A SOLUBLE DNA-DEPENDENT RNA POLYMERASE IN NUCLEI OF NON-DIVIDING ANIMAL CELLS

M.Ramuz, J.Doly, P.Mandel and P.Chambon

Institut de Chimie Biologique, Faculté de Médecine, Strasbourg, France

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Up to now the only animal sources of soluble DNA-dependent RNA polymerase were embryonic (Furth and Loh, 1963) and neoplastic tissues (Furth and Loh, 1964) and testis (Ballard and Williams-Ashman, 1964) which have a large number of dividing cells. The intracellular localization of the enzyme was not stated. In other tissues, such as rat liver (Weiss, 1960 - Busch et al., 1962 - Ro and Busch, 1964), rat prostate (Hancock et al., 1962), rat uterus (Gorski, 1964) and other organs (Mandel et al., 1964), RNA polymerase appeared to be a non soluble "aggregate" enzyme containing firmly bound DNA which could not be removed in a manner which would allow an exogenous DNA dependency test.

We now report that, besides the "aggregate" enzyme, nuclei from rat liver and other tissues also contain a soluble DNA-dependent RNA polymerase.

Material and Methods.

Rat liver nuclei prepared and lysed as previously described (Busch et al., 1962) were centrifuged for 15 min at 54,000 g. The pellet contained the "aggregate" enzyme while the soluble DNA-dependent RNA polymerase was found in the supernatant which we used as the source of enzyme in our studies. No more than 0.5 to 2 % of the total nuclear DNA, can be detected

in this supernatant fraction. A longer centrifugation (1 h at 100,000 g) did not result in any further appreciable deposition of material with enzymatic activity. Treatment of incubation mixtures and measurement of acid-insoluble radioactivity were carried out as already related (Busch et al., 1962). Nearest neighbor base frequencies were determined by counting the radioactivity of the individual 2'3'-nucleoside monophosphates rendered acid-soluble after alkaline hydrolysis (0.5 N KOH, 15 h at 37°). In these experiments the components of the reaction mixture (table I) were 20 fold increased in order to obtain enough labelled RNA. The 2'3'-nucleoside monophosphates were separated by high-voltage paper electrophoresis after charcoal treatment.

Results.

Some characteristics of the soluble liver nuclear DNA-dependent RNA polymerase are summarized in table I. Omission of DNA or of one of the nucleoside triphosphates, or addition of minute amounts of DNase or RNase markedly reduce the incorporation of the labelled nucleotide. Native DNA is a more efficient primer in the reaction than heat-denatured DNA. Omission of Mn^{++} or Mg^{++} results in practically no incorporation. At low concentration Mn^{++} is a better activator than Mg^{++}. Low concentrations of Actinomycin C_1 and inorganic pyrophosphate are very potent inhibitors. While the "aggregate" RNA polymerase activity is enhanced by raising the ionic strength (Goldberg, 1961), the present enzyme is inhibited by ammonium sulphate. The nucleotide incorporation is linear during the first 10 min, then increases but not linearly, at least up to 90 min. The optimal pH is 7.2.

The RNA nature of the reaction product is suggested by the

Table I. - Requirements for soluble nuclear liver RNA-polymerase.

labelled nucleotide	reaction mixture	μμmoles incorporated
Exp. 1 α-P^{32}-GTP	Complete	104.0
	Omit CTP	2.8
	Omit CTP, UTP, ATP	6.1
	Omit DNA	0.6
	Omit DNA, add denatured DNA	30.0
	Add inorganic phosphate 10 mM	95.0
	Add inorganic pyrophosphate 10 mM	0.0
Exp. 2 α-P^{32}-GTP	Complete	108.0
	Add Actinomycin C_1 10 μg	0.0
	Add Actinomycin C_1 1 μg	7.5
	Add DNase 2.5 μg	2.6
	Add RNase 2.5 μg	6.0
	Post-treatment 5 μg DNase (10 min at 37°)	89.0
	Post-treatment 5 μg RNase (10 min at 37°)	7.0
Exp. 3 α-P^{32}-UTP	Complete	131.0
	Omit ATP	21.4
	Omit Mn^{++}	1.6
	Omit Mn^{++}, add Mg^{++} 4 mM	19.0
	Add ammonium sulphate 6.6 % saturated	2.8

The complete reaction mixture (0.25 ml) contains : 160 mM Tris buffer pH 7.2, 4 mM $MnCl_2$, 8 mM 2-mercaptoethanol, 1 mM each of CTP, ATP, UTP, GTP, one labelled in α with P^{32}, 60 μg of calf thymus DNA and approximately 0.2 mg protein from the supernatant fraction. Incubation for 10 min at 37°.

fact that it is rendered acid-soluble by treatment with RNase, but not DNase (table I), and also by alkaline hydrolysis when nearest neighbor experiments are performed. In this case all of the 2'3'-ribonucleoside monophosphates are radioactive, no matter which α-P^{32}-labelled nucleoside triphosphate is added.

