The cytoskeleton of *Xenopus* oocytes and its role in development

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SUMMARY

Much is known about determinative events in early amphibian embryos, perhaps more than any other animal group. However, as yet, little attention has been focused on the cytoarchitecture of the oocyte, and the way in which this could regulate asymmetries in the egg, which in turn could lead to developmentally important interactions.

The changing cytoarchitecture of the *Xenopus* oocyte is described with the emphasis on the following:- firstly the polarity; the oocyte is not radially symmetrical at early stages of oogenesis, but shows marked polarity. Secondly, several cytoskeletal elements change their distribution during oogenesis, and again during maturation to form a fertilizable egg. Thirdly, monoclonal antibody methods show that the oocyte develops several asymmetries which are retained in the egg and early embryo, and may be lineage related.

INTRODUCTION

It is clear from many experiments on diverse animal species that regional and cell lineage specifications may be derived initially from asymmetries in the fertilized egg. Evidence for this is both direct and indirect. In the ascidian *Styela*, the cytoplasmic domain of the egg which ends up in developing muscle, is known to be enriched for actin mRNA (Jeffery, Tomlinson & Brodeur, 1983) probably held in the cytoskeleton (Jeffery, 1984). In *Xenopus*, analysis of regions of the egg required for later expression of the α-actin gene, suggests the importance of a particular central area of egg cytoplasm in this process (Gurdon, Mohun, Fairman & Brennan, 1985). Furthermore, the cells of the early blastula derived from the vegetal pole cytoplasm are responsible for inductive signals which establish both the embryonic mesoderm and the dorsal axis (Gimlich & Gerhart, 1984); once again implying the importance of asymmetries in the egg. Despite these, as well as a wealth of other data (see Jeffery & Raff, 1983 for several excellent reviews), little work has been carried out on the generation of such asymmetries during amphibian oogenesis, nor on the role of the cytoskeleton in oocyte cytoarchitecture. Even the origin of the germ plasm during oogenesis, the most celebrated example of localization, was only established recently, as will be described below.

Key words: oocyte, oogenesis, actin, tubulin, vimentin, cytokeratin, cytoplasmic determinants, germ plasm, *Xenopus laevis*

For these reasons, it is becoming increasingly important to study the developing cytoarchitecture of the oocyte, and the basis of cytplasmic regionalizations which develop during oogenesis. This article will review the existing rather fragmentary data on the cytoskeleton of the *Xenopus* oocyte, and give some recent examples of regionalization of the oocyte cytoplasm.

The germ line is, of course, almost continuous throughout the generations, and so any stage of the life cycle will contain some stage of germ cell differentiation. The cytoarchitecture described will therefore rather arbitrarily be restricted to the period of oogenesis between early previtellogenesis to the end of maturation. In *Xenopus*, oocytes are continually formed from a dividing stem cell population, called oogonia, throughout the reproductive period. Most of the period of oogenesis is spent with the chromosomes in prophase of the first meiotic division. Only when oogenesis is complete, and the oocyte ovulated, does it progress through the first meiotic division, during the events of maturation of the oocyte to form a fertilizable egg.

Oogenesis is divided by morphological criteria into six stages, denoted by the Roman numerals I to VI (Dumont, 1972). Details of these stages are described by Dumont but a brief synopsis is as follows.

Stage I. Previtellogenic, during this period the oocyte grows from about $50 \mu m$ to $300 \mu m$ in diameter.

Stage II. Vitellogenesis begins, and the oocyte goes at first cloudy then yellow, by this time it is about $450 \mu m$ in diameter.

Stage III. Pigment appears in the cytoplasm. The oocyte goes gradually dark grey, and grows to $600 \mu m$ in diameter.

Stage IV. The oocyte becomes visibly polarized, due to the pigment accumulating in the future animal hemisphere. It grows to $1000 \mu m$ in diameter.

*Stage V*. This is characterized by further growth to $1200 \mu m$ in diameter.

Stage VI. A white equatorial band appears, separating the animal and vegetal poles. The size is now maximal at $< 1200 \mu m$.

Fig. 1. Nomarski images of living oocytes at early (A), mid (B) and late (C) stage I and early stage II (D). The mitochondrial cloud is arrowed in (A), (B), (D) and can be seen spreading to one pole in (D). The connection of the mitochondrial cloud to the rest of the cytoplasmic matrix can be seen in (B). Mitochondrial aggregates around the nucleus, and connected to the cytoplasmic matrix, are seen in (C). Scale bars = $25 \mu m$.

Fig. 2. Living stage I oocyte stained with rhodamine 123 to show distribution of mitochondria. Staining is seen in the mitochondrial cloud, perinuclear aggregates, and in the follicle cells round the outside. Scale bar = $25 \mu m$.

Fig. 3. Anti-tubulin (kindly donated by Dr K. Fujiwara) staining stage I oocyte. Staining is seen throughout the cytoplasm, but highly concentrated in the cortex, the mitochondrial cloud, and perinuclear aggregates. Scale bar = $40 \mu m$.

