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Distribution of nuclear proteins during maturation of the *Xenopus* oocyte

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SUMMARY

The internal structure of the *Xenopus* oocyte is reorganized during the hormone-induced egg maturation. A cytological survey of the intracellular movements and changes is described. The behaviour of the nuclear lamina protein and of three nucleoplasmic proteins during these processes was studied by immunocytology. The proteins are finally deposited in the egg in different patterns brought about by their differential behaviour during the process of maturation.

INTRODUCTION

The structural organization of the egg provides the basis from which the complex processes of embryonic pattern formation are initiated. It has been a long and still unsettled dispute as to how elaborate the egg structure is and how far into embryogenesis individual processes are causally linked to the structural pattern of the egg. A localization of molecules of key importance for developmental decisions in specific egg regions has often been assumed and speculations focus on such components which might be involved in gene regulation. Nuclear proteins are the favourite candidates for such molecules.

Recent investigations have shown that the *Xenopus* oocyte nucleus contains a large number of proteins, which, after initiation of development, will be transferred to the nuclei of the embryo (Dreyer, Singer & Hausen, 1981; Dreyer, Wang, Wedlich & Hausen, 1983). During oocyte maturation, when the ultimate structure of the egg is being established, the nucleus breaks down and the nuclear proteins are released into the egg plasm. The germinal vesicle breakdown (GVBD), however, is not the only aspect of the maturation process. Numerous biochemical events were found to occur (for review see Masui & Clarke, 1979). The whole interior of the amphibian oocyte is remodelled by extensive cytoplasmic movements (Tschou-Su & Wang, 1958; Wittek, 1952). New cytological structures are being generated and others disappear (Brachet, Hanoq & van Gansen, 1970; Imoh & Miyazaki, 1984; Bluemink, Hage, van den Hoef & Dictus, 1983; Huchon, Crozet, Cantenot & Ozon, 1981; Imoh, Okamoto & Eguchi, 1983).

Key words: oocyte maturation, nucleoplasmin, lamin L III, Romeis fixative, yolk platelets, germinal vesicle, cytoskeleton.

The aim of this study is to describe the movements and the final localization of individual nuclear proteins in the mature egg and to understand their behaviour in the context of the whole of the structural reorganization of the egg. The internal features of the oocytes at different stages of maturation were monitored with conventional light microscope cytology. Some of our observations corroborate other investigations, although a comprehensive survey for *Xenopus* has not been compiled. To trace the nucleoplasmic proteins, immunohistological techniques were applied using monoclonal antibodies from a library described before (Dreyer *et al.* 1983). To assure that the proteins were kept in their original position during the histological processing, a very quick and rigorous fixation procedure had to be applied, with the drawback that only three antigens could be monitored. The antigenicity of the others was too strongly impaired by the fixative. One of the proteins studied is nucleoplasmin (Laskey, Honda, Mills & Finch, 1978; Krohne & Franke, 1980), the second maps close to protein 4 in a two-dimensional (2D) gel analysis (Dreyer & Hausen, 1983) and the third is a protein of 86 kD, which is localized in the nucleoli of somatic cells. The concentration of all three proteins in the germinal vesicle was high enough to be still detectable after their release into the egg plasm. In addition to these three nucleoplasmic proteins, a component of the nuclear envelope was analysed. By use of a monoclonal antibody described before (Stick & Hausen, in press), it was possible to study lamin L_{III}, the polypeptide constituting the nuclear lamina in the oocyte.

These four proteins are most probably not involved in differential gene expression. Thus, we do not expect that the observations reveal the processes by which the oocyte establishes a pattern of localized 'determinants', but the data suggest the presence of mechanisms which could be used to erect such a pattern during egg maturation.

MATERIALS AND METHODS

Oocytes were liberated from ovaries of adult females by collagenase treatment (Epping & Dumont, 1976). Stage VI oocytes were collected and incubated in 2 μg progesterone ml^{-1} in modified Barth's solution (MBS-H). Samples were taken at different times and fixed with Romeis fixative (25 ml saturated mercuric chloride; 20 ml 5 % trichloroacetic acid; 15 ml 37 % formaldehyde) (Romeis, 1968) for 2 h. They were transferred to 100 % ethanol for 2 h with one change, and then into 50 % Technovit 7100 monomer in ethanol for 1 h and into 100 % Technovit 7100 monomer overnight, before polymerization. Technovit is a glycolmethacrylate (GMA) derivative sold by Kulzer and Co., 6393 Wehrheim, FRG. 5 μm sections were obtained with Ralph's glass knives.

For immunohistological studies, samples were treated as above, but after the ethanol the samples were transferred to 50 % and then 100 % polyester wax (Steedman, 1957).

When the nuclear lamina was to be investigated the formaldehyde was omitted from the fixative.

10 μm sections were obtained from the wax blocks. The plastic sections were stained either with azure B (0·25 g l^{-1} in 0·1 M-citric acid–phosphate pH = 4·3; 4 h) or with azofuchsin/aniline blue/orange G (1 % azofuchsin in 1 % acetic acid for 5 min, then 0·5 % aniline blue, 2 % orange G in 8 % acetic acid for 5 min, then 0·5 % aniline blue in H_2O for 15 min).

The preparation of monoclonal antibodies against nuclear lamina and against the nucleoplasmic proteins, and the immunostaining procedure has been described (Stick & Hausen, 1985; Dreyer *et al.* 1981).

RESULTS

Light microscope cytology

Stage VI oocytes (Dumont, 1972) liberated from the ovary of adult frogs by digestion with collagenase were transferred to MBS-H containing 2 μg progesterone ml^{-1}. Samples were fixed after different times of incubation. Individual oocytes were embedded, sectioned and stained (see materials and methods).

To arrange the specimen in the correct chronological order, the maturation process had to be divided into a series of defined stages. Attempts to define stages according to a simple timing schedule proved to be of no use due to an inherent asynchrony in the oocyte population. Since staging by external criteria as proposed by Huchon *et al.* (Huchon *et al.* 1981) was found to be unreliable, the individual specimens were classified according to the internal criteria described by these authors. Stage a_1:a thick bundle of filaments, probably microtubular in nature, forms underneath the basal rim of the nucleus. Stage a_2:a yolk-free cylinder is formed underneath the nucleus extending into the centre of the oocyte. Stage b (Prometaphase I): the nucleus has dissolved and the chromatin is migrating to the animal pole as a clump of material. Stage c (Metaphase I): the chromosomes are arranged in the metaphase spindle at the animal pole. Stage d (Metaphase II): after formation of the first polar body, the chromosomes are arranged in the second metaphase spindle.

