

A monoclonal antibody against mouse oocyte cytoskeleton recognizing cytokeratin-type filaments

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SUMMARY

Monoclonal antibodies were raised to detergent-extracted cytoskeleton preparations of mouse oocytes. In immunofluorescence microscopy, one of the antibodies, OCS-1, localizes exclusively to epithelial cells in frozen tissue sections, including various simple and stratified epithelia. The antibody decorates a keratin-type of fibrillar, vinblastine-resistant network in various cultured, epithelial-type cells, but not in myoid or fibroblastoid cells.

In mouse oocytes and cleavage-stage embryos, the OCS-1 antibody gives a diffuse, spotty staining pattern. In blastocyst-stage embryos, the antibody reveals a keratin-type filamentous organization in the trophectoderm cells. In immunoelectron microscopy, the OCS-1 antibody decorates 10 nm-thick filaments, often identifiable as desmosome-attached tonofilaments, in detergent-treated trophectoderm cells.

The antigen(s) recognized by the OCS-1 antibody is apparently present in, or closely associated with, cytokeratin filaments. In addition to mouse oocytes and early embryos, a wide variety of epithelial cells in various species seem to share this antigen(s). The present results suggest that at the early stages, the cytokeratin-related antigen(s) defined by the OCS-1 antibody are stored in a non-fibrillar form which is then converted into a fibrillar network at the blastocyst stage. A pre-existing supply of cytokeratin-related protein may be essential for the development of the blastocyst.

INTRODUCTION

Formation of different types of intermediate filaments (IFs) is associated with cell differentiation, and IF subclasses serve as markers for different cell types (Lazarides, 1982; Osborn & Weber, 1983). Special interest should therefore be addressed to the IF cytoskeleton of germ cells and early embryonic cells. The first type of IF proteins expressed during early mouse development is cytokeratin (Jackson *et al.* 1980; Paulin, Babinet, Weber & Osborn, 1980; Lehtonen *et al.* 1983c), and another type of IF, vimentin, appears in the newly forming mesoderm cells late on embryonic day 8 (Jackson *et al.* 1981; Franke *et al.* 1982). In the preimplantation mouse embryo, the presence of cytokeratin filaments in the trophectoderm cells of the blastocyst has been confirmed by several authors (Brûlet, Babinet, Kemler & Jacob, 1980; Paulin *et al.* 1980; Jackson *et al.* 1980; Lehtonen *et al.* 1983c; Oshima *et al.* 1983). In a previous report, we have demonstrated, with electron microscopy, the presence of detergent-resistant

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10 nm-thick filaments already in 2-cell-stage embryos and also suggested that mouse oocytes contain cytokeratin polypeptides (Lehtonen *et al.* 1983c; see, however, Jackson *et al.* 1980). In an attempt to raise antibodies to the IF proteins of early embryos, mice were immunized with detergent-extracted cytoskeletal preparations of mouse oocytes. Here I describe one of the monoclonal antibodies obtained, the OCS-1 (oocyte cytoskeleton-1) antibody, and show that the recognized antigen(s) are present in or closely associated with cytokeratin filaments.

MATERIALS AND METHODS

Immunization of mice

Oocytes from CBA/T6T6 mice were used for immunization. The procedures for superovulating the females and for collecting the oocytes were as earlier described (Lehtonen *et al.* 1983c). Briefly, the oocytes were collected in medium, the granulosa cells were removed with hyaluronidase and the zona pellucida was removed with acid Tyrode's solution. The oocytes were then rinsed in NaCl-phosphate buffer (140 mM-NaCl, 10 mM-sodium phosphate, pH 7.2) and stored at -70°C . Cytoskeleton preparations were made by extracting the oocytes with 0.5% Triton X-100 in 50 mM-Tris-HCl, pH 7.2, supplemented with 1 mM-phenylmethylsulphonyl fluoride (PMSF; Sigma Chemical Co., St. Louis, MO), for 30 min at 0°C . Nine-week-old BALB/c mice were immunized intraperitoneally at 3- to 4-week intervals, each time with cytoskeleton preparations from about 350 oocytes emulsified in Freund's complete adjuvant.

Hybridomas and monoclonal antibodies

Three days after the fourth immunization, the spleens were dissociated and the cells fused with the myeloma cell line P3/NS-7/1-Ag4-1, using the procedure of Köhler & Milstein (1976). Hybrid selection was started 24 h later using standard techniques. Cultures were screened for antibody production by indirect immunofluorescence using methanol-fixed oocytes and a panel of cultured cells. Positive hybrids were cloned by using a mouth-controlled micropipette.

Antibody-containing ascites fluid was produced by injecting 10^7 cloned hybridoma cells into BALB/c mice intraperitoneally. The ascites fluid and the conditioned culture medium were stored at 4°C in the presence of 0.02% sodium azide. Alternatively, the ascites fluid was stored at -70°C . Both the ascites fluid and the conditioned culture medium were stable for at least 10 months.

Immunofluorescence microscopy

The tissues and cultured cells used in this study are listed in Tables 1 and 2, respectively. Preimplantation mouse embryos were collected and processed for stainings as previously described (Lehtonen, 1980; Lehtonen & Badley, 1980; Lehtonen *et al.* 1983c). The following cell lines were obtained from American Type Culture Collection (Rockville, MD): HeLa, A431, MDCK, PtK1, RD, WI-38 and 3T3. Human amniotic epithelial cells (Virtanen *et al.* 1981), human keratinocytes (Kariniemi *et al.* 1982) and normal human fibroblasts (Virtanen *et al.* 1981) were as previously described. The F9 and STO cell lines were obtained from Dr C.F. Graham (Department of Zoology, University of Oxford, Oxford, U.K.). The cells were cultured in Eagle's minimum essential medium supplemented with 10% foetal calf serum, as described elsewhere (Lehtonen, Lehto, Paasivuo & Virtanen, 1983b; Lehtonen *et al.* 1983c). In some experiments, the cells were exposed to $10\ \mu\text{g ml}^{-1}$ of vinblastine sulphate (Eli Lilly, Indianapolis, IN) for 3 h before fixation.

