CYCLIC AMP-INDUCED MORPHOLOGICAL TRANSFORMATION OF CELLS INFECTED BY TEMPERATURE-SENSITIVE MOUSE SARCOMA VIRUS

Expression of Transformation-Associated Markers

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ABSTRACT

Normal rat kidney (NRK) cells infected with a temperature-sensitive (ts) mutant of mouse sarcoma virus (NRK[MSV-1b]) express the transformed phenotype under permissive conditions, but acquire the normal phenotype when grown under restrictive conditions. Addition of 3′,5′ cyclic adenosine monophosphate (cAMP) to NRK(MSV-1b) cells grown at the restrictive temperature results in morphological transformation. To determine whether other markers associated with the transformed phenotype were coordinately expressed after cAMP exposure, concanavalin A (Con A) agglutinability, hexose transport rate, and incorporation of radioactively labeled fucose into fucolipid III and fucolipid IV (FL III and FL IV) of the cells were examined. NRK cells transformed by wild-type MSV or NRK(MSV-1b) grown under permissive conditions were agglutinated by low concentrations of Con A and exhibited relatively high maximal agglutination levels which were specifically inhibited by α-methyl-D-mannoside. In contrast, NRK(MSV-1b) cells grown under restrictive conditions were weakly agglutinated by Con A and exhibited reduced maximal agglutination levels, similar to uninfected NRK cells. Treatment of NRK(MSV-1b) cells at the restrictive temperature with cAMP resulted in morphological transformation and a change in the pattern of incorporation of labeled fucose into FL III and FL IV to one comparable to that of NRK(MSV-1b) cells at the permissive temperature or to NRK cells transformed by wild-type MSV. In contrast, cAMP treatment resulted in no increase in Con A agglutinability or 2-deoxy-D-[^{3}H]glucose transport relative to mock treated cultures. The results demonstrate that cAMP-induced morphological transformation and altered fucolipid composition of NRK(MSV-1b) cells are not correlated with alterations in hexose transport rate or Con A agglutinability.

We have recently reported (29) that 3′,5′ cyclic adenosine monophosphate (cAMP) affects the morphological transformation of normal rat kidney (NRK) cells transformed by a temperature-sensitive (ts) mutant of mouse sarcoma virus (MSV). Expression of the transformed phenotype

by cells grown at the permissive temperature is associated with increased levels of endogenous cAMP. Correspondingly, exogenous addition of cAMP or analogues of cAMP resulted in morphological transformation of cells infected by the ts mutant of MSV at the restrictive temperature. These findings demonstrated that intracellular cAMP levels directly influenced the morphological expression of transformation, in contrast to various reports which have shown an inverse relationship between cAMP levels and abnormal growth and morphological properties of virus-transformed cells (reviewed in references 20 and 35). Therefore, it was of interest to determine whether other markers associated with the transformed phenotype were coordinately expressed in the ts MSV-transformed cells after cAMP exposure. Previous studies have demonstrated alterations in hexose transport rate and fucolipid composition at the permissive temperature that paralleled changes observed with wild-type MSV-transformed NRK cells (17, 31). Our findings indicate that two markers of transformation – agglutinability by concanavalin A and hexose transport rate – are not correlated with cAMP-induced morphological transformation, whereas an alteration in the incorporation of radioactive fucose into fucolipids appears to parallel cAMP-induced morphological transformation.

MATERIALS AND METHODS

Cells

The cell lines used in this study have been described previously (27, 28) and included (*a*) NRK cells, (*b*) NRK cells transformed and productively infected with Moloney sarcoma-leukemia virus, NRK(MSV-MLV), (*c*) NRK cells transformed by a clonal isolate of MSV (NRK[MSV-1]), and (*d*) NRK cells transformed by a cold-sensitive mutant of MSV (NRK[MSV-1b]). The NRK(MSV-1b) cells express the transformed phenotype at the permissive temperature (39°C) but appear phenotypically normal at the restrictive temperature (33°C). Cell lines were grown in Eagle's minimal essential medium (MEM; Auto Pow, Flow Laboratories, Rockville, Md.) supplemented with 10% fetal calf serum. Cells were exposed to 0.4 mM 8-bromo cyclic AMP (8BrcAMP; ICN Pharmaceuticals, Inc., Irvine, Calif.) without replacing the growth medium as previously described (29). For morphological studies, cells were seeded at 5–8 × 10^5 per 60-mm Petri dish, incubated for 2 days, exposed to 8BrcAMP, and examined with a Zeiss phase-contrast microscope.

Concanavalin A (Con A) Agglutination

Falcon plastic flasks (75 cm^2; Falcon Plastics, Div. of BioQuest, Cockeysville, Md.) were seeded with 2–3 × 10^6 cells and used for agglutination studies 2–3 days later when the cells were subconfluent. Cell lines were tested for agglutinability as described by Burger (3). Briefly, the cells were rinsed five times *in situ* with Ca^{++} – and Mg^{++} – free phosphate-buffered saline (CMF-PBS) and five times with CMF-PBS containing 0.20 g of ethylenediaminetetracetic acid (EDTA) per liter prewarmed at 37°C. The cells were then incubated for 10 min at 37°C in CMF-PBS containing EDTA. Under these conditions, the cells readily detach from the flasks or are easily removed and clumps dispersed by gentle trituration. The cell suspension was centrifuged (450 *g*, 3 min), resuspended in 3 ml of CMF-PBS, recentrifuged, and eventually suspended and adjusted to 2 × 10^6 cells/ml in CMF-PBS, using an electronic Coulter Counter (Coulter Electronics Inc., Hialeah, Fla.). Equal volumes (0.3 ml) of cell suspension and Con A (Miles Laboratories Inc., Kankakee, Ill.) dilutions were mixed in a Falcon #3008 Multiwell tissue culture plate to give final Con A concentrations of 0, 2, 8, 30, 60, 125, and 250 μg/ml. Plates were incubated at room temperature for 20 min on a rotary platform, and agglutination was scored microscopically as the percentage of cells trapped in aggregates of three or more cells. Control experiments to demonstrate specificity of Con A agglutination contained 0.1 M α-methyl-D-mannoside (Sigma Chemical Co., St. Louis, Mo.).

