Dynamics of the control of body pattern in the development of *Xenopus laevis*

II. Timing and pattern in the development of single blastomeres (presumptive lateral halves) isolated at the 2-cell stage

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**SUMMARY**

*Xenopus* embryos have been selected in which the first cleavage plane is tending strongly to correspond with that of bilateral symmetry for the future larval pattern. The two blastomeres produced by this cleavage have been separated and allowed to develop as reciprocal pairs of presumptive lateral-half isolates. The early development and the time course of the gastrulation movements, and the qualitative and quantitative aspects of larval mesodermal patterns, have been studied in relation to synchronously fertilized controls. The result is restoration of bilateral symmetry for developmental potential, and production of a pair of small gastrulae and larvae with qualitatively complete body plans but 50% of control cell numbers each (see Kageura & Yamana, 1983). Most commonly, however, the resulting small patterns deviate from the normal range in their proportions in at least one of two ways. The notochord territory is rarely, if ever, under-represented (i.e. notochords contain at least 50% control cell numbers) but it is frequently over-represented, in some cases approximating that of control whole embryos. Reciprocal pairs of isolates thus tend, on average, to produce more notochordal tissue than they would have if developing normally. Secondly, the balance of cell population sizes assigned to somites up and down the axis is altered so that relatively anterior members of the series are less scaled down than those in trunk and tail regions. The mesodermal plan developed by a lateral-half isolate is thus characteristically intermediate between that of a small, harmonious larva (the proportional presumptive fates of the materials, apart from loss of bilaterality), and that of the mosaically developing presumptive dorsoanterior cell pair from the 4-cell stage, discussed in the previous paper. A minority of the lateral isolates does develop a harmonious and thus well-regulated body plan, however, and the normality or otherwise of the schedules of gastrulation timing in isolates seems a good predictor of their morphogenetic performance in this respect. The results are discussed as informing us about the dynamics of the interactions, prior to gastrular stages, that underlie the normal mesoderm development.

**INTRODUCTION**

Since the classical era of experimental embryology it has been widely accepted that if the amphibian pre-gastrular embryo is divided into two parts, each including material from a restricted region destined to contribute the anterior and dorsal mesodermal structures, then two harmonious-looking larval bodies can result (Spemann, 1902, 1938; Ruud & Spemann, 1923). A recent study separating

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Xenopus blastomeres at 2- to 8-cell stages has confirmed the finding for this relatively small, rapidly developing embryo (Kageura & Yamana, 1983). These authors also confirmed that an early restoration of the normal inside–outside blastula configuration, by new cell contacts, is necessary for the regulative events that restore bilaterally symmetrical capacities to the halves and thus redistribute developmental fates of the material. The basic result takes on additional interest in view of the overall conclusion from the study reported in Paper I of the present series, on isolated development of presumptive dorsoanterior and posterior egg parts. This indicated that positive specification for general regions of the body plan may be widespread around the margin of the egg soon after the ‘symmetrization’ events of the precleavage period, rather than confined to a dorsoanterior ‘reference’ localization as had been assumed. Formation of a truly harmonious body plan by each lateral half would on this view entail a positive adjusment of the developmental tendencies in the material, rather than simply the successful performance of a dynamic positional gradient system, originating at the dorsal reference location but organizing a reduced cell population on a smaller scale.

This paper reports the results of a series of blastomere separations at the 2-cell state in Xenopus, similar to those of Kageura & Yamana but with the additional monitoring of the time courses of gastrulation and quantitative investigation of the anatomical patterns at tailbud larval stages. Reciprocal pairs of presumptive lateral halves have been kept in agar wells beside the precisely equivalent, whole controls (see previous paper). The relative frequencies of different versions of the result obtained seem to differ from those of the Japanese authors, but it is hard to say whether this is a real difference or one appearing because of our pre-occupation with the rather precise performance, in terms of proportions, of the normal pattern formation. This leads us to be relatively sensitive observers of the deviations from normal pattern which the majority of our half-size embryos display, even though they are complete bodies in a qualitative sense.

Circumstantial evidence is presented that the more, or less, successful production of normal pattern at small size may be related to the geometrical events whereby normal early blastula configuration is restored in individual instances. The results are discussed together with the conclusions from 4-cell-stage separations (Paper I), as possibly informing us about the dynamics of the mechanism that first lays out a spatial gradation of properties across the fertilized egg, and about the way in which such an early gradation is actually used to realize a pattern. This, the previous and the following papers in the series are so inter-related that they should be read together, and together they cast great doubt on the validity of previous evidence for true regulative capacity by the system after size reduction operations at blastula stages (Cooke, 1975, 1981, 1982).

MATERIALS AND METHODS

Fertilization and treatment of eggs, selection of first cleavage planes passing near the vertical plane defined by sperm entry, demembranation and separation of daughter cells at the first cleavage were all carried out as described in detail for the 4-cell stage separations in the previous
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In certain reciprocal pairs of blastomeres small Nile-blue marks were placed at marginal sites near the sperm-entry plane and on the old, 'external'-type cell surface, by temporary insertion of a chip of impregnated agar into the snugly fitting well against each newly separated cell (see Fig. 4). In the great majority of experimental sets, development was slowed at 15°C between control 64-cell stage and the following morning when the temperature was restored to 20°C. The onset and detailed time course of gastrulation was then compared between isolates and identically treated co-fertilized controls (see Materials and Methods, Paper I). The position of any Nile-blue-marked regions in the structure of the early gastrula was also then noted.