The microscopic studies of GMA-embedded sections stained with azure B or with azofuchsin/orange G/aniline blue concentrated on a number of prominent features, which were followed through the process of oocyte maturation. The results are compiled in Fig. 1. The drawings in this figure were made from coloured photographs of the individual sections, with concomittant control of the section under the microscope.

1. *The yolk-size gradient.* The size of the largest yolk platelets of the different cytoplasmic regions was measured. The measurements within one field of observation showed a standard deviation of approximately 10 %. The data were inserted into the photographs and lines of equal yolk size were constructed by interpolation. As may be seen from Fig. 1-1, the size of the yolk in the oocyte is distributed in a

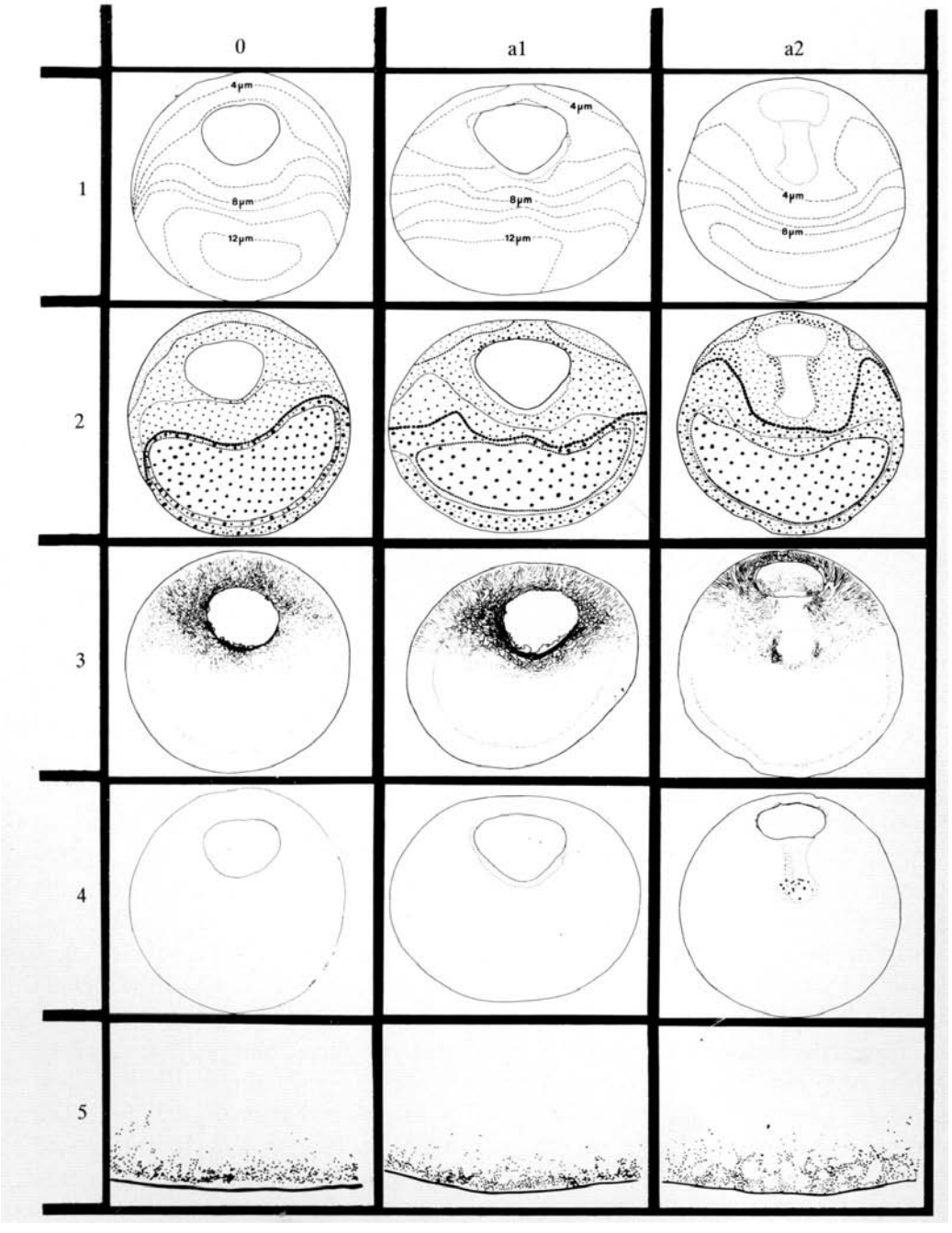
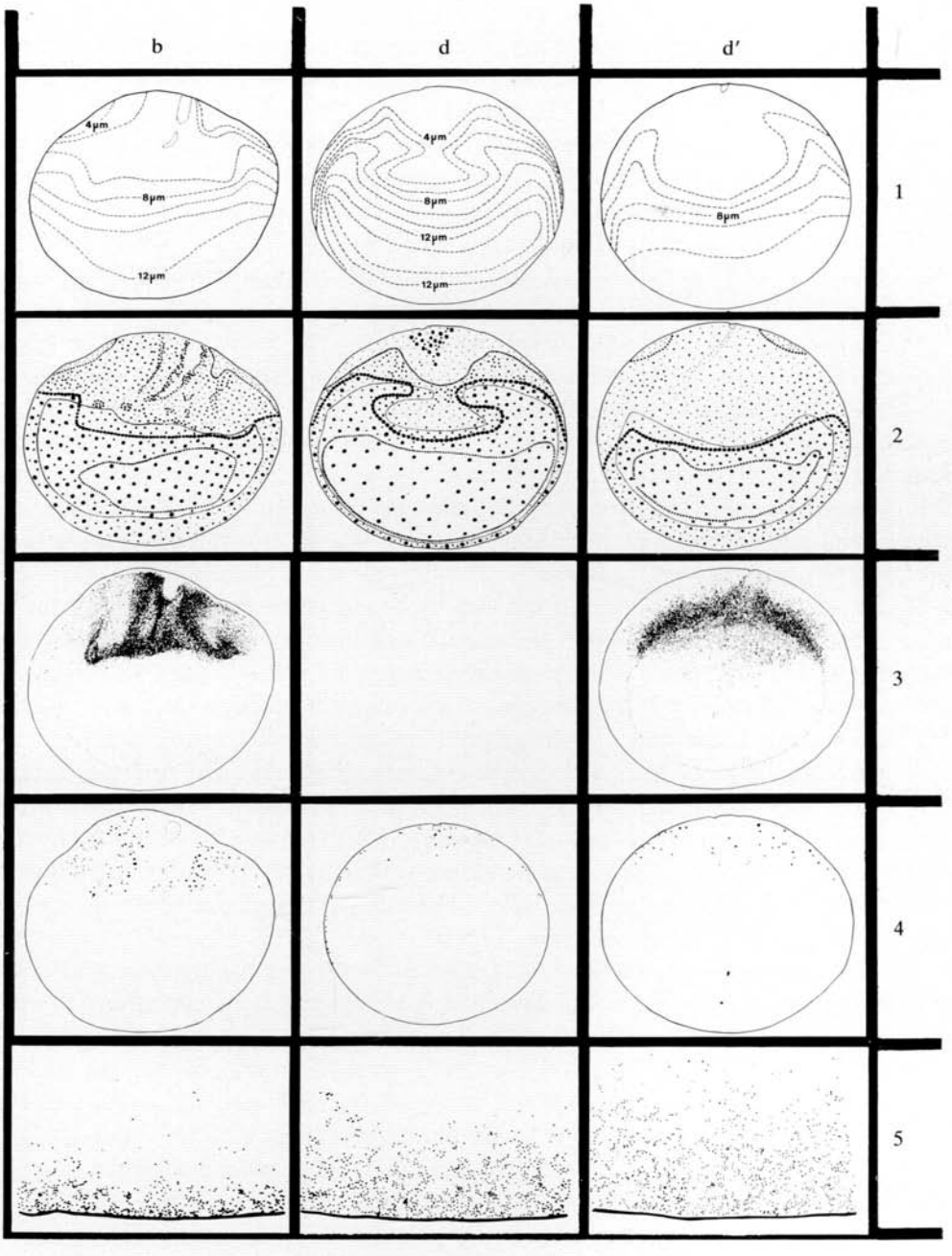