Hexose Transport

The rate of sugar uptake was determined by using 2-deoxy-D-[^{3}H]glucose (specific activity 10.0 Ci/mmol, New England Nuclear, Boston, Mass.) as previously described (17). Briefly, cells were seeded at 8 × 10^5 cells per 60-mm tissue culture dish and assayed for 2-deoxy-D-[^{3}H]glucose transport 1 day later. Cell cultures were washed three times with glucose-free Hanks' balanced salt solution (HBSS), prewarmed to 39°C, and labeled at 39°C for 10 min in 2 ml of glucose-free HBSS containing 0.25 μC/ml 2-deoxy-D-[^{3}H]glucose. Cultures were then washed three times with ice-cold glucose-free HBSS; the cells were scraped off with a rubber policeman into 0.5 ml of water, transferred to a small tube, and dispersed in a vortex mixer. An aliquot (0.1 ml) was mixed with 0.6 ml of NCS solubilizer (Amersham/Searle Corp., Arlington Heights, Ill.), toluene liquifluor added, and radioactivity determined in a Beckman LS-250 scintillation counter (Beckman Instruments, Inc., Silver Springs, Md.). The rate of sugar uptake was expressed as uptake of radioactivity per milligram protein in 10 min at 39°C. Protein was measured by the method of Lowry et al. (16).

Fucolipid Analysis

NRK, NRK(MSV-MLV), or NRK(MSV-1b) cells were seeded at 2–3 × 10^6 cells in plastic flasks (75 cm^2) at the appropriate temperature and utilized after 24 h. After the addition of 0.4 mM 8BrcAMP or of buffer and

[^{14}C]fucose (5 μCi/ml; 56.2 mCi/mmol; Amersham/Searle), the cells were incubated for an additional 24-h period. The monolayers were washed three times with PBS and dislodged by scraping. The scraped cell suspension was centrifuged at 1,500 *g* for 5 min, the pellet washed four times in cold PBS, and the lipid extracted in $CHCl_3$-CH_3OH as previously described (6). The lipid extract was taken to dryness under N_2, resuspended in $CHCl_3$-CH_3OH-pyridine-H_2O (15:6:4:1.5, by vol) and chromatographed in one dimension on silica gel thin-layer plates (Q-5, Quantum Industries, Fairfield, N. J.) sequentially in $CHCl_3$-CH_3OH-H_2O (60:35:8, by vol), followed by 2-propanol-NH_4OH (sp gr 88)-H_2O (7:2:1, by vol). The plates were scraped in 1-cm bands from origin to solvent front, and the radioactivity was measured by scintillation spectrometry. Fucolipids III (FL III) and IV (FL IV) are quantitatively located between fractions 5 and 10.

RESULTS

Effect of cAMP on Cell Morphology

The effect of treatment with 0.4 mM 8BrcAMP on cellular morphology is shown in Figs. 1 and 2. NRK(MSV-1b) cells grown at 33°C (Fig. 1*a*) appear flattened, elongated, and poorly refractile. After exposure to 8BrcAMP, the cells became highly refractile and polygonal to rounded in shape (Fig. 1*b*), characteristic of NRK(MSV-1b) cells grown at 39°C—the temperature permissive for expression of morphological transformation (Fig. 1*c*). Treatment of NRK(MSV-1b) cells grown at 39°C with 8BrcAMP resulted in a slight augmentation of cell rounding (Fig. 1*d*). These morphological alterations were obvious within 10 min after exposure to 0.4 mM 8BrcAMP, achieved maximum expression by 1 h, and resulted in morphological alterations of 100% of the NRK(MSV-1b) cells at 33°C.

Removal of the 8BrcAMP-containing medium and incubation of the cells in growth medium resulted in flattening and elongation of cells within 3 h (Fig. 1*e*), indicating that the 8BrcAMP-induced morphological alterations of NRK(MSV-1b) cells at 33°C were completely reversible and not simply a toxic response to the cAMP analogue. Further evidence for the lack of toxicity of 0.4 mM 8BrcAMP was obtained by comparing growth rates of NRK cells cultured in 0.4 mM 8BrcAMP-supplemented medium relative to control growth medium. The only detectable inhibitory effect of the 8BrcAMP-medium was to reduce the final saturation density of NRK cells by approximately 30%. The lack of growth inhibition by 8BrcAMP medium could not be attributed to degradation of 8BrcAMP by cAMP phosphodiesterase in the serum, because medium, removed from NRK dishes treated up to 3 days with 8BrcAMP, was capable of causing morphological transformation of NRK(MSV-1b) cells at 33°C.

As reported in an earlier analysis (29), treatment of NRK or NRK(MSV-MLV) cells grown at 37°C with 0.4 mM 8BrcAMP did not alter the morphological properties of these cells (Fig. 2*a*–*d*). Furthermore, 8BrcAMP treatment of NRK or NRK(MSV-MLV) cells grown at either 33°C or 39°C did not result in morphological changes.

