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► To cite this version:

Patrick Cormier, Agnès Boutet, Pierre Colas, Sandrine Ruchaud, Stéphane Bach, et al.. The Roscoff Biological Station in the era of cellular and molecular biology: Focus on the 50 last years of research on marine animals. *Cahiers de Biologie Marine*, 2023, 64 (1), pp.35-52. 10.21411/CBM.A.C705BF49 . hal-04254376

HAL Id: hal-04254376

<https://hal.science/hal-04254376v1>

Submitted on 26 Oct 2023

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The Roscoff Biological Station in the era of cellular and molecular biology: 50 years of research on marine animals.

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Running title: 50 years of research on marine animals

Keywords: marine metazoans, marine animals, marine station, MPF, mRNA translation, Evo-devo, electrophysiology

Abstract

In the context of the 150th anniversary of the Roscoff Biological Station, we describe here how cellular and molecular biology using marine metazoans has been developed at the station during the last 50 years. The discipline was first initiated by an interest in the endocrinology of marine invertebrates and allowed the characterization of the famous MPF in echinoderms. During this exciting period of time the biological station focused the interest of the international cell cycle scientific community. Then, discoveries made at the station played a founding role in developing applied research and creating companies offering innovative therapeutic solutions. Later, the scientific dynamic using marine metazoans has been perpetuated by studying the cellular and molecular mechanisms, such as ionic membrane transports or mRNA translation, that govern the life of cells and embryos. More recently, acquiring metazoan genomes has allowed researchers to analyze marine metazoan embryonic development in an evolutionary context. Finally, the Biological Station contributes to transfer this essential knowledge through international courses, which prepare the young generations for future scientific challenges.

Résumé:

Il y a tout juste 50 ans, la biologie cellulaire et moléculaire des métazoaires marins a intégré la Station Biologique de Roscoff. Dans le contexte de l'anniversaire des 150 ans de cette station, nous proposons ici de revisiter les temps forts de cette période. C'est en s'intéressant à l'endocrinologie des invertébrés marins qu'a été initiée la découverte chez les échinodermes du fameux MPF. La Station Biologique a alors focalisé l'intérêt de la communauté scientifique mondiale s'intéressant au contrôle du cycle cellulaire. Les découvertes faites à la station ont ainsi joué un rôle fondateur dans le développement de recherches appliquées et à la création de compagnies proposant des solutions thérapeutiques innovantes. La dynamique scientifique utilisant les métazoaires marins s'est perpétuée en étudiant les mécanismes cellulaires et moléculaires qui président à la vie des cellules et des embryons d'animaux marins. Plus récemment, l'acquisition de génomes de métazoaires marins a conduit les équipes à s'intéresser à la biologie évolutive du développement. Riche de ce dynamisme scientifique, la Station Biologique de Roscoff diffuse ces connaissances fondamentales au travers d'enseignements internationaux qui préparent les jeunes générations aux défis scientifiques à venir.

The interest of marine animal models for basic research

Since the beginning of the XXIst century, the study of marine animals has been rewarded with four Nobel Prizes. In 2000, Eric Kandel, Arvid Carlsson and Paul Greengard won the Nobel Prize in Medicine and Physiology for their findings on the mechanisms responsible for signal transmission in the nervous system (Camilli & Carew, 2000). In fact, Eric Kandel's experiments on the marine mollusk *Aplysia* had laid the basis for the study of the molecular logic underlying long-term memory (Pinsker et al., 1973). The following year, one of the laureates was Tim Hunt, whose work on sea urchin and clam embryos contributed decisively to the identification of molecular players regulating cell cycle progression (Evans et al., 1983; Dorée & Hunt, 2002). Clams have also been used as a model by Avram Hershko, who participated in the discovery of ubiquitin mediated protein degradation and was awarded with the 2004 Chemistry Prize (Sudakin et al., 1995). Finally, Roger Tsien, Martin Chalfie and Osamu Shimomura obtained the same award in 2008 for the discovery and development of green fluorescent proteins (the famous GFPs), which were first identified in cnidarians (Service, 2008; Zimmer, 2009). Alone, these great scientific achievements are sufficient to illustrate the importance of marine animal models and how their study can provide answers to multiple biological questions. But if the study of marine organisms has produced so many exciting discoveries, it is to a great extent thanks to institutions like the Roscoff Biological Station (SBR: Station Biologique de Roscoff), which has acted since its foundation as a powerful catalyzer of scientific advance.

The main objective of this article is to present a survey of the spectacular advances in cell biology that the SBR has contributed during the last fifty years. But instead of presenting them in a strict chronological order, we have chosen to describe separately the main thematic axes that have been developed at the Station. These axes are often linked to the implementation of new approaches that have been inspired by the most recent findings of molecular biology. Indeed, in the late 60's and early 70's the rise of molecular biology was about to influence all the domains of biology, including cell biology, embryology and of course, research applied to marine models.

In these early days, scientific expertise was gradually redirected towards a deeper understanding of the molecular mechanisms that control the life of cells and organisms,

often in detriment of more descriptive approaches based on micro- and macroscopic analyses. However, this paradigm change was not without some misgivings on the part of a few teasing colleagues, who use to dub as "the molecules" those scientists analyzing at the molecular level transduction pathways and cellular processes. Most criticisms were due to a more or less explicit rejection of the idea that the functioning of living beings could be entirely reduced to the properties of its molecular constituents. This hostility was far from being specific to the SBR, but was representative of the sometimes difficult cohabitation between molecular biology and disciplines concerning higher organization levels, such as physiology, ecology or evolutionary biology (Morange, 2003). Ironically, molecular approaches targeting a few specific genes and their products were just foreshadowing the dawn of a much more radical conceptual change, a revolution that accompanied the arrival of the holistic disciplines in the XXIst century.

All starts with an egg...

The gametes of marine invertebrates are in general easy to handle, thus permitting detailed microscopic observations of egg fertilization and first embryonic cleavages. This property allowed Pierre Guerrier to study the segmentation of spiralian eggs and generate his elegant descriptions of the movements executed by their cellular components. However, he became gradually interested in the role of translational control in the morphogenesis of the embryo and chose as a model the polychaete annelid *Sabellaria alveolata* (Guerrier, 1971) (**Figure 1A,B**). Global gene activity could be analyzed at the time by incubating embryos with antibiotics such as actinomycin D and puromycin, which are respectively powerful inhibitors of mRNA transcription and translation. The major conclusion of his studies was that the control exerted by polar lobe cytoplasm on the differentiation of apical tuft and post-trochal bristles could be related to the early translation of maternally inherited RNAs. His approach, which was resolutely molecular, was about to initiate a whole area of research that is still one of the major concerns of the Station. Eventually, the analysis of polar lobe formation on the determination of the dorso-ventral polarity of different mollusks –such as the limpet and the dentalium– continued in collaboration with the team of Jo Van den Biggelaard in the University of Utrecht (Guerrier et al., 1978). Guerrier's group had in fact contributed a major conceptual shift, making one of the first attempts to link the regulation of gene

expression to the mechanisms controlling morphogenetic events and cell fate specification in marine embryos.

Towards the characterization of M-phase Promoting Factor

The gametes of different echinoderms, such as starfish and sea urchins (**Figure 1C-F**), have been a popular experimental model since the end of the XIXth century. In the 50's, it became clear that a neural hormone similar to gonadotropin could control reproduction in the starfish, a finding that constituted the founding event of invertebrate reproductive endocrinology (Kishimoto, 2018). In these animals, meiosis resumption depends on the release of 1-methyl adenine (1-MeAde), a relay hormone produced by follicle cells upon reception of a peptide hormone of neural-origin that acted as an upstream signal. These findings revealed that gonadotropins control ovulation and oocyte maturation indirectly, with ovarian follicles acting as intermediates (Kishimoto, 2018). In the 70's, several teams were trying to understand the mechanism of action of 1-MeAde. At the SBR, Pierre Guerrier and Marcel Dorée became first interested in the features of the 1-MeAde receptors present in the oocytes of two local starfish, *Marthasterias glacialis* and *Asteria rubens* (Dorée et al., 1976). With Marc Moreau, a young electrophysiologist, they managed to show that calcium release from intra-cellular compartments and activation of a sodium pump mediated the oocyte response to 1-MeAde (Meijer & Guerrier, 1984). Furthermore, they demonstrated that 1-MeAde triggers a dramatic increase of the phosphorylation status of many proteins as early as 4 minutes after hormone addition (Guerrier et al., 1977). These results confirmed that an increase in protein phosphorylation plays a crucial role in the transduction mechanism controlling meiotic resumption upon 1-MeAde stimulation. Therefore, these findings opened immediately a new field of research, as nobody knew which factors were directly responsible for this phosphorylation wave.

