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# Actin-based forces driving embryonic morphogenesis in *Caenorhabditis elegans*

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Morphogenesis is the process by which multicellular organisms transform themselves from a ball of cells into an organized animal. Certain virtues of *Caenorhabditis elegans* make it an excellent model system for the study of this process: it is genetically tractable, develops as a transparent embryo with small cell-numbers, and yet still contains all the major tissues typical of animals. Furthermore, certain morphogenetic events are also amenable to study by direct manipulation of the cells involved. Given these advantages, it has been possible to use *C. elegans* to investigate the different ways in which the actin cytoskeleton drives the cellular rearrangements underlying morphogenesis, through regulated polymerization or actomyosin contraction. Recent insights from this system have determined the involvement in morphogenesis of key proteins, including the actin-regulating WASP and Ena proteins, potential guidance molecules such as the Eph and Robo receptors, and the cell-cell signaling proteins of the Wnt pathway.

## Addresses

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## Introduction

The transformation of a spherical mass of dividing cells into a fully formed animal with distinctive shape is a complex problem that has produced many different solutions throughout the animal kingdom. The nematode worm *Caenorhabditis elegans* has a simple vermiform shape, and so has the seemingly straightforward task of morphing from a ball of cells into a tube-like organism with properly positioned ectoderm, mesoderm and endoderm. The embryonic morphogenetic movements that occur during this transformation have been known since the early lineage experiments more than 20 years ago [1]; however, it is only recently that the cellular mechanisms that underpin these movements, and to a lesser extent the guidance cues that direct these cellular movements, have begun to be understood.

The first step in *C. elegans* morphogenesis is the rearrangement of the three germ layers during gastrulation. At the 28-cell stage, the two endoderm-precursor cells move from the ventral surface to the interior of the embryo (Figure 1a). These cells are followed by mesoderm and germ-line precursor cells in waves of migrations over the next three hours, until around a third of the embryonic cells are internalized [2]. After these movements have been completed, the gap left by these ingressions, known as the gastrulation cleft, is filled in by neuroblasts (i.e. neuronal precursors; Figure 1b).

