Blastomere derivation and domains of gene expression in the Spemann Organizer of *Xenopus laevis*

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SUMMARY

Spemann’s Organizer, located in the dorsal marginal zone of the amphibian gastrula, induces and differentiates dorsal axial structures characteristic of this and other vertebrates. To trace the cellular origins of the *Xenopus* Organizer, we labelled dorsal blastomeres of three of the four tiers (A, B and C) of the 32-cell embryo with green, red and blue fluorescent lineage tracers. A strong vegetalward displacement of labelled clones occurs between the late blastula and early gastrula stages but clones mix only slightly at their borders. The typical early gastrula Organizer is composed of approximately 10% A1 progeny in its animalmost region, 70% B1 progeny in the central region, and 20% C1 progeny in vegetal and deep regions. Variability in the composition of the early gastrula Organizer results from variability in the position of early cleavage planes and in pregastrulation movements. As the Organizer involutes during gastrulation, forming dorsal axial mesoderm, clonal boundaries are greatly dispersed by cell intermixing. Within a clone, deep cells are displaced and intermixed more than superficial cells. Variability in the distribution of progeny in the dorsal axial mesoderm of the late gastrula results mostly from variable intermixing of cells during gastrulation. Experiments to perturb later developmental events by molecular or embryonic manipulations at an early stage must take this variability into account along with the majority distributions of the fate map. Within the early gastrula Organizer, the genes *Xbra*, *goosecoid*, *noggin* and *xNR3* are expressed differently in the animal-vegetal and superficial-deep dimensions. In situ hybridization and lineage labelling define distinct regions of the dorsal marginal zone. By the end of gastrulation, dorsal axial mesoderm cells derived from the Organizer have altered their expression of the genes *Xbra*, *goosecoid*, *noggin* and *xNR3*. At a given stage, a cell’s position in the embryo rather than its lineage may be more important in determining which genes it will express.

Key words: fate map, gastrula, dorsal mesoderm, cell lineage, in situ hybridization, *Xenopus laevis*

INTRODUCTION

The essential role of the Organizer in vertebrate development has been realized since 1924 when Spemann and Mangold transplanted a region of the dorsal marginal zone of an amphibian gastrula embryo to the ventral marginal zone of a host embryo and found that it induced a secondary set of dorsal axial structures organized around the notochord differentiated by the graft tissue. The Organizer was defined in terms of this experimental effect. The reverse experiment, the removal of the Organizer from the embryo shortly before gastrulation, results in the development of a ventralized form lacking all chordate-characteristic dorsal structures (Stewart and Gerhart, 1990). Because this region is both necessary and sufficient for formation of the body axis, it has been the subject of much investigation to this day.

There are at least two inductive activities of the Organizer during gastrulation: (1) to dorsalize and organize the mesoderm of the lateroventral marginal zone to form somites, kidney and heart, and (2) to neuralize and pattern the ectoderm to form neural tissue (Gerhart et al., 1991; Harland, 1994). This activity defines the early gastrula Organizer in functional terms. These inductions occur coincidentally with morphogenetic movements which are also activities of the Organizer, in particular the spreading migratory movements of early involuted Organizer cells, and the convergent extension movements of later involuted cells as they lengthen the embryonic axis beneath the neural tube. After gastrulation, and well into neurulation, the Organizer continues to induce and pattern neural tissue in the dorsoventral dimension of the neural tube, and to induce somite differentiation into myotome, sclerotome and dermatome. This activity defines the late gastrula Organizer in functional terms. *Xenopus* Organizer cells ultimately contribute primarily to three differentiated cell types, pharyngeal endoderm, head mesoderm and notochord (Keller, 1975, 1976). Some Organizer cells may contribute to somites and heart but this is less certain. From classical surgical functional studies, it seems the Organizer contains at least two parts as gastrulation begins: a vegetal anterior leading part, the cells of which engage in spreading migration, induce anterior neural
plate and differentiate as head mesoderm; and an animal posterior trailing part that engages in convergent extension, induces trunk-tail parts of the neural plate and perhaps dorsalizes mesoderm, and eventually differentiates as notochord (Spemann, 1938; Hama et al., 1985). The boundaries of these subregions are not known and the inductive activity of the parts of the Organizer may change during gastrulation (Saha and Grainger, 1992), especially anterior parts may gain and then lose head-inducing properties. The superficial layer of cells of the stage 10 Organizer, which is destined for pharyngeal endoderm and archchenton roof, has axis-inducing properties as do the deep cell layers (Shih and Keller, 1992).

It has become possible in the past four years to address *Xenopus* embryonic organization in terms of regional gene expression, and to correlate this with regional function. A number of Organizer-specific genes have been identified: *Xbra* (Smith et al., 1991), *goosecoid* (Cho et al., 1991), *noggin* (Smith and Harland, 1992), *X-lim1* (Taira et al., 1992), *XF-D1* (Knochel et al., 1992), *forkhead1* (Dirksen and Jamrich, 1992), *chordin* (Sasai et al., 1994), *xNR3* (Smith et al., 1995) *Xnot1* (von Dassow, 1993) and the closely related *Xnot2* (Gont et al., 1993). Three of these, *goosecoid (gsc)*, *noggin (nog)* and *chordin* (Sasai et al., 1994) have axis-inducing activity in experimental situations. We report the regional expression of some of these genes within the Organizer, identifying subregions that may be correlated later with inductive and morphogenetic functions.

At the same time, we want to know the origins of different parts of the Organizer from different and interacting parts of the cleaving egg, and to this end we have determined the progeny contributions of cleavage stage blastomeres to early (stage 10) and late (stage 12) gastrula Organizers. Previous work provided fate maps of blastomere progeny contributions to various differentiated tissues and parts of the tadpole from the 16- (Moody, 1987b) and 32-cell embryo (Nakamura and Kishiyama, 1971; Cooke and Webber, 1985; Dale and Slack, 1987; Moody, 1987a). Other previous work has provided fate maps for progeny contributions of gastrula cells to neurula and tail bud stages (Keller, 1975, 1976). However, there has been little attention to intermediate stages, namely, progeny contributions of early blastomeres to gastrula and neurula stages, especially to the Organizer. Interpolation from previous maps is unreliable. Thus, we constructed an intermediate, or predifferentiated, fate map from the 32-cell stage to the early and late gastrula Organizers. Accurate fate maps are needed as the background for interpreting the consequences of removing, perturbing or injecting blastomeres.

Recently, Bauer et al. (1994) reported a survey of intermediate fates, but with no attention to Organizer subregions, and without correlating lineage data with region-specific gene expression. In order to reduce ambiguity introduced by embryo-to-embryo variability of cleavage planes, we have labelled dorsal blastomeres of individual 32-cell embryos with three different lineage tracers and examined the positions of progeny at early and late gastrula stages, analyzing serial sections of numerous embryos. Also, since cells move extensively at the start of gastrulation, we have taken care to identify embryos uniformly at stage 10–11 (Keller, 1991), before the surface layer involutes. We quantified the variability found in blastomere contributions to the Organizer even after careful scrutiny of embryos. Using a combination of lineage and in situ hybridization data, we describe the Organizer in terms of subregions of gene expression and lineage origin.

