Cell Configuration-related Control of Vimentin Biosynthesis and Phosphorylation in Cultured Mammalian Cells

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ABSTRACT The cell configuration-related control of a cytoskeletal protein (vimentin) expression was examined by varying cell shape between flat and spherical. Cultivation of cells in monolayer or in a spherical configuration on poly-2-hydroxyethylmethacrylate-coated plates revealed a preferential down regulation of vimentin synthesis during suspension culture. The mechanism(s) regulating the decrease in the expression of vimentin in spherical cells appears to be at the level of translation, because mRNAs extracted from monolayer and suspension cultured cells were equally active in directing vimentin synthesis in the rabbit reticulocyte cell-free system. When after prolonged suspension culture, the cells were allowed to reattach and spread, vimentin synthesis recovered rapidly to the control monolayer rate. The phosphorylation of vimentin was also reduced dramatically during suspension culture. However, unlike the rapid recovery of vimentin biosynthesis upon reattachment (<6 h), the recovery in the rate of vimentin phosphorylation was much slower (>20 h) and paralleled the recovery to the monolayer growth rate. Although the control of vimentin biosynthesis in suspension culture is a cell configuration-related process, the decrease in the rate of vimentin phosphorylation in suspension culture appears to be the result of the slower growth rate and may reflect the reported correlation between the rate of vimentin phosphorylation and the accumulation of cells in mitosis.

Evidence is accumulating that a wide variety of cell activities are modulated by cell configuration; Folkman and associates have postulated a central role for cell shape in growth control (21) and have demonstrated that cell proliferation correlates directly with the degree of cell spreading (22). Our studies on the control of macromolecular metabolism by cell configuration (1, 2, 18) have shown that protein synthesis depends on the establishment of cell contact with the substrate and that macromolecular metabolism in the nucleus (DNA, rRNA, mRNA production) requires extensive cell spreading (7). Studies in which cell shape was modulated by changing the composition of the extracellular matrix have revealed that the mitogenic response of cells also is cell shape dependent (23). Hay and Meier (24) and Benya and Shaffer (3) have shown that differentiation to a specific phenotype depends on cell configuration. Ben-Ze'ev and Raz (8) have reported that cytokinesis is highly dependent on cell shape, whereas nuclear division is less sensitive to alterations in cell morphology. I (4) recently demonstrated that cell configuration-induced restriction of growth control is a dominant phenomenon that cannot be overcome by infection with the DNA tumor virus SV40. Furthermore, the replication of DNA and RNA viruses is inhibited in parallel with cellular macromolecular metabolism, when the infected cell shape is varied from flat to spheroid (4).

Recent studies of cell structure and organization have revealed the existence of a proteinaceous subcellular structural framework or cytoskeleton serving primarily to maintain cell shape and to determine cell locomotion and chromosome movement. Therefore, studies related to cell configuration-dependent growth control have been directed toward the investigation of cytoskeletal protein gene expression (6, 13, 20, 41). These studies suggest that there is a correlation between the morphological state of the cell and the expression of the cytoskeletal genes. In a recent study Farmer et al. (19) showed that the expression of the actin genes in suspended and reattached fibroblasts is regulated at the levels of translation and mRNA production. Inasmuch as significant amounts...
of both actin and tubulin are found in the cell in assembled and disassembled forms, whereas vimentin is by and large found in an assembled form (26, 39), it was of interest to me to investigate the control of vimentin biosynthesis during manipulations in cell configuration.

In the present study I examined the control of vimentin synthesis and phosphorylation in monolayer cell cultures, in suspension, and in reattaching cell cultures. The results indicate that the rate of vimentin biosynthesis is selectively reduced during suspension culture by a posttranscriptional control mechanism and that upon reattachment vimentin synthesis is rapidly restored. The phosphorylation of vimentin, which is also greatly reduced during suspension culture, recovers much more slowly after reattachment, together with the recovery to the monolayer growth rate.

