THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0626

Mechanisms of convergence and extension by cell intercalation

Ray Keller[1*], Lance Davidson[1], Anna Edlund[1], Tamira Elul[2], Max Ezin[1], David Shook[1] and Paul Skoglund[1]

[1]*Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, VA 22903, USA*
[2]*Department of Physiology, Howard Hughes Medical Institute, University of California, San Francisco, CA 94143-6723, USA*

The cells of many embryonic tissues actively narrow in one dimension (convergence) and lengthen in the perpendicular dimension (extension). Convergence and extension are ubiquitous and important tissue movements in metazoan morphogenesis. In vertebrates, the dorsal axial and paraxial mesodermal tissues, the notochordal and somitic mesoderm, converge and extend. In amphibians as well as a number of other organisms where these movements appear, they occur by mediolateral cell intercalation, the rearrangement of cells along the mediolateral axis to produce an array that is narrower in this axis and longer in the anteroposterior axis. In amphibians, mesodermal cell intercalation is driven by bipolar, mediolaterally directed protrusive activity, which appears to exert traction on adjacent cells and pulls the cells between one another. In addition, the notochordal–somitic boundary functions in convergence and extension by 'capturing' notochordal cells as they contact the boundary, thus elongating the boundary. The prospective neural tissue also actively converges and extends parallel with the mesoderm. In contrast to the mesoderm, cell intercalation in the neural plate normally occurs by monopolar protrusive activity directed medially, towards the midline notoplate–floor-plate region. In contrast, the notoplate–floor-plate region appears to converge and extend by adhering to and being towed by or perhaps migrating on the underlying notochord. Converging and extending mesoderm stiffens by a factor of three or four and exerts up to 0.6 μN force. Therefore, active, force-producing convergent extension, the mechanism of cell intercalation, requires a mechanism to actively pull cells between one another while maintaining a tissue stiffness sufficient to push with a substantial force. Based on the evidence thus far, a cell–cell traction model of intercalation is described. The essential elements of such a morphogenic machine appear to be (i) bipolar, mediolaterally orientated or monopolar, medially directed protrusive activity; (ii) this protrusive activity results in mediolaterally orientated or medially directed traction of cells on one another; (iii) tractive protrusions are confined to the ends of the cells; (iv) a mechanically stable cell cortex over the bulk of the cell body which serves as a movable substratum for the orientated or directed cell traction. The implications of this model for cell adhesion, regulation of cell motility and cell polarity, and cell and tissue biomechanics are discussed.

Keywords: convergence; extension; gastrulation; *Xenopus*; morphogenesis; motility

1. INTRODUCTION

(a) *What are the convergence and extension movements?*

Convergence and extension are classical terms for narrowing and lengthening, respectively, of a cell population during morphogenesis (reviewed in Keller *et al.* 1991*a*,*b*) (figure 1*a*). These movements occur ubiquitously in metazoan development and probably account for more tissue distortion in embryogenesis than any other single process (Keller 1987; Keller *et al.* 1991*b*). Examples include archenteron elongation in echinoderms (Ettensohn 1985; Hardin & Cheng 1986), germ band extension (Irvine & Wieschaus 1994) and imaginal leg disc evagination in *Drosophila* (see Condic *et al.* 1991), *Hydra* regeneration (Bode & Bode 1984), nematode body axis elongation (Priess & Hirsh 1986; Williams-Masson *et al.* 1997), and elongation of dorsal axial embryonic tissues of ascidians (Cloney 1964; Miyamoto & Crowther 1985), fishes (Warga & Kimmel 1990; Kimmel *et al.* 1994; Concha & Adams 1998), birds (Schoenwolf & Alvarez 1989), mammals (Sausedo & Schoenwolf 1994), and amphibians (Vogt 1929; Schechtman 1942; Burnside & Jacobson 1968; Keller 1984; Keller *et al.* 1991*a*,*b*). Convergence can be coupled directly to extension with conservation of tissue volume, the decrease in width accounted for by a proportional increase in length (figure 1*a*). More often, however, convergence produces thickening as well as lengthening, the proportion of each depending on the specific case (figure 1*b*). For example, the notochordal and somitic mesoderm of the amphibian both converge, extend and thicken, but convergence results in less extension and more thickening in the case of the somitic mesoderm (see Keller *et al.* 1989*a*; Keller 2000). Thus the collective term

*Author for correspondence (rek3k@unix.mail.virginia.edu).

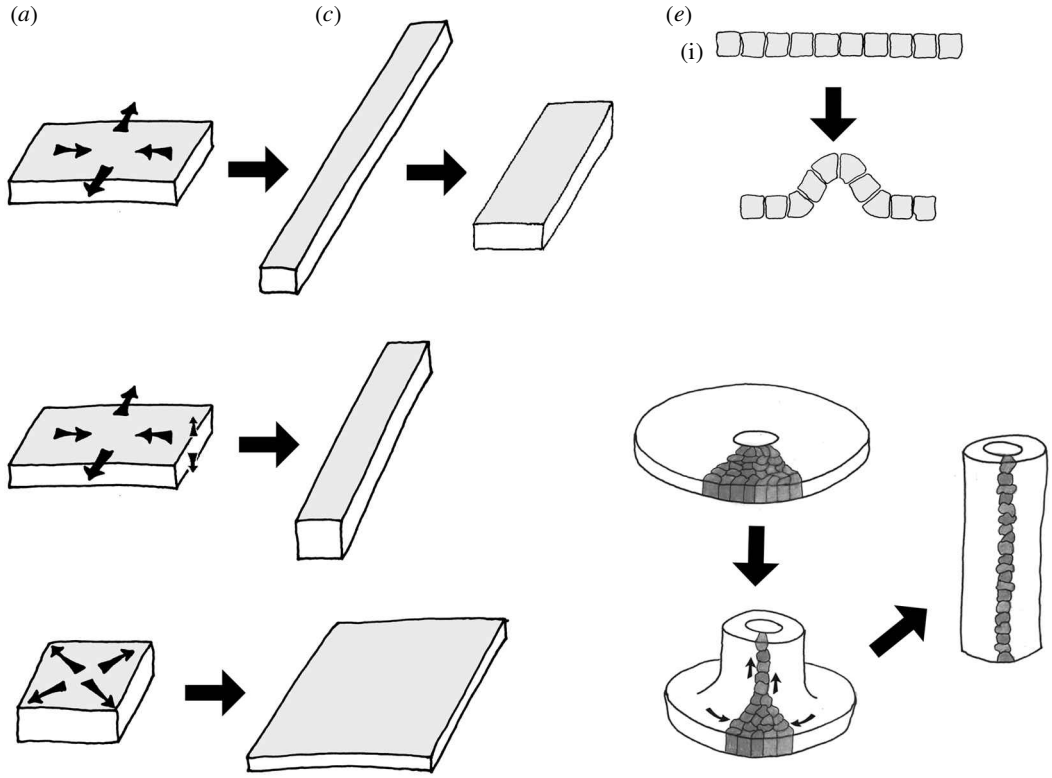

Figure 1. Different types of mass tissue movements, including (*a*) convergence and extension, (*b*) convergence, extension and thickening, (*c*) divergence and shortening, (*d*) epiboly, and (*e*) invagination in (i) sectional view, and (ii) in three dimensions.

'convergent extension' can be used for convenience, but it can be misleading unless one remembers that the relationship between convergence, extension and thickening can be and usually is more complex than this term suggests.

Convergence and extension movements are representative of a larger, more general class of 'mass movements' involving change in tissue proportions with approximate conservation of volume. Divergence and shortening (figure 1*c*), the reverse of convergent extension, occurs during *Drosophila* germ band retraction (Hartenstein & Campos-Ortega 1985). Epiboly, an increase in tissue area by thinning (figure 1*d*), occurs in the animal region of early amphibian (see Keller 1978, 1980) and teleost fishes embryos (Betchaku & Trinkaus 1978), to mention only two examples. Convergence and extension are often major components of what are usually identified as other types of morphogenic processes. For example, invagination of a disc of cells to form a tubular gut, such as primary invagination of a echinoderm vegetal plate to form the gut, is often portrayed as a simple bending in sectional view (figure 1*e*). In fact, such an invagination involves a form of convergent extension, the progressive narrowing of the circumference of the disc and extension along its radius (the length of the tube) (figure 1*e*).

Convergence and extension have been studied most in situations where tissue volume changes due to cell growth does not occur at all or is insignificant, such as in the early development of sea urchins (Hardin & Cheng 1986), amphibians (Burnside & Jacobson 1968; Keller 1986), and flies (Condic *et al.* 1991). However, these movements also occur in embryos that grow dramatically in early development, such as those of the mouse (Snow 1977), and in these cases, tissue volume changes may also come into play. Analysis of the mechanism of convergent extension in organisms that grow rapidly may reveal variations on the themes developed here, specifically the potential involvement of directed, anisotropic growth.

Convergence and extension movements, as well as related mass tissue movements, may be passive responses to forces generated elsewhere in the embryo or they may be active, force-producing processes. We will focus on the active movements here, although the passive ones are also important in morphogenesis, and misunderstanding them can result in erroneous conclusions. For example, it is a common assumption that if a mutation targeted to a particular tissue in the embryo disrupts convergent extension of that tissue, the movement is an active one. However, the movement could be a passive response to force generated elsewhere and the true function of the wild-type gene could be to increase the deformability of the tissue, allowing it to be stretched and narrowed by the active tissue.

(b) *Why study convergence and extension?*

These movements present a major challenge and an opportunity to understand cell function in the morphogenesis of integrated populations. They represent a category of morphogenic processes, the so-called 'mass movements' in which a tissue distorts by virtue of integrated cell behaviours within its boundaries, due to interactions among these cells, rather than interactions with external substrates. Little is known of the cellular, molecular and biomechanical mechanisms of these types of movements, an important deficiency in the light of their dramatic role in shaping embryonic body form in so many systems.

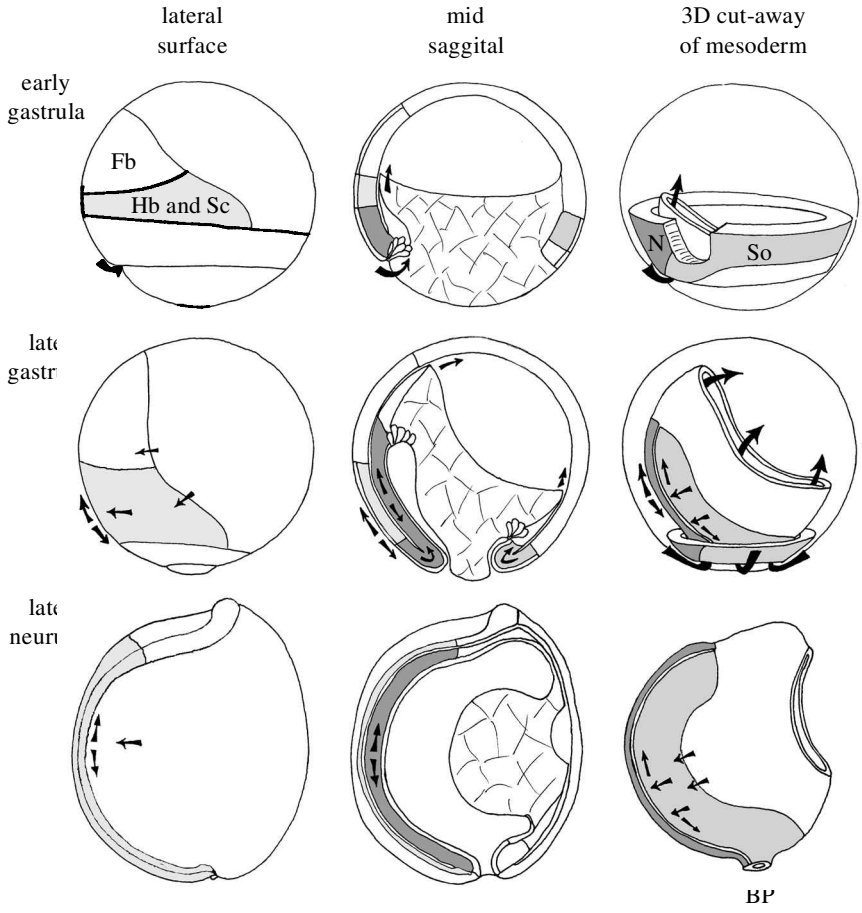

Figure 2. Expression of the convergence and extension movements during gastrulation and neurulation of *Xenopus laevis*. Tissues undergoing convergence and extension include the prospective hindbrain (Hb) and spinal cord (Sc) regions of the neural tissue (light shading), the prospective somitic mesoderm (So, medium shading), and the prospective notochord (N, dark shading). Also shown are the prospective forebrain (Fb), the prospective head mesoderm (HM), and the blastopore (BP).

The conceptual framework for analysis of cell interactions within integrated populations is poorly developed. It is difficult to visualize and interpret cell motility in embryos and thus little is known of the cell behaviour driving these movements. The common paradigm for analysis of cell function in morphogenesis is the study of motility of individual cells in culture at low density crawling on a rigid, transparent substratum. In contrast, most cell movements in embryos involve high densities of cells interacting with one another, or with the extracellular matrix between them. Moreover, the forces individual cells generate have largely local effects on their movement in culture, whereas these forces have both local effects and effects that are integrated over the cell population in embryos. The mechanism for global integration of locally generated forces and the molecular basis of the necessary cell and tissue biomechanical properties are poorly understood in both concept and experimental measurement. Finally, too often the paradigm for genetic and molecular analysis of tissue movements has been compositional rather than mechanistic. Experiments have been focused on interdicting the expression of a gene or the function of a molecule and showing that the gene or molecule it encodes is necessary for a morphogenic process to occur. Collectively, these experiments resolve the composition of the machine rather than how the machine works. Here we will discuss 'morphogenic machines', largely at the cell and cell population level. Cell biology has defined a large number of molecular machines for generating force, for generating cell polarity (Brunner & Nurse, this issue; Schwab *et al*., this issue), imparting mechanical integrity, for regulating motility (Hall & Nobes, this issue), and for imparting cell adhesion (see Takeichi *et al*., this issue), and contact behaviour (see Xu *et al*., this issue).

It is our goal here to define and characterize the cellular machines that connect these molecular machines to massive movements of large coherent cell populations in the embryo.

(c) *The paradox of convergence and extension: mobility in the presence of stiffness*

We will focus on what is emerging as the most common type of convergence and extension movements, those that occur by cell rearrangement. The paradox of this type of convergence and extension is that the cells actively intercalate between one another to produce a change in shape of the tissue while forming a stiff array that can distort surrounding, passive tissues. The major question to be addressed here is how can cells move with respect to one

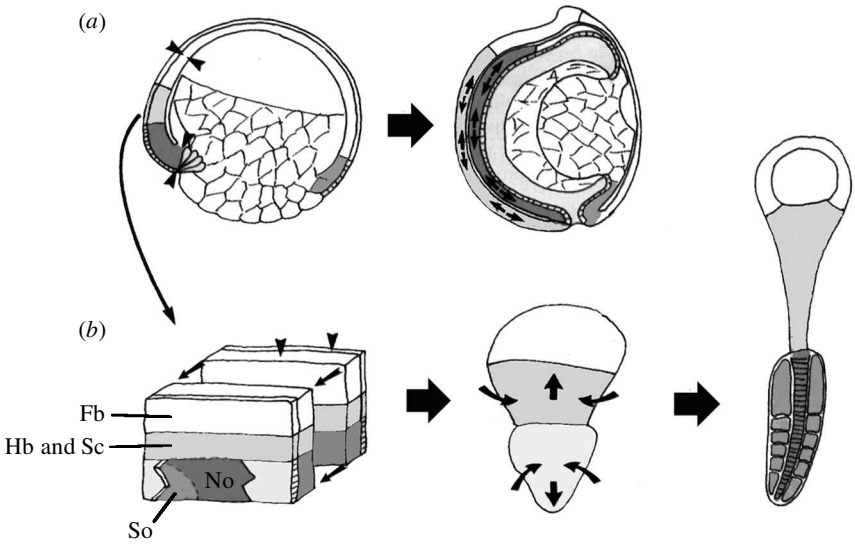

Figure 3. Comparison of the convergence and extension movements in (*a*) the whole embryo, and (*b*) an explant of the dorsal sector of the gastrula. Normally the prospective notochordal (No, dark shading) and somitic (So, medium shading) mesoderm involutes beneath the prospective posterior hindbrain (Hb) and spinal cord (Sc, light shading) and the mesodermal and neural tissues converge and extend posteriorly together (arrowheads in *a*). When the dorsal sectors of two early gastrulae are cut out and sandwiched with their inner, deep cell surfaces together, the explant converges, extends and differentiates in culture without forces generated in the rest of the embryo and without traction on an external substratum (*b*).

another and yet form a stiff tissue capable of pushing and distorting surrounding tissues?

We will discuss convergence and extension movements in the amphibian *Xenopus laevis* in more detail. It is one of two systems in which cell behaviours have been described. It offers at least two examples of convergent extension, one in the posterior mesodermal tissue and one in the posterior neural tissue, consisting of what appear to be variants of a basic mechanism. Analysis of these two systems may reveal common, and perhaps essential, features of the mechanism of convergent extension by cell intercalation. Finally, convergence and extension in amphibians is demonstrably an active, force-producing process, and it is the active forms of these movements that will be focused on here, although those examples of passive responses to external forces are interesting in their own right.

2. EXPRESSION AND FUNCTION OF CONVERGENT EXTENSION DURING FROG GASTRULATION AND NEURULATION

During gastrulation, the prospective somitic (figure 2, medium shading) and notochordal mesoderm (figure 2, dark shading) involute over the blastoporal lip, and as they do so, they converge in the mediolateral orientation, around the circumference of the blastopore, and extend in the anteroposterior orientation to form the elongated body axis (see Keller 1975, 1976; Keller *et al.* 1991*b*) (figure 2). Meanwhile, the posterior neural plate, consisting of the prospective spinal cord and hindbrain (figure 2, light shading) likewise converges in the mediolateral orientation and extends in the anteroposterior axis (figure 2), more or less coincident with the underlying converging and extending mesodermal tissue (Keller *et al.* 1992*a*). Similar movements occur in the urodele (Vogt 1929; Schechtman 1942; Jacobson & Gordon 1976), but they differ in their timing and the degree of tissue distortion.

