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Differential expression of type I and type II cyclic AMP-dependent protein kinases during cell cycle and cyclic AMP-induced growth arrest

(phosphorylation/Chinese hamster ovary cell/Rat-1 cell/viral transformation)

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ABSTRACT **The activation state of cyclic AMP-dependent protein kinase(s) (ATP:protein phosphotransferase, EC 2.7.1.37) is transiently increased 2-fold as a function of G_1 progression in mitotically synchronized Chinese hamster ovary cells. The cellular content of type I kinase increases concomitantly with the increase in general protein, whereas the activity of type II kinase increases as a function of time in G_1 to a maximum at the G_1/S border. In contrast, in the presence of dibutyryl-cyclic AMP, there is a decrease of type II kinase and a several-fold increase of type I kinase. In proliferating cells, the ratio of type I to type II was 0.37, while in the dibutyryl-cyclic AMP growth-arrested cells it was 3.96. The increase in type II kinase during G_1 transition and the increase in type I kinase during dibutyryl-cyclic AMP treatment were dependent on protein synthesis. A similar pattern of type I and type II kinase expression during cell cycle progression occurred in Rat-1 fibroblasts and Rat-1 cells transformed by Rous sarcoma virus. The inclusion of dibutyryl-cyclic AMP in the growth media promoted a marked increase in type I holoenzyme, which was inhibited by cycloheximide, and a decrease in type II kinase. Neither AMP nor sodium butyrate had any effect on cellular kinase levels, whereas 8-bromo-cyclic AMP mimicked the action of dibutyryl-cyclic AMP. Estimation of half-lives for the kinase types showed that there was little turnover of either type during normal G_1 progression, rapid turnover of both types as cells exited from mitosis, and selective turnover of type II upon addition of dibutyryl-cyclic AMP.**

Abnormally high cellular cyclic AMP (cAMP) concentrations caused by the addition of analogs of cAMP or phosphodiesterase inhibitors result in the arrest of cellular growth (reviewed in ref. 1). However, the addition of moderate concentrations may facilitate the growth of some cells (2–7). Furthermore, fluctuations in the cellular content of both cAMP (8–12) and its specific protein kinases (ATP:protein phosphotransferase, EC 2.7.1.37) (11–14) occur as a normal component of cell cycle traverse. In nondividing cells, cAMP has been implicated as the second messenger for trophic hormone action, mediating increases in general or specific protein and RNA synthesis (reviewed in refs. 15 and 16). The trophic process by which a cell increases its mass through increased RNA and protein synthesis is an essential aspect of the cell cycle in dividing cells, constituting the major events related to G_1 progression (16, 17). It appears, therefore, that there exists some major difference between the intracellular events promoted by high exogenously derived cellular cAMP content, which lead to growth arrest, and those that occur as the result of trophic hormone- or growth factor-triggered physiological changes in cAMP concentrations, which are associated with a growth-stimulatory response.

Two types of cAMP-dependent protein kinase exist (18); they contain the same catalytic subunit but have been postulated to play different functional roles as a result of their different activation and regulatory characteristics and intracellular localizations, which are a function of differences in the regulatory subunit (reviewed in ref. 19). The two types are differentially activated by trophic hormones (14, 20, 21) and differentially synthesized during changing developmental (22, 23) or endocrine (24) status or during hypertrophy (25). Therefore, we examined the activation, synthesis, and turnover of cAMP-dependent protein kinase in fibroblasts that were either proliferating or had their growth arrested by dibutyryl-cAMP (Bt_2cAMP) to see if any major differences in these primary effectors for cAMP action could be elucidated that could explain the apparently opposing consequences ascribed to the action of the cyclic nucleotide in proliferating cells.

MATERIALS AND METHODS

Cell Cultures. Chinese hamster ovary (CHO) cells were grown in monolayer in McCoys 5A medium/20% fetal calf serum (GIBCO) and were synchronized by selective detachment of cells blocked in metaphase by Colcemid addition as described (26). There was a G_1 phase of *ca.* 5 hr before the onset of DNA synthesis, as determined by [^{3}H]thymidine incorporation and autoradiography.