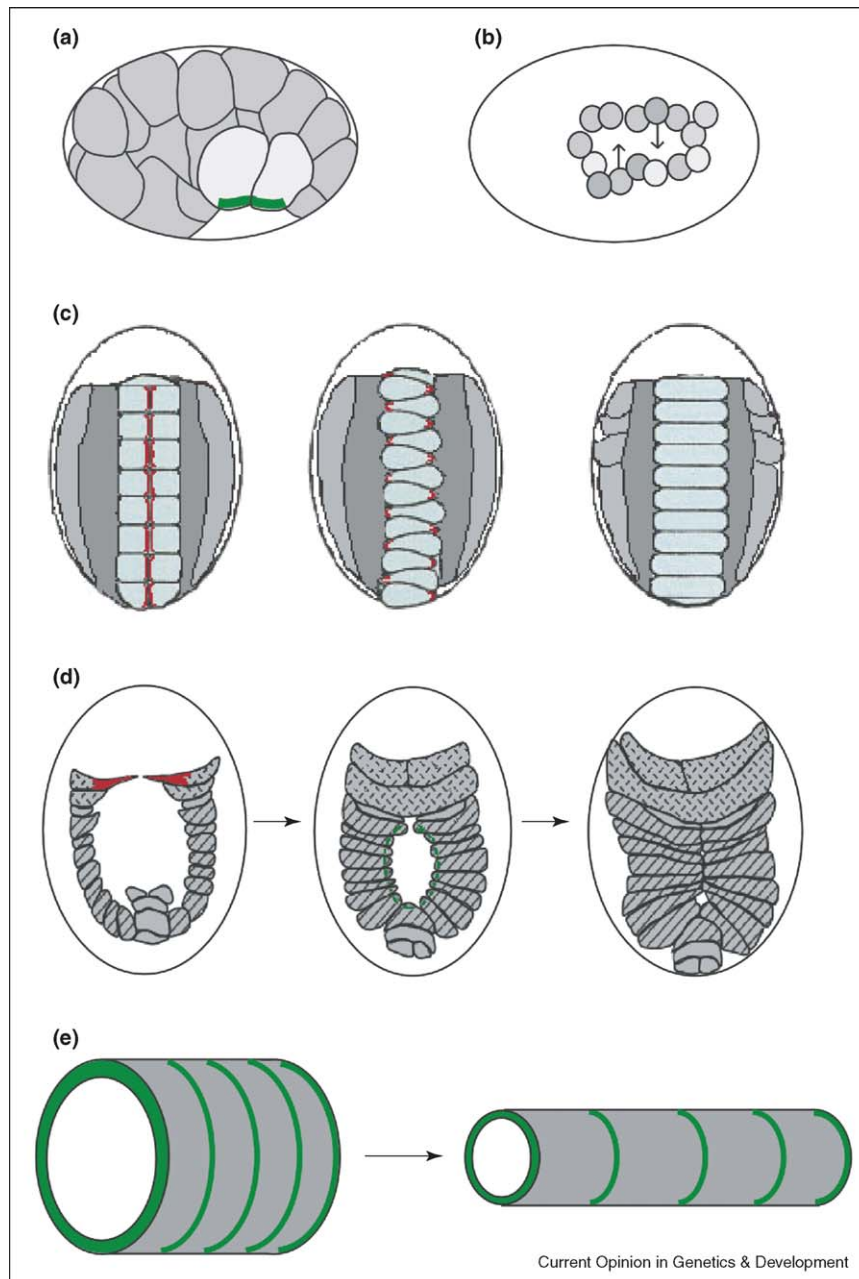
At the end of gastrulation, the hypodermal (epidermal) cells of the embryo are arranged in three lines (dorsal, lateral and ventral) on each side of the dorsal surface of the embryo. At this time, the dorsal lines of each side are adjacent, and the ventral lines only cover the lateral surfaces of the embryo. Once gastrulation is complete, the hypodermal cells spread ventrally, completely enclosing the embryo. The first movement of the hypodermal cells is the intercalation of the dorsal lines of cells from each side, in which the two lines merge to form a single, much longer row of cells on the dorsal surface (Figure 1c) [3]. After this, the ventral line of hypodermal cells spreads around the embryo, enclosing the whole embryo. Ventral enclosure is a two-step process. In the first step, an anterior pair of ventral hypodermal cells migrates from each side of the embryo, akin to epiboly, until the two pairs meet on the ventral surface, where they form adherens junctions. In the second step, the remaining hypodermal cells elongate towards the ventral midline, closing the ventral pocket (Figure 1d) [4]. Upon completion of enclosure, the embryo elongates by contraction of circumferential actin-bundles in the hypodermis, squeezing the embryo into a worm shape with a roughly four-fold longitudinal elongation of the embryo (Figure 1e) [5].

In this review, we discuss the different ways in which the actin cytoskeleton provides the mechanical forces that drive embryonic morphogenesis in *C. elegans* and how the actin-dependent events are regulated. We discuss two classes of actin-based morphogenetic movements: protrusive events, in which actin polymerization provides the force to alter cell morphology or drives cells forward; and contractile events, in which shortening of actomyosin bundles pulls cells and/or membranes together.

