the influence of motility. Adv. Space Res. 39, 1225–1232. 10.1016/j.asr.2006.10.009.
Beuls, E., van Houdt, R., Leys, N., Dijkstra, C., Larkin, O., Mahillon, J., 2009. *Bacillus thuringiensis* conjugation in simulated microgravity. Astrobiology 9, 797–805. 10.1089/ast.2009.0383.
Bhagwat, A.S., Lieb, M., 2002. Cooperation and competition in mismatch repair: very short-patch repair and methyl-directed mismatch repair in *Escherichia coli*. Mol. Microbiol. 44, 1421–1428. 10.1046/j.1365-2958.2002.02989.x.
Bhattacharyya, M., Martin, C., Smith, A., 1993. The importance of starch biosynthesis in the wrinkled seed shape character of peas studied by Mendel. Plant Mol. Biol. 22, 525–531. 10.1007/BF00015981.
Bijlsma R, Loeschcke V, editors, 1997. Environmental Stress, Adaptation and Evolution Birkhäuser Verl, Basel/Switzerland.
Bilandžija, H., Ma, L., Parkhurst, A., Jeffery, W.R., 2013. A potential benefit of albinism in Astyanax cavefish: downregulation of the oca2 gene increases tyrosine and catecholamine levels as an alternative to melanin synthesis. PLoS ONE 8, e80823. 10.1371/journal.pone.0080823.
Bizzarri, M., Monici, M., van Loon, J.J.W.A., 2015. How microgravity affects the biology of living systems. Biomed Res. Int. 2015, 863075. 10.1155/2015/863075.
Boever, P. de, Mergeay, M., Ilyin, V., Forget-Hanus, D., Van der Auwera, G., Mahillon, J., 2007. Conjugation-mediated plasmid exchange between bacteria grown under space flight conditions. Microgravity Sci. Technol. 19, 138–144. 10.1007/BF02919469.
Boto, L., 2010. Horizontal gene transfer in evolution: facts and challenges. Proc. Biol. Sci. 277, 819–827. 10.1098/rspb.2009.1679.
Boucher, Y., Douady, C.J., Papke, R.T., Walsh, D.A., Boudreau, M.E.R., Nesbø, C.L. et al., 2003. Lateral gene transfer and the origins of prokaryotic groups. Annu. Rev. Genet. 37, 283–328. 10.1146/annurev.genet.37.050503.084247.
Bowler, C., Allen, A.E., Badger, J.H., Grimwood, J., Jabbari, K., Kuo, A. et al., 2008. The *Phaeodactylum* genome reveals the evolutionary history of diatom genomes. Nature 456, 239–244. 10.1038/nature07410.
Boyko, A., Blevins, T., Yao, Y., Golubov, A., Bilichak, A., Ilnytskyy, Y. et al., 2010. Transgenerational Adaptation of *Arabidopsis* to Stress Requires DNA Methylation and the Function of Dicer-Like Proteins. PLoS ONE 5, e9514. 10.1371/journal.pone.0009514.
Braddock, M., 2019. From Target identification to Drug Development in Space: Using the Microgravity Assist. Curr. Drug Discov. Technol. 10.2174/1570163816666190112150014.
Braun, M., Buchen, B., Sievers, A., 1999. Electron microscopic analysis of gravisensing *Chara* rhizoids developed under microgravity conditions. FASEB J. 13, S113-S120.
Braun, M., Hauslage, J., Czogalla, A., Limbach, C., 2004. Tip-localized actin polymerization and remodeling, reflected by the localization of ADF, profilin and villin, are fundamental for gravity-sensing and polar growth in characean rhizoids. Planta 219, 379–388.
Braun, M., Limbach, C., 2006. Rhizoids and protonemata of characean algae: model cells for research on polarized growth and plant gravity sensing. Protoplasma 229, 133–142.
Braun, M., Richter, P., 1999. Relocalization of the calcium gradient and a dihydropyridine receptor is involved in upward bending by bulging of *Chara* protonemata, but not in downward bending by bowing of *Chara* rhizoids. Planta 209, 414–423.

Braun, M., Sievers, A., 1994. Role of the microtubule cytoskeleton in gravisensing *Chara* rhizoids. Eur. J. Cell Biol. 63, 289–298.
Brinckmann, E., 2007. Biology in Space and Life on Earth Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany.
Brown, J.R., 2003. Ancient horizontal gene transfer. Nat. Rev. Genet. 4, 121–132. 10.1038/nrg1000.
Brown, J.R., Doolittle, W.F., 1999. Gene descent, duplication, and horizontal transfer in the evolution of glutamyl- and glutaminyl-tRNA synthetases. J. Mol. Evol. 49, 485–495. 10.1007/PL00006571.
Brown, J.R., Zhang, J., Hodgson, J.E., 1998. A bacterial antibiotic resistance gene with eukaryotic origins. Curr. Biol. 8, R365-S12. 10.1016/s0960-9822(98)70238-6.
Brown, M.E., Antle, J.M., Backlund, P., Carr, E.R., Easterling, W.E., Walsh, M.K. et al. [Internet]. Climate Change, Global Food Security, and the U.S. Food System., 2015. Available from: http://www.usda.gov/oce/climate_change/FoodSecurity2015Assessment/FullAssessment.pdf.
Bruijn, F.J. de, 2016. Stress and Environmental Regulation of Gene Expression and Adaptation in Bacteria. John Wiley & Sons, Inc, Hoboken, NJ, USA.
Burch, C.L., Guyader, S., Samarov, D., Shen, H., 2007. Experimental estimate of the abundance and effects of nearly neutral mutations in the RNA virus phi 6. Genetics 176, 467–476. 10.1534/genetics.106.067199.
Burggren, W., 2016. Epigenetic Inheritance and Its Role in Evolutionary Biology: Re-Evaluation and New Perspectives. Biol. 5. 10.3390/biology5020024.
Bushart, T.J., Cannon, A.E., Ul Haque, A., San Miguel, P., Mostajeran, K., Clark, G.B. et al., 2013. RNA-seq analysis identifies potential modulators of gravity response in spores of *Ceratopteris* (Parkeriaceae): Evidence for modulation by calcium pumps and apyrase activity. Am. J. Bot. 100, 161–174. 10.3732/ajb.1200292.
Calarco, J.P., Borges, F., Donoghue, M.T.A., van Ex, F., Jullien, P.E., Lopes, T. et al., 2012. Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. Cell 151, 194–205. 10.1016/j.cell.2012.09.001.
Cappellesso, R., Nicole, L., Guido, A., Pizzol, D., 2015. Spaceflight osteoporosis: current state and future perspective. Endocrine regulations 49, 231–239. 10.4149/endo_2015_04_231.
Cardinale, B.J., Duffy, J.E., Gonzalez, A., Hooper, D.U., Perrings, C., Venail, P. et al., 2012. Biodiversity loss and its impact on humanity. Nature 486, 59–67. 10.1038/nature11148.
Carlton, J.M., Hirt, R.P., Silva, J.C., Delcher, A.L., Schatz, M., Zhao, Q. et al., 2007. Draft genome sequence of the sexually transmitted pathogen *Trichomonas vaginalis*. Sci. 315, 207–212. 10.1126/science.1132894.
Carroll, S.B., Grenier, J., Weatherbee, S., 2009. From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design. 2nd ed. John Wiley & Sons Ltd, Hoboken.
Casadesús, J., Low, D., 2006. Epigenetic gene regulation in the bacterial world. Microbiol. Mol. Biol. Rev. 70, 830–856. 10.1128/MMBR.00016-06.
Cerwinka, W.H., Sharp, S.M., Boyan, B.D., Zhau, H.E., Chung, L.W.K., Yates, C., 2012. Differentiation of human mesenchymal stem cell spheroids under microgravity conditions. Cell Regen. 1, 2. 10.1186/2045-9769-1-2.
Chang, D., Xu, H., Guo, Y., Jiang, X., Liu, Y., Li, K. et al., 2013. Simulated microgravity alters the metastatic potential of a human lung adenocarcinoma cell line. In Vitro Cell. Dev. Biol. -Animal 49, 170–177. 10.1007/s11626-013-9581-9.
Chapman, J.A., Kirkness, E.F., Simakov, O., Hampson, S.E., Mitros, T., Weinmaier, T. et al., 2010. The dynamic genome of *Hydra*. Nature 464, 592–596. 10.1038/nature08830.
Charlesworth, J., Eyre-Walker, A., 2006. The rate of adaptive evolution in enteric bacteria. Mol. Biol. Evol. 23, 1348–1356. 10.1093/molbev/msk025.
Chatani, M., Mantoku, A., Takeyama, K., Abduweli, D., Sugamori, Y., Aoki, K. et al., 2015. Microgravity promotes osteoclast activity in medaka fish reared at the international space station. Sci. Rep. 5, 14172. 10.1038/srep14172.
Chen, Z.-Y., Guo, S., Li, B.-B., Jiang, N., Li, A., Yan, H.-F. et al., 2019. Effect of Weightlessness on the 3D Structure Formation and Physiologic Function of Human Cancer Cells. Biomed Res. Int. 2019, 4894083. 10.1155/2019/4894083.
Cheng, X., Xu, X., Chen, J., Zhou, X., Cheng, L., Li, M. et al., 2014. Effects of simulated microgravity on *Streptococcus mutans* physiology and biofilm structure. FEMS Microbiol. Lett. 359, 94–101. 10.1111/1574-6968.12573.

Chengzhi, L., 2011. Agronomy in space–China's crop breeding program. Space Policy, 27(3), 157-164. https://doi.org/10.1016/j.spacepol.2011.06.001

Chiu, B., Wan, J.Z.-M., Abley, D., Akabutu, J., 2005. Induction of vascular endothelial phenotype and cellular proliferation from human cord blood stem cells cultured in simulated microgravity. Acta Astronaut. 56, 918–922. 10.1016/j.actaastro.2005.01.018.

Choi, I.-G., Kim, S.-H., 2007. Global extent of horizontal gene transfer. Proc. Natl. Acad. Sci. U. S. A. 104, 4489–4494. 10.1073/pnas.0611557104.

Chopra, V., Fadl, A.A., Sha, J., Chopra, S., Galindo, C.L., Chopra, A.K., 2006. Alterations in the Virulence Potential of Enteric Pathogens and Bacterial–Host Cell Interactions Under Simulated Microgravity Conditions. J. Toxicol. Env. Heal. A 69, 1345–1370. 10.1080/15287390500361792.

Ciancaglini, P., Simão, A.M.S., Bolean, M., Millán, J.L., Rigos, C.F., Yoneda, J.S. et al., 2012. Proteoliposomes in nanobiotechnology. Biophys. Rev. 4, 67–81. 10.1007/s12551-011-0065-4.

Clark, A.G., Fucito, C.D., 1998. Stress tolerance and metabolic response to stress in Drosophila melanogaster. Heredity 81 (Pt 5), 514–527. 10.1046/j.1365-2540.1998.00414.x.

Clarke, M.S.F., Sundaresan, A., Vanderburg, C.R., Banigan, M.G., Pellis, N.R., 2013. A three-dimensional tissue culture model of bone formation utilizing rotational co-culture of human adult osteoblasts and osteoclasts. Acta biomater. 9, 7908–7916. 10.1016/j.actbio.2013.04.051.

Clejan, S., O'Connor, K., Rosensweig, N., 2001. Tri-dimensional prostate cell cultures in simulated microgravity and induced changes in lipid second messengers and signal transduction. J. Cell Mol. Med. 5, 60–73. 10.1111/j.1582-4934.2001.tb00138.x.

Clejan, S., O'Connor, K.C., Cowger, N.L., Cheles, M.K., Haque, S., Primavera, A.C., 1996. Effects of simulated microgravity on DU 145 human prostate carcinoma cells. Biotechnol. Bioeng. 50, 587–597. 10.1002/(SICI)1097-0290(19960605)50:5<587:AID-BIT14>3.0.CO;2-G.

Clément, G., Slenzka, K., (eds.) 2006. Fundamentals of space biology, research on cells, animals and plants in space. Microcosm Press, El Secondo, CA.

Cordero, O.X., Hogeweg, P., 2009. The impact of long-distance horizontal gene transfer on prokaryotic genome size. Proc. Natl. Acad. Sci. U. S. A. 106, 21748–21753. 10.1073/pnas.0907584106.

Correll, M.J., Pyle, T.P., Millar, Katherine D. L., Sun, Y., Yao, J., Edelmann, R.E. et al., 2013. Transcriptome analyses of *Arabidopsis thaliana* seedlings grown in space: implications for gravity-responsive genes. Planta 238, 519–533. 10.1007/s00425-013-1909-x.

Corydon, T.J., Kopp, S., Wehland, M., Braun, M., Schütte, A., Mayer, T. et al., 2016a. Alterations of the cytoskeleton in human cells in space proved by life-cell imaging. Sci. Rep. 6, 20043. 10.1038/srep20043.

Corydon, T.J., Mann, V., Slumstrup, L., Kopp, S., Sahana, J., Askou, A.L. et al., 2016b. Reduced Expression of Cytoskeletal and Extracellular Matrix Genes in Human Adult Retinal Pigment Epithelium Cells Exposed to Simulated Microgravity. Cell Physiol. Biochem. 40(1-2), 1–17. 10.1159/000452520.

Courchesne, N.M.D., Parisien, A., Wang, B., Lan, C.Q., 2009. Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. J. Biotechnol. 141, 31–41. 10.1016/j.jbiotec.2009.02.018.

Cox, M.M., Battista, J.R., 2005. *Deinococcus radiodurans* - the consummate survivor. Nat. Rev. Micro. 3, 882–892. 10.1038/nrmicro1264.

Crabbé, A., Nielsen-Preiss, S.M., Woolley, C.M., Barrila, J., Buchanan, K., McCracken, J. et al., 2013. Spaceflight Enhances Cell Aggregation and Random Budding in *Candida albicans*. PLoS ONE 8, e80677. 10.1371/journal.pone.0080677.

Crabbé, A., Schurr, M.J., Monsieurs, P., Morici, L., Schurr, J., Wilson, J.W. et al., 2011. Transcriptional and proteomic responses of *Pseudomonas aeruginosa* PAO1 to spaceflight conditions involve Hfq regulation and reveal a role for oxygen. Appl. Environ. Microbiol. 77, 1221–1230. 10.1128/AEM.01582-10.

Crisp, P.A., Ganguly, D., Eichten, S.R., Borevitz, J.O., Pogson, B.J., 2016. Reconsidering plant memory: Intersections between stress recovery, RNA turnover, and epigenetics. Sci. Adv. 2, e1501340. 10.1126/sciadv.1501340.

Cropley, J.E., Suter, C.M., Beckman, K.B., Martin, D.I.K., 2006. Germ-line epigenetic modification of the murine A vy allele by nutritional supplementation. Proc. Natl. Acad. Sci. U. S. A. 103, 17308–17312. 10.1073/pnas.0607090103.

Cui, Y., Lu, C., Chen, B., Han, J., Zhao, Y., Xiao, Z. et al., 2018. Restoration of mandibular bone defects with demineralized bone matrix combined with three-dimensional cultured bone marrow-derived mesenchymal stem cells in minipig models. Journal of materials science. J. Mater. Sci.: Mater. Med 29, 147. 10.1007/s10856-018-6152-3.

Dagan, T., Artzy-Randrup, Y., Martin, W., 2008. Modular networks and cumulative impact of lateral transfer in prokaryote genome evolution. Proc. Natl. Acad. Sci. U. S. A. 105, 10039–10044. 10.1073/pnas.0800679105.

