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Mechanotransduction: Getting Morphogenesis Down Pat

Embryonic morphogenesis requires the coordination of forces across multiple tissues and their associated extracellular matrices. A new study reports a mechanical feedback loop in the *Caenorhabditis elegans* embryo between muscle and epidermis that may provide a model for understanding how tissues coordinate morphogenetic events in the embryo.

Jeff Hardin

Morphogenesis in animal embryos is a complicated business, both in time and in space. Cells in the embryo must coordinate movement and force production, and do so across multiple germ layers with intricate spatial relationships, often separated by multiple layers of extracellular matrix [1]. If the embryo is a quintessential example of D'Arcy Thomson's dictum that the organism is a "diagram of forces" at work within it [2], then understanding the mechanical interplay between multiple tissues during morphogenesis is a key challenge of current research at the interface between cellular and developmental biology. One difficulty is finding discrete morphogenetic events that are amenable to genetic and cellular analysis and that are sufficiently simple to begin to tease apart the rules that underlie this interplay. Examples exist, including the three germ layers of the amphibian embryo as it elongates [3] and the multiple tissues that contribute to dorsal closure in the *Drosophila* embryo [4]. Recent work by Zhang *et al.* [5] in the *Caenorhabditis elegans* embryo represents a fresh entry in this area.

The *C. elegans* embryo undergoes a dramatic elongation along its anterior–posterior (A–P) axis during late embryogenesis, becoming roughly four times longer along the A–P axis during this time. Unlike many organisms, in which convergent extension movements drive such dramatic elongation, elongation of the *C. elegans* embryo is accomplished largely by a coordinated change in shape of its epidermal (or hypodermal) cells [6]. This explosive cell shape change requires the construction of a remarkably ordered set of circumferential actin filament bundles in dorsal and ventral epidermal cells, and carefully tuned, cell-specific activation or suppression of non-muscle myosin activity within epidermal cells (Figure 1, left). Previous experiments showed that lateral (or seam) epidermal cells require elevated activity of the myosin regulatory light chain, MLC-4, presumably downstream of Rho and activated ROCK/LET-502 [7,8], whereas myosin activity is downregulated in other epidermal cells via a RhoGAP, RGA-2 [8], and the myosin phosphatase MEL-11 [7].

During the early steps of elongation, the cadherin–catenin complex is required for transmitting forces generated within the epidermal cells to adherens junctions at their surfaces. Zygotic loss of *hmp-1*/α-catenin function or the function of the other core components of the cadherin–catenin complex severely affects elongation, leading to dorsal folds in the epidermis and failure of morphogenesis early in elongation [9,10]. Recent work indicates that the actin-binding function of HMP-1 is crucial for these events; embryos that produce a carboxy-terminally deleted form of HMP-1 that lacks the actin-binding domain can form circumferential filament bundles, but fail to form a mechanically robust junctional-proximal actin network, leading to physical ripping of circumferential filaments away from the cell periphery [10].

Although actomyosin-mediated contractile forces, transmitted through the cadherin complex, are crucial for the early steps of elongation, later stages curiously depend on underlying muscle cells. Muscle cells are mechanically connected to the overlying epidermal cells, and ultimately to the cuticular exoskeleton through an elaborate set of epidermal attachment structures known as fibrous organelles (FOs). FOs consist of hemidesmosome-like attachments on the apical and basal surfaces of epidermal cells that are spanned by intermediate filaments (Figure 1, right). FOs transmit the forces produced by muscle contractions through the basal lamina to the epidermis and ultimately to the cuticle. Several clues gleaned over a period of many years indicated an intimate mechanical and functional connection between muscle and epidermis. Embryos that lack muscle activity, or in which attachment of muscle cells

