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Experimental analyses of cytoplasmic rearrangements which follow fertilization and accompany symmetrization of inverted *Xenopus* eggs

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SUMMARY

Cytoplasmic rearrangements which follow fertilization were monitored in inverted eggs. A set of yolk compartments was resolved by cytological analyses of both normally oriented and inverted eggs. Those compartments were characterized by their yolk platelet compositions and movement during egg inversion. In addition to the major yolk masses which contain either small, intermediate or large platelets, minor cytoplasmic compartments which line the egg cortex were also identified.

During egg inversion the yolk compartments shift. Those yolk mass shifts occurred only after the inverted egg was activated (by sperm, electrical or cold shock). The direction of shift of the major yolk components, rather than the sperm entrance site (as in normal orientation eggs), determines the dorsal/ventral polarity of the inverted egg. Among different spawnings the rate of shift varied. Eggs that displayed the fastest rate of shift exhibited the highest frequency of developmental abnormalities during organogenesis.

Isopycnic density gradient analysis of yolk platelets and blastula blastomeres showed that isolated yolk platelets and mid-blastula blastomeres are not of uniform buoyant density. Three major yolk platelet density bands were resolved. Large, intermediate, and small yolk platelets were found in all bands. The high density band had the largest proportion of the large yolk platelets and the low density fraction showed the largest proportion of the small yolk platelets.

Interpretation of novel observations on cytoplasmic organization provided criticisms of some earlier models. A new 'Density Compartment Model' was developed and presented as a coherent way to view the organization of the egg cytoplasm and the development of bilateral symmetry.

INTRODUCTION

Amphibian eggs display a distinct and easily recognized animal/vegetal (A/V) polarity. It is visible externally as the non-uniform distribution of surface pigments. The animal hemisphere, which normally opposes gravity in fertile

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eggs, is darkly pigmented. The vegetal hemisphere, which faces gravity, is almost devoid of pigment. Internally the polarity is most profoundly manifested in yolk platelet distributions. The animal hemisphere typically contains smaller-sized yolk platelets while the vegetal hemisphere is usually densely packed with relatively larger-sized platelets. Those features of amphibian egg polarity have been studied extensively since the earliest days of experimental embryology. Consequently, substantial background literature on many of the more pronounced cytological characteristics of egg polarity is available (see reviews by Pasteels, 1964; Nieuwkoop, 1977; Brachet, 1977; Gerhart, 1980; Malacinski, 1983).

Other components, such as oogenetically derived mRNA or regulatory molecules, may also be distributed in non-uniform patterns along the A/V axis. Although preliminary reports (Carpenter & Klein, 1982; Sagata, Okuyama & Yamana, 1981; Capco & Jeffery, 1981) suggest these possibilities, no definitive evidence exists.

Following fertilization, many of the major egg cytoplasmic components undergo a spatial rearrangement. The surface pigmentation pattern, for example, is altered. In anuran eggs (e.g. *Xenopus*, *Rana*) a contraction toward the animal pole occurs. That contraction involves the cortex and displaces the pigment granules associated with it (Elinson, 1975; Stewart-Savage & Grey, 1982). Some of the surface pigment eventually becomes concentrated around the sperm entrance site (Palecek, Ubbels & Rzehak, 1978), marking the future ventral side of the egg. The opposite side of the egg becomes less pigmented, and prior to the first cleavage a lightly pigmented 'gray crescent' area can often be observed (see Malacinski, Benford & Chung, 1975, for photographs).

Internally, rearrangements also follow fertilization. Some of the vegetal hemisphere's larger yolk platelets opposite the sperm entrance site appear to shift into the animal hemisphere to line the subcortical area of the dorsal side of the egg. That area has been termed the 'vitelline wall' (Pasteels, 1964) and has been implicated as a causative agent in the establishment of the dorsal/ventral (D/V) polarity of the egg (Pasteels, 1964; Gerhart *et al.* 1981). In the animal hemisphere a set of astral rays radiate from the sperm pronucleus and eventually extend throughout much of the animal hemisphere (Stewart-Savage & Grey, 1982; Ubbels, Hara, Koster & Kirschner, 1983). Concomitantly a patch of animal hemisphere cytoplasm which is relatively yolk free – the so-called 'dorsal yolk-free cytoplasm' – moves toward the dorsal side (Klag & Ubbels, 1975; Herkovits & Ubbels, 1979; Ubbels *et al.* 1983).

Which, if any, of those cytoplasmic rearrangements are prerequisites for normal pattern specification? Recently 'dorsal yolk free cytoplasm' movement has been dissociated from dorsalization (Ubbels *et al.* 1983). Although the results of artificial activation, manipulations, and the use of metabolic inhibitors suggest astral rays may be required for normal development (Ubbels *et al.* 1983), no direct tests of the requirement of astral ray growth has been devised. Likewise

no direct test of the requirements of vitelline wall formation of egg polarization has been provided. Furthermore, since those internal rearrangements are very subtle and, as will be documented in the present report, display considerable variation from one spawning to another, they are difficult to analyse.

To determine which of the internal rearrangements are associated with pattern formation, inverted rather than normally oriented eggs were chosen as a model system. Preliminary evidence (Radice, Neff & Malacinski, 1981; Neff & Malacinski, 1982) indicated that inverted eggs display a dramatic rearrangement of cytoplasmic components. In contrast to normally oriented eggs, the internal rearrangements can easily be tracked, and in several instances rearrangements could be correlated with specific pattern reversal elements (Chung & Malacinski, 1983).

Detailed analyses of several features of those inverted egg cytoplasmic rearrangements are presented in this report. The data provide novel insights into the manner in which the egg cytoplasm is organized, and led to the formulation of a 'density compartment' model to explain how cytoplasmic rearrangements specify pattern.

MATERIALS AND METHODS

Source of eggs and egg inversion

The procedures employed for artificial insemination and egg inversion are described in other recent publications from our laboratory (Neff *et al.* 1983; Chung & Malacinski, 1982) with the following modifications. Eggs were oriented with forceps before fertilization on 35 mm plastic (Falcon) petri dishes. Eggs that were inverted after natural rotation were initially oriented with their vegetal hemispheres facing gravity. After natural rotation the eggs were immobilized with 20 % (w/v) Ficoll (Type 400, Sigma, St. Louis, MO.) in 10 % Steinberg's solution (pH 7·35). At the appropriate time the dishes were inverted. The 20 % Ficoll solution was replaced with 10 % Steinberg's solution at the beginning of first cleavage. Eggs were cultured at 15 °C and staged according to the fraction of the interval between fertilization (T = 0) and first cleavage (T = 1·0) at the time of the relevant manipulation and observation.

Microscopy

Eggs were fixed and embedded as described previously (Neff *et al.* 1983). Before polymerization, eggs were oriented in embedding moulds so that the plane of sections would run parallel to or along the plane of bilateral symmetry. That plane is delineated by the sperm entrance point and the central point of the animal hemisphere. At first cleavage the furrow usually passes through these two points. Using a Porter-Blum ultramicrotome (MT-1) equipped with a glass knife, 0·5 μm or 1·0 μm sections were prepared. They were mounted semiserially on subbed slides and stained with toluidine blue.

The remaining halves of incompletely sectioned normal or inverted eggs which remained in epoxy-resin blocks were used for making macroscopical observations on the distribution and displacement of internal pigment granules. Likewise, observations on pigment displacement were made on intact, fixed, but unembedded eggs which were dehydrated and cleared in benzyl benzoate.

To differentiate between yolk compartments occupied by different types of yolk platelets and to localize the germ plasm, eggs were embedded in hydroxyl ethyl methacrylate embedding medium (Sorvall), serial sectioned (4 μm), and stained using a modified Heidenhain's azan stain (Clark, 1973). A 70 % ethanol destaining step was added and the staining times modified to stain the large yolk platelets red and the small and intermediate yolk platelets, the nuclei, and the germ plasm blue.

Yolk platelet isolation

The density of yolk platelets from unfertilized, chemically dejellied (1·75 % cysteine-HCl, pH 7·6) eggs were characterized on 32 % to 45 % metrizamide (Sigma, St. Louis) continuous density gradients (Radice *et al.* 1981). After centrifugation at 30 000 *g* (90 min), the bands were collected by piercing the sides of the centrifuge tube. They were diluted by 1:4 with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (Dulbecco & Vogt, 1954). The percent of yolk platelets in the following three size categories was determined for each band with an ocular reticule at 800× magnification: large (>8 μm long axis), intermediate (4–8 μm), and small (<4 μm).

Blastomere isolation

Dejellied morulae and early blastulae were dissociated in their vitelline membranes (0·75 % phosphate-buffered saline without Ca^{2+} and Mg^{2+}, pH 7·4). The blastomeres were isolated on a 45 % to 95 % Percoll (Pharmacia) continuous gradient at 15 °C (Neff & Malacinski, 1982).

Artificial activation – electrical activation

Freshly spawned eggs were manually oriented with their vegetal hemispheres facing gravity and then activated by a single 79 V pulse (1 to 2 s) in 100 % Steinberg's solution (pH 7·5).

Cold shock activation

Freshly spawned eggs were cold shocked on ice (0 °C) in either 100 % Steinberg's solution (pH 7·5) or 20 % Ficoll in 10 % Steinberg's solution (pH 7·5) for 5 to 30 min, depending on the sensitivity of the batch of eggs, and then incubated at 15 °C in 10 % Steinberg's solution. Under these conditions 100 % of the eggs showed evidence of activation such as animal hemisphere pigment pattern change (loss of maturation spot), egg rotation, and aborted cell cleavage.

RESULTS

Observations on normal orientation eggs

Brief review of the internal cytology of normally oriented eggs

As a starting point for experimental analyses, a general description of the changes in the cytology of naturally oriented *Xenopus* eggs between fertilization and first cleavage was carried out. In many instances data were collected which confirmed the observations reported previously by other workers. Several of the more recent earlier studies will, therefore, be cited directly in the foregoing section. In other cases, additions to the previous accounts were formulated and they will be described in detail.

