POLYSOMES IN *ESCHERICHIA COLI* DURING AMINO ACID STARVATION: STRUCTURAL CHANGE OBSERVED BY ELECTRON MICROSCOPY

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1. Introduction

Earlier experiments have shown that ribosomes are released from polysomes when they encounter a codon for which no charged tRNA is available [1, 2]. It was further shown that these ribosomes then reinitiate and resume translation [3, 4]. Inhibition of RNA synthesis by the addition of rifampin [5] resulted in the elimination of polysomes from amino acid starved cells [3]. These findings indicate that during amino acid starvation polysomes are broken down and reconstituted continually. The rate of breakdown of polysomes in starved cells was slower than in growing cells [3, 6] suggesting that when a ribosome reaches a codon specifying a missing amino acid there is a delay before its release from the mRNA.

It is conceivable that the rate with which new ribosomes go on the mRNA at the point of initiation of translation, and the rate of translocation are independent of the rate at which ribosomes are released from mRNA at the codon for which no charged tRNA is available. This possibility might result in a 'piling up' of ribosomes on mRNA during amino acid starvation when release is delayed. Alternatively, the process of release of ribosomes might feed back on either the process of initiation or the process of translocation. This effect bould be achieved by several mechanisms: (a) There might be a feed back mechanism regulating the rate of initiation by the rate of release and/or translocation. (b) The level of one or more factors required for initiation or for translocation might be limiting. If the release or movement of ribosomes are slowed down, due to their inability to translate at the codon for which charged tRNA is missing, the factor(s) might be tied up on ribosomes thus creating a shortage of free factors. (c) There are physical factors which limit the number of ribosomes per unit length of mRNA. If the distance between every two ribosomes moving along the mRNA corresponds to the smallest distance which they can physically occupy, then a slowing down of the release would result in a corresponding slowing down of initiation. If the rate at which new ribosomes go on mRNA is coupled to, or regulated by the rate of release of ribosomes from the same mRNA molecule, then polysomes from amino acid-starved cells should be similar to those from grow-

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ing cells in the number of ribosomes per unit length of mRNA. In such a case, amino acid deprivation is not expected to result in 'piling up' of ribosomes along the mRNA molecules or along certain parts of it.

2. Experimental

The following isogenic strains of *Escherichia coli* K-12 were used: 428 (*pro*$^-$, *his*$^-$, *thi*$^-$, *rel*$^+$) and 78 (*pro*$^-$, *his*$^-$, *thi*$^-$, *rel*$^-$).

For preparation of polysomes cells were grown in minimal medium [7] and treated as previously described [3, 8]. Fractions containing polysomes were collected from sucrose gradients and negatively contrasted with uranyl acetate.

3. Results and discussion

Typical polysomes from growing and from amino acid-starved cells are shown in Plate 1. Measurements of polysome length (summarized in fig.1) indicate

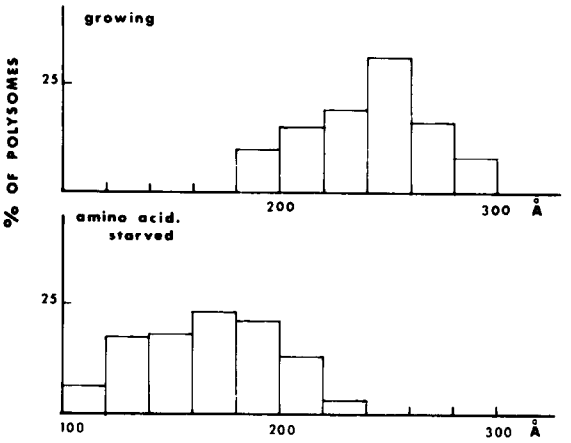

Fig.1. Distance between ribosomes in polysomes of growing and amino acid-starved cells. The distance between ribosomes (in Å) was obtained by projecting the Plates, measuring the length of each polysome and dividing it by the number of ribosomes. The distribution is in % of the polysomes with the given distance between their ribosomes. The total number of polysomes counted (100%) was 68 for the growing and 143 for the amino acid-starved (108 starved for histidine and 34 starved for proline). The distance between ribosomes was independent of the size of the polysome. Only polysomes in which all the ribosomes were in one line were measured.

