Polyamines and Cellular Adenosine 3':5'-Cyclic Monophosphate

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The effect of polyamines on the cellular concentrations of cyclic AMP was studied. It was shown that 1 μ M-spermine caused a decrease in cyclic AMP in chick-embryo heart cells, chick-embryo fibroblasts, neuroblastoma, glioma and neuroblastoma–glioma hybrid cells, grown in culture. A similar decrease was observed when polyamines were added to cells in the presence of a phosphodiesterase inhibitor or after stimulating the cells with various hormones. Noradrenaline was used in cultures of heart cells, prostaglandin E₁ and adenosine for neuroblastoma and neuroblastoma–glioma hybrids, whereas isoproterenol was used for the stimulation of glioma cells. Polyamines at higher concentrations were either without effect or caused a slight increase in cyclic AMP. Spermidine (10 μ M) also caused a decrease in cellular cyclic AMP, as did 0.1 μ M-putrescine. It is suggested that the effect of polyamines on cellular cyclic AMP may be explained by the effect of these polycations on the activity of cellular phosphodiesterase.

The naturally occurring polyamines spermine and spermidine and the diamine putrescine are widely distributed in biological material (Cohen, 1971; Bachrach, 1973) and are currently considered to play an important role in regulating growth, development and function of various types of cells (Caldarera & Moruzzi, 1970; Heby *et al.*, 1975a; Tabor & Tabor, 1976; Jänne *et al.*, 1978). Small changes in growth rates, progression through the mitotic cycle and responses to various hormones are frequently accompanied by striking alterations in the activity of the biosynthetic enzymes (Russell *et al.*, 1976; Clò *et al.*, 1976). The activity of ornithine decarboxylase (EC 4.1.1.17), the rate-limiting enzyme that catalyses the synthesis of putrescine, has been shown to increase markedly in response to factors that stimulate cell growth (Morris & Fillingame, 1974). The activity of ornithine decarboxylase also declines during growth cessation and when synthesis of proteins or nucleic acids is arrested (Williams-Ashman *et al.*, 1972; Heby *et al.*, 1975b). Other studies indicated that in many systems cyclic AMP is involved in the induction of ornithine decarboxylase, mainly after the administration of various hormones that activate adenylate cyclase, or other agents that cause the increase in cellular cyclic AMP (Byus & Russell, 1975).

On the other hand, the mechanisms that lead to the decrease in ornithine decarboxylase activity, and subsequently to the arrest of growth, remain obscure. It is tempting to speculate that polyamines, the products of ornithine decarboxylase, regulate cellular cyclic AMP concentrations and thereby exert a self-regulatory effect.

In this paper we show that polyamines may indeed be involved in the control mechanism of cellular cyclic AMP concentrations, and that exogenous polyamines, at low concentrations, in the range of those found in physiological fluids (Shimizu *et al.*, 1965; Bachrach, 1973; Jänne *et al.*, 1978), cause a decrease in cyclic AMP. This has been demonstrated in various cultures of normal and neoplastic cells, after the administration of serum, hormones and phosphodiesterase inhibitors.

Materials and Methods

Reagents

The following chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.): putrescine dihydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, 1-noradrenaline bitartrate, adenosine, 3-isobutyl-1-methylxanthine, DL-isoproterenol hydrochloride, hypoxanthine, aminopterin and thymidine. Prostaglandin E₁ was kindly provided by Dr. John Pike, Upjohn Co. (Kalamazoo, MI, U.S.A.). Cyclic [³H]AMP (34.4 Ci/mmol) was from New England Nuclear Corp. (Boston, MA, U.S.A.); reagents for cyclic AMP assay were from The Radiochemical Centre (Amersham, Bucks., U.K.; cyclic AMP kit, TRK 432). Sterile plastic dishes were from Falcon Plastic Co. (Los Angeles, CA, U.S.A.); Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] was from Eurobio Laboratories (Paris, France). Horse serum, calf serum and foetal calf serum were from Bio-Lab (Jerusalem, Israel). Dulbecco's

modified Eagle's minimum medium (cat. no. H-21), Eagle's minimum essential medium (cat. no. F-15) and medium 199 (cat. no. E-11) were from Grand Island Biological Co. (Grand Island, NY, U.S.A.).

