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Mitochondria: at the crossroads between mechanobiology and cell metabolism

Running title

Mitochondria, mechanics and metabolism

Authors

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Abstract

Metabolism and mechanics are two key facets of structural and functional processes in cells, such as growth, proliferation, homeostasis and regeneration. Their reciprocal regulation has been increasingly acknowledged in recent years: external physical and mechanical cues entail metabolic changes, which in return regulate cell mechanosensing and mechanotransduction. Since mitochondria are pivotal regulators of metabolism, we review here the reciprocal links between mitochondrial morphodynamics, mechanics and metabolism. Mitochondria are highly dynamic organelles which sense and integrate mechanical, physical and metabolic cues to adapt their morphology, the organization of their network and their metabolic functions. While some of the links between mitochondrial morphodynamics, mechanics and metabolism are already well established, others are still poorly documented and open new fields of research. First, cell metabolism is known to correlate with mitochondrial morphodynamics. For instance, mitochondrial fission, fusion and cristae remodeling allow the cell to fine-tune its energy production through the contribution of mitochondrial oxidative phosphorylation and cytosolic glycolysis. Second, mechanical cues and alterations in mitochondrial mechanical properties reshape and reorganize the mitochondrial network. Mitochondrial membrane tension emerges as a decisive physical property which regulates mitochondrial morphodynamics. However, the converse link hypothesizing a contribution of morphodynamics to mitochondria mechanics and/or mechanosensitivity has not yet been demonstrated. Third, we highlight that mitochondrial mechanics and metabolism are reciprocally regulated, although little is known about the mechanical adaptation of mitochondria in response to metabolic cues. Deciphering the links between mitochondrial morphodynamics, mechanics and metabolism still presents significant technical and conceptual challenges but is crucial both for a better understanding of mechanobiology and for potential novel therapeutic approaches in diseases such as cancer.

Keywords

Mitochondrial morphodynamics; mechanotransduction; oxidative phosphorylation; glycolysis; membrane tension; cytoskeleton; cancer

1 Introduction

Cells sense and process signals from their external environment to coordinate structural and functional processes such as growth, proliferation, homeostasis and regeneration. Both chemical and mechanical cues are known to be regulators of cell fate and behavior. Cells integrate information from their physical environment through the process of mechanotransduction, during which mechanical cues are translated into biochemical signals which further induce adaptive changes in cell functions. Extracellular mechanical stimuli such as compressive, tensile or shear stresses are transmitted into the cell through the coordinated activity of mechanosensors along mechanosignaling pathways. Previous studies have investigated and reviewed the role of the plasma membrane, mechano-activated ion channels, focal adhesion proteins, cytoskeletal filaments (actin, microtubule and intermediate filaments) and the nucleus in sensing and transducing mechanical stresses [Ingber, 2006; Orr et al., 2006; Jaalouk and Lammerding, 2009; Schwartz, 2010; Isermann and Lammerding, 2013; Douguet and Honoré, 2019; Kechagia et al., 2019; Uray and Uray, 2021], but the molecular mechanisms of mechanotransduction are still incompletely understood, in particular when intracellular compartments such as the endomembrane system are involved [Phuyal and Baschieri, 2020; Phuyal et al., 2023]. With the spurring growth of intracellular rheology, the mechanical properties of the cytoplasm, intracellular organelles and the cytoskeleton emerge as key players in both mechanosensing and mechanotransduction [Mathieu and Manneville, 2019].

Cellular metabolism, comprising the biochemical processes that produce or consume energy in a cell, underpins virtually every cellular process through catabolism and anabolism [Ferreira et al., 2020]. In catabolism, cells break down complex molecules, such as polysaccharides, lipids, proteins and nucleic acids, in smaller units, like monosaccharides, fatty acids, amino acids and nucleotides. These small molecules are then oxidized to release energy or supply other chemical reactions. Cells can also synthesize new macromolecules via anabolic pathways.

There is mounting evidence of a reciprocal regulation between cell mechanics and metabolism. Mechanical forces rewire the metabolism of cells to adapt to their mechanically constrained environment. In return, metabolic changes modulate mechanosensing and mechanotransduction. From an evolutionary standpoint, the hypothesis that cytoskeletal proteins derive from metabolic enzymes provides an intrinsic link between metabolism and mechanics [Evers et al., 2021]. External cues activate downstream signaling pathways. As reviewed in [Evers et al., 2021; Romani et al., 2021; Torrino and Bertero, 2022], all aspects of the subsequent cell response, including cytoskeletal remodeling, actomyosin contraction and transcriptional changes, are energy-consuming and can rewire metabolism. Resulting metabolic changes may in turn induce a reorganization of cellular components and processes impacting the mechanical properties of the cell.

Commonly referred to as the energy powerhouse of the cell, mitochondria are essential regulators of cell metabolism [Ernster and Schatz, 1981; Spinelli and Haigis, 2018]. From the oxidative degradation of carbohydrates, amino acids and fatty acids, they supply cells with energy in the form of adenosine triphosphate (ATP) through the citric acid cycle and oxidative phosphorylation (OXPHOS). Mitochondria also generate metabolic precursors for macromolecules, including lipids, proteins, DNA and RNA. They ensure calcium buffering and are the major intracellular sources of reactive oxygen species (ROS), which are a byproduct of cellular oxidative metabolism [Sena and Chandel, 2012; Hernansanz-Agustín and Enríquez, 2021]. Although ROS were mostly considered for their damaging oxidative effects and their contribution to mitochondrial dysfunction and cell death [Ott et al., 2007], there is now mounting evidence that they are important regulators of cell homeostasis. ROS are signaling molecules which not only regulate a wide range of physiological processes, but fluctuations of their levels also activate adaptive mechanisms, also termed mito-hormesis [Sena and Chandel, 2012; Yun et al., 2014; Pizzino et al., 2017]. In addition to these metabolic functions, mitochondria also regulate cell signaling and apoptosis [McBride et al., 2006].

The morphology of mitochondria is well characterized (Figure 1). The endosymbiotic theory of mitochondrial origin stating that mitochondria are ancient proteobacteria which developed a symbiotic relationship with archae more than 1.5 billion years ago, is now well confirmed [Roger et al., 2017; Boguszewska et al., 2020]. However, the exact lineage is still

debated [Martijn et al., 2018]. As a result, mitochondria possess their own DNA (mtDNA) and are limited by an inner membrane and an outer membrane. The outer mitochondrial membrane acts both as a protective physical barrier and an exchange interface by allowing the selective diffusion of preproteins, small hydrophilic metabolites and ions. The presence of specific transporters in the outer mitochondrial membrane ensures metabolite exchange between the intermembrane space and the cytosol [Becker and Wagner, 2018]. The outer mitochondrial membrane also contains large quantities of porins, integral membrane proteins allowing the diffusion of small molecules. In contrast, the inner mitochondrial membrane, which encloses a space containing mtDNA, ribosomes and various soluble compounds (e.g. enzymes, small organic molecules...) named the matrix, is highly impermeable, even to small metabolites. It is densely packed with the complexes of the OXPHOS machinery. Perhaps the most striking characteristic of mitochondria is their exceptional morphological plasticity. Indeed, the prokaryotic mitochondrial ancestor developed structural and functional mechanisms to adapt to the intracellular environment of its eukaryotic host. Across different tissues and within the same cell types, a large spectrum of mitochondrial morphologies has been observed, ranging from hyperfused or tubular to short tubes and fragmented shapes [Wai and Langer, 2016]. The cristae, which are folds of the inner mitochondrial membrane, display a wide range of morphologies, from simple tubular structures to large, flat lamella and vacuolated intercrystal spaces [Mannella et al., 1994].

ATP is the main source of high-energy phosphate bonds involved in essentially all cellular processes, including intracellular signaling, DNA synthesis and active transport [Dunn and Grider, 2021]. Most intracellular ATP is synthesized from two different processes: mitochondrial OXPHOS and cytosolic glycolysis. OXPHOS is carried out by the mitochondrial electron transport chain, a group of five enzyme complexes (I to V) bound to the inner mitochondrial membrane. This process has been thoroughly described at the molecular level [Ernster and Schatz, 1981; Saraste, 1999]. In a nutshell, the citric acid cycle or Krebs cycle, which takes place in the mitochondrial matrix, is a set of redox reactions of substrates derived from glucose, fatty acids and amino acids through different pathways. The Krebs cycle feeds the electron transport chain with reduced cofactors (NADH and FADH₂). The latter donate electrons to complexes I and II and the liberated energy is used by complexes I, III and IV to

pump protons to the intermembrane space. This proton motive force is finally used by complex V (ATP synthase) to synthesize ATP from ADP and inorganic phosphate. Cytosolic glycolysis, the other ATP-generating process, is a metabolic pathway that can occur under both aerobic and anaerobic conditions [Chaudhry and Varacallo, 2018]. This process yields two pyruvate molecules per glucose molecule and ultimately 32 ATP molecules in aerobic conditions where pyruvate enters mitochondria and is degraded through OXPHOS. In anaerobic conditions, pyruvate stays in the cytosol and is converted to lactate, producing *in fine* only 2 ATP molecules per glucose molecule (Figure 1).

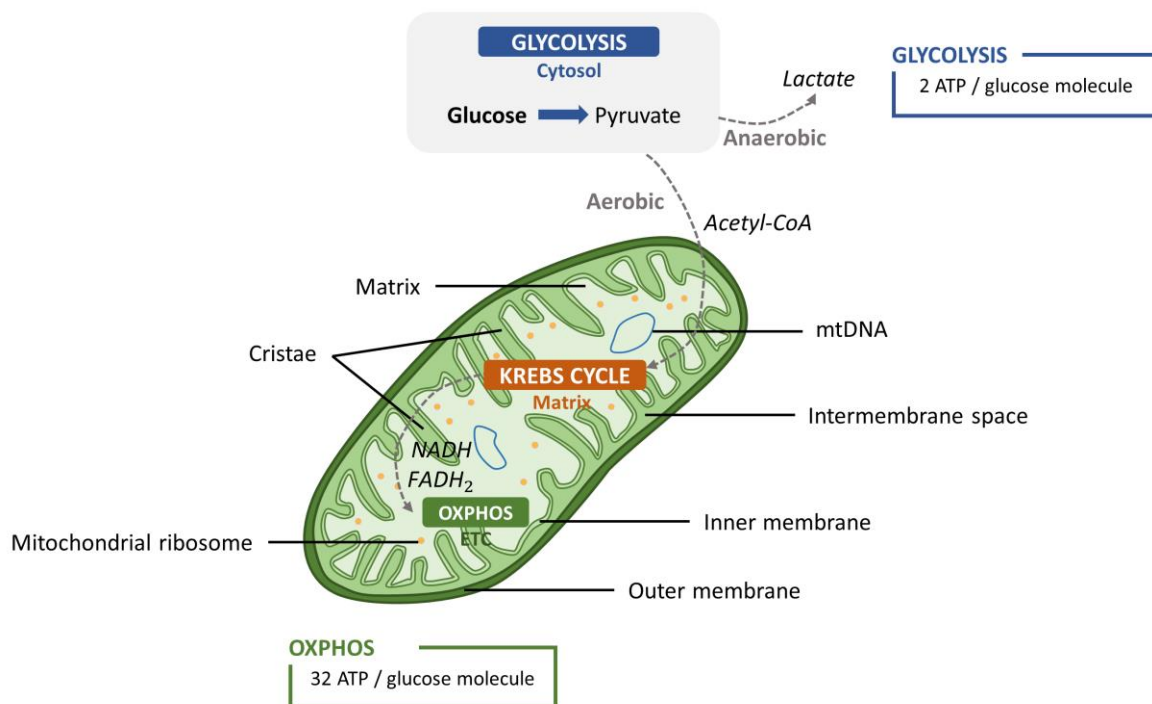


Figure 1: Schematic representation of the internal structure of a mitochondrion and the two main ATP synthesis processes in a eukaryotic cell: oxidative phosphorylation (OXPHOS) and cytosolic glycolysis. OXPHOS relies on the citric acid cycle or Krebs cycle, which is a set of redox reactions of substrates derived from glucose (through cytosolic glycolysis), fatty acid or amino acid oxidation.

Mitochondria are organized in an extensive network in the cell. Their morphology, motility and distribution across the cytosol, which we refer to here as mitochondrial

morphodynamics, are largely determined by cytoskeletal filaments [Korobova et al., 2013; Miragoli et al., 2016; Pernas and Scorrano, 2016; Bartolák-Suki et al., 2017; Casafuz et al., 2021; Fatima and Ananthanarayan, 2023]. Besides, mitochondria are involved in inter-organellar contact sites such as the endoplasmic reticulum (ER), lipid droplets and the Golgi apparatus, which can regulate their morphodynamics and functions [Murley and Nunnari, 2016; Lackner et al., 2019; Tábara et al., 2021]. The actin cytoskeleton is required for short-distance movements of mitochondria and for their immobilization at sites of high ATP demand, as reported in neurons [Boldogh and Pon, 2006]. As reviewed in [Schwarz and Leube, 2016], the distribution, anchorage and function of mitochondria at regions of high-energy demand are mostly ensured by intermediate filaments, such as neurofilaments in neuronal cells [Wagner et al., 2003], keratin in epithelial cells [Duan et al., 2009], vimentin in fibroblasts [Nekrasova et al., 2011] or desmin in muscle cells [Capetanaki et al., 2007]. The contribution of microtubules to mitochondrial directional transport is also well documented [Summerhayes et al., 1983; Morris and Hollenbeck, 1995; Boldogh and Pon, 2007; Saxton and Hollenbeck, 2012]. Mitochondria undergo anterograde and retrograde transport along microtubules, powered by kinesin and dynein motor proteins respectively [Casafuz et al., 2020]. Actin controls the positioning and redistribution of mitochondria during mitosis [Moore et al., 2021]. Translocation of mitochondria along cytoskeletal tracks also requires adaptor proteins to couple the three types of motor proteins - myosins for actin filaments and kinesins and dyneins for microtubules, with mitochondria. Miro GTPases are tail-anchored in the outer mitochondrial membrane and interact with both the mitochondrial intermembrane space bridging (MIB) complex and TRAK proteins to form the Miro-TRAK adaptor complex that recruits kinesin or dynein-dynactin motors [Kruppa and Buss, 2021; Zinsmaier, 2021].

The variety of mitochondrial lengths and shapes observed in different cell types and under different conditions stems from changes in fusion and fission rates [Griparic and van der Bliek, 2001; Youle and Van Der Bliek, 2012]. The processes of cristae remodeling, fission and fusion allow the mitochondria to constantly support and regulate their bioenergetic and biosynthetic functions. To preserve cellular homeostasis, eukaryotic cells activate autophagy in order to degrade unnecessary energy-consuming organelles and recycle their components [Reggiori and Klionsky, 2002]. Mitophagy, defined as the selective autophagy of mitochondria,

requires the fragmentation of mitochondria to complete their engulfment in autophagosomes of a restricted size [Rambold et al., 2011; Zorov et al., 2019]. Morphodynamic mechanisms underlying mitochondrial quality control regulation and mitophagy are reviewed in [Ni et al., 2015; Song et al., 2021; Tang et al., 2021].

A number of molecular complexes involved in mammalian mitochondrial cristae remodeling, fission and fusion have been identified. The detailed mechanisms of mitochondrial fission and fusion have been extensively reviewed previously [Chan, 2006; Pernas and Scorrano, 2016; Wai and Langer, 2016; Jones and Naylor, 2022]. High molecular weight guanosine triphosphatases (GTPases) are key regulators of mitochondrial morphodynamics. In mammalian cells, the canonical fission machinery involves the dynamin-related protein 1 (DRP1). ER tubules wrap around the mitochondrion [Friedman and Voeltz, 2011], initiating a pre-constriction which facilitates the recruitment of cytosolic DRP1 [Otera et al., 2010]. DRP1 colocalizes with mitochondrial constrictions and multimerizes into a spiral to further constrict the outer mitochondrial membrane with the use of the free energy of GTP hydrolysis [Labrousse et al., 1999; Smirnova et al., 2001; Francy et al., 2015]. Translocation of DRP1 is coordinated by a number of outer mitochondrial membrane proteins, such as fission protein 1 (FIS1), mitochondrial fission factor (MFF) and mitochondrial dynamics proteins of 49 and 51 kDa (MID49 and MID51) [Losón et al., 2013]. In ER-mediated fission, actin polymerization at contact sites between the ER and the mitochondrion involves both the ER-localized inverted formin 2 (INF2) and the actin-nucleating spire protein Spire1C and provides the mechanical force needed to sever the mitochondrion [Korobova et al., 2013; Li et al., 2015; Manor et al., 2015].

Recent studies shed light on alternative fission pathways which do not necessarily rely on the canonical fission machinery. Mitochondrial fission can be actin, DRP1 or ER-dependent or independent. Inhibitors of the electron transport chain, ATP synthase or permeability transition pore induce reversible mitochondrial fission which is dependent on both DRP1 and the actin cytoskeleton [De Vos et al., 2005]. Similarly, the fusion/fission balance of the mitochondrial network is regulated by actin cyclic polymerization and depolymerization at the level of mitochondrial subpopulations. In HeLa cells, actin transiently cycles onto all mitochondrial subsets, but preferentially assembles and disassembles on the outer membrane

of elongated mitochondria, thereby promoting DRP-1 dependent fission [Moore et al., 2016]. However, force-induced mitochondrial fission might be DRP1-dependent but actin-independent [Helle et al., 2017]. Conversely, an actin-dependent but DRP1-independent fission process can be mediated by the stress-responsive protein GJA1-20k, which localizes to the outer mitochondrial membrane and inhibits actin depolymerization [Shimura et al., 2021]. A recent study also showed that different components are involved depending on the positioning of the fission site – in the so-called midzone or in the peripheral regions – in relation with the fate of the mitochondria. Although both types of fission events require DRP1, in contrast with peripheral division which is followed by mitophagy, midzone fission recruits ER, actin and MFN [Kleele et al., 2021]. The requirement for DRP1 and actin regulators, such as Arp2/3, INF2 or Spire1C, thus seems to depend on the context of mitochondrial fission. For instance, the stiffness of the extracellular matrix (ECM) was recently shown to regulate actin perimitochondrial assembly and fission [Romani et al., 2022].

Another family of proteins involved in mitochondrial dynamics are mitofusins. Mitofusins 1 and 2 (MFN1 and MFN2) are key proteins required for fusion of two outer mitochondrial membranes, while Optic Atrophy 1 (OPA1) ensures cristae remodeling via the fusion of the inner mitochondrial membranes [Chen et al., 2003]. Their mRNA or protein expression levels are different among tissues [Eura et al., 2003].

Here we review the emerging relations between mechanics, metabolism and mitochondrial morphodynamics. We show that reciprocal links exist between these three elements (Figure 2). We examine these links in pairs, starting with the coupling between morphodynamics and metabolism (section 2), the links between mitochondrial morphodynamics and mechanics (section 3) and then the links between mechanics and metabolism at the level of mitochondria (section 4). While some of these links are well established, others are still poorly documented and open new fields of research. Finally, we identify the working hypotheses that may guide future experimental investigations on the emerging topic of mitochondrial mechanometabolism (section 5).

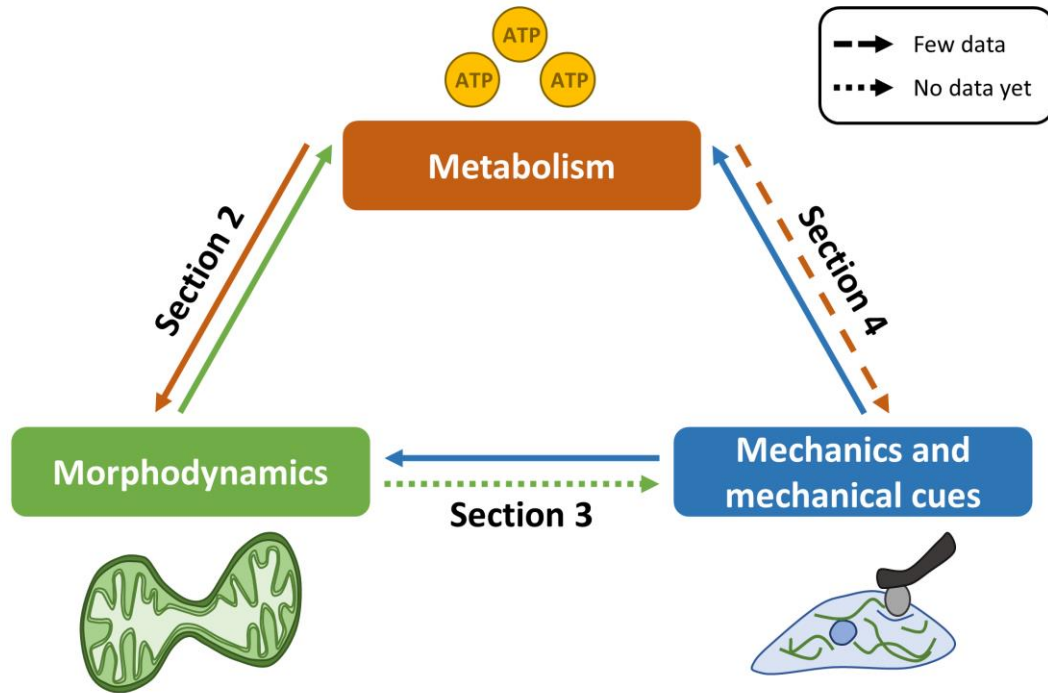


Figure 2: Established (solid arrows) or potential (dashed arrows) bidirectional links between metabolism, mitochondrial morphodynamics, mechanics and mechanical cues. The influence of metabolism on mechanics is supported by few data, while the impact of changes in mitochondrial morphodynamics on their mechanics is not yet highlighted in the literature.

