In vivo analysis of enveloping layer morphogenesis during epiboly of annual killifish.

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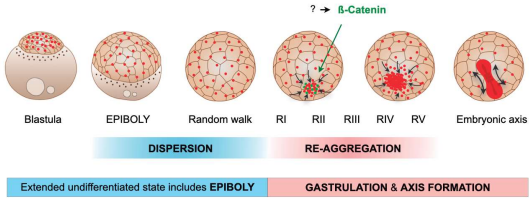
Abstract

The early stages of animal development are the most critical, as they define where the embryo will form and acquire its primordial body organisation. Research in model organisms highlights the fundamental role of extra-embryonic structures in providing the mechano-geometric constraints necessary to guide the initial steps of embryonic development. In teleosts, the early egg consists of a cellular domain that gives rise to the embryo proper, surrounded by two extra-embryonic tissues, the yolk syncytial layer and the enveloping layer (EVL). Both extra-embryonic tissues undergo epiboly movement, whereby they spread from the animal pole towards the vegetal pole and eventually engulf the entire egg. Using the annual killifish as a model, we studied the epibolic spreading of the EVL, a flat epithelial sheet consisting of 50-60 large multinucleted cells. Using light-sheet fluorescence microscopy, quantitative image-based approaches and assessment of actomyosin cytoskeleton dynamics, we reveal distinct spatial domains of EVL spreading along the animal-vegetal axis during epiboly, as well as different forms of epiboly closure. The EVL can undergo epiboly closure following an annular shape that seals at one point, or in a polygonal shape that leads to a zipper-like sealing. We have also started to analyse the relationship between the morphogenesis of the EVL and the formation of the future embryo.

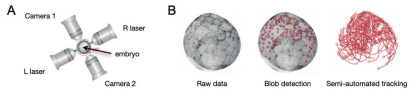
1. How does occur the early development in annual fish?



Unlike zebrafish, in annual Killifish, gastrulation and epiboly are separated in time. Gastrulation starts after epiboly is completed and the DCL are dispersed on the surface of the egg.
Gastrulation is marked by the formation of a cellular aggregate in an embryo pole (arrows), where nuclear ß-catenin becomes first restricted in the early re-aggregate by an unknown mechanism.

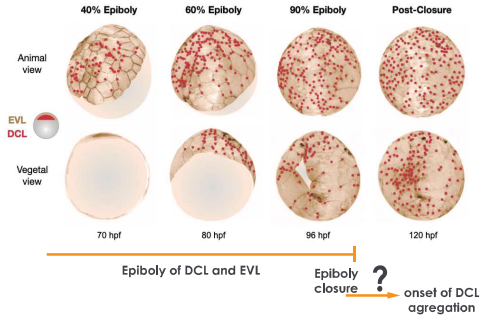
2. What is new in annual embryos epiboly?

Figure 1



We use multi-view light-sheet fluorescence microscopy (Figure1A) combined with cell tracking (Figure 1B), spinning disc microscopy (Figure 6), quantitative analysis of cell behaviour and assessment of actomyosin cytoskeleton.

Figure 2

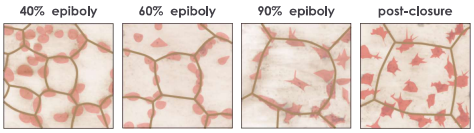


General view of annual fish epiboly and the onset of cell agregation: the DCL (red) and EVL (brown lines) spreads from the animal pole at low cell density to cover the egg surface

2.1 DCL cells change the migratory behaviour at the end of epiboly

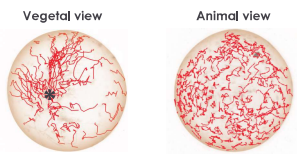
Immediately after the completion of epiboly, the DCL cells change their behaviour according to their position along de animal-vegetal axis. At the animal pole they start non-directional motion, like random walk (Figure 3) while at the vegetal pole, they begin a directional movement towards the epiboly closure zone (Figure 4).

Figure 3



The DCL cells (red) DCLs cease to move only at the edges of EVLs (brown lines).

