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# Oocytes and early embryos of *Xenopus laevis* contain intermediate filaments which react with anti-mammalian vimentin antibodies

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## SUMMARY

Previous studies have shown that *Xenopus* oocytes possess a cortical shell, which includes actin-containing microfilaments and cytokeratin-containing intermediate filaments. In this paper we show that oocytes of *Xenopus laevis* also contain filaments throughout their cytoplasm which are stained by several anti-vimentin antibody preparations. We also show that dramatic changes in pattern of these filaments occur during oocyte differentiation, first during vitellogenesis, and then during maturation of the oocyte to form an egg.

## INTRODUCTION

It is now becoming clear from immunocytochemical data that intermediate filament proteins collectively represent a large and complex multigene family, only certain members of which appear in different cell lineages (Anderton, 1981; Lazarides, 1980, 1982; Osborn & Weber, 1982). It is therefore of importance to study the ontogeny of intermediate filament proteins, in particular the spatial and temporal sequence of appearance of the five protein classes in early embryos. A certain amount of data have appeared recently in this respect, principally from mammalian and avian species.

In mouse embryos, two cytokeratin polypeptides have been found in the earliest cleavage stages (Lehtonen, Lehto, Paasivuo & Virtanen, 1983*a*) as well as at later blastula stages (Jackson *et al.* 1980, 1981; Paulin, Babinet, Weber & Osborn, 1980; Brulet, Babinet, Kemler & Jacob, 1980; Kemler *et al.* 1981). These proteins are followed by vimentin which appears in the primary mesenchyme cells during gastrulation, and then desmin is found in the embryo at a later stage (Franke *et al.* 1982*a*, 1982*b*). In the developing nervous system, vimentin is found in both presumptive glial and neural cells, and the tissue-specific neurofilament proteins and glial fibrillary acidic protein (GFAP) appear later in development (Schnitzer, Franke & Schachner, 1981; Raju, Bignami & Dahl, 1981; Bignami, Raju & Dahl, 1982).

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In chick embryos, studies of intermediate filament proteins have been largely confined to neurogenesis and myogenesis. Here the pattern of expression is similar to that found in mammalian embryos. Vimentin is found before either neurofilament proteins or GFAP in the developing nervous system, but is gradually replaced by these proteins in the neuron and glial lineages respectively (Tapscott *et al.* 1981; Holtzer *et al.* 1982; Bignami & Dahl, 1975; Bignami, Dahl & Seiler, 1980). Vimentin also precedes desmin expression in chick myogenesis (Bennett, Fellini, Toyama & Holtzer, 1979; Holtzer *et al.* 1982; Lazarides *et al.* 1982; Gard & Lazarides, 1980).

For some time it was thought that differentiating gametes (i.e. the germ line) did not contain intermediate filaments. However, recent reports from several laboratories have now demonstrated the presence of cytokeratin-containing intermediate filaments in both mouse (Lehtonen *et al.* 1983*b*) and frog (Gall, Picheral & Gounon, 1983; Franz *et al.* 1983) oocytes. It is therefore of great interest to determine whether other intermediate filament proteins are present, whether they are stored for use during early postfertilization development, whether they play a role in oocyte function, and the temporal and spatial pattern by which they are replaced by embryonic lineage-specific intermediate filament proteins.

The female frog germ line is an attractive system to study in this respect. The ovary contains many hundreds of oocytes, at all stages of oogenesis, representing a very large tissue mass for routine biochemistry. The events of oogenesis are well-characterized biochemically (Gurdon, 1974; De Robertis, Zeller, Carrasco & Mattaj, 1983; Woodland *et al.* 1983). Furthermore, the fully grown frog oocyte is a single very large cell (1·2–1·4 mm in diameter) with a complex and characteristic cytoarchitecture (Wischnitzer, 1966; Dumont, 1972; Mohun, Lane, Colman & Wylie, 1981). This can be disrupted by cytoskeleton-disrupting agents, which also cause assayable biochemical effects on protein translation, secretion and turnover (Colman *et al.* 1981). Frog oocytes therefore represent a potentially useful model system in which to study the role of intermediate filaments in the maintenance of cytoarchitecture and other aspects of cell physiology. We report here that oocytes of *Xenopus laevis* contain intermediate filaments from early previtellogenic stages to the fully grown state. These are identifiable by electron microscopy, immunoblotting and immunofluorescence cytochemistry. We show that vimentin is a major intermediate filament protein in these cells and demonstrate that the differentiation of the oocyte and its maturation to form the egg are accompanied by major changes in the pattern of vimentin-containing filaments.

## MATERIALS AND METHODS

### *Preparation for electron microscopy*

Pieces of ovary were excised from adult *Xenopus laevis* in normal saline and fixed in 1 % w/v glutaraldehyde in 0·1 M-phosphate buffer for 3 h. In order to

improve the fixation of cytoskeletal elements, 0·5 mg/ml saponin and 2 mg/ml tannic acid were added to the fixative after the method of Maupin & Pollard (1983). Stage-4 and -5 oocytes were cut in half to allow complete penetration. After washing in 0·1 M-phosphate buffer, samples were postfixed in 1 % w/v $OsO_4$ in 0·1 M-phosphate buffer for 2 h. They were then dehydrated through an ethanol series and embedded in Araldite. Blocks were sectioned on a Sorvall Ultramicrotome, stained with aqueous uranyl acetate and lead citrate and silver sections were viewed in a Philips 301 electron microscope.

### *Immunocytochemistry*

Adult *Xenopus laevis* ovaries were dissected into small fragments in Gurdon-modified Barth's solution (Barth-x, Gurdon, 1968). They were then fixed in 2 % w/v trichloroacetic acid, transferred to absolute ethanol and then to polyethyleneglycol 400 distearate wax (BDH) containing 1 % w/v cetyl alcohol, in which they were embedded (Dr P. Hausen, personal communication). Sections were cut at 16–18 °C, mounted on glass slides and stored desiccated. Sections were dewaxed in an acetone series and treated with the following sequence of solutions: 3 M-urea (1 min), phosphate-buffered saline (PBS) wash (2 × 5 min), a blocking buffer consisting of PBS containing 1 % w/v BSA (10 min), primary antibody solution (20 min), three washes in PBS plus 1 % BSA (5 min each), second antibody solution: a 1: 100 dilution of fluorescein-coupled goat anti-rabbit immunoglobulin (Nordic, 20 min), PBS (2 × 5 min washes); 0·004 % w/v eryochrome black (5 min) and finally one further wash with PBS. Stained sections were mounted in an aqueous u.v.-free mounting medium (Difco Ltd.) and examined under a Zeiss photomicroscope III using epifluorescence. Three different anti-vimentin antibody preparations were used by this method to stain *Xenopus* oocytes: a) a rabbit antiserum raised against hamster vimentin (kindly donated by Dr R. O. Hynes, See Hynes & Destree, 1978, for details), a purified IgG preparation from this antiserum was used in some of the studies reported here, b) a rabbit antiserum raised against calf lens vimentin (kindly donated by Dr F. Ramaekers, See Ramaekers *et al.* 1982), c) a monoclonal antibody raised against human vimentin (donated by Dr I. Virtanen, See Virtanen *et al.* 1981). All gave essentially the same staining pattern.

