

The epithelium of the dorsal marginal zone of *Xenopus* has organizer properties

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Summary

We have investigated the properties of the epithelial layer of the dorsal marginal zone (DMZ) of the *Xenopus laevis* early gastrula and found that it has inductive properties similar to those of the entire Spemann organizer. When grafts of the epithelial layer of the DMZ of early gastrulae labelled with fluorescein dextran were transplanted to the ventral sides of unlabelled host embryos, they induced secondary axes composed of notochord, somites and posterior neural tube. The organizer epithelium rescued embryos ventralized by UV irradiation, inducing notochord, somites and posterior neural tube in these embryos, while over 90% of ventralized controls showed no such structures. Combinations of organizer epithelium and ventral marginal zone (VMZ) in explants of the early gastrula resulted in convergence, extension and differentiation of dorsal mesodermal tissues, whereas similar recombinants of non-organizer epithelium and the VMZ did none of these things. In all cases, the axial structures forming in response to epithelial grafts were composed of labelled graft and unlabelled host cells, indicating an induction by the organizer epithelium of dorsal, axial morphogenesis and tissue differentiation among mesodermal cells that otherwise showed non-axial development.

Serial sectioning and scanning electron microscopy of control grafts shows that the epithelial organizer effect occurs in the absence of contaminating deep cells adhering to the epithelial grafts. However, labelled organizer epithelium grafted to the superficial cell layer contributed cells to deep mesodermal tissues, and organizer epithelium developed into mesodermal tissues when deliberately grafted into the deep region. This shows that these prospective endodermal epithelial cells are able to contribute to mesodermal, mesenchymal tissues when they move or are moved into the deep environment. These results suggest that in normal development, the endodermal epithelium may influence some aspects of the cell motility underlying the mediolateral intercalation (see Shih, J. and Keller, R. (1992) *Development* 116, 901-914), as well as the tissue differentiation of mesodermal cells. These results have implications for the analysis of mesoderm induction and for analysis of variations in the differentiation and morphogenetic function of the marginal zone in different species of amphibians.

Key words: epithelial mesenchymal interactions, gastrulation, *Xenopus*, induction, organizer.

Introduction

This paper addresses the relative contributions of the superficial epithelial and deep mesenchymal cells to the function of the *Xenopus* gastrula organizer. The dorsal involuting marginal zone (DIMZ), the Spemann organizer, of the *Xenopus* gastrula consists a superficial layer of prospective epithelial, endodermal cells and a deep region of several layers of non-epithelial, prospective mesodermal cells (Keller, 1975, 1976). There is little or no exchange of cells between these two regions during gastrulation (Keller, 1975; Smith and Malacinski, 1983), which distinguishes *Xenopus* from the urodeles (Vogt, 1929; Lundmark, 1986; Smith and Malacinski, 1983) and the other anurans studied thus far (Vogt, 1929; Purcell, 1989).

Since the organizer of *Xenopus* has epithelial and deep components, it is important to understand how each region contributes to the morphogenetic and patterning functions of the organizer. The epithelial and deep mesenchymal regions of the *Xenopus* organizer have different roles in the convergence and extension movements that narrow and elongate the dorsal, posterior axial tissues during gastrulation and neurulation. The epithelial cells intercalate mediolaterally to form a longer, narrower array (Keller, 1978), but they appear to do so passively (Keller, 1981, 1984; Keller and Danilchik, 1988; Wilson, 1990; Shih and Keller, 1992a). In contrast, the deep mesodermal cells actively intercalate between one another in both the radial and mediolateral directions by crawling on one another's surfaces (Keller et al., 1985a,b; 1989a,b; Wilson et al., 1989; Wilson

and Keller, 1991; Weliky et al., 1992; Shih and Keller, 1992a).

Although the epithelium does not generate the mechanical forces for convergence and extension, it appears to have a role in organizing the force-producing behaviors of the underlying deep mesodermal cells. Explants of dorsal mesoderm cells can converge and extend without the epithelial layer from the mid-gastrula stage or later but not before (Wilson 1990; Shih and Keller, 1992a). This observation suggests a necessary interaction with the epithelial layer during the first half of gastrulation. In addition, rotation of the epithelium 90 degrees with respect to the underlying deep cells in explants of the early gastrula affects the direction of convergence and extension of the explant (Shih, J. and Keller, R., unpublished observations). Finally, the deep cells immediately beneath the epithelium participate most strongly in the mediolateral intercalation process (Keller et al., 1989b; Shih and Keller, 1992a).

In this paper, we investigate how the epithelial layer affects deep mesodermal morphogenesis by making classical organizer grafts to the ventral sides of sibling embryos. We show that grafts of the dorsal marginal zone (DMZ) epithelium to the ventral marginal zone (VMZ) induces dorsal morphogenetic movements and tissue differentiation in ventral tissue, including convergence and extension, differentiation of a second set of somites, and the induction of a second nervous system, as in the classical organizer experiments (Spemann and Mangold, 1924; Spemann, 1938). The deep cells of the DMZ can also organize secondary axes from ventral tissue, but these axes are not as well-organized as those induced by the DMZ epithelium. This suggests that the deep and the superficial components of the organizer have different functions. These results further our understanding of how cell interactions within the organizer control the patterning and differentiation of mesodermal tissue fates and organize the cell behaviors that drive convergence and extension, as described in the companion articles (Shih and Keller, 1992a,b).

Materials and methods

Xenopus laevis eggs were ovulated, fertilized and dejellied by standard methods (see Kay and Peng, 1991). For FDX (fluorescein dextran) labeling, embryos were placed in 4% ficoll solution in full-strength Steinberg's and injected before first cell division with 10-15 nl of FDX10,000 (Sigma) at 25 mg per ml of 0.2 N KCl (Gimlich and Braun, 1985). For making ventralized embryos, the embryos were irradiated vegetally prior to first cell cleavage with short wavelength ultraviolet light from a UVP Mineralight lamp (model uvg-11) for 60 seconds, a dose that gave better than 90% grade 0 embryos on the dorsal axial index (DAI) of Kao and Ellinson (1988). Staging was according to Nieuwkoop and Faber (1967).

Microsurgery was done with a knife of eyelash hair and a hair-loop. The grafted epithelial layer is approximately that covering a standard DMZ explant (Keller and Danilchik, 1988); the size and position of the grafts will be shown for individual experiments described below. Grafting and explantation was done in Shih-modified Danilchik's solution (SMD; see Keller, 1991). Explants were cultured in SMD and whole embryos bearing grafts were transferred to 30% Steinberg solution during early neurula stages. Specimens were fixed in 4% formaldehyde in 2.5% DMSO-

PBS for histology and immunostaining. Whole-mount staining of somites was done with monoclonal antibody 12-101 (Kintner and Brockes, 1984) obtained from Dr Chris Kintner and the NIH Developmental Studies Hybridoma Facility and used according to the protocol developed by Wilson (Wilson, 1990; see Keller, 1991). Staining for N-CAM was done using a rabbit polyclonal primary antibody RO-16 (Jacobson and Rutishauser, 1986) visualized with an HRP-conjugated secondary antibody according the procedure of Patel and others (1989). Epifluorescence was done on a NIKON Diaphot inverted microscope. Scanning electron microscopy (SEM) was done as described previously (Keller et al., 1989a). Recordings of cell behavior were made using a Zeiss upright compound microscope linked to a DAGE-MTI 81 high resolution video camera. Images were processed, using frame averaging (16 frames), background subtraction and edge enhancement, with an Image One video image processor (Universal Imaging, Media, PA), and then recorded on a Panasonic TQ-2028F optical memory disk recorder (OMDR).

