PEPTIDYL-PUROMYCIN SYNTHESIS ON POLYSOMES FROM STARVING BACTERIA

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1. Introduction

When bacteria of stringent (*relA*$^+$) genotype are starved for any amino acid required for growth, they initiate a rapid program of metabolic adjustments termed the 'stringent response' [1–3]. No such response is observed in mutants harboring the relaxed (*relA*) genotype. In either type of strain, however, amino acid starvation results in the same drastic reduction of the rate of protein synthesis to only a few percent of the value measured in normally growing cells [4,5]. The persistent level of protein synthesis is then sustained by amino acid molecules arising from protein turnover [6].

The reduction of the rate of protein sysnthesis during limitation of amino acid supply is caused, on the one hand, by a significant reduction of the apparent rate of peptide chain elongation. Thus, the no. amino acids polymerized/s by starving cells is 4–5-fold lower than in growing cells, whatever the strain [7–9]. On the other hand, the inhibition of polypeptide assembly is connected with a decrease of the intracellular concentration of ribosomes active in protein synthesis to 30–40% of the normal value [9]. One would therefore expect that the proportion of polysomes in starved bacteria be also reduced to a constant 30–40% level. Some data indicate that polysome level is significantly reduced only in the case of starvation of stringent strains, and then only for certain amino acids, such as arginine or leucine which frequently occur in cellular proteins [10,11]. In contrast, when stringent strains are deprived of other amino acids, such as histidine or tryptophan which are rather rare in proteins, the level of polysomes is only slightly, or even not at all, affected [12,13]. This is also the case for relaxed mutants when deprived of any kind of amino acid [10–14].

Taken together, these observations therefore indicate that quite different levels of polysomes actually sustain the same low rate of residual protein synthesis with a similar assembly time of polypeptides. A possible explanation for such a paradoxical situation would be that only a low level of active polysomes, i.e., a relatively small proportion of ribosomes actively engaged in polypeptide formation, is necessary to assure residual protein synthesis. Consequently, when polysomes are present in a higher amount, a significant fraction of their ribosomes would not be involved in polypeptide formation, even though they still interact with messenger RNA molecules.

This present work was undertaken to check this hypothesis. The experimental approach is based on the fact that active ribosomes carrying peptidyl–tRNA can specifically react with puromycin to give peptidyl-puromycin [15–18]. This reaction was followed in the polysomal structures isolated from a stringent strain of *Escherichia coli* starved for 2 different amino acids. A parallel analysis of an otherwise isogenic relaxed strain was done. The results reported here provide evidence that polysomes whose level remains high during starvation contain a significant proportion of inactive ribosomes.

2. Materials and methods

The otherwise isogenic pair of *E. coli* strains CP 78 (*relA*$^+$) and CP 79 (*relA*) was used in all experiments. Both strains require histidine, threonine, arginine and leucine for growth. Cells were cultured under forced aeration at 37°C in a minimal Tris–glucose medium [14] supplemented with the 4 essential amino acids (50 μg/ml each). Starvation experiments were performed by transferring exponentially growing cells to fresh medium lacking either arginine of histidine.

Protein synthesis in normally growing or amino acid-starved cells was measured by the incorporation

of [^{14}C]proline (0.03 μCi and 11 μg/ml) into hot 5.5% trichloroacetic acid precipitates. Radioactivity was counted in scintillation fluid using a Tri-Carb Packard spectrometer.

The method for preparing crude lysates and isolated polysomes has been detailed [19]. Briefly, cells were harvested by quick chilling and centrifuged in the cold. Lysates were prepared by the lysozyme–EDTA technique and were layered onto 15–40% RNase-free sucrose gradients. After centrifugation for 150 min at 39 000 rev./min in a Beckman SW 41 Ti rotor, gradients were pumped through the continuous-flow cell of a recording spectrophotometer which monitored A_{260}. The proportion of polysomes in the total ribosomal material taken as 100% (polysomes + monosomes + ribosomal subunits) was determined from the absorbance tracing. In the experiments designed to analyze the reaction of polysomes with puromycin, cellular lysates were centrifuged through a 15–40% sucrose gradient supported by a 1 ml cushion of 55% sucrose in order to concentrate polysomes in the bottom part of the tube. Fractions were then collected and only polysomes larger than trimers were kept apart, pooled and used in the peptidyl-puromycin assay.

