The binding of adenosine(5')tetraphospho(5')adenosine to calf thymus histones measured by non-equilibrium dialysis

Gerald JUST and Eggehard HOLLER

Institut für Biophysik und Physikalische Biochemie der Universität, Universitätsstrasse 31, D-8400 Regensburg, German Federal Republic

Binding of adenosine(5')tetraphospho(5')adenosine (Ap_4A) to histones of calf thymus was investigated by non-equilibrium dialysis. Histone H1 interacts with the dinucleotide via two strong sites and competes with Mg^{2+} ions. Intrinsic dissociation constants were $1.6 \pm 0.1 \mu\text{M}$ and $11 \pm 1 \mu\text{M}$ for zero and 0.4 mM-Mg^{2+} concentration respectively. Binding of poly(dT) and of other nucleotides to histone H1 was measured in an $[^3\text{H}]\text{Ap}_4\text{A}$ -competition assay. The tendency to form complexes among nucleotides was highest for bisnucleoside tetraphosphates and decreased in the order $\text{poly(dT)} \gtrsim \text{Ap}_4\text{A} \sim \text{Gp}_4\text{G} > \text{Ap}_4 \gg \text{Ap}_3\text{A} \sim \text{Ap}_5\text{A} \gtrsim \text{ATP, GTP and dTTP}$. The co-ordination complex derived from Ap_4A and *cis*-diammine-dichloroplatinum(II) was not reactive. The other histones of calf thymus also bound Ap_4A with affinities decreasing in the order $\text{H4} \sim \text{H3} > \text{H1} > \text{H2b} > \text{H2a}$. Ap_4A stimulated the exchange of histone H1 between nucleosomes, but this effect was referred to ionic strength. It did not bind to assembled nucleosomes. Binding of Ap_4A to histone H1 was decreased by salt (NaCl). At physiological saline concentration the value of the dissociation constant is commensurable with the value of the Ap_4A concentration in the nucleus and thus indicative of complex-formation *in vivo*.

INTRODUCTION

Adenosine(5')tetraphospho(5')adenosine (Ap_4A) is readily synthesized by certain aminoacyl-tRNA synthetases in the presence of Zn^{2+} ions (Plateau *et al.*, 1981; Goerlich *et al.*, 1982) and is metabolized by specific hydrolases (Guranowski *et al.*, 1983; Zamecnik, 1983, and references cited therein). Its concentration rises during growth from $0.01 \mu\text{M}$ in resting cells to $5 \mu\text{M}$ in rapidly dividing cells (Zamecnik, 1983), with peak concentrations during the onset of DNA synthesis (Weinmann-Dorsch *et al.*, 1984). In stress situations, such as under heat shock or oxidation shock, the Ap_4A concentration can exceed the $100 \mu\text{M}$ level (Lee *et al.*, 1983; Varshavsky, 1983).

Among suggested functions in its role as a pleiotypic cell growth effector (Zamecnik, 1983) is the triggering of initiation of DNA synthesis, as in baby-hamster kidney cells (Grummt, 1978). Another function is inhibition of ADP-ribosylation of proteins in a histone H1-dependent fashion (Tanaka *et al.*, 1981; Yoshihara & Tanaka, 1981).

In the present investigation we have studied whether Ap_4A could bind to histone H1 and the other histones, and whether it could bind to assembled nucleosomes and effect an exchange of histone H1.

EXPERIMENTAL

Materials

Unfractionated histones were prepared from calf thymus of freshly slaughtered animals by the procedure of Panyim & Chalkley (1971) and separated by partition

chromatography on Bio-Gel P-100 by the method of Sommer & Chalkley (1973). Histone H1 and arginine-rich histone purchased from Sigma Chemical Co. were not preparatively resolved into subspecies. Purity of the protein fractions was assessed after denaturing polyacrylamide-gel electrophoresis in the presence of SDS (Laemmli, 1970). By these criteria, histones H1, H2a and H4 were more than 90% pure proteins, the histone H2b/H3 preparation contained more than 75% histone H2b, and arginine-rich histone (Sigma) contained more than 70% histone H3.

