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Genetics

Unusual alleles of *recB* and *recC* stimulate excision of inverted repeat transposons Tn*10* and Tn*5*

(inverted and direct repeats/precise and nearly precise excision/*recA*/λ recombination/χ)

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ABSTRACT **Precise and nearly precise excision of transposon Tn*10* occur by host-mediated processes unrelated to transposition. Both types of excision involve interactions between short (9 or 24 base-pair) direct repeat sequences at or near the termini of the transposon and are stimulated by the large (1,329-base-pair) inverted repeats that form the ends of Tn*10*. We describe here three mutations of *Escherichia coli* K-12, designated *texA*, that enhance excision of Tn*10* and of the structurally analogous transposon Tn*5*. Genetic mapping and complementation analysis show that these mutations are unusual alleles of the *recB* and *recC* genes that alter but do not abolish RecBC function. As Tn*10* excision normally does not depend on RecA or RecBC functions, *texA* mutations appear to provide another pathway for excision that depends on altered RecBC function; for one *texA* allele, excision has become dependent on RecA function as well. The available evidence suggests that *texA* mutations alter the stimulatory interaction between the inverted repeats of Tn*10*.**

The prokaryotic transposon Tn*10* is 9,300 base pairs long and has at its ends 1,329-base-pair inverted repeats of insertion sequence IS*10*. Tn*10* inserts into many different sites in a bacterial chromosome by a process that depends on IS*10*-encoded functions and on specific sites at the termini of the transposon (1). Insertion of Tn*10* is accompanied by duplication of a nine-base-pair target DNA sequence and the final product contains Tn*10* material inserted between direct repeats of the short target sequence.

Tn*10* can also be excised from the bacterial chromosome, either partially or completely. Three specific Tn*10*-associated excision events have been described (2, 3). Precise excision reconstructs the interrupted target chromosome to its original wild-type sequence and can be detected as reversion to wild type of a Tn*10* insertion mutation in a structural gene. The entire Tn*10* element and one copy of the 9 base-pair direct repeat are removed during precise excision. Nearly precise excision is a second specific deletion event involving short repeats near each end of Tn*10* and resulting in excision of all but 50 base pairs of Tn*10* material. This 50-base-pair remnant can itself be further excised to give a precise excision product.

Precise and nearly precise excision are closely related processes. In both cases, excision occurs between two short direct repeat sequences and results in deletion of all intervening material together with one copy of the direct repeat. The short direct repeats occur at either end of a lengthy inverted repeat formed by the IS*10* elements at the ends of Tn*10*, and in both cases these inverted repeats act in a structural way to stimulate excision. All of the host mutations analyzed thus far affect precise excision and nearly precise excision coordinately (2, 4). The third excision event, excision of the 50-base-pair remnant, appears to occur by an unrelated pathway.

Table 1. Bacterial and phage strains used

Strain	Genotype
Bacterial	
NK5661	W3110 *lacZ2900*::Tn*10*
VL101	W3110 *lacZ2900*::Tn*10 texA343*
VL323	W3110 *lacZ2900*::Tn*10 texA344*
VL211	W3110 *lacZ2900*::Tn*10 texA345*
JC5220	F⁻ *thr-1 leuB6 thi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL tsx-33*
JC7752	F⁻ *leu-307 trpE-9829 lac-301 rpsL-321 recB21 sbcB15*
JC5489	Same markers as JC5220 and also *supE44 recC22*
JC4456	Same markers as JC5220 and also *supE44 recC73*
AB2470	Same markers as JC5220 and also *supE44 recB21*
AFT159	Same markers as JC5220 and also *thyA recF143*
AFT314	*his-4 met recF143 rpsL thyA*
JC5488	Same markers as JC5220 and also *supE44*/F'15
JC5497	*recA67 rpsE lac sup⁺ his trp thy*/F'15*recB21*
V79	*his-4 met recF143 rpsL argA recC73*/F'15*recC73*
AFT407	W3110 *argA*::Tn*10*/F'15 *texA343 thyA*
AFT409	W3110 *argA*::Tn*10*/F'15 *texA344 thyA*
Phage λ	
λ 108	*red270 cI857*
λ 618	*gam210*
λ 620	*gam210 red270 cI857*
λ 112	+

Strains JC5220, JC5489, JC4456, JC7539, AB2470 (all derivatives of AB1157), and JC7752 were from A. J. Clark; JC5488 and JC5497, from D. Mount; V79, from D. W. Schultz; phage strains λ 108, λ 618, and λ 620 were from E. Signer. All other strains are derived from the work presented in this paper.

