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The function and mechanism of convergent extension during gastrulation of *Xenopus laevis*

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SUMMARY

The processes thought to function in *Xenopus* gastrulation include bottle cell formation, migration of cells on the roof of the blastocoel, and autonomous convergent extension of the circumblastoporal region. A review of recent and classical results shows that only the last accounts for the bulk of the tissue displacement of gastrulation, including spreading of the marginal zone toward the blastopore, involution of the marginal zone, and closure of the blastopore. Microsurgical manipulation and explantation studies, analysed by time-lapse video and cine microscopy, shows that the dorsal circumblastoporal region contains two regions which show either autonomous or semiautonomous convergent extension. The dorsal involuting marginal zone (IMZ) undergoes convergence (narrowing) and extension (lengthening) after its involution, beginning at the midgastrula stage and continuing through neurulation, such that it simultaneously extends posteriorly across the yolk plug and narrows the blastoporal circumference. Concurrently, the corresponding region of the overlying non-involuting marginal zone (NIMZ) begins a complementary convergent extension, but at a greater rate, which spreads vegetally to occupy surface area vacated by the IMZ. Tissue recombination experiments show that the deep cells of the dorsal IMZ bring about convergent extension. Labelling of small populations of these cells with a cell lineage tracer shows that convergent extension involves intercalation of deep cells to form a longer, narrower array. Direct time-lapse video and cine micrography of deep cells in cultured explants show that convergent extension involves radial and circumferential intercalation. Removal of the entire blastocoel roof of the early gastrula, including all or part of the NIMZ, shows that convergent extension of the IMZ alone can bring about its involution and blastopore closure. The role of convergent extension in gastrulation of other amphibians and other metazoans and its significance to related problems in early development are discussed.

INTRODUCTION

The roles of specific, regional processes

Amphibian gastrulation appears to involve all parts of the gastrula in a complex

Key words: gastrulation, convergent extension, marginal zone, notochord, mesoderm, fate map, cell movement, fluorescein-lysine-dextran (FLDx)

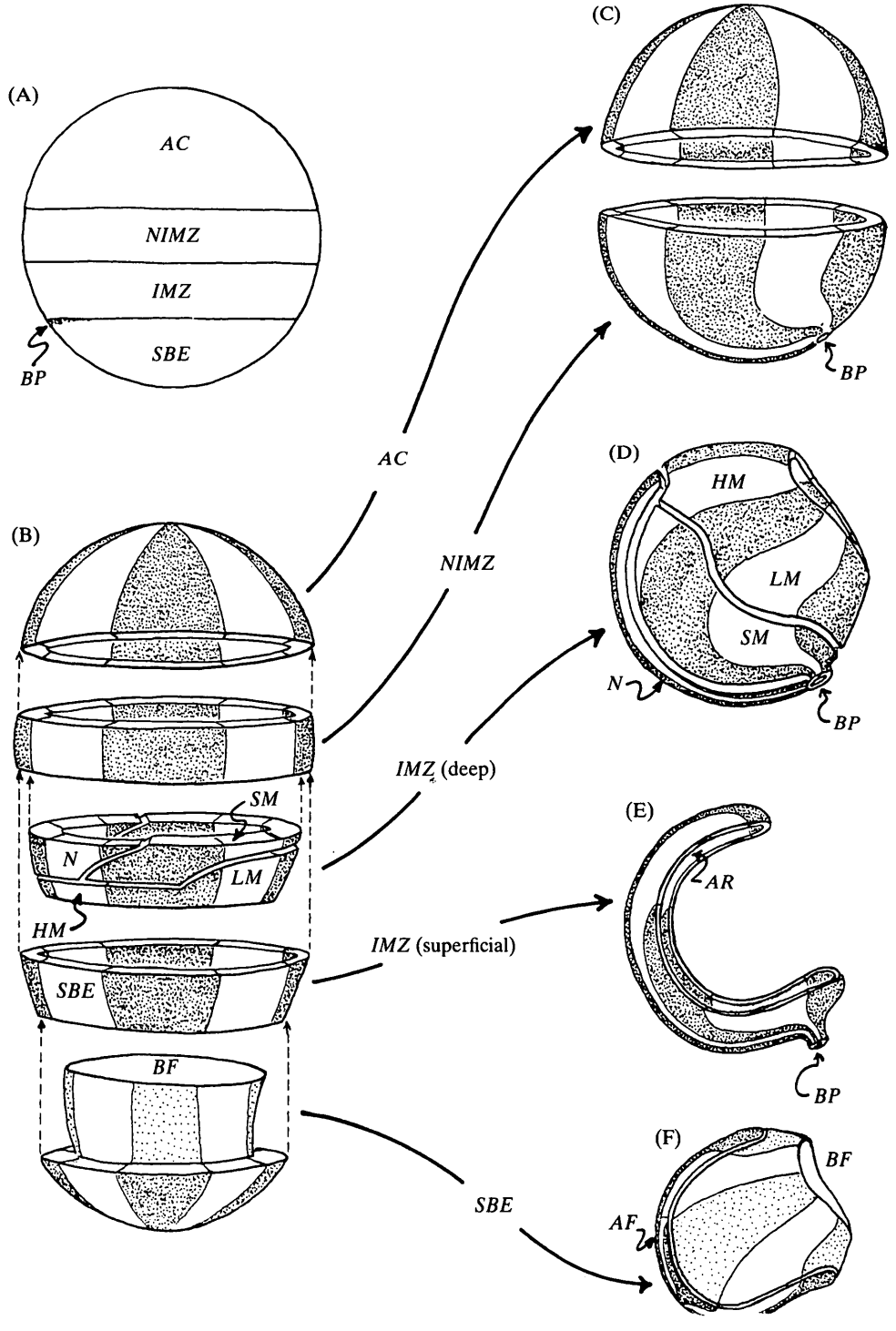
(A)
AC
NIMZ
IMZ
SBE
BP
(B)
SM
N
LM
HM
SBE
BF
(C)
BP
(D)
HM
LM
SM
N
BP
(E)
AR
BP
(F)
BF
AF
AC
NIMZ
IMZ (deep)
IMZ (superficial)
SBE

Fig. 1

integration of autonomous, local processes that together result in the distortion of the whole (reviewed in Spemann, 1938 and in Keller, 1985). These include epiboly of the animal cap region (Vogt, 1929; O. Mangold, 1923; Spemann, 1931; Holtfreter, 1933), the change in shape or the active migration of bottle or flask cells (Rhumbler, 1902; Holtfreter, 1943*a,b*; Baker, 1965; Perry & Waddington, 1966), migration of involuted cells toward the animal pole (Vogt, 1929; Holtfreter, 1943*b*; Nakatsuji, 1974, 1975*a*, *b*, 1976, 1984; Keller & Schoenwolf, 1977), and convergence and extension of the marginal zone (Vogt, 1922*a,b*; Spemann, 1902; O. Mangold, 1920; Lehman, 1932; Schechtman, 1942).

Although convergence and extension has largely been ignored in recent studies, with certain exceptions (Schechtman, 1942; Ikushima & Maruyama, 1971; Phillips, 1984; Keller, 1984), the classical literature shows it to be important (see Keller, 1985). There are several reasons to suspect that convergent extension may play a major and perhaps dominant role in gastrulation of *Xenopus* and perhaps of other amphibians as well. Vogt (1922*a,b*) recognized the capacity of the marginal zone to constrict and close the blastopore, and the dorsal marginal zone can autonomously undergo the narrowing (convergence) and lengthening (extension) (Spemann, 1902; O. Mangold, 1920; Lehman, 1932) characteristic of this region of the gastrula *in situ* (Vogt, 1929). Indeed, it seems to be the only one of the several processes that have been identified as playing a role in gastrulation that can account for blastopore closure. Migration of mesodermal cells animalward could result in shear at the interface between themselves and the overlying gastrular wall (Keller & Schoenwolf, 1977), but it is unlikely that this could explain either epiboly, involution, or blastopore closure (see Discussion and Keller, 1985). Bottle cell formation may be important in reorienting the convergent extension machinery in the dorsal sector

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Fig. 1. The early *Xenopus* gastrula (A) consists of the animal cap (*AC*), the non-involuting marginal zone (*NIMZ*), the involuting marginal zone (*IMZ*), and the sub-blastoporal endoderm (*SBE*). These are shown schematically on an exploded view of the early gastrula (B), and each is shown separately after gastrulation is complete (C–F). The dorsal sides of all figures are to the left, and (C) through (F) show successive layers of the neurula, from outside (C) to inside (F). During gastrulation (B–C), the AC expands uniformly and the NIMZ mostly on the dorsal side to form the entire outer shell of the neurula, consisting of prospective neural and epidermal ectoderm (C). In the early gastrula (B), the deep layer of the IMZ is a annulus consisting of prospective notochord (*N*), somitic mesoderm (*SM*), head mesoderm (*HM*), and lateral–ventral mesoderm (*LM*). During gastrulation, the annulus turns inside out to form the mesodermal mantle (D). The HM and LM spread, respectively, toward animal and ventral regions whereas the N and, to a lesser extent, the SM converge dorsally and elongate to form the dorsal, axial array of notochord and somites (D). The superficial layer of the IMZ consists of prospective endoderm (suprablastoporal endoderm, *SPE*) and lies outside the deep mesodermal annulus (B). During gastrulation, it turns inside out, converges dorsally, and elongates with the underlying mesoderm to form the archenteron roof (*AR*) at the end of gastrulation (E). The large mass of sub-blastoporal endoderm (*SBE*) is pushed inside and covered over by the IMZ during gastrulation to form the archenteron floor (*AF*). *BP*, blastopore; *BF*, blastocoel floor. From Keller, 1985, with permission.

of the early gastrula (see Keller, 1985) but removal of bottle cells after their formation does not affect gastrulation in any major way (see Cooke, 1975; Keller, 1981). These facts and others convinced us to try to define the role and mechanism of convergent extension in gastrulation of *Xenopus*.

