Development 1989 Supplement, 37–51 (1989)
Printed in Great Britain © The Company of Biologists Limited 1989

Cortical rotation of the *Xenopus* egg: consequences for the anteroposterior pattern of embryonic dorsal development

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Summary

We first review cortical–cytoplasmic rotation, a microtubule-mediated process by which the *Xenopus* egg, like other amphibian eggs, transforms its polarized cylindrical symmetry into bilateral symmetry within the first cell cycle after fertilization. This transformation, the earliest of many steps leading to dorsal development, involves the displacement of the egg's cortex relative to its cytoplasmic core by 30° in an animal–vegetal direction. As rotation is progressively reduced by microtubule-depolymerizing agents, embryos develop with body axes progressively deleted for dorsal structures at the anterior end. With no rotation, ventralized embryos are formed. In an effort to comprehend this progressive effect on embryonic organization, we go on to review subsequent developmental processes depending on rotation, and we propose, with evidence, that reduced rotation leads to a reduced number of vegetal dorsalizing cells, which induce during the blastula stage a Spemann organizer region of smaller than normal size. The reduced organizer then promotes a reduced amount of cell rearrangement (morphogenesis) at gastrulation. Reduced morphogenesis seems the proximate cause of the incompleteness of axial pattern, as shown further by the fact that embryos that are normal until the gastrula stage, if exposed to inhibitors of morphogenesis, develop body axes that are progressively less complete in their anterior dorsal organization the earlier their gastrulation had been blocked. We discuss why axial pattern might depend systematically on morphogenesis.

Key words: cortical rotation, *Xenopus laevis*, mesoderm induction, microtubules, gastrulation, polarity, FGF, TGFβ, anteroposterior pattern, dorsoventral pattern, lithium, D_2O, organizer.

I. Organization and reorganization of the egg:

The egg's initial symmetry arises in oogenesis

The unfertilized *Xenopus* egg displays polarized cylindrical symmetry around an axis connecting the poles of the animal and vegetal hemispheres (these hemispheres henceforth abbreviated as AH and VH). The two hemispheres differ as the result of various localization mechanisms segregating materials during oogenesis, perhaps in relation to the oocyte's inherent axis comprising the nucleus, centrosome and division bridge (Tourte *et al.* 1984; Wylie *et al.* 1985; Danilchik & Gerhart, 1987; Yisraeli *et al.* this volume). The boundary between hemispheres (the 'equator') is roughly identified externally as the limit of dark pigment of the animal half, although this marking may not quite coincide with the internal interface of materials specific to the two hemispheres. Oocyte organization corresponds roughly to the germ layer organization of the embryo: ectodermal tissues arise from AH materials, endodermal tissues from VH materials, and mesoderm and archenteron roof endoderm from equatorial materials (reviewed in Gerhart & Keller, 1986).

All pole-to-pole meridians of the egg surface are indistinguishable before fertilization. Sperm can be applied at any point in the animal hemisphere (Elinson, 1980; K. Hara, unpublished; J. Gerhart, unpublished), and that point of entry identifies a meridian that coincides approximately but not perfectly with the eventual ventral midline of the embryo. The opposite meridian falls on the dorsal midline of the embryo, or more correctly, on the midline of the neural plate. Many experiments, some discussed later, indicate that the egg has an initial capacity to develop dorsally at any, several, or even all, meridians, and the same is true of ventral development. Normal embryonic development arises from the proportioned, patterned use of this potential, normally in relation to the sperm entry point.

Bilateralization of the egg after fertilization

The *Xenopus* egg reorganizes its cytoplasmic contents in an interval between 45 and 90 min after fertilization, a G_2-like period of the first cell cycle (100 min, 18 °C), a period absent from the next 11 or 12 rapid cycles (35 min each). Within this interval, the egg behaves as if composed of two rigid units, a thin cortex (2–5 μm thick) and a large spherical core (1200 μm diameter). As diagrammed in Fig. 1, these *rotate* relative to one

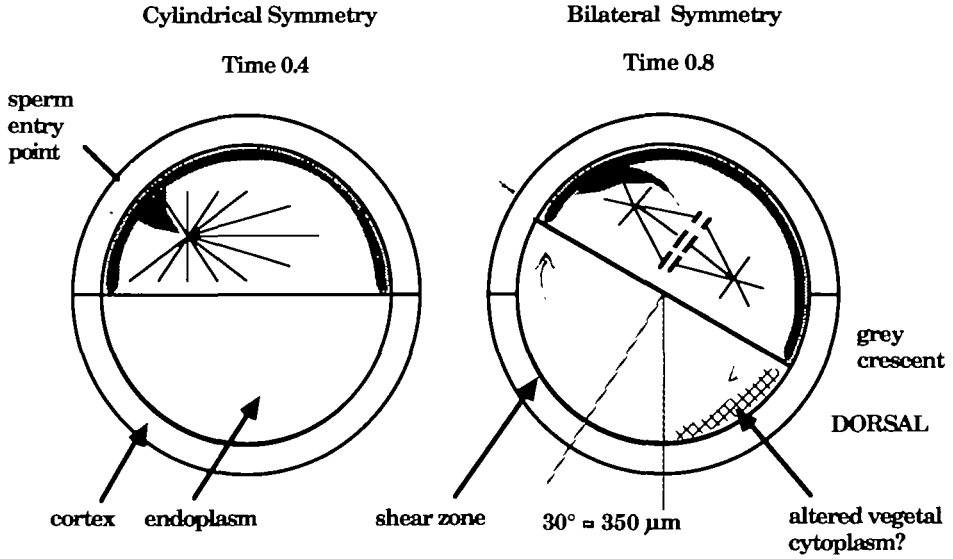

Fig. 1. Reorganization of the *Xenopus* egg cytoplasm by cortical rotation. On the left is shown a schematic cross section of an egg at time 0·4 (40% of the first cell cycle, lasting approximately 100 min at 18°C), before cortical rotation has occurred. The sperm has entered at the left, forming a large aster and migrating towards the egg's center. The egg's cortex is shown as a rigid peripheral unit, 2–5 μm in depth, containing the plasma membrane, cortical actin, and materials that will travel with it during rotation. The core is shown as a rigid spherical unit, 1200 μm in diameter, containing yolk platelets and most cytoplasmic contents of the egg. Note that the cortex and core both differ in their animal and vegetal portions. Prior to rotation, the two units are in register along all pole-to-pole meridians, and the egg has polarized cylindrical symmetry.

On the right is shown an egg cross-section at time 0·8, after cortical rotation has occurred. The egg surface has been immobilized during rotation, and the core has undergone the entire displacement of 30° of arc, which is 350 μm of linear distance. The core has rotated in an animal–vegetal direction, downward on the right and upward on the left, separated from the cortex by a shear zone a few microns thick. The rotation axis is at the center of the section, pointing out of and into the page. Rotation destroys cylindrical symmetry and generates bilateral symmetry: contacts between core and cortical materials differ now on the right and left. Downward movement on the right activates dorsal development; the future dorsal embryonic midline will coincide with the meridian of greatest displacement. The opposite movement (on the left side of the section) has no consequence for dorsal development; ventral development ensues on the left side, but this kind of development occurs even without rotation. It is not known what cytoplasmic modifications are directly caused on the right side by rotation. They presumably occur in the shear zone or at the contact sites between the two rigid units, perhaps just in the vegetal hemisphere, but perhaps in both hemispheres. Some amphibian eggs (e.g. *Rana* but not *Xenopus*) show a grey crescent at the equatorial level; these species have black pigment in the animal hemisphere cortex and grey pigment in the animal hemisphere core. Where the core rotates downward, grey pigment can be seen as a crescent through the pigmentless vegetal cortex. The grey crescent may have importance for dorsal development (there is no convincing evidence of this, except for topographic correlation) or it may just be a convenient marker of the time and direction of cortical rotation.

If the egg were free to orient in the gravitational field (as when floating in pond water), the core would remain in gravitational equilibrium, weighted by its dense vegetal yolk platelets, and the cortex would undergo the entire displacement.

another in an animal–vegetal direction, for 30° of arc, (a distance of 350 μm) around a new axis perpendicular to the animal–vegetal axis. Movement can be observed by marking the cortex or core with dye spots (Nile blue, fluorescent lectins, photobleached fluoresceinated yolk proteins; see Vincent *et al.* 1986) or by tracking at high magnification the few pigment granules embedded in the core's vegetal surface (Rowning, 1989). It accelerates from rest to full speed (8 μm min^{-1}) within 7 min, remains almost constant for 40 min, and then abruptly stops. Spot patterns remain sharp and coherent for at least an hour, demonstrating the rigidity of the cytoplasm and cortex. The two units just seem to glide over one another, separated by a shear zone of less than 5 μm in depth. If the cortex is immobilized, as is the case for eggs embedded in gelatin or agarose (as in Fig. 1), the core makes all the displacement, even though work must be done to lift its dense vegetal yolk mass out of gravitational equilibrium. If the cortex is free to move, as is the case for an egg floating in a pond, it makes all the displacement while the cytoplasmic core remains in gravitational equilibrium.