MATERIALS AND METHODS

Cell Culture: 3T3, BSC-1, B16-F1 melanoma, and SV101 (SV40-transformed 3T3 cells) cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in monolayer cultures or on poly(HEMA)-coated plastic dishes or on poly(dT)-cellulose chromatography (poly(HEMA), Hydron Laboratories, New Brunswick, NJ), coated plastic plates as described (7, 22). In brief, a 12% stock solution of poly(HEMA) were prepared in 95% ethanol, and each plastic plate (5 cm in diameter) was covered with 2.5 ml of a 10 -2 dilution of the stock poly(HEMA). The plates were incubated in a warm room at 37°C for 36-48 h to allow the evaporation of the ethanol. The cells were synchronized by incubating them twice in complete medium containing 2 mM thymidine for 16 h, with a 10-h release period between the thymidine blocks (8).

Radioactive Labeling: [35S]Methionine was used at a concentration of 100 µCi/ml (1.25 Ci/mmol, Amersham, UK) in methionine-deficient medium and 10% dialyzed fetal calf serum. For labeling with [32P]P, 0.5 mCi/ml of [32P]orthophosphoric acid (200 µCi/mmol) from the Nuclear Research Centre, Israel, was used in phosphate-free medium containing 10% dialyzed fetal calf serum. The pellet obtained after centrifugation was resuspended in 10 mM NaCl, 10 mM HEPES (pH 7.4), and 1.5 mM MgCl₂ containing 0.5 mg/ml DNase I and incubated at 4°C for 15 min. KCl was added to a final concentration of 0.6 M, and the pellet obtained after centrifugation for 5 min at 2,000 rpm at 4°C was used for further analysis. In some cases (see Table I) the DNase and high-salt step was omitted.

Cell Fractionation: Triton cytoskeletons were prepared by washing the cells with cold phosphate-buffered saline (PBS) and lysed in lysis buffer (0.5% Triton X-100, 50 mM NaCl, 300 mM sucrose, 10 mM PIPES [pH 6.8], 2.5 mM MgCl₂, and 1 mM phenylmethylsulfonyl fluoride at 4°C). The pellet, obtained after centrifugation at 10,000 g, was resuspended in 10 mM NaCl, 10 mM HEPES (pH 7.4), and 1.5 mM MgCl₂ containing 0.5 mg/ml DNase I, were analyzed on SDS acrylamide gels. Such analyses allow the determination of the rate of synthesis of a specific protein relative to total cell protein. The results shown in Fig. 2 B show that the rate of vimentin synthesis is reduced significantly during suspension culture as compared with its rate of synthesis in monolayer culture (Fig. 2 A). This decrease in vimentin expression is not the result of the rapid degradation of newly synthesized vimentin, because similar results were obtained in pulse-chase experiments in which cells were labeled for 10 min and chased for 60 min with unlabeled methionine (results not shown). The reduction in the rate of vimentin synthesis is gradually reversed (completed in ~6 h) when the cells are allowed to reattach to control plastic plates, and similar rates of vimentin synthesis were obtained after 6 h (Fig. 2 C) or 24 h (Fig. 2 D) of reattachment. To compare the mass of vimentin to its rate of synthesis, Triton cytoskeletons were prepared from [35S]methionine-labeled monolayer (Fig. 2 E), suspension-grown cells (Fig. 2 F), and B16 melanoma cells (Fig. 2 G) after 6 h of replating, because vimentin has been shown to be highly insoluble under these extraction conditions (6, 26), and equal amounts of radioactive proteins were analyzed on two-dimensional isoelectric focusing and SDS gels. The gels were stained with Coomassie blue (Fig. 2, E~G) and then autoradiography was performed on the same gels (Fig. 2, E~G). Although the rate of vimentin synthesis was reduced in suspension culture (cf. Fig. 2, E and F), the mass of nonradioactive vimentin in suspended cells was comparable to its amount in monolayer culture (Fig. 2, E' and F'). Immunofluorescence revealed similar amounts of vimentin in monolayer, suspended, and replated cells (results not shown). These results suggest that the total amount of vimentin is kept constant, probably by stabilization against turnover during suspension culture. In addition to the prominent reduction in the rate of vimentin biosynthesis during suspension culture, there also was some reduction in the rate of actin synthesis (cf. Fig. 2, A and B), in agreement with our recent study with 3T3 cells (see reference 19 below).