Different species-specific signals –sometimes hormones, in other cases fertilization itself– can trigger the entry into M-phase of mature oocytes (**Figure 2A,B**). Transplantation experiments involving the transfer of cytoplasm from M-phase oocytes into recipient G2-arrested eggs permitted the characterization of the so-called MPF (Maturation-Promoting Factor or M-phase Promoting Factor), an unknown cytoplasmic agent capable of inducing M-phase entry in absence of any other stimulation (**Figure 2C**). The purification of MPF and

its eventual characterization as a CDK1/Cyclin B heterodimer was an exciting period of time at the Marine Station (Dorée & Hunt, 2002). Marcel Dorée, Gérard Peaucellier and André Picard became experts in echinoderm microinjection techniques, an essential prerequisite to unravel the MPF identity. First, they observed in the maturing oocyte of the starfish *Marthasterias glacialis* a tight correlation between the oscillations of MPF activity and the general levels of protein phosphorylation (Dorée et al., 1983). Next, in collaboration with the Arago laboratory at Banyuls and the CRBM at Montpellier, they analyzed the role of protein synthesis and protease activity in orchestrating these oscillations (Picard et al., 1985). They showed that the concomitant oscillation of the respective activities of MPF and an M-phase-specific Histone 1 kinase (H1K) depended not only on protein synthesis but also on protein degradation, both during meiotic maturation and early embryonic development. These observations led them to propose that proteolysis is in fact essential for H1K inhibition when MPF activity starts decaying.

In 1988, Laurent Meijer's group could show that the H1K purified from starfish oocytes is a protein closely related to p34^{cdc2}, the product of the *cdc2* gene in the fission yeast *Schizosaccharomyces pombe* (Arion et al., 1988). H1K purification was thus greatly facilitated by its high affinity for the fission yeast protein p13^{suc1}, as the sea urchin H1K strongly binds to p13-Sepharose beads. One year later, they could show that MPF extracted from starfish oocytes co-purifies with the H1 histone kinase p34^{cdc2} and that the activation of this kinase was coupled to its dephosphorylation (Labbe et al., 1989) (**Figure 3**). MPF activity, M phase-specific histone H1 kinase, and p34^{cdc2} were in fact the same entity! This *tour de force* culminated when it was shown that Cyclin B is synthesized *de novo* during the first mitotic cell cycle of fertilized sea urchin eggs, and that this protein co-purifies with p34^{cdc2} in p13-Sepharose beads (Meijer, L. et al., 1989). In this way, MPF was finally shown to be a heterodimer comprising one molecule of Cyclin and one molecule of Cdc2 kinase (hence renamed as CDK, for Cyclin-Dependent Kinase).

All this pioneering work on MPF characterization has been instrumental to establish sea urchin eggs and embryos as reference models for the study of cell cycle progression. Thanks to this expertise, the Station's resident teams have contributed to a better understanding of the biochemical and cellular mechanisms controlling cell division. But that required entering

a so-called house-keeping process that turned out to be highly regulated: the world of mRNA translation (Mathews et al., 2000).

Discovering the molecular network regulating protein synthesis activation in response to fertilization.

Translational activity is remarkably low in unfertilized sea urchin eggs, and fertilization induces a dramatic increase of protein synthesis that occurs independently of mRNA transcription and ribosome biogenesis (reviewed in (Cormier et al., 2016)). Sea urchin thus provides a system where gene expression is not governed at the transcription level and relies on the translational mobilization of a large pool of maternal mRNAs during the first three cell divisions (Mathews et al., 2000). Besides, some of these mRNAs had already been identified, because the mitotic Cyclins A and B were known to be actively translated in response to fertilization (Evans et al., 1983).

In the late 90s, the group of Robert Bellé was studying several translation factors involved in *Xenopus* oocyte maturation, but was appealed by the properties of the echinoderm system and saw an opportunity to analyze the connections between translational regulation and cell cycle progression. The group thus left Paris to join the Marine Station, which offered excellent facilities for biochemical studies. But perhaps the most powerful drive for this new project was the research community that was orbiting around the sea urchin. The members of Bellé's group were indebted to Gérard Peaucellier, who taught them how to manipulate starfish and sea urchin eggs during the summer school of the Arago Laboratory in Banyuls. What an extraordinary privilege must have been learning microinjection techniques with someone as André Picard! And techniques kept further improving, thanks among others to Ina Arnone –who came several times to Roscoff from the Naples Zoological Station (SZN: Stazione Zoologica Anton Dohrn) with the support of a Training and Mobility of Researchers (TMR) program of the European Union– and to multiple exchanges with the local community of electrophysiologists.

The group started to unveil the molecular mechanisms activating mRNA translation upon fertilization (**Figure 4**). It soon became clear that a key event is the release of the mRNA cap-binding protein eIF4E (eukaryotic Initiation Factor 4E) from its repressor 4E-BP (eIF4E-

binding protein) (Cormier et al., 2001; Salaun et al., 2003). In absence of this repressor, eIF4E associates rapidly with a scaffolding protein known as eIF4G and forms a complex whose activity is essential for cap-dependent translation and completion of the first mitotic division (Oulhen et al., 2007).

In the sea urchin, 4E-BP degradation is triggered by fertilization in a Ca^{2+} and pH-dependent event and relies ultimately on the activation of mTOR (mechanistic Target Of Rapamycin), a central regulator of protein translation that plays a crucial role during the early development of these animals. However, new results soon demonstrated that eIF4E release is not the only mechanism for translational activation in the sea urchin egg. For instance, it could be shown that cell-cycle dependent phosphorylation of different proteins, such as a new isoform of eEF2 (eukaryotic Elongation Factor 2) or the initiation factor eIF2 α , are also required for the completion of the first cell cycle (Belle et al., 2011; Costache et al., 2012).

The activation of the translation machinery at fertilization leads to the production of newly synthesized proteins, which are encoded by a large pool of maternally inherited mRNAs. Polysome profiling allows the identification of the so-called translatoome, a comprehensive representation of the mRNAs associated with polysomes that provides a global picture of the cellular translational activity at a specific time point. The translatoome of the sea urchin *Paracentrotus lividus* has been analyzed during the egg-to-embryo transition by polysome profiling and RNA sequencing (Chassé et al., 2017, 2018). Polysomal recruitment at fertilization has proved to be a very selective process. As expected, mRNAs encoding for different cell cycle actors –such as Cyclin A and B– are rapidly recruited, but also many mRNAs coding for different proteins involved in RNA-Binding and translational activation. These results suggest that many mRNAs other than the mitotic cyclins must be translated to finely regulate the onset into mitosis. Interestingly, the *de novo* early translation of some key molecular players of translation, like eIF4B (eukaryotic Initiation factor 4B), can set in motion a positive regulatory loop that further reinforces translational activity in the fertilized egg (**Figure 4**). In fact, eIF4B can act as a stimulator of cap-dependent translation and its levels seem to have a direct impact on cleavage dynamics during the first steps of sea urchin embryo development (Pontheaux et al., 2022). Fertilization thus activates a

translation regulatory network (TIRN) that is tightly intertwined with cell division and that orchestrates the polysomal recruitment of a specific set of maternal mRNAs.