At a late tailbud larval stage (stage 30 Nieuwkoop & Faber, 1967), somite numbers segmented and body conformations in synchronous sets of experimental and controls were recorded by camera lucida under shifting oblique lighting. Quantitative assessment of the balance and proportions between parts in the newly founded mesodermal body pattern was carried out by cell counting on regularly distributed transverse sections. For details of fixation, embedding, sectioning at 7μm, staining with Feulgen/Light green/Orange G and counting of nuclei, see previous work (Cooke, 1979d, 1981, 1983). Certain series of horizontal sections were also made to corroborate earlier findings in this laboratory concerning the actual cellular texture (internuclear distance) in smaller and less elongated bodies as compared with controls of identical stage.

Observations

Body patterns

External body conformation was recorded at round stage 30 from 52 reciprocal pairs of 1st cleavage isolates. These had developed without any sign of abnormal relaxation of cellular tension near the site of original separation, or any subsequent abnormal exposure of blastocoel or 'internal' type cell surface to the medium. Members where only one isolate survived are not included, in order to facilitate interpretation of the observations. Body forms were always classified in comparison with the synchronous, equivalently treated controls.

74 individuals (75%) were classified as appearing significantly 'overbalanced' in anterior or dorsoanterior aspects of pattern. See Fig. 1B and C for examples. Although the anterior part of the body was constructed on a smaller scale than that of controls, it was less scaled down than the trunk and tail, giving a 'pigeon-chested' appearance. More posterior regions were characteristically slender, small and uptilted. In most examples the blastopore was nevertheless positioned ventrally to a small tailbud (Fig. 1B) but in a more extreme minority the whole conformation approached the 'jug-handle' appearance that was a common outcome in presumptive dorsoanterior isolates from the 4-cell stage (Paper I). In these bodies, a relatively very disproportionate head and heart region was accompanied by a thin, nearly radially symmetrical posterior extension containing little endoderm and terminated by the blastopore. In the less extreme majority, somites could be counted. The number segmented, and relative body position reached by the segmentation process, were never significantly different from control. Typically, however, the profile of relative somite sizes (depths) along the axes were quite abnormal (see below). At the other end of the spectrum of morphologies, a harmoniously balanced but small body confirmation was approached, with 18 embryos classifiable as having no apparent imbalance in
pattern (see Fig. 1D). As a population, therefore, the morphologies produced in these first cleavage separations spanned from external normality to a degree of imbalance resembling the mean (though never the extreme) of those produced by the isolated dorsoanterior cell pair. The commonest outcome, however, was a qualitatively complete pattern with a distinct overbalance in the direction of dorsal and anterior structures. There was no appearance of left–right asymmetry in major proportions, and only one case of situs inversus (mirror reversal of normal heart and gut handedness) was seen among more than 30 twin pairs examined at 40 s stages.

In each of the remaining six pairs, one individual showed qualitative imbalance in the reverse direction to that of the series as a whole, having either extreme cyclopia or anterior truncation at ear vesicle level, with histology revealing a notochord territory confined to the tail region. Since these were paired with examples which were all very dorsoanterior in morphology, in 5/6 cases more so than all others of this series, they may with reasonable confidence be attributed to the results of first cleavages (and sperm-entry points) deviating more widely than normal from the sagittal plane for pattern. Together with a few examples mentioned in Paper I they form a connecting series, in the egg regions partitioned and in their behaviour, between typical isolations at first and at second cleavages.

After exclusion of the presumed results of mistaken separations just described, the morphological outcomes for the reciprocal members of pairs in the series showed a positive rather than an inverse correlation. Thus a few instances of one extreme and one decidedly dorsoanterior body were seen (though none in which both members were extreme) and the commonest single outcome of the separation was two small larvae displaying the kind of moderately but significantly imbalanced body typified in Fig. 1B. The minor class of harmonious small bodies was also characteristically produced as reciprocal pairs (16/18). The abnormally balanced morphologies are thus due to events following the isolation per se, and not due to asymmetrical partitioning of the relevant regions of the egg structure. The variation in outcome as between pairs was presumably a reflection of variation in some property or properties of eggs as well as due to chance events, and there was a strong impression that each egg batch used in the experiments tended to produce its own degree of imbalanced development in first cleavage isolates.

In terms of somite pattern, although the commonest result for pairs was a regulation in the sense that two numerically complete sets of somite segments were produced, a relative overassignment of tissue to the more anterior somites in the smaller scale patterns was evident.

The mesodermal patterns of apparently harmonious and of dorsoanteriorly imbalanced bodies were analysed quantitatively in relation to within-set controls. In terms of the overall proportions of the mesodermal nuclei encountered in each histologically defined pattern element (see Cooke, 1981, 1983), these bodies fall remarkably within normal limits except with respect to notochord. But the recent fate-mapping work (Cooke & Webber, 1985; Webber & Cooke, in preparation) has revealed that we should not expect a lack of regulation after size reduction to
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Fig. 1. Body forms developing from presumptive lateral halves isolated at first cleavage. (A) Two control larvae at the stage used for examination of pattern. (B) Two small bodies qualitatively complete but with dorsal and anterior regions significantly over-represented in the pattern. (C) Two more severely imbalanced patterns. The balance of mesodermal pattern in such cases approaches that seen in some dorsoanterior isolates from the 4-cell stage (Fig. 4B, Paper I of this series). It is rare for both members of a reciprocal pair to exhibit pattern as imbalanced as that at lower right. (D) Two small bodies with harmoniously balanced pattern, having cellular proportions closely resembling those of controls but half their total cell number. fb, forebrain; ev, hindbrain level and ear vesicle; cg, cement gland; g, gill structure; e, eyecup; pn, pronephros; bp, proctodaeum (original blastopore). Somite segment numbers and positions indicated as visible in camera lucida. Scale bar represents 1 mm approx.