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

Cells were seeded on poly(HEMA)-coated plastic dishes with reduced adhesiveness to maintain a spheroid cell shape as demonstrated previously (7, 22). B16 melanoma cells were chosen because they are anchorage independent for growth (8) and can proliferate in monolayer or in suspension on poly(HEMA)-coated plates, although at different rates, as shown in Fig. 1 A. In monolayer culture these cells have a fibroblastic morphology (Fig. 1 B), but during suspension culture the spheroid cells form loosely attached aggregates (Fig. 1, C and D) that can easily be dispersed by pipetting, and the cells, when replated on control plastic plates, resume the monolayer growth rate after 16-20 h. Fig. 2 shows the biosynthetically labeled protein pattern of B16 cells in monolayer (Fig. 2 A), after 3 d of suspension culture on poly(HEMA)-coated plates (Fig. 2 B), and after 6 h (Fig. 2 C) and 24 h (Fig. 2 D) of replating. The cells were pulse-labeled for 1 h with [35S]methionine, and equal amounts of trichloroacetic acid-precipitable radioactive total cell proteins were analyzed on SDS acrylamide gels. Such analyses allow the determination of the rate of synthesis of a specific protein relative to total cell protein. The results shown in Fig. 2 B show that the rate of vimentin synthesis is reduced significantly during suspension culture as compared with its rate of synthesis in monolayer culture (Fig. 2 A). This decrease in vimentin expression is not the result of the rapid degradation of newly synthesized vimentin, because similar results were obtained in pulse-chase experiments in which cells were labeled with cold methionine and chased for 60 min with unlabeled methionine (results not shown). The reduction in the rate of vimentin synthesis is gradually reversed (completed in ~6 h) when the cells are allowed to reattach to control plastic plates, and similar rates of vimentin synthesis were obtained after 6 h (Fig. 2 C) or 24 h (Fig. 2 D) of reattachment. To compare the mass of vimentin to its rate of synthesis, Triton cytoskeletons were prepared from [35S]methionine-labeled monolayer (Fig. 2 E), suspension-grown cells (Fig. 2 F), and B16 melanoma cells (Fig. 2 G) after 6 h of replating, because vimentin has been shown to be highly insoluble under these extraction conditions (6, 26), and equal amounts of radioactive proteins were analyzed on two-dimensional isoelectric focusing and SDS gels. The gels were stained with Coomassie blue (Fig. 2, E~G) and then autoradiography was performed on the same gels (Fig. 2, E~G). Although the rate of vimentin synthesis was reduced in suspension culture (cf. Fig. 2, E and F), the mass of nonradioactive vimentin in suspended cells was comparable to its amount in monolayer culture (Fig. 2, E' and F'). Immunofluorescence revealed similar amounts of vimentin in monolayer, suspended, and replated cells (results not shown). These results suggest that the total amount of vimentin is kept constant, probably by stabilization against turnover during suspension culture. In addition to the prominent reduction in the rate of vimentin biosynthesis during suspension culture, there also was some reduction in the rate of actin synthesis (cf. Fig. 2, A and B), in agreement with our recent study with 3T3 cells (see reference 19 below).

The decrease in the biosynthesis of vimentin during suspen...
Figure 1 The morphology and growth rate of B16 melanoma cells in monolayer and in suspension culture. 5 x 10³ cells were seeded in multiwell Falcon (3008) plates on plastic (O) or on poly(HEMA)-coated wells (●). Cell number was determined by removing the cells in 2 mM EDTA and counting in a Coulter counter (Coulter Electronics Inc., Hialeah, FL) (A). Cell morphology is shown in monolayer (B) after 1 d (C) and after 3 d (D) in poly(HEMA)-coated wells. × 250.