Calf thymus nuclei and chromatin were prepared from freshly slaughtered animals by the procedure of Huang & Cole (1984). Soluble chromatin was prepared from nuclei after digestion with micrococcal nuclease. Concentrations were measured photometrically in the presence of 0.1 M-NaOH by using the absorption coefficient $\epsilon_{260} = 10 \text{ mg}^{-1} \cdot \text{cm}^2$ (Caron & Thomas, 1981).

$[^3\text{H}]\text{Ap}_4\text{A}$ (9 Ci/mmol) was obtained from Amersham-Buchler (Braunschweig, Germany) and was purified by ion-exchange chromatography (Pimmer *et al.*, 1976). Unlabelled nucleotides were from Sigma or Boehringer (Mannheim, Germany), all other chemicals of highest grade from Merck (Darmstadt, Germany). The adduct of Ap_4A with *cis*-diamminedichloroplatinum(II) has been prepared by the method of Segal *et al.* (1984) and purified similarly to Ap_4A (Pimmer *et al.*, 1976).

Non-equilibrium dialysis

Dissociation constants and stoichiometries were measured by non-equilibrium dialysis as described previously (Colowick & Womack, 1969; Güntner & Holler, 1979; Muise & Holler, 1985). A dialysis apparatus was employed that consisted of a $1500 \mu\text{l}$ (upper) reaction

Abbreviations used: Ap_3A , adenosine(5')triphospho(5')adenosine; Ap_4A , adenosine(5')tetraphospho(5')adenosine; Ap_5A , adenosine(5')pentaphospho(5')adenosine; Ap_6A , adenosine(5')hexaphospho(5')adenosine; Gp_4G , guanosine(5')tetraphospho(5')guanosine.

Fig. 1. Non-equilibrium dialysis

(a) Titration of $[^3\text{H}]\text{Ap}_4\text{A}$ with histone H1 measured by non-equilibrium dialysis. Values for c.p.m._i, the radioactivity in the effluent, were measured as a function of histone added at the positions of arrows. Radioactivity c.p.m.₀ refers to complete saturation of $[^3\text{H}]\text{Ap}_4\text{A}$ by the protein. Values were corrected for dilution. The concentration of bound dinucleotide, $[[^3\text{H}]\text{Ap}_4\text{A}]_b$, was calculated according to $[[^3\text{H}]\text{Ap}_4\text{A}]_b = [[^3\text{H}]\text{Ap}_4\text{A}]_0 (1 - \text{c.p.m.}_i / \text{c.p.m.}_0)$. The concentration of total dinucleotide, $[[^3\text{H}]\text{Ap}_4\text{A}]_0$, was $24 \mu\text{M}$ and that of MgCl_2 was 0.4 mM . (b) Stoichiometry of the H1- $[^3\text{H}]\text{Ap}_4\text{A}$ complex. The tangent method was used (Bartmann *et al.*, 1975). Conditions were those of Fig. 1 (a).

compartment connected via a Servapor dialysis membrane (from Serva; exclusion limit 10–15 kDa, membrane 8.8–18.8 mm² in diameter) with a 100 μl (lower) effluent compartment. Fractions (3 drops) of effluent were collected in 2 ml of scintillation cocktail (Packard Emulsifier Scintillator 299) at a rate of 66–80 $\mu\text{l}/\text{min}$ and their radioactivities counted in a Nuclear-Chicago Isocap 300 apparatus. The principle of the technique was to measure the radioactivity in the effluent, which was proportional to the concentration of the diffusible ligand in the (upper) reaction compartment.

Effluent fractions contained 5000–15000 c.p.m. radioactivity under the conditions employed in the present experiment. Dialysis displayed negligible depletion of radioactivity from the reaction compartment. All measurements were performed at $23 \pm 1^\circ\text{C}$ in 10 mM-Tris/HCl buffer, pH 7, containing 0.4 mM- MgCl_2 unless otherwise mentioned.

