

# Change of Cytokeratin Filament Organization during the Cell Cycle: Selective Masking of an Immunologic Determinant in Interphase PtK<sub>2</sub> Cells

WERNER W. FRANKE, ERIKA SCHMID, JENNIFER WELLSTEED, CHRISTINE GRUND, ORITH GIGI,\* and BENJAMIN GEIGER\*

*Division of Membrane Biology and Biochemistry, Institute of Cell and Tumor Biology, German Cancer Research Center, D-6900 Heidelberg, Federal Republic of Germany; and \*Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel*

**ABSTRACT** The organization of intermediate-sized filaments (IF) of the cytokeratin type was studied in cultures of PtK<sub>2</sub> cells in which typical IF structures are maintained during mitosis, using a monoclonal antibody (K<sub>G</sub> 8.13). This antibody reacts, in immunoblotting experiments, with the larger of the two major cytokeratin polypeptides present in these cells but, using standard immunofluorescence microscopy procedures, does not react with the cytokeratin filaments abundant in interphase cells, in striking contrast to various antisera and other monoclonal cytokeratin antibodies. In the same cell cultures, however, the antibody does react with cytokeratin filaments of mitotic and early postmitotic cells. The specific reaction with cytokeratin filaments of mitotic cells only is due to the exposure of the specific immunologic determinant in mitosis and its masking in interphase cells. Treatment of interphase cells with both Triton X-100 as well as with methanol and acetone alters the cytokeratin filaments and allows them to react with this monoclonal antibody. A similar unmasking was noted after treatment with buffer containing 2 M urea or low concentrations of trypsin. We conclude that the organization of cytokeratin, albeit still arranged in typical IF, is altered during mitosis of PtK<sub>2</sub> cells.

Intermediate-sized filaments (IF)<sup>1</sup> of vertebrates are cytoplasmic structures that are notoriously stable, both mechanically and chemically, and resist extractions in buffers of a broad range of ionic strengths and pH values (for reviews, see references 1–4). Different types of IF have been distinguished by subunit polypeptide composition and by immunological criteria and their specific expression has been related to routes of cell differentiation (1–3, 5–7). Yet they all share some common principles of morphology and homologies of amino acid sequence (1–4, 8–10). In spite of their remarkable stability, IF may undergo redistribution of filaments as well as rearrangements of subunit proteins in living cells. It has been described that arrays of vimentin filaments are reorganized during mitosis (11–15) and drug-induced perinuclear aggregation (11, 16–17) and similar observations have been made for cytokeratin IF in mitosis of some epithelial cells (6, 12, 18, 19). In most cases such re-distributions have been inter-

preted as altered distributions of intact IF that do not involve intrafilamentous changes such as disassembly and re-assembly of IF subunits, in agreement with electron microscopic observations of normal-looking IF in all stages of mitosis of various cultured cells of mesenchymal (11, 13, 15) and epithelial (19) origin. By contrast, certain epithelial cells exhibit a drastic, transient change of IF organization during mitosis in which IF are unravelled into different, yet still insoluble and polymeric structures that aggregate into variously sized, spheroidal masses containing cytokeratin (20–22). These observations suggest that the structural state of at least certain IF is physiologically regulated. In the present study, we describe observations indicating that systematic, though less conspicuous changes of cytokeratin IF organization also occur in mitotic cells which, at the electron microscopic level, maintain typical IF morphology such as cultured rat kangaroo (PtK<sub>2</sub>) cells. These observations have been made possible by the use of a monoclonal antibody, K<sub>G</sub> 8.13 (23), which recognizes a cytokeratin determinant, the exposure of which is modulated during the cell cycle.

<sup>1</sup> Abbreviations used in this paper: IF, intermediate-sized filaments; PtK<sub>2</sub>, cultured rat kangaroo cells.

## MATERIALS AND METHODS

**Cells:** PtK<sub>2</sub> cells were grown as previously described (19). For enrichment of mitotic stages some cell cultures were treated with 10<sup>-6</sup> M colcemid for 12–24 h (19).

**Antibodies:** The monoclonal murine antibody K<sub>G</sub> 8.13 (IgG<sub>2</sub>) has recently been described in detail (23). Conventionally prepared antibodies (IgG fractions or affinity-purified) obtained from guinea pigs and rabbits immunized with epidermal prekeratins from bovine muzzle have previously been described, including their reaction with IF of PtK<sub>2</sub> cells (3, 6, 18, 20, 24, 25). Guinea pig antibodies to vimentin have also been described (6, 24). To control the stainability of cytokeratin IF in all phases of the cell cycle of PtK<sub>2</sub> cells, we also used other monoclonal antibodies to cytokeratins (20).