Results

The effect of epithelial organiser grafts to the ventral marginal zone

The epithelial layer of the DMZ of a stage 10 embryo was grafted to the VMZ of a stage 10 host. The graft consisted of the superficial epithelium of the involuting marginal zone (IMZ) and the noninvoluting marginal zone (NIMZ), a patch of about 20-30 cells on an edge, centered on the dorsal midline (Fig. 1). Recipient embryos gastrulated at a rate

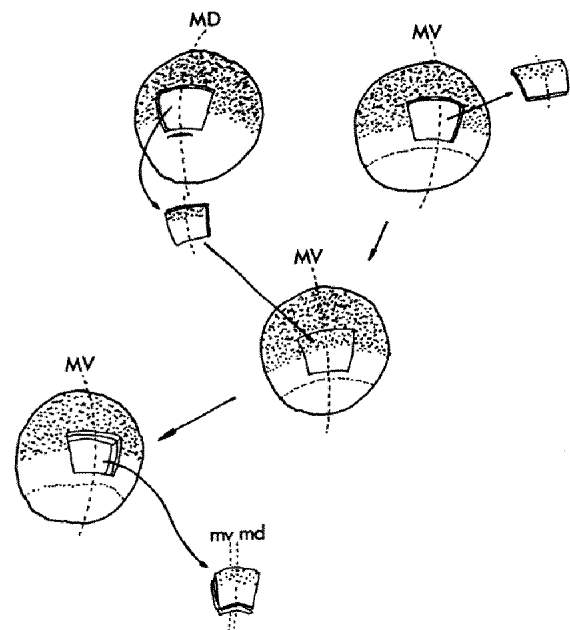


Fig. 1. The dorsal marginal zone epithelium was removed from a stage 10 gastrula and grafted to the prospective ventral side of the same stage recipient embryo, replacing the epithelial layer of the ventral marginal zone (top). For time-lapse OMDR recordings, we explanted the ventral marginal zone after the dorsal epithelium healed in and cultured the recombinant as an open faced explant in modified Danilchik's (bottom). MD, mid-dorsal line; MV, midventral line.

and in manner similar to unmanipulated controls. However, at stage 13 (the slit blastopore stage) secondary neural plates formed on the ventral sides (Fig. 2A,B). These were

smaller than those of the hosts, typically 50-70% as long, narrower at the anterior end, and lacking a brain region. The neural folds closed to form secondary neural tubes at

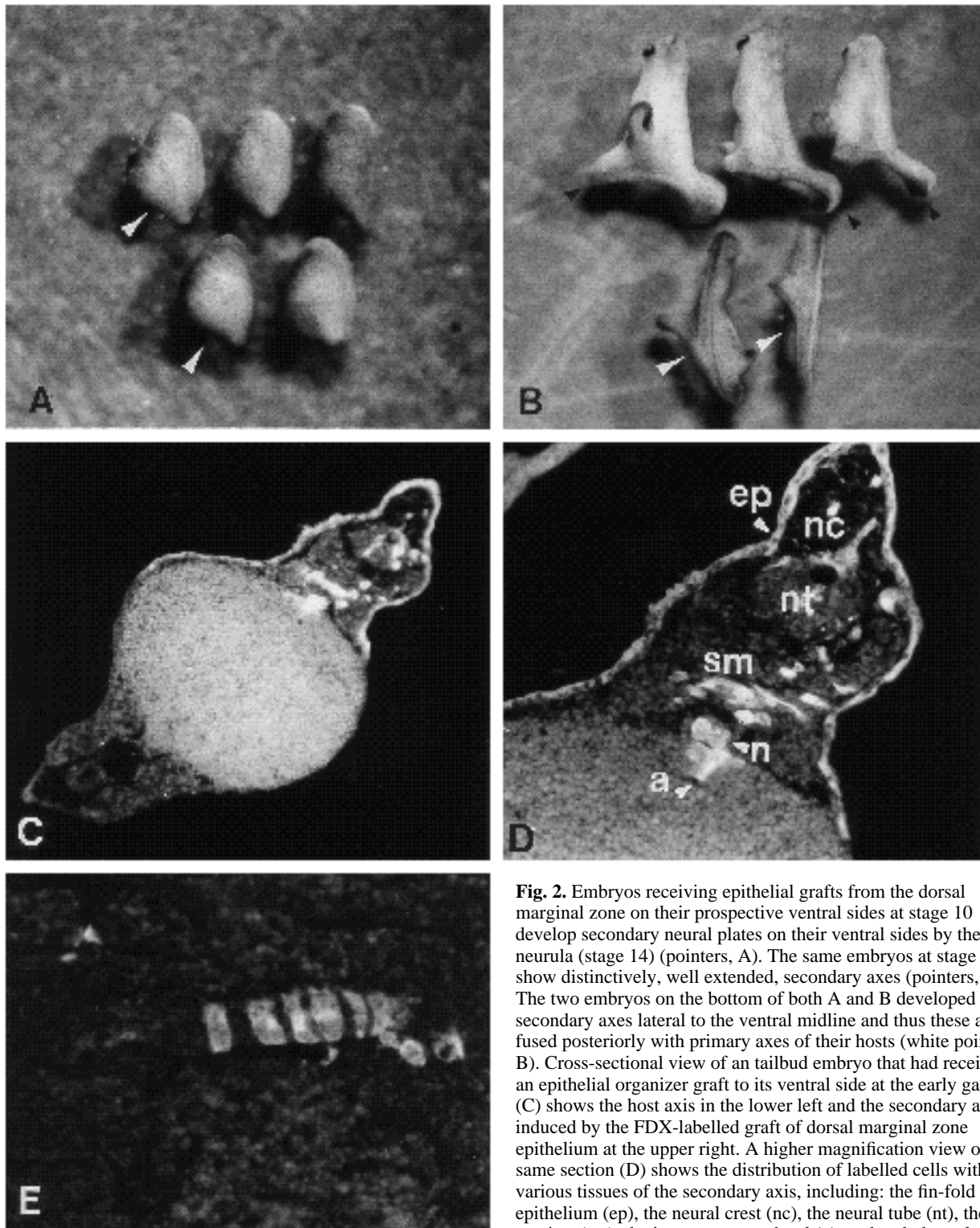


Fig. 2. Embryos receiving epithelial grafts from the dorsal marginal zone on their prospective ventral sides at stage 10 develop secondary neural plates on their ventral sides by the early neurula (stage 14) (pointers, A). The same embryos at stage 27-30 show distinctly, well extended, secondary axes (pointers, B). The two embryos on the bottom of both A and B developed secondary axes lateral to the ventral midline and thus these axes fused posteriorly with primary axes of their hosts (white pointers, B). Cross-sectional view of a tailbud embryo that had received an epithelial organizer graft to its ventral side at the early gastrula (C) shows the host axis in the lower left and the secondary axis induced by the FDX-labelled graft of dorsal marginal zone epithelium at the upper right. A higher magnification view of the same section (D) shows the distribution of labelled cells within various tissues of the secondary axis, including: the fin-fold epithelium (ep), the neural crest (nc), the neural tube (nt), the somites (sm), the immature notochord (n), and endodermal roof of the secondary archenteron (a). Labelled graft cells are found in all

the tissues. A longitudinal section (E) shows that the immature notochord is a rod composed of thick, disk-shaped cells derived from the host (unlabelled) and some from the graft (labelled), intercalated along the length of the notochord as expected from previous studies (Keller et al., 1989a).

