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Review Letter

POLYPEPTIDE ELONGATION AND tRNA CYCLING IN *ESCHERICHIA COLI*: A DYNAMIC APPROACH

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1. Introduction

Understanding the functioning of the genetic code in the prokaryote system is progressing rapidly. We present here a synthesis of the dynamic aspects of *Escherichia coli* protein biosynthesis, based on quantitative data to the extent possible. Previous work along this line was undertaken when few experimental data were available [1–3]. More recent authors have focused on incorporation errors [4–8] or the possible influence of mRNA secondary structure on ribosome movement [9,10]. Although quantities of most of the active molecules have now been estimated, measurement of the duration of many steps in this complex process is difficult. This is one justification for our theoretical approach. We have done Brownian motion computer simulations to determine the duration of the ribosomal A-site searches by tRNAs. Our main result is a dynamic interpretation of the observed relative quantities of ribosomes and the molecules which 'feed' them. We find that these quantities are optimized to maintain maximum polypeptide elongation rate at various cell growth rates. We also calculate the mean duration of several steps in the tRNA cycle.

2. Data supporting this analysis

In vivo quantities of molecules active in *E. coli* protein synthesis are shown in table 1 for a wide range of cell growth rates. Starting at 1.5 doublings/h, the polypeptide elongation rate becomes surprisingly constant although the concentration of all active molecules increases greatly. Total protein synthesis in the cell is enhanced by increasing the number of ribosomes while keeping a nearly constant ratio of tRNA, aminoacyl(aa)-tRNA ligases (synthetases) and elongation factors (EFTu, EFG, EFTs) to ribosomes. Several authors have emphasized this finding [11–14]. Our aim is to give it a dynamic interpretation.

It appears established that distributions for isoacceptor tRNAs and the 61 codons vary greatly from one system to another but are correlated [15–17]. Many workers have calculated the mean number of aa-tRNA discriminations that occur during an elongation cycle [3,6,18], as predicted by theoretical tRNA and codon distributions. Nevertheless, the distribution of the 39 tRNA species in *E. coli* is not known with precision; the codon distribution is better known since about 4000 codons have been sequenced. Hence we use here the mean number of aa-tRNA discriminations predicted by a uniform distribution of all tRNA species and of the codons grouped according to each isoacceptor tRNA. This value is 39, that is the same as the total number of tRNA species. Consequently, on the average a tRNA has previously interacted with 38 non-complementary codons at A-sites when it meets a cognate one.

3. tRNA cycling

We study here (fig.1) the mean duration of tRNA cycle steps for a medium value of cell growth rate: 2 doublings/h. Table 1 shows that a whole tRNA cycle takes 0.69 s. Let us follow a tRNA after its release from a ribosomal P-site. The first step is acylation. Analysis of *E. coli* tRNA resistance to periodate oxidation reveals, after weighting for amino acid frequencies in the *E. coli* average protein [15], that 83% of total tRNA is acylated [19]. Therefore acylation consumes 17% of a tRNA cycle (0.12 s) and 18 000

Table 1
Quantitative data for *Escherichia coli* protein synthesis

Cell growth rate[a] (dbl/h)	1	1.5	2	2.4	2.9	[Ref.]
Cell volume[b] (10^9 nm^3)	1.1	1.4	1.7	2.1	2.5	[25,26]
Genomes/cell	1.6	2.2	2.8	3.4	4.8	[11]
70 S/genome[c]	4500	7100	9400	11 200	13 700	[11–13]
tRNA/genome[d]	63 000	84 000	106 000	120 000	135 000	[11,12]
Synthetases/genome[e]	9400	12 000	13 700	15 100	16 000	[27]
aa Residues/genome (10^8)	3.4	4.0	4.0	4.0	4.0	[11]
tRNA/70 S	13.8	11.9	11.3	10.7	9.9	
Synthetases/70 S	2.1	1.7	1.5	1.3	1.2	
EFTu/70 S[f]	15.8	12.6	10.4	?	?	[28]
70 S-to-70 S distance[g] (nm)	54	45	40	38	34	
Elongation rate[h] (aa × 70 S^{-1} × s^{-1})	14.5	16.3	16.4	16.5	16.3	[11]
tRNA cycle[i] (s)	0.96	0.73	0.69	0.65	0.60	

Calculated quantities are: [a] growth rate at 37°C; [b] from cell diameter [25] and length [26]; [c] from the total quantity of RNA [11] and the ratio rRNA/RNA [12] assuming 77% of total rRNA exists in active (polypeptide elongating) ribosomes [13] and 1.8 × 10^6 as mol. wt of 79 S rRNA; [d] since only 2–4% of RNA is mRNA [11] and tRNA mol. wt is 25 000 we assume tRNA/RNA = 1 – rRNA/RNA; [e] we double the quantity obtained for 10 of these enzymes as recommended in [27]; [f] we adjust published values [28] since only 77% of rRNA is in active ribosomes; EFG, EFTs and 70 S are in equimolar quantities at all growth rates [29]; [g] the side of a cube containing one ribosome assuming uniform distribution in the cell of all ribosomes; [h] the average number of residues incorporated into polypeptides × s^{-1} × ribosome^{-1}: (cell growth rate) × log 2 × (residues/genome) × (70 S/genome)$^{-1}$ × (3600 s/h)$^{-1}$ as in [11]; [i] the mean time for a tRNA to complete a cycle: (tRNA/70 S) × (elongation rate)$^{-1}$

deacylated tRNAs along with 13 700 synthetases exist per genome (table 1). Considering the difficulty in recovering and quantifying 20 enzymes, this is roughly a stoichiometric ratio with a small excess of the substrate molecule, tRNA.