2 Cell metabolism correlates with mitochondrial morphodynamics

Cell metabolism regulates mitochondrial morphodynamics

Mitochondrial morphology is tissue-, cell- and cell compartment specific

An important observation advocating for links between cell metabolism and mitochondrial morphodynamics is that mitochondria exhibit a striking heterogeneity across tissues in terms of organization, density, morphology and function (Figure 3).

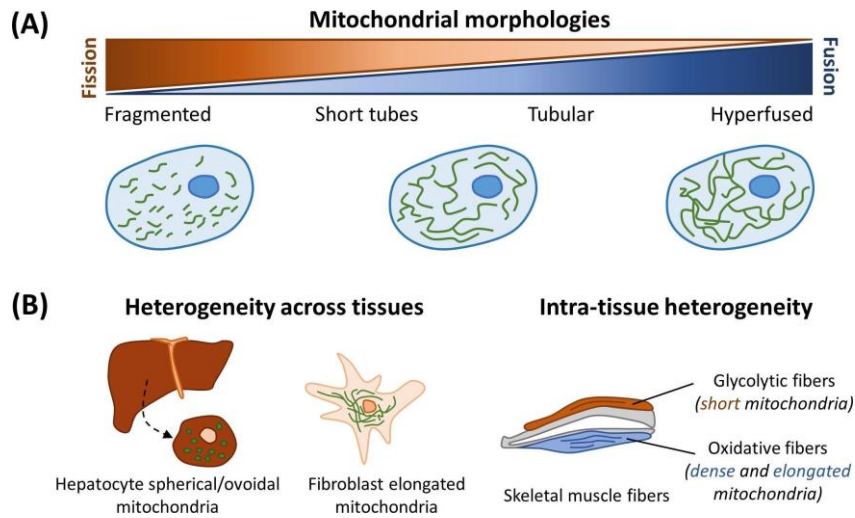


Figure 3: Links between mitochondria morphodynamics and metabolism. (A) Mitochondria exhibit a wide variety of shapes depending on the balance between fission and fusion rates. (B) Mitochondria display not only a substantial heterogeneity across tissues in terms of density and shapes, but also an intra-tissue heterogeneity, reflecting the coupling between their structure and their function.

Each tissue or organ presents specific energetic needs which correlate with their physiological functions. Mitochondrial morphodynamics thus reflects the tissue physiology and more specifically its bioenergetic efficiency and adaptation to nutrient availability [Kunz, 2003; Liesa and Shirihai, 2013; Wai and Langer, 2016]. In terms of spatial distribution and network organization, mitochondria can be uniformly or non-uniformly distributed in the cell, depending on the cell type [Collins et al., 2002; Kuznetsov et al., 2009; Faitg et al., 2021]. The cell-type specificity of mitochondrial morphology and intracellular distribution is reviewed in [Kuznetsov et al., 2009]. For instance, in differentiated podocytes, which have a complex structure of interdigitating foot processes, mitochondria are clustered around the nucleus and absent in the foot processes [Ozawa et al., 2015]. This heterogeneous distribution correlates with the spatial differences of energy metabolism: while both glycolysis and mitochondrial respiration support ATP production in the central area, glycolysis is predominant in the cortical area and notably regulates cell motility and lamellipodia formation. A sub-cellular regional

specificity is also observed in neurons: axonal mitochondria display much simpler morphologies and a volume of about half that of their dendritic counterparts, a feature associated with the need of highly mobile mitochondria in axons [Faitg et al., 2021].

Individual mitochondria can display very different shapes - elongated, rod-like and ovoid [Kuznetsov et al., 2009] – which have been characterized by a complexity index [Vincent et al., 2019]. Because different tissues are subjected to distinct microenvironments which may affect the distribution and shape of mitochondria, studies have been performed both *in situ* and *in vitro* [Kuznetsov et al., 2022]. A peculiar instance is the liver, which plays a pivotal role in essentially all metabolic processes by ensuring the homeostasis of carbohydrates, lipids and proteins. Hepatocyte mitochondria constantly regulate the flux of metabolites in response to changes in energy demand and nutrient supply, through liver-specific metabolic pathways such as glycogenolysis, gluconeogenesis or ketogenesis [Rui, 2014]. In cultured mammalian cells, hepatocyte mitochondria are ovoid and evenly distributed in the cytosol [Kuznetsov et al., 2009]. In terms of mitochondrial morphology, cultured fibroblasts are an opposite instance to hepatocytes, since they are predominantly elongated and form interconnected networks (Figure 3B) [Kuznetsov et al., 2009].

Intra-tissue heterogeneity is particularly emphasized in the two types of striated muscles, the skeletal and cardiac muscles, which both display a highly specialized structure: cardiomyocytes and skeletal muscle cells are surrounded by a sarcolemma and comprise myofibril bundles. Cells of both tissues display similar mitochondrial network patterns: spheroid interfibrillar mitochondria are evenly aligned in longitudinal rows, subsarcolemmal mitochondria are irregularly spread under the sarcolemma and perinuclear mitochondria are found clustered around the nucleus [Li et al., 2020]. Furthermore, skeletal muscle fibers differentiate into specific fiber types with distinct metabolic properties whether they rely on OXPHOS or on glycolysis [Bourdeau Julien et al., 2018]. Oxidative muscle fibers, characterized by slow contraction rates and high OXPHOS activity, are associated with a higher density and more elongated morphology of mitochondria, compared to the fragmented mitochondria in glycolytic fibers which perform rapid contractions [Mishra et al., 2015]. Interestingly, such morphological differences seem to stem from the fiber metabolic profile. Indeed, while genetically enhancing the expression of mitofusins to promote higher fusion rates

in glycolytic fibers does not switch them into oxidative fibers, increasing the respiratory capacity of the mice through endurance exercise induces a substantial switching of glycolytic fibers to oxidative ones and elongation of their mitochondria [Mishra et al., 2015].

Besides their spatial and morphological heterogeneity in mature tissues, mitochondria are subjected to changes during tissue differentiation and maturation [Porter et al., 2011; Kere et al., 2020], as reviewed in [Ding et al., 2021; Garben and Lee, 2021] for pluripotent stem cell-derived cardiomyocytes. Although cardiomyocyte mitochondria are fragmented and clustered around the nucleus at early embryonic stage, they then become long and rod-shaped after 13.5 weeks. In adult cardiomyocytes, elongated rod-shaped mitochondria are replaced by spheroid mitochondria which densely align along the myofibrils [Porter et al., 2011]. Mitochondrial structural and morphological remodeling notably coincides with the bioenergetic changes occurring during heart development, from anaerobic glycolysis at early stages of development to aerobic fatty acid oxidation after placenta maturation and blood circulation development [Porter et al., 2011].

Metabolism induces mitochondrial morphodynamic changes

To adapt to energetic demand and supply and sustain metabolic homeostasis, mitochondria undergo remodeling which further modifies their function (Figure 4). Both fast and longer-term mechanisms can regulate the mitochondrial networks in response to changes in nutrient scarcity or oversupply, as reviewed in [Babbar and Sheikh, 2013; Liesa and Shirihai, 2013; Gao et al., 2014].

As a protective response to nutrient or oxygen deprivation, mitochondria elongate to be spared from mitophagy and maintain sufficient ATP levels during starvation. Induction of autophagy by starvation in various cell types triggers mitochondrial elongation, resulting in tubular and interconnected networks [Gomes et al., 2011; Zemirli et al., 2018]. Interestingly, in mouse embryonic fibroblasts, starvation conditions can differentially affect mitochondrial morphodynamics: glucose reduction does not induce mitochondrial tubulation, whereas deprivation of either glutamine or amino acids, thus of a nitrogen source, is followed by an

increase in mitochondrial tubulation after six hours of starvation [Rambold et al., 2011]. Nutrient replenishment after starvation decreases tubulation and interconnection in the mitochondrial network, showing that starvation-induced elongation of mitochondria is a reversible mechanism [Rambold et al., 2011]. Mitochondria elongation also correlates with an increased cristae surface area. Cristae remodeling is crucial in modulating mitochondrial function as the proliferation of cristae folds under high energy demands may provide more surface area for OXPHOS processes [Pernas and Scorrano, 2016; Nielsen et al., 2017] and an optimal assembly of the respiratory chain supercomplexes [Cogliati et al., 2013], which both increase the mitochondrial respiratory efficiency. Electron cryo-tomography showed that elongated mitochondria have higher levels of the dimeric form of ATPase, associated with increased efficiency in ATP production [Strauss et al., 2008]. Different post-translational mechanisms induce mitochondrial elongation upon nutrient starvation. Nutrient starvation increases the intracellular levels of cyclic adenosine monophosphate (cAMP), thereby activating protein kinase A (PKA) [Beebe, 1994]. Mitochondrial elongation can then be promoted by increased PKA-mediated phosphorylation and inactivation of DRP1, which impairs mitochondrial fission [Gomes et al., 2011, Rambold et al., 2011].

Besides this starvation-induced mitochondrial elongation, nutrient oversupply correlates with mitochondrial network fragmentation in different cell types [Leininger et al., 2006; Molina et al., 2009; Wang et al., 2012; Kobayashi et al., 2020; Audzeyenka et al., 2021]. In dorsal root ganglia neurons exposed to high glucose, upregulation of DRP1 entails mitochondrial network fragmentation, which increases the number of mitochondria to support glucose excess [Leininger et al., 2006]. Similarly, in differentiated mouse podocytes, a five-day period of high glucose exposure also triggers mitochondrial fragmentation, consistent with a downregulation of MFN1 and MFN2 and an upregulation of DRP1 [Audzeyenka et al., 2021]. Hyperglycemia is proposed to induce Rho-associated coiled coil-containing protein kinase 1 ROCK1-mediated mitochondrial fission by promoting DRP1 recruitment in podocytes and endothelial cells [Wang et al., 2012]. Exposing primary β -cells within mouse islets to a nutrient-rich environment combining high levels of glucose and palmitate for 48 hours decreases their fusion rates and induces a fragmentation of their mitochondrial networks [Molina et al., 2009]. However, high levels of glucose alone without palmitate do not significantly affect the mitochondrial network connectivity [Molina et al., 2009], which echoes the glucolipototoxicity

hypothesis in pancreatic β -cells [Lytrivi et al., 2020]. It was proposed that mitochondrial fragmentation in β -cells within islets and INS-1 cells is partially due to increased nutrient-induced ROS production [Molina et al., 2009]. In various cell types at different developmental stages (primary neonatal and adult rodent cardiomyocytes and liver, myoblast and aortic cell lines), acute or sustained exposure to high levels of glucose trigger mitochondrial fragmentation [Yu et al., 2006; Yu et al., 2008; Yu et al., 2011; Kobayashi et al., 2020]. In neonatal rat ventricular cardiomyocytes, mitochondrial fragmentation is associated with decreased MFN1 expression and no change in DRP1 expression [Kobayashi et al., 2020]. Conflicting data have nevertheless been reported on the role of mitochondrial fission in response to hyperglycemia in cardiomyocytes. Following former studies [Yu et al., 2006; Yu et al., 2008], inhibiting DRP1-mediated fission by overexpression of a dominant-negative mutant form of DRP1, limits hyperglycemia-induced cardiomyocyte death. Contrasting results show that mitochondrial fragmentation may rather be a protective mechanism to cope with high glucose toxicity [Kobayashi et al., 2020].

ROS levels may have implications in mitochondrial morphodynamics. Various methods have been employed to modulate ROS levels, including hyperglycemia [Yu et al., 2011], acute exposure to hydrogen peroxide (H_2O_2) [Jendrach et al., 2008; Fan et al., 2010; Iqbal et al., 2014] or photoexcitation [Wu et al., 2011]. All led to a fragmentation of the mitochondrial networks, which appears reversible upon stopping the applied stress. The reversible nature of mitochondrial fragmentation after an acute, dose-dependent and non-cytotoxic ROS exposure is reminiscent of a transient upregulation of mitophagy to limit ROS levels. Oxidative stress may be better mitigated in some cells such as myocytes, since contracting skeletal muscle fibers physiologically releases ROS [Fan et al., 2010]. Although mitochondrial morphological changes may occur on the order of hours, Fan et al. reported no major transcriptional changes in the regulation of the main proteins of the fission and fusion machineries. Since a decrease of both the inner mitochondrial membrane potential and the maximum respiratory state have been reported to precede mitochondrial network fragmentation, the molecular mechanism could involve H_2O_2 inhibition of some proteins of the Krebs cycle [Fan et al., 2010]. DRP1 phosphorylation rather than transcriptional changes in this protein synthesis seem to partially mediate the morphological response of mitochondria in skeletal muscle myoblasts [Iqbal et al., 2014]. A recent study reporting mitochondrial fragmentation in primary neuronal cultures

induced by ROS-induced down-expression of Mortalin, a chaperone protein involved in quality control of proteins imported into the mitochondrial matrix, supports such a view [Ferré et al., 2021]. In hyperglycemic conditions, intracellular Ca^{2+} acts as an upstream signaling factor for mitochondrial fragmentation. Increase of intracellular Ca^{2+} relies on Ca^{2+} entry across the plasma membrane, but it remains unclear why high-glucose stimulation activates this influx [Yu et al., 2011]. Photoexcitation of an endogenous photoacceptor by high-fluence low-power laser irradiation in the electron transport chain triggers oxidative stress in ASTC-a-1 cells and COS-7 cells, followed by mitochondrial fragmentation through inhibition of fusion and enhanced fission [Wu et al., 2011].

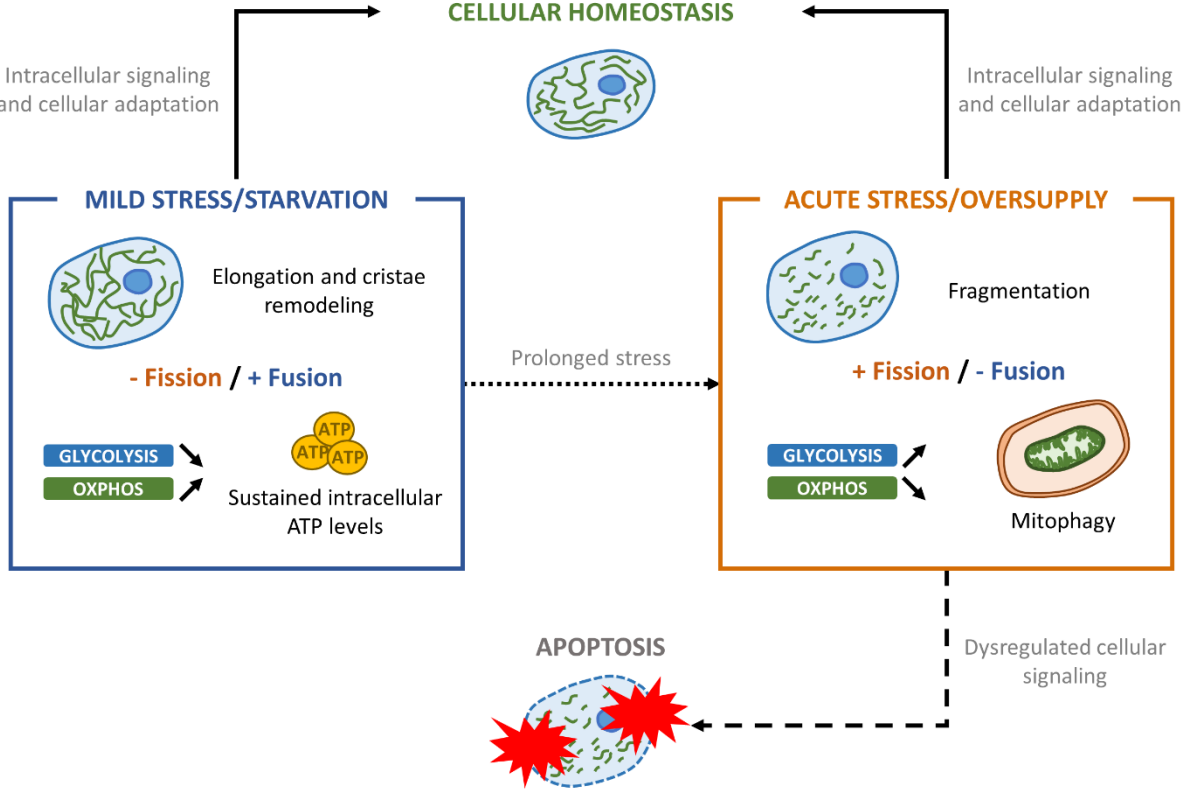


Figure 4: Mitochondrial responses to metabolic cues, depending on the nature, the intensity and the duration of the metabolic signal. Metabolic cues either induce protective mechanisms in mitochondrial dynamics to support cellular homeostasis or impairs cellular signaling and triggers apoptosis.

Metabolic needs impact mitochondria morphodynamics during cell adhesion and cell spreading

Cell adhesion and spreading are energy-consuming processes. The uptake of glucose required to generate ATP for cytoskeleton remodeling and cell adhesion-mediated contractility depends on substrate stiffness. On stiffer substrates (Young modulus $E = 20$ kPa) compared to soft substrates ($E = 1$ kPa), cell spreading induces an initial drop in intracellular ATP, which is associated with the formation of focal adhesions and the reorganization of the actin cytoskeleton [Xie et al., 2021]. AMP-activated protein kinase (AMPK) is subsequently activated by the drop in ATP concentration. AMPK is known to be activated by different types of metabolic stresses that deplete cellular energy levels, including nutrient starvation and hypoxia [Hardie et al., 2012; Kim et al., 2016]. Inhibition of AMPK correlates with significantly reduced glucose uptake even on stiff substrates, suggesting that substrate stiffness is only an exogenous mean to enhance glucose uptake by activating AMPK [Xie et al., 2021]. Surprisingly, AMPK activation following the drop in intracellular ATP during cell spreading on stiff substrates induces fragmentation of the mitochondrial network due to increased fission rates compared to cell spreading on soft substrates.

Overall, the nature, the intensity and the duration of the metabolic signal all have an impact on mitochondrial morphodynamics (Figure 4). Enhanced fission or fusion events can be part of a cytoprotective mechanism to allow cellular adaptation. However, too high levels of metabolic stress may lead to abnormal mitochondrial morphodynamics and cell damage.

Mitochondrial morphodynamics induces metabolic changes

While structural and functional remodeling of the mitochondrial network has been observed in response to metabolic inputs, conversely there is also mounting evidence that mitochondrial morphodynamics has dramatic effects on cell metabolism. Fission and fusion are coordinated processes controlling the mitochondrial network. Alterations of these mechanisms can spark

metabolic dysfunction. Mitochondrial quality control by fission and fusion is all the more central in post-mitotic tissues such as skeletal muscles, which cannot mitigate impaired mitochondria through cellular division. For example, disruption of mitochondrial morphodynamics was found to disturb signaling pathways regulating muscle mass and performance [Romanello and Sandri, 2021].

The predominance of either elongated or fragmented mitochondria in pathologies suggests that an imbalance between fission and fusion rates can lead to metabolic dysfunction. Loss- or gain-of-function studies targeting proteins associated with mitochondrial morphodynamics, as well as the use of cells harboring mutations leading to identified pathologies (such as the Charcot-Marie-Tooth disease), allowed to decipher how these proteins affect metabolism. For a comprehensive overview of how dysregulated activity of fusion- or fission-related proteins can impact cell metabolism, the reader is referred to [Babbar and Sheikh, 2013; Schrepfer and Scorrano, 2016; Zemirli et al., 2018; Eisner et al., 2018; Filadi et al., 2018; Dai and Jiang, 2019]. We summarize below some key instances related to MFN1, MFN2 and DRP1 mutations.

MFN1 and MFN2

Implication of MFN1 and MFN2 in the regulation of metabolism has been demonstrated in different physiological and pathological cell models [Bach et al., 2003; Bach et al., 2005; Chen et al., 2005; Pich et al., 2005; Loiseau et al., 2007; Kulkarni et al., 2016]. Although these GTPases cooperatively regulate mitochondrial outer membrane fusion, MFN1 plays a more prominent role in membrane tethering than MFN2 [Ishihara et al., 2004]. The latter is rather more strongly involved in mitochondrial metabolism, as it notably governs mitochondrial membrane potential, glucose, fatty acid and amino acid oxidation and OXPHOS, as reviewed in [Schrepfer and Scorrano, 2016]. Differently from MFN1, a small fraction of MFN2 has been observed to be located in ER membranes juxtaposed to outer mitochondrial membranes. MFN2 deletion is reported to induce an ER stress, through a mechanism still under debate [Yang et al., 2023], which can lead to a deterioration of cellular functions [Filadi et al., 2018].

