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Mechanisms of elongation in embryogenesis

Ray Keller

Here, I discuss selected examples of elongation in embryogenesis to identify common and unique mechanisms, useful questions for further work, and new systems that offer opportunities for answering these questions. Fiber-wound, hydraulic mechanisms of elongation highlight the importance of biomechanical linkages of otherwise unrelated cellular behaviors during elongation. Little-studied examples of elongation by cell intercalation offer opportunities to study new aspects of this mode of elongation. Elongation by oriented cell division highlights the problem of mitotic spindle orientation and the maintenance of cell-packing patterns in anisotropic force environments. The balance of internal cell-adhesion and external traction forces emerges as a key issue in the formation of elongate structures from compact ones by directed migration.

Introduction

The elongation of tissues plays a major role in embryogenesis and organogenesis. Well-known examples include convergent extension movements that are driven by cell intercalation during vertebrate gastrulation, in the ascidian notochord, during *Drosophila* germ band extension, and during echinoderm gut elongation (Keller, 2002). However, most work has focused on the early development of a few systems, and convergent extension is only one mechanism of elongation. Here, other mechanisms of elongation, and less well-known examples of convergent extension by cell intercalation that are instructive and have not received adequate attention, are discussed with the goal of identifying important, unexplored questions. The major conclusions are that specific outcomes of morphogenesis emerge from the global biomechanical integration of local, cellular force-generating processes. This integration follows the mechanical principles that are built in to each particular type of morphogenic machine. The challenge ahead is to integrate genetic and molecular manipulations with cell biological and biomechanical analyses to learn how genes encode the forces, and the cell and tissue material properties (Koehl, 1990) that transmit these forces, to generate the organized patterns essential for the heritable reproduction of form.

Fiber-wound, hydraulic systems

Fiber-wound, hydraulic systems either maintain their shape or change it as a function of two interacting components: an external winding of restraining, tension-resisting fibers and an internal compression-resisting fluid under pressure (Koehl et al., 2000). The geometry of the fiber-windings determines the morphological changes that occur when the internal pressure is increased. Two examples will be discussed here. The first is the vertebrate notochord, in which a swelling of vacuoles increases the pressure, and the mechanical output is determined by the angle of orientation of extracellular matrix (ECM) fibrils. The second is the nematode embryo, in which the fiber-windings themselves contract to cause an increase in pressure.

Department of Biology, University of Virginia, Charlottesville, VA 22901, USA.

e-mail: rek3k@virginia.edu

Elongation and straightening of the notochord

After participating in convergent extension during gastrulation and neurulation (Glickman et al., 2003; Keller et al., 1989), the notochord of amphibians and fish forms a curved cylinder, which continues to elongate and straighten to form a stiff but flexible rod that contributes to the elongation of the posterior body axis (Fig. 1A,B). It also serves as a flexible skeleton in larval swimming. In the amphibian, elongation and straightening occur in the early tailbud stage, as the notochord cells, which are initially flattened transverse to the length of the notochord (Keller et al., 1989), form vacuoles filled with proteoglycans, which hydrate and swell (Mookerjee et al., 1953; Waddington and Perry, 1953) (Fig. 1C,D). The cylinder of swelling cells is surrounded by a fibrillar ECM, or sheath, composed of collagen and glycosaminoglycans (GAGs) (Bruns and Gross, 1970; Hay, 1984; Kenney and Carlson, 1978; Mookerjee, 1953; Mookerjee et al., 1953), fibrillin (Skoglund et al., 2006) and fibronectin (Bruns and Gross, 1970; Davidson et al., 2004; Weber, 1961). In *Xenopus*, the fibrils are wound around the cylinder at an average angle of 54 degrees with respect to its long axis (Adams et al., 1990) (Fig. 1E, enlarged box). Isolated notochords are osmotically sensitive, and they can be driven through repeated cycles of stiffening, straightening and elongating, and softening, bending and shortening, by varying the osmotic strength of the medium (compare black and gray in Fig. 1E). The *Xenopus* notochord increases in volume, diameter and length, and it straightens and increases in flexural stiffness (resistance to bending) during early tailbud stages (Adams et al., 1990). This behavior requires the mechanical restraint of the sheath, and if it is enzymatically digested, a floppy array of swollen cells is produced, instead of a stiffening, straightening notochord (Adams et al., 1990) (Fig. 1F). Thus, the elongation, stiffening and straightening is due to osmotic pressurization of a fiber-wound cylinder, a fiber-wound hydraulic skeleton (Koehl et al., 1990).

The mechanical properties of curved, fiber-wound hydraulic systems have important, general implications for morphogenic mechanisms of this type. The role of the fiber angle on the behavior of curved hydraulic systems was analyzed empirically with physical models of the curved *Xenopus* notochord by Koehl and colleagues (Koehl et al., 2000) They built curved latex cylinders embedded with taffeta cloth, which provided reinforcing fibers at various angles, and inflated them at increasing pressures in a supporting water bath (Fig. 1G). They measured changes in the shape and the mechanical properties of these cylinders as a function of fiber angle (Fig. 1H-J). At fiber angles greater than 54 degrees, the observed angle of *Xenopus* notochord fibers, the notochord models narrowed, lengthened, and straightened on inflation, whereas they widened and shortened at angles less than 54 degrees (Fig. 1H). The straightening occurred at any fiber angle but was greater at larger fiber angles. Flexural stiffness, the resistance to bending and kinking (Fig. 1I), increased at all fiber angles but was greater at lower angles. On inflation, the ends of the cylinders exerted pushing forces above 54 degrees and pulling forces below this angle (Fig. 1J). Fiber angles above and below 54 degrees converged on this value as the inflation pressure increased, and as the cylinder changed shape (Koehl et al., 2000). Measurements of pushing work (pushing force $\times$ distance)

that could be done by the models showed that fiber angles greater than 54 degrees could do more work against a moderate load, but angles less than 54 degrees provided a greater flexural stiffness and resistance to bending or kinking under greater loads, which suggests that the *Xenopus* fiber angle is a compromise between these two performance parameters.

These experiments illustrate empirically that the mechanical performance of fiber-wound cylinders depends on an oriented cellular property (the angle of fibril deposition) and on a uniform cellular property (the hydraulic pressure due to osmotically active molecules). ECM fiber angle is a specifically patterned property, which results from intrinsic molecular properties, oriented secretion and assembly, and mechanical interaction with cells (cell traction) during or after assembly (Canty and Kadler, 2002; Canty and Kadler, 2005; Harris et al., 1980; Kadler, 2004; Kadler et al., 1996). Cell regulation of fiber angle, the mechanical significance of fiber angle, the contribution of the different fibril types, as well as fibril cross-linking and matrix remodeling (Davidson et al., 2004), are all parameters that should be studied further in specific morphogenic systems.

More generally, the fiber-wound notochord illustrates how morphogenic mechanisms are greater than the sum of their parts. Context-dependent, emergent properties result from biomechanical linkages between basic cellular processes, such as uniform vacuole inflation on the one hand and oriented fiber winding on the other, to produce a specific morphogenic outcome, which cannot be attained by either acting alone. Morphogenic specificity arises from this type of large-scale biomechanical integration of local, cellular force-generating processes (Hardin, 1990), an integration that follows the mechanical rules inherent to each morphogenic machine.

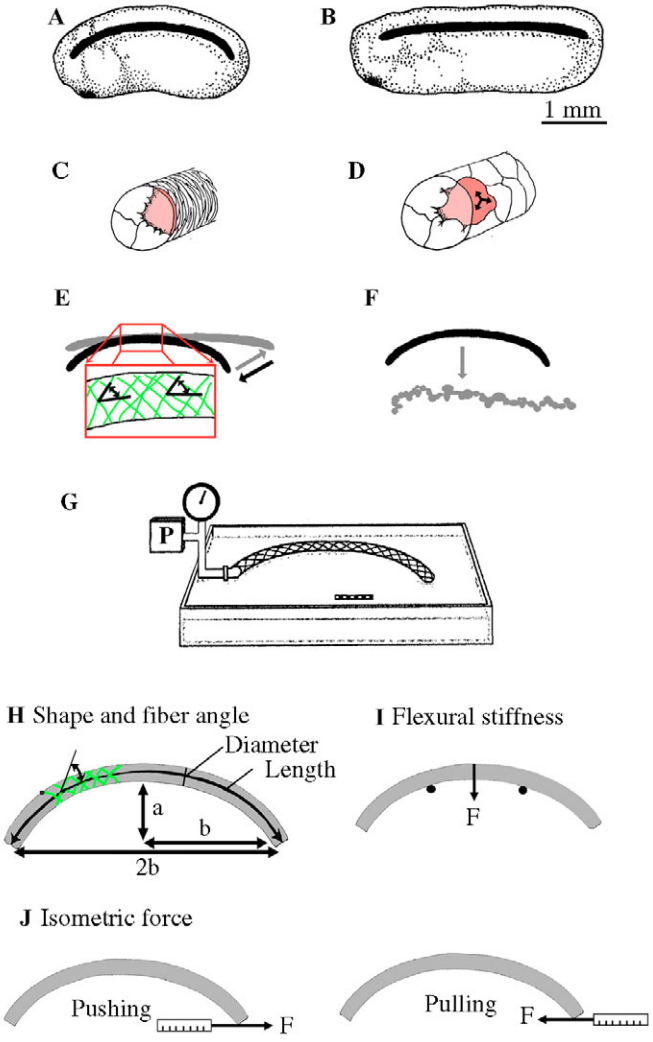

Fig. 1. Straightening and elongation of the notochord. (**A**,**B**) The amphibian (*Xenopus*) notochord straightens and elongates from the early (A) to late (B) tailbud stage (anterior is to the left, dorsal at the top). (**C**,**D**) The early tailbud notochord consists of transversely stacked flattened cells that are the shape of pizza slices (C), which vacuolate and swell during notochord elongation (D). (**E**) The notochord is encased in a sheath of extracellular matrix with an average fiber angle of 54° (fibers shown in green). Isolated intact notochords straighten and lengthen in solutions of low osmotic strength (gray), and bend and shorten in solutions of high osmotic strength (black). (**F**) The cells swell but notochord morphogenesis is lost when the sheath is enzymatically digested. (**G**) The mechanical behavior of fiber-wound hydraulic skeletons has been studied experimentally by varying the water pressure (P) inside latex tubes, which were embedded with taffeta fiber windings at varying angles and suspended in a water bath. (**H**) Geometric parameters were measured, including fiber angle, diameter, length, curvature (a/b) and shortening or elongation (2b). (**I**,**J**) Mechanical properties were measured, including flexural stiffness [the force (F) necessary to produce bending between two supports; I] and isometric force production in pushing and pulling (J). (A,B,E-J) Reproduced, with permission, from Koehl et al. (Koehl et al., 2000).

