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Analysis of embryonic induction by using cell lineage markers

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[Plate 1]

Three distinct inductive interactions have been demonstrated in early embryos of *Xenopus laevis*: mesoderm induction, dorsalization and neural induction. The experiments were done with grafts from embryos uniformly labelled with passive cell lineage markers, either FITC-lysine-dextran (FLDx) or horseradish peroxidase (HRP), which allow the provenance of regions to be determined down to the single cell level. In each case the fate of the target tissue in the presence of the appropriate inductor was quite different from the fate in normal development.

Only two mechanisms are known to be responsible for regional specification in early embryos: cytoplasmic localization and induction. Cytoplasmic localization involves the unequal partition of a determinant between daughter cells, and in a variety of types of embryo this seems to be important for the first step in the formation of the body plan (Subtelny & Konigsberg 1979). Induction, on the other hand, involves an interaction between two groups of cells: a signalling region and a responding region. The responding region embodies a competence to follow two or more developmental pathways, and the pathway a cell follows depends on its position in relation to the signalling centre (Wolpert 1969). Where there are several possible pathways the responding region becomes partitioned into a series of differently specified zones, and an interaction of this kind, having multiple outcomes, is sometimes described as a 'morphogenetic gradient'. In all animal types for which a reasonable body of experimental evidence exists it is clear that a sequence of inductive interactions plays the major role in the formation of the general body plan (Slack 1983).

Despite immense progress in our knowledge of the control of gene expression in terminal differentiation, molecular biology has so far made little contribution to our understanding of regional specification in early embryos. With respect to induction, although the phenomenon was first described eighty years ago (Lewis 1904), we still do not know the nature of the signals, how they are transmitted from cell to cell, the character of the initial responses and how these become stabilized as states of determination. In fact there has been a tendency in recent years to ignore this group of problems or even to argue that induction does not exist at all but is an artefact of cellular selection in conditions of rapid growth. Embryological textbooks, on the other hand, tend to accept certain of the classical experiments in an uncritical way and to relegate the whole complex process of body plan formation to a single event vaguely referred to as 'primary induction' or 'the organizer'.

The purpose of the present paper is to show that the formation of the body plan in *Xenopus* embryos involves at least three, and perhaps more, inductive steps. These are revealed unambiguously by the use of highly discriminating markers which have recently been introduced for the study of cell lineage: fluorescein-lysine-dextran (FLDx; Gimlich & Cooke 1983) and horseradish peroxidase (HRP; Weisblat *et al.* 1978; Jacobson & Hirose 1978).

In most of the experiments to be described the label is injected into fertilized eggs to produce uniformly labelled embryos. These are allowed to develop to the appropriate stage and then grafts are done between the labelled donors and unlabelled hosts. The compound embryos are allowed to develop until a suitable stage, usually tailbud, then they are fixed and sectioned, and the position and type of the labelled cells is identified. As both FLDx and HRP are of sufficient molecular mass not to diffuse through the gap junctions between cells, they mark all the progeny of the original labelled cells in the combination. They are both retained in position by aldehyde fixatives. FLDx is fluorescent and can be localized in ordinary paraffin sections. HRP is localized in frozen sections by a histochemical reaction for peroxidase (Mesulam 1976). It is important to understand that in amphibian embryos, as in most free living embryos, there is no net growth in early development; all the cell divisions are cleavage divisions in which the daughter cells are smaller than the mother. This means that passive lineage labels like FLDx or HRP do not become diluted as development proceeds. Similar studies on higher vertebrates require genetic labels because the embryos are growing from the earliest stages and passive labels will rapidly become diluted out.

