CONSEQUENCE OF AMBER MUTATION ON THE SIZE OF POLYRIBOSOMES

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There is evidence for a direct relationship between the size of polyribosomes and proteins synthesized thereon (Slayter, et al., 1963; Noll, et al., 1963; Padayatty and Rolfe). Kiho and Rich (1965) have shown that polyribosomes containing the lac messenger are smaller than normal when they are prepared from mutants with amber mutations in the lac operon. We have investigated the effect of amber mutation on the size of polyribosomes at late times after infection of su^- and su^+ strains of Escherichia coli with amber mutants of bacteriophage T4.

Methods.

Amber mutants of T4 phage were grown in a su^+ strain, E. coli CR63, purified by differential centrifugation, and suspended in M9 buffer (Adams, 1959). Spheroplasts were prepared from E. coli CR63 (su^+) and E. coli B (su^-) after infection with amber mutants and incubated for 28 min at 30° in a rich medium containing 1% Difco nutrient broth, 0.1% Difco casamino acids, 0.05% Difco yeast extract, 0.1% glucose, 10% Merck sucrose, 0.2% $MgSO_4$ and 0.005% L-tryptophan. Polyribosomes were prepared according to the method of Padayatty and Rolfe and the sedimentation coefficients of the polyribosome peaks were calculated according to the method of Martin and Ames (1961).

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Results and Discussion.

During late times after infection with wild type T4 phage, a polyribosome peak of sedimentation coefficient 280 S has been observed (Rolfe, 1965). Representative samples of polyribosome profiles from su⁻ and su⁺ strains infected with amber mutants in gene 23 are shown in Fig. 1. Polyribosome peaks after infection of E. coli B with amber B17 or C137 have lower S values than those of the su⁺ strain, E. coli CR63. Thus the effect of

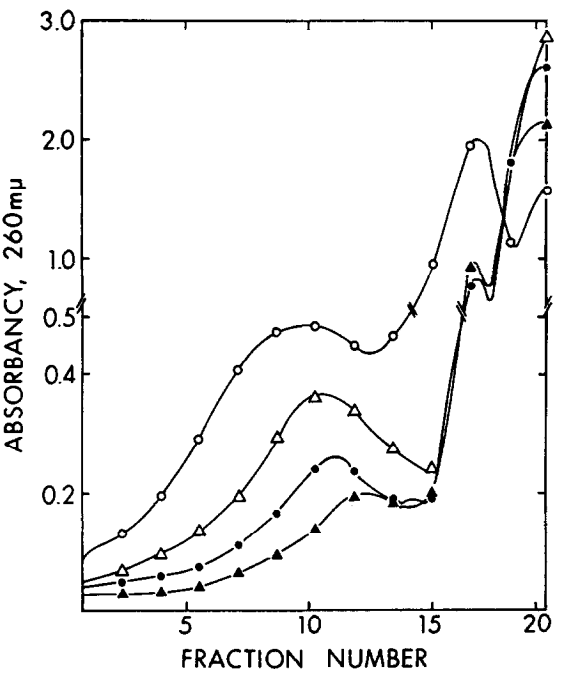

Fig. 1. Distribution of polyribosomes from su⁺ and su⁻ strains after infection with am B17 or am C137.

E. coli B or E. coli CR63, grown to 5 x 10^8 cells per ml in the medium of Fraser and Jerrel (1953), were harvested and infected with the T4 phage mutant. The cells were converted to spheroplasts within 15 min by lysozyme-EDTA treatment, suspended in a rich medium (described under Methods) and incubated at 30°. After 28 min they were harvested, lysed with DNase-Brij and 1 ml portions of the lysate almost free from cell membrane debris were centrifuged through 15-30% sucrose at 25,000 rpm for 2 hr in SW25.1 rotor of the Spinco Model L-2 ultracentrifuge. The tube was punctured, the solution was pumped at a uniform rate (Sigma motor pump, 20 drops per fraction), and the absorption at 260 mμ was recorded in a Gilford Spectrophotometer. O —— O am B17 in E. coli CR63; ● —— ● am B17 in E. coli B. △ —— △ am C137 in E. coli CR63; ▲ —— ▲ am C137 in E. coli B.

amber mutation on polyribosome size is suppressible in the permissive host, E. coli CR63, where the main polysome peak is at 280 S.