The resulting investigations show: 1) that convergent extension plays a powerful and major role in gastrulation; 2) that it is brought about by active intercalation of deep cells; 3) that the role of other processes in gastrulation are somewhat different than previously supposed; 4) and that our notions of the nature of regional morphogenetic processes and their interactions should be revised. Largely qualitative evidence and arguments to support these general conclusions will be presented here, along with information from previous studies. Detailed and quantitative data supporting refinements of the process described here will be presented elsewhere. For those interested in background, a history of the investigation of convergent extension, its submergence during the interest in bottle cells and mesodermal cell migration, and the necessity of its reconsideration are reviewed in Keller (1985).

MATERIALS AND METHODS

Procurement and preparation of embryos

Embryos of the African clawed frog, *Xenopus laevis*, were obtained, dejellied, and held for experimental manipulation as in previous studies (see Keller, 1981). Staging was carried out according to Nieuwkoop & Faber (1967). Staging designations for explants represent the stage of companion, intact embryos.

Microsurgery, cell labelling, and culture of explants

Microsurgery of gastrulae was carried out in modified Steinberg's solution (Steinberg's salts with 5 mM-HEPES buffer, pH 7·4) on a Permoplast base, using hair loops and knives of eye-brow hairs. Operated embryos were transferred to agarose-coated culture dishes soon after the operations. Local populations of cells in the early gastrula were labelled by injecting the precleavage embryo with fluorescein-dextran-amine [FDA] (see Gimlich & Cooke, 1983). At stage 10–10·25 (early gastrula), selected regions of labelled embryos were grafted to the corresponding regions of unlabelled embryos; these were allowed to develop to the appropriate stage, fixed in 2 % formaldehyde, serially sectioned at 10 μm in paraffin, and the degree of intercalation of labelled and unlabelled cells determined by fluorescence microscopy under epi-illumination. Background fluorescence of yolk is higher with paraffin processing than with plastic sections, but it is acceptable and permits routine serial sectioning. 'Sandwich explants' of circumblastoporal regions of the gastrula were made and cultured in modified Steinberg's as described in the Results. 'Open-faced' sandwich explants and embryos without blastocoel roofs were made and cultured as described in the Results. Both these preparations were cultured in a medium developed by one of us (Danilchik), following the measurements of the

ionic composition of the blastocoelic fluid of *Xenopus* (Gillespie, 1983). This medium will be referred to as 'Danilchik's Medium' (Appendix I). In this medium, explants undergo apparently normal morphogenesis and development. Thus direct observation of the normal activities of the deep cells during *Xenopus* gastrulation are possible for the first time.

Micrography, Scanning Electron Microscopy (SEM), and morphometric analysis. Time-lapse cinemicrography was carried out with an Arriflex or Bolex 16 mm camera and intervalometer on Kodak Plus X or Ektachrome Reversal film; videomicrography was done with a Panasonic time-lapse recorder, camera, and monitor. Standard or inverted microscopes were used with objectives from × 2·5 to 40 (water immersion) magnification under epi-illumination from heat-filtered tungsten or fibre-optic sources. Preparation for SEM was done as described previously (Keller & Schoenwolf, 1977) and the microscopy was done on a ISI microscope at 10 to 15 kV. Morphometrics were done with a Numonics digitizer and Apple II computer.

RESULTS

Prospective areas and morphogenetic movements

We shall briefly describe the patterns of morphogenetic movements to provide background and to set forth a terminology more appropriate for describing what follows than that commonly used. A schematic fate map of the early gastrula (Fig. 1A,B) and the subsequent distortions of each of the prospective areas (Fig. 1C–F) are shown in exploded views of the embryo (see also Keller, 1975, 1976). We have defined two regions in the marginal zone according to their behaviour in gastrulation. The involuting marginal zone [IMZ] is that region lying immediately above the site of formation of the blastoporal pigment line; it consists of the prospective endodermal roof of the archenteron in the superficial layer and of prospective mesoderm in the deep region (Keller, 1975, 1976). This region undergoes involution during gastrulation. The non-involuting marginal zone [NIMZ] lies adjacent to the involuting marginal zone and bounds it at the limit of involution. The limit of involution will come to lie at the rim of the closed blastopore at the end of gastrulation. Note that both the IMZ and NIMZ undergo convergence and extension in closing the blastopore, with the maximum occurring in the dorsal sector of each and increasing symmetrically in the animal–vegetal extent of each, toward the maximum represented by the limit of involution (Fig. 1). Here we shall refer to convergence and extension as convergent extension, because the two movements occur together during gastrulation.

The pattern of convergent extension in explants of the gastrula

The major distortion involved in the transition from the array of anlagen at the onset of gastrulation (Fig. 1B) to that at the neurula stage (Fig. 1C–F) is involution of the IMZ and the coordinate convergent extension of both the IMZ and NIMZ,

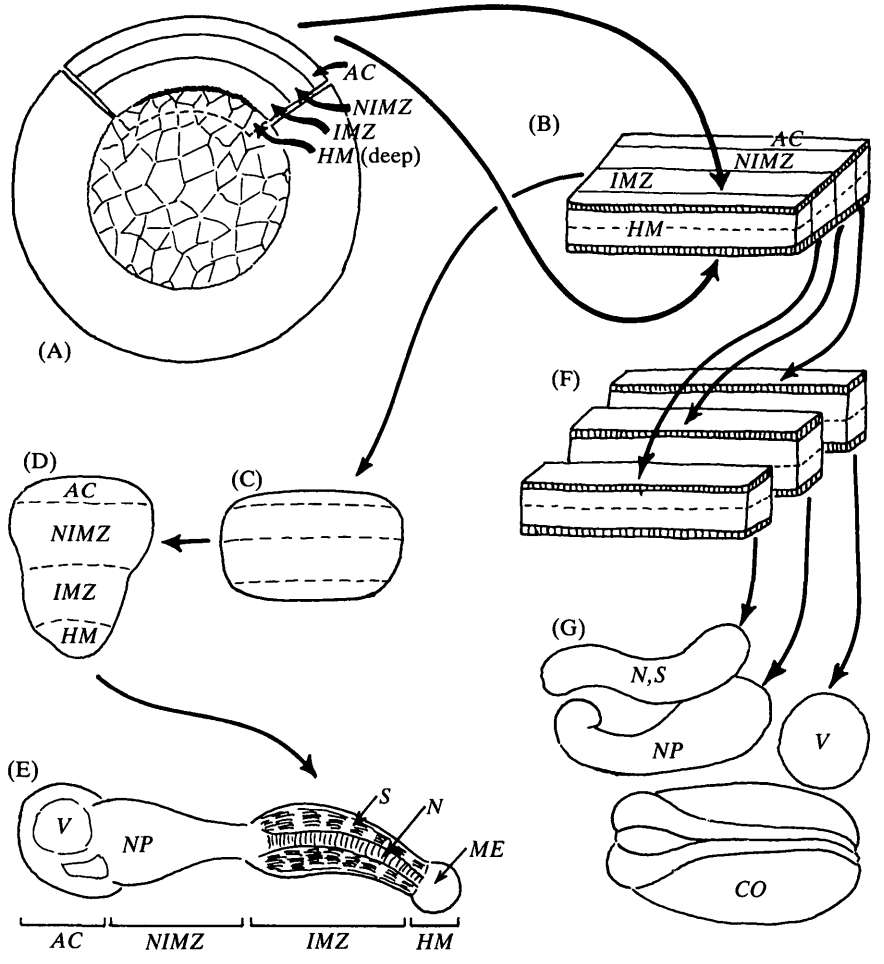


Fig. 2. The animal cap (*AC*), non-involuting marginal zone (*NIMZ*), involuting marginal zone (*IMZ*), and head mesoderm (*HM*) of the dorsal sector of the early gastrula (A), each show highly stereotyped behaviour and differentiation when two such regions are excised (A), sandwiched with their inner surfaces together (B), and allowed to develop through the early gastrula (C) and late gastrula (D) stages, to the late neurula stage, seen in sectional view (E). If these regions are separated after sandwiching (F) and allowed to develop independently to the late neurula stage (G), they show the same patterns of development. *CO*, control neurula; *ME*, mesenchyme; *N*, notochord; *NP*, notoplate; *S*, somitic mesoderm; *V*, fluid-filled vesicle.

particularly in their dorsal sectors. How does this occur? Previous microsurgical rearrangement experiments showed that the dorsal sector of the IMZ undergoes autonomous convergent extension, as expected from the abundant evidence in the classical literature (see Schechtman, 1942 and Keller, 1985). However, it occurs largely in the second half of gastrulation, after involution (Keller, 1984). It was proposed (Keller, 1984) that the dorsal sector of the mesodermal annulus is turned under (involuted) in the first half of gastrulation, probably in part by the mechanical

forces generated during bottle cell formation (see Keller, 1985) and in part as the result of migration of the leading head mesodermal cells (Schechtman, 1942). Then, at the midgastrula stage (stage 10·5), this region undergoes convergence to form a constriction ring that acts on the inner surface of the blastoporal lip and tends to roll the remaining, uninvoluted IMZ over the lip. The convergent extension, predominantly on the dorsal side, results in movement of the lip across the yolk plug. These coordinate actions result in involution of the remaining IMZ, eccentric closure of the blastopore, and the dorsal convergence and extension observed in fate maps (Keller, 1984).

