INCREASED DNA ASSOCIATED WITH RAT LIVER NUCLEOLI WHEN ISOLATED WITH SPERMIDINE

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SUMMARY

When rat liver nucleoli were isolated in the presence of 5 mM spermidine rather than 5 mM magnesium, the nucleolar-associated RNA polymerase activity was found to increase some 10-fold. The spermidine-isolated nucleoli contained more than twice the amount of DNA found in magnesium-isolated nucleoli. The protein concentrations and RNA content were similar. These findings suggest that spermidine stimulation of nucleolar DNA-dependent RNA polymerase may be due to its capacity to increase the amount of DNA available as template for the enzyme.

Polyamines are found ubiquitously in living organisms (1) and their synthesis and accumulation are enhanced in rapid growth systems (2-9). Observations have been made by numerous investigators that polyamine accumulation parallels RNA accumulation (3,4,10-12). Further, in the anucleolate mutant of Xenopus laevis, neither rRNA nor spermidine are accumulated after gastrulation (8). Autoradiographic studies indicate that ^{3}H-putrescine and ^{3}H-spermidine accumulate at the nucleolus, the site of rRNA synthesis (13,14).

In an attempt to elucidate the underlying mechanism of the relationship(s) between polyamines and rRNA, we have studied the effects of spermidine on nucleoli isolated from rat liver.

MATERIALS

Uridine-5-^{3}H-5'-triphosphate ($\geq$12Ci/mM) was obtained from New England Nuclear or from Amersham-Searle. Contaminants were removed by chromatography

in a solvent system consisting of isobutyric acid, H_2O and concentrated NH_4OH (66:33:1). After the chromatogram was dried, the purified UTP was visualized under ultraviolet light and eluted with distilled H_2O. The dTTP-γ-32^P (4 Ci/mM) was obtained from ICN. DNA, RNA, GTP, CTP, ATP, UTP, dTTP, dATP, dCTP, dGTP, pyruvate kinase, and phosphoenolpyruvate were obtained from Sigma. Spermidine, spermine, and dithiothreitol were obtained from Calbiochem. The α-amanitin was graciously donated by Prof. T. Wieland, Max Planck Institute, Munich, Germany. Female Sprague-Dawley rats (100-150 g each) were obtained from Zivic-Miller and were used as the source of liver tissue in all experiments.

METHODS

Isolation of the nucleus, the nucleolus, and nucleolar RNA polymerase

In two volumes of a 0.34 M sucrose solution containing either 5 mM magnesium or 5 mM spermidine as the stabilizing cation, 20 g of liver were homogenized at 2° using a glass-teflon homogenizer. Nuclei, isolated according to the method of Ro and Busch (15), were subjected to sonic disruption and the nucleoli released were purified in accordance with the published procedure (15). The resulting nucleolar preparation was free, essentially, of nuclear contamination, having less than 1 nucleus per 4000 nucleoli.

The nucleoli, in turn, were disrupted at high ionic strength and the RNA polymerase(s) were partially purified by ammonium sulfate fractionation followed by DEAE-Sephadex chromatography (16). This preparation was desalted by dialysis prior to assaying for enzyme activity.

RESULTS AND DISCUSSION

Although others have used low amounts of spermine along with high magnesium concentrations for the isolation of nuclei and nucleoli, this is the first time, to our knowledge, that a polyamine alone has been used to isolate nuclear and nucleolar fractions.

Table I. Effect of spermidine on nucleolar-associated RNA polymerase and on partially purified nucleolar RNA polymerase

Experiment	Fraction	Enzyme Activity
		(cpm/10^6 nucleoli)
A	Whole nucleoli isolated with 5 mM spermidine	486 ± 53
	Whole nucleoli isolated with 5 mM magnesium	54 ± 8
		(cpm/mg DNA)
B	Partially purified polymerase assayed with 5 mM spermidine	1500 ± 230
	Partially purified polymerase assayed with 5 mM magnesium	1630 ± 250

RNA polymerase assayed according to the method of Roeder and Rutter (16).

Each value represents the mean ± SE of 5 determinations in duplicate on 5 separate preparations.

(A) Assay mixture contained 20 μg calf thymus DNA (Sigma, Type I), 1.0 μmole KCl, 0.75 μmole NaF, 0.5 μmole PEP, 0.2 μmole 2-mercaptoethanol, 0.075 μmole each of GTP, CTP, and ATP; 0.0125 μmole UTP, 0.0005 μmole ^{3}H-UTP, 2.5 μg pyruvate kinase, 7 μmoles Tris HCl, pH 7.9; 0.2 μmole $MnCl_2$, and either 0.05 or 0.075 ml of nucleolar preparation in a final volume of 0.125 ml.

(B) Assay components were the same as (A), except that either 5 mM spermidine or 5 mM magnesium was added as indicated, and 0.075 ml of enzyme preparation was used.