Table II shows that the rate at which each nucleotide is incorporated reflects the composition of the DNA used in the

Table II. - Influence of the base composition of DNA on the incorporation of ribonucleotides.

DNA source	nucleotide incorporation in μμmoles				RNA		DNA
	CMP	GMP	UMP	AMP	$\frac{A+G}{C+U}$	$\frac{A+U}{G+C}$	$\frac{A+T}{G+C}$
Calf thymus	185	177	250	261	1.00	1.41	1.25*
M.Lysodeikticus	134	135	50	58	1.05	0.40	0.39**

Reaction mixtures and assay were as described in the legend of table I.

*Lehman et al., 1958 - **Lee et al., 1956.

reaction. Moreover determination of nearest neighbor sequencies performed with two DNAs and any of the four α-P^{32}-labelled nucleoside triphosphates (table III) provides strong evidence that the synthesized RNA is a replica of the DNA template and that the two strands of the RNA are of opposite polarity.

Discussion.

These results indicate that a nuclear DNA-dependent RNA polymerase is present in a soluble form in an animal tissue with a very low mitotic index. The characteristics of the enzyme are similar to those of the purified RNA polymerase isolated from bacteria (Hurwitz and August, 1963) or animal proliferating tissue (Furth and Loh, 1963 and 1964 - Ballard and Williams-Ashman, 1964). The determination of nearest neighbor frequencies shows that the in vitro mechanism of the DNA transcription is similar whether bacterial or animal RNA polymerase are used.

We have found the same soluble nuclear enzyme, with the same characteristics, in all rat organs so far investigated : brain, kidney, spleen, testis and prostate. The highest enzy-

Table III. - Nearest neighbor frequencies for DNA primer and synthesized RNA.

nearest neighbor sequence	Calf thymus				M.Lysodeikticus			
	DNA* primer		RNA** synthesized		DNA* primer		RNA** synthesized	
ApA, UpU	0.089	0.087	0.085	0.078	0.019	0.017	0.028	0.026
CpA, UpG	0.080	0.076	0.083	0.072	0.052	0.054	0.058	0.051
GpA, UpC	0.064	0.067	0.063	0.074	0.065	0.063	0.059	0.067
CpU, ApG	0.067	0.072	0.071	0.070	0.050	0.049	0.052	0.048
GpU, ApC	0.056	0.052	0.051	0.052	0.056	0.057	0.054	0.057
GpG, CpC	0.050	0.054	0.053	0.063	0.112	0.113	0.101	0.125
UpA	0.053		0.049		0.011		0.016	
ApU	0.073		0.072		0.022		0.027	
CpG	0.016		0.018		0.139		0.123	
GpC	0.044		0.046		0.121		0.108	

*For DNA, thymine is substituted for uracil. The values for DNAs are taken from Josse et al. (1961).

**Base-incorporation factors are derived from the radioactivity measurements in the experiment as described by Josse et al. (1961). These base-incorporation factors are : thymus DNA primed reaction A = 0.279, U = 0.272, G = 0.213, C = 0.236 - M.lysodeikticus primed reaction, A = 0.161, U = 0.159, G = 0.323, C = 0.357.

matic activity was found in testis : 0.75 mμmole P^{32}-GMP incorporated per mg protein for 10 min at 37° with calf thymus DNA as primer. This value, determined without any purification of the extract, is more than twice that reported for the soluble DNA-dependent RNA polymerase purified from testis by Ballard and Williams-Ashman (1964).

With α-P^{32}-UTP as labelled nucleoside triphosphate and calf thymus DNA as primer the total enzymatic activity of the supernatant fraction from liver nuclei equals the total activity of the corresponding "aggregate enzyme". This obviously is not more than an approximation since the determination of the enzymatic activity is related to the base composition of the DNA and to the nature of the labelled nucleoside triphosphate

used in the assay.

The relation between this soluble enzyme and the two RNA polymerase enzymatic activities reported by Widnell and Tata (1964a) in rat liver nuclei is very obscure. The enzymatic activity which is the most activated by Mn^{++} in their system is also stimulated by ammonium sulphate and presents an optimum pH of 8.5-9.0, while the soluble enzyme we described has a poor activity at pH 8.5-9.0 and is inhibited by ammonium sulphate like bacterial (Chambon, unpublished) and partially purified testis RNA polymerases (Ballard and Williams-Ashman, 1964).

The presence of a soluble DNA dependent RNA polymerase in liver will permit the study of the mechanism of the increase in RNA polymerase activity following partial hepatectomy (Busch et al., 1962 - Tsukada and Lieberman, 1964). Preliminary results indicate that this increase is related to an increase in active enzyme. The existence of a soluble RNA polymerase in other organs offers an opportunity to investigate the mechanism by which RNA polymerase activity is enhanced in these organs after hormonal (Barnabei and Sereni, 1964 - Gorski, 1964 - Hancock et al., 1962 - Weill et al., 1963 - Widnell and Tata, 1964b) and other treatments (Hawtrey and Nourse, 1964). These studies are now in progress.

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