Fig. 4. Anti-vimentin (kindly donated by Dr R. O. Hynes) staining of early (A), mid (B) and late (C) previtellogenic oocytes. Staining first appears in a perinuclear ring (A) and becomes concentrated in the mitochondrial cloud (B) perinuclear aggregates (C) and a network of increasing complexity through the cytoplasm (C). Scale bars : (A) = 20 μm, (B) = 47 μm, (C) = 50 μm.
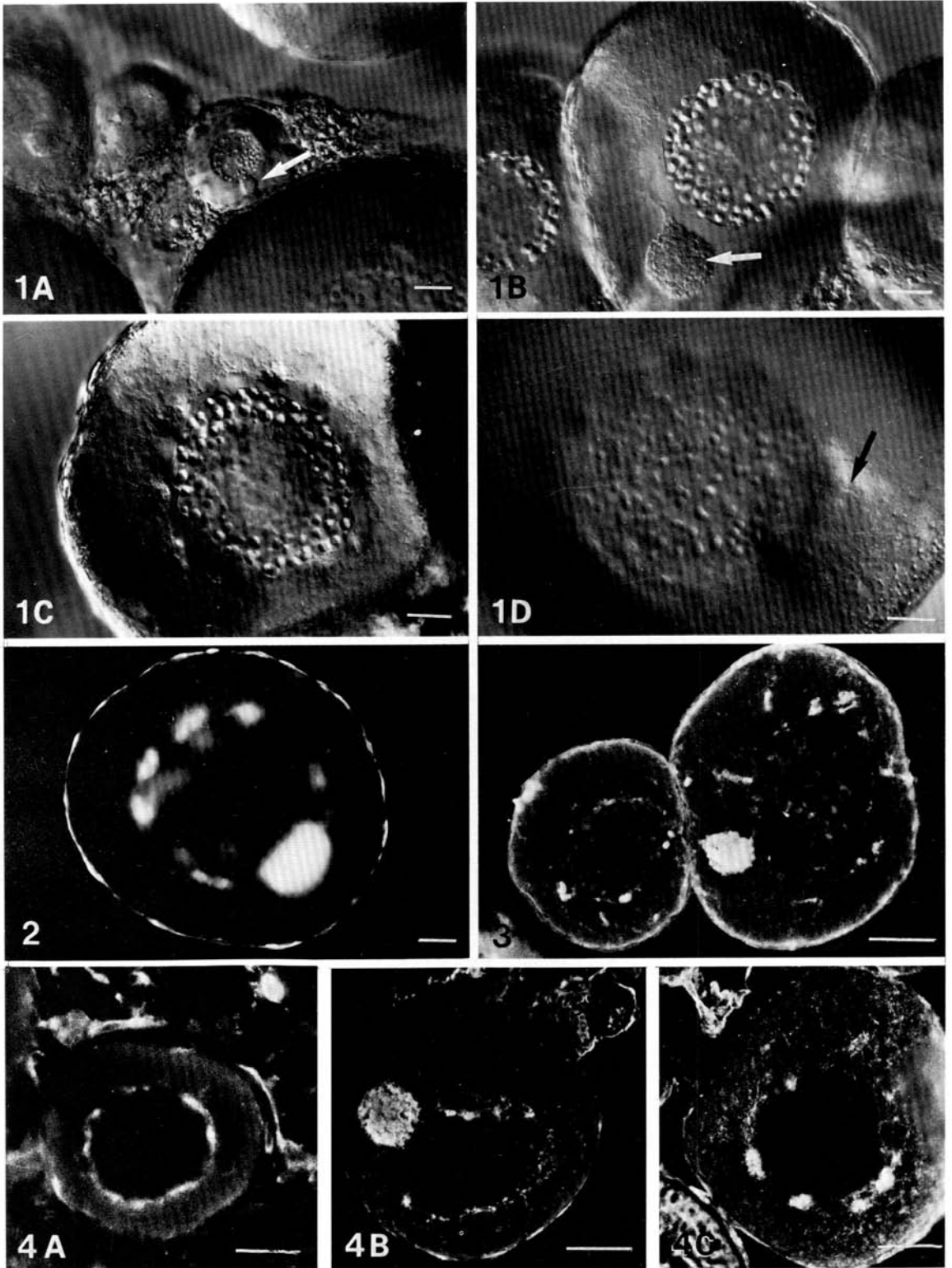
Figs 1–4

THE ROLE OF THE CYTOSKELETON IN THE GROWING OOCYTE

(i) *Previtellogenic stages*

The existence of a cytoskeletal framework, and the structures held in it, can be visualized directly using differential interference optics in the living stage I oocyte. Fig. 1 shows the progressive increase in complexity of the oocyte cytoplasm during stage I. First, a perinuclear ring of filamentous structures appears (Fig. 1A). The mitochondrial cloud is clearly seen in living oocytes, and is attached to this ring. As the oocyte grows, the mitochondrial cloud also grows, remaining attached to the perinuclear ring of filaments (Fig. 1B). At points around this ring, new aggregations of material appear, until a spherical arrangement of these encapsulates the nucleus (Fig. 1C). From these perinuclear masses, filamentous material extends outwards into the more peripheral cytoplasm. At the beginning of stage II, when the oocyte is becoming cloudy with accumulating lipid and yolk, the mitochondrial cloud breaks into smaller aggregates and moves towards the surface of the oocyte (Fig. 1D). The smaller perinuclear aggregates remain around the nucleus.

