The gene coding for lipoprotein signal peptidase (*lspA*) and that for isoleucyl-tRNA synthetase (*ileS*) constitute a cotranscriptional unit in *Escherichia coli*

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The *lspA* gene coding for lipoprotein signal peptidase is located very close to the *ileS* gene coding for isoleucyl-tRNA synthetase on the *Escherichia coli* chromosome. Deletions were generated in vitro from both ends of the 4.3 kb fragment that carries the *lspA* gene and the *ileS* gene, and the expression of the two genes was examined before and after insertion of the *trp* promoter-operator at one end. The results indicate that the *lspA* and *ileS* genes constitute a cotranscriptional unit in the order of promoter-*ileS-lspA*. The gene order of *dnaJ-rpsT-ileS-lspA-dapB* around 0.5 min on the *E. coli* chromosome map was also determined.

Lipoprotein signal peptidase *Signal peptidase* *Lipoprotein* *Isoleucyl-tRNA synthetase* *Protein secretion* ileS-lspA *operon*

1. INTRODUCTION

Lipoprotein signal peptidase catalyzes the cleavage of the signal peptide from the glyceride-containing precursors of lipoproteins during their secretion across the cytoplasmic membrane in *Escherichia coli* [1]. The *lspA* gene coding for the signal peptidase has been cloned [2,3] and mapped at about 0.5 min on the *E. coli* chromosome map near the *ileS* gene that codes for isoleucyl-tRNA synthetase [4].

Here we report that the *lspA* and *ileS* genes constitute one transcriptional unit.

2. MATERIALS AND METHODS

2.1. *Materials*

All restriction endonucleases and synthetic linker oligonucleotides (*Eco*RI, dGGAATTCC; *Xho*I, dCCTCGAGG) were from Takara Shuzo. Exonuclease BAL31 was from New England Biolabs, and S1 nuclease and the Klenow fragment of *E. coli* DNA polymerase I from Bethesda Research Laboratories.

2.2. *Bacteria and plasmids*

E. coli JE5506 used as a host cell was described in [5]. Plasmid pHY11 was described in [2]. Promoter vector plasmid pDR720 carrying the *trp* promoter-operator was obtained from P-L Biochemicals.

2.3. *Media*

Difco penassay broth medium no.3 was used in general. L-Broth was used to prepare competent cells. For induction of enzymes that are under the control of the *trp* promoter-operator, M9-0.2% glucose, supplemented per ml with 20 μg β-indoleacrylic acid, 2 μg thiamine and 5 μg each arginine, histidine and proline, was used. Cells harboring a plasmid were grown with tetracycline (10 μg/ml).

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Abbreviations: LP, major outer membrane lipoprotein; proLP, glyceride-containing precursor of LP; kb, kilobase pair; *trp*PO, *trp* promoter-operator

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2.4. *Enzyme assays*

The assay for signal peptidase was described in [6]. [^{35}S]Methionine-labeled proLP was used as substrate. Conversion of proLP to LP was analyzed on SDS-polyacrylamide slab gel by fluorography [7]. The assay for isoleucyl-tRNA synthetase was described in [8].

2.5. *DNA techniques*

Plasmid DNA was prepared as in [9]. Restriction endonuclease reactions were performed as in [10]. The BAL31 digestion was performed as proposed by the manufacturer. Protruding ends of BAL31 fragments were removed by S1 treatment as in [11]. Gel electrophoresis of DNA fragments was carried out on agarose gel (0.4 or 0.8%) or polyacrylamide gel (5%) with 50 mM Tris–borate (pH 8.3)–1 mM EDTA buffer. DNA fragments were eluted from polyacrylamide gel by electroelution and from agarose by the freeze and squeeze technique [11]. Transformation was carried out as in [12].

3. RESULTS AND DISCUSSION

3.1. *pHY11 carries both the* lspA *and* ileS *genes*

Plasmid pHY11 possesses the *lspA*-carrying 4.3 kb fragment derived from the *E. coli* chromosome [2]. Genetic complementation experiments in [4] did not support the existence of the *ileS* gene in this plasmid. However, the direct assaying of the isoleucyl-tRNA synthetase activity revealed that the plasmid carries the *ileS* gene in addition to the *lspA* gene (table 1). Since the M_r of the synthetase is about 112000 [13], the *ileS* gene must occupy at least 3.4 kb of the 4.3 kb fragment.

