*J. Embryol. exp. Morph.* **89, Supplement, 317–331 (1985)**
*Printed in Great Britain* © *The Company of Biologists Limited 1985*



# Cell lineage labels and region-specific markers in the analysis of inductive interactions

J. C. SMITH,

*Laboratory of Embryogenesis, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, U.K.*

L. DALE AND J. M. W. SLACK

*Laboratory of Experimental Morphology, Imperial Cancer Research Fund, Mill Hill Laboratories, Burtonhole Lane, London NW7 1AD, U.K.*

SUMMARY

This paper reviews work with cell lineage labels and cell-type specific markers in the analysis of inductive interactions in early amphibian development. Our results provide clear evidence for the existence of three such interactions. *Mesodermal induction* occurs in the early blastula and results from the action of vegetal pole cells on the animal hemisphere. At least two mesodermal rudiments are formed, one dorsal and one ventral. During the next interaction, which we call *dorsalization*, the ventral mesodermal rudiment becomes subdivided into several territories under the influence of the dorsal marginal zone, or organizer. Finally, during gastrulation, the involuting organizer induces neural tissue from the overlying ectoderm. This interaction is called *neural induction*.

Although these phenomena can readily be demonstrated under experimental conditions, direct evidence that they occur in normal development awaits an understanding of the molecular basis of induction.

## INTRODUCTION

It is normally supposed that regionalization along the two major axes, animal–vegetal and dorsoventral, of the fertilized amphibian egg is caused by cytoplasmic localizations. Differences between the animal and vegetal poles arise during oogenesis, and include a gradient in the sizes of yolk granules (large at the vegetal pole, small at the animal pole) and the fact that there is more non-yolky cytoplasm in the animal hemisphere (Gerhart, 1980). In addition the animal hemisphere is more heavily pigmented than the vegetal, and presumably there are other differences, some of which may cause the two halves of the embryo to behave differently during development. The dorsoventral polarity is, in *Xenopus laevis*, defined at fertilization. The dorsal side of the embryo appears opposite the site of sperm entry, and this is thought to be due to a reorganization of the egg cytoplasm which localizes specific determinants to that side (Gerhart *et al.* 1981).

*Key words*: induction, specification, mesoderm induction, dorsalization, neural induction, monoclonal antibodies, amphibia.

Thus cytoplasmic determinants are thought to define the principal polarities; but there is little evidence that the early specification of body pattern is any more fine-grained than that. There do not seem to be, for example, determinants in the egg for skin cells, nerve cells, or muscle cells. Rather, the basic body plan of the embryo is built on the framework of the embryonic axes by a series of inductive interactions (Slack, Dale & Smith, 1984). In this paper we shall describe experiments on three such interactions, making use of cell lineage labels and region-specific markers.

## CELL LINEAGE LABELS

Two of the advantages of amphibian embryos for embryological research are obvious: they are large, and they are accessible for experimentation at all developmental stages. Less obvious an advantage is the fact that amphibian embryos, unlike avian and mammalian embryos, do not grow during development. This means that passive cell lineage labels introduced by the experimenter do not become diluted during development. In retrospect it is hard to understand why such labels were not in use many years ago. As it is, the first was applied to amphibian embryos in 1978 by Jacobson & Hirose. This was the enzyme horseradish peroxidase (HRP) which can be injected into the cells of interest and detected later in frozen sections using standard histochemical techniques.

Other passive cell lineage labels followed: the Bolton-Hunter reagent radioiodinates the proteins of cells exposed to it and they can later be detected by autoradiography (Katz *et al.* 1982; Smith & Malacinski, 1983); tetramethylrhodamine isothiocyanate has been used to label single isolated blastomeres of *Xenopus laevis* (Heasman, Wylie, Hausen & Smith, 1984; this volume). Most popular, however, is fluorescein-lysine-dextran (FLDx) (Gimlich & Gerhart, 1984) and its counterpart rhodamine-lysine-dextran (RLDx). These molecules are injected into the cells of interest: the dextran prevents diffusion from cell to cell via gap junctions, the lysine renders the molecule fixable with aldehydes, and the fluorochromes make visible the progeny of the injected cells. Fluorescent cell lineage labels have the advantage over other detection systems that they do not obscure cytological detail in the injected cells. Furthermore, they can frequently be combined with region-specific markers which are observed by fluorescence at a different wavelength.

## REGION-SPECIFIC MARKERS

Until recently, identification of the phenotypes of cells in whole embryos or explants has had to rely on their position or their appearance in the light microscope. Over the last two years, however, we have been collecting and making reagents that allow unambiguous identification of cell types, even if the cells in question are in explants or surrounded by an inappropriate tissue. These are antibodies, or other reagents, which react with region-, or tissue-, specific markers.

We define a marker as a substance characteristic of a particular region or cell type, irrespective of its function. In the results reviewed in this paper we used four such reagents:

(1) A rabbit antibody raised against total keratin from human stratum corneum (Wu & Rheinwald, 1981). This antibody reacts with *Xenopus* epidermis and (weakly) with the notochord sheath.

(2) A rabbit antibody raised against adult *Xenopus laevis* myosin heavy chain (MHC2). This interacts specifically with muscle from stage 35 onwards (stages of Nieuwkoop & Faber, 1967).

(3) MZ15, a mouse monoclonal antibody raised against pig chondrocytes and characterized as recognizing keratan sulphate by radioimmunoassay with various proteoglycan fragments (Zanetti, Ratcliffe & Watt, 1985). This antibody interacts specifically with the notochord from stage 17 but also stains the ventral portion of the ear vesicle from stage 27. Staining of the notochord with MZ15 is enhanced by pretreating sections with chrondroitinase ABC (Smith & Watt, 1985).

(4) Fluorescein isothiocyanate–conjugated peanut lectin (FITC–PNA) interacts with the epidermis of *Xenopus* embryos from stage 13. It also stains, albeit weakly, some extracellular matrix components. This latter staining is enhanced by pretreating sections with neuraminidase (Slack, 1985).

The regional specificity of the first three reagents on sections of normal embryos is shown in Fig. 1; results with FITC–PNA are described in the paper in this volume by Slack, Cleine and Smith.