Histone H1 exchange

Exchange of histone H1 between short and long chromatin segments was measured by the method of Caron & Thomas (1981). The chromatin segments were prepared as described in the cited reference after 5–30 %-(w/v)-sucrose-gradient centrifugation (in 39 ml of 5 mM-Tris/HCl buffer, pH 7.5, containing 0.2 mM-EDTA and 1 mM-phenylmethanesulphonyl fluoride) of nuclease-digested nuclei in a Beckman L265B ultracentrifuge (SW27 rotor, 22000 rev./min for 22 h). Segments lacking histone H1 were prepared in the same way but with the

inclusion of 0.5 M-NaCl in the centrifugation buffer. After recording A_{260} , fractions of short (oligomers of three or four nucleosomes) and long segments (14 or 15 nucleosomes) were collected in accordance with Caron & Thomas (1981). Fractions were concentrated by dialysis against 30 % (w/v) poly(ethylene glycol) 20000 (Roth, Munich, Germany) in centrifugation buffer.

Reaction mixtures for histone H1 exchange (500 μl) contained 200 μg of histone H1-associated short segments and 200 μg of long segments devoid of histone H1, together with various amounts of Ap_4A and/or NaCl. The reaction mixture was fractionated after 24 h at 4°C by sucrose-gradient centrifugation as described above (11 ml tubes, SW40 rotor, 22000 rev./min at 17 h) into short and long segments. Fractions were analysed by polyacrylamide-gel electrophoresis under denaturing conditions in the presence of SDS in accordance with Thomas & Kornberg (1978). After staining with Coomassie Brilliant Blue R-250, gels were scanned with a Desaga densitometer equipped with a Hewlett-Packard integrator. Ratios of proteins in the histone H1 and H4 bands were calculated for the short and the long segments.

Other methods

Chromatography on polyethyleneimine-(PEI)-cellulose thin-layer sheets (Merck) was performed as described previously (Goerlich *et al.*, 1982) with minor modifications (Guranowski *et al.*, 1983). Automated radioactivity analysis was performed on a Berthold t.l.c. linear analyser LB 2820.

Fig. 2. Scatchard plot for Ap_4A -histone H1 binding and Mg^{2+} dependence

(a) Scatchard plot for the titration of histone H1 with $[^3H]Ap_4A$ in single-addition experiments. The concentration of histone H1 was $12 \mu M$, that of $[^3H]Ap_4A$ was $1-200 \mu M$, and that of $MgCl_2$ was $0.4 mM$. (b) Effect of Mg^{2+} on the stability of $H1 \cdot [^3H]Ap_4A$ complex. Values of the dissociation constant, $K_{diss. (obs.)}$, were determined in single-addition experiments. Concentrations were $12 \mu M$ -histone H1 and $11 \mu M$ - $[^3H]Ap_4A$.

RESULTS

Formation of complex with histone H1 at various concentrations

The concentration of bound $[^3H]Ap_4A$ was measured as a function of the concentration of added histone H1 (Fig. 1a). Application of the tangent method (Bartmann *et al.*, 1975) revealed that two molecules of bound $[^3H]Ap_4A$ bound per molecule of histone H1 (Fig. 1b).

A Scatchard (1949) plot was calculated in another example, taking into account that two molecules of Ap_4A bind a single molecule of histone H1. The data points indicated a single class of binding sites with an overall dissociation constant $K_{diss. (over.)} 4.5 \pm 0.2 \mu M$ and of an intrinsic dissociation constant of $2 \times K_{diss. (over.)} 9 \mu M$ (per binding site). Calculations of amounts of bound $[^3H]Ap_4A$ were based on the fact that 46% of the original radioactive material in the effluent was not Ap_4A and did not bind histone. It corresponded to $[^3H]AMP$ and $[^3H]ATP$, which were found to be almost unavoidable impurities even in repeatedly purified preparations of $[^3H]Ap_4A$. The mononucleotides were identified by t.l.c. and represented 2-3% of the total radioactivity. Such amounts of $[^3H]AMP$ and/or $[^3H]ATP$ in the reaction compartment were verified to produce the same radioactivity in the effluent as did the impurities. On an equal-concentration basis, the mononucleotides diffused 10-fold faster than did Ap_4A .

Reversibility of complex-formation

The complex of histone H1 and $[^3H]Ap_4A$ was collected by ultrafiltration (Amicon PM 10 membrane) and was shown by t.l.c. to contain intact $[^3H]Ap_4A$, ruling out the possibility of covalent attachment to histone. Further-

more, reversibility of complex-formation was verified by displacement of $[^3H]Ap_4A$ ($12 \mu M$) from the complex ($10 \mu M$) as a function of added unlabelled Ap_4A ($8-55 \mu M$) followed by the appearance of radioactivity in the dialysis effluent. The data points obtained in accordance with Güntner & Holler (1979) followed a linear dependence when plotted in accordance with Scatchard (1949) and a dissociation constant of $12 \mu M$ in agreement with above results.

