THE EFFECT OF INHIBITORS OF RNA AND PROTEIN SYNTHESIS ON DIBUTYRYL CYCLIC AMP MEDIATED MORPHOLOGICAL TRANSFORMATIONS OF CHINESE HAMSTER OVARY CELLS IN VITRO

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Received November 20, 1972;Revised December 5, 1972

Summary Morphological transformations of cultured Chinese hamster ovary cells (CHO-K1) produced by the addition and removal of dibutyryl cyclic AMP plus testosterone are unaffected by inhibition of either RNA synthesis or protein synthesis.

Introduction

In previous papers "reverse transformation" of the Chinese hamster ovary cell from a compact, randomly growing, knobbed, epithelial-like cell to a stretched, spindle-shaped, contact inhibited, fibroblast-like cell by the action of dibutyryl cyclic AMP and testosterone or other hormones has been described. It was also demonstrated that change in the opposite direction (i.e., conversion from fibroblast-like to epithelial-like morphology), "forward transformation," could be effected by simple removal of dibutyryl cyclic AMP plus testosterone or by treatment of fibroblast-like cells with agents which disrupt either the microfibrillar or microtubular structure of the cell, such as colcemide or cytochalasin B (1-3). In the present paper, the results of studies on the function of protein synthesis and/or RNA synthesis in these morphological changes are reported.

Materials and Methods

a) The Chinese hamster ovary cell (CHO-K1) was employed. The method of cultivation and the standard procedure used to determine cell conversion between epithelial-like and fibroblast-like morphologies have been described previously, as has the quantitative method used to score such interconversions (2,3).

b) Measurement of Macromolecule Synthesis. Protein synthesis was measured as the incorporation of ^{14}C-leucine (Schwarz/Mann, 312 mC/mmole) into cold CCl_3COOH-precipitable material. Incorporation was allowed for up to six hours in standard growth medium containing 10^{-4}M leucine plus 0.1 μC ^{14}C-leucine/ml in 35 mm plastic petri dishes. After the incorporation period, medium was removed and 1.0 ml of 0.1NNaOH was added. The samples were then incubated at 37° for 10 minutes after which 1.0 ml of ice-cold 10% CCl_3COOH was added and the samples were kept at 4°C for at least 30 minutes. Samples were then collected by filtration on nitrocellulose membrane filters (Millipore, HAWP, 0.45μ), washed with excess cold 5% CCl_3COOH, dried, and assayed for radioactivity by liquid scintillation counting in a toluene-based scintillation fluid in a Nuclear Chicago Mark I Scintillation Counter.

Incorporation of ^{14}C-uridine (Schwarz/Mann, uridine, 2-^{14}C, 50 mC/mmole) into RNA was measured by a modification of the above method. Uridine was added to standard medium at 10^{-5}M. ^{14}C-uridine was added at 0.3 μC/ml. After the desired period of incorporation, medium was removed and 1.0 ml of 0.5% sodium dodecyl sulfate was added. Processing was then continued as described above.

Experimental Results

1. Inhibition of Macromolecule Synthesis. Table I demonstrates the extent of inhibition of protein synthesis or RNA synthesis obtained, as measured by incorporation of ^{14}C-leucine or ^{14}C-uridine into cold CCl_3COOH precipitable macromolecules. The effect of protein synthesis inhibitors was maximal in less than 30 minutes, while RNA synthesis continued at a reduced rate for up to two hours in the presence of actinomycin D, after which incorporation ceased. Inhibition was equally effective with CHO-K1 cells in either the epithelial-like or fibroblast-like state.

Table I

Inhibition of Macromolecule Synthesis by Various Antibiotics During 6 Hours of Incubation

Antibiotic	^{14}C-Leucine Incorporation	^{14}C-Uridine Incorporation	% of Isotope Incorporation as Compared to Control
0	18,523	20,748	100
Puromycin (100μgm/ml)	206	-	1.1
Pactamycin (10^{-7}M)	608	-	3.3
Cycloheximide (20μgm/ml)	393	-	2.1
Actinomycin D (0.33μgm/ml)		1,432	6.9

2. Demonstration that Inhibitors of Protein Synthesis or RNA Synthesis do not affect Interconversions of the Epithelial-like and Fibroblast-like Morphologies. Figure 1A shows CHO-K1 cells in their normal epithelial-like state, while those cells shown in Figure 1B have been treated for six hours with 2 x 10^{-4}M dibutyryl cyclic AMP plus 5 μg/ml testosterone propionate and show the typical fibroblast-like morphology of reverse transformed CHO-K1 cells.