Fig. 1. Changes of the internal structure of oocytes during maturation. Histological sections of oocytes at different stages of maturation were photographed. Drawings were made from the photographs. 0, a_1, a_2, b, d are stages of oocyte maturation, d' is a stage corresponding to d shown to demonstrate variability. Lane 1: the size of the largest yolk platelets in the different regions was determined, and the lines of equal yolk size constructed by interpolation. Lane 2: the yolk platelets were arbitrarily divided into three



size classes, which are indicated by large (> 8 μm), medium (4–8 μm) and fine dots (< 4 μm). These drawings indicate the intermingling of the yolk. Lane 3: distribution of fibrillar material. Lane 4: distribution of yolk-free patches. Lane 5: inward migration of vegetal pigment granula at higher magnification.

fairly smooth gradient extending from 4 μm at the animal pole to 12 μm at the vegetal pole. Thus, specific yolk size values could be ascribed to every level of the animal vegetal axis of the oocyte. This finding enables us to trace movements of the cytoplasm during maturation by changes of the yolk distribution.

With progression of egg maturation this rather defined yolk pattern of the oocyte becomes distorted. Beginning with the appearance of the fibre bundle at the basal rim of the nucleus, the yolk from this region is dislocated sidewards. This process becomes even more obvious when the subnuclear yolk-free cylinder has formed. As the germinal vesicle moves towards the animal pole, the apical yolk also becomes displaced. When the germinal vesicle has disappeared, a zone of small yolk forms in the centre of the egg engulfed by regions of larger yolk. Some variability of these movements is common. An extreme case in which an egg shows the loss of most of the larger yolk from the animal hemisphere is presented in Fig. 1-1d.

2. *Intermingling of yolk size classes.* The above description might give the impression that the yolk-laden cytoplasm moves as a coherent viscous mass. That this is not so may be concluded from the following observations. The yolk platelets were categorized as large, medium and small. The distribution of yolk of these sizes in the different cytoplasmic regions is given in Fig. 1-2.

In the oocyte the zones of small and large yolk are well separated by the zone of medium sized yolk. The marginal and vegetal subcortical region is an exception to this, as it harbours all three yolk categories (Czołowska, 1969). When the large yolk moves upwards laterally it intermingles with the zone of medium sized yolk. At the end of maturation, the animal hemisphere contains yolk of all size classes.

Of particular interest is a collection of large and medium sized yolk platelets at the animal pole of the mature egg. They are in all probability derived from the centre of the cell from where they have moved upwards passing the subnuclear yolk-free zone of the nucleus. The location of these yolk platelets, probably in the course of movement, is depicted in Figs 1–2a_2. The vegetal cytoplasm seems not to be involved in any of these movements.

3. *Fibrillar structures.* Generally, such cytoplasmic movements are the result of the activity of the cytoskeletal system. Indications of this system may be observed even with the light microscope. Staining with azure B reveals a system of fibrillar structures arranged in an elaborate architecture (Figs 1–3). In the oocyte, the nuclear region appears as a centre from which a system of fibrils extends radially to the animal cortex and to a lesser extent in the vegetal direction. The vegetal half of the oocyte seems to be free of such directed fibrils. The vegetal ectoplasm is more intensely stained by azure B than the endoplasm.

The fibrillar system becomes more pronounced at stages a_1 and a_2 of the maturation process. The newly formed bundle of fibres, tangential to the basal rim of the nucleus, is found to be connected up with the existing network. Later on, fibrils intrude into the nucleus from the basal side. They seemingly keep continuity with the tangential fibrils and those lateral to the nucleus. The cytoplasmic fibrillar system gives the impression of being directed towards the animal pole. At later

stages, the distinct fibrils disappear finally leaving a fairly unstructured mass of remnants.

4. *Yolk-free patches*. During maturation, a number of '*yolk-free patches*' (Imoh & Miyazaki, 1984) of various sizes appear in the oocyte. They seem to originate in the subnuclear yolk-free zone. At later stages they spread over the animal hemisphere (Figs 1–4).

