Fate map for the 32-cell stage of *Xenopus laevis*

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**Summary**

A complete fate map has been produced for the 32-cell stage of *Xenopus laevis*. Embryos with a regular cleavage pattern were selected and individual blastomeres were injected with the lineage label fluorescein-dextran-amine (FDA). The spatial location of the clones was deduced from three-dimensional (3D) reconstructions of later stages and the volume of each tissue colonized by labelled cells in each tissue was measured.

The results from 107 cases were pooled to give a fate map which shows the fate of each blastomere in terms of tissue types, the composition of each tissue by blastomere, the location of each prospective region on the embryo and the fate of each blastomere in terms of spatial localization.

Morphogenetic movements up to stage 10 (early gastrula) were assessed by carrying out a number of orthotopic grafts at blastula and gastrula stages using donor embryos uniformly labelled with FDA.

Although there is a regular topographic projection from the 32-cell stage this varies a little between individuals because of variability of positions of cleavage planes and because of short-range cell mixing during gastrulation. The cell mixing means that the topographic projection fails for anteroposterior segments of the dorsal axial structures and it is not possible to include short segments of notochord or neural tube or individual somites on the pregastrulation fate map.

Key words: fate map, *Xenopus laevis*, FDA, fluorescein-dextran-amine, orthotopic grafts, blastomere.

**Introduction**

A fate map shows what will become of each region of an embryo in the course of normal development. It depicts in a prospective way both the morphogenetic movements and the fates of regions in terms of cell differentiation. Knowledge of the fate map is an essential precondition for virtually every type of embryological experiment for, unless the normal behaviour of cells is known, we cannot say whether the behaviour has been altered by grafting, isolation, treatment with substances and so on.

Many fate maps have been compiled for amphibian embryos over the years, perhaps the most famous being that of Vogt (1929) for the early gastrula stage of a variety of urodele and anuran species. Vogt made extensive use of both careful morphological description and of localized staining with the vital dyes nile blue and neutral red. Most subsequent studies have used the same methods, for example the maps of the axolotl by Pasteels (1942) and of *Xenopus* by Keller (1975, 1976). Although these fate maps have been of enormous help to the experimenter over the years they are limited by the shortcomings of the vital dye technique. In particular vital dyes are not cell autonomous but tend to spread and fade, so only the main concentration of dye remains visible at later stages. This means that cell mixing is not easily detected and that minority contributions from a particular region tend to be ignored.

An entirely new method of prospective marking was introduced by Weisblat, Sawyer & Stent (1978) which consisted of the injection of horseradish peroxidase into individual cells, allowing the embryo to develop to the desired stage, then staining for peroxidase activity. This method was rapidly applied to amphibian embryos by Jacobson and Hirose (Jacobson & Hirose, 1978, 1981; Hirose & Jacobson, 1979; Jacobson, 1983), who used serial frozen sections to build up 3D reconstructions of labelled clones. This was soon followed up by the introduction of fluorescent lineage labels which were superior to HRP by
virtue of lower toxicity, the possibility of using paraffin sections and the fact that the cytology of the labelled region can be seen as well as the label (Weisblat, Zackson, Blair & Young, 1980; Gimlich & Braun, 1985).

The early studies showed conclusively that injected lineage labels were truly cell autonomous, being passively transmitted to daughter cells on cleavage but failing to enter neighbouring cells over long periods of time. In embryos such as amphibians that do not increase in size during early development lineage labels are not diluted out and remain clearly visible for several days. They therefore offer great advantages for cell marking by comparison with vital dyes; in particular single cell resolution is possible and allows the detection of minority contributions and cell mixing; visualization in serial sections allows accurate 3D reconstructions to be built up; and deep tissues can be followed as easily as surface ones.

Lineage labels can be used for fate mapping in two distinct ways. First, a single identified blastomere can be injected at an early stage and its progeny followed to later stages. Second, embryos can be uniformly labelled by injection of the zygote and then orthotopic grafts performed between labelled and unlabelled embryos. Of course, the latter method is also appropriate to heterotopic grafts and we have made much use of these in our recent studies on mesoderm induction, dorsalization and neural induction (Dale, Smith & Slack, 1985; Smith & Slack, 1983; Slack, 1985).

The present study uses the blastomere injection method to produce a comprehensive fate map of the 32-cell stage of *Xenopus laevis* and orthotopic grafts to check morphogenetic movements up to stage 10 (early gastrula). We chose the 32-cell stage because it is the latest stage at which it is reliably possible to obtain regularly cleaving embryos, i.e. embryos arranged as four tiers of eight cells, and therefore to be confident of labelling the same volume element of the egg in repeated experiments. We chose *Xenopus laevis* because it is now clear that this is the species that the research community has chosen for a decisive attack on developmental mechanisms. *Xenopus* now occupies the position held by the mouse among mammals and *Drosophila* among insects, while *Rana* *pipiens*, the axolotl, *Pleurodeles waltl* and *Cynops pyrrhogaster* are all rapidly becoming relegated to the position of organisms for comparative and confirmatory work.

There have been some previous studies in which lineage labels have been used to fate map the 32-cell stage of *Xenopus*. Jacobson & Hirose (1981) published a detailed fate map for the brain and spinal cord, Gimlich (1986) published reconstructions for blastomeres C1 and D1, and Cooke & Webber (1985) published a fate map for the mesoderm based on injections at the 8- and 32-cell stage, but without reconstructions. Useful as these studies have been we felt the need for a complete fate map based on a large number of cases that would map all the blastomeres onto all the tissues and that would provide tissue-fate information as well as spatial information. The study is presented here and since its completion we have been making much use of the results in designing experiments on inductive interactions in early development. The results should also enable other investigators to perform experiments for which answers to the following types of questions are needed:

1. Can I find a blastomere that populates exclusively a given tissue?
2. Can I find a blastomere that does not normally contribute to a given tissue?
3. Which blastomeres do I need to label (ablate, isolate) if I want to cover the entirety of a given tissue?