Results presented in Table II demonstrate that neither protein nor RNA synthesis is necessary for complete expression of dibutyryl cyclic AMP plus testosterone induced reverse transformation. The data presented in Table III demonstrate that "forward transformation," the reversion of dibutyryl cyclic AMP plus testosterone induced fibroblast-like morphology to normal epithelial-like morphology upon removal of the reverse transforming agents, also goes to completion in the absence of protein synthesis.

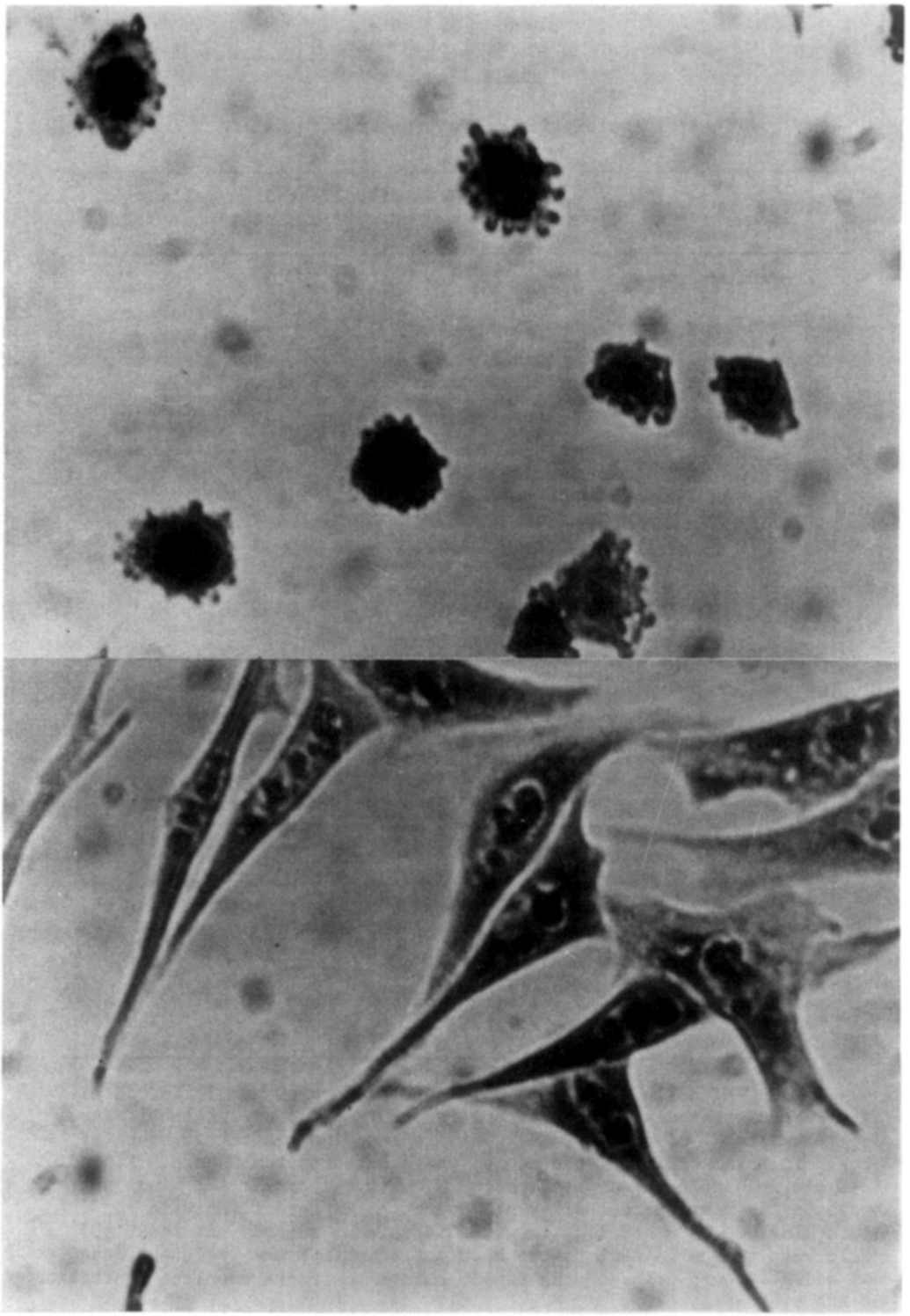

Figure 1A. Normal CHO-K1 cells growing in epithelial-like morphological state.

