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Original Article

Mesoderm induction by the mesoderm of *Xenopus* neurulae

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ABSTRACT Combinations were made between explants of mesoderm from the archenteron roof of early *Xenopus* neurulae and explants of ectoderm from mid-blastulae. In each combination one component was labeled with the fluorescent lineage label RDA(rhodamine-dextran-amine). Frequent and large mesoderm inductions, consisting mainly of muscle, were found where the presomite plate was used as the inducer. Less frequent and smaller mesoderm inductions were found when notochord was used as the inducer. We conclude that induced mesoderm can itself be active as a mesoderm inducing tissue. If this capability is acquired in the blastula then it follows that mesoderm induction must propagate from cell to cell and its spread be antagonized by some other factor.

KEY WORDS: *Xenopus laevis, mesoderm induction, homoeogenetic induction*

Introduction

It is well known that inductive interactions in development can either occur within a cell layer or between different cell layers (Slack 1983; Gurdon 1987). It is less well appreciated that the induced tissue itself may be a source of the inducing signal, giving the signal the ability to propagate across the competent field in the absence of long range diffusion. This phenomenon was first noted by Mangold and Spemann (1927) who found that explants of neuroepithelium could induce further neuroepithelium from newt gastrula ectoderm. They called the process "homoeogenetic induction" and its apparent autocatalytic property caused Needham (1942) to speculate on its resemblance to a viral infection.

In recent years a number of laboratories have made detailed studies on the developmentally earlier process of mesoderm induction, which occurs in the blastula and which leads to the formation of a ring of mesoderm around the equator of the embryo (review: Smith 1989). Considerable progress has been made in the identification of the possible morphogens (bFGF and XTC-MIF) and the biochemistry of the early responses. However we also need dynamical information in order to formulate a quantitative model of the process, and one of the most important things we need to know is whether, in fact, mesoderm induction can be self-propagating.

It has proved difficult to find this out, mainly because it is not possible to cut out from the blastula a piece of tissue which is guaranteed to contain mesoderm – but not endoderm – for use as an inducer in a combination experiment. A recent study by Cooke *et al.* (1987) addressed the problem by combining XTC-MIF induced ectoderm with uninduced ectoderm. They did obtain a number of inductions and concluded that homoeogenetic induction was occurring, although they could not absolutely exclude the possibility that the effect was due to residual free XTC-MIF carried over with the induced tissue. In the present paper we have adopted a different approach by examining the inductive ability of mesoderm from the archenteron roof of early neurulae. At this stage it is possible to dissect pure explants of mesoderm, uncontaminated by ecto or endoderm, and even to separate different parts of the archenteron roof, such as the notochord and presomite plate to examine their effects separately. Our results show that homoeogenetic induction of muscle by presomite plate does occur. There is also some induction of muscle by the notochord, but notochord seems to be a weaker mesoderm inducer than the presomite plate, while being a stronger neural inducer. Notochord is not induced either by notochord or by presomite plate. A recent study by

Abbreviations used in this paper: RDA, rhodamine-dextran-amine.

