In vivo analysis of cell migration and its relation to epiboly during gastrulation in annual killifish.

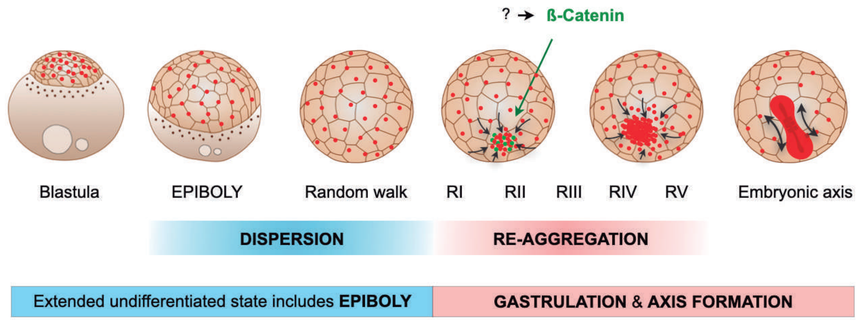
Carmen Gloria Lemus C.[1,2], Carlos F. Navarro[1, 2], Néstor Guerrero[1,2], Constanza Vásquez[1,2], Margarita Meynard[1,2], Cristina Bertocchi[4], Andrea Ravasio[4], Mauricio Cerda [1,2], Miguel L. Concha [1,2,3]

[1] ICBM, Facultad de Medicina, Universidad de Chile. [2] Instituto de Neurociencia Biomédica. [3] Centro de Gerociencia, Salud Mental y Metabolismo. [4] Pontificia Universidad Catolica.

Abstract

The early stages of animal development, and in particular gastrulation, are the most critical, as they define where the embryo will form and acquire its primordial body organisation. Research in model organisms highlights the critical role of extra-embryonic (ExEmb) 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 ExEmb tissues, the yolk syncytial layer and the enveloping layer (EVL). Both ExEmb 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 previously demonstrated that during the early stages of epiboly, embryonic cells migrate by mechanically interacting with the EVL, but whether this behaviour extends to the entire epiboly process and its relationship to later stages of embryo formation remains unknown. Here we begin to address this question by studying in vivo the relationship between embryonic cell migration and ExEmb tissues during epiboly and beyond. Using multi-view light-sheet fluorescence microscopy combined with cell tracking, quantitative analysis of cell behaviour and assessment of actomyosin cytoskeleton dynamics, we unveil distinct phases of embryo-ExEmb interactions: an early phase in which embryonic cell migration follows the morphogenetic transformations of the EVL, and a late phase in which these interactions disappear and embryonic cells initiate random and directed migration patterns with distinct spatial distribution and actomyosin accumulations. These findings provide new insights into the role of tissue mechanics and ExEmb tissues in guiding morphogenesis and patterning events during early embryonic development.

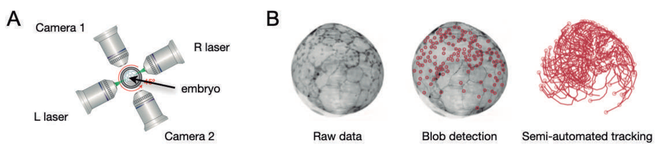
1. How does gastrulation occur in annual fishes?



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

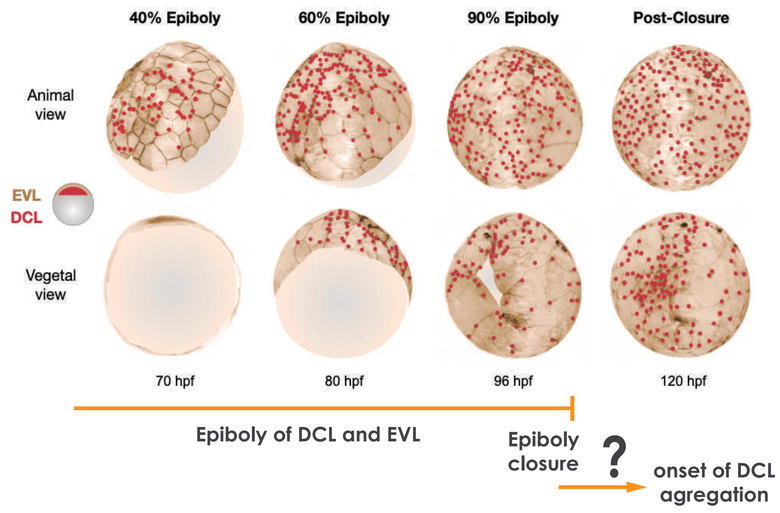
2. Is the end of epiboly related to the beginning of the re-aggregation?