Figure 1B. CHO-K1 cells treated for six hours with 2 x 10^{-4} M dibutyryl cyclic AMP plus 5 µgm/ml testosterone propionate. Cells were fixed and stained as in Table 1.

Table II

Effect of Antibiotics on Reverse Transformation

Medium	Fibroblast-like Cells (% of Total)
"E"-Medium	14
"F"-Medium	92
"E"-Medium + 10^{-7}M Pactamycin	15
"F"-Medium + 10^{-7}M Pactamycin	95
"E"-Medium + 100µgm/ml Puromycin	11
"F"-Medium + 100µgm/ml Puromycin	88
"E"-Medium + 20µgm/ml Cycloheximide	7
"F"-Medium + 20µgm/ml Cycloheximide	89
"E"-Medium + 0.33µgm/ml Actinomycin D	51*
"F"-Medium + 0.33µgm/ml Actinomycin D	93

*An unusual morphology was often found in these cultures making scoring difficult.

10^4 CHO-K1 cells were allowed to attach and grow at 37° in 0.3 ml of F12 supplemented with the macromolecular fraction of fetal calf serum equivalent to 5% of whole serum. This is E medium. After 18 hours of growth in this condition, the medium was changed to that shown. F. medium is E medium supplemented with 2 x 10^{-4}M dibutyryl cyclic AMP plus 5µgm/ml testosterone propionate. After six hours, cells were fixed with glutaraldehyde, stained lightly with Giemsa, and scored. At least 500 cells were scored in each case. When actinomycin D was used, the drug was added 4 hours prior to the medium change to insure complete inhibition of RNA synthesis.

Table III

Effect of Antibiotics on Forward Transformation

Time of Addition	Addition	Medium Change	Fibroblast-like Cells (% of total)
0	0	E → E	11
0	0	F → F	77
0	0	F → E	14
-30 min.	10^{-7}M Pactamycin	F → E	13
-30 min.	20μgm/ml Cycloheximide	F → E	7
-30 min.	100μgm/ml Puromycin	F → E	8
-4 hr.	0.33μgm/ml Actinomycin D	F → E	9

E medium and F medium are defined in Table II. 2×10^4 CHO-K1 cells were deposited in 35 mm plastic petri dishes and allowed to attach for 4 hrs. Then 2.0 ml of the appropriate medium (either E or F medium) was added for 16 hours. At this time the appropriate medium change was carried out. The time of medium change was zero time. Scoring was carried out after 6 hours of incubation. At least 500 cells were scored in each case.

Discussion

The simplest interpretation of these experiments is that both reverse transformation and forward transformation require no new protein synthesis but involve realignment of the pre-existing microtubular-microfibrillar system so as to provide the observed morphological changes. The involvement of the microtubular-microfibrillar system was suggested by the inhibition of and indeed reversal of reverse transformation which can be brought about by treatment of the cells with agents such as colcemide or cytochalasin B (1,3).

The possibility that the small amount of residual macromolecule synthesis observed in the conditions employed here is responsible for

the observed morphological changes is unlikely for the following reasons: 1) Protein synthesis can be inhibited by greater than 99.5% with 10^{-6}M pactamycin and reverse transformation is still observed. 2) Protein synthesis inhibitors can be added at least 60 minutes before reverse transformation is induced with no morphologically observable effect. 3) Actinomycin D can be added up to 16 hours prior to reverse transformation with no effect. The results reported here have also been confirmed using a technique allowing measurement of morphological change and measurement of macromolecule synthesis on the same cells and also by the use of time-lapse photomicrography (3,4). These results are consistent with preliminary results reported previously by this laboratory (2) and with reports from other laboratories working on different morphological transformations (5,6). However, in at least two cases in which different cells were employed a contradictory result has been reported (7,8).

We should point out that these results demonstrate only that morphological changes in response to the addition or removal of dibutyryl cyclic AMP plus testosterone can occur in the absence of protein or RNA synthesis. These results do not bear upon the question of whether the presence or absence of dibutyryl cyclic AMP (or dibutyryl cyclic AMP plus hormone) causes either a quantitative or a qualitative change in the complement of proteins synthesized by CHO-K1 cells which does not alter the cell morphology. This point is under investigation.

Acknowledgements: This work was aided by USPHS Grant 5-P01 HD02080 and by a Fellowship to D.P. from the Damon Runyon Memorial Fund No. DRF-662-AT. This publication is from the Eleanor Roosevelt Institute for Cancer Research and the Department of Biophysics and Genetics (No. 515), University of Colorado Medical Center, Denver, Colorado.

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