Inhibition by Cyclic Guanosine 3′:5′-Monophosphate of the Soluble DNA Polymerase Activity, and of Partially Purified DNA Polymerase A (DNA Polymerase I) from the Yeast *Saccharomyces cerevisiae*

Hans Eckstein

Institut für Physiologische Chemie der Universität, Martinistr. 52-UKE, D-2000 Hamburg 20

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Dedicated to Professor Dr. Joachim Kühnau *on the Occasion of His 80th Birthday*

cGMP, DNA Polymerase Activity, DNA Polymerase A, DNA Polymerase I, Baker's Yeast

DNA polymerase activity from extracts of growing yeast cells is inhibited by cGMP. Experiments with partially purified yeast DNA polymerases show, that cGMP inhibits DNA polymerase A (DNA polymerase I from Chang), which is the main component of the soluble DNA polymerase activity in yeast extracts, by competing for the enzyme with the primer-template DNA. Since the enzyme is not only inhibited by 3′,5′-cGMP, but also by 3′,5′-cAMP, the 3′:5′-phosphodiester seems to be crucial for the competition between cGMP and primer. This would be inconsistent with the concept of a 3′-OH primer binding site in the enzyme. The existence of such a site in the yeast DNA polymerase A is indicated from studies with various purine nucleoside monophosphates.

When various DNA polymerases are compared, inhibition by cGMP seems to be restricted to those enzymes, which are involved in DNA replication. DNA polymerases with an associated nuclease activity are not inhibited, DNA polymerase B from yeast is even activated by cGMP. Though some relations between the cGMP effect and the presumed function of the enzymes in the living cell are apparent, the biological meaning of the observations in general remains open.

Introduction

DNA polymerase activity from cell extracts of synchronized growing yeast shows a fluctuating behavior during the cell cycle [1], indicating a strong regulatory control of the enzyme during the cell growth. The fluctuations must be ascribed to DNA polymerase A [2, 3]. Their underlying mechanism as yet is unknown. Observations on striking short-time alterations of cyclic nucleotide levels during the cell cycle suggest a regulation by cyclic nucleotide-dependent protein modification. Though in most cases a correlation in time is reported between increasing cyclic nucleotide levels and transition from G_0 to G_1, or onset of mitosis, respectively [4–12], and also some inconsistent observations are made [13–15], increased levels of cAMP and of cGMP, respectively, also are found in some cases at the S phase [16, 17]. Furthermore, observations on a cAMP-dependent phosphorylation of nuclear proteins, such as DNA polymerase α, virus-induced reverse transcriptase, and other acidic nuclear proteins [18–20], indicate a distinct role of protein modification in the control of cell proliferation. The present paper describes the effect of cGMP on the DNA polymerase activity from yeast extracts, and the influence of cGMP on purified DNA polymerase preparations from several cell systems. Parts of the results were communicated recently in a preliminary form [21].

Reprint requests to Dr. H. Eckstein.

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Materials and Methods

A cell line, formerly isolated from baker's yeast (Germania Hefe, Deutsche Hefewerke, Hamburg, *cf.* [22]) was used for all experiments. Cells were grown in a modified Wickerham medium as described earlier [23]. DNA polymerase I from *E. coli* MR 600 (purified up to step 7 according to Jovin *et al.* [24] was purchased from Boehringer (Mannheim). DNase I from bovine pancreas (chromatographically pure, 2000 Kunitz-units/mg) was obtained from Merck (Darmstadt). [^{3}H]methyl thymidine 5′-triphosphate was from the Radiochemical Centre (Amersham), [8-^{3}H](N)-3′:5′-cyclic GMP (17.2 Ci/mmol) was from NEN, unlabeled nucleotides were purchased from Boehringer. Cyclic nucleotides were chromatographed before use on a Sephadex G-10 column, they were free of pyro-

phosphate. β-mercaptoethanol was from Fluka (Switzerland). Poly(dA-dT), Na-salt, was obtained from Boehringer. Salmon sperm DNA (highly polymerized, free of protein and salt) was from Serva (Heidelberg). ([^{14}C]methyl-thymine)-DNA from *Escherichia coli*, 1.55 μCi/OD_{260}, was purchased from New England Nuclear (Dreieichenhain, Germany).

