

Accumulation, organization and deployment of oogenetically derived *Xenopus* yolk/nonyolk proteins

ROSAMUND C. SMITH,^{1,*} ANTON W. NEFF²
AND GEORGE M. MALACINSKI¹

¹*Department of Biology and* ²*Medical Sciences Program, School of Medicine,
Indiana University, Bloomington, IN 47405, USA*

INTRODUCTION

The animal/vegetal (A/V) polarity of the typical amphibian egg is immediately recognizable by the distinct pigmentation differences between the darkly pigmented (animal) and lightly pigmented (vegetal) hemispheres. That polarity arises early in oogenesis. Prior to the vitellogenic period during which yolk platelets accumulate and the original (uniform) pigmentation pattern changes, a polarized distribution of several internal components can be detected. Ribosomal DNA accumulates within a localized area in the nucleus (Brachet, 1977). The Balbiani body (containing large numbers of mitochondria) is located outside and to one side of the nucleus, while the nucleoli are later segregated within the nucleus on the opposite side (Billett & Adam, 1976). By the end of the previtellogenic period the Balbiani body (mitochondrial cloud) has moved to the periphery, marking the future vegetal pole (Heasman, Quarmby & Wylie, 1984). During early vitellogenesis the distribution of yolk in an animal/vegetal gradient pattern may be related to the 'slightly off center' position of the germinal vesicle (Nieuwkoop, Johnen & Rzehak, 1985). The uneven accumulation along the A/V axis of mRNA (Capco & Jeffery, 1982; Phillips, 1982; Carpenter & Klein, 1982; King & Barklis, 1985; Rebagliati, Weeks, Harvey & Melton, 1985; Smith, 1986) could conceivably also be initiated by those early asymmetries in oocyte organelle distribution.

Nieuwkoop (1977) has speculated that those anuran egg A/V asymmetries in organelle distribution may be vertically transferred from generation to generation through the cytoplasmic continuity of the germplasm. The anuran germplasm is localized in the vegetal pole (Bounoure, 1934) and is easily visualized with contemporary histological techniques (e.g. Smith & Neff, 1985). It can be tracked through embryogenesis, and the cells that contain it (primordial germ cells) develop into the gametes of the next generation. The absence of a localized

* Author's present address: Department of Biological Sciences, Columbia University, New York 10027, USA.

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germplasm in urodele eggs, proposed by Sutasurya & Nieuwkoop (1974), necessitates ascribing to somatic ancestor cells the role of establishing and maintaining asymmetry in developing urodele oocytes and embryos (Nieuwkoop *et al.* 1985).

By the completion of vitellogenesis and physiological maturation the egg is radially symmetrical about an axis which extends through the animal and vegetal poles (Fig. 1A). Yolk platelets as well as nonyolk components are organized into zones or compartments, which will be described in detail below.

During the sperm entry and egg activation, events that make up the fertilization reaction, the radial symmetry of both the internal and external (cortical) cytoplasm is broken. This can occasionally be visualized superficially by the change in pigmentation pattern in the equatorial region of the egg. A crescent-shaped zone of pigmentation difference, the so-called 'grey crescent', appears. In some anuran eggs (e.g. *Xenopus*, but not *Rana*) the sperm entrance site (SES) can also be visualized since the pigment surrounding the site where the sperm penetrated the egg cortex coalesces and creates a dark spot (Palecek, Ubbels & Rzehak, 1978). The SES, which forms first, marks the future ventral site of the embryo. The grey crescent forms approximately 180° opposite it (Ancel & Vintemberger, 1948). Associated with grey crescent formation is an egg surface (membrane and cortex) movement (approx. 30°) relative to the deeper cytoplasm. This 'rotation of dorsalization' provides a reliable marker for the future dorsal side of the embryo (Vincent, Oster & Gerhart, 1986).

Internally, the radial symmetry of the egg cytoplasm is also broken. Substantial rearrangements of yolk platelets occur (Dalcq & Pasteels, 1937). Those rearrangements are most easily examined in an anuran egg such as *Xenopus* because the

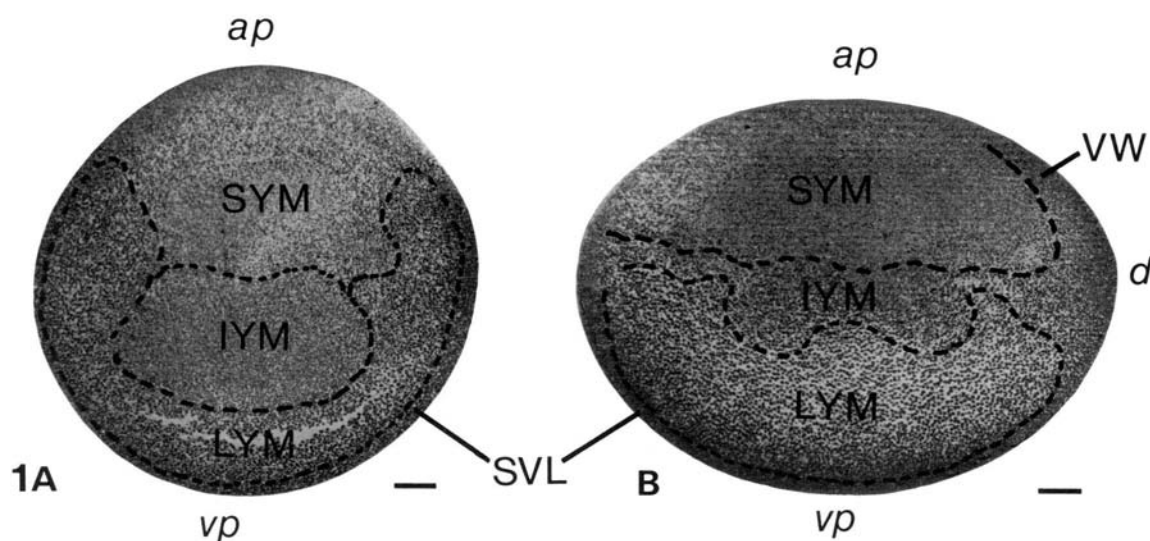


Fig. 1. *Xenopus* egg histological (plastic) sections (0.5 μ m). (A) Typical egg immediately following fertilization. (B) Egg at first cleavage showing vitelline wall on future dorsal side. LYM, large yolk mass; IYM, intermediate yolk mass; SYM, small yolk mass; SVL, subcortical vitelline layer; VW, vitelline wall; ap, animal pole; vp, vegetal pole; d, dorsal side. Bar, 100 μ m. (Modified from Neff *et al.* 1984b.)

SES, being easier to visualize than the grey crescent, can be used to correlate internal cytoplasmic alterations with external (e.g. cortical) changes. Fig. 1B illustrates the postfertilization shifts in yolk platelets. Those cytoplasmic rearrangements are permanent. The cleavage that follows fertilization partitions the egg cytoplasmic components to the cells in which they are presumably later employed.

By correlating surface changes (SES, grey crescent) and internal shifts with cell lineage studies and the later pattern of morphogenesis, it becomes apparent that the symmetry-breaking events that are triggered by the fertilization reaction specify pattern. Cytoplasmic shifts away from the SES and towards the grey crescent define the future dorsal side of the egg. The invagination of both surface and internal cells (gastrulation) is initiated on that side and eventually leads to differentiation of the dorsal and anterioposterior axis. Conversely, on the opposite side of the egg future ventral structures (e.g. endoderm-gut) develop. Experimental manipulation has confirmed the presumed causal relationship between cytoplasmic shifts and subsequent pattern specification. Ultraviolet irradiation of uncleaved eggs, which interferes with those shifts, presumably interferes with axis specification, while 90° rotation of uncleaved eggs repositions the axis (Scharf & Gerhart, 1980, 1983; Chung & Malacinski, 1980). Gentle centrifugation can direct polarity (Black & Gerhart, 1985) and cause twinning (Motomura, 1935; Gerhart, Black & Scharf, 1983).

ORGANIZATION OF INTERNAL CYTOPLASM

From the knowledge that cytoplasmic shifts in the uncleaved egg generate a bilateral symmetry (dorsal/ventral polarity) which persists through primary embryonic axis formation and subsequent morphogenesis, attention has recently focused on two major questions. (1) How are cytoplasmic components physically organized, so that they rearrange in such a regular and orderly fashion? (2) What mechanism acts to drive the rearrangements? Answers to the first question will be offered, and discussed in detail, in the next two sections. A brief review of the history of research in this area will first be provided, followed by a detailed discussion of studies performed in our own laboratory. Answers to the second question will be much more speculative than those to the first. In this instance, only a brief review of the literature that provides support for a few of the more plausible models will be given (in the third section below).