The next step is aa-tRNA binding to EFTu-GTP. There are 98 000 EFTu molecules per genome (table 1) and 79 000 tRNAs available for this reaction (83% of total minus those trapped in P-sites). The equilibrium constant (7 × 10^6 M^{-1}, [20]) and the two above quantities imply that practically all aa-tRNA is in a ternary complex: aa-tRNA · EFTu · GTP. We assume this binding is rapid.

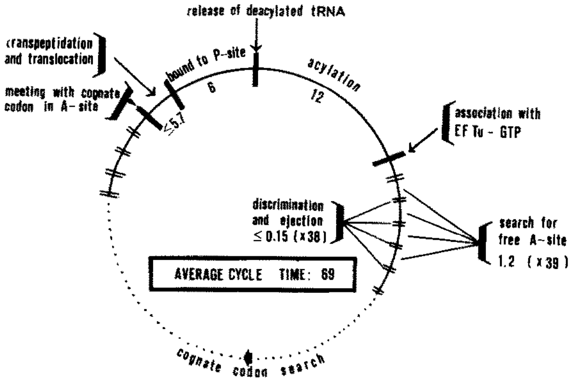

Fig.1. The tRNA cycle. Cell growth rate is 2 doublings/h; all values are averages expressed in 10^{-2} s.

Then the tRNA searches for a cognate codon at an A-site not yet occupied by a tRNA. As shown above, on the average the tRNA first encounters 38 such A-sites containing non-cognate codons and is discriminated each time. After cognate codon pairing, transpeptidation and translocation occur. We delimit the time of these steps in section 5. Finally, the tRNA remains at site P during a whole elongation cycle (6.1 × 10^{-2} s, table 1).

4. The A-site search

Assuming Brownian motion for tRNA, we are concerned here with determining the duration of an A-site search by a ternary complex in cells growing at 2 doublings/h. The diffusion constant of Dextran (15 000 mol. wt) in a bacterial cell extract at 37°C is 2.5 × 10^{-7} cm^2/s [1]. We adjust this value to the ternary complex (71 000 mol. wt) assuming proportionality to (mol. wt)$^{-1/3}$, thus obtaining 1.5 × 10^{-7} cm^2/s. Only membrane proteins, a minor fraction in *E. coli*, are synthesized on membrane-bound polysomes [21,22]. Moreover, active ribosomes are so numerous that considering them uniformly distributed in the whole cell leads to an average distance between neighbors of 40 nm (table 1), which is only slightly larger than the ribosome itself (25 × 30 × 24 nm, [23]). Hence this distribution is necessarily a good approximation of the overall polysome structure.

Codon–anticodon interaction occurs at site A; we represented the interaction zone by cubes of 5 nm on a side (close to the ternary complex size) with one side only permitting tRNA passage. Since it is impossible to compute analytically the time of first entering of a Brownian particle in one of these targets, we made 350 computer simulations. They show that the time to first collision has an exponential distribution with a mean of 7.6×10^{-4} s. This value is our estimate of the average duration of the Brownian path for the ternary complex up to initial collision with an A-site, whatever the codon present there. We consider below the probability of this site being already occupied by another tRNA.

5. Elongation cycle

We discuss here the consequences on the elongation cycle of our value for A-site search time. Let N be the tRNA/ribosome ratio (11.3, see table 1) and P the average fraction of ribosomes with tRNA at site A (hence involved in codon–anticodon matching or in transpeptidation or translocation). The analysis of section 3 shows that, on the average, there are $0.83 \times N - 1 - P$ ternary complexes not already engaged at site A per ribosome. These molecules are necessarily searching for free A-sites, that is ribosomes whose A-sites are not occupied by a tRNA. We have seen that, on the average, 39 such searches occur during each tRNA cycle. Hence, if C is the elongation cycle time (6.1×10^{-2} s), then $(0.83 \times N - 1 - P) \times C/39$ is the mean duration of a search.

On the other hand, our simulations reveal that the time (t) to collision with an A-site is 7.6×10^{-4} s if the possibility of the site already being occupied is neglected. This time is actually $t/(1 - P)$ s because $1 - P$ is the fraction of ribosomes with free A-sites, that is the probability that a given ribosome has a free A-site. Thus the equation:

$$(0.83 \times N - 1 - P) \times C/39 = t/(1 - P)$$

furnishes the relation between ribosomes with free and occupied A-sites. Introducing N, C and t values yields $P = 0.935$. This value reveals that the presence of $N = 11.3$ tRNAs obliges each ribosome to await tRNA arrivals only 6.5% (or $1 - P$) of the time. The remaining time (5.7×10^{-2} s $= P \times C$) is given to testing tRNA or to transpeptidation and translocation.

