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Effect of structure of the initiator codon on translation in *E. coli*

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A set of plasmids carrying different initiator codons – either AUG, or GUG, or UUG, or CUG (as a control) in the hybrid gene *lac*IZ – was constructed by using synthetic oligonucleotides. GUG and UUG codons were demonstrated to be 2–3-times less effective than AUG in translation initiation. Furthermore, the correlation between the efficiencies of different initiator codons in translation initiation proved to vary, depending on the phase of bacterial growth. The rarely occurring usage in nature of the initiator codons GUG and UUG is supposed to be due to the particular role played by the initiator triplets in regulation of gene expression.

Initiator codon; Translation regulation; Plasmid

1. INTRODUCTION

The initiator codons GUG and UUG have been found to occur 10- and 100-times more scarcely than AUG, respectively [1]. The seldom observed utilization of these triplets may be suspected as resulting from their low degree of efficiency. However, the minor initiator triplets have been shown to be essentially more effective than might have been expected based on statistical analyses [2–4]. Moreover, GUG and UUG are known to be involved in the initiation of synthesis of a number of major polypeptides in *E. coli*, e.g. of ribosomal proteins [5]. The question which thus arises concerns the circumstances in which such infrequent usage of minor initiator codons does indeed occur, despite its rarity. The use of these codons is supposed to occur not solely as the result of a decrease in the efficiency of gene expression, but also mainly as the consequence of regulation of specific gene expression depending on the physiological status of bacteria.

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2. EXPERIMENTAL

E. coli K-12ΔH1Δtrp producing temperature-sensitive P_R promoter-repressor c1857 was used [6]. Activation of pVRG operon transcription was achieved by increasing the temperature of cultivation. The efficiency of β-galactosidase synthesis was evaluated by means of assaying enzymatic activity [7], and via determination of the relative densities of protein zones separated on PAGE after Coomassie staining [8]. Oligonucleotides were synthesized as in [9]. The primary structure of DNA was analysed according to [10].

3. RESULTS AND DISCUSSION

Four pVRG plasmids containing different initiator codons – either AUG, or GUG, or UUG, or CUG (as a control) in the *lac*IZ cistron were constructed (fig.1). For pVRG construction, we used plasmids pVR41 [11], pCL47 [12] and four sets of oligodeoxyribonucleotide adapters, containing incomplete, cohesive *Eco*RI and *Bam*HI ends. The use of these adapters demands preliminary filling of the single-stranded end regions of DNA by the large fragment of DNA polymerase 1 from *E. coli*. This approach excludes the necessity of removing the adapter oligomers. Plasmids pVRG contain an operon-like structure under the control of the λ phage P_R promoter, where the small region of the

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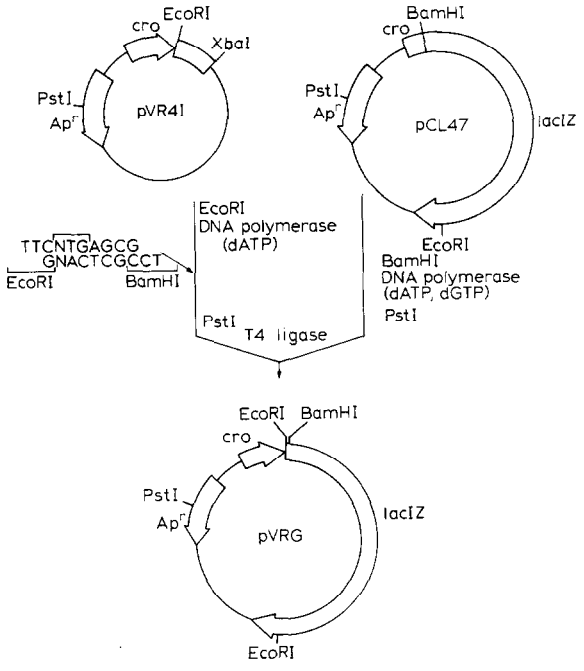

Fig. 1. Strategy of pVRG construction. Structures of synthetic deoxyoligonucleotides containing incomplete single-stranded *Eco*RI and *Bam*HI end regions are shown. N denotes a variable position in these deoxyribonucleotides. Deoxyribunucleotide triphosphates used for partial filling of the cohesive *Bam*HI or *Eco*RI ends of the plasmid fragments necessary for attachment of these synthetic oligonucleotides are indicated in parentheses.

