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Specific Protein Phosphorylation During Cyclic AMP-Mediated Morphological Reversion of Transformed Cells

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Treatment of transformed Chinese hamster ovary cells with dibutyryl cAMP or other agents that elevate cAMP results in the acquisition of growth and morphology characteristic of normal fibroblasts. The role of specific protein phosphorylation in this process of morphological reversion has been examined using metabolic labelling of Chinese hamster ovary (CHO) cells with ^{32}P-orthophosphate in the presence or absence of $N^6O^{2'}$-dibutyryladenosine 3′:5′-cyclic monophosphoric acid (Bt_2cAMP). Analysis of labelled cultures by SDS gel electrophoresis and radioautography demonstrate dramatic changes in the phosphorylation of only 2 cellular proteins during reverse transformation. A 55,000 dalton protein (pp55) was phosphorylated and a 20,000 dalton protein (pp20) was dephosphorylated. The time course of these events was consistent with the kinetics of morphological reversion. The lower molecular weight species, pp20, was dephosphorylated within 15–30 minutes, prior to all morphological changes except membrane tranquilization. The higher molecular weight protein, pp55, was maximally phosphorylated over 1–2 hours following addition of Bt_2cAMP, paralleling early stages in the establishment of fibroblastic form. The phosphorylated forms of pp20 and pp55 were both extracted from cellular cytoskeletons by 0.5% Triton X-100, but analysis of ^{35}S-methionine-labelled cultures suggested that unphosphorylated pp 20 may be bound to the cytoskeleton. Since pp20 was found to comigrate with the 20,000 dalton myosin light chain, it is possible that dephosphorylation of CHO cell myosin induced by cAMP may alter its interaction with actin microfilaments and modulate the assembly of stress fibers during morphological reversion.

Key words: cytoskeletons, cell growth, protein kinase, morphology, cyclic AMP, phosphorylation, transformation

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The oncogenic transformation of fibroblasts by chemicals, viruses, or spontaneously has received a great deal of attention in an attempt to understand the molecular mechanisms that mediate the conversion of a normal cell to a cancer cell. Transformed fibroblasts are altered in both growth and morphology [1]. They usually grow to higher saturation densities, escape from contact inhibition and substrate dependence, and have reduced serum requirements [2]. Among the morphological changes seen are a rounded shape, altered membrane topography with numerous zeiotic blebs and microvilli, enhanced agglutinability by lectins, a disorganized culture morphology [3], and a reduction in directed motility. Various cytoskeletal and exoskeletal changes have been reported, including a reduction in microfilament bundles [4], altered stability and amount of microtubules [5], and a reduction in fibronectin [6]. Despite many advances, the molecular basis for these changes in growth, shape, and motility remains obscure.

One model for studying the control of fibroblast transformation is reverse transformation (morphological reversion) [7, 8]. In some lines of transformed fibroblasts, including certain SV40 virus-transformed 3T3 cells [9], L929 cells [10], and Chinese hamster ovary (CHO) cells [11], treatment with cyclic AMP (cAMP) or agents that elevate intracellular cAMP results in the acquisition of a normal fibroblastic phenotype. The changes induced by cAMP include many features crucial to cell morphology and growth. Cells become oriented and polarized. Stress fibers and lamellar cytoplasm increase [12, 13], as does substrate adhesion. Blebs and microvilli disappear and agglutinability is reduced. Cells grow more slowly [14, 15]. In some cases, contact inhibition is restored. There is an increase in assembled microtubules [5,16–18], and the appearance of prominent microfilament bundles [13].

These pleiotropic effects of cAMP on transformed cells suggest that this molecule may be important in the regulation of cell transformation. Some transformed lines have lower cAMP levels than their normal counterparts [19]. Some normal cells show an increase in cAMP at high density or contact inhibition [20]. Furthermore, the cAMP level of several cell lines decreases during mitosis. Since mitotic cells have increased agglutinability, rounded shape, and other features of the transformed phenotype, parallels have been drawn between the mitotic cell and the transformed cell [21].

By what mechanism does cyclic AMP exert its varied effects on the growth and morphology of the transformed cell? The only defined mode of cAMP action in eukaryotes is the activation of cAMP-dependent protein kinases [22, 23]. As best defined in certain hormone-responsive tissues where cAMP serves as a second messenger [24], the activation of a cAMP-dependent kinase results in the phosphorylation of specific target proteins. Phosphorylation alters the activity of the protein, and, often through a cascade of events, the hormonal response is evoked. Studies with S49 lymphoma cell mutants have demonstrated that cAMP-dependent protein kinase also mediates the effects of cAMP on growth [25]. The growth of mutant cells that lack the kinase is no longer inhibited by cAMP. Recently, a few mutants of CHO defective in cAMP-dependent kinase have been isolated [26]. Cyclic AMP does not evoke a morphological response in these cells. Hence, the genetic evidence is that both morphological and proliferative alterations of reverse transformation are mediated by cAMP-dependent protein kinases.

The regulation of cell growth, morphology, and the expression of the transformed phenotype by protein phosphorylation is not restricted to cAMP-induced reverse transformation. Following infection of fibroblasts by RNA tumor viruses such as Rous sarcoma, rapid changes in morphology and growth occur (for a review see [2]). Studies with transformation-defective, temperature-sensitive mutants demonstrate that a single gene (src) is responsible

for expression of the transformed phenotype [27]. The product of this gene is a 60,000 dalton protein kinase [28]. Hence, in both cAMP-mediated reverse transformation, and RNA tumor virus-mediated transformation, the diverse alterations of morphology and growth may be initiated by the action of protein kinases that phosphorylate specific cellular proteins.

