STIMULATION OF THE ACTIVITIES OF SOLUBILIZED PIG LYMPHOCYTE RNA POLYMERASES BY PHYTOHAEMAGGLUTININ

Anne Cooke* and Melanie Brown
School of Biological Sciences, University of Sussex
Brighton, England

Received February 15, 1973

SUMMARY. DNA dependent RNA polymerase has been solubilised from pig peripheral blood lymphocytes. Using α amanitin, an inhibitor of the nucleoplasmic polymerase B activity, we have found that 20 hrs following lymphocyte stimulation with phytohaemagglutinin (PHA) the activities of both polymerase A and polymerase B are increased. As previously observed with intact nuclei a greater stimulation of polymerase A activity is observed at this time. Since the activity of these enzymes was assayed using exogenous template this indicates that PHA stimulates RNA synthesis by regulating the amounts and/or the activities of the polymerases.

Stimulation of human peripheral blood lymphocytes with phytohaemagglutinin** (PHA) has been shown to result in an increase in nuclear RNA polymerase activity[1,2,3,4]. Multiple RNA polymerases have been isolated from animal tissues[5,6,7]. These have been shown to exist in different nuclear compartments, to have different ionic requirements and different functional roles[5,8,9]. The bicyclic polypeptide α-amanitin obtained from the fungus, Amanita phalloides[10], has been shown to inhibit only the nucleoplasmic polymerase B activity; the nucleolar polymerase A is wholly resistant to this toxin[11].

Using this inhibitor it has been demonstrated that 20 hr following PHA stimulation the amanitin resistant activity of the lymphocyte nuclei is stimulated about 4-fold and the total polymerase activity about 2-fold[2,4]. The observed stimulation correlates well with the observed increases in ribosomal and total RNA synthesis following PHA stimulation[12,13]. The amanitin resistant polymerase activity measured in these nuclei appears to be involved in the synthesis of rRNA[4].

---

* Present address: Department of Microbiology, University of Illinois at the Medical Center, Chicago, Illinois 60612 U.S.A.

** Abbreviation: PHA, phytohaemagglutinin.

We have now solubilized the RNA polymerases of pig peripheral blood lymphocytes and demonstrated that 20 hr following PHA stimulation the activities of both the solubilized A and B polymerases are also increased. As observed with intact nuclei, a greater stimulation of polymerase A activity is observed at this time.

MATERIALS AND METHODS.

Pig peripheral blood, obtained from the abattoir, was defibrinated and red blood cells removed by sedimentation in 1% dextran at 37°. Lymphocytes were prepared from the supernatant with a cotton wool column as described for human cells by Cooper[14]. The lymphocytes were incubated at 2×10^6/ml in Eagles MEM supplemented with 10% autologous serum, 2 mM glutamine, and 50 units penicillin and 50 μg streptomycin per ml. Incubations were carried out for 20 hr with or without 3 μg/ml PHA-Q_1 (Wellcome Labs, Beckenham, Kent) in a humidified atmosphere of air containing 5% CO_2. Nuclei were prepared from stimulated and resting lymphocytes[4] and their capacity to form RNA *in vitro*[4] measured in the presence or absence of 1 μg/ml α-amanitin. The DNA dependent RNA polymerase was isolated from the nuclei according to the method of Roeder and Rutter[9] and the mixed polymerase activity assayed in the presence or absence of 2 μg/ml α-amanitin at optimal salt and divalent ion concentration and in the presence of excess exogenous calf thymus DNA. The reaction was almost completely dependent on the addition of exogenous DNA template, was sensitive to actinomycin D (5 μg/ml) and the rifamycin derivative AF013 (Gruppo Lepetit, Milano, Italy) which is said to inhibit the initiation of transcription[15].

RESULTS.