YOLK PLATELET COMPARTMENTS

The relative ease with which yolk platelets can be observed with conventional light microscopic histology has been exploited by at least three generations of embryologists. In each instance the yolk platelets of the fertile egg have been described as being stratified along the animal/vegetal axis into a series of non-overlapping zones (Table 1). The accuracy of the geometry of the various zones, as well as the total number of zones (up to 11; Dorfman & Cherdantsev, 1977)

Table 1. *Historical review of stratification of yolk platelets along the animal/vegetal axis of the amphibian egg*

Investigation	Species	Main observation
Born, 1885	<i>Rana</i>	Yolk platelets unevenly distributed
Pasteels, 1941	<i>Rana</i>	Yolk distribution is clearly discontinuous
Ancel & Vintemberger, 1948	<i>Rana</i>	3 zones; discrete pigmented central zone
Nieuwkoop, 1956	<i>Xenopus</i>	7 zones
Harris, 1964	<i>Ambystoma</i>	4 zones
Klag & Ubbels, 1975	<i>Discoglossus</i>	4 zones
Dorfman & Cherdantsev, 1977	<i>Rana</i>	11 zones
Herkovits & Ubbels, 1979	<i>Xenopus</i>	Yolk-free area of the small yolk platelet zone ('clear cytoplasm') identified
Neff <i>et al.</i> 1984b	<i>Xenopus</i>	Zones of yolk platelets shift as discrete 'density compartments'; some compartments mobile, others fixed
Phillips, 1985	<i>Xenopus</i>	3D map of platelets

have in our opinion, however, too often been exaggerated. Egg variability, for example, is a major consideration which is routinely overlooked. A detailed discussion of this potential problem has recently been offered (Malacinski & Neff, 1986). Among *Xenopus* spawnings, the geometry of the various yolk platelet zones in individual eggs varied substantially. Likewise, histological artifacts (e.g. differential shrinkage) associated with yolk-rich eggs are rarely considered.

In order to circumvent those difficulties our laboratory formulated an inverted egg model system using *Xenopus* eggs. This system provides an operational definition of yolk compartments, since the physical integrity of the various zones is elucidated as they reorient along the new gravity vector. Eggs were inverted (lightly pigmented vegetal hemisphere oriented so it opposed gravity) and activated by either sperm or electrical or cold shock. Time course analyses of the shift of the various yolk zones was monitored. An example of the data is included in Fig. 2. Not all the yolk oriented in accordance with the new gravity vector. Two zones or 'compartments', one that lines the original vegetal hemisphere and contains the germplasm (subcortical vitelline layer, SVL) and another that also remains in the original vegetal hemisphere (residual vitelline yolk mass, RVM) were also discovered (Fig. 3). The key findings from those studies (Neff, Wakahara, Jurand & Malacinski, 1984b) can be summarized as follows. (1) Yolk platelets are organized into a set of integral zones or 'compartments' (Fig. 1B). As those compartments become displaced in inverted eggs, little or no mixing occurs between the compartments. (2) Yolk platelets display nonuniform buoyant densities. Larger platelets are generally more dense than smaller platelets. Those density differences may contribute to the structural integrity of the compartments. (3) The direction of shift of the major yolk compartments determines the dorsal/ventral polarity of inverted eggs. This observation is consistent with the earlier observations that tilting a naturally oriented egg alters its dorsal/ventral polarity

(Ancel & Vintemberger, 1948). (4) Inverted eggs that display a symmetrical shift of major yolk compartments fail to involute. That is, if the compartments shift without breaking the radial symmetry of the egg cytoplasm, the embryo fails to gastrulate and eventually arrests (Neff *et al.* 1984b; Wakahara, Neff & Malacinski, 1986).

The above data have been integrated into a 'density compartment' model that proposes that rearrangements of the yolk compartments that follow fertilization specify pattern in early embryogenesis (Neff *et al.* 1984b). Several lines of evidence support the proposed role of compartment rearrangements in pattern specification. First, the egg rotation and centrifugation experiments referred to earlier implicate compartment rearrangements in D/V polarization. Second, the failure of involution to occur in inverted eggs that display symmetrical compartment shifts suggests that asymmetrical rearrangements are required for dorsal lip formation. Third, constantly rotating eggs on a horizontal clinostat so that a gravity vector is not established generates developmental catastrophes only when the clinostat rotation causes compartment mixing (Smith & Neff, 1986).

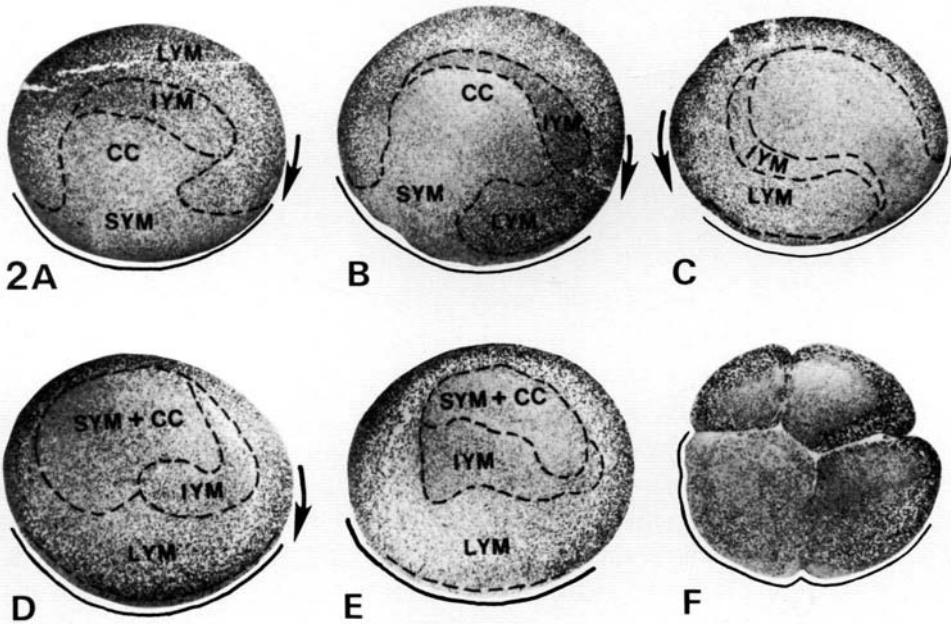


Fig. 2. Sequential shift of major yolk compartments in inverted eggs as observed in $0.5\ \mu\text{m}$ sections. The surface pigmentation of the egg's original animal hemisphere is revealed by the solid line. Dotted lines indicate the margins of various major yolk compartments. (A) $T = 0.07$ egg. Beginning of displacement of yolk compartments in direction of 'tilt' of the egg (arrow). (B) $T = 0.25$ egg. Further displacement of LYM. Its leading edge is deflected away from the animal hemisphere cortex (pointer). (C) $T = 0.50$ egg. (D) $T = 0.75$ egg. (E) $T = 1.0$ egg. (F) 8-cell-stage embryo. The yolk distribution pattern observed at $T = 1.0$ persists through early cleavage. cc, clear cytoplasm. Bars, $100\ \mu\text{m}$. $T = 0$, time of fertilization; $T = 1.0$, first cleavage. (From Neff *et al.* 1984b.)

