Differential Inhibitions of the Synthesis of Soluble and Particulate RNA*

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The origin of s-RNA, despite an extensive literature, remains obscure. One aspect of its synthesis, the origin of its unique methylated components, has been recently elucidated by the demonstration in our laboratory of the existence of an enzyme system which methylates the pre-formed polynucleotide chain (1,2). In investigations on the origin of the primary structure of s-RNA we have recently obtained evidence which seems to indicate divergent synthetic origins for s-RNA and p-RNA.

In E. coli K_{12} W_6 a genetic deletion renders the organisms incapable of suppressing RNA synthesis in the absence of amino acids (3,4). As a consequence accumulation of both s-RNA and p-RNA can continue for as long as three hours after the deprivation of the organisms of an essential amino acid. However, the rate of synthesis of the two

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species of RNA is altered from that which prevails in organisms in logarithmic growth phase. In several experiments which were designed for other aims a consistent pattern of a shift in favor of s-RNA synthesis was observed.

We have recently studied this trend quantitatively and find that the altered rate of synthesis is constant for both histidine and methionine requiring organisms both of which contain the deletion for control of RNA synthesis (4). (Table I).

Table I

Differential Rates of Incorporation of Uracil-2-C^{14} into s- and p-RNA in Cells with Relaxed Control of RNA

Organism	Specific Activity of RNA (CPM/μg Ribose)			
		1	2	
	Exp.	p-RNA	s-RNA	Ratio 1/2
E. coli K_{12} W_6 Minus Methionine	1	28	38	.7
	2	17	24	.7
	3	30	39	.8
	4	19	30	.6
	5	17	47	.4
E. coli K W_6 G-15* Minus Histidine	6	37	53	.7
E. coli K_{12} W_6 in Logarithmic Growth Phase	7	62	69	.9
	8	1.7×10^4	1.7×10^4	1.0
	9	1.0×10^4	1.1×10^4	0.9

* We are indebted to Dr. Gunther Stent for making this organism available to us.

The phenomenon is enhanced even further if the p-RNA is separated from s-RNA in the presence of 10^{-4}M Mg^{++}. Apparently a moiety of newly synthesized RNA can be shunted to either fraction depending on the binding by Mg^{++}.

Still another line of evidence for differential sensitivity of s-RNA and p-RNA synthesis was obtained from irradiation studies.

We have shown earlier that inhibition by u.v. irradiation of the incorporation of formate and uracil into the RNA of growing bacterial cells varies with the log of the u.v. dose (6,7). A detailed study of the effect of u.v. irradiation on RNA in growing cells revealed a significantly greater inhibition of the synthesis of p-RNA than of s-RNA over a wide range of irradiation dose. (Fig. 1.)

In order to minimize post irradiation cell repair which might obscure the initial damage, we investigated the effect of the u.v. irradiation on RNA synthesis in the organisms with the relaxed control over RNA synthesis. It is apparent from Fig. 2 that the impact of irradiation has different effects on the synthesis of the two species of RNA in organisms deprived of methionine. Inhibition of s-RNA synthesis shows a threshold type of curve, while inhibition of p-RNA synthesis has a direct relationship to dose. (The same pattern emerged with organisms which were starved of histidine.)

A more detailed study of uracil incorporation into the two RNA fractions at low u.v. dose levels revealed that

no decrease in s-RNA synthesis occurs up to u.v. dose levels which give 30% or more inhibition of p-RNA synthesis. (Fig. 3) Synthesis of the latter is inhibited throughout the dose level sequence, beginning with the lowest dose used.

