Gastrulation in *Drosophila*: the formation of the ventral furrow and posterior midgut invaginations

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Summary

The ventral furrow and posterior midgut invaginations bring mesodermal and endodermal precursor cells into the interior of the *Drosophila* embryo during gastrulation. Both invaginations proceed through a similar sequence of rapid cell shape changes, which include apical flattening, constriction of the apical diameter, cell elongation and subsequent shortening. Based on the time course of apical constriction in the ventral furrow and posterior midgut, we identify two phases in this process: first, a slow stochastic phase in which some individual cells begin to constrict and, second, a rapid phase in which the remaining unconstricted cells constrict. Mutations in the *concertina* or *folded gastrulation* genes appear to block the transition to the second phase in both the ventral furrow and the posterior midgut invaginations.

Key words: *Drosophila*, embryonic development, gastrulation.

Introduction

During *Drosophila* gastrulation, two major invaginations, the ventral furrow and the posterior midgut, internalize mesodermal and posterior endodermal precursor cells (Poulson, 1950; Turner and Mahowald, 1977; Campos-Ortega and Hartenstein, 1985). The ventral furrow (VF), arises as a lengthwise cleft on the ventral side of the embryo. Cells internalized by this invagination will give rise to mesoderm. About eight minutes after the ventral furrow begins to form, the posterior midgut (PMG) invagination is initiated at the posterior pole. This invagination internalizes cells that will become endoderm.

Although the VF and PMG invaginations occur in regions of the embryo that are governed by different systems of maternal pattern determinants (Nüsslein-Volhard *et al.* 1987; Govind and Steward, 1991), the cellular mechanisms underlying both invaginations appear to be similar (Turner and Mahowald, 1977). In both cases the initial invaginations are associated with a change in cell shape from columnar to trapezoidal. These cell shape changes occur rapidly in a single-cell-layered epithelium and are restricted to defined cell populations.

Further support that VF and PMG formation is governed by the same underlying cellular mechanisms might be obtained from mutations that specifically affect these invaginations, but leave other morphogenetic aspects of gastrulation unaffected. Two such useful loci with effects on both the VF and PMG invaginations are *folded gastrulation* (*fog*) and *concertina* (*cta*). The *folded gastrulation* locus was originally identified by a zygotic lethal mutation (Wieschaus *et al.* 1984; Zusman and Wieschaus, 1985); in contrast, *concertina* is a maternal effect gene whose product is supplied by maternal transcription during oogenesis (Schüpbach and Wieschaus, 1989; Parks and Wieschaus, 1991). In spite of the difference in their genetics, the most obvious defect in both mutants is a failure to form a posterior midgut invagination. Both loci also affect the morphology of the ventral furrow.

The following paper presents a detailed comparison of VF and PMG formation in wild-type embryos. This morphological comparison confirms the basic similarity of the two invaginations, and limits the differences between them to minor variations in overall sequence, the extent of cell shape changes, and possible contributing mechanical forces. Both the VF and the PMG are initially recognized as a region of cells undergoing characteristic changes in surface morphology. Cell apices first become flat and then form blebs or ruffles as the cell diameters constrict. These changes are associated with subsequent alterations in the lengths of the cells and are followed by the invagination of each primordium, in a manner characteristic to its initial shape and location.

The effects of strong (amorphic) alleles of *folded*
gastrulation and concertina on each of these invaginations is discussed. Previous analysis of folded gastrulation regarded the ventral defects as secondary consequences of the more severe defects in PMG formation (Zusman and Wieschaus, 1985). In this work we show that in both fog and cta the VF defects precede those in the PMG and as such are not secondary consequences of the latter. The PMG is more strongly affected than the VF by both mutations, suggesting that the two invaginations may employ the two gene activities in a slightly different manner. We conclude that embryos affected by these two mutations are similar in their gross morphology and present a model with a role for these two genes in coordinating cell shape changes during the invagination process.

Materials and methods

Genotypes and stocks used

Analysis of wild-type gastrulation is based on embryos collected from wild-type Ore-R Drosophila stocks. In addition, embryos of normal morphology from the mutant crosses outlined below served as controls for staging and fixation artifacts in those particular experiments. Embryos that lack pole cells were obtained from females homozygous for the temperature-sensitive allele oskK261, which had been maintained at 18°C to ensure normal abdominal development (Lehmann and Nüsslein-Volhard, 1986). To obtain gastrulae lacking maternally supplied concertina product, embryos were collected from homozygous ctaRC1° mothers, which had been allowed to mate with their heterozygous or homozygous brothers. All embryos from this cross show the cta phenotype.