These movements play major roles in gastrulation and body axis formation. During gastrulation, the forces of convergence form a hoop stress around the blastopore, which squeezes the blastopore shut in the normal anisotropic fashion, towards the ventral side of the embryo (Keller *et al.* 1992*b*). These forces also contribute to involution of the mesodermal and endodermal tissues (Shih & Keller 1992*a*; Keller *et al.* 1992*b*; Lane & Keller 1997), although the vegetal endodermal rotation movements, recently discovered by Winklbauer & Schürfeld (1999), account for much of the early involution movements. Finally, convergent extension also morphologically defines the anteroposterior body axis. The prospective anteroposterior body axis of vertebrates is remarkably short and wide in the pregastrula stages. The dorsal mesodermal and neural tissues converge and extend tremendously during gastrulation and neurulation, pushing the head away from the tail, and thereby morphologically defining the anteroposterior body axis that is so important in getting through life head first (figure 2).

3. AMPHIBIAN CONVERGENT EXTENSION IS AN ACTIVE, FORCE-PRODUCING PROCESS

The first step in analysis of an example of specific convergent extension movement is determining whether it is an active, force-producing process or a passive response to forces produced elsewhere in the embryo. The source of the forces causing these movements in amphibians remained a mystery until explants of tissue actively converged and extended in culture, mechanically isolated from the rest of the embryo (Schechtman 1942; Holtfreter 1944; Jacobson & Gordon 1976; Keller *et al.* 1985). In

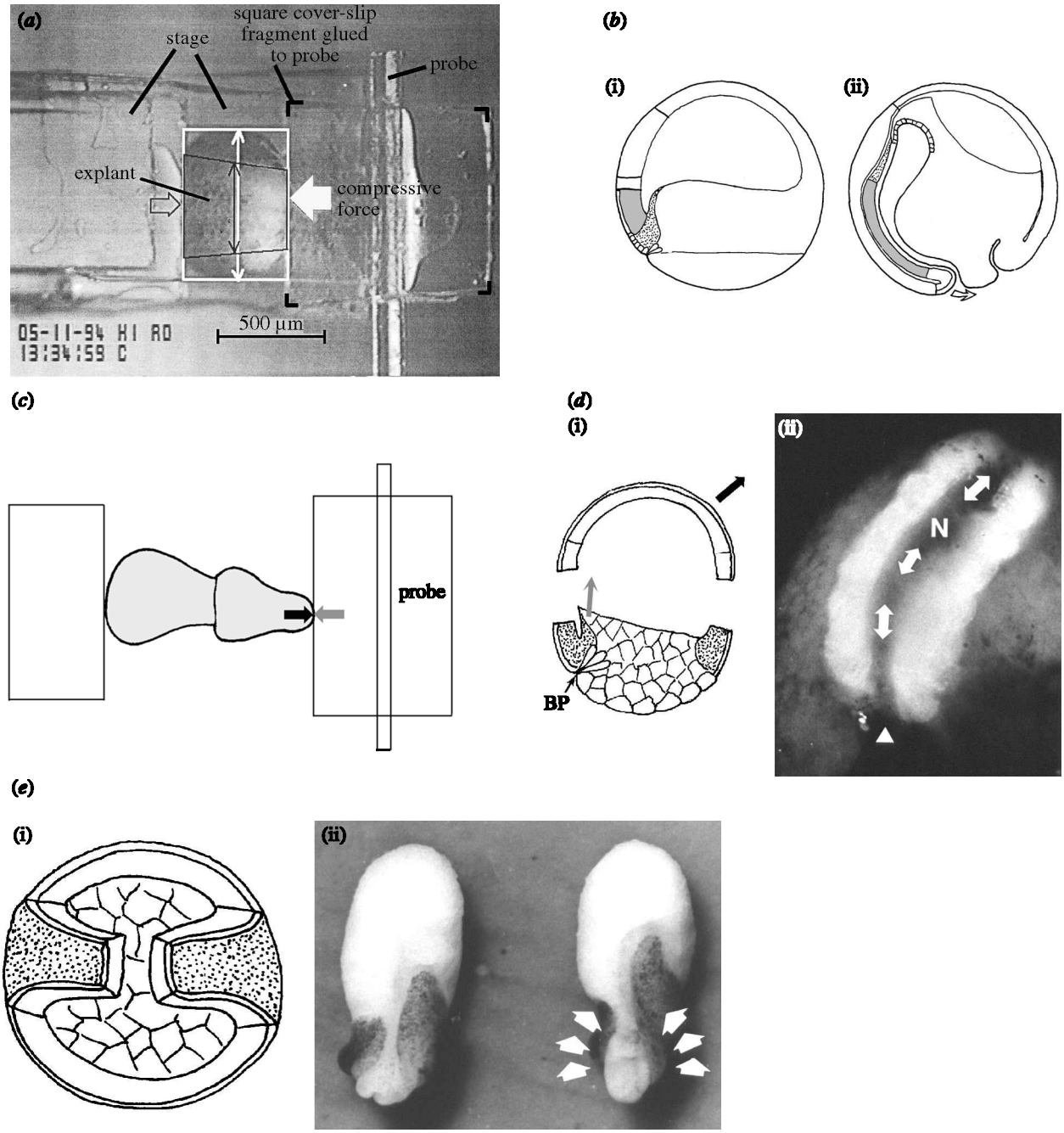

Figure 4. (*a*) An explant between the stage and the probe of a computer-controlled biomechanical measuring machine, the 'Histowiggler' during a compressive stress relaxation test for tissue stiffness (see Moore *et al.* 1995). (*b*) Compression stress relaxation tests compare stiffness (E in N m^{-2} after 180 s compression) of the mesoderm of the early gastrula before involution with stiffness of the same tissue at the late midgastrula stage, after involution. (i) Before involution, $E(180) = \sim 3$–4 N m^{-2}; (ii) after involution, $E(180) = \sim 14$–15 N m^{-2}. The tissues tested are shown in grey shading; the test was done with compression along the anteroposterior axis. The force of extension is measured with an isometric force measurement (*c*) in which a converging and extending explant (shaded) extends against the probe of the Histowiggler with a force (dark arrow) that is balanced by an opposing force from the Histowiggler probe (light arrow) such that the length of the explant does not increase. Force measurement, 1.0–1.2 µN for a sandwich of two and 0.5–0.6 µN for a dorsal mesoderm. (*d*) Convergence and extension of the mesoderm is independent of the blastocoel roof. When deprived of its normal migratory substrate, the blastocoel roof (i), dorsal notochordal (N) and somitic tissue (stained) converge and extend (arrows) without the overlying substrate (ii). The blastopore is indicated by BP or a triangle. (*e*) The extending axial and paraxial mesoderm controls the shaping of the embryo. When a blastocoel roof substrate is offered to the extending dorsal mesoderm, off-axis, at the sides of the embryo (dorsal is at the bottom of the figure) (i), the dorsal mesoderm extends up the midline of the embryo, and the 'substrates' on both sides are pulled dorsally, over the extending mesoderm (ii, arrows).

explants composed of two dorsal sectors of the early *Xenopus* gastrula sandwiched together, the prospective notochordal and somitic mesoderm and the prospective posterior neural tissues converge and extend by internally generated forces, independent of traction on the substrate and of forces generated elsewhere in the embryo (Keller *et al.* 1985; Keller & Danilchik 1988) (figure 3).

(a) *Tissues undergoing convergent extension form a stiff embryonic skeleton that defines the shape of the embryo*

The independent convergence and extension of sandwich explants in culture implies that these tissues must push and exert enough force to overcome their own internal resistance to distortion and to deform the neighbouring, passive tissues in the embryo as well. In fact, the converging and extending tissues stiffen to form an embryonic 'skeleton' capable of pushing forces that elongate the embryonic axis and take a leading role in defining the shape of the early embryo. Moore and associates (Moore 1992; Moore *et al.* 1995) developed a computer-controlled, biomechanical measuring device, the 'Histowiggler' (figure 4*a*), capable of measuring mechanical properties of embryonic tissues and the forces they generate. Uniaxial, compression, stress relaxation tests made with this machine showed that the dorsal mesodermal tissue stiffens in the axis of extension by a factor of three or four during extension (figure 4*b*), and isometric force measurements show that it is capable of pushing with a force of about 0.5 μN (Moore 1992; Moore *et al.* 1995) (figure 4*c*). The onset of this stiffness is regulated such that it occurs after involution of the mesodermal tissues. Patterning the expression of this increased stiffness to the post-involution region appears to be essential for gastrulation. When substantial pieces of the post-involution mesoderm are grafted back to the lip of the blastopore, they will not involute but will sit up on the lip of the blastopore like a canoe on the edge of a waterfall, apparently too stiff to turn the corner (R. Keller, unpublished data).

The role of the stiff, forcefully extending mesoderm in shaping the embryo is illustrated by experiments in which the roof of the blastocoel was removed (Keller & Jansa 1992). Under these conditions, the axial and paraxial mesodermal tissues involute and extend into the liquid medium without an overlying blastocoel roof to serve as a substratum (figure 4*d*). Moreover, these dorsal mesodermal tissues, rather than the blastocoel roof 'substratum', dominate the shaping of the embryo. If fragments of blastocoel roof are offered as substrata for migration in regions to the sides of the axis of extension, these tissues do not change their direction of extension to accommodate the substratum; rather the substratum is pulled over the stiffened, extending tissues (figure 4*e*).

The neural region can also push, although the force it generates has not been measured. When put in serial opposition with the mesodermal component by barricades at both ends of the extending explant, the neural region buckles in the face of extension of the mesodermal region, arguing that the neural region is the least stiff and thus probably weakest extender of the two. In the embryo, the mesodermal tissues are fastened to the overlying neural plate by a strong attachment of the notochord to the region of the neural plate overlying it, the 'notoplate' (Jacobson & Gordon 1976). These attached neural and mesodermal tissues extend together (A. Edlund and R. Keller, unpublished data; Keller *et al.* 1992*a*). In this situation, the force generated should be at least the sum of the forces generated separately, but may be greater if contact between them makes one, the other, or both more forceful in extension.

(b) *Biomechanics of other systems*

It is not clear whether many examples of convergence and extension are active or passive processes, and, if active, how much force they generate. The axial and paraxial tissues of fishes can extend as explants, in isolation from the embryo (see Laale 1982), and they can push into the yolk in teratogenized embryos (see Bauman & Sanders 1984), implying an internal, force-generating process. However, the notochord and somitic files in these explants are often kinked, as if they bucked in the process of extending, suggesting that the converging and extending tissues of the teleost fishes may be less stiff and extend with less force than those of *Xenopus*. The variation in design of early morphogenesis is substantial, even between closely related taxa of amphibians (Keller 2000), and thus mechanical variations of this type should be expected among the vertebrates. Imaginal leg discs of *Drosophila* (see Condic *et al.* 1991) converge and extend during evagination in culture, which indicates an active process. Experimental manipulation and finite element modelling suggests that the archenteron of echinoderms converges and extends by an internal, force-producing process (Hardin & Cheng 1986; Hardin 1988), and mutations affecting germ band elongation in *Drosophila* suggest but do not prove that the same may be true of this process (see Irvine & Wieschaus 1994).

4. AMPHIBIAN CONVERGENT EXTENSION OCCURS BY CELL INTERCALATION

Because convergent extension of axial structures of amphibians occurs in absence of cell growth and the appropriate changes in cell shape, Waddington (1940) suggested that these movements occur by the rearrangement of cells. In fact, morphological studies, tracings of cells with fluorescent labels, and time-lapse recordings of cell behaviour in both mesodermal (Keller & Tibbetts 1988; Keller *et al.* 1989*a*; Wilson *et al.* 1989; Wilson & Keller 1991; Shih & Keller 1992*a*,*b*) and neural regions of *Xenopus* (Keller *et al.* 1992*a*; Elul et al 1997; Elul & Keller 2000) have shown that these tissues converge and extend by two types of cell rearrangement. To understand these rearrangements it is important to know that the regions involved consist of a single layer of superficial epithelial cells and several layers of deep, mesenchymal cells (figure 5*a*). In the first half of gastrulation, the deep mesenchymal cells of the mesoderm (Wilson *et al.* 1989; Wilson & Keller 1991) and the posterior neural tissue (Keller *et al.* 1992*a*) undergo radial intercalation (Keller 1980) in which they intercalate along the radius of the embryo, normal to its surface, to produce a thinner array that is also longer in the prospective anteroposterior axis (figure 5*a*). Radial intercalation of several layers of deep cells to form one is also the cellular basis of the extension of the neural and mesodermal tissues in sandwich

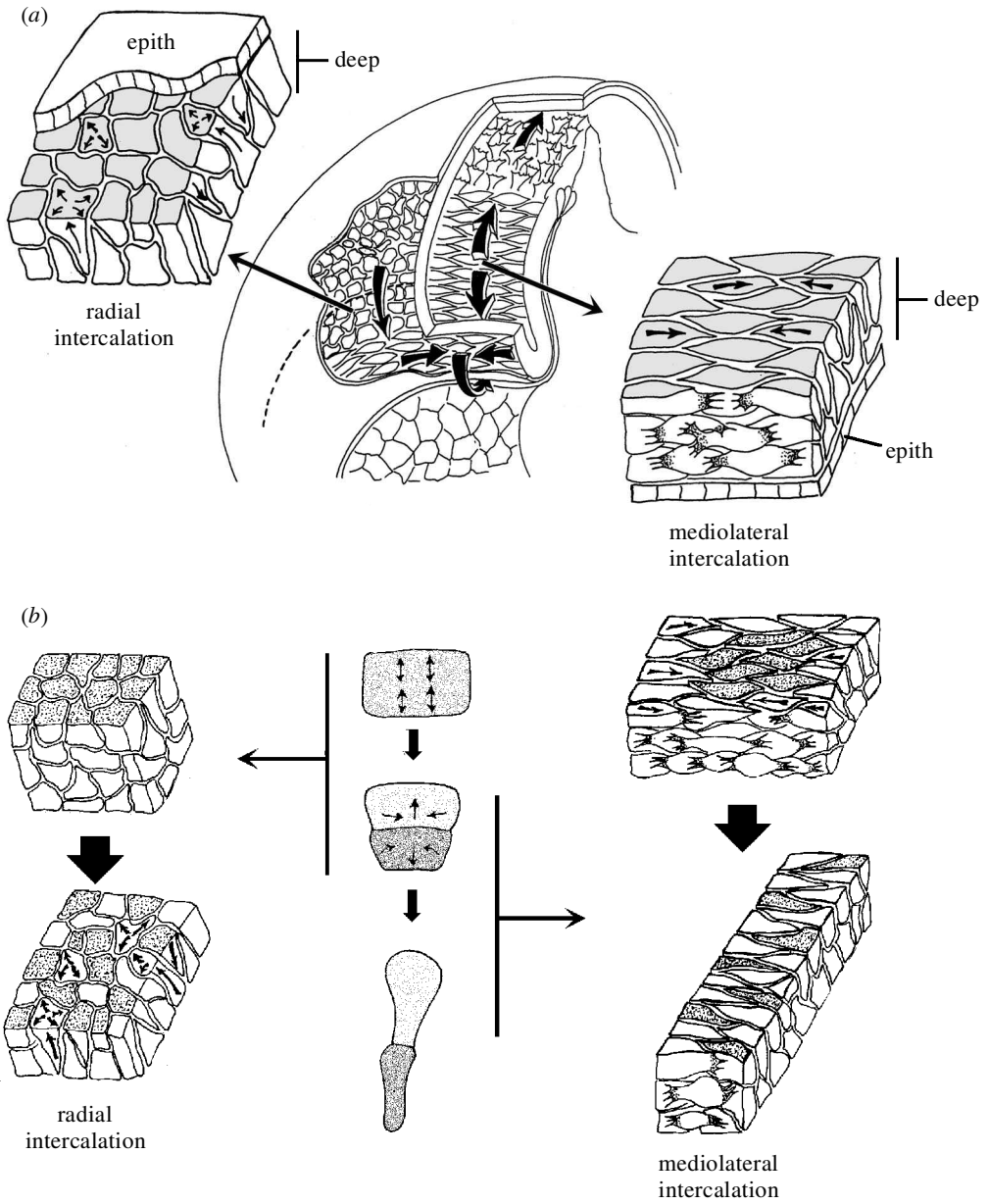

Figure 5. The expression of radial cell intercalation and mediolateral cell intercalation in (*a*) whole embryo, and (*b*) explants. Radial intercalation consists of several layers of deep cells intercalating along the radius of the embryo (normal to the surface) to form fewer layers of greater area. Mediolateral cell intercalation consists of multiple rows of deep cells intercalating along the mediolateral axis to form a longer, narrower array. Radial intercalation occurs first, followed by mediolateral intercalation in both the mesodermal and neural tissues. The overlying epithelial layer (epith) of the embryo is shown. From Keller *et al.* (1991*a*, *b*).

explants (Wilson & Keller 1991; Keller *et al.* 1992*a*) (figure 5*b*). Radial intercalation also occurs in the animal cap region of the embryo, but there the increase in area is more uniform in all directions, rather than primarily along one axis. The mechanism of this difference between the animal cap and the neural and mesodermal tissue is not known.

The superficial epithelial cells do not participate in radial intercalation in *Xenopus*, but spread and divide to accommodate the larger area of the spreading deep cell population (Keller 1978, 1980). This is not the case in many other species of amphibian, particularly the embryos of the urodeles in which the deep cells intercalate between the superficial epithelial cells to form one layer, according to the published work (reviewed in Keller 1986). The intercalation of deep mesenchymal cells outwards, into the epithelium, has never been observed directly, nor is the mechanism known by which this might happen while maintaining tight junctions and an intact physiological barrier.

Following radial intercalation, convergence and extension occurs as the deep cells of the prospective mesodermal and neural tissue undergo mediolateral intercalation by moving between one another along the mediolateral axis to produce a narrower, longer, and usually somewhat thicker array (Keller *et al.* 1989*a*, 1992*b*; Shih & Keller 1992*a*,*b*) (figure 5*a*,*b*). The superficial epithelial cells accommodate this narrowing and extension of the deep cell array by also

intercalating mediolaterally, as well as by dividing and spreading (see Keller 1978).