Untransformed Rat-1 cells and Rat-1 cells transformed by the B77 wild-type Rous sarcoma virus [Rat-1(wt/RSV)] were grown in monolayer in Dulbecco's modification of Eagle's medium/10% fetal calf serum (Flow Laboratories) and were synchronized in a nondividing state by a period of serum deprivation as described (27). Addition of fresh serum-containing media stimulated the cells to begin replicating their DNA after a lag of *ca.* 8 hr corresponding to G_1 phase.

Determination of the Activation Ratio of Cyclic AMP-Dependent Protein Kinase Activities. Suitable conditions for assessing the activation state of the total soluble cAMP-dependent protein kinase were determined after incubating cell monolayers 30 min at 37°C in the presence of 1 mM 3-isobutyl-1-methyl-xanthine (IBMX) to increase the intracellular cAMP levels prior to sonication in extraction buffer containing various concentrations of KCl. The inclusion of 100 mM KCl was found to result in the lowest control activity ratio (0.4) and preserve the highest IBMX-stimulated state (0.85).

Cells (2×10^6) were scraped, pelleted, and sonicated (twice for 30 s each) at 4°C in 500 μl 10 mM KPO_4 (pH 7.0) containing 5 mM NaF, 2 mM EDTA, 0.5 mM IBMX, and 100 mM KCl. A 50-μl aliquot of a $10,000 \times g$ (5 min) supernatant was assayed for endogenously activated protein kinase by incubating for 5 min at 30°C in a total volume of 75 μl containing 50 mM KPO_4

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Abbreviations: cAMP, adenosine 3′,5′-cyclic monophosphate; Bt_2-, dibutyryl-; 8-Br-, 8-bromo-; CHO, Chinese hamster ovary; RSV, Rous sarcoma virus; IBMX, 3-isobutyl-1-methylxanthine; Cyh, cycloheximide.

(pH 7.0), 0.5 mM IBMX, 5 mM NaF, 10 mM $MgCl_2$, 50 μg of F_2b histone, 0.1 mM ATP, and 1 μCi (3.7×10^4 becquerels) of [γ-^{32}P]ATP. Total protein kinase activity was assayed by including 10 μM cAMP in the reaction. Incorporation of ^{32}P into protein was determined by chromatography of a 50-μl aliquot of the reaction on silica gel-impregnated glass fiber sheets (28). The reactions were linear with respect to time and the number of cells extracted, and activation was inhibited more than 90% by the inclusion of the specific heat-stable inhibitor of cAMP-dependent protein kinase (29).

Determination of Cyclic AMP-Dependent Protein Kinase Holoenzyme. Cells ($5–10 \times 10^6$) were scraped, pelleted, and sonicated at 4°C in 1 ml of 5 mM Tris·HCl, pH 7.5, containing 2 mM EDTA and 3 mM NaF. The sonicates were centrifuged at $10,000 \times g$ for 5 min, and 0.7 ml of the resulting supernatant was applied to a DEAE-cellulose column (0.7×14 cm), which was washed with 20 ml of buffer and eluted with a linear gradient of 0–0.35 M NaCl in equilibration buffer, total volume 30 ml, as described (23).

Fig. 1 shows the elution profiles on DEAE-cellulose of cAMP-dependent protein kinase activity of CHO cell cytosol from early G_1 (1 hr after mitosis) and G_1/S (5 hr). Protein kinase type I eluted from the column between 0.06 and 0.12 M NaCl and type II eluted between 0.2 and 0.25 M NaCl. The elution profile of free catalytic subunit (0.015–0.035 M NaCl) did not overlap with that of type I enzyme. The completeness of reassociation of the kinase holoenzymes is demonstrated by the lack of any kinase activity eluting during the sample application, wash, or initial gradient fractionation. Both eluted peaks of kinase activity represented cAMP-dependent reassociated holoenzyme; they were dependent on added cAMP for maximal activity (8- to 20-fold stimulation) and were inhibited (more than 90%) by the addition of the specific heat-stable inhibitor of cAMP-dependent protein kinase (29). After chromatography,

