Identification and Characterization of ssb and uup Mutants with Increased Frequency of Precise Excision of Transposon Tn10 Derivatives: Nucleotide Sequence of uup in Escherichia coli

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A Lac* papillation assay was used to identify mutants (tex) of Escherichia coli that exhibit an increased frequency of precise excision of a lacZ::Tn10dKan insertion. Three tex strains had suffered mutations in the gene (ssb) encoding the essential single-stranded DNA-binding protein SSB, which resulted in the following alterations in the 177-residue protein: G4D; L10F, P24S; and V102M. The phenotypes of these ssb mutants indicated that they were largely unaffected in other functions mediated by SSB, such as DNA replication, recombination, and repair. Strains with multicopy ssb* exhibited a decreased frequency of Tn10dKan precise excision. Three other tex mutants had insertion mutations in the locus designated uup at 21.75 min on the linkage map. The nucleotide sequence of uup was determined, and the gene was inferred to encode a 625-amino-acid hydrophilic protein that belongs to the superfamily of ABC-domain proteins (with two pairs of the Walker A and B motifs), which are postulated to be involved in coupling ATP hydrolysis with other biological processes. The uup gene product shares extensive homology with the deduced sequences of two proteins of Haemophilus influenzae. The uup gene is also situated immediately upstream of (and is transcribed in the same direction as) the pararquat-inducible SoxRS-regulated pgi-5 gene, two reported promoters for which are situated within the uup coding sequence.

Insertion elements and transposons, including the transposable bacteriophage Mu, are so called because they have the ability to transpose and to insert themselves, as autonomous genetic entities, fairly randomly within the genomes of their hosts. Proteins, genetically referred to as transposases, that are encoded by genes included within the structure of the transposable elements, are essential for mediating the transposition of the cognate elements. Both in vivo and in vitro approaches have been used to study the mechanisms of transposition of several transposons. Transposition may be either nonreplicative (conservative) or replicative, and the latter may also include the formation and resolution of covalently joined cointegrate structures as intermediates. A characteristic feature in transposition is the generation, by duplication of the host sequence at the site of insertion, of a short stretch of directly repeated sequences (whose length is characteristic for any particular transposon) immediately flanking the ends of the transposable element (reviewed in reference 6).

Another property shared by many, if not all, transposons but whose mechanism is not as extensively studied or well understood is that of precise excision. This is a mutational event that results in the loss of the transposon sequence along with one repeat length and precise excision frequency has also been shown for the kanamycin resistance (Kan') element Tn5 (9, 10). Phage Mu does not have these inverted repeats, and precise excision of Mu is observed only under certain special circumstances (2).

Lundblad et al. have identified mutations, designated tex (for transposon excision), in several genes of the Escherichia coli host strain that increase the frequency of precise and nearly precise excisions of Tn10 (29, 30). The mutations include dominant alleles of recBC and recessive alleles of genes involved in methyl-directed mismatch repair such as mutS, mutH, mutL, dam, and uvrD. A temperature-sensitive mutation (ssb-113) in ssb, the gene encoding the E. coli single-stranded-DNA-binding protein SSB (27, 33), also confers a tex phenotype at the permissive growth temperature (29). The model proposed is that the formation of a stem-loop structure between the pair of inverted repeats permits the pair of flanking direct repeats to be brought in register for a “replication slippage” event that results in precise or nearly precise excision (8, 10, 13; reviewed in reference 43). Because the inverted repeats are not perfectly homologous, the stem-loop structure would be less stable in cells that are proficient in mismatch repair (13, 29). This model is supported by the findings that (i) precise and nearly precise excision frequencies are a function of the length of the inverted repeats (9, 13); (ii) mutations in the inverted repeat region that reduce the mispairing potential within the stem lead to an increase in these frequencies of excision (13); and (iii) the frequencies of precise and nearly precise excision are increased under conditions where the single-stranded template (which would more readily be able to form the stem-loop structure) is expected to be abundant, such as in the presence of an M13 ori sequence on the template (8) or during Tra-dependent synthesis of single-stranded DNA during conjugal transfer of an F' plasmid (34, 44).