### *Preparation of triton-insoluble proteins from 3T3 cells and rat brain*

Triton-insoluble proteins were prepared by the methods described by Pruss *et al.* (1981) using 1 mM-phenylmethylsulphonylfluoride and 1 mM-p-chloromercuribenzoate to inhibit proteolysis.

### *Production of eggs and embryos*

Synchronously developing batches of embryos were produced by artificial fertilization of eggs as described by Gurdon & Woodland (1975). Embryos were

classified by developmental stage as described by Nieuwkoop & Faber (1956). Unfertilized eggs were collected from females prior to addition of sperm.

### *Preparation of defolliculated oocytes*

Follicle cells were removed from the surface of oocytes by collagenase treatment. The efficiency of this procedure was monitored by scanning electron microscopy (see Mohun *et al.* 1981 for full description).

### *Immunoblotting*

Cellular proteins were separated electrophoretically in 10% w/v SDS-polyacrylamide slab gels, transferred onto nitrocellulose sheets and stained with antibodies as described previously (Pruss *et al.* 1981). Anti-IFA was used at a 1:10 dilution and was detected using a $^{125}$I-labelled rabbit anti-(mouse Ig) antibody. The rabbit anti-vimentin antisera were used at 1:100 dilution and the anti-hamster vimentin IgG preparation was used at a concentration of 12 μg/ml. Rabbit antibody binding was detected using $^{125}$I-labelled goat anti-(rabbit Ig) antibody.

## RESULTS

### 1. *Identification of intermediate filaments in oocytes by electron microscopy*

The adult *Xenopus* ovary contains oocytes of many different developmental stages ranging from small previtellogenic cells to full-grown oocytes (approximately 1·2–1·4 mm diameter). This enormous increase in volume takes some weeks and is largely due to the appearance and storage of yolk platelets (vitellogenesis), although other organelles, e.g. the nucleus, also increase in size. The general morphology of the previtellogenic oocyte is seen in Fig. 1. The nucleus is more or less central and the cytoplasm is homogeneous, with the exception of large phase-dense perinuclear masses which are particularly striking

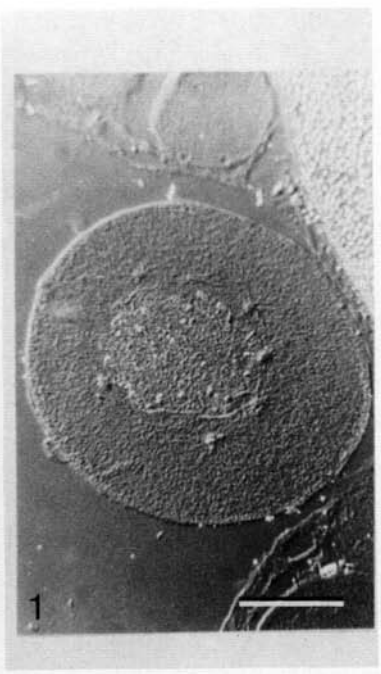
Fig. 1. Low-power view of previtellogenic oocyte. Oocyte from 7 μm thick section through polyester-wax-embedded ovary viewed with Nomarski optics. Bar 100 μm.

Fig. 2. Low-power view of stage-6 oocyte. Vertical section, 7 μm thick, through polyester-wax-embedded stage-6 oocyte, stained with 0·1 % toluidine blue in 0·1 % borax and viewed with bright-field optics. Bar 100 μm.

Fig. 3. Electron micrograph showing a branching bundle of intermediate filaments in a previtellogenic oocyte. Bar 0·2 μm.

Fig. 4. High-power electron micrograph of intermediate-sized filaments in a previtellogenic oocyte. Micrograph used for measurement of filament size. Individual filaments measure 15 nm in diameter. Bar 0·1 μm.

Fig. 5. Electron micrograph of intermediate filaments in follicle cells. The diameters of intermediate-sized filaments from oocytes were compared with intermediate-sized filaments (arrow) from follicle cells surrounding oocytes. Intermediate filaments from both cell types measured 15 nm diameter. Bar 0·1 μm.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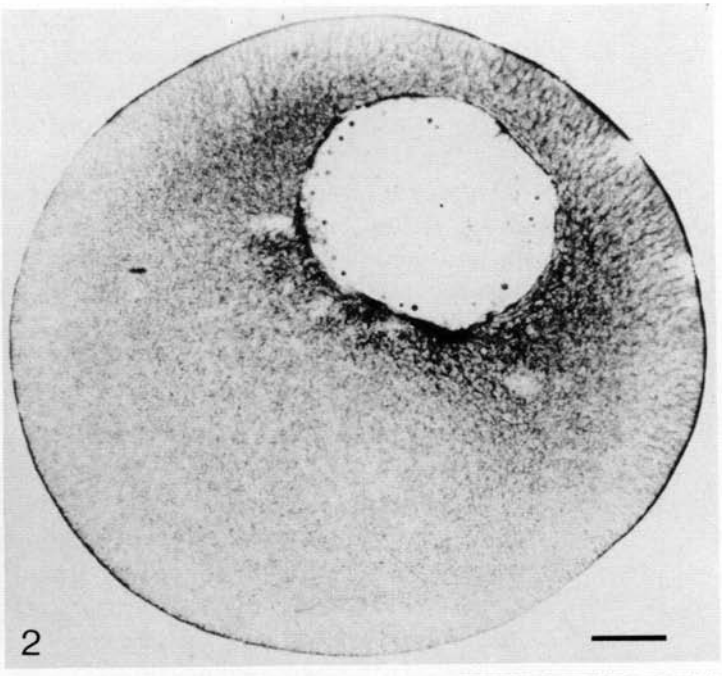
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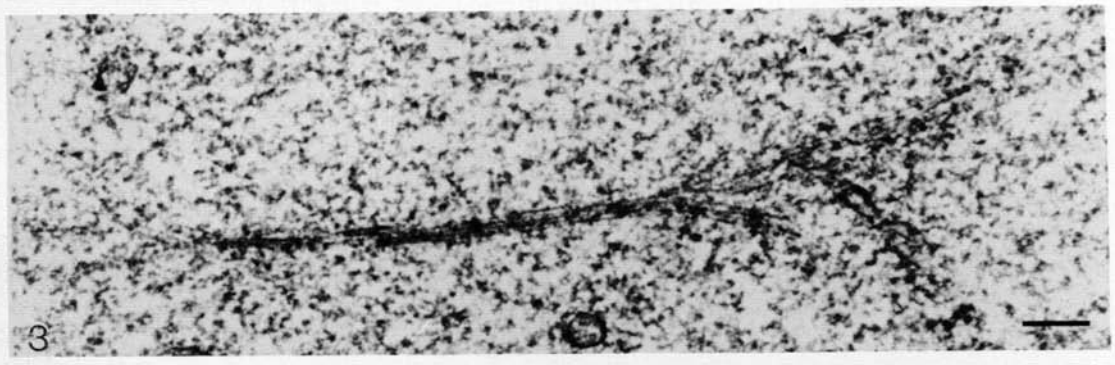
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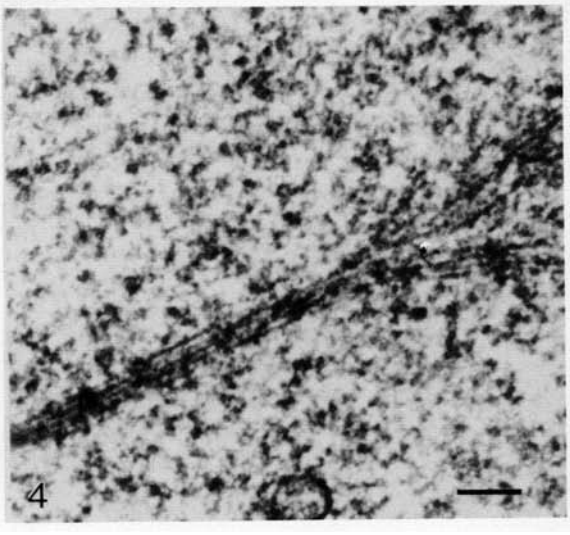
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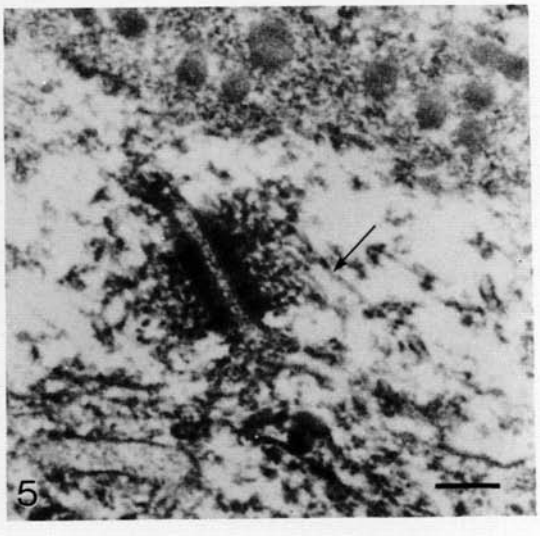
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in interference images. These are connected to the mitochondrial cloud, a characteristic feature of early germ-line cells (Billett, 1979). By the end of vitellogenesis, the oocyte has an elaborate cytoarchitecture (Fig. 2). The animal hemisphere, which contains the nucleus, is characterized by a layer of pigment immediately beneath the surface membrane and by the presence of small yolk platelets. These are arranged in large islands passing radially from just beneath the cell surface inwards towards the nucleus. Between these islands are yolk-free tracts of 'active' cytoplasm i.e. endoplasmic reticulum, Golgi, mitochondria and other non-yolk constituents. The vegetal pole is not so obviously organized and consists largely of a mass of yolk platelets larger than those in the animal pole (the 'vegetal yolk mass').