For indirect immunofluorescence microscopy, frozen tissue sections or cultured cells on coverslips were fixed with -20°C methanol for 10 min and washed in NaCl-phosphate buffer. The stainings also included double immunofluorescence experiments using rabbit vimentin antibodies (Virtanen *et al.* 1981; a generous gift from Dr I. Virtanen, Department of Pathology,

University of Helsinki, Helsinki, Finland). In these experiments, the specimens were first exposed to rabbit vimentin antibodies, followed by fluorescein isothiocyanate (FITC)-coupled goat anti-rabbit IgG (Dakopats, Copenhagen, Denmark), and then to OCS-1 antibody, followed by tetramethyl rhodamine isothiocyanate (TRITC)-coupled goat anti-mouse mixed Igs (N.L. Cappel Laboratories Inc., Cochranville, PA). Alternatively, TRITC-coupled goat anti-mouse IgM (Cappel) was used for visualization of OCS-1 reactivity. Rat monoclonal TROMA 1 antibodies, reacting only with cytokeratin (Kemler *et al.* 1981; a generous gift from Dr Rolf Kemler, Max-Planck-Institut, Tübingen, Federal Republic of Germany), were used in immunofluorescence experiments designed to study the SDS-sensitivity of the antigen(s) recognized by OCS-1 and TROMA 1 antibodies, respectively. The specimens were examined in a Zeiss Universal microscope equipped with an epi-illuminator III RS and filters for FITC and TRITC fluorescence.

Immunogold staining and electron microscopy

For indirect immunogold staining, blastocyst-stage embryos were treated with 0.05 % Triton X-100 in 50 mM-Tris-HCl, pH 7.2, supplemented with 1 mM-PMSF, for 5 min at 4°C. The specimens were fixed with 1 % paraformaldehyde in NaCl-phosphate buffer for 20 min and then incubated with 0.1 M-lysine in 50 mM-Tris-HCl, pH 7.2, followed by washing in 0.1 % bovine serum albumin (BSA; Sigma) in 20 mM-Tris/0.9 % NaCl, pH 8.2 (0.1 % BSA-buffer). The specimens were then incubated with the OCS-1 antibody, diluted 1:5 with 1 % BSA in 20 mM-Tris/0.9 % NaCl, pH 8.2 (1 % BSA-buffer), for 45 min at 20°C, washed in 0.1 % BSA-buffer, and incubated with colloidal gold-coupled goat anti-mouse IgM (approximate granule size 5 nm; Janssen Pharmaceutica, Beerse, Belgium), diluted 1:40 with 1 % BSA-buffer, for 60 min at 20°C. After washing in 0.1 % BSA-buffer, the specimens were fixed with 1 % glutaraldehyde in 0.1 M-phosphate buffer, fixed in 0.5 % OsO₄ in 0.1 M-phosphate buffer, dehydrated in ethanol and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate and examined in a Jeol Temscan electron microscope.

Gel electrophoresis and immunoblotting

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) and immunoblotting experiments were carried out as described elsewhere (Lehtonen *et al.* 1983 *a-c*; Lehtonen, Virtanen & Saxén, 1985).

RESULTS

Hybridomas

The immunogen used in this study was a Triton-extracted cytoskeleton preparation of mouse oocytes. Supernatants produced by hybridoma cells were screened by immunofluorescence on methanol-fixed oocytes and cultured cells. Hybridoma cells were obtained from 640 of 1920 wells, and 10 of these stained mouse oocytes and decorated a fibrillar network in cultured epithelial-type cells. Hybridoma cells from one of these wells were cloned. All of the 35 clones which were chosen produced antibodies which gave a similar immunofluorescence pattern on oocytes and cultured epithelial cells and were characterized as mouse IgM. One of the clones, OCS-1 (oocyte-cytoskeleton-1), was arbitrarily chosen for this study.

The immunoglobulin yield of OCS-1 ascites was up to 11 mg ml⁻¹, and the IgM fraction (Seppälä, Sarvas, Péterfy & Mäkelä, 1981) gave positive

immunofluorescence staining of cytokeratin filaments in MDCK cells at protein concentrations down to 500 ng ml⁻¹.

