Mechanisms, mechanics and molecular control of tissue folding during sea urchin gastrulation

Abstract

Tissue folding is a key process in the life of all animals. During embryogenesis, defects in tissue folding (which takes place for instance during neurulation or gastrulation) are usually lethal or can lead to strong birth defects, such as spina bifida. In adult animals, tissue folding is also pathologically relevant, as for instance in cancer formation, where epithelial buckling is induced before invasion. Better understanding the principles underlying tissue folding is thus critical, yet studying this process remains a great challenge. To understand tissue folding, it is important to tackle the mechanisms, probe the mechanics, and unveil the molecules controlling tissue and cell shape changes. The goal of the proposed project is to shed new light on tissue folding through the assessment of all these aspects. To this end, we will use as a model system the gastrulation process in the sea urchin *Paracentrotus lividus*. We will implement 4D multi-view light sheet and 2nd harmonic generation imaging to visualize and characterize the initial process of gastrulation in the sea urchin embryo and determine the mechanisms responsible for it. We will develop and implement infra-red laser dissection, micro-pipette aspiration and micro-indentation along with mathematical modeling to establish the key mechanics of tissue folding. Finally, molecular investigations will be conducted to determine the signaling pathways and transcription factors that control cell and tissue shape changes. Altogether this work will yield new insights into the fundamental process of tissue folding both at the mechanical and molecular levels.

Project

Historically, sea urchins are among the first animals that have been used for studying embryonic development[1]. In this context, sea urchins have been a very valuable experimental system for the analysis of gastrulation[2,3]. Sea urchin gastrulae combine a number of outstanding features making this system a unique model for studying the mechanisms and mechanics of tissue folding: (1) sea urchin gastrulae develop outside the mother and are transparent so that the entire outer and inner regions of the embryo can be perfectly imaged with or without fluorescent markers; (2) sea urchin gastrulae constitute a spherical monolayer epithelium composed of 1.000 cells and thus represent a very simple and appealing system for both experimentation and modeling; (3) signaling molecules and transcription factors controlling sea urchin embryonic development and tissue folding during gastrulation are known and can be easily knocked down to dissect their functions; (4) within the gastrula, the invaginating tissue is mechanically accessible, it can be partitioned, cells can be transplanted, and micro-pipetting and micro-indentation techniques can be applied, thereby enabling measurements of the mechanical properties of tissues. In sum, the sea urchin gastrula is a perfect playground for both biologists and biophysicists.

The sea urchin gastrula presents however one important challenge: it is difficult to image live using fluorescence microscopy techniques since it is fragile, highly photosensitive and since it swims at high speed, being propelled by cilia. Because of this technical challenge, in the last decades few mechano-biology studies have been carried out using this model organism. Recently, we have overcome this bottleneck by using a physical stabilization approach and a 3D digital stabilization method in tandem with light sheet microscopy to reduce photo toxicity. We are thus now capable of imaging in 4D the sea urchin gastrulae over several hours, i.e. from the initial folding of the vegetal plate to the final elongation of the archenteron within the blastocoel. The proposed project is thus a breakthrough in the study of tissue folding using the *P. lividus* gastrula as a model system.

In this context, the imaging and processing pipeline that we have already established allows to (1) reliably image the gastrulation movements of sea urchin embryos in 4D with a 200 nm isotropic resolution at a frequency of 1 image/min, (2) segment all 1.000 cells constituting the gastrula in 3D and (3) track the 3D segmented cells over time. Using this approach, the goal is now to extract precise morphological and kinematic information of the folding during sea urchin gastrulation and to use this information to rule out or advance different mechanistic hypotheses. These hypotheses will further be tested using advanced mechano-techniques, such as in-plane micro-indentation, micro-pipetting and infra-red (IR) femtosecond (fs) laser dissection coupled to 2nd harmonic generation imaging and *in silico* mathematical modeling. Finally, we will identify the molecular tookit(s) that underlie each of the cell and tissue shape changes identified using different molecular biology approaches. Altogether, this interdisciplinary study will allow to decipher how the biophysical forces that lead to tissue folding during sea urchin gastrulation are generated and controlled in space and time.

