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Intermediate filaments in the *Xenopus* oocyte: the appearance and distribution of cytokeratin-containing filaments

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SUMMARY

Cytokeratins have previously been shown to exist in the *Xenopus* oocyte cortex. Using three monoclonal antibodies against cytokeratins, we follow the appearance of cytokeratin-containing filaments, and their changes in distribution during oocyte differentiation and maturation. Cytokeratin-containing filaments are shown to change dramatically in distribution in the oocyte during its ontogeny.

INTRODUCTION

Since 1969, when intermediate filaments were first described, they have become established as a major component of the cytoskeleton, despite the continuing mystery of their function. They have been shown by biochemical and immunocytochemical data to comprise a related group of five classes of polypeptide, each of which is expressed in defined cell lineages (for reviews see Anderton, 1981; Lazarides, 1982; Osborn & Weber, 1982). There are several instances of different intermediate filament proteins being expressed in the same cell. These include the coexpression of vimentin and neurofilament protein in the developing neural tube of the chick embryo, (Jacobs, Choo & Thomas, 1982; Bignami, Raju & Dahl, 1982), vimentin and desmin in developing muscle cells (Lazarides *et al.* 1982), vimentin and glial filament acidic protein in some astrocytes (Tapscott *et al.* 1981; Yen & Fields, 1981; Schnitzer, Franke & Schachner, 1981), and vimentin and cytokeratin in the Reichert's membrane of early mouse embryos (Lane, Hogan, Kurkinen & Garrels, 1983; Lehtonen *et al.* 1983).

Until very recently, there has been uncertainty over whether cells of the germ line express intermediate filaments. However, recent reports suggest that both

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mouse (Lehtonen *et al.* 1983) and frog (Gall, Picheral & Gounon, 1983; Franz *et al.* 1983) oocytes contain cytokeratins. In addition, vimentin has been demonstrated in the frog oocyte (Godsave, Anderton, Heasman & Wylie, 1984).

In the anuran amphibian *Xenopus laevis*, fully grown oocytes, eggs, and blastulae have been shown to possess a cortical network of cytokeratin-containing filaments (Gall *et al.* 1983; Franz *et al.* 1983). In this paper we would like to extend these observations by presenting immunocytochemical data to show that cytokeratin filaments are not confined to the cortex of the fully grown oocyte, but form a complex cytoskeletal network throughout the cytoplasm. We also show that cytokeratin-containing filaments appear at previtellogenic stages, that their distribution changes as the oocyte differentiates and grows, and that a large-scale shift in cytokeratin distribution takes place during oocyte maturation, when most of the cytokeratin does become cortical. This may represent an important example of localization in the egg cytoplasm during maturation.

MATERIALS AND METHODS

(i) *Antibodies used*

In this work we have used three anti-cytokeratin monoclonal antibodies, which have been designated LE65, LP3K and LP1K. These antibodies have been shown to stain cytokeratins specifically in many cell types. LE65 which was raised against PtK1 cell cytoskeletons, reacts strongly with most soft and simple non-squamous internal epithelia and also reacts with intestinal epithelia in rats and mice (Lane, 1982) and with some cells in the early mouse embryo (Lane *et al.* 1983). Immunoprecipitation studies show that LE65 precipitates three major polypeptides within the size range of simple epithelial cytokeratins in parietal endoderm cells of mouse embryos (Lane *et al.* 1983). Tests on mammalian tissues have shown that LP1K and LP3K are also specific for epithelial cells (Lane *et al.* in preparation).

(ii) *Immunocytochemistry*

A variety of fixation and embedding techniques were used to stain histological sections of *Xenopus* oocytes, eggs, and gut. Fixation with aldehydes or 2% w/v trichloroacetic acid caused complete loss of antigenic activity. We therefore adopted the following protocol:- pieces of gut, ovary or unfertilized eggs were fixed in 100% ethanol for 48 h, rehydrated, infiltrated with 5% w/v sucrose in phosphate-buffered saline (PBS) for 6 h, 15% w/v sucrose in the same buffer for 2 h, and then embedded in $7\frac{1}{2}$% w/v gelatin (BDH, 300 Bloom) in 15% w/v sucrose in PBS. Sections were cut at −30 °C on a Bright cryostat, and air dried. Sections were rehydrated in PBS containing 1% v/v rabbit serum and 1% w/v BSA and stained with undiluted tissue culture supernatants containing anticytokeratin antibodies for 90 min at room temperature or 16 h at 4 °C. Antibody

incubations were followed by three 10 min washes in PBS containing 1 % v/v rabbit serum 1 % w/v BSA and the addition of rhodamine-conjugated rabbit anti-mouse immunoglobulin, 1:50 dilution in PBS (1 % w/v BSA, Nordic Ltd). After further washing, sections were mounted in u.v.-free water-soluble mounting medium (Difco Ltd.) and viewed in a Zeiss photomicroscope with epifluorescence attachment. Unfixed frozen sections were also taken for some staining reactions, and treated in the same way.

