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Identification and Characterization of *ssb* and *uup* Mutants with Increased Frequency of Precise Excision of Transposon Tn*10* 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*::Tn*10*dKan 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 Tn*10*dKan 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 paraquat-inducible SoxRS-regulated *pqi-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, generically 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 copy of the direct repeat at the target insertion site, so that the integrity of the original host sequence (before transposon insertion) is faithfully retained. It is known that both transposase and host-encoded homologous recombination functions are dispensable for precise excision (10, 13). In studies with derivatives of the tetracycline resistance transposon Tn*10*, Foster et al. (13) have demonstrated a correlation between the frequency of precise excision (or of a related event called nearly precise excision) and the length of the imperfect inverted repeats at the ends of the element. Most transposons possess such inverted repeats at their ends, and a similar relationship between repeat length and precise excision frequency has also been shown for the kanamycin resistance (Kan^r) element Tn*5* (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 Tn*10* (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

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the frequency of precise excision of Tn*10* and Tn*5* (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 *drpA* (24) have been reported to exhibit a decreased frequency of precise excision of transposon insertions. A phenomenon of UV-induced precise excision of Tn*10* 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 Tn*10* derivative (Tn*10*dKan, encoding Kan^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, phages, and plasmids. All the bacterial strains we used were derivatives of *E. coli* K-12 and are listed in Table 1. Phages P1*kc* and Mu *c*(Ts) were from our laboratory stocks. The transposon vehicle phages λ1098 (46) and λ1323 (18), used as vectors for the transposition of Tn*10*dTet, have been described previously. The ordered λ phage library of Kohara et al. (21) was kindly provided by K. Isono.

The plasmid vectors pBR322 (40), pBR329 (7), pCL1920 (25), and pBluescriptII KS (pBKS; Stratagene, La Jolla, Calif.) have been described previously; pCL1920 is derived from pSC101, whereas all the others are ColE1 derivatives. Plasmid pBR*ssb*$^+$ (given by L. E. Carlini) is a pBR322 derivative with a 0.8-kb *Bam*HI 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 *Eco*RI insert subcloned from the λ phage 637 of the collection of Kohara et al. (21), which carries the *ssb*$^+$ and *uvrA*$^+$ 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 A (supplemented with glucose or other indicated C source and the appropriate auxotrophic requirements) and Luria-Bertani (LB) medium (35). Unless otherwise indicated, the growth temperature was 37°C. Tetracycline, rifampin, ampicillin, and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) were used at 15, 100, 100, and 25 μg/ml respectively, and kanamycin, nalidixic acid, streptomycin, and spectinomycin were each used at 50 μg/ml.

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*::Tn*10*dKan. A population of mutagenized cells of strain GJ1821 (*lacZ*$^+$) was infected with a P1*kc* lysate prepared on a strain (GJ1854) carrying the closely linked *lacI*::Tn*10* and *lacZ*::Tn*10*dKan insertions, and Tet^r colonies were selected on LB agar plates supplemented with X-Gal and 0.1% lactose. The majority of Tet^r transductants were Lac$^-$ and exhibited a low frequency of Lac$^+$ papillation on these plates; putative *tex* mutants GJ1854.1 and GJ1854.2 were identified as Tet^r Lac$^-$ colonies that exhibited an increased frequency of Lac$^+$ papillation. An additional *tex* mutant, GJ1864, was obtained following direct nitrosoguanidine mutagenesis of the *lacZ*::Tn*10*dKan strain GJ1823.

In an alternative strategy, transposon insertion mutagenesis in the *lacZ*::Tn*10*dKan strain GJ1885 with phages λ1098 and λ1323 was performed as described previously (18, 35), and *tex* mutants were identified by screening on plates similar to those described above. Each of the two λ phages carries a Tet^r-encoding mini-Tn*10* derivative, and these derivatives differ from each other marginally in that the latter has two additional *Bam*HI sites located within the transposable elements (18, 46). For convenience, the two elements have been referred to in this study as Tn*10*dTet*1* and Tn*10*dTet*2*, respectively. In both phages, the transposase gene is situated outside the ends of the transposable element downstream of a *tac* promoter, with the gene in λ1323 being a variant that confers altered target site specificity on the enzyme (18).

