Properties of the primary organization field in the embryo of *Xenopus laevis*

III. Retention of polarity in cell groups excised from the region of the early organizer

By J. Cooke

From the Developmental Biology Group, University of Sussex

SUMMARY

An experiment is described whose results strengthen the classical conclusion, due to Spemann and co-workers, that amphibian gastrulation movements are co-ordinated and controlled by properties intrinsic to the invaginating mesodermal zone, rather than by interaction between this and any field of cell-guiding information, symmetrically disposed about the presumptive head ectodermal region in the animal hemisphere of the blastula/gastrula. The possibility remains, however, that the field of information coming to reside in the marginal mesodermal zone, is itself originally set up utilizing the animal pole as an origin, as well as the presumptive organizer site.

Experiments are then described where whole organizer apices, and also subapical squares of dorsal mesoderm from stage-10 donors, are implanted with presumptive polarity reversed 180° relative to that of the host. It is found that reasonably extensive migration, on the part of the graft and the influenced host tissue, is required for the individuation of recognizable axial structure, and that such migration is often prevented in reversed implants due to a retention of autonomous polarity in host and graft.

Reasons are suggested for the apparently greater autonomy, in this respect, of apical organizer plugs, but evidence is given that autonomy is nevertheless expressed even by squares of dorsal presumptive mesoderm of side ca. 0.1 mm. The significance of this observation, for theory concerning the nature of the cellular properties involved in the maintenance of embryonic fields, is discussed.

INTRODUCTION

Spemann and his co-workers had observed, in their original work upon the properties of the dorsal lip in amphibian gastrulae as organizer (see Spemann, 1938), that the transplanted pieces had a tendency to make their initial morphogenetic movements, after operations, in an autonomous manner, according to the orientation of the graft. Only later, due to the overriding effect of the invaginating host tissues, did the direction of immigration of most secondarily induced fields blend with that of the host. The final co-ordination was assumed by them to be due to a twisting of the immigrating tongue of cells from the

1 Author’s address: Developmental Biology Group, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG, Sussex, U.K.
graft, after its contact and fusion with the mesodermal cell layers of the gastrulating host.

These workers also performed the experiment of rotating the whole animal neurectodermal cap, down to the approximate level of the equator, through 180°, at the onset of gastrulation. Such an operation was often followed by successful re-healing of the cap and then normal morphogenesis. This led Spemann to assert that normal gastrulation was directed entirely by intrinsic symmetry of stretching and migratory properties in the mesodermal field, rather than by anything such as a pre-pattern or gradient of adhesive properties in the overlying neurectoderm, that might lead the immigrating mesodermal cells towards some goal point at the presumptive head-forming region.

In the present paper, results of a similar experiment are first reported, which strengthen the conclusion that gastrulation movements, and the final position of the mesodermal mantle, are achieved via polarity and stretching properties built up only in the presumptive mesodermal zone. Then the results are given of experiments, similar to those reported in the previous two papers of this series, but where an implanted, second organizer is reversed in orientation so that its presumptive migration polarity would tend to direct its invaginating cells towards the host vegetal pole. Further experiments show that the retention of an autonomous polarity of migration tendency, which is extreme in complete plugs of cells that include the very apex of the organizer, is also exhibited in sub-apical squares of presumptive axial mesoderm from just above the beginning dorsal lip.

MATERIALS AND METHODS

For the method of obtaining fertile eggs of *Xenopus*, the solutions used, and the details of the treatment of embryos during and after the operations, see Paper I (Cooke, 1972a) of this series (Materials and Methods). The first operations described here, where a complete plug of head organizer cells is used, are similar to the main series of operations described in Paper I, except that the graft is implanted with its smaller, presumptive mesodermal cells situated nearer the host vegetal pole (see Fig. 2). Controls were members of the main series of these operations.

Further types of operation are described as the results are presented. The EDTA treatment described in Paper I was particularly helpful in ensuring neatness of healing between host and graft cell sheets in the case of the subapical squares of mesoderm.

