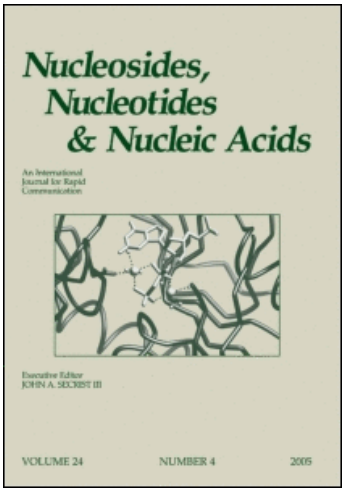

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Construction of a Translation Enhancer-Containing Vector for Gene Expression in a Prokaryotic Two-Cistron System

Klara R. Birikh[a]; Ekaterina N. Lebedenko[a]; Yuri A. Berlin[a]
[a] M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry Russian Academy of Sciences, Moscow, Russia

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CONSTRUCTION OF A TRANSLATION ENHANCER-CONTAINING VECTOR FOR GENE EXPRESSION IN A PROKARYOTIC TWO-CISTRON SYSTEM

Klara R. Birikh, Ekaterina N. Lebedenko and Yuri A. Berlin*

M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry
Russian Academy of Sciences, Moscow 117871, Russia

ABSTRACT: A prokaryotic two-cistron system for gene expression, based on the pGEM1 plasmid and containing a translation enhancer in the coding part of the first cistron, has been constructed. The gene to be expressed can be inserted into the vector by means of a PCR-mediated approach using type IIS restriction endonucleases (SDL method). Efficiency of the system is exemplified by the expression of a synthetic gene encoding human interleukin 1α.

One of the most widely used approaches to preparing eukaryotic peptides and proteins is based on the synthesis of the corresponding intronless gene, its insertion in a bacterial vector containing the regulatory elements necessary for gene transcription, followed by translation of the synthesized mRNA (for review see[1]). In this paper, the previously described method of DNA splicing by directed ligation (SDL)[2] has been used to construct a plasmid vector for artificial gene expression, exemplified by a gene encoding human mature interleukin 1α (IL1α) also synthesized by the SDL approach[2].

Expression of the synthetic gene for IL1α in the standard plasmid vector pGEM1 failed, probably because of the Shine-Dalgarno (SD) sequence in the 5' untranslated region of the corresponding transcript being included as a whole into the stem of a probable hairpin (Fig. 1A). In an attempt to overcome this problem, we

Dedicated to the memory of Dr. Roland K. Robins.

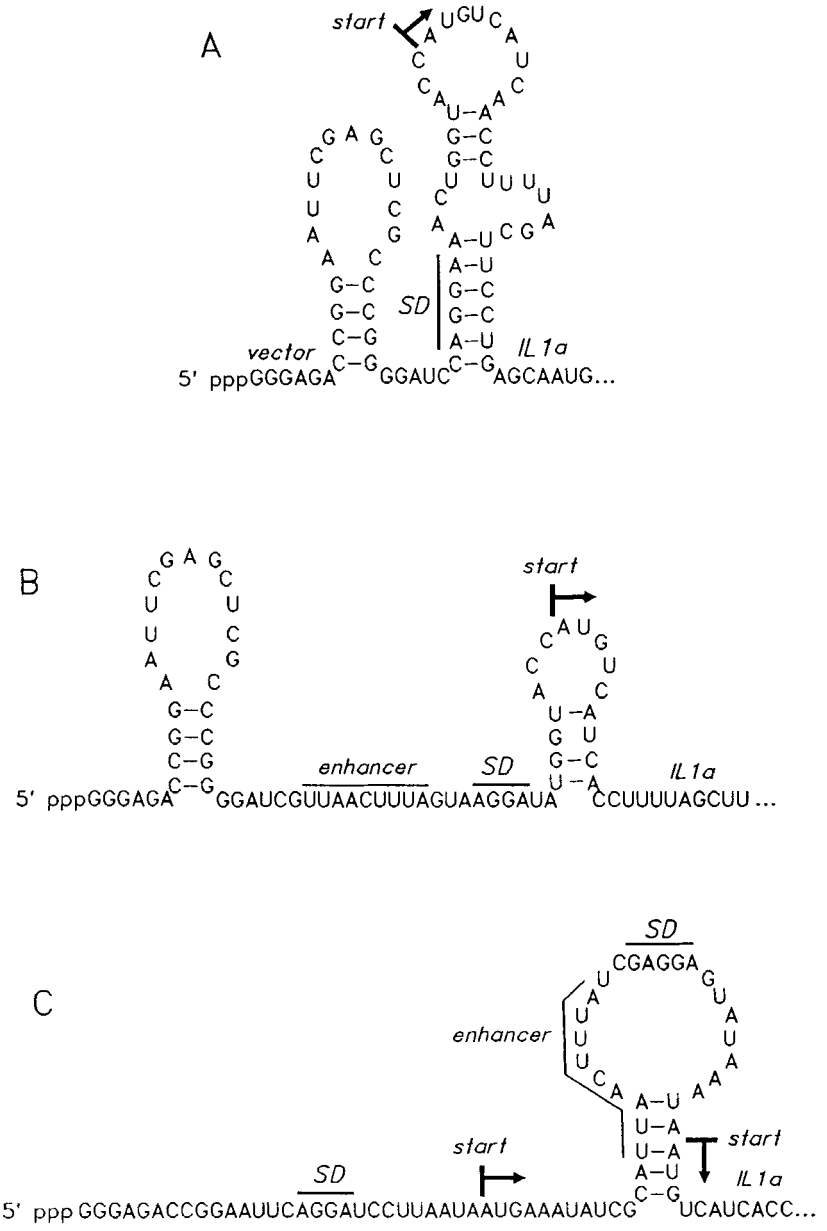
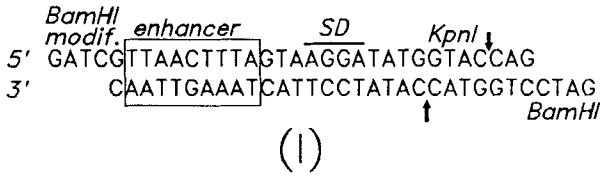


