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Tissue morphogenesis: how multiple cells cooperate to generate a tissue

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Genetic analysis in model organisms has recently achieved a detailed molecular description of many key cellular processes controlling embryonic morphogenesis. To understand higher order tissue morphogenesis, we now need to define how these processes become integrated across different cell groups and cell layers. Here, we review progress in this fast moving area, which was to a large degree made possible by novel imaging methods and the increasingly frequent use of modeling. Discussing examples from *Caenorhabditis elegans* and *Drosophila* embryos, two powerful and simple models, we highlight novel principles relying in part on mechanical tension, and outline the role of junctions as signal integrators.

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Genetic analysis in different species has revealed that embryonic morphogenesis depends on several basic cellular processes, such as cell polarity, the assembly of cell–cell or cell–ECM junctions, or cytoskeleton remodeling [1–6]. However, detailed knowledge of these processes cannot alone accurately depict higher order tissue morphogenesis, in part because most studies have neglected the potential influence of surrounding cells. At which level the inputs received from these neighboring cells are integrated remains poorly understood. Moreover, many basic cellular processes occur in parallel, but it is not entirely clear how and to what extent they temporally or spatially influence each other.

Here, we review attempts to integrate the contribution of different cell types to achieve morphogenesis. These studies highlight the importance of mechanical forces and tension, and point to junctions as a place where integration might occur.

Integrating the inputs from different epithelial cell groups

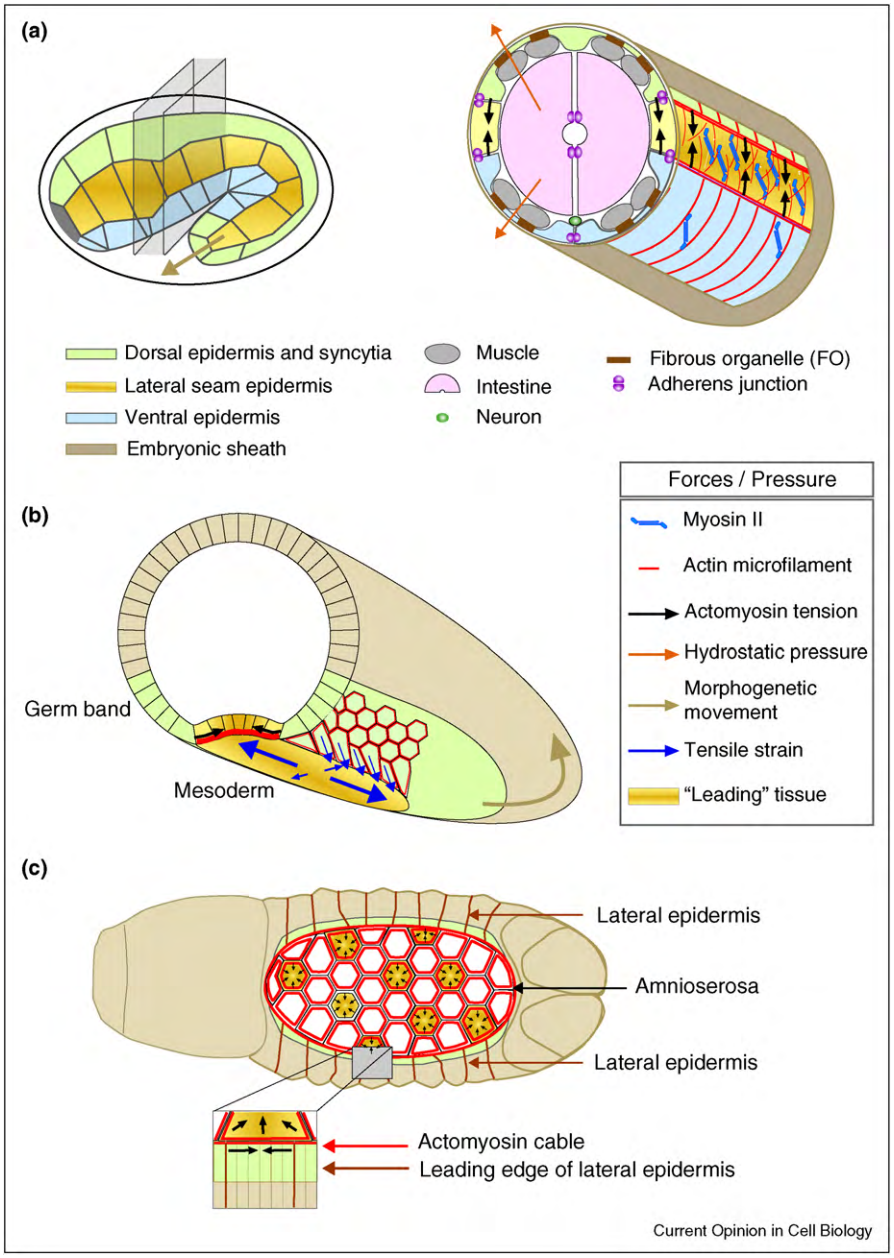
Integration in *C. elegans* of different epidermal cells during embryonic elongation