Several constituents of these structures are now known. Vital staining with rhodamine 123 which is known to be a specific stain for mitochondria in living cells (Johnson, Walsh & Chen, 1980), shows that the perinuclear aggregates and the structures that connect them, as well as the mitochondrial cloud, are rich in mitochondria (Fig. 2). Thus the mitochondrial cloud is not the only source of mitochondria in the developing oocytes, centres of mitochondrial replication or accumulation exist in a spherical array around the nucleus.

Two cytoskeletal elements are associated with these accumulations of mitochondria; tubulin (Fig. 3) and the intermediate filament protein vimentin (Godsave, Anderton, Heasman & Wylie, 1984*b*). Vimentin distribution starts as a perinuclear ring in the smallest stage I oocyte (Fig. 4A). It is present in large amounts in the mitochondrial cloud (Fig. 4B), the smaller perinuclear aggregates (Fig. 4C) and a network of filaments which increases in amount and complexity during stage I (Fig. 4A-C).

Intermediate filaments containing cytokeratin also appear in the oocyte during stage I (Godsave, Wylie, Lane & Anderton, 1984*a*). Initially cortical (Fig. 5A) they spread to form a capsule around the mitochondrial cloud, and appear to divide it into subcompartments (Fig. 5B). During late stage I and stage II, a network of

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Fig. 5. Anti-cytokeratin (kindly donated by Dr E. B. Lane) staining of early (A and B) stage I and vitellogenic stage III (C) oocytes. Cytokeratin filaments first appear cortically (A). They surround and compartmentalize the mitochondrial cloud (B), and eventually spread throughout the cytoplasm (C). Scale bars (A) and (B) = 40 μm, (C) = 50 μm.

Fig. 6. Anti-vimentin (A) and anti-tubulin (B) staining of the mitochondrial cloud fragments as it breaks down spreads to the future vegetal pole. Scale bars = 40 μm.

Fig. 7. Stage I oocytes treated with 10^{-1} M DAC for 18 h. (A) Mitochondrial pattern shown by rhodamine 123, (B) anti-vimentin pattern, (C) Nomarski image of living oocyte, (D) anti-tubulin pattern. Scale bars: (A) = 25 μm, (B), (C), (D) = 40 μm.
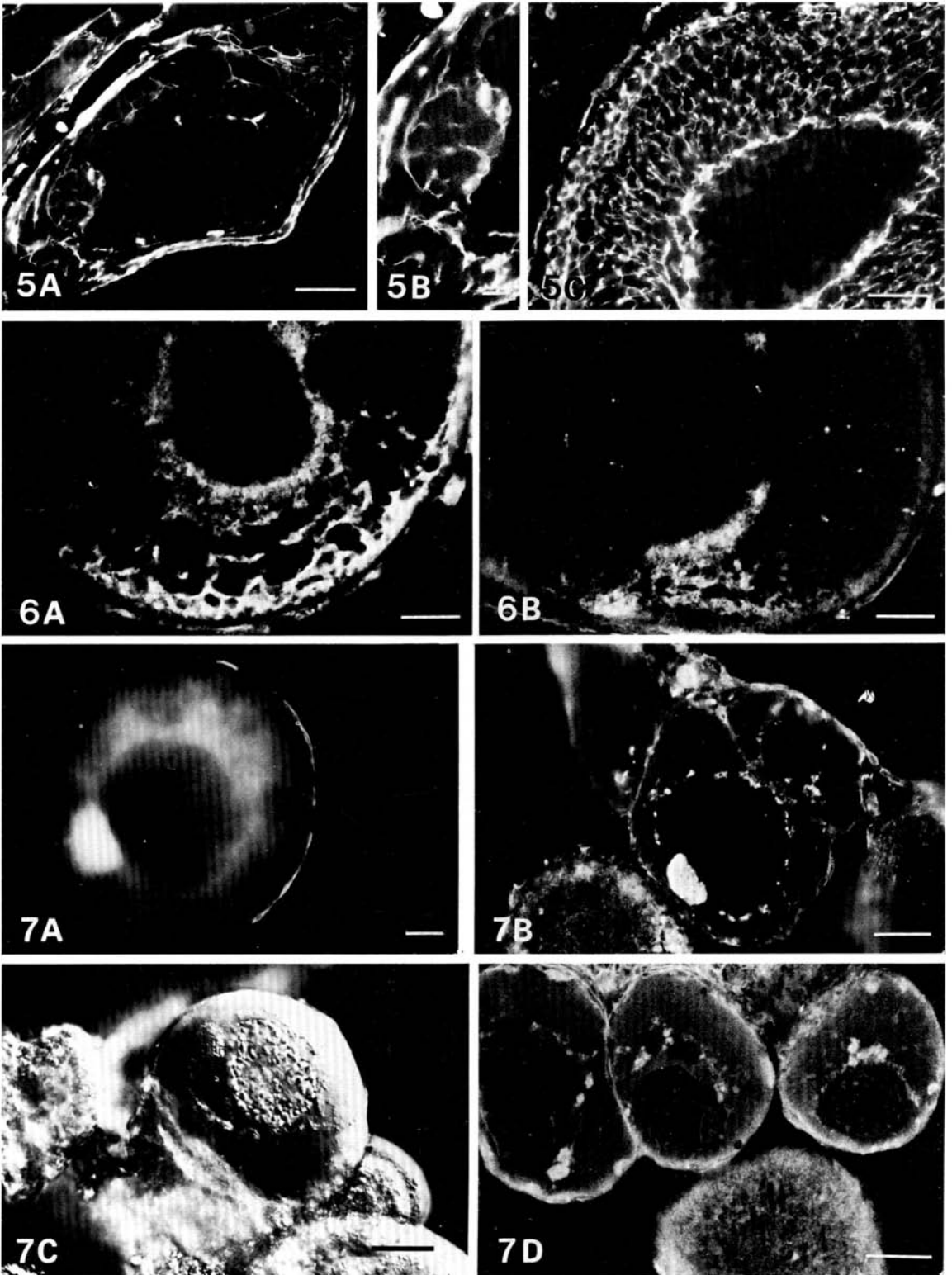
cytokeratin containing filaments spreads to fill the whole oocyte, with particularly high concentrations in the cortex and around the nucleus (Fig. 5C).