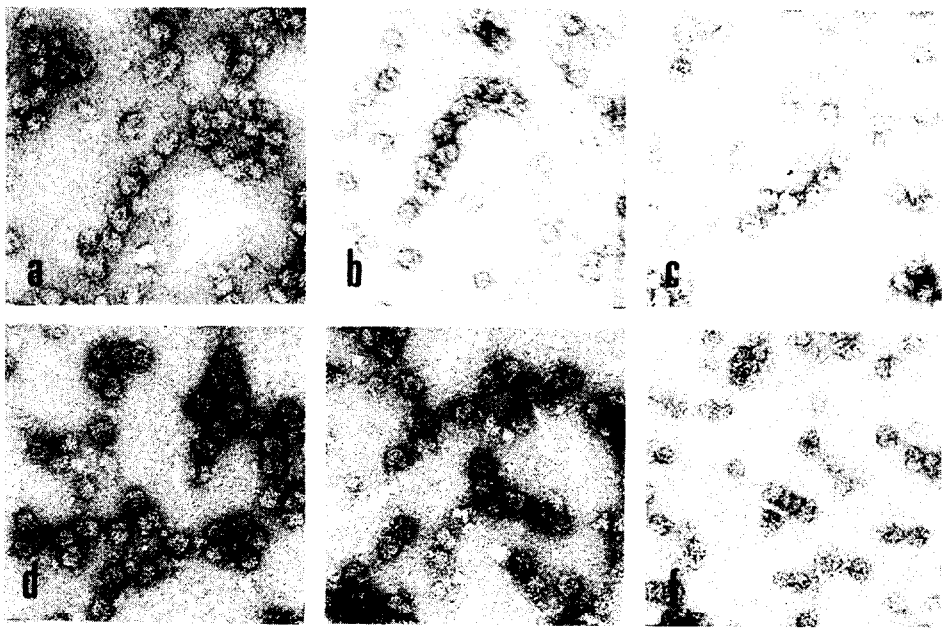

Plate 1. Polysomes from growing and amino acid-starved *E. coli*. Polysomes were obtained from *E. coli* K-12 strain 428 (*pro*$^-$, *his*$^-$, *thi*$^-$, *rel*$^+$) as described in Experimental. (a–c). Growing cells; (d, e). Cells starved for histidine for 30 min; (f) Cells starved for proline. Magnification: × 120 000.

that the number of ribosomes per unit length of polysome is significantly larger in amino acid-deprived cells than in growing cells. It was previously shown [3] that the size and the level of the polysomes extracted during deprivation of a particular amino acid is a function of the relative frequency with which that amino acid appears in cellular proteins. Thus, deprival of a commonly occurring amino acid results in a more extensive loss than deprival of a rare amino acid. However, in Electron Microscopy the number of ribosomes per unit length of mRNA was similar in cells starved for either histidine which is rare in proteins or proline which occurs in proteins more frequently. These findings suggest that the rates of initiation and of translocation are independent of the rate of release of ribosomes from mRNA. Slowing down of the release process results in 'piling up' of ribosomes on mRNA. Furthermore, the results indicate that ribosomes moving along mRNA do not follow each other at the smallest distance which is physically possible.

Relaxed strains [9], in contrast to stringent strains, maintain their polysomes even during starvation for common amino acids [1,2]. The presence of polysomes during starvation for common amino acids in relaxed strains suggested that the polysomes in these strains might be different than in stringent strains. This possibility was supported by the finding [3] that relaxed polysomes are broken down more slowly than polysomes in growing cells and in starved stringent cells after the addition of rifampin. We found that in two relaxed strains polysomes extracted during amino acid starvation are indistinguishable from polysomes extracted from growing cells. This finding further supports the notion that ribosomes (and polysomes) from relaxed strains are different than those from stringent strains. It should be noted that the average distance occupied on the mRNA by ribosomes from relaxed strains, both growing and amino acid-starved, was about 10% smaller than in stringent cells (230 Å as compared with 250 Å). However, the number of polysomes counted was statistically insignificant for proving a difference between strains with different *rel* allells in polysomes from growing cells.

An alternative hypothesis to explain polysome metabolism during amino acid starvation suggests that ribosomes 'glide' over an empty codon [10,11]. This hypothesis was supported by experiments using strains with temperature sensitive valyl-tRNA synthetase in wich deprivation of charged tRNA was achieved by elevating the temperature. In these experiments the rate of polysome breakdown was found to be similar in growing cells and in cells at the higher temperature in both relaxed and stringent strains. The difference in the results obtained from this experiment and our experiments might be due to several factors such as the difference of strains, the different ways by which deprivation of charged tRNA was obtained and the abundance of the amino acid involved in cellular proteins. At any rate the nature of polysomes from starved cells, as viewed by Electron Microscopy supports the notion that during amino acid starvation in stringent strains ribosomes are unable to 'glide' over an empty codon, but are 'piled up' at that site. However, the Elctron Microscope data did not exclude the possibility that in relaxed polysomes such 'gliding' takes place.

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References

[1] Morris, D. W. and DeMoss, J. A. (1966) Proc. Natl. Acad. Sci. USA 56, 262–268.
[2] Ron, E. Z., Kohler, R. E. and Davis, B. D. (1966) Proc. Natl. Acad. Sci. USA 56, 471–475.
[3] Ron, E. Z. (1971) J. Bacteriol. 108, 263–268.
[4] Brunchede, H. and Bremer, H. (1971) J. Mol. Biol. 57, 35–57.
[5] Geiduschek, P. and Haselkorn, R. (1969) Ann. Rev. Biochem. 78, 647–676.
[6] Gurgo, C., Craig, E., Schlessinger, D. and Afolayan, A. (1971) J. Mol. Biol. 62, 525–535.
[7] Davis, B. D. and Mingiolo, E. S. (1950) J. Bacteriol. 60, 17–28.
[8] Ron, E. Z., Kohler, R. E. and Davis, B. D. (1966) Science 153, 1119.
[9] Stent, G. S. and Brenner, S. (1961) Proc. Natl. Acad. Sci. USA 47, 2005–2014.
[10] Cozzone, A. J. and Donini, P. (1973) J. Mol. Biol. 76, 149–162.
[11] Cozzone, A. J. and Stent, G. S. (1973) J. Mol. Biol. 76, 163–179.