Preparing the stage for the study of translational regulatory networks

In the early 2000s, the sea urchin research community joined efforts to support the *Strongylocentrotus purpuratus* Genome project, and this organism became the first fully sequenced echinoderm (Sea Urchin Genome Sequencing et al., 2006). One of the outputs of this achievement is the Echinoderm genome database, which has been enriched with the genomes of other members of this phylum and has nowadays become an inescapable reference for many investigators (Kudtarkar & Cameron, 2017). All these non-chordate deuterostome genomes provide an essential resource for studies on evolution and both developmental and cellular biology (Pontheaux et al., 2021). Moreover, they have been instrumental for the characterization of the developmental gene regulatory networks (dGRNs), the routines that specify different fates in a forming embryo and their multiple circuits, loops and regulatory inputs (Martik et al., 2016).

The sea urchin genome contains roughly 23,300 genes, which closely represent all the vertebrate gene families without extensive redundancy. The Roscoff group took rapidly advantage of this resource and focused on the analysis of genes participating in translational regulation, identifying all the molecular components of the basic sea urchin translational toolkit (Morales et al., 2006). It turned out that most of the factors implicated in translational regulation are encoded in this animal by non-redundant genes, a feature that facilitates functional studies. This translational toolkit has thus become a reference to characterize in detail the TIRN that orchestrates protein synthesis during development.

Altogether, these findings indicate that translation activation depends on a complex network of coordinated events, a network that we have just begun to explore and whose properties can be modeled with the help of mathematicians, allowing the establishment of the first numerical models describing its most relevant properties (reviewed in (Moundoyi et al., 2018)). Clearly, the future challenge for the laboratory will be deciphering the molecular logic of these TIRNs and how they are linked to both cell cycle progression and the early events controlling patterning formation in a developing embryo.

In this new phase, the laboratory is taking advantage of all the resources that modern integrative biology can offer. For example, the development of a modern in-house imaging facility, equipped with optical and electron microscopes (Plateforme MerImage), allows to visualize the different cellular events that accompany cell cycle dynamics. Commercial antibodies against mammalian proteins have proved to be efficient tools in many cases, and they can be used to monitor dynamic processes such as microtubule reorganization or Cyclin B/CDK1 oscillations during embryonic divisions. In addition, the localization of different mRNAs can be also analyzed in whole mounts thanks to different *in situ* hybridization techniques, providing new insights into the principles determining transcript spatial distribution within the cell (**Figure 5**).

Concerning functional studies, microinjection represents so far the only efficient way to introduce reagents into the sea urchin eggs and blastomeres. Indeed, the development of injection methods at the SBR has facilitated the analysis of gene function and gene regulation during the first cleavages of sea urchin embryo. Reagents such as drugs, biologically active peptides, luciferase reporters, exogenous mRNAs and morpholinos interfering with the translation of endogenous transcripts have been applied successfully to study the roles of different molecular actors (Pontheaux et al., 2021). It is also very likely that the CRISPR-Cas 9 gene targeting technology could be adapted in a near future to the local sea urchin species, as this technique has already been implemented successfully in *Strongylocentrotus purpuratus* and *Temnopleurus reevesii* (Oulhen & Wessel, 2016; Yaguchi et al., 2020).

From fundamental researches to molecular screenings aimed at the characterization of new chemical tools and therapeutic compounds

The members of Laurent Meijer's group realized immediately that CDK1/Cyclin B is a universal regulator of cell cycle progression and they hypothesized that any inhibitor of this complex would be a potent antimitotic agent and thus a tool to fight cancer. They thus decided to take advantage of starfish oocytes as an abundant source of a native and highly active CDK1/cyclin B complex that could be easily purified by affinity chromatography (**Figure 6**). The kinase activity of this complex could be detected using a robust radiometric assay, which was optimized to allow the detection of different potent inhibitors. These

include the famous 2,6,9-tri-substituted purine compound, (R)-roscovitine (also known as CYC-202 or Seliciclib)(Meijer et al., 1997). (R)-roscovitine is now a powerful tool for biologists worldwide, which can inhibit pharmacologically its activity and study its multiple roles. At the same time, this molecule has reached phase II clinical evaluation against NSC lung cancer.

The seminal work carried out at the SBR on protein kinase inhibitors has contributed decisively to reveal the clinical interest of this class of compounds. Indeed, protein kinases are involved in multiple physiological processes and are often key mediators of cellular responses to different external stimuli. Nowadays, the human kinome (518 protein kinases) is one of the main targets of pharmacology and up to one third of the drug discovery projects worldwide focus on the identification of new protein kinase inhibitors (PKIs). As a result, the number of approved PKIs is growing steadily and in August 2022, 71 drugs targeting more than 20 different protein kinases have been approved by the FDA, the vast majority for treating malignancies. A complete and updated listing of PKIs in clinical trials can be found at www.icoa.fr/pkidb/.

In Roscoff, the discovery of (R)-roscovitine paved the way for massive screening campaigns, and currently a platform called KISSf (Kinase Inhibitor Specialized Screening facility) is specifically devoted to the identification of new PKIs. Taking advantage of the marine environment, the Roscoff's team has been actively exploiting the amazing chemodiversity of marine organisms. Collaborating with different chemists, they improved the bioactivity of original molecules and contributed to the discovery of new promising PKIs, such as leucettamine B (*Leucetta microraphis*), indirubins (Muricidae and Thaididae), hymenialdisine (Agelas) and meriolins (*Kirkpatrickia* and *Aplidium*), to name just a few (Meijer et al., 2003; Debdab et al., 2010). These discoveries led in addition to the development of a biotech company in 2007, ManRos Therapeutics "from sea to pharmacy", spawned by the Roscoff's team.

Following this line of research, the SBR hosts as well projects aimed at characterizing kinases involved in biological processes linked to human pathologies, including mitosis and regulated necrosis. The search for new anti-cancer drugs has thus contributed to a deeper

understanding of the physiological role of different specific essential mitotic kinases. For instance, Haspin is responsible for the phosphorylation of a specific histone mark during early mitosis (H3T3ph) and its activity is essential for Aurora B kinase activation. In turn, Aurora B contributes to faithful chromosome segregation and genetic stability. Not surprisingly, both kinases have emerged as promising therapeutic targets and several projects involving Roscoff's researchers and external collaborators have characterized and developed different families of specific inhibitors. These include the lamellarins (*Lamellaria*) –a series of potent Haspin inhibitors (Avula et al., 2022)– and different fragments of benzosceptrin B, a drug originally extracted from the marine Pacific sponge *Phakellia* that behaves as an atypical Aurora B kinase allosteric inhibitor (Juillet et al., 2021). All these compounds, often inspired by structures concealed within marine organisms, reinforce our therapeutical arsenal and provide valuable tools for fundamental research aimed at dissecting basic biological mechanisms and their role in disease (Papini et al., 2019).

In another biological context, the development at the SBR of a necrosis cellular assay has allowed the identification of new promising drugs, leading to the creation in 2019 of the SeaBeLife Biotech Company. Necroptosis is a specialized biochemical pathway of regulated necrosis and depends notably on the activity of the serine/threonine kinase RIPK1 (see (Galluzzi et al., 2018) for details on the classification of regulated cell death pathways). RIPK1 contributes to multiple human disorders involving necroptosis, including ischemia-reperfusion injury in the brain, heart and kidney, and a plethora of neurodegenerative, inflammatory and infectious diseases. Patented candidate RIPK1 inhibitors –sibiriline and nigratine (Le Cann et al., 2017), all identified at the SBR– are now developed by SeaBeLife Biotech to treat acute liver injury/acute liver failure (ALI/ALF) and other chronic diseases, such as age-related macular degeneration (AMD) and Parkinson's disease (PD). Some of these RIPK1 inhibitors are first-in-class polypharmacological compounds because they also inhibit regulated necrosis by ferroptosis (Delehouzé et al., 2022). Ferroptosis, an iron-dependent oxidative form of cell death associated with increased lipid peroxidation (Galluzzi et al., 2018), is an evolutionarily conserved cell death pathway in a wide set of species ranging from prokaryotes and plants to mammals. Interestingly a ferroptosis-like pathway exists also in marine cyanobacteria, suggesting that this regulatory circuit originated more than 2.7 billion years ago.

