

The nature of developmental restrictions in *Xenopus laevis* embryos

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INTRODUCTION

Fate maps of the late blastula stage of the *Xenopus laevis* embryo indicate that the cells of the vegetal pole area are destined to become part of the endoderm germ layer (Keller, 1975; Heasman, Wylie, Hausen & Smith, 1984). By labelling single cells from this region and transplanting them into the blastocoel cavity of host embryos, we have shown that the determinative process that restricts blastomeres to their normal fate occurs between the early blastula and early gastrula stages (Heasman *et al.* 1984). To progress towards an understanding of this process, we need to establish some fundamental points. In particular, the following issues are discussed here. (1) Is cell interaction required for determination to proceed? (2) What is the cellular nature of determination?

We have used the labelling and transplantation technique described previously (Heasman, Snape, Smith & Wylie, 1985; Heasman, Snape, Smith, Holwill & Wylie, 1985) to study these questions in relation to the mechanism of determination of vegetal pole cells in *Xenopus laevis*.

I. IS CELL INTERACTION REQUIRED FOR DETERMINATION TO PROCEED?

One of the oldest questions that has been asked by developmental biologists is whether heterogeneity is already present in the fertilized egg or whether it is established due to cell interaction during early embryogenesis. It has become clear, however, that embryos cannot be categorized rigidly into determinate and indeterminate types. For example, one of the most extreme cases of stereotyped determinate development is that of the nematode *Caenorhabditis elegans* (Sulston, Schierenberg, White & Thomson, 1983), where cell interactions have been shown to be necessary for the establishment of some cell types, including the vulval cells (Kimble, 1981) and 'hook' cells (Greenwald, Sternberg & Horvitz, 1983).

Similarly in sea urchin embryos, the weight of evidence suggests that blastomeres in different regions of the embryo become determined by different

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mechanisms. Culturing experiments indicate that the micromeres become committed autonomously, while in chimaeric recombinations cells from the other tiers of the 16-cell embryo are labile and subject to inductive interactions (reviewed by Angerer & Davidson, 1984). This heterogeneity in behaviour has not so far been attributed to the pattern of mRNAs or of protein constituents at the 16-cell stage. So far as can be detected, these molecules are homogeneously distributed in the embryo, (Ernst, Hough-Evans, Britten & Davidson, 1981; Tufaro & Brandhorst, 1979). However, germ-layer-specific molecules have been recognized by the hatching and mesenchyme blastula stages, using monoclonal antibodies (Wessel & McClay, 1985) and *in situ* hybridizations of cloned probes (Angerer & Davidson, 1984). The suggestion is that differences at the 16-cell stage may be too small to detect by the means available.

The three germ layers of *Xenopus* embryos also appear to be established by different mechanisms. Indeed, there is evidence from ligation, ablation and culture studies (Gurdon, Mohun, Fairman & Brennan, 1985) and from cell labelling and transplantation work (Gimlich, 1985) that the mesoderm layer forms as a result of initial cytoplasmic localizations in the fertilized egg enhanced later by interactions between vegetal and equatorial blastomeres. In contrast, there are reports from disaggregation and culture experiments that activation of muscle-specific α -actin genes is absolutely dependent on cell contacts at least until the 128-cell stage (Sargent, Jamrich & Dawid, 1986).

The nature of localized determinants is unknown. It has been shown that there is a small population of mRNAs in the fertilized egg that are regionally distributed and inherited by different blastomeres (Rebagliati, Weeks, Harvey & Melton, 1985). However, the developmental importance of these RNAs is not yet known.

The requirement for cell interaction in the determination of the endoderm layer is equally uncertain. Experiments using molecular markers show that dissociated cells transcribe endoderm-specific mRNAs on schedule, suggesting that cell interaction is not required (Sargent *et al.* 1986). However, there is evidence, particularly from work on urodele amphibians, that pharyngeal and dorsal endoderm form as a result of cell interactions between suprablastoporal cells and the vegetal mass (Nieuwkoop & Ubbels, 1972).

In recent experiments on *Xenopus laevis*, we have shown that, at the early blastula stage, cells of the vegetal pole are not committed to form endoderm. When transplanted into the blastocoels of host embryos single labelled cells produce progeny in ectoderm, mesoderm and endoderm germ layers (Heasman *et al.* 1984; Table 1; Fig. 1). They can thus be defined as pluripotent and the host blastocoel cavity can be defined as a 'neutral site', allowing cells inserted therein a free choice of entry into any of the prospective germ layer regions. This suggests that if cytoplasmic determinants are responsible for vegetal pole determination they do not act before the early blastula stage or their effect can be overridden.