The *C. elegans* embryo elongates fourfold along its antero/posterior (A/P) axis within three hours. Elongation relies on epidermal cell shape changes [7]. The length of each epidermal cell decreases along the dorsal/ventral (D/V) axis, and increases along the A/P axis (Figure 1a). As in other epithelia [3], the nonmuscle myosin II and its upstream regulators are essential for embryonic elongation (see Box 1) [8–10]. The epidermis comprises six rows of cells: two dorsal (D), two ventral (V), and two lateral cells. There are significant cellular and molecular differences among each row (Figures 1a and 2), which raises two questions. Is myosin II equally active in all cells, and do D/V cells and lateral cells bring an equal contribution to elongation?

Cell-specific rescue experiments show that MLC-4, the myosin regulatory light chain (MRLC), is mainly required in lateral cells during elongation [11•]. Hence, lateral cells require high tension, at least to initiate elongation. Furthermore, a systematic RNAi screen identified a Rho-GAP protein called RGA-2 as a negative regulator of MLC-4 only in D/V cells, where it maintains these cells under low tension (see Box 1) [12]. Altogether, these findings suggest that lateral cells provide the driving force for elongation, and that D/V cells deform concomitantly, but rather passively.

Finite element modeling of the worm embryo indicates that such a mechanism is compatible with the laws of physics [13••]. Ben Amar and coworkers considered that the embryo contains biological material endowed with an active response characterized by myosin II, and other material displaying a passive viscoelastic response [13••]. The latter include internal cells, which exert hydrostatic pressure on the outer epithelial layer when the embryonic diameter changes (Figure 1a), and circumferentially oriented microtubules in D/V cells, which can resist compression [14]. By solving the equations that predict the embryonic diameter at mid-body over time as a function of forces, their model predicts that elongation can proceed with no or very little myosin activity in D/V cells, but only if these cells contain microtubules [13••]. In agreement, colcemid and nocodazole (microtubule polymerization inhibitors) are known to reduce the extent

Figure 1



Leading cells in different morphogenetic processes. Many morphogenetic processes involve 'leading tissues/cells' (yellow) that exert a tensile strain to influence the behavior of other cells (light green or blue). **(a)** Left panel, lateral view of a *C. elegans* embryo (dorsal side up) during early elongation stage (1.5-fold stage). Right panel, cross-section of the area within the gray box; the outer embryonic sheath (light brown) was partially removed to reveal inner details. Lateral cells (yellow) contain more myosin II [9,10]; dorsal/ventral epidermal cells (blue and green) have thicker actin microfilament bundles and well-organized circumferential microtubules (not shown for the sake of clarity) [11•,14]. During early elongation, myosin II activity in lateral cells is the main driving force (black arrows) [11•,12]. The intestine (pink) and muscles, which occupy most of the inner part of the embryo, exerts hydrostatic pressure on the outer epidermal layer (orange arrows). **(b)** Lateral–ventral view and cross-section of a *Drosophila* embryo (dorsal side up) at the beginning of germband extension and gastrulation. The ventrolateral epidermis of the germband (green) extends along the anterior–posterior axis by a process of cell intercalation driven by myosin II. Meanwhile, the invaginating mesoderm (yellow) is under anterior–posterior tension (thick blue arrows). This tension, the origin of which is unknown, is transmitted as an A/P-directed strain in the attached germband. Drawing inspired by Ref. [26•]. **(c)** Dorsal view of a *Drosophila* embryo undergoing dorsal closure, which involves replacement of a transient tissue (amnioserosa) by the lateral epidermis. Bottom enlargement, lateral epidermis/amnioserosa interface; note the actomyosin cable (red) maintaining the leading edge under strong tension (black arrows). Amnioserosa cells constrict their apical surface in a ratchet movement (small black arrows), which pulls the leading edge dorsally. Adjacent amnioserosa cells do not constrict simultaneously [33••]. Drawing inspired from Ref. [52].