(iii) *Immunoblotting*

Cellular proteins, either total protein or triton-insoluble material, from *Xenopus laevis* swimming tadpoles 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).

RESULTS

1. *Specificities of antibodies used*

This was tested by staining adult frog gut sections prepared in the same way as, and stained in parallel with, the oocyte sections.

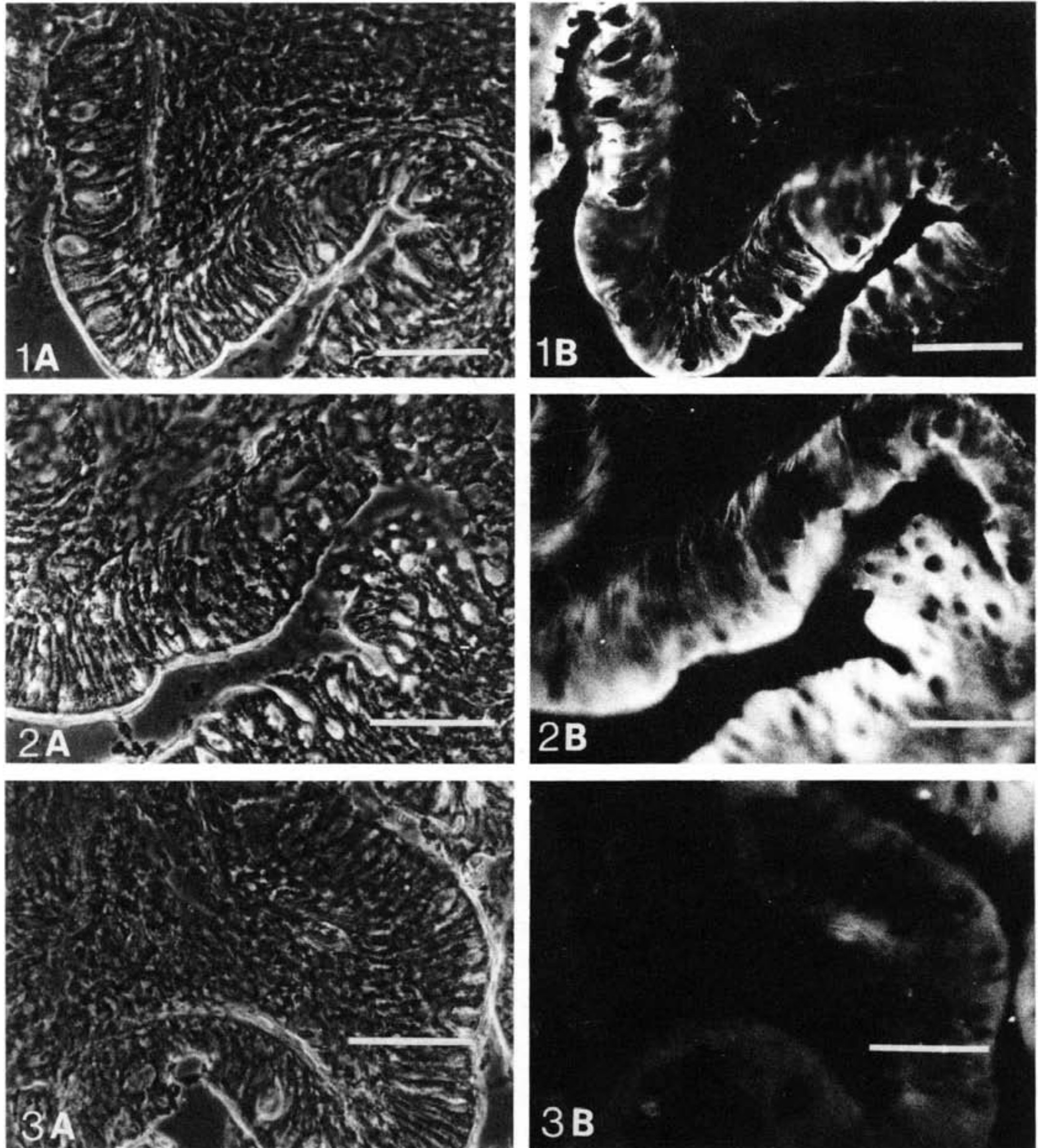
Figs 1–3 show adult *Xenopus* gut sections stained with LP3K and LE65. Control sections were incubated with tissue culture medium in place of the first antibody. Both antibodies stain the surface epithelium strongly, but do not react with the submucosal connective tissues and muscle layers. LP1K shows the same specificity (not shown).