Cell cultures

Rat glioma clone C6-BU-1 subcultures and N 115 clonal-line subcultures of mouse neuroblastoma C 1300 were kindly provided by Dr. M. Nirenberg, and grown in 60 mm dishes in Dulbecco's medium containing 5% calf serum (glioma) or 10% foetal calf serum (neuroblastoma), in a humidified atmosphere of CO₂/air (1:19), at 37°C.

Neuroblastoma × glioma hybrid NG 108-15, which was also kindly provided by Dr. M. Nirenberg, was cultured in 60 mm dishes in Dulbecco's medium, supplemented with 10% foetal calf serum, 0.1 mM-hypoxanthine, 1 μ M-aminopterin and 16 μ M-thymidine to prevent proliferation of the parent cells. The cultures were kept at 37°C, in CO₂/air (1:19).

Primary beating-heart-cell cultures, obtained from 8-10-day chick embryos as described by DeHaan (1967), were grown at 37°C in 90 mm dishes in 4.0 ml of Eagle's medium, containing 10% calf serum, 10% tryptose phosphate broth and 25 mM-Hepes, pH 7.4.

Secondary chick-embryo fibroblasts were prepared as previously described (Don & Bachrach, 1975) and grown in medium 199 supplemented with 5% foetal calf serum, 2% (w/v) glutamine, 15% tryptose phosphate broth and 25 mM-Hepes, at 37°C in CO₂/air (1:19). In all the media, penicillin (50 units/ml) and streptomycin (50 μ g/ml) were added to prevent bacterial contamination. All the cells were fed with fresh complete medium every other day until confluency was reached.

Experimental procedures

Confluent N 115 neuroblastoma, C6-BU-1 glioma and NG 108-15 hybrid cultures were washed twice with prewarmed (37°C) phosphate-buffered saline (0.8% NaCl; 0.02% KCl; 0.115% NaHPO₄; 0.02% KH₂PO₄, pH 7.2). Thereafter, 2 ml of incubation buffer (118 mM-NaCl, 4.7 mM-KCl, 2.5 mM-CaCl₂, 2 H₂O, 1.19 mM-KH₂PO₄, 1.19 mM-MgSO₄·7H₂O, 5.55 mM-glucose, 2.4 mM-NaHCO₃) was added to the cells. In some experiments 3-isobutyl-1-methyl-xanthine (0.1 mM), adenosine (0.5 mM), isoproterenol (0.1 mM), polyamines or prostaglandin E₁ (5 μ M) was also added. Prostaglandin E₁ was dissolved in ethanol; after dilution into the incubation buffer, the ethanol concentration was 0.5% (v/v) and had no effect on intracellular cyclic AMP content (Sharma *et al.*, 1975). Confluent chick-embryo fibroblast cultures were similarly treated, except that a serum-free medium containing the test agents was used instead of incubation buffer. In heart-cell confluent cultures, the effect of noradrenaline was studied by

adding 100 μ l of 40 μ M-noradrenaline (in a serum-free medium) to the cultures without changing the medium. When polyamines were added to the cultures, horse serum, which contains no amine oxidase activity (Blashko, 1962), was used instead of calf serum.

Cyclic AMP determination

At the end of each incubation period the incubation buffer was rapidly removed by aspiration from neuroblastoma, glioma or hybrid cultures. The medium was similarly removed from chick-embryo fibroblast cells and all cultures were immediately covered with 3 ml of boiling 50 mM-sodium acetate buffer (pH 4.0). The cells were scraped off the plates with a rubber policeman and lysed by freezing and thawing. After centrifugation at 13000g for 10 min, duplicate samples of the supernatant fractions were assayed for cyclic AMP, essentially as described by Gilman (1970), by using rat liver protein kinase as the binding protein prepared as follows. Rat liver (2-4 g) was homogenized in 0.05 M-Tris/HCl/2 mM-dithiothreitol buffer, pH 7.5 (20%, w/v). The supernatant obtained after centrifugation at 10000 rev./min for 45 min served as a crude binding protein. The pellets were dissolved in 0.1 M-NaOH, and protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

The metabolic activity of heart-cell cultures was stopped by decanting the incubation medium and washing the monolayers twice with cold saline solution (0.85% NaCl) followed by addition of ice-cold 0.6 M-HClO₄. The cells were harvested by scraping and centrifuged at 15000g for 10 min. The supernatant was used for cyclic AMP assay and the pellet was dissolved in 0.1 M-NaOH and assayed for protein as described above. Intracellular cyclic AMP was assayed by means of a cyclic AMP-assay kit from The Radiochemical Centre, after purifying the cyclic AMP as described by Meruelo & Edidin (1975).

