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Characterization of the *uup* Locus and Its Role in Transposon Excisions and Tandem Repeat Deletions in *Escherichia coli*

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Null mutations in the *Escherichia coli uup* locus (at 21.8 min) serve to increase the frequency of RecA-independent precise excision of transposable elements such as Tn*10* and to reduce the plaque size of bacteriophage Mu (Uup$^-$ phenotype). By the combined approaches of physical mapping of the mutations, complementation analyses, and protein overexpression from cloned gene fragments, we have demonstrated in this study that the Uup$^-$ phenotype is the consequence of the absence of expression of the downstream gene (*uup*) of a two-gene operon, caused either directly by insertions in *uup* or indirectly by the polar effect of insertions in the upstream gene (*ycbY*). The promoter for *uup* was mapped upstream of *ycbY* by primer extension analysis on cellular RNA, and assays of reporter gene expression indicated that it is a moderately active, constitutive promoter. The *uup* mutations were also shown to increase, in a RecA-independent manner, the frequencies of nearly precise excision of Tn*10* derivatives and of the deletion of one copy of a chromosomal tandem repeat, suggesting the existence of a shared step or intermediate in the pathways of these latter events and that of precise excision. Finally, we found that mutations that increase the frequency of precise excision of Tn*10* are divisible into two categories, depending upon whether they did (*uup*, *ssb*, *polA*, and *topA*) or did not (*mutHLS*, *dam*, and *uvrD*) also increase precise excision frequency of the mini-Tn*10* derivatives. It is suggested that the differential response of mini-Tn*10* and Tn*10* to the second category of mutations is related to the presence, respectively, of perfect and of imperfect terminal inverted repeats in them.

One of the features of mutations generated by the insertion of transposable elements is their ability to undergo true reversion, at characteristically low frequencies, by precise excision between the pair of host sequence-derived direct repeats that flank each insertion. Unlike other properties associated with transposons, precise excision is mediated by host-encoded functions and does not depend on the transposase encoded within each element (10, 13). In studies with the tetracycline resistance element Tn*10* (13, 25, 26), Kleckner and coworkers have identified precise excision as one of three related genetic rearrangements, the other two being nearly precise excision (in which a deletion event between two repeats internal to Tn*10* results in excision of all but 50 bp of the element, so that the target gene remains nonfunctional but there is relief of polarity on the expression of downstream genes in the operon) and precise excision of the 50-bp remnant of nearly precise excision. All three rearrangements are RecA independent and fall into the category of illegitimate recombination events.

The mechanism by which precise excisions occur is not known, nor is it clear what, if any, are the other non-transposon-related mutations, resulting from illegitimate recombination events in bacteria, that are mechanistically related to precise excision. Foster et al. (13) had provided early evidence that, whereas precise excision and nearly precise excision of Tn*10* may occur by very closely related pathways, precise excision of the 50-bp remnant appears to occur by a different mechanism. One model has been that precise excision occurs by a RecA-independent replication slippage event across the pair of direct repeats (of host-derived sequence) flanking the insertion and that the inverted repeats at the ends of the element facilitate the process (10, 13, 48). The inverted repeats may, for example, participate in formation of intrastrand stem-loop structure(s), although alternative structures involving interactions between the inverted repeats as duplex DNA have not been excluded (10, 13, 40, 48). The former possibility is supported by the findings that 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 (7) or during Tra-dependent synthesis of single-stranded DNA during conjugal transfer of an F′ plasmid (30, 49). An in vitro model that mimics precise excision and that is mediated by replication slippage has also been reported (6). Finally, a separate phenomenon of UV-induced transposon precise excision that appears to require functions encoded by the SOS regulon has also been described (22, 34).

Mutations (designated *tex*, for transposon excisions) in several host genes that increase the frequency of precise excisions have been identified (16, 25, 26, 38). One such locus is *uup* (16, 38), which maps at 21 min on the *Escherichia coli* chromosome and increases the precise excision frequency of both transposons Tn*5* and Tn*10*. Mutants in *uup* also exhibit a reduction in lytic growth of Mu bacteriophage. In an earlier study (38), we had described the isolation of several independent insertion mutations in the *uup* locus. Molecular cloning, complementation analysis, and nucleotide sequence determination of the gene identified by one of the disruptions had indicated that the Uup protein is cytosolic and belongs to the superfamily of ATP-binding cassette domain proteins (23).

In the present study, we have investigated the organization and regulation of the *uup* locus. Our results indicate that *uup* is part of a complex operon and that it is situated downstream of a conserved gene of apparently unrelated function (*ycbY*) with which it is cotranscribed from a moderately active, constitutive promoter. We also report that *uup* mutations increase the frequency of two other RecA-independent recombination

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TABLE 1. List of *E. coli* strains[a]