Some hemizygous folded gastrulation embryos were obtained from the heterozygous stock (yϕ6αfog4a6/FM7). In addition, to identify fog embryos unambiguously at early stages of gastrulation, we stained embryos from the following cross with an antibody to Sex lethal (Sxl) protein (Bopp et al., 1991):

C(1)DX, y f/Y × y ϕ6αfog4a6/FM7

y+Y mal126 is a Y-linked duplication carrying a wild-type allele of fog. All of the male embryos from this cross will be hemizygous for fog. They can easily be distinguished from their female siblings, which stain with Sxl antibody, and from the nullo- X embryos, which become abnormal during cellularization (Wieschaus and Sweeton, 1988).

For the detailed analysis of apical constriction in the ventral furrow presented in Fig. 4, embryos from mothers homozygous for the maternal effect mutation torso were used. Such embryos have ventral furrows that extend along the entire anterior–posterior length (Schüpbach and Wieschaus, 1986; Leptin and Grunewald, 1990), but are otherwise normal in their early stages. To obtain both wild-type and fog embryos from such females, the following cross was used:

C(1) DX, y f/Y × torsoPM31/torPM31 × y ϕ6αfog4a6/y+Y mal126

To identify the hemizygous fog embryos, embryos were stained with Sxl antibodies as above.

The concertina and folded gastrulation alleles used in this study are judged by genetic criteria to be amorphic. Hemizygous fog4a6 males are identical in phenotype to embryos hemizygous for deficiencies. The abnormalities observed in the progeny of homozygous ctaRC1° females are identical to those produced by insertion alleles which disrupt the coding sequence of the gene and are not significantly weaker than those produced by females heterozygous for ctaRC1° and a deficiency for the region (Parks and Wieschaus, 1991).

Observations of living embryos

To time the events of gastrulation, time-lapse videotapes were made of individual living embryos from a given cross at 400× following the procedure previously published (Wieschaus and Nüsslein-Volhard, 1986). Cellular movements during early gastrulation were also analyzed using epi-illumination and time-lapse recording as previously published (Merrill, Sweeton and Wieschaus, 1988).

Scanning electron microscopy

Embryos were collected at timed intervals, dechorionated, fixed with 25% glutaraldehyde in heptane and hand peeled in PBS. Embryos for cross section viewing in the SEM (scanning electron microscope) were cut in half after fixation, during hand peeling. Embryos were then post-fixed in 1% osmium tetroxide and dehydrated through an ethanol series. They were dried using Peldri II (Ted Pella Inc.), coated with gold palladium in a Denton Desk II sputter coater, and examined and photographed in a JEOL 840 SEM.

Sex lethal antibody (Bopp et al., 1991) was used to identify early fog embryos for scanning analysis. These embryos were fixed for 20 min in 4% formaldehyde and heptane. The vitelline membrane was then removed by shaking in heptane and methanol (Bopp et al., 1991). This fixation procedure is harsh and removes ruffles and blebs from the cell surface membrane. The embryos were stained with the standard procedures for Vectastain peroxidase kit (Vector Labs), and subsequently dehydrated and dried for SEM.

Sectioned material

Embryos sectioned for light and electron microscopic analysis were prepared following previously published protocols for fixation and embedding (Wieschaus and Nüsslein-Volhard, 1986). Light microscope sections were also made of embryos stained for twist antibody (gift from S. Roth) using the standard protocol for Vectastain kit (Vector Labs).

Results and Discussion

The ventral furrow is initiated immediately upon completion of cellularization of the ventral side

During the initial cleavage divisions in the Drosophila embryo, the nuclei replicate without intervening cytokinesis. After 13 rounds of division, there are approximately 6000 nuclei at the egg surface (Turner and Mahowald, 1976). By 15 min into the 14th cycle, a clear layer of cortical cytoplasm, which will be partitioned into cells, has formed. During the subsequent cellularization, membrane advances from the embryo surface between adjacent nuclei (Fullilove and Jacobson, 1971). When the membrane front reaches the centrally located yolk, cells are pinched off, although they retain cytoplasmic bridges to the underlying yolk (Rickoll, 1976). Due to a dorsal—ventral difference in the rate of cellularization, ventral cells finish cellularizing a few minutes before other regions of the embryo.