Immunofluorescence on frozen sections

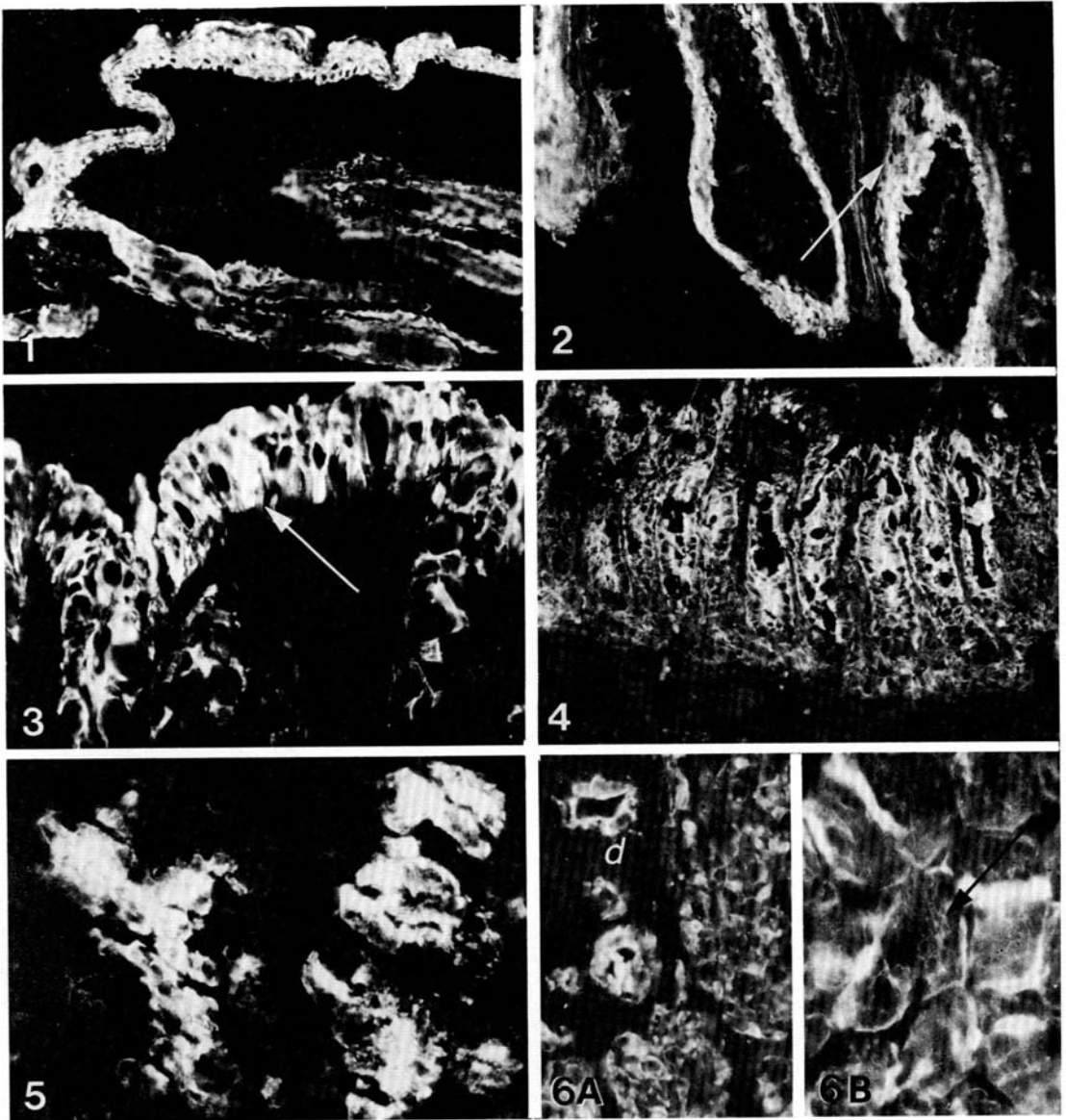
The results from immunostainings of different tissues with the OCS-1 antibody are summarized in Table 1. Generally, the antibody recognizes epithelial cells in a wide variety of tissues. Thus, both stratified epithelia (Figs 1, 2) and various simple epithelia, including glandular epithelia (Figs 3–6) reacted with the antibody. The OCS-1 antibody often revealed a fibrillar staining pattern in the epithelial cells (Figs 3, 6). In the tissues studied, the antibody never reacted with cell types other than the epithelial ones (Figs 1–4, 6A; Table 1).

Immunofluorescence on cultured cells

The results from immunostainings of different cell lines with the OCS-1 antibody are summarized in Table 2. The antibody decorated a fibrillar, keratin-type network in all cultured epithelial-type cells studied (Figs 7, 9, 10, 12), but not in myoid or fibroblastoid cells (Fig. 8). Some epithelial cell lines (A431, Fig. 10; HeLa, Fig. 12) contained both OCS-1-positive and -negative cells (Table 2). Variation in keratin expression in the A431 cell line has previously been observed (Nadakavukaren *et al.* 1984). Cultured murine F9 embryonal carcinoma cells were

Table 1. *Immunostaining of tissues by OCS-1 antibody*

Tissue	Positive staining
Human skin	Epidermal basal cells (strong) Epidermal suprabasal cells (weak) Hair follicles Sebaceous gland cells Sweat gland ductal cells
Human mammary gland	Ductal epithelium
Human intestine	Surface epithelium
Human thymus	Epithelial cells
Human kidney	Collecting ducts
Human uterus	Endometrium
Rabbit skin	Epidermal basal cells (strong) Epidermal suprabasal cells (weak) Hair follicles
Rabbit tongue	Epithelium
Rabbit ovary	Mesothelium
Rabbit intestine	Surface epithelium
Rabbit submaxillary gland	Ducts
Rabbit pancreas	Ducts
Rabbit liver	Parenchymal cells Bile ducts
Rabbit kidney	Collecting ducts
Rabbit bladder	Epithelium
Mouse intestine	Surface epithelium
Mouse 16-day embryo kidney	Collecting ducts



Figs 1-6. Reaction of the OCS-1 antibody with frozen tissue sections, indirect immunofluorescence microscopy.

Fig. 1. Rabbit skin. Basal and suprabasal layers of the epidermis as well as hair follicles are stained while the dermis remains unstained. $\times 200$.

