Drosophila morphogenesis: **Orchestrating cell rearrangements**

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Changes in shape of individual cells need to be coordinated to generate the movements of cell groups and sheets that are so important in morphogenesis. Recent results have shown that, during *Drosophila* gastrulation, multiple signalling pathways act to orchestrate the complex cell rearrangements.

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Many of the morphogenetic events during development involve coordinated movements of cell groups, which help to shape a tissue and indeed the whole organism. These movements are associated with extensive changes in the shapes of individual cells, which largely depend on the cytoskeleton and proteins associated with it. Equally important are the intercellular signals that coordinate the activities of individual cells within a group to achieve the orchestrated movement of a tissue. A major challenge in this field is to determine how cells are instructed so that they know whether to move, when to start moving and where to go. To understand these processes, it will be necessary to identify the extracellular signals that provide the instructions, as well as the membrane-bound receptors and intracellular molecules that transduce the instructions to the targets that mediate the cellular response — ultimately, a change in cell shape as a result of modification of the cytoskeleton.

Gastrulation is a good example of a developmental process in which coordinated cell movements and shape changes play an important role, one that is well suited to experimental dissection. During gastrulation, the basic body plan of an animal is established — according to Wolpert (quoted in [1]), it "is truly the most important time in life". In *Drosophila*, the early movement of the mesoderm is by far the best understood part of gastrulation [2]. This movement is initiated about three hours after egg laying by the invagination of the ventral furrow, which results in the formation of a tube-like structure in the interior of the embryo (Figure 1a,b). The tube then flattens and begins to disintegrate, the mesodermal cells resuming mitosis and spreading as a group on the underlying ectoderm (Figure 1c,d).

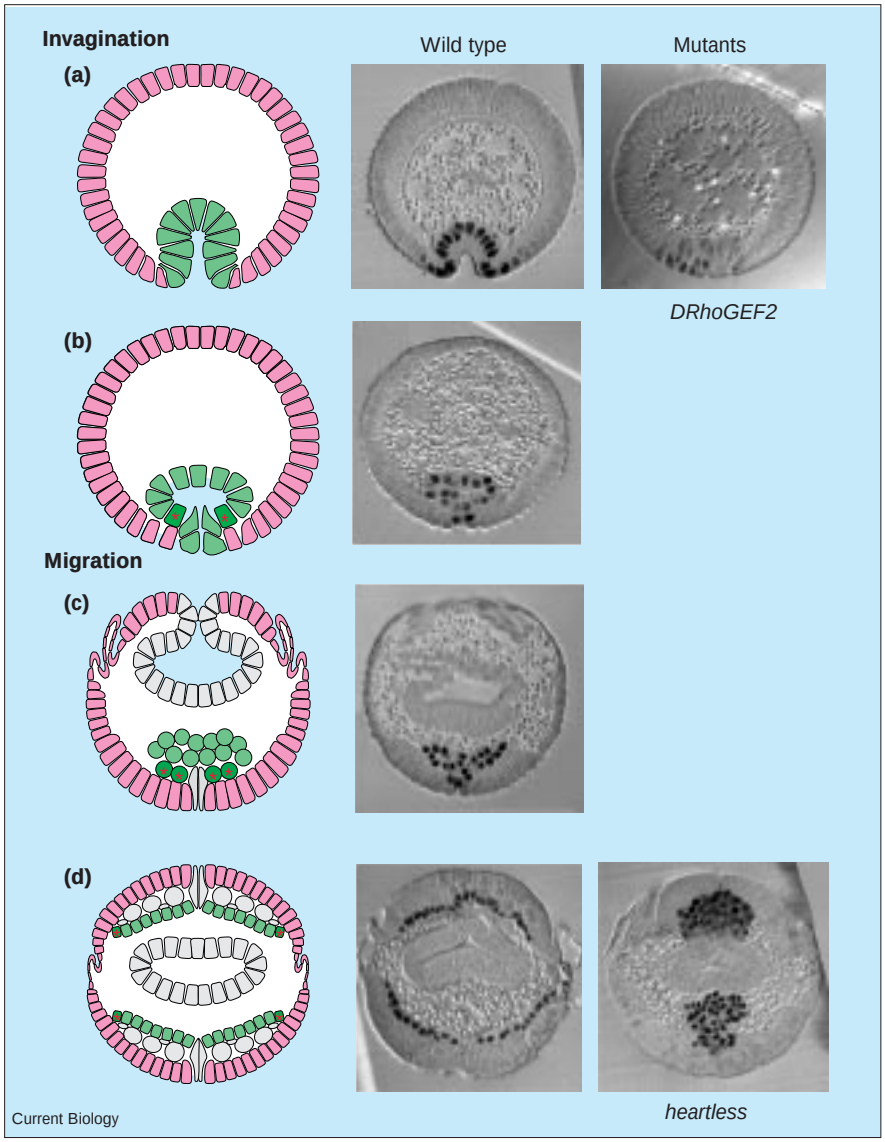
All three of these steps — formation of the furrow, flattening of the tube, and breakdown of the tube — are associated with coordinated cell shape changes. In the first two steps, the cell shape changes provide the trigger to drive the movement of an epithelial cell layer; in the third step, they enable the cells to abandon the epithelial sheet and to migrate. A number of genes have been identified recently that are involved in these steps, and studies of these genes and their products have provided new insights into the molecular mechanisms controlling coordinated cell shape changes.