show up as any marked change in tissue proportions between somite, pro-nephros and lateral plate. This is because somite, lateral plate mesoderm and pro-nephros are not specified at pregastrular stages by relative distance from an ‘organizing’ region, but are each disposed in the fate map as bands of tissue stretching around the equatorial regions. The axial position in the head-to-tail sense, to which material is set to contribute, is however related to distance from the centre of dorsalization. We might therefore expect instead, a quantitative imbalance in the
anteroposterior distribution especially of somite and lateral plate tissue, along the axis in ill-regulated patterns. In fact, the internal reconstructions do reveal an anatomical imbalance of this sort that correlates with the external classification of individuals, and show that their imbalanced appearance is no trivial mechanical consequence of small overall size or germ-layer disproportion etc. This will be described below after the simpler notochord imbalance has been dealt with. Both these abnormal features of the analysed sample are laid out in Table 1. Since overall proportionality for the most extensive tissue types is maintained, relative estimates of the total mesodermal cell populations as between experimentals and controls are justified and cell number in these small, quantitatively complete patterns co-incides closely with the expected 50% of that in whole sibling larvae. This is in line with the previous finding (Cooke, 1979, 1981) that foundation of a pattern on an abnormal small scale does not affect cell cycle before larval stages in these embryos (cp. mammals, Snow & Tam, 1979).

The only histological imbalance which is a regular feature of these patterns involves notochord, since more than half of the analysed sample has notochords that embrace 6-9% of their mesoderm as opposed to the 3.5-4.5% that is normal. These results have been ratified by inspection of cell packing in horizontally sectioned material. They show that the most restricted development of notochord normally seen in these isolates involves 50-60% of the control cell number (i.e. normal proportioning), and that it is common for at least one of the original egg halves to devote to notochord a cellular territory 70-90% as large as that which the whole egg would have devoted. Therefore the pairs of blastomeres, when isolated, have between them usually produced much more notochord than they would have in normal development (at least six of the nine pairs included in Table 1). But within this elevated range (leading to a mean of 72% of control notochord cells in bodies with 50% control mesoderm cells) the picture is complicated by the appearance in the table of a tendency to inverse correlation in the notochord development between reciprocal isolates.

Fig. 2 shows the relationship between absolute notochord size and total mesoderm size at pattern foundation, from whole eggs (extensive control series from the laboratory collection) and from the sample of isolates at the 2-cell stage. This is interesting but not straightforward. Mesoderm cell number at a constant tailbud stage varies across a 2-fold range among normal embryos, so that the upper end of the distribution of isolate-derived patterns overlaps in tissue size with the lower end of that of the control series. The range is probably related to variation in original egg (or blastomere) size (see following subsection and Discussion). In ‘whole’ developments, notochord size appears to regress linearly upon mesoderm size so that a strong tendency towards constancy of proportion is seen. But only a minority of the experimentally small patterns, those with the smallest notochords, preserve this proportionality within and below the lower end of the normal mesodermal size range. More typically, some feature of development in the isolated lateral blastomere elevates the relative size of the founded notochord territory even where the absolute amount of tissue is within
the normal range for embryos of the species. Notochords of experimental bodies were of essentially normal distribution up and down the axis.

It is only when the quantitative analysis of body pattern contributions is carried to a more intimate level than that of major histological elements, that the remaining abnormal feature in experimental mesoderms shows up. This abnormality, of balance with respect to somite sizes along the segment series, is itself variable. Its degree within individuals correlates with their prior classification as to general body form (see Table 1). Because of the way that nuclei are positioned within the populations of parallel, longitudinally stretched cells that constitute the larval somite segments, examination of transverse serial sections enables the numbers of segments to be counted as peaks in numbers of transected nuclei. Such examination also reveals a characteristic profile with respect to the relative numbers of cells composing somites (i.e. participating in their cross sections in depth and width), at different positions along the segment series. These relative somite sizes are a constant pattern feature for the normal larval body. Lineage tracing reveals that they are themselves related to the normal contributions from each pregastrular region to the various positions along the somite rows (Webber & Cooke, unpublished; Cooke & Webber, 1985, Paper I). The mean ratio of maximal nuclear numbers seen in somites 4–6 (ear vesicle/pronephros levels) to

Fig. 2. Relationship between notochord cell number and whole mesoderm cell number among normal embryos and in lateral-half isolates. The axes intersect at the zero origin so that the relative range of notochord and mesoderm sizes to be found at the stage of pattern estimation is seen. The significant preservation of proportionality (around 4%) in notochords of normal embryos of different sizes (open) is also seen. The isolates (filled), while overlapping with control mesoderm sizes at the bottom end of the latter range, are often deviant as regards notochord proportion. They may possess notochords of a size near normal for controls within their own egg batch.
those in somites 14–16 (normally among the largest) varied from 1:2.5 to 1:3.7 in controls of the various sets analysed. The variation in this control ‘somite ratio’ would appear to be a characteristic of development from various egg batches as it was much more consistent within each set, but it was not correlated with absolute mesoderm size within our restricted sample of normal bodies. In many experimental small patterns however, the somite ratio was much altered, the posterior trunk somites having cell populations little larger than those of the anterior ones. As seen in Table 1, the ratio varied from almost normal down to very abnormally reduced values among experimental patterns, and expressed rather well the variation that led to individuals being classified as balanced, mildly imbalanced or