MFN2 is downregulated in some metabolic diseases such as type 2 diabetes and obesity, leading to a fragmented mitochondrial network [Schrepfer and Scorrano, 2016; Vezza et al., 2022]. For instance, in primary fibroblasts derived from Charcot-Marie-Tooth disease type 2

patients who display MFN2 mutations, mitochondrial metabolism is greatly affected, with a reduction of both mitochondrial membrane potential and OXPHOS efficacy [Loiseau et al., 2007]. *In vitro* studies show that MFN2 knockdown or knockout models are associated with alterations of both mitochondrial and non-mitochondrial functions. MFN2 downregulation in either L6E9 skeletal myotubes or 10T1/2 mouse embryonic fibroblasts (mediated by an adenoviral vector encoding a mouse MFN2 antisense sequence) induces fragmentation of the mitochondrial network into independent clusters, along with a reduction of glucose oxidation and mitochondrial membrane potential and the inhibition of both pyruvate and palmitate oxidation [Bach et al., 2003]. These changes are mediated by a reduced expression of subunits of the complexes I, II, III and V of the OXPHOS system, as observed in MFN2-deficient obese rats [Pich et al., 2005]. More recent *in vivo* studies on mouse brown adipose tissues show that MFN2 knockout is associated with the disruption of brown adipose tissue thermogenic function [Boutant et al., 2017; Mahdavian et al., 2017]. More precisely, this impairment correlates with a reduced lipolytic capacity and fatty acid oxidation, which are controlled by mitochondrial uncoupling protein 1. Conversely, MFN2 upregulation in HeLa cells by transient transfection of a truncated form of human MFN2 results in the increase of glucose oxidation and mitochondrial membrane potential [Pich et al., 2005].

While MFN2 knockout consistently reduces mitochondrial membrane potential, glucose oxidation and OXPHOS, MFN1 knockout impacts differentially the metabolism depending on the tissues. Liver-specific deletion of the MFN1 gene in mice increases the dependence of hepatocytes on lipids as an oxidative energy source through fatty acid oxidation [Kulkarni et al., 2016]. Fed mice lacking MFN1 in proopiomelanocortin neurons display increased respiratory capacity through mitochondrial complex I and II, along with enhanced ROS production upon glucose stimulation [Ramírez et al., 2017]. Conversely, MFN1 knockout specific to brown adipose tissue does not alone alter mitochondrial bioenergetics [Boutant et al., 2017].

DRP1

In muscle cells, loss of DRP1 remodels the mitochondrial network inducing larger but fewer mitochondria. Functionally, reduction of mitochondrial respiration is observed, consistent with a downregulation of respiratory chain complexes subunits and alteration of calcium

homeostasis [Favaro et al., 2019]. Silencing DRP1 in HeLa cells induces abnormal branching and excessive budding of the mitochondrial network accompanied by an increase in mitochondrial membrane fluidity, which downregulates the expression of the molecular machinery required for ATP synthesis [Benard et al., 2007]. Of note, an increase in membrane fluidity from another origin (i.e. mediated by an intracellular amyloid beta-peptide (A β) accumulation) leads also to a decrease in the efficiency of mitochondrial ATP production [Aleari et al., 2005]. In DRP1 knockdown pancreatic MIN6 cells which express 50 % less DRP1 mRNA and 80 % less protein than control MIN6 cells, coupled mitochondrial respiration is reduced upon glucose stimulation, demonstrating an enhanced mitochondrial proton leak by DRP1 depletion [Kabra et al., 2017]. Conversely, overexpression of DRP1 in skeletal muscle of a transgenic mouse line does not significantly affect mitochondrial bioenergetics. While the rates of oxygen consumption in control and transgenic mice are similar, there is a slight but not significant increase of ATP levels produced by OXPHOS in transgenic mice [Touvier et al., 2015].

3 Mechanical cues and alterations in mitochondrial mechanical properties re-shape and reorganize the mitochondrial network

Changes in the mechanical properties of mitochondria in response to signaling cues should contribute to reshape and reorganize the mitochondrial network. It is indeed becoming increasingly clear that mitochondrial mechanics regulates their morphodynamics. In stark contrast, to our knowledge, evidence for the reciprocal link has not been demonstrated until now.

External forces reshape the mitochondrial network

External forces applied to the cell can modify cell functions in the process known as mechanotransduction. Several modules that transduce forces into intracellular signals have been

identified at the plasma membrane, including cell-substrate and cell-cell adhesions or stretch-activated ion channels [Mathieu and Manneville, 2019]. From a physical point of view, forces applied to membranes often change their tension. In the case of the plasma membrane, changes in osmotic pressure [Roffay et al., 2021], stresses applied by the underlying actomyosin cortex during cell motility [Sitarska and Diz-Muñoz, 2020] or stresses stemming from cell spreading on substrates [Raucher and Sheetz, 2000] modify membrane tension. Similarly, for mitochondrial membranes, forces applied inside (internal) or outside (external) the cell may affect membrane tension and, consequently, mitochondrial morphodynamics. However, how external forces are transmitted from the plasma membrane to mitochondria is still largely unexplored. As for other endomembranes [Phuyal and Baschieri, 2020] and for the nucleus, the cytoskeleton is probably a major actor. Several studies have shown that external mechanical stimulation of either intracellular or isolated mitochondria increase fission or fusion events [Phuyal et al., 2023]. A variety of mechanical stimuli - encounter with motile intracellular pathogens, external pressure applied by an atomic force microscope or cell migration across uneven microsurfaces - have been shown to induce mitochondrial fission [Helle et al., 2017].

Cell stretching

The mitochondrial response to mechanical cues has been particularly studied in cells which integrate mechanical stimuli, such as stretches, as part of their physiological functions. For instance, stretching lung fibroblasts induces an elongation of mitochondria at membrane area strains of 7-14 % and mitochondrial fission for strains above 30 % [Imsirovic et al., 2015]. Similar strains also result in local stretching of the mitochondria of pulmonary endothelial cells [Chapman et al., 2005]. Although the actomyosin cortex [Duda et al., 2019] and/or membrane reservoirs [Sinha et al., 2011] are likely to buffer a large fraction of the applied force on the whole cell, stretching or fission of mitochondria may occur due to their attachment to the cytoskeleton [Bartolák-Suki et al., 2017].

Moreover, the organization of the mitochondrial networks can be differently altered depending on the temporal pattern of the applied forces. To mimic irregular mechanical fluctuations, such as those arising from changes in blood pressure, Bartolák-Suki et al. have

shown in bovine aortic cells the spectacular effects of variable cyclic stretches that incorporate physiological levels of cycle-by-cycle variability in stretch amplitude, compared to monotonous stretch cycles of constant amplitudes [Bartolák-Suki et al., 2015]. The authors found that the average mitochondrial cluster area per cell increases from the unstretched control significantly more during variable stretch than monotonous stretch. The space-filling capacity of the network increases solely in variable stretch. Importantly, variable stretch enhances ATP production by increasing the expression of the catalytic domain of ATP synthase, the expression of cytochrome c oxidase and its tyrosine phosphorylation, and the expression of mitofusins and PGC-1 α . The precise mechanism leading to these results is still unknown, although actin and microtubule networks seem to respond differentially to monotonous stretch and variable stretch stimulations [Bartolák-Suki et al., 2015].

Plasma membrane disruption

The nature and the magnitude of external forces applied on cells can indirectly affect mitochondrial morphodynamics. A slowly increasing compression applied on primary bovine aortic endothelial cells using a spherical microindenter, without membrane rupture, is not sufficient to induce generalized mitochondrial fission [Gonzalez-Rodriguez et al., 2015]. In that case, fission is observed only for dynamic mitochondria and the threshold strain required for fission has not been measured yet. However, local deformation and rupture of the cell plasma membrane using a glass micropipette induce mitochondrial fission in primary bovine aortic endothelial cells [Gonzalez-Rodriguez et al., 2015]. The inner mitochondrial membrane potential decreases after plasma membrane disruption, which demonstrates a partial permeabilization of the inner mitochondrial membrane. The application of cytoskeleton disrupting agents, such as cytochalasin D or nocodazole, does not significantly modify these observations, suggesting that actin and microtubule filaments might only play a role in mitochondrial fission when the plasma membrane integrity is not compromised. However, the question arises whether mitochondrial fission is induced here by an apoptotic process following plasma membrane rupture, which triggers fast, Ca²⁺ signaling pathways. Previous studies have shown that fragmentation of mitochondria and loss of membrane potential are observed during apoptosis through Ca²⁺ signaling, involving permeabilization of the outer mitochondrial membrane and cytochrome c release from the intermembrane space of the mitochondria into

the cytosol [Karbowski and Youle, 2003; Gottlieb et al., 2003]. A more recent study on local disruption of plasma membrane by laser injury in primary mouse embryonic fibroblasts also reports extracellular calcium influx and DRP1-mediated mitochondrial fragmentation at the injury site a few seconds after plasma membrane disruption [Horn et al., 2020]. Injury-proximal mitochondrial fragmentation has a twofold function: it does not only preserve mitochondria from calcium overload and apoptosis, but also enables polarized mROS signaling to mediate plasma membrane repair.

Effect of pressure and osmotic shocks

Elevated hydrostatic pressure can affect mitochondrial structure and network morphology. In differentiated retinal ganglion cells, incubation for three days at elevated hydrostatic pressure (4000 Pa) results in mitochondrial fragmentation and cristae depletion [Ju et al. 2007; Ju et al., 2009]. These changes are associated with higher DRP1 translocation from the cytosol to mitochondria [Ju et al., 2007], increased cytosolic OPA1 and cytochrome c fractions [Ju et al., 2009]. Cytochrome c release might be triggered by either an OPA1 or DRP1-mediated mechanism after pressure treatment [Ju et al., 2009]. Similar results are observed in the optic nerve of glaucomatous mice, which spontaneously develop high intraocular pressure [Ju et al., 2008]. Conversely, in human umbilical vein endothelial cells cultured for 24 hours at a much higher hydrostatic pressure (40 kPa), mitochondria are more elongated than in control conditions, consistent with a decreased expression level of DRP1 and increased expression level of OPA1 [Xu et al., 2022].

Although sometimes difficult to interpret when not combined with other experimental techniques, osmotic shocks may also generate mechanical forces that impact on mitochondrial morphodynamics and mechanics. In monkey kidney epithelial cells, hypertonic conditions induced by high concentration of NaCl result in a reversible mitochondrial network fragmentation, which occurs from the very first five minutes and further develops within an hour [Copp et al., 2005]. This fragmentation is secondary to mitochondrial membrane potential dissipation. In the same study involving plasma membrane rupture by a glass micropipette in aortic endothelial cells [Gonzalez-Rodriguez et al., 2015], in hypertonic conditions, mitochondria are less fragmented after plasma membrane mechanical disruption than in isotonic conditions. Such a hypertonic shock prior to plasma membrane rupture may result in a

shrinking of the mitochondria, associated with a reduction of their membrane tension [Gonzalez-Rodriguez et al., 2015].

Substrate stiffness

The *in vitro* response of mitochondrial morphodynamics to substrate stiffness is a matter of debate and the molecular signaling pathways involved in the regulation of mitochondrial morphology are still under investigation. While some studies report mitochondrial fragmentation on stiff substrates [Tharp et al., 2021; Xie et al., 2021; Guo et al., 2023], stiffness-induced mitochondrial elongation has been evidenced in [Papalazarou et al., 2020, Chen et al., 2021, Romani et al., 2022, Li et al., 2023]. Such conflicting results could result from the different experimental conditions and cell types used in these studies.

In human mammary epithelial cells, it is proposed that solute carrier family 9 member A1-dependent ion exchange SLC9A1 mediates mitochondrial fission in stiffer conditions [Tharp et al., 2021]. In primary human lung fibroblasts, which perform durotactic migration, DRP1 and MFF-mediated mitochondrial fission is also enhanced on stiffer substrates mimicking the pulmonary fibrotic environment, although the mechanotransduction pathways are not clear yet [Guo et al., 2023].

Conversely, in human and mouse pancreatic cancer cells, elongated mitochondrial shapes are also observed on stiff substrates and even on glass [Papalazarou et al., 2020]. Along the same line, in human breast epithelial cells, a soft ECM induces DRP1-mediated mitochondrial fission [Romani et al., 2022]. Increased fission events follow the formation of peri-mitochondrial F-actin driven by Spire1C and Arp2/3 [Romani et al., 2022; Phuyal et al., 2023]. In mesenchymal stem cells, lung adenocarcinoma A549 cells and fibrosarcoma HT1080 cells, ECM stiffening induces mitochondrial elongation by both promoting fission and inhibiting fission [Chen et al., 2021]. Two integrin-dependent signaling pathways are involved, namely kindlin-2-dependent upregulation of fusion and PINCH-1-dependent suppression of DRP1 expression and inhibition of fission [Chen et al., 2021].

Internal forces reshape the mitochondrial network

Mitochondria are mechanically stimulated by forces generated within the cell. Because they are involved in inter-organellar and cytoskeletal contacts, mitochondria are continually subjected to tensile and compressive stresses [Feng and Kornmann, 2018]. There is mounting evidence that mitochondrial fission correlates with these intracellular mechanical cues.

Internal forces are produced by actin and microtubule-associated motor proteins that anchor mitochondria to the cytoskeleton [Boldogh and Pon, 2007]. In particular, the microtubule-based motor proteins dynein and kinesin move mitochondria along microtubule tracks and deliver piconewton forces which induce tension on mitochondrial membranes [Howard, 2002; Xie and Minc, 2020]. Moreover, recruiting molecular motors to the outer mitochondrial membrane via an optogenetic approach induces membrane deformation and pulls membrane tubules which systematically undergo DRP1-dependent and ER-dependent fission [Liu et al., 2022; Song et al., 2022]. The mechanisms underlying the preferential fission at the protruding tubules are not yet clear. On one hand, there is a higher probability that long mitochondrial tubules reach the ER and form contact sites [Liu et al., 2022]. On the other hand, mitochondrial tubules, which are thinner than the mitochondrial body diameter, have a higher local membrane curvature and may provide preferential fission sites by recruiting the fission machinery. This observation is echoing a previous *in vitro* study on curvature-dependent dynamin polymerization on tubules pulled from giant unilamellar vesicles as model membrane systems [Roux et al., 2010]. In order to deform the membrane into tubules, the force generated by dynamin polymerization has to overcome membrane tension. Taken together, these results further suggest that force-induced mitochondrial fission may involve the spontaneous or physically-mediated recruitment of the fission machinery. However, recent experiments performed with synthetic actin-based biomolecular devices on short (i.e. minute) time scales suggest that fission can also occur in the absence of DRP1 independently of the endogenous mitochondrial fission machinery [Nakamura et al., 2020; Miyamoto et al., 2021].

Physico-chemical cues regulate mitochondrial morphodynamics

Physico-chemical stimuli can affect the mechanical properties of mitochondria and change their

morphodynamics. The role of cardiolipin (CL), a unique negatively charged phospholipid found only in membranes of both bacteria and mitochondria, in mitochondrial function and dynamics has been thoroughly reviewed in [Paradies et al., 2019; Yang et al., 2022]. Besides the regulation of proteins and enzymes involved in mitochondrial metabolic functions, CL is substantial in both fission and fusion dynamics. In particular, it emerges as a key player of the inner mitochondrial membrane plasticity by contributing to cristae structure and physico-chemical properties [Joubert and Puff, 2021], and by interacting with fission and fusion proteins such as DRP1 [Stepanyants et al., 2015] and OPA1 [Liu et al., 2017; Ban et al., 2018; Ge et al., 2021]. For instance, CL is required for mediating heterotypic mitochondrial inner membrane fusion through its interaction with the long form of OPA1. The morphology of mitochondria can be drastically altered by changes in cardiolipin concentration. Low levels of cardiolipin are associated with enlargement and swelling of mitochondria, along with disorganization of the cristae structure which may be linked to a decrease in membrane fluidity [Nichols-Smith et al., 2004].

pH levels can also affect the deformability of mitochondria and their fission and fusion rates by perturbing the organization of the mitochondrial membranes [Wang et al., 2008; Gonzalez-Rodriguez et al., 2015; Yoneda et al., 2022]. A non-physiological pH stiffens isolated mitochondria, as deduced from the deformation of these organelles using the micropipette aspiration technique [Wang et al., 2008]. Acidification of the outer surface of the inner membrane is associated with a decrease in cristae rigidity, its unfolding and subsequent mitochondrial inner membrane swelling [Yoneda et al., 2022]. Since the inner mitochondrial membrane contains a large concentration of proteins, including proteins of the cristae junction complexes [Yang et al., 2022], such softening as the pH decreases may be due to a higher flexibility of these proteins and/or changes in lipid composition and charge. In giant unilamellar vesicles containing cardiolipin, a local modulation of the pH across the membrane creates or represses the formation of cristae-like invaginations [Khalifat et al., 2008].

Is mitochondrial membrane tension essential for mitochondrial fission?

The lipid double-membrane of mitochondria participates both in mitochondrial mechanics and

morphodynamics. The inner and outer mitochondrial membranes play specific functional roles and understanding how they respond to mechanical stimulation has been of recent interest.

The role of mitochondrial membrane tension in mitochondrial morphodynamics has recently drawn attention, with a recent work shedding light on the correlation between this physical parameter and fission events [Mahecic et al., 2021]. In this work, the authors followed the fluorescence lifetime of the mechanosensitive MitoFlipper probe as an indirect sensor of the mitochondrial membrane tension in COS-7 cells. In COS-7 cells, constriction sites enriched in DRP1 and/or overlapping with ER tubules do not always lead to fission. Moreover, these reversible constriction events ('reversals') do not result from differences in the recruitment of DRP1, Dyn2 and the colocalization between ER tubules and DRP1-enriched constriction sites. By comparing the occurrence of fission events with MitoFlipper fluorescence lifetimes and analytical membrane tension values, Mahecic et al. show that a higher membrane tension coincides with a greater probability of fission events. Mitochondrial constrictions under higher membrane tension (typically 6 $\mu\text{N/m}$) are more likely to undergo fission than constrictions under low tension (typically 4 $\mu\text{N/m}$). This study also suggests that fluctuations in membrane tension could play a critical role in mitochondrial fission by stochastically increasing the local membrane elastic energy and lowering the energy barrier for fission. The mechanisms underlying changes in mitochondrial membrane tension are however not clear (Figure 5). The observed reduction in mitochondrial membrane tension and fission upon inhibition of non-muscle myosin II using ML-7 or Blebbistatin, or depolymerization of microtubules using Nocodazole may be an indirect consequence of cytoskeletal-dependent changes. As reviewed in [Fatima and Ananthanarayan, 2023], results in [Mahecic et al., 2021] contrast with those in [Mehta et al., 2019], as depolymerization of microtubules in budding yeast upon deletion of kinesin-like proteins is correlated with a higher mitochondrial fission frequency. Furthermore, although in a different context, a decrease in plasma membrane tension was shown to favor abscission during cell division [Lafaurie-Janvore et al., 2013]. Overall, there are many pending questions to be answered: is an increase in mitochondrial membrane tension sufficient to induce fission? Is this membrane tension-induced fission DRP1-dependent, ER-dependent and actin-dependent? Are mitochondrial membrane tension changes rather a consequence of the recruitment of the canonical or non-canonical fission machinery?

In summary, mechanical forces, whether applied externally (by stretching the cell, applying pressure or modulating substrate stiffness) and transmitted to mitochondria, or applied internally directly on mitochondria (by the cytoskeleton or the fission machinery), can alter mitochondrial morphodynamics. During these mechanotransduction events, while signaling events involving not only proteins but also lipids play critical roles, physical parameters such as membrane curvature and membrane tension also emerge as key regulators.

