

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

IV. Timing and pattern in the development of twinned bodies after reorientation of eggs in gravity

JONATHAN COOKE

Laboratory of Embryogenesis, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK

Summary

The mesendodermal anatomy of twinned larval axes is described in relation to the normal single pattern, when twinning has been caused by experimental tilting of eggs before first cleavage. The formation of two origins for gastrulation movements (dorsal lips) and their relatively rapid spread and coalescence to give a circular blastopore, is a predictor of twin formation in individual embryos after treatment. The anatomy of twins where development has been disturbed from the outset in this way is appreciably different from that induced by the later operation of second dorsal lip implantation. It is also variable in a systematic way. The total sizes of cellular allocations to individual

notochords and prechordal head patterns are enhanced above normal if they arise relatively close together in the tissue, but significantly reduced if they arise far apart. These and other features of twinned patterns due to precleavage disturbance are discussed in terms of what they might indicate about the physicochemical nature of the body positional system. The results confirm that by a variety of rather simple, nonsurgical manipulations the relative amounts of territory in the egg devoted to different parts of the body can be greatly influenced.

Key words: gastrulation, precleavage events, pattern formation, *Xenopus laevis*, timing, gravity, reorientation, twinning.

Introduction

It is becoming clear from recent work on *Xenopus* development that two largely distinct processes occur in the early establishment of the body plan. One is the generation of mesoderm, due to instructions in the vegetal-to-animal direction in the blastula. Recently important steps may have been taken in the search for the nature of this mesoderm-inducing or 'vegetalizing' signal (Gurdon, Fairman, Mohun & Brennan, 1985; Smith, 1987) and it may well involve diffusing 'morphogens'. A second process involves the establishment of a set of 'body position values' that determine the proportions in which contributions to the head-to-tail axial pattern are produced. The present paper is one of a series exploring the properties of this primary positional system (Cooke & Webber, 1985a,b; Cooke, 1985a). There is no determination of tissue types at the individual cell level

before gastrulation and the conventional view is that the head-to-tail organization of the body is only finalized during the gastrulation movements. However, recent experiments of several types could be taken to indicate that the overall polarity, and then proportions, of the body structure become specified across the material in a very few hours following the start of development (e.g. Black & Gerhart, 1985, 1986; Scharf & Gerhart, 1980, 1983; Cooke, 1985b, 1986). According to this view, which has been developed in the present series of papers, there appears to be an early system of positional information (Wolpert, 1971), in that some continuously graded physiological variable, representative of position, is set up. But its behaviour is in some ways more reminiscent of a structural, biomechanical recording system than of the diffusion-controlled morphogen gradients that have more usually been considered to explain other examples of large-scale pattern formation.

I describe here the larval anatomy in twinned, partial body plans resulting from exposure of eggs to conflicting sets of influences from gravity, during the time when the proposed positional system is becoming established just before first cleavage. The effects are probably closely related to those of centrifugation at 90° to the normal gravitational field at around 40 % of the time to first cleavage (Black & Gerhart, 1986). The motivation for the present experiments was to extend the knowledge of the system's dynamics emerging from previously published work, which can be summarized as follows. The positional system acts like a series of levels of 'activation' in egg material, each leading to the development of different contributions to the body plan from dorsoanterior to posterior. Activation levels can be ranked from 'high' to 'low' in that they interact according to an asymmetrical or ratchet-like principle (Cooke & Webber, 1985*b*). The highest level present in a normal egg or embryo causes development of head (pharyngeal) endoderm and the induction of head mesoderm and notochord, with corresponding forebrain levels of neural structure. Intermediate levels lead to development of trunk structure including somite segments (but not notochord) and to progressively more posterior levels of nervous system pattern. The lowest degrees of activation cause derivatives normally seen in belly mesoderm only and cannot by themselves lead to induction of any somite mesoderm or nervous tissue. In isolated fragments of early embryos or in incompletely activated whole ones, the most activated material present reveals its level by developing autonomously, and by causing graded lower levels in the remaining material to harmonize with its own contribution. This leads to a partial, dorsoanteriorly truncated body pattern. Despite the stability against downward decay which intermediate levels of activation thus show when isolated from early stages, they cannot normally 'climb' or up-regulate to restore the proper upper boundary level, given in a complete system at the outset. A series of stably developing, but partial, body plans is therefore seen after a variety of manipulations that give systems deprived of the normal 'highest' position values once the brief setting-up period in the egg is over. The concept of graded levels of activation underlying body position and interacting in the above manner, is described and graphically illustrated in Cooke & Webber (1985*b*) and in Cooke (1985*a,b*). It should be noted, however, that a by-pass of the normal dynamics has recently been achieved (Kao, Masui & Elinson, 1986), in that initially incomplete activation of eggs can be substituted for by exposure to the lithium ion as late as the 32-cell stage.

Can the normal mechanism be deceived into recording profiles of position value that are permanently disturbed in the *opposite* sense to the dorsoanteriorly incomplete profiles just referred to; that is, ones possessing disproportionately large territories activated to 'high' dorsoanterior levels of body pattern? It is already clear that pairs of regions with high activation can survive stably in single eggs from early stages, giving twinned dorsoanterior pattern (Gerhart, Ubbells, Black, Hara & Kirschner, 1981). But if the 'ratchet' principle were to operate consistently, such regions should fail to regulate quantitatively in restricting the amount of tissue devoted to the corresponding pattern contributions during later development. Systematic overallocation to dorsal and anterior body structure should be maintained, and the resulting larval bodies should show corresponding reduction or incompleteness of posterior anatomy because of the unchanged amount of tissue within which pattern formation occurs (Cooke, 1979).