By this simple geometrical operation, the cylindrical symmetry of the egg transforms into bilateral symmetry (Fig. 1). Animal–vegetal organization is systematically modified by the slippage of concentric layers. Displacement is greatest on the midlines of rotation and decreases to zero at the rotation poles (located at opposite positions on the equator). On one rotation midline, the core moves in a vegetal direction relative to the cortex, or stated identically, the cortex moves animalward relative to the core. This midline coincides accurately (±10°) with the embryo's dorsal midline (Vincent *et al.* 1986). On the other rotation midline, which coincides

with the embryonic ventral midline, displacement has the opposite sense, and, as discussed later, this sense has no consequence for development since ventral development occurs even without rotation. The ventral midline just seems by default the farthest position from the dorsal midline.

This reorganization process has received many names: subcortical rotation, the rotation of symmetrization or cortical/cytoplasmic rotation. We will call it cortical rotation for brevity. It was first inferred by Banki in 1929 for axolotl eggs and described in detail by Ancel & Vintemberger (1948) for *Rana fusca* eggs, who recognized it as the process forming the grey crescent, a lightly pigmented subequatorial region marking the embryo's future dorsal side, including its organizer region. Cortical rotation has also been inferred in *Rana pipiens* eggs from grey crescent formation and from displacement of the polar body spot (Elinson, 1980). Rotation was not detected in *Xenopus* eggs until our studies with artificially marked eggs because *Xenopus* eggs rarely form a grey crescent; this is simply because the pigment granules reside entirely in the core during rotation. Probably most amphibian eggs make use of cortical rotation, as do the eggs of some fish (ancient groups such as lungfish and sturgeons) which display grey crescents and complete cleavage.

Cortical rotation correlates well with embryonic dorsoventral organization

Rotation is normally oriented toward the sperm entry point (SEP), as is the dorsoventral organization of the embryo. Løvtrop (1965) has proposed that the true dorsalizing process in *Xenopus* eggs may not be cortical rotation but a contraction of cortical materials toward the sperm entry point. However, there is now abundant evidence that rotation is the more crucial rearrangement for embryonic dorsoventral development, and that the sperm only affects embryonic organization by influencing (*via* its sperm aster) the direction of rotation:

(1) Under normal conditions of fertilization and development, the direction of rotation more accurately predicts the embryonic dorsal midline than does the SEP (Vincent *et al.* 1986).

(2) Eggs can be tipped out of gravitational equilibrium, or squeezed laterally in various directions before or during cortical rotation, and these treatments randomize the relation of the SEP to the embryonic axes, whereas the direction of rotation still accurately correlates (Gerhart *et al.* 1980; Vincent *et al.* 1986; Black & Vincent, 1988).

(3) Dispermic and trispermic eggs initiate a single direction of rotation which predicts the dorsal midline (Vincent & Gerhart, 1987).

(4) Artificially activated eggs, which lack an SEP, rotate in a unique direction and, if later transplanted with a nucleus and centrosome, will develop an embryo having its dorsal midline at the position predicted by the rotation direction (Vincent & Gerhart, 1987; J. Roberts & B. Rowning, unpublished).

(5) When fertilized eggs fail to engage in cortical rotation, they develop no dorsal embryonic structures, as discussed later, despite their SEP.

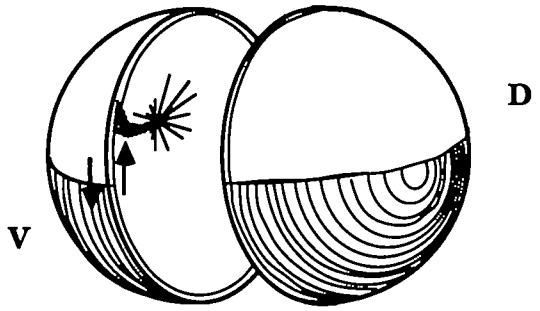

Fig. 2. Schematic diagram of parallel microtubules in the vegetal hemisphere of the *Xenopus* egg during cortical rotation. The egg is cut in the bilateral plane, showing the sperm aster in the animal hemisphere (upper half), and the parallel microtubules in the periphery of the vegetal hemisphere (lower half). The tubule array exists only in the interval from 0·4–0·9 of the first cell cycle, at the time of cortical rotation. The sheet of tubules is only 2–4 μm thick, starting approximately 2 μm from the egg surface. Tubules are aligned in the direction of rotation, shown by the arrows; their polarity is not known. Ventral (V) and dorsal (D) development will occur on opposite meridians, as indicated. Note that tubules describe tighter half-circles as the rotation poles ('hubs') are approached.

Taken in its entirety, the evidence is strong that cortical rotation is an indispensable step in normal dorsal development. The need for it can be bypassed under certain experimental conditions (e.g. the use of lithium ion), and it is certainly not sufficient by itself for dorsal development; but it seems to be a normal, essential, early member of a series of processes leading to dorsal development. The role of the sperm in influencing the direction of rotation will be discussed later.

Microtubules serve as tracks for cortical rotation

At the start of rotation, a thin layer of parallel, slightly sinuous, microtubules (MTs) appears in the vegetal hemisphere, approximately 2–5 μm beneath the egg surface (Elinson & Rowning, 1988). The MTs align in the direction of rotation, describing tighter semicircles nearer the rotation hubs (Fig. 2). They are dynamic and perhaps associate laterally. They disappear at the end of rotation. The array is probably anchored to the core (B. Rowning and T. Mitchison, unpublished). If true, the cortex would then have anchored to it the motor molecules (e.g. kinesin or dynein), which presumably crawl along the tracks of parallel MTs and generate the force to displace the rigid cortex relative to the rigid core. The polarity of the MTs is not known, and so we cannot yet speculate on the need for kinesin as opposed to dynein. However, since movement is unaffected by injected sodium vanadate (10–100 μM) combined with long wavelength UV irradiation of the vegetal egg surface (365 nm), we currently disfavor the possibility of a dynein-like motor molecule.

Many agents depolymerize MTs of the array, and all of these inhibit cortical rotation. These include colchi-

cine, vinblastine, nocodazole, cold shock and hydrostatic pressure. Also, UV-irradiation (254 nm) of the vegetal surface prevents MT polymerization (Grant & Wacaster, 1972; Malacinski *et al.* 1977; Elinson & Rowning, 1988), perhaps because GTP becomes covalently bound to tubulin (S. Roberts, 1989). Since all these agents inhibit rotation, we can assert that MTs are needed for rotation under normal conditions, probably as aligned tracks for movement. Cytochalasin D, in contrast, does not interfere with rotation, precluding a continuous role for dynamic microfilaments. Finally, cycloheximide doesn't inhibit rotation, even when applied before fertilization, indicating that the entire process is post-translational, depending only on preformed maternal proteins.

What aligns the microtubule array?

This is an important issue because as the array gains a unique directionality (one selected from 360° of possibilities), rotation is committed to that direction, producing a unique bilateral symmetry retained throughout the rest of development. Before rotation starts at 45 min postfertilization, there is no detectable MT array. Whatever controls the direction of MTs in the first few minutes of their polymerization, controls the orientation of subsequent embryonic development, under normal conditions.

We have had difficulty examining the earliest stages of polymerization because the array builds up so quickly. As rotation accelerates, a few short and poorly aligned MTs can be seen, but within a few minutes, rotation reaches full speed and the array is complete. To explain the rapid transition from a disordered to ordered array, we propose a *positive feedback model* with two provisions, namely: microtubules orient rotation, and rotation orients microtubules (Fig. 3). The first provision follows reasonably from what is known about MT function, for as the first few tubules polymerize with poor alignment, a single direction of rotation of the two rigid units (cortex *versus* core) must nonetheless emerge as the vector sum of forces generated along all MTs (their lengths and directions considered). MTs of opposed directions will cancel each other, while any slight excess of MT polarity in one direction will dictate the direction of rotation. At first rotation will be slow because the vector sum is small.

The second provision is more controversial: can rotation feed back on MT polymerization so that MTs tend to arise or persist in the direction of rotation? As background, we note evidence that the direction of MT polymerization in the periphery of cultured cells responds to the arrangement of elongated tubes of endoplasmic reticulum (Terasaki *et al.* 1986). Endoplasmic reticulum (ER) vesicles and tubes possess kinesin-like MAPs which associate with MTs during their transport and elongation (Vale, 1987), and these vesicle-associated MAPs could in principle stabilize the walls (rather than ends) of MTs, thus disfavoring MT depolymerization. If rotation could elongate and align ER in the shear zone between the rigid cortex and core, then the ER might in turn preferentially stabilize MTs in that direction. In this way, rotation might influence the direction of MT polymerization, through the mediation of shear-aligned ER tubes and vesicles. With increased MT alignment, rotation would then be faster, leading to still more alignment of the ER and more positive feedback on tubule polymerization. Soon MTs would lie overwhelmingly in the rotation direction, directing rotation and ensuring their own persistence in that direction. The egg would be fully committed to one direction of rotation, out of all directions initially possible (Fig. 3 right panel).