Nematode elongation

The nematode embryo is an ideal system in which to explore the genetic encoding of biomechanical information of this type. The oval-shaped embryo of the nematode *Caenorhaditis elegans* forms an elongate worm using the principle of the fiber-wound, pressurized cylinder, but, in this case, the pressure is generated by the circumferential contraction of a cytoskeleton, an event that is preceded by cell movements that form a circumferentially reinforced array of cells that develop this contractile force. The hypodermal cells (also called epidermal cells) initially lie dorsally in three rows per side, which are named the dorsal, lateral and ventral hypodermal cells from their eventual position (Priess and Hirsh, 1986; Simske and Hardin, 2001) (see Fig. 2A,D,G). First, the two rows of dorsal cells intercalate to form a narrower but longer single row (see Fig. 2A-C), which elongates the dorsum of the embryo, and gives it a convex dorsal curvature and a concave ventral curvature (Priess and Hirsh, 1986) (Fig. 2G-I). Then, the anterior, ventral hypodermal cells elongate and extend filopodia ventrally (Fig. 2D), and attach to their counterparts on the other side, thus encircling the embryo at this level (Fig. 2D,E,G,H). Following this event, and dependent upon it, the ventral ends of the posterior ventral hypodermal cells form a contiguous ‘purse string’ (Fig. 2E,H), which then zips up from anterior to posterior, and brings about ventral closure (Fig. 2F,I). The embryo is then surrounded by five rows of hypodermal cells, a single dorsal row formed by intercalation, two lateral rows (sometimes called ‘seam cells’), and two ventral rows that meet in the ventral midline (Fig. 2I).

The encircling cells are linked at their outer apices by belt desmosomes, which are intercellular adhesive organelles that link the cells together in a cylindrical, contractile array (Priess and Hirsh, 1986) (Fig. 2K-M). Bundles of microfilaments and microtubules become oriented circumferentially in the dorsal and ventral hypodermal cells, whereas the microfilaments in the lateral or seam cells form a less organized meshwork (Priess and Hirsh, 1986; Raich

et al., 1999) (Fig. 2K-M). A circumferential contraction occurs in the lateral cells, which decreases the diameter and lengthens the cylinder (Fig. 2K,L). The significance of having most of the circumferential contraction take place in the lateral cells is not understood; the dorsal and ventral hypodermal cells may serve primarily as circumferentially reinforced, tensile elements, and, to the degree that they lack a circumferential contraction of their own, they must be passively deformed by the hydraulic effect of the actively contracting lateral cells.

Wounding the hypodermis results in the extrusion of underlying deep cells, but only during elongation, indicating that internal pressure rises at this time. Elongation, and the rise in pressure are blocked by cytochalasin D, suggesting a hydraulic mechanism in which an actin microfilament-mediated contraction increases internal pressure (Priess and Hirsh, 1986). The contraction is myosin dependent. Mutations in the non-muscle myosin regulatory light chain, *mlc-4*, result in defective elongation, possibly because of failure of microfilament bundle contraction (Shelton et al., 1999). Mutations in the Rho kinase gene, *let-502*, a positive regulator of myosin, inhibit elongation, an effect that is suppressed by mutations in *mel-11*, which encodes a smooth muscle myosin phosphatase regulatory subunit, a negative regulator of myosin (Wissman et al., 1999; Wissman et al., 1997). Mutants or knockdowns of spectrins, proteins important in organizing the plasma membrane cytoskeleton, cause disorganized circumferential actin bundles and a slow initial elongation (up to the twofold stage) (Norman and Moerman, 2002; McKeown et al., 1998). Microtubules are oriented parallel to the microfilament bundles, and their disruption with nocodozole or other reagents results in poor elongation and surface abnormalities (sharp constrictions, broad swales) that do not appear if microfilaments are also disrupted. This implies that microtubules do not directly affect microfilament bundle function but modify or channel the forces that they develop (Priess and Hirsh, 1986). The circumferential tensile forces developed during elongation require circumferential tissue integrity to produce elongation, which, in turn, requires the cell adhesion molecule cadherin, as well as the cytoplasmic proteins β-catenin and α-catenin which link cadherins to the cytoskeleton (Costa et al., 1998; Williams-Masson et al., 1997). Without strong adhesions at the end of ventral closure, the entire hypodermis often retracts to the dorsal side (Raich et al., 1999), a behavior that reinforces the notion that complete encirclement is necessary for developing the circumferential forces that drive elongation (Priess and Hirsh, 1986; Simske and Hardin, 2001).

The embryonic sheath of extracellular matrix (ECM) functions in elongation, and the cuticle developing from it is necessary for the maintenance of the elongated body form (Costa et al., 1997; Priess and Hirsh, 1986). Attachment of the embryonic sheath to the hypodermis occurs only in the region where the hypodermal cells are immediately underlying the circumferential actin bundles. Trypsin digestion of the embryonic sheath results in the formation

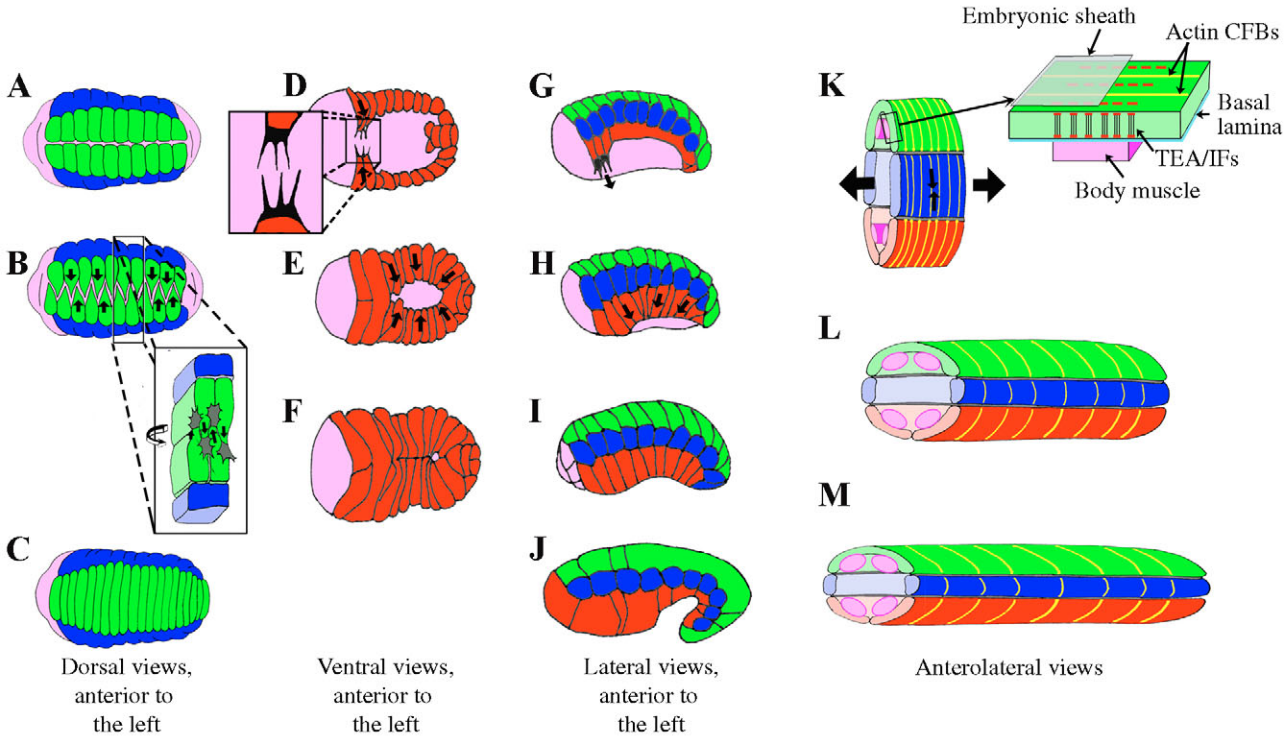

Fig. 2. Elongation of the nematode embryo. (**A-C**) The dorsal surface of the nematode embryo shows intercalation of dorsal hypodermal cells (green), which elongates the dorsal aspect of the embryo, giving it a comma shape (lateral view, G). B is an enlargement of A: the basal surfaces of the intercalating dorsal hypodermal cells show medially directed protrusions. (**D-F**) Ventral views and (**G-J**) lateral views of ventral closure, during which the ventral hypodermal cells (red) move across the underlying neuroblast cells (purple) and meet in the midline. (**K-M**) Hypodermal-mediated elongation beyond the comma stage involves a circumferential actin microfilament cytoskeleton (yellow) in the dorsal (green) and ventral (red) hypodermal cells, and a circumferential contraction of the lateral or seam hypodermal cells (blue). In regions overlying muscles (K and enlargement), trans-epithelial attachments (TEAs) develop and connect the underlying matrix and muscle (purple) to the overlying cuticle (embryonic sheath). TEAs consist of fibrous organelles (FOs) – electron-dense plaques similar to hemidesmosomes on the ECM/muscle side and on the embryonic sheath side of the hypodermis; FOs are connected by intermediate filaments that span the hypodermal cells. Anterior is to the right in A-J. (A-J) Adapted, with permission, from Simske and Hardin (Simske and Hardin, 2001); (K-M) adapted, with permission, from Ding et al. (Ding et al., 2004).

of deep furrows at the sites of the bundles, and elongation fails (Priess and Hirsh, 1986). This implies that the sheath is necessary for the distribution of the cytoskeletal contractile force across the surface of the cylinder. Proper elongation beyond the twofold stage also requires the interaction of the hypodermal cells with the underlying longitudinal muscles on the inside, but the mechanism of this effect is not understood. Trans-hypodermal connections of fibrous organelles (FOs), which consist of electron dense plaques similar to hemidesmosomes, and intermediate filaments, mechanically link the muscles on the inside to the embryonic sheath on the outside (Bosher et al., 2003; Ding et al., 2003; Hapiak et al., 2003; Woo et al., 2004) (Fig. 2K). Isoforms of spectraplakin function in different aspects of FO assembly and actin filament organization, and their loss of function results in the separation of the hypodermis from the embryonic sheath and from the longitudinal muscles, or a thickened epidermis (Bosher et al., 2003). Interactions of the hypodermal cells with the underlying longitudinal muscles are necessary for beyond twofold elongation. The muscle cells are necessary for FO localization in the hypodermal cells and hypodermal cells reciprocally affect muscle development (Hresko et al., 1999; Hresko et al., 1994). Abnormal muscle contractions, or abnormal muscle action potentials, result in abnormal morphology, including a block of elongation after the twofold stage (Lee et al., 1997). However, simple mechanical coupling of the contracting longitudinal muscles to the hypodermis does not explain elongation, as these muscles contract longitudinally and should shorten rather than elongate the embryo. There may be as yet unknown organizing signals from the muscle to the hypodermis. Also, the highly organized mechanical coupling of the muscle, the hypodermis, and the embryonic sheath may form an integrated system with unknown force-transducing properties, much as fiber windings channel the osmotic forces of the notochord.