Observations of the results of operations, made with the object of examining migration polarity in grafts, were carried out ×25 and ×50 under the dissecting microscope, at 20 min or 30 min intervals, and finally, after 24 h, when control embryos and hosts had organized sets of axial structures.
Table 1. The effect upon morphogenesis of rotation through 180°, at stage 10, of an asymmetric disc of animal pole tissue as in Fig. 1

<table>
<thead>
<tr>
<th></th>
<th>No. of operations</th>
<th>No. healing satisfactorily</th>
<th>No. normal at stage 26</th>
<th>Microcephaly and cyclopia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>(cutting without rotation)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

(1) Cap-reversal operations

Although, in Spemann’s total animal cap-reversal experiment, the presumptive head region or goal-point for the migration of the mesodermal mantle is eccentrically situated in the rotated cap of cells, the normal development observed following such an operation might conceivably be due to regulation, acting to restore the position of that goal-point which is in fact specified by a radially disposed field or gradient of cellular properties in the neurectoderm around it. Such a field could guide, for example, chemotactically or mechanically, the cells of the advancing edge of the mesodermal mantle during gastrulation.

In the experiment shown in Fig. 1, whose results are given in Table 1, an eccentrically situated disc of neurectoderm, embracing the animal pole and part of the presumptive head region, and at its original lower edge the equator of the
stage-10 gastrula, is cut out and rotated through 180°. Control embryos are cut and re-healed only. At this time the dorsal lip is visible, so that the disc cut out can be chosen to span symmetrically the dorsal meridian of the gastrula. Thus subsequently invaginating dorsal mesodermal cells will be presented with an opposed version of any graded information which may be supposed to exist in the neur ectoderm, radially disposed around the presumptive ‘goal’ region for the head mesoderm of the neurula. During the movements of gastrulation, they will encounter the normal goal region first.

The essentially normal development seen after this operation greatly strengthens the original conclusion, that the neur ectoderm contains no guiding information for the advancing mesodermal cells. The time allowable, from stage 10, for regulation leading to some restoration of the position of the goal-point in the neur ectoderm, would be very little (30 min to 1 h, at most). However, the results of this experiment do not affect the possibility that, during earlier blastula stages, the animal pole region of the embryo is utilized as an origin for the erection of positional information (see Wolpert, 1969) that is subsequently expressed as organization of the prospective mesodermal mantle at the onset of gastrulation. Experiments are in progress to test this possibility, but are rendered difficult by the unfavourable reaction of the early blastula to opening up of the blastocoel during cap-reversal operations.

(2) Organizer implantations

The whole stage-10 organizer plug, including dorsal lip, head-endoderm cells and bottle-shaped cells (see Paper I), was found to preserve its intrinsic migration tendencies very well indeed. Of 20 operations in which such a graft was placed in reverse orientation (see Fig. 2a) along the presumptive zone of marginal intucking, and at 130° or more to the host's dorsal axis to allow autonomy of expression, only one showed individuation of a recognizable secondary axis. In this case, there was evidence that raggedness of the graft as it healed in had led to its being turned through some distance by the movements of host cells, thus allowing axial elongation, as observed classically, sweeping into line with the host’s field. In the remaining 19 cases, two situations were observed:

(1) Graft carried passively beneath surface up to the anterior end, where a minimum degree of secondary individuation had occurred, sometimes making the host head asymmetric. Vital staining showed a completely compact position of the graft, with no elongation, or organization of surrounding cells.

(2) Graft staying at level of blastoporal rim, in a compact mass with cells attempting migration towards vegetal pole of host, in conflict with its own mesoderm. Result, a twisting of the host axis sideways in the neurula, giving rise to a large lateral yolk-plug. A lateral equivalent of ‘spina bifida’.

Ninety per cent of operations with normally orientated second organizers, situated at 130° or more from the hosts’ dorsal axes, show recognizable in-
Retention of polarity in excised cell groups

Fig. 2. Implantations in marginal zone, in normal and reversed animal/vegetal orientation, of (a) whole organizer plugs, and (b) subapical organizer squares. Vegetal view at stage 10 to show nature of operation. Site of extirpation of graft, and position of host organizer, represented at bottom, and sites of implantation in host margin at wide angle. Typical results of reverse operations also shown.