During the following 5 h, however, the behaviour of vegetal pole cells changes, so that when they are transplanted into host embryos an increasing proportion of them enter only the embryonic endoderm (their normal fate). By the early

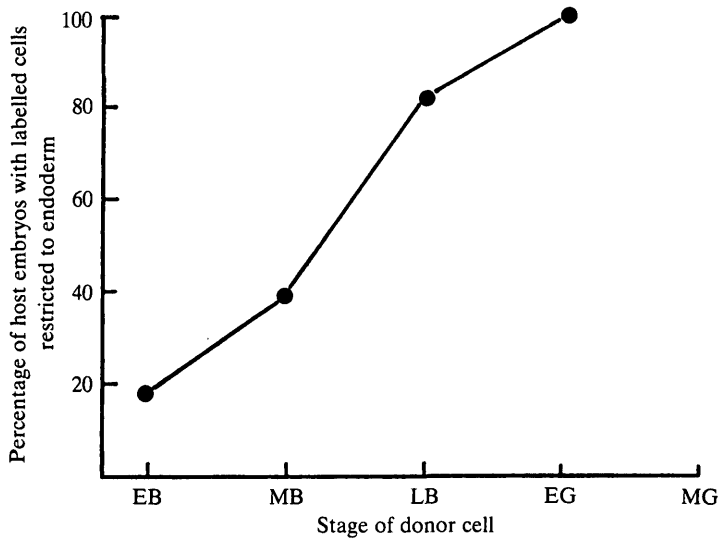


Fig. 1. Embryonic stage and the state of commitment of vegetal pole cells. EB, early blastula; EG, early gastrula; MB, midblastula; MG, midgastrula; LB, late blastula.

gastrula stage, all vegetal pole cells are found only in the endoderm of host embryos (Table 1; Fig. 1). The mechanism by which this change occurs is unknown. The most likely explanation is that during this period the blastomeres become increasingly able to sort to the correct germ layer primordium in the host embryo and do not colonize other areas. The absence of label in other areas supports this view. This progressive change in behaviour of vegetal pole cells is loosely defined as 'commitment'. We do not know whether it is accompanied by loss of the capacity to differentiate into other lineages, given appropriate stimuli, as in a grafting experiment. Vegetal pole cells at the early blastula stage can

Table 1. Results of transplanting single vegetal pole cells of various stages into blastula hosts

Stage of donor cells	Hosts with labelled cells	Number of host embryos with labelled cells in the following germ layers						
		end only	end mes ect	end mes only	end ect only	mes ect only	mes only	ect only
* Early blastula (stage 6-7)	14	4	5	1	3	1	—	—
* Midblastula (stage 8)	44	15	14	1	11	3	—	—
* Late blastula (stage 9)	47	38	1	—	6	—	2	—
* Early gastrula	22	22	—	—	—	—	—	—

* Includes data from Heasman *et al.* (1984).

colonize most (possibly all) regions of the host, where they can differentiate appropriately, and are therefore truly pluripotent. Early gastrula vegetal pole cells have become restricted in the area of the host embryo they can colonize and so always differentiate, under these conditions of testing, according to their normal fate. It is this particular restriction of developmental capacity that we are defining as 'commitment'.

If cytoplasmic localizations are responsible for commitment of vegetal pole cells in *Xenopus*, then it becomes interesting to know whether single vegetal pole cells can become more committed in isolation from the rest of the embryo or whether cell interactions are necessary. To do this, we dissociated cells from the vegetal poles of midblastula-stage embryos and cultured them in isolation until sibling embryos reached early gastrula stage. Initial experiments suggested that single cells did not become further committed in culture, although whole pieces of vegetal tissue did (Heasman *et al.* 1985).

More recently, we have asked the question whether the requirement for large numbers of cells is specific or not. The design of the experiment is illustrated in Fig. 2. Here the state of commitment of cells cultured in isolation was compared with that of cells cultured with a mass of cells dissociated from the animal cap and a similar-sized mass of cells collected from a very circumscribed zone at the vegetal pole of many embryos. As Table 2 shows, the animal cap cells were almost as successful as vegetal pole cells in causing the labelled blastomeres to become more committed in culture. While isolated cells became 68% committed to form endoderm, in combination with animal pole cells the level rose to 90% and with other vegetal cells to 99%. This suggests that the cell interaction requirement is a nonspecific tissue mass effect. A large number of cells is needed to condition the medium, providing a suitable environment in which commitment can proceed. These results indicate that a specific signalling centre or cell interaction is not necessary for commitment as it is unlikely that this could reside both in the animal cap and in the vegetal pole. The suggestion is that the change can proceed autonomously as long as suitable culturing conditions are provided.

