

INTERACTION OF POLY-L-LYSINE WITH CHROMATIN

Inhibition of *In Situ* RNA Synthesis

Mediated by *Escherichia coli* RNA Polymerase

N. UMIEL and W. PLAUT

From the Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706. Dr. Umiel's present address is The Hebrew University, Faculty of Agriculture, Rehovot, Israel.

ABSTRACT

RNA polymerase from *Escherichia coli* was used in conjunction with labeled nucleosides as an autoradiographic reagent to study the availability of template in the chromatin of fixed nuclei and chromosomes. Sequential treatments of the tissues with acid and poly-L-lysine were used to compare the effect of these treatments on the availability of template with the previously reported effects on the *in situ* priming for *Escherichia coli* DNA polymerase. Acid treatment was found to increase the *in situ* activity of both enzymes, while poly-L-lysine strongly inhibited the *in situ* reactions mediated by RNA and DNA polymerases. When the DNA polymerase reaction was previously carried out on alcohol-fixed chicken blood smears, leukocyte nuclei primed extensively for DNA synthesis. In contrast, we did not detect incorporation into intact nuclei of any cell type in alcohol-fixed blood smears that were treated with RNA polymerase.

INTRODUCTION

We have recently shown (Umiel and Plaut, 1972) that poly-L-lysine (PLL) inhibits the *E. coli* DNA polymerase catalyzed incorporation of deoxyribonucleotides *in situ* in fixed cytological preparations of nuclei and chromosomes and presented evidence in support of the hypothesis that an acid reversible interaction between PLL and chromatin is at least in part responsible for this inhibition. Moreover, in alcohol-fixed chicken blood smears, erythrocyte nuclei did not prime for the *E. coli* enzyme, while the nuclei of other cell types (e.g. thrombocytes and leukocytes) did prime and exhibited moderate to heavy incorporation of [³H]thymidine into the chromatin (see Fig. 5, Umiel and Plaut, 1972). These observations prompted us to carry out similar studies on RNA synthesis *in situ*, using RNA polymerase from *E. coli*.

Nuclei and chromatin isolated from eukaryotic

cells have been used in conjunction with bacterial RNA polymerase in a variety of *in vitro* studies aimed at elucidating the role of basic protein in the regulation of gene transcription (for reviews see Hnilica, 1967; Bonner, et al., 1968). Both histones and polylysine have been shown to be strong inhibitors of RNA polymerase-mediated RNA synthesis in all such systems. Similar results have been obtained in *in vitro* studies with DNA polymerase (Hnilica, 1967). The present communication describes the effects of poly-L-lysine and acid treatment of fixed chicken blood nuclei and *Drosophila* salivary gland chromosomes on the *in situ* incorporation of ribonucleotides with *E. coli* RNA polymerase. The results largely parallel those obtained with DNA polymerase in that acid treatment enhances incorporation and that polylysine inhibits this enhancement. The observations with

RNA polymerase differ from the former in that no intact nuclei of alcohol-fixed chicken blood smears show incorporation of RNA precursors without previous acid treatment while thrombocyte and lymphocyte nuclei do exhibit moderate to heavy deoxyribonucleotide incorporation when incubated with DNA polymerase without exposure to acid

MATERIALS AND METHODS

Preparation of Acid-Fixed Salivary Glands

Squashes of salivary gland chromosomes of *D. melanogaster* were prepared as described by Plaut et al. (1966). Glands were dissected from third instar larvae in Ephrussi-Beadle medium. The glands were fixed and squashed on gelatinized slides in 45% acetic acid, frozen in liquid nitrogen for cover slip removal, post-fixed in acetic acid-alcohol (1:3), and transferred to 75% ethanol through absolute and 95% ethanol. The slides were then brought down to water and air dried at room temperature. Glass tubing (19 mm ID) cut into 7-mm lengths was glued to the slides around the tissue with gelatin to form a well for enzymatic reactions

Preparation of Chicken Blood Smears

Fresh blood from adult chicken was smeared on gelatinized slides, fixed for 1 h in 70% ice-cold ethanol, transferred to 80% ethanol, and air dried. Acid treatment consisted of a 10-min exposure of the tissue to 0.1 N HCl at room temperature, followed by water rinsing and air drying.