Daiker, V., Häder, D.-P., Richter, P.R., Lebert, M., 2011. The involvement of a protein kinase in phototaxis and gravitaxis of *Euglena gracilis*. Planta 233, 1055–1062. 10.1007/s00425-011-1364-5.

Daiker, V., Lebert, M., Richter, P., Häder, D.-P., 2010. Molecular characterization of a calmodulin involved in the signal transduction chain of gravitaxis in *Euglena gracilis*. Planta 231, 1229–1236. 10.1007/s00425-010-1126-9.

Daubin, V., Moran, N.A., Ochman, H., 2003. Phylogenetics and the cohesion of bacterial genomes. Sci. 301, 829–832. 10.1126/science.1086568.

Davidson, E.H., 2006. The regulatory genome: Gene regulatory networks in development and evolution Academic, Burlington, MA, San Diego.

Davies, J., Davies, D., 2010. Origins and evolution of antibiotic resistance. Microbiol. Mol. Biol. Rev. 74, 417–433. 10.1128/MMBR.00016-10.

Dean, R., van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D. et al., 2012. The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13, 414–430. 10.1111/j.1364-3703.2011.00783.x.

DiFrancesco, J.M., Olson, J.M., 2015. The economics of microgravity research. npj Microgravity 1, 15001. 10.1038/npjmgrav.2015.1.

Ding, Y., Fromm, M., Avramova, Z., 2012. Multiple exposures to drought 'train' transcriptional responses in *Arabidopsis*. Nat. Commun. 3, 740. 10.1038/ncomms1732.

Dittrich, A., Grimm, D., Sahana, J., Bauer, J., Krüger, M., Infanger, M. et al., 2018. Key Proteins Involved in Spheroid Formation and Angiogenesis in Endothelial Cells After Long-Term Exposure to Simulated Microgravity. Cell. Physiol. Biochem. 45, 429–445. 10.1159/000486920.

Doron, L., Segal, N.'a., Shapira, M., 2016. Transgene Expression in Microalgae-From Tools to Applications. Front. Plant Sci. 7, 505. 10.3389/fpls.2016.00505.

Douliez, J.-P., Martin, N., Beneyton, T., Eloi, J.-C., Chapel, J.-P., Navailles, L. et al., 2018. Preparation of Swellable Hydrogel-Containing Colloidosomes from Aqueous Two-Phase Pickering Emulsion Droplets. Angew. Chem. 130, 7906–7910. 10.1002/ange.201802929.

Drake, J.W., 2006. Chaos and Order in Spontaneous Mutation. Genetics 173, 1–8.

Dröge, M., Pühler, A., Selbitschka, W., 1998. Horizontal gene transfer as a biosafety issue: A natural phenomenon of public concern. J. Biotechnol. 64, 75–90. 10.1016/s0168-1656(98)00105-9.

Duchêne, S., Holmes, E.C., Ho, S.Y.W., 2014. Analyses of evolutionary dynamics in viruses are hindered by a time-dependent bias in rate estimates. Proc. Biol. Sci. 281. 10.1098/rspb.2014.0732.

Duchêne, S., Holt, K.E., Weill, F.-X., Le Hello, S., Hawkey, J., Edwards, D.J. et al., 2016. Genome-scale rates of evolutionary change in bacteria. Microb. Genom. 2, e000094. 10.1099/mgen.0.000094.

Dunning Hotopp, J.C., Clark, M.E., Oliveira, D.C.S.G., Foster, J.M., Fischer, P., Muñoz Torres, M.C. et al., 2007. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes. Sci. 317, 1753–1756. 10.1126/science.1142490.

Dupuy, P., Grossin, F., Trotet, G., 1976. Methode de culture des Cyanophycees sur support mineral solide. C.R. Acad. Sci. 170, 44.

Dzieciol, A.J., Mann, S., 2012. Designs for life: protocell models in the laboratory. Chem. Soc. Rev. 41, 79–85. 10.1039/C1CS15211D.

Ebenezer, T.E., Zoltner, M., Burrell, A., Nenarokova, A., Novák Vanclová, A.M.G., Prasad, B. et al., 2019. Transcriptome, proteome and draft genome of *Euglena gracilis*. BMC Biol. 17, 11. 10.1186/s12915-019-0626-8.

Eichten, S.R., Schmitz, R.J., Springer, N.M., 2014. Epigenetics: Beyond Chromatin Modifications and Complex Genetic Regulation. Plant Physiol. 165, 933–947. 10.1104/pp.113.234211.

El Masri, D., Vissers, T., Badaire, S., Stiefelhagen, J.C.P., Vutukuri, H.R., Helfferich, P. et al., 2012. A qualitative confocal microscopy study on a range of colloidal processes by simulating microgravity conditions through slow rotations. Soft Matter 8, 6979. 10.1039/C2SM07217C.

Elena, S.F., Ekunwe, L., Hajela, N., Oden, S.A., Lenski, R.E., 1998. Distribution of fitness effects caused by random insertion mutations in *Escherichia coli*. Genetica 102-103, 349–358.
Eme, L., Gentekaki, E., Curtis, B., Archibald, J.M., Roger, A.J., 2017. Lateral Gene Transfer in the Adaptation of the Anaerobic Parasite *Blastocystis* to the Gut. Curr. Biol. 27, 807–820. 10.1016/j.cub.2017.02.003.
Eyre-Walker, A., 2006. The genomic rate of adaptive evolution. Trends Ecol. Evol. 21, 569–575. 10.1016/j.tree.2006.06.015.
Eyre-Walker, A., Keightley, P.D., 2007. The distribution of fitness effects of new mutations. Nat. Rev. Genet. 8, 610–618. 10.1038/nrg2146.
Eyre-Walker, A., Keightley, P.D., Smith, N.G.C., Gaffney, D., 2002. Quantifying the slightly deleterious mutation model of molecular evolution. Mol. Biol. Evol. 19, 2142–2149. 10.1093/oxfordjournals.molbev.a004039.
Fajardo-Cavazos, P., Leehan, J.D., Nicholson, W.L., 2018. Alterations in the Spectrum of Spontaneous Rifampicin-Resistance Mutations in the *Bacillus subtilis rpoB* Gene after Cultivation in the Human Spaceflight Environment. Front. Microbiol. 9, 192. 10.3389/fmicb.2018.00192.
Fajardo-Cavazos, P., Nicholson, W.L., 2016. Cultivation of Staphylococcus epidermidis in the Human Spaceflight Environment Leads to Alterations in the Frequency and Spectrum of Spontaneous Rifampicin-Resistance Mutations in the rpoB Gene. Front. Microbiol. 7, 999. 10.3389/fmicb.2016.00999.
Fang, A., Pierson, D.L., Koenig, D.W., Mishra, S.K., Demain, A.L., 1997a. Effect of simulated microgravity and shear stress on microcin B17 production by *Escherichia coli* and on its excretion into the medium. Appl. Environ. Microbiol. 63, 4090–4092.
Fang, A., Pierson, D.L., Mishra, S.K., Demain, A.L., 2000. Growth of *Steptomyces hygroscopicus* in rotating-wall bioreactor under simulated microgravity inhibits rapamycin production. Appl. Microbiol. Biotechnol. 54, 33–36.
Fang, A., Pierson, D.L., Mishra, S.K., Koenig, D.W., Demain, A.L., 1997b. Gramicidin S production by *Bacillus brevis* in simulated microgravity. Curr Microbiol 34, 199–204.
Fang, A., Pierson, D.L., Mishra, S.K., Koenig, D.W., Demain, A.L., 1997c. Secondary metabolism in simulated microgravity: beta-lactam production by *Streptomyces clavuligerus*. J. Ind. Microbiol. Biotechnol. 18, 22–25. 10.1038/sj.jim.2900345.
Felmingham, D., Washington, J., 1999. Trends in the antimicrobial susceptibility of bacterial respiratory tract pathogens--findings of the Alexander Project 1992-1996. J. Chemother. 11 Suppl 1, 5–21. 10.1179/joc.1999.11.Supplement-2.5.
Feng, S., Jacobsen, S.E., Reik, W., 2010. Epigenetic reprogramming in plant and animal development. Sci. 330, 622–627. 10.1126/science.1190614.
Fengler, S., Spirer, I., Neef, M., Ecke, M., Hauslage, J., Hampp, R., 2016. Changes in Gene Expression of *Arabidopsis thaliana* Cell Cultures Upon Exposure to Real and Simulated Partial-g Forces. Microgravity Sci. Technol. 28, 319–329. 10.1007/s12217-015-9452-y.
Fengler, S., Spirer, I., Neef, M., Ecke, M., Nieselt, K., Hampp, R., 2015. A whole-genome microarray study of *Arabidopsis thaliana* semisolid callus cultures exposed to microgravity and nonmicrogravity related spaceflight conditions for 5 days on board of Shenzhou 8. Biomed. Res. Int. 2015, 547495. 10.1155/2015/547495.
Ferl, R., Wheeler, R., Levine, H.G., Paul, A.-L., 2002. Plants in space. Curr Opin Plant Biol 5, 258–263.
Firth, C., Kitchen, A., Shapiro, B., Suchard, M.A., Holmes, E.C., Rambaut, A., 2010. Using time-structured data to estimate evolutionary rates of double-stranded DNA viruses. Mol. Biol. Evol. 27, 2038–2051. 10.1093/molbev/msq088.
Forterre, P., La Bouthier de Tour, C., Philippe, H., Duguet, M., 2000. Reverse gyrase from hyperthermophiles: probable transfer of a thermoadaptation trait from Archaea to Bacteria. Trends Genet. 16, 152–154. 10.1016/S0168-9525(00)01980-6.
Foster, P.L., 2000. Adaptive mutation: implications for evolution. Bioessays 22, 1067–1074. 10.1002/1521-1878(200012)22:12<1067:AID-BIES4>3.0.CO;2-Q.
Fournier, G.P., Gogarten, J.P., 2008. Evolution of acetoclastic methanogenesis in *Methanosarcina* via horizontal gene transfer from cellulolytic *Clostridia*. J. Bacteriol. 190, 1124–1127. 10.1128/JB.01382-07.

Francesca, S., Simona, G., Francesco, N.T., Andrea, R., Vittorio, R., Federico, S. et al., 2006. Downy mildew (*Plasmopara viticola*) epidemics on grapevine under climate change. Global Change Biol. 12, 1299–1307. 10.1111/j.1365-2486.2006.01175.x.

Freed, L.E., Langer, R., Martin, I., Pellis, N.R., Vunjak-Novakovic, G., 1997. Tissue engineering of cartilage in space. Proc. Natl. Acad. Sci. U. S. A. 94, 13885–13890. 10.1073/pnas.94.25.13885.

Freed, L.E., Vunjak-Novakovic, G., 1997. Microgravity tissue engineering. In Vitro Cell. Dev. Biol. - Animal 33, 381–385. 10.1007/s11626-997-0009-2.

Freed, L.F., Pellis, N.R., Searby, N., Luis, J. de, Preda, C., Bordonaro, J. et al., 1999. Microgravity cultivation of cells and tissues. Gravit. Space Biol., 12, 57–67.

Frerich, B., Zückmantel, K., Hemprich, A., 2006. Microvascular engineering in perfusion culture: immunohistochemistry and CLSM findings. Head Face Med. 2, 26. 10.1186/1746-160X-2-26.

Frerich, B., Zückmantel, K., Winter, K., Müller-Dürwald, S., Hemprich, A., 2008. Maturation of capillary-like structures in a tube-like construct in perfusion and rotation culture. Int. J. Oral Maxillofac. Surg. 37, 459–466. 10.1016/j.ijom.2008.01.014.

Fukuda, T., Fukuda, K., Takahashi, A., Ohnishi, T., Nakano, T., Sato, M. et al., 2000. Analysis of deletion mutations of the rpsL gene in the yeast *Saccharomyces cerevisiae* detected after long-term flight on the Russian space station Mir. Mutat. Res. Genet. Toxicol. Environ. Mutagen 470, 125–132. 10.1016/S1383-5742(00)00054-5.

Gao, H., Liu, Z., Zhang, L., 2011. Secondary metabolism in simulated microgravity and space flight. Protein Cell 2, 858–861. 10.1007/s13238-011-1125-z.

Garcia, A.M., Derventzi, A., Busuttil, R., Calder, R.B., Perez, E., Chadwell, L. et al., 2007. A model system for analyzing somatic mutations in *Drosophila melanogaster*. Nat. Meth. 4, 401–403. 10.1038/nmeth1027.

Garcia-Vallvé, S., Romeu, A., Palau, J., 2000. Horizontal gene transfer of glycosyl hydrolases of the rumen fungi. Mol. Biol. Evol. 17, 352–361. 10.1093/oxfordjournals.molbev.a026315.

Gelvin, S.B., 2003. Agrobacterium-mediated plant transformation: the biology behind the "gene-jockeying" tool. Microbiol. Mol. Biol. Rev. 67, 16-37, 10.1128/MMBR.67.1.16-37.2003.

Gendrel, A.-V., Colot, V., 2005. *Arabidopsis* epigenetics: when RNA meets chromatin. Curr. Opin. Plant Biol. 8, 142–147. 10.1016/j.pbi.2005.01.007.

Gingerich, P.D., 2001. Rates of evolution on the time scale of the evolutionary process. Genetica 112-113, 127–144.

Gladyshev, E.A., Meselson, M., Arkhipova, I.R., 2008. Massive horizontal gene transfer in bdelloid rotifers. Sci. 320, 1210–1213. 10.1126/science.1156407.

Godfray, H.C.J., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F. et al., 2010. Food security: the challenge of feeding 9 billion people. Sci. 327, 812–818. 10.1126/science.1185383.

Gogarten, J.P., 2003. Gene Transfer: Gene Swapping Craze Reaches Eukaryotes. Curr. Biol. 13, R53-R54. 10.1016/s0960-9822(02)01426-4.

Gogarten, J.P., Doolittle, W.F., Lawrence, J.G., 2002. Prokaryotic evolution in light of gene transfer. Mol. Biol. Evol. 19, 2226–2238. 10.1093/oxfordjournals.molbev.a004046.

Gomes, A.Q., Nolasco, S., Soares, H., 2013. Non-coding RNAs: multi-tasking molecules in the cell. Int. J. Mol. Sci. 14, 16010–16039. 10.3390/ijms140816010.

Gophna, U., Charlebois, R.L., Doolittle, W.F., 2004. Have archaeal genes contributed to bacterial virulence? Trends Microbiol. 12, 213–219. 10.1016/j.tim.2004.03.002.

Grimm, D., Bauer, J., Kossmehl, P., Shakibaei, M., Schöberger, J., Pickenhahn, H. et al., 2002. Simulated microgravity alters differentiation and increases apoptosis in human follicular thyroid carcinoma cells. FASEB J. 16, 604–606.

Grimm, D., Bauer, J., Ulbrich, C., Westphal, K., Wehland, M., Infanger, M. et al., 2010. Different responsiveness of endothelial cells to vascular endothelial growth factor and basic fibroblast growth factor added to culture media under gravity and simulated microgravity. Tissue Eng. Part A 16, 1559–1573. 10.1089/ten.tea.2009.0524.

Grimm, D., Egli, M., Krüger, M., Riwaldt, S., Corydon, T.J., Kopp, S. et al., 2018. Tissue Engineering Under Microgravity Conditions-Use of Stem Cells and Specialized Cells. Stem Cells Dev. 27, 787–804. 10.1089/scd.2017.0242.

Grimm, D., Grosse, J., Wehland, M., Mann, V., Reseland, J.E., Sundaresan, A. et al., 2016. The impact of microgravity on bone in humans. Bone 87, 44–56. 10.1016/j.bone.2015.12.057.

