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The system specifying body position in the early development of *Xenopus*, and its response to early perturbations.

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SUMMARY

Evidence is presented that the system setting up preliminary specifications for contributions to the axial body plan, across vegetal regions of the *Xenopus* embryo, acts in a widespread way at early stages. Mechanisms that regulate the spatial profile of this primary positional variable, and thus ensure the constancy and harmony of the body plans normally achieved, have lost this integrative ability by the 4-cell stage one hour after the plasm shifts that precede first cleavage and symmetrize the egg. Abnormal, partial or distorted profiles of the positional system across whole eggs or isolates, recorded by these times, are retained to give correspondingly partial or imbalanced mes/endodermal pattern at tailbud larval stages. There is evidence that subsequent 'back-up' positional interactions, which can heal gross positional discontinuities in isolated presumptive lateral half-eggs and so restore bilateral symmetry, also do this at the price of loss of complete pattern specification. This is probably because of an asymmetrical principle whereby relatively activated (dorsoanterior specified) material can raise the level of originally posterior material on contact, whereas the reverse interaction cannot occur. The observations are discussed in relation to apparently different behaviour in certain other amphibian embryos, and to our knowledge of other positional interactions, normal and also experimentally provoked, such as those that set up the germ layers.

INTRODUCTION

In the normal *Xenopus* embryo, the pattern of developmental tendencies that constitutes the body plan is regulated in a subtle way. Across a 2·5-fold natural variation in total mesoderm size at the time of pattern foundation, the architecture of the newly formed axis is remarkably constant in terms of the *proportions* of the tissue devoted to particular histologically recognized elements at particular positions in the anteroposterior sequence of the body. One extreme hypothesis would be that processes with the required character of regulation and spatial integration act over most of the time up to tailbud stages. Alternatively, such processes might be confined to very early times after fertilization and the events that activate and orientate development in the egg.

The basic interpretation of the development undergone by large isolates from urodele embryos, made at stages from egg to late blastula, has been that a quite

Key words: whole body pattern, blastomere isolation, precleavage events, gravity re-orientation, specification, *Xenopus*, fate map, FLDx.

restricted sector of the fertilized egg is capable of organizing a whole and harmonious, if small, body pattern in isolates that include its material (reviewed by Spemann, 1938, and Gerhart, 1980). Isolates lacking a share of this sector appear not to possess or maintain adequate information for anything other than radial, non-axial gastrulation. Such divergent modes of behaviour in sectors isolated from a system in which a positional signal gradient is due to be formed, viz. regulative arrival at a normal gradient profile across reduced available space or else loss of all information with which to break symmetry and form pattern, fit well with the predictions from a dominant and widely successful class of model mechanism that has been proposed for the arrangement of such gradients (Gierer & Meinhardt, 1972, reviewed by Meinhardt, 1982).

In this paper I wish to present the main evidences that at least for the rather small and rapidly developing egg type represented by *Xenopus*, the system specifying the axial body plan *in normal development* behaves from soon after fertilization as a rather stable positional 'memory', with the characteristics of a cell-structural rather than a diffusional mechanism. This system shows those subtle properties of integration and regulation, that normally ensure the relative accuracy of the plan, only while it is actually being set up over a very contained time period near the outset of development. Much of the data is in course of publication as individual experimental papers (Cooke & Webber, 1985*a, b* Cooke, 1985*a*) where the past literature to which these observations are relevant is more fully quoted. Here, I shall thus cite only samples from other work.

Much attention has recently been devoted to the fascinating set of events following fertilization in anuran eggs, essentially at precleavage stages, whereby the axial pattern is orientated, in relation to the egg meridian on which the sperm happened to penetrate (Gerhart *et al.* 1981, Scharf & Gerhart, 1980, 1983). Most of this work has been discussed in relation to the earlier amphibian literature where an initial localization within some part of the egg structure was supposed to constitute the sole reference point for subsequent specification of axial pattern, by being in some way the precursor for the 'organizer' (dorsal blastoporal lip) region (Spemann, 1938, Nieuwkoop, 1973). Thus it has been assumed that what is to be explained is just the creation of a vegetally situated localization, opposite the meridian of sperm entry, which then becomes the controlling origin for a sequence of inductions or for the diffusional build-up of a morphogen gradient. The results and observations to be reviewed below seem rather to require the interpretation that the organized movements in the plasm structure of the *Xenopus* egg, occurring in the second half of the precleavage interval after fertilization, lead fairly directly to a profile across the egg with respect to some quite stable variable (that might be termed 'degree of activation'). This functions as a coding system to specify contributions to the normal body plan. Those properties of this system that are already described in experimental papers will be reviewed only briefly (see Cooke & Webber, 1985*a*, *b*; Cooke 1985*a*, *b*). I shall present more fully a newer experiment, that attempts to perturb the early system of plasm movements after fertilization, thus carrying the result of these movements outside its normal narrow range of variability. This is followed by the observation of deviations from the normal range of pattern balance in larval mesoderms 30 h later; a striking illustration of the durability of what is set up in the hour-long period that registers the disturbance.

Since many observations are to be mentioned, the conclusions that seem indicated by each result will be given as it is described. Two principal phenomena might appear initially to be discrepant with these findings, and thus need discussion. They are a), induction of mesoderm *per se* by information coming from the endoderm-producing egg regions during blastula stages, and b), the undoubted capacity, up to late gastrula stages, for the developmental fates of cells to be changed altogether when materials from disparate initial positions in the system are artificially (usually surgically) juxtaposed. The ways in which these observations can indeed be reconciled will be mentioned at appropriate points. Possible causes of the discrepancy between the lack of overall regulative ability in response to the present kind of experiment, and the conclusions from past experiments with this embryo type (e.g. Cooke, 1981, 1983), are given full consideration in the discussions of the data papers referred to above.

