

Cleavage and Gastrulation in *Drosophila* Embryos

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The cytoskeleton guides early embryogenesis in *Drosophila*, which is characterized by a series of rapid synchronous syncytial nuclear divisions that occur in the absence of cytokinesis. Following these divisions, individual cells are produced in a process called cellularization, and these cells are rearranged during the process of gastrulation to produce an embryo composed of three primordial tissue layers.

Drosophila's Unusual Syncytial Blastoderm: an Overview

Like many other insects, early embryonic development in the fruitfly *Drosophila melanogaster* is rapid and occurs in a syncytium. The first 13 nuclear divisions are completed in just over 3 hours and occur in the absence of cytokinesis, producing an embryo of some 6000 nuclei in a common cytoplasm. At the interphase of nuclear 14, these nuclei are packaged into individual cells in a process known as cellularization. Soon after cellularization is completed, gastrulation commences. This rapid development is made possible by several attributes of the early embryo. First, the embryo is endowed with an abundance of maternally supplied products, which allows it to bypass zygotic transcription during the first several divisions. Transcription is not required until interphase of nuclear cycle 14. Second, nuclear division is uncoupled from cytokinesis. Third, the nuclear division cycles alternate between M and S phases, with extremely abbreviated G₁ and G₂ phases. Thus, the initial division cycles proceed rapidly, ranging from 10 to 25 minutes, compared to the typical cell cycle duration of 24 hours.

Once fertilization and fusion of the male and female pronuclei occur, the syncytial cycles are initiated (**Figure 1**). During these divisions, the nuclei undergo a precisely orchestrated pattern of migration and movements. During the first three divisions, the nuclei remain clustered in a ball at the anterior third of the embryo. During nuclear cycles 4 to 6, in a process called axial expansion, the nuclei become evenly distributed along the length of the embryo. Nuclear migration to the cortex begins during nuclear cycle 8 and the first nuclei arrive at the posterior end of the cortex at nuclear cycle 9. These nuclei are the germline precursors and push through the plasma membrane to form pole cells during nuclear cycle 10. Most of the remaining nuclei arrive synchronously at the cortex during interphase of nuclear cycle 10. These cortical nuclei undergo four more divisions

and finally cellularize during interphase of nuclear cycle 14. Immediately following completion of cellularization, gastrulation is initiated with the formation of the head and ventral furrows.

Space is limited during the syncytial divisions. This problem is particularly acute during the cortical divisions when thousands of nuclei are rapidly dividing in a confined monolayer. In spite of the crowding, embryogenesis is an extremely precise process. Much work over the last decade indicates that this is in large part due to a dynamic and responsive cytoskeleton guiding both the nuclear division cycles and morphogenesis. This review highlights classic and recent studies that illuminate the mechanisms driving these events.

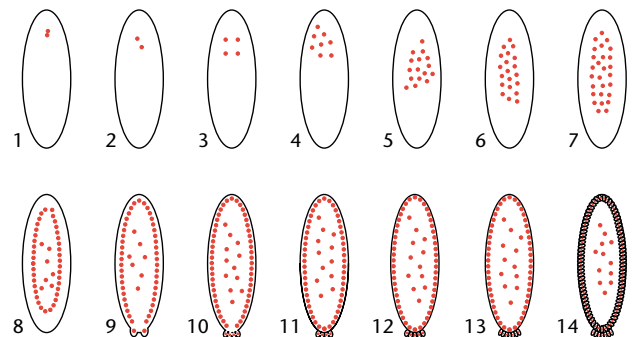


Figure 1 Early nuclear divisions and migration during *Drosophila* embryogenesis. Cycle 1 is initiated after fusion of the male and female pronuclei. During divisions 1–3, nuclei divide in a sphere at the anterior of the embryo. During divisions 4–6, nuclei divide and spread out along the anterior–posterior axis (axial expansion). Nuclei migrate to the cortex of the embryo during divisions 8–10 (cortical migration). Pole cells form at the posterior end of the embryo (cycle 9), while yolk nuclei remain in the interior. After most of the cortical nuclei complete four mitotic divisions, they are surrounded by membranes that invaginate from the surface and the cellular blastoderm is formed.

