Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2747–2751, April 1989
Genetics

Involvement of vimentin in the reverse transformation reaction

(intermediate filaments/genetic regulation/chromosomal domains/cAMP/differentiation)

DANIEL CHAN*†‡, ALISON GOATE§, AND THEODORE T. PUCK*†‡¶

*Eleanor Roosevelt Institute for Cancer Research, 1899 Gaylord Street, Denver, CO 80206; †University of Colorado Cancer Center, Denver, CO 80262; §Department of Neurochemistry, Institute of Neurology, London, England WC1 2NS; and ‡Department of Medicine, University of Colorado School of Medicine, Denver, CO 80262

Contributed by Theodore T. Puck, December 15, 1988

ABSTRACT **An organized cytoskeleton is required for the cAMP-induced reverse transformation reaction in CHO-K1 cells. In the course of the reaction a considerable fraction of the genome changes its nuclease sensitivity. The current paper presents the following evidence that cAMP-induced phosphorylation of vimentin is an early step in this reaction complex. (*i*) Vimentin is only slightly phosphorylated in transformed CHO-K1 cells but is heavily phosphorylated in normal fibroblasts. (*ii*) cAMP addition almost triples the vimentin phosphorylation of CHO-K1 cells but does not change that of normal cells. (*iii*) Vimentin phosphorylation is one of the earliest phenomena to occur after addition of cAMP to CHO-K1 cells, preceding the cell-stretching reaction and other manifestations of reverse transformation. (*iv*) Indirect immunofluorescence experiments demonstrate that vimentin appears as a condensed mass in transformed CHO-K1 cells but cAMP addition restores the filamentous structure characteristic of the normal fibroblast. (*v*) Other transformed cells unresponsive to reverse transformation by cAMP failed to demonstrate increased phosphorylation of vimentin on treatment with cAMP. These results support the proposed scheme that phosphorylation of cytoskeletal elements initiates a large-scale genetic regulatory action in which a substantial change in the spectrum of genome exposure and sequestration occurs. A function for intermediate filaments in reverse transformation is implied.**

Treatment of transformed CHO-K1 cells with cAMP derivatives causes loss of the malignant phenotype (1–5). cAMP treatment results in a pattern of events named reverse transformation (RT) that involves the following components: (*i*) The compact pleomorphic morphology of CHO-K1 cells is flattened and elongated to a smooth-surfaced fibroblastic form (1–3, 5). (*ii*) The hyperactive cell surface is tranquilized, and cells secrete fibronectin and undergo other changes (6–8). (*iii*) The cytoskeleton changes from a sparse and randomly oriented set of elements to one in which the fibers are dense and largely parallel to each other (9, 10). (*iv*) Growth control is initiated as shown by the inability of cells to multiply in suspension culture or to form multi-layered colonies ("pile-up") on solid substrates (6). (*v*) New patterns of protein synthesis and processing are established (11, 12). (*vi*) As shown by isolated nuclei treated with appropriate nucleases, chromatin undergoes structural changes in which a large and specific set of genes becomes more sensitive to hydrolysis (13–15). It has been demonstrated that the cytoskeletal microtubules and microfilaments play an essential part in this reaction complex (2, 6, 15).

cAMP, by activating its specific kinase, causes phosphorylation of particular proteins (16, 17). This laboratory proposed (15) that the resulting conformations of some of these proteins change the patterns of a set of connections between cytoskeletal proteins and specific points on the chromosomes, presumably through the intermediary action of nuclear matrix proteins and nuclear membrane elements. A new pattern of gene expression results providing products that are available for interaction with constituents of the cell and surrounding medium. Although some general evidence supporting this theory has been presented (14, 15, 18), no specific cytoskeletal proteins have yet been shown to play a role in RT.

To identify which specific cAMP-induced phosphorylation reactions are part of RT, phosphorylation gels were prepared from transformed CHO-K1 cells, and untransformed CHO-180 cells after growth in the absence or presence of cAMP. Candidate proteins were sought that exhibited the following properties: (*i*) The protein must be phosphorylated in the absence of exogenous cAMP in normal fibroblast cells and must be phosphorylated to a lower level in transformed CHO-K1 cells. (*ii*) Addition of cAMP should not appreciably increase the phosphorylation of the protein in the untransformed cell but phosphorylation of the protein should increase in transformed cells. This paper presents evidence that vimentin fulfills these criteria and also displays other behaviors supporting its role as a specific cytoskeletal protein involved in RT.

