EMBO MEMBER'S REVIEW

Gastrulation in *Drosophila*: the logic and the cellular mechanisms

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The egg contains a set of molecules that can be used to trigger cell-shape changes leading to morphogenetic movements. The temporally and spatially controlled activation of these molecules, and hence the choreography of gastrulation movements, is determined by region-specific expression of transcription factors which turn on a set of downstream targets whose products mediate the successive steps of gastrulation.

Keywords: cell shape/morphogenesis/RhoGTPase signal transduction/Snail/Twist

Introduction

One of the earliest events in the development of a multicellular organism is the process of gastrulation, i.e. the segregation of the primordia of the future internal tissues, the mesoderm and the endoderm, into the interior of the developing embryo. For gastrulation to occur, the territories to be internalized must first be defined, and morphogenetic movements then orchestrated to bring the cells into their final positions inside the embryo. Gastrulation thus involves both pattern formation and morphogenesis, as well as the coordination of cell behaviour. Not surprisingly, molecules known to be important for gastrulation range from transcription factors through cytoskeletal components to signalling molecules. Some of these pre-exist in the embryo, being supplied to the egg by the mother, while others are synthesized at the time when gastrulation begins, specifically in those cells that initiate gastrulation. In this review I summarize what is known about the molecules involved in the mechanics of gastrulation in *Drosophila*, and the rules by which they are deployed under the control of regionally expressed transcription factors.

Description of gastrulation in *Drosophila*

Gastrulation is the process that transforms a blastula or a blastoderm into a multilayered embryo with three germ layers. The territories from which the inner germ layers arise in *Drosophila* are precisely defined in the pre-gastrulation embryo (the blastoderm) (Figure 1). The mesoderm primordium is a band of cells on the ventral side of the embryo, encompassing ~80% of the length and ~20% of the circumference of the embryo. The endoderm primordium is split in two parts which lie anterior and posterior of the mesoderm primordium,

with the anterior endoderm primordium restricted to the ventral side of the embryo and the posterior endoderm primordium encompassing the whole posterior pole of the blastoderm. These mesodermal and endodermal primordia are internalized by infoldings of the blastoderm epithelium and later disperse into individual cells that spread out to form the germ layers by cell migration and rearrangement.

The invagination of the mesoderm is heralded by subtle changes in the shapes of the most ventrally located blastoderm cells (Leptin and Grunewald, 1990; Sweeton *et al.*, 1991). These cells first flatten on their apical (outer) sides while their nuclei, initially positioned directly underneath the apical cell cortex, begin to migrate basally (Figure 1B and C). More pronounced changes ensue over a period of 10–15 min. The cells progressively constrict their apical sides until they are wedge shaped, and finally shorten along their apical–basal axis. As a result of these changes, the blastoderm epithelium first forms an indentation, the ventral furrow, which is then completely internalized. Once inside the embryo, the mesoderm primordium loses its epithelial structure and disperses into single cells which divide, attach to the ectoderm and migrate out on the ectoderm to form a single cell layer. The anterior endoderm invaginates together with the mesoderm, as the most anterior part of the ventral furrow. A few minutes after the ventral furrow has begun to invaginate, a similar series of cell-shape changes begins to occur in the posterior endoderm primordium (usually known as the posterior midgut or PMG primordium). These cells also constrict at their apical ends and become wedge shaped, and eventually invaginate, while at the same time the posterior end of the embryo is pushed dorsally by independent ectodermal cell movements. The posterior endoderm remains epithelial for a longer period and will only disperse into individual cells much later. These cells then use the mesodermal cell layer as substratum for migration towards the middle of the embryo, where they will meet up with the cells of the anterior endoderm to form the continuous endodermal cell layer that will become the midgut epithelium (Reuter *et al.*, 1993; Tepass and Hartenstein, 1994).