Work plan

In sea urchins, tissue folding starts at the end of the mesenchyme blastula stage, once all skeletogenic mesoderm cells have ingressed within the blastocoel[1]. At that stage, the embryo is flat at the vegetal pole (see bottom side of the 0h embryo in Fig. 1). This flat area is referred to as the vegetal plate and is composed of non-skeletogenic mesoderm cells located in its center and surrounded by endoderm cells. In sea urchins, tissue folding is initiated by the non-skeletogenic mesoderm tissue[1]. During this first step, referred to as the primary invagination, the non-skeletogenic mesoderm cells change shape and the vegetal plate changes curvature. From flat it becomes concave and eventually moves towards the interior of the embryo (Fig. 1). However, although several mechanisms have been proposed in the past decades to account for primary invagination[4] it is still unclear which one(s) of these mechanisms is(are) at play. In addition, how does primary invagination take place at a mechanical level is still not well understood. Last, although several developmental genes are known to affect primary invagination[5,6,7], it remains unclear which mechanisms or mechanics they control. **The proposed project aims to define the mechanisms of primary invagination, to establish its related mechanics and implement a related mathematical model and to determine the role of identified developmental genes in cell and tissue shape changes.**

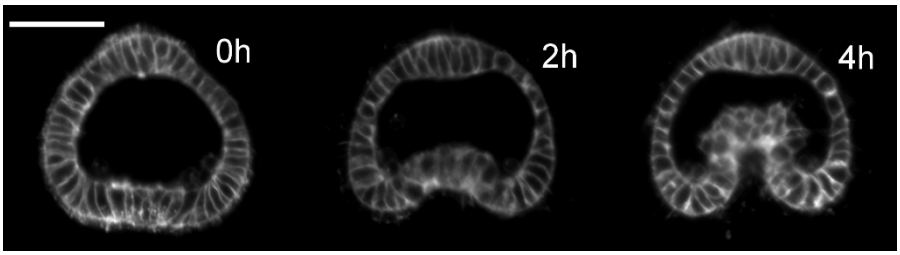

Figure 1: Vegetal plate folding in the sea urchin *P. lividus*: the tissue changes curvature from flat to concave and eventually moves towards the interior.
Scale bar is 50 µm.

***AIM 1*: Unraveling in space and time the potential mechanisms responsible for vegetal plate folding**

Experimental evidence from the 90's are indicative of six potential mechanisms that could underlie primary invagination during sea urchin gastrulation: apical-basal contraction, bottle cell formation, cell tractoring, contractile ring formation, extracellular matrix swelling and collective cell migration[4]. Here we propose to implement our imaging, processing and morphological analysis pipeline to precisely characterize the mechanisms of vegetal plate folding and ultimately rule out some of the proposed mechanisms.

Task 1. We will image and characterize over time the distinct cell and tissue shape changes taking place within the vegetal plate during its initial folding. To achieve this, we will inject fluorescent mRNA synthesized *in vitro* into sea urchin eggs, which allow the visualization of different components of the cells (e.g. cell membranes, actomyosin fibers, etc.). This information will be used to establish a database for 4D cell and tissue shape changes in which each cell will be segmented, tracked and analyzed with developed *ad hoc* image processing codes. This database will thereby provide the basis for cell shape quantification and cytoskeletal distribution on the principle of which we will rule out or advance mechanistic hypotheses.

Task 2. We will perform IR fs laser dissections to locally ablate the actomyosin network of the cells composing the vegetal plate or to disrupt the blastocoel matrix in the region just above the basal side of the vegetal plate. This will respectively enable us to assess the apical-basal contraction and collective migration hypotheses. The extracellular matrix is further expected to thicken at the vegetal plate during primary invagination[7]. We will thus measure the thickness of the extracellular matrix by using 2nd harmonic generation imaging. Furthermore, we will implement IR fs laser dissections to scalpel locally the extracellular matrix at the vegetal pole to directly test its role in tissue folding.