MATERIALS AND METHODS

Cell Strains. CHO-K1 is a subclone of a spontaneously transformed Chinese hamster cell line that arose in a culture of fibroblasts isolated from an ovarian biopsy (19). CHO-180 is a fibroblast line also derived from a Chinese hamster ovary that displays normal morphology and growth characteristics (monolayer growth on cell surfaces, no growth in suspension, and higher serum requirements for growth) (12–15). It has never deviated from normal fibroblastic behavior except that it appears to grow almost indefinitely in tissue culture. Cells were grown in F12 medium supplemented with 5% (vol/vol) fetal calf serum for CHO-K1 cells and with 15% for CHO-180 cells. Other nontransformed Chinese hamster fibroblasts originating from brain (CHB), heart (CHH), and skin (CHS) have also been used as control cells for CHO-K1 and are grown in F12 medium supplemented with 15% fetal calf serum. Several other transformed cells possessing vimentin were examined: HeLa, human cervical carcinoma; HT1080, human fibrosarcoma; RFS, human fibrosarcoma; HL60 and U937, human leukemia cells (obtained from ATCC); and CHB IV, a transformed Chinese hamster brain cell. These cells were grown in F12 medium supplemented with 10% fetal calf serum. All cultures were incubated at 37°C in an atmosphere of high humidity and 5% CO_2/95% air.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: RT, reverse transformation; Bt_2cAMP, $N^6,O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate.
¶To whom reprint requests should be addressed.

Labeling Procedures. Approximately 7 × 10^4 cells were plated in 35-mm plastic dishes (Lux Scientific) and were grown overnight before drug treatment. 8-Bromo-adenosine 3′,5′-cyclic monophosphate (1 mM) or N^6,$O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate (Bt_2cAMP) (3 mM) was added for various periods of time. Cells were labeled with [^{32}P]orthophosphate (ICN) at 0.5–1.0 mCi/ml (1 Ci = 37 GBq) in phosphate-free F12 medium plus fetal calf serum, in the presence or absence of drugs.

Two-Dimensional Gel Electrophoresis. Proteins were analyzed by one- and two-dimensional gel electrophoresis. One-dimensional slab gel electrophoresis, with a 10% polyacrylamide resolving gel and a 3% polyacrylamide stacking gel, was performed according to Laemmli (20). Two-dimensional gel electrophoresis was carried out as described by Garrels (21) using a pH 5–8 gradient in the first dimension (isoelectric focusing) and a 10% polyacrylamide resolving gel in the second dimension (molecular weight). Equivalent amounts (0.5–1 × 10^6 cpm) of each sample were loaded. After the electrophoresis, the slab gels were fixed in 10–20% (wt/vol) trichloroacetic acid, rinsed in distilled water and then in 3% (vol/vol) glycerol, and dried by using a Bio-Rad slab gel drier. Autoradiography was carried out by exposing the gel to Kodak X-OMAT-R film at −70°C for various periods of time. In particular experiments, quantitative analysis of the phosphorylated products was performed either by cutting out the appropriate spots of the dried gels and measuring the radioactivity in these in a liquid scintillation spectrophotometer or by scanning the autoradiographic film in a computerized Bio-Rad microdensitometer to measure the peak area. One or more nonvariant proteins on each gel were selected as a reference standard. Where more rapid, semiquantitative comparisons were required, spot intensity was judged by eye and evaluated as 0, 1+, 2+, 3+, or 4+; where 0 means no spot was visible; 1+ was the smallest clearly definable deposit; 2+ was an intensity of 2–5 times greater than 1+; 3+ was an intensity 2–4 times greater than 2+; and 4+ was an intensity of 2 or more times greater than 3+. These visual assessments, although semi-quantitative, were highly reproducible. Western blotting was carried out following Towbin *et al.* (22).

Immunofluorescence Microscopy. Cells were plated on 4- or 8-chamber glass slides, allowed to grow overnight at 37°C in 5% CO_2/95% air, and then treated with the appropriate drugs. The slides were rinsed three times with cold isotonic phosphate-buffered saline (PBS) and fixed in 100% (vol/vol) methanol at −20°C for 10 min followed by fixing for 2 min in acetone at −20°C. The slides were then treated with appropriate amounts of primary monoclonal antibodies (anti-vimentin, anti-actin, or anti-tubulin) at 37°C for 1 hr. They were then rinsed with PBS and incubated with appropriate secondary antibodies conjugated with fluorescein or rhodamine for another hour. After rinsing with PBS, slides were treated with *p*-phenylenediamine (23) and examined.

