Evidence for Cyclic GMP in the Yeast *Saccharomyces cerevisiae*, and Studies on Its Possible Role in Growth

Hans Eckstein

Institut für Physiologische Chemie der Universität, Martinistraße 52 – UKE, D-2000 Hamburg 20, Bundesrepublik Deutschland

Z. Naturforsch. **43c,** 386–396 (1988); received October 20, 1987

Cyclic GMP, Cyclic AMP, Phosphodiesterase Inhibitors, Cholera Toxin, Growth, Yeast

The yeast *Saccharomyces cerevisiae* is shown to be equipped with cyclic GMP, the level of which ranges from 6 pmol/10^9 cells with pressed baker's yeast to 21 pmol/10^9 cells with exponentially growing cells. In extracts from synchronized growing yeast, cyclic GMP increases stepwise, being doubled at the time of each mitosis. Theophylline and 3-isobutyl-1-methylxanthine induce a rapid increase of cyclic GMP, followed by a premature formation of the septal cell wall between mother cell and bud. The effects of 3-isobutyl-1-methylxanthine are reversible. Dibutyryl-cyclic GMP, and, after a pronounced lag, also dibutyryl-cyclic AMP, induce a premature cell division, too. Cholera toxin induces premature cell divisions without a preceding increase in cyclic GMP. Neither theophylline nor 3-isobutyl-1-methylxanthine, cholera toxin or one of the dibutyryl-cyclic nucleotides modify the growth rate of the culture. None of the agents has significant effects on the level of cyclic AMP. The results suggest that cyclic GMP possibly controls an early step of mitosis, whereas ADP-ribosylation might govern a subsequent event.

Introduction

Cyclic nucleotides act as effectors in the regulation of many metabolic processes by amplifying primary signals *via* activation of protein kinases, which results in modified protein functions. Whilst the participation of cyclic AMP in this process extensively is studied, the role of cyclic GMP is less clear. In animal cells, cyclic GMP seems involved in processes of very different kind, including specific functions of highly specialized cells as well as cell proliferation, in which cyclic GMP seems to act as a positive effector (for a review see [1, 2]).

As it is shown by several authors, the yeast *Saccharomyces cerevisiae* is provided with cyclic AMP and cyclic AMP-dependent protein kinases [4–18]. Cyclic AMP takes part in transfer and translation of signals by certain nutrients [4, 18], and it seems to be involved in the regulation of cell growth [10, 13–15]. In contrast to these findings, nothing is known hitherto about cyclic GMP in yeast, though most organisms are provided with cyclic GMP as well as with cyclic AMP. We recently reported that cyclic GMP inhibits the DNA synthesis by partially purified DNA polymerase A from yeast [3]. To understand the biological meaning of this observation we felt stimulated to look, whether *S. cerevisiae* also is equipped with cyclic GMP and cyclic GMP-dependent enzyme systems, and what relation exists to the cyclic AMP-dependent systems. Data are presented in this paper on the cyclic GMP levels in synchronized and asynchronously growing yeast, and on effects of dibutyryl-cyclic GMP, theophylline, MIX, and cholera toxin, on cyclic GMP level and cell proliferation.

Abbreviations: Cyclic GMP, 3′:5′-cyclic guanosine monophosphate; cyclic AMP, 3′:5′-cyclic adenosine monophosphate; dibutyryl-cyclic GMP, N_2, 2′-O-dibutyryl-3′:5′-cyclic GMP; dibutyryl-cyclic AMP, N_2, 2′-O-dibutyryl-3′:5′-cyclic adenosine monophosphate; MIX, 3-isobutyl-1-methylxanthine; EDTA, ethylenedinitrilotetraacetic acid disodium salt; Tris, tris(hydroxymethyl)aminomethane; RIA, radioimmunoassay; CT, cholera toxin; PDE, 3′:5′-cyclic nucleotide phosphodiesterase (EC 3.1.4.17); premature cell division, the premature formation of the septal cell wall between mother cell and bud.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/88/0500–0386 $ 01.30/0

Materials and Methods

Yeast strains

The same clone originally derived from baker's yeast (*Saccharomyces cerevisiae,* "Germania-Hefe", DHW Hamburg) as earlier [19] was used throughout all experiments with asynchronously growing cells. Synchronously growing yeast was prepared from baker's yeast (L'Hirondelle, Lesaffre, Marce en Barceul, France) by heat shock treatment as described earlier [20]. Yeast was grown in a modified Wickerham medium [20].

Chemicals

[2,8-^{3}H]Adenosine 3′,5′-cyclic phosphate (^{3}H-cyclic AMP, 37 MBq (1 mCi)/ml, 1332 GBq (36 Ci)/mmol, and [8-^{3}H]guanosine 5′-triphosphate (^{3}H-GTP), 37 MBq (1 mCi)/ml, 396 GBq (10.7 Ci)/mmol, were from Amersham-Buchler (Braunschweig). [8-^{3}H(N)]guanosine 3′:5′-cyclic phosphate (^{3}H-cyclic GMP, 37 MBq (1 mCi)/ml, 636.4 GBq (17.2 Ci)/mmol) was from New England Nuclear (Dreieich). Unlabeled cyclic nucleotides, their derivatives dibutyryl-cyclic AMP and dibutyryl-cyclic GMP, and other nucleotides were from Boehringer (Mannheim). Theophylline was purchased from SERVA (Heidelberg), 3-isobutyl-1-methylxanthine (MIX) and cholera toxin (from *Vibrio cholerae,* toxic potency 21.5 lb/μg protein) were obtained from SIGMA Co. (St. Louis, U.S.A.). Cholera toxin was activated immediately before use by incubation for 30 min at 31 °C according to [21]. Norit A was from SERVA (Heidelberg), it was activated according to [22]. 3′:5′-Cyclic nucleotide phosphodiesterase (EC 3.1.4.17) from bovine heart and other enzymes were from Boehringer (Mannheim). Triethylamine was from FLUKA (Buchs, Switzerland). Rabbit anti-cyclic AMP antiserum, prepared by Dr. M. Schumacher, was a kind gift from Prof. Hilz and Dr. W. Weber, Institut für Physiologische Chemie, Hamburg. Rabbit *anti*-cyclic GMP antiserum was obtained from Amersham-Buchler, Braunschweig; in some experiments with theophylline and with cholera toxin, a preparation from Dr. Schumacher kindly made available by Prof. Hilz was used.