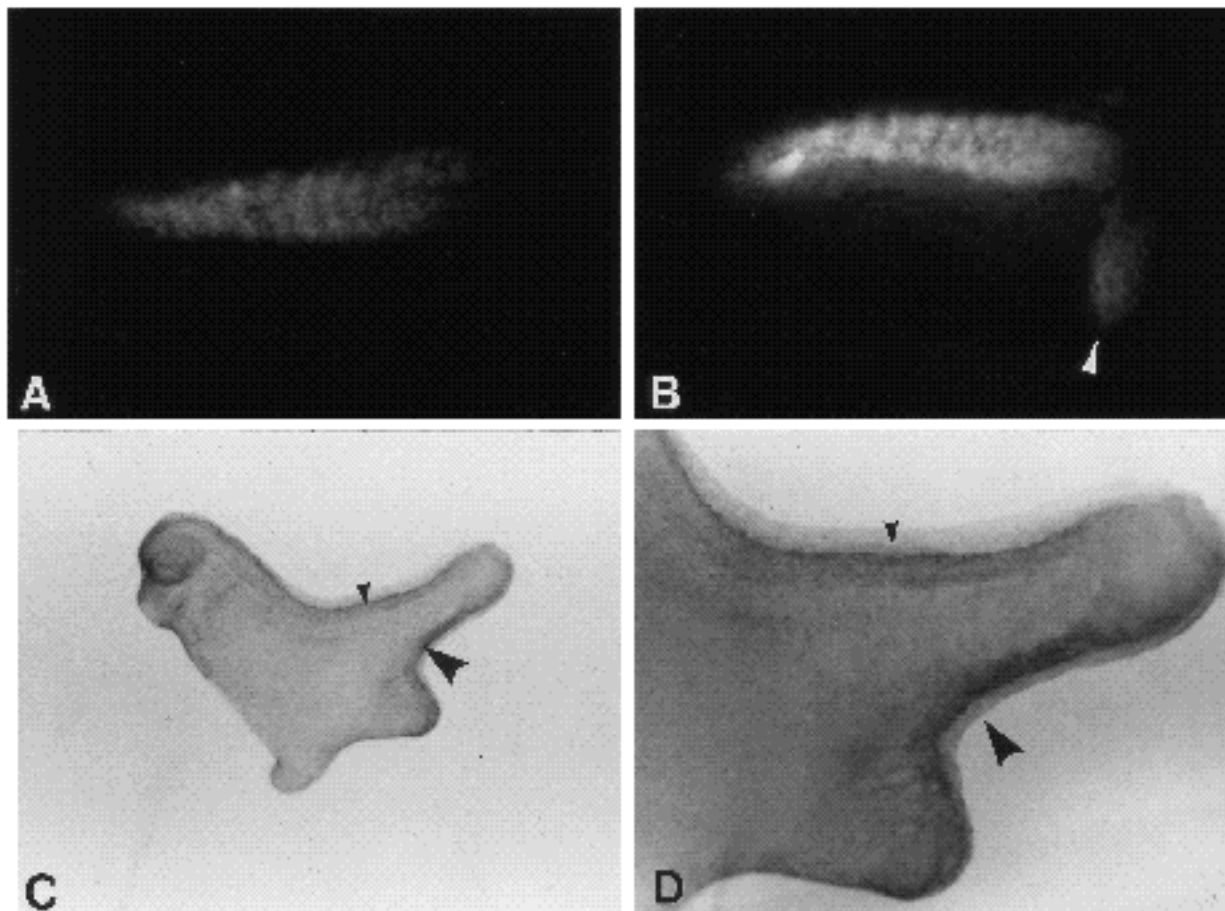


Fig. 3. Fluorescence micrographs show the location of somitic mesoderm in a stage 23 control (A) and in a stage 23 embryo that had received an epithelial organizer graft at the early gastrula stage (B). Whole-mount staining with the 12-101 monoclonal antibody, specific for somites, was done using a fluorescein-tagged secondary antibody. The anterior ends are to the left. The chevrons of somites of the normal axis are clearly visible in both, but the embryo that received dorsal epithelial graft on its prospective ventral side at stage 10 shows the presence of a secondary array of somites (pointer, B). Anti-N-CAM staining, using a horseradish-peroxidase-labelled secondary antibody, of such a double-axis embryo, shows the primary (small pointers) and secondary neural tubes (large pointers) at low magnification (C) and at high magnification (D).

about the same time as controls. By stage 30-35, melanocytes were visible on the sides of the secondary axes. Anterior head structures, such as forebrain and eyes were not induced. Control embryos, in which ventral marginal zone epithelium was first removed and then replaced, did not form secondary axes and were indistinguishable from normal embryos of the same stage.

Transverse and longitudinal serial sections of these double-axis embryos show the tissues and organization of the secondary axis (Fig. 2C-E). The outer surface forms an epidermal fin fold containing mesenchyme resembling the normal neural crest-derived fin-fold mesenchyme. A neural tube formed deep to the fin fold, followed by a layer of somitic mesoderm and then a notochord. Innermost was a monolayered epithelium, the endodermal archenteron roof. The secondary neural tube had no floorplate and was thickened ventrally. Its ventral surface was in contact with somitic mesoderm rather than the notochord, which normally induces the floorplate in amphibians (see Holtfreter and Hamburger, 1955). The neurocoel was well-defined posteriorly but was consistently absent anteriorly. The

somatic mesoderm segmented into a single file of somites beneath the neural tube rather than two bilateral files. Most of the somitic cells were aligned parallel to the secondary axes, as in the primary axes, although a few along the midline had a perpendicular orientation. The identity and position of the induced neural tube and the somites were confirmed with N-CAM and 12-101 antibody staining (Fig. 3). The secondary notochords appear to be arrested in a prevacuolation stage of development. The notochord cells consistently elongate and form an array parallel to one another and perpendicular to the embryonic anterior-posterior axis, each spanning the width of the notochord, an arrangement unique to the prevacuolation-notochord (Keller et al., 1989a) (Fig. 2E). However, these cells do not vacuolate, even by stage 30, whereas normal notochord cells are visibly vacuolated by stage 26. The grafted dorsal epithelium actually induces the ventral mesodermal cells to participate in forming secondary axes, since the bulk of both the somitic mesoderm and neural tissue in the secondary axes was derived from unlabelled host tissue. Labelled graft cells formed most of the endodermal archenteron lining, and

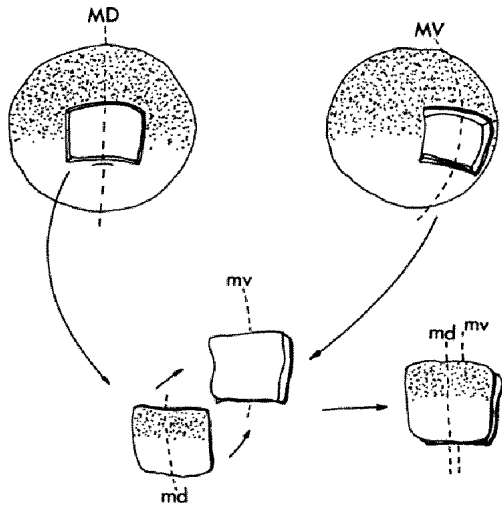


Fig. 4. The three-layer sandwich, used for assaying epithelial induction of convergence and extension in recombinant explants, was constructed by grafting the epithelium of the dorsal marginal zone on to the inner surface of either a ventral marginal zone explant or that from embryos ventralized by UV treatment in the first cell cycle. MD, middorsal line, MV, midventral line.

were scattered through the posterior neural tube, the finfold, the somites and the immature notochord (Fig. 2C-E).

The induction of morphogenetic behavior and tissue differentiation in explants

In the above experiments, the dorsal epithelium induces a second array of somites, independent of the primary axis. However, since ventral mesoderm normally contributes to posterior somites (Keller, 1976), it may be dorsalized to become somitic in fate by the host organizer (see Dale and Slack, 1987) but recruited into the second axis by the grafted dorsal epithelium. To learn whether the organizer epithelium can actually dorsalize the fate of ventral mesoderm, we grafted it to explants of ventral mesoderm that otherwise do not form somitic mesoderm. Three-layered sandwiches were made, in which ventral deep mesoderm was positioned between ventral epithelium on one side and organizer epithelium on the other (Fig. 4). These explants extended through the gastrula and neurula stages in a manner and at a rate comparable to normal DMZ sandwiches. They partitioned into a well-defined dorsal IMZ (DIMZ) region containing axial mesoderm and endoderm, and a dorsal NIMZ (DNIMZ) region containing neural tissue (Fig. 5A) like normal sandwich explants (see Keller and Danilchik, 1988; Keller et al., 1991a,b). Somitic mesoderm appeared in the mesodermal portion of the explant by stage 23 (Fig. 5B). Control explants in which ventral mesoderm was sandwiched between two layers of ventral epithelium, did not extend (Fig. 5C), nor did they make somitic mesoderm (Fig. 5D). Likewise, when dorsal epithelium was applied to ventral deep mesoderm in an 'open-faced' explant (Wilson and Keller, 1991), the IMZ converged, extended and formed somitic mesoderm (Fig. 6A,B). Controls, which consisted of recombining ventral epithelium and ventral deep cells, did not converge, extend or develop

somitic mesoderm (Fig. 6C,D). The location of somitic and notochordal mesoderm in a normal open-face explant is shown in Fig. 6E.

The effect of the dorsal epithelial organizer on embryos ventralized by UV

Although ventral mesoderm isolated with its own epithelium at stage 10 or 10+ did not differentiate somites or converge and extend in the above experiments, or in others (see Dale and Slack, 1987; Keller and Danilchik, 1988), it may be biased in this direction by the native organizer and thus needs only a weak inductive signal, provided by our dorsal epithelial grafts, to undergo dorsal-specific morphogenesis and differentiation. For a more stringent test of the dorsalizing capacity of the organizer epithelium, we used mesodermal tissue from ventralized embryos that do not differentiate dorsal tissues. Embryos were ventralized by UV irradiation of the vegetal region during the first cell cycle (Scharf and Gerhart, 1980), with a dose producing greater than 90% grade 0 embryos, graded according to the dorsal axial index (DAI) scale (Kao and Ellinson, 1988). DAI 0 embryos do not form any dorsal tissue types owing to the absence of organizer activity during development (Fig. 7A). However, grafts of organizer epithelia from normal embryos to the marginal zones of ventralized hosts in the early gastrula stage produce axial elongation and the posterior axial tissues of notochord, somites and neural tube (pointers, Fig. 7B). FDX-labelled epithelial grafts show that most of these dorsal tissues are derived from the ventralized host embryo (Fig. 7C). The epithelial graft induces notochord formation in ventralized embryos and was itself able to participate in notochord formation (Fig. 7C). The notochord in these embryos differs from normal notochords in that it is separated from the neural tube by somitic mesoderm (Fig. 7C). However, the cells in these notochords do possess the characteristic 'pizza slice' morphology (Keller et al., 1989a) and they vacuolate extensively by control stage 26, in contrast to those induced in the ventral side of a normal host (Fig. 2E). In these ventralized host embryos, as in the normal host embryos, only posterior axial tissues were induced; no anterior head structures, such as eyes and forebrain, were formed.

