POLYSOME-RIBOSOME DISTRIBUTION IN ISOGENIC RC^{str} and RC^{rel} STRAINS OF *ESCHERICHIA COLI*

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Study of the relative proportions of ribosomes and polysomes released by a standardized lysing procedure from isogenic RC^{str} and RC^{rel} strains of *Escherichia coli* shows that a 20-min period of amino acid starvation of RC^{str} bacteria reduces the fraction of ribosomes recovered in polysomes to about 60% of its value characteristic of exponentially growing cells; A similar starvation treatment of the RC^{rel} bacteria causes no appreciable reduction in the fraction of polysomal ribosomes.

1. Introduction

It has been proposed that the rate of net RNA synthesis in bacteria shows an inverse dependence on the intracellular concentration of free, i.e. non-polysomal ribosomes [1, 2]. Under this model the arrest of net RNA synthesis in amino acid starved RC^{str} *Escherichia coli* was explained by envisaging that amino acid starvation of an RC^{str} strain results in the breakdown of polysomes, thereby raising the level of free ribosomes and hence inhibiting net RNA synthesis. To explain the continued net RNA synthesis in RC^{rel} bacteria, it was envisaged that the RC^{rel} allele of the RC^{rel} gene allows polysome formation during amino acid starvation, thus avoiding the accumulation of free ribosomes and hence permitting the continuation of net RNA synthesis. More recent investigations [3] on the polysome content of several strains of *E. coli* during exponential growth and amino acid starvation have cast some doubt on the connection between the inhibition of net RNA synthesis and disappearance of polysomes, insofar as significant breakdown of polysomes was found to occur only in some but not in other amino acid starved RC^{str} strains and only upon starvation for some but not for other amino acids.

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2. Results and discussion

In order to eliminate these sources of variation, a re-examination of this phenomenon has been made using the otherwise isogenic *E. coli* strain pair CP78 (RC^{str}) and CP79 (RC^{rel}) [4]. It has to be noted that earlier investigations on the outlined problem have been made with related but not with isogenic strains of *E. coli*. Various strains of *E. coli* respond differently to the same lysing procedure, cf. [3]; moreover RC^{str} and RC^{rel} strains seem to have an altered cell envelope after different periods of amino acid starvation [5]. It is of great importance for the comparison of the polysome-ribosome-distributions of the two isogenic strains to choose a procedure for gently lysing the bacteria in which either strain, after exponential growth and after different periods of starvation, release about the same amount of its stable RNA. For this purpose the method described by Flessel et al. [6] was modified and the time of lysozyme and EDTA treatment at 0°C for the formation of spheroplasts was extended from two to five minutes. Longer times of treatment yielded a higher release of RNA in the following lysis but a lower ratio of polysomes to ribosomes. In order to arrest release of polysomal ribosomes from mRNA, chloramphenicol (CAP) was present during the lysing procedure at a final concentration of 100 μg/ml [7].

For a typical experiment bacteria were grown ex-

Table 1
Distribution of stable RNA after lysis.

Strain	Growth conditions at the moment bacteria were chilled	Cell debris %	Lysate %
CP78 (RC^{str})	Exponentially growing	38	62
	Arginine starvation for 5 min	28	72
	10 min	28	72
	20 min	29	71
	60 min	26	74
CP79 (RC^{rel})	Exponentially growing	37	63
	Arginine starvation for 5 min	41	59
	10 min	43	57
	20 min	57	43

The chilled cells were harvested by centrifugation at 10000 *g* for 10 min. After the supernatant was decanted, 0.3 ml of a CAP solution (2.0 mg/ml in H_2O) was added. The pellet was then resuspended with 4.5 ml of sucrose-salt solution (0.5 M sucrose, RNA-ase-free; 0.1 M tris-HCl, pH 8.0; 0.1 M NaCl). 0.6 ml of a fresh lysozyme solution (Mann, 1.0 mg/ml in sucrose-salt solution) and 0.4 ml of 0.14 M EDTA (pH 8.0) were added at 0°C in order to form spheroplasts. After 5 min at 0°C the magnesium concentration was restored to 10^{-2} M by the addition of 0.12 ml of 1 M $MgSO_4$. After centrifugation for 5 min at 5000 *g* and removal of the supernatant which contained 10 to 20% of the total radioactivity present in the unlysed bacteria, the spheroplasts were lysed with 0.5–1.0 ml of lysing medium (0.5% Brij 58; 0.5% sodium deoxycholate; 4 μg/ml DNAase; 100 μg/ml CAP; 0.05 M NH_4Cl; 0.01 M $MgSO_4$; 0.01 M tris-HCl, pH 7.5) at 0°C. The bacterial debris was removed by centrifugation for 15 min at 10000 *g* and the lysate was subjacted to sucrose density gradient centrifugation. In order to determine the percentage of stable RNA in the bacteria, cell debris and lysate, samples of the resuspended cells, debris and of the lysate were analysed for ^{14}C-uracil present in cold trichloroacetic acid (5%) precipitable material.

Table 2
Polysome-ribosome distribution after sucrose density gradient centrifugation.