Con A Agglutinability of Cells and Effect of cAMP

Fig. 3 presents agglutination dose-response curves of NRK, NRK(MSV-MLV), and NRK(MSV-1b) cells grown at the permissive or restrictive temperature for maintenance of transformation. NRK cells transformed by wild-type MSV-MLV were readily agglutinated by low concentrations of Con A, had a relatively high level of spontaneous agglutination, and exhibited maximal agglutination levels of approximately 65%. In contrast, uninfected NRK cells were weakly agglutinated by Con A with a maximal agglutination level of about 28%. NRK (MSV-1b) cells at the permissive temperature of 39°C exhibited agglutination dose-reponse curves similar to NRK(MSV-MLV) cells at lower Con A concentrations, but the extent of agglutination obtained with high lectin concentrations was about 45%. Con A agglutinability of phenotypically normal NRK(MSV-1b) cells grown at the restrictive temperature of 33°C closely resembled that of the uninfected NRK cell line. Specificity studies demonstrated that agglutinability by Con A was specifically inhibited by 0.1 M α-methyl-D-mannoside over a wide range of lectin concentrations (Fig. 3). In preliminary experiments with wheat germ agglutinin, we have observed that normal and transformed cells have agglutination levels similar to those reported here for Con A.

Fig. 3 demonstrates that, for all cell lines, the degree of agglutination was positively correlated with the Con A concentration up to a plateau level of maximal agglutination. This observation permits one to compare maximal agglutination levels of cell lines under various experimental conditions. Similar methods of expressing agglutination have been reported by Glimelius et al. (8). We have determined experimentally that maximal agglutination occurred at a Con A concentration of

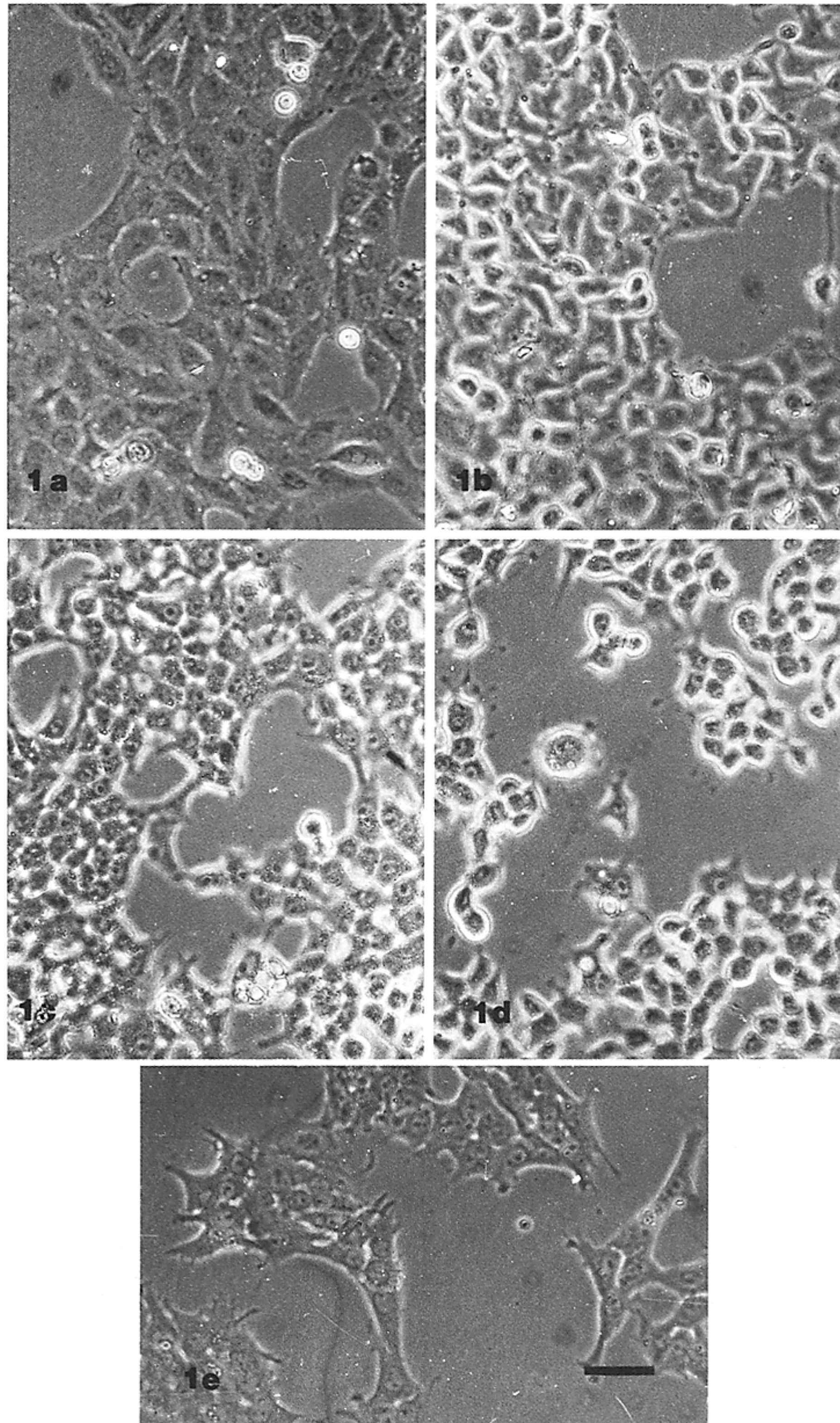

FIGURE 1 Effect of 8BrcAMP on the morphology of NRK(MSV-1b) cells. Cells were seeded at 5–8 × 10^5 per 60-mm Falcon tissue culture dish, incubated for 2 days at either 33 or 39°C, and treated for 2 h with 0.4 mM 8BrcAMP or mock treated without replacing the growth medium. NRK(MSV-1b) cells grown at (*a*) 33°C without 8BrcAMP treatment, (*b*) 33°C with 0.4 mM 8BrcAMP treatment, (*c*) 39°C without 8BrcAMP treatment, (*d*) 39°C with 0.4 mM 8BrcAMP treatment, and (*e*) treated as in *b*, and reversed for 3 h by replacing the medium with growth medium. Phase-contrast. Bar, 16 μm. × 614.