Immediately after sperm penetration eggs were fixed, sectioned and examined. Fig. 1A displays a typical cytological section. Yolk platelets are arranged in three major zones or masses. The following criteria were used to define these yolk masses; (1) predominant yolk platelet size, (2) ease of recognition in cytological sections, and (3) behaviour in inverted eggs (see following section). The large yolk mass (LYM) occupied by large yolk platelets (8–15 μm; largest diameter) is located in the vegetal hemisphere. The intermediate yolk mass (IYM) occupied by intermediate platelets (4–8 μm) is centrally located in the marginal zone. The small yolk mass (SYM) occupied by small platelets (<4 μm) takes up a major portion of the animal hemisphere. Subdivision into more than

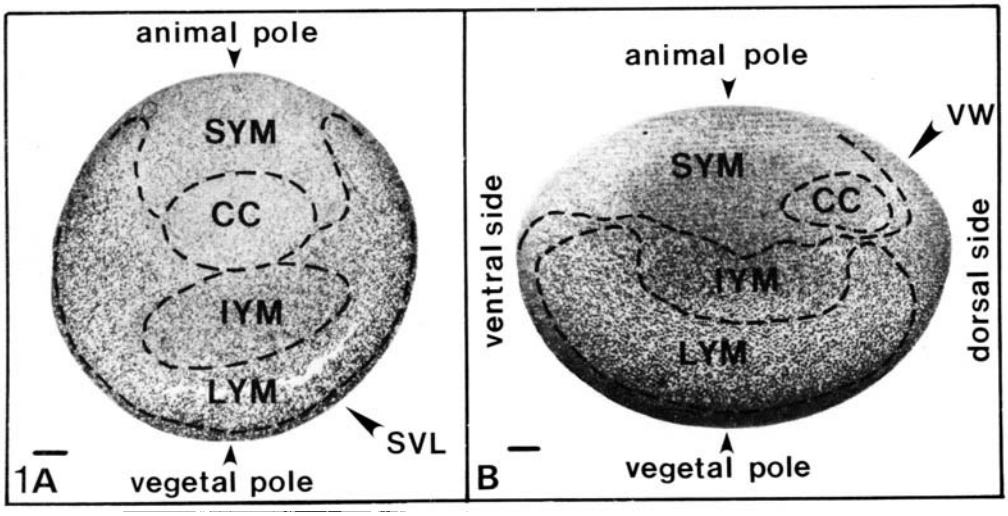


Fig. 1. Histological section (0·5 μm) of *Xenopus* eggs. (A) Typical egg immediately after fertilization (T = 0·07). (B) Section (0·5 μm) of egg at T = 1·0 (first cleavage). This egg displayed a vitelline wall on the side of the egg opposite the sperm entrance site (future dorsal side), and the clear cytoplasm towards the dorsal side. Abbreviations: CC = clear cytoplasm; LYM = large yolk mass; IYM = intermediate yolk mass; SYM = small yolk mass; SVL = subcortical vitelline layer; VW = vitelline wall. Magnification bars = 100 μm.

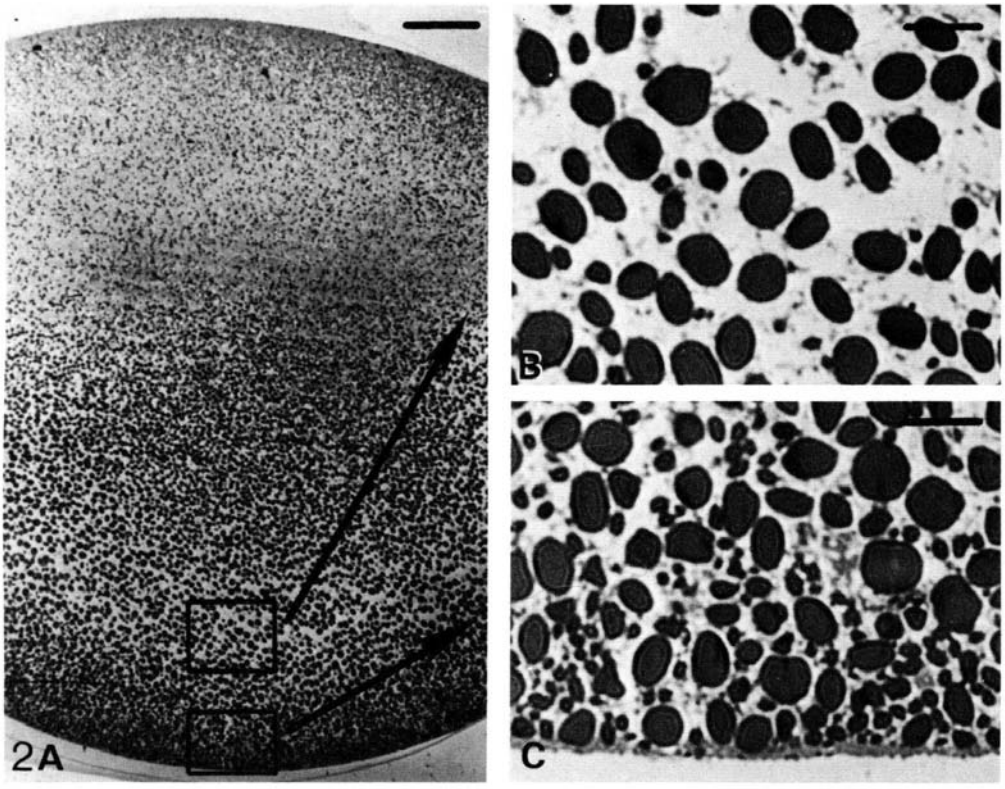


Fig. 2. 0·5 μm sections through naturally oriented egg at T = 1·0. (A) Low magnification (×100) view shows distribution of small, intermediate, and large yolk platelets. (B) Higher magnification view (×500) shows that major proportion of platelets in the LYM are relatively large. (C) High magnification (×500) view shows the 'subcortical vitelline layer' (SVL). It is characterized by the presence of small yolk platelets, proximity to the egg cortex, and location of germ plasm (see Fig. 7). Bars (A) = 100 μm, (B) and (C) = 200 μm.

three zones is possible. Harris (1964) denoted four yolk zones for axolotl eggs, Dorfman & Cherdantsev (1977) charted eleven yolk zones for *Rana* eggs, and Nieuwkoop (1956) described up to seven zones for *Xenopus* eggs. In the present studies, however, three major zones were easily recognized and they will be discussed when describing yolk platelet rearrangement. An area which is relatively free of yolk platelets – the 'clear cytoplasm' (Ubbels, 1977) was also recognized, and is labelled in Fig. 1A. The relative sizes of those various zones as well as their spatial distributions varied somewhat among the eggs of different spawnings.

In addition to those four major cytoplasmic zones, a minor yolk zone – heretofore not described – lines the vegetal cortex. It resides between the area occupied by the large yolk platelet mass and the cortex and is distinguished by the presence of substantial numbers of both intermediate and small-size platelets in addition to large-size platelets. That 'subcortical vitelline layer' (SVL) is

approximately 30–60 μm deep and extends from the vegetal pole approximately up to the equator (Fig. 1A, and B; Fig. 2A, B and C). The SVL may actually extend into part of the animal hemisphere (see below). The germ plasm resides in the vegetal hemisphere SVL (Fig. 7A). A distinction between the three major zones and the SVL is most easily observed in histological sections embedded in plastic methacrylate and stained with Heidenhain's azan. The large platelets stain red while the germ plasm, small, and intermediate platelets stain blue.

Those five major zones of cytoplasm can routinely be observed in sections of naturally oriented *Xenopus* eggs. Further characterization of those yolk zones, together with observations on two additional zones emerged during the course of the studies on inverted eggs (below).

At approximately T = 0·25 some eggs display an upward displacement of large yolk platelets in the subcortical region of the egg on the side opposite the sperm entrance site. At this relatively early time the upward displacement of yolk can only be seen in sections which pass either directly through or closely parallel to the sperm entrance site. That upward displacement of yolk probably represents the initial stage of 'vitelline wall' formation. By T = 0·50 further displacement toward the animal hemisphere of the large yolk platelets is observed. By T = 0·75 the formation of the vitelline wall is virtually complete (Fig. 1B). The so-called 'vitelline wall' is not a rigid, well defined structure as the term originally coined by Pasteels ('mur vitellin'; Pasteels, 1964) might imply. Rather, it appears to represent an extension of the large yolk platelet mass from the vegetal hemisphere into the animal hemisphere. Although it consists predominantly of large platelets, it also contains a significant proportion of intermediate and small platelets.

It should, however, be pointed out that a distinct vitelline wall, 180° opposite the sperm entrance site, is not a regular feature of fertile *Xenopus* eggs: First, 0·5 μm and 4 μm plastic sections through the sperm entrance site of T = 0·5 and T = 0·75 eggs or parallel to the cleavage furrow of T = 1·0 eggs from several females were carefully examined for the presence of the vitelline wall. Only approximately two thirds of those eggs displayed a distinct vitelline wall. Second, the size of the vitelline wall varied substantially, even among the eggs of a single spawning; and third, in some eggs from certain spawnings no vitelline wall was detected.

Buoyant density analysis of yolk platelets and individual blastomeres

The general stratification of yolk platelets along the A/V axis shown in Fig. 1A is largely maintained throughout early embryogenesis in naturally oriented eggs. That could be a reflection, at least in part, of different buoyant densities of various size yolk platelets.

To investigate that possibility, yolk platelets were gently isolated from uncleaved eggs and centrifuged in a continuous 32–45 % metrizamide gradient. The results indicate that yolk platelets do not display a uniform density (Fig. 3).

