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Disruption of the keratin filament network during epithelial cell division

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The behaviour of keratin filaments during cell division was examined in a wide range of epithelial lines from several species. Almost half of them show keratin disruption as described previously: by immunofluorescence, filaments are replaced during mitosis by a 'speckled' pattern of discrete cytoplasmic dots. In the electron microscope these 'speckles' are seen as granules around the cell periphery, just below the actin cortical mesh, with no detectable 10 nm filament structure inside them and no keratin filament bundles in the rest of the cytoplasm. A time course of the filament reorganization was constructed from double immunofluorescence data; filaments are disrupted in prophase, and the filament network is intact again by cytokinesis. The phenomenon is restricted to cells rich in keratin filaments, such as keratinocytes; it is unrelated to the co-existence of vimentin in many of these cells, and vimentin is generally maintained as filaments while the keratin is restructured. Some resistance to the effect may be conferred by an extended cycle time. Filament reorganization takes place within minutes, so that a reversible mechanism seems more likely than one involving *de novo* protein synthesis, at this metabolically quiet stage of the cell cycle.

Key words: cytoskeleton/electron microscopy/epithelia/keratin/mitosis

Introduction

Intermediate filaments, or 10 nm filaments, are set apart from the other cytoskeletal fibres (the actin and tubulin systems) by two characteristics, their biochemical heterogeneity and their relative insolubility under physiological conditions (see Anderton, 1981, for a recent review). Among the five tissue-specific classes of intermediate filaments, the epithelial keratin proteins (cytokeratins) represent extremes in both these aspects. In the elaborate filament networks of epithelial cells, the involvement with intercellular junctions (desmosomes) suggests a possible role in maintaining tissue integrity under physical stress. This, however, does not account for the modulation of keratin filament expression seen during normal differentiation or neoplastic transformation.

At cell division, all the major structural elements of the cell are substantially reorganized. The cell rounds up, and for 30 min or more, microtubules and actin microfilaments are redeployed for specific mitotic functions. The behaviour of intermediate filaments is more variable. By immunofluorescence, vimentin filaments, of mesenchymal cells *in situ* and many cell types in culture, appear to be interrupted as intact bundles in the plane of the cleavage furrow during cytokinesis (Blose, 1979), and in some epithelial lines the same happens to the keratin filaments (Aubin *et al.*, 1980). Other epithelial lines undergo a greater degree of keratin disruption (Horwitz *et al.*, 1981; see Lane and Klymkowsky, 1981), such that the immunofluorescence pattern of filamentous keratin is replaced during mitosis by a pattern of discrete cytoplasmic dots, giving the cells a 'speckled' appearance.

This mitotic reorganization of keratin is so far the only known situation in which intermediate filaments are repeatedly broken down and reconstructed by the cell. We have analysed this phenomenon in a large number of cell lines, and data from double immunofluorescence, time-lapse films, and electron microscopy suggest some possible mechanisms for this effect.

Results

Disruption of keratin filaments during mitosis was seen as a characteristic 'speckled' immunofluorescence staining pattern of discrete aggregates, from prophase to telophase (Figure 1), after which the filamentous pattern was restored. This effect was seen in 17 out of the 40 cell lines listed in Table I, after alcohol, acetone, or aldehyde fixations, although aldehydes diminished binding of the keratin antibodies. Two antibodies were used (see Materials and methods), a rabbit antiserum to the keratin-containing fraction of human callus (L14: Trejdosiewicz *et al.*, 1981) which reacts with most epithelia tested, including skin and bladder, but not with hepatocytes; and a mouse monoclonal antibody (LE61; Lane, 1982) to small acidic keratins of simple (not stratified squamous) epithelia, including liver and intestine, which does not bind to normal keratinocytes. The two keratin antibodies never gave conflicting data regarding mitosis; when both antibodies reacted with the same cell line, they both revealed the same keratin behaviour.

A DNA marker (Hoechst 33258) was used in parallel with immunofluorescence to identify stages of the cell cycle and to check for mycoplasma infection. All lines showing keratin disruption at mitosis were mycoplasma-free. To ensure against scoring dead cells, mortality rates were calculated from time-lapse films. In lines showing this effect, >80% of the dividing cells showed well-defined speckled keratin, while the mitotic mortality rate was always <5%.

Since the stimulus for keratin speckling is unknown, it was felt inadvisable to use drugs to accumulate dividing cells, so for electron microscopy mitotic cells were harvested by shaking them loose. Mitotic keratin aggregates have now been seen by electron microscopy in several cell lines, including L132, HeLa, RT112, and SVK14 from Table I, and in all cases the ultrastructure is similar. By combining the data from a large number of immunofluorescence experiments (Figure 1) and a smaller number of electron microscopical ones (Figures 4 and 5), a time course was constructed (Figure 2) for the filament disruption and reformation. Figure 3

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shows the proportion of cells which showed only speckled keratin (i.e., no filaments) at successive stages of mitosis. The mitotic cells not scored in Figure 3 either contained filament remnants with the speckles or, more usually, showed only diffuse immunofluorescence.