In vivo labeling and separation of cyclic GMP

Yeast cultures containing 10×10^6 cells/ml were grown with 462.5 KBq (12.5 μCi/ml, 1.168 nmol/ml) [8-^{3}H]GTP. After 210 min, the cells from 5 ml aliquots were sedimented by centrifugation and extracted with 3.0 ml $HClO_4$ as described below. The nucleotides (more than 95% of the radioactivity) were separated from the extract by adsorption to Norit A according to [22]. The Norit was sedimented by centrifugation, the nucleotides were eluted according to [23] by shaking 120 min at 37 °C with 10.0 ml of an ethanol–ammonia–water mixture (20 + 1 + 29), followed by 5 ml of the same solvent for 15 h at room temperature. The two Norit supernatants containing about 75% of the adsorbed radioactivity were pooled and evaporated to dryness in a rotary evaporator at 35 °C. The residue was redissolved with 250 μl water (= Norit eluate).

50 μl Norit eluate, or 50 μl supernatant from the PDE assays (see below) were supplied with 25 nmol cyclic GMP or cyclic AMP as a tracer, and applied to a silica gel thin-layer plate (Merck TLC plates silica gel 60). As a control, 25 nmol each of cyclic GMP, cyclic AMP, 5′-AMP, 5′-GMP, ATP, GTP, adenosine or guanosine, were applied separately. The chromatogram was developed by an isopropanol–water–ammonia mixture (7:2:1) according to [24]. The nucleotides were detected by their UV absorption, the spots were moistened with water, scraped off with a sharp spatula, and counted in a liquid scintillation spectrometer. Quench correction was performed by external standardization, and dpm incorporated into the acid-soluble fraction from 10^9 cells were calculated.

Degradation by phosphodiesterase

The assay contained, in a total volume of 85 μl: 0.82 mmol/l $MgCl_2$, 0.21 mmol/l Na_2-EDTA, 20.5 mmol/l Tris-HCl, 50 μl Norit eluate and 0.1 mg PDE, at a final pH = 7.5. Three kinds of control assays were performed: a) The assay was supplemented with 370 Bq (10 nCi, 0.5 pmol) [8-^{3}H]cyclic GMP. b) 370 Bq [8-^{3}H]cyclic GMP and 5 nmol unlabeled cyclic GMP were supplemented instead of the eluate. c) The enzyme was omitted. After incubation 60 min at 37 °C, the reaction was stopped by heating 5 min at 95 °C, the insoluble material was centrifuged off, and the clear supernatant was analyzed by thin-layer chromatography.

Radioimmunoassay

Cyclic AMP and cyclic GMP were estimated from yeast extracts by the RIA technique according to Steiner *et al.* [25] with some modifications. Growing yeast cells were harvested by centrifugation (10 min, $6000 \times g$, +2 °C) from aliquots of a culture containing 1×10^8 cells at zero time of incubation. The cyclic nucleotides were extracted with 2.0 ml $HClO_4$ (1 mol/l) 60 min at 37 °C. After cooling for 5 min in an ice-bath, the insoluble material was centrifuged off. The pH of the supernatant was adjusted to pH = 7.0–7.2 by addition of 390 μl KOH (5 mol/l) and 500 μl potassium phosphate buffer, 1 mol/l, pH =

7.4. The $KClO_4$-precipitate was centrifuged off, the clear supernatant (= "extract") was brought into the RIA. All estimations were done in duplicate.

a) Cyclic AMP-estimation: The radioimmunoassay contained, in a total of 400 µl: 370 Bq (10 nCi) ^{3}H-cyclic AMP, 0.6 mg gamma globulin, yeast extract containing 0.3–3.5 pmol cyclic AMP, 0.05 mol/l sodium phosphate pH 7.0, 0.085 mol/l NaCl. The reaction was started by addition of rabbit *anti*-cyclic AMP antiserum.

b) Cyclic GMP was estimated after acetylation by a slightly modified method from [26, 27]. 750 µl extract were supplemented with 370 Bq (10 nCi) ^{3}H-cyclic GMP, and mixed with 50 µl sodium acetate, 0.4 mol/l, pH = 6.2, and 50 µl "acetylation reagens". The reagens was freshly prepared from 0.5 ml acetic anhydride and 1.0 ml triethylamine. After 5 min, the assay was neutralized with 500 µl potassium phosphate buffer, 1 mol/l, pH = 7.4, and a little solid potassium bicarbonate. 100 µl 0.3% gamma globulin were added, and the reaction was started with 50 µl *anti*-cyclic GMP antiserum.