**Immunofluorescence Microscopy:** PtK<sub>2</sub> cells grown on glass coverslips to various densities (from 1 d after plating to confluency) were rinsed with phosphate-buffered saline at room temperature. The standard fixation procedure included dipping for 5 min in -20°C methanol and then for 1 min in -20°C acetone, followed by air-drying. Variations of this procedure included the following:

(a) Extended (2 or 5 min) or repeated (6 × 1 min) incubations in acetone. (b) Use of ethanol instead of methanol. (c) Rinsing in PBS, followed by incubation for 5 min in 10 mM Tris-HCl buffer (pH 7.2) containing 140 mM NaCl and 0, 1, or 5 mM MgCl<sub>2</sub> (cf. reference 26) before application of methanol and acetone. (d) The same as c, except methanol and acetone were at 0°C or at room temperature. (e) Rinsed cells incubated in Tris-buffer containing 140 mM NaCl and 1% Triton X-100 (with or without 1 mM MgCl<sub>2</sub>) for various periods of time (1, 2, 3, 4, 5, and 10 min), followed by an additional wash (5 min) in Tris-buffer, prior to application of first antibody (cf. reference 26). (f) The same as in e, except with fixation in -20°C methanol and -20°C acetone before application of first antibody. (g) The same as e, except with additional wash (5 min) in PBS. (h) As done in f, except methanol and acetone were at 4°C. (i) The same as in f, except incubation in methanol and acetone were both at room temperature. (j) After treatment with buffer containing Triton X-100 (as above) cells were incubated in high salt buffer (10 mM Tris-HCl, 140 mM NaCl, 1.5 M KCl, pH 7.2) for 5 min or 30 min, then rinsed in PBS containing 1 mM MgCl<sub>2</sub>, prior to direct application of first antibody. (k) The same as in j, except after rinsing in PBS the specimens were incubated in methanol and acetone of various temperatures (-20°C, 4°C, room temperature). (l) The same as in f, except 1% Nonidet P-40 was used instead of Triton X-100. (m) After standard treatment with methanol and acetone cells were dipped into PBS containing 2 M urea (ultrapure) for a few seconds, followed by washes in PBS. (n) Cells treated with methanol and acetone were dipped into PBS containing trypsin (~20 µg/ml), followed by washes in PBS.

Incubation with the respective first antibody was for 45 min at room temperature, followed by several washes in PBS, and incubation with second antibody. In the case of the murine antibodies second antibodies were either fluorescein- or rhodamine-labeled rabbit anti-mouse IgG (freshly prepared or purchased from Miles-Yeda, Rehovot, Israel) or rhodamine-labeled goat antibodies to mouse (Cappel Laboratories, Cochranville, PA). When guinea pig antibodies were used in the first place they were visualized with rhodamine- or fluorescein-conjugated goat or rabbit antibodies to guinea pig IgG (freshly prepared or purchased from Miles-Yeda or Cappel Laboratories). After incubation with the specific second antibodies specimens were rinsed twice in PBS, air-dried and mounted in Mowiol (Hoechst, Frankfurt, Federal Republic of Germany).

Photomicrographs were taken with a Zeiss photomicroscope III (Zeiss, Oberkochen, Federal Republic of Germany). For double label immunofluorescence, both the murine and the guinea pig antibodies were applied at the same time (cf. reference 27). Controls for specificity of the second antibodies used in double immunofluorescence were routinely included.

**Electron Microscopy:** Cells grown on cover slips were fixed and processed for electron microscopy of ultrathin sections as previously described (19). For immunoelectron microscopy cells were treated in the specific way used for immunofluorescence microscopy, then were incubated with the first antibody for 1 h at room temperature. After washing three times with PBS, goat antibodies to mouse IgG that had been coupled to 5 nm colloidal gold particles (Janssen Chemicals, Beerse, Belgium) were added in PBS and incubated for 2 h. After three washes with PBS, specimens were fixed with 2.5% glutaraldehyde for 15 min, followed by OsO<sub>4</sub>-fixation, and processed for ultrathin sectioning as described (28).

**Gel Electrophoresis and Detection of Antigens:** Polypeptides of whole PtK<sub>2</sub> cells and cytoskeletons made therefrom (29) were separated by gel electrophoresis, transferred on nitrocellulose paper sheets and examined by the immunoblotting technique as previously described (23).

## RESULTS

The monoclonal antibody K<sub>G</sub> 8.13 recognizes, in epithelia of man and cow, a determinant present in almost all members of the “basic subfamily” of cytokeratin polypeptides as well as in cytokeratin D (No. 18 of the human catalog; cf. reference 30) of man and cow (23). In cytoskeletons of PtK<sub>2</sub> cells, two major cytokeratin polypeptides have been identified, in addition to vimentin (3, 29, 31). One has an apparent *M<sub>r</sub>* value of 54,000 and is related to cytokeratin “A” of other species, the other is more acidic, has an electrophoretic mobility similar to that of actin and appears to be related to component “D” of higher mammalian species (3, 23, 29). Of these, antibody K<sub>G</sub> 8.13 reacts only with the larger cytokeratin (Fig. 1).

