



**HAL**  
open science

## Toward Multiscale Modeling of Molecular and Biochemical Events Occurring at Fertilization Time in Sea Urchins

Harold Moundoyi, Josselin Demouy, Sophie Le Panse, Julia Morales, Benoît Sarels, Patrick Cormier

► **To cite this version:**

Harold Moundoyi, Josselin Demouy, Sophie Le Panse, Julia Morales, Benoît Sarels, et al.. Toward Multiscale Modeling of Molecular and Biochemical Events Occurring at Fertilization Time in Sea Urchins. *Marine Organisms as Model Systems in Biology and Medicine*, 65, Springer International Publishing, pp.69-89, 2018, Results and Problems in Cell Differentiation, 10.1007/978-3-319-92486-1\_5 . hal-03834508

**HAL Id: hal-03834508**

**<https://hal.science/hal-03834508>**

Submitted on 30 Oct 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Towards multiscale modeling of molecular and biochemical events occurring at fertilization time in sea urchins.**

Harold Moundoyi<sup>1,2</sup>, Josselin Demouy<sup>1,3</sup>, Sophie Lèpanse<sup>4</sup>, Julia Morales<sup>1</sup>, Benoît Sarels<sup>1,2</sup>,  
Patrick Cormier<sup>1,†</sup>

<sup>1</sup> Sorbonne Universités, UPMC Univ. Paris 06, Laboratoire de Biologie Intégrative des Modèles Marins, CNRS UMR 8227, Station Biologique de Roscoff, F-29680 Roscoff

<sup>2</sup> Sorbonne Universités, UPMC Univ. Paris 06, Laboratoire Jacques-Louis Lions, CNRS UMR 7598, Univ. Paris-Diderot, F-75005 Paris

<sup>3</sup> Present address: Sorbonne Universités, UPMC Univ. Paris 06, CNRS, UMR 7232, Integrative Biology of Marine Organisms, Observatoire Océanologique, Banyuls-sur-Mer 66650, France.

<sup>4</sup> Sorbonne Universités, UPMC Univ. Paris 06, Plateforme d'Imagerie, CNRS, FR 2424, Station Biologique de Roscoff, Station Biologique de Roscoff, F-29680 Roscoff.

†Author for correspondence: (cormier@sb-roscoff.fr)

**Abstract:**

We review here previous theoretical and experimental works, which aim to model major events that occur at the time of fertilization in the sea urchin. We discuss works that perform experiments and develop hypotheses that link different scales of biological systems such as the intracellular  $\text{Ca}^{2+}$  concentration oscillations and the swimming behavior of sperm, the  $\text{Ca}^{2+}$  wave propagation and the fertilization membrane elevation of the egg, the mRNA translational activation and the completion of the first mitotic division of the early embryo. The aim of this review is on one hand, to highlight the value of systems biology for understanding the mechanisms associated with fertilization and early embryonic development in sea urchins. On the other hand, this review attempts to illustrate, for mathematicians and bioinformaticians, the potential that represent these molecular and cellular events for modeling clear physiological processes.

**Keywords:**

Sea urchin, fertilization, sperm swimming, egg activation,  $\text{Ca}^{2+}$  signaling pathway, mRNA translation, systems biology

## **Introduction**

The events that occur at fertilization and during the early embryonic development of the sea urchin represent, for biologists, a fascinating system of study to understand the molecular and biochemical mechanisms that preside at the beginning of the life of a metazoan. The succession of molecular, biochemical and cellular events that follow fertilization of the sea urchin egg gives the opportunity to approach their integration into different spatio-temporal contexts.

Sea urchin embryos have been extensively used in laboratories for more than one century and are particularly useful for studying fertilization, the crucial process by which most sexually reproducing individuals begin [1,2]. Sea urchins are gonochoric and gametes are very easy to obtain in large quantities. The sea urchin fertilization process is external and sperm swimming plays a key role for the navigation towards the egg so that the two mature and competent gametes may fuse. The size of the sea urchin egg ( $\approx 100 \mu\text{m}$  diameter) and their optical characteristics make them particularly interesting for manipulation, microinjection and observation under optical microscopy [3,4].

Therefore, it is easy to observe the elevation of the fertilization envelope first described in 1847 by Derbès as a “circular line” encompassing the egg following fertilization [5,6]. In advanced high school, community college, and university biology laboratory courses, fertilization envelope elevation serves classically as the first indication of sperm-mediated egg activation [7]. The induction by the sperm and the spread of the

fertilization envelope elevation around the egg is triggered by a calcium signaling process [8-10]. Calcium first increases at the point of sperm-egg interaction and crosses the egg as a wave to the antipode [11]. This calcium wave is responsible for sea urchin egg activation, which can be characterized in part by the fertilization envelope elevation, the increases of protein synthesis, and the entry into the cell cycle [12,13].

Unfertilized sea urchin eggs have completed their meiotic maturation and are physiologically blocked at the G1 stage of the cell cycle. Fertilization triggers entry into S-phase and completion of the first mitotic division of the embryonic division. Thanks to the large amount of eggs recovered and the synchrony of fertilization and cell cycle events, these gametes have been useful material for biochemical investigations of calcium-mobilizing second messengers, cell cycle actors and protein translation control triggered by fertilization [14-16]. The sea urchin embryo is also a major system for studies on the mechanisms of gene regulation in early development [17].

Because of the remarkable usefulness of the early embryos as a research model system for modern molecular, cell, and developmental biology, the genome of the sea urchin was sequenced [18]. This sequencing gave researchers the opportunity to learn a great deal about the components of key signaling pathways induced by fertilization [19-23]. Furthermore, the sea urchin genome sequence provides strong support for the “bilaterian regulatory toolkit” and gives new insight into the evolution of gene interactions that form the so-called Gene Regulatory Network regulating embryonic development embryogenesis [24,25].

The complex nature of the vast networks activated by fertilization that allow the beginning of the embryonic development and successive cell divisions represents an important challenge to understanding their dynamics, regulations and functions. The propagation of the calcium wave and its biological implication in terms of the activation

of the egg involve several mechanisms and sub-processes that connect intracellular molecular interactions to the scale of a cell. The quantitative modeling of such a multiscale system depends upon mathematical tools and the extensive use of computational simulations [26].

We review here previous theoretical and experimental works, which aim to model three of the main molecular, metabolic, sub-cellular and cellular events triggered by the sperm and the egg at the time of fertilization and during the first mitotic division of the sea urchin embryo. Providing a nontechnical overview of mathematical/ bioinformatic models using the sea urchin early developmental system, in different context of scales of time and space, we wish to demonstrate that the sea urchin represents an interesting system for multiscale modeling analyses (**Figure 1**).

#### **From $\text{Ca}^{2+}$ signaling pathway to sperm navigation.**

In many species eggs secrete chemoattractants from surrounding jelly layers to influence sperm swimming towards their source. The chemotactic behavior of sperm towards the egg is controlled by flagellar curvature alterations, which depend on intracellular  $\text{Ca}^{2+}$  concentration  $[\text{Ca}^{2+}]_i$  oscillations [27]. In *Strongylocentrotus purpuratus*, speract a decapeptide, which is present in and diffuses from the external layer of the egg, binds to a receptor located in the flagellar membrane of the sperm. It activates a finely regulated signaling pathway, which produces a train of intracellular  $\text{Ca}^{2+}$  concentration  $[\text{Ca}^{2+}]_i$  oscillations. The  $[\text{Ca}^{2+}]_i$  response is characterized by a sustained (tonic) increase in  $[\text{Ca}^{2+}]_i$  followed by superimposed fluctuations of that response (supratonic) (review in [28]). When speract binds the extracellular side of the receptor (SR), it activates latent guanylyl cyclase (GC) activity in the cytoplasmic side of the receptor, which produces cGMP [29]. The increase of cGMP triggers the opening of a cGMP-regulated  $\text{K}^+$  channel (KCNG) leading to a hyperpolarization of the membrane

potential ( $V$ ), which triggers the following four main processes: 1.) The  $\text{Na}^+/\text{Ca}^{2+}$  Exchanger (NCE) is activated and consequently induces a decrease in the  $[\text{Ca}^{2+}]_i$  level. 2.) The  $\text{Na}^+/\text{H}^+$  pump is activated and consequently the intracellular pH ( $\text{pH}_i$ ) increases. The  $\text{pH}_i$  elevation inhibits the GC activity and activates a soluble Adenylate Cyclase (AC), which produces cAMP. 3.) The elevation of cAMP activates a Hyperpolarization-activated and Cyclic Nucleotide gated channel (HCN), and a cAMP-dependent calcium channel, which tends to repolarize the membrane potential. 4.) Repolarization removes the inactivation of the high and low voltage-activated  $\text{Ca}^{2+}$  channels (HVA and LVA), triggering the opening of these channels and consequently depolarization and an increase of the  $[\text{Ca}^{2+}]_i$  level. The oscillation of  $[\text{Ca}^{2+}]_i$  requires a new hyperpolarization that is achieved by a  $\text{Ca}^{2+}$ -dependent  $\text{Cl}^-$  channel (CaCC) and a  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channel (CaKC) that are opened when the  $[\text{Ca}^{2+}]_i$  level is high. The processes are cyclically repeated to produce a train of  $[\text{Ca}^{2+}]_i$  oscillations. In parallel, basal  $[\text{Ca}^{2+}]_i$  levels are maintained by a constant passive  $\text{Ca}^{2+}$  extrusion through the Calcium pump (CaP) and the NCE. The cyclic alternation of hyperpolarization and repolarization generates the train of oscillations in  $[\text{Ca}^{2+}]_i$  that control the pattern of the sea urchin sperm swimming [30].

Based on the regulatory logic of the interactions rather than on their kinetic details, the group of Martinez-Mekler modeled the speract-activated  $\text{Ca}^{2+}$  signaling pathway network that controls the sperm motility [30]. They adopted a discrete dynamics formalism acting on a logic representation for the pathway. The logical signaling network consists of about twenty nodes representing the principal elements involved in the pathway (**Figures 2A and 2B**). Each node has its own regulatory function and for the construction of these regulatory functions, they used all biological knowledge of an electrophysiological nature available in the literature and their laboratory. In order to

study the dynamics of the system, they implemented a discrete formulation derived from a boolean approach. The behaviour of most of the nodes is binary, meaning inactive or active, present or absent, closed or open. A few nodes are required to be represented by three-state variables such as the  $[Ca^{2+}]_i$  (basal 0, tonic 1 and supratonic 2) and the membrane potential (hyperpolarized 0, resting 1 and depolarized 2). An example of the time evolution representation of the network is illustrated in **Figures 2C and 2D**. They validated their network model by comparing the outgoing dynamics of the system with previously available experimental data as well as with new experiments suggested by predictions. In all cases, the numerical simulations of the network model were in good agreement with experimental determinations. Interestingly, model prediction helped clarify the role of different elements in the network. In this context, the model was used to predict that the  $Ca^{2+}$  CatSper channel is the main  $Ca^{2+}$  channel involved in the regulation of sea urchin sperm motility [31].