Results

Effect of polyamines on chick-embryo cells

Primary chick-embryo heart cells were grown as described in the Materials and Methods section, and polyamines and/or noradrenaline, which is known to induce cyclic AMP accumulation in heart-cell cultures (Moura & Simpkins, 1975), were added after complete confluency was reached. Fig. 1(a) shows that 1 μ M-spermine, 10 μ M-spermidine and 0.1 μ M-putrescine caused a decrease in cellular cyclic AMP of 34%, 42% and 48% respectively, in cells incubated for 24 h with 1% horse serum. Cyclic AMP increased by 84% when cells were incubated with 1 μ M-noradrenaline for 20 min (Fig. 1b). This effect was, however, strongly decreased (by more than 50%) when polyamines were present in the incubation

Fig. 1. Effect of polyamines and serum on cyclic AMP contents in chick-embryo heart cells

(a) Confluent cultures, maintained in 1% horse serum for 24 h, were incubated for 80 min, with polyamines, added directly to the medium. (b) Experimental conditions were as in (a), but noradrenaline (1 μM) was added 60 min after the onset of the experiment. (c) Cells were incubated in a serum-free medium for 24 h, and fed with fresh medium containing 10% horse serum (▤). Cyclic AMP was assayed after 80 min. In the control experiment, no serum was added. (d) Experimental conditions were as in (c), except that noradrenaline and polyamines were added, as indicated, 20 min before stopping. The values are the means for four samples. Cells from four plates were pooled for each sample. Key: □, control; Sp, spermine (1 μM); Spd, spermidine (10 μM); Put, putrescine (0.1 μM). The s.d. was less than 10%.

Fig. 2. Kinetics of the effect of polyamines (a, spermine; b, spermidine; c, putrescine) on cyclic AMP content in noradrenaline-stimulated chick-embryo heart cells

Confluent cultures, maintained in 1% horse serum for 24 h, were incubated for 40 min with noradrenaline and/or polyamines (at the same final concentrations given in Fig. 1), directly added to the medium. Results are the means $\pm$ s.d. of four different samples. Cells from four plates were pooled for each sample. Key: Nor, noradrenaline; for other abbreviations see Fig. 1 legend.

medium. As expected, serum decreased the cyclic AMP in cells incubated for 24 h in a serum-free medium (Fig. 1c). Noradrenaline caused an increase in cyclic

AMP in serum-treated cells, but this increase was completely prevented by simultaneous addition of polyamines (Fig. 1d).

The kinetics of changes in cellular cyclic AMP concentrations, after addition of noradrenaline to confluent chick-embryo heart cells, are illustrated in Fig. 2. This hormone caused an increase in cellular cyclic AMP of 150% within 5min. After approx. 40min, the concentrations returned to the basal values (Fig. 2). All the polyamines tested caused a

decrease in the peak values of cyclic AMP. The difference between polyamine-treated and control cultures was most pronounced 10min after stimulation with noradrenaline. Fig. 2 also shows that polyamines enhanced the decline in cyclic AMP concentration. This observation may be interpreted by an increase in the rate of excretion or degradation

Table 1. *Effect of polyamines on cellular cyclic AMP contents of chick-embryo fibroblasts*
Cells were incubated with polyamines or 3-isobutyl-1-methylxanthine (IBMX), in a serum-free medium for 20min, and assayed for cyclic AMP. Values are the means of two determinations in duplicate. The S.E.M. was less than 10%.