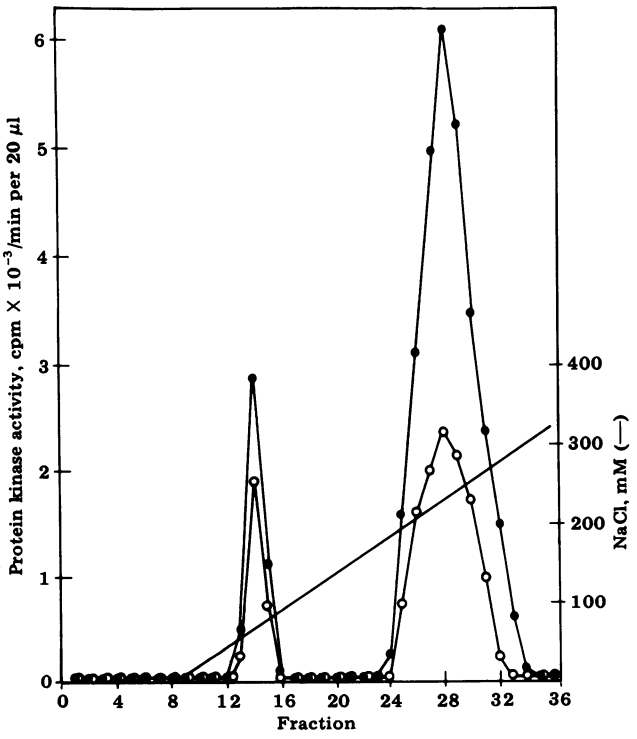

FIG. 1. DEAE-cellulose chromatography of cAMP-dependent protein kinase from CHO cells. Synchronized cells were allowed to progress for 1 hr (○; early G_1) or 5 hr (●; G_1/S) after mitotic release before being extracted, chromatographed, and assayed for phosphotransferase activity.

120–145% of the supernatant kinase activity was consistently recovered. Total activity of the two types of kinase was calculated by determination of the kinase activity (pmol/min) eluted in the respective peak, corrected for salt inhibition (type I, 40%; type II, 50%), and expressed per the number of cells or mg of protein applied in the original supernatant to the column. Protein was determined according to the method of Bradford (30).

Chemicals. cAMP, Bt_2cAMP, AMP, ATP, butyric acid, and histone F_2b were obtained from Sigma; IBMX from Aldrich; DEAE-cellulose (DE52) from Whatman; [γ-^{32}P]ATP from New England Nuclear; and the silica gel-impregnated glass fiber sheets (ITLC) from Gelman (Ann Arbor, MI).

RESULTS

Changes in Activation Ratio and Type of cAMP-Dependent Protein Kinase During CHO Cell Cycle Progression and Bt_2cAMP Growth Arrest. The activity ratio of soluble cAMP-dependent protein kinase increased from 0.4 to *ca.* 0.8 as a function of G_1 progression in mitotically synchronized CHO cells and then decreased below 0.4 near the G_1/S transition (Fig. 2*A*, *Inset*). The total amount of type I and type II activity also changed during cell cycle progression, fluctuating in an independent fashion (Fig. 2*A*). Type I kinase increased as a function of time to a cellular content at mitosis of *ca.* 300% that found in early G_1 cells (206.0 vs. 68.6 pmol/min per 10^6 cells, respectively). The consistency of the specific activity of type I indicated little change throughout the cell cycle (Table 1). In contrast, type II increased as a function of G_1 progression to a peak at the G_1/S border, followed by another increase in activity during late S and G_2 phase (Fig. 2*A*). At mitosis, the cellular content of type II kinase was 4 times greater than in early G_1 (375 vs. 108 pmol/min per 10^6 cells, respectively). The highest specific activity of type II occurred at the G_1 to S phase transition, and then decreased to an activity at mitosis twice that present during early G_1 (Table 1).

After the addition of 1 mM Bt_2cAMP, the activation state of the total cAMP-dependent protein kinase in the blocked cells increased and remained elevated throughout the 5 hr tested (Fig. 2*B*, *Inset*). Addition of the cAMP analog resulted in a selective disappearance of type II kinase and the marked accumulation of type I (Fig. 2*B*). Both changes were initiated within 2 hr. After 5 hr of incubation, type II had declined to 50% of the original postmitotic value, an activity lower than that detectable at any time during normal cell cycle progression. At the same time, type I increased to 300% of the original level,

Table 1. Type I and type II cAMP-dependent protein kinase expression during CHO cell cycle progression and cAMP growth arrest

Cells	Time, hr	Kinase specific activity, pmol/min per mg protein		I/II ratio
		Type I	Type II	
Cycling	2	668	836	0.8
	5	674	1816	0.37
	9	571	1120	0.51
	12	858	1534	0.56
+ Bt_2cAMP	2	724	551	1.32
	5	1307	330	3.96
	9	1466	300	4.89
	12	1413	320	4.42
+ AMP	5	668	1790	0.37
+ Na butyrate	5	680	1830	0.36

Specific activities of kinases in CHO cells plated for the times indicated after mitosis were determined by DEAE-cellulose chromatography.

