Development 116, 347-355 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

Xenopus maternal RNAs from a dorsal animal blastomere induce a secondary axis in host embryos

ALEXANDRA M. HAINSKI and SALLY A. MOODY*

Department of Anatomy and Cell Biology, University of Virginia School of Medicine, Box 439 Health Sciences Center, Charlottesville, VA 22908, USA

*Author for correspondence. Current address is Department of Anatomy, The George Washington University Medical Center, 2300 I Street, N.W., Washington, D.C., 20037, USA

Summary

The initial steps of dorsal axis formation are controlled by localized maternal determinants in *Drosophila*, and a similar process has been proposed in *Xenopus*. The present study demonstrates that there are axis-inducing RNA molecules located in a specific dorsal midline, animal blastomere (D1.1) of the 16-cell-stage embryo. This blastomere, although in the animal hemisphere at cleavage stages, populates most of the dorsal lip of the blastopore, the region of Spemann's organizer, during gastrulation, and is the major progenitor for dorsal mesodermal tissues. Cytosol from this blastomere causes ventral cells to take a more dorsal fate. RNA from this blastomere induces a secondary axis when injected into ventral blastomeres and restores the dorsal axis in UV-irradiated embryos. In *Xenopus*, activinβ_B, goosecoid and Xwnt-8 RNAs can ectopically induce a dorsal axis; however, none is a maternal transcript. Therefore, the D1.1 blastomere probably contains dorsal determinant(s) that are either maternal members of these gene families, or other presently unknown molecule(s). Regardless of the identity of the determinant(s), this study presents the first indication that *Xenopus* maternal RNAs in the dorsal animal hemisphere are able to organize the dorsal axis.

Key words: cytoplasmic determinants, mesoderm induction, Spemann organizer, gastrulation, lineage tracing, cell fate, dorsal axis formation.

Introduction

The establishment of the dorsal-ventral axis is a critical initial step in pattern formation. In amphibian embryos, this process is composed of many steps, beginning with a dorsal-ventral asymmetry established at fertilization and ending with the induction of the nervous system by Spemann's organizer (reviewed by Elinson and Kao, 1989; Wakahara, 1989). The earliest known step of dorsal axis formation in *Xenopus* involves the rotation of cortical cytoplasm in the fertilized egg away from the point of sperm entry (Vincent et al., 1986; Vincent and Gerhart, 1987). Many manipulations that inhibit this rotation block the development of dorsal mesoderm, and thus the dorsal axis (Scharf and Gerhart, 1980; Elinson and Rowning, 1988). Therefore, it is postulated that this rearrangement of cytoplasm localizes or activates cytoplasmic determinants to the future dorsal side of the embryo (Elinson and Kao, 1989; Gerhart et al., 1989; Danilchik and Denegre, 1991). Several hours after cortical rotation is completed, dorsal vegetal blastomeres induce overlying marginal cells to form dorsal mesoderm (Nieuwkoop, 1973; Gimlich and Gerhart, 1984; Dale and Slack, 1987b), probably through the action of secreted growth factors (Green et al., 1990). Finally, during gastrulation dorsal mesodermal cells that comprise Spemann's organizer involute and induce the overlying ectoderm to form the neural plate. Although several peptide growth factors have been shown to induce mesoderm from undifferentiated ectoderm (Slack et al., 1987; Rosa et al., 1988; Paterno et al., 1989; Asashima et al., 1991; Green et al., 1990; Thomsen et al., 1990; Sokol et al., 1991), developmentally relevant molecules that initiate the process of dorsal axis formation remain unknown.

In *Drosophila*, localized maternal morphogens have been shown to be responsible for determining the anterior-posterior and dorsal-ventral axes (reviewed by Nusslein-Volhard, 1991). Similar localized maternal messages or proteins in the amphibian embryo have been difficult to identify without mutants. However, dorsal-ventral asymmetries in cytoplasm arise after fertilization: a yolk-free, RNA-rich cytoplasm from the germinal vesicle consolidates in the dorsal animal hemisphere (Herkovits and Ubbels, 1979; Imoh, 1984) and the rearrangement during cortical rotation produces a "swirl" of deep and cortical yolk platelets that is unique to the dorsal side of the embryo (Danilchik and Denegre, 1991). These studies suggest that the cytoplasm on the dorsal side of the fertilized embryo is different from that on the ventral side, but its unique constituents are not known. Although there is evidence for differentially localized mRNAs in the animal-vegetal axis of

the oocyte (Ressom and Dixon, 1988; King and Barklis, 1985) and embryo (Carpenter and Klein, 1980; Capco and Jeffrey, 1981; Rebagliatti et al., 1985), no localized dorsal-specific messages have yet been reported.

Several studies suggest that important dorsal determinant molecules may be present in dorsal midline blastomeres of the cleavage stage embryo. (1) When 16- and 32-cell dorsal midline blastomeres are transplanted ventrally, their descendants either incorporate into the host dorsal axis (Gallagher et al., 1991) or they induce and contribute to a secondary dorsal axis (Gimlich, 1986; Kageura and Yamana, 1986; Kaguera, 1990; Takasaki and Konishi, 1989; Gallagher et al., 1991). (2) Equatorial and vegetal dorsal blastomeres can restore the dorsal axis in UV-irradiated embryos (Gimlich and Gerhart, 1984; Gimlich, 1986). (3) Dorsal midline blastomeres autonomously differentiate into dorsal axial tissues when cultured as explants (Gallagher et al., 1991). (4) Cytoplasm from a dorsal vegetal blastomere can induce a secondary axis when injected into a ventral vegetal blastomere (Yuge et al., 1990). Thus, it has been suggested that dorsal cleavage stage blastomeres contain informational molecules, which are maternally synthesized and activated at about the time of fertilization, whose function is to instruct recipient blastomeres to manifest a dorsal axial fate (Elinson and Kao, 1989; Wakahara, 1989).

In search of molecules with the function of instructing cells to form the dorsal axis, we developed a sensitive functional assay in the *Drosophila* tradition (Nusslein-Volhard et al., 1987), namely by transferring cytoplasm or RNA from specific regions of the wild-type embryo into ectopic regions in wild-type and mutant embryos, and assaying for phenotypic changes. We transferred cytoplasm and RNA from 16-cell-stage dorsal midline blastomeres, which normally give rise to the dorsal axis (Moody, 1987a), into ventral midline blastomeres, which normally do not contribute to these structures. We combined our injected material with a lineage tracer so as to identify any subtle changes in fate. This approach has allowed us to demonstrate that RNA from the dorsal, animal midline cell is a potent source of dorsal-axis-forming activity.