Convergent extension by cell intercalation

Convergent extension by cell intercalation is a diverse process that occurs in both mesenchymal and epithelial tissues. It is driven by different cellular mechanisms and regulated by several, different pathways in different systems. Here, systems that offer new opportunities to probe this diversity of mechanism are discussed.

Intercalation of nematode dorsal hypodermal cells

Intercalation of the dorsal hypodermal cells leads directly to the elongation of the dorsal side of the nematode embryo (Simske and Hardin, 2001). The initial interdigitation of dorsal cells is led by medially directed lamelliform protrusions of their deep or basal surfaces (Williams-Masson et al., 1998) (Fig. 2B, enlargement), indicating that these ends, rather than the apical ends, generate most of the forces for intercalation, perhaps by using the deeper parts of the embryo or ECM as a substrate. Potential substrates in this region have not been characterized, and the mechanism of adhesion or traction is not known. Die-1, a zinc-finger transcription factor, is essential for this intercalation (Simske and Hardin, 2001), but the downstream cellular components have not been identified. This system is particularly interesting because it is an intercalation of epithelial cells in which basal protrusive activity has been described in some detail, and thus it will be very useful to compare its mechanism and regulation with an epithelial system, such as the cells of the *Drosophila* germband, which are thought to rearrange by junctional remodeling rather than by basal protrusive activity (Bertet et al., 2004), and with mesenchymal ones, such as the dorsal mesoderm of frogs, that use bipolar protrusive activity (Keller et al., 2000).

Drosophila hindgut elongation

During germ band extension, the *Drosophila* proctodeal primordium, which includes the future hindgut epithelium, is internalized, and as the germ band retracts, the hindgut differentiates into the small and large intestines and elongates (Lengyel and Iwaki, 2002; Myat, 2005) (Fig. 3A,B). Most of the elongation is due to a circumferential intercalation of cells that decreases their number per cross section from about 50 to 12 (Johansen et al., 2002; Lengyel and Iwaki, 2002) (Fig. 3C). The evidence suggests that these cell intercalations are regulated by a graded activation of the JAK/STAT pathway (Johansen et al., 2002) (see Fig. 3D). Localized expression of *upd* RNA and Upd protein in the small intestine is thought to establish a gradient of Upd in the large intestine, presumably by diffusion, which in turn drives the graded activation of JAK/STAT (Fig. 3E). Deficient expression of Udp, Dome, Hop and Stat92E (see Fig. 3D), as well as expression of a dominant-negative form of Dome, produces shorter, wider hindguts (Johansen et al., 2002). Localized expression of Upd in the small intestine of *upd* mutants rescues elongation, whereas its uniform expression in the large intestine does not, nor does uniform expression of STAT in the large intestine.

From these and other results, Lengyel and Iwaki (Lengyel and Iwaki, 2002) propose that this activity gradient establishes an anteroposterior (AP) polarity vector in the intestine, which directly organizes circumferential cell intercalation, or forms an attractive cue that would direct cell migration anteriorly, which would somehow result in cell intercalation. The second mechanism seems improbable, as it is difficult to see how an anteriorly directed migration would produce circumferential intercalation. The first mechanism is favored by the fact that induction of transverse (mediolateral or circumferential) intercalation by a perpendicular (AP) signal is emerging as a common theme. AP signaling is necessary for mediolateral cell intercalation during germ band extension in *Drosophila* (Zallen and Wieschaus, 2004) and during convergent extension by mediolateral cell intercalation in *Xenopus laevis*, where this process may depend on AP differences in the adhesion, and sorting out behavior, of cells (Ninomiya et al., 2004). JAK/STAT signaling regulates several processes, including cell motility and adhesion, and its graded activity might set up local differences in cell adhesion that could also cue circumferential cell intercalation in the hindgut.

These and other possibilities should be studied further in this intriguing system. What are the downstream effectors and what cellular processes do they control? Does cell intercalation in the fly hindgut, an epithelium, occur via the bipolar protrusive activity seen in the intercalating mesenchymal cells of the frog (Shih and Keller, 1992a), by the myosin-mediated junctional remodeling seen in the intercalating epithelial germ band cells of *Drosophila* (Bertet et al., 2004), or by some other mechanism not yet described?

Malpighian tubule elongation

Malpighian tubules of *Drosophila* form at the junction of the mid and hindgut by the evagination of four stubby cylindrical buds (Fig. 4A), which elongate to form crescent-shaped tubes, and then thin and elongate further to form two anterior and two posterior tubules (Fig. 4A) (Myat, 2005; Skaer, 1989; Skaer and Arias, 1992). The initial elongation requires cell division (Janning et al., 1986; Skaer and Arias, 1992), which is stimulated through epidermal growth factor (EGF) signaling by a specialized cell, the tip cell, selected from among those residing at the end of the extending tubule by Notch signaling (Hoch et al., 1994; Myat, 2005). It is not known how efficiently the axis of cell division parallels the length of the tube,

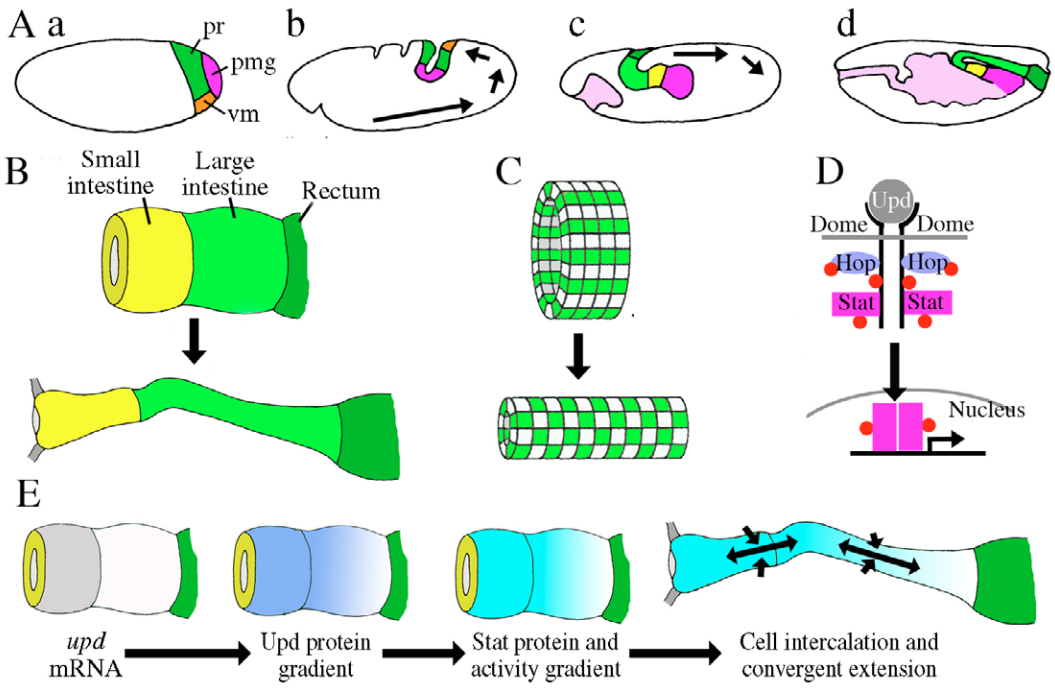

Fig. 3. Convergent extension of the *Drosophila* hindgut. (**A**, parts a,b) Sagittal views show invagination of the posterior midgut (pmg, purple), proctodeal ring (pr, green), and visceral mesoderm (vm, orange) during germ band extension. (A, parts c,d; **B**) During germ band retraction, the hindgut elongates (yellow, small intestine; green, large intestine). (**C**) This elongation occurs by circumferential cell intercalation. (**D**) Elongation is regulated by the JAK/STAT pathway, including the ligand Unpaired (Upd), the receptor Dome (Dome, Domeless or Master of Marelle), JAK (Hop, Hopscotch) and Stat (Stat92E or Marelle). (**E**) A model for regulation of hindgut convergent extension is shown, in which *unpaired* (*upd*) mRNA is expressed in the small intestine (gray), and the encoded protein diffuses posteriorly, establishing a gradient (lavender). Upd activates Stat and positively regulates Stat protein levels, resulting in a Stat protein and activity gradient (blue) that, in turn, regulates circumferential intercalation and the resulting convergent extension. Anterior is to the left in A-C,E. Adapted, with permission, from Lengyel and Iwaki (Lengyel and Iwaki, 2002) and Johansen et al. (Johansen et al., 2003).

and thus how much it contributes directly to elongation rather than widening. In the second phase of elongation, convergent extension by a circumferential intercalation reduces the number of cells in cross section from about eight to two (Skaer, 1989; Skaer and Arias, 1992) (Fig. 4B). Mesenchymal-to-epithelial transition (MET) and radial intercalation may also function in elongation. The tubules derived from the initial epithelial evagination consist of principal cells (Fig. 4C), but, later, mesenchymal cells from the presumptive visceral mesoderm migrate along the epithelial tubules, polarize as incipient epithelial cells, and integrate themselves into the wall of the tubule as stellate cells (Denholm et al., 2003) (see Fig. 4C).

