ADENYLATE CYCLASE AND CYCLIC AMP PHOSPHODIESTERASE OF NORMAL AND ROUS SARCOMA VIRUS TRANSFORMED CHICKEN EMBRYO FIBROBLASTS

Tom R. Russell and Wayne B. Anderson

Laboratory of Molecular Biology, National Cancer Institute, N.I.H., Bethesda, Maryland 20014

Cyclic AMP appears to regulate cell growth. Cyclic AMP levels are high in normal chicken embryo fibroblasts and drop to very low levels when the cells are transformed by the Bryan high-titer strain of Rous sarcoma virus. Cells infected with a temperature-sensitive mutant of the virus have normal levels of cyclic AMP at the nonpermissive (nontransforming temperature), but when the cells are shifted to the permissive (transforming) temperature the cyclic AMP levels rapidly fall to values that are found in transformed cells. Studies on the adenylate cyclase and cyclic AMP phosphodiesterase in normal and transformed chicken embryo fibroblasts have shown that the adenylate cyclase is greatly decreased in the transformed cells whereas the phosphodiesterase is increased. The decrease in adenylate cylcase activity is the result of an increase in the Km of the substrate and a loss of a magnesium ion activator site. The increase in phosphodiesterase activity is the result of an increase in total phosphodiesterase activity and a decrease in the negative cooperativity of plasma membrane bound phosphodiesterase. Thus the fall in cyclic AMP levels that occurs on transformation can be correlated with changes in the activity of adenylate cyclase and cyclic AMP phosphodiesterase.

Cyclic AMP appears to regulare a number of properties of normal fibroblasts grown in tissue culture such as adhesion to substratum, motility, morphology, and growth rate (1–4). Logarithmically growing normal cells and transformed cells have low levels of cyclic AMP when compared to nongrowing and contact-inhibited cells (4–6). Some of the abnormal properties of transformed cells can be reverted toward that of normal cells by the addition of the cyclic AMP analogue, dibutyryl cyclic AMP (7, 8). Studies were undertaken to attempt to correlate the decrease in cyclic AMP that occurs on transformation with the activities of the enzymes that synthesize and degrade cyclic AMP, adenylate cyclase and cyclic AMP phosphodiesterase.

In these studies we have utilized chicken embryo fibroblasts which are readily transformed by the Bryan high-titer strain of Rous sarcoma virus and by a temperature-sensitive mutant of this virus which was isolated and characterized by Bader and Brown (9). Normal chicken embryo fibroblasts contain relatively high levels of cyclic AMP (70–100 pmoles/mg nucleic acid) whereas transformed cells contain relatively low levels of cyclic AMP (less than 30 pmoles/mg nucleic acid). Cells infected with the temperature-sensitive mutant and grown at the nonpermissive (nontransforming) temperature (41°C) have cyclic AMP levels that are similar to normal cells but when the cells are shifted to

the permissive (transforming) temperature (36°C) the cyclic AMP levels fall to values identical to that of transformed cells (8). The rapid fall in cyclic AMP levels in the mutant infected cells precedes morphological transformation which is complete in four hours. Additional study has shown that adenylate cyclase activity is greatly reduced in cells transformed by the wild-type and temperature-sensitive virus while phosphodiesterase activity is enhanced in the transformed cells. Thus the drop in cyclic AMP levels that occurs on transformation can be correlated with the changes in the adeylate cyclase and cyclic AMP phosphodiesterase activities.

