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Synonymous Codon Selection Controls In Vivo Turnover and Amount of mRNA in *Escherichia coli bla* and *ompA Genes*

ATILIO DEANA,[1]* RICARDO EHRLICH,[1] AND CLAUDE REISS[2]

Laboratoire Structure et Dynamique du Génome, Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91198 Gif sur Yvette, France,[2] and Departamento de Bioquímica, Facultad de Ciencias, 11200 Montevideo, Uruguay[1]

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A number of silent codon changes were made in two *Escherichia coli* genes. For the *ompA* gene, the replacement of seven consecutive frequently used codons with synonymous infrequently used codons reduced the *ompA* mRNA level and its half-life. For the *bla* gene, the exchange of 24 codons for the most frequently used synonymous codons extended the *bla* mRNA half-life. A modification of ribosome traffic could account for these observations.

The level of expression of a gene depends on the amount and translation frequency of its full-length mRNA. The former is linked to promoter strength but also to mRNA turnover and to successful completion of transcription elongation, which may depend on transcription polarity (14). The action of RNases, which govern mRNA stability (3, 8), requires the substrate target to be exposed free for sufficient time. The ribosome traffic supported by the mRNA, defined as the frequency of ribosome passage and free space between consecutive ribosomes, and between RNA polymerase and the first translating ribosome, could in part control RNase activity and polarity (6, 7, 9, 10) and thereby gene expression. Neglecting context effects (17), the rate of translation of a given sequence is mainly the sum of those of its individual codons, which depend primarily on the availability of the cognate tRNAs (1, 13, 18). The rate-limiting factor of codon translation would then be the time taken until the arrival and binding of the first cognate tRNA, which to a first approximation is inversely proportional to the cellular concentration of that tRNA (12). These considerations suggest that substitutions of synonymous codons might introduce changes in translation rates affecting the ribosomal traffic and RNase accessibility to mRNA.

Here we report that in the *ompA* and *bla* genes of *Escherichia coli*, the exchange of several codons for synonymous ones, selected for their anticipated differences in translation rates and therefore their expected ability to modify the ribosome traffic, indeed greatly affected the amount and turnover of mRNA in vivo. The strongly expressed *ompA* (outer membrane protein A) gene of *E. coli* makes a rather systematic use of "fast" codons (among synonymous codons having at least two cognate tRNAs, the "fast" codon is the one having the most abundant cognate tRNA). We expect that silent mutations, made by substituting fast codons by synonymous "slower" ones, would reduce the translation rate. The silent mutations were made from codon 183 to 190 (see Fig. 1A), selected because they are far from the ribosome binding site and therefore should not interfere with translation initiation.

Plasmid pADM1 (pSC101 replication origin) carries the *ompA* gene from the *Pst*I-*Asn*I fragment of pTU100 (4). The silent mutations (fast codons 183 to 190 exchanged for synonymous slow ones) (Fig. 1A) were made by the method of Kunkel (11), resulting in plasmid pADM183-190. The *E. coli* strain BRE2413 (5) (*ompA*), was transformed by wild-type or mutated plasmids and grown at 37°C in LB. Rifampin was