Estimation of DNA polymerase activity

DNA polymerase activity was estimated in an assay modified from [1]. It contained in a total of 400 μl: 5 nmol dTTP, including 2.5 μCi [methyl-^{3}H]-dTTP, 50 nmol each of dATP, dGTP and dCTP, 100 nmol ATP, 500 nmol β-mercaptoethanol, 13.4 μg "activated" salmon sperm DNA, 4 μmol $MgCl_2$, 40 μmol Tris-HCl-buffer, pH = 7.6. The reaction was started by addition of 10 μl of enzyme solution and incubation at 37 °C. At 0, 5, 10, 15, 20, 25 and 30 min of incubation, 50 μl aliquots were transferred to filter papers (Schleicher & Schuell, No. 589[1], 20 × 20 mm) and immersed into icecold 10% TCA. The filters were washed twice with 5% TCA, twice with alcohol, and with diethylether, and transferred into 15 ml of a dioxan scintillation cocktail (4.0 g Szintimix III, Merck, 100 g naphthalene, Dioxan ad 1000 ml).

Radioactivity incorporated from [^{3}H]dTTP into the acid-insoluble material was counted in a "Betascint" liquid scintillation counter (Fa. Berthold, Wildbach, Germany), and nmol dTMP incorporated per 400 μl assay during the first 10 min of incubation were calculated. One unit of enzyme was defined as that amount, which catalyzes the incorporation of 1 nmol dTMP into acid-insoluble material per minute under the test conditions.

Protein was estimated by the biuret reaction, and by the Lowry method, respectively.

"Activation" of DNA

Salmon sperm DNA was "activated" by a 30 min incubation at 37 °C with DNase I. The reaction mixture contained, in a total volume of 560 μl: 500 μg DNA, 100 ng DNase I, 0.01 mol/l $MgCl_2$, 0.1 mol/l Tris-buffer pH 7.6. After incubation, the reaction was stopped, and DNase was inactivated by addition of 10 μmol Na_2-EDTA and heating 15 min at 95 °C. Finally the mixture was cooled slowly at room temperature. 15–20 percent of the DNA became acid-soluble by this procedure.

Estimation of DNA degrading activity

DNA degrading activity was tested in an assay containing in a total volume of 200 μl: 10 nCi [^{14}C]DNA, 500 nmol β-mercaptoethanol, 0.01 mol/l $MgCl_2$, 0.1 mol/l Tris-HCl-buffer, pH = 7.6, and enzyme. After incubation for 60 min at 37 °C, the mixture was cooled in an ice bath, 10mg bovine serum albumin (Schering, Berlin), and TCA (final concentration 5%) were added. The insoluble material was centrifuged off. The radioactivity was determined in the clear supernatant, it was refered to a standard assay with 1 μg DNase I.

Preparation of yeast DNA polymerase

If not stated otherwise, all operations were carried out at 0–4 °C. Yeast was harvested from cultures containing 20–25 × 10^9 growing cells/l by centrifugation 10 min at 16300 × *g*. After washing the cells twice with water and once with MET-buffer (0.25 mol/l D-mannitol, 1 mmol/l Na_2-EDTA, 0.02 mol/l Tris-HCl, pH = 7.6; *cf.* [25]), they were suspended with MET-buffer (1 × 10^9 cells/ml), and broken by shaking with glass beads (0.45 mm ∅) in a MSK cell homogenizer (B. Braun, Melsungen). Microscopic observation gave evidence that more than 99% of the cells were destroyed. The glass beads were separated by filtration, the cell debris was centrifuged off (15 min, 1500 × *g*, − 2 °C) and discarded. The supernatant (= "crude extract") was centrifuged 25 min at 30000 × *g*, − 2 °C. The resulting residue contained some DNA polymerase activity, which with reference to [26] preliminary was called "mitochondrial". The 30000 × *g* pellet was washed twice with MET-buffer, the supernatants were combined and centrifuged at 115000 × *g* (120 min, − 2 °C). In contrast to [3, 25], we again found the postmitochondrial DNA polymerase activity nearly completely in the 115000 × *g* supernatant (*cf.* also [1]). DNA polymerase A and B were purified from the 115000 × *g* supernatant in essential according to [25], but with the following modifications:

a) Enzyme activity was separated from the 115000 × *g*-supernatant by stepwise precipitation with a 4 mol/l ammonium sulfate solution containing 20 mmol/l Tris-HCl, 1 mmol/l Na_2-EDTA, and 2 mmol/l mercaptoethanol, pH = 7.5. DNA polymerase activity precipitated completely between 1.10 and 1.95 mol/l ammonium sulfate.