MESODERMAL INDUCTION

The first inductive interaction is the formation of a mesodermal region around the equator of the blastula as a result of the action of the vegetal region on the animal hemisphere (Nieuwkoop 1969, 1973). We have used the FLDx label to compare the development of the animal pole region *in situ* (its normal fate) with its development when combined with vegetal tissue (figure 1).
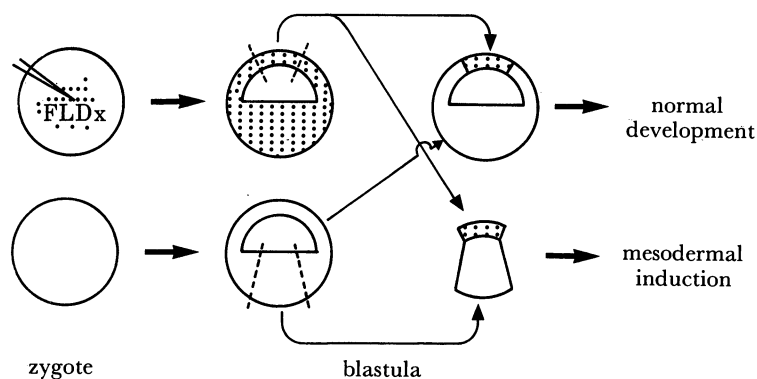

FIGURE 1. Comparison of the normal fate of the animal pole region with its fate after combination with vegetal pole tissue.

The normal fate of this region has been determined by orthotopic grafts at stage $7\frac{1}{2}$ (blastula) and stage 10 (early gastrula). When embryos derived from stage $7\frac{1}{2}$ grafts are examined at later stages, the labelled cells are found mainly in the epidermis, neural tube and neural crest. There are also many labelled cells in the mesenchyme of the head and a few in the myotomes and lateral mesoderm. It is likely that all these are of neural crest origin but we cannot positively exclude a small contribution to mesodermal structures. Embryos arising from stage 10 grafts contain labelled cells mainly in the epidermis and its derivitives such as lens, ear vesicle or lateral line (figure 2 (*a*) and (*b*), plate 1). There are a few labelled cells in the head mesenchyme
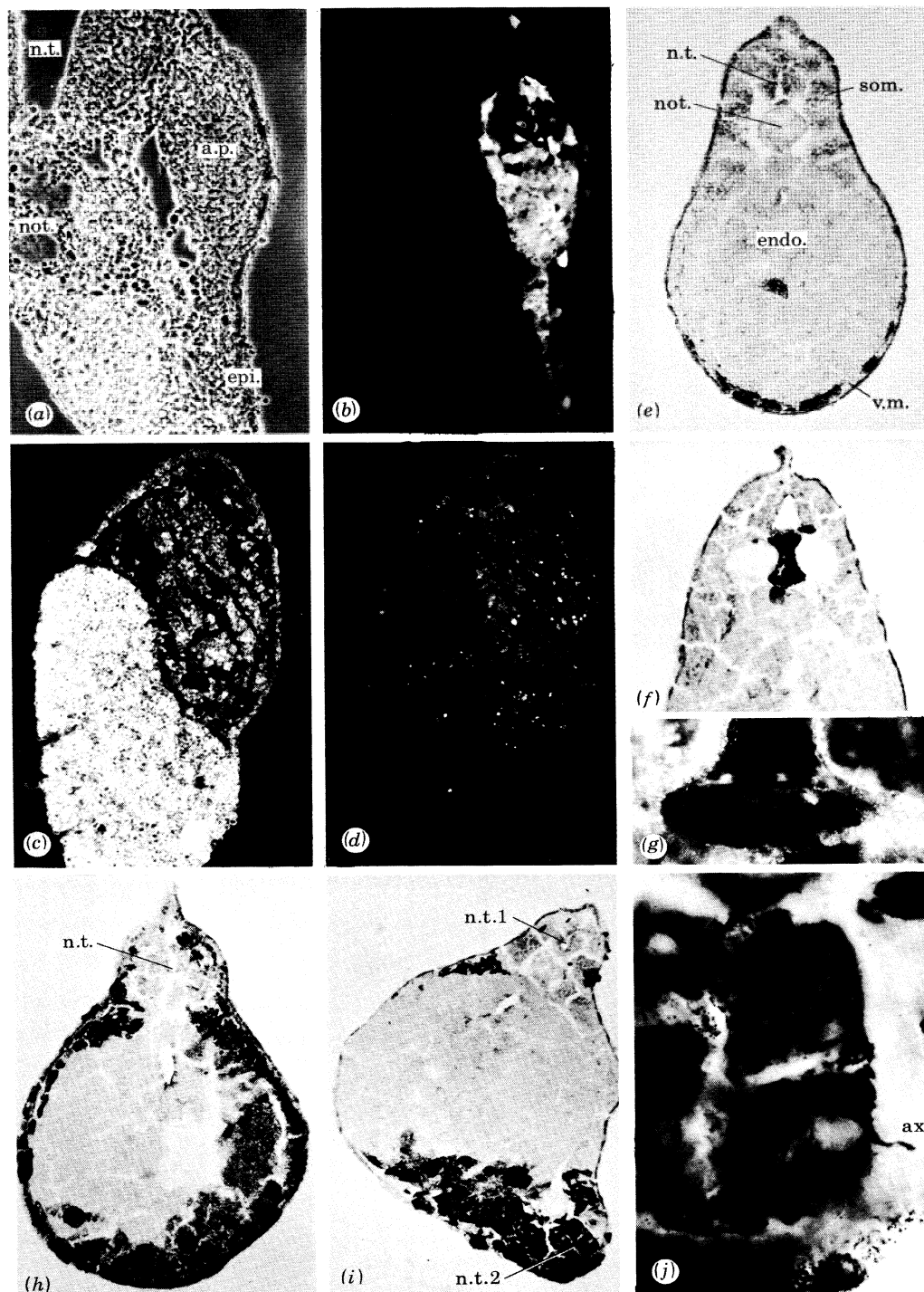
FIGURE 2. (*a*), (*b*) Normal fate of an FLDx labelled animal pole graft. These pictures show part of a tailbud stage *Xenopus* embryo in which the ear vesicle and epidermis are labelled. (*a*) Phase contrast. (*b*) Fluorescence: n.t., neural tube; not., notochord; a.p., auditory placord; epi., epidermis. (*c*), (*d*) Mesodermal induction, an animal–vegetal combination is shown in which the labelled tissue is mainly muscle. (*c*) Dark field. (*d*) Fluoresence. (*e*) Normal fate of HRP labelled ventral marginal zone (transverse section through trunk region). (*f*) HRP labelled v.m.z. after grafting to the dorsal mid-line at stage 10. The graft tissue has split the host notochord into two. (*g*) higher power to show extensive muscle differentation in the graft. (*h*) Normal fate of HRP labelled vegetal ventral blastomere pair. Note that the neural tube (n.t.) is unlabelled. (*i*) A similar embryo which received an organizer graft at stage 10. The secondary neural tube (n.t.2) is labelled and so must have arisen from progeny of the ventral vegetal blastomere pair. (*j*) High-power view of the secondary neural tube showing an axon (a.) growing into the surroundings.