Materials and methods

Embryos were obtained from natural matings of adult *Xenopus laevis*. Embryos were dejellied and selected at the 2-cell stage as previously described (Klein, 1987; Moody, 1987a) to identify reliably dorsal from ventral midline blastomeres. At the 16-cell stage, only embryos with stereotypic radial cleavage patterns were used (as described in detail in Moody, 1987a; Gallagher et al., 1991) in order to study consistently the same "packet" of cytoplasm in different experiments. The nomenclature used for the 16-cell blastomeres is that of Hirose and Jacobson (1979; Fig. 1). [For comparison to the nomenclature for 32-cell embryos established by Nakamura and Kishiyama (1971), D1.1 is the mother cell of A1 and A2; D2.1 is the mother cell of A3 and A4; V1.1 is the mother cell of D1 and D2; V2.1 is the mother cell of D3 and D4.]

Lineage labeling

To identify the clones of dorsal blastomeres during gastrulation, blastomeres D1.1 or D2.1 (Fig. 1) were injected with a lineage dye. In some cases, one cell was injected with 1 nl of 5% aqueous horseradish peroxidase (HRP, Boerhinger-Mannheim; Moody, 1987a). These embryos were fixed in a solution of 1% paraformaldehyde, 4% glutaraldehyde, 0.5% dimethylsulfoxide in 0.1 M phosphate buffer at stages 9-14 (Nieuwkoop and Faber, 1967), cut in half in the sagittal plane and histochemically reacted to display the HRP-labeled clones. The reaction medium consisted of 0.1% diaminobenzidine and 0.0001% hydrogen peroxide. In other cases, both cells were labeled; D1.1 was injected with 0.5% fluorescein-dextran-amine (FDA), and D2.1 was injected with 0.5% Texas Red-dextran-amine (TRDA; Molecular Probes). These embroyos were fixed between stages 8-12 in 4% paraformaldehyde, sectioned with a cryostat and tissue sections coverslipped with glycerol. At least three embryos from each stage were analyzed (n=57).

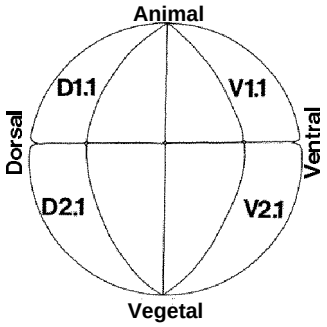

Fig. 1. The location and nomenclature of the 16-cell blastomeres used in this study. Nomenclature is that of Hirose and Jacobson (1979).

Injection of cytoplasm

To test whether the cytoplasm of the dorsal blastomeres contains a moiety that can alter the fate of ventral blastomeres, a soluble fraction of cytoplasm was prepared. Ten of each blastomere (either D1.1, D2.1, V1.1, or V2.1; Fig. 1) were dissected away from the rest of the embryo in 50% Steinberg's solution, collected in a microcentrifuge tube, and homogenized in 10-15 µl of 100% Steinberg's solution containing a final concentration of 1% HRP. The mixture was centrifuged at 4ºC for 10 minutes at 12,000 revs/minute. The blastomere homogenate separated into a top lipid layer, a middle cytosolic fraction and a pellet of membranes, organelles and nuclei. The cytosolic fraction was back loaded into a glass micropipette (5-10 µm tip diameter), and 10 nl of the solution was injected into one (unilateral) or two (bilateral) host blastomeres. Previous fate maps were made with 1-2 nl injections of 5% HRP (Moody, 1987a). We increased the volume of cytosolic injection to 10 nl in order to concentrate any putative cytoplasmic factors. As a control for this increased volume (but not increased HRP concentration), we also injected ventral blastomeres with 10 nl of 1% aqueous HRP.

RNA injections

Total RNA was isolated from each of the dorsal or ventral midline blastomeres (Fig. 1) according to the methods of Sambrook et al. (1989). Hundreds of each blastomere were homogenized in Buffer A with 0.1 volume Proteinase K (25 mg/ml; Boehringer-Mannheim), extracted with phenol-chloroform, and the aqueous phase precipitated in 2.5 volumes of 100% ethanol and 0.1 volume of 3 M sodium acetate (pH 5.2). The remaining yolk proteins were removed from the RNA by a 4 M LiCl precipitation. The RNA was visualized and quantitated by RNAase protection assays, using a probe for *Xenopus* α5 integrin (C. Whittaker and D. DeSimone, personal communication) and a probe for *Xenopus* elongation factor 1α (EF-1α; Kreig et al., 1989) to ensure that the

RNA in our injection material was not degraded significantly. From the OD_{260} (optical density) measurement of one extraction, we calculated the amount of total RNA recovered per blastomere. This figure was used to estimate the amount of RNA injected in all subsequent extractions based on the number of blastomeres homogenized and the volume of material injected. Injections of 3-4 ng of transfer RNA and RNA transcribed from a 2.2 kb fragment of a *Xenopus* fibronectin cDNA (DeSimone et al., 1992) were used as controls. The RNAs were mixed with aqueous HRP (final concentration was 1%), and 1.5-4 nl (depending upon the number of blastomeres used for the RNA extraction) were injected into the ventral midline blastomeres (V1.1 and V2.1; Fig. 1). These low volume injections do not, of themselves, alter the fate of recipient animal or vegetal blastomeres of the 16-cell-stage embryo.

Analysis of developmental fate

Embryos were allowed to develop to late tailbud stages (32-40; Nieuwkoop and Faber, 1967). They were fixed as described above and sectioned at 20 μm with a cryostat. Tissue was histochemically prepared for the visualization of HRP-labeled cells as described above. Serial sections were viewed at 400× and the locations of all labeled cells were mapped as described in detail elsewhere (Moody, 1987a). All tissue sections were analyzed so that subtle changes in fate could be detected.

UV irradiation

The procedure used for ultraviolet (UV) irradiation was modeled after that used by Scharf and Gerhart (1980). Artificially fertilized eggs were dejellied 20-30 minutes after the addition of sperm and their vegetal poles were exposed to UV light (254 nm; Mineral Lamp no. UVG-11, UVP Inc., San Gabriel, CA) for 30 seconds in the middle of their cleavage cycle (45 minutes post-fertilization). This procedure resulted in a high survival rate, but 80% of the surviving embryos developed without a dorsal axis (UV=5 according to Scharf and Gerhart, 1980; DAI=0 according to Kao and Elinson, 1988).