5. *Vegetal pigment granula*. Although the vegetal hemisphere is not so obviously affected by the movements of the yolk and the fibrillar network, some changes could be detected in the vegetal cortical zone. This zone contains pigment granula, though sparse as compared to the animal cortex. The granula are located in the vicinity of the outer membrane from where they move inwards during the later phases of maturation (Figs 1–5).

Immunofluorescent studies of nuclear proteins

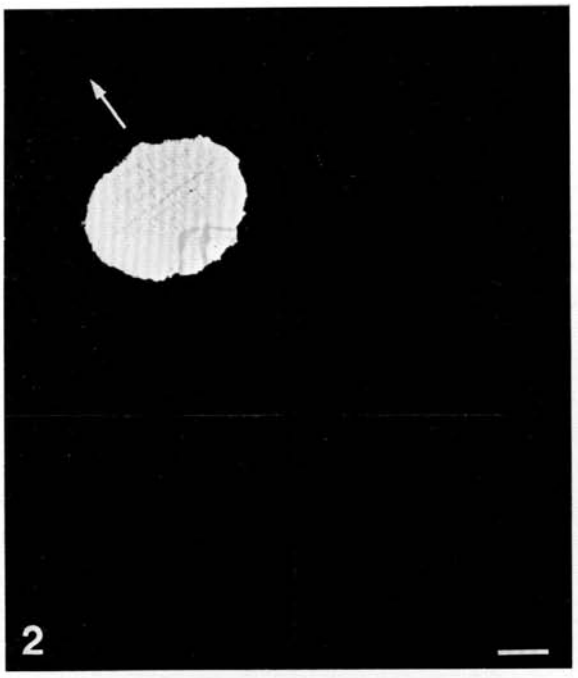
The studies have been performed with four different monoclonal antibodies. mAB L6-8A7 reacts with lamin L_{III}. This polypeptide forms the nuclear lamina in the oocyte and is found in a dissociated form in the egg plasma after maturation (Stick & Hausen, 1985). mAB b7-1D1 reacts with nucleoplasmin, a protein supposed to bind histones (Kleinschmidt *et al.* 1985) and to be involved in the generation of nucleosomes during cleavage (Laskey *et al.* 1978). This protein constitutes approximately 10 % of the total nucleoplasm of the oocyte. mAB b2-2B10 reacts on immunoblots with a polypeptide of 94 kD and, to a lesser extent, with the nuclear peptide 14 (for nomenclature see Dreyer & Hausen, 1983). mAB b6-6E7 binds to a protein (86 kD) that is located in the nucleoli and nucleoplasm of the oocyte and is later found in the nucleoli of somatic cells (Dreyer *et al.* 1983).

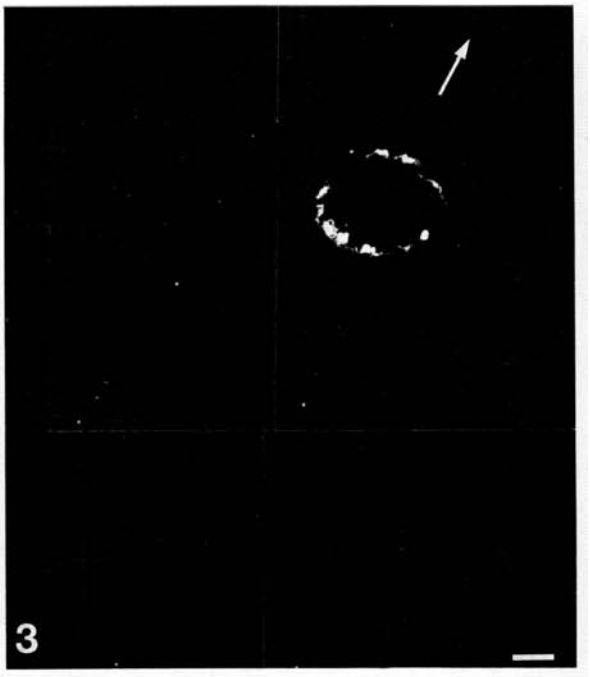
The latter three antibodies show a good reaction in immunofluorescence in sections of oocytes fixed and dehydrated according to Romeis, the same procedure which yields a high quality of structural preservation with GMA embedding. For immunostaining, however, the specimens had to be embedded in polyester wax.

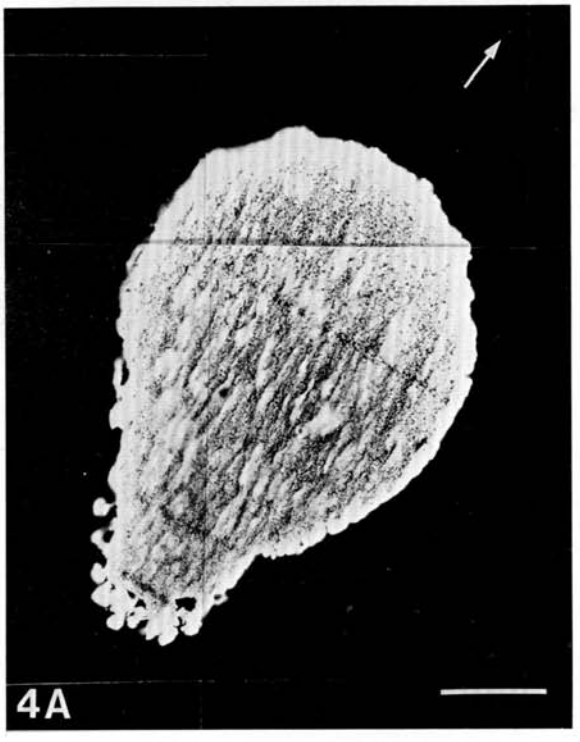
Lamin L_{III} loses the property of binding the antibody after treatment with Romeis fixative, so a milder procedure had to be used (see materials and methods). For this reason, the behaviour of the nuclear lamina and that of the nucleoplasmic proteins could not be compared in sections from the same specimen.

Fig. 2 shows a section of an oocyte stained with the anti-nucleoplasmin antibody, and Fig. 3 shows a section stained with anti-lamin antibody. The other two antibodies directed against nucleoplasmic proteins showed the same distribution as nucleoplasmin. These results indicate that lamin L_{III} is found exclusively in the nuclear envelope (Stick & Hausen, 1980; Stick & Krohne, 1982) and that the nucleoplasmic proteins are strictly confined to the nucleus. This was a precondition for our aim of tracing the movements of the antigens during the process of maturation.