Transposon tagging of *tex* point mutations. Random transpositions of Tn*10*dTet*1* into the chromosome of the *tex*$^+$ strain GJ1823 were generated following infection with λ1098 (35). A P1*kc* lysate prepared on the pool of Tet^r clones was used to infect the *tex* mutant GJ1864, and Tet^r transductants were selected on the Lac$^+$ papillation plates described above. Approximately 1% of the colonies exhibited a low frequency of Lac$^+$ papillation, suggesting that cotransduction of *tex*$^+$ with Tet^r in the cross occurred. One such clone was subsequently shown to have a Tet^r insertion (designated *zjc-904*::Tn*10*dTet*1*) approximately 80% linked to the *tex* locus in GJ1864 and the Tet^S derivatives of two other mutants GJ1854.1 and GJ1854.2. These *tex* mutations were also transduced into other strains with the aid of the same Tet^r insertion.

Measuring precise excision frequency in log phase. Cultures (5 ml) of *lacZ*::Tn*10*dKan strains were grown from an initial inoculum of around 10^3 cells to a density of 2×10^7 to 5×10^7 cells/ml in LB medium. The cells were harvested by centrifugation, washed once in minimal A buffer, and plated at appropriate dilutions on 0.2% lactose–minimal A plates to determine the numbers of Lac$^+$ revertants. Values reported are means of at least three independent determinations and are expressed as the fraction of the viable count simultaneously measured for each culture.

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

Strain	Genotype[a]	Source or reference
MG1655	Wild type	42
CAG12130	MG1655 *zcb-3111*::Tn*10*Kan	42
GJ1821	MG1655 *zbh-900*::Tn*10*dKan(Ts)*1*	38
GJ1823	GJ1821 *lacZ4525*::Tn*10*dKan	38
GJ1854	GJ1821 *lacI42*::Tn*10* *lacZ4525*::Tn*10*dKan	This work
GJ1854.1	GJ1854 *ssb-200*	This work
GJ1854.2	GJ1854 *ssb-201*	This work
GJ1864	GJ1821 *lacZ4525*::Tn*10*dKan *ssb-202*	This work
GJ1885	*ara zbh-900*::Tn*10*dKan(Ts)*1* *lacZ4525*::Tn*10*dKan	From CSH142 (35), in several steps
GJ1886	GJ1885 *uup-351*::Tn*10*dTet*1*	This work
GJ1887	GJ1885 *uup-352*::Tn*10*dTet2	This work
GJ1888	GJ1885 *uup-353*::Tn*10*dTet2	This work
GJ1889	GJ1885 Δ(*uup-351*::Tn*10*dTet*1*)	Tet^S selection from GJ1886
GJ1890	GJ1885 *ssb-200 zhc-904*::Tn*10*dTet*1*	This work
GJ1891	GJ1885 *ssb-201 zjc-904*::Tn*10*dTet*1*	This work
GJ1892	GJ1885 *ssb-202 zjc-904*::Tn*10*dTet*1*	This work
GJ1893	GJ1885 Δ(*uup-351*::Tn*10*dTet*1*) *ssb-200 zjc-904*::Tn*10*dTet*1*	This work

[a] Genotype designations are those of Berlyn et al. (1). All strains are F$^-$. The insertion mutations *lacI42*::Tn*10* (42) and *zbh-900*Tn*10*dKan(Ts)*1* (38) have been described previously.

Other genetic techniques. Procedures for transduction with phage P1*kc* (15), conjugation (35), mutagenesis with nitrosoguanidine (35), selection of Tet^S colonies (31), assay for cell survival following UV irradiation (35), measurement of spontaneous mutation frequency to Rif^r or Nal^r (35), and determination of burst size following phage Mu *c*(Ts) infection (17) were as described previously.