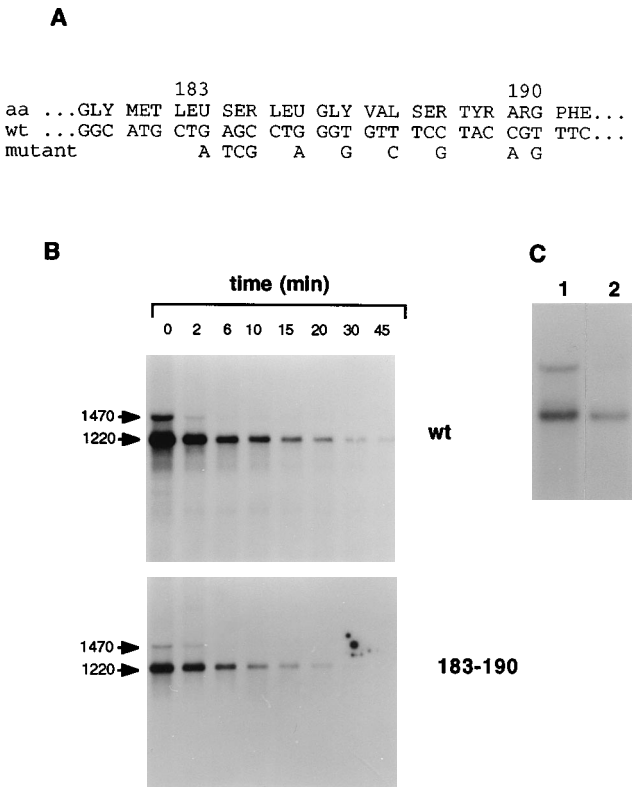

FIG. 1. (A) Wild type (wt) and silently mutated sequences of the *ompA* gene, codons 183 to 190, with the corresponding amino acid (aa) sequence. (B) Decay of wt and silently mutated mRNAs of the *ompA* gene, as observed by Northern blotting. Two bands are seen on the autoradiograms, indicated by arrows. They correspond to two transcripts of the *ompA* gene, the longer one (1,470 nucleotides) having initiated at the *lacZ* promoter located 250 bp upstream of the normal *ompA* promoter in our construction and the shorter one (1,220 nucleotides) starting at the latter. Only half-lives of transcripts initiated at the *ompA* promoter were measured. (C) Northern blot showing the amounts of mutated *ompA* mRNA (lane 2) and the wt mRNA (lane 1).

* Corresponding author. Mailing address: Dept. de Bioquímica, Facultad de Ciencias, Tristán Narvaja 1674, Montevideo 11200, Uruguay. Fax: (5982) 409973. Electronic mail address: atilio@ibiol.edu.uy.

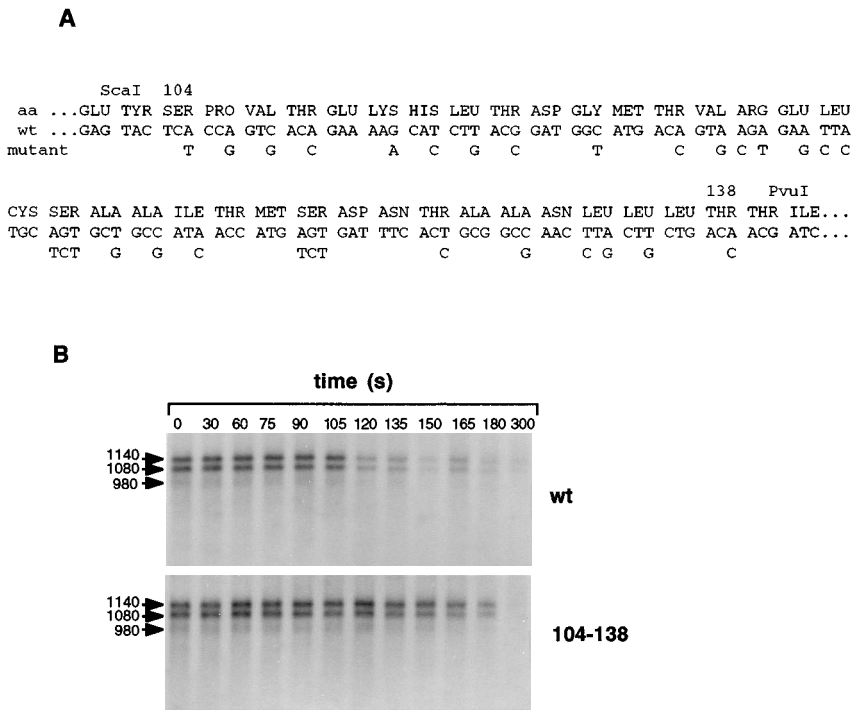

FIG. 2. (A) Wild type (wt) and silently mutated sequences of the *bla* gene, codon 104 to 138, with the corresponding amino acid (aa) sequence. (B) Decay of wt and silently mutated mRNAs of the *bla* gene, as observed by the Northern blotting. Three bands are seen on the autoradiograms, indicated by arrows. They correspond to the three transcripts initiated at the same promoter and terminated at the three known terminators.

added (500 μg/ml) to exponentially growing cells. Aliquots were removed (at times indicated on top of the lanes in Fig. 1B), and total RNA was extracted. Hybridizations were done with a uniformly labelled antisense RNA probe produced by in vitro transcription of the *Bam*HI-*Taq*I fragment (216 nucleotides) of the *ompA* gene, complementary to the 3′ end of the *ompA* transcript. The mRNA half-life was measured by plotting, on a semilog scale, the band intensities measured by densitometry versus time.

