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Escherichia coli ribosomes translate *in vivo* with variable rate

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The question of whether or not 'rare' codons are translated with the same rate as 'common' codons was investigated by measuring the translation time for two genes, *lacI* and *bla*, rich in rare codons, and comparing the results with the translation times measured on *fus*, *tsf*, *tuf* and *rpsA* which have very few rare codons. The rate of synthesis of the *lac* repressor was first measured with the up-promoter mutation *lacI*^{q1} present on the high copy number plasmid pBR322. In such a strain the average translation times for *lacI* and *bla* were 50% slower than the rate calculated from the translation time for the four ribosomal proteins. In a strain having *lacI*^{q1} on an F'*lac* episome this difference was much smaller, thus slow translation of genes rich in rare codons is exaggerated in strains with increased drain on the rare codon tRNAs. The data do not exclude that only a subset of the rare codons is translated more slowly. Translation times were also measured in cells growing in different media, and the translation chain growth rate was found to increase by ~40% going from acetate medium to a fully supplemented medium.

Key words: Escherichia coli/growth rate/rare codons/translation rate

Introduction

The first measurements of the macromolecular content of bacteria (Schaechter *et al.*, 1958) showed the protein content per genome equivalent of DNA to be fairly constant whereas the amount of RNA varied proportionally to the growth rate of the culture. Subsequent measurements of parameters related to bacterial growth, reviewed by Maaløe and Kjeldgaard (1966) and by Maaløe (1979), led to the conclusion that in *Escherichia coli* the difference in protein synthesis rates at different growth rates is caused by a difference in the number of ribosomes and not by variation in the translation rate of the ribosome. This conclusion was based on the average rate of translation or on the translation time of very few individual genes, e.g., *lacZ*, and the possibility of gene-specific translation times was not and could not be taken into consideration.

When the first structural genes were sequenced it was noted that different codons for a given amino acid were not used with the same frequency (Fiers *et al.*, 1976). Genes for proteins which are abundant in the cell, e.g., the ribosomal proteins, use almost exclusively a narrow set of codons (Post *et al.*, 1979) whereas genes for less abundant proteins, such as the *lac* repressor gene, have a broader spectrum of codons (Farabaugh, 1978). Of the codons rarely used in the abundant protein genes ('rare codons') some have low concentrations of the cognate tRNA in the cell (Ikemura, 1981), some have very high or very low affinity to the anticodon (Grosjean and Fiers, 1982) and some are not used, for no apparent reason. It was proposed by Grosjean and Fiers (1982) and by Gouy and Gautier (1982), that rare codons might be translated more slowly, but no direct evidence for a consequence of the presence of rare codons in a given mRNA has been demonstrated. The experiments reported here measure translation times of individual genes, rich or poor in rare codons, in cells growing in different media, and show that the steptime of the ribosome is not always constant.

Results

I have chosen to consider the following 22 codons as 'rare': TTA, TTG, CTT, CTC, *CTA*, *ATA*, GTC, TCA, TCG, CCT, *CCC*, ACA, ACG, GCC, AAT, CGA, *CGG*, AGT, *AGA*, *AGG*, *GGA* and *GGG*. These codons are used in ribosomal protein genes with a frequency of <10% of the number of codons for the same amino acid (Grosjean and Fiers, 1982). The codons listed above in italics have rare tRNA associated with them (Ikemura, 1981) and are called 'rare tRNA' codons. In the *tsf*, *tuf*, *rpsA* and *fus* genes the rare codons occur with a frequency of 5% whereas they amount to 23% and 28% of the codons in *lacI* and *bla*.

The method of measuring translation times for individual genes is a further development of that used by Bremer and Yuan (1968) with two-dimensional gels for separation of the cell extracts. A culture is pulsed with radioactive methionine and then chased with a large excess of the unlabeled amino acid. The length of the pulse must be short compared with the synthesis time of the protein. At intervals after the chase a number of samples is harvested. After separation of the total cell extracts, the kinetics of appearance of radioactivity in the finished polypeptide are followed. More and more label will end up in the finished protein until the first methionine in the molecule is chased into the completed protein and incorporation will then stop. The length of the protein is known, and this permits a direct calculation of the translation time of the individual protein. At 37°C the translation time for the average protein will be ~20 s and the experiments were therefore carried out at ~25°C. It is important that no protein synthesis is allowed to take place during the harvesting procedure. This condition is satisfied, as can be seen from Figure 1, where the synthesis curves for the individual proteins extrapolate back to no incorporation at zero time.