Immunofluorescence microscopy of PtK<sub>2</sub> cells in interphase with conventional cytokeratin antibodies of rabbits guinea pigs reveals an intricate meshwork of wavy cytokeratin fibrils characteristic of this cell type (not shown; cf. references 3, 6, 7, 12, 18, 20, 31, 32). The same fibrillar meshwork is also seen with various monoclonal murine antibodies (not shown; cf. references 20, 32, 33). In mitotic PtK<sub>2</sub> cells this meshwork is altered but fibrillar structures are still well discerned, in agreement with previous reports (12, 19, 20). By contrast, antibody K<sub>G</sub> 8.13 does not stain interphase PtK<sub>2</sub> cells but reacts only with mitotic stages (Fig. 2). This specific reaction in mitotic PtK<sub>2</sub> cells only is illustrated by double immunofluorescence microscopy in Fig. 3, a–d which also demonstrate that this reaction is not restricted to prophase-telophase stages of mitosis (Fig. 3, a and b), but extends to postmitotic stages of early G<sub>1</sub> phase of both daughter cells (Fig. 3, c and d). This specific reaction of antibody K<sub>G</sub> 8.13 with cytokeratin structures of perimitotic but not interphase PtK<sub>2</sub> cells is not restricted to mitoses that have rounded off but is also seen in mitotic cells that have remained flat, thus demonstrating that it is related to the mitosis as such but not the morphological shape change. It is further evident that this change in antigenic reaction with PtK<sub>2</sub> mitoses is also seen in thin cytoplasmic projections as they occur in some normal mitoses and, more frequently, in mitotic stages arrested by treatment with colcemid (data not shown).

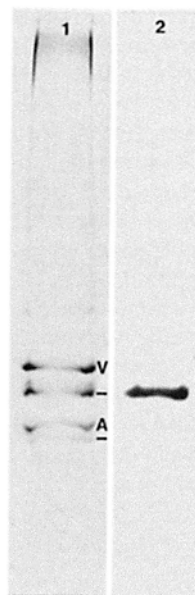


FIGURE 1 Cytoskeletal polypeptides obtained from PtK<sub>2</sub> cells after extraction with Triton X-100 and high salt buffer have been separated by SDS PAGE and stained with Coomassie Blue (lane 1) or blotted on nitrocellulose paper and allowed to react with monoclonal antibody K<sub>G</sub> 8.13 followed by reaction with <sup>125</sup>I-labeled protein A (lane 2, autoradiography). The two horizontal bars denote the two cytokeratins 1 (upper) and 2 (lower). V, vimentin; A, actin. Note that only cytokeratin 1 reacts with the antibody.

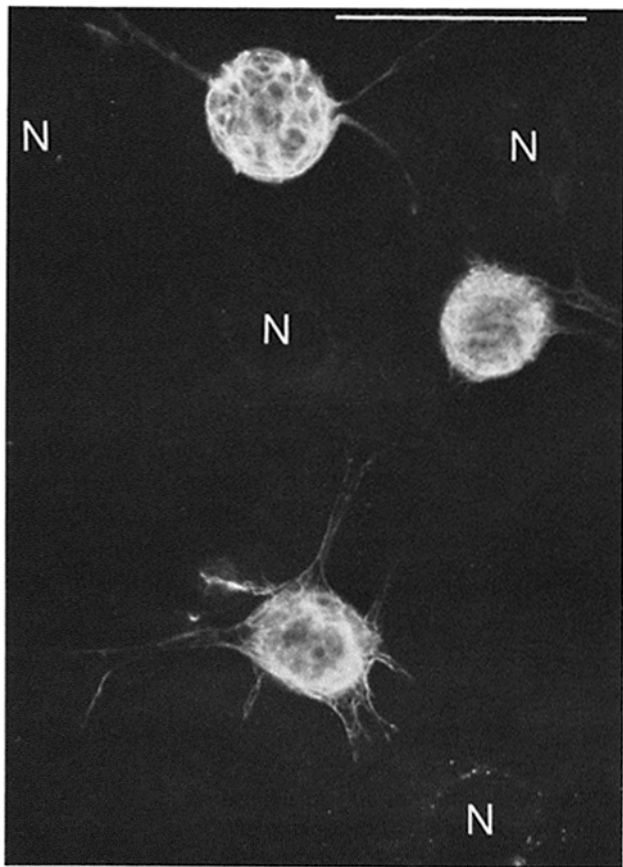


FIGURE 2 Immunofluorescence microscopy of mitoses-rich PtK<sub>2</sub> cell cultures after staining with murine monoclonal antibody K<sub>G</sub> 8.13. Note that antibody K<sub>G</sub> 8.13 reacts exclusively with mitotic and early postmitotic cells. Nuclei of unstained cells are indicated by N. Bar, 40 μm. × 820.