Three-layered sandwich explants, made by combining organizer epithelium with both layers of the marginal zone of completely ventralized embryos, converge, extend and form a mesodermal and a neural component (Fig. 7D). This behavior is comparable to that of sandwich explants of normal embryos (Keller and Danilchik, 1988) and to that of explants of deep ventral marginal zone and dorsal organizer epithelium from normal embryos. In contrast, control recombinants of ventral epithelium from normal early gastrulae and marginal zone from DAI 0 embryos neither extend nor differentiate into dorsal tissue types (data not shown).

Controls for deep cell contaminants

The effect of epithelial organizer grafts on ventral deep cells might be due to contaminating deep organizer cells. Early in these studies, an average of 10 (range 0 to 23) deep mesodermal cells remained on the inner surface of the grafted

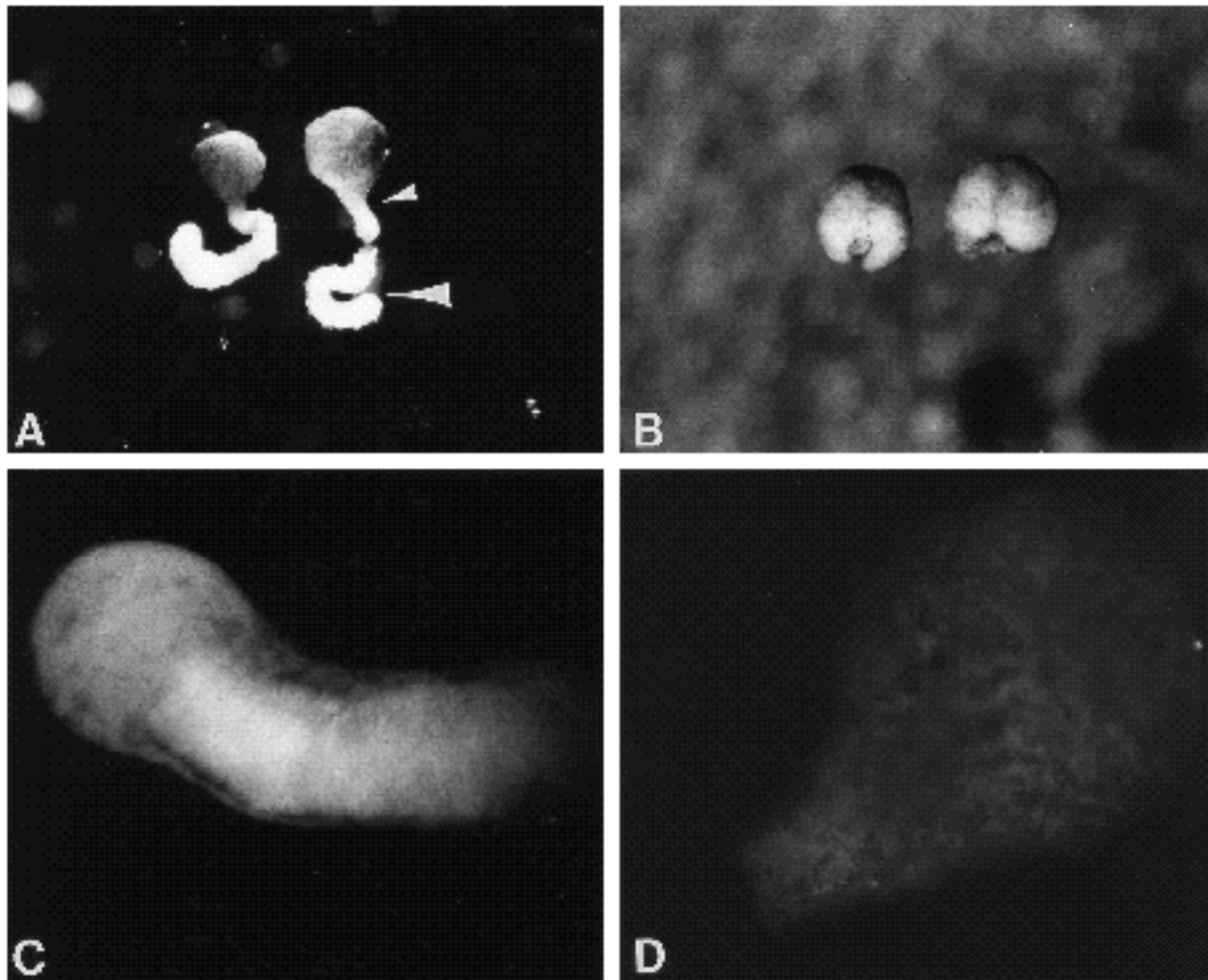


Fig. 5. Three-layer sandwiches of the ventral marginal zone and the epithelium of the dorsal marginal zone (A) extended dramatically by stage 23, partitioning into an IMZ, mesodermal extension (large pointer) and a NIMZ, neural extension (small pointer) as previously described for normal dorsal sandwich explants (Keller and Danilchik, 1988). Controls, consisting of three-layer explants of ventral marginal zone combined with ventral epithelium showed no signs of convergence and extension (B), and they did not stain with the 12-101 anti-somite antibody (not shown), indicating the absence of somitic mesoderm. The explants of ventral marginal zone and dorsal marginal zone epithelium show presence of somitic mesoderm in the mesodermal portion of the explant, indicated by 12-101 antibody staining (C). The NIMZ region (D) did not stain with this antibody.

organizer epithelium. With more practice, the epithelial grafts were made without any adherent deep cells, as assayed by SEM (Fig. 8A) and by serial sectioning (Fig. 8B) of epithelial sheets peeled off and fixed immediately. The early experiments having a few contaminating deep cells and the later ones having none gave the same results.

The effects of grafts of deep organizer cells

To evaluate the organizing role of the deep cells, clusters of 10-15, 15-20, 30-50 and 50-100 deep organizer cells were grafted beneath the VMZ epithelium at stage 10. For small grafts the number of cells was counted; for larger grafts, the number of cells was estimated by counting the cells along each edge of the block of tissue. Fifty or more deep organizer cells were necessary to induce a secondary axis approaching the size and completeness of axes induced by epithelial grafts (Fig. 9). Less than 50 cells typically

resulted in the formation of lumps containing notochord, made entirely of graft tissue. This notochord was surrounded by somitic mesoderm, most of which was derived from host tissue as determined by FDX labeling of the graft (Fig. 9F). These results show that the dorsal mesoderm of a stage 10 embryo can dorsalize ventral mesoderm without the aid of the epithelium. These experiments also show that deep cell contamination cannot account for the inductive property of the dorsal epithelium, since it took 4 to 5 times the average number of contaminating deep cells found in our early, most contaminated grafts to get comparable secondary axis formation. Axes organized by deep cells do not extend well and the somitic mesoderm is often discontinuous, disorganized and unsegmented (Fig. 9G), whereas axes organized by the epithelial endodermal cells extend well and contain a contiguous array of well-organised axial tissues.