DNA methods. The standard protocols of Sambrook et al. (40) were followed for experiments involving recombinant DNA, including plasmid manipulations and transformation, Southern blot hybridization with the Kohara mini-set-library, PCR, and DNA sequencing. Chromosomal DNA was isolated as previously described (32). The following pair of oligonucleotide primers was used for the PCR amplification of the *ssb* gene from chromosomal DNA preparations: 5′-GCG*GGATCC*ACGAACATGGC-3′ and 5′-TGCA*CTGCAG*ACAAATCAGAA-3′; each of the PCR products was digested with *Bam*HI and *Pst*I (the recognition sites in the primers are italicized) and cloned into pBKS before DNA sequence analysis. DNA sequencing was done by the dideoxy-chain termination method either manually with ^{32}P-labelled nucleotides (Bhabha Atomic Research Centre, Bombay, India), or on an automated DNA sequencer (Applied Biosystems model 377) as specified by the manufacturer.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper have been submitted to the EMBL, GenBank, and DDBJ databases and assigned accession no. Y09439.

RESULTS

Identification of new *tex* mutants. New *tex* mutants were identified in this study following mutagenesis (with either nitrosoguanidine or Tn*10*dTet) of *E. coli* strains carrying *lacZ4525*::Tn*10*dKan, 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 Tn*10* 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

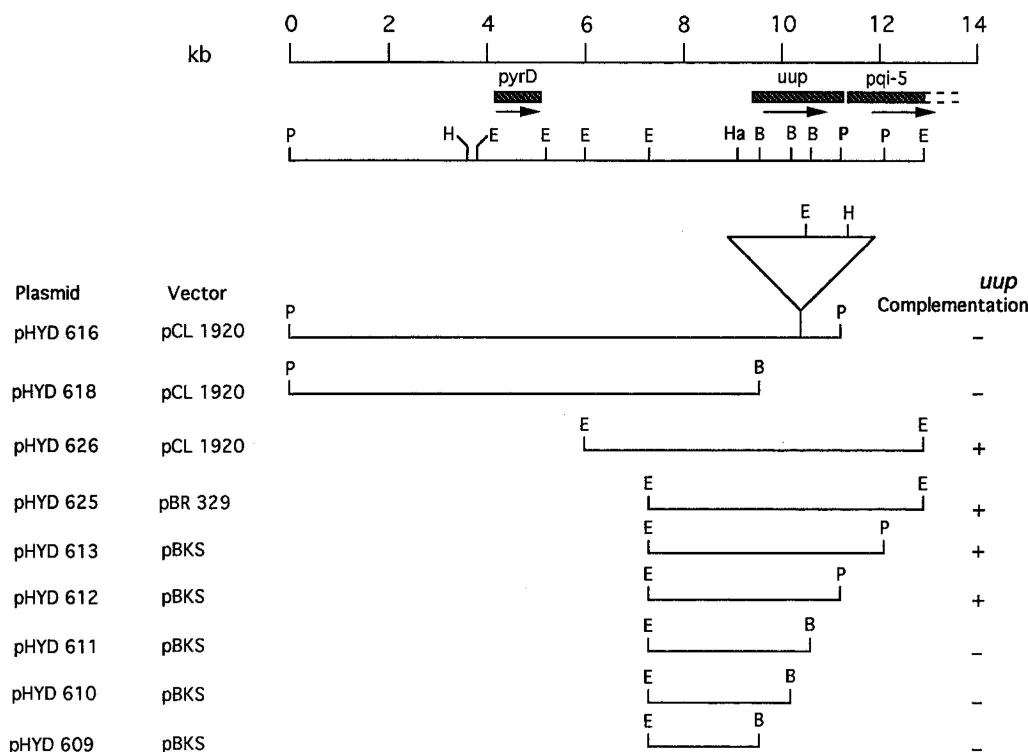

FIG. 1. Physical map of the min 21.5 to 21.8 region of the *E. coli* chromosome (1) and *uup* complementation results with plasmids carrying inserts derived from this region. Below the kilobase scale is depicted the physical map of this region for the enzymes *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), and *Pst*I (P). The positions and direction of transcription of the ORFs corresponding to *pyrD*, *pqi-5*, and *uup* (as deduced from this study) are marked. Marked in boldface type are the *Hae*II (Ha) and *Pst*I sites which defined the limits of the nucleotide sequence determined in this study; additional sites for *Hae*II exist but are not marked on the map. Each line aligned beneath the physical map represents the extent of chromosomal DNA cloned into a plasmid, whose numerical designation and vector derivation are indicated alongside. Also indicated is the ability of the plasmid to complement (+) or not to complement (−) the *uup-351* mutant for its Lac$^+$ papillation phenotype. The insert in plasmid pHYD616 carries the *uup-351*::Tn*10*dTet*1* insertion, represented by the inverted triangle.