Ventral furrow formation begins immediately after the ventral-most cells have completed cellularization
(see also Campos-Ortega and Hartenstein, 1985; Leptin and Grunewald, 1990). In living embryos, the onset of gastrulation can be recognized by a subtle disorganization in the regular array of the ventral nuclei. This involves a slight basal shift in some of the nuclei (see below), which is detectable in time-lapse videotapes within a minute of the time when the membrane front on the ventral side of the embryo reaches the central yolky cytoplasm. Changes in nuclear position are observed in at least some cells of all sectioned material fixed at stages when the membrane has reached the yolk. Morphological changes associated with gastrulation are not observed in wild-type embryos prior to cellularization. Thus, it is possible that completion of cellularization may trigger the initial movements of gastrulation, or be a prerequisite for their initiation.

The primordium of the ventral furrow is initially defined by cell surface flattening

The first sign of ventral furrow formation is a flattened zone about 18 cells wide and 60 cells long on the ventral side of the embryo (Leptin and Grunewald, 1990; these results). Within this domain, cells lose their rounded apical surface or 'cobblestone' appearance and become more closely apposed along their entire length (Fig. 1A,B). In contrast, cells in other regions of the blastoderm do not adhere closely to each other in the apical 15% of their length.

Flattening of the ventral zone begins immediately after cells in this region have completed cellularization and thus appears to occur concomitantly with the shifts in nuclear position described earlier (Fig. 2A). Cells on the ventral midline begin to flatten slightly earlier than more lateral cells; this is possibly due to the dorsal-ventral difference in rates of cellularization. Consequently, when the flattened domain first becomes discernible, it consists of 12 to 15 cells in width, but soon widens to 18 cells. At this point the flattened domain corresponds to the same number of cells that strongly express the twist antigen (Fig. 2B) (Thiisse et al. 1988; Leptin and Grunewald, 1990). In the anterior-posterior direction, the domain initially extends from approximately 30 to 70% of the egg length. It later lengthens to include 20 to 80% egg length, thereby including some cells anterior to the cephalic furrow (Fig. 1E-K).

We propose that during flattening the extent of contact between adjacent cells remains constant and, therefore, the flattening of the ventral zone is due to a loss of apical membrane. This loss of membrane pulls the apical surface flat and obliterates the intercellular spaces. This model derives from the observation that the area of cytoplasm above the nucleus appears to initially decrease as the cell flattens. As flattening continues, the nuclei begin to shift basally and the bases of the cells enlarge and are flattened onto the yolk sac (Fig. 2B, 3A). These observations suggest that flattening exerts a pressure on the underlying nuclei and cytoplasm, pushing the contents of the cell basally. Moreover, in early stages single flattened cells are observed whose neighbors still have a cobblestone appearance. All these observations are inconsistent with a model proposed for other systems, in which a 'zipping up' of cell contacts causes flattening (Gustafson and Wolpert, 1962; Ettensohn, 1985), since zipping up would be expected to result in increased cytoplasmic area above the nuclei, and should not be possible in single cells. The cellular mechanism that causes the flattening of the surface is not clear. However, since a ruffling or blebbing of the overlying membrane is not observed at this stage, (as might be expected if flattening were produced by a constriction of the cortex underlying the apical surface of each cell), flattening may be due to a direct loss of apical surface, as would occur if apical membrane was internalized into the cell.

Within the ventral zone, apical constriction is restricted to a central domain and occurs in two phases

Once cells have flattened, membranous blebs or ruffles (bulbous processes, Turner and Mahowald, 1976) begin to form on the surface of an approximately 12-cell wide band in the center of the ventral domain (Fig. 1C-F). These 12 cells with blebs are the only ones in the flattened ventral domain that undergo apical constriction (Leptin and Grunewald, 1990; these results). The blebs that are observed on the surface appear when the apices of these cells constrict and, once present, these blebs remain. Other cells in the ventral domain, but lateral to the midventral region (lateral-ventral cells), remain unconstricted with smooth apical surfaces (Fig. 3B-D). The blebs may indicate that contraction of the apical cytoskeleton plays a major role in generating the force responsible for the cell shape changes that occur in the ventral primordium. This view is consistent with the localization of myosin and actin in the apical cortex of the cells (Kiehart et al. 1990; Young et al. 1991).

Within the midventral region, apical constriction and associated shape changes do not occur simultaneously in all cells. Instead, individual cells begin to constrict in an apparently stochastic manner (Fig. 1C,D). To reconstruct the spatial and temporal sequence of these contractions more accurately, we used a vigorous fixation procedure which removes the blebs and projections, facilitating the measurement of cell diameters (see Material and methods). During the initial stages of constriction, the distribution of contracting cells appears scattered. Cells closest to the ventral midline are slightly more likely to initiate constriction than more laterally situated cells (Fig. 4A,B). However, we saw no large-scale deviations from randomness in the pattern of contractions. In particular, no waves of constriction spreading from a single well-defined initiation center (Odell et al. 1981) were obvious. The apparently random cell shape changes continue until about 40% of the cells are constricted.