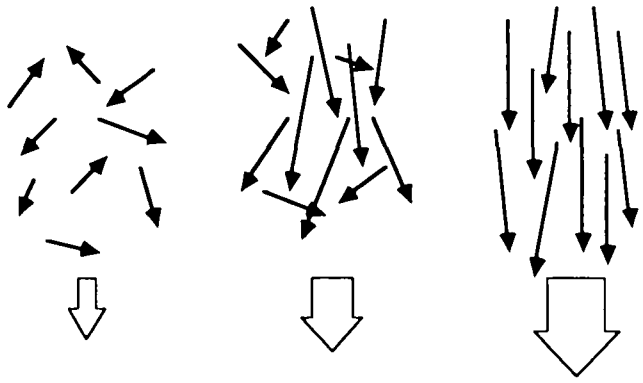

Fig. 3. Positive feedback model for the unidirectional aligning of vegetal microtubules (MTs) during rotation. In the left panel, MTs are first polymerizing in a small region of the egg's vegetal periphery, and rotation is just starting (approximately time 0·4). MTs are short and poorly aligned. Tubule polarity is indicated by the solid arrow heads. We assume MTs are anchored to the rigid core. Since kinesin or dynein-like motor molecules, which are assumed anchored to the cortex, must travel unidirectionally along MTs and exert force in the direction of each MT, rotation will start in whatever single direction constitutes the vector sum of all tubules (tubule direction and length considered). Only one rotation direction can result (see large open arrow) since there are only two rigid units. At first, rotation will be slow since the vector sum is small. Then, as rotation starts, cytoplasmic materials of the shear zone become partially aligned by shear flow (a proposal, not a fact), and these materials stabilize MTs that form in the direction of alignment, while other tubules break down. The identity of these materials is unknown. The center panel shows the MT array a few minutes later: MTs are more numerous, longer on average, and more aligned in the direction of movement. More force is generated in the direction of rotation and now displacement is faster (large open arrow). Faster rotation means more shear, leading to still more alignment of cytoplasmic materials, and still more stabilization of MTs in that direction. In the right panel, a few minutes later, MTs are still longer and better aligned, supporting full-speed displacement in the original direction. Since MT orientation defines the direction of rotation, and since rotation defines the direction of MT orientation, polymerization and rotation have a positive feedback relationship to one another. Due to positive feedback, the egg amplifies a small initial asymmetry (left panel) into a large one (right panel); the polymerization–rotation process tends strongly to disrupt cylindrical symmetry, as long as it starts in an animal–vegetal direction.

The initial bias can be small

According to this model, any initial slight departure from a perfectly random array of MT (a vector sum of zero) will quickly amplify into a large departure, the unidirectional array. What initial bias might allow a few more MTs to form in one direction than in others, thereby controlling the orientation of later embryonic organization? To begin with, one could argue that, even if there were no bias, the first MTs are very unlikely to take up a perfectly random orientation, and therefore the egg will always be able to escape from cylindrical symmetry, due to positive feedback. A near-random condition may be approached in carefully manipulated eggs which are artificially activated by needle puncture or electric shock. In these, the direction of rotation can't be predicted beforehand. Usually they succeed in rotating for the full distance at the correct time, though occasionally they choose a skewed or equatorial path. A reliable bias can be introduced in such an egg simply by tipping it out of gravitational equilibrium for 5–10 min, at some time before rotation, to achieve a small displacement of the core relative to the cortex in an animal–vegetal direction (Ancel & Vintemberger, 1948; Rowning, 1989). Remarkably this forced displacement, which occurs in the absence of MTs, can be terminated long before rotation begins (15–30 min before), and nonetheless, when MTs appear, they align in the direction of the earlier forced displacement. MT-mediated rotation then continues what tipping had started. Materials of the egg periphery must have been oriented by the forced movement; these must have persisted and later biased the direction of MT polymerization and/or stability. In addition to demonstrating an experimentally imposed bias, this is our best evidence that similar displacements might influence the direction of MT polymerization during the normal cortical rotation period, as part of the positive feedback mechanism, and that the influence makes use of something other than MTs.

Under normal conditions, though, the sperm provides the dominant bias (Manes and Barbieri, 1977). MTs of the sperm aster extend through the AH long before vegetal cortical MTs appear (Stewart-Savage & Grey, 1982; Ubbels *et al.* 1983). Perhaps MTs of the aster enter the vegetal hemisphere at an early time and support a small amount of cortical displacement, leaving materials of the shear zone slightly oriented in the same way as can gravitationally forced displacement. Any slight orientation of materials with respect to the SEP would suffice to bias the whole MT array to build up in that direction. Actually, the sperm's effect is not strong; if gravity or centrifugal force is used to displace materials in another direction in a fertilized egg, that displacement easily comes to provide the dominant bias (Black & Gerhart, 1985).

In general, once the array of MT becomes aligned and rotation reaches full speed, it is difficult to change direction (Vincent & Gerhart, 1987), although this can occasionally happen in eggs briefly subjected to low temperatures to reversibly depolymerize the MT array during rotation (Vincent & Gerhart, 1987) or can frequently happen in eggs compressed laterally during rotation to force movement into a new path (Black & Vincent, 1988). If the MT array is really dynamic and if positive feedback operates continuously to define MT directionality, these changes would be explicable.

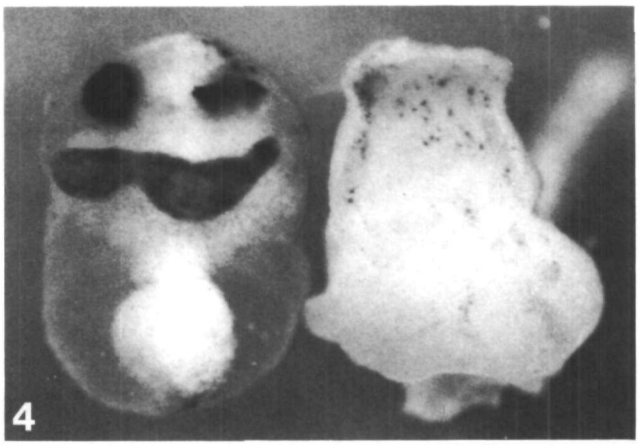

Fig. 4. Dorsalized and ventralized embryos of *Xenopus laevis*. The right embryo developed from an egg UV-irradiated (254 nm, 44 $\mu W\,cm^{-2}$, 2 min) at time 0·4 of the first cell cycle to prevent cortical rotation; that on the left from an egg treated with 60 % D_2O for 4 min at time 0·28. The right embryo lacks dorsal axial structures, but has differentiated a ciliated epidermis, red blood cells, coelomic compartments, and a short gut. The left embryo has a large heart, and circumferential bands of eye pigment and of cement gland. Both embryos retain the egg's cylindrical symmetry in their organization.

Dorsal development requires rotation

Embryonic organization is drastically altered when rotation is prevented. The egg, after it has been treated briefly (2–4 min) with nocodazole, cold shock, hydrostatic pressure, or UV irradiation (of the vegetal surface only) to prevent MT polymerization and cortical rotation (Vincent *et al.* 1987; Elinson & Rowning, 1988), cleaves normally but then gastrulates abnormally, forming a ventral-type of blastopore lip which is late and synchronous around the gastrula circumference (Scharf & Gerhart, 1980, 1983). Although the embryo does not neurulate or form dorsal components of the body axis, it still develops a limited set of ventral structures at all meridians, preserving the cylindrically symmetric organization of the oocyte (Fig. 4, right). Tissues of all three germ layers are present in these ventralized 'limit forms': ciliated epidermis, coelomic mesoderm and blood islands (up to 15 times the normal amount of red blood cells; Cooke and Smith, 1987), and a short gut. The total amount of mesoderm is the same as that of normal embryos; but dorsal mesoderm is missing (Cooke & Smith, 1987). Dorsal development clearly depends on rotation whereas ventral development does not.