Precise and nearly precise excision normally do not depend on either transposon-encoded functions or the host homologous recombination functions RecA, RecB, and RecC (refs. 2 and 4; this work). We describe here three mutations of *Escherichia coli* K-12, identified among a larger set of mutations that enhance Tn*10* excision, that lie in the *recB* and *recC* genes. These mutations, called *texA*, are unusual alleles of *recB* and *recC* that stimulate transposon excision above their normal RecBC-independent levels. *texA* mutations do not greatly reduce the frequency of homologous recombination but do alter host sensitivity to UV irradiation and growth of certain phage λ mutants. For one *texA* mutation, enhancement of excision is dependent on RecA function. These and other observations suggest that *texA* mutations may create another pathway(s) for transposon excision. Other properties of *texA* mutations suggest that they enhance excision by altering the interaction between inverted repeats.

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MATERIALS AND METHODS

Bacterial and Phage Strains. The genotypes of the *E. coli* strains and λ phages used in this work are described in Table 1.

E. coli *K-12 W3110 derivatives.* Isogenic strains listed in Table 2 were constructed by transducing each Tn*5*, Tn*10*, or Tn*9* insertion into NK6922 (W3110 *thyA*), followed by transduction to Thy$^+$ using as donors NK5661 (Tex$^+$), VL101 (*texA343*), VL323 (*texA344*), VL211 (*texA345*), JC7752 (*recB21*), and JC5489 (*recC22*). Tex$^-$ or Rec$^-$ transductants were identified by aberrant plating behavior of λ 620 (Table 3). Strains listed in Table 4 were constructed from derivatives of the isogenic strains of Table 2 by introduction of appropriate F'15 elements from JC5497, JC5488, V79, AFT407, and AFT409. Strains listed in Table 5 are isogenic derivatives of NK6927 (W3110 *lacZ2900*::Tn*10* *tet*^{R}::Tn*5* *thyA*); the presence of Tn*5* in the tetracycline-resistance gene of Tn*10* does not alter the frequency of precise or nearly precise excision of the *lacZ2900* element. Tex$^-$ or Rec$^-$ alleles were introduced first and RecA$^{-/+}$ derivatives were constructed by P1*vir* transduction using DF475 (*srl*::Tn*10* *recA56*) as donor.

AB1157 recF143 *derivatives.* Strains used in Table 4 were derived from AFT314, a derivative of AFT159 lacking some auxotrophic markers. *argA* and *argA thyA* derivatives were constructed, and Rec or Tex alleles were introduced into the Thy$^-$ versions by P1 cotransduction with *thyA* using as donors AFT229 (W3110 *lacZ2900*::Tn*10* *texA343*), AFT231 (W3110 *lacZ2900*::Tn*10* *texA344*), AB2470, and JC4456. F'15 derivatives were introduced into the resulting strains from JC5497, JC5488, V79, AFT407, and AFT409 by selection for Arg$^+$.

Media. Luria broth and minimal medium (M9) have been described by Miller (5); for solid media, 1.5% agar was added. λ broth, λ agar, and SM buffer have been described by Kleckner *et al.* (6). MacConkey agar (Difco) indicator plates were supplemented with 1% (wt/vol) lactose.

Isolation of Mutant Strains. Host mutants increased for the frequency of precise excision of Tn*10* were isolated from NK5661, after mutagenesis with ethylmethanesulfonate or with nitrosoguanidine using a Lac$^+$ papillation assay as described (2). Mutant strains were identified on MacConkey lactose indicator plates as single colonies that contained more Lac$^+$ papillae than normal. VL101 (*texA343*) was isolated from an ethylmethanesulfonate-mutagenized culture of NK5661; VL323 (*texA344*) and VL211 (*texA345*) were isolated from two independently nitrosoguanidine-mutagenized cultures.