Introductory article

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Fertilization and the Initiation of Mitotic Cycling

After mating, a *Drosophila* female typically stores 300–500 sperm in specialized sperm storage organs called the spermathecae and seminal receptacle. This supply of sperm enables the female to fertilize eggs for up to 2 weeks without remating. Mature oocytes are individually fertilized in the uterus. Sperm are released from the storage organs and enter the egg through a special opening at the anterior end of the egg called the micropyle. Although egg activation and fertilization occur simultaneously, egg activation does not depend on sperm entry but rather on dehydration of the egg. Completion of meiosis produces four haploid nuclei arranged perpendicularly with respect to the plasma membrane. The innermost nucleus migrates towards the interior while the remaining polar bodies form a cluster at the cortex encompassed by microtubules. Migration of the female pronucleus relies on the plus-end directed motor protein KLP3A, which may play a role in pushing the female pronucleus away from the polar body cluster. Pronuclear migration may also involve plus-end directed migration of the male pronucleus along the astral microtubules.

The first mitotic division in *Drosophila* is known as a gonomeric division because the maternal and paternal genomes do not become integrated until after anaphase. Once the male and female pronuclei reside next to each other, the newly assembled centrosomes separate to form the poles of the spindle. This is immediately followed by partial nuclear envelope breakdown and entry into metaphase. Large sections of the nuclear envelope remain intact, however, effectively separating the maternal and paternal chromosome complements. In addition to genetic material, the sperm also donates a centriole which nucleates maternally supplied centrosomal proteins to form the zygotic centrosome. Some of the maternally supplied components include centrosomin (Cnn), CP60, CP190 and gamma tubulin. In spite of its conspicuous presence, the role of the centrosome during the initial mitotic divisions remains unclear as embryos lacking functional centrosomes still organize spindles and proceed through mitosis (see next section).

Preblastoderm

Following pronuclear fusion the nuclei undergo four synchronous divisions in the anterior third of the embryo. During these early divisions, the centrosome plays a key role in generating astral microtubules that orient and prevent collisions between neighbouring syncytial nuclei. Embryos containing defective centrosomes that fail to produce the robust astral microtubule arrays develop spindles and undergo several rounds of mitosis but large

numbers of fused nuclei are often observed. This phenotype is similar to that of unfertilized *Sciara* embryos. *Sciara* embryos undergo syncytial development very similar to that of *Drosophila*. Unfertilized *Sciara* embryos lacking centrosomes form kinetochore microtubules and pole-to-pole microtubules, but not astral microtubules. The initial divisions occur normally, but by the third and fourth nuclear cycles, neighbouring telophase nuclei often collide and fuse. This suggests that as nuclear density increases, astral microtubules serve as fenders to prevent collisions. Supporting this interpretation is the fact that these collisions occur primarily during telophase when the astral microtubules are most prominent. The astral microtubules also may function to establish overall nuclear orientation and distribution. Even in the initial divisions prior to extensive nuclear fusions, the unfertilized embryos exhibit abnormal nuclear configurations.

Following nuclear cycle 4, nuclei undergo two distinct patterns of movement: axial expansion followed by cortical migration. At nuclear cycle 4, eight nuclei form a sphere at the anterior end of the embryo. During the next two cycles, the nuclei move axially to produce an even distribution interiorly along the length of the embryo. This process is sensitive to cytochalasin but not colcemid, indicating that the underlying mechanism requires microfilaments and not microtubules. Supporting this conclusion is the fact that expansion occurs during prophase and metaphase when astral and interpolar microtubules are minimal.