### **From $Ca^{2+}$ wave to fertilization envelope elevation.**

The view that calcium is essential for egg activation in metazoans and more specifically in marine invertebrate deuterostome has been extensively reviewed [8-10,32]. The hypothesis of  $Ca^{2+}$  release following fertilization of sea urchin was first tested in the mid-20<sup>th</sup> century [33]. Using the luminescent calcium sensor aequorin, calcium release triggered by ionophore treatment and fertilization was demonstrated in sea urchin eggs [12,34].

In sea urchin eggs two independent types of  $Ca^{2+}$  increases have been observed following fertilization. The first one is a small initial cortical flash, which is a consequence of an action potential-mediated influx of extracellular  $Ca^{2+}$ . This is followed by a second major cytosolic wave due to the release of  $Ca^{2+}$  from the intracellular stores



beginning from the sperm-interaction site and travelling through the depths of the cytoplasm across the egg [35,36]. The initial cortical flash does not initiate the subsequent  $\text{Ca}^{2+}$  wave, which is a distinct process triggered by the sperm. Furthermore, it is noteworthy to mention that in sea urchin a single wave of  $\text{Ca}^{2+}$  crosses the eggs, whereas the sperm triggers  $\text{Ca}^{2+}$  oscillations in ascidians and mammals [8,37,38].

Accumulation of experimental data gives insights on how sea urchin eggs form a calcium wave but some points still remain to be clarified [9,32,39,8]. It is generally accepted by the scientific community that just following fertilization, the  $\text{Ca}^{2+}$  rise occurs as a result of inositol 1,4,5-triphosphate ( $\text{IP}_3$ )-mediated release of  $\text{Ca}^{2+}$  from the endoplasmic reticulum [40]. However, studies show that in sea urchin eggs, other intracellular second messengers, including Nicotinic Acid Adenine Dinucleotide Phosphate (NAADP), cyclic Guanosyl Mono Phosphate (cGMP), cyclic ADP-ribose (cADPR) and Nitric Oxide (NO) increase at fertilization and could trigger  $\text{Ca}^{2+}$  release [41]. However, in contrast to  $\text{IP}_3$ , none of these second messengers are indispensable to the fertilization wave in the sea urchin egg.

According to their velocity, one can distinguish three different main classes of intracellular  $\text{Ca}^{2+}$  waves [42]. At 20°C, the fast waves move at about 5-30  $\mu\text{m/s}$ , the slow waves move at 0.1-1  $\mu\text{m/s}$ , and the ultrafast ones accompany electrical propagation processes such as action potential in many neurons and muscles. The  $\text{Ca}^{2+}$  wave at fertilization in sea urchin crosses the egg (100  $\mu\text{m}$  in diameter) over a period of 20 s and is consequently classified as fast wave [43,42]. Cytosolic  $\text{Ca}^{2+}$  waves can be broadly classified into two types: Type 1 waves propagate as sharp bands throughout the cell, with velocities and frequencies that are high enough to permit the propagation of several fronts at the same time [44]. Type 2 waves correspond to the progressive increase of the cytosolic level of  $\text{Ca}^{2+}$  throughout the cell, followed by a return to the

basal level in a quasi-homogeneous way [45,44]. The  $\text{Ca}^{2+}$  wave at fertilization in sea urchin corresponds to a type 2 wave [43].

The propagation of the cytosolic calcium wave through a diverse range of cells prompted biologists and mathematicians to model the system. Such a modeling requires a thorough knowledge of the propagation mechanism of the wave and of the different molecular actors and organelles involved. The observation of the sea urchin egg by electron microscopy makes it possible to visualize the actual complexity of the spatial distribution of diverse organelles (**Figure 3A**). Several organelles are present on the cortex of unfertilized eggs, including cortical granules [46], acidic vesicles [47,48] and the endoplasmic reticulum [47]. The endoplasmic reticulum is the site of ATP-dependent calcium sequestration and  $\text{IP}_3$ -induced calcium release and experiments have shown that it plays a major function in the solitary calcium wave triggered by fertilization of sea urchin eggs [40]. At the molecular level, the sea urchin “calcium toolkit” has been extensively described in the genome [49,18]. At any given time, the level of intracellular  $\text{Ca}^{2+}$  is the result of a balance between the “on” reactions that induce the introduction of  $\text{Ca}^{2+}$  into the cytoplasmic compartment and the “off” reactions that remove free-intracellular  $\text{Ca}^{2+}$  via a combination of buffers, pumps and exchangers [1]. The  $\text{IP}_3$ -receptor channel ( $\text{IP}_3\text{R}$ ) and the ryanodine receptor/channel (RyR), which is activated by cGMP or cADPR, are present in the endoplasmic reticulum membrane (**Figure 3B**). The involvement of the two molecules acting together or separately is always a point of discussion and it is still not clear which specific one would begin the cytosolic  $\text{Ca}^{2+}$  wave [32]. The classic process of calcium-induced calcium release (CICR) contributes to the explosive release during the fertilization wave [50,43]. CICR is a reaction-diffusion mechanism in which  $\text{Ca}^{2+}$  release is mediated by RyR and  $\text{IP}_3\text{R}$  present in the endoplasmic reticulum. The CICR theoretical model was originally proposed for

$[Ca^{2+}]_i$  oscillations [51,52]. The RYR and the  $IP_3R$  are sensitive to and consequently open in the presence of low  $Ca^{2+}$  concentration whereas higher mM concentrations inhibit them. In a widely used model, calcium waves are propagated by a reaction-diffusion process in which calcium ions progress outside the endoplasmic reticulum network-tubes and trigger further  $Ca^{2+}$  release from the ER by binding to an  $IP_3$  bound  $IP_3$  receptor and promoting further CICR. The SERCA pump (smooth endoplasmic reticulum calcium ATPase) drives the accumulation of  $Ca^{2+}$  in the endoplasmic reticulum [53]. The SERCA is present in sea urchin eggs [49].  $Ca^{2+}$  induces conformational changes in calsequestrin [54]. However these changes do not directly trigger  $Ca^{2+}$  release from this buffer protein and in most models, free  $Ca^{2+}$  in the reticulum is assumed not to be limiting. A complementary tandem model has been proposed, in which a process called proton-induced proton release (PIPR) propagates the  $Ca^{2+}$  wave within the reticulum while CICR propagates the  $Ca^{2+}$  wave outside the endoplasmic reticulum [55]. In this model,  $H^+$ -induced conformational changes in calsequestrin would induce calcium release. Finally, the rise of calcium to more than  $0.2\mu M$  in the cytoplasm is the primary cause of the activation and the elevation of the fertilization membrane in the sea urchin egg at fertilization [56].

A large variety of mathematical models have been developed for cellular  $Ca^{2+}$  oscillations [57]. Indeed, the reaction-diffusion processes mentioned above gave rise to an extensive research domain pertaining to the theory of partial differential equations (PDE). Such equations study the evolution of quantities depending upon several variables of interest, which can be for our focus the time and a two-dimensional or three-dimensional space variable [58,59]. It is widely accepted that chemical and biochemical reactions are particularly well represented by reaction-diffusion equations and systems as long as there is a great number of agents involved (mean-field limit). A

realistic theoretical model for repetitive  $\text{Ca}^{2+}$  wave generation and propagation has been developed and gives insights into the origin of the pacemaker in mature ascidian eggs [38]. Other models have focused on the rather typical single wave that is triggered at fertilization in *Xenopus* and sea urchin eggs [60,61]. Interestingly, in sea urchins, it has been developed a multiscale biophysico-chemical model, which simulates the events from the  $\text{Ca}^{2+}$  wave propagation induced by fertilization to the elevation of the fertilization membrane that represents a physical barrier against polyspermy [60]. Recently, taking the calcium wave triggered by fertilization of the sea urchin egg, exclusive mathematical approaches have been developed in order to know if travelling wave solutions of the reaction-diffusion equation can generate a travelling wave for the diffusion equation [62]. The next step is to describe, with mathematical tools, how such a  $\text{Ca}^{2+}$  wave generates the observed diffusion of the released contents of cortical granules between the cell membrane and the vitelline membrane.

### **From translation regulation to the first mitotic division following fertilization.**

The fertilization-induced transitory increase in cytoplasmic free intracellular  $\text{Ca}^{2+}$  concentration and the sustained rise in intracellular pH triggers the activation of the sea urchin eggs [63]. Under the term “egg activation”, are brought together many characteristic changes, both morphological and biochemical, that happen in a period of time comprised between the penetration of the sperm into the egg and the onset of the mitotic cell cycle of the embryo [39,10,32]. Sea urchin eggs are haploid cells blocked in G1 and fertilization triggers entry into S-phase and completion of the first mitotic division of the embryonic development. *De novo* protein synthesis is dispensable for the S-phase but is required for the onset of M-phase and subsequent embryonic cell cycles [64,65]. The mitotic cyclins A and B were first discovered in sea urchin as key proteins,

which are synthesized and degraded during the mitosis phase at each cell division [14]. Cyclins and their catalytic kinase partners CDKs (Cyclin-dependent Kinases) control cell cycle progression [66].

The protein synthesis rate of unfertilized sea urchin eggs remains remarkably low and fertilization is the starting signal, which triggers their awakening, and induces a dramatic increase of mRNA translation (review in [67]). This increase of mRNA translation is independent of mRNA transcription and ribosome biogenesis [68,69]. Therefore, sea urchin egg activation provides an example of regulated gene expression at the translational level using maternal mRNAs that does not follow the traditional scheme of gene regulation governed at the transcription level [70].