Strain	Genotype[b]	Source or reference
BL21(DE3)	*hsdS gal* (λDE3)	47
JJC520	*deo lacZ*DR$_{624}$ *lacY1* Cm^r	3
KM22	Δ(*recC ptr recB recD*)::(P$_{lac}$-*bet exo* Kan^r)	33
MC4100	Δ(*argF-lac*)*U169 rpsL150 relA1 araD139 flbB5301 deoC1 ptsF25*	Lab stock
MG1655	Wild type	CGSC[c]
GJ1885[d]	*ara zbh-900*::Tn*10*dKan(Ts)*1 lacZ4525*::Tn*10*dKan	38
GJ1886	GJ1885 *uup-351*::Tn*10*dTet*1*	38
GJ1887	GJ1885 *uup-352*::Tn*10*dTet*2*	38
GJ1888	GJ1885 *uup-353*::Tn*10*dTet*2*	38
GJ1934	MC4100 *recA56 srl-300*::Tn*10 zgb-910*::Tn*10*dCm	This work
GJ2240	KM22 Δ*ycbY*::Tet	This work
GJ2241	MC4100 *lacI*^{q} L8 *lacZ*$^+$ *zai-911*::Tn*10*dCm	This work
GJ2242	MC4100 (λ*cI857*)	This work
GJ2243	GJ1885 *topA72*::Tn*10*dTet*2*	This work
GJ2255	GJ1885 Δ*ycbY*::Tet	This work
GJ2256	MG1655 *lacZ*DR$_{624}$ Cm^r	This work
GJ2258	GJ1885 *recA56 srl-300*::Tn*10*	This work
GJ2259[e]	GJ1886 *recA56 srl-300*::Tn*10 zgb-910*::Tn*10*dCm	This work
GJ2260[e]	GJ1887 *recA56 srl-300*::Tn*10 zgb-910*::Tn*10*dCm	This work
GJ2261[e]	GJ1888 *recA56 srl-300*::Tn*10 zgb-910*::Tn*10*dCm	This work
GJ2265	GJ1885 *lacZ*$^+$	This work
GJ2268	GJ2265 *lacZ2900*::Tn*10*	This work
GJ2269	GJ2265 *lacZ4526*::Tn*10*dTet*2*	This work
GJ2272[f]	GJ2265 *uup-351*::Tn*10*dTet*1 lacI3098*::Tn*10*Kan *lacZ2900*::Tn*10*	This work
GJ2273[f]	GJ2265 *uup-351*::Tn*10*dTet*1 lacI3098*::Tn*10*Kan *lacZ4526*::Tn*10*dTet*2*	This work
GJ2274[f]	GJ2265 *ssb-200 zjc-904*::Tn*10*dTet*1 lacI3098*::Tn*10*Kan *lacZ2900*::Tn*10*	This work
GJ2275[f]	GJ2265 *ssb-200 zjc-904*::Tn*10*dTet*1 lacI3098*::Tn*10*Kan *lacZ4526*::Tn*10*dTet*2*	This work
GJ2276	GJ2265 *polA12 zih-3166*::Tn*10*Kan *lacZ2900*::Tn*10*	This work
GJ2278	GJ2265 *polA12 zih-3166*::Tn*10*Kan *lacZ4526*::Tn*10*dTet*2*	This work
GJ2280	GJ2265 Δ*uvrD288*::Kan *lacZ2900*::Tn*10*	This work
GJ2281	GJ2265 Δ*uvrD288*::Kan *lacZ4526*::Tn*10*dTet*2*	This work
GJ2282	GJ2265 *dam*::Tn*9 lacZ2900*::Tn*10*	This work
GJ2283	GJ2265 *dam*::Tn*9 lacZ4526*::Tn*10*dTet*2*	This work
GJ2284	GJ2265 *mutH471*::Kan *lacZ2900*::Tn*10*	This work
GJ2285	GJ2265 *mutH471*::Kan *lacZ4526*::Tn*10*dTet*2*	This work
GJ2288[f]	GJ2265 *mutS*::Tn*10*dTet*1 lacI3098*::Tn*10*Kan *lacZ2900*::Tn*10*	This work
GJ2289[f]	GJ2265 *mutS*::Tn*10*dTet*1 lacI3098*::Tn*10*Kan *lacZ4526*::Tn*10*dTet*2*	This work
GJ2290[f]	GJ2265 *mutL*::Tn*10 lacI3098*::Tn*10*Kan *lacZ2900*::Tn*10*	This work
GJ2291[f]	GJ2265 *mutL*::Tn*10 lacI3098*::Tn*10*Kan *lacZ4526*::Tn*10*dTet*2*	This work
GJ2292	*ara zbh-900*::Tn*10*dKan(Ts)*1 lacZ*DR$_{624}$ Cm^r	This work
GJ2293	GJ2292 *recA*::Kan	This work
GJ2294	GJ2292 *uup-351*::Tn*10*dTet*1*	This work
GJ2295	GJ2292 *uup-351*::Tn*10*dTet*1 recA*::Kan	This work

[a] BL21(DE3) is *E. coli* B. All other strains are K-12 derivatives.

[b] Genotype designations are as in the work of Berlyn (2). All strains are F$^-$. Allele numbers are indicated where they are known. The designations Tn*10*dTet*1* and Tn*10*dTet*2* have been described earlier (38). In the GJ strains listed, the following mutations were transduced from strains described earlier: *recA56* and *srl-300*::Tn*10*, GJ971 (43); *lacI*^{q} L8, NR3835 (45); *lacZ2900*::Tn*10*, NK5661 (26); *lacI3098*::Tn*10*Kan, CAG18420 (46); *ssb-200* and *zjc-904*::Tn*10*dTet*1*, GJ1890 (38); *polA12*, MM383 (CGSC no. 5022); *zih-3166*::Tn*10*Kan, CAG18601 (46); Δ*uvrD288*::Kan, SK6776 (52); *mutH471*::Kan, GW3773 (CGSC no. 7306); *mutS*::Tn*10*dTet*1*, CSH115 (31); and *mutL*::Tn*10*, ES1484 (CGSC no. 7050). Strains carrying the *dam*::Tn*9* and *recA*::Kan alleles were obtained from N. Kleckner and R. Jayaraman, respectively. The *zgb-910*::Tn*10*dCm and *zai-911*::Tn*10*dCm insertions were new alleles obtained in this study (with the aid of the transposon vehicle phage λNK1324 [17]), cotransducible 60 and 80%, respectively, with the *recA* and *lac* loci. The *lacZ4526*::Tn*10*dTet*2* insertion was also obtained in this study.

[c] CGSC, Coli Genetic Stock Center.

[d] The *zbh-900*::Tn*10*dKan(Ts)*1* allele confers kanamycin-resistance at 30 but not at 42°C (37).

[e] GJ2259, GJ2260, and GJ2261 were constructed by P1 transduction of GJ1886, GJ1887, and GJ1888, respectively, with a P1 lysate prepared on GJ1934. The presence of the *srl-300*::Tn*10* allele in these strains has not been verified.

[f] The *lacZ* allele in each of these strains was introduced by cotransduction with the linked *lacI3098*::Tn*10*Kan marker, with selection for kanamycin resistance at 42°C.

events, namely, nearly precise excisions and deletions of one copy of a chromosomal tandem repeat (tandem repeat deletion). Finally, our results permit classification of the *tex* mutations into two categories, depending upon their differential effects on Tn*10* derivatives with imperfect versus perfect terminal inverted repeats.

MATERIALS AND METHODS

Growth media and conditions. The defined and nutrient media were, respectively, minimal A medium (supplemented with glucose or other indicated C source and the appropriate auxotrophic requirements) and Luria-Bertani medium (31). Concentrations of antibiotics and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) used were as earlier described (37, 38).

Bacterial strains and plasmids. The genotypes of *E. coli* strains used in this study are listed in Table 1. Plasmids were constructed from the following vectors: the high-copy-number derivatives pBluescript II KS (pBKS; Stratagene, La Jolla, Calif.) and pET21b (Novagen, Madison, Wis.); a medium-copy-number pSC101 derivative, pCL1920 (21); and a very-low-copy-number IncW derivative, pMU2385 (51), carrying the *lacZ* reporter gene for promoter cloning experiments. The extent of *uup* locus carried on each of the plasmids is depicted in Fig. 1.

DNA methods. The standard protocols of Sambrook et al. (42) were followed for experiments involving recombinant DNA, including plasmid manipulations, gel electrophoresis, transformation, preparation of radiolabeled probes, Southern blot hybridization, and DNA sequence determination on double-stranded

plasmid DNA templates. The oligonucleotide primer 5′-TGGTCACCAACGC TTTTCCCGAG-3′, designed so as to read outward from a site immediately internal to the right terminal inverted repeat of Tn*10*dTet*2* (see Fig. 1), was used to determine the junction sequences of insertions generated with this element.