At stage a_1 when the bundle of tangential fibres has appeared, the nuclear envelope begins to decay at the basal side by forming small antigen-containing







Figs 2–4

evaginations (Fig. 4A and B). All three of the nucleoplasmic proteins give the same appearance. The space harbouring the bundle of filaments beneath the nucleus is apparently the site into which the proteins are released thereafter (Fig. 5). At later stages antigen b2-2B10 is deposited along the fibrils, which originate from the basal and lateral nuclear region into the apical cytoplasm (Figs 6, 7). Whereas at the beginning of this movement the apical region between nucleus and plasma membrane is still free of antigen (Figs 5, 6), this zone accumulates the antigen later on (Fig. 8). The pictures obtained for antigen b6-6E7 were essentially identical to those of b2-2B10.

The distribution of nucleoplasmin in the maturing oocyte differs from that of the above-described antigens. Fig. 9 depicts a staining with anti-nucleoplasmin antibody b7-1D1. This section was adjacent to the one shown in Fig. 6. The antigen is deposited basally and on both sides of the nucleus. The decoration of the fibrils is much less prominent than in the staining with b2-2B10. An unstained area extends in a wave-like fashion on both sides of the nucleus. At the stage where a prominent subnuclear yolk-free cylinder has formed, the antigen has spread over the whole animal half. The unstained wave-like band is now found in a more vegetal position (Fig. 10). At later phases of the nuclear breakdown the staining extends into the marginal zone at the border between the cortical ectoplasm and the endoplasm (Fig. 11, see also Fig. 13).

The nuclear lamina protein exhibits again a different pattern. As the nucleus disappears from the maturing oocyte, the lamina staining is seen in small patches which seem to be torn towards the centre of the cell in a coordinated fashion (Fig. 12).

To ensure that the ultimate pattern of protein distribution in the matured egg was not an artifactual result of the *in vitro* conditions, eggs were obtained from the coelom of females which had been injected with gonadotropin, and fixed immediately. Fig. 13 and Fig. 14 show staining with antibody b7-1D1 and b2-2B10 of such coelomic eggs. The difference between the distribution of these two antigens is obvious. Antigen b2-2B10 is found mainly in the animal hemisphere with a prominent accumulation at the animal pole. Some cytoplasmic patches show a preferential staining. On the other hand, b7-1D1 seems to surround the vegetal half forming a thin layer separating the cortical from the endoplasmic region. In the animal half it shows a distribution more uniform than b2-2B10.

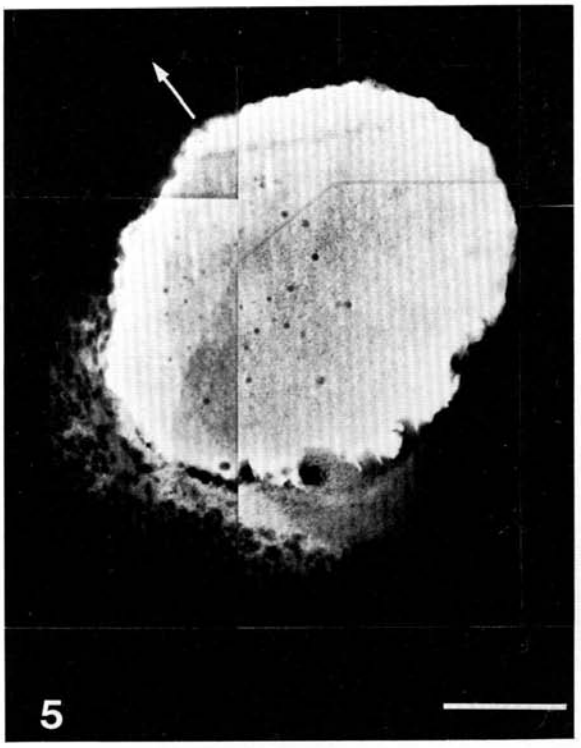
The lamina protein could not be detected in the eggs, probably due to its low concentration after its dispersion in the cytoplasm.

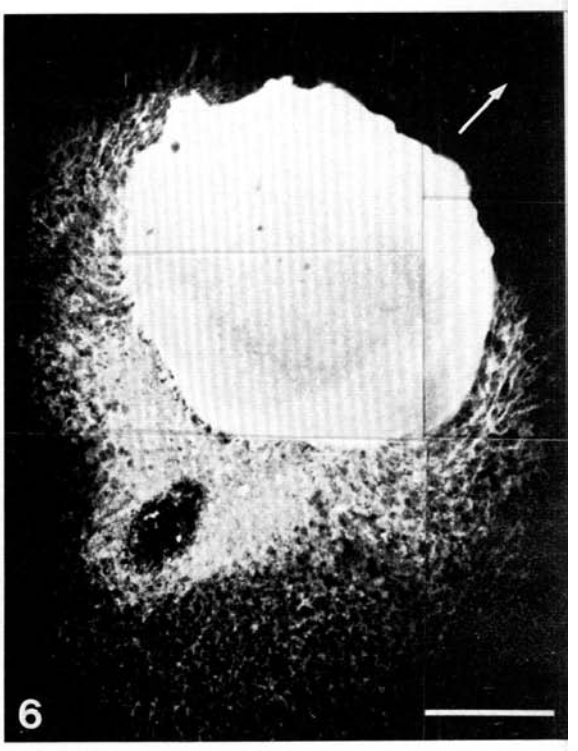
Fig. 2. Staining of a section of a mature oocyte with mAB b7-1D1 directed against nucleoplasmin.

Fig. 3. Staining of a section of a mature oocyte with mAB L6-8A7 directed against lamin L_{III}.

Fig. 4A. Stage a_1 of oocyte maturation. Section of the nucleus stained with antibody b7-1D1 directed against nucleoplasmin.

Fig. 4B. Corresponding phase contrast picture. Bars in all figures represent 100 μm, the arrows indicate the direction of the animal–vegetal axis.