METHODOLOGY

The strategy has been to compare patterns of mesodermal differentiation, following various early perturbations of whole eggs or in large egg fragments isolated at very early cleavage stages, with the normal patterns in synchronous control sibling embryos. In doing this we have been aided by three simple techniques. Firstly, a new level of normal fate mapping, that of reconstructing clonal body contributions from *in situ* lineage-labelled early blastomeres, has been employed. Secondly, the gentle separation of blastomeres by directing currents into the newly formed cleavage furrows from a micropipette has allowed the rapid reformation of blastulae by part-eggs, and the continuation of development essentially without the loss of tempo that occurs with the more severe techniques for making miniature embryos at later blastula stages. Finally, we have for some years been building up information as to the normal range of variation in proportions of the newly founded mesodermal pattern at the late tailbud axial stage 28. This is assayed both as percentages of the cells found in the principal histologically defined territories, and in terms of the more subtle, but highly controlled assignment of relative cell numbers to the structures at different axial positions (see introduction). At the stage chosen, all cells in the major region analysed (which excludes the prechordal region and the tailbud) are scorable as belonging to one of the four pattern elements notochord, somite body, pronephric system and lateral plate including blood-forming tissue. On the other hand, the proportions in which the founder cell populations for these parts were set aside have had little or *no* opportunity to be obscured by normal or compensatory growth. Measurement is based on equivalent serial sampling in transverse section of each embryo, including some 4–10 000 cells (an estimated 30% of each mesoderm). It thus reflects rather intimately the prior performance of the unknown pattern-regulating machinery in each individual.

Details of each technique are described in the original papers, and will only be further mentioned insofar as they help readers assess the meaning of experimental results to be described. The particular regime of abnormal orientation in 1 × gravity for precleavage embryos, that has been found to perturb the positional system, will however be fully described at the appropriate place.

RESULTS

4-cell-stage isolate patterns in relation to the mesodermal fate map

Fate mapping by *in situ* lineage labelling from 4- to 32-cell stages has revealed that the pattern of origin of materials for the normal mesoderm is somewhat different from that conventionally assumed. It is given in Figure 1A. Details of fate maps are only of importance where they help in interpreting the meaning of experiments, but this appears to be so in the present case and so the features of this map must briefly be discussed.

The salient features of the mesodermal fate map (see Fig. 1A) are as follows. With the exception of the notochord territory, which is confined to a sector (ideally) centred opposite the meridian of sperm entry, the future tail-to-head axis is better described as being laid out around the marginal zone from sperm entry region to the region opposite (the classical centre of dorsalization or organizer precursor), rather than from animal towards vegetal pole. At least for origin of endo/mesoderm, the sperm entry side of the egg is best described as a *posterior* side, and the opposite side as *dorsoanterior*. Only the relatively restricted presumptive notochord territory is laid out (and thus presumably induced) in head-to-tail sequence towards the animal pole from the end abutting on the vegetal prechordal and endodermal region. Because the lateral plate tissue is most strongly represented in the postcardiac region and is slight in the posterior trunk, at least half of the final lateral plate cell population usually derives from the side of the typical second cleavage plane opposite sperm entry. Similarly, because the posterior somites include larger ones, and contain much more aggregate tissue than those of the head and anterior trunk, considerably *more* than half the normal larval somite cells derive from the sperm-entry-side blastomere pair at the 4-cell stage.

For most of the axis, the final material for each somite segment is gathered from a considerable sector of the original egg material as shown, with *every* somite in the body contributed to by at least some material that abutts on the notochord territory and normally gives rise to the central part of the somite cross section. Material for the anterior four to eight small somites (varying with individual eggs) is contributed entirely from the dorsoanterior cell pair, while from here backwards in the body plan, increasing proportions of the laterally derived part of each successive segment are contributed from the 'posterior' cell pair. Thus, the bulk of the mesoderm for each successive segment, in head-to-tail order, is derived from egg and pregastrular