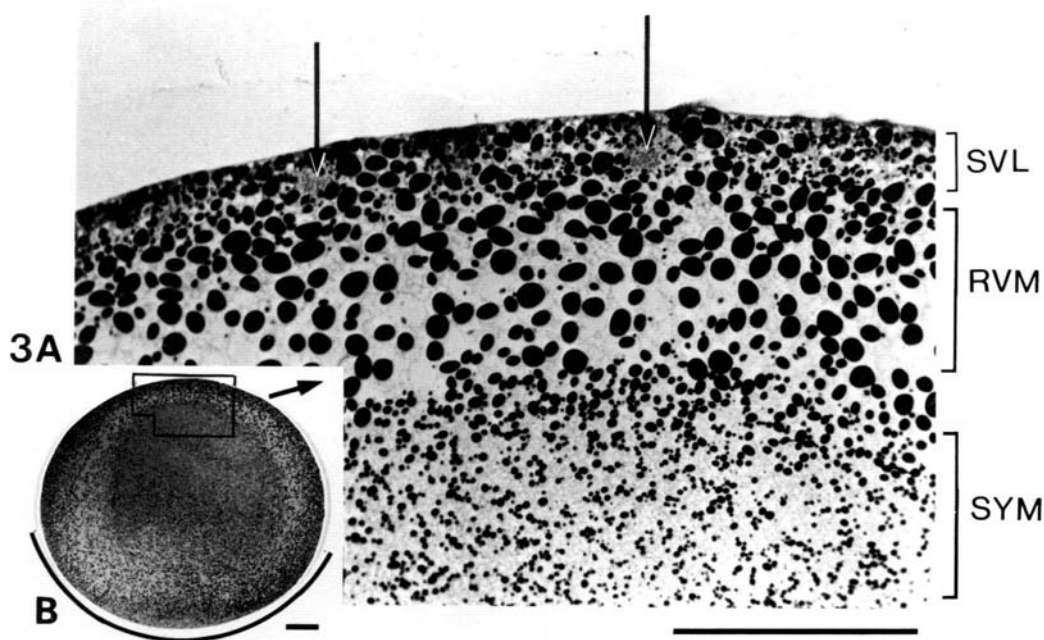


Fig. 3. Further examination of inverted $T=1.0$ egg revealed SVL and 'residual vitelline mass' (RVM). (A) SVL, germplasm (arrows), displaced SYM and RVM in original vegetal hemisphere ($\times 320$). Distinct separation between large yolk platelets (LYM) and SYM is apparent. (B) Solid line indicates the surface pigmentation of the egg's original animal hemisphere ($\times 38$). Bars, $100\mu\text{m}$. (Modified from Neff *et al.* 1984b.)

ORGANIZATION OF NONYOLK COMPONENTS

Because of the relative ease with which yolk platelets can be examined, their distribution and rearrangements in eggs and early embryos have been extensively studied (e.g. Table 1). Whether nonyolk proteins are similarly distributed and rearranged has, however, not been extensively analysed. That is, whether the compartment organization model extends to nonyolk proteins is not at all understood. We have begun an investigation into this matter in an attempt to determine (1) if yolk compartments serve as 'organization centres' for nonyolk proteins, and (2) whether nonyolk proteins track with yolk compartments during the cytoplasmic rearrangements that follow egg activation.

Accordingly, monoclonal antibodies (MoAb) prepared against a small number of nonyolk egg protein antigens were employed to locate and track them during oogenesis, egg activation and early embryogenesis.

PREPARATION OF MONOCLONAL ANTIBODIES

A two-dimensional (2D) gel electrophoresis separation of nonyolk proteins included in the vegetal half of unfertilized *Xenopus* eggs (Fig. 4) was prepared

according to previously published methods (Smith & Knowland, 1984). This gel was then Western blotted according to standard procedures. A small piece of nitrocellulose (approx. 1 cm^2) was cut out (Fig. 4) and used for antigen (Ag) presentation. Fig. 5 illustrates the protocol employed for implanting the nitrocellulose into a mouse and subsequent steps in monoclonal antibody (MoAb) production. Positive hybridoma wells (identified by indirect immunofluorescence of hybridoma supernates against histological sections of first cleavage eggs) were cloned by limited dilution.

From a screening of 182 clones 14 MoAbs were produced. Two (H8a and C7e) were chosen for detailed analysis while several of the remainder (e.g. F9b) were only occasionally studied. The criteria employed for choosing those two MoAbs are listed in Table 2. Western blots are illustrated in Fig. 6. MoAb C7e consistently displayed only a single major band (approx. $55\,000 M_r$) against fertilized egg extracts. MoAb H8a displayed a single major band (approx. $40\,000 M_r$) and a minor band (approx. $30\,000 M_r$). The primary antigens recognized by those MoAbs in egg extracts range, therefore, from $40\,000$ to $55\,000 M_r$. That range coincides with the upper and lower M_r range of the area of the 2D-gel employed for immunization (Fig. 4).

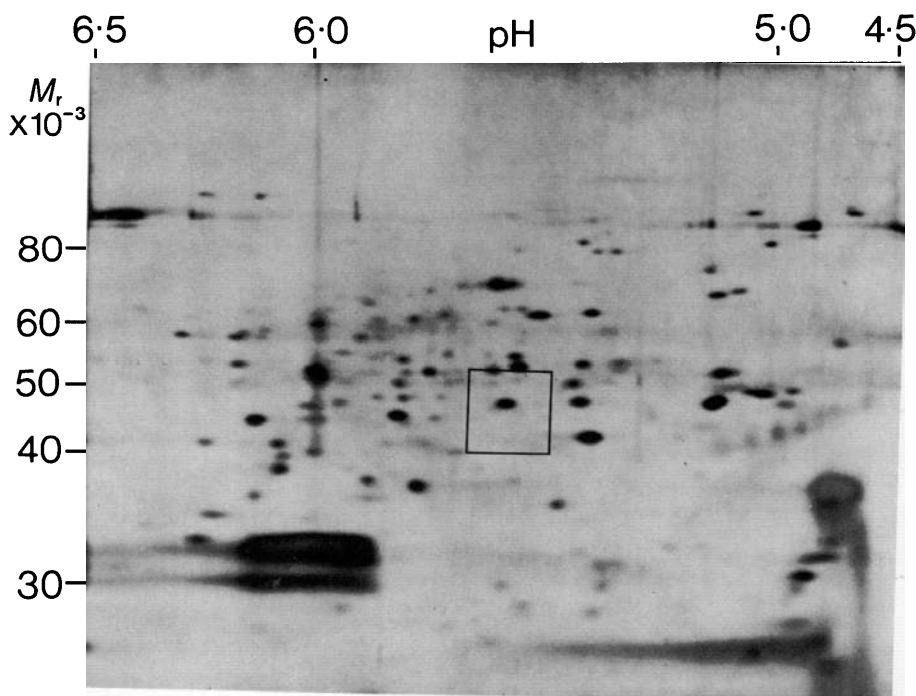


Fig. 4. 2D-gel electrophoresis separation of proteins from three vegetal halves of unfertilized *Xenopus laevis* eggs visualized by silver staining. Area of gel used for implantation is outlined. That area was estimated to contain less than $10\text{ }\mu\text{g}$ protein. It was selected for study because it had been analysed in previous studies (Smith, 1986).

DISTRIBUTION OF NONYOLK ANTIGENS IN FERTILE EGGS

Immunocytochemistry procedures demonstrated that both MoAbs strongly labelled the egg cytoplasm. Neither yolk platelets nor the vitelline membrane

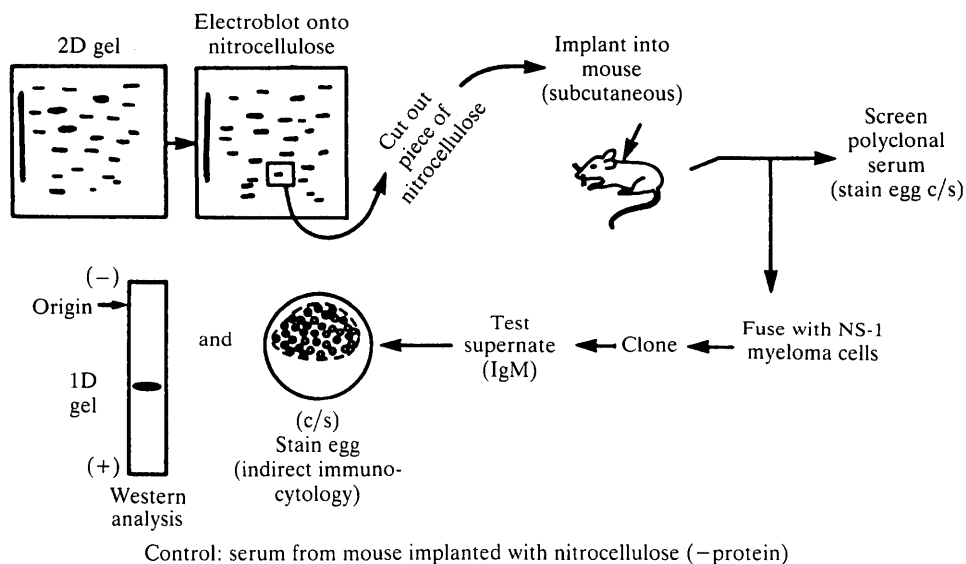


Fig. 5. Scheme for MoAb preparation. Nitrocellulose containing antigens was implanted subcutaneously into the back of the neck, just under the skin, of a mouse (Klymkowsky & Dutcher, 1985). This method provides continuous presentation of the antigen and permits the use of a single implantation of small amounts of antigen. Control sera were prepared from a mouse which received an implant of plain nitrocellulose. Serum was collected at 2-week intervals and tested for polyclonal Abs by indirect immunofluorescence against first cleavage eggs. Preimmune and control implanted sera were negative except for staining of vitelline membrane. Serum from mouse implanted with *Xenopus* proteins showed a response by 2 weeks: for example, animal hemisphere cytoplasm labelled as well as halos around vegetal yolk platelets and vitelline membrane. The response remained the same but increased in intensity over the next 4 weeks. At 7 weeks postimplantation the mouse was sacrificed and fusion of NS-1 myeloma and spleen cells performed.