Syncytial Blastoderm and Nuclear Migration

At nuclear cycle 7, the nuclei are evenly spaced in an ellipsoid in the interior of the embryo. During the next two nuclear cycles, the ellipsoid expands, driving the nuclei towards the plasma membrane. Expansion of the ellipsoid occurs only during telophase at a time when each nucleus contains a centrosome pair with extensive astral microtubule arrays. In contrast to axial expansion, microtubule inhibitors, but not microfilament inhibitors, disrupt cortical nuclear migration. These findings lead to a model in which the expansion force is generated through plus-end directed motors acting on neighbouring arrays of overlap microtubules. In this model, interactions between the centrosome-based astral arrays provide the force for migration. The nuclei do not play an active role in cortical migration; they are simply ‘passengers’ on the migrating centrosomes. This is in accord with observations in which centrosomes unassociated with nuclei migrate to the cortex. Currently, no microtubule-based motors have been implicated in the process of cortical migration.

Establishment of the yolk nuclei occurs during nuclear cycles 8 and 9. As described above, while the majority of the

nuclei are migrating towards the cortex, a set of nuclei remain in the interior. These are destined to become the yolk nuclei and initially they divide in synchrony with the migrating nuclei. Once the somatic nuclei reach the cortex, the yolk nuclei delay entering telophase of nuclear cycle 10 and their centrosome cycle becomes disrupted. As a result, the yolk nuclei become polyploid and then cease to divide at about nuclear cycle 11. Subsequently, the yolk nuclei no longer undergo mitotic divisions and their number remains at about 200 from nuclear cycles 11 through 14. Why these nuclei fail to migrate is unclear, but careful observation suggests that they were initially part of the ellipsoid of expanding nuclei that had receded into the interior. Although yolk nuclei are a conserved feature of insect development their function remains unknown.

Pole Cell Formation

While the majority of nuclei reach the cortex during interphase of nuclear cycle 10, a few nuclei reach the posterior cortex during interphase of nuclear cycle 9. These nuclei exert an outward force on the plasma membrane, producing localized protrusions known as pole buds. During telophase of this cycle, the buds pinch off and form a cluster of posteriorly localized pole cells, the germline precursors. Special properties of the cytoplasm limit these events to the posterior pole. The cytoplasm at the posterior pole contains morphologically distinct polar granules consisting of RNA and protein. Transplantation of this cytoplasm is sufficient to generate ectopic pole cell formation. The posterior pole also contains extra plasma membrane in the form of extensive microvillar projections and high levels of contractile components such as myosin. This suggests that the posterior region of the cortex is primed to form cells requiring only the proper stimulus.

The Cortical Divisions and Cellularization

After nine divisions in the interior of the embryo, syncytial divisions 10–13 occur in the actin-rich cortex just beneath the plasma membrane. Prior to the arrival of the nuclei and their tightly associated centrosomes, the actin is homogeneously distributed. The migrating nuclei and their associated centrosomes induce a dramatic redistribution in the actin (**Figure 2**). During interphase, the actin concentrates into apical caps centred above each cortical nucleus and its apically positioned centrosomes. As the nuclei progress into prophase, the centrosomes migrate toward opposite poles and the actin caps undergo a dramatic redistribution to form an oblong ring outlining each nucleus and its associated separated centrosome pair. At metaphase, the furrows invaginate to a depth of approximately 10 μm to form a half shell encompassing each spindle. (d and e) During late anaphase and telophase, the metaphase furrows rapidly regress.

