Differential Control of Cytokeratins and Vimentin Synthesis by Cell-Cell Contact and Cell Spreading in Cultured Epithelial Cells

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Abstract The expression of cytokeratins and vimentin was investigated in Madin-Darby bovine epithelial cells (MDBK) in culture under conditions of varied cell spreading and cell-cell contact. When extensive cell-cell contact was achieved by seeding cells at high density in monolayer, or in suspension culture in which multicellular aggregates formed, the cells synthesized high levels of cytokeratins and low levels of vimentin. In contrast, in sparse monolayer and suspension cultures where cell-cell contact was minimal, the cells synthesized very low levels of cytokeratins. The level of vimentin synthesis was high in sparse monolayer culture and was low in both sparse and dense suspension cultures. The ratio of cytokeratin to vimentin synthesis was not affected during the cell cycle, or when cell growth was inhibited by ara C and in serum-starvation-stimulation experiments. The variations in the synthesis of cytokeratins and vimentin under the various culture conditions were also reflected at the level of mRNA activity in a cell-free in vitro translation system and as determined by RNA blot hybridization with cDNA to vimentin and cytokeratins. The results suggest that control of cytokeratin synthesis involves cell-cell contact, characteristic of epithelia in vivo, while vimentin synthesis responds to alterations in cell spreading.

The growth-related activities of certain vertebrate cells in vivo and in vitro is mediated to a considerable extent through elaborate changes in cell morphology (for reviews see references 4 and 10). In some cells important cell functions such as cell proliferation (23) and differentiation (33) require the attachment and spreading of these cells on extracellular matrices, which result in cell shape changes (for review see reference 35). We have demonstrated that changes in cell shape may bring about responses at the level of macromolecular metabolism (2, 5, 7-9, 19). In recent years studies of cell structure revealed the existence of a structural subcellular cytoskeleton implicated primarily in maintaining cell shape and in determining cell locomotion (for recent reviews see reference 13). Elements of the cytoskeletal system (microtubules, microfilaments) are also presumptive candidates for playing a role in transducing signals exerted at the cell surface by growth factors to bring about the initiation of DNA synthesis (15, 16, 27, 46, 62, 63). Our studies on cell configuration related gene expression suggest that cytoskeletal genes respond to alterations in cell morphology. We have shown that the level of tubulin mRNA production depends on the level of unpolymerized tubulin (6) and that actin mRNA expression in suspended and reattached fibroblasts is regulated at the levels of translation and transcription and its level correlates with changes in growth (20). Studies from other laboratories have demonstrated a linkage between the regulation of actin synthesis and the organization of the microfilaments (61, 65). In a recent study I have shown that the biosynthesis of vimentin, the mesenchymal type intermediate filament protein, is selectively reduced during suspension culture in various cell lines (B16 melanoma, 3T3, BSC-1, and SV 3T3 cells) but is rapidly reversed upon reattachment of cells to the substrate (3).

In the present study I examined the synthesis of intermediate filament proteins in epithelial cells in culture in response to cell-cell interactions and cell shape changes, as a model system for the study of regulation of genes involved in morphological differentiation. Cultured epithelial cells such as the Madin-Darby kidney cells (bovine [MDBK] or canine [MDCK]) provide a useful model system for studying kidney epithelium function since they maintain in tissue culture...
epithelial specific architectural polarity and vectorial secretion and transport of water and ions (12, 48). Madin-Darby canine kidney (MDCK) cells were also shown to display vectorial viral budding (55). In vivo intermediate filament proteins are tissue type specific, namely different proteins of the same class are building the same structures. However, many permanent epithelial cell lines express, in addition to the epithelial specific cytokeratin type filaments, also the vimentin type mesenchymal filaments, not found usually in the epithelia of the tissue of their origin (1, 41, 43).

The present study demonstrates that in epithelial kidney cells that express both the cytokeratin type and vimentin type intermediate filaments, the rate of keratin synthesis is mediated by the extent of cell-cell contact, while the rate of vimentin synthesis responds to changes in cell shape.