Examination of detergent-extracted and tannic-acid-treated previtellogenic oocytes at the electron microscopic level shows the presence of intermediate filament bundles crossing the cytoplasm in various directions (Fig. 3). These bundles are of variable size and are sometimes seen to split into smaller structures following divergent paths. Each filament of a bundle measures approximately 15 nm in diameter in saponin-/tannic-acid-fixed material (Fig. 4). These figures are in agreement with those of Maupin & Pollard (1983) for intermediate filament sizes. In order to compare the size of oocyte intermediate filaments with that of known *Xenopus* intermediate filaments, photographs were taken, at the same magnification, of desmosome-associated filaments found between follicle cells surrounding the oocyte (Fig. 5). The intermediate filaments seen associated with these desmosomes are of the same diameter as those seen in the oocyte cytoplasm.

Examination of sections of previtellogenic oocytes by light microscopy shows them to contain characteristic phase-dense masses in the cytoplasm arranged around the nucleus (Fig. 1). When these oocytes are fixed with glutaraldehyde in the presence of saponin and tannic acid, and examined under the electron microscope the masses are found to contain mitochondria, microtubules, nuage (a generic name given to undefined fibrillogranular cytoplasmic masses in germline cells), and intermediate filament bundles (Figs 6 & 7).

In vitellogenic stages, the cytoplasm becomes much more crowded with organelles and cytoskeletal elements are difficult to see. However, in cortical areas of full-grown oocytes, intermediate filaments are visible in close association with mitochondria (Fig. 8), although their organization cannot be deduced by electron microscopy.

### 2. *Identification of intermediate filament proteins in oocytes and embryos by immunoblotting*

The ontogeny of intermediate filament polypeptides from oocytes through to swimming tadpoles was followed by immunoblotting using a monoclonal anti-intermediate filament antibody (anti-IFA, Pruss *et al.* 1981). This antibody has been shown to cross react with most (possibly all) intermediate filament