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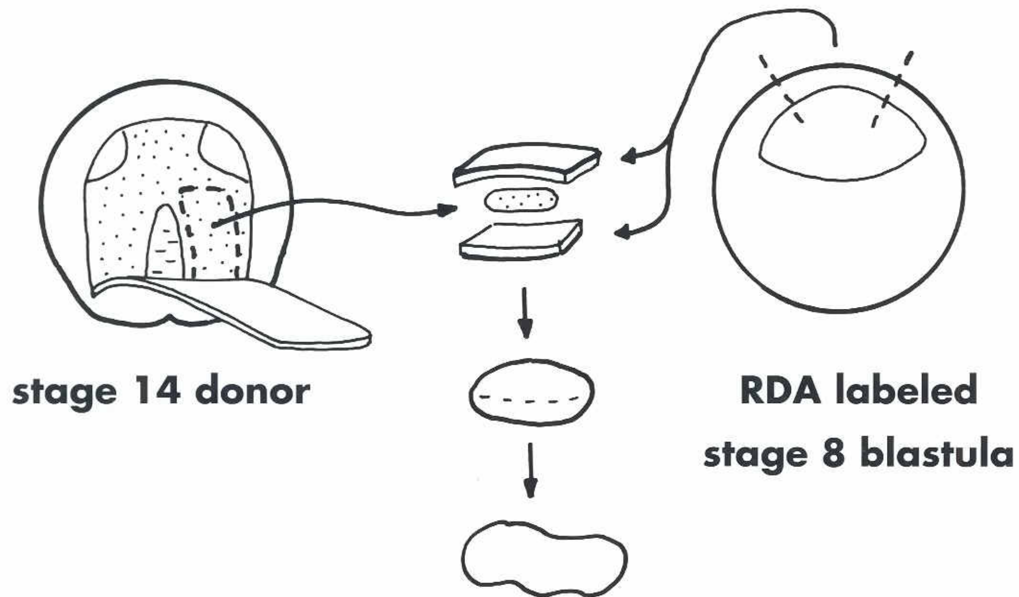

Fig. 1. Diagram of the type of operation performed in this study. *In this case unlabeled presomite plate from the early neurula is combined with RDA labeled ectoderm from a stage 8 blastula.*

Woodland and Jones (1988), although concerned with a different problem, also incidentally showed the mesoderm inducing activity of the posterior archenteron roof.

Three series of combinations were carried out: notochord + labeled ectoderm (Fig. 1), labeled notochord + ectoderm, and presomite plate + labeled ectoderm. The results are shown in Table 1. The RDA labeling was not distinct in many of the cases and we can therefore not be sure that all the "small muscle inductions " are really inductions rather than being donor-derived. However the "large inductions" are very large and could not have been formed by the small pieces of donor tissue, so we regard all of these as being genuine inductions.

The presomite plate developed into both muscle and mesenchyme and in two cases some notochord was present which was probably donor-derived and arose because the original tissue was contaminated with notochord cells. In most cases the presomite plate induced large masses of muscle, sometimes accompanied by mesenchyme. This is shown in Fig. 2. Blood cells were rare or absent in this series. Some neural inductions were found and although these tended to be small it does confirm our previous belief that at least part of the presomite plate has neural inducing properties.

TABLE I

EMBRYONIC TISSUE RECOMBINATIONS

Operation	Cases	Large muscle induction	Small muscle induction	Neural induction
Notochord + RDA-ectoderm	22	9	6	18
RDA-notochord + ectoderm	22	12	4	21
Presomite plate + RDA-ectoderm	16	13	2	11

The donor notochords developed into a mass of notochord and in about half the cases there was also a small, closely apposed area of muscle judged to be donor-derived from the cases where the RDA label was good. In most cases large neural inductions were produced. Large muscle inductions were found in about half the cases. We considered the possibility that the notochord itself was inactive and that all these were induced by contaminating donor-derived presomite plate. However many of the large inductions were found in the absence of any donor-derived muscle and so we conclude that, although it is weaker than the presomite plate, the notochord is nonetheless capable of inducing muscle. Mesenchyme but not blood was found in a number of cases. In Fig. 3 a case is shown in which the host tissue was RDA labeled and muscle was induced in the absence of donor muscle. This section also shows some small induced neural structures.

Results

The results clearly show that mesoderm from the archenteron roof can induce further mesoderm from