With intermediate amounts of rotation, the egg develops certain dorsal structures, in an interesting anteroposterior deletion series that depends on the amount of rotation. The more rotation, the more

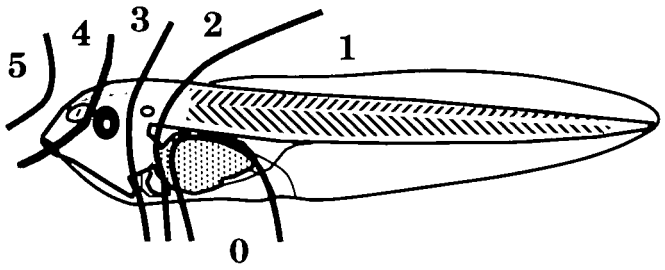

Fig. 5. Schematic diagram of the anteroposterior order of deletion of dorsal structures from the embryonic body axis as cortical rotation is reduced. Although the embryonic body axis can be truncated at any level, grades (or anatomical types) of embryos are defined to establish a scoring system, for the rough quantification of embryonic defects. This scale originated with Malacinski *et al.* (1977), was modified by Scharf & Gerhart (1980), and expanded to include excessively dorsalized embryos by Kao & Elinson (1988). Note that the scores described here are not interchangeable with those described in the earlier papers. A grade 5 embryo has all normal axial structures, whereas a grade 0 embryo lacks all dorsal structures, as well as some ventral ones such as the heart, gills, and probably some parts of the digestive system. However, grade 0 embryos still develop red blood cells and coelomic mesoderm (organized in two chambers), and are covered with ciliated epidermis (see Fig. 4). They digest their vegetal yolk in what may be a simple gut. Ventral structures of the normal belly region (i.e. excluding gills, heart, and anus) develop along all meridians of the egg, rather than just on the ventral-most. This is the 'limit form' obtained without cortical rotation, or without vegetal dorsalizing cells (a 'Nieuwkoop center'), or without a Spemann organizer. Intermediate grades have the structures of grade 0, plus more and more of the dorsal structures, as shown by the contour lines on the diagram. Embryonic structures are reduced and lost concertedly in the anteroposterior and dorsoventral directions. The pattern of progressive deletion can be more easily rationalized by examining the fate map of the early gastrula, than by examining the final larva.

anterior is the truncation level beyond which dorsal elements are missing from the body axis. Thus, while the direction of rotation defines the position of the dorsal midline, the extent of rotation defines the anterior extent of dorsal development. Fig. 5 shows the progression by which body parts are gained and lost.

While all eggs require rotation, they differ in the quantity required; some need at least 25° and others only 12° for development of the complete body axis (Fig. 6A). Normally eggs accomplish about 30° of rotation, an amount sufficient for the normal development of even the most insensitive members of the population. Presumably the difference reflects a variable effectiveness of rotation on its target, a component involved in a subsequent process of the series leading toward dorsal development.

Rotation without microtubules still allows dorsal development

When eggs are treated with anti-microtubule agents so that they have no tracks along which to effect rotation, they can nonetheless be forced to rotate artificially by tipping them out of gravitational equilibrium, while immobilizing the egg surface. The vegetally weighted core then slips relative to the cortex, driven by the artificial motive force of gravity. Tipped eggs, it turns out, are well rescued for dorsal development (Scharf and Gerhart, 1980), and the amount of forced displacement correlates with the amount of rescue. The anterior end of the body axis is rescued only in eggs experiencing the greatest displacements (Vincent & Gerhart, 1987). During the period of forced displacement in these eggs, MTs cannot be detected in the vegetal periphery. Rotation, driven by artificial means, seems sufficient to initiate dorsal development. Thus, we think that in normal eggs the parallel MT array functions only as tracks for cortical rotation in the first cell cycle, and that rotation itself, not the MTs, causes a dorsalizing change in the egg periphery.

What and where is the immediate target of rotation?

When rotation stops at time 0·85 of the first cell cycle, what cytoplasmic alteration persists, with consequences for later steps of dorsal development? Unfortunately we don't know, but we have considered two rather different possibilities: either a change of the contacts between the cortex and core of the egg, or an alignment and polarization of materials in the shear zone between the cortex and core.

Regarding the first, the animal core cytoplasm, for example, comes into contact with vegetal cortex on the prospective dorsal side during rotation. This kind of contact is not formed on the prospective ventral side. However, this contact may not be important since normal, and not excessively dorsalized, development is still obtained from eggs in which the core has been completely inverted at time 0·4 in the first cell cycle, bringing animal core cytoplasm into total contact with vegetal cortex (S. Black, unpublished). These eggs gastrulate normally from the hemisphere containing the vegetal yolk mass and animal cortex. The core alone may determine the animal–vegetal differences of development, and rotation-dependent modifications of the core may determine dorsoventral differences.

Regarding the second possibility, the zone between the core and cortex must experience high shear as the two rigid units move past one another for 350 μm while separated by only 2–5 μm. Shear could in principle lead to the alignment and deformation of materials within the zone. High voltage electron microscopy shows the shear zone to contain large numbers of membrane-bounded vesicles (Rowning, 1989). Perhaps these become elongated and aligned during rotation. If such vesicles are important in influencing the direction of tubule polymerization and hence the direction of rotation, as described earlier, shear forces would have to polarize them. Furthermore, aligned vesicles would have to retain different polarities on opposite sides of the egg if they are to promote dorsal development on just one side. To our knowledge, this proposal of polarized vesicles gains as yet no support from studies of other types of cells. Nonetheless, shear-dependent

