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COMPARISON OF NUCLEOTIDE SEQUENCES OF THE RPLJ LEADER IN ENTEROBACTERIA

Determination of primary structure and comparison of the mRNA secondary structure in the rplJL lider was carried out for five species of Enterobacteria. The highly conserved structural organisation was found.

Gene expression in the *rplJL* operon encoding ribosomal proteins *L10* and *L12* of *E. coli* is regulated autogenously at the level of translation by the feedback mechanism [1, 2]. The regulatory protein (*L10* or *L10-L12* complex) binds the *rplJ* leader about 150 nucleotides upstream of the start codon and results in translational block [3], hypothesized to occur because of mRNA secondary structure transition to a form in which the ribosome binding site is sequestered [3]. We observed a negative effect, typical for the feedback regulating proteins, when the *E. coli L10* was overproduced in *Salmonella* [4], *Klebsiella* [5] and *Citrobacter* [6]. We reasoned that the ability of the *E. coli L10* to regulate expression of heterologous *rplJL* operons was provided by the highly conserved structure of the protein binding site as well as of whole the *rplJ* leader. Comparison of the *rplJ* leader sequences from five species of *Enterobacteria* was aimed to find structural similarities of potential functional importance.

Fragments containing the *rplJ* 5′-terminal portion were isolated from the chromosomal DNA of *Citrobacter freundii* and *Serratia marcescens* by PCR technique [7] and cloned in *pUC* plasmid [8]. Sequencing was performed by the procedure [9]. Two recombinant phages kindly provided by M. Nomura, M13 018157 (with the rplK′AJL rpoBC′ DNA fragment from P. vulgaris) and M13 018155 (with the rplK′AJL′ fragment from S. marcescens), were used to subclone and sequence the respective rplJ leaders. Comparison of the determined rplJ leader sequences of C. freundii, P. vulgaris and S. marcescens with those of E. coli [10] and S. typhimurium [11], shown in Fig. 1., revealed the regions with highly and completely conserved structure. The E. coli rplJ leader contains five double-stranded regions (I—V) [12]. The mRNA secondary structure predicted for the sequenced leaders showed that similarly to E. coli five regions of base-pairing are typical also for rplJ leaders in other Enterobacteria (Fig. 2). Nucleotides in region IV ascribed to *L10* binding site [13—15] are completely conserved. Another structural similarity shared by all compared species is the GNAA tetra loop in stem-loop structure IV. Noteworthy, the GNAA and UNCG tetraloops can confer unusual stability to the stem [16]. The number of conserved base-pairings at foundations of all five stems is equal within all species. The conserved length of the single-stranded regions between stems IV and III (consisting of 3 nucleotides) and between stems II and I (consisting of 9 nucleotides) may be of structural importance. The single stranded region between stems III and IV, comprising 7 nucleotides in all but the E. coli leader, may have a similar function. In vitro structural analysis [12] failed to proove the conformational switch in the E. coli rplJ leader proposed to cause translational block [12]. Interestingly, that sequences presumed