During stage I, time-lapse films of living oocytes show this cytoarchitectural arrangement to be extremely stable, although individual filaments may waver around, the overall pattern of nucleus, mitochondrial mass, and mitochondrial aggregates does not change (Heasman, Quarmby & Wylie, 1984). As the mitochondrial cloud breaks down, the masses of tissue derived from it remain attached to each other by strands of material rich in vimentin (Fig. 6A) and tubulin (Fig. 6B). This network of mitochondrial masses remains in one region of the oocyte cortex throughout the rest of oogenesis. This region of the cortex becomes the vegetal pole (Heasman et al. 1984).

During stage I, therefore, the cytoskeleton of the oocyte first becomes obvious. The distribution of tubulin, cytokeratin, and vimentin become highly organized, and concomitantly an oocyte polarity becomes established, which will continue throughout oogenesis and be inherited as the animal/vegetal axis of the egg.

The fact that this polarity is sustained by the cytoskeleton was shown by treating stage I oocytes with 10^{-4} M-des-acetyl colchicine (DAC, Quarmby, Heasman and Wylie, unpublished observations). This analogue of colchicine was used because its effect on the cytoskeleton of fibroblasts has been found to be more reversible than that of colchicine (Thomson & Dabrowska-Bernstein, 1983) due to a lower binding affinity for tubulin. DAC had two major effects on the stage I oocyte, it disrupted the mitochondrial distribution (Fig. 7A, compare with Fig. 2), and the arrangement of both vimentin (Fig. 7B) and tubulin (Fig. 7D). Polarity of the oocyte was also lost. The mitochondrial cloud, associated with collapsed cell components became free to move around the cytoplasm (Fig. 7C).

The conclusion from this is that the very precise cytoarchitecture which appears during stage I is based on the arrangement of microtubules and vimentin-containing filaments.

One interesting observation here was that the integrity of the mitochondrial cloud itself was resistant to colchicine. This is probably due to the fact that it is supported by cytokeratin filaments, which are not disrupted by colchicine or its analogues.

(ii) Vitellogenic stages

The cytoskeletal assembly formed during stage I is used as a scaffolding for organelle accumulation during vitellogenesis. As yolk platelets appear during stage II and later, they are laid down in a network of vimentin-containing filaments which is at first evenly distributed over the oocyte cytoplasm. However, its distribution becomes more polarized as vitellogenesis proceeds, and eventually the animal and vegetal hemispheres come to have different patterns of vimentin (Fig. 8A and 8B Godsave et al. 1984b). Similarly, the cytokeratin pattern changes throughout vitellogenesis (Godsave et al. 1984a). Early in vitellogenesis staining is seen around the cortex and around the nucleus, with sparse fine filaments crossing the cytoplasm

between them. As vitellogenesis proceeds, when yolk platelets are laid down progressively from the cortex inwards, the cytokeratin accumulates most in the regions of yolk-free cytoplasm, quite the opposite of the vimentin pattern. In the full-grown oocyte, cytokeratin remains in the pattern of a cortical shell and a perinuclear shell. In the animal hemisphere relatively straight fibres run radially between these in yolk-free tracts of cytoplasm, (Fig. 8C) whereas in the vegetal hemisphere there is an irregular sparse randomly arranged distribution (Fig. 8D). Throughout oogenesis the distributions of vimentin and cytokeratin are different. Vimentin is concentrated in the yolky cytoplasm between the nucleus and the cell surface, whereas cytokeratin is found both outside and inside this vimentin-rich area as two shells, connected by rather sparse linking filaments.