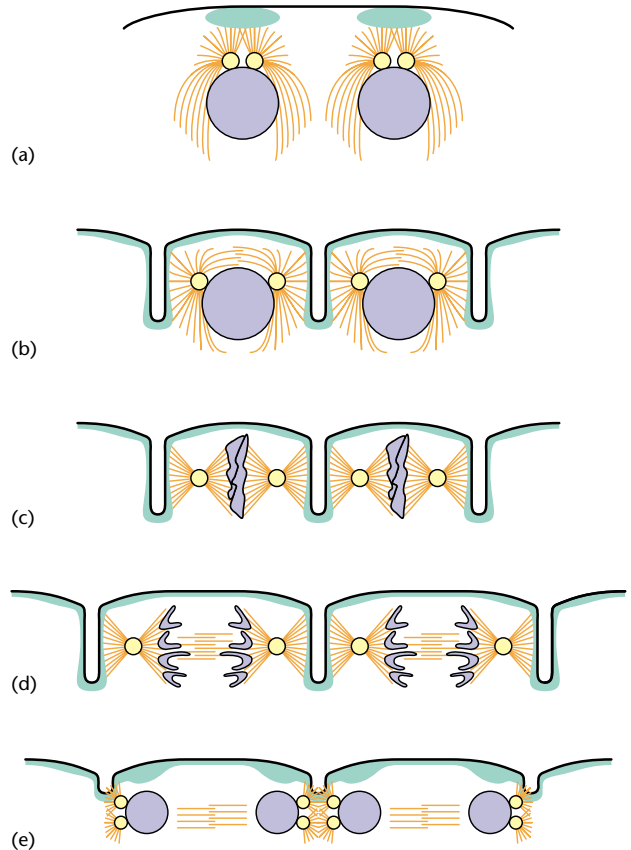


Figure 2 Metaphase furrow formation. (a) During interphase, the actin (green) concentrates into apical caps centred above each cortical nucleus (blue) and its apically positioned centrosomes (yellow). (b) As the nuclei progress into prophase, the centrosomes migrate toward opposite poles and the actin caps undergo a dramatic redistribution to form an oblong ring outlining each nucleus and its associated separated centrosome pair. (c) At metaphase, the furrows invaginate to a depth of approximately 10 μm to form a half shell encompassing each spindle. (d and e) During late anaphase and telophase, the metaphase furrows rapidly regress.

These rings are equivalent in composition to conventional cytokinesis contractile rings and include actin, myosin II, spectrins, anillin, septins and formins. In addition, they are closely associated with the plasma membrane and are required for invagination of furrows around the spindles (these are referred to as metaphase or pseudocleavage furrows). At metaphase, the furrows invaginate to a depth of approximately 8 μm to form a half shell encompassing each spindle. During late anaphase and telophase, the metaphase furrows rapidly regress. Centrosome duplication occurs during late anaphase and the newly formed centrosome pairs are again located apically in the next interphase and the actin caps reform. The centrosome pair serves as a focus for the reforming actin caps.

This alteration between interphase actin caps and metaphase furrows occurs through interphase of nuclear cycle 14. At this point, the nuclei remain in interphase and

cellularization occurs. Furrow formation relies on an inverted microtubule basket that originates from an apically positioned centrosome pair and encompasses the elongated interphase nuclei (Figure 3). These microtubules guide invagination of the cellularization furrows through an initial slow phase and a second fast phase. Once the