Control of gastrulation: genetics of early embryogenesis

What are the molecules that control these processes? Most of the components that have been shown to be essential during *Drosophila* gastrulation were identified genetically. To be able to discuss their roles it is first necessary briefly to review the essentials of the genetics of early embryonic development in *Drosophila*.

As the egg develops in the female gonad, it is filled with RNA and protein produced by nurse cells and encoded

Fig. 1. Four stages of gastrulation. (A) Diagrams of whole embryos indicating the regions of the mesodermal, endodermal and ectodermal primordia. At the cellular blastoderm stage (~3 h of development at 25°C) the primordia lie at the surface of the embryo (top). Fifteen minutes later, the prospective mesoderm has formed a furrow on the ventral side of the embryo (second embryo). A few minutes later, the posterior part of the endoderm has invaginated and the germ band has begun to extend onto the dorsal side of the embryo (third embryo). Approximately 45 min after the beginning of gastrulation the mesoderm is fully internalized and has begun to spread to form a single cell layer (bottom embryo). (B) Diagrams of cross sections of embryos at the same stages as those shown in (A). Colours mark regions or cells in which events relevant for gastrulation occur. Top: expression domains of Twist (red) and Snail (blue). Twist is shown as protein in the nucleus and Snail as RNA in the cytoplasm only to be able to show both in one cell. Second embryo, orange: Fog and Concertina (and probably myosin and actin) activity in apical constrictions. Third embryo, yellow: unknown activity in cell shortening. Last embryo, green: Cell division, and FGF-receptor activity in cell spreading. (C) Changes in an individual mesodermal cell in the embryos shown on the left.

by the maternal genome. These molecules are not only needed for the specification and growth of the egg, but continue to direct developmental processes after fertilization, allowing the embryo to develop for >2 h, nearly 15% of the embryonic period. During this period, transcription of genes from the zygotic genome is not necessary (Merrill *et al.*, 1988). Since the morphogenetic

processes that occur at this time (14 nuclear division cycles, migration of nuclei, the formation of the primary germ cells) do not depend on zygotic transcription, they are not affected by the genetic constitution of the zygote, but only by that of the mother. Zygotic gene activity is first required for the conversion of the syncytium resulting from the nuclear cleavages into a cellular epithelium, and

Fig. 2. Maternal and zygotic gene activities directing the gastrulation events shown in Figure 1.

immediately afterwards for gastrulation (Zusman and Wieschaus, 1985; Merrill *et al.*, 1988).

The maternal dowry to the egg contains a number of molecules known from other systems to affect cell shape (Figure 2). These include the actin cytoskeleton and its regulatory molecules. Two of the six actin isoforms in *Drosophila* are synthesized in the germ line during oogenesis, and both their RNAs and the proteins are present in the oocyte. A large number of actin-binding proteins have been purified from early embryos (Miller *et al.*, 1989), and recently the small GTP-binding protein Rho and its exchange factor RhoGEF have been shown to be active in the early embryo and to be provided maternally (Barrett *et al.*, 1997; Häcker and Perrimon, 1998). Cytoplasmic myosin is also produced maternally and can be visualized at the sites where the actin cytoskeleton is assumed to play critical morphogenetic roles. For example, during subdivision of the syncytial blastoderm into individual cells, myosin is located at the tips of the plasma membrane furrows as they are drawn into the embryo (Young *et al.*, 1991). However, a few minutes later when the ventral furrow begins to invaginate, myosin becomes concentrated at the apical sides of the invaginating cells as they begin to constrict. The role of many of these proteins cannot be analysed genetically, because they are required for the production of the oocyte and therefore germ line cells in which their genes are mutated fail to produce any eggs at all.

However, not all of the molecules produced during oogenesis are needed for both oogenesis and early development. Some are used exclusively in the early embryo, while others are used in the early embryo and again later during development, at the latter point as products of the zygotic genome. For both cases genetic screens have revealed genes that are essential for gastrulation.