After incubation of the assays at 4 °C for 18 h, the protein was precipitated with 2.0 ml 4 mol/l $(NH_4)_2SO_4$, and centrifuged off (20 min, 12,000 × *g*, +2 °C). The residue was washed twice with 2.67 mol/l $(NH_4)_2SO_4$, and redissolved with 1.2 ml 10^{-3} mol/l NaOH. The radioactivity was estimated by liquid scintillation counting, pmol cyclic nucleotides/10^9 cells were calculated by means of standard curves.

More than 95% from cyclic AMP or cyclic GMP, which was added to yeast cells together with the $HClO_4$, were recovered. The yield of cyclic AMP and cyclic GMP increased proportionally to the number of cells extracted, up to 10^9 cells. Under standard conditions, cyclic GMP is bound by the *anti*-cyclic AMP antiserum to an extent of less than 1% of cyclic AMP. In presence of 1 pmol cyclic AMP, no cyclic GMP binding could be detected, even with a 10-fold excess of cyclic GMP. Likewise, no reaction between *anti*-cyclic GMP antiserum and cyclic AMP was seen, even with cyclic AMP in a 1000-fold excess.

Others

Growth was controlled by counting the cells and buds under a phase-contrast microscope. The number of budding cells is expressed as percent of the total cell number. DNA was estimated from the acid-insoluble cell residue by the Burton reaction [28]. Protein was measured by the biuret reaction.

Results

When growing yeast cells are incubated for 210 min with ^{3}H-GTP, about 5% of the incorporated radioactivity are found in the acid-soluble cell fraction. Separation of this material by thin-layer chromatography (TLC) exhibits radioactive fractions comigrating with cyclic GMP, and with cyclic AMP, respectively, showing more label associated with cyclic GMP than with cyclic AMP. Incubation of the labeled acid-soluble material with cyclic nucleotide phosphodiesterase prior to separation by TLC results in a marked loss of radioactivity from both the cyclic GMP- and the cyclic AMP-fractions (Table I).

Cyclic GMP also is detected in the acid-soluble cell fraction from yeast by means of a RIA with rabbit *anti*-cyclic GMP antiserum. The amount of cyclic GMP found by this system increases proportionally to the amount of cell extract used. We found no interaction of the antiserum with cyclic AMP (*cf.* Materials and Methods).

Different cyclic GMP levels are observed with yeast from different metabolic states (Table II). With logarithmically growing cells, about three times as much cyclic GMP is found as with pressed baker's yeast (which represents nutrient depleted resting cells). When logarithmically grown yeast is incubated under aerobic conditions, but only with glucose and

Table I. Detection of cyclic nucleotides from yeast extracts by thin-layer chromatography after labeling of growing yeast cells with [^{3}H]GTP. Growing yeast cells (log phase) were incubated with 8[^{3}H]GTP, extracted with perchloric acid, and the labeled nucleotides were separated, as described in "Materials and Methods". Cyclic GMP and cyclic AMP were detected by UV-absorption, and identified by their R_F-values (the R_F-values of the corresponding nucleoside 5′-monophosphates are 0.12–0.14, those of the triphosphates are zero, the nucleosides migrate near the solvent front). (−) PDE: After incubation in a phosphodiesterase assay, but without enzyme; (+) PDE: After treatment with 3′:5′-cyclic nucleotide phosphodiesterase. Mean from three experiments.

Nucleotide	R_F	dpm/10^9 cells (−) PDE	(+) PDE
cyclic GMP	0.30	18653	7183
cyclic AMP	0.44	13594	5167

Table II. Cyclic GMP and cyclic AMP in extracts from yeast kept in different metabolic states. a) Commercially available baker's yeast ("Germania Hefe", DHW, Hamburg), washed twice with water, and resuspended with water. b) Yeast taken from a growing culture (log phase), washed twice with water, and incubated over night at 31 °C with 80 g/l glucose in 0.05 mol/l potassium phosphate, pH = 6.5 (final cell density 1×10^6 cells/ml). c) Cells handled as in b), but incubated with vigorous stirring and aeration. d) Yeast grown in parallel overnight up to 10×10^6 cells/ml. Aliquots containing about 100×10^6 cells were taken from each assay, the cells were extracted and the cyclic nucleotides estimated, as described in "Materials and Methods". Average values from two experiments (eight single data), ± standard deviation.

	pmol/10^9 cells		Cyclic GMP / Cyclic AMP
Assay	Cyclic GMP	Cyclic AMP	
a) pressed baker's yeast	5.9 ± 2.8	346.4 ± 109.1	0.017
b) with glucose + phosphate, but starved of O_2	7.6 ± 2.5	579.1 ± 134.4	0.013
c) with glucose + phosphate, aerated	9.3 ± 1.4	654.2 ± 120.1	0.014
d) log phase growth	20.6 ± 6.2	1712.9 ± 286.0	0.012

potassium phosphate as substrates, the cyclic GMP level decreases to about 45%. Additional O_2-starvation results in a further decrease to about 37%. It should be noted that similar alterations occur with cyclic AMP. The relation cyclic GMP: cyclic AMP is slightly changed in an opposite manner, showing somewhat higher values with pressed baker's yeast.

With synchronized growing yeast, the level of cyclic GMP appears constant throughout the greater part of each of at least three consecutive cell cycles. But each time, when the buds reach their final size and are separated from the parent cells by formation of the new cell wall, cyclic GMP is doubled. In contrast to this stepwise increase of cyclic GMP, cyclic AMP is augmented nearly steadily (Fig. 1).