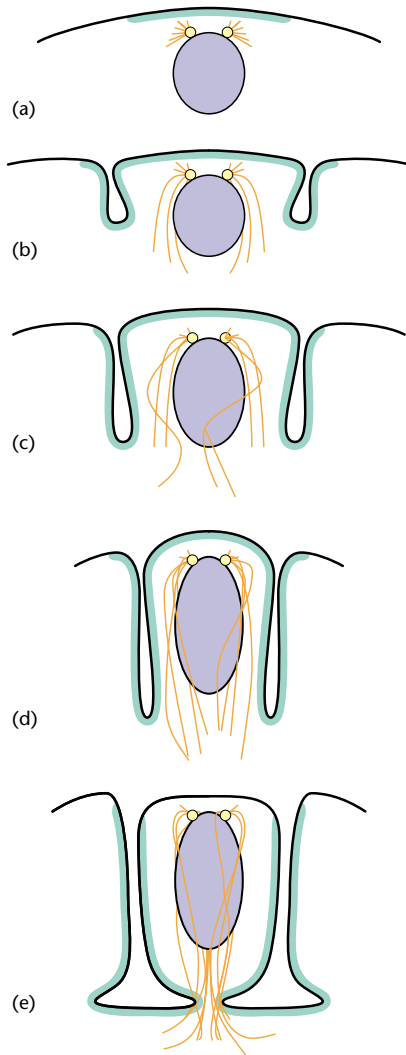


Figure 3 Formation of the cellularization furrows. (a) At $t = 0$, cellularization begins with actin (green) concentrated at the cortex above each nucleus and apical centrosome pair (yellow). Aster microtubules are present (red). (b) At $t = 10$, the plasma membrane starts to invaginate and actin is concentrated at the cortex and the leading edge of the invaginating furrow. Microtubules rapidly elongate towards the cytoplasm. (c) $t = 20$, slow phase. Microtubules have elongated further into the cytoplasm forming a basket-like structure around the elongating nucleus. Actin is still concentrated at the cortex above the nuclei and along the invaginating furrow. (d) $t = 40$, rapid phase. Furrows invaginate rapidly once they have passed the fully elongated nuclei. Towards the conclusion of cellularization. (e) $t = 60$, furrows have fully extended to the inner yolk layer within the cytoplasm and begin to pinch off using a contractile ring composed of actin and other furrow components.

furrows reach a depth of about $35\ \mu\text{m}$ they pinch off to form individual mononucleate cells.

A key difference between these specialized forms of cytokinesis and conventional cytokinesis is the timing of furrow formation (Figure 4). Conventional cytokinesis furrows form during anaphase and telophase. In contrast, metaphase furrows are present from early prophase through anaphase. Cellularization furrows form during the prolonged interphase of nuclear cycle 14. The difference in the timing in which the furrows are established results in a profound difference in the position of the furrow with respect to the centrosome/spindle complex. Establishment of a conventional cleavage furrow occurs after sister centrosomes have separated and a bipolar spindle has formed. The furrow forms at a point midway and perpendicular to the centrosome/spindle axis. In contrast, metaphase and cellularization furrows are

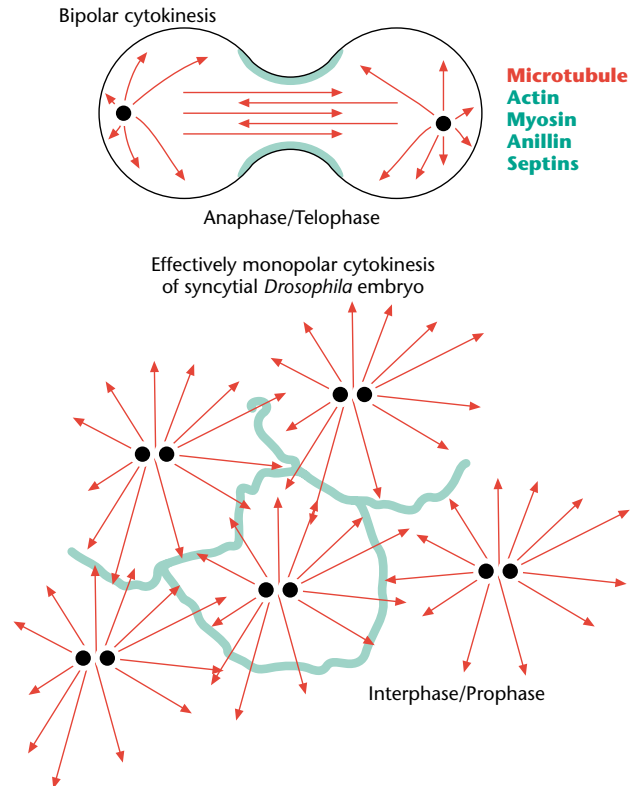


Figure 4 A key difference between metaphase furrows and conventional cytokinesis is the timing of furrow formation. (a) In conventional cytokinesis, furrow formation occurs during anaphase and telophase and after sister centrosomes have separated and a bipolar spindle has formed. The furrow forms at a point midway and perpendicular to the centrosome/spindle axis. (b) Metaphase furrows are present from early prophase through anaphase and are established prior to the separation of sister centrosomes. Consequently they are established around effectively monopolar microtubular arrays. The difference in furrow position may be a direct result of a difference in the pattern of microtubular arrays between a monopolar and bipolar systems.