3.2. *Construction of deletions of the 4.3 kb fragment*

To determine the location of the *lspA* and *ileS* genes in the 4.3 kb fragment, deletions were constructed as shown in fig.1. First, one of the *Eco*RI ends of the 4.3 kb was converted to a *Xho*I end through partial digestion with *Eco*RI (fig.1A). The site of conversion was confirmed by restriction analysis with *Eco*RI and *Stu*I. Deletions from the *Xho*I end and those from the *Eco*RI end of the 4.3 kb fragment were then constructed as shown in fig.1B. The extent of deletions in individual plasmids was determined by analyzing the *Xho*I-*Eco*RI fragments on 0.8% agarose and the results are shown in fig.2.

Table 1

Effect of deletions on the *ileS* expression as determined from the isoleucyl-tRNA synthetase activity

Plasmid	Synthetase activity
pHY11	920
pHY10T	2120
pYK110T	5670
pYK160T	100
pYK210T	4820
pYK220T	3970
pYK230T	50
pYK250T	10

All values were subtracted for that without plasmid (440 cpm). [^{14}C]Isoleucine (spec. act. 50 μCi/mmol) was used as substrate, and radioactivity incorporated into isoleucyl-tRNA was counted. The enzyme activity was expressed as cpm/μg envelope protein per 2 min. pYK10T was constructed from pYK10 by *trp*PO insertion at the *Xho*I site

Unexpectedly, none of the deletions from either the *Xho*I site or the *Eco*RI site exhibited the signal peptidase activity, despite the fact that the *ileS* gene must occupy a larger portion of the 4.3 kb fragment. One plausible explanation is that the *lspA* and *ileS* genes constitute a cotranscriptional unit having a common promoter near one end of the 4.3 kb fragment and the *lspA* gene near the other end with the *ileS* gene in between.

3.3. *Effect of insertion of the* trp *promoter-operator on* lspA *and* ileS *expression*

To examine the possibility discussed above, insertion of the *trp* promoter-operator (*trp*PO) into either the *Xho*I site (fig.1C) or the *Eco*RI site was attempted to investigate restoration of the activity of the two enzymes. When *trp*PO was inserted into the *Xho*I site, restoration of the enzyme activity was observed. The results are shown in table 1 and fig.3, and summarized in fig.2. The defect in *ileS* and *lspA* expression, that was caused by a small deletion at the *Xho*I end, was overcome by the *trp*PO insertion (pYK110T), indicating that this region functions as the promoter for both genes.