Precise and Nearly Precise Excision Assays. Experiments were carried out as described in Foster *et al.* (2). Within any particular experiment, individual clones having more than 10 times the average frequency of revertants were excluded as "jackpots" (7). For a given strain, average excision frequencies vary no more than 2- to 4-fold from one experiment to another.

RESULTS

Identification of TexA Mutants. The isolation and partial characterization of 39 mutations of *E. coli* K-12 that increase the frequency of precise and nearly precise excision of Tn*10* have been described (2, 4). These mutations are called *tex* for Tn*10* excision. General screening of Tex$^-$ mutants revealed three mutations, designated *texA*, whose specific phenotype suggested they might be altered in RecBC function: these three mutants plated λ Gam$^-$ phages with drastically reduced efficiency but λ Gam$^+$ phages with normal efficiency. The only known function of the γ gene product is to efficiently inhibit, by direct protein–protein interaction, all of the known activities of the RecBC enzyme (8). The nature and properties of these three *texA* mutations form the basis of this report.

Each of the three *texA* mutations increases precise excision of Tn*10*, precise excision of the structurally similar transposon Tn*5*, and nearly precise excision of Tn*10*. Excision is enhanced from 1.3- to 140-fold depending on the particular *texA* allele (Table 2). The degree of enhancement of excision varies with the site of the transposon; this variation has been observed for all mutants of *E. coli* that increase transposon excision (2, 9). Excision of Tn*9*, a transposon having direct rather than inverted repeats at its ends (10), is not enhanced. The effects of the *texA* mutations on phage plating are shown in Table 3: each mutation decreases plating of λ Gam$^-$ phages and abolishes plating of λ Red$^-$ Gam$^-$ phages but has no effect on λ Red$^-$ plating behavior. The three *texA* mutations are very different from previously isolated *recB* and *recC* mutations, including nonsense mutations, which improve plating of λ Gam$^-$ phages (11) and have no effect on the frequency of precise or nearly precise excision (Tables 2 and 3).

Mapping and Complementation Analysis. By P1-mediated transductional crosses, the *texA* mutations have been localized to the genetic interval between the *thyA* and *argA* genes, a 0.5-min interval that includes the *recB* and *recC* genes (12, 13). Two factor crosses place *texA343* near *recC* and *texA344* near *recB* (Fig. 1). Three factor crosses place both of these *texA* mutations and *recB* and *recC* mutations between *thyA* and *argA* (data not shown). The third *texA* allele, *texA345*, is linked to *thyA* (33%) but has not been further mapped. In these and other mapping experiments, *texA* mutations have been monitored by their enhancement of transposon excision, by their inhibition of λ Red$^-$ Gam$^-$ phage growth, and by a third phenotype, sensitivity to UV irradiation (see below). All three of these TexA phenotypes

Table 2. Effect of *texA*, *recB*, and *recC* on transposon excision

	Tn*10*						
	Precise excision		Nearly precise excision	Tn*5* precise excision		Tn*9* precise excision	
Tex/Rec genotype	*lacZ2900*	*nadA6933*	(*lacZ2900*)	*met6000*	*trp6006*	*pheA6060*	*leu5783*
Tex$^+$*Rec*$^+$	1.0 (0.2×10^{-9})	1.0 (4.6×10^{-9})	1.0 (0.8×10^{-6})	1.0 (5.0×10^{-9})	1.0 (8.3×10^{-9})	1.0 (5.8×10^{-6})	1.0 (2.1×10^{-8})
texA343	140.0	7.5	13.0	12.0	130.0	1.0	3.6
texA344	60.0	2.5	16.0	7.0	40.0	1.0	2.0
texA345	25.0	1.3	2.8	8.0	35.0	0.9	1.0
recB21	0.5	0.7	2.8	NT	NT	0.9	1.1
recC22	1.0	0.3	0.6	NT	NT	1.6	1.0

Excision frequencies for mutant strains are expressed relative to those for wild-type strains, for which absolute values are given in parentheses.