<table>
<thead>
<tr>
<th>Set</th>
<th>Pair</th>
<th>External body classification (see Fig. 1)</th>
<th>Notochord size (% of mean within set control cell no.)</th>
<th>Somite ratio (Mean max. nuclei/section seen in somites 14–16 as a multiple of those seen in somites 4–6)</th>
<th>Control range of somite ratio (see previous column)</th>
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severely imbalanced at the time of fixation. Pattern balance as revealed by this somite ratio among experimental small embryos was again uncorrelated with their absolute sizes. The tendency to overbalance in the direction of notochord production correlates at most poorly with this second tendency to pattern imbalance within individuals, in a way consistent with the positive correlation between pair members as regards external classification and the absent or negative correlation for notochord size. Fig. 3A,B show, in schematic exploded view, the distortions in balance of axial anatomy typically found after isolated development of 1st cleavage egg halves.

The nervous system was well developed histologically and architecturally in all genuinely lateral half-isolates. In examples showing the more extreme dorso-anterior mesodermal imbalance, the brain and accessory head inductions though smaller than normal were over-represented in relation to spinal cord.

Early development and time schedules of gastrulation

The positions of cleavage planes immediately following the isolations were usually deviant from those to be expected in the halves of intact embryos. Most often, the patch of naked new cell membrane from the separation was helped to occlude itself from the surface by this altered pattern, but the latter was not invariant. The naked membrane, although no longer apparent by control 64-cell stage if development was to continue healthily, must in some cases have 'converted' to outer-type surface, or have been internalized by local adjustment of blastomeres (see vital staining results below). At onset of stage 10 (the beginning dorsal lip) 65-70% of control cell numbers were counted around the marginal zone in isolates of each set. The range of control numbers obtained by such surface cell counting was however variable between eggs of different batches, with originally large eggs producing gastrulae of greater diameter and apparent cell number.

Controls for gastrulation timing were always siblings in which blastomeres had been re-annealed after near-total separation as for experimentals, since this process alone is known to delay measurably the onset of stage-10. With this precaution followed, the onset of dorsal lip formation in the faster member of each experimental pair was always within 15 min on either side of mean control onset. In about one third of all pairs stage-10 onset was synchronous within a few minutes in the members, and only in those exceptional isolates which were to form apically incomplete patterns (oblique cleavages, see last subsection) was lip formation delayed by as much as 25 min. In the remaining pairs, the gap varied between about 10 and 20 min. Of 12 individuals showing a notochord cell population close to 50% of control (i.e without imbalance for notochord specification), 10 cases associated with satisfactory gastrulation records came from pairs in which one partner had shown a relatively late (20 min behind the other, and appreciably later than control) stage-10 onset.

More than two thirds of all isolates, having begun lip formation within 15 min of controls, showed a precipitate time course of spread in the surface 'bottling'
activity around the marginal zone. This led to completion of a ring-shaped blastopore within 50–65 min of onset, at 20°C, as opposed to the 95–100 min typical for control gastrulation. It is thus typical for an isolate, even if stage-10 begins 15 min later than in a control, to complete the blastoporal ring some 15 min ahead of it. The most rapidly gastrulating examples tended to go on also to perform rapid closure of the blastopore, and to produce a nipple or extended area round the closed blastopore which led to an abnormal pear-shaped configuration before any neural plate formation (see also Paper I of the series). 63 of the 74 individuals classified as pattern imbalanced (anterior-heavy) came from pairs where at least one member (usually both) had pursued this significantly foreshortened schedule of gastrulation.
The remaining recorded small gastrulae, having started activity across the same time range as those discussed so far, appeared then to pursue a schedule parallel with their controls in terms of lip development, lateral spread and completion of an annular zone of bottling activity. In view of their size, this involved an appreciably slower spread of the wavefront of new cell activity through tissue, on a per cell basis. 16 of the 18 embryos classified as of balanced morphology came from pairs where at least one member (usually both) had pursued this extended, thus control-like schedule of gastrulation. No clear counter examples to the overall correlation between mode of gastrulation and later balance of pattern were seen, the remaining cases either lacking adequate records or coming from egg batches of the type where at least the surface aspects of the gastrulation progression are less well organized and coherent than is usual. Although a binary classification has been made, the records show a continuum of timing behaviour by small gastrulae in relation to their controls. But in keeping with the positive within-pair correlations for pattern balance in isolates, the occurrence of similar gastrulation schedules in members of pairs was much more frequent than that of markedly different schedules.