## INDUCTIVE INTERACTIONS

### *States of specification*

The sequence of inductive interactions that occurs during development can be monitored by observing the states of specification of different parts of the embryo at different stages (Slack, 1983). In practice, states of specification are defined by removing cells from the embryo and allowing them to develop in the neutral environment of a simple buffered salts solution. The cells are said to be 'specified' to form the structure(s) they eventually become. If this structure is not the one the cells form in the course of normal development, this suggests that they were removed from the embryo before being subjected to the appropriate inductive signal.

Two significant changes occur in the states of specification of cells in the early amphibian embryo. In *Xenopus* these occur around the 64-cell stage and during gastrulation. Before the 64-cell-stage isolates of cells from the dorsal marginal zone differentiate poorly in culture, but from this stage on they differentiate well, forming notochord, muscle, pronephros and blood (Nakamura, 1978). As development proceeds there is a tendency, pronounced by the beginning of gastrulation, for 'dorsal' structures – notochord and somite – to form from the dorsal marginal zone,

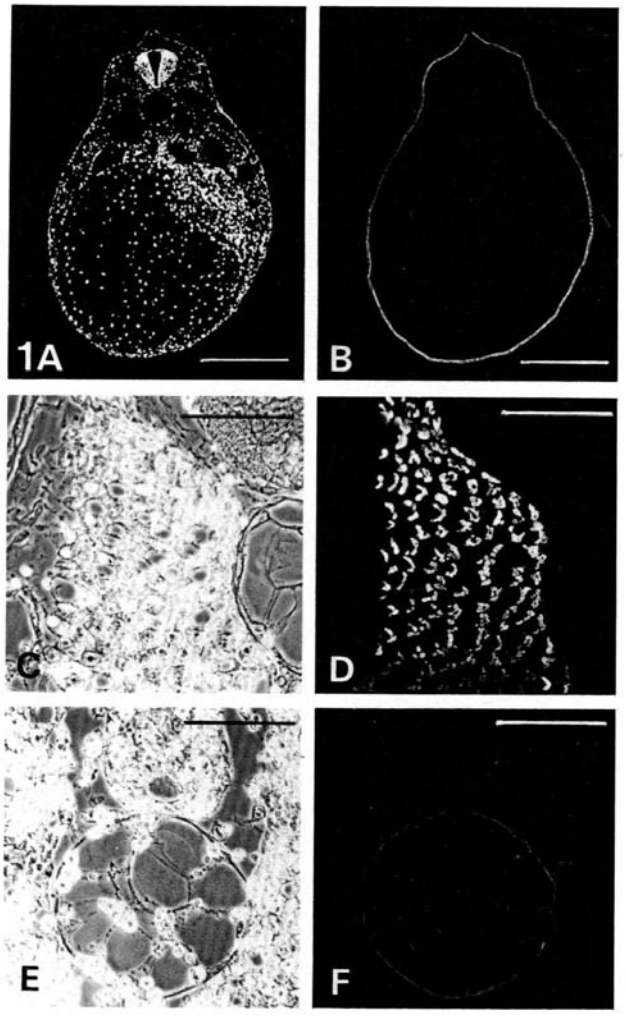


Fig. 1. Region-specific markers in embryos of *Xenopus laevis*. (A), (B) anti-keratin antibody stains epidermis. (A) DAPI staining to show nuclei; (B) indirect immunofluorescence with the anti-keratin antibody. (C), (D) MHC2 stains somitic muscle. (C) phase contrast; (D) indirect immunofluorescence with MHC2. (E), (F) MZ15 stains the notochord. (E) phase contrast; (F) indirect immunofluorescence with MZ15. Scale bar in (A) and (B): 400 $\mu$m. Scale bar in (C), (D), (E) and (F): 100 $\mu$m.

and for 'ventral' structures – mostly blood cells, mesenchyme and mesothelium, to form from the ventral marginal zone (Slack & Forman, 1980). Meanwhile, until the onset of gastrulation, isolates from the animal pole give rise to epidermis-like tissue. However, by the mid- to late- gastrula stage the former animal pole region covers virtually the whole embryo; now, those on the lateral and ventral sides of the embryo still form epidermis on isolation, but those on the dorsal side are specified to form neural structures (Spemann, 1938). These changes in states of specification can be accounted for by the following sequence of inductive interactions.

*Mesodermal induction*

Isolation experiments (above) suggest that the first inductive interaction in amphibian development occurs around the 64-cell stage and results in the formation of a mesodermal rudiment around the equator of the embryo. Work by Nieuwkoop (1969, 1973) indicates that the mesoderm is induced from the animal hemisphere by a signal from the vegetal region. When animal pole isolates are cultured alone they form epidermis, while vegetal pole isolates fail to differentiate. However, when the two regions are combined large amounts of mesoderm are formed and cell marking experiments show that this comes from the animal pole component. The implication is that a similar interaction occurs between the animal and vegetal pole regions in normal development.