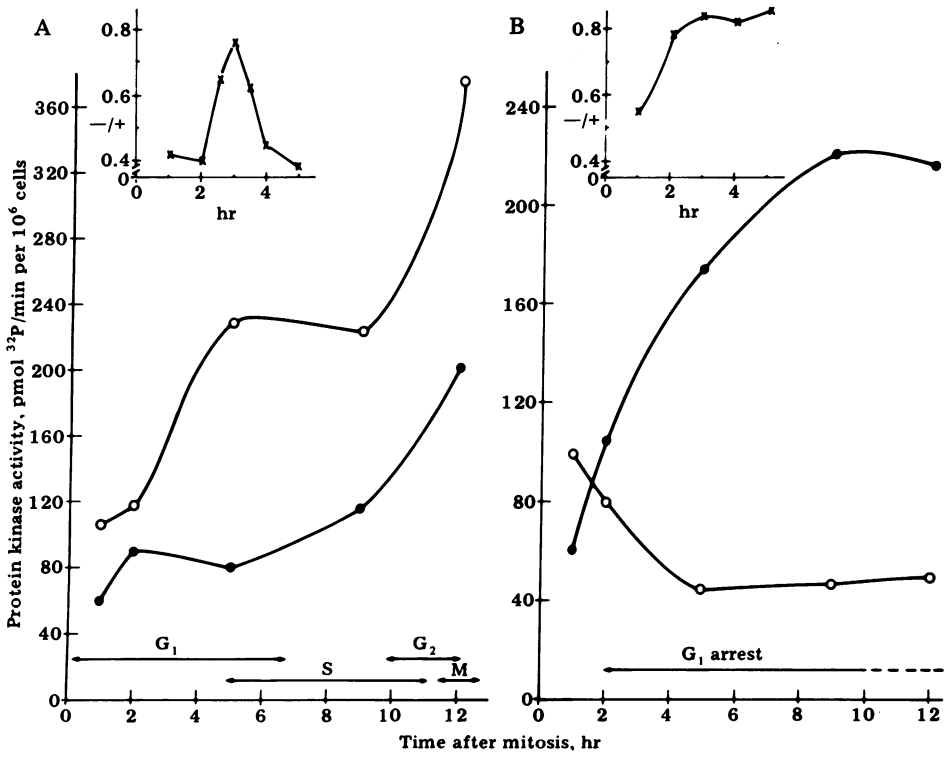

FIG. 2. Expression of cAMP-dependent protein kinase during cell cycle progression and Bt_2cAMP-induced growth arrest. Mitotically synchronized cells were plated in the presence (*B*) or absence (*A*) of 1 mM Bt_2cAMP. At the times indicated, cells were extracted and chromatographed on DEAE-cellulose to determine the pool sizes of type I (●) and type II (○) kinases. (*Inset*) At the times indicated, cells were extracted in the presence of 100 mM KCl and assayed for activated (−cAMP) and total (+cAMP) protein kinase activity. The −/+ ratio gives the fraction of the total cellular cAMP-dependent kinase in an activated state.

an activity greater than that reached in the cycling cell. These opposing changes were also demonstrable when the holoenzyme activities were expressed per mg of soluble cellular protein (Table 1). Thus, at a time when the untreated cells are entering S phase (5 hr), the balance between the two kinase types has been inverted (Table 1). In the proliferating cells, type II kinase predominates over the activity of type I so that the type I-to-type II ratio is 0.37. In the growth-arrested cells the content of type I is greater than that of type II so that the type I-to-type II ratio is 3.96. The decreased content of type II was evident throughout 12 hr of incubation, while type I holoenzyme continued to increase. Neither AMP nor sodium butyrate had any effect on the activity of either type of kinase (Table 1).