The results show that at 37°C, the silent mutations reduced the half-life from 4.5 min (*ompA*) to 3.7 min (*ompA183-190*) (Fig. 1B). The steady-state amount of mRNA was reduced to a 70% (Fig. 1C). The half-life values are the averages from two independent experiments and are accurate within 5 to 10 s.

It is noteworthy that, for bacteria growing in the same medium, the half-life of the wild-type *ompA* mRNA measured at 37°C (4.5 min) differs significantly from that measured at 30°C (15 min; data not shown). The latter corresponds to the half-life measured at 30°C also by von Gabain et al. (19). The turnover of the *ompA* mRNA displays a strong dependence on growth temperature (unpublished results).

In contrast to the *ompA* gene, the *bla* (β-lactamase) gene from Tn*3* contains a fair amount of slow codons. This gene therefore offers the opportunity to examine the effect of the introduction of synonymous fast codons on the local and global translation rate. The *bla* gene of plasmid pBRDT (pBR322 deleted from *Eco*RI to *Bam*HI, thus having a single promoter transcribing the *bla* gene) was mutated by the exchange of codons 104 to 138 of the *bla* coding sequence by synonymous, frequent codons, yielding plasmid pBR104-138 (a gift from Delin Zhu [20]); the sequence of *bla104-138* is given in Fig. 2A. *E. coli* HB101 was transformed with these plasmids and grown at 30°C in LB. Decay kinetics were measured as for the *ompA* assay. For the Northern blot (Fig. 2B) an RNA probe (266 nucleotides) complementary to the 5′ end of the *bla* transcript (corresponding to the *Ssp*I-*Aha*II fragment of pBR322) was produced by in vitro transcription. The mRNA half-life was 3.0 min for *bla* and 5.0 min for *bla104-138*. We measured the *bla* mRNA decay at 30°C to have accuracy comparable to that of the data obtained for the *ompA* mRNA at 37°C.

The silent mutations introduced were aimed at disturbing the ribosome traffic. In order to estimate the contribution of the ribosomal traffic to the mRNA stability, the latter was measured in the extreme situation where the mRNA is free of translating ribosomes. The antibiotic kasugamycin inhibits the initiation of translation in *E. coli* without affecting its elongation (16). mRNA decay experiments in the presence of kasugamycin (1 mg/ml added 1 min before addition of rifampin) using the Northern blot technique (not shown) indicate that the *ompA* and *ompA183-190* mRNAs had similar half-lives (2.0 and 2.3 min, respectively, at 37°C). Similar results were also obtained with *bla* and *bla104-138* mRNAs (54 and 66 s, respectively, at 30°C). The absence of translating ribosomes not only reduces the mRNA stability but also abolishes the differential effect introduced by the silent mutations.

The results suggest that in the case of the *ompA* gene, ribosomes efficiently protect the mRNA against endoribonucleases. The replacement of codons 183 to 190 by synonymous, slow ones presumably increases the translation time of this sequence. This could result in a queue of ribosomes just upstream from codon 190 and much less dense ribosome traffic downstream. The mRNA segment 3′ to codon 190 would then be exposed to RNase digestion for extended time intervals (the time required by the translation pause). Also, the length of the free mRNA segment between the transcribing RNA polymerase and the first translating ribosome may have become larger downstream from codon 190. This could allow the rho factor, which needs 70 to 80 contiguous nucleotides for interaction

(2), to find enough space and time to terminate elongation of the mutated mRNA prematurely (14).