In three egg batches certain new 2-cell isolates were given a small Nile-blue mark, on ‘exterior’-type membrane near the equator, close to where the 1st cleavage had passed through or near the sperm entry position (Fig. 4A). Of these, 13 were observed the next day to have the mark at or to one side of the stage-10 dorsal lip (Fig. 4B). Ten were healthy, and all completed the precipitate type of gastrulation, and the six survivors to the stage of pattern classification were all of dorsoanterior imbalanced type (Fig. 1B or C). A further three embryos showed the mark lateroposteriorly, i.e. far from the dorsal lip at stage 10 (Fig. 4C). These gastrulated on the extended, control type of time schedule and the two that survived developed ‘balanced’ morphology (Fig. 1D). This very limited direct evidence supports the inference, from observing development immediately after separations, that lateral half-eggs may restore the blastula configuration in two basic ways (Fig. 4D,E). These have rather different consequences for the geometrical redistribution of those egg regions originally defined by their relationship with the sperm entry meridian and thus the ‘centre of dorsalization’. By itself, the placement of a single mark cannot give strong evidence as to whether the site of dorsal lip formation in the material was itself altered by the reorganization in lateral isolates. Observation of pigment patterns and cell-size discontinuities in relation to these graded properties in whole sibling blastulae strongly indicates, however, that the actual centre of dorsalization shifts little if at all, whereas material elsewhere is geometrically reorganized to varying extents.

**Discussion**

The small larval bodies produced by separations at the 2-cell stage are as bilaterally symmetrical as a control series. In contrast to the presumptive
Fig. 4. Modes of reorganization in 2-cell-stage isolates. (A) Pair of newly separated isolates to show position of vital stain marks near the sperm entry meridian, thus opposite the centre of dorsalization where the dorsal blastoporal lip would normally form. (B) View from vegetal aspect of stage-10 gastrulae with newly formed dorsal lips, derived from isolates as in A. Tissue bearing the vital stain is usually at one side of the dorsal midline, suggesting that presumptively posterior material has been rejoined to dorsoanterior material as would happen in the left diagram of Fig. 4C. These events may be linked to the precipitate schedule of gastrulation, and the imbalanced specification map and thus body pattern shown in Fig. 3B. Alternatively the stained territory sometimes remains far from the 'organizer' region, suggesting that the resymmetrization has been accomplished more as in the right diagram of Fig. 4C. This may produce small embryos that gastrulate more nearly in parallel with full-sized controls, and produce more balanced body plans. (C) *Camera lucida* sketches, from animal pole aspect, of half-morulae re-establishing a blastula configuration in two apparently different ways. The left-hand, more frequent mode involves the inrolling of material to reform a blastocoel rudiment using the original, abnormally exposed 'inner'-type cell membrane. Two or three rounds of cleavage planes following separation are vertical, and inclined towards the contracting zone of abnormally exposed membrane. In the right-hand, less frequent mode, a new blastocoel rudiment is constructed with less massive geometrical reorganization. Exposed 'inner' membrane is removed from the surface by local contraction and blastomere rotation. Cleavages again tend to be vertical for two or three cycles, but do not centre upon the zone of separation.
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dorsoanterior isolates described in the previous paper, many of them are also sufficiently normal in their geometry and tissue-type balance for relative estimates of their overall mesodermal cell numbers to be well founded. Their patterns appear to contain rather exactly the 50% of within-set control cell numbers that would be predicted if the cell cycle is autonomous, and independent of experimentally imposed rearrangements, in embryogenesis of this type (Cooke, 1979a, 1981, 1982). Taken together with estimates of surface cells countable around control and experimental beginning gastrulae, the results indicated that both 1st and 2nd cleavage isolates enter gastrulation and then found axial patterns with only the numbers of descendent cells that they would have contributed to a whole development.

Eggs nevertheless vary greatly in size, and early cell counting in vivo together with all the quantitative work on larval patterns suggests that embryos of precisely equivalent morphological stage can vary over a 2½- to 3-fold range in cell number from the onset of gastrulation. Egg size–cell number relations would repay proper study with techniques of disaggregation and direct cell counting, but observations already tell us that cell number at stages of axial morphogenesis is more controlled by original egg size than by a cleavage schedule of fixed average generation number. Experiments of Kobayakawa & Kubota (1981) and Newport & Kirschner (1982) help us to understand why this might be so. Initially, the amphibian cell cycle is synchronous and clock like (Hara et al. 1980), but before gastrulation, positive control and desynchronization set in. The cell numbers with which individual embryos or early blastomere isolates arrive at this control point, and enter gastrulation, may be largely governed by the ratio of nuclear numbers to volumes of the cytoplasm, which is equipped with a finite reservoir of some control molecule in the egg.

Our principal morphological finding is that presumptive lateral half isolates, having begun development with a representative sample of the egg’s structure, usually develop a body whose mesoderm is imbalanced in pattern in that anterior contributions to the body are over-represented. Over-representation of a restricted dorsal component, the notochord, is also frequent, and its under-representation rare. The reader is referred to the fate map and the body forms of dorsoanterior isolates from the 4-cell stage, described in Paper I of the series (Cooke & Webber, 1985). Such reference would suggest that the pattern of mesoderm usually specified in lateral-half isolates lies somewhere on a spectrum in between the original fates of the material (i.e. a balanced whole although initially unilaterally distributed), and that which would derive from mosaic specification by the normal dorsoanterior half of the egg material. The extent of the pattern imbalance, from extreme ‘anteriorized’ to the almost normal, harmonious small body plan, correlates with the time course of gastrulation after its onset in each experimental individual—precipitate, or a relatively extended, normal schedule. Both these features are possibly associated with differing modes in which the spherical blastula configuration can be re-achieved after the blastomere isolation, though the evidence for this is incomplete.
Since the pattern imbalance seen is so variable, the present spectrum of results may actually differ from that achieved by Kageura & Yamana (1983), due to the particular females or egg batches used or to other unknown conditions. But the relatively late larval stage at which their characteristic results are illustrated makes it impossible to judge whether those bodies considered normal by them, are actually examples that our minute concern with pattern would reveal to be outside the normal range of balance. Since all the anatomy is at least represented, in the great majority of the patterns we obtain, there is no a priori reason why development should not be able to proceed to tadpole, metamorphic and frog stages as reported for their small larvae by the other authors.