EXPERIMENTAL RESULTS

Vimentin Phosphorylation. The most striking and reproducible change in cAMP-induced phosphorylation in CHO-K1 cells, which is not duplicated in CHO-180 cells, was in the phosphorylation of vimentin. Fig. 1 shows changes in the intensity of phosphorylation of the vimentin spot on a two-dimensional gel from transformed CHO-K1 cells and

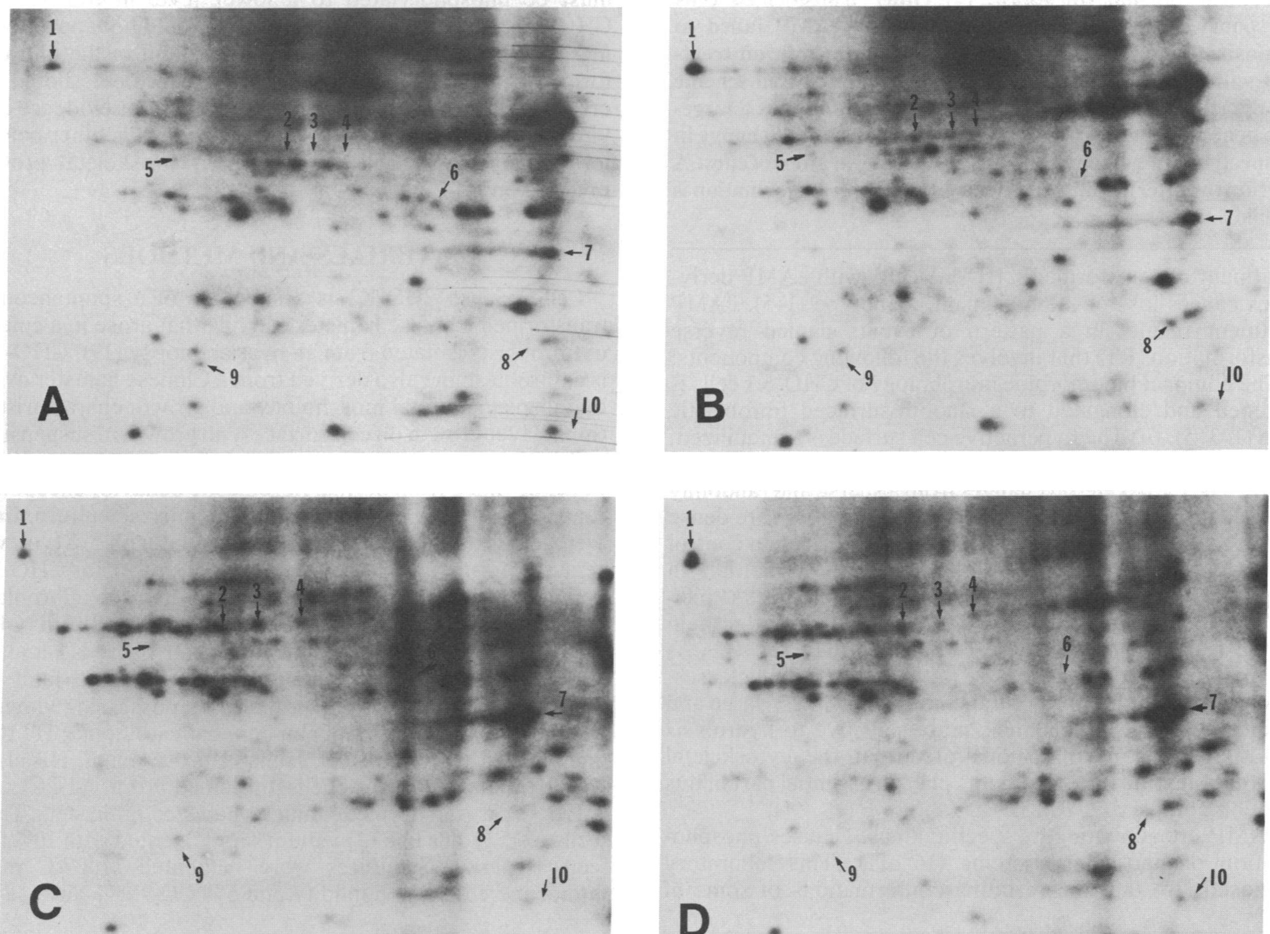

FIG. 1. Two-dimensional gel electrophoresis of ^{32}P-labeled proteins from CHO-K1 cells (*A*), CHO-K1 cells plus 3 mM Bt_2cAMP (*B*), CHO-180 untransformed fibroblasts (*C*), and CHO-180 cells plus 3 mM Bt_2cAMP (*D*). Spot 7 at 58 kDa with a pI value of 5.6 is vimentin.