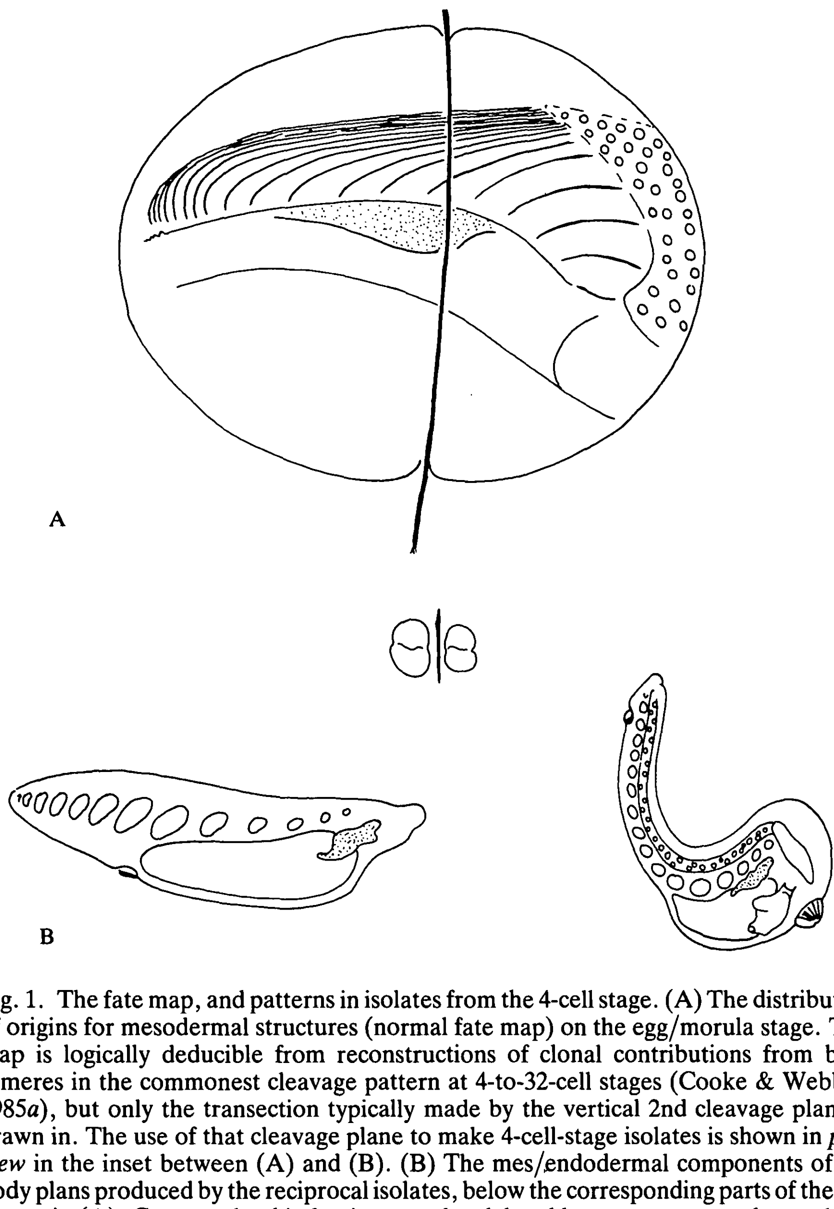

Fig. 1. The fate map, and patterns in isolates from the 4-cell stage. (A) The distribution of origins for mesodermal structures (normal fate map) on the egg/morula stage. This map is logically deducible from reconstructions of clonal contributions from blastomeres in the commonest cleavage pattern at 4-to-32-cell stages (Cooke & Webber, 1985*a*), but only the transection typically made by the vertical 2nd cleavage plane is drawn in. The use of that cleavage plane to make 4-cell-stage isolates is shown in *plan view* in the inset between (A) and (B). (B) The mes/endodermal components of the body plans produced by the reciprocal isolates, below the corresponding parts of the egg shown in (A). Cement gland induction, prechordal and heart structure and notochord are only, but always, seen in the dorsoanterior isolate. The posterior isolate gives more variable development but the pattern shown, corresponding to truncation of the complete body plan *at* the normal transection by the cleavage plane, is frequent. Pattern part codes; notochord, small open circles; pronephros, stippled; endoderm, solid outline. Somite segment prospective regions (and the corresponding numbers of formed segments) are indicated in accurate relation as between the fate map and the partial body plans seen, though the real number is extended posteriorly in all versions of the pattern by the multiple small somites of the tail bud region.

positions progressively closer to the sperm entry meridian. Pronephros is contributed to more from the posterior cell pair than from the dorsoanterior pair, but rarely made exclusively by the former, its territory lying athwart the typical cleavage plane position.

Fertilized eggs have been manipulated and selected (see Gerhart *et al.* 1981) to maximize the likelihood that the first two cleavage planes are set orthogonally and symmetrically in relation to the hidden pattern-forming events, using the visible sperm entry point as a marker. Some such eggs were used for blastomere injection at this 4-cell and at subsequent stages, to found marked clones and allow the fate mapping just described. Others have been subjected to gentle separation of the two, non-equivalent pairs of blastomeres defined by the second cleavage. These are each bilaterally symmetrical in appearance, but usually consist of a smaller, less pigmented pair and a larger, darker pair. Figure 1 inset, and 1B (diagrammatic body plans) show the cell separation, and the patterns of mesoderm developing in the successfully healing small blastulae and gastrulae that result.

The successful dorsoanterior isolates always make small blastulae which commence gastrulation on schedule in relation to synchronous controls, but which then proceed rapidly to completion of an annular zone of active gastrulation so that there is precipitate closure of the yolk plug. The partial larval mesodermal pattern ensuing is close, both qualitatively and quantitatively in terms of the cellular sizes of notochord, lateral plate and somite segment contributions, to just that contribution to total body pattern which would have derived from the corresponding sector had the whole egg been left to develop. Regulation – the adjustment of cells' fates, and hence the sizes of newly founded structures so that a more nearly 'whole' or harmonious pattern is produced – is never seen in such isolates. Note that this means that while the number of somite bodies segmented is that characterizing the whole body pattern (see Fig. 1A), the anterior few are essentially normal sized whereas the remainder of the series is progressively reduced to small, juxtanotochordal cell groups.

Regulation is also absent from the behaviour of the reciprocal 'posterior' cell-pair isolates. About half of such isolates, apparently depending upon egg type and egg batch, pursue a late onset schedule of gastrulation activity which spreads from a definite origin but runs parallel with that of the sperm-entry-side half in synchronous control gastrulae. They correspondingly develop an axial, but anteriorly truncated and notochordless body pattern. A substantial pronephric formation, and hindbrain/ear vesicle levels of neural induction can occur at the front end of such patterns, but the somite series is always truncated by at least three segments anteriorly and the somite bodies are bridged under the neural tube. The remainder of these isolates develop mesodermal part-patterns that correspond to increasingly restricted sectors of the normal fate map centred around the posterior or sperm entry meridian. The limiting case, seen only in a minority of instances, is a radially symmetrical structure lacking somite segment organization, after a belated episode of gastrulation that takes place in a radially synchronous manner. Throughout the results, the extent of the partial pattern to be produced by individual isolates may be quite well predicted from the timing of onset and spread in externally visible gastrulation activity in relation to that in cofertilized siblings.

Figure 2A and B represent the behaviour indicated by this experiment in terms of a signal variable (or the recorded trace of such a variable) that comes to exist around the egg by the stage when the isolates are made, whose 'levels' or degrees of intensity are used in some way to specify the contributions to the normal whole body plan that will be made by endoderm and mesoderm. The relative stability or durability in the absence of continued contact between normal 'boundary states' of such a system, but the lack of any positive restorative or regulatory ability, are in striking contrast to the behaviour predicted if the mechanism were a prolonged, dynamic 'diffusional' one, controlled only from a special localization initially set up in the egg. The divergent modes of behaviour (see Fig. 2C, either regulation or total loss of information) that would be expected of a reaction or source-diffusion-controlled pattern field after early transection, are in contrast with the apparent behaviour of the specifying system in many *Xenopus* eggs. The discrepancy is the more striking in view of the very early, essentially uncellularized stage at which the physical continuity in the fate map is interrupted. The experiment has studied only

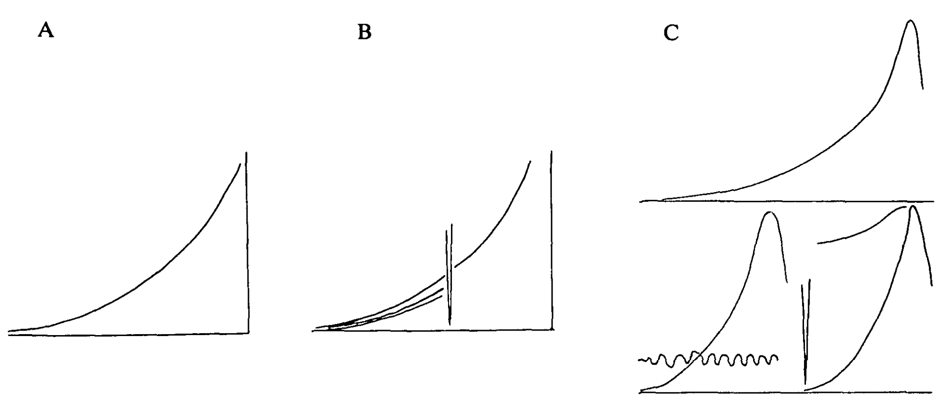

Fig. 2. The dynamic behaviour of the *Xenopus* positional system, and the expected behaviour from reaction-diffusion controlled morphogen systems. (A) An arbitrarily drawn profile to represent the spatial gradation in intensity of a signal variable set up across the relevant part of the *Xenopus* egg, used to code position and set the mes/endodermal contributions to the normal axial body plan. (B) Representation of the behaviour of the positional profile in response to transection by preparation of isolates across the second cleavage plane. The dorsoanterior -specifying (right hand) sector nearly always remains unchanged, while the posterior sector may do this but may behave in ways that indicate various degrees of 'relaxation', or loss of recorded information in different individual cases. The phenomenon is known in the literature of insect embryology as the 'gap' phenomenon. Regulation (see C) is not observed. (C) Two diagrams that represent the behaviour expected, in response to such early transection of a pattern-forming field, in the case of a reaction-diffusion, or source-diffusion controlled morphogen concentration gradient as the signalling system. A divergent behaviour, leading to regulation or to profound loss of graded information, is expected in one or both isolated sectors.