FIG. 1. Hypothetical secondary structures of 5' areas of transcripts from the T7 promoter of plasmids pGEM1-IL1α (A), pGEM1-ENH-IL1α (B) and pGEM1-MCENH-IL1α (C) (OLIGO program).

modified the ribosome-binding site in pGEM1 by introducing an additional regulatory element, the translation enhancer 5' TTAACTTTA from the leader mRNA of gene 10 (g10l) of bacteriophage T7.[3] For that goal, the synthetic duplex (I), containing the enhancer, SD sequence, and a KpnI site for subsequent gene cloning, was inserted into the pGEM1 BamHI site to give the pGEM1-ENH vector.



(I)

A gene coding for IL1α[2], modified at the proximal terminus by a polymerase chain reaction (PCR) to introduce a KpnI site, was inserted into the KpnI/BamHI pGEM1-ENH vector to yield the plasmid pGEM1-ENH-IL1α. Analysis of the plausible secondary structure of the expected transcript from the T7 promoter (Fig. 1B) suggests it to contain a hairpin in the area of translation initiation. At the same time, both the SD sequence and the ATG codon would seem to be located within single-stranded segments, indicating that the mRNA's secondary structure would not be detrimental to translation initiation. In fact, the desired protein was synthesized in the E.coli BL21(DE3) strain transformed with the pGEM1-ENH-IL1α plasmid, in the presence of rifamycin. Its content in the total cellular protein as determined by densitometry of a Coumassie stained SDS-PAGE gel, was 15% (Fig. 2, lane 1). The protein was identified by its electrophoretic mobility (M 18.5 kDa), which was confirmed by immunoblotting with polyclonal antibodies to IL1α (data not shown).

Since a mRNA's secondary structure in the area of translation initiation and, therefore, the level of translation, largely depends on the sequence of the proximal part of the gene to be expressed, the efficiency of protein expression from pGEM1-ENH constructs can vary widely among cloned genes. To prepare a more universal vehicle for efficient translation, we made use of coupled translation in a

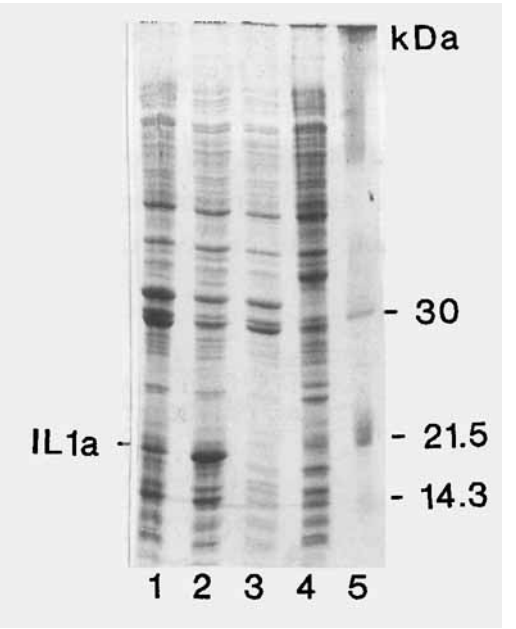
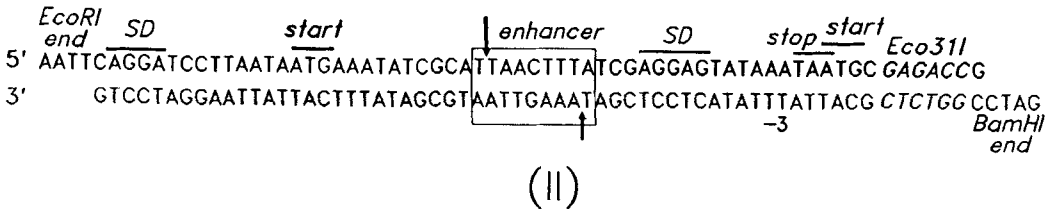