established prior to the separation of sister centrosomes. Consequently, they are established around effectively monopolar microtubular arrays. The difference in furrow position may be a direct result of a difference in the pattern of microtubular arrays between a monopolar and bipolar system. In spite of this timing difference, it is likely that many of the mechanisms that establish and drive furrow progression are shared between the two systems. Both processes are precisely coordinated with the cell cycle and result in the invagination of the plasma membrane. In both, actin is closely associated with the plasma membrane and rearrangements in the actin cytoskeleton are essential for plasma membrane invagination.

Rapid Nuclear Division and the Cell Cycle

A number of studies demonstrate that cell cycle checkpoints are present and operational during the syncytial cortical divisions. This is unexpected because checkpoint-induced cell cycle delays would disrupt division synchrony necessary for normal syncytial development. However, in contrast to checkpoints that rely on delays to correct the division errors, it appears that many of the checkpoints operating in the syncytial embryo rely on cell cycle delays to eliminate abnormal division products. For example, in embryos bearing an abnormally long chromosome or in X-irradiated embryos, individual nuclei often delay anaphase initiation. The telophase products of these delayed nuclei recede into the interior and are removed from the dividing population of cortical nuclei. When these nuclei recede into the interior their centrosomes remain on the cortex. The net result is an efficient mechanism of eliminating abnormal nuclei and maintaining the integrity of the dividing population of cortical nuclei.

Overview of Gastrulation

Gastrulation is initiated immediately after cellularization is completed and requires massive, coordinated movements that transform the monolayer of cells into layers that will become endoderm, mesoderm and ectoderm. These are the tissue primordia from which the larval organs will arise. Endoderm will give rise to the midgut; mesoderm to muscles; and ectoderm to epidermis, central nervous system and fore-and hind-gut. By the time gastrulation begins, the developmental fates of many cells have already been specified (**Figure 5**). For example, the ventral-most cells give rise to mesoderm; the lateral cells to ectoderm; and the cells at the posterior end to endoderm. Through the morphogenetic movements of gastrulation, cells that contribute to mesoderm and endoderm are brought to

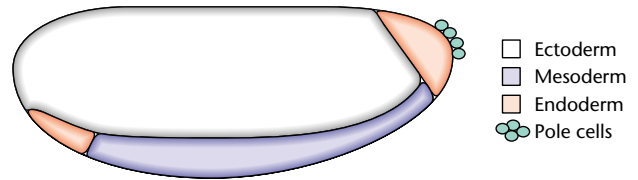


Figure 5 Fate map of the blastoderm, prior to gastrulation; anterior is to the left and dorsal on top. Ectoderm gives rise to epidermis, nervous system, and fore-and hind-gut; mesoderm to muscles; endoderm to midgut; and pole cells to the germline. This diagram is modelled after figure 1a in Leptin M (1999) *EMBO Journal* 18: 3187–3192.

the interior of the embryo by invagination and cells that contribute to ectoderm are spread over the surface of the embryo through cell migration and rearrangement.

Cell Shape Changes

Cell shape change is the driving force behind many of the morphogenetic movements that occur during gastrulation. At the end of cellularization, all somatic cells are columnar with apically located nuclei. Cells that are brought into the embryo interior through invagination undergo a stereotypical series of changes in shape: apical flattening, apical constriction, and cell shortening (**Figure 6**). During apical flattening, the apical cell surface decreases in area and the extent of cell–cell contact between neighbouring cells increase. The function of apical flattening is unknown. It may be required for apical constriction or tethering of the cells that will invaginate. Apical constriction results in elongation of the cells and displaces the nuclei basally. Importantly, the constriction of only a subset of cells produces a force that pulls neighbouring cells toward the area of constriction, producing a shallow groove. The cells then shorten and expand laterally, becoming wedge shaped. This expansion produces the force that causes the initial groove to invaginate.

