The role and regulation of cell adhesion in zebrafish germ-cell migration

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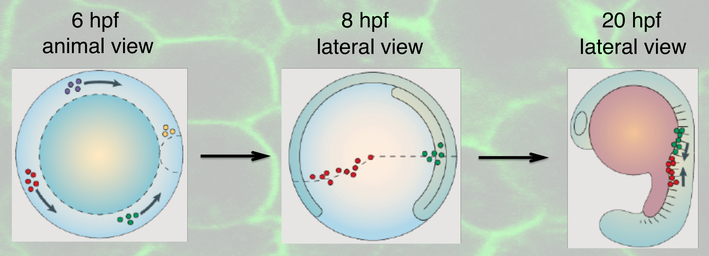
INTRODUCTION

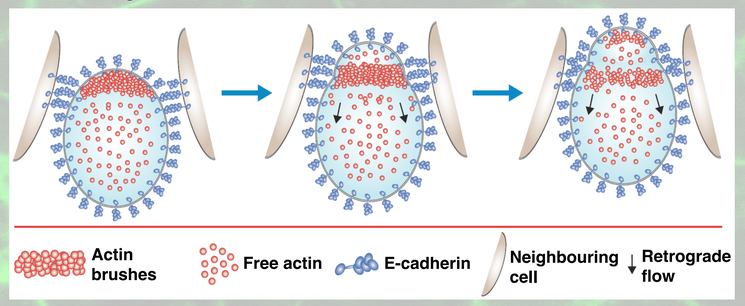
Primordial germ cell migration in zebrafish



In zebrafish embryos, primordial germ cells (PGCs) are specified and start to migrate towards the forming gonad guided by the chemokine Cxcl12 (movie1). Here, we utilise PGCs migration as a model to investigate how cell-cell adhesion, as mediated by E-cadherin, facilitates cell motility. (hpf = hours post fertilization)

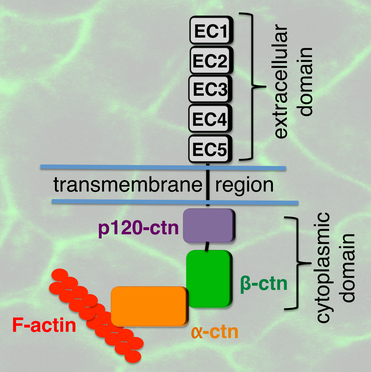
A model for traction generation

Zebrafish germ cells migrate using spherical protrusions termed blebs (movie2). At the base of the blebs, actin-rich structures (called actin brushes) are formed and exhibit retrograde flow ([1]). We propose that E-cadherin is linked to the actin brushes at the cell front and mediates the traction forces between the germ cells and the surrounding tissue.



E-cadherin structure

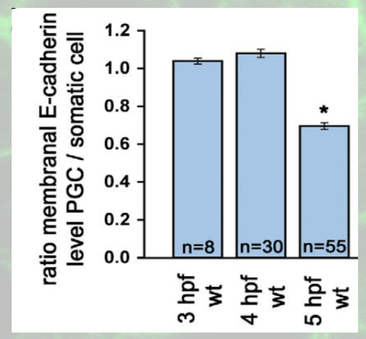
E-cadherin connects the actin cytoskeleton within the cells to the extracellular environment. This connection involves additional proteins collectively known as Catenins (ctn), which interact with the cytoplasmic domain of E-cadherin.



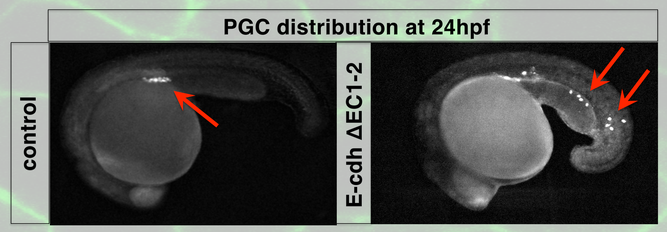
The mechanisms controlling E-cadherin function and the distribution of the forces around the cell perimeter of PGCs are not known.

Cell-cell adhesion in PGCs migration

1. The level of E-cadherin is reduced when PGCs initiate migration. When PGCs start to migrate at 5hpf a slight reduction in E-cadherin levels can be observed ([2]). This finding is consistent with the idea that E-cadherin is involved in the ability of PGCs to become motile.