Addition	Cyclic AMP content	
	(pmol/mg of protein)	(% of control)
None (control)	104	100
Spermine (1 μM)	75	72
Spermine (10 μM)	127	122
Spermine (100 μM)	150	144
Spermidine (10 μM)	75	72
IBMX (100 μM)	157	151
IBMX (100 μM)+spermine (1 μM)	105	101
IBMX (100 μM)+spermine (10 μM)	115	110
IBMX (100 μM)+spermine (100 μM)	211	203
IBMX (100 μM)+spermidine (10 μM)	114	109
IBMX (100 μM)+putrescine (0.1 μM)	37	35

Fig. 3. *Effect of polyamines on cyclic AMP contents of N 115 neuroblastoma cells*
Confluent cultures were treated with incubation buffer with or without 0.1 mM-3-isobutyl-1-methylxanthine (IBMX). Polyamines (spermine, Sp; spermidine, Spd) or 5 μM-prostaglandin E₁ (PGE₁) were added as indicated. After incubation for 20 min at 37°C, the cells were assayed for cyclic AMP. Values are the means of two determinations in duplicate. The S.E.M. was less than 10%. The first column of each group is for no added polyamine.

Table 2. *Effect of polyamines on adenosine-stimulated N 115 neuroblastoma cells*

Confluent cultures were treated for 20min with incubation buffer containing 0.1mM-3-isobutyl-1-methylxanthine (IBMX). Polyamines or 0.5mM-adenosine were added as indicated. Values are the means of two determinations in duplicate. The S.E.M. was less than 10%.

Addition	Cyclic AMP content	
	(pmol/mg of protein)	(% of control)
IBMX (control)	144	100
IBMX+spermine (1 μ M)	63	43
IBMX+spermine (10 μ M)	138	95
IBMX+spermine (100 μ M)	223	154
IBMX+adenosine	252	175
IBMX+adenosine+spermine (1 μ M)	135	93
IBMX+adenosine+spermine (10 μ M)	184	127
IBMX+adenosine+spermine (100 μ M)	283	196

Fig. 4. *Effect of spermine on cyclic AMP contents of NG 108-15 neuroblastoma x glioma hybrids*

Experimental conditions and abbreviations were as given in Fig. 3. Values are the means of two determinations in duplicate. The S.E.M. was less than 10%.

of cyclic AMP. This interpretation is in line with the finding (Fig. 2) that polyamines also caused a decrease in cyclic AMP in unstimulated cells. In addition to chick-embryo heart cells, polyamines also affected chick-embryo fibroblasts. Table 1 demonstrates that spermine at 1 μ M and spermidine at 10 μ M were effective in decreasing cyclic AMP in chick-embryo fibroblasts in the presence or absence of 3-isobutyl-1-methylxanthine. Spermine at 10 μ M and putrescine at 0.1 μ M caused a decrease in cyclic AMP in the presence of phosphodiesterase inhibitor, whereas a high concentration of spermine (100 μ M) caused the accumulation of cyclic AMP in chick-embryo fibroblasts (Table 1).

Effect of polyamines on neural neoplastic cells

Polyamines affected cyclic AMP concentrations,

not only in normal chick-embryo cells, but also in cultures of neoplastic cells. Fig. 3 shows that cellular cyclic AMP concentrations fell by more than 50% when spermine (1 μ M or 10 μ M) was added to cultured neuroblastoma cells. Spermine at higher concentrations (100 μ M) hardly affected the concentrations of cellular cyclic AMP. These results were practically identical when spermine was added to neuroblastoma cells stimulated by 3-isobutyl-1-methylxanthine or prostaglandin E₁. Again, 1 μ M-spermine was most active in decreasing cellular cyclic AMP (Fig. 3). The effect of prostaglandin E₁ on cellular cyclic AMP was more pronounced when the cells were treated with the hormone in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine. In this case, too, 1 μ M-spermine or 10 μ M-spermidine led to a decrease in cellular cyclic AMP. Spermidine at higher concentrations triggered the increase in cellular cyclic AMP concentrations (Fig. 3). In another experiment, adenosine was added to neuroblastoma cells in the presence of 3-isobutyl-1-methylxanthine. In this experiment, too, spermine at 1 μ M caused a significant decrease in cellular cyclic AMP, whereas at 100 μ M a slight increase in cyclic AMP was observed (Table 2).

