Tagore echoed the consonance between life and death with the words "Death is not extinguishing the light; it is only putting out the lamp because the dawn has come". Future research into alternative cell death mechanisms should illuminate the diverse ways of 'putting out' the cell after it has accomplished its developmental goal.

References

1. Meier, P., Finch, A., and Evan, G. (2000). Apoptosis in development. Nature *407*, 796–801.
2. Kerr, J.F.R., Wyllie, A.H., and Currie, A.R. (1972). Apoptosis - basic biological phenomenon with wide-ranging implications in tissue kinetics. Brit. J. Cancer *26*, 239–257.
3. Lockshin, R.A., and Williams, C.M. (1965). Programmed cell death–I. cytology of degeneration in the intersegmental muscles of the Pernyi silkmoth. J. Insect Physiol. *11*, 123–133.
4. Edinger, A.L., and Thompson, C.B. (2004). Death by design: apoptosis, necrosis and autophagy. Curr. Opin. Cell Biol. *16*, 663–669.
5. Kroemer, G., and Martin, S.J. (2005). Caspase-independent cell death. Nat. Med. *11*, 725–730.
6. Abraham, M.C., Lu, Y., and Shaham, S. (2007). A morphologically conserved nonapoptotic program promotes linker cell death in *Caenorhabditis elegans*. Dev. Cell *12*, 73–86.
7. Mazzalupo, S., and Cooley, L. (2006). Illuminating the role of caspases during Drosophila oogenesis. Cell Death Differ. *13*, 1950–1959.
8. Clarke, P.G. (1990). Developmental cell death: morphological diversity and multiple mechanisms. Anat. Embryol. (Berl) *181*, 195–213.
9. Van Cruchten, S., and Van Den Broeck, W. (2002). Morphological and biochemical aspects of apoptosis, oncosis and necrosis. Anat. Histol. Embryol. *31*, 214–223.
10. Lettre, G., and Hengartner, M.O. (2006). Developmental apoptosis in *C. elegans*: a complex CEDnario. Nat. Rev. Mol. Cell Biol. *7*, 97–108.
11. Kimble, J., and Hirsh, D. (1979). The postembryonic cell lineages of the hermaphrodite and male gonads in *Caenorhabditis elegans*. Dev. Biol. *70*, 396–417.
12. Ellis, H.M., and Horvitz, H.R. (1986). Genetic control of programmed cell death in the nematode *C. elegans*. Cell *44*, 817–829.
13. McCall, K. (2004). Eggs over easy: cell death in the Drosophila ovary. Dev. Biol. *274*, 3–14.
14. Foley, K., and Cooley, L. (1998). Apoptosis in late stage *Drosophila* nurse cells does not require genes within the H99 deficiency. Development *125*, 1075–1082.
15. Witkus, E.R., Altman, L.G., and Taparowsky, J.A. (1980). An investigation of the presence of smooth endoplasmic reticulum and GERL during vitellogenesis in the ovary of Drosophila melanogaster. Exp. Cell Biol. *48*, 373–383.
16. Sulston, J.E., Albertson, D.G., and Thomson, J.N. (1980). The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. Dev. Biol. *78*, 542–576.
17. Yin, V.P., and Thummel, C.S. (2005). Mechanisms of steroid-triggered programmed cell death in *Drosophila*. Semin. Cell Dev. Biol. *16*, 237–243.
18. Soller, M., Bownes, M., and Kubli, E. (1999). Control of oocyte maturation in sexually mature *Drosophila* females. Dev. Biol. *208*, 337–351.
19. Syntichaki, P., Xu, K., Driscoll, M., and Tavernarakis, N. (2002). Specific aspartyl and calpain proteases are required for neurodegeneration in *C. elegans*. Nature *419*, 939–944.
20. Varbanov, M., Espert, L., and Biard-Piechaczyk, M. (2006). Mechanisms of CD4 T-cell depletion triggered by HIV-1 viral proteins. AIDS Rev. *8*, 221–236.

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Morphogenesis: Joining the Dots to Shape an Embryo

In the study of morphogenesis, how upstream signalling events are intricately linked to downstream cytoskeletal organisation is not entirely understood. Recent work in the *Drosophila* embryo has begun to shed light on this problem.

Sarah Woolner

During the development of an embryo, its cells and tissues must be bent, tugged and sculpted into shape during numerous morphogenetic events. The general mechanisms used for morphogenesis are shared across the animal world and can be reactivated in adulthood to close a wound or during tumour metastasis. A key player in all morphogenetic events is the actin cytoskeleton, which in combination with non-muscle myosin II (actomyosin), can provide the contractile force to shape a single cell or a whole tissue.

