A Rare 920-Kilobase Chromosomal Inversion Mediated by IS1 Transposition Causes Constitutive Expression of the yiaK-S Operon for Carbohydrate Utilization in Escherichia coli

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The regulator of the yiaK-S operon, currently assigned a carbohydrate utilization function in Escherichia coli, is inactivated by a genome rearrangement that leads to the constitutive expression of the operon. The yiaK-S constitutive cells acquire the ability to utilize the rare pentose l-lyxose. Restriction analysis and sequencing of the constitutive expression of the operon. The yiaK-S constitutive expression of the operon. The regulator gene indicates that it is disrupted by foreign DNA. The insert consists of a large inverted fragment of DNA of 920 kilobases flanked by two IS1 elements with opposite polarity. One corresponds to that found naturally at min 0.4 of the bacterial chromosome and the other to a new copy transposed into the regulator gene located at min 80.6. This insertion-inversion could be the result of the intramolecular transposition mechanism itself, a gene rearrangement rarely originated by IS1. Alternatively, it could be attributed to the homologous recombination between the IS1 at min 0.4 and the IS1 transposed intermolecularly into the yiaK-S regulator gene. The participation of a rare IS1-mediated inversion in the evolution of a stable phenotype is thus identified.

Most known mobile IS1 can be inserted at a large number of chromosomal sites. They can subsequently be recombined and excised precisely or imprecisely, and they can engender inversions or deletions in neighboring chromosomal regions (1, 2). The presence of these insertion elements in the chromosome of Escherichia coli and other bacteria such as Salmonella typhimurium or Bacillus subtilis originally became apparent as a result of the transposition of these sequences from their natural positions into functional genes, which can lead to recognizable mutant phenotypes. As a corollary, their derived deletions, inversions, and other gene rearrangements could also result in phenotypic changes that could normally be stable and easily identifiable.

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inversions documented so far. Here the *yiaK*-S operon (locus *lyx* at 80.6 min of the *E. coli* chromosome) (19), has been found to be constitutively expressed in the mutant strain JA134 selected for its ability to grow on the rare pentose L-lyxose (20). The constitutive expression of the operon has been shown to be the result of a chromosomal inversion secondary to an *IS* transposition into the repressor gene of the *yiaK*-S operon. The metabolic consequences of *yiaK*-S repressor disruption were found to be stable.

**MATERIALS AND METHODS**

*Bacterial Strains and Cell Growth*—The bacterial strains used in this work are *E. coli* K12 derivatives. ECL1 is Hfr*C phoA8 relA1 tonA22 T2* (λ) (21). JA134 is *Lxy* +* rhAB* but is otherwise isogenic to ECL1 (20). JA161 obtained from ECL1 in this work is *yiaK::cat*. NM539 is F− supF, *hsdR* +*mk* +, *lacY* +*P2* (22), and JC7623 is *arg thi thr leu pro his strA* +.*::bar*.

**Preparation of Cell Extracts and Enzyme Activities**—Cell extracts were prepared as described previously (24). L-Xylulose kinase activity was determined from the rate of NADH oxidation. The reaction mixture consisted of 100 mM Tris-HCl buffer (pH 8.0), 4 mM L-xylulose, 1 mM ATP, 1.75 mM phosphoenolpyruvate, 3.5 mM MgCl2, 0.25 mM NADH, 0.35 mM reduced glutathione and 2 units/ml of pyruvate kinase and lactate dehydrogenase.

**DNA Manipulation**—Chromosomal DNA was obtained using the genomic DNA purification kit (Promega, Madison, WI). Plasmid DNA was routinely prepared by boiling the method (25). For a large scale preparation, a crude DNA sample was purified through a column (Qiagen GmbH, Düsseldorf, Germany). Other standard DNA manipulations were performed essentially as described by Sambrook et al. (26).

**Library Construction**—Chromosomal DNA from strains ECL1 and JA134 were totally digested with *SalI* and then ligated with the λ EMBl4 cleaved with *SalI* as described by Sambrook et al. (26). The packaging of ligation mixtures in *vitro* was performed by using the Packagene extract system (Promega) and propagated on NM539 (P2) for Spi selection of recombinant phages. For strain JA134 a partial Sau3A genomic library was also prepared in a *BamHI* digested EMBl4 phage.

