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Nucleotide Sequence Analysis of the Inversion Termini Located within IS*3* Elements $\alpha_3\beta_3$ and $\beta_5\alpha_5$ of *Escherichia coli* K-12

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This paper presents the first detailed structural analysis of termini of an inversion mediated by recombination between *Escherichia coli* native IS elements. The complete nucleotide sequence of the inversion termini in the lactose region of *Escherichia coli* K-12 confirms our previous suggestion that the inversion occurred by homologous recombination between $\alpha_3\beta_3$ and $\beta_5\alpha_5$ IS*3* elements (D. J. Savic, J. Bacteriol. 140:311–319, 1979; D. J. Savic, S. Romac, and S. D. Ehrlich, J. Bacteriol. 155:943–946, 1983). The data show a slight structural divergence of $\alpha_3\beta_3$ and $\beta_5\alpha_5$ elements, but they do not reveal new sequences within recombined IS*3* elements that could influence the expression of nearby genes.

Genetic rearrangements of the inversion type are rarely found in the bacterial chromosome (1, 15). It was suggested that their rarity may be due to mechanistic limitations which may prevent physical contacts between specific sites (15). In an analysis of the literature, lethal consequences, rather than mechanistic limitations, were sought as the primary cause of the rarity of inversions (3). This speculation was based on the hypothesis that the genome of *Escherichia coli* has evolved a physical organization of coding sequences that optimizes relative gene dosage and also avoids head-on collisions between replication forks and RNA polymerases. Finally, the deleterious effect of inversions having at least one endpoint in the third of the chromosome surrounding replication termini were attributed to the disruption of a long-range chromosomal organization essential for nucleoid organization (11).

Until very recently, there were only three reports of unselected, natural inversions (5, 13, 18). However, the recent discovery of two large inversions in a single strain (1) throws into question previous hypotheses and suggests that inversions may not be so rare. The termini of a spontaneous, approximately 75-kb inversion in the *lac* region appear to map within mutually inverted IS*3* elements (14, 17). Similarly, breakpoints of the two recently discovered inversions are associated with mutually inverted IS*1* and IS*5* elements (1).

Whatever the advantage of a particular gene order may be, selective pressure is evidently not strong enough to prevent the survival of strains which have experienced some large inversions. The questions that one may ask at this point are whether large inversions confer some selective advantage in some cases and, if so, what mechanisms ensure creation of the new quality?

Nucleotide sequence analyses of different IS*3* elements show that they may not be identical (16). Therefore, one could postulate that homologous recombination between incompletely homologous $\alpha_3\beta_3$ and $\beta_5\alpha_5$ IS*3* elements, which created the above-mentioned inversion in the lactose region (14), may result in homogenization of nonhomologous nucleotides by mismatch correction in the recombinational bridge (9, 10). For instance, that mechanism may be used to equalize promoter activity and inactivity between different alleles of outwardly directed promoters located inside inverted repeats of β domains and IS*3* elements (6, 16). Such homogenization of promoter activities could cause alterna-

TABLE 1. Bacterial strains and vectors used in this study

Strain or vector	Description	Reference(s)
Strains		
DY100	W3100 *thyA deoC lacZ118 metE rpsL polA1 azi*(Res) *val*(Res) *supE42*	20
SY99	As DY100 with an inversion in the *lac* region	13, 14
MC4100	*araD39* Δ(*argF lac*)*U169 rpsL150 relA1 deoC1 ptsF25 rbsR flbB5301*	4
Vectors		
pBR322	Chimeric cloning vehicle	2
pHC79	Chimeric cloning vehicle	8
pUC18 and pUC19	Chimeric cloning and sequencing vectors	19
M13mp18 and M13mp19	Chimeric cloning and sequencing vectors	19
pHV1005	$\beta_5\alpha_5$-*proC Bam*HI segment from DY100 inserted into pHC79	14
pHV1007	$\beta_3\alpha_5$-*proC Bam*HI segment from SY99 inserted into pBR322	14
pHV2800	$\alpha_3\beta_3$ *Bam*HI fragment from DY100 inserted into pBR322	This work
pHV2801	$\alpha_3\beta_5$ *Bam*HI fragment from SY99 inserted into pBR322	This work