regional specification for body position, not a system of determination, since amphibian embryonic cells are known not to become committed to developmental fates until many hours and 10–15 cell cycles later (see discussion, and Heasman *et al.* this volume). The system's behaviour in terms of fixity of information for pattern at the time of transection (Fig. 2B), is comparable to that shown by various insect embryos ligatured at precellularized or cellularizing blastoderm stages when some ten cell cycles have occurred (Sander, 1975).

These results also have an important consequence for our thinking about induction of the mesoderm *per se*, by information passing towards the animal pole from presumptive endoderm during morula or blastula stages (see Gimlich, and Smith *et al.* this volume). There has been a tendency to assume that normal specification for pattern of mesodermal development is by signals emanating from a 'dorsal' mesodermal region, induced above the original vegetally located centre of dorsalization in eggs, and is slow and progressive. Thus the assumed sequence has been a) vegetal dorsoanterior localization in endodermal mass, progressively controlling both endodermal body pattern and that of dorsal mesoderm by intercellular signalling (i.e. inductions or physiological gradients), and then b) control of the remaining mesodermal body pattern by similar events originating at the secondary mesodermal centre, the originally named 'organizer' of Spemann in the suprablastoporal lip region. The *Xenopus* 4-cell-stage isolate behaviour however, namely the partitioning of the developed pattern rather closely following the partition made by the appropriate cleavage plane in the *mesodermal* fate map, could not follow if this species were organized in this way. Mesoderm pattern appears to be the result of vegetal → animal inductive influences occurring around much of the morula or blastula.

Development of lateral half isolates from the two-cell stage: evidence for a back-up mechanism of slower positional interactions.

Detailed familiarity with the normal range of pattern balances found after embryogenesis in *Xenopus* has made possible the detection that a further 'classical' finding does not hold true for this species. If eggs selected for symmetry of cleavage and sperm entry as before are separated into two blastomeres at first cleavage, the resulting isolates make small blastulae and gastrulae that maintain their schedules of morphogenetic activity in relation to controls. There is restoration of bilateral symmetry in each of the half-sized body patterns that result, and the *situs inversus* (mirror reversal of left/right anatomical asymmetry) seen in one pair member after twinning by early gastrular constriction in urodele embryos, is rarely if ever observed after this much earlier twinning procedure. Nevertheless, the balance of mes/endodermal body pattern in such *Xenopus* twin members is usually deviant from the normal range in a number of ways. The degree of pattern imbalance seen, i.e. the imperfection of 'regulation', is positively correlated as between reciprocal pair members, and is thus due in some way to the dynamics of the system rather than to chance asymmetries in partitioning of specific egg plasms.

The nature of the pattern imbalance is summarized in Fig. 3. It consists of a relative overdevotion of material to parts of the body plan that, in normal development, are specified in egg regions relatively far from the sperm entry position and thus close to the dorsoanterior centre. There is a corresponding *extra* miniaturization of the scale of pattern formation in parts of the axial plan usually deriving from the slower gastrulating, sperm-entry-side regions.

In anatomical terms, most lateral half-isolate bodies have a *relatively* overlarge notochord rudiment, so that each pair of such bodies produce more notochord tissue together than the egg from which they were derived would have done. They also have an abnormal somite balance index (see introduction), in that the cell numbers in segments at the relatively posterior (14–16 somite) position are more scaled down than are those in anterior ones. The head, brain and chest are disproportionately large and the tailbud small, even though all the normal segment series is represented as in dorsoanterior isolates.

There is a certain amount of evidence that in the version of lateral half-isolate development that produces this imbalance, cellular material derived from near the original sperm entry position is repositioned by alterations in the cleavage planes and cell contacts so as to lie adjacent to material from the opposite, dorsoanterior original position. The reacquisition of bilateral symmetry by these isolates requires massive change of fates in material around the zone of new junction. The evidence from the final pattern abnormalities, together with our knowledge of the normal fate map, suggests that this reorganization occurs according to an asymmetrical principle of spatial interaction with respect to body position value in the tissue. We have termed this the 'ratchet principle'. It appears that material which received a relatively 'low' or posterior level of activation, in the original rapidly acting positional specification system, can have that level 'raised' and thus its contribution to pattern made more anterior, by subsequent interactions if juxtaposed to more dorsoanterior specified material. Gross discontinuity, and right/left asymmetry, of pattern is thus avoided. But the dynamics of the whole system do not appear to contain any true regulatory principle, whereby a new, fully posterior specified region is then instituted to restore a complete, balanced body pattern. Such integration seems only to be a property of the more rapid events that set up the initial system in the whole egg, whose properties are examined in the previous and in the following experimental sections.