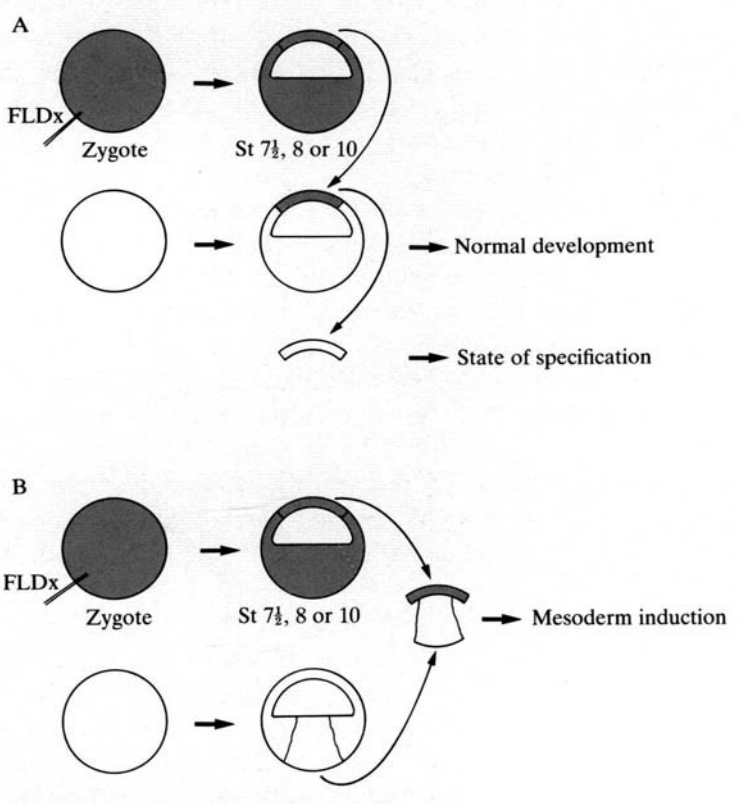


Fig. 2. Mesodermal induction in *Xenopus laevis*: the experimental design. (A) Studying the normal fate and state of specification of animal pole tissue. To study normal fate, orthotopic grafts of animal pole tissue were made from FLDx-labelled donors into unlabelled recipients. Donor and recipient were at the same developmental stage. To study its state of specification, animal pole tissue was allowed to develop in isolation. (B) Mesodermal induction is demonstrated by combining FLDx-labelled animal pole tissue with vegetal pole tissue from unlabelled embryos. Usually such combinations were between explants of the same developmental stage, but some heterochronic combinations were also made.

We have now repeated this work, and, making use of cell lineage labels and tissue-specific markers, extended it to demonstrate that mesodermal induction is a genuine instructive phenomenon that supresses epidermal differentiation and results in the formation of *bona fide* mesodermal cell types. The experimental design is shown in Fig. 2 and the detailed quantitative results are given by Dale, Smith & Slack (1985). Stages are those of Nieuwkoop & Faber (1967).

The normal fate of a 60° region surrounding the animal pole at stages $7\frac{1}{2}$ (early blastula), 8 (mid blastula) and 10 (early gastrula) was established by orthotopic grafts between embryos uniformly labelled with FLDx and unlabelled embryos (Fig. 2A). At stage 10 all the cells from this region were found in ectodermal derivatives, mostly epidermis but also some head mesenchyme. At stages $7\frac{1}{2}$ and 8 labelled cells were present in the neural tube, another ectodermal derivative, as well as epidermis, but they were also found in mesodermal structures, including notochord, somite and lateral plate (Fig. 3A). At stage $7\frac{1}{2}$ about 14 % of the cells were found in mesoderm and at stage 8, 3 %. These results are in slight contrast to the behaviour of isolated animal pole regions (Fig. 2A) which at all three stages differentiate exclusively as epidermis, all cells staining with the anti-keratin antibody (Fig. 3B).

We demonstrated mesodermal induction by combining labelled animal pole regions with unlabelled vegetal pole regions (Fig. 2B). At stages $7\frac{1}{2}$ and 8 the animal tissue responded by forming large amounts of mesodermal structures, significantly more than they would have formed in the course of normal development (Fig. 3C, D). The histological identification of muscle and notochord was confirmed by use of the antibodies MHC2 and MZ15, respectively. Similarly, use of the antikeratin antibody showed that many cells had been diverted from epidermal differentiation.

At stage 10 no mesoderm was formed in the combinations and by combining stage-$7\frac{1}{2}$ animal poles with stage-10 vegetal poles and *vice versa* we found that this was because the animal poles had lost the ability to respond to the vegetal pole.

At stages $7\frac{1}{2}$ and 8, the response of the animal pole could be classified as 'dorsal' or 'ventral', a distinction based on the behaviour in isolation of marginal zone tissue from early gastrulae (Slack & Forman, 1980; see above). Boterenbrood & Nieuwkoop (1973) had previously shown, in the axolotl, that dorsovegetal material would induce 'dorsal' type mesoderm, and ventrovegetal cells would induce 'ventral' mesoderm. In our experiments, we found that the same is true in *Xenopus*.

These results suggest that after fertilization, the vegetal hemisphere is subdivided into at least two regions, dorsal and ventral, and that these characters are transmitted to the animal hemisphere as part of the overall mesodermal induction process.

The next interaction for which we have evidence suggests that the ventral mesoderm becomes subdivided into different territories under the influence of the dorsal region, or 'organizer'.