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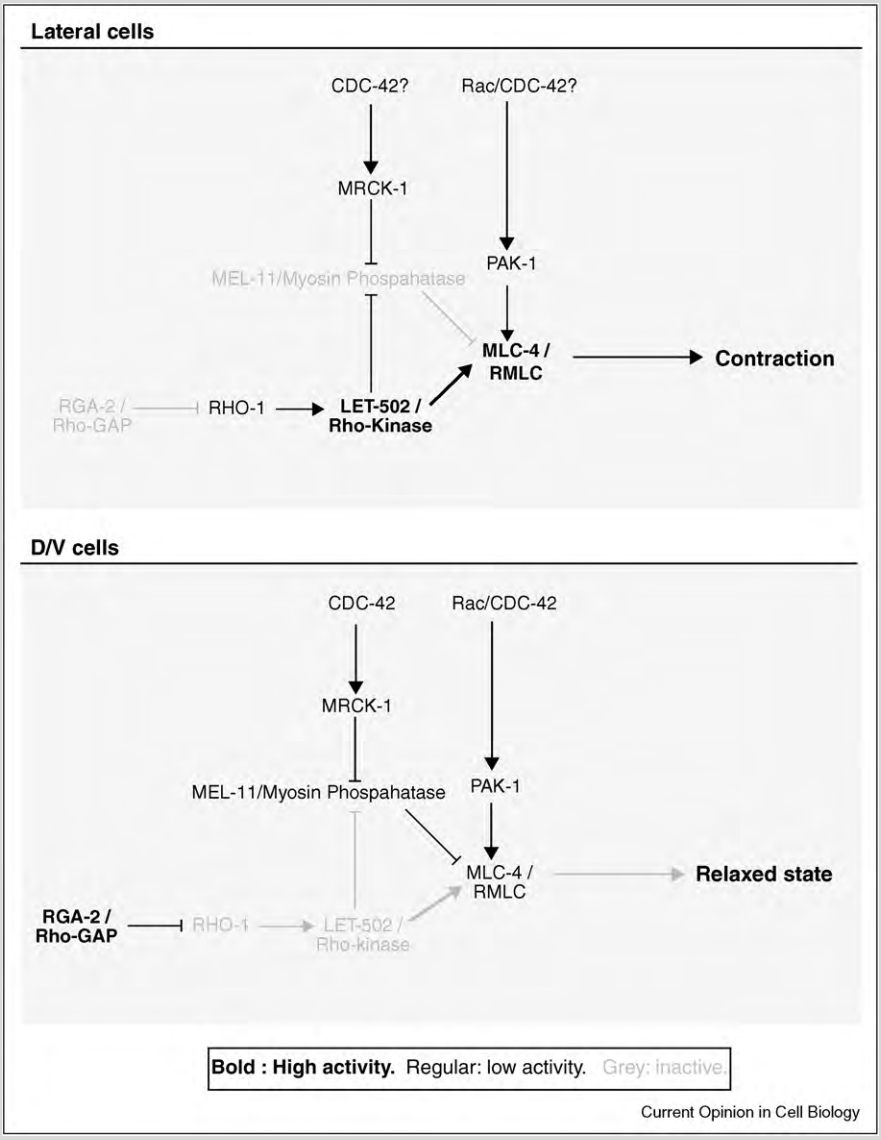
Box 1 Myosin II activation in *C. elegans*