Prophase

Filament disruption was first seen in prophase, (Figure 1), i.e., only when there was clear evidence from the DNA staining of chromosome condensation. The time of onset of the effect was somewhat variable; in HEp2 cells the filaments started to break up early in prophase and recovered in cyto-

Table I. Keratin filament disruption seen during mitosis in epithelial cell lines

Cell line	Keratin speckles at mitosis	Tissue of origin of cell line	Immunofluorescence[d]			Reference[e]
			LE61	L14	Vimentin	
T24	+	Bladder/human[c]	+ + +	+ +	+ + +	Bubenik *et al.*, 1974
RT112	+	Bladder/human[c]	+ + +	+ + +	+	Marshall *et al.*, 1977
HeLa 21	+	Cervix/human[c]	+ + +	ND	+ +	Gey *et al.*, 1952
TR146	+	Cheek/human[c]	+ + +	+ + +	+	Rupniak *et al.*, 1982
SVK14	+	Epidermis/human[b]	+ + +	+ + +	−	Taylor-Papadimitriou *et al.*, 1982
TR131	+	Larynx/human[c]	−	+ + +	+	Rupniak *et al.*, 1982
TR138	+	Larynx/human[c]	+ + +	+ + +	−	Rupniak *et al.*, 1982
HEp2*	+	Larynx/human[c]	+ + +	+ +	+ +	Moore *et al.*, 1955
L132	+	Embryo lung/human[a]	+ + +	+ +	+ + +	Davies and Bolin, 1960
CaMa 1	+	Mammary gland/human[c]	+	ND	ND	Dobrynin, 1963
BT20	+	Mammary gland/human[c]	+ + +	+	−	Lasfargues and Ozzello, 1958
T-47D	+	Mammary gland/human[c]	+ + +	+	+	Keydar *et al.*, 1979
SKBR3	+	Mammary gland/human[c]	+ + +	+ +	−	Fogh and Trempe, 1975
MCF-7	+	Mammary gland/human[c]	+ + +	+ +	−	Soule *et al.*, 1973
FaDu	+	Pharynx/human[c]	+ +	ND	ND	Rangan, 1972
A431	+	Vulva/human[c]	+ +	+ +	−	Giard *et al.*, 1973
EJ	−	Bladder/human[c]	+ + +	+ +	+ + +	Marshall *et al.*, 1977
RT4	−	Bladder/human[c]	+ + +	+ + +	+ +	Marshall *et al.*, 1977
HT29	−	Colon/human[c]	+ +	+ +	+	Fogh and Trempe, 1975
LS-174T	−	Colon/human[c]	+ + +	−	−	Tom *et al.*, 1976
LoVo	−	Colon/human[c]	+ +	−	+ +	Drewinko *et al.*, 1976
734B	−	Mammary gland/human[c]	+ + +	+ + +	−	Soule *et al.*, 1973
fR2	−	Mammary gland/human[b]	+	+	+ + +	Chang *et al.*, 1982
TR126	−	Tongue/human[c]	+ +	+	+ +	Rupniak *et al.*, 1982
MB67C	−	Bladder/murine[b]	ND	+ +	ND	Summerhayes, 1979
MB63	−	Bladder/murine[b]	+ +	+ +	ND	Summerhayes, 1979
MB63C	−	Bladder/murine[b]	ND	+ +	ND	Summerhayes, 1979
MB48B	−	Bladder/murine[b]	+ + +	+ +	ND	Summerhayes, 1979
RAG	−	Kidney/murine[c]	+	+ +	ND	Klebe *et al.*, 1963
CMT64	−	Lung/murine[c]	+ + +	+ + +	−	Franks *et al.*,. 1976
NMuMG	−	Mammary gland/murine[a]	+ + +	+ + +	ND	Owens *et al.*, 1974
CSG255	−	Salivary gland/murine[b]	ND	+ + +	ND	Wigley, 1979
CSG211	−	Salivary gland/murine[b]	+ +	+ +	+ +	Wigley, 1979
CSG120/7	−	Salivary gland/murine[b]	ND	+ + +	ND	Knowles and Franks, 1977
CSG121/M	−	Salivary gland/murine[b]	ND	+ + +	ND	Knowles and Franks, 1977
CSG205	−	Salivary gland/murine[b]	ND	+ + +	ND	Wigley, 1979
MDCK	−	Kidney/canine[a]	+ + +	+ + +	+ + +	Madin and Darby, 1981
PtK1	−	Kidney/marsupial[a]	+ + +	+ + +	+ +	Walen and Brown, 1962
PtK2	−	Kidney/marsupial[a]	+ + +	+ + +	+ + +	Walen and Brown, 1962
CV-1	−	Kidney/monkey[a]	+	−	+ + +	Jensen *et al.*, 1964
BS-C-1	−	Kidney/monkey[a]	+	−	+ + +	Hopps *et al.*, 1963

[a]Line arose spontaneously *in vitro* from 'normal' material.
[b]Derived by experimental transformation *in vitro*.
[c]Line derived from a carcinoma.
[d]Scored subjectively from −, negative, to + + +, intensely positive.
[e]Reference to characterisation of cell lines.
(*: may be HeLa contaminated, but morphology of our HEp2 and HeLa strains are distinct.)