Culture of explants of the dorsal sector of the early gastrula verifies this notion of how convergent extension functions in gastrulation. The dorsal sectors of two early gastrulae, consisting of a superficial epithelium and a layer of deep cells, four to six cells thick, were excised and sandwiched with their inner surfaces together (see Ikushima & Maruyama, 1971), and allowed to heal (Fig. 2A, 3B). Four regions can be recognized in the explants, based on their morphogenesis 1) the animal cap (AC); 2) the non-involuting marginal zone (NIMZ); 3) the involuting marginal zone (IMZ); 4) the head mesoderm (HM). After explantation, epithelial edges heal together (Fig. 3A) and no major change in the shape of the explant occurs until the midgastrula stage. Then the IMZ begins to narrow and lengthen as does the NIMZ (Fig. 2C–D; Fig. 3B). Both regions continue their convergent extension through the late gastrula stage (Fig. 3C) and into the late neurula stage (Fig. 2E; Fig. 3D). The AC and HM become spherical and knob-like (Fig. 2E, 3D). The IMZ differentiates into a central notochord, flanked by somitic mesoderm (Fig. 2E,3E). The NIMZ forms an elongated structure of small pleiomorphic cells that are similar in appearance to the cells of the early neural tube. This region is similar in behaviour to the notoplate, which is that part of the neural anlage in contact with the notochord (Jacobson, 1982). The AC invariably inflates one or more fluid-filled vesicles surrounded by cells histologically similar to those of the notoplate. The HM region forms a ball of mesenchyme cells by the early tail-bud stage (not shown). These regions appear to have some measure of autonomy with respect to one another and the rest of the gastrula by the early gastrula stage; they can be cut apart from one another at a stage equivalent to stage 10·25, and each piece will follow its normal course of behaviour and differentiation (Fig. 2F,G). Note that both IMZ and NIMZ may form the full length of the notochordal–notoplate region of the corresponding control neurula (Fig. 2G). Similar explants of the lateral and ventral sectors show progressively less capacity for convergent extension during the gastrula stages. In these sectors, convergent extension appears to occur only in the IMZ, and it does not continue into the neurula stages (data not shown). Similar behaviour of dorsal, lateral and ventral sectors of the IMZ were observed by Schechtman (1942) in the anuran *Hyla regilla* and in many other classical investigations (see Keller, 1985 for a review).

Tracings from time-lapse cinemicrographic frames of explants filmed simultaneously from the top (tangential or surface view; Fig. 4) and from the side (not

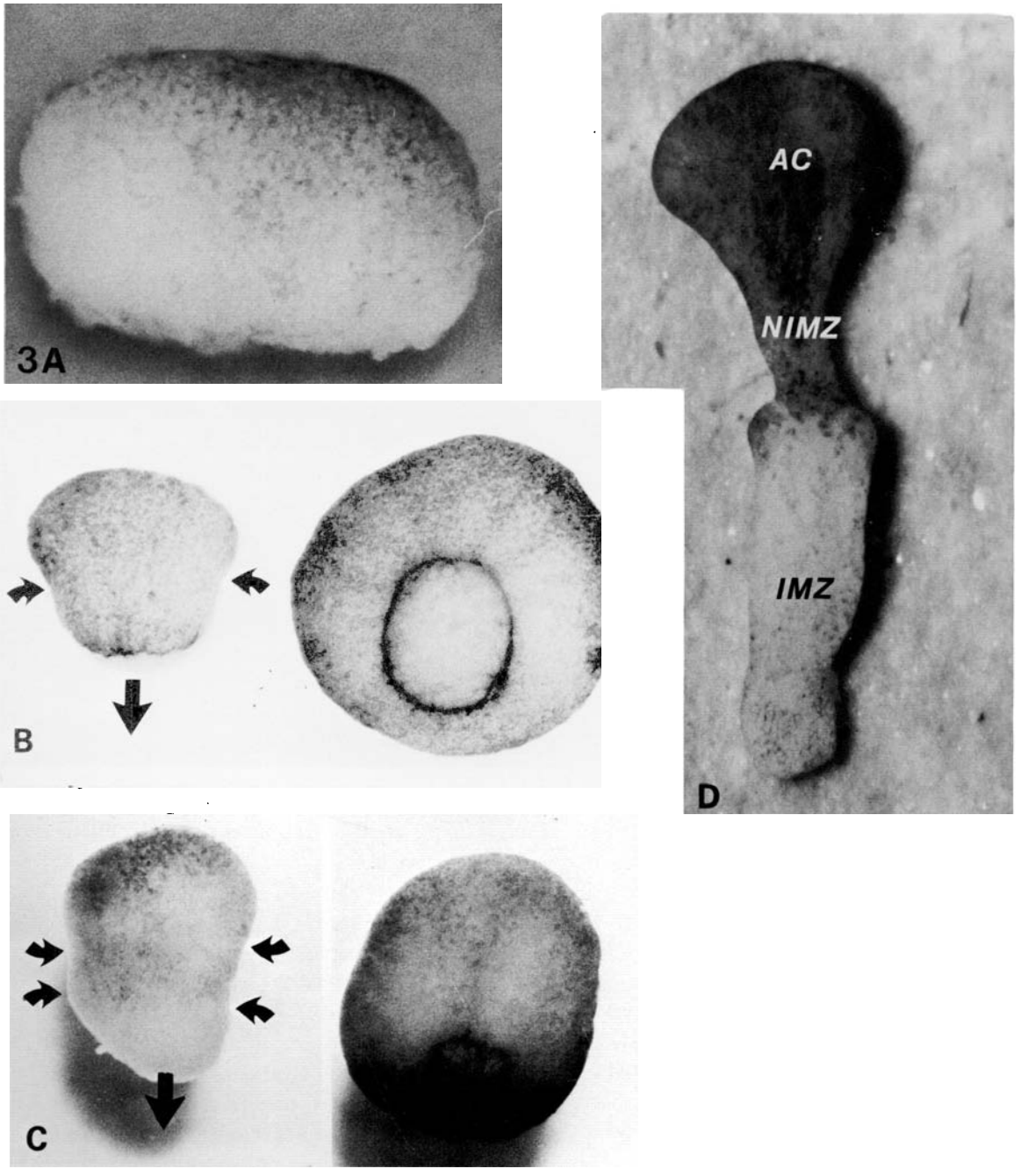
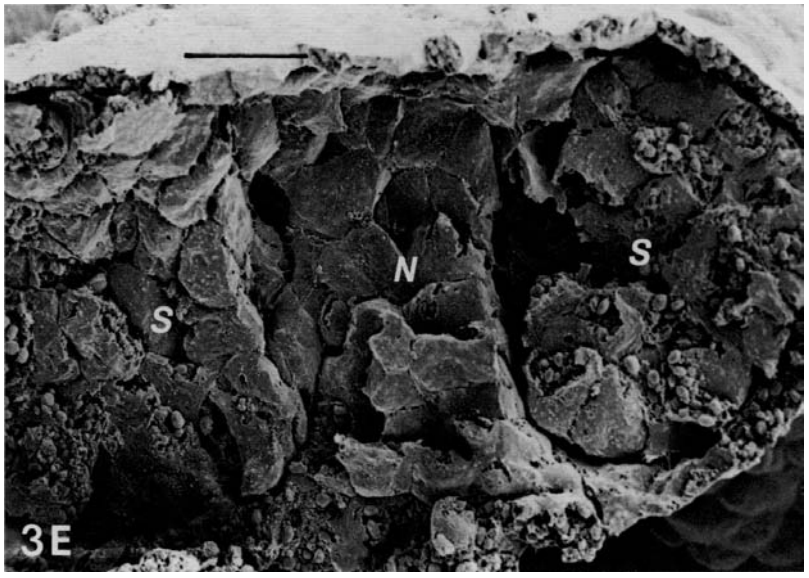


Fig. 3. Light micrographs show an explant (left) 20 mins after construction, at stage 10+ (A), an explant and control embryo at stage 11 (B) and 11·5 (C), and an explant at stage 17 (D). A scanning electron micrograph (E) shows the internal structure of the IMZ region of an explant at the late neurula stage. *AC*, animal cap; *IMZ*, involuting marginal zone; *NIMZ*, non-involuting marginal zone; *N*, notochord; *S*, somitic mesoderm. Magnifications: A, 52; B, C, 30; D, 37.



shown), show the distortion involved in convergent extension. When convergent extension begins, the thickness of the explant increases near the animal end (prospective posterior end) of the IMZ as the lateral regions move toward the midline, and the vegetal end (prospective anterior end) begins extension (Fig. 4, t = 0–1 h). Likewise, the NIMZ begins extension, without thickening, in the opposite direction from the IMZ (Fig. 4, t = 0–1 h). Extension continues through the late gastrula stage (Fig. 4, t = 1·5–3 h) and into the late neurula stage (Fig. 4, t = 6 h). The mean rate of extension of the IMZ is 2·6 μm min^{-1} and that of the NIMZ is 3·6 μm min^{-1} (means of eight and six explants, respectively). The rate and degree of convergent extension of each region varies from explant to explant. Good to excellent convergent extension (approximating that shown) occurred in 52 of 84 cases; in 19 cases convergent extension was fair, and it was apparent but poor in 13 cases.