Construction of a chromosomal *ycbY* deletion-insertion mutant. A 1.2-kb *Bcl*I fragment that spans the promoter and proximal third of the *ycbY* open reading frame (ORF) was excised from plasmid pHYD633, and in its stead was ligated a 2.7-kb *Bam*HI fragment (derived from Tn*10*dTet*2*) comprising the *tetA* and *tetR* genes. The resulting plasmid pHYD650 thus carries both a deletion and a tetracycline resistance insertion in *ycbY*. The Δ*ycbY*::Tet mutation was recombined into the chromosome of the *recD* strain KM22 as described elsewhere (33), following transformation with 1 μg of a gel-purified 5.3-kb fragment from pHYD650 that carries the mutation and flanking DNA from the *ycbY-uup* locus. In order to control for the possibility that the mutation might be lethal, the linear transformation with the fragment from pHYD650 was attempted in both KM22 and KM22/pHYD646 (where pHYD646 is expected to provide $ycbY^+$ and uup^+ functions even after disruption of the chromosomal locus); equal numbers of Tet^r transformants were obtained with both recipient strains. A P1 lysate prepared on one KM22 Tet^r transformant, GJ2240, was then used to transduce the *lacZ*::Tn*10*dKan strain GJ1885, as well as the pHYD646 derivative of GJ1885, to Tet^r. Once again, Tet^r transductants were obtained in both strains at equal frequencies, and a 100% linkage was observed in GJ1885 between Tet^r and the Uup^- phenotype (data not shown). One such GJ1885 derivative was designated GJ2255.

Measuring mutation frequencies. In strains where reversions to $lacZ^+$ were being examined, Lac^+ papillation tests (37, 38) were employed to obtain rapid and qualitative estimates of mutation frequency. The general procedure for quantitative determination of mutant frequencies in cultures was as described by Fijalkowska and Schaaper (12). Briefly, the mutant frequency was calculated as the ratio of the median number of mutants in a series of (four to eight) cultures to the average number of viable cells per culture. The median frequency was chosen so as to avoid the problem of disproportionate contributions by mutational jackpots in individual cultures. In agreement with earlier reports (12, 13), threefold or greater differences in mutation frequencies between different strains were clearly reproducible in these experiments. In all the experiments involving selection for utilization of lactose, melibiose, or phenyl-β-D-galactoside as C source, appropriate minimal plates prespread with 10^9 cells of the Δ*lac* strain MC4100 (or its derivatives carrying the plasmid vector pBKS, pCL1920, or pMU2385 for surviving appropriate antibiotic supplementation) as scavenger were used.

The frequency of precise excision of the kanamycin resistance insertion in *lacZ*::Tn*10*dKan strains was measured following selection for Lac^+ revertants; all complementation experiments involving plasmid-borne genes were done in *recA* strains. The frequency of nearly precise excision of *lacZ*::Tn*10*dKan was measured following selection for polarity relief mutants capable of expressing LacY permease and growth on melibiose as sole C source at 42°C (31), on plates additionally supplemented with isopropyl-β-D-thiogalactopyranoside (IPTG) and X-Gal so that the ratio of white Lac^- Mel^+ colonies (nearly precise-excision mutants) to blue Lac^+ Mel^+ colonies (precise-excision mutants) could be determined; typically, this ratio was at least 200:1. All Mel^+ Lac^- colonies tested had also lost the Kan^r marker in *lacZ* and were capable of reverting in a subsequent step to Lac^+ (on Lac^+ papillation medium), indicating that they had uniformly suffered nearly precise excision of the *lacZ*::Tn*10*dKan element. The frequency with which the 50-bp remnant of Tn*10*dKan in *lacZ* following nearly precise excision undergoes precise excision was measured exactly as for precise excision of *lacZ*::Tn*10*dKan itself.

Tandem repeat deletion frequency was measured by selection for Lac^+ revertants of the strain GJ2292 (or its derivatives). GJ2292 carries a 624-bp in-frame duplication within the *lacZ* gene ($lacZDR_{624}$) and a chloramphenicol resistance marker gene near *lacZ* and is similar to strain JJC520, which had been used earlier by Michel and coworkers (3), except that the former is $lacY^+$ whereas the latter also carries the *lacY1* mutation. GJ2292 was constructed in two steps as follows. (i) A P1 phage lysate prepared on JJC520 was used to infect strain MG1655, and a double selection was imposed for Cm^r and Mel^+ at 42°C (that is, for the marker closely linked to $lacZDR_{624}$ and for $lacY^+$, respectively), followed by screening for Lac^- colonies; one transductant so recovered was designated GJ2256. (ii) In the second step, P1 transduction to Cm^r with a lysate prepared on GJ2256 was used to replace the *lacZ*::Tn*10*dKan allele in GJ1885 with $lacZDR_{624}$, and the resulting strain was designated GJ2292.

lacI mutation frequencies were determined in strain GJ2241 or its *uup* derivatives following selection for growth on 0.05% phenyl-β-D-galactoside as sole C source (in the absence of IPTG), as described elsewhere (45). The frequency of occurrence of spontaneous deletions in the *att*λ-*gal* locus was measured using a strain (GJ2242) carrying a λ*c*I*857* prophage or its *uup* derivatives, following selection for survivors at 42°C on minimal A-glycerol medium supplemented with 1 mM 2-deoxy-D-galactose.

Isolation of a *topA* insertion mutant as *tex*. Random insertions of transposon Tn*10*dTet*2* were generated in the chromosome of the *lacZ*::Tn*10*dKan strain GJ1885 after infection with the transposon vehicle phage λNK1323, as described elsewhere (17). Tet^r colonies were screened on Luria-Bertani–lactose–X-Gal agar plates by the method earlier described (38), for clones that exhibited increased Lac^+ papillation frequency following precise excision of the Tn*10*dKan insertion in *lacZ*. One *tex* mutant so identified also showed extremely poor growth characteristics on both defined and rich media and was designated GJ2243. The Tn*10*dTet*2* insertion was cloned as part of a 12-kb *Pst*I fragment from the chromosome of GJ2243 in the plasmid vector pCL1920, and the resulting plasmid was designated pHYD661. A radiolabeled probe prepared from DNA of plasmid pHYD661 hybridized to the recombinant λ phages 253 and 254 of the ordered Kohara miniset phage library (20, 41). Data from physical mapping of pHYD661 permitted the inference that the Tn*10*dTet*2* insertion in GJ2243 is located in *topA* (encoding topoisomerase I). Determination of the junction sequence (using pHYD661 as template) confirmed that the insertion had occurred immediately preceding the first base of codon 481 in the 865-residue-long *topA* ORF. The insertion allele in GJ2243 has been designated *topA72*::Tn*10*dTet*2*.