Another locus, called uup, has also been identified at 21 min on the E. coli linkage map, and mutations in this locus increase
the frequency of precise excision of Tn10 and Tn5 (17). However, neither its function nor its mechanism of action has been characterized in detail. In contrast, cells deficient in the protein integration host factor (34) and mutants with mutations in a locus designated dprA (24) have been reported to exhibit a decreased frequency of precise excision of transposon insertions. A phenomenon of UV-induced precise excision of Tn10 that requires SOS functions has also been described (26).

In this study, we have used a Lac\(^+\) papillation screen with a strain carrying an insertion of a transposition-defective Tn10 derivative (Tn10Kan, encoding Kan\(^{\text{r}}\)) in lacZ to identify new tex mutations. We describe the characterization of new mutations in ssb and in a gene we believe to be uup, which were isolated by this approach. The nucleotide sequence analysis of uup is also reported.

**MATERIALS AND METHODS**

**Bacterial strains, plages, and plasmids.** All the bacterial strains we used were derivatives of *E. coli* K-12 and are listed in Table 1. Phages P1Ck and Mu (Ts) were from our laboratory stocks. The transposon vehicle phages \(\lambda\)1098 (46) and \(\lambda\)1323 (18) (used as markers for the transposition of Tn10, have been described previously. The ordered \(\lambda\) phage library of Kohara et al. (21) was kindly provided by K. Isoe. The plasmid vectors pBR322 (40), pBR329 (7), pCL1920 (25), and pliucucrRRI (pBSK Streptagene, La Jolla, Calif.) have been described previously. pCL1920 is derived from pSC101, whereas all the others are CoE1 derivatives. Plasmid pBR\(\text{ab}^{\star}\) (given by L. E. Carlini) is a pBR322 derivative with a 0.8-kb BamHI insert (obtained from plasmid pBRZ151 [4]) carrying the ssb\(^+\) gene. Plasmid PHYD620 was constructed in this study as a pCL1920 derivative with a 7-kb EcoRI insert subcloned from the \(\lambda\) phage 637 of the collection of Kohara et al. (21), which carries the ssb\(^{+}\) and mrr\(^+\) genes. Plasmids carrying various regions of the uup locus that were constructed in this study are described in the text and depicted in Fig. 1.

**Growth media and conditions.** The defined and nutrient media were, respectively, minimal media supplemented with glucose or other indicated C source and minimal A (supplemented with glucose or other indicated C source and streptomycin or spectinomycin) and Luria-Bertani (LB) medium (35). Unless otherwise indicated, the growth temperature was 37°C. Tetracycline, the appropriate auxotrophic requirements, and LB medium were each used at 50 \(\mu\)g/ml. All the bacterial strains we used were described previously. The ordered \(\lambda\) phage library of Kohara et al. (21) was kindly provided by K. Isoe. The standard protocols of Sambrook et al. (40) were followed. The plasmid vectors pBR322 (40), pBR329 (7), pCL1920 (25), and pliucucrRRI (pBSK Streptagene, La Jolla, Calif.) have been described previously. pCL1920 is derived from pSC101, whereas all the others are CoE1 derivatives. Plasmid pBR\(\text{ab}^{\star}\) (given by L. E. Carlini) is a pBR322 derivative with a 0.8-kb BamHI insert (obtained from plasmid pBRZ151 [4]) carrying the ssb\(^+\) gene.

**Isolation of tex mutants.** The strategy of localized nonmutagenesis (14) was used in obtaining nitrosoguanidine-induced trans-acting tex mutants that exhibited an increased frequency of precise excision of lacZ::Tn10Kan. A population of mutagenized cells of strain GJ1821 (lac\^-\) was infected with a P1c lysate prepared from a strain carrying dKan (bpBSK Streptagene, La Jolla, Calif.) and cloned previously. The ordered \(\lambda\) phage library of Kohara et al. (21) was kindly provided by K. Isoe.

