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Convergent Extension: The Molecular Control of Polarized Cell Movement during Embryonic Development

Review

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During development, vertebrate embryos undergo dramatic changes in shape. The lengthening and narrowing of a field of cells, termed convergent extension, contributes to a variety of morphogenetic processes. Focusing on frogs and fish, we review the different cellular mechanisms and the well-conserved signaling pathways that underlie this process.

One of the attractions of working on embryos is the fascination of watching them change their shape. Despite the temptation to study morphogenesis, most developmental biologists have concentrated on the molecular mechanisms that control cell fate specification in embryos, and the enormous progress in understanding transcriptional control and intercellular signaling is obvious. However, morphogenesis has not been ignored. This review concentrates on our current understanding of one of the mechanisms by which the architecture of the vertebrate body plan is reorganized during early development. The process of convergent extension (Table 1), in which a tissue narrows along one axis and lengthens in a perpendicular axis, occurs during gastrulation, neurulation, axis elongation, and organogenesis in both vertebrate and invertebrate embryos.

In chordate animals, convergent extension occurs in small populations of cells (e.g., the ascidian notochord; see Miyamoto and Crowther, 1985) and also in quite large populations of cells, such as the dorsal mesoderm and neural ectoderm of frogs and fish (Solnica-Krezel et al., 1995; Keller et al., 2000). Convergent extension was recognized as a morphogenetic process over 100 years ago. T.H. Morgan (1895) alludes to the process, and after watching the movements of needles implanted in developing fish embryos, Sumner concluded matter-of-factly that the "net result of this heaping up of cells toward the embryonic axis is the continued elongation of the embryo" (Sumner, 1904). Using dye marking of amphibian embryos, Smith suggested a similar connection between the elongation of the anteroposterior axis and coincident translocation of lateral tissue toward the dorsal midline (Smith, 1914).

The organisms where convergent extension has been studied most are those where the movements of cells are easy to see, and the external development and large size of frog and fish embryos has made them excellent candidates. We will synthesize what is known about the mechanisms of convergent extension in frogs and fish, and then discuss recent papers investigating the molecular control of convergent extension.

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Convergent Extension Is Only One Component of the Axis Elongation Machinery

It is important to note at the outset that convergent extension is only one of a suite of morphogenetic engines at work during early embryonic development. For example, directed migration of the head mesoderm contributes to the elongation of dorsal mesoderm in *Xenopus* (Winklbauer and Nagel, 1991; Keller and Jansa, 1992). In addition, uniform radial intercalation (Table 1) of cells causes thinning and spreading of the mesoderm and ectoderm (epiboly) in both fish and frogs (Keller, 1980; Warga and Kimmel, 1990). An additional morphogenetic engine, also mediated by radial intercalation, makes a significant contribution to axis elongation in frog embryos (Keller, 1980; Wilson and Keller, 1991; Marsden and DeSimone, 2001). Whether similar radial intercalations contribute to axis elongation in fish embryos is unclear. Finally, oriented cell divisions are associated with axis elongation in fish (Concha and Adams, 1998). Continued examination of all of the movements and cellular mechanisms in many different organisms will be needed to appreciate the regulatory logic and evolutionary constraints that determine the form of the vertebrate embryo.

Frogs and Fish Use Different Cellular Mechanisms to Accomplish Convergent Extension

At a descriptive level, the cell behaviors that drive animal morphogenesis fall into a limited number of classes (Locascio and Nieto, 2001). At one extreme are migratory events involving the directed movement of individual cells or small groups of cells across a relatively stationary substrate, such as a basement membrane or adjacent tissue (Figure 1A). Examples include leucocyte chemotaxis or germ cell migration. On the other hand, there are also tissue morphogenesis events, in which coordinated cell shape changes transform a tissue with little translocation of any one cell (Figure 1B). Examples include hinge-point formation in the vertebrate neural tube and initiation of blastopore formation in the frog embryo. Finally, there is an intermediate class where individual cells in a tissue move relative to neighboring cells in the same tissue, rearranging in order to reshape the population as a whole (Figure 1C).

While both frogs and fish engage in convergent extension, there are important and often overlooked differences in the overall process in these two animals. In frog embryos, only cell rearrangement has been implicated in convergent extension. On the other hand, fish use both cell rearrangement and directed migration.

Mediolateral Cell Intercalation Drives Convergent Extension in *Xenopus*

Vogt may have been the first to suggest that convergent extension involved active rearrangement of cells: "a longitudinal staggering of cell-complexes" (Vogt, 1922).

Table 1. Some Useful Definitions

Boundary capture: The anchoring of mediolaterally intercalating cells to a tissue boundary. Cells attach firmly to the boundary but continue to express protrusive activity on the cell face away from the boundary, exerting traction on neighboring cells and pulling them toward the boundary.
Convergent extension: We define convergent extension as the concomitant narrowing and lengthening of a tissue, regardless of the underlying cellular mechanism. Indeed, as is discussed in this review, convergent extension is accomplished by different cellular mechanisms in different animals and tissues.
Dorsal convergence: The directed migration of cells in the lateral regions of the fish embryo toward the dorsal midline. The underlying yolk cell or yolk syncytial layer provides the substrate for this migration. This is one of two components of convergent extension in the fish. There appears to be no dorsal convergence in the frog.
Intercalation: The interdigitation of cells (see Figure 1C). Intercalation can occur either actively or passively. In active cases such as mediolateral intercalation in *Xenopus*, cells crawl between one another using attachment to other cells in the same population as a substrate for movement. In passive cases, cells can intercalate in response to external tension or stretching.
Mediolateral intercalation: Intercalation of cells specifically along the mediolateral axis. This type of intercalation need not be dorsally directed, only mediolaterally oriented. Mediolateral intercalation is the driving force for convergent extension in *Xenopus*, and is also an important contributor in fish.
Protrusions/protrusive activity: In lamellipodia or filopodia, the generation of lamellipodial and filopodial cell protrusions used for cell movement.
Radial intercalation: The intercalation of cells along the radial axis of the embryo resulting in the thinning and spreading of a tissue. Distinct mechanisms of radial intercalation drive epiboly and the "thinning extension" of dorsal tissues. This type of cell rearrangement is quite distinct from convergent extension, but contributes significantly to axis elongation.