Genetic analysis suggests that at least three protein kinases act in parallel to activate myosin II: LET-502 (Rho-kinase), PAK-1 (p21-activated kinase-1), and MRCK-1 (myotonic dystrophy kinase-related Cdc42-binding kinase-1). As observed in epithelia in other species, LET-502/Rho-kinase is the main myosin regulatory light chain (MRLC) activator although other kinases can also activate it [3]; for instance in *Drosophila* eye disk, Rho-kinase acts in parallel to an undefined kinase [15]. In *C. elegans*, a strong *let-502* mutation combined with mutations in *pak-1* or *mrck-1* completely blocks elongation [11•]; either PAK-1 or MRCK-1 alone is dispensable for elongation. PAK-1 is likely to directly phosphorylate MLC-4/RMLC since a phosphomimetic form of MLC-4 can significantly suppress the elongation defect of *let-502; pak-1* double mutants [11•]. In contrast, MRCK-1 appears to activate MLC-4 indirectly, by inhibiting the myosin-binding subunit of myosin phosphatase known as MEL-11 [11•]. Biochemical analysis in vertebrates is consistent with these genetic results [16]. Since RGA-2 can block myosin II activation through LET-502 in D/V epidermal cells, it indicates that D/V myosin II might still be activated at lower levels probably through PAK-1 and MRCK-1. Hence, D/V epidermal cells are probably maintained under low tension, rather than completely lacking myosin II activity.



of elongation [14] (as predicted in the model), and to generate misshapen embryos.

Integration of *Drosophila* gastrulation and germband extension

Germband extension and gastrulation occur concomitantly during *Drosophila* embryogenesis (Figure 1b). The germband extends along the A/P axis by a mechanism involving cell intercalation, mediated by a polarized enrichment of myosin II at junctions oriented along the D/V axis [17–19,20••]. Recently, Sanson and colleagues found that mesoderm invagination also contributes to germband extension [21•]. Using novel image analysis tools to describe cell deformation [22•], they found that

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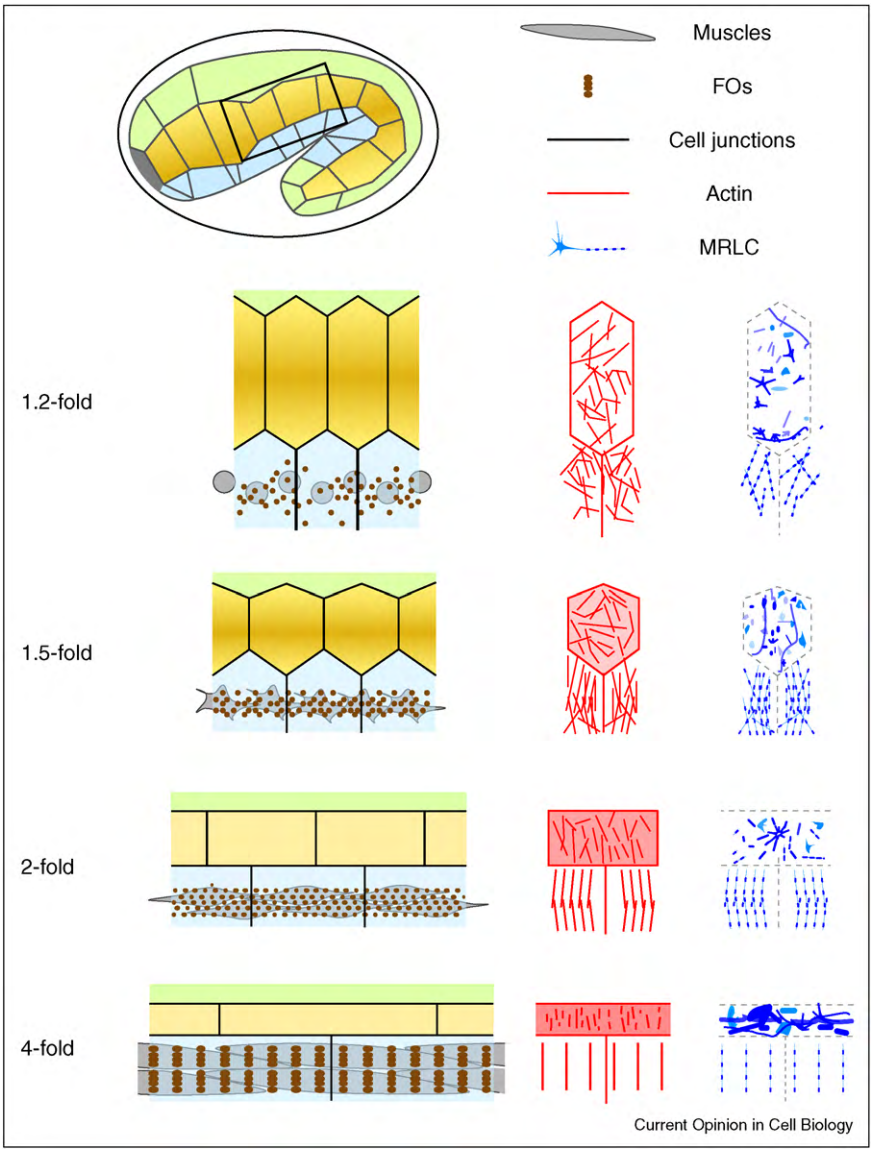
Figure 2