**Identification of new tex mutations.** New tex mutants were identified in this study following mutagenesis (with either nitrosoguanidine or Tn10KanTet) of *E. coli* strains carrying lacZ4525::Tn10Kan, as described above. The frequency of precise excisions in mutagenized colonies was scored by a Lac\(^+\) papillation assay, modified from that described previously (22, 36), on LB agar plates supplemented with lactose and X-Gal. Colonies that exhibited an increased Lac\(^+\) papillation frequency were identified as putative tex mutants and further characterized.

**RESULTS**

**Identification of new tex mutants.** New tex mutants were identified in this study following mutagenesis (with either nitrosoguanidine or Tn10KanTet) of *E. coli* strains carrying lacZ4525::Tn10Kan, as described above. The frequency of precise excisions in mutagenized colonies was scored by a Lac\(^+\) papillation assay, modified from that described previously (22, 36), on LB agar plates supplemented with lactose and X-Gal. Colonies that exhibited an increased Lac\(^+\) papillation frequency were identified as putative tex mutants and further characterized.

**ssb mutations that increase the precise excision frequency of Tn10 derivatives.** Three tex mutants (GJ1854.1, GJ1854.2, and GJ1864) obtained as described above following nitrosoguanidine mutagenesis were shown, based on the following lines of evidence, to have suffered mutations in ssb, the gene encoding

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**Table 1. List of E. coli K-12 strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype*</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GJ1655</td>
<td>Wild type</td>
<td></td>
</tr>
<tr>
<td>CAG12130</td>
<td>MG1655 zcb-3111:::Tn10Kan</td>
<td>42</td>
</tr>
<tr>
<td>GJ1821</td>
<td>MG1655 zbc-900::Tn10Kan(Ts)-</td>
<td>38</td>
</tr>
<tr>
<td>GJ1823</td>
<td>GJ1821 lacZ4525::Tn10Kan</td>
<td>38</td>
</tr>
<tr>
<td>GJ1854</td>
<td>GJ1821 lacZ4525::Tn10Kan</td>
<td>38</td>
</tr>
<tr>
<td>GJ1854.1</td>
<td>GJ1854 ssb-200</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1854.2</td>
<td>GJ1854 ssb-201</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1864</td>
<td>GJ1821 lacZ4525::Tn10Kan xban-202</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1885</td>
<td>ara zbc-900::Tn10Kan(Ts)-</td>
<td>From CSH142 (35), in several steps</td>
</tr>
<tr>
<td>GJ1888</td>
<td>GJ1885 uap-353::Tn10KanTet1</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1887</td>
<td>GJ1885 uap-353::Tn10KanTet2</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1889</td>
<td>GJ1885 uap::Tn10KanTet</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1890</td>
<td>GJ1885 ssb-200 zch-904::Tn10KanTet1</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1891</td>
<td>GJ1885 ssb-201 zch-904::Tn10KanTet1</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1892</td>
<td>GJ1885 ssb-202 zch-904::Tn10KanTet1</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1893</td>
<td>GJ1885 uap::Tn10KanTet1</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1893</td>
<td>GJ1885 uap::Tn10KanTet2</td>
<td>This work</td>
</tr>
<tr>
<td>GJ1894</td>
<td>GJ1885 uap::Tn10KanTet2</td>
<td>This work</td>
</tr>
</tbody>
</table>

* Genotype designations are those of Berrily et al. (1). All strains are F\(^-\). The insertion mutations lacZ4525::Tn10 (42) and zbc-900(Tn10Kan(Ts)-) (38) have been described previously.
the 177-amino-acid essential SSB protein. The mutant alleles have been designated ssb-200, ssb-201, and ssb-202 respectively.

