NUCLEOLI OF THIOACETAMIDE-TREATED LIVER AS A MODEL FOR STUDYING REGULATION OF PRERIBOSOMAL RNA SYNTHESIS

Michael W. Andersen, N. Raghuveera Ballal and Harris Busch

Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030

Received July 22, 1977

SUMMARY

Nucleoli isolated from livers of rats injected intraperitoneally with one dose of thioacetamide had a five-fold increase in the rate of RNA synthesis *in vitro* when compared with livers of rats treated with saline or CCl_4. The stimulation was maximal 24 hours after treatment and decreased to control values 73 hours after treatment. The enhanced level of nucleolar activity was maintained at that level when thioacetamide was injected daily. Along with the increase in the endogenous activity there was a 7-fold increase in the "free" RNA polymerase I activity determined by blocking the bound enzyme with actinomycin D (7). The nucleoli of the thioacetamide-treated rats offer a useful model of modulation of ribosomal gene function.

INTRODUCTION

Previous studies from this laboratory indicated that the nucleolus exhibits marked changes in the protein pattern when the cells were stimulated to proliferate such as in the regenerating rat liver (1) or during nucleolar hypertrophy induced by administration of thioacetamide (2). Recently, it was shown that isolated nucleoli retain specificity of expression of preribosomal RNA *in vitro* and thus offer a good system for studying the modulation of nucleolar activity (3). Inasmuch as administration of thioacetamide induces rapid increase in liver nucleolar activity (4), studies with isolated nucleoli from thioacetamide-treated livers should provide valuable information on mechanisms of regulation of preribosomal RNA synthesis.

ISSN 0006-291X

Earlier reports from this laboratory showed that preribosomal RNA synthesis increases in isolated nucleoli after injection of thioacetamide into rats (5). This report indicates that the RNA synthetic activity of isolated nucleoli of thioacetamide-treated rat livers increases almost five-fold within 24 hours after a single injection of the drug and can be maintained at a high level by repeated daily injections of thioacetamide. The enhanced activity returned to normal levels 73 hours after a single injection. The increased rate of synthesis in vitro parallels the increased rate observed in vivo (6) and the increased activity is accompanied by increases in both endogenous (bound) enzyme activity as well as the activity of the "free" (unbound) RNA polymerase I molecules (7).

MATERIALS AND METHODS

Materials - Labeled nucleotides used, ^{3}H guanosine 5'-triphosphate (13 Ci/mM and 14 Ci/mM) and ^{3}H-uridine 5'-triphosphate (10 and 12 Ci/mM), were purchased from Schwarz/Mann. Unlabeled nucleotides were purchased from Sigma Chemical Co. Ultrapure, enzyme grade sucrose was a product of Schwarz/Mann. Thioacetamide was purchased from Fisher Chemical Co. Actinomycin D and poly d(A-T) were products of Sigma Chemical Co.

Protein and DNA Determination - Protein was determined by the method of Lowry (8) and DNA by the method of Burton (9).

Thioacetamide Treatment and Isolation of Nucleoli - Male Sprague-Dawley rats (180-200 g) were injected intraperitoneally with thioacetamide in 0.15 M NaCl (50 mg TA/kg body wt). Rats were sacrificed by decapitation and nucleoli isolated by a modification of the procedure described earlier (10). Livers were perfused with TNKM (0.05 M Tris, 0.13 M NaCl, 25 mM KCl, and 2.5 mM $MgCl_2$). They were homogenized in a solution containing 2.3 M sucrose-10 mM $MgCl_2$ and centrifuged at 20,000 x g for 80 minutes. Isolated nuclei were washed once in 0.88 M sucrose and resuspended in 0.34 M sucrose-0.05 mM $MgCl_2$ solution for sonication.

The sonicate was layered over 0.88 M sucrose and nucleoli were sedimented by centrifugation at 1200 x g for 20 minutes. The sedimented nucleoli were resuspended in TGMED (0.05 M Tris, 50% glycerol, 1 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, pH 7.9) containing 0.1 mM PMSF and were stored at -80°C.