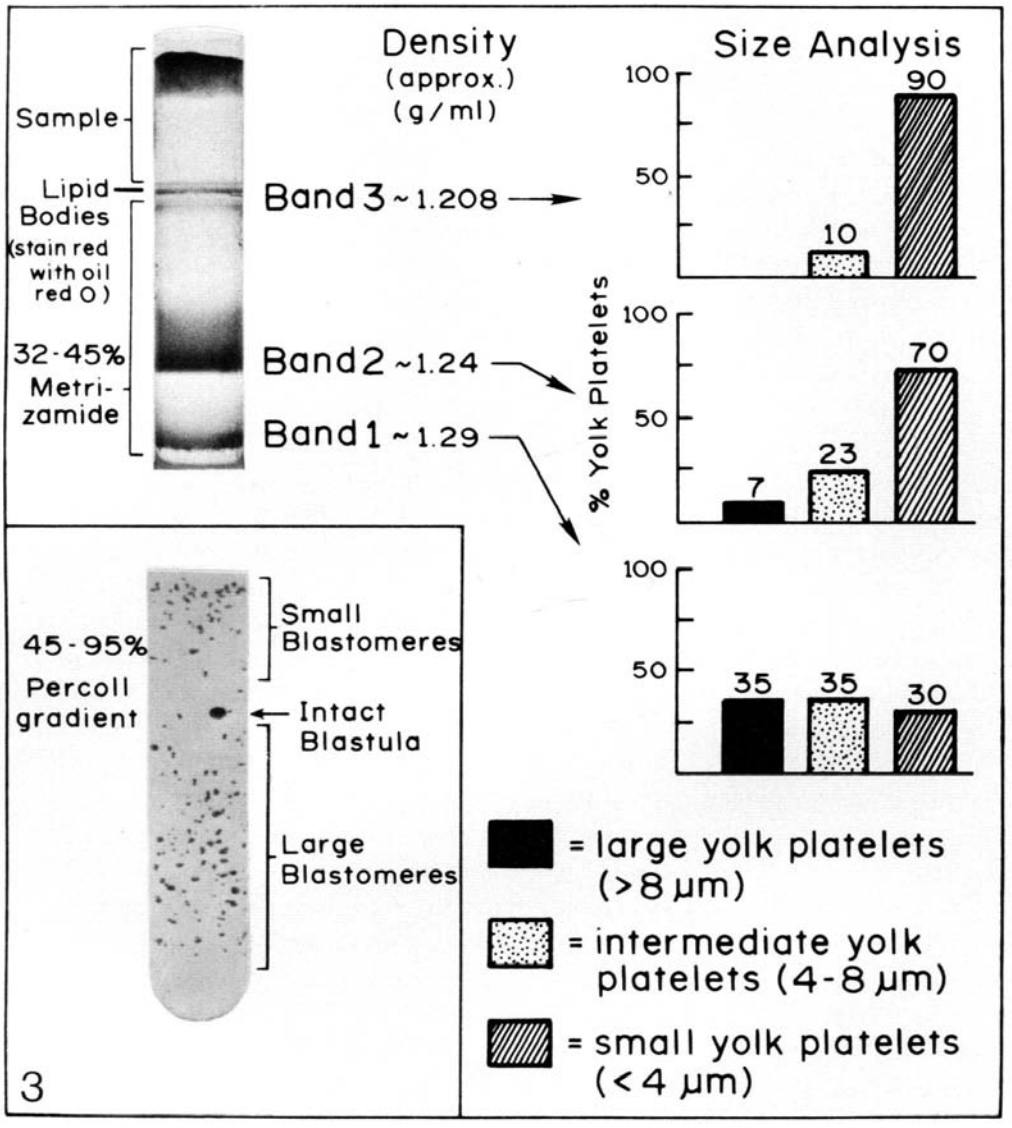


Fig. 3. Continuous 32–45 % metrizamide gradient of unfertilized *Xenopus* egg cytoplasm. Bands represent major density classes of yolk platelets derived from an 8 egg sample. Size distributions of platelets in each band are shown in bar graphs. The photograph of the Percoll gradient (Neff & Malacinski, 1982), in the insert, displays the distribution of individual blastomeres which were dissociated from 5 mid-blastula embryos.

At least three density classes can be resolved on isopycnic gradients. Microscopic examination of samples of each of the three major density classes revealed that although the most-dense class (Band 1 – approximately 1·29 g/ml) contains more larger size platelets than the other classes (Bands 2 and 3), significant numbers (65 %) of the dense platelets are of either small or intermediate size. Those

observations present the possibility that the small- and intermediate-size platelets observed in the SVL may be just as dense as the adjacent larger platelets of the vegetal hemisphere.

When mid-blastula-stage embryos were dissociated into single cells and run on a 45–95 % Percoll (Pharmacia) gradient a stratification of blastomeres was observed. Smaller animal hemisphere blastomeres were less dense than the larger vegetal hemisphere blastomeres. The whole embryo was of intermediate density (Fig. 3). The animal/vegetal distribution of small (usually less dense) to large (more dense) yolk platelets determines the blastomere yolk platelet composition and is most likely responsible for the intrinsic buoyant density of individual blastomeres of the embryo.

Observations on inverted eggs

Dorsal/ventral polarity in inverted eggs

In normally oriented *Xenopus* eggs the dorsal side (involution site) usually forms opposite the side the sperm entered (Palecek *et al.* 1978). In eggs that are rotated 90 ° off-axis that relationship between sperm entrance site and dorsal side is altered. The dorsal side forms on the side which opposed gravity during the rotation period (Scharf & Gerhart, 1980; Chung & Malacinski, 1981). Gravity orientation appears, therefore, to influence the establishment of dorsal/ventral polarity, at least in rotated eggs.

In order to study D/V polarity in inverted embryos, fertile eggs were inverted

Female no.	Time inverted	Location of sperm entrance site / direction of tilt			Involution site			
								
135	T=0·25	13	2	3	18	0	0	0
45	T=0·40	10	3	3	16	0	0	0
101	T=0·45	4	3	1	8	0	0	0
No. of eggs		27	8	7	42	0	0	0
%		64	19	17	100	0	0	0

Fig. 4. Location of sperm entrance site, direction of tilt, and position of involution site in eggs inverted after the natural rotation which follows activation. The location of the sperm entrance site is depicted on the down-tilt, up-tilt side, and perpendicular to the tilt of the inverted egg respectively. Virtually all eggs which are inverted after natural rotation display a 'tilt' to 165 °–175 ° off-axis, rather than remaining precisely 180 ° off-axis.

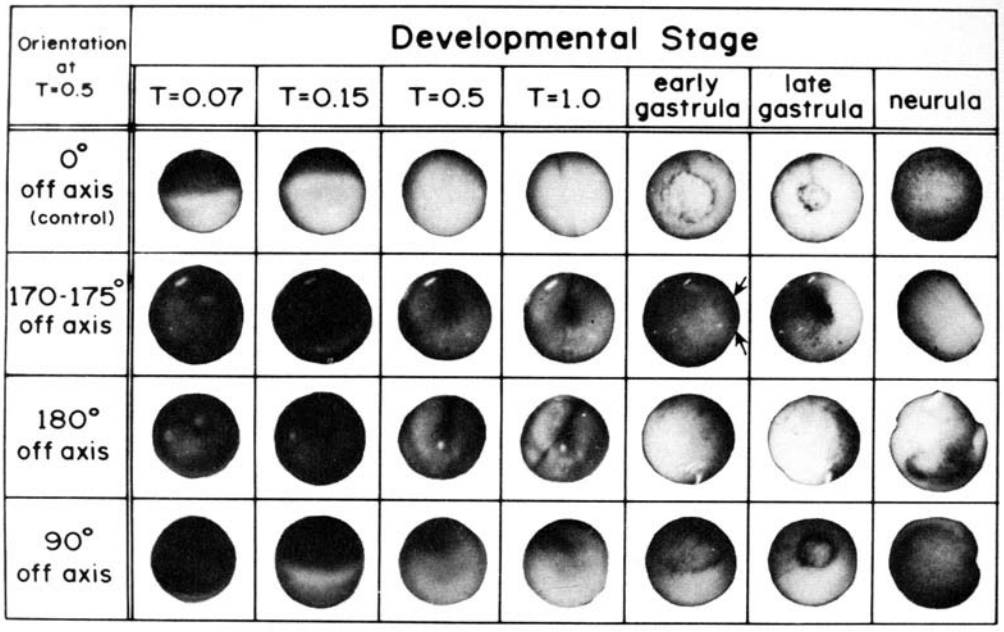


Fig. 5. Symmetrization in eggs inverted prior to fertilization. Photographs of hemisphere which faced gravity. Inverted eggs display coalescence of pigment prior to cleavage (see also Fig. 10). Involution site was on the 'down side' of the tilt in 170–175° off-axis egg (arrows). That embryo displayed complete reversal of surface pigmentation pattern at neurula stage so it is lightly pigmented, compared to control embryo. 180° off-axis egg exhibited abnormal involution site and developmental arrest at neurulation. Invagination of surface cells was often delayed in inverted eggs. Embryos which were 180° off-axis manifested more delay than 170–175° off-axis embryos. That delay can be observed in the above photographs (compare 170–175° embryo with 180° embryo). 90° off-axis egg exhibited partial reversal of pigmentation pattern at neurulation.

after they had undergone their natural rotation response to activation (T = 0·2). Those eggs offer one important advantage over eggs that are inverted prior to fertilization; they display a prominent sperm entrance site. Symmetrization in inverted eggs was monitored by scoring three parameters; location of the sperm entrance site, direction of tilt from 180° off-axis to approximately 165° off-axis, and location of involution site at gastrulation. Three important observations emerged from an analysis of the data included in Fig. 4. First, virtually all eggs that were inverted after fertilization displayed some tilt (egg's equator not horizontal). Curiously, the direction of tilt was not strictly controlled by the location of the sperm entrance point. Second, the dorsal/ventral polarity of the embryo was determined not by the sperm entrance site, but rather by the direction of tilt. It will be demonstrated later by the data in Figs 6, 8, and 12 that the direction of tilt determines the direction of a series of internal yolk shifts. Third, the involution site was always located in the original vegetal hemisphere or marginal zone.