2. *Staining of frog oocyte sections*

Cytokeratin staining is first found during stage I (Dumont, 1972) as sparse cortical threads. Fig. 4 shows an oocyte of approximate diameter 100 μm, where these threads are only just beginning to appear. In later previtellogenic oocytes, more obvious cortical and subcortical areas are found (Figs 5, 6). Fig. 5 shows a grazing section through the cortex of a previtellogenic oocyte in which a fine network of filaments is stained. The mitochondrial cloud appears also to be surrounded by a 'capsule' of cytokeratin filaments, from which filaments dip inwards towards the middle of the cloud, partially separating it into segments (Fig. 6).

During early vitellogenic stages, cytokeratins appear around the nuclear membrane. From this thin line of staining, radially arranged filaments project towards the cortex (Fig. 7). As vitellogenesis proceeds, the yolk spreads towards the centre of the oocyte. The perinuclear array of cytokeratin becomes much more dense. At this stage there are three distinct areas, concentrically arranged in the oocyte (Fig. 8). The most central area around the nucleus contains a dense network of filaments, outside this is the yolky area where cytokeratin filaments

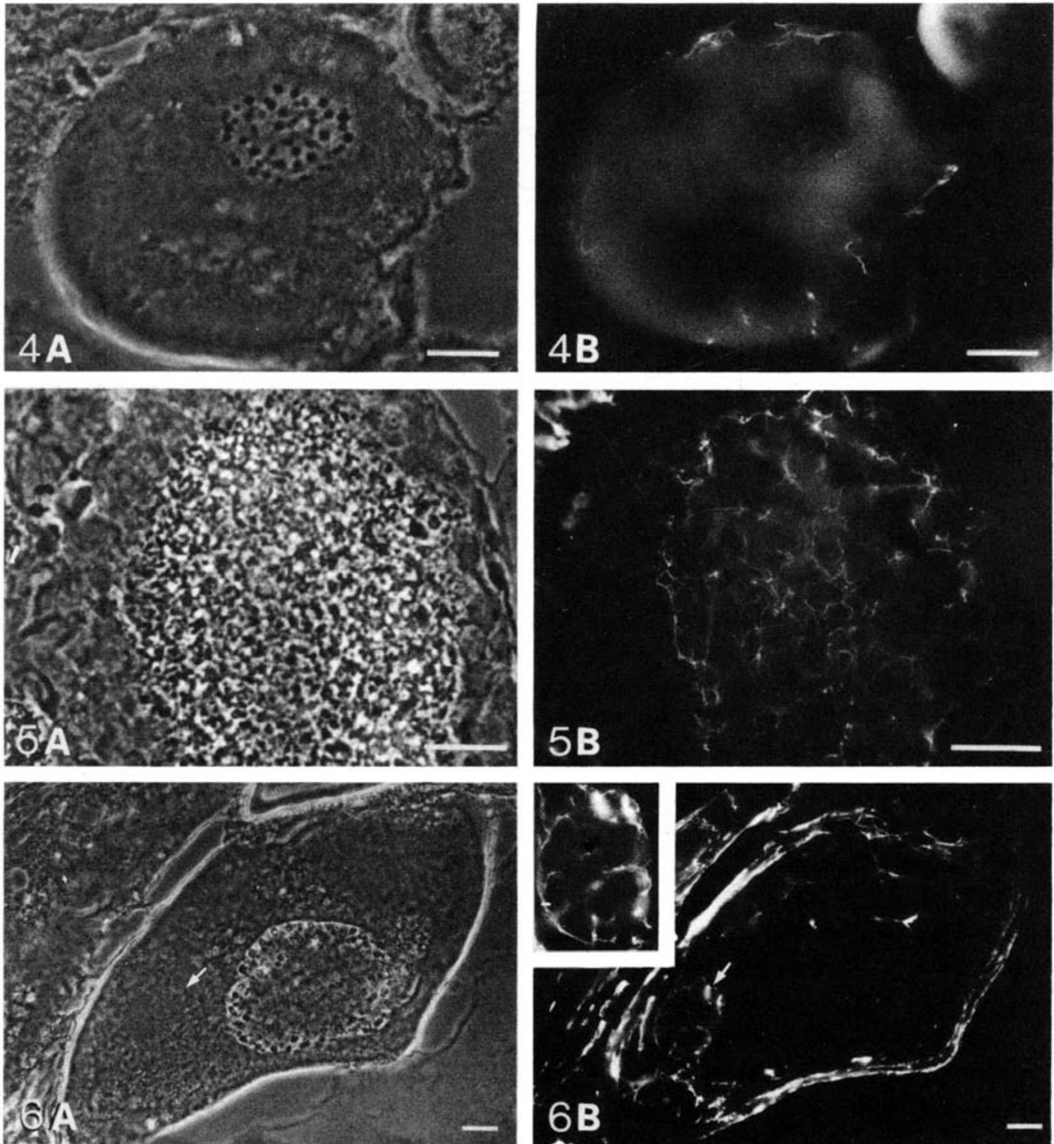
are sparse, and the outermost cortical layer contains a dense network of filaments, most of which are circumferentially arranged. Controls in which primary antibody is replaced by tissue culture supernatant show no staining (Fig. 9). At the end of vitellogenesis (Figs 10 & 11), there are differences between animal and