These observations of a specific reaction of antibody K<sub>G</sub> 8.13 with cytokeratin filaments of mitotic but not interphase PtK<sub>2</sub> cells suggest that this reaction is the result of the masking of the determinant recognized in denatured cytokeratin (Fig. 1) during interphase. The strong staining of cytokeratin fibrils in interphase PtK<sub>2</sub> cells with various other conventional and monoclonal antibodies demonstrates the accessibility of the cytokeratin filaments to murine immunoglobulins. Therefore, we examined various preparative conditions which, in interphase cells, might unmask the determinant recognized by K<sub>G</sub> 8.13. It has been found that when cells are extracted first with buffer containing Triton X-100 or Nonidet P-40 and then with methanol and acetone as described under methods *f-l* above significant fibrillar staining with antibody K<sub>G</sub> 8.13 is seen in interphase PtK<sub>2</sub> cells (Fig. 4, *a-c*). Positive reaction is seen in all cells although the distinctiveness of the fibrillar fluorescence is often variable from cell to cell in the same culture. Double label immunofluorescence microscopy of PtK<sub>2</sub> cells treated in this way with guinea pig antibodies and with murine monoclonal antibody K<sub>G</sub> 8.13 shows that the fibrillar structures stained with antibody K<sub>G</sub> 8.13 are identical to those stained by cytokeratin antibodies that are positive also with cells processed according to the standard procedure. Inclusion of millimolar concentrations of Mg<sup>2+</sup> or Ca<sup>2+</sup> in the various buffers has not resulted in significant differences of this unmasking effect. This unmasking of the determinant

recognized by antibody K<sub>G</sub> 8.13 has been most effective and uniform when both methanol and acetone were used at 4°C or at room temperature rather than at -20°C. Unmasking of the determinant recognized by antibody K<sub>G</sub> 8.13 has also been achieved by brief treatments with 2 M urea and trypsin.

Electron microscopic examination has confirmed that antibody K<sub>G</sub> 8.13 specifically binds to bundles of cytokeratin filaments. Positive decoration on IF bundles is seen in mitotic cells as well as in interphase cells treated with both the detergent and the organic solvents (data not shown).

## DISCUSSION

Our results present the case of a specific cell cycle-dependent re-arrangement of IF structures that is recognized by the selective masking and unmasking of a specific immunologic determinant in cytokeratin filaments. Selective masking has been discussed by Lazarides et al. (34) as a likely explanation of an unexpected reaction of another type of IF, desmin, with a monoclonal antibody (D76) that reacts with desmin only in later stages of myotube differentiation but not in early myotubes. However, direct proof by experimental unmasking of this determinant has not been presented for this case. Our observations in PtK<sub>2</sub> cells demonstrate cell cycle-dependent masking of an IF determinant by experimental masking. This occurrence of a selective and complete masking of a determinant in an IF system should be taken as a reason for concern and caution in interpreting negative results obtained in immunolocalization studies, especially when monoclonal antibodies are used. The phenomenon described here may also explain unexpected heterogeneities of reactivity in interphase cells of the same culture (for examples, see references 21, 33, 35, 36). It may also be relevant for some observations in frozen tissue sections, and failures to certain monoclonal or conventionally obtained antibodies to react with fibrils of some epithelial cells but not with those of others (e.g., references 6, 7, 25, 32, 33, 37, 38) may reflect differential masking rather than differences of expression. Woodcock-Mitchell et al. (39) have presented an example of a monoclonal antibody that does not bind to its cytokeratin determinant in suprabasal layers of epidermis and have suggested that this might be due to selective masking in situ. We propose to include, as controls for possible masking of the type described here, immunoblotting tests on proteins from the specific cell colonies or layers under question as well as treatments with unmasking reagents as they have been successful in this study.

Antibody K<sub>G</sub> 8.13 reacts with a determinant of a group of cytokeratin polypeptides present in diverse mammalian species (23). Interestingly, this determinant is differently exposed during interphase in cultured cells from various species. Whether this different reactivity of the same determinant in different cells reflects functional differences or only tolerance of changes in an unimportant region of this molecule is not known.

The accessibility of the cytokeratin determinant recognized in PtK<sub>2</sub> cells during mitosis but not during most of the interphase indicates that the organization of cytokeratin filaments is not constant but goes through systematic and dynamic changes, both when the cell enters mitosis and when it re-establishes its interphase architecture. PtK cells maintain intermediate-sized filament structures during mitosis, although changes in their display have been noticed by electron