Mediolateral intercalation is patterned in space and time, occurring progressively in both the mesodermal and neural tissue in the second half of gastrulation and through neurulation. It originates in the anterior, lateral regions of both tissues, and spreads posteriorly and medially (Shih & Keller 1992*a*; Domingo & Keller 1995; Elul & Keller 2000) (see § 9). Mediolateral intercalation of mesodermal cells occurs mostly at and beyond the blastoporal lip in the post-involution region, whereas radial intercalation of mesodermal cells occurs predominantly in the pre-involution region (figure 5*a*,*b*). However, there are exceptions to this rule. In late gastrulation and early neurulation, the prospective somitic mesodermal cells of the lateral and ventral margins of the blastopore undergo radial intercalation, followed by mediolateral intercalation in the post-involution region (Wilson *et al.* 1989). Also, the rapid spreading of the mesoderm–endoderm just after its rotation and application to the roof of the blastocoel appears to involve radial intercalation (Winklbauer & Schürfeld 1999). Mediolateral intercalation appears to be coincident with the stiffening of the post-involution mesoderm described in § 9(a),(c).

(a) *Mechanisms in other systems*

Most examples of convergent extension appear to occur by cell intercalation, although other processes may be involved as well. Cell intercalation contributes to convergent extension of the sea urchin archenteron (Ettensohn 1985; Hardin & Cheng 1986; Hardin 1989), the germ band of *Drosophila* (Irvine & Wieschaus 1994), and the axial, paraxial, and neural structures of ascidians (Cloney 1964; Miyamoto & Crowther 1985), fishes (Warga & Kimmel 1990), birds (Schoenwolf & Alvarez 1989), and mammals (Sausedo & Schoenwolf 1994). In contrast, the imaginal leg disc of *Drosophila* undergoes convergent extension mostly by cell shape change (Condic *et al.* 1991), although cell intercalation may be involved as well. Likewise, change in cell shape, as well as intercalation, plays a role in nematode morphogenesis (Priess & Hirsh 1986; Williams-Masson *et al.* 1997). In addition to cell intercalation, orientated cell division is a factor in convergent extension of the neural plate of birds (Schoenwolf & Alvarez 1989), the early morphogenesis of the fish embryo (Concha & Adams 1998), and *Drosophila* germ band morphogenesis (Hartenstein & Campos-Ortega 1985).

Cell growth could also potentially contribute to increased length during convergence and extension in some systems, such as the mouse, which develop an early yolk sac placenta and thus have an external source of nutrients that allows them to grow substantially during gastrulation and neurulation (Snow 1977). Amphibians, which have no large external supply of nutrients but rely instead on intracellular stores of yolk, do not show a general increase in cellular volume prior to feeding (see Tuft 1962), the exception being the osmotic swelling of the notochordal cells in the tailbud stages (see Adams *et al.* 1990). Teleost fishes (Warga & Kimmel 1990) and birds (see Schoenwolf & Alvarez 1989) probably also do not grow significantly prior to the development of the circulatory system.

5. MECHANISMS OF MEDIOLATERAL CELL INTERCALATION

To understand convergence and extension by cell intercalation we must understand the motility that cells use to move themselves between one another in the face of internal and external mechanical loads. A description of a specific cellular motility, a 'cellular machine', is needed. The cellular basis of mediolateral cell intercalation will be discussed exclusively here, although similar cell motility and mechanical principles may be involved in radial intercalation (see Keller 1980; Davidson & Keller 1999).

What do cells do in order to intercalate? Resolving cell behaviour in a cell population presents several challenges. First, the *Xenopus* embryo consists of an outer epithelial layer and several layers of deep, non-epithelial, mesenchymal-like cells, raising the question of the relative contribution of each to the forces producing convergent extension. For both mesodermal (Shih & Keller 1992*b*) and neural tissue (Elul *et al.* 1997), the deep, mesenchymal cell population alone can produce convergent extension. The superficial epithelial layer of the *Xenopus* embryo has not shown convergence and extension by itself, in explants, but this could be an artefact of explantation and culture conditions. Thus whether the epithelial layer can also contribute to force generation when attached to the deep region in the embryo is not known. For the present, the deep region is the only layer that has been demonstrated to converge and extend by itself.

To visualize deep cell behaviour, the 'open-faced explant' and the 'deep cell explant' were designed to expose these deep cells to modern imaging methods (Keller *et al.* 1985; Keller & Danilchik 1988; Shih & Keller 1992*a*,*b*). The open-faced explant consists of culturing one of the dorsal mesodermal–neural units making up the sandwich explant (figure 3) alone, with the deep cells facing the microscope objective. The deep cell explant consists of just the deep cells of the same embryonic region, isolated at the midgastrula stage (Shih & Keller 1992*b*). In addition, a solution mimicking the composition of the blastocoel fluid (Danilchik's solution) was developed in order to support normal deep cell behaviour in culture (for the original version see Keller *et al.* (1985) and Keller & Danilchik (1988); for a more recent version see Sater *et al.* (1993)).

Low-light fluorescence microscopy and time-lapse recordings of deep cells labelled with fluorescent markers revealed the protrusive activity and cell behaviour underlying mediolateral cell intercalation in the notochord (Keller *et al.* 1989*a*), in the dorsal mesoderm before and after formation of the notochordal–somitic boundary (NSB) (Shih & Keller 1992*a*,*b*), and in the neural tissue under several conditions of patterning (Elul *et al.* 1997; Elul & Keller 2000). From these studies, several major types of cell behaviours were found to underlie active, substrate-independent cell intercalation: (i) a bipolar, mediolaterally orientated protrusive activity in the mesoderm; (ii) a boundary-mediated, boundary-capture mechanism in the notochordal mesoderm; (iii) a monopolar, medially directed protrusive activity in the neural tissue; and (iv) a bipolar, mediolaterally orientated protrusive activity in the neural tissue, which occurs only under abnormal tissue interactions and probably represents a

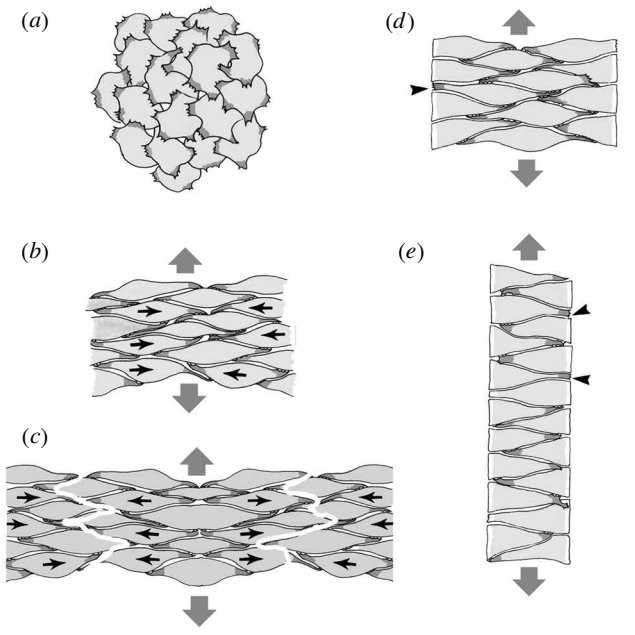

Figure 6. The cell behaviours of mediolateral cell intercalation. (*a*) Cells are initially multipolar and show random, rapid protrusive activity in the early gastrula. (*b*) At the mid-gastrula stage, they show bipolar, medially and laterally orientated protrusive activity, extending protrusions that appear to exert traction on adjacent cells. As a result, they elongate in the mediolateral direction and appear to pull themselves between one another along the mediolateral axis. At the late midgastrula stage, the notochordal–somitic boundary (NSB) begins to form. The decision as to where it will form is made (white line, *c*), but initially it has no morphological identity. Then the boundary takes form as it straightens and the sides of the cells facing the boundary, or entering the boundary, spread on the boundary and cease protrusive activity (light shading, *d*). Once they touch the boundary, they rarely leave it again (boundary capture). The internal notochord cells continue to intercalate to form a narrower array, and they progressively come into contact with the boundaries. (*d*,*e*) As the invasive, tractive ends of the internal bipolar cells contact the boundary (dark shading, arrowhead), they spread on the boundary and become quiescent (light areas). Redrawn from Keller *et al.* (1992*b*).

latent pattern of cell motility that is not used in the embryo.

(a) *Mesodermal cell intercalation: the bipolar, mediolaterally orientated mode*

The prospective dorsal mesodermal cells of *Xenopus* early gastrula initially show multipolar protrusive activity in all directions (figure 6*a*). At the midgastrula stage, when mediolateral cell intercalation and the resulting convergent extension begin, the cells locally adopt a strongly bipolar morphology and show protrusive activity orientated strongly in the medial and lateral directions (figure 6*b*). These protrusions appear to adhere directly to adjacent cells and result in traction on their surfaces. As a result, the cells become elongated and their long axes become aligned parallel to the mediolateral axis of the embryo (Shih & Keller 1992*a*,*b*) (figure 6*b*). The mediolaterally orientated traction of neighbouring cells on one another appears to pull them between one another, thus producing a forceful mediolateral intercalation that serves to narrow and elongate the tissue (figure 6*b*).

(b) *Mesodermal cell intercalation: the monopolar, boundary-capture mode*

At the late midgastrula stage, the NSB forms within this array of bipolar, mediolaterally intercalating cells. At this point, a second, boundary-associated mechanism of mediolateral cell intercalation comes into play. Tracings of the NSB backwards in time-lapse recordings (Shih & Keller 1992*a*) show that the prospective boundary is an irregular line, passing in zig-zag fashion between the future notochordal and somitic cells. The boundary takes form as this line straightens and a smooth interface is formed between the notochordal and somitic cells (figure 6*c*). Notochordal cells that initially form the boundary bleb for a short time and then cease protrusive activity at their boundary ends, forming a flat surface in the plane of the boundary (figure 6*d*). Very few of these cells leave the boundary once they are in contact with it, and thus they are 'captured' by the boundary, resulting in its elongation (Shih & Keller 1992*a*) (figure 6*d*,*e*). Meanwhile, the protrusive activity characteristic of the former bipolar state continues at the inner (medial) ends of these cells, which is thought to exert traction on the interior notochordal cells, thus pulling them into the boundary. The cells in the interior of the notochord continue to intercalate using the bipolar mode, thus progressively converging the notochord and bringing its interior cells into contact with the boundary where they also undergo 'boundary capture'. These processes continue until ultimately the notochord has extended greatly and converged to less than two cells in width (figure 6*e*) (see Keller *et al.* 1989*a*). Note that this mode of cell intercalation involves modification of the bipolar orientated form to a monopolar, directional form of intercalation behaviour in which one end of the cell, the boundary end, is quiescent and the other continues traction on adjacent bipolar cells that have not yet contacted the boundary. Boundary capture was invoked originally by Jacobson and associates as a boundary-mediated mechanism of elongating the nervous system (see Jacobson *et al.* 1985, 1986; Jacobson & Moury 1995).

(c) *Misunderstandings*

Several points of confusion have emerged over the years since these behaviours were first described (Shih & Keller 1992*a*,*b*). First, the bipolar mode of mediolateral cell intercalation is an independent mechanism of intercalation that occurs before the boundary-capture mechanism comes into play. Its first expression is in the vegetal alignment zone (VAZ) (discussed in § 9(a)), which forms before the notochordal–somitic mesodermal boundary. Moreover, the bipolar mode continues within the interior, non-boundary regions of both the somitic and notochordal regions after formation of the NSB. Second, evidence thus far suggests that the bipolar mode consists of an orientated behaviour, not a directional behaviour. There is no evidence that the medial and lateral ends of the cells differ in any way, nor is there evidence that they are undergoing a 'directed migration'. In fact, their activities appear to be balanced and equivalent in the medial and lateral directions, and we make the

argument below (§6(g)) that this balance is necessary for convergent extension to occur. Thus the behaviour is orientated parallel to the mediolateral axis but it is not directional. In contrast, the monopolar mode of intercalation is a directional behaviour with a stabilized lateral end and the tractional protrusive activity pointed medially. Third, the exercise of the bipolar mode within the notochord will inevitably bring all the internal notochord cells into contact with the boundary without any bias in the motility of these cells toward the boundary. Fourth, it is an attractive idea that the bipolar, internal notochordal cells recognize a signal emanating from the boundary at some point in their inevitable movement towards it, and accelerate their movement in that direction. However, there is no evidence supporting this notion. If such an acceleration occurs, it must occur very close to the boundary and will require a more detailed statistical analysis of rates of movement with distance from the boundary than has been done this far.

(d) *Neural cell intercalation: pattern-specific modes—the bipolar and the monopolar modes*

As noted above, the neural tissue of *Xenopus* first extends and thins by radial intercalation and then actively converges and extends using mediolateral cell intercalation (Keller *et al.* 1992*b*). As with the mesoderm, the cell behaviour driving radial intercalation has not been observed and remains unknown.

Turning to the mediolateral intercalation, Elul and associates (Elul *et al.* 1997; Elul & Keller 2000) found that the deep neural cells are capable of two mechanisms of convergence and extension, depending on degree of vertical interaction with the underlying mesoderm. Deep neural tissue explanted into culture from a late midgastrula shows no morphological or behavioural regionalization (figure 7*a*,*c*). In contrast, deep neural tissue explanted with the underlying notochordal and somitic mesoderm shows the normal subdivision into a medial notoplate, which is that part of the neural plate overlying the notochord (see Jacobson & Gordon 1976), and a lateral neural plate (figure 7*b*,*d*).

The deep neural explant undergoes mediolateral cell intercalation and convergent extension using a bipolar form of cell behaviour similar to that seen in the mesoderm (figure 7*c*). However, it differs in having a greater frequency of formation of new protrusions per hour compared with mesodermal cells (Elul *et al.* 1997). It is also more episodic; instead of elongating mediolaterally and maintaining that shape, as the mesodermal bipolar cells do, the neural cells extend protrusions, elongate and then shorten again in more of an inchworm fashion (see Elul *et al.* 1997). These differences could reflect differences in how forces are generated by the cells. The mesodermal cells may exert relatively continuous traction, using short, local extensions, attachment, and retraction of protrusions, with the retractive force reflecting a local contractile event and short episodes of traction. In contrast, the neural cells appear to extend further, shorten more, and involve more of the whole cell in an active contraction.

The explants of deep neural tissue made with underlying mesoderm show a dramatically regionalized cell behaviour, the regions corresponding to the notoplate,

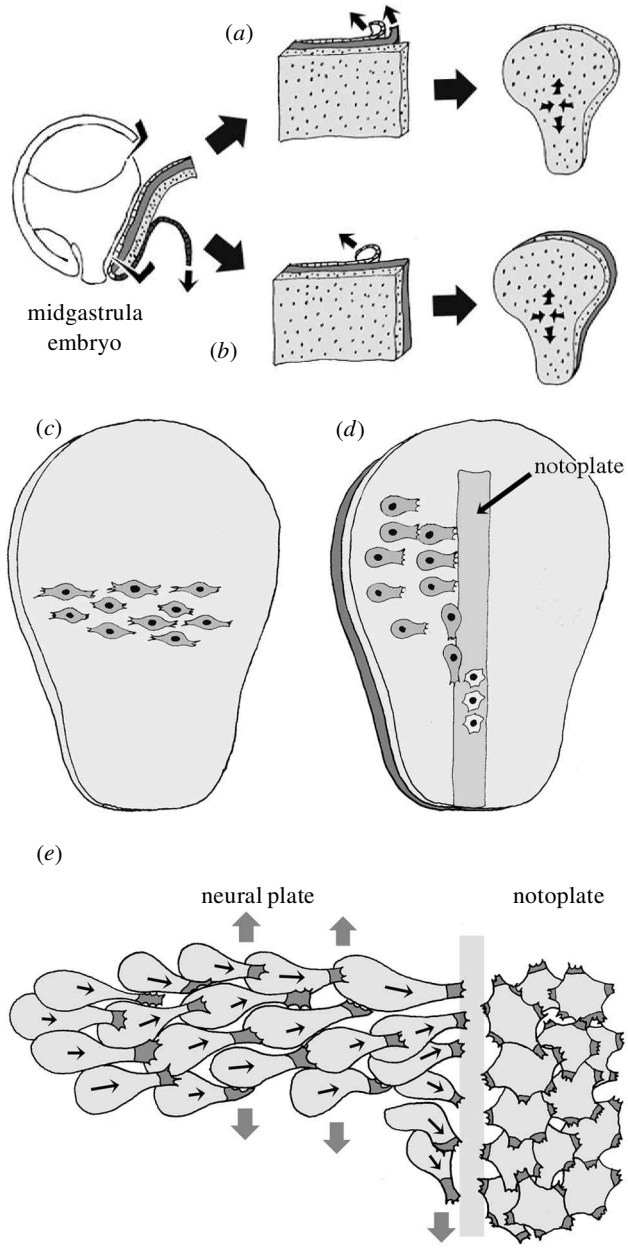

Figure 7. Two types of explants were used to study neural cell intercalation. (*a*) The deep layer of the midgastrula neural plate was isolated by removing both the underlying mesoderm and the outer epithelial layer of the neural plate. (*b*) The deep neural-over-mesoderm explant was made by excising the dorsal mesoderm along with the neural plate and removing both the outer neural epithelial layer and the endodermal epithelial layer from the mesoderm. (*c*, *d*) Both explants expose the deep cells to time-lapse recordings of cell behaviour. (*c*) In deep neural explants, convergence and extension occurs as the cells intercalate using a bipolar mediolaterally orientated protrusive activity. (*d*) In the deep neural-over-mesoderm explants convergence and extension occur as deep neural cells show a monopolar, medially directed protrusive activity in the lateral neural plate. In the notoplate, the cells show multipolar, randomly orientated protrusive activity. (*e*) In these explants, monopolar, medially directed protrusions appear to exert traction on more medial cells and pull themselves between medial neighbours to form a longer, narrower array. At the neural plate–notoplate boundary (shaded bar), some of the neural plate cells turn posteriorly but none enters the domain of the notoplate. From Elul & Keller (2000).

the neural plate, and the boundary between them (Elul & Keller 2000) (figure 7*d*). The deep neural plate cells, located on both sides of the midline notoplate, show a monopolar, medially directed protrusive activity (figure 7*d*,*e*). The monopolar medially directed protrusive activity appears to exert traction on adjacent cells and pull the cells between one another in a fashion similar to that seen in the bipolar mode, but in this case the motility is directed towards the midline (figure 7*d*,*e*). In contrast, the midline notoplate cells show a multipolar, randomly orientated protrusive activity (Elul & Keller 2000) (figure 7*d*,*e*).