II. WHAT IS THE CELLULAR NATURE OF COMMITMENT?

The results discussed above and elsewhere indicate that the process of commitment at the vegetal pole of *Xenopus* embryos happens gradually between the blastula and gastrula stages of development. During this time the embryo cell number increases from 128 to 20 000 cells and, as no growth occurs, cell size becomes progressively smaller. There is no obvious correlation of the timing of commitment with the midblastula transition events described by others (Newport & Kirschner, 1982). One central and unresolved problem is the nature of the commitment process in each of these cells. There are at least two possible models that would explain the gradual progression we see. First, a switch could occur in

Table 2. Results of culturing vegetal pole cells from midblastula embryos single and in clumps. A, B, C, D refer to the experimental manipulations shown in Fig. 2

Source of donor cells	Number of hosts with labelled cells	The number of hosts with labelled cells in the following combinations of germ layers										
		end only	end mes ect	end mes only	end ect only	mes ect only	mes only	ect only	mes ect only	mes only	ect only	
(A) st. 8 veg. pole straight back in	22	10 (45%)	—	—	4	—	—	—	3	5	—	—
(B) veg. clone 1	4	4	—	—	—	—	—	—	—	—	—	—
veg. clone 2	14	9	—	—	5	—	—	—	—	—	—	—
veg. clone 3	9	7	—	—	1	—	—	—	—	1	—	—
veg. clone 4	9	6	—	—	1	—	—	—	—	2	—	—
veg. clone 5	9	5	—	—	—	—	—	—	—	4	—	—
Total	45	31 (68%)	—	—	7	—	—	—	—	7	—	—
(C) single veg. cells + animal pole cells	10	—	—	—	—	—	—	—	—	—	—	—
clone 1	9	9	—	—	—	—	—	—	—	1	—	—
clone 2	9	8	—	—	—	—	—	—	—	1	—	—
Total	19	17 (90%)	—	—	—	—	—	—	—	2	—	—
(D) Clump of veg. pole cells	33	32 (99%)	—	—	—	—	—	—	—	1	—	—