When the nucleoli were isolated in the presence of 5 mM spermidine rather than 5 mM magnesium, it was observed that the nucleolar-associated RNA polymerase was 10-fold more active (Table I, Experiment A). This activity was insensitive to inhibition by 1 μM α-amanitin. Neither spermidine nor magnesium were used in the actual assay for RNA polymerase activity. However, after partial purification of the nucleolar RNA polymerase, its activity assayed in the presence of 5 mM spermidine was similar to that assayed in the presence of 5 mM magnesium (Table I, Experiment B). These data were interpreted to mean that spermidine was not affecting the polymerase activity by interaction with the enzyme itself, and that we had purified away the factor or factors upon

Table II. Effects of isolation of rat liver nucleoli with spermidine upon their DNA, RNA, and protein content

Fraction	pg protein/no	pg RNA/no	pg DNA/no
Nucleoli isolated with 5 mM spermidine	8.2	1.2	1.0
Nucleoli isolated with 5 mM magnesium	7.5	0.99	0.41

Nucleoli were isolated by the method of Ro and Busch (15).

DNA content was assayed according to the method of Burton (17), RNA by the method of Schneider (18), and protein by the method of Lowry et al. (19), except in the spermidine fractions in which the microbiuret method was used (20).

pg = picograms; no = nucleolus.

which spermidine was acting in order to cause enhanced nucleolar RNA polymerase activation of the intact nucleoli.

Analysis of the levels of RNA, DNA, and protein in the nucleolar fractions indicated that there were indeed dramatic differences between the fractions isolated with 5 mM spermidine and those isolated with 5 mM magnesium. The spermidine-isolated nucleoli, for example, contained twice as much DNA (Table II) as those isolated with magnesium. The amount of DNA found in association with the magnesium-isolated nucleolus is in good agreement with data from other laboratories (21). However, the amount of DNA associated with the spermidine-isolated nucleolus is higher and is similar to the amount associated with the nucleolus of a Walker tumor cell. Further, electron micrographs of nuclei isolated with 5-10 mM spermidine and spermine show extra chromatin caps (22), and these caps may be extra DNA bound by polyamines to the nucleolus.

The DNA-dependent RNA polymerase found in the nucleolar fraction of rat liver appears to be rather specific for mammalian DNA as illustrated in Table III. The activity measured with the addition of bacterial DNA or other nonmammalian DNAs was about 25% of the efficiency found with mammalian DNA.

Table III. Relationship of origin of DNA to nucleolar DNA-dependent RNA polymerase activity

Origin	cpm incorporated
Calf thymus	19,906
Salmon	5,833
Soft roe	4,700
Herring	3,900
Cl. perfringens	5,900
E. coli	4,725

RNA polymerase assayed according to the method of Roeder and Rutter (16).

With the exception of the origin of the DNA, the assay mixture was the same as shown on Table I. A 0.05 ml aliquot of a nucleolar preparation isolated with 5mM spermidine was used in each assay. Each value is the mean of 4 determinations.

On the basis of these findings we feel that it is reasonable to propose that the increase in nucleolar RNA polymerase activity in response to spermidine reported herein, which has been reported by other workers (23), is due to the increased amount of DNA found associated with the nucleolus when spermidine is present. Further studies are in progress to determine the nature of this DNA.

REFERENCES

1. Tabor, H. and Tabor, C. W., Pharmacol. Rev. 16, 245 (1964)
2. Jänne, J., Raina, A. and Siimes, M., Acta Physiol. Scand. 62, 352 (1964)
3. Dykstra, W. G. and Herbst, E. J., Science 149, 428 (1965)
4. Caldarera, C. M., Barbiroli, B. and Moruzzi, G., Biochem. J. 97, 84 (1965)
5. Russell, D. and Snyder, S. H., Proc. Nat. Acad. Sci. USA 60, 1420 (1968)
6. Jänne, J. and Raina, A., Acta Chem. Scand. 22, 1349 (1968)
7. Russell, D. H., Ann. N. Y. Acad. Sci. 171, 772 (1970)
8. Russell, D. H., Proc. Nat. Acad. Sci. USA 68, 523 (1971)
9. Russell, D. H. and Lombardini, J. B., Biochim. Biophys. Acta 240, 273 (1971)
10. Raina, A., Jänne, J. and Siimes, M., Biochim. Biophys. Acta 123,197 (1966)
11. Raina, A. and Telaranta, T., Biochim. Biophys. Acta 138, 200 (1967)
12. Jänne, J., Acta Physiol. Scand. Suppl. 300, 1 (1969)
13. Gfeller, E. and Russell, D. H., Anat. Rec. 166, 306 (1970)
14. Gfeller, E. and Russell, D. H., Z. Zellforsch 120, 321 (1971)

15. Ro, T. S. and Busch, H., Cancer Res. 24, 1630 (1964)
16. Roeder, R. G. and Rutter, W. J., Nature 224, 234 (1969)
17. Burton, K., Biochem. J. 62, 315 (1956)
18. Schneider, W. C., J. Biol. Chem. 161, 293 (1945)
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem. 193, 265 (1951)
20. Zamenhof, S., in Sidney P. Colowick and Nathan O. Kaplan (Editors), Methods in Enzymology, Vol. 3, 696 (1957)
21. Busch, H. and Smetana, K. The Nucleolus, Academic Press, New York, p. 174 (1970)
22. Gfeller, E., Russell, D. H., Levy, C. C., Taylor, R. L., and Stern, D. N., Z. Zellforsch, in press.
23. Caldarera, C. M., Moruzzi, M. S., Barbiroli, B. and Moruzzi, G., Biochem. Biophys. Res. Commun. 33, 266 (1968)