In the next future, the high chemodiversity of marine environment has to be explored (e.g. the marine sponges and associated microbiomes) to characterize new chemical probes (inducers and/or inhibitors). This endeavour will lead to the discovery of new non-canonical cell death modalities and contribute to improve human therapies.

The knowledge acquired at the SBR on the molecular mechanisms regulating cell cycle progression has also led to a renewed interest in human pathologies that are still poorly understood. In 2008, perpetuating a long-standing tradition, one of Pierre Guerrier alumni initiated a research project on CDK10, which was at the time among the most mysterious members of the CDK family and had no assigned cyclin subunit. This CDK10 binding partner turned out to be Cyclin M, which enabled to reveal the *in vitro* kinase activity of a functional CDK10/CycM heterodimer. This novel protein kinase was shown to phosphorylate the ETS2 oncoprotein, thereby controlling its stability. Mutations on the Cyclin M gene were known to cause a severe human developmental disorder called STAR syndrome, whose etiology was totally unknown. Cells isolated from a STAR patient display increased ETS2 protein levels and were shown to have a deficient CDK10/CycM kinase (Guen et al., 2013). Soon after, the same group showed that this kinase is involved in the regulation of actin dynamics and ciliogenesis (the formation of primary cilia), through a PKN2-RhoA signaling axis. Kidney cells isolated from a STAR patient were shown to display abnormal cilia, which suggested that the STAR syndrome is a ciliopathy (*i.e.* a pathology caused by ciliary defects) (Guen et al., 2016). This group has also contributed to the characterization of a new syndrome discovered in Israel, caused by mutations on the CDK10 gene (Guen et al., 2018). This work adds to the growing list of human developmental disorders caused by mutations on genes coding for CDKs, which demonstrates the pivotal roles played by these protein kinases during development (Colas, 2020).

Finally, we cannot overlook the importance of marine organisms as models for ecotoxicology. For example, one of the world's best-selling agrochemical compounds was shown to perturb many molecular events involved in sea urchin early development, including CDK1/Cyclin B activation and mRNA transcription and translation (Marc et al., 2002, 2005). These findings challenged the notion that these compounds are innocuous and

warned the public opinion on the negative consequences that could derive from their massive utilization. Nobody could have anticipated that the sea urchin embryo would become one day the protagonist of such a heated social debate!

Electrophysiology at the Roscoff marine Station

By recording in the 70s the effect of acetylcholine on the membrane potential from the electric organ of *Torpedo mamorata*, Marc Moreau introduced electrophysiology approaches in the Biological Station (Moreau & Changeux, 1976). He strengthened the local implantation of electrophysiology actively collaborating with Pierre Guerrier and analyzing the role of different cations, such as Ca^{2+} and Na^+ , during the meiotic maturation of starfish oocytes (Meijer & Guerrier, 1984).

Electrophysiology was further developed in the SBR by Henri and Marie Goudeau, who analyzed the main events accompanying oocyte meiosis and egg fertilization in several marine invertebrates (**Figure 7**). For example, they characterized the channels that are physiologically responsible for the oocyte membrane excitability in the ascidian *Phallusia mammillata* (Goudeau et al., 1992). They recorded as well the egg electrical response to fertilization and managed to identify the channels involved (Goudeau & Goudeau, 1993). In addition, they also showed by voltage clamp experiments that the egg electrical response to fertilization mediates a block of polyspermy (Goudeau & Goudeau, 1993).

The international visibility of electrophysiology at the SBR kept increasing with the arrival in the late 90s of the team of Serge Thomas and Stéphane Égée. This group has focused on the study of membrane ionic transport in erythrocytes of different species. They managed to adapt modern electrophysiology techniques, such as whole-cell and single-cell patch-clamp, to both fish and human erythrocytes. In this way, they could demonstrate the presence of ATP-sensitive K^+ channels and Ca^{2+} -gated K^+ channels in the red blood cell membrane of the lamprey (*Petromyzon marinus*) (Lapaix et al., 2002).

From developmental biology to Evo-Devo

Throughout its history, the Station has attracted internationally renowned researchers who fully developed their activity within its walls or just joined for the summer season. These scientists were coming to the Biological Station to find live material (**Figure 8**) and good

conditions for experimental work. They have contributed decisively to its worldwide reputation in the fields of cell biology, development and evolutionary biology.

At the beginning of the XXth century, Yves Delage was taking already advantage of the Station facilities to take a new look at the embryogenesis of sponges and analyze the processes of parthenogenesis in sea urchins. Jean Brachet, a renowned biochemist from the University of Brussels, describes Roscoff in the 30's as an extremely exciting place, where the great biologists of the time arrived full of enthusiasm (Brachet, 1975). Among these, he mentions the Swede Sven Hörstadius, renowned for his embryological experiments in sea urchins, and the geneticist Boris Ephrussi, who started his career working also with these organisms. Jean Brachet could also discuss at leisure with his friends André Lwoff and Jacques Monod, who received the Nobel Prize in Medicine and Physiology in 1965 for their work on gene activity regulation in virus and bacteria. And several outstanding investigators have continued visiting the SBR in more recent times. Among them, it is worth to mention two Swiss developmental biologists: Walter Gehring, who participated to the discovery of the homeobox genes (McGinnis et al., 1984), and Volker Schmid, who demonstrated that the mononucleated striated muscle cells of the tiny jellyfish *Podocoryne carnea* constitute a unique model system to analyze cell transdifferentiation (Schmid & Alder, 1984). And we must not forget the contributions of Billie Swalla and William Jeffery to the study of anural development in ascidians (Swalla & Jeffery, 1990).

In 2006, the completion of the sea urchin *Strongylocentrotus purpuratus* genome has placed this echinoderm at the forefront of model organisms, providing new insights into the signaling pathways and the gene regulatory networks involved in embryo patterning. Ever since, the number of marine animal genomes has been growing at a steady pace and now includes representatives of many different phyla, such as the vertebrates (*Fugu rubripes*), tunicates (*Ciona intestinalis*, *Ciona savignyi* and *Oikopleura dioica*) or cnidarians (*Nematostella vectensis* and *Aurelia aurita*), to quote just a few examples. Most of these organisms have become valuable experimental model systems and have completely changed our view of the links between phylogenetic position and genetic complexity. Their study not only allows to propose evolutionary scenarios explaining metazoan diversity, but

also to identify new molecular players and biological processes that have immediate practical applications for the benefit human biology and human society.

The Station has contributed to this endeavor in the past and will certainly continue doing so in the future. Resident groups have made important efforts to understand how evolutive changes sustain diversification of forms and structures, contributing to the development of Evo-Devo, also known as evolutionary developmental biology. The interest of the Station for this discipline stemmed out of the initiative of Jo Van den Biggelaard and André Adoutte, who proposed an advanced course in Evolutionary Developmental Biology at the SBR in 1999, thanks to the support of the TMR program. Over the years, the content of this course has been modified to focus on marine organisms and to cover a wide range of biological fields (neurobiology, cell biology, Evo-Devo, modelling of organism growth, stem cell biology, cell cycle regulation). This teaching is now called the Schmid Training Course (STC) and is part of master programmes of Sorbonne Université in collaboration with other foreign universities. Besides local financial support and contributions from partner universities, the course also received a grant from EuroMarine (European Marine research Network) in 2015, confirming its integration into the marine biology community in Europe. More recently, the teaching staff of the STC decided to create a web platform dedicated to research on all their favourite marine organisms with the aim to provide online support for students and promote autonomous learning. The platform called DigitalMarine was funded by the European Union through the strategic partnership Erasmus programme and allows now hybrid training (partially off-campus distance learning via the platform, partially on-campus learning at the marine station) for the STC (Flom et al., 2022). This funding was also the opportunity to edit a Handbook of Marine Model Organisms in Experimental Biology depicting state of the art knowledge and ongoing research for each model (reviewed in (Adameyko, 2022)). Contributions from marine biologists outside the STC network were asked to cover a full spectrum of marine model organisms. The SBR is thus a major place for research in biology where students benefit from suitable training to understand why and how to use marine organisms in life sciences.