Any correlation of relative notochord size alone with the mode of early development in individuals is obscured by a large, arbitrary component in the notochord outcome which we suspect is dominated by a different factor. It is most easily understood by assuming that the first cleavage rarely bisects exactly the relatively restricted vegetal egg sector that normally induces notochord, and that the relative amount of this original territory in each isolate has its own strong effect on final notochord extent. Certainly if the mid-dorsal region is excluded to an extent that delays the onset of gastrulation in the isolate beyond about 25 min, anterior regions of notochord begin to be omitted from the pattern, and even lesser delays are associated with the smaller notochords of the series. The negative correlation for notochord between pair members is thus to be expected, but since the whole range of notochord proportion is elevated above normal we are not at this stage dealing with a mosaic system of plasms for specification of mesodermal territories. The general mode of organization is evidently such that isolation as a 2-cell blastomere leads to an increase beyond normal expectation in the proportion of the material that finally becomes devoted to notochord. The types of imbalance recorded are not the results of development at small size per se. Notochord proportion and anteroposterior balance of contributions to somite are not functions of mesoderm size across the range seen from whole developments, even though this overlaps with that of isolates. Some regulative mechanism can usually normalize these features against size, but our results indicate, surprisingly in terms of traditional ideas, that the lateral egg half at first cleavage is already past a stage when this mechanism can operate fully! The abnormalities seen must somehow be a consequence of the adjustment of development from that of a presumptive lateral half to that of a bilaterally symmetrical body. We assume that prechordal (head) mes-endoderm is really the boundary or 'high' point of the field of activation established in the egg, but have only worked quantitatively on notochordal levels of pattern. Heads (including brain) of isolates showing the more extreme of our quantified imbalances always seem disproportionately large.

Observations from this, the previous and the following papers in the series (Cooke & Webber, 1985; Cooke, 1985) build a strong case that the onset time and the subsequent time course of gastrulation activity as it sweeps in a wavefront across the embryo (see Zeeman, 1974) correlates in individuals with the normality or otherwise of the final pattern. Something that could be called rate of
development is a continuous variable that is set locally in tissue at an early stage, and correlates with its assigned position value in contributing to pattern. We cannot yet say whether this rate variable is itself part of the mechanism whereby the original spatially distributed signal in eggs is used to specify the contributions of material to the pattern. Developmental rate and fate may merely be intimately correlated, parallel expressions of position value. It is scarcely possible however to separate them experimentally, and even the slowing of developmental rate by physiological shock beyond certain narrow limits is associated with failure to form pattern regions normally associated with the fastest developing region (see Paper I).

We can use the present observations in conjunction with those of the other papers to continue describing the dynamics of the early system of spatial variation that is finally translated into pattern. We can say that this system normally ensures a particular profile of position value across the egg independently of its size. This is expressed as balanced pattern for the species, assessed by external body form, normal percentage of mesoderm cells incorporated into each histologically defined body element, and normality of the relative amounts of somite tissue used in each part of the axis – the ‘somite index’. Fig. 5A,B, a repeat of Fig. 7A from Paper I but drawing attention to the size-invariant profile, may be taken to express the distribution of tissue position value in terms of a hypothetical variable graded round the margin of the egg. We know the variable to be very stable in material in the sense that levels are preserved when the field is physically transected early on as in frontal (i.e. 4-cell) isolations. But the reorganization necessary to restore bilaterality to a lateral half-egg results in a more or less abnormal, truncated profile (Fig. 5C, compare with Fig. 3C,D), expressed as anterodorsally over-balanced pattern and a foreshortened wavefront passage time in gastrulation owing to omission of mesoderm with the late schedule of onset in movement that goes with specification for posterior contributions. The centre of dorsalization seems to be dominant in this reorganization as it keeps its position value intact. This is reminiscent of how in frontal separations, the isolate containing that centre normally preserves its developmental information in a way that approaches mosaicism, whereas the posterior isolate may do this but frequently does not. We also note how the extent of pattern imbalance in the reorganized lateral isolate is related, in all probability, to the geometrical events whereby the reorganization has occurred in individual cases (Fig. 4D,E). Imbalance may be most severe in cases where material originally furthest from the centre of dorsalization has been brought into contact with that centre.