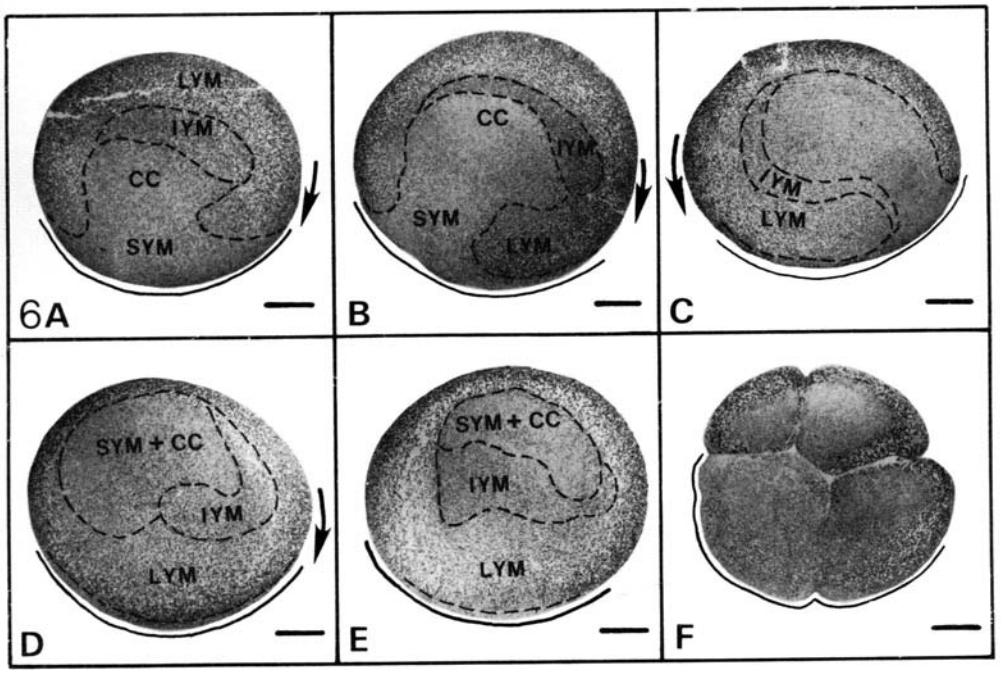


Fig. 6. Time course of the shift of major yolk masses in inverted eggs (0·5 μm sections). The surface pigmentation (melanin) of the egg's original animal hemisphere is indicated by the solid line. Dotted lines reveal the margins of various major yolk zones. (A) T = 0·07 egg. Beginning of displacement of yolk masses in direction of 'tilt' of the egg (arrow). (B) T = 0·25 egg. Further displacement of large yolk mass. Its leading edge is deflected away from the animal hemisphere cortex (pointer). (C) T = 0·50 egg. Direction of yolk shift always correlates with tilt orientation (Figs 4 and 5) of eggs. (D) T = 0·75 egg. The large yolk mass has completed its movement to the original animal hemisphere. It does not, however, make direct contact with the egg cortex. (E) T = 1·0 egg. Yolk shifts are complete. (F) 8-cell-stage embryo. The yolk distribution pattern observed at T = 1·0 persists through early cleavage. These particular eggs belong to the IMC class which is explained in Fig. 12. Bar = 200 μm.

A similar observation regarding the location of the involution site and the direction of tilt emerged from an analysis of symmetrization in eggs that were inverted prior to fertilization. Most of those eggs also displayed a small degree of tilt, so they were usually approximately 165° off-axis. Some eggs, however, remained precisely 180° inverted. A total of 32 eggs from four females were photographed from T = 0·1 to the gastrula stage. Fig. 5 contains a series of photographs of typical eggs that developed in normal, 90° off-axis and inverted orientations after T = 0·5. As was the case for the eggs described in Fig. 4, the involution site was always located on the 'down side' of the tilt. Eggs which remained in a 180° off-axis orientation either failed to involute, or displayed a substantial lag in morphogenesis. They always developed abnormally. That observation prompted an analysis of the internal cytology of inverted eggs. It will

be demonstrated later (see Fig. 8) that the series of internal yolk shifts in those 180° off-axis eggs is symmetrical, unlike the asymmetrical yolk shifts that will be described for 'tilted' (165–175° off-axis) eggs.

Cytology of inverted eggs

A preliminary report from this laboratory revealed that a major rearrangement of yolk platelets occurs in inverted eggs (Radice *et al.* 1981). Significantly, yolk platelets of inverted eggs appear to redistribute as discrete zones, with little mixing between, for example, the large and small yolk masses (Neff & Malacinski, 1982). The manner in which yolk platelets rearrange in inverted eggs was investigated in detail in an attempt to further understand the structural basis of the organization of the egg cytoplasm. As will be revealed below, close inspection of sections of inverted eggs provided new insights into the manner in which yolk platelets are organized. Furthermore, an explanation can be offered for the failure of 180° off-axis eggs to develop properly.

The time course of the shift of the major zones of cytoplasm in sections of inverted eggs is given in Fig. 6. At T = 0·07 the beginning of the yolk shift was observed (compare Fig. 6A with Fig. 1A). All three major yolk zones shift as coherent masses. The direction of the shift of the large yolk mass (arrows in Fig. 6) was always related to the tilt orientation of the egg. By T = 0·25 a distinct shift of the large and intermediate yolk masses in the direction of gravity was detected. The small yolk mass (including the 'clear cytoplasm') was displaced in the opposite direction (away from gravity). As can be seen in Fig. 6B (pointer), the leading edge of the large yolk mass appeared to be deflected away from the egg cortex during its downward shift. It will be demonstrated below (Fig. 7) that the animal hemisphere cortex is lined by an integral mass of yolk platelets, much the same way the vegetal hemisphere cortex is lined by the SVL. By T = 0·75 (Fig. 6D) the shift of most of the large yolk mass to the original animal hemisphere is complete, although direct contact between that large yolk mass and the animal hemisphere cortex still is not made. An approximately 50 μm deep zone of small and intermediate size yolk platelets similar to the SVL lines the animal hemisphere cortex. The movement of the small yolk mass and 'clear cytoplasm' was difficult to monitor in 0·5 μm plastic sections. In 8 μm paraffin sections, however, the 'clear cytoplasm' appeared to become localized in the geometric centre of the egg. It was usually surrounded by the small yolk mass.

At first cleavage (T = 1·0) the inverted egg cytoplasm (Fig. 6E) displays a superficial similarity to the yolk distribution pattern observed in the normal orientation egg illustrated in Fig. 1A. Close inspection of high magnification views of T = 1·0 eggs revealed, however, that the SVL remained localized adjacent to the cortex in the original vegetal hemisphere. The original animal hemisphere which now contains the large yolk mass remained lined with the small and intermediate platelets similar to those found in the SVL. This yolk distribution pattern persisted through early cleavage (Fig. 6F).

Detailed examination (Fig. 7) of inverted T = 1·0 eggs revealed more clearly the presence of the SVL (containing the germ plasm) in the original vegetal hemisphere, the presence of a similar zone lining the animal hemisphere cortex, and the existence of yet another zone of large yolk platelets in the vegetal hemisphere. The latter zone occupied an area between the SVL and the small yolk mass. It consisted predominately of large platelets and represents a residual

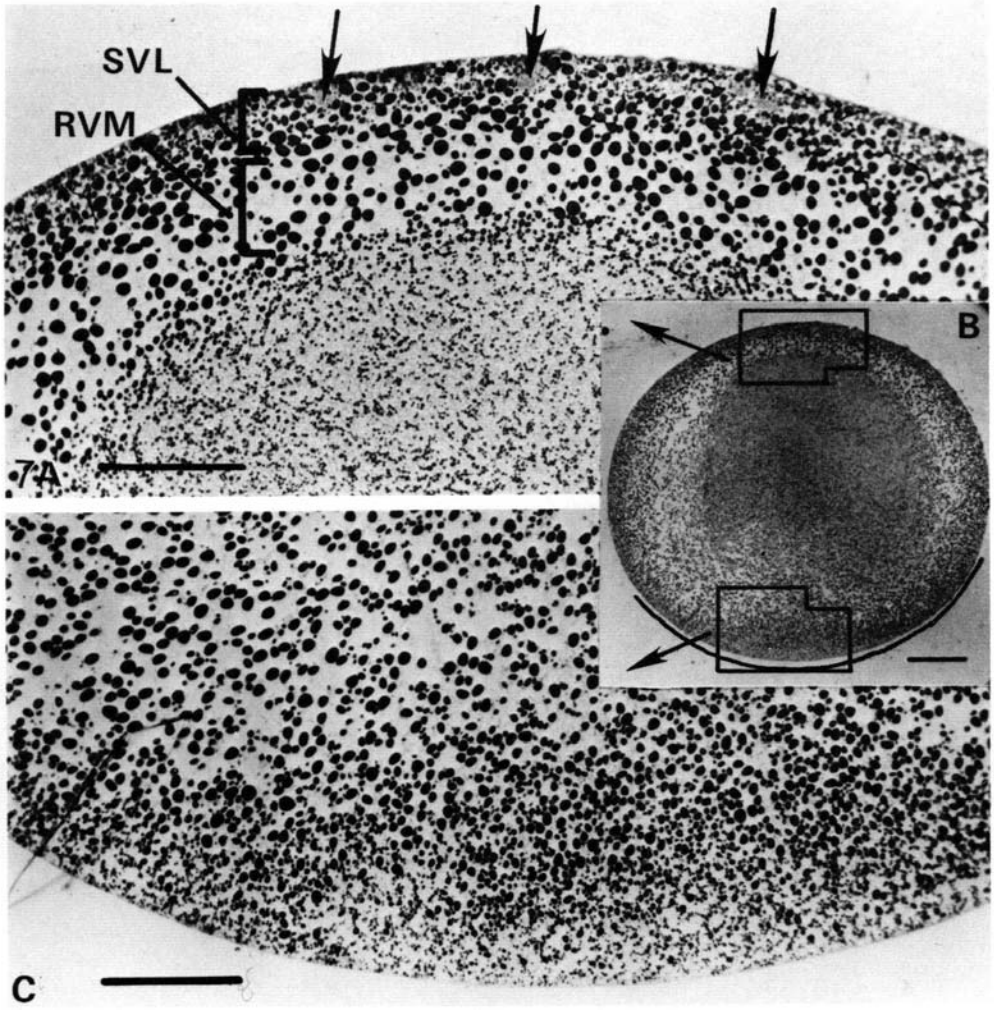


Fig. 7. Detailed examination of inverted T = 1·0 egg revealed SVL and 'residual vitelline mass' (RVM). (A) SVL, germ plasm (arrows), and RVM in original vegetal hemisphere (×195). (B) Distinct separation between large yolk mass and 'central cytoplasm' is apparent. Solid line indicates the surface pigmentation of the eggs original animal hemisphere. (C) Layer of small and intermediate size yolk platelets lines the cortex of the original animal hemisphere (×195). That layer is clearly visible because of presence of large yolk mass adjacent to it. Bars (A) and (C) = 100 μm, and (B) = 200 μm.