Figs 1–2. Immunofluorescence labelling of frozen sections of *Xenopus* gut with anti-cytokeratin antibodies. Phase contrast and fluorescence pairs are shown: Fig. 1A,B, LP3K staining; Fig. 2A,B, LE65 staining. Bar = 50 μm.

Fig. 3. Immunofluorescence labelling of adult *Xenopus* gut with control tissue culture medium. (A) phase contrast; (B) fluorescence. Bars = 50 μm.

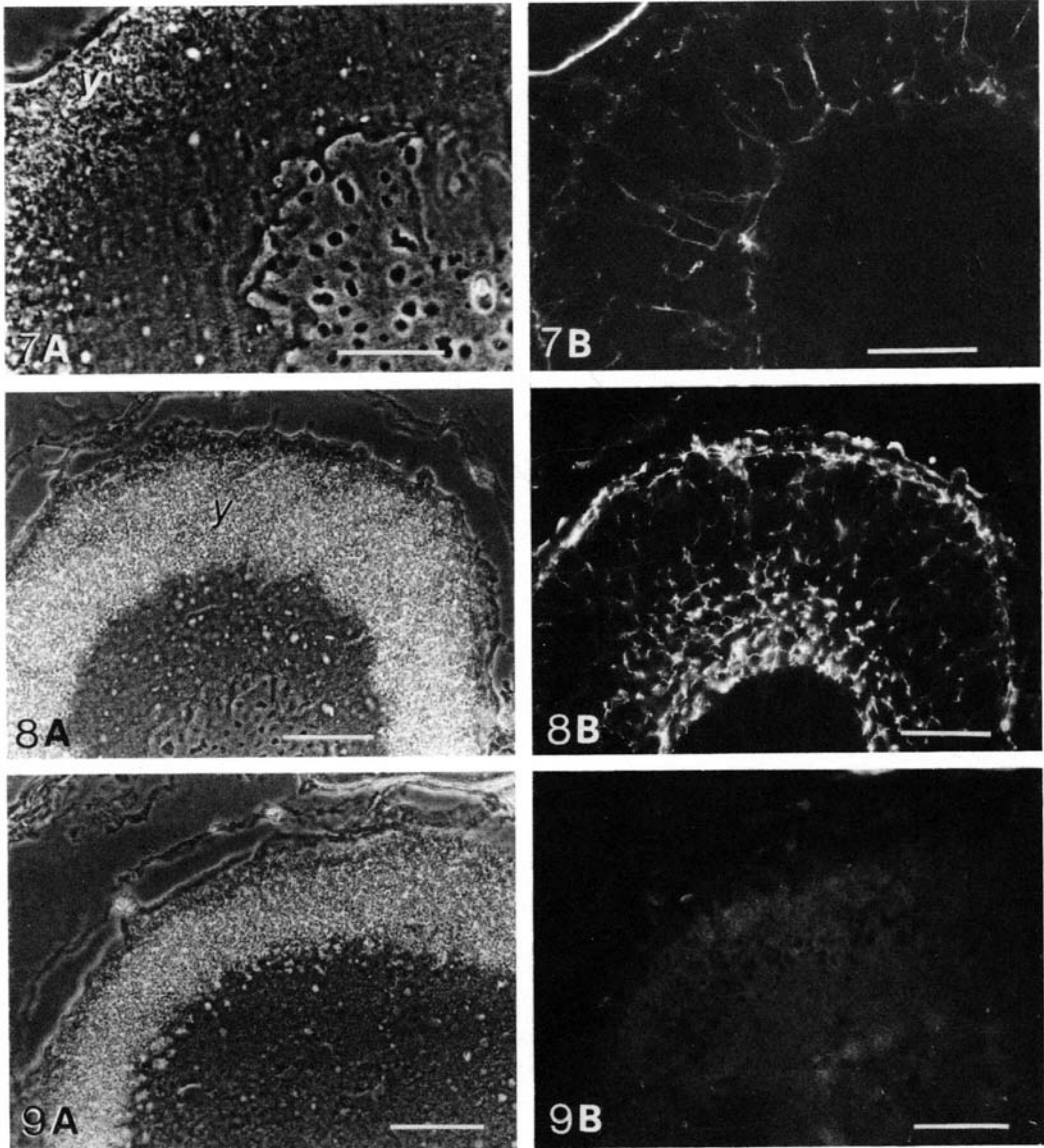
vegetal hemispheres. In the animal hemisphere (Fig. 10) fine filaments project radially from the nucleus through the yolk-free tracts towards the cortex. In the