Table 3. Plating behavior of λ mutants on TexA, RecB and RecC mutant strains

Tex/Rec genotype	λ *gam210* plaque size*	Efficiency of plating	
		λ *gam210 red270*	λ *red270*†
Tex⁺Rec⁺	Small	1.0 (small plaques)	1.0
texA343	Minute	$<10^{-3}$	1.0
texA344	Minute	$<10^{-3}$	1.0
texA345	Minute	$<10^{-2}$	1.0
recB21	Large	1.0 (large plaques)	1.0
recC22	Large	1.0 (large plaques)	1.0

Plaque morphology and efficiency of plating were determined by spotting appropriate dilutions of phage stocks on bacterial lawns prepared from midlogarithmic cultures. Phage dilutions were chosen such that individual plaques would be observed for plating efficiencies ranging from 1.0 to 0.001 relative to the *Tex⁺* parental strain. Strains were isogenic derivatives of NK5661 listed in Table 2.
*Efficiency of plating is 1.0 for all strains.
†Plaque morphology is normal for all strains.

cosegregate in transductional crosses: no recombinants with only one or two of the three TexA phenotypes have been observed among the hundreds of transductants scored. We conclude that all three phenotypes are the result of a single (or more than one tightly linked) mutation(s) located between *thyA* and *argA*.

Complementation tests show that *texA343* is an allele of the *recC* gene while *texA344* is an allele of the *recB* gene. Merodiploids containing appropriate combinations of *texA*, *recB*, *recC*, and wild-type alleles were constructed using F'15 episomes carrying the *thyA-recC-recB-argA* region. These merodiploids have been tested for Tn*10* precise excision, for plating efficiency of λ Red⁻ Gam⁻ phages, and for a third phenotype, activity of χ sites in λ recombination, described below. Precise excision tests have been used in only a limited number of cases because the F episome alone enhances Tn*10* excision (Table 4; ref. 15), thus complicating data interpretation. As shown in Table 4, *texA343*/F'*recC73* and *texA344*/F'*recB21* merodiploids have the same phenotypes as the corresponding *texA*/F'*texA* homozygous merodiploids and *texA* haploids (compare lines 2, 3, 11, 13, 16, and 17), whereas *texA343*/F'*recB21* and *texA344*/F'*recC73* strains are the same as the corresponding *texA*/F'Rec⁺ strains (compare lines 12, 14, 18, and 19). The assignment of *texA343* and *texA344* to separate genes is further supported by complementation between these two mutations: heterozygous merodiploids (*texA343*/F'*texA344* and *texA344*/F'*texA343*) are more similar to wild type than to the corresponding homozygous diploids (compare lines 6, 11, 15, 16, and 20). The same conclusions were reached from complementation experiments using cloned *recB* or *recC* genes on multicopy plasmids (16). Merodiploid analysis of the weakest *texA* mutation, *texA345*, indicates that this mutation is an allele of *recB* (data not shown).

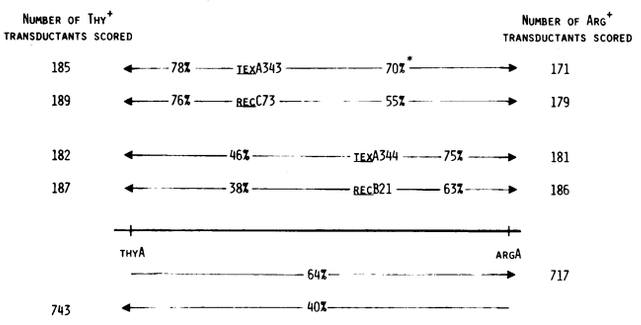

FIG. 1. Two-factor co-transduction frequencies of *texA*, *recB*, and *recC* mutations with *thyA* and *argA*. P1 stocks grown on AFT229 (*texA343*), AFT231 (*texA344*), JC4456 (*recC73*) and AB2470 (*recB21*) were used to transduce AFT325 (AB1157 *recF143 thyA argA*). Thy⁺ or Arg⁺ transductants were selected and examined for acquisition of TexA or RecBC phenotypes by screening for increased sensitivity to UV light and aberrant plating behavior of a λ Red⁻ Gam⁻ phage. The arrowhead is at the selected marker; the percentage of selected recombinants inheriting the unselected marker is shown.
*This co-transduction frequency is reproducibly higher than expected for a *recC* mutation; however, complementation analysis indicates that *texA343* is an allele of *recC* (see Table 4).