Endogenous RNA Polymerase Activity - Assays for endogenous RNA polymerase activity of whole nucleoli were carried out at

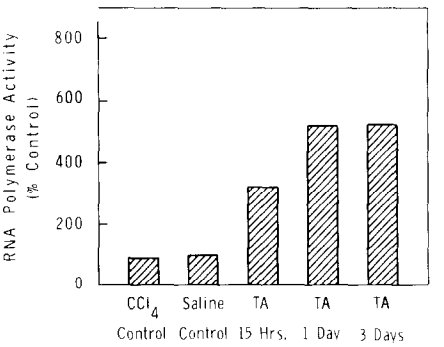

Figure 1 Effect of daily injections of thioacetamide upon RNA synthetic ability of isolated liver nucleoli. Assays were performed as described in Materials and Methods and activities were expressed as pmoles of GMP incorporated per μg nucleolar DNA.

30° in 0.1 ml assay mixture containing 0.05 M Tris (pH 8.0), 0.1 M KCl, 5 mM KF, 0.625 mM each of unlabeled CTP, UTP and ATP, 0.015 mM unlabeled GTP, 1 μCi of ^{3}H-GTP (13 Ci/mM), 5 mM $MgCl_2$, 5 mM DTT, 0.5 mM PMSF and 10 mg Bentonite. The assay mixture was incubated at 30°C for 10 minutes, terminated by pipetting aliquots of the mixture onto Whatman DE81 filter discs which were assayed for radioactivity in a liquid scintillation counter (11).

"Free" RNA Polymerase Activity - To assay the "free" (unbound) RNA polymerase activity of nucleoli, conditions were the same except that 2 mM $MnCl_2$ was substituted for $MgCl_2$ and ^{3}H-UTP (12 Ci/mM) was added in place of ^{3}H-GTP; also, poly d(A-T) (200 μg/ml) and actinomycin D (20 μg/ml) were used in the assay. Bentonite was omitted since it almost completely inhibited "free" enzyme activity.

RESULTS

Nucleoli of livers of thioacetamide-treated rats had a five-fold increase in activity (per μg DNA) within 24 hours after a single injection (Fig. 1). With repeated daily injections of thioacetamide for 3 days, the activity was maintained at five-fold over saline controls. To determine whether this increase reflected hepatotoxicity, nucleoli of livers of rats treated with CCl_4 (1 ml of a 20% solution, i.p.) were analyzed (12); in this case, the endogenous activity was the same as the saline control.

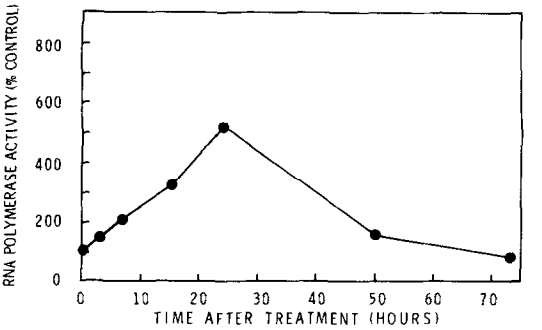

Figure 2 Effect of a single injection of thioacetamide upon the endogenous RNA synthetic activity of isolated liver nucleoli. Rats were sacrificed at different times after one injection and liver nucleoli were isolated and assayed as described in Materials and Methods. Activities were expressed as pmoles of GMP incorporated per μg nucleolar DNA.

The stimulation of RNA synthesis by thioacetamide is shown in Figure 2. Nucleolar RNA synthesis was most active at 24 hours after injection but significant stimulation occurred by 7 hours after injection. However, 50 hours after one injection, the stimulation of liver nucleolar RNA synthesis markedly decreased and at 73 hours after injection, the activity was not significantly different from the control.

The endogenous activity assay primarily indicates the amount of polymerase molecules already involved in synthesis (3,13-15). To determine whether thioacetamide also increased the free enzyme activity, the technique of Yu (7) was employed; the engaged enzyme was inhibited with actinomycin D and the activity of free enzyme was determined with poly d(A-T) as template. Table I shows there was nearly a seven-fold increase in the activity of "free" enzyme upon thioacetamide treatment for 3 days. Thus, the increase in endogenous activity parallels an increase in the unbound RNA polymerase I activity.

TABLE I

EFFECT OF THIOACETAMIDE TREATMENT ON NUCLEOLAR ENDOGENOUS (BOUND) AND FREE RNA POLYMERASE ACTIVITY

Treatment	Endogenous Activity[1]	Free RNA Polymerase Activity[2]
Saline Control	2.97*	5.94
TA 3 Days	15.5	42.5

*Values are the mean of 2 to 5 experiments.