The first sign of any change in cell shape during formation of the ventral furrow is apical flattening of the group of cells that go on to develop into the mesoderm. This flattening of all the presumptive mesoderm cells is followed by the very rapid apical constriction of a subset of them, at first of just a few random cells and then of all the central-most cells, which start to invaginate first. These cell shape changes result in a ventral furrow that moves inwards (Figure 1a). Two genes, *folded gastrulation* (*fog*), which encodes what is probably a secreted protein, and *concertina* (*cta*), which encodes what is likely to be a Gα subunit of a trimeric G protein, have been implicated as components of a signalling pathway that coordinates the concerted constrictions of the cells of the presumptive mesoderm [3–5].

Two independent lines of work [6,7] have identified another, apparently central, component of this putative signalling pathway — the product of the recently identified *Drosophila* gene *DRhoGEF2*. Unlike *fog* and *cta*, *DRhoGEF2* is indispensable for furrow formation during gastrulation. The loss of maternal and zygotic *DRhoGEF2* expression results in striking defects in ventral furrow formation: mesodermal cells fail to flatten their apical surfaces, and the surfaces of only very few of the prospective mesoderm cells undergo constriction. This inability to undergo cell shape changes results in a complete failure to form a ventral furrow (Figure 1).

From its sequence, the *DRhoGEF2* gene product is predicted to be a guanine nucleotide exchange factor (GEF), a link with the Ras superfamily of small GTPases. Some of the members of this superfamily, notably Rho, Rac and Cdc42, are known to be key regulators of the actin cytoskeleton (reviewed in [8]). GTPases cycle between an inactive state, when bound to GDP, and an active state, when bound to GTP, in which they regulate the activity of effector proteins. GEFs promote the exchange of GDP by GTP on their target GTPases, thereby shifting them into the active state. The sequence of DRhoGEF2 indicates that it is likely to be a regulator specifically of Rho GTPases. This is supported by the observation that, in

Figure 1



Drosophila gastrulation, showing the invagination and migration of the presumptive mesoderm demonstrated in transverse cross sections of *Drosophila* embryos. In the drawings on the left (modified from [13]), ectodermal cells are coloured pink, and mesodermal cells green; red asterisks indicate those mesodermal cells in which MAP kinase is activated in response to FGF signalling. The panels on the right show histological sections; the nuclei of the presumptive mesodermal cells are labeled with antibodies against the Twist protein. In a dynamic process that takes less than two hours, the mesoderm first invaginates into the interior of the embryo (a,b) and then migrates as a coherent cell group over the underlying ectoderm (c,d). Two different mutant phenotypes are depicted on the far right, in which mesoderm morphogenesis is affected in distinct processes. In *DRhoGEF2* mutant embryos, invagination of the mesoderm is blocked (micrograph courtesy of K. Barrett and J. Settleman); in *heartless* mutant embryos, migration of the mesoderm does not occur.

embryos expressing a dominant-negative form of the Rho1 GTPase that inhibits the activity of the endogenous enzyme, cells fail to undergo shape changes. The embryonic phenotype is comparable to that obtained in the absence of *DRhoGEF2* activity, a phenocopying that is not achieved by expression of dominant-negative versions of the other *Drosophila* GTPases, Rac or Cdc42.