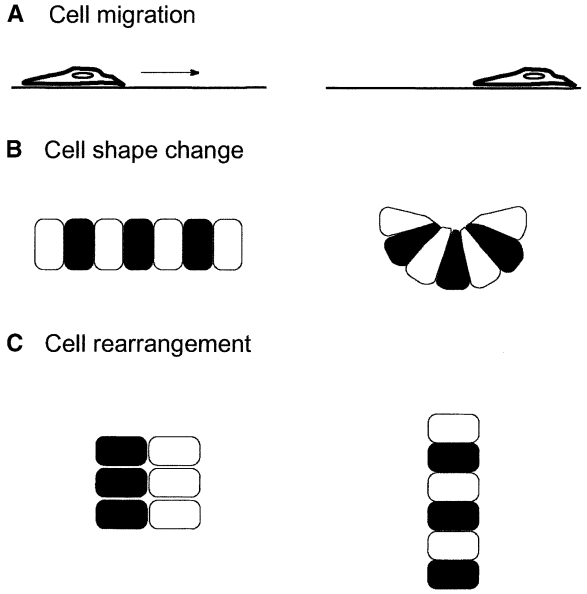

Figure 1. Categories of Morphogenetic Movement

(A) Directed cell migration, in which a single cell or small cohorts of cells move in a directed manner across a relatively stationary substrate.

(B) Coordinated cell shape change in a population, in which many cells engage in a similar shape change effecting the movement of the tissue as a whole. Note that individual cells do not change position relative to their neighboring cells.

(C) Cell rearrangement, in which cells exert traction on neighboring cells in order to change their positions relative to one another, thus reshaping the population.

Schectman and Holtfreter each showed that dorsal tissues removed from the embryo and cultured in isolation narrowed and elongated just as they would in an intact embryo, demonstrating that the motive force for convergent extension was generated within the tissue, and was not driven by other movements in the embryo (Schectman, 1942; Holtfreter, 1944). Holtfreter noted that mesodermal cells aligned mediolaterally—perpendicular to the axis of explant elongation—and thus extension could not be driven by change in cell shape but must be due to the rearrangement of motile cells (Holtfreter, 1943, 1944). This insight, that understanding morphogenesis requires understanding the behaviors of individual cells, remains critical.

More recently, Ray Keller and colleagues have studied convergent extension in *Xenopus*. To view the cell behaviors that drive convergent extension, they have taken advantage of the autonomous movements of explanted *Xenopus* tissue (reviewed in Keller et al., 1992a, 2000). Time-lapse recordings demonstrated that during convergent extension of the mesoderm, cells actively align and intercalate between one another along the mediolateral axis (Table 1; Wilson and Keller, 1991). This rearrangement of cells leads directly to the elongation of the tissue along the anteroposterior axis (Figure 1C; Shih and Keller, 1992a). Studies of intact gastrulae also revealed the mediolateral alignment and intercalation of these cells (Keller and Schoenwolf, 1977; Keller and Tibbetts, 1989), thus verifying the explant system.

Polarized Cell Behavior Drives Mediolateral Intercalation in *Xenopus* Mesoderm

Mesodermal cells engaged in convergent extension follow a stereotypical sequence of polarized behavior (Figure 2A; Shih and Keller, 1992a, 1992b). Before gastrulation movements are evident, cells transiently extend lamellipodial protrusions in random orientations (Figure 2A, top panel). As gastrulation proceeds, protrusive activity (Table 1) becomes more stable and polarized in the mediolateral axis (Figure 2A, middle panel). Similar oriented cell protrusions are observed in whole embryos (Keller and Schoenwolf, 1977). These mediolaterally biased, stable protrusions are thought to be firmly attached to neighboring mesoderm cells (Figure 2A, middle panel) and to generate the traction required for mediolateral intercalation (Keller et al., 2000). Indeed, failure of convergent extension follows disruption of either the polarity or stability of these protrusions (Wallingford et al., 2000). The traction of these cells upon one another explains why convergent extension does not require an external substrate and can occur in explanted tissues.

The Paradox of Individual Cell Movement versus Collective Cell Movement in Mediolateral Intercalation in *Xenopus* Mesoderm

Naively, it may be simplest to consider that convergent extension could be driven by an attraction of cells toward the dorsal midline, but this is not what occurs in

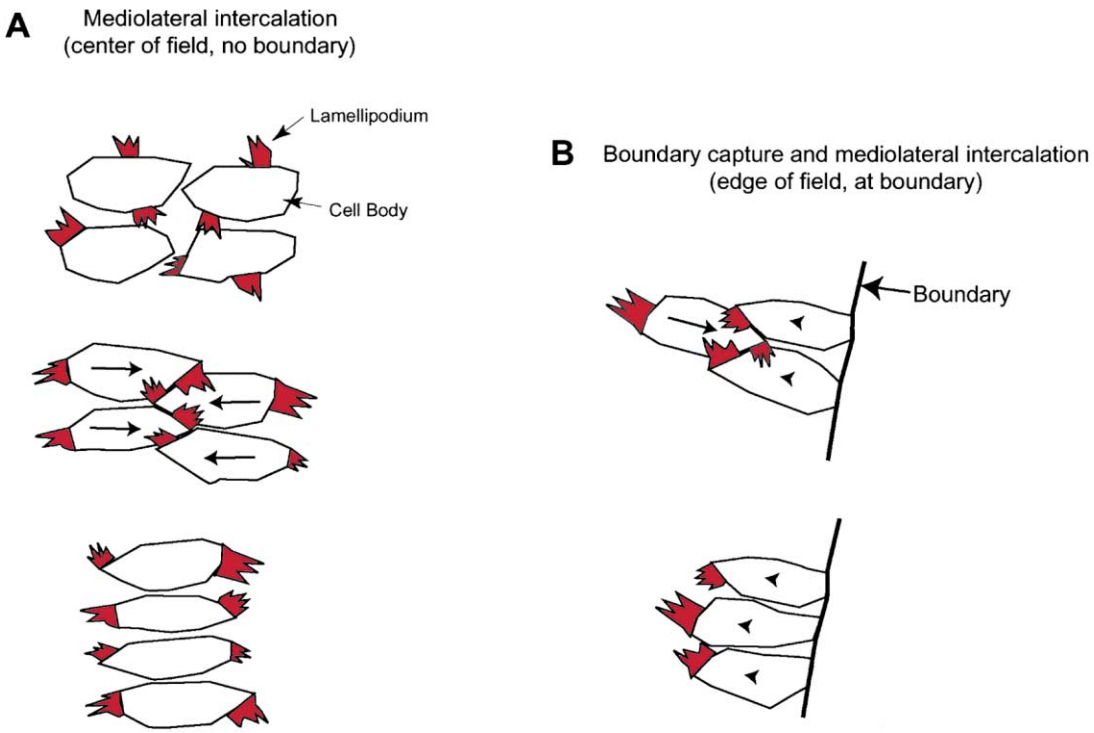

Figure 2. In *Xenopus* Mesoderm, Mediolateral Intercalation Occurs via a Stereotypical Sequence of Cell Behaviors

(A) Prior to the onset of gastrulation, cells extend and retract lamellipodia randomly. As convergent extension begins, cells align mediolaterally, and stabilize lamellipodia at their medial and lateral ends. The stable lamellipodia attach to mediolaterally neighboring cells and exert traction, pulling the cells between one another. This type of behavior is displayed by cells in the middle of the notochord or somite field.