There follows a working hypothesis about the dynamic of the early positional system which may account for the phenomena, and also relates the system to morphallactic pattern formation generally. In the precleavage period, asymmetrical mechanical events occur whereby various semicohesive plasms or regions in the deep vegetal part of the egg are shifted in relation to its more superficial layers. The way in which these activate pattern is not yet understood but various views of them are discussed in Gerhart et al. (1981), Kirschner et al. (1980), Neff et
Fig. 5. Properties of the system specifying body position, suggested by observations on lateral-half isolates. (A) Representation, as a spatial gradation in a property, of the system specifying position value across the egg according to normal fates of mes-endodermal regions (as in Fig. 7A of Paper I in this series). Results from 4-cell isolates show that in many eggs this is set up quite rapidly and stably, at or after the events of precleavage. (B) The constancy of pattern balance against normal variation in egg and mesoderm size, is evidence for some integrative system within the egg. This may be of a mechanical nature in the first instance (see Fig. 5D), and refined later by interactions that more resemble the interplay of diffusing morphogens (see e.g. Cooke, 1983). Such an integrative mechanism results in normally balanced profiles of specification in whole eggs which are no bigger than the lateral-half isolates resulting from other eggs (see Fig. 2). (C) If material containing the newly set-up system of position values is reorganized so that regions having the lowest levels of activation are brought abnormally into contact with those having the highest, a 'ratchet principle' of slow interaction (see Discussion) may cause the position value profile to come to a new equilibrium that implies only partial or imbalanced pattern (Fig. 3), regardless of system size. This is because, while signals can slowly spread to 'raise' the level of less-activated (posterior) material in the new configuration, as shown by the dotted original profile and the arrows, the reciprocal interaction does not occur. There appears also to be no true regulative capacity whereby the missing lowest levels of position value are then restored, in the 'French Flag' (Wolpert, 1971) sense. (D) A simple physical model to illustrate possible events of the late precleavage period which accomplish normal specification for position. A bilaterally symmetrical system of shift between egg-cell components appears to be set up. The extent or sign of shift or shear, between components attached to a surface cytoskeletal array and the specific plasms in deeper-lying more-fluid egg regions, may be somehow recorded at each location as a particular level of activation or position value for future development. In this particular model a graded property around the vegetal pole of the egg cortex (represented by stripe width) interacts with the interface between two fluid plasms. In natural fertilization, the plasm interface may be preserved horizontal by gravity (●), while the egg's organized contractile system rotates the cortical shell with respect to it. The model is illustrative of a possible class of events rather than a serious working hypothesis.
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al. (1984) and Scharf & Gerhart (1983). Here, it is proposed that these shifts set up a graded system of what we call 'activation' across some crucial part of the egg apparatus. This system extends round much or all of the subequatorial region and is used as a variable that positively assigns position values or specifications as to expected contributions by mes-endoderm derived from each egg meridian. A normally balanced profile of specifications tends to be given regardless of egg size because the egg is a sphere containing a mechanically coherent system. Thus a maximum displacement of some kind between two components at a particular meridian (say, opposite sperm entry), mechanically implies displacements of particular relative extents, or even different signs, at the other positions round the egg in a bilaterally symmetrical manner. Fig. 5D, where the egg is modelled simply as a shell filled by bodies of largely immiscible viscous liquids of different relative densities, serves to illustrate the behaviour which might be seen in such a system.

We view the position values, given in this way, as then behaving like the relatively stable component in most positional information models (Wolpert, 1971). But they have been registered in some structural way by events that occur over a very early, brief interval in the egg, rather than arrived at by the dynamics of morphogen diffusion etc. across the available space which would require a much more prolonged time course (Crick, 1970). Seen in comparative evolutionary terms, the Xenopus egg appears to be a mechanism that by-passes the operation of morphogen diffusion gradients that are probably used in many developing systems, but arrives at the same regulated profile of position value by some other, mechanical transduction system. The stable behaviour of many dorsoanterior and posterior isolates, and of partially u.v.-impaired embryos, is our evidence for both the widespread early completion of the informational system and its relatively stable form. This contrasts with the older idea (which may or may not remain appropriate for other amphibian egg types) that the centre of dorsalization alone is initially specified, and then acts as source or boundary in a prolonged build-up of the graded information elsewhere.

We now need to explain the disharmony caused by the reorganization following separations of 2-cell blastomeres. Even the most extreme tipping of whole precleavage eggs so as to reinforce or exaggerate their endogenous movements by means of gravity does not lead to patterns nearly as imbalanced as those reported in this paper (unpublished results). This may be a problem for simple-minded mechanical theories as to the positional coding system's nature, but it does mean that inadvertent exaggeration of the initial events is unlikely to underlie the present results. We suggest that an interaction rule which might be called the 'ratchet principle' with respect to activation level or position value, that has been documented in other systems, is operating here. According to this principle there is an asymmetrical dynamic behaviour in the system. When more activated regions ('higher' position values) are brought into contact with lower ones to cause a positional disparity such that the system is not at equilibrium, then the more activated region keeps its status and promotes or 'raises' the adjoining material's value to be nearer its own, whereas the converse interaction does not occur. The
particular intercellular communication that accomplishes this may be of the kind that operates in positional (morphogen) gradients generally, and different from the original rapid-acting mechanical apparatus of the *Xenopus* egg system. Such interaction can be quite slow (Smith, 1979; Meinhardt, 1977), but in *Xenopus* development it may have 10 h or more in which to act, since we know that immunity of territories from such ‘promotion’ by newly adjacent tissue (i.e. their determination as opposed to initial specification) does not occur until some time during gastrulation itself (Smith & Slack, 1983; Forman & Slack, 1980).