Amber mutations result in the premature termination of polypeptide chains and the lengths of the fragments depend on the distance of the mutation from the end of the gene corresponding to the N-terminus of the protein (Sarabhai, et al., 1964). These results, however, do not prove that the mutation acts directly at the translation level, since peptide fragments might have resulted from the production of m-RNA fragments. Imamoto and Yanofsky (1967) have shown that amber mutation has an effect on transcription producing smaller messengers. However, the effects of amber mutation may be expressed at the level of translation since such mutations were "suppressed" when the phase of reading of the genetic message was altered (Brenner and Stretton, 1965).

Sedimentation coefficients of polyribosome peaks obtained from su^- and su^+ strains after infection with various ambers are shown in Table 1. The S values of polyribosome peaks from the su^- strain after infection with amber mutants in gene 23 were consistently lower than the polyribosome peak of 280 S obtained from E. coli B infected with T4 phage or from the su^+ strain infected with amber mutants. Amber mutation in addition to its chain terminating effect may cause dissociation of ribosomes (unloading) from ribosome-messenger complex. Since the order of mutation on the genetic map as deduced from peptide fragments is (H11, C140, B17), B272, H32, B278, C137, H36, A489 (Sarabhai, et al., 1964), it is evident that there is little correlation between the size of the polyribosome peak and the position of the mutation. Thus, the "unloading" effect seems to be dependent on factors other than the position of the amber codon on the messenger.

Polyribosomes involved in the synthesis of hemoglobin of 17,000 daltons contain 5-6 ribosomes (Slayter, et al., 1963); those involved in the synthesis of a protein of 30,000 daltons contain 8-12 ribosomes (Noll, et al.,

Table 1

Sedimentation Coefficients of Polyribosome Peaks from Spheroplasts of *E. coli* B Infected with T4 Amber Mutants

Gene	Mutant	S-Value	Gene	Mutant	S-Value
23	H11	215	20	N50	277
				B8	270
	C140	225			
			21	N90	315
	B17	242		N76	270
	(287 from CR63)				
			22	B270	252
	B272	243			
			24	B26	273
	H32	215			
			31	N54	277
	C137	225			
	(280 from CR63)		7	B16	315
	H36	225	8	B23	270
	A489	252			

1963), and those involved in the synthesis of the T4 phage head protein of 42,000 daltons contain 11-12 ribosomes (Padayatty and Rolfe). Thus, a peptide of approximately 3000 to 4000 daltons containing 25 to 35 amino acids corresponds to one ribosome. If the molecular weight of peptides synthesized by ambers do not differ by more than 3000 to 4000 daltons, the number of ribosomes per polysome may not differ. Since there was not much difference in S values of the polyribosome peaks from su^- host infected with amber mutants, except for B17, B272 and A489, it may be speculated that the differences between the molecular weights of peptide fragments of these mutants are less than 3000 to 4000 daltons. However, the S values of the polyribosome peaks of amber B17, B272, and A489 were significantly higher than those from H11, C140, H32, C137 and H36. This may indicate that the environment of the amber codon is more important in ribosome-messenger attachment than the distance of the amber codon from the chain initiation codon on the messenger.

Mutants of genes 20 to 24, 30 and 31 of T4 phage are characterized by the absence of heads in lysates of infected su⁻ cells; these genes thus appear to be involved in head protein synthesis or assembly (Epstein, et al., 1963). There is now further evidence that gene 23 specifically controls the structure of the head protein and that these proteins are unaffected by mutations in other genes in the same cluster. The appearance of normal polyribosome peaks after infection with mutants other than in gene 23 (Table 1) indicates the synthesis of normal head protein, even though heads do not appear in the lysate. These results confirm that the products of genes 20 to 22, 24 and 31 are necessary for assembly of the head protein even though head proteins are made (Epstein, et al., 1963).

Normal sized polyribosome peaks were obtained from a su⁻ strain infected with mutants in genes 7 and 8 showing they are not involved in the synthesis of phage head protein. Indeed, mutations in genes 7 and 8 do not cause any effect on the production of heads in the lysates.

Summary.

The sedimentation coefficients of polyribosome peaks from su⁻ strain were lower than that from su⁺ strain during late times after infection with T4 phage head amber mutants (gene 23). This may be due to (i) production of smaller messenger RNA molecules or (ii) "unloading" effect on ribosomes by the amber codon. The amber effect on polyribosomes size is suppressed in the su⁺ strain. The non-correlation between the size of a polyribosome peak from the su⁻ strain and the position of the amber mutation may indicate that environment of the amber codon plays a significant role in ribosome-messenger attachment. Mutants in genes 20 to 22, 24 or 31 produced normal polyribosome peaks in su⁻ strain, showing that they do not interfere in phage head protein synthesis, even though involved in assembly of head.

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