Grimm, D., Infanger, M., Westphal, K., Ulbrich, C., Pietsch, J., Kossmehl, P. et al., 2009. A delayed type of three-dimensional growth of human endothelial cells under simulated weightlessness. Tissue Eng. Part A 15, 2267–2275. 10.1089/ten.tea.2008.0576.

Grossi, G., Goglio, P., Vitali, A., Williams, A.G., 2019. Livestock and climate change: impact of livestock on climate and mitigation strategies. Animal Front. 9, 69–76. 10.1093/af/vfy034.

Guljamow, A., Jenke-Kodama, H., Saumweber, H., Quillardet, P., Frangeul, L., Castets, A.M. et al., 2007. Horizontal gene transfer of two cytoskeletal elements from a eukaryote to a cyanobacterium. Curr. Biol. 17, R757-9. 10.1016/j.cub.2007.06.063.

Haddock, S.H.D., Moline, M.A., Case, J.F., 2010. Bioluminescence in the sea. Annu. Rev. Mar. Sci. 2, 443–493. 10.1146/annurev-marine-120308-081028.

Häder, D.-P., Braun, M., Grimm, D., Hemmersbach, R., 2017. Gravireceptors in eukaryotes-a comparison of case studies on the cellular level. npj Microgravity 3, 13. 10.1038/s41526-017-0018-8.

Häder, D.-P., Richter, P., Lebert, M., 2006a. Signal transduction in gravisensing of flagellates. Signal Transduct. 6, 422–431. 10.1002/sita.200600104.

Häder, D.-P., Richter, P., Ntefidou, M., Lebert, M., 2005. Gravitational sensory transduction chain in flagellates. Adv. Space Res. 36, 1182–1188.

Häder, D.-P., Richter, P., Schuster, M., Dachev, T., Tomov, B., Georgiev, P. et al., 2009a. R3D-B2-Measurement of ionizing and solar radiation in open space in the BIOPAN 5 facility outside the FOTON M2 satellite. Adv. Space Res. 43, 1200–1211.

Häder, D.-P., Richter, P.R., Schuster, M., Daiker, V., Lebert, M., 2009b. Molecular analysis of the graviperception signal transduction in the flagellate *Euglena gracilis*: Involvement of a transient receptor potential-like channel and a calmodulin. Adv. Space Res. 43, 1179–1184. 10.1016/j.asr.2009.01.029.

Häder, D.-P., Richter, P.R., Strauch, S.M., Schuster, M., 2006b. Aquacells - flagellates under long-term microgravity and potential usage for life support systems. Micrograv. Sci. Technol. 18, 210–214.

Hagen, B. ten, Kümmel, F., Wittkowski, R., Takagi, D., Löwen, H., Bechinger, C., 2014. Gravitaxis of asymmetric self-propelled colloidal particles. Nat. Commun. 5, 4829. 10.1038/ncomms5829.

Hagiwara, H., Higashibata, A., Ogawa, S., Kanazawa, S., Mizuno, H., Tanaka, R., 2018. Effectiveness of endothelial progenitor cell culture under microgravity for improved angiogenic potential. Sci. Rep. 8, 14239. 10.1038/s41598-018-32073-2.

Hall, C., Dietrich, F.S., 2007. The reacquisition of biotin prototrophy in Saccharomyces cerevisiae involved horizontal gene transfer, gene duplication and gene clustering. Genetics 177, 2293–2307. 10.1534/genetics.107.074963.

Hamilton, G., 1998. Multicellular spheroids as an in vitro tumor model. Cancer Lett. 131, 29–34. 10.1016/S0304-3835(98)00198-0.

Hammond, T.G., Hammond, J.M., 2001. Optimized suspension culture: the rotating-wall vessel. Am. J. Physiol. Renal Physiol. 28, 12–25.

Hammond, T.G., Stodieck, L., Birdsall, H.H., Becker, J.L., Koenig, P., Hammond, J.S. et al., 2013. Effects of microgravity on the virulence of *Listeria monocytogenes*, *Enterococcus faecalis*, *Candida albicans*, and methicillin-resistant *Staphylococcus aureus*. Astrobiology 13, 1081–1090. 10.1089/ast.2013.0986.

Han, J., Dai, J., 2016. Microgravity may help future organ/tissue manufacture. Science China. Life Sci. 59, 850–853. 10.1007/s11427-016-5079-5.

Hao, W., Golding, G.B., 2008. Uncovering rate variation of lateral gene transfer during bacterial genome evolution. BMC Genomics 9, 235. 10.1186/1471-2164-9-235.

Harada, K., Obiya, Y., Nakano, T., Kawashima, M., Miki, T., Kobayashi, Y. et al., 1997. Cancer risk in space due to radiation assessed by determining cell lethality and mutation frequencies of prokaryotes and a plasmid during the Second International Microgravity Laboratory (IML-2) Space Shuttle experiment. Oncol. Rep. 4, 691–695. 10.3892/or.4.4.691.

Hashemi, B.B., Penkala, J.E., Vens, C., Huls, H., Cubbage, M., Sams, C.F., 1999. T cell activation responses are differentially regulated during clinorotation and in spaceflight. FASEB J. 13, 2071–2082. 10.1096/fasebj.13.14.2071.

Hauslage, J., Cevik, V., Hemmersbach, R., 2017. Pyrocystis noctiluca represents an excellent bioassay for shear forces induced in ground-based microgravity simulators (clinostat and random positioning machine). npj Microgravity 3, 1. 10.1038/s41526-017-0016-x.

Hausmann, N., Fengler, S., Hennig, A., Franz-Wachtel, M., Hampp, R., Neef, M. et al., 2014. Cytosolic calcium, hydrogen peroxide and related gene expression and protein modulation in *Arabidopsis thaliana* cell cultures respond immediately to altered gravitation: parabolic flight data. Plant Biol. J. 16, 120–128. 10.1111/plb.12051.

Hayashi, T., Makino, K., Ohnishi, M., Kurokawa, K., Ishii, K., Yokoyama, K. et al., 2001. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res. 8, 11–22. 10.1093/dnares/8.1.11.

He, X., Liu, M., Lu, J., Xue, H., Pan, Y. Space Mutation Breeding: A Brief Introduction of, UK Global Science Books, UK, 2006.

Hemmersbach, R., Simon, A., Waßer, K., Hauslage, J., Christianen, P.C.M., Albers, P.W. et al., 2014. Impact of a high magnetic field on the orientation of gravitactic unicellular organisms--a critical consideration about the application of magnetic fields to mimic functional weightlessness. Astrobiology 14, 205–215. 10.1089/ast.2013.1085.

Hemmersbach, R., Strauch, S.M., Seibt, D., Schuber, M., Häder, D.-P., 2006. Comparative studies on gravisensitive protists on ground (2D and 3D clinostats) and in microgravity. Microgravity Sci. Technol. 18, 257–259.

Herranz, R., Anken, R., Boonstra, J., Braun, M., Christianen, P.C.M., Geest, M. de et al., 2013. Ground-Based Facilities for Simulation of Microgravity: Organism-Specific Recommendations for Their Use, and Recommended Terminology. Astrobiology 13, 1–17. 10.1089/ast.2012.0876.

Hershberg, R., 2015. Mutation--The Engine of Evolution: Studying Mutation and Its Role in the Evolution of Bacteria. Cold Spring Harb. Perspect. Biol. 7, a018077. 10.1101/cshperspect.a018077.

Hoekstra, H.E., Coyne, J.A., 2007. The locus of evolution: evo devo and the genetics of adaptation. Evol. 61, 995–1016. 10.1111/j.1558-5646.2007.00105.x.

Hoekstra, H.E., Hirschmann, R.J., Bundey, R.A., Insel, P.A., Crossland, J.P., 2006. A single amino acid mutation contributes to adaptive beach mouse color pattern. Sci. 313, 101–104. 10.1126/science.1126121.

Hoffmann, A.A., HERCUS, M.J., 2000. Environmental Stress as an Evolutionary Force. BioSci. 50, 217.

Hoffmann, A.A., Merilä, J., 1999. Heritable variation and evolution under favourable and unfavourable conditions. Trends Ecol. Evol. 14, 96–101. 10.1016/S0169-5347(99)01595-5.

Holliday, R., 2006. Epigenetics: a historical overview. Epigenetics 1, 76–80. 10.4161/epi.1.2.2762.

Holt, K.E., Baker, S., Weill, F.-X., Holmes, E.C., Kitchen, A., Yu, J. et al., 2012. *Shigella sonnei* genome sequencing and phylogenetic analysis indicate recent global dissemination from Europe. Nat. Genet. 44, 1056–1059. 10.1038/ng.2369.

Horne, S.D., Chowdhury, S.K., Heng, H.H.Q., 2014. Stress, genomic adaptation, and the evolutionary trade-off. Front. Genet. 5, 92. 10.3389/fgene.2014.00092.

Horneck, G., Bucker, H., Dose, K., Martens, K.D., Bieger, A., Mennigmann, H.D. et al., 1984a. Microorganisms and biomolecules in space environment experiment ES 029 on Spacelab-1. Adv. Space Res. 4, 19–27.

Horneck, G., Bucker, H., Reitz, G., Requardt, H., Dose, K., Martens, K.D. et al., 1984b. Life Sci.: Microorganisms in the Space Environment. Sci. 225, 226–228. 10.1126/science.225.4658.226.

Horneck, G., Klaus, D.M., Mancinelli, R.L., 2010. Space Microbiology. Microbiol. Mol. Biol. Rev. 74, 121–156. 10.1128/MMBR.00016-09.

Huang, B., Liu, N., Rong, X., Ruan, J., Huang, Y., 2015. Effects of simulated microgravity and spaceflight on morphological differentiation and secondary metabolism of *Streptomyces coelicolor* A3(2). Appl. Microbiol. Biotechnol. 99, 4409–4422. 10.1007/s00253-015-6386-7.

Huang, J., 2013. Horizontal gene transfer in eukaryotes: the weak-link model. BioEssays 35, 868–875. 10.1002/bies.201300007.

Huang, J., Gogarten, J.P., 2008. Concerted gene recruitment in early plant evolution. Genome Biol. 9, R109. 10.1186/gb-2008-9-7-r109.

Huang, L., Raats, D., Sela, H., Klymiuk, V., Lidzbarsky, G., Feng, L. et al., 2016. Evolution and Adaptation of Wild Emmer Wheat Populations to Biotic and Abiotic Stresses. Annual Rev. Phytopathol. 54, 279–301. 10.1146/annurev-phyto-080614-120254.

Hughes-Fulford, M., Chang, T.T., Martinez, E.M., Li, C.-F., 2015. Spaceflight alters expression of microRNA during T-cell activation. FASEB J. 29, 4893–4900. 10.1096/fj.15-277392.

Ichijo, T., Yamaguchi, N., Tanigaki, F., Shirakawa, M., Nasu, M., 2016. Four-year bacterial monitoring in the International Space Station-Japanese Experiment Module "Kibo" with culture-independent approach. npj Microgravity 2, 16007. 10.1038/npjmgrav.2016.7.

Ilyin, V.K., 2005. Microbiological status of cosmonauts during orbital spaceflights on Salyut and Mir orbital stations. Acta Astronaut. 56, 839–850.

Infanger, M., Kossmehl, P., Shakibaei, M., Baatout, S., Witzing, A., Grosse, J. et al., 2006. Induction of three-dimensional assembly and increase in apoptosis of human endothelial cells by simulated microgravity: Impact of vascular endothelial growth factor. Apoptosis 11, 749–764. 10.1007/s10495-006-5697-7.

Ingber, D., 1999. How cells (might) sense microgravity. FASEB J. 13 Suppl, S3-15. 10.1096/fasebj.13.9001.s3.

Ingber, D.E., 2014. Mechanobiology, Tissue Development and Organ Engineering. In: Principles of Tissue Engineering: Elsevier. p. 309–322.

Ingram, M., Techy, G.B., Saroufeem, R., Yazan, O., Narayan, K.S., Goodwin, T.J. et al., 1997. Three-dimensional growth patterns of various human tumor cell lines in simulated microgravity of a NASA bioreactor. In Vitro Cell. Dev. Biol. -Animal 33, 459–466. 10.1007/s11626-997-0064-8.

Interagency Coordination Group on Antimicrobial Resistance [Internet]. No time to Wait: Securing the future from drug-resistant Infections. A report to the Secretary-General of the United Nations, 2019. Available from: http://oie.int/fileadmin/Home/eng/Media_Center/docs/pdf/IACG2019/IACG_final_report_EN.pdf.

Ito, H., 2014. Plant Models of Transgenerational Epigenetic Inheritance. In: Transgenerational Epigenetics: Elsevier. p. 147–161.

J. Majima, H., P. Indo, H., Tomita, K., Iwashita, Y., Suzuki, H., Masuda, D. et al., 2009. Bio-Assessment of RISK in Long-Term Manned Space Exploration-Cell Death Factors in Space Radiation and/or Microgravity: A Review. Biol. Sci. Space 23, 43–53. 10.2187/bss.23.43.

Jain, R., Rivera, M.C., Moore, J.E., Lake, J.A., 2002. Horizontal gene transfer in microbial genome evolution. Theor. Popul. Biol. 61, 489–495.

Janosi, I.M., Czirok, A., Silhavy, D., Holczinger, A., 2002. Is bioconvection enhancing bacterial growth in quiescent environments? Environ..Microbiol. 4, 525–531.

Jeong, A.J., Kim, Y.J., Lim, M.H., Lee, H., Noh, K., Kim, B.-H. et al., 2018. Microgravity induces autophagy via mitochondrial dysfunction in human Hodgkin's lymphoma cells. Sci. Rep. 8, 14646. 10.1038/s41598-018-32965-3.

Jessup, J.M., Frantz, M., Sonmez-Alpan, E., Locker, J., Skena, K., Waller, H. et al., 2000. Microgravity culture reduces apoptosis and increases the differentiation of a human colorectal carcinoma cell line. Nat. Med. 36, 367. 10.1290/1071-2690(2000)036<0367:MCRAAI>2.0.CO;2.

Jin, F., Zhang, Y., Xuan, K., He, D., Deng, T., Tang, L. et al., 2010. Establishment of three-dimensional tissue-engineered bone constructs under microgravity-simulated conditions. Artif. Organs 34, 118–125. 10.1111/j.1525-1594.2009.00761.x.

Jin, J., Chen, H., Cai, W., 2015. Transcriptome Analysis of *Oryza sativa* Calli Under Microgravity. Microgravity Sci. Technol. 27, 437–453. 10.1007/s12217-015-9432-2.

Jin, T., Chen, Y., Jarboe, L.R., 2016. Evolutionary Methods for Improving the Production of Biorenewable Fuels and Chemicals. In: Biotechnology for Biofuel Production and Optimization: Elsevier. p. 265–290.

Juergensmeyer, M.A., Juergensmeyer, E.A., Guikema, J.A., 1999. Long-term exposure to spaceflight conditions affects bacterial response to antibiotics. Microgravity Sci. Technol. 12, 41–47.

Kacena, M.A., Manfredi, B., Todd, P., 1999. Effects of space flight and mixing on bacterial growth in low volume cultures. Microgravity Sci. Technol. 12, 74–77.

Kalpana, D., Im, C., Lee, Y.S., 2016. Comparative growth, cross stress resistance, transcriptomics of Streptococcus pyogenes cultured under low shear modeled microgravity and normal gravity. Saudi J. Biol. Sci. 23, 24–33. 10.1016/j.sjbs.2015.02.004.