Although the changes in the types of cAMP-dependent protein kinase after Bt_2cAMP addition were dramatic, the total cellular contents of cAMP-dependent phosphotransferase activity were similar in cycling and arrested cells. Therefore, the possibility of an interconversion of the regulatory subunits resulting in the changes in the kinase holoenzyme types was considered. However, as shown in Table 2, the increases in kinase holoenzyme types during both normal G_1 transition and the Bt_2cAMP-arrested state were a result of apparent protein synthetic events. The inclusion of cycloheximide (Cyh) in the culture media blocked both the increase in type II that occurred at the G_1-to-S phase transition and the increase in type I stimulated by the addition of Bt_2cAMP. In contrast, the decrease in type II promoted by the cAMP analog was not affected by the protein synthesis inhibitor.

Alterations in Kinase Holoenzymes as a Function of Cell Cycle and Bt_2cAMP Growth Arrest in Normal and Transformed Rat-1 Cells. Rat-1 fibroblasts and their RSV-transformed counterparts were synchronized in a nongrowing state by a period of serum deprivation and stimulated to progress through the cell cycle by the addition of serum-containing medium. Rat-1 fibroblasts in the serum-deprived state have comparable amounts of the two holoenzymes, but, during the 8-hr lag after serum addition before the fibroblasts start to synthesize DNA, the cellular content of type II kinase increased 360% while that of type I increased 35%. Similar cells transformed by RSV have a greater cellular content of both types of kinase in the serum-deprived state (Fig. 3*B*). This increased amount of phosphotransferase activity is also apparent when calculated on the basis of cellular protein; the transformed cells contained 3 nmol/min per mg protein of type I and 7.1 of type II, while the wild-type had 1.1 and 1.3, respectively. A further difference in the transformed fibroblast was a high type II holoenzyme content relative to type I maintained during the serum-deprived state with no further increase in type II during G_1 transition.

The kinase holoenzyme changes in both the normal and transformed Rat-1 fibroblasts after the addition of Bt_2cAMP are similar to those seen in CHO cells. The inclusion of the cAMP analog in the serum-containing medium promoted a 2.5-fold increase in type I kinase in Rat-1 cells, while the cellular content of type II decreased to 36% of that after serum alone (Table 3, experiment A). Extraction of the particulate fraction from control or Bt_2cAMP-treated cells with 0.2% Triton X-100 (31) solubilized only an additional 2% of the total cAMP-dependent protein kinase activity as assessed by inhibition by the

Table 2. Increases in type I and type II protein kinase during CHO cell cycle or Bt_2cAMP growth arrest are dependent on protein synthesis

Cell cycle phase	Time, hr	Kinase activity, pmol/min per 10^6 cells	
		Type I	Type II
Early G_1	1	68.6	108.0
G_1/S	5	84.2	227.6
G_1/S + Cyh	5	60.0	115.9
Bt_2cAMP arrest	5	185.2	54.4
Bt_2cAMP arrest + Cyh	5	92.0	61.5

Cells were incubated after mitotic exit in the presence of 1 mM Bt_2cAMP or Cyh at 25 μg/ml prior to fractionation by DEAE-cellulose chromatography.