**Isolation of RNA and Northern Blot Hybridization**—For preparation of total RNA, cells of a 25-ml culture grown to an *A*~590~ of 0.5 were collected by centrifugation at 5,000 × g for 10 min and processed as described by Belasco et al. (29). Northern blot hybridization was performed with each RNA sample (10 μg) by following the procedure described previously by Moraledo et al. (30).

**RESULTS**

**Expression of yiaK-S Operon**—Mutant strain JA134 is an ECL1 derivative selected for its ability to grow on the rare sugar L-lyxose, which does not support growth of wild-type strain. This mutant was characterized by constitutively expressed *yiaK*-S operon. Lines show the inserts of the indicated plasmids. CAT corresponds to a cassette-encoding chloramphenicol acetyltransferase used for site-directed mutagenesis of *yiaJ*.

**Isolation of RNA and Northern Blot Hybridization**—For preparation of total RNA, cells of a 25-ml culture grown to an *A*~590~ of 0.5 were collected by centrifugation at 5,000 × g for 10 min and processed as described by Belasco et al. (29). Northern blot hybridization was performed with each RNA sample (10 μg) by following the procedure described previously by Moraledo et al. (30).

**Inactivation by CAT Insertion**—The chloramphenicol resistance cassette CAT19 was used in the gene inactivation experiments (31) by inserting it into the restriction site indicated below. This cassette had no terminator in the downstream region of the CAT gene and thus did not cause polarity when inserted in the same orientation as the interrupted gene. The plasmid-carrying inactivated gene was linearized by *SalI* digestion and used to transform strain JC7623 to chloramphenicol resistance. This strain efficiently recombines linear DNA into its chromosome (32). *Plov* lysates obtained from the selected chloramphenicol resistance recombinants were used to transduce the CAT insertions into the parental strain ECL1 or the mutant strain JA134.

**PCR**—Reactions were performed on purified chromosomal DNA with Vent DNA polymerase (New England Biolabs, Beverly, MA) according to the manufacturer’s protocol and 100 pmol of each of the oligonucleotide primers in 100 μl of reaction mixture. The reaction routinely consisted of 30 cycles. The primers used to amplify the junction region proximal to the origin (min 0) in the mutant strain were: REG2 (*GACTTTCATCCATGCGG*) and HIS1 (*GAAAATCATCACCTACGGC*) and those used to amplify the distal junction region were REG1 (*CGCGATGTGACCTTGCG*) and 2SK1 (*AGCCTGGCCTTTTGATGCG*). For Southern analysis covering the full-length of inverted DNA, over 50 probes of 1 kb at intervals of approximately 15 kb were prepared by PCR using pairs of primers of 18–20 bp (not shown), which were designed following the *E. coli* genome sequence in GenBankTM. PCR products were resolved by 2% agarose gel electrophoresis and purified by QiAquick PCR purification kit (Qiagen GmbH).

**RESULTS**

**Expression of yiaK-S Operon**—Mutant strain JA134 is an ECL1 derivative selected for its ability to grow on the rare sugar L-lyxose, which does not support growth of wild-type strain. This mutant was characterized by constitutively expressing the L-xylulose kinase activity involved in the L-lyxose metabolism (20). The gene encoding L-xylulose kinase was cloned in plasmid pJC2 (Fig. 1). Here we have attempted the sequencing of the insert present in pJC2. This sequence displayed total identity with that of the open reading frame located between positions 6123 and 7619 in the entry AE000435 (section 325 of 400). The L-xylulose kinase gene was thus identified as part of a gene cluster, which according to its location at 80.6 min of the *E. coli* genome has been designated as *yiaD-S* (33). Based on the genome analysis by Sofia et al. (34) this cluster is formed by nine genes encoding products for carbohy-
drate metabolism (yiaK to yiaS), transcribed clockwise, and one (yiaJ) transcribed anticlockwise (Fig. 1).

Northern analysis of total RNA of strain JA134 using probes of the structural genes yiaP and yiaR showed that transcription of the operon was detected in all growth conditions used.

Fig. 2A displays a typical experiment with a yiaP internal probe. The probe was 32P-labeled by the random primed method (26) using a 360-bp AgeI-XhoI fragment as template. The pattern of transcript molecules always showed several bands due to fragmentation of the long messenger RNA. In contrast, no transcript was found in wild-type strain preparations run in parallel. Determination of l-xylulose kinase in crude extracts of the same cultures used for isolation of RNA displayed similar high activity values of 105–115 nmol/min/mg. These results allowed us to use l-xylulose kinase as a reporter activity of the expression of the yiaK-S operon.