Binding parameters were also determined in single-addition experiments, in which the reactants were exposed to each other during a minimum in time (10-15 min). In this case steady-state dialysis was first established for $[^3H]Ap_4A$ alone and then histone added. Data plots in accordance with Scatchard (1949) were linear for $1-28 \mu M$ - $[^3H]Ap_4A$ and $12 \mu M$ -histone H1, following a stoichiometry value of 1.8 ± 0.1 mol of Ap_4A /mol of histone H1 and a dissociation constant of $11 \pm 1 \mu M$ (Fig. 2a), again in agreement with the results above. At elevated concentrations of the dinucleotide, the amount of bound Ap_4A was higher than estimated for the above parameters (Fig. 2a). This is expected if additional binding of Ap_4A occurs to other sites with less affinity. Indeed, more than two binding sites on histone H1 have been suggested on the basis of c.d. measurements (Holler, 1984).

Mg^{2+} ions and ionic strength

The dissociation constant was measured as a function of Mg^{2+} concentration (single-addition experiments with $MgCl_2$ in the reaction and effluent buffers). The affinity decreased in a fashion that was linear below $1 mM$ - Mg^{2+} , following a slope of 2.8×10^2 (Fig. 2b). In a separate experiment at zero concentration of Mg^{2+} the intrinsic

dissociation constant of $1.6 \pm 0.1 \mu\text{M}$ and the stoichiometry of 2 mol of Ap_4A /mol of histone H1 was established.

In order to understand the results in Fig. 2(b), we assume that Mg^{2+} and Ap_4A form a complex with low binding affinity for histone H1 (eqn. 1). The value for the dissociation constant in Fig. 2(b) is calculated according to eqn. (2).

$$K_{\text{diss. (obs.)}} = \frac{([\text{Ap}_4\text{A}] + [\text{Mg}^{2+} \cdot \text{Ap}_4\text{A}]) \cdot [\text{H1}]}{[\text{H1} \cdot \text{Ap}_4\text{A}] + [\text{H1} \cdot \text{Mg}^{2+} \cdot \text{Ap}_4\text{A}]} \quad (2)$$

Introducing the concentration of Mg^{2+} and the dissociation constants defined in eqn. (1), one obtains eqn. (3):

$$K_{\text{diss. (obs.)}} = \frac{K_1 \cdot (K_{\text{Mg}^{2+}} + [\text{Mg}^{2+}])}{K_1 K_{\text{Mg}^{2+}} / K_0 + [\text{Mg}^{2+}]} \quad (3)$$

The initial slope of the dependence in Fig. 2(b) is $K_0/K_{\text{Mg}^{2+}}$ according to eqn. (3) and for $K_1 \gg K_0$ and $K_1 \cdot K_{\text{Mg}^{2+}}/K_0 \gg [\text{Mg}^{2+}]$. With above values for K_0 and the slope, the dissociation constant $K_{\text{Mg}^{2+}}$ of $57 \mu\text{M}$ is calculated. A value of $28 \mu\text{M}$ has been reported under somewhat different conditions (pH 8.0; Holler *et al.*, 1983). The dinucleotide binds Mg^{2+} to a second site with a dissociation constant of 6.2 mM (Holler *et al.*, 1983). Introducing this into above equations would result in a square concentration term for Mg^{2+} and thus to a parabolic concentration-dependence, as seen in Fig. 2(b) at elevated concentrations.

The effect of ionic strength was probed for various concentrations of NaCl at constant concentrations of histone H1 ($10 \mu\text{M}$) and $[^3\text{H}]\text{Ap}_4\text{A}$. The measurements were complicated by an increase in diffusion rates through the dialysis membrane, which had to be decreased in size in order to prevent considerable depletion of $[^3\text{H}]\text{Ap}_4\text{A}$ in the reaction chamber. Dissociation constants were $10 \mu\text{M}$, $13 \mu\text{M}$, $34 \mu\text{M}$, $48 \mu\text{M}$ and $55 \mu\text{M}$ in the presence of zero, 10 mM-, 25 mM-, 50 mM- and 100 mM-NaCl respectively. At physiological ionic strength (150 mM) the complex could be no longer reliably detected. From a plot of the above data the value of the dissociation constant of $100 \pm 10 \mu\text{M}$ was estimated by graphical extrapolation.