Gastrulation in embryos lacking the blastocoel roof

The autonomy and the timing of convergent extension in the gastrula (Keller, 1984), as well as evidence in the literature (Keller, 1985), argue strongly that convergent extension alone can account for the bulk of the distortion of the gastrula and that other processes such as bottle cells formation and mesodermal cell migration on the roof of the blastocoel play ancillary roles (see Keller, 1985). This notion was tested and verified by removing the blastocoel roof and studying gastrulation in its absence.

The 'blastocoel roof' was removed from early gastrulae by cutting circumferentially, parallel to the prospective blastoporal pigment line, through the wall of the blastocoel and lifting off the roof (Fig. 5A–C). The animal–vegetal level (the 'latitude') of the cut was varied to include: i) only the AC; ii) the AC and part of the NIMZ; or iii) the AC, NIMZ and part of the IMZ. The parts of the embryo were

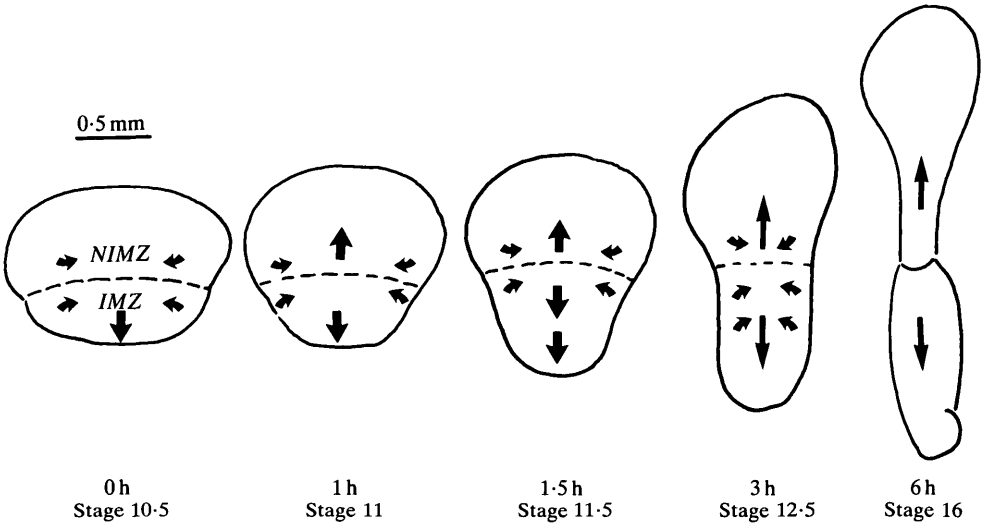


Fig. 4. Tracings of a dorsal sector explant in tangential (surface) view, from a time-lapse cinemicrographic record. The progress of convergent extension of the NIMZ and IMZ through the gastrula stages (10·5 to 12·5) and neurula stages (16) are shown. The arrows indicate the directions of movements.

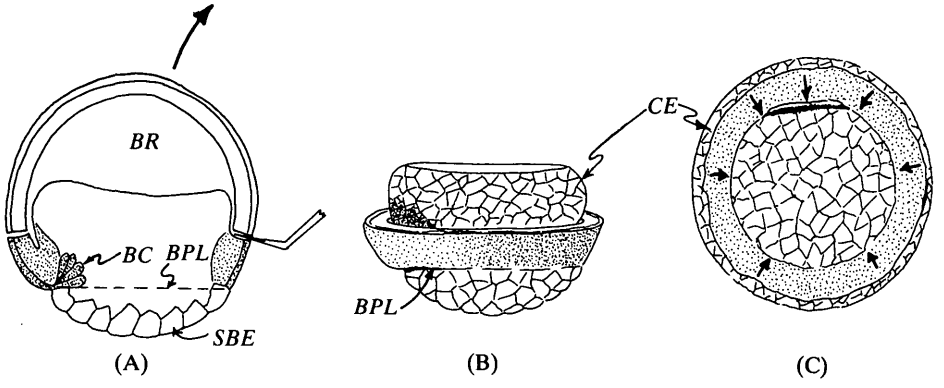


Fig. 5. The blastocoel roof (*BR*) of the early gastrula of *Xenopus* was removed by cutting through the wall of the gastrula parallel to and some distance above the site of formation of the blastoporal pigment line (*BPL*), which is at the perimeter of the sub-blastoporal endoderm (*SBE*), as shown in a midsagittal diagram of the early gastrula (A). The resulting embryo, shown in lateral (B) and vegetal (C) view, has an annulus of marginal zone material (shaded) encircling a central column of endoderm consisting of a central endodermal core (*CE*) and the SBE. In succeeding stages of development, the marginal zone will involute (arrows) and the blastopore will close. The level of cuts were varied such that the remaining annulus consisted of both IMZ and NIMZ, IMZ only, and only the vegetal-most (prospective anterior-most) IMZ.

cultured in Danilchik's medium. Time-lapse videomicrography of the vegetal regions of these embryos shows that involution of the IMZ and closure of the blastopore occur as fast or faster than in the controls (Fig. 6). Embryos with the NIMZ remaining show involution of the IMZ, closure of the blastopore (Fig. 6,

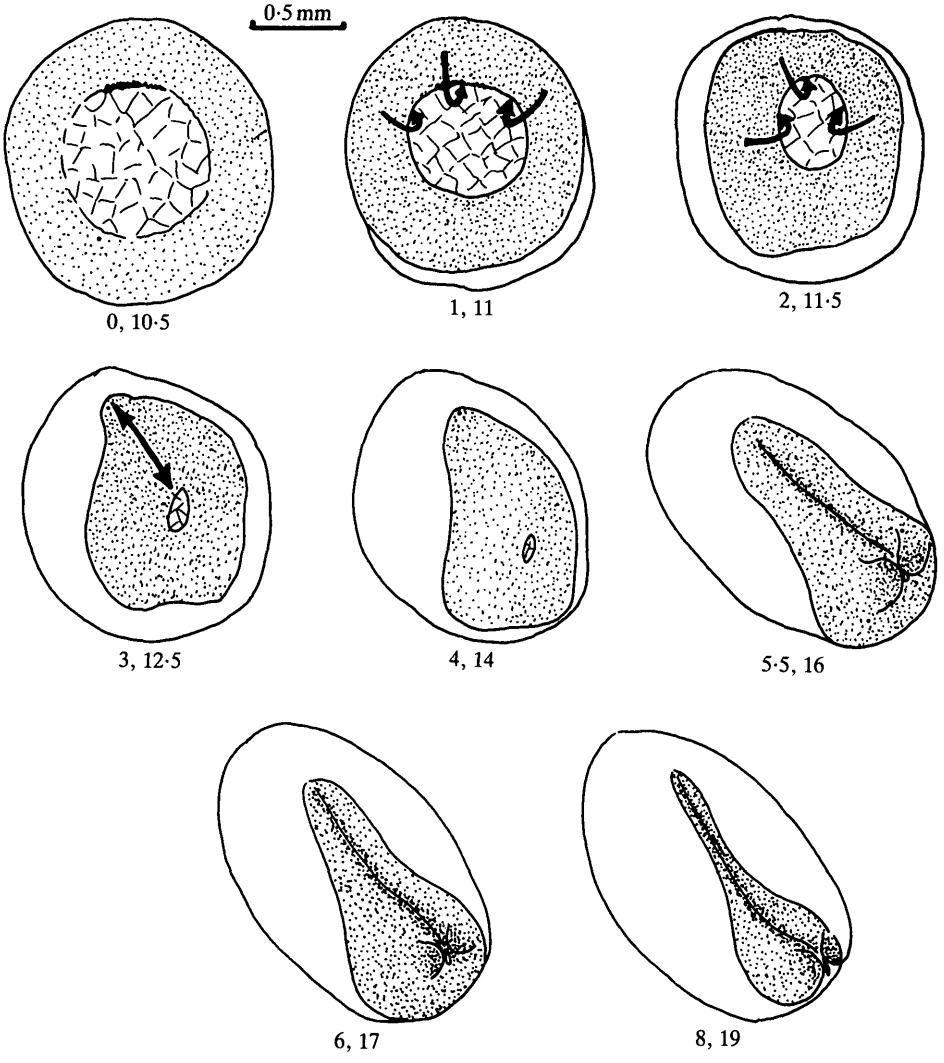
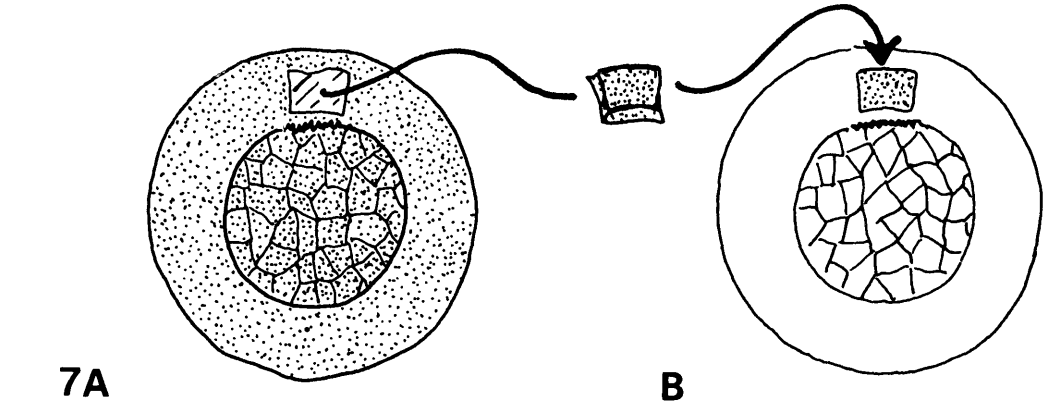
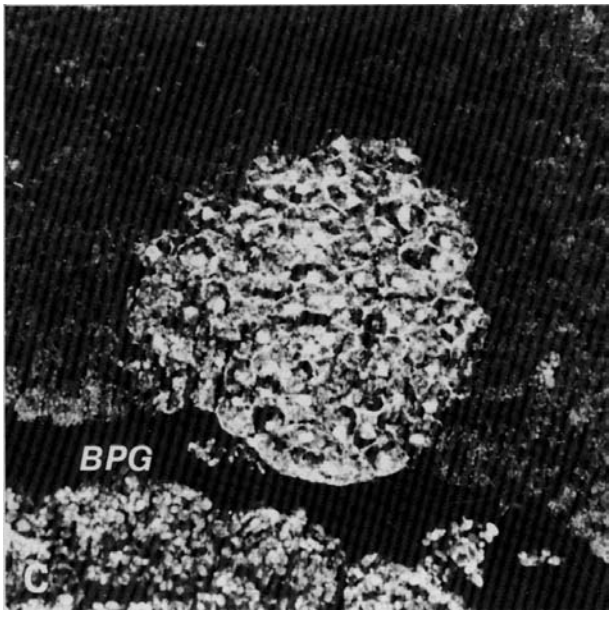


