

REVIEW

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How we are shaped: The biomechanics of gastrulation

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Abstract Although it is rarely considered so in modern developmental biology, morphogenesis is fundamentally a biomechanical process, and this is especially true of one of the first major morphogenic transformations in development, gastrulation. Cells bring about changes in embryonic form by generating patterned forces and by differentiating the tissue mechanical properties that harness these forces in specific ways. Therefore, biomechanics lies at the core of connecting the genetic and molecular basis of cell activities to the macroscopic tissue deformations that shape the embryo. Here we discuss what is known of the biomechanics of gastrulation, primarily in amphibians but also comparing similar morphogenic processes in teleost fish and amniotes, and selected events in several species invertebrates. Our goal is to review what is known and identify problems for further research.

Key words Biomechanics · gastrulation · morphogenesis · vertebrate · *Xenopus laevis* · zebrafish · chick · evolution · development

Introduction

To understand the biomechanics of gastrulation we must answer a number of questions. First, when and where do cells move? This is known with sufficient accuracy in few, if any systems. Second, which cells generate force and which cells are passively molded by forces generated elsewhere? Third, what are the mechanical properties of cells and tissues that determine the effect of these forces?

The specificity of outcome is rarely a property of the cells generating the force alone, but a contextual property arising from the mechanical environment. The composite mechanical properties of cells and extracellular matrix determine the resistance of tissues to deformation, the geometry of their deformation, and the efficiency of transmission of forces over distance. Fourth, what are the contributions of the cytoskeleton, cell adhesion, cell shape, cell packing-pattern, and extracellular matrix to these mechanical properties, and how are these properties genetically encoded? We know little or nothing about these parameters in most cases. Fifth, how are these forces, and the tissue mechanical properties that transmit them, patterned in space and time such that they produce a specific outcome? Cell and tissue polarity is important in determining how cells exert force in a particular orientation, and how they lay down organized extracellular matrix with patterned mechanical properties. Finally, how do mechanical forces feed back on cell regulatory processes to modify subsequent morphogenesis and differentiation?

From this perspective, morphogenesis is the problem of how genes encode forces and mechanical properties. Patterning is usually thought of in terms of regulating expression of genes controlling cell differentiation rather than regulating expression of genes that control the patterns of force and tissue mechanical properties important in morphogenesis. However, the magnitude, timing and direction of force generation, and the tissue mechanical properties, such as viscosity and stiffness, that regulate how far, over what time, and in what geometry locally generated forces are transmitted, are in themselves phenotypes. These phenotypes are controlled immediately by the distribution, organization, and function of protein complexes, and ultimately by the genes encoding these proteins. Connecting protein function with macroscopic tissue deformation is a basic problem of biomechanics, and it must be understood in terms of

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the *function of morphogenic machines*, machines at the subcellular, cell, or tissue (cells and their extracellular matrix) level that generate and transmit forces that deform tissues. Developmental biologists have been notably successful in identifying the *components* of morphogenic machines, using molecular and genetic interdiction experiments that cause morphogenesis to arrest, fail or otherwise go awry, as assayed macroscopically. We must go beyond these compositional analyses towards more functional analyses of morphogenic machines by applying the concepts, skills, methods, and approaches of cell biology, biophysics and bioengineering towards what is essentially a biomechanical problem.

In studying gastrulation, we must also understand variation in *morphogenic design* and *morphogenic constraint*, the functional limitations of a particular type of morphogenic machine. Gastrulation movements are not the same from species to species, with many different strategies for generating the same basic body plan. Embryos use a limited stock of cell behaviors, but they use them in different combinations, in different geometric and mechanical contexts, and with different timings. In some cases they execute the same global movements with different cell behaviors, whereas in others they execute different global movements with the same or similar cell behavior, used in different ways. This diversity of morphogenic mechanism is driven by the evolution of diverse reproductive strategies and life histories, which may entail the evolution of different egg sizes, amounts and proportions of yolk, and developmental rates. As these parameters change, the performance limits of a particular morphogenic design will be exceeded, and it will fail with increasing frequency unless it also evolves. Understanding the correlated co-evolution of mechanical environment and morphogenic mechanism will provide insights into the dependence of the latter on the former. The key to understanding gastrulation in the broadest and deepest sense lies in understanding its cell biological and biomechanical design, not human or supernatural design, but a set of mechanisms produced stochastically by evolution: what is, is what works.

***Xenopus* Gastrulation**

Summary of Cell Movements

Gastrulation in tail-less (Anuran) amphibians, represented here by the predominant model system, *Xenopus laevis*, primarily involves *involution* of the IMZ (*involuting marginal zone*; Fig. 1A,E,I), which turns inward and back on itself, and subsequently moves across the blastocoel roof (black arrows, Fig. 1F-G, J-K). The IMZ is an annulus of tissue consisting of a superficial epithelial sheet of cells and a thicker, underlying deep region of mesenchymal cells. The superficial IMZ consists of prospective notochord (pink), a small amount of

prospective somitic mesoderm (red), prospective endoderm (yellow), and the prospective bottle cell endoderm (dark green; Fig. 1A-C, E-H). The multi-tiered region of deep mesenchymal cells consists of prospective notochord (magenta), prospective somitic mesoderm (red), and prospective head, heart and lateroventral mesoderm (orange; Fig. 1E-H, I-L). The amount of prospective somitic mesoderm in the superficial IMZ varies greatly among species of amphibian and has considerable biomechanical significance (see p. 187). The IMZ lies at the periphery of the large core of vegetal endoderm (light green, Fig. 1E-H).

Involution begins dorsally with the formation of a local depression, the blastoporal groove, by *invagination*, defined as the bending inward of an epithelial sheet, as the cuboidal prospective bottle cells undergo apical constriction and become wedge shaped (Figs. 1A-B, E-F, 2A-C). The shallow blastoporal groove marks the site of the initiation of gastrulation but contributes little to the eventual depth of the archenteron. Much larger changes are wrought at the same time by the recently described *vegetal rotation* movements (Winklbauer and Schürfeld, 1999). Throughout late blastula stages and gastrulation, the apices of the vegetal endodermal cells gradually contract (Keller, 1975), pushing their basal ends animally and drawing the IMZ vegetally. At the onset of gastrulation, the central vegetal endodermal cells are already moving up (animally) towards the blastocoel floor and those on the floor are moving peripherally (dorsally) against the dorsal blastocoel roof. This composite set of movements rotates the entire IMZ and initiates the involution of its vegetal edge around the inner blastopore lip (straight black arrows, Fig. 1E-F; I-J; Fig. 2 A,B). Both bottle cell formation and vegetal rotation begin dorsally and proceed laterally and ventrally. Much of the early involution thought due to the formation of bottle cells (Hardin and Keller, 1988) occurs instead because of these more substantial vegetal rotation movements (see p. 178). Vegetal rotation brings the mesendoderm (the leading edge of the mesodermal mantle and the associated endoderm) in apposition to the overlying blastocoel roof (Fig. 1F). Once there it migrates directionally toward the animal pole, using the overlying roof of the blastocoel as a substrate (Holtfreter, 1943a,b; 1944; Nakatsuji, 1975; Nakatsuji and Johnson, 1982; Winklbauer, 1990; Davidson et al., 2002).

Involution of the IMZ alone does not close the blastopore, nor account for the anterior-posterior elongation of the body axis. These events are accomplished largely by *convergence* and *extension*. The IMZ first *thins* in the radial direction and *extends* in the animal-vegetal direction (white arrow, Fig. 1E-F), coincident with vegetal rotation. Then, beginning in the midgastrula, the post-involution prospective notochordal and somitic mesoderm and the overlying pos-

terior neural plate narrows (*converges*) in the mediolateral (circumblastoporal) direction and elongates (*extends*) in the anterior-posterior direction (white arrows, Fig. 1G-H, K-L). These paired movements, often called *convergent extension*, elongate the body axis and contribute to involution and blastopore closure (see Keller, 1984, 1986; Keller et al., 1991, 2000). Throughout the blastula and gastrula stages, the animal region of the embryo expands or undergoes *epiboly*, spreading vegetally into the region vacated by the IMZ as the latter is internalized. Epiboly occurs by thinning and spreading of the blastocoel roof in all directions (Fig. 1A-C).

Invagination: The “bottle” cell morphogenic machine

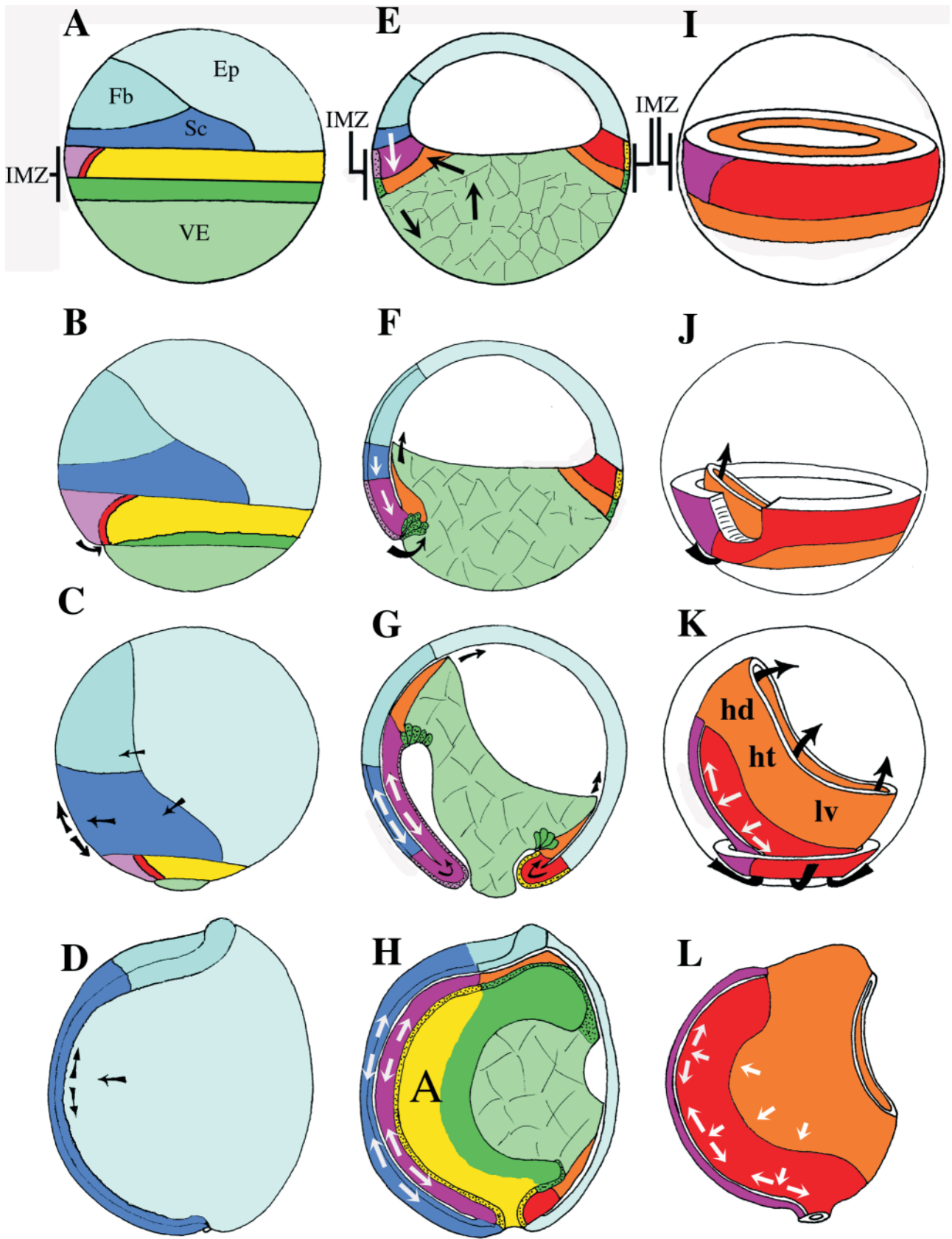
The prospective bottle cells (dark green, Fig. 2A-B) actively constrict their outer, apical surface, elongate in the apical-basal direction, and become wedge-shaped, which results in bending of the epithelial sheet to form a shallow *invagination* (see Hardin and Keller, 1988; black arrows, Fig. 2A,B). This type of cell behavior is of general significance as it occurs in many systems (see Ettensohn, 1985b), including amphibian gastrulation (see Holtfreter, 1943a, b; Baker, 1965; Perry and Waddington, 1966), vertebrate neurulation (Burnside and Jacobson, 1968; Schoenwolf and Smith, 1990; Bush et al. 1990), echinoderm primary invagination (Gustafson and Wolpert, 1963; Ettensohn, 1984; Davidson et al., 1995), gastrulation in nematodes (Nance and Priess, 2002; Lee and Goldstein, 2002), and in *Drosophila* mesoderm formation (see Leptin and Grunewald, 1990; Sweeton et al., 1991). The biomechanics of the relation of the cell shape change to the tissue bending has been established by physical (Spek, 1918, Lewis, 1947) and mathematical modeling (Odell et al., 1981; Hardin and Keller, 1988; Davidson et al., 1995), but much remains unknown about how these cells function in the embryo.

Comparison of bottle cell behavior in the embryo and in isolated explants, free from surrounding mechanical constraints, shows that their effect is context-dependent. As the bottle cells constrict their apices, the surrounding epithelium passively expands toward the site of the forming bottle cells (Fig. 2A-B, 2F; Hardin and Keller, 1988). Also, most of the contraction of the bottle cell apices is in the animal-vegetal direction, rather than in the mediolateral (circumblastoporal) direction. This anisotropy is reflected in the characteristic elongated shape of the constricted apices (Fig. 2F), which are aligned circumferentially around the vegetal endoderm. When prospective bottle cells form in explants without adjacent tissues, or in embryos from which the vegetal endoderm has been removed, they contract isotropically, forming circular rather than elongated apices (Fig. 2G; Hardin and Keller, 1988). The large mass of vegetal en-

doderm serves as a relatively undeformable anchorage on the vegetal side of the bottle cells, which focuses most of the effect of apical constriction towards stretching the relatively deformable IMZ vegetally. The resistance of the vegetal endoderm to deformation, compared to that of the IMZ, is also responsible for restricting the contraction of bottle cell apices in the mediolateral direction. These behaviors support the notion that bottle cell apical constriction is an isotropic force-generating process that acts in a mechanically anisotropic environment, which channels its effect towards displacing the outer IMZ vegetally.

The effectiveness of apical constriction in bending the cell sheet is related to the resistance of the cell to apical-basal elongation (see Hardin and Keller, 1988). Apical constriction results in apical-basal elongation and wedging, and moderate bending of the epithelium in the embryo (Fig. 2H-I) but relatively little apical-basal elongation, a rotund cell shape, and extreme bending of the epithelium in explants without the resistance of adjacent tissues (Fig. 2H-J). This behavior suggests that resistance to apical-basal elongation is important in bending a cell sheet by apical constriction; the greater the resistance to elongation, the greater the bending of a tissue with a given resistance to bending. If apical-basal resistance to elongation is very small, or resistance of the surrounding tissue to bending very high, apical constriction should result in columnarization (Fig. 2H-K). If these columnarized cells then actively rounded up, or shortened their apical-basal dimension (Fig. 2K-L), the cells should secondarily adopt the bottle or wedge shape, or the rotund shape, and bend the epithelium moderately (Fig. 2L-I), or severely (Fig. 2I-J). Thus regulation of resistance to apical-basal elongation is an important molecular and genetic control point. Basolateral cortical tension would resist apical-basal elongation, with higher tension driving the cell toward the spherical shape and severe bending (Fig. 2J) and lower tension allowing extreme apical-basal elongation (columnarization) and little or no bending (Fig. 2K). A specialized apical-basal reinforcement of the cytoskeleton could also resist elongation, either by an active contraction or an increased elastic resistance to stretching in this dimension.

In *Xenopus*, cell wedging and bending of the cell sheet coincide with apical constriction, whereas in most systems, bending occurs in two phases, the second perhaps reflecting an active apical-basal shortening. In the first phase, apical constriction occurs with apical-basal elongation, resulting in *columnarization* or *palisading*. In the second phase, apical-basal shortening occurs, resulting in cell wedging and bending of the epithelium (Fig. 2H-K, L-I). This two phase mechanism drives bending of cell sheets in a number of cases, including the thickening of the vegetal plate followed by primary invagination in echinoderms (see Gustafson and Wolpert, 1963), the thickening of the neural plate prior to cell wedging and rolling of this structure into a tube (see Burnside and



Jacobson, 1968; Jacobson and Gordon, 1976), and as a first step in *Drosophila* mesoderm formation (Leptin and Grunewald, 1990; Sweeton et al., 1991).

Multiple phases of cell behavior during invagination: lessons from *Drosophila*

We diverge here to consider briefly a very instructive example from invertebrates. Prospective mesodermal cells of *Drosophila* provide a good example of this multiphased process (see Leptin and Grunewald, 1990; Sweeton et al. 1991; Kam et al., 1991; Oda and Tsukita, 2000). First, the apices of some of the cells flatten, followed by the onset of a slow apical constriction. After apical constriction has begun in about 40% of the cells, which are scattered at random through the presumptive invaginating tissue, a fast phase of apical constriction occurs over all the cells, simultaneously, in a coordinated fashion, coincident with apical-basal elongation. Then, in the third and final phase, the cells shorten their apical-basal axes, broaden their basal ends, and become wedge-shaped; as a result, the epithelium bends (invaginates; see Leptin and Grunewald, 1990; Sweeton et al.,

1991; Kam et al., 1991). This secondary shortening and rounding is most likely caused by an active apical-basal contraction or an active increase in basolateral cortical tension. A less likely mechanism is that the elastic resistance to apical-basal elongation is initially too small to produce cell wedging and bending of the sheet of cells under the resistance of the surrounding tissues. Once the resistance to bending is relaxed below a threshold level, elastic recoil can drive apical-basal shortening, producing wedging of the bottle cells and bending of the epithelial sheet. In this case, active force generation would occur prior to actual bending, during columnarization, with elastic energy stored during the stretching of the apical basal axis.

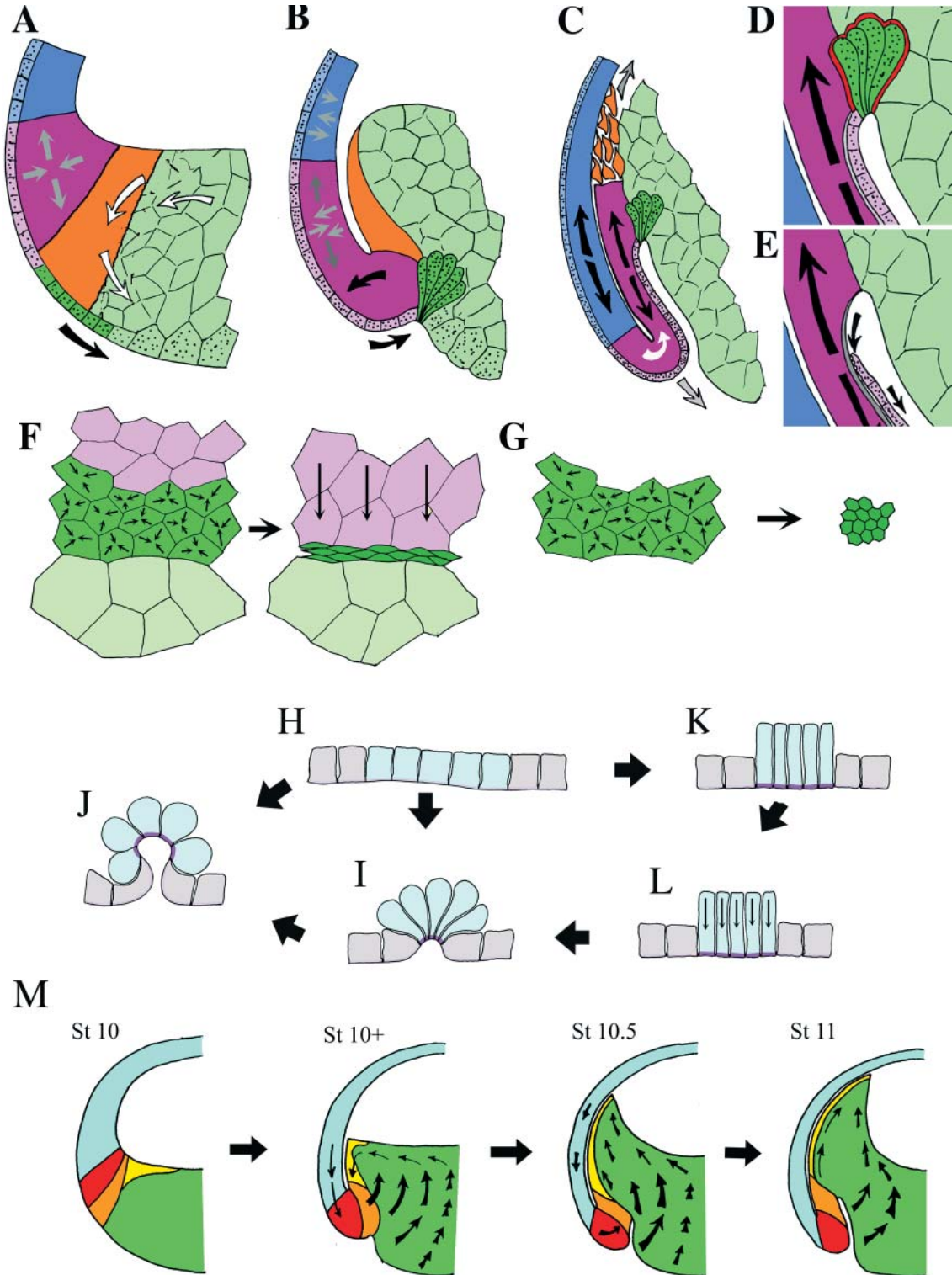
The mechanism of restricting apical-basal extension, or active apical-basal shortening, is largely unexplored, despite the fact that the work described above and modeling studies (Davidson et al., 1995) show these parameters to be important in invagination. A potentially important parameter, cortical deformability of cells, varies in developmental contexts, such as during *Fundulus* (teleost) development (Tickle and Trinkaus, 1973), but this parameter has not been studied in bottle cells of any system. Reinforcement of the cytoskeleton along the apical-basal axis could result in greater elastic resistance to elongation during apical constriction, and could generate an active contraction along this axis. An active apical-basal contraction must occur to produce wedged cells from columnar ones, in systems having the two-phase process of apical constriction and columnarization, followed by apical-basal shortening and cell wedging. Perry and Waddington (1966) suggested that an organized cytoskeleton might lie parallel to the long axis of the bottle cells of newts, and longitudinally-oriented microtubules are found in the elongated neck regions of the bottle-shaped primary mesenchyme cells of sea urchins during their ingression (Katow and Solursh, 1980) and in *Drosophila* epithelial cells (Foe et al., 2000). Finally, loss of adhesion to adjacent cells could also contribute to cell rounding, shortening and basal expansion (Gustafson and Wolpert, 1963), but these potentially important parameters have also not been investigated sufficiently.