Table I. Purification of yeast DNA polymerases.

Fraction	Total units	Specific activity [units/mg protein]	Yield [%]
Crude extract	32.5	0.039	100.0
30 000 × *g* residue	0.56	0.088	1.7
30 000 × *g* supernatant	43.0	0.056	132.3
115 000 × *g* supernatant	57.3	0.074	176.3
ammonium sulfate – ppt. + dialysis	33.2	0.221	102.2
Enzyme A			
DEAE-cellulose + ammonium sulfate – ppt. + dialysis	9.5	1.87	29.2
DNA-cellulose	4.8	217.48	14.8
Enzyme B			
DEAE-cellulose + ammonium sulfate – ppt. + dialysis	0.81	0.914	2.5
DNA-cellulose	0.40	32.4	1.2

b) After separation of DNA polymerases A and B by anion exchange chromatography (step 5 in ref. [25], the enzymes were precipitated by 2 mol/l ammonium sulfate and dialysed against PEMEG-buffer (0.03 mol/l potassium phosphate, 1 mmol/l Na_2-EDTA, 2 mmol/l mercaptoethanol, 10% (w/v) glycerol, pH = 7.5).

The resulting DNA polymerase A fraction was free of measurable DNA degrading activity. In this point, and with respect to the behaviour at anion exchange chromatography, our preparation corresponds to DNA polymerase A from Wintersberger [25]. It also appears comparable to DNA polymerase I (step Va) from Chang [27].

Further purification of DNA polymerases A and B, resp., was achieved by chromatography on a DNA cellulose column (12×175 mm) according to [28]. The enzymes were eluted by 0.6 mol/l NaCl in TEMEG-buffer containing 6.09 mg/l phenylmethylsulfonylfluoride. Referring to the activity in the crude extract, enzyme A was purified about 5600 times, enzyme B 830 times (Table I)*.

* The degree of purity by far exceeds that obtained by Potuzak *et al.* [28]. On the other hand, the data cannot be compared with those from Chang [27], since this author, using a more complicated preparation method, works at differing test conditions.

Table II. Effects of cyclic nucleotides on the soluble DNA polymerase activity from 115 000 × *g*-supernatants, and their modification by assay conditions. All nucleotides were 0.25 mmol/l. The % values are related to corresponding controls without cGMP. All experiments were done in triplicate.

Assay conditions	Incorporation	
	[pmol dTMP]	[%]
standard	14.81	100.0
standard + cGMP	9.48 ± 0.44	64.0 ± 3
standard + cAMP	22.04 ± 1.04	148.8 ± 7
standard + GMP	20.10 ± 0.89	135.7 ± 6
standard + AMP	12.37 ± 0.59	83.5 ± 4
10 × [dNTP], + cGMP	6.86 ± 0.59	62.8 ± 4
10 × [protein], + cGMP	31.65 ± 0.74	107.5 ± 5
4 × [DNA], + cGMP	12.21 ± 0.75	96.0 ± 5
5 mmol/l theophylline + cGMP	6.26 ± 0.43	45.1 ± 3

Results

In a first attempt to get some information about an influence of cyclic nucleotides on yeast DNA polymerases we tested the effects of cAMP and cGMP on the soluble DNA polymerase activity from yeast extracts. As pointed out in Table II, the results were quite different: cGMP (0.25 mmol/l) inhibits the enzyme activity from 115000 × *g*-supernatants, but cAMP activates the DNA synthesis.

These effects are in contrast to those induced by the corresponding 5′-nucleoside monophosphates, since GMP enhances the enzyme activity, whereas AMP brings about a slight inhibition.