These findings stimulated us for the following experiments:

Addition of 10^{-3} mol/l dibutyryl-cyclic GMP to an asynchronously growing culture results in a decrease of the percentage of budding cells about 40 min later (Fig. 2). Examination under the microscope showed that this decrease is brought about by a premature formation of the septal cell wall between mother cell and bud (in the following designated as "premature cell division"), occurring before the daughter cells have reached their final size (Fig. 6A). Only after division the cells grow up to their "normal" volume. The increase in cell number and consequently the growth rate of the culture, is not affected significantly. Neither sodium butyrate, a possible inhibitor of phosphodiesterase, nor 5′-GMP in equivalent amounts induce a similar effect. With dibutyryl-cyclic AMP, the number of budding cells decreases, too, as a result of such premature cell divisions. But in this case, the effects become manifest considerably later than with dibutyryl-cyclic GMP.

When exponentially growing yeast is exposed to 5×10^{-3} mol/l theophylline, a possible inhibitor of cyclic nucleotide phosphodiesterase, a significant increase of the level of cyclic GMP above the control is observed between 60 and 80 min ($p < 0.05$, Fig. 3). The level of cyclic AMP shows no essential modification, and also the augmentation of DNA and protein (not shown) remains unaffected. But the percentage of budding cells temporarily declines significantly ($p < 0.001$, Table IV) by nearly one third after about 90 min. Like with dibutyryl-cyclic GMP, this decrease is brought about by premature cell division (Fig. 6C), without any change in the growth rate of the culture. Additionally, numerous cells grow to a remarkably enlarged volume.

With 1×10^{-3} mol/l 3-isobutyl-1-methylxanthine, another inhibitor of phosphodiesterase, cyclic GMP significantly ($p < 0.05$) is raised over the control just after 20 min and with a second peak at 80 min of growth (Fig. 4). The level of cyclic AMP shows no deviation from the control, nor differ DNA and protein augmentation from control. But again, the percentage of budding cells decreases significantly ($p < 0.001$, Table IV), by about one third between 30 and 60 min of incubation. Like with the previous agents, the decrease comes about by premature cell divisions (Fig. 6B), without change in the growth rate of the culture.

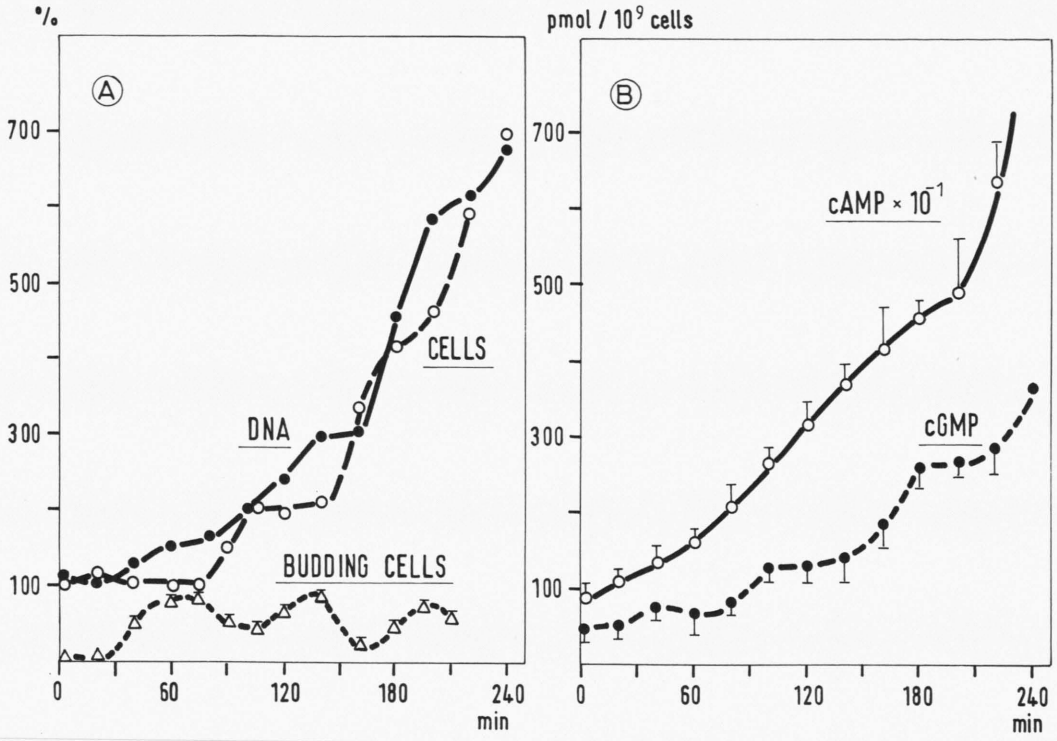


Fig. 1. Cyclic nucleotides, DNA, and growth in synchronized yeast. Cyclic GMP and cyclic AMP were estimated in duplicate from extracts from synchronized growing cells by the RIA technics, and calculated as pmol/10^9 cells at zero time of growth. Mean from five experiments, + standard deviation. Average standard errors: Cyclic GMP 15.3%, cyclic AMP 11.7%, DNA 2.1%.