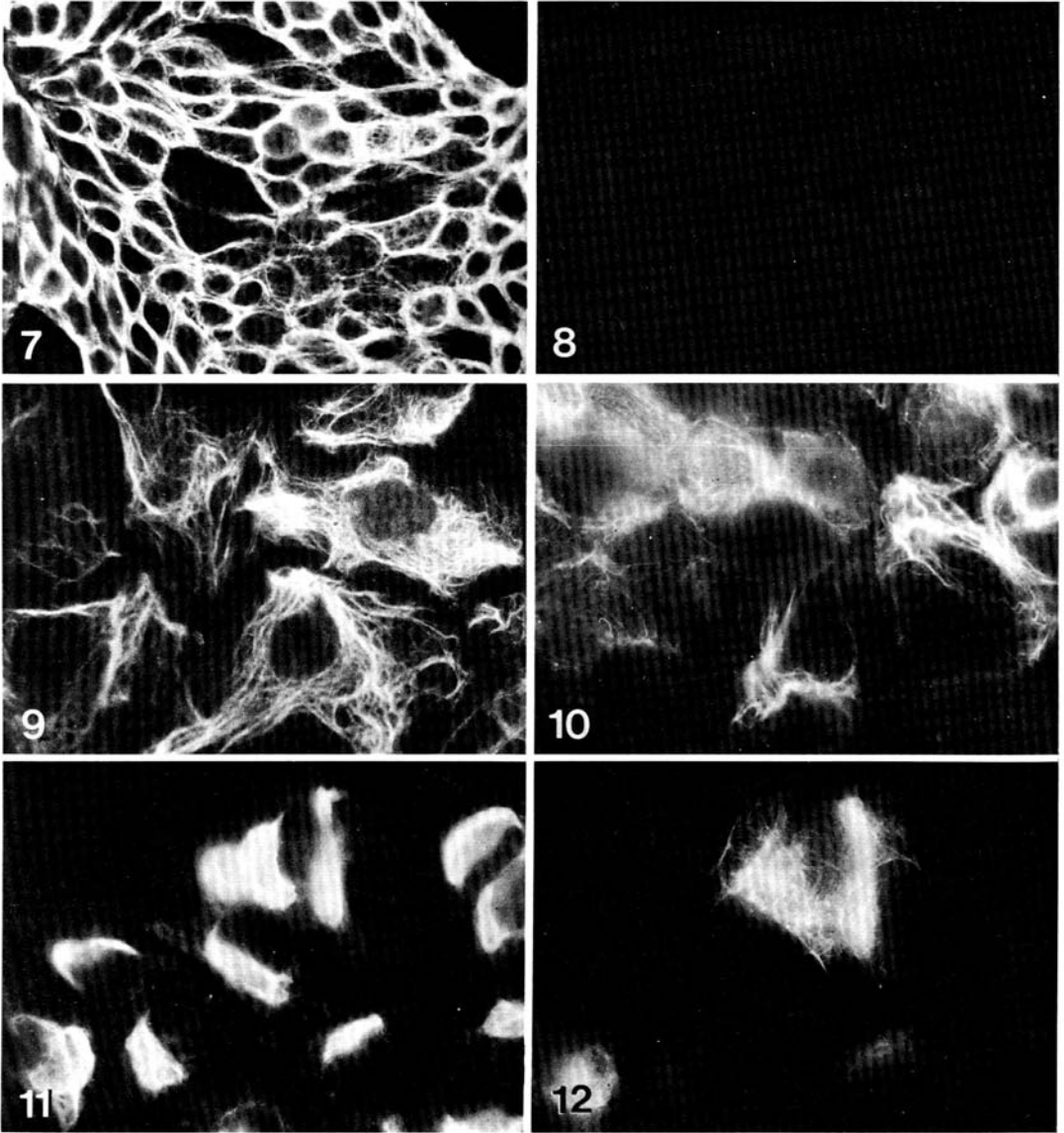
Fig. 2. Rabbit hair follicles. The staining pattern in some of the epithelial cells is fibrillar (arrow). The dermis remains unstained. $\times 200$.

Fig. 3. Rabbit colon. Note the fibrillar staining in some of the epithelial cells (arrow). $\times 400$.

Fig. 4. Mouse ileum. The epithelium shows a positive reaction while the sub-epithelial part of the gut wall (lower part of the figure) remains unstained. $\times 200$.

Fig. 5. Rabbit pancreas. Duct epithelium reacts with the antibodies. $\times 400$.

Fig. 6. Rabbit liver. Both the bile ducts (*d*, Fig. 6A) and the hepatocytes show affinity for the antibody. Note the fibrillar staining pattern in the hepatocytes (arrow, Fig. 6B). Fig. 6A, $\times 250$; Fig. 6B, $\times 450$.



Figs 7–12. Reaction of the OCS-1 antibody with cultured cells.

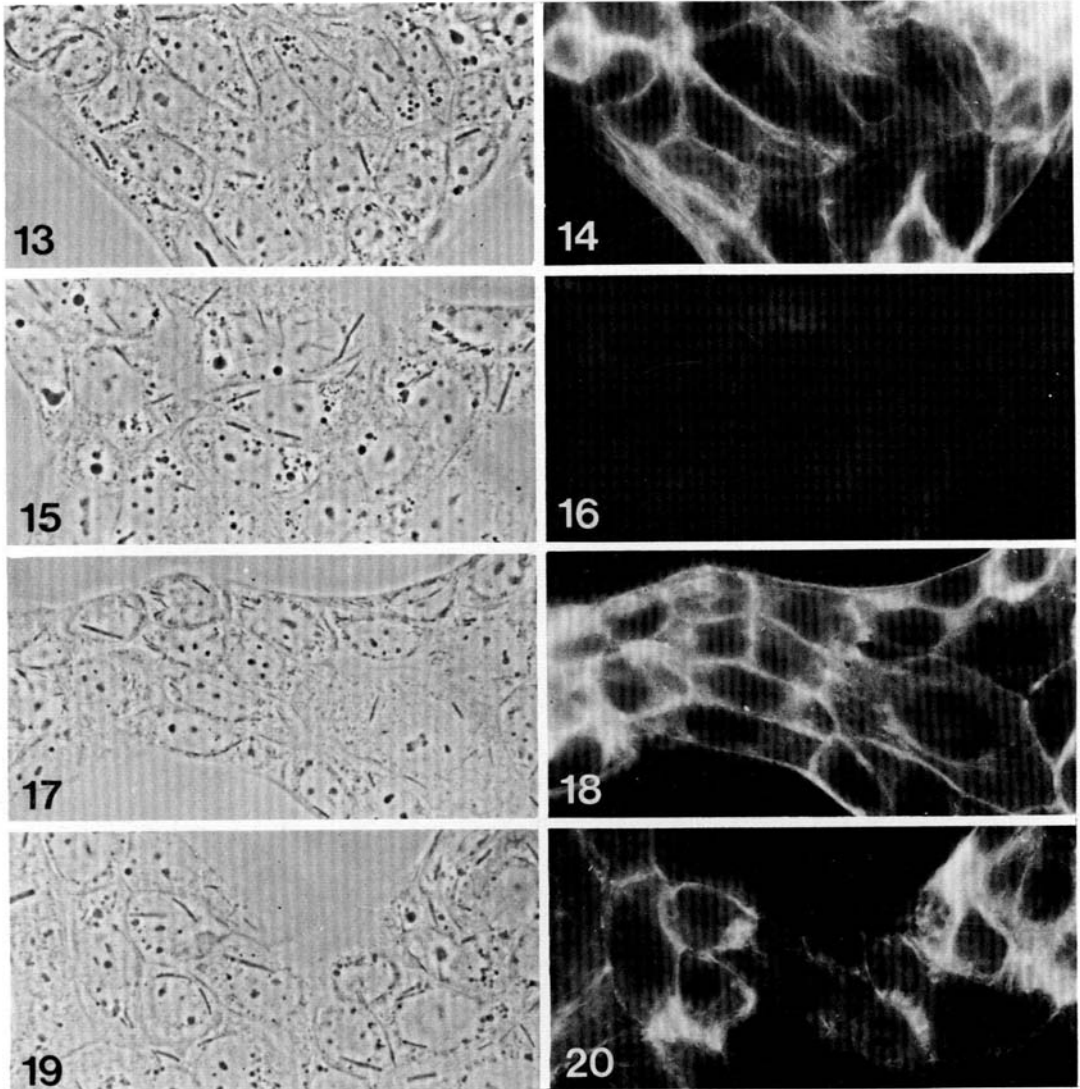
Figs 7, 9, 10. A keratin-type of fibrillar network revealed by the OCS-1 antibody in cultured MDCK, PtK1, and vinblastine-treated A431 cells, respectively. Note that some of the A431 cells (Fig. 10) do not show OCS-1-positive filaments. Fig. 7, $\times 250$; Figs 9, 10, $\times 400$.