2. Interfering with E-cadherin activity in the germ cells adversely affects their motility. Germ cells expressing a dominant negative form of E-cadherin (ΔEC1-2 E-cadherin) show a strong inhibition of migration ([1]) (movie3).

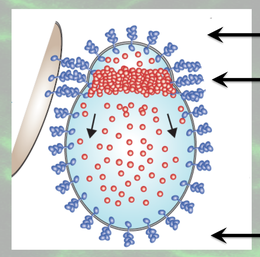


3. The adhesion forces between germ cells and somatic cells depend on E-cadherin. In a dual micropipette aspiration assay it was shown that the adhesion force between isolated germ cells and mesodermal cells decreases dramatically in the absence of E-cadherin ([1]) (movie4).

	Adhesion Force
Control	17.4 – 40.0 nN
E-cdh KD	1.0 – 2.5 nN

QUESTIONS

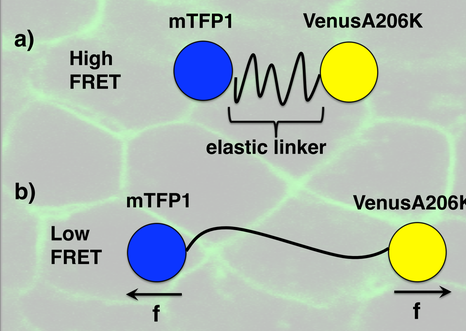
What is the connection between the actin brushes, adhesion and traction force generation?



How is the traction force distributed?

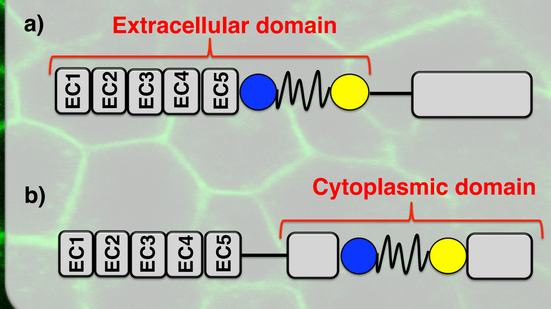
METHODS

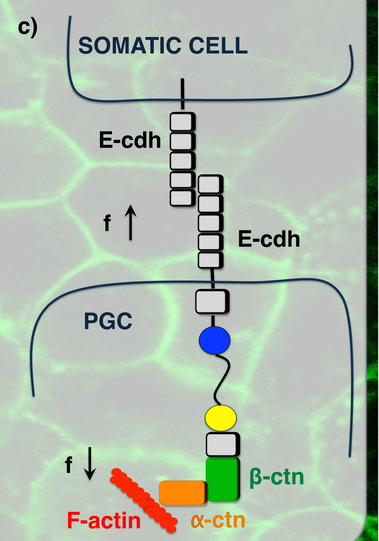
Mechano-sensors will be used to monitor the tension within E-cadherin molecules that are engaged in homophilic interactions and are linked to the actin cytoskeleton.



Molecular-tension sensor module for mapping force across E-cadherin. a) An elastic domain, derived from the spider silk protein Flagelliform, is inserted between two fluorophores that undergo efficient FRET (fluorescent resonance energy transfer; ([3])). b) When force across the biosensor extends the elastic linker, FRET efficiency decreases (f=force).

Scheme of E-cadherin-tension sensors. The sensors consist of the FRET construct inserted in the extracellular domain (a) or the cytoplasmic domain (b) of E-cadherin. Low FRET is expected when E-cadherin is stretched due to interactions with another E-cadherin molecule and the actin cytoskeleton in live, migrating cells (c).





SUMMARY AND OUTLOOK

According to our current model, in migrating PGCs E-cadherin molecules are linked to the actin brushes and to neighbouring cells mediating the traction forces required to translocate the cell body. To test this hypothesis, we need to assess whether differential pulling forces are exerted on E-cadherin at different regions of the cell perimeter (e.g., front, back, sides). To this end, we will express the E-cadherin tension sensors in PGCs and monitor force distribution around wild-type cells, as well as in cells in which the cytoskeleton is manipulated (for example, at different states of myosin contractility).

References

1. Kardash E, *et al. Nature Cell Biology*, 1, 47-53 (2010).
2. Blaser H, Reichman-Fried M, *et al. J. Cell Sci*, 118, 4027-4038 (2005).
3. Grashoff C, *et al. Nature*, 466, 263-266 (2010).