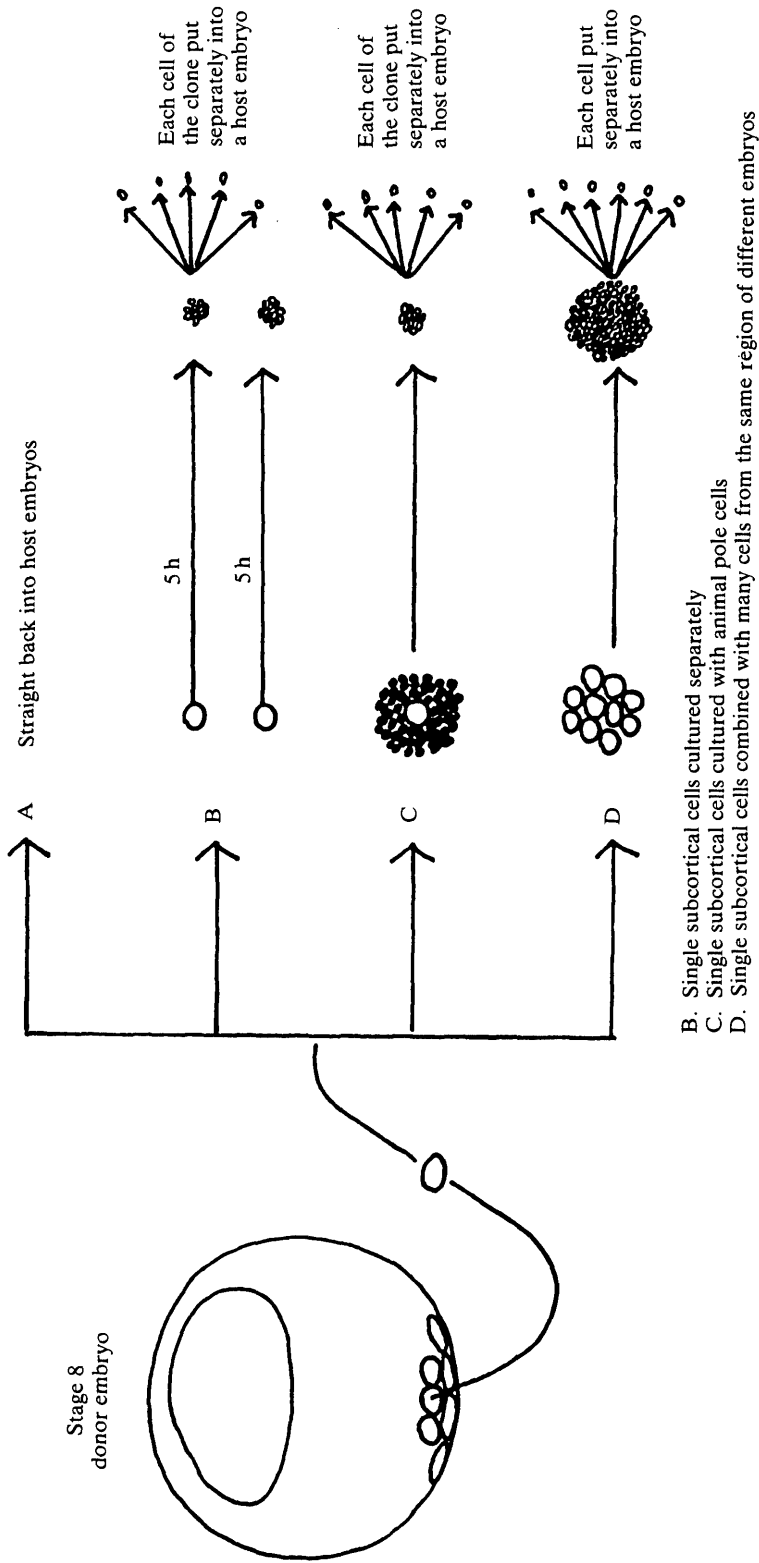


Fig. 2. The design of an experiment to test the importance of cell interaction in determination.

each cell at the vegetal pole. In its simplest form, individual cells are either pluripotent or stably switched to endoderm only, the change happening rapidly but at different times in each cell of the population. According to this model, when the cells are 40% committed at the midblastula stage, the interpretation is that four out of every ten blastomeres on average are committed to endoderm while the rest are still pluripotent. By the late blastula stage eight out of ten are committed. Examples of such deterministic processes are found in the invariant development of nematode (Sulston *et al.* 1983) and leech embryos (Stent, Weisblat, Blair & Zackson, 1982).

Second, an alternative strategy by which gradual commitment could occur is to consider that there is no simple switch but that all cells of the population are at the same state of commitment at any time point and that they progress together towards the committed state. The transition between the uncommitted and committed states may be governed by a single rate-limiting process. Such stochastic models have been suggested to best explain the kinetics of terminal differentiation of haemopoietic stem cells (Till, McCulloch & Siminovitch, 1963), erythroid cells (Levenson & Housman, 1981) and melanoma cells (Bennett, 1983), and also to explain the variability of G₁ phase of mitosis in mammalian cells (Smith & Martin, 1973; Brooks, 1985). A simple example of such a probabilistic model for *Xenopus* blastomeres is to suggest that, during the early blastula stage, cells in the vegetal pole start to synthesize a substance X on their surfaces. As X accumulates, the cells become progressively more able to sort back to the endoderm germ layer of the host embryo when placed in the blastocoel cavity. According to this model, 40% commitment to form endoderm at the midblastula stage would be interpreted as a 40% chance that each cell has to sort to the endoderm germ layer. By the early gastrula stage, so much X has accumulated that all the cells from this region will return to it when transplanted elsewhere.

To distinguish between these two possibilities, it is necessary to assay single cells of the population in order to discover their states of commitment at the mid- and late-blastula stages. The strategy that we have used is to isolate vegetal pole cells at the midblastula stage and culture them singly for 5 h, during which time each cell forms a clone. If the deterministic model is correct then four out of ten isolated cells, on average, will be already committed and this state should be stably inherited by the daughter cells, producing whole clones committed to endoderm only. If the probabilistic model applies, then all the clones would be expected to be mixed. Table 2 summarizes the clonal analysis data drawn from this experiment. Only one out of seven clones analysed consisted of cells all committed to endoderm. A similar experiment has produced the same result of one out of seven clones committed to endoderm. According to the deterministic theory, at least 40% of the clones should be committed to endoderm. While many more clonal analysis data are required, the evidence to date favours the probabilistic model of determination and makes it seem possible that there is a rate-limiting component responsible for commitment. By introducing specific inhibitors of macromolecule

synthesis into the culture conditions of vegetal pole cells we may be able to analyse the nature of this rate-limiting component.

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