The rationale we propose assumes that RNases and the transcription termination factor rho (15) could find proper target sites in the regions of reduced ribosome traffic or poor transcription-translation coupling.

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REFERENCES

1. **Andersson, S. G. E., and C. G. Kurland.** 1990. Codon preferences in free-living microorganisms. Microbiol. Rev. **54:**198–210.
2. **Bear, D. G., P. S. Hicks, K. W. Escudero, C. L. Andrews, J. A. McSwiggen, and P. H. von Hippel.** 1988. Interactions of *Escherichia coli* transcription termination factor rho with RNA. II. Electron microscopy and nuclease protection experiments. J. Mol. Biol. **199:**623–635.
3. **Belasco, J. G., and C. F. Higgins.** 1988. Mechanisms of mRNA decay in bacteria: a perspective. Gene **72:**15–23.
4. **Bremer, E., E. Beck, I. Hindennach, I. Sonntag, and U. Henning.** 1980. Cloned structural gene (*ompA*) for an integral outer membrane protein of *Escherichia coli* K-12. Mol. Gen. Genet. **179:**13–20.
5. **Bremer, E., T. J. Silhavy, M. Maldener, and S. T. Cole.** 1986. Isolation and characterisation of mutants deleted for the *sulA-ompA* of the *Escherichia coli* K-12 chromosome. FEMS Microbiol. Lett. **33:**173–178.
6. **Deana, A.** 1993. Etude du rôle de l'utilisation des codons synonymes dans l'expresion du gène *ompA* d'*Escherichia coli*. Ph.D. thesis, Université de Paris VII.
7. **Deana, A., and C. Reiss.** 1993. Messenger RNA stability of the *E. coli ompA* gene is affected by synonymous codon usage. C. R. Acad. Sci. Paris **316:**628–632.
8. **Deutscher, M. P.** 1985. *E. coli* RNases: making sense of alphabet soup. Cell **40:**731–732.
9. **Iost, I., and M. Dreyfus.** 1995. The stability of *Escherichia coli lacZ* mRNA depends upon the simultaneity of its synthesis and translation. EMBO J. **14:**3252–3261.
10. **Folley, L. S., and M. Yarus.** 1989. Codon context from weakly expressed genes reduce expression *in vivo*. J. Mol. Biol. **209:**359–378.
11. **Kunkel, T. A.** 1985. Site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:**488–492.
12. **Kurland, C. G.** 1987. Strategies for efficiency and accuracy in gene expression. Trends Biochem. Sci. **12:**126–128.
13. **Pedersen, S.** 1984. *Escherichia coli* ribosomes translate *in vivo* with variable rate. EMBO J. **3:**2895–2898.
14. **Richardson, J. P.** 1991. Preventing the synthesis of unused transcripts by rho factor. Cell **64:**1047–1049.
15. **Richardson, J. P.** 1993. Transcription termination. Biochem. Mol. Biol. **28:** 1–30.
16. **Schneider, E., M. Blundell, and D. Kennell.** 1978. Translation and mRNA decay. Mol. Gen. Genet. **160:**121–129.
17. **Sørensen, M. A., and S. Pedersen.** 1991. Absolute *in vivo* translation rates of individual codons in *Escherichia coli*. The two glutamic acid codons GAA and GAG are translated with three-fold difference in rate. J. Mol. Biol. **222:**265–280.
18. **Varenne, S., J. Buc, R. Lloubes, and C. Lazdunski.** 1984. Translation is a non-uniform process. Effect of tRNA availability on the rate of elongation of nascent polypeptide chains. J. Mol. Biol. **180:**549–576.
19. **von Gabain, A., J. G. Belasco, J. L. Schottel, A. C. Y. Chang, and S. N. Cohen.** 1983. Decay of mRNA in *Escherichia coli*: investigation of the fate of specific segments of transcripts. Proc. Natl. Acad. Sci. USA **80:**653–657.
20. **Zhu, D.** 1989. Etude experimentale du rôle des codons synonymes dans l'expression de la β-lactamase chez *E. coli*. Ph.D. thesis, Université de Paris VII.