The presence of cortical cytokeratin in the oocyte, as well as the egg, has also been demonstrated elegantly by immuno-gold staining at EM level (Gall, Picheral & Gounon, 1983). Whilst the presence of cortical actin arranged as a submembranous microfilamentary array which extends into the cores of the microvilli, has been known for some years (Franke *et al.* 1976).

The degree to which the cytoarchitecture of the full-grown oocyte, and its function, are dependent on the cytoskeleton, was tested by treatment with cytochalasin B and colchicine (Colman *et al.* 1981). In this study, the effects were analysed of the two drugs separately and together on both cytoarchitecture at light and electron microscope level, and the ability of the oocyte to secrete proteins synthesized under the direction of exogenous mRNA. Cytochalasin and colchicine together severely reduced secretion of casein, ovalbumin and lysozyme synthesized on injected mRNAs. Cytochalasin had no effect, whereas colchicine caused a smaller, temperature-dependent reduction. The two drugs had mutually distinct effects on oocyte cytoarchitecture. In untreated oocytes the animal hemisphere consists of a highly organized array of organelles. In the cortex are the microvilli, evenly spaced and containing cores of microfilaments, the cortical granules, and pigment granules. Beneath this cortical layer the cytoplasm is divided into regions of yolk-free cytoplasm radiating outwards from the nucleus, between which are islands of yolk platelets (Colman *et al.* 1981; Mohun, Lane, Colman & Wylie, 1981). Cytochalasin causes the pigment layer to bunch into dense aggregates, leaving other areas pigment-free. Colchicine treatment causes the pigment layer to sink deeper into the cytoplasm, and disruption of the organization of the deeper subcortical cytoplasm. Together, the drugs act synergistically. The nucleus loses its shape and floats up towards the surface. The cortical microfilament network and microvilli are also disrupted most with both drugs, though colchicine alone has considerably less effect than cytochalasin alone. Colchicine treatment is known to disrupt vimentin-containing filaments, as well as microtubules (Blose & Chako, 1976; see Anderton, 1981 for review), so the effects of colchicine on the deeper cytoplasm almost certainly reflect this fact, given that the distribution of vimentin closely parallels the general cytoarchitecture of the subcortical animal hemisphere cytoplasm (see Fig. 8A).

Much more work on disruption of the cytoskeleton, preferably with more specific reagents, is required before the full picture emerges as to how the detailed cytoarchitecture is maintained by the different cytoskeletal elements.

(iii) *The oocyte nucleus*

Little work has been done on the existence or role of cytoskeletal elements in the oocyte nucleus. However, several recent reports make this an interesting future area for research. Firstly actin has been shown to be a major protein of amphibian oocyte nuclei (Clark & Rosenbaum, 1979; Krohne & Franke, 1980; Gounon & Karsenti, 1981). Secondly, the injection of anti-actin antibodies directly into the oocyte nucleus causes collapse of the lateral loops of the lampbrush chromosomes and inhibits transcription (Scheer, Hinssen, Franke & Jockusch, 1984). The spatial and temporal pattern by which actin could organize the chromosomal array and its function in the nucleus is unknown.

EVIDENCE FOR REGIONALIZATIONS DURING OOGENESIS WHICH ARE INHERITED BY THE EMBRYO

It is obvious that many structures are regionalized in the oocyte, and play a role in early development which could be described as housekeeping. Examples of these would be cortical granules and pigment granules synthesized during oogenesis and localized to the cortex, asymmetrically in the case of pigment granules. There is less evidence in amphibian oocytes for regionalization of cytoplasm into areas which play a role in establishing cell lineages or regions. It might be envisaged that two types of such regionalization could occur; firstly a determinative one, where a particular regional cytoplasm causes regional or cell lineage restriction in the cells which inherit it. Secondly, a facilitative regionalization could occur, where a particular area of cytoplasm contains a maternal store of molecules useful to a certain cell lineage which becomes determined in that region of the embryo by some other mechanism.

Three examples of fairly large-scale regionalization will be given here, although which of the above types they conform to is not yet known.

Fig. 8. (A) and (B) show anti-vimentin staining of late vitellogenic oocyte in animal (A) and vegetal (B) hemispheres. V = position of vegetal pole, A = position of animal pole. (C) and (D) show anti-cytokeratin staining in animal and vegetal hemispheres respectively. Scale bars (A) and (B) = $25\,\mu$m, (C) and (D) = $50\,\mu$m.