The mechanism of apical constriction is poorly understood. Cultured retinal pigmented epithelial cells constrict using a circumapical microfilament bundle (Owaribe et al. 1981; Owaribe and Masuda, 1982). However, the electron dense material filling the apical ends of the bottle cells (Baker, 1965; Perry and Waddington, 1966) suggests the contraction of an actin meshwork spanning the entire apical surface. The fact that the apices of amphibian bottle cells remain flat rather than bulging outward during constriction (Hardin and Keller, 1988) is also consistent with this notion, as is the flattening that precedes apical constriction in *Drosophila* ventral furrow formation, described above, and apical flattening of ingressing cells of the gastrulating nematode (Nance and

Fig. 1 Diagrams show presumptive fates and movements of gastrulation in right lateral view (A-D), in midsagittal plane (E-H), and in a 3 dimensional diagram of the involuting marginal zone (IMZ; I-L). As gastrulation begins, the lower black arrow in B and F shows the pulling of the IMZ vegetally and rotation inward as the result of bottle cell (green) apical constriction. At the same time, internally the Winklbauer vegetal rotation movements (black arrows, E) rotate the dorsal IMZ, applying the leading edge mesoderm (cf. orange in E and F, I and J) against the blastocoel roof and involute a substantial amount of the axial mesoderm (notochord, cf. magenta in E and F, I and J). The mesoderm then migrates anteriorly across the blastocoel roof (upper black arrow, F, J). In early gastrulation, the dorsal IMZ undergoes thinning and extension by radial intercalation (white arrows, E, F). After involution, the dorsal IMZ undergoes convergence and extension (white arrows, G, K) coordinately with the overlying neural tissue (black arrows, C), closing the blastopore and elongating the dorsal anterior-posterior axis (cf. second to last row, C, G, K and last row D, H, L). Prospective fates: epidermis (light blue, Ep); forebrain (blue, Fb); spinal cord (dark blue, Sc), superficial endoderm (yellow), superficial endoderm-bottle cells (green); vegetal endoderm (light green, VE); notochord (magenta); somitic mesoderm (red); leading edge mesoderm (orange) of the head (hd), heart (ht) and lateral-ventral body (lv). Note that the superficial layer of the IMZ contains presumptive notochord (about 20% of the total; pink in A, B, C) and a small amount of presumptive somitic mesoderm (1-3 cells per somite, red in A, B, C), which ingresses from the roof of the gastrocoel during neurulation to form the endodermally lined archenteron (A in H). The processes of bottle cell formation, vegetal endodermal rotation, involution and convergence and extension all begin dorsally and proceed laterally and finally ventrally. Dorsal is to the left and vegetal at the bottom. (Based on Keller, 1975, 1976; Shook and Keller, unpublished; and Winklbauer and Schürfeld, 1999.).

Preiss, 2002). Calcium and calmodulin appear to be involved in apical constriction (Lee and Nagele, 1985; Ferreira and Hilfer, 1993). Non-muscle myosin is localized at the constricting apices of the cells during *Drosophila* ventral furrow formation (Leptin and Grunewald, 1990;

Young et al., 1991) and prior to and during cell ingression in nematode gastrulation (Nance and Preiss, 2002). Expression of dominant negative Rho1 or loss of DRhoGEF2, an activator of Rho, block change in shape of the mesoderm cells and their subsequent ingression



in the *Drosophila* ventral furrow (Barrett et al., 1997; Hacker and Perrimon, 1998). Absence of p190RhoGAP inhibits neural apical constriction and neural tube formation (Brouns et al., 2000). The evidence points toward active, regulated actomyosin-mediated contraction, perhaps regulated by Rho, the Rho activated kinase, ROCK, and its substrate, the non-muscle myosin regulatory light chain (see Settleman, 2001).

The effects of mutants of *twist* and *snail*, transcription factors necessary for *Drosophila* mesoderm formation, highlight the complex regulation of apical constriction and at the same time the necessity of coordinated apical constriction. No apical constriction occurs at all in *snail* mutants, suggesting that this gene is essential for the process. In *twist* mutants, some cells show transient constriction, and these cells are located in small domains to which *snail* expression has been restricted by defective *twist* (Leptin and Grunewald, 1990; Costa et al., 1994). Restoring *snail* function in *twist* mutants results in broader but uncoordinated apical constriction, and irregular invagination (Ip et al., 1994). Coordinated apical constriction is essential, as one might expect, for the

mechanics of making an appropriate invagination. In mutants of two other genes, *fog* (*folded gastrulation*; Costa et al., 1994), which encodes a secreted molecule, and *cta* (*concertina*; Parks and Wieschaus, 1991), which encodes a heterotrimeric G protein α subunit, the first phase of uncoordinated apical flattening and constriction occurs but produces a poor invagination in absence of the secondary, fast, coordinated phase of apical constriction, which never occurs in these mutants (see Oda and Tsukita, 2000). Costa and others (1994) proposed a model in which *fog* binds an unidentified receptor, which then activates *cta*, which in turn, may activate Rho through DRhoGEF2 (Barrett et al., 1997). Ectopic expression of *fog* and *cta* shows that these molecules can induce apical flattening and constriction independent of the mesodermal cell phenotype, with *cta* being downstream of *fog* (Morize et al., 1998). Whether homologous components function similarly in other invaginating systems is not known. These experiments provide a promising beginning to understanding how the apical constriction is regulated, and particularly how it is coordinated, a coordination essential for its mechanical function.

Other models of bottle cell function

What is the contribution of the bottle cells, as benders of the epithelial sheet, to gastrulation of amphibians? The local bending of the epithelium does not add much to the eventual depth of the archenteron, because most of the depth of the archenteron is due to extension of the IMZ backwards, vegetally, over the vegetal endoderm (see p. 185; and Keller et al., 2000). Surgical removal of the bottle cells or the prospective bottle cells of *Xenopus*, does not block gastrulation but decreases the reliability of gastrulation by causing poor initiation of involution and higher frequencies of protruding yolk plugs and exogastrulation (Keller, 1981; Hardin and Keller, 1988). In their role as benders of an epithelial sheet, bottle cells appear to make a significant but not the major contribution to gastrulation. Part of this contribution is probably due to the pulling of the outer IMZ vegetally, thus aiding the rotation of the IMZ, driven in larger part by the vegetal rotation movements described above.

Amphibian bottle cells may serve as active or passive towing devices. Holtfreter (1943a, b) observed that dissociated bottle cells maintain their apical-basal polarity and migrate with the basal end leading when cultured in alkaline medium on glass. He postulated the newly formed bottle cells actively migrate toward the inside, stimulated by the observed alkaline pH of the blastocoel fluid, and that they pull the adjacent superficial cells inside by virtue of their common apical adhesion (Holtfreter, 1943a, b). However, bottle cells never actually move very far with respect to the neighboring mesodermal cells

Fig. 2 Diagrams illustrate the effect of the Winklbauer vegetal endodermal rotation movements (white arrows, A) and bottle cell apical constriction (black arrow, A) in moving the leading edge mesendoderm (orange) from the floor of the blastocoel and against the blastocoel roof (A-B) and the initiation of the involution of the IMZ (black arrows, B). This is followed by convergence and extension of the neural and mesodermal tissue (black arrows, C) and migration of the leading edge mesendoderm (gray arrow, C). The presumptive bottle cells (green) undergo apical constriction and apical-basal elongation (A-C) and form a high adhesion attachment (red, D) that anchors the superficial epithelial layer of the archenteron to the underlying mesoderm (magenta, D); when they are removed, the epithelium is not anchored and slips backwards (E). Bottle cell apical constriction stretches and pulls the superficial IMZ (pink, F) vegetally, rather than pulling the vegetal endodermal cells (light green, F) animally. In the embryo, apical constriction is highly anisotropic with little contraction in the circumferential (mediolateral direction) and much contraction in the animal-vegetal direction, resulting in an elongated shape of the bottle cells (F). But in explants of bottle cells without adjacent tissues, apical constriction is isotropic, occurring in all directions equally (G), suggesting that the intrinsic apical constriction is isotropic (arrows, F, G) but is modulated by an anisotropic environment. Apical constriction of presumptive bottle cells in the embryo results in cell wedging and a moderate bending of the epithelial sheet (invagination; H-I), but presumptive bottle cells explanted alone undergo less apical-basal elongation, become more rotund, and produce extreme bending (H-J). Apical constriction without resistance to apical-basal elongation, or extreme resistance of the surrounding tissues to deformation is predicted to produce columnarization (H-K). Subsequent apical-basal shortening would secondarily produce bending and invagination (K-L-I), perhaps in the extreme (I-J). The progress of the Winklbauer vegetal endodermal rotation movements are illustrated diagrammatically (M). (Based on Keller, 1978, 1981; Hardin and Keller, 1988; and Winklbauer and Schürfeld, 1999. M is modified from Winklbauer and Schürfeld, 1999.)

until late gastrulation when they contribute to archenteron expansion by respreading and flattening (Hardin and Keller, 1988). It is the mesendodermal cells that actually move by migrating on the roof of the blastocoel (see p. xx). Instead of actively towing other cells, the bottle cells of *Xenopus* serve as highly adhesive, static anchor points that attach the epithelial archenteron roof to the underlying, anteriorly migrating mesendoderm. When the bottle cells are surgically removed, the epithelial layer of the IMZ tends to slip backward (Fig. 2D-E), as if it were most tightly anchored to the anterior mesendoderm by the bottle cells (Keller, 1981). If the epithelial layer is microsurgically peeled off the deep IMZ of the early gastrula, it comes off easily everywhere except at the bottle cells where it is stuck too tightly to remove (R. Keller, unpublished observation). The mechanism of this unusually strong adhesion is not known.

Ironically, the largest contribution of *Xenopus* bottle cells to morphogenesis seems to be during their respreading, rather than their formation. Beginning dorsally (presumptive anterior) at the late midgastrula stage, and proceeding laterally and finally ventrally (presumptive posterior) in reverse of the order of their formation, the bottle cells respread to form a large area everywhere on the periphery of the archenteron. This respreading is their major contribution to the depth of the archenteron, and when they are cut out, the archenteron is truncated peripherally (Keller, 1981; Hardin and Keller, 1988). Nothing is known about the cell biological or biomechanical processes underlying this dramatic respreading, but cytoskeletal, behavioral, and adhesive changes would be expected.

To summarize, *Xenopus* bottle cells demonstrate that the result of a local cell behavior is dependent on the larger context, and, in turn, not all aspects of local cell behavior are intrinsic properties. Instead, some are modified by the mechanical context (Hardin and Keller, 1988). An intrinsically isotropic apical constriction can result in an anisotropic deformation of surrounding tissues and will itself become anisotropic in a geometrically or mechanically anisotropic environment. An anisotropic environment channels the effect of an isotropic force generator into toward a specific population of cells. The specificity of this morphogenic machine lies in its biomechanical context, as well as the intrinsic mechanics of apical constriction-apical-basal elongation. Bottle cells may have multiple morphogenic functions in their life history, some more important than others. In this regard, little is known about the important respreading of bottle cells in *Xenopus*.

The Cryptic Machine of Amphibian Gastrulation: Vegetal Endodermal Rotation

Instead of the obvious bottle cells, the major mover of early gastrulation is the long ignored vegetal endo-

derm. The large vegetal endodermal cells were shown to undergo apical shrinkage (Keller, 1975) and were initially thought to undergo some ingression (Schechtman, 1934). However, later work showed that there was no true ingression of vegetal endoderm in any amphibian (Schechtman 1935; Ballard, 1955; Keller 1978). The vegetal endoderm was otherwise viewed as passive, some contributing to the gut but most destined to be digested as food for more important cells in later development. However, Winklbauer and Schürfeld (1999) discovered that while bottle cells are just forming on the outside, vegetal endodermal cells begin a massive rotation. Cells near the center of the vegetal endoderm on the dorsal side move anteriorly, toward the floor of the blastocoel, and those on the floor of the blastocoel move dorsally, toward the blastocoel wall, while those at the dorsal periphery of the vegetal endodermal mass move vegetally (stg 10⁻–10⁺, Fig. 2M). As this occurs, the outer IMZ is drawn vegetally by the apically contracting epithelial vegetal endoderm cells (Keller, 1975), aided by the constricting bottle cells, while the inner, vegetal part of the IMZ, lying at the periphery of the blastocoel floor, is rolled against the overlying roof of the blastocoel (stg 10⁻–10⁺, Fig. 2M), forming the cleft of Brachet. This tissue, consisting of prospective anterior mesendoderm, attaches to and migrates directionally on the roof of the blastocoel (see p. xx). These movements begin dorsally and then progress laterally and ventrally (Winklbauer and Schürfeld, 1999), presaging the progression of all subsequent movements of gastrulation. These movements occur in explants and therefore are thought to be active and intrinsic to the vegetal endoderm. The cell behaviors, biomechanics, and regulation of this most recently identified tissue movement are largely unknown. Vegetal rotation is important in a number of ways. It accounts for most of the initial involution and formation of the cleft of Brachet and cryptic internal onset of gastrulation noted by Nieuwkoop and Florschütz (1950), and perhaps much of the total involution of the IMZ (Winklbauer and Schürfeld, 1999), with the formation of the bottle cells (see Hardin and Keller, 1988) playing a smaller role. Their limited role is suggested by the fact that their formation, and the resulting blastoporal groove, are not reliable indicators of the progress of the internal rotation of the IMZ and the apposition of the leading edge mesendoderm to the roof of the blastocoel (Poznanski and Keller, 1997). Although it has been viewed as passive and an obstacle to be enclosed during gastrulation, rather than as a contributor to gastrulation, the vegetal endoderm contains a major force-generating machine, making it part of the solution rather than part of the problem of gastrulation. In this regard, vegetal rotation may play an especially important role in gastrulation of yolky eggs with massive amounts of vegetal endoderm (see p. 187).

Migration of the Leading Edge Mesendoderm

As vegetal rotation applies the prospective mesendoderm to the blastocoel roof (stg 10⁺, Fig. 2M), the mesendoderm attaches to and migrates across the roof of the blastocoel (see Nakatsuji, 1975; Keller and Schoenwolf, 1977; Winklbauer, 1990; Davidson et al., 2002; Fig. 2A-C; upper gray arrow, 2C). The mechanical consequence is a shear force at the interface of the outer gastrula wall and the involuted tissue, tending to pull the former vegetally and the latter animally. This migration depends on an integrin-mediated cell interaction with a fibronectin-containing matrix deposited on the inner surface of the blastocoel roof (Nakatsuji and Johnson, 1982; Boucaut and Darribère, 1983a, b; 1984; Shi et al., 1989; Smith et al., 1990; Ramos and DeSimone, 1996; Winklbauer and Keller, 1996). Migration is directed from the vegetal to animal ends of substrates conditioned by animal caps (Nakatsuji and Johnson, 1983; Winklbauer and Nagel, 1991; Nagel and Winklbauer, 1999). These directional cues depend on the fibrillar nature of the matrix for their activity. Cell aggregates migrate directionally on fibrillar matrix deposited on a substrate whereas this directionality is lost on non-fibrillar matrix deposited in the presence of cytochalasin treatment or RGD-containing peptides inhibiting integrin binding to matrix (Winklbauer and Nagel, 1991).

The migration of this population of cells is an example of what J.P. Trinkaus (1984a) called a “cell stream“, a population of cells maintaining contact with one another while translocating on an external substratum, in this case the blastocoel roof. The cellular and biomechanical mechanisms underlying such group cell migration are poorly understood. Davidson and associates (2002) have shown that three force generating processes are involved in mesendoderm migration. First, these cells migrate as monopolar cells, in a “shingled” arrangement such that the leading edges are apposed to the roof of the blastocoel and the trailing edges are within the mass (Winklbauer et al., 1996; Davidson et al., 2002; Fig. 3). Both the leading edge cells and the submarginal cells exert traction on the substrate and provide force for directional migration of the cell population (red arrows, Fig. 3). Second, the entire mass of mesendoderm cells actively extends in its anterior-posterior dimension and thins in the radial direction by *radial intercalation* (black arrows, Fig. 3). Finally, there is an additional, contextual force, probably generated by lateral association of cells in the leading edge mesendoderm as they converge from all sides toward a point on the underside of the animal cap (see Fig. 1K,L). This enhancement can be recreated in culture. If 4 explants of mesendoderm are placed in contact around an enclosed space, mimicking the situation in the embryo, the rate of advance of the leading edge equals that seen in the embryo and is faster than the advance seen in a single explant under the same conditions (Davidson et al.,

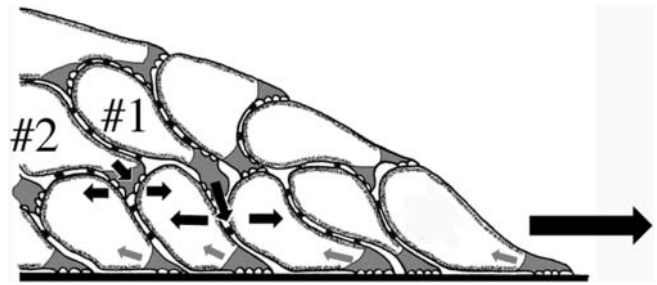


Fig. 3 Two processes driving the extension and migration of the leading edge mesendoderm are illustrated diagrammatically. The mesendoderm cells form migratory protrusions and exert traction (gray arrows) on the matrix found on the blastocoel roof or in cell culture (black line) which moves them en masse animally (large black arrow). At the same time, cells further from the matrix substrate (e.g. Cells #1 and #2) intercalate radially, between cells closer to the substrate, forcing an extension in the anterior-posterior direction (black arrows). (Based on Davidson et al., 2002.)

2002). The mechanism of this aggregate enhancement in the round is not understood and should be explored further, as it illustrates the potency of integrative, population-level, contextual behavior to enhance or transform the effect of local cell behavior. Directionality is also contextually enhanced. Explanted aggregates of mesodermal cells migrate directionally on conditioned substrate whereas single cells cannot (Winklbauer et al., 1992). Such mechanisms may be universal in metazoan morphogenesis and their recognition and characterization in other systems is a challenge for future work.

Epiboly

The animal cap expands in area and spreads vegetally during blastulation and gastrulation (Fig. 1A-D; Vogt, 1929; Keller, 1975, 1978). In *Xenopus*, this movement involves spreading and division of superficial cells and *radial intercalation* of multiple layers of deep cells to form fewer layers of greater area (Keller, 1978, 1980; Fig. 4A). In urodeles, indirect evidence suggests intercalation of all layers, including intercalation of deep cells between the superficial epithelial cells (Holtfreter, 1943b; Fig. 4B), but this has not been observed directly.

There is no direct evidence that epiboly of the animal cap is a force-producing process in amphibians. In natural or experimentally induced exogastrulae the animal cap bears numerous folds, which might suggest that an actively spreading animal cap throws itself into folds in the absence of involution of the IMZ (Boucaut et al., 1984). However, these animal caps are very thick, their inner surfaces are smooth, and the blastocoel is small, suggesting that the folds are formed by collapse and thickening of the inner cell layers of the blastocoel roof rather than its expansion (see Keller, 1986). Attempts to

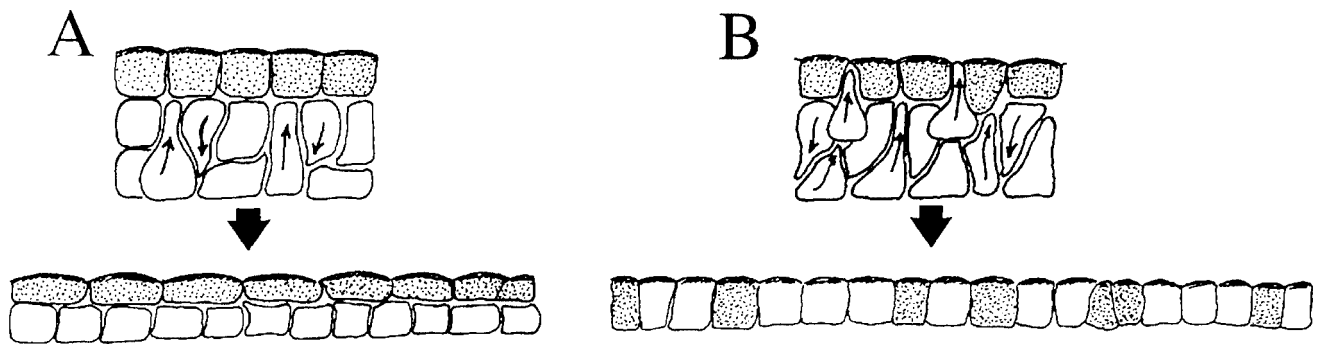


Fig. 4 In *Xenopus*, epiboly, or spreading of the animal cap, occurs by intercalation of several layers of deep mesenchymal cells between one another along the radius of the embryo (radial intercalation) to produce fewer layers of greater area while the cells in the overlying epithelial layer spread and divide (A). In the urodele

(tailed) amphibians radial intercalation was thought to be more extreme, resulting in the intercalation of deep cells between one another and finally intercalation of deep cells into the overlying epithelial sheet (B). (Based on Keller, 1978, 1980; and Holtfreter, 1944).

get the animal cap to undergo epiboly in culture without the aid of external substrates or attachments have failed (R. Keller, unpublished results), which could either mean that it is not an active process or that the conditions in culture did not support the process.

Keller (1980) proposed that an active wedging of several tiers of deep cells between one another during radial intercalation provides the forces driving epiboly while the overlying epithelial cells are passively stretched by the spreading deep region. Increased affinity or adhesion between the undersides of the overlying epithelial cells and the adjacent surfaces of the intercalating deep cells would act as a “boundary capture” mechanism, assuring that any deep cell that intercalated between others and made contact with this favorable adhesion site would likely remain there. Alternatively, adhesion of deep cells to the fibronectin-containing matrix on the inner surface of the blastocoel roof could function in the same way. The early radial intercalation and epiboly of the animal cap occurs in the blastula stage (Keller, 1978, 1980), prior to deposition of the fibronectin-containing matrix from stage 10 onward (see Fig. 2 of Winklbauer and Stoltz, 1995), making the first mechanism more likely than the second at this early stage. The late radial intercalation underlying the final stages of animal cap epiboly and the initial extension of the dorsal IMZ during early gastrulation (Wilson and Keller, 1991) occurs as the fibronectin-containing matrix is being deposited and continues at later stages in the thickened collar of involuted mesoderm at the ventral side of the late gastrula blastopore (Wilson et al., 1989; Keller et al., 1989). In an elegant paper, Marsden and DeSimone (2002) showed that this late stage radial intercalation is dependent on integrin-mediated interactions with fibronectin. Therefore, there appear to be two types of radial intercalation. The first occurs early, is isotropic, results in epiboly, and is independent of cell-fibronectin interactions. The second occurs later, is anisotropic, results in

extension, and is dependent on cell-matrix interactions. The second process is an active, force producing process (Keller and Danilchik, 1988), but it is not known if this is the case for the early epibolic process.