The present strategy for forcing the positional system outside its normal, well-controlled performance was based upon recent knowledge concerning mechanical aspects of the precleavage events that initiate body pattern formation (Scharf & Gerhart, 1980, 1983; Gerhart *et al.* 1981; Scharf, Vincent & Gerhart, 1984; Vincent, Oster & Gerhart, 1986; Black & Gerhart, 1985, 1986). Artificially imposed and maintained tilt of the precleavage egg can subvert the effects of its own spontaneous movements in establishing the axis for development. Apparently normal body patterns are then set up, but with the egg meridian whose material was raised highest in gravity, rather than that opposite the sperm entry position, now establishing the dorsal-anterior midline. This is presumed to occur because downward sliding of the relatively dense, deeper-lying material relative to the rotated surface, under the force of gravity, creates or triggers a system of mechanical events which pre-empts the effect of those that the egg normally generates. Either the spontaneous or the gravitationally imposed plasm shifts can somehow provide a set of 'position values' around the egg's meridians for future development.

Towards the end of the precleavage interval and of the normal period of active plasm movement, progressive 'gelling' of some component of egg structure acts to record and render more permanent the net results of all preceding reorganization (see e.g. Elinson, 1984). It therefore seemed that experimental regimes involving reorientation in gravity *late* in this sensitive period might lead to the setting up of positional profiles less well regulated than those resulting from either the endogenous mechanism or experimental tilt acting alone. This would be especially true if the egg's own mechanism and the new,

later tendencies to movement of its contents under gravity were acting in conflict at a wide angle. Such conflict was achieved by exposing eggs to the regime of manipulations illustrated in Fig. 1 and described fully in its legend.

Imbalanced allocation of tissue to the body plan is indeed produced at significant frequency by the regime of gravity reorientation described. It takes two main forms, which can be understood as versions of one kind of distortion. The commoner form, a dorsoanteriorly overbalanced single axis, has already been adequately described (Cooke, 1985c, 1986). The other form is a profoundly twinned body pattern, with notochords separate right to their origins at the blastoporal collar even if running parallel in the tissue, and an overall imbalance in favour of anterior levels of structure. These twin forms have been alluded to in previous publications, but here I present the quantitative data on their mesodermal anatomy. They are significantly different in internal pattern from twins seen after second dorsal lip implantation into normal late blastulae (Cooke, 1972; Gimlich & Cooke, 1983), but directly comparable with those seen after certain regimes of exposure to 30g in the centrifuge (Black & Gerhart, 1986 – small sample from S. D. Black examined). They are essentially pairs of partial body patterns sharing a normal-sized or slightly small total mesoderm tissue, each pattern being to some extent incomplete in posterior contributions. The quantitative variation, in the cellular extents of allocations to those parts that are represented, has certain systematic features among the set of bodies examined. These are described and illustrated in this paper, and reveal that the early positional system involves interactions that operate across considerable distance within the material.

Materials and methods

Perturbation of axis formation

Synchronously fertilized and rapidly dejellied eggs (see Cooke & Webber, 1985a) were orientated under a layer of 5.5% Ficoll in 1/10th amphibian saline. Their sperm entry points (s.e.p.), visible one third of the way through pre-cleavage, were initially down-rotated some 30° relative to the spontaneous gravity-orientated position. Then, as illustrated in Fig. 1, they were finally up-tilted some 70–100° along a meridian near that of sperm entry. The up-tilt was imposed late in pre-cleavage and maintained until the 8-cell stage.

Quantitative study of the proportions of the mesodermal cells in the parts of axial patterns

This was carried out on stage-30 larvae by standardized nuclear counting on sections as elsewhere in this series of studies. Due to the distorted geometry that accompanies twin formation and is more exaggerated in the present than