The only gene known so far whose product is made during oogenesis and whose function appears absolutely specific for gastrulation is *concertina* (*cta*), identified in a screen for maternal effect mutations (Parks and Wieschaus, 1991). The Cta protein is a putative α -subunit of a heterotrimeric G protein. In the absence of wild-type Cta, the cell-shape changes in the ventral furrow and the posterior endoderm primordium do not proceed normally. Although cells appear to be able to undergo all aspects of the typical cell-shape changes they do so more slowly and in a less coordinated fashion. The cells of the ventral furrow nevertheless eventually become internalized and generate a normal mesodermal cell layer, but in the PMG primordium the delay and the lack of coordination result in a complete failure to invaginate. This in turn means that the PMG cannot be pushed onto the dorsal side, and the germ band cannot extend normally but folds itself up like a concertina—hence the name of the mutant.

If a gene product that is supplied to the embryo by the mother is also produced and needed during later stages of development, then homozygous mutant females cannot develop. Thus, embryos lacking the gene product can only be obtained from homozygous mutant germ line cells made by mitotic recombination in a heterozygous (i.e. phenotypically wild type and therefore alive) mother. By screening the progeny of such mutant germ lines, many new genes were discovered whose products were essential for embryogenesis, among them one that was essential for gastrulation (Perrimon *et al.*, 1996). This gene, which had also been identified in a screen for interaction partners for the small GTPase Rho, codes for a Rho-GTP exchange factor, termed DRhoGEF2 (Barrett *et al.*, 1997; Häcker and Perrimon, 1998). Embryos lacking this protein develop normally until cellularization. Thus none of the actin-dependent processes during oogenesis and very early embryogenesis appear to require Rho; however, gastrulation is completely blocked. Neither the ventral cells nor the cells in the PMG primordium undergo apical constriction and they remain on the surface of the embryo. This phenotype can also be induced by expressing a dominant negative form of the GTP-binding protein Rho in the embryo, confirming that the major cell-shape changes of gastrulation are indeed triggered by signalling through Rho. The recent finding that RhoGEFs may be activated through their interactions with G α subunits (Hart *et al.*, 1998; Kozasa *et al.*, 1998) raises the tantalizing idea that *Drosophila* gastrulation is triggered by the gastrulation specific Cta activating DRhoGEF2. However, this is not consistent with the phenotypes of the two mutants, which differ: in the ventral furrow DRhoGEF is essential for the cell-shape changes while Cta is only involved in optimizing, coordinating or accelerating them. Thus, a mechanism must exist in ventral cells that activates Rho in the absence of Cta. Conversely, even when a constitutively active form of Cta is expressed throughout the whole embryo, apical constrictions are seen only in ventral cells (Morize *et al.*, 1998). There must therefore also be some input into the mechanism triggering apical constriction from other sources as well, leading directly to the question of temporal and spatial control of gastrulation.

The temporal and spatial control of cell-shape changes

All of the molecules discussed so far are present in the egg from the beginning of development and are ubiquitously distributed. Why then do gastrulation movements begin only after 4 h, and why only in a subset of the cells of the embryo? Clearly, the molecules must be specifically activated in these cells, and so the two regions in which the cell-shape changes occur must be different from the rest of the embryo. The rules and mechanisms that govern the specification of these regions are in fact well understood (reviewed in St Johnston and Nüsslein-Volhard, 1992). Briefly, the maternal systems that pattern the egg result in regional differences in the expression of transcription factors. Along the dorso-ventral axis, the maternal protein Dorsal is distributed in a gradient in the blastoderm nuclei that reaches its highest point in the most ventral nuclei. Dorsal activates the expression of two transcription factors, Twist and Snail, in a band of ventral cells that include the mesoderm primordium. However, Twist and Snail alone do not define the precise position of the mesoderm, since both genes are also expressed in cells that do not contribute to the mesoderm. Specifically, the anterior-posterior extent of the ventral furrow does not coincide with the anterior and posterior borders of the Twist and Snail expression domains, but is set by genes that subdivide the anterior-posterior axis (Reuter and Leptin, 1994). Two of these, *huckebein* and *tailless*, are expressed at the anterior and posterior tips of the embryo (Pignoni *et al.*, 1990; Brönner *et al.*, 1994). The expression domain of *huckebein* defines the anterior and posterior borders of the mesoderm and the ventral furrow, and in *huckebein* mutants, the furrow extends over the whole length of the egg (Brönner, 1994; Reuter and Leptin, 1994). Embryos mutant for *twist* or *snail* fail to produce a functional mesoderm, while *huckebein* defines the anterior and posterior endodermal primordia, and together with *tailless*, the posterior endodermal invagination.