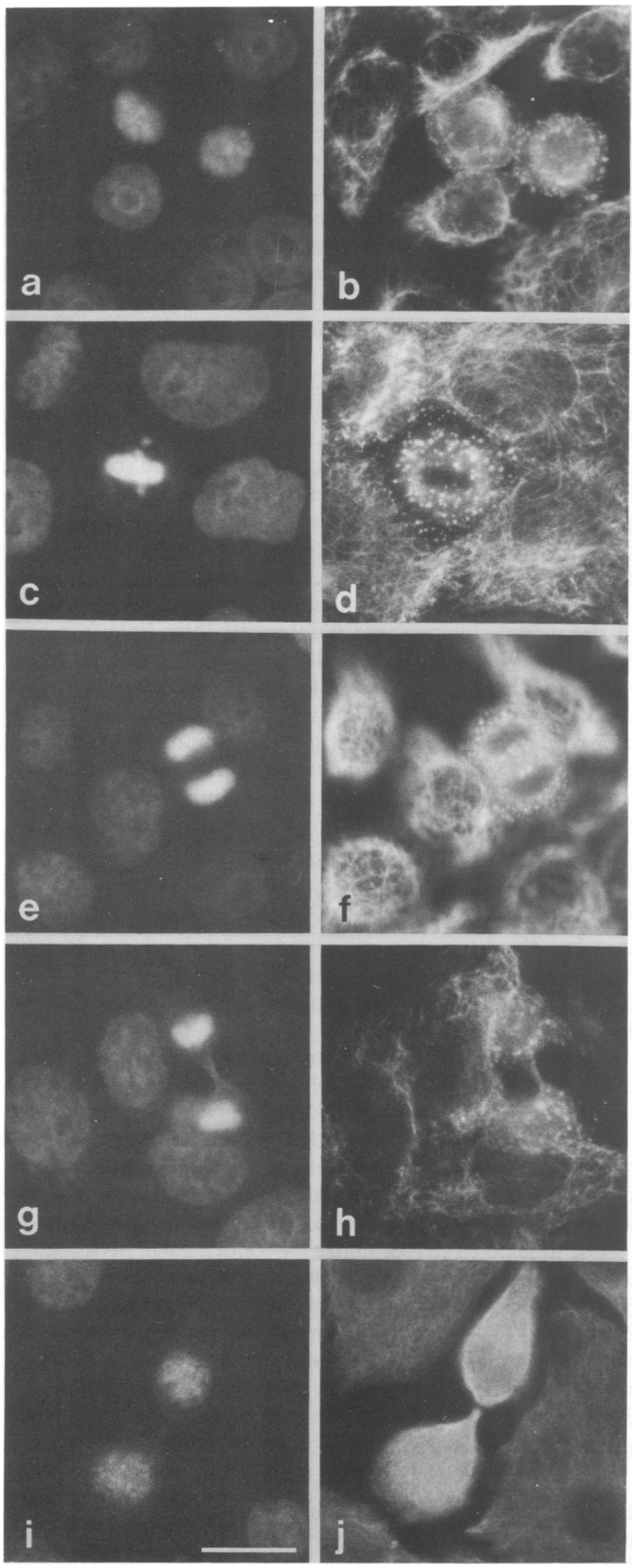

Fig. 1. Keratin 'speckling': filament disruption and reassembly during mitosis. Left (**a,c,e,g,i**) = DNA staining with Hoechst 33258. Right (**b,d,f,g,h**) = same field, indirect immunofluorescence of keratin filaments using monoclonal antibody LE61. **a,b** = prophase (L132 cells); **c,d** = metaphase (HEp2 cells); **e,f** = anaphase (L132 cells); **g,h** = telophase (HEp2 cells); **i,j** = cytokinesis (BT20 cells). Only dividing cells show 'speckled' keratin pattern. Scale bar = 20 μm.

kinesis, whilst L132 cells developed aggregates at the end of prophase and recovered in telophase. As the nuclear envelope disintegrated and the cells progressed towards metaphase, electron microscopy showed cells to contain no thick tonofilament bundles in the cytoplasm, but instead a number of homogeneous, near-spherical bodies (Figure 4). Partial filament disruption could be seen by immunofluorescence as comma-like configurations of antibody-labelled aggregates with filamentous appendages.

Metaphase to anaphase

The speckled keratin pattern is shown in Figure 1 at various stages of mitosis; metaphase and anaphase are easily recognizable by the chromatin configurations. By electron microscopy, the speckles were readily observable as aggregates of up to 2 μm in diameter (Figure 5). These aggregates were never membrane-bound; they were more compact and less electron-dense than the chromosomes, and easily distinguished from them. The peripheral aggregates were just below the cortical actin mesh, and slightly flattened against it, suggesting that some interaction may be holding them there. Small trans-cortical filamentous connections with the plasma membrane were often seen, and were sometimes associated with small patches of electron-dense material inside and outside the membrane. Aggregates were also seen towards the spindle, away from the plasma membrane.