Sea urchin eggs are relevant models that have helped to address the control of gene expression at the translational level in relation to cell cycle regulation in physiological conditions [67,71]. While the large and rapid increase in the rate of protein synthesis following fertilization of the sea urchin egg has long been a paradigm of translational control, the mechanisms by which mRNA translation is increased following fertilization and how those controls contribute to proper development have only started to be understood at the beginning of 2000. The multiple ways in which the overall rates of mRNAs translation can be controlled in response to the fertilization of sea urchin eggs have been reviewed recently [67]. The availability of the sea urchin genome has allowed the identification of the “translational toolkit” in this model system [18,22]. Thanks to accumulated knowledge on cap-dependent initiation of translation, we have obtained an integrative model for translation initiation using a programming environment for system biology coupled with model validation (named BIOCHAM (the BIOChemical Abstract Machine)) [72]. This model is described by abstract rules and contains 51 reactions involved in 74 molecular complexes. The model proved to be coherent with

the biological knowledge as judged from specification tests and simulations performed with computational tree logic (CTL) as well Boolean simulations. The numerical simulation from BIOCHAM showed that cap-dependent translation was almost undetectable in unfertilized egg and strongly increased following fertilization, after a delay corresponding to the time to initiate the translational machinery, in agreement with biological observations. Furthermore, we showed that the combination of our model of translation regulation with the BIOCHAM version [73] of the cell cycle regulation [74] can be used for analysis of the cap-dependent translational machinery in relationships with the cell-cycle control. However, while this model constitutes a basis to which new information can be progressively supplemented, this type of model is not actually usable for driving hypotheses for further experimentations. Furthermore, the BIOCHAM model contains too many reactions and protein interactions, for which kinetics parameters remain undetermined, to allow quantitative simulations of dynamic changes occurring following sea urchin egg fertilization. It is therefore important to investigate the strategy of extracting a minimal core model containing a small number of actors playing a crucial role in the process.

Three translation factors have been experimentally shown to be activated *in vivo* in response to egg fertilization leading to an increase of protein synthesis (**Figure 4A**): one elongation factor, eEF2 (eukaryotic Elongation Factor 2) and two initiation factors, eIF2 (eukaryotic Initiation Factor 2) and the cap-binding protein eIF4E (eukaryotic Initiation Factor 4E).

The protein eEF2 is considered to be the major regulatory target for controlling elongation rate [75]. The activity of this elongation factor is inhibited by phosphorylation. The phosphorylation/activation status of eEF2 depends on the activity of an unusual calcium-calmodulin kinase, which targets specifically eEF2 and is

consequently called eEF2 kinase [76]. Multiple phosphorylation sites positively or negatively regulate eEF2 kinase activity. PKA (Protein Kinase A) and AMPK (AMP-activated-Protein Kinase) activate while mTOR (mechanistic Target Of Rapamycin) and ERK (Extracellular-signal-Regulated Kinase) inhibit the kinase [75,76]. The kinase that phosphorylates eEF2 is therefore considered as an integrating target of several signaling pathways that leads to protein synthesis regulation through the phosphorylation of its unique substrate eEF2. We reported the existence of a new isoform of eEF2 protein whose phosphorylation is required for the completion of the first cell cycle of early development in sea urchin eggs [77]. Interestingly, the eEF2 isoform is re-phosphorylated with a cell-cycle dependent behaviour. We proposed that eEF2 is phosphorylated in a spatio-temporal context at the M-phase stage and that a localized pool of eEF2 would be involved in the elongation pauses in sea urchin [78,77]. However, no kinetic parameter is yet available to integrate this molecular actor in a minimal core model of the translation activity occurring at fertilization in sea urchin.

At the initiation level, we showed that eIF2 plays a key role in the increase of mRNA translation triggered by fertilization in sea urchin eggs [79]. The translation factor eIF2 is a heterotrimeric complex composed of three subunits ( $\alpha$ ,  $\beta$  and  $\gamma$ ). When phosphorylated, the  $\alpha$  subunit of eIF2 sequesters eIF2B and consequently inhibits the translational initiation and global protein synthesis, while inducing the translation of specific mRNAs [80]. We demonstrated that eIF2 $\alpha$  phosphorylation contributes to the regulation of protein synthesis required for the first mitotic division in sea urchin embryos [79]. GCN2, the most ancestral eIF2 $\alpha$  kinase, is an attractive candidate for the phosphorylation regulation of eIF2 $\alpha$  in sea urchin eggs. Active GCN2 in unfertilized eggs would phosphorylate and consequently inhibit eIF2 $\alpha$ . Fertilization would inhibit GCN2 allowing eIF2 $\alpha$  to contribute to the protein synthesis increase [67,79]. The regulation of

translation initiation by eIF2 and its phosphorylation have been integrated in our model of translation regulation with the BIOCHAM version [72], but again the lack of kinetic data in the sea urchin does not allow us to integrate eIF2 $\alpha$  in a model for quantitative simulations.

Finally, the earlier event resulting in the dramatic increase in the rate of global protein synthesis triggered by fertilization in sea urchin eggs was reported to be due to the release of eIF4E from its repressor 4E-BP (eIF4E-Binding Protein) [81,15]. In all eukaryotic cells, eIF4E recognizes and binds to the 5' cap structure (m<sup>7</sup>GpppN, where N is any nucleotide). When available, eIF4E associates with eIF4G (eukaryotic Initiation Factor 4G), a large scaffolding protein that interacts with eIF4A (eukaryotic Initiation Factor 4A), an RNA-dependant ATPase and RNA helicase [82]. We showed that, in the sea urchin *Spharechinus granularis*, fertilization allows rapid eIF4E association with eIF4G and we provided evidence that eIF4E/eIF4G complex formation is functionally important for the completion of the first mitotic division following fertilization, implicating cap-dependent translation in this process [83]. A still intriguing discovery is that fertilization triggers dramatic post-translational eIF4G modifications, which seem to be independent of the mTOR kinase but dependent on the Ca<sup>2+</sup> signaling pathway [83,84].

4E-BP competes with eIF4G and consequently 4E-BP inhibits cap-dependent translation. A single 4E-BP protein ortholog exists in sea urchin [22,18], while the vertebrate genome duplications gave rise to three classes of 4E-BPs, 4E-BP1, 4E-BP2 and 4E-BP3 in mammals [85]. 4E-BPs bind to eIF4E through a bipartite interface that consists of the eIF4E-binding domain YXXXXL $\Phi$  and a “non-canonical” eIF4E-binding motif [86]. Using Small Angle X-Ray Scattering (SAXS), we showed that while sea urchin 4E-BP is intrinsically disordered in the free state, it undergoes a dramatic compaction of 4E-BP



when it interacts with eIF4E [87], in agreement with the dynamic model of human 4E-BP2 associations with eIF4E [88]. The binding of 4E-BPs to eIF4E is controlled by the phosphorylation state [89,90]. Hypophosphorylated 4E-BPs bind to eIF4E, whereas hyperphosphorylated forms do not. The mTOR kinase phosphorylates 4E-BP and leads to its dissociation from eIF4E [91,92]. While phosphorylation may result in electrostatic repulsion with the negative surface of eIF4E [93], using phospho-mimetics mutants of sea urchin 4E-BP unable to weaken 4E-BP / eIF4E binding, we hypothesized that additional post-translational modifications are required [94]. Finally, it has been demonstrated that multisite phosphorylation induces folding on the intrinsically disordered human 4E-BP2, resulting in a dramatic decrease of 4E-BP2 affinity for eIF4E [88].

By monitoring 4E-BP following egg fertilization and during embryonic development of sea urchin we were the first to describe down- and up-regulation of the 4E-BP protein level in physiological conditions [95,81]. We demonstrated that the 4E-BP degradation triggered by the fertilization of sea urchin eggs is under the control of  $Ca^{2+}$  and pH-dependent events [84]. The control of 4E-BPs stability is now recognized as a supplementary means to regulate eIF4E availability and consequently to control cap-dependent translation activity [81,96-98]. However, it is unlikely that the mechanism of 4E-BP degradation observed in sea urchin corresponds to the one observed in mammalian cells (reviewed in [67]). The 4E-BP degradation induced by fertilization of sea urchin eggs is inhibited either by rapamycin treatment or PP242, a novel and specific ATP-competitive inhibitor of mTOR kinase [99,81]. Taken together, these data indicate a major role of mTOR-mediated regulation of translation initiation at the early embryonic development.

The concentrations of 4E-BP and eIF4E in unfertilized eggs were determined by immunoblotting followed by densitometric quantification and the kinetic parameters for 4E-BP / eIF4E interaction were determined by surface plasmon resonance [100]. These different data allowed us to build a highly simple reaction model for the regulation of protein synthesis following fertilization in sea urchin [100]. The model simulates the physiological changes in the total 4E-BP amount observed during time after fertilization and we demonstrated that a reduced model involving 4E-BP, eIF4E and eIF4G is satisfactory to fully simulate the cap-dependent translational changes occurring at fertilization (**Figure 4B**). Furthermore, the model postulates that two changes occurring at fertilization are required to fit with experimental data: an increase of 4E-BP / eIF4E complex dissociation and an important activation of the 4E-BP degradation mechanism. While, as expected, the model predicts that 4E-BP / eIF4E complex destabilization is controlled by the kinase mTOR, surprisingly the mechanism of 4E-BP degradation itself seems also strongly affected by the kinase. Therefore, it would be of great interest to characterize the 4E-BP degradation mechanism occurring at fertilization and to search whether similar mechanisms exist in other organisms and other physiological regulations.

For a great number of protein neo-synthesized from the different available maternal mRNAs, the rise in proteins synthesis occurs at a constant rate following fertilization. Cyclin B protein translation follows a kinetic curve that is different from the global protein synthesis and consequently we developed a mathematical model to analyse the dissimilarity in the biosynthesis kinetic patterns [101]. We hypothesized that two forms of *cyclin B* mRNA, distinct by their immediately- or not-immediately-availability to be translated, are present in sea urchin eggs. The model predicts that translation of the not-immediately-available form of mRNA is largely dominant compared to the immediately-

available form of cyclin B mRNA. This model should help provide insights into the signaling utilized for the control of the biosynthesis and accumulation of cyclin B protein triggered by fertilization and required for the completion of the first mitotic division of the sea urchin embryo [99,102].

### **Conclusions and perspectives**

The models that we have presented in this review simulate the basic features of three independent events that accompany fertilization in the sea urchin system. The different mathematical approaches allow the unveiling of processes, which are hard to detect only by experimental procedures. The most important challenges are now to develop the multi-scale implications of these modelings.