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*::Tn*10*dTet*1*) 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*::Tn*10* (data not shown).

(ii) Two different plasmids carrying the cloned *ssb*$^+$ gene, pBR*ssb*$^+$ 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 *ssb*$^+$ 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); and *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*::Tn*10*dKan 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 Tn*10*dKan. 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 Tn*10*dTet*1* 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

TABLE 2. *ssb* and *uup* effects on frequency of precise excision of *lacZ*::Tn*10*dKan during exponential growth

Strain	Relevant genotype	No. of Lac$^+$ revertants (per 10^8 cells)
GJ1885	Wild type	86
GJ1890	*ssb-200*	1,050
GJ1891	*ssb-201*	400
GJ1892	*ssb-202*	425
GJ1886	*uup-351*	490
GJ1893	*ssb-200 uup-351*	2,400
GJ1885/pHYD620	*ssb*$^+$ (5 copies/cell)	38
GJ1885/pBR*ssb*$^+$	*ssb*$^+$ (20 copies/cell)	24

TABLE 3. UV irradiation tolerance and spontaneous point mutation frequencies in *tex* mutants

Strain	Relevant genotype	Fractional survival after exposure to UV for[a]:		No. of spontaneous mutants (per 10^9 cells)	
		20 s	60 s	Rif^r	Nal^r
GJ1885	Wild type	0.5	0.20	14	2
GJ1890	*ssb-200*	0.24	ND[b]	12	2
GJ1891	*ssb-201*	0.5	0.20	10	<1
GJ1892	*ssb-202*	0.4	0.31	3	4
GJ1886	*uup-351*	0.22	0.18	5	3
GJ1893	*ssb-200 uup-351*	ND	ND	5	<1

[a] 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 *uvrD* mutant strain was $<2 \times 10^{-4}$.

[b] ND, not determined.

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

We tested whether the *tex* mutations in *ssb* conferred additional phenotypes associated with SSB 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^r or Nal^r, 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 *tex* mutant phenotype of the new *ssb* derivatives was also unaffected by NaCl or sucrose supplementation of the medium (data not shown), in contrast to the osmoremedial nature of several *ssb* mutations that have been characterized earlier (33).

Multicopy *ssb*$^+$ effect on precise excisions. We observed a gene dosage effect of *ssb*$^+$ on the frequency of precise excisions of *lacZ*::Tn*10*dKan (Table 2). Thus, this frequency was lower in the strain with plasmid pHYD620 (which carries *ssb*$^+$ on a pSC101 vector; approximately 5 copies/cell) than in the haploid *ssb*$^+$ strain, and it was even further reduced in the strain with plasmid pBR*ssb*$^+$ (which carries *ssb*$^+$ on a pBR322 vector, approximately 20 copies/cell). The possible significance of these results is discussed below.

Identifying *tex* mutants following Tn*10*dTet insertion mutagenesis. Three independent Tn*10*dTet insertion derivatives (GJ1886 to GJ1888) of strain GJ1885 were identified in the Lac$^+$ papillation screen as *tex* mutants. In all three cases, we were able to demonstrate 100% linkage in P1 transduction between Tet^r and the increased Lac$^+$ papillation phenotype. The mutants exhibited an approximately fivefold increase in precise excision of *lacZ*::Tn*10*dKan during exponential growth, without significant alteration in the frequency of occurrence of spontaneous point mutations or in UV sensitivity (data for GJ1886 are given in Tables 2 and 3; data not shown for GJ1887 and GJ1888). As with the *ssb* 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 *zcb-3111*::Tn*10*Kan insertion in strain CAG12130 (42) (data not shown).