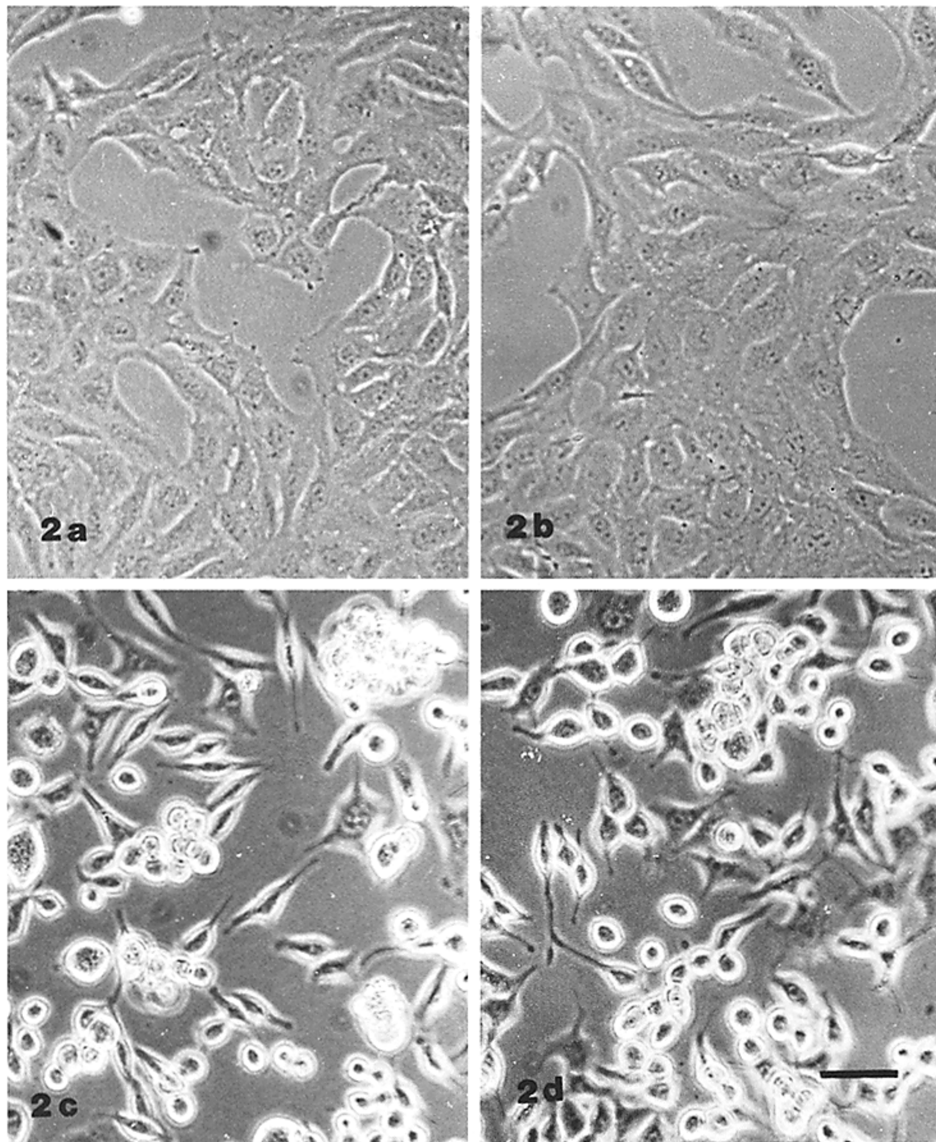

FIGURE 2 Effect of 8BrcAMP on the morphology of NRK and NRK(MSV-MLV) cells. Cultures were seeded at 5–8 × 10^5 per 60-mm Falcon tissue culture dish, incubated for 2 days at 36°C, and treated for 2 h with 0.4 mM 8BrcAMP or mock treated without replacing the growth medium. NRK cells grown (*a*) without 8BrcAMP treatment, and (*b*) with 0.4 mM 8BrcAMP treatment. NRK(MSV-MLV) cells grown (*c*) without 8BrcAMP treatment, and (*d*) with 0.4 mM 8BrcAMP treatment. Phase-contrast. Bar, 16 μm. × 614.

125 μg/ml under standard conditions of constant cell density, temperature, and incubation time. Therefore, for routine purposes of comparison, the extent of agglutination estimated by 125 μg of Con A/ml represents maximal Con A-induced cell agglutination.