The initial phase of constriction is followed by an extremely rapid transition phase during which the remaining cells in the midventral zone constrict. The transition phase is so rapid that when a large collection of embryos was fixed during early gastrulation, extremely few were caught at intermediate stages of
Fig. 1. Scanning electron micrographs of ventral furrow formation. In the early phase of ventral furrow formation, the primordium is identified as a flattened zone (A,B). The midventral cells within this zone begin to constrict stochastically (C,D). As the cell apices constrict, membrane is extruded creating blebs on the surface (E,F). The outlines of constricting cells become lost beneath this blebbing (F). As more cells constrict, a shallow groove forms and the more lateral cells are drawn towards the ventral midline (G–J). As the ventral furrow forms, it extends anteriorly to the deepening cephalic furrow. The sides of the furrow are brought together as it invaginates into the interior of the embryo to give rise to mesoderm (K,L).
Fig. 2. Light microscope sections of ventral furrow and posterior midgut. Initiation of flattening in the ventral furrow primordium (A). Note the tightly apposed flattened surfaces and subtle nuclear shifts in the ventral zone defined by arrowheads, as opposed to the more lateral cells. Also note the asynchrony in the onset of constriction in neighboring cells (arrow). A slightly later stage, twist stained embryo (B) with 18–20 darkly staining nuclei paralleling the flattened domain. twist antibody also weakly stains two cells on either side of the band of 18–20 cells, bringing the total number of staining cells to 22–24. These additional two cells are eventually stretched toward the midline as the ventral furrow forms, but they remain ectodermal and later intercalate over the furrow. Their stretched appearance makes it difficult at midgastrulation stages to distinguish them from the marginal flattened zone, and contributes to the apparent increasing width of the flattened zone (from 18 to 22 cells) (see table in Fig. 5). Note the descending position of the nuclei at this stage. At the shallow groove stage (C) each nucleus has moved basally in correlation with the extent of the cell’s apical constriction. Note the intermediate stages of constriction. A sagittal section of a midgastrula (D) shows cells of the posterior midgut primordium with flattened surfaces located anterior and posterior to the pole cells. Margins of the flattened zone are indicated by the arrowheads. In a later stage gastrula, all the cells within the primordium are elongated when the PMG appears ledge-shaped (E). The cells beneath the pole cells are not constricting. Note also the differential position of the nuclei. Embryos homozygous for oskar (F), do not make pole cells. Subsequently, all cells within the posterior midgut primordium constrict their apices and shift their nuclei basally.

constriction with more than 40% of the cells constricted. Instead, in the next detectable stage, all the midventral cells have already begun to constrict and their apical surfaces have been pulled into a broad, shallow groove on the ventral midline (Fig. 4C,D). The shallow indentation formed in this second phase of constriction is visible in time-lapse videotapes about four to five minutes after the completion of cellularization (Fig. 5). At this stage, the constriction occurs predominantly along the medial–lateral axis, leaving
Fig. 3. Scanning electron micrographs of cell shape changes during ventral furrow formation. Gastrulation process: Stage 1 (A) flattening and initial random apical constrictions; Stage 2 (B,C,D) shallow groove associated with continued constriction and elongation; Stage 3 (E,F) cell shortening and invagination; Stage 4 (G,H) furrow internalization and closing.
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Fig. 4. Apical constriction in the ventral furrow. SxL stained fog⁺ and fog⁻ embryos from torso mothers were used to identify constricted cells in the ventral furrow. Constriction begins in random cells at early stages in wild-type embryos (A,B). In the tracings B, and F, dots mark cells which have reduced apical diameters of 3 μm or less in the shortest dimension. Cells with reduced apices in tracing D are easily distinguishable from the unconstricted cells. Within the sections of the midventral domain shown; B contains 39% constricting cells by this criterion, D has 86%, and F has 55%. The bar is equal to 10 μm. After a rapid transition, most cells in the midventral region have begun constriction, forming a shallow groove (C,D). Of the fog⁺ embryos analyzed: 19 had less than or equal to 40% constricted cells, 1 was 46% constricted, and 14 were in the shallow groove stage with approximately 90–100% constricted cells. For fog embryos, a more or less continuous percentage range is observed, but never are 100% of the cells constricted. Embryos mutant for fog show intermediate constriction phenotypes late in gastrulation (E,F). The fog embryo (E) is about the same age as the wild-type (C).