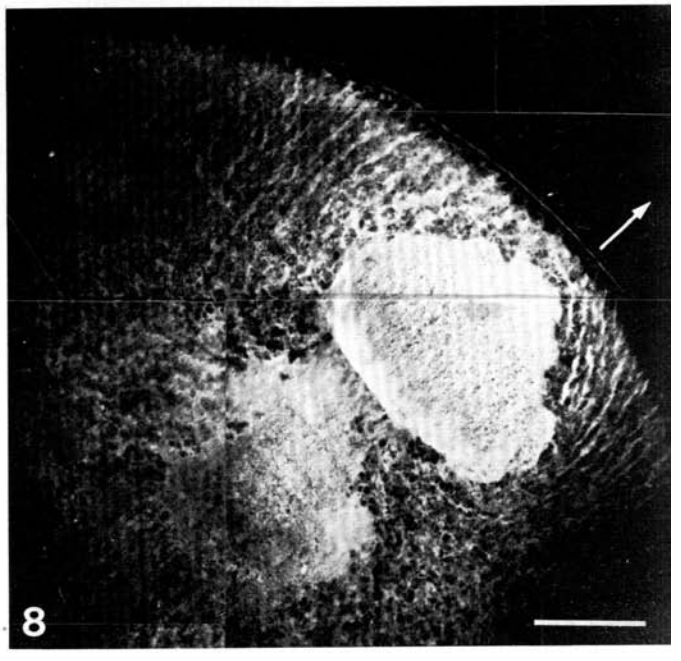


Fig. 5. Stage a_1 of oocyte maturation. Section through the nucleus stained with mAB b2-2B10.
Fig. 6. Early stage a_2 of oocyte maturation. Section through the nucleus stained with mAB b2-2B10.
Fig. 7 and Fig. 8. Stage a_2 of oocyte maturation. Sections through the nuclear area stained with mAB b2-2B10.

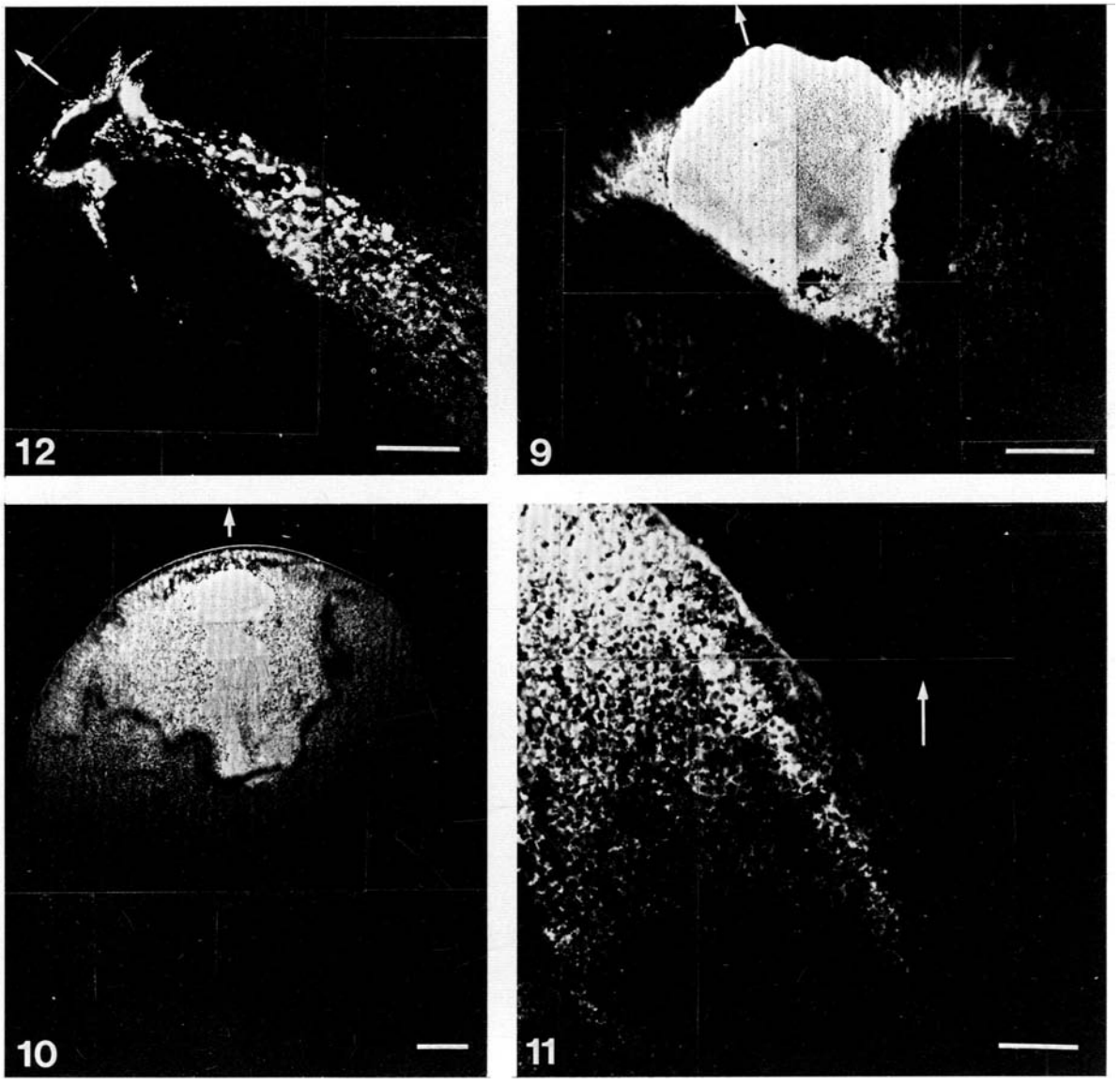
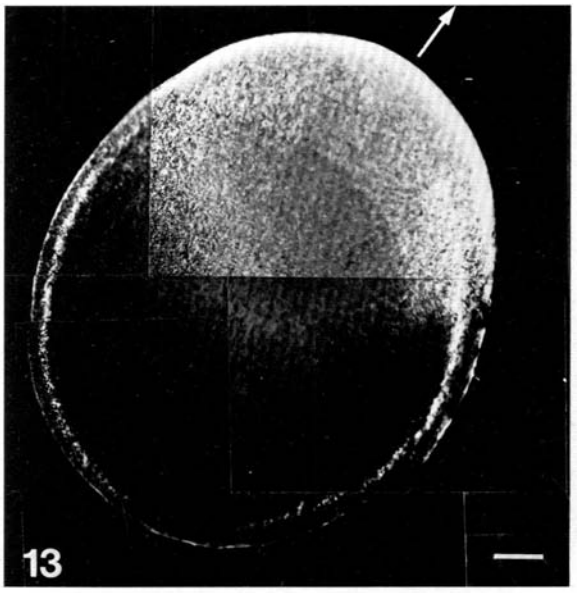


Fig. 9. Early stage a_1 of oocyte maturation. Section adjacent to the one in Fig. 6 stained with mAB b7-1D1.
Fig. 10. Stage a_2 of oocyte maturation. Section stained with mAB b7-1D1.
Fig. 11. Stage c after GVBD. Section of the lateral cortical region stained with mAB b7-1D1.
Fig. 12. Stage b of oocyte maturation. Section through the nuclear area stained with mAB L6-8A7 directed against lamin L_{III}.