Specificity

Nucleotides and histones were examined in single-addition experiments for their ability to form complexes with histone H1 and Ap_4A respectively. Concentrations were $12 \mu\text{M}$ each of histone H1 and $[^3\text{H}]\text{Ap}_4\text{A}$, and either $125 \mu\text{M}$ or $250 \mu\text{M}$ unlabelled nucleotide. In the case of $[^3\text{H}]$ -labelled ATP, GTP, dTTP, *cis*-diamminedichloroplatinum(II) adduct of $[^3\text{H}]\text{Ap}_4\text{A}$ (each $12 \mu\text{M}$) and $50 \mu\text{M}$ -histone H1 as reactants (Ap_4A absent), binding was not detectable. The results (Table 1) indicate a decreasing

Fig. 3. Exchange of histone H1 between long and short segments of chromatin

The donor short segments are indicated by open symbols, the acceptor long segments by filled symbols. Data points in the upper panel refer to the sum of the amounts of histone H1 relative to histone H4 in the donor and acceptor segments. Amounts of histone H1 relative to histone H4 refer to amounts of proteins detected after polyacrylamide-gel electrophoresis under denaturing conditions in the presence of SDS. Ionic strength was calculated for 4 elementary charges per molecule of Ap_4A . Squares refer to NaCl (Ap_4A absent), triangles to Ap_4A (NaCl absent) and circles to Ap_4A in the presence of 5 mM-NaCl.

tendency for binding in the following order: $\text{poly(dT)} \gtrsim \text{Ap}_4\text{A} \sim \text{Gp}_4\text{G} > \text{Ap}_4 \gg \text{Ap}_3\text{A} \sim \text{Ap}_5\text{A} \gtrsim \text{ATP, GTP and dTTP}$, where the concentration of poly(dT) was measured in terms of mononucleotides. In the case of varied concentrations of histones ($5\text{--}14 \mu\text{M}$) the overall dissociation constants were $33 \pm 4 \mu\text{M}$, $14 \pm 4 \mu\text{M}$, $0.9 \pm 0.1 \mu\text{M}$, $0.8 \pm 0.1 \mu\text{M}$ for histones H2a, H2b, H3 and H4 respectively. The number of binding sites was not determined in these cases. Thus the ability to form complexes with Ap_4A decreases in the order of $\text{H4} \sim \text{H3} > \text{H1} > \text{H2b} > \text{H2a}$.

Exchange of histone H1 between segments of chromatin

The potency of Ap_4A to compete with DNA was examined in a measurement of its effect on the exchange of histone H1 between short and long chromatin segments in accordance with Caron & Thomas (1981). In Fig. 3 the amounts of histone H1 in the donor short segments (upper curves) and in the acceptor long chromatin segments (lower curves) are shown as a function of effector concentrations. Ap_4A clearly stimulated exchange. When plotted as a function of Ap_4A in terms of ionic strength, the stimulation coincided with that for NaCl or NaCl plus Ap_4A . The relative amounts of

Table 1. Competition of nucleotides and poly(dT) with [³H]Ap₄A to bind histone H1

Concentrations were 12 μ M-histone H1 and 12 μ M-[³H]Ap₄A in 10 mM-Tris/HCl buffer, pH 7, containing 0.4 mM-MgCl₂.