Treatment with PLL

PLL type I (mol wt 130,000) and PLL type II (mol wt 2860) purchased from Sigma Chemical Co., (St. Louis, Mo.), were used in aqueous solution (400 μ g/ml) to treat the slides. A drop (0.020 ml) of PLL solution was placed on the tissue and covered with a 22-mm² cover slip to insure uniform distribution and to minimize evaporation. After 10 min at room temperature the cover slip was removed with a stream of water. The slides were washed three times, 10 min each wash, in deionized water, air dried, and glass rings were glued on. Several slides were treated with hydrochloric acid (0.1 N HCl for 10 min at room temperature, followed by washing five times in deionized water and air drying) after the treatment with PLL.

RNA Synthesis in Situ

Unlabeled ATP, GTP, CTP, and UTP, as well as RNA polymerase (from *E. coli* strain K-12, sp act 205 U/mg) were purchased from Sigma Chemical Co.

and [³H]UTP (sp act 13 Ci/mmol) from Schwarz Bio Research Inc., Orangeburg, N. Y. The incubation mixture (Hurwitz, 1963) contained (per 0.5 ml) 25 μ moles of pH 7.5 malate buffer, 5 μ moles 2-mercaptoethanol, 10 μ moles MnCl₂, 80 nmols each of ATP, GTP, and CTP, 0.15 nmols of [³H]UTP, and 5 U of RNA polymerase. In control incubations the enzyme was omitted from the reaction mixture. Slides were incubated at 38°C for 40 min. The enzymatic reaction was stopped by removing the reaction mixture from the well and replacing it with 5% ice-cold trichloroacetic acid (TCA). After 10 min the TCA was removed from the well and the glass ring was separated from the slide. The slides were washed three times in cold TCA followed by ethanol-ether (1:1) and ether, 10 min for each wash. The slides were then air dried.

Treatment with DNase I and RNase

Bovine pancreas RNase and DNase I were purchased from Worthington Biochemical Corp., Freehold, N. J. The RNase was heated in aqueous solution at 100°C for 15 min to inactivate contaminating DNase. The incubation mixture for DNase I (0.5 ml/well) contained 41 μ mol acetate buffer, pH 5.0, 2 μ mol MgSO₄, and 150 μ g DNase I. The incubation mixture (0.5 ml/well) for RNase contained 41 μ mol acetate buffer, pH 5.0, and 150 μ g RNase. All incubations were carried out at 37°C for either 30 min or 3 h, and stopped as in the case of the RNA polymerase incubations.

Autoradiography and Microscopy

Slides were coated with K5 liquid emulsion (Ilford Ltd., Ilford, Essex, England), exposed for 24 days at 4°C, and developed in D-19 (Eastman Kodak Co., Rochester, N. Y.). Microscope observations were done with phase optics.

RESULTS

Squashed Salivary Glands (Acid-Fixed)

RNA polymerase catalyzed extensive incorporation of [³H]ribonucleotides into chromosomal material and to a lesser extent into the nucleolar mass (Fig. 1 D). No incorporation was detected when RNA polymerase was omitted from the incubation mixture (Fig. 1 A) or when the tissue was exposed to PLL before incubation with the enzyme (Fig. 1 E). When PLL treatment was followed by exposure of the tissue to acid before incubation with the enzyme [³H]ribonucleotide incorporation was restored (Fig. 1 F). The incorporated radioactivity was removed from the chromosomes by a short RNase treatment (30 min) but not by DNase