The Development and Evolution of Vertebrates team invested in the creation of new methods and tools to study at a functional level two marine vertebrate models: the lamprey

Petromyzon marinus and the catshark *Scyliorhinus canicula*. The Station played once again a key role, furnishing catshark embryos and juveniles of different stages and thus fostered pioneering approaches using these animals (Coolen et al., 2008). As a result, it has been shown that the asymmetry of epithalamus is controlled by the morphogen Nodal (Lagadec et al., 2015). These observations provide important insights into the ancient origin of epithalamic asymmetry, indicating that a nodal-dependent process operated already in the forebrain of the common ancestor of vertebrates. But adult catsharks display other interesting features, as they are still able to generate new nephrons, the functional units of the kidney. This regeneration capacity is shared by other Chondrichthyes but has been lost in mammals. A specific research program has thus been developed to study the catshark renal progenitors in both embryos and juvenile, using among other techniques spatial transcriptomics. Future work will focus on changes affecting translational control in this specific cell population and the activity of the mTOR signaling pathway, which is involved in a wide range of metazoan regeneration processes (Lund-Ricard et al., 2020).

This research project illustrates, once again, how basic research focused on marine animals can result in medical applications and perhaps future therapies permitting the regeneration of adult mammalian kidney and other organs. But the potential of marine organisms is still huge, and that concerns also non-conventional metazoan organisms, like the acoel worm *Symsagittifera roscoffensis* (Martinez et al., 2021). The so-called Roscoff worm, which is intimately bound to the history of the Station, presents in fact a unique feature: its ability to regenerate a full brain following complete destruction. The study of its nervous system during early embryonic development has already revealed that the genetic mechanisms underlying this property are evolutionary conserved, challenging the notion that the nervous system of acoels is primitive (Arboleda et al., 2018). But this strange creature offers also an opportunity to study a wide repertoire of genetically encoded traits that determine interactions with photosymbionts, photo- and geotaxis, circa-tidal rhythmicity and social interactions.

In the new era of integrative biology, the Biological Station of Roscoff will thus continue fostering new discoveries and unveil the many secrets that marine animals still hold.

Acknowledgments We apologize to those investigators whose work was not cited or discussed here due to space limitation. We are indebted to the all the members of the Marine and Diving Facility, the Aquarium Service of the Roscoff Marine Station and the Marine Biological Resource Center (MBRC). In particular, we would like to thank Gaétan Schires, Sébastien Henry and Ronan Garnier for taking care of our favourite models. We thank all the members of the LBI2M unit for many stimulating scientific discussions. We are also grateful to the imaging facility of the FR2424 (Plateforme MerImage, Biogenouest, Roscoff Marine Station). Research in the authors' laboratories is supported by La Ligue contre le Cancer (coordination du Grand Ouest, comités Finistère, Côtes d'Armor, Deux-Sèvres and Morbihan), the Groupama Foundation, the Fondation d'Entreprise Grand Ouest, IBI SA (Infrastructures en sciences du vivant: biologie, santé et agronomie), Biogenouest (Western France life science and environment core facility network supported by the Conseil Régional de Bretagne), the SATT Ouest Valorisation, the Région Bretagne, the Cancéropôle Grand Ouest (Marine molecules, metabolism and cancer network), and the Conseil Général du Finistère.

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Figure captions:

Figure 1: Typical metazoans used in cell studies at the SBR. **(A)** The colonies of *Sabellaria alveolata* are collected in the shallow waters of the seashore. **(B)** A female *S. alveolata* releases many white oocytes when extracted from its sandy tube and stressed by an osmotic shock. **(C)** Starfish adult specimens (**Asteria rubens* and ***Marthasterias glacialis*) are collected by divers in the Brest area. **(D)** Light micrograph of an isolated living oocyte from *Marthasterias glacialis*; The germinal vesicle (VG) contains a nucleolus. **(E)** A sea urchin adult specimen (*Sphaerechinus granularis*) wanders on a sandy substrate. **(F)** Light micrograph of an isolated early embryo of *S. granularis*. The two-cell stage embryo is surrounded by the fertilization envelope (FE). **(G)** A catshark adult specimen (*Scyliorhinus canicula*) swims over the rocky substrate. **(H)** Catshark embryo is protected by a horny capsule with long tendrils.

Figure 2: Entry into M-phase of echinoderm mature oocytes and demonstration of MPF. **(A)** Starfish oocytes are arrested in prophase I (PI) of meiosis. The 1 methyl-adenine (1-MeAde) triggers the meiotic maturation by activating the maturation-promoting factor (MPF) in the oocyte cytoplasm. MPF activity rises upstream of metaphase I (MI) and metaphase II (MII) and drops during the anaphase of both meiotic divisions. Optimal fertilization occurs naturally between MI and MII. **(B)** Sea urchin eggs are blocked at the G1 stage of the cell cycle after completion of their meiotic divisions. Fertilization triggers the activation of the MPF and the entry into the first mitotic division (M) of the embryonic development. **(C)** MPF is demonstrated by cytoplasmic transfer from maturation-inducing hormone-treated maturing oocytes into untreated immature oocytes, which in turn enter into meiotic maturation. The MPF activity does not decrease through multiple successive transfers into immature oocytes. (GV) germinal vesicle; (N) haploid pronucleus; (GVBD) germinal vesicle breakdown.

Figure 3: Activation of the CDK1/cyclin B complex at entry into M-phase and its subsequent inactivation at the exit from M-phase in sea urchin eggs and starfish oocytes. Cell events following fertilization in sea urchin eggs and oocyte meiotic maturation in starfishes are respectively shown at the top and the bottom of the diagram. The time scale between the

two systems is not taken into account. The molecular events describing the activation of the CDK1/Cyclin B heterodimer are shown in the middle of the diagram. (1) Sea urchin egg fertilization triggers the synthesis of the Cyclin B protein, which associates with CDK1. (2) In starfish meiotic egg maturation, CDK1/Cyclin B complex activation is associated with CDK1 phosphorylation status. CDK1 activation requires its phosphorylation on threonine 161 site (T161) and dephosphorylation on two inhibitory sites, threonine 14 and tyrosine 15 (T14 and Y15). Active CDK1/Cyclin B complex and active MPF activity are the same entity. (3) CDK1/Cyclin B complex is phosphorylated by the kinase Wee1 on the two inhibitory sites, and consequently, the heterodimer is maintained inactive. In starfish oocytes, the hormone addition induces an initial activation of CDK1/Cyclin B. (4) Subsequently, active CDK1/Cyclin B phosphorylates the Cdc25 and Wee1 for activation and inhibition, respectively. Hence, the Wee1-Cdc25 balance is further reversed via an auto-catalytic loop, leading to the autoregulatory activation of the CDK1/Cyclin B complex and MPF. (5) At exit from M-Phase, active CDK1/Cyclin B activates the anaphase-promoting complex (APC/cyclosome), leading to poly-ubiquitination of CDK1-associated cyclin B. (6) Subsequently, the proteasome proteolyzes Cyclin B, resulting in CDK1/Cyclin B inactivation, M-phase exit and entry into anaphase. G1, Gap1; S, S-phase (DNA synthesis); M, M-phase of the first embryonic division; Anaph., anaphase; PI, Prophase of the first meiotic division; GVBD, germinal vesicle breakdown; MI, M-phase of the first meiotic division. Anaph. I, anaphase of the first meiotic division.

Figure 4: Diagram illustrating the components involved in the translational activation induced by sea urchin egg fertilization. (1) Sperm entry triggers a Ca^{2+} wave, a sustained rise of pH, and an entry of amino acids. (2) Ca^{2+} rise activates the mTOR pathway, which triggers phosphorylation of 4E-BP (eIF4E-binding protein) and eEF2K (eukaryotic elongation factor 2 kinase). (3) mTOR phosphorylation of 4E-BP results in its degradation and the release of eIF4E, which is free to associate with eIF4G and stimulate global cap-dependent translation of maternal mRNAs. (4) eEF2K phosphorylation releases eEF2 activity. (5) GCN2 is inhibited by the increase in amino acids; consequently, eIF2 is dephosphorylated and active for the initiation step. (6) All signaling pathways converge to activate global and specific protein synthesis at post-fertilization time. (7) The analysis of mRNAs recruited into active polysomes 1-hour post-fertilization showed that 18% of the maternal mRNAs are newly

recruited. Among these, mRNAs encoding for different cell cycle players (e. g. mitotic cyclins and CDK1) and translational regulators (e. g. eIF4B) are newly translated.

