Gene expression in the polycistronic operons of *Escherichia coli* heat-labile toxin and cholera toxin: a new model of translational control

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A new model is proposed based on the suggestion that stable local secondary structures of mRNA interfere with ribosome movement on mRNA and consequently reduce the translation rate. This model accounts for a different level of translation for each cistron in the polycistronic mRNA of *Escherichia coli* heat-labile toxin (LT) and cholera toxin. We also conclude that the mRNA secondary structures have been conserved during the evolution of the toxin genes for its functional importance.

Escherichia coli *heat-labile toxin* *Cholera toxin* *Polycistronic operon* *mRNA secondary structure* *Translational control*

1. INTRODUCTION

Reports on the regulation of bacterial gene expression at the level of translation are accumulating [1–5]. However, this aspect of gene expression remains imprecisely understood. *E. coli* heat-labile enterotoxin (LT) and *Vibrio cholerae* enterotoxin (cholera toxin, CT) are final products from polycistronic operons which have evolved from a common ancestry [6]. In both cases the holotoxin consists of one molecule of subunit A (having an ADP-ribose transferase sequence) and five molecules of subunit B (having the ability to bind to the receptor) [7–9]. In these operons, a single cistron has been identified for each subunit; the A cistron encoding subunit A is located proximal to the B cistron encoding subunit B [10–13] with a short overlapping sequence ATGA [14,15]. Previous experiments with the LT operon have shown that more of subunit B is produced in *E. coli* minicells than of subunit A [16], suggesting that control of the formation of holotoxin (1A5B) takes place mainly at the level of translation. This paper describes secondary structures of mRNA which could allow the B cistron to be translated more efficiently than the A cistron.

2. METHODS

The free energy of the optimal secondary structure in a 100-base-long sequence was calculated according to the method of Zuker and Stiegler [17] and using the free energy data compiled by Salser [18]. The free energy for a complex between a Shine-Dalgarno sequence and the complementary sequence at the 3′-end of 16 S rRNA was also calculated using free energy values compiled by Salser [18]; in this calculation, chain association energy, which depends on a concentration of rRNA and mRNA, was assumed to be 0 kcal/mol. Frequency (f) of use of optimal codons was calculated according to the method of Ikemura [2] using the data described in [19]: f = number of optimal codons/sum of numbers of optimal and nonoptimal codons.

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3. RESULTS AND DISCUSSION

Ribosomes move on mRNA and there is a space between adjacent ribosomes. These free spaces on mRNA, which are unattached to ribosomes, are approx. 100 bases in length (kindly calculated by Dr Hideo Yamagishi, Kyoto University, Kyoto, using the *E. coli* data [20]). If intrastrand base-pairing takes place between invertedly aligned homologous sequences which are present within those unbound spaces on mRNA, the resultant secondary structures (e.g., stem-and-loop structures) could interfere with ribosome movement on mRNA and consequently reduce the translation rate. This possibility was tested with the polycistronic mRNAs of LT and CT, by calculation of a free energy.

In the nucleotide sequences of the LT operon [10,13] and the CT operon [11,12], there exist many and various sequences which can potentially pair with each other to form stem-and-loop structures (not shown). Fig.1 shows the free energy values calculated for such local secondary structures from a running 100-base-long nucleotide sequence of the LT and CT operons. Stable local secondary structures were found predominantly in the A cistron and the average free energy of the local secondary structures in the A cistron was significantly lower than that for the local secondary structures in the B cistron. This was true for both the LT and CT operons. Moreover, even when the free energy was calculated for the secondary structures over a shorter sequence, of 50 bases in length, similar results were obtained. On average the free energy (kcal/mol) for the local secondary structures in the A and B cistrons was -7.7 and -4.7 for the LT operon and -8.4 and -4.7 for the CT operon, respectively. These observations are consistent with the observed subunit composition (1A5B) in the holotoxin, and thus could be an important determinant of the differential rate of expression of each cistron in the polycistronic mRNAs of LT and CT. In addition, we conclude that this structural feature of mRNA has been conserved during the evolution of the toxin (LT and CT) genes, as it plays an important role in the translational control.

It has been reported that codon usage [2,19] and the Shine-Dalgarno sequence [21] are involved in a translational control. Data with those factors in the case of the LT and CT operons are summarized in table 1. With respect to codon usage, no significant difference is confirmed between the A and B cistrons in terms of the frequency of use of the op-