Fig. 6. Tracings from a time-lapse videomicrographic record of the vegetal or dorsal aspects of embryos developing without the animal cap show the progress of involution, closure of the blastopore, and convergent extension under these conditions. The IMZ–NIMZ is shaded and movements are indicated by arrows. The time elapsed is given in hours, followed by the approximate developmental stage of control embryos.

0–4 h), and convergence and extension of the dorsal sector (Fig. 6, 2–8 h). Moreover, if part of the animal-most (prospective posterior) IMZ is also removed, constriction of the blastopore ceases once all the IMZ is involuted. From the late gastrula stage onward, the course of development of these embryos depends on whether or not there is dorsal NIMZ available for interaction with the involuted IMZ. If dorsal NIMZ is left on the embryo, it comes into apposition with the IMZ in the late gastrula and the two undergo convergent extension together in the





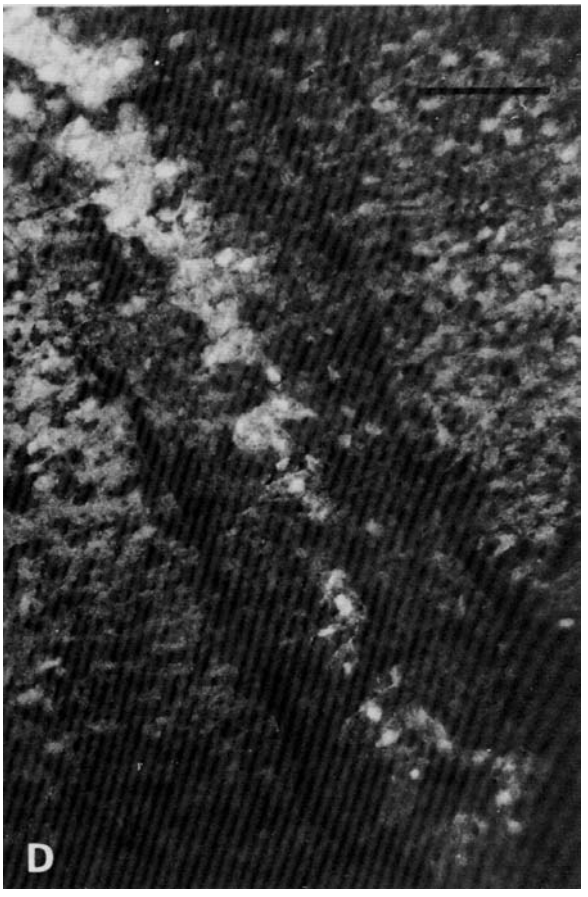
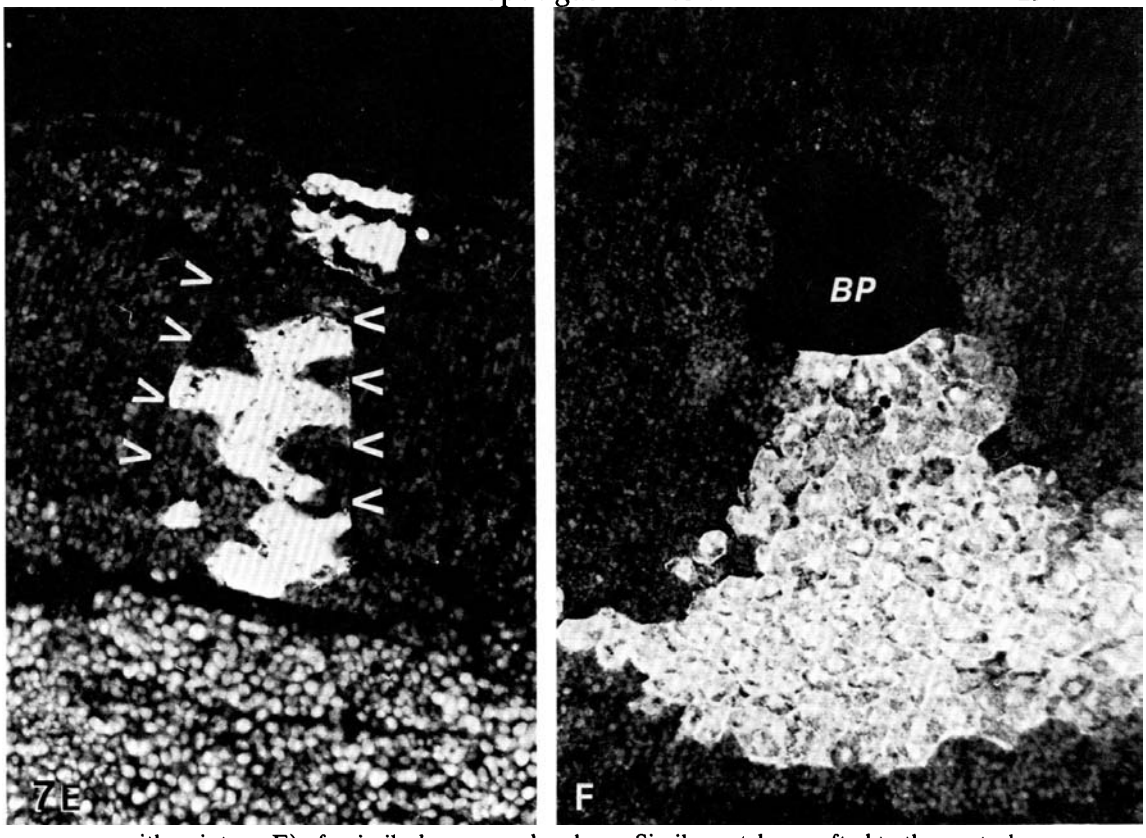


Fig. 7. A small patch of an early gastrula labelled with FDA (A) was grafted to an unlabelled embryo (B). Fluorescence microscopy of serially sectioned gastrulae 20 mins later shows the grafted patch (C). By stage 14, labelled cells from such a patch are intercalated with unlabelled cells of the host and are scattered the full length of the notochord (D). Intercalation can also be seen in cross section of the notochord (outlined



with pointers, E) of a similarly prepared embryo. Similar patches grafted to the ventral region, which shows much less convergent extension, remain nearly contiguous (F). Magnifications: A–F ×.

neurula stages (Fig. 6, 2–8 h). Under these conditions, they form a stable array of dorsal axial structures, including notochord, somites, an archenteron and ventral part of the neural tube. Without the NIMZ, the IMZ involutes and undergoes convergent extension, but it sinks into the endoderm and its position and direction of extension may vary considerably.

The mechanism of convergent extension

The convergent extension and involution of the IMZ depends on the activities of its deep, non-epithelial cells (Keller, 1981). On the other hand, this behaviour does not depend on the character of the superficial epithelium: this tissue can be replaced with epithelium from other non-extending regions or reoriented without effect (see Keller, 1984, 1985). What do the deep cells do to bring about convergent extension? Waddington (1940, page 109) pointed out that the elongation of the dorsal sector of the amphibian embryo is not accompanied by overall change in cell shape and suggested that this distortion is brought about by cell rearrangement. Such is the case. By grafting a small patch of IMZ cells from an embryo labelled with FDA to an unlabelled embryo (Fig. 7A,B) and examining the sectioned embryo with

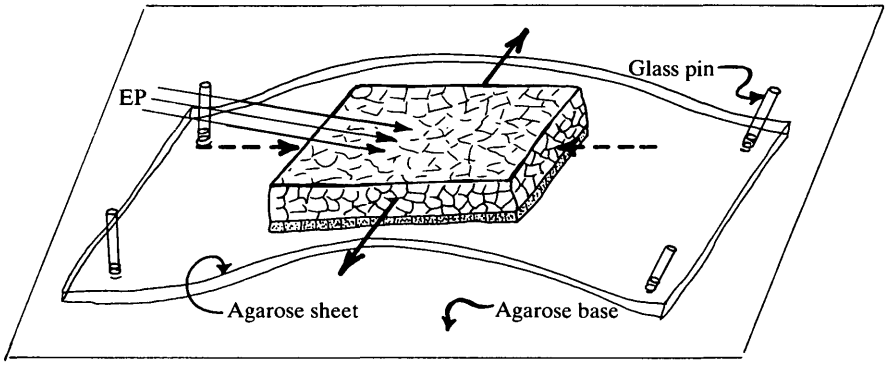


Fig. 8. For direct observation and micrography of cell behaviour during convergent extension, "open-faced" explants were made as shown and cultured in Danilchik's medium. Explants were placed epithelial side down for observation from above, or epithelial side up for use with an inverted microscope. EP, low-angle epi-illumination; solid arrow, extension axis; dashed arrow, convergence axis.

fluorescence microscopy at later stages, the circumferential intercalation of labelled graft and unlabelled host cells is apparent. Shortly after grafting a labelled patch to an unlabelled embryo, the patch boundaries are contiguous, with few isolated cells (Fig. 7C). In contrast, by the early neurula stage, extensive mixing has occurred in the circumferential direction such that labelled cells are strung nearly the full length of the notochord and have intercalated with many unlabelled cells (Fig. 7E). This evidence shows that intercalation of many rows of cells to form fewer rows of greater length is coincident with convergent extension. In contrast, a corresponding experiment done in the ventral sector of the gastrula, where little convergent extension occurs, reveals no intercalation (Fig. 10F).