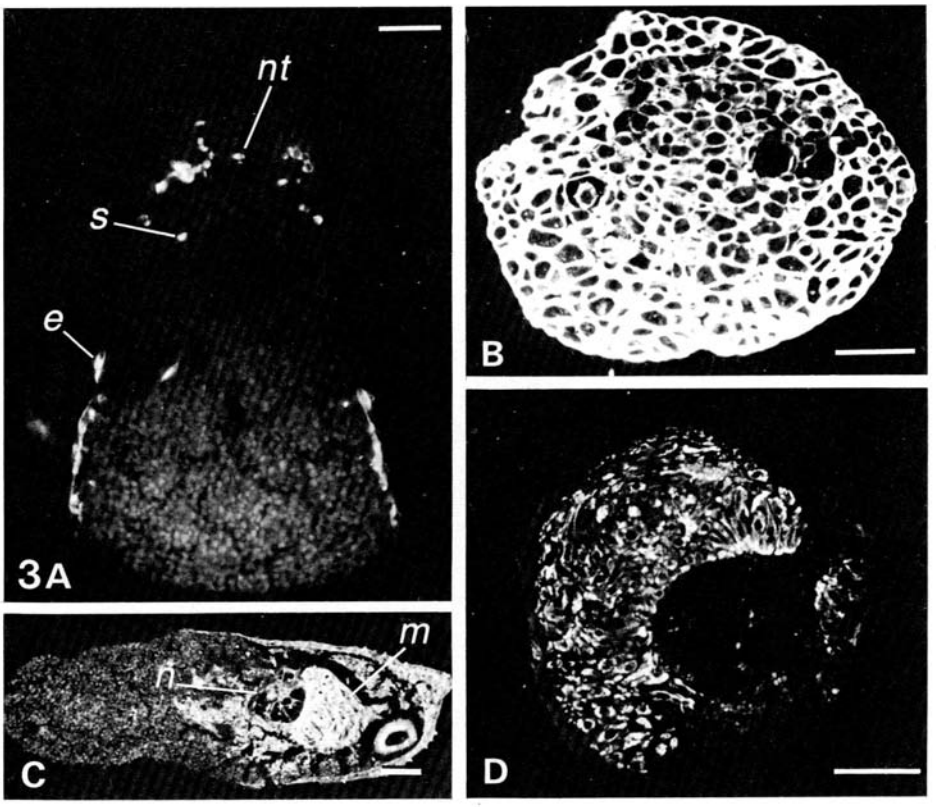


Fig. 3. Mesodermal induction in *Xenopus laevis*. (A) The normal fate of an FLDx-labelled stage- 7½ animal pole region grafted into an unlabelled host. Labelled cells are found in the epidermis (*e*), neural tube (*nt*) and somites (*s*). (B) The state of specification of a similar animal pole region is studied by allowing it to develop in isolation. All the cells stain with an antibody to keratin. (C) An FLDx-labelled stage- 7½ animal pole region was combined with an unlabelled vegetal pole region. The animal pole tissue differentiated as notochord (*n*) and muscle (*m*). (D) A similar experiment using unlabelled animal pole tissue which stains with MHC2, an antibody against myosin heavy chain. Scale bar in (A) is 50 μm; scale bar in (B), (C) and (D) is 100 μm.

*Dorsalization*

By the beginning of gastrulation the *Xenopus* marginal zone has acquired several dorsoventral states of specification. Isolated dorsal marginal zones (DMZ's) form predominantly notochord and neural tissue, while successive lateral positions form large masses of muscle and then small masses of muscle with abundant kidney tubules; finally, blood cells surrounded by mesothelium and mesenchyme are formed from the ventral marginal zone (VMZ) (Slack & Forman, 1980). Recently, we have combined FLDx-labelled VMZ's with unlabelled DMZ's and *vice versa*. We find that the ventral tissue becomes 'dorsalized' by this procedure (that is, it produces large amounts of muscle and no blood cells) but that the dorsal tissue remains dorsal (L. Dale, unpublished observations; see Fig. 4). This observation

confirms the earlier report by Slack & Forman (1980), using interspecies combinations, and also that of Smith & Slack (1983) in which horseradish peroxidase-labelled VMZ's were grafted into the dorsal midline of unlabelled host embryos. It should be noticed that dorsalization occurs at stage 10 and perhaps later during gastrulation; it occurs at a later stage than, and is thus distinct from, mesodermal induction.

We have argued (Slack & Forman, 1980; Smith & Slack, 1983; Slack *et al.* 1984) that dorsalization of the marginal zone is the main interaction at work in the 'organizer' graft of Spemann & Mangold (1924). Grafts of HRP-labelled dorsal marginal zones to the ventral marginal zones of unlabelled early gastrulae result in double-dorsal embryos where the contribution made by the grafted DMZ is, as in normal development, notochord with a little somite (Fig. 5A, B). It seems probable that the somite tissue in these secondary embryos comes from dorsalization of adjacent ventral marginal zone, although in this experiment we cannot rule out the possibility that some of it derives from the migration of cells determined to be somite in the host axis (Cooke & Smallcombe, personal communication).

The organizer graft also reveals the third inductive interaction involved in the formation of the body plan – neural induction.

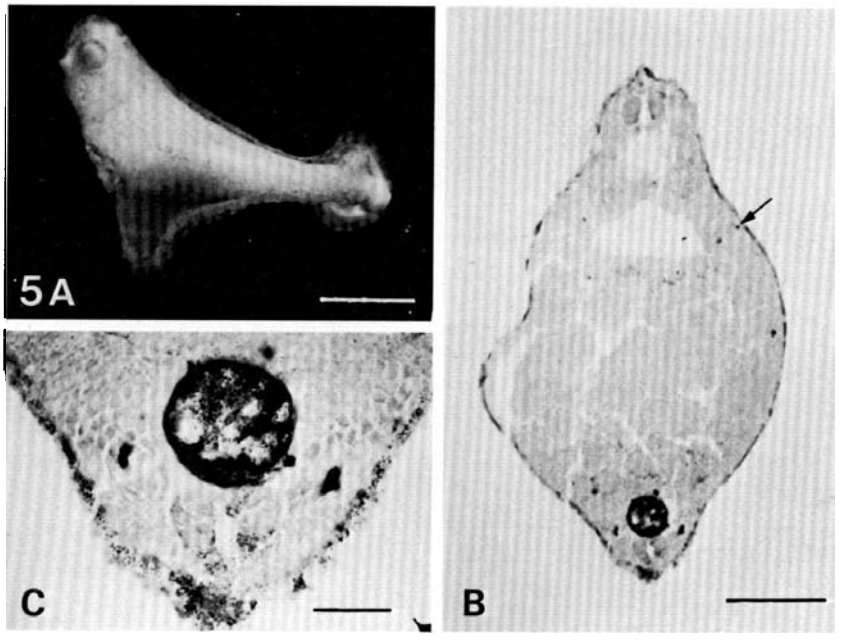


Fig. 5. The result of an organizer graft. An HRP-labelled dorsal marginal zone was grafted to the ventral marginal zone of an unlabelled host. (A) shows the double-dorsal duplicated embryo thus formed. (B) A section through the embryo. Notice that only the notochord and a few cells in the somites of the secondary embryo are derived from the graft. There is also a single labelled cell in the host's axial structures (arrow). (C) Higher power of the notochord, somites and neural tube of the secondary axis. (A) scale bar is 1 mm; (B) scale bar is 200 $\mu$m; (C) scale bar is 50 $\mu$m.