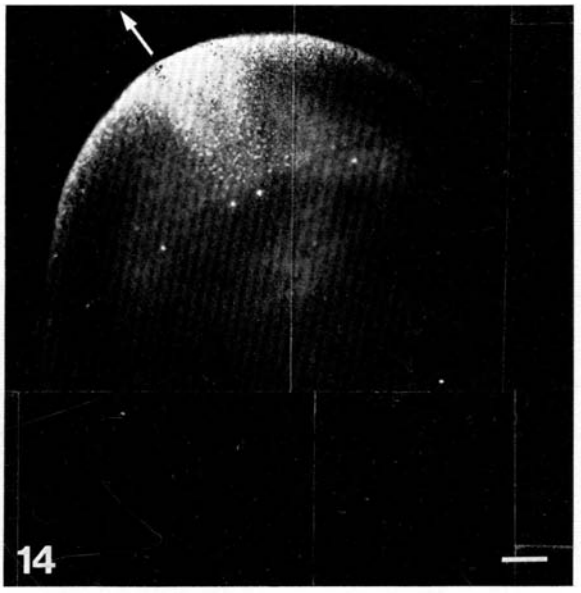


Fig. 13. Section through a coelomic egg (stage d) stained with b7-1D1.
Fig. 14. Section through a coelomic egg (stage d) stained with b2-2B10.

DISCUSSION

Many of the features summarized in Fig. 1 have been described before, although different species have been studied by different investigators. As a precondition for the immunohistological work, we have attempted to construct this summary for *Xenopus*.

Although an intermingling of yolk platelets of different size classes exists in some regions of the oocyte, the underlying pattern of a smooth gradient of yolk size can be recognized if only the largest yolk platelets are taken into account. This observation may give us some clues for future considerations as to how the intracellular pattern of yolk distribution is being organized. In the present context it allows us to trace the cytoplasmic movements in the course of maturation as the individual cytoplasmic regions are labelled by a specific yolk size value.

The appearance of the yolk-free cylinder (Tchou-Su & Wang, 1958; Brachet *et al.* 1970; Huchon *et al.* 1981) in the centre of the cell during germinal vesicle breakdown is apparently counterbalanced by a corresponding upward shift of the yolk mass along the cortical region of the cell (Tchou-Su & Wang, 1958). When this movement comes to a rest after the breakdown of the germinal vesicle, the remnants of the central yolk-free cylinder become filled with some small yolk from the animal hemisphere, generating a region poor in yolk which is surrounded by larger yolk. This central yolk-free cytoplasm has been thought to play an important role in the dorsalization events occurring after fertilization (Herkovits & Ubbels, 1979; Ubbels, 1978; Klag & Ubbels, 1975).

No movements of the yolk could be recognized in the vegetal half of the cell. The mass of large yolk platelets in the central region and the zone of mixed yolk in the cortical region apparently stay in place during the whole maturation process. The observation that an intermingling of yolk from different regions occurs in the animal half during the maturation movements suggests that the individual yolk platelets are not embedded in a coherent cytoplasmic matrix (Colombo, 1983), but that different cytoplasmic regions may penetrate each other or that yolk granules can move independently. The transient yolk-free cylinder contains masses of smooth endoplasmic reticulum (Huchon *et al.* 1981). It seems to be the zone from which the yolk-free patches arise (Imoh & Miyazaki, 1984).

Numerous other events, which are not recorded in Fig. 1, are known to occur during maturation. These include the migration of the chromatin to the animal pole, the disintegration of the nucleoli and the dislocation of the nucleolar DNA (Brachet *et al.* 1970). That all regions of the cell are affected by these events is seen by the inward migration of the vegetal pigment granula and the disintegration of annulate lamellae (Imoh *et al.* 1983; Kessel & Subtelny, 1981) throughout the cell at the time of germinal vesicle breakdown.

These intracellular movements are probably provoked and controlled by cytoskeletal elements. The zone of fibrillar material at the basis of the nucleus, from where the fibrils invade the nuclear area at the climax of the germinal vesicle breakdown, contains numerous microtubules and is possibly organized by a microtubular organization centre localized in this region (Huchon *et al.* 1981; Jessus *et al.* 1984). The fibrils are seemingly connected to or in close contact with an elaborate network of fibrillar material, a prominent part of which is represented by the fibres extending radially from the nucleus to the animal cortex. These fibrils were found to contain vimentin (Godsave, Anderton, Heasman & Wylie, 1984*a*) and cytokeratin (Godsave, Wylie, Lane & Anderton, 1984*b*). The disintegration of these structures during maturation (see Fig. 1) is accompanied by a dislocation of the cytokeratin to the cortex and the integration of the vimentin into a fine network in the interior of the cell (see Wylie *et al.* this volume). These observations underline the dynamic behaviour of the cytoskeleton during these processes. The decrease in the viscosity of egg plasm during maturation may be related to these events (Merriam, 1971).

The behaviour of individual protein species in this interplay of cellular components was studied by immunohistology. The interpretation of these results depends critically on the knowledge of whether the proteins under study retain their antigenicity and whether they are kept in their original position during processing. The latter point is the more critical since the oocytes and eggs do not exhibit the internal compartmentation by cell membranes found in tissues, and so the soluble nucleoplasmic proteins may easily be displaced.

Previously, we have reported that during egg maturation several nucleoplasmic antigens attained a similar distribution, which was comparable to that of b2-2B10 in the present study (Dreyer *et al.* 1983). Since, in the previous work, weaker

fixatives had been applied, nucleoplasmin could not be studied because it is easily redistributed under many conditions. Several fixatives and embedding procedures have therefore been newly investigated. All these experiences have led us to the conclusion that Romeis fixative was the best suited to the antigens studied. We also know from the GMA-embedded sections that structural preservation is good.