What are these target proteins? Until recently, no identification of specific cellular phosphoproteins that are altered during reverse transformation had been made. The identification and characterization of such proteins would provide new insight into the mechanism by which cAMP-dependent phosphorylation exerts its pleiotropic effects on the growth and morphology of transformed cells. In this paper we present studies on CHO cells metabolically labelled with radioactive phosphate in the presence or absence of agents that elevate intracellular cyclic AMP. We demonstrate that reverse transformation is accompanied by major changes in the phosphorylation of only a very few cellular proteins, and we suggest that these proteins may mediate the effects of cAMP on the growth and morphology of transformed cells.

METHODS

Cell Culture

CHO-K1A cells were maintained in plastic cultureware in F-10 nutrient mixture, supplemented with 10% calf or fetal calf serum (all from GIBCO). To induce reverse transformation, N^6, $O^{2'}$-dibutyryl adenosine 3′:5′-cyclic monophosphoric acid (Bt_2cAMP) and testosterone propionate (both from Sigma) were added to the medium at final concentrations of 1 mM and 15 μM, respectively, from concentrated stock solutions.

Metabolic Labelling

Cells were labelled for 10 min with ^{32}P-orthophosphate (New England Nuclear) at a final concentration of 1–4 mCi/ml in conditioned F-10 that contained 5% the usual phosphate concentration plus 10% serum. Newly synthesized proteins were detected in cells labelled with ^{35}S-methionine (New England Nuclear) at a final concentration of 2–10 μCi/ml for 10 min or 3 h in conditioned MEM, containing 5% the normal methionine concentration plus 10% serum.

Fractionation of Cells

Cytoskeletons were isolated from metabolically labelled cells by extraction for 5 min at room temperature with 100 mM PIPES, pH 6.9, 1 mM EGTA, 1 mM $MgCl_2$, 10 mM TAME, and 0.5% Triton X-100. The detergent-resistant cytoskeletons were collected in 1X SDS sample buffer [29]. The detergent soluble material was clarified by a 3-min spin in an Eppendorf microcentrifuge and mixed with 1/10 volume of 10X SDS gel sample buffer [29].

To separate cytoplasm, membranes, and nuclei, ^{32}P-labelled cells were suspended in 15 mM PIPES, pH 6.8, 10 mM TAME, 15 mM sodium pyrophosphate, 10 mM NaF, 1 mM $CaCl_2$, and 1 mM PMSF. After Dounce homogenization, the nuclei were pelleted at 1100g for 3 min. The nuclei were then cleaned of contaminating membranes by 2 washes in the isolation buffer containing 0.5% Triton X-100, and dissolved in 1X SDS sample buffer. The 1100g supernatant, which contained cytoplasm and membranes, was spun at 4°C at 100,000g for 1 h. The membrane pellet was dissolved in 1X SDS sample buffer and the cyotplasmic supernatant was mixed with 1/10 volume of 10X sample buffer.

SDS Gel Electrophoresis and Radioautography

A Tris-glycine-SDS slab gel system [29] was used for 7%–12% acrylamide gradients. For 15% gels, the identical buffers were used, but the acrylamide:bis ratio was doubled [30]. Before electrophoresis, 2-mercaptoethanol was added to all samples to 1/10 volume, and the samples were boiled. The TCA-insoluble radioactivity was determined by adsorption on GF/C filters (Whatman), and approximately 20,000 cpm of ^{32}P or 200,000 cpm of ^{35}S were applied per well. Marker proteins and their sources were as follows: skeletal muscle mysoin heavy chain and actin (Sigma); BSA and E coli RNA polymerase (Bochringer Mannheim); neurotubulin, prepared as described [31]; myosin light chain, gift of Dr. Robert Adelstein. After electrophoresis, gels were stained with coomassie brilliant blue R, destained, photographed, dried onto filter paper, and mounted against Kodak XR-2 X-ray film for 1–7 days. Following development, some radioautographs were scanned with a Joyce-Loebel microdensitometer. For quantitation, each scan was cut out and weighed. Then, the peaks of interest were cut out, weighed, and compared to the whole scan weight. Corrections were made for peak overlap.

Microscopy

For the time courses, living cells were photographed on a Leitz Diavert Microscope through a 20X, 0.32 NA phase objective on Tri-X film. For indirect immunofluorescence, we isolated cytoskeletons as described here and stained them with anti-myosin or anti-tubulin as we have reported [13]. Rhoadamine-labelled IgG fraction of goat anti-rabbit IgG (Cappel) was used as the fluroescent second antibody. Fluorescence and phase contrast micrographs were taken on Tri-X by a Leitz Orthomat camera, mounted on a Leitz Ortholux II microscope, equipped with Zeiss 63X, 1.4 NA and Leitz 25X, 0.75 NA phase-fluorescence objectives.

For scanning electron microscopy, cells were grown on plastic coverslips for 16 h in the presence or absence of Bt_2cAMP. The cells were then fixed for 24 h at 4°C in PBS containing 2% glutaraldehyde, and postfixed in 2% OsO_4 in PBS for 1 h at room temperature. Following dehydration in increasing concentrations of ethanol, the cells were critical-point dried in CO_2 from 100% ethanol. The cells were shadowed by platinum-palladium evaporation, and viewed in a JEOL JSM35 scanning electron microscope. Photographs were taken on 4″ × 5″ Kodak Tri-X film.