To determine whether cAMP-mediated morphological transformation was associated with an enhanced degree of Con A agglutination, NRK(MSV-1b) cells grown at 33 or 39°C were exposed to 0.4 mM 8BrcAMP and then assayed for Con A agglutination. The results presented in Table I demonstrate that 8BrcAMP-induced morphological transformation of NRK(MSV-1b) cells

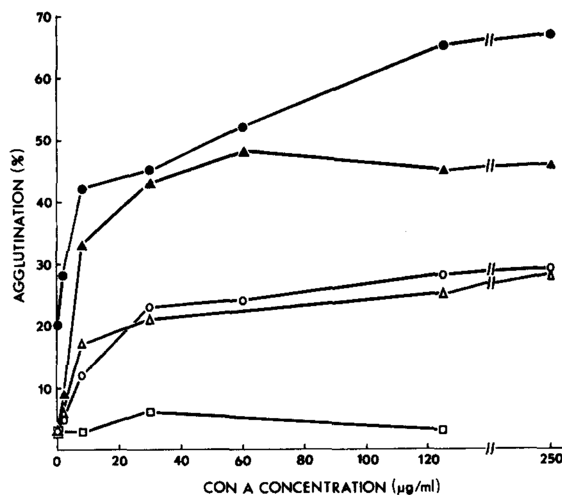

FIGURE 3 Effect of Con A concentration on the agglutination of NRK and MSV-transformed NRK cells. Falcon plastic flasks (75 cm^2) were seeded with 2–3 × 10^6 cells and used for agglutination studies 2–3 days later when the cells were subconfluent. Agglutinability of (●—●), NRK(MSV-MLV) cells; (○—○), NRK cells; (▲—▲), NRK(MSV-1b) cells grown at 39°C; (△—△), NRK(MSV-1b) cells grown at 33°C. (□—□), agglutination in the presence of 0.1 M α-methyl-D-mannoside of NRK(MSV-1b) cells grown at 39°C.

at 33°C was not associated with increased levels of maximal Con A agglutination. Treatment of NRK(MSV-1b) cells at 39°C with 0.4 mM 8BrcAMP resulted in minimal morphological changes and no alteration in the levels of Con A agglutination. The degree of Con A agglutination was not affected by 8BrcAMP exposure times, which varied in separate experiments from 2 to 16 h. Treatment of NRK and NRK(MSV-MLV) with 0.4 mM 8BrcAMP neither induced morphological changes nor altered the levels of Con A agglutination (Table I). In additional experiments, we have observed no morphological changes or significantly altered levels of Con A agglutination in 8BrcAMP or mock treated NRK or NRK(MSV-MLV) cells, regardless of whether the cells were grown at 33, 36, or 39°C (data not shown), indicating that growth temperature per se did not determine the agglutinability of cells by Con A. The results demonstrate that cAMP does not affect Con A agglutinability, in spite of its effects on the morphology of NRK(MSV-1b) cells grown at the restrictive temperature.

Effect of cAMP on 2-Deoxy-D-[^{3}H]Glucose Transport of Cells

The rate of 2-deoxy-D-[^{3}H]glucose transport was measured in 8BrcAMP or mock treated NRK(MSV-1b) cells grown at 33 or 39°C, and in control cultures of uninfected NRK and transformed NRK(MSV-1) cells (Table II). As reported previously (17), uptake of 2-deoxy-D-glucose is enhanced four- to sixfold in untreated, morphologically transformed cell lines, NRK(MSV-1) and NRK(MSV-1b) at 39°C, but only slightly increased in NRK(MSV-1b) at 33°C, relative to uninfected NRK cells. Treatment of NRK(MSV-1b) cells grown at 33°C with 0.4 mM 8BrcAMP for 2 h resulted in morphological transformation, but uptake of 2-deoxy-D-glucose was unchanged relative to mock treated cultures (Table II). In control experiments, 8BrcAMP treatment of NRK(MSV-1b) grown at 39°C and NRK(MSV-1) and NRK cells grown at 36°C resulted in no significant changes in 2-deoxy-D-glucose transport. In additional experiments, 2-deoxy-D-[^{3}H]glucose transport of NRK and NRK(MSV-MLV) was unaffected by growth of the cells at 33, 36, or 39°C. These results demonstrate that 8BrcAMP-induced morphological

TABLE I

Agglutination by Concanavalin A of Morphologically Transformed and Nontransformed NRK Cells

Cell line	Transformed morphology	Maximal agglutination*
		%
NRK(MSV-lb)		
33°C	−	25
33°C + 0.4 mM 8BrcAMP	+	20
NRK(MSV-1b)		
39°C	+	45
39°C + 0.4 mM 8BrcAMP	+	43
NRK(MSV-MLV)		
36°C	+	65
36°C + 0.4 mM 8BrcAMP	+	59
NRK		
36°C	−	28
36°C + 0.4 mM 8BrcAMP	−	27

Plastic flasks (75 cm^2) were seeded with 2–3 × 10^6 cells and used for agglutination studies 2–3 days later when the cells were subconfluent. NRK(MSV-lb) cells were grown at either 33 or 39°C, whereas NRK and NRK(MSV-MLV) cells were grown at 36°C. All cells were exposed to 0.4 mM 8BrcAMP or mock treated without replacing the growth medium. 2 h later, the cells were examined microscopically for morphological transformation and were tested for Con A agglutinability.

* Agglutination level in the presence of 125 μg Con A/ml calculated from agglutination dose-response curves with constant cell density, temperature, and incubation time.