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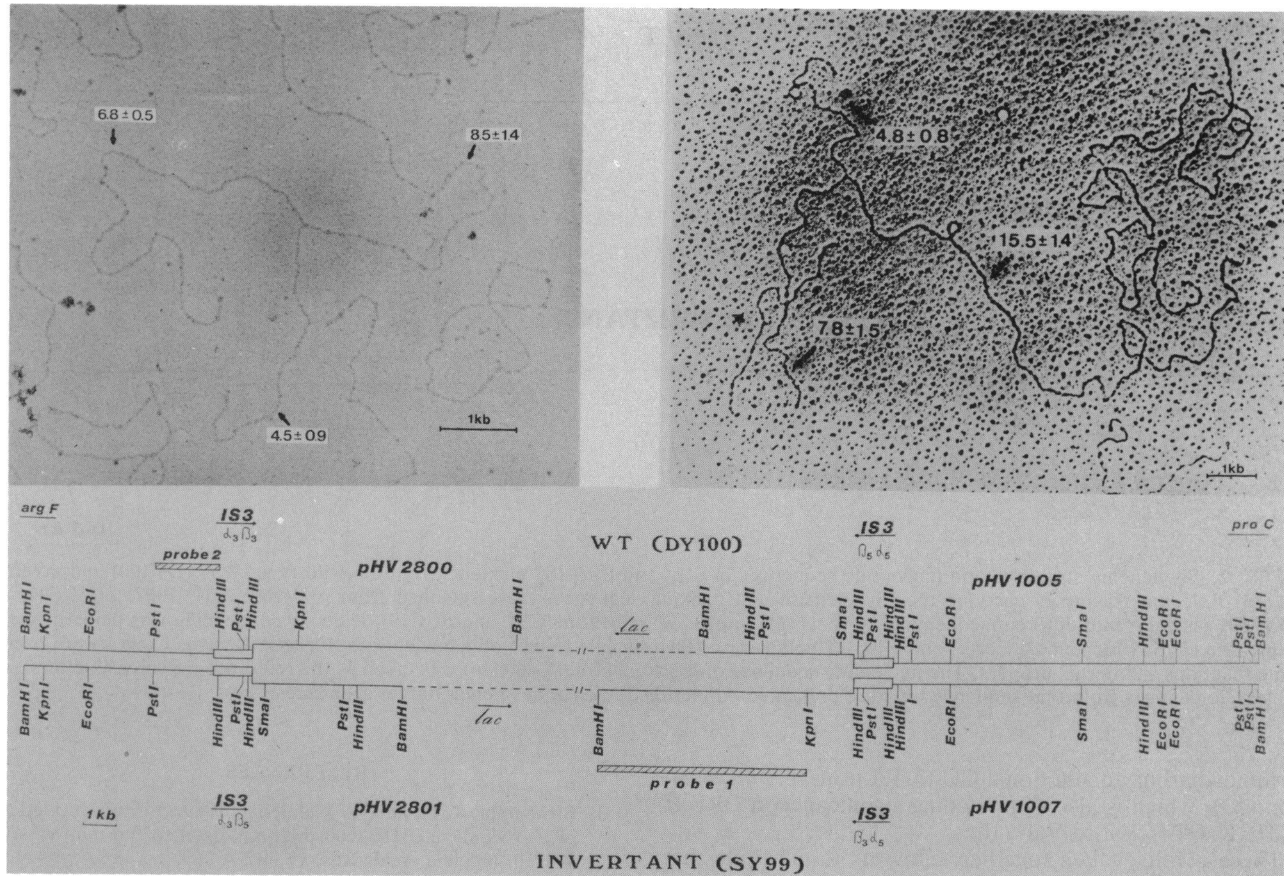