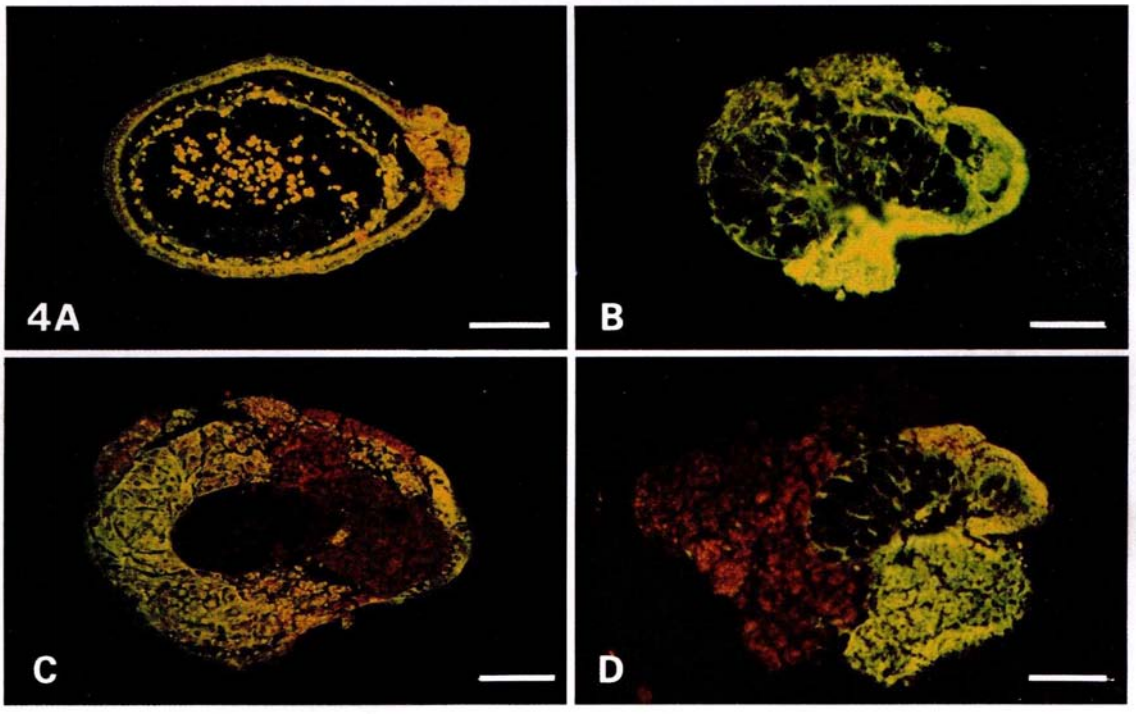


Fig. 4. Dorsalization of ventral marginal zone (VMZ) tissue. (A) An FLDx-labelled VMZ was allowed to develop in isolation: it formed blood cells, mesenchyme and mesothelium. (B) An FLDx-labelled dorsal marginal zone (DMZ) formed predominantly notochord. (C) When an FLDx-labelled VMZ was combined with an unlabelled DMZ it formed large amounts of muscle, wrapped around notochord tissue derived from the DMZ. (D) The complementary experiment, FLDx-labelled DMZ combined with unlabelled VMZ, confirms that the dorsal tissue is not 'ventralized'; it still forms notochord. Scale bars 200 $\mu$m for A and B, 100 $\mu$m for C and D.

*Neural induction*

In the double-dorsal embryos produced by the organizer graft the secondary neural tubes clearly derive from host tissue (Spemann & Mangold, 1924; Smith & Slack, 1983; Gimlich & Cooke, 1983; Jacobson, 1984). It has long been suspected that these neural tubes arise from the action of the involuting dorsal mesoderm on the overlying ectoderm – i.e. by neural induction. Until recently, however, two objections to this conclusion have remained to be satisfied (Jacobson, 1982). First, it needed to be shown that the cells in the secondary neural tube were cells that would normally have formed ventral epidermis, and that they had not migrated from the 'primary' neural tube. And secondly, it needed to be shown that the cells in the secondary neural tube were genuine neuroepithelial cells and not epidermal cells rolled into a cylinder.

Experiments by Gimlich & Cooke (1983), Jacobson (1984), and Slack *et al.* (1984) have established beyond doubt that the cells in the secondary neural tube would otherwise have formed epidermis. These authors injected cell lineage markers (FLDx or HRP) into ventral cells of early *Xenopus* embryos that normally contribute almost exclusively to epidermis. Then, when these embryos reached the beginning of gastrulation they grafted organizers – dorsal marginal zones – into the ventral marginal zones. The second neural tubes that resulted were composed largely of labelled cells, so they clearly came from cells which should have made epidermis, and not from cells of the primary neural tube which had migrated to the ventral side. We approached the question of whether the secondary neural tube was composed of neuroepithelial cells by using a reagent for an epidermis-specific marker, fluoresceinated peanut lectin (FITC–PNA) (Slack, 1985). The same ventral cells injected by Gimlich & Cooke (1983) and Jacobson (1984) were injected with rhodamine-lysine-dextran (RLDx), and, at the beginning of gastrulation, an unlabelled organizer was grafted to the VMZ of these host embryos. Again, it was seen that the secondary neural tubes contained labelled cells; however, they did not stain with FITC–PNA, indicating that they had been diverted from epidermal differentiation, presumably towards neuroepithelium.

It should be noted that, like mesodermal induction, the process of neural induction includes the transmission of regional information. It was shown by Mangold (1933) and Horst (1948) that different regions of the involuted mesoderm induce specific parts of the nervous system. The mechanism by which the involuted mesoderm acquires these craniocaudal states of specification is still unknown.

## ROLES OF INDUCTIVE INTERACTIONS IN NORMAL DEVELOPMENT

The inductive interactions we have described can only be observed by placing cells and tissues into abnormal positions with respect to each other. We cannot be certain, therefore, whether they occur in normal development or whether they are manifestations of the cellular properties of specified states once they have been

created by some other means. This is not a problem unique to early amphibian development, of course: an interaction analogous to the dorsalization of ventral marginal zone has been demonstrated in the chick limb bud (Tickle, Summerbell & Wolpert, 1975), but it is still not known whether the so-called zone of polarizing activity plays a role in normal development (Saunders, 1977; Smith, 1979; Summerbell, 1979).

The best evidence that inductive interactions occur in normal amphibian development comes from changes in specified states, particularly at the 64-cell stage, when cells are specified to become mesoderm, and during gastrulation, when cells become specified to form neuroepithelium. At present, evidence is limited as to the creation of dorsoventral specified states in the mesoderm; clearly some specification occurs along with mesodermal induction, but it seems possible that further subdivision happens at later stages.