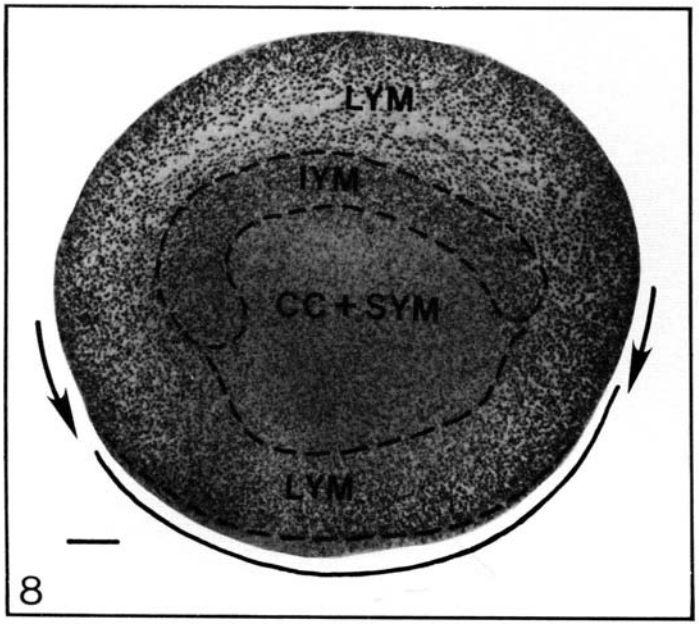


Fig. 8. Yolk rearrangement in inverted T = 0·50 egg which was 180° off-axis (×63). The yolk shift was symmetrical (indicated by arrows), in contrast to yolk shifts in slightly tilted eggs shown in Fig. 6. The eggs original animal hemisphere is indicated by the solid line. As indicated by the data in Fig. 5, such 180° off-axis eggs exhibited abnormal involution and developmental arrest. Bar = 100 μm.

mass of large platelets left behind when the large yolk mass shifted away from the original vegetal hemisphere. Accordingly, it was termed 'residual vitelline mass' (RVM).

During the course of the present investigation approximately 300 fertile inverted eggs were examined for yolk shift. Virtually all eggs displayed the substantial rearrangements shown in Figs 6 and 7. Only two features of the rearrangement process just described varied among different batches of eggs. First, the shift of the large yolk mass was symmetrical in inverted eggs which were 175–180° inverted. Fig. 8 shows a 0·5 μm section of a T = 0·50 egg which was oriented 180° off-axis. The symmetrical shift of the large yolk mass is clearly visible. Second, the rate at which the shift of the major yolk zones occurred varied among eggs (see below).

In summary, the foregoing cytological studies revealed that (1) major yolk zones shift as coherent masses; (2) some less prominent yolk zones (SVL, RVM) which are recognized in inverted eggs do not respond to gravity orientation; (3) the major yolk zones shift asymmetrically in 165°–175° inverted eggs; (4) the direction of shift is determined by the orientation of the egg's tilt; and (5) 175°–180° off-axis eggs display a symmetrical yolk shift (as well as abnormal morphogenesis).

A consequence of the yolk rearrangements described above can be seen in

Fig. 9. In tailbud embryos (approximately stage 35) which developed from inverted eggs, large yolk platelets were frequently observed in the epidermis, neural tube, and notochord. Most likely, those large platelets represent yolk which was

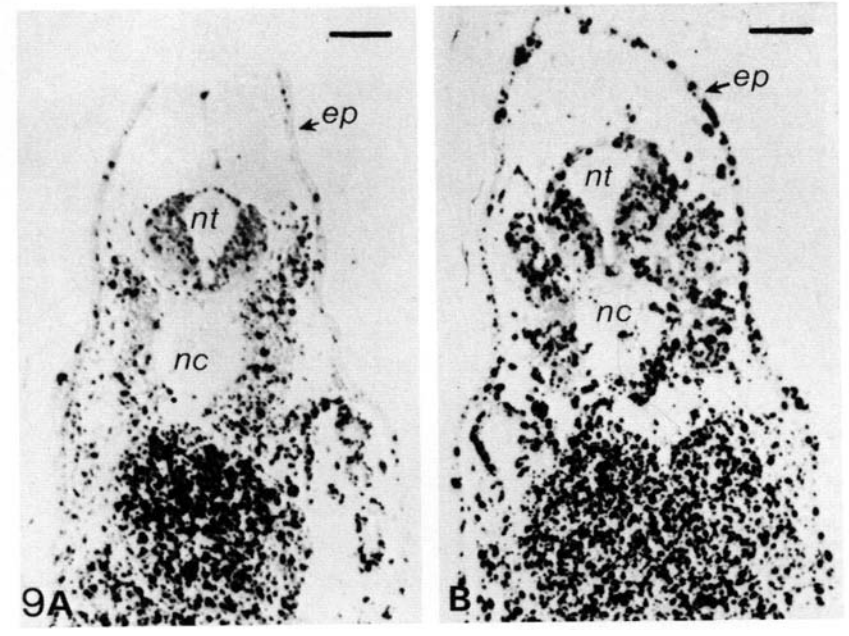


Fig. 9. Histological analysis of tailbud embryos which developed from inverted eggs revealed the presence of large yolk platelets (black bodies 8 to 15 μm in diameter) in epidermis, neural tube, and notochord. (A) Control, normal orientation embryo. (B) Inverted embryo. Abbreviations: ep = epidermis; nt = neural tube; nc = notochord. Bars = 100 μm.

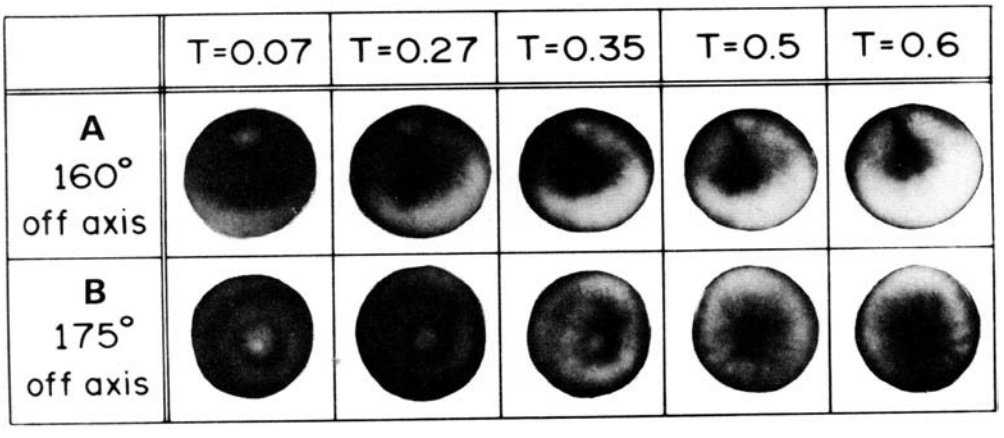


Fig. 10. Time course of movement of inverted egg's original animal hemisphere pigment towards a single dark spot. (A) Inverted egg which was slightly tilted (approximately 165° off-axis). (B) 175–180° off-axis egg. Eggs were photographed from below.

contained in the RVM. During morphogenesis it was included in the blastomeres which developed into ectodermal and mesodermal tissues.

Displacement of surface pigment in inverted eggs

Detailed descriptions of the changes in the external pigmentation pattern which follow normal fertilization and accompany symmetrization of *Xenopus* eggs have been published previously (e.g. Rzehak, 1972; Palecek *et al.* 1978). Observations made in our laboratory confirm those earlier findings and will not be described here. It was observed, however, that the external pigmentation of the activated, inverted egg differed from normal orientation eggs. Since pigment pattern can serve as an early marker of D/V polarity in normal orientation eggs, detailed observation was made of pigmentation in inverted eggs. During the interval between T = 0·2–0·5 the animal hemisphere pigment typically coalesced to form a single main patch or line of dark pigment (Fig. 10). The pigment that usually surrounds both the sperm entrance site and maturation spot was included in that intense pigment patch. The grey crescent, which can occasionally be observed on normal orientation eggs, was never observed on inverted eggs.

In addition to the coalescence of the surface pigment, a displacement of some

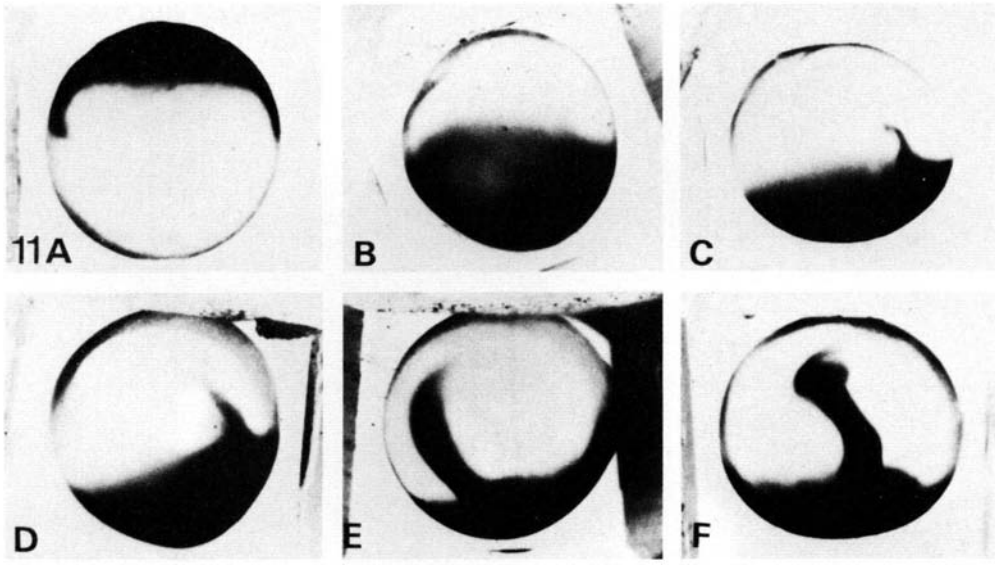


Fig. 11. Inward displacement (to the deep cytoplasm) of animal hemisphere surface pigment in inverted eggs. Medial photographs of plastic embedded eggs sliced in half along the mid-sagittal plane. (A) Control, normal orientation egg at T = 0·5. (B) Inverted egg at T = 0·07; (C) T = 0·25; (D) T = 0·50; (E) T = 0·75; and (F) T = 1·0. The internalized pigment appeared to originate from the area around the intense pigment patch which includes the polar body emission site (maturation spot). Comparisons of 'cleared' whole eggs with sectioned eggs revealed that the inward displacement of pigment was distinct and separate from the sperm penetration pigment track.