Other techniques. Procedures for transduction with P1 phage (15); determination of burst size following phage Mu *c*(Ts) infection (16); IPTG-mediated overexpression, and identification by gel electrophoresis, of the products of genes cloned into pET plasmid vectors and introduced into the BL21(DE3) strain (47); and measurement of β-galactosidase activities in cultures (31) were as described previously. Strains were made *recA* following transduction either to Cm^r with a P1 lysate prepared on strain GJ1934 that carries a Tn*10*dCm insertion 60% linked to the *recA* locus or to Kan^r with a lysate prepared on a *recA*::Kan mutant.

RESULTS

Insertions in both *uup* and *ycbY* confer a Uup^- phenotype. In an earlier study (38), we had described the isolation and mapping by phage P1 transduction of three independent Tn*10*dTet insertion mutations (*uup-351*, −*352*, and −*353*) to a single chromosomal locus. The cloning, physical mapping, and sequence analysis of the gene designated *uup* that had been rendered null by the *uup-351*::Tn*10*dTet*1* insertion was also described. Plasmids bearing the cloned uup^+ gene complemented all three mutants, and conversely, a plasmid with the *uup-351* insertion complemented none of them.

The physical map and organization of genes in the vicinity of *uup* deduced from the genome sequence of *E. coli* (4) are shown in Fig. 1. In this study, we determined, by Southern blot analysis of genomic DNA prepared from the *uup-352* and −*353* mutants (GJ1887 and GJ1888, respectively), the physical positions of the cognate Tn*10*dTet insertions in them. For this purpose, a radiolabeled probe prepared from an internal fragment (*Eco*RI-*Hin*dIII) of the *tet* gene was used, and the data are presented in Fig. 2.

The fact that, for both mutants, the size of the hybridizing fragment following *Eco*RI-*Pst*I digestion was approximately 1.8 kb smaller than that following digestion with *Eco*RI alone permitted the inference that the insertions were situated in the interval between the *Eco*RI and *Pst*I sites that delimit the major portion of the two ORFs *ycbY* and *uup* (Fig. 1), in the common orientation shown. From the size of the *Eco*RI-*Pst*I hybridizing fragment, the position of each insertion within this interval was calculated and is marked in Fig. 1 (along with that of the *uup-351* insertion characterized earlier, for comparison). The results indicated that the *uup-352* insertion is located downstream of *uup-351* in the *uup* gene, approximately 0.5 kb from the 3′ end. On the other hand, *uup-353* is an insertion in the anonymous ORF *ycbY* situated immediately upstream of, and in the same orientation as, *uup*. The Southern hybridization data from the *Hin*dIII-*Pst*I chromosomal digests (Fig. 2) were consistent with these conclusions. Thus, insertions in two adjacent ORFs, *ycbY* and *uup*, appear to confer a Uup^- phenotype.

We also cloned the chromosomal *Pst*I fragment encoding Tet^r from the *uup-353* mutant strain GJ1888 into the vector pCL1920, and the resulting plasmid was designated pHYD638 (Fig. 1). Analysis of restriction digests of pHYD638 (data not shown) provided confirmation for the fact that the Tn*10*dTet insertion in the plasmid is in *ycbY*. The exact site of *uup-353* mutation in pHYD638 was identified by DNA sequencing across the junction of the Tn*10*dTet*2* insertion, and the data