The phenotypes of all Tex⁻/F'Tex⁺ merodiploids and of *texA343*/F'*recB21* and *texA344*/F'*recC73* merodiploids are intermediate between those of *texA*/F'*texA* and Tex⁺/F'Tex⁺ merodiploids (lines 6, 9, 10, 12, 14, 18, and 19 of Table 4). Since nonsense alleles of *recB* and *recC* are fully recessive (ref. 12 and lines 6–8 of Table 4), we interpret this result to mean that the *texA* alleles are partially dominant with respect to wild type.

Effects of *texA* Mutations on Homologous Recombination. *texA* mutations have little or no effect on the frequency of cellular homologous recombination. Previously isolated *recB21* and *recC73* mutations reduce the frequency of recombinants obtained in P1-mediated transductional crosses by factors of 12 to 15 and the frequency of recombinants in Hfr-mediated conjugational crosses by factors of 30 to 100 (12, 13). In contrast, *texA* mutations reduce recombination proficiency by a factor of no more than 2 in both types of crosses (data not shown; ref. 17).

texA mutations do, however, exert significant effects on growth and recombination of phage λ. Although λ⁺ grows well in TexA strains, growth of λ Gam⁻ is significantly inhibited and growth of λ Red⁻ Gam⁻ is virtually abolished (Table 3). The *Gam* gene product is a specific inhibitor of the RecBC enzyme, while the *Red* genes are responsible for phage-specified homologous recombination. λ DNA can be packaged into mature phage particles only from dimeric or concatemeric substrate molecules (18, 19). In a λ Red⁻ Gam⁻ infection, the only pathway for production of packageable (dimeric) progeny DNA molecules is *recA*-dependent homologous recombination between intact or partially replicated monomers; normal rolling-circle replication intermediates are eliminated by the RecBC enzyme and the phage recombination system is absent. The severe growth inhibition of Red⁻ Gam⁻ phages in TexA hosts could be explained if the mutant RecBC enzyme retains its ability to block rolling-circle replication and, in addition, has acquired the ability to interfere with or has lost the ability to promote residual recombinational pathways for dimer formation. We report below that TexA mutants contain nearly wild-type levels of one activity of the RecBC enzyme.

There is also an apparent reduction in the activity of the genetic element, χ, as assayed by infection of TexA mutant strains with λ Red⁻ Gam⁻. χ sites stimulate homologous recombination near themselves in λ crosses (20). This stimulation is seen only when the recombination is mediated by the host RecBC pathway (14). In wild-type strains, the frequency of recombinants with exchanges in a χ-containing interval is increased about 6-fold relative to the frequency of recombinants in the same interval without χ; in TexA mutant strains, this increase is only about 2-fold (Table 4). It is possible that the RecBC enzyme interacts directly with χ and that the *texA* mutations have altered this interaction. However, other explanations are also possible. For example, intermediates or products of RecBC-promoted (χ-stimulated) recombination events may be frequently and selectively destroyed in TexA hosts. Such a possibility is suggested by the apparent reduction in the formation of packageable recombi-