Coordinated structural reorganization of different cell types during *C. elegans* embryonic elongation. Summary of the main changes undergone by epidermal cytoskeletal and junctional elements, and by body-wall muscles during *C. elegans* embryonic elongation (beginning of elongation is onefold stage; end of elongation is fourfold stage). The region boxed in black (upper panel) is shown at four stages, with muscle cells underneath. The three vertical columns describe fibrous organelles (FO, left), actin filament (middle), and myosin II changes when epidermal cells extend and muscles become contractile. 1.2-fold stage: FO components accumulate at muscle-adjacent areas, and gradually form a single band of puncta. Meanwhile, epidermal actin filaments remain short and disoriented throughout the epidermis, with higher accumulation at cell junctions. From 1.5-fold to 2-fold stages: dorsal and ventral epidermal actin filaments gradually form thick parallel circumferential bundles; those in lateral cells remain relatively short and fuzzy. After the 1.7-fold stage, when muscles become contractile, FOs progressively align into short parallel stripes along the dorsal and ventral epidermis. Rho-kinase activity becomes dispensable beyond the twofold stage, suggesting that lateral cells may not play the same leading role beyond that stage (pale yellow) [12]. Twofold to threefold stages: circumferential actin bundles seem discontinuous in the region corresponding to where FOs localize. Beyond the threefold stage: FO stripes colocalize with circumferential actin bundles. The nonmuscle myosin II reorganizes along actin filaments in D/V cells, but remains apparently disorganized and forms transient foci or cables in lateral cells during elongation.

the gastrulating mesoderm [23] submits germband cells to an A/P-directed strain, which induces germband cells to change their shapes during the first part of germband extension [21•]. This A/P-directed strain is strong enough to initially compensate for the lack of cell intercalation in *Krüppel* mutants and promote partial germband extension [21•,24].

Separate work by Eric Wieschaus and colleagues provides a potential explanation for the origin of this tensile force.

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Mesodermal cells invaginate through apical constriction in a polarized ratchet process [25••]. Constriction occurs mainly along the ventral–lateral direction, which reflects the mechanical properties of the entire tissue [26•]. Indeed, when cell adhesion is partially compromised, blocks of mesodermal cells separate along the A/P-direction, revealing a global A/P-directed tension [26•]. Hence, as both tissues are connected, the mesoderm could transmit this tensile force to the germband and create the A/P-directed strain observed in germband cells [21•]. Further work will be required to determine whether the ventral furrow is the source of A/P-directed tension in the germband, or if its role is to transmit A/P forces generated elsewhere in the embryo, for instance through contraction of the dorsal side of the embryo or through posterior midgut invagination [24].

Integration of *Drosophila* dorsal epidermal cell progression and amnioserosa constriction

During late embryogenesis, lateral epidermal cells extend dorsally to establish novel junctions at the dorsal midline with their contralateral homologs, and replace the amnioserosa (Figure 1c) [27–29]. Pioneering experiments by Kiehart *et al.* had established that dorsal closure depends on two cells types, the dorsal-most epidermal cells (or leading edge) and the amnioserosa [27,30]. Combined with genetic analysis of myosin II mutants [31], their laser ablation experiments revealed that dorsal closure progresses under the influence of two main forces: first, a pulling force exerted on the leading edge by amnioserosa cells when they constrict their apical surface; second, a contractile force exerted by an actomyosin cable present within the leading edge cells in contact with the amnioserosa (Figure 1c) [27,30,32].