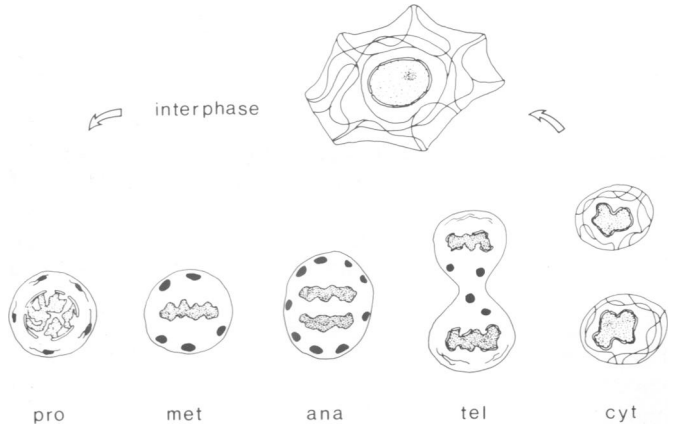

Fig. 2. Schematic representation of time course of filament disruption during mitosis.

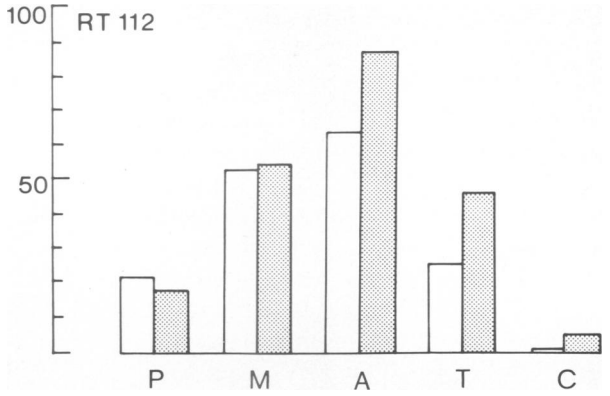

Fig. 3. Percentage of mitotic cells showing well-defined 'speckled' keratin (no filaments) at sequential stages of mitosis in one cell line (RT112) counted from immunofluorescence using L14 antiserum, stippled bars, and LE61 monoclonal antibody, open bars. Sample sizes were 450 dividing cells per antibody. P, prophase; M, metaphase; A, anaphase; T, telophase and C, cytokinesis.

Keratin aggregation reached its peak in anaphase. Using the antiserum L14, a completely speckled keratin pattern (no filaments) was seen in 86% of anaphase cells in RT112 cells (Figure 3). Counts of cells stained with the monoclonal LE61 gave a slightly lower percentage, as more of the dividing cells showed diffuse staining. This is probably due to some obscuring of the LE61 binding site in the reorganized keratin material, whereas the polyclonal antiserum contains antibodies to sites that are not all masked, and therefore strains more of the cells.

In electron micrographs of metapase and anaphase cells, no filamentous structures of 10 nm or similar diameter were see in the focal aggregates, but the finely fibrous texture suggested that protofilamentous substructure was retained, which might facilitate the rapid filament reconstruction seen during telophase and cytokinesis.

Telophase to cytokinesis

Returning filament structure in the cytoplasm (Figure 5b) accompanied nuclear envelope reconstruction. In early telophase some cells in a culture showed only diffuse keratin immunofluorescence (cf., prophase). Fragments of reforming filament bundles were first seen in the distal cytoplasm beyond the daughter nuclei, while keratin focal aggregates persisted longer in the region proximal to the incipient cleavage furrow. At cytokinesis, the nuclear envelope was intact, and the filament network appeared to be fully reconstructed as seen by immunofluorecence; keratin 'speckles' were not seen after cell separation was complete.

Differences between cell lines

We have only seen mitotic focal aggregation of intermediate filaments in keratin-containing cells.

In addition to the cell lines listed in Table I, a number of mesenchymal cells were examined for any signs of filament reorganization. These lines included 3T3 (mouse), Rat-1 (sic), NF4, He117, and SV80 (human fibroblasts), and NIL-8 and BHK21 (hamster). No keratin was detectable by immunofluorescence in any of these, and the vimentin intermediate filaments were divided between daughter cells as described previously for mesenchymal cells (Hynes and Destree, 1978; Blose, 1979; Aubin *et al.*, 1980), i.e., by severing of intact bundles. BHK21 cells also contain the muscle-specific desmin type of filaments, and using an antiserum kindly provided by E.D. Frank (Frank and Warren, 1981) we saw that desmin also behaved like vimentin during division.