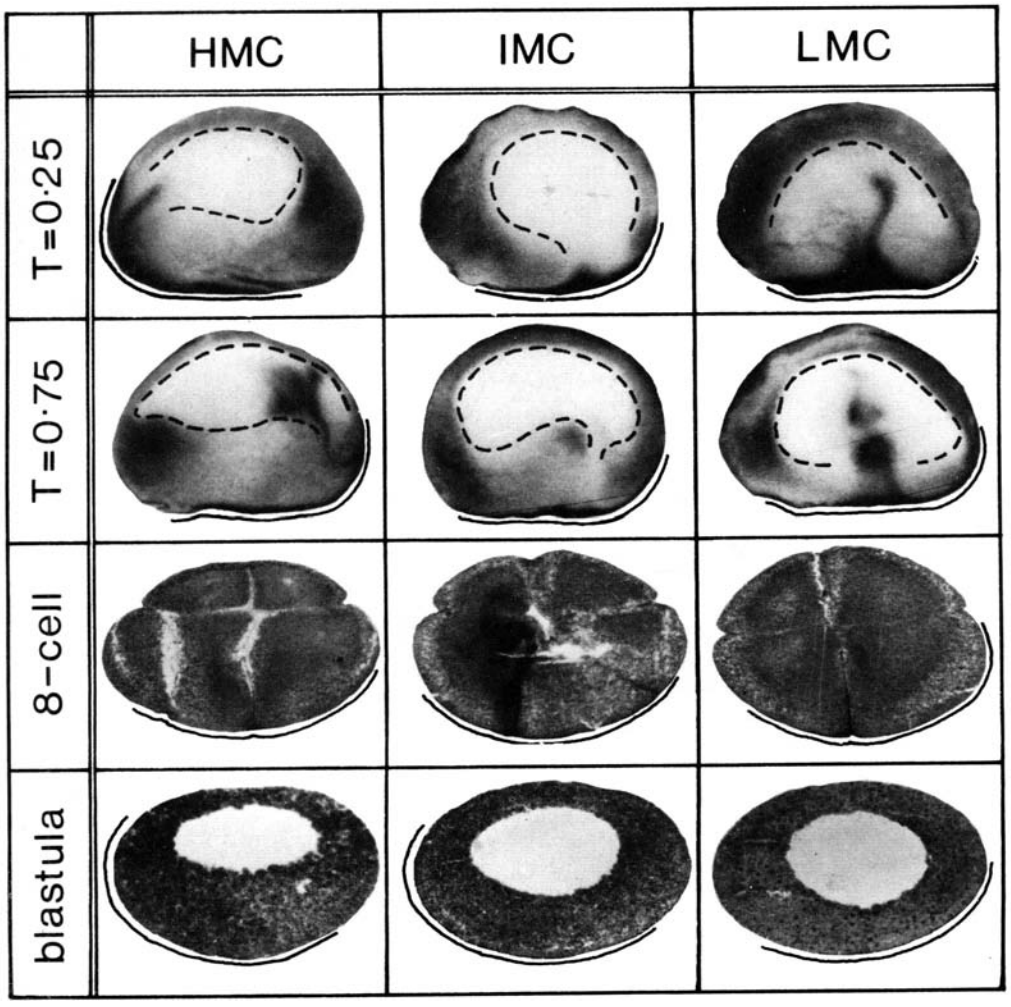


Fig. 12. Rate of shift of yolk masses in eggs from different spawnings. At various times after fertilization eggs were fixed, sliced, and microscopically examined for extent of yolk shift. Three classes of eggs based upon rate of yolk shift were identified. 'High mobility cytoplasm' (HMC) eggs displayed a substantial shift of the large yolk mass by T = 0·25; 'Low mobility cytoplasm' (LMC) eggs displayed a slower rate of shift which was not even complete by T = 0·75; 'Intermediate mobility cytoplasm' (IMC) eggs exhibited a substantial shift by approximately T = 0·75. Pigment displacements of the type shown in Fig. 11 can be seen in each type of egg. At the 8-cell stage the relative sizes of the blastomeres varied. HMC animal blastomeres were similar in size to normal orientation egg blastomeres. LMC animal blastomeres were larger. IMC animal blastomeres were intermediate in size between HMC and LMC eggs. At the mid-blastula stage the location of the blastocoel varied among the three classes of eggs. The dotted lines show the distribution of the large yolk platelets. The solid lines show the surface pigmentation of the eggs original animal hemisphere. Internalized pigment can be seen in HMC and LMC eggs.

of that surface pigment to the deep internal cytoplasm was often observed. Using cleared whole eggs (see Materials and Methods) it was possible to track conveniently the inward movement of the surface pigment. A time-course analysis of that inward displacement of surface pigment is provided in Fig. 11. The direction of the inward displacement usually (e.g. Fig. 11E) deviated away from the moving front of the mass of large yolk platelets, but not always (e.g. Fig. 11F).

The internalization of surface pigmentation was, however, characteristically asymmetrical, and provided further evidence that a substantial redistribution of the egg cytoplasm occurs in inverted eggs.

Rate of shift of major yolk masses in inverted eggs

During the course of observing yolk shifts and pigment displacements it became apparent that the rate of shift/displacement varied among batches of eggs. That variation could explain the variability in the pattern of morphogenesis displayed by different batches of inverted eggs (Wakahara, Neff & Malacinski, 1983). A scoring system was developed to characterize the batch-dependent continuous variation of the rate of shift of the large yolk mass in inverted eggs into three classes. It is described in the legend to Fig. 12. The three classes of eggs in the scoring system are illustrated in Fig. 12. Eggs from different spawnings usually had either high mobility cytoplasm (HMC), intermediate mobility cytoplasm (IMC), or low mobility cytoplasm (LMC; see Fig. 12 legend for definition). Typically, most of the eggs from a single spawning belonged to only one of those classes.

Each of the classes displayed different patterns of early morphogenesis (Fig. 12). HMC eggs exhibited a complete reversal of the small/large blastomere pattern at the 8-cell stage. LMC eggs, however, cleaved into nearly similar-size blastomeres. At the mid-blastula stage HMC embryos exhibited a normal internal morphology, including a large blastocoel in the hemisphere which opposed gravity (original vegetal hemisphere). In contrast, LMC embryos contained a centrally located blastocoel. Examination of later pattern formation in HMC and LMC embryos revealed that survival (Fig. 13), external morphological characteristics (e.g. pigmentation pattern, Fig. 13), and internal features (e.g. primordial germ cell development – Wakahara *et al.* 1983) differed between those two extreme classes of embryos. IMC eggs displayed intermediate amounts of survival and morphogenetic pattern reversal.

The HMC, IMC, or LMC character of eggs can be ascribed to physiological rather than genetic effects. Individual females were respawned over a 6-month period. Female No. 65 spawned HMC eggs in the first spawning, LMC eggs in the second spawning, and IMC eggs in the third spawning. Female No. 37 first shed IMC eggs, and then HMC eggs; female No. 24, LMC, then HMC eggs; and female No. 101, HMC, then IMC eggs.