Table II

Rate of 2-Deoxy-D[³H]Glucose Transport in Morphologically Transformed and Nontransformed NRK Cells

Cell line	Transformed morphology	CPM uptake in 10 min/mg protein × 10^{-3}
NRK(MSV-1b)		
33°C	−	46
33°C + 0.4 mM 8BrcAMP	+	42
NRK(MSV-1b)		
39°C	+	99
39°C + 0.4 mM 8BrcAMP	+	110
NRK(MSV-1) 36°C	+	178
NRK(MSV-1) 36°C + 0.4 mM 8BrcAMP	+	151
NRK 36°C	−	28
NRK 36°C + 0.4 mM 8BrcAMP	−	28

Petri dishes (60 mm) were seeded with 8 × 10^5 cells and 1 day later were exposed to 0.4 mM 8BrcAMP or mock treated without replacing the growth medium. 2 h later, the cells were examined microscopically for morphological transformation, and 2-deoxy-D-[³H]glucose uptake was measured as described in Materials and Methods.

transformation of NRK(MSV-1b) cells at 33°C does not alter 2-deoxy-D-glucose uptake. To determine whether variable exposure times to 8BrcAMP might affect the rate of hexose transport, NRK(MSV-1b) cells grown at 33°C were treated for 1, 2, 4, and 24 h with 0.4 mM 8BrcAMP and assayed for 2-deoxy-D-glucose uptake. Under conditions where 100% of the cAMP-treated cells were morphologically transformed, there were no significant alterations in hexose uptake relative to mock treated cultures.

Effect of 8BrcAMP on the Incorporation of [¹⁴C]Fucose into FL III and FL IV of NRK(MSV-1b) Cells at Restrictive Temperature

The addition of 8BrcAMP to NRK(MSV-1b) cells at the restrictive temperature results in a decrease in the incorporation of [¹⁴C]fucose into FL IV (Fig. 4*a* vs. *b*). FL IV is the least chromatographically mobile, presumably most complex fucolipid. The relative incorporation of [¹⁴C]fucose into FL III vs. FL IV in the 8BrcAMP-treated cells is comparable to that observed with NRK(MSV-1b) cells grown at 39°C (Fig. 4*d*). Moreover, addition of 0.4 mM 8BrcAMP to NRK(MSV-1b) cells at 39°C (Fig. 4*c*) does not enhance the incorporation of label into FL IV. Similar experiments of longer duration, i.e., 48 or 72 h, gave comparable results. Addition of 8BrcAMP to NRK cells (Fig. 4*e* and *f*) or to NRK(MSV-MLV) cells (Fig. 4*g* and *h*) at 33 or 39°C does not significantly alter the incorporation of labeled fucose into FL III and FL IV; however, in a 24-h pulse more label per milligram protein is incorporated into NRK and NRK(MSV-MLV) cells than into NRK(MSV-1b) cells.

DISCUSSION

Morphological transformation of cells by avian and mammalian sarcoma viruses is associated with a number of phenotypic changes including increased agglutinability by plant lectins (22), increased rate of hexose transport (11), an alteration in the composition of surface membrane glycoproteins and glycolipids (10, 33), and altered levels of endogenous cAMP (20). That these phenotypic changes are associated with the transformation process has been demonstrated with the aid of ts viral mutants (39). Cells infected with the

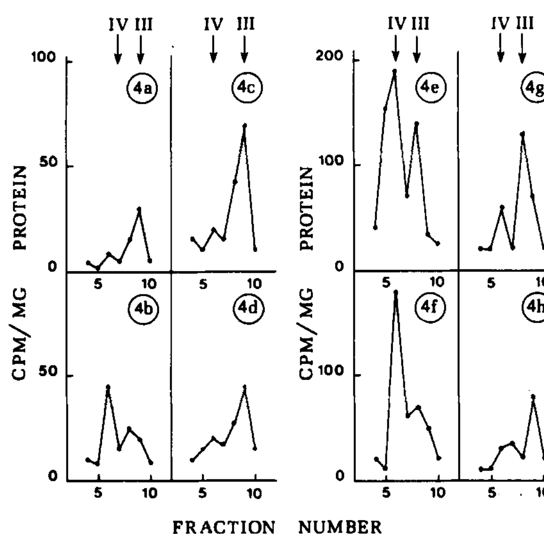

Figure 4 [¹⁴C]Fucose patterns of NRK(MSV-1b), NRK, and NRK(MSV-MLV) cells. NRK(MSV-1b) cells grown at (*a*) 33°C plus 0.4 mM 8BrcAMP, (*b*) 33°C without 8BrcAMP, (*c*) 39°C plus 0.4 mM 8BrcAMP, and (*d*) 39°C without 8BrcAMP. NRK cells grown at 33°C (*e*) with 0.4 mM 8BrcAMP, and (*f*) without 8BrcAMP. NRK(MSV-MLV) cells grown at 33°C (*g*) with 0.4 mM 8BrcAMP, and (*h*) without 8BrcAMP. Similar results were obtained if the cells were grown at 39°C. For details of cell culturing, fucose labeling, lipid extraction, and analysis of FL III and FL IV, see Materials and Methods.

viral mutants under permissive conditions express the transformed phenotype, but acquire the normal phenotype when grown under restrictive conditions. Recently, we demonstrated, in NRK cells infected with a cold-sensitive mutant of MSV, that cAMP mediated the morphological transformation of NRK(MSV-1b) cells grown at the restrictive temperature (29). Thus, based on morphological criteria, cells acquired the transformed phenotype either by a temperature shift to the permissive condition or by the addition of cAMP. Therefore, we asked whether other markers associated with transformation were also expressed after cAMP treatment. In this report, we have demonstrated that the cAMP-induced morphological changes are paralleled by alterations in fucolipid metabolism, but are not accompanied by changes in hexose transport rate or Con A agglutinability of the cells. The independent expression of these transformation-associated markers indicates that cAMP treatment does not simply complement the ts transforming function in these cells, but rather induces morphological changes and alterations in fucolipid composition without altering membrane sites for sugar transport or Con A agglutination. The association of cAMP-induced morphological transformation with a block in the incorporation of radioactive fucose into FL IV and a parallel increased incorporation into FL III is similar to the results obtained in previous studies with both RNA and DNA virus-transformed cells (30–32), and provides additional evidence that the expression of this biochemical marker is correlated with morphological transformation.