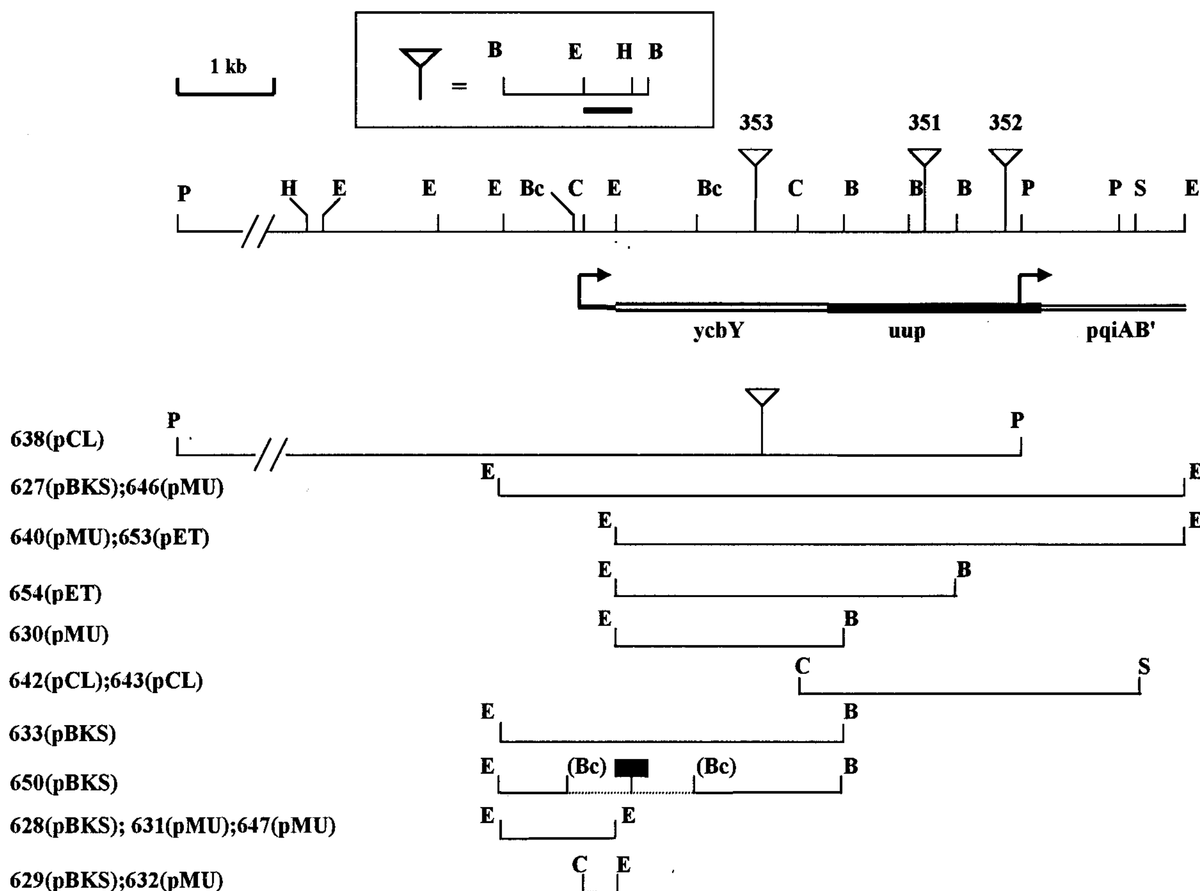

FIG. 1. Extents of insert DNA from *E. coli uup* locus in plasmids used in this study. On top is depicted, to the indicated scale, the position of recognition sites for the enzymes *Bam*HI (B), *Bcl*I (Bc), *Cla*I (C), *Eco*RI (E), *Hin*dIII (H), *Pst*I (P), and *Sal*I (S); for *Bcl*I, *Cla*I, and *Sal*I, only the relevant sites have been marked. Also depicted are the positions of the *uup-351*, *-352*, and *-353*::Tn*10*dTet insertions (as inverted triangles). Immediately beneath is depicted the alignment of the *ycbY*, *uup*, *pqiA*, and *pqiB′* ORFs and the promoters (as hooked arrows) within *uup* and upstream of *ycbY* identified, respectively, by Roe's group (18) and in this study. The *uup* locus sequence is from the work of Blattner et al. (4) and is corrected from that reported earlier (18, 38). The inset shows a map, drawn to one-half scale, of Tn*10*dTet in the orientation present in each of the three *uup* insertions, and the solid bar marks the fragment used for radiolabeled probe preparation in the Southern blot of Fig. 2; the inset map is that of Tn*10*dTet*2* present in *uup-352* and *uup-353*, whereas the Tn*10*dTet*1* element in *uup-351* lacks the pair of *Bam*HI sites shown (17, 38). Each line aligned beneath the *uup* locus physical map represents the extent of chromosomal DNA, delimited by the cut sites marked, that has been cloned into a plasmid(s) whose numerical pHYD designation(s) (and vector derivation[s] in parentheses) is indicated alongside. Abbreviations for plasmid vectors: pCL, pCL1920; pET, pET21b; and pMU, pMU2385. The interrupted line segment in the insert of pHYD650 depicts deletion of DNA between the parenthetical *Bcl*I sites marked, and the filled rectangle indicates insertion at this site of the 2.7-kb Tet^r-encoding *Bam*HI fragment from Tn*10*dTet*2* (see inset). Digestions at the *Bcl*I sites and at the right *Cla*I site marked were done on DNA prepared from a *dam* strain.

indicate that the insertion has occurred between bases 1 and 2 of codon 417 in the 702-residue-long *ycbY* ORF.

Plasmid pHYD638 (bearing the *uup-353* insertion) failed to complement the Uup$^-$ phenotype of the chromosomal *uup-351* and *−352* insertions in the downstream *uup* gene (Table 2). Taken together with our earlier result (38) that a plasmid carrying *ycbY*$^+$ and the *uup-351* insertion in *uup* also does not complement the chromosomal insertion (*uup-353*) in *ycbY*, we conclude that *ycbY* and *uup* constitute a single operon and that the failure of the *ycbY* insertion to complement mutations in *uup* is because of a polarity effect associated with the former.

That both the ORFs *ycbY* and *uup* encode proteins of the expected size was established with the aid of an IPTG-inducible T7 RNA polymerase-based in vivo expression system (Fig. 3). Following induction with IPTG, a pair of closely migrating protein bands of approximately 73,000 in M_r was detected from a template (pHYD653) that included both ORFs (Fig. 3, lane 5), whereas the lower band of this doublet was replaced by one of approximately 47,000 in M_r when a template (pHYD654) with a truncated *uup* ORF was used (Fig. 3, lane 7). The deduced M_rs of the *ycbY* and *uup* gene products are, respectively, 78,854 and 72,066; the deduced M_r of the truncated *uup′* product expected to be synthesized from pHYD654 is 45,112.

***ycbY* expression is not required for Uup$^+$ phenotype.** In the next set of experiments, we examined whether the Uup$^-$ phenotype associated with the *ycbY* insertion was because of (i) merely a polarity effect of the insertion on expression of the downstream *uup* gene or (ii) the need for *ycbY* as well in conferring the Uup$^+$ phenotype. For this purpose, we constructed a pair of plasmids (pHYD642 and pHYD643) in which a fragment carrying *uup*$^+$ without an intact *ycbY* had been cloned in either orientation into a site in the vector pCL1920. Both plasmids were able to complement the transposon precise excision phenotype of the mutant carrying the *uup-353* insertion in *ycbY* (Table 2) as well as its Mu plaque size phenotype, suggesting that *ycbY* itself is not required for the Uup$^+$ function.

The *uup-353* insertion is situated approximately two-thirds into *ycbY*, and it is possible that the resulting truncated protein was necessary and sufficient, along with *uup*$^+$, for the Uup$^+$

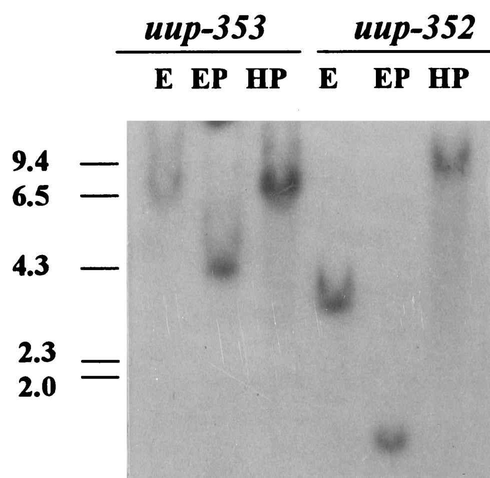

FIG. 2. Mapping of *uup-352* and *-353* insertions. Reproduced is the autoradiograph following Southern blot hybridization to electrophoresed DNA from strains GJ1887 (*uup-352*) and GJ1888 (*uup-353*) after digestion with *Eco*RI (E), *Eco*RI-*Pst*I (EP), or *Hin*dIII-*Pst*I (HP) of a radiolabeled probe prepared from the *Eco*RI-*Hin*dIII Tn*10*dTet fragment described for Fig. 1. At the left are shown the positions of migration of DNA markers of the indicated size in kilobases. Calculated sizes (in kilobases) of the hybridizing fragment for each digest of the *uup-352* and *uup-353* mutants were, respectively, 3.2 (E), 1.5 (EP), and 10.1 (HP) and 6.0 (E), 4.2 (EP), and 7.2 (HP).