Unlabelled competing nucleotide	Bound [³ H]Ap ₄ A (μ M)	Relative amount of complex
None	6.67 $\pm$ 0.14	1.00
Ap ₄ (160 μ M)	4.27 $\pm$ 0.08	0.64 $\pm$ 0.01
Ap ₃ A (250 μ M)	5.78 $\pm$ 0.20	0.87 $\pm$ 0.04
Ap ₄ A (125 μ M)	4.44 $\pm$ 0.08	0.67 $\pm$ 0.04
Ap ₄ A (250 μ M)	3.11 $\pm$ 0.12	0.47 $\pm$ 0.02
Ap ₅ A (125 μ M)	6.44 $\pm$ 0.12	0.97 $\pm$ 0.02
Ap ₅ A (250 μ M)	Precipitate	—
Ap ₆ A (125 μ M)	Precipitate	—
Gp ₄ G (125 μ M)	4.44 $\pm$ 0.09	0.67 $\pm$ 0.01
Gp ₄ G (250 μ M)	2.89 $\pm$ 0.12	0.43 $\pm$ 0.01
Poly(dT) (120 μ M)	2.49 $\pm$ 0.05	0.37 $\pm$ 0.01
Poly(dT) (145 μ M)	Not detectable	0
Pt adduct of Ap ₄ A (250 μ M)	6.70 $\pm$ 0.15	1.00 $\pm$ 0.02

histone H1 (with amounts of histone H4 as reference, curves in the top of Fig. 3) for short and long segments together was invariant, indicating that the relative yield of chromatin-bound histone H1 was not diminished in the presence of Ap₄A. We consider this as evidence that Ap₄A did not succeed in competition with DNA for histone H1, and that the stimulation of exchange was merely a salt effect. We verified by non-equilibrium dialysis experiments that Ap₄A did not bind to fully assembled nucleosomes (1–11 μ M-[³H]Ap₄A and 3–22 μ M-histone-H1-containing nucleosomes, 0.4 mM-MgCl₂). The dinucleotide was conserved intact, as indicated by t.l.c. of the reaction mixture.

DISCUSSION

As a basic protein, histone H1 is apt to bind to an acidic molecule such as Ap₄A via electrostatic attraction, the view being supported by the measured salt effects. However, the interaction with the present nucleotides exhibits specificity for $\alpha\omega$ -bisnucleotides of tetraphospho chains as in Ap₄A and Gp₄G, thus reflecting the length of the oligophosphate chain and the presence of nucleobases at its ends.

Histone H1 contains two strong and probably several weak binding sites (Fig. 2; Holler, 1984). With regard to competition, the strong sites interact with poly(dT) and are probably in association with DNA of chromatin.

The overall dissociation constant of the H1·Ap₄A complex is 4.5 ± 0.2 μ M (0.4 mM-MgCl₂). Inhibition by Ap₄A of histone-H1-dependent ADP-ribosylation in the presence of poly(ADP-ribose) polymerase has been reported under similar experimental conditions to follow an inhibition constant of 5.1 μ M (Tanaka *et al.*, 1981). Our results support previous suggestions that Ap₄A binds to histone H1, where it serves as a primer of poly(ADP-ribose) polymerization (Yoshihara & Tanaka, 1981). The reported antagonism by Mg²⁺ is consistent with our finding that MgAP₄A is a poor if any ligand of histone H1 (Fig. 2b). Histone H1 as well as Mg²⁺ influence the structure of Ap₄A (Holler *et al.*, 1983; Holler, 1984), and a different structural effect is seen from platination of Ap₄A (Segal *et al.*, 1984). Whether the structural effects or the diminished charges in the

metal complexes of Ap₄A leads here to the loss in binding remains to be investigated.

Recent studies by Morioka & Shimada (1986) as well as by Weinmann-Dorsch & Grummt (1986) have indicated that the higher fraction of Ap₄A in the cell was located in the nucleus. Using their amounts of Ap₄A per nucleus and the dimensions of nuclei between 3 and 10 μ m, we calculate the concentration of Ap₄A to be 25–2000 μ M. By comparison, the value of the dissociation constant of the histone H1·Ap₄A complex is 100 μ M at physiological ionic strength and in the presence of 0.4 mM-Mg²⁺. It suggests that Ap₄A could interact with free histone H1 and possibly with the other histones under conditions prevailing *in vivo*. One interaction could be that as a primer of poly(ADP-ribose) polymerization (Yoshihara & Tanaka, 1981). Other possible roles are in histone modification or in the assembly of nucleosomes in terms of an assembly factor, as has been discussed for polymers (Laskey & Earnshaw, 1980; Nelson *et al.*, 1981). The extent of interaction with histone H1 and possibly with the other histones varies as a function of the cellular concentration of free Mg²⁺. This may be low at times of high concentrations of nucleoside 5'-triphosphates, as during the S-phase of the cell cycle, and could thus be a regulating factor. According to our results, however, Ap₄A cannot promote nucleosome disassembly. The dinucleotide also binds to other nuclear proteins, of which the DNA polymerase-associated Ap₄A-binding protein has received attention (Grummt *et al.*, 1979; Rapaport *et al.*, 1981; Zourgi *et al.*, 1986).