Figure 5: Cellular and biochemical events accompanying cell cycle dynamics during the first mitotic division in *Paracentrotus lividus* embryos. **(A)** Confocal microscopy images illustrating the different cellular events that rhythm the first mitotic division. The top row displays embryos stained by *in situ* hybridization with *cyclin B* (*CycB*) mRNA specific probes, revealing the subcellular localization of this transcript (b/w signal). Its levels remain constant, but its association with microtubules determines its dynamic distribution during cell division. The embryos shown in the second row were stained with antibodies against α -tubulin, revealing the organization of the cell cytoskeleton (shown in green). Visible in late prophase embryos, the mitotic spindle determines cell polarity and orchestrates the segregation of chromosomes (shown in red in all the pictures). The embryos were also stained with a specific antibody recognizing a phosphorylated form of PP1c and endogenous phosphatase involved in cell cycle progression (blue signal). PP1c is a direct target of CDK1, and its phosphorylation status reflects the activity of the CDK1/Cyclin B complex. The levels of ph-PP1c increase gradually during the early phases of mitosis, reach a maximum during metaphase and decay abruptly during anaphase. The third and fourth rows correspond respectively to the α -tubulin and ph-PP1c individual channels, shown here in b/w. **(B)** Western blots of embryonic protein extracts prepared at different time points during the first embryonic cleavage. The blot in the top panel has been incubated with an antibody against Cyclin B. Active translation of the *CycB* mRNA results in increasing levels of Cyclin B protein and enhanced CDK1 activity until cells reach anaphase when Cyclin B protein is degraded. By 135 minutes post-fertilization, Cyclin B protein levels rise again, heralding the second mitotic division. A specific antibody reveals that the levels of CDK1 remain stable throughout the first cleavages (lower panel).

Figure 6: Screening for CDK1/Cyclin B inhibitors using marine resources. **(1)** Native active CDK1/Cyclin B enzymes are purified from extracts of M-phase starfish oocytes. The complex is purified by affinity chromatography on recombinant p9^{CKShs1} immobilized on a Sepharose matrix. **(2)** The purified enzyme phosphorylates histone H1 as a substrate. The activity can be detected by various methods, from radiometric to luminescent-based assays. **(3)** For the

screening of chemical libraries, the activity is assayed in the presence of the tested compound X. (4) Hits are defined as a compound that inhibits the enzyme's activity dose-dependent manner. The structure of the well-known substituted purine CDK inhibitor (R)-roscovitine is depicted.

Figure 7: Electrical responses to fertilization in decapod crustacean and ascidian eggs. (A) Fertilization potential of a crab egg (*Maia squinado*). Fertilization (sperm symbol) leads to a rapid hyperpolarization correlated to a change in membrane permeability, as shown by the decrease of membrane resistance R_M after gamete fusion ensuring block of polyspermy (from (Goudeau & Goudeau, 1989)). (B) Change in membrane permeability in crab egg upon fertilization. Prior fertilization, crab egg permeability is dominated by chloride (light blue square, as shown by external Cl^- removal). Sperm fusion triggers fertilization potential (* and light gray square) by a drastic change in membrane permeability for K^+ (dark blue square). Blue arrows indicate when the microelectrode penetrated the egg. $[\text{Cl}^-]_o=0$, seawater without chloride ; $[\text{K}^+]_o=487$, seawater where extracellular Na^+ is replaced by K^+ ; W(ASW) return to ionic nominal conditions by flushing eggs with artificial seawater ; * fertilization potential (From (Goudeau et al., 1984)). (C) Time course of the fertilization potential of a dechorionated *Phallusia mammilata* egg. Contrary to crab eggs, fertilization potential consists of an initial rapid regenerative depolarization (1) depending on Na^+ permeability, followed by a phase of membrane depolarization (2). Finally, the membrane depolarization is shown at an expanded time (a) with typical oscillations leading to the resumption of meiosis and expulsion of the 1st and 2nd polar bodies (b) ((from (Goudeau et al., 1992)).

Figure 8: Examples of metazoans that are studied at the SBR. (A) Ascidiars (*Phallusia mammillata* and *Ciona intestinalis*). (B) Sea urchin (*Paracentrotus lividus*). (C) Cuttlefish (*Sepia officinalis*). (D) Juvenile catshark (*Scyliorhinus canicula*).

