CHARACTERIZATION OF RNA SYNTHESIZED AT HIGH IONIC STRENGTH BY RAT LIVER AGGREGATE RNA POLYMERASE

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SUMMARY

RNAs synthesized *in vitro* at high ionic strength by rat liver nuclear aggregate RNA polymerase were characterized by DNA-RNA hybridization. Results indicate that all the RNA species which were synthesized *in vitro* and which hybridized with rat liver DNA were also present *in vivo* in rat liver nucleus. Only a fraction of these *in vitro* synthesized RNAs were found *in vivo* both in nucleus and cytoplasm. These results suggest that RNA synthesis catalyzed *in vitro* at high ionic strength by the aggregate RNA polymerase retains most of the specificity of RNA synthesis *in vivo*.

Animal nuclei contain an insoluble RNA polymerase (aggregate RNA polymerase) which synthesizes mainly G-C rich RNAs at low ionic strength (1). In the presence of substances able to dissociate the histones from the DNA template, there is a high increase in the synthesis of A-U rich RNAs, mostly as a result of transcription of parts of extranucleolar chromatin (1). That these regions of the genome are also transcribed *in vivo*, is suggested by the fact that RNA polymerase molecules which synthesize the A-U rich RNAs are found in a tight DNA-RNA polymerase-RNA complex (transcription complex) (1). These observations suggest that during the preparation of nuclei, some rearrangements could occur in the chromatin, leading, at low ionic strength, to the obstruction of parts of the genome previously transcribed *in vivo*. Such an interpretation leads to the prediction that RNA

species synthesized *in vitro* at high ionic strength must have their *in vivo* counterpart. We present here results of hybridization experiments showing that the RNAs synthesized *in vitro* at high ionic strength by the nuclear aggregate RNA polymerase are also present *in vivo*.

MATERIAL AND METHODS

Only sterile solutions were used. Rat liver nuclei were prepared according to Chauveau. The aggregate RNA polymerase was prepared and incubated at high ionic strength (0.4 M ammonium sulphate) for 1 h as already described (1) with α^{32}P-GTP of very high specific activity (400 µC/µmole) as the labelled nucleotide. Nuclear RNA was extracted by the hot phenol-SDS method (2) with a DNAase-Pronase step and a final gel filtration on Sephadex G 200 (3). The same method was used for the isolation of *in vitro* synthesized RNA. This RNA was the product of an asymmetrical transcription since less than 1 °/₀ of the total RNA was resistant to RNAase treatment after self-annealing experiments. Cytoplasmic RNA was prepared according to Brawerman (4). All RNA preparations were checked for their DNA content by the method of Burton (5). The contamination was always less than 1 °/₀. DNA was extracted and purified from rat liver nuclei as described by Stévenin *et al.* (3).

DNA-RNA hybridization was conducted according to Gillespie and Spiegelmann (6) at 67°C in 0.3 or 0.4 ml. The salt concentration was 4 SSC. Filters were washed and treated by RNAase (10 µg/ml) before counting in a Packard scintillation counter. Blank filters containing T_4DNA or no DNA gave similar counts which were substracted from the experimental values.

RESULTS

Figure 1 shows a typical saturation curve. Double reciprocal plotting indicates that saturation of DNA would occur at a RNA/DNA ratio of about 100. To be meaningful, hybridization experiments conducted with animal nucleic acids have to be done as close as possible to the saturation level. First, because qualitative differences between two RNA populations are lowered when working at a low RNA/DNA input, and secondly, because it was shown that unspecific competitions could occur at low RNA/DNA input (7). For practical reasons, our experiments were performed at about half saturation of DNA by the labelled RNA.

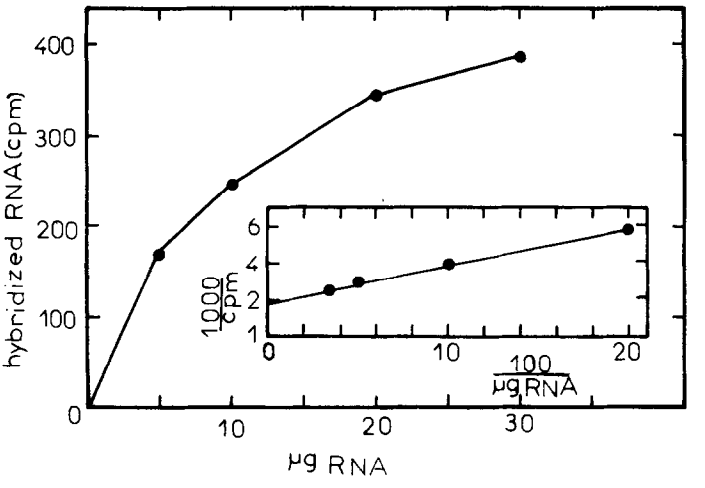

Fig. 1a - Saturation curve of 1.5 µg of rat liver DNA with ^{32}P RNA synthesized *in vitro* at high ionic strength. RNA specific activity was 3,000 counts/min/µg.

1b - Double reciprocal plot of 1a.

