Evidence for the ability of L10 ribosomal proteins of *Salmonella typhimurium* and *Klebsiella pneumoniae* to regulate *rplJL* gene expression in *Escherichia coli*

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Genes *rplJ*, coding for ribosomal protein L10 of *Salmonella typhimurium* and *Klebsiella pneumoniae*, have been cloned on pUC plasmid. The resultant multicopy recombinant plasmids were detrimental for the growth of normal *JM*101 *E. coli* host cells and harmless for the mutant *JF*3029 host. This negative effect is the evidence for the ability of heterologous L10 proteins to regulate expression of *rplJL* genes in *E. coli.* Nucleotide sequence was determined completely for *S. typhimurium rplJL'* DNA portion and partially for *rplJL'* genes of *K. pneumoniae.* According to the nucleotide sequence data obtained three amino acid substitutions differ L10 proteins of *S. typhimurium* and *E. coli* and the long range, providing for the coupled translations of L10 and L7/L12 cistrons in *E. coli* mRNA is also valid for *S. typhimurium* and *K. pneumoniae.*

1. INTRODUCTION

Genes *rplKAJL-rpoBC*, encoding ribosomal proteins L11, L1, L10 and L7/L12 and RNA-polymerase β and β' subunits, are clustered in the six species of *Enterobacteria*: *E.coli*, *Shigella*, *Salmonella*, *Citrobacter*, *Klebsiella*, *Serratia* and *Proteus* [1]. Expression of genes in the *rplKA* and *rplJL* operons of *E.coli* is controlled through translational feedback, exerted by the regulatory proteins L1 and L10 [2]. Experimental evidence has been obtained for the ability of proteins L1 of *Serratia marcescens* and *Proteus vulgaris* to regulate expression of the *E.coli rplKA* operon [3]. While studying the structure and functional topology of *E.coli* protein L10 [4] we have compared it to the homologous proteins of other bacteria, and in particular examined the possibility that these proteins could regulate expression of *rplJL* genes in *E.coli.* Cloning of an *rplJL-rpoBC'*-containing fragment of *S. typhimurium* DNA resulted in the unstable recombinant plasmid pNL1 [5]. Our experimental practice in cloning the homologous *E.coli* fragment in plasmid pUC and phage M13 suggested that superproduction of proteins L10 and L7/L12 can affect the viability of host *E.coli* cells [6]. Superproduction of L10 as well as other regulatory ribosomal proteins from a multicopy plasmid is detrimental to growth or even lethal for normal host cells, due to blocking of the expression of *rplJL* genes [7]. This effect can be overcome by maintenance of the respective recombinant DNA construct in a host containing mutations in the L10 target sequence which render cells tolerant to high level production of protein L10 [8]. Cloning of the *rplJL* genes from *S. typhimurium* and *K. pneumoniae* was carried out to test the possibility that the encoded L10 proteins could influence the expression of the *rplJL* operon genes in *E.coli.* We supposed that if they can exert a feedback effect in *E.coli*, heterologous L10 proteins would produce the growth-inhibiting effect when overexpressed from the multicopy recombinant plasmids in normal host cells. We also expected that this effect, if produced, could be overcome by maintenance of the respective plasmids in mutant *E.coli* JF3029 cells, insensitive to high level expression of *E.coli* L10 protein.

2. MATERIALS AND METHODS

Routine techniques described in the manual of Maniatis et al. [9]. were used for recombinant DNA construction, isolation and analysis. To clone the *rplJ* gene of *S. typhimurium*, the pNL1 recombinant plasmid [5], carrying the *rplA* 'P_{L10}-*rpoBC*' region of *Salmonella typhimurium* DNA, was used. For large scale preparation, pNL1 was maintained in mutant *JF*3029 host. The fragment containing P_{L10}-*rplJ* was isolated by *Eco*RI digestion of pNL1 and further ligated to *Eco*RI digested and calf intestine phosphatase treated pUC19 DNA [10]. The ligation mixture was introduced into normal *JM*101 and mutant *JF*3029 *E.coli* host cells [10]. Selection in *JM*101 host was carried out by *Lac*$^-$ phenotype of recombinant clones. To visualize the growth-detrimental effect, pMW12 and pMW14 were maintained in *JM*101 and *JF*3029 host *E.coli* cells, which were kindly provided by J. Friesen. 1.5 ml of overnight culture was used for mini-scale preparation and comparison of recombinant plasmid DNA yields.