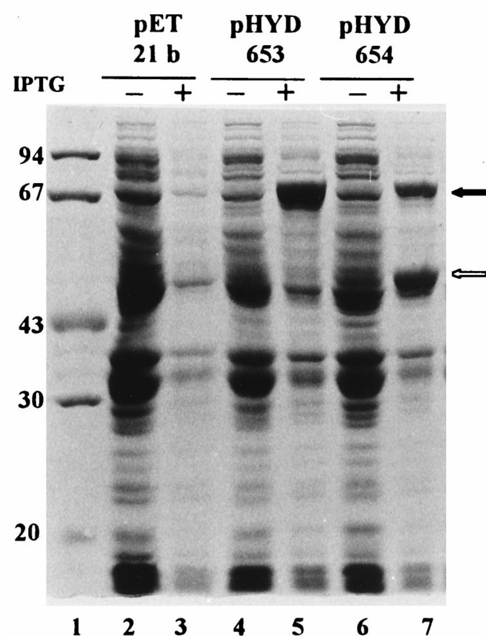

FIG. 3. Polypeptides encoded by the *ycbY-uup* operon. Protein extracts prepared from uninduced (−) and IPTG-induced (+) cultures of BL21(DE3) derivatives carrying the plasmid pET21b, pHYD653, or pHYD654 were subjected to gel electrophoresis on lanes as indicated and visualized by staining with Coomassie blue. Lane 1 represents marker proteins of indicated sizes in kilodaltons. Solid and open arrows identify bands of 73,000 and 47,000, respectively, in M_r that are discussed in the text.

phenotype. To exclude this possibility, we constructed (as described above) a chromosomal *ycbY* deletion-insertion mutant GJ2255, in which a Tet^r cassette had replaced 1.2 kb of sequence encompassing the promoter and proximal one-third of the *ycbY* gene (Fig. 1). Strain GJ2255 was Uup$^-$, and it too was complemented to Uup$^+$ by either of the plasmids pHYD642 and pHYD643 described above which expressed *uup*$^+$ alone without *ycbY* (data not shown). The results therefore established that the *ycbY* insertions confer a Uup$^-$ phenotype only because of their polar effect on expression of the downstream *uup* gene.

As described below, the physiologically relevant promoter for chromosomal *uup* expression is that situated upstream of *ycbY*. We therefore believe that the expression of *uup*$^+$ from the pair of complementing plasmids pHYD642 and -643 is directed from promoters situated in the vector. Likewise, positive complementation (observed by us earlier [38]) of *uup* mutations, by a multicopy plasmid-borne fragment which includes *uup*$^+$ and all but the first codon of *ycbY* but not bearing the upstream promoter region, is because of read-through into *uup* from a promoter in the vector or of a fortuitous weak internal promoter in *ycbY* that is phenotypically relevant only in the multicopy state. The same fragment, when borne on a single-copy-number plasmid (pHYD640), failed to complement the *uup-353* mutant strain, whereas a larger fragment that included the promoter (pHYD646) successfully complemented the mutant (Table 2).

TABLE 2. Plasmid complementation analysis of *uup* mutants[a]

Plasmid	Normalized precise excision frequency of *lacZ*::Tn*10*dKan in derivatives of strain:			
	GJ2258 (wild type)	GJ2259 (*uup-351*)	GJ2260 (*uup-352*)	GJ2261 (*uup-353*)
pCL1920	1.0	7.1	9.8	6.6
pHYD638	0.7	ND[b]	5.5	7.8
pHYD642	0.8	0.8	0.9	0.9
pHYD643	0.8	1.2	1.2	1.0
pMU2385	1.0	6.5	ND	12.0
pHYD640	1.1	6.0	ND	8.3
pHYD646[c]	0.9	0.6	0.8	0.9
pBKS	1.0	7.4	4.2	6.0
pHYD627	0.05	0.03	0.03	0.06

[a] The median frequency of Lac$^+$ revertants per plated cell was measured for each of the strain derivatives carrying the indicated plasmids and is expressed as a relative frequency value normalized to the corresponding vector plasmid control in the wild-type strain GJ2258 (taken as 1.0). The actual median mutant frequencies for the three control strains GJ2258/pCL1920, GJ2258/pMU2385, and GJ2258/pBKS were, respectively, 3.3×10^{-6}, 5×10^{-6}, and 2.1×10^{-6}.

[b] ND, not determined.

[c] Lac$^+$ revertants for the pHYD646 derivatives were selected on minimal medium containing phenyl-β-D-galactoside as sole C source and IPTG.

Characterization of the *ycbY*−*uup* operon promoter. We cloned several fragments from the *ycbY-uup* region upstream of the *lacZ* reporter gene in a single-copy-number plasmid, in order to examine promoter activities and regulation in vivo. Each of the fragments extended either upstream or downstream of the *Eco*RI site that cleaves at codon 2 of *ycbY*. Two upstream fragments (in plasmids pHYD632 and pHYD647) that extended respectively up to a *Cla*I site (0.29 kb) and an *Eco*RI site (1.2 kb) exhibited promoter activities of comparable strength (Table 3), suggesting that the promoter for the *ycbY-uup* operon is situated downstream of the *Cla*I site. Experiments of primer-extension analysis on total cellular RNA from *uup*$^+$ strains (data not shown) were also consistent with the existence of a single transcription start site 172 bases upstream of the *ycbY* ORF.

The promoter activity identified in either of the plasmids pHYD632 and pHYD647 above was not subject to regulation in vivo by any of the following agents or mutations tested:

TABLE 3. Promoter activity in fragments from *uup* locus[a]

Plasmid	Enzyme sp act
pMU2385	3.3
pHYD630	4.4
pHYD631	30
pHYD632	114 (105)
pHYD640	35 (472)
pHYD647	144 (130)

[a] The specific activity of β-galactosidase was determined for transformants of strain MC4100 carrying the *lacZ* promoter-cloning vector pMU2385 or its derivatives, and values are given in Miller units (31). Values in parentheses indicate the specific activity for the indicated strains in cultures supplemented with 0.5 mM paraquat.

growth rate, pH, SOS response, oxidative stress, *recA*, *rpoS*, *hns*, *oxyR*, or *soxR*; neither was it subject to autoregulation by the products of *ycbY* and *uup* themselves (data not shown). Promoter-*lac* expression was unaffected in a strain carrying multiple copies of the same region on another compatible plasmid (pHYD627 or pHYD628), suggesting that a titratable positive or negative regulatory factor did not exist (data not shown). A plasmid (pHYD631) bearing the 1.2-kb *Eco*RI fragment in inverted orientation (relative to pHYD647) upstream of the *lacZ* reporter gene also exhibited a weak and constitutive promoter activity (Table 3), which we believe represents the promoter for the divergently transcribed ORF *ycbX* upstream of *ycbY*.

Two downstream fragments from the *Eco*RI site at the start of the *ycbY* ORF were also cloned into the *lacZ* reporter gene plasmid. A 2.3-kb fragment encompassing all of *ycbY* and the 5′ end of *uup* exhibited negligible promoter activity (pHYD630 [Table 3]), consistent with other results above showing that the *ycbY* and *uup* genes constitute a single unit of transcription. A 5.7-kb fragment that extended further downstream displayed promoter activity that was inducible by paraquat (pHYD640 [Table 3]); this observation is in accord with the findings of Koh and Roe (18, 19) that a paraquat-inducible promoter for the downstream *pqi-5* gene in this complex operon is situated within the *uup* ORF (Fig. 1).