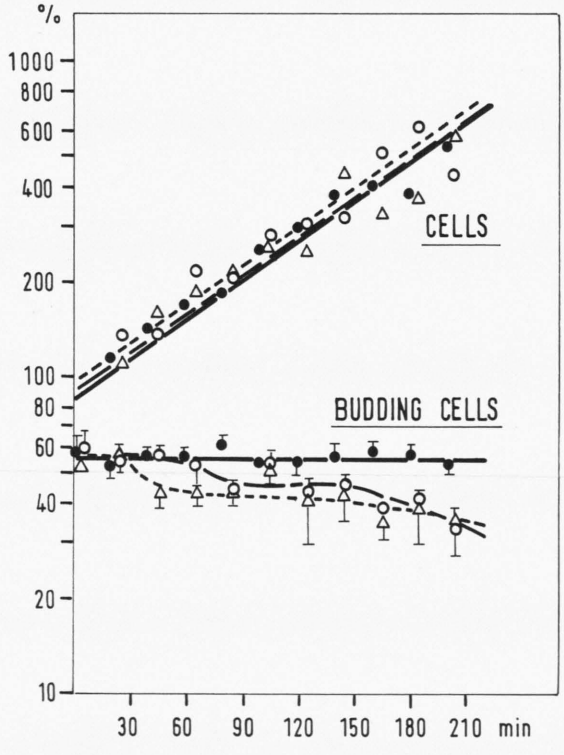


Fig. 2. Effects of dibutyryl-cyclic GMP and dibutyryl-cyclic AMP on asynchronously growing yeast. Aliquots from an asynchronously growing culture containing 8×10^6 cells/ml were supplemented with 10^{-3} mol/l dibutyryl-cyclic GMP, and dibutyryl-cyclic AMP, respectively, and the incubation continued. Cell number and proportion of budding cells were estimated in duplicate at the indicated times, as described in Materials and Methods. Mean from three experiments, + standard deviation. ●—● Control, △—△ + dibutyryl-cyclic GMP, ○—○ + dibutyryl-cyclic AMP.

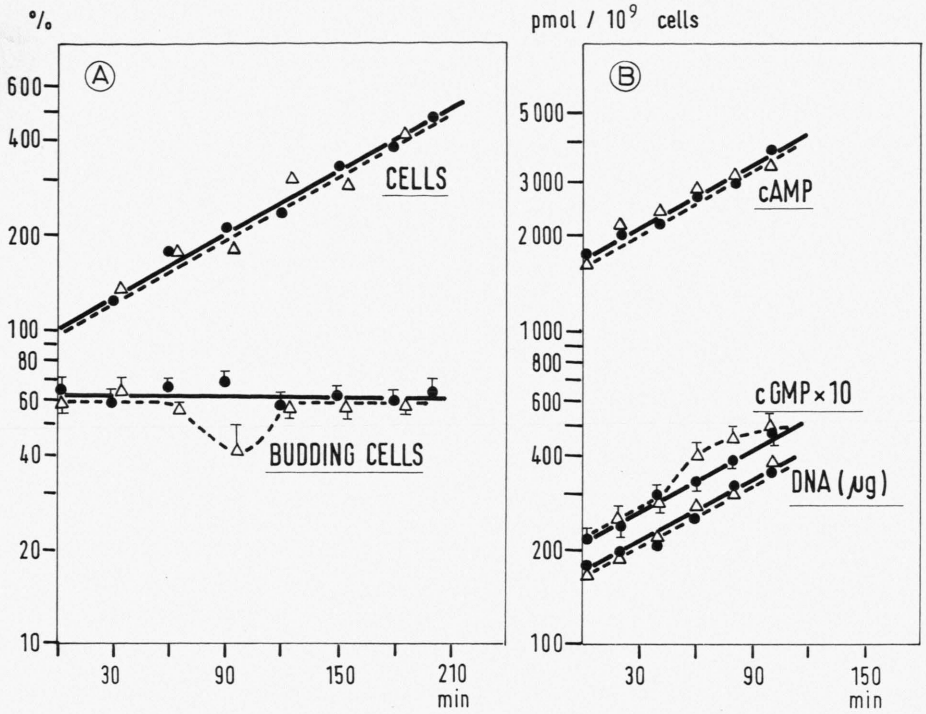


Fig. 3. Effects of theophylline on the level of cyclic GMP, cyclic AMP, and DNA, and on the cell proliferation of asynchronously growing yeast. Aliquots from an asynchronously growing culture containing 18×10^6 cells/ml were diluted to 10×10^6 cells/ml with aerated growth medium at 31 °C, supplemented with 5×10^{-3} mol/l theophylline, and incubated further. Extracts were prepared in duplicate at the indicated times, and the cyclic nucleotides were estimated in duplicate from each extract. Mean from two experiments, + standard deviation. A: Cell growth. B: Increase in cyclic nucleotides and DNA. ●—● Control, △—△ + theophylline.

When yeast cells grown with MIX are washed with cold growth medium and then reincubated with fresh medium under growth conditions, the cyclic GMP level approximates to the control just at zero time of reincubation (Table III), being augmented henceforth like in the control. The percentage of budding cells starts to recover to normal values after a 40 min delay. Unfortunately, changing the medium for itself also brings about a temporary delay in budding, as seen with the control.

A rapid decrease of the percentage of budding cells also is induced by cholera toxin (Fig. 5 and 6D, Table IV), again by premature cell division without change in the growth rate. But unlike the other effectors, this decrease is not preceded by an increase of the cyclic GMP level. Only when the reduced number of buds is manifest, a transient slight increase of cyclic GMP (at 40–60 min) is seen, which but is not significant. The level of cyclic AMP likewise does not differ from the control.

The effects of the various agents on percentage of budding cells and levels of cyclic nucleotides are summarized in Table IV. The average values from the control differ significantly from those obtained with dibutyryl-cyclic GMP, MIX, CT, and theophylline (at 90 min). Except with CT, an increase of the cyclic GMP level precedes the premature cell division, whereas no change of the cyclic AMP level is found.

