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trp operon induction during the expression in *E. coli* of two IFN-γ sequences

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Two nucleotide sequences coding for mature human immune interferon (IFN-γ) and differing from each other by nine N-terminal nucleotides were expressed in *E. coli* under the control of a trp promoter. The longer gene variant after the ATG initiatory codon contained a TGT TAC TGC sequence, which was absent in the shorter gene. When expressed in *E. coli* under the direction of identical transcription and translation regulatory elements, these genes showed different susceptibility to induction.

Complementary DNA; Interferon-γ; Gene variant; Gene expression; Tryptophan promoter; Transcriptional control

1. INTRODUCTION

Expression efficiency in a host cell is a function of many factors, such as promoter efficiency, the secondary structure of the RNA to be synthesized, the Shine-Dalgarno sequence structure, composition and the distance from the ATG codon, and of course the structure of the gene itself [1–4]. Numerous studies on foreign gene expression are now in progress, but we are still somewhat far from a true understanding of the process and the factors governing it.

Here we report the expression of two gene variants coding for mature IFN-γ and differing in structure by nine nucleotides [5,6]. Gene expression was directed by identical regulation sequences taken from the trp operon and a synthetic Shine-Dalgarno sequence. The small difference in the encoding part of the gene was shown to affect considerably its expression in the cell.

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2. MATERIALS AND METHODS

2.1. *Construction of the expressing plasmids (fig.1)*

cDNA coding for the synthesis of IFN-γ was obtained by reverse transcription of lymphocyte mRNA induced by staphylococcal enterotoxin. The cDNA obtained was cloned in the *Pst*I site of a pBR322 in *E. coli* K-802 [7].

The sequences coding for the two variants of mature IFN-γ [5,6] were constructed using cDNA fragments and synthetic oligonucleotides containing the ATG initiating codon and Shine-Dalgarno sequence. Gene expression was controlled by a previously cloned trp promoter [8]. Recombinant plasmids were used to transform *E. coli* strain MH-1.

2.2. *Cultivation of bacterial cells carrying pIFN-γ-trp-1 and pIFN-γ-trp-2*

E. coli harbouring pIFN-γ-trp-1 and pIFN-γ-trp-2 were grown overnight in LB medium containing 5 μg/ml tetracycline. The overnight culture was diluted in M-9 medium supplemented with 0.2% casamino acids and 5 μg/ml tetracycline in a 1 : 100

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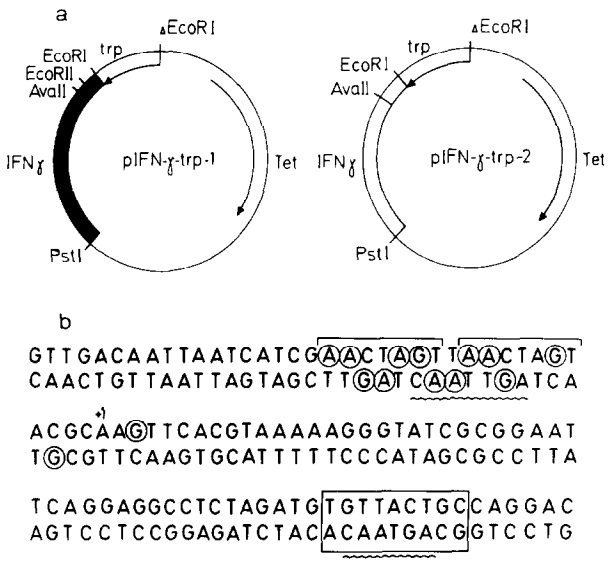

Fig.1. (a) Restriction maps of pIFN-γ-trp-1 and pIFN-γ-trp-2. (b) The primary structure of a stretch of expressing pIFN-γ-trp-1 and pIFN-γ-trp-2 comprising the trp promoter and IFN-γ gene joining segment. The nonanucleotide that is present in pIFN-γ-trp-1 and absent in pIFN-γ-trp-2 is boxed; homologous sequences in the nonanucleotide and trp operator are underlined with a wavy line; repeats in the operator are shown by square brackets over the line. Nucleotides to be protected against methylation in the repressor-operator complex [11] are encircled. Transcription starting point is shown by +1.

ratio. After 30 min incubation at 37°C several samples were supplemented with 3β-indoleacrylic acid to a final concentration of 20 μg/ml.

2.3. *IFN-γ activity assay*

IFN-γ activity in crude cell lysates was assayed either according to its antiviral action on diploid human fibroblasts [3] or radioimmunologically using a Celltech IFN-γ-IRMA kit.

2.4. *SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of cell lysates*

A bacterial cell pellet from 1.5 ml suspension was kept for 5 min at 100°C in 200 μl solution containing 10 mM Tris-HCl (pH 7.0), 3% SDS, 5% 2-mercaptoethanol and 10% glycerol. The obtained lysates in 5 μl portions were separated by 4–30% gradient SDS-PAGE. The gels were stained with Coomassie blue R-250.