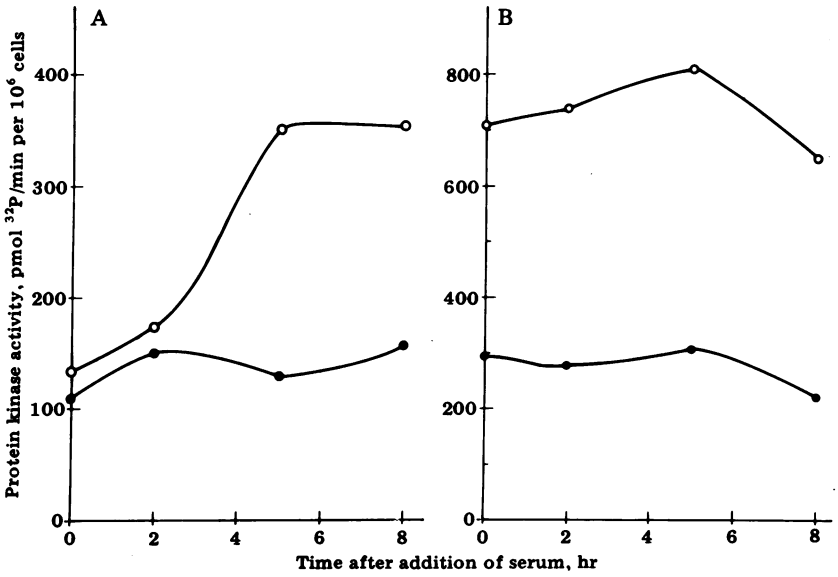

FIG. 3. cAMP-dependent protein kinase during G_1 progression of normal and transformed cells. Quiescent Rat-1 (*A*) and RSV-transformed Rat-1 (*B*) cells were stimulated by the addition of fresh serum-containing media. At the times indicated, cells were extracted and chromatographed on DEAE-cellulose to determine the pool sizes of type I (●) and type II (○) kinases.

specific heat-stable inhibitor (29). Therefore, the decrease in detectable type II kinase activity after Bt_2cAMP was not the result of the transfer of either the holoenzyme or catalytic subunit to a particulate cellular fraction. The increase in type I was blocked by the inclusion of Cyh and partially inhibited by the addition of actinomycin D. However, the decrease in type II was even more pronounced in the presence of inhibitors (11% of control). The increase in type II during normal G_1 progression of the Rat-1 cell also was inhibited by Cyh or actinomycin D. Type I kinase increased in the transformed Rat-1 cells after Bt_2cAMP addition, although to a lesser extent, while type II holoenzyme was markedly decreased (Table 3, experiment B). The increase in type I was blocked by Cyh but not actinomycin D. In both cell types, the addition of 8-BrcAMP mimicked the effect of Bt_2cAMP in increasing type I and decreasing type II.

Table 3. Bt_2cAMP-stimulated synthesis of type I and turnover of type II cAMP-dependent protein kinase in Rat-1 and Rat-1(wt/RSV) cells

Cells	Kinase activity, pmol/min per 10^6 cells: Type I	Type II	I/II ratio
Experiment A (Rat-1 cells)			
Quiescent	102	132	0.77
Serum	134	262	0.52
+ Cyh	84	120	0.70
+ actinomycin D	64	82	0.78
Serum + Bt_2cAMP	274	94	2.92
+ Cyh	104	30	3.47
+ actinomycin D	190	24	7.92
Serum + 8-BrcAMP	340	92	3.70
Experiment B [Rat-1(wt/RSV) cells]			
Quiescent	300	710	0.42
Serum	225	640	0.35
Serum + Bt_2cAMP	415	120	3.45
+ Cyh	236	51	4.63
+ actinomycin D	488	171	2.85
Serum + 8-BrcAMP	402	107	3.76

Quiescent Rat-1 and Rat-1(wt/RSV) cells or cells incubated for 8 hr after the addition of fresh serum-containing media in the presence of 1 mM Bt_2cAMP, 8-BrcAMP, 25 μg of Cyh per ml, or 10 μg of actinomycin D per ml where indicated were extracted and chromatographed on DEAE-cellulose to determine the pool sizes of type I and type II kinases.

Turnover of cAMP-Dependent Protein Kinase Holoenzymes. The rapid disappearance of type II kinase after the addition of Bt_2cAMP suggested that under certain conditions there could be selective turnover of the kinase holoenzymes. This possibility was evaluated in mitotic cells released from Colcemid block, G_1 transiting cells, and Bt_2cAMP-arrested cells as shown in Table 4. After release of CHO cells from mitosis, the specific activity of both types of kinase decreased by 2 half-lives within 60 min (experiment A). In cells plated to progress through the cycle to which Cyh was added at 1 hr, there was little turnover of either holoenzyme during a subsequent 4-hr incubation (experiment B). There also was little turnover of type I kinase when the Cyh was added after Bt_2cAMP; however, during the same time, the type II kinase decreased to less than ⅓ (experiment C), giving an estimated half-life of, at most, 2 hr. Similar independent changes in half-lives for the two kinases could be calculated for Rat-1 cells during G_1 transition or growth arrest (experiment D).