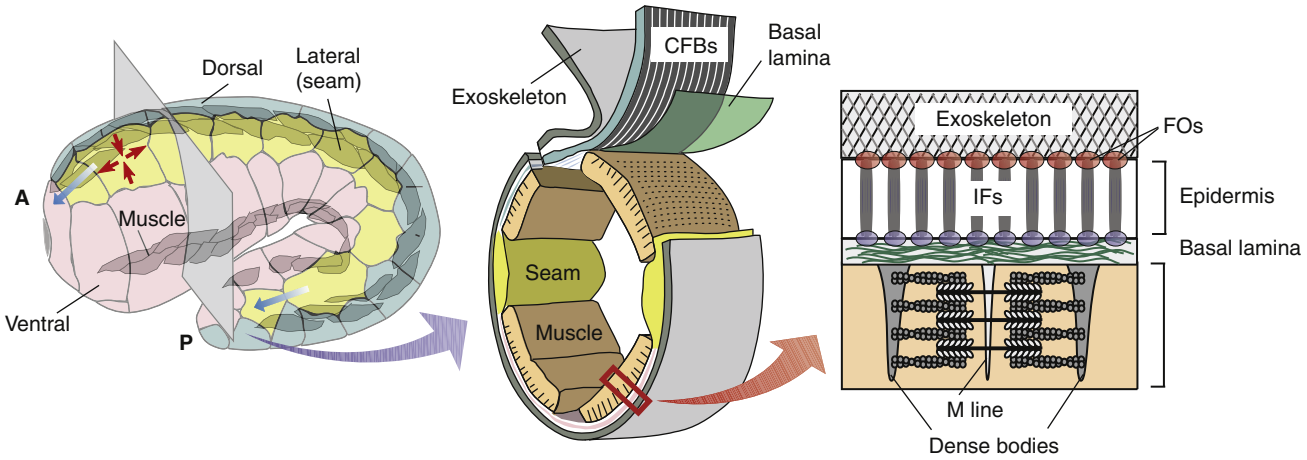

Figure 1. Muscle and epidermis in the *C. elegans* embryo.

(Left) A *C. elegans* embryo at the outset of elongation, showing the epidermal cells on the exterior. Pink, dorsal; yellow, lateral (seam); green, ventral. Muscles are shown underneath the epidermis. Seam cells elongate, increasing their length along the anterior–posterior axis at the expense of the dorsoventral dimension (red arrows), elongating the embryo (blue arrows). A, anterior; P, posterior. (Center) In cross-section, the epidermis lies beneath an exoskeleton (the embryonic sheath). Dorsal and ventral epidermal cells produce circumferential actin filament bundles (CFBs). Between the muscle and epidermis is a thin layer of basal lamina. (Right) Intermediate filaments connect to apical and basal attachment structures in the epidermis (fibrous organelles, FOs). Muscle dense bodies, which are the *C. elegans* equivalent of vertebrate Z discs, contain focal adhesion proteins, such as integrins, that allow attachment to the basal lamina. See [12] for details on muscle anatomy.

to the extracellular matrix is abrogated either through loss of integrin-based attachments (e.g., through loss of *pat-2*/α-integrin or *pat-3*/β-integrin) or via perturbation of deposition of extracellular matrix components such as *unc-52*/perlecan, are paralyzed and arrest after elongating to twice their original length — the Pat phenotype, for Paralyzed at two-fold [11] (reviewed in [12]). Moreover, muscle cells induce correct spatial organization of FOs [13]. Conversely, mutations that disrupt the organization of FOs, such as *let-805*/myotactin [13], *vab-10*/spectraplakin [14], *vab-19*/Kank and *eps-8* [15], or conditions that alter the abundance of FO proteins [16], also cause defects in later elongation.

All of these data suggested that muscle and epidermis engage in crosstalk during elongation, but why this interplay is so important has long been puzzling. Zhang *et al.* [5] begin to tease apart this story by elucidating at least one pathway involved in this crosstalk. Starting from a previously published RNA interference-based screen for genes whose knockdown results in enhancement of morphogenetic defects in weak *vab-10*/spectraplakin mutants [16], Zhang *et al.* [5] focused on *C. elegans* p21-activated kinase (PAK-1). They found that PAK-1 phosphorylates the intermediate filament protein IFA-3, which in turn regulates organization and integrity of FOs. In muscle mutants, CED-10/Rac activity decreases, and PAK-1 activity is concomitantly reduced, which can be partially rescued by introducing activated CED-10 and MLC-4. Taken together, these results suggest that Rac activates PAK-1. Based on work in other systems (e.g. [17]), Zhang *et al.* [5] then looked for effects on FOs in weak *vab-10* mutants after removing the function of the p21-activated kinase-interacting exchange factor (PIX-1) and G-protein-coupled receptor kinase-interacting protein (GIT-1), both FO proteins themselves. The results were similar to loss of PAK-1.