Table 4. Complementation analysis of *texA*, *recB*, and *recC* mutations

	Genotype	Efficiency of plating of λ Gam⁻ Red⁻*	χ activity in λ recombination†	Precise excision *lacZ*::Tn*10*‡
1	*Tex*⁺*Rec*⁺	1.0	6.1	0.13×10^{-9}
2	*texA343*	<0.001	1.8	20.0×10^{-9}
3	*texA344*	<0.001	2.0	NT
4	*recB21*	NT	1.1	NT
5	*recC73*	NT	1.1	NT
6	*Tex*⁺/F'15	0.6	6.8	0.5×10^{-9}
7	*Tex*⁺/F'15*recB21*	1.0	7.1	1.4×10^{-9}
8	*Tex*⁺/F'15*recC73*	1.0	9.3	1.0×10^{-9}
9	*Tex*⁺/F'15*texA343*	<0.01	5.6	13.0×10^{-9}
10	*Tex*⁺/F'15*texA344*	0.1	5.7	NT
11	*texA343*/F'15*texA343*	<0.001	1.8	NT
12	*texA343*/F'15*recB21*	0.3	4.3	NT
13	*texA343*/F'15*recC73*	<0.001	1.6	NT
14	*texA343*/F'15*Tex*⁺	0.1	6.7	10.0×10^{-9}
15	*texA343*/F'15*texA344*	<0.01	4.6	NT
16	*texA344*/F'15*texA344*	<0.001	2.8	NT
17	*texA344*/F'15*recB21*	<0.001	2.5	NT
18	*texA344*/F'15*recC73*	0.25	5.7	NT
19	*texA344*/F'15*Tex*⁺	0.2	5.9	NT
20	*texA344*/F'15*texA343*	<0.001	4.6	NT

Strains used for efficiency of plating and precise excision assays were derived from NK5661 and strains used for χ activity assays were derived from AFT159.
*Plating of λ 620 (*gam210 red270 cI857*) was determined as described in Table 3.
†Phage crosses were carried out and χ activities were determined as described by Stahl and Stahl (14).
‡Precise excision assays were carried out as described in *Materials and Methods*.

nants during λ Rec⁻ Gam⁻ infections of TexA strains.

Excision Is *recA*-Dependent in One *texA* Strain. Transposon excision in wild-type strains occurs at normal frequencies in the absence of RecA function (ref. 2; Table 5). However, for one *texA* allele, the enhancement of transposon excision is *recA* dependent: stimulation of precise and nearly precise excision by *texA344* is abolished by the introduction of a *recA56* mutation. In contrast, *texA343* increases the frequency of excision in both *Rec*⁺ and *recA56* strain backgrounds (Table 5). The *recA* dependence of *texA344* is not due to an inability of *texA344 recA56* to induce SOS functions: the enhancement of excision by *texA344* and *texA343* is not reduced by the presence of a *lexA3* mutation, which abolishes the ability of a cell to induce *lexA*-repressed genes after DNA damage (ref. 21; data not shown).

Other Effects of *texA* Mutations. The *recB* and *recC* genes together code for exonuclease V, an ATP-dependent double-strand DNase (22, 23); standard *recBC* mutants lack this exonuclease activity. In extracts of *recB21* and *recC73* strains, the level of the ATP-dependent double-strand DNase activity of exonuclease V (assayed essentially as described in ref. 24) is <2% that of wild type, whereas extracts of *texA343* and *texA344* strains have 140% and 120%, respectively, of wild-type levels. Other *in vitro* activities of the RecBC enzyme, such as single-strand DNase activity and DNA unwinding activity, have not been tested.

Table 5. Effect of *recA* on TexA enhancement of precise and nearly precise excision

Tex/*Rec* genotype	Relative excision frequency of *lacZ*::Tn*10*	
	Precise excision	Nearly precise excision
Tex⁺	1.0	1.0
texA343	140.0	16.0
texA344	30.0	16.0
recA56 Tex⁺	1.0	1.0
recA56 texA343	160.0	9.9
recA56 texA344	0.2	1.7

The absolute precise excision frequencies for the *Tex*⁺ and *Tex*⁺ *recA56* strains are 0.12 and 0.3×10^{-9}, respectively; the absolute nearly precise excision frequencies are 2.6 and 1.7×10^{-6}, respectively.

Mutations altering recombination proficiency often simultaneously alter sensitivity to UV. In a *recF143* strain background, the UV sensitivity of TexA mutants is intermediate between that of standard RecBC mutants and that of RecBC⁺; in a *RecF*⁺ background, TexA mutants are not appreciably more UV sensitive than Tex⁺ (data not shown). TexA mutants also differ from standard recBC mutants in viability. Many of the cells in standard recBC mutant cultures are unable to give rise to a visible colony; the viability, measured as the ratio of microscopically visible cells to colony-forming units, can be as low as 20% (25). In a *recF143* background, the viability of fresh overnight cultures of *texA343* was 1.0, while those of *recB21* and *recC73* were 0.3 and 0.2, respectively.