Convergence and Extension

The prospective notochordal and somitic mesoderm and the overlying posterior neural plate, converge (narrow) in their mediolateral (circumblastoporal) dimension and extend (lengthen) in their anterior-posterior dimension during gastrulation and neurulation (Vogt, 1929; Keller, 1975; 1976). These movements lengthen the anterior-posterior body axis, drive involution, lead to the asymmetric closing of the blastopore, and result in the formation of an elongated archenteron (reviewed in Keller, 1986 and Keller et al., 2000; white arrows, left panels, Fig. 5A, B). These movements are active, force-producing, and locally-generated as shown by the fact that explants of the mesodermal (Schechtman, 1942; Holtfreter, 1944; Keller and Danilchik, 1988) and neural tissues (Keller and Danilchik, 1988; Keller et al., 1992; Elul and Keller, 2000) converge and extend in culture unattached to an external substrate or to other parts of the embryo (right panels, Fig. 5A, B). Cell tracing (Keller and Tibbetts, 1989) and timelapse recording of living cells with epi-illumination (Keller et al., 1985a, b; Wilson et al., 1989; Wilson and Keller, 1991), or fluorescently-labeled cells (Keller et al., 1989; Shih and Keller, 1992a,b) showed that convergence and extension involve two types of cell intercalation. First, several layers of deep cells intercalate along the radius of the embryo (*radial intercalation*) to produce fewer layers (*thinning*) of greater length (*extension*; middle panel, Fig. 5A), and then the deep cells intercalate mediolaterally (*mediolateral intercalation*) to produce a narrower (*convergence*), longer (*extension*) array (middle panel, Fig. 5B). Radial

intercalation predominates in the first half of gastrulation (Fig. 5A) and mediolateral intercalation predominates in the second half of gastrulation and through neurulation (fig. 5B) in both the dorsal mesodermal tissue and in the prospective posterior neural tissue (spinal cord and hindbrain).

During mesodermal mediolateral cell intercalation,

protrusive activity becomes polarized with large lamelliform protrusions at the medial and lateral ends of the cells and small filiform protrusions at their anterior and posterior surfaces (red cell, Fig. 6A; Fig. 6B). The medial and lateral protrusions appear to exert traction on adjacent cells, and generate tension in the mediolateral axis. The cells become mediolaterally elongated,

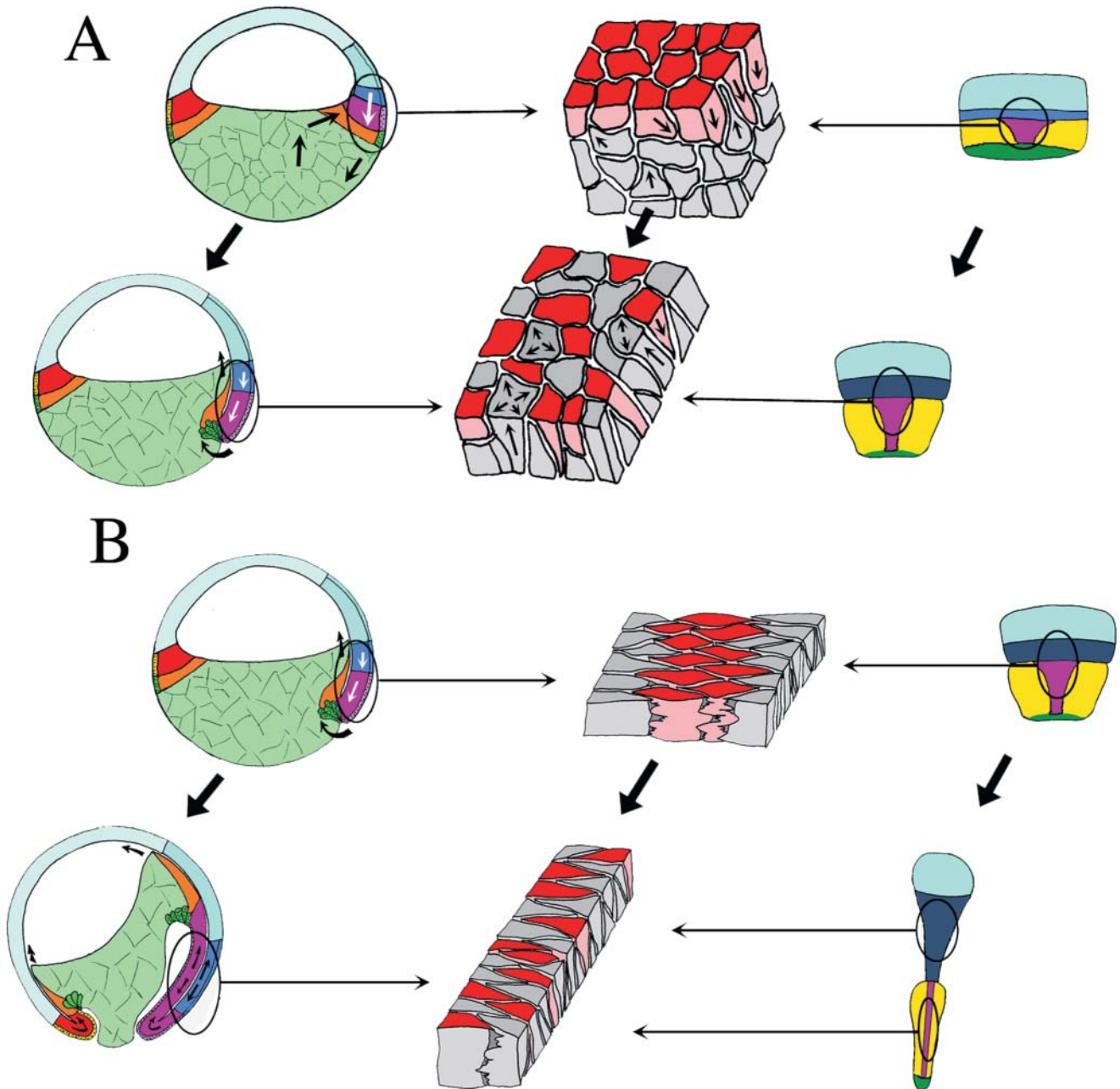
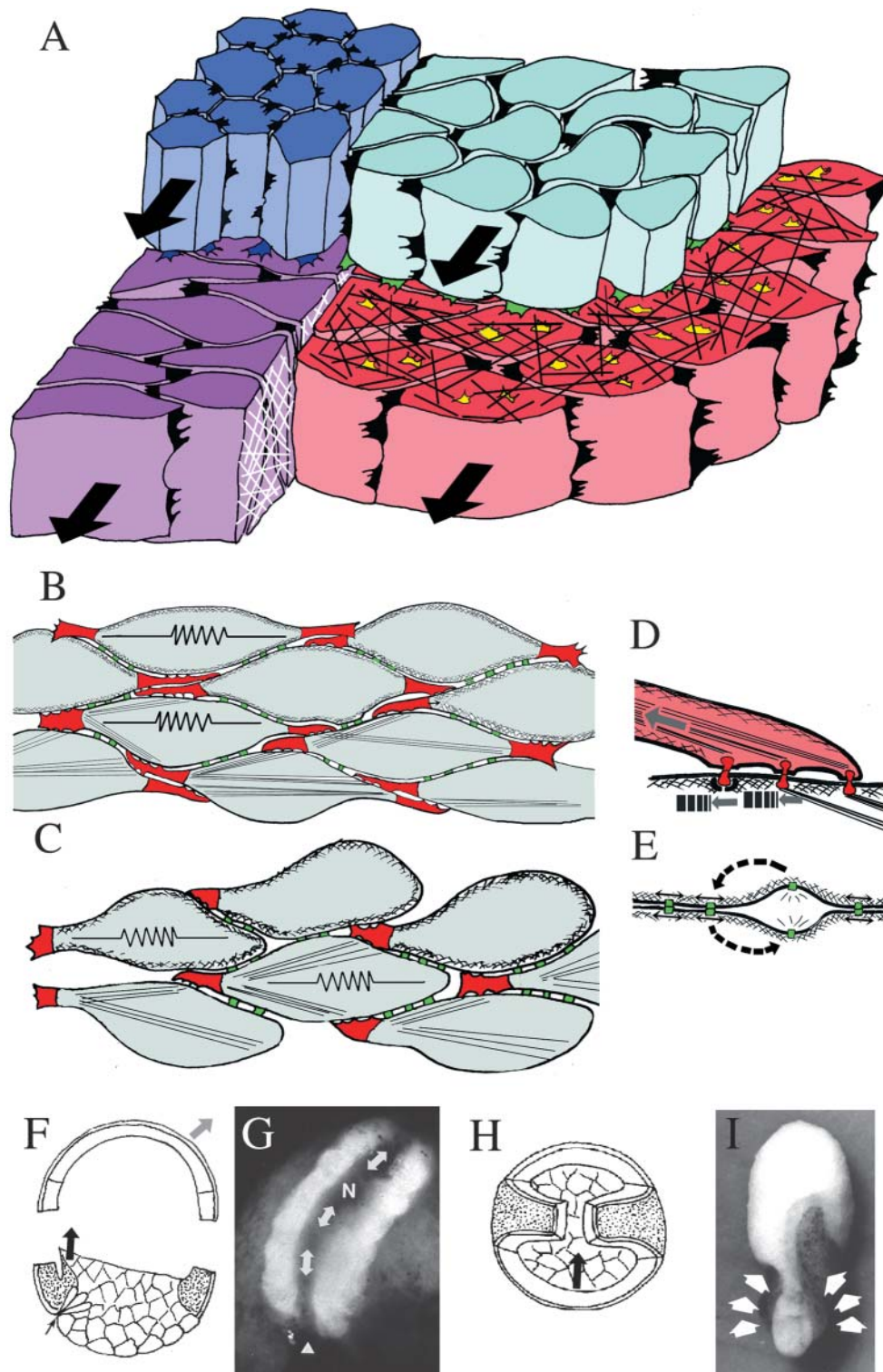


Fig. 5 Early in gastrulation, the dorsal deep mesoderm and posterior neural tissue undergoes a thinning and extension (white arrows, left panel, A) that is driven by radial intercalation of multiple layers of deep cells for fewer layers of greater area (center panel, A). From the midgastrula stage onward, these same tissues undergo convergence and extension (black arrows, bottom

left panel, B), which is driven by mediolateral cell intercalation (middle panel, B). These movements of thinning and extension and convergence and extension also occur independent of other tissues in explants (right panel, A, B) (Based on Keller and Danilchik, 1989; Wilson and Keller, 1991; Shih and Keller, 1992b; Keller et al., 1992c).



oriented parallel to one another, and move between one another along the mediolateral axis, producing a longer, narrower array (red cells, Fig. 6B; see Keller et al., 1992c). Mediolateral intercalation occurs in posterior neural tissue in a similar fashion, with one major difference. Instead of being bipolar, the neural cells have their

protrusive activity heavily biased toward the medial ends (Elul and Keller, 2000; blue cells, Fig. 6A,C). This “monopolar”, medially-directed protrusive activity is dependent on the presence of a midline notoplate/notochord (cf. Elul et al., 1997 and Elul and Keller, 2000; Ezin, M., 2002), and is thought to drive cell intercalation

Fig. 6 A diagram illustrates the behavior of cells during convergence and extension (A): neural cells (light blue) show monopolar, medially directed protrusive activity; somitic mesodermal cells (red) and notochordal cells (magenta) show bipolar, mediolaterally directed protrusive activity; notoplate cells (dark blue) show multipolar protrusive activity. These protrusive activities are thought to drive intercalation of cells along the mediolateral axis, producing convergence, which in turn, results in extension along the anterior-posterior axis (black arrows). A fibronectin-containing matrix (black lines) lies between the neural and somitic mesodermal tissue and a fibrillin matrix (white lines) lies between the notochord and somitic mesoderm. Cell intercalation (B, C) is thought to be driven by cell traction on adjacent cells mediated by large lamelliform protrusions (red) directed medially and laterally in the case of the mesoderm (B) and directed medially in the case of the neural tissue (C). These protrusions use adjacent cells as substrates. The tension generated by this oriented or polarized protrusive activity pulls the cells between one another mediolaterally, and results in a force-producing extension in the transverse direction. The tractive protrusions must be attached to adjacent cells in a fashion that does not allow excessive slippage or shear (D). At the same time, the cells must be attached to one another by “stiffening adhesions” (green), adhesions that are dynamic and allow cells to shear past one another in the short term but in the long term, allow cell intercalation (E). The dorsal mesoderm (presumptive notochord and somitic mesoderm) and vegetal endoderm form the “skeleton” of the embryo. When the blastocoel roof is removed (F), convergence and extension closes the blastopore and elongates the body axis independent of an overlying substrate (G). If offered substrate off-axis (H), the substrate is pulled over the extending axis but can not divert the extension of the axis (I). (From Keller et al., 1989; Shih and Keller, 1992a, b; Elul and Keller, 1997; Keller and Jansa, 1992.)

by the same cell-cell traction mechanism as the bipolar mode (Fig. 6C).

A number of components in the planar cell polarity (PCP), or planar tissue polarity pathway that regulates polarity of cells in *Drosophila* (Adler, 2002), have also been implicated in regulation of convergence and extension in *Xenopus*. These include the secreted signaling ligand Wnt11 (Tada and Smith, 2000) and the 7-pass transmembrane Wnt receptor Frizzled (Djiane et al., 2000). Wnt/Frizzled signaling appears to regulate convergence and extension through regulation of the Rho family GTPases that control cytoskeletal organization, cell polarity, and protrusive activity (Settleman, 2001). Frizzled signals through Dishevelled (Sokol, 1996; Wallingford et al. 2000; Wallingford and Harland, 2001; Habas et al., 2001), which interacts downstream with DAAM1 (Dishevelled associate activator of morphogenesis), and it, in turn, regulates RhoA (Habas et al., 2001) and Rho Kinase (Marlow et al., 2002). Wnt11 also signals via Frizzled and Dishevelled to activate Rac, independent of activation of Rho, in a manner necessary for convergence and extension (Habas et al., 2003). Wnt11/Frizzled signaling also regulates Cdc42 activity through a G-protein coupled mechanism, the Wnt/Ca pathway, and Cdc42 activity appears to be important in both convergence and extension (Choi and Han, 2002) and tissue

separation (Winklbauer et al., 2002). A 4-pass transmembrane protein, Strabismus, is also involved (Darken et al., 2002; Goto and Keller, 2002; Park and Moon, 2002). It has been shown that this pathway affects protrusive activity (Wallingford et al., 2000), but whether this pathway acts during radial intercalation with its effects cascading through the subsequent process of mediolateral intercalation, or whether it acts directly on mediolateral intercalation is not known.

The Biomechanics of Convergence and Extension

During convergence and extension, the tissue forms a stiff self-supporting structure that nevertheless is self-deforming and can push by actively intercalating its component parts, the cells. Converging and extending mesodermal tissues stiffen by a factor of 3 to 4 in the anterior-posterior axis and generate a pushing force of about a half micronewton (Moore et al., 1995; Moore, 1992). How can the cells exert traction on one another and shear between one another and yet maintain adhesion to one another sufficient to form a stiff beam capable of exerting a pushing force? The cell-substrate traction mechanism proposes to account for these apparently conflicting properties (see Keller et al., 2000). The key element in this mechanism is that the cells both exert traction on one another and at the same time serve as movable substrates for that traction (Fig. 6B,C). Large bipolar or monopolar protrusions exert traction on the adjacent cell bodies (red, Fig. 6A, B), and the adjacent cell bodies serve as a substratum, a substratum on which these “tractoring” protrusions do not slip substantially (Fig. 6B,D). The cells are held together in a stiff but dynamic array by many small contact points, called “stiffening” protrusions, along the elongated anterior and posterior sides of the cells (green, Fig. 6C,E). Recent high resolution confocal imaging shows that these contacts also are constantly being made and broken (Fig. 6E; Davidson, L., unpublished observations), suggesting a locally dynamic adhesion. At any instant, on the whole, a large number of these adhesions lock the cells into a rigid array, but locally, periodic breakdown of these adhesions allows local shearing of cells past one another and invasion of the intercalating, tractoring protrusions between cells. Dynamic adhesion provides an escape from the paradox of intercalation: one might think that cell adhesion must be decreased to allow rearrangement, but to form a stiff beam that can support a compression load and push, the cells must be tightly linked. The tissue can be both stiff and self-deforming by cell intercalation if adhesions are very strong while they persist, but they have a physiologically regulated turnover. It is important for this mechanism that adhesions be actively disassembled as a physiological process, rather than being broken by

mechanical stress. The predominant cadherin in the intercalating mesodermal cell population is C-cadherin, and it has been proposed that the activity of C-cadherin goes down during convergence and extension, perhaps allowing the cells to intercalate (Brieher and Gumbiner, 1994; Zhong et al., 1999). Axial and paraxial protocadherin likewise appear to have roles in convergence and extension, perhaps also by modulating adhesion (Kim et al., 1998; also see Yamamoto et al., 1998). Cdc42 also appears to regulate cell adhesion during gastrulation (Choi and Han, 2002; Winklbauer et al., 2002). It is not known if specific cadherins or protocadherins, or specific levels of their activity, are associated with the polarized tractive lamelliform protrusions or the stabilizing filiform contacts. It is also not known how the proposed dynamic adhesion process, with rapid, regulated turnover, would appear in the adhesion assays used to characterize cadherin and protocadherin function. There is much to learn about the biomechanics of cell adhesion during this process.

The extracellular matrix and cell-matrix interactions also are likely to play a mechanical role in convergence and extension. A fibronectin-containing fibrillar matrix is formed on the blastocoel roof, and it comes to lie between the somitic mesoderm and neural tissue (black lines, Fig. 6A; Nakatsuji and Johnson, 1983; Komazaki, 1988; Darribère et al., 1990; Winklbauer and Stoltz, 1995). Both the intercalating neural cells (green protrusions, Fig. 6A) and the intercalating mesodermal cells (yellow protrusions, Fig. 6A) interact with this matrix. It is not known whether the cells carry this matrix with them or use it as a substratum and leave it behind as they converge medially. Removal of the animal cap, and therefore this matrix layer, does not stop convergence and extension (Fig. 6F-G). However, the extending mesoderm may make a new matrix. Inhibiting integrin-mediated cell interactions with matrix retards blastopore closure and embryo elongation, perhaps by affecting convergence and extension (Ramos and DeSimone, 1996). Inhibition of cell-matrix interactions also blocks radial intercalation (Marsden and DeSimone, 2002). Whether the full extent of the effect of inhibiting cell-matrix interactions on convergence and extension is due to blocking radial intercalation, or whether mediolateral intercalation also depends on these interactions is not known.

A fibrillin-containing matrix is found in the notochordal-somitic mesodermal boundary (white, Fig. 6A). Fibrillin is a large extracellular matrix molecule that is a normal component of the 10 nm microfibrils, and it has multiple and complex roles in morphogenesis (Sakai et al., 1986; Ramirez et al., 1992; 1993; Zhang et al., 1994). Mutations in the human fibrillins result in Marfan syndrome congenital contractural arachnodactyly, diseases involving defects in the connective tissue, skeletal, and circulatory systems (Ramirez et al., 1992, 1993; Wang et al., 1996). Fibrillin protein

is found at the midgastrula stage in the newly formed notochordal-somitic mesodermal boundary of *Xenopus*, and expression of truncated dominant inhibitory forms of fibrillin disrupt fibril assembly, affect cell behavior, a result in poorly-extended, kinked notochords, suggesting an effect on the stiffness of the extending notochord (Skoglund, P., unpublished work).

Regardless of whether or not these and other matrix components are active participants in convergent extension, extracellular matrix is a major contributor to the mechanical properties of tissues, even in early embryos (Davidson et al., 1999). Therefore, it must be deformed, remodeled, ignored and left behind, or destroyed in order for the tissues to undergo convergence and extension. As with adhesion, the biomechanics of extracellular matrix remains an important and relatively uncharacterized player in convergence and extension.

The cause of the developmentally regulated 3–4 fold stiffening of the extending mesoderm is not known. Cells do not rearrange during the compression or the relaxation phase of the compression-stress relaxation test, regardless of whether it is done before or after the stiffening (Moore et al., 1995). Therefore, the increased stiffness is not due solely to greater resistance of the cells to rearrangement. Increased stiffness of the cells themselves, presumably by cytoskeletal changes, or in the matrix between the cells, or both, could account for the increased stiffness. Increased adhesion could occur as well but this does not seem to be a deciding factor, as cell-cell or cell-matrix adhesion appears to be stronger than the cytoskeleton and restricts cell rearrangements under an externally applied load both before and after the stiffening. Adhesion is always strong enough to prevent cell rearrangement, and therefore loads applied to tissue are transferred through adhesions to the individual cells, resulting in their deformation rather than their rearrangement. Adhesion and the cytoskeleton may change together, in a mutual interdependence; as adhesion receptors are increased in number or activity, the adhesion-linked cytoskeleton may be enhanced by a similar amount, but with the latter being the weak link. The role of cell-cell and cell-matrix interactions in stiffening the extending tissues remains a major unresolved issue in understanding the biomechanics of convergence and extension by cell intercalation.

The mediolaterally polarized cell protrusive activity may be enhanced biomechanically by mediolateral tension in a positive biomechanical feed-back loop. The large medial and lateral lamelliform protrusions appear to exert traction on adjacent cells and generate tension in the mediolateral axis. Cell behavior (Shih and Keller, 1992a) and microsurgical manipulation of the embryo (Keller, 1984; Keller et al., 1992c) suggests that the elongated anterior and posterior sides of the cells are under mediolateral tension, which may sup-

press lamelliform tractive protrusions along these sides and enhance polarity. Stretching cultured mammalian cells results in suppression of lamellipodia along the sides elongated by the stretching, and results in increased lamellipodial activity at the short ends (Katsumi et al., 2002; also see Lee et al., 2002). This effect is due to inhibition of the activity of the small GTPase Rac, probably mediated by regulation of GAP activity (Katsumi et al., 2002). Such a mechanism would have the potential of making the bipolar lamelliform protrusive activity of the intercalating cells self-reinforcing; an initial polarization of lamellipodia to medial and lateral ends would stretch the cells mediolaterally, and the tension in the long sides would feed back to suppress lamelliform protrusive activity there and enhance the initial polarization.