FIG. 1. Heteroduplexes and restriction enzyme maps of *Bam*HI IS*3*-harboring segments from the wild type (WT) (pHV1005 [14] and pHV2800) and invertant (pHV1007 [14] and pHV2801). The *Bam*HI-*Kpn*I fragment contained in the *Bam*HI fragment of the existing clone carrying the right terminus of inversion (14) was recloned into pUC18. It was used as a hybridization probe for identification of the clone carrying the IS*3* element, i.e., the postulated left end of the invertable segment of the parental strain DY100. When obtained, this left-end clone was used as a source for constructing the probe (*Pst*I-*Hin*dIII fragment) for identification of the clone harboring the left terminus of the inversion in SY99. Cosmid libraries of both strains were prepared in the *proAB-lac* deletion-carrying recipient MC4100. The inserts of chromosomal DNA were produced by partial digestion with *Bam*HI, and the insert site of pHC79 vector was opened with the same enzyme. All DNA manipulations were essentially the same as previously described (12), except that packaging of cosmids was carried out as suggested by the manufacturer of the packaging kit (Amersham). Transfer of bacterial colonies from original plates to nylon filters and processing of filters were performed as specified by the manufacturer (Amersham). Radioactive labeling (^{32}P) of purified probe fragments and colony hybridization were carried out as previously described (12). As expected, recombinant cosmids from positive clones contained several extra *Bam*HI fragments. The relevant IS*3*-carrying *Bam*HI fragments were identified after their hybridization with the original probes and by hybridization with an IS*3*-specific probe (Table 1). Both fragments were eluted and cloned into pBR322. These clones (pHV2800 and pHV2801) were further used for restriction enzyme, nucleotide sequence, and heteroduplex analyses. The existing clones of the right terminus (pHV1005 and pHV1007 [14]) were carried in parallel for sequencing and heteroduplex analyses. The approximate distance between inversion termini is 75 kb (17).

tive expression of genes proximal to β_3 and β_5 ends in derivative strains.

To test this possibility and to verify the exact position of the inversion termini, we performed a structural analysis of the inversion termini, including an analysis of nucleotide sequences of $\alpha_3\beta_3$ and $\beta_5\alpha_5$ IS*3* elements flanking the inverted segment in both the parental strain DY100 and the invertant SY99 (Table 1). The combined restriction maps of IS*3* ($\alpha_3\beta_3$)-harboring *Bam*HI fragments from the wild type and the invertant were identical between the "left" *Bam*HI end and the more distal of the two *Hin*dIII sites situated within IS*3*. On the other hand, the two maps were completely divergent between the same *Hin*dIII site and the other *Bam*HI end (Fig. 1). These divergent map sections were identical to restriction maps of their counterparts in pHV1005 and pHV1007 (Fig. 1). The homologies between the parental and invertant segments at both termini were verified by electron-microscopic analysis of heteroduplexes between pHV1005 and pHV1007 and between pHV2800 and pHV2801 (Fig. 1). Both analyses strongly indicated that inversion termini are situated within IS*3* elements.

That this is indeed the case was confirmed by nucleotide sequence analyses of all four IS*3* elements and flanking DNA. The results (Fig. 2) show that the sequences we obtained closely resemble the sequence of the IS*3* element from the chromosome of the strain C600 (16). An exception is a CG-to-GC transversion at position 1130 of all IS*3* elements, with no coding significance in open reading frame I (ORF I) or ORF III (CGC-to-CGG, Arg-to-Arg; GTC-to-GTG, Val-to-Val [16]). However, the IS*3* elements at the left