Results

The normal site of dorsal axial determinants

Fate maps of cleavage-stage embryos have shown that most of the dorsal axial tissues of the tailbud embryo, i.e., notochord, perinotochordal somite and central nervous system, arise from dorsal midline blastomeres (Cooke and Webber, 1985; Dale and Slack, 1987a; Moody, 1987a,b). A recent review has proposed that putative dorsal determinants lie mostly in the vegetal half of the dorsal midline at cleavage stages (see Fig. 3 in Elinson and Kao, 1989). However, we find that during gastrulation the clone of the dorsal *animal* cell (D1.1) is the major occupant of the region known as Spemann's organizer, the dorsal lip of the blastopore. In the late blastula, part of the D1.1 clone has moved vegetally to the equatorial region (Fig. 2A). At early stage 10, when the site of mesoderm involution is just visible, the D1.1 clone has extended vegetally to the dorsal lip (Fig. 2B). At the site of involution, D2.1 descendants are sparsely intermixed with those of the animal blastomere, but most of the D2.1 clone is more vegetal and deep (Fig. 2C). At stage 11, the D1.1 clone constitutes the majority of involuting mesodermal cells and of the overlying ectoderm destined to become nervous system (Fig. 2D), whereas D2.1 contributes many fewer cells to the involuting mesoderm (Fig. 2E). The D1.1 clone in the stage 13 embryo extends throughout the rostral-caudal extent of the dorsal axis, including neural ectoderm and notochordal tissues (Fig. 2F). The D2.1 clone at this stage occupies some of the dorsal axial mesoderm, but very little of the neural ectoderm (not shown), as is expected from the published fate maps of tailbud embryos. These results suggest that the *animal* dorsal blastomere is the most likely site for dorsal axial determinants, since its descendants are the major constituents of Spemann's organizer.

Cytoplasmic transfers

In *Drosophila*, the existence of axial determinants was first identified by transferring cytoplasm from one pole of the embryo to another, and observing whether cell fates changed (Nusslein-Volhard et al., 1987). We repeated this approach in *Xenopus* by transferring wild-type cytoplasm from dorsal midline blastomeres (D1.1 and D2.1; Fig. 1), which normally produce large amounts of dorsal axial tissues (e. g., CNS, notochord, head somites and pharynx; Moody, 1987a), to ventral midline blastomeres (V1.1 and V2.1; Fig. 1), which normally do not contribute to these dorsal structures. We were not able to transfer whole cytoplasm plus lineage dye into ventral blastomeres because the viscosity of the cytoplasm clogged our ultrathin pipettes (5-10 μm). For this reason, we fractionated blastomeres, combined their cytosolic material with 1% HRP and injected this mixture into ventral blastomeres to map the fate of their progeny. Control injections included lineage dye only and cytosol from the same blastomere as the one injected. Control injections at volumes of 20-30 nl resulted in irregular and asynchronous cleavages, dead progeny, abnormal gastrulation movements and defective embryos. Control injections at a volume of 10 nl caused no fate changes in V2.1, but caused blastomere V1.1 to populate midbrain (7.6% of 13 embryos), retina (38.4%) and notochord (15.3%), tissues that are never labeled when V1.1 is injected with only 1-4 nl of lineage tracers (Moody, 1987a). Also, the percentage of embryos that had labeled cells in the forebrain increased from 10% to 31%. These results caution that the volume injected into a single blastomere must be adequately controlled since changing the volume of a cell can significantly, but non-specifically, change its fate.

Nonetheless, some small changes in fate ($P<0.1$, χ^2 test) were observed when ventral midline cells were injected with 10 nl of cytosol from dorsal midline blastomeres. Cytosol from D1.1 caused V1.1 ($n=52$) to contribute more often to forebrain (44.2% versus 30.7%) and hindbrain (17.3% versus 0), and V2.1 ($n=72$) to contribute more often to branchial arch mesoderm (34.7% versus 3.7%) and notochord (19.7% versus 0). Cytosol from D2.1 caused V1.1 ($n=23$) to contribute more often to hindbrain (13% versus 0), but caused no changes in V2.1 ($n=37$). This last result is in contrast to the dramatic secondary axes reported by Yuge et al. (1990). Perhaps this difference is because Yuge et al. (1990) directly transferred 20-30 nl of whole cytoplasm into the V2.1 cell. This swift large volume injection

may have allowed them to transfer an appropriate threshold of critical molecules, which were diluted or lost during our fractionation procedure.

RNA transfers

The cytoplasmic transfers suggest that D1.1 contains a low level of a cytoplasmic factor that can change the fate of ventral blastomeres to a more dorsal type, but because large volume control injections also could cause fate changes, it was not possible to inject more of this factor to elicit a more dramatic fate change. To concentrate the activity and to identify whether it was due to localized RNAs, the four midline blastomeres were dissected separately and total RNA was extracted from each of them. RNAase protection assays using a probe for *Xenopus* α5 integrin and for EF-1α demonstrated that the isolation procedures did not result in substantial degradation of the RNA (data not shown). In those cases in which RNA from D1.1 was injected into V2.1, a secondary axis frequently was induced (Fig. 3). These axes always included posterior structures and occasionally (9.5% of cases) included head structures. Tissue sections from these embryos show that progeny from the injected V2.1 blastomere populated the CNS, notochord, branchial arches, somitic muscle and foregut roof in the secondary axis (Fig. 4). Labeled V2.1 progeny rarely (6% of cases) were found in the host CNS or notochord. The induction of a secondary axis derived from V2.1 was dependent upon the concentration of D1.1 RNA that was injected (Table 1), and was specific for D1.1 RNA; it was not elicited by injection of RNA from blastomeres D2.1, V2.1 or V1.1, or by the injection of transfer RNA or *Xenopus* fibronectin mRNA (Table 1). Interestingly, secondary axes never were detected when the same D1.1 RNA preparation was injected into V1.1.

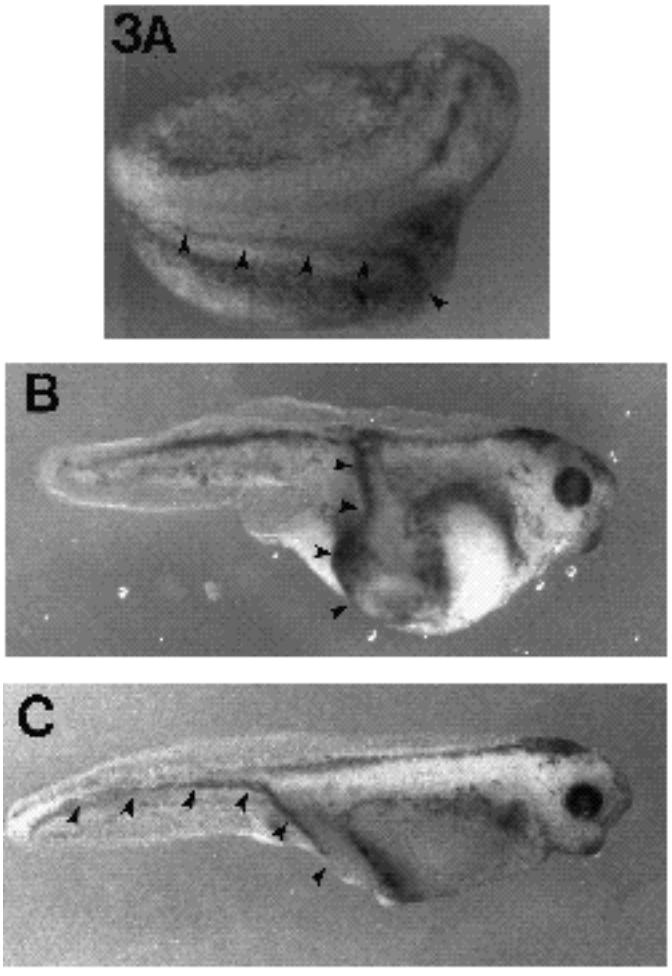

Fig. 3. Examples of embryos that developed a secondary dorsal axis after being injected at the 16-cell-stage with about 4 ng total RNA from blastomere D1.1. (A) Dorsal view of a stage 20 neurula. The primary neural tube is to the top and the secondary neural tube is marked with arrowheads. (B) Side view of a stage 40 embryo with a large secondary axis (arrowheads) that diverges ventrally at the gut and ends in a swelling that contained brain structures. (C) Side view of a stage 40 embryo with a smaller secondary axis (arrowheads).