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Gradual Reduction in Vimentin Synthesis during Suspension Culture

Whereas in the anchorage-dependent 3T3 cells in suspension culture the rate of protein synthesis is reduced to 10% of the monolayer level, B16 melanoma cells in suspension continue to synthesize proteins at rates similar to the monolayer rate (8). Fig. 4 shows that the reduction in the level of vimentin biosynthesis is gradual in B16 melanoma cells during suspension culture. Equal amounts of radiolabeled proteins from monolayer cultures (Fig. 4a) or from suspension cultures after

1 (Fig. 3, lanes G and H) and in SV40-transformed 3T3 cells (SV 101) (not shown), and to a lesser extent in CHO cells that grow equally well in suspension and in monolayer (not shown).
FIGURE 2 Reversible down regulation of vimentin biosynthesis during suspension culture of B16 cells. B16 melanoma cells were labeled with [35S]methionine for 60 min in monolayer (A and E), after 3 d in suspension culture (B and F), and after 6 (C and G) and 24 h (D) of reattachment. Equal amounts of TCA-precipitable total cell proteins were analyzed on 7-17% SDS acrylamide gels (A-D), on Triton cytoskeletons were run on two-dimensional gels. The autoradiograms (E-G) or the Coomassie blue (E'-G') protein patterns are shown (a, actin; v, vimentin).

FIGURE 3 Modulation of vimentin biosynthesis by cell configuration in 3T3 and BSC-1 cells. 3T3 cells were labeled with [35S]methionine for 60 min in monolayer (A and E), after 3 d in suspension (B and F), and after 4 (C) and 8 h (D) of reattachment. BSC-1 cells were labeled for 60 min with [35S]methionine in monolayer (G) and after 3 d in suspension culture (H). Equal amounts of TCA-precipitable radioactive proteins were analyzed on 10% acrylamide gels (A-D) or by two-dimensional gel electrophoresis (E-H) (a, actin; v, vimentin).

FIGURE 4 Gradual decrease in vimentin biosynthesis in suspension culture in B16 melanoma cells. (A) B16 melanoma cells from monolayer (a and a'), after 1 (b and b'), 2 (c and c') and 3 d (d and d') in suspension culture, and after 6 (e and e') and 24 h (f and f') of replating were labeled with [35S]methionine for 2 h. Equal amounts of TCA-precipitable radioactive proteins were used for immune precipitation with antivimentin antibody (a-f) or with nonimmune serum (a'-f'). M, 14C-methylated molecular weight markers: myosin, phosphorylase b, bovine serum albumin, ovalbumin, and lactoglobulin A. (B) Planimetric determination of the amount of vimentin in the gels shown in A. The arrow indicates the time of reattachment to control plastic.
from flat to spheroid results, most probably, from a posttranscriptional control mechanism.

**Phosphorylation of Vimentin as a Function of Cell Shape and Cell Cycle**

Vimentin is known as a phosphoprotein (12, 31), and most recent studies have shown an increase in the rate of phosphorylation of vimentin during mitosis (10, 17, 40) and during hormonal stimulation (14).

I have analyzed the level of vimentin phosphorylation in monolayer, suspended, and reattaching cells, and the results are shown in Fig. 7. Vimentin phosphorylation was assayed by labeling an equal number of cells for 2 h with $^{32}$PO$_4$ and the Triton cytoskeletons prepared from each sample were subjected to two-dimensional gel electrophoresis. The gels were stained with Coomassie blue and then exposed to x-ray films at 4°C. The rate of vimentin phosphorylation is significantly reduced after 3 d in suspension culture (Fig. 7, lane a), but, unlike the rate of vimentin biosynthesis, the level of phosphorylation did not recover to the monolayer level (Fig. 7, lane d) after 8 h of replating (Fig. 7, lane b). Only after 24 h of replating (Fig. 7, lane c) did vimentin phosphorylation recover from the low suspension rate. The Coomassie blue staining pattern (Fig. 7, lanes a'–d') gives an estimate of the mass of total vimentin in each sample. The decreased rate of vimentin phosphorylation might result from a difference in the rate of phosphate uptake and its use in the cell. Therefore, it is possible that vimentin phosphorylation is regulated by a posttranscriptional mechanism.[1]