Kamal, K.Y., Herranz, R., van Loon, J.J.W.A., Medina, F.J., 2018. Simulated microgravity, Mars gravity, and 2g hypergravity affect cell cycle regulation, ribosome biogenesis, and epigenetics in *Arabidopsis* cell cultures. Sci. Rep. 8, 6424. 10.1038/s41598-018-24942-7.

Kaur, P., Ward, B., Saha, B., Young, L., Groshen, S., Techy, G. et al., 2011. Human breast cancer histoid: an in vitro 3-dimensional co-culture model that mimics breast cancer tissue. J. Histochem. Cytochem. 59, 1087–1100. 10.1369/0022155411423680.

Kay, G.L., Sergeant, M.J., Zhou, Z., Chan, J.Z.-M., Millard, A., Quick, J. et al., 2015. Eighteenth-century genomes show that mixed infections were common at time of peak tuberculosis in Europe. Nat. Commun. 6, 6717. 10.1038/ncomms7717.

Keeling, P.J., Palmer, J.D., 2008. Horizontal gene transfer in eukaryotic evolution. Nat. Rev. Genet. 9, 605–618. 10.1038/nrg2386.

Kelleher, J.E., Raleigh, E.A., 1995. On the regulation and diversity of restriction in *Escherichia coli*. Gene 157, 229–230. 10.1016/0378-1119(94)00678-L.

Kenific, C.M., Wittmann, T., Debnath, J., 2016. Autophagy in adhesion and migration. J. Cell Sci. 129, 3685–3693. 10.1242/jcs.188490.

Kennemann, L., Didelot, X., Aebischer, T., Kuhn, S., Drescher, B., Droege, M. et al., 2011. *Helicobacter pylori* genome evolution during human infection. Proc. Natl. Acad. Sci. U. S. A. 108, 5033–5038. 10.1073/pnas.1018444108.

Khraiwesh, B., Zhu, J.-K., Zhu, J., 2012. Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta 1819, 137–148. 10.1016/j.bbagrm.2011.05.001.

Kim, H.J., Lee, J., Choi, J.-H., Bahinski, A., Ingber, D.E., 2016. Co-culture of Living Microbiome with Microengineered Human Intestinal Villi in a Gut-on-a-Chip Microfluidic Device. J. Vis. Exp (114): 54344. 10.3791/54344.

Kim, H.W., Rhee, M.S., 2016. Influence of Low-Shear Modeled Microgravity on Heat Resistance, Membrane Fatty Acid Composition, and Heat Stress-Related Gene Expression in *Escherichia coli* O157:H7 ATCC 35150, ATCC 43889, ATCC 43890, and ATCC 43895. Appl. Environ. Microbiol. 82, 2893–2901. 10.1128/AEM.00050-16.

Kim, W., Tengra, F.K., Shong, J., Marchand, N., Chan, H.K., Young, Z. et al., 2013a. Effect of spaceflight on Pseudomonas aeruginosa final cell density is modulated by nutrient and oxygen availability. BMC Microbiol. 13, 241. 10.1186/1471-2180-13-241.

Kim, W., Tengra, F.K., Young, Z., Shong, J., Marchand, N., Chan, H.K. et al., 2013b. Spaceflight promotes biofilm formation by Pseudomonas aeruginosa. PLoS ONE 8, e62437. 10.1371/journal.pone.0062437.

Kim, Y.J., Jeong, A.J., Kim, M., Lee, C., Ye, S.-K., Kim, S., 2017. Time-averaged simulated microgravity (taSMG) inhibits proliferation of lymphoma cells, L-540 and HDLM-2, using a 3D clinostat. Biomedical Eng. Online 16, 48. 10.1186/s12938-017-0337-8.

Kimbrough, J.M., Salinas-Mondragon, R., Boss, W.F., Brown, C.S., Sederoff, H.W., 2004. The fast and transient transcriptional network of gravity and mechanical stimulation in the *Arabidopsis* root apex. Plant Physiol 136, 2790–2805.

King, J.L., Jukes, T.H., 1969. Non-Darwinian evolution. Sci. 164, 788–798. 10.1126/science.164.3881.788.

King, J.R., Edgar, S., Qiao, K., Stephanopoulos, G., 2016. Accessing Nature's diversity through metabolic engineering and synthetic biology. F1000Res. 5. 10.12688/f1000research.7311.1.

King, M.C., Wilson, A.C., 1975. Evolution at two levels in humans and chimpanzees. Sci. 188, 107–116. 10.1126/science.1090005.

Kishore, S.P., Stiller, J.W., Deitsch, K.W., 2013. Horizontal gene transfer of epigenetic machinery and evolution of parasitism in the malaria parasite *Plasmodium falciparum* and other apicomplexans. BMC Evol. Biol. 13, 37. 10.1186/1471-2148-13-37.

Kiss, J.Z., 2000. Mechanisms of the early phases of plant gravitropism. Crit. Rev. Plant Sci. 19, 551–573.

Kiss, J.Z., Wolverton, C., Wyatt, S.E., Hasenstein, K.H., van Loon, J.J.W.A., 2019. Comparison of Microgravity Analogs to Spaceflight in Studies of Plant Growth and Development. Front. Plant Sci. 10, 1577. 10.3389/fpls.2019.01577.

Klaus, D., Simske, S., Todd, P., Stodieck, L., 1997. Investigation of space flight effects on *Escherichia coli* and a proposed model of underlying physical mechanisms. Microbiol. 143 (Pt 2), 449–455. 10.1099/00221287-143-2-449.

Klaus, D.M., 2001. Clinostats and bioreactors. Gravit. Space Biol. Bull.14(2):55-64.

Klaus, D.M., 2004. Gravitational influence on biomolecular engineering processes. Gravit. Space Biol. Bull. 17, 51–65. 10.1016/S1389-0344(04)00014-0.

Klaus, D.M., Benoit, M.R., Nelson, E.S., Hammond, T.G., 2004. Extracellular mass transport considerations for space flight research concerning suspended and adherent in vitro cell cultures. J Gravit. Physiol. 11, 17–27.

Kloesges, T., Popa, O., Martin, W., Dagan, T., 2011. Networks of gene sharing among 329 proteobacterial genomes reveal differences in lateral gene transfer frequency at different phylogenetic depths. Mol. Biol. Evol. 28, 1057–1074. 10.1093/molbev/msq297.

Klymchuk, D.O., 2007. Structural and functional organization of mitochondria in soybean root statocytes under microgravitation. Tsitol. Genet. 41, 30–35.

Kohn, F., Hauslage, J., Hanke, W., 2017. Membrane Fluidity Changes, A Basic Mechanism of Interaction of Gravity with Cells? Microgravity Sci. Technol. 29, 337–342. 10.1007/s12217-017-9552-y.

Kondo, N., Nikoh, N., Ijichi, N., Shimada, M., Fukatsu, T., 2002. Genome fragment of *Wolbachia* endosymbiont transferred to X chromosome of host insect. Proc. Natl. Acad. Sci. U. S. A. 99, 14280–14285. 10.1073/pnas.222228199.

Koonin, E.V., Makarova, K.S., Aravind, L., 2001. Horizontal gene transfer in prokaryotes: quantification and classification. Annu. Rev. Microbiol. 55, 709–742. 10.1146/annurev.micro.55.1.709.

Koonin, E.V., Mushegian, A.R., Galperin, M.Y., Walker, D.R., 1997. Comparison of archaeal and bacterial genomes: computer analysis of protein sequences predicts novel functions and suggests a chimeric origin for the archaea. Mol. Microbiol. 25, 619–637. 10.1046/j.1365-2958.1997.4821861.x.

Koonin, E.V., Wolf, Y.I., 2008. Genomics of bacteria and archaea: the emerging dynamic view of the prokaryotic world. Nucleic Acids Res. 36, 6688–6719. 10.1093/nar/gkn668.

Kopp, S., Krüger, M., Bauer, J., Wehland, M., Corydon, T.J., Sahana, J. et al., 2018. Microgravity Affects Thyroid Cancer Cells during the TEXUS-53 Mission Stronger than Hypergravity. Int. J. Mol. Sci. 19. 10.3390/ijms19124001-.

Kopp, S., Warnke, E., Wehland, M., Aleshcheva, G., Magnusson, N.E., Hemmersbach, R. et al., 2015. Mechanisms of three-dimensional growth of thyroid cells during long-term simulated microgravity. Sci. Rep. 5, 16691. 10.1038/srep16691.

Kordyum, E., 2003. A role for the cytoskeleton in plant cell gravisensitivity and Ca2+-signaling in microgravity. Cell Biol. Internat. 27, 219–221. 10.1016/S1065-6995(02)00315-3.

Kordyum, E.L., 2014. Plant cell gravisensitivity and adaptation to microgravity. Plant Biol. 16 Suppl 1, 79–90. 10.1111/plb.12047.

Kordyum, E.L., Chapman, D.K., 2017. Plants and microgravity: Patterns of microgravity effects at the cellular and molecular levels. Cytol. Genet. 51, 108–116. 10.3103/S0095452717020049.

Kossmehl, P., Shakibaei, M., Cogoli, A., Infanger, M., Curcio, F., Schönberger, J. et al., 2003. Weightlessness induced apoptosis in normal thyroid cells and papillary thyroid carcinoma cells via extrinsic and intrinsic pathways. Endocrinol. 144, 4172–4179. 10.1210/en.2002-0171.

Kossmehl, P., Shakibaei, M., Cogoli, A., Pickenhahn, H., Paul, M., Grimm, D., 2002. Simulated microgravity induces programmed cell death in human thyroid carcinoma cells. J. Gravit. Physiol. 9, 295-306.

Krüger, J., Richter, P., Stoltze, J., Strauch, S.M., Krüger, M., Daiker, V. et al., 2019a. Changes of Gene Expression in *Euglena gracilis* Obtained During the 29th DLR Parabolic Flight Campaign. Sci. Rep. 9, 14260. 10.1038/s41598-019-50611-4.

Krüger, M., Kopp, S., Wehland, M., Bauer, J., Baatout, S., Moreels, M. et al., 2019b. Growing blood vessels in space: Preparation studies of the SPHEROIDS project using related ground-based studies. Acta Astronaut. 159, 267–272. 10.1016/j.actaastro.2019.03.074.

Krüger, M., Melnik, D., Kopp, S., Buken, C., Sahana, J., Bauer, J. et al., 2019c. Fighting Thyroid Cancer with Microgravity Research. Int. J. Mol. Sci. 20. 10.3390/ijms20102553.

Ku, C., Martin, W.F., 2016. A natural barrier to lateral gene transfer from prokaryotes to eukaryotes revealed from genomes: the 70 % rule. BMC Biol. 14, 89. 10.1186/s12915-016-0315-9.

Ku, C., Nelson-Sathi, S., Roettger, M., Sousa, F.L., Lockhart, P.J., Bryant, D. et al., 2015. Endosymbiotic origin and differential loss of eukaryotic genes. Nature 524, 427–432. 10.1038/nature14963.

Kucsko, G., Maurer, P.C., Yao, N.Y., Kubo, M., Noh, H.J., Lo, P.K. et al., 2013. Nanometre-scale thermometry in a living cell. Nature 500, 54–58. 10.1038/nature12373.

Kunz-Schughart, L.A., 1999. Multicellular tumor spheroids: intermediates between monolayer culture and in vivo tumor. Cell Biol. Internat. 23, 157–161. 10.1006/cbir.1999.0384.

Kunz-Schughart, L.A., Freyer, J.P., Hofstaedter, F., Ebner, R., 2004. The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. J. Biomol. Screen. 9, 273–285. 10.1177/1087057104265040.

Kunz-Schughart, L.A., Heyder, P., Schroeder, J., Knuechel, R., 2001. A heterologous 3-D coculture model of breast tumor cells and fibroblasts to study tumor-associated fibroblast differentiation. Exp. Cell Res. 266, 74–86. 10.1006/excr.2001.5210.

Kuo, C.-H., Ochman, H., 2009. The fate of new bacterial genes. FEMS Microbiol. Rev. 33, 38–43. 10.1111/j.1574-6976.2008.00140.x.

Kurland, C.G., Canback, B., Berg, O.G., 2003. Horizontal gene transfer: a critical view. Proc. Natl. Acad. Sci. U. S. A. 100, 9658–9662. 10.1073/pnas.1632870100.

Kuzminov, A., 1999. Recombinational Repair of DNA Damage in *Escherichia coli* and Bacteriophage λ. Microbiol. Mol. Biol. Rev. 63, 751–813.

Lam, K.S., Gustavson, D.R., Pirnik, D.L., Pack, E., Bulanhagui, C., Mamber, S.W. et al., 2002. The effect of space flight on the production of actinomycin D by *Streptomyces plicatus*. J. Ind. Microbiol. Biotechnol. 29, 299–302. 10.1038/sj.jim.7000312.

Lam, K.S., Mamber, S.W., Pack, E.J., Forenza, S., Fernandes, P.B., Klaus, D.M., 1998. The effects of space flight on the production of monorden by *Humicola fuscoatra* WC5157 in solid-state fermentation. Appl. Microbiol. Biotechnol. 49, 579–583.

Lapchine, L., Moatti, N., Gasset, G., Richoilley, G., Templier, J., Tixador, R., 1986. Antibiotic activity in space. Drugs Exp. Clin. Res. 12, 933–938.

Law, J.A., Jacobsen, S.E., 2009. Molecular biology. Dynamic DNA methylation. Sci. 323, 1568–1569. 10.1126/science.1172782.

Lawal, A., Kirtley, M.L., van Lier, C.J., Erova, T.E., Kozlova, E.V., Sha, J. et al., 2013. The effects of modeled microgravity on growth kinetics, antibiotic susceptibility, cold growth, and the virulence potential of a *Yersinia pestis* ymoA-deficient mutant and its isogenic parental strain. Astrobiology 13, 821–832. 10.1089/ast.2013.0968.

Leandro, L.J., Szewczyk, N.J., Benguría, A., Herranz, R., Laván, D., Medina, F.J. et al., 2007. Comparative analysis of *Drosophila melanogaster* and *Caenorhabditis elegans* gene expression experiments in the European Soyuz flights to the International Space Station. Adv. Space Res. 40, 506–512. 10.1016/j.asr.2007.05.070.

Lebert, M., Häder, D.-P., 1996. How *Euglena* tells up from down. Nature 379, 590.

Lebert, M., Häder, D.-P., 1997a. Behavioral mutants of *Euglena gracilis*: functional and spectroscopic characterization. J Plant Physiol 151, 188–195.

Lebert, M., Häder, D.-P., 1997b. Effects of hypergravity on the photosynthetic flagellate, *Euglena gracilis*. J Plant Physiol 150, 153–159.

Lee, M.C., Israelsson, U.E., 2001. New microgravity fundamental physics research areas in the ISS era. Acta Astronaut. 48, 597–602. 10.1016/S0094-5765(01)00029-7.

Lee, S.-M., Young, E.M., Alper, H.S., 2015. Remaining Challenges in the Metabolic Engineering of Yeasts for Biofuels. In: Direct Microbial Conversion of Biomass to Advanced Biofuels: Elsevier. p. 209–237.

Lelkes, P.I., Galvan, D.L., Hayman, G.T., Goodwin, T.J., Chatman, D.Y., Cherian, S. et al., 1998. Simulated microgravity conditions enhance differentiation of cultured PC12 cells towards the neuroendocrine phenotype. In Vitro Cell. Dev. Biol. -Animal 34, 316–325. 10.1007/s11626-998-0008-y.

Lerat, E., Daubin, V., Ochman, H., Moran, N.A., 2005. Evolutionary origins of genomic repertoires in bacteria. PLoS Biol. 3, e130. 10.1371/journal.pbio.0030130.