Table 2. Criteria employed for choice of H8a and C7e MoAb for detailed study

Character	Rationale
Antigen isolated from 2D-gel	Provides information on size and pI of antigen
Single major band on Western blot of first cleavage egg proteins	Indicates high degree of specificity of MoAb (see Fig. 6)
Enrichment (in animal hemisphere)	Useful for analysing relationship to yolk compartments
Staining pattern unchanged re fixative; reproducible among egg batches	Indicates antigen distribution pattern is not an artifact of fixation and subsequent processing of eggs; increases the research potential of the MoAbs

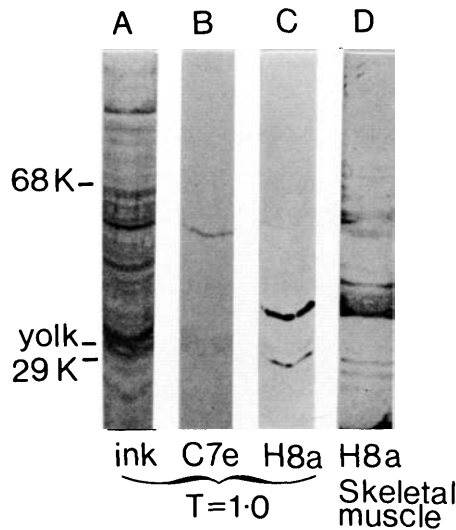


Fig. 6. Western blot analysis of specificity of C7e and H8a MoAbs. Protein extracts were prepared from first cleavage eggs (Smith & Knowland, 1984) and separated on a 12 % SDS-polyacrylamide gel. Each lane contained a protein extract equivalent to 1½ eggs. Lane A represents a control (ink staining of blotted proteins) to demonstrate that transfer of a wide molecular weight range of protein had occurred. Lane B displays staining with C7e; lane C, stained with H8a. Lane D contains adult leg muscle protein extract stained with H8a.

labelled. Furthermore, both displayed a distinct enrichment in the animal hemisphere. From fertilization onwards nuclei were not labelled. Fig. 7 illustrates those features of the labelling patterns. Often, asymmetries in the intensity of the animal hemisphere labelling were observed.

In contrast to the staining patterns included in Fig. 7, an overall labelling was displayed by several other clones (Fig. 8).

Further evidence that H8a and C7e antigens are indeed enriched in the animal hemisphere was obtained by extracting proteins from both animal and vegetal caps and performing a Western analysis. As shown in Fig. 9, MoAb C7e detects more antigen in the animal cap than in the vegetal cap. Since the labelling pattern of H8a is very similar to C7e (Fig. 7) it is highly likely that the antigen recognized by this MoAb is likewise enriched in the animal hemisphere cytoplasm. Preliminary immunoblot data (not shown) of H8a against animal and vegetal cap proteins support that expectation.

Comparison of the C7e and H8a cytological labelling patterns to the yolk compartments (Fig. 1) revealed the following. (1) The 'small yolk mass' labels strongly, while the 'intermediate yolk mass' labels less intensely. (2) The portion of the 'large yolk mass' that borders the IYM also labels (less intensely). (3) Labelling of the more vegetal LYM and the vegetal 'subvitelline layer' is either greatly reduced or absent.

Taken together, the immunocytology and Western data suggest that the region of the egg cytoplasm recognized by MoAbs C7e and H8a defines a nonyolk

compartment. To investigate this possibility the behaviour of those antigens was analysed in inverted eggs. Inverted egg studies permit a determination of whether the antigens move with respect to the gravity vector, as do yolk compartments (Fig. 2), and also whether the antigen-enriched area behaves as an integral mass,

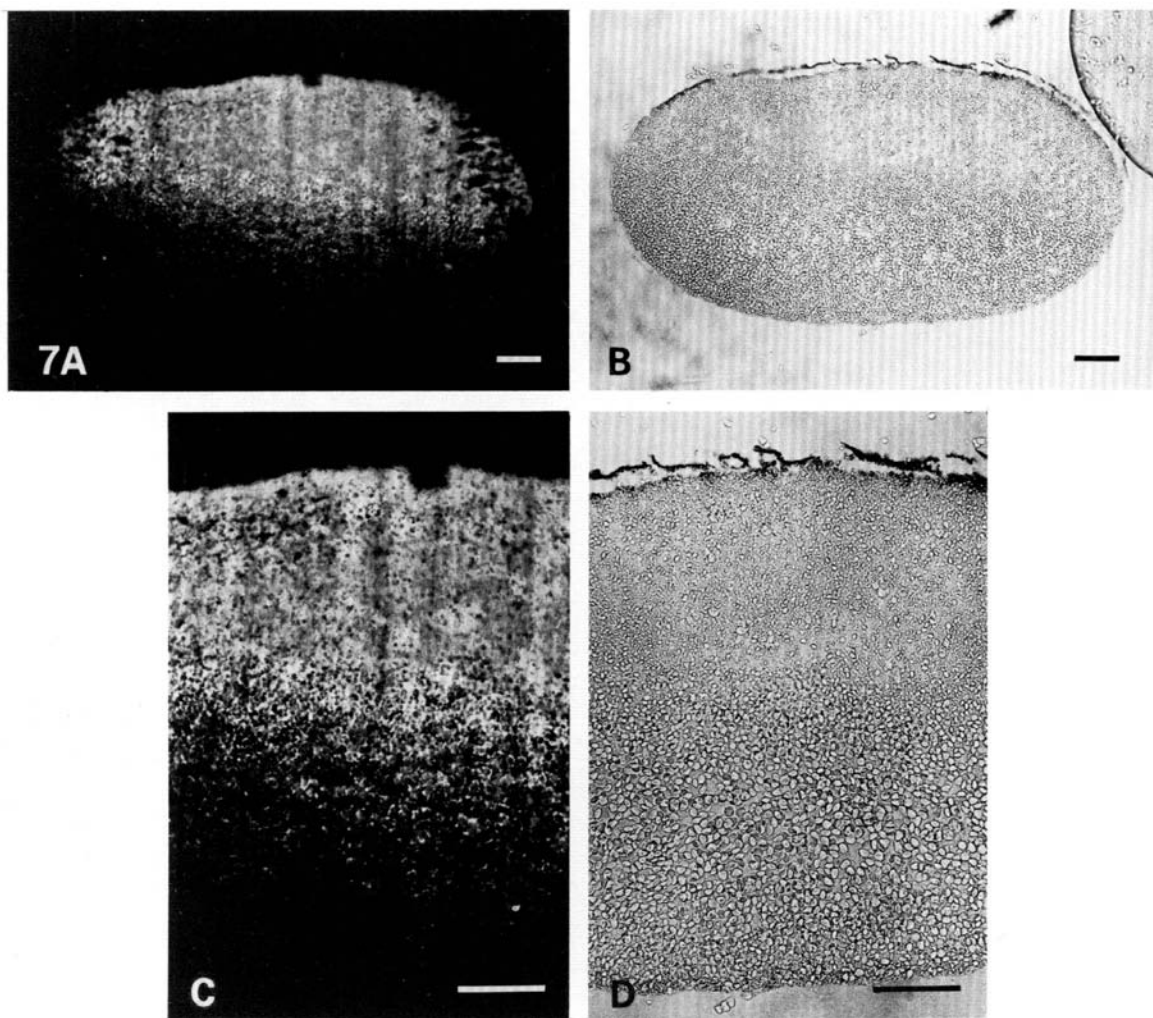


Fig. 7. Indirect immunofluorescence labelling pattern (fertile early first cleavage eggs) of MoAb H8a. A and C are fluorescence micrographs and B and D are corresponding phase-contrast micrographs. Note that the antigen recognized by the H8a MoAb is not restricted to a single compartment. Enlargements (C,D) show the labelling of the SYM, IYM and the adjacent LYM. MoAb C7e showed a similar pattern. This labelling pattern was reproducible in a variety of fixatives (e.g. 5% trichloroacetic acid, 0.5% glutaraldehyde, 4% paraformaldehyde). Eggs were fixed in methanol, paraffin embedded, and sectioned ($5\mu\text{m}$) by standard histological methods. Standard indirect immunofluorescence utilizing undiluted supernate as the primary Ab and FITC-conjugated rabbit anti-mouse IgG (whole molecule, Sigma) (1:100 dilution) was used as the secondary Ab (Smith & Neff, 1985). Bars, $100\mu\text{m}$.