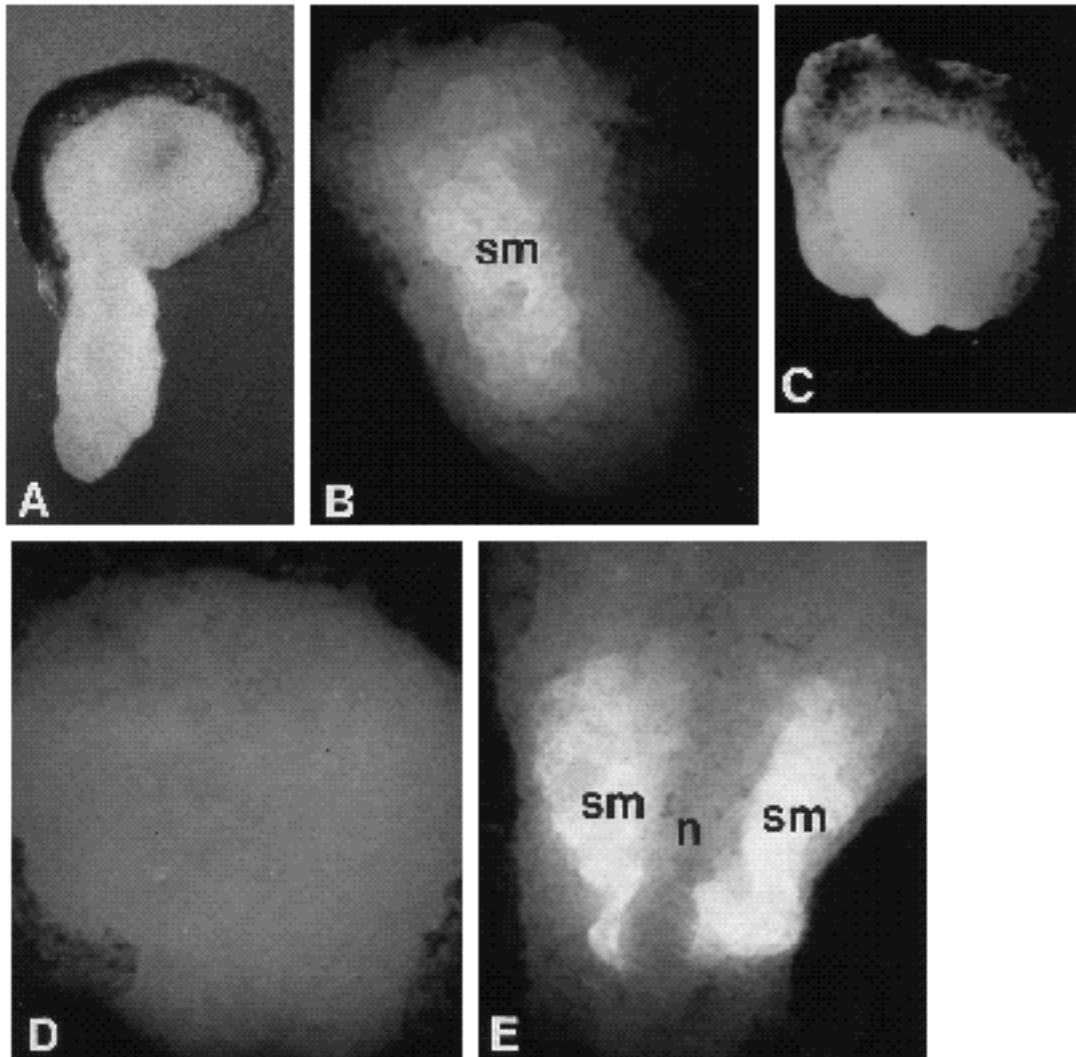


Fig. 6. Open-faced explants of ventral deep cells and dorsal epithelial cells show convergence and extension (A), and differentiation of somitic tissue, as shown by 12-101 monoclonal antibody staining (B). In contrast, explants composed of ventral deep cells and ventral epithelial cells do not converge and extend (C) and do not show the somite-specific staining (D). Explants of dorsal epithelial cells and dorsal deep cells (E) show somites (sm) developing on both sides of a notochord (n), in contrast to the single column of somitic mesoderm and absence of notochord when dorsal epithelium is combined with ventral deep cells (B).

Contributions of the grafted epithelial layer to mesodermal tissues

Grafts of labelled organizer epithelium consistently contribute labelled cells to deep mesodermal tissues in the induced second axes of whole embryos (Figs 2C,D, 7C) and in explants (not shown), despite there being essentially no contaminating deep mesodermal cells in the later grafts. In normal development, the superficial layer of the IMZ contributes only to the surface layer lining of the archenteron (Nieuwkoop and Florshutz, 1950; Keller, 1975; Smith and Malacinski, 1983). To learn if grafting itself causes surface epithelial cells to ingress, the epithelium of the DMZ, and in some cases of much larger areas, of normal stage 10 embryos was replaced with that from the same region of FDX-labelled embryos, taking care to ensure that no contaminating deep cells were carried along. Serial sections of embryos fixed an hour after grafting shows large areas of

a single layer epithelial cells (Fig. 10). However, several cells have made large protrusions on their basal ends and appear to be entering the deep region (pointers, Fig. 10). At the tailbud stage of such embryos, a number of labelled cells from the same type of graft are found in the notochord and somites (Fig. 10B). To learn if unmanipulated, epithelial cells ingress under normal conditions, time-lapse recordings were made of the epithelial surface of five sandwich explants of the DMZ. In all five, rapid cell division occurred from stage 10 to stage 10.5, followed by cell shape changes and rearrangements previously associated with convergence and extension in time-lapse films of whole embryos (Keller, 1978). Only two cells ingressed in one recording. Thus, little if any ingression occurs in unmanipulated epithelium during gastrulation and neurulation, a finding consistent with previous observations (Keller, 1975, 1978).