RESULTS

The morphological reversion of CHO cells by cAMP is illustrated in the scanning electron and phase contrast micrographs of Figure 1. There is a rapid tranquilization of the membrane involving a loss of surface blebs and microvilli. The cells become flattened, and polarized, and they develop an extensive array of stress fibers. CHO-K1A, the clone used in our laboratory, also shows a reduction in growth rate and contact inhibition of growth in response to cAMP (not shown). Since the known effect of cAMP in eukaryotic cells occurs via the activation of protein kinases, CHO cells were metabolically labelled with ^{32}P-orthophosphate to identify cellular proteins phosphorylated during Bt_2cAMP-induced morphological reversion. Following SDS-polyacrylamide gel electrophoresis and radioautography, two altered phosphoproteins were identified in Bt_2cAMP-treated cells. A protein of 55,000 daltons (pp55) was phosphorylated, while a protein of 20,000 daltons (pp20) was dephosphorylated (Fig. 2). No major changes in either the phosphorylation or synthesis of other cellular proteins were detected. A comparison

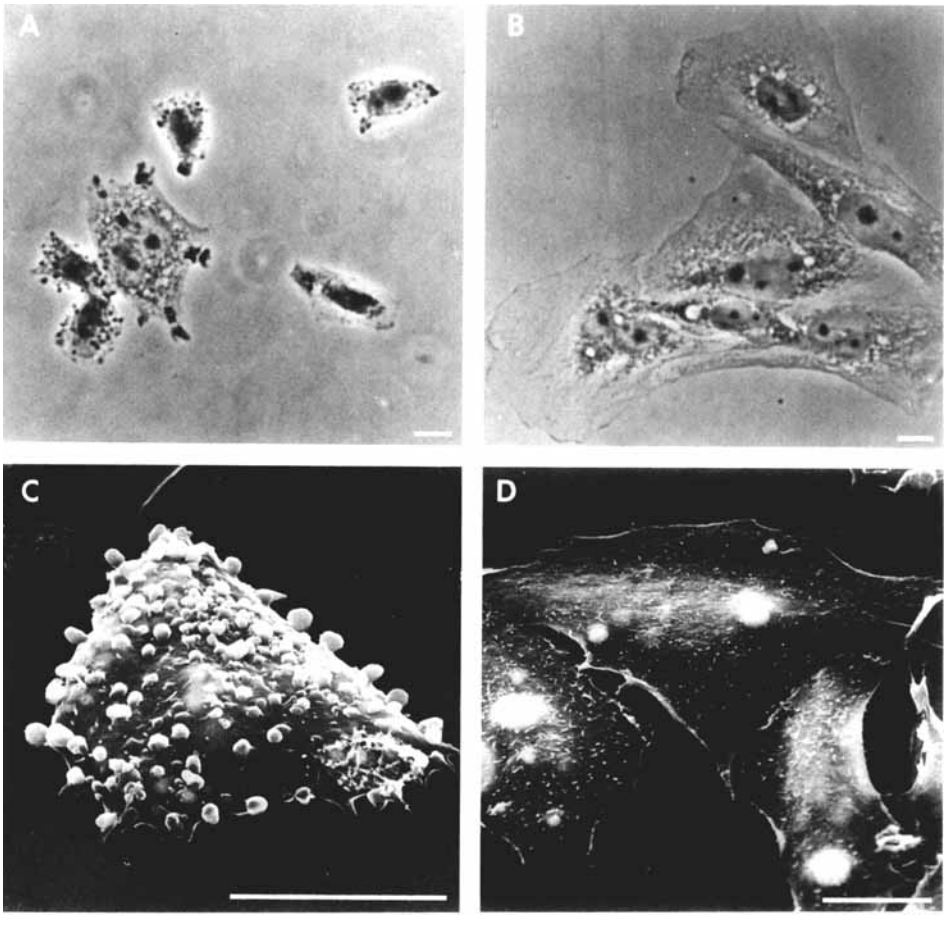

Fig. 1. CHO cells viewed by phase contrast (A and B) and scanning electron (C and D) microscopy. (A) and (C) untreated cells. (B) and (D) cells treated with Bt_2cAMP for 36 h. Bars equals 10 μm.

with proteins of known molecular weight indicated that pp20 comigrates with the 20,000 dalton myosin light chain from smooth muscle, while pp55 migrates between α- and β-tubulin (Fig. 3).

The progression of events in morphological reversion occurs over 8 h (Fig. 4A). After addition of Bt_2cAMP, blebs are rapidly lost (15 min–1 h). This is followed by the appearance of actomyosin bundles visible in phase contrast or immunofluorescence (1–3 h) and the polarization of the cells to a fibroblastoid morphology (3–5 h). Some further shape change occurs up to 6–8 h. Examination of the phosphorylation of pp55 during this period showed that the phosphorylation increases during the first 2 h of morphological reversion to a level that is maintained as long as Bt_2cAMP is present (Fig. 4B). The other protein, pp20, is dephosphorylated within 30 min after the addition of Bt_2cAMP. For unknown reasons, it is slowly rephosphorylated even in the continued presence of the cyclic nucleotide (Fig. 4B).