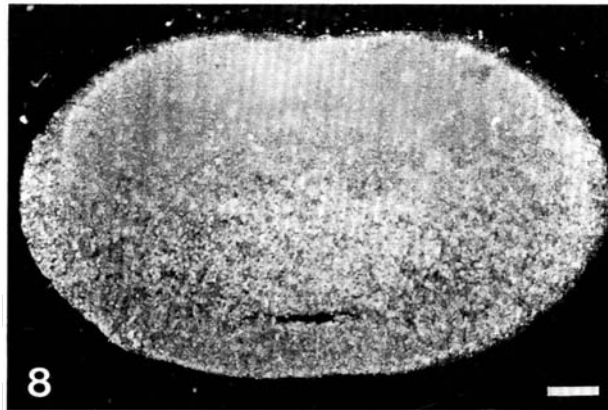


Fig. 8. Overall labelling pattern displayed by several clones obtained from the same myeloma fusion which yielded H8a and C7e. This staining pattern is markedly different, even at various dilutions of MoAb, from those shown in Fig. 7. Bar, 100 μ m.

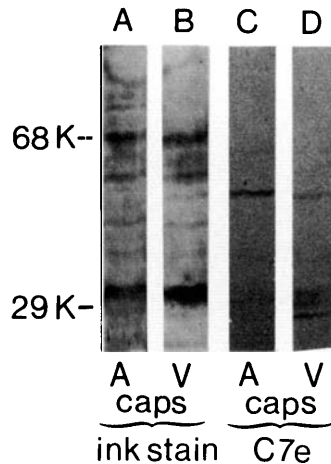


Fig. 9. Immunoblots of proteins extracted from animal (top third) and vegetal (bottom third) fertile egg caps, demonstrating animal hemisphere enrichment of C7e antigens. Equal amounts of extracted proteins were run in each gel lane, and transferred to nitrocellulose. Lanes A and B illustrate ink staining of proteins transferred from animal and vegetal caps, respectively. Lane C represents an immunoblot of C7e against animal cap proteins (see lane A), and lane D against vegetal cap proteins (see lane B).

like the yolk compartment. The effect of egg inversion on the distribution of antigens recognized by C7e and H8a is illustrated in Fig. 10. Both antigens displayed similar behaviour. By first cleavage they had moved (as a mass) into the original vegetal hemisphere, and there displayed much the same geometry as they did in their original animal hemisphere location. Neither antigen, however, penetrated the yolk compartments that line the original vegetal hemisphere (SVL and RVM – see Fig. 3).

The displacement of these proteins against the gravity vector as a coherent mass provides the first indication that nonyolk protein components are organized into

compartments. From the immunocytology and Western blot data (Figs 7, 9) it can furthermore be deduced that this nonyolk compartment is organized separately from the major yolk compartments, since its location transcends at least a portion of each of the major yolk compartments.

ACCUMULATION OF NONYOLK ANTIGEN DURING OOGENESIS

Further information concerning the separate organization of yolk and nonyolk compartments was obtained by tracking C7e and H8a antigen accumulation during oogenesis. A stage series is provided in Fig. 11. Stage I and II oocytes display an overall cytoplasmic labelling pattern, indicating that antigen accumulation and distribution begins prior to yolk platelet deposition, which begins at approximately stage 3. In fact, the smallest oocytes we could reliably identify (approx. 30 μm diameter) showed cytoplasmic labelling. MoAb C7e did not stain the nucleus of

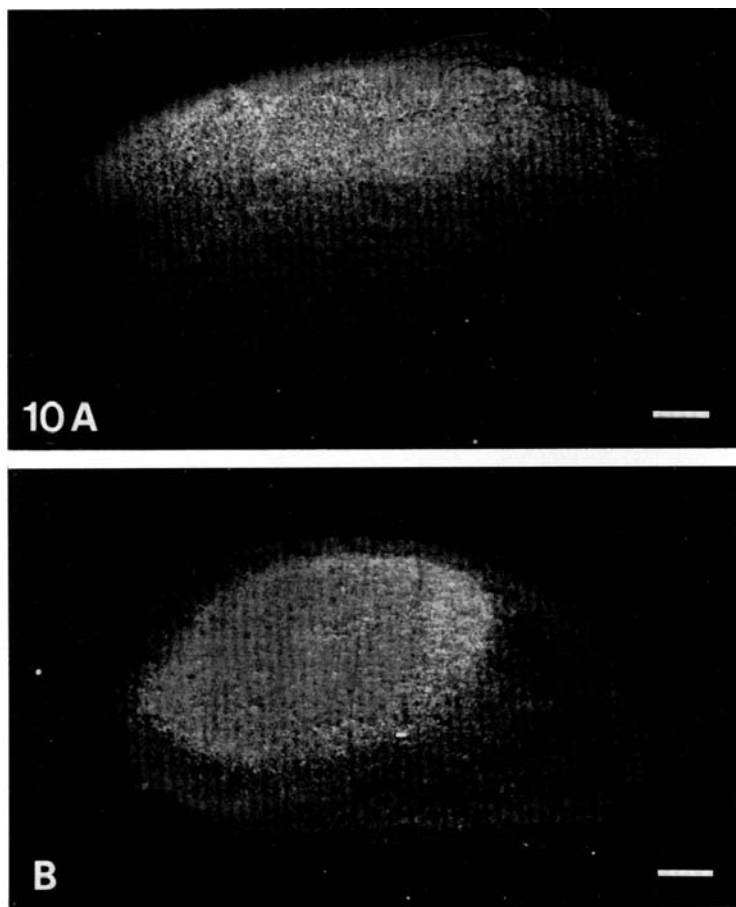


Fig. 10. Displacement of H8a antigen from the original animal hemisphere of a naturally oriented egg (A) to the opposing gravity (original vegetal) hemisphere of an inverted fertile egg (B). Note the SVL and RVM of the inverted egg do not stain. Bars, 100 μm .