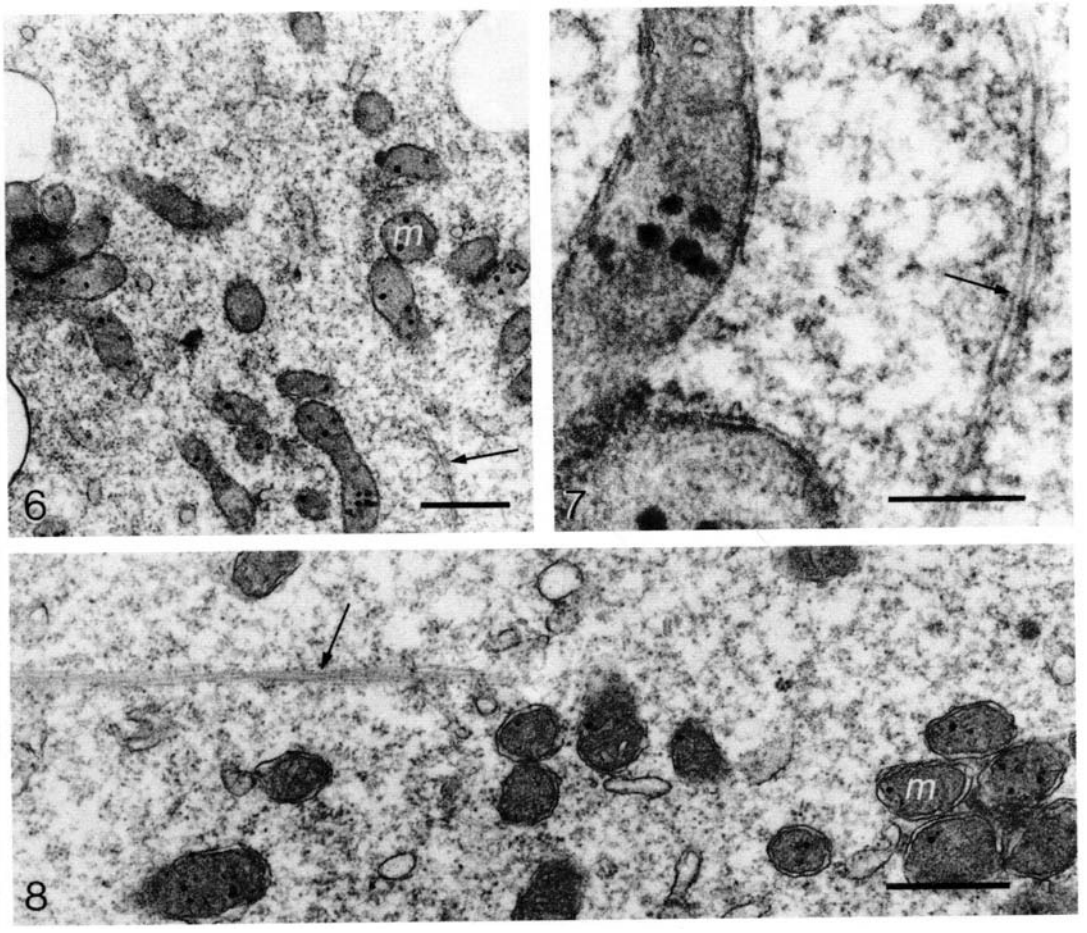
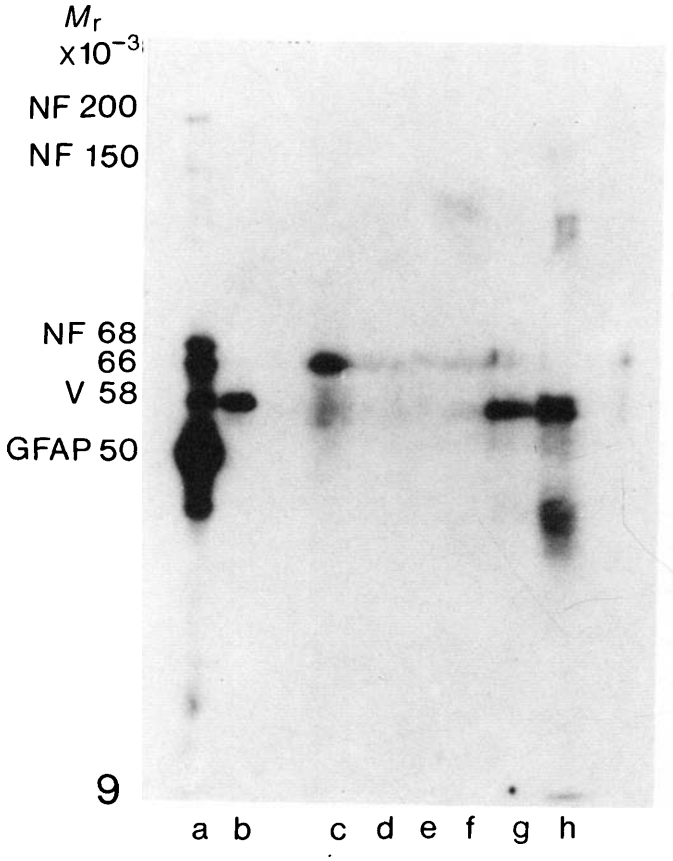



Fig. 6. Low-power electron micrograph of a mitochondrial cloud in a previtellogenic oocyte. Intermediate-sized filaments (arrow) can be seen in this mitochondria (*m*)-rich region. Bar 0·5 μm.

Fig. 7. High-power electron micrograph of part of a mitochondrial cloud. An intermediate filament bundle is indicated (arrow). Bar 0·1 μm.

Fig. 8. Intermediate filaments in a vitellogenic oocyte. Intermediate filament bundle (arrow) in a yolk free area of the animal pole of a full-grown oocyte. Many mitochondria (*m*) are also present in this area. Bar 0·5 μm.

polypeptides from a wide variety of cell types and species. It therefore represents a useful initial probe with which to study *Xenopus* oocytes and embryos to see if any recognizable intermediate filament polypeptides are present. The various samples were run on a single SDS-PAGE slab gel and transferred to nitrocellulose and then processed with antibodies (Fig. 9). Track A illustrates the pattern of reactivity of anti-IFA on Triton-X-100-insoluble protein from rat brain. This tissue is enriched in GFAP, neurofilaments and vimentin. These are all labelled. Anti-IFA has been found to stain a protein of 66000 relative molecular mass ($M_r$) in a number of tissues (Pruss *et al.* 1981) and a band of this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NF 200
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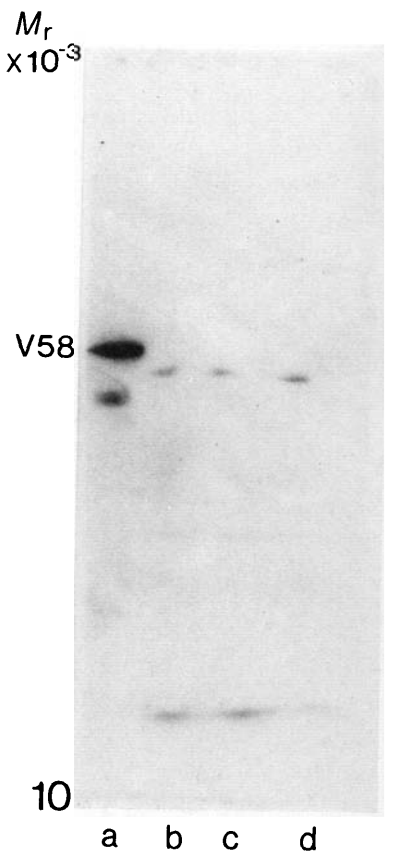
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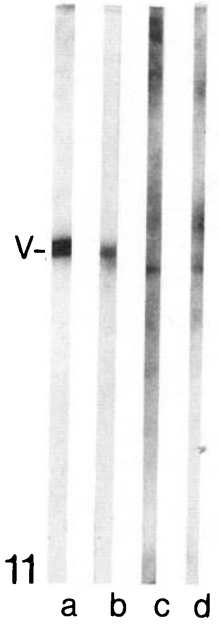
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a b c d

$M_r$ is stained in rat brain by this antibody. Labelling below GFAP is presumed to be of proteolytic products of the intermediate filaments (Pruss *et al.* 1981). Track *b* is of Triton-X-100-insoluble 3T3 cellular proteins and shows strong labelling of vimentin but only weak binding to the 66 000 $M_r$ protein. Defolliculated oocytes (track *c*) show strong labelling of the 66 000 $M_r$ component and probable multiple bands around the vimentin region. *Xenopus* vimentin has been reported to have a $M_r$ of 55 000 compared to mouse vimentin (58 000) (Nelson & Traub, 1982). The cytokeratin detected by Franz *et al.* (1983) in *Xenopus* oocytes by immunoblotting exhibits a $M_r$ of 56 000 and may also form a component of the staining of this region. Fertilized eggs and stages 10 and 16 (tracks *d–f*) are similar in pattern to oocytes but at stage 22 (track *g*) a band in the position of *Xenopus* vimentin is the most strongly labelled with relatively weaker 66 000 $M_r$ protein. In swimming tadpoles (stage 46, track *h*) the pattern is more complex, indicating that other intermediate filament proteins are being synthesized by this stage.