More direct evidence comes from actual observation of the cell behaviour during convergent extension. If the dorsal sector of the gastrula is explanted as shown for the construction of sandwich explants, but left as an 'open-faced' sandwich, cultured in Danilchik's medium (Fig. 8), the behaviour of the deep cells can be seen directly and recorded by video or cine micrographic methods, using a compound microscope with a $\times 40$ water immersion objective and epi-illumination. In this preparation, the epithelial wound-healing response is held at bay and the explant is not distorted by the attempts of the free edge to heal with itself. The IMZ sector of the explant converges, extends, and differentiates into notochord and somitic mesoderm under full view of the experimenter. As suggested by the surface distortion of the sandwich explants, deep cells of 'open-faced' explants move medially at the posterior IMZ and forward at the vegetal end of the explant. This is accompanied by breaking of contacts between cells and their separation to form short-lived fractures that extend between several to many cells. At high magnification, individual cells intercalate between one another in the circumferential (Fig. 9A) and radial (Fig. 9B) directions. In the late gastrula–early neurula stage, the boundaries of the notochord become apparent and the notochordal cells undergo circumferential

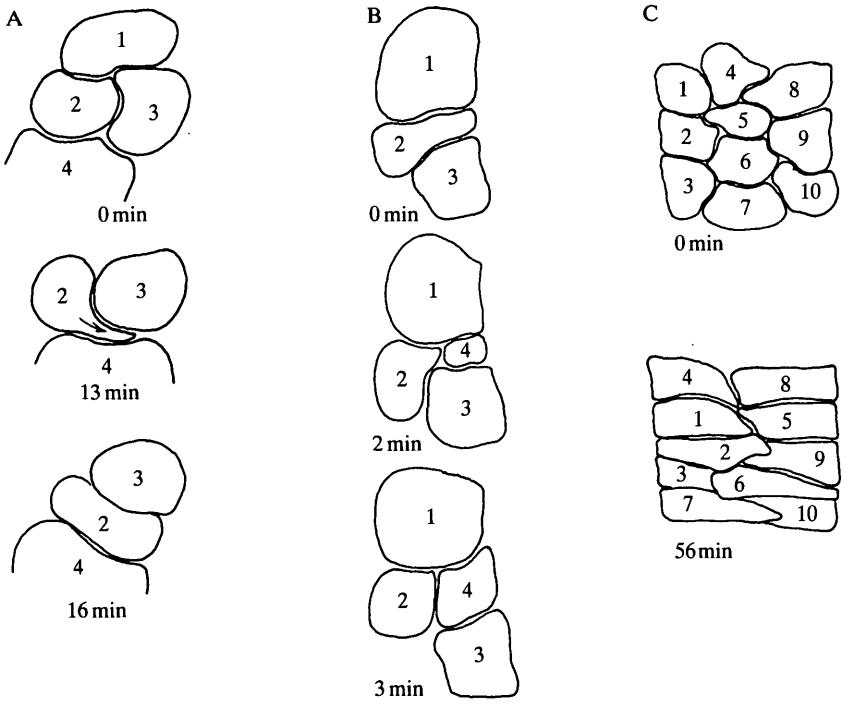


Fig. 9. Tracings of deep cells of the IMZ, recorded by time-lapse videomicrography of an "open-faced" explant culture, show circumferential intercalation (A) and radial intercalation (B) during convergent extension, and intercalation of notochord cells in the neurula (C). The axis of extension is vertical and that of convergence is horizontal in all figures.

intercalation to form the 'stack of coins' array characteristic of this tissue (Mookerjee, Deuchar, & Waddington, 1950).

The mechanism of intercalation and its relationship to production of force appropriate to produce elongation and narrowing of the explant is not yet clear. The fracturing observed may be simply a mechanism for releasing internal stresses; alternatively, it may be organized and produce spaces into which cells can move. All observed intercalation events in the gastrula-stage explants involved dramatic cytoplasmic flow, as revealed by movement of yolk platelets. Such flow often passed distally, through the core of the cell protrusion leading the intercalation, and at the distal end of the protrusion it turned peripherally in a 'fountain-head' and joined what appeared to be a cortical region stably attached to adjacent cells.

The convergent extension of these open-faced explants occurs without traction on an external substratum. The cells do not attach to the agarose base. The notochord apparently elongates with considerable force. In some preparations left overnight, the notochord continues to elongate, forms its characteristic sheath, and may throw itself into bends, some of which push laterally through the somites, on one side.

DISCUSSION

Classical evidence relating to convergent extension

There is abundant evidence in the classical literature supporting a major role of convergent extension in gastrulation, and a rekindling of interest in this morphogenetic process is long overdue. Spemann (1902) and Vogt (1922*a*) demonstrated the capacity for the marginal zone to constrict. Schechtman (1942) showed that the dorsal marginal zone has the capacity to extend and converge and argued that this behaviour plays a major role in closure of the blastopore. A variety of microsurgical manipulations (Mangold, 1920; Spemann, 1931; Lehman, 1932; Holtfreter, 1933; Schechtman, 1942) and explantations (Holtfreter, 1938*a*,*b*; Schechtman, 1942; Ikushima, 1959, 1961; Ikushima & Maruyama, 1971) of the dorsal marginal zone show some tendency to form elongated, narrowed structures. However, the lack of a continuous time-lapse record of shape change and malformation of the explants, probably due to effects of wound healing, make it difficult to determine from these experiments, when (gastrula or neurula stage) and where (prior to or after involution) convergent extension takes place. Although the common view seems to have been that convergent extension of the marginal zone pushes material toward the lip from the outside, Holtfreter (1944, page 187), with typical insight, clearly suggested that it might also be a postinvolution process.

Several geometric and mechanical facts argue that convergent extension plays the major role in driving gastrulation. Those regions of the gastrula that distort greatly lie in a narrow annulus immediately above the blastopore (the IMZ and NIMZ), and their dorsal sectors show the greatest change in shape (Keller, 1975, 1976). Traction of postinvolution cells on the roof of the blastocoel could produce shearing at the interface of the two tissues and perhaps move the outside vegetally and the inside toward the animal pole (see Keller & Schoenwolf, 1977), but this would not account for the forces necessary for blastopore closure nor for the convergent extension movements in the circumblastoporal region. Lastly, removal of one member of the pair of tissues involved in this traction, but blastocoel roof, as described here and by Holtfreter (1933), fails to hinder involution, blastopore closure, or convergent extension, whereas appropriate manipulation of the circumblastoporal region results in immediate and local blockage of all these processes (see Schechtman, 1942; Keller, 1981, 1984). These facts suggest that the 'main engine' of gastrulation lies in the region just above the blastopore and involves the autonomous capacity of this sector to converge and extend.

Proposed function of convergent extension in gastrulation

In contrast to other mechanisms, convergent extension of the circumblastoporal region can account for the bulk of the distortion of the gastrula. The proposed mechanism can best be visualized by considering a diagram of the dorsal sector of the gastrula, viewed from inside the blastopore, looking out (Fig. 10). The vegetal edge of the dorsal sector of the mesodermal torus is turned over the lip in the early