(B) Near the notochord-somite boundary cells display boundary capture, in which cells attach stably to the boundary and continue to exert traction on neighboring cells away from the boundary, pulling the cells between one another.

Xenopus mesoderm. Observations instead indicate that movement of individual cells is only biased mediolaterally, and that any one cell is just as likely to move medially as laterally (Figure 3). The presence of cell protrusions on both medial and lateral sides of intercalating cells is consistent with the gross movement of cells in both medial and lateral directions (Shih and Keller, 1992a).

This polarized, but not directed, cell movement results in convergence of mesodermal cells to the midline because "boundary capture" (Table 1) occurs at the interface of notochord and somite. For example, when notochord cells reach this boundary, they become anchored there; protrusive activity is eliminated on the cell face contacting the boundary (Shih and Keller, 1992a, 1992b). However, captured cells continue to extend protrusions on the face away from the boundary and still generate traction on neighboring cells, pulling these neighbors toward the boundary (Figures 2B and 3). Continued repacking of cells in the center of the notochord field then narrows the tissue toward the dorsal midline. So long as the cells have a mediolateral bias in the orientation of their repacking and so long as cells exhibit boundary capture, the tissue will converge and extend (Figure 3; see Keller et al., 1992a, 2000).

A Slightly Different Mechanism Drives Neural Convergent Extension in *Xenopus*

The discussion so far has described convergent extension as it occurs in the *Xenopus* mesoderm. The hindbrain and spinal cord also narrow and elongate dramatically during neurulation (Jacobson, 1994), and this convergent extension of the neural epithelium also occurs autonomously in explants (Keller and Danilchik, 1988). Like the mesoderm, neural convergent extension is driven by cell intercalation (Keller et al., 1992b). Time-lapse analysis revealed that normal neural cell intercalation was driven by medially directed, monopolar protrusive activity and by boundary capture at the border between the neural plate and notoplate (Elul et al., 1997; Elul and Keller, 2000). What accounts for the difference in cell behaviors between mesoderm and ectoderm remains an open question and highlights our ignorance not only of the directional cues, but also of the mechanism by which a cell can choose a bipolar or monopolar organization.

Convergent Extension in Fish Involves Multiple, Distinct Cellular Mechanisms

While the process of convergent extension in frogs and fish appears outwardly very similar (the axis narrows mediolaterally and lengthens anteroposteriorly), the details of tissue and cell rearrangements differ substantially. For example, while the data from *Xenopus* argue that convergent extension in the frog occurs exclusively via mediolateral intercalation (Shih and Keller, 1992a), an uncoupling of convergence from extension has long been acknowledged during fish development. During the early stages of gastrulation in the fish, cells move from lateral regions toward the dorsal midline, piling up into the thickened embryonic shield (Morgan, 1895; Sumner, 1904; Oppenheimer, 1936).

Modern labeling techniques and time-lapse analysis

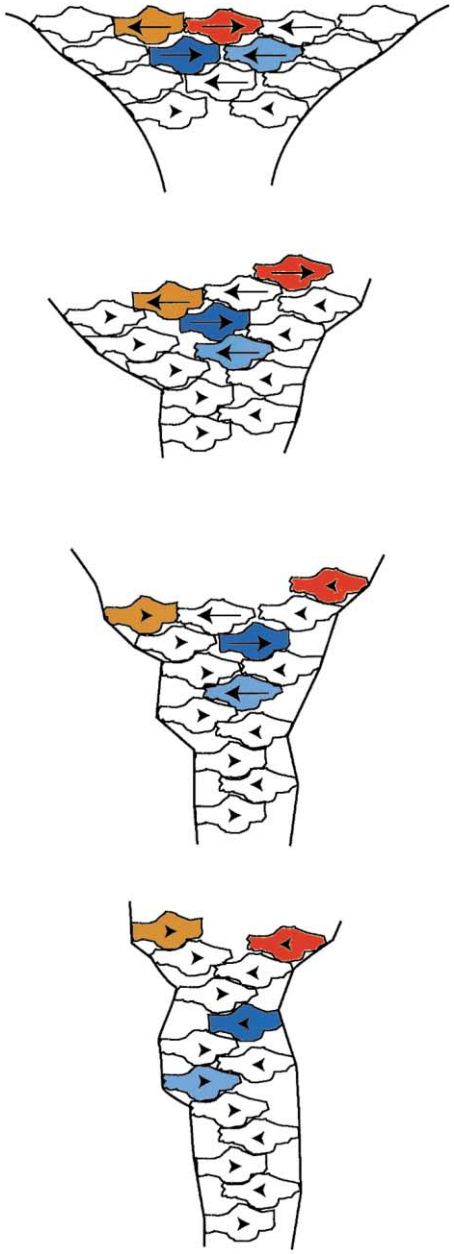

Figure 3. Convergent Extension in *Xenopus* Mesoderm Involves Mediolateral Intercalation and Boundary Capture

Arrows indicate the direction of movement of each intercalating cell; arrowheads indicate the medially directed traction of boundary-captured cells. The direction of movement of intercalating cells is biased mediolaterally, but not directed. Cells intercalate in both medial and lateral directions. For example, the blue cells move medially at first, then pass one another and diverge laterally until they reach a boundary. The brown and orange cells diverge laterally from the outset, yet are eventually brought closer together as the boundaries converge. Note that all boundary-captured cells exert traction medially, bringing the boundaries closer together. Also note that all the cells involved are in continuous close contact.