1. Endogenous activity was measured as described in Materials and Methods using ^{3}H-GTP as precursor and expressed as pmoles GMP incorporated per μg DNA.

2. Free enzyme activity was measured in the presence of actinomycin D (20 μg/ml), with saturating amounts of poly d(A-T) template (100-200 μg/ml) using ^{3}H-UTP as the precursor and expressed as pmoles of UMP incorporated per μg nucleolar DNA.

DISCUSSION

In eukaryotes, preribosomal RNA synthesis occurs in the nucleolus and isolated nucleoli provide a useful *in vitro* system for the study of regulation of single gene expression. Ribosomal RNA synthesis is highly responsive to change in physiological state of the cell (4) and it is of interest to investigate the mechanisms involved in such modulation of transcriptional activity. Thioacetamide-treated liver nucleoli appear to offer a useful tool for such studies inasmuch as RNA is greatly enhanced after a single injection and the activity rapidly reverts to normal levels. The increased rate of RNA synthesis by isolated nucleoli closely parallels that observed *in vivo* (6), thus

indicating that the isolated nucleoli retain their altered characteristics in vitro.

The enhanced endogenous activity which is a measure of the bound RNA polymerase I molecules is accompanied by a similar increase in the activity of the "free" RNA polymerase I enzyme. Whether the increased activity is due to an increase in the number of RNA polymerase molecules or due to an "activation" of the pre-existing molecules is not certain from this data. The increase in the endogenous activity indicate that in the activated state more enzyme molecules initiate synthesis on the rDNA template. This increase might be due to an increase in the number of "initiation factors" and/or an increase in the number of active RNA polymerase molecules (16).

ACKNOWLEDGMENTS

We wish to thank Mrs. Rose K. Busch for supplying the rats and Mr. James W. Bremer for technical assistance. These studies were supported by the Cancer Research Center Grant CA-10893, awarded by the National Cancer Institute, DHEW, the Pauline Sterne Wolff Memorial Foundation, a gift from Dr. and Mrs. O. A. Breiling, a generous gift from Mrs. Jack Hutchins, and the Bristol-Myers Fund.

REFERENCES

1. Ballal, N. R., Kang, Y-J., Olson, M. O. J., and Busch, H. (1975) J. Biol. Chem. 250, 5921-5925.
2. Ballal, N. R., Goldknopf, I. L., Goldberg, D. A., and Busch, H. (1974) Life Sci. 14, 1835-1845.
3. Ballal, N. R., Choi, Y. C., Mouche, R., and Busch, H. (1977) Proc. Natl. Acad. Sci. USA 74, 2446-2450.
4. Busch, H. and Smetana, K. (1970) The Nucleolus, pp. 492-497, Academic Press, New York.
5. Villalobos, Jr., J. G., Steele, W. J., and Busch, H. (1964) Biochim. Biophys. Acta 91, 233-238.
6. Steele, W. J. (1966) The Cell Nucleus, pp. 203-220, Taylor & Francis, London.
7. Yu, F-L. (1974) Nature 251, 344-346.
8. Lowry, O. H., Rosebrough, N. T., Farr, A. L., and Randell, R. J. (1951) J. Biol. Chem. 193, 265-275.
9. Burton, K. (1968) Meth. Enzymol. 12(B), 163-166.

10. Busch, H. and Smetana, K. (1970) The Nucleolus, pp. 512-547, Academic Press, New York.
11. Blatti, S. P., Ingles, C. J., Lindell, T. J., Morris, P. W., Weaver, R. F., Weinberg, F., and Rutter, W. J. (1970) Cold Spr. Harb. Symp. Quant. Biol. 35, 649-658.
12. Castro, J. A., Gomez, M. I. Diaz, de Castro, C. R., de Fenos, O. M., de Ferreyva, E. C., and D'Acosta, N. (1975) Res. Comm. Chem. Path. Pharm. 10, 93-103.
13. Grummt, I. (1975) Euro. J. Biochem. 57, 159-167.
14. Udvardy, A. and Seifart, K. H. (1976) Euro. J. Biochem. 62, 353-363.
15. Cox, R. F. (1973) Euro. J. Biochem. 39, 49-61.
16. Yu, F. L. and Feigelson, P. (1972) Proc. Natl. Acad. Sci. USA 69, 2833-2837.