TRANSCRIPTION-DEPENDENT RELEASE OF A MAMMALIAN RNA POLYMERASE FROM ITS ENDOGENOUS TEMPLATE

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Summary: RNA polymerase activity is released from the insoluble fraction of mouse kidney nuclei during synthesis of RNA *in vitro*. Conditions that inhibit transcription also inhibit the release of RNA polymerase.

In isolated eucaryotic nuclei, enzymatic activity characteristic of RNA polymerase is generally found in an insoluble (so-called "aggregate") form (1-3). The usual interpretation is that the enzyme molecules are tightly bound to the chromatin, probably to the template sites on which they function. However, if a molecule of RNA polymerase *in vivo* is to transcribe more than one non-adjacent segment of DNA, or a given segment more than once, the molecule must pass from one physical location on the chromatin to another.

We have found that the RNA polymerase of nuclei isolated from mouse kidneys can transcribe exogenous DNA as well as endogenous DNA. We present evidence that the polymerase is released from its endogenous template, and that this release depends on transcription.

METHODS

Kidneys were removed from male Charles River mice and decapsulated. Nuclei were prepared according to the method of Blobel and Potter (4), but substituting 2.0 M sucrose for 2.3 M. Nuclei were resuspended in TMD buffer (0.05 M Tricine (N-Tris hydroxymethyl methyl glycine), pH 7.9 at 0°, 2 mM $MgCl_2$, 1 mM dithiothreitol, 25% (v/v) glycerol) by gentle homogenization in a Dounce homogenizer, and stored in small aliquots at

at -70°. Each kidney typically yielded 200-400 µg of DNA in the final suspension of nuclei.

Standard reaction mixtures for incorporation of [^{3}H]UTP into RNA (see Fig. 1) were incubated at 30°, and incorporation of [^{3}H]UTP was measured by the filter paper disc method (5).

Mouse liver DNA was prepared by the method of Marmur (6). Other DNA's were purchased from Sigma Chemical Company. Synthetic d(A-T) copolymer was from Miles Laboratories and ^{3}H-labeled compounds from New England Nuclear Corp.

RESULTS AND DISCUSSION

Figure 1 shows that addition of calf thymus DNA stimulated the incorporation of [^{3}H]UTP into RNA by isolated nuclei at 0.05 M $(NH_4)_2SO_4$. This enhanced incorporation continued for at least 24 hours.

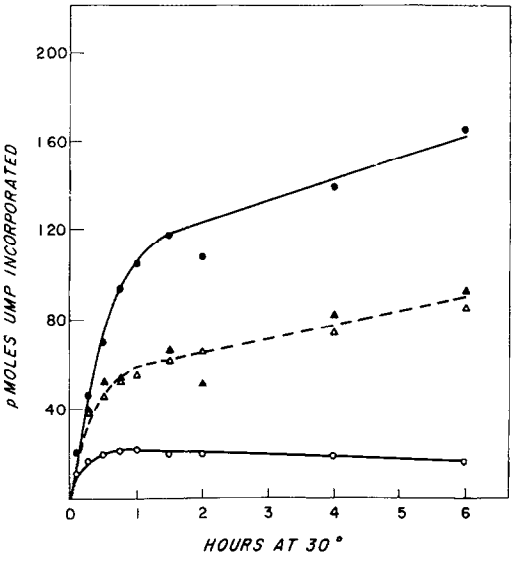

Figure 1. Effect of exogenous DNA on incorporation of [^{3}H]UTP into RNA by isolated nuclei *in vitro*. Reaction mixtures contained the following in 0.5 ml: 0.1 M Tricine, pH 8.25; 2 mM $MnCl_2$; 5 mM $MgCl_2$; 0.05 M $(NH_4)_2SO_4$; 5 mM creatine phosphate; creatine phosphokinase, 50 µg/ml; 5 mM dithiothreitol; ATP, CTP, GTP, 0.5 mM each; [^{3}H]UTP, 0.05 mM, 500 mC/mmole; heat denatured calf thymus DNA, 0 or 100 µg; isolated mouse kidney nuclei containing 47 µg DNA. Symbols: nuclei assayed at 0.05 M $(NH_4)_2SO_4$ with (●) and without (○) added DNA; at 0.5 M $(NH_4)_2SO_4$ with (▲) and without (△) added DNA. Each point represents the average of two assays.

During the first few minutes, the incorporation was almost linear in the presence of added DNA, but not in its absence (Fig. 2). The initial velocity, however, may be the same with and without added DNA. Incorporation was higher at 0.5 M $(NH_4)_2SO_4$ than at 0.05 M $(NH_4)_2SO_4$, but was not stimulated by added DNA. Under these conditions the physical state of the system is visibly different: the reaction mixture is not a suspension of nuclei, but a gel.