The boundary between the notoplate and neural plate is not a physically well-defined border like that seen between the notochordal and somitic mesoderm (see Shih & Keller 1992*a*). However, cells do not cross the boundary in either direction after stage 11, the midgastrula stage, in explants (Elul & Keller 2000) or in the embryo (A. Edlund and R. Keller, unpublished data). Cells of the neural plate that approach the notoplate–neural plate boundary either stop, or they turn posteriorly and show protrusive activity in that direction (figure 7*e*). It is possible that this behaviour results in a shearing force of the neural plate against the notoplate at the boundary between them, tending to pull the neural plate posteriorly, but there is no evidence for or against this notion.

(e) *The notoplate: attachment, directional spreading and towing*

The notoplate is an isolated cell population after stage 11, the midgastrula stage, when it nominally consists of a deep and a superficial layer. Deep cells will not cross the notoplate–neural plate boundary in explants (Elul & Keller 2000) or in embryos after this stage (A. Edlund and R. Keller, unpublished data). In dissections of living embryos, the deep cells of the notoplate are tightly joined to the underlying notochord from stage 11 to 11.5 (late midgastrula) onwards, whereas the lateral neural plate is much more easily removed from the underlying somitic mesoderm (R. Keller, unpublished data). As the deep layer of cells of the two-layered neural plate undergoes radial intercalation to form the single layer of cells making up the neural tube, nearly the entire floor plate is formed from these tightly attached deep cells of the notoplate (A. Edlund and R. Keller, unpublished data). The notoplate cells show multipolar protrusive activity in all directions. It is difficult to see how the notoplate would actively converge and extend using this type of isotropic protrusive activity. When challenged with notochord and somitic tissue as substrates, the deep notoplate cells, as well as the intact tissue, spread on the notochord (R. Keller and A. Edlund, unpublished data). Thus one possibility is that in the embryo the notoplate cells may spread posteriorly on the notochord as the notochord elongates in that direction, and thus be towed along by the underlying notochord. The neural plate, attached to the lateral aspect of the notoplate, would then, in turn, be towed along by the notoplate. This towing would be in addition to its endogenous convergent extension driven by the monopolar mode of intercalation described above (§5(d)). These are speculations. The contribution of passive towing of the neural tissue by virtue of its attachment to the extending notochord, the contribution of active migration of the notoplate posteriorly on the extending notochord, and the contribution of active extension of the notoplate–neural plate should be determined as part of a comprehensive mechanism of neural extension.

(f) *Differences between the bipolar and monopolar modes of neural cell intercalation*

The medially directed, monopolar mode of neural cell intercalation differs in several ways from the bipolar, mediolaterally orientated mode. First, the monopolar mode results in a conservative pattern of cell intercalation with mostly nearest neighbours intercalating with one another at any one time. In contrast, the bipolar mode results in a promiscuous pattern of intercalation in which cells mix with neighbours further afield and more often (Elul & Keller 2000). Second, the bipolar mode seems less efficient than the monopolar mode in producing convergent extension. Extension is usually less in the deep neural explant, which uses the bipolar mode, than in the deep neural tissue plus mesoderm explant, which uses the monopolar mode. This difference may be due to inherent properties of the two modes of neural cell intercalation. In the bipolar mode, the neighbour changes appear more chaotic and involve relatively rapid, long-distance movements of cells first in one direction and then the other along the mediolateral axis, often resulting in exchange of places along the mediolateral axis without producing net convergence and extension. We think that the bipolar mode depends on balanced traction, which may make it inherently a less stable and efficient mechanism of intercalation than the monopolar mode, which, of course, does not require balanced traction (see §6(a)). On the other hand, the bipolar mode, as it is expressed in the mesoderm, appears to be more efficient at producing convergent extension than the neural bipolar mode. One explantation for this difference may be that the neural bipolar mechanism is a latent mechanism, needed as an underpinning for the definitive monopolar mode but never used alone in normal development (see below), and not capable of producing efficient convergent extension by itself.

Alternatively, or perhaps in addition, the deep neural-over-mesoderm explants may extend better than the deep neural explants because of the possible towing of the notoplate by the underlying notochord or the possible active posterior spreading of the notoplate referred to above. Resolving these issues depends on being able to design an explant in which the mesoderm is allowed to induce the monopolar intercalation behaviour, but then is removed so that the notochord cannot act as a substrate or as a towing device. This is now possible.

Finally, the episodic bipolar, mediolaterally orientated mode of neural cell intercalation is probably not used in normal development of the embryo. This mode of intercalation behaviour occurs only in explants developing under the transient planar and vertical signals from the organizer tissue that function during the first half of gastrulation (Elul *et al.* 1997; Poznanski *et al.* 1997; Elul & Keller 1999). The monopolar, directional protrusive activity emerges only with persistent, vertical interactions between mesoderm and the overlying neural tissue. Thus in the embryo, the bipolar mode is either not used, or

used very briefly during midgastrulation before the monopolar mode comes into play.

Why then is the bipolar mode of neural cell intercalation expressed at all under the influence of transient interactions in the early gastrula stage? If it is used little or not at all in the embryo, why has the subcellular machinery essential for its expression persisted, and why does it emerge as a functioning mode of cell intercalation when deep neural tissue develops under abnormal signalling? Perhaps the bipolar mode is preserved because expression of the monopolar mode depends on components of the bipolar machinery. If so, the bipolar mode may be a latent mechanism, never used for its own sake but retained only as a prerequisite for the monopolar mode. Under the simplified planar and transient signalling regime under which the deep cell explant develops, the bipolar neural mode of intercalation re-emerges as an independent mechanism. We view the normal mechanisms of morphogenesis as products of much evolutionary history, with parts of some earlier versions of morphogenic machines used as underpinnings for the extant mechanisms. Manipulating signalling pathways and tissue interactions in embryos may uncover these latent machines as separate mechanisms, which perhaps no longer work all that well by themselves.

(g) *Local motility, global displacement: the efficiency of cell intercalation as a mechanism of convergent extension*

Convergent extension by cell intercalation is a highly efficient mechanism of translating local cell traction into global displacement of cells. There is the small, local displacement of a given cell with respect to its neighbours, due to its own motility, and there is the much larger global displacement of the cell that occurs by virtue of the cell's membership in the aggregate. The integration of small local movements over the tissue makes the latter movements very large. If each cell moves an average of half a cell diameter with respect to its neighbours in the mediolateral direction, the length of the tissue will be doubled and its width halved. For this reason, the first direct visualization in time-lapse recordings of cell intercalation as a mechanism of convergent extension was subtle; looking locally, not much seemed to be happening, but the displacement of the cells overall was great (Keller *et al.* 1989*a*; Shih & Keller 1992*a*,*b*). Note that in the *Xenopus* mesoderm, cell shape change is counterproductive in producing convergent extension; the cells are elongated in the dimension of convergence and shortened in the dimension of extension. Nevertheless, multiple rounds of cell intercalation are very effective and efficient in elongating and narrowing the axial and paraxial tissues.

Finally, a cell's apparent speed and direction of movement is determined by the reference point used in visualizing converging and extending tissues. If, in the case of the extending neural tissue, for example, the position of the forebrain is held constant, cells progressively more posterior in the axis will appear to move posteriorly at greater rates, although the local motility of the cells may be exactly the same as those cells further anteriorly that appear to be moving more slowly. If the posterior end of the neural plate is used as the reference point, cells appear to move anteriorly, and the rate of displacement in regard to anteroposterior position is reversed. These considerations should be taken in to account when describing examples of convergent extension.

6. A CELL–CELL TRACTION MODEL OF CELL INTERCALATION

Here we hope to identify the important properties of cells and the interactions between cells that will allow them to intercalate forcefully and yet maintain a stiff array capable of generating the pushing forces described above (§3(a)). Concepts about how the motility and the adhesion of cells are related to a force-producing convergent extension on the part of the tissue as a whole are poorly developed. To address this deficiency we will describe a model of cell intercalation, specifying parameters of cell adhesion, cell traction, and cell contact behaviour that are based on and account for experimental observations of cell intercalation. We consider the mechanisms proposed to be working hypotheses to guide further observations and experiments.

It seems wise at this point to consider both cell–cell and cell–matrix-mediated intercalation mechanisms, because both seemed to be involved. Supporting the cell–cell traction model, the intercalating cells appear to be exerting traction directly on the surfaces of neighbouring cells in both the mesoderm (Shih & Keller 1992*a*,*b*) and in the neural plate (Elul *et al.* 1997; Elul & Keller 2000). In addition, there is evidence that specific protocadherins in the axial (Yamamoto *et al.* 1998) and paraxial mesoderm (Kim *et al.* 1998) may be involved in convergent extension, which, in its simplest interpretation, implies a cell–cell-mediated process. In scanning electron micrographs (SEMs) connecting protrusions seem to be applied directly to adjacent cells without intervening matrix (figure 9; Keller *et al.* 1992*b*). However, there is also evidence that cell–matrix interactions are involved in NSB-mediated intercalation (P. Skoglund, unpublished data; see §8), and perhaps in other regions of the mesoderm as well.

(a) *Cell–cell traction model requires regionalized function: tractive protrusions at the ends of the cells and the cell body serving as movable substrata*

Both the monopolar and bipolar modes of cell intercalation require the cell body to serve as a substratum, thus providing stable anchorage for the tractive protrusions that pull the cells between one another (figure 8*a*,*b*). Moreover, each cell must restrict its tractive protrusive activity to the medial and lateral ends in the case of the bipolar mode (figure 8*a*), and to the medial ends in the case of the monopolar mode (figure 8*b*). Thus each cell has a dual function: one or both ends of the cell exerts traction on a substratum, and at the same time, the surfaces of the cell bodies, especially the apposed anterior and posterior surfaces, serve as 'movable substrata'. Intercellular traction must be confined to the end(s) of the cells. When 'tractoring' is spread over the cell body, such as in the cortical tractor model of cell movement discussed in §6(f), the substrate function of the cell body is pre-empted, no stable substratum is available, and the cell–cell traction model will not work. Based

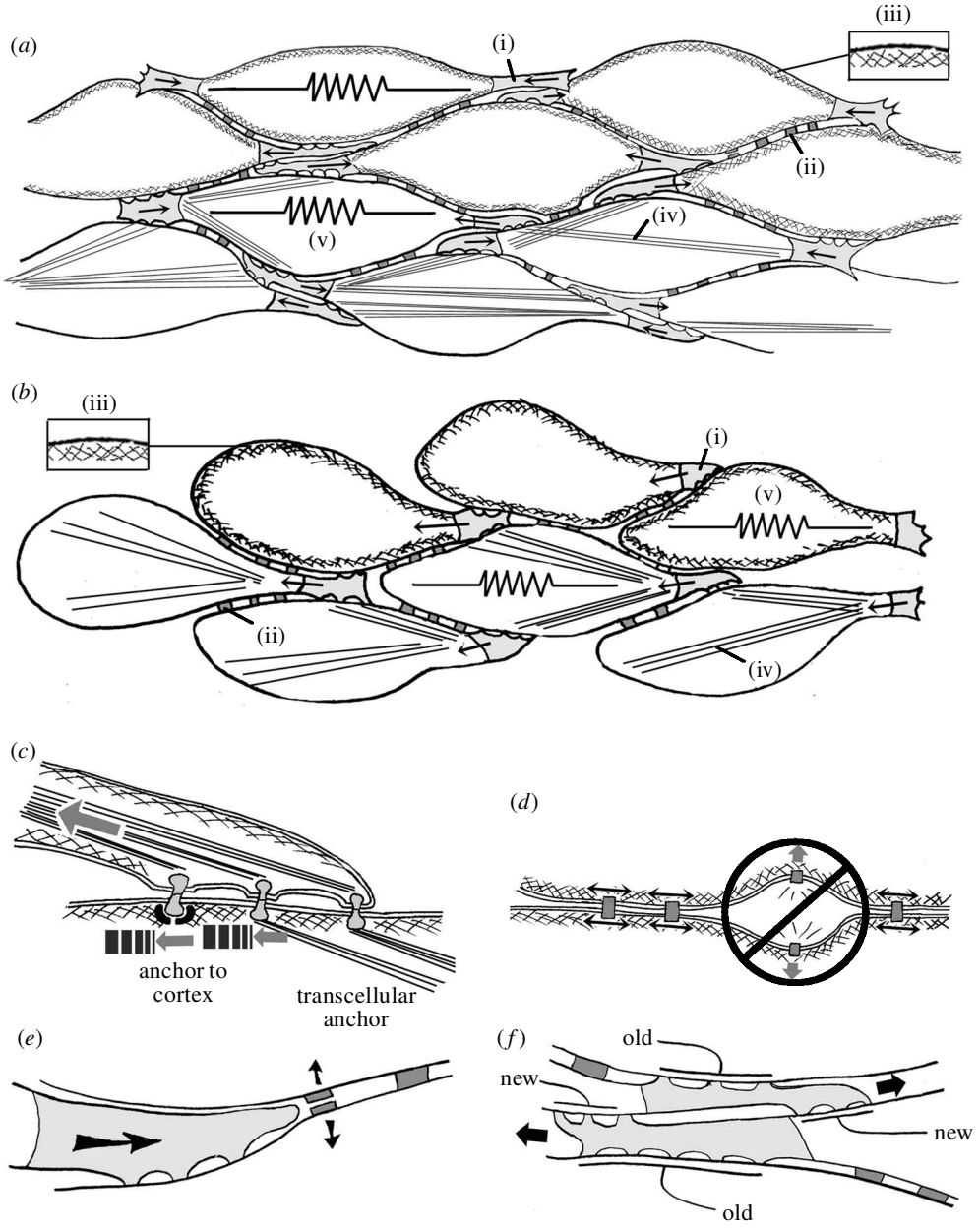

Figure 8. The cell–cell traction model of mediolateral cell intercalation for (*a*) the bipolar, and (*b*) the monopolar modes of intercalation. The elements of this model are (i) large tractive protrusions (light shading, *a*, *b*; details in *c*) at both the medial and lateral ends of the cells in the bipolar mode of intercalation (*a*) or at the medial ends of the cells in the monopolar mode (*b*); (ii) many small 'stiffening adhesions' across the cell body (dark shading in *a*, *b*; details in *d*); (iii) a stiff cortex of the cell body which serves as a substrate by offering adhesions to tractive protrusions that resist tangential displacement (*c*), or (iv) cytoskeletal elements spanning the cell between tractive adhesions (*a*, *b*); and (v) an elastic or contractile element of the cytoskeleton spanning the length of the cell. The large tractive protrusions should induce disassembly of the stiffening adhesions (*e*) but pass one another without interacting (*f*).

on time-lapse recordings of mesodermal cell behaviour (Shih & Keller 1992*a*,*b*), the tractive force appears to be generated locally. These cells show short advances and retractions of the tractive protrusions at the medial and lateral ends, tugging on and distorting neighbouring cells in the process. In the neural cell intercalation behaviour, which is made up of larger, more episodic extension and retraction cycles, traction may involve contraction of a larger part of the cell body. These cells show a greater change in aspect ratio in a cyclic fashion (see Elul *et al.* 1997), suggesting that the traction-generating machinery may involve more of the cell body. Nevertheless, the traction seems to be confined largely to the protrusions at one or both ends, rather than along the entire cell body, based on distortions of adjacent cells in response to protrusive activity of a given cell.

(b) *The cell body as a substratum: an attachment for tractive protrusions that will resist tangential deformation* (*shear with respect to the cell surface*)

As a substrate, the cell surface must offer resistance to deformation parallel to the cell surface (resistance to shear) under the traction of adjacent cells (figure 8*c*). The stiffness that defines the substrate function of the cell

body could be a permanent property of the cell cortex, or this property could be contact induced, specifically by contact of a tractive protrusion on a cell body. Such resistance to shear must involve anchorage of the cell adhesion molecules to the cytoskeleton. We envisage that the relevant cytoskeleton could be a cortical cytoskeleton, perhaps permanently in place, or a transcellular cytoskeleton induced by the tractive adhesions as they advance (figure 8*a*,*b*). We do not know how the cytoskeleton of the mesodermal and neural cells is organized in the three-dimensional tissue array while they are intercalating. Visualizing the cytoskeleton in these large yolky cells is difficult but such studies should be done.

Arguing from results in other systems, the resistance to deformation on the part of the cell body could be induced by the forces exerted on the cell body by the tractive protrusions. The attachment of integrin matrix receptors to the cytoskeleton is strengthened by resistance offered to the movement of a matrix-coated bead (Choquet *et al.* 1997). The identity of the receptor–ligand system involved in the adhesion of the putative tractive protrusions to the cell body are not known, and therefore it is not known if such a response is likely among intercalating cells. However, time-lapse recordings of pairs of fluorescently labelled, intercalating cells often show repeated attachments and tugging on one another, with the substrate cell body seemingly offering greater resistance on repeated tuggings (R. Keller, unpublished data). These observations are anecdotal and should be pursued in a formal way, perhaps using two-colour fluorescent time-lapse imaging to distinguish better the deformation of the cell exerting traction and the one offering its cell body as substrate.

A better way to approach the problem of both tractive force and resistance of the substratum to shear would be to test both using mechanical manipulation of beads coated with the appropriate ligands. However, an important missing piece of information for analyses of this type is the identity of the cell adhesion system involved in the traction events. This information is needed because the response of the system to mechanical stress is likely to be specific to the adhesion system. The analysis of mechanical aspects of regulating adhesion molecule or matrix receptor location, function and attachment to the cytoskeleton promises to be complex (see Evans *et al.* 1991; Lee *et al.* 1993; Choquet *et al.* 1997), and perhaps all the more interesting in the specialized mechanical environment of the intercalating cells.

A common and important element in our model of both monopolar and bipolar modes of intercalation is that each cell spends a high proportion of time functioning as a tension-bearing element in a chain of tension-bearing elements. The tensile elements forming the cortex or spanning the cell cytoplasm, must link the traction sites of other cells on the body of a given cell to that cell's own tractive protrusions or to traction sites of another cell pulling from the opposite direction (figure 8*a*,*b*). A novel feature of this chain is that the links (the cells) can move, thus shortening the chain, but the movement of the links must not break the chain. In the case of the bipolar mode, this means that the cells must show continuous, balanced traction on the medial and lateral ends. If the traction of a cell becomes substantially unbalanced, it will move in the direction of the strongest traction, and the chain is essentially broken. And if many cells become unbalanced, randomly, in the medial or lateral direction, the cells will simply exchange places, rather than shorten the chain. In the case of the monopolar mode, traction must be strongly and constantly biased in the medial direction. Again, if cells show mixed, medially directed and then laterally directed traction, a medially or laterally directed 'migration' will occur, again resulting in exchange of places without extension-producing intercalation.