Adenylate Cyclase of Normal and Virus Transformed Cells

The activity of adenylate cyclase is decreased at least 50% in chicken embryo fibroblasts transformed by the Bryan high-titer strain of Rous sarcoma virus when assayed at low (0.2 mM) ATP concentration (10). The enzyme from both normal and transformed cells is an integral part of the plasma membrane and exhibits maximal activity at pH 7.8. When the cells are transformed by the virus, two significant changes occur in adenylate cyclase activity which account for the decreased activity and which can lead to the lower levels of cyclic AMP found in these cells. First, transformation results in an altered Km for substrate (Mg ATP). The apparent Km ATP is 0.23 mM in normal cells and 1.1 mM in cells transformed with wild-type virus. Second, the enzyme from transformed cells shows an altered response to magnesium. Adenylate cyclase from untransformed cells shows a progressive increase in activity with increasing magnesium ion concentration through 20–40 mM. In contrast, the enzyme from transformed cells is saturated at 4–6 mM magnesium. These results might indicate that the enzyme has at least two types of binding sites for magnesium: one at the catalytic site and perhaps one or more regulatory or stimulatory sites. If this is the case, it would appear that transformation results in a loss of magnesium activator sites. The enzyme from cells infected with the temperature-sensitive mutant and grown at the nonpermissive temperature (42°C) has properties similar to that from normal cells. When these cells are shifted to the permissive temperature (36°C), adenylate cyclase activity is reduced and the enzyme behaves as that from wild-type virus transformed cells. When cells infected with the temperature-sensitive virus are shifted from 41° to 36°C,adenylate cyclase activity decreases rapidly and falls to one-half that of normal cells 30 minutes after the temperature shift.

Cyclic AMP Phosphodiesterase of Normal and Transformed Cells

Cyclic nucleotide phosphodiesterase of normal chicken embryo fibroblasts is found in both particulate and soluble fractions. The particulate enzyme appears to be a component of the plasma membrane. The enzyme is specific for cyclic AMP and exhibits negative co-operative kinetic regulation. The activity and Km of the plasma membrane enzyme can be altered by trypsin treatment of intact chicken embryo fibroblasts but the catalytic site of the phosphodiesterase does not reside on the cell exterior. Thus the plasma membrane phosphodiesterase whose active site is inside the cell can be altered by an agent that acts on the cell exterior. The supernatant fraction of homogenized chicken embryo fibroblasts contains two phosphodiesterase activities that are spearable on DEAE cellulose (11). One fraction hydrolyzes both cyclic AMP and cyclic GMP whereas the other fraction is specific for cyclic AMP. When the cells are transformed by the Bryan

high-titer strain of Rous sarcoma virus, the specific activity of the phosphodiesterase is increased and the negative cooperativity of the plasma membrane is decreased. Such a decrease in negative cooperativity leads to an increase in the ability of the phosphodiesterase to hydrolyze cyclic AMP. When the cells are infected with the temperature-sensitive mutant and grown at the nonpermissive (nontransforming) temperature, the phosphodiesterase has similar characteristics to that of the normal cells. However, four hours after shifting to the permissive (transforming) temperature the specific activity of the phosphodiesterase is doubled. The increase in phosphodiesterase activity is seen in both the membrane and soluble fractions (12). The plasma membrane enzyme also has a decreased negative cooperativity constant as is seen in the enzyme from wild-type transformed cells. The increase in total phosphodiesterase activity and the decrease in the negative cooperativity constant of the plasma membrane enzyme lead to an increase in the ability of the phosphodiesterase to lower cyclic AMP levels.

A number of mechanisms might be proposed to explain the alteration in cyclic AMP metabolism which results with transformation by the Bryan high-titer strain of Rous sarcoma virus. One attractive possibility is that a specific viral transformation factor might interact with the cell membrane and cause a reduction in the activity of the membrane-bound adenylate cyclase which leads to a decreased synthesis of cyclic AMP. Transformation also directs an increase in total phosphodiesterase activity apparently to insure that cyclic AMP levels remain low to maintain the transformed state. With the temperature-sensitive mutant the viral transformation factor is inactive at the nonpermissive temperature but becomes active immediately upon shifting to the permissive temperature. Proof of this mechanism requires isolation of the putative viral transformation factor followed by experiments that show that this factor has a direct effect on the activity of adenylate cyclase.

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