URACIL-2-C^{14} INCORPORATION INTO RNA FRACTIONS OF GROWING E.coli $K_{12}W_6$ AFTER IRRADIATION

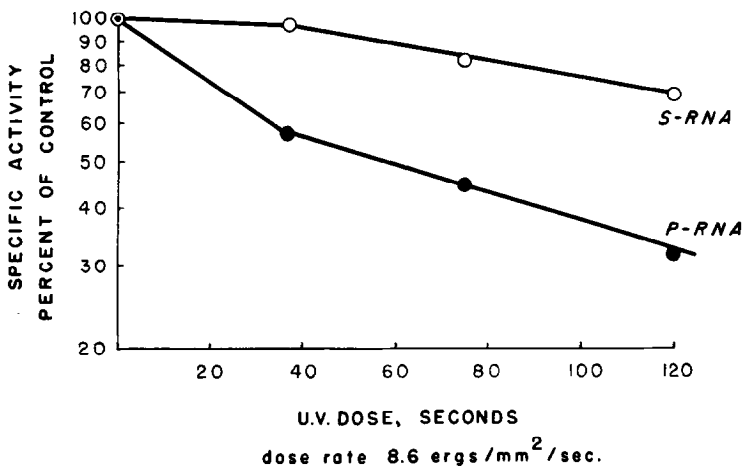

Figure 1

The irradiations were carried out in a glass tray on a shaker at the indicated dose rate on 300 ml aliquots of cells in logarithmic growth phase at a population of $2x10^8$ cells per ml. Uracil-2-C^{14} (10-15 µg per ml) was added at the cessation of irradiation and the cultures were incubated for 20' at 37°C. The cells were then rapidly chilled, harvested and washed. RNA fractions were isolated by the method cited (5). Specific activity is expressed as CPM per ug ribose.

These data seem to indicate that different targets are involved in u.v. radiation damage to the synthesis of s-RNA and p-RNA. Whether this is a difference in localization within the cell or a difference in enzymes, cofactors, or even of primer for the synthetic sequences remains to be determined.

URACIL-2-C^{14} INCORPORATION INTO RNA
OF STARVING E.coli $K_{12}W_6$ AFTER IRRADIATION

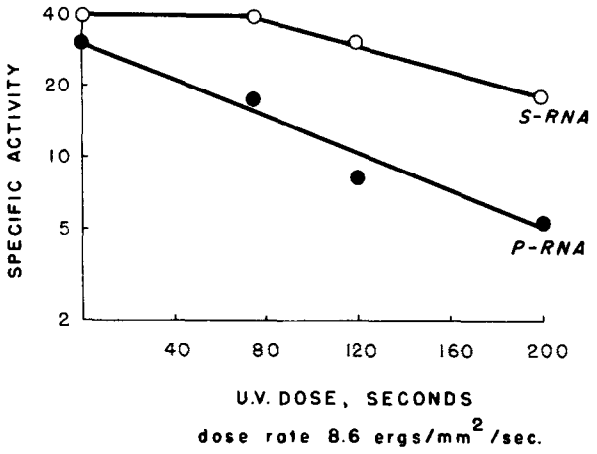

Figure 2

Washed cells from cultures in logarithmic growth phase were suspended in medium lacking methionine and were irradiated at various dose levels. Uracil-2-C^{14} (10-15 ug per ml) was added and the cultures were incubated at 37° for 2 hours. Thereafter the cells were processed as described under Figure 1.

INCORPORATION OF URACIL-2-C^{14} INTO
RNA FRACTIONS
OF IRRADIATED STARVING E.coli $K_{12}W_6$

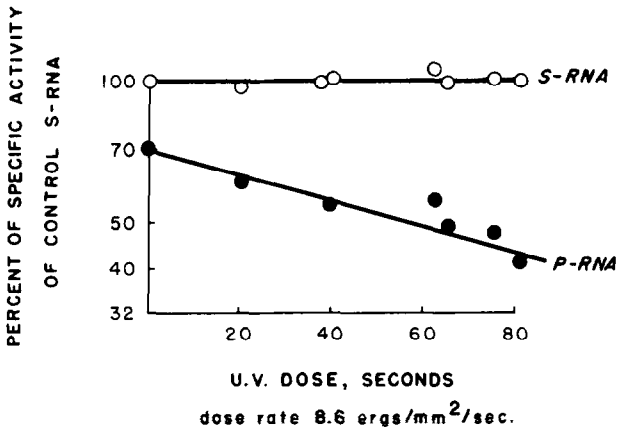

Figure 3

For Experimental details see Figure 2.

For details of starvation see (3). In each experiment organisms were permitted to starve of the amino acid for 2 hours in presence of 12-15 μg/ml of C^{14} uracil. s- and p-RNA were isolated by the method of Lacks and Gros (5). In Experiment 5 the concentration of Mg^{++} was 10^{-4} M. In all others it was 10^{-2} M.

REFERENCES

1. Borek, E., Mandel, L. R., and Fleissner, E. Fed. Proc., 21, 379 (1962)
2. Fleissner, E., and Borek, E., Proc. Natl Acad. Sci., U.S., 48, 1199 (1962)
3. Borek, E., Ryan, A., and Rockenbach, J., J. Bact., 69, 460 (1955)
4. Stent, G. S., and Brenner, S., Proc. Natl Acad. Sci., U.S., 47, 2005 (1961)
5. Lacks, S., and Gros, F., J. Mol. Biol., 1, 301 (1959)
6. Wainfan, E., and Borek, E., Int. J. Rad. Biol., 4, 327 (1962)
7. Wainfan, E., and Borek, E., Abstr. 142nd Am. Chem. Soc. Meeting, 40C (1962)