Several genes necessary for this elongation have been identified (reviewed by Myat, 2005), but the underlying cell behaviors driving the intercalation, and their regulation, remain unknown. Is intercalation in this system regulated by one of the pathways important in other systems, or by yet another one? Does it follow the emerging rule of requiring signaling perpendicular to the axis of intercalation, or does it violate this rule? How much elongation, if any, the MET and radial intercalation of stellate cells produces, and how these processes are regulated are also unknown.

Drosophila ovariole development

Convergent extension by cell intercalation in *Drosophila* has been studied only in epithelial sheets (the germ band, the hindgut, Malpighian tubules, the imaginal disc), but development of the terminal filaments, the interfollicular, and the basal stalks of the *Drosophila* ovariole offer opportunities to study convergent extension by a mesenchymal cell intercalation in a genetically tractable system (Godt and Laski, 1995). In the late larval and early pupal stages, an AP stratified mass of somatic and primordial germ cells is separated into about 20 ovarioles as the apical cells move posteriorly between them (Godt and Laski, 1995) (Fig. 5A-E). The process begins as the presumptive terminal filament cells become

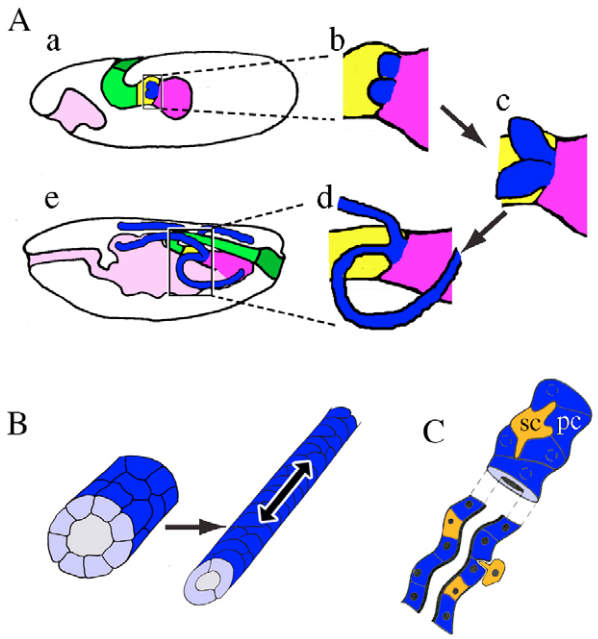

Fig. 4. *Drosophila* Malpighian tubule elongation. (**A**) Malpighian tubules form as evaginations from the hindgut-midgut junction (blue, parts a,b), and first elongate by cell division (parts b,c), and later by circumferential cell intercalation (parts c-e). Anterior is to the left. (**B**) Cell intercalation reduces the circumference from about eight cells to two cells, while elongating the tubule commensurately. (**C**) Mesenchymal cells from the visceral mesoderm (orange) intercalate into the primary cells (pc) to form the stellate cells (sc). (A) Adapted, with permission, from Lengyel and Iwaki (Lengyel and Iwaki, 2002); (C) Adapted, with permission, from Jung et al. and Denholm et al. (Jung et al., 2005; Denholm et al., 2003).

fusiform, align parallel to one another, intercalate, and form stacks as their dorsal and ventral ends come together, possibly because of strong adhesion at these sites (Fig. 5A, parts a,b). These ends stain strongly for actin filaments, and for Armadillo, a cytoplasmic (catenin-like) linker of the cytoskeleton to cadherins. As the stacks mature, the apical cells, which are initially mesenchymal, form an epithelium and invade between and surround the stacks (Fig. 5A, parts c,d). The terminal filament cells then become flattened and disc-shaped (Fig. 5A, parts c,d), redistribute Armadillo to their flattened anterior and posterior surfaces, and apply their narrow, circumferential surfaces against the basal surface (perhaps a basal lamina) of the newly invading epithelium (Fig. 5A, part d). As the epithelium invades posteriorly, it encloses and separates similar arrays of germ cells and somatic cells (Fig. 5B,C), and the basal stalk

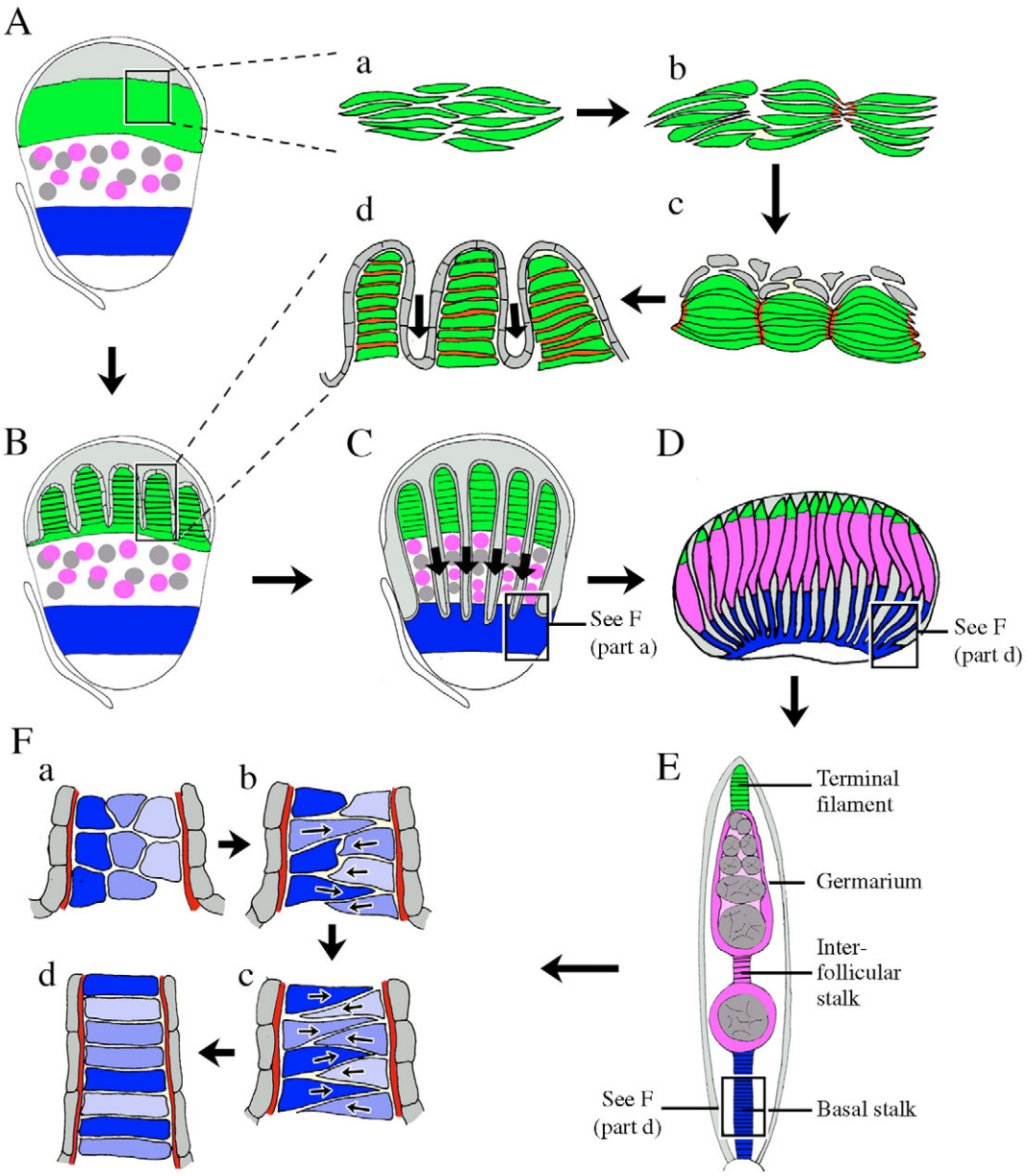

Fig. 5. The development of the *Drosophila* ovary. (**A**) At puparium formation, the ovary is an anteroposteriorly stratified structure, consisting of apical cells (light gray), presumptive terminal filament (TF) cells (green), germ cells (dark gray), somatic cells (magenta) and presumptive basal stalk cells (blue); anterior is to the top and lateral to the left in all diagrams. About 20 ovarioles are formed as the TF cells intercalate laterally into stacks of elongated cells in register anteroposteriorly, which then separate into stacks of discs (A, parts a-d, **B**). As they do so, the apical cells form an epithelial sheet and move posteriorly between the stacks of discs, separating them into TFs (A, parts a-d). (**C**) The posteriorward (arrows) invasion of the apical cell epithelium then separates the germ cells and associated somatic cells and, finally, basal stalk cells into ovarioles (**D**,**E**). (**F**) The apical cell epithelium with an underlying basal lamina (red) separates the basal stalk cells into arrays of several cells in diameter (part a). These cells intercalate transversely to form a longer, narrower array, thus forming the elongated basal stalks and completing the separation of the ovarioles (parts b,c). A similar process occurs among the somatic cells associated with the germ cells, to elongate the interfollicular stalk, which separates the newly formed follicles from the germarium. Anterior is to the top and posterior to the bottom in all figures. Adapted, with permission, from Godt and Laski (Godt and Laski, 1995).

cells (Fig. 5C,D), thus completing the separation of ovarioles (Fig. 5E). Instead of forming stacks directly, the basal cells form an array about three cells across, bounded at the perimeter by the basal lamina of the intruding apical epithelium (Fig. 5F, part a). The rounded cells then flatten in the AP dimension and elongate transversely, with their ends bounding the basal lamina and flattened on it (Fig. 5F, parts b,c). Their inner ends then intercalate and extend the cell array to form the elongated basal stalk (Fig. 5F, parts c,d). At about 2.5 days after puparium formation, an ongoing process of interfollicular stalk formation occurs between the germarium and the successively maturing follicles, using a cell intercalation mechanism similar to that which forms the basal stalks.

The terminal filament cells express Bab from the *bric-a-brac* (*bab)* locus (Couderc et al., 2002; Sahut-Barnola et al., 1995), and *bab* mutants have defective terminal filaments, interfollicular stalks and basal stalks, and reduced or absent ovarioles. Mosaics of mutant and wild-type cells show that *bab* has a cell autonomous role in the formation of these structures (Godt et al., 1993; Godt and Laski, 1995). The *bab* locus consists of two genes that encode nuclear proteins that regulate several morphogenic processes, but their downstream, cell biological mechanisms are unknown.