Fig. 9. Shows the germinal ·granules found in the germ plasm of the egg (A) and mitochondrial cloud of stage I oocyte (B). The increased contrast seen in (B) is due to the addition of saponin and tannic acid in the fixative (See Heasman *et al.* 1984 for details). Anti-vimentin staining of the germ plasm of the fertilized egg (C) and 32-cell-stage embryo (A) shows that the vimentin-containing germ plasm islands aggregate during this period. Scale bars: (A) = 150 nm, (B) = 300 nm, (C) = $50\,\mu$m, (D) = $40\,\mu$m.
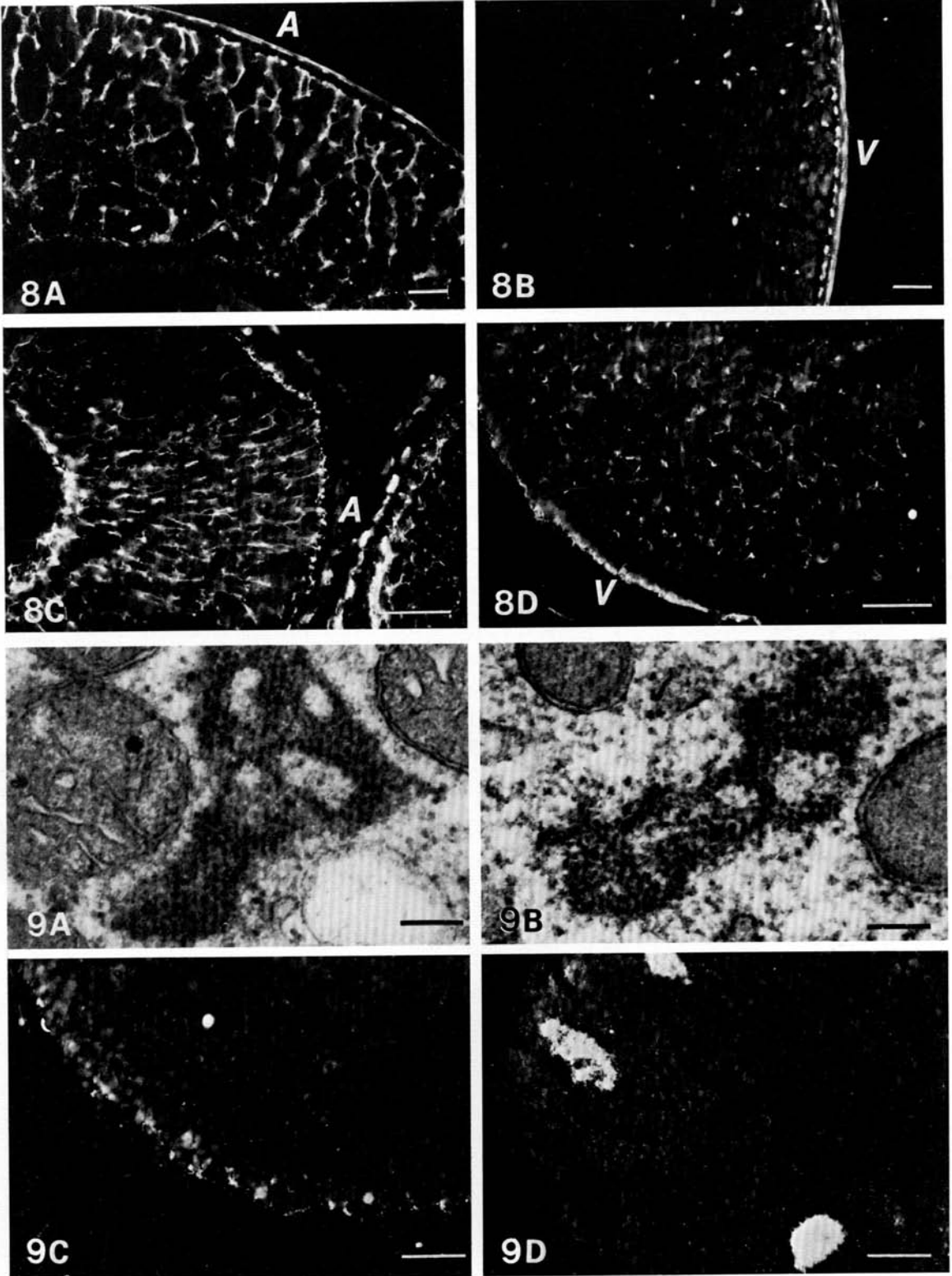
(i) *Intermediate filament localization*

When the full-grown oocyte is stimulated to undergo maturation, dramatic changes take place in the distributions of both vimentin and cytokeratin. In the case of vimentin, the differences in pattern between animal and vegetal pole are eliminated, and it adopts an even distribution throughout the egg, which is inherited by all of the blastomeres (Godsave *et al.* 1984*b*). It is tempting to speculate that vimentin is the scaffolding which supports organelle structure in the oocyte, egg, and blastomeres derived from it.

In contrast to this, cytokeratin apparently becomes redistributed to the cortex during maturation (Godsave *et al.* 1984*a*). Its position in the cortical cytoplasm immediately beneath the submembraneous microfilament layer has been accurately shown by EM immunocytochemistry (Gall *et al.* 1983). The cortical pattern of cytokeratin inherited by the egg means that as cleavage proceeds the most superficial cells will inherit the bulk of the cytokeratin. This is presumably important in the epithelial functions of this outer layer. It is therefore tempting to speculate that cytokeratins, whether or not they have a function in the oocyte, represent a maternal store of localized protein which is important in the early differentiation of the most superficial cells of the blastula to form a functional epithelium.

(ii) *Germ plasm*

It has been known for many years that germ plasm is localized to the vegetal pole of the egg, and is inherited by a small number of vegetal pole blastomeres (usually about four). Some of the progeny of these blastomeres retain the germ plasm and become the progenitor cells of the germ line (see Smith & Williams, 1979; Smith, Michael & Williams, 1983 for review). However, neither the time of synthesis of germ plasm components, nor the time of distribution to the vegetal pole, have been established.