To this end we have presented the data in several forms. Tables of figures show both percentage contributions of individual blastomeres to tissues and also the percentage composition of tissues by blastomere. Drawings of archetypal cases show typical spatial distributions of particular injections; layer graphs show the average contribution over a series of cases to each tissue at each anteroposterior level; stylized embryos, like the classical fate map, map tissues onto blastomeres; and finally pie diagrams show visually the composition of each tissue in terms of individual blastomere contribution. This may seem a lot of information compared to the classical fate map which is expressed as a single drawing, but it is necessary both because of the essential indeterminacy of the map and because of the various different uses to which we expect the data to be put by other workers.

In addition, the orthotopic grafts at blastula and early gastrula stages confirm and extend the data presented in our previous papers and show how the fate map changes from stage 6 to stage 10.

**Materials and methods**

**Preparation of specimens**

Eggs were obtained from female *Xenopus laevis* injected the previous evening with 500 i.u. human chorionic gonadotrophin (‘Pregnyl’: Organon). They were fertilized artificially using the macerated testes of a dead male. Embryos were then chemically dejellied with 2 % cysteine hydrochloride (pH 7.8–8.1), thoroughly washed and transferred into 5 % NAM (Slack, 1984) in Petri dishes coated with 1 % agar (Noble agar: Difco). Note that in dilutions of NAM only the salts are diluted, not the buffers or antibiotic. All embryos were staged according to Nieuwkoop & Faber (1967).
Dorsoventral polarity was ascertained at the 8-cell stage when dorsal micromeres are often less pigmented than ventral micromeres (Nieuwkoop & Faber, 1967). Embryos displaying a regular cleavage pattern were then marked in the dorsal equatorial zone with a crystal of Nile red (Kirschner & Hara, 1980). The accuracy of this method was checked by a prospective study on eight egg batches by measuring the angle between the Nile red stain and the dorsal blastopore at the early gastrula stage. In 83% of cases (n = 70) they were coincident and in 95% of cases the stain was within 60° of the dorsal blastopore lip.

Stage-6 (32-cell) embryos were selected in which the blastomeres were regularly arranged as four tiers of eight cells. The proportion of such eggs tends to depend on the female: some lay batches with a high proportion of regular cleavages while some lay batches with few if any regular cleavages. For this sort of work it is therefore worth keeping records and setting aside the more suitable females specifically for blastomere injection. The nomenclature used for each blastomere at this stage is that of Nakamura & Kishiyama (1971) and is shown in Fig. 1A; tiers A to D run from animal to vegetal and columns 1 to 4 run from dorsal to ventral. In each embryo a single blastomere was injected with 2 nl of the lineage label fluorescein-dextran-amine (FDA: Gimlich & Braun, 1985) at 100 mg ml⁻¹ in water. During and after injection they were kept in 5% NAM containing 5% w/v Ficoll (type 400: Pharmacia) to prevent leakage of cytoplasm (Newport & Kirschner, 1982). Injections were performed using a Burleigh inchworm to drive a 10 μl pressure-tight syringe connected by a liquid-filled tube to the injection needle. The external diameter of the needle tip was about 20 μm.

The fate of the animal pole region and of dorsal, dorsolateral, ventrolateral and ventral fragments of early gastrula marginal zones (DMZ, DLMZ, VLMZ and VMZ, respectively, Fig. 1B,C) was established by orthotopic grafts between labelled and unlabelled embryos. Labelled embryos were produced by injecting zygotes with 20 nl of FDA and grafts were performed in full-strength NAM using ground forceps, hair loops and electrolytically sharpened tungsten needles. Following wound healing embryos were gradually returned to 5% NAM to prevent exogastrulation.

All specimens were cultured until stages 28-30 (early tailbud) and fixed in 4% paraformaldehyde in 70% PBSA overnight at 4°C. A small number of cases with abnormal external morphology was discarded. The embryos were then washed in 70% PBSA, dehydrated in an ethanol: butanol series, embedded in paraffin wax (m.p. 56°C) and sectioned transversely at 10 μm. After dewaxing in xylene, rehydrated specimens were stained with 0.5 μg ml⁻¹ DAPI (4,6-diamidino-2-phenyl indole: Boehringer) for 1 min. All specimens were then dehydrated, cleared in xylene and mounted in DPX. Some examples of the cases used are shown in Fig. 2 and show the sharp discrimination between labelled and unlabelled cells.