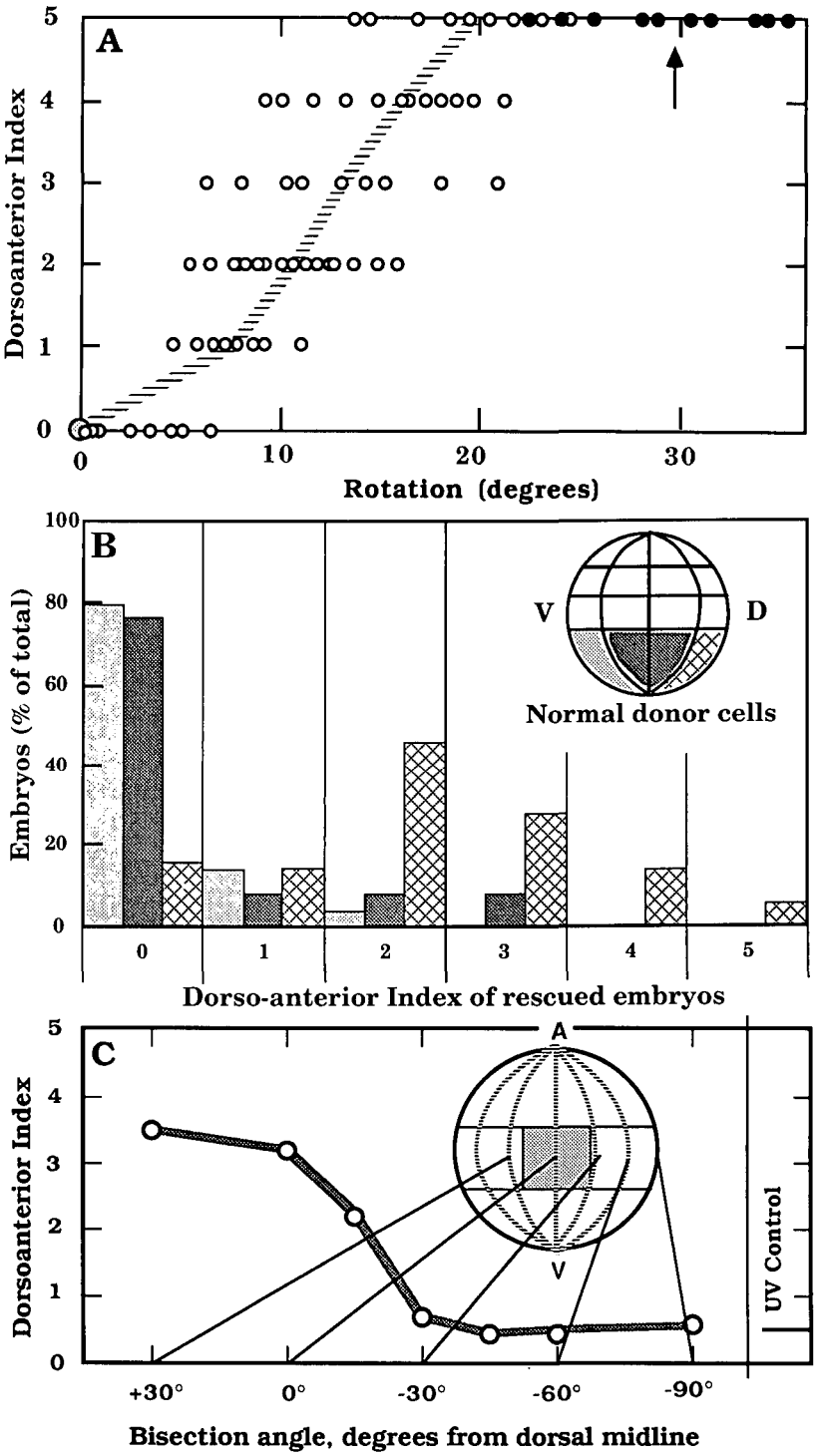

Fig. 6. The anterior completeness of the embryonic body axis depends on the amount of cortical rotation, of vegetal dorsalizing induction and of the organizer.
Panel A: Cortical rotation. Details are in Vincent & Gerhart (1987), from which these data are replotted. *Xenopus* eggs were UV-irradiated at times before or during cortical rotation. The amount of rotation of each egg was measured as the angular displacement (degrees of arc, shown on the horizontal scale) of a hexagonal array of nile blue spots applied to the vegetal surface of the egg. The open circles report the movement of the cytoplasmic core. The egg surface was immobilized by resting the eggs in tight-fitting wells in agarose. Eggs were removed from the wells at gastrulation and allowed to develop to stage 40, for scoring of the dorsoanterior completeness of the body axis, recorded as the dorsoanterior grade, on the vertical scale of the figure. Each data point is an individual egg and embryo. Filled circles report the degree of cortical rotation of ten control eggs and the vertical arrow indicates the average, about 30°.
Panel B: Vegetal dorsalizing induction. Details are in Gimlich & Gerhart (1984), from which these data are replotted. Prospective host eggs were UV-irradiated early in the first cell cycle to prevent cortical rotation. Such eggs would develop to give mostly grade 0 embryos, lacking all dorsal axial structures. At the 64-cell stage, two cells were removed from the tier of cells nearest the vegetal pole (the D tier) and were replaced by two cells taken from the corresponding tier of a normal (non-irradiated) embryo of the same age. Donor cells came from the dorsal midline, the lateral position, or the ventral midline, as indicated in the inset. Dorsalmost cells were most effective at rescue, giving larvae with dorsal axial structures complete to the ear vesicle level or more anterior (Grades 3, 4, 5).
Panel C: The organizer. Details are in Stewart & Gerhart (1989) from which these data are replotted. Normal late blastula embryos (taken 30–60 min before stage 10) were cut in half in an animal–vegetal plane. The plane of cutting was chosen to split the embryo on the bilateral plane or at various angles from that plane. In this way a half embryo (called the 'test half') could be made to contain different amounts of the organizer region, which is centered on the prospective dorsal midline. (Eggs had been tipped and marked in the first cell cycle so that the prospective dorsal midline of the blastula was accurately known). The test half was combined with a cut half of an embryo from an egg which had been UV-irradiated in the first cell cycle to prevent cortical rotation, and which therefore would lack an organizer. Such recombinates develop well and achieve bilateral symmetry in all cases, although the anterior truncation level of the embryo's body axis varies with the amount of organizer present. Half an organizer is usually sufficient for development of an entire body axis up to the level of the hindbrain or even further (Grades 3, 4, 5). At an angle of 30° from the bilateral plane, there is too little organizer in the test half to allow dorsal development, and the recombinate develops just as would an intact UVed embryo. Thus, the marginal zone of the normal late blastula, except for the 60°-wide organizer, lacks capability for autonomous dorsal development, although it is competent to produce somites, heart, and kidney if allowed to interact with the organizer during gastrulation.

polarization is worth mentioning as a very different possibility from that of altered core–cortex contacts.

In addition to our ignorance of what is affected, we don't know where the effect of rotation is first registered, namely, in the AH or VH, or both, even though we know that the effect must occur near the prospective dorsal midline, probably in the shear zone between the core and cortex. There are several indications that the vegetal hemisphere contains the target: Elinson & Pasceri (1989) have recently shown that UV-irradiated oocytes give rise to eggs that form vegetal microtubules and that rotate, but do not thereafter develop dorsal structures; these are presumably damaged in materials that rotation would normally activate. The target of irradiation is in the vegetal hemisphere of the oocyte. By the 16- or 32-cell stage, the vegetal hemisphere is certainly specialized for promoting dorsal development; it contains a dorsalizing inductive quadrant (discussed later) centered on the midline of greatest rotational displacement.

However, Cardellini (1988) has presented evidence that the animal hemisphere is initially specialized near the prospective dorsal midline, presumably by rotation, and that it passes its effect to the vegetal hemisphere at the 8- to 16-cell stage. Kageura & Yamana (1984) have also found cases where dorsal animal blastomeres, if transplanted at the 8-cell stage, can initiate dorsal development independent of the state of vegetal blastomeres. (We have unfortunately not been able to reproduce these results; in our hands, the animal hemisphere carries no dominant dorsalizing bias at the 8-cell stage, and the vegetal hemisphere is already fixed in its control of dorsal development.) Furthermore, London *et al.* (1988) report that AH cells at the 8-cell cleavage stage differ on the prospective dorsal and ventral sides: isolated and cultured AH prospective ventral cells can autonomously express at a later time an antigen characteristic of normal embryonic epithelium, whereas prospective dorsal AH cells cannot. Non-expression is characteristic of the neural plate region of the intact embryo, a region that normally develops from the AH prospective dorsal cells. However, the isolated AH prospective dorsal cells do not form a neural plate autonomously. Thus, there is evidence that the animal hemisphere is regionally modified at least to some extent by rotation.

At this time, the most inclusive interpretation would be that the entire dorsal meridian of the egg, in both hemispheres, is modified by rotation, and the particular local developmental expression of this modification will differ depending on the animal, equatorial, or vegetal level. Finally, rotation may only initiate a first transient cytoplasmic change, soon leading to other longer-lasting modifications. In this connection, it is observable that pigmentation differences continue to develop in the animal hemisphere until at least the 4-cell stage, that deep cytoplasmic materials of the AH circulate in relation to the direction of rotation, and that cortical materials ingress into the egg interior along new cleavage furrows which differ in detail on the prospective dorsal and ventral sides (Phillips, 1985; M. Danilchik and J. Degnen, unpublished). It remains to be seen how many of these changes are indispensible for dorsal development, and how many are epiphenomena of rotation.

Excessive dorsoanterior development

When eggs are centrifuged twice in opposite directions, to achieve two forced displacements, they frequently develop as twins, with dorsal midlines at positions related to two force vectors (Black & Gerhart, 1986). A 10 min interval must separate the two centrifugations in order for both displacements to have effect; with shorter intervals only the second displacement initiates dorsal development. The existence of these twins shows that the egg has the capacity to initiate dorsal development in excess of the normal amount.

The egg can accomplish still more dorsoanterior development, at the expense of ventroposterior development, if treated with D_2O early in the first cell cycle (Scharf *et al.* 1989). In the extreme, such embryos possess a large heart, a core of unextended notochord, and circumferential bands of eye pigment and cement gland. They lack somites and red blood cells. They retain the cylindrical symmetry of the unfertilized egg, with dorsal development initiated on all meridians. In exaggerating dorsoanterior development, they define the opposite end of the anatomical spectrum from ventralized embryos (Fig. 4, left). Partially affected eggs develop as embryos with enlarged heads and trunks, but lacking tails, or as enlarged heads lacking trunks and tails (Scharf *et al.* 1989). As discussed later, similar embryos arise from blastulae treated with lithium ion at the 64- to 128-cell stage (Kao & Elinson, 1988). Cooke and Smith (1988) estimate that hyperdorsalized embryos contain the same total amount of mesoderm as normal embryos, but dorsal mesoderm has been increased at the expense of its ventral counterpart.

These hyperdorsal forms arise only if eggs are treated with D_2O before cortical rotation. Once rotation begins, D_2O has no effect. D_2O causes a precocious polymerization of MTs in the vegetal periphery, in a dense random array that persists into the rotation period. The D_2O-stabilized MTs are crucial since any subsequent treatment that eliminates them also eliminates hyperdorsal development. The action of D_2O remains a puzzle to us. Even though unified cortical rotation is strongly inhibited, probably because the random MT array cannot acquire unidirectionality, there may occur multiple local displacements of cortex and core materials. The egg behaves as if it had rotated in many directions, and among the mildly affected cases, twinning occurs frequently.

The existence of hyperdorsal embryos shows that the egg has maternal resources for more extensive dorsal development than it normally uses. Presumably this potential resides at all meridians of the egg's circumference, in a latent state, just as does the potential for ventral development. Unified cortical rotation normally activates the use of this potential in just one region, while leaving the remainder of the circumference for

ventral development, thereby establishing the proportions of the normal embryonic pattern.

II. The quantitative dependence of later events on cortical rotation

Why does the *quantity* of cortical rotation in the first cell cycle delimit the anterior completeness of the embryonic body axis? To consider this relationship, we will sketch three steps of later development in which the same truncation series of embryos can be generated by experimental interference. We propose that the amount of cortical rotation determines: (1) the number of vegetal dorsalizing cells which in turn, by way of induction in the blastula period, determine (2) the number of the cells of the Spemann organizer region which in turn, by way of inductive interactions in the gastrula period, determine (3) the extent of morphogenesis and patterning during gastrulation.

Vegetal dorsalizing induction

As Nieuwkoop discovered (1973), cells of the vegetal hemisphere of the midblastula embryo (4000-cell stage) induce neighboring cells of the animal hemisphere to behave as marginal zone cells at gastrulation and to differentiate ultimately as many types of mesoderm and as archenteron roof endoderm. The dorsalmost quadrant of the vegetal hemisphere differs from other vegetal regions: it induces the organizer quadrant of the marginal zone, which initiates much of the morphogenesis of gastrulation, and which usually forms the notochord, a central mesodermal tissue of the embryonic dorsal midline. Dorsal development beyond the blastula stage depends on the presence of these special vegetal cells.