Effect of *uup* on other types of genetic rearrangements. We had earlier shown that the frequency of occurrence of spontaneous point mutations to rifampin resistance or nalidixic acid resistance is not altered in *uup* mutant strains (38). We have now examined the effect of *uup* on several other types of spontaneous genetic rearrangements that fall under the broad category of RecA-independent recombination events, using the assays described above. We observed that the frequencies of nearly precise excision of Tn*10*dKan, as well as of tandem repeat deletion in *lacZ*, were elevated approximately four- to ninefold in both the *recA*$^+$ *uup* and *recA uup* strains (Table 4). On the other hand, the *uup* mutations did not affect the frequencies of occurrence of (i) spontaneous mutations in *lacI* (roughly 70% of which are caused by insertion or deletion of a 4-bp sequence at a site where this sequence is present in three tandem repeats in the *lacI*$^+$ gene [11, 45]), (ii) deletions in the *att*λ*-gal* locus, or (iii) precise excision of the 50-bp remnant following nearly precise excision of *lacZ*::Tn*10*dKan (data not shown). The last finding is consistent with an earlier report that precise excision of the Tn*10* remnant might occur by a mechanism which is different from that mediating precise excision or nearly precise excision (13).

***uup*$^+$ gene dosage effect on genetic rearrangements.** While undertaking the complementation experiments, we observed that strains carrying the multicopy *uup*$^+$ plasmid pHYD627

TABLE 4. *uup* effects on nearly precise excisions and tandem repeat deletions[a]

uup genotype	Normalized frequency in strain			
	Nearly precise excision		Tandem repeat deletion	
	recA$^+$	*recA*	*recA*$^+$	*recA*
Haploid *uup*$^+$	1.0	1.4	1.0	1.3
uup-351	4.2	9.2	4.2	8.3
Haploid *uup*$^+$ (with pBKS vector)	1.1	1.2	ND[b]	1.5
Multicopy *uup*$^+$	0.02	0.03	ND	0.03

[a] The median frequency of mutants obtained per plated cell was measured for each of the strains and is expressed as a relative frequency value normalized to that in the haploid *uup*$^+$ *recA*$^+$ derivatives, GJ1885 and GJ2292, for nearly precise excisions and tandem repeat deletions, respectively (each taken as 1.0). The actual median mutant frequencies for the two strains were 4.7×10^{-4} and 3.3×10^{-5}, respectively. The other strains used for the nearly precise excision studies were GJ1886 (*uup-351 recA*$^+$), GJ2258 (*uup*$^+$ *recA*), and GJ2259 (*uup-351 recA*), and those for the tandem repeat deletion studies were GJ2294 (*uup-351 recA*$^+$), GJ2293 (*uup*$^+$ *recA*), and GJ2295 (*uup-351 recA*). Multicopy *uup*$^+$ derivatives carried the plasmid pHYD627.

[b] ND, not determined.

exhibited lower frequencies of precise excision (approximately 20- to 30-fold) than did the isogenic strain GJ2258 that was haploid *uup*$^+$ (Table 2). The frequencies of nearly precise excision and of tandem repeat deletion were also reduced around 30- to 50-fold in the multicopy *uup*$^+$ strains bearing plasmid pHYD627, compared to the values in strains carrying the vector pBKS (Table 4). Furthermore, the plaque size of Mu *c*(Ts) when plated on the multicopy *uup*$^+$-bearing strain was significantly larger than that on the haploid *uup*$^+$ strains (data not shown). The relevance of these observations to our understanding of the possible physiological role and mechanism of *uup* function is discussed below.

***tex* mutations fall into two categories.** In the course of these studies, we have also found that the various *tex* mutations described so far can be classified into two categories (Table 5). An example of the first category is *uup*, which increases the precise excision frequency of both Tn*10* (16) (Table 5) and the

TABLE 5. *tex* effects on precise excisions of Tn*10* and mini-Tn*10*

Strain pair[a]	Relevant genotype	Normalized precise excision frequency of strain type[b]:	
		lacZ::Tn*10*	*lacZ*::Tn*10*dTet*2*
GJ2268; GJ2269	Wild type	1.0	1.0
GJ2272; GJ2273	*uup-351*	12.6	13.3
GJ2274; GJ2275	*ssb-200*	16.2	20.0
GJ2276; GJ2278[c]	*polA12*	5.1	9.6
GJ2280; GJ2281	Δ*uvrD288*::Kan	13.6	0.8
GJ2282; GJ2283	*dam*::Tn*9*	5.5	1.7
GJ2284; GJ2285	*mutH471*::Kan	3.0	0.9
GJ2288; GJ2289	*mutS*::Tn*10*	5.4	1.0
GJ2290; GJ2291	*mutL*::Tn*10*	8.3	1.2

[a] The first and second strain numbers in each pair correspond, respectively, to the Tn*10* and Tn*10*dTet*2* derivatives.

[b] The median frequency of Lac$^+$ revertants per plated cell was measured for each strain and is expressed as a relative frequency value normalized to that in the appropriate wild-type control strains GJ2268 and GJ2269 (taken as 1.0). The actual median mutant frequencies for these two strains were 1.5×10^{-9} and 2×10^{-9}, respectively.

[c] Because *polA12* is a Ts allele (52), cultures of GJ2276 and GJ2278 were grown in glucose minimal medium at 42°C prior to plating for selection of Lac$^+$ revertants.

mini-Tn*10* derivatives such as Tn*10*dKan (38) (Table 2), Tn*10*dTet (Table 5), and Tn*10*dCm (data not shown). Other examples of the first category include mutations in *ssb* (38) (Table 5) and *polA* (27) (Table 5), the genes encoding the single-stranded DNA binding protein SSB and DNA polymerase I, respectively.

Mutations in *topA*, the gene encoding topoisomerase I, also belong to this first category, because they have earlier been shown to increase precise excision of Tn*10* (25), and we found in this study that a new *topA* insertion (*topA72*::Tn*10*dTet*2*), obtained as described above, increased the median frequency of precise excision of *lacZ*::Tn*10*dKan 20-fold in the strain GJ2243 (1.1×10^{-4} per cell, compared to 5×10^{-6} in the control strain GJ1885). Strains with *topA* null mutations are known to accumulate suppressors in loci encoding the gyrase subunits, in order to compensate for the excessive supercoiling of DNA in these strains (9, 36, 39); the following features of *topA72*, however, lead us to suggest that the observed *tex* effect is caused by topoisomerase I deficiency itself rather than by the additional suppressor mutation(s). (i) In P1 transduction experiments employing the *topA72* strain GJ2243 as donor, no Tet^r transductants could be recovered in either the MC4100 or MG1655 strain backgrounds, whereas slow-growing Tet^r colonies were obtained at the normal expected frequency in strain GJ1885 after 2 days' incubation. This result suggested that different *E. coli* strains differ in their ability to tolerate *topA* disruption, some being killed (as has also been reported earlier [9]) and others exhibiting behavior analogous to *Salmonella enterica* serovar Typhimurium in that they are sick yet retain viability (39). (ii) When the selection for *topA72* transductants of GJ1885 (which carries *lacZ*::Tn*10*dKan) was undertaken on Lac^+ papillation medium, the vast majority of the slow-growing colonies exhibited a clear hyperpapillation phenotype, suggesting that the increase in precise excision frequency is a trait that accompanies inheritance of the *topA* mutation itself.