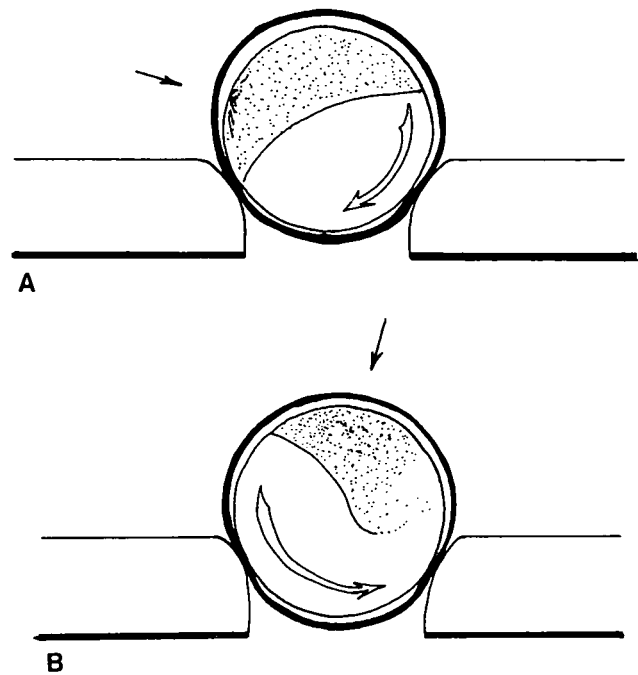


Fig. 1. An experimental manipulation of eggs in 1g that can permanently disturb the body positional system. Fertilized batches of eggs were allowed to proceed with their normal events while held close to the natural gravity orientation, with the perivitelline space collapsed by 5.5% Ficoll (Sigma, M_r 70 000) so that any reorientation within their retaining wells would be maintained. Their surfaces were artificially downward rotated about 30° along the sperm-entry meridian (s.e.m.), which strengthens the statistical tendency to alignment of the endogenous plasm shift, and the ultimate midline of pattern, with s.e.m. (A). At times 65–85% of the way to first cleavage (i.e. 65–85 min after fertilization at 19°C), experimentals were retilted through 70–100° so as to give upward displacement along a meridian near, though not on, the s.e.m. (B). The point of sperm entry is shown by a small arrow in each diagram. Such a manoeuvre carried out at earlier time points would give midlines of pattern widely, but slightly less than 180°, displaced from that expected on the basis of s.e.p. Carried out at later times, when the egg may be close to recording the values around its vegetal regions of some unknown cellular variable related to plasm shift, the procedure may result in the recording of abnormal configurations of 'position value'. Curved arrows, shown inside the outlines of eggs in the plane of rotation, indicate the endogenous and the experimentally imposed tendencies for deeper-lying material to slide across the 'surface' structure. At the 8-cell stage, after 1 h tilt at 19°C, experimentals and the controls (30° s.e.p. down tilt only) were returned to free gravity orientation in 1/10 strength saline for development to axial larval stages. It is noteworthy that in this regime, due to rapid use of Ficoll on dejellied eggs, they are at no time exposed to prolonged reorganization by 1g along with deformation away from a spherical shape, this also being known to cause twin formation (Gerhart *et al.* 1981).

in previously studied types of twin (Cooke, 1981), the sectional series scanned was expanded to cover mesoderm of the prechordal category and the tailbud. Percentage estimates of the proportions of various pattern parts are thus not directly comparable with those given for control embryos in past papers. In addition, certain axes of twins and their controls were sectioned in a horizontal plane, to check on the extent to which the spacing of nuclei in particular tissues in this plane of section was affected by twinning of the pattern. The slight distortions of the textures of tissues caused by twinning proved not to affect the validity of estimates of pattern proportions, in terms of cell number (Abercrombie, 1946).

Results

When batches of synchronously fertilized *Xenopus* eggs are dejellied gently with cysteine HCl, avoiding mechanical deformation, then orientated normally in gravity according to procedures in Paper I of this series (Cooke & Webber, 1985a), a remarkably constant balance of axial mesodermal anatomy is attained among individuals at the larval stage. This suggests a well-regulated spatial profile of activation levels or 'position values' across the normal egg. Eight experimental populations of about 80 eggs each were exposed to the régime described in Fig. 1, by performing the s.e.p.-up tilt of each individual at some point during the 20-min interval from 65 to 85% of the expected time from fertilization to first cleavage. These were kept to stage 30 and each compared with a control population (no s.e.p.-up tilt) of siblings. In general in experimental populations, from blastula stages onwards, abnormality of the cortical pigment field in relation to the free gravity orientation was observed at high frequency. This was followed by abnormal positioning of the zone of blastopore formation in relation both to gravity and to cortical pigmentation, as if the yolk-plug surface and the epidermis finally surrounding the proctodaeum of larvae had inherited the egg surface normally confined to the animal pole.