Lerminiaux, N.A., Cameron, A.D.S., 2019. Horizontal transfer of antibiotic resistance genes in clinical environments. Can. J. Microbiol. 65, 34–44. 10.1139/cjm-2018-0275.

Lewis, M.L., Reynolds, J.L., Cubano, L.A., Hatton, J.P., Lawless, B.D., Piepmeier, E.H., 1998. Spaceflight alters microtubules and increases apoptosis in human lymphocytes (Jurkat). FASEB J. 12, 1007–1018.

Li, H.-F., Chen, X.-P., Zhu, F.-H., Liu, H.-Y., Hong, Y.-B., Liang, X.-Q., 2013. Transcriptome profiling of peanut (*Arachis hypogaea*) gynophores in gravitropic response. Funct. Plant Biol. 40, 1249. 10.1071/FP13075.

Liao, B.-Y., Weng, M.-P., Zhang, J., 2010. Contrasting genetic paths to morphological and physiological evolution. Proc. Natl. Acad. Sci. U. S. A. 107, 7353–7358. 10.1073/pnas.0910339107.

Limbach, C., Hauslage, J., Schäfer, C., Braun, M., 2005. How to activate a plant gravireceptor. Early mechanisms of gravity sensing studied in characean rhizoids during parabolic flights. Plant Physiol. 139, 1030–1040. 10.1104/pp.105.068106.
Lind, M.I., Spagopoulou, F., 2018. Evolutionary consequences of epigenetic inheritance. Heredity 121, 205–209. 10.1038/s41437-018-0113-y.
Løbner-Olesen, A., Skovgaard, O., Marinus, M.G., 2005. Dam methylation: coordinating cellular processes. Curr. Opin. Microbiol. 8, 154–160. 10.1016/j.mib.2005.02.009.
Loftus, B., Anderson, I., Davies, R., Alsmark, U.C.M., Samuelson, J., Amedeo, P. et al., 2005. The genome of the protist parasite *Entamoeba histolytica*. Nature 433, 865–868. 10.1038/nature03291.
Lynch, M., 2006. The origins of eukaryotic gene structure. Mol. Biol. Evol. 23, 450–468. 10.1093/molbev/msj050.
Lynch, M., 2010a. Evolution of the mutation rate. Trends Genet. 26, 345–352. 10.1016/j.tig.2010.05.003.
Lynch, M., 2010b. Rate, molecular spectrum, and consequences of human mutation. Proc. Natl. Acad. Sci. U. S. A. 107, 961–968. 10.1073/pnas.0912629107.
Lynch, M., Sung, W., Morris, K., Coffey, N., Landry, C.R., Dopman, E.B. et al., 2008. A genome-wide view of the spectrum of spontaneous mutations in yeast. Proc. Natl. Acad. Sci. U. S. A. 105, 9272–9277. 10.1073/pnas.0803466105.
Lynch, S.V., Mukundakrishnan, K., Benoit, M.R., Ayyaswamy, P.S., Matin, A., 2006. *Escherichia coli* Biofilms Formed under Low-Shear Modeled Microgravity in a Ground-Based System. Appl. Environ. Microbiol. 72, 7701–7710. 10.1128/AEM.01294-06.
Ma, X., Pietsch, J., Wehland, M., Schulz, H., Saar, K., Hübner, N. et al., 2014. Differential gene expression profile and altered cytokine secretion of thyroid cancer cells in space. FASEB J. 28, 813–835. 10.1096/fj.13-243287.
Ma, X., Wehland, M., Schulz, H., Saar, K., Hübner, N., Infanger, M. et al., 2013. Genomic approach to identify factors that drive the formation of three-dimensional structures by EA.hy926 endothelial cells. PLoS ONE 8, e64402. 10.1371/journal.pone.0064402.
Ma, Y., Cheng, Z., Wang, W., Sun, Y., 2007. Proteomic analysis of high yield rice variety mutated from spaceflight. Adv. Space Res. 40, 535–539. 10.1016/j.asr.2007.05.028.
Mackiewicz, P., Bodył, A., Moszczyński, K., 2013. The case of horizontal gene transfer from bacteria to the peculiar dinoflagellate plastid genome. Mob. Genet. Elements 3, e25845. 10.4161/mge.25845.
MacLean, R.C., Buckling, A., 2009. The distribution of fitness effects of beneficial mutations in *Pseudomonas aeruginosa*. PLoS Genet. 5, e1000406. 10.1371/journal.pgen.1000406.
MacLean, R.C., Torres-Barceló, C., Moxon, R., 2013. Evaluating evolutionary models of stress-induced mutagenesis in bacteria. Nat. Rev. Genet. 14, 221–227. 10.1038/nrg3415.
Manley, S., Cipelletti, L., Trappe, V., Bailey, A.E., Christianson, R.J., Gasser, U. et al., 2004. Limits to gelation in colloidal aggregation. Phys. Rev. Lett. 93, 108302. 10.1103/PhysRevLett.93.108302.
Mann, V., Grimm, D., Corydon, T.J., Krüger, M., Wehland, M., Riwaldt, S. et al., 2019. Changes in Human Foetal Osteoblasts Exposed to the Random Positioning Machine and Bone Construct Tissue Engineering. Int. J. Mol. Sci. 20. 10.3390/ijms20061357.
Manzano, A.I., van Loon, Jack JWA, Christianen, P.C.M., Gonzalez-Rubio, J.M., Medina, F.J., Herranz, R., 2012. Gravitational and magnetic field variations synergize to cause subtle variations in the global transcriptional state of *Arabidopsis* in vitro callus cultures. BMC Genomics 13, 105. 10.1186/1471-2164-13-105.
Mao, X.W., Pecaut, M.J., Stodieck, L.S., Ferguson, V.L., Bateman, T.A., Bouxsein, M. et al., 2013. Spaceflight Environment Induces Mitochondrial Oxidative Damage in Ocular Tissue. Radiat. Res. 180, 340–350. 10.1667/RR3309.1.
Marcet-Houben, M., Gabaldón, T., 2010. Acquisition of prokaryotic genes by fungal genomes. Trends Genet. 26, 5–8. 10.1016/j.tig.2009.11.007.
Marin, B., Nowack, E.C.M., Glöckner, G., Melkonian, M., 2007. The ancestor of the *Paulinella* chromatophore obtained a carboxysomal operon by horizontal gene transfer from a *Nitrococcus*-like gamma-proteobacterium. BMC Evol Biol 7, 85. 10.1186/1471-2148-7-85.
Martinez, E.M., Yoshida, M.C., Candelario, T.L.T., Hughes-Fulford, M., 2015. Spaceflight and simulated microgravity cause a significant reduction of key gene expression in early T-cell activation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 308, R480-8. 10.1152/ajpregu.00449.2014.

Martzivanou, M., Babbick, M., Cogoli-Greuter, M., Hampp, R., 2006. Microgravity-related changes in gene expression after short-term exposure of *Arabidopsis thaliana* cell cultures. Protoplasma 229, 155–162. 10.1007/s00709-006-0203-1.

Martzivanou, M., Hampp, R., 2003. Hyper-gravity effects on the *Arabidopsis* transcriptome. Physiol. Plant 118, 221–231.

Masiello, M.G., Cucina, A., Proietti, S., Palombo, A., Coluccia, P., D'Anselmi, F. et al., 2014. Phenotypic switch induced by simulated microgravity on MDA-MB-231 breast cancer cells. Biomed. Res. Int. 2014, 652434. 10.1155/2014/652434.

Masson-Delmotte, V., Zhai, P., Pörtner, H.O., Roberts, D., Skea, J., Shukla, P.R. et al. 1. IPCC, 2018: Global warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty, 2018.

Mauclaire, L., Egli, M., 2010. Effect of simulated microgravity on growth and production of exopolymeric substances of *Micrococcus luteus* space and earth isolates. FEMS Immunol. Med. Microbiol. 59, 350–356. 10.1111/j.1574-695X.2010.00683.x.

Mazars, C., Brière, C., Grat, S., Pichereaux, C., Rossignol, M., Pereda-Loth, V. et al., 2014a. Microgravity induces changes in microsome-associated proteins of *Arabidopsis* seedlings grown on board the international space station. PLoS ONE 9, e91814. 10.1371/journal.pone.0091814.

Mazars, C., Brière, C., Grat, S., Pichereaux, C., Rossignol, M., Pereda-Loth, V. et al., 2014b. Microsome-associated proteome modifications of *Arabidopsis* seedlings grown on board the International Space Station reveal the possible effect on plants of space stresses other than microgravity. Plant Signal. Behav. 9, e29637. 10.4161/psb.29637.

McKenzie, G.J., Harris, R.S., Lee, P.L., Rosenberg, S.M., 2000. The SOS response regulates adaptive mutation. Proc. Natl. Acad. Sci. U. S. A. 97, 6646–6651. 10.1073/pnas.120161797.

McKenzie, J.A., Batterham, P., 1994. The genetic, molecular and phenotypic consequences of selection for insecticide resistance. Trends Ecol. Evol. 9, 166–169. 10.1016/0169-5347(94)90079-5.

McPherson, A., DeLucas, L., 1999. Crystal-growing in space. Science 283, 1459.

Melnik, D., Krüger, M., Kopp, S., Wehland, M., Bauer, J., Infanger, M. et al., 2018. Microgravity-based Modulation of VEGF Expression in Human Thyroid Carcinoma Cells. Front. Physiol. 9. 10.3389/conf.fphys.2018.26.00002.

Mermel, L.A., 2012. Infection Prevention and Control During Prolonged Human Space Travel. Clin. Infect. Dis. 56, 123–130. 10.1093/cid/cis861.

Millar, K.D., Johnson, C.M., Edelmann, R.E., Kiss, J.Z., 2011. An endogenous growth pattern of roots is revealed in seedlings grown in microgravity. Astrobiology 11, 787–797. 10.1089/ast.2011.0699.

Millar, K.D., Kiss, J.Z., 2013. Analyses of tropistic responses using metabolomics. Am. J. Bot. 100, 79–90. 10.3732/ajb.1200316.

Monciardini, P., Iorio, M., Maffioli, S., Sosio, M., Donadio, S., 2014. Discovering new bioactive molecules from microbial sources. Microbial biotechnology 7, 209–220. 10.1111/1751-7915.12123.

Monk, M., 1995. Epigenetic programming of differential gene expression in development and evolution. Develop. Genet. 17, 188–197. 10.1002/dvg.1020170303.

Morabito, C., Guarnieri, S., Catizone, A., Schiraldi, C., Ricci, G., Mariggiò, M.A., 2017. Transient increases in intracellular calcium and reactive oxygen species levels in TCam-2 cells exposed to microgravity. Sci. Rep. 7, 15648. 10.1038/s41598-017-15935-z.

Moreno-Villanueva, M., Wong, M., Lu, T., Zhang, Y., Wu, H., 2017. Interplay of space radiation and microgravity in DNA damage and DNA damage response. npj Microgravity 3, 14. 10.1038/s41526-017-0019-7.

Morrison, M.D., Fajardo-Cavazos, P., Nicholson, W.L., 2019. Comparison of *Bacillus subtilis* transcriptome profiles from two separate missions to the International Space Station. npj Microgravity 5, 1. 10.1038/s41526-018-0061-0.

Motamedi, M.R., Szigety, S.K., Rosenberg, S.M., 1999. Double-strand-break repair recombination in *Escherichia coli*: physical evidence for a DNA replication mechanism in vivo. Genes Dev. 13, 2889–2903.

Müller, G.B., 2003. Embryonic motility: environmental influences and evolutionary innovation. Evol. Dev. 5, 56–60. 10.1046/j.1525-142X.2003.03009.x.

Musilova, Z., Cortesi, F., Matschiner, M., Davies, W.I.L., Patel, J.S., Stieb, S.M. et al., 2019. Vision using multiple distinct rod opsins in deep-sea fishes. Sci. 364, 588–592. 10.1126/science.aav4632.
Nakamura, Y., Itoh, T., Matsuda, H., Gojobori, T., 2004. Biased biological functions of horizontally transferred genes in prokaryotic genomes. Nat. Genet. 36, 760–766. 10.1038/ng1381.
Nasir, A., Le Bail, A., Daiker, V., Klima, J., Richter, P., Lebert, M., 2018. Identification of a flagellar protein implicated in the gravitaxis in the flagellate *Euglena gracilis*. Sci. Rep. 8, 7605. 10.1038/s41598-018-26046-8.
Nasir, A., Strauch, S.M., Becker, I., Sperling, A., Schuster, M., Richter, P.R. et al., 2014. The influence of microgravity on *Euglena gracilis* as studied on Shenzhou 8. Plant Biol. J. 16, 113–119. 10.1111/plb.12067.
Nassef, M.Z., Kopp, S., Wehland, M., Melnik, D., Sahana, J., Krüger, M. et al., 2019. Real Microgravity Influences the Cytoskeleton and Focal Adhesions in Human Breast Cancer Cells. Int. J. Mol. Sci. 20. 10.3390/ijms20133156.
Nedelcu, A.M., Miles, I.H., Fagir, A.M., Karol, K., 2008. Adaptive eukaryote-to-eukaryote lateral gene transfer: stress-related genes of algal origin in the closest unicellular relatives of animals. J. Evol. Biol. 21, 1852–1860. 10.1111/j.1420-9101.2008.01605.x.
Neef, M., Ecke, M., Hampp, R., 2015. Real-time Recording of Cytosolic Calcium Levels in *Arabidopsis* thaliana Cell Cultures during Parabolic Flights. Microgravity Sci. Technol. 27, 305–312. 10.1007/s12217-015-9461-x.
Nei, M., 1987. Molecular evolutionary genetics Columbia University Press, New York.
Nei, M., 2005. Selectionism and neutralism in molecular evolution. Mol. Biol. Evol. 22, 2318–2342. 10.1093/molbev/msi242.
Nei, M., 2013. Mutation-driven evolution Oxford University Press, Oxford.
Nei, M., Rooney, A.P., 2005. Concerted and birth-and-death evolution of multigene families. Annu. Rev. Genet. 39, 121–152. 10.1146/annurev.genet.39.073003.112240.
Nelson, K.E., Clayton, R.A., Gill, S.R., Gwinn, M.L., Dodson, R.J., Haft, D.H. et al., 1999. Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of *Thermotoga maritima*. Nature 399, 323–329. 10.1038/20601.
Ness, R.W., Morgan, A.D., Colegrave, N., Keightley, P.D., 2012. Estimate of the spontaneous mutation rate in *Chlamydomonas reinhardtii*. Genetics 192, 1447–1454. 10.1534/genetics.112.145078.
Nevo, E., 2011. Evolution under environmental stress at macro- and microscales. Genome Biol. Evol. 3, 1039–1052. 10.1093/gbe/evr052.
Newton, A.C., 2016. Exploitation of Diversity within Crops-the Key to Disease Tolerance? Front. Plant Sci. 7, 665. 10.3389/fpls.2016.00665.
Nickerson, C.A., Ott, C.M., Mister, S.J., Morrow, B.J., Burns-Keliher, L., Pierson, D.L., 2000. Microgravity as a novel environmental signal affecting *Salmonella enterica* serovar Typhimurium virulence. Infect. Immun. 68, 3147–3152.
Nickerson, C.A., Ott, C.M., Wilson, J.W., Ramamurthy, R., Pierson, D.L., 2004. Microbial responses to microgravity and other low-shear environments. Microbiol. Mol. Biol. Rev. 68, 345–361.
Nikawa, T., Ishidoh, K., Hirasaka, K., Ishihara, I., Ikemoto, M., Kano, M. et al., 2004. Skeletal muscle gene expression in space-flown rats. FASEB J. 18, 522–524. 10.1096/fj.03-0419fje.
Noireaux, V., Libchaber, A., 2004. A vesicle bioreactor as a step toward an artificial cell assembly. Proc. Natl. Acad. Sci. U. S. A. 101, 17669–17674. 10.1073/pnas.0408236101.
Nordin, S.M., Nordin, K.J., 2017. Food, the source of Nutrition. WN 8, 87. 10.26596/wn.20178187-94.
Ntefidou, M., Iseki, M., Watanabe, M., Lebert, M., Häder, D.-P., 2003. Photoactivated adenylyl cyclase controls phototaxis in the flagellate *Euglena gracilis*. Plant Physiol. 133, 1517–1521.
Ochman, H., Lawrence, J.G., Groisman, E.A., 2000. Lateral gene transfer and the nature of bacterial innovation. Nature 405, 299–304. 10.1038/35012500.
Ohta, T., 1992. The Nearly Neutral Theory of Molecular Evolution. Annu. Rev. Ecol. Syst. 23, 263–286. 10.1146/annurev.es.23.110192.001403.
Ohyabu, Y., Kida, N., Kojima, H., Taguchi, T., Tanaka, J., Uemura, T., 2006. Cartilaginous tissue formation from bone marrow cells using rotating wall vessel (RWV) bioreactor. Biotechnol. Bioeng. 95, 1003–1008. 10.1002/bit.20892.
Okabe, K., Inada, N., Gota, C., Harada, Y., Funatsu, T., Uchiyama, S., 2012. Intracellular temperature mapping with a fluorescent polymeric thermometer and fluorescence lifetime imaging microscopy. Nat. Commun. 3, 705. 10.1038/ncomms1714.