We extended these studies to earlier stages in an attempt to connect cortical rotation to the founding of this specialized vegetal quadrant. At the 32- to 64-cell blastula stage, two vegetal cells can be removed from the prospective dorsal midline, as predicted by the direction of cortical rotation, and these cells can be transplanted into another blastula of the same age, for example, replacing two vegetal cells of a blastula derived from a UV-irradiated egg, one that would on its own develop only ventral structures. From these grafted embryos arise well-rescued tadpoles, with nearly complete body axes containing normal dorsal structures of the head, trunk and tail. When graft cells are preloaded with a fluorescent lineage tracer, we see that progeny cells of the graft do not populate the rescued embryo's dorsal structures, but only the ventral yolk mass. Graft cells induce neighboring equatorial cells of the host to develop the entire body axis. Gastrulation begins near the graft, and the dorsal midline of the rescued embryo is centered on the meridian of grafting (Gimlich & Gerhart, 1964; Gimlich, 1986).

Rescuing graft cells can only be obtained from positions close to the prospective dorsal midline of the donor, within 45° or less of the midline; cells from lateral or opposite vegetal positions do not rescue (Figure 6B). The effective donor region includes the two vegetal tiers of cells present at the 32- to 64-cell stage, that is, from the equator to the vegetal pole. The inductive strength of cells of the two tiers differs from embryo to embryo, perhaps because cleavage furrows are variably related to the locus of specialized cytoplasm (Gimlich, 1986). In the absence of other names, we call this the 'vegetal dorsalizing region', or the 'organizer inducing region', or the 'Nieuwkoop center'.

The normal embryo requires cortical rotation for this region's formation, and the region arises on the meridian of greatest cytoplasmic displacement, on the side where the core moves vegetally *vis à vis* the cortex. While any part of the VH is latently capable of forming a dorsalizing center, only one region actually does in normal development: that part affected by the single, unidirectional rotation. As discussed before, rotation may directly activate cytoplasm of the vegetal dorsalizing center, presumably bordering the shear zone or at new contact regions between the cortex and core, or it may activate materials of the animal hemisphere, which secondarily activate the vegetal center, as suggested by Cardellini (1988). If the latter, the steps of transfer are probably complete by the 8- to 16-cell stage.

The rest of the vegetal hemisphere, unaffected by rotation, also engages in an induction of the marginal zone, but this does not lead to organizer formation or to differentiation of dorsal mesoderm. It leads to the formation of the 'indifferent' or 'ventral' portion of the marginal zone. This type of ventralizing induction does not depend on rotation, for it occurs in embryos prevented from rotation by MT depolymerizing agents. As discussed elsewhere in this Volume (see chapters by Slack *et al.*; Smith *et al.*; Yisraeli *et al.*), the VH may exert its inductive effects by secreting growth-factor-like proteins. By these accounts, cortical rotation might be expected to enable cells of the vegetal dorsalizing region to specifically release a TGF-β homolog, whereas all vegetal cells even without rotation would release an FGF-like material. At this time we have no idea how cortical rotation might alter the secretory properties of cells derived from one region. Since TGF-β and FGF homologs are present as proteins even in the unfertilized egg (Kimelman *et al.* 1988; Slack & Isaacs, 1989; Dale *et al.* 1989; Tannahill & Melton, 1989), their release could involve the region-specific activation of a step of processing or externalization, rather than of transcription or translation.

As an aside, lithium ion deserves special mention because it obviates the need for cortical rotation and for a Nieuwkoop center. Eggs that have failed to rotate can be rescued to form near-normal embryos by an injection of lithium chloride into equatorial cells at the 16- to 32-cell stage (tier C; Busa & Gimlich, 1989). Uniform exposure of such eggs to lithium solutions leads to the development of cylindrically symmetric hyperdorsalized forms resembling D_2O embryos (Kao & Elinson, 1988). Apparently lithium acts not by stimulating vegetal cells to release organizer inducing (dorsalizing) factors, but by sensitizing animal hemisphere cells to

respond to the vegetal ventralizing inductors *as if* they were dorsalizing inductors, i.e. to an FGF homolog as if it were a TGF-β homolog (Nieuwkoop, 1970; Kao & Elinson, 1989; Slack *et al.* 1988; Cooke & Smith, 1988). Hence, the embryo circumvents the normal requirements for cortical rotation and the Nieuwkoop center. Given this interesting response to lithium ion, it seems plausible that the normal vegetal dorsalizing inductor, like lithium, just enhances the response of neighboring AH cells to the ubiquitous vegetal ventralizing inductor, and these responding cells become the organizer. In this regard, TGF-β1 is known to synergize the FGF-dependent response by explanted AH cells to produce dorsal mesoderm (Kimelman & Kirschner, 1987).

Rescue by cells of the Nieuwkoop center is often incomplete

Vegetal dorsalizing cells do not always promote complete rescue when grafted into UVed recipients. When the body axis is incomplete, it is always truncated from the anterior end, and the same scoring scale can be used as for embryos inhibited in cortical rotation (see Fig. 5 and 6B). Grafts with one, two, or four cells from the C and D tiers of the donor give increasingly frequent well-rescued embryos. Truncated embryos of the same types can also be produced from normal blastulae by eliminating cells from the Nieuwkoop center of the 32-cell blastula (Gimlich, 1986). The elimination of more cells leads to greater anterior defectiveness in the final embryos.

These results, while not well quantified yet, imply that later dorsal development depends systematically on the amount of Nieuwkoop center present in the blastula stage embryo. Since experimental perturbations of vegetal induction produce the same final body patterns as do perturbations of rotation, we suggest that the amount of rotation determines the amount of vegetal dorsalizing induction. With no rotation, there is no such induction. Intermediate rotation gives intermediate induction. Two opposite rotations lead to two opposite Nieuwkoop centers. And after D_2O treatment, most of the vegetal hemisphere becomes a fully active Nieuwkoop center. Presumably the amount of rotation determines the amount of secretion of a specific inductor protein from cells of the Nieuwkoop center. Despite this quantitative correspondence, however, we can't yet discern why the anterior end of the body axis is the end missing in deficient embryos.

The amount of organizer

By the end of the blastula period, the marginal zone is sufficiently patterned for the initiation of normal gastrulation. In *Xenopus*, the marginal zone's organizer region, which arises directly above the vegetal dorsalizing region, is approximately 60° wide; whereas the indifferent part of the marginal zone is 300° wide, arising above the non-Nieuwkoop part of the VH (Smith & Slack, 1983; Dale & Slack, 1987; Stewart & Gerhart, 1989).

The organizer can be reduced in size by surgery at the late blastula stage. This allows us to test the effects on development of subnormal amounts of organizer. As one way to do this, the late blastula (stage 9, approx. 15 000 cells, 30–60 min before blastopore formation) is cut vertically on the bilateral plane (i.e. in the animal–vegetal direction through the organizer region), and the left or right half is recombined with half an embryo of equal age, cut from an egg which had been UV-irradiated in the first cell cycle to prevent its rotation and to preclude its formation of a Nieuwkoop center. This latter half, lacking an organizer, would on its own be incapable of any dorsal development. Although such recombinates have each just half an organizer, they develop remarkably well and produce near-perfect bilateral embryos, in many cases with near-complete body axes containing normal dorsal structures of the head, trunk and tail. One side of the body is contributed by the would-be ventralized half, as shown by lineage tracing. Still, some recombinates give bilateral embryos with incomplete body axes (Fig. 6C), and when dorsal structures are missing, the body axis is truncated from the anterior end, just as we have seen before, and the same scoring system can be used.

In the same kind of operation, the organizer can be intentionally cut off center (e.g. 15° from the midline), to include a still smaller amount of organizer in the rescuing normal half embryo used to make a recombinate with a UVed half. When this is done, the recombinate tends to have still fewer anterior structures (Fig. 6C), though always in a bilateral arrangement to which the UVed half contributes one side. The relationship seems monotonic: the less organizer, the less anterior development.

Finally, when the cut departs 30° or more from the bilateral plane, the piece lacking the dorsal meridian has no capacity for rescue; the recombinate develops as a cylindrically symmetric ventralized embryo. The organizer, at least in its capacity to initiate dorsal axial development, apparently extends just 30° to either side of the dorsal meridian, i.e. it is 60° wide. The remaining marginal zone material of a normal late blastula resembles the marginal zone of a ventralized embryo, with no autonomy for dorsal development, even though this part is destined in normal development to produce the bulk of the mesodermal tissues of the embryo, namely, the somites, kidney and heart, in fact, almost all mesoderm except the notochord and some head mesenchyme. This indifferent region must interact with organizer cells during gastrulation to generate its substantial part of the normal embryo's axial organization. The major share of definitive embryonic organization, along both the dorsoventral and anteroposterior axes, must be established during and/or after gastrulation, as shown by the performance of UVed halves brought into contact with an organizer, and by the performance of normal indifferent marginal zone regions deprived of an organizer.