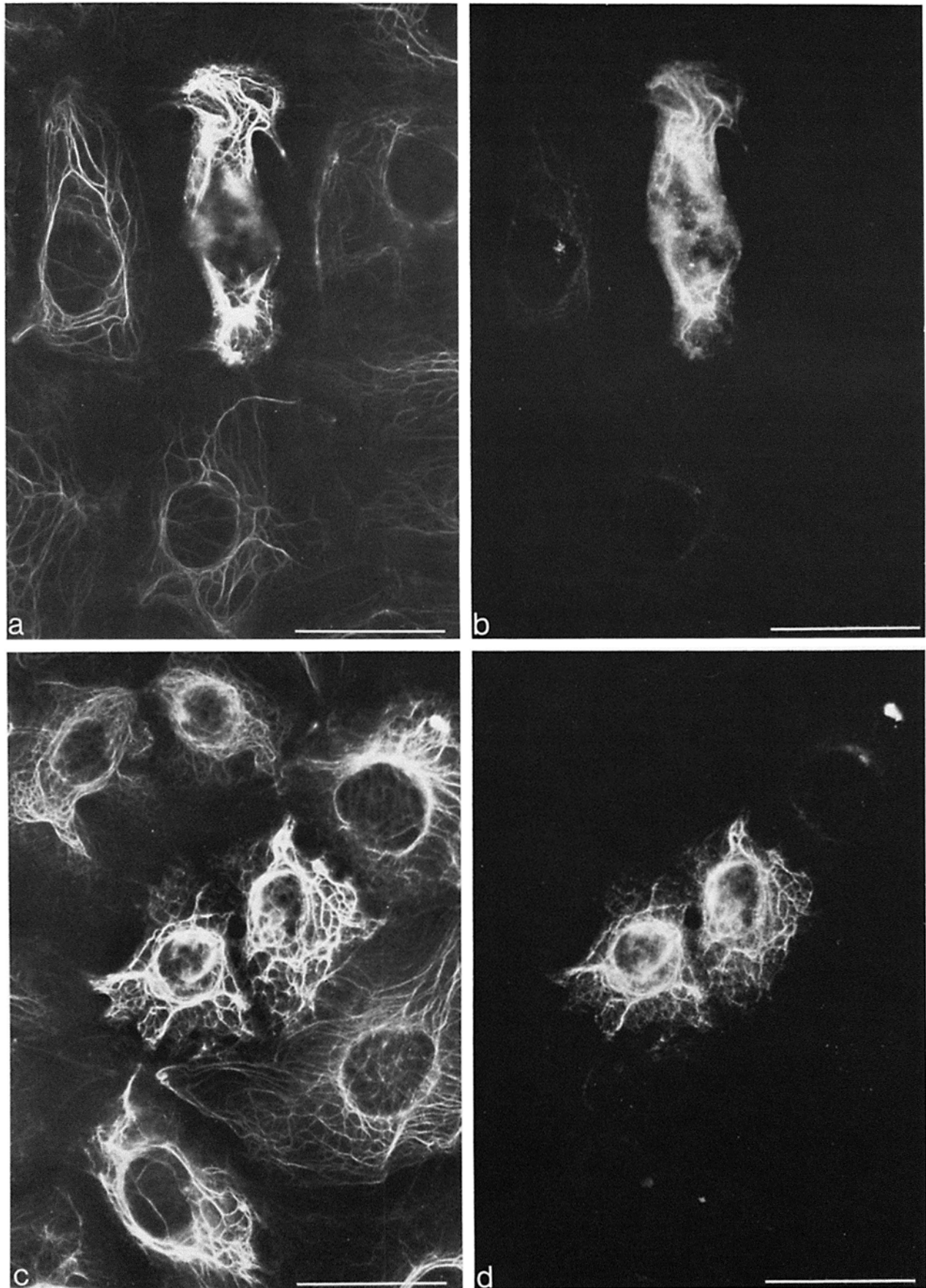


FIGURE 3 Double label immunofluorescence microscopy of PtK<sub>2</sub> cells, using guinea pig antibodies against cytokeratin (a and c; same as in Fig. 2, a-c) and murine antibody K<sub>C</sub> 8.13 (b and d). Note that antibody K<sub>C</sub> 8.13 reacts only with filaments of mitotic cells (a and b) and postmitotic cells of early 61 phase. (c and d). Bars, 30  $\mu$ m.  $\times$  900.