As shown in Fig. 10 immunoblotting analysis of oocyte proteins was also carried out using an IgG prepared from an anti-vimentin serum (Hynes & Destree, 1978). For details of anti-vimentin antibodies used see Materials and Methods. Track *a* shows the reaction of this antibody on total protein of 3T3 cells. The major band corresponds to vimentin. The band of lower $M_r$ is probably a result of proteolytic degradation of vimentin. Tracks *b* and *c* contain total protein of defolliculated oocytes and whole ovary respectively, which both show a band of slightly lower $M_r$ than 3T3 cell vimentin. A protein of the same mobility is also seen in unfertilized egg protein (not shown) and in stage-46 swimming tadpole protein (track *d*). The lower $M_r$ of *Xenopus* than mouse vimentin is in agreement with previously published results (Nelson & Traub, 1982). In each sample of *Xenopus* protein there are also several minor bands of much lower $M_r$ than vimentin, probably the result of proteolysis in the samples.

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Fig. 9. Nitrocellulose blotting of *Xenopus* oocytes and embryos with anti-IFA. Immunoblotting was carried out as described in Materials and Methods and the nitrocellulose sheet was stained with anti-IFA. Tissues stained were in lane a, triton X-100-insoluble material from rat brain; lane b, triton X-100-insoluble material from 3T3 cells; lane c, defolliculated oocytes; lane d, fertilized eggs; lane e, stage-10 embryos; lane f, stage-16 embryos; lane g, stage-22 embryos; lane h, stage-46 embryos. The relative molecular masses marked are those of rat brain intermediate filament proteins known to react with anti-IFA (Pruss *et al.* 1981). Abbreviations: *NF*: neurofilament; *V*: vimentin; *GFAP*: glial fibrillary acidic protein.

Fig. 10. Nitrocellulose blotting of *Xenopus* oocytes, ovary and tadpoles with anti-vimentin IgG. Anti-vimentin IgG (Hynes & Destree, 1978) was used to stain a nitrocellulose blot containing electrophoretically separated total protein from: track a, 3T3 cells; track b, defolliculated oocytes; track c, whole ovary; track d, stage-46 tadpoles. 3T3 cell vimentin band is labelled *V*.

Fig. 11. Nitrocellulose blotting of total proteins from 3T3 cells, tracks a and b; and defolliculated oocytes (tracks c and d) with anti-hamster vimentin serum (Hynes & Destree, 1978), tracks a and c and anti-calf lens vimentin serum (Ramaekers *et al.* 1982) tracks b and d. The position of the 3T3 cell vimentin band is labelled *V*.

Samples of whole anti-vimentin rabbit sera (Hynes & Destree, 1978; Ramaekers *et al.* 1982) also stained vimentin from 3T3 cells and a band in defolliculated oocytes of slightly lower $M_r$ than 3T3 cell vimentin, presumably *Xenopus* vimentin; additionally they showed weak labelling of the 66000 $M_r$ protein detected with anti-IFA (Fig. 11): anti-vimentins have previously been reported to react with a protein of this $M_r$ (Moll, Von Bassewitz, Schultz & Franke, 1982).

### 3. *The distribution of vimentin in developing oocytes and early embryos, as seen by immunofluorescence microscopy*

Three different anti-vimentin antibodies from different laboratories were used to stain histological sections of *Xenopus* oocytes (see Materials and Methods). All of these show the conventional vimentin tissue specificity when used to stain adult *Xenopus* gut prepared and treated in the same way as the oocytes (see Fig. 12). The controls used in the immunofluorescence studies described in this paper were for rabbit antibodies, non-immune rabbit serum or IgG at the same concentration as the primary antibody preparation and for monoclonal anti-vimentin, tissue culture medium. In Fig. 13, the reactivity of non-immune rabbit serum with sections of TCA-fixed adult gut is shown.

All of the antibodies give essentially indistinguishable patterns of staining on *Xenopus* oocytes. The only difference in staining between them is that one (the anti-human vimentin monoclonal) stains all nuclear membranes as well as cytoplasmic filaments. Nuclear membranes of 3T3 cells are not stained (not shown) however, and so this result is assumed to be due to a coincidental cross-reaction of the monoclonal antibody with a frog nuclear membrane protein.

The distribution of vimentin changes markedly during oocyte differentiation and maturation. The earliest stage at which vimentin can be seen is early stage I (staging according to Dumont, 1972) where in oocytes of about 70 $\mu$m in diameter, a fine perinuclear ring can be made out (not shown). As the oocyte grows in size through stage I, more vimentin-containing strands are found distributed through the cytoplasm. The mitochondrial cloud, which is connected to the vimentin-positive strands, is also stained (Fig. 14). At later previtellogenic stages, staining appears in the perinuclear sphere of cytoplasmic masses, rich in mitochondria (Fig. 15, see also Fig. 1). A fine filamentous network is also seen spreading throughout the cytoplasm. During vitellogenesis, the vimentin is excluded from areas containing yolk platelets. These latter are formed, or aggregate after formation, into discrete columns in the cytoplasm, initially in the cortical area, and spreading as vitellogenesis proceeds, towards the nucleus. Vimentin staining is found around the outside of these columns throughout the cytoplasm (Fig. 16). A similar cell stained with control rabbit serum is shown in Fig. 17.

By late vitellogenic stages (Dumont stages V and VI) when the cytoarchitecture of the oocyte is established (see Fig. 2) vimentin staining is seen in