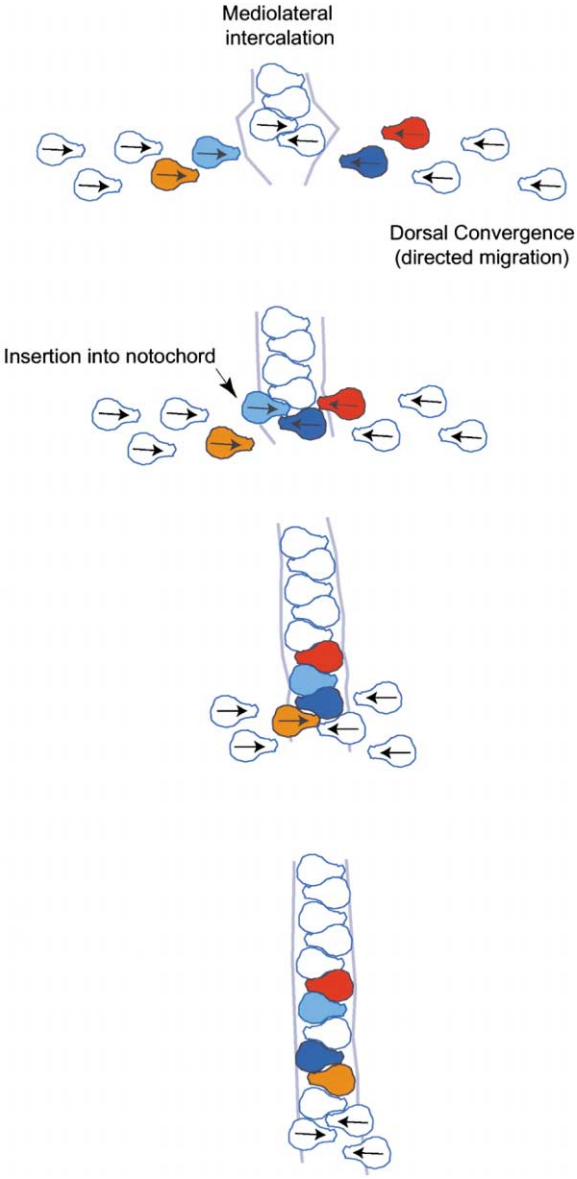

Figure 4. Convergent Extension in Teleosts Involves Dorsally Directed Migration in Lateral Regions and Mediolateral Intercalation at the Midline

Arrows indicate the direction of cell movement. Note that all cells move medially. Lateral cells migrate dorsally as individuals using the underlying YSL as a substrate. These cells are not tightly packed and do not rearrange (e.g., on the right side, the blue and orange cells remain in constant relative position). Upon reaching the midline, cells insert into the notochord field and make close contact with other cells. Intercalation at the midline results in repacking (e.g., once the blue and orange cells reach the midline, they become separated by other intercalating cells). Normal axis elongation in fish requires intercalation at the midline, dorsal migration of lateral cells, as well as additional extension motors, such as epiboly.

of living fish embryos have demonstrated that cells in the zebrafish germ ring are loosely packed as they move dorsally across the yolk cell during gastrulation (Figure 4). When they reach the dorsal side at late gastrulation, cells change their neighbors by mediolateral intercalation (Figure 4; Warga and Kimmel, 1990). This is consistent with the analysis of other teleosts (*Fundulus* and the rosy barb), where little rearrangement occurs among cells moving dorsally in the germ ring or in paraxial regions; only when cells approach the midline and enter

the axial tissue do they undergo mediolateral intercalation (Thorogood and Wood, 1987; Trinkaus et al., 1992; Wood and Thorogood, 1994). It is important to note that while we present a synthesis of cell behaviors and movements in this review based upon observations made in these three fish, differences do exist between these species.

Together, the data suggest that there are at least two distinct components to the overall mechanism of convergent extension in fish (Kane and Warga, 1994; Solnica-Krezel et al., 1995). The first component, termed "dorsal convergence" (Table 1), entails the *directed* migration of individual cells and small groups of cells toward the dorsal midline. This process does not require cell rearrangement; it is a migratory event (Figure 4). The second component, which does involve rearrangement, is mediolateral intercalation of cells at the dorsal midline (Figure 4). The separability of dorsal convergence, mediolateral intercalation, and anteroposterior extension in zebrafish is clearly highlighted by mutant phenotypes in which convergence and extension are affected to different degrees and also implicates additional morphogenetic engines in axis elongation in the fish (see Solnica-Krezel et al., 1996; Sepich et al., 2000; Myers et al., 2002).

Cell Biological Basis of Convergence and Intercalation in Fish

The protrusive activity of cells engaged in dorsal convergence in *Fundulus* has been described in detail. In the more lateral regions, individuals and clusters of cells have medially directed protrusions during dorsal migration, probably using the surface of the underlying yolk cell as a substrate (Trinkaus, 1973; Trinkaus and Erickson, 1983). This type of dorsally directed migratory motility in lateral regions stands in contrast to the mediolateral intercalations observed throughout the dorsolateral extent of the *Xenopus* mesoderm (Keller and Danilchik, 1988; Shih and Keller, 1992b). Indeed, there seems to be no counterpart to the dorsal convergence movements during *Xenopus* gastrulation, though as we have seen, mediolateral intercalation with boundary capture produces a narrowing of the tissue and consequently brings the cells toward the dorsal midline (Figure 3).

A transition of cell behavior from migration to intercalation has been observed in time-lapse movies of the rosy barb; cells migrate right up to the presumptive notochord-somite boundary, where they actively insert into the notochord by intercalation (Wood and Thorogood, 1994). Somewhat later, the notochord-somite boundary begins to restrain cell crossing and may act in boundary capture for narrowing of the notochord. This addition of cells into the notochord and their subsequent intercalation likely contributes to elongation. Unlike *Xenopus*, however, this process alone is not sufficient to account for elongation even in the most dorsal regions of the fish; additional engines must be involved (Kane and Warga, 1994; Wood and Thorogood, 1994). In *Fundulus* and zebrafish, the distinction between convergence and intercalation may be less clear, as cells migrate dorsally with increasing speed, indicating a continuously changing behavior (Trinkaus, 1998; Sepich et al., 2000; Myers et al., 2002).

The subcellular behaviors underlying mediolateral intercalation at the midline in the fish have yet to be described in detail, though it is likely that many behaviors are shared between fish and frog. For example, cells in the paraxial mesoderm, notochord, and dorsal ectoderm do align mediolaterally during fish gastrulation (Wood and Thorogood, 1994; Concha and Adams, 1998; Topczewski et al., 2001). However, since *Xenopus* mesodermal and neural cells intercalate using bipolar and monopolar protrusive activity, respectively (Keller et al., 2000), it is difficult to predict how teleost cells may behave.