Figs 4–6. Immunofluorescence labelling of previtellogenic oocytes with antibodies to cytokeratins. Bars = 20 μm. Fig. 4. Early previtellogenic oocyte from a frozen section of ethanol-fixed ovary stained with LE65. (A) phase contrast; (B) fluorescence. Fig. 5. Grazing section through the cortex of a previtellogenic oocyte from a sample of unfixed frozen ovary, stained with LP1K. (A) phase contrast; (B) fluorescence. Fig. 6. Previtellogenic oocyte exhibiting a mitochondrial cloud (arrows) stained with LE65. (A) phase contrast; (B) fluorescence. Inset shows mitochondrial cloud at twice the magnification of that in the parent figure. Note its cortical shell of staining, and partial division into segments.

vegetal hemisphere (Fig. 11) there is a fine network arranged apparently randomly. There is a cortical shell of staining all around the oocyte.

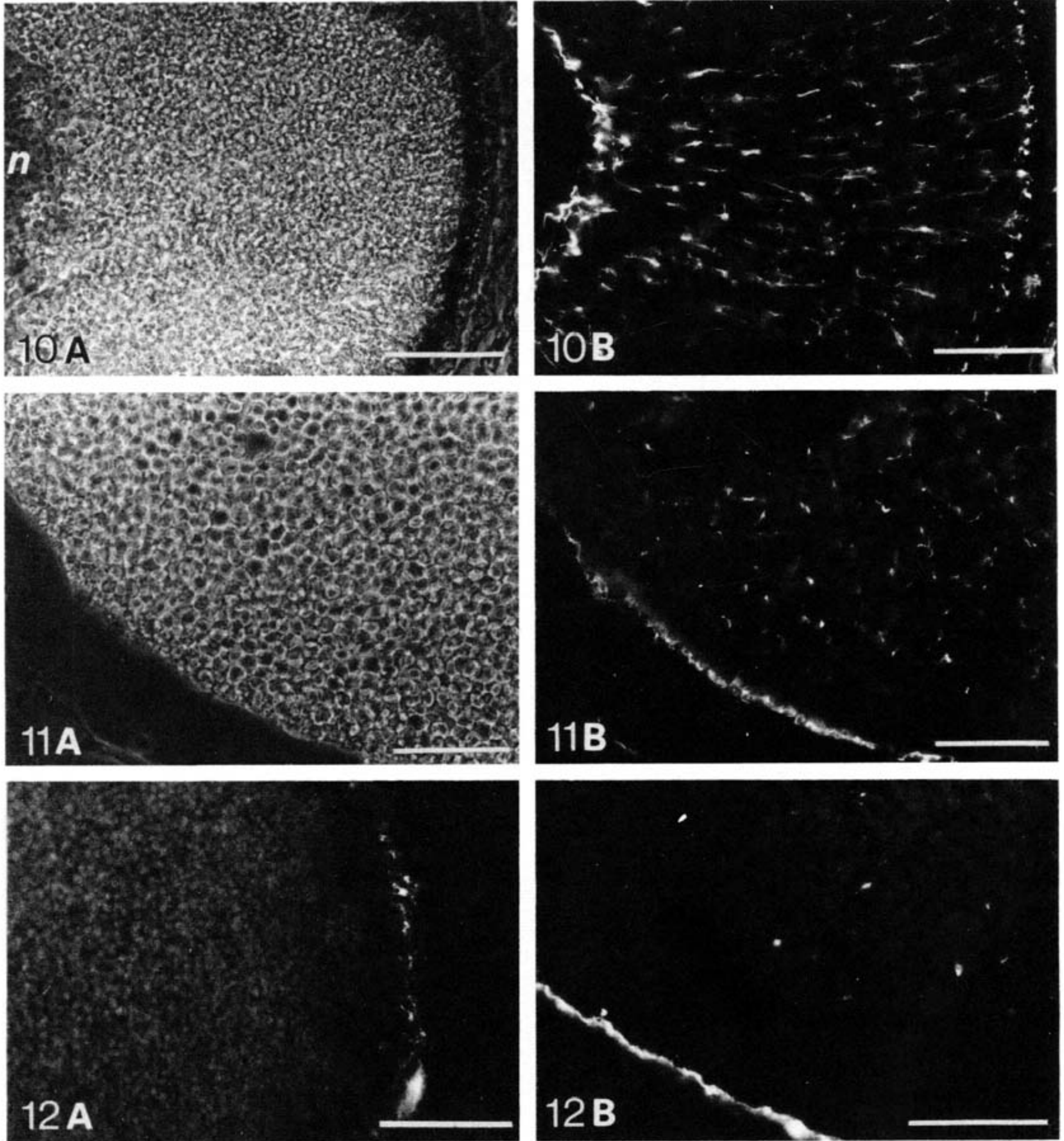
The fate of this complex array of cytokeratin in the fully grown oocyte was