Observations of the development of large numbers of HMC and LMC eggs

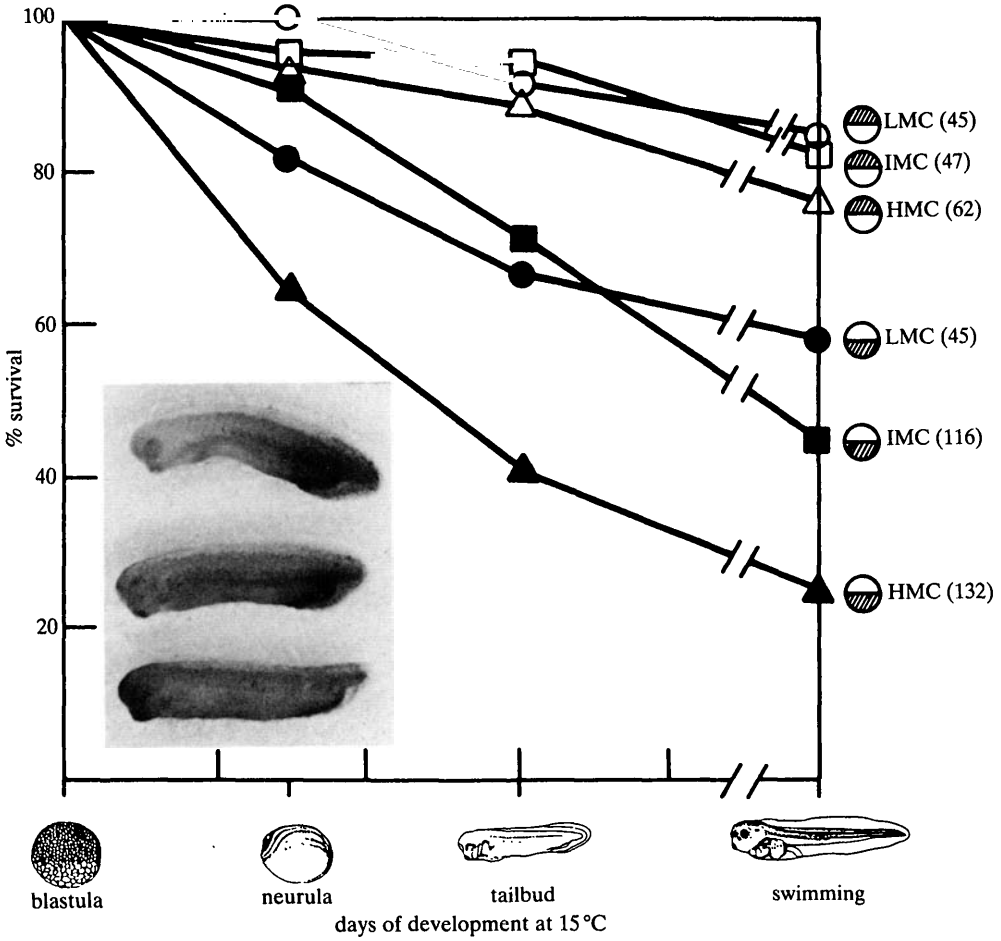


Fig. 13. Survival and pattern reversal in HMC, IMC, and LMC eggs. Survival of normally oriented as well as inverted eggs of each class is indicated by data in the graph. HMC eggs showed lowest survival frequency. Numbers in parentheses represent sample size. Insert contains photographs of external pigmentation pattern of HMC inverted embryos (top), LMC inverted embryos (middle), and normally oriented control embryos (bottom). The pattern of gastrulation apparently differs between HMC and LMC eggs. The external pigmentation pattern of HMC embryos is largely reversed (lightly pigmented epidermis). LMC embryos exhibit only partial reversal of the external pigmentation pattern.

from several spawnings provided the following additional information. Inverted HMC eggs exhibited a greater tendency to remain 180° off-axis than inverted LMC eggs (which usually tilted and developed in a 165°–175° off-axis orientation). Perhaps the relatively rapid yolk shift of HMC eggs inhibits the events that generate tilt. In the absence of tilt a symmetrical distribution of cytoplasmic components results (Fig. 8). Conceivably, the lack of asymmetry of the egg cytoplasm leads in turn to involution failure (Fig. 5) and the low survival of HMC eggs indicated in Fig. 13.

Yolk mass shifts in artificially activated eggs

No displacements of either external pigment or the major internal yolk masses occurs in inverted unfertilized eggs (Radice *et al.* 1981). A stabilization mechanism such as a cytoskeleton probably maintains the egg cytoplasm in a rigid configuration. To determine whether sperm penetration is a prerequisite for the yolk mass shifts illustrated in Figs 6, 8, and 12, artificially activated eggs were analysed. Electrical activation was chosen first, for as will be demonstrated below, eggs responded by uniformly orienting in one direction. That feature permitted additional detailed observations of external pigment changes and extent of tilt. Fig. 14 contains photographs of typical electrically activated inverted eggs which were fixed at the equivalent (for fertilized eggs) of T = 0·75. Virtually all inverted electrically activated eggs exhibited a substantial (or in some cases, complete) shift of the major yolk masses.

Next, the effect of cold shock activation was analysed. In contrast to the directional nature of electrical activation (Fig. 14A), cold shock is uniformly administered. Mature unfertilized, inverted eggs from several females were treated with varying levels (5–30 min) of cold shock (0 °C). Virtually all batches of eggs exhibited a substantial yolk shift of the type shown in Figs 6 and 12 at one or another level of cold shock. In addition to the shift of the yolk masses, cold shocked eggs routinely displayed the pigment displacements illustrated in Figs 10 and 11. Although uniformly administered, activation by cold shock generated an intense pigment patch in the original animal hemisphere which was offset

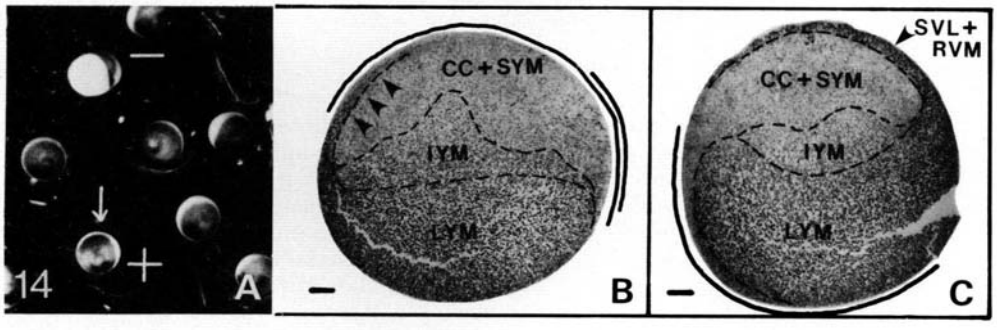


Fig. 14. Effects of electrical activation on yolk mass distribution in mature, unfertilized inverted egg. Eggs were activated in 100 % Steinberg's solution by a single pulse of 79 V. (A) Top view of a batch of eggs which were oriented prior to activation so the animal hemisphere opposed gravity. Asymmetry of surface pigmentation is apparent. An intense dark patch of pigment faced the + pole. (B) 0·5 μm section through pigment patch of normally oriented activated egg at the equivalent (for fertilized eggs) of T = 0·75. This particular egg is indicated by the arrow in (A). The original animal hemisphere (surface pigment) is shown by a single solid line. The pigment patch is shown by the double solid line. This egg displayed some tilt, and also a distinct vitelline wall (arrows). (C) Inverted egg showing displacements of yolk masses (compare with HMC egg in Fig. 12). The SVL and RVM are clearly visible. Bar = 100 μm.

from the animal pole. Coincidently, the yolk shift was usually asymmetrical in character, as shown in Fig. 6, rather than symmetrical, as in Fig. 8. Curiously, both electrically and cold activated eggs displayed an asymmetrical yolk shift. It can therefore be postulated that the unfertilized egg's radial symmetry is easily broken, even by an apolar stimulus such as cold shock. The unfertilized egg's predisposition toward bilateral symmetrization indicates that during oogenesis and maturation the components for polarity are built into the egg cytoplasm. Contributions to the polarization mechanism which are provided, under natural conditions, by the sperm should be considered dispensible.

DISCUSSION

An important observation which emerged from the survey of yolk mass shifts that follow activation of both normal and inverted eggs is the substantial variability among eggs of different spawnings. The basis of that variability is most likely physiological rather than genetic, as repeated spawnings of individual females demonstrated. One way to circumvent potential difficulties in data interpretation is to screen eggs carefully and attempt to select for study those which fit the stereotypic character of the radially symmetrical *Xenopus* egg (e.g. Ubbels *et al.* 1983). An alternative approach was, however, employed in the present studies. An effort was made to understand the basis of the variability of the egg's response to inversion.

By carrying out a comprehensive analysis of several aspects of the internal cytology and external morphogenesis of large numbers of both normal and inverted eggs a variety of novel observations were made. The *first* concerns the vitelline wall. The most striking finding was its *variability in normal orientation eggs*. It was not detectable in some eggs, and its prominence varied in other eggs. A fully formed vitelline wall of the type described by Pasteels (1953) (Fig. 1B) was not always observed. Data from inverted eggs (e.g. Figs 6, 7, and 12) call into question its functional significance, as it was never recognized in inverted eggs. Furthermore, the structural integrity of the subcortical vitelline layer (SVL) and residual yolk mass (RVM) would interfere with its formation in inverted eggs. The large yolk mass that is displaced to the egg's original animal hemisphere is prevented from re-organizing to mimic normal orientation eggs by the SVL and RVM (e.g. Fig. 6B, see pointer). Yet morphogenesis often proceeds normally in inverted eggs, especially in those with an LMC type cytoplasm (Fig. 13). The role of vitelline wall/cortex interactions of the type originally proposed by Dalcq & Pasteels (1937) for pattern specification should, therefore, be questioned.

The *second* novel observation concerns the 'compartment-like' character of the various yolk masses. Vegetal yolk mass rearrangements along the inner cortex of *Rana fusca* eggs were originally described by Born (1885). Those observations were, however, never pursued in detail or related to pattern formation. Analysis of histological sections of inverted eggs provides a direct test of the

structural integrity of the yolk compartments. Each of the major compartments maintains its identity in inverted eggs. Significant mixing of yolk compartments was never observed even in HMC eggs (Fig. 12). The description of yolk platelet rearrangements in inverted *Xenopus* eggs presented in this report differs substantially from the *Rana* model developed earlier by Pasteels (1941). In that model the large platelets were considered to be displaced through the centre of the egg rather than along the cortex, as described in this report. Perhaps the composition and arrangement of the various yolk compartments differ between those anuran species. Another major difference is the fact that in the *Xenopus* egg the movement of the large platelets away from the hemisphere that opposes gravity (i.e. the original vegetal hemisphere) is not complete. The epidermis, neural tube, and notochord of inverted embryos contain, therefore, some large yolk platelets (Fig. 9).

Correlations of yolk compartment shifts in inverted eggs with subsequent patterns of morphogenesis led us to formulate a 'density compartment' model for early pattern specification which will be discussed in detail below.

A *third* original observation was the discovery of the SVL and RVM. The existence of the SVL probably would have gone unnoticed if it did not contain the germ plasm. The failure of the germ plasm to rearrange in inverted eggs (Neff *et al.* 1983) is probably due to the rigid structural integrity of the SVL. The very existence of the SVL and the fact that it maintains the germ plasm in its original location presents an intriguing possibility for speculation: Perhaps the cortex plays a definitive role in pattern specification by anchoring the SVL to it. Since the germ plasm is rigidly stabilized within the SVL, it tracks with the original vegetal hemisphere cortex, regardless of the orientation of the egg with respect to gravity. So, by anchoring the SVL, the vegetal cortex may function to specify the site of the early development of primordial germ cells.

Fig. 15. Diagrammatic repesentation of the density compartment model. Four major steps are considered to be involved in the construction and deployment of the density compartments. The major yolk compartments serve as a paradigm and are therefore illustrated above. Other density compartments probably also exist, but were not detected with the histological methods employed in the present study.