Although many embryonic cells express *DRhoGEF2*, not all of them undergo cell shape changes. How is the activity of *DRhoGEF2* restricted to a subset of cells? This restriction might involve *fog*, which is specifically expressed in the ventral furrow. Cells that normally do not receive the *fog* signal, such as those on the dorsal side of the embryo, are induced to constrict their apical surfaces upon ectopic expression of *fog* [3,9]. Strikingly, this ectopic cell shape change is abolished in the absence of *DRhoGEF2*, indicating that DRhoGEF2 is essential for transduction of a signal provided by Fog [6]. These results provide evidence that, in wild-type embryos, it is the restricted distribution of the Fog signal that induces cell shape changes only in those cells destined to invaginate, although many more cells have the ability to undergo such changes. Fog is probably not the only signal molecule, however, because although furrow invagination is delayed in its absence, it does eventually occur.

Once the ventral invagination has formed a tube inside the embryo, the mesodermal cells undergo a second highly coordinated change in shape. This results in the flattening and subsequent disintegration of the tube (Figure 1). This process depends on the activity of one of

the two fibroblast growth factor (FGF) receptors that have been identified in *Drosophila*, a receptor tyrosine kinase encoded by the gene *heartless* (reviewed in [10]). In embryos lacking *heartless* function, mesodermal cells fail to spread out on the underlying ectoderm (Figure 1). The nature and the source of the signal that activates the Heartless receptor in these cells are both still unknown.

Some clues about the expression of the Heartless ligand have been obtained by monitoring those cells in which the receptor is activated. This is achieved using an antibody against the phosphorylated, active form of the mitogen activated protein (MAP) kinase, which is on the FGF receptor intracellular signalling pathway. Despite the fact that the Heartless receptor is expressed in all cells of the mesodermal tube, only those few cells that contact the ectoderm show MAP kinase activation [11,12] (Figure 1b,c). Later, when the cells have dispersed and migrate on the ectoderm, only those cells on the leading edge contain the activated form of MAP kinase [11] (Figure 1d).

A new paper has now revealed another component of the *Drosophila* FGF signalling pathway that is required for mesoderm morphogenesis. This is the product of the gene *downstream of FGF receptor* (*dof*), which as its name implies acts downstream of Heartless but upstream of Ras [12]. The *dof* gene is normally expressed in all the cells of the presumptive mesoderm, and loss of *dof* function prevents formation of an evenly spread mesodermal cell layer on the ectoderm, a phenotype reminiscent to that of *heartless* mutants.

It is notable that *dof* is also required in tracheal morphogenesis, which depends on the second *Drosophila* FGF receptor, Breathless, and is not required to transduce signals from other receptor tyrosine kinases. Dof is not notably similar in sequence to any known protein, and its sequence suggests that it does not bind directly to the Heartless receptor. Dof does have several consensus sites for interaction with proteins containing phosphotyrosine-binding Src homology 2 (SH2) domains, suggesting that it might act to recruit a signalling complex to the activated Heartless receptor — perhaps via interaction with an as yet unidentified linker protein — resulting in activation of the MAP kinase pathway. In support of this, in the absence of *dof*, the normal activation of MAP kinase in the mesodermal cells that contact the ectoderm fails to occur [12].

What do these two distinct processes — invagination of the ventral furrow and initiation of mesodermal cell migration — have in common? First, that the cell shape changes are restricted to a selected group of cells. Second, that this restriction is imposed by the distribution of an extracellular signal that induces the cell shape changes by activating an intracellular signalling pathway. And third, that the end result of activation of the intracellular signalling pathway is very likely to be a direct modification of the cytoskeleton. The identification and characterisation of the missing links in this process will hopefully teach us how common and specific components are used by the cells to coordinate their movements.

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