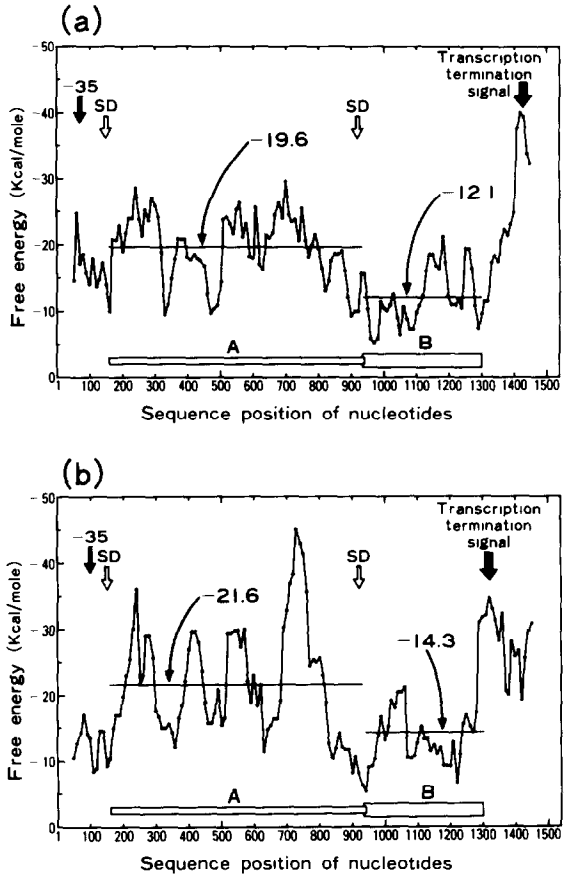

Fig.1. Free energy distribution of a local secondary structure along the nucleotide sequences of the LT operon (a) and the CT operon (b). The free energy of the secondary structures in a 100-base-long sequence was plotted againsts the middle nucleotide position in the 100-base-long sequence. This calculation was carried out repeatedly by sliding 10 nucleotides along the nucleotide sequences of the operons. A and B each represent cistrons of the respective subunits and the box indicates the position of the cistron. Horizontal lines represent average free energy values for the local secondary structures in the cistrons A and B. The positions of the RNA recognition sequence (-35), Shine-Dalgarno sequence (SD) and possible rho-independent transcription termination signal, with a typical stem-and-loop structure, are indicated by an arrow and have been described in [10,13,14] in the case of the LT operon, and in [11,15] in the case of the CT operon.

Table 1

Summary of sequence information affecting the translational expression

Operon	Cistrons (product)	Average free energy for local secondary structures (kcal/mol)	Frequency (f)[a] of use of optimal codons	Shine-Dalgarno sequence[b] (shown in large letters) and free energy for a 16 S rRNA complex (kcal/mol)
LT	*toxA* (subunit A)	−19.6	0.38	−5.1 UAAGuuuuccucg *toxA* → aug 16 S rRNA: 3′ åùùċ cucca
	toxB (subunit B)	−12.1	0.44	−6.9 GGAaugaauu *toxB* → aug 16 S rRNA: 3′ auuċċù cca
CT	*ctxA* (subunit A)	−21.6	0.41	−10.6 AGGGAGcauuau *ctxA* → aug 16 S rRNA: 3′ aùùċċùċ ca
	ctxB (subunit B)	−14.3	0.40	−12.0 UAAGGAugaauu *ctxB* → aug 16 S rRNA: 3′ åùùċċù cca
Cloacin DF13	*clo* (cloacin DF13)	−27.8	0.58	−9.50 AAGAGGaaaacgau *clo* → aug 16 S rRNA: 3′ aùùċùċċ a
	imm (immunity protein)	−17.6	0.51	−13.4 UAAGAGGUaaau *imm* → aug 16 S rRNA: 3′ åùùċùċċà

[a] The f values for the cistrons in the LT and CT operons have been described in [6]; in that paper, the f values were calculated using the data described in [2]

[b] Shine-Dalgarno sequences have been described in [13,14] for LT, [15] for CT, and [24] for cloacin DF13

timal codons; the frequency of use of the optimal codons is closely related to the amount of the gene product (protein) in *E. coli* [2]. In contrast, the Shine-Dalgarno sequence may play a role in the translation of the A and B cistrons at a different level. As shown in table 1, the A and B cistrons of LT and CT are preceded by a Shine-Dalgarno sequence which can potentially pair with the complementary sequence at the 3′-end of 16 S rRNA [21]. In terms of the Shine-Dalgarno complementarity, the sequence for the B cistron is more efficient than that for the A cistron (this has been pointed out previously [13,15]). However, we do not know at present how effectively this contributes to the differential control of translation of the A and B cistrons. In a certain instance [22,23], no direct correlation is found between the extent of the Shine-Dalgarno complementarity and the level of translation.

Interestingly, even in the case of the polycistronic operon of cloacin DF13, secondary structures of mRNA could play an important role

in a translational control for each cistron (table 1); in the cloacin DF13 operon, the immunity protein-coding cistron (*imm*) is located distal to the cloacin DF13-coding cistron (*clo*) [24], and more immunity protein is produced than cloacin DF13 [24,25].

The present data also suggest that, with respect to the in vitro synthesis of genes, to obtain maximum gene expression at the level of translation it is important to minimize stable local secondary structures in the coding sequences.

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