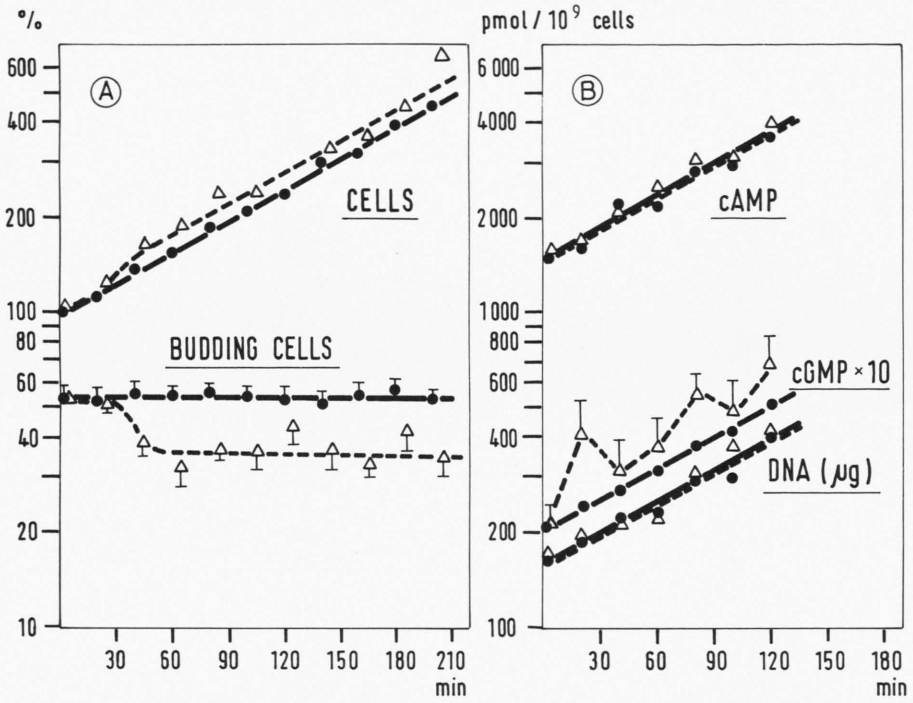


Fig. 4. Effects of MIX on the level of cyclic GMP, cyclic AMP, and DNA, and on the cell proliferation of asynchronously growing yeast. Aliquots from an asynchronously growing culture containing 7.5×10^6 cells/ml were supplemented with 10^{-3} mol/l MIX, and the incubation continued. The cyclic nucleotides were estimated in duplicate from cell extracts prepared in duplicate at the indicated times. Mean from three experiments, + standard deviation. A: Cell growth. B: Increase in cyclic nucleotides and DNA. ●—● Control, △—△ + MIX.

Table III. Restoration to normal values of cyclic GMP level and budding after removal of MIX. Asynchronously growing yeast was incubated with MIX, as described in Fig. 4. After 85 min growth, 50 ml aliquots were diluted with 125 ml fresh, cold growth medium, the cells were spun down and washed three times with 125 ml each of cold growth medium. The yeast then was resuspended with 125 ml fresh growth medium and reincubated at growth conditions. Cyclic GMP was estimated as described in Fig. 4. Mean from 5 experiments ± standard deviation. I = control, II = preincubated with MIX.

Reincubation	% Budding cells		pmol Cyclic GMP/10^9 cells	
	I	II	I	II
0 min	57.8 ± 3.1	39.5 ± 5.4	23.7 ± 5.0	22.1 ± 11.3
20 min	53.7 ± 6.4	39.5 ± 5.3	18.3 ± 10.4	16.5 ± 4.7
40 min	48.8 ± 2.6	51.6 ± 3.6	24.2 ± 9.6	28.7 ± 7.9
60 min	45.8 ± 7.7	51.4 ± 11.6	38.6 ± 11.1	35.5 ± 16.2
80 min	56.9 ± 3.4	62.4 ± 5.3	46.8 ± 19.0	44.5 ± 12.3