**Posttranscriptional Control of Vimentin Synthesis during Suspension Culture**

To investigate the level at which the expression of the vimentin gene is regulated during suspension culture, poly(A)-containing RNA was isolated from monolayer cultures and 3-d suspension cultures of B16 melanoma cells, and the RNA was translated in the cell-free system of the rabbit reticulocytes. Equal amounts of trichloroacetic acid–precipitable radioactive proteins were analyzed on SDS gels before (Fig. 6, A and H) or after immunoprecipitation with antivimentin antibody (Fig. 6, D and F). The results shown in Fig. 6, D and F indicate that the activity of the mRNA coding for vimentin synthesis remains unchanged during suspension culture, because similar amounts of radioactive vimentin were synthesized in the cell-free system, as shown also by the two-dimensional gel analysis (Fig. 6, H and I). Thus, the reduction in the rate of vimentin biosynthesis when cell shape is varied from flat to spheroid results, most probably, from a posttranscriptional control mechanism.

**FIGURE 5** Vimentin biosynthesis is cell cycle independent. BSC-1 cells were synchronized by a double (16-h) thymidine block with a 10-h release between the thymidine blocks. After release from the second thymidine block, 5 × 10$^5$ cells were labeled with $^{35}$S-methionine for 1-h periods (A), and total cell protein was analyzed on 7–17% gradient acrylamide SDS gels, or the cells were labeled with 10 $\mu$Ci/ml $^3$H]thymidine for 1 h (B), and the TCA-precipitable radioactivity in 10$^5$ cells was determined. (v, vimentin; a, actin; h, histones).

**FIGURE 6** In vitro translation of mRNA from monolayer and suspended B16 melanoma cells. Poly(A)-containing RNA from monolayer and suspended cells was prepared and translated in a cell-free reticulocyte lysate as described in Materials and Methods. Equal amounts of TCA-precipitable in vitro synthesized proteins with mRNA from monolayer (A, D, E, H) and from 3-d suspended cells (B, F, C, I) were analyzed on 7–17% gradient acrylamide SDS gels before (A, B) or after immunoprecipitation with antivimentin antibody (D, F) and nonimmune serum (E, G) or by two-dimensional gel electrophoresis (H, I). C, control, endogenous translation product; M, $^{14}$C-methylated protein molecular weight markers as shown in Fig. 4. v, vimentin; a, actin.

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and RNA. The incorporation into TCA-precipitable material was determined 6 h of replating following 3 d of suspension for 3 d (d a' and a'), or after 8 (b and b') and 20 h (c and c') of replating after suspension culture were labeled for 2 h with 0.5 mCi/ml H32PO4, and vimentin-enriched Triton cytoskeletons were prepared as described in Materials and Methods. The gels were stained with Coomassie blue (a'-d') and then exposed to x-ray films at 4°C (a-d). v, vimentin; a, actin.

TABLE I

| Culture Conditions | [35S]Metionine | H32PO4 |
|--------------------|----------------|--------|
| Monolayer          | 155,034        | 305,655|
| Suspension for 3 d | 18,350         | 278,916|
| 6 h of replating following 3 d of suspension | 110,269 | 346,774|

8-16 melanoma cells grown in monolayer, in suspension for 3 d, and after 6 h of replating were labeled for 2 h with [35S]methionine or H32PO4. Triton cytoskeletons enriched in vimentin were prepared from each preparation, except that the DNase and high-salt step was omitted with the H32PO4-labeled cells to allow determination of phosphate incorporation into DNA and RNA. The incorporation into TCA-precipitable material was determined in 5 x 10^6 cells.