The stiff, extending axial and paraxial mesoderm, along with the vegetal endoderm, dominates the shape of the early embryo, and thus forms an embryonic “skeleton”. Axial and paraxial mesoderm extend as a stiff beam, which elongates the anterior-posterior axis of the embryo, independent of traction on an external substrate. When their normal substrate, the blastocoel roof, is removed, they extend as they would in the intact embryo (Fig. 6F-G; Keller and Jansa, 1992). When the blastocoel roof is offered as a substrate only on one side, the other side, or both sides (Fig. 6H), the extending axis does not deviate from its normal course, but instead the substrate is pulled on top of the extending axial and paraxial mesoderm (Fig. 6H-I; Keller and Jansa, 1992). The vegetal endoderm also serves as part of this embryonic skeleton. The axial mesoderm and paraxial mesoderm are anchored to the vegetal endoderm where the lateral paraxial mesoderm meets the vegetal endoderm, an anchorage perhaps mediated in part by the bottle cells. Convergence of the axial and paraxial mesoderm during gastrulation and neurulation pulls these tissues closer to and across the vegetal endoderm. Then, during tailbud stages, the vegetal endoderm actively extends (see Drawbridge and Steinberg, 2000; Larkin and Danilchik, 1999) through a cell intercalation mediated mechanism. Although the axial and paraxial mesoderm in the blastocoel roofless embryos can behave independent of its substratum, the overlying neural plate, it is normally reinforced in its extension by the coordinate, parallel extension of the posterior neural plate. Inhibition of neural but not mesodermal convergent extension with dominant inhibitory Dishevelled results in arch-backed embryos, which argues that both neural and mesodermal components are actively elongating the embryo independently (Wallingford and Harland, 2001).

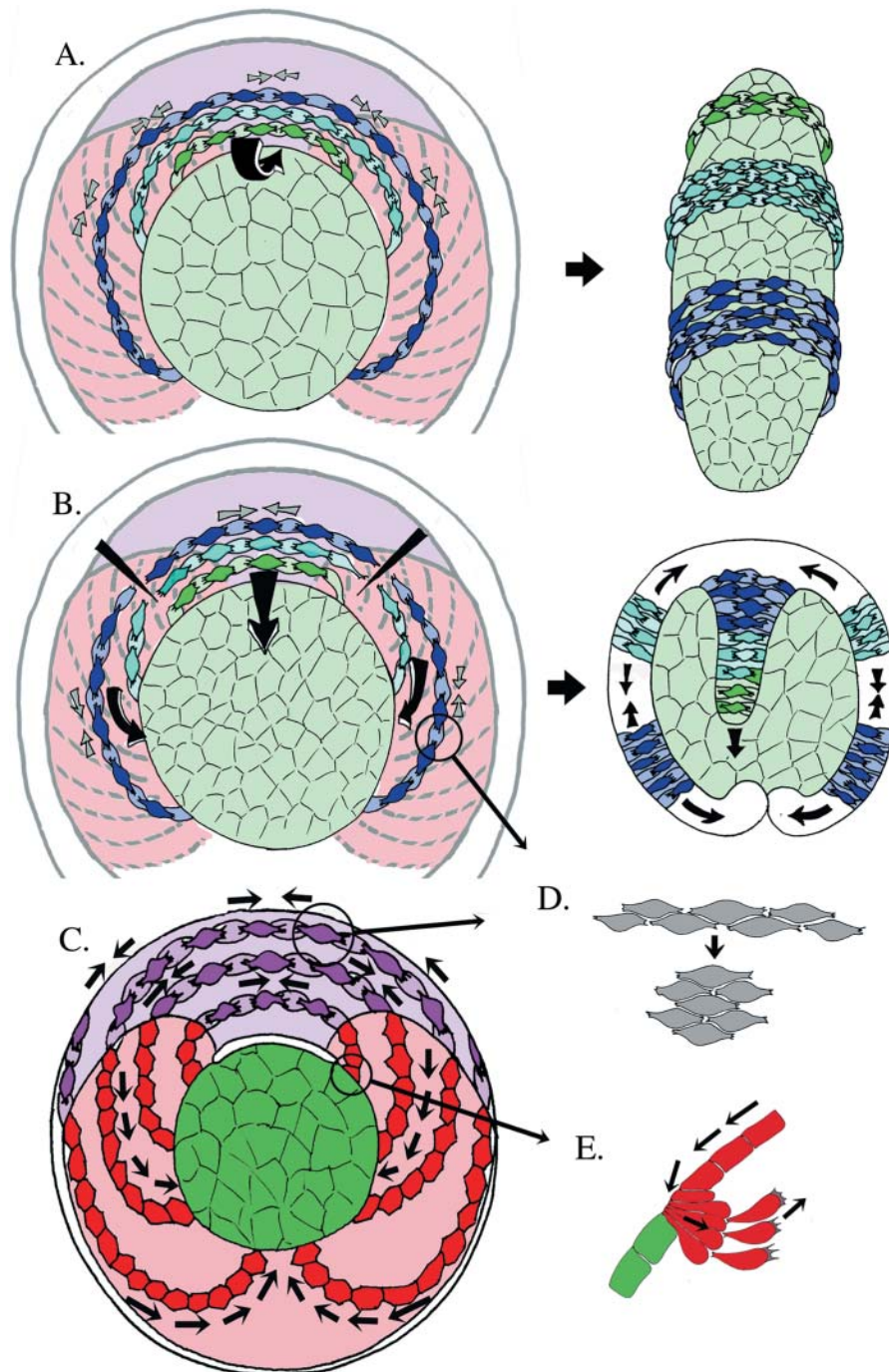
The hoop stress developed by the progressive expression of mediolateral intercalation behavior (MIB) drives involution and the asymmetric blastopore closure that is essential for the formation of a dorsally-located, elongate archenteron. Expression of MIB is

progressive, beginning anteriorly and laterally in the axial/paraxial mesoderm, and spreading medially and posteriorly (Shih and Keller, 1992b; Domingo and Keller, 1995; Lane and Keller, 1997). This progression and its mechanical consequences can be visualized as a series of arcs spanning the dorsal lip with each end anchored at the vegetal endoderm (Fig. 7A). As successive arcs are “converged” or shortened by MIB (gray arrows, Fig. 7A, left panel), hoop stress is generated across the dorsal lip, which in turn produces a perpendicular, tensile force that tends to pull the IMZ over the lip and turn it outside in (*involution*; curved arrow, Fig. 7A, left panel). The arcs, which are narrower and longer after convergent extension, involute and span the archenteron roof (Fig. 7A, right panel). The progression of MIB is, of course, continuous, and the discrete arcs in the diagrams are only used to visualize the process.

If the hoop stress across the dorsal lip is broken microsurgically (Schechtman, 1942; Keller, 1984), the secondary, perpendicular force that contributes to involution is lost, and the isolated dorsal portion of the IMZ extends straight out across the yolk plug but it does not involute (Fig. 7B,C). The isolated lateral parts of the IMZ also do not involute but converge against the vegetal endoderm and extend parallel to its edge; as a result the blastopore reopens and expands to form a “ring embryo” (Fig. 7B, C). In this instance, as with the bottle cells (cf. p. 173), the geometric and biomechanical context of a local cell behavior is important in determining the specificity of its macroscopic result. The progressivity of MIB is important in determining the outcome of local cell behavior. For example, lithium-induced dorsalization of embryos results in simultaneous convergence and extension of all parts of the IMZ, rather than the dorsally initiated, progressive pattern of normal embryos. As a result, “proboscis” embryos are often produced; these have axial mesoderm that does not involute but extends straight out (Kao and Elinson, 1988). A local cell behavior (*MIB*) acts in a global context (*the laterally anchored arc-pattern*) that transforms the effect of the local behavior into a specific, global morphogenic result (*involution*). In this situation, the specificity of morphogenesis lies in distributed information that is integrated by the biomechanical and geometric properties of the tissues. For example, MIB can produce convergence as a primary product. But the amount of this convergence that is channeled into tissue extension and how much into tissue thickening is related to another piece of morphogenic “information” – the resistance of the tissue to thickening, which in turn appears to be a function of continued tendency for radial intercalation (Keller and Davidson, 2003). And convergence contributes to blastopore closure and involution, but only if the process of convergence is exercised progressively and only if the ends of the

converging arcs of tissue are anchored at the edges of the vegetal endoderm. If this additional information is not present, first in the form of a progressive patterning and second in the form of a mechanical linkage, the tissue normally involuting will extend straight out and blastopore closure and involution will fail (see

Keller, 1984; Keller et al. 1992, 2000). Therefore, genetic regulation of the specificity in this system will involve genes encoding the proteins regulating the local process of MIB, and those encoding the proteins that establish the progressive patterning of the MIB, specifically, the arc-like, progressive pattern of expres-



sion, and finally those genes encoding the proteins that impart the mechanical properties of the tissue that transmit the hoop stress and pattern the lateral anchorage points.

Comparative Biomechanics of Amphibian Gastrulation

Urodele Gastrulation Uses A Different Cell Behavior To Generate The Same Mechanical Forces

Morphogenesis at the blastopore of a typical anuran amphibian, such as *Xenopus laevis* resembles that at the blastopore of a typical urodele amphibian, such as *Ambystoma mexicanum* or *Taricha granulosa*. But this morphogenesis is driven by very different underlying cell behaviors, which nevertheless produce the same biomechanical result – a hoop stress arcing across the dorsal lip. Analysis of whole embryos and dorsal explants of *Ambystoma mexicanum* and *Taricha granulosa* (Shook et al., 2002) shows that the notochordal region

of the dorsal IMZ undergoes arc-shortening (convergence), as well as extension, during gastrulation in a fashion similar to that shown in *Xenopus* explants of the same type, most likely by cell intercalation (magenta cells, Fig. 7C; Fig. 7D). But unlike *Xenopus*, these urodeles have considerably more prospective somitic mesoderm in the epithelial, surface layer of the preinvolution, lateral IMZ (red cells, Fig. 7C). Whereas *Xenopus* generates arcs of hoop stress by cell intercalation throughout the axial and paraxial mesoderm, these epithelial prospective somitic cells of urodeles generate arc-shortening by ingressing from the epithelial sheet. They first constrict their apices, which shortens the “arc”, and then leave the surface layer by ingression at the vegetal (endodermal) edge of the field of somitic tissue, further shortening the arc. They subsequently undergo an epithelial-mesenchymal transition (EMT) (Fig. 7C,E). This behavior is very similar to that of cells ingressing through the primitive streak of avian and mammalian embryos (cf. Fig. 7E and 10B).

The urodele uses the same *mechanical principle* of arc-shortening across the dorsal lip of the blastopore as *Xenopus*, but uses a different *cell behavior* in the somitic regions (apical constriction and ingression at one edge; Shook et al., 2002). The expression of apical constriction and ingression in urodeles and the expression of MIB in *Xenopus* progress across the IMZ in nearly identical patterns (cf. Shih and Keller, 1992b; and Shook et al., 2002). This progression probably reflects the activity of a conserved, underlying pattern generator that uses different downstream outputs (cell behaviors) to create similar patterns of stress in the two types of amphibians.

Fig. 7 Cell intercalation and convergence generate hoop stresses arcing across the dorsal lip of the blastopore, which are thought to function in driving involution and closing the blastopore asymmetrically over the ventral vegetal region, thereby producing the elongated archenteron. In a vegetal view of the early *Xenopus* gastrula (left panel, A), the bipolar, mediolaterally intercalating mesodermal cells can be visualized as chains of cells that form a series of arcs, anchored at both ends near the vegetal endoderm (light green cells, A). Local cell intercalation shortens these arcs (gray arrows, A; D), beginning with the presumptive most anterior (green), and progressing to the midbody level (light blue) and finally posteriorly (dark blue). As each arc is shortened by mediolateral cell intercalation, it tends to pull the IMZ over the lip (black arrow, A) and turn it outside in (involution). Each arc eventually shortens mediolaterally (converges) and becomes thicker antero-posteriorly (extends), and after involution, spans the roof of the archenteron transversely (right panel, A). If these arcs are broken experimentally at the onset of gastrulation (left panel, B), convergence and extension of the isolated middorsal part and the laterally parts occur (right panel, B), but involution fails and the blastopore does not close because of lack of continuity of the arcs (right panel, B). In the urodele gastrula, arc-shortening appears to occur as a result of two cell behaviors, rather than one (C-E). In the notochord, convergence (and extension) and arc-shortening occurs as in *Xenopus*, probably by cell intercalation (magenta area, C;D). In contrast, arc-shortening occurs in the large area of superficial epithelial presumptive somitic mesoderm (pink area, C) by removing the cells from the surface layer (right panel, C). They undergo an apical contraction and ingress into the deep region (E). Note that these behaviors are represented as separated arcs for illustrative purposes only; in fact, both the mediolateral cell intercalation behavior in *Xenopus* and ingression in the urodele occur as continuous, progressive processes. (Based on Schechtman, 1942; Keller, 1981, 1984; Shih and Keller, 1992b; Domingo and Keller, 1995; Shook et al., 2002). Illustrations A and B are modified from Keller et al. (2000).

Biomechanics of Gastrulation of Large, Yolky Eggs

Most amniote eggs with larger proportions of yolk also have proportionately larger prospective vegetal endodermal regions. Therefore their IMZs, which are typically located at the upper edge of the vegetal endoderm, are found nearer the animal pole (Fig. 8A; see Elinson and Beckham, 2002) and must cover a much larger area of vegetal endoderm before closing the blastopore. How is this accomplished? Of the different morphogenic machines described above, some of them have the potential to work well in closing over the endoderm of such eggs and others do not. For example, application of the convergence-generated hoop stress in the yolky sturgeon egg relatively early in gastrulation, as in *Xenopus*, would result in squeezing of the equator of the yolk rather than rolling the IMZ inside (Fig. 8B-C; see Bolker, 1993). In fact, the IMZ of the sturgeon, a chondrosteian fish, moves very far vegetally (Fig. 8D-E) before converging and constricting and closing the blastopore (Fig. 8E-F; Bolker, 1993). This extended vegetal movement has been attributed to a very strong thinning and epiboly of the animal cap, perhaps by an exceptionally robust phase of

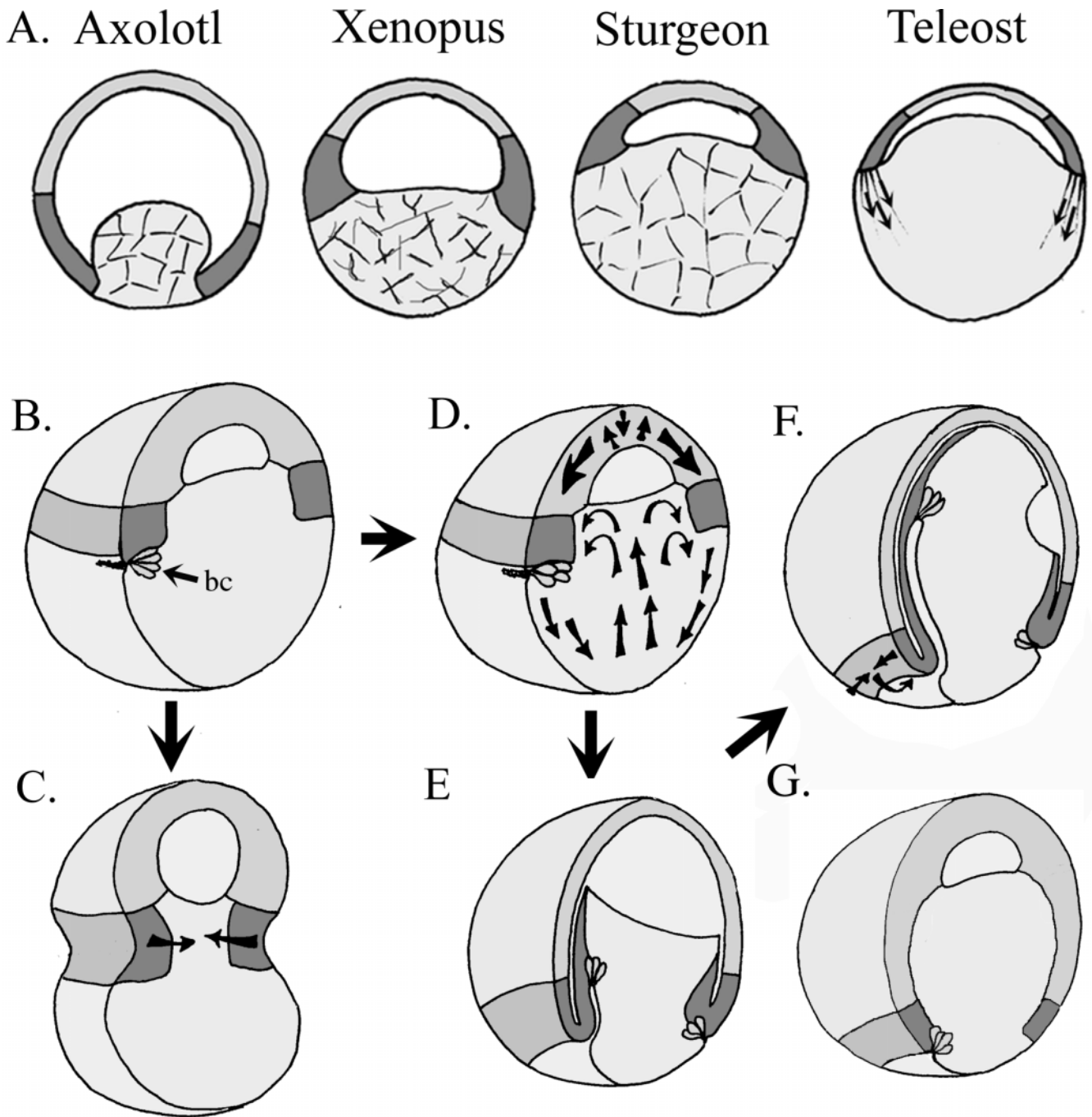


Fig. 8 Diagrams illustrate increasing amounts of yolk in eggs of the axolotl, *Xenopus*, the sturgeon, and the teleost fish, with the result that the IMZ (dark gray) is displaced progressively farther animally (A). Mechanisms of using convergence and extension to enclose the yolky vegetal endoderm during gastrulation must be modified to be successful in the face of larger amounts of yolk. If convergence occurs in IMZs located at or above the equator (B), it will constrict the embryo and the embryo will fail to gastrulate

(enclose the yolky endoderm; C; e.g. Bolker, 1993). Instead, embryos of this type, represented by the sturgeon, undergo an extensive epiboly (see Bolker, 1993) and probably very strong Winklbauer vegetal rotation movements that result in movement of the IMZ vegetally, below the equator (D-E), prior to convergence and extension (E-F). The alternate mechanism for enclosing the yolk is to uncouple the formation of the IMZ from yolk deposition.

radial intercalation of the originally very thick animal cap (upper black arrows, Fig. 8D; Bolker, 1993). But this does not account for the decreasing area of vegetal

endoderm lying below and in the path of the movement of the IMZ. It is unlikely that epiboly is forceful enough to compress the vegetal endoderm ahead of the IMZ. It

is more likely that the vegetal rotation movement described in *Xenopus* (p. 178) occurs very strongly in the sturgeon and moves the IMZ far vegetally and into position for convergence, extension, and blastopore closure (Fig. 8D-E).

Our prediction is that large yolky eggs having their IMZs near the animal pole will use vegetal endodermal rotation to move the IMZ vegetally prior to convergence and extension. These eggs should also have thick, multi-tiered animal caps and IMZs that undergo extensive radial intercalation (extension without convergence) prior to undergoing convergent extension. *Eleutherodactylus coqui*, a large-egged, direct developing anuran amphibian (see Elinson, 1987; Elinson et al., 1990) may be similar to the sturgeon in this regard. *Gastrotheca riobambae*, an egg-brooding, marsupial frog with a very large yolky egg, also appears to use such a strategy. It forms a disc-like blastoderm very near the vegetal pole, despite having a large yolky egg, and it gastrulates to form a small symmetrical archenteron near the vegetal pole. It does so without axial extension or notochord formation, both of which are delayed until later stages (del Pino and Elinson, 1983; Elinson and del Pino, 1985; del Pino, 1996). This species is described as having strong epibolic and embolic movements, suggesting that the IMZ may originate animally and move vegetally, although there is no good description of this process. As an alternative strategy to moving the IMZ vegetally, it is possible that some species with large yolky eggs have evolved a mechanism to uncouple yolk deposition from the IMZ position. These species would form the IMZ near the vegetal pole despite having a lot of yolk (Fig. 8G). Instead of modulating the morphogenic movements, this strategy would involve modulation of early patterning of the egg, perhaps by localization of determinants important in specifying the IMZ and the site of the blastopore (bottle cells) such as VegT for example (see Kurth and Hausen, 2000; Shinagawa and Kobayashi, 2000; Elinson and Bockham, 2002). No species having this strategy has been described thus far.

Comparison of urodele (*Ambystoma* and *Taricha*) and anuran (*Xenopus*) gastrulation suggests that comparative analyses of gastrulation should focus on the mechanisms of morphogenic machines and the design and the sufficiency of biomechanical strategies. *Xenopus* and *Ambystoma* have roughly the same type of egg, with a relatively vegetally positioned IMZ, and therefore a similar biomechanical strategy, convergent arcs of cells generating hoop stress, works for both. However, the cellular behaviors used to generate this shared biomechanical strategy are not conserved, the urodeles using two cell behaviors (intercalation and ingression) to accomplish what *Xenopus* accomplishes with one (intercalation). In eggs having more yolky endoderm, these same cell biological and biomechanical mechanisms may be modified in their use (used in new ways, in different degrees, or with altered timing), or abandoned in favor of new

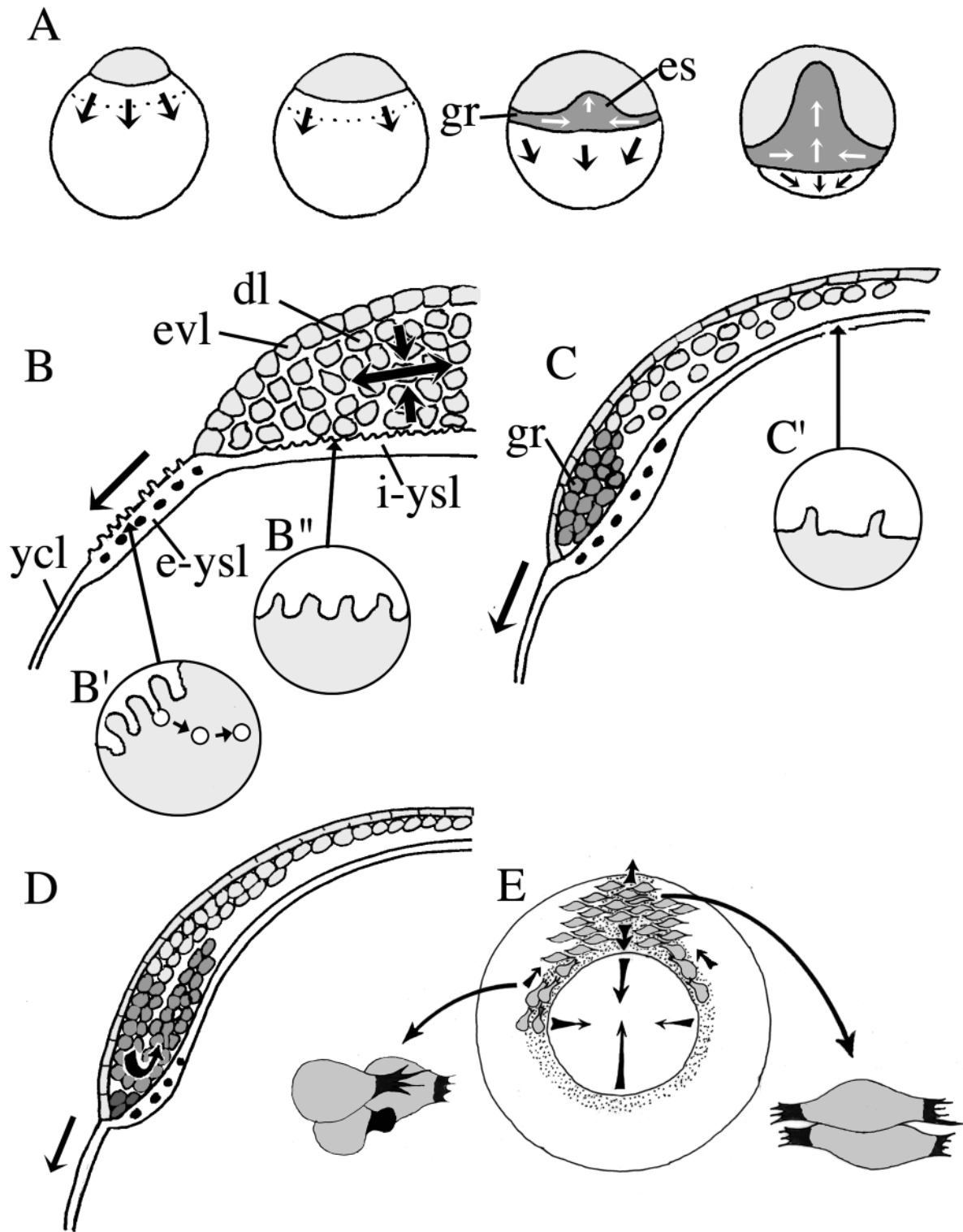
mechanisms. The sturgeon may be an example of modified use. The convergent extension movements used early in *Xenopus* and *Ambystoma* are also used in the sturgeon, but they appear to be delayed until the IMZ is moved below the equator, probably by the same processes of vegetal rotation and epiboly seen in *Xenopus*, but exaggerated in duration and extent. An example of abandonment of mechanisms and substitution of new ones in the face of even larger amounts of uncleaved yolk, and an IMZ located far animally, may occur in the teleost, which brings a new player to the scene— an extraembryonic motor for epiboly, and perhaps extension (arrows, Fig. 8A, far right).