We can now extend our causal chain of quantitative relationships by one more step since progressive reductions in the amount of organizer material in the late blastula result in embryonic body axes progressively truncated from the anterior end. We suggest that the

amount of rotation defines the amount of Nieuwkoop center, which defines the amount of organizer. With no rotation, there is no vegetal dorsalizing induction and no organizer. Partial rotation leads to partial induction and a reduced organizer. However, despite our causal chain, we haven't answered why, when the organizer is reduced in size, the anterior end of the embryonic axis is missing.

Definitive axis formation

Gastrulation transforms egg organization into embryonic organization. In this process, cells of the marginal zone generate the dorsoventral and anteroposterior dimensions of the body axis as the result of their migration, repacking and manifold interactions (Keller & Danilchik, 1988; Wilson & Keller, 1989). In undisturbed development, the first cells to gastrulate are those of the organizer region, which comprises the 'dorsal lip' of the blastopore. Cells of the organizer region are special in their ability, as a population, to converge toward the prospective dorsal midline and to extend in the anteroposterior direction. They induce neighboring cells of the indifferent marginal zone to undertake similar movements and they induce ectodermal cells to undertake neurulation. Eventually they contribute to dorsoanterior elements of the body axis, such as pharyngeal endoderm, head mesenchyme, archenteron roof, notoplate and notochord (see Gerhart & Keller, 1986, for a review).

Because organizer cells are so special, we initially assumed that they are fully determined at the start of gastrulation, not only for their gastrulation movements and induction abilities, but also for their ultimate cell type differentiations. Fate maps show that the first invaginated cells of the organizer tend to populate the most anterior parts of the body, and later ones contribute more posteriorally. The organizer might have within it a cryptic anteroposterior and a dorsoventral pattern of cellular differences, a pattern that would propagate to the rest of the embryo during morphogenesis. By this view, the organizer would itself be already fully organized at the start of gastrulation, a miniature mosaic of the future embryo's axial organization. In many respects, this interpretation could explain the series of systematically truncated embryos produced by our experimental interventions just described. For example, reduced rotation and reduced vegetal induction of the organizer might just lead to the formation of an organizer lacking the most anterior–dorsal parts of its cryptic pattern, if we assume that qualitative differences within the organizer originate from quantitative differences set up by vegetal induction. In such manipulated embryos, gastrulation does in fact start later, and cells accomplish less convergence and extension. Thus, we thought it plausible that the diminished organizer just lacked cells for organizing the most anterior dorsal parts of the body axis. Of course, it must be admitted that the results on reducing the size of the organizer by surgery at the start of gastrulation would not readily fit this interpretation.

The situation is considerably more subtle, though, as shown by the results of experiments in which embryos are allowed to develop normally up to and into the gastrula stage (so as to have a well-organized organizer), and then at various times are prevented from continuing gastrulation. Inhibitors of *Xenopus* gastrulation are not easy to find: there is no significant effect from RGD peptides, *p*-nitrophenyl-xylosides, or β-aminopropionitrile, agents that arrest gastrulation in sea urchins (Wessel & McClay, 1987; Lane & Solursh, 1988) and *Pleurodeles* (Boucaut *et al.* 1984). However, polysulfonated compounds such trypan blue and suramin (germanin) are very effective (Waddington & Perry, 1956). These are powerful teratogens in mammals, acting at the primitive streak stage; they animalize sea urchins by arresting gastrulation (Beck & Lloyd, 1966). Suramin disrupts the binding of protein ligands to cell surface receptors, the examples including growth factors (FGF and TGF-β, Coffey *et al.* 1987), vitellogenin, and LDL (Peacock *et al.* 1988). High doses of trypan blue or suramin (20 μM in the blastocoel) stop convergent extension movements *in vivo*, though allowing the blastopore to close. The agents effectively arrest these same movements of cells in explanted preparations of the organizer (Danilchik *et al.* 1989).

The clearest demonstration of their systematic effect on axis formation comes when these agents are injected into the blastocoel *at different times* during the 6 h period of gastrulation (stages 10 to 13): embryos thereafter develop body axes truncated at different anteroposterior levels depending on the time of injection (Figure 7 and 8). If injected when the dorsal lip is first invaginating, the resulting embryo develops no head or trunk, and sometimes no tail as well (Fig. 8B). Somites and notochord are absent; the embryo resembles the cylindrically symmetric ventralized forms seen when rotation fails or when the Nieuwkoop center or organizer is ablated. If the agents are injected midway in gastrulation, embryos develop without heads, but with trunks and tails (Fig. 8A). Finally, if injected at the end of gastrulation, the embryo is completely unaffected, even though the agents are present throughout the periods of neurulation and cell differentiation. Thus, the polysulfonated agents are not indiscriminately toxic, but act in a stage-specific way during gastrulation, inhibiting some process perhaps unique to gastrulation, or most exaggerated or critical at that time.

Gastrulation is the latest stage at which we have been able to produce the familiar series of truncated anatomies for which we can use the same dorsoanterior index scoring system (Fig. 5). In preliminary experiments with injected fluorescent lineage tracers, we find that the leading cells of the dorsal marginal zone (the organizer region) differentiate into whatever parts of the body axis are most anterior in the truncated embryo. Also, in preliminary experiments, the extent of gastrulation (the length of the archenteron) correlates with the completeness of the body axis. In contradiction to the expectations of the miniature mosaic model, organizer cells do not seem to have a fixed fate at the start of gastrulation but seem to undergo a progressive dorsoanteriorization in relation to the time

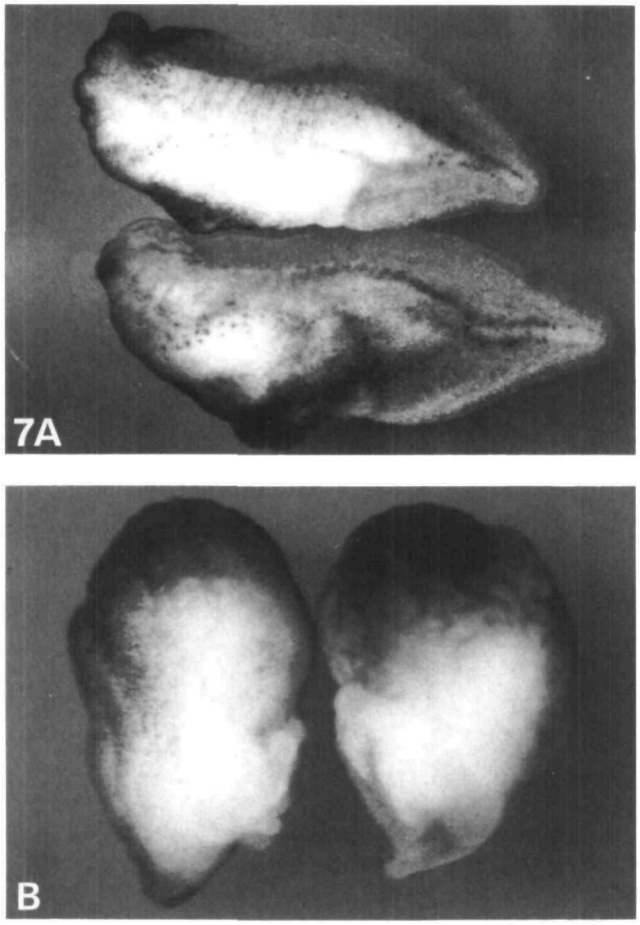

Fig. 7. Embryos blocked in gastrulation by polysulfonated compounds develop truncated body axes similar to those developed by UV-irradiated eggs. Eggs were allowed to develop normally until the gastrula stage, and were then injected with trypan blue (TB; 40 μM stock, 30 nl injected in the blastocoel). Panel A: Two grade 2 embryos at the equivalent of stage 32 are shown. The lower one is from a midgastrula (stage 11) injected with TB, the upper from a UVed egg. Panel B: Two grade 0 to 1 embryos. The left embryo is from a UVed egg and the right, from an early gastrula (stage 10) injected with TB. The similarities allow us to use the DAI scoring scale (Fig. 5) for TB-injected embryos.