(i) A new Tet r insertion (zjc-904::Tn10dTet1) was obtained as described above that was 75 to 85% cotransducible with the tex mutations in each of the three strains. Conjugational and transductional mapping experiments with strains from the mapping kit of Singer et al. (42) permitted localization of the three mutations to the vicinity of the ssb locus at 92 min on the E. coli linkage map, 30% cotransducible with malF::Tn10 (data not shown).

(ii) Two different plasmids carrying the cloned ssb1 gene, pBRssb1 and pHYD620, were able to complement the tex mutant phenotype upon introduction into each of the three mutant strains (data not shown). The Lac+ papillation frequency in the corresponding transformants was the same as that in the transformants of the control ssb1 strain (see below).

(iii) The ssb gene from each of the three mutants was amplified by PCR, and its nucleotide sequence was determined. The following transition mutations were identified (nucleotide numbering as in reference 33; the corresponding amino acid position and change in SSB are denoted in parentheses):

- ssb-200, G to A at position 41 (G4D);
- ssb-201, G to A at position 337 (V102M);
- ssb-202, C to T in each of two positions 58 and 100 (L10F, P24S).

The magnitude of the tex mutant phenotype for each of the three ssb alleles was determined by measurement of the reversion frequency of lacZ::Tn10dKan to Lac+ in exponentially growing cultures, as described above (Table 2). The mutations conferred a 5- to 12-fold increase in the frequency of precise excision of Tn10dKan. Interestingly, the ssb-202 allele displayed a growth temperature-dependent tex phenotype, with a markedly elevated excision frequency at 42°C and a near-parental level at 30°C (data not shown). All three alleles also increased the frequency of precise excision of a Tn10dTet1 insertion in lacZ (data not shown). The mutant phenotypes were unaffected by the introduction of a recA mutation, nor were they complemented by the ssb-homologous gene carried on the sex factor F (5; data not shown).

tex mutations in ssb do not affect other functions of SSB. SSB is essential for cell viability, and its function has been implicated in DNA replication, homologous recombination, mismatch repair, and excision repair. ssb mutants that are differentially affected in various functions of the protein have

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**TABLE 2. sbb and uup effects on frequency of precise excision of lacZ::Tn10dKan during exponential growth**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>No. of Lac+ revertants (per 10^9 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GJ1885</td>
<td>Wild type</td>
<td>86</td>
</tr>
<tr>
<td>GJ1890</td>
<td>sbb-200</td>
<td>1,050</td>
</tr>
<tr>
<td>GJ1891</td>
<td>sbb-201</td>
<td>400</td>
</tr>
<tr>
<td>GJ1892</td>
<td>sbb-202</td>
<td>425</td>
</tr>
<tr>
<td>GJ1886</td>
<td>uup-351</td>
<td>490</td>
</tr>
<tr>
<td>GJ1893</td>
<td>sbb-200 uup-351</td>
<td>2,400</td>
</tr>
<tr>
<td>GJ1885/pHYD620</td>
<td>sbb+ (5 copies/cell)</td>
<td>38</td>
</tr>
<tr>
<td>GJ1885/pBRssb</td>
<td>sbb+ (20 copies/cell)</td>
<td>24</td>
</tr>
</tbody>
</table>
TABLE 3. UV irradiation tolerance and spontaneous point mutation frequencies in tex mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Fractional survival after exposure to UV ltr&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. of spontaneous mutants (per 10&lt;sup&gt;8&lt;/sup&gt; cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 s</td>
<td>60 s</td>
</tr>
<tr>
<td>GJ1885</td>
<td>Wild type</td>
<td>0.5</td>
<td>0.20</td>
</tr>
<tr>
<td>GJ1890</td>
<td>ssb-200</td>
<td>0.24</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GJ1891</td>
<td>ssb-201</td>
<td>0.5</td>
<td>0.20</td>
</tr>
<tr>
<td>GJ1892</td>
<td>ssb-202</td>
<td>0.4</td>
<td>0.31</td>
</tr>
<tr>
<td>GJ1886</td>
<td>uup-351</td>
<td>0.22</td>
<td>0.18</td>
</tr>
<tr>
<td>GJ1893</td>
<td>ssb-200 uup-351</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Exponentially growing cultures were subjected to UV irradiation, as described previously (35), from a 15-W germicidal lamp at a distance of 37 cm for 20 or 60 s as indicated. Values are given as the ratios of surviving cells in each culture to that in an unirradiated aliquot. At the lower dose, the value for a control <i>nuvD</i> mutant strain was <2 × 10<sup>-4</sup>.<sup>b</sup> ND, not determined.