## Protrusive events: actin polymerization drives morphogenesis

Actin polymerization can provide forces that drive the formation of membrane protrusions, and this can alter

Figure 1



Morphogenetic movements during *C. elegans* embryogenesis. **(a)** Side view of a 28-cell stage embryo during gastrulation. Endoderm precursors (in pale grey) move from the ventral surface into the embryonic interior. **(b)** Dorsal view of embryo undergoing gastrulation cleft closure. Neuroblasts migrate to fill the cleft left by gastrulation. **(c)** Dorsal view of embryonic hypodermis during dorsal intercalation, showing the ventral (outermost), lateral (intermediate) and dorsal (central) lines of hypodermal cells. The two dorsal lines of hypodermal cells merge to form a single line of cells [33]. **(d)** Ventral view of embryonic hypodermis during ventral enclosure. The ventral line of hypodermal cells migrates to enclose the embryo in two steps: (i) the anterior cells migrate towards the midline; then (ii) the posterior cells extend towards the midline to close the ventral pocket. Adapted from Williams-Masson *et al.* [3]. **(e)** Elongation of the embryo. Circumferential actin bundles in the hypodermis act to squeeze the embryo. For all stages, sites of actin polymerization (red) and actomyosin contraction (green) are marked.

cellular morphology or drive cell movements [6]. Biochemical studies investigating the formation of actin filaments have demonstrated how actin polymerization and nucleation of branches by the Arp2/3 complex can

ratchet the plasma membrane forward [7]. Studies of actin-binding proteins in cell culture systems have shown how these raw filaments are bundled or cross-linked into 3D structures, providing the compressive strength that

produces the extension of membrane protrusions such as lamellae and filopodia [8]. Upstream regulators such as the Rho family GTPases Rac and Cdc42 act to coordinate new actin polymerization and the formation of these protrusive structures [9].

During *C. elegans* embryonic morphogenesis, there are three situations in which actin polymerization is likely to provide the impetus for cell rearrangements: the initial epibolic migrations of the anterior ventral hypodermis around the ventral surface of the embryo (Figure 1d); the closure of the gastrulation cleft by neuroblasts (Figure 1b); and the intercalation of the dorsal hypodermal cells (Figure 1c). The best understood of these is the migration of the ventral hypodermis, when two anterior hypodermal cells extend actin-rich filopodia and flat lamellae-like membrane protrusions as they migrate ventrally. Evidence that these morphogenetic movements require actin-driven cell-crawling comes from studies in which actin polymerization is inhibited. Treatment of embryos with cytochalasin D, which inhibits actin polymerization, or using RNAi to deplete Arp2/3 components, thereby inhibiting Arp2/3-nucleated actin polymerization, prevents the formation of the actin-rich protrusions in the hypodermal cells and disrupts ventral enclosure [3,10,11]. Rac and Cdc42 act as master regulators of Arp2/3-nucleated actin polymerization [9]. Embryos deficient in one of the *C. elegans* Rac homologues, CED-10, have defects in ventral enclosure [12]. Rac and Cdc42 increase Arp2/3 activity through a complex of proteins including the WASP family members WASp (Wiskott-Aldrich syndrome protein) and WAVE (WASP family verprolin-homologous protein) [13], and using RNAi to deplete these proteins [11] or their interacting protein WIP (WASP-interacting protein) [14] also causes defects in hypodermal cell migration. Furthermore, GEX-2 and GEX-3, which are known to be part of the Rac-WAVE complex in other systems [15,16], have been recognized as being essential for ventral enclosure [12]. Finally, regulation of intracellular calcium levels might be important for the formation of the protrusive actin structures in these cells, because depletion of an inositol 1,4,5-triphosphate receptor reduces formation of filopodia and lamella in the migrating ventral hypodermal cells and prevents ventral enclosure [17].

Although less is known about the closure of the gastrulation cleft by neuroblasts, many of the same molecules required for anterior ventral hypodermal cell migration are required for gastrulation cleft closure. Embryos deficient in WSP-1 or WVE-1 (the WASp and WAVE homologues, respectively) have wider, more persistent gastrulation clefts, indicative of impaired migration of the neuroblasts [18<sup>\*</sup>]. UNC-34 is the *C. elegans* homologue of Ena, a protein that modulates actin polymerization by regulating barbed-end-capping [19], and recent studies have shown that if UNC-34 is depleted at the same time

as WSP-1 then the embryos have very large ventral clefts [20], further suggesting that regulated actin polymerization is important for gastrulation cleft closure.