To sequence the *S. typhimurium* gene *rplJ* a series of increasingly large deletions extending from the 3′ end of the inserted *rplJL*′-containing fragment was constructed. Subclones of pMW12 were made as shown in Fig. 1. Deletion of pMW12 *Ecl*136II(*Sac*I)-*Sna*BI fragment, *Hind*III-B fragment, *Sma*I-B fragment, and *Pst*I-

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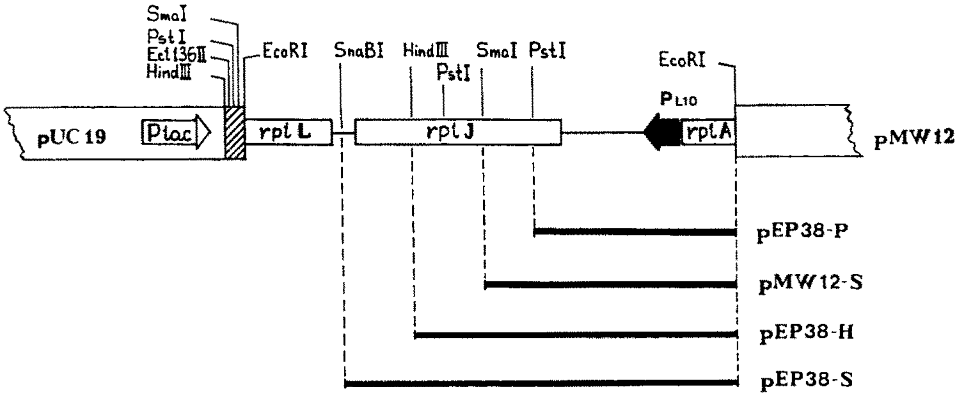

Fig. 1. Subcloning of pMW12 for DNA sequencing. Enzymes used to generate deletions extending from the 3′-side of *rplJL′* insert are indicated. Bold lines show the remaining *rplJ* portions. The polylinker region of the vector pUC plasmid is hatched.

B + C fragments resulted in construction of pEP38-S, pEP38-H, pMW12-S and pEP38-P respectively.

DNA primary structure analysis was carried out according to [11]. Direct and reverse DNA primers as well as almost all of the restriction enzymes used were purchased from ESP Fermentas (Vilnius).

To clone the *Klebsiella pneumoniae rplJ* gene, chromosomal DNA was isolated from *K. pneumoniae* according to [12], digested with *Eco*RI and electrophoresed. The DNA band with the mobility corresponding to that of the 2,15 kbp P_{L10}-*rplJ* containing *E.coli* chromosomal DNA fragment, was eluted and ligated to *Eco*RI opened pUC19. The ligation mixture was introduced into *E.coli JM*101 and 200 Lac$^-$ clones were chosen for mini-scale plasmid DNA screening. The 882 bp *Bsp*RI fragment, containing the coding region of *E.coli rplJ* gene, was digested with Cfr9I(*Xma*I) and the protruding 5′-ends were labelled with ^{32}P-dCTP by Klenow DNA polI treatment. Nine clones gave positive signals when dot-hybridized to the *E.coli rplJ* coding region containing the *Bsp*RI 882 bp fragment.