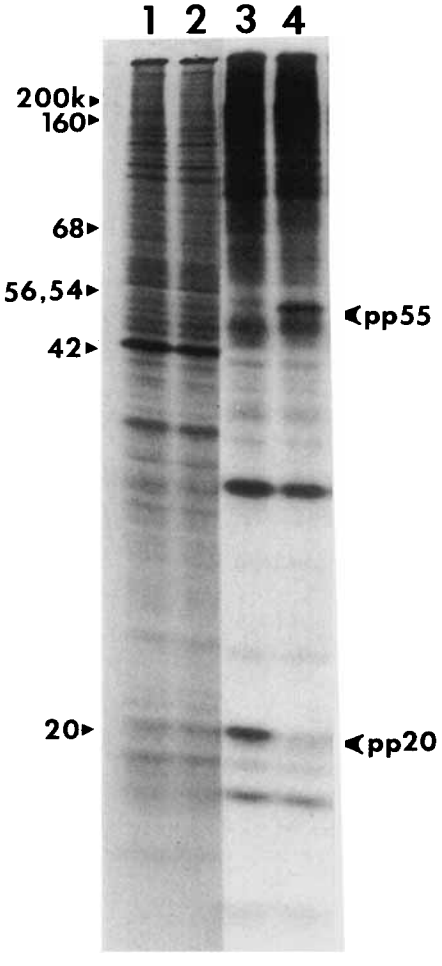

Fig. 2. Changes in cellular phosphoproteins accompany reverse transformation by Bt_2cAMP: 15% acrylamide gel radioautograph. Cells were labelled for 10 min with ^{35}S-methionine (lanes 1 and 2) or ^{32}P-orthophosphate (lanes 3 and 4). Controls: 1 and 3; Bt_2cAMP-treated for 2 h: 2 and 4. Molecular weights in kilodaltons of selected marker proteins (see Fig. 3) are at the left side of the gel.

When Bt_2cAMP is removed from the medium, intracellular cAMP levels fall rapidly and cells at once begin to revert to their original transformed morphology (Figure 5A). This loss of the fibroblastic phenotype is accompanied by dephosphorylation of pp55 and phosphorylation of pp20 (Fig. 5B). The time course is such that escape from morphological reversion precedes dephosphorylation of pp55 but is accompanied by phosphorylation of pp20 (Figure 5B). Hence, phosphorylation and dephosphorylation of these 2 phosphoproteins occurs in roughly the same temporal sequence as the morphological event of reverse transformation. A graphical representation of these changes is shown in Figure 6 using data obtained from quantitative densitometry of radioautographs. Because of the brief (10 min) labelling

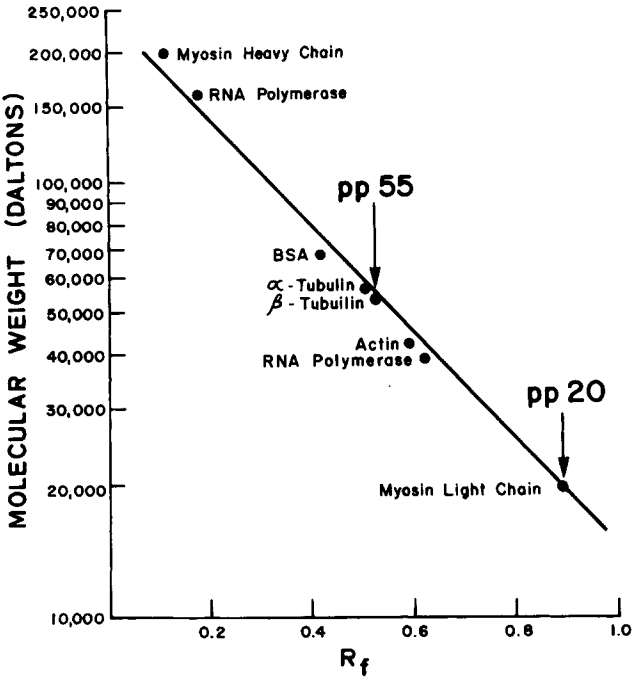

Fig. 3. Molecular weight determination of pp20 and pp55 on 7%–12% acrylamide gradient gel. Marker protein: myosin heavy and light chains (200,000 and 20,000 daltons); E coli RNA polymerase (160,000 and 39,000 daltons); BSA (68,000 daltons); actin (42,000 daltons); α- and β-tubulin (56,000 and 54,000 daltons).

times used in these experiments, it was possible that changes in specific activity of protein bound phosphate due to nonequilibration of cellular ATP pools could account for the alterations in pp20 and pp55. This is unlikely since the vast majority of cellular phosphoproteins show no change in apparent phosphorylation. Also, recent experiments in which cells are labelled to equilibrium with $^{32}PO_4$ before the addition of cAMP give the same changes in pp20 and pp55 observed with short labelling times (not shown).

The morphological manifestations of reverse transformation are very likely a consequence of changes in the cytoskeleton and possibly in membrane adhesion sites. Consequently, we have determined whether either pp55 or pp20 is physically associated with detergent-resistant cytoskeletons. Such cytoskeletons were prepared in a buffer that preserves microtubules, microfilament bundles, intermediate filaments and the nucleus (Figure 7A, B, C), but extracts most membrane and soluble proteins. When such cytoskeletons from Bt_2cAMP-treated cells metabolically labelled with ^{32}P-orthophosphate were analyzed, we found that the phosphorylated forms of both pp55 and pp20 were extracted and not retained in the cytoskeleton. However, a substantial fraction of the ^{35}S-methione-labelled protein that comigrates with pp20 was cytoskeletal.