Table 1. Comparison of vimentin phosphorylation in presence and absence of Bt_2cAMP in transformed CHO-K1 cells and untransformed CHO-180 fibroblasts

Cells	Relative phosphorylation − Bt_2cAMP	Relative phosphorylation + Bt_2cAMP
CHO-K1	1.0	2.8 ± 0.4
CHO-180	8.5 ± 0.6	8.8 ± 0.6

These results were reproducible in more than 20 experiments. Relative vimentin phosphorylation intensity is normalized with respect to the control CHO-K1 cells (mean ± SEM). Cells were preincubated with 3 mM Bt_2cAMP for 4 hr where indicated.

untransformed CHO-180 cells. In standard growth medium vimentin is clearly less phosphorylated in CHO-K1 cells than in nontransformed fibroblasts. Addition of cAMP to CHO-K1 cells for 4 hr markedly increases the phosphorylation of vimentin though still not to the extent in untreated normal cells. However, treatment of normal cells with cAMP produced no observable change in vimentin phosphorylation. These changes, which are readily visible to the eye, were confirmed by densitometric measurements on autoradiograms and by cutting out the corresponding spots on the gel itself and measuring the radioactivity in a scintillometer (Table 1). That the spot in question actually corresponds to vimentin was demonstrated by its molecular weight and isoelectric point and by the specific binding of antivimentin monoclonal antibodies only to this spot on Western blots (22).

Other normal Chinese hamster fibroblasts originating from brain, heart, and skin resembled CHO-180 cells in that they showed high vimentin phosphorylation that was identical in the presence or absence of added cAMP derivatives. Finally, when a series of human–CHO-K1 hybrids containing various human chromosomes was cultured in the absence of cAMP, all hybrids exhibiting a rounded compact morphology had low vimentin phosphorylation, whereas the one hybrid exhibiting a stretched and fibroblastic morphology had high vimentin phosphorylation.

Time Relationships. Various manifestations of the RT reaction occur at characteristic times from 15 min to 72 hr after addition of cAMP derivatives to CHO-K1 cultures (1–15). The increase in vimentin phosphorylation and the increase in the number of stretched cells at various times after addition of 5 mM Bt_2cAMP to a CHO-K1 cell culture were measured. The data of Fig. 2 indicate that vimentin phosphorylation increases within 15 min after the addition of cAMP; the initial slope is maximal and is maintained for more than an hour, after which the rate slows; and the peak is

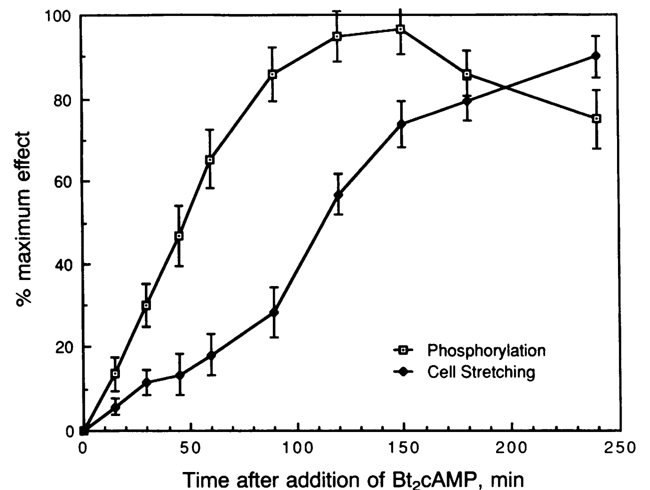

FIG. 2. Vimentin phosphorylation and cell stretching in CHO-K1 cells versus length of Bt_2cAMP treatment.

reached in 2 hr. However, the maximum rate of increase in percent of stretched cells is not achieved until 90 min; at that point the slope of the percent-stretched-cell curve is similar to the maximum slope of the phosphorylation curve; at 120 min the phosphorylation curve has peaked whereas the cell-stretching curve continues to increase beyond 180 min. These data indicate that the phosphorylation of vimentin cannot be a consequence of the cell-stretching reaction, of the gene exposure reaction, or of the reaction resulting in fibronectin deposition at the cell membrane, since all of these latter phenomena occur appreciably later than vimentin phosphorylation (1, 2, 7, 13, 14). Therefore, vimentin phosphorylation is one of the earliest manifestations of the RT reaction and could well be the cause of any or all of the subsequent phenomena in this complex of reactions (15).