**MATERIALS AND METHODS**

**Embryo culture and staging**

Adult female *X. laevis* were injected with 800 i.u. of human chorionic gonadotropin 12-18 hours before spawning. Eggs were squeezed from females and fertilized with sperm released from macerated testis in a minimum volume of one-third strength Ringer’s (R/3), pH 7.8 (33 mM NaCl, 0.6 mM KCl, 0.67 mM CaCl2, 0.33 mM MgCl2, 1.16 mM Hepes, pH adjusted with NaOH). After cortical contraction, approxi-mately 10 minutes postfertilization at 22°C, the dishes of eggs were flooded with R/3, pH 7.8. Embryos were left undisturbed until the onset of first cleavage. At first cleavage, embryos were dejellied in 2% cysteine- HCl, pH adjusted to 8.0 with NaOH. Regular cleaving embryos were transferred to a Nile Blue labelling buffer (5% Ficoll in equal volumes R/3, pH 7.8 and 100 mM sodium phosphate buffer, pH 7.8) Equal volume drops of 1% Nile Blue and 100 mM sodium carbonate were mixed in a Petri dish; crystals were picked up with a fire polished glass pipette and held against the dorsal midline surface of an embryo for a few seconds, marking the dorsal side of the embryo with Nile Blue at the 2-cell stage (Kirschner and Hara, 1980). After Nile Blue labelling, embryos were cultured in R/3, pH 7.8, 5% Ficoll at either 22°C or 15°C. After microinjection at the 32-cell stage, embryos were cultured in R/3, pH 7.8, containing 50 µg/ml gentamycin, with or without 5% Ficoll.

Embryos were staged according to Nieuwkoop and Faber (1967) with further finer distinctions during gastrula stages (Keller, 1991).

**Selection of regular cleaving embryos**

We selected embryos in which the first cleavage bisected the light-dark pigmentation of the animal hemisphere. The paler side of the animal hemisphere predicts the dorsal side of the embryo (Vincent and Gerhart, 1987; Vincent et al., 1986) and, when the animal hemisphere pigment is bisected, the first cleavage plane predicts the future midline of the embryo (Masoh, 1990). Regularly cleaving embryos with clear pigmentation asymmetry were labelled with Nile Blue (above). 90-98% of Nile Blue label marks were within 15° on either side of the dorsal lip at stage 10. At the 8-cell stage, we eliminated embryos that did not have a clean horizontal equatorial cleavage. Further selection was made at the 16- and 32-cell stages so that only embryos with regular cleavage patterns were used, typically 2-10% of a given spawning.

The nomenclature for blastomere designations at the 32-cell stage is that of Nakamura and Kishiyama (1971; Fig. 1).

**Microinjection of lineage label**

Dorsal Nile Blue marks enabled us to rapidly orient 32-cell embryos in agarose injection wells (Kroll and Gerhart, 1994). Injections were performed in R/3, pH 6.5, 5% Ficoll on a cooled stage (15°C). Sutter microcapillary pipettes were pulled on a Sutter needle puller. Tips were broken off to a diameter of less than 5 µm. Injections were executed with a Narishige micromanipulator and a Tritech microinjection controller.

For dorsal injections, embryos were injected with three lineage labels at stage 6. The A1 blastomere to the left or right of the dorsal midline was injected with 1-2 nl of fluorescein dextran amine, *M*<sub>r</sub> 10×10<sup>3</sup> (FDA) at a concentration of 50 mg/ml; the B1 blastomere on the same side was injected with 1-2 nl of Texas Red dextran amine, *M*<sub>r</sub> 10×10<sup>3</sup> (TrxRDA) at 35-50 mg/ml; and the C1 blastomere on the same side was injected with 2-3 nl of Cascade Blue dextran amine, *M*<sub>r</sub> 10×10<sup>3</sup> (CBDA) at 100 mg/ml. All lineage tracers were from Molecular Probes, dissolved in dH<sub>2</sub>O and filtered through a 0.22 µm filter before use. These volumes and concentrations balanced brightness of label and minimized cell damage. Three needles were used,
each containing a different lineage label; 4-30 embryos were injected with one label before switching needles.

Ventral injections were performed in the same way into ventral blastomeres (A4, B4, C4) at stage 6.

For animal cap injections, three tier A blastomeres were injected with 1-2 nl of label at the above concentrations: the tier A ventral cell (A4) with FDA; the tier A ventrolateral cell (A3) with CBDA; the tier A dorsal cell (A1) with TmRDA.

All manipulations of fluorescently labelled embryos were performed with minimal light exposure.

**Embryo fixation and sample preparation**

Embryos were fixed without removal of vitelline envelopes at stage 6 (immediately following injection), stage 9 (late blastula), stage 10 (early gastrula) or stage 12 (late gastrula) in MEMFA (0.1 M MOPS, 2 mM EGTA, 1 mM MgSO\(_4\), adjusted to pH 7.4 with NaOH and 3.7% formaldehyde; Harland, 1991) overnight at 4°C. Fixed embryos were dehydrated through an ethanol series, cleared in histosol and embedded in paraffin. Embryos were oriented in hot paraffin using a dissecting microscope so that the plane of section would be parallel to the dorsal midline of the embryo. Thick (13 μm) serial sagittal sections were cut using an AO 820 rotary microtome. Sections were mounted on microscope slides treated with Gatenby-Cowdry adhesive (720 ml dH\(_2\)O, 15 g gelatin, 1 g chromium potassium sulfate, 300 ml 95% methanol, 70 ml acetic acid), dewaxed in xylenes, rehydrated through an ethanol series and mounted in Aqua/polymount (Poly-sciences).

Triple exposure photographs were taken on a Zeiss Axioshot microscope with Kodak Ektachrome Plus Professional 100 slide film using filters optimized for each fluorophore.

**Analysis of dorsal lineage label at stages 10 and 12**

Ten labelled embryos were analyzed at stage 10, and nine at stage 12, from no fewer than five females. Samples with errors in the plane of sectioning or showing injection damage were discarded. Embryos were serially sectioned and all sections for each embryo were counted; the median section was taken to be the midline. Three sections (Fig. 3) were serially sectioned and all sections for each embryo were counted; those section positions include roughly a lateral half of the dorsal marginal zone, from the midline to one lateral edge. Progeny distributions within sections were analyzed on a standardized grid because each embryo and section differs slightly in size and shape. A standardized section (based on the anatomy of several real sections) was drawn on paper and subdivided with a grid (Fig. 3). Then we projected a color slide of each real section onto a different piece of paper and made a tracing of the outline of the section and labelled regions within. The grid was then scaled to fit each tracing using the animal-vegetal axis, the dorsal lip, and the floor of the blastocoel as landmarks at stage 10, and the animal-vegetal axis, tip of the archenteron, boundary between surface and deep cells, the yolk plug, and the dorsal lip as landmarks at stage 12. Equal grid spacing and numbers of grid areas were maintained between these landmarks so that all sections of each stage could be equated with the standardized section. The resulting maps contain the data from 30 sections at stage 10 (three sections from each of ten embryos). For stage 12, we mapped the 18 sections from the midline and the first parasagittal section (65 μm from the midline) directly to one standardized section, but did not include the third section position (135 μm from the midline) because it was on the edge of the archenteron and included more lateral than midline structures.

