# Scientific Report - Short Visit Grant Ref. Number 4912

I visited the group of prof. Maria Leptin at EMBL Heidelberg in Germany. For the past year assist. prof. Primoz Ziherl and I have been working on a mechanical model of embryonic development in the fruit fly together with prof. Leptin and Matteo Rauzi, who is a member of her group. Our work focuses on epithelial folding which is a common process in the development of animals. This morphological change can displace cells from within a planar sheet to a new location, thereby creating new layers of cells or organ primordia. Epithelial folding is often the first step in gastrulation, where it initiates the transition from a simple epithelial sphere or ovoid called the blastula to a more complex, multilayered embryo. A well-studied example of tissue folding is the formation of a furrow on the ventral midline of the fruit fly (*Drosophila melanogaster*) embryo during gastrulation. Before the ventral furrow forms, the Drosophila embryo is an elongated ellipsoidal one-cell-thick epithelium of about 6000 cells coating the central yolk mass and surrounded by a shell called the vitelline membrane. During the first stage of furrow formation, cells in a band along the ventral side change their shapes and the ventral tissue folds inwards.
Our 2D model of a tubular epithelium resembling the early *Drosophila* embryo consists of a single layer of identical cells with energy associated with the tension of cell cortex. Depending on the tension of the apical, basal, and lateral sides of the cells we nicely reproduce embryo shapes observed *in vivo*. Matteo Rauzi has preformed experiments with *Drosophila* embryos and using various imaging techniques our purpose has been to compare experimental embryo shapes to the model shapes.

Purpose of visit:
My visit at EMBL Heidelberg was important as I was able to finally meet prof. Leptin and Matteo Rauzi in person since our collaboration was thus far based only on our communication via phone and email. The goals of my visit were to:
• become better acquainted with the experimental work done in the Leptin group,
• connect the model with experimental facts even more,
• revise and resubmit our manuscript that resulted from our collaboration and was submitted to Biophysical Journal (Hočevar et al., *A model of epithelial invagination driven*

*by collective mechanics of identical cells*).

Work carried out during the visit:

During my visit I performed numerical computation and made several predictions that allowed us to connect the model with experimental facts even better. I also revised and resubmitted our manuscript.

I also had a chance to get first hand contact with experiments and learn about the experimental work done by the Leptin group. Using SPIM (Selective Plane Illumination Microscopy) I was shown how fruit fly embryos are observed. By selecting an embryo at the right stage of development and placing it into a liquid-filled chamber I was able to see how this powerful microscopy tool reveals the 3D structure of the embryo. I became acquainted with basic principles of the microscope and by the end of my visit I was able to perform some basic microscopy myself.

Description of the main results obtained:

Before my visit we connected experimental data to our model by comparing the model shapes to *in vivo* fruit fly embryo shapes. We showed that some of the solutions of the model are characterized by a single deep groove and nicely correspond to observed *in vivo* shapes. We also demonstrated that in the model an increase in the cross-sectional area of a subset of cells is sufficient to ensure that the infolding occurs at a predetermined section of the epithelium. We showed that this variation of cell parameters across the epithelium is also observed *in vivo* and it is sufficient to make it fold at a specific site.

During my visit we were able to connect the model with experimental facts even more by comparing the role of the model vitelline membrane to the *in vivo* case. The vitelline membrane is modeled by an exponentially increasing pressure $p_0$ that is exerted on all parts of the epithelium whose distance from the center exceeds the radius of the resting vitelline membrane. We relate this external model pressure to a measurable mechanical property like the *in vivo* compliance of the vitelline membrane. While I was at EMBL Heidelberg we performed quantitative experiments using kymograph analysis to show that the vitelline membrane stretches very little during ventral furrow formation. However the thickness of the interstitial layer between the apical cell sides and the vitelline membrane is reduced during invagination (Fig. 1). At the onset of gastrulation the thickness of the

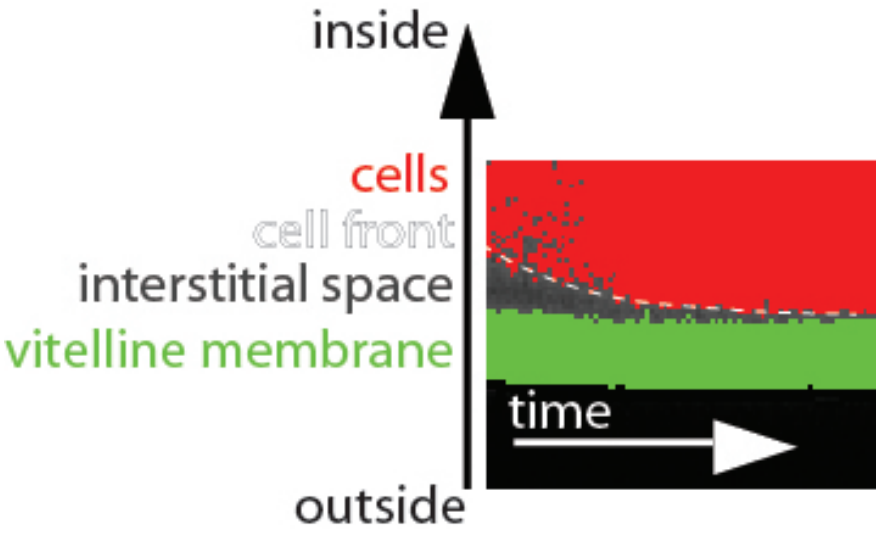


FIG. 1: The vitelline membrane (green) stretches very little during gastrulation. However the fluid in the interstitial space between the apical side of the cells (white dashed line) and the vitelline membrane is compressed during the process. The thickness of the interstitial layer reduces by about $2\mu$m, which amounts to about 2% of the vitelline membrane radius. The model vitelline membrane pressure $p_0$ at which the model vitelline membrane radius stretches by about 2% is $p_0 = 10^4 p_{int}$.