been characterized. Conditional-lethal mutants also exist, the best characterized of which are the <i>ssb</i>-1 (H55Y) and <i>ssb</i>-113 (P176S) derivatives (for reviews, see references 27 and 33).

We tested whether the <i>tex</i> mutations in <i>ssb</i> conferred additional phenotypes associated with <i>ssb</i> function. None of the three mutants was temperature sensitive for growth, and the growth rate of each in LB medium was at least 90% of that of the wild-type strain (data not shown). All of them also exhibited normal frequencies of spontaneous mutations to Rif<sup>c</sup> or Nal<sup>c</sup>, and the fraction of surviving cells in each of them following exposure to UV irradiation was roughly the same as that in the parent (Table 3).

The <i>tex</i> mutant phenotype of the new <i>ssb</i> derivatives was also unaffected by NaCl or sucrose supplementation of the medium (data not shown), in contrast to the osmoremedial nature of several <i>ssb</i> mutations that have been characterized earlier (33).

**Multicopy <i>ssb</i><sup>*</sup> effect on precise excisions.** We observed a gene dosage effect of <i>ssb</i><sup>*</sup> on the frequency of precise excisions of lacZ<sup>c</sup>:{Tn10dKan} (Table 2). Thus, this frequency was lower in the strain in which the plasmid pHYD620 (which carries <i>ssb</i><sup>+</sup> on a pSC101 vector; approximately 5 copies/cell) than in the haploid <i>ssb</i> strain, and it was even further reduced in the strain with plasmid pHRS<sup>b</sup> (which carries <i>ssb</i> on a pBR322 vector, approximately 20 copies/cell). The possible significance of these results is discussed below.

**Identifying <i>tex</i> mutants following Tn10dTet insertion mutagenesis.** Three independent Tn10dTet insertion derivatives (GJ1886 to GJ1888) of strain GJ1885 were identified in the Lac<sup>c</sup> papillation screen as <i>tex</i> mutants. In all three cases, we were able to demonstrate 100% linkage in P1 transduction between Tet<sup>c</sup> and the increased Lac<sup>c</sup> papillation phenotype. The mutants exhibited an approximately fivefold increase in spontaneous point mutation frequencies and in UV sensitivity (data for GJ1886 are given in Tables 2 and 3; data not shown for GJ1887 and GJ1888). As with the <i>ssb</i> mutants, these phenotypes were also RecA independent (data not shown). Results of mapping experiments indicated that each of the three insertions is situated at around 21 min on the genetic map, approximately 85% cotransducible with the chromosomal Tn10Kan insertion in strain CAG12130 (42) (data not shown).

Based on the map position, two genes, <i>helD</i> (encoding helicase IV) and <i>uup</i> (discussed above), were considered as plausible candidates for the <i>tex</i> insertion mutations. It has been speculated earlier that <i>helD</i> and <i>uup</i> might even represent the same gene (28). To test whether the <i>tex</i> mutations were insertions in <i>helD</i>, we cloned the <i>helD<sup>+</sup></i> gene on a 3.5-kb <i>PstI</i> fragment taken from phage 223 of the collection of Kohara et al. (21) into plasmid vector pCL1920 and introduced the resulting plasmid (pHYD621) by transformation into the three Tn10dTet insertion mutants. None of the mutants was complemented by the <i>helD<sup>+</sup></i>, thereby excluding this gene as the candidate for the <i>tex</i> insertion mutations.