In further subcellular fractionation studies, the distribution of pp55 and pp20 in membrane, nucleus, and cytoplasm was determined (Table I). Phosphorylated pp20 was entirely cytoplasmic, but about 35% of phsophorylated pp55 was membrane bound. This membrane-

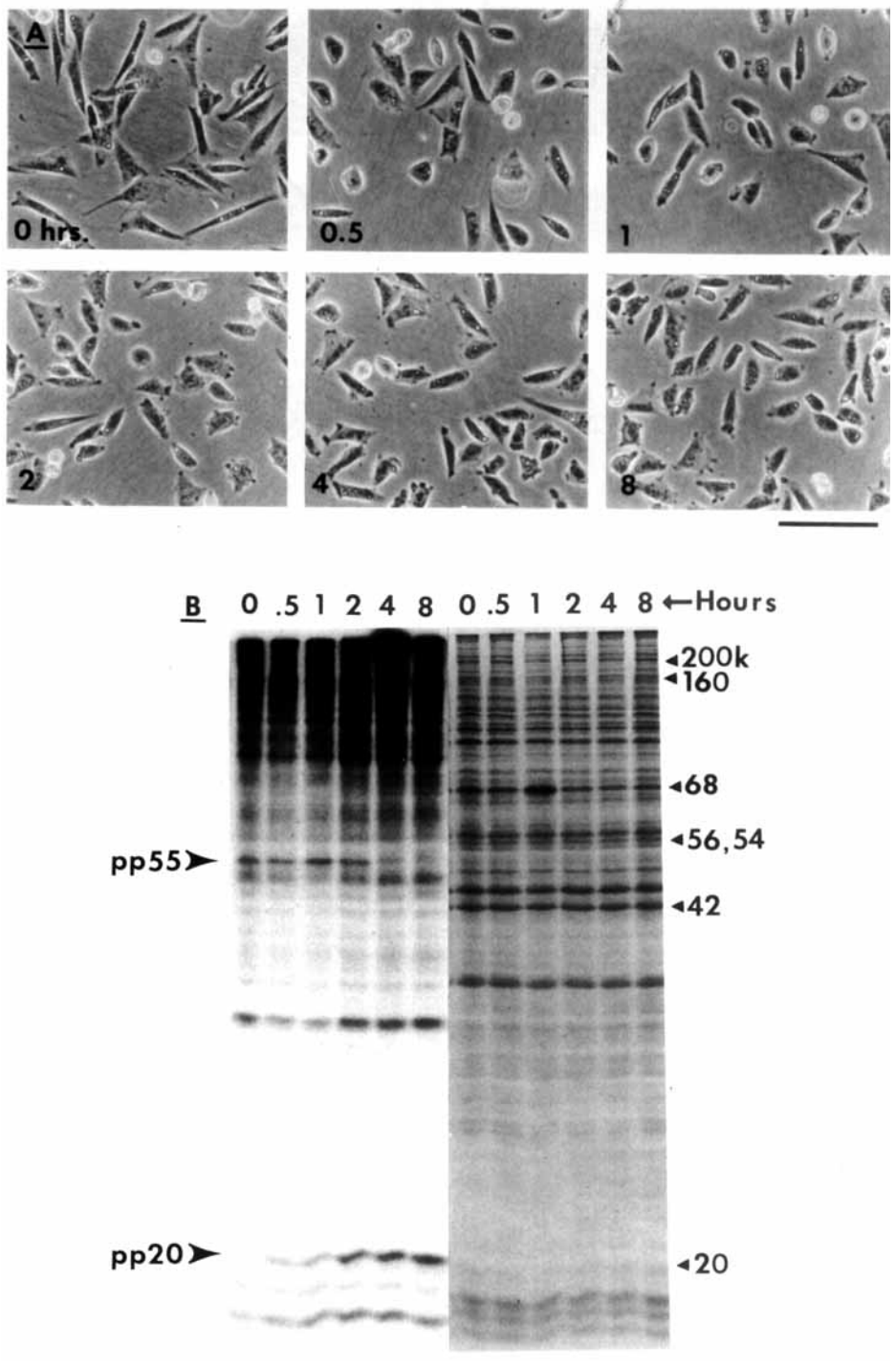

Fig. 4. Phosphorylation levels of pp20 and pp55 correlate with morphological reversion. A) phase contrast time course of living cells undergoing morphological reversion. Bar equals 100 μm. B) phosphorylation time course of the same cells; 15% gel. Left side is ^{32}P radioautograph; right side shows the coomassie blue pattern of the same lanes. See Fig. 3 for a description of molecular weight markers.

