POLYSOME LEVEL AND STABILITY IN STRINGENT *E. Coli* STRAINS UNDER AMINOACYL-tRNA DEPRIVATION.

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SUMMARY. - Under aminoacyl-tRNA deprivation polysome level is reduced to a different degree in various stringent *E. Coli* strains. Polysomes which are maintained in such deprived cells undergo continual turnover. The higher polysome level measured in certain strains seems to be due to a higher ability of these strains to assemble polysomes rather than to a lower rate of polysome decay.

INTRODUCTION

Numerous studies have been made of polysome level in relaxed (*rel*$^-$) and stringent (*rel*$^+$) *E. Coli* strains deprived of a required aminoacid. The data obtained in most laboratories (1-7) agree to indicate that the level of polysomes remains unchanged in aminoacid deprived *rel*$^-$ strains as compared to the level of polysomes in normally growing cells. However, conflicting results have been reported on polysome level in aminoacid deprived *rel*$^+$ strains. Indeed, in some experiments (1,2) polysomes were shown to remain intact under deprivation whereas, in other experiments (3-7), polysomes were shown to be rapidly converted into monosomes and ribosomal subunits. In an attempt to explain these discrepancies it has been suggested that the extent of polysome breakdown in aminoacid deprived *rel*$^+$ cells might be dependent both on the bacterial strain used and on the aminoacid withheld (1). Furthermore it has been reported that the nature of the withheld aminoacid determines whether polysomes break down or remain intact during aminoacid deprivation (2,6) and moreover that the level of polysomes in *rel*$^+$ strains deprived of a particular aminoacid is a function of the relative abundance of that aminoacid in cellular proteins (6).

In the present work, the availability of various *E.Coli* strains harboring a temperature sensitive aminoacyl-tRNA synthetase led us to reinvestigate the effect of aminoacid deprivation on polysome level in *rel*$^+$ cells. Deprivation was achieved by shifting cells to the non-permissive temperature thus preventing aminoacylation of a particular species of tRNA. Such a treatment appeared to us more accurate than depriving an auxotroph strain of an aminoacid essential for growth since it has been shown that, in the latter case, a limited supply of aminoacids remains available inside the cell due to protein degradation (8-11).

In addition, polysome stability was measured in *rel*$^+$ strains under aminoacyl-tRNA deprivation. For this purpose, the decay of polysomes was studied under rifampin treatment and the process of their assembly was studied after they had been previously converted to monosomes by starving cells for glucose.

EXPERIMENTAL

Three pairs of otherwise isogenic *rel*$^+$ and *rel*$^-$ strains of *E. Coli* were used, kindly supplied by Dr. G.S. Stent. All strains are derivatives of the same strain D2 and are arginine auxotrophs (12-14). Strains 10B6 *rel*$^+$ and 10B6 *rel*$^-$, strains 9D5 *rel*$^+$ and 9D5 *rel*$^-$, and strains 10B3 *rel*$^+$ and 10B3 *rel*$^-$ are temperature-sensitive respectively for valyl-t-RNA synthetase, alanyl-tRNA synthetase and phenylalanyl-tRNA synthetase. The permissive temperature for all six strains is 30° C, and the non-permissive temperature is 43° C.

Cells were grown at 30° C with forced aeration in a minimal citrate-phosphate medium (15) supplemented with 100 µg/ml arginine and 2 mg/ml glucose. Glucose starvation experiments were performed as previously described (7) under conditions allowing exponential growth to approximately 4.10^8 cells/ml.

The method of preparing crude lysates and isolated polysomes has been reported (16). Cells were harvested by centrifugation and resuspended in a sucrose-buffer solution containing 0.5 M RNase-free sucrose, 0.016 M Tris-HCl buffer, pH 8.1, and 0.05 M KCl. Protoplasts were formed by lysozyme action (1 mg/ml) in presence of 0.2 % EDTA, pH 8.0, and lysates were prepared in a buffer solution containing 0.5 % Brij 58, 0.5 % sodium deoxycholate and 5 µg/ml RNase-free DNase. Lysates

were then layered onto 15 to 40 % sucrose gradients in 0.05 M NH_4Cl, 0.01 M $MgCl_2$ and 0.01 M Tris-buffer solution at pH 7.8. Gradients were centrifuged in a Beckman SW 41 Ti rotor for 150 min at 39,000 revs/min and pumped through the flow cell of a Beckman recording spectrophotometer which monitored the optical density at 260 nm.

The amount of ribosomal material present in polysomes was then determined by measuring the area under the absorbancy tracing and was expressed as a percentage of the total amount of ribosomal material (polysomes + monosomes + ribosomal subunits).