**Scoring and analysis**

The specimens were scored on a Zeiss photomicroscope equipped with the appropriate filter sets for fluorescein and DAPI fluorescence. Each specimen was analysed every tenth section to determine the absolute volume of each tissue type derived from individual blastomeres labelled at stage 6. This was calculated by dividing the field of view (magnification ×250) into squares of side 9-25 μm using an eyepiece graticule and counting those squares in which at least 50% of the area contained FDA. Only embryos with a normal internal morphology and no cell debris were included. The distribution of labelled blastomeres was also reconstructed on a standard set of diagrams representing transverse sections at eight locations along the anterior-posterior axis of the embryo (Fig. 5). For each specimen the distribution and volume of labelled tissues were compared both with other specimens in which the same blastomere was injected and specimens in which the sister blastomere was injected. This was to ensure that no specimens in which both of the sister blastomeres had been labelled by a single injection were included for further analysis. This can

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**Fig. 1.** (A) Nomenclature of blastomeres at the 32-cell stage. D, dorsal; V, ventral; AP, animal pole; VP, vegetal pole. (B) Orthotopic grafts at stage 10 viewed from vegetal pole. (C) Orthotopic grafts viewed in median section. AP, animal pole; VP, vegetal pole; DMZ, dorsal marginal zone; DLMZ, dorsolateral marginal zone; VLMZ, ventrolateral marginal zone; VMZ, ventral marginal zone.
happen because a cytoplasmic bridge persists for some time between sister blastomeres even though the cleavage appears complete from the exterior. Similarly a comparison between neighbouring blastomere sets was used to remove those cases in which the wrong blastomere had clearly been labelled, presumable as a result of the inaccuracies inherent in the method of assessing the dorsoventral polarity (see above). 107 cases passed all the tests and were included in the analysis presented below. They came from numerous egg batches and included at least five cases for each blastomere. It should be noted that we have chosen absolute volume rather than cell number to score the specimens. The reasons for this are that cell number is constantly increasing and cell division rates differ between tissues, so we felt that volume was the right measure for construction of a fate map.

Ten tissue types were scored in this analysis: epidermis, brain, spinal cord, head mesenchyme, trunk mesenchyme, notochord, somites, anterior mesoderm, lateral plate and endoderm. Although the definition of many of these tissue types is selfexplanatory, some need further explanation. 'Mesenchyme' means tissue of loose mesenchymal appearance whereas 'mesoderm' means a coherent cell sheet. Two clearly defined markers were used to divide the embryo into three regions, the liver diverticulum (level of somite 2) was used to define the boundary between head and trunk and the proctodeum to define the boundary between trunk and tail. Brain and head mesenchyme are defined by their location in the head; spinal cord and trunk mesenchyme by their location in the trunk and tail. Anterior mesoderm is similarly defined by its location in the head, it included cells of the prechordal plate and heart but not notochord and somites. Lateral plate mesoderm comprises the pronephros and blood islands as well as the lateral plate proper. We define 'ectoderm' as comprising brain, spinal cord and epidermis and 'mesoderm' as comprising notochord, somites, anterior mesoderm, lateral plate and mesenchymes. In reality the mesenchymes probably have a mixed origin with a substantial neural crest contribution.

Layer and pie diagrams were generated on an Olivetti M24 Personal Computer using the spreadsheet and graphics of The Smart Software System (Innovative Software). The stipple diagrams presented in Fig. 4 were generated on the Olivetti using a BASIC program written in the laboratory. Software limitations required the number of tissue types to be reduced to six for the layer graphs presented in Fig. 6. This was achieved by placing data for the brain and spinal cord into a single category: neural tube, and data for the head and trunk mesenchyme, anterior mesoderm and lateral plate in a single category: lateral plate. Furthermore since individual embryos are of different lengths, the data were normalized to a standard size. Data in the layer graphs are presented as a percentage of head–proctodeum distance, 100% being the location of the proctodeum. Since most of the variability results from the variable length of the tail, data for the head/abdomen and tail were normalized separately with a standard length of 30% chosen for the tail. So the anteroposterior axis of the embryo is represented on these diagrams by 130 units, each unit being 1% of head–proctodeum distance. The liver diverticulum, chosen by us to divide head from trunk, is located at 35–40% on this scale.

Results

Tier and column contributions

Tables 1–4 show averaged results for 107 blastomere injections. Table 1 gives the absolute volumes (1 unit represents 8570 μm3) for each tissue derived from each blastomere. The mean total of fixed and sectional embryos was found to be very close to the mean sum of all contributions from 32 blastomeres, which shows that nothing is being missed or counted twice. Since little net growth occurs over the developmental period studied (Tuft, 1962) there should be a good conservation of relative volume between different parts of the embryo. Probably the exceptions to this are the notochord, whose cells become extensively vacuolated, and the mesenchymes in which there is significant extracellular space. For these tissues the prospective regions at the 32-cell stage will presumably be smaller than the volumes given in Table 1. The assignment of tissue types to germ layers is inevitably subjective but following the convention described above the ecto-, meso- and endoderm are respectively derived from 20, 22 and 58% of the embryo volume at the 32-cell stage.

Tables 2–4 are expressed as percentages rather than absolute volumes. Table 2 breaks down the contribution to each tissue type by halves, tiers and columns of the 32-cell stage embryo. It should be noticed that although dorsal and ventral halves contribute approximately equal volumes, the animal 'hemisphere' contributes only one-third as much as the vegetal 'hemisphere'. This is because C tier blastomeres are about twice the size of A or B tier and D tier blastomeres twice the size again. This fact is readily apparent when a section of an early cleavage embryo is examined but is not so obvious from
Fig. 3. Pie diagrams showing the percentage volume composition of each tissue by blastomere.
the outside. In terms of tissue contributions the table is selfexplanatory but it is perhaps worth drawing attention to the origin of the somites since they loom large in many contemporary studies. The somites are derived almost entirely from the B and C tiers with about one-third coming from the former and two-thirds from the latter. Although they end up on the dorsal side, the majority of the somite volume is derived from the ventral half of the early embryo. For the mesoderm as a whole, 28% comes from the animal and 72% from the vegetal hemisphere.