(c) *The paradox of stiffness and pushing in the presence of neighbour exchange requires a special type of adhesion, the 'stiffening adhesion'*

As noted above (§3(a)), the dorsal mesodermal tissue is stiffer in the anteroposterior axis by a factor of three to four during convergent extension (Moore *et al.* 1995), which allows it to push with a force of about 0.5 μN (Moore 1992). Stiffness is necessary because if a tissue is to push, it must resist bending under the load imposed by internal resistance and the resistance of surrounding tissues. To form a stiff tissue, the cells composing the tissue must resist deformation, and they must resist rearrangement under an external load. However, at the same time, mediolateral cell intercalation must occur in order for the tissue to extend.

How then do cells of a tissue exchange neighbours while the tissue remains stiff enough to support a compression load? The cells must be attached tightly to one another in such a way that they resist exchange of neighbours under external loads but can exchange neighbours as a result of the internally generated, tractive forces proposed above. This requires a second, special type of cell–cell adhesion, the stiffening adhesion.

Stiffening adhesions are predicted to lie along the anterior and posterior surfaces of the cell bodies (figure 8*a*,*b*) where they join cells together, fore and aft, and resist separation normal to the surface of the cell (figure 8*d*). However, these stiffening adhesions must offer little resistance to tangential movements (parallel to the cell surface) in order to allow the cells to slide by one another during cell intercalation (figure 8*d*). Moreover, as a cell advances between two others, and exchanges neighbours, it must assemble new stiffening adhesions with its new neighbours. Thus at the ends of the cells there should be zones of regulated assembly (or disassembly) of the stiffening adhesions. Finally, the stiffening adhesions must also allow the tractive protrusions to pass through them, and thus they should show a contact-induced disassembly on approach or on contact by the large, advancing, tractive protrusions that pull cells between one another (figure 8*e*). It is important to emphasize that we do not expect this disassembly to be due to mechanical forces pulling the adhesions apart, but a contact-mediated controlled process of disassembly.

(d) *Rules of contact-mediated regulation of protrusive activity and adhesion*

The invasive, tractive protrusions should not show mutual contact inhibition of advance or contact-induced retraction, when meeting head-on, but should be able to pass by one another (figure 8*f*). Likewise, they should not show contact inhibition by the cells' surfaces serving as

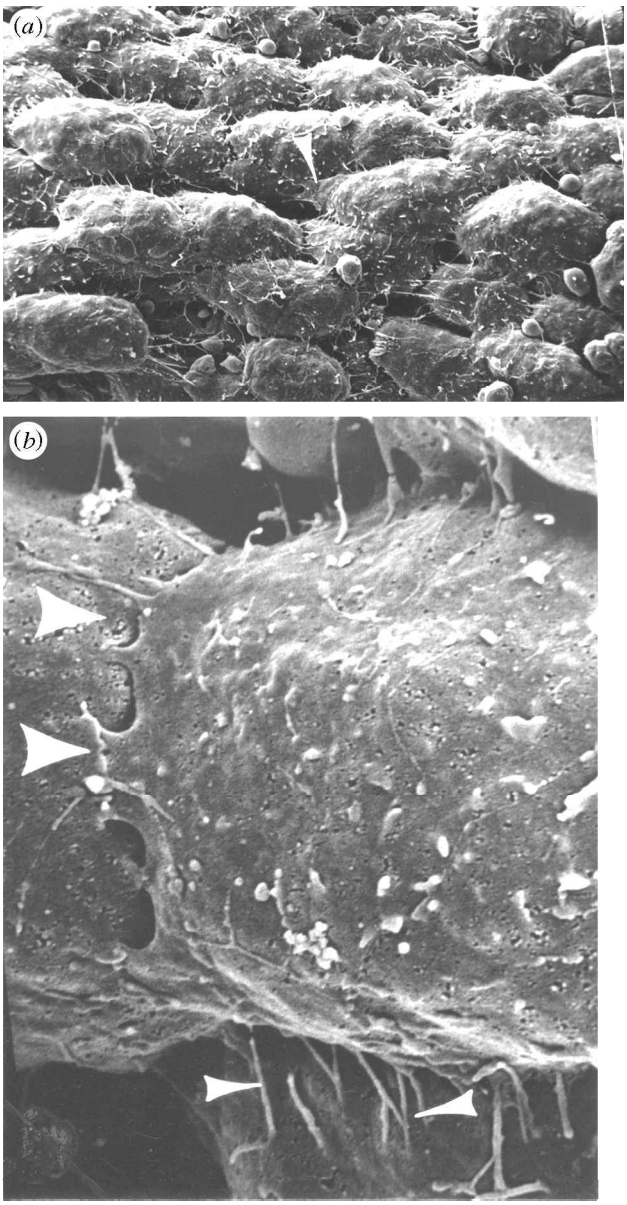

Figure 9. Scanning electron micrographs at (*a*) low and (*b*) high magnification show large lamelliform tractive protrusions at the medial and lateral ends of elongated and aligned mesodermal cells (arrowhead, *a*; large arrowheads, *b*), and numerous small adhesions, mediated by filopodia, at the anterior and posterior surfaces of these cells (small arrowheads, *b*). From Keller *et al.* (1989*b*).

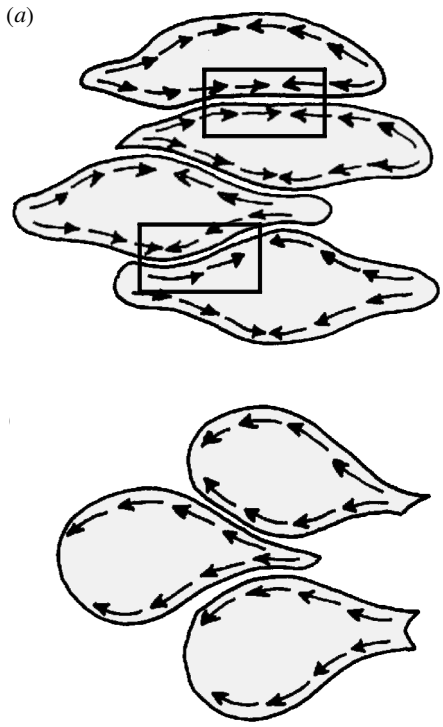

Figure 10. The cortical tractor model of cell motility results in parallel tractoring in the same direction (top box in *a*; right box in *b*) in both (*a*) bipolar, and (*b*) monopolar modes of cell intercalation. Opposing directions of tractoring occur only at partial overlap of bipolar cells (lower box in *a*). The arrows indicate the pattern of tractoring of the cell cortex; bipolar cells are assumed to tractor from both ends to the middle.

substrata; if so, they could not advance across these surfaces. Finally, the tractive protrusions should be able to transfer their adhesion from one cell to the next as the opportunity arises (figure 8*f*).

Is there evidence for these stiffening adhesions? Deep mesodermal cells have large numbers of small filiform protrusions attaching the long anterior and posterior surfaces of adjacent cells to one another as seen in SEM (Keller *et al.* 1992*b*) (figure 9*a*,*b*). These intercellular contacts may not exist as protrusions in the embryo. In the embryo the cells appear to be tightly packed together and low-light fluorescence microscopy of labelled cells reveals few such filiform protrusions on these surfaces (Shih & Keller 1992*a*). Instead, these protrusions may reflect the presence of small local adhesions between tightly packed cells, which are then pulled out into filiform structures (figure 9*b*) during the shrinkage associated with SEM, which can be 10% or more in this system (see Keller & Schoenwolf 1977). These numerous contact points on the anterior and posterior surfaces of these elongated cells could serve as the stiffening adhesions between cells regardless of whether or not they exist as filiform protrusions.

The large lamelliform protrusions at the medial and lateral ends of bipolar mesodermal cells seen in low-light fluorescence time-lapse recordings are also seen in SEM (figure 9*a*,*b*). These we believe to be the tractive protrusions.

(e) *Common criticisms of the cell traction–cell substrate mechanism*

There are a number of objections that are commonly raised against the cell traction–cell substrate mechanism of cell intercalation described here. One of these is that the two halves of the substrate cell in the bipolar model are somehow different in polarity, one end pointing one way and the other end pointing the other way, and that somehow the tractive protrusions will fail to produce further movement when they pass beyond the midpoint of an elongated cell. However, no such polarity is implied in the model or observed in cell behaviour. The model proposes that the cell body is an unpolarized substratum, uniform from end to end (figure 8*a*,*b*). The important

element in the model is that the cells maintain a continuous structural organization to transmit tension over part or most of its mediolateral length, in either direction, as the tractive protrusion of another cell crawls along its surface from either end to the other; there is no reason to suppose that this function involves polarization (figure 8*a*,*b*). This objection arises from assuming that the traction of a bipolar cell occurs over the whole cortex of the cell and in opposite directions from both ends towards the middle (figure 10*a*). As discussed below (§6(f)), this type of cortical tractoring over large areas of the cell presents problems for both the bipolar and monopolar modes of intercalation. But cortical tractoring is not seen in time-lapse recordings; instead the traction seems localized to the protrusions at the ends of the cells (Elul & Keller 2000) (see §6(g)).

A second objection is that some unusual circumstance will arise when a tractive protrusion reaches the end of its substrate cell and transfers to another cell. This objection seems to arise from the assumption that there will be, or may be, a critical reversal of polarity at this transfer point. But again, the cell–cell traction model proposed here does not imply such a polarity, and there is no evidence that these cells are polarized in such a fashion. As a tractive protrusion reaches the end of its substrate cell, it has the opportunity to transfer to an adjacent cell and continue exerting traction on the new substrate cell (figure 8*f*). We do not envisage the substratum function of the cell body as offering less resistance to traction in one direction or the other over the whole cell body. Indeed, review of cell behaviour in low-light fluorescence time-lapse films of neural and mesodermal cells intercalating does not betray any obvious differences in protrusive behaviour as a cell advances along another cell (R. Keller, unpublished data). In terms of the model, the chains of tension are not broken when a cell transfers its tractive adhesion to another substrate cell, but is remodelled such that a given medial segment might be hooked up with a new and different lateral segment.

A third objection is that when cells come to lie parallel and at the same mediolateral position, they are using one another for traction and thus cannot move with respect to one another. But, in fact, time-lapse recordings show that any two such cells are available as substrates for cells medial and lateral to them, and in turn can use these cells as substrates because of the partial interdigitation of their medial and lateral ends. Therefore the question of their mutual traction is largely irrelevant. Given the maximum density packing of the cells and the partial interdigitation of these fusiform cells, tractive protrusions on a given cell have many opportunities to jump over to other substrate cell bodies when reaching the limits of the current substrate cell. And again, traction appears localized to the end or ends of the cells, rather than spread over the cell body.

(f) *The 'cortical tractor' model of cell motility as a counter-example*

The 'cortical tractor' model of cell motility provides a good counter-example to the localized traction mechanism of cell intercalation described here because it does not restrict traction to localized protrusions at the ends of cells. The cortical tractor model proposes that cells move forward by tractoring their cortex backwards over much or all of the cell body from the leading edge of the cell (figure 10*b*) (Jacobson *et al*. 1985, 1986, fig. 8). Jacobson and co-workers have used the cortical tractoring model of cell motility to explain the rolling of the neural plate into a trough and to explain the elongation of the notoplate–neural plate boundary by cell intercalation and boundary capture in the urodelean amphibian (Jacobson *et al*. 1986). In this application, the notoplate cells at the boundary with the neural plate show cortical tractoring toward the boundary, thus pulling interior notoplate cells into the boundary where they, in turn, adopt this behaviour (Jacobson *et al*. 1986, fig. 8).

Although successful in this application to morphogenesis of the urodele neural plate, invoking the cortical tractor across the whole field of *Xenopus* deep neural cells as a mechanism of moving the cells towards the midline illustrates the need for localized traction, traction restricted to the ends of the cells. Applying the cortical tractor model to the monopolar mode, it appears that all the tractoring surfaces would abut one another and attempt to use one another as a source of traction (figure 10*b*). And of course, they would fail to move, because for each cell, the substrate is moving in the same direction as the applied traction. Another cell could not enter between any two other cells likewise because of parallel tractoring. Applied to the bipolar mode, the cortical tractor might actually work well initially in the stages of partial overlap of two intercalating cells, because the approaching ends of bipolar cells would be of opposite tractoring directions. But tractoring would become progressively less effective as the cells near complete overlap when a greater part of their adjacent surfaces display parallel tractoring (figure 10*b*). Again, the important difference between the cortical tractor mode of cell motility and the localized traction mode of cell motility described here is that the entire surface of the cell is serving as a tractor and as a substrate in the cortical tractor model, whereas these two functions are separated spatially on the cell in the localized traction model.

(g) *Cell behaviour observed during intercalation*

The localized cell–cell traction mechanism fits the observations of the behaviour of intercalating cells in both the mesoderm and neural tissue. First, the observed protrusive activity of intercalating cells is local and confined to the ends of the cells, both ends in the case of bipolar mode in both mesodermal (Shih & Keller 1992*a*) and neural tissue (Elul *et al*. 1997; Elul & Keller 2000), and the medial end in the case of the monopolar, neural mode (Elul & Keller 2000). In the latter case, the cells often have small lateral protrusions, perhaps representing retraction fibres off the trailing edge of the cells. There is, thus far, no evidence of global cortical tractoring amongst intercalating mesodermal or neural cells in *Xenopus*. When both substrate and tractive cell are labelled, and thus can be seen in recordings, the putative tractive protrusions at the ends of the cells appear to tug on and distort the adjacent cells, implying that these protrusions are, in fact, tractive. Similar protrusions seen on various types of cells cultured on deformable rubber sheets are associated with traction, visualized by deformation of the sheets (Harris 1980). Time-lapse recordings of intercalating mesodermal

cells in explants suggests that the most convergent extension occurs when there is balanced bipolar protrusive activity. When these cells appear to be unbalanced in their traction, they 'migrate' medially or laterally, as individuals, exchanging places, and little convergent extension is produced (R. Keller, unpublished data). Mediolaterally orientated rows of cells at specific anteroposterior levels are occasionally observed to slide medially and laterally as a unit, an observation consistent with the idea that intercalating cells form mediolaterally orientated chains under tension that span the mediolateral axis and serve as the organizational unit for producing convergent cell intercalation. Finally, the tractive protrusions do not show contact inhibition of one another but instead pass by one another when meeting head-on, and attach to the cell bodies behind one another. The predicted contact induced disassembly of the stiffening cell adhesions along the anterior and posterior sides of the cells has not be observed directly, but this may be because of the close apposition of the cells. The fact that cells seen intercalating in time-lapse recordings are found to have large numbers of lateral contacts in SEM, suggests that these contacts offer little resistance to shearing of cells past one another.

(h) *Cell polarity and cell intercalation behaviour are context dependent*

Efforts to dissociate the intercalating mesodermal cells and culture them as individuals on defined substrates have failed thus far to produce very much useful information about the cell intercalation behaviour. When cultured on fibronectin, these cells, attach, spread and become transiently multipolar, then bipolar, and back again to multipolar, displaying none of the highly organized behaviour seen in the explants (R. Keller, unpublished data). Immediate reaggregation of dissociated dorsal mesodermal cells failed to produce convergent extension (R. Keller, unpublished data), a result also obtained long ago by Holtfreter (1944). Whatever the basis of the bipolar and monopolar intercalation behaviours, it is contextual and requires maintenance of intercellular communication, either by contact or by signalling over longer distances. It also does not appear to be self-organizing in a dissociated, scrambled population of cells. The overlying epithelial layer of the organizer appears to have a role in setting up the organization necessary for cell intercalation in the deep region in the first half of gastrulation (Shih & Keller 1992*c*), but we do not know the mechanism of this effect.

The polarization of cell morphology and protrusive activity in the bipolar mode, the monopolar mode, and in the boundary-capture mode is very strong when observed in the intact explant. In the context of the explant, these cells are large volume, rectangular containers of yolk platelets in which it has been difficult to resolve details of cytoskeletal organization that underlies this dramatic polarity of behaviour and morphology. Also, little is known about the motility regulating factors (see Hall & Nobes, this issue) that are expected to control the type and distribution of protrusive activity. We must develop methods to study the cytoskeleton and its regulation in the context of the explant, or resolve the contextual cues that will allow expression of intercalation behaviour in a simpler context where the cytoskeleton can be studied more easily. Or we must study these aspects in one or more of the systems mentioned above that shows similar intercalation behaviour but is more amenable to visualization and analysis of the cytoskeleton.

7. FUNCTION OF CELL ADHESION MOLECULES IN CELL INTERCALATION

(a) *Cadherins*

Are there specific cell–cell adhesion systems associated with either the tractive protrusions or the putative stiffening adhesions holding the cells together, or do cell adhesion molecules serve only a general function of holding the cells of the embryo together with the specificity of the polarized cell behaviour lying elsewhere? A number of types of cadherins, calcium-dependent cell adhesion molecules, are found in early *Xenopus* development, including maternally derived cadherins (EP- or C-cadherin and XB- or U-cadherin), E-cadherin, which comes on at gastrulation in the ectoderm, and N-cadherin, which replaces E-cadherin in the neural ectoderm (see Levine *et al*. 1994; Heasman *et al*. 1994; Herzberg *et al*. 1991). Another cadherin, *Xenopus* cadherin-11 (Xcadherin-11), is expressed at the gastrula stage in mesenchymal cells (Hadeball *et al*. 1998). One of these, EP- or C-cadherin, is the major cadherin expressed in the early embryo and it is the primary cell adhesion molecule binding the cells of blastula and early gastrula together (Heasman *et al*. 1994; Lee & Gumbiner 1995). It appears to be downregulated in functional activity when convergent extension is induced experimentally in animal caps, suggesting that decreased cadherin-mediated cell–cell adhesion is associated with increased morphogenic movements (Brieher & Gumbiner 1994). Expression of a dominant inhibitory form of this cadherin, consisting of the deletion of the cytoplasmic tail, by RNA injections, blocked involution and closure of the blastopore when expressed in the dorsal sector of the marginal zone but not when expressed in the animal cap or ventral sector of the marginal zone (Lee & Gumbiner 1995). In this study, expression of wild-type C-cadherin rescued the effect of the dominant negative form when co-expressed, and generated gastrulation defects when expressed alone, somewhat different from and more severe than those of the dominant negative form. A monoclonal antibody to the ectodomain of C-cadherin, AA5, is a strong activator of C-cadherin-mediated adhesion of early frog blastomeres and inhibits the elongation of activin-treated animal caps, arguing that a decrease in C-cadherin-mediated adhesion is necessary for convergent extension (Zhong *et al*. 1999). These studies argue that C-cadherin is playing a role in convergence and extension but it is not clear specifically what that role might be. It appears to be downregulated in activity in *in vitro* assays, but that does not necessarily mean that the same occurs in the context of the intercalating cells of the axis; in that context, it may be regulated locally, and it is only a default, lower-adhesion state that is expressed in disassembled axial tissues. The general problem with arguing that downregulation of adhesion is a major element in mesodermal convergent extension is that the cells must stick together in order to form a stiff array that is able to push in the fashion described in § 3). Therefore, if specific cadherin-mediated

adhesion is decreased, cell–cell adhesion must be increased by a different cadherin, by a different cell adhesion molecule, or by cell matrix adhesion in order to form a stiff extension.