MATERIALS AND METHODS

Cell Culture: The Madin-Darby bovine kidney (MDBK) (45) established cell line was obtained from Dr. B. Geiger and the cells were grown in Dulbecco's modified Eagle medium with 10% fetal bovine serum. The cells were plated either on Falcon tissue culture dishes or on poly-L-lysine-coated plates. Poly-L-lysine-coated dishes were prepared by covering the dishes with 2 mg/cm² of polylysine (BDH, Poole, England) as described (57). Poly-L-lysine was not required, 0.6 M KCl was substituted with 100 mM NaCl. The Triton-cytoskeletons were prepared by scraping PBS washed cells into a buffer containing 0.5% Triton X-100, 0.6 M KCl, 14 mM 8-mercaptoethanol, 2.5 mM EGTA, 1 mM phenylmethyl-sulfonyl fluoride, and 10 mM HEPES, pH 7.4. (EGTA, 8-mercaptoethanol and phenylmethylsulfonyl fluoride were included to minimize the Ca²⁺-dependent action of a protease that degrades the intermediate type filaments (51, 57). In some experiments the cells were seeded on poly-L-lysine-coated dishes in medium containing 1.35% methyl cellulose as described (8). Cell growth was arrested by inhibiting DNA synthesis with 25 μg/ml of ara C or by treating the cell cultures for 24 h-periods with 2 mM thymidine with a 24 h-release period between the two treatments. After the second thymidine block the growth in the majority of cells was arrested as assayed by DNA and histones synthesis (5). The cells were stimulated into growth by the addition of fresh medium. In the serum deprivation induced arrest of cell growth, the cells were seeded in 0.5% serum for 48 h and then stimulated into the growth phase with 10% serum.

Cell Fractionation: Triton-cytoskeletons were prepared by scraping PBS washed cells into a buffer containing 0.5% Triton X-100, 0.6 M KCl, 14 mM 8-mercaptoethanol, 2.5 mM EGTA, 1 mM MgCl₂, 1 mM phenylmethyl-sulfonyl fluoride, and 10 mM HEPES, pH 7.4. Triton-insoluble fraction was pelleted by sedimentation for 3 min in an Eppendorf centrifuge. The pellet was analyzed by SDS gel electrophoresis according to Laemmli (40), or by two-dimensional gel electrophoresis according to O'Farrell (52).

RNA Slot-Blot Hybridization: Serial (1:2) dilutions of poly(A)⁺ cytoplasmic RNA starting with 5 μg RNA per slot, were applied in 125 μl of 20 × SSC (1 × SSC is 1.5 M NaCl plus 0.15 M sodium citrate) to nitrocellulose paper (Schleicher and Schuell, Inc., Keene, NH) using a slot-blot apparatus designed in our Institute. Nick translated °P-labeled rat vimentin cDNA (17), a mixture of pka and pkb human cytokeratins, cDNA (28) or tubulin cDNA (33) were prepared as described (20). Hybridization with 2 × 10⁶ cpm per blot was performed in 5 × SSC, 10 × Denhard's solution (0.2% BSA, 0.2% Ficoll, 0.2% polyvinylpyrrolidine) and 0.1% SDS at 68°C for 36 h in sealed plastic bags. The blots were then washed several times at room temperature in 2 × SSC and then for 2 h at 35°C in 0.1 × SSC and 0.1% SDS with frequent changes of the washing buffer. Finally the blots were exposed to X-ray film for 2-20 h at -70°C.

Immunofluorescence and Immunoblotting: Immunofluorescent staining for vimentin and cytokeratins was performed with rabbit antivimentin antibody, or with a broadly cross-reactive monoclonal antibody against cytokeratins. Cells on glass coverslips were fixed and permeabilized by a 20-min incubation in absolute methanol at -20°C and then rinsed five times in acetone at -20°C. The cells were then incubated for 30 min at room temperature with the first antibody, washed in PBS, and incubated for 30 min with the second rhodamine goat anti-rabbit IgG or the rhodamine goat anti-mouse IgG and viewed in a Zeiss photomicroscope III. Rabbit antivimentin antibody was the generous gift of Dr. R. Hynes (Massachusetts Institute of Technology, Cambridge, MA). Cytokeratin antibody (K3.8.13.2) (31) and the IgGs were a gift of Dr. B. Geiger (The Weizmann Institute, Rehovot). Immunoblotting with monoclonal antikeratin antibodies followed by °P-labeled goat anti-mouse antibody was performed as described previously (31).