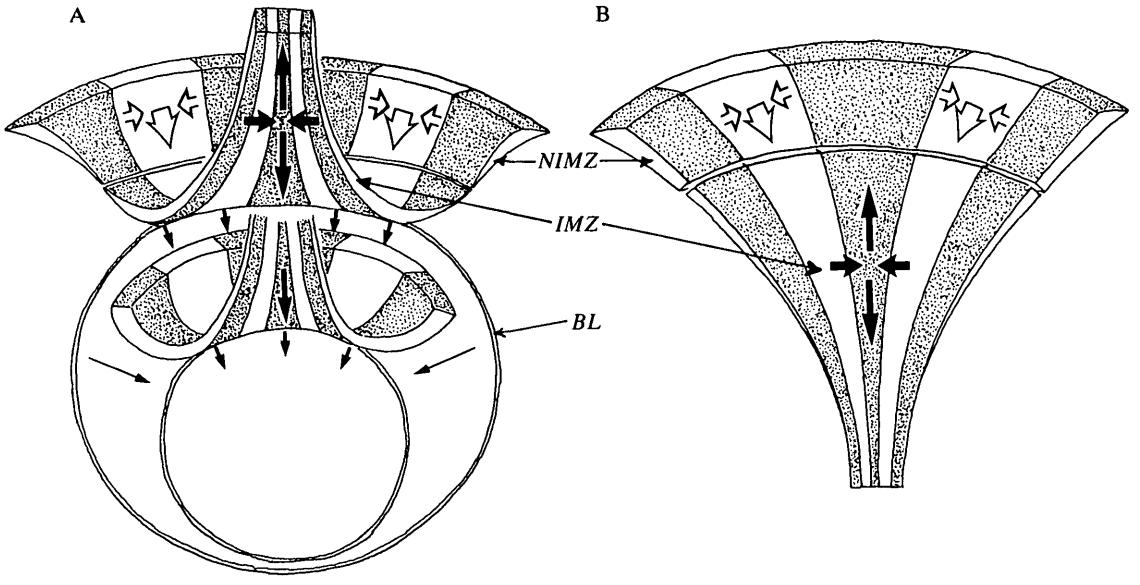


Fig. 10. The function of convergent extension of the involuting marginal zone (*IMZ*) and non-involuting marginal zone (*NIMZ*) in normal gastrulation (A) and exogastrulation (B) is shown diagrammatically as seen from the inside of the circumblastoporal region of the gastrula. The IMZ undergoes convergent extension (solid arrows) in the postinvolution position and thus simultaneously pushes vegetally and constricts the blastopore (*BL*). On the outside, the NIMZ undergoes convergent extension (open arrows) and thus pushes vegetally, toward the blastopore. If the IMZ is not turned over the lip and directed inward by the early midgastrula stage, its convergent extension movement is directed outward, in series with that of the NIMZ (B) rather than parallel to it (A). From Keller (1985) with permission.

gastrula stage, probably due to distortions associated with formation of the bottle cells (see Keller, 1985) and perhaps due to the invasive, migratory behaviour of the leading mesodermal cells in this region (Schechtman, 1942). At the midgastrula stage, the involuted part of the IMZ begins convergent extension posteriorly and thus, in one stroke, constricts and pushes vegetally on the inside of the blastoporal lip (Fig. 10A). This tends to roll preinvolution cells of the IMZ over the lip where they join in the convergent extension movement. At some point, the NIMZ also begins convergent extension and feeds cells vegetally to take up positions vacated by the involution of the IMZ. The maximum constriction of the blastopore and thus the limit of involution is fixed by the maximum degree of convergent extension accomplished by the adjacent, posterior regions of the IMZ and NIMZ. If the mesodermal annulus is not turned under at the onset of convergent extrusion, the convergent extension machinery is directed outward and produces an exogastrula (Fig. 10B).

Several facts suggest a special role of the dorsal NIMZ in this process. Firstly, convergent extension is characteristic of only the dorsal sector of the NIMZ that normally is in contact with the notochord and because the floor plate of the

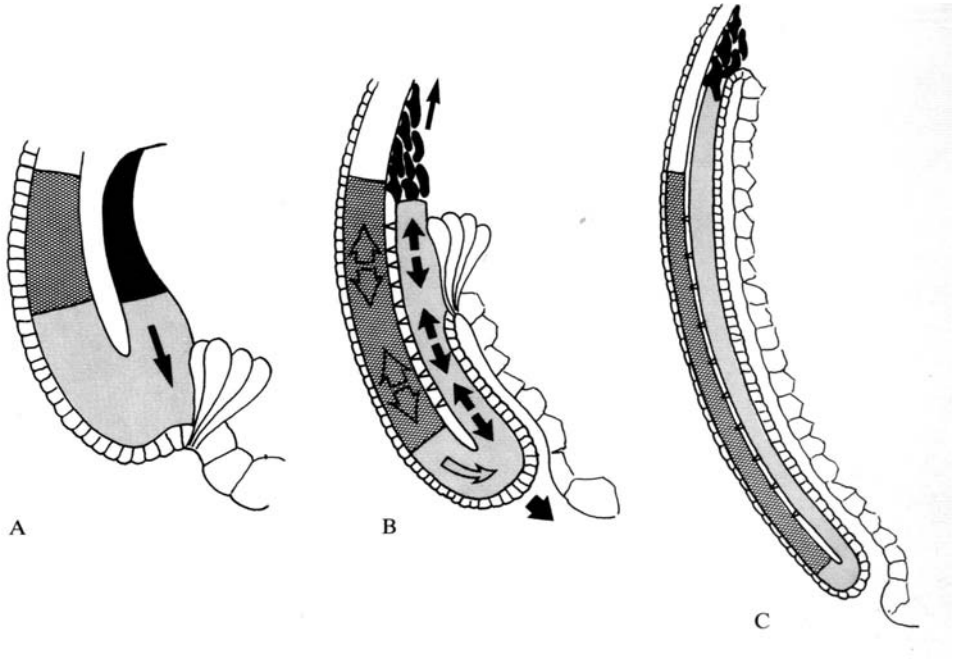


Fig. 11. A summary of the behaviour of the dorsal IMZ (light shading), the dorsal NIMZ (dark shading), and the dorsal leading mesoderm (very dark shading) is shown in diagrams of the early gastrula (stage 10·25–10·5, A), midgastrula (stage 11–11·5, B) and the late gastrula and neurula (stage 12·5–16, C). See the text for an explanation.

posterior neural plate; this special region of the neural plate has been called the notoplate (see Jacobson, 1982). Secondly, the dorsal NIMZ does not show convergent extension when the dorsal IMZ–NIMZ is rotated or repositioned in the embryo, whereas the dorsal IMZ does (Keller, 1984). The NIMZ may show convergent extension but may be too weak to overcome the mechanical resistance of the uncooperative tissues around it. Alternatively, some tissue relationship is necessary for the exercise of convergent extension may be disturbed in these circumstances. Thirdly, convergent extension of the dorsal NIMZ does not appear to occur in the 'open-faced' explants, described below. Lastly, in embryos lacking an animal cap region, also described below, the NIMZ often does not extend if it is not in contact with the underlying involuted part of the IMZ. The common feature in all these failures of convergent extension of the NIMZ is the absence of contact of its basal region with itself or with the IMZ. Is convergent extension of NIMZ dependent on contact of its basal (deep) surface with other tissues, and if so, with what tissues and under what conditions? Extension of notoplate in *Taricha* becomes independent of the notochord at the early neurula stage (Jacobson, 1982). Dissections of living *Xenopus* gastrulae show the notoplate (dorsal NIMZ) and underlying notochordal region are tightly connected and nearly inseparable from stage 11 onward. Is convergent extension of the dorsal NIMZ regulated by basal contact with the involuted

IMZ (notochordal mesoderm)? This would explain the sensitivity of its behaviour to basal contact and would also insure coordination of convergent extension in the two regions.

We summarize our current thinking on how the convergent extensions of the two regions are coordinated in Fig. 11. First, the leading edge of the dorsal mesodermal annulus is turned under by the action of the bottle cells and perhaps the migration of the leading prospective head mesoderm (see Schechtman, 1942) (Fig. 11A). Second, convergence, which constricts the blastoporal lip, and extension, which pushes it vegetally across the yolk plug, begin in the involuted IMZ at the midgastrula stage and proceed vegetally in the IMZ toward its uninvoluted region (solid arrow in IMZ, Fig. 11). As this occurs, the cells of the uninvoluted IMZ are recruited into the process of convergent extension, and the resulting constriction forces, developed because of convergence, tend to roll these cells over the lip (open arrow in IMZ, Fig. 11B). The involuted IMZ makes contact basally with the NIMZ, and the NIMZ begins convergent extension at a rate faster than the involuted IMZ (open arrows in NIMZ, Fig. 11B). Coordinated convergent extension of the two accomplish involution, blastopore closure, and organization of a stable array of dorsal, axial structures [Fig. 11C]. Basal contact with the involuted IMZ, which appears to be a necessary condition for convergent extension of the dorsal NIMZ, is strong and resists physical separation *in vivo* only at stage 11 and beyond. Throughout, the traction of the leading mesodermal cells on the roof of the blastocoel may assist in bringing about relative movement of the involuted material and the outer, gastrular wall (Fig. 11B,C), although such behaviour does not appear to be necessary for involution or blastopore closure. This model is consistent with the results of Schechtman (1942) on *Hyla*, with previous results on perturbation of the marginal zone (Keller, 1981, 1984), with the behaviour of the explants and embryos without blastocoel roofs described here, and with the behaviour of the notoplate during neurulation of *Taricha* (Jacobson, 1982).