At the onset of maturation the nuclear envelope becomes highly involuted at the basal region of the nucleus, with the lateral and apical regions still remaining unaffected. At later times when the nuclear lamina is disintegrating the patches of lamina antigen are drawn in a coordinated way towards the centre of the cell. It should be emphasized that another nuclear element, the chromatin, is displaced to the opposite direction at the same time.

The nucleoplasmic proteins behave very differently during the maturation process. When the basal nuclear envelope assumes the lobed appearance, the individual lobes or cristae are filled with nucleoplasmic protein. The proteins are then released and apparently retained by the fibrous material basally adjacent to the nucleus. From then on nucleoplasmin and the antigens b2-2B10 and b6-6E7 take up deviating pathways. The latter are seen preferentially associated with the fibrillar material extending from the nuclear area into the cytoplasm. The microscopic picture always gives the impression that the proteins move along the fibres towards the animal pole region where they finally accumulate in their highest concentration. Later in the course of maturation and up to the egg stage both antigens are found enriched in cytoplasmic patches, which might be identical with those described above. The quality of the wax sections was not sufficient to prove this point.

Antibody staining of nucleoplasmin revealed much less association of this protein to the fibrils. As it moves out of the nuclear area, a sharply defined unstained borderline appears, which separates the area containing the bulk of the protein from the remainder of the cell. This border moves vegetally until it disappears at the egg stage. As yet there is no satisfactory explanation for this rather strange phenomenon. It demonstrates, however, a further structural feature of the egg during the maturation process. Starting from the animal half, later in maturation the nucleoplasmin antigen begins to move along the borderline between the cortical zone and the endoplasm until finally an antigen-containing layer completely surrounds the interior of the egg vegetally.

These results indicate that different nuclear proteins behave differently during maturation, finally resulting in distinct patterns of distribution. The structured distribution of the antigens during the different phases of maturation, makes it unlikely that diffusion alone is the driving force of these movements.

Several other reports also indicate differential material localizations in the amphibian oocyte and egg. Moen & Namenwirth (1977) reported on the differential distribution of proteins along the animal–vegetal axis in *Xenopus* eggs. Capco and Jeffery (Capco & Jeffery, 1982; Capco, 1982) found a zone of polyA-containing RNA in the cortical region of the oocyte. Interestingly, during maturation, the

polyA$^+$ RNA disappears from this zone, when the nucleoplasmin takes this position. In the axolotl egg a differential distribution of proteins from the germinal vesicle was found (Jäckle & Eagleson, 1980). DNA polymerase α moves from the germinal vesicle to the endoplasmic reticulum (Nagano, Okano, Ikegami & Katagiri, 1982).

All these observations underline the ability of the oocyte to establish spatial patterns of molecular distribution. Such distribution may be the basic pattern from which the morphogenetic processes start. This ooplasmic localization hypothesis has received much attention again in recent times not only for the classical mosaic egg such as that of ascidians (Jeffery, 1984) but also for the amphibian egg (Gurdon, Mohun, Fairman & Brennan, 1985; Kageura & Yamana, 1983). How far the pattern of distribution in the unfertilized egg contributes to such morphogenetic localizations, is still an open question, since other sets of cytoplasmic movements are initiated at fertilization and the first cleavage divisions.

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DISCUSSION

Speaker: P. Hausen (Tubingen)

Question from Elizabeth Jones (Warwick):
In the last few slides you showed the distribution of nucleoplasmin in the cortical region – does that correspond with the region that you mapped by yolk distribution and is there a difference in the mobility of this region?

Answer:
I think that the nucleoplasmin in fact marks the region of the cortical yolk. We don't know whether there is any higher fluidity of this cytoplasm but there is a connection to the animal half. It could be tested with some inert molecules injected into the ooplasm. Maybe somebody has done this already.

Question from J. Tata (NIMR, London):
Was there movement of yolk platelets or were we seeing some kind of temporal pattern of laying down yolk?

Answer:
This gradient which we find might give us some clues as to how the pattern of yolk is being created during oogenesis. Very definitely, within the 6 h before maturation, there is no new synthesis or new production of existing yolk. If you look at this gradient-like pattern, you see the massive movement of the cytoplasm but within this movement – individual yolk platelets can move over fairly long distances and so must be released from their normal surroundings.

Question from D. Smith (Purdue):
Some of the proteins leaving the germinal vesicle may bind specific proteins already organized in the oocytes at stage 6. If one goes back to earlier stages and induces germinal vesicle breakdown, are the distributions the same?

Answer:
We did not think about it and therefore have done nothing.

Question from J. Cooke (NIMR, London):
In Figure 10 you showed us a rather dramatic wave-like darkening around the antigen. Have you looked at this with respect to the other antigens, and is it some sort of limiting barrier?

Answer:
It is difficult to speculate until we have more data. We should, of course, ask the

question whether all antibodies against nucleoplasmin show this pattern, because that would tell us whether it is a zone which does not contain the nucleoplasmin or a zone where the nucleoplasmin is modified in such a way that the antibody does not recognise it.

Question from B. Goodwin (Open University):
You have got Hans Meinhardt quite close to you. I wonder if he disputes your interpretation? He can produce virtually any pattern at all using diffusion. How would you make a conclusive argument to eliminate diffusion?

Answer:
I can't, I'm sorry. The interpretation I gave you was the interpretation of these results. This type of methodology gives you a lot of data, but very soft data. We can contrast this with chemical research which gives less data but of harder character.

Question from R. Laskey (Cambridge):
Have you considered injecting tracer molecules directly into the nucleus to look at their pattern of escaping from the nucleus during breakdown?

Answer:
Yes, we have tried to inject HRP but I am not sure about the significance of the pattern we got. In fact it distributed more to the inside of the cell rather than the cortex, but there is the problem of fixation. As the fixative moves into the oocyte different regions are exposed for different times and the HRP might have been lost from some areas.

Question from H. Woodland (Warwick):
What is the nucleoplasmin distribution in the early embryo and when does it become re-associated with the nuclei?

Answer:
It associates with nuclei very early in the cleavage stage.