Each cell surface longer in anterior-posterior axis. This gives the cells an overall wedge-shaped rather than cone-shaped (or symmetrically pointed apex) morphology (Fig. 4C,D). This allows the furrow to contract into a groove without substantially decreasing its length. At earlier stages, apical constriction is uniform.
Fig. 5. Cell shapes change over time during gastrulation. The height of cells in the ventral furrow is based on examination of cross sections in the SEM. Embryos with similar morphology were grouped and their approximate age determined by comparison with time-lapse video tapes of living embryos. Timing of gastrulation events can vary from embryo to embryo by several minutes. Temperature can also affect the rate of development. These considerations, along with the rapidity with which early gastrulation events occur make it difficult to establish an absolute time line for cell shape changes in the ventral furrow. However, due to the small time window within which all these events occur, the margin for error remains only about two minutes. The onset of gastrulation (0 min.) was designated as the time at which the cell membranes reach the yolk sac on the ventral side of the embryo. Cell counts for all stages were compiled from 36 embryos viewed in cross section by SEM. twist staining cell counts for all stages were compiled from 180 sections viewed by light microscopy.

Around the entire cell circumference (Fig. 4A,B). The apparent polarity of the later stage constrictions may reflect mechanical constraints on the primordium, i.e. lateral cells may be less resistant to being pulled than anterior and posterior cells. The embryo's overall shape may also create structural constraints upon cell shape changes.

Apical constriction within the ventral furrow primordium initially results in shifts in nuclear position and cell elongation

As the apical diameters of cells in the midventral region continue to decrease, the density of blebs increases and obscures the outlines of single cells (Fig. 1E-H). Cell apices of varying widths are observed within the primordium in cross sections, suggesting that constriction is a gradual process (Fig. 3A,B). As the apical surfaces of the midventral cells constrict, the displaced cytoplasm contributes to an elongation of the cells (Figs 3B-D and 5). Upon completion of cellularization, the length of cells at the ventral midline is approximately 17% of the outer diameter of the egg. As constriction occurs, the cells lengthen to as much as 29% of the egg diameter (Fig. 5). Because of the elongation of the cells, apical constriction does not initially translate into a change from a columnar to a trapezoidal cell shape (Fig. 3A–D). In fact, the total
basal width of the midventral primordium does not increase until elongation has ceased.

In a manner associated with the movement of cytoplasm, the nuclei move basally (Fig. 2A–C). Although nuclei are first observed to shift during flattening, they do not drop very far during that process. Only when considerable apical constriction has occurred do the nuclei reach their full depth of about two-thirds cell length (Fig. 2C). All nuclei in the 12-cell midventral region appear to be fully descended when the apical diameters have decreased approximately 50%. This occurs approximately 6 min after the onset of gastrulation, as measured by correlating furrow depth in fixed embryos with time-lapse videotapes (Fig. 5). All cells that are not in the constricted midventral region retain their nuclei at approximately one-third of cell length from the egg surface.

We propose that the elongation of the cells and the shift in nuclear positions are passive responses to the constriction of the cell apices. This view is supported by observations of nuclear movements in the PMG (see below). Another apparent consequence of the apical constriction of the midventral cells is that the entire ventral side of the embryo is drawn towards the ventral midline. The apical ends of the lateral-ventral cells are 'bent' toward the ventral midline, while their basal regions retain their original positions and orientations (Fig. 3). These observations are consistent with a model in which apical constriction generates the force which pulls these cells toward the midline.

**Furrow invagination is associated with cell shortening**

The transformation of the ventral surface of the embryo from a shallow furrow to an invagination occurs very rapidly (between 8 and 11 min after the onset of gastrulation). It is associated with a shortening of the constricting cells of the midventral region. These cells, which had elongated to 29% of the diameter of the egg, shorten to approximately 15% of egg diameter by the time these invaginated cells have formed a tube (Fig. 5). Because the apical ends of the midventral cells remain constricted as the cells shorten, the resultant redistribution of cytoplasm would cause the basal ends of the cells to expand (Fig. 3E–G). We propose, therefore, that it is the lengthwise cell shortening that results in the cell shape change from columnar through trapezoidal to triangular. This transforms the shallow furrow into an invagination (Fig. 3G). Alternative models might regard the widening of the cell base as the active component and cell shortening as a secondary passive response.

Germband extension begins within a minute or two after the midventral cells begin to shorten, about 9.5 min after the onset of gastrulation. The furrow continues to invaginate deeper into the embryo as the germband extends. During this period, the average number of cell surfaces observed in the VF cross sections of fractured SEM samples decreases (Fig. 3G,H). This suggests that cells within the furrow are sliding by each other as extension occurs (Fig. 5).