Prostaglandin E₁ also led to an increase in cyclic AMP in cultures of glioma-neuroblastoma hybrids (clone NG 108-15). Again, 1 μ M-spermine was inhibitory, whereas higher concentrations were less effective (Fig. 4). The effect of polyamines on the concentrations of cyclic AMP in glioma cells was very similar to that observed with chick-embryo heart cells (cf. Figs. 1 and 5). In glioma cells, 3-isobutyl-1-methylxanthine caused an increase in cellular cyclic AMP; this effect was prevented by spermine (100-1 μ M) and spermidine (10 μ M). A slight stimulation was obtained with 1mM-spermidine. Putrescine also prevented the increase in cellular cyclic AMP at relative low concentrations (Fig. 5). Isoproterenol

Fig. 5. Effect of polyamines on cyclic AMP contents of C6-BU-1 glioma cells
Experimental conditions were as given in Fig. 3, except that cells were treated for (a) 5 min or (b) 20 min. Values are the means of two determinations in duplicate. The S.E.M. was less than 10%. Abbreviation: Put, putrescine (for other abbreviations and symbols see Fig. 3 legend).

Table 3. Effect of polyamines on cyclic AMP contents of isoproterenol-stimulated C6-BU-1 glioma cells
Confluent cultures were treated for 20 min with incubation buffer containing 0.1 mM-3-isobutyl-1-methylxanthine (IBMX) and 0.1 mM-isoproterenol (isop.). Polyamines were added as indicated. Values are the means of two determinations in duplicate. The S.E.M. was less than 10%.

Addition	Cyclic AMP content	
	(pmol/mg of protein)	(% of control)
IBMX+isop. (control)	1950	100
IBMX+isop.+spermine (1 μ M)	1404	72
IBMX+isop.+spermine (10 μ M)	1587	81
IBMX+isop.+spermidine (10 μ M)	1570	80
IBMX+isop.+spermidine (100 μ M)	1849	94
IBMX+isop.+putrescine (0.1 μ M)	946	48
IBMX+isop.+putrescine (1 μ M)	1672	85

caused the accumulation of cyclic AMP in glioma cells incubated in the presence of 3-isobutyl-1-methylxanthine. Spermine (10 μ M and 1 μ M) pre-

vented this accumulation, as did 10 μ M-spermidine and 0.1 μ M-putrescine (Table 3). Polyamines at higher concentrations did not cause a decrease in cellular

cyclic AMP, and a slight increase was observed when putrescine was used at a concentration of $10\mu\text{M}$ (results not shown).

Discussion

The activity of ornithine decarboxylase may be regulated by (a) changes in the half-life of the enzyme; (b) induction or activation of ornithine decarboxylase antizyme, which neutralizes the activity of the enzyme; (c) changes in the activity of adenylate cyclase and protein kinase; (d) modulation of cellular cyclic AMP concentration.

Numerous studies in recent years indicate that, in rapidly proliferating systems, the half-life of ornithine decarboxylase increases from 12–20 min to 45–60 min. This has been demonstrated during the progression from the quiescent to the proliferative state (Hogan & Murden, 1974) and during the transformation of cells by either oncogenic viruses (Bachrach, 1976) or carcinogenic compounds (O'Brien, 1976). This change in enzyme stability may lead to an apparent increase in activity, even when the rate of enzyme synthesis remains constant (Tabor & Tabor, 1976).

Antizyme is a protein which is induced (or activated) when exogenous polyamines are added to cultured cells (Heller *et al.*, 1976). This is a possible mechanism for the inactivation of the existing enzyme, and may lead to an immediate arrest of polyamine biosynthesis.

If this mechanism operates under physiological conditions, then polyamines (which are formed after the increase in ornithine decarboxylase activity) exert a self-regulatory effect.

In many animal cells the induction of ornithine decarboxylase is mediated by cyclic AMP, which in turn activates protein kinase. In these cases, the activities of adenylate cyclase and protein kinase fluctuate during the cell cycle (Jungmann & Russell, 1977; Bachrach *et al.*, 1978). It has been demonstrated that polyamines affect the activity of adenylate cyclase (Shah *et al.*, 1975; Atmar *et al.*, 1976; Caldarera *et al.*, 1976; Sheth *et al.*, 1976; Wright *et al.*, 1976) and of cyclic AMP-dependent protein kinase (Takai *et al.*, 1976; Murray *et al.*, 1976; Mäenpää, 1977; Hochman *et al.*, 1978), apparently by interacting with its catalytic subunit (Hochman *et al.*, 1978). This may be another self-regulatory mechanism which controls the synthesis of polyamines.