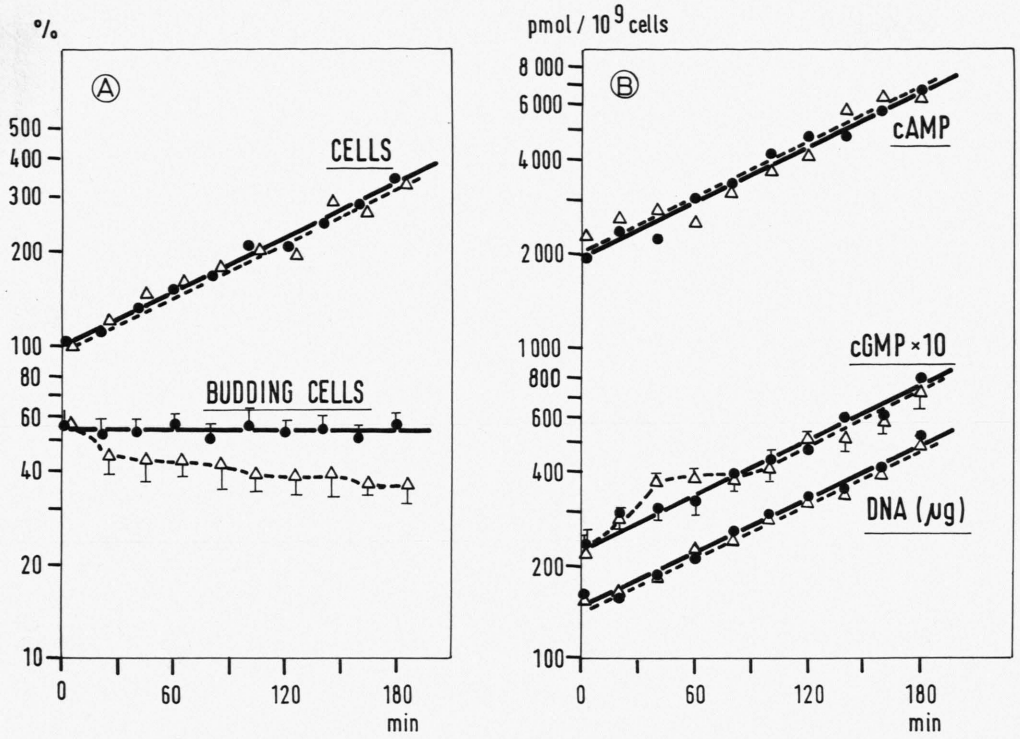
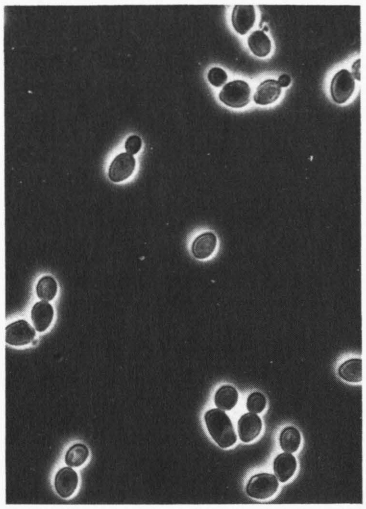


Fig. 5. Effects of cholera toxin on the cell proliferation and on levels of cyclic GMP, cyclic AMP, and DNA in asynchronously growing yeast. Aliquots from an asynchronously growing culture containing 25×10^6 cells/ml were diluted to 10×10^6 cells/ml with aerated growth medium at 31 °C, supplemented with 10 µg/ml cholera toxin, and the incubation continued. The cyclic nucleotides were estimated in duplicate from extracts prepared in duplicate at the indicated times. Mean from four experiments, + standard deviation. A: Cell growth. B: Cyclic nucleotides and DNA. ●—● Control, △—△ + cholera toxin.

Table IV. Number of budding cells in cultures supplemented with different agents. The average per cent of budding cells in each experimental series is calculated and compared with its respective control. The significance of the deviation from control is estimated by Student's t-test.

	Per cent budding cells (mean)	Standard deviation	Probability p	Early increase of cyclic GMP	cyclic AMP
Control	55.5	3.05	–		
dibutyryl-cyclic AMP	47.5	8.83	<0.001		(+)
dibutyryl-cyclic GMP	43.3	6.58	<0.001	(+)	
GMP	52.8	3.57	>0.05		
Na-butyrat	54.1	4.22	>0.05		
theophylline	53.1	3.67	>0.05	+	
at 90 min incubation with theophylline	40.1	1.59	<0.001	+	
MIX	39.4	7.27	<0.001	+	
Cholera toxin	38.7	5.35	<0.001		



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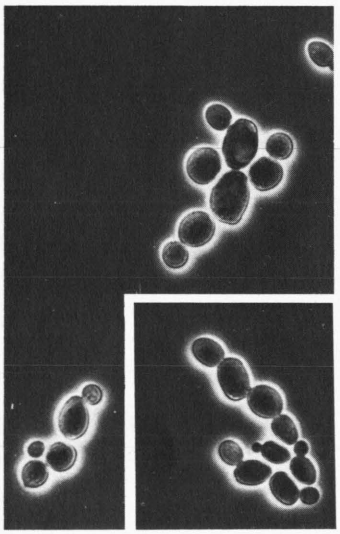

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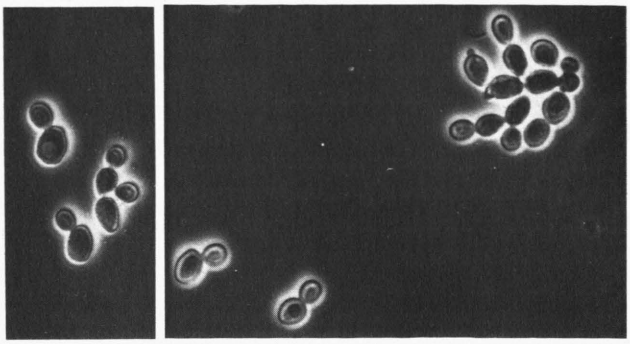

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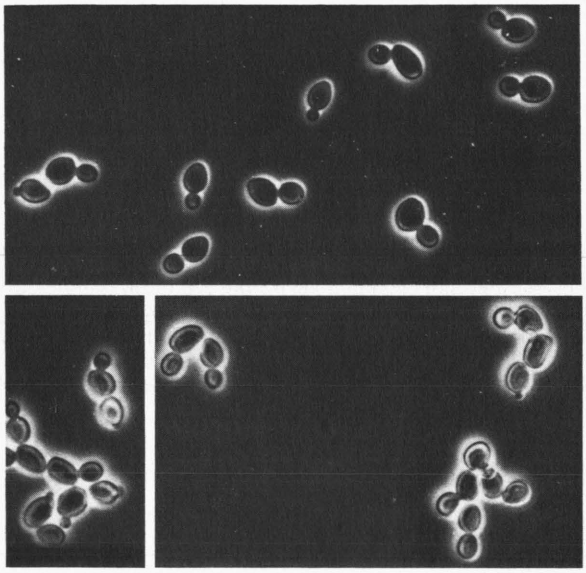