Changes in Phosphorylation of Other Proteins. Table 2 shows other changes in as yet unidentified proteins whose phosphorylation changes are highly reproducible. The data suggest the proteins fall into three groups. Group A contains seven proteins. In CHO-K1 cells, group A proteins show cAMP-induced changes in phosphorylation that are, within the limits of the resolving power of these experiments, similar or identical to changes found in nontransformed CHO-180 fibroblasts. We tentatively conclude that these proteins are not directly responsible for the RT process. It is of interest that, although most of these proteins acquire new or increased phosphorylation in the presence of cAMP, proteins

Table 2. Changes in protein phosphorylation on addition of Bt_2cAMP to CHO-K1 and CHO-180 cells

Protein group	Spot	Molecular mass, kDa	pI value	Phosphorylation CHO-K1 Control	Phosphorylation CHO-K1 3 mM Bt_2cAMP	Phosphorylation CHO-180 Control	Phosphorylation CHO-180 3 mM Bt_2cAMP
A	1	97.4	6.8	+	++	+	++
	5	67	6.6	+	++	+	++
	6	61	5.9	+	−	+	−
	8	45	5.7	−	+	−	+
	9	41	6.5	+	−	+	−
	10	36	5.5	−	+	−	+
	12	25	5.8	−	+	−	+
B	2	70	6.35	−	+	+	+
	3	70	6.30	−	+	+	+
	4	70	6.20	−	+	+	+
	7*	58	5.6	+	++	+++	+++
C	11	32	6.55	+++	++	+	+

pI values are expressed as the mean, where the SEM is ± 0.2 (n = 10). Symbols are explained in *Materials and Methods*.
*Vimentin.

6 and 9 are phosphorylated in the absence of cAMP but lose this phosphorylation in the presence of the drug.

Group B contains four proteins, including vimentin, and constitutes a set of proteins whose phosphorylation in transformed CHO-K1 cells is increased in the presence of cAMP but in the untransformed CHO-180 fibroblasts shows no cAMP-induced change, within the resolving power of our methodology. Proteins 2, 3, and 4, showing a vimentin-like response, may well represent a homolog family. Group C contains a single member, C-11 (which turned out to be phosphorylated on tyrosine). Addition of cAMP to transformed CHO-K1 cells reduced the intensity of C-11 phosphorylation slightly, but there was no discernible effect on C-11 in untransformed CHO-180 cells.

Preexisting Vimentin Is Phosphorylated. CHO-K1 cells were treated with [^{32}P]orthophosphate and cycloheximide (20 μg/ml) for 1 hr before the addition of Bt_2cAMP. Under these conditions, protein synthesis falls to extremely low levels (24). The phosphorylation of vimentin in cells pretreated with cycloheximide was increased by cAMP addition to an extent indistinguishable from that in cells not treated with cycloheximide, implying that phosphorylation of preexisting vimentin is induced by cAMP. Earlier studies (24) demonstrated that cycloheximide does not inhibit cAMP-induced CHO-K1 cell stretching.

Cells treated with reagents disrupting microtubules (colchicine and griseofulvin) or microfilaments (cytochalasin B) to prevent the morphological changes and the gene exposure of RT (1, 2, 6, 14) failed to prevent the cAMP-induced increase in vimentin phosphorylation. These results suggest that the phosphorylation of vimentin represents a stage in RT that occurs earlier than steps, such as gene exposure and cell stretching, that are prevented by disruption of microtubular and microfibrillar elements (1, 6, 14).

