Lipid biosynthetic genes and a ribosomal protein gene are cotranscribed

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Abstract By using insertional mutagenesis we demonstrated that the *rpmF* gene encoding ribosomal protein L32, the *plsX* gene encoding a protein involved in membrane lipid synthesis and several fatty acid biosynthetic genes (*fabH*, *fabD* and *fabG*) are cotranscribed. Organization of these genes into an operon may play a role in the coordinate regulation of the synthesis of ribosomes and the cell membranes.

Key words: *plsX* gene; Lipid biosynthetic gene; Ribosomal protein gene; Cotranscription; Insertional mutagenesis; *Escherichia coli*

1. Introduction

The rate of ribosome production in *Escherichia coli* is controlled in relation to bacterial growth rate (for review, see [1]). The synthesis rates of ribosomal proteins and rRNAs are strictly regulated so that the pools of free ribosomal components are small. Genes for the 52 ribosomal proteins are organized into at least 20 operons. Many of them contain genes for essential cellular processes including protein secretion, DNA replication, transcription and translation. The organization of these genes and the ribosomal protein genes into polycistronic transcription units is related to their coordinate regulation.

Recently we established the physical locations of genes surrounding the *plsX* gene of *E. coli* which encodes a protein involved in membrane lipid synthesis [2]. The *rpmF* gene encoding ribosomal protein L32 is located just upstream of the *plsX* gene and several fatty acid biosynthetic (*fab*) genes are located just downstream of the *plsX* gene (Fig. 1). Northern and promoter activity analysis suggested that the *rpmF-plsX-fab* genes comprise an operon (Oh and Larson, manuscript in preparation). In the present study, the effect of polar insertions into different sites of the *rpmF-plsX-fab* region was used to demonstrate cotranscription of the *rpmF*, *plsX*, *fabH*, *fabD* and *fabG* genes.

2. Materials and methods

Escherichia coli K-12 strain DH5αF′[F′ φ80d*lacZ*ΔM15 Δ(*lac*ZYA-*arg*F)U169 *deo*R *rec*A1 *end*A1 *hsd*R17(r_k^-,m_k^+) *sup*E44 λ^- *thi*-1 *gyr*A96 *rel*A1] (Gibco BRL, Gaithersburg, MD, USA) was used as the host for DNA manipulations. Plasmid pSP417 [3] was used as the vector for construction of operon fusions and plasmid pHP45Ω [4] was the source of the spectinomycin omega cassette. As a source of DNA containing different parts of the *rpmF-plsX-fab* region we used an extensive plasmid collection generated in our laboratory. For plasmid DNA purification, Wizard Minipreps DNA Purification System was employed (Promega, Madison, WI, USA). DNA fragments for cloning were isolated from agarose gel by using Wizard PCR Preps DNA Purification System (Promega). All other standard molecular biology techniques were used, as described elsewhere [5]. β-Galactosidase activity encoded by the various *lacZ* fusions was assayed as described by Miller [6]. β-Galactosidase activity was measured at least in triplicate and the results given are the average of these data.

The complete nucleotide sequence of the *rpmF-plsX-fab* region was compiled from a number of sequences deposited in GenBank (for the accession numbers see [2]). Mapping of the restriction sites was carried out by using PC/GENE computer program [7].

3. Results and discussion

In order to determine which genes of the *rpmF-plsX-fab* region are cotranscribed we constructed a series of transcriptional fusions between different parts of the region and *lacZ* in the plasmid vector pSP417 designed for construction of transcriptional fusions. Then, the interposon Ω carrying a spectinomycin resistance gene (Sp^r) flanked by transcriptional termination signals in inverted orientations was inserted into different positions of the fusions. Strain DH5αF′ was transformed with the recombinant plasmids, and the level of *lacZ* expression was measured. The structure of each fusion and corresponding β-galactosidase activity are summarized in Fig. 1.