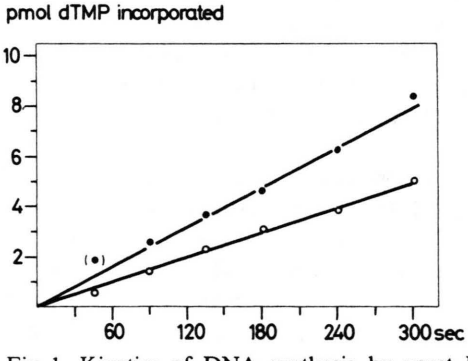

Fig. 1. Kinetics of DNA synthesis by yeast DNA polymerase under cGMP. DNA polymerase assays were prepared as described in "Materials and Methods", but with a tenfold concentration of [^{3}H]dTTP. The cGMP concentration was 0.25 mmol/l. The reaction was started by addition of enzyme (1.5 µg protein) and incubation at 37 °C. At the times indicated, aliquots of 50 µl were taken off and treated as described in "Materials and Methods".
●——● Control; ○——○ +cGMP.

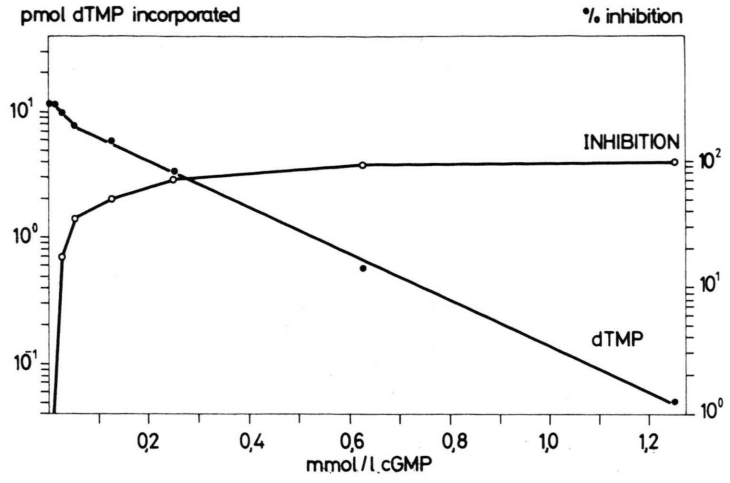

Fig. 2. Inhibition of yeast DNA polymerase A by cGMP. Yeast DNA polymerase A was assayed, as described in "Materials and Methods", and incubated in presence of the indicated concentrations of cGMP. Inhibition was calculated from a reference assay without cGMP.
●——● pmol dTMP;
○——○ inhibition (%).

Studies on the kinetics of the cGMP effect demonstrate (Fig. 1) that the DNA synthesis is inhibited by cGMP even after the first minute of incubation. The degree of inhibition remains constant for at least ten minutes.

The inhibition by cGMP of the DNA synthesis can be removed by raising the concentration of primer-template DNA, or yeast extract, respectively, but not by elevated precursor concentrations. The effect of cGMP is strengthened in presence of theophylline, a known inhibitor of cyclic nucleotide degrading phosphodiesterase. Theophylline alone has a slight effect on DNA synthesis by yeast extracts only with concentrations above 10 mmol/l.

Two questions arise from these findings: 1) Does the inhibition by cGMP of the soluble yeast DNA polymerase activity reflect a cGMP-mediated modification of enzyme protein during the incubation? 2) Which of the three yeast DNA polymerases is the target?

The main component of the soluble DNA polymerase activity from yeast extracts is DNA polymerase A. This enzyme is inhibited by cGMP concentrations above 0.01 mmol/l (Fig. 2). 50% reduction of the enzyme activity is obtained by 0.1 mmol/l cGMP. In contrast to enzyme A, yeast DNA polymerase B is activated by 0.25 mmol cGMP/l (Table III). On the other hand, no significant cGMP effect is observed with the DNA polymerase activity from 30000 × *g* residues. From other DNA polymerases, the activity of DNA polymerase I from *Escherichia coli* is not changed by 0.25 mmol cGMP/l, whereas a clear inhibition of the DNA synthesis with DNA polymerase α from bovine liver embryonic cells is observed.

In view of the different situation with the various yeast DNA polymerases, we restricted the following investigations to enzyme A.