Immunofluorescence Studies. Untransformed fibroblasts treated with fluorescent antibodies to vimentin in the indirect immunofluorescent procedure revealed a characteristic pattern of intermediate filaments traversing the entire cell length (Fig. 3*a*), as described by other investigators (25–30). These patterns were unchanged when these cells were treated with cAMP derivatives (Fig. 3*b*). When these untransformed cells were pretreated with colchicine in either the absence or presence of Bt_2cAMP, the intermediate filaments formed collapsed bundles with distinct ropy elements (Fig. 3 *c* and *d*).

In contrast, untreated transformed CHO-K1 cells lacked the characteristic intermediate filament structure of the normal fibroblast as shown by immunofluorescence with vimentin antibodies (Fig. 3*e*). Treatment of these cells with Bt_2cAMP before addition of the antibodies restored the typical intermediate filament pattern characteristic of normal fibroblasts, although the density of this filamentous pattern was somewhat less than that of the normal fibroblast (Fig. 3*f*). Moreover, in the absence of cAMP, colchicine had little effect on the vimentin immunofluorescence pattern (Fig. 3*g*). In the presence of Bt_2cAMP, colchicine again produced the typical collapsed ropy bundles of intermediate filaments (Fig. 3*h*). These data demonstrate that intermediate filaments are disrupted in transformed CHO-K1 cells as compared to normal fibroblasts, are intimately involved in the integrity of the microtubular system, and appear to require cAMP-