**Controls for D1 leakage**

In several cases, small amounts of C1-injected lineage label leaked into the adjacent D1 blastomere, presumably because cytokinesis was incomplete at the time of injection despite external appearances. On such occasions, the label in D1 and its progeny was very faint, and usually did not show up in photographs above the background auto-fluorescence. To ensure that we had not scored such descendants of D1 as C1 descendants, we performed single lineage label injections into D1 blastomeres, and observed the position of the D1 descendants in at least 5 embryos at each stage 10 and 12. Without performing the same extensive analysis we did for A1, B1 and C1, we confirmed that we had not scored D1 progeny labelled by C1 leakage as C1 progeny (data not shown).

**Analysis of other lineage-labelled embryos**

Dorsally injected stage 9, and ventrally injected stages 10 and 12, embryos were processed and sectioned as described above, but not subjected to the same degree of analysis. Twenty three stage 9 dorsally injected, sectioned embryos from three different females were examined; the three section positions (midline, 65 μm and 130 μm away from the midline) were photographed in two embryos.

Sections from five ventrally injected stage 10 and seven ventrally injected stage 12 embryos were viewed. Two of each stage were photographed at the three section positions.

Four stage 10 and five stage 12 animal-cap-injected embryos were sectioned and viewed. Two of each were photographed at the three section positions.

**In situ hybridization**

Embryos were fixed as described above except that vitelline envelopes were removed prior to fixation. Digoxigenin-labelled antisense probes to Xbra (Smith et al., 1991), Gsc (Cho et al., 1991), Nog (Smith and Harland, 1992) and \(xN\)R3 (Smith et al., 1995) were used for in situ hybridization in whole-mount albino and lineage-labelled pigmented embryos according to Harland (1991) with the following modifications for the antibody washes and incubations (from T. Doniach, personal communication). After the overnight hybridization, washes were performed without CHAPS detergent. The embryos were washed twice in MAB (100 mM maleic acid, 150 mM NaCl, neutralized with NaOH to pH 7.5) for 15 minutes, then in MAB plus 2% blocking reagent (Boehringer-Mannheim) for 1 hour. The antibody incubations were performed in MAB plus block and 20% lamb serum, either overnight at 4°C, or 4 hours at room temperature. The embryos were washed five times in MAB at room temperature. BM-purple (Boehringer-Mannheim) with 5 mM levamisole was the staining substrate for the alkaline phosphatase reaction. Stained embryos were embedded, serially sectioned at 20 μm and mounted as described above. Sections were photographed using DIC (differential interference contrast) optics to visualize the in situ staining and fluorescence optics to view lineage label as described above.

When in situ hybridization was performed on lineage-labelled embryos, the combined conditions were suboptimal for each signal; good fixation for tissue integrity in sections were unfavorable for good hybridization, and the hybridization procedure and staining product often reduced fluorescence of the lineage label.

**RESULTS**

**Working definition of the Organizer’s location**

To describe our results, we have spatially defined the Organizer at stage 10 and 12, based on previous (Spemann and Mangold, 1924; Spemann, 1938; Stewart and Gerhart, 1990) and current (J. S. Zoltewicz, personal communication) functional and anatomical descriptions (Keller, 1991) of the dorsal marginal zone: at stage 10—, the beginning of gastrulation as seen externally, the Organizer occupies a quadrangle from the level of the floor of the blastocoel (approximately the future limit of involution) to that of the pigment line of the dorsal
blastopore lip, about 20 cell diameters in this animal-vegetal dimension and about 60° wide mediolaterally. It is approximately five cells thick in the radial direction, from the surface to the depth of the blastocoel wall. This region, which has functional Organizer activity, corresponds to the dorsal involuting marginal zone (IMZ) which will involute during gastrulation to form the dorsal (prechordal and chordal) mesoderm and pharyngeal endoderm. Since the exact latitude of the limit of involution cannot be predicted because of extensive convergent extension, we take the floor of the blastocoel as a provisional boundary between IMZ and non-involuting marginal zone (NIMZ), marking the animal boundary of the Organizer.

Fig. 1. 32-cell blastomere designations and dorsal injections. Dorsal is to the right. (A) Diagram of one-half of a stage 6 embryo indicating the tiers A-D, animal to vegetal, and the blastomere nomenclature of Nakamura and Kishiyama (1971). (B) Schematic diagram of dorsal lineage label injections at the 32-cell stage. (C) Parasagittal section of a stage 6 embryo in which A1 was injected with FDA, B1 with TxRDA and C1 with CBDA. Scale bar is 160 μm.

Fig. 2. The gastrula fates of the cleavage stage dorsal blastomeres. (A-C) Early gastrula, stage 10--; (D-F) late gastrula, stage 12. (A,D) Sagittal sections; (B,E, and C,F) parasagittal sections 65 and 130 μm, from the midline, respectively. The green (FDA) cells are A1 progeny; the red (TxRDA) cells are B1 progeny. The blue (CBDA) cells are C1 progeny. Dorsal is to the right. The dorsal lip is indicated by an arrow in A-C. Scale bar is 160 μm.
stage 12, Organizer activity is mostly confined to the presumptive notochord, which lies along the dorsal midline. However, for the purposes of our analysis, we included the anterior (head) mesoderm and some of the more lateral dorsal mesoderm, extending into the presumptive somite regions.

**Contributions of dorsal blastomeres to the stage 10 Organizer**

The stage 10 embryo in Fig. 2A-C is representative of the ten analyzed. Green-labelled cells are descendants of A1; red-labelled cells are descendants of B1 and blue-labelled cells of C1. When compared with the 32-cell embryo, this stage 10–/10 embryo reveals the strong vegetal displacement of labelled clones of all three dorsal tiers with respect to their original positions at the 32-cell stage (Fig. 1C). Labelled clones have intermixed slightly at their borders, but borders are still apparent. In the sagittal section (Fig. 2A), descendants of the C1 blastomere are adjacent to the dorsal lip and descendants from the B1 blastomere are about 4 cell diameters away. In this section, several A1 descendants are present in the IMZ, surrounded by B1 progeny. Thus, descendants from all three dorsal tiers populate the dorsal marginal zone, Spemann's Organizer. The parasagittal section, approximately 65 μm from the midline (Fig. 2B), is similar. This section has more label than the sagittal or more lateral parasagittal sections because only one member of a bilateral pair of dorsal blastomeres was injected at the 32-cell stage. A1 and B1 descendants have shifted vegetally into the marginal zone compared to their starting positions. Many C1 cells are deeper than the marginal zone and will remain part of the endodermal yolk mass according to gastrula fate maps (Keller, 1976, 1991). The C1 surface area is reduced approximately 70% from stage 6, probably due to the apical constriction of the bottle cells at the dorsal lip (Keller, 1978). The lower boundary of the Organizer, defined by the blastopore, always forms within C1 progeny, i.e., bottle cells are always C1 progeny. The third section analyzed for each embryo was a parasagittal section 130 μm from the midline. In this embryo (Fig. 2C), at the outer lateral edges of the dorsal marginal zone seen in this section, the C1 descendants populate most of the areas directly adjacent to the dorsal lip and B1 descendants make up a correspondingly smaller amount of the Organizer. A lateral half of the Organizer can be entirely accounted for by progeny of three labelled dorsal blastomeres, a result not revealed when mapping with fewer than three lineage labels simultaneously.