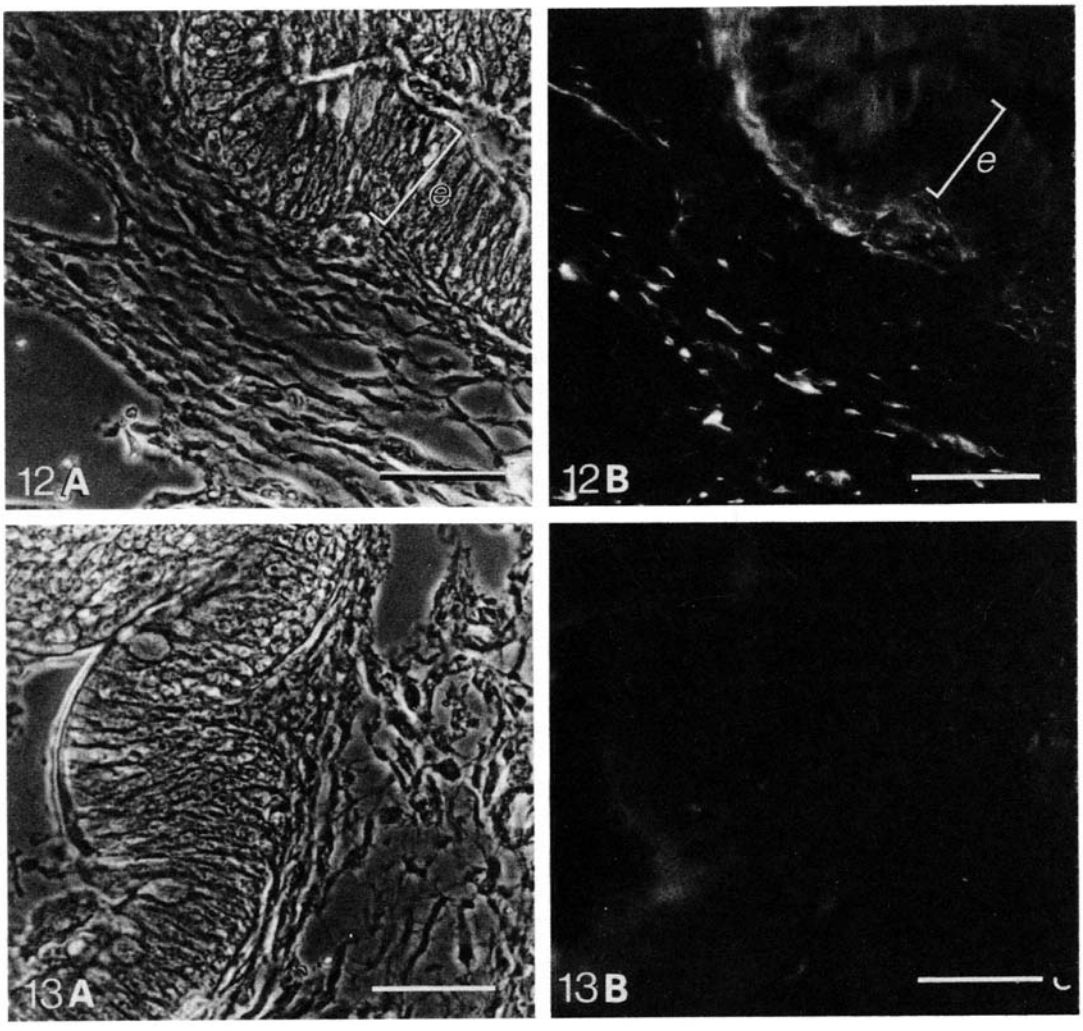
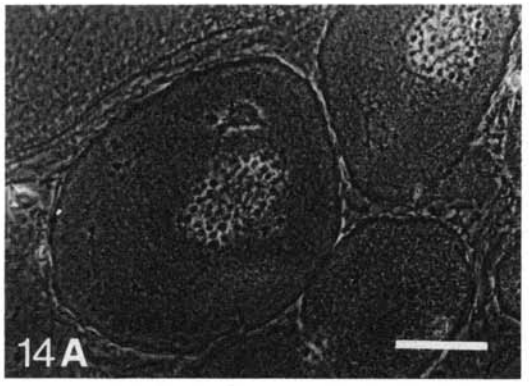



Fig. 12. Immunofluorescence labelling of adult *Xenopus* gut with anti-vimentin serum. Wax sections were stained with rabbit anti-vimentin antiserum (Ramaekers *et al.* 1982) as described in Materials and Methods. Paired phase contrast (A) and fluorescence (B) photographs are shown. The epithelium is marked (*e*). Only the underlying connective tissue is stained. Bar 50 $\mu$m.

Fig. 13. Immunofluorescence labelling of adult *Xenopus* gut with control serum. Wax sections of gut were stained with non-immune rabbit serum. (A) phase contrast (B) immunofluorescence. Bar 50 $\mu$m.

asymmetric fashion between animal and vegetal poles. In the animal pole (Fig. 18) vimentin is confined to the yolk-free areas of cytoplasm which divide the yolk platelets into columns. The thick strands of vimentin-containing cytoplasm do not extend right to the animal pole surface. A cortical layer containing pigment can be seen separating them from the surface membrane of the oocyte. In the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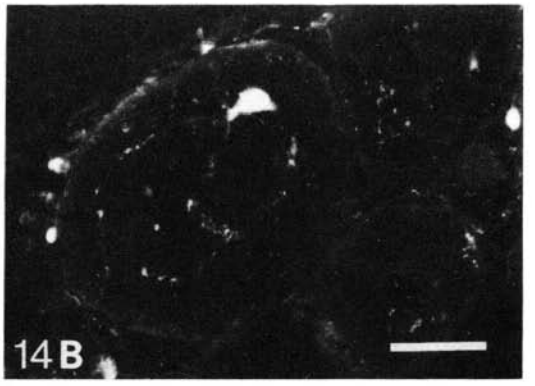
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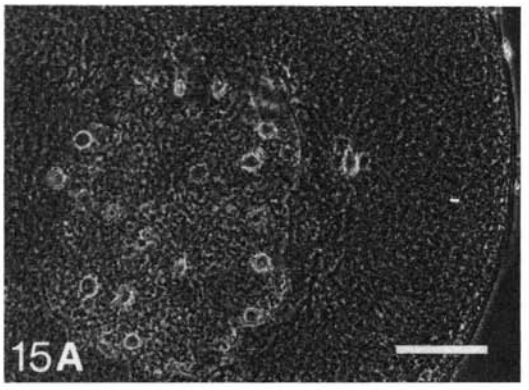
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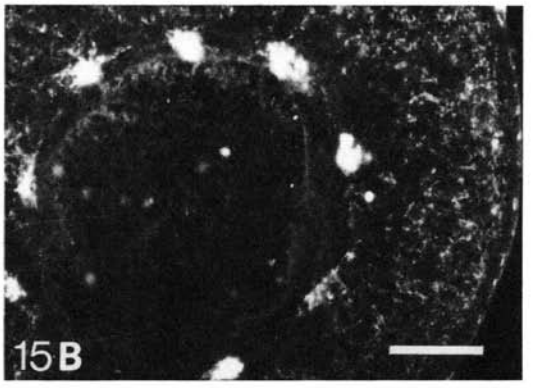
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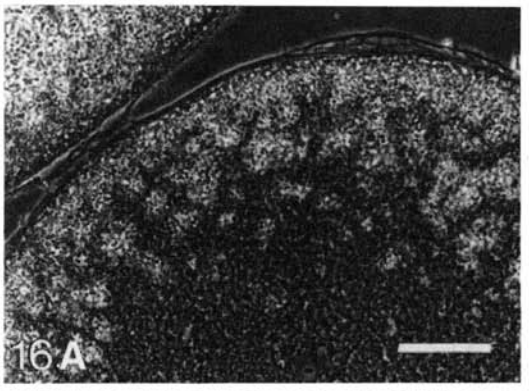
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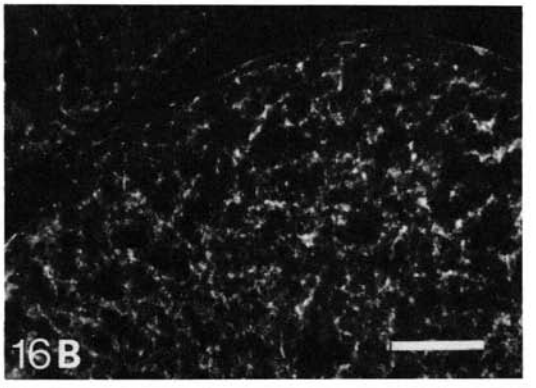
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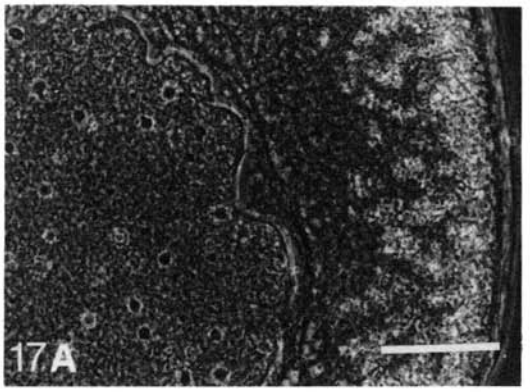
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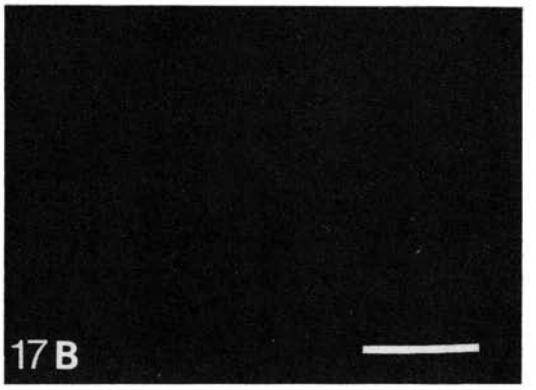
17B