On the other hand, our results support the interpretation that the Tet<sup>c</sup> insertions have occurred in the <i>uup</i> gene. (i) The observed <i>tex</i> mutant phenotypes and map position of the mutations are similar to those reported previously for the <i>uup</i> mutation (17). (ii) As for the previously described <i>uup</i> mutation (17), we also found that the burst size following phage Mu<sup>c</sup> (Ts) infection, as well as the size of phage Mu<sup>c</sup>(Ts) plaques, was reduced in the Tet<sup>c</sup> insertion strains (data not shown). We have accordingly designated the mutations <i>uup-351</i>, <i>uup-352</i>, and <i>uup-353</i>, respectively, although the original <i>uup</i> mutants are not available for undertaking complementation studies.

A double mutant (GJ1893) carrying <i>tex</i> mutations in each of the <i>ssb</i> and <i>uup</i> genes, constructed by P1 transduction, exhibited a higher frequency of excision of lacZ<sup>c</sup>:{Tn10dKan} than did either of the single mutants alone but remained unaffected in the frequency of spontaneous point mutation to Rif<sup>c</sup> or Nal<sup>c</sup> (Tables 2 and 3).

**Molecular cloning and physical localization of <i>uup</i>.** The <i>uup-351</i>:{Tn10dTet} insertion was cloned on a 14-kb <i>PstI</i> fragment from a preparation of GJ1886 chromosomal DNA into the plasmid vector pCL1920 by selection for the Tet<sup>c</sup> marker (with 5 μg of tetracycline/ml, in addition to the vector marker for resistance to streptomycin and spectinomycin) following ligation and transformation. The recombinant plasmid (pHYD616) so obtained was shown to hybridize to λX220, λX221, and λX222 from the panel of Kohara et al. (21). Alignment of the physical map of pHYD616 with that of the <i>E. coli</i> chromosome in this region permitted us to place the site of Tn10dTet insertion at 21.8 centisomes on the <i>E. coli</i> physical map (1), immediately upstream of (and counterclockwise from) the <i>pqi-5</i> gene (19) (Fig. 1).

A 5.7-kb EcoRI fragment cloned from λX222 into the EcoRI site of plasmid vector pBR329 (resulting in plasmid pHYD625 [Fig. 1]) was able to complement the <i>uup-351</i>:{Tn10dTet} mutant for its Lac<sup>c</sup> papillation phenotype (data not shown). A series of deletion subclones of the EcoRI insert fragment was constructed (in the plasmid vector pBK5) and tested for complementation. The results, depicted in Fig. 1, indicated that the complementing region is present within a 4.0-kb EcoRI-Pst<sup>I</sup> fragment carried on plasmid pHYD612.

Plasmids pHYD625 and pHYD612 were also able to complement the <i>uup-352</i>:{Tn10dTet} and <i>uup-353</i>:{Tn10dTet} mutants for their Lac<sup>c</sup> papillation phenotype, providing support to the conclusions from genetic mapping experiments that the three insertions define a single locus. Furthermore, when plasmid pHYD616 (carrying the <i>uup-351</i>:{Tn10dTet} insertion) was introduced into the <i>uup-352</i> and <i>uup-353</i> mutants, the resulting transformants continued to display a <i>tex</i> mutant phenotype, indicating that there was no complementation between the three alleles.