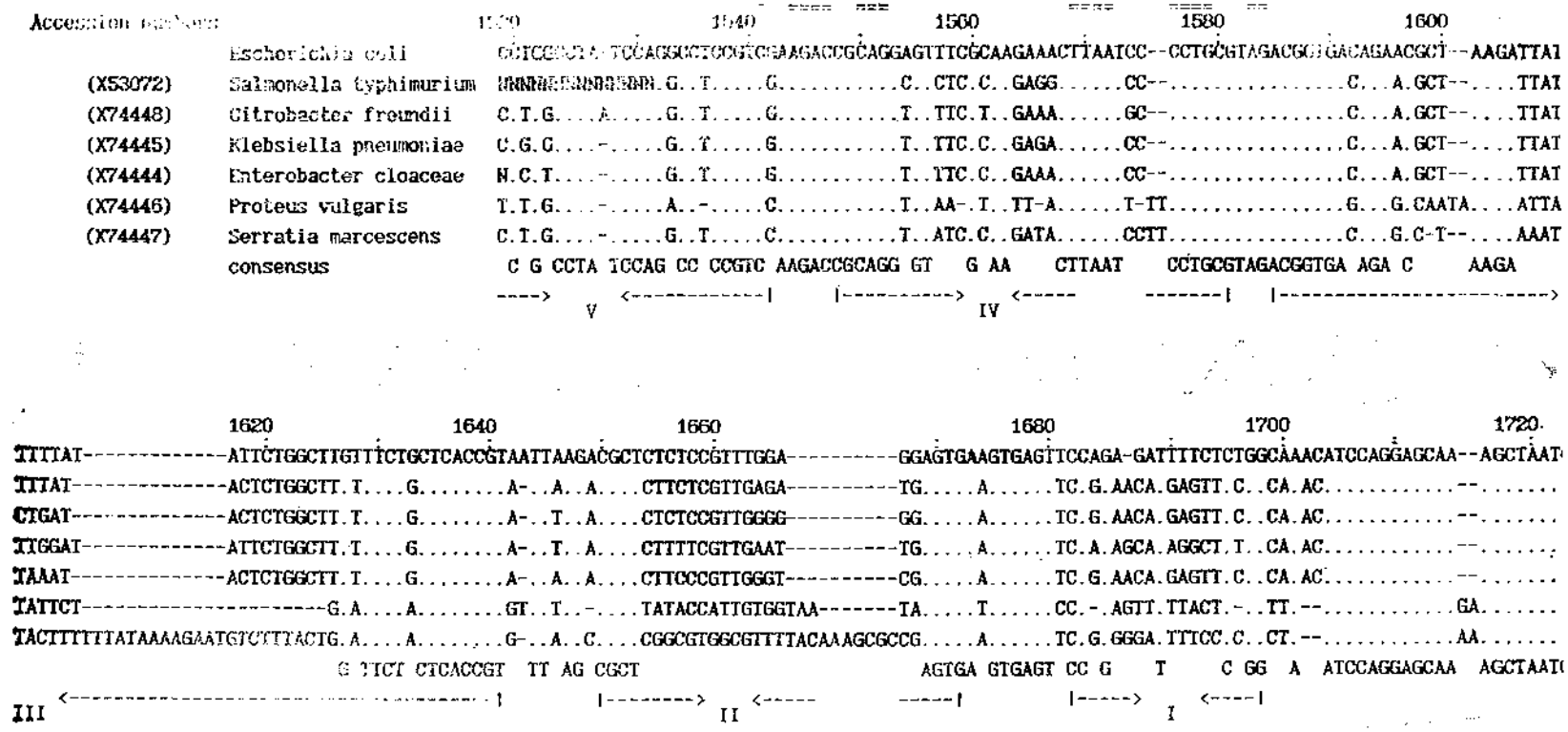

Fig. 1. Alignment of *rplJ* leader sequences. Nucleotide numbers correspond to *E. coli* [10]. *L10* binding site [13—15] is overlined. (·) — denotes identi matches; (—) denotes gaps introduced to optimize the alignment. Arrows with roman numerals correspond to regions of base-pairing (I—V) in the *E. li rplJ* leader [12]. In the «consensus» sequence latters denote conserved nucleotides. *K. pneumoniae* and *E. cloacea rplJ* leaders sequenced late, has been discussed but completely support consensus mRNA secondary structure proposed.

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necessary for the switch are completely conserved in all the leaders, as if pointing out another possible functional role. Nucleotides C1548, G1590, G1594, C1634 and G1640 were shown to be important for the functional role of the E. coli leader and are conserved among the analyzed species. Also concerved are A1571 and A1572 in the bulge loop of region