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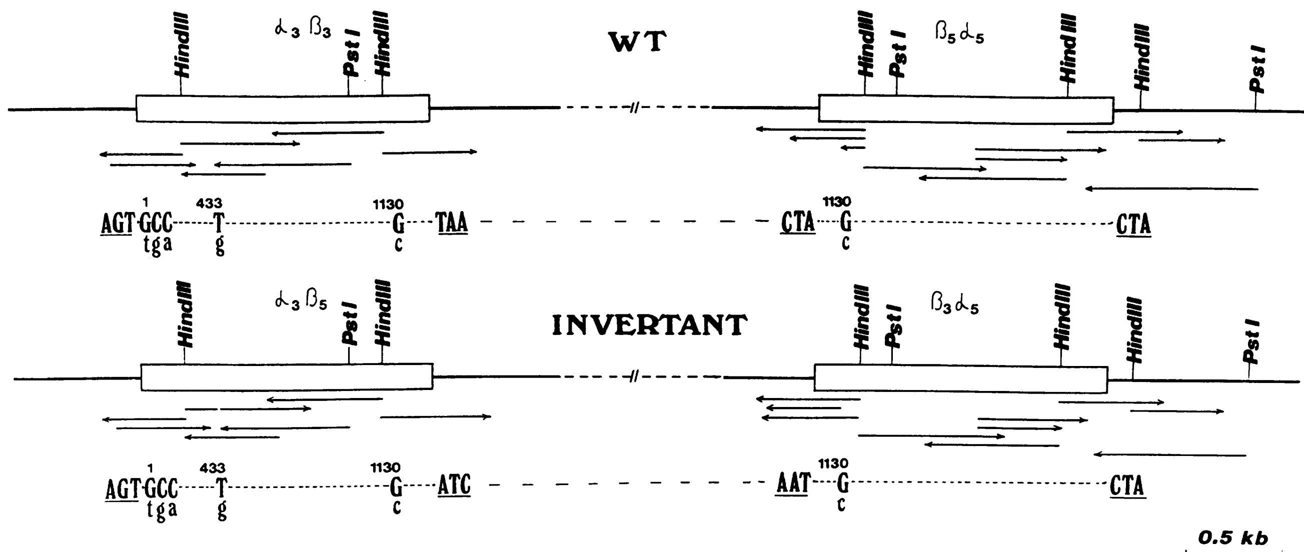

FIG. 2. Sequencing strategies and nucleotide sequences of $\alpha_3\beta_3$ and $\beta_5\alpha_5$ IS*3* elements from the wild type (WT) (DY100) and invertant (SY99). Relevant fragments carrying the IS*3* element and flanking sequences were recloned from pHV1005, pHV1007, pHV2800, and pHV2801 plasmids into different sequencing systems (M13mp18, M13mp19, pUC18, and pUC19). Dideoxy sequencing was performed with Sequenase (U.S. Biochemical Corp.) and with T7 polymerase (Pharmacia); 15-mer oligonucleotides (Genetic Designs Corp.) were used in some reactions as internal primers. The nucleotide sequence of IS*3* from strain C600 (16) was taken as the reference (lowercase letters), and nucleotide changes from that sequence only are presented. Underlined nucleotides denote target sequences.

terminus harbor an additional GC-to-TA transversion (position 433), which is also of no coding significance in ORF II (GTG-to-GTT, Val-to-Val) (16).

These results, taken together, allow us to conclude that the recombinational event leading to this inversion occurred within IS*3* elements $\alpha_3\beta_3$ and $\beta_5\alpha_5$, somewhere between position 433 and the inside end of the elements. That the recombination occurred within IS elements was also verified by the nucleotide sequence analysis of the flanking regions: the sequence bordering the β_3 domain in the wild type was identical to its counterpart bordering the β_3 domain in the invertant; the sequence bordering the β_5 domain in the wild type was identical to the sequence bordering the β_5 domain in the invertant (several hundred nucleotides were analyzed [results not presented]). These data also show a complete disparity of the 3-bp duplicates of the target sequence bordering the $\alpha_3\beta_3$ element (Fig. 2). At present, it is not possible to deduce the structure of the original target sequence, the number of accumulated mutations, or the meaning of these changes.

The available data fail to demonstrate the creation within recombined IS*3* elements of new sequences which would be expected to change the expression of nearby genes. However, this need not mean that inversions of this type represent neutral events of no adaptive value. The possibility that changes in DNA superhelicity and overall topology of the inverted segment could affect the activity of genes (7) in this region should also be taken in consideration.

Nucleotide sequence accession number. The nucleotide sequences of IS*3* ($\alpha_3\beta_3$) and IS*3* ($\beta_5\alpha_5$) have been deposited in the GenBank nucleotide sequence data bases with the accession numbers M55510 and M55511, respectively.

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