To determine if *rpmF* and *plsX* are cotranscribed, the *Sal*I–*Ssp*I DNA fragment containing the *g30k* gene for a 30-kDa protein with unknown function, the *rpmF* gene and the 5′ part of the *plsX* gene was inserted into pSP417, yielding plasmid pSP419. This fragment was chosen for construction of the fusion because we recently showed that the *rpmF* gene is transcribed from the three promoters downstream of the *Sal*I site located within coding and non-coding parts of *g30k* (manuscript in preparation). Expression of *lacZ* from recombinant plasmid pSP419 was compared to that obtained from the same plasmid with an Ω cassette inserted at the unique *Hin*dIII site just downstream of *rpmF* and 61 bp upstream of the *plsX* start codon (plasmid pSP422). Cotranscription of *rpmF* and *plsX* was indicated since *lacZ* expression was abolished in the case of pSP422. Although the mechanism of PlsX action is not established, it is known that the *plsX50* mutation together with *plsB26* encoding a defective *sn*-glycerol-3-phosphate acyltransferase is required for conferral of a glycerol-3-phosphate-auxotrophic phenotype [8]. Since *sn*-glycerol-3-phosphate acyltransferase catalyzes the initial reaction of membrane phospholipid synthesis in *E. coli*, PlsX may play an important role in the whole process. Cotranscription of *plsX* and *rpmF* may suggest coordinate regulation of the synthesis of ribosomes and membranes.

To find out if the *fabH* gene following the *plsX* gene is cotranscribed together with *rpmF* and *plsX*, insertional mutagenesis of the *g30k-rpmF-plsX-fabH-lacZ* fusion (plasmid pSP418) was performed. Ω insertion at the *Hin*dIII site upstream of the *plsX* gene decreased, but did not abolish *lacZ*

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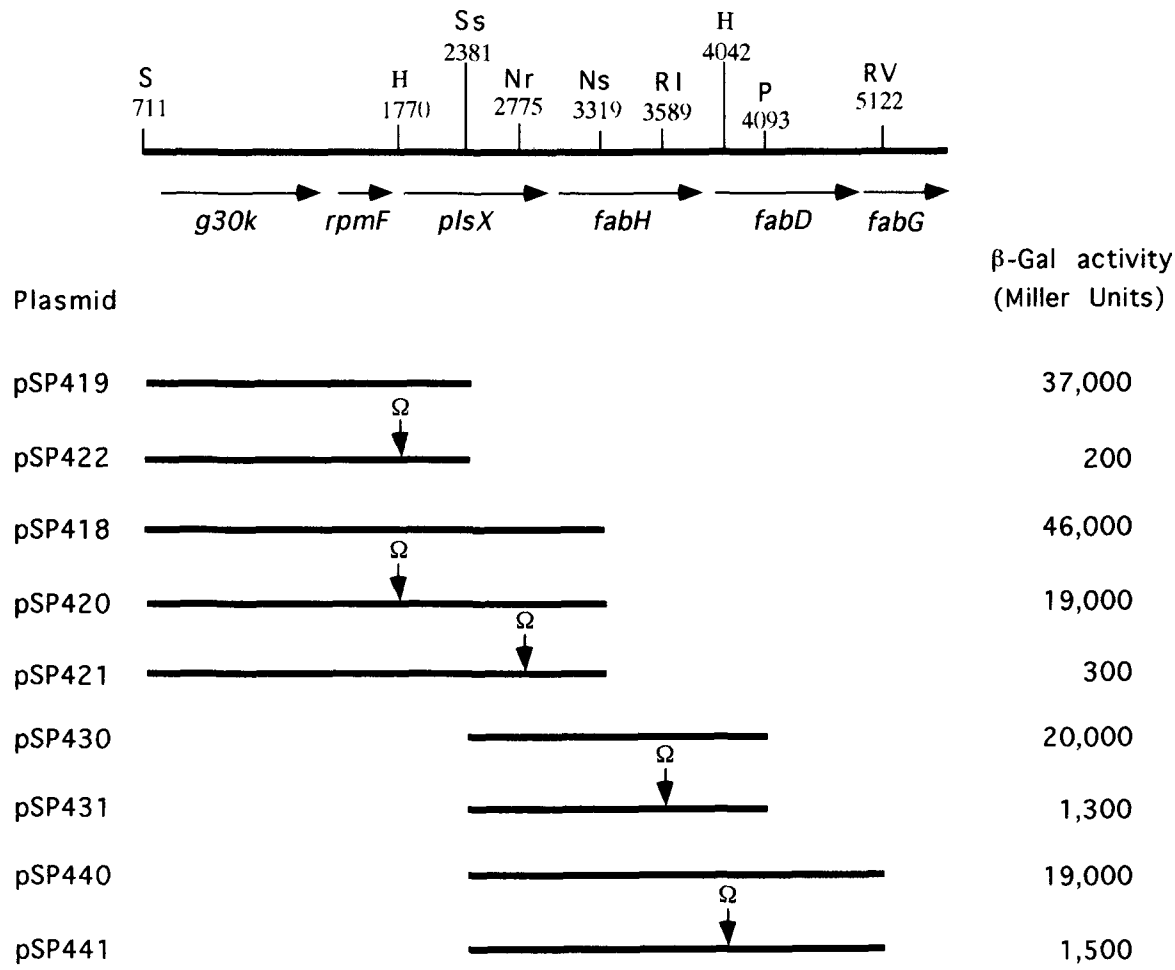