The second category of *tex* mutations consists of those which were earlier known (25, 26) to increase the precise excision frequency of Tn*10* and confirmed to be so in this study (Table 5) but which do not affect the precise excision frequency of the mini-Tn*10* derivatives (Table 5). Included in this category are *mutH*, *mutL*, *mutS* (encoding the MutHLS proteins involved in methyl-directed mismatch repair), *dam* (encoding DNA adenine methylase), and *uvrD* (encoding DNA helicase II). The mechanistic implications of this bipartite classification of the *tex* mutations are discussed below.

DISCUSSION

Operonic arrangement at the *E. coli uup* locus. The following lines of evidence obtained in this study establish that *uup* is cotranscribed with another ORF (*ycbY*) from a promoter situated upstream of *ycbY*. (i) An insertion mutation in chromosomal *ycbY* confers a Uup^- phenotype, because of a polarity effect on *uup* expression. (ii) Likewise, a plasmid (pHYD638) carrying the *ycbY-uup* locus with the insertion in *ycbY* failed to complement chromosomal *uup* mutations. (iii) Only one promoter capable of transcribing uup^+ in the haploid state was identified in experiments involving either genetic complementation or *lacZ* reporter gene expression, and this promoter is situated upstream of *ycbY*. (iv) Finally, the coordinate production of proteins corresponding to both the *ycbY* and *uup* ORFs in the T7 RNA polymerase-based expression system is consistent with the notion that they constitute a single transcriptional unit. The *ycbY-uup* genes are also part of a more complex operon that includes downstream genes such as *pqiA* and *pqiB* for which additional promoters exist that are embedded in the *uup* ORF (Fig. 1).

Although *uup* mutants exhibit phenotypes related to transposon excisions, tandem repeat deletions, and phage Mu growth, the normal physiological function of the Uup gene product remains unknown. The fact that *ycbY* does not share the known *uup* mutant phenotypes suggests that the two genes perform unrelated functions, notwithstanding their organization together in a single operon. Analysis of the database of microbial genome sequences (obtained from the website of The Institute for Genomic Research at http://www.tigr.org) reveals that the orthologs of the two genes are placed together in an operon in *Yersinia pestis*, *S. enterica* serovar Typhi, and *S. enterica* serovar Paratyphi A; on the other hand, the genes are still clustered but separated by about 240 bp in *Vibrio cholerae* and *Shewanella putrefaciens*, whereas they are widely separated on the chromosomes of *Actinobacillus actinomycetemcomitans*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*. A similar dichotomy, with conservation of gene order in closely related genera and dispersal in the more distant ones, has been reported recently for the complex operon that includes the *fpg* and *mutY* genes in *E. coli* (14).

A shared step in pathways of transposon excisions and tandem repeat deletions? We found that *uup* mutations increase the frequency of three different RecA-independent genetic rearrangements, namely, transposon precise excisions and nearly precise excisions and deletions of a tandem chromosomal repeat. This observation implies the existence of a shared step in the pathways of these events which is influenced by the Uup product. That precise excision and nearly precise excision share similar mechanisms has previously also been suggested by Kleckner and coworkers on the basis of other lines of genetic evidence (13, 25, 26).

The exact role played by Uup in these genetic rearrangements is as yet unclear. There is prior evidence to support the notion that each of these three categories of mutations is the consequence of a RecA-independent slippage event (between the pair of direct repeats) during replication, either simple or involving sister-strand chromatid exchange (3, 5, 7, 10, 13, 44). The role of the Uup product (whose deduced sequence suggests that it is cytosolic and belongs to the ATP-binding cassette family of proteins [23]) might then be to actively destabilize the looped and misaligned intermediate that is expected to precede the postulated slippage event. The fact that multicopy uup^+ strains exhibit a still lower frequency of transposon excisions and tandem repeat deletions in comparison with haploid uup^+ strains suggests that the Uup-sensitive intermediate contributes to the rearrangement events even in the latter. Likewise, the data for Mu phage growth in *uup*, haploid uup^+, and multicopy uup^+ strains (references 16 and 38 and this study) suggest a dose-dependent effect of Uup on burst size of phage-infected cells, but the mechanism is unknown. At the same time, it must be noted that the mechanistic pathways, at least for precise excision and tandem repeat deletion, are not identical; for example, we have found that mutations in *rep* and *priA*, genes encoding the DNA helicase Rep and the primosome assembly protein PriA, respectively, do not affect the frequency of precise excision (data not shown), although they are known to increase that of tandem repeat deletion (3, 44).

Two categories of *tex* mutations. It has been shown earlier (10, 13) that the frequency of precise excision is determined in part by the length and degree of matching of the inverted repeat sequence at the ends of the transposable element. Other workers have also shown that the occurrence of spontaneous deletions in vivo and in vitro between short stretches of direct repeats is facilitated by the presence of palindromic

sequences within the deletion interval (1, 6, 8, 35, 50, 53). These results have led to the hypothesis that an interaction between the inverted repeats represents an intermediate in the precise excision (or deletion) pathway.

Our results indicate that the *tex* mutations affecting precise excision of Tn*10* fall into two categories: the first, such as *uup*, *ssb*, *topA*, and *polA*, increasing precise excision of both Tn*10* and mini-Tn*10* and the second, such as *mutHLS*, *dam*, and *uvrD*, increasing precise excision of Tn*10* but not of mini-Tn*10*. It may be noted that (i) a common feature of the genes (in particular, *mutH*, *-L*, and *-S*) of the second category is their involvement in mismatch repair (32) and (ii) a major distinction between Tn*10* on the one hand and the various mini-Tn*10* derivatives on the other is that the inverted repeats in the former possess several mismatches whereas those in the latter are perfectly matched (17). The present categorization therefore provides additional support for the hypothesis that during precise excision the inverted repeats interact as intrastrand stem-loop or snapback structures (rather than as a pair of duplex DNA stems), because it explains why a functional MutHLS system reduces precise excision only of elements in which the palindromes are imperfect. The snapback model may also explain the broad-specificity *tex* nature of *topA* and *ssb* mutations, the former because of increased supercoiling that would favour cruciform extrusion (28) and the latter because of the heightened permissiveness for interactions between single-stranded regions of DNA in the mutant strains (24, 29).

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