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Morphological Transformation of Chinese Hamster Cells by Dibutyryl Adenosine Cyclic 3′:5′-Monophosphate and Testosterone

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ABSTRACT **Treatment of Chinese hamster ovary cells *in vitro* with dibutyryl adenosine cyclic 3′:5′-monophosphate converts the culture from one of compact, randomly oriented cells that grow in multilayers to a monolayer of elongated, fibroblast-like cells growing parallel to one another. Testosterone propionate, which has a similar though smaller effect at high concentrations and after prolonged incubation, potentiates the action of dibutyryl cyclic AMP even when added at very low concentrations. The transformation is recognizable within one hour, affects cells throughout all or most of the life cycle, and is completely reversible. Both cell forms can reproduce, with approximately the same generation time. Agents like colcemid and vinblastine, which inhibit assembly of microtubules, prevent the transformation to the fibroblast-like form. It is postulated that the dibutyryl cyclic AMP and testosterone act by promoting organization of microtubules from protein monomers.**

The molecular basis of morphological characteristics that mammalian cells assume under different conditions of growth *in vitro* and *in vivo* is still largely obscure. We describe here a reversible transformation in structure of mammalian cells cultivated *in vitro* that is mediated by dibutyryl adenosine cyclic monophosphate ($But_2A>p$) and testosterone, and present evidence that this metamorphosis involves action of cellular microtubules.

METHODS AND MATERIALS

The Chinese hamster ovary clone, CHO-Kl, previously described was used in all of these experiments (1). The cells were routinely grown in F12 medium (2) supplemented with 10% fetal calf serum at 37°C and harvested by the standard trypsinization procedure (3). Aliquots of the washed cells were added to the plastic Petri dishes (Falcon) in which the experiments were performed. The growth medium utilized in the actual experiments was F12 supplemented with the macromolecular fraction of fetal calf serum in an amount equivalent to 10% of whole serum, the small-molecule components of the serum being excluded in order to assure better molecular definition of the cell environment. The generation time of these cells in this medium is approximately 12 hr (4).

The chemicals used and their sources are as follows: Adenosine cyclic 3′:5′-monophosphoric acid (which was brought to pH 7.0 with NaOH) and $N^6,O^{2'}$-dibutyryl A>p (sodium salt), Sigma Chemical Co.; colcemid, CIBA Pharmaceutical Products, Inc., testosterone propionate (Δ^4-androsten-3-one 17β-propionate), Mann Research Laboratories; vinblastine sulfate, Eli Lilly and Co.

Dibutyryl A>p was used in the expectation that it would penetrate cells more effectively than unsubstituted A>p and perhaps might also be less susceptible to the hydrolytic action of phosphodiesterase inside the cell (5). Aqueous stock solutions of 0.01 M But_2 A>p were made up and preserved in the freezer until use. Stock testosterone solutions were made up at a concentration of 1.5×10^{-3} M in ethanol. Control experiments were always performed to ensure that none of the effects observed could be due to traces of the solvent carried along with the drugs employed.

RESULTS

Action of $But_2A>p$ alone

A>p itself had no action on these cells and neither did sodium butyrate. However, $But_2A>p$ produced a marked change in morphology (Fig. 1). In the absence of the agent the cells are compact, well separated, and poorly oriented. In its presence the cells elongate to the spindle shape characteristic of the mammalian fibroblast, and line up in parallel fashion to produce a highly oriented colony typical of the fibroblast (6). These differences in growth pattern cause the two types of colonies to be distinctly recognizable macroscopically as well as microscopically, as shown in Fig. 2.

Comparison of Figs. 1*A* and *B* reveals another important aspect of this transformation: colonies grown in the presence of $But_2A>p$ are contact-inhibited and the cells are strictly confined to growth in a monolayer, whereas in the absence of this agent, cell growth in three dimensions occurs, producing colonies with the general shape of a derby hat.