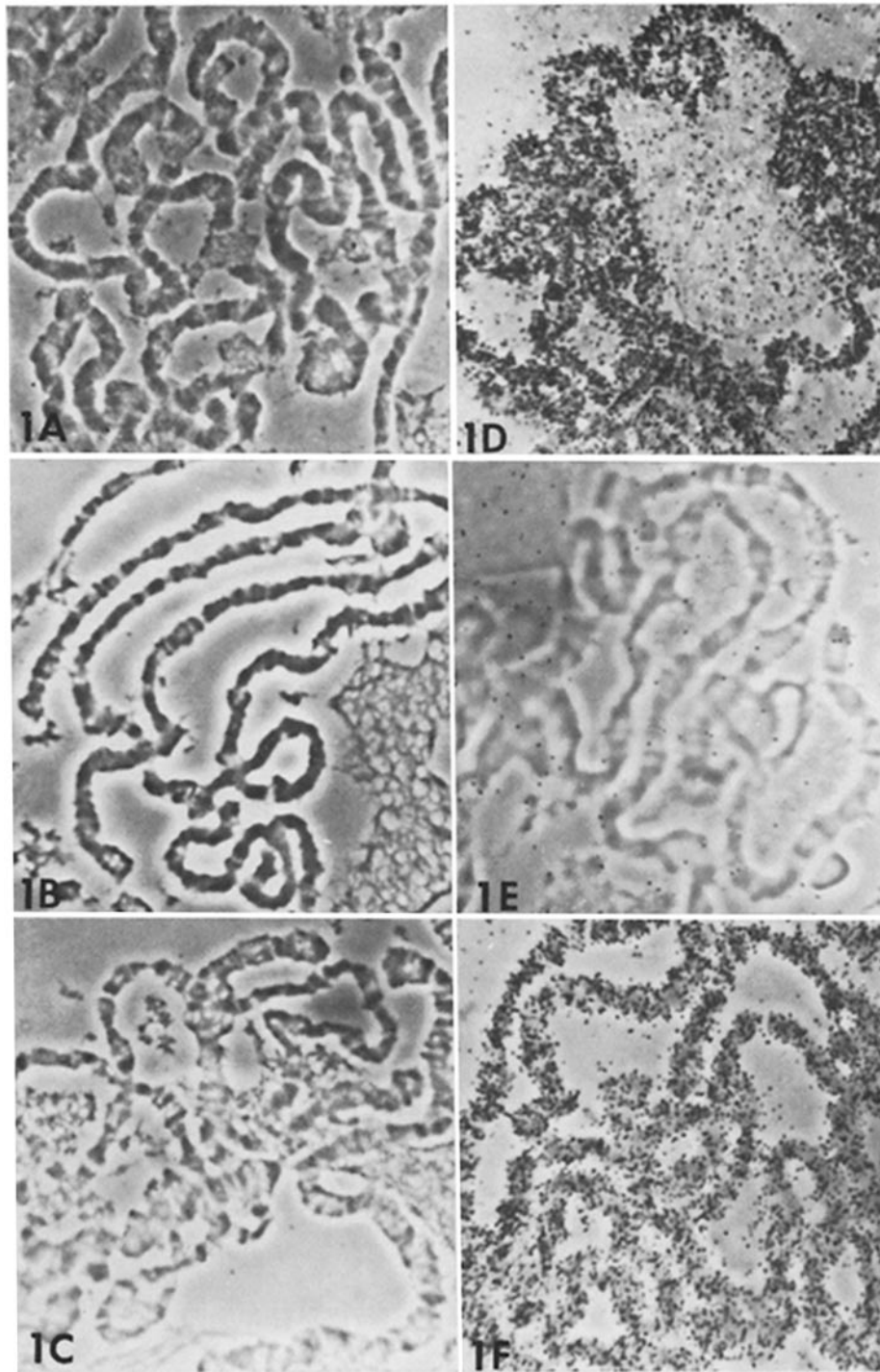


FIGURE 1 Inhibition of *E. coli* RNA polymerase by PLL *in situ* using *Drosophila melanogaster* salivary gland chromosomal DNA as template. All incubation mixtures contained ^3H UTP, ATP, CTP, and GTP. Figs. 1 A, 1 B, and 1 C, control incubation omitting RNA polymerase. Fig. 1 A, no polylysine treatment. Fig. 1 B, pretreatment with PLL type I. Fig. 1 C, pretreatment with PLL followed by acid treatment. Figs. 1 D, 1 E, and 1 F incubation mixture containing RNA polymerase; Fig. 1 D, no polylysine treatment. Fig. 1 E, pretreatment with PLL type I. Fig. 1 F, pretreatment with PLL followed by acid treatment. $\times 1000$.