Table IV summarizes the results from experiments, where the concentrations of single components of the enzyme assay were raised. As demon-

Table III. Effect of cGMP on the activity of DNA polymerases from various sources. DNA polymerase preparations from different organisms were incubated in a DNA polymerase assay as described in "Materials and Methods". cGMP was supplemented as indicated. The assays with yeast DNA polymerase B contained no ATP. DNA polymerase α from bovine embryonic liver cells (sedimentation quotient approximately 7 s; original specific activity: 12 nmol dTMP incorporated/mg protein at 31 °C in 30 min) was a gift from Prof. Hofschneider, Munich. It was kindly made available by Dr. Dahlmann, Hamburg.

	cGMP	Incorporation/ 10 min	
	[mmol/l]	[pmol dTMP]	[%]
yeast DNA polymerase A	–	13.05	100.0
(1.5 µg protein/assay)	0.25	3.80	29.2
yeast DNA polymerase B	–	4.04	100.0
(2.5 µg protein/assay)	0.25	13.49	334.0
30000 × *g* residue	–	4.44	100.0
from yeast extracts	0.25	3.88	87.5
(160 µg protein/assay)			
E. coli DNA polymerase I	–	11.46	100.0
(25 milliunits/assay)	0.25	12.17	106.2
DNA polymerase α from	–	10.11	100.0
liver embryonic cells	0.25	4.00	39.6
(3.5 µg protein/assay)			

Table IV. Inhibition by cGMP of yeast polymerase A with modified assay conditions. Yeast DNA polymerase A was incubated in parallel with and without cGMP at modified concentrations of substrates, primer-template DNA, and enzyme, respectively, as indicated.

Assay	cGMP	Incorporation/ 10 min	
	[mol/l]	[pmol dTMP]	[%]
standard	–	10.48	100.0
standard	0.25×10^{-3}	3.14	30.0
10 fold dNTP concentration	–	9.43	100.0
	0.25×10^{-3}	2.97	31.5
4 fold enzyme concentration	–	36.31	100.0
	0.25×10^{-3}	12.24	33.7
5 fold DNA concentration	–	7.09	100.0
	0.25×10^{-3}	8.24	116.2
standard + 5 mmol/l theophylline	–	8.81	100.0
	0.25×10^{-3}	2.49	28.3

strated, the inhibition by cGMP of the yeast polymerase A is abolished only when the primer-template DNA concentration is raised. It should also be noticed, that theophylline, which clearly intensifies the cGMP-effect with cell extracts, does not influence the inhibition of the purified enzyme.

The competition for the enzyme between cGMP and the primer-template DNA becomes evident from studies on the relation between reaction speed of DNA synthesis and primer-template concentration in the assay (Fig. 3). But at very low DNA concentrations, the reaction velocity in presence of cGMP appears too high for a regular competitive inhibition, possibly indicating a more complicated mode of interaction between enzyme, primer DNA and inhibitor. Nevertheless, the presentation of the data by a Lineweaver-Burk plot indicates, that K_M is about 8.0 µg DNA/ml for the uninhibited reaction, but 45.5 µg DNA/ml in the case of inhibition, whereas V_{max} is about 12 pmol dTMP incorporated in 10 minutes in both cases.

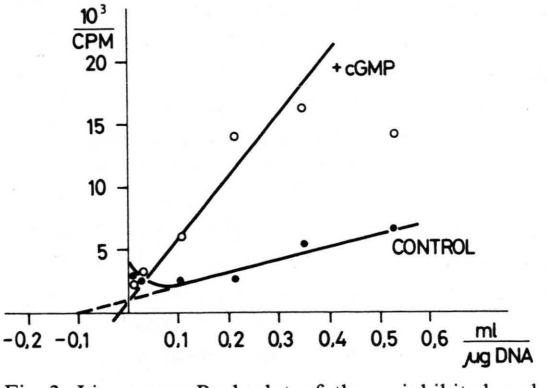

Fig. 3. Lineweaver-Burk-plot of the uninhibited and the inhibited DNA-polymerase A-reaction. Yeast DNA polymerase A was incubated in a DNA polymerase assay as described in "Materials and Methods". At each indicated DNA-concentration assays were prepared in parallel with and without 0.25 mmol/l cGMP. dTMP incorporation per assay was estimated, the reciprocal values were calculated and plotted against the reciprocal of DNA concentration as proposed by Lineweaver and Burk [29].