Okubo, T., Tsuchida, A., Okuda, T., Fujitsuna, K., Ishikawa, M., Morita, T. et al., 1999. Kinetic analyses of colloidal crystallization in microgravity — aircraft experiments. Colloids **Surf**. A Physicochem. Eng. Asp. 153, 515–524. 10.1016/S0927-7757(98)00474-9.

Okubo, T., Tsuchida, A., Takahashi, S., Taguchi, K., Ishikawa, M., 2000. Kinetics of colloidal alloy crystallization of binary mixtures of monodispersed polystyrene and/or colloidal silica spheres having different sizes and densities in microgravity using aircraft. Coll. Polymer Sci. 278, 202–210. 10.1007/s003960050033.

Orsini, S.S., Lewis, A.M., Rice, K.C., 2017. Investigation of simulated microgravity effects on Streptococcus mutans physiology and global gene expression. npj Microgravity 3, 4. 10.1038/s41526-016-0006-4.

Ou, X., Long, L., Wu, Y., Yu, Y., Lin, X., Qi, X. et al., 2010. Spaceflight-induced genetic and epigenetic changes in the rice (*Oryza sativa* L.) genome are independent of each other. Genome 53, 524–532. 10.1139/G10-030.

Pacello, F., Rotilio, G., Battistoni, A., 2012. Low-Shear Modeled Microgravity Enhances Salmonella Enterica Resistance to Hydrogen Peroxide Through a Mechanism Involving KatG and KatN. Open Microbiol. J. 6, 53–64. 10.2174/1874285801206010053.

Pál, C., Papp, B., Lercher, M.J., 2005. Adaptive evolution of bacterial metabolic networks by horizontal gene transfer. Nat. Genet. 37, 1372–1375. 10.1038/ng1686.

Palazzo, A.F., Lee, E.S., 2015. Non-coding RNA: what is functional and what is junk? Frontiers in genetics 6, 2. 10.3389/fgene.2015.00002.

Parsons, P.A., 2005. Environments and evolution: interactions between stress, resource inadequacy and energetic efficiency. Biol. Rev. Camb. Philos. Soc. 80, 589–610. 10.1017/S1464793105006822.

Patel, M.J., Liu, W., Sykes, M.C., Ward, N.E., Risin, S.A., Risin, D. et al., 2007. Identification of mechanosensitive genes in osteoblasts by comparative microarray studies using the rotating wall vessel and the random positioning machine. J. Cell. Biochem. 101, 587–599. 10.1002/jcb.21218.

Patil, V.S., Zhou, R., Rana, T.M., 2014. Gene regulation by non-coding RNAs. Crit. Rev. Biochem. Mol. Biol. 49, 16–32. 10.3109/10409238.2013.844092.

Paul, A.-L., Amalfitano, C.E., Ferl, R.J., 2012a. Plant growth strategies are remodeled by spaceflight. BMC Plant Biol. 12, 232. 10.1186/1471-2229-12-232.

Paul, A.-L., Ferl, R.J., Meisel, M.W., 2006. High magnetic field induced changes of gene expression in *Arabidopsis*. Biomagn. Res. Technol. 4, 7. 10.1186/1477-044X-4-7.

Paul, A.-L., Manak, M.S., Mayfield, J.D., Reyes, M.F., Gurley, W.B., Ferl, R.J., 2011. Parabolic flight induces changes in gene expression patterns in *Arabidopsis thaliana*. Astrobiology 11, 743–758. 10.1089/ast.2011.0659.

Paul, A.-L., Popp, M.P., Gurley, W.B., Guy, C., Norwood, K.L., Ferl, R.J., 2005. *Arabidopsis* gene expression patterns are altered during spaceflight. Adv. Space Res. 36, 1175–1181. 10.1016/j.asr.2005.03.066.

Paul, A.-L., Zupanska, A.K., Ostrow, D.T., Zhang, Y., Sun, Y., Li, J.-L. et al., 2012b. Spaceflight transcriptomes: Unique responses to a novel environment. Astrobiology 12, 40–56. 10.1089/ast.2011.0696.

Paul, A.-L., Zupanska, A.K., Schultz, E.R., Ferl, R.J., 2013. Organ-specific remodeling of the *Arabidopsis* transcriptome in response to spaceflight. BMC Plant Biol. 13, 112. 10.1186/1471-2229-13-112.

Pérez-Losada, M., Porter, M.L., Tazi, L., Crandall, K.A., 2007. New methods for inferring population dynamics from microbial sequences. Infect. Genet. Evol. 7, 24–43. 10.1016/j.meegid.2006.03.004.

Perrin, J., 1916. Atoms Constable, London & Co.

Philpott, D.E., Popova, I.A., Kato, K., Stevenson, J., Miquel, J., Sapp, W., 1990. Morphological and biochemical examination of Cosmos 1887 rat heart tissue: Part I--Ultrastructure. FASEB J. 4, 73–78.

Pickens, L.B., Tang, Y., Chooi, Y.-H., 2011. Metabolic engineering for the production of natural products. Annu. Rev. Chem. Biomol. Eng. 2, 211–236. 10.1146/annurev-chembioeng-061010-114209.

Pietsch, J., Gass, S., Nebuloni, S., Echegoyen, D., Riwaldt, S., Baake, C. et al., 2017. Three-dimensional growth of human endothelial cells in an automated cell culture experiment container during the SpaceX CRS-8 ISS space mission - The SPHEROIDS project. Biomaterials 124, 126–156. 10.1016/j.biomaterials.2017.02.005.

Pitzschke, A., Hirt, H., 2010. New insights into an old story: *Agrobacterium*-induced tumour formation in plants by plant transformation. EMBO J 29, 1021–1032. 10.1038/emboj.2010.8.
Pletser, V., 2004. Short duration microgravity experiments in physical and life sciences during parabolic flights: the first 30 ESA campaigns. Acta Astronaut. 55, 829–854. 10.1016/j.actaastro.2004.04.006.
Polz, M.F., Alm, E.J., Hanage, W.P., 2013. Horizontal gene transfer and the evolution of bacterial and archaeal population structure. Trends Genet. 29, 170–175. 10.1016/j.tig.2012.12.006.
Popova, A.F., 2003. Comparative characteristic of mitochondria ultrastructural organization in *Chlorella* cells under altered gravity conditions. Adv. Space Res. 31, 2253–2259.
Prasad, B., Lein, W., Lindenberger, C.P., Buchholz, R., Vadakedath, N., 2019a. An optimized method and a dominant selectable marker for genetic engineering of an industrially promising microalga—*Pavlova lutheri*. J. Appl. Phycol. 31, 1163–1174. 10.1007/s10811-018-1617-9.
Prasad, B., Lein, W., Thiyam, G., Lindenberger, C.P., Buchholz, R., Vadakedath, N., 2019b. Stable nuclear transformation of rhodophyte species *Porphyridium purpureum*: advanced molecular tools and an optimized method. Photosynth. Res. 140, 173–188. 10.1007/s11120-018-0587-8.
Prasad, B., Vadakedath, N., Jeong, H.-J., General, T., Cho, M.-G., Lein, W., 2014. *Agrobacterium tumefaciens*-mediated genetic transformation of haptophytes (*Isochrysis* species). Appl. Microbiol. Biotechnol. 98, 8629–8639. 10.1007/s00253-014-5900-7.
Pross, H.D., Kiefer, J., 1999. Repair of cellular radiation damage in space under microgravity conditions. Radiat. Environ. Biophys. 38, 133–138. 10.1007/s004110050149.
Qian, A., Zhang, W., Xie, L., Weng, Y., Yang, P., Wang, Z. et al., 2008. Simulated weightlessness alters biological characteristics of human breast cancer cell line MCF-7. Acta Astronaut. 63, 947–958. 10.1016/j.actaastro.2008.01.024.
Rakyan, V.K., Chong, S., Champ, M.E., Cuthbert, P.C., Morgan, H.D., Luu, K.V.K. et al., 2003. Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. Proc. Natl. Acad. Sci. U. S. A. 100, 2538–2543. 10.1073/pnas.0436776100.
Reid, J.B., Ross, J.J., 2011. Mendel's genes: toward a full molecular characterization. Genetics 189, 3–10. 10.1534/genetics.111.132118.
Rest, J.S., Mindell, D.P., 2003. SARS associated coronavirus has a recombinant polymerase and coronaviruses have a history of host-shifting. Infect. Genet. Evol. 3, 219–225. 10.1016/j.meegid.2003.08.001.
Rétaux, S., Casane, D., 2013. Evolution of eye development in the darkness of caves: adaptation, drift, or both? EvoDevo 4, 26. 10.1186/2041-9139-4-26.
Rettberg, P., Eschweiler, U., Strauch, K., Reitz, G., Horneck, G., Wanke, H. et al., 2002. Survival of microorganisms in space protected by meteorite material: results of the experiment 'EXOBIOLOGIE' of the PERSEUS mission. Adv. Space Res. 30, 1539–1545.
Rhodes, F.H., 1983. Gradualism, punctuated equilibrium and the Origin of Species. Nature 305, 269–272. 10.1038/305269a0.
Ricard, G., McEwan, N.R., Dutilh, B.E., Jouany, J.-P., Macheboeuf, D., Mitsumori, M. et al., 2006. Horizontal gene transfer from Bacteria to rumen Ciliates indicates adaptation to their anaerobic, carbohydrates-rich environment. BMC Genomics 7, 22. 10.1186/1471-2164-7-22.
Richards, T.A., Dacks, J.B., Campbell, S.A., Blanchard, J.L., Foster, P.G., McLeod, R. et al., 2006. Evolutionary origins of the eukaryotic shikimate pathway: gene fusions, horizontal gene transfer, and endosymbiotic replacements. Eukaryot. Cell 5, 1517–1531. 10.1128/EC.00106-06.
Richter, P., Lebert, M., Tahedl, H., Häder, D.-P., 2001. Calcium is involved in the gravitactic orientation in colorless flagellates. J. Plant Physiol. 158, 689–697.
Richter, P., Ntefidou, M., Streb, C., Lebert, M., Häder, D.-P., 2002. Cellular perception and transduction mechanisms of gravity in unicellular organisms. Curr. Top. Plant Biol. 3, 143–154.
Roberts, D., 1985. IS10 transposition is regulated by DNA adenine methylation. Cell 43, 117–130. 10.1016/0092-8674(85)90017-0.
Rocha, E.P., 2008. Evolutionary patterns in prokaryotic genomes. Curr. Opin. Microbiol. 11, 454–460. 10.1016/j.mib.2008.09.007.
Rosenberg, S.M., 2001. Evolving responsively: adaptive mutation. Nat. Rev. Genet. 2, 504–515. 10.1038/35080556.

Rosenzweig, J.A., Ahmed, S., Eunson, J., Chopra, A.K., 2014. Low-shear force associated with modeled microgravity and spaceflight does not similarly impact the virulence of notable bacterial pathogens. Appl. Microbiol. Biotechnol. 98, 8797–8807. 10.1007/s00253-014-6025-8.

Rosenzweig, J.A., Chopra, A.K., 2012. The effect of low shear force on the virulence potential of *Yersinia pestis*: new aspects that space-like growth conditions and the final frontier can teach us about a formidable pathogen. Front. Cell. Infect. Microbiol. 2, 107. 10.3389/fcimb.2012.00107.

Royall, C.P., van Roij, R., van Blaaderen, A., 2005. Extended sedimentation profiles in charged colloids: the gravitational length, entropy, and electrostatics. J. Phys.: Condens. Matter 17, 2315–2326. 10.1088/0953-8984/17/15/005.

Rutherford, S.L., 2000. From genotype to phenotype: buffering mechanisms and the storage of genetic information. Bioessays 22, 1095–1105. 10.1002/1521-1878(200012)22:12<1095:AID-BIES7>3.0.CO;2-A.

Rutherford, S.L., 2003. Between genotype and phenotype: protein chaperones and evolvability. Nat. Rev. Genet. 4, 263–274. 10.1038/nrg1041.

Ruyters, G., Braun, M., 2014. Plant biology in space: Recent accomplishments and recommendations for future research. Plant Biol. 16 Suppl 1, 4–11. 10.1111/plb.12127.

Ruyters, G., Friedrich, U., 2006. From the Bremen drop tower to the international space station ISS - ways to weightlessness in the German Space Life Sciences Program. Signal Transduct. 6, 397–405.

Safari, M.S., Poling-Skutvik, R., Vekilov, P.G., Conrad, J.C., 2017. Differential dynamic microscopy of bidisperse colloidal suspensions. npj Microgravity 3, 21. 10.1038/s41526-017-0027-7.

Sahana, J., Nassef, M.Z., Wehland, M., Kopp, S., Krüger, M., Corydon, T.J. et al., 2018. Decreased E-Cadherin in MCF7 Human Breast Cancer Cells Forming Multicellular Spheroids Exposed to Simulated Microgravity. Proteomics 18, e1800015. 10.1002/pmic.201800015.

Sakai, S., Mishima, H., Ishii, T., Akaogi, H., Yoshioka, T., Ohyabu, Y. et al., 2009. Rotating three-dimensional dynamic culture of adult human bone marrow-derived cells for tissue engineering of hyaline cartilage. J. Orthop. Res. 27, 517–521. 10.1002/jor.20566.

Salmi, M.L., Roux, S.J., 2008. Gene expression changes induced by space flight in single-cells of the fern Ceratopteris richardii. Planta 229, 151–159. 10.1007/s00425-008-0817-y.

Salmi, M.L., Ul Haque, A., Bushart, T.J., Stout, S.C., Roux, S.J., Porterfield, D.M., 2011. Changes in gravity rapidly alter the magnitude and direction of a cellular calcium current. Planta 233, 911–920. 10.1007/s00425-010-1343-2.

Salzberg, S.L., White, O., Peterson, J., Eisen, J.A., 2001. Microbial genes in the human genome: lateral transfer or gene loss? Sci. 292, 1903–1906. 10.1126/science.1061036.