Recent analysis describing cell shape changes across the entire dorsal surface considerably refined the initial model of dorsal closure. Brunner and colleagues found that amnioserosa cells exhibit pulsed contractions of their apical surface [33••]. Adjacent amnioserosa cells pulse asynchronously, suggesting that, as for the mesoderm [25••], constriction involves two steps, first reduction of the apical surface, and then maintenance of the constricted perimeter [33••]. During the initial phase, the actomyosin cable at the leading edge displays an oscillatory behavior in synchrony with amnioserosa cells [33••]. When an outer amnioserosa cell constricts, it pulls dorsally the adjacent leading edge cell and deforms its neighbors. In response, the actomyosin cable progressively strengthens and stiffens, which prevents in a ratchet-like process the ventral-ward movement of the leading edge, and locally dampens the oscillatory behavior of amnioserosa cells [33••]. Interestingly, central amnioserosa cells pulse for longer than outer amnioserosa cells in contact with the epidermis [33••]. A parallel quantitative analysis also concludes that there are different phases of amnioserosa contractions, and that there is a radial gradient of amnioserosa contraction [22•,34•].

Dorsal closure thus suggests the existence of several tension-sensing mechanisms within the amnioserosa and leading edge cells that serve to stabilize cells in a given position, or to synchronize their behavior. A recent study has outlined that myosin II cable formation is regulated by tension in a positive feedback loop during morphogenesis [35•]. This could account for both the sequential arrest of the pulsatile behavior among amnioserosa cells, and the stiffening of the actomyosin cable at the leading edge. More generally, tension also mediates the function of barriers inhibiting cell mixing across compartment boundaries [36•,37•].

In the three processes discussed above, the behavior of one cell type influences another cell type. In each case, adherens junctions are prime candidates to relay tensile forces [26•]. These processes highlight how understanding higher order morphogenesis requires integrating the behavior of many cells within and outside of the tissue. Future challenges will involve dissecting the mechanisms that sense tension to modulate myosin activity, actin microfilament bundling or anchoring, and potentially yet other cellular processes. In this respect, E-cadherin/β-catenin and nectin/afadin adhesion complexes [26•,38], and other actin-binding proteins modulating junction stability [39,40] are prime mechano-sensing candidates.

Integrating inputs from different tissue layers

A mature organ often includes an epithelial layer and a smooth muscle layer, which generally interact through a shared extracellular matrix (ECM). When simultaneously recruited to the organ primordium, these different cell types must coordinately change their shapes during morphogenesis. Very little is known about this coordination.

Epidermis–muscle interactions in *C. elegans*

The *C. elegans* body-wall muscles contact the dorsal and ventral epidermis through an ECM, and are required for late embryonic elongation [7,41]. Muscles attach to an extracellular exoskeleton covering the apical surface of the epidermis through *trans*-epithelial attachments called fibrous organelles [42]. Each fibrous organelle is composed of two hemidesmosome-like complexes, one at the apical and another at the basal plasma membranes of the epidermis, which are connected by intermediate filaments (Figures 1 and 2; reviewed in Ref. [42]). Below, we discuss how these organelles can potentially integrate inputs from muscles and the epidermis.

C. elegans body-wall muscles become contractile once the embryo has reached the so-called 1.7-fold stage [43]. Mutants with defective muscles arrest elongation as paralyzed embryos at the twofold stage (Pat phenotype)

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[41]. There is no satisfactory molecular explanation yet to account for the Pat phenotype, but indirect data indicate that muscles could affect fibrous organelle assembly or maintenance. Indeed, these organelles are absent when muscles are locally missing [44]. This in turn might explain how muscles impact on elongation, since most hemidesmosome-like components are essential for epidermal integrity and embryonic elongation beyond the 1.7-fold stage [42]. In severe fibrous organelle mutants, circumferential actin bundles present in D/V epidermal cells (see Figure 2) are less regularly patterned and occasionally fragmented during late elongation [45–47], suggesting that hemidesmosome-like components could act to maintain the circumferential actin bundles, which are essential for elongation [14]. Indeed, fibrous organelles progressively organize into short parallel circumferentially oriented stripes [43,48•], which appear to coincide in position with stripes formed by circumferential actin bundles at the twofold stage (Figure 2) (HZ, unpublished observations). Thus, proteins within the fibrous organelle, especially the actin-binding protein EPS-8 [47] or the VAB-10B/MACF isoform [45], might help maintain circumferential actin bundles at late stages. Moreover, in normal twofold embryos, circumferential actin bundles are frequently interrupted at the position of fibrous organelles [11•], indicating they may serve as anchoring sites for circumferential actin bundles.