Careful light and electron microscopical analysis of oocytes has now shown that the germ plasm becomes concentrated in the mitochondrial cloud of the stage I oocyte, and becomes distributed to the future vegetal pole during mitochondrial cloud breakdown at the end of stage I and early stage II (Heasman *et al.* 1984). Fig. 9 shows the fine structure of germinal granules found in the germ plasm of the egg

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Fig. 10. Shows staining patterns in oocytes and embryos with the monoclonal antibodies VC4 (A and B), VC1 (C–E), and MC3 (F–H). (A) and (B) show VC4 staining in the central region of the full-grown oocyte and gastrula respectively. The superficial region, seen in (A) is unstained. The distribution of VC1 is shown in the vegetal hemisphere of the stage VI oocyte (C), the animal hemisphere of the fertilized egg (D), and in the swimming tadpole (E). In (E) primordial germ cell is stained (straight arrow) as are the mesonephric ducts and the gut lining (curved arrow).

The distribution of MC3 is shown in the previtellogenic oocyte (F), swimming tadpole (G) and larval oesophagus (H). In (G) only a primordial germ cell is stained. In (H) only certain cells of the oesophagus at the same stage are stained. Scale bars (A)–(G) = 50 μm, (H) = 30 μm.
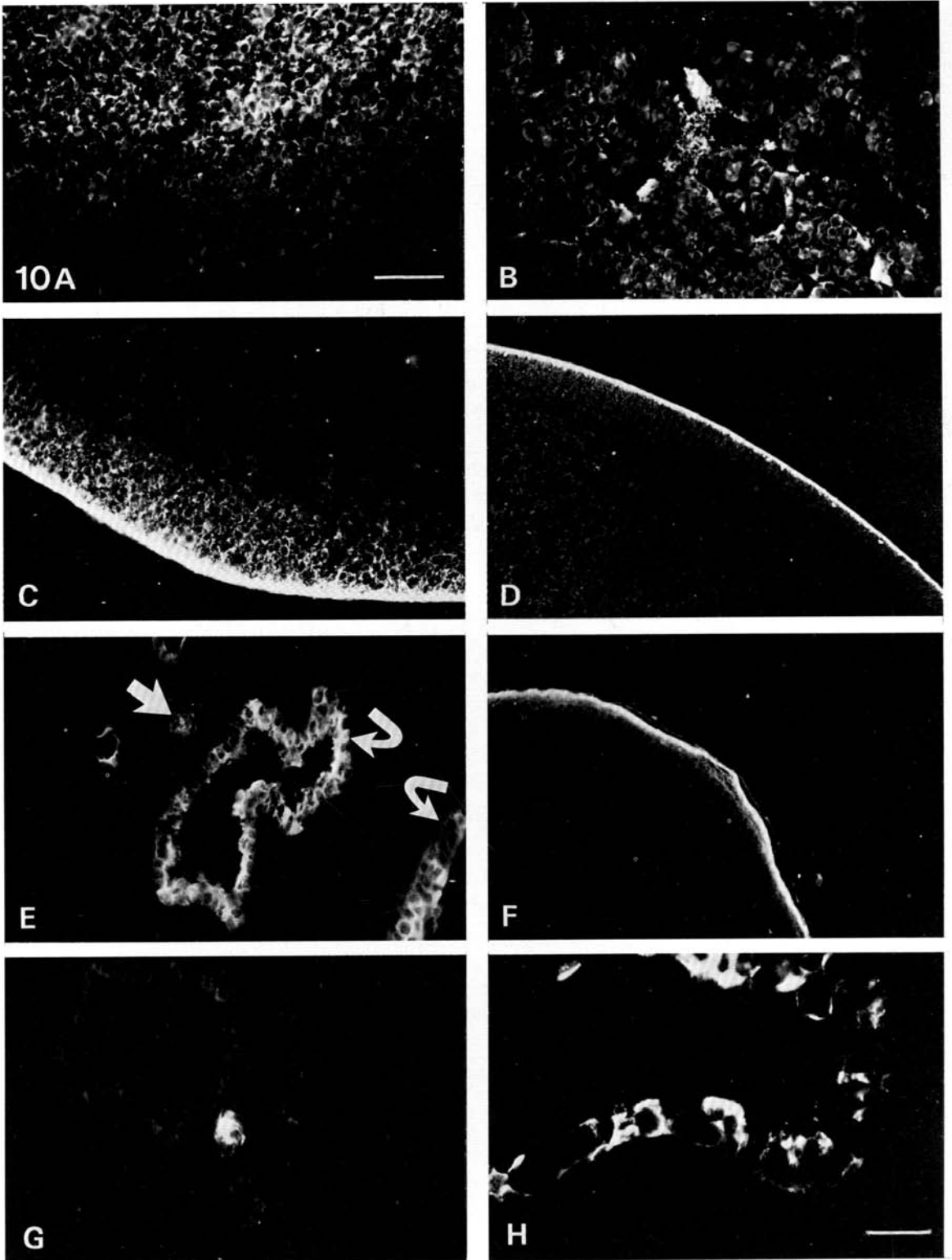
Fig. 10

(Fig. 9A), compared with the electron-dense masses which accumulate in the mitochondrial cloud of the stage I oocyte (Fig. 9B). Only the mitochondrial cloud has these masses, the other smaller mitochondrial aggregates do not. When the mitochondrial cloud breaks down, the electron-dense masses are found in the smaller aggregates derived from the cloud, and now localized in one segment of the surface. They are still found in one segment of the oocyte cortex when the animal/vegetal polarity obvious from pigment changes at stage IV. This segment of the cortex is the vegetal pole.