Various green fluorescent protein (GFP)-based tools now allow for the observation of changes in the organisation of actin, myosin and cell adhesions in the lead-up to and during morphogenetic events [1–3]. Genetic studies have also revealed a great deal about the upstream events that are required for morphogenesis, such as local signalling pathways or the transcription of specific morphogenetic regulators. However, the biggest gaps in our knowledge concern the links between these upstream events and the downstream changes in the organisation of actomyosin and cell-cell adhesions. Bridging these gaps will be crucial to gaining a complete understanding of how tissues and organs are shaped during development. Recent work using *Drosophila* embryogenesis as a model system has made sizable steps towards doing just that [4–7].

One well studied example of morphogenesis is gastrulation, the process whereby the different cell layers of the embryo are laid down. In the *Drosophila* embryo, gastrulation begins with the invagination of a group of cells on the ventral surface of the embryo, forming the ventral furrow (reviewed in [8]). The cells of the ventral furrow are internalized and will later undergo an epithelial to mesenchymal transition to form the mesoderm precursors of the embryo. Ventral furrow formation is driven by the apical constriction of the ventral cells, which causes them to change from cuboidal to wedge-shaped — a transition that forces them inside the embryo (Figure 1A). The apical

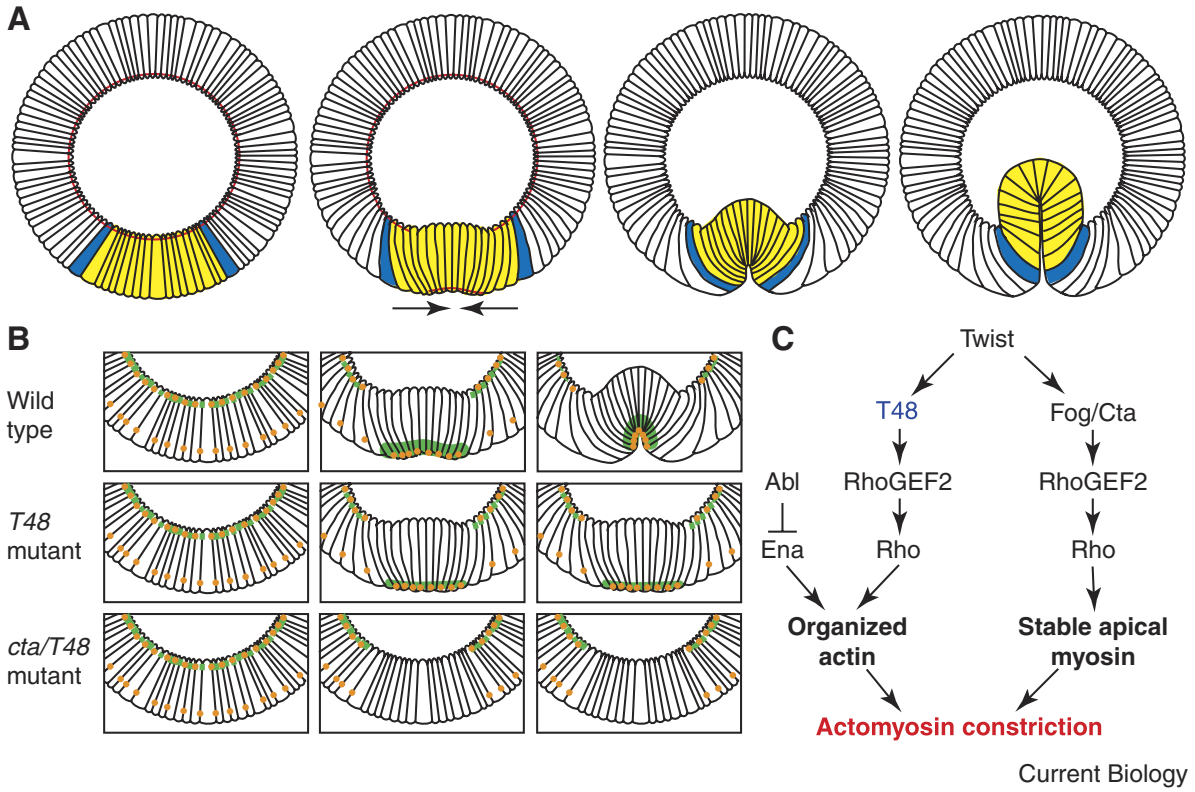

Figure 1. Ventral furrow formation and actomyosin regulation during *Drosophila* gastrulation.