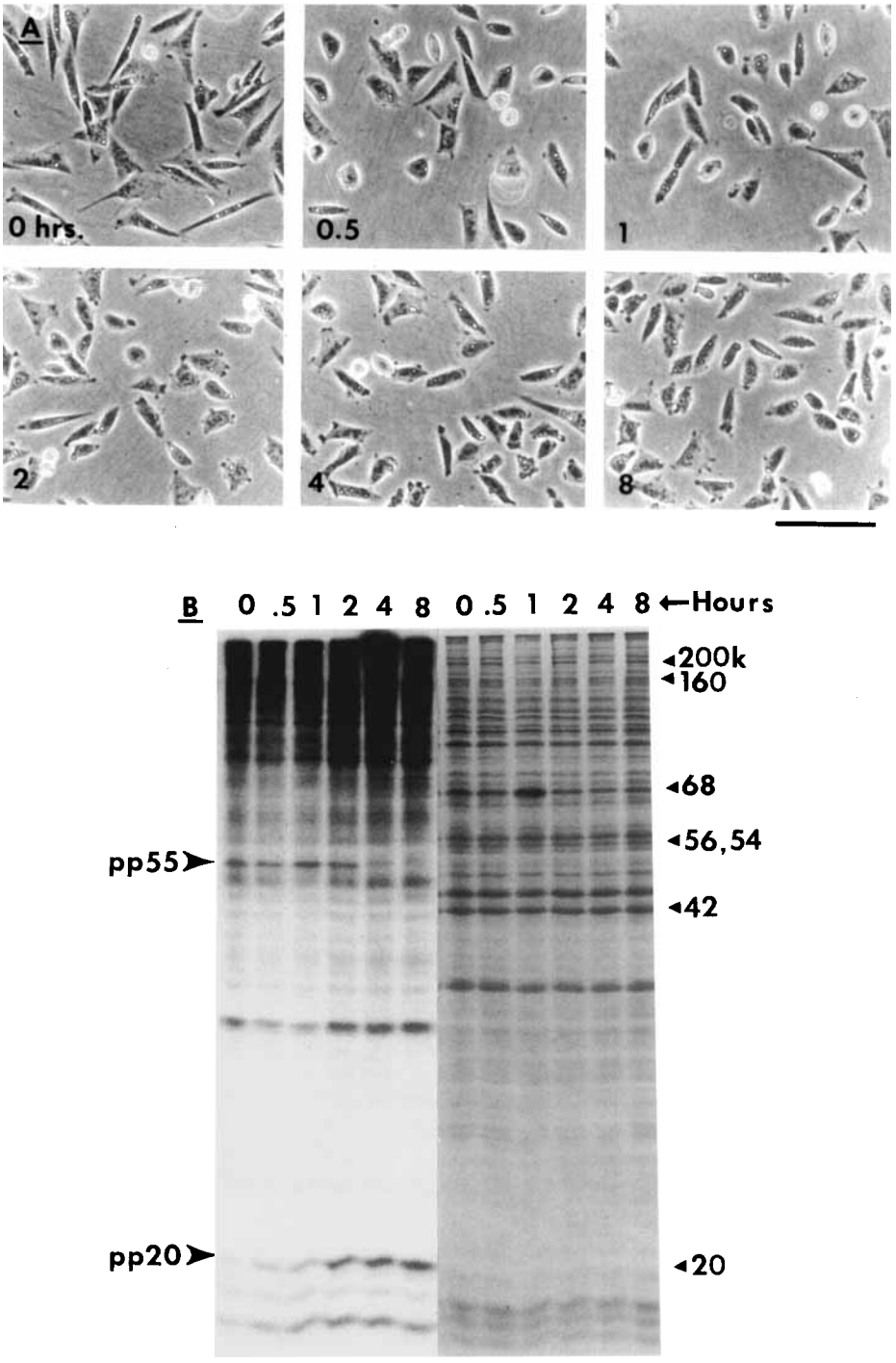

Fig. 5. Morphological reversion and cAMP effects on pp20 and pp55 are fully reversible. Cells were treated with Bt_2cAMP for 3 h and transferred to conditioned basal medium. A) phase contrast micrographs of living cells returning to epithelioid morphology. Bar equals 10 μm. B) phosphorylation time course of the same cells; 15% gel. Left side is ^{32}P radioautograph, right side shows coomassie blue patterns of the same samples. See Fig. 3 for a description of molecular weight markers.

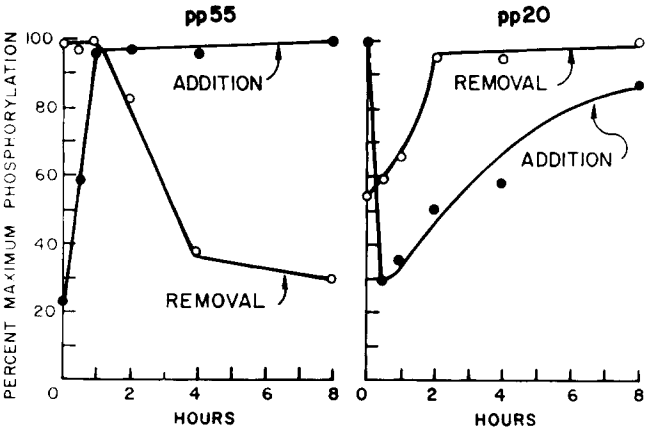

Fig. 6. Quantitative changes in levels of pp55 and pp20 following addition or removal of Bt_2cAMP. The data were acquired by quantitative densitometry (see Methods) of the radioautographs shown in Fig. 4 and 5.

bound pp55 is probably not exposed on the external surface of the cells since both pp20 and pp55 are completely resistant to trypsin in intact cells, although they are digested by proteases in cell homogenates.

In an attempt to correlate pp20 and pp55 with either growth or morphology during reverse transformation, we examined the effect of various inhibitors on their phosphorylation. Both actinomysin D and cycloheximide abolished the cAMP-dependent phosphorylation of pp55 but did not affect the dephosphorylation of pp20 (Table II). Neither inhibitor prevented morphological reversion. Hence, it is unlikely that pp55 mediates the morphological effect of cAMP on CHO cells. Since macromolecular synthesis is required for the cAMP effect on growth, pp55 may be involved in this event. Inhibitors of microtubule or microfilament function abolish the morphological reversion. They do not, however, interfere with either the phosphorylation of pp55 or the dephosphorylation of pp20 (Table II). Thus the integrity of the cytoskeleton is not essential for the cAMP effect on pp20 or pp55.