Sambandam, Y., Townsend, M.T., Pierce, J.J., Lipman, C.M., Haque, A., Bateman, T.A. et al., 2014. Microgravity control of autophagy modulates osteoclastogenesis. Bone 61, 125–131. 10.1016/j.bone.2014.01.004.

Sanford, G.L., Ellerson, D., Melhado-Gardner, C., Sroufe, A.E., Harris-Hooker, S., 2002. Three-dimensional growth of endothelial cells in the microgravity-based rotating wall vessel bioreactor. In Vitro Cell Dev. Biol. Animal 38, 493–504.

Sani, E., Herzyk, P., Perrella, G., Colot, V., Amtmann, A., 2013. Hyperosmotic priming of *Arabidopsis* seedlings establishes a long-term somatic memory accompanied by specific changes of the epigenome. Genome Biol. 14, R59. 10.1186/gb-2013-14-6-r59.

Sanjuán, R., Moya, A., Elena, S.F., 2004. The distribution of fitness effects caused by single-nucleotide substitutions in an RNA virus. Proc. Natl. Acad. Sci. U. S. A. 101, 8396–8401. 10.1073/pnas.0400146101.

Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., Nelson, A., 2019. The global burden of pathogens and pests on major food crops. Nat. Ecol. Evol. 3, 430–439. 10.1038/s41559-018-0793-y.

Saylo, M.C., Escoton, C.C., Saylo, M.M., 2011. Punctuated equilibrium vs. phyletic gradualism. Int. J. BioSci. Biotechnol. 3, 27–42.

Schöb, C., Kerle, S., Karley, A.J., Morcillo, L., Pakeman, R.J., Newton, A.C. et al., 2015. Intraspecific genetic diversity and composition modify species-level diversity-productivity relationships. New Phytol. 205, 720–730. 10.1111/nph.13043.

Schüler, O., Hemmersbach, R., Böhmer, M., 2015. A Bird's-Eye View of Molecular Changes in Plant Gravitropism Using Omics Techniques. Front. Plant Sci. 6, 1176. 10.3389/fpls.2015.01176.

Schüler, O., Krause, L., Görög, M., Hauslage, J., Kesseler, L., Böhmer, M. et al., 2016. ARADISH - Development of a Standardized Plant Growth Chamber for Experiments in Gravitational Biology Using Ground Based Facilities. Microgravity sci. Technol. 28, 297–305. 10.1007/s12217-015-9454-9.

Scoville, H., 2019. Gradualism vs. Punctuated Equilibrium. Available from: https://www.thoughtco.com/gradualism-vs-punctuated-equilibrium-1224811.

Searles, S.C., Woolley, C.M., Petersen, R.A., Hyman, L.E., Nielsen-Preiss, S.M., 2011. Modeled microgravity increases filamentation, biofilm formation, phenotypic switching, and antimicrobial resistance in *Candida albicans*. Astrobiology 11, 825–836. 10.1089/ast.2011.0664.

Senatore, G., Mastroleo, F., Leys, N., Mauriello, G., 2018. Effect of microgravity & space radiation on microbes. Future Microbiol. 13, 831–847. 10.2217/fmb-2017-0251.

Sharif, T., Martell, E., Dai, C., Kennedy, B.E., Murphy, P., Clements, D.R. et al., 2017. Autophagic homeostasis is required for the pluripotency of cancer stem cells. Autophagy 13, 264–284. 10.1080/15548627.2016.1260808.

Sharma, A., Singh, R.S., Gupta, G., Ahmad, T., Kaur, B., 2019. Metabolic Engineering of Enzyme-Regulated Bioprocesses. In: Advances in Enzyme Technology: Elsevier. p. 293–323.

Shi, F., Zhao, T.-Z., Wang, Y.-C., Cao, X.-S., Yang, C.-B., Gao, Y. et al., 2016. The Impact of Simulated Weightlessness on Endothelium-Dependent Angiogenesis and the Role of Caveolae/Caveolin-1. Cell. Physiol. Biochem. 38, 502–513. 10.1159/000438646.

Sieber, K.B., Bromley, R.E., Dunning Hotopp, J.C., 2017. Lateral gene transfer between prokaryotes and eukaryotes. Exp. Cell. Res. 358, 421–426. 10.1016/j.yexcr.2017.02.009.

Sieber, M., Kaltenbach, S., Hanke, W., Kohn, F.P.M., 2016. Conductance and Capacity of Plain Lipid Membranes under Conditions of Variable Gravity. J. Biomed. Sci. Eng. 09, 361–366. 10.4236/jbise.2016.98031.

Sikavitsas, V.I., Bancroft, G.N., Mikos, A.G., 2002. Formation of three-dimensional cell/polymer constructs for bone tissue engineering in a spinner flask and a rotating wall vessel bioreactor. J. Biomed. Mater. Res. A 62, 136–148. 10.1002/jbm.10150.

Smillie, C.S., Smith, M.B., Friedman, J., Cordero, O.X., David, L.A., Alm, E.J., 2011. Ecology drives a global network of gene exchange connecting the human microbiome. Nature 480, 241–244. 10.1038/nature10571.

Sniegowski, P., Raynes, Y., 2013. Mutation rates: how low can you go? Curr. Biol. 23, R147-9. 10.1016/j.cub.2013.01.018.

Soanes, D., Richards, T.A., 2014. Horizontal gene transfer in eukaryotic plant pathogens. Annu. Rev. Phytopathol. 52, 583–614. 10.1146/annurev-phyto-102313-050127.

Soni, A., O'Sullivan, L., Quick, L.N., Ott, C.M., Nickerson, C.A., Wilson, J.W., 2014. Conservation of the Low-shear Modeled Microgravity Response in Enterobacteriaceae and Analysis of the trp Genes in this Response. Open Microbiol. J. 8, 51–58. 10.2174/1874285801408010051.

Soucy, S.M., Huang, J., Gogarten, J.P., 2015. Horizontal gene transfer: building the web of life. Nat. Rev. Genet. 16, 472–482. 10.1038/nrg3962.

Sprenger, H.J., Marquardt, P., Tadros, T.F., 1993. The scope of microgravity experiments in colloid science. Adv. Colloid Interface Sci. 46, 343–347. 10.1016/0001-8686(93)80048-G.

Stamenković, V., Keller, G., Nesic, D., Cogoli, A., Grogan, S.P., 2010. Neocartilage formation in 1 g, simulated, and microgravity environments: implications for tissue engineering. Tissue Eng. Part A 16, 1729–1736. 10.1089/ten.tea.2008.0624.

Stanhope, M.J., Lupas, A., Italia, M.J., Koretke, K.K., Volker, C., Brown, J.R., 2001. Phylogenetic analyses do not support horizontal gene transfers from bacteria to vertebrates. Nature 411, 940–944. 10.1038/35082058.

Stout, S.C., Porterfield, D.M., Briarty, L.G., Kuang, A., Musgrave, M.E., 2001. Evidence of Root Zone Hypoxia in *Brassica rapa* L. Grown in Microgravity. Int. J. Plant Sci. 162, 249–255. 10.1086/319585.

Strauch, S.M., Richter, P., Schuster, M., Häder, D.-P., 2010. The beating pattern of the flagellum of *Euglena gracilis* under altered gravity during parabolic flights. J. Plant Physiol. 167, 41–46. 10.1016/j.jplph.2009.07.009.

Streb, C., Richter, P., Ntefidou, M., Lebert, M., Häder, D.-P., 2002. Sensory transduction of gravitaxis in *Euglena gracilis*. J. Plant Physiol. 159, 855–862.

Stutte, G.W., Monje, O., Hatfield, R.D., Paul, A.-L., Ferl, R.J., Simone, C.G., 2006. Microgravity effects on leaf morphology, cell structure, carbon metabolism and mRNA expression of dwarf wheat. Planta 224, 1038–1049. 10.1007/s00425-006-0290-4.

Sung, W., Ackerman, M.S., Miller, S.F., Doak, T.G., Lynch, M., 2012. Drift-barrier hypothesis and mutation-rate evolution. Proc. Natl. Acad. Sci. U. S. A. 109, 18488–18492. 10.1073/pnas.1216223109.

Suparta, W., Zulkeple, S.K., 2014. Spatial Analysis of Galactic Cosmic Ray Particles in Low Earth Orbit/Near Equator Orbit Using SPENVIS. J. Phys.: Conf. Ser. 495, 12040. 10.1088/1742-6596/495/1/012040.

Tahedl, H., Richter, P., Lebert, M., Häder, D.-P., 1998. cAMP is involved in gravitaxis signal transduction of *Euglena gracilis*. Microgravity Sci. Technol. 11, 173–178.

Tan, C., Wang, H., Zhang, Y., Qi, B., Xu, G., Zheng, H., 2011. A proteomic approach to analyzing responses of *Arabidopsis thaliana* root cells to different gravitational conditions using an agravitropic mutant, pin2 and its wild type. Proteome Sci. 9, 72. 10.1186/1477-5956-9-72.

Taylor, G.R., 1974. Space Microbiology. Annu. Rev. Microbiol. 28, 121–137. 10.1146/annurev.mi.28.100174.001005.

Thatcher, J.W., Shaw, J.M., Dickinson, W.J., 1998. Marginal fitness contributions of nonessential genes in yeast. Proc. Natl. Acad. Sci. U. S. A. 95, 253–257. 10.1073/pnas.95.1.253.

Thewissen, J.G.M., Cooper, L.N., George, J.C., Bajpai, S., 2009. From Land to Water: the Origin of Whales, Dolphins, and Porpoises. Evo. Edu. Outreach 2, 272–288. 10.1007/s12052-009-0135-2.

Timmery, S., Hu, X., Mahillon, J., 2011. Characterization of Bacilli isolated from the confined environments of the Antarctic Concordia station and the International Space Station. Astrobiology 11, 323–334. 10.1089/ast.2010.0573.

Tirumalai, M.R., Karouia, F., Tran, Q., Stepanov, V.G., Bruce, R.J., Ott, C.M. et al., 2017. The adaptation of *Escherichia coli* cells grown in simulated microgravity for an extended period is both phenotypic and genomic. npj Microgravity 3, 15. 10.1038/s41526-017-0020-1.

Tirumalai, M.R., Karouia, F., Tran, Q., Stepanov, V.G., Bruce, R.J., Ott, C.M. et al., 2019. Evaluation of Acquired Antibiotic Resistance in *Escherichia coli* Exposed to Long-Term Low-Shear Modeled Microgravity and Background Antibiotic Exposure. mBio. 10. 10.1128/mBio.02637-18.

Todd, P., 1989. Gravity-dependent phenomena at the scale of the single cell. ASGSB Bull. 2, 95–113.

Tomita, Y., Seki, T., Fukaya, N., Nishikawa, S., Sato, N., Imai, M. et al., 2018. Crystallization of Charged Colloids under Microgravity during Aircraft Parabolic Flights. Int. J. Microgravity Sci. Appl. 35, 1–6.

Treangen, T.J., Rocha, E.P.C., 2011. Horizontal transfer, not duplication, drives the expansion of protein families in prokaryotes. PLoS Genet 7, e1001284. 10.1371/journal.pgen.1001284.

Tucker, D.L., Ott, C.M., Huff, S., Fofanov, Y., Pierson, D.L., Willson, R.C. et al., 2007. Characterization of *Escherichia coli* MG1655 grown in a low-shear modeled microgravity environment. BMC Microbiol. 7, 15. 10.1186/1471-2180-7-15.

Ulbrich, C., Pietsch, J., Grosse, J., Wehland, M., Schulz, H., Saar, K. et al., 2011. Differential gene regulation under altered gravity conditions in follicular thyroid cancer cells: relationship between the extracellular matrix and the cytoskeleton. Cell. Physiol. Biochem. 28, 185–198. 10.1159/000331730.

Ulbrich, C., Wehland, M., Pietsch, J., Aleshcheva, G., Wise, P., van Loon, J.J.W.A. et al., 2014. The impact of simulated and real microgravity on bone cells and mesenchymal stem cells. Biomed Res. Int. 2014, 928507. 10.1155/2014/928507.

Ulbrich, C., Westphal, K., Baatout, S., Wehland, M., Bauer, J., Flick, B. et al., 2008. Effects of basic fibroblast growth factor on endothelial cells under conditions of simulated microgravity. J. Cell Biochem. 104, 1324–1341. 10.1002/jcb.21710.

van der Graaf, A., Wardenaar, R., Neumann, D.A., Taudt, A., Shaw, R.G., Jansen, R.C. et al., 2015. Rate, spectrum, and evolutionary dynamics of spontaneous epimutations. Proc. Natl. Acad. Sci. U. S. A. 112, 6676–6681. 10.1073/pnas.1424254112.

van Loon, J.J.W.A., 2007. Some history and use of the random positioning machine, RPM, in gravity related research. Adv. Space Res. 39, 1161–1165. 10.1016/j.asr.2007.02.016.

van Mulders, S.E., Stassen, C., Daenen, L., Devreese, B., Siewers, V., van Eijsden, R.G.E. et al., 2011. The influence of microgravity on invasive growth in *Saccharomyces cerevisiae*. Astrobiology 11, 45–55. 10.1089/ast.2010.0518.

Vandenbrink, J.P., Herranz, R., Poehlman, W.L., Alex Feltus, F., Villacampa, A., Ciska, M. et al., 2019. RNA-seq analyses of *Arabidopsis thaliana* seedlings after exposure to blue-light phototropic stimuli in microgravity. Am. J. Bot. 106, 1466–1476. 10.1002/ajb2.1384.

Vasiliou, S.K., Diamandis, E.P., Church, G.M., Greely, H.T., Baylis, F., Thompson, C. et al., 2016. CRISPR-Cas9 System: Opportunities and Concerns. Clin. Chem. 62, 1304–1311. 10.1373/clinchem.2016.263186.

Veen, S.J., Antoniuk, O., Weber, B., Potenza, M.A.C., Mazzoni, S., Schall, P. et al., 2012. Colloidal aggregation in microgravity by critical Casimir forces. Phys. Rev. Lett. 109, 248302. 10.1103/PhysRevLett.109.248302.

Velkov, V.V., 2002. New Insights into the Molecular Mechanisms of Evolution: Stress Increases Genetic Diversity. Mol. Biol. 36, 209–215. 10.1023/A:1015365805383.

Vico, L., Collet, P., Guignandon, A., Lafage-Proust, M.-H., Thomas, T., Rehailia, M. et al., 2000. Effects of long-term microgravity exposure on cancellous and cortical weight-bearing bones of cosmonauts. Lancet 355, 1607–1611. 10.1016/S0140-6736(00)02217-0.

Vidyasekar, P., Shyamsunder, P., Arun, R., Santhakumar, R., Kapadia, N.K., Kumar, R. et al., 2015. Genome Wide Expression Profiling of Cancer Cell Lines Cultured in Microgravity Reveals Significant Dysregulation of Cell Cycle and MicroRNA Gene Networks. PLoS ONE 10, e0135958. 10.1371/journal.pone.0135958.

Vogel, C., Chothia, C., 2006. Protein family expansions and biological complexity. PLoS Comput. Biol. 2, e48. 10.1371/journal.pcbi.0020048.

Vorselen, D., Roos, W.H., MacKintosh, F.C., Wuite, G. J. L., van Loon, J.J.W.A., 2014. The role of the cytoskeleton in sensing changes in gravity by nonspecialized cells. FASEB J. 28, 536–547. 10.1096/fj.13-236356.

Vos, M., Hesselman, M.C., te Beek, T.A., van Passel, M.W.J., Eyre-Walker, A., 2015. Rates of Lateral Gene Transfer in Prokaryotes: High but Why? Trends Microbiol. 23, 598–605. 10.1016/j.tim.2015.07.006.