Figure 1

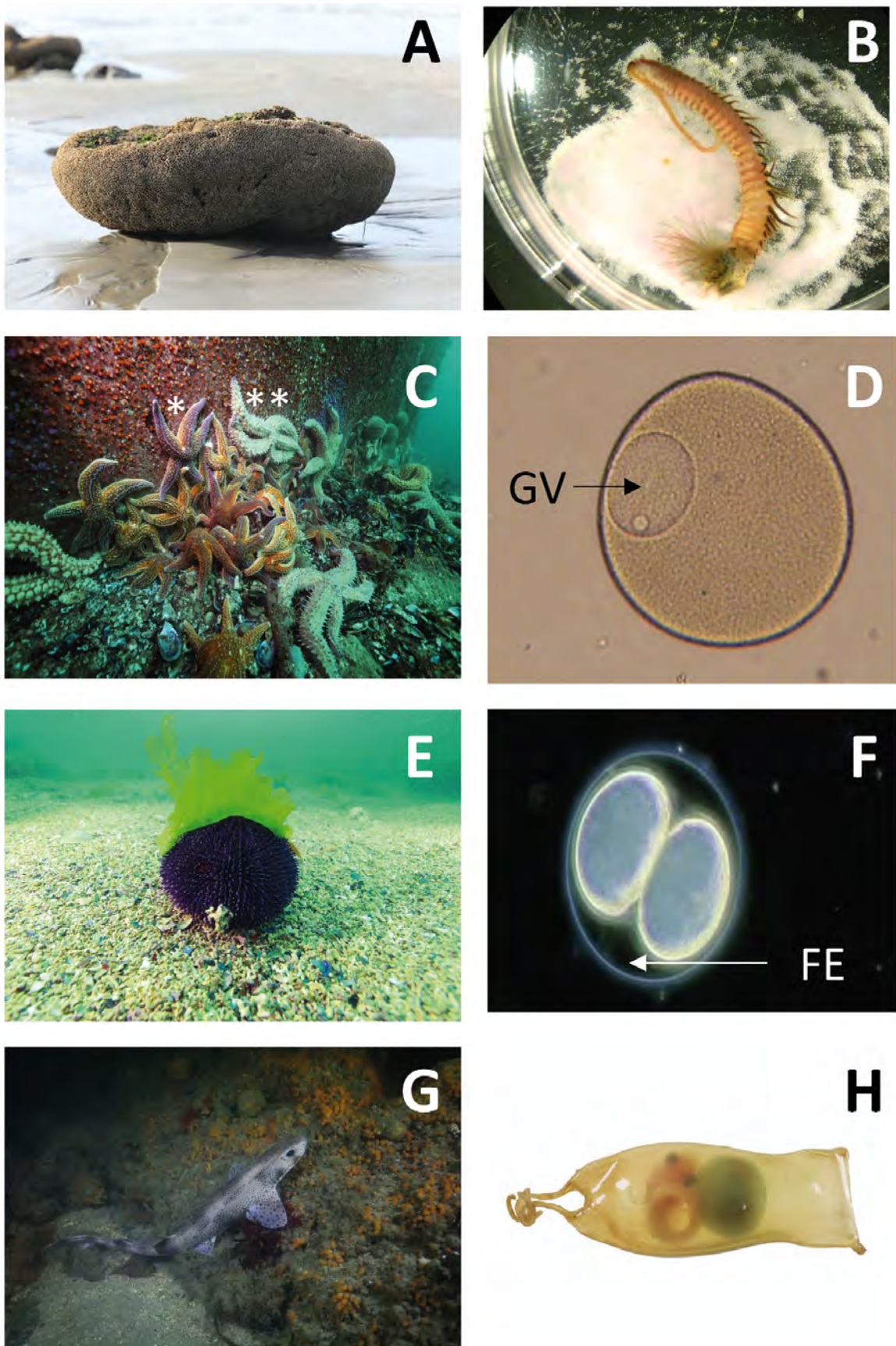


Figure 2

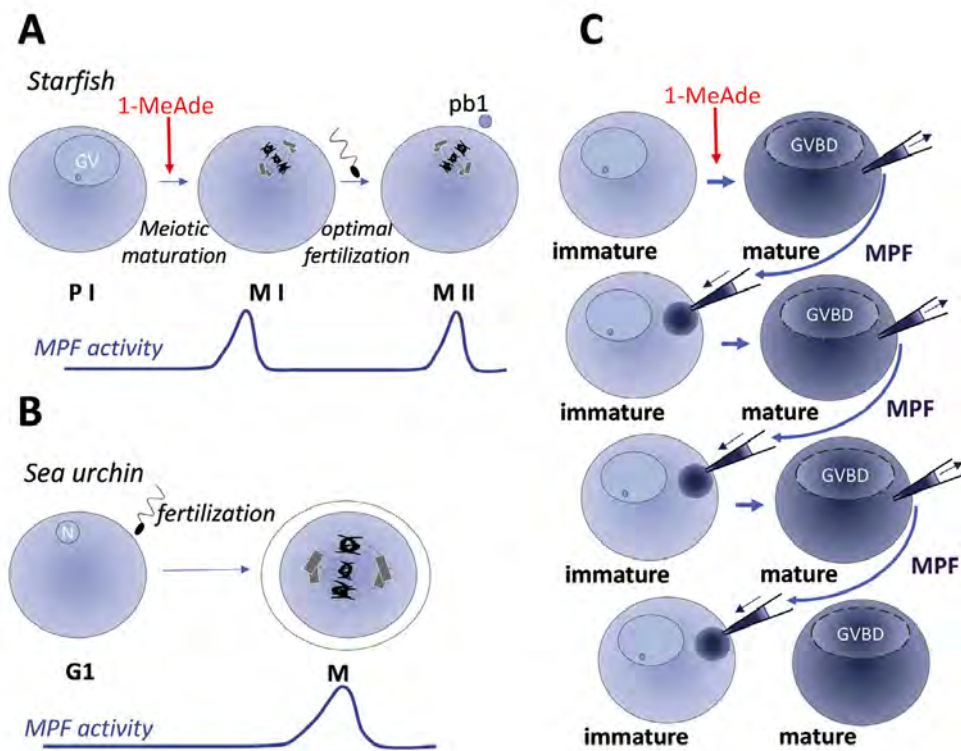


Figure 3

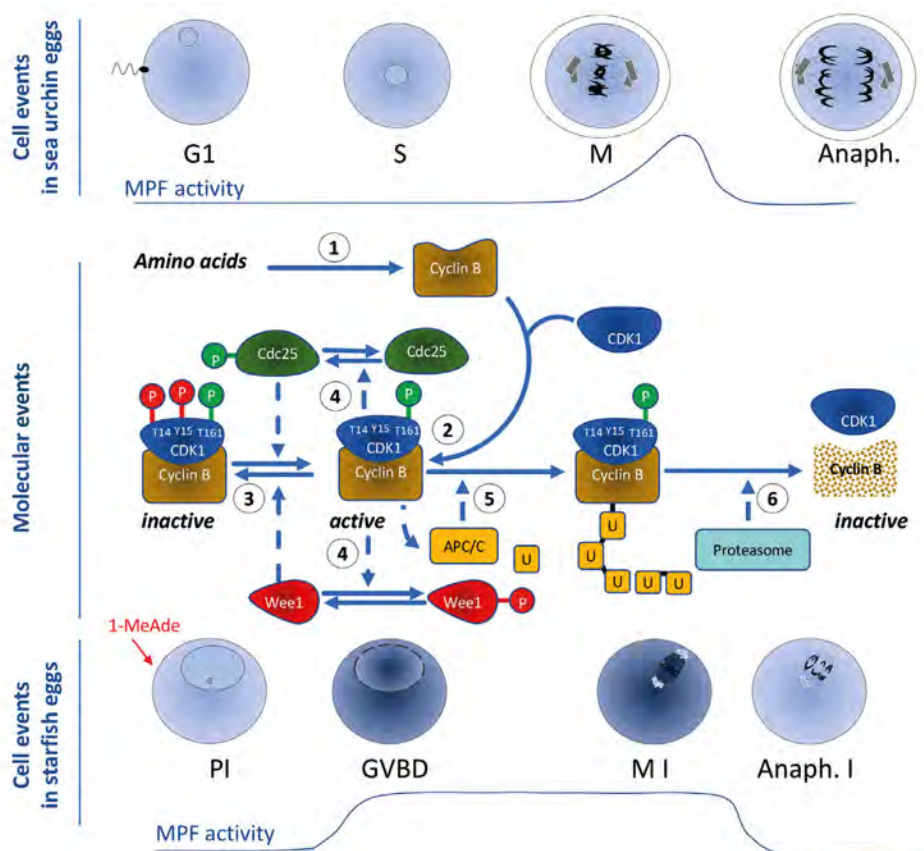


Figure 4