To test the reproducibility of the result the experiment had to be carried out several times. In addition, the experiment was repeated in acetate, in glucose and in rich medium supporting slow, medium and fast growth, respectively.

Figure 1 shows the results for four of the six proteins. In both slow and fast growing cultures the translation time of the *lacI* and the *bla* mRNA is 30–50% slower than expected from the translation time of the ribosomal proteins measured in the same experiment. What is also evident is that the chain growth rate is different for both classes of proteins in the three media. The results are summarized in Table I.

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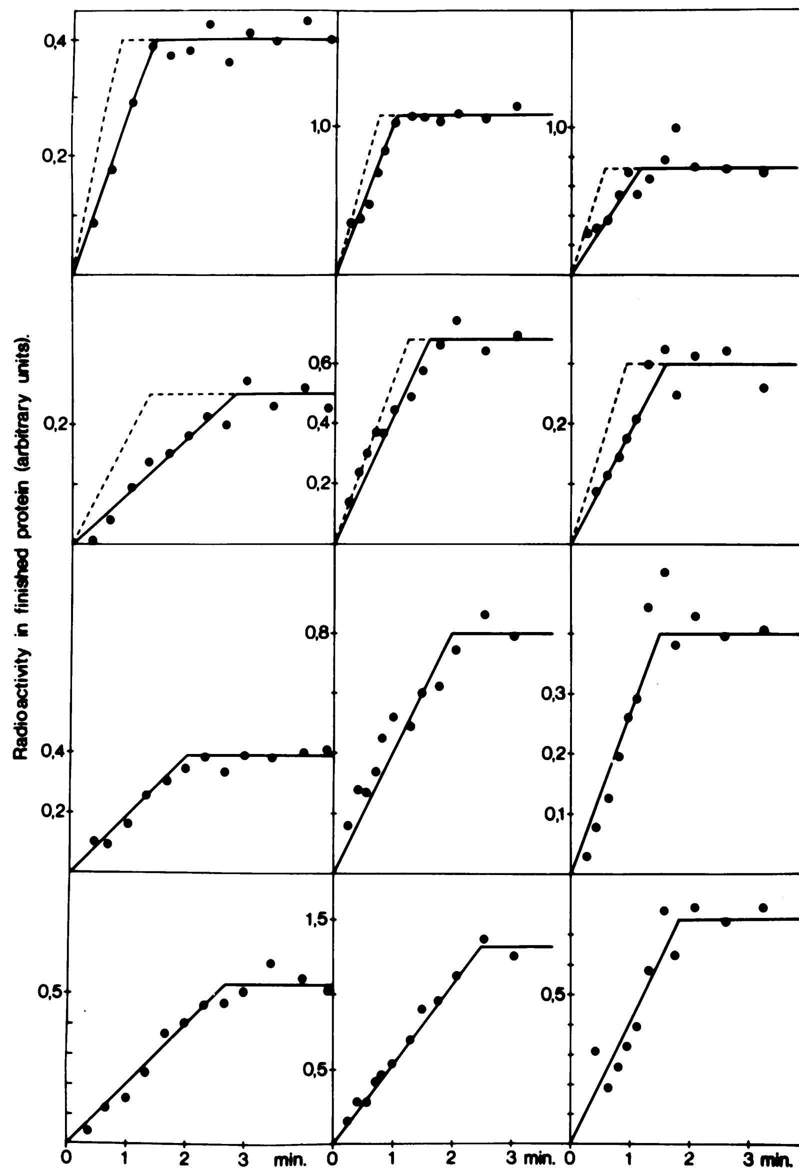

Fig. 1. Synthesis of the *bla, lacI, rpsA* and *fus* gene products (from top to bottom, respectively) in a pulse-chase experiment. **Left column**, acetate medium; **center column**, glucose medium; **right column**, rich medium. The dotted lines in the *bla* and *lacI* panels indicate the calculated curves for these genes if synthesized with the average of the chain growth rate measured on the *tuf, rpsA* and *fus* genes in the same experiment. Strain NF929/pR2172 was used.

Assuming that the codon usage for 98% of all genes is the same as for the ribosomal genes and assuming that the remaining 2% have the codon usage typical of the repressor genes, it can be calculated that a cell making 5% additional proteins from genes with the 'repressor type' codon usage, will experience a 40% increase in the drain on the rare tRNAs. In constructing the strain to measure the translation time for the *lacI* and *bla* mRNA one might therefore have created a situation where the effect of rare codons is more severe than in a wild-type strain. The experiment was therefore carried out with a strain with the *lacI*^{q1} present on an F′*lac* episome. Figure 2 shows that the translation time for the *lacI* gene seems to be very close to the time expected from the *fus* gene translation time.