The morphological transformation caused by $But_2A>p$ occurs in a period much shorter than the cellular generation time. A clear effect is observed with 10^{-3} M $But_2A>p$ after only 1 hr. Within 3 hr the great majority of the cells have undergone recognizable morphological change and in about 5 hr the cell elongation has reached the point where the ratio of the longest to the shortest cell dimension is 13.8 ± 4.6 as compared to the value of 3.9 ± 1.3 in the control culture. It can therefore be concluded that cells in all or most of the phases of the life cycle are susceptible to this action of the drug.

Smaller concentrations of $But_2A>p$ produce smaller and more slowly developing effects. The action of concentrations

Abbreviations: A>p, adenosine cyclic 3′:5′-monophosphate; $But_2A>p$, $N^6O^{2'}$-dibutyryl adenosine cyclic 3′:5′-monophosphate.

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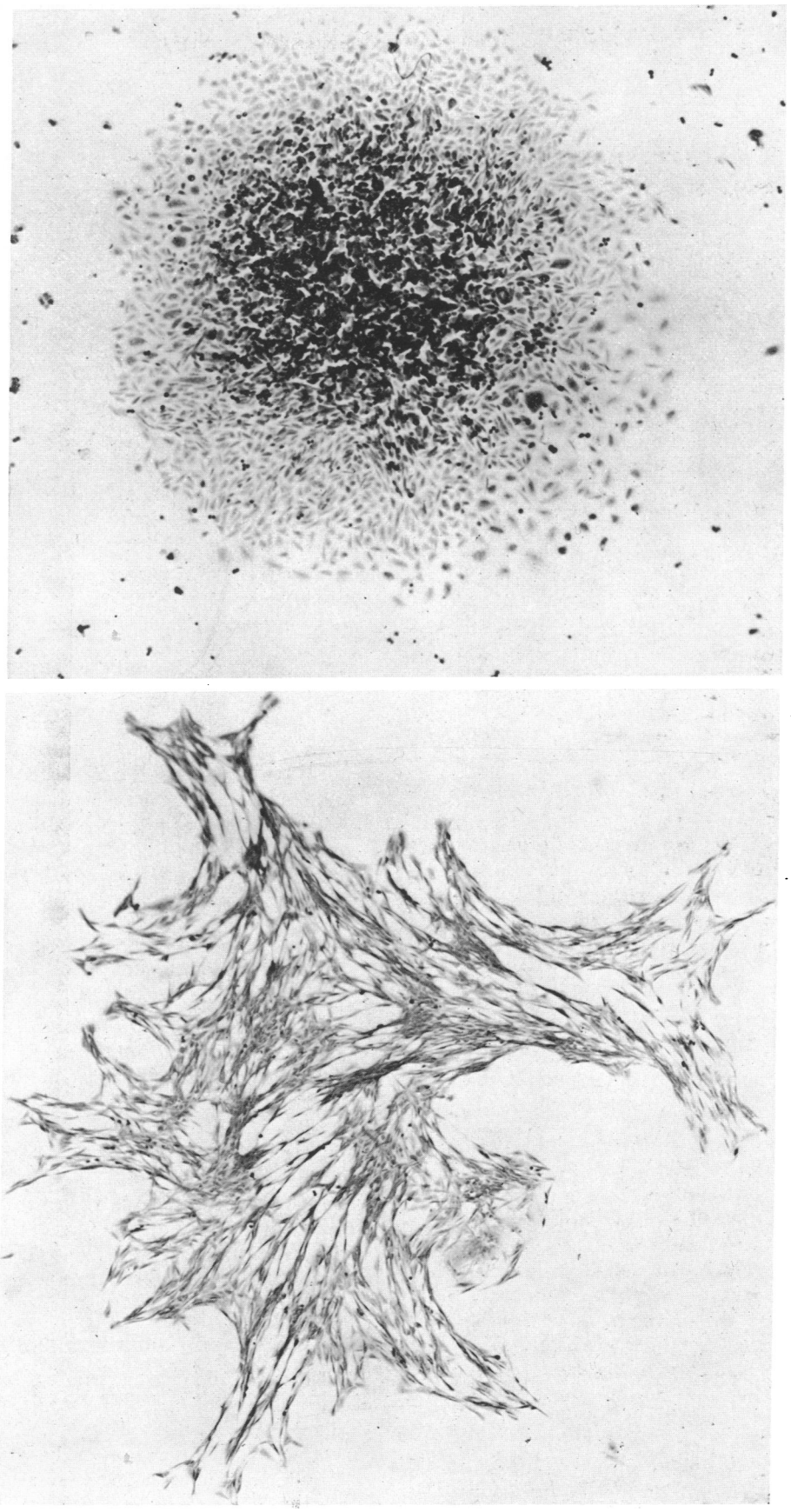