**Origins of the tissue types**

Table 3 shows how each tissue type is made up by contribution from the individual blastomeres on one side of the body. It is clear that no tissue arises only from a single blastomere at this stage and many tissues arise from quite an extended area. This table contains the essential information for workers who wish to label, isolate or ablate the entirety of the prospective region for a given tissue. So more than 95% of the following tissues can be labelled by the outside. In terms of tissue contributions the table is selfexplanatory but it is perhaps worth drawing attention to the origin of the somites since they loom large in many contemporary studies. The somites are derived almost entirely from the B and C tiers with about one-third coming from the former and two-thirds from the latter. Although they end up on the dorsal side, the majority of the somite volume is derived from the ventral half of the early embryo. For the mesoderm as a whole, 28% comes from the animal and 72% from the vegetal hemisphere.

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<table>
<thead>
<tr>
<th>Tissue</th>
<th>A1-4</th>
<th>B3-4</th>
<th>C3-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>A1-2</td>
<td>B1-2</td>
<td>C2</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>A1-2</td>
<td>B1-3</td>
<td>C1-3</td>
</tr>
<tr>
<td>Notochord</td>
<td>B1</td>
<td>C1-2</td>
<td></td>
</tr>
<tr>
<td>Somite</td>
<td>B1-4</td>
<td>C2-4</td>
<td></td>
</tr>
<tr>
<td>Blood-forming region</td>
<td>C3-4</td>
<td>D3-4</td>
<td></td>
</tr>
</tbody>
</table>

The same information is displayed in the pie diagrams of Fig. 3, which give a clearer visual...
impression of the number of contributing blastomeres and which arranges them in descending order.

In Table 4 the fate map is presented from the point of view of the blastomeres rather than the final embryo. It shows the proportions by which each blastomere becomes the various tissue types. This is quite different from Table 3, for example the D blastomeres each contribute about 20% to the total endoderm, but more than 95% of each D blastomere becomes endoderm. The same information is shown pictorially on the stylized embryo diagrams of Fig. 4 and this shows also the spatial disposition of the prospective regions.

The A tier is shown to become almost entirely ectoderm, 92% to 8% 'mesoderm' much of which is actually mesenchyme. A4 is the blastomere that comes closest to having a single fate in terms of tissue type, 99% of its volume becoming epidermis. This property is very desirable for experiments on either mesodermal or neural induction which are carried out on intact embryos since if A4 is lineage labelled a contribution to epidermis, particularly on the ventral side, and to CNS on the dorsal side. Only the most dorsal blastomeres, B1 and C1, make large contributions to the notochord while all the B tier and C2–4 contribute heavily to somite. The C tier makes a

| Table 2. Composition by percentage of total volume of each tissue in terms of tiers and columns of blastomeres at the 32-cell stage |
|---|---|---|---|---|---|---|---|---|
| Tissues | Epi | Br | SC | HM | TM | No | So | AM | LP | En | Total |
| Animal | 89 | 90 | 65 | 49 | 35 | 46 | 34 | 23 | 1 | 0 | 23 |
| Vegetal | 11 | 10 | 35 | 51 | 65 | 54 | 66 | 77 | 99 | 100 | 77 |
| Dorsal | 24 | 97 | 81 | 84 | 12 | 100 | 42 | 83 | 7 | 55 | 52 |
| Ventral | 76 | 3 | 19 | 16 | 88 | 0 | 58 | 17 | 93 | 45 | 48 |
| A tier | 63 | 51 | 17 | 33 | 6 | 1 | 2 | 0 | 0 | 0 | 12 |
| B tier | 27 | 39 | 48 | 16 | 29 | 45 | 32 | 23 | 1 | 0 | 12 |
| C tier | 10 | 10 | 35 | 49 | 63 | 54 | 64 | 55 | 87 | 18 | 28 |
| D tier | 0 | 0 | 0 | 2 | 2 | 0 | 1 | 22 | 12 | 82 | 49 |
| Column 1 | 9 | 60 | 28 | 27 | 2 | 90 | 7 | 27 | 6 | 30 | 26 |
| Column 2 | 15 | 37 | 54 | 57 | 10 | 10 | 35 | 56 | 1 | 26 | 26 |
| Column 3 | 3 | 18 | 16 | 50 | 0 | 30 | 16 | 27 | 21 | 23 |
| Column 4 | 38 | 0 | 1 | 1 | 30 | 0 | 28 | 1 | 66 | 24 | 25 |

In this and subsequent tables, any deviation of summed percentages from 100 is due to computational rounding errors.

| Table 3. Composition by percentage of total volume of each tissue in terms of individual blastomeres at the 32-cell stage |
|---|---|---|---|---|---|---|---|
| Blastos | Epi | Br | SC | HM | TM | No | So | AM | LP | En | Total |
| A1 | 8 | 34 | 6 | 5 | 0 | 1 | 0 | 0 | 0 | 0 | 3 |
| A2 | 11 | 14 | 9 | 17 | 0 | 0 | 1 | 0 | 0 | 0 | 3 |
| A3 | 26 | 2 | 2 | 11 | 6 | 0 | 1 | 0 | 0 | 0 | 4 |
| A4 | 17 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| B1 | 1 | 19 | 14 | 4 | 0 | 42 | 5 | 3 | 0 | 0 | 3 |
| B2 | 2 | 1 | 1 | 11 | 5 | 3 | 10 | 13 | 0 | 0 | 3 |
| B3 | 3 | 0 | 7 | 1 | 1 | 21 | 0 | 10 | 7 | 0 | 3 |
| B4 | 14 | 0 | 1 | 0 | 2 | 0 | 7 | 0 | 0 | 0 | 3 |
| C1 | 0 | 1 | 3 | 17 | 2 | 46 | 1 | 17 | 4 | 8 | 7 |
| C2 | 0 | 8 | 24 | 29 | 4 | 7 | 23 | 29 | 1 | 6 | 8 |
| C3 | 3 | 1 | 8 | 4 | 30 | 0 | 19 | 8 | 19 | 2 | 5 |
| C4 | 6 | 0 | 4 | 0 | 28 | 0 | 21 | 1 | 63 | 2 | 8 |
| D1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 7 | 1 | 21 | 13 |
| D2 | 0 | 0 | 0 | 1 | 0 | 1 | 3 | 0 | 20 | 12 |
| D3 | 0 | 0 | 0 | 1 | 0 | 1 | 7 | 19 | 12 |
| D4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 22 | 13 |
| Totals | 13 | 4 | 2 | 2 | 1 | 3 | 10 | 2 | 5 | 58 | 100 |
significant contribution to endoderm, particularly on the dorsal side. By contrast the D tier forms almost entirely endoderm and like blastomere A4 approaches monospecificity.