Of the ten stage 10 embryos analyzed, five had an Organizer like the example in Fig. 2A-C (approximately 10% A1, 70% B1 and 20% C1). In three others, B1 descendants reach the dorsal lip and populate more of the Organizer, and the C1 descendants make up correspondingly less (approximately 15% A1, 80% B1, 5% C1). However, in the remaining two, the B1 descendants populate even less, and C1 progeny more.

**Fig. 3.** Progeny locations of dorsal blastomeres at early (stage 10–) and late (stage 12) gastrula. (A,B) Stage 6-10 fate maps; (C,D) stage 6-12 fate maps. (A,C) Modal maps; (B,D) statistical maps. Green indicates regions populated by A1 progeny; red indicates regions populated by B1 progeny and blue indicates regions populated by C1 progeny. Location of progeny and their ultimate fates are described in text for Figs 2, 3. Scatter in B is due to variation between embryos; scatter in D primarily is due to cell mixing. Dorsal is to the right. Data include 30 sections for stage 10 and 18 for stage 12. See text for details on construction of fate maps.
of the DMZ and Organizer (approximately 5% A1, 45% B1, 50% C1). This variation presumably reflects variable cleavage planes relative to cytoplasmic materials determining the level of the blastopore and reflects variable cell movements preceding gastrulation.

Contributions of dorsal blastomeres to the stage 12 Organizer

At this stage, the end of gastrulation, all cells that will involute have done so (with the exception of tail structures; Gont et al., 1993). The progeny composition of the entire dorsal mesoderm, from the most anterior head mesoderm above the tip of the archenteron, to the most posterior chordal mesoderm at the interior edge of the dorsal lip, can be seen (Keller, 1975, 1976, 1991). Fig. 2D-F is an example of a stage 12 embryo used to construct the stage 12 fate map. Again a sagittal and two parasagittal sections are shown. The extensive intermixing of cells between different labelled populations is most striking. Most lineage label is in the first parasagittal section (Fig. 2E). As expected, the movements of convergent extension during gastrulation (Keller et al., 1985) have significantly narrowed and elongated the region in which label is distributed. This has led to intercalations of cells with different labels and no label.

The most anterior mesoderm (prospective head mesoderm; Keller, 1976) in all three sections is made up mostly of C1 descendants. A few B1 progeny contribute to the head mesoderm, especially as seen in Fig. 2E. Along the length of the remaining dorsal mesoderm, the chordal mesoderm is composed of a mixture of B1 and C1 descendants. At the anterior end, C1 progeny predominate and at the posterior end B1 progeny predominate. In the most lateral section (Fig. 2F), the B1 progeny extend less anteriorly; this region of paraxial mesoderm is mostly presomitic (Keller et al., 1989). In sections closer to the midline (Fig. 2D,E), the chordal mesoderm that will form the notochord (Keller, 1976) and retain inductive activity at this stage is made up mostly of B1 descendants. The notochord will contain mostly progeny of animal hemisphere blastomeres above the first horizontal cleavage of the 8-cell embryo. In this embryo, there are not many A1 progeny in the dorsal mesoderm, but in other embryos analyzed at stage 12 (7 of 9), at least 5 A1-derived cells (of over a hundred total) populate the interior lip region and the most posterior chordal mesoderm. Two of nine have A1 progeny even in the mid-region of the chordal mesoderm. This can be seen in the stage 12 fate map (Fig. 3D).

Within individual embryos, the deep cells of the marginal zone shear away from the superficial cell layer and are more likely to involute (Fig. 2D-F). Near the tip of the archenteron, blue C1 progeny in the involuted superficial layer that will line the pharynx (Keller, 1975) are next to deep red cells derived from B1 (Figs 2D-F; 4G,E). Many non-involuted B1 progeny occupy the neurectoderm surface layer. Likewise, involuted A1 progeny are always found in deep layers (Fig. 3B; and data not shown).

In contrast to the minimal intermingling of labelled clones at stage 10, clonal boundaries at stage 12 are obliterated by extensive intermixing. Descendants from individual dorsal blastomeres are spread along much of the embryonic axis. B1 descendants are the most extreme case; they can contribute to prospective head mesoderm, notochord and neural plate (a non-involuted tissue) in a single embryo. Approximately a third of the B1 progeny remain uninvolted. This intermixing and diversity of fates is consistent with what is known about gastrulation movements (Keller et al., 1985; Jacobs and Fraser, 1994) and with previous fate maps (Keller, 1975; Dale and Slack, 1987; Moody, 1987b).

Modal and statistical fate maps: stage 6 to stages 10 and 12

Because of the substantial variation among embryos, a quantitative analysis was done to create two kinds of composite fate maps of dorsal blastomere progeny locations at gastrula stages.

Modal maps in Fig. 3A and C were constructed to represent locations of the majority of each labelled population at stage 10 and 12, respectively, showing where progeny of each blastomere predominate in most embryos. To do this, each grid area was assigned one lineage label colour when that label occupied the majority of the grid area in the majority of the embryos. In a few grid areas, no one label was in a clear majority, and the box was split between two (or in one case three) labels. For stage 10 (Fig. 3A), this “winner takes all” assignment of labels to grid areas gives the average position of majority progeny and of clonal boundaries; hence it is called a Modal map. The procedure tends to sharpen boundaries. Borders between labelled areas are thus clear in vivo and in the map. However, it is important to remember that, within each individual embryo (Fig. 2), some mixing between labelled populations is not reflected in the summary. For stage 12 (Fig. 3C), many grid areas had to be split despite the boundary enhancement of the Modal map. This reflects extensive cell intermixing and dispersal of clonal boundaries.

The statistical maps in Fig. 3B and D were constructed to better represent variation among embryos (especially at stage 10) and the variable extent of cell intermingling (especially at stage 12). These include minority contributions of labelled cells in each grid area. To do this, each grid area was assigned lineage label colors in proportion to the relative area and frequency of each label (or no label) in that grid box in the 30 sections at stage 10 and 18 sections at stage 12. Minority and majority contributions were thus recorded. For stage 10 (Fig. 3B), where clonal boundaries in individual embryos are apparent, the slight scatter of the statistical map is due to variation in boundary positions between embryos. For stage 12 (Fig. 3D), the considerable scatter, which is greater than seen in the comparable modal map, is due to some variation between embryos but mostly to variable cell intermixing, which is extreme in regions of convergent extension. For instance grid areas near the lip are solid red in Fig. 3C, but contain some green in Fig. 3D, because although the majority of cells in these regions are red, some green cells are nearly always present.

Both kinds of maps confirm the qualitative impressions stated before: the three tiers that started out more or less vertically stacked, A upon B upon C, are rearranged extensively by the beginning of gastrulation (stage 10–). The stack becomes a “U” shape, with C1 cells doubled back upon the line, losing most of their surface exposure as bottle cells undergo apical constriction. This first indication of involution at stage 10 becomes exaggerated at stage 12 when the stack has been completely doubled back so that C1 descendants now underlie A1 descendants. Pregastrulation movements make gastrula fate maps (Keller, 1975, 1976) non-superimposable on
the 32-cell embryo because the blastomere positions at stage 6 do not coincide with the positions of their progeny at stage 10.

