OCCURRENCE OF TERMINATION RIBOSOMES AS FREE 70 S PARTICLES

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The physiological significance and even the existence of 70 S ribosomes in bacteria are still under discussion because of conflicting results from several laboratories. These results, based on the ribosome profiles obtained after sucrose gradient centrifugations, vary according to the culture medium [1], the lysis conditions [2–7] and the ionic composition of the sucrose solutions employed.

In 1966 Mangiarotti and Schlessinger [2] proposed that most or all the 70 S particles which appeared in bacterial lysates were produced by polyribosome breakdown. This conclusion seems unlikely in the light of later studies [3–6, 8]. More recently several groups [3, 7, 8] have obtained evidence that a portion of the monomers found were free 70 S ribosomes liberated from polysomes upon termination of translation.

Other investigations [4] have shown that 70 S ribosomes almost disappeared when NaCl was substituted for KCl in the lysing buffer and sucrose solutions. This fact has been interpreted as an indication that the monomers obtained in the presence of K^+ were artificially formed by association of subunits during or after lysis. Moreover Varricchio has found 4–7 percent of the total ribosomes as initiation monosomes. He and other workers [9, 5, 6] have suggested that the ribosome profile obtained in Na^+-containing gradients was a good picture of the *in vivo* distribution and that during the ribosome cycle the subunits were released from polyribosomes at the end of each round of translation.

The ribosome distribution pattern has been further investigated in our laboratory using lysates of cells in exponential growth or treated with trimethoprim. We have some new evidence supporting the existence of free 70 S ribosomes in bacteria.

Extracts from *Escherichia coli* were obtained and analyzed according to methods already described [8]. The ribosome profiles were very similar in gradients without monovalent salts or containing either NaCl or KCl. In all cases the monomer peak amounted to 15–20% and the subunits to 8–12% of the total ribosomes. This pattern did not change even after the addition of aurintricarboxylic acid to the lysing media in order to prevent the attachment of mRNA to subunits [10, 11] and therefore the formation of artificial initiation monosomes.

In another series of experiments the culture medium and the method of lysis described by Phillips et al. [4, 5] were used. In contrast with the results obtained using our methods it could be shown that the same extract gave different ribosome profiles according to the ionic composition of the sucrose gradients. When they contained either K^+ or no monovalent cation a sharp and prominent 70 S peak was evident. However, when NaCl was substituted for KCl the amount of 70 S ribosomes was greatly reduced with a concomitant accumulation of 50 S particles and a slight increase of 30 S subunits.

The effect of cations on the ribosome pattern was more remarkable when $MgSO_4$ was used instead of magnesium acetate. Thus, confirming the results of Phillips et al. [4] the monomer peak was small in gradients containing Na^+ and SO_4^{2-}, whereas it was larger in the presence of Na^+ and acetate or chloride ions.

Fig. 1 shows the combined effect of cations and anions on the ribosome profiles obtained by centrifugation of the same bacterial lysate. In all cases the monomer peak was high in the presence of K^+. (A small portion of these 70 S ribosomes might have arisen from a partial degradation of polysomes [8,9].) The amount of monomers decreases when Na^+

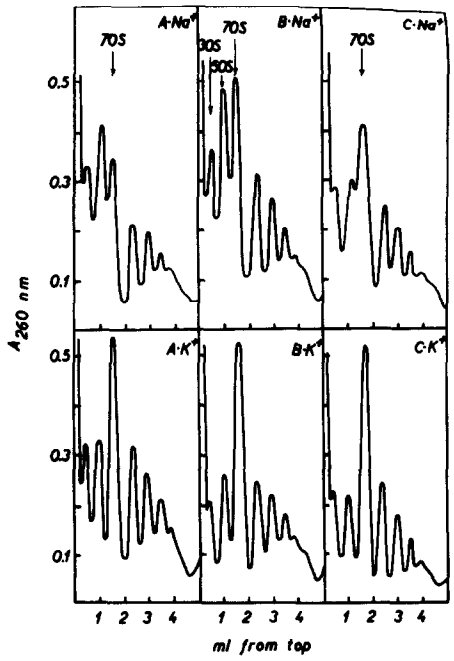

Fig. 1. Effect of different ions on the ribosomal distribution pattern. *E. coli* Hfr 3000 was grown to 2×10^8 cells/ml, harvested and lysed as described by Phillips et al. [4, 5]. Aliquots of the extract were layered on 4.6 ml of 15–40% linear sucrose gradients containing 20 mM tris-HCl, pH 7.5, 10 mM Mg salt (sulfate, acetate or chloride as indicated) and 50 mM NaCl or KCl. After 80 min centrifugation at 50,000 rpm in a Spinco SW–50L rotor, absorbance at 260 nm was continuously recorded with a Gilford spectrophotometer. A, B and C correspond to gradients containing $MgSO_4$, magnesium acetate and $MgCl_2$ respectively.