Molecular Regulation of Convergent Extension

Like the cell movements, the regulatory apparatus controlling morphogenesis has been a topic of study for decades. Early studies focused on metabolic chemistry (see Needham, 1942), while more recent work has centered on the molecular biology of morphogenesis and how distinct cell movements are elicited in response to cell fate specification (Ho, 1992; Smith and Howard, 1992). Now experiments with molecules directly coordinating cell motile behaviors are revealing that the mechanisms which control morphogenesis may be highly conserved throughout the chordates. Much of this work has focused on noncanonical Wnt signaling pathways (McEwen and Peifer, 2000). The first indications that such signals governed convergent extension movements came from experiments with members of the Wnt4, Wnt5a, and Wnt11 family of secreted glycoproteins. Unlike the canonical Wnts, these molecules do not strongly activate the β-catenin pathway (Du et al., 1995). However, overexpression studies demonstrated that Wnt5a and Wnt4 disrupt convergent extension in both frogs and fish, without dramatically affecting cell fate (Moon et al., 1993; Ungar et al., 1995). Later experiments revealed that while Wnt-5a activates Frizzled receptors, it actually inhibits the canonical Wnt pathway and elicits intracellular calcium release (the Wnt/Ca^{2+} pathway; see below; Torres et al., 1996; Slusarski et al., 1997). Many other Wnt signaling components were also shown to disrupt convergent extension, including Dishevelled and Frizzled (Sokol, 1996; Deardorff et al., 1998; Shi et al., 1998; Djiane et al., 2000; Medina et al., 2000).

The Planar Cell Polarity (PCP) Pathway Regulates Convergent Extension in Both Frogs and Fish

The clear role of polarized cell behavior within the plane of tissues undergoing convergent extension recalls the polarity of structures in the insect cuticle, where the outgrowth of hairs and bristles is coordinated. In *Drosophila*, this so-called planar cell polarity is controlled by a noncanonical Wnt signaling pathway, the PCP cascade. This pathway uses Wnt components such as Frizzled and Dishevelled, but then diverges and does not involve GSK-3, Axin, or β-catenin (Figure 5). Instead, PCP signaling involves a different set of transducers, including Strabismus (Stbm), Prickle, and JNK (Figure 5; Shulman et al., 1998; Boutros and Mlodzik, 1999; Adler and Lee, 2001). Interestingly, the *Drosophila* PCP cascade has not been associated with a localized Wnt signal (Yang et al., 2002). Combined with similar requirements for cell polarity, the established role of noncanonical Wnt signals in convergent extension hinted at the

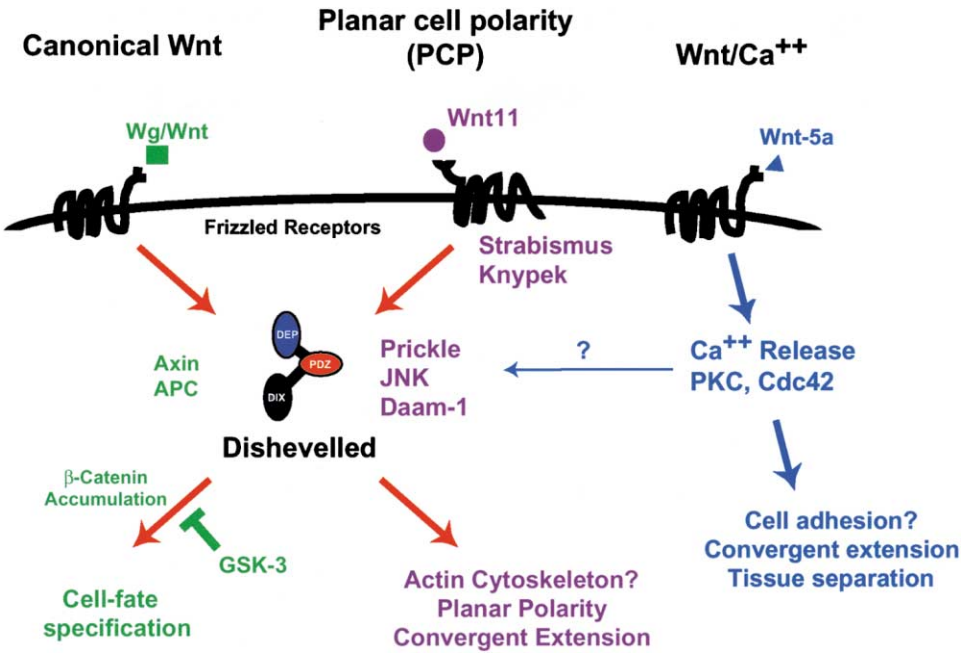

Figure 5. Canonical Wnt, PCP, and Wnt/Ca^{2+} Signaling Pathways

PCP pathway members are shown in purple, Wnt components in green, and Wnt/Ca^{2+} components in blue.

existence of a PCP signaling function previously unrecognized in vertebrates. Several recent experiments have now borne this out.

Deletion constructs of Dishevelled can discriminate between the various signaling properties of the protein (Axelrod et al., 1998; Boutros et al., 1998; Rothbächer et al., 2000), and clear indications of a requirement for PCP signaling in convergent extension came from experiments using similar deletion constructs in vertebrate embryos (Heisenberg et al., 2000; Tada and Smith, 2000; Wallingford et al., 2000). For example, mutants of Dishevelled which specifically disrupt PCP signaling but remain functional for Wnt signaling in *Drosophila* were found to disrupt convergent extension when expressed in *Xenopus* (Wallingford et al., 2000) or zebrafish (Heisenberg et al., 2000). In the converse experiment, an interfering mutant of Xwnt-11 severely disrupts convergent extension, and deletions of Dishevelled which are not competent to signal through the canonical Wnt pathway are nonetheless able to rescue the effects of the mutant Xwnt-11 (Tada and Smith, 2000). Likewise, convergent extension defects in the *silberblick* zebrafish mutant, which encodes Wnt-11, could also be rescued by this mutant Dishevelled (Heisenberg et al., 2000).