This system offers some unique opportunities to study new aspects of convergent extension by cell intercalation. The intercalation of terminal filament cells into bundles and then their expansion to form stacks of discs appear to involve polarized adhesions, first at their dorsal and ventral ends, and then at their flattened anterior and posterior surfaces. Also, the posterior invasion of the apical cell population between the ovariole primordia involves a MET and is led by the basal surface of the newly formed epithelium, a rather unique geometry of invasion about which nothing is known. The association of the invading apical epithelium, and its basal lamina, with the process of intercalation of basal stalk cells, which appear to be mesenchymal initially, offers a unique opportunity to study the epithelial and matrix regulation of cell intercalation behavior. The outer ends of basal stalk cells are in apposition to the epithelial basal lamina, and their subsequent intercalation behavior at this boundary closely parallels vertebrate notochord cell behavior at the notochordal-somitic boundary (Shih and Keller, 1992b), which is also matrix-filled (Skoglund et al., 2006). Shared features include the formation of the tapered, interdigitated, inner ends, the stable apposition of the blunt ends of the cells against the basal lamina and the intercalation of inner cells into the boundary, all features that parallel the boundary-mediated quiescence and capture of notochord cells in contact with the notochord/somite boundary in *Xenopus* (Keller et al., 1989; Shih and Keller, 1992b). Do the basal stalk cells and frog notochord cells use similar types of motility to intercalate, and how do the regulatory pathways differ?

In summary, this system offers many interesting behaviors to study, including the polarization of adhesions, MET, epithelial invasive behavior, epithelial-mesenchymal signaling, and signaling from the ECM during cell intercalation. Most importantly, it is, to date, the only mesenchymal cell intercalation system in *Drosophila* in which extensive genetic approaches are available. Comparing this system to the intercalation of *Drosophila* epithelial cells should be most interesting.

Elongation by oriented cell division

Oriented cell division, with or without cell growth, can result in elongation. The linear dimensions of a cuboid cell are the cube root of its volume, and therefore on halving its volume by cytokinesis, the daughters, oriented end-to-end, are 1.6 the length and 0.8 the width and thickness of the parent cell. But localized cell division, even accompanied by cell growth, may result in a large spheroid mass rather than elongation, unless a serial order arrangement of post-division cells is maintained.

Oriented cell division and maintenance of serial order is a fundamental feature of the body elongation of the leech (Huang and Weisblat, 1996; Stent and Weisblat, 1982), an annelid worm. Five large teloblasts on each side of the embryo undergo repetitive, highly unequal divisions to produce bandlet cells, which maintain their serial end-to-end order to form five bandlets: four ectodermal bandlets and one mesodermal bandlet (Fig. 6A). These adhere to one another shortly after leaving their parent teloblasts, and form a germinal band on each side (see Fig. 6A). As the bandlets extend by the addition of cells posteriorly, the germinal bands bow vegetally and ventrally

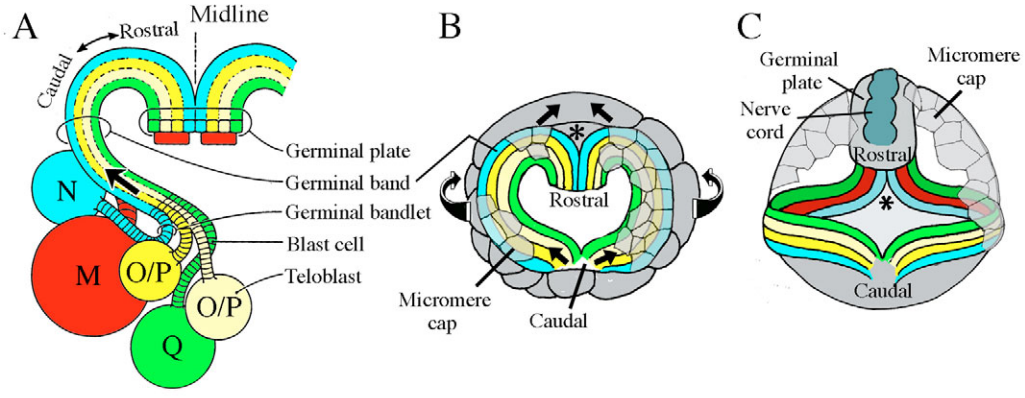

Fig. 6. Elongation of the leech body plan from teleoblasts. (**A**) The teleoblasts of the leech (annelid) embryo produce elongating germinal bandlets through the sequential production of blast cells by highly unequal divisions. On each side, four ectodermal bandlets (N, O/P and Q) come into lateral apposition, while the mesodermal (M) bandlet comes into apposition from beneath. The five bandlets form the germinal band, which extends across the embryo (arrow, A) as bandlet cells are added posteriorly by highly unequal cell divisions of the parent teloblasts. The germinal bands anneal with their contralateral partner in a rostrocaudal sequence at the midline to form the germinal plate. (**B**,**C**) As the germinal bandlets extend by cell division and anneal, they, and the micromere cap overlying them, expand over the surface of the embryo in epiboly (B, curved arrows, early stage 8, animal view; C, late stage 8, rostral view). (A) Reproduced, with permission, from Nelson and Weisblat (Nelson and Weisblat, 1991); (B,C) reproduced, with permission, from Smith et al. (Smith et al., 1996). Asterisks indicate apposition of germinal bands.

across the large, underlying macromeres (see Fig. 6B). They adhere to one another at the midline, and zip up, beginning anteriorly and progressing posteriorly (Fig. 6B,C), to form the germinal plate, which forms the body axis as each bandlet makes specific contributions to the differentiation of the 32 segments of the leech.

The crucial features of this mechanism of elongation are the orientation of the divisions and the end-to-end cell packing of cuboidal cells parallel to the AP axis. The cuboidal, gridiron packing pattern, rather than the common, stable, near-hexagonal packing pattern, implies a highly anisotropic, polarized pattern of adhesion between neighbors in a column (intra-bandlet adhesion), and also between columns (inter-bandlet adhesion), of cells, as the bandlet cells maintain both their end-to-end order and their lateral registry between the bandlets during the complex bending and movements of the germinal bands. The bandlets do show specific amounts of inter-bandlet, AP slippage during their elongation and in making segment contributions, and the different bandlets vary in their adhesion to one another (Huang and Weisblat, 1996). It is not known whether the movements of the germinal bands are due to: internally generated, pushing forces; active migration on surrounding tissues; forces generated by surrounding tissues; or a combination of these forces. In any case, the rows and columns of cells may be reinforced with specialized and polarized adhesions.

Oriented division also contributes to elongation in the short (or intermediate) germ band insects, such as the grasshopper (*Schistocerca*) and the flour beetle (*Tribolium*), and in *Parhyale*, a crustacean, in which body segments form progressively from a posterior growth zone, rather than nearly simultaneously, as in *Drosophila* (Sander, 1976; Browne, 2005). Oriented cell division may also have a role in elongating the body axis of some vertebrates. Cell division is reported to be biased in the AP axis, the axis of extension, in the notochord and neural plate of bird and mouse embryos (Schoenwolf and Alvarez, 1989; Schoenwolf and Alvarez, 1992; Schoenwolf and Yuan, 1995), in the extending primitive streak of the bird (Wei and Mikawa, 2000), and in dorsal tissues of the teleost fish (Concha and Adams, 1998; Gong et al., 2004). In the fish, orientation requires the planar cell polarity (PCP) pathway (Gong et al., 2004), which is also necessary for oriented cell intercalation in vertebrates (Keller, 2002; Myers et al., 2002; Wallingford et al., 2002), and for epidermal and eye cell polarity *Drosophila* (Mlodzik, 2002).

These studies raise several important issues. The end-to-end, side-by-side packing of cuboidal cells violates the general rule for cell packing in near-hexagonal arrays. The mechanisms of how adhesive surfaces, the cytoskeleton, or cell-contact behavior establish and maintain cuboidal gridiron packing patterns are unknown. Also, for oriented cell division to produce force that actively elongates the tissue against external loads, the spindle poles must be oriented parallel to the axis of extension, despite being under compression in this axis. But cells tend to orient their mitotic spindle parallel to their long axis [Hertwig's Rule (see Wilson, 1900)], which in a compressed cell is usually transverse to the axis of compression. Thus forcing the spindle to orient perpendicular to compressive forces may require unknown and specialized mechanisms beyond those orienting spindle axes in mechanically isotropic environments. However, the degree of active pushing during the elongations described above, as opposed to the passive stretching by other tissues, has not been measured. Also, even during active elongations, specialized local environments may protect dividing cells from these compressive forces and allow division in an isotropic environment. These issues deserve attention.

Elongation by following tracks

Many organ systems elongate by following signals embedded in tracks. Two systems discussed here raise questions about how the mechanism of the cell-substrate-mediated guidance is coordinated with internal cell-cell rearrangements that either accommodate the elongation or assist in driving it.