Figure 1



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

Figure 2

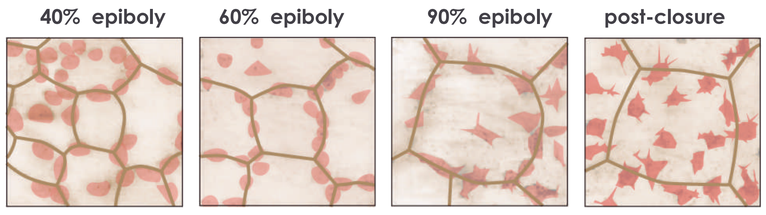


General view of annual fish epiboly and the onset of cell agregation: the DCL (red) and EVL (brown lines) spreads from the animal pole at low cell density to cover the egg surface

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

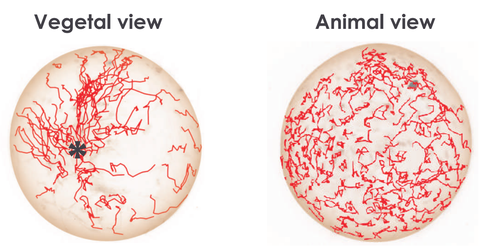
Immediately after the completion of epiboly, the DCL cells change their behaviour according to their position along de animal-vegetal axis. At the animal pole they start non-directional motion, like random walk (Figure 3) while at the vegetal pole, they begin a directional movement towards the epiboly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

Figure 3



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

Figure 4

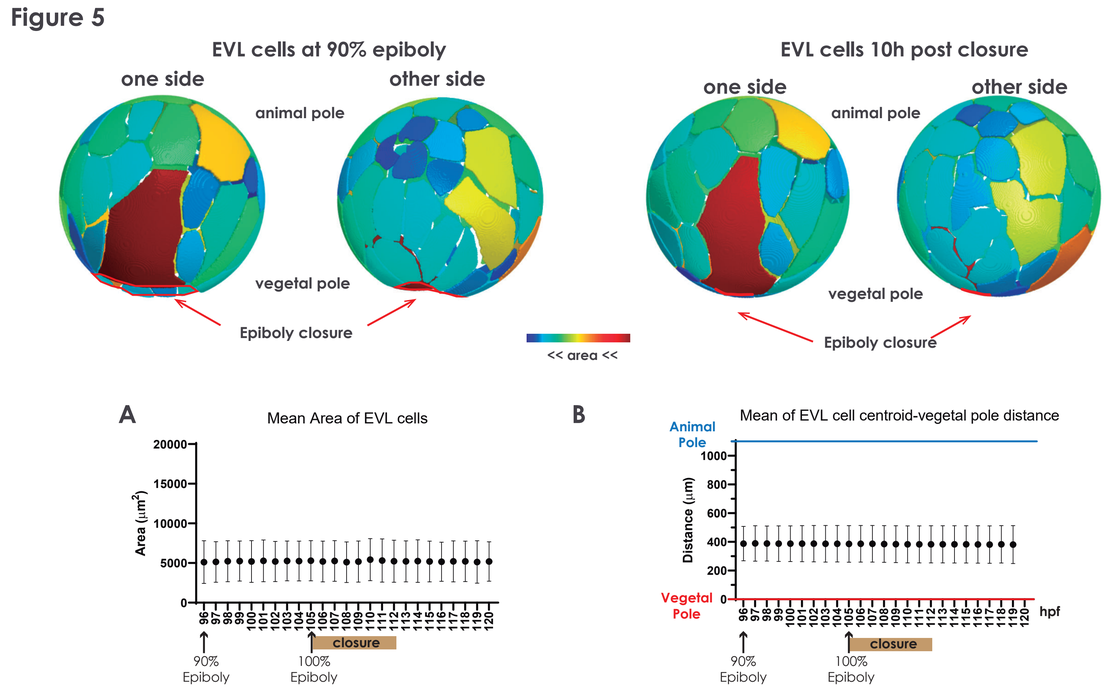


Tracking of DCL cells (red) immediately after epiboly closure (times 96-120 hpf).
* SIte of epiboly closure