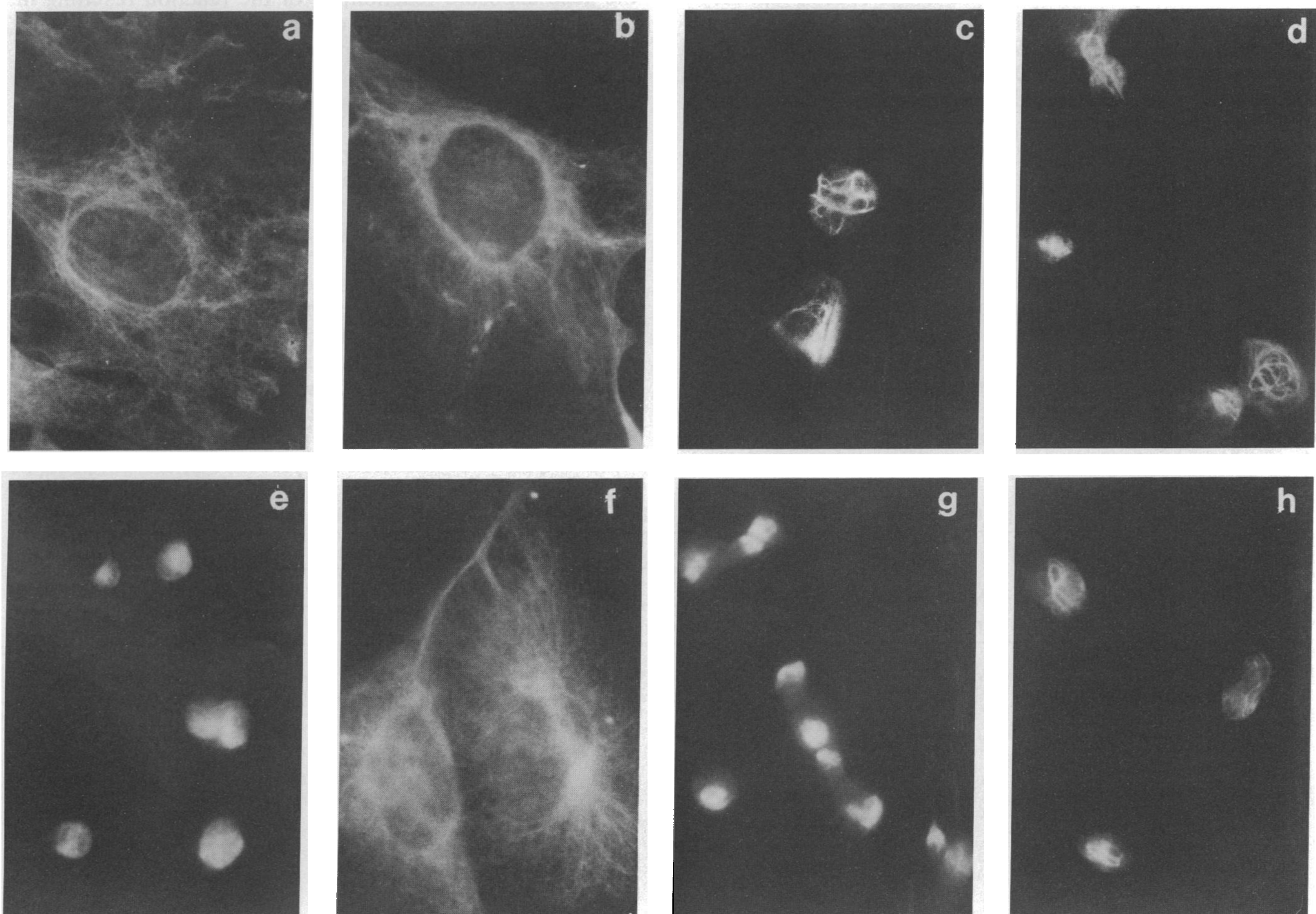

FIG. 3. (*a–d*) Indirect immunofluorescence of vimentin in CHO-180 cells. (*a*) Control. (*b*) Cells treated with 3 mM Bt_2cAMP. (*c*) Cells treated with colchicine. (*d*) Cells treated with Bt_2cAMP then with colchicine. (*e–h*) Indirect immunofluorescence of vimentin in CHO-K1 cells. (*e*) Control. (*f*) Cells treated with Bt_2cAMP. (*g*) Cells treated with colchicine. (*h*) Cells treated with Bt_2cAMP first and then with colchicine.

induced action to re-form the organized structures necessary for completion of the RT reaction pathway. They also show that the vimentin filaments of the reverse transformed cell are not quite as dense as those of the normal cell, so that RT may not lead to the complete recovery of the normal phenotype.

Behavior of Transformed Cells That Are Nonresponsive to cAMP. A series of vimentin-containing transformed cells (HeLa, HT1080, RFS, HL60, U937, and CHBIV) that fail to exhibit the typical response of CHO-K1 cells when treated with cAMP was identified and examined. None of these transformed cells showed any discernible increase in phosphorylation of vimentin when treated with cAMP. This finding supports the interpretation that these cells differ from CHO-K1 cells in the mechanism by which they were transformed.

DISCUSSION

Space does not permit discussion of the impressive literature by Lockwood and coworkers (4, 31, 32) and Gottesman and coworkers (5, 8), among others, on cAMP-induced protein phosphorylation on CHO and other cells. Although these papers include some studies on vimentin phosphorylation, none has implicated vimentin in the RT reaction. It is noteworthy that Ben-Ze'ev (33) and Fey *et al.* (34) found phosphorylation changes in vimentin in some transformed cells, though they did not relate these changes to cAMP effects. Our present work demonstrates that at least 12 proteins are affected by cAMP treatment. The most striking change found so far is that affecting vimentin. Three other 70-kDa proteins show vimentin-like changes and require further investigation.

Vimentin phosphorylation by cAMP could be the primary, or one of the primary, reactions of the RT complex: It begins along with the earliest group of changes (i.e., within minutes) after addition of cAMP derivatives, whereas other aspects of the reaction complex are not observed until much later (1–15). Previous studies had demonstrated (1, 6, 14) a definitive role only for the microtubules and the microfilaments in the complex of reactions associated with RT. The current experiments demonstrate that the integrity of all three elements of the cytoskeleton is involved in this process.

This laboratory has postulated (14, 15) that RT consists of a series of reactions initiated by phosphorylation of one or more cytoskeletal components that produces an altered conformation followed by reorganization of the cytoskeleton and by a consequent change in the binding pattern of various associated molecules to specific sites on the chromosomes, resulting in a different pattern of gene sequestration and exposure. The present studies indicate that vimentin fits the postulated role of such an initiator molecule. The fact that other transformed cells that fail to respond to cAMP by RT also do not exhibit a cAMP-induced increase in vimentin phosphorylation further supports this interpretation. The report of Georgatos and Blobel (35) showing vimentin attachment to the nuclear membrane is of interest in this connection.

The function of vimentin in cells of mesenchymal origin has been a mystery (18, 28–30, 36). We have demonstrated that the cell cytoskeleton is an essential component of the gene regulatory mechanism initiated by cAMP in CHO-K1 cells. Phosphorylation of vimentin may be one of the earliest reactions in this pathway. These experiments demonstrating that the intermediate filaments as well as the microtubules and microfilaments are involved in the RT reaction suggest as a working hypothesis that the intermediate filaments in other tissues play similar roles in gene regulation (and presumably differentiation) as that demonstrated here in the CHO fibroblast. We propose that desmin, the cytokeratins, and the glial and neuronal filamentous proteins play roles in gene regulation in muscle, epithelial, glial, and neuronal cells, respectively, analogous to that of vimentin in fibroblasts, but each of the different types of intermediate filaments may well regulate a gene set that may be different in different cell types (18, 28, 29).