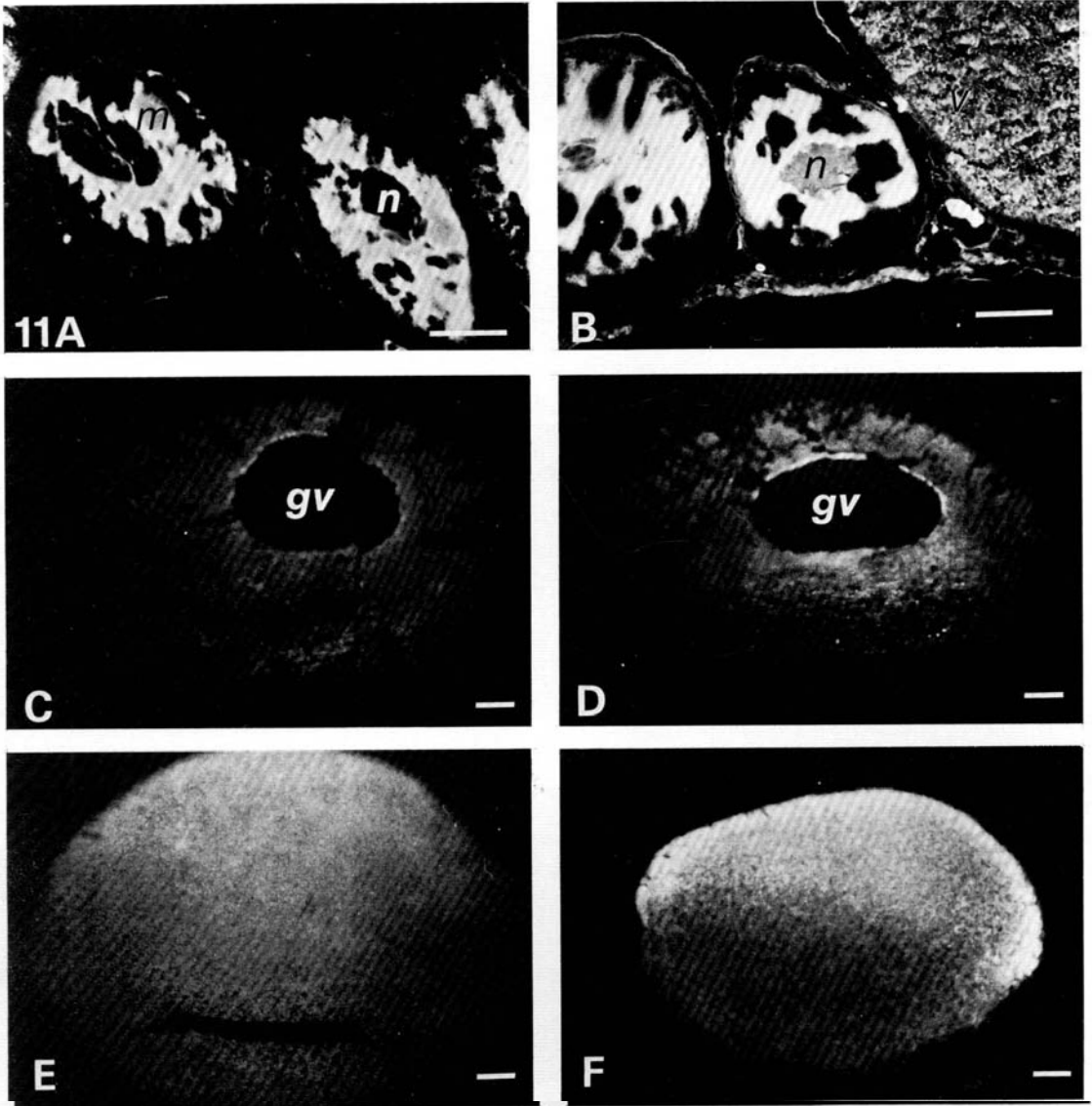


Fig. 11. Immunocytochemical distribution of H8a and C7e antigen distribution during oogenesis. A and B show previtellogenic oocytes, C and D are stage VI (Dumont, 1972), and E and F mature oocytes. A,C,E were stained with MoAb C7e, while B,D,F were labelled with MoAb H8a. *gv*, germinal vesicle; *n*, nucleus; *mc*, mitochondrial cloud; *v*, vegetal portion of stage VI oocyte. Empty spaces within oocytes and between *gv* and cytoplasm are a shrinkage artifact of methanol fixation. Bars, 100 μ m.

previtellogenic oocytes. In contrast, H8a labelled the nucleus (not including the nucleoli). That labelling difference is, however, not surprising since Western blot data (Fig. 6) demonstrated that those antibodies recognize proteins of different molecular weights. Labelling of the nucleus by H8a gradually decreases as the

oocyte enlarges so that nuclei of stage VI oocytes appear devoid of antigen, and virtually identical to nuclei stained with C7e. This apparent differential labelling of early oocyte nuclei by the two antibodies was quantified with a photometer (Table 3). Whether the decrease in nuclear labelling by H8a as oogenesis proceeds is due to degradation or export from the nucleus, or dilution as the germinal vesicle volume increases, is unknown. Clearly, however, the antigen is found in the nucleus during the early stages of oogenesis when RNA synthesis is occurring at a maximal rate.

The labelling of oocyte cytoplasm by C7e and H8a continues through oogenesis. Prior to yolk deposition the labelling of cytoplasm is uniform. The mitochondrial cloud (Balbiani body) of previtellogenic oocytes stains, but to a lesser extent than the general cytoplasm. Follicle cells label at an intensity no higher than background, suggesting that these antigens are synthesized within the oocyte rather than transferred from somatic cells.

As yolk platelets are deposited in growing oocytes the MoAbs stain the cytoplasm between the yolk platelets. The A/V polarity in the labelling patterns seen in early first cleavage eggs is built up gradually during oogenesis. The labelling begins as an overall pattern in the early oocyte and eventually changes to an A/V polarization in the mature oocyte. The mechanisms that generate that redistribution are unknown.

FATE OF H8a AND C7e ANTIGENS IN EMBRYOGENESIS

The fate of these antigens was elucidated by examining histological sections of third cleavage, blastula, gastrula, neurula and tailbud embryos (Fig. 12). The following observations were made. (1) Both antigens were recognized at all stages. The presence of antigen in tailbud embryos suggests that resynthesis may occur. (2) The labelling pattern in the various pretailbud-stage embryos could be pre-

Table 3. *Quantification of antigen distribution in oocytes*

Antibody	Previtellogenic oocytes		Stage VI oocytes	
	Cytoplasm	Germinal vesicle	Perinuclear cytoplasm	Germinal vesicle
H8a	30* (12-56)† n = 28‡	15 (4-23) n = 28	38 (28-53) n = 5	8 (7-9) n = 4
C7e	28 (15-36) n = 20	6 (1-10) n = 20	32 (22-43) n = 7	7 (4-9) n = 4

* Relative units with background amount = 0.

† Range.

‡ Sample size.

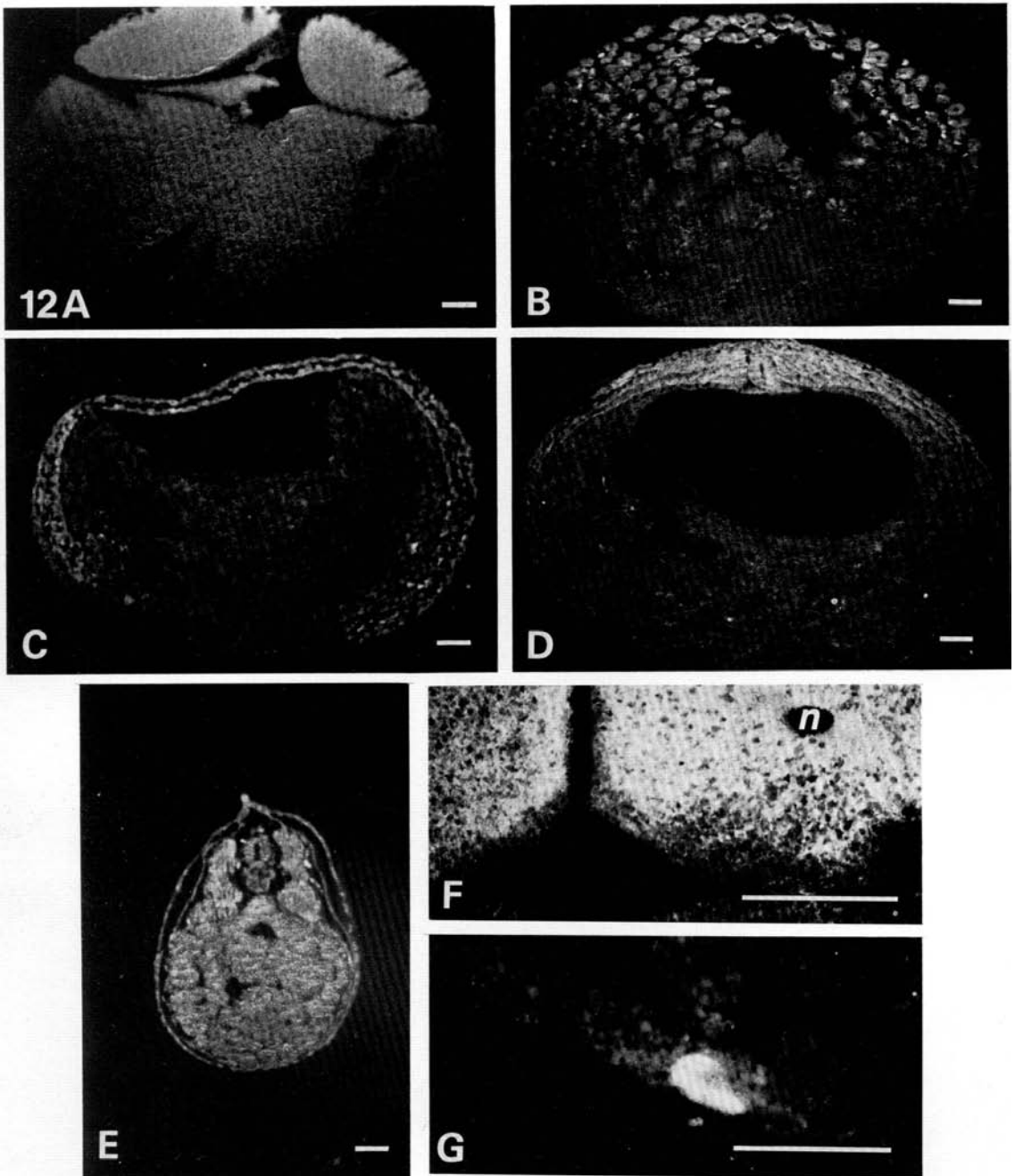


Fig. 12. Distribution of C7e antigen during postfertilization embryogenesis. (A) Third cleavage stage showing animal hemisphere enrichment; (B) blastula depicting continued animal hemisphere enrichment; (C) gastrula illustrating staining of ectoderm; (D) neurula showing dorsal enrichment; (E) tailbud depicting overall labelling; (F) enlarged central region of another third cleavage stage embryo showing absence of nuclear labelling (*n*, nucleus). (G) Enlargement of vegetal region of a blastula embryo showing labelling of the germplasm. H8a antigens exhibited a similar distribution during postfertilization development including absence of nuclear labelling and staining of the germplasm (data not shown). Bars, 100 μ m.

dicted from classical fate maps. Ectoderm and mesoderm label intensely, while endoderm labels only faintly. The general intensity of the label appeared to increase at the tailbud stage, and included increased labelling of the endoderm. (3) Nuclei were never labelled. (4) Strong perinuclear labelling was observed in animal and vegetal cells from early cleavage through the gastrula stage. (5) Both MoAbs labelled the germplasm brightly at the 8-cell and blastula stages.