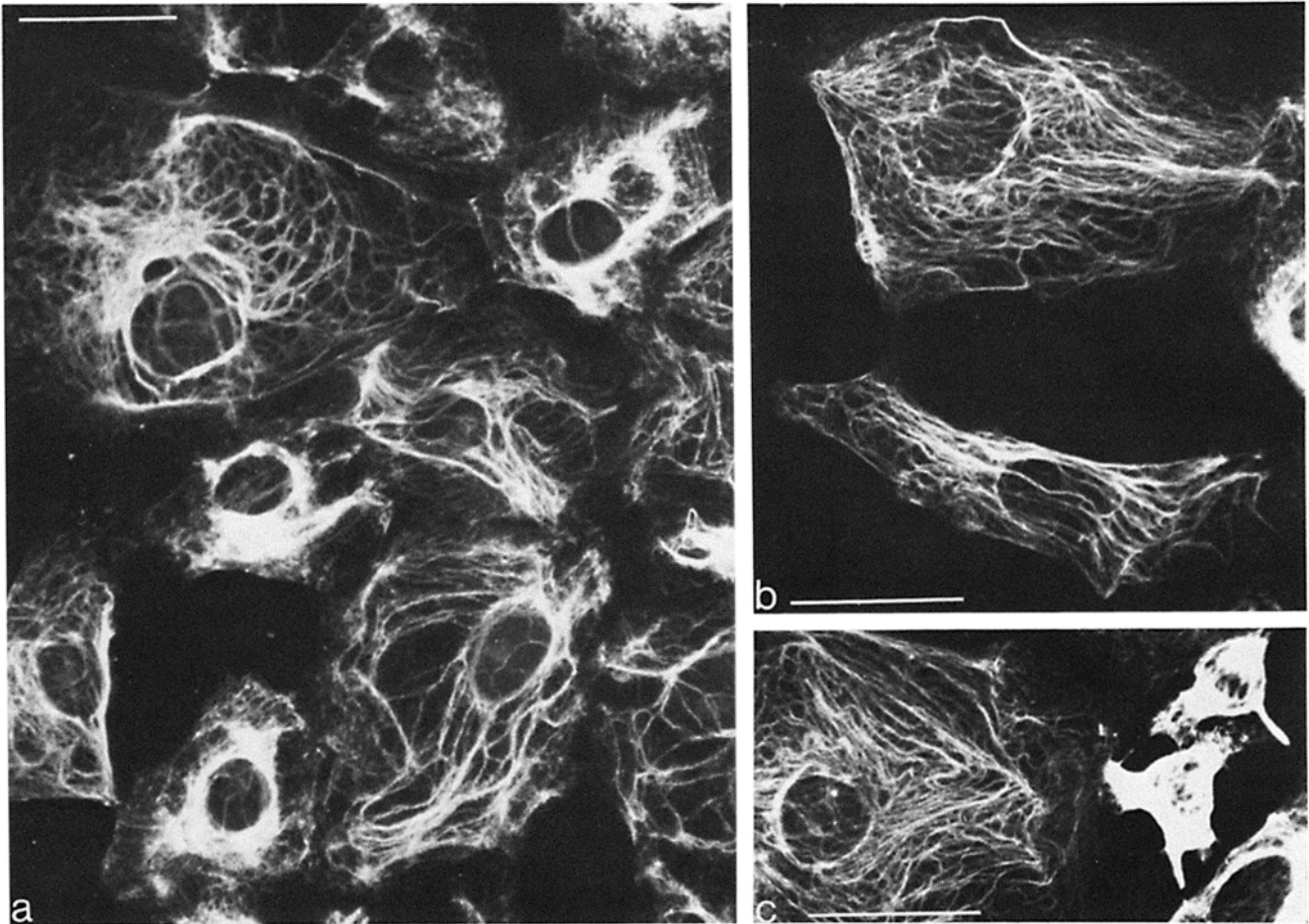


FIGURE 4 Immunofluorescence microscopy of PtK<sub>2</sub> cells treated sequentially with Triton X-100 as well as methanol and acetone using monoclonal antibody K<sub>G</sub> 8.13 (a-c). Note that cyokeratin filaments positively stained with antibody K<sub>G</sub> 8.13 are recognized in cells extracted in this way (a, survey; b, a large and a small cell; c, an interphase cell, in the left, and two mitotic cells in the upper right). Bars, 30  $\mu$ m. (a)  $\times$  700; (b)  $\times$  750; (c)  $\times$  750.

microscopy (19) as well as immunofluorescence microscopy (14, 18), different from many other epithelial cells in which cyokeratin filaments are transformed into spheroidal aggregates of non-IF structures (20-22). Now our observations that cyokeratin filaments of mitotic PtK<sub>2</sub> cells are not identical to those of interphase PtK<sub>2</sub> cells and can be clearly distinguished by monoclonal antibody K<sub>G</sub> 8.13 suggest that perimitotic changes of IF organization may be much more common, albeit not necessarily as dramatic as in those cells which transiently transform their cyokeratin filaments into non-fibrillar aggregates (20-22).

Our experiments do not allow us to decide whether the masking of the K<sub>G</sub> 8.13 determinant in cyokeratin filaments of PtK<sub>2</sub> interphase cells is due (i) to the specific association with a noncyokeratin protein or (ii) to an intrinsic change in the arrangement of the cyokeratin polypeptides. Treatment with Triton X-100 or Nonidet P-40 does not detectably change the electron microscopic appearance of these filaments (cyokeratins are even capable of reconstituting intermediate-sized filaments *in vitro* in the presence of 1% Triton X-100, data not shown). The various treatments used for unmasking could "loosen" the specific polypeptide arrangement within IF or extract a masking component. It is also possible that the perimitotic changes in the K<sub>G</sub> 8.13 determinant of PtK<sub>2</sub> cells are related to modifications of cyokeratins (for reports

of increased phosphorylated of vimentin in mitosis, see references 40, 41). The observed changes in IF organization may have, beyond the structural aspect, functional importance: the surface pattern of IF which, in the living cell, provide a large area for potential structure-bound reactions may not be constant and inactive but may be involved in the regulation of other cellular activities.