N-cadherin is expressed in the neural tissue of *Xenopus* during the period of its convergent extension (Detrick *et al.* 1990). Expression of N-cadherin along with β-galactosidase to mark the expressing cells suggested that N-cadherin overexpression prevents the normal amount of cell mixing. Expression of N-cadherin also affected neural tube formation, ranging from its failure to close to affects on its cellular arrangement (Detrick *et al.* 1990). It is not known whether these phenotypes were due to effects on convergent extension and the type of cell intercalation described above for the neural tissue, or other, prior events in early development. Rapid and extensive radial and mediolateral intercalations are involved in reorganization of the dorsal neural tube during its closure (Davidson & Keller 1999); perhaps these intercalations were affected in these experiments. Interestingly, another cell adhesion molecule, the calcium-independent neural cell adhesion molecule N-CAM, which is normally expressed in the neural tube, has no apparent effect on early neural development when overexpressed (Kintner 1988). Expression of extracellularly truncated of E- and EP-cadherins in *Xenopus* (Broders & Thiery 1995) and Pleurodeles (Delarue *et al.* 1998) embryos disrupted the convergent extension movements by affecting the patterns of cell intercalation. RNA encoding cadherin-11 is expressed in the animal cap and marginal zone of the *Xenopus* gastrula and later in the sclerotome, lateral plate mesoderm, and neural crest but not the converging and extending tissues of notochordal and somitic mesoderm (Hadeball *et al.* 1998). Overexpression of *Xenopus* cadherin-11 results in failure of convergent extension in explants, abnormalities of the neural tube and notochord and head in the embryo (Hadeball *et al.* 1998).

These experiments suggest an involvement of cadherins at some level in convergent extension but do not characterize their role. The challenge is to determine which cell adhesion molecules have general roles in convergent extension, to determine which, if any, have specific roles in convergent extension, and to characterize these roles in terms of cell and tissue biomechanics.

(b) *The role of protocadherins*

Two protocadherins, paraxial protocadherin (PAPC) and axial protocadherin (AXPC) (Kim *et al.* 1989; Yamamoto *et al.* 1998) have been implicated more specifically in convergent extension. In *Xenopus*, PAPC-encoding RNA is first expressed in all posterior mesoderm of the late blastula and early gastrula, and when the mesoderm involutes, it disappears from the notochord but persists in the somitic mesoderm. In contrast, AXPC-encoding RNA is expressed in the notochord as PAPC RNA disappears in this tissue. AXPC and PAPC show mutually exclusive specific cell adhesion properties in dissociation, reaggregation and sorting out studies. Expression of a secreted, extracellular domain of PAPC has a dominant negative effect and disrupts convergence of paraxial mesoderm into the normal somite files of *Xenopus*, mimicking to some degree the defects of somitic mesoderm convergence seen in the effect of the mutation *spadetail* in the zebrafish (Kimmel *et al.* 1989). The dominant negative PAPC disrupts elongation of animal cap explants induced with activin, but the normal PAPC potentiates expression of convergent extension movements and adoption of the elongate shape typical of the intercalating mesodermal cells in animal caps treated with subthreshold levels of activin (Kim *et al.* 1998).

Surprisingly, truncations of most of the intracellular domain did not have a dominant inhibitory effect, as would be predicted from the behaviour of classical cadherins of this type. Instead, the truncated form showed greater positive effects on cell adhesion than the normal molecules, suggesting that the cytoplasmic tails of these molecules may function in decreasing adhesion (Kim *et al.* 1998). These results suggest involvement in regulation of cell adhesion during convergent extension, and perhaps regulation of the expression of cell intercalation behaviour. Kim and associates suggest that a specific region of the intracellular domain of PAPC may associate with an unidentified protein to lower cell adhesion, making possible the transient cell associations necessary during cell intercalation. Could PAPC function in the 'stiffening adhesions', postulated above, by promoting high adhesion of the anterior and posterior surfaces of the intercalating mesodermal cells, but yet disassemble these adhesions in a regulated way, as an intercalating cell leaves one neighbour, and reassemble an adhesion as a new one is acquired?

(c) *Possibilities*

In principle, it is now possible to test what specific role a cadherin, a protocadherin, a cytoskeletal element, or regulator of protrusive activity, might have in the context of the cell–cell traction model described above (§6), using time-lapse recordings of cell behaviour and biomechanical measurements together to evaluate the protrusive activity, the stiffness of the tissue, and response of the cells to mechanical manipulation. In practice, application of these sorts of analyses requires working with the explants, which are difficult to make, and visualizing cell behaviour with low-light time-lapse fluorescence microscopy, which requires sophisticated equipment and knowledge of imaging. And biomechanical analyses are likewise difficult. Nevertheless, with the appropriate application of these methods, it will soon be possible to determine not only whether a molecule is necessary for convergent extension, but what specific function it plays.

8. CELL–MATRIX MODEL OF CELL INTERCALATION

An alternative to, or perhaps an addition to the cell–cell traction model of cell intercalation is a cell traction-on-extracellular matrix model for active cell intercalation. Formally, fibrils of an extracellular matrix could occupy the spaces between the intercalating cells and serve as a substratum for an orientated or directed migration (figure 11*a*). To produce cell intercalation, the matrix-mediated migration would have to result in wedging of the cells between one another to make a longer array along the anteroposterior axis. The matrix might be interstitial, as shown here, or as a mat on one or both surfaces of the intercalating cell array. Of course, in either case, the matrix would have to be deformable or capable

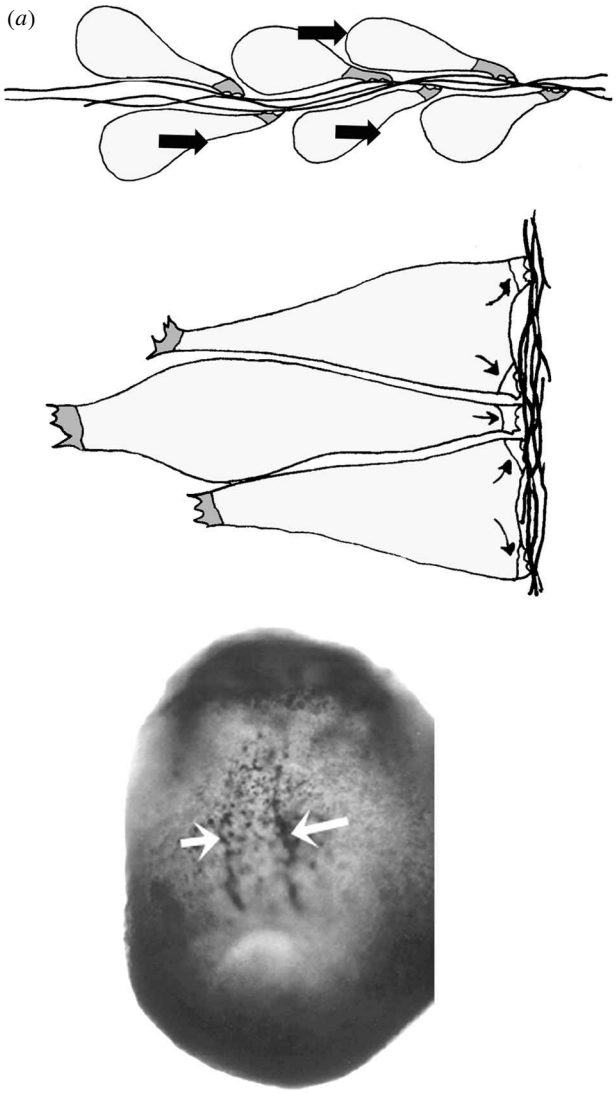

Figure 11. (*a*) An extracellular matrix might function in cell intercalation by providing a non-cellular substrate on which cells could crawl directionally in the case of the monopolar mode of cell intercalation. (*b*) Matrix may prevent cells from crossing the NSB and also control the boundary-capture mode of cell intercalation by offering a substrate on which cells could adhere and spread. In both cases, matrix remodelling would have to occur for large extensions. Antibody staining shows the presence of fibrillin protein in the early NSB (pointers) at the midgastrula stage viewed from the dorsal side (*c*); the blastopore is at the bottom.

of being remodelled in the course of convergent extension in order to allow the tissue to lengthen and narrow. An interstitial matrix or a mat of matrix could serve as a substratum for a monopolar, medially directed cell crawling of the type seen in the neural tissue, but it is not obvious how such a matrix would serve as a substratum for the bipolar mechanism of cell intercalation seen in the mesoderm and in neural tissue under minimal interactions with mesoderm. Formally, the model of the bipolar mode of intercalation seems to work only as a cell–cell traction model. Finally, an extracellular matrix could be involved in the 'boundary capture' described in §5(b)) for the NSB; in this model, cells would crawl on the matrix, perhaps directionally, until reaching the boundary where they would spread on the matrix in the boundary and then cease their motility (figure 11*b*).

What is the evidence for extracellular matrix function in convergent extension? An external substratum, such as the overlying blastocoel roof, is not essential for convergent extension of the mesodermal tissue of *Xenopus* (see Shih & Keller 1992*b*; Keller & Jansa 1992). Likewise, the neural tissue can converge and extend without the underlying mesoderm (Keller & Danilchik 1988; Elul & Keller 2000). Most matrix research has focused on the prominent fibronectin-containing matrix lining the blastocoel roof and its role in mesodermal migration (see Winklbauer & Keller 1996). Whether this matrix is needed for neural extension is not known. Because it is presumably retained, at least initially, in the deep neural explants, it could be used as a substratum for neural convergent extension. Although the mesodermal cells do not need the organized fibrillar matrix on the blastocoel roof to converge and extend, a fibrillar system of matrix might lie between the mesodermal cells and serve as a substrate for the traction of these cells. Arguing against this notion is the fact that no such fibrillar system has been characterized in either the neural or mesodermal tissue.

However, it is possible that extracellular matrix components function in a non-fibrillar form. Molecular fibronectin, as opposed to organized fibrils, is found throughout the mesodermal tissue through gastrulation and beyond (D. DeSimone, personal communication). Whether this form of fibronectin functions in the mesodermal mediolateral intercalation behaviour (MIB) is not known. Arguing against this notion is the fact that antibodies and peptides blocking the integrin-mediated migration of mesodermal cells on the blastocoel roof have much less or no affect on convergent extension movements (Ramos & DeSimone 1996; Ramos *et al.* 1996; Winklbauer & Keller 1996). But it is not known whether the antibody and peptide reagents used in these experiments penetrate between the densely packed mesodermal cells of the extending axis.

There is extracellular matrix in the NSB and this matrix may have a function in setting up the boundary, blocking cell movements across the boundary, and perhaps even organizing the boundary-mediated cell capture described above (§5(b)) (figure 11*b*). Extracellular matrix is abundant in the notochordal sheath, and this structure arises from the notochordal–somitic mesodermal boundaries that develop as morphologically visible structures at midgastrula stage (Shih & Keller 1992*a*). Molecules such as laminin (Fey & Hausen 1990), fibronectin (Lee *et al.* 1984) and elastin (P. Skoglund, unpublished results) are found by immunofluorescence in the notochordal sheath at neurula stages, but have not been detected at earlier stages of boundary formation.

However, the matrix molecule fibrillin is found in the forming boundary at midgastrula stages (figure 11*c*; P. Skoglund, unpublished data). Fibrillin is a component of microfibrils (Sakai *et al.* 1986), widely distributed 10–12 nm diameter, extensible extracellular fibres that can serve mechanical functions, for example, in ligaments of the human eye (reviewed in Ramirez *et al.* 1993). Therefore this molecule is a strong candidate for involvement in the changing biomechanical properties that dorsal mesoderm exhibits as these boundaries form. In addition,

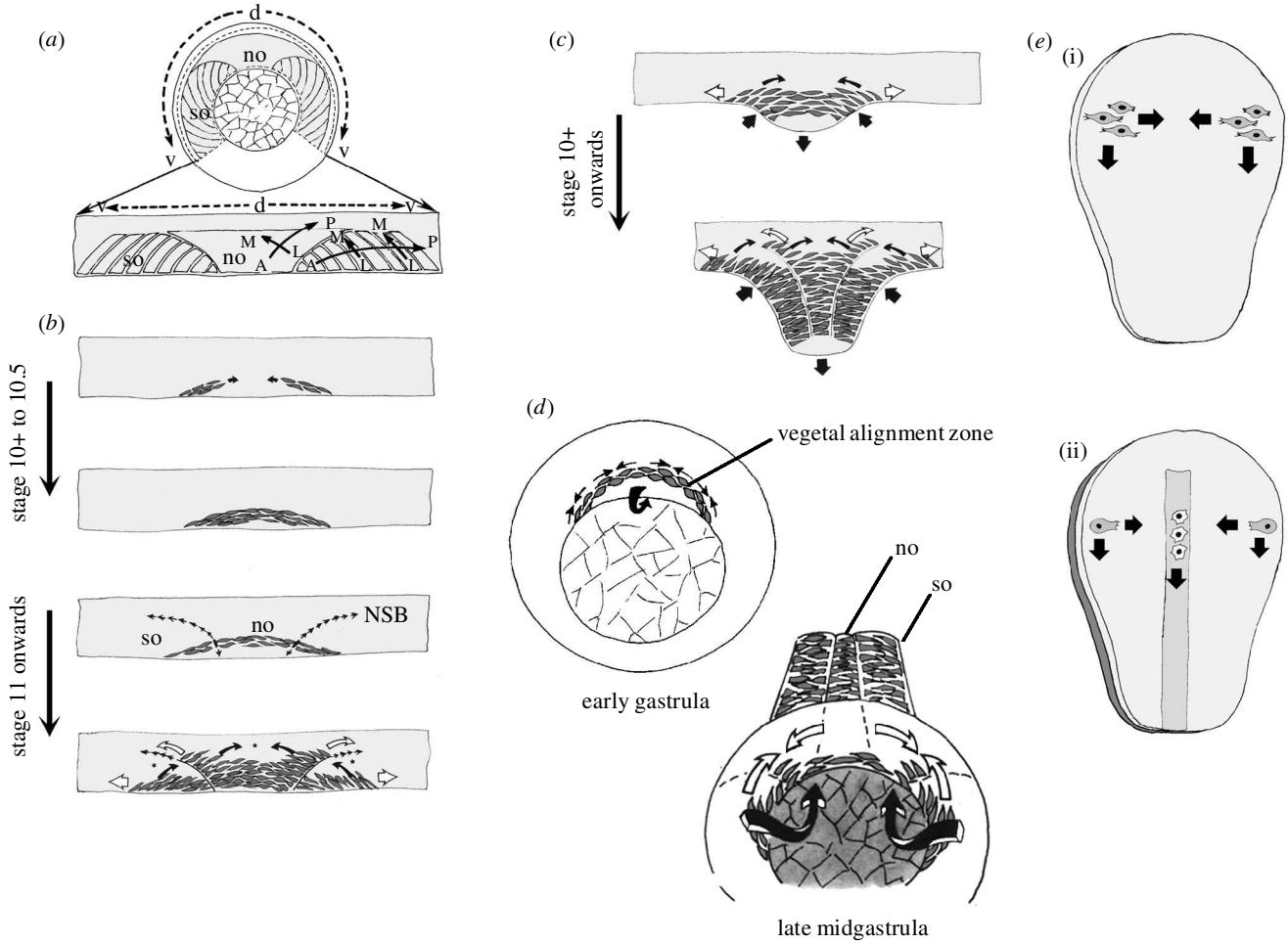

Figure 12. To visualize the pattern of expression of mediolateral intercalation behaviour (MIB), (*a*) explants of a large part or all of the involuting marginal zone were made and cultured in two ways. Some explants were cultured beneath a restraining cover-slip, such that they did not undergo convergence and extension (*b*), or more loosely restrained, and allowed to converge and extend (*c*). Under these conditions, the progress of MIB was determined from time-lapse recordings and represented here by fusiform cell profiles (*b*, *c*). (*d*) The expression of MIB in the whole embryo at the early gastrula and late midgastrula is also shown. The prospective anterior (A) to posterior (P) and lateral (L) to medial (M) axes, as well as the dorsal (d) and ventral sides (v) in the somitic and notochordal fields are indicated in (*a*). Note that the progress of MIB is from anterior to posterior and from lateral to medial. (*e*) Similar anterior-to-posterior and lateral-to-medial expression of MIB occurs in the neural plate and notoplate. Also shown is the notochord (no), the somitic mesoderm (so), and the NSB. (i) Deep neural plate; (ii) deep neural plate over the mesoderm.

because cells have receptors for fibrillin (Pfaff *et al.* 1996; Sakamoto *et al.* 1996), fibrillin is a candidate molecule for a role in regulating the boundary-capture mode of cell behaviour seen there.

Extracellular matrix functions in the straightening and stiffening of the notochord in the tailbud stages. The dense fibrillar matrix of the notochordal sheath develops in the late neurula through the tailbud stages and forms a fibre-wound tube. The notochord cells then vacuolate and generate pressure within the matrix-wound tube, which results in stiffening and straightening of the notochord in this period (see Adams *et al.* 1990).

9. PATTERNING OF MEDIOLATERAL INTERCALATION BEHAVIOUR: PROGRESSIVE ANTERIOR-TO-POSTERIOR AND LATERAL-TO-MEDIAL EXPRESSION

Expression of mediolateral intercalation behaviour (MIB) is spatially and temporally regulated in both the mesodermal and neural tissues. Expression occurs from anterior to posterior and from lateral to medial in both tissues, such that the two undergo parallel movements, the mesodermal on the inside and the neural on the outside of the blastoporal lip (Keller *et al.* 1992*a*). This progressive organization is essential for the function of both neural and mesodermal convergent extension machines in gastrulation.