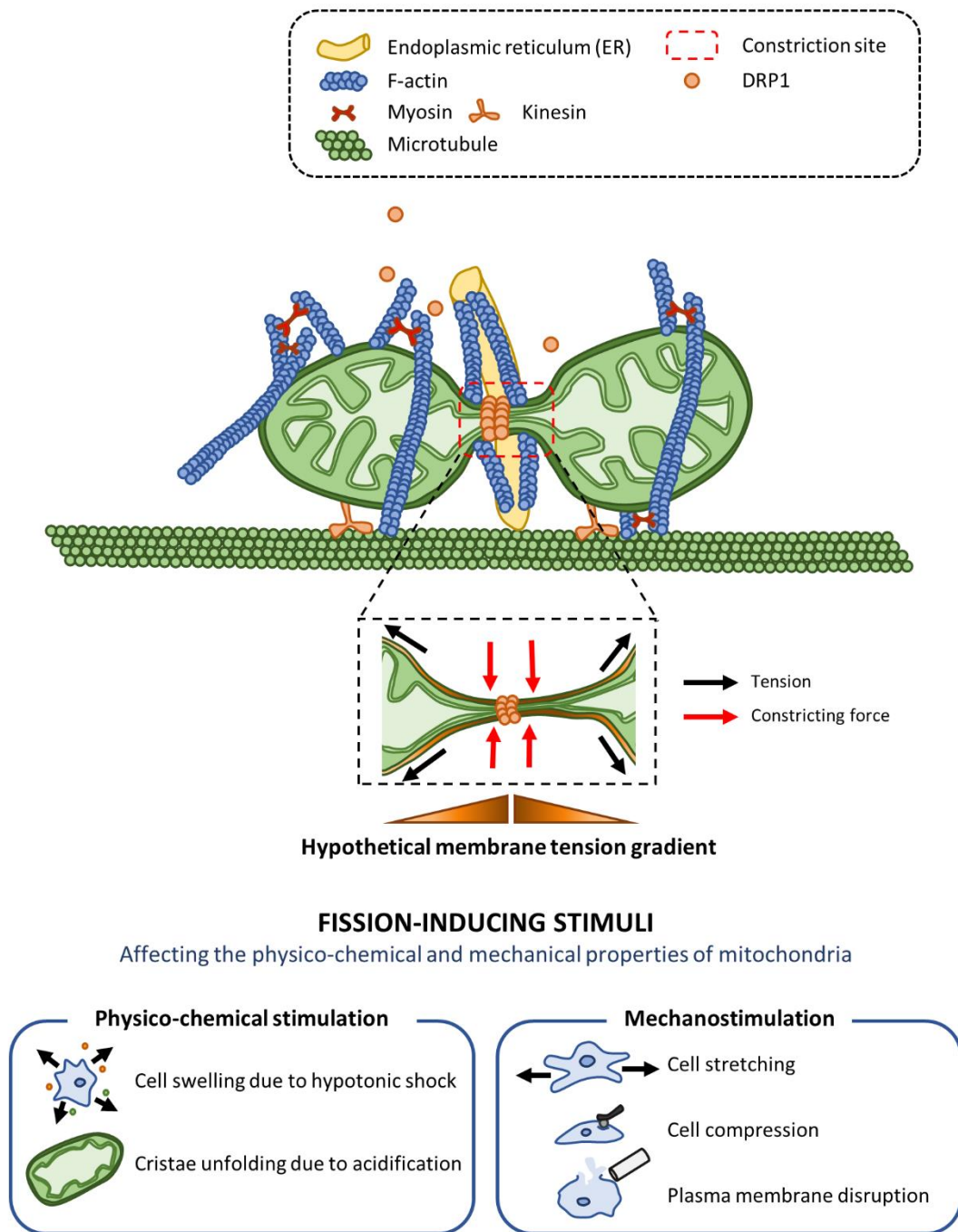


Figure 5: Schematic representation of the actin-dependent and DRP1-dependent mitochondrial fission process and the variety of fission-inducing stimuli contributing to the increase in mitochondrial membrane tension. A hypothetical membrane tension gradient is represented in the boxed region of the constriction site, with the maximum value localized at the level of the DRP1 helical structure. The recruitment of DRP1 at a pre-constricted fission site, the association with the endoplasmic reticulum (ER) and the coordinated activity of actin filaments

generate tensile and bending forces. The recruitment of DRP1 by mitochondrial adaptor proteins is not represented. DRP1 assembles into a helical structure to further constrict the mitochondrion and may generate a membrane tension gradient.

4 Mitochondrial mechanics and metabolism are reciprocally regulated

How cellular mechanics and metabolism are connected at multiple scales, from molecular mechanosensors to the cytoskeletal networks and the organelles, is a topic of growing interest [Evers et al., 2021]. We focus here on the interplay between mechanics and metabolism at the level of mitochondria. The links between mechanical cues, mitochondrial mechanics and their metabolic functions are still poorly understood. While it is clear that the physical integrity of the mitochondrial membrane is crucial for OXPHOS, the mechanical properties of mitochondria and/or physical cues could also influence mitochondrial metabolic processes including ATP or ROS production (Figure 6).

Mechanical cues and mechanosensitive pathways regulate metabolism at the level of mitochondria

Physical cues regulate cell metabolism

OXPHOS is sensitive to physical variations of the mitochondrial environment. Two early studies investigated the effects of osmotic pressure on the respiration of rat liver mitochondria [Sitaramam et al., 1989; Sitaramam and Rao, 1991]. Based on the generation and use of the proton electrochemical gradient in the respiratory chain, the activity of the complexes of the OXPHOS system in mitochondrial membranes was assessed by the well-established polarographic technique [Chance and Williams, 1955; Wenchich et al., 2003; Li and Graham, 2012]. Mitochondria subjected to hyperosmotic pressure undergo structural shrinking and

inhibition of respiration. Additionally, the mitochondrial respiratory chain complexes display a differential osmotic sensitivity [Sitaramam et al., 1989].

Applied forces change cell metabolism

Mitochondrial metabolic responses strongly depend on the nature of the applied forces. This is particularly true for primary bovine aortic smooth muscle cells subjected to sinusoidal stretches with either a constant or a variable amplitude, compared to unstretched conditions [Bartolák-Suki et al., 2015]. Stretching cells induces a reorganization of the mitochondrial network, but the bioenergetic responses depend on the stretch pattern. A variable amplitude enhances ATP production compared to a constant amplitude, as a consequence of the increase in the expression of the ATP synthase catalytic domain and of cytochrome c oxidase [Bartolák-Suki et al., 2015]. These results indicate that the temporal pattern of stretching is sensed by mitochondria and promotes ATP production through OXPHOS.

Flow-generated mechanical forces play a critical role in cellular metabolism. In particular, the luminal surface of the endothelium is constantly exposed to shear stress induced by the blood circulation. Endothelial cells need a constant supply of energy to perform various processes such as the trafficking of blood-borne cells, nutrients and hormones, angiogenesis and the maintenance of blood fluidity. The shear stress magnitude and the flow pattern can significantly vary. For instance, during exercise, the shear stress can increase from 1.5 Pa to 3 Pa in human arteries [Tang et al., 2006]. Changes in the magnitude and the pattern of hemodynamic flow induce mitochondrial remodeling and metabolic reprogramming. As reviewed in [Hong et al., 2022], several studies have focused on metabolic reprogramming within endothelial cells subjected to laminar shear stress or oscillatory shear stress. Laminar shear stress is associated with an increase in mitochondrial respiration, a decrease in glycolysis and an upregulation of lipid metabolism [Kim et al., 2014; Doddaballapur et al., 2015; Feng et al., 2017; Wu et al., 2017; Venturini et al., 2019]. High laminar shear stress induces activation of the anti-inflammatory transcription factor, Kruppel-like factor Klf2 [Coon et al., 2022], which downregulates gene expression of glycolytic enzymes [Doddaballapur et al., 2015]. Interestingly, either mitophagy or an antioxidant response can activate Klf2, which shows that the MEKK2/3-MEK5-ERK5-Klf2 signaling pathway is an instance of mito-hormesis. Hypoxia

inducible factor-1 α (HIF-1 α) and protein kinase AMP-activated/AMP-activated protein kinases (PRKA/AMPK) are the major transcriptional regulators activated by oscillatory shear stress and increase the expression of glycolytic enzymes [Wu et al., 2017; Yang et al., 2018]. Enhanced glycolysis under oscillatory shear stress may be a protective metabolic adaptation providing endothelial cells sufficient energy to counteract the disturbed flow. Interestingly, upregulation of aerobic glycolysis in endothelial cells of atheroprone arteries promotes endothelial proliferation which is essential for repair [Doddaballapur et al., 2015]. Flow-generated mechanical forces also affect the mitochondrial metabolic response in metastatic cancer cells [Park et al., 2022]. Two breast cancer cell lines with different levels of aggressiveness (MDA-MB-231 and MCF-7 cells) display distinct ATP production pathways when exposed to shear stress. Although shear stress increases oxygen consumption in both cell lines, the more invasive MDA-MB-231 cells rely more on aerobic glycolysis than on OXPHOS, characterized by higher lactate production, when compared to MCF-7 cells [Park et al., 2022]. Overall, shear stress enhances oxygen consumption and a non-monotonous fluid flow induces a switch towards a more glycolytic behavior. However, how fluid shear stress affects the mechanical properties of mitochondria remains to be understood.

Mechanical stimulation also impacts ROS production. Cyclic mechanical strain of 25 % on human umbilical vein endothelial cells significantly increases mitochondrial ROS production compared to unstretched conditions, suggesting that endothelial cells detect cyclic strain and transmit mechanical stimuli via the actin-cytoskeleton to the mitochondria, thus triggering ROS production [Ali et al., 2004]. Similarly, cyclic mechanical strain increases ROS production in pulmonary endothelial cells [Chapman et al., 2005]. Strains above a threshold value of 15 % trigger an increase in ROS production. This threshold level is of the same order of magnitude as physiological strain levels experienced by lungs *in vivo* during breathing [Birukov et al., 2003]. The mechanisms linking ROS production and the magnitude of stretching still have to be clarified.

Mechanotransduction regulates metabolic changes

Modulation of the physical and mechanical properties of the microenvironment and notably of the ECM induces metabolic changes through adhesion-mediated mechanosignaling [Papalazarou et al., 2020; Park et al., 2020; Romani et al., 2021; Tharp et al., 2021; Romani et al., 2022]. ECM stiffness sensing by integrin- and cadherin-mediated adhesions triggers intracellular signaling cascades and subsequent rearrangements of the cytoskeleton and focal adhesions. Both the expression and activity of metabolic enzymes can be affected by mechanosignaling-induced cytoskeleton remodeling. Recent findings related to the links between ECM architecture and mitochondrial functions have been reviewed in [Yanes et al., 2022].

Using Seahorse extracellular flux analysis and different ECM hydrogels, the influence of the ECM stiffness on OXPHOS and glycolysis can be assessed in a high-throughput fashion [Bertero et al., 2016; Chakraborty et al., 2021; Romani et al., 2022]. While an overall increase in glycolysis and decrease in OXPHOS have been observed in stiff conditions, differences in the basal or maximal levels of glycolysis and OXPHOS have been observed in stiff conditions [Bertero et al., 2016; Romani et al., 2022]. In pulmonary arterial endothelial cells and smooth muscle cells, basal glycolysis increases on a stiff matrix (Young modulus $E = 50$ kPa) compared to a soft matrix ($E = 1$ kPa), while the rates of basal oxygen consumption, ATP-dependent oxygen consumption and maximal oxygen consumption decrease [Bertero et al., 2016]. The results by [Romani et al., 2022] report a significant increase in the maximal respiratory capacity in human mammary epithelial cells in stiff conditions. In dendritic cells, changes in substrate stiffness also induce a similar metabolic reprogramming, reflected by an upregulation of glucose uptake, glycolysis gene expression and maximal glycolytic and respiratory capacity on stiff hydrogels ($E = 50$ kPa) [Chakraborty et al., 2021]. Since this switch from OXPHOS to glycolysis with increased stiffness is not sufficient to sustain the growing energetic needs during proliferation, the two main anaplerotic pathways, glutaminolysis and carboxylation of pyruvate to oxaloacetate, are also concomitantly activated to replenish carbon intermediates [Bertero et al., 2016]. In human mammary epithelial cells, mitochondrial oxygen consumption is impaired on a stiff ECM ($E = 60$ kPa) compared to a soft ECM ($E = 400$ Pa), due to the downregulation of some electron transport chain subunits [Tharp et al., 2021]. Together with this reduction of mitochondrial oxygen consumption, these cells display higher levels of lactate and lower levels of metabolites involved in two cytosolic metabolic processes, namely the upstream glycolysis and pentose-phosphate pathway. Mechanistically, in all these

different cell types, remodeling of the cell bioenergetics in response to increased ECM stiffness requires the mechanoactivation of downstream Hippo-signaling pathway transcriptional co-activators Yes-Associated Protein 1 (YAP) and TAZ [Dupont et al., 2011; Bertero et al., 2016; Chakraborty et al., 2021; Romani et al., 2022].

Understanding how cancer cells fine-tune their energy production and consumption in response to the heterogeneous mechanical properties of the microenvironment is of relevance to further identify the mechanisms regulating proliferation and migration. Indeed, during the metastatic cascade, they are subjected to changes in stiffness, from the stiff environment of primary tumors to often softer sites of secondary metastases. In MDA-MB-231 metastatic breast cancer cells, both the collagen matrix density and fiber orientation influence the intracellular ATP:ADP ratio [Zanotelli et al., 2018]. In denser collagen matrices where cell migration is impaired, the ATP:ADP ratio is higher than in less dense gels. Moreover, in aligned collagen matrices, a facilitated cell migration goes hand in hand with a decrease of the ATP:ADP ratio. These results show that impaired motility in more challenging environments induces an increase in ATP consumption and production to compensate for higher energetic needs related to cytoskeleton and focal adhesion remodeling. In mouse KPC and human PANC-1 pancreatic cancer cells, ECM stiffening induces a metabolic shift that could support both cell invasion and migration [Papalazarou et al., 2020]. While a softer matrix favours aerobic glycolysis, a stiffer substrate engages sustained ATP production through the TCA-cycle and OXPHOS, which correlates with higher levels of ROS. Inhibition of the mitochondrial ATP synthase and OXPHOS by oligomycin A reduces cancer cell invasion, which points to a substantial role of mitochondrial respiration in driving cell motility [Papalazarou et al., 2020]. On a soft ECM, MCF10AneoT human mammary epithelial cells treated with ROCK and myosin light chain kinase (MLCK) inhibitors and D2.OR mouse metastatic breast cancer cells both show an increase in cytoplasmic, mitochondrial and membrane lipid ROS, predominantly of mitochondrial origin [Romani et al., 2022]. Increased levels of ROS upregulate the transcriptional factor nuclear factor erythroid 2-related factor (NRF2) which exerts a feedback control on ROS. Activation of DRP1 on soft ECM regulates ROS levels and subsequently enables cells to overcome oxidative stress [Romani et al., 2022]. Using the recently synthesized Peroxymycin probe [Chung et al., 2018], Tharp et al. observed an increase in intracellular ROS on stiffer substrates, which could regulate the expression of heat shock factor 1 (HSF1) target

genes [Tharp et al., 2021]. They suggested that HSF1 in turn represses ROS-induced oxidative stress by limiting oxygen consumption. Alternatively, there is evidence that the YAP/TAZ pathway is involved in the regulation of the HSF1 target genes [Luo et al., 2020]. However, the increase in ROS on stiff substrates observed in [Tharp et al., 2021] using Peroxymycin is conflicting with the results from the same study using the more common probe DCFDA and with other studies using DCFDA, MitoSOX and Cystine-FITC [Romani et al., 2022] or CellRoxOrange [Yang et al., 2019]. These results suggest that a stiff ECM induces a decrease, rather than an increase, in ROS production. Future studies should help clarify this issue.

Metabolic activity controls mitochondrial mechanics

Although crucial for our understanding of the mechano-metabolic feedback control of mitochondria, very little is known about the mechanical adaptation of mitochondria in response to metabolic cues. A theoretical approach has shown that mechanical parameters of the inner mitochondrial membrane, such as its surface tension, bending rigidity and elastic modulus of Gaussian curvature, are affected by metabolic-driven changes of the proton-motive force, such as proton leakage and mitochondrial membrane potential [Chvanov, 2006]. How different metabolic cues alter the electrochemical fluxes and thus the mechanical properties of mitochondria still remain to be explored.

In a recent optomechanical study, the mechanical properties of mitochondria isolated from rat glial cells were measured using real-time fluorescence and deformability cytometry [Komaragiri et al., 2022]. Results show that mitochondrial mechanics correlates with intracellular superoxide levels. Exposing cells to H_2O_2 reduces the size of mitochondria and significantly increases their deformation in the microfluidic channel. Mitochondrial stiffness is reduced with increasing H_2O_2 concentrations. This elegant study provides a first step towards elucidating how mitochondrial mechanics depends on metabolic cues.

Oxidative phosphorylation (OXPHOS)

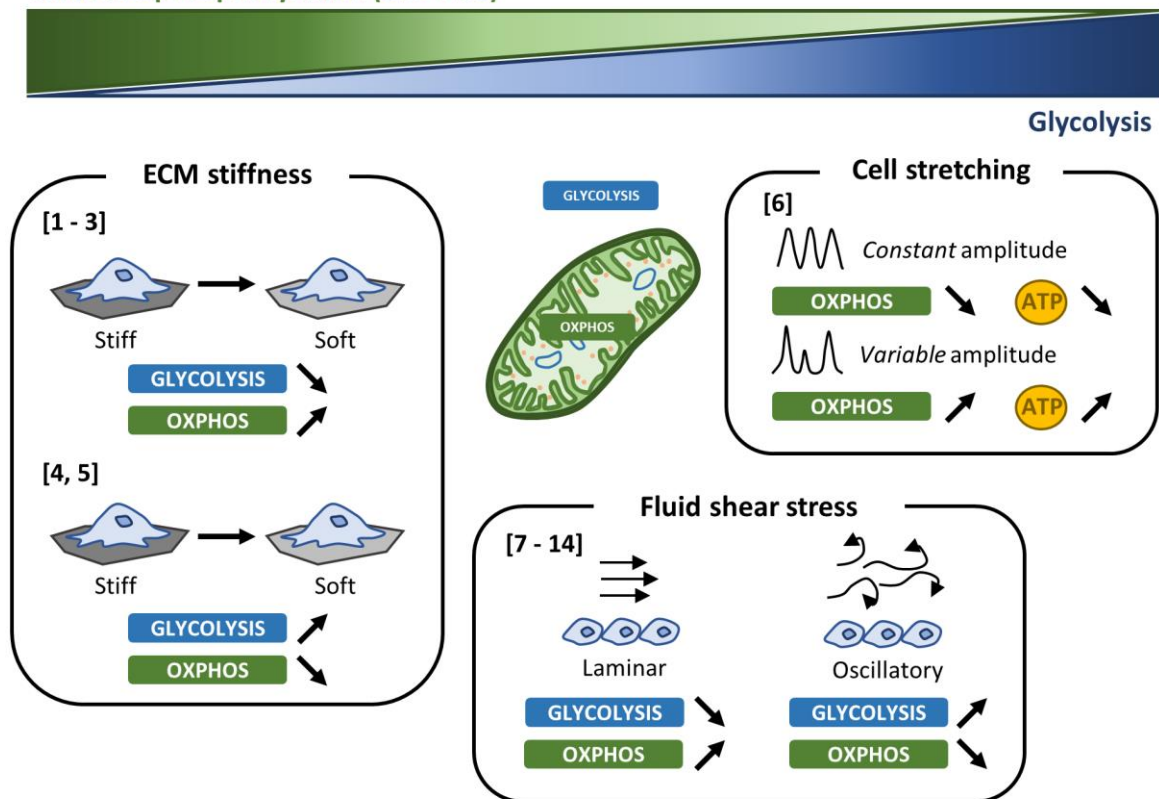


Figure 6: Mitochondrial metabolic adaptation to mechanical cues. The contribution of OXPHOS and glycolysis in ATP production is regulated by the nature and the magnitude of mechanical forces exerted on the cell. References indicated on the figure are : [1] Bertero et al., 2016, [2] Chakraborty et al., 2021, [3] Romani et al., 2022, [4] Papalazarou et al., 2020, [5] Tharp et al., 2021, [6] Bartolák-Suki et al., 2015, [7] Kim et al., 2014, [8] Doddaballapur et al., 2015, [9] Feng et al., 2017, [10] Wu et al., 2017, [11] Yang et al., 2018, [12] Venturini et al., 2019, [13] Coon et al., 2022, [14] Park et al., 2022.

5 Discussion and perspectives

Bidirectional links exist between mitochondrial

morphodynamics, mechanics and metabolism

In the past decade, there has been a rising interest in organellar mechanosensing and mechanotransduction. The role of mitochondria in the cross-talk between mechanotransduction and cell metabolism has just begun to receive some attention. It is becoming clear that mitochondria can be described as dynamic systems which constantly adjust their response to mechanical and chemical cues. Recent studies have cast light on one or several aspects of the tripartite interactions between mitochondrial morphodynamics, mechanics and metabolism. Grasping such complex correlations not only allows to understand the physiological contribution of mitochondria as key organelles but is also relevant in pathological conditions.

Despite the intercellular and intracellular heterogeneity of mitochondrial morphodynamics, which goes hand in hand with the physiological functions of cells and tissues, it is possible to identify similar effects induced by mechanical and physico-chemical stimuli. When cells are exposed to stress, whether in the form of intracellular variations in metabolite fluxes or in the form of forces exerted by the environment, mitochondria change their morphology, their dynamics and their organization. These changes at the level of the mitochondria have important implications for their functions and for cell metabolism. Mechanistically however, it is not yet clear how different internal or external stimuli can trigger similar morphological and metabolic changes.