and lateral plate which may be derived from the neural crest. At both stages therefore the labelling is predominantly of ectodermal rather than mesodermal structures, the main difference being that more neural tube and neural crest is labelled at blastula as compared with gastrula stage.

The fate of the labelled cells in the combinations is quite different. In combinations made at stage $7\frac{1}{2}$ most cases contain a large proportion of labelled muscle (figure 2 (c) and (d), plate 1), and about half of the cases also contain a mass of labelled notochord. Most of the undifferentiated yolky part of the combinations is unlabelled, showing that it is derived from the vegetal component. However, many cases contain a few labelled cells in this region as well. It is clear that the developmental pathway of the animal pole tissue has been altered by its new environment and that a large proportion differentiates into mesodermal rather than ectodermal derivatives. The same experiment conducted with stage 10 animal pole tissue gave only a few positive cases suggesting that the competence of this tissue is lost around the beginning of gastrulation. It should be noted that *isolated* animal pole tissue in the same medium has often been shown to form only epidermis (see, for example, Slack & Forman 1980).

One can never prove that interactions demonstrated by abnormal graft–host combinations also occur in normal development, but the most economical hypothesis for the normal formation of the mesoderm is that it arises by induction from the animal hemisphere and that its equatorial position is a result of proximity of this region to the vegetal hemisphere.