In Vitro Translation: Cells were washed in PBS and scraped into 10 mM Tris (pH 7.4), 1.5 mM MgCl₂, 150 mM NaCl. The cells were then lysed by the addition of NP-40 to 0.5% and after vigorous mixing the nuclei were sedimented. To the supernatant an equal volume of 7 M urea, 0.35 M NaCl, 1% SDS, 0.01 M EDTA was added followed by an equal volume of phenol and chloroform. After two successive extractions with chloroform the RNA was precipitated with ethanol. The poly(A) containing RNA was prepared by oligo(GT)-cellulose chromatography and equal amounts of poly(A)° RNA were used for translation in a reticulocyte cell-free system (Amersham Corp., United Kingdom) as described (19).

RESULTS

Changes in Vimentin and Cytokeratins Synthesis in Monolayer, Suspension, and Reattaching Cells

MDBK cells were seeded either on plastic tissue culture dishes (Fig. 1A) or on poly(HEMA) coated dishes (Fig. 1B), that reduce the adhesiveness of the substrate (2, 3, 7, 23). Cells plated on thick poly(HEMA) films are unable to attach and spread onto the films but they do attach to each other and form large aggregates that are tightly packed together and contain epithelial specific intercellular junctions (of adherence and desmosomal type) as revealed by electron microscopy (Ben-Ze'ev, A., unpublished results, and reference 56). Analysis by high resolution two dimensional gel electrophoresis of [°³5]methionine-labeled Triton-cytoskeleton proteins from monolayer cultures (Fig. 1C) and cell cultures suspended for 3 d (Fig. 1D) reveals a dramatic decrease in vimentin synthesis during suspension culture, in agreement with our previous findings in fibroblasts (3). If the cells are allowed to reattach and spread on control tissue culture plates for 6 h, vimentin synthesis rapidly recovers (Fig. 1E) from its low synthesis rate in suspension. Unlike vimentin synthesis, which is markedly affected by these manipulations in culture conditions, the synthesis of the two cytokeratins (no. 8 and 18) characteristic to this cell line (57) is not affected by these dramatic alterations in cell morphology. A reversible reduction in the Triton-cytoskeleton-associated actin is also observed, in agreement with previous studies with fibroblasts (20). Similar turnover rates for vimentin and cytokeratins synthesis were obtained with [°³5]methionine labeling in pulse-chase experiments, suggesting that the reduction in vimentin labeling is not the result of a rapid degradation of newly synthesized vimentin in suspension culture. The same electrophoretic profiles described in Fig. 1, C and E were obtained also when the gels were loaded with radioactive cytoskeletal proteins from equal counts of total trichloroacetic acid-precipitable protein, since the Triton-insoluble fraction constituted an equal percent of total radioactive cell proteins (~35%) with very little variations between the various culture conditions.

Variations in Cytokeratins and Vimentin Synthesis in Sparse and Dense Cultures in Monolayer and in Suspension

In the experiments described in the previous section the cells were grown under conditions that enable the formation of extensive cell-cell interactions (Fig. 1) in both the monolayer and suspension cultures. These conditions are favorable for the expression of normal epithelial morphology i.e., structural and functional polarization. The synthesis of the cytokeratins and of vimentin was also followed under conditions
Figure 1 Cytokeratin and vimentin synthesis in monolayer and suspension cultures of MDBK by [35S]methionine labeling of cells. Semiconfluent MDBK cells (A and C) or cells after 3 d suspension in culture (B and D) or cells after 6 h of replating following 3 d suspension in culture (E) were labeled with 100 μCi/ml [35S]methionine for 2 h and equal amounts of TCA-precipitable radioactivity from Triton-cytoskeletons were analyzed by two-dimensional gel electrophoresis. v, vimentin; a, actin; 8, 18, cytokeratins of 53 and 44 kd, according to the nomenclature of Moll et al. (49). (A and C) x 250.