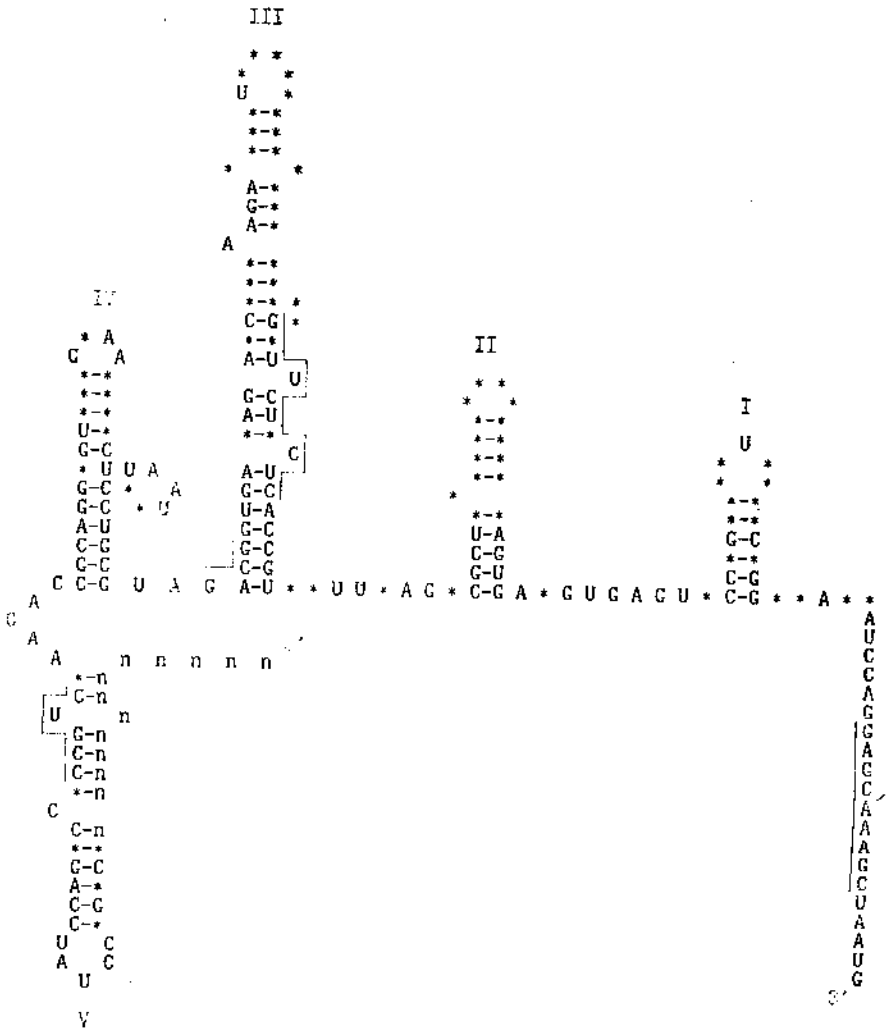

Fig. 2. A consensus mRNA secondary structure of enterobacterial *rplJ* leader based on the secondary structure of the *E. coli* leader mRNA [12] and alignment of five enterobacterial leader sequences. Latters denote conserved nucleotides. (*) — denote variable nucleotides, *n* — not determined. Sequences presumed to provide structure transition of the *E. coli rplJ* leader to the untranslatable form [3] are marked

IV. In the E. coli leader these two bases are strongly protected by *L10-L12* complex [12]. Besides the general similarity forming the ground for the consensus secondary structure (Fig. 2) rightful for all compared rplJ leaders, additional similarities can be found within two subgroups: Escherichia, Salmonella, Citrobacter and Proteus, Serratia.

In E. coli translation regulation of *L10-L12* mRNA by the feedback mechanisms requires at least two structural elements. The rplJ leader region binds the regulatory protein which results in translational block. Repression of *L12* translation is achieved via the long-range interaction between the *L10* and *L12* cistrons [17]. Comparison of the sequenced 5′-portions of the rplJ coding sequences (results not shown) revealed the

complete conservation of the rplJ sequence necessary for translational coupling. Therefore, phylogenetic comparison lends evidence for similarity of structural requisites of the feedback regulation mechanism in Enterobacteria.

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ПОРІВНЯННЯ НУКЛЕОТИДНИХ ПОСЛІДОВНОСТЕЙ ЛІДЕРІВ rplJL ОПЕРОНІВ ЕНТЕРОБАКТЕРІЙ

Резюме

Проведено визначення нуклеотидної послідовності та порівняння структур лідерних областей *rplJ* оперонів п'яти ентеробактерій. Виявлено високу консервативність структурової організації.

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