The discrete dynamics model for the speract-activated signaling network in sea urchin sperm allowed the prediction of novel characteristics of the signaling pathway [31]. During chemotaxis, sperm swim in a chemoattractant gradient by accommodating the flagellar waveform and consequently the swimming path. The rate of change in  $[Ca^{2+}]_i$  regulates chemotaxis [103]. Therefore, the model proposed by Martinez et al. represents a solid basis of knowledge and a possible simulation to understanding how the temporal synchronization of the signaling pathway controls the fine-tuning required for influencing the movement of the sperm in response to the spatio-temporal gradient of chemoattractant secreted by the egg.

Fertilization offers the opportunity to study  $Ca^{2+}$  waves and their mechanisms of propagation in the context of a clear physiological function. Mathematical models have been developed for cellular  $Ca^{2+}$  waves propagation triggered by fertilization. Generally speaking, wave phenomena in the cell are ubiquitous, and their mathematical modeling is a growing field of research. This type of mathematical construction could be useful to

better understand the dynamics of the fertilization membrane elevation induced by the  $\text{Ca}^{2+}$  wave. Protein kinases C (PKCs), multifunctional calmodulin-dependent protein kinases (CaMKs) and the phosphatase calcineurin are present in the sea urchin egg [49]. Now, a crosstalk between simulation and experimentation should help us to predict which among these  $\text{Ca}^{2+}$  transducers represent potential candidates to link the  $\text{Ca}^{2+}$  wave to subsequent translational regulations and more general cellular events that occur at fertilization time in sea urchin.

The mTOR-signaling pathway plays a crucial role in the fine-tuning regulation of *cyclin B* mRNA translation following fertilization in sea urchin. Translatome analysis, by achieving polysome profiling in combination with high-throughput sequencing technologies [104], should allow us to identify the mRNAs that are specifically translated under the mTOR pathway triggered by fertilization and consequently should help in establishing translational regulatory networks that regulate the early events of the sea urchin embryo. As is the case for modeling and analysis of gene regulatory networks [105], computational and mathematical methods should allow us accurate predictions of the behaviour of the translational regulatory network following fertilization in sea urchin.

Finally, fine-scale spatial and temporal control of translation regulation could play a major role in relation to the cell cycle in general and with regard to spindle formation in particular [106-108,102]. Taking advantages of the quasi-spherical shape and constant volume of the cell and the integration of knowledge about the translational machinery at different scales (molecular, structural and intracellular compartments), the sea urchin egg system opens up a promising field of research for systems biology that focuses on translational regulation in relationships with cell cycle control.

## Acknowledgements

We apologize to those whose work was not cited or discussed here because of the broad scope of this review and space limitation. We would like to thank Remi Dumollard (Observatoire Océanologique, 06230 Villefranche-sur-Mer, France.) for calcium wave discussions and for proofreading the manuscript; Benoît Perthame (Sorbonne Université, Laboratoire Jacques-Louis Lions, Paris France) for mathematical and modelling approaches discussion. We thank Dr. Gustavo Martinez-Mekler for his permission to use figures from reference [30]. Special thanks to Huixin Lu (University of Toronto, Canada) for proofreading and correcting the manuscript. Research in the authors' laboratories is supported by "La Ligue contre le Cancer (coordination du Grand Ouest (comités Finistère, Côtes d'Armor, Deux-Sèvres, Morbihan)", the "Région Bretagne", and the "Conseil Général du Finistère". Brittany Regional Council Research Grant (Région Bretagne, project MoDyst) and the French Ministry of Research through the LABEX "CALSIMLAB" (ANR-11-LABX-0037) for Ph.D. Fellowship to H.M. and the ANR blanche project "KIBORD" (ANR-13-BS01-0004).

## References

1. Briggs E, Wessel GM. In the beginning...animal fertilization and sea urchin development. *Dev Biol.* 2006; 300:15-26. doi:10.1016/j.ydbio.2006.07.014
2. Santella L, Vasilev F, Chun JT. Fertilization in echinoderms. *Biochem Biophys Res Commun.* 2012; 425:588-594. doi:10.1016/j.bbrc.2012.07.159
3. Stepicheva NA, Song JL. High throughput microinjections of sea urchin zygotes. *J Vis Exp.* 2014:e50841. doi:10.3791/50841
4. Angione SL, Oulhen N, Brayboy LM, Tripathi A, Wessel GM. Simple perfusion apparatus for manipulation, tracking, and study of oocytes and embryos. *Fertility and sterility.* 2015; 103:281-290 e285. doi:10.1016/j.fertnstert.2014.09.039
5. Wong JL, Wessel GM. Renovation of the egg extracellular matrix at fertilization. *Int J Dev Biol.* 2008; 52:545-550. doi:10.1387/ijdb.072557jw

6. Derbès A. Observations sur le mécanisme et les phénomènes qui accompagnent la formation de l'embryon chez l'oursin comestible. *Ann Sci Nat Zool.* 1847; 8:19
7. Vacquier VD. Laboratory on sea urchin fertilization. *Mol Reprod Dev.* 2011; 78:553-564. doi:10.1002/mrd.21360
8. Whitaker M. Calcium at fertilization and in early development. *Physiol Rev.* 2006; 86:25-88. doi:10.1152/physrev.00023.2005
9. Ramos I, Wessel GM. Calcium pathway machinery at fertilization in echinoderms. *Cell Calcium.* 2013; 53:16-23. doi:10.1016/j.ceca.2012.11.011
10. Costache V, McDougall A, Dumollard R. Cell cycle arrest and activation of development in marine invertebrate deuterostomes. *Biochem Bioph Res Co.* 2014; 450:1175-1181. doi:10.1016/J.Bbrc.2014.03.155
11. Hafner M, Petzelt C, Nobiling R, Pawley JB, Kramp D, Schatten G. Wave of free calcium at fertilization in the sea urchin egg visualized with fura-2. *Cell Motil Cytoskeleton.* 1988; 9:271-277. doi:10.1002/cm.970090309
12. Steinhardt RA, Epel D. Activation of sea-urchin eggs by a calcium ionophore. *Proc Natl Acad Sci U S A.* 1974; 71:1915-1919
13. Poenie M, Alderton J, Tsien RY, Steinhardt RA. Changes of free calcium levels with stages of the cell division cycle. *Nature.* 1985; 315:147-149
14. Evans T, Rosenthal ET, Youngblom J, Distel D, Hunt T. Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell.* 1983; 33:389-396
15. Cormier P, Pyronnet S, Morales J, Mulner-Lorillon O, Sonenberg N, Belle R. eIF4E association with 4E-BP decreases rapidly following fertilization in sea urchin. *Dev Biol.* 2001; 232:275-283. doi:10.1006/dbio.2001.0206
16. Morgan AJ, Galione A. Preparation and use of sea urchin egg homogenates. *Methods in molecular biology.* 2014; 1128:161-173. doi:10.1007/978-1-62703-974-1\_10
17. Davidson EH, Cameron RA, Ransick A. Specification of cell fate in the sea urchin embryo: summary and some proposed mechanisms. *Development.* 1998; 125:3269-3290
18. Sea Urchin Genome Sequencing C, Sodergren E, Weinstock GM, Davidson EH, Cameron RA, Gibbs RA, Angerer RC, Angerer LM, Arnone MI, Burgess DR, Burke RD, Coffman JA, Dean M, Elphick MR, Ettensohn CA, Foltz KR, Hamdoun A, Hynes RO, Klein WH, Marzluff W, McClay DR, Morris RL, Mushegian A, Rast JP, Smith LC, Thorndyke MC, Vacquier VD, Wessel GM, Wray G, Zhang L, Elsik CG, Ermolaeva O, Hlavina W, Hofmann G, Kitts P, Landrum MJ, Mackey AJ, Maglott D, Panopoulou G, Poustka AJ, Pruitt K, Sapojnikov V, Song X, Souvorov A, Solovyev V, Wei Z, Whittaker CA, Worley K, Durbin KJ, Shen Y, Fedrigo O, Garfield D, Haygood R, Primus A, Satija R, Severson T, Gonzalez-Garay ML, Jackson AR, Milosavljevic A, Tong M, Killian CE, Livingston BT, Wilt FH, Adams N, Belle R, Carbonneau S, Cheung R, Cormier P, Cosson B, Croce J, Fernandez-Guerra A, Genevriere AM, Goel M, Kelkar H, Morales J, Mulner-Lorillon O, Robertson AJ, Goldstone JV, Cole B, Epel D, Gold B, Hahn ME, Howard-Ashby M, Scally M, Stegeman JJ, Allgood EL, Cool J, Judkins KM, McCafferty SS, Musante AM, Obar RA, Rawson AP, Rossetti BJ, Gibbons IR, Hoffman MP, Leone A, Istrail S, Materna SC, Samanta MP, Stolc V, Tongprasit W, Tu Q, Bergeron KF, Brandhorst BP, Whittle J, Berney K, Bottjer DJ, Calestani C, Peterson K, Chow E, Yuan QA, Elhaik E, Graur D, Reese JT, Bosdet I, Heesun S, Marra MA, Schein J, Anderson MK, Brockton V, Buckley KM, Cohen AH, Fugmann SD, Hibino T, Loza-Coll M, Majeske AJ, Messier C, Nair SV, Pancer Z, Terwilliger DP, Agca C, Arboleda E, Chen N, Churcher AM, Hallbook F, Humphrey GW, Idris MM, Kiyama T, Liang S, Mellott D, Mu X, Murray G, Olinski RP, Raible F, Rowe M, Taylor JS, Tessmar-Raible K, Wang D, Wilson

KH, Yaguchi S, Gaasterland T, Galindo BE, Gunaratne HJ, Juliano C, Kinukawa M, Moy GW, Neill AT, Nomura M, Raisch M, Reade A, Roux MM, Song JL, Su YH, Townley IK, Voronina E, Wong JL, Amore G, Branno M, Brown ER, Cavalieri V, Duboc V, Duloquin L, Flytzanis C, Gache C, Lapraz F, Lepage T, Locascio A, Martinez P, Matassi G, Matranga V, Range R, Rizzo F, Rottinger E, Beane W, Bradham C, Byrum C, Glenn T, Hussain S, Manning G, Miranda E, Thomason R, Walton K, Wikramanayake A, Wu SY, Xu R, Brown CT, Chen L, Gray RF, Lee PY, Nam J, Oliveri P, Smith J, Muzny D, Bell S, Chacko J, Cree A, Curry S, Davis C, Dinh H, Dugan-Rocha S, Fowler J, Gill R, Hamilton C, Hernandez J, Hines S, Hume J, Jackson L, Jolivet A, Kovar C, Lee S, Lewis L, Miner G, Morgan M, Nazareth LV, Okwuonu G, Parker D, Pu LL, Thorn R, Wright R. The genome of the sea urchin *Strongylocentrotus purpuratus*. *Science*. 2006; 314:941-952. doi:10.1126/science.1133609