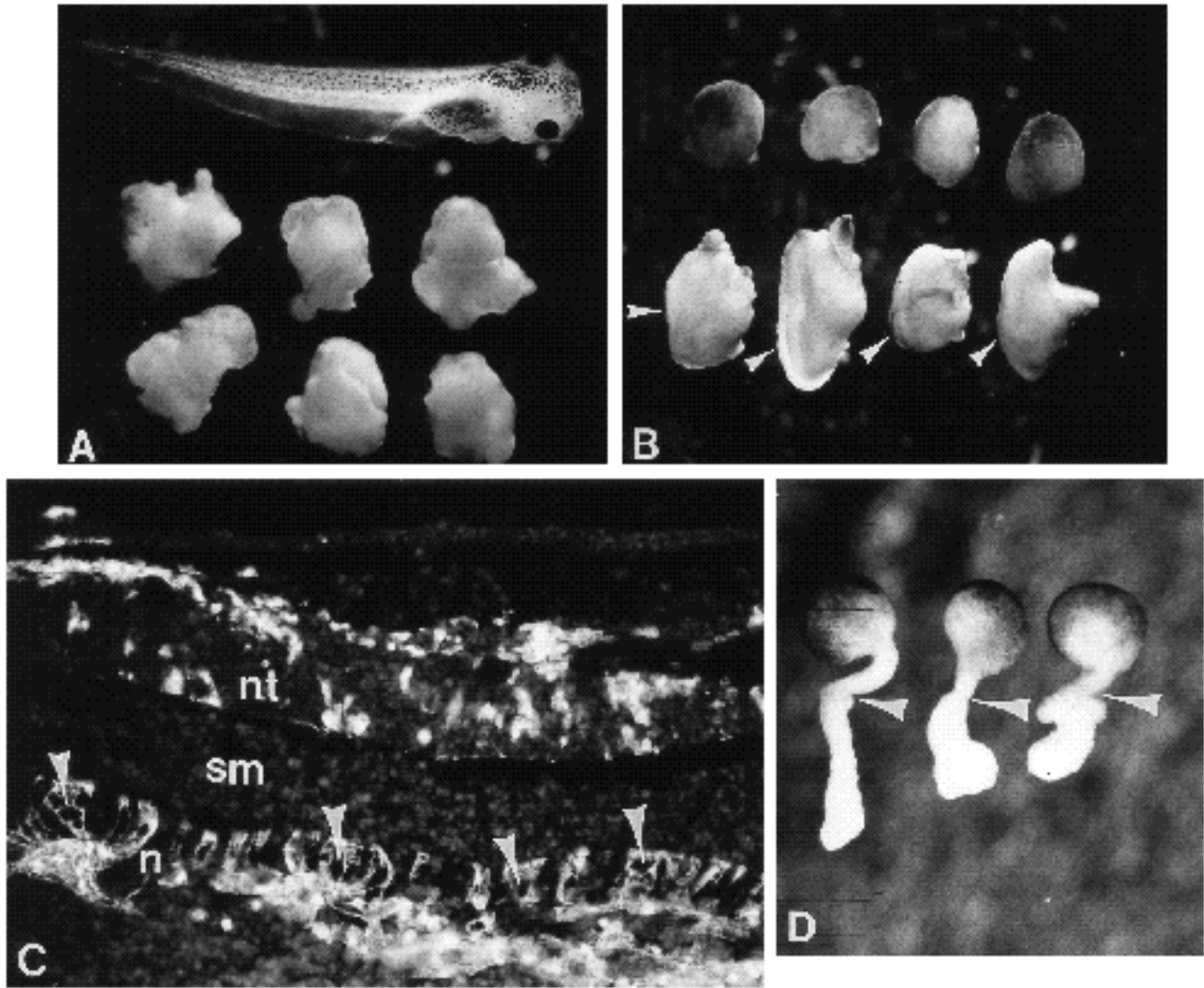


Fig. 7. Six examples of DAI grade 0 embryos produced by UV irradiation are shown at stage 41 (A). None contained axial tissues as assessed by histological analysis for vacuolated notochord cells and 12-101 staining for somites. A control, normal embryo is shown at the top. At stage 28 (B), a comparison of four control DAI grade 0 embryos (top) shows a spherical morphology and absence of axial elongation, whereas four embryos that received grafts of dorsal marginal zone epithelium at the early gastrula stage (bottom) show the elongation characteristic of the induced, posterior axial structures (pointers). A histological section (C) shows these induced axes to contain: somites (sm), primarily from the host, neural tube (nt), from both the host and donor cells, and notochord (n) from both host and donor cells. In the notochord, unlabelled host cells were intercalated with labelled cells derived from the graft, which had vacuolated as shown by the appearance of characteristic label-free spaces within cells (pointers, C). Three-layer sandwiches made of marginal zone from ventralized DAI grade 0 embryos and dorsal marginal zone epithelium from a normal embryo (D) result in extension of these explants and partitioning into the mesodermal, IMZ component (below the pointers) and the neural, NIMZ component (above the pointers), typical of normal dorsal sandwich explants (Keller and Danilchik, 1988). Staining with the 12-101 antibody shows somitic mesoderm in their mesodermal portion (not shown). Recombinants of ventralized mesoderm with either ventral epithelium or with DAI grade 0 marginal zone epithelium show neither extension nor staining with the 12-101 for somites (not shown).

Grafts of epithelial organizer cells to the deep mesodermal region

The grafted superficial cells that ingressed appear to have changed from an endodermal to a mesodermal fate. To explore further the possibility that prospective epithelial endodermal cells can make mesoderm, small squares of DMZ epithelial cells, roughly 4-8 cells on a side, from FDX-labelled stage 10 gastrulae were grafted to the DMZ of unlabelled stage 10 hosts, placing them *between* the

epithelial layer and the deep mesoderm (Fig. 11). This 'forced ingression' resulted in labelled cells distributed throughout the entire length of the notochord and somites, and in the posterior neural tube (Fig. 12). The morphologies of labelled cells in these locations were indistinguishable from those of their unlabelled neighbors, indicating that these prospective endodermal epithelial cells had participated in the formation of axial mesodermal structures.

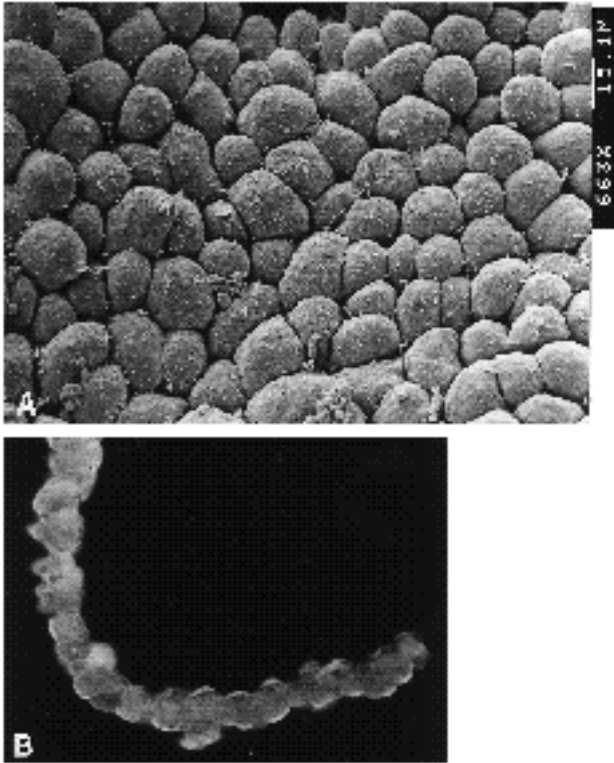


Fig. 8. A scanning electron micrograph of the inner surface of an epithelial organizer graft shows the absence of any adhering deep cells (A). Serial sections of an epithelial organizer graft (B) show that the graft is indeed a monolayer.

Discussion

The dorsal endoderm epithelium has organizer properties

Three experiments show that the DMZ epithelium of the early gastrula of *Xenopus* has organizer properties. First, grafts of dorsal epithelium to the ventral side of normal early gastrulae, comparable to the original organizer grafts of Mangold and Spemann, induce secondary embryonic axes on the ventral side. Second, grafts of DMZ epithelium rescue axis formation in embryos ventralized by UV irradiation. Third, DMZ epithelium grafted to ventral explants induce the ventral mesoderm to converge, extend and make somites, behaviors that are not observed in ventral explants lacking DMZ epithelium. This effect represents an induction of tissue fates and movements since much of the secondary axes were derived from the host, as in the case of whole organizer grafts (Spemann and Mangold, 1924; Gimlich and Cooke, 1983). Since much of the ventral mesoderm of *Xenopus* normally converges, extends and forms posterior somites, albeit later than the dorsal mesoderm (Keller, 1976; Wilson et al., 1989), the organizer epithelium grafted to the VMZ may be recruiting these cells into a second axis *without* altering their tissue fate. However, the grafts of organizer epithelium to explants of the VMZ and to UV-ventralised embryos *induced* ventral mesoderm to express movements and tissue fates that were not expressed at all without the organizer epithelium.

Differences between deep and superficial organizer activity

Deep cells of the DIMZ show organizer activity when grafted beneath the VMZ epithelium. But deep cells lack the capacity for spatial organization shown by the epithelium, since the amount of extension and the tissue organization was poor in axes induced by the deep cells compared to those induced by the epithelium. The grafted epithelium may impose directional cues directly on large areas of deep cells beneath it, thus enabling this large, planar population to produce an organized extension. In contrast, the clump of grafted deep cells probably passes the organizing signal in all directions, without or with minimal directional cues, resulting in the weak spatial organization and convergence and extension that were observed. In addition to these differences in the geometry of presentation, the deep and epithelial organizer signals may differ qualitatively. The epithelial organizer can induce ventral cells to undergo at least the initial stages of notochord formation, whereas the deep organizer cells cannot induce notochord at all (see Shih, 1991).

Evidence that the endodermal epithelium normally organizes the deep mesodermal cell behaviors driving convergence and extension of the axial mesoderm

Several facts argue that the endodermal epithelium of the DIMZ organizes the highly patterned protrusive activity driving convergence and extension of deep axial mesodermal cells (see Shih and Keller, 1992a,b). First, deep cells immediately beneath the endodermal epithelium show the bipolar, mediolateral protrusive activity that drives the cell elongation, alignment, and mediolateral intercalation underlying convergence and extension (Shih and Keller, 1992a,b), whereas the deep cells farther from the epithelium show these behaviors weakly or not at all during gastrulation (Wilson and Keller, 1991) (Fig. 13). Second, the deep cells next to the epithelium show a progression of these cell activities from anterior to posterior and from lateral to medial in both notochordal and somitic tissues (Shih and Keller, 1992b), whereas this progression appears only in rudimentary form among the deep cells farther from the epithelium (Wilson and Keller, 1991). Third, convergence and extension and the associated repertoire of cell behaviors occur only in the notochordal and somitic tissues, which remain in contact with the endodermal epithelium throughout gastrulation (Hardin and Keller, 1988; Keller and Tibbetts, 1989; Wilson et al., 1989; Wilson and Keller, 1991). In contrast, the mesoderm at the leading edge of the mesodermal mantle, which loses contact with the overlying endodermal epithelium as the bottle cells form (Hardin and Keller, 1988), migrates and spreads on the blastocoel roof (Keller and Tibbetts, 1989; see Winklbauer, 1990) (Fig. 13). Lastly, the DMZ epithelium induces only the converging and extending posterior structures, such as notochord, somites, hindbrain and spinal cord.

Summary of tissue interactions regulating cell intercalation

The tissue interactions regulating cell intercalation are summarized in Fig. 13. (1) The normal dorsal endodermal epithelial effect on deep mesodermal cell behavior occurs in the early gastrula stage, prior to stage 10.5 (Fig. 13A).