In our experiments, we used various systems, in order to establish the generality of the phenomenon. We used differentiated chick-embryo cells (primary heart cells) and undifferentiated fibroblasts. In addition, we tested the effect of polyamines on various tumour-cell lines derived from the nervous system. These lines differ from each other in their responsiveness to various hormones.

Data presented in this paper clearly show that exogenous polyamines, at relatively low concen-

trations, cause a decrease in cellular cyclic AMP. On the other hand, polyamines at higher concentrations bring about the accumulation of cyclic AMP. It is of special interest that putrescine is most active in this respect, and a decrease in cellular cyclic AMP is obtained even with a $0.1\mu\text{M}$ solution of the diamine. It appears that the effect is general one, as polyamines affect cyclic AMP concentrations in various cells, such as chick-embryo heart cells (Fig. 1) and fibroblasts (Table 1) as well as in cultures of glioma (Fig. 5), neuroblastoma (Fig. 3) and neuroblastoma-glioma hybrid cells (Fig. 4). These changes are obtained after stimulating the cells with different hormones such as noradrenaline, isoproterenol and prostaglandin E_1 , and chemicals such as adenosine and phosphodiesterase inhibitors.

It has been shown that exogenous polyamines are taken up and metabolized by cultured cells (Siimes & Jänne, 1967; Clark & Fuller, 1975; Kremzner *et al.*, 1975). A substantial increase in the intracellular polyamines is observed when extracellular concentrations rise to the endogenous concentrations (Clark & Fuller, 1975; Heller *et al.*, 1978) and furthermore exogenous polyamines appear not to enter the same pool as amines synthesized within the cell (Russell *et al.*, 1970). These findings, together with the observed sensitivity of most cells to exogenous polyamine in the presence of much higher endogenous concentrations (Mitchell *et al.*, 1978; Heller *et al.*, 1978), make it difficult at present to assess the reason for different effects on cyclic AMP of increasing concentrations of exogenous polyamines. We are tempted to suggest that this phenomenon might be related to a different availability of free and more active polycations inside the cell. However, considering the inverse correlation between growth rate and cyclic AMP concentrations (Otten *et al.*, 1972; Kram *et al.*, 1973; Bombik & Burger, 1973), the opposite effects elicited by low or high exogenous polyamine concentrations are consistent with their observed ability to act as promoters (Ham, 1964; Clô *et al.*, 1976) or inhibitors (Halevy *et al.*, 1962; Gahl *et al.*, 1976) of cell growth, respectively.

The changes in cellular cyclic AMP concentrations caused by exogenous polyamines seem to have some physiological relevance. Our preliminary experiments, performed on heart-cell cultures, indicate that the cyclic AMP-mediated induction of ornithine decarboxylase activity by noradrenaline (Bachrach, 1975) is prevented by low exogenous polyamine concentrations. This was not the case when serum, whose action is not mediated by cellular cyclic AMP (Hogan *et al.*, 1974), was used as inducer (C. M. Caldarera & C. Clô, unpublished work). Similarly, it has been suggested that exogenous polyamines affect the rate of lipolysis in fat-cell cultures, by changing the cellular concentration of cyclic AMP (Lockwood *et al.*, 1971).

The following mechanisms should be considered to explain the effect of polyamines on cellular cyclic AMP.

(a) Polyamines regulate adenylate cyclase activity.

(b) Polyamines control the release of cyclic nucleotides by the cells.

(c) Polyamines affect the binding of hormones to cell receptors.

(d) Polyamines affect phosphodiesterase activity.

The effect of polyamines on adenylate cyclase activity has been well documented (see above). On the other hand, little is known about the effect of polyamines on the release or uptake of cyclic AMP by animal cells. It is unlikely that polyamines affect the specific binding of each hormone to its respective receptors. The effect of polyamines on the basal cyclic AMP concentrations (Fig. 2) also suggests that the polyamine effect is not due to impaired hormone binding. Preliminary studies indicate that polyamines regulate phosphodiesterase activity (C. Clô, C. M. Caldarera & U. Bachrach, unpublished work). Those experiments suggest that the concentration of cellular cyclic AMP is controlled by more than a single mechanism, and stress the importance of the polyamines in regulating the cell cycle.

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