To see whether the presence of the *lacI* and *bla* genes on pBR322 had other measurable effects on the cell, the growth rate of the cultures was determined. The ratio between the ampicillin-sensitive and ampicillin-resistant bacteria was followed in mixtures of NF929 and NF929/pBR322 or NF929/pR2172 growing for many generations. In the experiment with NF929 plus NF929/pBR322 the ratio changed from 1:1 to 3.6:1 in 19 h. With NF929 plus NF929/pR2172 the ratio changed from 1:1 to 9:1 in 22 h. The values are corrected for loss of plasmid, which was high for pR2172. NF929 has a growth rate of 60 min in glucose medium, and from the above ratios the generation time of NF929/pBR322 and NF929/pR2172 could be calculated to be 62.5 min and 66 min, respectively. The same results were obtained with the corresponding MC1000 derivatives.

Discussion

The rate of translation of codons in the *lacI* and *bla* genes was found to be ~50% slower than that of the codons in ribosomal protein genes, when the *lacI* and *bla* genes were present on a high copy-number plasmid (Figure 1). This slow translation rate correlates with the high content of rare codons in these two genes and if, indeed, the codon usage was the cause

Table I. Steptime in different media at 25°C

Gene	Acetate s/amino acid	Glucose s/amino acid	Rich s/amino acid
lacI	0.47	0.28	0.27
bla	0.39	0.29	0.31
Average	0.43 (7.0)	0.29 (10.3)	0.29 (10.3)
tuf	0.30	0.22	0.20
rpsA	0.23	0.21	0.16
fus	0.25	0.23	0.17
Average	0.26 (11.5)	0.22 (13.6)	0.18 (17.0)

The calculated chain growth rates at 37°C (amino acids per s) are shown in parentheses. The temperature dependence of the chain growth rate is assumed to be a factor of 3, i.e., the temperature-dependent difference of the growth rate of the culture.

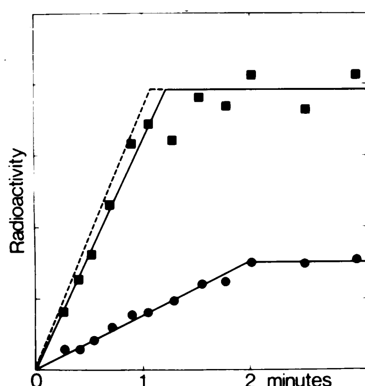

Fig. 2. Synthesis of the *lacI* (■) and the *fus* gene (●) products in a pulse-chase experiment. The curves represent the average of two independent experiments with NF1830 grown in glucose medium. The dotted line is the calculated curve if the *lacI* gene was translated with the chain growth rate of the *fus* gene.

of the slow translation, each rare codon would be translated with a rate of 0.6 s/amino acid compared with 0.2 s/amino acid for the common codons in this experiment. If the effect was caused by the 'rare tRNA codon' subset of the rare codons, each of such codons would take ~2 s to translate. Other mechanisms, such as slow translation of a particularly difficult secondary structure of the mRNA, could also explain this result, but the experiment in Figure 2 shows this not to be the case. When the experiment is performed in a strain where the content of proteins with rare codons is approximately the same as in a wild-type cell, the *lacI* gene is translated at about the normal rate. The slow translation of the *lacI* and *bla* genes in Figure 1 seems therefore to be an artificial result, caused by an increased drain on the rare tRNAs, although the scattering of the points was larger than in Figure 1 due to the very low amount of *lac* repressor present in this strain.