Going beyond correlation between mitochondrial morphodynamics, mechanics and metabolism

Given the numerous pathways which contribute to mitochondrial morphodynamics and which may be interconnected and redundant, it is challenging to interpret the reciprocal effects of mechanics and metabolism and to go beyond simple correlation. Determining whether alterations in mitochondrial shapes and dynamics are a cause or a consequence of an impairment of their mechanical properties and/or metabolic functions, requires to precisely identify and monitor sequential mechanosensitive signaling pathways. A systematic exploration of the direct bidirectional links between mitochondrial morphodynamics, mechanics and metabolism is still

lacking. For instance, whether mitochondrial morphological changes impact their mechanical or mechanosensing properties is not demonstrated yet, even if disrupting mitochondrial morphodynamics is known to have dramatic effects on cell metabolism which could in turn regulate mitochondrial mechanics.

Another limitation of the studies performed so far is the variability of the results depending on the cell type or the technique. A large spectrum of cell types (*e.g.* endothelial cells, muscle cells, endocrine cells, cells from connective tissues) has been investigated separately. It would be interesting to compare the response of mitochondria to a given mechanical or metabolic cue in different cell types with the same technique. In addition to the nature and the magnitude of the environmental stress, the metabolic and mechanical phenotypes of the cell have to be taken into account. The relative contributions of OXPHOS and cytosolic glycolysis and the mechanical properties of the cell, such as its visco-elasticity, may modulate the response of the cell to both mechanical and physico-chemical cues.

Studying mechanotransduction and metabolism at mitochondria is technically challenging

Mitochondria are extremely dynamic organelles in both physiological and pathological conditions. They undergo fission and fusion in response to mechanical and metabolic cues. Studying the mechanobiology of mitochondria is technically challenging as it requires intracellular micro-manipulation hence to develop new techniques or to adapt existing methods [Fuller and Arriaga, 2003].

One key issue is whether mitochondria should be studied individually and/or collectively to fully characterize their mechanobiology. In cells, the three cytoskeletal networks not only support the reorganization of mitochondrial networks and the directional transport of mitochondria within the cell, but can also transmit internal and external forces to these organelles [Bartolák-Suki et al., 2017; Phuyal et al., 2023]. Therefore, since they are tethered to cytoskeletal components, mitochondria are constantly under tension in cells [Feng et al., 2019]. In contrast, mitochondria are rounder when isolated *in vitro* [Gonzalez-Rodriguez et al., 2015]. In cells, they also form interconnected networks and contact other organelles such as the

ER [Rizzuto et al., 1998; Rowland and Voeltz, 2012; Csordás et al., 2018]. Studying isolated mitochondria can thus provide clues for understanding their mechanobiology, as mechanical parameters such as the viscosity of the mitochondrial matrix impacts on the organization of the mitochondrial network [Yang et al., 2013] and correlates with higher H₂O₂ levels in multiple cell types from various organisms [Fan et al., 2021; Li et al., 2019]. However, it is certainly not sufficient to fully characterize their properties in a cellular environment. Besides the traditional differential centrifugation methods to isolate mitochondria [Bustamante et al., 1977; Wieckowski et al., 2009; Djafarzadeh and Jakob, 2017], microfluidics allows efficient and damage-free isolation of mitochondria. A recent study introduced a curved 3D-microfluidic device which exploits the balance between the inertial lift force and the Dean drag force in a size-dependent fashion to separate mitochondria from cellular debris [Lee et al., 2022].

Whether isolated *in vitro* or in live cells, studying the molecular mechanisms that govern the responses of mitochondria to mechanical and metabolic signals remains a technical challenge. The development of non-invasive and possibly high-throughput intracellular mechano-stimulators and probes is required to address this question in live cells. Microfluidics has been used to examine the mechano-response of mitochondria to large and global stresses. For instance, microchannels with constrictions that are less than 1 μm wide, which is the typical diameter of an ovoidal mitochondrion, were designed to study the mitochondrial response to cell squeezing [Feng et al., 2019]. Real-time fluorescence and deformability cytometry is another high-throughput microfluidic technique that allows to measure mitochondrial size, deformation and Young's modulus using a hydrodynamic flow field [Komaragiri et al., 2022]. Mechano-stimulation can also be performed in a highly targeted manner by exerting local forces on mitochondria with magnetic, optical or acoustic tweezers, although with a lower throughput than microfluidics [Norregaard et al., 2017].

As emphasized in section 3, membrane tension seems to be a key mechanical property underpinning mitochondrial morphodynamics. Probing mitochondria is difficult because of their inner membrane and negative membrane potential. They are impermeable to most molecules targeting intracellular compartments [Ma et al., 2020]. Molecules with lipophilic and cationic moieties are thus commonly used to target the mitochondria. Flipper probes can indirectly report membrane tension or lipid order through their fluorescence lifetime [Colom et al., 2018; Goujon et al., 2019; Roffay et al., 2022]. These fluorogenic mechanophores

spontaneously insert between lipid hydrophobic tails. They measure changes in lipid bilayer organization through their conformational changes and the associated variations in their fluorescence properties. Flipper probes that selectively label mitochondria have been designed [Colom et al., 2018; Goujon et al., 2019] and have already been shown to be extremely pertinent tools to report mitochondrial mechanics in live cells [Mahecic et al., 2021].

Deciphering the cross-talk between mechanics and metabolism requires the combination of mechanical and metabolic measurements. Techniques for bulk metabolic analysis performed on a large number of cells [TeSlaa and Teitell, 2014] should be made compatible with high-throughput mechanical measurements. To compare glucose uptake and lactate production for glycolytic flux quantification, chromatography combined with mass spectrometry is widely used, allowing labeled glucose-derived carbon tracing in glycolytic intermediates [Luo et al., 2007]. To evaluate OXPHOS through oxygen consumption rate, Clark-type micro-oxygen electrodes are commonly used [Li and Graham, 2012]. However, these techniques do not allow the simultaneous measurement of glycolysis and OXPHOS in cells. Alternatively, it is possible to measure both the oxygen consumption rate and the extracellular acidification rate using Seahorse flux analyzer [Little et al., 2020; Gu et al., 2021]. This technique provides data on the energetic demand of cells under basal conditions, the maximal capacity of the electron respiratory chain, the glycolytic capacity of cells and their ability to adapt to changes in energy demand. Combining such techniques with mechanical measurements and/or mechanical stimuli as in previous studies on the role of substrate stiffness [Bertero et al., 2016; Chakraborty et al., 2021; Romani et al., 2022] should be instrumental for a better understanding of the links between cell mechanics and cell metabolism at the mitochondrial level.

Is the mechano-morpho-metabolic signature of mitochondria a potential diagnostic tool in pathologies?

Mutations in mitochondrial DNA or in nuclear genes encoding mitochondrial proteins can cause so-called primary mitochondrial diseases. Such genetic alterations notably impair the electron transport chain machinery and subsequently OXPHOS [Wallace, 2010, Niyazov et al., 2016]. In contrast to these primary mitochondrial diseases, secondary mitochondrial

dysfunctions rather stem from multifactorial disorders, such as diabetes, neurodegenerative disorders or cancers [Pich et al., 2005; Loiseau et al., 2007; Camara et al., 2010; Zambrano et al., 2022]. The importance of investigating the mechanometabolic signature of mitochondria in pathologies is twofold. First, although many diseases do not necessarily stem from a mitochondrial dysfunction, the latter may contribute to their progression. Second, disruption of mitochondrial function was shown to contribute to the etiology of distinct pathologies through common pathways, such as mitochondrial fission and fusion dynamics [Murphy and Hartley, 2018]. Given the reciprocal regulation between cell metabolism and mitochondrial mechanics, targeting the latter aspect in therapeutic approaches can possibly improve the outcome of multiple diseases.

Characterizing the morphological and mechanical properties of mitochondria, in addition to their metabolic functions, emerges as a tool for cancer detection, prognosis and possibly therapy. One striking historical example suggesting a causative relationship between metabolic disruption and tumorigenesis is the *in vitro* induction of malignancy in rat heart fibroblasts subjected to intermittent anaerobiosis [Goldblatt and Cameron, 1953]. The focus on mitochondria in cancer was initiated by Warburg's discovery that most cancer cells undergo aerobic glycolysis as their main ATP production pathway, while decreasing their dependence on mitochondrial OXPHOS [Warburg, 1956; Gatenby and Gillies, 2004]. Biochemical processes within mitochondria of cancer cells were later shown to be critical for both tumorigenesis and metastasis [Vyas et al., 2016; Zong et al., 2016; Scheid et al., 2021]. How cancer cells adapt their bioenergetics in response to fluctuating metabolic needs during migration and invasion, processes which combine protrusive and contractile forces through e.g. actin dynamics and actomyosin contractility, is well reviewed in [Zanotelli et al., 2021]. Along this line, Garde et al. reported very recently that ATP bursts are produced by enhanced mitochondria recruitment at the front of anchor cells crossing the uterine and vulval basal membranes to sustain a local increase in glucose import, showing that energy import, processing and generation are interlinked with the invasive machinery [Garde et al., 2022]. There is also mounting evidence that cancer is coupled with an alteration of mitochondrial dynamics. In particular, an imbalance of mitochondrial fission and fusion dynamics, yielding a fragmented mitochondrial network, is often observed in cancer cells [Senft and Ze'ev, 2016]. In lung cancer, fragmentation of mitochondria is triggered by high expression or enhanced

activation of DRP1 and/or downregulation of MFN2 [Rehman et al., 2012]. Hypoxia promotes the migratory activity of glioblastoma U251 cells through upregulation of DRP1 [Wan et al., 2014]. Similarly, in breast cancer cells, increased mitochondrial fission through an upregulation of DRP1 may be correlated to cell motility and invasion by promoting lamellipodia formation at the leading edge of the cell [Zhao et al., 2013]. Conversely, downregulation of DRP1 results in mitochondria elongation and enhanced apoptosis in HCT116 and SW480 human colon cancer cells [Inoue-Yamauchi and Oda, 2012]. Besides the metabolic and morphodynamic changes occurring during tumorigenesis and metastasis, cancer cells are subjected to a myriad of well identified mechanical cues from their microenvironment, including solid stresses, interstitial fluid pressure, tissue material properties and shear stress in the vasculature [Nia et al., 2020; Colin et al., 2021]. However, how the physical interactions between cancer cells and their environment tune the morphodynamics, metabolic functions and mechanics of mitochondria remains to be elucidated.

These observations raise many questions. Is mitochondrial fission necessary for migration in all cancer cells? Can increased mitochondrial fragmentation be considered as a diagnostic biomarker for metastasis? Because mitochondrial morphodynamics depends on the mechanical properties of mitochondria, is it possible to identify a common mechanophenotype for mitochondria from cancer cells of the same grade or similar aggressiveness? Technical and conceptual advances are clearly required to convert mitochondrial mechanobiology into an efficient diagnostic and prognostic method. Associating different techniques at multiple scales to probe the morphological, metabolic and mechanical properties of mitochondria should provide clues for a better understanding of tumor progression and other pathologies.

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Conflict of interest statement

The authors have declared no conflict of interest.

References

- [Aleari et al., 2005] Aleari, A., Benard, G., Augereau, O., Malgat, M., Talbot, J., Mazat, J., Letellier, T., Dachary-Prigent, J., Solaini, G., and Rossignol, R. (2005). Gradual alteration of mitochondrial structure and function by β -amyloids: importance of membrane viscosity changes, energy deprivation, reactive oxygen species production, and cytochrome c release. *Journal of Bioenergetics and Biomembranes*, 37(4):207–225.
- [Ali et al., 2004] Ali, M. H., Pearlstein, D. P., Mathieu, C. E., and Schumacker, P. T. (2004). Mitochondrial requirement for endothelial responses to cyclic strain: implications for mechanotransduction. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 287(3):L486–L496.
- [Audzeyenka et al., 2021] Audzeyenka, I., Rachubik, P., Typiak, M., Kulesza, T., Topolewska, A., Rogacka, D., Angielski, S., Saleem, M.A. and Piwkowska, A. (2021). Hyperglycemia alters mitochondrial respiration efficiency and mitophagy in human podocytes. *Experimental Cell Research*, 407(1), 112758.
- [Babbar and Sheikh, 2013] Babbar, M., & Sheikh, M. S. (2013). Metabolic stress and disorders related to alterations in mitochondrial fission or fusion. *Molecular and cellular pharmacology*, 5(3), 109.
- [Bach et al., 2003] Bach, D., Pich, S., Soriano, F. X., Vega, N., Baumgartner, B., Oriola, J., Daugaard, J. R., Lloberas, J. o., Camps, M., Zierath, J. R., et al. (2003). Mitofusin-2 determines mitochondrial network architecture and mitochondrial metabolism: a novel regulatory mechanism altered in obesity. *Journal of Biological Chemistry*, 278(19):17190–17197.
- [Bach et al., 2005] Bach, D., Naon, D., Pich, S., Soriano, F. X., Vega, N., Rieusset, J., ... & Zorzano, A. (2005). Expression of Mfn2, the Charcot-Marie-Tooth neuropathy type 2A gene, in human skeletal muscle: effects of type 2 diabetes, obesity, weight loss, and the regulatory role of tumor necrosis factor α and interleukin-6. *Diabetes*, 54(9), 2685-2693.
- [Ban et al., 2018] Ban, T., Kohno, H., Ishihara, T., & Ishihara, N. (2018). Relationship

between OPA1 and cardiolipin in mitochondrial inner-membrane fusion. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1859(9), 951-957.

[Bartolák-Suki et al., 2015] Bartolák-Suki, E., Imsirovic, J., Parameswaran, H., Wellman, T. J., Martinez, N., Allen, P. G., Frey, U., and Suki, B. (2015). Fluctuation-driven mechanotransduction regulates mitochondrial-network structure and function. *Nature Materials*, 14(10):1049–1057.

[Bartolák-Suki et al., 2017] Bartolák-Suki, E., Imsirovic, J., Nishibori, Y., Krishnan, R., and Suki, B. (2017). Regulation of mitochondrial structure and dynamics by the cytoskeleton and mechanical factors. *International Journal of Molecular Sciences*, 18(8):1812.

[Becker and Wagner, 2018] Becker, T. and Wagner, R. (2018). Mitochondrial outer membrane channels: emerging diversity in transport processes. *BioEssays*, 40(7):1800013.

[Beebe, 1994] Beebe, S. J. (1994, August). The cAMP-dependent protein kinases and cAMP signal transduction. In *Seminars in cancer biology* (Vol. 5, No. 4, pp. 285-294).

[Benard et al., 2007] Benard, G., Bellance, N., James, D., Parrone, P., Fernandez, H., Letellier, T., and Rossignol, R. (2007). Mitochondrial bioenergetics and structural network organization. *Journal of Cell Science*, 120(5):838–848.

[Bertero et al., 2016] Bertero, T., Oldham, W.M., Cottrill, K.A., Pisano, S., Vanderpool, R.R., Yu, Q., Zhao, J., Tai, Y., Tang, Y., Zhang, Y.Y. and Rehman, S. (2016). Vascular stiffness mechanoactivates YAP/TAZ-dependent glutaminolysis to drive pulmonary hypertension. *The Journal of clinical investigation*, 126(9), 3313-3335.

[Birukov et al., 2003] Birukov, K.G., Jacobson, J.R., Flores, A.A., Ye, S.Q., Birukova, A.A., Verin, A.D. and Garcia, J.G. (2003). Magnitude-dependent regulation of pulmonary endothelial cell barrier function by cyclic stretch. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 285(4), L785-L797.

[Boguszewska et al., 2020] Boguszewska, K., Szewczuk, M., Kazmierczak-Baran'ska, J., and Karwowski, B. T. (2020). The similarities between human mitochondria and bacteria in the context of structure, genome, and base excision repair system. *Molecules*,

25(12):2857.

[Boldogh and Pon, 2006] Boldogh, I. R. and Pon, L. A. (2006). Interactions of mitochondria with the actin cytoskeleton. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1763(5-6):450–462.

[Boldogh and Pon, 2007] Boldogh, I. R. and Pon, L. A. (2007). Mitochondria on the move. *Trends in Cell Biology*, 17(10):502– 510.

[Bourdeau Julien et al., 2018] Bourdeau Julien, I., Sephton, C. F., and Dutchak, P. A. (2018). Metabolic networks influencing skeletal muscle fiber composition. *Frontiers in Cell and Developmental Biology*, 6:125.

[Boutant et al., 2017] Boutant, M., Kulkarni, S.S., Joffraud, M., Ratajczak, J., Valera-Alberni, M., Combe, R., Zorzano, A. and Cantó, C. (2017). Mfn2 is critical for brown adipose tissue thermogenic function. *The EMBO journal*, 36(11), 1543-1558.

[Bustamante et al., 1977] Bustamante, E., Soper, J. W., and Pedersen, P. L. (1977). A high-yield preparative method for isolation of rat liver mitochondria. *Analytical Biochemistry*, 80(2):401–408.

[Camara et al., 2010] Camara, A. K., Lesnefsky, E. J., and Stowe, D. F. (2010). Potential therapeutic benefits of strategies directed to mitochondria. *Antioxidants & Redox Signaling*, 13(3):279–347.

[Capetanaki et al., 2007] Capetanaki, Y., Bloch, R. J., Kouloumenta, A., Mavroidis, M., and Psarras, S. (2007). Muscle intermediate filaments and their links to membranes and membranous organelles. *Experimental Cell Research*, 313(10):2063– 2076.

[Casafuz et al., 2020] Casafuz, A. F., De Rossi, M. C., and Bruno, L. (2020). Intracellular motor-driven transport of rodlike smooth organelles along microtubules. *Physical Review E*, 101(6):062416.

[Casafuz et al., 2021] Casafuz, A. B. F., De Rossi, M. C., and Bruno, L. (2021). Morphological fluctuations of individual mitochondria in living cells. *Journal of Physics: Condensed Matter*, 34(9):094005.

- [Chakraborty et al., 2021] Chakraborty, M., Chu, K., Shrestha, A., Revelo, X.S., Zhang, X., Gold, M.J., Khan, S., Lee, M., Huang, C., Akbari, M. and Barrow, F. (2021). Mechanical stiffness controls dendritic cell metabolism and function. *Cell reports*, 34(2), 108609.
- [Chan, 2006] Chan, D. C. (2006). Mitochondrial fusion and fission in mammals. *Annual Review of Cell and Developmental Biology*, 22:79–99.
- [Chance and Williams, 1955] Chance, B. and Williams, G. R. (1955). A simple and rapid assay of oxidative phosphorylation. *Nature*, 175(4469):1120–1121.
- [Chapman et al., 2005] Chapman, K. E., Sinclair, S. E., Zhuang, D., Hassid, A., Desai, L. P., and Waters, C. M. (2005). Cyclic mechanical strain increases reactive oxygen species production in pulmonary epithelial cells. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 289(5):L834–L841.
- [Chaudhry and Varacallo, 2018] Chaudhry, R. and Varacallo, M. (2018). Biochemistry, glycolysis. In *StatPearls [Internet]*. StatPearls Publishing.
- [Chen et al., 2003] Chen, H., Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E., and Chan, D. C. (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *The Journal of Cell Biology*, 160(2):189–200.
- [Chen et al., 2005] Chen, H., Chomyn, A., and Chan, D. C. (2005). Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *Journal of Biological Chemistry*, 280(28):26185–26192.
- [Chen et al., 2021] Chen, K., Wang, Y., Deng, X., Guo, L., and Wu, C. (2021). Extracellular matrix stiffness regulates mitochondrial dynamics through PINCH-1-and kindlin-2-mediated signalling. *Current Research in Cell Biology*, 2:100008.
- [Chvanov, 2006] Chvanov, M. (2006). Metabolic control of elastic properties of the inner mitochondrial membrane. *The Journal of Physical Chemistry B*, 110(45):22903–22909.
- [Cogliati et al., 2013] Cogliati, S., Frezza, C., Soriano, M. E., Varanita, T., Quintana-Cabrera, R., Corrado, M., ... & Scorrano, L. (2013). Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell*, 155(1), 160–171.