DISCUSSION

Genetic mapping and complementation analyses show that the three mutations originally designated *texA* are in fact alleles of *recB* and *recC* and these alleles have been renamed *recC343*, *recB344*, and *recB345*.

The phenotypes of *texA* mutations are most easily accounted for by the assumption that they qualitatively alter (rather than abolish) one or more activities of the RecBC enzyme. Five properties of the *texA* mutations support this view. First, *texA* mutations enhance transposon excision and severely inhibit growth of λ Rec⁻ Gam⁻ phages, whereas previously isolated *recB* and *recC* mutations, including nonsense mutations, do not affect excision and improve growth of λ Red⁻ Gam⁻ phages. Second, TexA strains show nearly wild-type levels of recombination in Hfr- and P1-mediated crosses, which are significantly reduced by standard *recB* and *recC* mutations. Third, growth of bacteriophage λ on a TexA strain requires γ function, which specifically inhibits the RecBC nuclease. Fourth, *texA* alleles are partially dominant to wild-type alleles in F' merodiploids, in contrast to previously isolated recessive *recB* and *recC* alleles; since this partial dominance is virtually eliminated in plasmid com-

plementation experiments in which the wild-type *RecB*$^+$ or *RecC*$^+$ genes are present in many extra copies (16), this eliminates the possibility that the TexA phenotype is due solely to an increase in the amount of either the *recB* or *recC* gene products. And finally, extracts from TexA strains contain wild-type levels of the RecBC-specified ATP-dependent exonuclease activity.

Other *recC* mutants, obtained as intragenic pseudorevertants of a *recC* missense mutant, also have the Tex phenotype (17). The properties of these mutants, like those described here, indicate that the Tex phenotype results from a qualitative alteration in the RecBC enzyme. One property common to the mutant RecBC enzymes may be responsible for their various phenotypes.

Tn*10* excision normally does not depend on the host RecABC pathway of homologous recombination, because excision is not affected by standard (null) mutations in any of these three genes (Tables 2 and 5). In sharp contrast, TexA enhancement of excision appears to depend on an altered form of the RecBC nuclease, and for one *texA* allele this enhancement is dependent on RecA function. Thus, we infer that Tn*10* excision in TexA strains involves new pathway(s) that are not significant in wild-type hosts.

Tn*10* and Tn*5* excision is normally stimulated by the presence of long inverted repeats at the ends of the element: reduction in the length of these repeats reduces the frequency of excision (2, 26). Although *texA* mutations stimulate excision of both Tn*10* and Tn*5*, none of the *texA* mutations enhances excision of any Tn*9* insertion tested (Table 2). Unlike Tn*10* and Tn*5*, the ends of Tn*9* are long direct repeats of the insertion sequence IS*1* (10). Although there are other differences between Tn*9* and the other elements (10), this observation is consistent with the possibility that *texA* mutations stimulate Tn*10* excision by altering the interaction between the inverted repeat IS*10* elements.

The genetic and biochemical properties of wild-type RecBC enzyme suggest several particular mechanisms by which an altered RecBC enzyme could stimulate either the initial interaction between inverted repeats or the conversion of the resulting structures to mature excision products: (*i*) Under appropriate *in vitro* conditions, RecBC enzyme promotes the formation of single-stranded loops from duplex DNA in which the ends of the single-stranded region are held together, presumably in a DNA–protein complex (27, 28). This activity brings together in single-stranded form two regions of a DNA strand that are normally thousands of base pairs apart. Such an activity could promote formation of cruciform structures or single-strand snapbacks, and *texA* mutations might enhance or alter this activity. (*ii*) RecBC enzyme can also produce double-stranded molecules with single-stranded tails *in vitro*. Such tails should be able to invade a homologous duplex region with the assistance of RecA protein (29). The *recA*-dependent allele *texA344* might increase excision by increasing the formation of single-stranded tails of one of the inverted repeats that could invade the other (duplex) inverted repeat, producing a cruciform structure. (*iii*) It has been suggested that the role of the RecBC enzyme in homologous recombination involves specific interaction of the protein with Holliday recombination structures (30). Since the structure of a Holliday junction is identical to the structure at the center of a cruciform, it is possible that enhanced Tn*10* excision results from altered interaction of the TexA nuclease with such structures.