3. RESULTS

The *rplJ* gene of *Salmonella typhimurium* was isolated in the *Eco*RI-E fragment from the pNL1 recombinant plasmid reported to be highly unstable in normal *HB*101 *E.coli* host cells [5]. We assumed that instability of the pNL1 plasmid might result from the negative effect of *S. typhimurium* L10 superproduction on normal host *E.coli* cells. If so, this effect was evidence of the ability of this heterologous r-protein to regulate expression of *rplJL* genes in *E.coli*. We supposed that stability of pNL1 would be increased by introducing it into mutant *E.coli JF*3029 host cells, insensitive to high level production of *E.coli* L10 protein. In fact, in *JF*3029 cells pNL1 could be maintained stably and prepared in high yield. The strategy of *S. typhimurium rplJ* gene cloning was based on the identity of *E.coli* and *S. typhimurium Eco*RI digestion patterns. This made highly probable the location of *S. typhimurium rplJ* gene on the *Eco*RI-E-fragment of pNL1. This fragment was isolated and cloned in pUC19 [10]. Similarly to pEP20, carrying the *E.coli* P_{L10}-*rplJ* containing DNA fragment [4], all the 20 recombinant pMW12 plasmids analyzed carried the inserted *Eco*RI fragment in the opposite orientation to that of the vector's P_{lac}. The pMW12 was growth detrimental to normal *JM*101 *E.coli* cells and could be maintained stably in mutant *JF*3029 host. We were interested in the possible effect of the alternative orientation of *S. typhimurium* and *E.coli* P_{L10}-*rplJ* containing fragments cloned in pUC19. For this reason mutant *JF*3029 *E.coli* cells were transformed by the products of ligation of *Eco*RI opened pUC19 DNA and the respective *rplJ*-containing fragments. Plasmids pEP20-1 and pMW12-1 with the alternative orientation of *E.coli* and *S. typhimurium* P_{L10}-*rplJ* DNA regions were obtained in *JF*3029 cells, though both plasmids were growth-detrimental even for this mutant host. Thus, the slight increase of both *E.coli* and *S. typhimurium rplJ* genes' expression resulting from their additional transcription driven from P_{lac}, though at its leaky constitutive level, caused a similar dramatic effect. The same feature has been observed by increasing the expression of genes encoding other regulatory ribosomal proteins of *E.coli*, for example, rps4 (r-protein S4) (D. Draper, personal communication).

The ability of *S. typhimurium* L10 protein to regulate expression of *E.coli rplJL* genes suggested that the structures of *S. typhimurium* and *E.coli* L10 proteins are similar. Data obtained by DNA sequencing (Fig. 2) confirmed the striking similarity of *S. typhimurium* and *E.coli* [13] *rplJ* gene coding regions. Twelve nucleotide substitutions in the structural portion of gene rplJ were observed, though the deduced primary structure of *S. typhimurium* L10 protein differs by only three amino acid substitutions ($Ala_{62} \rightarrow Val$, $Pro_{67} \rightarrow Gln$ and $Ala_{74} \rightarrow Thr$) from that of *E.coli* [13]. It has been proposed [14] that long range interactions resulting in formation of mRNA secondary structure, lead to masking of the *rplL* ribosome binding site and coupling of translation of L10 and L7/L12 cistrons in *E.coli* L10-L7/L12 bicistronic mRNA. The nucleotide sequence of the *rplJL′* genes determined here confirms that the same mRNA secondary structure is possible also for *S. typhimurium* L10-L7/L12.