Recent observations by Willingham and Pastan (36–38) have led to the hypothesis that the agglutinability of cells is regulated through the modulation of cell surface microvilli by cAMP, and that cAMP decreases agglutinability by decreasing the formation of microvilli and altering the intracellular distribution of microfilaments and microtubules. Others have been unable to confirm these findings, and have suggested that the presence or absence of microvilli alone does not explain differences in agglutinability (4, 18). The proposal that transformed cells are highly agglutinable because their low cAMP levels result in the formation of numerous surface microvilli is not supported by our data. NRK(MSV-1b) cells grown under conditions permissive for expression of the transformed phenotype have elevated levels of endogenous cAMP (29), numerous surface membrane blebs and microvilli (34), and an increased agglutinability relative to the cells grown under restrictive conditions. Furthermore, the acquisition of the transformed morphology without an increase in agglutinability after cAMP treatment of NRK(MSV-1b) cells at 33°C indicates that cAMP-mediated morphological alterations are not necessarily associated with altered agglutinability.

Although the precise role of cAMP in regulating cell morphology is yet to be determined, several studies have shown that treatment of transformed cells with derivatives of cAMP restores the normal morphological appearance of the cells and stimulates the assembly of cytoplasmic microtubules (2, 13, 14, 21, 37) and microfilaments (37). The paradoxical effects of cAMP in NRK cells infected with a ts transformation mutant of MSV are similar to cAMP-induced rounding and morphological transformation observed in cultured adrenal tumor cells (15) and in NRK cells transformed by a ts mutant of Rous sarcoma virus (25), and suggest that cAMP may be involved in disaggregation of cytoskeletal elements. The reports of cAMP-stimulated phosphorylation of microtubules (9) and microtubule-associated protein (26) suggest the possibility that phosphorylation could affect the disassembly of microtubules. Obviously, other factors are involved in controlling microtubule assembly (19), and several studies have demonstrated a relationship between cAMP-mediated phenomena and calcium (5, 7, 12, 23, 24). Recent electron microscope observations of NRK(MSV-1b) cells grown under restrictive conditions have revealed an altered form of cell-to-cell adherens junctions in which the microfilaments appear disorganized (1). These findings demonstrated a relationship between decreased numbers of microfilaments and expression of the transformed phenotype and suggested that the perturbed assembly of microfilaments in NRK(MSV-1b) cells may be an intermediate stage in the loss of adherens junctions during viral transformation. We propose that cAMP treatment of these cells results in further disassembly of microfilaments, resulting in morphologically transformed cells.