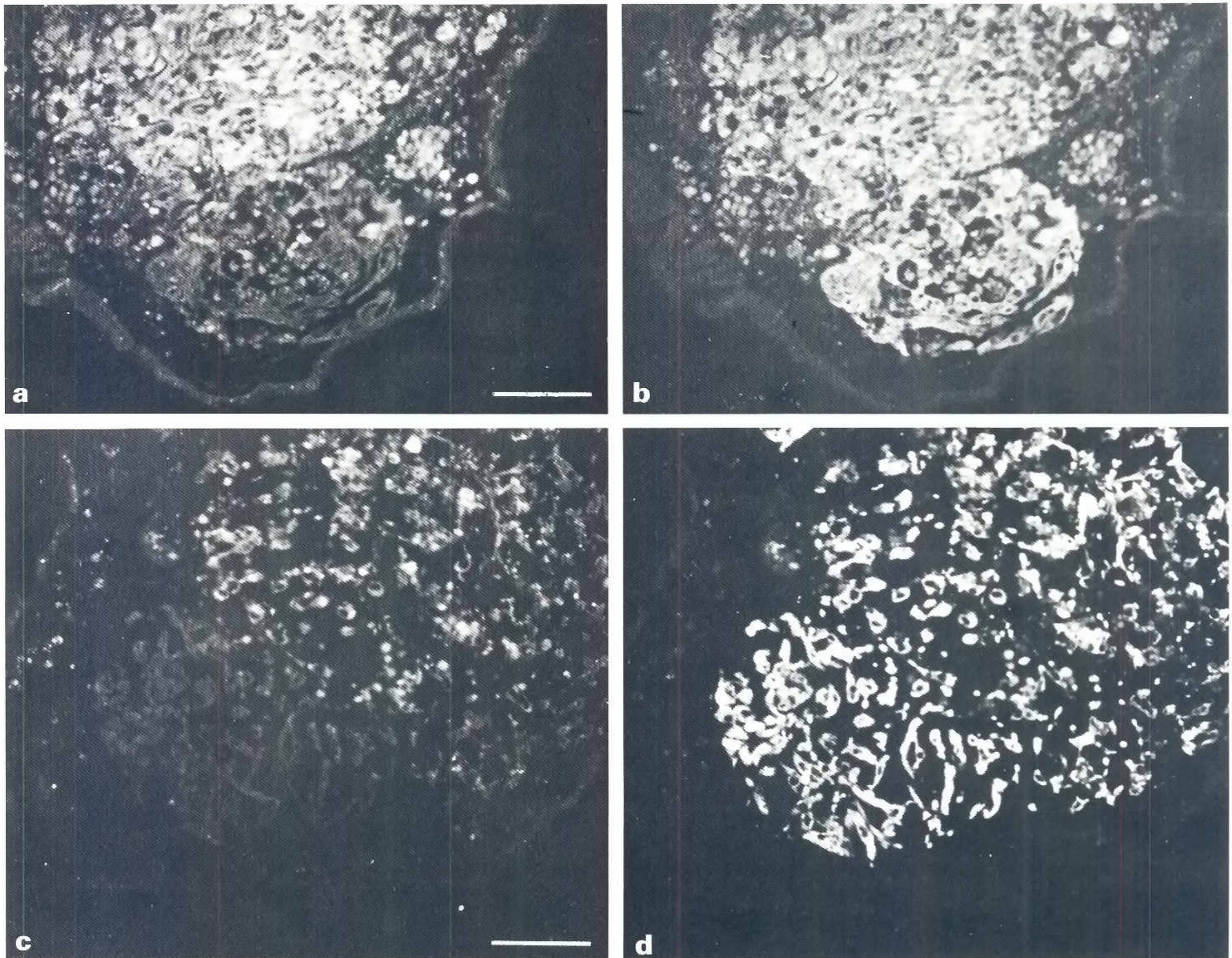

Fig. 2. Induction of muscle by presomite plate. *Two cases are shown and for each the left plate* **(a,c)** *shows the RDA label which identifies the tissue as being of ectodermal origin and the right* **(b,d)** *the 12/101-fluorescein label which identifies tissue as muscle. In both cases the inducer tissue gave rise to the lower, RDA unlabelled, part of the muscle mass. In these cases the outer epidermis was also RDA labeled, but in a granular and sparse manner not easily seen on the photographs. Scale bars 100 microns.*

competent blastula ectoderm. In the case of the presomite plate the induced tissue is like the inducer and so we can conclude that homeogenetic induction definitely occurs. In the case of the notochord there is no further induction of notochord, and although the induction of other mesodermal tissues definitely occurs, it seems inferior to that produced by presomite plate or by vegetal tissue from blastulae. By contrast the neural inductions produced by the notochord are florid and numerous while those produced by the presomite plate are small and few.