The amphibian pronephric duct

The amphibian pronephric duct rudiment segregates from the anterior intermediate mesoderm, which lies just lateral to the somitic mesoderm, as an oblong mass of cells. This rudiment forms the duct by extending a solid cord of cells posteriorly to the cloaca by following a path on the intermediate mesoderm toward the cloaca (Drawbridge and Steinberg, 1996; Poole and Steinberg, 1977; Poole and Steinberg, 1981) (Fig. 7A). Later, the cord hollows to form the duct that serves the kidney tubules, which develop later from the intermediate mesoderm. Vital dye staining and microsurgical manipulations show that the urodele duct extends posteriorly from the mass of the rudiment, rather than developing in situ from underlying cells (Drawbridge and Steinberg, 1996; Poole and

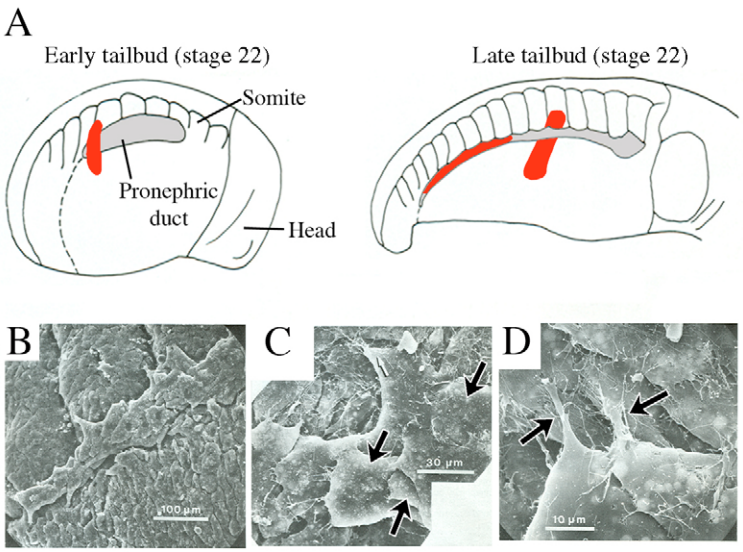

Fig. 7. Pronephric duct formation and posterior elongation in the urodele amphibian. (**A**) Dye marks (red) placed across the duct (dark gray) and adjacent tissues at the early tailbud stage show posterior extension of the duct by the late tailbud stage, rather than development in situ from underlying mesoderm. (**B**,**C**) Scanning electron micrographs (SEMs) show the posterior end of the pronephric duct at mid-tailbud (B), and the overlapping of anterior cells on more posterior ones (C, arrows). (**D**) SEM showing protrusions of pronephric duct cells attached to underlying intermediate mesodermal cells (arrows). Posterior is to the left in A and to the lower left in B. (A) Adapted, with permission, from Poole and Steinberg (Poole and Steinberg, 1981); (B-D) adapted, with permission, from Poole and Steinberg (Poole and Steinberg, 1977).

Steinberg, 1981) (Fig. 7A). The leading tip cells make lamellipodial and filopodial contacts with the underlying cells, which may indicate an active migration (Poole and Steinberg, 1981) (Fig. 7B-D). Ablating potential sources of long-range guidance cues, including the cloaca, does not block duct elongation, suggesting that primary guidance cues are provided locally (see Poole and Steinberg, 1982; Zackson and Steinberg, 1987). The behavior of primordia grafted ectopically on the flank shows that the guidance cues are polarized toward the posterior, and they may take the form of a wave that progresses posteriorly in synchrony with somite segmentation, and perhaps also coordinated with waves of changes in cell adhesion, of matrix deposition, and of cell intercalation in the flank mesoderm (Gillespie and Armstrong, 1986; Zackson and Steinberg, 1986; Zackson and Steinberg, 1988; Zackson and Steinberg, 1989; Poole and Steinberg, 1982). Poole and Steinberg (Poole and Steinberg, 1982) proposed that the duct tip lies within and is guided by a wave of graded change in adhesiveness of the somitic and flank mesoderm. Reorienting the epidermis, or matrix-conditioned microcarriers, can reorient or block duct advance, indicating that the overlying epidermis and the ECM also has a role in guidance (Drawbridge et al., 1995; Morris et al., 2003). Cell surface alkaline phosphatase activity (Zackson and Steinberg, 1989), the ECM protein laminin and its receptor α6β1 integrin, and glial cell line derived neurotrophic factor (GDNF) and its co-receptor GFRα-1, have all been implicated in guidance of the duct (Drawbridge et al., 2000; Morris et al., 2003), but how the functions of these factors are related to one another, and to the putative guiding wave of adhesive changes, and whether they have instructive or permissive roles remain unresolved.

A most interesting problem posed by this system is that traction of the tip cells must be accompanied by deformation of the initial compact arrangement of cells for the elongation to proceed. The pronephric primordium is initially four to five cells wide, and three to four cells deep, and, as it extends, it narrows by cell rearrangement to one to two cells wide and deep, rather than by cell shape changes or cell division (see Overton, 1959; Poole and Steinberg, 1977; Poole and Steinberg, 1981). The duct cells could passively rearrange in response to traction at the tip, but it seems unlikely that tensile forces could be transmitted along the full length of the duct. Post-tip cells could also migrate directionally along the duct pathway, thereby generating tensile, stretching forces all along the length of the duct. If so, migration must occur at progressively slower rates toward the anterior, otherwise the entire primordium would move posteriorly. Moreover, guidance along the length of the duct is not consistent with the local nature of the guidance cues. If there were an active, force-producing cell intercalation, it would have to be kept in step with the rate of tip migration, and blocked tips are not accompanied by a buckling of the duct. An active, tension-regulated mechanism of regulating cell intercalation might account for all observations. In such a mechanism, the cells would actively rearrange, but only in response to terminally or sub-terminally generated tensile forces, which would open up spaces in the tissue-packing pattern and locally stimulate intercalation. Coordinate regulation of tractional forces at the tip and regulation of active extension or passive deformation, such that an extending primordium is properly shaped remains a key problem in this system and many others, and one that must be solved to understand how the relatively simple elongation of a duct is engineered.

The pronephric duct of the anuran *Xenopus laevis* forms similarly, but, in addition, the cells of the underlying intermediate mesoderm are recruited in situ to join the duct at increasingly large numbers as the duct moves posteriorly (Cornish and Etkin, 1993). This addition of cells from an adjacent tissue is reminiscent of the addition of stellate cells to *Drosophila* Malpighian tubules. Local recruitment of cells by a migrating, or otherwise extending, anlagen may be an important and widely used mode of enhancing elongation by other mechanisms, and this too remains a largely unexplored issue.

The lateral line

The lateral line system provides an instructive contrast to the pronephric duct. It consists of linear arrays of organs (neuromasts) that develop in species-specific patterns over the body surface of fish and amphibians. The neuromasts, which consist of receptor cells bearing sensory cilia, supporting cells and innervating neurons, sense water currents (Ghysen and Dambly-Chaudiere, 2004; Winklbauer, 1989). Cephalic placodes generate lateral line primordia, which migrate beneath or within the epidermis in species-specific paths across the body (Winklbauer, 1989). The posterior lateral line placode originates just posterior to the otic placode and migrates along the horizontal myoseptum to the tip of the tail. In contrast to the pronephric duct, the entire primordium migrates, and, at intervals, cells segregate from its posterior aspect and are deposited as primary neuromasts. In zebrafish, CXCR4, a cytokine receptor involved in the guidance of several cell types, is expressed in the cells of the primordium, and it is essential for migration. Its ligand, SDF1 (stromal-derived factor 1), is expressed in the pathway but not on either side (David et al., 2002; Li et al., 2004). The repulsive semaphorin, Sema3A1, is expressed on either side of the horizontal myoseptum pathway, indicating that repulsion may also play a role in guidance (Shoji et al., 1998). Growth cones of the sensory neurons follow the migrating primordium and remain in contact with it. Their migration is guided by the movement of the primordium; they stop when it stops and follow it when it is diverted to abnormal paths (David et al., 2002). The segregation and deposition of neuromasts from the migrating primordium is independent of the surrounding tissues in the axolotl (Smith et al., 1990; Winklbauer, 1989) and zebrafish (Gompel et al., 2001). In zebrafish, neuromast deposition involves downregulation of CXCR4 in the posterior of the primordium, which presumably stops their movement, allowing the rest of the primordium to migrate away from the cells that stop.

A comparison of the similarities and differences between pronephric duct and lateral line primordia highlight some important questions. Both follow tissue-embedded tracks of guidance cues, but, in the latter, the entire primordium migrates, whereas in the former, only the posterior part responds. Is this because only the posterior part of the primordium recognizes the track, or because only this region activates migratory protrusive activity? The posterior mass of the pronepheric primordium accommodates the migration of the tip by elongating in nearly a uniform fashion without breaking, whereas in the lateral line, when a trailing edge mass of cells downregulates its guiding receptor and ceases moving directionally, they break off. This difference may also involve the local downregulation of intercellular adhesion. The contrasting morphogenesis of these primordia appears to be governed by a balance of internal tissue integrity and external traction. How these parameters are integrated is a major unsolved mystery.

Conclusions

A common feature of all elongations is the use of oriented or polarized cell behaviors, structural properties, or tissue material properties, but the cellular and biomechanical mechanisms, and the signaling systems underlying them, are diverse. In regard to cellular

biomechanics, many morphogenic strategies have been exploited. The use of hydraulic mechanisms for active elongation, hereto largely ignored in the field, illustrates the emergence of morphogenic specificity from the distributed nature of morphogenic information and from the multilevel, biomechanical integration of forces. The elongation of compact structures by guided traction on external substrates requires the integration of external and internal forces, and modulation of the internal structural and material properties of the tissue by unknown mechanisms. It is not known how oriented division contributes to elongation, nor is it clear that this process is an active, force-producing component of elongation. Circumstantial evidence suggests that most of the systems described above may be active, force-producing processes, rather than passive responses to forces generated elsewhere, but this has been tested only in the case of the vertebrate dorsal mesoderm (Moore, 1994; Moore et al., 1995). Epithelial and mesenchymal cell intercalations appear to occur by substantially different mechanisms, with the limited evidence thus far suggesting that the former occur by junctional remodeling and the latter by directed protrusive activity, but too few systems have been studied. The *Drosophila* hindgut and ovary, and the dorsal hypodermal cells of the nematode, offer new opportunities to explore the validity of this generalization. In regard to the regulation of elongation by cell intercalation, the geometry of the signals that organize intercalation may be more conserved than are the specific signaling molecules. Cell intercalation in the *Drosophila* hindgut, in the *Drosophila* germ band and in the vertebrate dorsal mesoderm, all seem to require a global organizing signal parallel to the axis of elongation, and transverse to the axis of intercalation, but, in terms of signaling pathways, the vertebrate mesoderm requires the PCP pathway, whereas the *Drosophila* germ band does not (Lengyel and Iwaki, 2002; Ninomiya et al., 2004; Zallen and Wieschaus, 2004). Again, too few systems have been studied to support generalizations, and much remains to be learned about the diversity of signaling strategies, as well as the specific pathways involved. The less-studied systems described above offer new opportunities in this regard.

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References

Adams, D. S., Keller, R. and Koehl, M. A. (1990). The mechanics of notochord elongation, straightening and stiffening in the embryo of Xenopus laevis. *Development* **110**, 115-130.

Bertet, C., Sulak, L. and Lecuit, T. (2004). Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* **429**, 667-671.