Cell Movements

Like other events during embryonic development, gastrulation is highly dynamic, involving vast cell movement and rearrangement. As discussed above, the end result of gastrulation is the internalization of endodermal and mesodermal precursors and the spreading of ectodermal precursors over the surface of the embryo. This is accomplished by three major cell movement events: ventral furrow invagination, posterior midgut invagination and germ band extension. The majority of mesodermal cells are internalized via ventral furrow invagination while endodermal cells are internalized via a combination of ventral furrow invagination and posterior midgut invagination.

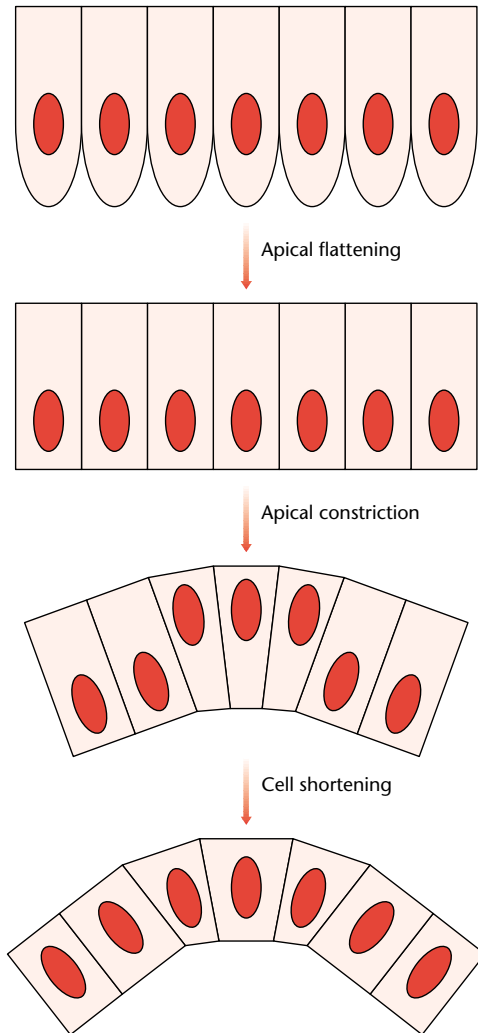


Figure 6 Cell shape changes drive ventral furrow and posterior midgut invagination. The cells are initially columnar with apically located nuclei. First, apical flattening increases cell–cell contact between neighbours. Then, a subset of cells constrict at their apical ends, causing the cell sheet to dimple and form a groove. Finally, the cells shorten and become wedge-shaped, pulling further neighbouring cells toward the area of constriction, and thus forming the invagination.

The ectodermal cells are transported to their final destinations via germ band extension.

The ventral furrow is the first visible landmark of gastrulation. It arises from a strip of cells at the ventral midline. As described above, the cells in this region undergo a sequence of cell shape changes that produce a groove in the region. Further cell shape changes deepen the groove, forming an open tube. At its completion, the edges of this invagination seal to internalize the tube of cells. The posterior midgut invagination begins soon after the ventral furrow closes. Like ventral furrow formation, invagination requires sequential cell shape changes. While the cell shape