Fig. 1. Structure and analysis of transcriptional fusions. The indicated restriction fragments were cloned upstream of the promoterless *lacZ* gene of pSP417. Ω denotes the spectinomycin resistance omega cassette containing transcriptional terminators. DH5αF′ cells were transformed with plasmids carrying the fusions and β-galactosidase activity was measured as described in section 2. Background β-galactosidase activity for DH5αF′(pSP417) was 50 U. Numbering of nucleotides starts from the first base of the *PstI* site located within the *orfX* gene [2]. Restriction sites are abbreviated as follows: S, *SalI*; H, *Hin*dIII; Ss, *Ssp*I; Nr, *Nru*I; Ns, *Nsi*I; RI, *Eco*RI; P, *Pvu*II; RV, *Eco*RV. Only those restriction sites used for cloning or insertional mutagenesis are indicated.

expression (plasmid pSP420) while the insertion within the 3′ part of the *plsX* gene at *Nru*I abolished expression of *lacZ* (plasmid pSP421). These results indicate the presence of an additional promoter within the *plsX* gene that contributes to *fabH* transcription. The extent of the polar effect revealed that this promoter, in multicopy plasmids, provides approximately 40% of the *fabH* transcription.

Similar insertional mutagenesis was performed for *fabH* and *fabD* (plasmids pSP430 and pSP431) and for *fabD* and *fabG* (pSP440 and pSP441). The strong polar effects of insertions at either the *Eco*RI site (plasmid pSP431) or the *Hin*dIII site (plasmid pSP441) showed that the *fabH* transcripts continue into *fabD* and *fabG* and all three genes are cotranscribed. *fabH* encodes β-ketoacyl-ACP synthase III that may be a potential regulator of fatty acid biosynthesis in bacteria [9]. Malonyl CoA-ACP transacylase encoded by *fabD* provides malonyl-ACP, the key intermediate of fatty acid synthesis [10]. Mutants deficient in malonyl CoA-ACP transacylase require both saturated and unsaturated fatty acids for growth [11]. *fabG* encodes 3-ketoacyl-ACP reductase acting on an elongation step of fatty acid biosynthesis [10].

Based on the results of analysis of all the fusions shown in Fig. 1, we concluded that the *rpmF* gene and the *plsX-fab* genes are cotranscribed. This is the only known example where lipid biosynthetic genes and a ribosomal protein gene comprise an operon. Such organization is likely to play an important role in the coordinate regulation of ribosome and cell membrane synthesis. Further studies concerning transcriptional organization and regulation of the *rpmF-plsX-fab* operon are in progress.

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