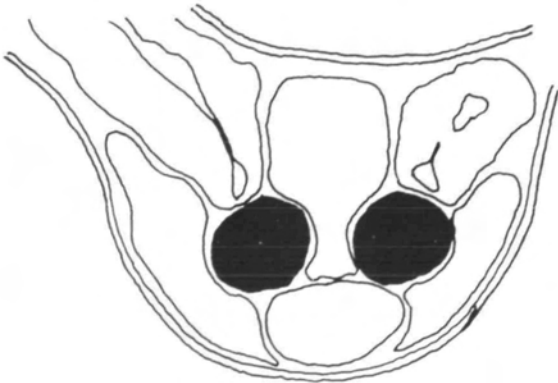
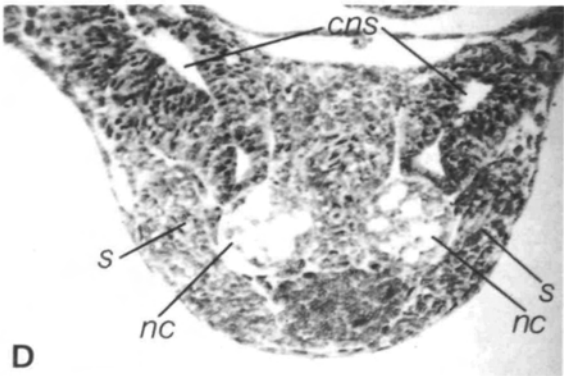
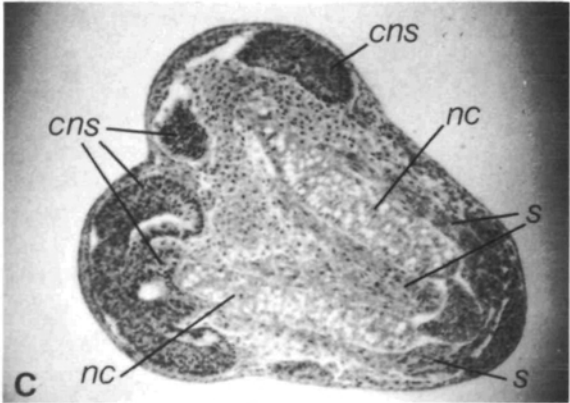
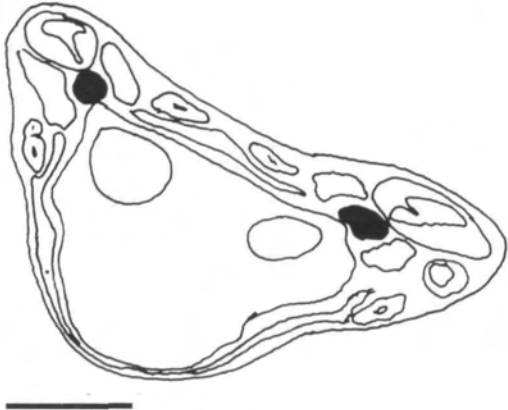
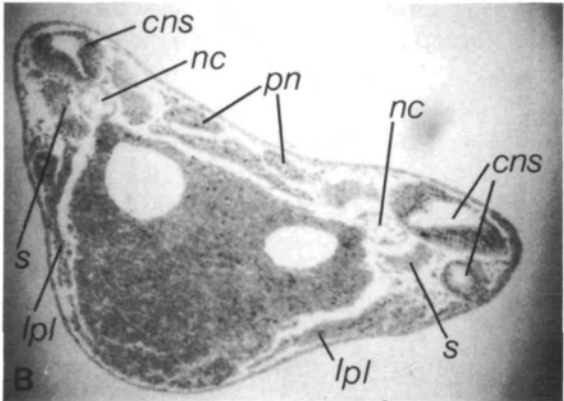
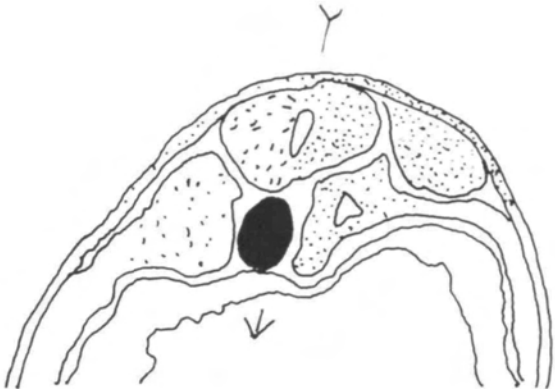
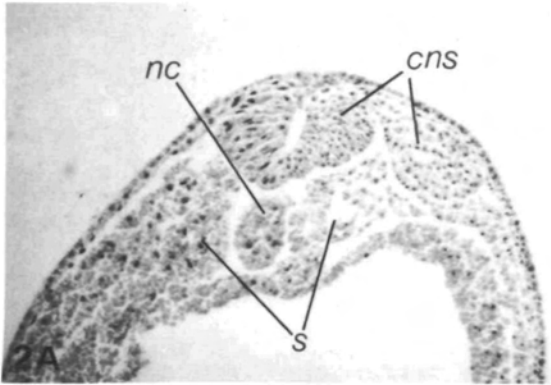
In addition, 34 twin morphologies were observed (between two and eight per population in six of eight experiments), whereas only one was seen among 600 controls. When gastrulae were sorted according to normality or otherwise of the pigment and blastopore position, however, the imbalanced and twinned morphologies that finally developed were not concentrated among those visibly reorganized at the earlier stages. Indeed the two egg batches showing the greatest (>50%) incidence of visibly aberrant pigment distribution gave fewest abnormal larval body balances and no twins. Sectioning of normally proportioned bodies that had shown grossly asymmetrical pigmentation and surface cell-size distribution, revealed that the distribution of different-sized yolk

platelets and of blastomere volume had become quite unlinked from the normal relationship with contributions to anatomical structure. Fig. 2A shows a late neurula embryo in which one neural fold was white and the other heavily pigmented, where quite different cell sizes are found across a sharp boundary in the morphological midline of neurectoderm and mesoderm. Thus the genesis of particular structures from regions of egg substance other than normal, after early disturbance, is an aberration distinct from the generation of quantitatively imbalanced anatomy.

20 twins have been analysed internally for architecture of the endoderm and pattern balance of mesoderm, in relation to members of their control populations and to experimentally tilted but normal-appearing siblings. The latter class, the majority (80%) of each experimental population whose morphology appears normal to external inspection, proves not to be detectably different from controls or from the general population on internal analysis. Results from three sibling sets of twins and controls, analysed at stage 30, are laid out in Table 1. However, as should be realized from the following paragraphs, only some aspects of their pattern can readily be tabulated.

The almost pyramidal shape of the twinned bodies (Fig. 2B–D) is due to profound duplication of the anterior gut architecture and large allocation of mesoderm and neurectodermal areas to development of the heads. In addition, the dual set of dorsal convergence movements in chorda–mesodermal areas acts to squeeze the considerable lateral plate allocation of tissue forward into a pronounced concentration between the heads and gill regions. Somite-forming columns are usually fused on one or both

Fig. 2. Effects on embryo structure, of perturbation in gravity before 1st cleavage. (A) Transverse section of a neurula-stage embryo that showed abnormal pigmentation and external cell-size distribution in relation to anatomy. Note the sharp discontinuity in size (and other ooplasmically inherited characters) of cells across the midline in neurectoderm and mesoderm structures. Such embryos are frequent after regimes of early disturbance in 1g, but are not characteristically the individuals which go on to show the quantitative imbalances of axial pattern as in B–D. (B) Transverse section of anterior trunk levels in a twin with widely separated (near opposed) dorsal midlines. Note sizes of notochord sections. (C,D) Transverse sections through twinned embryos with lesser angles between original dorsal midlines of pattern. Note the more massive construction of each notochord and the slender form of more posterior axial region. The histological appearance of each section is clarified by the accompanying tracing. *nc*, notochord; *lpl*, lateral plate; *s*, somite; *pn*, pronephros; *cns*, part of central nervous system, including ear vesicle. Bar, 300 μ m.



sides, not far behind the normal pronephric level (e.g. somites 7–9). The pronephroi, which are rarely more than two in number, are very reduced in cellular allocation. More usually, on the side with the lesser tissue distance between dorsal midlines and thus the more anterior level of somite fusion, pronephros is absent. The posterior trunk levels are usually thin columns of nervous system, paired notochords and a sheath of somite tissue, all 'lateral plate' mesoderm having been pressed anteriorly. The tailbud is either single and very small, or absent, with the gut formations sharing a single, near-terminal blastopore. Whatever their relative sizes, notochords are always anatomically discrete right back to their roots in the latest-recruited mesoderm posteriorly. Cardiac development is usually minimal, even though two appropriate regions are often present.