19. Bradham CA, Foltz KR, Beane WS, Arnone MI, Rizzo F, Coffman JA, Mushegian A, Goel M, Morales J, Genevriere AM, Lapraz F, Robertson AJ, Kelkar H, Loza-Coll M, Townley IK, Raisch M, Roux MM, Lepage T, Gache C, McClay DR, Manning G. The sea urchin kinome: a first look. *Dev Biol*. 2006; 300:180-193. doi:10.1016/j.ydbio.2006.08.074

20. Byrum CA, Walton KD, Robertson AJ, Carbonneau S, Thomason RT, Coffman JA, McClay DR. Protein tyrosine and serine-threonine phosphatases in the sea urchin, *Strongylocentrotus purpuratus*: identification and potential functions. *Dev Biol*. 2006; 300:194-218. doi:10.1016/j.ydbio.2006.08.050

21. Fernandez-Guerra A, Aze A, Morales J, Mulner-Lorillon O, Cosson B, Cormier P, Bradham C, Adams N, Robertson AJ, Marzluff WF, Coffman JA, Genevriere AM. The genomic repertoire for cell cycle control and DNA metabolism in *S. purpuratus*. *Dev Biol*. 2006; 300:238-251. doi:10.1016/j.ydbio.2006.09.012

22. Morales J, Mulner-Lorillon O, Cosson B, Morin E, Belle R, Bradham CA, Beane WS, Cormier P. Translational control genes in the sea urchin genome. *Dev Biol*. 2006; 300:293-307. doi:10.1016/j.ydbio.2006.07.036

23. Song JL, Wong JL, Wessel GM. Oogenesis: single cell development and differentiation. *Dev Biol*. 2006; 300:385-405. doi:10.1016/j.ydbio.2006.07.041

24. Arnone MI, Andrikou C, Annunziata R. Echinoderm systems for gene regulatory studies in evolution and development. *Curr Opin Genet Dev*. 2016; 39:129-137. doi:10.1016/j.gde.2016.05.027

25. Howard-Ashby M, Materna SC, Brown CT, Tu Q, Oliveri P, Cameron RA, Davidson EH. High regulatory gene use in sea urchin embryogenesis: Implications for bilaterian development and evolution. *Dev Biol*. 2006; 300:27-34. doi:10.1016/j.ydbio.2006.10.016

26. Meier-Schellersheim M, Fraser ID, Klauschen F. Multiscale modeling for biologists. *Wiley Interdiscip Rev Syst Biol Med*. 2009; 1:4-14. doi:10.1002/wsbm.33

27. Bohmer M, Van Q, Weyand I, Hagen V, Beyermann M, Matsumoto M, Hoshi M, Hildebrand E, Kaupp UB. Ca<sup>2+</sup> spikes in the flagellum control chemotactic behavior of sperm. *EMBO J*. 2005; 24:2741-2752. doi:10.1038/sj.emboj.7600744

28. Darszon A, Guerrero A, Galindo BE, Nishigaki T, Wood CD. Sperm-activating peptides in the regulation of ion fluxes, signal transduction and motility. *Int J Dev Biol*. 2008; 52:595-606. doi:10.1387/ijdb.072550ad

29. Galindo BE, Beltran C, Cragoe EJ, Jr., Darszon A. Participation of a K(+) channel modulated directly by cGMP in the speract-induced signaling cascade of *strongylocentrotus purpuratus* sea urchin sperm. *Dev Biol*. 2000; 221:285-294. doi:10.1006/dbio.2000.9678

30. Espinal J, Aldana M, Guerrero A, Wood C, Darszon A, Martinez-Mekler G. Discrete dynamics model for the speract-activated Ca<sup>2+</sup> signaling network relevant to sperm motility. *PLoS One*. 2011; 6:e22619. doi:10.1371/journal.pone.0022619
31. Espinal-Enriquez J, Priego-Espinosa DA, Darszon A, Beltran C, Martinez-Mekler G. Network model predicts that CatSper is the main Ca<sup>2+</sup> channel in the regulation of sea urchin sperm motility. *Sci Rep*. 2017; 7:4236. doi:10.1038/s41598-017-03857-9
32. Swann K, Lai FA. Egg Activation at Fertilization by a Soluble Sperm Protein. *Physiol Rev*. 2016; 96:127-149. doi:10.1152/physrev.00012.2015
33. Mazia D. The release of calcium in Arbacia eggs on fertilization. *J Comp Physiol*. 1937; 10:14
34. Steinhardt R, Zucker R, Schatten G. Intracellular calcium release at fertilization in the sea urchin egg. *Dev Biol*. 1977; 58:185-196
35. Parrington J, Davis LC, Galione A, Wessel G. Flipping the switch: how a sperm activates the egg at fertilization. *Dev Dyn*. 2007; 236:2027-2038. doi:10.1002/dvdy.21255
36. Whitaker MJ, Steinhardt RA. Ionic regulation of egg activation. *Q Rev Biophys*. 1982; 15:593-666
37. Sardet C, Roegiers F, Dumollard R, Rouviere C, McDougall A. Calcium waves and oscillations in eggs. *Biophys Chem*. 1998; 72:131-140
38. Dupont G, Dumollard R. Simulation of calcium waves in ascidian eggs: insights into the origin of the pacemaker sites and the possible nature of the sperm factor. *J Cell Sci*. 2004; 117:4313-4323. doi:10.1242/jcs.01278
39. Runft LL, Jaffe LA, Mehlmann LM. Egg activation at fertilization: where it all begins. *Dev Biol*. 2002; 245:237-254. doi:10.1006/dbio.2002.0600
40. Terasaki M, Sardet C. Demonstration of calcium uptake and release by sea urchin egg cortical endoplasmic reticulum. *J Cell Biol*. 1991; 115:1031-1037
41. Kuroda R, Kontani K, Kanda Y, Katada T, Nakano T, Satoh Y, Suzuki N, Kuroda H. Increase of cGMP, cADP-ribose and inositol 1,4,5-trisphosphate preceding Ca(2+) transients in fertilization of sea urchin eggs. *Development*. 2001; 128:4405-4414
42. Jaffe LF. Classes and mechanisms of calcium waves. *Cell Calcium*. 1993; 14:736-745
43. Galione A, McDougall A, Busa WB, Willmott N, Gillot I, Whitaker M. Redundant mechanisms of calcium-induced calcium release underlying calcium waves during fertilization of sea urchin eggs. *Science*. 1993; 261:348-352
44. Dupont G, Goldbeter A. Properties of intracellular Ca<sup>2+</sup> waves generated by a model based on Ca(2+)-induced Ca<sup>2+</sup> release. *Biophys J*. 1994; 67:2191-2204. doi:10.1016/S0006-3495(94)80705-2
45. Tsien RW, Tsien RY. Calcium channels, stores, and oscillations. *Annu Rev Cell Biol*. 1990; 6:715-760. doi:10.1146/annurev.cb.06.110190.003435
46. Vacquier VD. The isolation of intact cortical granules from sea urchin eggs: calcium ions trigger granule discharge. *Dev Biol*. 1975; 43:62-74
47. Sardet C. The ultrastructure of the sea urchin egg cortex isolated before and after fertilization. *Dev Biol*. 1984; 105:196-210
48. Morgan AJ. Sea urchin eggs in the acid reign. *Cell Calcium*. 2011; 50:147-156. doi:10.1016/J.Ceca.2010.12.007
49. Roux MM, Townley IK, Raisch M, Reade A, Bradham C, Humphreys G, Gunaratne HJ, Killian CE, Moy G, Su YH, Etensohn CA, Wilt F, Vacquier VD, Burke RD, Wessel G, Foltz KR. A functional genomic and proteomic perspective of sea urchin calcium signaling and egg activation. *Dev Biol*. 2006; 300:416-433. doi:10.1016/j.ydbio.2006.09.006



50. McDougall A, Gillot I, Whitaker M. Thimerosal reveals calcium-induced calcium release in unfertilised sea urchin eggs. *Zygote*. 1993; 1:35-42
51. Friel DD.  $[Ca^{2+}]_i$  oscillations in sympathetic neurons: an experimental test of a theoretical model. *Biophys J*. 1995; 68:1752-1766. doi:10.1016/S0006-3495(95)80352-8
52. Goldbeter A, Dupont G, Berridge MJ. Minimal model for signal-induced  $Ca^{2+}$  oscillations and for their frequency encoding through protein phosphorylation. *Proc Natl Acad Sci U S A*. 1990; 87:1461-1465
53. Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol*. 2003; 4:517-529. doi:10.1038/nrm1155
54. Wang S, Trumble WR, Liao H, Wesson CR, Dunker AK, Kang CH. Crystal structure of calsequestrin from rabbit skeletal muscle sarcoplasmic reticulum. *Nat Struct Biol*. 1998; 5:476-483
55. Jaffe LF. A proton-led model of fast calcium waves. *Cell Calcium*. 2004; 36:83-87. doi:10.1016/j.ceca.2003.12.004
56. Hamaguchi Y, Hiramoto Y. Activation of sea urchin eggs by microinjection of calcium buffers. *Exp Cell Res*. 1981; 134:171-179
57. Schuster S, Marhl M, Hofer T. Modelling of simple and complex calcium oscillations. From single-cell responses to intercellular signalling. *Eur J Biochem*. 2002; 269:1333-1355
58. Desvillettes L, Fellner K, Tang BQ. Trend to Equilibrium for Reaction-Diffusion Systems Arising from Complex Balanced Chemical Reaction Networks. *SIAM Journal on Mathematical Analysis*. 2017; 49:2666-2709. doi:10.1137/16m1073935
59. Desvillettes L, Fellner K. Entropy methods for reaction-diffusion systems. vol Dynamical systems and differential equations. Proceedings of the 6th AIMS International Conference, suppl.2007.
60. Duran AL, Santamaria-Holek I. Multiscale modeling of exocytosis in the fertilization process. *J Phys Chem Biophys*. 2014; 4:161. doi:10.4172/2161-0398.1000161
61. Wagner J, Fall CP, Hong F, Sims CE, Allbritton NL, Fontanilla RA, Moraru, II, Loew LM, Nuccitelli R. A wave of IP<sub>3</sub> production accompanies the fertilization  $Ca^{2+}$  wave in the egg of the frog, *Xenopus laevis*: theoretical and experimental support. *Cell Calcium*. 2004; 35:433-447. doi:10.1016/j.ceca.2003.10.009
62. Moundoyi H, Moussa A, Perthame B, Sarels B. Analytical examples of diffusive waves generated by a traveling wave. *Applicable Analysis*. 2017; 96:1596-1615. doi:10.1080/00036811.2017.1314463
63. Epel D. The initiation of development at fertilization. *Cell differentiation and development : the official journal of the International Society of Developmental Biologists*. 1990; 29:1-12
64. Dube F. Effect of reduced protein synthesis on the cell cycle in sea urchin embryos. *Journal of cellular physiology*. 1988; 137:545-552. doi:10.1002/jcp.1041370321
65. Wagenaar EB. The timing of synthesis of proteins required for mitosis in the cell cycle of the sea urchin embryo. *Experimental cell research*. 1983; 144:393-403
66. Malumbres M. Cyclin-dependent kinases. *Genome biology*. 2014; 15:122
67. Cormier P, Chassé H, Cosson B, Mulner-Lorillon O, morales J. Translational Control in Echinoderms: The calm before the storm. In: editors Evolution of the protein synthesis machinery and its regulation. Springer International Publishing Switzerland; 2016. p. 413-434.