Additional evidence of a PCP pathway in convergent extension has come from the identification of PCP-specific genes in *Xenopus* and zebrafish. For example, a homolog of the *Drosophila* PCP gene *Strabismus* (*Stbm*) is expressed in cells undergoing convergent extension, and disruption of Stbm function inhibits convergent extension in both frogs and fish (Wolff and Rubin, 1998; Darken et al., 2002; Goto and Keller, 2002; Park and Moon, 2002). A *Xenopus* homolog of another *Drosophila* PCP gene, *Prickle*, is also expressed in tissues undergoing convergent extension (Gubb et al., 1999; Wallingford et al., 2002). JNK, though not specific to the pathway, is also required for both PCP signaling in *Drosophila* and for convergent extension in *Xenopus* (Boutros et al., 1998; Yamanaka et al., 2002). Interestingly, additional vertebrate players in this pathway have recently been identified and shown to be involved in convergent extension, including the glypican *knypek* (Topczewski et al., 2001) and the formin homology protein Daam-1 (Habas et al., 2001).

A final line of evidence implicating PCP signaling in convergent extension is the subcellular localization of Dishevelled protein (Figure 6). Membrane association of Dishevelled is required for *Drosophila* PCP, and Dishevelled is membrane localized in tissues where PCP signaling is active (Axelrod, 2001). In *Xenopus*, Dishevelled is localized to the cell membrane specifically in cells undergoing convergent extension, but is cytoplasmic in other cells (Wallingford et al., 2000). Stbm has been shown to be involved in the translocation of Dishevelled to the membrane (Park and Moon, 2002), perhaps indicating a mechanism by which Stbm regulates convergent extension. In the fly wing, Dishevelled is not only localized to the membrane, but is also concentrated at the distal vertex of the cells (Axelrod, 2001). It will be interesting to look for such polarized localization of Dishevelled protein during convergent extension.

PCP Signaling Establishes Mediolateral Cell Polarity during Convergent Extension

J.P. Trinkaus wrote that "...in order to understand how gene transcription and translation relate to gastrulation (and other morphogenetic processes), we must know the discrete cellular changes involved" (Trinkaus, 1969). Indeed, analyses of cell behaviors during convergent extension have revealed a strong connection between

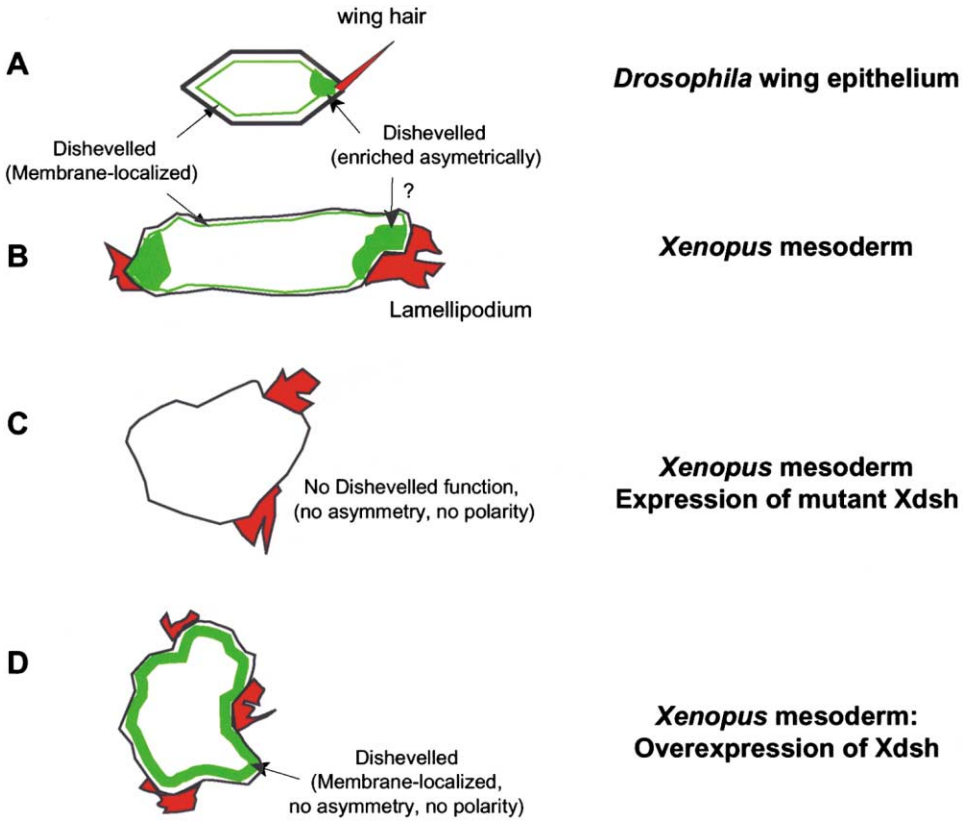

Figure 6. PCP Signaling and Cell Polarity

PCP signals control cell polarity in *Drosophila* wing epithelial cells (A) and in *Xenopus* mesoderm cells (B–D). Polarity is manifested by the wing hair or by lamellipodial protrusions, respectively. In both cases, overexpression and loss-of-function of PCP signals result in depolarization.

PCP signaling and mediolateral cell polarity. Time-lapse confocal imaging of cells engaged in mediolateral intercalation revealed that disruption of Dishevelled signaling severely disrupts mediolateral cell polarity (Wallingford et al., 2000). In cells expressing mutant Xdsh, both mediolateral alignment of cells and the directionality of lamellipodial protrusions are randomized (Wallingford et al., 2000). Likewise, overexpression of the PCP gene *Stbm* disrupts mediolateral cell polarity and convergent extension in both mesoderm and ectoderm in *Xenopus* (Goto and Keller, 2002). Together, these data experimentally correlate PCP signals, mediolateral polarity, cell intercalation, and convergent extension.

Similar disruptions of cell polarity are observed following manipulation of PCP signaling in the zebrafish, but consistent with the multiple components of convergent extension in the fish, more than one phenotype is observed. For example, mutation of *knypek* impairs both components of fish convergent extension (Solnica-Krezel et al., 1996; Topczewski et al., 2001). Tracing of cell populations at the midline suggested a defect in mediolateral intercalation, while tracing of lateral mesoderm cells revealed a defect in dorsal convergence (Topczewski et al., 2001). Analysis of individual cells showed that the mediolateral polarity of paraxial mesoderm cells engaged in dorsal convergence is disrupted in *knypek* fish (Topczewski et al., 2001). Since it is not yet clear to what extent mediolateral intercalation and dorsal convergence are interdependent, it may be that the primary defect lies in one process, and the effect on the other is a secondary consequence. Alternatively, it is attractive to think that PCP signals may control both dorsal convergence and mediolateral intercalation in the fish.