3. RESULTS AND DISCUSSION

Heterological gene expression in host cells depends heavily on a number of factors that are not known sufficiently. Some factors, however, have been investigated fairly well; for example, for *E. coli* these are the promoter power, mRNA stability, primary and secondary structure of the ribosome-binding site and protein stability [9]. However, very often a system optimized for the expression of one gene proves to be quite unsuitable for other genes [1,10].

In our studies of IFN-γ cDNA expression in *E. coli* we came upon quite a surprising fact: the two very similar gene variants coding for two also similar IFN sequences exhibited quite different inducibility under identical expression conditions. These two gene variants were derived in the following way: at first on the basis of cDNA sequence data it was believed that the IFN-γ N-terminal sequence was Cys-Tyr-Cys-Gln... [5]; however, it was later shown that the first three amino acid

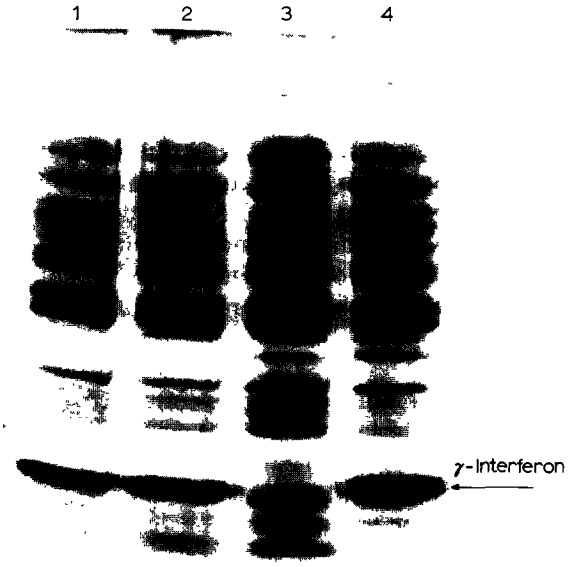

Fig.2. SDS-PAGE separation of total proteins from bacterial cells containing pIFN-γ-trp-2 (lanes 1,2) and pIFN-γ-trp-1 (lanes 3,4). Induction in a medium not containing trp (lanes 1,3). Induction by 3β-indoleacrylic acid (lanes 2,4).

residues did not belong to mature IFN-γ, which actually began with Gln [6].

We have constructed both gene sequences, the longer and the shorter one, and introduced them into the expressing plasmids under the control of identical regulation elements (fig.1a,b), so that the constructions differed exactly by nine nucleotides coding for the Cys-Tyr-Cys sequence.

Unexpectedly, it was found that the longer gene variant (pIFN-γ-trp-1) required much more stringent induction conditions than did the shorter gene (pIFN-γ-trp-2) (fig.2). Thus, to induce the shorter gene it was sufficient to reduce the trp content in the nutrient medium, whereas the longer gene required addition of 3β-indoleacrylic acid.

The first reason for this might be that during the cloning procedure one of the regulatory elements in either the longer or shorter plasmid vector suffered some sort of mutation thus affecting the induction requirement.

To rule out this possibility, we performed a gene exchange in the expressing plasmids, so that the promoter, regulating the short gene expression, was joined to the longer gene and vice versa, as shown in fig.3.

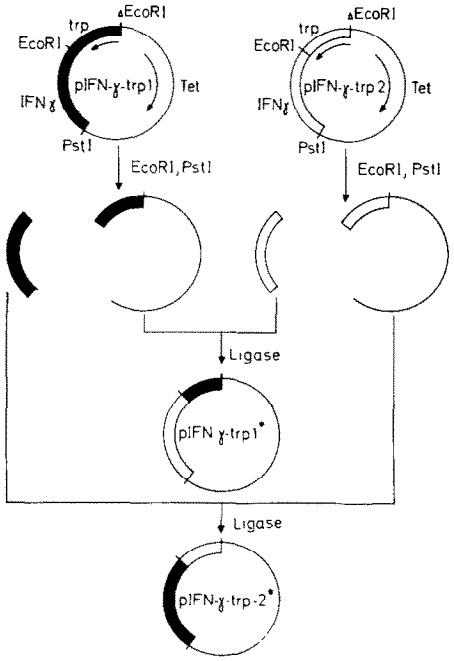

Fig.3. Schematic representation of structural gene exchanges in pIFN-γ-trp-1 and pIFN-γ-trp-2.

Notwithstanding the reconstruction, the longer gene had to be induced under more rigorous conditions, thus making it clear that the described behaviour was an inherent property of the gene, and was apparently caused by the nine extra nucleotides.

This nonanucleotide sequence could act either at the transcription level, or later at the post-transcriptional step, though the second possibility looked less probable.

It may well be that this nonanucleotide sequence by some mechanism affected the binding constant of trp repressor with trp operator. This nonanucleotide is indeed to some degree homologous to the repeated sequence of the trp operator (fig.1b) containing nucleotides which apparently take part in trp repressor binding [11].

Hence, it could act as an additional operator, for example by firmly binding the repressor protein, or by taking part in the repressor transport to the main operator by the sliding mechanism proposed by Von Hippel [12].

We hope that the investigations which are now in progress will enable us to understand the mechanism of the observed effect.

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