The stage 10 Organizer is clearly made up of cells from three tiers. A large number of the C1 descendants within the involuting marginal zone will contribute to the most anterior mesoderm (head mesoderm) at stage 12 and some to anterior chordal mesoderm; some C1 cells will also form the endodermal lining of the pharynx and archenteron roof (Keller, 1975, 1976). B1 descendants make up the largest fraction of the stage 10 Organizer in both the modal and statistical maps. These cells will contribute to head mesoderm and to chordal mesoderm, forming the bulk of the notochord at stage 12. A few A1-derived cells lie deep within the most animal part of the stage 10 Organizer and will contribute to posterior notochord at stage 12, but most A1 descendants lie within the non-involuting marginal zone and will contribute to neural structures and epidermis.

**Pregastrula locations of dorsal blastomere progeny**

Because labelled cells changed locations between stages 6 and 10, we examined intermediate stages to see when this displacement occurred. Stage 9 late blastula embryos were fixed approximately an hour before the first appearance of the dorsal lip at 22°C. We examined twenty-three sectioned embryos. All were similar to the one shown in Fig. 4A, a parasagittal section, 65 μm from the midline. The sagittal and other parasagittal section are not significantly different. Dorsal blastomere descendants have been displaced vegetally slightly from their positions at the time of injection (Fig. 1C), but have not moved as far vegetally as by stage 10 (Fig. 4C for comparison). A few cells have spread into neighboring clones, but the borders are more even and closer to the original shape of the injected blastomeres than at stage 10. It appears that most of the cell mixing is due to cell divisions (Keller, 1978) and that pronounced movement of these dorsal cells toward the vegetal pole does not occur until gastrulation begins (Keller, 1980). The pre gastrula stage 9 dorsal marginal zone (between the level of the blastocoel floor and prospective blastopore) is still made up mostly of C1 descendants with only a small contribution from B1 descendants. The cells whose progeny will form the bulk of the gastrula Organizer (Stewart and Gerhart, 1990) are still high in the animal hemisphere. Therefore downward displacement of the dorsal blastomeres occurs between stages 9 and 10.

**Gastrula locations of ventral blastomere progeny**

We examined the progeny positions of ventral blastomeres at gastrula stages for comparison with the dorsal blastomeres. Labels were injected at the 32-cell stage as on the dorsal side: A4 with FDA (green), B4 with TxA (red), C4 with CBDA (blue).

Cells derived from ventral blastomeres are not displaced as far vegetally at stage 10 (Fig. 4B) and clones intermix only slightly compared to dorsal counterparts (Fig. 4C). C4 progeny occupy a large area of the embryo surface at the time when C1 progeny are displaced vegetally and involuted, occupying fewer surface positions. This is consistent with the initiation of gastrulation movements on the dorsal side. Locations of ventral labelled cells at stage 10 (Fig. 4B) appear more similar to locations of dorsal blastomere progeny at stage 9 than at stage 10 (Fig. 4A,C). C4 descendants make up most of the ventral marginal zone at stage 10, with only small contributions from A4 and B4. This anticipates the non-involuting locations of these cells at stage 12 (Fig. 4D,E). Involuting tissue of the ventral marginal zone is derived mostly from C4, while B4 and A4 progeny make minor equal contributions. As expected (Keller and Danilchik, 1988) less tissue involutes, less cell intermixing occurs and less lengthening of the axis in a vegetal direction occurs on the ventral side (Fig. 4D-G). Labelled clones on the ventral side are not nearly as dispersed and intercalated with one another and with unlabelled clones by stage 12. But some of the same shearing that occurs on the dorsal side is apparent on the ventral side (Fig. 4E,G). Only deep labelled cells involute. A significant number of C4 progeny remain on the non-involuting surface in contrast to C1 progeny which all involute. It is difficult to conclude whether the deep and most anteriorly placed A4 and B4 progeny arrive in their stage 12 positions by involution or if they cross the boundary between the non-involuting surface layer and the mesoderm directly, without going around the blastopore lip. If they arrive there by involution, then deep cells must move significantly further than surface cells. If they arrive there by crossing the boundary, it would contrast markedly with mesoderm formation on the dorsal side. In a preliminary confocal study of whole-mount lineage-labelled embryos, the displacement of ventral clones vegetally, begins at stage 10-10.5, when the ventral blastopore lip first appears, at least an hour after the corresponding dorsal movements (data not shown).

In contrast to a recent report (Bauer et al., 1994), we do not see extensive contribution of A4 and B4 ventral progeny to the dorsal animal cap at either stage 10 or 12. At stage 10 (Fig. 4B), one to four scattered cells derived from A4 (out of at least 100 cells) may just pass the animal pole onto the dorsal side by a few cell diameters. Cells never moved in large blocks (Fig. 4B). B4 descendants were never seen to move dorsal to the animal pole. Of seven examined, the stage 12 embryo in Fig. 4D is the most extreme example of dorsally displaced A4 progeny, a few of which just reach the presumptive neural region. B4 progeny are not displaced dorsally. Bauer and colleagues (1994) examined fewer embryos less completely and may have inadvertently focused on a few anomalous cases.

**Animal cap analysis**

To assess further the possibility that ventral animal hemisphere blastomeres contribute to dorsal ectoderm, we injected ventral tier A (A4) with FDA, ventrolateral tier A (A3) with CBDA, and dorsal tier A (A1) with TxA, and examined these embryos at stages 10 and 12. Most (three of four) looked like the stage 10 embryo shown in Fig. 5, in which no cells derived from A4 are on the dorsal side. In the fourth embryo examined at stage 10, a few A4 progeny had passed the pole to the dorsal side. Note that ventrolateral blastomere progeny may inhabit the pole region and intermix with both ventral and dorsal blastomere progeny (Fig. 5). This observation explains the absence of dorsal (A1) progeny at the pole as labelled dorsal clones are displaced vegetally (Figs 2A-C; 3A; 4D). For all five embryos examined at stage 12, no A4 progeny progressed further dorsally than those in Fig. 4D.

**Tailbud stage fates**

We allowed several dorsally injected embryos to develop to tailbud stages 35/36 to confirm that blastomere fates were consistent with those expected from previous 32-cell Xenopus fate
maps (Dale and Slack, 1987; Moody, 1987b). Fig. 6 is a transverse section of the head of a stage 35/36 embryo. A1 (green) progeny contribute to the brain and eye; and B1 (red) progeny to the notochord, loose mesothelium and some neural structures in the eye and brain. Clearly B1 progeny are greatly scattered, stretching from anterior neural positions to anterior notochord positions. C1 descendants (blue) are located in the mesothelium of the head (head mesoderm) and in the pharynx (not shown). These fates are indeed consistent with those previously described (Dale and Slack, 1987; Moody, 1987b).