Some researchers might not like to use the term "homoeogenetic induction" for the induction of muscle by notochord, and it certainly does not seem to be the same as the original organizer-inducing signal from the dorsovegetal region of the blastula endoderm. The two candidate morphogens for early *Xenopus* development, FGF and XTC-MIF, show "ventral inducing" and "organizer inducing" activity, respectively (Smith 1989). In terms of this distinction, the mesoderm inductions seen in the present study would be classified as "FGF-like" rather than "XTC-like", leading us to speculate that the newly formed mesoderm emits bFGF but not factors of the XTC-MIF type. However we have no direct evidence on this and the chemical nature of the process must remain a matter for future investigation.

The time from the onset of gastrulation, when mesoderm induction is thought to finish, until the onset of

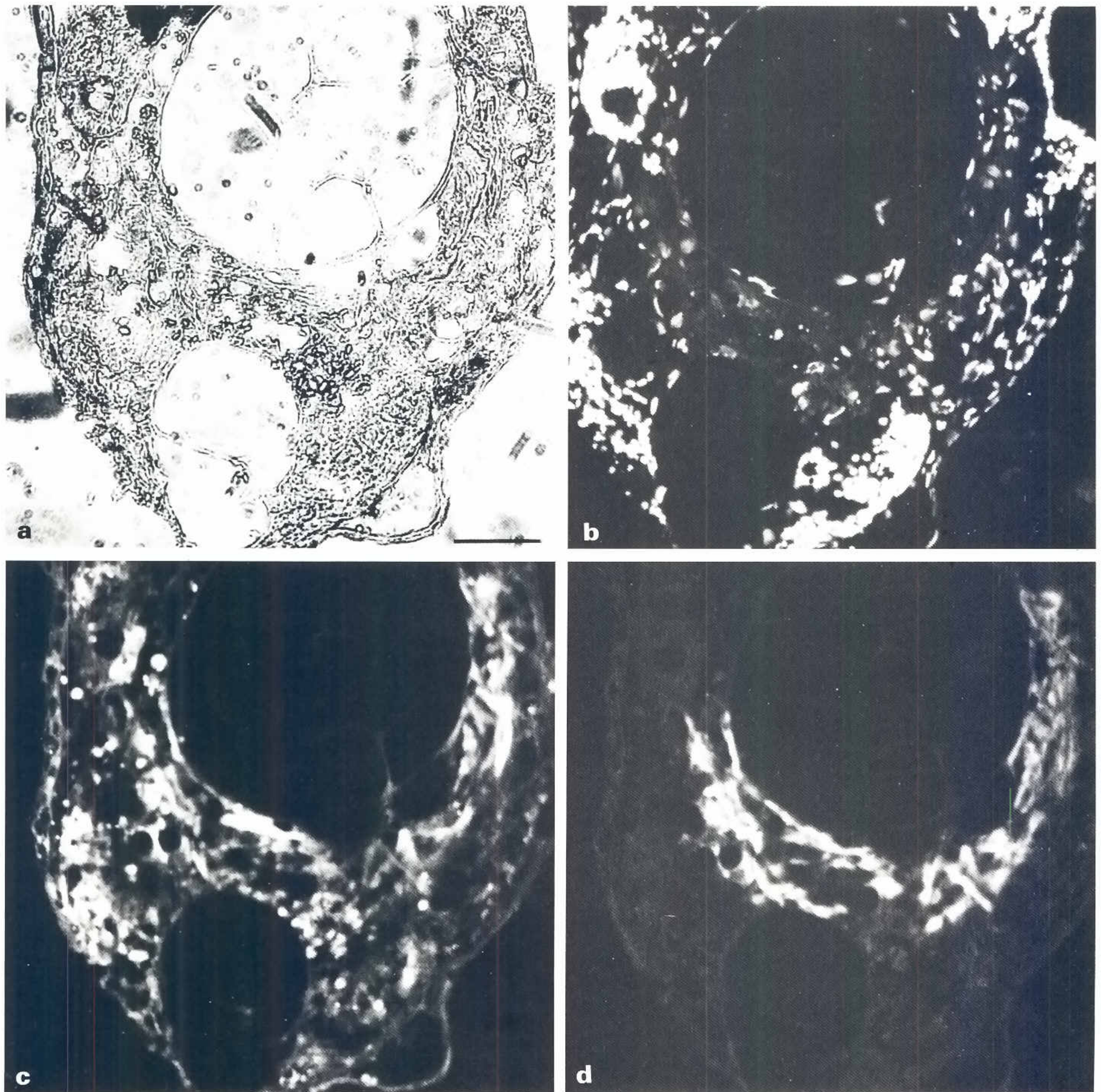

Fig. 3. Inductions provoked by notochord. **(a)** *phase view showing two clumps of notochord (joined out of the plane of section). Scale bar 100 microns.* **(b)** *DAPI stain showing the characteristic dense packed nuclei of two induced neural structures (n).* **(c)** *RDA label, showing that all tissue in this section except the notochord is of ectodermal origin.* **(d)** *12/101-fluorescein label showing a patch of induced muscle.*

neurulation, the stage of our donor tissues, is about 5-6 hours at normal laboratory temperatures. We cannot prove that the mesoderm inducing capacity of the mesoderm is acquired at the time of its formation rather than at some later time during gastrulation, but this does seem the simplest possibility.

If mesoderm induction can in fact propagate in a homoeogenetic manner we need to explain why its progress is eventually stopped and the whole animal hemisphere does not become mesodermalized. Gurdon (1989) has proposed that this is because the ectoderm cells lose their competence before the signal has travelled very far, and this is supported by our recent finding that cell surface FGF receptor density falls sharply from stage 8 to stage 10 (Gillespie *et al.