Figure 4

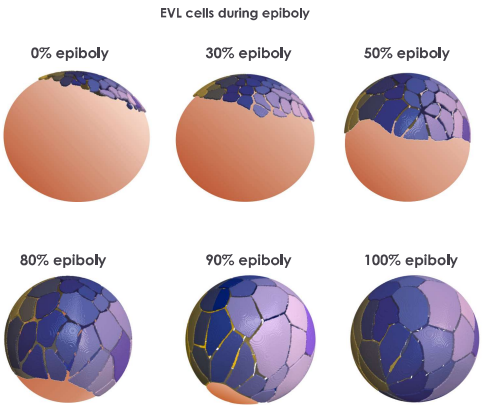


Tracking of DCL cells (red) immediately after epiboly closure (times 96-120 hpf).
* Site of epiboly closure

2.2 EVL cells spread over the surface of the egg until the end of epiboly

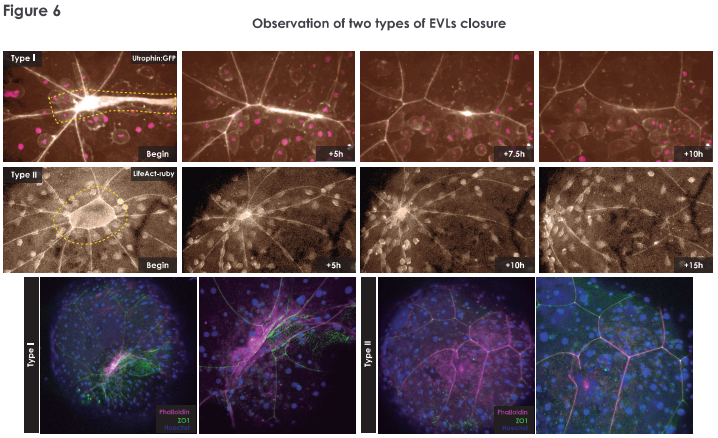
View of annual embryo epiboly by 3D-Reconstruction showing the spreading of EVL cells on the surface of the egg (Figure 5).

Figure 5



The closure of the epiboly involves EVL cells coming together, contacting and undergoing epithelial sealing (Figure 6). This process takes about 10 hours.

Figure 6

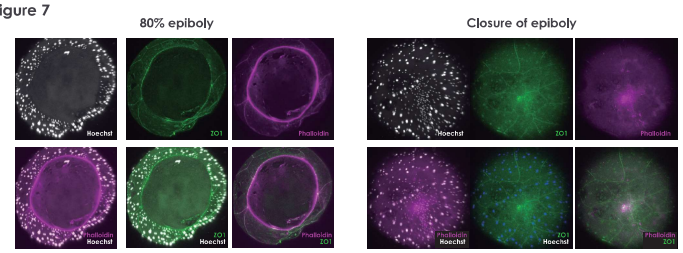


Time lapse using spinning disc microscopy (25x) showing two types (I and II) of epiboly closure in an embryo injected with Utrophin:GFP mRNA and H2A-RFP mRNA (Top, in sepia borders and magenta, respectively) or with LifeAct-ruby mRNA (Middle, in sepia borders). Bottom, Inmunofluorescense of phaloidin and ZO1 in the two types of epiboly closure).

2.3 Actin accummulates and persists at the site of epiboly closure

Previous to the end of epiboly, there is an increasing accumulation of F-actin both in the EVL cells closest to the closure edge and at the closure zone.
In time, this accumulation shows a graded spatial distribution being higher at the center of the closure zone and decreasing away from it.

Figure 7



Inmunofluorescence of phaloidin and ZO1 relative to Hoechst nuclear staining during epiboly closure.

3. Discussion/Conclusions

The end of epiboly appears as a critical period in which: **(1)** DCL cells seem to be released from the mechanical influence of EVL-EVL cell junctions and begin new cell migration patterns. **(2)** The presence of DCL directional migration towards the region of epiboly closure suggests that guidance cues are produced in this closure zone. **(3)** The EVL cells join together in the closure zone (in a long period) coinciding with F-actin enrichment in time and space.
The close relationship between the onset of DCL directional migration and the process of closure of EVL cells suggests that both processes are related with the formation of the initial re-aggregate and future embryo.

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