I measured the TCA-precipitable phosphate incorporated into macromolecules (DNA, RNA, and protein) in Triton cytoskeletons and compared that to the incorporation of [35S]methionine into preparations highly enriched in vimentin (Triton cytoskeleton treated with DNase and high salt) from an equal number of monolayer, suspended, and reattaching cells. The results shown in Table I clearly demonstrate that whereas the rate of incorporation of [35S]methionine into vimentin-enriched cytoskeletons was reversibly reduced during suspension, the rate of incorporation of radioactive phosphate into TCA-precipitable macromolecules was not altered during suspension culture. This implies that the low rate of vimentin phosphorylation during suspension culture is not a result of altered phosphate uptake or incorporation into macromolecules.

Because the rate of vimentin phosphorylation does not recover in parallel with the recovery of the rate of vimentin biosynthesis but rather recovers in parallel with the recovery of the rate of DNA synthesis to the monolayer growth rate (>20 h after replating, results not shown), I analyzed the rate of vimentin phosphorylation during the cell cycle. BSC-1 cells, which react with a preferential decrease in the rate of vimentin biosynthesis during suspension culture (see Fig. 3, G and H), were synchronized by the double thymidine block (see Fig. 5). Equal numbers of cells were labeled with 32PO4 for 2 h in G1 before removal of the second thymidine block (Fig. 8A), during mid-S phase, between 3 and 5 h after removal of the thymidine block (Fig. 8B), and in late S phase, between 8 and 10 h after removal of the thymidine block (Fig. 8C). Triton cytoskeletons were prepared and analyzed by two-dimensional gel electrophoresis. The data in Fig. 8 clearly show a great increase in the rate of vimentin phosphorylation in late S phase (Fig. 8C), when a significant number of cells are in mitosis. An additional protein (X) with a molecular weight of about 115,000 is also phosphorylated in parallel with vimentin. The Coomassie blue pattern (Fig. 8, A'-C') of the gels shows a preferential proteolysis of vimentin in mid- and late-S phases (Fig. 8, B' and C') as compared with G1 phase (Fig. 8A'), but only the main vimentin spot is phosphorylated. These results suggest that the lower rate of vimentin phosphorylation observed in suspended cells, which recovers after reattachment and spreading in parallel with the recovery to the monolayer growth rate, may be related to the extended cell cycle in cells during suspension culture.

DISCUSSION

In this paper I report on the cell configuration-related control of the biosynthesis and phosphorylation of a major cytoskeletal protein, vimentin. Studies with whole-cell extracts or with Triton cytoskeletons prepared under gentle extraction conditions indicate that relatively large pools of disassembled (or unpolymerized) precursors for actin cables and microtubules are found in the cell (16, 35). In contrast, intermediate filaments are insoluble under these conditions (26), and various studies (39, 42) have shown that vimentin-containing filaments cannot be solubilized under conditions that completely depolymerize microfilaments and microtubules.

Although during certain alterations in cell morphology accompanied by changes in the organization of the cytoskeleton the cells react by changing the balance between unpolymized and polymerized cytoskeletal proteins or by changing their intracellular location (36, 37), during other morphological changes the cells appear to react by regulating the expression of the cytoskeletal gene proteins (6, 13, 41).

Because vimentin-containing filaments are highly insoluble under most conditions and because in the cell there appears to be no reservoir of unpolymerized vimentin, the recently described temporary phosphorylation of vimentin during mitosis (10, 17, 40) has been interpreted as being necessary for the altered organization of the intermediate filaments during mitosis (9, 46).