Identification and Inactivation of the Regulator Gene—The putative role of yiaJ gene as regulator of yiaK-S was approached by characterization and inactivation of this gene. Sequence analysis of the regulator gene product and comparison with other regulator sequences showed a helix-turn-helix motif of binding to DNA and a second motif of unknown function (Fig. 3). Both motifs were also found in regulator proteins such as glyceral activator (streptomycetes), acetate repressor (S.}

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**Fig. 2.** Northern blots of total RNA from mutant strain JA134 and wild-type strain ECL1. Cells were grown on casein acid hydrolysate in the absence (−) or in the presence (+) of l-lyxose. Blots were hybridized with 32P-labeled fragments corresponding to the structural gene yiaP (A) or the regulator gene yiaJ (B). Size of the transcripts in kb according to markers used in the experiment are indicated.

**Fig. 3.** Nucleotide sequence and amino acid translation of the yiaJ gene encoding the repressor of the yiaK-S operon. 5'-proximal shaded region corresponds to the helix-turn-helix motif, whereas the 3'-proximal one corresponds to the IeII family consensus of undetermined function. Target sequence for the IS1 transposition is boxed, and the position where the gene has been interrupted by the insertion in strain JA134 is indicated by the arrow. The BglII restriction site used for site-directed mutagenesis is labeled.
typhimurium), or Kdg repressor involved in pectinolysis in Erwinia chrysanthemi, all belonging to the known "IclR" family (35).

The proposed regulator gene upstream of the operon was inactivated by a CAT cassette insertion in wild-type cells. To this end a clone encompassing the complete operon was isolated from a SalI chromosomal library of wild-type strain ECL1 prepared as indicated under "Materials and Methods." The clone was selected by hybridization with a probe\(^{32}\)P-labeled by the random primed method (26) using as template a 520-bp XhoI-EcoRV internal fragment obtained from pJC2. The 3.2-kb HindIII fragment containing the proposed regulator gene was subcloned in pBR322 (plasmid pJB1). Inactivation of the putative regulator gene was carried out by insertion of CAT cassette in the BglII restriction site present in plasmid pJB1 insert, yielding plasmid pJB2 (Fig. 1) and the mutant gene transferred to the wild-type ECL1 background yielding strain JA161. The correct integration of the CAT resistance gene was confirmed by Southern blot analysis (not shown).

The effects of the inactivation of the putative regulator gene were analyzed in cell extracts of strain JA161 by measuring the l-xylulose kinase activity, which was found to be present in growth conditions that did not yield this same activity in wild-type cells. Furthermore, Northern analysis of total RNA of strain JA161 using probes of the structural genes yiaP and yiaR (not shown) were indistinguishable from those obtained with total RNA of strain JA134. When a BglII-AgeI probe internal to the regulator gene was used, Northern analysis showed a single constitutive transcript in RNA preparations of strain ECL1 (Fig. 2B) and no transcript in the CAT mutated strain JA161 (not shown). The derived constitutivity in the expression of the yiaK-S operon in strain JA161 indicated that the yiaJ had regulatory functions and that this system is under the control of a repressor protein encoded by this gene.

Characterization of the Mutation in the Regulator Gene of Strain JA134 —The constitutive expression of yiaK-S operon in strain JA134 thus suggested that the mutation responsible could affect this operon regulatory region. To characterize the mutation, a probe\(^{32}\)P-labeled by the random primed method using a 640-bp EcoRV-HindIII fragment belonging to yiaK (see Fig. 1) was used to hybridize Southern blots of the wild-type and mutant JA134 DNA digested with SalI, BamHI, or HindIII. The hybridization pattern obtained in these experiments for each of the indicated restriction enzymes was clearly different in the wild type and mutant strain (Fig. 4). These results are consistent with the presence of an insertion or deletion in the nearby region encompassing the regulator and the yiaK-S operon promoter.

Furthermore, the search of the regulator gene transcript using a BglII-AgeI probe internal to the regulator gene, showed no band of hybridization (Fig. 2B), consistent with the type of proposed mutation that could abolish initiation of transcription or synthesize an anomalous messenger not detected with the probe used.