Regional gene expression in the Organizer

We compared the expression of four Organizer-specific genes Xbra, xNR3, nog and gsc, between stages 10 and 12 in lineage-

Fig. 4. Comparison of the locations of dorsal and ventral cleavage stage blastomere progeny at pregastrula and gastrula stages. Late blastula, stage 9 dorsally injected embryo (A). Early gastrula stage 10−, ventrally injected (B) and dorsally injected (C). Late gastrula, stage 12, ventrally injected (D-E) and dorsally injected (F-G). (E,G) Close-ups of the involuted ventral and dorsal mesoderm from D and F, respectively. Green (FDA) cells are descendants of A1 (A,C,F,G) and A4 (B,D,E); red cells (TxRDA) are descendants from B1 (A,C,F,G) and B4 (B,D,E); blue cells (CBDA) are descendants from C1 (A,C,F,G) and C4 (B,D,E). All sections are parasagittal, approximately 65 μm from the midline; dorsal is to the right, except in E and G, dorsal is at the top with anterior to the left. The dorsal lip is indicated by an arrow in C. Scale bar for A-C,D,F is 220 μm; scale bar for E, G is 97 μm.

Fig. 5. Animal blastomere injections. Early gastrula, stage 10−, parasagittal section, 65 μm from the midline. Green (FDA) cells are progeny of A4; blue (CBDA) cells are progeny of A3; and red (TxRDA) cells are progeny of A1. B is a close-up of the animal cap region in A. Scale bar is 220 μm in A and 97 μm in B.
Composition of the Organizer labelled, sectioned embryos and find significant differences in their regional expression, summarized in Table 1 and Fig. 9.

Xbra
This *Xenopus* homologue of the mouse *Brachyury* or *T* gene (Smith et al., 1991) encodes a DNA-binding protein (Kispert and Herrmann, 1993) expressed in the notochord and tailbud, as in zebrafish (Schulte-Merker et al., 1992). The gene product is involved in posterior mesoderm formation (Herrmann, 1991; Rashbass et al., 1991). In *Xenopus*, Xbra expression is an immediate early response to mesoderm induction, occurring in the entire marginal zone of the early gastrula (Smith et al., 1991), not just in the Organizer. In the late gastrula expression is found along the dorsal midline and in a circumblastoporal ring (Green et al., 1992).

At stage 10, Xbra is expressed strongly in deep, and less strongly in superficial, cells of the middle of the Organizer (Fig. 7A-C). Expression is excluded from cells at the level of the blastocoel floor and from the four or five cell layers closest to the blastopore. About half the cells of the Organizer express this gene, mostly deep C1 progeny and a few B1 progeny. Xbra expression in the lateral and ventral marginal zone (Fig. 7C) was also limited to sub-regions of these areas (Smith et al., 1991; Lemaire and Gurdon, 1994).

At stage 12, Xbra is expressed in involuted dorsal mesoderm of the presumptive notochord and in involuted presumptive pharyngeal endoderm (Fig. 8A,B) by a mixture of B1 and C1 descendants mostly, and a few A1 descendants in positions near the lip. Xbra expression also occurs in non-involuted tissue surrounding the closing blastopore (mixture of A1 and B1 progeny), tissue destined to form posterior tail mesoderm (Fig. 7C) (Gont et al., 1993). Some of the same cells that expressed Xbra on the dorsal side at stage 10 may still express it at stage 12, but it is clear that more dorsal cells express at stage 12 than at stage 10. Therefore many cells have transcribed new Xbra message during gastrulation (Table 1).

xNR3
The gene encoding this TGF-β family member is expressed in the dorsal marginal zone of the *Xenopus* embryo (Fig. 6). Green (FDA) cells are descendants from A1; red cells (TxRDA) are descendants from B1; and blue cells (CBDA) are descendants from C1. Scale bar is 80 μm.

![Fig. 6. Tailbud fates of dorsal blastomeres. A transverse section of the right side of the head of a stage 35/36 tailbud. The brain is indicated by short arrow, the notochord by curved short arrow and the eye by long arrow. Green (FDA) cells are descendants from A1; red cells (TxRDA) are descendants from B1; and blue cells (CBDA) are descendants from C1. Scale bar is 80 μm.](image-url)
embryo from stage 9 to stage 12 (Smith et al., 1995). At stage 10, xNR3 is expressed in superficial cells of the dorsal marginal zone (Fig. 7D-F; Smith et al., 1995), with expression highest in cells just above the lip, but with significant expression in cells below the lip. B1 progeny express the highest amount of xNR3, but some C1 and D1 progeny also express xNR3. At stage 12 (Fig. 8C,D; Smith et al., 1995) xNR3 message has nearly disappeared, but remains readily detectable in a few isolated cells on the exterior surface of the lip. No involted tissue at either stage 10 or 12 expresses xNR3.

Noggin
This gene, expressed in deep and superficial cells throughout the Organizer (Fig. 7G,H,I), encodes a secreted protein that dorsalizes lateral-ventral mesoderm and induces neural tissue from ectoderm (Smith and Harland, 1992; Smith et al., 1993; Lamb et al., 1993). Expression is strongest near the dorsal lip (Fig. 7G,I). Like xNR3, nog is also expressed in sub-blastoporal cells. Near the level of the blastocoel floor, expression is restricted to, or stronger in, deep cells than in superficial ones (Fig. 7F). nog expression is mostly confined to B1 and C1 progeny, with sub-blastoporal expression in D1 progeny.

At stage 12, (Fig. 8E-G) nog is expressed only in the deep involted mesoderm (presumptive notochord), whereas Xbra is also expressed in the involted superficial layer (prospective gut endoderm). The majority of expressing cells are B1 progeny; some C1 and A1 progeny may also express nog at the anterior and posterior ends of the axis respectively. At the end of gastrulation, nog is not limited to mesodermal cell types; it is also expressed in the anterior neural plate (Fig. 8G). A subset of the cells expressing nog at stage 10 probably continue expressing at stage 12, while some cells switch it off (e.g. sub-blastoporal cells), and others begin to express nog during gastrulation (Table 1).

goosecoid
This homeobox gene, which is expressed in the Organizer (Fig. 7G,H,I), encodes a secreted protein that dorsalizes lateral-ventral mesoderm and induces neural tissue from ectoderm (Smith and Harland, 1992; Smith et al., 1993; Lamb et al., 1993). At stage 10, its expression is confined to deep cells of the middle to vegetal (anterior) region of the Organizer, extending to, but not below, the blastopore (Fig. 7J,K). Cells expressing gsc are mostly C1 progeny, as expected from their positions (Fig. 7K). Detection of stage 12 gsc expression in lineage-labelled embryos was difficult. However, Fig. 8H,I reveals expression in the anterior involted mesoderm, head mesoderm and maybe some anterior chordal mesoderm. These would be mostly C1 progeny.

Thus the four genes are expressed in different regions of the stage 10 Organizer with respect to superficial and deep layers of cells and with respect to animal-vegetal levels (Fig. 9). The Organizer may have a more complex organization than that revealed by surgical functional experiments thus far.