The posterior midgut primordium undergoes cell shape changes similar to those that take place in the ventral furrow

The posterior midgut is formed by a disc-shaped primordium located at the posterior end of the embryo (Poulson, 1950). These somatic cells at the posterior pole finish cellularizing after ventral cells and before dorsal cells (Fig. 2D). As in the ventral furrow, cell shape changes begin immediately upon completion of cellularization. The cells of the posterior midgut primordium, like those of the ventral furrow primordium, first become distinct by the loss of a cobblestone-like appearance due to the flattening of the apical surface (Figs 2D, 6A,B). As in the VF there is a shift in nuclear position associated with this surface flattening. The cells of the PMG also subsequently undergo apical constriction which coincides with the formation of membranous blebs on the cell surfaces (Fig. 6C,D). Apical constriction in the posterior midgut primordium is initially associated with an elongation of the cells and a slight widening of their bases (which were initially thinner than their apices) causing them to become evenly columnar. Based on time-lapse videos, the length of the PMG cells appears to increase by approximately 38% from the completion of cellularization to the migration of the PMG dorsally to form a horizontal ledge upon which the pole cells sit (Fig. 2E). For comparison, the cell length change in the ventral furrow cells is about 50%. The apical constriction of the PMG cells is also associated with a basal movement of the nuclei. As in the ventral furrow, when the apical diameter of PMG cells has been reduced by approximately 50%, the nuclei have descended their full extent, about two-thirds the length of the cell. As the apical constriction of cells dorsal to the pole cells continues, this region of the PMG domain begins to curl or indent, in a manner analogous to the shallow groove observed during the corresponding stages of ventral furrow formation. The cells adjacent to constricting regions are stretched at their apical surfaces like the lateral-ventral cells of the ventral domain (Fig. 6C–F). Like VF cells, the PMG cells also shorten once they have elongated.

The major structural differences between the posterior midgut and the ventral furrow are that the PMG primordium contains fewer cells and is circular in shape. Moreover, the initial PMG apical constrictions are focused in a narrowly defined region of the flattened primordium and then progress in a defined pattern (Fig. 6). This is unlike the rapid constriction transition of the entire midventral region of the VF domain. The first cells to constrict in the PMG primordium are those closest to the dorsal side of the pole cell cluster (Fig. 7A). Later, constrictions are observed in the other dorsal cells of the primordium (Fig. 7B), then laterally across the PMG domain, and eventually ventral to the pole cells. These last, posterior-most cells of the primordium begin to constrict 14 min into gastrulation. The delay in constriction of the ventral portion of the PMG may, in part, reflect constraint on the cells in this
Fig. 6. Scanning electron micrographs of posterior midgut formation. Flattened cells of the posterior midgut primordium (A,B) still retain early surface filaments making cell outlines indiscernible. At a slightly later time (C,D), membranous blebs are indicative of the apical constriction of cells adjacent to the pole cells (arrowhead) and at midpoint in the PMG domain dorsal-anterior to the pole cells (arrow). As apical constriction continues, dense blebbing is observed in a circle around the pole cells (E,F) as the posterior midgut takes on a characteristic ledge shape (see Fig. 2E). Posterior midgut begins transformation into a cup-shaped invagination (G,H) as its ventral margin moves onto the dorsal side of the embryo as a result of germband extension. The hindgut and posterior end of the ventral furrow have begun to close over. As the germband continues to extend, the posterior midgut becomes internalized and starts to close over (I,J). The elevated and unconstricted surfaces of the central cells of the PMG can be seen where pole cells have been mechanically removed (K). In contrast, an oskar embryo (L) at a similar age shows all cell surfaces in the PMG constricting.
Fig. 7. Embryos mutant for folded gastrulation and concertina fail to make a posterior midgut. The process of flattening and constriction is visible in vigorously fixed early and later wild-type embryos (A,B). A fog embryo (C) of similar age to (B) shows the failure of folded gastrulation mutants to constrict cells in the PMG. Embryos A–C were Sxl stained for unambiguous fog identification. Dorsal views of cta gastrulae (D,E,F) and fog gastrulae (H,I,J). Lateral views at later gastrulation stages of cta (G) and fog (K), show a similar characteristic 'folded' phenotype.
region of the embryo by the invaginating ventral furrow (Figs 1K, 2E).