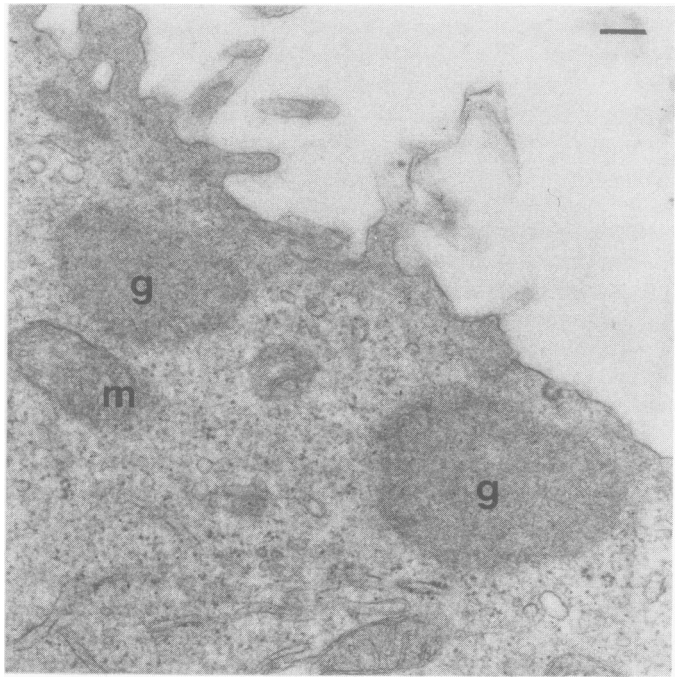

Fig. 4. Detail from periphery of L132 cell at metaphase; the keratin 'speckles' of immunofluorescence are clearly visible as granules, g, situated just below the cortical actin mesh. m = mitochondrion; scale bar = 0.2 μm.

Of the lines we examined, we observed keratin disruption only in human cells (Table I: 17/27), and all the 'speckling' cell lines possessed a well-developed keratin filament network during interphase.

No relationship was observed between mitotic speckling and organ of origin, but all of the lines derived from keratinocytes (8/8) show the effect. Keratinocytes are defined here as the cells of stratified squamous epithelia, and so this group includes the lines from epidermis, tongue, pharynx, oesophagus, vulva, and almost certainly cervix and larynx. These cells have the most abundant keratin filaments, and probably have the highest number of desmosomes.

Epithelial cell lines are often aneuploid, but a comparison of the modal chromosome numbers of these lines (see e.g., American Type Culture Collection, 1981) revealed no correlation with keratin speckling, although DNA content is known to influence the timing of the cell cycle (Grosset and Odartchenko, 1976). The tumour origin of all but one of the human lines was noted, but the mouse lines were also derived by transformation (*in vitro*), and the one human 'normal' line, L132, is aneuploid and has a highly transformed phenotype.

Cell morphology and behaviour

Different epithelial lines remain flattened to different extents during mitosis. The suggestion by Horwitz *et al.* (1981) that keratin disruption may be associated with a rounded metaphase morphology is contradicted by our observations that some of the speckling cells remained very flat, e.g., HEp2 and TR146.

Several cell lines were followed throughout the cell cycle by time-lapse cinematography, full details of which will be published elsewhere. A positive, but incomplete, correlation was observed between keratin 'speckling' and a fast cell cyle time: lines with a mean cycle time below ~30 h tend to show keratin reorganization during mitosis, and the lines with the longest cycle times tend not to.

The time taken to reorganize the tonofilament network was also estimated from films. Cell rounding up (prophase) and particularly cell separation (telophase to cytokinesis) were both rapid (3–6 min), and these phases correspond to the times of filament reorganizatin. The keratin changes, at least network reformation, are thus effected within time spans of 3–6 min.

Cytoskeleton composition

It seems unlikely that the redistribution of actin and tubulin at mitosis is related to the variability in the keratin redistribution, since their organization is similar in all these lines. Keratin proteins are known to be very heterogeneous (Franke *et al.*, 1981), and amongst the lines used here there are many differences in the expression of keratin antigens, as seen by immunofluorescence with L14 and LE61 and also with other monoclonal antibodies to different keratin determinants, (unpublished data). However, no correlation was seen between these antigenic differences and the differences in susceptibility to keratin filament breakdown.

Some of the cell lines (EJ, BS-C-1, CV-1, and fR2) have a contracted and degenerate keratin cytoskeleton (see Lane and Klymkowsky, 1981), and these cells showed no sign of keratin speckling. Vimentin was the dominant intermediate filament system in these lines, and all the filaments behaved like vimentin during mitosis.

Many of the lines with abundant keratin filaments also contained vimentin at the time of testing, but this appeared to have no influence on susceptibility to keratin speckling (Table I). Although with double immunofluorescence we occasionally saw focal vimentin staining superimposed on the keratin aggregates in dividing RT112 and HEp2 cells, in all other cases examined vimentin was filamentous throughout mitosis, even when keratin was broken down in the same cells (Figure 6).

Discussion

The phenomenon observed by immunofluorescence and electron microscopy reflects a transient change in state of keratin filaments, a change specifically associated with