68. Epel D. Protein synthesis in sea urchin eggs: a "late" response to fertilization. *Proceedings of the National Academy of Sciences of the United States of America*. 1967; 57:899-906
69. Brandhorst BP. Two-dimensional gel patterns of protein synthesis before and after fertilization of sea urchin eggs. *Developmental biology*. 1976; 52:310-317
70. Mathews MB, Sonenberg N, Hershey JW. Origins and principles of translational control. In: Sonenberg N, Hershey JW, Mathews MB, editors *Translational control of gene expression*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2000. p. 1-31.
71. Cormier P, Pyronnet S, Salaun P, Mulner-Lorillon O, Sonenberg N. Cap-dependent translation and control of the cell cycle. *Prog Cell Cycle Res*. 2003; 5:469-475
72. Belle R, Prigent S, Siegel A, Cormier P. Model of cap-dependent translation initiation in sea urchin: a step towards the eukaryotic translation regulation network. *Molecular reproduction and development*. 2010; 77:257-264. doi:10.1002/mrd.21142
73. Calzone L, Fages F, Soliman S. BIOCHAM: an environment for modeling biological systems and formalizing experimental knowledge. *Bioinformatics*. 2006; 22:1805-1807. doi:10.1093/bioinformatics/btl172
74. Tyson JJ. Modeling the cell division cycle: cdc2 and cyclin interactions. *Proc Natl Acad Sci U S A*. 1991; 88:7328-7332
75. Proud CG. Signalling to translation: how signal transduction pathways control the protein synthetic machinery. *Biochem J*. 2007; 403:217-234. doi:10.1042/BJ20070024
76. Proud CG. Regulation and roles of elongation factor 2 kinase. *Biochem Soc Trans*. 2015; 43:328-332. doi:10.1042/BST20140323
77. Belle R, Pluchon PF, Cormier P, Mulner-Lorillon O. Identification of a new isoform of eEF2 whose phosphorylation is required for completion of cell division in sea urchin embryos. *Dev Biol*. 2011; 350:476-483. doi:10.1016/j.ydbio.2010.12.015
78. Monnier A, Morales J, Cormier P, Boulben S, Belle R, Mulner-Lorillon O. Protein translation during early cell divisions of sea urchin embryos regulated at the level of polypeptide chain elongation and highly sensitive to natural polyamines. *Zygote*. 2001; 9:229-236
79. Costache V, Bilotto S, Laguerre L, Belle R, Cosson B, Cormier P, Morales J. Dephosphorylation of eIF2alpha is essential for protein synthesis increase and cell cycle progression after sea urchin fertilization. *Dev Biol*. 2012; 365:303-309. doi:10.1016/j.ydbio.2012.03.002
80. Jackson RJ, Hellen CU, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nature reviews Molecular cell biology*. 2010; 11:113-127. doi:10.1038/nrm2838
81. Salaun P, Pyronnet S, Morales J, Mulner-Lorillon O, Belle R, Sonenberg N, Cormier P. eIF4E/4E-BP dissociation and 4E-BP degradation in the first mitotic division of the sea urchin embryo. *Dev Biol*. 2003; 255:428-439
82. Gingras AC, Raught B, Sonenberg N. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. *Annual review of biochemistry*. 1999; 68:913-963. doi:10.1146/annurev.biochem.68.1.913
83. Oulhen N, Salaun P, Cosson B, Cormier P, Morales J. After fertilization of sea urchin eggs, eIF4G is post-translationally modified and associated with the cap-binding protein eIF4E. *J Cell Sci*. 2007; 120:425-434. doi:10.1242/jcs.03339
84. Oulhen N, Mulner-Lorillon O, Cormier P. eIF4E-binding proteins are differentially modified after ammonia versus intracellular calcium activation of sea urchin unfertilized eggs. *Mol Reprod Dev*. 2010; 77:83-91. doi:10.1002/mrd.21110

85. Gillespie KM, Bachvaroff TR, Jagus G. Expansion of eIF4E and 4E-BP Family Members in Deuterostomes. In: editors Evolution of the protein synthesis machinery and its regulation. Springer International Publishing Switzerland; 2016. p. 165-185.
86. Peter D, Igreja C, Weber R, Wohlbold L, Weiler C, Ebertsch L, Weichenrieder O, Izaurralde E. Molecular Architecture of 4E-BP Translational Inhibitors Bound to eIF4E. *Molecular cell*. 2015; 57:1074-1087. doi:10.1016/j.molcel.2015.01.017
87. Gosselin P, Oulhen N, Jam M, Ronzca J, Cormier P, Czjzek M, Cosson B. The translational repressor 4E-BP called to order by eIF4E: new structural insights by SAXS. *Nucleic acids research*. 2011; 39:3496-3503. doi:10.1093/nar/gkq1306
88. Bah A, Vernon RM, Siddiqui Z, Krzeminski M, Muhandiram R, Zhao C, Sonenberg N, Kay LE, Forman-Kay JD. Folding of an intrinsically disordered protein by phosphorylation as a regulatory switch. *Nature*. 2015; 519:106-109. doi:10.1038/nature13999
89. Pause A, Belsham GJ, Gingras AC, Donze O, Lin TA, Lawrence JC, Jr., Sonenberg N. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. *Nature*. 1994; 371:762-767. doi:10.1038/371762a0
90. Gingras AC, Kennedy SG, O'Leary MA, Sonenberg N, Hay N. 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. *Genes & development*. 1998; 12:502-513
91. Fonseca BD, Smith EM, Yelle N, Alain T, Bushell M, Pause A. The ever-evolving role of mTOR in translation. *Seminars in cell & developmental biology*. 2014; 36:102-112. doi:10.1016/j.semcdb.2014.09.014
92. Merrick WC. eIF4F: a retrospective. *J Biol Chem*. 2015; 290:24091-24099. doi:10.1074/jbc.R115.675280
93. Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK. Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF4G. *Molecular cell*. 1999; 3:707-716
94. Oulhen N, Boulben S, Bidinosti M, Morales J, Cormier P, Cosson B. A variant mimicking hyperphosphorylated 4E-BP inhibits protein synthesis in a sea urchin cell-free, cap-dependent translation system. *PLoS One*. 2009; 4:e5070. doi:10.1371/journal.pone.0005070
95. Salaun P, Boulben S, Mulner-Lorillon O, Belle R, Sonenberg N, Morales J, Cormier P. Embryonic-stage-dependent changes in the level of eIF4E-binding proteins during early development of sea urchin embryos. *J Cell Sci*. 2005; 118:1385-1394. doi:10.1242/jcs.01716
96. Lin TA, Kong X, Saltiel AR, Blackshear PJ, Lawrence JC, Jr. Control of PHAS-I by insulin in 3T3-L1 adipocytes. Synthesis, degradation, and phosphorylation by a rapamycin-sensitive and mitogen-activated protein kinase-independent pathway. *J Biol Chem*. 1995; 270:18531-18538
97. Elia A, Constantinou C, Clemens MJ. Effects of protein phosphorylation on ubiquitination and stability of the translational inhibitor protein 4E-BP1. *Oncogene*. 2008; 27:811-822. doi:10.1038/sj.onc.1210678
98. Yanagiya A, Suyama E, Adachi H, Svitkin YV, Aza-Blanc P, Imataka H, Mikami S, Martineau Y, Ronai ZA, Sonenberg N. Translational homeostasis via the mRNA cap-binding protein, eIF4E. *Molecular cell*. 2012; 46:847-858. doi:10.1016/j.molcel.2012.04.004
99. Chasse H, Mulner-Lorillon O, Boulben S, Glippa V, Morales J, Cormier P. Cyclin B Translation Depends on mTOR Activity after Fertilization in Sea Urchin Embryos. *PLoS One*. 2016; 11:e0150318. doi:10.1371/journal.pone.0150318

100. Laurent S, Richard A, Mulner-Lorillon O, Morales J, Flament D, Glippa V, Bourdon J, Gosselin P, Siegel A, Cormier P, Belle R. Modelization of the regulation of protein synthesis following fertilization in sea urchin shows requirement of two processes: a destabilization of eIF4E:4E-BP complex and a great stimulation of the 4E-BP-degradation mechanism, both rapamycin-sensitive. *Frontiers in genetics*. 2014; 5:117. doi:10.3389/fgene.2014.00117
101. Picard V, Mulner-Lorillon O, Bourdon J, Morales J, Cormier P, Siegel A, Belle R. Model of the delayed translation of cyclin B maternal mRNA after sea urchin fertilization. *Mol Reprod Dev*. 2016. doi:10.1002/mrd.22746
102. Mulner-Lorillon O, Chasse H, Morales J, Belle R, Cormier P. MAPK/ERK activity is required for the successful progression of mitosis in sea urchin embryos. *Dev Biol*. 2017; 421:194-203. doi:10.1016/j.ydbio.2016.11.018
103. Alvarez L, Dai L, Friedrich BM, Kashikar ND, Gregor I, Pascal R, Kaupp UB. The rate of change in Ca(2+) concentration controls sperm chemotaxis. *J Cell Biol*. 2012; 196:653-663. doi:10.1083/jcb.201106096
104. Chasse H, Boulben S, Costache V, Cormier P, Morales J. Analysis of translation using polysome profiling. *Nucleic acids research*. 2017; 45:e15. doi:10.1093/nar/gkw907
105. Karlebach G, Shamir R. Modelling and analysis of gene regulatory networks. *Nat Rev Mol Cell Biol*. 2008; 9:770-780. doi:10.1038/nrm2503
106. Cormier P. Translation regulator ballet in meiotic spindle. *Cell Cycle*. 2017; 16:733-734. doi:10.1080/15384101.2017.1304732
107. Jansova D, Koncicka M, Tetkova A, Cerna R, Malik R, Del Llano E, Kubelka M, Susor A. Regulation of 4E-BP1 activity in the mammalian oocyte. *Cell Cycle*. 2017; 16:927-939. doi:10.1080/15384101.2017.1295178
108. Susor A, Jansova D, Anger M, Kubelka M. Translation in the mammalian oocyte in space and time. *Cell Tissue Res*. 2016; 363:69-84. doi:10.1007/s00441-015-2269-6

## Figure legends

### **Figure 1: Graphic representation of different biological scales occurring at fertilization time and during early developmental embryogenesis in sea urchin.**

From left to right are illustrated the different biological scales, the time scales, the experimental approaches and their associated modeling techniques. ODE, ordinary differential equation; PDE, partial differential equation. (Adapted from Meier-Schellersheim [26] for sea urchin early developmental system).