Preloading occurs during oogenesis. A cytoskeleton is postulated to stabilize the compartments. Release of density compartments is promoted by activation. A change in the structure of the cytoskeleton probably follows egg activation. Artificial activation can substitute for natural fertilization in the density compartment release step. Symmetry breaking can result from any of a number of stimuli, including sperm entrance, aster growth, cortex contraction, or 'tilt'. Novel compartment-neighbour interactions form, which lead to new metabolic activities and subsequent activity of dorsal and ventral morphogenetic determinants.

Animal/vegetal polarity is viewed in the same terms as dorsal/ventral polarity. Density compartments which are stratified along the animal/vegetal axis (like the yolk components illustrated above) are released at fertilization. These compartments are involved in primary embryonic organizer activity and competence of neural induction.

In order to generate a framework for understanding the above observations, and to provide guidelines for further research a comprehensive model was developed. It is described in detail below.

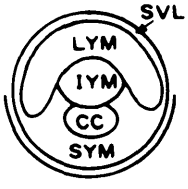
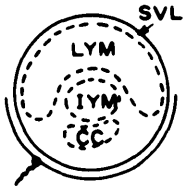
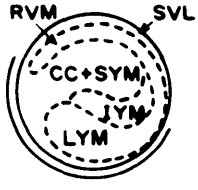
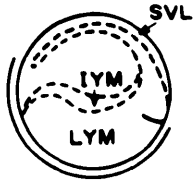
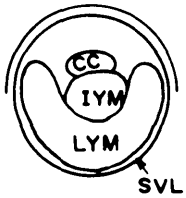
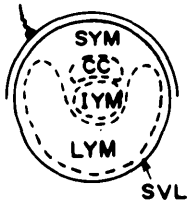
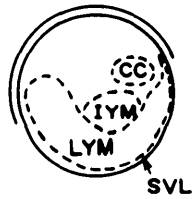
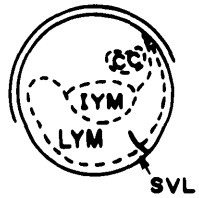
	Density compartment model			
	Preloading (radial symmetry)	Release (activation)	Symmetry breaking	D/V polarity
process in inverted eggs				
supporting data	description of major yolk zones Fig. 1,2,3,7	ability of yolk masses to move following fertilization and artificial activation – Fig. 1,6, 8,11,14	shift of yolk masses in direction of "tilt" Fig. 4,6,11,12, 14	involution site determined by egg "tilt" Fig. 4,5
postulated process in normal orientation eggs				
	density compartments established during oogenesis and maturation	release of compartments by fertilization and artifical activation	asymmetric shift of compartments in response to sperm aster growth, "tilt", cortical contraction, etc.	involution site determined by new compartment associations and/or asymmetries
selected supporting literature references	Nieuwkoop, 1956; Ubbels, 1977.	Herkovits and Ubbels, 1979; Radice et al., 1981.	Born, 1885; Pasteels, 1964; Ubbels et al., 1983; Slack, 1982.	Ancel and Vintemberger 1948; Scharf and Gerhart, 1980.

Fig. 15

Density compartment model

From the descriptions and analyses presented in this report a comprehensive 'density compartment model' has been formulated. This model attempts to interpret the present data in terms of a single, unified theory. The model can be reconciled with observations reported by others, and also explains animal/vegetal and dorsal/ventral pattern specification as the product of similar subcellular mechanisms. The model provides guidelines for future research. Its salient features are summarized in Fig. 15 and described below.

The cytoplasm of the unfertilized egg is viewed as containing several spatially distinct zones or masses. They differ from one another in several ways, including size, shape, composition, intrinsic viscosity, and intrinsic buoyant density. Those zones are referred to as 'density compartments'. Density compartments in the present context represent physical masses or domains in contrast to 'compartments' in insect genetics (Lawrence, 1975), which imply units of function. In addition to the yolk zones, other density compartments or subcompartments that are not detectable with the conventional cytological methods employed in this report may also exist. The clearly visible yolk zones provide, however, a suitable example for describing the characteristics of density compartments. The density compartments become organized and stabilized during oogenesis and egg maturation. The physiologically mature, unfertilized egg is considered to be radially symmetrical with respect to the distribution of density compartments. The various density compartments are probably stabilized internally as well as in relation to one another by a cytoskeleton. The rigidity of the cytoskeleton is most likely responsible for the fact that until activation the cytoplasm of an inverted egg is stable towards gravity perturbation. The cytoskeleton may also stabilize individual compartments of activated eggs such as the SVL.

Egg activation, whether achieved via natural fertilization or artificial mechanisms (e.g. pricking, electrical shock, cold shock, or calcium ionophore) releases the density compartments from one another, perhaps by altering the cytoskeleton. The density compartments then shift to assume natural buoyant relationships. At a minimum, the density compartments stabilize the large egg's cytoplasm insuring that the egg establishes initially only a single initial animal/vegetal axis and after fertilization only a single emerging dorsal/ventral axis. The density compartments thus provide a *passive* (low energy) method to insure that the necessary morphogenetic events are properly localized, and that the egg cytoplasm responds predictably to extrinsic and intrinsic forces such as gravity, sperm aster growth, cortical contraction and natural egg tilt. In addition, the exciting possibility exists that the density compartments play an *active* role in early embryo pattern formation; the bilateral symmetrization (dorsal/ventral polarization) of the egg might be a direct consequence of density compartment shifts. As the density compartments relocate and perhaps undergo some mixing at the compartment margins, new compartment–neighbour interactions could

emerge. Those novel compartment interactions could be visualized as generating new metabolic activities which regulate pattern specification during early embryogenesis. Localized morphogenetic information might, for example, be generated. Alternatively, gradients of morphogens of the type offered as explanations of early insect pattern specification (Meinhardt, 1982) may be set up.

Compartments or subcompartments might also be preloaded during oogenesis in orientations which reflect inverted densities. Upon fertilization those compartments then shift to achieve natural buoyant density relationships. For example, the SVL may be less dense than the overlying LYM. The vegetal cortex and adherent SVL could amplify the initial egg asymmetries brought about by initial egg tilt, sperm aster growth, or cortical contraction and provide an upward driving force opposite the sperm entry site. The subsequent downward flow of the LYM and adherence of the SVL to the cortex could create a yolk platelet wall which mimics 'Born's crescent'. The yolk platelet composition of the SVL and the refractory behaviour of the SVL to gravity in inverted eggs are consistent with this model.

A key feature of the model is the breaking of the oogenetic pattern of radial symmetry of the unfertilized egg to generate dorsal/ventral polarity. Under natural conditions a stimulus such as sperm entrance may initially suffice to provide a direction to the shifting density compartments. Other stimuli, including sperm aster growth (Ubbels *et al.* 1983), or cortical contractions (Hara, Tydeman & Hengst, 1977) might also participate in the symmetry breaking process (Slack, 1982). Physical perturbations such as the natural tilt (Ancel & Vintemberger, 1948) observed in some eggs probably promote the symmetry breaking process. Mechanical rotation of an egg to a 90° off-axis orientation (Scharf & Gerhart, 1980; Chung & Malacinski, 1981) most likely reinforces the natural compartment shift mechanism. In some cases severe gravity disturbances (e.g. centrifugation – Motomura, 1935) no doubt simply overwhelm the natural symmetrization process and either realign or bifurcate some of the density compartments. Apolar or twinned embryos which result from gravity perturbations and centrifugation (Gerhart *et al.* 1981) are understood within the context of the density compartment model.

The density compartment model explains each of the phenomena described in this report. Differences in yolk platelet densities (Fig. 3); release of density compartments by activation as well as natural fertilization (Figs 6, and 14); shifting of yolk masses as integral zones in inverted eggs; symmetrical shifting of yolk zones 180° off-axis eggs (Fig. 8); relationship between rate of shift of density compartments and subsequent pattern of morphogenesis (Figs 12 and 13), are all accounted for by the 'density compartment model'. Furthermore, animal/vegetal polarity and dorsal/ventral polarity are viewed in a coherent way. Animal/vegetal polarity is considered to involve density compartments that are in principle similar to those related to dorsal/ventral polarity. Animal/vegetal polarity is visualized as being primarily involved in early embryonic cleavage pattern specification, germ plasm segregation, and blastocoel formation. During

later pattern specification animal/vegetal polarity is involved in primary embryonic axis formation. That is, animal/vegetal polarity regulates the activity of dorsal marginal zone cells in such a manner that primary embryonic organizer activity develops in the cells that surround the invagination site. Animal/vegetal polarity also guides the development of the animal hemisphere's capacity to respond to the action of the primary embryonic organizer.

Experimental evidence to support this role of animal/vegetal polarity is available. The reversal of developmental competence from the original animal hemisphere to the vegetal hemisphere in inverted eggs was recently observed (Chung & Malacinski, 1983). Primary embryonic organizer activity was, however, not reversed. Developmental competence can therefore be uncoupled from the development of primary organizer activity. Those observations of inverted eggs are explained by the density compartment model as representing the complete reversal of some compartments (e.g. those for competence), but not others (e.g. those for primary organizer activity). Several features of the model can be subjected to direct test. For example, clinostat (simulated hypogravity) (Nace & Tremor, 1981) or space lab (microgravity) experiments could be employed to examine whether density compartment shifts are required for normal pattern specification. In addition, microsurgical manipulation of the internal cytoplasm could be carried out. Withdrawal or transfer of various density compartments might succeed in identifying density compartments which are associated with specific morphogenetic events (e.g. gastrulation).

Additionally, using monoclonal antibody protocols developed by Dreyer, Singer & Hausen (1982) it should be possible to track the movement, in inverted eggs, of non-yolk proteins. Should the movements of those proteins resemble yolk displacements, a sound basis for extending the density compartment model to potential regulatory proteins would be provided.

Some of the germinal vesicle proteins tracked in the Dreyer *et al.* (1982) studies remained localized in the egg's animal hemisphere cytoplasm. Later they moved into the nuclei of the developing embryo. By tracking those proteins in inverted eggs information might be obtained regarding whether those specific proteins are required for pattern specification.

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