Reciprocally, fibrous organelles appear to integrate input from the actomyosin machinery. In *let-502*/Rho-kinase, *mlc-4*/RMLC or *spc-1*/α-spectrin mutants, which affect the epidermal cytoskeleton, fibrous organelles form stripes that occupy a wider area than normal [49]. Consequently, muscles occupy a wider area too, showing that the epidermis feeds back to regulate muscles [49]. A potential mechanism to account for the contribution of the epidermal cytoskeleton on fibrous organelles comes from a recent study in which we characterized an E3 ubiquitin-ligase (EEL-1) as a hemidesmosome assembly factor [48•]. In *eel-1* mutants, the hemidesmosome-like membrane receptor LET-805 is twice as abundant as wild type, which, combined with a weak *vab-10A* mutation, prevents normal fibrous organelle maturation [48•]. Their maturation from puncta to stripes (Figure 2) presumably requires hemidesmosome disassembly and reassembly. Increased LET-805 levels might delay fibrous organelle disassembly, and in turn affect their ability to withstand muscle tension and epidermal extension. In support of this model, slowing down the rate of elongation by reducing β_H-spectrin levels in the epidermis to provide more time for the fibrous organelle disassembly/reassembly process, partially rescues the defects of *vab-10A(weak); eel-1* double mutants [48•].

The ECM at the muscle–epidermal interface plays a major role in coordinating epidermal and muscle shape change. The *C. elegans* perlecan homolog UNC-52, which is a major ECM component secreted by the epidermis, impacts on fibrous organelle organization. A slight reduction of UNC-52 deposition is enough to affect fibrous organelle structure [48•]. Some other ECM proteins appear to indirectly affect embryonic elongation and fibrous organelle organization, because they affect muscle assembly. For instance, when the F-spondin homolog SPON-1 is absent, UNC-52/perlecan distribution becomes fragmented, which in turn progressively alters fibrous organelles [50].

Altogether, the epidermis, ECM, and muscles form a feedback network, which controls embryonic elongation. Furthermore, fibrous organelles appear to be well positioned to integrate multiple signals and influence other cellular processes. Future studies should help define how they could do so.

Lumenal epithelial/myoepithelial interactions in branching mammary gland

Interactions across tissue layers in mammalian morphogenesis have been mainly depicted in terms of signaling through growth factors and morphogens. Recently Ewald *et al.* described an interaction between different epithelial layers that might not go through classical growth factors/morphogen signaling during mammary gland branching morphogenesis [51••]. They developed a 3D-model of breast explants in matrigel and observed that ducts contain a luminal epithelial layer and a surrounding myoepithelial layer expressing smooth muscle actin. Timelapse movies revealed that duct elongation involves the collective migration of the multilayered epithelium at the ductal tip, but not leading cell extensions or protrusions. Interestingly, new branches occur in areas lacking myoepithelial cells, raising the exciting possibility that the myoepithelial layer could restrain duct elongation and regulate branching [51••]. Further molecular dissection of this promising system should reveal how myoepithelial cells might influence epithelial behavior, and whether this interaction bears any resemblance to worm elongation.

In conclusion, novel image analysis methods [22•,33••], modeling [33••], and the input of physics to the interpretation of biological processes [13••], coupled to more conventional developmental biology approaches in which multiple cell types were considered, have been key to progress in this area. Further progress will depend in part on the development of biosensors that can measure stress and stiffness *in vivo*, as well as on the identification of the molecules involved in sensing or transducing tension.

Note added in proofs

Two recents papers establish that alphaE-catenin is a tension sensor at adherens junctions. Under tension, it undergoes a conformational change resulting in vinculin recruitment [53,54].

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