### **Figure 2: Dynamic of the signaling pathway induced by speract in sea urchin sperm.**

**A)** Schematic diagram illustrating the components involved in the speract signaling pathway. **B)** Cascade events induced by the binding of speract to its receptor. **C)** Representation of the logical signaling network without deletions (all nodes present) **D)** Representation of the logical signaling pathway after the elimination of PDE. (from [30] with permission of Matinez-Mekler G.).

### **Figure 3: Mechanisms of egg activation and fertilization envelope elevation.**

**A)** Transmission electron micrograph showing partial views of two *Sphaerechinus granularis* sea urchin eggs, one before fertilization (on the left) and one after fertilization (on the right). (Nu) nucleus; (CG) cortical granules; (FE) fertilization envelope. **B)** Schematic drawing illustrating the main mechanisms involved in the fertilization envelope (FE) elevation. Sperm entry triggers, by a still unidentified process, the increase of cytosolic IP<sub>3</sub>, which induces the release of Ca<sup>2+</sup> from the endoplasmic reticulum (ER) to the cytoplasm. CICR propagates the wave outside of the reticulum. The drawing of the ion-releasing macromolecular complex (black and white diamonds)

represents calsequestrin as shown in Figure 1 of reference [55] and Figure 6 of reference [54]. The  $\text{Ca}^{2+}$  wave triggers egg activation and initiates the exocytosis of cortical granules (CG) and elevation of the fertilization envelope.

**Figure 4: Signaling pathways and translation factors showed to be involved in the translational control triggered by fertilization. A)** Schematic diagram illustrating the components involved in translation activation induced by fertilization. Sperm entry induces the  $\text{Ca}^{2+}$  wave, a sustained rise in intracellular pH and an entry of amino acids.  $\text{Ca}^{2+}$  activates the mTOR pathway, which induces the phosphorylation and degradation of 4E-BP, allowing eIF4E to associate with eIF4G and consequently to engage the global cap-dependent translation of mRNAs. The activated mTOR phosphorylates and inhibits eEF2 kinase. The inhibition of eEF2 kinase allows eEF2 activation and the elongation step of the mRNAs translation. GCN2 is inhibited by the increase in amino acids; consequently eIF2 $\alpha$  is dephosphorylated and active for the initiation step. **B)** Simulation of the concentration modifications of translation factors following fertilization of the sea urchin egg. Using the minimal model involving “eIF4E”, eIF4G “ and “4E-BP”, the parameters determined in the unfertilized eggs and the changes predicted in the fertilized eggs of the kinetic of concentration changes of all the parameters were simulated as described in reference [100].