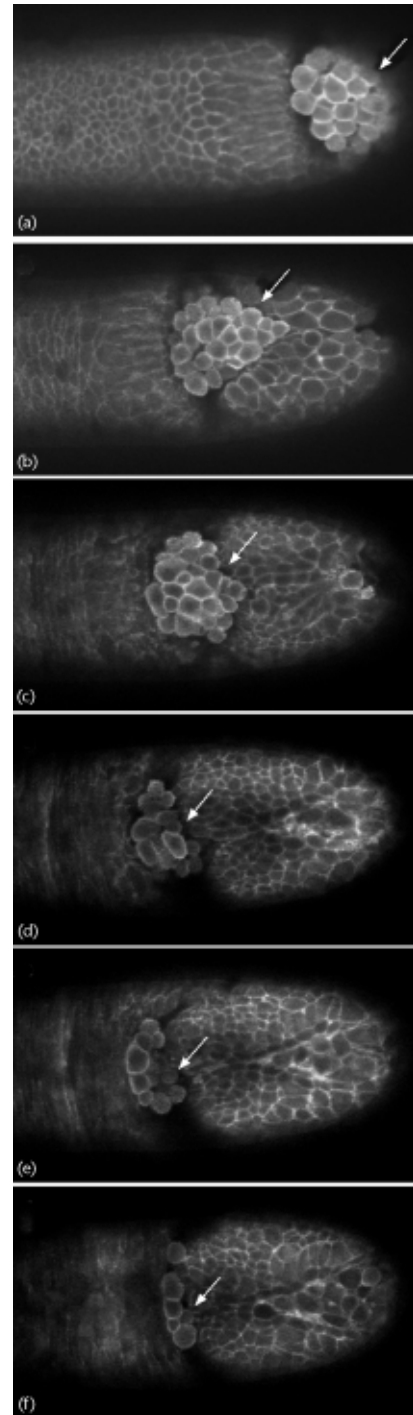


Figure 7 Time-lapse analysis of posterior midgut invagination in a living embryo. This is the dorsal view of a transgenic embryo, carrying the green fluorescent protein tagged to the actin cytoskeleton, which shows the outlines of individual cells. (a) At $t = 0$, the posterior midgut invagination (PMG, arrow) is visible at the posterior end of the embryo. (b) At $t = 10$ min, the PMG is transported towards the anterior end. (c–f, at 10 min intervals) The PMG is invaginated.

changes along the ventral midline produce a linear groove, these same cell shape changes along the posterior pole form a 'shallow cup' which encompasses the pole cells and thus internalizes these germ cell precursors along with the endodermal primordia. The posterior midgut is then transported anteriorly along the dorsal side, first by contractions on the dorsal side of the embryo and then by extension of the germ band (Figure 7).

The germ band gives rise to the segmented portions of the larva and is composed of ectoderm and the newly internalized mesoderm. Thus, the cells that comprise the germ band include dorsal epidermis, neurogenic ectoderm, mesectoderm and mesoderm. The germ band initially occupies the posterior two-thirds of the embryo on the dorsolateral surface; its anterior boundary is defined by the transient head furrow and its posterior boundary at the ventral furrow/posterior midgut boundary. During extension, the germ band is transported over the posterior end of the embryo and reaches a maximum extent of approximately 70% egg length (measured from the posterior pole) on the ventral side. Germ band extension occurs in two phases, an initial fast phase and a slow phase. During the fast phase, the germ band extends to 60% egg length over a 30-minute time course. During the slow phase, the germ band reaches its maximal anterior extent of 70% egg length over a 70-minute time course. When fully extended, the germ band is twice as long and half as wide as its initial dimensions. Germ band extension is thus driven by cell rearrangement. It has been proposed that cell intercalation is the mechanism by which the germ band extension occurs.

Mitotic Domains

During gastrulation, mitotic synchrony is lost and at least 25 distinct groups of cells can be identified based on when they enter mitosis. Generally, each group comprises two bilaterally positioned clusters of neighbouring cells that enter mitosis simultaneously. Each group of cells is known as a mitotic domain and each has a characteristic cycle 14

interphase duration and a specific location on the embryo surface. Timing of entry into mitosis in each of these domains is controlled by *string*, a conserved phosphatase, which activates CDK1, driving cells into mitosis. The cells that constitute a domain often undergo the same morphogenetic movements and have similar cell morphology. For example, mitotic domain 10 is composed of cells from the ventral furrow while mitotic domain 22 is composed cells from the posterior midgut invagination. Based on these characteristics, it has been proposed that cells are partitioned into mitotic domains based on their cell fate. This hypothesis has been shown to be correct for some domains but not for others. It has also been proposed that by partitioning the embryo into mitotic domains, mitosis and morphogenetic movements can occur simultaneously in different parts of the embryo, thus allowing development to proceed rapidly.

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