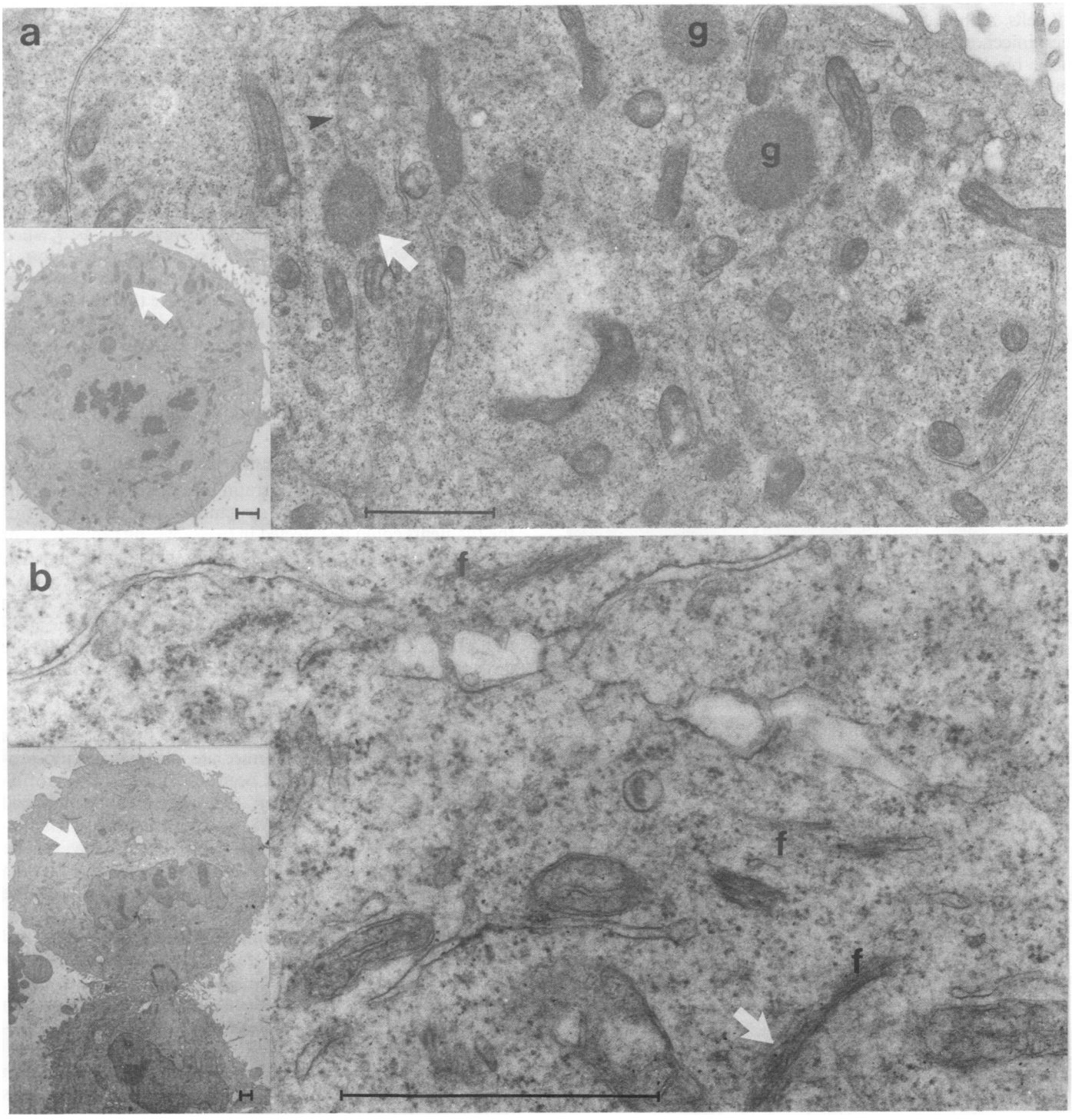

Fig. 5. L132 cells **(a)** in late prophase, just after nuclear envelope has broken down, and filamentous material (arrowhead) is still associated with the forming keratin granular aggregates, g; and **(b)** at cytokinesis, when the nuclear envelope is once more complete, and fragments of keratin fibres, f, are now visible in the cytoplasm. Insets show whole cells; large white arrow indicates marker structures. m = mitochondria; c = chromatin. Scale bars = 1 μm.

mitosis. In spite of the apparent insolubility of keratin filaments, some epithelial cells restructure the whole system within minutes. Ultrastructurally similar aggregates of tonofilament material have been observed in cold-shocked epidermis of fish (Schliwa and Eutener, 1979) and also in the disruption of keratin filaments induced by microinjection of antibody in PtK2 cells (Lane and Klymkowsky, 1981), but neither of these effects appear to be related to the cell cycle. A report by Brecher (1975) suggested a similar phenomenon, but the granules he observed in PtK cells appear at a different time in the cell cycle, and we have never seen 'speckling' in PtK cells. Although the aggregates induced by cold shock and microinjection are less regular in size and shape than those induced by mitosis, these observations confirm that keratin tonofilaments are susceptible to morphological rearrangements *in situ*.

Keratin speckling in mitosis was not seen in all the epithelial lines examined, and in occasional preparations it was absent from cells that normally showed the effect. Clearly therefore this cannot be an obligate element of epithelial cell division. A mitotic cell must be able to split its filaments between the two daughters, but since filaments can be severed more succinctly (as for vimentin), the question arises as to the reason for this exaggerated filament disruption. Breaking the keratin filaments may help to ease the extra cell into the epithelium matrix, since while a single cell's desmosomes are no longer connected across the cytoplasm by its tonofilament network, the positions of these anchorage junctions could be adjusted. Membrane characteristics are altered during mitosis (de Laat *et al.*, 1977; Quintart *et al.*, 1979), and the radical shape change which accompanies cell division in some of these lines must interfere in some way with membrane compartmentalization and cell polarity. *In situ*, cell division within an epithelium may be subjected to physical restrictions which may not apply to tissues with a less compact organisation.