interstitial layer amounts to about 2% of the vitelline membrane radius and it is compressed to almost zero thickness during invagination. We can thus consider that what we refer to as the vitelline membrane in the model is *in vivo* the vitelline membrane together with the interstitial fluid. We thus infer that the model pressure $p_0$ which describes the real system properly is the one at which the model vitelline membrane radius stretches by about 2% during invagination. For model vitelline membrane pressure $p_0 = 10^2 p_{int}$ the radius of the membrane of the invaginated shape is about 10% larger than the radius of the resting model vitelline membrane. Here $p_{int}$ is the pressure in the yolk before tissue buckling. For $p_0 = 10^4 p_{int}$ the radius stretches by about 2%, whereas for $p_0 = 10^6 p_{int}$ it stretches by about 0.5%. The case with $p_0 = 10^4 p_{int}$ is thus comparable to the *in vivo* case. This is consistent with the comparison of model shapes to *in vivo* fruit fly embryo shapes where the best fit was also obtained at $p_0 = 10^4 p_{int}$.

Another thing we tested while I was visiting is the assumption in the model that cell volumes are constant during epithelium folding. We experimentally measured the dimensions

of cells and the yolk during invagination and the results show that during the first stages of invagination the dimensions change with time. We started to measure the dimensions of the system at the onset of gastrulation and we see that during the first stages of the process cells elongate by about 25 % in the lateral direction, thus the epithelium thickens at the expense of the yolk which reduces in size (data is shown in Fig. 2). This curve eventually flattens and cells no longer change in size. To interpret our measurements correctly one needs to bear in mind that gastrulation is preceded by another process — cellularization. During cellularization cells are formed by elongating along the lateral direction. The observed cell elongation that we measure (shown in Fig. 2) is thus a remnant of cellularization that partly overlaps in time with furrow formation. Shortly after the onset of gastrulation, cell elongation is completed and cell volumes stay constant during the rest of gastrulation. Since our work is focused on modeling fold formation, it is reasonable not to take into account the changes of cell and yolk size.

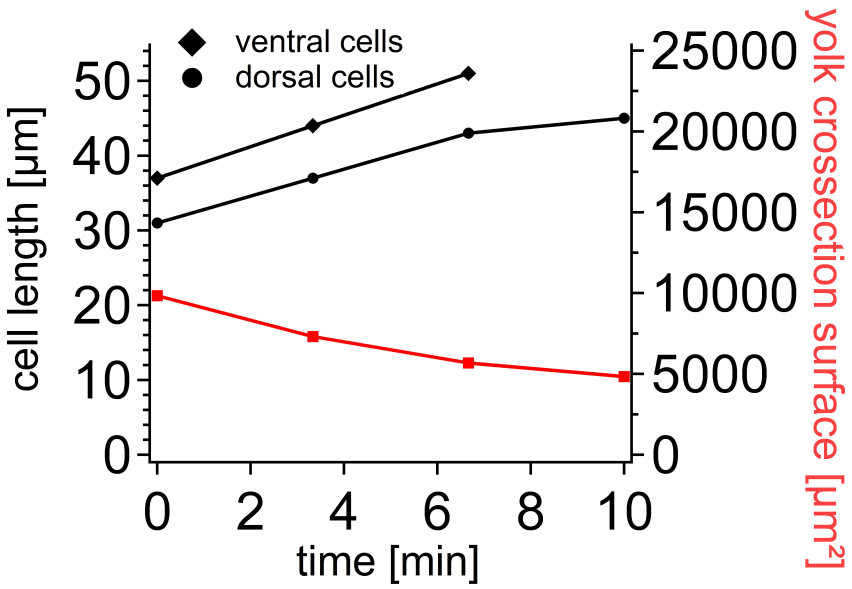


FIG. 2: We begin to measure cell and yolk dimensions at the onset of gastrulation (time 0) and we see that cells elongate in the lateral direction during the first few stages of gastrulation (black curves). At the same time the yolk somewhat shrinks in size (red curve). These changes in cell and yolk size represent the last stages of cellularization. After cellularization is completed cell volumes do not change anymore.

During my visit I also revised our manuscript and included all the new data we obtained (Figs. 1 and 2). Together with the response to reviewers' comments I resubmitted the manuscript.

Projected publications/articles resulting or to result from your grant:
The manuscript "A model of epithelial invagination driven by collective mechanics of identical cells" by A. Hocevar Brezavscek, M. Rauzi, P. Ziherl, and M. Leptin was resubmitted to Biophysical Journal.