***AIM 2*: Measuring and modeling the mechanical properties of tissues responsible for vegetal plate folding**

Biophysical forces play a key role during tissue folding[8]. Established techniques like micro-pipette aspiration and more recent technologies such as in-plane micro-indentation (implemented for the first time on sea urchin embryos in our lab) allow the measurement of mechanical properties of tissues. These analyses can further be used to establish mathematical models that can provide additional mechanistic insights, which can be back-tested experimentally[9]. Our second aim thus is to determine the forces at play during sea urchin primary invagination through both experimental procedures and mathematical modelling.

Task 1. We will map the mechanical properties of tissues during sea urchin primary invagination both in space and time by using micro-pipette aspiration and in-plane cantilever micro-indentation technologies (Fig. 2). Tissues will be probed at the apical and basal side, both at the animal and vegetal pole, to provide a complete map of the mechanical properties of the gastrula over time. This information will further subsequently be used to build the mathematical model.

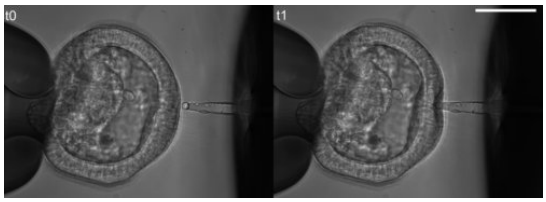

Figure 2: Cantilever micro-indentation of the animal pole of a gastrulating sea urchin embryo for local tissue visco-elasticity measurement.

Task 2. A mathematical model will be developed that will be a combination of 3D vertex model simulations and continuum calculations, to demonstrate physically how cell and tissue shape changes within the vegetal plate can induce folding. In the continuum theory, the epithelium will be represented as a spherical elastic sheet that exhibits bulk and shear elasticity and surrounds a compressible fluid. The goal here is to use this continuum representation to analytically show fundamental properties of the proposed mechanism(s). We will then make use of an apical (and eventually lateral) 3D vertex model, to quantitatively compare the predicted deformations with experiments and to take into account nonlinear effects. The *in silico* model will be back-tested experimentally.

***AIM 3:* Studying the role of key developmental genes in controlling vegetal plate folding**

Over the past decades, many studies have been conducted to decipher the molecular toolkit (or gene regulatory network) underlying sea urchin embryonic development. These studies have unveiled a number of developmental genes (i.e. signaling molecules and transcription factors) part of the regulatory cassette of primary invagination[7]. However, it still remains unknown, which specific tissue or cell mechanisms or mechanics these genes control. Here, as a first step, we aim to define the specific function of the transcription factor Brachyury (Bra) and of the G-protein coupled receptor Frizzled5/8 (Fzd5/8), both of which have already been shown to impair primary invagination when knock-down[5,6].

Tasks. Knockdowns of Bra and Fzd5/8 will be induced by microinjection into sea urchin eggs of mRNAs synthesized *in vitro* and encoding dominant-negative forms of the molecules, as well as of morpholino antisense oligonucleotides directed against each molecule. Tissue folding will be recorded under each experimental condition using the imaging procedure and pipeline we developed. Results from these analyses will be compared to the 4D database (AIM1) and to the mathematical model (AIM 2).

Altogether, this study will shine new lights on the mechanisms, mechanics and molecules at play during sea urchin primary invagination, a knowledge that will contribute to a more universal understanding of tissue folding.

Partners

This project will be co-supervised by **Jenifer Croce** (sea urchin development and genetics) at the Laboratoire de Biologie du Développement de Villefranche-sur-Mer and **Matteo Rauzi** (embryo imaging and mechanical experimentations) at the Institut de Biologie de Valrose in Nice. It will further benefit from collaborations with **Gregoire Malandain** (image processing and analysis) at the INRIA in Sophia Antipolis and **Guillaume Salbreux** (mathematical modelling) at the Francis Crick institute in London.

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