Based on the map position, two genes, *helD* (encoding helicase IV) and *uup* (discussed above), were considered as plausible candidates for the *tex* insertion mutations. It has been speculated earlier that *helD* and *uup* might even represent the same gene (28). To test whether the *tex* mutations were insertions in *helD*, we cloned the *helD*$^+$ gene on a 3.5-kb *Pst*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 Tn*10*dTet insertion mutants. None of the mutants was complemented by *helD*$^+$, thereby excluding this gene as the candidate for the *tex* insertion mutations.

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

A double mutant (GJ1893) carrying *tex* mutations in each of the *ssb* and *uup* genes, constructed by P1 transduction, exhibited a higher frequency of excision of *lacZ*::Tn*10*dKan than did either of the single mutants alone but remained unaffected in the frequency of spontaneous point mutation to Rif^r or Nal^r (Tables 2 and 3).

Molecular cloning and physical localization of *uup*. The *uup-351*::Tn*10*dTet*1* insertion was cloned on a 14-kb *Pst*I fragment from a preparation of GJ1886 chromosomal DNA into the plasmid vector pCL1920 by selection for the Tet^r 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 λ220, λ221, and λ222 from the panel of Kohara et al. (21). Alignment of the physical map of pHYD616 with that of the *E. coli* chromosome in this region permitted us to place the site of Tn*10*dTet*1* insertion at 21.8 centisomes on the *E. coli* physical map (1), immediately upstream of (and counterclockwise from) the *pqi-5* gene (19) (Fig. 1).

A 5.7-kb *Eco*RI fragment cloned from λ222 into the *Eco*RI site of plasmid vector pBR329 (resulting in plasmid pHYD625 [Fig. 1]) was able to complement the *uup-351*::Tn*10*dTet*1* mutant for its Lac$^+$ papillation phenotype (data not shown). A series of deletion subclones of the *Eco*RI insert fragment was constructed (in the plasmid vector pBKS) and tested for complementation. The results, depicted in Fig. 1, indicated that the complementing region is present within a 4.0-kb *Eco*RI-*Pst*I fragment carried on plasmid pHYD612.

Plasmids pHYD625 and pHYD612 were also able to complement the *uup-352*::Tn*10*dTet*2* and *uup-353*::Tn*10*dTet2 mutants for their Lac$^+$ 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 *uup-351*::Tn*10*dTet*1* insertion) was introduced into the *uup-352* and *uup-353* mutants, the resulting transformants continued to display a *tex* mutant phenotype, indicating that there was no complementation between the three alleles.

Nucleotide sequence of *uup*. We determined the nucleotide sequence of a 2,055-bp region of the wild-type *E. coli* chromosome between a *Hae*II site and a *Pst*I site (marked in Fig. 1) in the vicinity of the position of the *uup-351*::Tn*10*dTet*1* 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 *pqi-5* reported