DORSALIZATION

We call the next interaction 'dorsalization'. It probably starts concurrently with mesodermal induction but can be demonstrated at a later stage and so should be regarded as a distinct, although overlapping, process.

The experiment shown in figure 3 is designed to compare the normal fate of the *ventral marginal zone* of the early gastrula with its course of development after transplantation to a dorsal environment. The normal fate is confined to the ventroposterior part of the tailbud stage embryo. The graft shown, which somewhat exceeds the ventral mesodermal anlage, contributes labelled cells to lateral mesoderm and blood islands, epidermis and yolky endoderm (figure 2 (e), plate 1). A few labelled cells sometimes are also found in the myotomes but this is less than 1 % of the total. The course of development of ventral tissue grafted to the dorsal marginal zone is quite different. The morphogenetic movements of the host keep the graft as a tightly
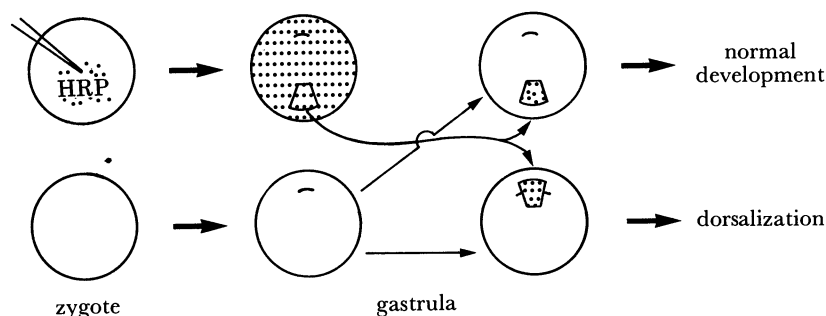

FIGURE 3. Comparison of the normal fate of the ventral marginal zone with its fate after grafting into the dorsal mid-line.

coherent mass of cells in between a bifurcated host notochord. Most of the identifiable cells in the labelled region are myotomal (figure 2 (*f*) and (*g*), plate 1) and in a few cases there is even a small contribution of labelled cells to the notochords. It therefore exhibits a characteristically dorsal mesoderm type of differentiation rather than its normal fate of ventral mesoderm.

We have argued elsewhere (Slack & Forman 1980; Smith & Slack 1983; Slack 1983) that the dorsalization of the marginal zone is the essential interaction revealed by the organizer graft of Spemann & Mangold (1924) and that the remainder of the secondary embryo is formed by subsequent interactions such as neural induction.

Neural induction

In the course of gastrulation the dorsal marginal zone becomes stretched over most of the craniocaudal extent of the embryo and comes to underly the dorsal ectoderm. At this stage the dorsal mesoderm, together with the hypochordal plate, makes up the archenteron roof; it has long been known that it influences the overlying ectoderm to form neural plate in the interaction called neural induction (Saxen 1980).

Neural induction is most elegantly demonstrated by the experiment shown in figure 4, which essentially is similar to that described by Gimlich & Cooke (1983). Here, a particular pair of
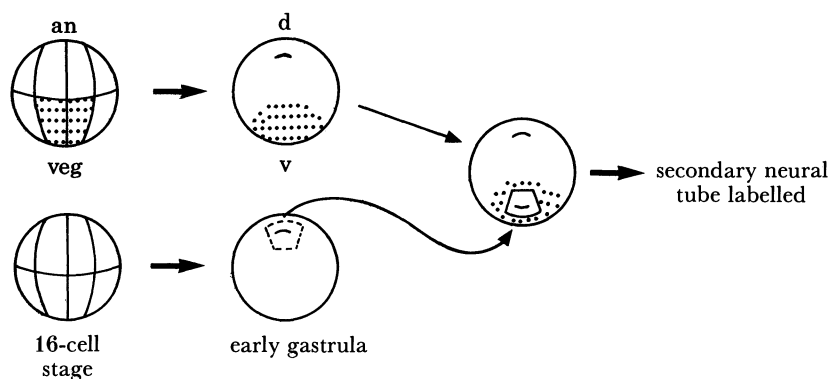