Rescue of UV-irradiated eggs

UV irradiation of the fertilized egg prior to the first cleavage furrow prevents rotation of the cortical cytoplasm and results in embryos without a dorsal axis (Scharf and Gerhart, 1980; Kao and Elinson, 1988; Fig. 5A). After UV irradiation alone (Table 2) or followed by injection with 10 nl of water (data not shown), over 80% of the embryos developed without a dorsal axis. When UV-treated eggs were injected late in the first cleavage cycle (60-70 minutes post-fertilization) with RNA isolated from D1.1 85% developed some dorsal axial structures (Table 2). About half of these developed partial axes, which consisted of a normal trunk and tail region and occasional anterior bulges, and about half developed a complete dorsal axis, which consisted of

Table 1. *Percentage of embryos having a secondary axis after injection of total RNA*

Donor RNA					
	Amount injected	Host V2.1		Host V1.1	
Source	(ng)	(*n*)	2° Axis	(*n*)	2° Axis
D1.1	0.08-0.8				
	unilateral	(6)	0	(10)	0
	bilateral	(7)	0	(13)	0
	2.8				
	unilateral	(19)	10.5	(4)	0
	bilateral	(20)	35.0	(16)	0
	3.6-4.8				
	unilateral	(21)	47.6	(9)	0
	bilateral	(21)	52.4	(10)	0
D2.1	3.6-4.8				
	unilateral	(13)	0	(14)	0
	bilateral	(16)	0	(14)	0
V2.1	3.6-4.8				
	unilateral	(12)	0	(9)	0
	bilateral	(17)	0	(15)	0
V1.1	3.6-4.8				
	unilateral	(17)	0	(14)	0
	bilateral	(14)	0	(15)	0
transfer RNA	3.6-4.8				
	unilateral	(10)	0	(10)	0
	bilateral	(10)	0	(10)	0
fibronectin RNA	3.6-4.8				
	unilateral	(7)	0	(9)	0
	bilateral	(9)	0	(10)	0