All four genes code for transcription factors. Twist, an HLH protein, acts as a transcriptional activator for mesodermal genes. In *twist* mutants, many mesodermal differentiation markers, such as the cell adhesion molecules N-cadherin and PS2 α -integrin, muscle specific proteins like β 3-tubulin, muscle myosin and Nautilus (the *Drosophila* homologue of vertebrate myogenic genes) are not expressed (Leptin, 1991). In contrast, the main function of Snail appears to be to repress the expression of genes in the mesoderm that are destined to be active in and restricted to the ectoderm, such as crumbs, E-cadherin and Delta. In *snail* mutant embryos the expression domains of these genes invade the mesodermal region (Kosman *et al.*, 1991; Leptin, 1991).

The absence of any one of these genes in the embryo results in serious gastrulation defects, detectable at the earliest stages of cell-shape changes. Instead of the normal succession of apical flattening and constriction, displacement of nuclei and cell shortening along the apical-basal axis, only some of these processes occur in mutant embryos (Leptin and Grunewald, 1990; Sweeton *et al.*, 1991). In *snail* mutants, the ventral epithelium becomes very thin, suggesting that shortening of cell occurs, but

no apical constriction is seen at all, and no furrow is formed. In *twist* mutants, ventral cells become narrow at their apical ends and nuclei move away from the apical side. As a result, a narrow and shallow furrow is formed. However, neither strong apical constriction nor cell shortening occurs, and the furrow fails to invaginate fully. Since Twist is required for the maintenance of Snail expression in the mesoderm (though not for its initial activation; Leptin, 1991), the defects in *twist* mutants might in part be due to a reduction in Snail function. Indeed, when additional Snail is provided in the prospective mesoderm, the Twist mutant phenotype is alleviated (Ip *et al.*, 1994). However, the ventral furrow is not restored to a normal appearance, suggesting that Twist also has Snail-independent functions. This is confirmed by the analysis of embryos mutant for both genes. In such embryos, no processes characteristic for the mesoderm cells occur at all, and the gene expression patterns of the ventral cells are identical to those in the neighbouring neural ectoderm. Thus, Twist and Snail jointly control the activation of the molecules which mediate cell-shape changes in the ventral furrow. The cell-shape changes in the PMG primordium are not affected; they are controlled by Huckebein and Tailless (Costa *et al.*, 1993), but their regulation has not been studied in the same detail as for the ventral cells.

Linking pattern formation and intracellular morphogenetic mechanisms

As described above, we understand how the fates of the cells that drive gastrulation are determined, which transcription factors control the effective cell-shape changes, and we know at least some of the molecules that mediate the cell-shape changes.

What we do not understand is the link between these levels of control or precisely what triggers the intracellular regulators. It is obvious that at least one of the links must be a transcriptional target of Twist or Snail. A large number of target genes for Twist are known, but almost all of them are required only for later steps in the differentiation of the mesoderm or its derivatives. Only one, *folded gastrulation* (*fog*), is known to be involved in gastrulation (Zusman and Wieschaus, 1985; Sweeton *et al.*, 1991; Costa *et al.*, 1994). *fog*, which codes for a secreted protein, is first expressed transiently in the prospective ventral furrow, and a few minutes later in the posterior pole of the embryo. Embryos mutant for *fog* show the same phenotype as those lacking functional Concertina protein, suggesting that *fog* and *cta* act in the same pathway, probably with Fog functioning as a localized ligand for the as yet unidentified receptor that activates the G α protein Cta. In this pathway the temporal and spatially controlled transcription of *fog* therefore provides an explanation for the temporal and spatial specificity of the activation of the ubiquitous component Cta. However, since Fog and Cta are not essential for ventral furrow formation, this cannot be the whole story. Even in the absence of Fog, cell-shape changes are initiated in the correct cells and at the right time, demonstrating the presence of other temporal and spatial controls. Furthermore, the *fog* and *cta* mutant phenotypes are much weaker than those of *twist*, *snail* or *DRhoGEF*, suggesting that at