Figure 4. An organizer graft into a host whose vegetal–ventral blastomere pair was labelled at the 16 cell stage.

blastomeres from the ventral side of the 16 cell embryo are labelled with HRP. These blastomeres never normally contribute descendants to the neural tube but do contribute heavily to the ventral epidermis (figure 2 (*h*), plate 1). At stage 10, an organizer graft is done: an unlabelled dorsal marginal zone piece is grafted into the prospective mid-ventral blastopore lip. Because of the dorsalizing signal from the graft, the host develops as a double dorsal embryo and the neural tube of the *secondary* embryo now contains many labelled cells, some clearly identifiable as neurons (figure 2 (*i*) and (*j*), plate 1). Evidently this tissue must have been caused to become neural rather than epidermal by the proximity of the secondary archenteron roof.

Neural induction is probably a good deal more complicated than is often implied. It has been shown that different regions of the archenteron roof induce different regions of the central nervous system and that the neural plate itself acquires the ability to induce further ectoderm in a regionally specific manner (Mangold 1933; Ter Horst 1948; Nieuwkoop 1952). This

regional specificity is not, however, shared by the pure chemical substances that can bring about neural tube formation in ectoderm of certain species (Needham 1942). The regional specificity of neural induction implies that the dorsal mesoderm becomes subdivided into a series of differently specified zones along the craniocaudal axis during the process of gastrulation, and that this hierarchy of states is in some way transmitted to the overlying ectoderm (Slack 1983).

Conclusions

We are led to conclude from the type of data presented above that at least three inductive interactions occur during the formation of the basic body plan in *Xenopus*: mesodermal induction; dorsalization; and neural induction. All of these interactions seem to involve multiple outcomes from the responding tissue. The sequence of events which we think is compatible with the main body of work on the early amphibian embryo (reviewed by Nakamura & Toivonen 1978; Slack 1983) is shown in figure 5.
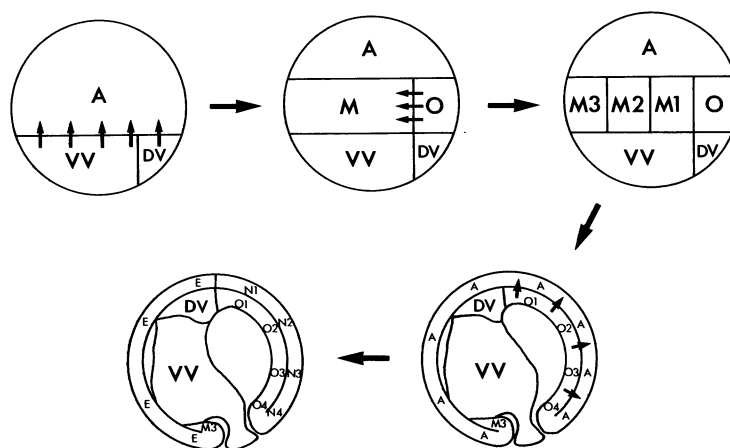

FIGURE 5. Inductive interactions in early amphibian development.

The initial regional differences in the egg seem to arise by cytoplasmic localization, along the egg axis during oögenesis and along the dorsoventral axis in the cytoplasmic reorganization following fertilization (Scharf & Gerhart 1983; Ubbels *et al.* 1983). A consequence of these events is that the vegetal hemisphere becomes a signalling centre and induces at least two mesodermal territories (dorsal and ventral) from the equatorial region of the competent animal hemisphere tissue (Boterenbrood & Nieuwkoop 1973; Gimlich & Gerhart 1984). Dorsalization then results in the formation of a blood forming and a myotomal region in the marginal zone, and possibly a pronephric region as well, under the influence of the dorsal lip region (Slack & Forman 1980; Cooke 1982). The formation of a series of determined regions along the craniocaudal axis seems to be associated in some way with the gastrulation movements but no intercellular signalling has yet been demonstrated at this step. Finally, neural induction occurs as a series of interactions between the regions of two closely opposed tissue layers, the archenteron roof and the dorsal ectoderm. By this stage the basic body plan of the embryo exists in the form of determined rudiments arranged in the correct spatial pattern. This body plan acts as the scaffolding for the cell migrations and local interactions involved in organogenesis and terminal cell differentiation.