In most resymmetrized presumptive lateral halves, therefore, we assume that we have embryos where the slow-acting system that restores continuity of position values after geometrical reorganization, has done this at the price of truncating the specification map so that values normally achieved furthest from the dorsoanterior centre are deleted. This involves no loss in somite segment numbers represented (see Fig. 1A), but an omission of the more laterally derived parts of trunk and tail somite cross sections and much posterior lateral plate. There is corresponding over-representation of notochord and of head pattern in the available tissue, because each reorganized small body now includes some material respecified with the latter

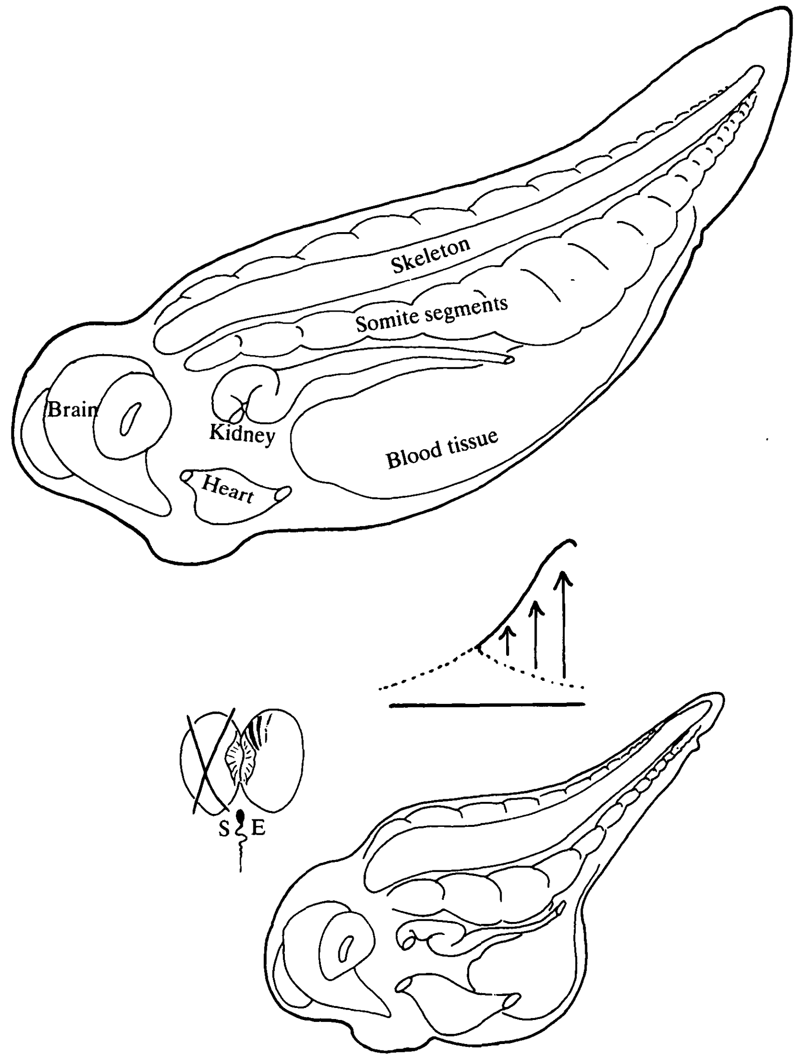

Fig. 3. Pattern imbalance in the 2-cell-stage isolate (presumptive lateral half egg). The upper diagram represents the normally balanced body plan seen from front left. Note proportions, positions of lateral plate, size of tailbud and particularly the relative sizes of somite cell populations at different axial positions (measured as the 'somite index', see text). Below is shown the typical alteration in pattern balance seen in the small body developed from presumptive lateral half egg isolates (see inset). Head endo/mesoderm, head inductions and primitive skeleton (notochord) are over-represented because scaled down much less than the other structures. Somite ratio is altered in favour of anterior somites because, although all the normal segment series is represented, trunk and tail somites are scaled down much more than the normally small anterior ones. The positional profile sketch between the two body plans indicates how, if materials from the posterior-specified extreme of the original egg are newly joined to dorsoanterior extremes in the geometrical reorganization after blastomere separation, an asymmetrical principle of spatial interaction may cause a new, partial profile that would specify the imbalanced body seen (c.p. Fig. 1A).

position values in addition to the material with these specifications inherited from the original egg at blastomere separation. The gradient profile inset of Fig. 3, to be compared with those of Fig. 2, illustrates this hypothesis as to the genesis of dorso-anteriorly imbalanced body patterns by the 'ratchet' principle of back-up positional interactions in *Xenopus*.

Perturbations of the precleavage events. Twinned and imbalanced single bodies.

Results so far described indicate an early-acting positional system in *Xenopus* that displays durability and stability more reminiscent of a structural record than of a dynamic diffusion/metabolism-maintained signalling or memory system. In particular the system indicates an asymmetrical principle of behaviour in that material, once having been activated to particular position values by the early rather rapid events, can frequently maintain such values without 'decay' to development that indicates lesser levels of activation, yet can only be further 'anterodorsalized' by new contact with material of higher originally given levels. This places limits on the system's capacity for later correction of non-ideal spatial profiles achieved by the early-acting system. Failure to correct deficient profiles in the upward sense is shown by the series of incomplete patterns formed after u.v. irradiation, (Scharf & Gerhart, 1980), and in posterior isolates from 4-cell-stage embryos, while failure to down-regulate imbalanced profiles is shown by those lateral half isolates that are nevertheless bigger than some whole eggs. A dramatic recent demonstration of the brevity of the developmental period during which real regulation occurs has come from exposure of fertilized eggs to regimes of abnormal orientation in gravity during the precleavage period, followed by the normal quantitative analysis of the tailbud-stage mesoderm. It is already known that certain such regimes of holding eggs in a tilted or inverted position can 'fool' the activation system into production of a certain incidence of twinning, but full examination also reveals a significant incidence of systematic distortion of balance in whole-sized, single patterns, relative to normal synchronous siblings.

The experiment was prompted by the literature concerning the early, mechanical reorganization in eggs that is required for axis formation, the normal control of axis orientation by sperm entry positon, and the possibility of subverting that control by holding eggs tilted in gravity (Scharf & Gerhart, 1980, 1983; Elinson, 1984; Gerhart *et al.* 1981; Neff, Wakahara, Jurand & Malacinski, 1984). In this laboratory, populations of about 100 sibling fertilized eggs have been immobilized in natural gravity orientation in fitting wells (see Cooke & Webber, 1985*a*), then rotated so as to lower the sperm entry points some 30°, all before halfway through precleavage (by fert. + 50 mins at 18 °C). Subgroups of them have then been retilted by some 90–100°, so as to place the sperm entry side *uppermost* in gravity, at intervals from 65 to 90 % of the time to first cleavage, but with the angle between sperm entry position and the new meridian of maximum downward tilt being somewhat less than 180°. All embryos have been restored to normal conditions (15 % saline, 20 °C) and left to develop side by side from 8-cell stage onwards. When done before the 60 %