Another difference between the two invaginations is that PMG cells never constrict to a point and remain broader at their apices than do VF cells. The posterior pole is drawn on to the dorsal side by the combination of germ band extension, the contraction of the embryo's dorsal side (Rickoll and Counce, 1980), and the apical constriction of the dorsal-most cells of the PMG. This transformation of the posterior of the embryo from a convex curve into a ledge shape occurs about 14 min into gastrulation (Fig. 6E–H). It follows the start of germ band extension by 4.5 to 5 min. As germ band extension continues on the ventral side of the embryo, the posterior end of the germ band is brought up and over the PMG primordium, bringing the PMG into the interior of the embryo (Fig. 6I). At this time, the elongated cells of the PMG begin to shorten. Although this shortening is similar to that occurring in the ventral furrow, the simultaneous elongation of the germ band makes it difficult to determine the role of cell shortening in the invagination of the midgut.

The PMG precursors beneath the pole cells do not undergo the same cell shape changes as the rest of the cells in the posterior midgut primordium

At the onset of gastrulation, the pole cells sit in a shallow 'nest' of somatic cells at the posterior pole of the embryo. When the somatic cells of the PMG flatten, the pole cells jut out from their nested position and sit prominently upon the surface of the flattened cells (Figs 2D, 6A,B). Although the PMG precursors beneath the pole cells flatten, their apices fail to constrict and their nuclei do not move basally (Figs 2E, 6K). However, in spite of their failure to constrict these cells elongate, but become thin at the base unlike the other cells of the PMG. It is possible they are stretched passively as the yolk sac is being pushed away from them by the elongation of the constricting cells around them. These cells retain their unconstricted morphology until the PMG has moved dorsally and assumed a cup shape, at which time all the cells of the PMG begin to shorten. The pole cells no longer appear attached to their underlying somatic cells and the latter no longer constrict their apices to the same extent as their neighbors (not shown).

To test if the pole cells hinder the initial constriction of the cells upon which they sit, we examined gastrulation in embryos which do not make pole cells. They were obtained from homozygous oskar mothers (Lehmann and Nüsslein-Volhard, 1986). In these embryos, all the cells of the posterior midgut primordium produce dense blebs, constrict their apices, and shift their nuclei basally to the full extent (Figs 2F, 6L). This result is consistent with a model where pole cell attachment normally prevents apical constriction. Moreover, it also suggests that nuclear migration is a passive response to constriction, since only if and when cells in the center of the primordium are allowed to constrict do their nuclei move basally.

Mutations in folded gastrulation and concertina produce very similar effects on the ventral furrow and posterior midgut

Putative null mutations in concertina and folded gastrulation cause very similar abnormalities during gastrulation. Mutant embryos make normal cellular blastoderms and initiate gastrulation normally. The cells on the ventral side form a flattened zone identical in morphology to that of the wild-type. They also show normal expression patterns of twist protein (Parks and Wieschaus, 1991; M.C., data not presented), indicating that the genes probably do not affect dorsal–ventral patterning. After the ventral domain of mutant embryos has flattened, apparently random cells within that domain begin to constrict apically as in the wild-type. These constricted cells are more frequent near the ventral midline and are restricted to the same central domain of 12 cells (Fig. 8). These results suggest that the ventral primordium and its subdomains are established normally.

In contrast to wild-type embryos, this initial phase of constriction is not followed by a more rapid phase of initiating constriction in the remaining cells. In fog embryos, random isolated cells in the midventral region continue to initiate constrictions in the same slow pattern characteristic of the initial phase (Fig. 8G–J). In cta embryos, it is probable that these second phase constrictions occur at an even slower rate than in fog embryos (Fig. 8A–D). The absence of a rapid phase of constriction results in many mutant embryos with intermediate levels of constricted cells only rarely observed in the wild-type (greater than 40% but less than 100%) (Fig. 4E,F). In both mutants, many cells in the midventral domain never constrict. An abnormal ventral furrow eventually forms, but closes over later than in the wild-type and often non-uniformly along its length, since the uncorrected cells seem to impede its closing (Fig. 8).

The phenotypes of cta and fog in the ventral furrow are qualitatively identical. The cta phenotype is, however, stronger than that of fog. The difference is frequently obvious in the morphology of fixed embryos and reflects the fact that more cells remain unconstricted in cta embryos than in fog embryos. In fog mutant embryos, the normal number of high-level twist expressing cells are usually brought into the invagination, whether or not they are constricted apically. In cta embryos, however, many of the more lateral twist expressing cells never invaginate. There is also greater time delay in ventral furrow invagination. In time-lapse videotapes of living gastrulae from cta mothers, the ventral furrow becomes visible about 12–13 min after the completion of ventral cellularization, compared with 4–5 min in the wild-type. The values obtained for fog embryos are intermediate: 8.5 min. The difference is not one of allelic strength since the fog allele used in this study produces a phenotype identical to chromosomal deletions that eliminate the fog gene.

