SYNTHESIS OF TRANSFER RNA IN RAT LIVER

RELATIVE RESISTANCE TO ACTINOMYCIN*

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Actinomycin D has been shown to inhibit incorporation of inorganic P^{32} into high molecular weight RNA of rat liver cytoplasm to a much greater extent than into 4 S RNA (Merits, 1963). In transfer RNA from the livers of actinomycin-treated rats, most of the P^{32} has been found in the terminal -pCpCpA ribonucleotides, and incorporation has been ascribed to end turnover (Merits, 1963; Harel et al, 1964). In the course of studies on the effects of actinomycin D (Revel and Hiatt, 1964) in rats given orotic acid-C^{14} as an RNA precursor we have found radioactivity in pseudouridylic and uridylic, as well as cytidylic acids of transfer RNA. These observations exclude turnover of the terminal sequence of ribonucleotides as the sole basis for RNA labeling in liver cytoplasm of actinomycin-treated rats.

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Methods.

Actinomycin D was administered intraperitoneally to white male rats weighing 200-250 g, and the effects on RNA labeling were determined by injecting orotic acid-C^{14}, 6.5 mc/mmole, and measuring C^{14} incorporation into RNA, as described previously (Revel and Hiatt, 1964). Liver cytoplasmic RNA was isolated after treatment with sodium dodecyl sulfate and phenol (Hiatt, 1962), and transfer RNA was separated by passage through Sephadex G 200 (Boman and Hjerten, 1962). Non-radioactive transfer RNA was added as carrier, and the preparations were hydrolyzed in 0.5 N KOH at 37° for 16 hours. The perchloric acid-soluble 2' (3')-ribonucleotides were neutralized with KOH, and the insoluble potassium perchlorate was removed by centrifugation at 4°C. Ribonucleotides were fractionated by chromatography on Dowex 1 x8 columns, with the elution system described by Cohn (1960). Ribonucleotides were identified by spectrophotometry and by paper chromatography (Cohn, 1960), concentrated by lyophilization, and counted in a liquid scintillation counter (Packard, Tricarb). Specific activities of individual ribonucleotides were calculated on the basis of the molar base ratios for liver transfer RNA reported by Lipschitz and Chargaff (1960).

Results

Thirteen hours following administration of orotic acid-C^{14} to rats previously given actinomycin D, 1.5 mg per kg, almost no label was found in the RNA of liver cytoplasmic ribosomes, while the specific activity of the soluble RNA fraction was approximately one-third of that observed in the control (Table 1). In order to

TABLE 1

Inhibition of Rat Liver Cytoplasmic RNA Labeling

by Actinomycin D

Cytoplasmic RNA Fraction#	Actinomycin D mg per kg 1.5*	5**
	C^{14} incorporati per cent of cont	
High molecular weight	4	1
4 S	36	9

*Actinomycin D in propylene glycol was injected intraperitoneally 17 hours, and orotic acid-C^{14} 13 hours prior to sacrifice. The control animals received propylene glycol.

**Actinomycin D was injected 11 hours, and orotic acid-C^{14} 10 hours prior to sacrifice.

#From sucrose gradient analyses (Revel and Hiatt, 1964).

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distinguish isotope in the terminal -pCpCpA ribonucleotides from uniform labeling of the entire molecule, the distribution of C^{14} in the pyrimidine residues was studied. As shown in Table 2, the ratios of the specific activities of cytidylic, uridylic, and pseudouridylic acids to that of RNA in the control (Breitman, 1960) were virtually identical to those in treated animals. (It should be emphasized that demonstration of isotope in pseudouridylic acid is essential to establish internal labeling, for Daniel and Littauer (1963) have shown that a purified rat liver enzyme can incorporate uridylic, as well as cytidylic acid into the terminal sequence of

TABLE 2

Isotope Distribution in 4 S RNA from Livers of Normal and Actinomycin-Treated Rats*

Sample	Specific Activity		Ratio S.A.# Nucleotide / S.A. RNA	
	Control	Actinomycin D	Control	Actinomycin D
	c.p.m. per μmole			
Total RNA	10,500	3,800		
2' (3')-CMP	10,340	3,640	.99	.96
2' (3')-UMP	31,538	10,940	3.00	2.88
2' (3')-ψUMP	42,000	15,311	4.00	4.03

*Rats were injected with actinomycin, 1.5 mg per kg, in 0.2 ml propylene glycol, or with propylene glycol (control) 17 hours, and orotic acid-C^{14} 13 hours prior to sacrifice.

#Specific activity.

transfer RNA.) The fact that the amount of isotope in transfer RNA is further reduced by a larger dose of actinomycin (Table 1) is additional evidence that the incorporation observed at low levels of the drug results from DNA-dependent RNA synthesis (Reich et al, 1962; Acs et al, 1963), rather than from end turnover.

Discussion.

Following the administration of isotopic precursors of RNA to normal rats, the 4 S RNA of liver cytoplasm is labeled much earlier than is ribosomal RNA (Hiatt, 1962). However, 24 hours

after administration of the precursor, both fractions are equally labeled. This suggests that while one component of the 4 S RNA turns over rapidly, the bulk of this material is synthesized (or labeled) at approximately the same rate as ribosomal RNA.

Several studies (Scholtissek, 1962; Littauer, 1961) demonstrate turnover of the terminal -pCpCpA ribonucleotides of transfer RNA, presumably the process which accounts for its early labeling. Indeed, some workers have ascribed to this process all of the labelin of RNA observed in the livers of rats given actinomycin D. The present work differs from previous studies in that the RNA was examined at a later time after the administration of the isotopic precursor, a time when end turnover would presumably take place from a pool of non-radioactive precursors (Hurlbert and Potter, 1952) In addition, in the studies of Merits (1963) and of Harel et al, (196 P^{32} was used as the RNA precursor. On the other hand, less than 50 per cent of labeled nucleoside incorporated into 4 S RNA of actinomycin-treated L cells was in terminal positions (Franklin, 1963 Further, Perry (1964) reported internal labeling in the low molecular weight RNA of Hela cells exposed to actinomycin D and cytidine-H^3.

Several explanations may be offered to account for the results presented. Because of the difference in size of the DNA templates, actinomycin may inhibit the synthesis of transfer RNA to a lesser degree than that of ribosomal (Franklin, 1963). A similar explanation has been proposed for continued transfer RNA synthesis by E. coli subjected to ultra-violet irradiation (Sibatani and Mizuno, 1963; Kroes et al, 1963). The small size of transfer RNA

or the fact that, in contrast to ribosomal RNA, its active form requires no accompanying protein, may facilitate its more ready transfer to cytoplasm from the nucleus, where some RNA synthesis is observed in the livers of treated animals (Revel and Hiatt, 1964). Finally, it should be noted that while transfer RNA is likely synthesized in the nucleus (Goodman and Rich, 1962; Giacomoni and Spiegelman,1962), the site and mechanism of entry of pseudouridylic acid into the S-RNA molecule remain to be defined (Srinivasan and Borek, 1964).

Summary

Labeling of the RNA in rat liver cytoplasmic ribosomes is more profoundly inhibited by actinomycin than is labeling of transfer RNA. Isotope in the latter fraction is found in pseudouridylic and uridylic, as well as cytidylic acids. This observation indicates that turnover of terminal ribonucleotides cannot account for all of the label incorporated into the transfer fraction.

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