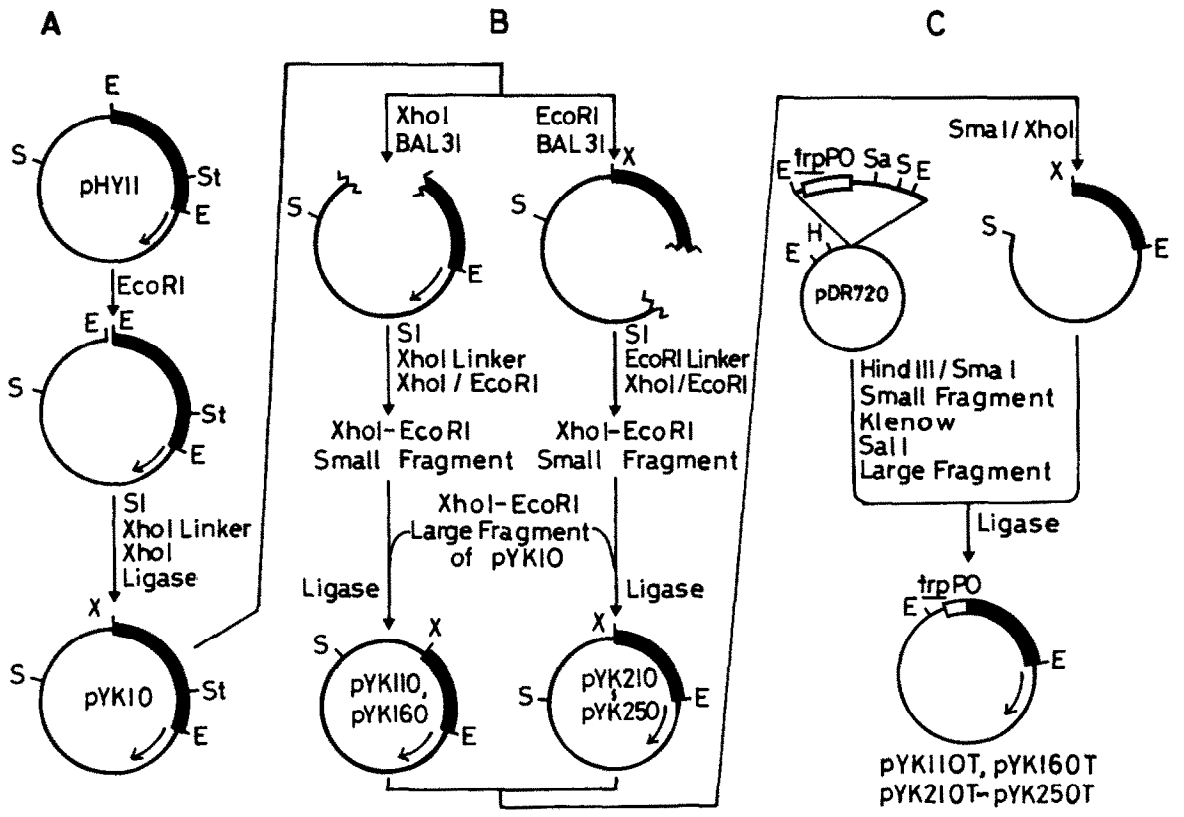

Fig.1. In vitro deletion mutagenesis of the 4.3 kb *ileS-lspA* region of the *E. coli* chromosome (A,B) and insertion of *trp*PO (C). Solid bars, the *ileS-lspA* region derived from the *E. coli* chromosome. The *tet* genes responsible for tetracycline resistance are indicated by arrows. S, St, E, X, H and Sa represent *Sma*I, *Stu*I, *Eco*RI, *Xho*I, *Hin*dIII and *Sal*I, respectively. A *trp*PO-carrying plasmid is represented by adding 'T' to the name of the plasmid from which it was derived.

Further deletion did not allow restoration by *trp*PO of the *ileS* function but did allow that of the *lspA* function (pYK160T). Contrary to this, a small deletion extending from the *Eco*RI site suppressed *lspA* expression, but not *ileS* expression (pYK210T, pYK220T). The *ileS* product (M_r 112000) was also examined on polyacrylamide gel and the results were consistent with those in table 1 (not shown).

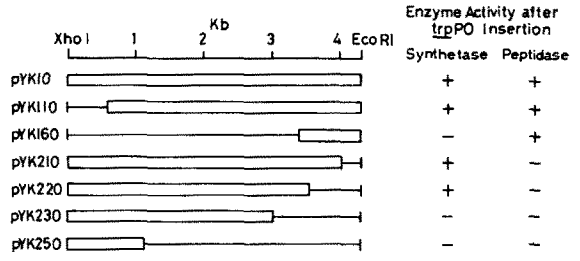

Fig.2. Deletions and their effect on synthesis of lipoprotein signal peptidase and isoleucyl-tRNA synthetase. Bars represent deletions in individual plasmids. Enzyme data were those for plasmids having *trp*PO at the *Xho*I site (taken from table 1 and fig.3).

3.4. *Genomic organization of* lspA *and* ileS

The results presented above strongly support the following genomic organization: (i) The promoter region responsible for the transcription of both the *ileS* and *lspA* genes is localized very close to the *Xho*I end of the 4.3 kb *Xho*I-*Eco*RI fragment; (ii) This region is followed by the coding region of the *ileS* gene encompassing about 3.4 kb; (iii) The coding region of the *lspA* gene is localized near the *Eco*RI end of the fragment. Recently, we sequenced the right half of the *Xho*I-*Eco*RI fragment and identified part of the *ileS* gene and the

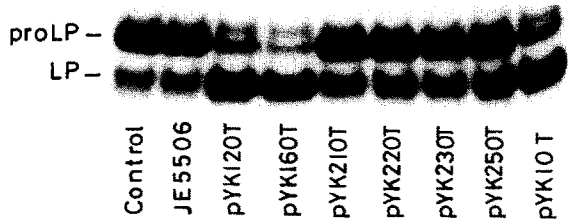

Fig.3. Effect of deletions on the *lspA* expression as determined from the signal peptidase activity. An envelope fraction was prepared from JE5506 harboring an indicated plasmid and conversion from proLP to LP was assayed. [^{35}S]Methionine-labeled proLP was used as substrate. Control, without enzyme (envelope).

entire coding region of the *lspA* gene (unpublished). The results were perfectly consistent with the genomic organization proposed here. In [4], the gene order around the *lspA* and *ileS* genes was suggested to be *dnaJ-rpsT-lspA-ileS-dapB* based on genetic complementation analysis. Taking the genomic organization revealed here into consideration, we propose the correct order of *dnaJ-rpsT-ileS-lspA-dapB*.

The physiological importance of the *ileS-lspA* cotranscriptional unit, if any, is not yet known. Isoleucine is a common constituent of signal peptides. The coordinated synthesis of the synthetase and the signal peptidase might have something to do with the regulation of secretory protein synthesis. Alternatively, the *ileS* protein might be involved in the protein secretion process in addition to its functioning in protein synthesis.

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