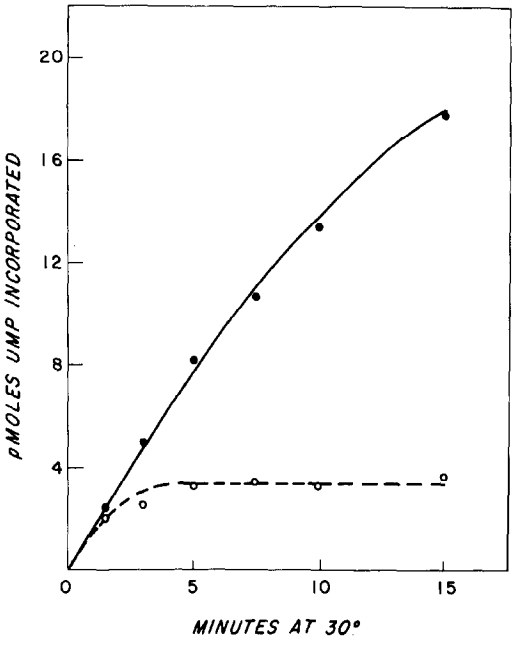

Figure 2. Initial period of incorporation. Conditions and symbols are as described in Fig. 1. Points represent the average of three assays.

To determine whether the exogenous DNA was transcribed, assays were performed with a variety of DNA's, and with [^{3}H]CTP as well as [^{3}H]UTP. The ratio of nucleotides incorporated (UMP/CMP) was compared with the ratio of adenine (A) to guanine (G) in the DNA used (calculated from data in reference 7). Two major results are shown in Table 1. First, although the ratios were not identical, the tendency was toward a higher ratio of UMP/CMP incorporated when the DNA's had a higher ratio of A/G. In the presence of d(A-T) almost no CMP was incorporated. Second, neither

Table 1. Incorporation of [^{3}H]UTP and [^{3}H]CTP into RNA by isolated nuclei with exogenous DNA's having different proportions of adenine and guanine

		Ratio of incorporation: pmoles UMP/pmoles CMP			
	Expt. No.	1A	1B	2	3
DNA source	A/G ratio (Ref. 7)				
None		.30	.45	.41	.43
Mouse liver	1.48				.84
Calf thymus	1.3 - 1.4	.53	.96	.77	.90
Salmon sperm	1.3 - 1.4	.38	.99		.80
E. coli	0.89	.40	.62	.70	.79
M. lysodeikticus	0.39	.48	.34	.45	.43
Synthetic d(A-T)	-			87.4	77.3

Components of the assay mixture were in the proportions described in Fig. 1. In experiment 1, the assay volume was 0.25 ml; one sample was taken at 2 minutes, (A), another at 9 hours (B). Assays of 0.125 ml were incubated for 9 and 16 hours in experiments 2 and 3, respectively. Each figure is the average of three assays.

the composition nor the presence of exogenous DNA appreciably affected the ratio of incorporation during the first two minutes (experiment 1A). The ratio in the first two minutes was about the same as that found in the absence of added DNA and was lower than that found at longer periods of incorporation with all the DNA's except *M. lysodeikticus* (in which A/G = .39). During this initial period, the incorporation seems to be directed by the endogenous DNA, regardless of the presence or absence of exogenous DNA.

The apparent ability of the RNA polymerase in the isolated nuclei to transcribe exogenous DNA suggests that functional enzyme molecules can detach from the endogenous template. To test this hypothesis, standard reaction mixtures without DNA were incubated 30 minutes, then separated into soluble and insoluble fractions by centrifugation.

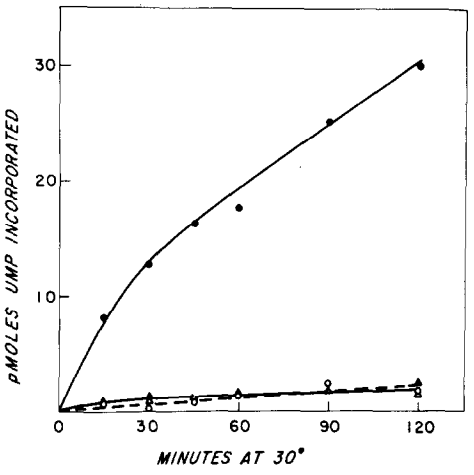

Figure 3. RNA polymerase activity in soluble and insoluble fractions of pre-incubated nuclei. Standard reaction mixtures as described in Fig. 1 were incubated 30 minutes at 30° then centrifuged 30 minutes at 40,000 x g. Supernatant and pellet fractions were assayed for incorporation of [^{3}H]UTP into RNA under standard conditions. Symbols: supernatant assayed with (●) and without (o) added DNA; pellet assayed with (▲) and without (Δ) added DNA.