Labelling of adult tissues was briefly examined in order to establish whether the antigens recognized by C7e and H8a are embryo specific. Table 4 summarizes the data. Most adult tissues displayed labelling. In all cases nuclei were negative.

The increase in general labelling intensity at the tailbud stage and the increase in endoderm labelling along with the labelling of the majority of adult tissues raised the possibility that embryonic or adult antigens recognized by C7e and H8a may differ from the oocyte (maternal) antigens. Preliminary evidence supports this idea. Immunoblots of proteins extracted from adult skeletal muscle, using the same procedure employed for embryos, displayed a number of bands recognized by the antibody (Fig. 6, lane D). Those proteins (or glycoproteins?) are probably related to the antigen recognized in uncleaved eggs, at least to the extent that they contain similar epitopes.

Table 4. *Distribution of C7e and H8a antigens in adult tissues*

Tissue	MoAb	
	H8a	C7e
Skeletal muscle	+++	++
Heart	++	±
Liver	+	±
Kidney	+	±
Gut	n.d.	+
Oviduct (ciliated cells)	++	++
Lung	±	++
Spinal cord/brain	+++	+++
Testes	++	++

± to +++ indicate relative amount of antigens; n.d., not determined.

Table 5. *Summary of evidence that the egg cytoskeleton is involved in compartment organization and rearrangement*

Reference	Comment
Sawai, 1979	Cortical layer contains autonomous programme for contraction
Ubbels <i>et al.</i> 1983	Sperm aster enlargement rearranges animal hemisphere cytoplasm
Elinson, 1985	Microtubules involved in dorsal/ventral polarization
Shinagawa, 1985	Endoplasm contains factors responsible for periodic reorganization
Vincent <i>et al.</i> 1986	In egg surface-immobilized fertile egg there is an active movement of the yolk cytoplasm against the gravity vector, presumably driven by the cytoskeleton

POSSIBLE ROLE OF CYTOSKELETON IN COMPARTMENT ORGANIZATION AND REARRANGEMENTS

The mature oocyte contains large amounts of microtubule (tubulin) (Elinson, 1985) and microfilament (actin) proteins. The extent that they are organized into a cytoskeletal network which permeates the various yolk/nonyolk cytoplasmic compartments is, however, unclear. Some reports claim that an extensive microtubule cytoskeleton is absent (e.g. Burgess & Schroeder, 1979). The observations that compartments become mobilized when an inverted infertile egg is cold shocked (Neff *et al.* 1984), and that the cytoplasm displays a viscosity change following egg activation during fertilization (Elinson, 1983) indicate that most likely some sort of microtubular cytoskeleton, perhaps obscured by yolk platelets, exists. It is certain, however, that microfilaments are abundant in the cortical layer (Gall, Picheral & Govnon, 1983). Also, actin shells appear to be associated with yolk platelets (Colombo, 1983), supporting the possibility that the yolk compartments may be organized by a cytoskeleton. Table 5 summarizes several of the models that employ the cytoskeleton to account for the organization and rearrangement of the egg cytoplasm that follow egg activation and accompany early pattern specification. The evidence that those mechanisms play cause/effect roles in specifying pattern (e.g. dorsal/ventral polarity) remains, however, indirect.

FUTURE DIRECTIONS

Although there is no reasonable basis for speculating that yolk platelets themselves specify early pattern, they may act indirectly by guiding the cytoplasmic rearrangements that follow symmetry breaking and persist through early embryogenesis (e.g. Fig. 12). Evidence that this may indeed be the case comes from experiments in which yolk compartment displacement was monitored in the inverted egg model. Yolk compartment rearrangements preceded reversal of animal/vegetal pattern specification (Neff, Malacinski, Wakahara & Jurand, 1983).

Additional, more direct, evidence will be forthcoming when *Xenopus* eggs are fertilized and monitored during the microgravity of space flight (Souza & Black, 1985). Yolk platelet density varies directly with size (Neff, Smith, Chung & Malacinski, 1984a). Estimates as to whether those density differences drive rearrangements, and the extent to which those rearrangements are prerequisites for pattern formation may emerge from analysis of eggs reared in microgravity.

Likewise, the extent to which nonyolk compartments such as those recognized by C7e and H8a MoAbs must remain intact and/or shift may also be forthcoming from microgravity experiments. Although the amphibian egg has traditionally been viewed as 'regulative' instead of highly 'mosaic' (reviewed by Davidson, 1976), the discovery of the compartment recognized by C7e and H8a suggests that perhaps less discrete, but nevertheless still important, mosaic-like features of amphibian eggs remain to be elucidated. In fact, Cooke & Weber (1985) have recently provided new evidence that dorsoanterior/posterior features of the

amphibian embryo appear to be specified by mosaic cytoplasmic determinants. The discovery of nonyolk compartments, recognized by MoAbs C7e and H8a, as well as the general approach employed in the studies described in this report, may serve to elucidate other mosaic-like features of the amphibian egg.

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REFERENCES

- ANCEL, P. & VINTEMBERGER, P. (1948). Recherches sur le determination de la symetrie bilaterale dans l'oeuf des amphibiens. *Bull. biol. Fr. Belg.* **31**, Suppl. 1-182.
- BILLETT, F. S. & ADAM, E. (1976). The structure of the mitochondrial cloud of *Xenopus laevis* oocytes. *J. Embryol. exp. Morph.* **33**, 697-710.
- BLACK, S. D. & GERHART, J. C. (1985). Experimental control of the site of embryonic axis formation in *Xenopus laevis* eggs centrifuged before first cleavage. *Devl Biol.* **108**, 310-324.
- BORN, G. J. (1885). Biologische Untersuchungen. 1. Über den Einfluss der Schwere auf das Froschei. *Arch. mikrosk. Anat. EntwMech.* **24**, 475-545.
- BOUNOURE, L. (1934). Recherches sur la liguée germinale chez la grenouille rousse aux premiers stades du développement. *Annls Sci. nat. 10e Sér.* **17**, 67-148.
- BRACHET, J. (1977). An old enigma: the grey crescent of amphibian eggs. *Curr. Top. devl Biol.* **11**, 133-186.
- BURGESS, D. R. & SCHROEDER, T. E. (1979). The cytoskeleton and cytomusculature in embryogenesis - an overview. *Methods Achiev. exp. Pathol.* **8**, 171-189.
- CAPCO, D. G. & JEFFERY, W. R. (1982). Transient localizations of messenger RNA in *Xenopus laevis* oocytes. *Devl Biol.* **89**, 1-12.
- CARPENTER, C. D. & KLEIN, W. H. (1982). A gradient of Poly (A)⁺ RNA sequences in *Xenopus laevis* eggs and embryos. *Devl Biol.* **91**, 43-49.
- CHUNG, H.-M. & MALACINSKI, G. M. (1980). Establishment of the dorsal/ventral polarity of the amphibian embryo: use of ultraviolet irradiation and egg rotation as probes. *Devl Biol.* **80**, 120-133.
- COLOMBO, R. (1983). Actin in *Xenopus* yolk platelets: a peculiar and debated presence. *J. Cell Sci.* **63**, 263-270.
- COOKE, J. & WEBBER, J. A. (1985). Dynamics of the control of body pattern in the development of *Xenopus laevis*. I. Timing and pattern in the development of dorsoanterior and posterior blastomere pairs, isolated at the 4-cell stage. *J. Embryol. exp. Morph.* **88**, 85-112.
- DALQ, A. & PASTEELS, J. (1937). Une conception nouvelle des bases physiologiques de la morphogénèse. *Archs Biol., Paris* **48**, 669-710.
- DAVIDSON, E. H. (1976). *Gene Activity in Early Development*, 2nd edn. New York: Academic Press.
- DORFMAN, Y. & CHERDANTSEV, V. G. (1977). Struktura morfogeneticheskikh dvizheniy gastrulyatsii u beskhostykh amfibi. Soobscheniye I. Destabilizatsiya ooplazmaticheskoy segregatsii i drobleniya pod dystviyem klinostatirovaniya. *Ontogenez* **8**, 238-249.
- DUMONT, J. N. (1972). Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morph.* **136**, 153-180.
- ELINSON, R. P. (1983). Cytoplasmic phases in the first cell cycle of the activated frog egg. *Devl Biol.* **100**, 440-451.
- ELINSON, R. P. (1985). Changes in levels of polymeric tubulin associated with activation and dorsoventral polarization of the frog egg. *Devl Biol.* **109**, 224-233.
- GALL, L., PICHERAL, B. & GOVNON, P. (1983). Cytochemical evidence for the presence of intermediate filaments and microfilaments in the egg of *Xenopus laevis*. *Biol. Cell* **47**, 331-342.
- GERHART, J., BLACK, S. & SCHARF, S. (1983). Cellular and pancellular organization of the amphibian embryo. *Modern Cell Biology* **2**, 483-507.