least one link remains to be identified. In the simplest scenario, the protein product of a direct target gene of Twist and Snail would provide additional input upstream of Rho, and serve to activate RhoGEF in parallel with the signal conveyed by Cta.

Later events

The formation of the ventral furrow and the posterior midgut invagination is only the first step in gastrulation. The invaginated mesoderm then has to undergo a transition from its epithelial to a mesenchymal state, the cells divide and migrate out on the underlying ectoderm. The first step, the transition to a mesenchymal cell type is not understood, although it is possible that a switch from E-cadherin to N-cadherin might play a role (Oda *et al.*, 1998). E-cadherin is supplied maternally, and its expression is also activated in the embryo as soon as zygotic transcription begins (Oda *et al.*, 1994). However, it is repressed in the mesoderm by Snail. Simultaneously, Twist activates N-cadherin expression in the mesoderm. The fact that the switch from E-cadherin to N-cadherin is controlled by Twist and Snail suggests that it may be important for mesoderm morphogenesis.

The next step of mesoderm morphogenesis, the first round of mitosis of the mesodermal cells, is triggered by the expression of the tyrosine phosphatase String/cdc25 (Edgar and O'Farrell, 1989), which is yet another transcriptional target of Twist. This cell division normally has no direct implications for gastrulation, as the mesodermal cell layer is formed even without cell division. However, if cells divide prematurely, for example as a result of premature String/cdc25 activity, severe disruptions of ventral furrow formation ensue (Foe *et al.*, 1993). Clearly, cells cannot undergo the essential cell-shape changes of gastrulation at the same time as dividing, and *Drosophila* appears to have evolved mechanisms for delaying the division until the embryo has completed the invagination of the mesoderm (T.Seher and M.Leptin, unpublished).

Finally, two further genes whose expression depends on Twist are required for mesodermal migration. One, *heartless*, encodes an FGF-receptor homologue (Shishido *et al.*, 1993; Beiman *et al.*, 1996; Gisselbrecht *et al.*, 1996), while the other, *dof/hbr*, codes for an intracellular protein implicated in signal transduction downstream of the FGF-receptor Heartless (Michelson *et al.*, 1998; Vincent *et al.*, 1998). This suggests that a homologue of FGF (or a functional analogue) is produced somewhere to induce mesoderm spreading, but as yet no candidate has been identified. As with the *fog* signalling pathway during ventral furrow formation, the FGF signalling pathway utilizes components (those of the MAP kinase module) that pre-exist in all cells of the embryo during mesodermal cell spreading by activating them at a specific time and place (see Figure 2).

Conclusion

The events described here may appear to be a special case of morphogenesis with little relevance for other developmental events or for other species. Indeed, even other insect species go about gastrulation in different ways. However, the cellular phenomena and the molecules

involved are clearly general, and even the way in which they are deployed will probably turn out to be not just a quirk of *Drosophila* development, but a paradigm for a general set of rules. Thus, most cells contain a large set of proteins that can serve a wide range of purposes, and that are triggered during development to do morphogenetic work at specific times and in specific places by extracellular signals, or by newly synthesized gene products. *Drosophila* gastrulation may be a case in which a pathway can eventually be fully reconstructed from pattern formation through specific cell signalling to intracellular changes leading to morphogenetic cell behaviour.

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