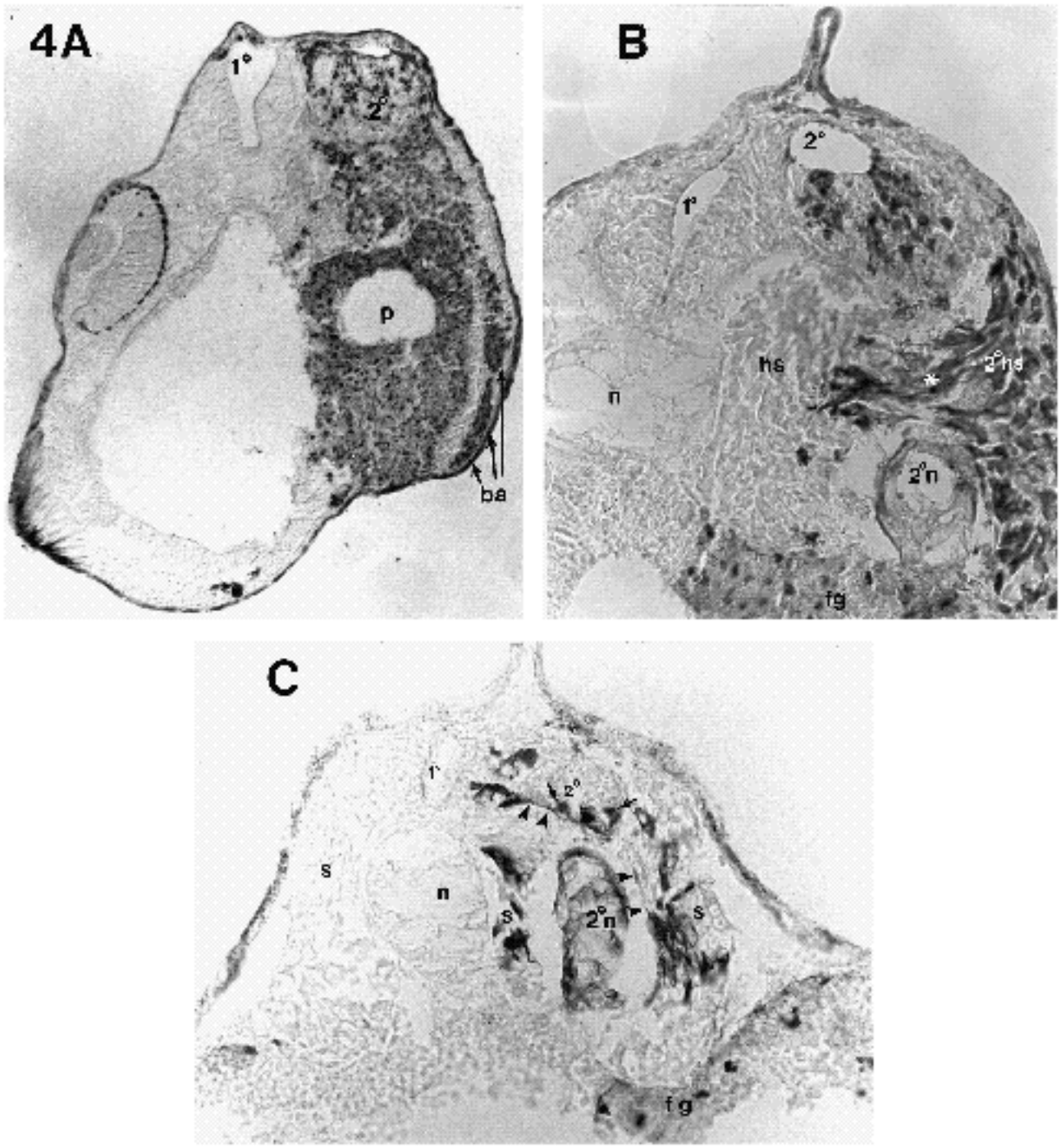

Fig. 4. Transverse sections of stage 36 embryos that developed a secondary dorsal axis after both V2.1 blastomeres were injected with about 4 ng total RNA (plus HRP) from blastomere D1.1. The majority of structures in the secondary axis are populated by progeny from the injected V2.1 blastomere, as indicated by the lineage dye; unlabeled cells are progeny of other blastomeres. (A) At the level of the midbrain, no labeled cells are in the primary CNS (1°), but they significantly contribute to the secondary CNS (2°). Note also that pharynx (p) is populated mainly by labeled cells and branchial arches (ba) are populated by both labeled and unlabeled cells. (B) At the level of the spinomedullary junction, many labeled cells are in the secondary CNS (2°), secondary head somite (2°hs), secondary notochord (2°n) and roof of the foregut (fg). These all are structures associated with the dorsal axis. Note the somitic tissue (*) between the secondary notochord and secondary CNS. (C) At the level of the caudal trunk, the secondary spinal cord contains motoneurons (small arrows) whose axons (arrowheads) project to clonally related somitic muscle cells. The secondary notochord is populated only by labeled cells, whereas the somitic muscle (s) in the secondary axis and in the shared somite is populated by both labeled and unlabeled cells. The roof of the foregut (fg) contains numerous labeled cells.

Table 2. *Percentage of embryos with dorsal axes after UV irradiation*

	Status of dorsal axis		
	None	Partial*	Complete†
UV irradiation alone	80.2	13.5	6.3
UV plus D1.1 RNA	14.6	46.3	39.1

*Partial dorsal axis is defined as a normal trunk and tail with either no head or only a few bulges in the cephalic region (see bottom right specimens in Fig. 5B).

†Complete dorsal axis is defined as a complete head with at least one retina and a normal or a shortened torso (see upper left specimens in Fig. 5B).

a trunk and a head with at least one retina (Table 2; Fig. 5B).

Discussion

Recently it was shown that the D1.1 blastomere is important for normal development of the dorsal axis. It can autonomously differentiate when cultured as an explant, and induce a secondary axis when transplanted to the ventral vegetal midline of a host embryo (Gallagher et al., 1991), suggesting that this blastomere contains intrinsic information regarding its dorsal fate, perhaps in the form of maternal dorsal determinants. The present study shows that the progeny of D1.1 populate the dorsal lip of the blastopore and are the first to involute during gastrulation; thus, descendants of D1.1 constitute Spemann's organizer. A cytosolic fraction from D1.1 causes some progeny of the ventral blastomeres to populate dorsal axial structures, and RNAs isolated from D1.1 induce a secondary dorsal axis and rescue UV-irradiated embryos. These results demonstrate that maternal RNA molecules contained within a specific dorsal animal blastomere are important for the establishment of the dorsal axis in *Xenopus*, and support the hypothesis that maternal dorsal determinants are located in the animal hemisphere of the early embryo (Sokol and Melton, 1991).

Proposed site for presumptive dorsal determinants

Molecular differences between the dorsal and ventral half of the *Xenopus* embryo have been difficult to demonstrate, even though dorsal and ventral blastomeres clearly differentiate into different structures both *in vivo* and *in vitro*. With regards to proteins, the dorsal and ventral cells have been shown to synthesize a different repertoire of proteins during cleavage stages (Miyata et al., 1987; Klein and King, 1988) and during gastrulation (Smith and Knowland, 1984). Further, there is immunohistochemical evidence that a 70×10^3 M_r maternal protein is enriched on the dorsal side at cleavage stages (Suzuki et al., 1991). However, none of these proteins has been identified and, although the differential distribution of protein in the early embryo probably is due to localization of translatable maternal mRNA, there are no reports yet of maternal mRNAs localized along the dorsal-ventral axis. Several studies have identified differentially localized mRNAs in the animal-vegetal axis (Carpenter and Klein, 1980; Capco and Jeffrey, 1981; King and Barklis, 1985; Phillips, 1985; Rebagliatti et al., 1985; Ressom and Dixon, 1988), but our study provides the first evidence for maternal RNAs that might be dorsally localized at early cleavage stages.

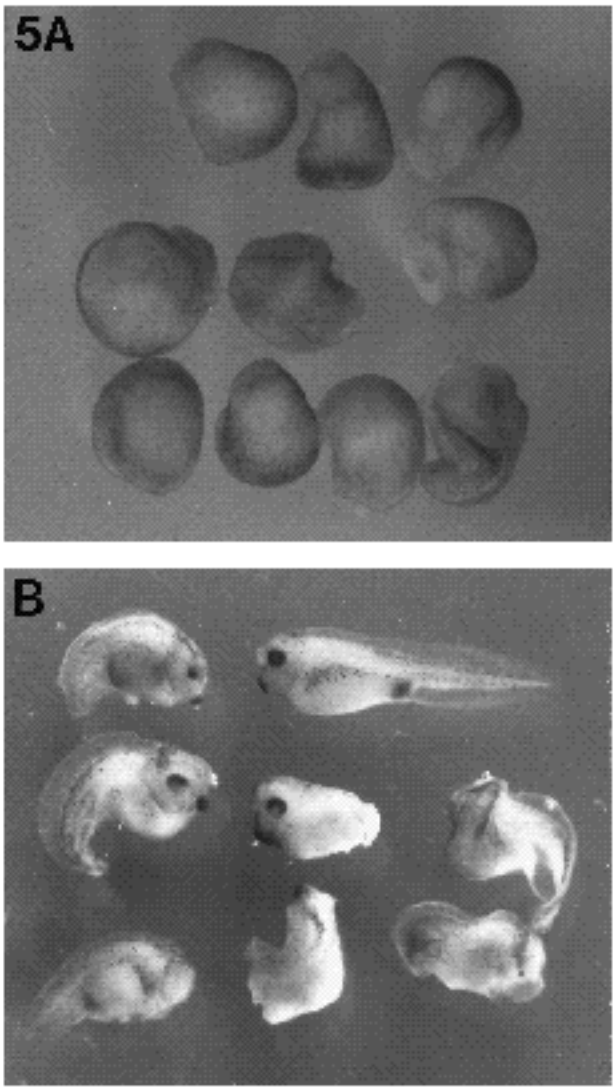

Fig. 5. Ultraviolet irradiation of embryos during the first cell cycle results in embryos without a dorsal axis. (A) With our protocol for irradiation, 80% of the embryos had morphologies as illustrated in this photomicrograph. The remaining 20% were normal in appearance. (B) The dorsal axis is restored in UV-irradiated embryos after injection of D1.1 RNA during the first cleavage cycle. Some embryos (four in the upper left) are completely rescued, as demonstrated by head structures including at least one retina. Some embryos (four lower right embryos) are partially rescued, as demonstrated by dorsal axial trunk and tail structures.