Fig. 2. SDS-PAGE (15%) analysis of crude lysates of cells harboring IL1α gene-containing plasmids.

1 - pGEM1-ENH-IL1α
2 - pGEM1-MCENH-IL1α
3 - pGEM1-IL1α
4 - pGEM1 (control)
5 - molecular weight markers

two-cistron system[5]. As the above-mentioned enhancer is apparently capable of promoting translation both from a downstream and an upstream initiation codon[3], we decided to place the enhancer in the coding part of a first cistron in an attempt to increase the translation level of the two cistrons. The structure of the first cistron (with the initiation codon of a second cistron), which was assembled from four synthetic oligonucleotides with the use of T4 DNA ligase and isolated by PAGE, is presented by the 67-meric DNA (II) (the arrows show the boundaries of the ligated segments).



(II)

This minicistron, encoding none of particular amino acid sequences, was coined with some data on the optimization of translation taken into account. It consists of eleven codons (besides the initiation and termination codons), most often used by E.coli[6] and, preferably, A/T rich[7]. Positions of the enhancer and an SD-sequence at an octanucleotide distance from the initiation codon of the second cistron were adjusted to provide an optimal combination of the codons, as well as the presence of a TTAA sequence within codons 5 and 6 of the first cistron and an A residue in position "-3" with regard to the second cistron[8]. The 5' untranslated domain is the ribosome-binding site from the E.coli lpp mRNA[9]. In the distal part of the duplex (II), the first cistron's termination codon and the second cistron's initiation codon are partially overlapped (a TAATG sequence at the joining site of the two cistrons), which is essential for efficient coupled translation[10]. An Eco31I site was introduced next to this sequence for the subsequent joining of the distal end of the duplex (II) and the proximal end of a second cistron by the SDL method.

Insertion of the duplex (II) into the EcoRI/BamHI pGEM1 plasmid led to the pGEM1-MCENH vector for gene cloning and expression in a two-cistron system. In this system the initiation translation area of the first cistron, which considerably contributes to the level of translation not only of the first but also the second cistron (the gene to be expressed), forms, together with the coding part of the first cistron, a constant domain, whose transcript must possess a defined secondary structure. It is therefore low probable that a different secondary structure, including a transcript of the coding part of the gene to be expressed, can arise which would hinder its translation, as it occurred in the case of the pGEM1-IL1α plasmid

(see Fig. 1A). Thus, in the two-cistron construction the structural organization of the first cistron's translation initiation area would not depend on the nucleotide sequence of the gene to be expressed, which makes the pGEM1-MCENH vecor versatile if not universal for prokaryotic systems.

We used this vector for cloning and expression of the human mature IL-1α gene[2] (it was inserted into the Eco31I/HindIII sites), which failed to be expressed in the pGEM1 vector because of an unfavorable secondary structure of the mRNA in the ribosome-binding site, but was expressed in the pGEM1-ENH vector, i.e. in pGEM1 modified by inserting a translation enhancer (see above) . In the transcript of the resultant two-cistron construction pGEM1-MCENH-IL1α, the translation initiation site of the first cistron is available for ribosome binding, whereas a weak hairpin (ΔG = -1 kcal/mole) (Fig. 1C), comprising the initiation codon of the second cistron, is located at the translated area of the first cistron and must be easily unwinded by ribosome. In fact, we observed an elevated level of expression (27% of the total protein) of the IL1α gene in the pGEM1-MCENH-IL1α plasmid (Fig. 2, lane 2), even higher than in case of the monocistronic construction pGEM1-ENH-IL1α.

Thus, the pGEM1 plasmid was transformed into a versatile enhancer-containing vector for gene expression in a two-cistron system, its efficiency being exemplified by the synthesis of human mature IL-1α.

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