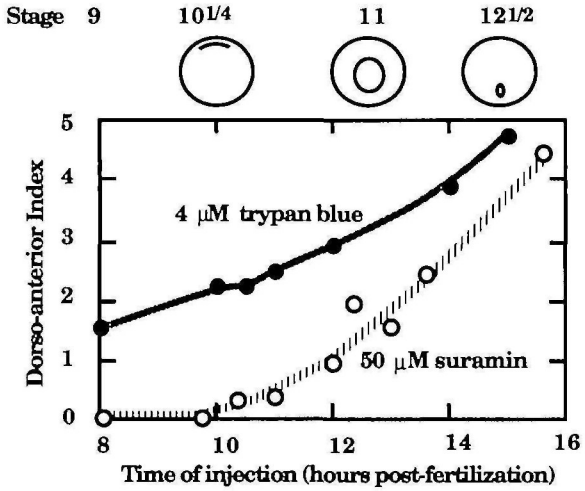

Fig. 8. The later gastrulation is blocked, the more anteriorly complete is the embryo's body axis. Eggs developed normally until the late blastula stage (8 h postfertilization, approx. 10 000 cells) and thereafter were each injected at various times with 30 nl of stock solutions of either 40 μM-trypan blue or 500 μM-suramin, delivered into the blastocoel. Estimated intrablastocoelic concentrations of the agents are indicated on the figure. Suramin (at a high dose) abruptly stops convergent extension movements, although the blastopore succeeds in closing. Trypan (at an intermediate dose) reduces but does not stop these movements. When control embryos reached stage 35–40, experimental embryos were scored. The dorsoanterior index for the treated populations (10 embryos averaged per data point) is shown on the vertical scale, graphed against the time of injection. Note that at stage 10, the embryos are still sensitive to the agents but by stage 12½ to 13 (the start of neurulation), they are completely resistant. The sensitive period to these agents falls exactly in the period of gastrulation. During gastrulation (hours 10–16), the later the injection, the more anterior will be the completeness of the eventual body axis. Across the top of the figure are shown vegetal views of embryos at the stage injected, to indicate the progress of blastopore closure.

and/or extent of morphogenesis (Gerhart *et al.* 1984). At the start of gastrulation, organizer cells seem determined only in their potentiality for starting a series of steps leading eventually to anterior dorsal development, steps that can be inhibited experimentally by polysulfonated compounds. If the steps are not completed, the potential is not fulfilled and the cells differentiate as posterior ventral tissues.

As further evidence that cell fates change during gastrulation, we find that trypan blue and suramin, injected at the gastrula stage, can even reverse the dorsoanteriorizing effects of lithium ion (applied at the 64- to 128-cell stage). Doubly treated embryos develop trunks and tails, and usually not heads; whereas with lithium alone they develop heads, but not trunks and tails (Doniach *et al.* 1989).

In comparing embryos from all our different stage-specific interferences with development, we see that the completeness of the embryonic body axis always correlates well with the extent of the morphogenetic movements of gastrulation; anteriormost parts of the axis emerge only when morphogenesis is most extensive, as diagrammed in Fig. 9. This is true no matter whether gastrulation is incomplete because it starts late and stops on time (as is the case with rotation-deficient embryos), or because it starts on time but ends early (as in the case with trypan and suramin-injected embryos). Gastrulation itself is the last developmental process that we can connect quantitatively to the amount of cortical rotation in the first cell cycle. During gastrulation, quantity seems finally converted to quality.

The organizer of the early gastrula is probably *not* a mosaic of terminally determined cell types. It may instead be a mosaic of cells of different potentials for arriving at the external and/or internal conditions needed for the differentiation of the different antero-posterior cell types predicted by fate maps. Cells of high

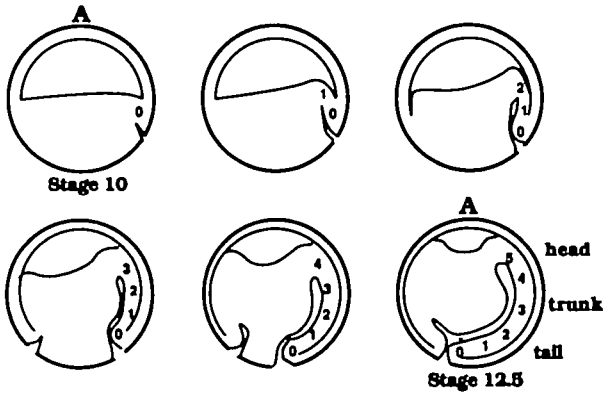

Fig. 9. Progressive anteriorization of the embryonic body axis during *Xenopus* gastrulation. In all our experimentally perturbed embryos (excluding D_2O effects), the truncation level of the final body is related to the extent of gastrulation movements, regardless of whether gastrulation starts normally at stage 10 but is then ended at different times (by trypan blue or suramin), or whether gastrulation starts later (due to reduced amounts of organizer in the marginal zone, due to inhibited rotation, or reduced numbers of vegetal dorsalizing cells, or surgical removal of parts of the organizer), while ending at the same time (probably stage 13).

The six panels are schematic cross-sections of embryos, showing different stages of the 6 h period of gastrulation, from stage 10 (upper left) and to stage 12·5–13 (lower right). The animal pole (A) is at the top of all figures. The blastocoel is the space in the animal hemisphere. At the start of gastrulation, some cells of the organizer part of the marginal zone (right subequatorial level) begin invaginating first, followed in sequence by cells more distant laterally and vertically. The '0' in the upper left section indicates that cells of the organizer, if their morphogenesis were stopped at that point, would differentiate ventral structures, like the parts of a grade 0 embryo (see Fig. 5). In the top center panel, leading cells of the organizer have moved farther inward, followed by dorsal marginal zone cells which were initially farther from the blastopore. The leading cells are now in a condition to differentiate as posterior dorsal structures, which they would do if gastrulation were stopped at this point, while cells behind them have just reached the '0' condition. These conditions, which may reflect internal or external (or both) states of the cells, change progressively with the time and/or distance of morphogenesis (see subsequent panels). Leading cells are unique in that they have the potential for progressing through the full range of conditions, finally reaching condition '5' (lower right) sufficient for anterior dorsal differentiation. Cells farther back in the migration series make less progress, because they start later and are less active. While cells of the marginal zone would be initially graded in their potential for progress toward anterior dorsal development, all would need to accomplish extensive morphogenesis to fulfill this potential. Thus, inhibitors of morphogenesis always have a posteroventralizing effect on the final pattern.

potential would initiate morphogenesis earlier and more vigorously than those of low potential, the latter not having the option to progress very far in the series of conditions for dorsoanterior differentiation; they would be limited to producing posterior parts of the pattern, although even in this they would fail if morphogenesis were inhibited.

Why is the extent of gastrulation related to the anterior completeness of the axial pattern?

This inquiry dates back to Spemann (1938) and Vogt who considered the connection between dynamic determination (a cell's morphogenetic behavior at gastrulation) and its material determination (its final cell type differentiation). We don't know the answer, but have considered two possibilities, for each of which we will give an example:

(1) Morphogenesis does not itself accomplish patterning but creates conditions upon which a separate patterning process operates. This possibility is inherent in the double gradient idea of Saxen and Toivonen (1961). All cells would have an intrinsic capacity for anterior differentiation, but many are suppressed by a posteriorizing (or caudalizing or tail-inducing) center located in those regions of the marginal zone gastrulating last. Suppression, and therefore fates, would be graded with distance from the center: the closest cells becoming tail, and more distant ones, trunk. Only the earliest, farthest advancing marginal zone cells and the most distant neurectoderm would escape the effects of the center and would become anterior. According to this view, if we inhibit gastrulation movements by any means, we should expect to prevent cells from escaping the posteriorizing center, i.e. from acquiring the external conditions needed for their anterior differentiation. The head end of the body axis would be deleted and the completeness of the axial pattern would be quantitatively related to morphogenesis, just as we find.

(2) Morphogenesis and axial patterning may depend jointly on yet another agency such as intercellular signalling. This possibility resembles ones for cell aggregation and slug migration in *Dictyostelium* (see chapter by Johnson *et al.* this Volume). At the start of gastrulation, organizer cells might be unique in their high intensity of intercellular signalling, due to prior stimulation from the Nieuwkoop center. Such a cell might have three responses to signals: to move, to emit more signal, and to keep a running sum of the amount of received signal, a sum that would determine the cell's anteroposterior–dorsoventral fate. If the response duration were short, repeated signal reception would be needed by cells to keep signalling and responding. Cell fate would change during gastrulation in concert with movement, both depending on continued intercellular signalling. The earliest invaginating cells would have the greatest potential for movement and anterior fates since they could maintain the longest period of signalling. Non-organizer cells of the marginal zone might receive enough signals from organizer cells to begin signalling, moving, and changing fates. Signalling could propagate through the marginal zone and neurectoderm. By this view, polysulfonated inhibitors of gastrulation would be inhibitors of signalling; they would terminate both morphogenesis and progress towards anterior fates since these jointly depend on continued signalling. Parenthetically, another agent that affects

signal transduction, namely lithium ion, also reduces morphogenetic movements and gives headless embryos, if applied in the gastrula stage (Hall, 1942; J. Slack, personal communication; an effect not to be confused with hyperdorsalization by treatment of the blastula). By this view, reduced rotation and vegetal induction would lead to a diminished organizer, one with a lowered level of signalling, and morphogenesis and anterior patterning would consequently diminish in concert.

These rather different interpretations have equal plausibility at present. Both confer to gastrulation, as a process, an inherent dynamic organization by which anterior dorsal fates arise in relation to the increasing quantity of morphogenesis, and both give to the early steps of development, such as cortical rotation and vegetal induction, the significance of setting the initial conditions of gastrulation, conditions that may limit its operation.

This work was supported by USPHS grant GM 19363 and NSF grant DCB-8517548.

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