Strain	Growth conditions at the moment bacteria were chilled	Poly-somes %	Mono-somes %	50 and 30 S particles %
CP78 (RC^{str})	Exponentially growing	42	35	23
	Arginine starvation for 5 min	31	49	20
	10 min	29	56	15
	20 min	26	57	17
	60 min	18	60	22
CP79 (RC^{rel})	Exponentially growing	41	35	24
	Arginine starvation for 5 min	49	29.5	21.5
	10 min	44	32	24
	20 min	37	38	25

Sucrose density gradients were analysed as described in fig. 1. The amount of stable RNA in the several areas of the gradients, polysomes, 70 S (monosomes) and 50 and 30 S particles, was calculated as percentage of the total ^{14}C-uracil radioactivity present in all three areas.

ponentially at 37°C in minimal medium supplemented with the amino acids required for growth (100 μg/ml) and thiamine (2 μg/ml). The culture was labelled with ^{14}C-uracil (0.01 μCi/ml, 0.033 μg/ml) and the cells were then grown for three more generations in order to label the stable RNA. For amino acid starvation were

then filtered, washed and resuspended in minimal medium lacking arginine, and further incubated for various periods of time. Bacterial metabolism was stopped by pouring 10 ml of the cell suspension into twice the volume of frozen minimal medium which contained CAP (200 μg/ml). The bacteria were then centrifuged in the cold and after their conversion to spheroplasts lysed with the neutral detergent Brij 58 [6]. Bacterial debris was removed by centrifugation and the lysate to which phage φ X 174 had been added as a sedimentation velocity marker was subjected to sucrose density gradient centrifugation (table 1). As can be seen from table 1, from about two thirds to three quarters of the stable RNA was released into the lysate under all cultural conditions, except after prolonged starvation of the RC^{rel} cells where the release amounted to little less than one half of the total stable RNA.

The polysome-ribosome distribution of three typical lysates, as revealed by their sucrose-density gradient centrifugation profiles, are presented in fig. 1. It can be seen in panel a of fig. 1 that about 42% of the ribosomes isolated from exponentially growing RC^{str} bacteria appeared in the polysome region; the rest behaved as monosomes, with a considerable fraction being present in the 50 S and 30 S areas. Panel b of fig. 1 shows that starvation of these bacteria for the required arginine for 20 min reduces the polysome content of the lysate to about one third of its exponential growth value. Finally, panel c shows that after an equivalent 20 min starvation the polysome content of RC^{rel} bacteria is higher than that of the isogenic RC^{str} cells. Table 2 presents in summary form the nine different lysates referred to in table 1. A shift to smaller polysomes was observed from the beginning of starvation on in either strain.

It can be seen that these results agree with earlier published results [2], inasmuch as the polysome content of the RC^{str} strain falls within the first 20 min of amino acid starvation to about 60% of its exponential value and experiences a further decrease during the next 40 min. By contrast, the RC^{rel} strain contains more polysomes immediately after the onset of starvation than while growing exponentially. But after 20 min of starvation of the RC^{rel} strain some net conversion of polysomes to monosomes has set in also here. It appears that the fraction of 50 S and 30 S particles remains nearly constant at the transition from exponential growth to amino acid starvation in either strain.

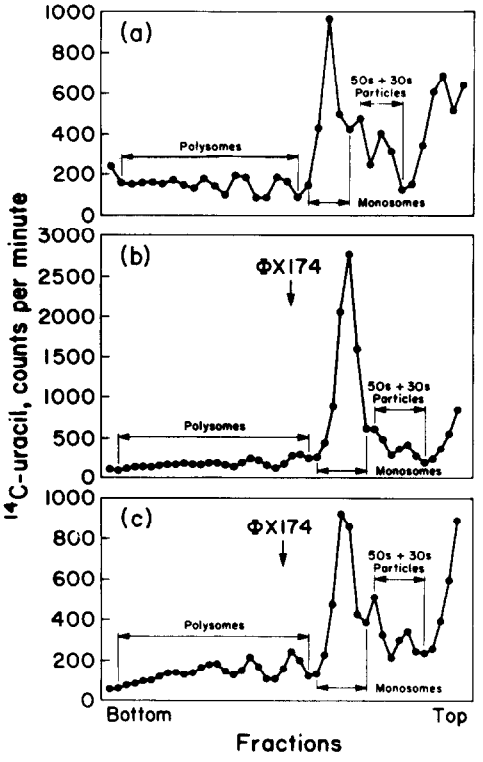

Fig. 1. Sucrose density gradient centrifugation. The lysate was layered (while avoiding any shearing forces) on top of a 28 ml gradient, buffered 30–15% sucrose solution, containing tris HCl, 0.01 M, pH 7.5; 0.01 M $MgCl_2$; 0.05 M NH_4Cl. After centrifugation for 4 hr at 25000 rpm (Spinco, SW 25.1) in the cold, fractions were collected and, after additions of 200 μg BSA, analysed for ^{14}C-uracil labelled cold trichloroacetic acid (5%) precipitable material. The position of phage φ X 174 was determined by plating samples of the fractions on *E. coli* C and plaque counting [8]. a) CP78 (RC^{str}), exponentially growing. 35 fractions. b) CP78 (RC^{str}), after 20 min of starvation for arginine. 47 fractions. Notice scale in b). c) CP79 (RC^{rel}), after 20 min of starvation for arginine. 47 fractions.

In RC^{str} strains of *E. coli* RNA synthesis ceases immediately after the onset of amino acid starvation. According to the proposed model of the regulation of RNA synthesis by free ribosomes the conversion of polysomes to free ribosomes must be equally rapid. Neither these data nor the results described earlier [1, 2] offer very good support for this model since the kinetics of the conversion of poly-

somes to monosomes are relatively slow compared to the cessation of net RNA synthesis. Nevertheless, it seems likely that the differential polysome-monosome dynamics of amino acid starved, isogenic RC^{str} and RC^{rel} cells does reflect some basis aspect of their manner of regulation of RNA synthesis.

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