Teleost Gastrulation

Cell Movements

The teleost egg of the type seen in *Danio rerio*, the zebrafish, and *Fundulus heteroclitus*, the killifish, has a very large amount of uncleaved yolk and a blastoderm, including the IMZ, initially above the equator (Fig. 8A, far right). The egg undergoes partial cleavage to form a blastula consisting of an enveloping layer (EVL) epithelium, attached at its edge to the large uncleaved yolk cell, and several to many layers of deep, non-epithelial cells (Fig. 9A, B). The multinucleate *yolk syncytial layer* (YSL) lies adjacent to the blastoderm and initially extends beyond its margins as the *external YSL* (E-YSL; Fig. 9B; see Betchaku and Trinkaus, 1978). The remaining, anucleate superficial part of the yolk cell beyond the E-YSL is the *yolk cytoplasmic layer* (YCL; Fig. 9B).

Beginning in the blastula stage and continuing through gastrulation, the EVL undergoes epiboly, expanding over the external YSL, then over the YCL, and eventually enclosing the yolk entirely (Fig. 9A, B). As this occurs, several layers of deep mesenchymal cells likewise move vegetally with the EVL, and they undergo radial intercalation to produce a thinner array of greater area as they do so (Kimmel and Law, 1985; Warga and Kimmel, 1990; Concha and Adams, 1998; black arrows, Fig. 9B). As radial intercalation occurs, the yolk cell “domes” upward under the thinning and spreading blastoderm. At 50 to 70 percent of epiboly, gastrulation begins and the deep cells of the embryonic shield/germ ring involute (Warga and Kimmel, 1990; Thorogood and Wood, 1987) or ingress (Shih and Fraser, 1995) at the margin of the blastoderm, usually referred to as the epiblast, and move deep where they form the hypoblast (Fig. 9C, D). Internalized cells form the *germ ring* (gr, Fig. 9C) beneath the vegetal edge of the EVL. The germ ring thickens on the future dorsal side to form the *embryonic shield* (es, Fig. 9A), the equivalent of the dorsal lip of the blastopore of the amphibian. These dorsal tissues converge and extend to form an elongated, nar-



row axis underlying the neural plate in a manner at least superficially similar to the movements described above for amphibians (Fig. 9E). Laterally, however, the movements differ from those in amphibians. The deep cells move toward the dorsal midline, as a migratory “stream” of cells with increasing rates of translocation

as they approach the midline (Trinka et al., 1992; Trinka, 1998; Sepich et al., 2000; Myers et al., 2002; Fig. 9E). On reaching the paraxial region, they participate in convergence and extension of the paraxial tissue and become elongated and aligned mediolaterally (see Topczewski et al., 2001 and Jessen et al., 2002). They re-

Fig. 9 Diagrams illustrate teleost gastrulation in dorsal view (A). The blastoderm (gray) undergoes epiboly (black arrows, A) and eventually encloses the entire uncleaved yolk cell. As epiboly occurs, the germ ring (gr) forms at the vegetal margin of the blastoderm, and cells in the germ ring converge dorsally (white medially directed arrows) as a thickened region, the embryonic shield (es) forms there. Cells in the epiblast of the embryonic shield involute or ingress to form the dorsal hypoblast, and the two layers undergo convergence and extension (white arrows, A). In midsagittal view (B), the early blastoderm consists of an outer epithelial sheet, the enveloping layer (evl), and a multilayered deep layer (dl). The blastoderm is attached to a part of the large uncleaved yolk cell called the yolk syncytial layer (ysl). The internal ysl (I-ysl) lies beneath the blastoderm, and the external ysl (e-ysl) lies just outside the blastoderm. Peripheral to the e-ysl is the yolk cytoplasmic layer (ycl), a thin layer of cytoplasm surrounding the large volume of yolk. The blastoderm is attached tightly at its margin by a junctional complex between the marginal cells of the evl and the ysl (see Betchaku and Trinkaus, 1978). In epiboly, the blastoderm is pulled vegetally by virtue of its attachment to the ysl/ycl (arrow, B). The membrane ahead of the advancing evl in the e-ysl and ycl is endocytosed (B') and the I-ysl expands by flattening of microfolds (cf. B'' and C'). The deep cells undergo radial intercalation and spread to form a thinner array of larger area, the epiblast, during epiboly (B-C; see Warga and Kimmel, 1990). As epiboly proceeds, cells of the epiblast involute or ingress to form the hypoblast (D; see Shih and Fraser, 1995; Warga and Kimmel, 1990). During gastrulation, cells in the lateral region of the germ ring migrate dorsally toward the embryonic shield using a polarized protrusive activity (E, left inset) whereas those in the embryonic shield and those joining the embryonic shield from the germ ring undergo convergence and extension, showing an mediolaterally elongate morphology similar to that seen in the amphibian (E, right inset; see Marlow et al. 2002).

semble their counterparts in the frog in this regard, presumably as a result of a similar MIB, although their protrusive activity has not yet been described.

Epiboly

Most of what is known about the biomechanics of teleost epiboly is due to elegant and classic studies by J.P. Trinkaus (1951; 1984b). He showed that when the blastoderm is detached from the YSL during epiboly, the YSL continues to move vegetally whereas the blastoderm retracts initially and then fails to spread on its own. This behavior suggests that forces driving epiboly are not generated within the blastoderm but in the E-YSL/YCL. The EVL is attached to the external YSL at its margins with a junctional complex that is contiguous with a well-developed actin cytoskeleton within the YSL/YCL, which is thought to be involved in towing the EVL vegetally during epiboly (Betchaku and Trinkaus, 1978). Microtubules have also been implicated in epiboly (Strahle and Jesuthasan, 1993; Solnica-Krezel, 1994). Betchaku and Trinkaus (1986) also showed that as the EVL advances in epiboly, the membrane ahead of it, first that of the E-YSL, followed by that of the YCL, is internalized into vesicles, while the area of the internal YSL

expands with the spreading of numerous microvilli that originally carpeted this surface (Fig. 9B, C, B'; cf. B'' and C').

The deep cells also move vegetally with the EVL/YSL during epiboly but the biomechanics of this movement is not understood. As the EVL and I-YSL are stretched during epiboly, the deep cells could move passively by virtue of their attachment to these layers. Alternatively, they could actively crawl vegetally into the space made available by the movement of the EVL/YSL. Thirdly, the “doming” up of the yolk cell against the under side of the deep blastomeres could force them to undergo passive radial intercalation and spreading. The fourth possibility is that the radial intercalation of the deep cells described by Kimmel and associates (Kimmel and Law, 1985; Warga and Kimmel, 1990) is an active, force-producing radial intercalation that is independent of the yolk cell-driven epiboly of the EVL and the doming of the yolk cell, but normally coordinated with these processes. Failure of the blastoderms detached from the YSL/YCL to spread on their own in Trinkaus's experiments does not rule out an active spreading, as the conditions may not have been sufficient to allow normal behavior. Or active radial intercalation of deep cells might occur but does not generate sufficient force to stretch a passive EVL/I-YSL by itself, without assistance from the extraembryonic yolk cell machine. A cell biological analysis of mutants should illuminate this issue. In the zebrafish mutant, *half-baked*, epiboly is incomplete, with the deep blastomeres stopping part way through the process, but the EVL-YSL continues to move ahead (Kane et al., 1996). This result shows that deep and superficial cell epiboly can be affected differentially, and offers the opportunity on further study to resolve some of these issues. Does *half-baked* knock out the attachment of the deep cells to the overlying and underlying cell layers whose epiboly is unaffected by the mutation? Or does it knock out an active motility that moves these cells actively along behind the advancing EVL/YSL? Or does it knock out an active pushing process driven by an active radial intercalation of the type seen in the frog?

Convergence and Extension

Convergence and extension movements of the axial and paraxial tissues have been described in the zebrafish (Warga and Kimmel, 1990) and in *Fundulus* (Trinkaus et al., 1992; also see Oppenheimer, 1959). Glickman and associates (2003) show that in wild type embryos, the axial (notochordal) domain undergoes convergence and extension by mediolateral cell intercalation with an efficient translation of convergence into extension. The paraxial region also shows convergence and extension, but with less efficient transformation of convergence into extension, the extension being about three-fold less

than in the notochord, perhaps due to greater thickening in the paraxial region. The faster extending notochord shears posteriorly with respect to the slower extending paraxial tissue, paralleling the faster and more efficient extension and posterior shearing of the notochord with respect to the slower, less efficiently extending paraxial mesoderm in frogs (Wilson et al., 1989).

Cells in the lateral region of the teleost germ ring migrate directionally toward the embryonic shield, using both blebbing and lamelliform-filiform protrusive activity, perhaps using the overlying EVL or underlying YSL, or even one another, as substrates (Trinka et al., 1992; Trinka, 1998; Sepich et al., 2000). These convergence movements constitute a directed migration and the cells do not rearrange in an organized fashion to produce an active extension in the lateral regions of the embryo in either the zebrafish or *Fundulus*. Convergence is accompanied by increasing extension as the lateral cells approach and enter the paraxial region in the zebrafish (see Myer et al., 2002), and the cells take on the mediolaterally elongate, aligned morphology characteristic of intercalating cells (Topczewski et al., 2001; Jessen et al., 2002; Glickman et al., 2002), suggesting that they undergo the same type of MIB seen in the frog, although the protrusive activity underlying cell intercalation has not been described in the zebrafish.

In the lateral region of directed migration, the biomechanical strategy of the teleost appears to differ considerably from that of *Xenopus*. The “arc shortening” that pulls dorsal tissues toward ventral anchor points at the periphery of the vegetal endoderm in *Xenopus* (see Fig. 7A-B) does not occur. Instead, the lateral cells of the teleost converge on the dorsal midline by a directed migration, vacating ventral regions, rather than pulling the dorsal tissues ventrally (Fig. 9E). It is noteworthy that this feeding of ventrolateral cells into the posterior paraxial region is not unique to the fish but also occurs in *Xenopus* but by a different mechanism. In *Xenopus*, the late involuting presumptive posterior somitic cells of the ventral IMZ form a thick collar just inside the ventral blastoporal lip after blastopore closure. As the fast-extending notochord shears posteriorly with respect to the anterior paraxial mesoderm, it pushes the blastopore posteriorly into this collar, and the cells of the collar stream around both sides of the notochord and join the slower extending paraxial mesoderm where they add to its length by accretion and by convergent extension (see Wilson et al., 1989; Keller et al., 1989).

The above discussion would lead one to believe that convergence generates no hoop-stress in the lateral and ventral germ ring of the teleost. However, there is evidence for circumferential tension parallel to the edge of the blastoderm, although perhaps not necessarily generated by the deep cells in the germ ring. Again, in the mutant half-baked, epiboly of the deep cells is halted at about 50% epiboly, while the EVL/YSL moves ahead, but a constriction of the embryo occurs at the site of the

deep cell germ ring (see Kane et al., 1996). Exactly when and how this constriction forms, and whether it is due solely to forces developed in the deep germ ring is not known.

The margin of the EVL and YSL decreases in circumference from 50% epiboly to its completion. Constriction forces at this margin would tend to pull the blastoderm vegetally and aid epiboly. The marginal EVL cells, as well as submarginal cells, intercalate mediolaterally to accommodate the decrease in circumference as epiboly passes over the equator and approaches the vegetal pole (Keller and Trinka, 1987). Although there is no evidence that this is an active, force-producing event, these cells do have many interlacing protrusions at their margins, which might suggest an active movement (Keller and J. P. Trinka, 1987; Zalik et al., 1999).

Is convergent extension of axial and paraxial mesoderm driven by forces generated within these tissues, or is it entirely or partly driven by external forces? Two potential external sources of force can be identified. Until the end of epiboly, the yolk cell epibolic motor could passively stretch (extend) the axial and paraxial mesoderm. The underlying I-YSL undergoes convergent extension in parallel with the overlying blastoderm, based on movement of the I-YSL nuclei, and this movement could contribute to convergent extension of the overlying blastoderm if these tissue were attached to the I-YSL (D’Amico and Cooper, 2001). Glickman and associates (2002) show, in their analysis of the *no tail (ntl)* mutant, that extension occurs in absence of the organized MIB that drives mediolateral cell intercalation, and thus convergence, and they suggest that there is another machine for extension. They observe that cells appear to initiate but cannot execute organized MIB in *ntl*, possibly because of lack of traction on adjacent cells. Nevertheless, the cells rearrange chaotically and the notochord extends, probably passively, due to towing by external forces. Other observations suggest that there is an active component to teleost extension but that it may not be sufficient for normal morphogenesis without assistance from one or more of these external sources of force. In *half-baked*, the epiboly of the deep cells is halted while the EVL/YSL advances ahead, and within this population of deep cells, a notochord develops, but it is bent and buckled (Kane et al., 1996). This suggests that the notochord actively extends but is not stiff enough to extend straight without buckling in an environment that does not provide appropriate external forces. After treating zebrafish embryos with a teratogen that blocks epiboly of the EVL/YSL, Bauman and Sanders (1984) observed kinked and bent notochords, and extension of the notochord beyond the boundaries of the halted EVL/YSL into the large yolk cell. This again suggests that the teleost notochord is an active extender but is prone to buckling when unassisted. Finally, the development of bent notochords in explants of zebrafish dorsal tissues (Laale, 1982) supports this view as well. These

observations suggest that active convergent extension occurs in the teleost but that external forces are provided by the epibolic advance of the EVL/YSL and the I-YSL extraembryonic extension motor (D'Amico and Cooper, 2001). These external forces could explain the paradoxical uncoupling of extension from convergence in mutants in which the latter fails but the first continues (Glickman et al., 2002; Marlow et al., 2002).

In both frogs and zebrafish, axial convergent extension appears dependent on interactions with paraxial tissue. In the frog, the notochord normally extends faster and shears posteriorly with respect to the notochord (see Wilson et al., 1989; Keller et al., 1989), but it will not extend when isolated from the slower extending somitic mesoderm (Wilson et al., 1989). In contrast, the somitic mesoderm actively extends, even when separated from the notochord (Wilson et al., 1989). A similar dependence of notochord extension on somitic mesoderm in the teleost was uncovered by Heisenberg and others (2000), in their analysis of the mutant *Silberblick* (Wnt 11). They found that the paraxial (somatic) mesoderm has an effect on axial (notochordal) tissue that is necessary for its extension, and this effect is dependent on Wnt 11 activity in the somitic mesoderm, but Wnt 11 activity in the notochord is not required.

The fact that convergence and/or extension is blocked by mutations in several genes expressed in the paraxial mesoderm and results in abnormal morphology and intercalation of cells in the paraxial mesoderm suggests that paraxial mesoderm is a major force-producing tissue, in addition to its support of axial extension. These include *spadetail* (Molven et al., 1990) and mutations of the planar cell polarity genes *silberblick* (*Wnt11*) of the planar cell polarity pathway (Heisenberg et al., 2000), *trilobite* (*Strabismus/Van Gogh*; Jessen et al., 2002), and a glypican modulator of non-canonical Wnt signaling, *Knypek* (Topczewski et al., 2001), all of which show failure of the typical mediolateral cell elongation and alignment to one degree or another, and disrupted cell intercalation patterns.

In summary, major tasks in understanding the biomechanics of fish convergence and extension include determining the relative contribution of internally and externally generated forces. Also, what are the external machines? The prime candidates are the extraembryonic epibolic machine and the autonomous extension of the underlying I-YSL (D'Amico and Cooper, 2001). Finally, are the forces generated by mediolaterally polarized protrusive activity and mutual cell traction, similar to what is thought to occur in the frog?

Mesendoderm Migration

A major unanswered question is whether or not there is an equivalent to mesendoderm migration and the generation of shear forces at the interface of the epiblast and

hypoblast by this mechanism in the teleost. What is the nature of the interface between the epiblast and the hypoblast? The equivalent boundary in *Xenopus* is defined at the gastrula stage by a fibronectin-containing matrix that serves as a substrate for mesendoderm migration (see p. 179). Is there such a matrix layer in fish? Does this interface in the teleost show the same sort of Frizzled-mediated tissue separation behavior seen in *Xenopus* (Winklbaauer et al., 2001; Wacker et al., 2000; Winklbaauer and Keller, 1996)? Do the hypoblast cells show an animally-directed migration on the undersurface of the epiblast, or on the outer surface of the I-YSL, of the type shown by mesendoderm cells on the blastocoel roof in *Xenopus* (see p. 179)? All of these interfaces are potential sites of force-generation or mechanical linkage.

Amniote Gastrulation

Cell Movements of Chick Gastrulation

We will focus largely on the mechanics of two major movements in chick gastrulation: extension of the primitive streak and regression of Hensen's node. Before these movements begin, the egg of the typical avian undergoes partial cleavage to form a circular blastoderm overlying a subgerminal cavity on one side of a very large, uncleaved yolk cell. At the onset of primitive streak formation, the blastoderm consists of an outer layer, the *epiblast*, and a second, deeper extraembryonic layer, the *secondary hypoblast*, both overlying a central, relatively clear area (the *area pellucida*) above the germinal cavity, bounded peripherally by an opaque area (the *area opaca*) attached to the yolk cell at its margins (Fig. 10A,B). The perimeter of the blastoderm is called the *marginal belt*.

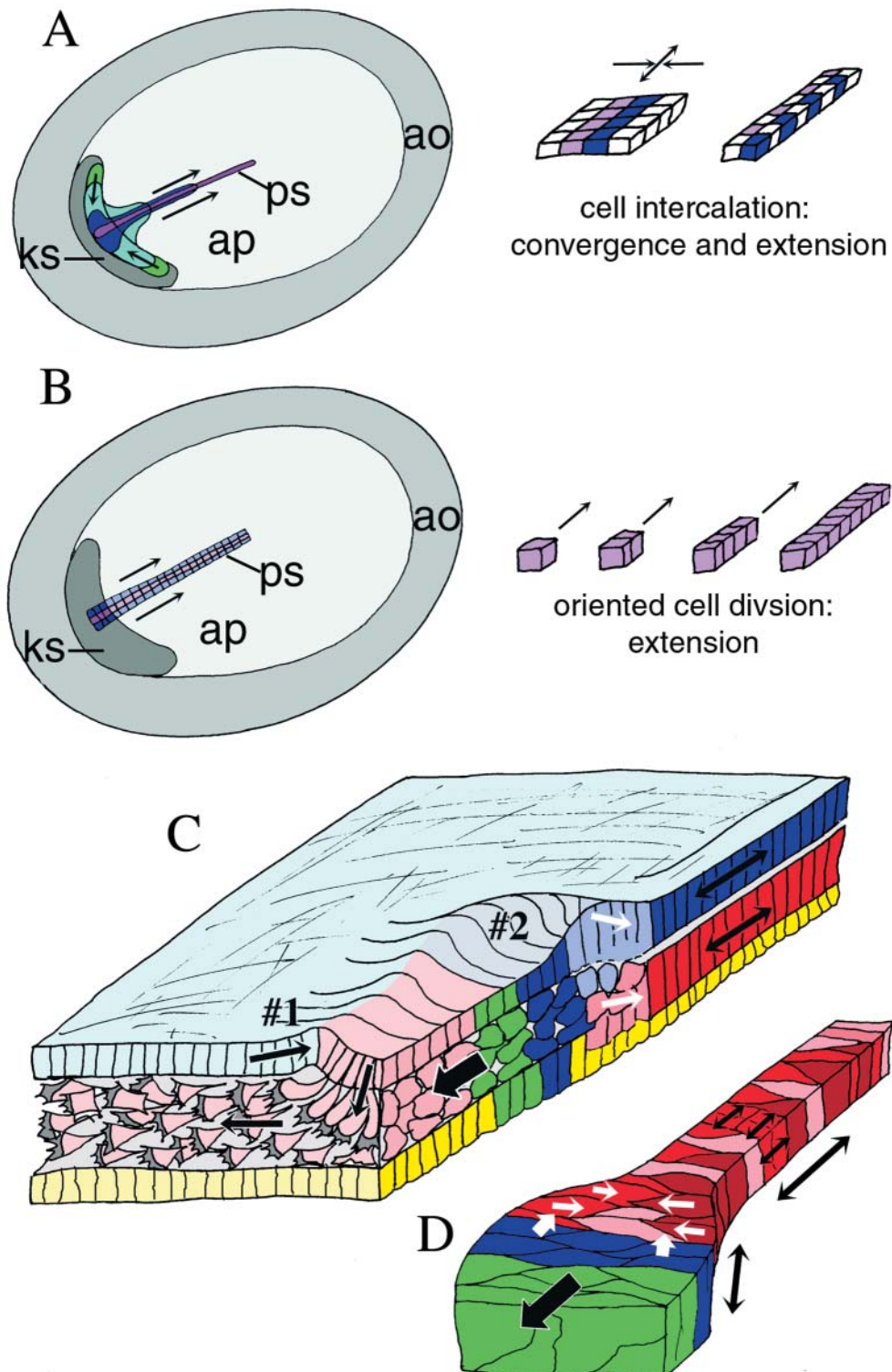
At the future posterior end of the embryo, there is a thickened, crescent-shaped region below the epiblast, called *Koller's sickle*. From the epiblast above Koller's sickle, an elongate, thickened structure, the *primitive streak*, extends anteriorly (Fig. 10A,B; see Lawson and Schoenwolf, 2001a,b and Bachvarova et al., 1998). A raised, thickened area, called the node, or Hensen's node, the equivalent of Spemann's Organizer, and analogous to the dorsal blastoporal lip of the amphibian embryo, forms at the anterior end of the extending primitive streak. It forms partially from cells at the anterior end of the streak and from others originally lying anterior to the primitive streak, which are added to the streak as it extends (Lawson and Schoenwolf, 2001a). The thickening of the streak occurs as part of its formation; the epiblast cells columnarize, undergo an epithelial-to-mesenchymal transition (EMT), and ingress to form the deeper layers of the primitive streak (Lawson and Schoenwolf, 2001b).