Table 4. Turnover rates of type I and type II cAMP-dependent protein kinases during different cell cycle stages

Cells	Time, hr	Type I activity*	$t_{1/2}$,† hr	Type II activity*	$t_{1/2}$,† hr
Experiment A					
CHO	0	206		425	
	1	52	0.5	133	0.58
Experiment B					
CHO	1	68.6		108	
+ Cyh	5	65	None	117	None
Experiment C					
CHO	1	93.6		158	
+ Bt_2cAMP + Cyh	5	113.6	None	43.4	2
Experiment D					
Rat-1	4	120		252	
+ Cyh	8	102	>10	162	6.5
+ Bt_2cAMP + Cyh	8	128	None	30	1.3

Control and 1 mM Bt_2cAMP-treated cells were incubated for the times indicated after mitosis (CHO cells) or serum addition (Rat-1 cells) in the presence or absence of Cyh at 25 μg/ml.

* Activity as determined by DEAE-cellulose chromatography and expressed as pmol/min per 10^6 cells.

† Half-life estimated as a minimal value based on the loss in holoenzyme activity during the cellular incubation under the indicated conditions.

DISCUSSION

This study provides evidence that changes in the cellular cAMP level may regulate the differential synthesis and turnover of type I and type II cAMP-dependent protein kinase. The addition of a cAMP analog to cultured fibroblasts results in growth arrest concomitant with a marked increase in the amount of type I kinase and a simultaneous decrease in the amount of type II. This phenomenon is not restricted to one cell type: it occurred in CHO cells, in Rat-1 cells, and in RSV-transformed Rat-1 cells. The addition of exogenous Bt_2cAMP to neuroblastoma cells in culture has previously been shown to increase the cellular content of cAMP-binding proteins (32, 33). Recently, this increase has been shown to be specific to the type I regulatory subunit and to occur in the absence of any increase in the catalytic subunit (34). Cho-Chung and coworkers (35, 36) have shown in an estrogen-dependent mammary tumor that administration of Bt_2cAMP or ovariectomy causes an induction of type II kinase in association with tumor regression.

The expression of type I and type II kinase in response to the addition of a cAMP analog in G_1 is markedly different from that during normal cell cycle progression. The cAMP-promoted changes result in the almost complete inversion of the expression of type I and type II kinase. These studies do not eliminate the possibility that the changes detected in holoenzyme pool size might be the result of changes in cochromatographing modifiers (i.e., activators or inhibitors) of the kinase or of changes in affinity of the specific types for either cAMP or histone. However, the chromatographic system employed will separate the one known inhibitor of the kinase and, to date, changes in the kinetic constants of the enzyme have not been shown to occur in other cell types.

cAMP is well established as a second messenger for the action of trophic hormones on differentiated cells, acting as a positive regulator of the process by which the cell increases general or specific protein and RNA synthesis (15, 16). The trophic process is also an essential aspect of the cell cycle of growing cells, in which it is coupled with DNA synthesis to coordinate cell doubling prior to division. However, the elucidation of cAMP as a positive effector of trophic processes in proliferating cells has been complicated by the fact that it can function as a negative effector of progression of cells into or through the DNA synthetic phase. The mechanism for exerting two effector roles for cAMP apparently lies in the temporally discrete activation of the appropriate type of kinase. Concanavalin A stimulation of human lymphocytes results in the selective activation of only type I kinase during pre-S phase, whereas the simultaneous activation of both type I and type II as the result of concomitant Bt_2cAMP addition was associated with an inhibition of DNA synthesis (14). In concanavalin A-stimulated mouse lymphocytes, a pre-S phase increase in cAMP is absolutely required for the proliferative response (12). However, if the elevated endogenous level of the cyclic nucleotide is prolonged by 8-BrcAMP addition, cells are blocked prior to S phase. The studies cited above and the data from the present study strongly suggest that type I kinase and type II kinase can exert different influences on the proliferative response. External manipulation of activation can be positive or negative to growth control as a function of the amounts of each type of kinase present initially or the alterations in relative amounts in response to the administration of cAMP analogs.