**DISCUSSION**

**Formation of the dorsal marginal zone and Spemann’s Organizer**

Pregastrula

We labelled three blastomeres on the dorsal side of the 32-cell embryo and examined progeny locations at several times until the completion of gastrulation. A1 and B1 blastomeres at the time of injection are within the animal hemisphere, above the first horizontal cleavage; the C1 blastomere is below the first horizontal cleavage, straddling the equator (Fig. 1). From late cleavage through blastula stages, cells containing animal hemisphere cytoplasm are displaced peripherally and slightly vegetally as the blastocoel volume increases and cells divide. The animal cap becomes thinner; the bulk of the cellularized animal hemisphere cytoplasm moves into the marginal zone (Fig. 1C versus Fig. 4A; see also Keller, 1978, 1980; Tuft, 1965). This displacement probably reflects local radial intercalations because labelled clones remain coherent and minimally intermixed (Fig. 4A). Cell division and radial intercalation characterize this pregastrula morphogenesis (Keller, 1978, 1980). By stage 9 the labelled clones have been displaced vegetally and are still coherent, and progeny in the surface and deep layers remain superimposed (Fig. 4A). The displacement of the animal hemisphere cells into the marginal zone prior to gastrulation occurs to about the same extent on the dorsal and ventral sides of the embryo (Fig. 4A,B) (Keller, 1980).

**Beginning of gastrulation**

Gastrulation is marked by active cell movements beginning on the dorsal side. By the time that the dorsal pigment line of the blastopore lip is visible (stage 10–), the animal cap cells and dorsal marginal zone cells have been strongly displaced to more vegetal positions, partially due to the reduction of surface area by the apical constrictions of bottle cells (Keller, 1980), causing C1 progeny to involute around an “internal blastopore lip” and taking B1 descendants within a few cell diameters of the bottle cells (Figs 2A-C; 3A). Labelled clones begin intermixing, indicating active cell movement in addition to overall tissue displacement. At stage 10–, the Organizer, defined as the dorsal marginal zone from the blastocoel floor to the blastopore lip, 4 or 5 cell layers deep and 60° wide, is composed of C1 progeny just above the dorsal lip, B1 progeny through most of its volume and A1 progeny at its most animal boundary (Figs 2A-C; 3A). The cellular composition of this geometrically defined zone is greatly different at stage 10 compared to stages 6 and 9 (Figs 1C,B; 4A).

The Organizer is formed by cells containing cytoplasm originating mostly above the first horizontal cleavage (B1), but it

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A “+” indicates gene expression in cells of the indicated lineage or position at that stage; “++” indicates heavy expression or expression in many cells. A “−” indicates absence of gene expression. Parentheses “( )” indicate lineage is inferred.
is uncertain what is required by these blastomeres to form the Organizer. Our results do not allow us to distinguish between (1) dorsal animal blastomere autonomy as a result of cortical rotation and (2) vegetal induction by the Nieuwkoop Center.

In contrast to a recent report (Bauer et al., 1994) that the stage 10 Organizer is made up almost exclusively of B1-derived cells, we find that C1 descendants make up as much as 45% of the Organizer in some embryos, and typically make up 20% of the Organizer at the beginning of gastrulation, mostly occupying the vegetal, leading part, prospective for head mesoderm. This is consistent with the location of C1 progeny in head mesoderm in our stage 12 embryos and with gastrula fate maps (Keller, 1975, 1976). Paradoxically, Bauer et al. (1994) report C1 descendants in head mesoderm at stage 13 though not in their stage 10 marginal zone. There are several possibilities to explain the discrepancy between our stage 10 data and theirs. Their summary diagram depicts a later gastrula, stage 10+ (Keller, 1991), when prospective anterior Organizer has already involuted significantly and they may have overlooked the significant contribution of C1 progeny to the Organizer. Their study involved fewer embryos and less extensive analysis of variation, and so their examples may have fortuitously contained only embryos with few C1 progeny in the Organizer, as happens in a minority of our cases. Or in emphasizing the consensus that B1 progeny populate the majority of the stage 10 Organizer, they may have under-represented the variation and the contributions from the A1 and C1 blastomeres.

The dorsal displacement of ventral animal blastomere progeny as described by Bauer et al. (1994) was undetectable among our twelve ventrally injected and nine animal cap injected embryos, even though we specifically looked for such cell behavior. However, lateral or off-angle (oblique to the midline) sections produce the illusion of a dorsal position for some ventral (A4) progeny (data not shown). We see progeny contributions from the lateral blastomere to the dorsal animal cap (Fig. 5). Unlike embryos described by Bauer et al. (1994) animal cap expansion appears uniform in our embryos, at least in a dorsal-ventral direction (also observed by Keller; 1978). We see no evidence for epiboly significantly altering the cytoplasmic composition of the animal cap in a ventrodorsal direction and thus no rationale for this contributing to changes in animal cap competence.

End of gastrulation
At the end of gastrulation, stage 12, when involution is complete (except for perhaps fewer than 5 cell diameters; Gont et al., 1993), extensive intermixing of labelled clones with one another and with unlabelled lateral clones has occurred (Figs 2D-F; 3C,D; 4F,G). This reflects the migratory cell behavior of head mesoderm cells, and the radial and mediolateral convergence and extension of the chordal mesoderm cells during gastrulation (Keller et al., 1989). Positions of labelled progeny in stage 12 relative to positions in stage 10 are consistent with previous predictions of cell locations between these two stages (Keller, 1975, 1976). Significant shear between deep and superficial cells leads to the more anterior cells of a labelled clone being in deep positions compared to the superficial positions of the more posterior cells of the clone. This means the limit of involution is not the same for superficial and deep cells of the marginal zone. The limit is higher, more animal, for deep than for superficial cells. The stage 12 Organizer at its anterior end is made up of C1 and B1 descendants; whereas the middle and posterior are mostly B1 descendants. A1 descendants may occupy the most posterior regions. At this stage neighboring cells are just as likely to be from two different clones as from the same one; boundaries between different clones are indistinct. This is in contrast to the more contiguous relationship of labelled cells at stage 10.

Ventral marginal zone
From the 32-cell stage through cleavage and early blastula stages, the movements and positions of ventral labelled clones appear equivalent to those on the dorsal side, with gradual displacement of cells vegetally as the blastocoel expands (Fig. 4B, data not shown). Because gastrulation begins on the dorsal side, the cellular composition of the ventral and dorsal marginal zones differ at stage 10. The C4 progeny still occupy most of the ventral marginal zone (Fig. 4B), while the B1 progeny occupy most of the dorsal marginal zone (Fig. 4C). Only at stage 10.5 when the ventral lip appears are ventral labelled clones displaced vegetally towards the lip (data not shown). The ultimate extent of involution and vegetally directed elongation on the ventral side is much less than on the dorsal side. C4 cells make up most of the involuted mesoderm, with only a few contributions from A4 and B4 progeny (Fig. 4D,E). We infer from the position of labelled cells at stage 12, that ventral mesoderm may be formed not merely by involution, but by some cells ingressing directly from the ectodermal layer to the mesodermal without going around the blastopore lip. This would mean that the morphogenesis of mesoderm formation is different on the dorsal and ventral sides. More thorough investigation of ventral tissue morphogenesis is required to demonstrate this ingression.

Gene expression domains subdivide the Organizer
The Organizer – defined by its inductive activity and ability to organize the body axis during the morphogenesis of gastrulation – is a set of cells with changing members and gene expression repertoire. The geometric region of the dorsal marginal zone from stage 6 to 10 also changes its cellular composition. C1 progeny populate the greater part of the stage 9 dorsal marginal zone when Organizer-specific gene expression begins. Not much is known about the changes in regional gene expression between the late blastula and early gastrula, but we have shown that the cellular composition of the dorsal marginal zone changes between these two stages. By stage 10, B1 progeny make up the majority of the Organizer and most highly express the Organizer genes examined (Table 1), whereas C1 progeny have mostly been displaced inward.