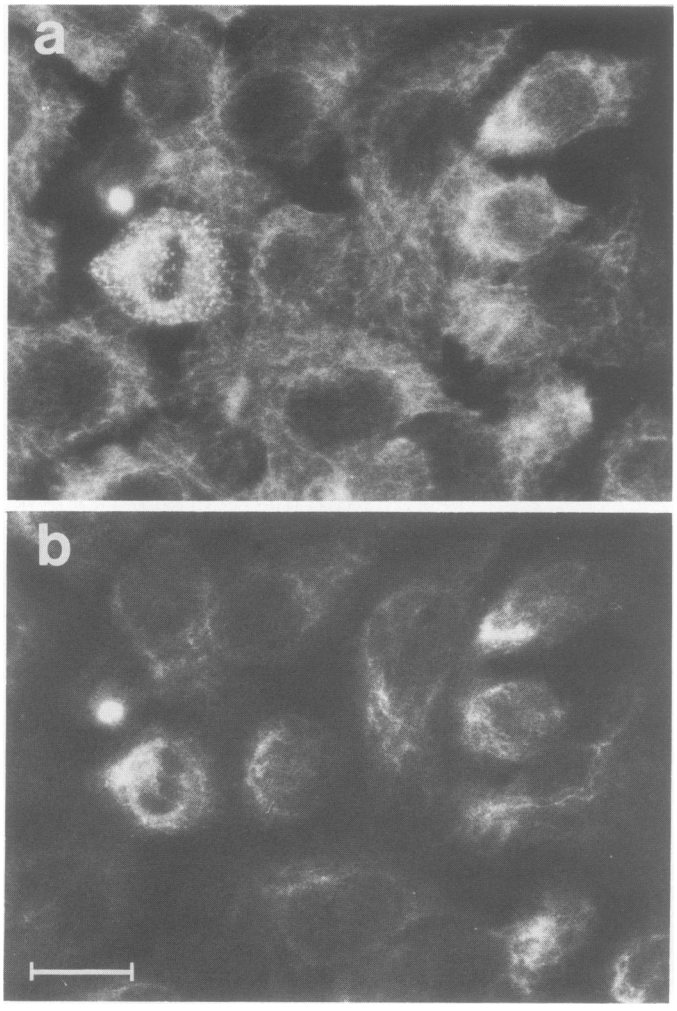

Fig. 6. Independent distribution of keratin [(**a**), with LE61] and vimentin [(**b**), with rabbit anti-vimentin serum] during division in HEp2 cells; the cell in metaphase shows the 'speckled' keratin pattern but the vimentin is still filamentous. Scale bar = 20 μm.

Differential susceptibility to keratin speckling

Of the variables between the cell lines which we have been able to examine, three factors may be associated with a susceptibility to keratin speckling, i.e., abundant cytokeratin filaments in interphase, possibly the species of origin, and possibly also a short cell cycle time. While we have so far not detected mitotic speckling in any of the mouse lines, this is probably an accident of sampling, and the data on cell cycle times still require further analysis. However, the association of speckling with keratin-rich lines is of interest, since most, if not all, of these lines are derived from tissues that show some degree of stratification *in situ*. It is possible that the ability to disrupt the filament network during cell division may be important for the development of stratification in an epithelium.

Although speckling at mitosis is only seen in the keratin-rich lines, the presence of vimentin in such lines appears to be irrelevant (Table I, Figure 6).

Mechanisms for a rapid, transient keratin reorganization

Within the fully formed keratin aggregates at mitosis, the intact intermediate filament structure is lost. Disruption of the filaments could be effected in two ways: by proteolysis, or by perturbation of interactions holding the polymer together, e.g., by charge changes on the keratin molecules. Because a minimal mixture of two keratin polypeptides can form 10 nm filaments *in vitro* (Steinert and Idler, 1976), without the addition of any further associated proteins, one assumes that the disrupting influence is acting directly on the keratin molecules.

Proteolysis by a specific enzyme has already been suggested as a mechanism for cleaving vimentin bundles at cytokinesis (Blose, 1979), and some intermediate filament-specific proteolytic enzymes have indeed been identified, for neurofilaments (Schlaepfer and Hasler, 1979) and for vimentin and desmin (Nelson and Traub, 1981). Reconstruction of the filament network after proteolysis would presumably require some replacement protein synthesis, but during cell division protein synthesis drops to about a third of its interphase rate (Fan and Penman, 1970), although the overall pattern of synthesis appears to be essentially unchanged (Milcarek and Zahn, 1978). The speed of keratin reorganization, together with the reduced metabolic activity of the cell during division, argues against a mechanism dependent on *de novo* protein synthesis either for destruction or reconstruction of the filament network.

The difference in timing between the keratin reorganization and the vimentin cleavage suggests that the mechanisms underlying the two phenomena are not the same. While vimentin cleavage is only seen at cytokinesis and the filament break is very restricted, the keratin disruption affects the whole cell and starts in late prophase. The keratin disruption

is probably triggered by events specific to the beginning of mitosis.