(A) The sequence of major steps (from left to right) in ventral furrow formation are shown as cross-sections through the *Drosophila* embryo (top = dorsal; bottom = ventral). Expression of the transcription factors Twist (blue) and Snail (overlapping Snail and Twist expression shown in yellow) define the ventral domain. The ventral cells accumulate non-muscle myosin II (red) at their apical surface and begin to constrict apically and thus invaginate. (B) A schematic representation of some of the key findings of Kölsch *et al.* [5], shown as close-ups of the ventral domain, progressing in time from left to right. In wild-type embryos, prior to the start of ventral furrow formation, RhoGEF2 (green) and Armadillo/ß-Catenin (orange) are found along the basal surface (facing the interior of the embryo) of the ventral cells, Armadillo is also found in a sub-apical region in all cells. The formation of the ventral furrow coincides with the relocalisation of RhoGEF2 and Armadillo: vanishing from the basal surface and accumulating apically. In the *T48* mutant, apical RhoGEF2 recruitment is reduced and the ventral cells do not fully constrict. In embryos lacking both T48 and Cta, RhoGEF2 is absent from the apical surface of the ventral cells and the furrow fails to form. (C) A possible model for the control of ventral furrow formation, in which a second pathway acts in parallel to Fog/Cta signalling, via T48 (adapted from [6]).

constriction of the ventral cells is brought about by the local accumulation of actin and myosin II at the apical end of the cells [1].

The upstream control of *Drosophila* gastrulation has been well characterised by genetic analyses. Two transcription factors, Twist and Snail, define the ventral domain and induce the expression of specific target genes [9]. One such target gene is *folded gastrulation* (*fog*), which encodes a secreted ligand [10]. The Fog signal operates through Concertina (Cta), a heterotrimeric G-protein subunit [11], to activate RhoGEF2 [12,13]. RhoGEF2 is an activator of the small GTPase Rho1, a potent regulator of the actin cytoskeleton and myosin. It thus provides a link between the upstream signalling events and the downstream changes in actomyosin organisation. Indeed, in *RhoGEF2* mutants, myosin II no longer accumulates apically in the ventral cells and these cells no longer constrict and, as a consequence, the ventral furrow does not form [7,12]. Superficially, this seems to be a reasonably complete explanation of how the ventral furrow is formed, but there are some problems with this description. Firstly, genetic studies show that, while *RhoGEF2* null mutants display a strong gastrulation defect, the phenotypes of *fog* and *cta* mutants are much weaker, as some cell constriction occurs and the ventral cells are still internalized [11,12,14]. These differences suggest that RhoGEF2 may be involved in a second pathway, independent of Fog and Cta. Secondly, while we can explain why it is only the ventral cells that constrict — only these cells receive the Fog signal — we can't explain why constriction, and the accumulation of myosin II and actin that it requires, is limited to just the apical side of these cells. The recent discovery by Kölsch *et al.* [5] of T48 — a transmembrane protein that can recruit RhoGEF2 and adherens junctions to the apical surface of ventral cells and acts in parallel to Fog/Cta — may go some way to answering these questions.

T48 was originally discovered in a genetic screen for genes that control gastrulation [15]. The *T48* gene encodes a predicted protein with a signal peptide and a transmembrane domain. In keeping with a possible role in gastrulation, T48 is expressed in the mesoderm of the fly embryo under the control of Twist [5]. Furthermore, T48 localises to the apical membrane of the blastoderm, the site to which myosin II and RhoGEF2 relocalise as ventral furrow formation begins. The carboxyl terminus of T48 contains a consensus sequence for interaction with PDZ domains and Kölsch *et al.* [5] use a protein interaction algorithm to predict PDZ domain containing proteins that are most likely to interact with T48. One interactor identified this way is RhoGEF2 and its interaction with T48 is confirmed by the authors using *in vitro* binding assays. Crucially, Kölsch *et al.* [5] find that GFP-tagged RhoGEF2 in fly S2 cells relocalises from the cytoplasm to the plasma membrane after the addition of T48, indicating that T48 can mediate the recruitment of RhoGEF2 to the plasma membrane.

What about the function of T48 in the embryo? Kölsch *et al.* [5] demonstrate a requirement for T48 in the early stages of gastrulation and, specifically, for the relocalisation of RhoGEF2 to the apical membrane of ventral cells. In wild type embryos before gastrulation, RhoGEF2 is found along the basal surface of the blastoderm epithelium (Figure 1B). Just as gastrulation is about to begin, RhoGEF2 disappears basally and, shortly after, high levels of RhoGEF2 accumulate at the apical side of the ventral cells, which begin to flatten apically. This pattern of relocalisation from the basal to apical edge of the ventral cell mirrors that observed with adherens junctions and myosin II. In contrast to the wild type, *T48* loss-of-function embryos do not show such a dramatic concentration of RhoGEF2 at the apical side of their ventral cells and, although these cells do begin to flatten, the ventral furrow does not invaginate properly. The phenotype of *T48* mutants is similar to that caused by loss of Fog or Cta: ventral cells still change shape to some degree and furrow formation is not completely abolished — a weaker phenotype than seen in the *RhoGEF2* null, which completely fails to form a furrow. This suggests that T48 and Fog/Cta signalling are both contributing to the apical recruitment of RhoGEF2 in ventral cells. This appears to be the case, as Kölsch *et al.* [5] find that embryos lacking both T48 and Cta have a much stronger gastrulation phenotype, failing to accumulate RhoGEF2 apically and showing no ventral invagination (Figure 1B). These results indicate that T48 and Fog/Cta are acting in parallel pathways to localise RhoGEF2 to the apical end of ventral cells.