The slow-acting ratchet principle may function in normal development to smooth out or otherwise normalize the inherently variable profiles of activation left by the primary system, thus accounting for the undoubtedly small variability in final pattern in relation to the variable apparent intensity of the precleavage movements among eggs. It would also act, abnormally, to make recovery of positional profiles within the normal range impossible after resymmetrization in one major class of lateral-half isolates. This is the commonest class in which it seems (in our laboratory) that bilaterality is restored by repositioning the blastomeres so as to join material originally specified as posterior onto that specified as dorsoanterior (see Fig. 4). In such cases, the ratchet principle will act to ‘promote’ the posterior component according to its new position relative to the apex of the activation system. This will act to delete progressively that part of the specification map that contributes most to posterior and lateral axial structure, meanwhile adding to that which specifies head and notochord development, in material now next to the original centre of dorsalization. A new equilibrium will be reached, but there is now no evidence that any such slow communication system, behaving like a diffusion gradient, will perform regulation in the sense of restoring both normal boundary values to the field independently of its size. In the absence of such true regulative ability, the most posterior specified region remaining to the isolate will be that which was some halfway round the original egg, because this will now be situated opposite the centre of dorsalization, or apex. The latter will have increased in relative extent, and re-achieved symmetry, by local upgrading of the newly adjacent material. An altered and incomplete specification profile like that of Fig. 5C will result, around each side of the reorganized body. Note that the disposition of the normal map of contributions around the meridians of the egg (see fate map of Paper I) is such that this will result not in any loss of somite segment number, but in a change in the proportional amounts of the material specified as due to populate segments at various positions in the series. See the altered body plan of Fig. 3B. In each individual case, the new equilibrium attained for the ‘activation’ profile in this type of reorganized isolate would be expected to raise somewhat the amount of material finally devoted to notochord, in relation to the amount of the original territory inherited from the whole egg at isolation. A majority of isolates re-achieving the blastular form in other ways, as in Fig. 4C and E, will preserve normal or near-normal profiles of specification. In this version of the isolate development also, the slow ‘back-up’ mode of interaction will ‘replace’ the information for the missing side of the embryo. But material which was
originally furthest from the apex of activation now remains in that relative position, so that the system ultimately retains a more balanced set of specifications. In the present experimental series, such an outcome has been reflected mainly in balanced body conformation and near-normal 'somite index' despite small size.

The results described in both this and the previous paper imply that much less by way of regulation is possible after the earliest stages in the *Xenopus* embryo than had been believed. We have used the term 'mosaicism', and it has been noted that the capacity to produce numbers of somite segment contributions according to normal fate is preserved, frequently in posterior and characteristically in dorsoanterior and lateral-half isolates. This must not be taken to imply literally the structural specification into a large and well-controlled number of 'segment precursors' or other organ rudiments at anything like the egg or 4-cell stage. We can have no molecular basis or other reason for such a belief. Some theory of epigenesis such as those of Cooke & Zeeman (1975) or Flint et al. (1978), which postulate interactions between general body position and some other factor, remains necessary to account for somite morphogenesis and the co-ordination of segment numbers.

The phenomena described in the present paper add to those discussed in the previous one as evidence for the validity of the concept of non-equivalence (Lewis & Wolpert, 1976) or early regionalization within mesodermal material, primarily as a coding for axial position within the body plan rather than for the development of particular histodifferentiations. The normal fate (and as we now believe, specification) map is distorted or truncated, in a similar way but to varying extents, in all those isolates that include a 'dorsalizing centre'. They lack the capacity to re-achieve a full gradation of states around the remainder of their marginal zones. The geographical layout of the presumptive somite region in the map is such that it is reasonable to believe, in these truncated systems, that some material destined for every segment of the series remains, but that increasing proportions of that for the segments posterior to about number 6 are missing. The evidence for the detailed nature of non-equivalence resides in the fact that, although they are geometrically isomorphic with (and in the same overall size range as) normal blastulae and gastrulae, such experimental bodies finally offer somite morphogenesis where the relative sizes of the newly founded somites are systematically abnormal as just described. This is best thought about not in terms of precise somite number (see above) but in terms of assignment of somite tissue to body position, bearing in mind that each somite contributes several tissue types to the body.

A development similar to that undergone by the smaller types of amphibian egg may well be primitive to the vertebrate subphylum, being most obviously derivable from that of other chordates such as tunicates. It is suited to the rapid establishment of a functional body plan without growth, utilizing the whole of a relatively but not extremely large egg cell. The blastodisc type of development seen in teleosts and amniotes must utilize a slower means of symmetry – breaking
and establishment of positional information, by some process that arises in a part of the already expanded cell population which is not structurally predetermined before cleavage. This necessarily slower process may rely entirely on interactions corresponding to morphogen gradients, reaction–diffusion or other intercellular signalling modes. These signalling modes might be retained as the slow 'back-up' interactions, obeying the ratchet principle, which we have invoked in the *Xenopus* system. In this system, however, they can never be used to generate levels of activation 'higher' (i.e. more dorsoanterior) than the highest already instigated within the individual by the action of the early rapid system before cleavage. Individuals in which that early system was impaired in function can only be rescued at later stages from incomplete body formation by literally grafting in tissue of an apical degree of activation. This feature of our system is mentioned in a comparative sense at the end of a following paper (Cooke, 1983), which deals specifically with the lack of capacity for 'apical restoration'.

Elucidation of the molecular assemblies involved in both the early rapid and the slower 'diffusion' type mechanisms for establishing position values is an important task for the future. But of more universal importance would be elucidation of the mechanism of nuclear response to these variables, that converts them into stable programmes for genomic expression as development proceeds. This system of nuclear response to primary positional variables must surely be universal, whatever the individual strategy for spatial organization, at least across vertebrates and probably throughout the metazoans.

REFERENCES


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