A number of cell surface receptors that are known to regulate cell movements in other systems have been implicated in controlling the cell migrations during gastrulation cleft closure and hypodermal enclosure. Eph (ephrin) receptors have been shown to regulate cell migrations in other systems and are known to be able to regulate Rho family GTPases, including Rac [21,22]. VAB-1, the *C. elegans* Eph receptor, is expressed in the migrating neuroblasts, and embryos with mutations in *vab-1* or its ephrin ligands have defects in gastrulation cleft closure and hypodermal cell migration [23–26]. It is likely that there is redundancy between VAB-1 and other guidance molecules, because the strongest *vab-1* mutants only give a partially penetrant effect on gastrulation cleft closure and hypodermal enclosure. Other potential guidance cues that might regulate the hypodermal cell migrations include genes encoding members of the semaphorin family [27,28], the Kallmann syndrome gene *kal-1* [29], and a Lar-like receptor tyrosine phosphatase gene *ptp-3* [30].

Recent data have shown that mutations in another gene, *sax-3*, which encodes the *C. elegans* homologue of the neuronal receptor Robo, cause a similar gastrulation cleft phenotype to that of the *vab-1* mutants, with around 30% of clefts failing to close. *vab-1/sax-3* double homozygous mutants have a very strong phenotype, with 100% of embryos failing to close their gastrulation clefts [31<sup>\*\*</sup>]. Additionally, *vab-1* and *sax-3* display gene-dose sensitivity, implying that Robo and Eph receptors act together in these migrations. Although mutations in *slt-1*, the gene encoding the Robo ligand, do not cause any morphogenesis defects, ventral hypodermal cells in *slt-1/vab-1* double homozygous mutant embryos often fail to begin migrating around the embryo, implying that SLT-1 might function redundantly with VAB-1 in guiding these migrations. Robo is expressed in the same neuroblasts as the Eph receptor, and the cytoplasmic tails of these two receptors can physically interact [31<sup>\*\*</sup>], so it will be interesting to understand how members of these two receptor–ligand families modulate the cell migrations during these morphogenetic movements. Both are capable of regulating cell migrations through interactions with members of the Rho family of small GTPases and other actin modulators such as Ena (UNC-34) [21,22,32].

The third morphogenetic movement that involves protrusive actin structures is dorsal intercalation, in which the two dorsal rows of hypodermal cells interdigitate, forming a longer, single row of cells. As the cells interdigitate, they send out basal processes that protrude between the adjacent cells. It has been hypothesized that these protrusive structures are driven by actin polymerization and

that this drives the intercalation of the two adjacent rows. Supporting this hypothesis, the Rac effectors GEX-2 and GEX-3 are required for this process [12]. Little is known about the regulation of these cell movements; they occur in the absence of the adjacent (lateral) rows of hypodermal cells [4], suggesting they are intrinsic to the dorsal hypodermal cells; however, mutations in *sax-3*, the Robo receptor, have recently been shown to give mild defects in cell morphology of the intercalating cells, independently of *vab-1* [31\*\*]. A transcription factor, DIE-1, has been shown to be required for the process [33], as have an APC homologue [34] and a fer-like receptor [35]; but, to date, the mechanism by which these proteins regulate these movements is not understood.