This work was supported by the Lucille P. Markey Charitable Trust and Grant HD 02080 from the National Institutes of Health. T.T.P. is an American Cancer Society Professor.

1. Hsie, A. W. & Puck, T. T. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 358–361.
2. Hsie, A. W., Jones, C. & Puck, T. T. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 1648–1652.
3. Pastan, I. & Willingham, M. (1978) *Nature (London)* **274**, 645–650.
4. Bloom, G. S. & Lockwood, A. H. (1980) *J. Supramol. Struct.* **14**, 241–254.
5. LeCam, A., Nicolas, J. C., Singh, T. J., Cabral, F., Pastan, I. & Gottesman, M. M. (1981) *Proc. Natl. Acad. Sci. USA* **256**, 933–941.
6. Puck, T. T. (1971) *Proc. Natl. Acad. Sci. USA* **74**, 4491–4495.
7. Nielson, S. E. & Puck, T. T. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 985–989.
8. LeCam, A., Gottesman, M. M. & Pastan, I. (1980) *J. Biol. Chem.* **255**, 8103–8108.
9. Porter, K., Puck, T. T., Hsie, A. W. & Kelley, D. (1974) *Cell* **2**, 145–158.
10. Meek, W. D. & Puck, T. T. (1979) *J. Supramol. Struct.* **12**, 335–354.
11. Gabrielson, E. G., Scoggin, C. & Puck, T. T. (1982) *Exp. Cell Res.* **142**, 63–68.
12. Rumsby, G. & Puck, T. T. (1982) *J. Cell. Physiol.* **111**, 133–139.
13. Schonberg, S., Patterson, D. & Puck, T. T. (1983) *Exp. Cell Res.* **145**, 57–62.
14. Ashall, F. & Puck, T. T. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5145–5149.
15. Ashall, F., Sullivan, N. & Puck, T. T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3908–3912.
16. Krebs, E. G. & Beavo, J. A. (1979) *Annu. Rev. Biochem.* **48**, 923–959.
17. Lincoln, T. M. & Corbin, J. D. (1978) *J. Cyclic Nucleotide Res.* **44**, 3–14.
18. Steinert, P. M. & Roop, D. R. (1988) *Annu. Rev. Biochem.* **57**, 593–625.
19. Tjio, J. H. & Puck, T. T. (1958) *J. Exp. Med.* **108**, 259–268.
20. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
21. Garrels, J. I. (1979) *J. Biol. Chem.* **254**, 7961–7977.
22. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
23. Platt, J. L. & Michael, A. F. (1983) *J. Histochem. Cytochem.* **31**, 840–842.
24. Patterson, D. & Waldren, C. A. (1973) *Biochem. Biophys. Res. Commun.* **50**, 566–573.
25. Lin, J. C. & Feramisco, J. R. (1981) *Cell* **24**, 185–193.
26. Klymkowsky, M. W. (1981) *Nature (London)* **291**, 249–251.
27. Gawlitta, W., Osborn, M. & Weber, K. (1981) *Eur. J. Cell Biol.* **26**, 83–90.
28. Osborn, M. & Weber, K. (1983) *Lab. Invest.* **48**, 372–394.
29. Traub, P. (1985) *Intermediate Filaments: A Review* (Springer, Berlin).
30. Wang, E., Fischman, D., Liem, R. & Sun, T. T., eds. (1985) *Ann. N.Y. Acad. Sci.* **455**.
31. Lockwood, A. H., Trivette, D. D. & Pendergast, M. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **46**, 909–919.
32. Bloom, G. S. & Lockwood, A. H. (1980) *Exp. Cell Res.* **129**, 31–45.
33. Ben-Ze'ev, A. (1983) *J. Cell Biol.* **97**, 858–865.
34. Fey, S. J., Larsen, P. M. & Celis, J. E. (1983) *FEBS Lett.* **157**, 165–169.
35. Georgatos, S. D. & Blobel, G. (1987) *J. Cell Biol.* **105**, 105–125.
36. Lazarides, E. (1982) *Annu. Rev. Biochem.* **51**, 219–250.