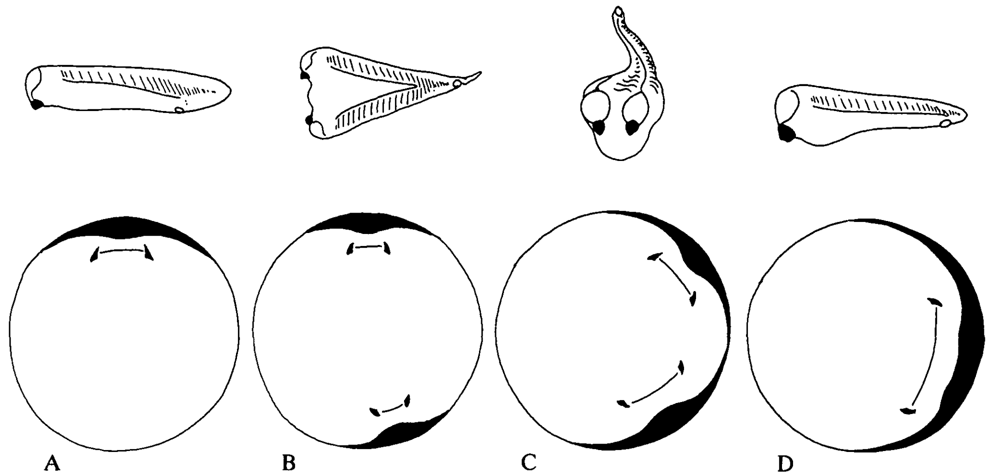

Fig. 4. Possible interpretation of the series of twinned and imbalanced single body patterns seen after tilting eggs in 1 × gravity at around 80 % of the precleavage interval after fertilization. (A–D) outlines of eggs in section normal to animal/vegetal axis, at a level where the proposed positional system registers graded differences around its structure as a result of plasm shifts. Outline thickness is used to represent achieved position values at and above an arbitrary threshold in the system corresponding to some anterior and dorsal level of pattern contribution. (A) represents the normally balanced system of activation, leading to a harmonious body plan organized in approximate relation to sperm entry site. (B) and (C) are twinned activation systems set up by late tilt of eggs in gravity, when one centre of dorsalization had already been instigated by the egg's own mechanisms but the structure elsewhere is still labile for the required plasm interactions. Data on such twin patterns indicate that if the initial angle between such centres is wide, each is of more restricted scale than normal when the egg equilibrates. If the angle is smaller, each tends to be of normal or supranormal extent (Cooke, in preparation). (D) is an imbalanced single system caused by late tipping, where pattern territories corresponding with the designated activation levels and above are made by more of the egg than normal, while posterior contributions to pattern are necessarily under-represented. This may occur when plasm shifts *across* egg meridians under conflicting influences of gravity and the egg's own unfinished movements. See text for further remarks.

time point, this procedure almost always results in normal larval body patterns orientated strongly in relation to the tilt (high sperm entry side dorsal), but as late as the 90 % time point, the sperm entry meridian is almost always left near the posterior pattern midline as in the natural activation. The present experimental egg populations, in comparison with synchronous similarly treated, but untilted control sibling populations, give rise to significant numbers of profoundly twinned bodies (c.4 %, Fig. 4B, C) and to a significant though variable incidence (1–15 %) of single bodies with strikingly imbalanced external morphology. Much material appears to be devoted to anterior trunk and to ventral head structure, and relatively little to posterior trunk and tail (Fig. 4D). The rates of arrival at gastrulation, around the marginal zone, predict the configuration of body pattern. The plan-view diagrams of eggs given in Fig. 4 are meant to represent, by the thickness and spread