We thank Caecilia Kuhn and Klaus Mähler for expert technical help.

The work has been supported in parts by the Deutsche Forschungsgemeinschaft (grant Fr 308/15).

Received for publication 20 April 1983, and in revised form 28 June 1983.

#### REFERENCES

1. Anderton, B. H. 1981. Intermediate filaments: a family or homologous structures. *J. Muscle Res. Cell Motil.* 2:141-166.
2. Lazarides, E. 1982. Intermediate filaments: a chemically heterogeneous, developmentally regulated class of proteins. *Annu. Rev. Biochem.* 51:219-250.
3. Franke, W. W., E. Schmid, D. L. Schiller, S. Winter, E. D. Jarasch, R. Moll, H. Denk, B. W. Jackson, and K. Illmensee. 1982. Differentiation-related patterns of expression of proteins of intermediate-size filaments in tissues and cultured cells. *Cold Spring Harbor Symp. Quant. Biol.* 46:431-453.
4. Steinert, P., R. Zackroff, M. Aynardi-Whitman, and R. D. Goldman. 1982. Isolation and characterization of intermediate filaments. *Methods Cell Biol.* 24:399-419.
5. Bennett, G. S., S. A. Fellini, J. M. Croop, J. J. Otto, J. Bryan, and H. Holtzer. 1978. Differences among 100-Å filament subunits from different cell types. *Proc. Natl. Acad.*