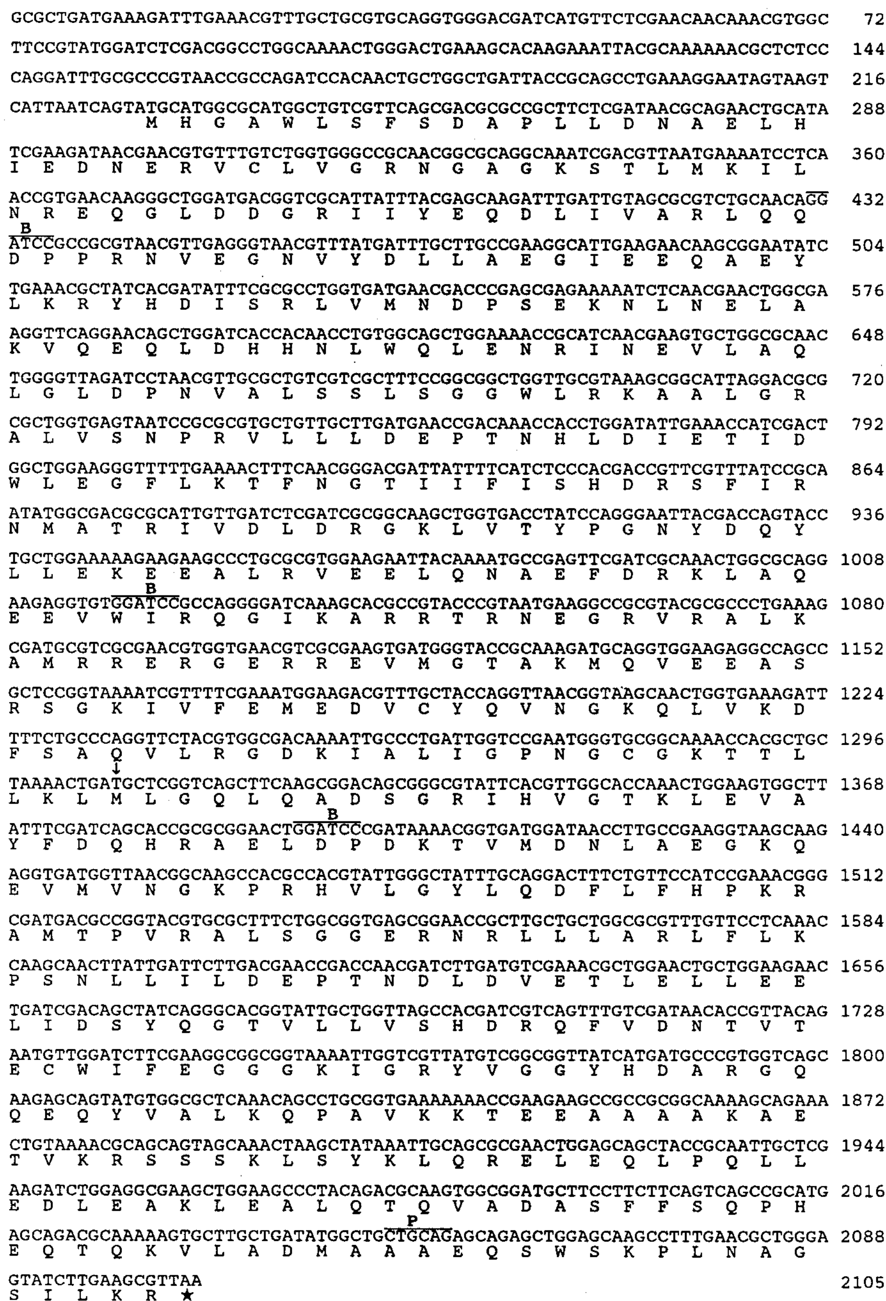

FIG. 2. Nucleotide sequence of the *uup* locus in *E. coli*. The determined sequence proceeds from a *Hae*II site to a *Pst*I site 2,055 bp away (both marked in Fig. 1). The last 50 bp of sequence beyond the *Pst*I site is taken from the sequence submission by Koh and Roe (19) (see the text). Nucleotide 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*::Tn*10*dTet*1* insertion is shown by a vertical arrow. Restriction sites for *Bam*HI (B) and *Pst*I (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).

previously (19). We also determined, by sequencing, the position of Tn*10*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 Tn*10*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 HIHI1300; accession no. L45934) in *Haemophilus influenzae* (12) (Fig. 3). Upon alignment of the corresponding polypeptide sequences (Fig. 3), the predicted N terminus of the *uup* gene product is just 5 residues away from that of the product encoded by

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                                                WA
UUP           MHGAWLSFSDAPLLDNAELHIEDNERVCLVGRNGAGKSTLMKILN  45
HI1300 [M]ALISLTNGYLSFSDAPLLDHAELHIEPNECVCLVGRNGAGKSTLLKIIA  50

UUP        REQGLDDGRIIYEQDLIVARLQQDPPRNVEGNVYDLLAEGIEEQAEYLKR  95
HI1300     GDVLMDDGKIQYEKDLVVSRLEQDPPRNAQGNIFDYVAEGVGHLTDLLKE  100

UUP        YHDISRLVMNDPSEKNLNELAKVQEQLDHHNLWQLENRINEVLAQLGLDP  145
HI1300     YHQISVQLEENYSDQILSQLEQVQAKLEHADGWRFENKINEVLLKLGLNP  150
                                         WB
UUP        NVALSSLSGGWLRKAALGRALVSNPRVLLLDEPTNHLDIETIDWLEGFLK  195
HI1300     NTKLSALSGGWLRKAALARALVCDPDVLLLDEPTNHLDVEAIEWLENFLL  200