FIG. 1. Effect of $But_2A>p$ on the morphology of Chinese hamster ovary cells. *A*, colony grown from a single CHO cell on standard medium for 6 days; *B*, colony grown in the presence of 10^{-3} M $But_2A>p$. Magnification, ×50.

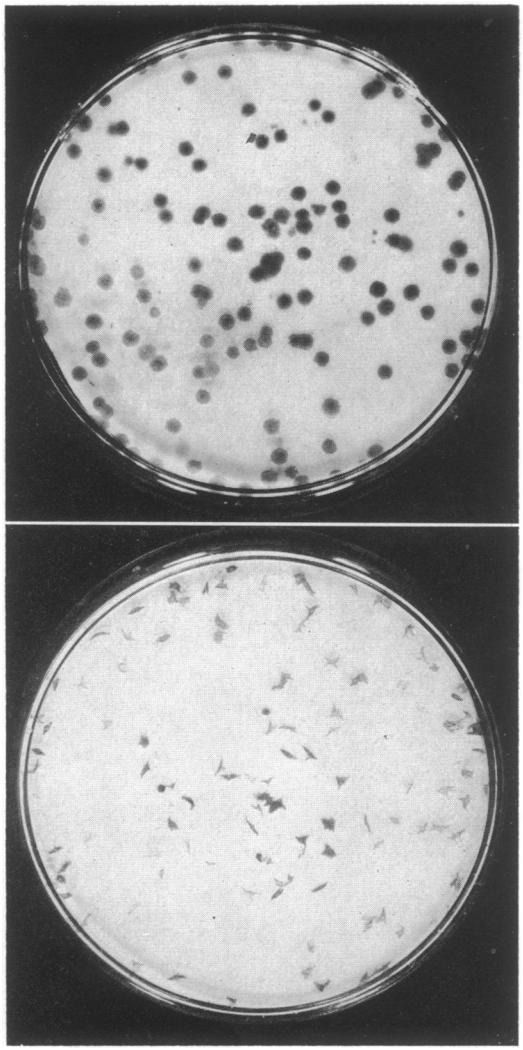

FIG. 2. Macroscopic appearance of the colonies without (*above*) and with (*below*) 10^{-3} M But$_2$A>p. Magnification, × 2.

of But$_2$A>p as low as 10^{-4} M can still be recognized. In a concentration of 10^{-3} M, But$_2$A>p does not appreciably alter the plating efficiency of the cells, although it increases the generation time of the cells by approximately 30%. At a concentration of 3×10^{-4} M excellent transformation to the fibroblast-like form is achieved and neither the growth rate nor the plating efficiency is affected.

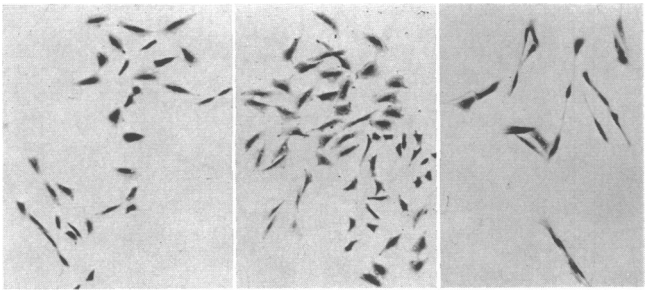

FIG. 3. Synergistic effect of testosterone and But$_2$A>p. Cells were incubated in standard medium for 20 hr with (*left*) But$_2$A>p, 10^{-4} M; (*middle*) testosterone propionate, 1.5×10^{-5} M; (*right*) But$_2$A>p, 10^{-4} M + testosterone, 1.5×10^{-5} M. Magnification, ×125.