Although protein or RNA determinants of the dorsal axis have not yet been identified, functional assays have demonstrated dorsal-forming activity in both dorsal animal and dorsal vegetal blastomeres of the early embryo. The evidence that indicates that the dorsal vegetal hemisphere contains these determinants includes the following facts. (1) The cortical rotation that occurs after fertilization causes a

Table 3. *Comparison of D1.1 RNA dorsalizing activity to that of zygotic mRNAs*

(A) Secondary axis formation after RNA injection into ventral vegetal blastomere

RNA injected	% secondary axis
Activinβ_B*	35
Xwnt-8*	86
Goosecoid†	75
D1.1	50

(B) UV-rescue by RNA injection

RNA injected	% dorsal axis (complete and partial)
Activinβ_B*	22
Mutated activin*	16
Xwnt-8‡	95
D1.1	85

*Data from Sokol et al. (1991).
†Data from Cho et al. (1991).
‡Data from Smith and Harland (1991).

net displacement of cytoplasm into the dorsal vegetal quadrant (Gerhart et al., 1989; Danilchik and Denegre, 1991). (2) When cytoplasm is removed from the dorsal vegetal midline, embryos develop with deficient dorsal axes (Wakahara, 1986). (3) Cytoplasm removed from D2.1 and injected into V2.1 induces a secondary axis in 37% of the cases (Yuge et al., 1990). (4) 16- and 32-cell dorsal vegetal blastomeres induce dorsal mesoderm in animal cap explants (Jones and Woodland, 1987; Dale and Slack, 1987b). (5) Transplanting the 32-cell dorsal midline vegetal blastomeres (either bilateral tier-3 or tier-4) into their equivalent ventral midline tier causes a secondary axis to form in almost 100% of the cases (Kageura, 1990). (6) 32-cell dorsal midline vegetal blastomeres (tier-3 plus tier-4) rescue UV-irradiated embryos (Gimlich and Gerhart, 1984; Gimlich, 1986). These results and the fact that the dorsal lip of the blastopore develops in the dorsal vegetal hemisphere suggest that this region is the most likely site of dorsal determinants.

Other studies, however, suggest that presumptive dorsal determinants are located in the dorsal animal hemisphere as well. (1) Cytoplasmic rearrangements that occur on the dorsal half of the embryo during the first cell cycle bring core yolk platelets next to cortical yolk platelets so that both types of platelets are contained in dorsal blastomeres (Danilchik and Denegre, 1991). In addition, a unique yolk-free cytoplasm (Herkovits and Ubbels, 1979), which is rich in RNA (Imoh, 1984), is located in the dorsal animal hemisphere. This cytoplasm is similar in appearance to the specialized cytoplasm that coalesces in the vegetal pole and contains germ cell lineage determinants (reviewed by Ressom and Dixon, 1988). (2) D1.1, rather than its vegetal neighbor, is the major progenitor of dorsal axial tissues in the embryo (Moody, 1987a). (3) D1.1 and its animal cap daughter induce a secondary axis when transplanted into the ventral vegetal midline, and autonomously differentiate into dorsal axial structures when cultured as explants (Gallagher et al., 1991). (4) The dorsal half of a stage 8 animal cap is predisposed to differentiate into dorsal structures (Sokol and Melton, 1991). The present study additionally demonstrates that D1.1, and not D2.1, is the major progenitor of Spemann's organizer, and that RNA isolated from D1.1, and not D2.1, induces a secondary axis in normal embryos and rescues the dorsal axis in UV-irradiated embryos. Therefore, important maternal molecules that can initiate dorsal axis formation also are located in the dorsal animal hemisphere.

What are the likely candidate molecules for dorsal determinants?

Recently, several RNA molecules have been shown to induce a secondary body axis when injected into ventral blastomeres (Table 3). Activinβ_B mRNA can induce a partial secondary axis (Thomsen et al., 1990), but cannot rescue UV-irradiated mutants (Sokol et al., 1991). Xwnt-8 mRNA is a more potent dorsalizing molecule than activin mRNA because it induces a complete secondary axis and effectively restores the dorsal axis in UV-irradiated embryos (Table 3; Smith and Harland, 1991; Sokol et al., 1991). Goosecoid, a dorsal-lip-specific mRNA with a homeodomain region (Blumberg et al., 1991), also induces a secondary axis when injected into host embryos (Table 3; Cho et al., 1991). However, none of these mRNAs is detectable until the onset of zygotic transcription (Thomsen et al., 1990; Blumberg et al., 1991; Christian et al., 1991a), and therefore cannot be involved in the initial events of dorsal axis formation. More likely, they interact with molecules downstream of the maternal determinants that initiate this process.

The RNAs isolated from D1.1 in our experiments induce a secondary dorsal axis when injected into a ventral vegetal blastomere at a potency about midway between that of activin β_B and goosecoid (Table 3). D1.1 RNAs also rescue UV-irradiated mutants with an activity nearly as great as that of Xwnt-8 (Table 3). However, D1.1 RNAs are maternal in source, suggesting that they are likely to be molecules involved in the first steps of forming the dorsal axis. Although our study does not identify the specific RNA molecule, it is possible that a maternal member of the gene families mentioned above is involved. It is interesting that D1.1 RNA induced a secondary axis only when injected into V2.1; no secondary axes formed when the same RNA was injected into the ventral *animal* blastomere (V1.1). Similarly, Xwnt-8 RNA does not induce a secondary axis when injected into a ventral *animal* blastomere (Christian et al., 1991b; Sokol et al., 1991). These results suggest that the instructive molecules cannot function in the animal hemisphere, either because their target sites are not present, their action is inhibited by some other moiety or they must be present in a higher concentration than was delivered. A correlation to this phenomenon is found in blastomere transplantation studies: the D1.1.1 blastomere (also called A1) induces a secondary axis in 67% of cases when it is transplanted into the ventral *vegetal* midline (Gallagher et al., 1991), but it only does so in 48% of the cases when transplanted into the ventral *animal* midline (Kageura, 1990). These results suggest that the ventral vegetal quadrant provides a more permissive environment for dorsal blastomeres, or their active molecules, to manifest their dorsal developmental program.

In summary, the concept that the initial steps of dorsal axis formation are controlled by localized maternal deter-

minants has been demonstrated in *Drosophila* (Nusslein-Volhard, 1991) and often proposed in *Xenopus* (Elinson and Kao, 1989; Wakahara, 1989). Activinβ_B, goosecoid and Xwnt-8 RNAs can ectopically induce a dorsal axis in *Xenopus*, but none is a maternal transcript. The present study demonstrates that there is a similar activity located in a specific dorsal blastomere of the early cleavage stage embryo, and that the activity is due to RNA. Perhaps the determinant(s) are maternal members of those gene families, or are some as yet unidentified RNA. The identification of the specific determinant(s) contained in D1.1 is our next step in understanding the pathway for this critical developmental event.

We are grateful for the helpful discussions with Dr Robert Grainger, the generous gifts of probes and advice from Dr Douglas DeSimone, and the assistance of R. Ward Morgan and Charles Whittaker. This research was supported by NIH grant NS23158.