Ingression of cells from the epiblast continues

through gastrulation as the epithelial presumptive endodermal and mesodermal cells in the epiblast move medially toward the streak, undergo an EMT within it, and migrate as mesenchymal cells into the deeper layers (arrows at #1, Fig. 10C), eventually leaving only presumptive ectoderm in the epiblast. Of the cells moving out of

the streak into deeper layers, the first ones insert into the hypoblast to form the embryonic endoderm and the remainder form a third layer, the mesoderm, between the endoderm and epiblast (Fig. 10C).

The prospective extraembryonic mesoderm, the heart, lateral, and ventral mesoderm, and somitic mesoderm



ingress in roughly that order with early ingressing, presumptive anterior and lateral mesoderm being located in the epiblast near the streak and the last-ingressing presumptive somitic mesoderm being located farther laterally in the epiblast. Presumptive medial tissues are generally located in the anterior streak and presumptive lateral ones in the posterior streak. Cells also move out of Hensen's node and anteriorly to form the notochord, endoderm, and some heart mesoderm (#2, Fig. 10C). Finally, as the notochord extends out of the node anteriorly, the node regresses, moving back through the primitive streak (large arrow, Fig. 10C). See Schoenwolf and others (1992), Psychoyos and Stern (1996), and Lopez-Sanchez and others (2001) for descriptions of these movements. The floor plate also extends from this region, either being derived from a region just anterior to the node or from the node itself (cf. Lopez-Sanchez et al., 2001 and Charrier et al. 1999).

Extension of the Primitive Streak

The tension of the primitive streak represents a precocious extension along the future anterior-posterior axis as an early step in gastrulation. It is not clear that there is an equivalent morphogenic movement in the amphibian or fish. The early onset of extension in the first

half of amphibian gastrulation shows no similarity to primitive streak extension beyond the fact that it is an extension. In fact, the frog tissue thins and extends whereas the extending primitive streak extends and thickens. It appears that this amniote, and perhaps others as well, has moved a major part of anterior-posterior extension of the body plan into the first step of gastrulation in the form of streak elongation, whereas in amphibians and fish, this step first occurs during convergence and extension, most of it (in frogs) coming in the second half of gastrulation. In the bird, this first extension seems to involve presumptive anterior, lateral, ventral and extraembryonic mesodermal cells that ingress early and subsequently spread out widely, rather than continue convergence and extension in the gastrula and neurula stages. In contrast, the earliest extension in most amphibians and teleost fish involves axial and paraxial mesoderm. In the avian embryo, extension continues during gastrulation and neurulation in the form of notochord and neural plate extension. Some, and perhaps most of this extension is absorbed in regression of the node back through the previously-extended primitive streak.

Labeling cells in the region above the anterior part of Koller's sickle shows convergence of these labeled patches of cells toward the midline and extension of the streak anteriorly, indicating that the formation of the streak is essentially a convergence and extension process (Lawson and Schoenwolf, 2001a; Fig. 10A). However, virus-mediated marking of cells in the center of this region shows formation of anterior-posteriorly oriented linear arrays of cells that appear to be generated by oriented cell division, a conclusion supported by an anterior-posterior bias in orientation of mitotic spindles (Wei and Mikawa, 2000; Fig. 10B). These two versions of streak extension are not necessarily incompatible. Only one round of cell intercalation appears to occur, thus halving the width and doubling the length of the streak (Lawson and Schoenwolf, 2001a). One round of intercalation would probably not show up as the break-up of labeled, linear arrays of cells generated by oriented cell division unless the labeled array were quite narrow. Oriented cell division also plays a role in elongation of the neural plate (Schoenwolf and Alvarez, 1989; Sausedo et al., 1997) and the notochord in both avian (Sausedo and Schoenwolf, 1993) and mouse embryos (Sausedo and Schoenwolf, 1994), suggesting that this process has a large role in early amniote morphogenesis.

Notochord Extension and Node Regression

One of the most intriguing and mysterious processes in avian gastrulation is the regression of Hensen's node posteriorly through the primitive streak. Notochord extension in both avians (Sausedo and Schoenwolf, 1993) and mammals (Sausedo and Schoenwolf, 1994;

Fig. 10 Avian gastrulation begins with formation of the primitive streak (ps, A, B). Two mechanisms have been suggested for extension or progression of the primitive streak: convergence and extension of a region of the epiblast overlying the anterior part of Koller's sickle (ks; A; Lawson and Schoenwolf, 2001a, b), and oriented cell division (B; Wei and Mikawa, 2001). Gastrulation occurs as presumptive endodermal and mesodermal cells of the epiblast undergo an epithelial-mesenchymal transition (EMT) in the primitive streak, ingress, and insert into the hypoblast to form the endoderm (yellow) or move laterally to form the mesoderm (arrows, #1, C). At the anterior end of the primitive streak is a thickened region, Hensen's node (#2, C). The midline of the neural tissue and the underlying notochord extend anteriorly from Hensen's node, as these structures undergo coordinate convergence and extension (double-headed, black arrows, C) and cells are added posteriorly (white arrows, C). Coordinate with this extension, the node regresses or moves posteriorly (large black arrow, C), replacing the primitive streak with neural tissue and notochord. Hensen's node is site of many complex behaviors, some of which are not yet characterized. It has a generative zone (blue, D), which feeds cells anteriorly into the notochord (white arrows, D). There these cells undergo mediolateral cell intercalation (white arrows). Finally, in the notochord, the cells undergo cell division oriented preferentially in the anterior-posterior axis (small black arrows, D). Lastly, and perhaps most mysteriously, there is a region at the posterior of the node (green, D) that is necessary for the movement of the node posteriorly. Similar processes shown in D for the extension of the mesoderm apply to the midline neural tissue. (Based on Charrier et al., 1999; Sausedo and Schoenwolf, 1993, 1994; Lawson and Schoenwolf, 2001a, b; Wei and Mikawa, 2001).

Smith et al., 1994) occurs by three cellular processes: the majority of the extension is by addition (*accretion*) of cells to the posterior end of the notochord (thick white arrows, Fig. 10D); these cells are supplied by cell division in the node (blue zone, Fig. 10D; see Charrier et al., 1999). In addition, cell division within the notochord or notochordal plate (in mammals) is preferentially oriented parallel to the anterior-posterior axis, resulting in contribution to the length of the notochord rather than its width (small black arrows, Fig. 10D). Lastly, the cells undergo mediolateral intercalation as they move out of the wide node and into the notochord or notochordal plate (white arrows, Fig. 10D). The overlying neural tissue, which undergoes convergence and extension coordinately with the notochord, also elongates by anterior-posteriorly oriented cell division as well as by cell intercalation (Schoenwolf and Alvarez, 1989; Sausedo et al., 1997; Tuckett and Morriss-Kay, 1985).

It is likely that convergence and extension of the notochord and overlying notoplate (floor plate) of avians is an active process, based on the fact that in blastoderms transected posterior to the node, the node regresses posteriorly, beyond the cut edge (Waddington, 1932), suggesting that the notochord/notoplate actively pushes the node posteriorly. The relative contributions of accretion, cell intercalation, and oriented division toward generating the forces for this extension are not known. It is also possible that in addition the node actively pulls itself posteriorly. Again, in blastoderms transected posteriorly to the node, the primitive streak appears to retract posteriorly from the cut margin in the posterior piece of blastoderm (Waddington, 1932). Such an active pulling would require some specialized behavior on the part of the cells at the posterior part of the node (green zone, Fig. 10C; 10D). There is evidence that the posterior region of the node is necessary for regression of the node (Charrier et al., 1999; see green region, Fig. 10C, D), but the mechanism of this activity is unknown. Cells at the posterior and posterior-lateral edges of the node could exert directional traction on surrounding tissues. Alternatively, the node might be pulled posteriorly by the activity of primitive streak cells, perhaps by an active removal of cells of the primitive streak at a locus just behind the node.

Other changes associated with node regression are independent of the node and its movement. The entire posterior blastoderm (area pellucida) elongates during notochord extension, but notochord extension and node regression does not cause this general elongation; if the node is removed and no notochord is laid down the elongation still occurs (Bellairs, 1971; see Bellairs, 1986). Conversely, lengthening of the posterior blastoderm does not appear to drive regression and notochord extension; embryos grown on agar do not elongate but regression occurs (Stern and Bellairs, 1984). The mechanism of lengthening the posterior blastoderm is not

known; perhaps some extra embryonic machine pulls on the posterior blastoderm, or perhaps some form of convergence and extension within the epiblast is involved. Such a convergence would also serve to move cells toward the primitive streak.

Little is known about how the epithelial epiblast cells move toward the primitive streak prior to their ingression. It is likely that apical constriction, followed by ingression in the primitive streak generates tension that pulls the epithelial epiblast toward the midline, much as bottle cell formation and ingression pulls cells toward the bilateral primitive streak of the urodele (Shook et al., 2002). After their ingression, traction of the laterally migrating mesodermal cell stream on the overlying epiblast might tend to pull the epiblast toward the midline. These are some possibilities that should be investigated. It is clear that there is much that we do not know about the biomechanics of the fascinating movements of avian (and mammalian) gastrulation.

Oriented Cell Division As A Force Generator

Is the cell division oriented parallel to the axis of extension in the primitive streak of bird, and in the notochord and neural plate of birds and mammals, a force generating process? Sausedo and colleagues (1997) observed that nonrandom, rostrocaudal orientation of spindles persisted in isolated neuroepithelia, suggesting that orientation is an intrinsic property not dependent on forces generated by adjacent tissues. To produce a pushing force, there must be mechanisms to orient the mitotic spindles against a compressive load. It has been known for some time (see discussion in Chapter VIII, p. 362, Wilson, 1900) that compression tends to reorient the spindle transverse to the axis of the applied pressure and therefore reorients cell division to be perpendicular to, rather than parallel to axis of compression. Therefore, if cell division is to occur parallel to the axis of extension, the asters and spindle must be constrained in a specialized way to resist reorientation. Progress has been made on understanding the molecular events underlying the polarized, asymmetric division of cells (see Strome, 1993), particularly the involvement of the planar cell polarity (PCP) pathway in regulating the polarized division of epithelial neural precursors in *Drosophila* (see Jan and Jan, 2000; Wodarz, 2001). However, the mechanical environment of these divisions is unknown and it is not known to what degree these mechanisms can maintain the orientation of cell division in an otherwise mechanically unfavorable orientation. Do similar mechanisms, or different ones not yet described, underlie orientation of cell division against a mechanical load? Does the planar cell polarity pathway control orientation of cell division during extension in birds and mammals as it appears to control polarized protrusive activity during cell intercalation in fish and frogs?

Sea Urchin Gastrulation

Although it is not our goal to review the biomechanics of gastrulation in the many invertebrate model systems, we will briefly review some studies of sea urchin gastrulation, which has been studied biomechanically in some depth, and illustrates some key problems that will be faced elsewhere as well.

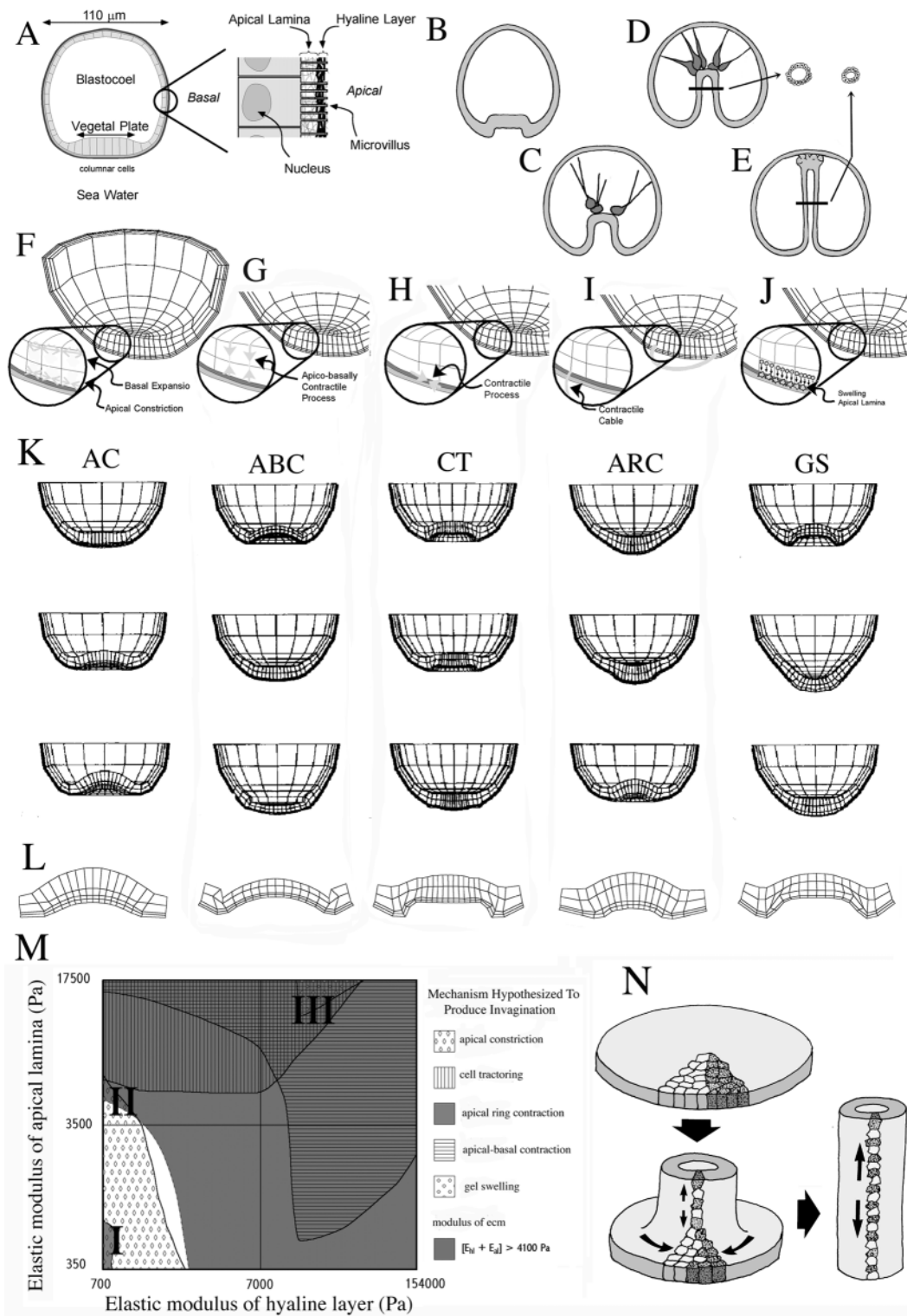
Primary Invagination

Studies of primary invagination reveal the possible involvement of multiple mechanisms in what seems to be a relatively simple bending of an epithelial sheet, and also shows the difficulty of distinguishing between them. At late blastula of the sea urchin embryo consists of a single layer of epithelial cells that is lined on the blastocoel side by a thin basal lamina and on the outside by a thicker, double layer of matrix consisting of an inner layer, the apical lamina, and an outer hyaline layer (Fig. 11A, and enlargement). During primary invagination, a thickened and flattened disc of epithelial cells, the vegetal plate, bends inward to form a blind tube about 5 to 10 microns deep (Fig. 11B-C). This short, blind tube later elongates and narrows, during secondary invagination, to form the archenteron (see Ettensohn, 1984, 1985a,b) (Fig. 11C-E). Microsurgically isolated vegetal plates undergo primary invagination (Moore and Burt, 1939), so the forces must be generated within the isolated plates, either by the cells or by the extracellular matrix associated with the vegetal plate.

Several mechanisms have been proposed to account for these movements. The first two involve basal expansion, relative to the apical aspect of the cell, resulting in an inward bending of the vegetal plate (Fig. 11F, G). Nakajima and Burke (1996) and Kimberly and Hardin (1998) proposed that the apices of the cells actively constrict as their basal regions expand and thereby drive the bending of the sheet (Fig. 11F). These studies identified intense localization of actin to circumapical bands and the apical ends of a subpopulation of cells within the vegetal plate that become bottle-shaped during invagination. Gustafson and Wolpert (1963) proposed that the apical-basal shortening model (Fig. 11G) in which decreased intercellular adhesion at the basal ends, or active contractile forces generated parallel to the apico-basal axis within the lateral cell cortex, causes the basal ends to round up and shorten, thus bending the plate. This model was supported by time-lapse films of the sea urchin *Psammechinus miliaris*, which showed that cells in the center of the vegetal plate reduced their intercellular contacts and rounded up at their basal ends. Because they remain attached to the extracellular matrix apically, loss of basolateral adhesion in the presence of cortical tension in the cell alone, or with an active apical-basal

contraction, would result in rounding, apical-basal shortening, and bending of the sheet. Burke and others (1991) proposed a cell tractor model in which the lateral vegetal plate cells try to crawl toward the center of the vegetal plate, using the inner surface of the external extracellular matrix as a substrate (Fig. 11H). As they do so, the plate is compressed and buckles inward. This model is supported by observations that in *Strongylocentrotus purpuratus* cells lateral to the vegetal plate: 1) have protrusions directed at the center of the vegetal plate; 2) have their apical ends skewed toward the center of the plate; 3) move four to five cell diameters toward the center of the vegetal plate during primary invagination; 4) primary invagination is blocked when embryos are cultured with antibody Fab fragments to glycoprotein substrates localized in the apical ECM, possibly implicating the apical lamina as a substrate for cell tractoring (see Burke et al., 1991). Davidson and others (1995) proposed the apical contractile ring model to account for the vegetally skewed cells observed in the peripheral vegetal plate. In this model, cells arrayed in an annular ring around the vegetal plate would undergo apical constriction, causing a contractile ring to form. Tension in this ring would cause the vegetal plate to come under compression, and with appropriate relative stiffnesses of the cells and extracellular matrix, it would buckle inward to form an invagination (Fig. 11I). Lane and others (1993) proposed the "gel swelling" hypothesis for primary invagination in which cells in the vegetal plate secrete a hydrophilic proteoglycan into the apical lamina of the late mesenchyme blastula (Fig. 11J). As the condensed granules are secreted into the inner apical lamina, they swell, and the differential swelling of the apical lamina relative to the non-swelling hyaline layer creates a bending moment throughout the vegetal plate that then buckles the plate inward. This mechanism is supported by several facts: 1) in *Strongylocentrotus purpuratus* such a proteoglycan is secreted in the right place at the right time; 2) experimentally induced, premature secretion of this proteoglycan results in precocious invagination; 4) blocking secretion of the proteoglycan prevents primary invagination whereas blocking actin-mediated contraction or motility with cytochalasin does not (see Lane et al., 1993). The evidence supports each of these mechanisms, to some degree, and in one or another species of echinoderm, but it does not support definitive conclusions as to which one, or which combination of mechanisms drives invagination in any species.

Davidson and others (1995) used finite element simulation to determine the importance of the composite stiffness of the cell layer, the apical lamina, and the hyaline layer, in each of the five mechanisms (Fig. 11F-J). They varied these mechanical properties over a biologically relevant parameter space and found that all five mechanisms would produce an invagination at some combination of cellular and extracellular matrix stiffness (Fig. 11K). They predicted the cell shape *changes* ex-



pected for each invagination (Fig. 11L) and mapped the mechanical parameter space for the cell and extracellular matrix stiffness at which each of these mechanisms would occur (Fig. 11M). For example, the gel-swelling hypothesis requires a stiff apical extracellular matrix and highly deformable cells (Fig. 11M). They then measured

the composite elastic modulus of the cellular and extracellular matrix layers of the blastula wall of *Strongylocentrotus purpuratus* embryos and compared this modulus with the modulus of embryos from which the extracellular matrix was removed and in with the modulus of embryos in which the cell cytoskeleton (both actin

Fig. 11 Processes relevant to the primary invagination of the vegetal plate and elongation of the archenteron across the blastocoel are illustrated. A schematic (A) shows the late mesenchyme blastula with the thickened vegetal plate. The primary invagination of the vegetal plate to form the archenteron is illustrated in sectional views at an early stage (B) and at completion (C). Secondary invagination consists of two phases of elongation of the archenteron, the first from about one third to two thirds the distance across the blastocoel (C-D) and the second from about two thirds this distance to contact with the animal region (D-E). Secondary mesenchyme cells are illustrated in red. Cross-sectional views (in D, E) show the decrease in number of cells comprising the circumference of the archenteron. Finite element simulations were done of apical contraction (F), apical-basal contraction (G), cell tracting (H), contractile ring contraction (I), and gel swelling (J). The results of finite element simulations are shown (K). Each vertical row shows the outcome for a finite element simulation (apical contraction, AC; apical-basal contraction, ABC; cell tracting CT; apical ring contraction, ARC; and gel swelling, GS). The horizontal rows show the results for the locations I (top row), II (middle row), and III (bottom row) in the mechanical parameter space (M). Shapes of the vegetal plate after “successful” invagination are shown for each of the finite element models (L). A map of the parameter space comparing “basins” where each finite element model can produce “successful” invaginations of 12 microns or more (see various crosshatching patterns) and the limits of the same mechanical properties of *S. purpuratus* measured experimentally are shown (M). The formation of the echinoderm archenteron, and tubes like it, involves transforming a disc into a tube, which involves a progressively greater decrease in circumference of the tissue toward the base, a decrease that may often involve cell intercalation (N).

microfilaments and microtubules) were disrupted (Davidson et al., 1999). These results showed that the composite stiffness of blastula wall is due largely to the apical extracellular matrix in this species with little contribution from the actin-based or microtubule based cytoskeleton of the cells. With these values for cell and extracellular matrix stiffness, neither the apical constriction nor the apical ring contraction hypothesis is a viable mechanism for this species, whereas the cell tracting, the gel swelling, and the apical-basal shortening mechanisms are possible. In other species, of course, the mechanical properties may be different, and therefore different mechanisms may be possible. Also, the measurements of stiffness were done on the mesenchyme blastula, just before primary invagination, for technical reasons, and the properties of the cells or matrix could change during gastrulation. These studies are only a beginning; in this and other systems, experimental manipulation must be done along with mechanical simulations and measurements of forces to which of the many plausible mechanisms actually work in a particular case.