RESULTS AND DISCUSSION

In a control experiment polysome level was measured in exponentially growing relaxed cells and compared to that in aminoacyl-tRNA deprived relaxed cells. It can be seen (Table I) that, in all three strains used, no loss of polysomes occurs after 60 min deprivation even though an evident shift towards smaller polysomes is observed (not shown here).

A similar set of experiments was performed with strains 10B6 *rel*$^+$, 9D5 *rel*$^+$ and 10B3 *rel*$^+$. In each case, cells were grown in exponential phase then shifted to the non-permissive

TABLE I - Polysome level in relaxed cells.

Source of polysomes	Strain		
	10B6 *rel*$^-$	9D5 *rel*$^-$	10B3 *rel*$^-$
Exponential	58.2	57.4	61.3
Deprived	59.6	62.7	63.9

Polysomes were prepared from either exponentially growing cells (exponential) or cells shifted for 60 min to 43° C (deprived). Polysome level is expressed as the percentage of the total amount of extracted ribosomal material. Mean values from 4 experiments are given.

temperature and polysome level was measured as a function of time (Fig. 1). In all three strains aminoacyl-tRNA deprivation results first in a gradual reduction of the polysome level. Then kinetic curves reach a plateau after about 30 min and polysome level remains unchanged for an additional 30 min period at least.

The percentage of polysomes persisting in 10B6 *rel*$^+$ strain under valyl-tRNA deprivation (34 %) is similar to that measured in 9D5 *rel*$^+$ strain under alanyl-tRNA deprivation (32 %) but it is much higher than in 10B3 *rel*$^+$ strain under phenylalanyl-tRNA deprivation (13 %). It thus appears, in agreement with previous studies (1,6), that the level of polysomes in stringent strains during deprivation of an aminoacid depends on the nature of that particular aminoacid. However our results conflict partially with those of Ron (6) since no permanent relationship between the level of polysomes during deprivation and the

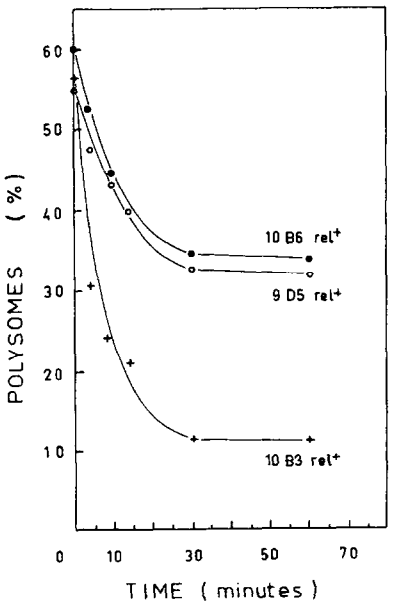

Fig. 1 - Polysome level in deprived stringent cells. Exponentially growing cells were shifted to 43° C (zero time). Samples were collected at various times thereafter, lysates were prepared and the percentage of polysomes was determined as described in Experimental.

frequency of the missing aminoacid in cellular proteins is observed. Indeed, polysome level is in fact identically high in cells deprived of either valine or alanine, two aminoacids equally very frequent in proteins (17). But it is much lower in cells deprived of phenylalanine notwithstanding this amino-acid is much less frequent in proteins than both valine and arginine. Such differing results might be due to the technique used to effect aminoacid deprivation.

In order to check whether polysomes which are maintained in aminoacyl-tRNA deprived cells are stable structures, rifampin, an inhibitor of RNA synthesis, was added to cultures previously shifted to 43° C for 45 min. The percentage of polysomes was then measured as a function of time of drug action. It appears (Fig. 2) that polysome level is maintained or slightly increased after about 1 min of rifampin treatment, then polysomes decay