### Contractile events: actomyosin contraction drives morphogenesis

Although polymerization of actin fibers enables pushing or protrusive forces to be exerted, it is also possible to use an existing actin meshwork and the motor protein myosin to exert a contractile or pulling force [36]. In *C. elegans* morphogenesis, the earliest example of this occurs within the ingressing endoderm precursors during gastrulation (Figure 1a). The two endoderm-precursors start on the ventral surface of the embryo at the 28-cell stage, and during an extended cell cycle they ingress into the embryonic interior, becoming completely enclosed by their neighbors. The ingressing cells show an apical accumulation of the myosin II heavy-chain protein NMY-2 (non-muscle myosin II) just prior to and during the cell movements [2]. The directions of these movements are intrinsic to the ingressing cells, rather than to their neighboring cells, and the ingressing cells undergo a constriction of their apical domains [37], suggesting that actomyosin contraction at the exterior surface of the endoderm precursors drives these early gastrulation movements. Correct apicobasal polarity of the endoderm precursors is required for this apical constriction. In the absence of the polarity protein PAR-3, the accumulation of myosin is delayed, and there is a substantial delay in the ingression of the endoderm cells [38].

Actomyosin contraction can be regulated by the phosphorylation state of myosin. Myosin II consists of six subunits: a single pair of heavy chains (NMY-2 in early *C. elegans* embryos) and two pairs of light chains. One pair of light chains (known as regulatory light chains, or MLC-4 in the *C. elegans* early embryo) can be phosphorylated at serine 18, and this has a dramatic effect, increasing the actin binding affinity and motor activity of myosin [39]. Preliminary data suggest that during gastrulation, phosphorylated myosin can be detected in the apical domain of the ingressing endoderm-precursors, confirming that myosin is becoming activated during gastrulation. This activation occurs downstream of a cell–cell signaling pathway involving Wnt and Frizzled (JY Lee, DJ Marston, and B Goldstein unpublished).

As described earlier, protrusive forces act early in ventral enclosure to bring the anterior hypodermal cells together on the ventral surface of the embryo. Once this has happened, the posterior hypodermal cells in the ventral pocket elongate as the ventral surface of the embryo zippers closed (Figure 1d) [3]. Little is known about the mechanisms of this event. It might work in a fashion analogous to dorsal closure in *Drosophila*, in which a similar pocket is closed. In *Drosophila*, an actin cable runs along each side of the pocket's opening, and contraction of this cable contributes to closure by a 'purse-string' mechanism [40]. In *C. elegans*, there is an enrichment of filamentous actin at the leading edge of the hypodermal cells closing the ventral pocket, consistent with a purse-string mechanism [3]. The presence of filopodia at the leading edge implies that there is also a role for a protrusive, crawling mechanism in these cells, although it has been proposed that these filopodia are required for adhesion of the hypodermal cells rather than for providing locomotive force [41].

The final contractile event of *C. elegans* morphogenesis gives rise to the most dramatic shape-change of the embryo. At the completion of ventral enclosure, contraction of circumferential actin-bundles within the hypodermis squeezes the bean-shaped embryo, driving a four-fold increase in length of the embryo, and a commensurate decrease in circumference (Figure 1e) [5]. Using cytochalasin D or depleting subunits of myosin or myosin regulators to destabilize the actin cytoskeleton can inhibit elongation [5,42,43]. During elongation, two enzymes have been shown to act antagonistically to regulate phosphorylation of MLC-4 and, therefore, the contractile state of the actin bundles. The first is a myosin phosphatase, MEL-11, which negatively regulates phosphorylation of MLC-4, and in a zygotic null this leads to a striking hyper-elongation of the embryo [44–46]. The second is a serine–threonine kinase, LET-502, which is the homologue of Rho-associated kinase (ROK) and is required to activate MLC-4 [44–46]. LET-502 might directly phosphorylate MLC-4 and thereby activate contraction. Alternatively, LET-502 can phosphorylate MEL-11, and this can activate MLC-4 and thus contraction indirectly by sequestering the MEL-11 phosphatase away from the contraction site [42]. Supporting the latter hypothesis, whereas LET-502 zygotic null embryos fail to elongate, embryos lacking both MEL-11 and LET-502 elongate normally, suggesting that these proteins antagonize each other [42,44–46]. The fact that these double embryos still complete elongation implies that there must be an additional, unidentified myosin light-chain kinase. Other genes that are involved in this pathway and that, when mutated, cause weak mutant phenotypes include the PP2c phosphatase *fem-2*, the Rho GTPases *ced-10* (Rac-like) and *mig-2* (Rho/Rac-like), the PI3 kinase *age-1*, and the insulin receptor *daf-2* [45,46].