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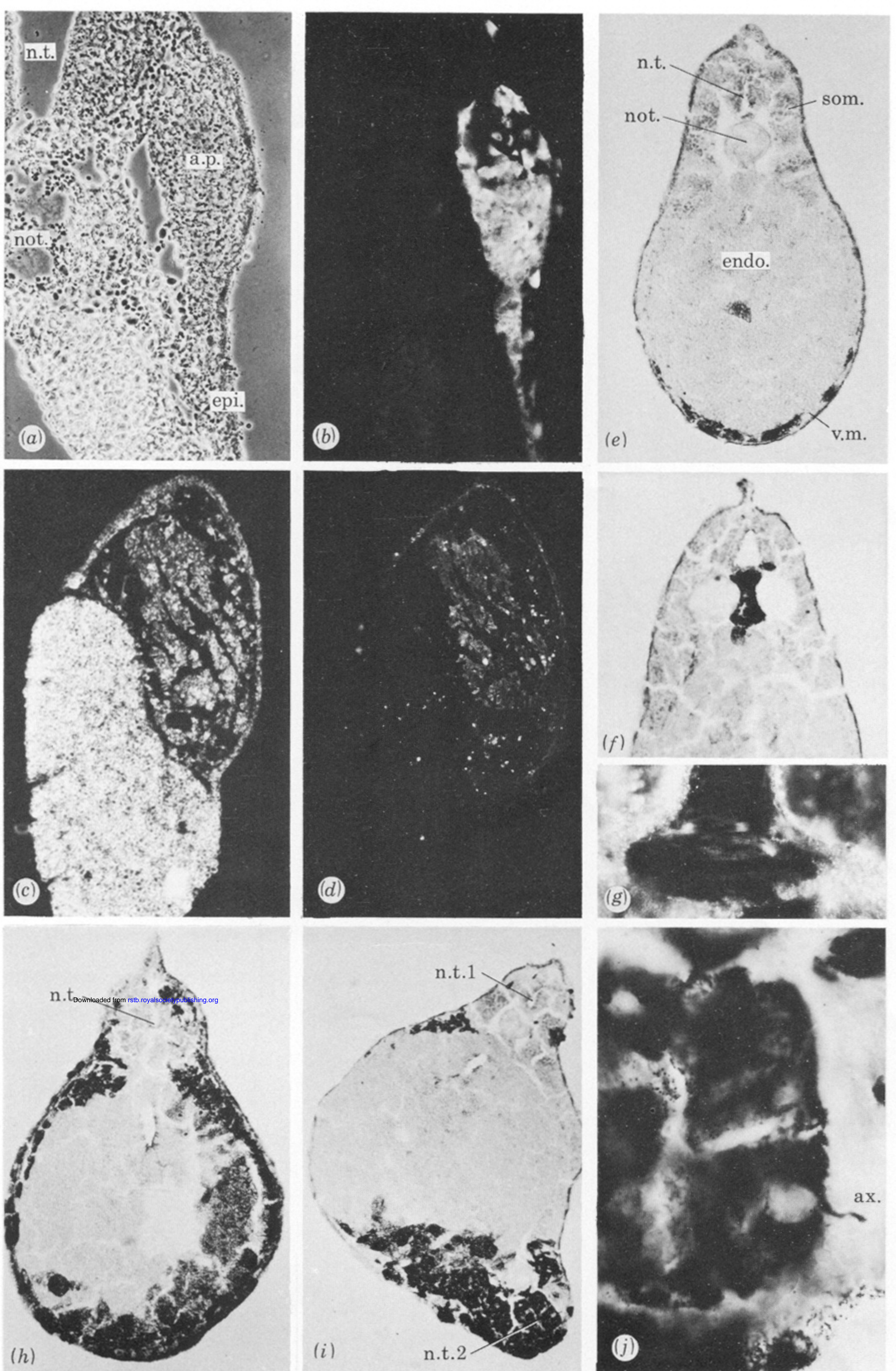
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