Fig. 8. Cultured RD cells, a negative staining result with the OCS-1 antibody. $\times 400$.

Figs 11, 12. Vinblastine-treated HeLa cells, double staining. A typical coiling pattern of fluorescence is revealed by antibodies to vimentin (Fig. 11), while a fibrillar network in the same cells reacts with the OCS-1 antibody (Fig. 12). Note that only a proportion of HeLa cells shows affinity to the OCS-1 antibody. $\times 400$.

negative for OCS-1, but upon retinoic acid treatment (Lehtonen *et al.* 1983a), differentiated cells with distinct, fibrillar OCS-1 staining appeared in the cultures (data not shown).

In vinblastine-treated MDCK and HeLa cells, double immunofluorescence revealed a typical coiling pattern of vimentin filaments, while a distinct keratin-



Figs 13–20. The effect of SDS on immunofluorescence with TROMA 1 (Figs 13, 14; 17, 18) and OCS-1 (Figs 15, 16; 19, 20) antibodies. Methanol-fixed vinblastine-treated MDCK cells. Exposure of the cells to 0.1% SDS before the application of antibodies does not affect the binding of TROMA 1 antibodies (Figs 13, 14), whereas that of OCS-1 antibodies (Figs 15, 16) is mostly abolished. When antibodies are applied before exposure to SDS, a nearly unaffected fibrillary staining is obtained with both TROMA 1 (Figs 17, 18) and OCS-1 (Figs 19, 20) antibodies. $\times 400$.

Table 2. *Immunostaining of cultured cells by OCS-1 antibody*

Cell type	Fibrillar staining
Human cervical carcinoma (HeLa)	+, -*
Human epidermoid carcinoma (A431)	+, -*
Human amniotic epithelial cells	+
Human keratinocytes	+
Dog kidney epithelial cells (MDCK)	+
Rat kangaroo kidney epithelial cells (PtK1)	+
Normal human fibroblasts (WI-38)	-
Normal human embryonic fibroblasts	-
Human embryonal rhabdomyosarcoma (RD)	-
Mouse fibroblasts (3T3; STO)	-
Mouse embryonal carcinoma (F9)	-

* Both positive and negative cells detected.

type of fibrillar network resisted the treatment and reacted with the OCS-1 antibody (Figs 10–12, 19–20).

Immunoblotting experiments and SDS-sensitivity of the antigen(s) recognized by the OCS-1 antibody

Immunoblotting (Lehtonen *et al.* 1983a–c; Lehtonen *et al.* 1985) was used in an attempt to further characterize the antigen(s) recognized by the OCS-1 antibody. However, these experiments with oocytes and various cultured cells always gave negative results. For immunoblotting, the cell material is solubilized in SDS. The oocytes used for raising the OCS-1 antibody were not treated with SDS, and it was therefore suspected that the antigenic site(s) recognized by the OCS-1 antibody might be SDS-sensitive. The following experiments were done to examine this possibility.

Cultured MDCK cells were fixed in methanol at -20°C for 10 min, exposed to 0.1 % SDS in NaCl–phosphate buffer at 4°C for 2 min, washed in NaCl–phosphate buffer and then stained with TROMA 1 and OCS-1 antibodies. In these experiments, the staining pattern with TROMA 1 remained unaffected (Figs 13, 14) whereas the binding of OCS-1 was mostly abolished (Figs 15, 16). When the cells were first stained with the antibodies and then exposed to SDS, a nearly

Figs 21–24. Mouse preimplantation stages stained with the OCS-1 antibody.