*, 1989). The other possibility is that the induced tissue emits an inhibitor which is so highly diffusible that it rapidly spreads out with a near uniform concentration across the whole field. This means that when a certain fraction of the field has become induced the inhibitor concentration will have built up to a level capable of stopping any further spread. The appeal of this type of mechanism is that it can explain proportion regulation since the final size of the induced zone is scaled to the size of the whole. Proportion regulation is a well known feature of early amphibian development (Cooke 1981), and good evidence has recently been obtained for its occurrence in mesoderm induction (Cooke 1989), but it must be said that there is as yet no direct evidence for the secretion of an inhibitor by the induced tissue.

Materials and Methods

Xenopus embryos were produced by artificial fertilization as described by Godsave *et al.* (1988). Either donors or hosts were labeled at the 2 cell stage by microinjection of both blastomeres with 15 microlitres of 10% rhodamine-dextran-amine (RDA) as described by Dale and Slack (1987). In this study "donor" means the provider of the archenteron roof tissue and "host" means the provider of the ectoderm. The donors were allowed to develop to stage 14 and then dissected in 0.01% trypsin as described by Slack (1984). Either the notochord with adhering hypochordal plate, or one presomite plate freed of endoderm, was used as the inducer. This was wrapped in two animal pole explants from stage 8 blastulae to form a sandwich as shown in Fig. 1.

The sandwiches were cultured for three days at 25°C until control embryos had reached stage 40-43 and were then fixed in paraformaldehyde, embedded in polyethylene glycol distearate (or in Surgipath wax), sectioned at 5 microns (8 microns for Surgipath), stained with the muscle-specific antibody 12/101 (Kintner and Brockes 1984) followed by FITC-anti mouse IgG, and counterstained with DAPI. Details of the immunohistochemical methods can be found in Dale *et al.* (1985). The sections were examined by fluorescence microscopy using the fluorescein channel for the muscle, the rhodamine channel to identify cell provenance, and the DAPI channel to identify cell nuclei.

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