Returning to tRNA cycling (fig.1), we see that each of the 39 A-site searches takes 1.2×10^{-2} s ($t/(1 - P)$). Transpeptidation and translocation can take no more than 5.7×10^{-2} s ($P \times C$). The upper limit, given rapid transpeptidation and translocation, for discrimination and ejection of an aa-tRNA with a non-cognate anticodon is therefore 0.15×10^{-2} s.

6. Dependence of elongation rate on tRNA concentration

During the elongation cycle, only the delays in tRNA arrival at the ribosome depend on the concentration of the ternary complex; the other steps (A-site interactions, transpeptidation and translocation) do not depend on this concentration. Hence knowledge of the overall duration of these other steps ($P \times C = 5.7 \times 10^{-2}$ s) allows us to compute the possible performance of the translating machinery with the same number of ribosomes as in a cell growing at 2 doublings/h and various quantities of acylated tRNAs (fig.2). The position on this curve of the physiological

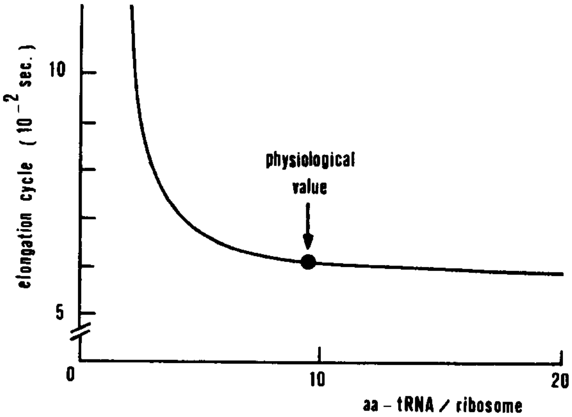

Fig.2. Elongation cycle dependence on aa-tRNA concentration. Length of the elongation cycle (C) is plotted versus the aa-tRNA:ribosome ratio (A). With P as the fraction of ribosomes having tRNA at site A: $(A-1-P) \times C/39 = t/(1 - P)$, as shown in section 5. The duration of all steps in this cycle other than the waitings for ternary complex arrival at the A-site does not depend on tRNA concentration, hence the product $F = P \times C$ is constant whatever the value of A: $F = 5.7 \times 10^{-2}$ s as shown in section 6. These two equations define C as a function of A:

$$C = 2F/\{A + 39t/F - [(A + 39t/F)^2 - 4(A - 1)]^{1/2}\}$$

value shows that the ribosomes are working very close to their maximum speed: more acylated tRNAs, together with more synthetases and elongation factors, would not significantly increase the elongation rate.

7. Conclusion and discussion

Because of the constancy of elongation rate and amount of tRNAs, synthetases and elongation factors per ribosome at various cell growth rates, it has been suggested that the overall protein synthesis rate is determined exclusively by the total number of active ribosomes [13]. Our analysis supports the following interpretation of this suggestion:

> In fast growing cells, the quantities of all 'ribosome feeding' molecules are just large enough to maintain the observed maximum incorporation rate of amino acids. Numbers of both EFTu and synthetases are indeed in nearly stoichiometric relationship to the tRNAs available for interaction with them; the resulting quantity of ternary complexes (aa-tRNA · EFTu · GTP) is such that the waiting time for tRNA arrivals is a small fraction of the elongation cycle duration.

No accurate estimate of the duration of tRNA discrimination relative to transpeptidation and translocation can be derived from available data. Experiments measuring the influence of isoacceptor tRNA distribution on elongation rate would help resolve this important problem.

Our analysis depends on the tRNA and codon distributions because they define the average number of non-cognate discriminations during an elongation cycle. The waiting time for ternary complex arrival is indeed determined by the total tRNA concentration, but is not influenced by tRNA species distribution. Hence our result is not critically dependent on the simplifying assumptions made on these distributions.

This work was also motivated by a desire to see if the overall process of protein synthesis could be 'rationalized'. No contradictions have appeared, but some uncertainties remain:

(i) We assumed that tRNA discrimination occurs with a probability of 1 when a ternary complex collides with an A-site provided the site is not already occupied by another tRNA.

(ii) We did not estimate the fraction of tRNA tied up in complementary anticodon–anticodon couples [24].

(iii) The time consumed in non-specific interactions between molecules, particularly between tRNA and synthetases, remains uncertain.

Of course, a better estimate of the diffusion constant for the ternary complex in the cytoplasm (not in distilled water!) would permit refining this synthesis.

A comprehensive review of prokaryotic protein synthesis has appeared since submission of this manuscript [30].

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Note added in proof

Our nucleic acid sequence bank now includes 17 bacterial genes (4240 codons) [31].

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