The dorsoventral aspect of the fate map shows a number of important features. The central nervous system comes from the dorsoanimal quadrant and much of the epidermis from the ventroanimal quadrant. The origin of the spinal cord is rather more diffuse than that of the brain and this is probably associated with the lack of a fine-grained mapping to postgastrulation anterior–posterior body levels (to be discussed below). The mesodermal tissues show a clear topographic mapping from dorsal to ventral in the order notochord, somite, lateral plate, and it should be remembered that lateral plate, as defined here, includes the ventral blood islands. There is a very small contribution to lateral plate from dorsal blastomeres (column 1) which is consistent across many specimens. Even smaller contributions from column 2 fail to reach the 1% threshold for inclusion on the diagram but we do not know whether this apparent gap in the prospective region is real since the volumes involved are so small. Although not shown on these diagrams, the pronephros is usually labelled by injection of C3, around the centre of the somite-forming region, and the heart by C1 and C2.
A true fate map not only shows what cell type each region will form but also where in the body it will end up. The spatial trajectories of volume elements shown in a fate map allow us to make an accurate reconstruction of the morphogenetic movements of gastrulation.

### Table 4. Percentage of the volume of each individual blastomere that enters the various tissues

<table>
<thead>
<tr>
<th>Blastomere</th>
<th>n</th>
<th>Epi</th>
<th>Br</th>
<th>SC</th>
<th>HM</th>
<th>TM</th>
<th>No</th>
<th>So</th>
<th>AM</th>
<th>pLP</th>
<th>En</th>
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<tbody>
<tr>
<td>A1</td>
<td>6</td>
<td>34</td>
<td>56</td>
<td>4</td>
<td>4</td>
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<tr>
<td>A2</td>
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<td>55</td>
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<td>5</td>
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</tr>
<tr>
<td>B1</td>
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In some respects the important entries in Table 4 and Fig. 4 are the zeros because these show the blastomeres that do not normally contribute as much as 1% of their volume to the tissue concerned. These are the ones that can be labelled in experiments on induction where a change of fate is looked for.

Several minor structures, that form parts of the major categories considered up to now, were separately counted in terms of whether or not they were labelled in each of the 107 specimens. This is not a rigorous quantitative procedure but gives a good idea of where the prospective regions lie on the fate map.

**Structure**  | Usually labelled by | Sometimes labelled by
--- | --- | ---
Sucker | A1 | A2, A3, B1, B2
Lens | A1, A2 | B2
Retina | A1, A2, B1, B2 | A3
Otic vesicle | A2, A3, B3 | B2, C3
Heart | C1, C2 | B1, D1, D2
Pronephros | C3 | D3

Noteworthy in these results is the rather large prospective region for the retina which derives from the whole dorsoanimal quadrant and, as will be shown below, from the contralateral A1 and B1 as well. The heart is seen to be a dorsal structure in the fate map although its final position is midventral. The pronephros, on the other hand, comes from the ventral half of the embryo and ends up in a dorso-lateral position.

**Spatial aspects of the fate map**

A true fate map not only shows what cell type each region will form but also where in the body it will end up. The spatial trajectories of volume elements shown in a fate map allow us to make an accurate reconstruction of the morphogenetic movements of gastrulation.

Two means have been adopted here to display these movements. In Fig. 5 are shown drawings of individual cases. These show the spatial contributions accurately but are only individual cases, chosen by us as being typical of the blastomere concerned. In Fig. 6 are shown layer graphs which are averages for all the cases of each blastomere injected. These give an accurate indication of anteroposterior contributions but not of other features such as the dorso-ventral contribution or the shapes of the clones. In these diagrams the ordinates represent the percentage contribution of a blastomere to a 5-unit-long segment of the body. This means that the sum of ordinates at a given level is not expected to add up to 100%; it is the area under the ordinates on one diagram that adds up to 100%.

There is a general tendency for dorsal blastomeres to project to the anterior and ventral ones to the posterior. The A tier projects to head and trunk, the D tier to trunk and tail. The situation is more complex for the B and C tiers because the dorsal blastomeres in particular contribute progeny to the dorsal axial structures along the whole length of the body and the B and C blastomeres are in general more spread out than the A or D ones.