(a) *Patterning of MIB in the mesoderm*

Expression of MIB in the prospective mesoderm is best visualized in explants of the entire involuting marginal zone (IMZ) of the embryo (figure 12*a*) (Shih & Keller 1992*a*). The expression of the bipolar mode of MIB is represented diagrammatically as elongated, fusiform shapes (figure 12). First, consider the expression of MIB in explants that are not allowed to converge and extend (figure 11*b*). MIB is first expressed in the anterior, lateral prospective somitic mesoderm, and progresses medially as

arcs, which meet in the midline to form the VAZ (figure 12*b*, solid arrows, stage 10+ to 10.5). The NSB then forms at the midgastrula stage within the VAZ, and it proceeds posteriorly (figure 12*b*, small arrows, stage 11 onwards). Then, MIB is expressed from anterior to posterior in the lateral region of somitic field and from anterior to posterior in the lateral, boundary region of the notochordal mesoderm (figure 12*b*, solid arrows, stage 11 onwards). From this lateral origin, MIB progresses medially towards the medial aspect of both the somitic field and the notochordal field (figure 12*b*, solid arrows, stage 11 onwards). If the explants are not allowed to converge and extend, medial, posterior regions of both the prospective somitic and notochordal mesodermal fields fail to express MIB and differentiate into their respective tissues (figure 12*b*, asterisks, stage 11 onwards).

If the explants are allowed to converge and extend, the expression of MIB follows the same pattern from anterior to posterior and lateral to medial in both notochordal and somitic regions (figure 12*c*). However, the convergence brings the lateral boundaries closer to the medial parts of both tissues and under these conditions, MIB spreads throughout the medial parts of both the notochordal and somitic regions. This fact, and the fact that MIB progresses medially from a lateral origin, suggests that the lateral boundaries of these tissues might be the sources of the signals organizing MIB as well as cell fate. This notion is supported by the fact that if the lateral boundaries are not allowed to converge, and move closer together, the medial cells do not differentiate MIB or proper tissue fates (Shih & Keller 1992*a*; Domingo & Keller 1995). If the tissue boundaries are sources of signals of limited range, convergent extension would have to be self-reinforcing; convergence of the lateral, signalling boundaries would bring more cells into range, allowing more convergence, and so on.

These results also suggest that the signalling systems organizing MIB and perhaps final cell fate are extant during gastrulation, and that cell fate is not yet determined in the early gastrula stage. To test these notions, labelled cells from the notochordal, somitic and epidermal regions of early to midgastrula stages were grafted into the notochordal region of early gastrula whole embryos and explants (Domingo & Keller 1995) (figure 13*a*). Their motility was recorded with time-lapse imaging and their cell fate determined by marker expression (Domingo & Keller 1995) (figure 13*b*). These grafted cells express MIB and differentiate as notochord in the same anteroposterior and lateral–medial order as the native notochordal cells (figure 13*b*). Thus the notochordal cells are not pre-programmed to express MIB in the observed pattern at the outset of the behaviour, but signals operating during gastrulation, probably emanating from the boundary regions, organize MIB during gastrulation. Moreover, in Domingo's experiments, cells of epidermal prospective fate grafted into the notochordal region were converted to notochord, as well as induced to express MIB, showing that these cells were exposed to mesodermal cell fate-inducing signals as well as MIB-inducing signals during gastrulation.

Mediolateral cell intercalation appears to be regulated by the Wnt signalling pathway. A dominant inhibitory form of *Xenopus* dishevelled, which is involved in the Wnt signalling pathway, inhibits convergent extension (Sokol 1996), as does a dominant-inhibitory form *Xenopus* frizzled-8, another, upstream component of the pathway (Deardorff *et al.* 1998). Evidence for the involvement of the 'planar polarity', non-canonical Wnt signalling pathway in controlling cell motility of convergent extension is described in Smith (this issue).

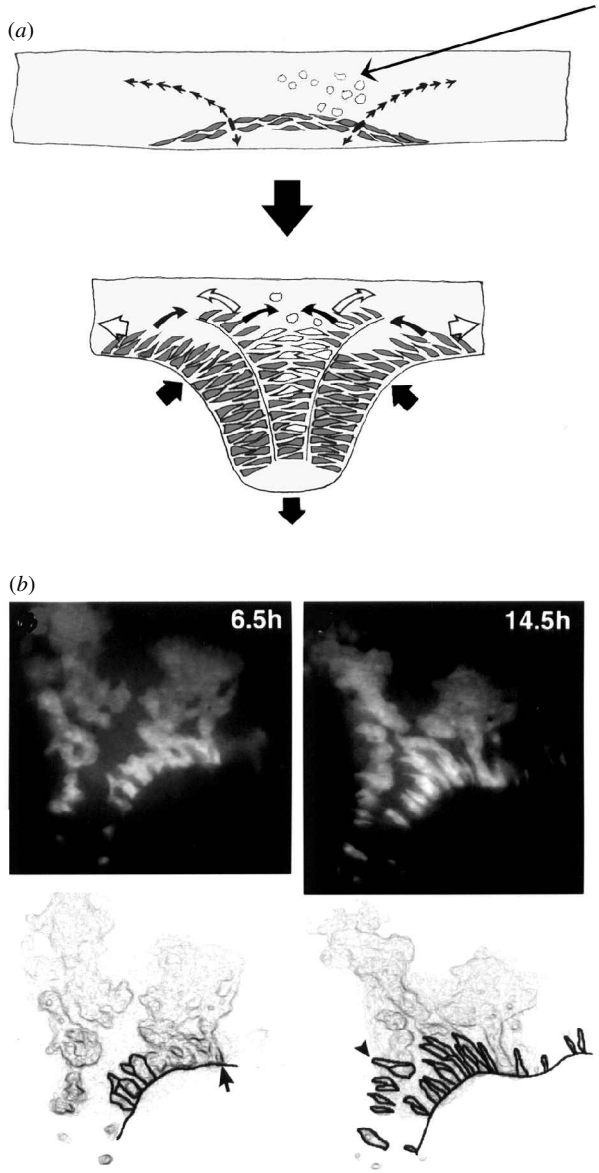

Figure 13. Labelled cells from another prospective notochordal, somitic, or epidermal tissue of a gastrula stage embryo were grafted into the notochordal region of an unlabeled early gastrula (long arrow, *a*). (*b*) The onset of MIB was then recorded with time-lapse fluorescence microscopy. Expression of MIB in the labelled cells occurred from lateral to medial (solid arrows, *a*) and from anterior to posterior (open arrows, *a*). From Domingo & Keller (1995).

(b) *Are there two zones of MIB organization?*

In an attempt to learn if microtubules were involved in development of cell polarity during MIB, Lane & Keller (1997) found evidence for two zones of organization of MIB and cell fate. If microtubules are disrupted with the

drug nocodozole in early gastrulation (i.e. before the VAZ forms), the VAZ never forms, convergent extension never occurs, involution never occurs, and a small amount of notochord and somitic tissue differentiates in the anterior (VAZ) region of the mesoderm. This mesoderm differentiates in the pre-involution position, and the remaining posterior axial and paraxial mesoderm does not express MIB or differentiate. If microtubules are disrupted after the VAZ has formed, the mesodermal cells express MIB, converge and extend, and differentiate as notochord and somite without microtubules. This includes the posterior axial and paraxial mesoderm. Absence of microtubules could prevent expression of MIB during formation of the VAZ in a number of ways, and thus no clear interpretation of this result can be made. However, the surprising and important result is that expression of MIB in the notochordal and somitic regions posterior to the VAZ does not require microtubules, provided the VAZ has formed. Although the anterior, VAZ region and the remaining posterior part of the axis appear to be identical in the type of MIB expressed and in the differentiation notochord and somites, these regions may represent two units of axial organization, each operating on different mechanisms at some level (see Lane & Keller 1997).

(c) *Patterning of MIB in the neural tissue*

The neural versions of MIB also originate in the anterior lateral region of the prospective neural plate and progress posteriorly and medially from this origin (Elul & Keller 2000). This progression occurs in both the deep neural explants and in the deep neural over mesoderm explants and applies to the both the bipolar mode and the monopolar mode of neural plate cell intercalation and to the notoplate-specific, multipolar behaviour (figure 11*e*). These progressions of cell behaviours follow the same pattern as the cell divisions and neuronal differentiation in the neural plate (see Hartenstein 1989).

These findings have implications for understanding neural patterning. The convergent extension movements can be induced by planar signals from Spemann's organizer (Keller *et al*. 1992*c*). However, if these signals are proceeding from the organizer toward the lateral boundaries of the neural plate, as would be expected, and expression of neural MIB follows the order in which the cells received these signals, then the expression of MIB should be from posterior to anterior and medial to lateral. Instead, the cell behaviours are organized medially from the lateral boundary and posteriorly from the anterior boundary, mimicking the pattern seen in the mesoderm. The simplest explanation is that inducing signals from the organizer interact with opposing signals from the epidermal region to establish a neural plate boundary (see review by Harland & Gerhart 1997). After the boundary is established, MIB is organized by secondary signals proceeding inwardly or posteriorly from this boundary.

(d) *The neural midline and the monopolar mode of cell intercalation*

The monopolar medially directed protrusive activity suggests that it might be stimulated by a signal emanating from a midline structure, the obvious one being the notoplate early, and following that, the floor plate derived from it. Elul & Keller (2000) observed a stronger polarization of the protrusive activity of the lateral neural plate cells as these cells approached the notoplate, which is consistent with a polarizing signal emanating from the notoplate and therefore stronger near it than further away. Arguing against this notion, however, is the observation that embryos without notochords, and apparently without floor plates, appear to develop relatively normal neural tubes (Malacinski *et al*. 1981). Whether the nervous systems of these embryos converged and extended by the normal monopolar mode or the bipolar mode is not known. Perhaps they used the less efficient bipolar mode, but its deficiencies were offset by towing forces from the underlying mesoderm.

An alternative mechanism is that the notoplate–floor plate, despite its prominent position, has nothing to do with organizing the monopolar MIB but instead the underlying somitic mesoderm imposes a medial directionality on the overlying neural cells. In this mechanism, somitic mesoderm, rather than notoplate–floor plate, would be necessary for medially polarized protrusive activity. Experiments are underway to test these possibilities.

(e) *Function of progressive expression of MIB*

The progressive expression of MIB is essential for proper gastrulation. The first expression of MIB, the VAZ, forms an arc across the dorsal lip of the gastrula, and as MIB shortens this arc, a hoop stress is generated across the dorsal lip, pulling it vegetally over the bottle cells, and thus contributing to involution (figure 12*d*, early gastrula). The endodermal rotation movements described recently by Winklbauer & Schürfeld (1999) initiate involution and set up the blastoporal groove below the VAZ, and drive much of the early involution. After this time, MIB is expressed in the postinvolution region, progressively posteriorly from the now involuted VAZ, and as a result, a wave of convergence, and resulting hoop stress, proceeds posteriorly on the inside of the blastopore (figure 12*d*, mid–late gastrula), driving continued involution and squeezing the blastoporal lip toward closure at a point over the ventral region of the vegetal endoderm. Because the mesoderm is expressing MIB posteriorly from the anterior, postinvolution region, and the neural tissue is expressing MIB posteriorly from an anterior origin in the prospective hindbrain on the outside of the gastrula, these tissues are expressing convergent extension in the same order and more or less in spatial correspondence (see Keller *et al*. 1992*a*).

(f) *Mechanical consequences of the bipolar and monopolar modes of cell intercalation*

The bipolar mode of MIB has no inherent directionality but only an orientation; the mediolaterally orientated protrusive activity tends to shorten the array of cells, pulling the ends together, without a bias as to which end, or perhaps both, is to move the most in accommodating the shortening. The anchorage points and deformability of the neighbouring, anchoring tissues determine these parameters. In the embryo, the mesodermal cells expressing MIB in progressively more posterior regions can be visualized as forming arcs that are anchored at each end near the margins of the large vegetal endodermal mass and span the across the IMZ (figure 14*a*). It should be

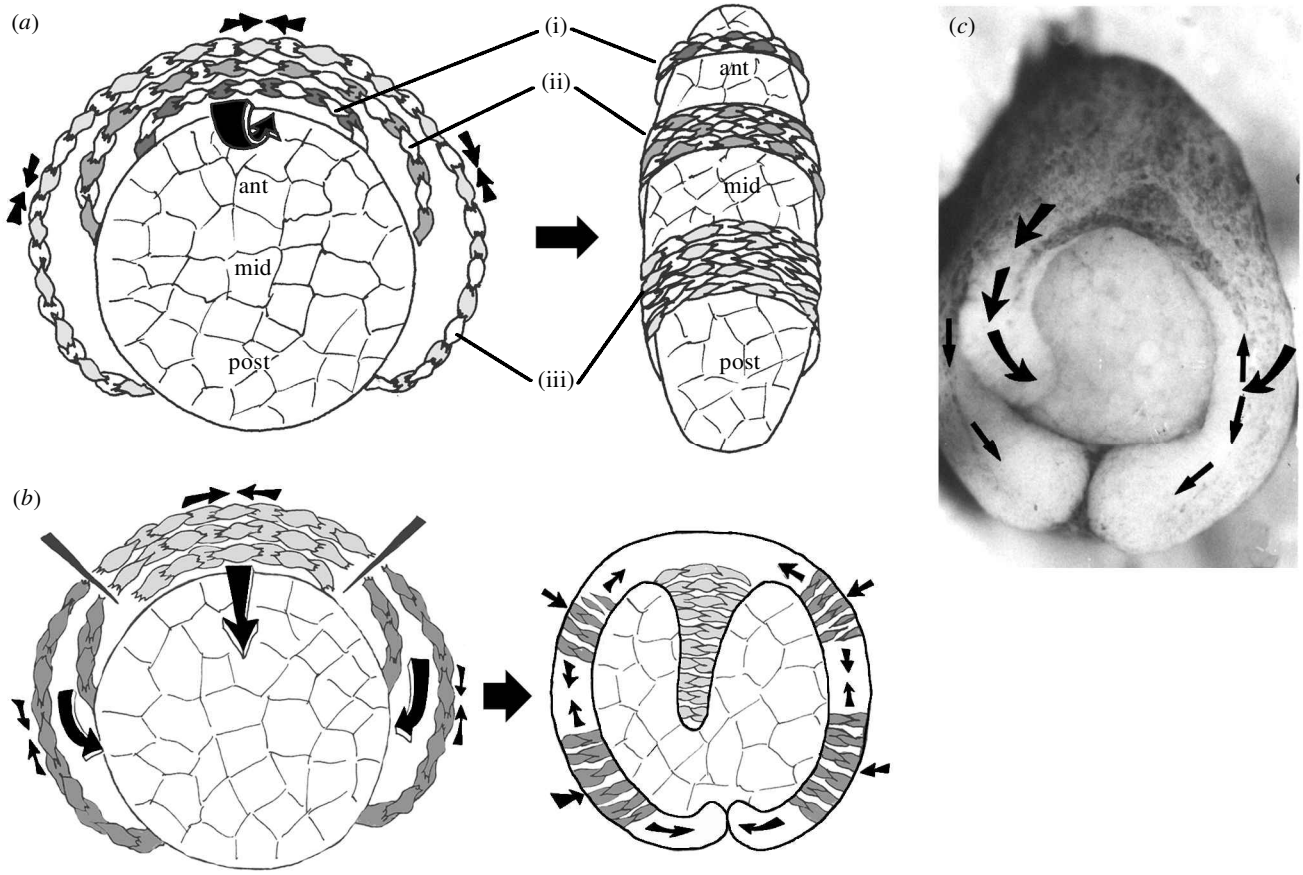

Figure 14. The mechanism of progressive mediolateral intercalation of mesodermal cells in bringing about involution of the marginal zone. (*a*) The mediolateral intercalation of mesodermal cells is illustrated as a series of arcs of intercalating cells, a prospective anterior (ant) one (i), a prospective middle (mid) one (ii), and a prospective posterior (post) one (iii), all anchored at each end at appropriate points near the large vegetal endodermal mass (*a*, left). During gastrulation these arcs are shortened by mediolateral cell intercalation, beginning with (i), and following with (ii) and (iii). As each successive arc is shortened, the corresponding region of the marginal zone is rolled over the lip by the hoop stress passing across the dorsal and lateral lips of the blastopore (*a*, right). (*b*) If these arcs are cut dorsolaterally, the isolated medial segments are no longer anchored laterally but have free ends. These arc segments shorten by convergence, pulling each, unanchored end medially, and as a result they extend a proboscis-like structure that normally would have involuted, out into the medium (*b*, right; *c*). Likewise, the isolated lateral segments now have a free medial end and an anchored lateral end (*b*). These arcs converge toward the vegetal endoderm, bringing their free medial ends towards the vegetal endoderm, and they extend around the circumference of the vegetal endoderm, causing the embryo to reopen its blastopore. (*c*) A light micrograph shows an embryo of this type, often called a 'ring embryo'.

kept in mind that in the embryo, the process is continuous; we only portray the process as separated arcs for clarity of illustration. As MIB progresses posteriorly, these arcs shorten in sequence, and thus generate a hoop stress across the IMZ, progressing posteriorly, as described above. In this case, neither end moves, but rather the arcs shorten and the IMZ is pulled across the blastoporal lip (figure 13*a*).

If the arcs are broken somewhere along their length on both sides, the medial segments of the arcs of MIB are unanchored and thus pull dorsally, where they converge, extend, and form an isolated extension, and, at the same time, fail to involute (figure 14*b*,*c*, light shading) (Keller 1981). The isolated lateral segments of the broken arcs in these experiments have their medial ends unanchored, and therefore they pull these ends towards (converge towards) the vegetal endoderm and extend in the perpendicular direction, parallel to the endodermal margin (figure 13*b*,*c*, dark shading). Such movement results in reopening of the partially closed blastopore (figure 13*c*). This type of embryo appears often in the classical literature, where it is known as a 'ring embryo', and it forms in cases where the continuity of the arcs of convergence are broken (see Keller 1986).

In the case of the monopolar mode of MIB in the neural tissue, the medially directed protrusive activity tends to pull the cells to the midline, thus pulling the edges of the neural plate towards the middle. The lateral anchorage of the neural plate is in the epidermis, which appears to be deformable, and thus it stretches to allow the edges of the neural plate to move medially. Note that there is no inherent reason why the bipolar mode would not work for convergent extension of the neural tissue as long as the epidermis is deformable.