UUP        TFNGTIIFISHDRSFIRNMATRIVDLDRGKLVTYPGNYDQYLLEKEEALR  245
HI1300     DFQGSIVFISHDRSFIRKMATRIVDLDRGQLVSYPGNYDLYLTTKEENLR  250

UUP        VEELQNAEFDRKLAQEEVWIRQGIKARRTRNEGRVRALKAMRRERGERRE  295
HI1300     VEALQNELFDKRLAQEEVWIRQGIKARRTRNEGRVRALKVMREERRQRRD  300

UUP        VMGTAKMQVEEASRSGKIVFEMEDVCYQVNGKQLVKDFSAQVLRGDKIAL  345
HI1300     VMGTAKLQLDTSSRSGKIVFEMEDVSYEIAGKTLLKDFSTTILRGDKIAL  350
             WA
UUP        IGPNGCGKTTLLKLMLGQLQADSGRIHVGTKLEVAYFDQHRAELDPDKTV  395
HI1300     VGPNGCGKTTFIKLLLGEIQPTSGKIRCGTKLEIAYFDQYRADLDPEKTV  400

UUP        MDNLAEGKQEVMVNGKPRHVLGYLQDFLFHPKRAMTPVRALSGGERNRLL  445
HI1300     MDNVADGKQDIEINGVKRHVLGYLQDFLFPPKRAMTPVKALSGGERNRLL  450
                      WB
UUP        LARLFLKPSNLLILDEPTNDLDVETLELLEELIDSYQGTVLLVSHDRQFV  495
HI1300     LAKLLLKPNNLLILDEPTNDLDVETLELLEEILTDYQGTLLIVSHDRQFI  500

UUP        DNTVTECWIFEGGGKIGRYVGGYHDARGQQEQYVALK----QPAVKKTE-  540
HI1300     DNTATECYLFEGKGHLNKYVGGFFDAKQQQANFWASKAVEEQAKAKKSEP  550

UUP        ---EAAAAKAETVKRSSSKLSYKLQRELEQLPQLLEDLEAKLEALQTQVA  587
HI1300     LKEESAVKNDRTSKPKSVKLSYKEQRELEQLPQLLEELETKITVLQAEIA  600

UUP        DASFFSQPHEQTQ---KVLADMAAAEQSWSKPLNAGSILKR          625
HI1300     DPAFFQQAHDITDAKLKALADTEAELETAFLRWEELEEKKNLVEGKA    647
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FIG. 3. Similarity between the aligned polypeptide sequences (denoted in the one-letter amino acid code) derived from the ORF of *uup* in *E. coli* (UUP) and that of the HI1300 gene in *H. influenzae* (HI1300). Amino acid numbering is indicated at the right end of each line. Each position of identity and of conservative substitution (within one or another of the following groups: D, E, N, Q; H, K, R; S, T; A, G; I, L, M, V; or F, W, Y) between the two sequences is represented, respectively, by a vertical line and a colon. Where necessary, gaps have been introduced in the sequence to maximize the homology in alignment. The two pairs of Walker motifs (WA and WB) in each sequence are overlined (45). The putative initiator methionine (bracketed) for the HI1300 product is encoded by a GTG codon.

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 *Pst*I site up to which the sequence was determined in this study. (The sequence of this extended region has been adopted from the *pqi-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; this region of the protein also shares no homology with the HI1300 product (Fig. 3). All other complementation results described in Fig. 1 are consistent with the *uup* ORF assignment deduced above.

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 predicted by hydropathic 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*::Tn*10*dKan 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 increased 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^+ (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^+ 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-113* mutation (which is *tex* at the permissive growth temperature) has been reported to encode a product with increased helix-destabilizing activity (27, 33).

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 *uup* gene is quite homologous to HI1300, a gene of unknown function identified in *H. influenzae* (12). It is intriguing that *H. influenzae* also has a second discrete gene, designated HI1342 (EMBL database ID HIHI1342; accession no. L45975), at a different chromosomal location, whose sequence is 96% identical to the 3′ region of HI1300 and which encodes a potential polypeptide that is

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 *uvrD* (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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