Several lines of evidence suggest that mechanical tension exerted by muscle cells is required to maintain GIT-1 localization to FOs. First, without muscle contraction, GIT-1 does not accumulate at cell boundaries, intermediate filaments are not phosphorylated, and FOs become disorganized. Second, the requirement for muscle contraction can be dispensed with if external force is applied via direct micromanipulation of embryos. This purely mechanically induced response is reminiscent of experiments performed in *Drosophila* showing that *twist* expression is upregulated in response to the compressive forces of germ band extension [18].

This work is an exciting starting point for unraveling mechanotransduction between the epidermis and muscle, and opens several new avenues for future study. First, PAK-1 presumably works alongside additional components that are yet to be identified, because loss of PAK-1 alone has no effect on elongation. One possibility is that other kinases work in parallel with PAK-1. Zhang *et al.* [5] have developed an experimental paradigm that should help in identifying some of these additional components in the future. Second, recent evidence in vertebrates indicates that adherens junctions (AJs) are capable of engaging in mechanotransduction, in part via tension-dependent conformational changes in α-catenin [19]. It will be interesting to see how AJ-dependent mechanotransduction events interrelate with those at FOs. Finally, classical experiments indicate that transmission of forces within epidermal cells during elongation requires not just actin, but microtubules [20], in addition to the IF-based apparatus found at FOs. How forces are distributed among all of these cytoskeletal elements is an exciting area for future study; presumably multivalent linker proteins, such as various VAB-10 isoforms [14], might interconnect different polymer

systems. What is clear is that the work of Zhang *et al.* [5] makes a unique contribution to addressing the important question of mechanical integration during morphogenesis.

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Animal Cognition: Monkeys Recall Previously Seen Images

A recent study has found that rhesus macaques can recall newly presented shapes: this demonstration of recall in non-human primates suggests that some animals have recollection processes similar to those of humans.

Bennett L. Schwartz

Consider, as a few novelists have done, that the only witness to a serious crime is a non-human primate [1,2]. Can a rhesus monkey or other primate bring an image to mind of the criminal? And how might that monkey relay that information to investigators? Implicit in these questions is the question of whether or not, like humans, animals can have recollective experiences [3]. Recollective experience refers to the notion that we maintain conscious images, thoughts, or feelings that refer to past events [4]. Much research with humans has shown that, in order to demonstrate recollective experience, a person must be able to recall the past event, not simply recognize it [5,6]. Until now, however, no research has even been able to demonstrate recall, and therefore conscious recollective experience, in a non-human animal. Basile and Hampton [7] have addressed precisely this question in a study reported in this issue of *Current Biology*.

In humans, recall tests are easy to conduct, because all you need to do is ask, and we can respond verbally. In some cases, when a visual memory is required, some people can make accurate drawings from memory. As Basile and Hampton [7] point out, however, animals can neither talk nor draw; consequently, all past research looking at animal memory has involved recognition tests, in which the animal must match their memory with a physically present signal. In match-to-sample tasks, for example, animals must choose an image or sound that they were exposed to earlier [8,9]. That is, in the visual domain, the animals must choose between an image that was presented earlier and a novel image. Similarly, in the auditory domain, an animal must choose between two sounds presented sequentially (or simultaneously), one of which was presented earlier [10]. Note that, in a delayed match-to-sample task, the to-be-remembered stimulus is presented to the animal at the time of test, and the animal must choose to accept it or reject it. In their new work, however, Basile and Hampton [7] used touch screen technology to demonstrate that monkeys, like humans, can remember images that are absent at the time of test.

Consider an experiment in primate memory conducted by Hoffman and colleagues [11]. In a delayed match-to-sample test, rhesus macaques saw a picture presented for three seconds; after a delay of either one second or 10 seconds, the monkey saw the same image and a new image not seen before. The monkeys had to touch the image