From Table I it can be seen that following PHA stimulation there is a significant increase in both the amanitin resistant and sensitive polymerase activity of pig peripheral blood lymphocytes. The changes in nuclear polymerase activity correspond closely to those previously observed in nuclei from

TABLE I

COMPARATIVE ESTIMATION OF CHANGES IN NUCLEAR AND SOLUBILIZED RNA POLYMERASE ACTIVITY IN RESTING AND 20-HR STIMULATED LYMPHOCYTES

	Increase in total polymerase activity $\frac{+PHA}{-PHA} \pm$ SEM	Increase in amanitin resistant activity $\frac{+PHA}{-PHA} \pm$ SEM	Increase in amanitin sensitive activity $\frac{+PHA}{-PHA} \pm$ SEM
Nuclear RNA Polymerase activity	2.0 ± 0.1 (13)	5.0 ± 0.8 (13)	1.6 ± 0.1 (13)
Solubilized RNA Polymerase activity	2.8 ± 0.5 (11)	5.0 ± 0.8 (11)	2.3 ± 0.6 (11)

Figures in parentheses represent the number of experiments. Nuclei were isolated and incubated as described in the text in the presence or absence of 1 μg/ml α-amanitin. The solubilized RNA polymerase was isolated by a modification of the procedure described by Roeder and Rutter[9]. Sonication at high ionic strength was carried out in a volume of 2 ml in a cooled vessel. Following reduction of the ionic strength, chromatin removal by centrifugation and ammonium sulphate fractionation, the enzyme was solubilized in 0.9 ml of 0.05 M Tris-HCl (pH 7.9), 5 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM EDTA, 25% glycerol (w/v) and dialyzed overnight at 4° C against 0.05 M $(NH_4)_2SO_4$ in the same buffer. After dialysis the suspension was cleared by centrifugation and 40 μl aliquots of the enzyme assayed in the standard assay mixture containing 42 mM $(NH_4)_2SO_4$, 2 mM MnCl $(CH_3COO)_2Mg$, 50 mM Tris-HCl, pH 7.9, 100 μg DNA, 0.75 mM CTP, GTP, ATP and 1 μc ^{3}H-UTP (1 Ci/m mole). The reaction volume was 0.24 ml. After incubation for 30' at 37° in the presence or absence of 2 μg/ml α-amanitin the reaction was stopped by the addition of 2 ml 10% TCA containing 20 mM sodium pyrophosphate, with 0.2 mg calf thymus DNA being used as carrier. The TCA insoluble radioactivity was determined by the standard procedure[4]. In some experiments only 20 μl of enzyme were used in a final assay volume of 0.12 ml which contained 50 μg DNA and 0.5 μc ^{3}H-UTP. Statistical analysis of the data was carried out using the students "t" test.

human peripheral blood lymphocytes[2,4]; there being a greater stimulation of polymerase A activity following PHA. At all times following PHA, however, most of the RNA was synthesized by the amanitin sensitive polymerase.

The total solubilized polymerase activity also increased following PHA. The pattern of changes observed in the solubilized polymerase activity was similar to that of the whole nuclear preparations, there being again a greater stimulation of polymerase A activity after PHA. There was no detectable change in the optimal divalent cation concentration measured in the presence or absence of α-amanitin. A peak of activity appeared in the salt concentration curve at 42 mM $(NH_4)_2SO_4$ following PHA stimulation (Fig. 1). This peak was noted both in the total and in the amanitin resistant

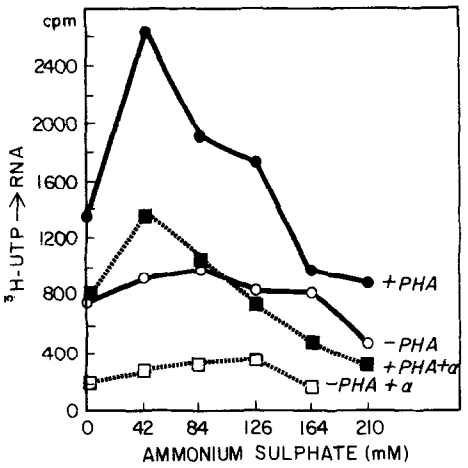

Figure 1. Effect of ionic strength on RNA polymerase activity
20 μl aliquots of the dialyzed enzymes were assayed as described in Table I for the standard assay except the ammonium suplhate concentration was varied as indicated. Incubations were also carried out in the presence or absence of 2 μg/ml α-amanitin as indicated in the figure.