The twins included in Table 1 are representative of the range of quantitative variation (see below). Only the notochords and prechordal regions are discrete and thus scorable as separate percentages of the total mesoderms, but the overall percentages of somite and notochord and the distributions of cell numbers into

somites at different axial positions show that these bodies are really pairs of dorsoanterior part-patterns (see Cooke & Webber, 1985*a,b*). These part-patterns are built on scales that range from near normal, leaving no tissue for any posterior pattern, to the significantly reduced, leaving enough tissue space for reduced, shared posterior pattern. The quantitative evidence for such interpretation follows.

The notochord and the prechordal region, the only elements of mesodermal pattern to be entirely duplicated, are those we know to be derived from restricted sectors at the dorsoanterior extreme in the normal specification map. The somite segments which are represented separately in each axis, the anteriormost ones, are those which are atypically slender and few celled in construction in the normal axial plan. The remaining segments, confluent between dorsal midlines on one or both sides, are near normal in number. The fused mass of somite at these more posterior levels is accompanied by little or no lateral plate in the same cross-sections, but abundant lateral plate is found to exist between the diverging axes anteriorly. The extension of the section series to

Table 1. Percentage allocation to axial mesodermal structures in twins and in control sibling larvae

		(Relative mesoderm size)	Prechorda	Notochord	Somite	Pronephros	Lateral plate	Tailbud
Set 1.	C	(6.5)	3.1	4.0	42.2	5.9	42.0	2.8
	C	(7.6)	2.9	3.8	43.5	6.5	40.4	2.9
	C	(6.3)	3.2	4.8	40.3	7.0	42.0	2.7
	C	(7.3)	3.0	4.7	42.7	5.4	41.1	3.1
	E	(5.5)	1.5, 0.5	5.5, 3.3	44.1	2.1	42.2	0.8
	E	(4.3)	1.4, 2.1	3.3, 3.9	43.2	—	44.9	1.2
	E	(4.1)	3.7, 4.1	10.1, 8.9	35.2	—	38.0	—
Triplet	E	(3.7)	7.4 total	8.2, 2.9, 5.4	40.3	1.7	35.8	—
Set 2.	C	(5.8)	4.0	4.7	39.4	5.7	43.4	2.8
	C	(6.5)	3.7	5.4	40.5	6.6	41.1	2.7
	C	(6.1)	3.5	4.6	40.8	7.0	41.0	3.1
	E	(5.6)	2.4, 2.1	3.1, 3.8	44.1	2.6	40.2	1.7
	E	(4.3)	3.4, 4.2	7.5, 7.9	36.0	—	41.0	—
	E	(4.4)	4.1, 3.4	10.6, 8.2	34.1	—	39.6	—
Set 3.	C	(6.4)	3.9	4.1	40.5	7.0	41.5	3.0
	C	(6.8)	3.8	3.6	42.7	5.7	41.0	3.2
	C	(7.2)	4.1	4.3	43.1	6.2	39.9	2.4
	C	(7.4)	3.7	3.7	40.9	5.5	43.5	2.7
	C	(7.0)	3.8	3.7	41.6	5.9	41.9	3.1
	E	(5.4)	4.0, 2.8	5.5, 3.5	41.0	3.4	37.7	2.1
	E	(6.1)	1.0, 1.6	3.8, 3.6	43.1	4.0	40.7	2.2
	E	(4.6)	3.7, 4.5	8.0, 10.7	32.1	—	41.0	—
	E	(4.2)	4.0, 4.1	8.5, 8.9	34.2	—	40.3	—
E	(4.9)	3.2, 3.0	7.7, 5.2	40.3	1.0	37.4	2.2	

Figures in brackets in the first column (relative mesoderm size) are the numbers of thousand nuclei encountered in sampling each embryo at every 10th section throughout the mesodermal axis. They represent a constant proportion of the absolute cell numbers in mesoderms within each sibling set, this proportion being around 20% in view of nuclear diameter, internuclear distance and section thickness (Abercrombie, 1946).

cover the entire lateral plate in both experimental and control bodies has confirmed that the strong representation of this tissue seen in the twins is real, and not just an artifact of the geometrical distortion of the axes. Somite tissues is often somewhat deficient in relation to lateral plate in twin bodies as a whole, especially when notochords and prechordal regions are large (set1E3, set2E3 and set3E3,4 of Table 1). This histological balance is characteristic of anterior parts of the normal axis, where slender, few-celled somites are specified and where lateral plate is relatively massive. In pattern formation terms, the parts of the somite still represented in the fused axes posteriorly are only those derived from egg meridians rather near the notochord territory in normal development (see the 'fate map' of Cooke & Webber, 1985a, fig. 2).