Insertion-Inversion into the Repressor—The absence of transcript of the regulator gene reported above was the result of a mutation in this gene and is not compatible with a mutation in the promoter region of the structural genes. For this reason the nature of the mutation was studied in the yiaJ regulator gene. A clone of the mutated regulator was isolated from a SalI library of strain JA134, using a probe prepared with the internal BglII-AgeI fragment (Fig. 1). Its SalI insert was transferred to Bluescript vector yielding clone pJB3 (Fig. 5). Restriction analysis of the insert in plasmid pJB3 indicated that foreign DNA sequences were present in the regulator.

To further identify the DNA fragment not belonging to the

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**Fig. 4. Southern blot analysis of the yiaJ region.** Comparison of Southern blot patterns between genomic DNA of strain ECL1 (lanes 1) and genomic DNA of strain JA134 (lanes 2) digested with SalI, BamHI, or HindIII. Blots were hybridized with a \(^{32}\)P-labeled probe corresponding to yiaK sequences (see text). Fragment size markers in kb are indicated on the right side of the panel.

**Fig. 5. Diagram of the inversion-insertion mutation in yiaJ gene of strain JA134.** The map shows the location in the genomic DNA (open bar) of the inverted region (thick line) with a long nonrepresented interval (dashed line). For better comprehension the relevant restriction sites, as well as the minute positions are indicated on the DNA. The extent and direction of transcription of different genes and IS1 copies (black arrows) and of the interrupted gene (shaded arrow) are also presented below. In the upper part, the sequence of the junction points is shown with the IS1 sequence boxed and the transposition-generated repeats derived from the target regulator sequences in bold. The bottom part displays the extent of the inserts in the plasmids used in the analysis of the junction regions (thin lines). A, AgeI; B, BamHI; Bg, BglII; H, HindIII; S, SalI; and V, EcoRV.
regulator gene, different subclones of plasmid pJB3 were prepared and their terminal ends were sequenced. Computational analysis showed 100% similarity with sequences corresponding to IS186, and to the ant, dnaJ and dnaK genes, all located at 0.4 min. The shortest clone (pJB4) containing the fragment with the joining site for regulator and foreign DNA was obtained and the corresponding insert was totally sequenced. Comparison of the sequences of pJB4 clone with GenBankTM sequences allowed us to locate an IS1 insertion element between sequences corresponding to the regulator gene and those corresponding to the 0.4 min, which were found to be inverted with respect to their natural genome order (see Fig. 5).

To determine the other joining site, not included in pJB3, a clone from a Sau3A library of strain JA134 was isolated using a probe prepared with the yiaK internal fragment EcoRV-HindIII. Likewise, a 2-kb internal HindIII fragment of the Sau3A clone was transferred to Bluescript vector, yielding clone pJB5. Restriction analysis of the insert in plasmid pJB5 indicated again that DNA sequences not belonging to the regulator region were present. Sequencing of clone pJB6, derived from pJB5, showed sequences of the regulator and of another IS1 element of the other junction point in the interrupted repressor gene. This implied an IS1 transposition into the repressor. This insertion element sits in this case between the 5’-end part sequences of the regulator and sequences identical to those located between positions 20227 and 20460 in the 0–2.4 min region (accession number D10483). The IS1 transposed displayed opposite polarity and a totally identical sequence with the IS1 naturally found in the 0.4 min of the E. coli chromosome (36). The two IS1 elements had the transposition-generated repeats derived from the target regulator sequences on only one side.

The size of the inversion and possible changes in the gene order inside the inverted fragment were checked by Southern analysis. To this end, PCR fragments of about 1 kb were used to prepare 32P-labeled probes every 15 kb along the putative inverted region. Comparison of the patterns in the Southern experiments using digested DNA from wild-type strain ECL1 and mutant strain JA134 with BamHI, EcoRI, SalI, PstI or HindIII restriction enzymes displayed no differences. It was thus established that the inversion was up to 920 kb in length and that no other internal rearrangement occurred in the inverted fragment.

To confirm the specific mutation two oligomers flanking each of the junction sites of the inversion (see “Materials and Methods”) were used in amplification of genomic DNA obtained from wild type (strain ECL1) and mutant strain JA134. A PCR product of amplification was found when mutant DNA, but not wild-type DNA, was the template.