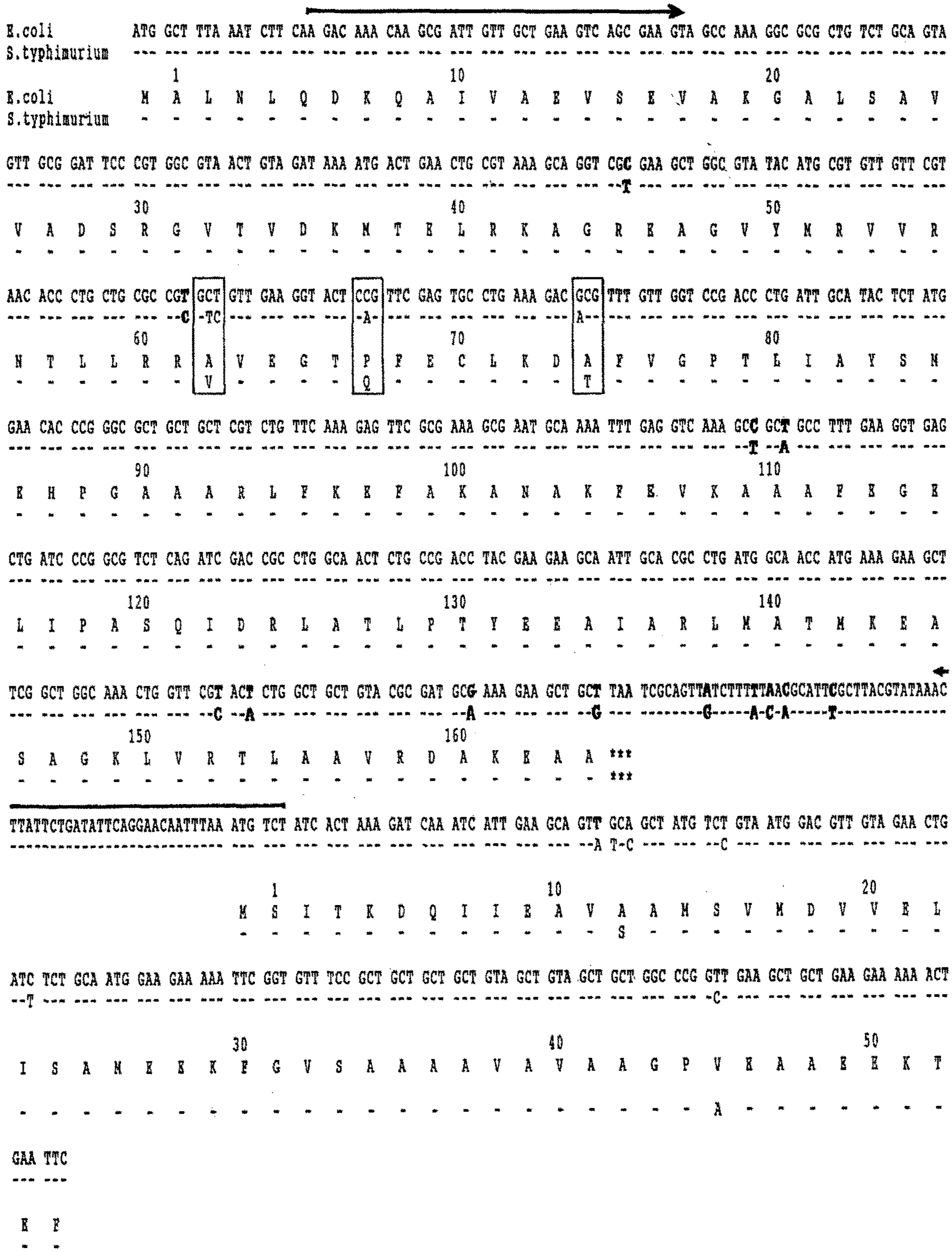

Fig. 2. Comparison of nucleotide and deduced amino acid sequences of *S. typhimurium* and *E.coli* (as determined by Post et al. [13]) *rplJL'* genes. Bold horizontal arrows indicate *rplJ* and *rplL* 5'-terminal regions, involved in long range interaction on L10-L7/L12 mRNA [14].

