A Mutation in a Rous Sarcoma Virus Gene That Controls Adenosine 3',5'-Monophosphate Levels and Transformation

(Received for publication, December 21, 1971)

Jacques Otten,* John Bader,† George S. Johnson,* and Ira Pastan*†

From the National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

SUMMARY

Chick embryo fibroblasts infected with a temperature-sensitive mutant of Rous sarcoma virus (chicken embryo fibroblasts (RSV-Ta)) have normal morphology at 40.5° but after a transfer to 36° show the morphological characteristics of transformed cells. This transformation is prevented by N9',2'-O-dibutyryl adenosine 3',5'-monophosphate plus theophylline. Chicken embryo fibroblasts (RSV-Ta) have the same high endogenous levels of adenosine 3',5'-monophosphate (cyclic AMP) at 40.5° as uninfected fibroblasts; however, when shifted to 36° the cyclic AMP levels fall to a value similar to that of the fibroblasts infected with wild type RSV. This suggests that an early event in transformation leads to a decreased level of cyclic AMP, and the decreased level of cyclic AMP results in some of the unique properties of transformed cells.

Cyclic adenosine 3',5'-monophosphate has an important role in controlling many properties of cultured fibroblasts. Its actions are most evident with transformed cells appearing spontaneously or after infection by oncogenic viruses. When transformed cells are treated with a cyclic AMP analogue, B*+cAMP, many of their abnormal properties return to or towards normal. B*+cAMP, cyclic AMP, or the phosphodiesterase inhibitor theophylline has been found to restore cell growth rate (1-4), change cellular morphology (4-6), increase adhesiveness to substratum (7), decrease motility (8), decrease agglutinability by concanavalin A (9), and decrease the saturation density of contact-inhibited cells (4). B*+cAMP has also been found to restore density-dependent inhibition of growth in two transformed cell lines (9), but many others are unaffected (4).

When cyclic AMP levels in transformed and normal fibroblasts were measured, an inverse relationship between the growth rate and the intracellular cyclic AMP concentration was observed (10). Further, cyclic AMP levels were found to rise in cells displaying contact inhibition of growth, but failed to rise in noncontact-inhibited cells even when grown to a high cell density (10).

These studies imply that an early event in the establishment of transformation leads to lower than normal cyclic AMP levels, and it is the inability of cells to maintain normal cyclic AMP levels that gives them many of the properties characteristic of transformed cells.

The actions of cyclic AMP described above have been investigated on cell lines maintained in culture for many generations. It is well known that cells maintained in culture lose their resemblance to normal cells. Therefore, it seemed important to study cells recently established in culture, and in which transformation could be rapidly induced in most of the cells under study. Bader and Brown (11) have recently described the isolation of a number of temperature-sensitive mutants of the Bryan strain of Rous sarcoma virus. The morphological response characteristic of transformation occurs when the cells are shifted from 40.5° to 36°. At 40.5° the cells are long and spindly, indistinguishable from normal chick embryo fibroblasts (11). However, within 1 to 2 hours after the temperature is lowered to 36°, the cells lose their spindly shape, and vacuoles appear around the nucleus. Chick embryo fibroblasts infected with one of the mutants, RSV-Ta, were examined with regard to several aspects of cyclic AMP metabolism.

We first studied the effect of treating chicken embryo fibroblasts (RSV-Ta) with B*+cAMP and theophylline. When these substances were added to an infected culture about 2 hours before the temperature was lowered from 40.5° to 36°, the cells retained their normal morphology for the following 12 to 24 hours (Fig. 1); longer times were not examined. Phosphodiesterase inhibitors by themselves, 1 mM theophylline, 0.2 mM papaverine, and 1 mM 1 methyl 3 isobutylxanthine partially blocked the transformation, and the latter two in combination with 1.2 mM B*+cAMP also completely blocked it. Adenosine monophosphate failed to block transformation.