Figs 7–8. Immunofluorescence labelling of vitellogenic oocytes with antibodies to cytokeratins. Bars = 50 μm. Fig. 7. Early vitellogenic oocyte stained with LP3K. (A) phase contrast; (B) fluorescence. *y*, yolk platelets. Fig. 8. Mid-vitellogenic oocyte stained with LE65. (A) phase contrast; (B) fluorescence.

Fig. 9. Immunofluorescence labelling of a vitellogenic oocyte with control tissue culture medium. (A) phase contrast; (B) fluorescence. Bars = 50 μm.

followed by staining sections of oocytes at the end of maturation (induced *in situ* by hormone injection into the female *Xenopus*). Fig. 12 shows areas of the animal (Fig. 12A) and vegetal (Fig. 12B) hemispheres of unfertilized and



Figs 10–11. Immunofluorescence labelling of fully grown oocytes with LE65. Bars = 50 μm. Fig. 10. Animal hemisphere containing nucleus (*n*). (A) phase contrast; (B) fluorescence. Fig. 11. Vegetal hemisphere. (A) phase contrast; (B) fluorescence.

Fig. 12. Immunofluorescence labelling of the animal hemisphere of an unfertilized egg using LP3K (A) and vegetal hemisphere of a fertilized egg using LE65 (B). Bars = 50 μm.

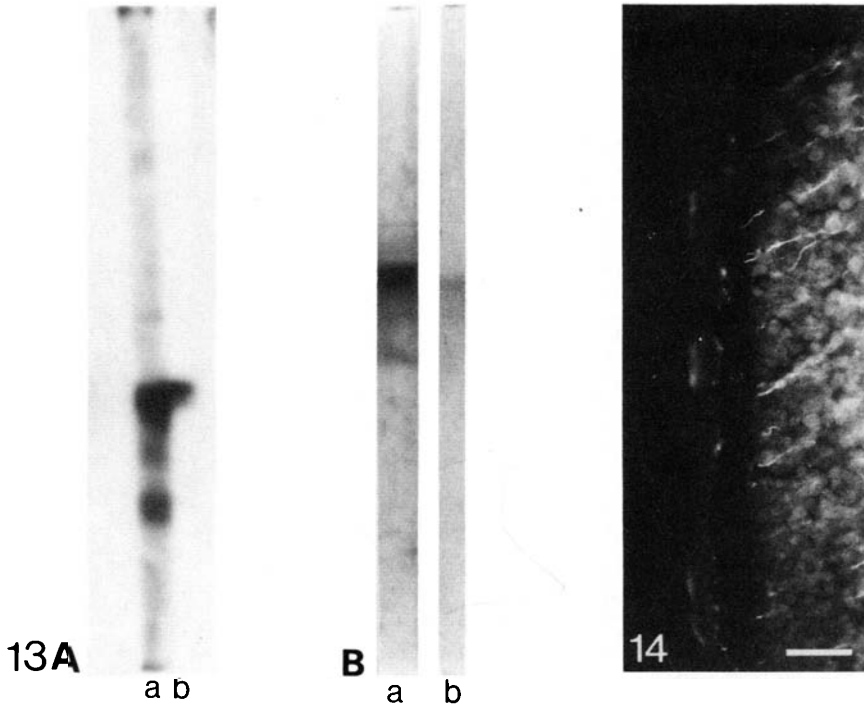


Fig. 13. Nitrocellulose blotting of *Xenopus* tadpole protein with anti-IFA and anti-cytokeratin monoclonal antibodies. (A) Triton-insoluble proteins stained with anti-(intermediate filament antigen) antibodies (anti-IFA), track a; and LP1K anti-cytokeratin antibodies, track b. (B) total tadpole protein stained with anti-IFA, track a; and LE65 anti-cytokeratin antibodies, track b.

Fig. 14. Immunofluorescence labelling of cytokeratin in a section of an unfixed fully grown oocyte using LP1K. Only the cortex and subcortical regions of the oocyte are shown. Bar = 20 μm.

fertilized eggs respectively. The staining is now found in a thin cortical shell around the whole egg.

The pattern of staining at all of these stages was identical with all three anti-cytokeratin monoclonal antibodies.