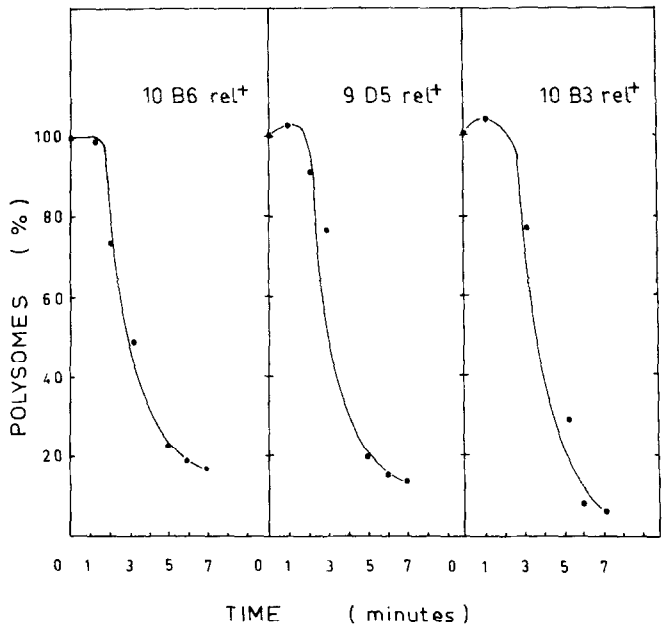

Fig. 2 - Polysome decay in deprived stringent cells in the presence of rifampin. After 45 min deprivation at 43° C rifampin was added (zero time) to a final concentration of 0.25 mg/ml and the percentage of polysomes was determined at the indicated times. For each strain, results are expressed as the percentage of the polysome level present at the time rifampin was added.

rapidly at a similar rate in all three strains since, in each case, about 80 % of polysomes are broken down after 5 min. This finding suggests that the level of polysomes persisting in deprived cells is due to breakdown and reassembly rather than to the stability of preexisting polysomes.

Therefore a study was made of the process of polysome assembly in deprived cells. For this purpose cells were first

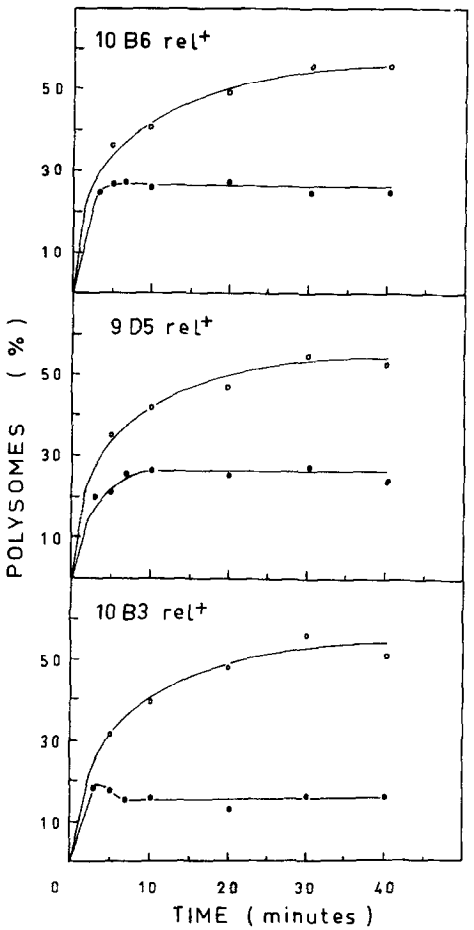

Fig. 3 - Polysome reassembly in stringent cells. After 90 min of glucose starvation, glucose was readded to a final concentration of 0.2 % (zero time) and each culture was divided into two portions. The first portion was maintained at 30° C (open symbols) and the second portion was quickly shifted to 43° C (filled symbols). The percentage of polysomes was determined at both temperatures after the indicated times.

grown at 30° C then subjected to 90 min of glucose starvation as indicated in Experimental section. Under these conditions polysomes were converted to monosomes (7,18). Then such polysome-free cells were shifted to 43° C, glucose was restored to them and polysome reassembly was studied as a function of time. In a control experiment polysome reassembly was also studied at 30° C after glucose readdition. It can be seen (Fig. 3) that, in all three strains, polysome reassembly is essentially complete after about 5 min at 43° C *i.e.* in the absence of protein synthesis. Although the initial rate appears identical in the three strains, the degree of polysome reassembly is quite different as shown by the height of the plateau after 20 min : 28 to 30 % polysomes in 10B6 *rel*$^+$ and 9D5 *rel*$^+$ strains, but only 14 % polysomes in 10B3 *rel*$^+$ strain. These values are similar to those obtained in Fig. 1 after shifting the cells to 43° C for 30 min. As expected, the polysome level attained during reassembly at 30° C is practically the same as in normally growing cells although the plateau is not reached before 20 to 30 min.

The results presented in this report indicate that the polysomes which are observed in aminoacyl-tRNA deprived stringent cells are in a state of turnover. In addition, the higher polysome level measured in certain deprived strains seems to be due to a higher ability of these strains to assemble polysomes rather than to a lower rate of polysome decay. However it is still not clear whether interaction between ribosomes and messenger RNA during the assembly process under aminoacyl-tRNA deprivation occurs specifically at physiological initiation sites or at other sites as well. This problem is currently under investigation.

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