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1. Friedman, D. L., Johnson, R. A. & Zeilig, C. E. (1976) in *Advances in Cyclic Nucleotide Research*, eds. Greengard, P. & Robison, G. A. (Raven, New York), Vol. 7, pp. 69–111.
2. MacManus, J. P. & Whitfield, J. F. (1970) *Exp. Cell Res.* **58**, 188–191.
3. Filosa, S., Pictet, R. & Rutter, W. J. (1975) *Nature (London)* **257**, 702–705.
4. Raff, M. C., Hornby-Smith, A. & Brockes, J. B. (1978) *Nature (London)* **273**, 672–673.
5. Short, J., Tsukada, K., Rudert, W. A. & Lieberman, I. (1975) *J. Biol. Chem.* **250**, 3602–3603.
6. Green, H. (1978) *Cell* **15**, 801–811.
7. Marcelo, C. L. (1979) *Exp. Cell Res.* **120**, 201–210.
8. MacManus, J. P., Franks, D. J., Youdale, T. & Braceland, B. M. (1972) *Biochem. Biophys. Res. Commun.* **49**, 1201–1207.
9. Hibasami, H., Tanaka, M., Nagai, J. & Ikeda, T. (1977) *Aust. J. Exp. Biol. Med. Sci.* **55**, 379–383.
10. Zeilig, C. E., Johnson, R. A., Sutherland, E. W. & Friedman, D. (1974) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **33**, 1391.
11. Costa, M., Gerner, E. W. & Russell, D. H. (1978) *Biochim. Biophys. Acta* **538**, 1–10.
12. Wang, T., Sheppard, J. R. & Foker, J. E. (1978) *Science* **201**, 155–157.
13. Costa, M., Gerner, E. W. & Russell, D. H. (1976) *J. Biol. Chem.* **251**, 3313–3319.
14. Byus, C. V., Klimpel, G. R., Lucas, D. O. & Russell, D. H. (1977) *Nature (London)* **268**, 63–64.
15. Jungmann, R. A. & Russell, D. H. (1977) *Life Sci.* **20**, 1787–1798.
16. Russell, D. H. & Haddox, M. K. (1979) in *Advances in Enzyme Regulation*, ed. Weber, G. (Oxford Univ. Press, London), Vol. 17, pp. 61–87.
17. Prescott, D. M. (1976) *Reproduction of Eukaryotic Cells* (Academic, New York).
18. Corbin, J. D., Keely, S. L. & Park, C. R. (1975) *J. Biol. Chem.* **250**, 218–225.
19. Nimmo, H. G. & Cohen, P. (1977) *Adv. Cyclic Nucleotide Res.* **8**, 145–226.
20. Schwoch, G. (1978) *Biochem. J.* **170**, 469–477.
21. Podesta, E. J., Dufau, M. L., Solano, A. R. & Catt, K. J. (1978) *J. Biol. Chem.* **253**, 8994–9001.
22. Lee, P. C., Radloff, D., Schweppe, J. S. & Jungmann, R. A. (1976) *J. Biol. Chem.* **251**, 914–921.
23. Haddox, M. K., Roeske, W. R. & Russell, D. H. (1979) *Biochim. Biophys. Acta* **585**, 527–534.
24. Fuller, D. J. M., Byus, C. V. & Russell, D. H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 223–227.
25. Byus, C. V., Chubb, J. M., Huxtable, R. J. & Russell, D. H. (1976) *Biochem. Biophys. Res. Commun.* **73**, 694–702.
26. Haddox, M. K., Scott, K. F. F. & Russell, D. H. (1979) *Cancer Res.* **39**, 4930–4938.
27. Haddox, M. K., Magun, B. E. & Russell, D. H. (1980) *Cancer Res.* **40**, 604–608.
28. Huang, K. P. & Robinson, J. C. (1976) *Anal. Biochem.* **72**, 593–599.
29. Walsh, D. A., Ashby, C. D., Gonzalez, D., Calkins, D., Fischer, E. H. & Krebs, E. G. (1971) *J. Biol. Chem.* **246**, 1977–1985.
30. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
31. Corbin, J. D. & Keely, S. L. (1977) *J. Biol. Chem.* **252**, 910–919.
32. Prashad, N. & Rosenberg, R. N. (1978) *Biochim. Biophys. Acta* **539**, 459–469.
33. Prasad, K. N., Sinha, P. K., Sahu, S. K. & Brown, J. L. (1976) *Cancer Res.* **36**, 2290–2296.
34. Walter, Y., Costa, M. R. C., Breakefield, X. O. & Greengard, P. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3251–3255.
35. Cho-Chung, Y. S., Clair, T. & Zubialde, J. P. (1978) *Biochem. Biophys. Res. Commun.* **85**, 1150–1155.
36. Bodwin, J. S., Clair, T. & Cho-Chung, Y. S. (1978) *Cancer Res.* **38**, 3410–3413.