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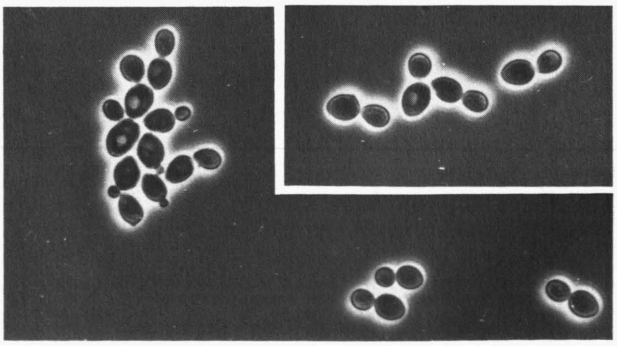

D

Fig. 6. Photomicrographs of yeast after incubation with different effectors. Exponentially growing yeast was incubated with dibutyryl-cyclic GMP, MIX, theophylline, and cholera toxin, as described in Figs 2–5. Photomicrographs were made with a phase-contrast microscope (Zeiss PM 3) at 200 magnifications after A: 40 min dibutyryl-cyclic GMP, B: 60 min MIX, C: 80 min theophylline, D: 40 min CT. E = control. Final enlargement 750 ×.

Discussion

It has been shown by Watson *et al.* [5] that yeast, like other eukaryotic organisms and bacteria, contains cyclic AMP. The intracellular level of cyclic AMP is increased by intracellular acidification following addition of fermentable sugars to the medium, thereby controlling the activity of several enzymes of the carbohydrate metabolism *via* a cyclic AMP-dependent protein kinase [18, 29–31]. Our studies concerning the incorporation of radioactive label into the acid-soluble fraction of yeast cells grown with [^{3}H]GTP, and analyses of cell extracts by the RIA technics show that the yeast *Saccharomyces cerevisiae* like many other organisms is equipped not only with cyclic AMP, but also with cyclic GMP. The intracellular level of cyclic GMP depends on the metabolic state of the cells, showing very low values with pressed baker's yeast, somewhat higher values at incubation with glucose under conditions of O_2-starvation, but about 3-fold higher values with aerobically growing cells. Similar results are obtained with cyclic AMP, the molar ratios between both cyclic nucleotides therefore show only minor differences under the different metabolic conditions. The higher levels of both cyclic nucleotides under growth conditions thus primarily would reflect the higher metabolic activity in these cells.

Incubation of exponentially growing cells with theophylline or with MIX, substances which possibly inhibit cyclic nucleotide phosphodiesterase, results in a significant increase of the cyclic GMP level. Additionally, a marked decrease of the percentage of budding cells is observed, which is not due to a delay in budding but to an earlier completion of mitosis by formation of the septal cell wall. Both effects are reversible, at least with MIX. Such a premature cell division also is seen, when growing yeast is incubated with cholera toxin, or with dibutyryl-cyclic GMP, and after a more pronounced lag also with dibutyryl-cyclic AMP. As can be seen not only from the undisturbed increase in cell number, but also from the unaffected augmentation of DNA and protein, the growth rate of the culture remains unimpaired by the effectors tested. Nevertheless, the different substances seem to exert their effects by different ways, as indicated by the formation of remarkably enlarged cells by theophylline. As a whole, the persistent decrease in the proportion of budding cells thus cannot be explained simply by a transition of the cells to G_0 or stationary phase.

Surprisingly, the inhibitors of cyclic nucleotide phosphodiesterase show no effects on the level of cyclic AMP, even when examined with shorter time-intervals (not shown here). It cannot be excluded, however, that there are short-time effects, which rapidly are compensated by counter-regulation. Temporary short-time effects by theophylline are reported by Van der Plaat *et al.* [30], but they could not explain the lasting premature cell division.

The observed effects of dibutyryl-cyclic GMP and of phosphodiesterase inhibitors on cyclic GMP level and mitosis suggest that cyclic GMP in yeast might contribute to the control of that chain of events, by which the cells traverse the mitosis. This would be in agreement with results from animal cell systems, where cyclic GMP as a positive effector seems to be involved in processes controlling cell proliferation [32–40]. Our results that cholera toxin also induces a premature formation of the septal cell wall between mother cell and bud, but without an outstanding increase in the level of cyclic nucleotides, must not be inconsistent with this hypothesis. Cholera toxin activates in vertebrate cells the membrane bound adenylate cyclase by ADP-ribosylation of the N-protein [41–44]. It also has been found to ADP-ribosylate certain cytoskeletal and cytosolic proteins, especially tubulin [45, 46]. Extranuclear microtubules in yeast appear to be involved in bud growth and in processes of nuclear division [47]. On the other hand, the membrane bound adenylate cyclase in yeast but is controlled by an N-protein [7, 11], but no ADP-ribosylation of the N-protein could be detected. However, ADP-ribosylation of multiple particulate proteins from yeast by cholera toxin was observed [11]. Further extensive studies are necessary to enlighten the regulatory function of cyclic GMP and of ADP-ribosylation in growing yeast cells.

Acknowledgements

Prof. Hilz, Dr. W. Weber, and Dr. M. Schumacher supported this work by the generous gift of rabbit anti cyclic GMP antiserum and rabbit *anti*-cyclic AMP antiserum. The excellent technical assistance of B. Flügge is gratefully acknowledged.

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