The situation is even more complicated in the *silberblick* (Wnt-11) mutants, which also display defects in axis elongation. Results of cell labeling experiments reveal that while the anteroposterior repacking of cells is inhibited, cell movement toward the dorsal midline and the distribution of cells relative to one another along the mediolateral axis are unaffected (Heisenberg et al., 2000). It is difficult to assess where the defect lies, and it remains at least possible that some part of the defect here lies in an as yet unidentified morphogenetic engine in the fish. Nonuniform radial intercalation like that observed in frog gastrulae is an excellent candidate (Keller, 1980; Wilson and Keller, 1991), especially since Dishevelled has been implicated in this process (Marsden and DeSimone, 2001).

PCP Signaling and Morphogenesis: So Many More Questions

PCP signaling coordinates planar polarity in static epithelia in flies and likely in vertebrates as well (Eaton, 1997). In convergent extension, PCP signaling regulates polarity in a mesenchymal population as cells move and repeatedly change neighbors. What now needs to be addressed is the mechanism by which PCP signals are

integrated into the different programs. One common thread is that in each case, actin-rich structures are formed at specific cell faces (e.g., distal prehairs in *Drosophila* wing epithelia; Wong and Adler, 1993) or mediolateral lamellipodia in *Xenopus* mesoderm (Shih and Keller, 1992a; Figure 6). Coupled with its presence at the cell membrane, a picture is emerging of Dishevelled as a regulator of the cytoskeleton. Such communication is likely to be very direct, as expression of mutant Dishevelled severely disrupts lamellipodial stability (Wallingford et al., 2000). Indeed, Daam-1 links Dishevelled to the actin cytoskeleton via small GTPases, and depletion of Daam-1 blocks convergent extension (Habas et al., 2001). Continued examination of the interplay of PCP signals with the machinery of cell motility will be critical.

Also of great interest is the origin of polarity in this system. In both convergent extension and *Drosophila* epithelia, the source of polarizing information remains elusive. Asymmetric localization of Frizzled and Dishevelled are important (Axelrod, 2001; Strutt, 2001), but how this asymmetry is established has yet to be discovered. Such polarization of intracellular signaling components may explain why both gain- and loss-of-function manipulations of Dishevelled or Frizzled disrupt planar polarity in flies (Krasnow and Adler, 1994; Axelrod et al., 1998) and why both gain- and loss-of-function manipulations of *Xenopus* Dishevelled or Frizzled disrupt mediolateral cell polarity and convergent extension (Djiane et al., 2000; Wallingford et al., 2000, 2001b). In the total absence of a protein, there will be no polarity (Figure 6C), and likewise if there is too much of a protein it may overwhelm localization machinery and become uniformly distributed about the membrane (Figure 6D). In either case, polarity would be lost.

Finally, how particular movements are patterned relative to cell fates will continue to be of great interest. For example, the mesoderm-inducing factors activin, FGF, and BMP-4 each elicit different combinations of morphogenetic cell behaviors, including convergent extension (Howard and Smith, 1993). The finding that *Xenopus* Wnt-11 is a target of the mesoderm-specifying transcription factor Brachyury thus provides a link between cell fate specification and the acquisition of convergent extension cell behaviors (Conlon and Smith, 1999; Tada and Smith, 2000). While Xwnt-11 is required for convergent extension, its mRNA expression pattern is not consistent with a role in establishing polarity directly (Heisenberg et al., 2000; Tada and Smith, 2000). It is attractive to consider that there may be localized expression of the protein, but it is equally possible that the Wnt signal provides a permissive environment for interpreting an independent polarizing signal. Indeed, planar polarity in the *Drosophila* wing has not been correlated with any Wnt ligand, though it is critically dependent upon Frizzled and Dishevelled (Rulifson et al., 2000).

Activation of canonical Wnt signals does not elicit morphogenetic cell behaviors, but dramatically alters those behaviors evoked by mesoderm-inducing factors (Howard and Smith, 1993). Indeed, canonical Wnt/β-catenin signaling is required to maintain proper cell fate during convergent extension, and may contribute to morphogenesis by regulating the expression of genes such as *Xnr3* and *Stat-3*, which may influence convergent extension movements more directly (Smith et al., 1995; Kuhl et al., 2001; Yamashita et al., 2002). The interplay between cell fate and cell movement remains one of the murkiest areas of developmental biology, but the recent progress in understanding convergent extension will certainly help us to understand these interactions.

The Wnt/Ca^{2+} Pathway and Convergent Extension

As mentioned above, the earliest indications of a role for Wnt signaling in convergent extension came from experiments with Wnts which activate what is now known to be the Wnt/Ca^{2+} pathway (Figure 5; Kuhl et al., 2000). It is not yet clear to what extent the Wnt/Ca^{2+} and PCP pathways overlap, but recent studies suggest an important role for Wnt/Ca^{2+} signaling during gastrulation. For example, cell aggregation assays related the inhibitory effect of Wnt5a on convergent extension to repression of calcium-dependent cell adhesion (Torres et al., 1996). Expression of the small GTPase Cdc42 also inhibits cell adhesion and convergent extension, and a dominant-negative mutant of Cdc42 blocks the effects of Wnt5a (Choi and Han, 2002). As reduction of adhesion between cells may be a necessary step during convergent extension (Brieher and Gumbiner, 1994), these data suggest a role for Wnt/Ca^{2+} and Cdc42 in this process. On the other hand, pharmacological agents which disrupt the Wnt/Ca^{2+} pathway do not inhibit convergent extension (Winklbauer et al., 2001), making the physiological function of Wnt/Ca^{2+} and cell adhesion during convergent extension unclear. The failure of proper tissue separation between mesoderm and ectoderm during gastrulation in embryos lacking Wnt/Ca^{2+} signaling suggests quite a different role (Winklbauer et al., 2001), though it should be pointed out that these different effects are not mutually exclusive and Wnt/Ca^{2+} signaling may serve a variety of functions during morphogenesis. Indeed, one study suggests that Wnt/Ca^{2+} may in fact serve as a modulator of both PCP and canonical Wnt pathways and in this way regulate convergent extension (Kuhl et al., 2001). Further exploration of these possibilities will be highly illuminating.