Fig. 3 shows two embryos, the upper one, at late neural plate stage, an example of a reversed organizer implantation where the graft had been carried forward but had not individuated further. The lower one is a case of the implantation of a reversed, whole organizer at a higher equatorial level of the late blastula host rather than in the marginal zone. Here, where the autonomous migration tendencies of the graft have been allowed some expression before encountering host material, it has formed a blastopore-like pocket of ingestion, and extended far enough to organize some structure with a polarity opposite to that of the host, and having head-like morphology. The extreme conflict between graft and host migration tendencies is evident in the photograph.

In order to discover whether the observed autonomy of migration polarity, in the organizer region, was a special property of the very apical cells, which the others would follow by some form of contact guidance, or whether it was intrinsic to isolated portions of the non-apical dorsal mesodermal field, a series
The upper one is an example of result type I for this operation (graft carried passively forward, see text, p. 50). The lower has had implantation at a more animal site in the gastrula host, showing up the antagonism between graft and host mesodermal migration tendencies.

Table 2. The results of implantation of (a) whole stage-10 organizer plugs, and (b) squares of mid-dorsal presumptive mesoderm from immediately above the stage-10 dorsal lip, into hosts of stages 9 and 10; see Fig. 2 for geometry of operations

<table>
<thead>
<tr>
<th></th>
<th>Recognizable secondary axis individuated</th>
<th>No recognizable secondary axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole organizers in normal orientation</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>Whole organizers in reversed orientation</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Subapical squares in normal orientation</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>Subapical squares in reversed orientation</td>
<td>18</td>
<td>7</td>
</tr>
</tbody>
</table>

of operations of the type shown in Fig. 2b was made. These operations were similar to the basic one, but used as grafts squares of presumptive dorsal mesoderm, from just above stage-10 dorsal lips, cut on all four sides and with orientation preserved. At implantation, which was always at 130° or more to the
Retention of polarity in excised cell groups

host's dorsal midline, they replaced a similar sized, excised square from the host cell sheets. The results are given in Table 2.

In accord with classical observations, such squares tended not to commence activity until the region of their origin was already intucking during the gastrulation of synchronous controls. In some cases, where initially pregastrular hosts were used, further delaying effects were observed, causing the healed-in implant to rest apparently quiescent for up to two hours. Eventually, in all observed cases (32) however, only that side of the square that had originally been nearest the dorsal lip in the donor, began immigration activity, underneath the rest of the square, together with some cellular elongation of the type described in Paper I of this series. The initial result was always a blastopore-like pocket leading in under the graft on the side that had originally been most apical, with radial stretching, and elongation, among the graft cells near it. Healing between cell sheets of graft and host was often very good, at least at the visible surface layer, a pigmentation difference then being the only mark of the host-graft boundary, except on the blastopore-forming side.

As seen in Table 2, for 180°-reversed squares of mesoderm, the results in terms of final axial induction fall into two categories. Where a secondary axis was formed, this swept either outwards or, more usually, inwards from the site of intucking, towards the host midline, to run parallel with the main axis of elongation in host, as in Fig. 2b. The impression gained was that the host gastrulation movements had been able to slew round the immigrating tongue of cells from the graft, which had not been placed so as to be very precisely in opposition to the host mesoderm. Once this had occurred, invagination to form the secondary axial field could be extensive, with normal involvement of host material, but such axes were always incomplete in structure, due to the subapical nature of the graft.

In cases where no secondary differentiations were observed, the graft tissue had characteristically been constrained as a compact mass, because of the opposition between its own migration tendencies and those of the host. Often, the graft as shown in Fig. 2b was raised into a loop of tissue because of the intrinsic tendency to elongation of its cells on the pocket side, these being nevertheless mechanically prevented from intucking by the opposed migratory host mesoderm. In two cases, following the operation, such raised loops of tissue were observed to develop progressively over the next 5–6 h. In these instances, the graft had been placed very precisely in opposition to the host's field, and also symmetrically midventral to the host's own axis.