that are unfavorable for the formation of cell-cell interactions. The cells were either seeded densely (Fig. 2A) to encourage the formation of cell-cell interactions, or very sparsely (Fig. 2B), to minimize such interactions. Although immunofluorescent staining with both an antibody against vimentin (Fig. 3, A and C) and with the monoclonal antibody Ko8.13.2 against a wide range of cytokeratins (31), (Fig. 3, B and D) showed staining of sparse and dense monolayer culture, Coomassie Blue-staining of cytoskeletal proteins derived from equal numbers of cells from sparse (Fig. 3 E) and dense (Fig. 3 F) monolayer cultures, showed a high content of cytokeratin number 8 in dense cultures and a high content of vimentin in sparse cultures. On SDS Laemmli gels cytokeratin number 18 and actin co-migrate, but these proteins could be partially separated on two dimensional gels due to slight differences in their pI (see below). Furthermore, the immunoblot with the wide range antikeratin antibody Ko8.13.2 shows that in dense monolayer cultures both cytokeratins 8 and 18 are expressed at equimolar amounts (Fig. 3 G). Analysis of the cytokeratins and vimentin synthesis by SDS- or by two dimensional high resolution-gel electrophoresis of [35S]methionine-labeled Triton-cytoskeletons of sparse and dense cultures revealed that in densely seeded cells in either monolayer (Fig. 4 lanes A, C, and E, and lanes A', C', E') or suspension (Fig. 4 lanes B, D, F and lane F') cultures the rate of vimentin synthesis rapidly decreases while the rate of synthesis of both cytokeratins 8 and 18 increases as shown on the two dimensional gels (Fig. 4, A', C', E', F'). In contrast, sparsely seeded cells maintain even after 5 d of culture a high ratio of vimentin vs. cytokeratin synthesis (compare Fig. 4 lanes G and H). Similarly, in sparsely seeded cells in suspension culture, if methyl cellulose was added to the medium to avoid formation of cell aggregates (Fig. 2C) there was no significant increase in the synthesis of cytokeratins and vimentin synthesis was reduced (Fig. 4I). If methyl cellulose was not added, and the same number of cells was seeded as in Fig. 4I, cell aggregates formed and the synthesis of cytokeratins was induced (Fig. 4J, compare with Fig. 4I). These results imply that while the synthesis of cytokeratins in both monolayer and suspension cultures is correlated with extensive cell-cell contact, the synthesis of vimentin responds to changes in cell spreading. Thus under conditions that severely reduce cell spreading like in dense cell cultures (compare Fig. 2, A and B) or in suspension culture (see Fig. 2C) vimentin synthesis is inhibited.

Variations in Vimentin and Cytokeratins Synthesis are Reflected at the Level of mRNA Concentration

Fig. 5 demonstrates that the difference obtained in the synthesis of cytokeratins in sparse and dense cultures are also
FIGURE 2. Morphology of MDBK cells grown in dense monolayer cultures or in sparse monolayer and suspension cultures. MDBK cells were seeded at either $5 \times 10^5$ cells per 35-mm dish (A), or at $10^6$ cells per 10-cm dish on monolayer (B) or on poly(HEMA)-coated plates in medium containing 1.35% methyl cellulose (C). The cells were photographed 3 d after seeding. × 250.