DISCUSSION

In many cell types, cyclic AMP has been implicated as a regulator of cell growth, transformation, and morphology [8, 32]. Fluctuations in intracellular cAMP often accompany the transition from growth to quiescence; from the interphase portion of the cell cycle to mitosis; and from normal to malignant growth. The general trend, particularly in fibroblasts, is that cyclic AMP levels are reduced in the replicating, transformed, or mitotic cell and elevated in the quiescent, normal, or interphase cell. In some cases, corresponding fluctuations in cyclic nucleotide-dependent protein kinase activity occurs.

Certain transformed cell lines show direct morphological and growth responses to cyclic AMP, thus providing a cellular model for the action of the nucleotide. As an approach to understanding the molecular basis for the pleiotropic effect of cAMP on cellular growth and form, we have begun to identify cellular proteins whose state of phsophorylation changes

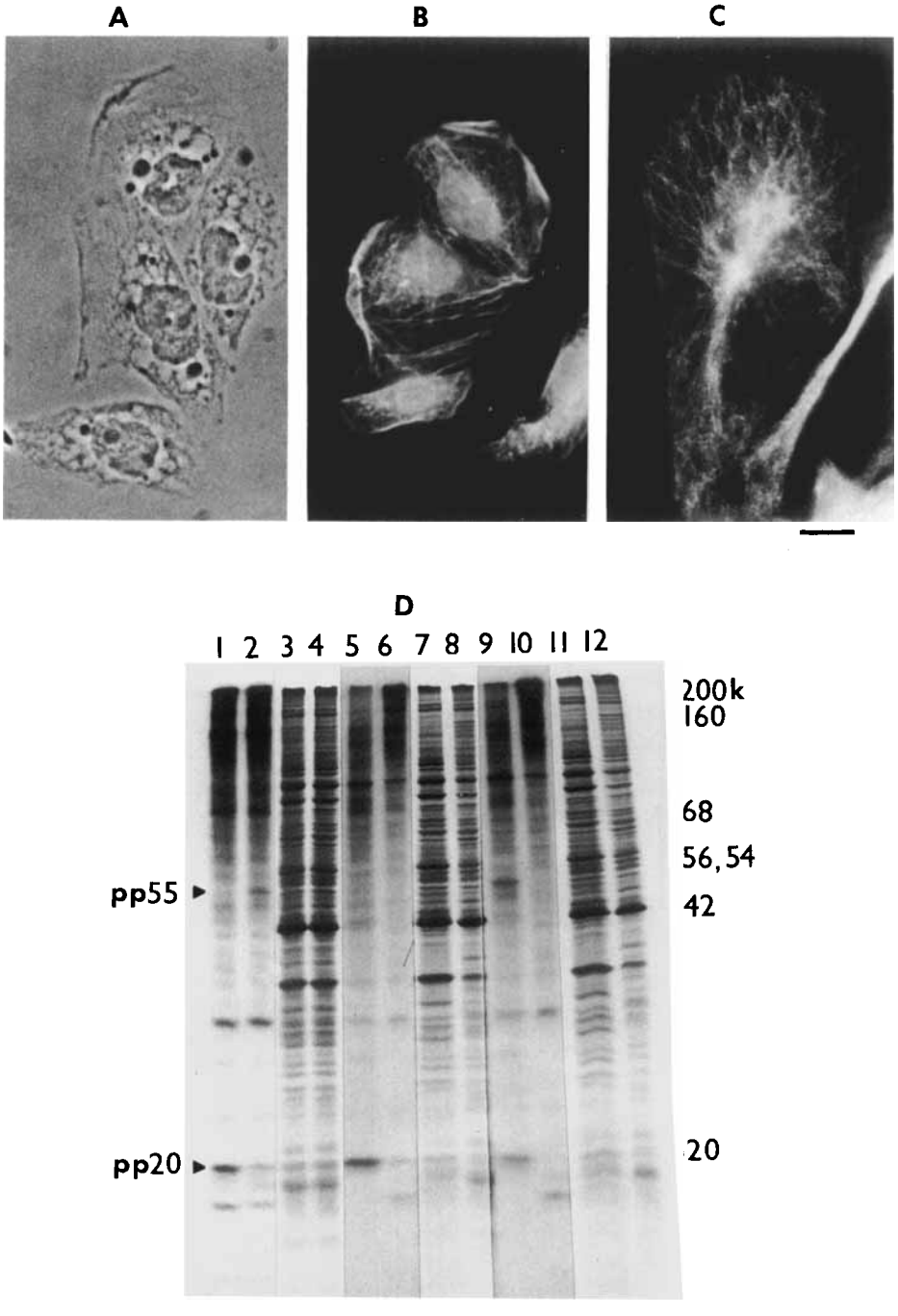

Fig. 7. Distribution of pp20 and pp55 in isolated cytoskeletons and detergent extracts. A) phase contrast micrograph of isolated cytoskeleton. B and C) Immunofluorescence micrographs of isolated cytoskeletons stained with anti-myosin in (B) or anti-tubulin (C). Bar equals 10 μm. D) Radioautographs of 15% gel. Cells were labelled for 10 min with ^{32}P (lanes 1 and 2, 5 and 6, 9 and 10) or ^{35}S (lanes 3 and 4, 7 and 8, 11 and 12). The first 4 lanes show whole cells (1 and 3: controls; 2 and 4: treated for 3 h with Bt_2cAMP). Lanes 5–8 show fractions from control cells (5 and 7: detergent soluble; 6 and 8: cytoskeletons). The last 4 lanes are fractions from reverse transformed cells (9 and 11: detergent soluble; 10 and 12: cytoskeletons).