Mesoderms tend to be of smaller total cell number in these twins, and this also is most pronounced in individuals where the heads (and notochords) are constructed on larger scales with correspondingly small-scale posterior axes (set1E4 triplet, set2E2,3 and set3E3,4). This again is evidence for the double-anterior nature of their pattern, in that the normal mesodermal cross-section is fewer celled, overall, at anterior than at posterior trunk levels. Thus embryos that had produced opposed pairs of dorsoanterior partial mesoderms, without any intervening adjustment of mitotic schedule (Cooke, 1979), would tend to show somewhat diminished mesodermal cell numbers.

There are two interesting modes of variation among the twin bodies examined. The first concerns the proportional amount of the material that is devoted to dorsoanterior pattern parts and this is systematically related to the positioning of the dorsal midlines (see Fig. 2B compared with C,D). In mesoderm, 'anterior' pattern is expressed as prechorda and notochord. Variation in head sizes is readily apparent externally but hard to quantify as a mesodermal cell type. Variation in notochord size, which seems to correlate well with the 'head-heaviness' or otherwise of individual axes, is scorable as cell number. Twinned embryos vary from those in which each notochord is larger than the normal single structure (e.g. set1E3, set2E3), to those in which each is relatively scaled down so that the mesoderm as a whole has only a moderate overallocation to this tissue (set2E1, set2E2). Because embryos of the former type with the more anterior levels of pattern fusion tend to have the smaller mesoderms overall, the effect of their overdevotion to notochord shows up all the more strongly when expressed in percentage terms as in Table 1, but the increase in notochord size is absolute. The embryos in which heads and notochords are scaled down in construction (Fig. 2)

are those where the dorsal midlines are most nearly opposed, arising 180° apart in the tissue, so that the amounts of mesoderm and of structure between notochords are relatively similar on either side of the cross-section. These are also the bodies in which the levels of axial fusion are most posterior in terms of segment numbers. This might be expected, both because somite axes fuse more anteriorly on the side of shorter 'tissue distance' in asymmetrically twinned axes and because the scaled-down head parts suggest that more nearly complete pattern is present in each axis. It is also empirically observed, in support of this interpretation, that the most symmetrically opposed pairs of part-patterns allow the most development of the tailbud and of the massive, more lateral cross-section of posterior trunk somites. In bodies where two large heads and massive notochords lie rather parallel in the structure, having relatively little tissue between them on their 'inner' sides, the posterior pattern is least represented. Their trunk regions thus tend to consist of narrow, curved hook-like axes with a terminal blastopore, there being no tailbud (Fig. 2D).

The second mode of variation to which attention must be drawn, is the frequency with which the two part-patterns are constructed on different scales within the available material. This occurs where both axes are morphologically complete at the dorsoanterior extreme, but also in cases where the smaller of the two (or, more rarely, both) shows the 'subapical' syndrome. The latter, an absence of the prechordal, cement gland and forebrain-inducing level of structure, has often been seen in twinned forms after dorsal lip grafting (Cooke, 1972, 1973), or in minimal axial deficiencies caused by mild u.v.-irradiation of the precleavage egg (e.g. Scharf & Gerhart, 1980; Cooke, 1985a). Although it is seldom extreme, measurable and systematic inequality of scale between the two part-patterns proves to be the rule rather than the exception among twins produced by the present gravity perturbations.

One body with three separate notochords, forebrains and sets of eyes, and two 'hyperdorsal' almost radially symmetrical bodies were produced in these experiments. In the latter, mesoderm is very reduced but seems to consist largely of prechorda and massive anterior lateral plate, with merely a sheath of somite muscle around the massive notochord.

In three of the experiments the entire population was inverted in 5.5% Ficoll at a late blastula stage, to pin embryos vegetal surface uppermost so that direct comparison of the time courses of gastrulation could be made. It was found that the appearance of two discrete initiations of invagination ('dorsal lips') in 14 individuals, and their relative positioning around the marginal zone, predicted the occurrence and the

configuration of twinned bodies at larval stages. Such initiations subsequently spread to give confluence and a circular blastopore ahead of the controls (see also Cooke, 1985c). Pairs of separate dorsal lips were not seen closer together than about 90° of angular distance on meridians of the embryo, and were then rather broad at their initiation. Only the three cases in which two lips were almost 180° -opposed produced the opposed-looking twin form with substantial posterior body and tailbud, and small head parts. In these, each initial 'dorsal lip' was of normal, or even narrower than normal, angular width for the egg batch in question.

Fig. 3 offers an interpretation of these twin forms in terms of the normal 'fate map' or projection of the body plan onto the material of the pregastrular embryo, suggesting the altered use of that material in

the case of various twin forms. In order better to represent the varying angular displacements between dorsal midlines of pattern, the fate map is reprojected from the normal unilateral view (Fig. 3A) to be an equivalent of those projections of the globe in which all lines of longitude are shown in one plane, and the entire equator is a straight line. It should be noted that, while this knowledge of the fate map allows us to understand why the development from opposed 'centres of dorsalization' gives the most nearly complete pattern posteriorly (Fig. 3C), it does not itself explain any effects of twinning on the sizes of anterior and dorsal duplicated parts.