2.2 EVL cells stop increasing area by the end of epiboly

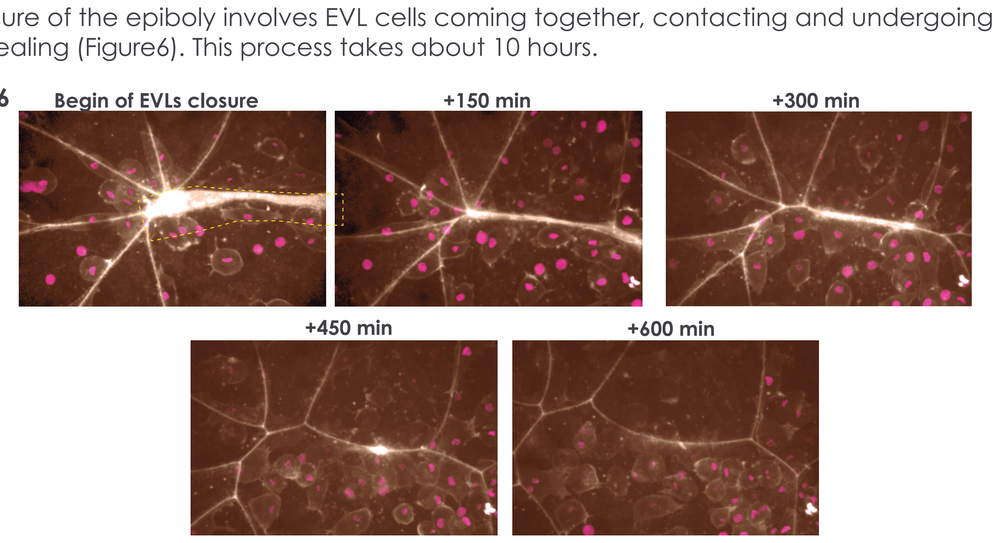
When epiboly reaches 100%, EVL cells stop their increase in surface area (Figure 5 & graphic A), stabilising their positions in reference to the vegetal pole (graphic B).

Figure 5



The closure of the epiboly involves EVL cells coming together, contacting and undergoing epithelial sealing (Figure6). This process takes about 10 hours.

Figure 6

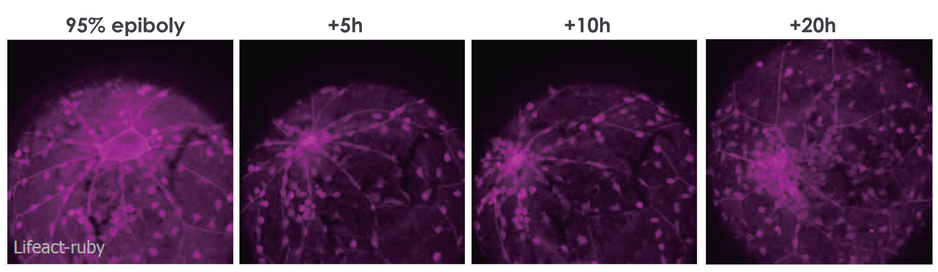


Time lapse using spinning disc microscopy (25x) showing the closure of epiboly in an embryo inyected with Utrophin:GFP mRNA (sepia borders) and H2A-RFP mRNA, to label F-actin and nuclei, respectively.

2.3 F-actin accummulate and persist at the site of epiboly closure

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

Figure 7



Time lapse using confocal microscopy showing the end of epiboly in an embryo inyected with LifeAct-ruby mRNA (magenta) as reporter of F-actin.

3. Discussion/Conclusions

The end of epiboly appear as a critical period in which:
1. DCL cells appear 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 for cell migration are produced in this closure zone.
3. The EVL cells join together in the closure zone (in a long period) that matches 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 process are related to the formation of the initial re-aggregate and future embryo.

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