FIG. 4. Reversal by colcemid of cell elongation produced by But$_2$A>p. *Left*, control; *middle*, 10^{-3} M But$_2$A>p for 5.0 hr; *right*, 10^{-3} M But$_2$A>p and 6.7×10^{-7} M colcemid for 5.0 hr. Magnification, ×125.

The reaction is completely reversible: On removal of the But$_2$A>p and addition of fresh growth medium to cells converted to the elongated form by a 4-hr exposure to 10^{-3} M of the agent, the cells revert to their original, compact form within approximately 1 hr.

Combined effect of But$_2$A>p and testosterone

Testosterone propionate itself, in any concentration tested, produces no immediate effect on cell elongation. Even after 8 hr of incubation, no cell elongation is evident in the presence of 4.5×10^{-5} M of the hormone, although an elongation effect can be recognized after 3 days of incubation with this concentration. However, simultaneous addition of testosterone and But$_2$A>p yields a highly exaggerated cell elongation, greater than the maximum achievable with either agent alone (Fig. 3). When 1.5×10^{-5} M of testosterone is combined with 3×10^{-5} M of But$_2$A>p, cell elongation clearly results even though each agent singly produces no detectable effect in these concentrations. With 10^{-4} M But$_2$A>p, the action of 1.5×10^{-6} M of testosterone can be recognized. The action of the combination of testosterone and But$_2$A>p is also rapidly and completely reversible.

Effect of colcemid on the transformation

The microtubules have been regarded as playing an important role in the maintenance of cellular morphologic characteristics. The production of a marked cell elongation suggests that the formation of linear structures may be involved. We therefore determined whether the morphologic transformation produced by But$_2$A>p, with or without testosterone, could be prevented by agents that inhibit assembly of cellular microtubules. Colcemid, an inhibitor of the polymerization of microtubular protein (7), was added in a concentration of 6.7×10^{-7} M to cell monolayers along with But$_2$A>p and testosterone. Elongation was prevented (Fig. 4). Vinblastine, another specific inhibitor of microtubule aggregation (8), also prevented the elongation.

DISCUSSION

These experiments demonstrate three processes: an action of But$_2$A>p in converting a compact, nonoriented, noncontact-inhibited cell into an elongated, highly oriented, highly contact-inhibited form; a strongly synergistic action of testosterone propionate on this action; and an antagonistic effect on this action of colcemid and vinblastine, agents that inhibit the assembly of microtubules from their monomeric protein components.

These findings appear relevant to a number of different biological phenomena. Since the $But_2A>p$ can transform cells throughout all or most of their life cycle, its action may simply involve promotion of microtubular assembly whenever the necessary precursors already exist in the cell. In that case this compound may be active in promoting a variety of cellular morphologic transformations that depend upon assembly of microtubules, and may also be useful as an indicator that the necessary precursor molecules are already available. Preliminary experiments, to be described elsewhere, indicate that new protein synthesis is not necessary for this action and that cytochalasin B, a compound that presumably specifically inhibits microfibril formation, also prevents the morphological conversion produced by $But_2A>p$ and testosterone.

The synergistic action of testosterone on this morphologic transformation is of considerable interest. The sensitivity to testosterone of the system consisting of the Chinese hamster ovary culture in the presence of 10^{-4} M $But_2A>p$ exceeds that of any other manifestation known to us of this hormone in tissue culture.

One of the most intriguing aspects of this phenomenon is the reversible restoration of contact inhibition to these cells as a result of the treatment described. Morphological changes of fibroblast-like, contact-inhibited cells to a more compact, randomly oriented, and noncontact-inhibited form have been found in mammalian cells after transformation with polyoma virus, x-irradiation, or certain chemical carcinogens (9). However, the changes produced by these latter agents are not reversible, as is that described here.

The fact that cells can multiply with the same generation time in the compact or elongated form makes it clear that the changes studied here do not affect any of the rate-limiting steps of the life cycle. Undoubtedly, however, other biochemical reactions will be affected by this change. Whether they are the same as those induced by other cell-stretching agents such as fetuin (10) remains to be investigated.

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