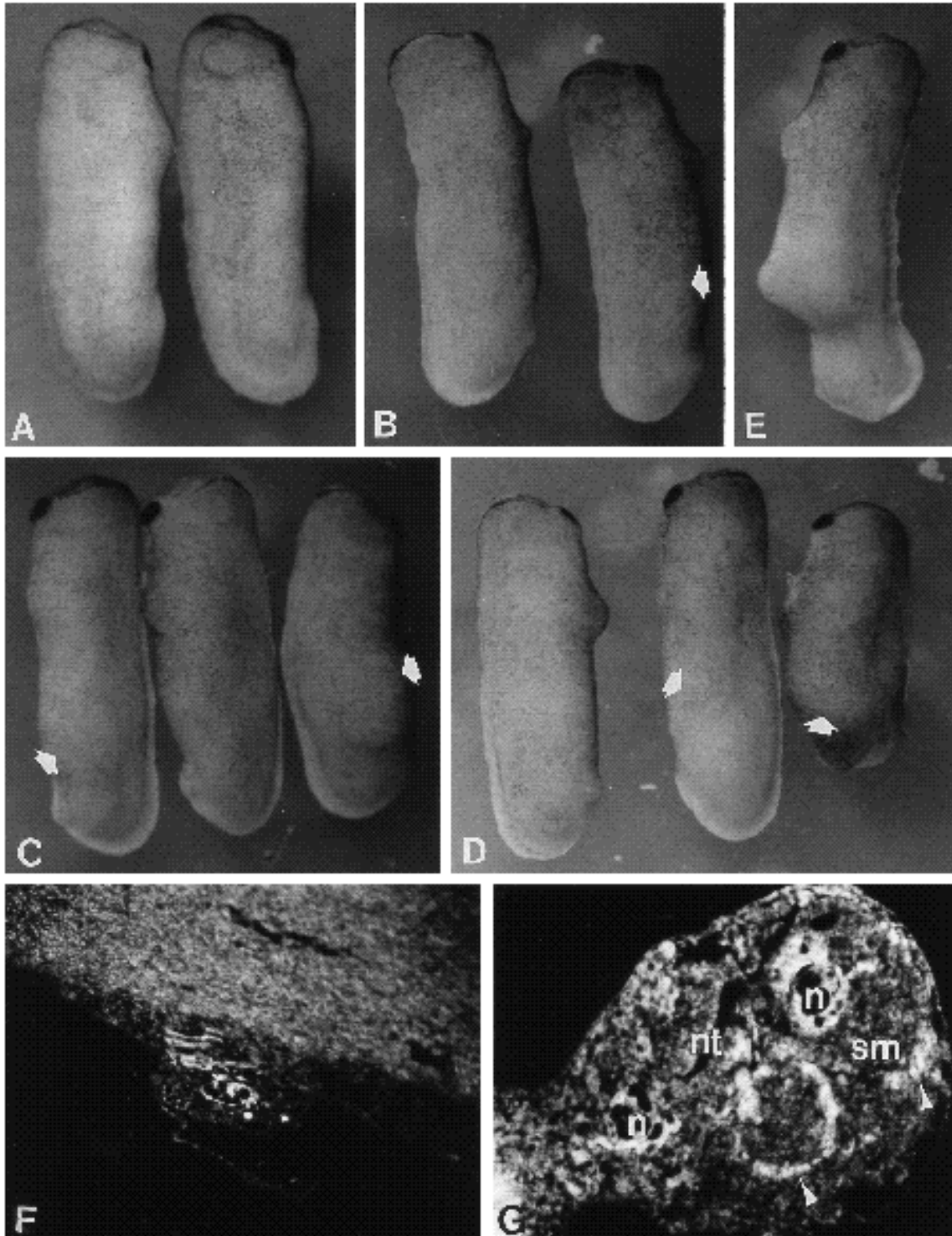


Fig. 9. Control embryos (A) are compared with embryos receiving deep cells grafted to their ventral marginal zones in numbers of 10-15 (B), 15-20 (C), 30-50 (D), and 50-100 (E). More than 50 deep cells were required to form a secondary axis comparable to the epithelium-induced axes. Less than 50 cells formed lumps ventrally or ventrolaterally (arrows) composed of small amounts of notochord and somites from the graft, occasionally associated with induced somitic mesoderm (F). A cross section through the anterior quarter of a secondary axis induced by grafting approximately 100 stage 10 dorsal mesoderm cells into the ventral marginal zone of recipients at the same stage resulted in the formation of an extended but disorganized axis (G). In cross section, notochord (n), though well-vacuolated, often formed multiple strands amid unsegmented and poorly aligned somitic mesoderm (sm). The neural tube (nt) thinned to form floor plate when adjacent to notochord but it often failed to span the length of the axis. In addition, there were often many unidentifiable graft cells scattered within the axial array (pointers).

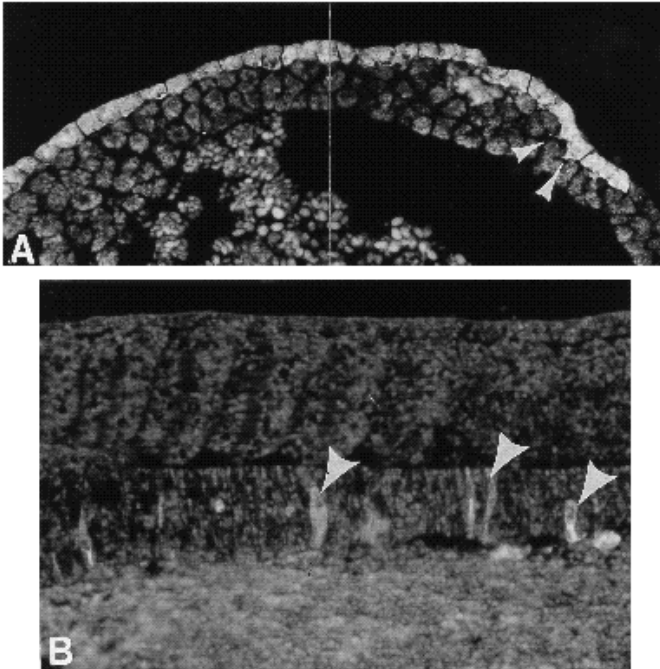


Fig. 10. The dorsal marginal zone epithelium was replaced with labelled dorsal marginal epithelium from another embryo (A). After a half hour, only a few signs of ingression are seen, such as protrusions at the inner ends of some cells (white pointers). Later, sagittal sections of the neurula and tailbud stages (B) show labelled, ingressed cells in the notochord (pointers).

Deep mesoderm explanted without its epithelium at stage 10 fails to converge and extend (Wilson, 1990; Shih and Keller, unpublished work), whereas by stage 10.5, mesoderm explanted without epithelium does converge, extend and show the motility characteristic of convergence and extension (Shih and Keller, 1992a). (2) The signal from the epithelium, whatever its nature, initially organises cell intercalation among the nearest, most superficial mesodermal cells, allowing the deeper cells to participate in migration (Fig. 13A,B). Mediolateral intercalation behavior is expressed (Shih and Keller, 1992a) and patterned (Shih and Keller, 1992b) strongly among the deep cells next to the epithelium but weakly among the deep mesodermal cells farther from the epithelium during gastrulation (Wilson, 1990; Wilson and Keller, 1991). Although the deeper cells initially undergo migratory behavior (Winklbauer, 1990; Winklbauer and Nagel, 1991), they subsequently all show mediolateral intercalation behavior (Keller et al., 1989a), perhaps because the organising signal penetrates to deeper layers and because the deeper cells move closer to the epithelium by radial intercalation (Fig. 13C). (3) The signal could be passed to deeper cells directly from the epithelium or it could be passed from deep cell to deep cell. The latter seems likely, since we show here that deep cells also have organizing activity. (4) The mesoderm can simultaneously converge and extend and also 'shear' or migrate animally with respect to the ectoderm through most of gastrulation. Since mediolateral intercalation is organized by the epithelium from the outside, the inner-most mesodermal cells and those out ahead of the bottle cells, which are

deprived of contact with the epithelium during bottle cell formation (Hardin and Keller, 1988), are free to participate in active migration on the blastocoel roof during most of gastrulation (Winklbauer, 1990). (5) Finally, in the late gastrula and through neurulation, all the axial (notochordal and somitic) mesodermal cells participate in convergence and extension (red cells, Fig. 13C), but reduction of migratory behavior in the late gastrula has no consequence, since the little shearing that occurs between the dorsal mesoderm and hindbrain-spinal cord after this time may be largely driven by relative rates of mesodermal and neural extension (Keller et al., 1992a).