Discussion

The inclusion of the large, apical endodermal cells of the stage-10 organizer plug in grafts appears to confer on them a much greater autonomy of expression of their intrinsic migration tendencies (compare results in Table 2). One explana-
tion for this would be that, in reversed implantations, these large, apical cells are much less susceptible to deflexion, during their attempted vegetalward migration, than is an advancing tongue of the smaller cells derived from sub-apical dorsal mesoderm. In whole organizers, all the cells of the graft, through their tight adhesion to this apical material, would be immune from physical re-orientation by advancing host cells. The adhesion made between the inner, yolky cells of grafted whole organizers and the host mesoderm, gives the impression of being good, and extensive. Thus these cells might be held as a compact mass by a balance of forces due \( a \) to their tendency toward locomotion across, and in an opposite direction to, the still external grafted mesodermal cells above them, as in normal gastrulation, and \( b \) to the symmetrical constraint imposed by the coherent sheet of host cells splitting to migrate around them. Prevention of migration due to the latter may be caused by intrinsic polarity perception on the part of the graft cells for the cells passing them (i.e. part of some normal, intrinsic polarity resident in these cells and perceptible by them, involved perhaps in the co-ordination of migration of cells in sheets), or simply by mechanical factors. We are not aware of any evidence, in the literature on cell behaviour, for anything that need be interpreted as intrinsic polarity perception on the part of cell surfaces in contact.

Subapical squares of organizer mesoderm tend not to express so permanently, in terms of morphogenesis, intrinsic polarity retained within them, since they tend to have their fields of immigration turned and swept in towards the host midline. However, at the level of initial, observed cell behaviour, the results show that such polarity is retained, though so far this has only been tested through a period of some 2 h after explantation, and before gastrulation activity of the graft, begins. Future experiments should be able to extend this period of testing, in order to determine the time limits for retention of original polarity on the part of these squares, when quiescent and situated in opposed tissue.

Now, the squares used in these grafting experiments are of side length of the order of 0·1–0·15 mm, or 15 cell diameters in the gastrular marginal zone, and consist of three or four cell layers — including the pigmented external cells. Such groups of cells are able to maintain a gradient of some nature, that controls the order of immigration for a period of the order of 2 h, whilst in contact with host material having a reversed gradient, either of similar mean value to that within themselves, or (since implants are far from the host dorsal midline) perhaps of much lower average value. If their polarity is only the expression of a substance gradient, with cells behaving passively in a diffusion field, then one of the following must be true; either \( a \) gradient adjustment due to diffusion amongst cells of the sheet formed by graft and host, or between cells and the bathing medium between excision and implantation, must be highly restricted: or \( b \) The real coefficient for diffusion, of the substance involved, must be very low: i.e. it is a very high molecular weight substance.

The former effect, a restriction upon diffusion interaction between cells,
Retention of polarity in excised cell groups could be due either to restricted diffusion as such, i.e. only special site available for transfer between adjacent cells, or to each cell’s acting as a homeostat tending to maintain some previously attained concentration of the substance within itself, as in the ‘sonk’ type of model due to Lawrence (see Lawrence, 1971).

An alternative hypothesis is that, as part of the structure of the field leading to normal gastrulation, single cells develop intrinsically polar properties, such as locomotory polarity (as distinct from passive chemotaxis) which can be expressed when several cells maintain contact with one another, or else a polarity of active transport which is sufficiently powerful to maintain such a local region of opposed gradient over the length of time observed.

It can be noted here that these results cannot be explained by any theory where a phase-shift field, of the type imagined by Goodwin & Cohen (1969), is directly responsible for polarity in cells of the gastrula, under the influence of the organizer apex as a propagation centre. On such a simple model, small territories of implanted cells should reverse their graded properties to assimilate into the host field much more rapidly than observed, provided that the host contains a region more nearly apical than any in the graft (as in the case for the experiments with subapical squares as implants). Hybrid models are still possibly, however, where a phase-shift mechanism is imagined to cause initial setting up and regulation of the boundaries of a positional information field, and then to cause ‘translation’ of the information into some less easily labile form, such as a substance gradient with restricted diffusion, or maintenance of concentrations due to cell homeostasis.

The experiments on subapical squares seem to show that at least by the onset of gastrulation, positional information has come to reside in some ‘memory’ form in individual cells or small groups of cells, which then display considerable tenacity in maintaining such information on a local level. This concept has now to be fitted in with the findings of classical amphibian embryology, and of experiments to be reported in the next paper of this series, that whole embryonic fields can regulate their boundary values to restore missing apical parts, when given periods of only a few hours longer than those dealt with in this paper.

REFERENCES


*Manuscript received 2 December 1971, revised 8 February 1972*