- HARRIS, T. M. (1964). Pregastrular mechanisms in the morphogenesis of the salamander *Ambystoma maculatum*. *Devl Biol.* **10**, 247–268.
- HEASMAN, J., QUARMBY, J. & WYLIE, C. C. (1984). The mitochondrial cloud of *Xenopus* oocytes: the source of germinal granule material. *Devl Biol.* **105**, 458–469.
- HERKOVITS, J. & UBBELS, G. A. (1979). The ultrastructure of the dorsal yolk-free cytoplasm and the immediately surrounding cytoplasm in the symmetrized egg of *Xenopus laevis*. *J. Embryol. exp. Morph.* **51**, 155–164.
- KING, M. L. & BARKLIS, E. (1985). Regional distribution of maternal messenger RNA in the amphibian oocyte. *Devl Biol.* **112**, 203–212.
- KLAGE, J. J. & UBBELS, G. A. (1975). Regional morphological and cytochemical differentiation in the fertilized egg of *Discoglossus pictus* (anura). *Differentiation* **3**, 15–20.
- KLYMKOWSKY, M. W. & DUTCHER, S. K. (1985). Nitrocellulose-immobilization antigen presentation used to generate monoclonal antibodies against *Chlamydomonas reinhardtii* basal bodies. *J. Cell Biol.* **99**, 695a.
- MALACINSKI, G. M. & NEFF, A. W. (1986). Effects of gravity perturbation on developing animal systems. *Adv. Space Res.* (in press).
- MOTOMURA, J. (1935). Determination of the embryonic axis in the eggs of amphibia and echinoderms. *Scient. Rep. Tohoku Univ.* (Ser. 4) **10**, 211–245.
- NEFF, A. W., MALACINSKI, G. M., WAKAHARA, M. & JURAND, A. J. (1983). Pattern formation in amphibian embryos prevented from undergoing the classical 'rotation response' to egg activation. *Devl Biol.* **97**, 103–112.
- NEFF, A. W., SMITH, R., CHUNG, H.-M. & MALACINSKI, G. M. (1984a). Influence of clinostat rotation on fertilized amphibian egg pattern specification. *The Physiologist* **27** S, 139–140.
- NEFF, A. W., WAKAHARA, M., JURAND, A. & MALACINSKI, G. M. (1984b). Experimental analysis of cytoplasmic rearrangements which follow fertilization and accompany symmetrization of inverted *Xenopus* eggs. *J. Embryol. exp. Morph.* **80**, 197–224.
- NIEUWKOOP, P. D. (1956). Are there direct relationships between the cortical layer of the fertilized egg and the development of the future axial system in *Xenopus laevis* embryos? *Pubbl. Staz. zool. Napoli* **28**, 241–249.
- NIEUWKOOP, P. D. (1977). Origin and establishment of embryonic polar axes in amphibian development. *Curr. Top. devl Biol.* **11**, 115–132.
- NIEUWKOOP, P. D., JOHNEN, A. G. & ALBERS, B. (1985). *The Epigenetic Nature of Early Chordate Development*. Cambridge: Cambridge University Press.
- PALEČEK, S., UBBELS, G. A. & RZEHA, K. (1978). Changes of the external and internal pigment pattern upon fertilization in the egg of *Xenopus laevis*. *J. Embryol. exp. Morph.* **45**, 203–214.
- PASTEELS, J. (1941). Recherches sur les facteurs initiaux de la morphogenese chez les Amphibiens anoures. V. Les effets de la pesanteur sur l'oeuf de *Rana fusca* maintenu en position anormale avant la formation du croisant gris. *Archs Biol., Paris* **52**, 321–361.
- PHILLIPS, C. R. (1982). The regional distribution of poly(A) and total RNA concentrations during early *Xenopus* development. *J. exp. Zool.* **223**, 265–275.
- PHILLIPS, C. R. (1985). Spatial changes in Poly (A) concentrations during early embryogenesis in *Xenopus laevis*: analysis by *in situ* hybridization. *Devl Biol.* **109**, 299–310.
- REBAGLIATI, M. R., WEEKS, D. L., HARVEY, R. P. & MELTON, D. A. (1985). Identification and cloning of localized maternal RNAs from *Xenopus* eggs. *Cell* **42**, 769–777.
- SAWAI, T. (1979). Cyclic changes in the cortical layer of non-nucleated fragments of the newt's egg. *J. Embryol. exp. Morph.* **51**, 183–193.
- SCHARF, S. & GERHART, J. C. (1980). Determination of the dorsal–ventral axis in eggs of *Xenopus laevis*: complete rescue of UV-impaired eggs by oblique orientation before first cleavage. *Devl Biol.* **79**, 181–198.
- SCHARF, S. & GERHART, S. (1983). Axis determination in eggs of *Xenopus laevis*: a critical period before first cleavage, identified by the common effect of cold, pressure and ultra-violet irradiation. *Devl Biol.* **99**, 75–87.
- SHINAGAWA, A. (1985). Localization of the factors producing the periodic activities responsible for synchronous cleavage in *Xenopus* embryos. *J. Embryol. exp. Morph.* **85**, 33–46.
- SMITH, R. C. (1986). Protein synthesis and messenger RNA levels along the animal–vegetal axis during early *Xenopus* development. *J. Embryol. exp. Morph.* **96**, 15–35.

- SMITH, R. C. & KNOWLAND, J. (1984). Protein synthesis in dorsal and ventral regions of *Xenopus laevis* embryos in relation to dorsal and ventral differentiation. *Devl Biol.* **103**, 355–368.
- SMITH, R. C. & NEFF, A. W. (1985). Use of the fluorochrome propidium iodide for the identification of *Xenopus* germ plasm during immunofluorescence studies. *Anat. Rec.* **213**, 518–519.
- SMITH, R. & NEFF, A. W. (1986). Organization of *Xenopus* egg cytoplasm: response to simulated microgravity. *J. exp. Zool.* (in press).
- SOUZA, K. A. & BLACK, S. D. (1985). Amphibian fertilization and development in microgravity. *The Physiologist* **28**, S93–S94.
- SUTASURYA, L. A. & NIEUWKOOP, P. D. (1974). The induction of the primordial germ cells in urodeles. *Wilhelm Roux Arch. EntwMech. Org.* **175**, 199–220.
- UBBELS, G. A., HARA, K., KOSTEV, C. H. & KIRSCHNER, M. W. (1983). Evidence for a functional role of the cytoskeleton in determination of the dorsoventral axis in *Xenopus laevis* eggs. *J. Embryol. exp. Morph.* **77**, 15–37.
- VINCENT, J. P., OSTER, G. F. & GERHART, J. C. (1986). Kinematics of gray crescent formation in *Xenopus* eggs: the displacement of subcortical cytoplasm relative to the egg surface. *Devl Biol.* **113**, 484–500.
- WAKAHARA, M., NEFF, A. W. & MALACINSKI, G. M. (1986). Development of delayed gastrulae and permanent blastulae from inverted *Xenopus laevis* eggs. *Acta Embryol. Morph. exp.* (in press).