So, how can we fit this together to form a coherent picture of ventral furrow formation? Fox and Peifer [6] suggest a multi-pathway model, whereby the Fog/Cta pathway regulates apical myosin via RhoGEF2, whilst a second pathway — also operating through RhoGEF2 and assisted by the cytoskeletal regulators Abl and Ena — organises apical actin [6]. It seems likely that with T48 Kölsch *et al.* [5] have uncovered the regulator of RhoGEF2 in this second pathway (Figure 1C). Intriguingly, in another invagination event — during formation of the spiracles, the respiratory organ of the fly larva — Simões *et al.* [4] showed that RhoGEF2 and RhoGEF64C are specifically localised to the apical edge of invaginating cells. It will be interesting to discover whether the T48 machinery employed for gastrulation is reused for this later step.

In conclusion, recent work in the *Drosophila* embryo has begun to identify solid links between upstream signalling pathways and downstream changes in the organization of the cytoskeleton during morphogenesis. A general mechanism is emerging, whereby the local control of the cytoskeleton is mediated via the targeted recruitment of cytoskeletal regulators, such as the RhoGEFs.

References

1. Young, P.E., Pesacreta, T.C., and Kiehart, D.P. (1991). Dynamic changes in the distribution of cytoplasmic myosin during Drosophila embryogenesis. Development *111*, 1–14.
2. Lecuit, T. (2005). Adhesion remodeling underlying tissue morphogenesis. Trends Cell Biol. *15*, 34–42.
3. Jacinto, A., and Baum, B. (2003). Actin in development. Mech. Dev. *120*, 1337–1349.
4. Simoes, S., Denholm, B., Azevedo, D., Sotillos, S., Martin, P., Skaer, H., Hombria, J.C., and Jacinto, A. (2006). Compartmentalisation of Rho regulators directs cell invagination during tissue morphogenesis. Development *133*, 4257–4267.
5. Kölsch, V., Seher, T., Fernandez-Ballester, G.J., Serrano, L., and Leptin, M. (2007). Control of Drosophila gastrulation by apical localization of adherens junctions and RhoGEF2. Science *315*, 384–386.
6. Fox, D.T., and Peifer, M. (2007). Abelson kinase (Abl) and RhoGEF2 regulate actin organization during cell constriction in Drosophila. Development *134*, 567–578.
7. Nikolaidou, K.K., and Barrett, K. (2004). A Rho GTPase signaling pathway is used reiteratively in epithelial folding and potentially selects the outcome of Rho activation. Curr. Biol. *14*, 1822–1826.
8. Pilot, F., and Lecuit, T. (2005). Compartmentalized morphogenesis in epithelia: from cell to tissue shape. Dev. Dyn. *232*, 685–694.
9. Leptin, M., and Grunewald, B. (1990). Cell shape changes during gastrulation in Drosophila. Development *110*, 73–84.
10. Costa, M., Wilson, E.T., and Wieschaus, E. (1994). A putative cell signal encoded by the folded gastrulation gene coordinates cell shape changes during Drosophila gastrulation. Cell *76*, 1075–1089.
11. Parks, S., and Wieschaus, E. (1991). The Drosophila gastrulation gene concertina encodes a G alpha-like protein. Cell *64*, 447–458.
12. Barrett, K., Leptin, M., and Settleman, J. (1997). The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in Drosophila gastrulation. Cell *91*, 905–915.
13. Hacker, U., and Perrimon, N. (1998). DRhoGEF2 encodes a member of the Dbl family of oncogenes and controls cell shape changes during gastrulation in Drosophila. Genes Dev. *12*, 274–284.
14. Sweeton, D., Parks, S., Costa, M., and Wieschaus, E. (1991). Gastrulation in Drosophila: the formation of the ventral furrow and posterior midgut invaginations. Development *112*, 775–789.
15. Seher, T.C., and Leptin, M. (2000). Tribbles, a cell-cycle brake that coordinates proliferation and morphogenesis during Drosophila gastrulation. Curr. Biol. *10*, 623–629.

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