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REFERENCES

- Bartmann, P., Hanke, T. & Holler, E. (1975) *J. Biol. Chem.* **250**, 7668–7674
- Caron, F. & Thomas, J. O. (1981) *J. Mol. Biol.* **146**, 513–537
- Colowick S. P. & Womack, F. C. (1969) *J. Biol. Chem.* **244**, 774–780
- Goerlich, O., Foeckler, R. & Holler, E. (1982) *Eur. J. Biochem.* **126**, 135–142

- Grummt, F. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 371–375
- Grummt, F., Walzl, G., Jantzen, H. M., Hamprecht, K., Huebscher, U. & Kuenzle, C. C. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6081–6085
- Guranowski, A., Jakubowski, H. & Holler, E. (1983) *J. Biol. Chem.* **258**, 14784–14789
- Güntner, C. & Holler, E. (1979) *Biochemistry* **10**, 2028–2038
- Holler, E. (1984) *Biochem. Biophys. Res. Commun.* **120**, 1037–1043
- Holler, E., Holmquist, B., Vallee, B. L., Taneja, K. & Zamecnik, P. (1983) *Biochemistry* **22**, 4924–4933
- Huang, H.-C. & Cole, R. D. (1984) *J. Biol. Chem.* **259**, 14237–14242
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Laskey, R. A. & Earnshaw, W. C. (1980) *Nature (London)* **286**, 763–767
- Lee, P. C., Bochner, B. R. & Ames, B. N. (1983) *J. Biol. Chem.* **258**, 6824–6834
- Morioka, M. & Shimada, H. (1986) *Exp. Cell Res.* **165**, 429–440
- Muise, O. & Holler, E. (1985) *Biochemistry* **24**, 3618–3622
- Nelson, T., Wiegand, R. & Brutlag, D. (1981) *Biochemistry* **20**, 2594–2601
- Panyim, S. & Chalkley, R. (1971) *J. Biol. Chem.* **246**, 4206–4215
- Pimmer, J., Holler, E. & Eckstein, F. (1976) *Eur. J. Biochem.* **67**, 171–176
- Plateau, P., Mayaux, J. F. & Blanquet, S. (1981) *Biochemistry* **20**, 4647–4653
- Rapaport, E., Zamecnik, P. C. & Baril, E. F. (1981) *J. Biol. Chem.* **256**, 12148–12151
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–663
- Segal, E., Girault, J.-P., Muzard, G., Chottard, G., Chottard, J.-C. & Lepecq, J. B. (1984) *Biopolymers* **23**, 1623–1635
- Sommer, H. R. & Chalkley, R. (1973) *Biochemistry* **13**, 1022–1027
- Tanaka, Y., Matsunami, N. & Yoshihara, K. (1981) *Biochem. Biophys. Res. Commun.* **99**, 837–843
- Thomas, J. O. & Kornberg, R. D. (1978) *Methods Cell Biol.* **18**, 429–440
- Varshavsky, A. (1983) *Cell* **34**, 711–712
- Weinmann-Dorsch, C. & Grummt, F. (1986) *Exp. Cell Res.* **165**, 550–554
- Weinmann-Dorsch, C., Hedl, A., Grummt, I., Albert, W., Ferdinand, F.-J., Friis, R. R., Pierron, G., Moll, W. & Grummt, F. (1984) *Eur. J. Biochem.* **138**, 179–185
- Yoshihara, K. & Tanaka, Y. (1981) *J. Biol. Chem.* **256**, 6756–6761
- Zamecnik, P. (1983) *Anal. Biochem.* **134**, 1–10
- Zourgi, L., Tharaud, D., Solari, A., Litvak, S. & Litvak, L. (1986) *Biochim. Biophys. Acta* **846**, 222–232

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