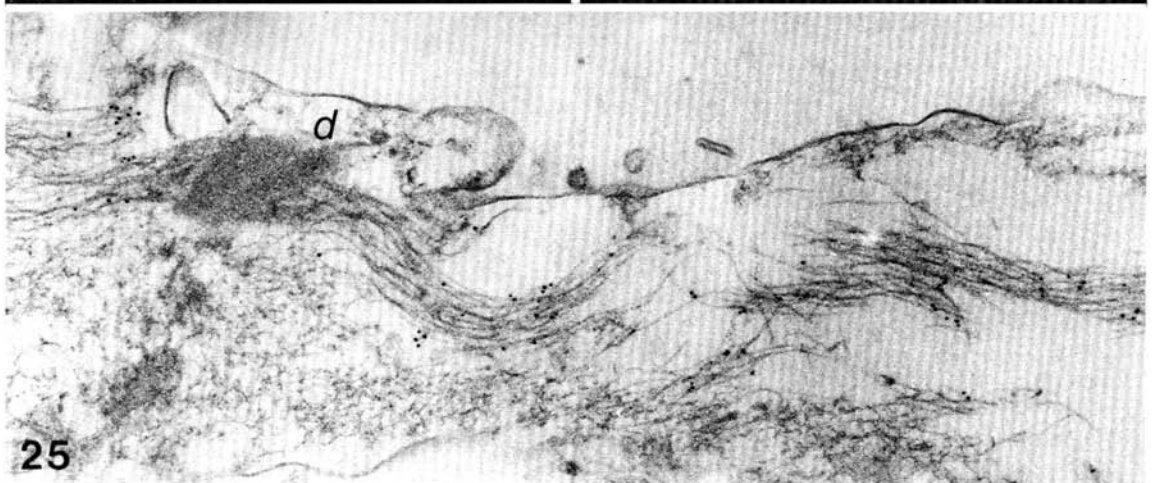
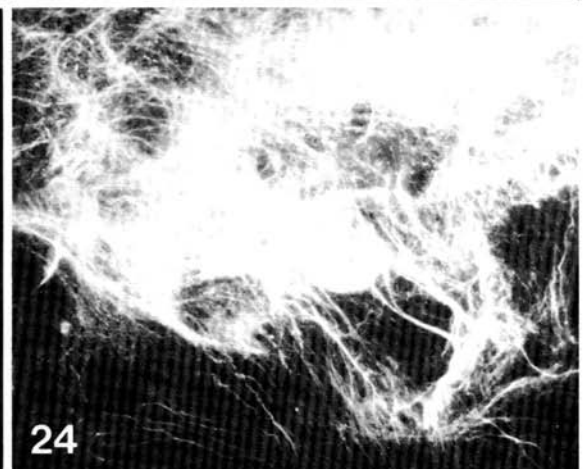
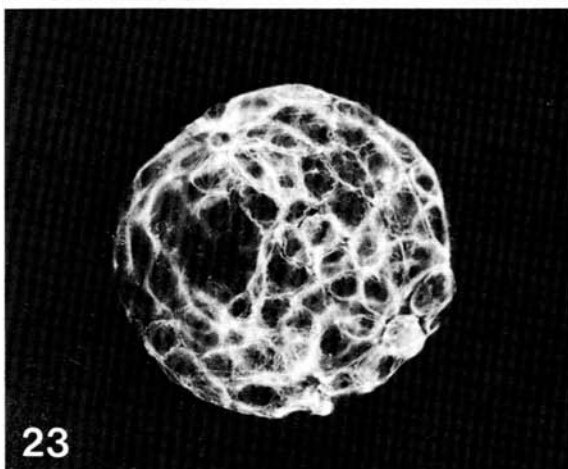
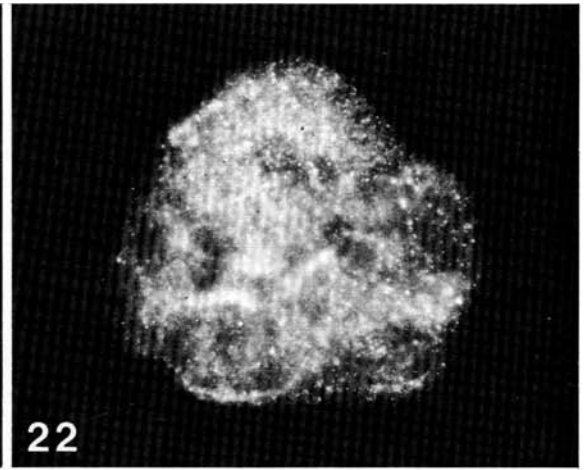
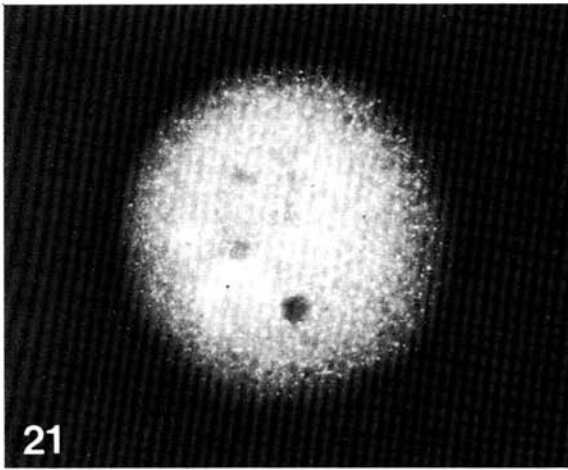
Fig. 21. An oocyte, focused at the middle of the cell. Note the diffuse, spotty pattern of fluorescence. $\times 450$.

Fig. 22. A morula. The general staining pattern is the same as in the oocyte (Fig. 21). $\times 400$.

Fig. 23. A blastocyst. Note the fibrillar pattern of fluorescence in the trophectoderm cells. $\times 350$.

Fig. 24. A blastocyst outgrowth. The flattened trophectoderm cells show a distinct keratin-type network. $\times 450$.

Fig. 25. A detergent-extracted mouse blastocyst stained with the OCS-1 antibody, indirect immunogold method. The label is always closely associated with 10 nm-thick filaments, most of which apparently represent tonofilaments attached to a desmosome (*d*). $\times 44\,000$.



unaltered staining was observed with both the TROMA 1 (Figs 17, 18) and the OCS-1 (Figs 19, 20) antibody. These results suggest that the antigenic site(s) recognized by the OCS-1 antibody are altered by SDS.

Immunostainings on oocytes, cleavage-stage embryos, and blastocysts

The OCS-1 antibody gave a diffuse, spotty staining pattern in mouse oocytes and cleavage-stage embryos (Figs 21, 22). A distinct fibrillar organization was revealed in the trophectoderm cells of expanded blastocysts (Fig. 23) and blastocyst outgrowths (Fig. 24).