vegetal pole, where cytoarchitectural detail is not as obvious as in the animal pole, vimentin is distributed in apparently random loci in the vegetal yolk mass and irregularly around the cortical cytoplasm of the vegetal pole (Fig. 19). Staining is also seen in the outer thecal layers.

In order to study the fate of the highly organized array of vimentin in the oocyte, unfertilized eggs, fertilized eggs and early cleavage-stage embryos were stained. The pattern of staining was similar in each case (unfertilized egg staining not shown). Fig. 20 shows the pattern of anti-vimentin staining in the fertilized egg. The dense aggregates seen in the animal pole have dispersed to give a more or less even distribution of fine vimentin-containing filaments throughout the whole egg. This pattern is inherited by each blastomere of the early cleavage stages (Fig. 21).

## DISCUSSION

There have been many previous studies of frog oocyte ultrastructure. However, these have mostly been devoted to the relationship between the oocyte and its follicle (Wischnitzer, 1966; Dumont & Brummet, 1978; Browne, Wiley & Dumont, 1979), or to ultrastructural aspects of the uptake of yolk precursors and their conversion to stored yolk (Wallace & Dumont, 1968; Brummett & Dumont, 1976; Wallace, 1983). Studies of the cytoskeleton of frog oocytes have been limited mainly to the microfilament cores of their numerous microvilli, and to the subsurface filament network (Wartenburg, 1964; Franke *et al.* 1976). This cortical region is now known also to contain cytokeratin filaments (Gall *et al.* 1983; Franz *et al.* 1983).

In considering the frog oocyte cytoskeleton, it is important to bear in mind that the full-grown oocyte is a single large cell, 1·2–1·4 mm in diameter, which has a complex cytoarchitecture consisting of cytoplasmic constituents maintained in a defined array (Mohun *et al.* 1981). Disruption of the cytoskeleton with colchicine and cytochalasin causes profound alterations to this array (Colman *et al.* 1981). It is clear that maintenance of the structure of such a large cell must depend on more than a subcortical web of filaments.

From the results presented in this paper, it seems certain that vimentin-containing intermediate filaments play a role in oocyte cytoarchitecture. Three

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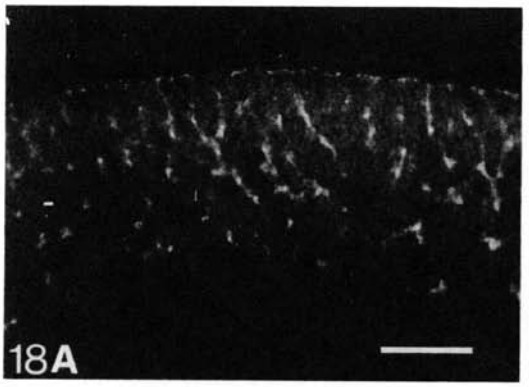
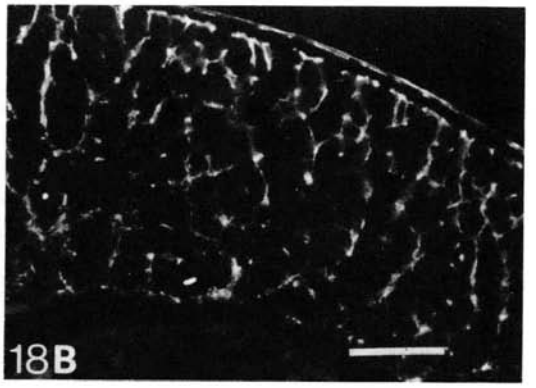
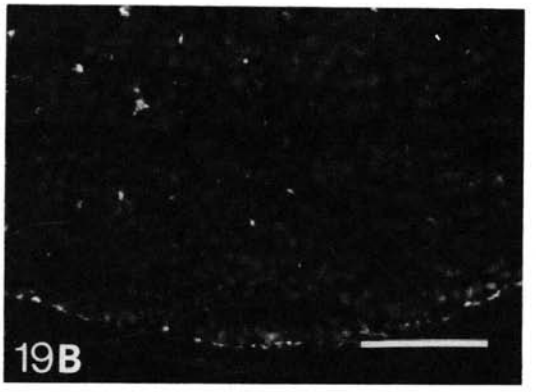
Figs 14–16. Immunofluorescence labelling of oocytes with anti-vimentin antisera. Wax sections of adult ovary were stained with anti-vimentin antisera as described in Materials and Methods. Paired phase-contrast and fluorescence photographs are shown of: Fig. 14A,B, small previtellogenic oocytes stained with rabbit anti-vimentin serum (Ramaekers *et al.* 1982) bar 50 $\mu$m. Fig. 15A,B, a larger previtellogenic oocyte stained with rabbit anti-vimentin serum (Hynes & Destree, 1978) bar 50 $\mu$m.

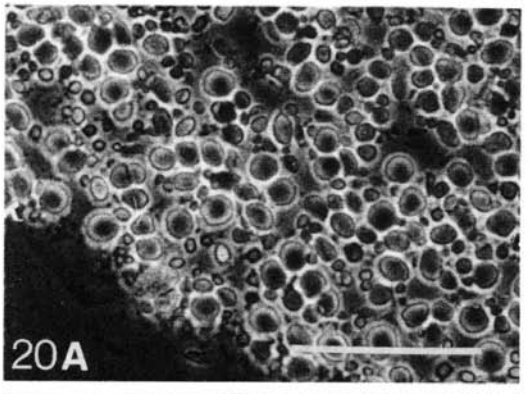
Fig. 17. Immunofluorescence labelling of an early vitellogenic oocyte with control rabbit serum. Wax sections of adult ovary were stained with non-immune rabbit serum. (A) phase contrast; (B) immunofluorescence. Bar 50 $\mu$m.