References

Asashima, M., Nakano, H., Sugino, H., Nakamura, T., Eto, Y., Ejima, D., Nishimatsu, S., Ueno, N., and Kinoshita, K. (1991). Presence of activin (erythoid differentiation factor) in unfertilized eggs and blastulae of *Xenopus laevis*. *Proc. Natl. Acad. Sci. USA* **88**, 6511-6514.

Blumberg, B., Wright, C. V. E., De Robertis, E. M., and Cho, K. W. Y. (1991). Organizer-specific homeobox genes in *Xenopus laevis* embryos. *Science* **253**, 194-196.

Capco, D. G. and Jeffrey, W. R. (1981). Regional accumulation of vegetal pole poly $(A)^+$ RNA injected into fertilized *Xenopus* eggs. *Nature* **294**, 255-257.

Carpenter, C. D. and Klein, W. H. (1980). A gradient of poly $(A)^+$ RNA sequences in *Xenopus laevis* eggs and embryos. *Dev. Biol.* **91**, 43-49.

Cho, K. W. Y., Blumberg, B., Steinbelsser, H., and De Robertis, E. M. (1991). Molecular nature of Spemann's organizer: the role of the *Xenopus* homeobox gene goosecoid. *Cell* **67**, 1111-1120.

Christian, J. L., Gavin, B. J., McMahan, J. A. and Moon, R. T. (1991a). Isolation of a cDNA partially encoding four *Xenopus* Wnt-1/Int-1 related proteins and characterization of their transient expression during development *Dev. Biol.* **143**, 230-234.

Christian, J. L., McMahon, J. A., McMahon, A. P. and Moon, R. T. (1991b). Xwnt-8, a Xenopus Wnt-1/int-1-related gene responsive to mesoderm-inducing growth factors, may play a role in ventral mesoderm patterning during embryogenesis. *Development* **111**, 1045-1055.

Cooke, J. and Webber, J. A. (1985). Dynamics of the control of body pattern in the development of *Xenopus laevis. J. Embryol. Exp. Morph.* **88**, 85-112.

Dale, L. and Slack, J. M. W. (1987a). Fate map for the 32-cell stage of *Xenopus laevis. Development* **99**, 527-551.

Dale, L. and Slack, J. M. W. (1987b). Regional specification within the mesoderm of early embryos of *Xenopus laevis. Development* **100**, 279-295.

Danilchik, M. V., and Denegre, J. M. (1991). Deep cytoplasmic rearrangements during early development in *Xenopus laevis. Development* **111**, 845-856.

DeSimone, D. W., Norton, P. A. and Hynes, R. O. (1992). Identification and characterization of alternately spliced fibronectin mRNAs expressed in early *Xenopus* embryos. *Dev. Biol.* **149**, 357-369.

Elinson, R. P. and Kao, K. R. (1989). The location of dorsal information in frog early development. *Dev. Growth Differ.* **31**, 423-430.

Elinson, R. P. and Rowning, B. (1988). A transient array of parallel microtubules in frog eggs: potential tracks for a cytoplasmic rotation that specifies the dorso-ventral axis. *Dev. Biol.* **128**, 185-197.

Gallagher, B. C., Hainski, A. M. and Moody, S. A. (1991). Autonomous differentiation of dorsal axial structures from an animal cap cleavage stage blastomere in *Xenopus. Development* **112**, 1103-1114.

Gimlich, R. L. and Gerhart, J. C. (1984). Early cellular interactions promote embryonic axis formation in *Xenopus laevis. Dev. Biol.* **104**, 117-130.

Gimlich, R. L. (1986). Acquisition of developmental autonomy in the equatorial region of the *Xenopus* embryo. *Dev. Biol.* **115**, 340-352.

Gerhart, J., Danilchik, M., Doniach, T., Roberts, S., Rowning, B., and Stewart, R. (1989). Cortical rotation of the *Xenopus* egg: consequences for the anteroposterior pattern of the embryonic dorsal development. *Development* **107**, Supplement, 37-51.

Green, J. B. A., Howes, G., Symes, K., Cooke, J., and Smith, J. C. (1990). The biological effects of XTC-MIF: quantitative comparison with *Xenopus* bFGF. *Development* **108**, 173-183.

Herkovits, J. and Ubbels, G. A. (1979). The ultrastructure of the dorsal yolk-free cytoplasm and the immediately surrounding cytoplasm in the symmetrized egg of *Xenopus laevis. J. Embryol. Exp. Morph.* **51**, 155-164.

Hirose, G. and Jacobson, M. (1979). Clonal organization of the central nervous system of the frog. I. Clones stemming from individual blastomeres of the 16-cell and earlier stages. *Dev. Biol.* **71**, 191-202.

Imoh, H. (1984). Appearance and distribution of RNA-rich cytoplasms in the embryo of *Xenopus laevis* during early development. *Dev. Growth Differ.* **26**, 167-176.

Jones, E. A. and Woodland, H. R. (1987). The development of animal cap cells in *Xenopus*: a measure of the start of animal cap competence to form mesoderm. *Development* **101**, 557-563.

Kao, K. R., and Elinson, R. P. (1988). The entire mesodermal mantle behaves as Spemann's organizer in dorsoanterior enhanced *Xenopus laevis* embryos. *Dev. Biol.* **127**, 64-77.

Kageura, H. (1990). Spatial distribution of the capacity to initiate a secondary embryo in the 32-cell stage embryo of *Xenopus laevis. Dev. Biol.* **142**, 432-438.

Kaguera, H. and Yamana, K. (1986). Pattern formation in 8-cell composite embryos of *Xenopus laevis. J. Embryol. Exp. Morph.* **91**, 79-100.

King, M. L. and Barklis, E. (1985). Regional distribution of maternal messenger RNA in the amphibian oocyte. *Dev. Biol.* **112**, 203-212.

Klein, S. L. (1987). The first cleavage furrow demarcates the dorsal-ventral axis in *Xenopus* embryos. *Dev. Biol.* **120**, 299-304.

Klein, S. L. and King, M. L. (1988) Correlations between cell fate and the distribution of proteins that are synthesized before the midblastula transition in *Xenopus. Roux's Arch. Dev. Biol.* **197**, 275-281.

Kreig, P. A., Varnum, S. M., Wormington, W. M. and Melton, D. A. (1989). The mRNA encoding elongation factor 1-α (EF-1α) is a major transcript at the midblastula transition in *Xenopus. Dev. Biol.* **133**, 93-100.

Miyata, S., Kageura, H., and Kihara, H. K. (1987). Regional differences of proteins in isolated cells of early embryos of *Xenopus laevis. Cell Differ.* **21**, 47-52.

Moody, S. A. (1987a). Fates of the blastomeres of the 16-cell stage *Xenopus* embryo. *Dev. Biol.* **119**, 560-578.