Bosher, J. M., Hahn, B.-S., Legouis, R., Sookhareea, S., Weimer, R. M., Gansmuller, A., Chisholm, A. D., Rose, A. M., Bessereau, J.-L. and Labouesse, M. (2003). The Caenorhabditis elegans vab-10 spectraplakin isoforms protect the epidermis against internal and external forces. *J. Cell Biol.* **161**, 757-768.

Browne, W. E., Price, A. L., Gerberding, M. and Patel, N. H. (2005). Staging of embryonic development in the amphipod crustacean, Parhyale hawaiensis. *Genesis* **42**, 124-149.

Bruns, R. and Gross, J. (1970). Studies on the tadpole tail. I. Structure and organization of the notochord and its covering layers in Rana catesbiana. *Am. J. Anat.* **128**, 193-224.

Canty, E. G. and Kadler, K. E. (2002). Collagen fibril biosynthesis in tendon: a review and recent insights. *Comp. Biochem. Physiol.* **133A**, 979-985.

Canty, E. G. and Kadler, K. E. (2005). Procollagen trafficking, processing and fibrillogenesis. *J. Cell Sci.* **118**, 1341-1353.

Concha, M. L. and Adams, R. J. (1998). Oriented cell divisions and cellular morphogenesis in the zebrafish gastrula and neurula: a time-lapse analysis. *Development* **125**, 983-994.

Cornish, J. and Etkin, L. (1993). The formation of the pronephric duct in Xenopus involves recruitment of posterior cells by migrating pronephric duct cells. *Dev. Biol.* **159**, 338-345.

Costa, M., Draper, B. W. and Priess, J. R. (1997). The role of actin filaments in patterning the Caenorhabditis elegans cuticle. *Dev. Biol.* **184**, 373-384.

Costa, M., Raich, W., Agbunag, C., Leung, B., Hardin, J. and Priess, J. R. (1998). A putative catenin-cadherin system mediates morphogenesis of the Caenorhabditis elegans embryo. *J. Cell Biol.* **141**, 297-308.

Couderc, J. L., Godt, D., Zollman, S., Chen, J., Li, M., Tiong, S., Cramton, S. E., Sahut-Barnola, I. and Laski, F. A. (2002). The bric a brac locus consists of two paralogous genes encoding BTB/POZ domain proteins and acts as a homeotic and morphogenetic regulator of imaginal development in Drosophila. *Development* **129**, 2419-2433.

David, N. B., Sapede, D., Saint-Etienne, L., Thisse, C., Thisse, B., Dambly-Chaudiere, C., Rosa, F. M. and Ghysen, A. (2002). Molecular basis of cell migration in the fish lateral line: role of the chemokine receptior CXCR4 and of its ligand, SDF-1. *Proc. Natl. Acad. Sci. USA* **99**, 16297-16302.

Davidson, L. A., Keller, R. E. and DeSimone, D. W. (2004). Assembly and remodeling of the fibrillar fibronectin matrix during gastrulation and neurulation in *Xenopus laevis*. *Dev. Dyn*. **231**, 888-895.

Denholm, B., Sudarsan, V., Pasalodos-Sanchez, S., Maddrell, S. H. P., Baylies, M. K. and Skaer, H. (2003). Dual origin of the renal tubules in Drosophila: Mesodermal cells integrate and polarize to establish secretory function. *Curr. Biol.* **13**, 1052-1057.

Ding, M., Goncharov, A., Jin, Y. and Chisholm, A. D. (2003). C. elegans ankyrin repeat protein VAB-19 is a component of epidermal attachment structures and is essential for epidermal morphogenesis. *Development* **130**, 5791-5801.

Ding, M., Woo, W.-M. and Chisholm, A. D. (2004). The cytoskeleton and epidermal morphogenesis in C. elegans. *Exp. Cell Res.* **301**, 84-90.

Drawbridge, J. and Steinberg, M. (1996). Morphogenesis of the axolotl pronephric duct: a model system for the study of cell migration in vivo. *Int. J. Dev. Biol.* **40**, 709-713.

Drawbridge, J., Wolf, A. E., Delgado, Y. L. and and Steinberg, M. S. (1995). The epidermis is a source of directional information for the migrating pronephric duct in Ambystoma mexicanum embryos. *Dev. Biol.* **172**, 440-451.

Drawbridge, J., Meighan, C. M. and Mitchell, E. A. (2000). GDNF and GFRa-1 are components of the axolotl pronephric duct guidance system. *Dev. Biol.* **228**, 116-124.

Ghysen, A. and Dambly-Chaudiere, C. (2004). Development of the zebrafish lateral line. *Curr. Opin. Neurobiol.* **14**, 67-73.

Gillespie, L. L. and Armstrong, J. B. (1986). Morphogenetic waves in the development of the lateral mesoderm in the Mexican axolotl (Ambystoma mexicanum) and their relationship to pronephric duct migration. *J. Exp. Zool.* **237**, 327-338.

Glickman, N. S., Kimmel, C. B., Jones, M. A. and Adams, R. J. (2003). Shaping the zebrafish notochord. *Development* **130**, 873-887.

Godt, D. and Laski, F. A. (1995). Mechanisms of cell rearrangement and cell recruitment in Drosophila ovary morphogenesis and the requirement of bric a brac. *Development* **121**, 173-187.

Godt, D., Couderc, J. L., Cramton, S. E. and Laski, F. A. (1993). Pattern formation in the limbs of Drsophila: bric-a-brac is expressed in both a gradient and a wave-like pattern and is required for specification and proper segmentation of the tarsus. *Development* **119**, 799-812.

Gompel, N., Cubedo, N., Thisse, C., Thisse, B., Dambly-Chaudiere, C. and Ghysen, A. (2001). Pattern formation in the lateral line of zebrafish. *Mech. Dev.* **105**, 69-77.

Gong, Y., Mo, C. and Fraser, S. (2004). Planar cell polarity signalling controls cell division orientation during zebrafish gastrulation. *Nature* **430**, 689-693.

Hapiak, V., Hresko, M. C., Schriefer, L. A., Saiyasisongkhram, K., Bercher, M. and Plenefisch, J. (2003). mua-6, a gene required for tissue integrity in Caenorhabditis elegans, encodes a cytoplasmic intermediate filament. *Dev. Biol.* **263**, 330-342.

Hardin, J. (1990). Context-sensitive cell behaviors during gastrulation. *Semin. Dev. Biol.* **1**, 335-345.

Harris, A. K., Wild, P. and Stopak, D. (1980). Silicone rubber substrata: a new wrinkle in the study of cell locomotion. *Science* **208**, 177-179.

Hay, E. (1984). Collagen and embryonic development. In *The Role of the Extracellular Matrix in Development* (ed. R. Trelstad), pp. 379-409. New York: Alan Liss.

Hoch, M., Broadie, K., Jackle, H. and Skaer, H. (1994). Sequential fates in a single cell are established by the neurogenic cascade in the malpighian tubules of *Drosophila*. *Development* **120**, 3439-3450.

Hresko, M. C., Williams, B. D. and Waterston, R. H. (1994). Assembly of body wall muscle and muscle cell attachment structures in Caenorhabditis elegans. *J. Cell Biol.* **124**, 491-506.

Hresko, M. C., Schriefer, L. A., Shirmankar, P. and Waterston, R. H. (1999). Myotactin, a novel hypodermal protein involved in muscle-cell adhesion in Caenorhabditis elegans. *J. Cell Biol.* **146**, 659-672.

Huang, F. Z. and Weisblat, D. (1996). Cell fate determination in an annelid equivalence group. *Development* **122**, 1839-1847.