Secondary Invagination

Mechanical analysis has also been useful in studying secondary invagination. Secondary invagination elongates

and narrows the short primary invagination such that it extends across the blastocoel and contacts the overlying inner surface of the blastocoel roof (Fig. 11C-E). Dan (1952), Dan and Okazaki (1956) and Gustafson and Wolpert (1963) observed that filopodia extend from the secondary mesenchyme cells at the tip of the archenteron and they attach to the overlying roof of the blastocoel (also see Hardin, 1988; Hardin, 1989; Hardin and McClay, 1990; Miller et al., 1995). They proposed that these filopodia actively shorten and pull on the archenteron, and thus passively stretch it into a more elongate structure. Finite element computer simulations predicted that filopodial towing of this type would bend the roof of the blastocoel inward, but the roof does not bend very much, casting doubt on this mechanism (Hardin and Cheng, 1986). Laser ablation of these filopodia showed that they were essential for the last half of secondary invagination but not the first half (Hardin, 1989). These results suggested that secondary invagination is actually composed of two phases, a first phase that is independent of secondary mesenchyme-generated filopodial tension, although the filopodia are normally present at this stage, and a second phase that is dependent on filopodial tension.

What then is the motive force for the first phase of secondary invagination? Etensohn (1984) and Hardin and Cheng (1986) showed that archenteron elongation is accompanied by circumferential cell intercalation to form a longer archenteron of smaller diameter and fewer cells in circumference (Fig. 11D, E). Archenteron elongation in the sea urchin is an example of convergence and extension of a cylinder by cell intercalation. A pattern of polarized protrusive activity of cells in the elongating archenteron has been suggested, based on static cell morphology (see Hardin, 1988), but it is unclear how this putative behavior is related mechanically to convergence and extension, and live imaging of cell behavior has not yet been done. These experiments also show the power of using mechanical simulations to test the plausibility of a mechanism, and to generate new hypotheses, and the power of using experimental mechanical interdictions, such as laser ablation, to test these hypotheses, and to characterize previously unsuspected morphogenic machines.

Simple cell wedging has long been the favored and plausible but untested mechanism of these types of invaginations, but more analysis suggests many ways that cells can and do function in this type of tubulogenesis. Why? These types of invaginations are often thought of as epithelial foldings, and they are usually represented in two dimensional, sectional views (Fig. 11B-E), a geometry in which simple cell wedging comes easily to mind. But in reality, they represent transformation of a flattened disc into an elongate tube, a much more complex geometry that also involves convergence, and perhaps, in many cases, cell intercalation (Fig. 11N). Viewed it this way, it is not

surprising that embryos have invented many cellular mechanisms to drive this process.

Conclusion

Biomechanics forms the rationale of morphogenesis and provides a theoretical and experimental framework in which its genetic, molecular, and cellular basis, as well as its evolution, can be understood. The repertoire of cell behaviors in gastrulation is fairly small in terms of general categories (cell crawling, cell intercalation (a specialized form of crawling), cell shape change, changes in cell adhesion, epithelial-mesenchymal transition, cell division, growth), and they are conserved in gastrulation of many species across the phylogenetic landscape. What varies between species is the specific combination, the geometry, the timing, and the mechanical linkages of these basic cell behaviors. Cell ingression, or removal from an epithelial layer, has very different mechanical consequences in echinoderm primary mesenchyme formation, movement of cells through the primitive streak of amniotes, and during somitic mesoderm ingression in the urodele amphibian, all because of the geometric and biomechanical contexts. Cell intercalation is used in *Xenopus* and cell ingression is used in the urodele amphibian to shorten arcs, but the biomechanical result is the same in both cases. Extension is produced variously by cell intercalation, oriented division, and cell shape change. Cell intercalation can produce convergence or thinning, depending on its polarity, and convergence can produce thickening or extension, depending on the mechanical context. This diversity is an inevitable consequence of mapping changes in gene function onto the biomechanical landscape during evolution. Biomechanics provides the selection test, the rationale, for evolution of morphogenic processes and strategies. As genetic changes occur, their morphogenic fitness is determined by how well they work to drive a specific morphogenic machine in a biomechanically-defined context. What emerges is what works, a biomechanically sufficient morphogenic strategy. Morphogenic developmental constraints are defined in terms of the specific, biomechanical system in which the function of genetic change is evaluated, not in terms of largely undefined notions of how conservative or radical differences in fate maps and in cell movements might be.

There are many challenges for the future. First, the power of biomechanical analysis should be brought to bear on genetic and molecular analyses of morphogenesis. Cell behaviors, their spatial organization and their progressions, the organization of tissues and assembly of extracellular matrix, the mechanical properties of cells and tissues, and their capacity for force-generation, should all be thought of as genetically-controlled phenotypes. Too many molecular interdictions bring the massive morphogenic movements of embryogenesis and

tissue repair to grinding halts, or block their beginning, but reveal little about the mechanism, other than identifying a molecule or gene as a necessary component. This is necessary, useful, and reassuring information, but reveals little about how morphogenic machines work. Applying the advances of the last several decades in imaging live cells to molecular and genetic interdiction studies have been helpful in defining molecular function in terms of cell behavior. A deeper understanding of morphogenesis will come when we can define molecular function in terms of the magnitude and patterns of forces and mechanical properties of cells and tissues. Second, we must develop the methods and conceptual framework to measure and analyze these biomechanical properties in standard engineering terms. Formulation of hypotheses should be done in quantitative biomechanical terms and data should be presented in standard engineering format so they can be understood, compared, and used by all (see Koehl, 1990). This will require better methods of making direct measurements of mechanical properties of embryonic cells and tissues, and the forces they generate. We have very little such data on the biomechanics of cells and cell populations in developing morphogenic systems (see Koehl, 1990; Adams et al., 1990; Moore, 1992; Moore et al., 1995; Davidson et al., 1999). Our discussion above identifies plausible biomechanical mechanisms, based largely on the behaviors of tissues and cells under various experimental conditions, but very few are formulated, modeled, and tested in quantitative terms. To do so is the main challenge for the future if we are to understand how the information encoded in the genome is translated into patterned forces and ultimately into very specific change in form that comprises gastrulation, other changes in form, and human diseases of morphogenesis.

References

- Adams, D., Keller, R.E. and Koehl, M.A.R. (1990) The mechanics of notochord elongation, straightening, and stiffening in the embryo of *Xenopus laevis*. *Development* 110:115–130.
- Adler, P. (2002) Planar signaling and morphogenesis in *Drosophila*. *Dev. Cell* 2:525–535.
- Bachvarova, R.F., Skromme, I. and Stern, C.D. (1998) Induction of primitive streak and Hensen's node by the posterior marginal zone. *Development* 125:3521–3534.
- Baker, P. (1965) Fine structure and morphogenetic movements in the gastrula of the tree frog, *Hyla regilla*. *J. Cell Biol.* 24:95–116.
- Ballard, W. (1955) Cortical ingression during cleavage of amphibian eggs, studied by means of vital dyes., *J. Exp. Zool.* 129: 7–79.
- Barrett, K., Leptin, M. and Settleman, J. (1997) The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. *Cell* 91:905–915.
- Bauman, M. and Sanders, K. (1984) Bipartite axiation follows incomplete epiboly in zebrafish embryos treated with chemical teratogens. *J. Exp. Zool.* 230:363–376.
- Bellairs, R. (1971) *Developmental processes in higher vertebrates*. Logos Press, London.
- Bellairs, R. (1986) The primitive streak. *Anat. Embryol.* 174:114.

- Betchaku, T. and Trinkaus, J.P. (1978) Contact relations, surface activity, and cortical microfilaments of marginal cells of the enveloping layer and of the yolk syncytial and yolk cytoplasmic layers of *Fundulus* before and after epiboly. *J. Exp. Zool.* 206: 381–426.
- Betchaku, T. and Trinkaus, J.P. (1986) Programmed endocytosis during epiboly of *Fundulus heteroclitus*. *Am. Zool.* 26:193–199.
- Bolker, J. (1993) The mechanism of gastrulation in the white sturgeon. *J. Exp. Zool.* 266:132–145.
- Boucaut, J.-C. and Darribère, T., Boulekbache, H. and Thierry, J.-P. (1984) Antibodies to fibronectin prevent gastrulation but do not perturb neurulation in gastrulated amphibian embryos. *Nature (Lond.)* 307:364–367.
- Boucaut, J.C. and Darribère, T. (1983a) Fibronectin in early amphibian embryos. Migrating mesodermal cells contact fibronectin established prior to gastrulation. *Cell Tissue Res.* 234:135–45.
- Boucaut, J.C. and Darribère, T. (1983b) Presence of fibronectin during early embryogenesis in amphibian *Pleurodeles waltlii*. *Cell Differ.* 12: 77–83.
- Brieher, W.M. and Gumbiner, B.M. (1994) Regulation of C-cadherin function during activin induced morphogenesis of *Xenopus* animal caps. *J Cell Biol.* 126:519–527.
- Brouns, M.R., Matheson, S.F., Hu, K., Delalle, I., Caviness, V.S., Silver, J., Bronson, R. T. and Settleman, J. (2000) The adhesion signaling molecule p190RhoGAP is required for morphogenetic processes in neural development. *Development* 127:4891–4903.
- Burke, R.D., Myers, R.L., Sexton, T.L. and Jackson, C (1991) Cell movements during the initial phase of gastrulation in the sea urchin embryo. *Dev. Biol.* 146:542–557.
- Burnside, M.B. and Jacobson, A. (1968) Analysis of morphogenetic movements in the neural plate of the newt *Taricha torosa*. *Dev. Biol.* 18:537–552.
- Bush, K.T., Lynch, F.J., DeNittis, A.S., Steinberg, A.B., Lee, H.Y. and Nagele, R.G. (1990) Neural tube formation in the mouse: a morphometric and computerized three-dimensional reconstruction study of the relationship between apical constriction of neuroepithelial cells and the shape of the neuroepithelium. *Anat. Embryol.* 181:49–58.
- Charrier, J.-B. and Teillet, M.-A. and Lapointe, F. and Le Douarin, N.M. (1999) Defining subregions of Hensen's node essential for caudalward movement, midline development, and cell survival. *Development* 128:4771–4783.
- Choi, S.-C. and Han, J.-K. (2002) *Xenopus* Cdc42 regulates convergent extension movements during gastrulation through Wnt/ Ca^{2+} signaling pathway. *Dev. Biol.* 244:342–357.
- Concha, M. and Adams, R. (1998) Oriented cell divisions and cellular morphogenesis in the zebrafish gastrula and neurula: a time-lapse analysis. *Development* 125:983–994.
- Costa, M., Wilson, E.T. and Wieschaus, E. (1994) A putative cell signal encoded by the folded gastrulation gene coordinates cell shape changes during *Drosophila* gastrulation. *Cell* 76:1075–1089.
- Dan, K. (1952) Cyto-embryological studies of sea urchins. II. Blastula stage. *Biol. Bull.* 102:74–89.
- Dan, K. and Okazaki, K. (1956) Cyto-embryological studies of sea urchins. III. Role of the secondary mesenchyme cells in the formation of the primitive gut in sea urchin larvae. *Biol. Bull.* 110:29–42.
- Darken, R., Scola, A., Rakeman, A., Das, G., Mlodzik, M. and Wilson, P. (2002) The planar polarity gene *strabismus* regulates convergent extension movements in *Xenopus*. *EMBO J.* 21:976–985.
- Darribère, T., Guida, K., Larjava, H., Johnson, K.E., Yamada, K., Thierry, J.-P. and Boucaut, J.-C. (1990) In vivo analysis of integrin $\beta 1$ subunit function in fibronectin matrix assembly. *J. Cell Biol.* 110:1813–1823.
- Davidson, L.A., Koehl, M.A.R. and Keller, R. and Oster, G. (1995) How do sea urchins gastrulate? Distinguishing between mechanisms of primary invagination using biomechanics. *Development* 121:2005–2018.
- Davidson, L., Oster, G., Keller, R., Koehl, M. (1999) Measurements of mechanical properties of the blastula wall reveal which hypothesized mechanisms of primary invagination are physically plausible in the sea urchin *Strongylocentrotus purpuratus*. *Dev. Biol.* 209:221–238.
- Davidson, L., Hoffstrom, B., Keller, R. and DeSimone, D. (2002) Mesendoderm extension and mantle closure in *Xenopus laevis* gastrulation: Combined roles for integrin $\alpha 5 \beta 1$, fibronectin, and tissue geometry. *Dev. Biol.* 242:109–129.
- D'Amico, L. and Cooper, M. (2001) Morphogenetic domains in the yolk syncytial layer of axiating zebrafish embryos. *Dev. Dyn.* 222:611–624.
- del Pino, E. (1996) The expression of Brachyury (T) during gastrulation in the marsupial frog *Gastrotheca riobambae*. *Dev. Biol.* 177:64–72.
- del Pino, E. and Elinson, R. (1983) A novel developmental pattern for frogs: gastrulation produces an embryonic disc. *Nature* 306:589–591.
- Djiane, A., Riou, J., Umbhauer, M., Boucaut, J. and Shi, D. (2000) Role of frizzled 7 in the regulation of convergent extension movements during gastrulation in *Xenopus laevis*. *Development* 127:3091–3100.
- Domingo, C. and Keller, R. (1995) Induction of notochord cell intercalation behavior and differentiation by progressive signals in the gastrula of *Xenopus laevis*. *Development* 121:3311–3321.
- Drawbridge, J. and Steinberg, M.S. (2000) Elongation of axolotl tailbud embryos requires GPI-linked proteins and organizer-induced, active, ventral trunk endoderm cell rearrangements. *Dev. Biol.* 223:27–37.
- Elinson, R. (1987) Fertilization and aqueous development of the Puerto-Rican terrestrial-breeding frog, *Eleutherodactylus coqui*. *J. Morphol.* 193:217–224.
- Elinson, R. and del Pino, E. (1985) Cleavage and gastrulation in the egg-brooding, marsupial frog, *Gastrotheca riobambae*. *J. Embryol. Exp. Morphol.* 90:223–232.
- Elinson, R., del Pino, E., Townsend, F., Cuesta, F. and Eichhorn, P. (1990) A practical guide to the developmental biology of terrestrial-breeding frogs. *Biol. Bull.* 179:163–177.
- Elinson, R. and Beckham, Y. (2002) Development in frogs with large eggs and the origin of amniotes. *Zool.* 105: *in press*.
- Elul, T., Koehl, M. and Keller, R. (1997) Cellular mechanism underlying neural convergent extension in *Xenopus laevis* embryos. *Dev. Biol.* 191:243–258.
- Elul, T. and Keller, R. (2000) Monopolar protrusive activity: a new morphogenic cell behavior in the neural plate dependent on vertical interactions with the mesoderm in *Xenopus*. *Dev. Biol.* 224:3–19.
- Ettensohn, C.A. (1984) Primary invagination of the vegetal plate during sea urchin gastrulation. *Am. Zool.* 24:571–88.
- Ettensohn, C.A. (1985a) Mechanisms of epithelial invagination. *Quart. Rev. Biol.* 60:289–307.
- Ettensohn, C.A. (1985a) Gastrulation in the sea urchin embryo is accompanied by the rearrangement of invaginating epithelial cells. *Dev. Biol.* 112:383–390.
- Ezin, M. (2002) Regulation of neural cell motility by the mesoderm. Ph.D. Thesis, University of Virginia, Charlottesville, VA.
- Fernandez, J.M., Villalon, M. and Verdugo, P. (1991) Reversible condensation of mast cell secretory products in vitro. *Biophys. J.* 59:1022–1027.
- Ferreira, M.C. and Hilfer, S.R. (1993) Calcium regulation of neural fold formation: visualization of the actin cytoskeleton in living chick embryos. *Dev. Biol.* 159:427–440.
- Foe, V.E., Field, C.M. and Odell, G.M. (2000) Microtubules and mitotic cycle phase modulate spatiotemporal distributions of F-actin and myosin II in *Drosophila* syncytial blastoderm embryos. *Development* 127:1767–1787.

- Glickman, N., Kimmel, C., Jones, M. and Adams, R. (2003) Shaping the zebrafish notochord. *Development* 130:873–887.
- Goto, T. and Keller, R. (2002) The planar cell polarity gene *strabismus* regulates convergence and extension and neural fold closure in *Xenopus*. *Dev. Biol.* 247:165–181.
- Gustafson, T. and Wolpert, L. (1963) The cellular basis of morphogenesis and sea urchin development. *Int. Rev. Cytol.* 15:139–214.
- Hacker, U. and Perrimon, N. (1998) DRhoGEF2 encodes a member of the Dbl family of oncogenes and controls cell shape changes during gastrulation in *Drosophila*. *Genes Dev.* 12:274–284.
- Habas, R., Kato, Y. and He, X. (2001) Wnt/Frizzled activation of Rho regulates vertebrate gastrulation and requires a novel formin homology protein Daam1. *Cell* 107:843–854.
- Hardin, J. (1988) The role of secondary mesenchyme cells during sea urchin gastrulation studied by laser ablation. *Development* 103:317–324.
- Hardin, J. (1989) Local shifts in position and polarized motility drive cell rearrangement during sea urchin gastrulation. *Dev. Biol.* 136(2):430–445.
- Hardin, J.D. and Cheng, L.Y. (1986) The mechanisms and mechanics of archenteron elongation during Sea Urchin Gastrulation. *Dev. Biol.* 115:490–501.
- Hardin, J. D. and Keller, R.E. (1988) The role of bottle cells in gastrulation of *Xenopus*. *Development* 103:210–230.
- Hardin, J. and McClay, D.R. (1990) Target recognition by the archenteron during sea urchin gastrulation. *Dev. Biol.* 142:86–102.
- Habas, R., Dawid, I. and He, X. (2003) Coactivation of Rac and Rho by Wnt/Frizzled signaling is required for vertebrate gastrulation. *Gene Develop.* 17:295–309.
- Heisenberg, C.P., Tada, M., Rauch, G.J., Saude, L., Concha, M.L., Geisler, R., Stemple, D.L., Smith, J.C. and Wilson, S.W. (2000) Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* 405:76–81.
- Holtfreter, J. (1943a) Properties and functions of the surface coat in amphibian embryos. *J. Exp. Zool.* 93:251–323.
- Holtfreter, J. (1943b) A study of the mechanics of gastrulation. Part I. *J. Exp. Zool.* 94:261–318.
- Holtfreter, J. (1944) A study of the mechanics of gastrulation. Part II. *J. Exp. Zool.* 95:171–212.
- Ip, Y., Maggert, K. and Levine, M. (1994) Uncoupling gastrulation and mesoderm differentiation in the *Drosophila* embryo. *EMBO J.* 13:5826–5834.
- Jacobson, A.G. and Gordon, R. (1976) Changes in the shape of the developing vertebrate nervous system analyzed experimentally, mathematically, and by computer simulation. *J. Exp. Zool.* 197:191–246.
- Jan, Y.-N. and Jan, L.Y. (2000) Polarity in cell division: What frames their fearful asymmetry? *Cell* 100:599–602.
- Jessen, J., Topczewski, J., Bingham, S., Sepich, D., Marlow, F., Chandrasekhar, A. and Solnica-Krezel, L. (2002) Zebrafish trilobite identifies new roles for *Strabismus* in gastrulation and neuronal movements. *Nat. Cell Biol.* 4:610–615.
- Kam, Z., Minden, J.S., Agard, D.A., Sedat, J.W. and Leptin, M. (1991) *Drosophila* gastrulation: analysis of cell shape changes in living embryos by three-dimensional fluorescence microscopy. *Development* 112:365–370.
- Kane, D.A., Hammerschmidt, M., Mullins, M., Maischein, H.-M., Brand, M., Van Eeden, F.J.M., Furutani-Seiki, M., Granato, M., Haffter, P., Heisenberg, C.-P., Jiang, Y.-J., Kelsh, R., Odenthal, J., Warga, R. and Nusslein-Volhard, C. (1996) The zebrafish epiboly mutants. *Development* 123:47–55.
- Kao, K. and Elinson, R. (1988) The entire mesodermal mantle behaves as Spemann's organizer in dorsoanterior enhanced *Xenopus laevis* embryos. *Dev. Biol.* 127:64–77.
- Katow, H. and Solorsh, M. (1980) Ultrastructure of primary mesenchyme cell ingression in the sea urchin *Lytechinus pictus*. *J. Exp. Zool.* 213:231–246.
- Katsumi, A., Milanini, J., Kiousses, W., del Pozo, M., Kaunas, R., Chien, S., Hahn, K. and Schwartz, M. (2002) Effects of cell tension on the small GTPase Rac. *J. Cell Biol.* 158:153–164.
- Keller, R.E. (1975) Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. I. Prospective areas and morphogenetic movements of the superficial layer. *Dev. Biol.* 42:222–241.
- Keller, R.E. (1976) Vital dye mapping of the gastrula and neurula of *Xenopus laevis*. II. Prospective areas and morphogenetic movements of the deep layer. *Dev. Biol.* 51:118–137.
- Keller, R.E. (1978) Time-lapse cinemicrographic analysis of superficial cell behavior during and prior to gastrulation in *Xenopus laevis*. *J. Morph.* 157:223–248.
- Keller, R.E. (1980) The cellular basis of epiboly: An SEM study of deep-cell rearrangement during gastrulation in *Xenopus laevis*. *J. Embryol. Exp. Morph.* 60:201–234.
- Keller, R.E. (1981) An experimental analysis of the role of bottle cells and the deep marginal zone in gastrulation of *Xenopus laevis*. *J. Exp. Zool.* 216:81–101.
- Keller, R. (1984) The cellular basis of gastrulation in *Xenopus laevis*: active, postinvolution convergence and extension by mediolateral interdigitation. *Am. Zool.* 24:589–603.
- Keller, R.E. (1986) The cellular basis of amphibian gastrulation. In: Browder, L. (ed) *Developmental Biology: A Comprehensive Synthesis*, Vol. 2 *The Cellular Basis of Morphogenesis*. Plenum Press, N.Y., pp 241–327.
- Keller, R. (2002) Shaping the vertebrate body plan by polarized embryonic cell movements. *Science* 298:1950–1954.
- Keller, R.E. and Schoenwolf, G.C. (1977) An SEM study of cellular morphology, contact, and arrangement, as related to gastrulation in *Xenopus laevis*. *Wilhelm Roux's Arch.* 182:165–186.
- Keller, R.E., Danilchik, M., Gimlich, R. and Shih, J. (1985a) Convergent extension by cell intercalation during gastrulation of *Xenopus laevis*. In: Edelman, G. M. (ed) *Molecular Determinants of Animal Form*, UCLA Symposia on Molecular and Cellular Biology, New Series 31. Alan Liss, Inc., ??, pp 111–141.
- Keller, R.E., Danilchik, M., Gimlich, R. and Shih, J. (1985b) The function and mechanism of convergent extension during gastrulation of *Xenopus laevis*. *J. Embryol. Exp. Morph.* 89 (Suppl.):185–209.
- Keller, R.E. and Trinkaus, J.P. (1987) Rearrangement of enveloping layer cells without disruption of the epithelial permeability barrier as a factor in *Fundulus* epiboly. *Dev. Biol.* 120:12–24.
- Keller, R.E. and Danilchik, M. (1988) Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus laevis*. *Development* 103:193–209.
- Keller, R.E. and Tibbetts, P. (1989) Mediolateral cell intercalation in the dorsal axial mesoderm of *Xenopus laevis*. *Dev. Biol.* 131:539–549.
- Keller, R.E., Cooper, M., Danilchik, M., Tibbetts, P. and Wilson, P. (1989) Cell intercalation during notochord development in *Xenopus laevis*. *J. Exp. Zool.* 251:134–154.
- Keller, R., Shih, J., Wilson, P. and Sater, A. (1991) Pattern and function of cell motility and cell interactions during convergence and extension. In: Gerhart, J. (ed) *Xenopus*. 49th Symp. Soc. Develop. Biol. Cell-Cell Interactions in Early Development. Wiley and Sons, N.Y., pp 93–107.
- Keller, R., Shih, J. and Domingo, C. (1992) The patterning and functioning of protrusive activity during convergence and extension of the *Xenopus* organizer. In: C. D. Stern and P. W. Ingham (eds) *Development 1992 Supplement – Gastrulation*. ??, ??, pp 81–91.
- Keller, R. and Jansa, S. (1992) *Xenopus* gastrulation without a blastocoel roof. *Develop. Dynamics* 195:162–176.
- Keller, R., Davidson, R., Edlund, A., Elul, T., Ezin, M., Shook, D. and Skoglund, P. (2000) Mechanisms of convergence and extension by cell intercalation. *Phil. Trans. R. Soc. Lond. B* 355:897–922.
- Keller, R. and Davidson, L. (2003) Cell crawling, cell behavior, and biomechanics during convergence and extension. In: Ridley