In other situations, limitation of the tRNA supply to the ribosome increases the rate of synthesis of ppGpp and induces the ribosome to mistranslate (Cashel and Gallant, 1969; Parker and Friesen, 1980). The synthesis of ppGpp was not measured, but no mistranslations, resulting in multiple, stuttering spots were observed. In contrast to amino acid starvation, the plasmid carrying the genes rich in rare codons would be expected to decrease the availability of both charged and uncharged tRNA, i.e., not to interfere with the charging level of the tRNA. The level of ppGpp might, therefore, not rise at all. It is conceivable that absence of mistranslations may be caused by rare tRNA codons being misread by wobbling in the third position, which means by the corresponding common codon tRNA that inserts the correct amino acid in the protein. The cells containing pBR322 and pR2172 were found to have reduced the growth rate to a degree that correlated with the expression of the rare codon proteins. Slow translation does not necessarily reduce the yield of protein from an mRNA. A reduced synthesis would only be expected if the ribosome binding site was masked because the rare codons were located early. The reduction in the growth rate of the culture is most likely caused by another mechanism, i.e., the expense of energy used for proofreading the rare codons with the 'correct common codon' tRNA. Results from Kurland's laboratory (Ruusala *et al.*, 1984) show that the energy of proofreading can constitute a large fraction of the energy used for translation and the additional proofreading could therefore lead to a reduction in the growth rate of the cell. Implicit in the above speculations is the assumption that the cell is unable to regulate its content of rare tRNAs to suit the demand.

The data in Figure 2 show that *lacI* and *fus* are translated at about the same rate in the haploid cell. However, the data do not exclude translation of the rare tRNA codons with one third of the rate of that of the common codons, and the rare tRNA codons might, therefore, still have any of the functions discussed previously (Pedersen, 1984). If so, drain of the rare codon tRNAs might create problems when high copy number plasmids are used to obtain high expression of proteins with an unusual codon usage. Randall *et al.* (1980) also found evidence for translational pauses in the synthesis of the periplasmic maltose binding protein, but the position of these pauses was not correlated with the presence of rare codons.

The chain growth rate for translation varies with the growth rate of the culture also for proteins using the common codons (Figure 1), which argues against the earlier conviction of our laboratory. Examining more closely the previous data, e.g., those of Jacobsen (1974) cited by Maaløe (1979), I found background problems to have influenced the conclusion. The data in the square-root plot (Schleif *et al.*, 1973) clearly show a 35% increase in the chain growth rate when the chemostat culture is shifted to the normal glucose medium. Engbæk *et al.* (1973) showed that the translation time for β-galactosidase in both rich medium and in acetate medium is longer than the translation time in glucose, and they concluded that chain growth rate was unaffected by the medium; but in the case of the rich medium their conclusion is based on a limited set of data. The results in Table I are very similar to those found indirectly for overall translation by Forchhammer and Lindahl (1971) and for β-galactosidase induction by Dalbow and Young (1975). We may now conclude that the chain growth rate for translation varies continuously with the growth rate of the bacteria, increasing by ~40% when the bacterial growth rate changes from acetate to rich medium, as predicted by Ehrenberg and Kurland (1984).

The variation of the chain growth rate and the possibility that pools of at least some of the tRNAs can be drained quite easily suggest that the ribosome *in vivo* is not always working at saturating substrate concentrations.

Materials and methods

Strains and plasmids

Escherichia coli NF929: *thr leu argH pyrE thi relA*$^+$ *relC*$^+$ *spoT*$^+$ (Fiil *et al.*, 1977). The maxicell CSR603: *thr1 leuB6 proA2 argE3 phr1 recA1 uvrA6 thi1 ara14 lacY1 galK2 xyl5 mtl1 rpsL31 tsx33 supE44* (Sancar *et al.*, 1979). MC1000: F$^-$ *ara-leu*Δ7679 *araD*139 *lac*ΔX74 *galE galK thi rpsL* (Casadaban and Cohen, 1980). NF1815: MC1000 *recA*1. NF1830: NF1815/F'*lacI*^{q1}

lacZ::Tn*5*.

pBR322 (Bolivar *et al.*, 1977); pR2172: pBR322 with a partial *Hind*II fragment containing the *lacI* gene with the *lacI*^{q1} up-promoter mutation was obtained from J.Hays, University of Maryland, Baltimore, USA.

Growth and labeling of cells

Steptime experiments were carried out at 24.6±0.2°C in the A + B medium (Clark and Maaløe, 1967) supplemented with 0.5 μg/ml thiamine and 50 μg/ml of the required amino acids and with 0.4% acetate, 0.2% glucose, or with 0.4% glucose plus an amino acid mixture with all amino acids except methionine (Neidhardt *et al.*, 1977). The growth rates in the three media were 0.16, 0.34 and 0.48 doublings per hour, respectively, a reduction by a factor of about three from the growth rate at 37°C. All cultures had been growing exponentially for more than four generations at the low temperature before the experiment started.

Maxicell experiments were performed as described by Christiansen and Pedersen (1981).