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1. Foster, T. J., Davis, M. A., Roberts, D. E., Takeshita, K. & Kleckner, N. (1981) *Cell* **23**, 201–213.
2. Foster, T. J., Lundblad, V., Hanley-Way, S., Halling, S. M. & Kleckner, N. (1981) *Cell* **23**, 215–227.
3. Ross, R. D., Swan, J. & Kleckner, N. (1979) *Cell* **16**, 733–738.
4. Lundblad, V. & Kleckner, N. (1982) in *Molecular and Cellular Mechanisms of Mutagenesis*, eds. Lemontt, J. F. & Generoso, W. M. (Academic, New York), pp. 245–258.
5. Miller, J. H. (1972) *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
6. Kleckner, N., Barker, D., Ross, D. & Botstein, D. (1978) *Genetics* **90**, 427–450.
7. Luria, S. E. & Delbruck, M. (1943) *Genetics* **28**, 491–511.
8. Sakaki, Y., Karu, A. E., Linn, S. & Echols, H. (1973) *Proc. Natl. Acad. Sci. USA* **70**, 2215–2219.
9. Hopkins, J. D., Clements, M. & Syvanen, M. (1983) *J. Bacteriol.* **153**, 384–389.
10. Kleckner, N. (1981) *Annu. Rev. Genet.* **15**, 341–404.
11. Zissler, J., Signer, E. & Schaefer, F. (1971) in *The Bacteriophage Lambda*, ed. Hershey, A. D. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 455–468.
12. Willetts, N. E. & Mount, D. W. (1969) *J. Bacteriol.* **100**, 923–934.
13. Emmerson, P. T. (1968) *Genetics* **60**, 19–30.
14. Stahl, F. W. & Stahl, M. M. (1977) *Genetics* **86**, 715–725.
15. Hopkins, J. D., Clements, M. B., Liang, T.-Y., Isberg, R. R. & Syvanen, M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2814–2818.
16. Hickson, I. D. & Emmerson, P. T. (1983) *J. Bacteriol.* **156**, 901–903.
17. Schultz, D. W., Taylor, A. F. & Smith, G. R. (1983) *J. Bacteriol.* **155**, 664–680.
18. Enquist, L. W. & Skalka, A. (1973) *J. Mol. Biol.* **75**, 185–212.
19. Stahl, F. W., McMilin, K. D., Stahl, M. M., Malone, R. E., Nozu, Y. & Russo, V. E. A. (1972) *J. Mol. Biol.* **68**, 57–67.
20. Stahl, F. W. (1979) *Annu. Rev. Genet.* **13**, 7–24.
21. Little, J. W. & Mount, D. W. (1982) *Cell* **29**, 11–22.
22. Tomizawa, J.-I. & Ogawa, H. (1972) *Nature (London) New Biol.* **239**, 14–16.
23. Hickson, I. D. & Emmerson, P. T. (1981) *Nature (London)* **294**, 578–580.
24. Eichler, D. C. & Lehman, I. R. (1977) *J. Biol. Chem.* **252**, 499–503.
25. Capaldo-Kimball, F. & Barbour, S. D. (1971) *J. Bacteriol.* **106**, 204–212.
26. Egner, C. & Berg, D. E. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 459–463.
27. Taylor, A. F. & Smith, G. R. (1980) *Cell* **22**, 447–457.
28. Telander-Muskavitch, K. M. & Linn, S. (1982) *J. Biol. Chem.* **257**, 2641–2648.
29. DasGupta, C., Wu, A. M., Kahn, R., Cunningham, R. P. & Radding, C. M. (1981) *Cell* **25**, 507–516.
30. Faulds, D., Dower, N., Stahl, M. M. & Stahl, F. W. (1979) *J. Mol. Biol.* **131**, 681–695.