- [Colin et al., 2021] Colin, F., Gensbittel, V., & Goetz, J. G. (2021). Biomechanics: a driving force behind metastatic progression. *Comptes Rendus. Biologies*, 344(3), 249-262.
- [Collins et al., 2002] Collins, T. J., Berridge, M. J., Lipp, P., & Bootman, M. D. (2002). Mitochondria are morphologically and functionally heterogeneous within cells. *The EMBO journal*, 21(7), 1616-1627.
- [Colom et al., 2018] Colom, A., Derivery, E., Soleimanpour, S., Tomba, C., Molin, M. D., Sakai, N., ... & Roux, A. (2018). A fluorescent membrane tension probe. *Nature chemistry*, 10(11), 1118-1125.
- [Coon et al., 2022] Coon, B.G., Timalina, S., Astone, M., Zhuang, Z.W., Fang, J., Han, J., Themen, J., Chung, M., Yang-Klingler, Y.J., Jain, M. and Hirschi, K.K. (2022). A mitochondrial contribution to anti-inflammatory shear stress signaling in vascular endothelial cells. *Journal of Cell Biology*, 221(7), e202109144.
- [Copp et al., 2005] Copp, J., Wiley, S., Ward, M. W., & Van Der Geer, P. (2005). Hypertonic shock inhibits growth factor receptor signaling, induces caspase-3 activation, and causes reversible fragmentation of the mitochondrial network. *American Journal of Physiology-Cell Physiology*, 288(2), C403-C415.
- [Csordás et al., 2018] Csordás, G., Weaver, D., and Hajnóczky, G. (2018). Endoplasmic reticulum–mitochondrial contactology: structure and signaling functions. *Trends in cell biology*, 28(7):523–540.
- [Dai and Jiang, 2019] Dai, W. and Jiang, L. (2019). Dysregulated mitochondrial dynamics and metabolism in obesity, diabetes, and cancer. *Frontiers in Endocrinology*, 10:570.
- [De Vos et al., 2005] De Vos, K. J., Allan, V. J., Grierson, A. J., & Sheetz, M. P. (2005). Mitochondrial function and actin regulate dynamin-related protein 1-dependent mitochondrial fission. *Current Biology*, 15(7), 678-683.
- [Ding et al., 2021] Ding, Q., Qi, Y., & Tsang, S. Y. (2021). Mitochondrial biogenesis, mitochondrial dynamics, and mitophagy in the maturation of cardiomyocytes. *Cells*, 10(9), 2463.
- [Djafarzadeh and Jakob, 2017] Djafarzadeh, S. and Jakob, S. M. (2017). Isolation of intact

- mitochondria from skeletal muscle by differential centrifugation for high-resolution respirometry measurements. *JoVE (Journal of Visualized Experiments)*, (121):e55251.
- [Doddaballapur et al., 2015] Doddaballapur, A., Michalik, K. M., Manavski, Y., Lucas, T., Houtkooper, R. H., You, X., Chen, W., Zeiher, A. M., Potente, M., Dimmeler, S., et al. (2015). Laminar shear stress inhibits endothelial cell metabolism via KLF2-mediated repression of PFKFB3. *Arteriosclerosis, thrombosis, and vascular biology*, 35(1):137–145.
- [Douguet and Honoré, 2019] Douguet, D. and Honoré, E. (2019). Mammalian mechano-electrical transduction: structure and function of force-gated ion channels. *Cell*, 179(2):340–354.
- [Duan et al., 2009] Duan, S., Yao, Z., Zhu, Y., Wang, G., Hou, D., Wen, L., and Wu, M. (2009). The Pirh2–keratin 8/18 interaction modulates the cellular distribution of mitochondria and uv-induced apoptosis. *Cell Death & Differentiation*, 16(6):826–837.
- [Duda et al., 2019] Duda, M., Kirkland, N. J., Khalilgharibi, N., Tozluoglu, M., Yuen, A. C., Carpi, N., ... & Mao, Y. (2019). Polarization of myosin II refines tissue material properties to buffer mechanical stress. *Developmental cell*, 48(2), 245-260.
- [Dunn and Grider, 2021] Dunn, J. and Grider, M. H. (2021). Physiology, adenosine triphosphate. In *StatPearls [Internet]*. StatPearls Publishing.
- [Dupont et al., 2011] Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S. and Elvassore, N. (2011). Role of YAP/TAZ in mechanotransduction. *Nature*, 474(7350), 179-183.
- [Eisner et al., 2018] Eisner, V., Picard, M., & Hajnóczky, G. (2018). Mitochondrial dynamics in adaptive and maladaptive cellular stress responses. *Nature cell biology*, 20(7), 755-765.
- [Ernster and Schatz, 1981] Ernster, L. and Schatz, G. (1981). Mitochondria: a historical review. *The Journal of Cell Biology*, 91(3):227s–255s.
- [Evers et al., 2021] Evers, T. M., Holt, L. J., Alberti, S., and Mashaghi, A. (2021). Reciprocal regulation of cellular mechanics and metabolism. *Nature Metabolism*, 3(4):456–468.
- [Eura et al., 2003] Eura, Y., Ishihara, N., Yokota, S., & Mihara, K. (2003). Two mitofusin proteins, mammalian homologues of FZO, with distinct functions are both required for mitochondrial fusion. *The Journal of Biochemistry*, 134(3), 333-344.

- [Faitg et al., 2021] Faitg, J., Lacefield, C., Davey, T., White, K., Laws, R., Kosmidis, S., Reeve, A.K., Kandel, E.R., Vincent, A.E. and Picard, M. (2021). 3D neuronal mitochondrial morphology in axons, dendrites, and somata of the aging mouse hippocampus. *Cell reports*, 36(6), 109509.
- [Fan et al., 2010] Fan, X., Hussien, R., & Brooks, G. A. (2010). H₂O₂-induced mitochondrial fragmentation in C2C12 myocytes. *Free Radical Biology and Medicine*, 49(11), 1646-1654.
- [Fan et al., 2021] Fan, L., Zan, Q., Wang, X., Wang, S., Zhang, Y., Dong, W., Shuang, S., and Dong, C. (2021). A mitochondria- specific orange/near-infrared-emissive fluorescent probe for dual-imaging of viscosity and H₂O₂ in inflammation and tumor models. *Chinese Journal of Chemistry*, 39(5):1303–1309.
- [Fatima and Ananthanarayanan, 2023] Fatima, N. U., & Ananthanarayanan, V. (2023). Mitochondrial movers and shapers: Recent insights into regulators of fission, fusion and transport. *Current Opinion in Cell Biology*, 80, 102150.
- [Favaro et al., 2019] Favaro, G., Romanello, V., Varanita, T., Andrea Desbats, M., Morbidoni, V., Tezze, C., Albiero, M., Canato, M., Gherardi, G., De Stefani, D., et al. (2019). Drp1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. *Nature Communications*, 10(1):1–17.
- [Feng and Kornmann, 2018] Feng, Q. and Kornmann, B. (2018). Mechanical forces on cellular organelles. *Journal of Cell Science*, 131(21):jcs218479.
- [Feng et al., 2017] Feng, S., Bowden, N., Fragiadaki, M., Souilhol, C., Hsiao, S., Mahmoud, M., Allen, S., Pirri, D., Ayllon, B. T., Akhtar, S., et al. (2017). Mechanical activation of hypoxia-inducible factor 1 α drives endothelial dysfunction at atheroprone sites. *Arteriosclerosis, thrombosis, and vascular biology*, 37(11):2087–2101.
- [Feng et al., 2019] Feng, Q., Lee, S. S., and Kornmann, B. (2019). A toolbox for organelle mechanobiology research—current needs and challenges. *Micromachines*, 10(8):538.
- [Ferré et al., 2021] Ferré, C. A., Thouard, A., Bétourné, A., Le Dorze, A. L., Belenguer, P., Miquel, M. C., ... & Szelechowski, M. (2021). HSPA9/Mortalin mediates axo-protection and modulates mitochondrial dynamics in neurons. *Scientific reports*, 11(1), 17705.

- [Ferreira et al., 2020] Ferreira, L. M., Li, A. M., Serafim, T. L., Sobral, M. C., Alpoim, M. C., and Urbano, A. M. (2020). Intermediary metabolism: An intricate network at the crossroads of cell fate and function. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1866(10):165887.
- [Filadi et al., 2018] Filadi, R., Pendin, D., & Pizzo, P. (2018). Mitofusin 2: from functions to disease. *Cell death & disease*, 9(3), 330.
- [Francy et al., 2015] Francy, C. A., Alvarez, F. J., Zhou, L., Ramachandran, R., and Mears, J. A. (2015). The mechanoenzymatic core of dynamin-related protein 1 comprises the minimal machinery required for membrane constriction. *Journal of Biological Chemistry*, 290(18):11692–11703.
- [Friedman and Voeltz, 2011] Friedman, J. R. and Voeltz, G. K. (2011). The er in 3d: a multifunctional dynamic membrane network. *Trends in cell biology*, 21(12):709–717.
- [Fuller and Arriaga, 2003] Fuller, K. M. and Arriaga, E. A. (2003). Advances in the analysis of single mitochondria. *Current Opinion in Biotechnology*, 14(1):35–41.
- [Gao et al., 2014] Gao, A. W., Cantó, C., & Houtkooper, R. H. (2014). Mitochondrial response to nutrient availability and its role in metabolic disease. *EMBO molecular medicine*, 6(5), 580-589.
- [Garbern and Lee, 2021] Garbern, J. C., & Lee, R. T. (2021). Mitochondria and metabolic transitions in cardiomyocytes: lessons from development for stem cell-derived cardiomyocytes. *Stem Cell Research & Therapy*, 12(1), 1-25.
- [Garde et al., 2022] Garde, A., Kenny, I. W., Kelley, L. C., Chi, Q., Mutlu, A. S., Wang, M. C., & Sherwood, D. R. (2022). Localized glucose import, glycolytic processing, and mitochondria generate a focused ATP burst to power basement-membrane invasion. *Developmental Cell*, 57(6), 732-749.
- [Gatenby and Gillies, 2004] Gatenby, R. A. and Gillies, R. J. (2004). Why do cancers have high aerobic glycolysis? *Nature reviews cancer*, 4(11):891–899.
- [Ge et al., 2021] Ge, Y., Boopathy, S., Nguyen, T. H., Lugo, C. M., & Chao, L. H. (2021). Absence of cardiolipin from the outer leaflet of a mitochondrial inner membrane mimic

- restricts Opa1-mediated fusion. *Frontiers in Molecular Biosciences*, 8, 769135.
- [Goldblatt and Cameron, 1953] Goldblatt, H. and Cameron, G. (1953). Induced malignancy in cells from rat myocardium subjected to intermittent anaerobiosis during long propagation in vitro. *The Journal of Experimental Medicine*, 97(4):525– 552.
- [Gomes et al., 2011] Gomes, L. C., Benedetto, G. D., and Scorrano, L. (2011). During autophagy mitochondria elongate, are spared from degradation and sustain cell viability. *Nature Cell Biology*, 13(5):589–598.
- [Gonzalez-Rodriguez et al., 2015] Gonzalez-Rodriguez, D., Sart, S., Babataheri, A., Taresté, D., Barakat, A. I., Clanet, C., and Husson, J. (2015). Elastocapillary instability in mitochondrial fission. *Physical Review Letters*, 115(8):088102.
- [Gottlieb et al., 2003] Gottlieb, E., Armour, S. M., Harris, M. H., & Thompson, C. B. (2003). Mitochondrial membrane potential regulates matrix configuration and cytochrome c release during apoptosis. *Cell Death & Differentiation*, 10(6), 709-717.
- [Goujon et al., 2019] Goujon, A., Colom, A., Strakova, K., Mercier, V., Mahecic, D., Manley, S., Sakai, N., Roux, A., and Matile, S. (2019). Mechanosensitive fluorescent probes to image membrane tension in mitochondria, endoplasmic reticulum, and lysosomes. *Journal of the American Chemical Society*, 141(8):3380–3384.
- [Griparic and van der Blik, 2001] Griparic, L. and van der Blik, A. M. (2001). The many shapes of mitochondrial membranes. *Traffic*, 2(4):235–244.
- [Gu et al., 2021] Gu, X., Ma, Y., Liu, Y., and Wan, Q. (2021). Measurement of mitochondrial respiration in adherent cells by Seahorse XF96 Cell Mito Stress Test. *STAR protocols*, 2(1):100245.
- [Guo et al., 2023] Guo, T., Jiang, C.S., Yang, S.Z., Zhu, Y., He, C., Carter, A.B., Antony, V.B., Peng, H. and Zhou, Y. (2023). Mitochondrial fission and bioenergetics mediate human lung fibroblast durotaxis. *JCI insight*, 8(1).
- [Hardie et al., 2012] Hardie, D. G., Ross, F. A., and Hawley, S. A. (2012). AMPK: a nutrient and energy sensor that maintains energy homeostasis. *Nature Reviews Molecular Cell Biology*, 13(4):251–262.

- [Helle et al., 2017] Helle, S. C. J., Feng, Q., Aebersold, M. J., Hirt, L., Grüter, R. R., Vahid, A., Sirianni, A., Mostowy, S., Snedeker, J. G., Šaric', A., et al. (2017). Mechanical force induces mitochondrial fission. *Elife*, 6:e30292.
- [Hernansanz-Agustín and Enríquez, 2021] Hernansanz-Agustín, P. and Enríquez, J. A. (2021). Generation of reactive oxygen species by mitochondria. *Antioxidants*, 10(3):415.
- [Hong et al., 2022] Hong, S.-G., Shin, J., Aldokhayyil, M., Brown, M. D., and Park, J.-Y. (2022). Mitochondrial and metabolic adaptations to exercise-induced fluid shear stress in endothelial cells. *Exercise and Sport Sciences Reviews*, 50(3):145–155.
- [Horn et al., 2020] Horn, A., Raavicharla, S., Shah, S., Cox, D., & Jaiswal, J. K. (2020). Mitochondrial fragmentation enables localized signaling required for cell repair. *Journal of Cell Biology*, 219(5).
- [Howard, 2002] Howard, J. (2002). Mechanics of motor proteins. In *Physics of Biomolecules and Cells*, pages 69–94. Springer.
- [Imsirovic et al., 2015] Imsirovic, J., Wellman, T. J., Mondoñedo, J. R., Bartolák-Suki, E., and Suki, B. (2015). Design of a novel equi-biaxial stretcher for live cellular and subcellular imaging. *PLoS One*, 10(10):e0140283.
- [Ingber, 2006] Ingber, D. E. (2006). Cellular mechanotransduction: putting all the pieces together again. *The FASEB journal*, 20(7):811–827.
- [Inoue-Yamauchi and Oda, 2012] Inoue-Yamauchi, A. and Oda, H. (2012). Depletion of mitochondrial fission factor DRP1 causes increased apoptosis in human colon cancer cells. *Biochemical and Biophysical Research Communications*, 421(1):81–85.
- [Iqbal et al., 2014] Iqbal, S., & Hood, D. A. (2014). Oxidative stress-induced mitochondrial fragmentation and movement in skeletal muscle myoblasts. *American Journal of Physiology-Cell Physiology*, 306(12), C1176-C1183.
- [Isermann and Lammerding, 2013] Isermann, P. and Lammerding, J. (2013). Nuclear mechanics and mechanotransduction in health and disease. *Current Biology*, 23(24):R1113–R1121.

- [Ishihara et al., 2004] Ishihara, N., Eura, Y., & Mihara, K. (2004). Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. *Journal of cell science*, 117(26), 6535-6546.
- [Jaalouk and Lammerding, 2009] Jaalouk, D. E. and Lammerding, J. (2009). Mechanotransduction gone awry. *Nature Reviews Molecular Cell Biology*, 10(1):63–73.
- [Jendrach et al., 2008] Jendrach, M., Mai, S., Pohl, S., Vöth, M. and Bereiter-Hahn, J. (2008). Short-and long-term alterations of mitochondrial morphology, dynamics and mtDNA after transient oxidative stress. *Mitochondrion*, 8(4), 293-304.
- [Jones and Naylor, 2022] Jones, M. D., & Naylor, K. (2022). Simple to Complex: The Role of Actin and Microtubules in Mitochondrial Dynamics in Amoeba, Yeast, and Mammalian Cells. *International Journal of Molecular Sciences*, 23(16), 9402.
- [Joubert and Puff, 2021] Joubert, F. and Puff, N. (2021). Mitochondrial cristae architecture and functions: Lessons from minimal model systems. *Membranes*, 11(7):465.
- [Ju et al., 2007] Ju, W.K., Liu, Q., Kim, K.Y., Crowston, J.G., Lindsey, J.D., Agarwal, N., Ellisman, M.H., Perkins, G.A. and Weinreb, R.N. (2007). Elevated hydrostatic pressure triggers mitochondrial fission and decreases cellular ATP in differentiated RGC-5 cells. *Investigative ophthalmology & visual science*, 48(5), 2145-2151.
- [Ju et al., 2008] Ju, W.K., Kim, K.Y., Lindsey, J.D., Angert, M., Duong-Polk, K.X., Scott, R.T., Kim, J.J., Kukhmazov, I., Ellisman, M.H., Perkins, G.A. and Weinreb, R.N. (2008). Intraocular pressure elevation induces mitochondrial fission and triggers OPA1 release in glaucomatous optic nerve. *Investigative ophthalmology & visual science*, 49(11), 4903-4911.
- [Ju et al., 2009] Ju, W.K., Kim, K.Y., Lindsey, J.D., Angert, M., Patel, A., Scott, R.T., Liu, Q., Crowston, J.G., Ellisman, M.H., Perkins, G.A. and Weinreb, R.N. (2009). Elevated hydrostatic pressure triggers release of OPA1 and cytochrome C, and induces apoptotic cell death in differentiated RGC-5 cells. *Molecular vision*, 15, 120.
- [Kabra et al., 2017] Kabra, U.D., Pfuhlmann, K., Migliorini, A., Keipert, S., Lamp, D., Korsgren, O., Gegg, M., Woods, S.C., Pfluger, P.T., Lickert, H. and Affourtit, C. (2017). Direct Substrate Delivery Into Mitochondrial Fission–Deficient Pancreatic Islets Rescues

Insulin Secretion. *Diabetes*, 66(5), 1247-1257.

- [Karbowski and Youle, 2003] Karbowski, M., & Youle, R. J. (2003). Dynamics of mitochondrial morphology in healthy cells and during apoptosis. *Cell Death & Differentiation*, 10(8), 870-880.
- [Kechagia et al., 2019] Kechagia, J. Z., Ivaska, J., and Roca-Cusachs, P. (2019). Integrins as biomechanical sensors of the microenvironment. *Nature Reviews Molecular Cell Biology*, 20(8):457–473.
- [Kere et al., 2020] Kere, M., Liu, P. C., Chen, Y. K., Chao, P. C., Tsai, L. K., Yeh, T. Y., ... & Ju, J. C. (2020). Ultrastructural characterization of porcine growing and in vitro matured oocytes. *Animals*, 10(4), 664.
- [Khalifat et al., 2008] Khalifat, N., Puff, N., Bonneau, S., Fournier, J.-B., and Angelova, M. I. (2008). Membrane deformation under local pH gradient: mimicking mitochondrial cristae dynamics. *Biophysical journal*, 95(10):4924–4933.
- [Kim et al., 2014] Kim, B., Lee, H., Kawata, K., and Park, J.-Y. (2014). Exercise-mediated wall shear stress increases mitochondrial biogenesis in vascular endothelium. *PLOS One*, 9(11):e111409.
- [Kim et al., 2016] Kim, J., Yang, G., Kim, Y., Kim, J., and Ha, J. (2016). AMPK activators: mechanisms of action and physiological activities. *Experimental & Molecular Medicine*, 48(4):e224–e224.
- [Kleele et al., 2021] Kleele, T., Rey, T., Winter, J., Zaganelli, S., Mahecic, D., Perreten Lambert, H., Ruberto, F. P., Nemir, M., Wai, T., Pedrazzini, T., et al. (2021). Distinct fission signatures predict mitochondrial degradation or biogenesis. *Nature*, 593(7859):435–439.
- [Kobayashi et al., 2020] Kobayashi, S., Zhao, F., Zhang, Z., Kobayashi, T., Huang, Y., Shi, B., ... & Liang, Q. (2020). Mitochondrial fission and mitophagy coordinately restrict high glucose toxicity in cardiomyocytes. *Frontiers in Physiology*, 11, 604069.
- [Komaragiri et al., 2022] Komaragiri, Y., Panhwar, M. H., Fregin, B., Jagirdar, G., Wolke, C., Spiegler, S., and Otto, O. (2022). Mechanical characterization of isolated mitochondria under conditions of oxidative stress. *Biomicrofluidics*, 16(6):064101.