Figure 2 indicates that, in competition experiments, unlabelled rat liver nuclear RNA completely inhibits hybridization of labelled RNA synthesized *in vitro* at high ionic strength. On the contrary when competition is carried out with non labelled rat

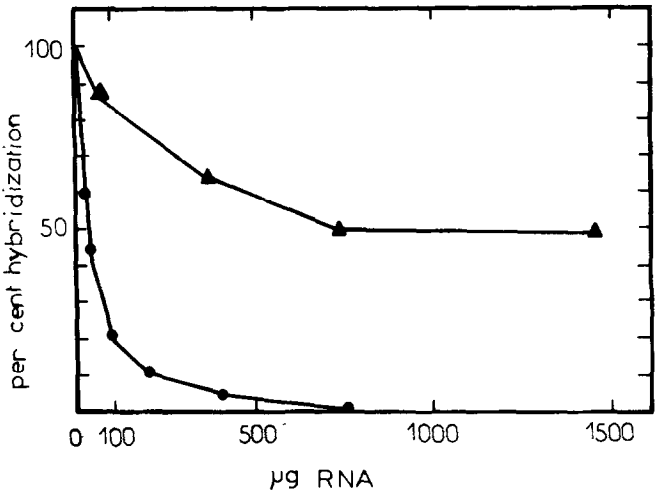

Fig. 2 - Competition between unlabelled liver RNA and ^{32}P labelled RNA synthesized in vitro at high ionic strength. Filters containing 1.5 µg of rat liver DNA reacted with 10 µg of labelled RNA and increasing quantities of unlabelled RNA. Input label was 65,000 · 100 °/₀, hybridization was 365 counts/min.
●——● rat liver nuclear RNA.
▲——▲ rat liver cytoplasmic RNA.

liver cytoplasmic RNA, a plateau of inhibition is reached at a value of about 50 °/₀.

Results presented in table 1 show that in opposition to the results of Birnboim et al. (8), an inhibition of hybridization of labelled RNA is observed when unlabelled competitor RNA is prehybridized with DNA. This suggests that the competition observed when both RNAs were mixed in the incubation was a true inhibition. The slightly smaller inhibition values obtained by preincubation can be accounted for by a loss of cold hybrid during the washing and subsequent incubation, as suggested by results presented in table 1.

DISCUSSION

Our results suggest very strongly that the RNA species syn-

TABLE 1

Exp.	Rat liver DNA/filter	Labelled RNA per incubation	CONDITIONS OF INCUBATION			counts/min.
			0 - 18 h	18 h	18 - 24 h	
1	1.5 µg	15 µg	4 SSC	Wash 60°	^{32}p RNA	89
			75 µg nuclear RNA	"	^{32}p RNA	46
			300 µg nuclear RNA	"	^{32}p RNA	38
			1450 µg cytoplasmic RNA	"	^{32}p RNA	71
			4 SSC	"	75 µg nuclear RNA + ^{32}p RNA	35
			4 SSC	"	300 µg nuclear RNA + ^{32}p RNA	16
			4 SSC	"	1450 µg cytoplasmic RNA + ^{32}p RNA	51
			^{32}p RNA	"	4 SSC	70
2	3 µg	10 µg	4 SSC	Wash 60°	^{32}p RNA	85
			80 µg nuclear RNA	"	^{32}p RNA	38
			4 SSC	"	80 µg nuclear RNA + ^{32}p RNA	28
			^{32}p RNA	"	4 SSC	76
3	1.5 µg	10 µg	^{32}p RNA	stop	-	318
			^{32}p RNA + 400 µg E.coli RNA	"	-	281
			^{32}p RNA + 1600 µg E.coli RNA	"	-	323

^{32}p RNA was synthetized *in vitro* as described under "Methods". Filters were washed with 4 SSC when indicated. Experiments 1, 2, and 3 were completely separated experiments.

thesized at high ionic strength by the aggregate RNA polymerase are also present *in vivo*. Similar results were recently obtained using whole nuclei instead of aggregate enzyme (9). It must be stressed that rare species of RNA would not be detected in such experiments, due to the well known complexity of animal genomes. Moreover we cannot demonstrate that all the species synthesized *in vivo* are synthesized *in vitro* at high ionic strength, since the low amount of RNA synthesized in our incubations does not permit to perform the reverse competition, i.e. cold RNA synthesized *in vitro* competing for labelled RNA made *in vivo*. Nevertheless, the *in vitro* synthesis of RNA species which are nuclear restricted together with species present both in the nucleus and cytoplasm indicate that the *in vitro* transcription catalysed at high ionic strength by the aggregate RNA polymerase retains most of the specificity of RNA synthesis *in vivo* (10, 11). The present results support previous hypothesis (1), namely that the increase of RNA synthesis and the change in base composition observed when incubating at high ionic strength would restore the *in vivo* pattern rather than induce an artificial derepression of the genome related to the dissociation of histones from DNA as previously suggested (12, 13). The *in vitro* transcription at low ionic strength would then be limited by some artifactual aggregation obscuring the actual *in vivo* pattern of transcription. This conclusion must be kept in mind when giving a physiological interpretation to the variations of RNA polymerase activities induced by various treatments of animals.

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