Vukanti, R., Model, M.A., Leff, L.G., 2012. Effect of modeled reduced gravity conditions on bacterial morphology and physiology. BMC Microbiol. 12, 4. 10.1186/1471-2180-12-4.

Vurro, M., Bonciani, B., Vannacci, G., 2010. Emerging infectious diseases of crop plants in developing countries: impact on agriculture and socio-economic consequences. Food Sec. 2, 113–132. 10.1007/s12571-010-0062-7.

Wang, H., 2006. A proteomic approach to analysing responses of *Arabidopsis thaliana* callus cells to clinostat rotation. J. Exp. Bot. 57, 827–835. 10.1093/jxb/erj066.

Wang, H., Yan, Y., Rong, D., Wang, J., Wang, H., Liu, Z. et al., 2016. Increased biofilm formation ability in *Klebsiella pneumoniae* after short-term exposure to a simulated microgravity environment. MicrobiologyOpen 5, 793–801. 10.1002/mbo3.370.

Ward, M.J., Gibbons, C.L., McAdam, P.R., van Bunnik, B.A.D., Girvan, E.K., Edwards, G.F. et al., 2014. Time-Scaled Evolutionary Analysis of the Transmission and Antibiotic Resistance Dynamics of *Staphylococcus aureus* Clonal Complex 398. Appl. Environ. Microbiol. 80, 7275–7282. 10.1128/AEM.01777-14.

Watkins, R.F., Gray, M.W., 2006. The frequency of eubacterium-to-eukaryote lateral gene transfers shows significant cross-taxa variation within amoebozoa. J. Mol. Evol. 63, 801–814. 10.1007/s00239-006-0031-0.

Wayne, M., Miyamoto, M.M., 2006. In Evolutionary Genetics: Concepts and Case Studies Oxford Univ. Press, New York.

Wei, L., Diao, Y., Qi, J., Khokhlov, A., Feng, H., Yan, X. et al., 2013. Effect of change in spindle structure on proliferation inhibition of osteosarcoma cells and osteoblast under simulated microgravity during incubation in rotating bioreactor. PLoS ONE 8, e76710. 10.1371/journal.pone.0076710.

West-Eberhard, M.J., 2003. Developmental plasticity and evolution Oxford University Press, New York.

White, R.J., Averner, M., 2001. Humans in space. Nature 409, 1115–1118. 10.1038/35059243.

Wiedenbeck, J., Cohan, F.M., 2011. Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiol. Rev. 35, 957–976. 10.1111/j.1574-6976.2011.00292.x.

Wilson, J.W., Ott, C.M., Quick, L., Davis, R., zu Bentrup, Kerstin Höner, Crabbé, A. et al., 2008. Media Ion Composition Controls Regulatory and Virulence Response of *Salmonella* in Spaceflight. PLoS ONE 3, e3923. 10.1371/journal.pone.0003923.

Wilson, J.W., Ott, C.M., Ramamurthy, R., Porwollik, S., McClelland, M., Pierson, D.L. et al., 2002. Low-Shear modeled microgravity alters the Salmonella enterica serovar typhimurium stress response in an RpoS-independent manner. Appl. Environ. Microbiol. 68, 5408–5416. 10.1128/AEM.68.11.5408-5416.2002.

Wilson, J.W., Ott, C.M., zu Bentrup, K. H., Ramamurthy, R., Quick, L., Porwollik, S. et al., 2007. Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. Proc. Natl. Acad. Sci. U. S. A. 104, 16299–16304. 10.1073/pnas.0707155104.

Winn, R.N., Norris, M.B., Brayer, K.J., Torres, C., Muller, S.L., 2000. Detection of mutations in transgenic fish carrying a bacteriophage lambda cII transgene target. Proc. Natl. Acad. Sci. U. S. A. 97, 12655–12660. 10.1073/pnas.220428097.

Wion, D., Casadesús, J., 2006. N6-methyl-adenine: an epigenetic signal for DNA-protein interactions. Nat. Rev. Micro. 4, 183–192. 10.1038/nrmicro1350.

Wohlleben, W., Mast, Y., Stegmann, E., Ziemert, N., 2016. Antibiotic drug discovery. Microb. Biotechnol. 9, 541–548. 10.1111/1751-7915.12388.

Wolff, S.A., Coelho, L.H., Karoliussen, I., Jost, A.-I.K., 2014. Effects of the Extraterrestrial Environment on Plants: Recommendations for Future Space Experiments for the MELiSSA Higher Plant Compartment. Life 4, 189–204. 10.3390/life4020189.

World Health Organization [Internet]. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis, 2017. Available from: http://www.who.int/medicines/areas/rational_use/PPLreport_2017_09_19.pdf?ua=1.

Wright, B.E., 2004. Stress-directed adaptive mutations and evolution. Mol. Microbiol. 52, 643–650. 10.1111/j.1365-2958.2004.04012.x.

Xianfang, W., Long, Z., Weixu, D., Chunhua, L., 2004. Study of space mutation breeding in China. Appl. Life Sci. 18, 241–246.

Xu, B., Li, C., Zheng, Y., Si, S., Shi, Y., Huang, Y. et al., 2015. Simulated microgravity affects ciprofloxacin susceptibility and expression of acrAB-tolC genes in *E. coli* ATCC25922. Int. J. Clin. Exp. Pathol. 8, 7945–7952.

Xu, D., Guo, S., 2013. Identification of Conserved miRNAs in *Solanum Lycopersicum* Response to Long-term RPM-treatment. JOLST, 136–141. 10.12720/jolst.1.2.136-141.

Xu, G., Guo, C., Shan, H., Kong, H., 2012. Divergence of duplicate genes in exon-intron structure. Proc. Natl. Acad. Sci. U. S. A. 109, 1187–1192. 10.1073/pnas.1109047109.

Xu, P., Chen, H., Jin, J., Cai, W., 2018. Single-base resolution methylome analysis shows epigenetic changes in *Arabidopsis* seedlings exposed to microgravity spaceflight conditions on board the SJ-10 recoverable satellite. npj Microgravity 4, 12. 10.1038/s41526-018-0046-z.

Yadav, D., Tanveer, A., Malviya, N., Yadav, S., 2018. Overview and Principles of Bioengineering. In: Omics Technologies and Bio-Engineering: Elsevier. p. 3–23.

Yang, J., Barrila, J., Roland, K.L., Ott, C.M., Nickerson, C.A., 2016. Physiological fluid shear alters the virulence potential of invasive multidrug-resistant non-typhoidal *Salmonella Typhimurium* D23580. npj Microgravity 2, 16021. 10.1038/npjmgrav.2016.21.

Yang, S.-T., Liu, X., Zhang, Y., 2007. Metabolic Engineering – Applications, Methods, and Challenges. In: Bioprocessing for Value-Added Products from Renewable Resources: Elsevier. p. 73–118.

Yao, J., Weng, Y., Dickey, A., Wang, K.Y., 2015. Plants as Factories for Human Pharmaceuticals: Applications and Challenges. Int. J. Mol. Sci. 16, 28549–28565. 10.3390/ijms161226122.

Yilmaz, A., Grotewold, E., 2010. Components and mechanisms of regulation of gene expression. Methods Mol. Biol. 674, 23–32. 10.1007/978-1-60761-854-6_2.

Yoshimura, K., Matsuo, Y., Kamiya, R., 2003. Gravitaxis in *Chlamydomonas reinhardtii* studied with novel mutants. Plant Cell Physiol. 44, 1112–1118.

Yuge, L., Kajiume, T., Tahara, H., Kawahara, Y., Umeda, C., Yoshimoto, R. et al., 2006. Microgravity potentiates stem cell proliferation while sustaining the capability of differentiation. Stem Cells Dev. 15, 921–929. 10.1089/scd.2006.15.921.

Zanchi, D., Poulain, C., Konarev, P., Tribet, C., Svergun, D.I., 2008. Colloidal stability of tannins: astringency, wine tasting and beyond. J. Phys.: Condens. Matter 20, 494224. 10.1088/0953-8984/20/49/494224.

Zanoni, M., Pignatta, S., Arienti, C., Bonafè, M., Tesei, A., 2019. Anticancer drug discovery using multicellular tumor spheroid models. Expert Opin. Drug Discov. 14, 289–301. 10.1080/17460441.2019.1570129.

Zhang, H., Li, Y., Zhu, J.-K., 2018. Developing naturally stress-resistant crops for a sustainable agriculture. Nat. Plants 4, 989–996. 10.1038/s41477-018-0309-4.

Zhang, J., Wang, S., Wang, Y., 2016. Biobutanol Production From Renewable Resources. In: Elsevier. p. 1–68.

Zhang, X., Nan, Y., Wang, H., Chen, J., Wang, N., Xie, J. et al., 2013. Model microgravity enhances endothelium differentiation of mesenchymal stem cells. Die Naturwissenschaften 100, 125–133. 10.1007/s00114-012-1002-5.

Zhang, Y., Wang, L., Xie, J., Zheng, H., 2015. Differential protein expression profiling of *Arabidopsis* thaliana callus under microgravity on board the Chinese SZ-8 spacecraft. Planta 241, 475–488. 10.1007/s00425-014-2196-x.

Zhang, Y., Zheng, H.Q., 2015. Changes in Plastid and Mitochondria Protein Expression in *Arabidopsis* Thaliana Callus on Board Chinese Spacecraft SZ-8. Microgravity Sci. Technol. 27, 387–401. 10.1007/s12217-015-9431-3.

Zhao, J., Ma, H., Wu, L., Cao, L., Yang, Q., Dong, H. et al., 2017. The influence of simulated microgravity on proliferation and apoptosis in U251 glioma cells. In Vitro Cell. Dev. Biol. -Animal 53, 744–751. 10.1007/s11626-017-0178-6.

Zhau, H.E., Goodwin, T.J., Chang, S.M., Baker, T.L., Chung, L.W., 1997. Establishment of a three-dimensional human prostate organoid coculture under microgravity-simulated conditions: evaluation of androgen-induced growth and PSA expression. In Vitro Cell. Dev. Biol. -Animal 33, 375–380. 10.1007/s11626-997-0008-3.

Zhaxybayeva, O., Gogarten, J.P., Charlebois, R.L., Doolittle, W.F., Papke, R.T., 2006. Phylogenetic analyses of cyanobacterial genomes: quantification of horizontal gene transfer events. Genome Res. 16, 1099–1108. 10.1101/gr.5322306.

Zhaxybayeva, O., Nesbø, C.L., Doolittle, W.F., 2007. Systematic overestimation of gene gain through false diagnosis of gene absence. Genome Biol. 8, 402. 10.1186/gb-2007-8-2-402.

Zhou, J., Sun, C., Wang, N., Gao, R., Bai, S., Zheng, H. et al., 2006. Preliminary report on the biological effects of space flight on the producing strain of a new immunosuppressant, Kanglemycin C. J. Ind. Microbiol. Biotechnol. 33, 707–712. 10.1007/s10295-006-0118-z.

Zhou, M., Sng, N.J., LeFrois, C.E., Paul, A.-L., Ferl, R.J., 2019. Epigenomics in an extraterrestrial environment: organ-specific alteration of DNA methylation and gene expression elicited by spaceflight in *Arabidopsis thaliana*. BMC Genomics 20, 205. 10.1186/s12864-019-5554-z.

Zhu, J., Jeong, J.C., Zhu, Y., Sokolchik, I., Miyazaki, S., Zhu, J.-K. et al., 2008. Involvement of *Arabidopsis* HOS15 in histone deacetylation and cold tolerance. Proc. Natl. Acad. Sci. U. S. A. 105, 4945–4950. 10.1073/pnas.0801029105.

Zhu, J., Li, M., Rogers, R., Meyer, W., Ottewill, R.H., Russel, W.B. et al., 1997. Crystallization of hard-sphere colloids in microgravity. Nature 387, 883–885. 10.1038/43141.

Zong, W., Zhong, X., You, J., Xiong, L., 2013. Genome-wide profiling of histone H3K4-tri-methylation and gene expression in rice under drought stress. Plant Mol. Biol. 81, 175–188. 10.1007/s11103-012-9990-2.

Figure Legends

Fig. 1. A simplified scheme of self-guided evolution. Diverse genetic variants of strains can be achieved by subjecting cells in microgravity and radiation in combination with other biotic and abiotic stressors. Suitable mutants selected according to defined parameters and isolated subsequently. Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Fig. 2. Scheme of horizontal gene transfer between non-related organisms. Horizontal gene exchange is common in closely related organisms but also occurs between distantly related organisms. Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Fig. 3. Stress-induced epigenetics in plants. Epigenetic bring about new traits without altering the genetic code. Epigenetic regulation increases variation within a species in times of environmental stresses or signals (biotic and abiotic factors), thereby provide ways for adaptation to the arising selection pressures.

Fig. 4. The central dogma of evolution. In biology and ecology, environmental factors or stressors (abiotic or biotic components) affect living organisms, leading to adaptive changes over time employing evolution, which includes mutation, gene flow (horizontal gene transfer), genetic drift, epigenetic regulation, and natural selection as mechanisms of change.

Fig. 5. Simplified representation of parameters cells or organisms encounters in outer space. Lack of gravity on board of a spacecraft considerably changes the environment in which thermal convection, mechanical load as well as hydrostatic does not exist. This has significant impacts on cells in terms of gas, nutrient, and heat transfer. Space unique environment provides a combination of stressors (e.g., high radiation and weightlessness/microgravity) that organisms have never experienced on Earth. Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Fig. 6. Different facilities for the investigation of altered gravitational conditions Platforms include aircraft-based parabolic flights, 3D and 2D clinostats, random-positioning machine (RPM), sounding rocket, and drop tower. Clinostat and RPM constantly change the position of the test object to the vector of gravity. With drop tower, aircraft-based parabolic flights, and sounding rockets, microgravity conditions can be achieved ranging from few seconds to several minutes.

Fig. 7. Applications of microgravity-based research on human cells. (A) MCF-7 breast cancer cells form unique ring-like structures when grown for 14 days in s-µ*g* produced by an RPM. **(B)** Schematics of cancer research using microgravity. In µ*g*, cancer cells detach from the growth surface and form 3D MCS composed of less aggressive, more differentiated cells. Using Omics research and bioinformatics, scientists try to elucidate the mechanisms behind the alteration of the phenotype to mimic these effects with drugs. The results may be used in the development of novel cancer therapies. Furthermore, µ*g* provides a suitable culture environment for scaffold- and necrosis-free tissue engineering of **(C)** cartilage, **(D)** bone, and **(E)** blood vessels. Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Fig. 8. Simplified scheme of the physiological mechanism involved in the gravitaxis of *Euglena gracilis*. If a vertically upward swimming cell (A) deviates from its vertical movement path (B and C), mechano-sensitive membrane patches are activated due to forces generated by the weight of the cell body (C). This triggers a physiological signal cascade (orange insert) in which calcium channels, a particular calmodulin (CAM 2, activated by increasing concentrations of calcium ions), a not yet

identified adenylate cyclase are involved. Increased levels of cAMP, synthesized from activated adenylate cyclase activates a protein kinase A, which most likely phosphorylates flagellar proteins. This results in reorientation strokes of the flagella, aligning the cell back to the vertical position (D). In addition, a recently identified flagellar protein EgPCDUF4201 is involved in gravitaxis, but its role in the signal transduction chain is yet to be determined. The Figure is adopted and modified according to (Nasir et al., 2018). Parts of the figure were drawn by using pictures from Servier Medical Art (http://smart.servier.com/), licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).