Induction of neural structures

In our experiments, as in those of Spemann and Mangold (1924), parts of the secondary neural axes were induced from the ventral tissue of the host. Neural induction could occur indirectly, if the grafted dorsal epithelium first dorsalised ventral mesoderm and then the dorsalised mesoderm induced neural structures. Alternatively, the NIMZ portion of the grafted dorsal epithelium may directly induce the underlying ventral ectodermal cells to become neural. Neural convergence and extension can be induced in ecto-

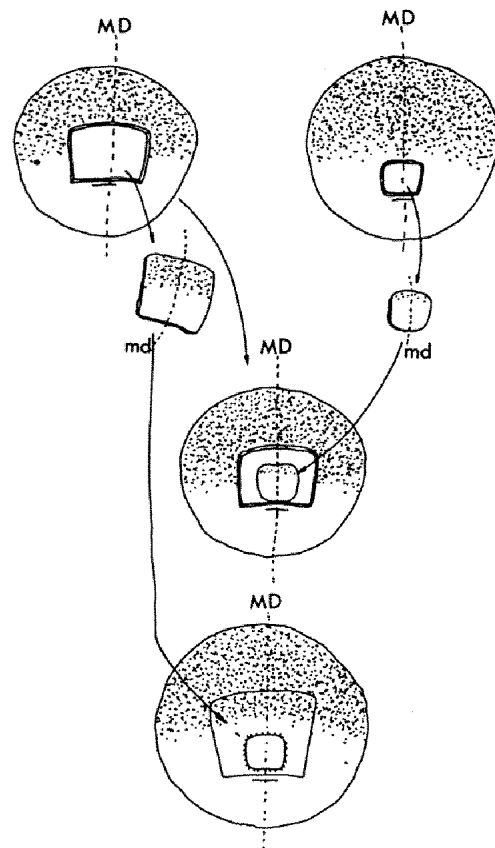


Fig. 11. This diagram shows the operation of inserting a patch of labelled epithelium of the dorsal marginal zone (prospective endoderm) between the dorsal marginal zone epithelium and the dorsal mesoderm of another unlabelled embryo. The covering graft of epithelium must be larger than the grafted endodermal patch to prevent problems of healing associated with having coincident epithelial boundaries.

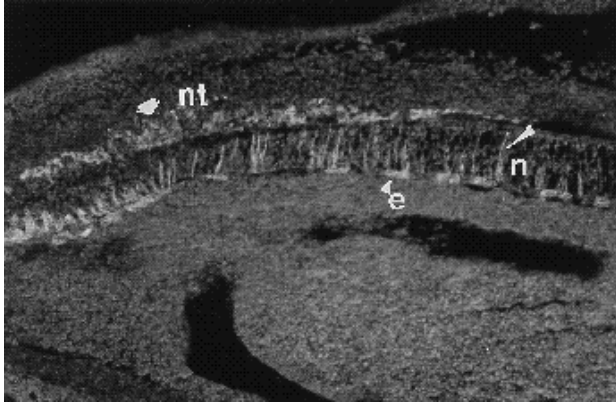


Fig. 12. Inserting a patch of labelled organizer epithelium between the dorsal epithelium and mesoderm of an unlabelled embryo at the early gastrula stage resulted in the graft cells (prospective endoderm) contributing to the notochord (large pointer, n), somites, neural tube (arrow, nt), and some endoderm (small pointer, e) along the length of the axis.

derm by signals passing anally from the DIMZ, through the plane of the tissue ('planar signals') (Keller et al., 1992a,b) (Fig. 13A). Thus the secondary neural tissue could be induced by planar signals, as well as by vertical ones produced when the dorsalized ventral mesoderm involutes beneath the ventral ectoderm.

The prospective endodermal epithelium of the IMZ can form mesodermal structures

Although the epithelium of the DIMZ normally forms only surface endoderm (Nieuwkoop and Florshutz, 1950; Keller 1975; Smith and Malacinski, 1983), we show that it can differentiate into notochordal and somitic mesoderm if it comes to lie in the deep region, either because it was destabilized during grafting and subsequently ingressed, or because it was deliberately grafted to the deep region. This suggests that regulation of ingression may be an important step in mesoderm formation. The amount and type of mesoderm present in the superficial layer of the marginal zone and the pattern of its ingression varies greatly among the amphibians (Vogt, 1929; Purcell, 1989, 1992), presumably because of differences in how the vegetal endoderm induces mesodermal and endodermal fates in the marginal zone (see Nieuwkoop, 1969a,b; Sudarwati and Nieuwkoop, 1971). In species containing both types of tissues in the superficial layer, cells may be committed to an endodermal or mesodermal fate, and as a result either remain superficial or ingress, respectively. Alternatively, all surface cells may have dual potential, having been committed to a 'mesendoderm' fate during induction by the vegetal endoderm in the blastula stage, and then the choice between mesodermal and endodermal fate is made during gastrulation on the basis of whether or not ingression occurs. In this scheme, regulation of the process of ingression would be the key step in determining tissue fate.

In *Xenopus*, the ingression of grafted epithelial organizer cells into the induced secondary neural tubes was expected, since in normal development, the epithelial neural epidy-

mal cells (the lining of the newly formed neural tube) ingress and intercalate into the deep neural ectodermal cells to form a monolayered neural tube (Schroeder, 1970; Hartenstein, 1990).

Ventral mesoderm of normal embryos and 'ventralized' mesoderm respond differently to organizer signals

The epithelium of the DIMZ consistently induces the ventral mesoderm of a normal embryo to form notochords that stop developing at a characteristic, prevacuolation stage, in contrast to the mature, vacuolated ones induced in UV 'ventralized' embryos. The native organizer in normal embryos may inhibit ventral mesoderm from responding to the grafted organizer epithelium to make complete notochords. UV-ventralized embryos, which lack organizers, would also lack this inhibition, leaving the mesoderm free to respond to the grafted organizer by making a mature notochord. Cooke (1981, 1983, 1989) presented evidence for just such an overall tissue-proportioning rule in which establishment of a tissue dorsally would inhibit formation of the same ventrally. There also is evidence for inhibitory interactions between organizing centers in the early development of birds (Eyal-Giladi and Khaner, 1989; Khaner and Eyal-Giladi, 1989).

The nature of epithelial organizer activity

The nature of the epithelial organizing activity and how it differs from that associated with deep cells is not known. We do not know whether the epithelial effect requires direct cell-cell contact, whether it is mediated by diffusible components, or whether it is carried by matrix-associated molecules. If secreted molecules are involved, they might simply be stuck to the surface of the epithelial cells and transferred to the graft site, in which case, either the deep cells or the epithelium, or both, may make the inducing substance or substances. It is not known whether the epithelium provides specific, directional cues for the organization of deep mesodermal cell protrusive activity or whether it supplies a supporting environment for the self-organization of this behavior within the mesoderm. The differences in the inductive properties of deep and superficial organizer described above and elsewhere (Shih, 1991) suggest that deep and superficial organizers have different mechanisms underlying their different roles.

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Fig. 13. A schematic diagram summarizes the tissue interactions affecting convergence and extension by cell intercalation. The endodermal epithelium (yellow) acts on the underlying deep mesodermal cell population (solid arrows, A), resulting in strong expression of the *mediolateral intercalation behavior* (see Shih and Keller, 1992a) among cells close to the epithelium (red cells, A), whereas those cells farther away express mediolateral intercalation behavior weakly or not at all (orange cells, A), leaving them free to migrate on the blastocoel roof (B). Note that the cells forming the leading edge of the mesodermal mantle were deprived of contact with the overlying epithelium (the bottle cells, shown in green), as a result of bottle cell formation (Hardin and Keller, 1988). These lead the migration on the blastocoel roof (Winklbauer, 1990). Once its involution has been initiated by bottle cell formation and the migration of the leading edge mesoderm (see Keller and Winklbauer, 1992 for a review), the deep mesodermal cells express mediolateral intercalation behavior in an anterior-to-posterior progression (see Shih and Keller, 1992b) that results in convergence and extension (open arrows in the mesoderm, B) and continued involution. Note that beneath the endodermal epithelium more mesodermal cells express mediolateral intercalation behavior as development proceeds. Radial intercalation brings them closer to the epithelium (Wilson and Keller, 1991), which may continue to organize mediolateral intercalation behavior, or because the deep cells expressing mediolateral intercalation behavior can themselves induce its expression in other deep cells. Finally, in the early neurula, all the axial mesodermal cells express mediolateral intercalation behavior (see Keller et al., 1989; Wilson et al., 1989) (C), although they can also express migratory behavior in culture (see Winklbauer and Nagel, 1991). The leading edge mesodermal cells, deprived of epithelial influence early in gastrulation, express strong migratory behavior and regain epithelial contact only when the bottle cells respread beneath them late in gastrulation (Hardin and Keller, 1988). The dorsal mesoderm acts on the prospective hindbrain-spinal cord ectoderm by signals passing through the plane of the tissue (planar induction) (pointer, A), resulting in convergence and extension of the hindbrain-spinal cord (open arrows in the ectoderm, B), which also occur by cell intercalation (see Keller et al., 1992a,b).