Preliminary biochemical analysis of the specificities of LP1K and LE65, two of the antibodies used in immunofluorescence experiments, was carried out using the immunoblotting technique. Reaction of proteins from swimming tadpoles with LP1K (Fig. 13A) and LE65 (Fig. 13B) on blots shows that both antibodies stain a protein of similar relative molecular mass to the major tadpole band stained by anti-(intermediate filament antigen) antibody (anti-IFA) a monoclonal antibody which reacts with most, if not all, intermediate filament proteins (Pruss *et al.* 1981). In Fig. 9 of the accompanying paper (Godsave *et al.* 1984) it was shown that the major anti-IFA-reactive protein from tadpoles has a slightly lower relative molecular mass (M_r) than mammalian vimentin (58 000) and that proteins of similar relative molecular mass from defolliculated oocytes

are also stained by anti-IFA. These findings are consistent with those of Franz *et al.* (1983) who demonstrated, by immunoblotting, the presence of a 56 000 M_r cytokeratin in *Xenopus* oocytes and eggs.

It is almost certain that the protein stained by LP1K and LE65 is a cytokeratin. LE65 has been well characterized in mammalian tissues and is also known to stain cytokeratins in the same M_r range in embryonic mouse tissue (Lane, Goodman & Trejdosiewicz, 1982; Lane *et al.* 1983). In *Xenopus* we have found by immunofluorescence that LE65 and LP1K react specifically with the epithelial layer of adult gut (see Fig. 1 for LE65 staining, LP1K staining not shown). Also in fully grown *Xenopus* oocytes, the staining patterns of both antibodies are consistent with those observed by Franz *et al.* (1983), i.e. cortical and subcortical filaments are stained (Figs 10, 11 & 14).

DISCUSSION

In the accompanying paper, we reported the presence, and described the distribution of vimentin in the *Xenopus* oocyte. Here we document the same phenomena for cytokeratin-containing filaments. It is clear from these studies that cytokeratin and vimentin co-exist during most of the differentiative stages of the female germ line. This co-existence of two intermediate filament types has been documented in several other cell types during normal differentiation (Lane *et al.* 1983; Schnitzer *et al.* 1981; Tapscott *et al.* 1981; Bignami *et al.* 1982), and in some fully differentiated tissues of adult animals (Dräger, 1983; Schnitzer *et al.* 1981; Lazarides *et al.* 1982). Also some cell types start to express vimentin when maintained in tissue culture (Franke *et al.* 1979; Lane *et al.* 1982; Virtanen *et al.* 1981). It has also been reported that cells of some human metastatic epithelial tumours will produce vimentin as well as cytokeratin during growth in cavity fluids *in vivo* (Ramaekers *et al.* 1983). There are, however, important differences between vimentin and cytokeratin in the *Xenopus* oocyte, both in their time of appearance and in distribution at different stages of oocyte development. Vimentin appears first, being found in a perinuclear ring at the earliest previtellogenic stages. Cytokeratins are first detectable at later previtellogenic stages than vimentin, and are initially cortical. At no stage of oocyte growth and differentiation are the two intermediate filament types codistributed. Obvious examples of this during early oocyte stages are the positive staining of the mitochondrial cloud and the perinuclear mitochondrial masses with anti-vimentin, whereas each seems to be encapsulated and compartmentalized, but not, itself stained, with anti-cytokeratin. Distribution of the two types is almost mutually exclusive during early vitellogenesis, when vimentin is more concentrated towards the cortex, whereas cytokeratin is mainly localized nearer to the nucleus. The other obvious difference is the lack of cortical vimentin, but the presence of cortical cytokeratin all through oocyte development.

The different distributions of two intermediate filament types in a differentiating oocyte raises intriguing questions concerning their function. The oocyte is an attractive system in which to study this, since it is a large cell and can easily be injected with specific reagents.

All three of the anti-cytokeratin monoclonal antibodies used gave the same staining pattern in oocytes. By analogy with their specificities in mammalian tissues each probably cross reacts with different polypeptides of the cytokeratin family. It will be interesting to determine biochemically the full spectrum of cytokeratins in oocytes and early embryos of *Xenopus*.

Both vimentin and cytokeratin undergo dramatic changes in distribution during oocyte maturation, resulting in distributions which clearly allow differences in their inheritance by blastomeres whose fate will be to enter different germ layers. Cytokeratins become cortical in the mature egg and will be inherited by the superficial layer of blastomeres; whereas vimentin becomes a fine network distributed throughout the egg, and is inherited equally by all blastomeres. This is an impressive example of localization in the amphibian egg.

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REFERENCES

ANDERTON, B. H. (1981). Intermediate filaments: a family of homologous structures. *J. Muscle Res. Cell Motil.* **2**, 141–166.

BIGNAMI, A., RAJU, T. & DAHL, D. (1982). Localization of vimentin, the non-specific intermediate filament protein, in early differentiating neurons. *Devl Biol.* **21**, 286–295.