A competition of cGMP and primer-template DNA for the enzyme probably concerns the primer binding site of DNA polymerase. The following experiments were conducted with the aim to elucidate the mechanism of the interaction between cGMP and the enzyme protein.

1. [³H]dTTP was replaced in the assay by [³H]cGMP. As to be expected, no radioactivity was incorporated into the acid-insoluble material (Table V).
2. When poly(dA-dT) instead of "activated" DNA was taken for a primer-template, the DNA polymerase reaction again was inhibited considerably by cGMP (Table VI).
3. A remarkable inhibition of yeast DNA polymerase A is produced not only by 3′,5′-cGMP,

Table V. Incorporation of cGMP by yeast DNA polymerase A into acid-insoluble material. The standard DNA polymerase assay and other details are described in "Materials and Methods". Incorporation of cGMP was estimated by applying [³H]cGMP instead of [³H]dTTP.

Assay	Marker	cGMP	Incorporation	
		[mol/l]	[pmol]	[%]
standard	2.5 µCi [³H]dTTP	–	7.26	100.0
standard	2.5 µCi [³H]dTTP	0.25×10^{-3}	2.07	28.5
standard	10 µCi [³H]cGMP	0.25×10^{-3}	0	–
standard	10 µCi [³H]cGMP	1.46×10^{-6}	0	–

Table VI. Inhibition of yeast DNA polymerase A by cGMP and cAMP, with poly(dA-dT) as a primer-template. Assay conditions were as described in "Materials and Methods", but DNA was replaced by equaivalent amounts of poly(dA-dT).

Assay conditions	Incorporation	
	[pmol dTMP]	[%]
Control	2.39	100.0
+ 0.25 mmol/l cGMP	0.54	22.4
+ 0.25 mmol/l cAMP	1.80	75.3

Table VII. Inhibition of yeast DNA polymerase A by purine nucleoside monophosphates. Standard DNA polymerase assays were supplemented with 0.25 mmol/l each of the indicated nucleotides, and incubated as described in "Materials and Methods".

Assay conditions	Incorporation	
	[pmol dTMP]	[%]
standard	11.72	100.0
+ 3′,5′-cGMP	4.94	42.1
+ 2′,3′-cGMP	10.22	87.2
+ 5′-GMP	7.07	60.3
+ 3′-GMP	12.11	103.3
+ 3′,5′-cAMP	7.54	64.3
+ 2′,3′-cAMP	11.84	101.0
+ 5′-AMP	8.63	73.6
+ 3′-AMP	12.25	104.5

but also by 5′-GMP (Table VII). 3′-GMP remains without effect. A slight but scarcely significant inhibition is observed also with 2′,3′-cGMP. Similar results were obtained with AMP analoga: 3′,5′-cAMP and 5′-AMP inhibit yeast DNA polymerase A significantly, whereas 3′-AMP, like 2′,3′-cAMP, remains without an effect.

Discussion

The observation, that the soluble DNA polymerase activity from extracts from growing yeast is changed in opposite directions by cAMP and cGMP (*cf.* Table II) suggests the idea of a regulation of this enzyme activity during the yeast cell cycle by a cAMP- and/or cGMP-dependent modification of the enzyme protein. This assumption is supported by the findings, that cAMP shows a peak at that time when in synchronized growing yeast DNA polymerase activity increases and DNA synthesis starts [1, 2, 16, 23]. A sharp cGMP peak at the end of [^{3}H]thymidine incorporation by the slime mould Physarum polycephalum [17] also points into this direction, though observations on the cGMP level during the cell cycle of other cell systems [5, 30] are inconsistent. However, the fact, that partially purified DNA polymerase A, the main component of the soluble DNA polymerase activity in yeast extracts [2, 3, 27], is inhibited, too, by cGMP, markes it rather unlikely, that a cGMP-mediated protein modification in the assay might be crucial for the decay of the enzyme activity.