Mitosis-specific phsphorylations in late G2 are seen on many proteins, and are probably important in restructuring the nucleus and cytoplasm for cell division. Tubulin phosphorylation (Piras and Piras, 1975) and phosphorylation of histones H1 and H3 (Gurley *et al.*, 1978; Matsui *et al.*, 1979) can be correlated with major structural rearrangements for spindle formation and chromatin condensation, respectively. Our observations suggest that the nuclear envelope and the keratin filament network are both disrupted at about the same time, and they may respond to a similar triggering event. The lamin proteins of the matrix lining the internal face of the nuclear envelope are reversibly depolymerized at mitosis, at the same time as phosphorylation alters their charge (Gerace and Blobel, 1980). Vimentin is also phosphorylated above its interphase level during mitosis (Robinson *et al.*, 1981; Bravo *et al.*, 1982; Evans and Fink, 1982), and Bravo *et al.* have reported a small increase in vimentin solubility at this time. These authors also demonstrated mitosis-associated phosphorylation of a number of cytoskeleton proteins other than vimentin in HeLa cells (which have keratins and do show speckling), and at least three of these are interpretable as keratin proteins from their size, charge, and insolubility.

If the keratin filaments are held intact by charges at or near to their phosphorylation sites, then a phosphorylation-induced charge shift could be sufficient to dissociate these structures into protofilaments. This could then allow non-linear reassociation to form the aggregates which we observed, from which the protofilaments could be rapidly reassembled into filaments as soon as dephosphorylation restored the charges to their interphase equilibrium state. This hypothesis does not explain how the intact filaments are subsequently organised into the complex reticular meshwork so characteristic of cytokeratins, but this is another problem which might usefully be investigated using epithelial mitosis as a model system.

We deduce that this keratin reorganization is taking place close to its threshold limiting conditions in the tissue culture environment, since we see so much variability in the phenomenon. For this reason, this should be a potent system in which to investigate keratin filament regulation.

Materials and methods

Cell culture

All the lines (see Table I) were grown in Dulbecco's modified Eagle's medium with 10% foetal calf serum (FCS), except for the following: the four TR lines were grown in Ham's F12 with 10% FCS, BT20 in minimal essential medium (MEM) + 10% FCS + 10 μg/ml insulin; CaMa 1 in MEM + 15% FCS; SVK14 in RPMI 1640 (Gibco) + 10% FCS + 5 μg/ml hydrocortisone + 50 ng/ml cholera toxin. All media had penicillin (100 IU/ml) and streptomycin (100 μg/ml) added.

Immunofluorescence microscopy

Two anti-keratin antibodies were used for immunofluorescence. The first, L14, was a rabbit antiserum raised against the keratin fraction prepared from human callus by three cycles of dissolution (with 8 M urea) and repolymerization (with 5–10 mM Tris buffer), (Trejdosiewicz *et al.*, 1981). The antiserum was affinity-purified against the same antigen preparation, after which it reacted with keratin filaments in many cell lines and frozen sections of most epithelia tested (including skin), with the exception of liver parenchyma (negative) and intestine (very weak). It recognizes two major bands in the mid-size mol. wt. region of keratin polypeptides from RT112 cells (L.K. Trejdosiewicz, unpublished observations). L14 was routinely used diluted 1:10. The other reagent, LE61, is a mouse monoclonal antibody which recognizes a determinant on low mol. wt. (40–43 kd) acidic keratins characteristic of simple epithelia as opposed to stratified squamous epithelia, (Lane, 1982). LE61 was used as culture supernatant, containing ~50 μg/ml specific immunoglobulin.

A rabbit antiserum to calf lens vimentin was also used (diluted 1:25), which was generously provided by F.C.S. Ramaekers (Ramaekers *et al.*, 1981). Chromatin condensation was monitored in the immunofluorecence preparation using the DNA intercalating fluorochrome, Hoechst 33258. In most experiments the dye was added to the culture medium 20 min before fixing the cells, at a final concentration of 0.0004%; using the dye on fixed cells gave the same results. The cells, grown on plastic or glass, were rapidly rinsed with phosphate buffered saline [pH 7.4; without Mg^{2+} or Ca^{2+}; (PBS)] before fixing for immunofluorescence with 1:1 methanol/acetone at 4°C for 10 min. Antibody incubations were all for 20 min at room temperature, and PBS was used for washing. The second antibody layers were fluorescein-conjugated goat anti-rabbit IgG (Miles-Yeda; 1:40) and rhodamine-conjugated goat anti-mouse IgG, (Cappel Laboratories; 1:40), as appropriate. Specimens were mounted with Gelvatol 20-30 (Monsanto) or Uvinert (BDH) and examined with a Zeiss Photomicroscope III.

Electron microscopy

Cell samples were enriched for mitotic figures by pipetting of monolayer cultures ('shake-off') and collecting the loosened cells by centrifugation. Cells were then fixed in 2.5% glutaraldehyde in FCS (40 min, room temperature) followed by washing and post-fixation with 1% aqueous osmium tetroxide, (45 min, room temperature). After washing in distilled water, the specimens were block-stained with uranyl acetate, dehydrated through alcohols and embedded in Durcupan. Thin sections were stained with lead citrate, and examined in a Zeiss EM10, using operating voltages of 60 or 80 Kv.

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Note added in proof

Since this manuscript was submitted, an independent report describing this phenomenon in another cell line has appeared (Franke *et al.* (1982) *Cell,* **30**, 103-113); where the two reports can be correlated, our observations are in good agreement with these authors.