- [Korobova et al., 2013] Korobova, F., Ramabhadran, V., and Higgs, H. N. (2013). An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. *Science*, 339(6118):464–467.
- [Kruppa et al., 2021] Kruppa, A. J., & Buss, F. (2021). Motor proteins at the mitochondria–cytoskeleton interface. *Journal of cell science*, 134(7), jcs226084.
- [Kulkarni et al., 2016] Kulkarni, S. S., Joffraud, M., Boutant, M., Ratajczak, J., Gao, A. W., Maclachlan, C., Hernandez-Alvarez, M. I., Raymond, F., Metairon, S., Descombes, P., et al. (2016). Mfn1 deficiency in the liver protects against diet-induced insulin resistance and enhances the hypoglycemic effect of metformin. *Diabetes*, 65(12):3552–3560.
- [Kuznetsov et al., 2009] Kuznetsov, A. V., Hermann, M., Saks, V., Hengster, P., & Margreiter, R. (2009). The cell-type specificity of mitochondrial dynamics. *The international journal of biochemistry & cell biology*, 41(10), 1928-1939.
- [Kuznetsov et al., 2022] Kuznetsov, A. V., Javadov, S., Margreiter, R., Hagenbuchner, J., & Ausserlechner, M. J. (2022). Analysis of mitochondrial function, structure, and intracellular organization in situ in cardiomyocytes and skeletal muscles. *International Journal of Molecular Sciences*, 23(4), 2252.
- [Labrousse et al., 1999] Labrousse, A. M., Zappaterra, M. D., Rube, D. A., and van der Bliek, A. M. (1999). C. elegans dynamin-related protein drp-1 controls severing of the mitochondrial outer membrane. *Molecular cell*, 4(5):815–826.
- [Lackner et al., 2019] Lackner, L. L. (2019). The expanding and unexpected functions of mitochondria contact sites. *Trends in cell biology*, 29(7), 580-590.
- [Lafaurie-Janvore et al., 2013] Lafaurie-Janvore, J., Maiuri, P., Wang, I., Pinot, M., Manneville, J.-B., Betz, T., Balland, M., and Piel, M. (2013). ESCRT-III assembly and cytokinetic abscission are induced by tension release in the intercellular bridge. *Science*, 339(6127):1625–1629.
- [Lee et al., 2022] Lee, C., Chen, Y., Wang, P., Wallace, D. C., and Burke, P. J. (2022). A three-dimensional printed inertial microfluidic platform for isolation of minute quantities of vital mitochondria. *Analytical Chemistry*.
- [Leininger et al., 2006] Leininger, G. M., Backus, C., Sastry, A. M., Yi, Y. B., Wang, C.

- W., & Feldman, E. L. (2006). Mitochondria in DRG neurons undergo hyperglycemic mediated injury through Bim, Bax and the fission protein Drp1. *Neurobiology of disease*, 23(1), 11-22.
- [Li et al., 2015] Li, S., Xu, S., Roelofs, B. A., Boyman, L., Lederer, W. J., Sesaki, H., and Karbowski, M. (2015). Transient assembly of f-actin on the outer mitochondrial membrane contributes to mitochondrial fission. *Journal of Cell Biology*, 208(1):109–123.
- [Li et al., 2019] Li, H., Xin, C., Zhang, G., Han, X., Qin, W., Zhang, C.-w., Yu, C., Jing, S., Li, L., and Huang, W. (2019). A mitochondria-targeted two-photon fluorogenic probe for the dual-imaging of viscosity and H₂O₂ levels in Parkinson's disease models. *Journal of Materials Chemistry B*, 7(27):4243–4251.
- [Li et al., 2020] Li, A., Gao, M., Jiang, W., Qin, Y., & Gong, G. (2020). Mitochondrial dynamics in adult cardiomyocytes and heart diseases. *Frontiers in Cell and Developmental Biology*, 8, 584800.
- [Li et al., 2023] Li, Y., Xiong, Z., Jiang, Y., Zhou, H., Yi, L., Hu, Y., Zhai, X., Liu, J., Tian, F. and Chen, Y. (2023). Klf4 deficiency exacerbates myocardial ischemia/reperfusion injury in mice via enhancing ROCK1/DRP1 pathway-dependent mitochondrial fission. *Journal of Molecular and Cellular Cardiology*, 174, 115-132.
- [Li and Graham, 2012] Li, Z. and Graham, B. H. (2012). Measurement of mitochondrial oxygen consumption using a clark electrode. In *Mitochondrial Disorders*, pages 63–72. Springer.
- [Liesa and Shirihai, 2013] Liesa, M. and Shirihai, O. S. (2013). Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. *Cell Metabolism*, 17(4):491–506.
- [Little et al., 2020] Little, A. C., Kovalenko, I., Goo, L. E., Hong, H. S., Kerk, S. A., Yates, J. A., Purohit, V., Lombard, D. B., Merajver, S. D., and Lyssiotis, C. A. (2020). High-content fluorescence imaging with the metabolic flux assay reveals insights into mitochondrial properties and functions. *Communications biology*, 3(1):1–10.
- [Liu et al., 2017] Liu, R., & Chan, D. C. (2017). OPA1 and cardiolipin team up for mitochondrial fusion. *Nature cell biology*, 19(7), 760-762.

- [Liu et al., 2022] Liu, X., Xu, L., Song, Y., Li, X., Wong, C.-Y., Rong, C., Feng, J., Chow, H.-M., Yao, S., Gao, S., et al. (2022). Mechanical force induces DRP1-dependent asymmetrical mitochondrial fission for quality control. *bioRxiv*.
- [Loiseau et al., 2007] Loiseau, D., Chevrollier, A., Verny, C., Guillet, V., Gueguen, N., Pou De Crescenzo, M.-A., Ferré, M., Malinge, M.-C., Guichet, A., Nicolas, G., et al. (2007). Mitochondrial coupling defect in Charcot–Marie–Tooth type 2A disease. *Annals of Neurology*, 61(4):315–323.
- [Losón et al., 2013] Losón, O. C., Song, Z., Chen, H., & Chan, D. C. (2013). Fis1, Mff, MiD49, and MiD51 mediate Drp1 recruitment in mitochondrial fission. *Molecular biology of the cell*, 24(5), 659-667.
- [Luo et al., 2007] Luo, B., Groenke, K., Takors, R., Wandrey, C., and Oldiges, M. (2007). Simultaneous determination of multiple intracellular metabolites in glycolysis, pentose phosphate pathway and tricarboxylic acid cycle by liquid chromatography–mass spectrometry. *Journal of Chromatography A*, 1147(2):153–164.
- [Luo et al., 2020] Luo, M., Meng, Z., Moroishi, T., Lin, K. C., Shen, G., Mo, F., ... & Guan, K. L. (2020). Heat stress activates YAP/TAZ to induce the heat shock transcriptome. *Nature cell biology*, 22(12), 1447-1459.
- [Lytrivi et al., 2020] Lytrivi, M., Castell, A. L., Poitout, V., & Cnop, M. (2020). Recent insights into mechanisms of β -cell lipo- and glucolipotoxicity in type 2 diabetes. *Journal of molecular biology*, 432(5), 1514-1534.
- [Ma et al., 2020] Ma, C., Xia, F., and Kelley, S. O. (2020). Mitochondrial targeting of probes and therapeutics to the power- house of the cell. *Bioconjugate Chemistry*, 31(12):2650–2667.
- [Mahdavian et al., 2017] Mahdavian, K., Benador, I.Y., Su, S., Gharakhanian, R.A., Stiles, L., Trudeau, K.M., Cardamone, M., Enríquez-Zarralanga, V., Ritou, E., Aprahamian, T. and Oliveira, M.F. (2017). Mfn2 deletion in brown adipose tissue protects from insulin resistance and impairs thermogenesis. *EMBO reports*, 18(7), 1123-1138.
- [Mahecic et al., 2021] Mahecic, D., Carlini, L., Kleele, T., Colom, A., Goujon, A., Matile, S., Roux, A., and Manley, S. (2021). Mitochondrial membrane tension governs fission. *Cell*

Reports, 35(2):108947.

- [Mannella et al., 1994] Mannella, C. A., Marko, M., Penczek, P., Barnard, D., and Frank, J. (1994). The internal compartmentation of rat-liver mitochondria: tomographic study using the high-voltage transmission electron microscope. *Microscopy Research and Technique*, 27(4):278–283.
- [Manor et al., 2015] Manor, U., Bartholomew, S., Golani, G., Christenson, E., Kozlov, M., Higgs, H., Spudich, J. and Lippincott-Schwartz, J. (2015). A mitochondria-anchored isoform of the actin-nucleating spire protein regulates mitochondrial division. *Elife*, 4, e08828.
- [Martijn et al., 2018] Martijn, J., Vosseberg, J., Guy, L., Offre, P., & Ettema, T. J. (2018). Deep mitochondrial origin outside the sampled alphaproteobacteria. *Nature*, 557(7703), 101-105.
- [Mathieu and Manneville, 2019] Mathieu, S. and Manneville, J.-B. (2019). Intracellular mechanics: connecting rheology and mechanotransduction. *Current opinion in cell biology*, 56:34–44.
- [McBride et al., 2006] McBride, H. M., Neuspiel, M., and Wasiak, S. (2006). Mitochondria: more than just a powerhouse. *Current Biology*, 16(14):R551–R560.
- [Mehta et al., 2019] Mehta, K., Chacko, L. A., Chug, M. K., Jhunjhunwala, S., & Ananthanarayanan, V. (2019). Association of mitochondria with microtubules inhibits mitochondrial fission by precluding assembly of the fission protein Dnm1. *Journal of Biological Chemistry*, 294(10), 3385-3396.
- [Miragoli et al., 2016] Miragoli, M., Sanchez-Alonso, J. L., Bhargava, A., Wright, P. T., Sikkil, M., Schobesberger, S., Diakonov, I., Novak, P., Castaldi, A., Cattaneo, P., et al. (2016). Microtubule-dependent mitochondria alignment regulates calcium release in response to nanomechanical stimulus in heart myocytes. *Cell Reports*, 14(1):140–151.
- [Mishra et al., 2015] Mishra, P., Varuzhanyan, G., Pham, A. H., and Chan, D. C. (2015). *iCell metabolism*, 22(6):1033–1044.
- [Miyamoto et al., 2021] Miyamoto, T., Uosaki, H., Mizunoe, Y., Han, S.I., Goto, S., Yamanaka, D., Masuda, M., Yoneyama, Y., Nakamura, H., Hattori, N. and Takeuchi, Y. (2021). Rapid manipulation of mitochondrial morphology in a living cell with iCMM. *Cell*

Reports Methods, 1(4), 100052.

- [Molina et al., 2009] Molina, A. J., Wikstrom, J. D., Stiles, L., Las, G., Mohamed, H., Elorza, A., Walzer, G., Twig, G., Katz, S., Corkey, B. E., et al. (2009). Mitochondrial networking protects β -cells from nutrient-induced apoptosis. *Diabetes*, 58(10):2303–2315.
- [Moore et al., 2016] Moore, A. S., Wong, Y. C., Simpson, C. L., and Holzbaur, E. L. (2016). Dynamic actin cycling through mitochondrial subpopulations locally regulates the fission–fusion balance within mitochondrial networks. *Nature communications*, 7(1):1–13.
- [Moore et al., 2021] Moore, A.S., Coscia, S.M., Simpson, C.L., Ortega, F.E., Wait, E.C., Heddleston, J.M., Nirschl, J.J., Obara, C.J., Guedes-Dias, P., Boecker, C.A. and Chew, T.L. (2021). Actin cables and comet tails organize mitochondrial networks in mitosis. *Nature*, 591(7851), 659-664.
- [Morris and Hollenbeck, 1995] Morris, R. and Hollenbeck, P. (1995). Axonal transport of mitochondria along microtubules and f-actin in living vertebrate neurons. *The Journal of Cell Biology*, 131(5):1315–1326.
- [Murley and Nunnari, 2016] Murley, A., & Nunnari, J. (2016). The emerging network of mitochondria-organelle contacts. *Molecular cell*, 61(5), 648-653.
- [Murphy and Hartley, 2018] Murphy, M. P. and Hartley, R. C. (2018). Mitochondria as a therapeutic target for common pathologies. *Nature Reviews Drug Discovery*, 17(12):865–886.
- [Nakamura et al., 2020] Nakamura, H., Rho, E., Deng, D., Razavi, S., Matsubayashi, H. T., & Inoue, T. (2020). ActuAtoR, a molecular tool for generating force in living cells: Controlled deformation of intracellular structures. *BioRxiv*, 2020-03.
- [Nekrasova et al., 2011] Nekrasova, O. E., Mendez, M. G., Chernouvanenko, I. S., Tyurin-Kuzmin, P. A., Kuczmariski, E. R., Gelfand, V. I., Goldman, R. D., and Minin, A. A. (2011). Vimentin intermediate filaments modulate the motility of mitochondria. *Molecular Biology of the Cell*, 22(13):2282–2289.
- [Ni et al., 2015] Ni, H. M., Williams, J. A., & Ding, W. X. (2015). Mitochondrial dynamics and mitochondrial quality control. *Redox biology*, 4, 6-13.

- [Nia et al., 2020] Nia, H. T., Munn, L. L., & Jain, R. K. (2020). Physical traits of cancer. *Science*, 370(6516), eaaz0868.
- [Nichols-Smith et al., 2004] Nichols-Smith, S., Teh, S.-Y., and Kuhl, T. L. (2004). Thermodynamic and mechanical properties of model mitochondrial membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1663(1-2):82–88.
- [Nielsen et al., 2017] Nielsen, J., Gejl, K. D., Hey-Mogensen, M., Holmberg, H.-C., Suetta, C., Krstrup, P., Elemans, C. P., and Ørtenblad, N. (2017). Plasticity in mitochondrial cristae density allows metabolic capacity modulation in human skeletal muscle. *The Journal of physiology*, 595(9):2839–2847.
- [Niyazov et al., 2016] Niyazov, D. M., Kahler, S. G., and Frye, R. E. (2016). Primary mitochondrial disease and secondary mitochondrial dysfunction: importance of distinction for diagnosis and treatment. *Molecular Syndromology*, 7(3):122– 137.
- [Norregaard et al., 2017] Norregaard, K., Metzler, R., Ritter, C. M., Berg-Sørensen, K., and Oddershede, L. B. (2017). Manipulation and motion of organelles and single molecules in living cells. *Chemical reviews*, 117(5):4342–4375.
- [Orr et al., 2006] Orr, A. W., Helmke, B. P., Blackman, B. R., and Schwartz, M. A. (2006). Mechanisms of mechanotransduction. *Developmental Cell*, 10(1):11–20.
- [Otera et al., 2010] Otera, H., Wang, C., Cleland, M. M., Setoguchi, K., Yokota, S., Youle, R. J., and Mihara, K. (2010). Mff is an essential factor for mitochondrial recruitment of Drp1 during mitochondrial fission in mammalian cells. *Journal of Cell Biology*, 191(6):1141–1158.
- [Ott et al., 2007] Ott, M., Gogvadze, V., Orrenius, S., and Zhivotovsky, B. (2007). Mitochondria, oxidative stress and cell death. *Apoptosis*, 12(5):913–922.
- [Paradies et al., 2019] Paradies, G., Paradies, V., Ruggiero, F.M. and Petrosillo, G. (2019). Role of cardiolipin in mitochondrial function and dynamics in health and disease: molecular and pharmacological aspects. *Cells*, 8(7), 728.
- [Ozawa et al., 2015] Ozawa, S., Ueda, S., Imamura, H., Mori, K., Asanuma, K., Yanagita, M., & Nakagawa, T. (2015). Glycolysis, but not Mitochondria, responsible for intracellular ATP distribution in cortical area of podocytes. *Scientific reports*, 5(1), 18575.

- [Papalazarou et al., 2020] Papalazarou, V., Zhang, T., Paul, N. R., Juin, A., Cantini, M., Maddocks, O. D., ... & Machesky, L. M. (2020). The creatine–phosphagen system is mechanoresponsive in pancreatic adenocarcinoma and fuels invasion and metastasis. *Nature metabolism*, 2(1), 62-80.
- [Park et al., 2020] Park, J. S., Burckhardt, C. J., Lazcano, R., Solis, L. M., Isogai, T., Li, L., Chen, C. S., Gao, B., Minna, J. D., Bachoo, R., et al. (2020). Mechanical regulation of glycolysis via cytoskeleton architecture. *Nature*, 578(7796):621–626.
- [Park et al., 2022] Park, H.-A., Brown, S. R., Jansen, J., Dunn, T., Scott, M., Mnatsakanyan, N., Jonas, E. A., and Kim, Y. (2022). Fluid shear stress enhances proliferation of breast cancer cells via downregulation of the c-subunit of the F1Fo ATP synthase. *Biochemical and Biophysical Research Communications*, 632:173–180.
- [Pernas and Scorrano, 2016] Pernas, L. and Scorrano, L. (2016). Mito-morphosis: mitochondrial fusion, fission, and cristae remodeling as key mediators of cellular function. *Annual Review of Physiology*, 78:505–531.
- [Phuyal and Baschieri, 2020] Phuyal, S. and Baschieri, F. (2020). Endomembranes: Unsung heroes of mechanobiology? *Frontiers in Bioengineering and Biotechnology*, 8:597721.
- [Phuyal et al., 2023] Phuyal, S., Romani, P., Dupont, S., & Farhan, H. (2023). Mechanobiology of organelles: illuminating their roles in mechanosensing and mechanotransduction. *Trends in Cell Biology*, S0962-8924(23)00084-3
- [Pich et al., 2005] Pich, S., Bach, D., Briones, P., Liesa, M., Camps, M., Testar, X., Palacín, M., and Zorzano, A. (2005). The Charcot–Marie–Tooth type 2A gene product, Mfn2, up-regulates fuel oxidation through expression of OXPHOS system. *Human Molecular Genetics*, 14(11):1405–1415.
- [Pizzino et al., 2017] Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., and Bitto, A. (2017). Oxidative stress: harms and benefits for human health. *Oxidative Medicine and Cellular Longevity*, 2017.
- [Porter et al., 2011] Porter Jr, G. A., Hom, J. R., Hoffman, D. L., Quintanilla, R. A., de Mesy Bentley, K. L., & Sheu, S. S. (2011). Bioenergetics, mitochondria, and cardiac myocyte differentiation. *Progress in pediatric cardiology*, 31(2), 75-81.

- [Rambold et al., 2011] Rambold, A. S., Kostecky, B., Elia, N., and Lippincott-Schwartz, J. (2011). Tubular network formation protects mitochondria from autophagosomal degradation during nutrient starvation. *Proceedings of the National Academy of Sciences*, 108(25):10190–10195.
- [Ramírez et al., 2017] Ramírez, S., Gomez-Valades, A.G., Schneeberger, M., Varela, L., Haddad-Tovolli, R., Altirriba, J., Noguera, E., Drougard, A., Flores-Martínez, Á., Imbernon, M. and Chivite, I. (2017). Mitochondrial dynamics mediated by mitofusin 1 is required for POMC neuron glucose-sensing and insulin release control. *Cell metabolism*, 25(6), 1390-1399.
- [Raucher and Sheetz, 2000] Raucher, D. and Sheetz, M. P. (2000). Cell spreading and lamellipodial extension rate is regulated by membrane tension. *The Journal of Cell Biology*, 148(1):127–136.
- [Reggiori and Klionsky, 2002] Reggiori, F. and Klionsky, D. J. (2002). Autophagy in the eukaryotic cell. *Eukaryotic Cell*, 1(1):11–21.
- [Rehman et al., 2012] Rehman, J., Zhang, H. J., Toth, P. T., Zhang, Y., Marsboom, G., Hong, Z., Salgia, R., Husain, A. N., Wietholt, C., and Archer, S. L. (2012). Inhibition of mitochondrial fission prevents cell cycle progression in lung cancer. *The FASEB Journal*, 26(5):2175–2186.
- [Rizzuto et al., 1998] Rizzuto, R., Pinton, P., Carrington, W., Fay, F. S., Fogarty, K. E., Lifshitz, L. M., Tuft, R. A., and Pozzan, T. (1998). Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca²⁺ responses. *Science*, 280(5370):1763–1766.
- [Roffay et al., 2021] Roffay, C., Molinard, G., Kim, K., Urbanska, M., Andrade, V., Barbarasa, V., Nowak, P., Mercier, V., García- Calvo, J., Matile, S., et al. (2021). Passive coupling of membrane tension and cell volume during active response of cells to osmosis. *Proceedings of the National Academy of Sciences*, 118(47):e2103228118.
- [Roffay et al., 2022] Roffay, C., Arcos, J. M. G., Chapuis, P., Lopez-Andarias, J., Colom, A., Tomba, C., Di Meglio, I., Matile, S., Roux, A., and Mercier, V. (2022). Technical insights into fluorescence lifetime microscopy of mechanosensitive flipper probes. *bioRxiv*.
- [Roger et al., 2017] Roger, A. J., Muñoz-Gómez, S. A., and Kamikawa, R. (2017). The origin

- and diversification of mitochondria. *Current Biology*, 27(21):R1177–R1192.
- [Romanello and Sandri, 2021] Romanello, V. and Sandri, M. (2021). The connection between the dynamic remodeling of the mitochondrial network and the regulation of muscle mass. *Cellular and Molecular Life Sciences*, 78(4):1305–1328.
- [Romani et al., 2021] Romani, P., Valcarcel-Jimenez, L., Frezza, C., and Dupont, S. (2021). Crosstalk between mechanotransduction and metabolism. *Nature Reviews Molecular Cell Biology*, 22(1):22–38.
- [Romani et al., 2022] Romani, P., Nirchio, N., Arboit, M., Barbieri, V., Tosi, A., Michielin, F., Shibuya, S., Benoist, T., Wu, D., Hindmarch, C.C.T. and Giomo, M. (2022). Mitochondrial fission links ECM mechanotransduction to metabolic redox homeostasis and metastatic chemotherapy resistance. *Nature Cell Biology*, 24(2), 168-180.
- [Roux et al., 2010] Roux, A., Koster, G., Lenz, M., Sorre, B., Manneville, J. B., Nassoy, P., & Bassereau, P. (2010). Membrane curvature controls dynamin polymerization. *Proceedings of the National Academy of Sciences*, 107(9), 4141-4146.
- [Rowland and Voeltz, 2012] Rowland, A. A. and Voeltz, G. K. (2012). Endoplasmic reticulum–mitochondria contacts: function of the junction. *Nature reviews Molecular cell biology*, 13(10):607–615.
- [Rui, 2014] Rui, L. (2014). Energy metabolism in the liver. *Comprehensive physiology*, 4(1), 177.
- [Saraste, 1999] Saraste, M. (1999). Oxidative phosphorylation at the fin de siecle. *Science*, 283(5407):1488–1493.
- [Saxton and Hollenbeck, 2012] Saxton, W. M. and Hollenbeck, P. J. (2012). The axonal transport of mitochondria. *Journal of cell science*, 125(9):2095–2104.
- [Scheid et al., 2021] Scheid, A. D., Beadnell, T. C., and Welch, D. R. (2021). Roles of mitochondria in the hallmarks of metastasis. *British Journal of Cancer*, 124(1):124–135.
- [Schrepfer and Scorrano, 2016] Schrepfer, E., & Scorrano, L. (2016). Mitofusins, from mitochondria to metabolism. *Molecular cell*, 61(5), 683-694.
- [Schwartz, 2010] Schwartz, M. A. (2010). Integrins and extracellular matrix in

mechanotransduction. *Cold Spring Harbor perspectives in biology*, 2(12):a005066.