Fig. 3 shows that the soluble fraction contained RNA polymerase activity that was dependent on added DNA, while virtually no activity was found in the insoluble fraction. In another experiment, described in Table 2, the RNA polymerase activity was initially associated with the insoluble fraction of the nuclei, but passed into the soluble fraction after incubation.

Table 2. Distribution of RNA polymerase activity between soluble and insoluble fractions of nuclei

	Total enzyme units	
	Supernatant	Pellet
First homogenate, before incubation	23	175
After 30 minutes at 30°	327	40

Isolated nuclei were broken by vigorous homogenization in TMD buffer and one third of the homogenate was centrifuged 30 minutes at 40,000 x g. To the remaining homogenate, all components of the standard assay mixture except DNA were added. This mixture was incubated 30 minutes, then centrifuged. Each supernatant and pellet was assayed in triplicate for RNA polymerase. One enzyme unit gives an initial velocity of incorporation of one pmole/minute.

Table 3. Release of RNA polymerase from insoluble fraction of nuclei

Conditions of incubation	First step: pmoles UMP incorporated	Second step: soluble enzyme units
Control	20.9	14.8
No incubation	1.3	4.3
Control	33.3	30.2
Incubated 5 minutes at 30°	13.0	24.4
Not incubated	0.4	11.5
Incubated 30 minutes at 0°	3.2	9.3
Nucleoside triphosphates omitted	-	15.3
Control	12.9	23.4
+ actinomycin D, 0.1 μg/ml	9.6	15.6
" , 5 μg/ml	3.6	3.1

Aliquots of the insoluble (pellet) fraction of broken nuclei were incubated under the conditions indicated, and the incorporation of [^{3}H]UTP into RNA was measured on a small sample (first step). The remainder was centrifuged 30 minutes at 40,000 x g, and the RNA polymerase activity of the soluble fraction was determined (second step). Control conditions: standard assay without added DNA, incubated 30 minutes at 30°. In the third experiment, actinomycin D was removed by dialysis after the first step. Each figure is the average of two (first step) or three (second step) determinations.

The experiments summarized in Table 3 were designed to determine whether this release of RNA polymerase was dependent on transcription. The results show that conditions that reduced the incorporation of [^{3}H]UTP in the first step also reduced the polymerase activity found in the supernatant fraction in the second step. Transcription would therefore seem to be necessary for the release of RNA polymerase from the insoluble fraction of the nuclei; it is not clear, however, whether an enzyme molecule must actually engage in transcription before it can be released. This phenomenon is not restricted to the kidney; we have obtained the same results with nuclei isolated from mouse liver.

Roeder and Rutter (8) have attributed the early cessation of RNA synthesis in isolated nuclei to inactivation of some essential factor in the transcription apparatus. Our results suggest that this factor may be the enzyme-template complex itself, which dissociates during

transcription in vitro. The phenomenon may also explain the success of several groups (9-11) in preparing soluble RNA polymerase by incubating what is essentially an "aggregate enzyme" preparation (1).

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REFERENCES

1. Weiss, S.B., Proc. Natl. Acad. Sci. U.S., 46, 1020 (1960).
2. Goldberg, I.H., Biochim. Biophys. Acta, 51, 201 (1961).
3. Nakagawa, S., and White, A., J. Biol. Chem., 245, 1418 (1970).
4. Blobel, G., and Potter, V.R., Science, 154, 1662 (1966).
5. Mans, R.J., and Novelli, G.D., Arch. Biochem. Biophys., 94, 48 (1961).
6. Marmur, J., J. Molec. Biol., 3, 208 (1961).
7. Sober, H.A., ed., Handbook of Biochemistry, Cleveland, The Chemical Rubber Company, 1968.
8. Roeder, R.G., and Rutter, W.J., Biochemistry, 9, 2543 (1970).
9. Cunningham, D.D., et al., Biochim. Biophys. Acta, 145, 834 (1968).
10. Jacob, S.T., et al., Biochem. Biophys. Res. Commun., 32, 831 (1968).
11. Goldberg, M.L., and Moon, H.D., Arch. Biochem. Biophys., 141, 258 (1970).