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REFERENCES

1. Altenburg, B. C., K. Somers, and S. Steiner. 1976. Altered microfilament structure in cells transformed with a temperature-sensitive transformation mutant of murine sarcoma virus. *Cancer Res.* **36:**251–257.
2. Brinkley, B. R., G. M. Fuller, and D. P. Highfield. 1975. Cytoplasmic microtubules in normal and transformed cells in culture: analysis by tubulin antibody immunofluorescence. *Proc. Natl. Acad. Sci. U. S. A.* **72:**4981–4985.
3. Burger, M. M. 1974. Assays for agglutination with lectins. *Methods Enzymol.* **32:**615–621.
4. Collard, J. G., and J. H. M. Temmink. 1976. Surface morphology and agglutinability with concanavalin A in normal and transformed murine fibroblasts. *J. Cell Biol.* **68:**101–112.
5. Durham, A. C. H. 1974. A unified theory of the control of actin and myosin in nonmuscle movement. *Cell.* **2:**123–136.
6. Gacto, M., and S. Steiner. 1976. Fucolipid metabolism as a function of cell population density in normal and murine sarcoma virus-transformed rat cells. *Biochim. Biophys. Acta.* **444:**11–22.
7. Gillespie, E. 1971. Colchicine binding in tissue slices: decrease by calcium and biphasic effect of adenosine-3',5'-monophosphate. *J. Cell Biol.* **50:** 544–549.
8. Glimelius, B., B. Westermark, and J. Pontén. 1974. Agglutination of normal and neoplastic human cells by Concanavalin A and *Ricinus communis* agglutinin. *Int. J. Cancer.* **14:**314–325.
9. Goodman, D. B. P., H. Rasmussen, F. DiBella, and C. E. Guthrow, Jr. 1970. Cyclic adenosine 3':5'-monophosphate-stimulated phosphorylation of isolated neurotubule subunits. *Proc. Natl. Acad. Sci. U. S. A.* **67:**652–659.
10. Hakamori, S. 1975. Structures and organization of cell surface glycolipids: dependence on cell growth and malignant transformation. *Biochim. Biophys. Acta.* **417:**55–89.
11. Hatanaka, M. 1974. Transport of sugars in tumor cell membranes. *Biochim. Biophys. Acta.* **355:**77–104.
12. Henneberry, R. C., P. H. Fishman, and E. Freese. 1975. Morphological changes in cultured mammalian cells: prevention by the calcium ionophore A23187. *Cell.* **5:**1–9.
13. Hsie, A. W., and T. T. Puck. 1971. Morphological transformation of Chinese hamster cells by dibutyryl adenosine cyclic 3':5'-monophosphate and testosterone. *Proc. Natl. Acad. Sci. U. S. A.* **68:**358–361.
14. Johnson, G. S., R. M. Friedman, and I. Pastan. 1971. Restoration of several morphological characteristics of normal fibroblasts in sarcoma cells treated with adenosine-3':5'-cyclic monophosphate and its derivatives. *Proc. Natl. Acad. Sci. U. S. A.* **68:**425–429.
15. Kwan, C. N., and R. M. Wishnow. 1974. Adenosine 3',5'-cyclic monophosphate and adrenal tumor cell morphology. *In Vitro (Rockville).* **10:**268–273.
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:**265–275.
17. May, J. T., K. D. Somers, and S. Kit. 1973. Temperature-dependent alterations in 2-deoxyglucose uptake in rat cells transformed by a cold-sensitive murine sarcoma virus mutant. *Int. J. Cancer.* **11:**377–384.
18. Nicolson, G. L. 1974. The interactions of lectins with animal cell surfaces. *Int. Rev. Cytol.* **39:**89–190.
19. Olmsted, J. B., and G. G. Borisy. 1973. Microtubules. *Annu. Rev. Biochem.* **42:**507–540.
20. Pastan, I., and G. S. Johnson. 1974. Cyclic AMP and the transformation of fibroblasts. *Adv. Cancer Res.* **19:**303–329.
21. Porter, K. R., T. T. Puck, A. W. Hsie, and D. Kelley. 1974. An electron microscope study of the effects of dibutyryl cyclic AMP on Chinese hamster ovary cells. *Cell.* **2:**145–162.
22. Rapin, A. M. C., and M. M. Burger. 1974. Tumor cell surfaces: general alterations detected by agglutinins. *Adv. Cancer Res.* **20:**1–91.
23. Rasmussen, H. 1970. Cell communication, calcium ion, and cyclic adenosine monophosphate. *Science (Wash. D.C.).* **170:**404–412.
24. Rasmussen, H., D. B. Goodman, and A. Tenenhouse. 1972. The role of cyclic AMP and calcium in cell activation. *Crit. Rev. Biochem.* **1:**95–148.
25. Rieber, M., and J. Bacalao. 1976. A cyclic adenosine 3':5'-monophosphate-mediated effect of cholera toxin on high molecular weight glycoprotein species of malignant cells. *Cancer Res.* **36:**3178–3184.
26. Sloboda, R. D., S. A. Rudolph, J. L. Rosenbaum, and P. Greengard. 1975. Cyclic AMP-dependent endogenous phosphorylation of a microtubule-associated protein. *Proc. Natl. Acad. Sci. U. S. A.* **72:**177–181.
27. Somers, K., and S. Kit. 1971. Clonal isolation of murine sarcoma virus (MSV): characterization of virus produced from transformed cells. *Virology.* **46:**774–785.
28. Somers, K., and S. Kit. 1973. Temperature-dependent expression of transformation by a cold-sensitive mutant of murine sarcoma virus. *Proc. Natl. Acad. Sci. U. S. A.* **70:**2206–2210.
29. Somers, K. D., M. Rachmeler, and M. Christensen. 1975. Cyclic AMP-mediated transformation of rat cells transformed by temperature-sensitive mouse sarcoma virus. *Nature (Lond.).* **257:**58–60.
30. Steiner, S., P. J. Brennan, and J. L. Melnick. 1973. Fucosylglycolipid metabolism in oncornavirus-trans-

formed cell lines. *Nat. New Biol.* **245:**19–21.
31. Steiner, S. M., J. L. Melnick, S. Kit, and K. D. Somers. 1974. Fucosylglycolipids in cells transformed by a temperature-sensitive mutant of murine sarcoma virus. *Nature (Lond.).* **248:**682–684.
32. Steiner, S., and M. R. Steiner. 1975/1976. Fucolipid patterns of highly and weakly tumorigenic simian virus 40 and Herpes simplex virus-transformed cell lines. *Intervirology.* **6:**32–41.
33. Warren, L., J. P. Fuhrer, and C. A. Buck. 1973. Surface glycoproteins of cells before and after transformation by oncogenic viruses. *Fed. Proc.* **32:**80–85.
34. Weeks, B. A., and K. D. Somers. 1975. Surface membrane topography of cells transformed by wild type and cold-sensitive murine sarcoma virus (MSV). *Abstr. Annu. Meet. Am. Soc. Microbiol.* 215.
35. Willingham, M. C. 1976. Cyclic AMP and cell behavior in cultured cells. *Int. Rev. Cytol.* **44:**319–363.
36. Willingham, M. C., and I. Pastan. 1974. Cyclic AMP mediates concanavalin A agglutinability of mouse fibroblasts. *J. Cell Biol.* **63:**288–294.
37. Willingham, M. C., and I. Pastan. 1975. Cyclic AMP and cell morphology in cultured fibroblasts: effects on cell shape, microfilament and microtubule distribution, and orientation to substratum. *J. Cell Biol.* **67:**146–159.
38. Willingham, M. C., and I. Pastan. 1975. Cyclic AMP modulates microvillus formation and agglutinability in transformed and normal mouse fibroblasts. *Proc. Natl. Acad. Sci. U. S. A.* **72:**1263–1267.
39. Wyke, J. A. 1974. The genetics of C-type RNA tumor viruses. *Int. Rev. Cytol.* **38:**67–109.