For actomyosin contraction to drive cell movements, the actin fibers must be anchored to the cell membranes, and the cells must be attached to each other through cell–cell adhesions. Members of the cadherin family of homophilic adhesion proteins play important roles in morphogenesis in other species [47], anchoring actin filaments to sites of cell–cell adhesion, although recent data have cast doubt on the precise mechanism by which this occurs [48•,49•]. In *C. elegans*, the classic cadherin complex is enriched at sites where the ventral hypodermal cells meet after the initial migrations of enclosure, and at the points where the circumferential actin-bundles form during elongation [50]. Members of the cadherin complex have been shown to be required for both ventral enclosure and elongation of the embryo. Embryos lacking the E-cadherin homologue HMR-1 fail in both enclosure and elongation because the two sides of the ventral hypodermis fail to adhere after migration around the embryo [50]. Zygotic nulls of  $\alpha$ -catenin and  $\beta$ -catenin (HMP-1 and HMP-2, respectively) fail to elongate because the circumferential actin-bundles pull apart; furthermore, removal of the maternal contribution leads to additional enclosure defects [50]. The *hmp-1* adhesion defects are increased in both penetrance and severity by depleting another catenin, p120 catenin [51]. The discs-large complex of proteins is also important for hypodermal cell–cell adhesions during elongation. Circumferential actin-bundles are organized normally in embryos mutant for these proteins, but the cells become detached after enclosure, and elongation fails [52–56]. Additionally, a tetraspanin adhesion protein, VAB-9, which is similar to members of the claudin family, is also found at sites of adherens junctions, and *vab-9* mutants enhance the defects caused by loss of the discs-large complex [57].

Spectrin protein complexes are also found at the ends of the circumferential actin-bundles and are important for organizing the actin–membrane interaction. Disrupting spectrin complexes produces short worms, and it was recently shown that the spectrin complex is essential for converting changes in actin organization into changes in epithelial cell shape during elongation [58,59,60•]. Interestingly, the short phenotype is suppressed by knocking down an ankyrin-repeat protein, VAB-19, implying that these proteins act antagonistically in regulating the circumferential actin-bundles [61].

## Conclusions

We have discussed how the actin cytoskeleton drives morphogenetic movements throughout *C. elegans* development. Regulated actin polymerization might provide pushing forces in multiple situations, driving cell rearrangements through changes in cell morphology, such as those that occur during dorsal intercalation and in cell migrations, as in ventral enclosure. Conversely, actomyosin contraction is used to draw cells together, such as in the cell movements of gastrulation, and to drive the larger

reorganization of the whole embryo during elongation. Many of the morphogenetic movements seen in *C. elegans* involve rearrangements that are similar to morphogenetic movements seen in other organisms. For example, ventral enclosure has many similarities to dorsal enclosure in *Drosophila* [62], whereas dorsal intercalation resembles vertebrate convergent extension [63]. It will be interesting to see how deeply these similarities run, and in particular to determine whether mechanistic or regulatory pathways have parallel uses.

Investigating the molecular mechanisms of morphogenetic movements and cell migrations during the development of such a genetically tractable organism might give insight into morphogenesis of more complex systems, because it will facilitate the identification of novel molecular players in both regulatory and mechanistic elements, which might otherwise be difficult to identify. These can then be applied in other experimental systems such as the biochemical and cell culture-based systems mentioned earlier, and it is hoped that this interplay of information can help to understand how fine regulation of the actin cytoskeleton and cell migrations occurs both during normal development and aberrantly in disease situations.

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