It is important to remember when looking at the drawings that morphogenetic movements during neurulation to some extent obscure the dorsoventral relationship of parts of the body plan. The rolling up and closure of the neural tube brings the lateral parts of the neural plate to a dorsal position and the dorsal convergence of somites brings the ventral part of the somite up around both sides of the dorsal part (Hamilton, 1969; and see fig. 2C in Cooke & Webber, 1985). It is the floor of the neural tube and the
Fig. 5. For legend see p. 542
Fig. 5. For legend see p. 542
Fig. 5. 3D reconstructions of typical cases representing each of the 16 blastomere injections.
midsomite that represent the dorsal midline structures in the neurula, i.e. those immediately adjacent to the notochord. When this is realized it is clear that blastomeres C1 and particularly B1 are properly designated prospective dorsal while A1 is prospective dorsoanterior. Conversely B4 and C4 are properly designated prospective ventroposterior while A4 is prospective ventral-trunk level.

**Cell mixing**

This is an aspect of normal development whose importance was not realized until the adoption of lineage labels and has been particularly stressed in the publications of Jacobson. The layer graphs and reconstructions show that there is a clear topographic projection from early to late stages but it is important to understand that superimposed on this is a short-range mingling of cells that results in a pepper and salt mixture of labelled and unlabelled cells. This is shown clearly in the photographs of Fig. 2. Mixing is particularly obvious in the epidermis, neural tube, somites, lateral plate and mesenchymes. It is not obvious in the notochord because the extensive vacuolation of the cells tends to concentrate the label in the periphery, but close inspection reveals mixing there too. Indeed since blastomeres B1 and C1 frequently contribute to the entire length of the notochord it is clear that there must be mixing. There is rather less mixing in the endoderm and very little in the trunk region where morphogenetic movements are minimal. For any labelled patch the mixing tends to be most extreme at the anterior and posterior ends. This is presumably because of the vast elongation of dorsal axial structures during gastrulation and the resulting amplification of local cell displacements at the periphery of a clone. However, even in the central region of a labelled patch of epidermis, neural tube or somite there is a small admixture of unlabelled cells.

Although the cell mixing is not so extreme as to obliterate the large-scale topographic projection, it is sufficient to render untenable the idea of a prospective region for a small structure such as a single somite. This point will be further elaborated below.

**Orthotopic grafts**

Grafts of the animal pole region were carried out at three stages (7½, about 256 cells; 8, about 1024 cells; and 10, first appearance of blastopore lip). The variability between cases is comparable to the blastomere injections at the earlier stages but is minimal at stage 10. Reconstructions of archetypal cases are shown in Fig. 7. In the blastula stage grafts there is extensive labelling of the epidermis accompanied by some contribution to neural tube and to somites along much of the length of the body. Comparison with the blastomere injection results suggests that the grafts are sampling heavily from the descendants of A2, 3 and 4 and more lightly from B3 and 4. This ventral bias is not surprising because the early asymmetry of pigmentation between dorsal and ventral sides means that the centre of the pigmented hemisphere in the blastula is perhaps 30° ventral to the position of the animal cross furrow at the 4-cell stage.

The stage-10 AP grafts are of lower volume than the blastula grafts and the label is almost entirely confined to epidermis in the anteroventral part of the body. The reduction of volume is due to the thinning of the animal cap resulting from the epibolic spreading which commences around stage 8. The effect is to bring the prospective regions for the brain and the tail down into the equatorial zone so contributions to these structures are no longer seen.

Orthotopic grafts at stage 10 were also carried out around the equatorial region so as to study dorso-ventral levels of the marginal zone. Both pre- and postinvolution tissue is included in the grafts together with a little ecto- and endoderm. Reconstructions of typical cases are shown in Fig. 8. The DMZ grafts are very similar to C1 and less similar to B1 injections, labelling anterior endoderm and notochord along the whole body axis. We deduce that the dorsal lip region is composed largely of C1 and B1 progeny. The VMZ grafts contribute heavily to posterior lateral plate, posterior endoderm and to a limited extent to posterior somite. These resemble C4 with an admixture of B4 and D4. The DLMZ grafts label dorsal (i.e. adjacent to notochord) somite along the entire body length suggesting derivation from B2 and C2. The VLMZ grafts label ventral somite and some lateral plate in the trunk and tail suggesting an origin from C3 with some B4 and C4. The pronephros was only labelled in a minority of cases and approximately equally by DLMZ and VLMZ grafts. This suggests that the prospective region lies in the gap between the grafts, about 90° from the dorsal midline.

**Discussion**

**Statistical character of the fate map**

From a theoretical point of view it is important to understand that fate maps for most types of animal embryo are not entirely deterministic (Slack, 1983). Only a very few invertebrates, such as *Caenorhabditis*, have a completely reproducible cleavage pattern and hence an exact fate map (Sulston, Schierenberg, White & Thompson, 1983). Even the ascidians, long thought to have an invariant cell lineage, are now known to have some indeterminacy (Nishida & Satoh, 1983, 1985). On the other hand there are some cases, such as the early stages of fish development, where there is no topographic projection at all to later
Fig. 6. Distribution of the progeny of each blastomere along the anteroposterior axis. The ordinates are the percentages of the total volume populated by a particular blastomere that lie within each 5% slice of snout–proctodeum length.
Each graph is an average of all the cases of the blastomere injection concerned. In these diagrams 'neural tube' includes brain and spinal cord and 'lateral plate' includes head mesenchyme, anterior mesoderm, trunk mesenchyme and lateral plate.
stages and the cells seem to become randomly mixed (Wourms, 1965; Ballard, 1973). In such organisms there can be no fate map until a stage of development at which reproducible behaviour commences.