The reaction mixture for the determination of peptidyl-puromycin formation was similar to that in [16]. It contained in a final volume of 2.7 ml the following components: 40 mM Tris–HCl (pH 7.8), 10 mM $MgCl_2$, 50 mM KCl, 1 mM ATP, 0.1 mM GTP, 1 mM phosphoenol pyruvate, 0.3 mg pyruvate kinase, 6 mM mercaptoethanol, 8.4–11.4 mg protein from S_{225} supernatant fraction, 6–7 A_{260} units of polysomes and 0.6 μCi [^{3}H]puromycin (5.7 Ci/mmol spec. act.; The Radiochemical Centre, Amersham). Incubation was done at 37°C. Aliquots of 200 μl each were withdrawn after various times, treated with 1 ml 10% trichloroacetic acid and kept at 4°C for 60 min. Precipitates were collected by filtration through Whatman GF/C discs, washed with 5% trichloroacetic acid and absolute ethanol to remove unreacted puromycin, then dried and counted for radioactivity in toluene scintillation fluid. All data were corrected for zero-time controls.

3. Results

In a first series of control experiments, the extent of residual protein synthesis during amino acid starvation of bacteria was measured and compared to that in exponentially growing cells. The stringent strain CP 78 and the relaxed strain CP 79 were deprived separately of arginine or histidine, and the amount of radioactive proline incorporated into the trichloroacetic acid-precipitable material was determined, in each case, at various times after the onset of starvation. The results presented in fig.1 indicate that in either type of strain, and whatever the nature of the withheld amino acid, the rate of residual protein synthesis is reduced to the same low value of $\lesssim$3% of the control.

The effects of amino acid starvation on the level of polysomes were analyzed. The corresponding kinetic curves are shown in fig.2. With respect to starvation of the stringent strain, two different patterns are discernible.

(i) In the absence of arginine, a commonly occurring amino acid in cellular proteins [20], the level of polysomes is considerably reduced within the first 10 min then reaches a steady-state absolute value of ~25%. Considering that the proportion of polysomes in unstarved cells is ~60%, this percentage for arginine-starved cells fits well with the relative 30–40% of normal value expected from the measurement of the intracellular concentration of ribosomes active in protein synthesis during starvation [9].

(ii) Quite different results were obtained when the same stringent strain was deprived of histidine, which is less frequent in cellular proteins than arginine [20].

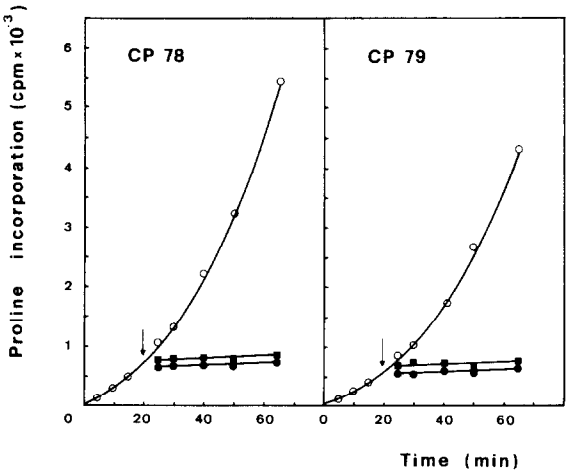

Fig.1. Protein synthesis in amino acid-starved bacteria. Cells of strain CP 78 or strain CP 79 were grown in exponential phase for 20 min in the presence of radioactive proline, then either maintained in a complete culture medium as a control (○), or starved for histidine (■) or arginine (●). Aliquots were withdrawn after the indicated times, subjected to hot trichloroacetic acid precipitation and their radioactivity was counted.

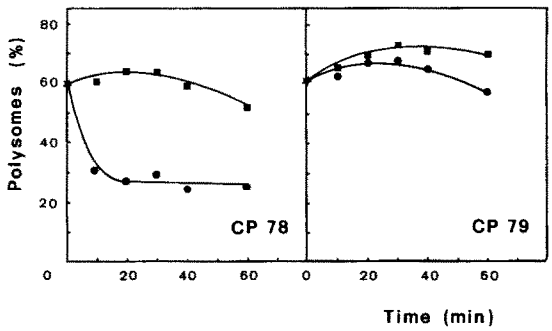

Fig.2. Polysome level in amino acid-starved bacteria. Exponentially growing cells of strain CP 78 or strain CP 79 were starved for either histidine or arginine (zero time). Polysomes were extracted after the indicated times and their proportion in the total ribosomal material was determined. Means from 4–6 expt are given. The average deviation was ± 7% of expressed values. Histidine starvation (■); arginine starvation (●).