Moody, S. A.(1987b). Fates of the blastomeres of the 32-cell stage *Xenopus* embryo. *Dev. Biol.* **122**, 300-319.

Nakamura, O. and Kishiyama, K. (1971). Prospective fates of blastomeres at the 32-cell stage of *Xenopus laevis* embryos. *Proc. Japan Acad.* **47**, 407-412.

Nieuwkoop, P. D. (1973). The "organization center" of the amphibian embryo: its spatial organization and morphogenic action. *Adv. Morphogen.* **10**, 1-39.

Nieuwkoop, P. D. and Faber, J. (1967). *Normal Table of* Xenopus laevis (*Daudin*). Amsterdam: North-Holland.

Nusslein-Volhard, C. (1991). Determination of the embryonic axes of *Drosophila*. *Development* Supplement **1**, 1-10.

Nusslein-Volhard, C., Frohnhofer, H. G. and Lehman, R. (1987). Determination of anteroposterior polarity in *Drosophila. Science* **238**, 1675-1681.

Paterno, G. D., Gillespie, L. L., Dixon, M. S., Slack, J. M. W., and Heath, J. K. (1989). Mesoderm-inducing properties of INT-2 and kFGF, two oncogene-encoded growth factors related to FGF. *Development* **106**, 79-83.

Phillips, C. R. (1985). Spatial changes in poly(A) concentrations during early embryogenesis in *Xenopus laevis*: analysis by *in situ* hybridization. *Dev. Biol.* **109**, 299-310.

Rebagliatti, M. R., Weeks, D. L., Harvey R. P., and Melton, D. A. (1985). Identification and cloning of localized maternal RNA's from *Xenopus* eggs. *Cell* **42**, 769-777.

Ressom, R. E. and Dixon, K. E. (1988). Relocation and reorganization of germ plasm in *Xenopus* embryos after fertilization. *Development* **103**, 507-518.

Rosa, F., Roberts, A. B., Danielpour, D., Dart, L. L., Sporn, M. B., and Dawid, I. B. (1988). Mesoderm induction in amphibians: the role of TGF-β2-like factors. *Science* **329**, 783-785.

Sambrook, J., Fritisch, E. F. and Maniatis, T. (1989). *Molecular Cloning, A Laboratory Manual*. 2nd edition. Cold Spring Harbor, NY: Cold Spring Harbor Press.

Scharf, S. R. and Gerhart, J. C. (1980). Determination of the dorsal-ventral axis in eggs of *Xenopus laevis:* complete rescue of uv-impaired eggs by oblique orientation before first cleavage. *Dev. Biol*. **79**, 181-198.

Slack, J. M. W., Darlington, B. G., Heath, J. K., and Godsave, S. F. (1987). Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors. *Nature* **326**, 197-200.

Smith, R. C. and Knowland, J. (1984). Protein synthesis in dorsal and ventral regions of *Xenopus laevis* embryos in relation to dorsal and ventral differentiation. *Dev. Biol*. **103**, 355-368.

Smith, W. C. and Harland, R. M. (1991). Injected Xwnt-8 RNA induces a complete body axis in *Xenopus* embryos. *Cell* **67**, 753-765.

Sokol, S., Christian, J. L., Moon, R. T., and Melton, D. A. (1991). Injected Wnt RNA induces a complete body axis in *Xenopus* embryos. *Cell* **67**, 741-752.

Sokol, S. and Melton, D.A. (1991). Preexistent pattern in *Xenopus* animal pole cells revealed by induction with activin. *Nature* **351**, 409-411.

Suzuki, A. S., Manabe, J., and Hirakawa, A. (1991). Dynamic distribution of region-specific protein during oogenesis and early embryogenesis of *Xenopus laevis. Roux's Arch. Dev. Biol*. **200**, 213-222.

Takasaki, H. and Konishi, H. (1989). Dorsal blastomeres in the equatorial region of the 32-cell *Xenopus* embryo autonomously produce progeny committed to the organizer. *Dev. Growth Differ*. **31**, 147-156.

Thomsen, G., Woolf, T., Whitman, M., Sokol, S., Vaugh, J., Vale, W., and Melton, D. A. (1990). Activins are expressed early in *Xenopus* embryogenesis and can induce axial mesoderm and anterior structures. *Cell* **63**, 485-493.

Yuge, M., Kobayakawa, Y., Fujisue, M., and Yamana, K. (1990). A cytoplasmic determinant for dorsal axis formation in an early embryo of *Xenopus laevis. Development* **110**, 1051-1056.

Vincent, J. -P., Oster, G. F., and Gerhart, J. C. (1986). Kinematics of grey crescent formation in *Xenopus* eggs: The displacement of subcortical cytoplasm relative to the egg surface. *Dev. Biol*. **113**, 484-500.

Vincent, J. -P. and Gerhart, J. C. (1987). Subcortical rotation in *Xenopus* eggs: an early step in embryonic axis specification. *Dev. Biol*. **123**, 526-539.

Wakahara, M. (1986). Modification of dorsal-ventral polarity in *Xenopus laevis* embryos following withdrawal of egg contents before first cleavage. *Dev. Growth Differ*. **28**, 543-554.

Wakahara, M. (1989). Specification and establishment of dorsal-ventral polarity in eggs and embryos of *Xenopus laevis. Dev. Growth Differ*. **31**, 197-207

(Accepted 26 June 1992)

dev9083 colour tip-in

Fig. 2. Blastomeres D1.1 and D2.1 were injected with lineage tracers at the 16-cell stage, and their clones were mapped during gastrulation. All micrographs are oriented with animal pole to the left and dorsal to the top (orientation marker is in panel E). (A) At stage 8.5, the D1.1 clone (yellow FDA label) has extended into the vegetal equatorial region. The limits of the embryo are highlighted with white arrowheads. b, blastocoel. (B) At early stage 10, the D1.1 clone (brown HRP label) has extended to the initial involution site of the dorsal blastoporal lip (arrow). (C) At early stage 10, the initial involution site of the dorsal blastoporal lip (arrow) is flanked by clones from both midline cells (D1.1 clone contains yellow FDA label; D2.1 clone contains red TRDA label). The D1.1 clone occupies most of the surface region that will involute. (D) At stage 11, a significant amount of the dorsal blastoporal lip (arrow) and involuting mesoderm (m) is occupied by descendants of D1.1 (brown HRP label). (E) At stage 11.5, the dorsal blastoporal lip (dl) is a mosaic of descendants from D1.1 (yellow FDA label) and D2.1 (red TRDA label), and those cells from the animal blastomere (D1.1) predominate. Large arrow points to involution site. an, animal; d, dorsal; v, ventral; veg, vegetal. (F) At stage 13, the entire length of the dorsal axis (da) is heavily populated by descendants of D1.1 (brown HRP label). a, archenteron; y, yolk plug.