In addition to their effects on ventral furrow formation, both cta and fog cause abnormalities in posterior midgut formation (Fig. 7D–K). In contrast to
Fig. 8. Mutations in concertina and folded gastrulation cause abnormal ventral furrow formation. Defects in the formation of the ventral furrow are similar for both concertina (A–F) and folded gastrulation (G–L). Note the prominent margins of the ventral zone (D,J).

the ventral furrow, the effects of both mutations are equally strong. The PMG primordium flattens normally and cells immediately anterior to the pole cells begin to constrict as in the wild-type (Fig. 7C). However, the number of constricted cells elsewhere in the PMG remains very small. There is no invagination of the
PMG in these mutants, and the entire primordium remains a flat plate of cells on the embryo surface. Thus the absence of cell shape changes and the morphological consequences to the embryo are more severe in the PMG than in the ventral region of the embryo.

Conclusions

In wild-type development, the first morphological changes in gastrulation occur after the embryo has been subdivided into distinct domains that give rise to mesoderm and the posterior endoderm. During subsequent development the cells in the two domains show common behaviors that result in characteristic morphogenetic movements.

Flattening does not occur synchronously in all the cells within the primordium. Similar asynchrony is observed in other processes during gastrulation, e.g. the initiation of apical constriction in the VF and PMG. Although there is a bias towards earlier constriction of the cells close to the ventral midline and in the cells adjacent to the cells beneath the pole cells, there is no obvious global pattern such as a wave of constriction from any single initiation point. Instead, at the single-cell level, the behaviors appear quite stochastic. This stochastic behavior is observed in the initial flattening of the VF primordium and becomes even more prominent during the initial apical constriction which represents the first phase of both ventral furrow and posterior midgut formation. This behavior may reflect local variability in the synthesis of gastrulation-specific components. The asynchrony has no obvious functional significance, although it does argue that many of the processes associated with Drosophila gastrulation can occur in single cells.

Our observations suggest that in both primordia, the process alternates between slow periods of gradual change and periods of rapid transition. We have divided the process into four phases: (1) flattening and initial random apical constrictions; (2) a shallow groove stage associated with increased constriction and cell elongation; (3) cell shortening and the subsequent invagination; (4) closing over of the invagination and germband extension. Each of the first three phases is separated from the phase that preceded it by a rapid transition. These rapid transitions were recognized in part because it is difficult to catch embryos at intermediate morphological stages.

What controls and coordinates these transitions may be different for each one. The rapid loss of the cobblestone appearance associated with transition to the first phase (flattening) may be induced by the completion of cellularization. It is also possible that flattening is permissible only when the mechanical stresses associated with cellularization are absent. During the flattening phase some cells have already begun apical constriction. In such embryos, we almost exclusively detect ventral zones in which less than 40% of the cells have begun to constrict. In the transition to phase 2, the remaining cells constrict. The rapidity of this transition suggests that once a sufficient fraction of the cells has begun to constrict apically, the probability of the remaining cells initiating constriction is enhanced.

It is this rapid transition to the groove stage that is affected in fog and cta mutants. It is attractive to interpret this defect in the light of the recent finding that cta encodes a putative alpha subunit of a G-protein (Parks and Wieschaus, 1991). Thus the phenotype of cta (and by analogy, that of fog) may result from a failure of intercellular communication during gastrulation. However, even if we extrapolate from the G-alpha protein homology to a role for cell communication in the constriction process (Stryer and Bourne, 1986), the morphological data presented in this paper does not identify the source of the signal or its immediate target. For example, in the VF, the source may be the constricting cells, and their targets may be the cells that have not yet constricted, as proposed by Parks and Wieschaus (1991). Alternatively, the signal for the transition might be a diffusable cue from elsewhere in the embryo, which would initiate the rapid constriction of the remaining cells. Moreover, since the behavior of the ventral contracting region can be influenced by cells in other areas of the embryo, the target of the signal might even be outside the VF and PMG primordia.

Any model would have to accommodate two additional observations. (1) Both cta and fog have more adverse effects on the posterior midgut than the ventral furrow, since almost none of the PMG cells constrict. This would suggest that more PMG cells than VF cells are dependent on these genes to constrict. (2) In the ventral furrow, the phenotype of cta is stronger than fog, even though both mutants are nulls. One possible explanation for the difference is that cta is absolutely required in the VF and that other gene products can partially substitute for fog.

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