From the data described above, and the results of others, we have proposed the following sequence of cytoplasmic localizations and inductive interactions (Fig. 6). During oogenesis differences arise between the animal (A) and vegetal (V) halves of the egg. Fertilization results in a subdivision of the vegetal half into a dorsovegetal (DV) and a ventral vegetal (VV) region. During mesodermal induction the dorsovegetal region induces the 'organizer' (O) from the animal half of the embryo and the ventral region induces a general ventral mesoderm (M) which is subdivided into several regions ('dorsalization') under the influence of the organizer. During gastrulation the organizer acquires craniocaudal states of determination (O1–O4) and these, together with a general neural induction signal, are transmitted to the overlying ectoderm. This produces neuroepithelium with homologous craniocaudal states (N1–N4) while the uninduced ectoderm becomes epidermis (E).

This sequence of events is compatible with the inductive events we know can occur in abnormal situations, but evidence that it occurs in reality awaits an understanding of the molecular basis of induction.

## THE MOLECULAR BASIS OF INDUCTION

It has to be admitted that we know virtually nothing about the biochemical basis of inductive interactions. It may be seen from the foregoing, however, that we expect there to be four components to the solution (see also Smith, 1985). First we need to identify the cytoplasmic determinants that establish the early polarity of the embryo; second, the nature of the inductive signals that pass between cells; third, how the cells store the memory of receiving those signals; and fourthly, how the 'memory' is translated into the specific cell activities which form the body plan: cell division, cell locomotion, and the expression of genes characteristic of different cell types. Several of these topics are discussed elsewhere in this volume. For the future, we intend to concentrate on mesodermal induction, for which a putative 'morphogen' has already been isolated. The 'vegetalizing factor' is a protein of molecular weight 30,000 isolated from chick embryos. When it is applied, as a

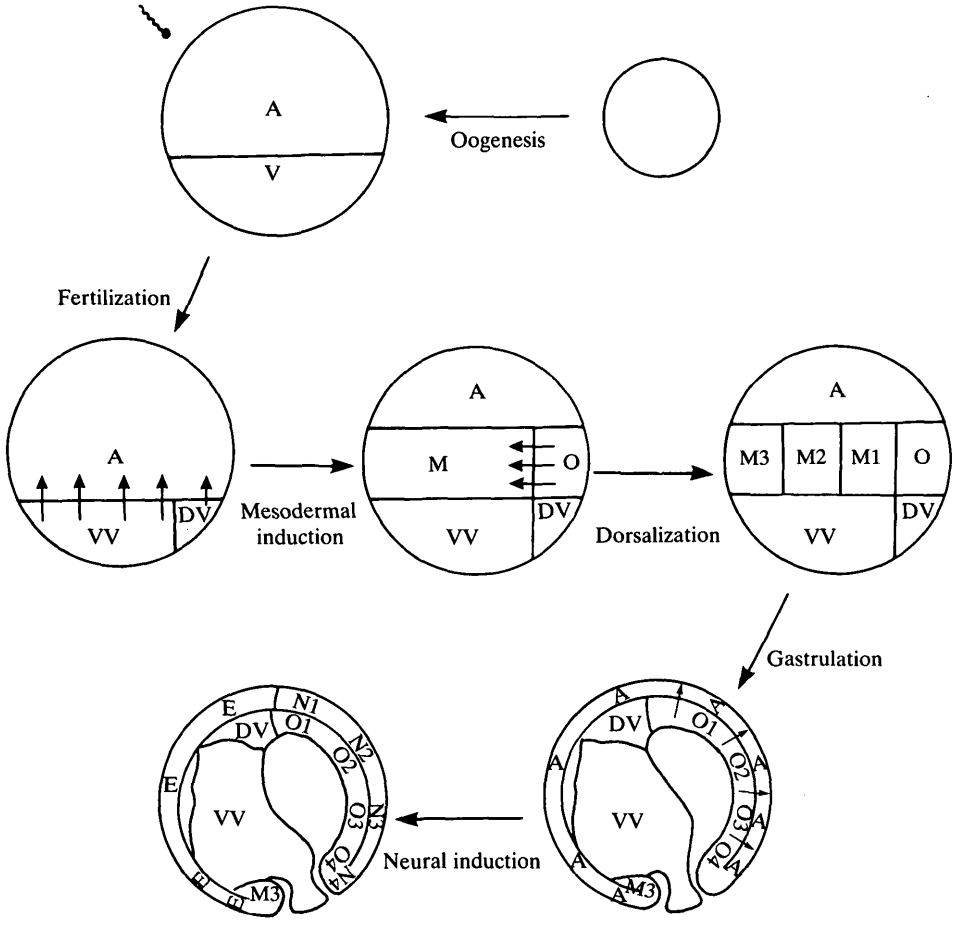


Fig. 6. The sequence of cytoplasmic localizations and inductive interactions during early amphibian development. During oogenesis differences arise between the animal (A) and vegetal (V) halves of the egg. Fertilization results in a subdivision of the vegetal half into dorsovegetal (DV) and ventral vegetal (VV) regions. During mesodermal induction the dorsovegetal region induces the organizer (O) from the animal half of the embryo and the ventral region induces a general ventral mesoderm (M) which is subdivided into several regions during dorsalization, under the influence of the organizer. During gastrulation the organizer acquires craniocaudal positional values (O1 to O4) and these are transmitted to the overlying ectoderm as part of the neural induction process. This produces neuroepithelium with homologous craniocaudal positional values (N1 to N4) while the uninduced animal pole material becomes epidermis (E). Reproduced, with permission, from *Science Progress*.

pellet, to amphibian blastula ectoderm it causes the differentiation of a variety of mesodermal tissues, therefore mimicking the effect of the vegetal pole (Born *et al.* 1972; Schwartz, Tiedemann & Tiedemann, 1981; see Fig. 7). Extracts with similar activity have been made from early *Xenopus* embryos (Faulhaber & Lyra, 1974). At present, nothing is known about the spatial and temporal distribution of molecules with vegetalizing activity, but our proposed sequence of inductive