- Sci. USA.* 75:4364-4368.
6. Franke, W. W., E. Schmid, Osborn, M., and K. Weber. 1978. Different intermediate-sized filaments distinguished by immunofluorescence microscopy. *Proc. Natl. Acad. Sci. USA.* 75:5034-5038.
  7. Sun, T.-T., C. Shih, and H. Green. 1979. Keratin cytoskeletons in epithelial cells of internal organs. *Proc. Natl. Acad. Sci. USA.* 76:2813-2817.
  8. Steinert, P. M., W. W. Idler, and R. D. Goldman. 1980. Intermediate filaments of baby hamster kidney (BHK-21) cells and bovine epidermal keratinocytes have similar ultrastructures and subunit domain structures. *Proc. Natl. Acad. Sci. USA.* 77:4534-4538.
  9. Geisler, N., and K. Weber. 1981. Comparison of two immunologically distinct intermediate filament proteins by amino acid sequence analysis. Desmin and vimentin. *Proc. Natl. Acad. Sci. USA.* 78:4120-4123.
  10. Geisler, N., and K. Weber. 1982. The amino acid sequence of chicken muscle desmin provides a common structural model for intermediate filament proteins. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1649-1656.
  11. Blose, S. H., and S. Chacko. 1976. Rings of intermediate (100-Å) filament bundles in the perinuclear region of vascular endothelial cells. *J. Cell Biol.* 70:459-466.
  12. Aubin, J. E., M. Osborn, W. W. Franke, and K. Weber. 1980. Intermediate filaments of the vimentin-type and the cytokeratin-type are distributed differently during mitosis. *Exp. Cell Res.* 129:149-165.
  13. Blose, S. H. 1979. Ten-nanometer filaments and mitosis: maintenance of structural continuity in dividing endothelial cells. *Proc. Natl. Acad. Sci. USA.* 76:3372-3376.
  14. Zieve, G. W., S. R. Heidemann, and J. R. McIntosh. 1980. Isolation and partial characterization of a cage of filaments that surrounds the mammalian mitotic spindle. *J. Cell Biol.* 87:160-169.
  15. Blose, S. H., and A. Bushnell. 1982. Observations on the vimentin-10-nm filaments during mitosis in BHK21 cells. *Exp. Cell Res.* 142:57-62.
  16. Goldman, R. 1971. The role of three cytoplasmic fibers in BHK-21 cell motility. I. Microtubules and the effects of colchicine. *J. Cell Biol.* 51:752-762.
  17. Holtzer, H., S. Fellini, N. Rubinstein, J. Chi, and K. Strahs. 1976. Cells, myosin and 100 Å filaments. *Cold Spring Harbor Conf. Cell Proliferation.* 3 (Book A):823-839.
  18. Franke, W. W., K. Weber, M. Osborn, E. Schmid, and C. Freudenstein. 1978. Antibody to prekeratin. Decoration of tonofilament-like arrays in various cells of epithelial character. *Exp. Cell Res.* 116:429-445.
  19. Franke, W. W., C. Grund, M. Osborn, and K. Weber. 1978. The intermediate-sized filaments in rat kangaroo PtK<sub>2</sub> cells. I. Morphology in situ. *Cytobiol.* 17:365-391.
  20. Horwitz, B., H. Kupfer, Z. Eshhar, and B. Geiger. 1981. Reorganization of arrays of prekeratin filaments during mitosis. *Exp. Cell Res.* 134:281-290.
  21. Franke, W. W., E. Schmid, C. Grund, and B. Geiger. 1982. Intermediate filament proteins in nonfilamentous structures: transient disintegration and inclusion of subunit proteins in granular aggregates. *Cell.* 30:103-113.
  22. Lane, E. B., S. L. Goodman, and L. K. Trejdosiewicz. 1982. Disruption of the keratin filament network during epithelial cell division. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1365-1372.
  23. Gigi, O., B. Geiger, Z. Eshhar, R. Moll, E. Schmid, S. Winter, D. L. Schiller, and W. W. Franke. 1982. Detection of a cytokeratin determinant common to diverse epithelial cells by a broadly cross-reacting monoclonal antibody. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1429-1437.
  24. Franke, W. W., E. Schmid, S. Winter, M. Osborn, and K. Weber. 1979. Wide-spread occurrence of intermediate-sized filaments of the vimentin-type in cultured cells from diverse vertebrates. *Exp. Cell Res.* 123:25-46.
  25. Franke, W. W., E. Schmid, C. Freudenstein, B. Appelhans, M. Osborn, K. Weber, and T. W. Keenan. 1980. Intermediate-sized filaments of the prekeratin type in myoepithelial cells. *J. Cell Biol.* 84:633-654.
  26. Osborn, M., and K. Weber. 1977. The detergent-resistant cytoskeleton of tissue culture cells includes the nucleus and the microfilament bundles. *Exp. Cell Res.* 106:339-349.
  27. Schmid, E., D. L. Schiller, C. Grund, J. Stadler, and W. W. Franke. 1983. Tissue type-specific expression of intermediate filament proteins in a cultured epithelial cell line from bovine mammary gland. *J. Cell Biol.* 96:37-50.
  28. Kreis, T. E., B. Geiger, E. Schmid, J. L. Jorcano, and W. W. Franke. 1983. Synthesis and specific assembly of keratin filaments after microinjection of mRNA for epidermal keratin into non-epithelial cells. *Cell.* 32:1125-1137.
  29. Franke, W. W., D. L. Schiller, R. Moll, S. Winter, E. Schmid, I. Engelbrecht, H. Denk, R. Krepler, and B. Platzer. 1981. Diversity of cytokeratins: differentiation specific expression of cytokeratin polypeptides in epithelial cells and tissues. *J. Mol. Biol.* 153:933-959.
  30. Moll, R., W. W. Franke, D. L. Schiller, B. Geiger, and R. Krepler. 1982. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell.* 31:11-24.
  31. Osborn, M., W. W. Franke, and K. Weber. 1980. Direct demonstration of the presence of two immunologically distinct intermediate-sized filament systems in the same cell by double immunofluorescence microscopy. *Exp. Cell Res.* 125:35-46.
  32. Lane, E. B. 1982. Monoclonal antibodies provide specific intramolecular markers for the study of epithelial monofilament organization. *J. Cell Biol.* 92:665-673.
  33. Lane, E. B., and M. W. Klymkowsky. 1982. Epithelial tonofilaments: investigating their form and function using monoclonal antibodies. *Cold Spring Harbor Symp. Quant. Biol.* 46:387-402.
  34. Lazarides, E., B. L. Granger, D. L. Gard, C. M. O'Connor, J. Breckler, M. Price, and S. I. Danto. 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.
  35. Debus, E., K. Weber, and M. Osborn. 1982. Monoclonal cytokeratin antibodies that distinguish simple from stratified squamous epithelia: characterization on human tissues. *EMBO (Eur. Mol. Biol. Organ.) J.* 1:1641-1647.
  36. Lazarides, E. 1978. The distribution of desmin (100 Å) filaments in primary cultures of embryonic chick cardiac cells. *Exp. Cell Res.* 112:265-273.
  37. Gown, A. M., and A. M. Vogel. 1982. Monoclonal antibodies to intermediate filament proteins of human cells: unique and cross-reacting antibodies. *J. Cell Biol.* 95:414-424.
  38. Tseng, S. C. G., M. J. Jarvinen, W. G. Nelson, J.-W. Huang, J. Woodcock-Mitchell, and T.-T. Sun. 1982. Correlation of specific keratins with different types of epithelial differentiation: monoclonal antibody studies. *Cell.* 30:361-372.
  39. Woodcock-Mitchell, J., R. Eichner, W. G. Nelson, and T.-T. Sun. 1982. Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. *J. Cell Biol.* 95:580-588.
  40. Bravo, R., J. V. Small, S. J. Fey, P. M. Larsen, and J. E. Celis. 1982. Architecture and polypeptide composition of HeLa cytoskeletons. Modification of cytoarchitectural polypeptides during mitosis. *J. Mol. Biol.* 154:121-143.
  41. Evans, R. M., and L. M. Fink. 1982. An alteration in the phosphorylation of vimentin-type intermediate filaments is associated with mitosis in cultured mammalian cells. *Cell.* 29:43-52.