In amphibians there is quite a high degree of topographic projection from the fertilized egg onwards, but there is also an element of indeterminacy which means that a particular volume element of cytoplasm will not occupy exactly the same position in later stages of all individuals. The sources of this indeterminacy are twofold. First, the cleavages are not very reproducible and even when cases with a pattern of four tiers of eight cells are selected out, as for the present study, there will be slight differences in the cytoplasmic volume element included in a particular blastomere. Second, there is a degree of cell mixing which occurs during gastrulation and this will expand the volume of the domain partially populated by descendants of a particular blastomere above that of the original blastomere. The reproducibility of experiments is greater for orthotopic grafts at stage 10 despite inevitable slight errors produced by the operation. This suggests that the grafted needle is more accurate than the cleavage furrows at dividing up the embryo, although the same sort of cell mixing remains apparent.

These considerations mean that the final fate map is a statistical construction based on an average of many individual cases. The prospective region for a given structure is actually larger than the structure itself and appears to shrink as differentiation takes place because instead of counting a large region which contributes some progeny to the structure we then count only the final volume. It is not clear to what extent the classical workers were aware of this but it is certainly not apparent from the published fate maps which give an impression of complete determinacy by plotting many tissues or structures onto a single diagram of an early stage. In the present study the statistical character of the fate map is recognized and expressed in the stipple patterns of Fig. 4. Each

![Fig. 7. 3D reconstructions of typical cases resulting from orthotopic grafts of animal pole regions.](image-url)
Fig. 8. 3D reconstructions of typical cases resulting from orthotopic grafts of marginal zone regions.
Comparison with previous results

The most direct comparison of the blastomere injection fate map with previous work can be made with that of Nakamura & Kishiyama (1971) and Nakamura, Takasaki & Nagata (1978), who studied the 32-cell stage of *Xenopus* with vital dyes. There is quite good agreement about the prospective regions of the notochord, neural tube and posterior lateral plate. The main discrepancy concerns the somites. We find a major contribution to somites from B2-4 and C2-4 while in their first paper they show the somites coming only from C2 and C3 and in the second only from B2, C2 and C3. We also find a significant endodermal contribution from C2, mentioned in their second but not the first study, and would regard the area of epidermis populated by A3 and A4 as rather larger than shown by their diagrams.

These differences are to be expected in view of the different methods used. It is not possible entirely to label a blastomere by vital staining, the periphery and deeper regions inevitably being less well stained, whereas with a lineage label the fill is uniform. Moreover, as emphasized above, minority cell contributions can be seen with a lineage label but not with vital dyes. Since the discrepancies all lie in the direction of wider contributions detected with the lineage label we feel that our fate map is the correct one.

The study of Jacobson & Hirose (1981) used injections of HRP to fate map the central nervous system. A large number of cases was studied and reconstructions were presented as well as a composite fate map. Their map shows significant contributions to brain and spinal cord from A1–3 and B1–3. We agree with this except for A3 where we find the contribution to neural crest to be rather larger than to dorsal neural tube. The reconstructions shown for A2, B2 and B3 are very similar to ours. Their reconstructions for A1 and B1 are almost identical to each other whereas we find that although there is much overlap A1 tends to populate a more lateral part of the neural tube (dorsal brain) and B1 a more midline part of the neural tube (ventral brain) as well as more spinal cord. We have confirmed the population of ventral retina on the contralateral side by progeny of A1 and B1, as originally described by Jacobson & Hirose (1978, 1981).

Jacobson's results have been presented in terms of lineage restriction by an unknown mechanism into seven compartments in the animal-dorsal quadrant. The compartments are thought to be established between the 256- and 512-cell stage, so consistent lineage restriction would not be expected until the 512-cell stage, although it might be observed following injection at an earlier stage if a clone happened to lie entirely within the future compartment boundary.

We have seen no evidence of this from our injections at the 32-cell stage. The only boundaries to be observed consistently are (i) at the dorsal midline of the neural tube arising from neural tube closure, (ii) between somite and underlying endoderm in the trunk due to separation of mesoderm and endoderm during gastrulation, and (iii) within the endoderm, due to retention of the original cleavage lines between progeny of the D tier.

Gimlich (1986) presented reconstructions of cases injected bilaterally in blastomeres C1 and D1. The results are almost identical to ours although we would regard the typical contribution of C1 to somites as somewhat lower than shown in his figure.

Cooke & Webber (1985) published a fate map for the mesoderm shown on an idealized 16-cell stage. We agree with the disposition of notochord and somites as blocks of tissue deriving from B1/C1 and B2–4/C2–4, respectively, although we regard a higher proportion as coming from the C tier than the B tier. The accord is less good when we consider the actual disposition of the somites. Our reconstructions show that the dorsal part of each somite along the whole body length comes from B2/C2, the ventral part in the trunk from B3/C3 and the ventral part in the tail from B4/C4. Their map shows C2, C3 and C4 forming ventral somite from anterior to posterior along the length of the body. There are a number of possible reasons for the discrepancies. First, there is the respective sizes of the data bases: Cooke & Webber analysed 40 cases from 4- to 32-cell stages, we used 107 cases all at the 32-cell stage. Second, Cooke & Webber's cases were tipped after fertilization to reinforce the dorsoventral polarity and this may affect the later disposition of cytoplasmic zones relative to cleavage planes. Third, they counted cell number rather than volume and it is possible that animal-derived cells are still a little smaller than vegetal-derived ones by the early tailbud stage. Finally, we have different views about the extent of the topographic projection from early stages. We find very little mapping of B1/2 to posterior versus C1/2 to anterior. Rather the progeny of both blastomeres tend to be arranged along the entire length of the body with B1/C1 mixed in the dorsal midline and B2/C2 mixed alongside. All four blastomeres show a larger contribution to anterior than posterior. There is thus no real possibility of filling in individual somites or anteroposterior levels on the fate map at this stage since a rather small region around the dorsal blastopore lip becomes extended into an extremely long thin dorsal midline and the topographic
projection is lost in the cell mixing that occurs in the course of gastrulation.