The most likely implications of these observations are two fold. Firstly the animal/vegetal polarity is established very early in oogenesis, before it becomes obvious by accumulation of pigment in the animal hemisphere. The rigid cytoarchitectural pattern which develops during stage I supports this view. Secondly, the real role of the mitochondrial cloud during anuran oogenesis is probably in the concentration of germ plasm elements, and their distribution to the correct region of the egg. Urodele eggs, which do not have germ plasm in the vegetal pole, and where PGCs arise from the equatorial zone, do not have a mitochondrial cloud in the stage I oocyte (C. C. Wylie, unpublished observations). They do, however, have the smaller mitochondrial aggregates surrounding the nucleus. It is most likely that these are the source of most of the maternal store of mitochondria in both species.

(iii) *Regionally distributed antigens*

Recent work in our laboratory (D. Brown, unpublished observations), has shown the presence of regionalized antigens in the *Xenopus* oocyte. These were identified by monoclonal antibodies raised against *Xenopus* oocyte and egg cytoplasm. Three of these show interesting specificities. The antibody VC4 reveals an antigen which appears during vitellogenesis at stage IV. By stage VI it is localized only to the central region of the oocyte (Fig. 10A). The antigen is completely absent from the superficial $100\,\mu$m of the oocyte. This pattern is inherited by the egg, so that only the central blastomeres inherit VC4 (Fig. 10B). These blastomeres are found beneath the blastocoel as it forms, and enter the embryonic endoderm. The antigen is gradually lost until it disappears entirely from the embryo before the swimming tadpole stage. Its slow disappearance suggests that it is synthesized in the oocyte but not by the embryo. Further work is in progress to test this. Presumably, the molecule carrying the VC4 antigen plays some role in this region of the egg during the earliest stages of development.

The antibody VC1 has an opposite distribution to VC4. It is found only in the superficial $100\,\mu$m of the full-grown oocyte, and excluded from the central region stained by VC4 (Fig. 10C). VC1 is also present in the previtellogenic oocytes as a thin cortical ring. Its pattern in the full-grown oocyte is inherited by the embryo so that only the cortex of the egg stains (Fig. 10D). At each cleavage division, however, VC1 is found in the cortical region of each blastomere, so is inherited or is synthesized by each blastomere. During later development, it is expressed in the germ line, which stains extremely strongly (Fig. 10E) as well as other endodermal

derivatives such as the liver and gut lining. It is also present in a subpopulation of single cells in the skin.

MC3 is an antibody whose distribution is similar to VC1. Its pattern in the previtellogenic oocyte is shown in Fig. 10F. Its only major difference is that during larval stages it only stains the germ line, and some of the cells lining the oesophagus (Figs 10G, H). This antibody should prove useful in tracing germ-line cells.

Neither the identity, nor the roles, of any of these three antigens are yet known, but are the subject of current research. However, they are given here as examples of molecules synthesized during oogenesis, and inherited in a highly regionalized fashion by the embryo. Studies like this seem likely to reveal many asymmetries in the egg, some of which may be the basis of early developmental decisions.

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DISCUSSION

Speaker: C. Wylie (St. Georges)

Question from J. Gurdon (Cambridge):
Is the animal–vegetal polarity of yolk a consequence of the position of the mitochondrial cloud location?

Answer:
No, I don't think the initial pattern of accumulating yolk bears any relationship to the position of mitochondrial cloud; it first appears as a cortical shell all the way round the oocyte.

Question from G. Malacinski (Indiana):
There are many other amphibian species that lack a mitochondrial cloud and yet develop an animal–vegetal polarity. Others, of course, lack classical germ plasm but nevertheless have a mitochondrial cloud.

Answer:
I didn't mean to imply that the mitochondrial cloud actually was responsible for establishing the vegetal pole; in fact its constant position during stage I indicates that animal–vegetal polarity is already established. We looked at axolotl oocytes provided by Jonathan Slack and they have no mitochondrial cloud but they do have the perinuclear masses of mitochondria. The main point is that the mitochondrial cloud is not actually the maternal store of mitochondria as was once thought, its more likely role is as a store of germ plasm and a means of its localization in the oocyte cytoplasm.

Question from J. Slack (ICRF, London):
You refer to the external surface of the oocyte later becoming a differentiated epithelium. Which epithelium in the embryo were you thinking of?

Answer:
I was thinking of outer cells of the blastula which form tight junctions and essentially keep things out.