The cellular basis of convergent extension

It is not clear how intercalation is related to generation of the force that produces convergent extension. It is clear that the explants, either the sandwiches or the 'open-faced' sandwiches do not require an external substratum and therefore must generate the force from within themselves. It is also clear from Waddington's (1940) argument and from our direct and indirect observations that intercalation of cells to form a longer, narrower array is the geometrical basis of convergent extension. Although cells of the superficial layer (Keller, 1978) and the deep region rearrange, those in the former do so passively (Keller, 1978, 1981). Therefore, deep cells must provide the motive force for convergent extension. We have two notions about how they might do so. The act of intercalation might be directly involved in generating the forces which produce elongation. In this mechanism, cells would actively force their way between their lateral neighbours (circumferential intercalation) or between their deep neighbours (radial intercalation), probably using the

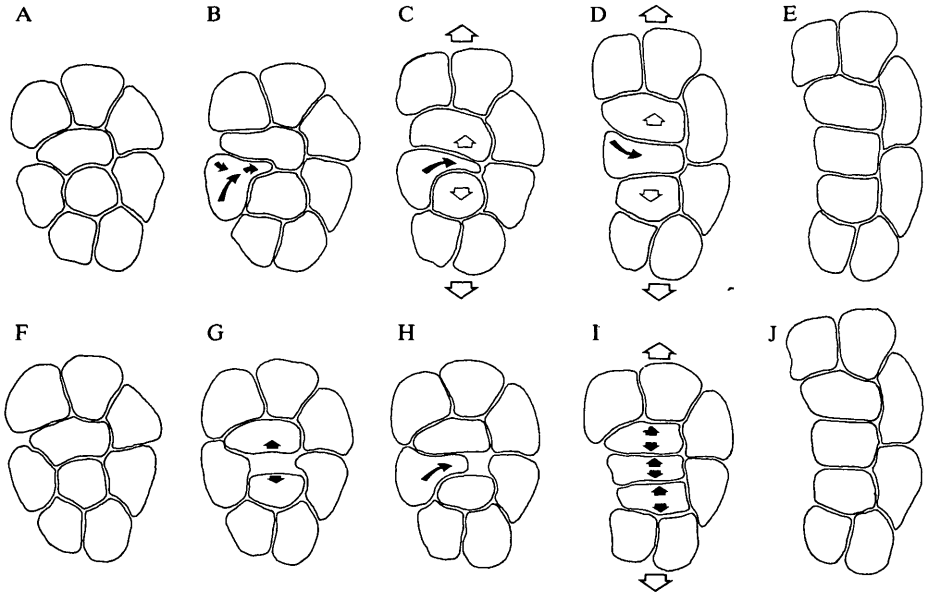


Fig. 12. Alternative relationships between cell intercalation and convergent extension are shown in diagrams of deep cells. In the first case (A–E), intercalation occurs by forceful cytoplasmic flow of the intercalating cell (solid arrows, B–D) between its neighbours, which are forced apart (open arrows) to produce extension (open arrows, C–E). Alternatively (F–J), the adjacent cells could change in shape and open a space (solid arrows, G) and the intercalating cell moves into the space unresisted (solid arrow, H). Then all cells return to their original shape (solid arrows, I) and produce convergent extension (I–J).

pattern of cytoplasmic flow seen in many of the cells [Fig. 12A–E]. Alternatively, intercalation might be involved indirectly. Local contractions might pull adjacent cells apart, producing microfractures between cells. Then adjacent cells might invade the free space and thus narrow the array; once intercalated, all cells would then forcefully adopt their original shape and produce the elongation (Fig. 12F–J). In the first mechanism, the intercalation would presumably occur against the mechanical resistance of adjacent cells and directly produce convergent extension. In the second, intercalation occurs in an island of tissue where compressive forces have been relieved locally by active contraction, and intercalation occurs unresisted. Then active change in cell shape produces the driving force.

The role of the blastocoel roof and mesodermal migration

The removal of the blastocoel roof shows what one would expect from geometrical and mechanical arguments: the active migration of involuted material contributes little, directly, to the major distortions of the embryo. Holtfreter did this experiment in 1933, on *Hyla*, with essentially the same results as reported here (Holtfreter, 1933; see Keller, 1985). This does not mean that mesodermal

migration has no role in gastrulation. The invasive migration of the head mesoderm in the blastocoel roof may function in the initial involution of the powerful convergent extension machinery in the prospective notochordal region of *Hyla* and thus prevent exogastrulation or formation of an excrescence (see Schechtman, 1942). Although it is not necessary for involution of convergent extension, traction of the mesodermal cells on the blastocoel roof may aid in bringing about the relative movement of the involuted part of the gastrula and the outer gastrular wall. In particular, the mesoderm that does not undergo convergent extension, such as head mesoderm, heart mesoderm, and the ventral mesoderm that forms the circulatory system over the yolk, may not move properly, maintain its position, or differentiate properly without an overlying substratum of blastocoel roof ectoderm. Lastly, the association of the dorsal involuted IMZ (differentiating notochord) with the overlying dorsal NIMZ (notoplate) appears to be necessary for stabilizing the position and shape of the dorsal, axial structures.

What are the relative roles of convergent extension and mesodermal migration in urodeles? The urodele marginal zone shows convergent extension (Vogt, 1922*a*, 1929*b*; Holtfreter, 1938*a*, 1944). Moreover, a large area of lateral (somitic) and ventral mesoderm leaves the superficial layer during gastrulation and migrates away from the circumblastoporal region (see Vogt, 1929*b*; Holtfreter, 1944). The convergent extension of the notochordal region and the act of removing the lateral mesodermal cells from the circumblastoporal array, prior to their migration, would presumably generate constriction forces that might act to bring about involution, even in absence of the blastocoel roof of *Ambystoma* (see Lewis, 1948, 1952). Thus migration might indirectly assist convergence of the lateral and ventral circumblastoporal region of urodele gastrulae, and thus contribute to constriction of the blastopore, by moving cells out of a tissue array (reviewed in Keller, 1985). Migration may also contribute directly in urodeles, at least to a greater extent than in *Xenopus*. Mesodermal cells have been observed to migrate in opened gastrulae of *Ambystoma* (Kubota & Durston, 1978). Urodele mesodermal cell morphology and their distribution during dissections suggests that they have a stronger association with the overlying blastocoel roof than corresponding cells in *Xenopus* (Nakatsuji, 1984). Moreover, the fibrillar matrix on the blastocoel roof, which forms the putative substratum for mesodermal cell migration, is better-developed in urodeles than in anurans (Nakatsuji & Johnson, 1983). These facts suggest that the several urodeles studied might depend more on mesodermal cell migration than *Xenopus*. Injection of anti-fibronectin antibodies (Boucaut, Darribère, Boulekbache & Thiery, 1984) or a synthetic peptide perhaps containing the cell recognition sequence of fibronectin (Boucaut *et al.* 1984*b*), result in failure of mesodermal cells to adhere and migrate on the blastocoel roof, but it also results in collapse and thickening of the blastocoel roof and other abnormalities of gastrula shape that are difficult to associate solely with failure of mesodermal cells to migrate. More extensive experiments in the vein of the brief but pioneering work by

Lewis(1948, 1952), demonstrating the relative contributions of mesodermal cell migration and convergent extension to the process of urodele gastrulation, should be done.

Unanswered questions concerning convergent extension

Our results have raised several major questions. Firstly, what protrusive activity and contact behaviour is involved in intercalation? Our working hypothesis is that intercalating protrusions form and extend by cytoplasmic flow through the central core of the protrusion to its distal end; there it turns peripherally and gels to form a stable cortical shell that is attached to and can exert traction on adjacent cells. Secondly, what is the underlying cause of the directional character of convergent extension? Cell intercalation is directional; it occurs circumferentially and radially. Does this directionality result from polarity of protrusive activity or of some other aspect of cell behaviour involved in intercalation? Holtfreter (1944) suggested that the original tissue organization is necessary for elongation, based on the fact that dissociated and reaggregated cells of the dorsal marginal zones would form notochord tissue but would not show convergent extension. Thirdly, what are the differences in cell behaviour underlying convergent extension of the dorsal IMZ and NIMZ? There is little superficial resemblance of morphology and arrangement of cells between these two regions during their convergent extension. Different cellular mechanisms may be involved in each. Fourthly, is the convergent extension in the gastrula and neurula stages similar in cell behaviour and mechanics? Differences in cell behaviour observed thus far suggest that the intercalation occurring during the formation of the 'stack of coins' array in the notochord may be different from that occurring in earlier stages.

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APPENDIX I

Ionic concentrations in Danilchik's medium were chosen to reflect the mean free intercellular ionic activities in *Xenopus* embryos reported by Gillespie (1983). Final concentrations are (mM): sodium, 95·0; potassium, 4·5; calcium, 1·0; magnesium, 1·0; chloride, 55; bicarbonate, 18–19; sulphate, 1·0; isethionate, approximately 20; gluconate, 4·5; bicine, (N,N-bis [2-hydroxyethyll-glycine], 5·0. pH at 21 °C = 8·3. Salt concentrations used to make the medium are (mM): sodium chloride 53·0; sodium bicarbonate, 15·0; potassium gluconate, 4·5; magnesium sulphate, 1·0; calcium chloride, 1·0. These salts are dissolved in glass-distilled water (about 90 % of the final volume). The pH is then adjusted to 8·3 with a measured volume of 1·0 M-sodium carbonate, the total sodium concentration is then calculated, and the final sodium concentration is brought to 95·0 mM with sodium isethionate. The final volume is then completed with glass-distilled water.

DISCUSSION

Speaker: R. Keller (Berkeley)

Question from J. Cooke (NIMR, London):
If you dissect out the presumptive notochord, vitally stain or otherwise mark one particular part, and then let it make a notochord in a cultured situation, is it programmed to form a rod with those cells in the particular place where they would normally be, or is it just programmed to expand?

Answer:
There is a rough correspondence between the position in the initial explant and in the final notochord. Locally, there is cell mixing but overall the system keeps its topography. Now, if you put mechanical stress on these things, they will change their response: they will make split notochords; they will put notochords out on one side. It is altered in some way and we don't know how. We really don't know the details of how the process is ordered, nor do we know where the polarity comes from.

Question from R. Gordon (Manitoba):
Will the notoplate elongate by itself?

Answer:
In the open-faced explants the notoplate never elongates, but if it has basal contact with itself or with the notochord then it will elongate.

R. Gordon
Can I correct something? I introduced the term "notoplate" over Jacobson's objections.