**Nucleotide sequence of <i>uup</i>.** We determined the nucleotide sequence of a 2,055-bp region of the wild-type <i>E. coli</i> chromosome between a <i>HaeII</i> site and a <i>PstI</i> site (marked in Fig. 1) in the vicinity of the position of the <i>uup-351</i>:{Tn10dTet} insertion. The sequence data are presented in Fig. 2. The last 662 bp of the determined sequence is identical (barring four frameshift errors) to the sequence near the 5′ end of <i>pqi-5</i> reported
previously (19). We also determined, by sequencing, the position of \( \text{Tn}^{10} \text{dTet}^{1} \) insertion in \( uup \). Conceptual translation of the nucleotide sequence in all reading frames allowed us to infer that the open reading frame (ORF) beginning at base position 228, with the potential to encode a 625-amino-acid polypeptide (Fig. 2), represents the coding region of the wild-type \( uup \) gene: (i) it is by far the longest ORF present in this region; (ii) the ORF is interrupted, beyond base position 1306, by the \( \text{Tn}^{10} \) \( \text{dTet}^{1} \) insertion representing the \( uup-351 \) allele; and (iii) the polypeptide sequence deduced from the ORF exhibits nearly 80% similarity (66% identity, and an additional 13% representing conservative amino acid substitutions) along its entire length to the product of the hypothetical gene HI1300 (EMBL database ID HI1300; accession no. L45934) in \( \text{Haemophilus influenzae} \) (12) (Fig. 3).

FIG. 2. Nucleotide sequence of the \( uup \) locus in \( \text{E. coli} \). The determined sequence proceeds from a \( \text{Hae}II \) site to a \( \text{PstI} \) site 2,055 bp away (both marked in Fig. 1). The last 50 bp of sequence beyond the \( \text{PstI} \) site is taken from the sequence submission by Koh and Roe (19) (see the text). Nucleotide numbering is indicated at the right end of each line. The deduced translation product of the \( uup \) ORF is denoted in the one-letter amino acid code. The position of ORF disruption by the \( uup-351 \)::\( \text{Tn}^{10} \text{dTet}^{1} \) insertion is shown by a vertical arrow. Restriction sites for \( \text{BamHI} \) (B) and \( \text{PstI} \) (P) are overlined. The following four corrections between positions 1394 and 2055 in the sequence were identified from that reported earlier (19) and are included in the figure (all single-base insertions; nucleotide positions indicated in parentheses): G (1431), C (1521), G (1532), and G (1832).
predicted by hydrophatic analysis to be a largely hydrophilic protein (reference 23 and data not shown). The deduced polypeptide sequence of uup, like that of the H. influenzae HI1300 gene, has two pairs of characteristic Walker A and Walker B motifs, which are believed to form the ATP-binding pockets in the protein (11, 16, 45) (Fig. 3).

DISCUSSION
Several novel tex mutations in ssb affect the N-terminal domain of SSB. By using a screen for mutations that lead to an increased frequency of precise excision of a lacZ::Tn10Kan insertion, we have obtained novel variants of the E. coli SSB protein. The majority of ssb mutants that have been studied earlier have alterations either in the central domain of SSB, which is implicated in tetramerization and DNA binding, or in the C-terminal domain, which is postulated to be involved in interactions with other proteins; these mutants also appear to be defective, to a greater or lesser extent, in all functions of SSB (4, 27, 33). Only one alteration in the N-terminal region of SSB has been previously characterized (G15D), and strains carrying this protein are temperature sensitive for growth and are extremely UV sensitive (33, 41).

In contrast, two of the ssb mutants obtained in this study (the G4D and L10F P24S mutants) carry alterations in this less well understood N-terminal domain and are also not drastically affected in replication, recombination, or DNA repair. The mechanism by which transposon excision in these mutants is not clear, primarily because we are ignorant of the exact mechanism of precise excision itself. It must be noted that precise excision is a stochastic event that occurs approximately once in a million cells even in the tex mutant strains, whereas the SSB protein is required stoichiometrically in each of the other functions in which it is implicated. Nevertheless, an analysis of the biochemical properties of the SSB mutant proteins identified in this work will be valuable.

One of the first points that needs clarification is whether the tex mutations in ssb represent loss-of-function or gain-of-function alleles. The complementation results obtained by us would suggest that the tex alleles are recessive to ssb<sup>+</sup> (although it must be noted that the latter was present on a multicopy plasmid in these experiments). The finding that there is an inverse correlation between ssb<sup>+</sup> mutation (which is implicated in tetramerization and DNA binding, or interaction with other proteins; these mutants also appear to be defective, to a greater or lesser extent, in all functions of SSB) and that precise excision is a stochastic event that occurs approximately once in a million cells even in the tex mutant strains, whereas the SSB protein is required stoichiometrically in each of the other functions in which it is implicated. Nevertheless, an analysis of the biochemical properties of the SSB mutant proteins identified in this work will be valuable.