reflected at the level of mRNA activity in a cell-free in vitro translation system. mRNA from sparse cell cultures (Fig. 5A) directs the synthesis of relatively low levels of cytokeratins and high levels of vimentin as compared with dense monolayer (Fig. 5C) or suspension cultures (Fig. 5D). The shift from a high vimentin low keratin pattern to a high keratin
low vimentin pattern in dense cell cultures takes between 3 to 5 d as demonstrated by the in vitro translation assay (compare Fig. 5, B and C), in agreement with results obtained with the [35S]methionine-labeled cell cultures (Fig. 4, A–F). Fig. 5 lanes E–H show that the differences obtained in the level of translational activity in vitro of the vimentin and cytokeratins mRNAs under the various culture conditions is due to changes in the amount of the respective RNA sequences as demonstrated by the RNA slot-blot hybridization with 32P-labeled cloned cDNAs to vimentin (17) and cytokeratins (28). Poly(A)+ RNA from sparse monolayer cultures (Fig. 5 E) contains between four to six times more vimentin RNA sequences than dense monolayer cultures (Fig. 5 F). In contrast, dense monolayer cultures (Fig. 5 G) contain between six to eight times more cytokeratins RNA sequences than sparse monolayer cultures (Fig. 5 H), as determined by densitometer tracing of the slot-blot. When an equivalent RNA blot was hybridized with 32P-labeled cloned cDNA to tubulin (32) similar amounts of this RNA sequences were detected in both sparse (Fig. 5 J) and dense (Fig. 5 J) monolayer cultures. Thus the decrease or the increase in the mRNA activity for vimentin and cytokeratins in an in vitro assay is paralleled in this study by a corresponding decrease or increase in the amount of the same mRNA under the various culture conditions.

Vimentin vs. Cytokeratin Synthesis during the Cell Cycle in the Presence of ara C and Under Serum-Starvation-Stimulation Conditions

For the analysis of proteins by SDS- or by two dimensional gel-electrophoresis the Triton-cytoskeleton fractions were prepared, since these are highly enriched in the insoluble intermediate filaments fraction. Analysis of the Triton-soluble supernatant of the experiment described in Fig. 4 (lanes A–F) did not reveal detectable amounts of soluble cytokeratins or vimentin, suggesting that the observed changes in the level of synthesis of the cytoskeleton-associated intermediate filaments did not result from partial depolymerization of these filaments.

The level of DNA and protein synthesis per cell as determined by short pulses with [35S]methionine or [3H]thymidine was similar in sparse as compared with semiconfluent monolayer cultures. The uptake of radioactive amino acid and nucleotides did not change under the various culture conditions (results not shown). However there was an approximately twofold decrease in the level of DNA synthesis in dense monolayer cultures and a tenfold decrease in suspension cultures. Similarly, there was a decrease in the levels of protein synthesis per same amount of total cell protein in the dense monolayer and suspension cultures (40 and 70%, respectively)
FIGURE 4 Variations in the levels of vimentin and cytokeratin synthesis in sparse and dense monolayer and suspension cultures. MDBK cells were seeded in dense monolayer (5 x 10^5 cells per 35-mm plate, lanes A, C, and E) or suspension (B, D, and F) cultures or in sparse (10^4 cells per 35-mm dish) monolayer cultures (G and H) or in sparse suspension cultures either with (I) or without (J) methylcellulose in the medium. The [35S]methionine-labeled Triton-cytoskeletons were analyzed after 1 d (A, B, A', and G), 3 d (C, C', and D), or 5 d (E, F, E', F', H, I, and J) by either SDS gel electrophoresis (lanes A-H) or by two dimensional gel electrophoresis (lanes A'-F', I and J). a, v, 8, and 18 are as described in Fig. 1.