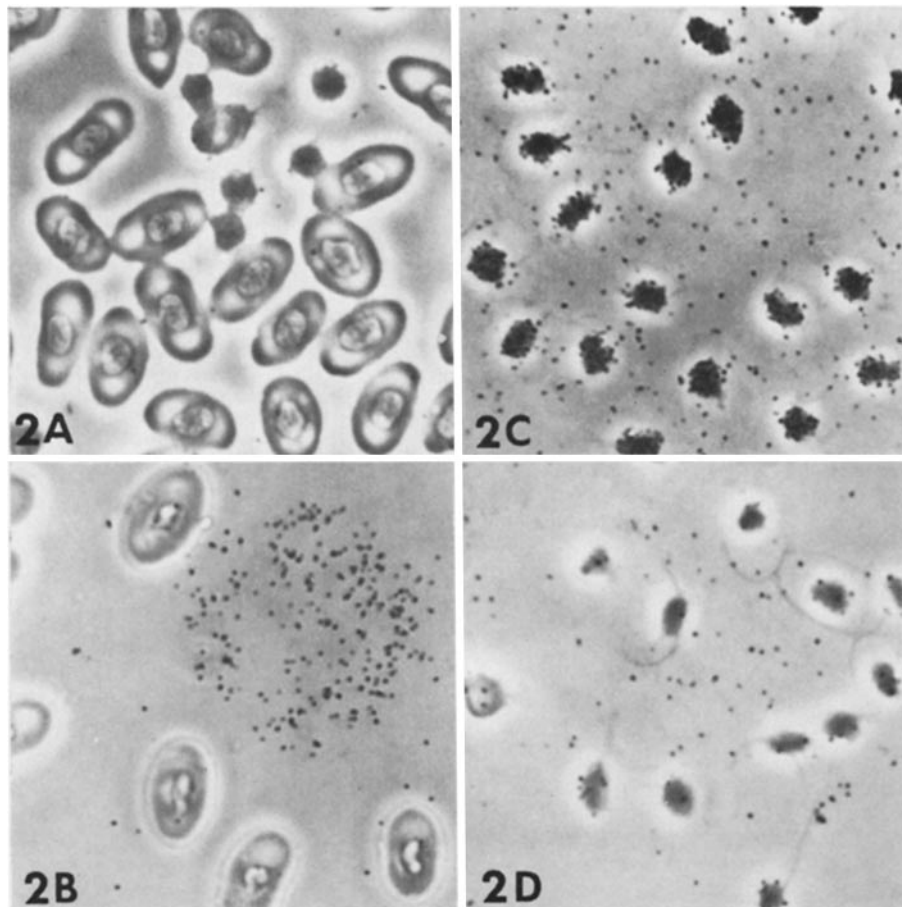


FIGURE 2 Template activity for *E. coli* RNA polymerase, by chromatin in fixed chicken blood smears (treatments are listed in sequential order of application; see text for details). Fig. 2 A, alcohol-fixed blood smears, incubated for 40 min with RNA polymerase. Fig. 2 B, same as in Fig. 2 A, but showing incorporation into an island of spread chromatin. Fig. 2 C, alcohol-fixed, acid-treated, and incubated with RNA polymerase. Fig. 2 D, alcohol-fixed, acid-treated, PLL-treated, and incubated with RNA polymerase. $\times 1000$.

or buffer. However, some of the label in the chromosomes was lost during prolonged incubation (3.5 h) in either DNase or buffer.