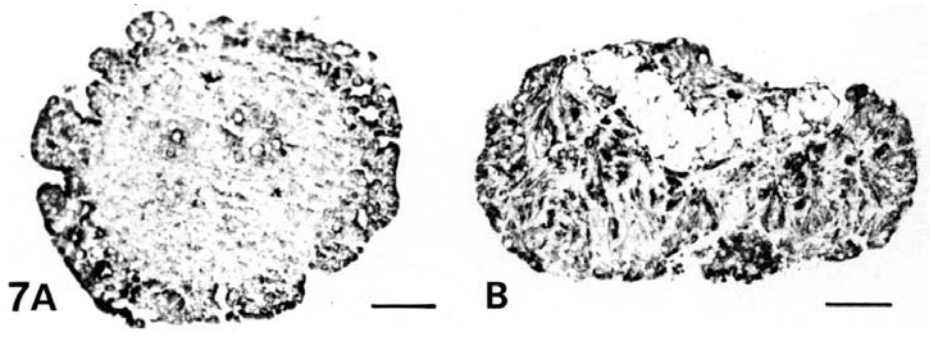


Fig. 7. The effect of the vegetalizing factor. In (A), a piece of animal pole tissue from a stage-8 *Xenopus* embryo was allowed to develop in isolation for three days before being fixed and sectioned. It formed 'atypical epidermis', and cells in the centre of the explant are beginning to die. In (B), a similar piece of tissue was combined with a pellet of vegetalizing factor kindly supplied by Dr. H. Tiedemann. The tissue differentiated as notochord and muscle. Scale bar in both figures is 200 $\mu$m.

interactions (Fig. 6) makes firm predictions as to where and when such molecules should appear. If the predictions are confirmed this will provide strong evidence for the reality of mesodermal induction in normal development.

We are grateful to Jim Rheinwald for supplying the anti-keratin antibody and Fiona Watt for MZ15.

## REFERENCES

BORN, J., GEITHE, H. P., TIEDEMANN, H., TIEDEMANN, H. & KOCHER-BECKER, U. (1972). Isolation of a vegetalizing inducing factor. *Hoppe-Seylers Z. Physiol. Chem.* **353**, 1075–1084.

BOTERENBROOD, E. C. & NIEUWKOOP, P. D. (1973). The formation of the mesoderm in urodelean amphibians. V. Its regional induction by the endoderm. *Wilhelm Roux Arch. EntwMech. Org.* **173**, 319–332.

DALE, L., SMITH, J. C. & SLACK, J. M. W. (1985). Mesoderm induction in *Xenopus laevis*: a quantitative study using a cell lineage label and tissue specific antibodies. *J. Embryol. exp. Morph.* **89**, 289–313.

FAULHABER, I. & LYRA, L. (1974). Ein Vergleich der Induktions–fähigkeit von Hüllenmaterial der Dotterplattchen– und der Microsomenfraktion aus Furchungs – sowie Gastrula – und Neurulastadien des Kraltenfrosches *Xenopus laevis*. *Wilhelm Roux Arch. EntwMech. Org.* **176**, 151–157.

GERHART, J. C. (1980). Mechanisms regulating pattern formation in the amphibian egg and early embryo. In *Biological Regulation and Development* (ed. R. F. Goldberger). pp 133–293. New York: Plenum.

GERHART, J., UBBELS, G., BLACK, S., HARA, K. & KIRSCHNER, M. (1981). A reinvestigation of the role of the grey crescent in axis formation in *Xenopus laevis*. *Nature* **292**, 511–516.

GIMLICH, R. L. & COOKE, J. (1983). Cell lineage and the induction of second nervous systems in amphibian development. *Nature* **306**, 471–473.

GIMLICH, R. L. & GERHART, J. C. (1984). Early cellular interactions promote embryonic axis formation in *Xenopus laevis*. *Devl Biol.* **104**, 117–130.

HEASMAN, J., WYLIE, C. C., HAUSEN, P. & SMITH, J. C. (1984). Fates and states of determination of single vegetal pole blastomeres of *Xenopus laevis*. *Cell* **37**, 185–194.

HORST, J. TER (1948). Differenzierungs und Induktionsleistungen verschiedener Abschnitte der Medullarplatte und der Urdarmdaches von *Triton* im Kombinat. *Wilhelm Roux Arch. EntwMech. Org.* **143**, 275–303.

JACOBSON, M. (1982). Origins of the nervous systems in amphibians. In *Neural Development* (ed. N. C. Spitzer) pp 45–99. New York: Plenum.

JACOBSON, M. (1984). Cell lineage analysis of neural induction: origins of cells forming the induced nervous system. *Devl Biol.* **102**, 122–129.

JACOBSON, M. & HIROSE, G. (1978). Origin of the retina from both sides of the embryonic brain: a contribution to the problem of crossing at the optic chiasma. *Science* **202**, 637–639.

KATZ, M. J., LASEK, R. J., OSDOBY, P., WHITTAKER, J. R. & CAPLAN, A. I. (1982). Bolton-Hunter reagent as a vital stain for developing systems. *Devl Biol.* **90**, 419–429.

MANGOLD, O. (1933). Uber die Induktions – Fähigkeit der verschiedenen Bezirke der Neurula von Urodelen. *Naturwissenschaften* **21**, 761–766.

NAKAMURA, O. (1978). Epigenetic formation of the organizer. In *Organizer – a Milestone of a Half-century from Spemann* (ed. Nakamura, O. & Toivonen, S.) pp 179–220.

NIEUWKOOP, P. D. (1969). The formation of mesoderm in Urodelean amphibians. I. Induction by the endoderm. *Wilhelm Roux' Arch. EntwMech. Org.* **162**, 341–373.

NIEUWKOOP, P. D. (1973). The "organization center"of the amphibian embryo, its origin, spatial organization and morphogenetic action. *Adv. Morphogen.* **10**, 1–39.

NIEUWKOOP, P. & FABER, J., EDS. (1967). *Normal Table of Xenopus laevis (Daudin)*. 2nd ed. Amsterdam: North-Holland.