(g) *Specificity of morphogenesis involves biomechanical integration of distributed information*

It is the global context of local cell behaviour that determines the specificity of the morphogenic effect. It is the geometry and the lateral anchorage points of the arc-like

expression patterns of MIB in the mesoderm that determine the specific effects of MIB. Taken alone, as in the sandwich explants (figure 3), the arc-like array MIB-expressing cells will produce simple convergence and extension. In contrast, if anchored at the edges of the vegetal endoderm, the arc-like pattern of progressive MIB expression will produce involution as well. The specificity of this type of morphogenesis depends on distributed information, information that is integrated by the biomechanical properties and geometry of the tissues to determine the output and significance of local cell behaviour. This has major implications for genetic and molecular analysis of morphogenesis.

We offer this paper in remembrance of Nigel Holder. We were not fortunate enough to know him personally, but his example, his published work, and his many less-definable but important contributions to the tenor of the field have gained our admiration and deep respect.

REFERENCES

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

Bauman, M. & Sanders, K. 1984 Bipartite axiation follows incomplete epiboly in zebrafish embryos treated with chemical teratogens. *J. Exp. Zool.* **230**, 363–376.

Betchaku, T. & 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 during epiboly. *J. Exp. Zool.* **206**, 381–426.

Bode, P. & Bode, H. 1984 Formation of pattern in regenerating tissue pieces of *Hydra attenuata*. III. The shaping of the body column. *Devl Biol.* **106**, 315–325.

Brieher, W. & Gumbiner, B. 1994 Regulation of C-cadherin function during activin induced morphogenesis of *Xenopus* animal caps. *J. Cell Biol.* **126**, 519–527.

Broders, F. & Thiery, J.-P. 1995 Contribution of cadherins to directional cell migration and histogenesis in *Xenopus* embryos. *Cell Adhesion Commun.* **3**, 419–440.

Burnside, M. B. & Jacobson, A. 1968 Analysis of morphogenetic movements in the neural plate of the newt *Taricha torosa*. *Devl Biol.* **18**, 537–552.

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

Choquet, D., Felsenfeld, D. P. & Sheetz, M. P. 1997 Extracellular matrix rigidity causes strengthening of integrin–cytoskeleton linkages. *Cell* **88**, 39–48

Cloney, R. 1964 The development of the ascidian notochord. *Acta Embryol. Morphol. Expo.* **7**, 111–130.

Condic, M., Fristrom, J. & Fristrom, D. 1991 Apical cell shape changes during *Drosophila* imaginal leg disc elongation: a novel morphogenetic mechanism. *Development* **111**, 23–33.

Davidson, L. & Keller, R. 1999 Neural tube closure in *Xenopus laevis* involves medial migration, directed protrusive activity, cell intercalation, and convergent extension. *Development* **126**, 4547–4556.

Deardorff, M., Tan, C., Conrad, L. & Klein, P. 1998 Frizzled-8 is expressed in the Spemann organizer and plays a role in early morphogenesis. *Development* **125**, 2687–2700.

Delarue, M., Saez,. F., Boucaut, J.-C., Thiery, J.-P. & Broders, F. 1998 Medial cell mixing during axial morphogenesis of the amphibian embryo requires cadherin function. *Devl Dynam.* **213**, 248–260.

Detrick, R. J., Dickey, D. & Kintner, C. 1990 The effects of N-cadherin misexpression on morphogenesis in *Xenopus* embryos. *Neuron* **4**, 439–506.

Domingo, C. & Keller, R. 1995 Induction of notochord cell intercalation behavior and differentiation by progressive signals in the gastrula of *Xenopus laevis*. *Development* **121**, 3311–3321.

Elul, T. & Keller, R. 2000 Monopolar protrusive activity: a new morphogenic cell behaviour in the neural plate dependent on vertical interactions with the mesoderm in *Xenopus*. *Devl Biol.* (In the press.)

Elul, T., Koehl, M. & Keller, R. 1997 Cellular mechanism underlying neural convergent extension in *Xenopus laevis* embryos. *Devl Biol.* **191**, 243–258.

Ettensohn, C. 1985 Gastrulation in the sea urchin embryo is accompanied by the rearrangement of the invaginating epithelial cells. *Devl Biol.* **112**, 383–390.

Evans, E., Berk, D. & Leung, A. 1991 Detachment of agglutinin-bonded red blood cells. I. Forces to rupture molecular-point attachments. *Biophys. J.* **59**, 838–848.

Fey, J. & Hausen, P. 1990 Appearance and distribution of laminin during development of *Xenopus laevis*. *Differentiation* **42**, 144–152.

Hadeball, B., Borchers, A. & Wedlich, D. 1998 *Xenopus* cadherin-11 (Xcadherin-11) expression requires the Wg/Wt signal. *Mech. Devl* **72**, 101–113.

Hardin, J. D. 1988 The role of secondary mesenchyme cells during sea urchin gastrulation studied by laser ablation. *Development* **103**, 317–324.

Hardin, J. D. 1989 Local shifts in position and polarized motility drive cell rearrangement during sea urchin gastrulation. *Devl Biol.* **136**, 430–445.

Hardin, J. D. & Cheng, L. Y. 1986 The mechanism and mechanics of archenteron elongation during sea urchin gastrulation. *Devl Biol.* **115**, 490–501.

Harland, R. & Gerhart, J. 1997 Formation and function of Spemann's organizer. *A. Rev. Cell Dev. Biol.* **13**, 611–667.

Harris, A. 1980 Silicone rubber substrata: a new wrinkle in the study of cell locomotion. *Science* **208**, 177–179.

Hartenstein, V. 1989 Early neurogenesis in *Xenopus*: the spatio–temporal pattern of proliferation and cell lineages in the embryonic spinal cord. *Neuron* **3**, 399–411.

Hartenstein, V. & Campos-Ortega, J. A. 1985 Fate mapping in wild-type *Drosophila melanogaster*. *Roux's Arch. Devl Biol.* **194**, 181–193.

Heasman, J., Crawford, A., Goldstone, K., Garner-Hamrick, P., Gumbiner, B., McCrea, P., Kintner, C., Noro, C. Y. & Wylie, C. 1994 Overexpression of cadherins and underexpression of β catenin inhibit dorsal mesoderm induction in early *Xenopus* embryos. *Cell* **79**, 791–803.

Herzberg, F., Wildermuth, V. & Wedlich, D. 1991 Expression of Xbcad, a novel cadherin, during oogenesis and early development of *Xenopus*. *Mech. Devl* **35**, 33–42.

Holtfreter, J. 1944 A study of the mechanics of gastrulation. II. *J. Exp. Zool.* **95**, 171–212.

Irvine, K. D. & Wieschaus, E. 1994 Cell intercalation during *Drosophila* germband extension and its regulation by pair-rule segmentation genes. *Development* **120**, 827–841.

Jacobson, A. & 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.

Jacobson, A. & Moury, J. D. 1995 Tissue boundaries and cell behavior during neurulation. *Devl Biol.* **171**, 98–110.

Jacobson, A., Odell, G. & Oster, G. 1985 The cortical tractor model for epithelial folding: application to the neural plate. In *Molecular determinants of animal form, University of California, Los Angeles, symposia on molecular and cellular biology (new series)*, vol. 31 (ed. G. M Edelman), pp. 143–167. New York: Liss.

Jacobson, A., Oster, G. F., Odell, G. M. & Cheng, L. Y. 1986 Neurulation and the cortical tractor model for epithelial folding. *J. Embryol. Exp. Morphol.* **96**, 19–49.

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. *Devl 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. *Devl 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. Morphol.* **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. Morphol.* **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. E. 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 *Developmental biology: a comprehensive synthesis*. 2. *The cellular basis of morphogenesis* (ed. L. Browder), pp. 241–327. New York: Plenum.

Keller, R. 1987 Cell rearrangement in morphogenesis. *Zool. Sci.* **4**, 763–769.

Keller, R. 2000 The origin and morphogenesis of amphibian somites. *Curr. Top. Devl Biol.* **47**, 183–246.

Keller, R. E. & Danilchik, M. 1988 Regional expression, pattern and timing of convergence and extension during gastrulation of *Xenopus laevis*. *Development* **103**, 193–209.

Keller, R. & Jansa, S. 1992 *Xenopus* gastrulation without a blastocoel roof. *Devl Dynam.* **195**, 162–176.

Keller, R. E. & 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. & Tibbetts, P. 1989 Mediolateral cell intercalation in the dorsal axial mesoderm of *Xenopus laevis*. *Devl Biol.* **131**, 539–549.

Keller, R. E., Danilchik, M., Gimlich, R. & Shih, J. 1985 The function and mechanism of convergent extension during gastrulation of *Xenopus laevis*. *J. Embryol. Exp. Morphol.* **89** (Suppl.), 185–209.

Keller, R. E., Cooper, M., Danilchik, M., Tibbetts, P. & Wilson, P. 1989*a* Cell intercalation during notochord development in *Xenopus laevis*. *J. Exp. Zool.* **251**, 134–154.

Keller, R., Shih, J. & Wilson, P. 1989*b* Morphological polarity of intercalating deep mesodermal cells in the organizer of *Xenopus laevis* gastrulae. In *Proceedings of the 47th Annual Meeting of the Electron Microscopy Society of America* (ed. G. W. Bailey), pp. 840–841. San Francisco, CA: San Francisco Press.

Keller, R., Shih, J., Wilson, P. & Sater, A. 1991*a* Pattern and function of cell motility and cell interactions during convergence and extension in *Xenopus*. In *49th Symposium of the Society for Developmental Biology: cell–cell interactions in early development* (ed. J. Gerhart), pp. 93–107. Wiley.

Keller, R., Shih, J. & Wilson, P. 1991*b* Cell motility, control and function of convergence and extension during gastrulation of *Xenopus*. In *Gastrulation: movements, patterns, and molecules* (ed. R. Keller, W. Clark & F. Griffin), pp. 101–119. Plenum.

Keller, R., Shih, J. & Sater, A. 1992*a* The cellular basis of the convergence and extension of the *Xenopus* neural plate. *Devl Dynam.* **193**, 199–217.

Keller, R., Shih, J. & Domingo, C. 1992*b* The patterning and functioning of protrusive activity during convergence and extension of the *Xenopus* organizer. *Development* **1992** (Suppl.), 81–91.

Keller, R., Shih, J., Sater, A. & Moreno, C. 1992*c* Planar induction of convergence and extension of the neural plate by the organizer of *Xenopus*. *Devl Dynam.* **193**, 218–234.

Kim, S.-H., Yamamoto, A., Bouwmeester, T., Agius, E. & DeRobertis, E. M. 1998 The role of paraxial protocadherin in selective adhesion and cell movements of the mesoderm during *Xenopus* gastrulation. *Development* **125**, 4681–4691.

Kimmel, C., Kane, D., Walker, C., Warga, R. & Rothman, M. 1989 A mutation that changes cell movement and cell fate in the zebrafish embryo. *Nature* **337**, 358–362.

Kimmel, C., Warga, R. & Kane, D. 1994 Cell cycles and clonal strings during formation of the zebrafish central nervous system. *Development* **120**, 265–276.

Kintner, C. 1988 Effects of altered expression of the neural cell adhesion molecule, N-CAM, on early neural development in *Xenopus* embryos. *Neuron* **1**, 545–555.

Laale, H. 1982 Fish embryo culture: observations on axial cord differentiation in presomitic isolates of the zebrafish *Brachydanio rerio* (Hamilton–Buchanan). *Can. J. Zool.* **60**, 1710–1721.

Lane, M. C. & Keller, R. 1997 Microtubule disruption reveals that Spemann's organizer is subdivided into two domains by the vegetal alignment zone. *Development* **124**, 895–906.

Lee, C. H. & Gumbiner, B. M. 1995 Disruption of gastrulation movements in *Xenopus* by a dominant-negative mutant for C-cadherin. *Devl Biol.* **171**, 363–373.

Lee, G., Hynes, R. & Kirschner, M. 1984 Temporal and spatial regulation of fibronectin in early *Xenopus* development. *Cell* **36**, 729–740.

Lee, T. L., Lin, Y. C., Mochitate, K. & Grinnell, F. 1993 Stress-relaxation of fibroblasts in collagen matrices triggers ectocytosis of plasma membrane vesicles containing actin, annexins II and VI, and beta 1 integrin receptors. *J. Cell Sci.* **105**, 167–177.

Levine, E., Chung, H. L., Kintner, C. & Gumbiner, B. 1994 Selective disruption of E-cadherin function in early *Xenopus* embryos by a dominant negative mutant. *Development* **120**, 901–909.

Malacinski, G. M. & Youn, B.-W. 1981 Neural plate morphogenesis and axial stretching in 'notochord-defective' *Xenopus laevis* embryos. *Devl Biol.* **88**, 352–357.

Miyamoto, D. & Crowther, R. 1985 Formation of the notochord in living ascidian embryos. *J. Embryol. Exp. Morphol.* **86**, 1–17.

Moore, S. W. 1992 Direct measurement of dynamic biomechanical properties of amphibian embryonic tissues. PhD thesis, University of California, Berkeley, CA, USA.

Moore, S., Keller, R. & Koehl, M. 1995 The dorsal involuting marginal zone stiffens anisotropically during its convergent extension in the gastrula of *Xenopus laevis*. *Development* **121**, 3131–3140.

Pfaff, M., Reinhardt, D. P., Sakai, L. Y. & Timpl, R. 1996 Cell adhesion and integrin binding to recombinant human fibrillin-1. *FEBS Lett.* **384**, 247–250.

Poznanski, A., Minsuk, S., Stathopoulos, D. & Keller, R. 1997 Epithelial cell wedging and neural trough formation are induced planarly in *Xenopus*, without persistent vertical interactions with mesoderm. *Devl Biol.* **189**, 256–269.

Priess, J. R. & Hirsh, D. I. 1986 *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. *Devl Biol.* **117**, 156–173.

Ramirez, F., Pereira, L., Zhang, H. & Lee, B. 1993 The fibrillin–Marfan syndrome connection. *BioEssays* **15**, 589–594.

Ramos, J. W. & DeSimone, D. W. 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.

Ramos, J. W., Whittaker, C. A. & Desimone, D. W. 1996 Integrin-dependent adhesive activity is spatially controlled by inductive signals at gastrulation. *Development* **122**, 2873–2883.

Sakai, L. Y., Keene, D. R. & Engvall, E. 1986 Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. *J. Cell Biol.* **103**, 2499–2509.

Sakamoto, H., Broekelmann, T., Cheresh, D. A., Ramirez, F., Rosenbloom, J. & Mecham, R. P. 1996 Cell-type specific recognition of RGD- and non-RGD-containing cell binding domains in fibrillin-1. *J. Biol. Chem.* **274**, 4916–4922.

Sater, A., Steinhardt, R. A. & Keller, R. 1993 Induction of neuronal differentiation by planar signals in *Xenopus laevis*. *Devl Dynam.* **197**, 268–280.

Sausedo, R. A. & Schoenwolf, G. C. 1994 Quantitative analyses of cell behaviors underlying notochord formation and extension in mouse embryos. *Anat. Rec.* **239**, 103–112.

Schechtman, A. M. 1942 The mechanics of amphibian gastrulation. I. Gastrulation-producing interactions between various regions of an anuran egg (*Ityla regila*). *Univ. Calif. Publ. Zool.* **51**, 1–39.

Schoenwolf, G. C. & Alvarez, I. 1989 Roles of neuroepithelial cell rearrangement and division in shaping of the avian neural plate. *Development* **106**, 427–439.

Shih, J. & Keller, R. 1992*a* Patterns of cell motility in the organizer and dorsal mesoderm of *Xenopus*. *Development* **116**, 915–930.

Shih, J. & Keller, R. 1992*b* Cell motility driving mediolateral intercalation in explants of *Xenopus laevis*. *Development* **116**, 901–914.

Shih, J. & Keller, R. 1992*c* The epithelium of the dorsal marginal zone of *Xenopus* has organizer properties. *Development* **116**, 887–899.

Snow, M. 1977 Gastrulation in the mouse: growth and regionalization of the epiblast. *J. Embryol. Exp. Morphol.* **42**, 293–303.

Sokol, S. 1996 Analysis of Dishevelled signalling pathways during *Xenopus* development. *Curr. Biol.* **6**, 1456–1467.

Tuft, P. 1962 The uptake and distribution of water in the embryo of *Xenopus laevis* (Daudin). *J. Exp. Biol.* **39**, 1–19.

Vogt, W. 1929 Gestaltanalyse am Amphibienkein mit örtlicher Vitalfärbung. II. Teil. Gastrulation und Mesodermbildung bei Urodelen und Anuren. *Wilhelm Roux Arch. EntwMech. Org.* **120**, 384–706.

Waddington, C. H. 1940 *Organizers and genes.* Cambridge University Press.

Warga, R. & Kimmel, C. 1990 Cell movements during epiboly and gastrulation in the zebrafish. *Development* **108**, 569–580.

Williams-Masson, E. M., Malik, A. N. & Hardin, J. 1997 An actin-mediated two-step mechanism is required for ventral enclosure of the *C. elegans* hypodermis. *Development* **124**, 2889–2901.

Wilson, P. & Keller, R. 1991 Cell rearrangement during gastrulation of *Xenopus*: direct observation of cultured explants. *Development* **112**, 289–300.

Wilson, P., Oster, G. & Keller, R. E. 1989 Cell rearrangement and segmentation in *Xenopus*: direct observation of cultured explants. *Development* **105**, 155–166.

Winklbauer, R. & Keller, R. E. 1996 Fibronectin, mesoderm migration, and gastrulation in *Xenopus*. *Devl Biol.* **177**, 413–426.

Winklbauer R. & Schürfeld, M. 1999 Vegetal rotation, a new gastrulation movement involved in the internalization of the mesoderm and endoderm in *Xenopus*. *Development* **126**, 3703–3713.

Yamamoto, A., Amacher, S., Kim, S.-L., Geissert, D., Kimmel, C. & DeRobertis, E. M. 1998 Zebrafish paraxial protocadherin is a downstream target of spadetail involved in morphogenesis of gastrula mesoderm. *Development* **125**, 3389–3397.

Zhong, Y., Brieher, W. & Gumbiner, B. 1999 Analysis of C-cadherin regulation during tissue morphogenesis with an activating antibody. *J. Cell Biol.* **144**, 351–359.