The inhibition by cGMP of yeast DNA polymerase A cannot be explained simply by artefacts: The effect of cGMP is not overcome by additional equimolar amounts of magnesium ions (not shown here). Furthermore, various conditions being effective as a magnesium trap do not essentially influence the incorporation of dTMP (*cf.* Table IV). The non-uniform results with various DNA polymerases (*cf.* Table III) and with various nucleotides (*cf.* Table VI and VII) also exclude an explanation by an artefact.

The competition between cGMP and primer-template DNA (*cf.* Fig. 3) for the enzyme primarily should be considered with regard to the mechanism of DNA binding. As was shown with DNA polymerase I from *Escherichia coli* [31], a free 3′-hydroxyl group and a 5′-phosphate in the ribose moiety are basic requirements for competition between nucleotides and DNA primer. The fact, that yeast DNA polymerase A is inhibited by 5′-GMP and by 5′-AMP, but not by the corresponding 3′-nucleotides nor by their 2′,3′-cyclic derivatives (Table VII) illustrates, that a similar mechanism must be postulated also for this enzyme. The inhibition by 3′,5-cyclic nucleotides, however, needs an additional explanation. Since this inhibition apparently occurs independently from the purine base, the crucial point seems to be the 3′:5′-phosphodiester configuration. It is rather surprising and not as yet understandable, how this configuration, though there is no free 3′-hydroxyl group, allows a competition for the primer binding site of yeast DNA polymerase A.

cGMP inhibits not only DNA polymerase A from yeast, but also DNA polymerase α from liver embryonic cells. This corresponds well to the similarity between yeast DNA polymerase I and eucaryotic DNA polymerase α as stated by Chang [27]. Both enzymes presumably are responsible for DNA replication [3, 32]. Another situation exists with the nuclease bearing yeast DNA polymerase B (yeast DNA polymerase II from Chang). This enzyme, which exhibits properties of the procaryotic DNA polymerases II and III [27], is activated by cGMP. Like these, yeast DNA polymerase B might be involved in repair and gap filling reactions [3].

Last but not least the insensitiveness against cGMP of the "mitochondrial" DNA polymerase activity from yeast $30000 \times g$-residues and of DNA polymerase I from *E. coli* should be mentioned, though the identity of this activity with the yeast mitochondrial enzyme was not established. Again, both

enzymes bear nuclease activity [24, 26]. In summary, the different sensibility of the DNA polymerases from yeast against cGMP could have some bearing on the presumed differential biological role of these enzymes.

An inhibition of DNA polymerase A by cGMP or other low molecular compounds *in vivo* could contribute essentially to the rapid decrease of the enzyme activity at the end of the S phase. It must be outlined, however, that the cGMP concentrations needed here apparently exceed significantly the known "physiological" values, though the level of cGMP in yeast cells, and its behavior during the cell cycle not as yet are investigated. But as compared with cAMP levels in yeast (*cf.* [16]), cGMP per milligramme enzyme protein in the assay producing a 50% inhibition is about the 10^5 fold, though cGMP per milliliter assay differs only by a factor of 10. Furthermore, an effect of cGMP would be rather unspecific, since cAMP also inhibits the yeast DNA polymerase A. It is not clear, how the enzyme activity could be influenced *in vivo:* With several cell systems low cAMP levels during the S phase are reported [9, 17, 33], but also contradictory observations are made, including yeast [16, 30, 34, 35]. A similarly complex situation exists in the literature with regard to cGMP [5, 17, 30]. Thus, further investigations are necessary to solve this problem.