Calcium Waves and Convergent Extension

In light of the role played by Wnt/Ca^{2+} signaling, it is interesting to consider that calcium signals coordinate cell motility in a variety of settings. Indeed, such a role in gastrulation has long been suggested by the dynamics of calcium storage and release during early amphibian development (Stableford, 1967; Brick and Weinberger, 1984). Modern imaging approaches have now revealed long-range intercellular calcium waves during gastrulation in fish and frogs which are correlated with the execution or coordination of convergent extension movements (Gilland et al., 1999; Wallingford et al., 2001a).

Using *f*-aequorin luminescence in early zebrafish embryos, rhythmic waves of calcium release were observed during gastrulation (Gilland et al., 1999). These waves initiate in the dorsal blastoderm margin and travel either around the germ ring or along the anteroposterior axis of the embryo (Gilland et al., 1999). Calcium waves were also observed during convergent extension of dorsal mesoderm explants in *Xenopus* using a calcium-sensitive

dye and confocal microscopy (Wallingford et al., 2001a). In *Xenopus*, calcium waves are accompanied by tissue contractions which are very similar to contractions previously described during convergent extension in explants and in intact embryos (Keller and Hardin, 1987).

The function of the calcium waves is unclear. Waves and tissue contractions are not associated with immediate changes in cell rearrangement (Keller and Hardin, 1987; Wallingford et al., 2001a), suggesting that calcium waves may not play an instructive role in convergent extension. Nonetheless, calcium waves initiate in tissues engaged in convergent extension and not in other regions of the embryo (Gilland et al., 1999; Wallingford et al., 2001a). Furthermore, disruption of calcium wave activity by pharmacological agents blocks convergent extension (Wallingford et al., 2001a).

Together, the data suggest a permissive role for calcium signals in convergent extension, perhaps serving as a molecular clutch or cue, allowing a particular signaling pathway to be activated. This possibility is made quite attractive by recent studies with Nkd, a calcium binding regulator of Wnt signals. Nkd is an EF hand protein which binds to Dishevelled, and misexpression of Nkd inhibits canonical Wnt signals while activating JNK. Intriguingly, misexpression of Nkd also disrupts planar polarity in the fly and convergent extension in *Xenopus* (Zeng et al., 2000; Rousset et al., 2001; Yan et al., 2001).

The signal initiating calcium waves also remains a mystery. Activation of the Wnt/Ca^{2+} pathway is one obvious candidate, but expression of a potent dominant-negative Frizzled-8 receptor had only a very mild effect on calcium wave activity (Wallingford et al., 2001a). Another very promising possibility is that FGF signaling may lie upstream of calcium wave activity. FGF signaling is required during gastrulation in *Xenopus* (Kroll and Amaya, 1996), and *Xenopus* Sprouty-2 is an intracellular antagonist of FGF-induced calcium release which inhibits convergent extension when misexpressed (Nutt et al., 2001). Further investigation of events both upstream and downstream of these calcium waves will be required for a comprehensive understanding.

Convergent Extension during Animal Morphogenesis: Just Variations on a Theme?

As we have seen, despite some critical differences between convergent extension in frogs and fish, a similar molecular mechanism controls the process in both animals. While this review focuses on convergent extension of the mesoderm of frogs and fish, this morphogenetic process is quite prevalent across a variety of tissues and animals, and similar regulatory mechanisms seem to be at work in most cases. For example, convergent extension of neural tissues in *Xenopus* requires PCP signaling for its regulation (Wallingford and Harland, 2001), and may also involve calcium waves (Leclerc et al., 2000). Likewise, notochord elongation in the primitive ascidians involves polarized protrusive activity, mediolateral intercalation, and PCP signaling (Miyamoto and Crowther, 1985; Keys et al., 2002; Munro and Odell, 2002). Another process of cell intercalation elongates epithelial tubes such as the sea urchin archenteron and *Drosophila* hindgut (Hardin, 1996; Lengyel and Iwaki, 2002). In the urchin, cell intercalation biased circumferentially around the lumen elongates and narrows the archenteron (Hardin, 1989), highlighting the idea that cell intercalation can drive elongation in the absence of an obvious midline to converge toward. Extension of the germband in *Drosophila* by cell intercalation may be another example where there is no biased movement toward a source of signal (Irvine and Wieschaus, 1994).

Several lines of evidence indicate that lessons learned from the frog and fish will be broadly applicable to morphogenesis in other vertebrates. Compelling evidence for a similar mechanism of convergent extension in mice comes from the phenotype of the classical mutation *looptail*. This mutation disrupts a homolog of *Strabismus* (Kibar et al., 2001; Murdoch et al., 2001), a gene required for *Drosophila* PCP and also for convergent extension in fish and frogs. Strikingly, mice homozygous for the *looptail* mutation display all of the attributes of frog or fish embryos in which convergent extension has been blocked: short anteroposterior axes, wide notochords, and broad, open neural tubes (Smith and Stein, 1962; Wilson and Wyatt, 1992; Greene et al., 1998), strongly supporting a conserved role for convergent extension in mammals and a role for PCP signaling in controlling the process.

Consistent with this idea, cell rearrangements do contribute to extension of the notochord and the neural tube of mice and chicks (Schoenwolf and Alvarez, 1989; Sausedo and Schoenwolf, 1993, 1994; Sulik et al., 1994). On the other hand, since directed cell divisions are also associated with axis elongation in these animals, it is interesting that PCP signaling components also orient cell divisions in *Drosophila* (Adler and Taylor, 2001; Bellaiche et al., 2001). It is tempting to speculate that the PCP pathway may regulate axis elongation by controlling the polarity of cell divisions during early development. The well-defined pattern of polarized cell divisions in the zebrafish epiblast during gastrulation and neurulation provides an excellent system in which to test this possibility (Concha and Adams, 1998).

Together, the findings discussed here provide the rough beginnings of a comprehensive understanding of the molecular control of at least one morphogenetic process involved in chordate development. But there are still many unanswered questions. By exploiting the complementary advantages of a variety of organisms and by combining embryological and molecular manipulations with dynamic analysis, we can hope to understand more. T.H. Morgan once wrote of early embryonic morphogenesis: "I have not been able to picture to myself clearly the cell-migrations that bring about or are involved in this process. The phenomenon is a most important one, and I regret exceedingly that I have not mastered the situation" (Morgan, 1895). One hundred and seven years later, progress has been made, but admitting that we too have not mastered the situation is an important first step toward a complete understanding.

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