To clone the *rplJ* gene of *Klebsiella pneumoniae*, chromosomal DNA was isolated, as described in section 2 and further digested with *Eco*RI. The hybridization analysis of the *rplKAJL* genes of *E.coli* and *Klebsiella* reported by Tittawella [1] demonstrated the presence of a similar 2,15 kbp *Eco*RI-fragment (containing the *rplA'*-P_{L10}-*rplJL'* region in *E.coli* DNA). The pMW14 recombinant plasmid was found growth detrimental for

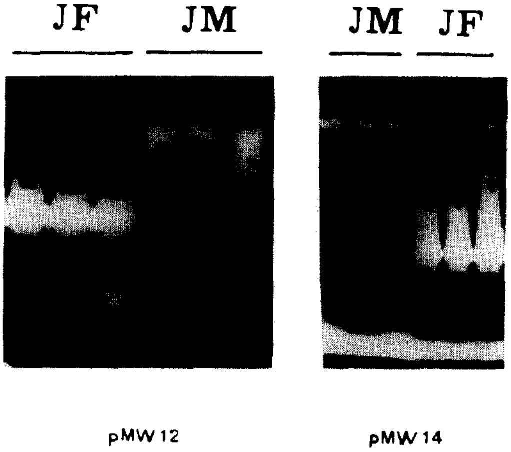

Fig. 3. Growth-detrimental and harmless maintenance of the recombinant pW12 and pMW14 plasmids in normal *JM*101 and mutant *JF*3029 *E.coli* host cells illustrated by comparison of mini-preps of plasmid DNA.

normal *JM*101 *E.coli* cells and harmless for the mutant *JF*3029 host (Fig. 3). This fact indicated that protein L10 of *Klebsiella pneumoniae* can regulate expression of the *E.coli* chromosomal *rplJL* genes. A partial sequence of the *Klebsiella rplJL'* DNA region, carried by pMW14 (manuscript in preparation) revealed the presence of the second *Pst*I cleavage site in the coding region of *rplJ*, as in *Salmonella*. The intergenic *rplJL* regions of *Klebsiella* and *Salmonella* DNA also possess strikingly similar nucleotide substitutions, as compared to the corresponding region of *E.coli* DNA.

4. DISCUSSION

This paper describes cloning of *S. typhimurium* and *K. pneumoniae rplJ* genes on multicopy pUC plasmid with the aim of studying the effect of high level expression of heterologous L10 proteins on the host *E.coli* cells. Maintenance of both pMW12 and pMW14 recombinant plasmids, containing *rplJ* genes of *Salmonella typhimurium* and *Klebsiella pneumoniae*, respectively, was growth-detrimental for normal *E.coli* cells. Both recombinant plasmids, expressing the heterologous L10 proteins appeared harmless for the mutant *E.coli JF*3029 host, insensitive to high level production of *E.coli* L10 protein. The same phenomenon is characteristic for *E.coli* L10 overproducing plasmids and indicates, therefore, the ability of these heterologous proteins to regulate expression of *rplJL* genes in *E.coli*. According to its primary structure deduced from the nucleotide sequence of the *rplJ* gene, protein L10 of *Salmonella* differs from *E.coli* L10 by 3 amino acid substitutions, $\text{Ala}_{62}\rightarrow\text{Val}$, $\text{Pro}_{67}\rightarrow\text{Gln}$, $\text{Ala}_{74}\rightarrow\text{Thr}$. These residues are, thus, non-essential for the regulatory function of L10 protein. A long range interaction resulting in the masking of the *rplL* ribosome binding site and coupled translation of proximal and distal cistrons of L10-L7/L12 mRNA was proposed for *E.coli* [14]. No changes were observed in the nucleotide sequence of the translation initiation regions of *Salmonella typhimurium* and *Klebsiella pneumoniae rplJ* and *rplL* genes, as compared to *E.coli*. Thus, the same mRNA secondary structure and mechanism of coupled translation of both cistrons may be valid for L10-L7/L12 encoding mRNA in *Salmonella typhimurium* and *Klebsiella pneumoniae*.

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