- A., Peckham M. and Clark P. (eds.) "Cell Motility". John Wiley and Sons, Ltd., in press.
- Kim, S., Yamamoto, A., Bouwmeester, T., Agius, E. and De Robertis, E. (1998) The role of paraxial protocadherin in selective adhesion and cell movements of the mesoderm during *Xenopus* gastrulation. *Development* 125:4681–4690.
- Kimberly, E.L. and Hardin, J. (1998) Bottle cells are required for the initiation of primary invagination in the sea urchin embryo. *Dev. Biol.* 204:235–250.
- Kimmel, C. and Law, R. (1985) Cell lineage of zebrafish blastomeres. III. Clonal analysis of the blastula and gastrula stages. *Dev. Biol.* 108:94–101.
- Koehl, M.A.R. (1990) Biomechanical approaches to morphogenesis. *Sem. Dev. Biol.* 1:367–378.
- Kurth, T. and Hausen, P. (2000) Bottle cell formation in relation to mesodermal patterning in the *Xenopus* embryo. *Mech. Dev.* 97:117–131.
- Laale, H.W. (1982) Fish embryo culture: observations on axial cord differentiation in presomitic isolates of the zebrafish *Brachydanio rerio*. *Can. J. Zool.* 60:1710–1721.
- Lane, M.C. and Solursh, M. (1991) Primary mesenchyme cell migration requires a chondroitin sulfate/dermatan sulfate proteoglycan. *Dev. Biol.* 143:389–397.
- Lane, C. and Keller, R. (1997) Microtubule disruption reveals that Spemann's organizer is subdivided into two domains by the vegetal alignment zone. *Development* 124:895–906.
- Lane, M.C., Koehl, M.A.R., Wilt, F. and Keller, R. (1993) A role for regulated secretion of apical extracellular matrix during epithelial invagination in the sea urchin. *Development* 117:1049–1060.
- Larkin, K. and Danilchik, M. (1999) Ventral cell rearrangements contribute to anterior-posterior axis lengthening between neurula and tailbud stages in *Xenopus laevis*. *Dev. Biol.* 216:550–560.
- Lawson, A. and Schoenwolf, G.C. (2001a.) Cell populations and morphogenetic movements underlying formation of the avian primitive streak and organizer. *Genesis* 29:188–195.
- Lawson, A. and Schoenwolf, G.C. (2001b) New insights into critical events of avian gastrulation. *Anat. Rec.* 262:238–252.
- Lee, J.-Y. and Goldstein, B. (2003) Mechanisms of cell positioning during *C. elegans* gastrulation. *Development* 130:307–320.
- Lee, H. and Nagele, R.G. (1985) Possible involvement of calmodulin in apical constriction of neuroepithelial cells and elevation of the neural folds in the chick. *Experientia* 41:1186–1188.
- Leptin, M. and Grunewald, B. (1990) Cell shape changes during gastrulation in *Drosophila*. *Development* 110:73–84.
- Lewis, W.H. (1947) Mechanics of invagination. *Anat. Rec.* 97:139–156.
- Lopez-Sanchez, C., Garcia-Martinez, V. and Schoenwolf, G.C. (2001) Localization of cells of the prospective neural plate, heart and somites within the primitive streak and epiblast of avian embryos at intermediate primitive-streak stages. *Cells, Tissues, Organs* 169:334–346.
- Marlow, F., Topczewski, J., Sepich, D. and Solnica-Krezel, L. (2002) Zebrafish rho kinase 2 acts downstream of wnt11 to mediate cell polarity and effective convergence and extension movements. *Curr. Biol.* 12:876–884.
- Marsden, M. and DeSimone, D. (2001) Regulation of cell polarity, radial intercalation and epiboly in *Xenopus*: novel roles for integrin and fibronectin. *Development* 128:3635–3647.
- Miller, J., Fraser, S.E. and McClay, D. (1995) Dynamics of thin filopodia during sea urchin gastrulation. *Development* 121:2501–2511.
- Molven, A., Wright, C.V., Bremiller, R., De Robertis, E.M., Kimmel, C.B. (1990) Expression of a homeobox gene product in normal and mutant zebrafish embryos: evolution of the tetrapod body plan. *Development.* 109:279–288.
- Moore, A.R. and Burt, A.S. (1939) On the locus and nature of the forces causing gastrulation in the embryos of *Dendroaster excrucicus*. *J. Exp. Zool.* 82:159–171.
- Moore, S. (1992) Direct measurement of dynamic biomechanical properties of amphibian embryonic tissues. PhD Thesis, University of California, Berkeley CA, USA.
- Moore, S., Keller, R. and Koehl, M. (1995) The dorsal involuting marginal zone stiffens anisotropically during its convergent extension in the gastrula of *Xenopus laevis*. *Development* 121:3131–3140.
- Morize, P., Christiansen, A., Costa, M., Parks, S. and Wieschaus, E. (1998) Hyperactivation of the *folded gastrulation* pathway induces specific cell shape changes. *Development* 125:589–597.
- Myers, D.C., Sepich, D.S. and Solnica-Krezel, L. (2002) Bmp activity gradient regulates convergent extension during zebrafish gastrulation. *Dev Biol* 243:81–98.
- Nagel, M. and Winklbauer, R. (1999) Establishment of substratum polarity in the blastocoel roof of the *Xenopus* embryo. *Development* 126:1975–1984.
- Nakatsuji, N. (1975) Studies on the gastrulation of amphibian embryos: Cell movement during gastrulation in *Xenopus laevis* embryos. *Wilhelm Roux Arch.* 178:1–14.
- Nakatsuji, N. and Johnson, K. (1982) Movement and guidance of migrating mesodermal cells in *Ambystoma maculatum* gastrulae. *J. Cell Sci.* 56:207–222.
- Nakatsuji, N. and Johnson, K. (1983) Conditioning of a culture substratum by the ectodermal layer promotes attachment and oriented locomotion by amphibian gastrula mesodermal cells. *J. Cell Sci.* 59:43–50.
- Nakajima, Y. and Burke, R.D. (1996) The initial phase of gastrulation in sea urchins is accompanied by the formation of bottle cells. *Dev Biol* 179:436–446.
- Nance, J. and Priess, J. (2002) Cell polarity and gastrulation in *C. elegans*. *Development* 129:387–397.
- Nieuwkoop, P. and Florschütz, P.A. (1950) Quelques caractères spécifiques de la gastrulation et de la neurulation de l'oeuf de *Xenopus laevis*, Daud. et de quelques autres Anoures. *Arch. Biol.* 61:113–150.
- Oda, H. and Tsukita, S. (2000) Real-time imaging of cell-cell adherens junctions reveals that *Drosophila* mesoderm invagination begins with two phases of apical constriction of cells. *J. Cell Sci.* 114:493–501.
- Odell, G., Oster, G., Alberch, P. and Burnside, B. (1981) The mechanical basis of morphogenesis. *Dev. Biol.* 85:446–462.
- Ohashi, T., Kiehart, D.P. and Erickson, H.P. (1999) Dynamics and elasticity of the fibronectin matrix in living cell culture visualized by fibronectin-green fluorescent protein. *Proc. Nat. Acad. Sci. USA* 96:2153–2158.
- Oppenheimer, J. (1959) Extraembryonic transplantations of sections of the *Fundulus* embryonic shield. *J. Exp. Zool.* 140:247–267.
- Owaribe, K., Kodama, R. and Eguchi, G. (1981) Demonstration of contractility of circumferential actin bundles and its morphogenetic significance in pigmented epithelial cells in vitro and in vivo. *J. Cell Biol.* 90:507–514.
- Owaribe, K. and Masuda, H. (1982) Isolation and characterization of circumferential microfilament bundles from retinal pigmented epithelial cells. *J. Cell Biol.* 95:310–315.
- Park, M. and Moon, R.T. (2002) The planar cell-polarity gene *stbm* regulates cell behaviour and cell fate in vertebrate embryos. *Nat. Cell Biol.* 4:20–25.
- Parks, S. and Wieschaus, E. (1991) The *Drosophila* gastrulation gene *concertina* encodes a G alpha-like protein. *Cell* 64:447–458.
- Perry, M. and Waddington, C.H. (1966) Ultrastructure of the blastoporal cells in the newt. *J. Embryol. Exp. Morphol.* 15:317–330.
- Poznanski, A. and Keller, R. (1997) The role of planar and early vertical signaling in patterning the expression of Hoxb-1 in *Xenopus*. *Dev. Biol.* 184:351–366.
- Psychoyos, D. and Stern, C.D. (1996) Fates and migratory routes of primitive streak cells in the chick embryo. *Development* 122:1523–1534.

- Ramirez, F., Lee, B. and Vitale, E. (1992) Clinical and genetic associations in Marfan syndrome and related disorders. *Mt Sinai J. Med.* 59:350–356.
- Ramirez, F., Pereira, L., Zhang, H. and Lee, B. (1993) The fibrillin-Marfan syndrome connection. *Bioessays* 15:589–594.
- Ramos, J. and DeSimone, D. (1996) *Xenopus* embryonic cell adhesion to fibronectin: position-specific activation of RGD/Synergy site-dependent migratory behavior at gastrulation. *J. Cell Biol.* 134:1–14.
- Sakai, L., Keene, D. and Engvall, E. (1986) Fibrillin, a new 350kD glycoprotein, is a component of extracellular microfibrils. *J. Cell Biol.* 97:2499–24509.
- Sausedo, R.A. and Schoenwolf, G.C. (1993) Cell behaviors underlying notochord formation and extension in avian embryos: quantitative and immunocytochemical studies. *Anat. Rec.* 237:58–70.
- Sausedo, R.A. and Schoenwolf, G.C. (1994) Quantitative analyses of cell behaviors underlying notochord formation and extension in mouse embryos. *Anat. Rec.* 239:103–112.
- Sausedo, R.A., Smith, J. and Schoenwolf, G.C. (1997) Role of non-randomly oriented cell division in shaping and bending of the neural plate. *J. Comp. Neuro.* 381:473–488.
- Schechtman, A.M. (1934) Unipolar ingression in *Triturus torosus*: A hitherto undescribed movement in the pregastrula stages of a urodele. *Univ. Calif. Publ. Zool.* 39:309.
- Schechtman, A.M. (1935) Mechanism of ingression in the egg of *Triturus torosus*. *Proc. Soc. Expt. Biol. Med.* 32:1072–1073.
- Schechtman, A.M. (1942) The mechanics of amphibian gastrulation. I. Gastrulation-producing interactions between various regions of an anuran egg (*Hyla regilla*). *Univ. Calif. Publ. Zool.* 51:1–39.
- Schoenwolf, G.C. and Alvarez, I. (1989) Roles of neuroepithelial cell rearrangement and division in shaping of the avian neural plate. *Development* 106:427–439.
- Schoenwolf, G.C. and Smith, J.L. (1990) Mechanisms of neurulation: traditional viewpoint and recent advances. *Development* 109:243–270.
- Schoenwolf, G.C., Garcia-Martinez, V. and Dias, M.S. (1992) Mesoderm movement and fate during avian gastrulation and neurulation. *Dev. Dyn.* 193:235–248.
- Sepich, D., Myers, D., Short, R., Topczewski, J., Marlow, F. and Solnica-Krezel, L. (2000) Role of the zebrafish trilobite locus in gastrulation movements of convergence and extension. *Genesis* 27:159–173.
- Settleman, J. (2001) Rac'n Rho: the music that shapes a developing embryo. *Dev. Cell* 1:321–331.
- Shi, D.L., Darribere, T., Johnson, K.E. and Boucaut, J.C. (1989) Initiation of mesodermal cell migration and spreading relative to gastrulation in the urodele amphibian *Pleurodeles waltlii*. *Development* 105:351–364.
- 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*. *Development* 116:915–930.
- Shih, J. and Fraser, S. (1995) Distribution of tissue progenitors within the shield region of the zebrafish gastrula. *Development* 121:2755–2765.
- Shinagawa, A. and Kobayashi, S. (2000) Localization and behavior of putative blastopore determinants in the uncleaved *Xenopus* egg. *Dev. Growth Diff.* 42:581–591.
- Shook, D., Majer, C. and Keller, R. (2002) Urodeles remove mesoderm from the superficial layer by subduction through a bilateral primitive streak. *Dev. Biol.* 248:220–239.
- Smith, J.L., Gesteland, K.M. and Schoenwolf, G.C. (1994) Prospective fate map of the mouse primitive streak at 7.5 days of gestation. *Dev. Dyn.* 201:279–289.
- Smith, J.C., Symes, K., Hynes, R.O., DeSimone, D. (1990) Mesoderm induction and the control of gastrulation in *Xenopus laevis*: The roles of fibronectin and integrins. *Development* 108:229–238.
- Sokol, S. Y. (1996) Analysis of Dishevelled signalling pathways during *Xenopus* development. *Curr. Biol.* 6:1456–1467.
- Solnica-Krezel, L. and Driever, W. (1994) Microtubule arrays of the zebrafish yolk cell: organization and function during epiboly. *Development* 120:2443–2455.
- Spek, J. (1918) Differenzen im quellungszustand der plasmakolloide als eine ursache der gastrulainvagination, sowie der einstülpungen und faltungen von zellplatten überhaupt. *Kolloidchemische Beihefte* 9:259–399.
- Spiegel, E. and Howard, L. (1983) Development of cell junctions in sea-urchin embryos. *J. Cell Sci.* 62:27–48.
- Stern, C. and Bellairs, R. (1984) The roles of node regression and elongation of the area pellucida in the formation of somites in avian embryos. *J. Embryol. Exp. Morphol.* 81:75–92.
- Strahle, U. and Jesuthasan, S. (1993) Ultraviolet radiation impairs epiboly in zebrafish embryos: evidence for a microtubule-dependent mechanism of epiboly. *Development* 119:909–919.
- Strome, S. (1993) Determination of cleavage planes. *Cell* 72:3–6.
- Sweeton, D., Parks, S., Costa, M. and Wieschaus, E. (1991) Gastrulation in *Drosophila*: the formation of the ventral furrow and posterior midgut invaginations. *Development* 112:775–789.
- Tada, M., Smith, J.C. (2000) Xwnt11 is a target of *Xenopus* Brachyury: regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* 127:2227–2238.
- Thorogood, P. and Wood, A. (1987) Analysis of in vivo cell movements using transparent tissue systems. *J. Cell Sci.* 8(Suppl.):395–413.
- Tickle, C.A. and Trinkaus, J.P. (1973) Change in surface extensibility of *Fundulus* deep cells during early development. *J. Cell Sci.* 13:721–726.
- Topczewski, J., Sepich, D., Myers, D., Walker, C., Amores, A., Lele, Z., Hammerschmidt, M., Postlethwait, J. and Solnica-Krezel, L. (2001) The zebrafish glypican knypek controls cell polarity during gastrulation movements of convergent extension. *Dev. Cell* 1:251–264.
- Trinkaus, J.P. (1984a) Cells Into Organs: Forces that Shape the Embryo. Prentice-Hall, Englewood Cliffs, N. J.
- Trinkaus, J.P. (1984b) Mechanisms of *Fundulus* epiboly – a current view. *Am. Zool.* 24:673–688.
- Trinkaus, J.P. (1951) A study of the mechanism of epiboly in the egg of *Fundulus heterochilus*. *J. Exp. Zool.* 118:269–320.
- Trinkaus, J.P. (1998) Gradient in convergent cell movement during *Fundulus* gastrulation. *J. Exp. Zool.* 281:328–335.
- Trinkaus, J.P., Trinkaus, M. and Fink, R. (1992) On the convergent cell movements of gastrulation in *Fundulus*. *J. Exp. Zool.* 261:40–61.
- Tuckett, F. and Moriss-Kay, G.M. (1985) The kinetic behaviour of the cranial neural epithelium during neurulation in the rat. *J. Embryol. Exp. Morph.* 85:111–119.
- Vogt, W. (1929) Gestaltanalyse am Amphibienkeim mit örtlicher Vitalfärbung. II. Teil. Gastrulation und Mesodermbildung bei Urodelen und Anuren. *Wilhelm Roux Arch. EntwMech. Org.* 120:384–706.
- Wacker, S., Grimm, K., Joos, T. and Winklbauer, R. (2000) Development and control of tissue separation at gastrulation in *Xenopus*. *Dev. Biol.* 224:428–439.
- Waddington, C.H. (1932) Experiments on the development of chick and duck embryos, cultivated in vitro. *Phil. Trans. Royal Soc. Lond., Series B* 221:179–230.
- Wallingford, J.B., Rowning, B.A., Vogeli, K.M., Rothbacher, U., Fraser, S.E. and Harland, R.M. (2000) Dishevelled controls cell polarity during *Xenopus* gastrulation. *Nature* 405:81–85.
- Wallingford, J.B. and Harland, R.M. (2001) *Xenopus* Dishevelled signaling regulates both neural and mesodermal convergent extension: parallel forces elongating the body axis. *Development* 128:2581–2592.

- Wang, M., Clericuzio, C. and Godfrey, M. (1996) Familial occurrence of typical and severe lethal congenital contractural arachnoidactyly caused by missplicing of exon 34 of fibrillin-2. *Am. J. Hum. Genet.* 59:1027–1034.
- Warga, R. and Kimmel, C. (1990) Cell movements during epiboly and gastrulation in zebrafish. *Development* 108:569–580.
- Wei, Y. and Mikawa, T. (2000) Formation of avian primitive streak from spatially restricted blastoderm: evidence for polarized cell division in elongating the streak. *Development* 127:87–96.
- Wilson, E.B. (1900) *The Cell in Development and Inheritance*. 2nd ed. The Macmillan Company, N.Y.
- Wilson, P., Oster, G. and Keller, R.E. (1989) Cell rearrangement and segmentation in *Xenopus*: Direct observation of cultured explants. *Development* 105:155–166.
- Wilson, P. and Keller, R. (1991) Cell rearrangement during gastrulation of *Xenopus*: Direct observation of cultured explants. *Development* 112:289–300.
- Winklbauer, R. (1990) Mesodermal cell migration during *Xenopus* gastrulation. *Dev. Biol.* 142:155–168.
- Winklbauer, R. and Keller, R. (1996) Fibronectin, mesoderm migration, and gastrulation in *Xenopus*. *Dev. Biol.* 177:413–426.
- Winklbauer, R., Medina, A., Swain, R. and Steinbeisser, H. (2001) Frizzled-7 signalling controls tissue separation during *Xenopus* gastrulation. *Nature* 413:856–860.
- Winklbauer, R. and Nagel, M. (1991) Directional mesoderm cell migration in the *Xenopus* gastrula. *Dev. Biol.* 148:573–589.
- Winklbauer, R., Nagel, M., Selchow, A. and Wacker, S. (1996) Mesoderm migration in the *Xenopus* gastrula. *Int. J. Dev. Biol.* 40:305–311.
- Winklbauer, R. and Schürfeld, M. (1999) Vegetal rotation, a new gastrulation movement involved in the internalization of the mesoderm and endoderm in *Xenopus*. *Development* 126:3703–3713.
- Winklbauer, R., Selchow, A., Nagel, M. and Angres, B. (1992) Cell interaction and its role in mesoderm cell migration during *Xenopus* gastrulation. *Dev. Dyn.* 195:290–302.
- Winklbauer, R. and Stoltz, C. (1995) Fibronectin fibril growth in the extracellular matrix of the *Xenopus* embryo. *J. Cell Sci.* 108:1575–1586.
- Wodarz, A. (2001) Cell polarity: No need to reinvent the wheel. *Curr. Biol.* 11:R975–R978.
- Yamamoto, A., Amacher, S., Kim, S., Geissert, D., Kimmel, C. and DeRobertis, E. (1998) Zebrafish paraxial protocadherin is a downstream target of spadetail involved in morphogenesis of gastrula mesoderm. *Development* 125:3389–3397.
- Young, R.E., Pesacreata, T.C. and Kiehart, D.P. (1991) Dynamic changes in the distribution of cytoplasmic myosin during *Drosophila* embryogenesis. *Development* 111:1–14.
- Zalik, S.E. and Kam, E. and Geiger, B. (1999) Cell adhesion and the actin cytoskeleton of the enveloping layer in the zebrafish during epiboly. *Biochem. Cell Biol.* 77:527–542.
- Zhang, H., Apfelroth, S., Hu, W., Davis, E., Sasanguineti, C., Bonadio, J., Mecham, R. and Ramirez, F. (1994) Structure and expression of fibrillin-2, a novel microfibrillar component preferentially located in elastic matrices. *J. Cell Biol.* 124:855–863.
- Zhong, Y., Briehner, W.M. and Gumbiner, B.M. (1999) Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. *J. Cell Biol.* 144:351–359.