SAUNDERS, J. W. JR., (1977). The experimental analysis of chick limb bud development. In *Vertebrate Limb and Somite Morphogenesis*. (ed. D. A. Ede, J. R. Hinchcliffe and M. Balls), pp 1–24. Cambridge and London: Cambridge University Press.

SCHWARTZ, W., TIEDEMANN, H. & TIEDEMANN, H. (1981). High performance gel permeation of proteins. *Mol. Biol. Rep.* **8**, 7–10.

SLACK, J. M. W. (1983). *From Egg to Embryo: Determinative Events in Early Development.* Cambridge and London: Cambridge University Press.

SLACK, J. M. W. (1985). Peanut lectin receptors in the early amphibian embryo: regional markers for the study of embryonic induction. *Cell*, **41**, 237–247.

SLACK, J. M. W., DALE, L. & SMITH, J. C. (1984). Analysis of embryonic induction by using cell lineage markers. *Phil. Trans. R. Soc. Lond.* **B 307**, 331–336.

SLACK, J. M. W. & FORMAN, D. (1980). An interaction between dorsal and ventral regions of the marginal zone in early amphibian embryos. *J. Embryol. exp. Morph.* **56**, 283–299.

SMITH, J. C. (1979). Evidence for a positional memory in the development of the chick wing bud. *J. Embryol. exp. Morph.* **52**, 105–113.

SMITH, J. C. (1985). Solving the organizer. *BioEssays* **2**, 277–280.

SMITH, J. C. & MALACINSKI, G. M. (1983). The origin of the mesoderm in an anuran, *Xenopus laevis* and a urodele. *Ambystoma mexicanum. Devl Biol.* **98**, 250–254.

SMITH, J. C. & SLACK, J. M. W. (1983). Dorsalization and neural induction: properties of the organizer in *Xenopus laevis*. *J. Embryol. exp. Morph.* **78**, 299–317.

SMITH, J. C. & WATT, F. M. (1985). Biochemical specificity of the *Xenopus* notochord. *Differentiation* (in press).

SPEMANN, H. (1938). *Embryonic Development and Induction.* (Reprinted in 1967 by Hafner, New York).

SPEMANN, H. & MANGOLD, H. (1924). Uber Induktion von Embryonenanlagen durch Implantation artfremder Organisatoren. *Wilhelm Roux Arch. EntwMech. Org.* **100**, 599–638.

SUMMERBELL, D. (1979). The zone of polarizing activity: evidence for a role in normal development. *J. Embryol. exp. Morph.* **50**, 217–233.

TICKLE, C., SUMMERBELL, D. & WOLPERT, L. (1975). Positional signalling and specification of digits in chick limb morphogenesis. *Nature* **254**, 199–202.

WU, Y. & RHEINWALD, J. G. (1981). A small (40kd) keratin filament protein made by some cultured human squamous cell carcinomas. *Cell* **25**, 627–635.

ZANETTI, M. RATCLIFFE, A. & WATT, F. M. (1985). Two subpopulations of differentiated chondrocytes identified with a monoclonal antibody to keratan sulphate. *J. Cell Biol.* **101**, 53–59.

DISCUSSION

*Speaker: J. Smith*

*Question from J. Cooke (NIMR, London)*:
I am wondering about dorsalization. You seem very happy with the idea that it plays a major role in normal development. In the experiment that you were talking about, you do show a spatial heterogeneity around the marginal zone with respect to what bit of the total mesodermal pattern is being induced by different regions of the vegetal tissue. But on the other hand, we know that body patterns which contain a lot of somite but no notochord can develop from isolated ventral blastomeres, or from mildly u.v.- irradiated eggs.

*Answer*:
I have two points to make about this. Firstly, what we are able to see as being induced by the ventral vegetal and the dorsal vegetal tissue look as though they represent only two sorts of induction: you either get notochord/muscle or you get red cells/mesothelium/mesenchyme, so you aren't getting the whole spectrum of mesodermal tissues. There is room for some sort of interaction later to create the rest of the observed diversity. The other thing is that, as you have shown [Cooke this volume], if you separate the embryo at 4-cell stage into the dorsal half and the ventral half, frequently you see the ventral half making what it would have made in normal development. However, it is also true, isn't it, that it often makes much less than it would make in normal development and, indeed, sometimes makes very little at all. So, when we come to discussing the role of dorsalization in normal development, it is rather difficult to say. At the moment, I would only suggest that our experiments are illuminating the dynamical properties of the dorsal–ventral axis as they exist. It is hard to know what 'play a part in normal development' really means in this context.

*Question from C. Wylie (St Georges, London)*:
In your dorsalization experiments, if the hypothesis is that the dorsal marginal zone is signalling "dorsal" to the next bit of tissue along, then you might expect that with more bits of ventral marginal zone you might get intermediate types of mesoderm, like kidney, and that might distinguish it from an induction of muscle as a terminal cell type by the notochord.

*Answer*:
Jonathan Slack should answer this one.

*J. Slack (ICRF, London)*:
Although the recent series of experiments, as described, gave very little kidney, the series I did some years ago which were interspecies combination, i.e. Axolotl and

*Xenopus*, gave a great deal of kidney, and it did seem to me that a short or weak dorsalizing signal gave you a lot a kidney while a long or strong signal gave a lot of muscle, although you don't seem to be able to dorsalize the VMZ right up to notochord.

*Question from H. Grunz (Essen)*:
Perhaps I should remind you of the Yamada experiments when he combined several pieces of marginal zone and obtained a greater diversity of tissues from the fused pieces than from isolated ones. Perhaps dorsalization arises simply from the correlation of the two pieces?

*Answer*:
I was aware of these experiments and I was also aware that J. Slack had controlled for mass effects by fusing together up to 3 ventral marginal zones and found they still behaved as if they were ventral marginal zones [Slack & Forman, *J. Embryol. exp. Morph.* **56** , 283 (1980)], so I don't think that is an effect in these experiments.

*Question from H. Grunz (Essen)*:
Also I think the self-differentiation of the notochord does not represent the most dorsal specification, the most dorsal is neural tissue as shown by the isolation experiments of Holtfreter-Ban.

*Answer*:
I think our marginal zone explants are more vegetal than hers and include little ectoderm, so you wouldn't expect much neural tissue to develop.