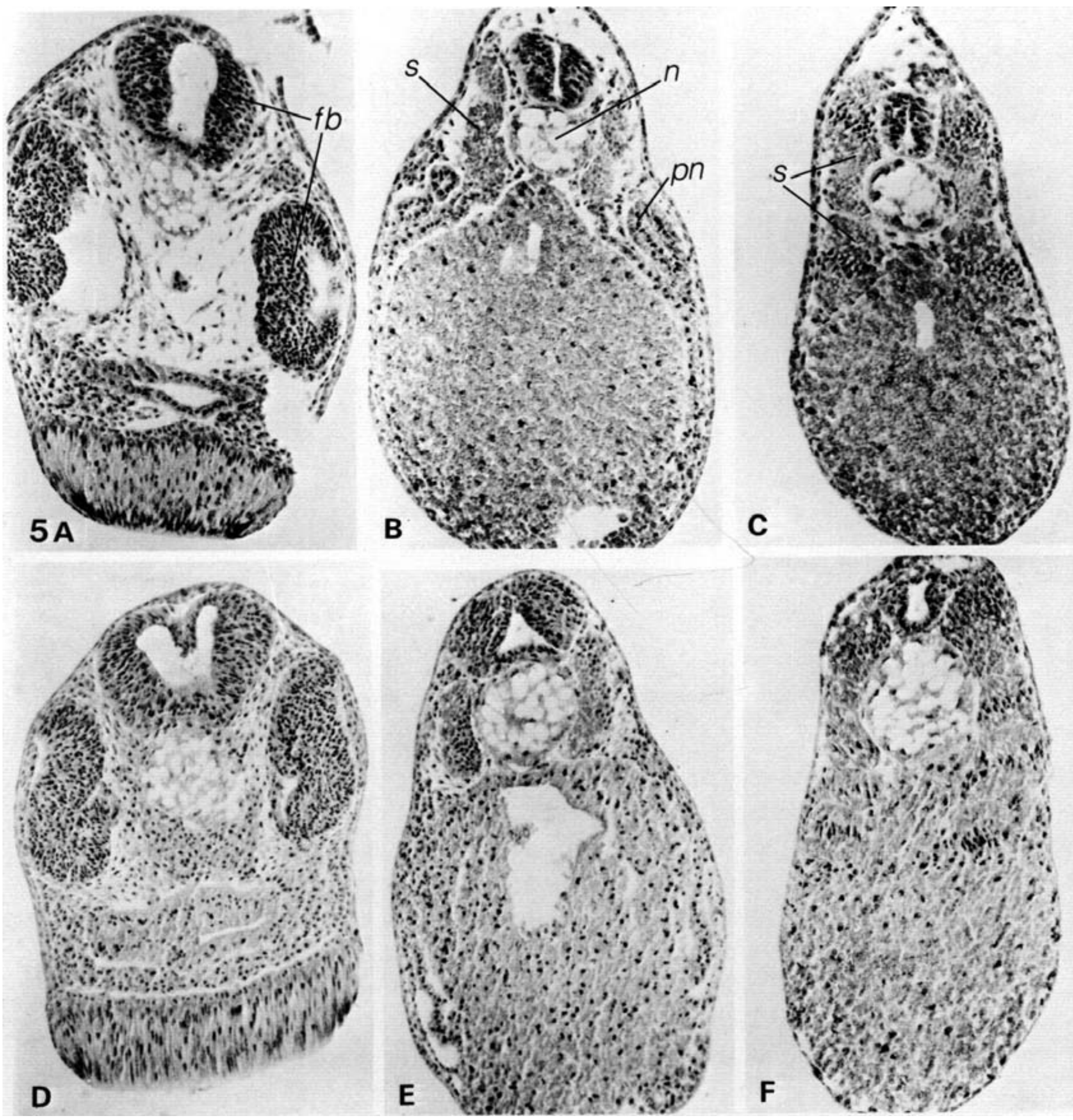

Fig. 5. Transverse sectional appearances of a normal and an imbalanced body, from sibling embryos after a 1 × gravity tilting experiment. A–C, normal body; D–F, dorsoanteriorly overbalanced body. A, D, level of forebrain, eyes and cement gland induction. B, E, level of the pronephros. C, F, mid-trunk (around somite 10). Note massiveness of cement gland area, and also massive notochord, ill-defined and small pronephros, and small trunk level somites of the abnormal body. *cg*, cement gland; *fb*, forebrain and eyecup profile; *n*, notochord; *pn*, pronephros at largest level of section; *s*, somite; *lpl*, lateral plate.

of their supposed sectors of high activation, the distribution of specification for the faster rates of gastrulation and the more anterodorsal body contributions in twins and in imbalanced bodies (Fig. 4D). It appears that under strongly conflicting

Table 1: *Pattern balance in a sibling set after an experimental regime of gravity reorientation to eggs*

(% mesoderm cells)	NC	S	PN	LPL	somite ratio (see text)
Cont. 1 (no tipping)	5·6	43·4	6·0	45·0	3·4
Cont. 2 (no tipping)	6·3	47·1	5·3	41·3	3·1
Cont. 3 (no tipping)	5·7	45·6	5·5	43·2	3·5
Cont. 4 (tip, but normal)	5·7	46·7	5·0	42·6	2·9
Cont. 5 (tip, but normal)	6·0	44·1	5·6	44·3	3·5
Exp. 1 (deviant gastrulation and morphology)	8·9	37·4	3·2	50·5	2·1
Exp. 2 (deviant gastrulation and morphology)	9·8	44·0	4·3	41·9	2·4
Exp. 3 (deviant gastrulation and morphology)	10·1	38·6	3·9	47·4	1·7
Exp. 4 (deviant gastrulation and morphology)	11·4	35·0	2·1	51·5	1·0
Exp. 5 (deviant gastrulation and morphology)	9·5	39·5	3·8	49·4	1·8

influences at wide angular displacements a single centre of activation, while embracing an abnormally large egg sector, may nowhere fully attain the levels characterizing the normal apex of the system. This shows up in detectably late onset gastrulation and, as will be mentioned, in the body pattern.

Figure 5 shows photographs of sections through a normal and a pattern-imbalanced single individual. Individuals appearing externally similar to these experimentally provoked ones are almost never seen in undisturbed egg batches, and controls that most approach the appearance have not been found to have internally measurable imbalance as given in Table 1. Table 1 shows pattern in a sample sibling set of normals and anterior-heavy larvae after a tilting experiment. Twins and imbalanced singles after these procedures form a series, as the most severely imbalanced, externally single bodies show internally an incipient duplication of notochord and prechordal endo/mesodermal structure. 11 twins and more than 20 singles have been analysed to date, in relation to a larger number of normal siblings. The series will be described in detail in a future paper. They show, to varying extents, the following deviant features of mesodermal pattern.

1) The notochord territory is significantly enlarged, often dramatically so, to give a notochord including up to 60–100 % more cells than in controls. This excess is concentrated anteriorly in the axis, with the tissue cross section reducing progressively to normal scale at the last-developing region in the tailbud.

2) The 'somite ratio', expressing the relative sizes of cell populations used to found segments at the pronephric level and at the posterior trunk levels 10–15 somites further back, is reduced because the small anterior segments are of normal or even above normal cross section, whereas the usually more massive posterior ones are reduced. This can be striking, with ratios of less than unity when control values are more than two.

3) Lateral plate tends on aggregate to be over-represented in the mesoderm population, while aggregate somite is reduced. The relatively anterior, postcardiac lateral plate where massive blood islands develop, accounts for most of the lateral plate overbalance. In individuals, massive lateral plate is correlated with massive notochord and deficient somite cell populations. This observation is striking in view of the usual belief that in mesodermal histogenesis notochord is a 'dorsal' differentiation, somite is 'induced' by proximity to notochord, while lateral plate results essentially from failure to achieve either of the other two developments.

4) The pronephric rudiment is usually few celled. This effect is variable, but present if the other features are marked. It recalls the small or absent pronephros in the dorsoanterior isolate part-pattern, and the frequent absence of detectable pronephros in the profoundly twin patterns caused by the early disturbances being discussed (unpublished results).

5) The tailbud is very small and the blastopore or proctodaeum thus more nearly terminal in position. The total analysed (postcephalic) mesoderm is sometimes significantly small for embryos of the egg batch, and in these cases the head (too complex for analysis) is seen to be correspondingly large. A different version of the syndrome, however, is seen on external and internal evidence *not* to have large head structure apart from the ventral area of cement gland induction, and the prechordal head mesoderm and forebrain/eye induction are found actually to be reduced in cellular size in some patterns showing the largest notochord proportions.

Taken together, these deviations from the normal range of primary body forms constitute a syndrome that can be interpreted both in terms of our revised understanding of the normal fate map for mes/endoderm, and in view of other experimental evidence about early specification for body position in the egg. The notochord-heavy but sometimes head-deficient pattern with abnormally small trunk somite segments, tailbud and pronephros, is what might be expected if the original spatial profile in a positional variable, given by the rapid-acting 'structural' system in the egg, were distorted as shown in Fig. 4D. This involves a 'flattening' of the profile so that relatively dorsoanterior activated regions occupy considerably more of the margin than normal (with corresponding deletion of specification levels normally found near the sperm entry position), but perhaps with a deficiency in the levels of the normal anteriormost apex of pattern that correspond with prechordal head territories. It appears that, if a particular level of the early variable be taken to correspond with a certain relatively anterodorsal contour of the normal fate map, then the disturbed eggs record an abnormally wide sector of vegetal material as activated to a level above this and a correspondingly diminished sector as below it.