**Orthotopic grafts**

The orthotopic grafting results can be compared with a previous study of the DMZ and VMZ in this laboratory (Smith & Slack, 1983) and with Keller's vital stain map for the stage-10 embryos (Keller, 1975, 1976).

The DMZ grafts are identical to those of Smith & Slack while the VMZs populate slightly more of the ventroposterior somites. The reason for this is probably that the VMZ grafts in the present study were larger, perhaps 45–50° of circumference compared to 30° in the previous study. Both studies show clearly the massive dorsal convergence movements that occur during gastrulation and neurulation. The DMZ grafts become squeezed mainly into the notochord while the VMZ grafts expand to occupy as much as 270° of the circumference at posterior levels of the tailbud embryo. Comparison of the 32-cell fate map with the stage-10 grafts shows that a slight but perceptible dorsal convergence has already occurred. This is most clearly seen by considering the pronephros. At the 32-cell stage this comes mainly from C3 with smaller contributions from B3 and D3. At stage 10 the pronephros maps to a position about 90° from the dorsal midline suggesting a shift of the prospective region of about 20–30° in a dorsal direction. A more substantial morphogenetic movement in the blastula is the epibolic spreading of the animal cap and this is clearly apparent from inspection of the AP grafts carried out at stages 7, 8 and 10. The third important morphogenetic movement which is not shown in the present study but is discussed by Keller (1976) is the involution of the mesoderm which is also well under way by stage 10. So three important components of the gastrulation movements have progressed to some extent before the conventional onset of gastrulation at the appearance of the blastopore lip. A more logical stage to define as the onset of gastrulation might be the 512-cell stage when there is a noticeable increase of cell adhesiveness and the epiboly of the animal cap commences. This would mean that the famous 'mid blastula transition' of Newport & Kirschner (1982) should really be called the 'early gastrula transition' since it occurs at the 4096-cell stage.

It is difficult to make precise comparisons between our orthotopic grafts and the fate map of Keller. Keller's map is on the one hand more detailed than ours since he used large numbers of small stain marks and attempted to map three distinct levels within the marginal zone (superficial, preinvolution, postinvolution), but on the other hand suffers from a lack of 3D reconstructions to show exactly where the marks end up. Judging from his fig. 2 in the 1976 paper showing the prospective mesoderm, the agreement about tissue types colonized is very good. When we look at fig. 3 in the 1975 paper, showing a variety of superficial marks, the agreement is good for dorsal and ventral grafts and slightly discordant for DLMZ and VLMZ. Our DLMZ graft, centred on 60° from the dorsal midline, labels parts of the archenteron lining corresponding to his 45° surface marks. Our VLMZ centred on 120° corresponds to his 135° surface marks. This discrepancy is quite small and once again is probably due to the vigour of dorsal convergence movements since all our grafts were performed as soon as the dorsal lip appeared whereas Keller's experiments extended up to the stage of a 120° blastopore.

The last comparison that is perhaps worth mentioning is the study by Cleine & Slack (1985) on the early gastrula of the axolotl. This also involved orthotopic grafts of AP, DMZ, LMZ and VMZ and the results were quite similar to those reported here. It has become fashionable to contrast urodeles and anurans with regard to various aspects of the fate map, for example the different contributions of superficial tissue to the mesoderm (Smith & Malacinski, 1983) but the fate maps based on grafts that extend right through from surface to blastocoel seem remarkably similar.

**Relevance of the fate map to developmental mechanisms**

It cannot be stressed too often that a fate map is a description of normal events and cannot in itself be used to prove the existence of a particular mechanism. Only experimental intervention can do this. However, once a fate map is known it may turn out to exclude certain possibilities. The very existence of a fate map is sufficient to exclude a model in which cell types differentiate at random from a population of pluripotential stem cells and the origins of spatial organization become entirely a problem of cell sorting. On the other hand the statistical character of the fate map rules out an extreme determinant model in which all cell types arise in particular positions from 'grainy' determinants in the egg. Although the extreme versions of both these models are ruled out, we cannot of course exclude some role for cell sorting to refine a 'grainy' pattern and we cannot exclude the existence of one or a few determinant model in the basic axial organization of the egg. Indeed abundant experimental evidence supports both these concepts (Townes & Holtfreter, 1955; Gerhart, 1981).

A more subtle question concerns the nature of the determined states set up by the onset of gastrulation.
By ‘determination’ we mean irreversible and heritable cellular commitment. Studies by Jacobson and by Keller (1978) show that cell mixing commences around the beginning of gastrulation. If the fate map shows that cell mixing between two regions normally occurs then the boundary cannot be determined until after this time. This is the case for the anteroposterior axis in the dorsal midline of the mesoderm for, as discussed above, we find very little mapping of C1/2 to a position anterior to B1/2 and yet this would be expected for in the absence of cell mixing the progeny of C1/2 would involute first and end up anterior. We must conclude that determination of anteroposterior body levels does not commence until the beginning of gastrulation and that the cell states represented in the late blastula are the product of vegetal–animal and dorsal–ventral gradients of positional information, as discussed in our previous papers (Dale et al. 1985; Smith, Dale & Slack, 1985). As in so many other cases in embryology, the loss of anterior parts following treatments such as u.v. irradiation or LiCl does not mean that an anterior determinant has been destroyed, but simply that the preconditions for the establishment of a complete anteroposterior axis have been interfered with.

References


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