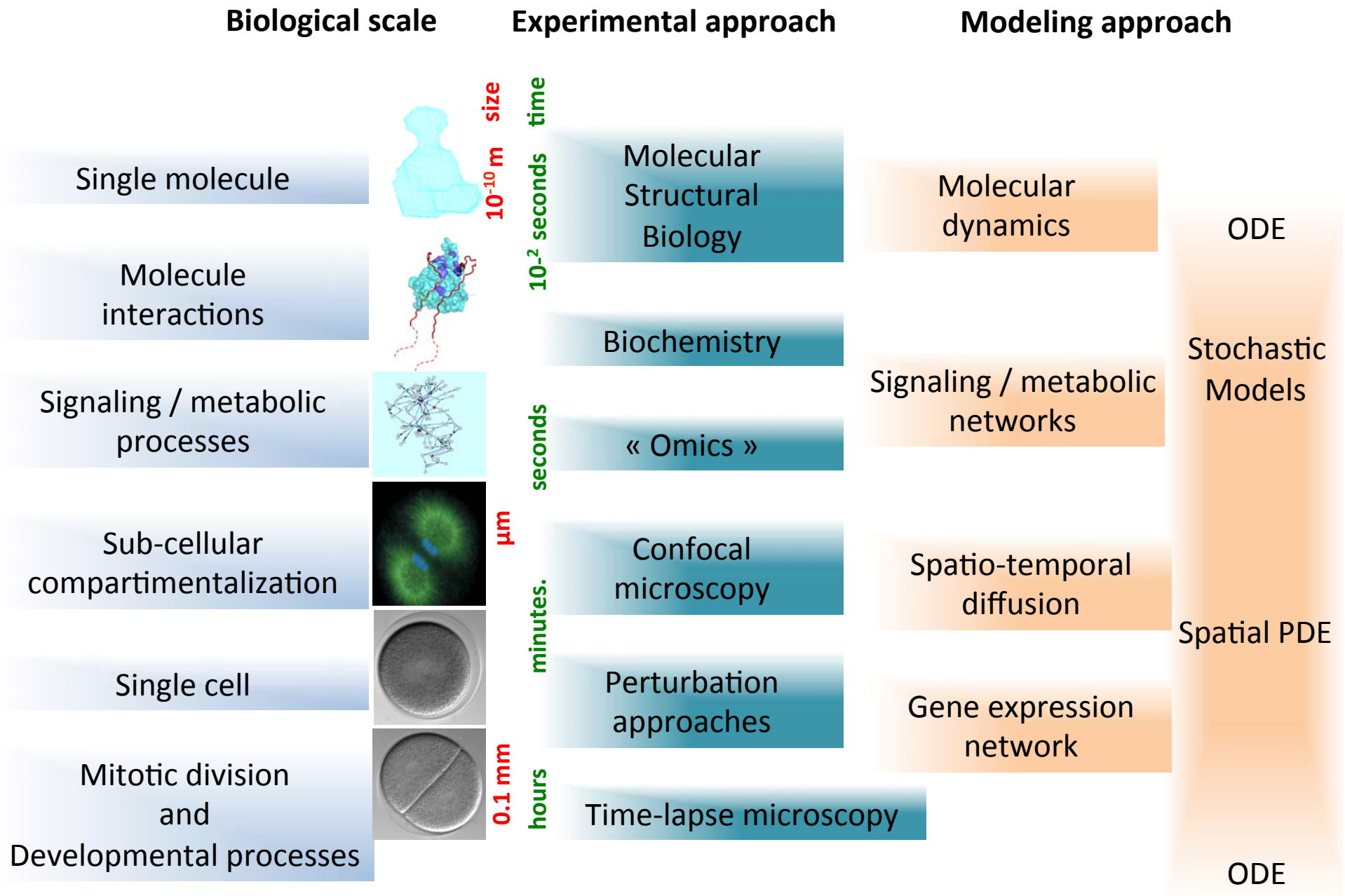


Figure 1

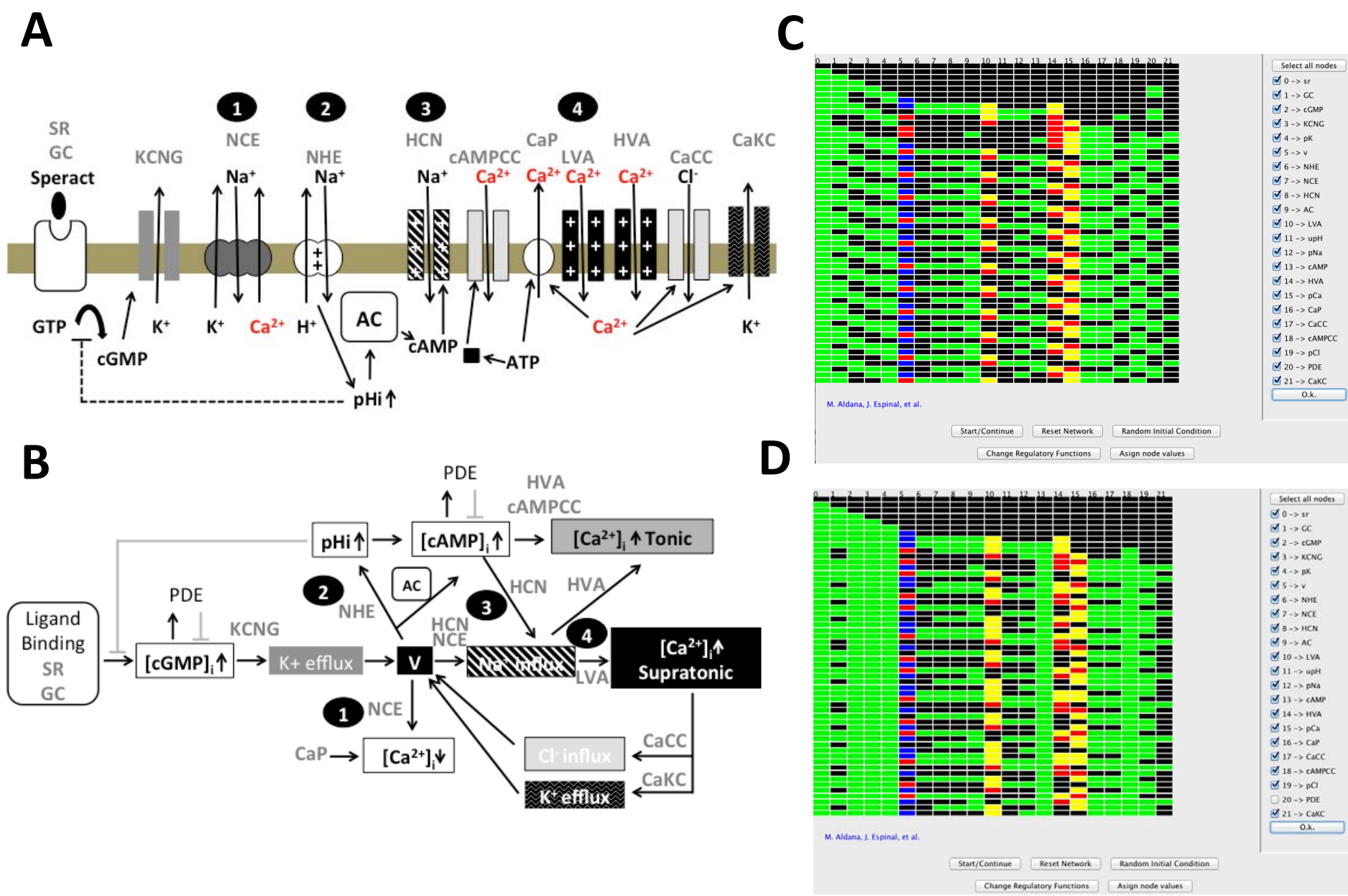


Figure 2



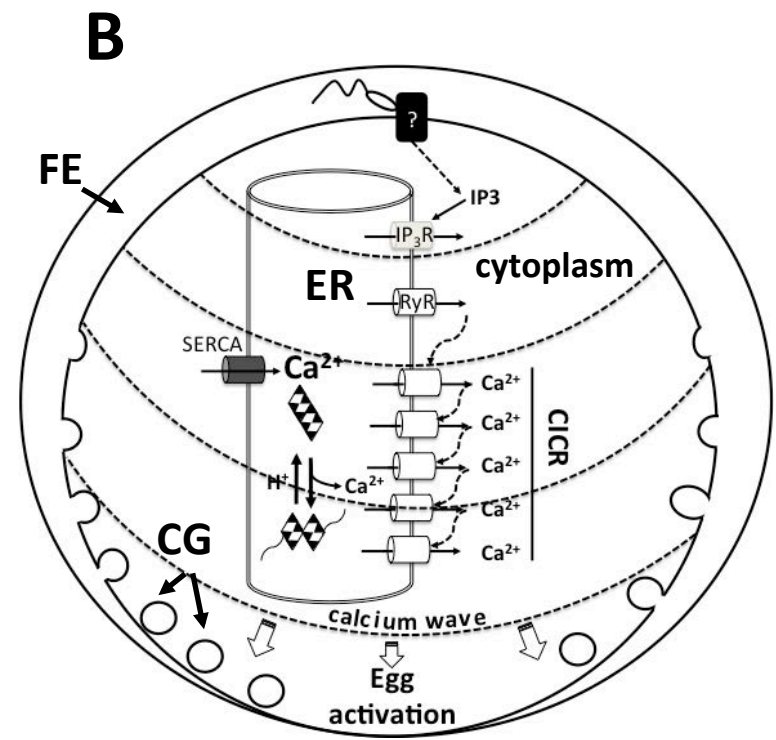
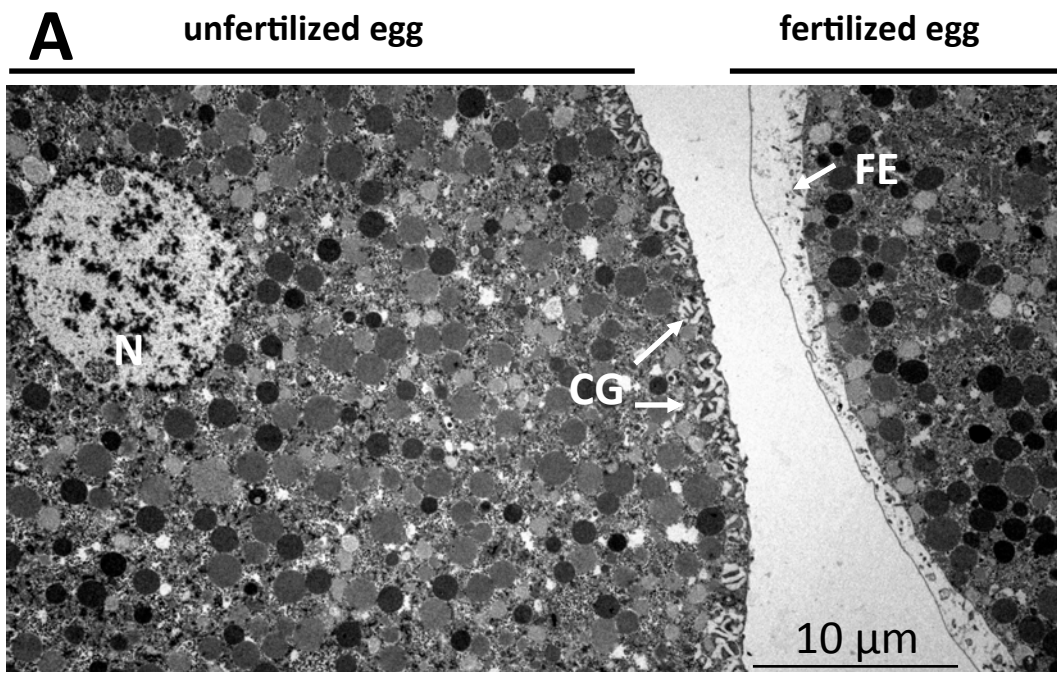


Figure 3

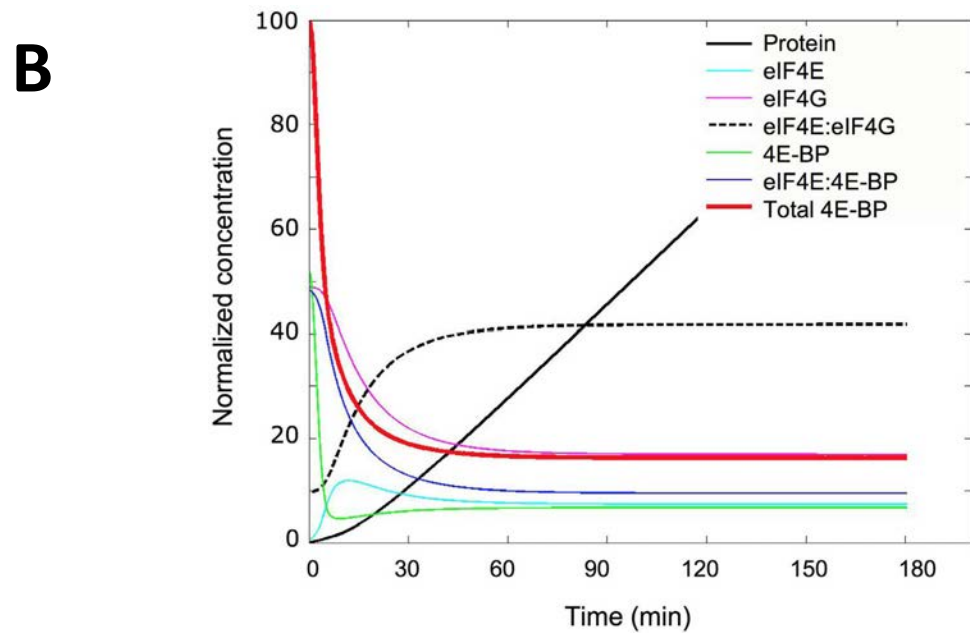
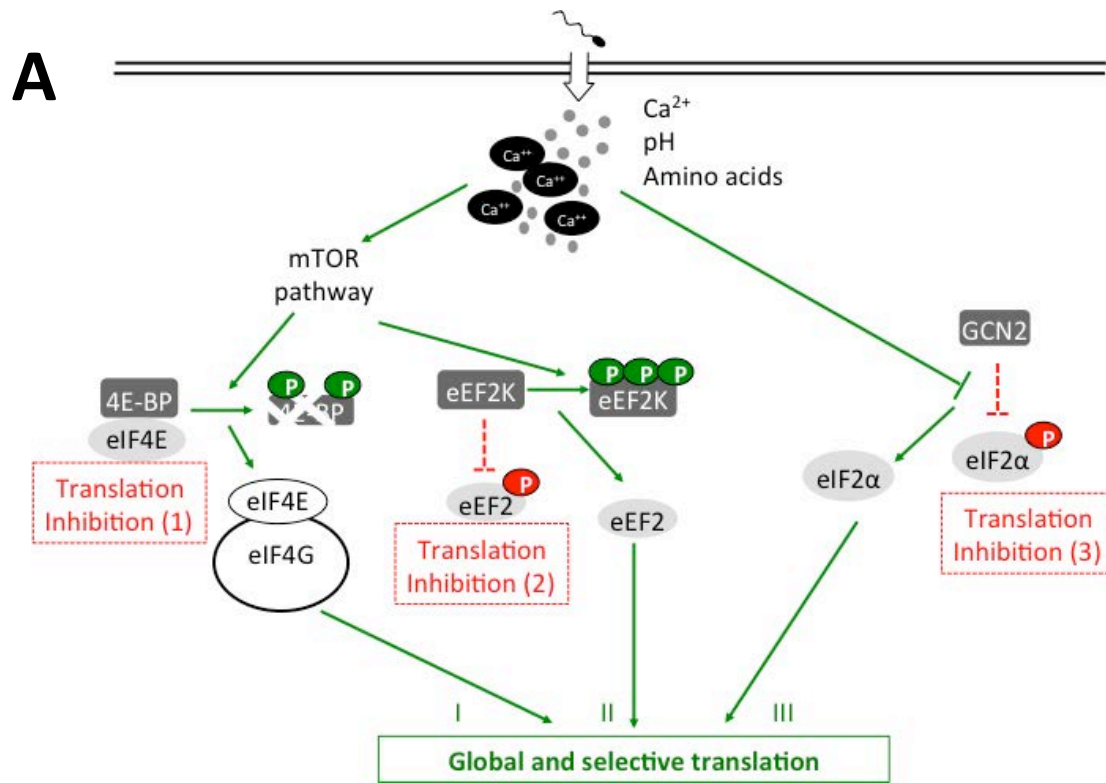


Figure 4