Features of the uup gene product. A search for similar proteins in the SWISSPROT database, with the aid of the FASTA program (37), revealed that the uup gene product belongs to the family of ATP-binding cassette (ABC)-domain proteins, which are postulated to be involved in the coupling of ATP hydrolysis with other biological processes (11, 16). Many members of this family are involved in substrate import, export, or channelling across membranes, but the uup product itself is

HI1300. At the nucleotide sequence level, the similarity between uup and HI1300 is around 65% (data not shown), indicating that the two genes are evolutionarily homologous.

It may be noted that the uup ORF has a 50-bp extension at its 3′ end beyond the PsrI site up to which the sequence was determined in this study. (The sequence of this extended region has been adopted from the pgi-5 sequence submission by Koh and Roe [19].) The complementation results with plasmid pHYD612 (Fig. 1) indicate that the C-terminal 16 amino acids encoded by this region of the ORF are dispensable for function; these mutants also appear to be defective, to a greater or lesser extent, in all functions of SSB (4, 27, 33). Only one alteration in the N-terminal region of SSB has been previously characterized (G15D), and strains carrying this protein are temperature sensitive for growth and are extremely UV sensitive (33, 41).

Canceill and Ehrlich (3) have established an in vitro replication slippage system that may be a mimic of the reactions of precise excision and nearly precise excision in vivo, and in this system the addition of native SSB stimulates replication slippage. In contrast, Rosche et al. (39) have used triplet repeat amplification as a measure of replication slippage to show that cells lacking SSB exhibit increased slippage. It will be of interest to determine how the tex SSB variants isolated in this study would affect the slippage in each of these two test systems.

Role of uup in precise excision. Unlike the case with ssb, it is reasonably certain with uup that the tex mutations represent loss-of-function alleles. The complementation results obtained by us would suggest that the tex alleles are recessive to ssb<sup>+</sup> (although it must be noted that the latter was present on a multicopy plasmid in these experiments). The finding that there is an inverse correlation between ssb<sup>+</sup> gene dosage and precise excision frequency (Table 2) also suggests that the tex mutations represent loss-of-function alleles. On the other hand, the ssb<sup>-</sup> mutation (which is tex at the permissive growth temperature) has been reported to encode a product with increased helix-destabilizing activity (27, 33).
virtually the same as the C-terminal 460 amino acids of the HI1300 product (13). In our case, Southern blotting with the Kohara miniset library of E. coli identified only one locus hybridizing to the uup probe (data not shown).

The uup gene is situated immediately upstream of and is transcribed in the same direction as the pqi-5 gene, which has been identified as a SoxRS-controlled paraquat-inducible locus but whose function has not been determined (19). The P1 and P2 promoters mapped for pqi-5 fall within the coding region of uup (19, 20). The possibility that uup transcripts also extend into pqi-5 needs to be examined. It may be noted in this context that although the structures of several multicistronic operons are conserved between E. coli and H. influenzae (1, 12), there is no pqi-5 homolog in the vicinity of either the HI1300 or HI1342 gene in the latter organism.

At present, the only clue to the function of uup is the presence of the nucleotide-binding motif (45) in the deduced sequence of the polypeptide encoded by the gene. Given that tex mutations have been found in helicase-encoding genes such as recBC (30) and mvrD (28, 29), we looked for but were unable to identify helicase motifs in the uup gene product. The fact that the tex effects of ssb and uup mutations are additive (Table 2) might indicate the existence of distinct pathways for precise excision. Additional studies are therefore required to clarify the roles of uup in precise excision and other aspects of host cell metabolism.

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