---

polymerase activities. It is possible that this shift in optimum may be due to an increased proportion of polymerase A activity or it may be that the properties of polymerase A change after PHA. Stein and Hausen have

isolated a factor which causes such a shift in optimal ionic strength of their solubilized polymerase B from calf thymus[16]. The stimulation observed was independent of the enzyme concentration over the range used.

Preliminary experiments have been carried out on the fractionation of mixed polymerases on a DEAE cellulose (DE-52) column using batch elution of polymerase A and B activity with 0.15 M $(NH_4)_2SO_4$ and 0.3 M $(NH_4)_2SO_4$ respectively. Following PHA there is a marked increase in the amanitin resistant activity eluted at 0.15 M $(NH_4)_2SO_4$ and a lesser increase in the amanitin sensitive activity eluted at 0.3 M $(NH_4)_2SO_4$.

DISCUSSION.

It has been proposed by several workers that the increased polymerase activity observed in nuclei following PHA stimulation could be attributed to an increased availability of the genome for transcription[3,17,18,19]. While the results presented here do not exclude the latter possibility, the fact that the increased polymerase activity observed in the experiments is noted using excess exogenous calf thymus DNA as template indicates that if such "gene activation" does occur it is not the only operative mechanism for increasing transcription during transformation. The increased polymerase activity found in these experiments could be due to either an increase in the number of polymerase molecules or an increase in the activity of pre-existing molecules. Further characterization of the isolated polymerases may help to resolve this issue.

It is interesting to note that the effect of PHA on polymerase activity appears to be wholly analogous to the effect of cortisol on rat liver polymerase activity[20]. An underlying mechanism of transcriptive control might, therefore, be being utilized.

ACKNOWLEDGEMENT.

We thank Dr. J. Kay for many helpful discussions and the Science Research Council for a post-doctoral fellowship for A.C.

REFERENCES.

1. Handmaker, S.D., and Graef, J.W., Biochim. Biophys. Acta 199:95 (1970).
2. Cooke, A., Kay, J.E., and Cooper, H.L., Biochem. J. 125:74P (1971).
3. Pogo, B.G.T., J. Cell Biol. 53:635 (1972).
4. Cooke, A., and Kay, J.E., Exptl. Cell Res., in press.
5. Roeder, R.G., and Rutter, W.J., Nature 224:234 (1969).
6. Chesterton, C.J., and Butterworth, P.H.W., FEBS Lett. 12:301 (1971).
7. Kedinger, C., Nuret, P., and Chambon, P., FEBS Lett. 15:169 (1971).
8. Jacob, S.T., Sajdel, E.M., and Munro, H.N., Biochem. Biophys. Res. Commun. 38:765 (1970).
9. Roeder, R.G., and Rutter, W.J., Proc. U.S. Nat. Acad. Sci. 65:675 (1970).
10. Wieland, T., Science 159:946 (1968).
11. Kedinger, C., Gniazdowski, M., Mandel, J.L., Gissinger, F., and Chambon, P., Biochem. Biophys. Res. Commun. 38:165 (1970).
12. Cooper, H.L., J. Biol. Chem. 244:1946 (1969).
13. Cooper, H.L., J. Biol. Chem. 244:5590 (1969).
14. Cooper, H.L., J. Biol. Chem. 243:34 (1968).
15. Butterworth, P.H.W., Cox, R.F., and Chesterton, C.J., Eur. J. Biochem. 23:229 (1971).
16. Stein, H., and Hausen, P., Cold Spring Harbor Symp. Quant. Biol. 35:709 (1970).
17. Kleinsmith, L.J., Allfrey, V.G., and Mirsky, A.E., Proc. U.S. Nat. Acad. Sci. 55:1182 (1966).
18. Pogo, B.G.T., Allfrey, V.G., and Mirsky, A.E., Proc. U.S. Nat. Acad. Sci. 55:805 (1966).
19. Killander, D., and Rigler, R., Expt. Cell Res. 39:710 (1965).
20. Sajdel, E.M., and Jacob, S.T., Biochem. Biophys. Res. Commun. 45:707 (1971)