[Schwarz and Leube, 2016] Schwarz, N. and Leube, R. E. (2016). Intermediate filaments as organizers of cellular space: how they affect mitochondrial structure and function. *Cells*, 5(3):30.

[Sena and Chandel, 2012] Sena, L. A. and Chandel, N. S. (2012). Physiological roles of mitochondrial reactive oxygen species. *Molecular cell*, 48(2):158–167.

[Senft and Ze'ev, 2016] Senft, D. and Ze'ev, A. R. (2016). Regulators of mitochondrial dynamics in cancer. *Current Opinion in Cell Biology*, 39:43–52.

[Shimura et al., 2021] Shimura, D., Nuebel, E., Baum, R., Valdez, S.E., Xiao, S., Warren, J.S., Palatinus, J.A., Hong, T., Rutter, J. and Shaw, R.M. (2021). Protective mitochondrial fission induced by stress-responsive protein GJA1-20k. *Elife*, 10, e69207.

[Sinha et al., 2011] Sinha, B., Köster, D., Ruez, R., Gonnord, P., Bastiani, M., Abankwa, D., Stan, R.V., Butler-Browne, G., Védie, B., Johannes, L. and Morone, N. (2011). Cells respond to mechanical stress by rapid disassembly of caveolae. *Cell*, 144(3), 402-413.

[Sitaramam et al., 1989] Sitaramam, V., Sambasivarao, D., and Mathai, J. C. (1989). Differential effects of osmotic pressure on mitochondrial respiratory chain and indices of oxidative phosphorylation. *Biochimica et Biophysica Acta (BBA)- Bioenergetics*, 975(2):252–266.

[Sitaramam and Rao, 1991] Sitaramam, V. and Rao, N. (1991). Oxidative phosphorylation in rat liver mitochondria: Influence of physical. *Indian Journal of Biochemistry and Biophysics*, 28:401–407.

[Sitarska and Diz-Muñoz, 2020] Sitarska, E. and Diz-Muñoz, A. (2020). Pay attention to membrane tension: Mechanobiology of the cell surface. *Current Opinion in Cell Biology*, 66:11–18.

[Smirnova et al., 2001] Smirnova, E., Griparic, L., Shurland, D.-L., and Van Der Bliek, A. M. (2001). Dynamin-related protein drp1 is required for mitochondrial division in mammalian cells. *Molecular biology of the cell*, 12(8):2245–2256.

[Song et al., 2021] Song, Y., Xu, Y., Liu, Y., Gao, J., Feng, L., Zhang, Y., Shi, L., Zhang, M.,

- Guo, D., Qi, B. and Zhang, M. (2021). Mitochondrial quality control in the maintenance of cardiovascular homeostasis: the roles and interregulation of UPS, mitochondrial dynamics and mitophagy. *Oxidative Medicine and Cellular Longevity*, 2021, 1-12.
- [Song et al., 2022] Song, Y., Huang, P., Liu, X., Zhao, Z., Wang, Y., Cui, B., and Duan, L. (2022). Light-inducible deformation of mitochondria in live cells. *Cell Chemical Biology*, 29(1):109–119.
- [Spinelli and Haigis, 2018] Spinelli, J. B. and Haigis, M. C. (2018). The multifaceted contributions of mitochondria to cellular metabolism. *Nature cell biology*, 20(7):745–754.
- [Stepanyants et al., 2015] Stepanyants, N., Macdonald, P. J., Francy, C. A., Mears, J. A., Qi, X., & Ramachandran, R. (2015). Cardiolipin's propensity for phase transition and its reorganization by dynamin-related protein 1 form a basis for mitochondrial membrane fission. *Molecular biology of the cell*, 26(17), 3104-3116.
- [Strauss et al., 2008] Strauss, M., Hofhaus, G., Schröder, R. R., and Kühlbrandt, W. (2008). Dimer ribbons of ATP synthase shape the inner mitochondrial membrane. *The EMBO journal*, 27(7):1154–1160.
- [Summerhayes et al., 1983] Summerhayes, I., Wong, D., and Chen, L. B. (1983). Effect of microtubules and intermediate filaments on mitochondrial distribution. *Journal of cell science*, 61(1):87–105.
- [Tábara et al., 2021] Tábara, L.-C., Morris, J. L., and Prudent, J. (2021). The complex dance of organelles during mitochondrial division. *Trends in Cell Biology*, 31(4):241–253.
- [Tang et al., 2006] Tang, B. T., Cheng, C. P., Draney, M. T., Wilson, N. M., Tsao, P. S., Herfkens, R. J., & Taylor, C. A. (2006). Abdominal aortic hemodynamics in young healthy adults at rest and during lower limb exercise: quantification using image-based computer modeling. *American Journal of Physiology-Heart and Circulatory Physiology*, 291(2), H668-H676.
- [Tang et al., 2021] Tang, C., Cai, J., Yin, X. M., Weinberg, J. M., Venkatachalam, M. A., & Dong, Z. (2021). Mitochondrial quality control in kidney injury and repair. *Nature Reviews Nephrology*, 17(5), 299-318.

- [TeSlaa and Teitell, 2014] TeSlaa, T. and Teitell, M. A. (2014). Techniques to monitor glycolysis. *Methods in Enzymology*, 542:91–114.
- [Tharp et al., 2021] Tharp, K. M., Higuchi-Sanabria, R., Timblin, G. A., Ford, B., Garzon-Coral, C., Schneider, C., Muncie, J. M., Stashko, C., Daniele, J. R., Moore, A. S., et al. (2021). Adhesion-mediated mechanosignaling forces mitohormesis. *Cell Metabolism*, 33(7):1322–1341.
- [Torrino and Bertero, 2022] Torrino, S. and Bertero, T. (2022). Metabo-reciprocity in cell mechanics: feeling the demands/feeding the demand. *Trends in Cell Biology*. 32(7):624-636
- [Touvier et al., 2015] Touvier, T., De Palma, C., Rigamonti, E., Scagliola, A., Incerti, E., Mazelin, L., Thomas, J.L., D'antonio, M., Politi, L., Schaeffer, L. and Clementi, E. (2015). Muscle-specific Drp1 overexpression impairs skeletal muscle growth via translational attenuation. *Cell death & disease*, 6(2), e1663-e1663.
- [Uray and Uray, 2021] Uray, I. P. and Uray, K. (2021). Mechanotransduction at the plasma membrane-cytoskeleton interface. *International Journal of Molecular Sciences*, 22(21):11566.
- [Venturini et al., 2019] Venturini, G., Malagrino, P. A., Padilha, K., Tanaka, L. Y., Laurindo, F. R., Dariolli, R., Carvalho, V. M., Cardozo, K. H. M., Krieger, J. E., and Pereira, A. d. C. (2019). Integrated proteomics and metabolomics analysis reveals differential lipid metabolism in human umbilical vein endothelial cells under high and low shear stress. *American Journal of Physiology-Cell Physiology*, 317(2):C326–C338.
- [Veza et al., 2022] Veza, T., Díaz-Pozo, P., Canet, F., de Marañón, A. M., Abad-Jiménez, Z., García-Gargallo, C., ... & Víctor, V. M. (2022). The role of mitochondrial dynamic dysfunction in age-associated type 2 diabetes. *The World Journal of Men's Health*, 40(3), 399.
- [Vincent et al., 2019] Vincent, A.E., White, K., Davey, T., Philips, J., Ogden, R.T., Lawless, C., Warren, C., Hall, M.G., Ng, Y.S., Falkous, G. and Holden, T., 2019. Quantitative 3D mapping of the human skeletal muscle mitochondrial network. *Cell reports*, 26(4), pp.996-1009.
- [Vyas et al., 2016] Vyas, S., Zaganjor, E., and Haigis, M. C. (2016). Mitochondria and cancer. *Cell*, 166(3):555–566.

- [Wagner et al., 2003] Wagner, O. I., Lifshitz, J., Janmey, P. A., Linden, M., McIntosh, T., and Leterrier, J.-F. (2003). Mechanisms of mitochondria-neurofilament interactions. *Journal of Neuroscience*, 23(27):9046–9058.
- [Wai and Langer, 2016] Wai, T. and Langer, T. (2016). Mitochondrial dynamics and metabolic regulation. *Trends in Endocrinology & Metabolism*, 27(2):105–117.
- [Wallace, 2010] Wallace, D. C. (2010). Mitochondrial DNA mutations in disease and aging. *Environmental and Molecular Mutagenesis*, 51(5):440–450.
- [Wan et al., 2014] Wan, Y.-Y., Zhang, J.-F., Yang, Z.-J., Jiang, L.-P., Wei, Y.-F., Lai, Q.-N., Wang, J.-B., Xin, H.-B., and Han, X.-J. (2014). Involvement of Drp1 in hypoxia-induced migration of human glioblastoma U251 cells. *Oncology Reports*, 32(2):619–626.
- [Wang et al., 2008] Wang, S., Jiang, C., Zhang, Y., Chen, J., Wang, B., Chen, Q., and Long, M. (2008). Membrane deformability and membrane tension of single isolated mitochondria. *Cellular and Molecular Bioengineering*, 1(1):67–74.
- [Wang et al., 2012] Wang, W., Wang, Y., Long, J., Wang, J., Haudek, S.B., Overbeek, P., Chang, B.H., Schumacker, P.T. and Danesh, F.R. (2012). Mitochondrial fission triggered by hyperglycemia is mediated by ROCK1 activation in podocytes and endothelial cells. *Cell metabolism*, 15(2), 186-200.
- [Warburg, 1956] Warburg, O. (1956). On the origin of cancer cells. *Science*, 123(3191):309–314.
- [Wenchich et al., 2003] Wenchich, L., Drahota, Z., Honzik, T., Hansikova, H., Tesarova, M., Zeman, J., and Houstek, J. (2003). Polarographic evaluation of mitochondrial enzymes activity in isolated mitochondria and in permeabilized human muscle cells with inherited mitochondrial defects. *Physiological Research*, 52(6):781–788.
- [Wieckowski et al., 2009] Wieckowski, M. R., Giorgi, C., Lebiedzinska, M., Duszynski, J., and Pinton, P. (2009). Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells. *Nature Protocols*, 4(11):1582–1590.
- [Wu et al., 2011] Wu, S., Zhou, F., Zhang, Z., and Xing, D. (2011). Mitochondrial oxidative stress causes mitochondrial fragmentation via differential modulation of mitochondrial fission–fusion proteins. *The FEBS journal*, 278(6):941–954.

- [Wu et al., 2017] Wu, D., Huang, R.-T., Hamanaka, R. B., Krause, M., Oh, M.-J., Kuo, C.-H., Nigdelioglu, R., Meliton, A. Y., Witt, L., Dai, G., et al. (2017). HIF-1 α is required for disturbed flow-induced metabolic reprogramming in human and porcine vascular endothelium. *Elife*, 6:e25217.
- [Xie et al., 2021] Xie, J., Bao, M., Hu, X., Koopman, W. J., and Huck, W. T. (2021). Energy expenditure during cell spreading regulates the stem cells responses to matrix stiffness. *bioRxiv*, page 852400.
- [Xie and Minc, 2020] Xie, J. and Minc, N. (2020). Cytoskeleton force exertion in bulk cytoplasm. *Frontiers in Cell and Developmental Biology*, 8:69.
- [Xu et al., 2022] Xu, K., Zhang, J., Ma, W., Huang, H., Yan, S., Wang, L., and Zhang, W. (2022). A facile cell culture device for studying nuclear and mitochondrial response of endothelial cells to hydrostatic pressure. *Chinese Chemical Letters*.
- [Yanes et al., 2022] Yanes, B., & Rainero, E. (2022). The interplay between cell-extracellular matrix interaction and mitochondria dynamics in cancer. *Cancers*, 14(6), 1433.
- [Yang et al., 2013] Yang, Z., He, Y., Lee, J.-H., Park, N., Suh, M., Chae, W.-S., Cao, J., Peng, X., Jung, H., Kang, C., et al. (2013). A self-calibrating bipartite viscosity sensor for mitochondria. *Journal of the American Chemical Society*, 135(24):9181–9185.
- [Yang et al., 2018] Yang, Q., Xu, J., Ma, Q., Liu, Z., Sudhakar, V., Cao, Y., Wang, L., Zeng, X., Zhou, Y., Zhang, M., et al. (2018). PRKAA1/AMPK α 1-driven glycolysis in endothelial cells exposed to disturbed flow protects against atherosclerosis. *Nature Communications*, 9(1):1–17.
- [Yang et al., 2019] Yang, H., Cheam, N. M. J., Cao, H., Lee, M. K. H., Sze, S. K., Tan, N. S., & Tay, C. Y. (2019). Materials stiffness-dependent redox metabolic reprogramming of mesenchymal stem cells for secretome-based therapeutic angiogenesis. *Advanced Healthcare Materials*, 8(20), 1900929.
- [Yang et al., 2022] Yang, Z., Wang, L., Yang, C., Pu, S., Guo, Z., Wu, Q., Zhou, Z. and Zhao, H., 2022. Mitochondrial membrane remodeling. *Frontiers in Bioengineering and*

Biotechnology, 9, 786806.

[Yang et al., 2023] Yang, X., Zhuang, J., Song, W., Shen, W., Wu, W., Shen, H., & Han, S. (2023). Mitochondria-associated endoplasmic reticulum membrane: Overview and inextricable link with cancer. *Journal of Cellular and Molecular Medicine*, 27(7), 906-919.

[Yoneda et al., 2022] Yoneda, M., Aklima, J., Ohsawa, I., and Ohta, Y. (2022). Effects of proton pumping on the structural rigidity of cristae in mitochondria. *Archives of Biochemistry and Biophysics*, 720:109172.

[Youle and Van Der Blik, 2012] Youle, R. J. and Van Der Blik, A. M. (2012). Mitochondrial fission, fusion, and stress. *Science*, 337(6098):1062–1065.

[Yu et al., 2006] Yu, T., Robotham, J. L., & Yoon, Y. (2006). Increased production of reactive oxygen species in hyperglycemic conditions requires dynamic change of mitochondrial morphology. *Proceedings of the National Academy of Sciences*, 103(8), 2653-2658.

[Yu et al., 2008] Yu, T., Sheu, S. S., Robotham, J. L., & Yoon, Y. (2008). Mitochondrial fission mediates high glucose-induced cell death through elevated production of reactive oxygen species. *Cardiovascular research*, 79(2), 341-351.

[Yu et al., 2011] Yu, T., Jhun, B. S., & Yoon, Y. (2011). High-glucose stimulation increases reactive oxygen species production through the calcium and mitogen-activated protein kinase-mediated activation of mitochondrial fission. *Antioxidants & redox signaling*, 14(3), 425-437.

[Yun et al., 2014] Yun, J., & Finkel, T. (2014). Mitohormesis. *Cell metabolism*, 19(5), 757-766.

[Zambrano et al., 2022] Zambrano, K., Barba, D., Castillo, K., Noboa, L., Argueta-Zamora, D., Robayo, P., Arizaga, E., Caicedo, A., and Gavilanes, A. W. (2022). Fighting Parkinson's disease: The return of the mitochondria. *Mitochondrion*. 64:34-44

[Zanotelli et al., 2018] Zanotelli, M. R., Goldblatt, Z. E., Miller, J. P., Bordeleau, F., Li, J., VanderBurgh, J. A., ... & Reinhart-King, C. A. (2018). Regulation of ATP utilization during metastatic cell migration by collagen architecture. *Molecular biology of the cell*, 29(1), 1-9.

[Zanotelli et al., 2021] Zanotelli, M. R., Zhang, J., & Reinhart-King, C. A. (2021).

Mechanoresponsive metabolism in cancer cell migration and metastasis. *Cell metabolism*, 33(7), 1307-1321.

[Zemirli et al., 2018] Zemirli, N., Morel, E., and Molino, D. (2018). Mitochondrial dynamics in basal and stressful conditions. *International Journal of Molecular Sciences*, 19(2):564.

[Zhao et al., 2013] Zhao, J., Zhang, J., Yu, M., Xie, Y., Huang, Y., Wolff, D. W., Abel, P. W., and Tu, Y. (2013). Mitochondrial dynamics regulates migration and invasion of breast cancer cells. *Oncogene*, 32(40):4814–4824.

[Zinsmaier, 2021] Zinsmaier, K. E. (2021). Mitochondrial Miro GTPases coordinate mitochondrial and peroxisomal dynamics. *Small GTPases*, 12(5-6), 372-398.

[Zong et al., 2016] Zong, W.-X., Rabinowitz, J. D., and White, E. (2016). Mitochondria and cancer. *Molecular Cell*, 61(5):667– 676.

[Zorov et al., 2019] Zorov, D. B., Vorobjev, I. A., Popkov, V. A., Babenko, V. A., Zorova, L. D., Pevzner, I. B., Silachev, D. N., Zorov, S. D., Andrianova, N. V., and Plotnikov, E. Y. (2019). Lessons from the discovery of mitochondrial fragmentation (fission): a review and update. *Cells*, 8(2):175.

Figure legends

Figure 1 Schematic representation of the internal structure of a mitochondrion and the two main ATP synthesis processes in a eukaryotic cell: oxidative phosphorylation (OXPHOS) and cytosolic glycolysis. OXPHOS relies on the citric acid cycle or Krebs cycle, which is a set of redox reactions of substrates derived from glucose (through cytosolic glycolysis), fatty acid or amino acid oxidation.

Figure 2 Established (solid arrows) or potential (dashed arrows) bidirectional links between metabolism, mitochondrial morphodynamics, mechanics and mechanical cues. The influence of metabolism on mechanics is supported by few data, while the impact of changes in mitochondrial morphodynamics on their mechanics is not yet highlighted in the literature.

Figure 3 Links between mitochondria morphodynamics and metabolism. (A) Mitochondria exhibit a wide variety of shapes depending on the balance between fission and fusion rates. (B) Mitochondria display not only a substantial heterogeneity across tissues in terms of density and shapes, but also an intra-tissue heterogeneity, reflecting the coupling between their structure and their function.

Figure 4 Mitochondrial responses to metabolic cues, depending on the nature, the intensity and the duration of the metabolic signal. Metabolic cues either induce protective mechanisms in mitochondrial dynamics to support cellular homeostasis or impairs cellular signaling and triggers apoptosis.

Figure 5 Schematic representation of the actin-dependent and DRP1-dependent mitochondrial fission process and the variety of fission-inducing stimuli contributing to the increase in mitochondrial membrane tension. A hypothetical membrane tension gradient is represented in

the boxed region of the constriction site, with the maximum value localized at the level of the DRP1 helical structure. The recruitment of DRP1 at a pre-constricted fission site, the association with the endoplasmic reticulum (ER) and the coordinated activity of actin filaments generate tensile and bending forces. The recruitment of DRP1 by mitochondrial adaptor proteins is not represented. DRP1 assembles into a helical structure to further constrict the mitochondrion and may generate a membrane tension gradient.

Figure 6

Mitochondrial metabolic adaptation to mechanical cues. The contribution of OXPHOS and glycolysis in ATP production is regulated by the nature and the magnitude of mechanical forces exerted on the cell. References indicated on the figure are : [1] Bertero et al., 2016, [2] Chakraborty et al., 2021, [3] Romani et al., 2022, [4] Papalazarou et al., 2020, [5] Tharp et al., 2021, [6] Bartolák-Suki et al., 2015, [7] Kim et al., 2014, [8] Doddaballapur et al., 2015, [9] Feng et al., 2017, [10] Wu et al., 2017, [11] Yang et al., 2018, [12] Venturini et al., 2019, [13] Coon et al., 2022, [14] Park et al., 2022.