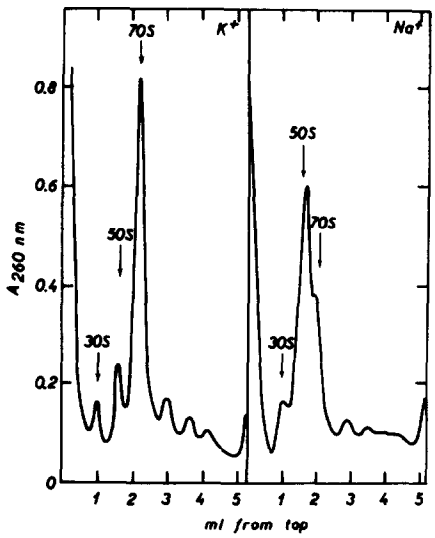

Fig. 2. Behaviour of free 70 S ribosomes in sucrose gradients with KCl or NaCl. Trimethoprim (50 μg/ml) was added to an exponentially growing culture of *E. coli* and 5–10 min later cells were harvested and lysed as described in fig. 1. Extracts were analyzed by gradients centrifugation in sucrose solution containing 20 mM tris–HCl, pH 7.5, 10 mM $MgSO_4$ and 50 mM KCl or NaCl.

was used and varied with different anions as pointed out before.

In order to enhance the ionic effects described, trimethoprim was added to a growing culture of *E. coli*. This substance blocks protein synthesis initiation without inhibiting elongation of polypeptide chain [12, 13]. A few minutes after this addition the polysomes almost disappeared and the final products of translation (termination ribosomes) accumulated. The same result could be obtained by slow cooling of the culture without any additions [8]. An extract of cells treated with trimethoprim gave very different ribosome patterns after centrifugation on gradients with K^+ or Na^+. When sucrose solutions containing KCl or no monovalent cation were used, the monomer peak was very high and sharp. On the contrary with Na^+ a tailing profile was obtained, with an evident decrease of 70 S particles (fig. 2). These results could indicate that ribosomes were transformed during centrifugation; a simple dissociation seems unlikely since the amount of 30 S subunits was almost constant.

Our results strongly suggest that bacterial lysates contain at least two kinds of native 70 S ribosomes: a) Initiation monosomes, active and fairly stable, which are formed by subunits, mRNA and either fMet–tRNA or peptidyl–tRNA; b) free monomers or termination ribosomes, rather unstable [14, 15], which can be partially or totally transformed into another particle of lower sedimentation coefficient according to the conditions used in lysis and gradient centrifugation. If these two kinds of 70 S particles do exist it is to be expected that after a short pulse with radioactive uridine only the initiation ribosomes would become labelled. Therefore the radioactivity in the 70 S region should be about the same when K^+ or Na^+–containing gradients were used. Moreover the peak of monomers (measured by absorbance) should

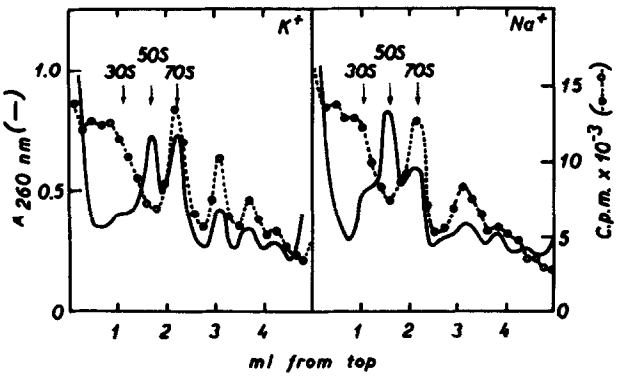

Fig. 3. Sucrose gradient analysis of pulse labelled *E. coli* ribosomes. An exponentially growing culture was labelled for 20 sec with ^{3}H-uridine (60 μCi/ml, sp. act. 11.1 Ci/mmole). The pulse was terminated and extracts were prepared according to Phillips et al. [4, 5]. The sucrose solutions used in gradient centrifugations were as described in fig. 2. Cold trichloroacetic acid-insoluble radioactivity was measured in a Packard scintillation counter.

decrease in gradients with Na^+ because of the instability of termination ribosomes. Both predictions are confirmed in the experiment shown in fig. 3.

The ribosomal distributions obtained with the different methods described up to the moment may not reflect the *in vivo* state, but it should be mentioned that solutions with K^+ rather than Na^+ reproduce more exactly the normal physiological conditions in bacteria. Recently, van Dijk - Salkinoja et al. [7] have used solutions with a composition very close to the intracellular medium and they have obtained *B. licheniformis* extracts with 68% polysomes, 20% monomers and 12% subunits.

Our findings seem to indicate that termination ribosomes are present in bacterial lysates as free 70 S particles. Moreover, Na^+ ion which only exists in bacteria in trace amounts [16], could change the conformation of 70 S ribosomes giving rise to modified particles with lower sedimentation coefficient. This type of "unfolded" ribosomes was already described in *E. coli* [17], liver [18] and reticulocytes [19]. Further experiments on these particles are in progress in our laboratory.

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