as compared with semiconfluent cultures. I therefore examined the ratio between vimentin and the cytokeratin synthesis in semiconfluent and sparse monolayer cultures under conditions that inhibit DNA synthesis. Semiconfluent (Fig. 6 K) and sparse cell cultures (Fig. 6 L) were treated for 24 h with ara C and equal counts per minute of total cell protein from the radioactive Triton-cytoskeletons labeled with [35S]methionine were analyzed on SDS gels. The results show that neither in the semiconfluent (Fig. 6, lanes I and K) nor in the sparse cell cultures (Fig. 6, lanes J and L) was the ratio between vimentin and cytokeratin synthesis affected by the inhibition of DNA synthesis. Similarly, when growth was arrested in a semiconfluent culture (Fig. 6, lane A) by the double thymidine block as shown by lack of histones synthesis and then the cells were stimulated into S phase, as demonstrated by the induction of DNA (not shown) and histones synthesis (Fig. 6, lanes B–H), the ratio between vimentin and cytokeratin synthesis was not altered. Similar results were also obtained with semiconfluent cells that were arrested for 48 h by serum deprivation (Fig. 6, lane M) which were then stimulated into growth with 10% serum (Fig. 6, lane N; compare levels of histones synthesis to lane M). In these studies the levels of histones synthesis served as an internal marker to the level of DNA synthesis in addition to the radioactive labeling with [3H]thymidine. Thus, while some of the variations in the intermediate filaments protein synthesis could be correlated with changes in the rate of cell proliferation, as also suggested in a recent study with mesothelial cells (14), Figs. 4 and 6 suggest in addition that the synthesis of cytokeratins correlates with extensive cell-cell contact, while vimentin synthesis correlates with cell spreading.

DISCUSSION

The present study demonstrated a different control of two types of intermediate filaments co-expressed in cultured epithelial kidney cells. Co-expression of vimentin intermediate type filaments in addition to the tissue type specific intermediate filaments is common among established cell lines in vitro (22, 24, 41, 60, 64). In contrast, co-expression of vimentin and cytokeratins in vivo is very rare and was previously reported only in neoplastic cells of epithelial origin (11, 39, 53). Recently, however, Lane et al. (42) and Lehtonen et al. (44) reported on the co-expression of cytokeratins and vimentin in the parietal endoderm of early mouse embryo. These cells constitute an individual population of motile cells that migrate out from the epithelium and settle on the Reichert membrane apart from each other, and it was therefore suggested that the expression of vimentin may be related to the reduced cell-cell contact and the independent existence of a cell. In addition in a recent study Connell and Rheinwald (14) have demonstrated such co-expression in mesothelial
cells in vivo and suggested to be the consequence of this cell type's derivation from embryonic mesoderm, which is also the embryonic origin of kidney epithelial cells used in the present study. Our study, using an in vitro model system, is compatible with the above studies and demonstrates that the regulation of cytokeratin expression is dependent on extensive cell-cell contact. The areas of cell-cell contact in epithelia are characterized by various intercellular junctions (21, 59). The desmosome type junctions are the most relevant to this study, since cytokeratin fibrils often terminate in the desmosomes at the intercellular boundary (34). The formation of desmosomes in epithelia and in embryonal development is usually accompanied by the formation of cytokeratin-type intermediate filaments that anchor at the desmosomal plaques (26, 36, 60). These areas of cytokeratin-type intermediate filaments and desmosomal plaque interaction do not contain vimentin filaments that are also expressed in the same cell (25; for MDBK cells see reference 30). In sparse monolayer cultures of MDBK cells, which we have used in this study, it has been demonstrated that desmosomes are characteristically observed only along areas of cell-cell contact, but not on free edges of cells (26, 37). In addition, using double immunofluorescence characterization, it was shown in MDBK cells that cytokeratin fibrils terminate in the desmosomal plaques characterized by antidesmoplakin antibodies (30). It is possible therefore that the increase in the extent of cell-cell interaction in dense cell cultures might induce the formation of desmosomes with the concomitant enhancement of the biosynthesis of cytokeratin proteins. In line with this suggestion thus, in both dense monolayer and suspension cultures, where extensive cell-cell interaction occurred, we observed an accentuated synthesis of cytokeratins, whereas sparse cell cultures, in either monolayer or suspension, expressed only low levels of cytokeratins. It is noteworthy that Rodriguez-Boulan et al. (56) have recently demonstrated that single cell suspension cultures of MDCK cells have lost the characteristic vectorial budding of viruses and viruses assembled in these cells in a nonpolarized fashion over most of the cell surface. However, in cell aggregates in suspension cultures where they found formations of junctional complexes, the polarity of viral budding was maintained as in monolayer cultures.