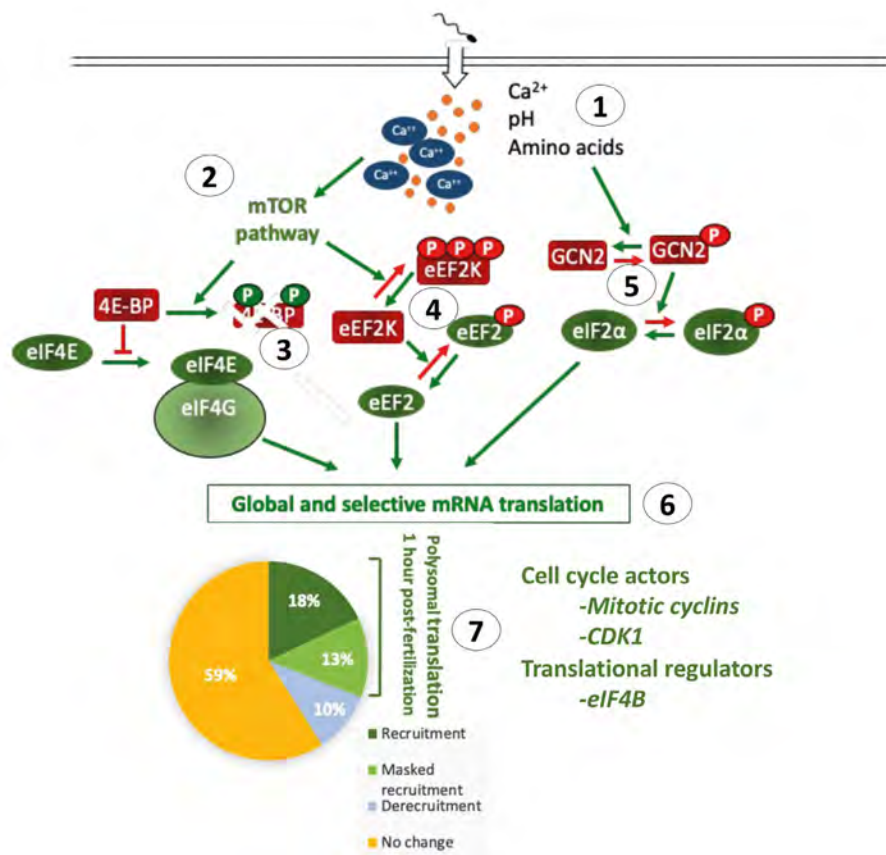


Figure 5

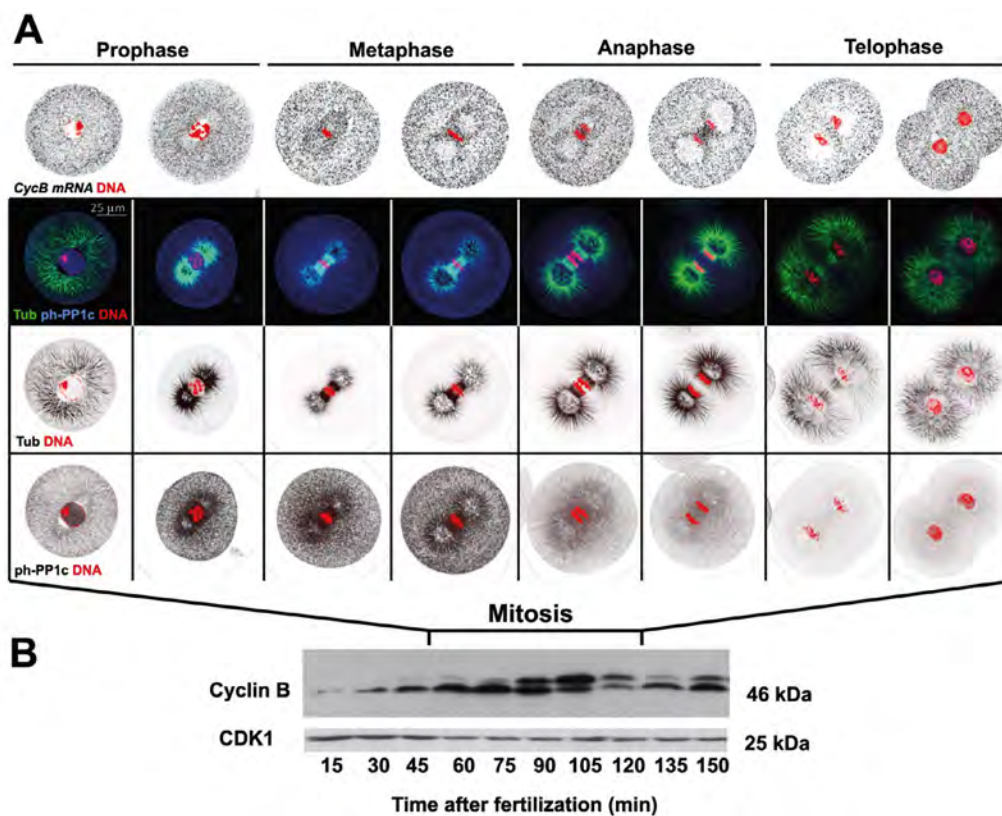


Figure 6

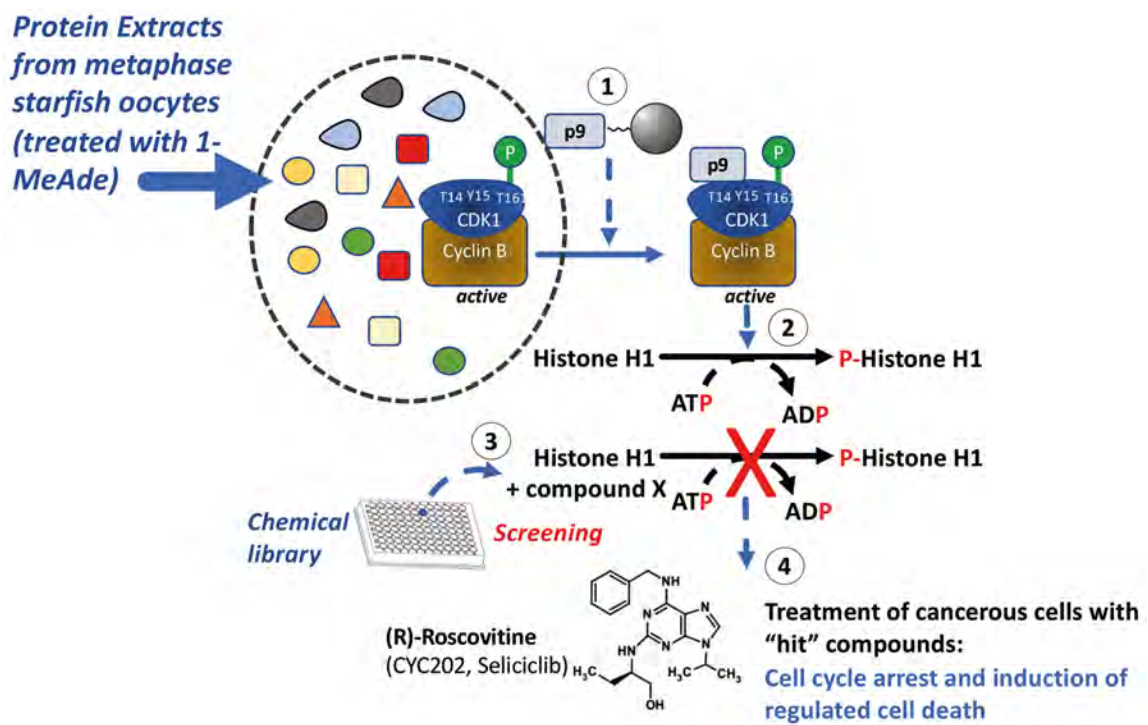


Figure 7

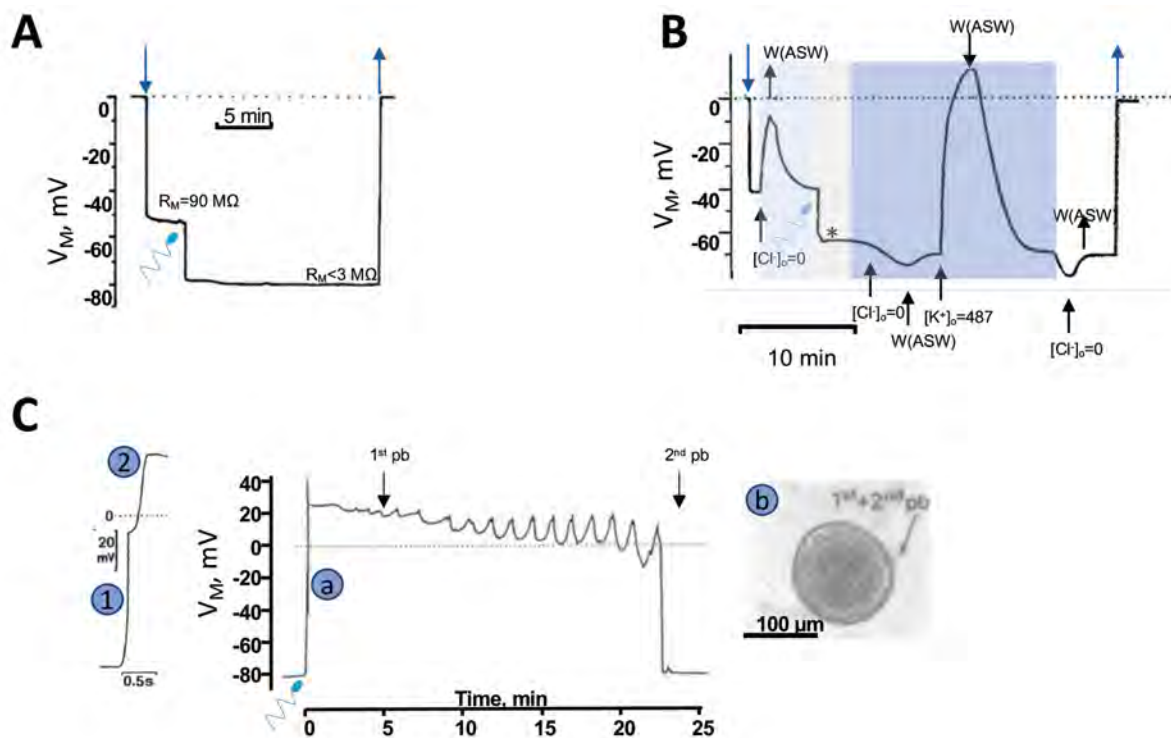


Figure 8