The expression patterns of four genes Xbra, gsc, xNR3 and nog delineate Organizer subregions (Figs 7, 9). The region of overlap between xNR3 and nog expression defines a vegetal (anterior), superficial domain of the stage 10 Organizer, extending below the dorsal lip. It is rarely considered that Organizer inductive functions extend below the blastopore. A more animal (posterior) superficial domain is defined by the region of overlapping expression of Xbra, nog and, possibly, xNR3. Furthermore, within the deep cells of the stage 10 Organizer, two gene expression domains exist, a vegetal (anterior) domain defined by nog and gsc overlap and an animal (posterior) domain defined by nog and Xbra. These
Organizer subregions do not coincide with lineage boundaries of the 32-cell stage blastomeres.

At stage 12, cell configurations and gene expression within the Organizer are significantly altered from stage 10 (Figs 8, 9). nog, Xbra, gsc and xNR3 expression domains are more discreet and less overlapping. Roughly two domains are defined within the deep cells of the stage 12 Organizer. gsc expression corresponds with the most anterior mesoderm (mostly C1 progeny), the prechordal plate head mesoderm; at stage 10, this tissue had also expressed nog. The overlap of nog and Xbra expression defines a domain of involuted tissue corresponding to deep chordal mesoderm posterior to the prechordal plate (mostly B1 progeny with some C1 and A1 progeny). Xbra expression alone defines the superficial involuted tissue fated to become endoderm. The remaining stage 12 expression domains of the “Organizer” genes, Xbra, xNR3 and nog are no longer within the Organizer. A second domain of noggin expression appears in the anterior neural plate. Xbra expression extends to deep and superficial layers of non-involuting tissue on the exterior of the blastopore; Xbra continues expression in the tailbud, potentially a later site of Organizer function. xNR3 is only expressed in a few superficial non-involuting cells.

Finer mapping by in situ hybridization with multiple probes simultaneously in a single embryo (Jowett and Lettice, 1994; Knecht et al, 1995) will better resolve the gene expression boundaries. For instance, at stage 10, gsc and Xbra expression appear to be mutually exclusive; and we have drawn the gsc and nog domains as non-overlapping at stage 12 (Fig. 9), even though they may overlap at their edges (Fig. 8E,G,H). However, our data cannot resolve these boundaries and potential overlaps conclusively.

At stage 12, C1 and B1 descendants compose most of the dorsal mesoderm, but some cells that belonged to the Organizer at stage 10 may not be present in the dorsal mesoderm, and the remaining cells are extensively rearranged with respect to nearest neighbors and to their anterior-posterior position in the embryo. Likewise, molecular expression patterns within these cells have altered. For instance, between stages 10 and 12, noggin expression is reduced from the entire Organizer to a subset of the dorsal mesoderm formed by cells of the Organizer. Therefore any anterior-posterior positional information present at stages 9 and 10 must be plastic enough at the cellular level to allow reassignment between early and late stages, during and after morphogenesis has altered cell neighbor relationships. The combination of our lineage and in situ hybridization results demonstrates that a cell’s position or location within the embryo at a given stage is more likely than its lineage relationships to determine which genes are expressed.

Consequences for experimental manipulations

Fate mapping of the Xenopus embryo by lineage-labelling blastomeres is inherently imprecise because cleavage planes vary between embryos without effect on development. Embryos with irregular cleavage planes, or those in which cleavage planes are intentionally re-oriented (Black and Vincent, 1988), develop normally, implying that normal development does not depend upon segregation of the early cytoplasm into particular blastomeres or upon lineage relationships. Fate maps from early embryos would ideally map cytoplasmic localities, not lineages. Cells are marked because there is no way to mark and follow cytoplasmic localities. Thus, it is important to recognize that it is the region of the embryo, the cytoplasm enclosed by a given blastomere, that is really mapped. For intermediate and final fate maps to be valuable to the researcher (e.g. for targeting of constructs or manipulations), regular cleaving embryos must be used if the position of early blastomeres is to have any predictive value of their progeny locations at later stages.
lineage data compared between stages 10 and 12 also confirm expression (dimension was about 5 cells deep, and Organizer gene visional boundary of the early gastrula Organizer in the radial re-evaluation of stage 10 Organizer boundaries. Our pro-

Our cell lineage and regional gene expression data require a precise examination of stage-specific Organizer function and anatomy is necessary to be conclusive.

The organization of the Organizer

We have examined only four genes, and a rapidly increasing number are known to be expressed in and sometimes restricted to the Organizer. The cellular and molecular organization of the Organizer is already complex at stage 10, reviving the possibility of separate (head versus trunk/tail) Organizers (reviewed in Gerhart et al., 1991) supported by recent results in the mouse (Shawlot and Behringer, 1995). Nevertheless, this stage 10 organization must allow for the variability between embryos and rearrangement of cells during the morphogenesis of gastrulation. Further mapping of gene expression domains, how they overlap or are excluded from one another, and how the domains change from the late blastula and continuing through the end of gastrulation can be combined with functional tests of gene and tissue activity to further clarify the organization of the Organizer.

Because classical genetics cannot be used to study Xenopus development, injection of overexpression (Vize et al., 1991) or dominant negative (Hemmati-Brivanlou and Melton, 1992; Amaya et al., 1991; MacNicol et al., 1993) constructs has been used for molecular perturbation. To perturb developmental events by injection or manipulation, it is important to know the blastomere fates of the cleavage stage embryo at the stage of interest. For instance to affect the majority of the Organizer at stage 10, the B1 blastomere is the best target. Progeny of B1 are most likely to occupy the stage 10 Organizer and to express the Organizer-specific genes, nog, gsc and xNR3. This is not obvious as one might assume from 32-cell blastomere positions that the C1 progeny would occupy most of the Organizer at stage 10. However, to hit the head mesoderm position of the Organizer, C1 is the better target.

Organizer boundaries

Our cell lineage and regional gene expression data require a re-evaluation of stage 10 Organizer boundaries. Our pro-

visional boundary of the early gastrula Organizer in the radial dimension was about 5 cells deep, and Organizer gene expression (Xbra and gsc) falls within this boundary. The lineage data compared between stages 10 and 12 also confirm that these cells will involute to form the dorsal mesoderm. The failure of all B1 progeny to involute, even though they were vegetal to our initial placement of the animal boundary at the floor of the blastocoel, indicate this placement was too high. Likewise, the boundaries of the most animal expressing genes examined in the marginal zone, Xbra and nog, are two to three cells below the floor of the blastocoel. If the functional Organizer is bounded by the limit of involution, and these genes represent Organizer function, then the animal boundary of the Organizer must be 2 to 4 cells below the floor of the blastocoel. The dorsal lip is the canonical vegetal boundary to the Organizer, but at least two “Organizer” genes, nog and xNR3, are expressed below the lip, in non-involuting D1 progeny. The expression patterns of Xbra, nog, gsc and xNR3 may represent the true Organizer boundaries, but a more precise examination of stage-specific Organizer function and anatomy is necessary to be conclusive.

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