For accurate determination of the relative growth rate of NF929, NF929/pBR322, NF929/pR2172 and the corresponding set of NF1830 strains, cultures which had been growing exponentially overnight in glucose minimal medium were spun down. The plasmid-containing strains had been growing in the presence of 100 μg/ml ampicillin and all pellets were washed twice with antibiotic-free medium, and resuspended in such a medium. After growth at 37°C for 2 h, mixtures of the strain with and without a plasmid were prepared with about twice as many plasmid-containing cells. Control cultures were prepared with only the plasmid-containing strain. The ratio between ampicillin-resistant bacteria and the total number of bacteria was then followed by plating for many generations during which all cultures were kept in the exponential growth phase by dilution.

At time zero 13 ml culture at OD_{436} 0.6−0.8 received 300 μCi carrier-free [^{35}S]methionine with a specific activity of 1000−1200 Ci/mmol. 6−8 s later non-radioactive methionine was added to 200 μg/ml. This chase prevented almost immediately further incorporation as shown previously for the glucose medium (Pedersen, 1984) and this was also the case in the acetate and the rich medium (data not shown). At frequent intervals during the next few minutes 12 1-ml samples were harvested into ice-cold tubes with 300 μg chloramphenicol dissolved in 30 μl ethanol. 10 μl were precipitated with 5% trichloroacetic acid, and the incorporated radioactivity determined. The remaining sample was centrifuged for 2 min in the cold and the pellet frozen at −80°C. The complete harvesting procedure lasted <6 min for all 12 samples.

The reference cells were labeled at 37°C with [^{3}H]leucine (Figure 1) or with [^{3}H]lysine (Figure 2) (20 μCi/ml at OD_{436} 0.1) and harvested three doubling times later.

2D-gel analysis

The samples were thawed on ice and reference cells were added to each sample to give a ^{3}H/^{35}S ratio of ~5. The cells were then opened by sonication and treated with 2 μg of RNase A and DNase I, and electrophoresis was carried out as described (O'Farrell, 1975) with a pH 3−10 ampholine mixture in the first and a 10% SDS-polyacrylamide gel in the second dimension.

After electrophoresis the gels were dried and the spots located by autoradiography. A number of spots were cut out, digested with H_2O_2 as described (Pedersen *et al.*, 1976) and the radioactivity determined. The data are given as the ^{35}S/^{3}H ratio in a spot divided by the ^{35}S/^{3}H ratio in the total cell extract loaded on the gel. The resultant curve gives the accumulation of radioactivity in arbitrary units for each protein (Reeh *et al.*, 1976).

The positions of EF-Ts, EF-Tu, EF-G and ribosomal protein S1 were previously determined (Pedersen *et al.*, 1978). The plasmids pBR322 and pR2172 were transformed into the maxicell and the approximate positions of the *lac* repressor and β-lactamase were determined by comparing the position of radioactive spots from a maxicell experiment with the stainable proteins from the cell extract. These positions were then verified by labeling NF929, NF929/ pBR322 and NF929/pR2172 during exponential growth and comparing the three autoradiograms. Both proteins have mol. wts. and isoelectric points as predicted from the sequence, mol. wt. 40 000, pH 8, and mol. wt. 25 000, pH 6.5 for the *lacI* and *bla* gene products, respectively. In these strains both proteins are abundant, each constituting ~3% of total protein. In the experiment shown in Figure 2 the *lac* repressor is only ~0.05% of total protein and the location of the *lacI* gene spot had to be ascertained by running a *lac* deletion strain in parallel to the experiment and comparing the autoradiograms.

The DNA sequences of the six proteins have been determined (An *et al.*, 1981; An and Friesen, 1980; Farabaugh, 1978; Schnier *et al.*, 1982; Sutcliffe, 1978; Yokota *et al.*, 1980; Zengel *et al.*, 1984). The N-terminal methionine is removed in β-lactamase, EF-Tu and EF-G (Sutcliffe, 1978; Arai *et al.*, 1980; Ovchinnikov *et al.*, 1982), and therefore the number of amino acids from the first methionine in the molecule to the end is as follows: 220 for β-lactamase, 282 for EF-Ts, 302 for EF-Tu, 360 for the *lac* repressor, 556 for ribosomal protein S1 and 654 for EF-G.

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Note added in proof

Translation times for the *lacI* and *fus* genes have now been measured in NF929/F′*lacI*^{q1}. The results are identical with those for NF1830 shown in Figure 2.