TABLE 1. Subcellular Distribution of pp20 and pp55

Fraction[a]	%Total pp20	%Total pp55
Cytoplasm	100%	49%
Membrane	0%	34%
Nucleus	0%	17%

[a]Cells were fractionated as described in Methods. The proportions of pp20 of pp20 and pp55 in each fraction were determined by a combination of liquid scintillation counting and quantitative densitometry of SDS gel radioautographs.

TABLE II. Effects of Biosynthetic and Cytoskeletal Inhibitors on pp20 and pp55 in CHO Cells Treated With Bt_2cAMP*

Inhibitor	Morphological reversion	pp20 Dephosphorylation	pp55 Phosphorylation
None	+	+	+
Actinomycin D (1 μg/ml)	+	+	–
Cycloheximide (20 μg/ml)	+	+	–
Colcemid (1 μM)	–	+	+
Vinblastine (0.1 μM)	–	+	+
Cytochalasin B (1 μg/ml)	–	+	+

*All inhibitors were present for the duration of the experiment. Bt_2cAMP was added 1 h after actinomycin D or cycloheximide. Cells were labelled 1 and 4 h later with ^{32}P. The cytoskeletal inhibitors were added either 2h before Bt_2cAMP or at the same time, the results being indistinguishable. Cells were labelled 3 h after Bt_2cAMP addition. Morphological reversion was assayed by phase contrast microscopy of the living cells, and phosphorylation was determined by SDS gel radioautography.

during reverse transformation of CHO cells by cAMP. Since cAMP exerts its biological effects through the activation of protein kinases, we feel it is reasonable to assume that the substrates of the cAMP-dependent kinases will be the molecular effectors of reverse transformation. To date, we have identified two phosphoproteins that undergo major changes during reverse transformation. Metabolic-labelling studies with $^{32}PO_4$ indicate that a 55,000 MW protein is phosphorylated while a 20,000 MW protein is dephosphorylated. Although we are inclined to attribute these events to altered protein kinase activity (discussed below), effects on protein phosphatase activity or on turnover of the phosophoproteins cannot at present be ruled out. The dephosphorylation of pp20 following Bt_2cAMP addition begins

immediately and is complete within 15–30 min. Thus, this event precedes all the earliest morphological events except membrane tranquilization. Phosphorylation of pp55 proceeds with much slower kinetics and is not complete until 1–2 h after the onset of morphological reversion. Such a time course might indicate that only newly synthesized pp55 is a substrate for the protein kinase. Consistent with this notion is the observation that protein synthesis is requred for pp55 phosphorylation. At present, we do not know the identity or function of pp55, although we believe it may be involved in growth regulation.

We feel that the shape changes of morphological reversion are determined in large measure by alterations in the cytoskeleton (together, perhaps, with an increase in membrane-substratum adhesiveness). Immunofluorescent and electron microscopic studies in our laboratory have demonstrated that during reverse transformation there is a major rearrangement of actin and myosin from a diffuse cytoplasmic form to a structured distribution along stress fibers [13]. There is also an increase in the cytoplasmic microtubule array [18]. Consequently, cytoskeletal proteins or proteins that modulate cytoskeletal assembly are likely targets for the action of protein kinases that affect morphology. A further, dramatic indication of such a relationship is our recent observation that antibodies to the catalytic and regulatory subunits of cAMP-dependent protein kinases show specific localization patterns on the actomyosin and cytoplasmic microtubule networks in interphase cells and on the mitotic spindle during mitosis [33, 39]. Consequently, we have been particularly concerned with the relationship of pp55 and pp20 to the cytoskeleton. In this context, it is of interest that reverse transformation of CHO cells by cAMP brings about the dephosphorylation of pp20. Obviously, when thinking about cAMP-induced dephosphorylation, other considerations apply besides activation of cAMP-dependent protein kinases. There is an intimate relation between cAMP and intracellular calcium [34], and there is a class of protein kinases activated by calcium through its interaction with the ubiquitous protein, calmodulin (CDR). One such enzyme is myosin light chain kinase, an enzyme that transfers phosphate to the 20,000 dalton subunit of myosin [35]. Interestingly, this calcium-activated kinase is believed to be under negative regulation by a cAMP-dependent protein kinase [36]. Such "yin-yang" effects could conceivably account for the dephosphorylation of pp20. Our studies indicate that pp20 comigrates with this myosin light chain. Should it be confirmed that pp20 is a myosin light chain, it then becomes of considerable interest that the unphosphorylated species of pp20 remains associated with detergent-resistant cytoskeletons (Fig. 7). By this model, phosphorylated myosin, present in CHO cells, would interact primarily with single actin microfilaments, eg, on membranes. Dephosphorylation of myosin, as a consequence of elevated intracellular cAMP, would induce myosin to cross-link actin filaments into bundles, or to bind preferentially to microfilament bundles preformed by the phosphorylation of other actin binding proteins, eg, filamin [37]. Interestingly, Hoar et al [38] have shown that phosphorylation of the 20,000 myosin light chain produces tension in smooth muscle fibers. This observation correlates nicely with our suggestion that phosphorylated myosin in untreated CHO cells interacts with actin filaments primarily for the generation of motile force rather than the establishment of a rigid cytoskeleton. We know that such a redistribution of myosin from a diffuse to a stress fiber-associated state occurs [13]. It remains to be determined whether differential dephosphorylation of the myosin light chain during morphological reversion is responsible for the redistribution of myosin and the formation of a rigid actomyosin cytoskeleton that establishes fibroblastic morphology in transformed cells exposed to cyclic AMP.

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