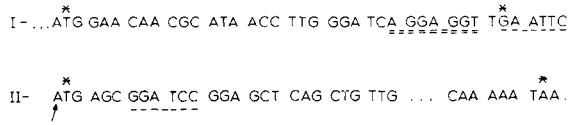

Fig. 2. Organization of pVRG operon-like structure. Cistrons: I, cro; II, *lac*IZ. Initiator and terminator codons are indicated by asterisks. *Eco*RI and *Bam*HI, sites are underlined. The Shine-Dalgarno sequence of the second cistron is double underlined. The arrow designates the variable position in the primary structure of pVRG.

cro gene might have been the first cistron, with the β-galactosidase gene being the second. The synthesis of cro was terminated just upstream of the enzyme gene initiator triplet that favoured co-translation of these cistrons. The primary structures of the pVRG plasmids differ in one position only (fig.2).

β-Galactosidase production in bacteria was evaluated over 2 h after promoter activation. In cells transformed by pVRG-AUG the enzyme content amounted to 4.7% of total cell proteins. Plasmids pVRG-GUG and pVRG-UUG were 2.5-times less efficient in *lac*IZ cistron expression as compared with pVRG-AUG. The efficiency of GUG and UUG codons proved to be practically equal ($P < 0.1$) in the expression system employed. The secondary structures of the two-cistron mRNAs constructed according to [13] were identical. Therefore, this was difficult to conceive as being responsible for the different translation activities. The correlation between the efficiencies of initiator codons AUG, GUG, and UUG corresponds to a ratio of 100:35:40 in this case (table 1).

A similar correlation between codons UUG and AUG has been shown to vary from 1:2 to 1:6 depending on the gene expression system used and possibly on the conditions for bacterial growth [4]. The physiological status of bacteria may also exert a variable degree of influence on the efficiency of protein synthesis controlled by different initiator codons. To examine the possibility of codon-dependent alteration of translation efficiency in *E. coli*, β-galactosidase production in cells transformed by pVRG was additionally measured over 5 and 20 h after the onset of induction. These additional points were chosen because in our experiments bacteria progressed from the logarithmic phase to stationary status over 5 h (known to be due to the shifting of physiological processes in cells, particularly with alteration in the number of functionally active ribosomes).

The additional data listed in table 1 demonstrate that the efficiency of protein synthesis during bacterial growth varies according to the structure of the initiator codon. The correlation between codon efficiency over 5 h after induction may be expressed as 100:27:30, whereas the value over 20 h is 100:37:60. The dynamics of β-galactosidase synthesis coded by pVRG may have been altered along with the changes of initiator codons.

General considerations prove the efficiency of ribosome interaction with minor initiator codons to decrease essentially with the decrease in number of active ribosomes. Indeed, the efficiency of β-galactosidase synthesis coded by pVRG-GUG and pVRG-UUG decreased over 5 h after induction. However, the maximum level of enzyme produc-

Table 1

Alteration of the efficiency of pVRG-coded β-galactosidase production depending on the time after P_R promoter induction

Plasmid	Time after activation of P_R promoter (h)			Increase in production[a]
	2	5	20	
pVRG-AUG	100[a]	130	150	1.5
pVRG-GUG	35	35	55	1.6
pVRG-UUG	40	40	90	2.3
pVRG-CUG	1	ND	ND	–

[a] This level of β-galactosidase production was taken to represent 100

[b] Ratio of the levels of protein synthesis observed 20 and 2 h after promoter induction

For all values excluding those specified otherwise in the text: $P<0.05$

tion was attained over 20 h (table 1). The wave-like oscillation in level of translation must have resulted from the organization of the structure of the pVRG operon. Translation of the first cistron of the operon was in all cases initiated with AUG by free ribosomes, however initiation of β-galactosidase synthesis might occur via two mechanisms: interaction with free ribosomes, and re-initiation. One mechanism might have been replaced by another with a different degree of efficiency according to structure of the initiator codon. This process could essentially exert an influence at the translational level.

The present data suggest that the rarity of utilization of minor initiator codons is caused by their particular role in the regulation of gene expression. Indirect evidence in favour of this suggestion may be provided by simple enumeration of *E. coli* proteins of which the synthesis is initiated on the UUG codon, namely adenylate cyclase [4], respiratory NADH dehydrogenase [14], carbamoyl-phosphate synthetase [2] and also ribosomal protein S20 [15]. The initiator codon UUG may play a key role in the regulation of expression of these genes as well as the even scarcer codon AUU that participates in regulation of IF3 synthesis [16].

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