The present study shows that the control of vimentin biosynthesis is related to cell configuration and that the mechanism controlling the vimentin gene expression in response to altered cell configuration appears to be posttranscriptional. The dramatic alterations in cell morphology introduced by suspension culture initiate a large and preferential decrease in the rate of vimentin biosynthesis as compared with other cell
was not altered by dibutyryl cyclic AMP or vanadate (12, 17), the accumulation of cells in mitosis. In these studies the increased rate of phosphorylation of vimentin during mitosis is not paralleled by a related alteration in the rate of vimentin mRNA synthesis. This is somewhat different from the regulation of actin synthesis during suspension culture described in this paper. It is possible, therefore, that in the anchorage-independent cells the sensitivity to changes in cell morphology is limited to the anchorage-independent cell fraction and that the anchorage-dependent cells are the least sensitive to alterations in cell morphology. It is possible, therefore, that in the anchorage-independent cells the sensitivity to changes in cell morphology is limited to the anchorage-independent cell fraction and that the anchorage-dependent cells are the least sensitive to alterations in cell morphology.

The decrease in the rate of vimentin synthesis during suspension culture described in this paper appears to be controlled at the level of translation, because the mRNA activities for vimentin synthesis measured in monolayer and suspension cultures and assayed in a cell-free system are very similar. This is somewhat different from the regulation of actin synthesis in suspended and reattaching 3T3 fibroblasts. In these cells, using a cDNA clone, we found a preferential decrease in actin synthesis during suspension culture at the level of translation and a great preferential increase in actin synthesis during reattachment as a result of increased levels of translatable mRNA sequences in the cytoplasm (19). A cDNA clone for vimentin recently obtained by Dodemont et al. (15) will soon be available, and it will enable a more accurate quantitation of the levels of vimentin mRNA in suspended and reattaching cells.

Recent studies (17, 40) have demonstrated that the accumulation of phosphorylated vimentin in cells correlates with the accumulation of cells in mitosis. In these studies the increased rate of phosphorylation of vimentin during mitosis was not altered by dibutyryl cyclic AMP or vanadate (12, 17), suggesting that changes in cyclic AMP levels are probably not involved in the alterations in vimentin phosphorylation. The decrease in the rate of vimentin phosphorylation observed in suspension culture most probably is related to the extended cell cycle of cells in suspension. This change in the cell cycle results in a decrease in the number of cells undergoing mitosis per unit of time. In addition, unlike the rapid recovery of vimentin biosynthesis upon reattachment (<6 h), the recovery in the rate of vimentin phosphorylation is slow (>20 h) and parallels the recovery of the rate of DNA synthesis to the control monolayer rate. Thus, my finding of the cell shape-related alterations in vimentin phosphorylation may support the possibility that vimentin phosphorylation is a control mechanism by which intermediate filament organization is altered during mitosis (9, 46). It is also possible that both the down regulation of vimentin biosynthesis and part of the decrease in the rate of vimentin phosphorylation are the result of the alterations in cell shape or that phosphorylated vimentin is preferentially degraded in suspension.

The close relationships between the cell cytoarchitecture and the control of the expression of the cytoskeletal protein genes described in this and other papers (6, 13, 19, 20, 41) are more than obvious regulatory mechanisms. Because intermediate filaments play a central role in the organization of the cytoplasm (for reviews see references 29 and 30) and because cytoskeletal elements may interact directly and/or indirectly with the cell surface (5, 45), these studies may have implications for the proposed relationship between cell configuration and growth control (4, 7, 22, 34).

I gratefully acknowledge the technical assistance of Tamara Kreizman, and it is my pleasure to thank David Wolf for helping with the in vitro translation, Dr. Avraham Raz for determining the 8 melanoma growth rate and morphology and for many helpful discussions, and Dr. Benny Geiger for the critical reading of the manuscript.

This study was supported, in part, by grants from the Israel-USA Binational Foundation and the Israel Academy of Sciences and Humanities-Basic Research Foundation. A. Ben-Ze'ev is the incumbent of the Ruth and Leonard Simon career development chair.

Received for publication 2 February 1983, and in revised form 20 April 1983.
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