Janning, W., Lutz, A. and Wissen, D. (1986). Clonal analysis of the blastoderm

anlage of the Malpighian tubules in *Drosophila melanogaster*. *Roux Arch. Dev. Biol.* **195**, 22-32.
Johansen, K. A., Iwaki, D. D. and Lengyel, J. A. (2003). Localized JAK/STAT signaling is required for oriented cell rearrangement in a tubular epithelium. *Development* **130**, 135-145.
Jung, A. C., Denholm, B., Skaer, H. and Affolter, M. (2005). Renal tubule development in Drosophila: a closer look at the cellular level. *J. Am. Soc. Nephrol.* **16**, 322-328.
Kadler, K. E. (2004). Matrix loading: assembly of extracellular matrix collagen fibrils during embryogenesis. *Birth Defects Res.* **72**, 1-11.
Kadler, K. E., Holmes, D. F., Trotter, J. A. and Chapman, J. A. (1996). Collagen fibril formation. *Biochem. J.* **316**, 1-11.
Keller, R. (2002). Shaping the vertebrate body plan by polarized embryonic cell movements. *Science* **298**, 1950-1954.
Keller, R., Cooper, M. S., Danilchik, M., Tibbetts, P. and Wilson, P. A. (1989). Cell intercalation during notochord development in Xenopus laevis. *J. Exp. Zool.* **251**, 134-154.
Keller, R., Davidson, L., Edlund, A., Elul, T., Ezin, M., Shook, D. and Skoglund, P. (2000). Mechanisms of convergence and extension by cell intercalation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 897-922.
Kenney, M. and Carlson, E. (1978). Ultrastructural identification of collagen and glycosaminoglycans in notochordal extracellular matrix in vivo and in vitro. *Anat. Rec.* **190**, 827-850.
Koehl, M. A. R. (1990). Biomechanical approaches to morphogenesis. *Semin. Dev. Biol.* **1**, 367-378.
Koehl, M. A. R., Adams, D. S. and Keller, R. E. (1990). Mechanical development of the notochord in *Xenopus* early tail-bud embryos. In *Biomechanics of Active Movement and Deformation.* Vol. H42 (ed. N. Akkas), pp. 471-485. Berlin, Heidelberg: Springer-Verlag.
Koehl, M. A. R., Quillin, K. J. and Pell, C. A. (2000). Mechanical design of fiber-wound hydraulic skeletons: the stiffening and straightening of embryonic notochords. *Am. Zool.* **40**, 28-41.
Lee, R. Y. N., Lobel, L., Hengartner, M., Horvitz, R. and Avery, L. (1997). Mutations in the alpha 1 subunit of an L-type voltage-activated Ca2+ channel cause myotonia in Caenorhabditis elegans. *EMBO J.* **16**, 6066-6076.
Lengyel, J. A. and Iwaki, D. D. (2002). It takes guts: the Drosophila hindgut as a model system for organogenesis. *Dev. Biol.* **243**, 1-19.
Li, Q., Shirabe, K. and Kuwada, J. (2004). Chemkine signaling regulates sensory cell migration in zebrafish. *Dev. Biol.* **269**, 123-136.
McKeown, C., Pratis, V. and Austin, J. (1998). sma-1 encodes a β_H-spectrin homolog required for *Caenorhabditis elegans* morphogenesis. *Development* **128**, 2087-2098.
Mlodzik, M. (2002). Planar cell polarization: do the same mechanisms regulate Drosophila tissue polarity and vertebrate gastrulation? *Trends Genet.* **18**, 564-571.
Mookerjee, S. (1953). An experimental study of the development of the notochordal sheath. *J. Embryol. Exp. Morphol.* **1**, 411-416.
Mookerjee, S., Deuchar, E. M. and Waddington, C. H. (1953). The morphogenesis of the notochord in amphibia. *J. Embryol. Exp. Morphol.* **1**, 399-409.
Moore, S. W. (1994). A fiber optic system for measuring dynamic mechanical properties of embryonic tissues. *IEEE Trans. Biomed. Eng.* **41**, 45-50.
Moore, S. W., Keller, R. E. and Koehl, M. A. R. (1995). The dorsal involuting marginal zone stiffens anisotropically during its convergent extension in the gastrula of *Xenopus leavis*. *Development* **121**, 3130-3140.
Morris, A. R., Drawbridge, J. and Steinberg, M. S. (2003). Axolotl pronephric duct migration requires an epidermally derived, laminin 1-containing extracellular matrix and the integrin receptor a6b1. *Development* **130**, 5601-5608.
Myat, M. M. (2005). Making tubes in the Drosophila embryo. *Dev. Dyn.* **232**, 617-632.
Myers, D. C., Sepich, D. S. and Solnica-Krezel, L. (2002). Convergence and extension in vertebrate gastrulae: cell movements according to or in search of identity? *Trends Genet.* **18**, 447-455.
Nelson, B. H. and Weisblat, D. A. (1991). Conversion of ectoderm to mesoderm by cytoplasmic extrusion in leech embryos. *Science* **253**, 435-438.
Ninomiya, H., Elinson, R. P. and Winklbauer, R. (2004). Antero-posterior tissue polarity links mesoderm convergent extension to axial patterning. *Nature* **430**, 364-367.
Norman, K. and Moerman, D. (2002). A spectrin is essential for morphogenesis and body wall muscle formation in *Caenorhabditis elegans*. *J. Cell Biol.* **157**, 665-677.
Overton, J. (1959). Studies on the mode of outgrowth of the amphibian pronephric duct. *J. Embryol. Exp. Morphol.* **7**, 86-93.
Poole, T. J. and Steinberg, M. S. (1977). SEM-aided analysis of morphogenetic movements: development of the amphibian pronephric duct. *IITRI/SEM/1977* **2**, 43-52.
Poole, T. J. and Steinberg, M. S. (1981). Amphibian pronephric duct morphogenesis: segregation, cell rearrangement and directed migration of the Ambystoma duct rudiment. *J. Embryol. Exp. Morphol.* **63**, 1-16.
Poole, T. and Steinberg, M. S. (1982). Evidence for the guidance of pronephric duct migration by a craniocaudally traveling adhesion gradient. *Dev. Biol.* **92**, 144-158.
Priess, J. R. and Hirsh, D. I. (1986). Caenorhabditis elegans morphogenesis: the role of the cytoskeleton in elongation of the embryo. *Dev. Biol.* **117**, 156-173.
Raich, W. B., Agbunag, C. and Hardin, J. (1999). Rapid epithelial-sheet sealing in the Caenorhabditis elegans embryo requires cadherin-dependent filopodial priming. *Curr. Biol.* **9**, 1139-1146.
Sahut-Barnola, I., Godt, D., Laski, F. A. and Couderc, J. L. (1995). Drosophila ovary morphogenesis: analysis of terminal filament formation and identification of a gene required for this process. *Dev. Biol.* **170**, 127-135.
Sander, K. (1976). Specification of the basic body pattern in insect embryogenesis. *Adv. Insect Physiol.* **12**, 125-238.
Schoenwolf, G. C. and Alvarez, I. S. (1989). Roles of neuroepithelial cell rearrangement and division in shaping of the avian neural plate. *Development* **106**, 427-439.
Schoenwolf, G. C. and Alvarez, I. S. (1992). Role of cell rearrangement in axial morphogenesis. *Curr. Top. Dev. Biol.* **27**, 129-173.
Schoenwolf, G. C. and Yuan, S. (1995). Experimental analyses of the rearrangement of ectodermal cells during gastrulation and neurulation in avian embryos. *Cell Tissue Res.* **280**, 243-251.
Shelton, C., Carter, J., Ellis, G. and Bowerman, B. (1999). The nonmuscle myosin regulatory light chain gene *mlc-4* is required for cytokinesis, anterior-posterior polarity, and body morphology during *Caenorhabditis elegans* embyrogenesis. *J. Cell Biol.* **146**, 439-451.
Shih, J. and Keller, R. (1992a). Cell motility driving mediolateral intercalation in explants of *Xenopus laevis*. *Development* **116**, 901-914.
Shih, J. and Keller, R. (1992b). Patterns of cell motility in the organizer and dorsal mesoderm of *Xenopus laevis*. *Development* **116**, 915-930.
Shoji, W., Yee, C. S. and Kuwada, J. (1998). Zebrafish semaphorin Z1a collapses specific growth cones and alters their pathway in vivo. *Development* **125**, 1275-1283.
Simske, J. S. and Hardin, J. (2001). Getting into shape: epidermal morphogenesis in Caenorhabditis elegans embryos. *BioEssays* **23**, 12-23.
Skaer, H. (1989). Cell division in malpighian tubule development in *Drosophila* melanogaster is regulated byt a single tip cell. *Nature* **243**, 566-569.
Skaer, H. and Arias, A. M. (1992). The wingless product is required for cell proliferation in the Malpighian tubule anlage of *Drosophila melanogaster. Development* **116**, 745-754.
Skoglund, P., Dzamba, B., Coffman, C. R., Harris, W. A. and Keller, R. (2006). Xenopus fibrillin is expressed in the organizer and is the earliest component of matrix at the developing notochord-somite boundary. *Dev. Dyn.* (in press).
Smith, C. M., Lans, D. and Weisblat, D. A. (1996). Cellular mechanisms of epiboly in leech embryos. *Development* **122**, 1885-1894.
Smith, S. C., Lannoo, M. J. and Armstrong, J. B. (1990). Development of the mechanoreceptive lateral-line system in the axolotl: placode specification, guidance of migration, and the orgin of neuromast polarity. *Anat. Embryol.* **182**, 171-180.
Stent, G. S. and Weisblat, D. (1982). The development of a simple nervous system. *Sci. Am.* **246**, 136-146.
Waddington, C. H. and Perry, M. (1953). The ultrastructure of the developing urodele notochord. *Proc R. Soc. Lond. B Biol. Sci.* **156**, 459-482.
Wallingford, J. B., Fraser, S. E. and Harland, R. M. (2002). Convergent extension: the molecular control of polarized cell movement during embryonic development. *Dev. Cell* **2**, 695-706.
Weber, R. (1961). Simlar pattern of fine structure in the basement lamella of the skin and the external sheath of the notochord in Xenopus larvae. *Experientia* **17**, 365.
Wei, Y. and Mikawa, T. (2000). Formation of the avian primitive streak from spatially restricted blastoderm: evidence for polarized cell division in the elongating streak. *Development* **127**, 87-96.
Williams-Masson, E. M., Malik, A. N. and Hardin, J. (1997). An actin-mediated two-step mechanism is required for ventral enclosure of the C. elegans hypodermis. *Development* **124**, 2889-2901.
Williams-Masson, E. M., Heid, P. J., Lavin, C. A. and Hardin, J. (1998). The cellular mechanism of epithelial rearrangement during morphogenesis of the Caenorhabditis elegans dorsal hypodermis. *Dev. Biol.* **204**, 263-276.
Wilson, E. B. (1900). *The Cell in Development and Inheritance*. Norwood, MA: The Macmillan Company, Norwood Press.
Winklbauer, R. (1989). Development of the lateral line system in Xenopus. *Prog. Neurobiol.* **32**, 181-206.
Wissman, A., Ingles, J., McGhee, J. and Mains, P. (1997). *Caenorhabditis elegans* LET-502 is related to Rho-binding kinases and human myotonic dystrophy kinase and interacts genetically with a homolog of the regulatory subunit of smooth muscle myosin phosphatase to affect cell shape. *Genes Dev.* **11**, 409-422.

Wissman, A., Ingles, J. and Mains, P. (1999). The caenorhabditis elegans mel-11 myosin phosphatase regulatory subunit affects tissue contraction in the somatic gonad and the embryonic epidermis and genetically interacts with the Rac signaling pathway. *Dev. Biol.* **209**, 111-127.

Woo, W. M., Goncharov, A., Jin, Y. and Chisholm, A. D. (2004). Intermediate filaments are required for C. elegans epidermal elongation. *Dev. Biol.* **267**, 216-229.

Zackson, S. and Steinberg, M. S. (1986). Cranial neural crest cells exhibit directed migration on the pronephric duct pathway: further evidence for an in vivo adhesion gradient. *Dev. Biol.* **117**, 343-353.

Zackson, S. and Steinberg, M. S. (1987). Chemotaxis or adhesion gradient? Pronephric duct elongation does not depend on distant sources of guidance information. *Dev. Biol.* **124**, 418-422.

Zackson, S. and Steinberg, M. S. (1988). A molecular marker for cell guidance information in the axolotl embryo. *Dev. Biol.* **127**, 435-442.

Zackson, S. and Steinberg, M. S. (1989). Axolotl pronephric duct cell migration is sensitive to phosphatidylinositol-specific phospholipase C. *Development* **105**, 1-7.

Zallen, J. and Wieschaus, E. (2004). Patterned gene expression directs bipolar planar polarity in Drosophila. *Dev. Cell* **6**, 343-355.