In electron microscopy of blastocysts, stained with the OCS-1 antibody by using the immunogold method, the gold particles were regularly found closely, and exclusively, associated with 10 nm-thick filaments, often identifiable as desmosome-attached tonofilaments (Fig. 25).

DISCUSSION

The OCS-1 antibody was raised against oocytes extracted with 0.5 % Triton X-100. After this treatment, paracrystalline arrays with closely associated 10 nm-thick filaments are the major cytoplasmic component of the cell ghosts (Lehtonen *et al.* 1983c; Lehtonen & Virtanen, 1985).

Characterization of the OCS-1 antibody

The present results suggest that the antigen(s) recognized by the OCS-1 antibody localizes in or is closely associated with cytokeratin filaments. Thus the distribution of the antigen(s) seems to be restricted to epithelial tissues and cells, and the fibrillar network decorated by OCS-1 is of the type characteristic of cytokeratin organization. Furthermore, the fibrillar organization revealed by the OCS-1 antibody is vinblastine-resistant, a feature of intermediate filaments of cytokeratin type only. Finally, in electron microscopy the OCS-1 antibody specifically labels desmosome-attached 10 nm-thick filaments, i.e. cytokeratin-containing tonofilaments.

The antigen(s) recognized by the OCS-1 antibody seem to be sensitive to SDS. This precluded the identification of the antigen(s) by immunoblotting which employs SDS to solubilize the cell material. Similarly, immunoprecipitation experiments involving solubilization of IF proteins in high concentrations of SDS or urea were unsuccessful (data not shown). It has been previously suggested that some monoclonal antibodies recognize SDS-sensitive sites (Gown & Vogel, 1982; Nadakavukaren *et al.* 1984). Generally, it is clear that different preparative procedures may affect the expression of various epitopes, and that this may result in 'assay-specificity' of monoclonal antibodies (Haaijman *et al.* 1984).

Cytokeratins are a family of IF polypeptides which in different epithelia appear in different specific combinations (Moll *et al.* 1982). Correspondingly, cytokeratin antibodies with varying staining specificities have been described. Interestingly, the OCS-1 antibody reacts with a wide variety of epithelial cells of different

species. The antigenic determinant defined by this antibody thus seems to be associated with many members of the cytokeratin family. The OCS-1 antibody recognizes only cytokeratin-type of IFs, in contrast with the antibody described by Pruss *et al.* (1981) which reacts with all five classes of IFs. In immunostaining experiments, the antigenic determinant defined by the OCS-1 antibody could not be blocked with TROMA 1 antibodies or with three independent polyclonal antibodies against cytokeratin polypeptides (data not shown).

Intermediate filament proteins and early embryogenesis

It has been suggested that germ cells and early embryo cells may not contain IFs of any type (Jackson *et al.* 1980; Osborn & Weber, 1983). In contrast with this, a distinct band of vimentin has recently been shown to encircle the head of human sperm cells (Virtanen, Badley, Paasivuo & Lehto, 1984), and *Xenopus laevis* oocytes and eggs exhibit a highly organized network of IFs of cytokeratin type (Gall, Picheral & Gounon, 1983; Franz *et al.* 1983; Godsave, Wylie, Lane & Anderton, 1984*b*) and vimentin type (Godsave, Anderton, Heasman & Wylie, 1984*a*). Mouse oocytes and early embryos also contain 10 nm-thick filaments (Lehtonen *et al.* 1983*c*; Lehtonen & Virtanen, 1985; Van Blerkom, 1983), but our previous and present observations suggest that their cytokeratin-related proteins may largely be in a non-fibrillar form.

The OCS-1 antibody decorates distinct spot structures in paraformaldehyde-fixed, detergent-treated oocytes and cleavage-stage cells. These structures remain to be identified in immunoelectron microscopy. The major cytoplasmic structures resisting detergent extraction in these cells are the paracrystalline arrays, which occupy about 25 % of the cytoplasm in oocytes (Garcia, Pereyra-Alfonso & Sotelo, 1979) and diminish gradually during development. The distribution of the spots stained by OCS-1 would be compatible with the suggestion that the paracrystalline arrays react with the antibody. The composition of these structures is not known (Pikó & Clegg, 1982), but they are closely associated with 10 nm-thick filaments (Lehtonen *et al.* 1983*c*; Lehtonen & Virtanen, 1985; Van Blerkom, 1983). It would be tempting to suggest that the paracrystalline arrays contain cytokeratin-related protein in a storage form which might then be converted into a filamentous form at the blastocyst stage. A pre-existing supply of IF protein may be essential for the development of the blastocyst.

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REFERENCES

- BRÔLET, P., BABINET, C., KEMLER, R. & JACOB, F. (1980). Monoclonal antibodies against trophectoderm-specific marker during mouse blastocyst formation. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4113–4117.