These findings suggest that a viral function controls the level of cyclic AMP in the cell. Therefore, the level of cyclic AMP was measured in RSV-Ta-infected cells. In the infected cells grown at 40.5°, the level of cyclic AMP was in the range of normal cells, but when the cells were shifted to 36° for 12 hours, the cyclic AMP level fell dramatically (Table I). In cells transformed by wild type RSV, the cyclic AMP levels were low in cells grown at both 36° and 40.5°. In contrast, the levels of cyclic AMP in normal fibroblasts were high and not affected by the change in temperature.

We next examined the kinetics of the change in cyclic AMP levels in cells infected with the mutant virus and found that within 20 min after changing from 40.5 to 36°, the level of cyclic AMP had fallen by about 50%. The level of cyclic AMP remained in this range for about 6 hours, and after 12 hours the levels of cyclic AMP had decreased further. It is evident from a comparison of Tables I and II that the level of cyclic AMP in the phenotypically normal cell varies. The data in Table I were obtained with cells grown to a higher density than those in Table II. Previously, we reported that cells displaying density dependent inhibition of growth have an increase in cyclic AMP levels at confluence, whereas transformed cells have a decrease in cyclic AMP levels at high cell density (10).
RSV-Ta is a virus which is capable of infecting cells and replicating at both 40.5° and 36°. However, at 40.5° the virus is unable to transform the cell. As soon as 20 min after the temperature is lowered to 36°, cyclic AMP levels have fallen by 50%, whereas morphological transformation is only evident after 1 to 2 hours. The rapid fall in cyclic AMP levels indicates that a temperature-sensitive function, a product of the transformation region of the virus, lowers cyclic AMP levels. Current efforts are directed towards the identification of the temperature-sensitive step in cyclic AMP metabolism.

It was previously shown that many of the characteristics of transformed cells are returned towards normal when they are treated with Bu-cAMP and theophylline (1-9). Further, in transformed cell lines containing an adenylate cyclase which can be stimulated by prostaglandin E1 (12), prostaglandin E2 also causes phenotypic reversion (3, 13). In this paper we have demonstrated that expression of a viral function lowers cyclic AMP levels before morphological transformation is evident. This finding strongly suggests that a fall in cyclic AMP levels results in some of the unique properties of transformed cells.

REFERENCES

1. BURK, R. R. (1968) Nature 219, 1272
2. RYAN, W. L. AND HEIDRICK, M. L. (1968) Science 162, 1484
3. JOHNSON, G. S., PERRY, C., OTTEN, J., MORGAN, W. D., AND PASTAN, I. (1971) J. Clin. Invest. 50, 50a
4. JOHNSON, G. S., AND PASTAN, I. (1972) J. Nat. Cancer Inst., in press
5. JOHNSON, G. S., FRIEDMAN, R. M., AND PASTAN, I. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 425
6. HAY, A. W., AND PECK, T. T. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 358
7. JOHNSON, G. S., AND PASTAN, I. (1972) Nature New Biol., in press
8. JOHNSON, G. S., AND PASTAN, I. (1972) Nature 235, 54
9. SHEPPARD, J. R. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 1316
10. OTTEN, J., JOHNSON, G. S., AND PASTAN, I. (1971) Biochem. Biophys. Res. Commun. 44, 1102
11. BADER, J. P., AND BROWN, N. R. (1971) Nature New Biol. 234, 11
12. PERRY, C. V., JOHNSON, G. S., AND PASTAN, I. (1971) J. Biol. Chem. 246, 6785
13. JOHNSON, G. S., AND PASTAN, I. (1971) J. Nat. Cancer Inst. 47, 1367

Acknowledgment—We thank G. D. Searle and Co. for supplying 1-methyl-3-isobutylxanthine. J. Otten is a recipient of National Institutes of Health Fellowship 1 FO5TW 1714-02 and G. S. Johnson is a Leukemia Society of American Special Fellow.