Chicken Blood Smears (Alcohol-Fixed)

In the blood smears, observations were made on the incorporation of [3 H]uridine into nuclei of different cell types (erythrocytes vs thrombocytes and leukocytes), whenever cell types were distinguishable. In addition we observed in some treatments a large number of "islands" of localized high grain density (Fig. 2 B). These islands probably represent labeling of spread chromatin which

originated from burst nuclei. Such islands of labeled spread chromatin were not detected when the RNA polymerase was omitted from the reaction mixture (Table I, group 1). The effects of the different treatments on the incorporation of [3 H]-ribonucleotides in blood smears are outlined in Table I. Alcohol-fixed blood smears which were incubated with RNA polymerase (Table I, group 2) did not exhibit any detectable incorporation into any cell type (Fig. 2 A). However, a large number of islands of labeled spread chromatin were found (Fig. 2 B). In all cases a treatment with PLL (Table I, groups 3 and 7) inhibited the RNA

TABLE I
The Effect of PLL and Acid Treatment on E. coli RNA Polymerase Activity In Situ, Using Chicken Blood Smear Preparations

Slide group	Sequential treatment of slides (left to right)*				Grain density in autoradiographs		
	0.1 N HCl	PLL	0.1 N HCl	RNA polymerase	Erythrocytes	Lymphocytes	Spread chromatin islands†
1	—	—	—	—	None	None	None
2	—	—	—	+	None	None	High
3	—	+	—	+	None	None	None
4	—	+	+	+	Low	Medium	High
5	+	—	—	—	None§	None§	None
6	+	—	—	+	Low to high§	Low to high§	High
7	+	+	—	+	Very low§	Very low§	None
8	+	+	+	+	Low to high§	Low to high§	High

* All fresh blood smears were air dried and then alcohol-fixed before further treatments.

† See Fig. 2 B.

§ Cell types could not be identified; acid treatment before air drying removed some of the cytoplasm and obliterated the differences between cell types

polymerase, while when the PLL treatment was followed by an acid treatment (Table I, groups 4 and 8) the [³H]ribonucleotide incorporation mediated by RNA polymerase was restored

DISCUSSION

Incubation of acid-fixed or acid-treated cytological preparations with *E. coli* RNA polymerase and [³H]UTP resulted in the incorporation of radioactive ribonucleotide into chromosomal material. The removability of the label by a short (30-min) treatment with RNase but not by DNase or buffer indicates that the labeled chromosomal material is RNA. The removal of some radioactivity during prolonged (3.5 h) incubation in buffer may suggest that some of the RNA synthesized is of low molecular weight, soluble in buffer but not in 5% TCA; alternatively this loss of label could result from the activity of endogenous RNase.

The *in situ* synthesis of RNA mediated by *E. coli* RNA polymerase was inhibited when the tissues were treated with PLL before incubation with the enzyme. This inhibition was removed when PLL treatment was followed by acid treatment (Table I, groups 4 and 8). We have reported similar results for the *in situ* reaction mediated by *E. coli* DNA polymerase previously (Umiel and Plaut, 1972). The effect of acid treatment in both sets of experiments is interpretable in terms of the removal by acid of basic proteins from chromatin (Dick and

Johns, 1968). It should also be noted that the effects of basic proteins or polypeptides and acid treatment on the *in situ* enzymatic activity of both DNA and RNA polymerase are in good agreement with results obtained from *in vitro* studies (see Hnilica, 1967 for review).