Discussion

Contrast with late, surgically induced twinning

The information determining the layout of body pattern is probably confined, initially, to the yolky endoderm-producing egg hemisphere, but transmitted to mesoderm before gastrulation (Sudarwati & Nieuwkoop, 1971; Gimlich & Gerhart, 1984; Dale, Smith & Slack, 1985). Following a duplication of the developing system from the outset, as in the present

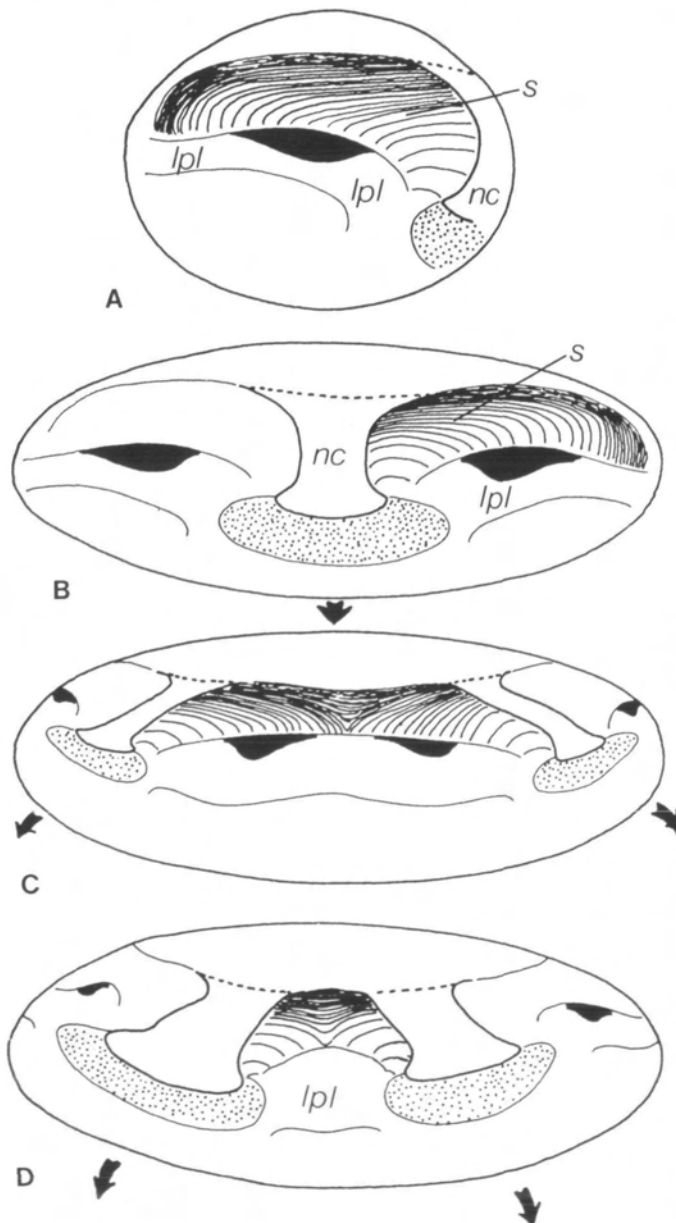


Fig. 3. The nature of twin forms after perturbation of the egg in gravity, in relation to the normal map for origin of mesodermal structures. (A) Current version of the 'normal fate map' for origin of mesodermal body plan from egg cleavage stages, as conventionally displayed with dorsoanterior to the right. (B) The fate map of (A) displayed as centred on the dorsal meridian, future head-end downwards (i.e. viewed as from the right in A). The layout of future somite segments on only one side is shown. Note that the entire circumference of the egg (length of the normal body axis) is represented, even though this is necessarily distorted in the same way as flat projections of the continents of the globe. (C) A hypothetical fate map, projection equivalent to B, but for formation of a pair of maximally separated, twin partial patterns. Note the reduced extents of origins for chordal and anterior structures, the possibility of separate but reduced pronephroi, and the relatively posterior axial level (i.e. high somite number) at which axial patterns fuse their somite territories. (D) A hypothetical map equivalent to C, but for a case of partial patterns with dorsal midlines considerably closer together on their 'insides'. Note the exaggerated extents of chordal and anterior regions, the reduction or loss of pronephric territory, the reduced numbers of somites anterior to the axial junction and the tendency to enlarged representation of lateral plate. These quantitative features of the experimental patterns are reflected in relative mesodermal cell numbers at the larval stage. *nc*, notochord; *s*, layout of origin of somite segments; *lpl*, lateral plate. Prechordal region is stippled, and pronephric territory, black.

work, a double-anterior partial pattern plus notochord duplication is seen rather than a double-dorsal one. This is not surprising in view of the normal way in which mesendodermal materials from around the egg are deployed in the head-to-tail sequence (Cooke & Webber, 1985a). Although the distinction is not sharp, *ab initio* twins contrast as a population with those produced by grafting a second dorsal blastoporal lip, or mesodermal 'organizer' into late blastulae. The latter more often have an appreciable tail region or even twinned tailbuds, together with substantial separate sets of trunk somites. In those, the gut mass shows only a rather minor involvement in the second axial pattern, whereas in the twin forms in the present study the endoderm pattern, the original site of the information, is often profoundly doubled anteriorly. The double-anterior character of the present twins is also revealed by their tendency to small mesoderm size and to relative excess of lateral plate tissue.

The nature of precleavage twinning and the egg's positional system

Early disequilibrium in gravity can evidently affect, profoundly and diversely, the relative amounts of the egg allocated to the different levels of body structure, either by distorting the single pattern or by production of duplicated partial forms. This fact, together with results obtained by more complex but controlled regimes of double centrifugation during precleavage (Black & Gerhart, 1986), makes it hard to argue that particular 'plasms' for body regions, of set relative sizes, are preformed and literally deployed in the correct spatial array by the egg's inner movements after fertilization. In the limiting case, either by total prevention of these movements or by a less well understood but opposite distortion of them, the egg can be caused to devote itself entirely either to ventral-posterior or to dorsal-anterior extremes of the normal pattern (see Scharf & Gerhart, 1983; Vincent, Oster & Gerhart, 1986; Scharf *et al.* 1984 and the two 'hyperdorsal' embryos of the present results). In addition to the embryos described here, the present regime of gravity disturbance also produced a low (1%) incidence of single and twinned axes with severe incompleteness anteriorly as in cases of impairment by u.v. light. In these, no notochord or head structure but only single or paired axes of somite tissue and spinal cord induction were produced.