Because of the 'ratchet' principle that operates in any more prolonged, subsequent positional interactions, and the lack of ability for regulative restoration of lower boundary states of the normal system, this disturbed outcome of the early events can never be restored to normal by such eggs. An unknown and possibly extended period elapses before cellular commitments and differentiations are normally made in the inner two germ layers, in response to this early positional record.

In this experiment, any abnormal events engendered by a conflict between the endogenous activity of eggs and the effect of gravity last at most one hour and a half, until the 8-cell stage. A brief early experience thus registers as a subtle but considerable and systematic departure from the normal balance with which mes/endoderm is apportioned to the structures of the body plan. This constitutes dramatic evidence that the kinetics and thus mechanism of the establishment of pattern in at least this species are very different from those that have been assumed as general to vertebrate embryogenesis.

DISCUSSION

As with other vertebrates, cell lineage as such is no part of the mechanism for early spatial organization in the embryo of *Xenopus*. The work discussed has merely made use of the relative consistency of a particular, common pattern of early cleavage, and of the fact that some important part of the structure of the activated egg is non-fluid and contains recorded position value so that the materials of the vegetal and marginal regions are used in a geographically very predictable way in founding the body plan.

We do not know at what point in normal development the primary cellular variable is utilized to affect the nuclei of cells inheriting it and thus begin differential programs of transcriptional activity. This is probably not prior to the midblastula transition (see Newport & Kirschner, 1982), when significant interphase and chromatin decondensation is first seen, and the differential primary information for pattern may be carried and expressed by extranuclear mechanisms for much longer than that (see e.g. Slack, 1983). Under experimental circumstances, isolated cells or small tissue pieces translocated to foreign environments in the embryo will still conform with a 'majority decision' made by their surroundings in choosing a final state of differentiation, even in some cases after having begun their development according to originally specified fate (see contributions by Gurdon, Slack, Nieuwkoop and Smith to this volume). This emphasizes that we are dealing with a signal that relies, for its integrity and the maintenance of large scale pattern, on normal tissue continuity and cell social behaviour over amounts of material considerable in relation to the size of the whole embryo (as in the large isolates, for instance). In lineage terms, most cells of the embryo, including probably most mesodermal cells, must make the structures they do as the result of experiencing specific regimes of inductive signals transmitted along the vegetal-to-animal dimension from those relatively restricted regions that *do* truly inherit the primary positional system. The production and receipt of such intercellular signals (as opposed to the differentiation in response to them) may or may not involve nuclear activity. This paper has been about the surprising earliness and brevity of the time period for the inception and regulation of the primary positional variable itself.

Two salient questions are; what is the molecular nature of this primary positional variable and the record of its levels in material, and what is the principle of cellular organization whereby it is finally 'interpreted' as specific schedules of inductive and transcriptional activity and the corresponding specifications and commitments (Slack, 1983)? As to the first question we can as yet say little. Its behaviour has little resemblance to that expected of a diffusing morphogen. Cellularization as such is not necessary to the mechanism that records its graded levels, and something cell structural is indicated both by its persistence and by the nature of the events (plasm shifts) that appear to set it up. Even the upper boundary levels of the activation system do not behave like a fixed quantity of some special plasm or substance in eggs, since embryos that have developed twinned profiles in response simply to tilting the egg in 1 × gravity often have more than twice as wide a sector as normal, in aggregate, devoted to head mes/endoderm pattern and notochord induction. As to the second question our only clue at present is the intimate correlation that exists, in all normal and experimental developments, between the rate (at each temperature) with which material arrives at the point of participation in the gastrulation movements, and the region of the mes/endodermal body plan which it goes on to form. Far from being instrumental in specifying the fate of mesoderm, as in some models, the time of participation in gastrulation by presumptive mesoderm is preprogrammed locally, and is itself a function of the latter's regional specification before gastrulation begins. Presumptive mesoderm probably receives inductive signals progressively later at positions progressively nearer the sperm entry meridian and the animal pole, i.e. at locations where progressively more posterior pattern contributions are specified within it. Cells' perception of time of receipt of inductive stimuli in relation to intrinsic 'age' (i.e. to physiological elapsed time since onset of development), presents itself as a possible coding principle for specifying tissue value in mesoderm. Such measurement of the relative rates of two cellular subprocesses, one endogenous and one caused to vary by the effect of a positional variable, could form a general mechanism for translation of an initial spatial gradation into a pattern of specified states.

The primary positional system in some other vertebrate embryo types, probably including some amphibians, must utilize different machinery from that studied here in the *Xenopus* egg. Such machinery behaves more in conformity with the expectations from diffusion-based systems, and corresponds perhaps with the 'back-up' or ratchet interactions that serve to heal artificial discontinuities in the positional system that we have described. We cannot yet say, however, whether the classically studied amphibian system, the large urodele egg, is really dominated by this slower, more regulative mechanism as classical reports indicate, or whether differences in methods of study account for the apparent differences in behaviour.

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DISCUSSION

Speaker: J. Cooke (NIMR London)

Question from B. Goodwin (Open University):
To what extent do you feel that the observations you have presented are consistent with the interpretation that the boundary values are included in the cortex or in very superficial layers of the egg?

Answer:
I purposely steered clear of suggesting any one physical subdomain of the egg as being dominant. Most of the structural studies that are now being done on eggs tend to suggest there is more possibility of mechanical preservation of information relatively near the surface of these enormous cells than in the middle. But I wouldn't say that the alternatives are ruled out.

Question from N. Holder (Kings College, London):
Have you done the separation at right angles: between the animal and vegetal quartets of the 8-cell stage?

Answer:
We have done very few of these separations. I think John Gurdon and his colleagues have done them. What we have been doing is paying a lot of attention to the division of the *fate map* that is made by the third cleavage. I think we can say already that most of the mesodermal pattern comes from the cells of the animal quartet, suggesting that it really is set up by inductive transfer of information from underneath. We have not done separations. I think you can easily find out what the result of those are but you shouldn't ask me, you should ask John Gurdon. [The vegetal tier forms muscle, the animal ties does not. See Gurdon *et al.* this volume – Ed.]

Question from G. Malacinski (Indiana):
Could we return to your separated blastomeres at the 2-cell stage? Is the repositioning of material which you describe subcellular or supracellular, and when does it occur?

Answer:
It occurs as the egg goes on the cleave. The fact of separating blastomeres means that the subsequent cleavage planes don't occupy the same positions in the egg material as they would have, and the way in which they deviate acts over the next 2 or 3 h to bring about the reorganisation of material.