The *in situ* syntheses mediated by *E. coli* DNA and RNA polymerase differed in one interesting respect: when DNA polymerase was used on alcohol-fixed chicken blood smears, leukocyte and thrombocyte nuclei exhibited moderate to heavy priming activity, even without acid treatment, in the presently reported set of experiments with RNA polymerase, no incorporation was detected in intact blood cells of any type without previous exposure to acid. We did observe islands of autoradiographic grains not associated with cells in alcohol-fixed blood smear preparations after incubation with RNA polymerase (as well as with DNA polymerase) and offer the following considerations in support of the conclusion that these islands represent spread chromatin from burst nuclei: (a) similar islands of grains were observed in autoradiographic preparations of blood smears after incubation with either polymerase and the appropriate labeled nucleoside triphosphates as well as after treatment with [³H]actinomycin D, a sensitive marker for DNA (Plessmann-Camargo and Plaut, 1967; Ebstein, 1967), (b) fluorescence microscopy of alcohol-fixed blood smears after staining with

acridine-orange or quinacrine revealed fluorescing areas unassociated with intact cells which were similar in size, shape, and distribution to the islands of grains. Similar islands of grains without apparent association with nuclei had been reported by Modak and Bollum (1970) for the *in situ* reaction mediated by calf thymus terminal transferase.

The observation that spread chromatin can act as template for RNA polymerase *in situ* while that condensed in intact nuclei does not, even though exposed to the same preparative treatment, urges caution in extrapolating data on fixed or extracted chromatin to *in vivo* conditions (see also Mirsky, 1971, Hotta and Stern, 1966; Von Borstel et al., 1967). The RNA polymerase reaction may well be sensitive to changes in the state of chromatin which are not yet amenable to experimental control. In reviewing *in vitro* studies on isolated chromatin and RNA polymerase, Hnilica (1967) had in fact suggested that discrepancies among such studies may be due to minor changes during the isolation procedures and that the absence of such discrepancies in similar investigations with DNA polymerase implied a lesser sensitivity of that enzyme to such changes. The differences between DNA and RNA polymerase reported here must also be viewed in that context.

These qualifications notwithstanding, *in situ* experiments do provide a useful tool with which to study the template activity of chromatin of different cell types and in different states of condensation. The inhibiting effect of basic protein or polypeptides is clearly demonstrable as is a difference between spread and condensed chromatin in the case of RNA polymerase. The extent to which

these observations are relevant to the control of transcription and replication in the living cell is subject to future experimental evaluation as is the possible existence of control mechanisms which escape detection by this approach.

We gratefully acknowledge the excellent technical assistance of Karen S. Voss and financial support through a research grant from National Institutes of Health (GM 15611) and the research committee of the Graduate School of the University of Wisconsin.

Received for publication 19 July 1972, and in revised form 14 August 1972.

REFERENCES

- BONNER, J., M. E. DAHMUS, D. FAMBROUGH, R. C. HUANG, K. MARUSHIGE, and D. Y. H. TUAN. 1968. *Science (Wash. D. C.)* 159:47.
- DICK, C., and E. W. JOHNS. 1968. *Exp. Cell Res.* 51: 626.
- EBSTEIN, B. S. 1967. *J. Cell Biol.* 35:709.
- HNILICA, L. S. 1967. *In Progress in Nucleic Acid Research and Molecular Biology.* 7:25.
- HOTTA, Y., and H. STERN. 1966. *Nature (Lond.)* 210: 1043.
- HURWITZ, J. 1963. *In Methods in Enzymology* VI. 23.
- MIRSKY, A. E. 1971. *Proc. Natl. Acad. Sci. U. S. A.* 68:2945.
- MODAK, S. P., and F. J. BOLLUM. 1970. *Exp. Cell Res.* 62:421.
- PLAUT, W., D. NASH, and R. FANNING. 1966. *J. Mol. Biol.* 16:35.
- PLESSMANN-CAMARGO, E., and W. PLAUT. 1967. *J. Cell Biol.* 35:713.
- UMIEL, N., and W. PLAUT. 1972. *J. Cell Biol.* 54. In press.
- VON BORSTEL, R. C., S. P. MODAK, and F. J. BOLLUM. 1967. *J. Cell Biol.* 35(2, Pt 2):137A(Abstr.).