In most of what follows, further interpretation of the meaning of these pattern abnormalities is made in line with the overall view of early *Xenopus* development coming from previous papers in this series. This is that 'position value' for body pattern is independent of the setting up of germ layers or determination of tissue types and is somehow encoded around the

structure of the egg as a continuous variable during precleavage and early cleavage stages. On this view, the present manipulations are leading to a certain incidence of abnormal profiles of position value, established long before their expression as atypical gastrulation and balance of tissue differentiation. Quite different interpretations are of course possible, if it is believed that the cell movements and other interactions of gastrulation themselves set up the overall proportions of the body, rather than being expressions of the prior balance of specification in mesoderm. But it is emphasized that all embryos were returned to free equilibrium in gravity after an hour by the 8-cell stage, so that it is very hard to conceive of the morphological abnormalities being direct mechanical effects of the original rotation, for instance expressed as abnormality in rate or extent of mesoderm involution. However, there may be other ways in which abnormal positioning of material within the egg could ultimately influence mesoderm motility. The external appearance of embryos, during gastrulation, is certainly more suggestive of precocious and abnormally vigorous than of delayed or deficient cell movements in cases due to become twinned or dorsoanteriorly overbalanced bodies.

If we return to the overall concept of a body positional system that has motivated this series of papers, the present results confirm that there is an early 'recording' aspect to the system that obeys the ratchet principle set down in Paper II (Cooke & Webber, 1985b). That is, a distorted positional profile will be retained as such in the material if it deviates from normal by having an over-representation of the more highly activated, dorsoanterior levels of specification. Values once recorded are not lost in favour of 'lower' ones. The overall results continue to suggest, on the other hand, that if the early events have been disturbed in an individual in such a way as to attenuate them, the 'reduced' positional profile is also retained; there is no restoration of 'headwards' values by up-regulation (the minor class of apically incomplete bodies referred to above). The results also give evidence for the rather local and perhaps mechanical, rather than diffusible, nature of the variables involved. This evidence is the asymmetry in partitioning of the available tissue into the two axial patterns, as well as the frequent incompleteness, at the 'upper' end, of one or both limbs of the doubled gradient profile. The maintenance of spatially asymmetrical profiles of the crucial variable setting contributions to pattern, after only transient disturbance during a brief 'setting up' period, is on the whole a behaviour difficult to achieve in model systems postulating diffusion after initial symmetry breaking events as the main agent of spatial organization (e.g. Turing, 1952; Gierer & Meinhardt, 1972).

The suggestion from this and other recent work that the recording of *Xenopus* egg position values is of a mechanical nature should not imply that there is no interaction of any sort across distance within the egg's structure. This is not the case, as is evident from the systematic relationship between positions occupied by twin centres of activation in particular eggs, and the sizes or scales of construction of the partial patterns due to each centre. It should be noted that even after dorsal lip implantation into blastulae, where the site was opposite the host dorsal midline, a detectable mutual scaling down of the duplicated pattern parts resulted. But it is now suggested that this geometry-dependent interactive effect is fundamentally to be understood in mechanical terms, rather than in terms of specific, diffusing inhibitors as was earlier proposed (Cooke, 1983). Since there can be no experimental evidence for this on a more cell-biological level, however, it is not useful to formalize it into a theory for the *Xenopus* primary positional system.

A single rather precisely proportioned pattern is reliably produced in normal eggs of a range of sizes within the species. The underlying mechanism must therefore have profound regulatory or buffering capacity against disturbance while that pattern is being initiated and orientated. In view of this, not much can be deduced from the normality of a majority of embryos after regimes that nevertheless record permanent disturbances in a significant minority. We can only try in an opportunistic way to build up a picture of the dynamics of the positional system from quantitative study of those deviant individuals that are produced. The present manipulations occur during a time when some crucial component of the cytoplasm is 'gelling' in a way that might record local values of a variable related to the self-activated plasm shifts (Neff, Wakahara, Jurand & Malacinski, 1984; Elinson, 1984; Vincent *et al.* 1986). Thus, only in a few eggs might an ambiguous or doubled configuration be caused. Other experiments, involving forces of many *g* (Black & Gerhart, 1985, 1986), may override more of the normal controls, as they give twinning at high though still incomplete incidence when carried out during particular time windows in the precleavage interval.

As in all previous observations in the present series of work, the rates at which cells on each meridian of the embryo undergo their hidden progress towards onset of gastrulation activity are correlated with the particular part of the normal axial plan that they will contribute. Distorted, doubled and/or incomplete sets of body structure are invariably presaged by corresponding doubling and transformation in the spatially organized time course of gastrulation. The

evidence is thus strengthened that the rate of development, in this sense, is almost a part of 'position value', or of what Lewis & Wolpert (1975) term *nonequivalence* in developing tissue. The ultimate task is to elucidate some molecular transduction machinery. An initial variable around the egg vegetal-marginal region, perhaps of a mechanically induced kind in view of the events that have so far been visualized, results in stable metabolic variation or in differential accessibility or activation of stored macromolecules that unlocks the various contributions to body pattern. It has recently been shown that egg activation and polarization to give grossly normal larvae can occur under conditions that simulate microgravity (Neff, Malacinski & Chung, 1985). This is not incompatible with the present observations that have introduced eggs to unusual experiments in 1 *g*, since it is clear at least that gravity is normally *one* of the interacting forces that set up the positional system.

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