Stability of Newly Acquired Phenotype—The constitutivity of the yiaK-S operon expression subsequent to the IS1 transposition into its regulator implied phenotypic consequences reflected by the acquisition of the ability to utilize l-lyxose. The stability of this mutation is of obvious interest for its evolutionary significance. To determine the stability of the constitutive expression of yiaK-S operon in strain JA134, the permanence of the l-lyxose utilization as a reporter phenotype was followed in its offspring. To this end, a culture of 1 colony-forming unit/ml of strain JA134 was grown on LB broth up to 5 × 10^6 colony-forming unit/ml. This culture was diluted and reincoculated for a second growth to the same cell densities. The experiment allowed more than 30 generations without any selective pressure for l-lyxose utilization. At the end of the culturing, 5 × 10^3 colony-forming units were plated on glycerol and replica-plated on l-lyxose. All colonies kept the l-lyxose positive phenotype.

**DISCUSSION**

Among the multiple changes that may originate phenotypic modifications, we present a case in which the regulator gene of an operon is interrupted by an inserted DNA fragment. This fragment, of an unusual length of approximately 920 kb, is flanked by two IS1 elements. Fine analysis of the sequences of plasmids pJB4 and pJB6 suggested that the insertion into the regulator was the result of an intramolecular transposition, leaving the IS1 elements with opposite polarity. Furthermore, an inverted gene order of the foreign DNA is seen in plasmid pJB3, whereas sequences of the repressor and 0.4 min region are found adjacent to each of the terminal ends of the IS1 in plasmid pJB5. Attending to these observations, it is concluded that a genome inversion from 0.4 to 80.6 min, interrupting the regulator gene (yiaJ), has taken place in mutant strain JA134. The indicated region could be inverted in the process of IS1 intramolecular transposition itself, as a consequence of ligation in opposite polarity of IS1 ends with the staggered break ends in the target (yiaJ) gene, precisely located in 80.6 min (Fig. 6).

However, as indicated by Turlan and Chandler (17) for IS1 and by Weinert et al. (37) for IS903 these insertion-inversion events, although possible in intramolecular transposition, are very infrequent if present at all. The mutation presented here, despite the scarce occurrence of this type of rearrangements, indicates its potential participation in genome evolution. At present we have no information on the frequency of this or similar mutations.

Despite the relatively well known genetic organization of IS1, its mechanism of transposition and consequently the re-
sulting rearrangement products are poorly understood. As has been widely discussed (1, 37) another speculative mechanism cannot be ruled out as an alternative to the process described above. Transposition, intermolecular in this case, and subsequent recombination between IS1 at min 0.4 and the IS1 newly inserted into the repressor, could also promote one such rearrangement. Since the products of any of the two mechanisms are indistinguishable, including the distribution of the transposition-generated repeats of the neighbor target sequences, we cannot at present discriminate between them. Nevertheless, the requirement of two independent events in this second proposed mechanism argues against the probability of its occurrence in the selection process.

It has been pointed out by several authors that insertion-inversion events that change the location of certain genes with respect to the origin of replication may be detrimental or lethal (38). This is seen as one of the functional barriers to inversion detection. To explain this, several functional consequences are invoked. They are, among others, changes in copy number of some fundamental genes, obstructive orientation impeding replication, or disturbance of the symmetrical positioning of the origin, requiring one of the growing forks to copy more than half of the chromosome. The fragment inverted in mutant strain JA134 spanned from 80.6 to 0.4 min and thus involved the replication origin oriC located at 84.5 min. It is of interest that in our study, not only was the insertion-inversion mutant viable and able to be isolated, but also growth on different carbon sources and conditions were indistinguishable from that obtained with wild-type strain.

In this model a genome rearrangement (insertion-inversion) of specific phenotypic consequences is presented as well as the mechanism leading to it. This is in contrast to most of the similar or larger insertion-inversions of unknown origin and undetermined phenotypic consequences. The constitutivity of yiaK-S operon, as indicated by the ability to utilize L-lyxose, is a stable genetic trait indicating that the mutation inactivating the repressor does not easily revert. In this context it is of interest to consider that reversion of insertion mutations such as the one described here are likely to be imperfect, leaving the IS1 element or other parts of the transposed DNA in place. These reversions would maintain the target gene interruption, giving rise to stable phenotypes.

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