DUMONT, J. N. (1972). Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals. *J. Morph.* **136**, 153–180.

DRÄGER, U. C. (1983). Coexistence of neurofilaments and vimentin in a neurone of adult mouse retina. *Nature* **303**, 169–172.

FRANKE, W. W., SCHMID, E., WINTER, S., OSBORN, M. & WEBER, K. (1979). Widespread occurrence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates. *Expl Cell Res.* **123**, 25–46.

FRANZ, J. K., GALL, L., WILLIAMS, M. A., PICHERAL, B. & FRANKE, W. W. (1983). Intermediate-size filaments in a germ cell: Expression of cytokeratins in oocytes and eggs of the frog *Xenopus*. *Proc. natn. Acad. Sci., U.S.A.* **80**, 6254–6258.

GALL, L., PICHERAL, B. & GOUNON, P. (1983). Cytochemical evidence for the presence of intermediate filaments and microfilaments in the egg of *Xenopus laevis*. *Biol. Cell* **47**, 331–342.

GODSAVE, S. F., ANDERTON, B. H., HEASMAN, J. & WYLIE, C. C. (1984). Oocytes and early embryos of *Xenopus laevis* contain intermediate filaments which react with anti-mammalian vimentin antibodies. *J. Embryol. exp. Morph.* **83**, 00–00.

JACOBS, M., CHOO, Q. L. & THOMAS, C. (1982). Vimentin and 70K neurofilament protein coexists in embryonic neurones from spinal ganglia. *J. Neurochem.* **38**, 969–977.

LANE, E. B. (1982). Monoclonal antibodies provide specific intramolecular markers for the study of epithelial tonofilament organization. *J. Cell Biol.* **92**, 665–673.

LANE, E. B., GOODMAN, S. L. & TREJDOSIEWICZ, L. K. (1982). Disruption of the keratin filament network during epithelial cell division. *EMBO J.* **1**, 1365–1372.

LANE, E. B., HOGAN, B. L. M., KURKINEN, M. & GARRELS, J. I. (1983). Co-expression of vimentin and cytokeratins in parietal endoderm cells of early mouse embryo. *Nature* **303**, 701–704.

LAZARIDES, E. (1982). Intermediate filaments: a chemically heterogeneous developmentally regulated class of proteins. *Ann. Rev. Biochem.* **51**, 219–250.

LAZARIDES, E., GRANGER, B. L., GARD, D. L., O'CONNOR, C. M., BRECKLER, J., PRICE, M. & DANT, S. I. (1982). Desmin and vimentin-containing filaments and their role in the assembly of the Z disk in muscle cells. *Cold Spring Harbor Symp. Quant. Biol.* **46**, 351–378.

LEHTONEN, E., LEHTO, V.-P., VARTIO, T., BADLEY, R. A. & VIRTANEN, I. (1983). Expression of cytokeratin polypeptides in mouse oocytes and preimplantation embryos. *Devl Biol.* **100**, 158–165.

OSBORN, M. & WEBER, K. (1982). Intermediate filaments: cell type-specific markers in differentiation and pathology. *Cell* **31**, 303–306.

PRUSS, R. M., MIRSKY, R., RAFF, M. C., THORPE, R., DOWDING, A. J. & ANDERTON, B. H. (1981). All classes of intermediate filaments share a common antigenic determinant defined by a monoclonal antibody. *Cell* **27**, 419–428.

RAMAEKERS, F. C. S., HAAG, D., KANT, A., MOESKER, O., JAP, P. H. K. & VOOIJS, G. P. (1983). Coexpression of keratin- and vimentin-type intermediate filaments in human metastatic carcinoma cells. *Proc. natn. Acad. Sci., U.S.A.* **80**, 2618–2622.

SCHNITZER, J., FRANKE, W. W. & SCHACHNER, M. (1981). Immunocytochemical demonstration of vimentin in astrocytes and ependymal cells of developing and adult mouse nervous system. *J. Cell Biol.* **90**, 435–447.

TAPSCOTT, S. J., BENNETT, G. S., TOYAMA, Y., KLEINHART, F. & HOLTZER, H. (1981). Intermediate filament proteins in the developing chick spinal cord. *Devl Biol.* **86**, 40–54.

VIRTANEN, I., LEHTO, V.-P., LEHTONEN, E., VARTIO, T., STENMAN, S., KURKI, P., WAGER, O., SMALL, J. V., DAHL, D. & BADLEY, R. A. (1981). Expression of intermediate filaments in cultured cells. *J. Cell Sci.* **50**, 45–63.

YEN, S. H. & FIELDS, K. L. (1981). Antibodies to neurofilament, glial filament and fibroblasts intermediate filament proteins bind to different cell types of the nervous system. *J. Cell Biol.* **88**, 115–126.

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