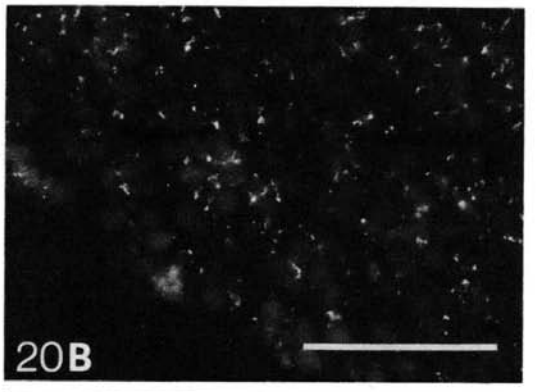

18A

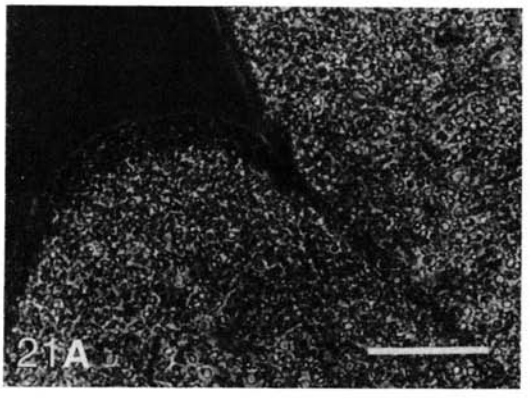

18B

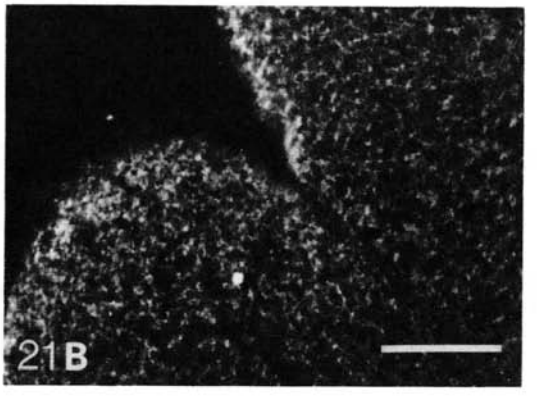

19A


19B


20A


20B


21A


21B

well-characterized anti-vimentin antibody preparations (Hynes & Destree, 1978; Ramaekers *et al.* 1982; Virtanen *et al.* 1981) were used to demonstrate the presence of this intermediate filament protein in oocytes. Also, anti-IFA stained material of the $M_r$ of *Xenopus* vimentin on Western blots. In addition, it was found to bind to a 66 000 $M_r$ protein; probably another important intermediate filament-associated protein in oocytes (Pachter, Moraru & Liem, 1983). Many cell types contain a protein of $M_r$ 66 000 which cross reacts with anti-IFA, though its role remains unknown.

In the previtellogenic oocyte, vimentin is found distributed throughout the cytoplasm as a network of filaments. The majority is associated with large cytoplasmic masses surrounding the nucleus. One interesting point to note is that other authors (Franz *et al.* 1983) have not seen staining of the oocyte sections with different anti-vimentin-antisera. The explanation of this probably lies in the methods used. In the work cited above, unfixed tissue was used. In our studies we found only a small degree of staining with anti-vimentin in unfixed tissue or after fixation in aldehyde or with absolute ethanol. The very bright staining shown in the results section here was found after fixation with TCA, a strong denaturing agent. The continued tissue specificity seen with all the antibodies used with this fixation method, as well as the blotting data, leave little doubt that vimentin is present in oocytes. However, the requirement for a particular fixation method is puzzling. One possible explanation is that the determinants with which the anti-vimentins cross react are masked in some way in *Xenopus* oocytes, and only exposed by the denaturation of themselves, or adjacent molecules. Masking of antigenic determinants has previously been suggested as an explanation for unexpected patterns of reactivity of several anti-intermediate filament protein antibodies (Woodcock-Mitchell, Eichner, Nelson & Sun, 1982; Lazarides *et al.* 1982) and it has been demonstrated that antigen masking is the cause of an anti-cytokeratin monoclonal antibody staining only at certain stages of the cell cycle in $PtK_2$ cells (Franke *et al.* 1983).

We cannot at the moment exclude the possibility that some of the immunofluorescence staining of oocytes seen is due to the $66 \times 10^3$ $M_r$ protein recognized by $\alpha$-IFA. However, this polypeptide is not recognized on immunoblots by the

Figs 18–21. Immunofluorescence staining of late vitellogenic oocytes and early embryos with anti-vimentin antibodies. Wax sections of adult ovary, fertilized eggs and 4-cell embryos were stained with anti-vimentin antibodies as described in Materials and Methods. Fig. 18 shows fluorescence photographs of the animal pole of (A) an oocyte at an advanced stage of vitellogenesis stained with the IgG preparation from anti-hamster vimentin (Hynes & Destree, 1978) and (B) a fully grown oocyte stained with rabbit anti-vimentin serum (Hynes & Destree, 1978). Fig. 19A,B, show phase-contrast and fluorescence exposures of the vegetal pole of a fully grown oocyte stained with the IgG preparation of $\alpha$-hamster vimentin serum; Fig. 20A,B, show the vegetal pole of a fertilized egg stained with rabbit anti-vimentin serum (Hynes & Destree, 1978). Fig. 21A,B, show the cleavage furrow in the animal hemisphere of a 4-cell embryo stained with rabbit anti-vimentin serum (Hynes & Destree, 1978). Bar 50 $\mu$m.

IgG preparation of $\alpha$-hamster vimentin under the conditions used. The $66 \times 10^3$ $M_r$ protein has recently been reported to be present in intermediate filaments in a variety of cell types and may bind to intermediate filament polypeptides of several classes (Pachter *et al.* 1983).

It is known that *Xenopus* oocytes contain pools of stored protein for use during early development e.g. non-polymerized tubulin (Pestell, 1975) and actin (Franke *et al.* 1976; Sturgess *et al.* 1980). We are currently studying the state of vimentin present in oocytes to see if there is a large soluble fraction.

The fact that the mitochondrial-rich masses are foci of vimentin and that their dispersal coincides with the appearance of a vimentin-containing web of filaments during early vitellogenesis, suggests that the cytoskeleton and particularly intermediate filaments play a major role in the developing cytoarchitecture of the oocyte. Intermediate filaments appear to be codistributed with mitochondria, a finding in agreement with previous studies in other cell types (Lee, Morgan & Wooding, 1979; Toh, Lolait, Mathy & Baum, 1980; Mose-Larsen *et al.* 1982; Summerhayes, Wong & Chen, 1983).

Following maturation of the oocyte to an egg and its subsequent fertilization, there is a profound change in the organization of vimentin, which presumably indicates equally important changes in other components of the cytoskeleton. In contrast to the fully grown oocyte, the egg contains a fine network of vimentin filaments throughout the cytoplasm and this pattern is inherited by the blastomeres formed by its first cleavages. This change in pattern during oocyte maturation is presumably due to redistribution of vimentin, although *de novo* synthesis is not ruled out by our results. Thus the changes in cytoarchitecture required to distribute the cytoplasm of the oocyte into the egg are paralleled by a change in distribution of vimentin. To what extent the former is dependent upon the latter will have to await physiological experiments.

In conclusion therefore, it seems most likely from the changing pattern of vimentin-containing filaments seen during oogenesis, fertilization and early cleavage, that intermediate filaments play a role in the maintenance of the highly asymmetrical cytoarchitecture of the egg and early embryo. Whether they initiate this architecture is unknown.

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