The synthesis of the vimentin type intermediate filament protein co-expressed in these cells appears to be sensitive to cell shape changes as reported previously in fibroblasts (3).
FIGURE 6 Synthesis of vimentin and cytokeratins in synchronized MDBK cells, in cells treated with inhibitors of DNA synthesis, and in serum-starved and -stimulated cells. The growth of semiconfluent MDBK cells was arrested (A) by the double thymidine block as described in Materials and Methods. The cells were labeled for 1-h intervals with [35S]-methionine before (A) and after stimulation into growth between 1 to 7 h (B-H) after release from the second thymidine block. Semiconfluent (I and K) or sparse (J and L) MDBK cultures were treated for 24 h with 25 μg/ml ara C (K and L) or left untreated (I and J), or semiconfluent cells were starved in 0.5% serum for 48 h (M), or 24 h after stimulation into growth with 10% serum (N) were labeled for 2 h with [35S]methionine. (The serum starved cells were labeled with [35S]methionine without serum in the labeling medium.) The Triton cytoskeleton (low-salt) fractions were analyzed, a, actin; v, vimentin; 18, 8, cytokeratins as described in Fig. 1; h, histones.

and in preadipocytes (58). In these studies it was demonstrated that cell rounding is accompanied by a dramatic decrease in the synthesis of vimentin. The present study shows that vimentin synthesis decreases in suspension culture in both sparse and dense kidney epithelial cell cultures, and also in very dense monolayer cultures, where the projected cell area is extensively reduced (compare Fig. 2, A and B). While the role of vimentin-type intermediate filaments in determining cell shape is not clear, since it was shown that microinjection of antivimentin antibody that disrupts vimentin filaments does not alter cell shape (38), several studies suggest that cells can spread out on a substrate to form the arborized processes when plated in the presence of cytochalasin B, which disrupts the actin filaments (7, 47). The pseudopodial arborized projections characteristic of cells treated with or seeded in the presence of cytochalasin B consist of parallel aligned intermediate filaments, but do not contain microfilaments and microtubules (47), suggesting a role for vimentin filaments in determining cell morphology. Furthermore, in a recent study Dulbecco et al. (18) have demonstrated that when a "wound" is introduced in a confluent monolayer culture of 3T3 cells, the vimentin containing intermediate filaments align in the front part of the cell's body in the direction of cell-movement when the cells spread out to fill the gap.

The differences in the rates of synthesis of cytokeratins and vimentin observed in [35S]methionine-labeled cells were also obtained in the reticulocytes cell-free in vitro translation systems directed with mRNA isolated from cells under the various culture conditions. This suggests that a reduction in the amount of the corresponding mRNA level may be responsible for the decrease in the rate of the cytoskeletal protein synthesized as was indeed obtained in RNA blot hybridizations to cloned vimentin and cytokeratins cDNAs. In addition, pulse-chase experiments with [35S]methionine labeling, Coomassie-Blue staining of gels and the immunofluorescence staining experiments suggest that there is no massive turnover of these proteins during changes in their rate of synthesis.

In a recent study Connell and Rheinwald (14) reported on a growth level related control of cytokeratins and vimentin synthesis in mesothelial cells where cytokeratin synthesis paralleled a slow growth level, while vimentin synthesis was correlated with a rapid growth level.

In the present study the level of vimentin synthesis also correlated with a rapid growth level, but in addition it correlated with changes in cell shape. In a previous study we demonstrated, that vimentin synthesis was reduced in the anchorage independent B16 melanoma cell line (54) when these cells were placed in suspension culture (3). In addition, the transition to a high cytokeratin/low vimentin synthesis correlated only partially with a slow growth level, because in suspension culture, where growth was arrested in both sparse and dense cell cultures, only when multicellular aggregates formed in dense cell cultures was the synthesis of cytokeratins induced (c.f. Fig. 4). Furthermore, by arresting growth in sparse and dense cultures with inhibitors of DNA synthesis or by the double thymidine block or in serum-starvation and -stimulation experiments the ratio between cytokeratins to vimentin synthesis could not be altered. Thus while these experiments do not exclude the role that the cell growth level can play in the control of vimentin and cytokeratins synthesis.
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