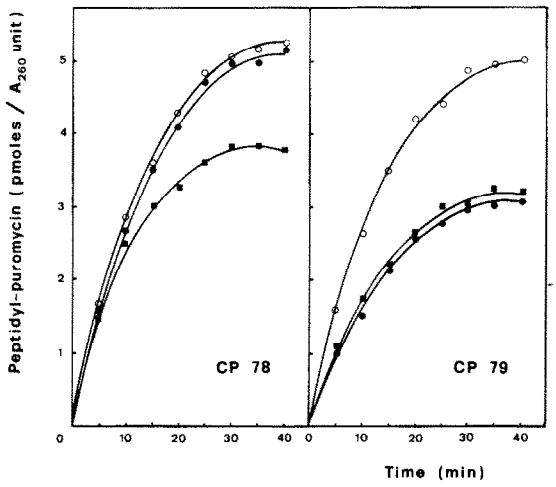

Fig.3. Peptidyl-puromycin synthesis on polysomes from exponentially growing and amino acid-starved bacteria. The amount of radioactive puromycin incorporated into trichloroacetic acid-precipitable material was determined, as a function of time, using polysomes isolated from growing CP 78 or CP 79 cells (○), or cells starved for histidine (■) or arginine (●). Results are expressed as the no. pmol puromycin incorporated/ A_{260} unit of polysome. Means from 3–4 experiments are given. The average deviation was ± 8% of expressed values.

In that case, no significant reduction of polysome level was observed. On the contrary, the proportion of persisting polysomes increased somewhat during the first 30 min. A similar situation was encountered when the relaxed strain was starved for arginine or histidine, in the sense that no reduction of polysome level was detected (fig.2). These results are in agreement with [12,14].

The activity in protein synthesis of ribosomes present on polysomes was then analyzed through their capacity to react with the aminoacyl-tRNA analogue puromycin [17,18]. The formation of peptidyl-puromycin was studied using purified polysomes rather than total cellular lysates in order to minimize interference with various non-polysomal populations: initiation complexes, i.e., ribosomes attached to messenger RNA and formylmethionyl-tRNA, or ribosomes arising from polysome breakdown during extraction, or else ribosomes detached from messenger RNA by physiological run-off. Polysomes were isolated from stringent and relaxed cells either in exponential phase of growth or after 30 min of amino acid starvation. They were incubated in the presence of radioactive puromycin and the av. no. counts/'polysomal' ribosome attributable to peptidyl-puromycin was determined. The results in fig.3 indicate that maximal peptidyl-puromycin synthesis occurred when using either polysomes from stringent or relaxed growing cells, or polysomes from arginine-starved stringent cells. In every case, 5 pmol radioactive puromycin were incorporated into trichloroacetic acid-precipitable material per A_{260} unit of polysome after 40 min incubation. The reaction was less extensive when using polysomes from histidine-starved stringent cells since the amount of radioactive puromycin incorporated per A_{260} unit of polysome after 30–40 min was significantly reduced by ~25%. An even more severe reduction, by 35–40%, was measured in the case of polysomes isolated from relaxed cells starved for arginine as well as for histidine.

By comparing the data from fig.2 and 3, it can be concluded that polysomes which are maintained in a relatively low proportion in arginine-starved stringent bacteria, appear to contain essentially ribosomes active in protein synthesis, just like polysomes of unstarved cells. By contrast, when polysomes are maintained at a high level during starvation, they seem to be composed of a mixed population of both active and inactive ribosomes, the latter being unable to react with puromycin.

4. Discussion

The main result of this study is that the level of polysomes in amino acid-starved bacteria does not

reflect their capacity to synthesize proteins. The possible existence of inactive ribosomes on certain polysomes had been suggested from experiments showing that polysomes of starved cells may be partially dissociated into ribosomal subunits in the presence of Na^+ [21]. Such salt treatment is known indeed to induce the dissociation of inactive ribosomes of the 'run-off' type, but not that of active ribosomes of the 'complexed' type carrying peptidyl-tRNA [22,23]. This interpretation is now favored by the results reported here using a different experimental procedure, i.e., the measurement of peptidyl-puromycin synthesis.

The low level of polysomes persisting in arginine-starved stringent bacteria, and the high level maintained in histidine-starved stringent cells or in relaxed cells starved for any amino acid, sustain the same constant low rate of residual protein synthesis. Therefore, when the polysome population is seen to decline under starvation, this is likely to be due to the preferential release of inactive ribosomes from messenger RNA.

On the other hand, since it has been demonstrated that polysome turnover continues to take place under starvation, in all cases [3,12], it would appear that inactive as well as active ribosomes can be continuously reassembled into polysomes. Whether the 2 classes of ribosomes are present on the same or on different polysomes remains, however, to be determined.

Acknowledgements

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