- FRANKE, W. W., GRUND, C., KUHN, C., JACKSON, B. S. & ILLMENSEE, K. (1982). Formation of cytoskeletal elements during mouse embryogenesis. III. Primary mesenchymal cells and the first appearance of vimentin. *Differentiation* **23**, 43–59.
- FRANZ, K., GALL, L., WILLIAMS, M. A., PIPHERAL, B. & FRANKE, W. W. (1983). Intermediate-sized 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., PIPHERAL, 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.
- GARCÍA, R. B., PEREYRA-ALFONSO, S. & SOTELO, J. R. (1979). Protein-synthesizing machinery in the growing oocyte of the cyclic mouse. *Differentiation* **14**, 101–106.
- GODSAVE, S. F., ANDERTON, B. H., HEASMAN, J. & WYLIE, C. C. (1984a). Oocytes and early embryos of *Xenopus laevis* contain intermediate filaments which react with anti-mammalian vimentin antibodies. *J. Embryol. exp. Morph.* **83**, 169–187.
- GODSAVE, S. F., WYLIE, C. C., LANE, E. B. & ANDERTON, B. H. (1984b). Intermediate filaments in the *Xenopus* oocyte: the appearance and distribution of cytokeratin-containing filaments. *J. Embryol. exp. Morph.* **83**, 157–167.
- GOWN, A. M. & VOGEL, A. M. (1982). Monoclonal antibodies to intermediate filament proteins of human cells: unique and cross-reacting antibodies. *J. Cell Biol.* **95**, 414–424.
- HAAIJMAN, J. J., DEEN, C., KRÖSE, C. J. M., ZIJLSTRA, J. J., COOLEN, J. & RADL, J. (1984). Monoclonal antibodies in immunocytology: a jungle full of pitfalls. *Immunology Today* **5**, 56–58.
- JACKSON, B. W., GRUND, C., SCHMID, E., BÜRKI, K., FRANKE, W. W. & ILLMENSEE, K. (1980). Formation of cytoskeletal elements during mouse embryogenesis. Intermediate filaments of the cytokeratin type and desmosomes in preimplantation embryos. *Differentiation* **17**, 161–179.
- JACKSON, B. W., GRUND, C., WINTER, S., FRANKE, W. W. & ILLMENSEE, K. (1981). Formation of cytoskeletal elements during mouse embryogenesis. II. Epithelial differentiation and intermediate-sized filaments in early postimplantation embryos. *Differentiation* **20**, 203–216.
- KARINIEMI, A.-L., LEHTO, V.-P., VARTIO, T. & VIRTANEN, I. (1982). Cytoskeleton and pericellular matrix organization of pure adult human keratinocytes cultured from suction-blister roof epidermis. *J. Cell Sci.* **58**, 49–61.
- KEMLER, R., BRÛLET, P., SCHNEBELN, M.-T., GAILLARD, J. & JACOB, F. (1981). Reactivity of monoclonal antibodies against intermediate filament proteins during embryonic development. *J. Embryol. exp. Morph.* **64**, 45–60.
- KÖHLER, G. & MILSTEIN, C. (1976). Derivation of specific antibody producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* **6**, 511–519.
- LAZARIDES, E. (1982). Intermediate filaments: a chemically heterogeneous, developmentally regulated class of proteins. *Ann. Rev. Biochem.* **51**, 219–250.
- LEHTONEN, E. (1980). Changes in cell dimensions and intercellular contacts during cleavage-stage cell cycles in mouse embryonic cells. *J. Embryol. exp. Morph.* **58**, 231–249.
- LEHTONEN, E. & BADLEY, R. A. (1980). Localization of cytoskeletal proteins in preimplantation mouse embryos. *J. Embryol. exp. Morph.* **55**, 211–225.
- LEHTONEN, E. & VIRTANEN, I. (1985). Evidence for the presence of cytokeratin-like protein in preimplantation mouse embryos. *Ann. N. Y. Acad. Sci.* (in press).
- LEHTONEN, E., LEHTO, V.-P., BADLEY, R. A. & VIRTANEN, I. (1983a). Formation of vinculin plaques precedes other cytoskeletal changes during retinoic acid-induced teratocarcinoma cell differentiation. *Expl Cell. Res.* **144**, 191–197.
- LEHTONEN, E., LEHTO, V.-P., PAASIVUO, R. & VIRTANEN, I. (1983b). Parietal and visceral endoderm differ in their expression of intermediate filaments. *EMBO J.* **2**, 1023–1028.
- LEHTONEN, E., LEHTO, V.-P., VARTIO, T., BADLEY, R. A. & VIRTANEN, I. (1983c). Expression of cytokeratin polypeptides in mouse oocytes and preimplantation embryos. *Devl Biol.* **100**, 158–165.
- LEHTONEN, E., VIRTANEN, I. & SAXÉN, L. (1985). Reorganization of intermediate filament cytoskeleton in induced metanephric mesenchyme cells is independent of tubule morphogenesis. *Devl Biol.* **108**, 481–490.

- MOLL, R., FRANKE, W. W., SCHILLER, D. L., GEIGER, B. & KREPLER, R. (1982). The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* **31**, 11–24.
- NADAKAVUKAREN, K. K., SUMMERHAYES, I. C., SALCEDO, B. F., RHEINWALD, J. G. & CHEN, L. B. (1984). A monoclonal antibody recognizing a keratin filament protein in a subset of transitional and glandular epithelia. *Differentiation* **27**, 209–220.
- OSBORN, M. & WEBER, K. (1983). Biology of disease. Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology. *Lab. Invest.* **48**, 372–394.
- OSHIMA, R. G., HOWE, W. E., KLIER, F. G., ADAMSON, E. D. & SHEVINSKY, L. H. (1983). Intermediate filament protein synthesis in preimplantation murine embryos. *Devl Biol.* **99**, 447–455.
- PAULIN, D., BABINET, C., WEBER, K. & OSBORN, M. (1980). Antibodies as probes of cellular differentiation and cytoskeletal organization in the mouse blastocyst. *Expl Cell Res.* **130**, 297–304.
- PIKÓ, L. & CLEGG, K. B. (1982). Quantitative changes in total RNA, total poly(A), and ribosomes in early mouse embryos. *Devl Biol.* **89**, 362–378.
- PRUSS, R. M., MIRSKY, R., RAFF, M. C., THORPE, R., DOWNING, 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.
- SEPPÄLÄ, I., SARVAS, H., PÉTERFY, F. & MÄKELÄ, O. (1981). The four subclasses of IgG can be isolated from mouse serum by using protein A-sepharose. *Scand. J. Immunol.* **14**, 335–342.
- VAN BLERKOM, J. (1983). Posttranslational modification and cellular reorganization during resumption of arrested meiosis—computer assisted analysis. *Table Ronde Roussel Uclaf No 47, Cellular Diversification in the Early Mouse Embryo*, 6–7 October 1983, Paris.
- VIRTANEN, I., BADLEY, R. A., PAASIVUO, R. & LEHTO, V.-P. (1984). Distinct cytoskeletal domains revealed in sperm cells. *J. Cell Biol.* **99**, 1083–1091.
- 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.

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