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[1] H. Eckstein, V. Paduch, and H. Hilz, Eur. J. Biochem. **3,** 224–231 (1967).
[2] J. Golombek, W. Wolf, and E. Wintersberger, Molec. Gen. Genetics **132,** 137–145 (1974).
[3] E. Wintersberger, Eur. J. Biochem. **50,** 41–47 (1974).
[4] M. M. Burger, B. M. Bombik, B. Mcl. Breckenridge, and J. R. Sheppard, Nature (New Biol.) **239,** 161–163 (1972).
[5] W. E. Seifert and P. S. Rudland, Nature **248,** 138–140 (1974).
[6] N. D. Goldberg, M. K. Haddox, S. E. Nicol, T. S. Acott, C. E. Zeilig, and D. B. Glass, ICN, UCLA Symp. Molecular and Cellular Biol., **Vol II,** W. A. Benjamin, Mass. 1975.
[7] J. R. Sheppard and D. M. Prescott, Exp. Cell Res. **75,** 293–296 (1972).
[8] D. H. Russell and P. J. Stambrook, Proc. Nat. Acad. Sci. USA **72,** 1482–1486 (1975).
[9] A. J. T. Millis, G. A. Forrest, and D. A. Pious, Exp. Cell Res. **83,** 335–343 (1974).
[10] G. Wong, J. Pawelek, M. Sansone, and J. Morowitz, Nature **248,** 351–354 (1974).
[11] C. E. Zeilig, R. A. Johnson, D. L. Friedman, and E. W. Sutherland, J. Cell Biol. **55,** 296 a (1972).
[12] C. E. Zeilig, R. A. Johnson, E. W. Sutherland, and D. L. Friedman, Fed. Proc. **33,** 948 a (1974).
[13] J. Oey, A. Vogel, and R. Pollack, Proc. Nat. Acad. Sci. USA **71,** 694–698 (1974).
[14] W. Moens, A. Vokaer, and R. Kram, Proc. Nat. Acad. Sci. USA **72,** 1063–1067 (1975).
[15] Z. Miller, E. Lovelace, M. Gallo, and J. Pastan, Science **190,** 1213–1215 (1975).
[16] C. D. Watson and D. R. Berry, FEMS Microbiol. Lett. **1,** 175–179 (1977).
[17] J. R. Lovely and R. J. Threlfall, Biochem. Biophys. Res. Comm. **71,** 789–795 (1976).
[18] V. G. Allfrey, A. Inous, J. Kern, E. M. Johnson, and G. Vidali, Cold Spring Harbour Symp. Quant. Biol. **38,** 785–801 (1974).
[19] S. R. Reisher, R. J. Rutman, and S. Erhan, Cytobios **12,** 57–60 (1975).
[20] S. G. Lee, M. V. Miceli, R. A. Jungmann, and P. P. Hung, Proc. Nat. Acad. Sci. USA **72,** 2945–2949 (1975).
[21] H. Eckstein, Abstr. Frühjahrstag. Ges. Biol. Chem., in: Hoppe-Seyler's Z. Physiol. Chem. **360,** 254 (1979).
[22] H. Eckstein, S. Ahnefeld, and K. Albiez-Loges, Z. Naturforsch. **29 c,** 272–282 (1974).
[23] H. Hilz and H. Eckstein, Biochem. Z. **340,** 351–382 (1964).
[24] T. M. Jovin, P. T. Englund, and L. LeRoy Bertsch, J. Biol. Chem. **244,** 2996–3008 (1969).
[25] U. Wintersberger and E. Wintersberger, Eur. J. Biochem. **13,** 11–19 (1970).
[26] U. Wintersberger and E. Wintersberger, Eur. J. Biochem. **13,** 20–27 (1970).
[27] L. M. S. Chang, J. Biol. Chem. **252,** 1873–1880 (1977).
[28] H. Potuzak and E. Wintersberger, FEBS Letters **63,** 167–170 (1976).
[29] H. Lineweaver and D. Burk, J. Am. Chem. Soc. **56,** 658 (1934).
[30] W. Seifert and P. S. Rudland, Proc. Nat. Acad. Sci. USA **71,** 4920–4924 (1974).
[31] J. E. Hubermann and A. Kornberg, J. Biol. Chem. **245,** 5326–5334 (1970).
[32] A Weissbach, Ann. Rev. Biochem. **46,** 25–47 (1977).
[33] J. R. Dickinson, M. G. Graves, and B. E. P. Swoboda, FEBS Letters **65,** 152–154 (1976).
[34] Ch. E. Zeilig, R. A. Johnson, E. W. Sutherland, and D. L. Friedman, J. Cell Biol. **71,** 515–534 (1976).
[35] Ch. E. Zeilig and N. D. Goldberg, Proc. Nat. Acad. Sci. USA **74,** 1052–1056 (1977).