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Suppressibility of *recA, recB,* and *recC* Mutations by Nonsense Suppressors

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Mutations in the *recA, recB,* and *recC* genes of *Escherichia coli* K-12 were surveyed to ascertain whether or not they are suppressed by nonsense suppressors. Several mutations which map in or near the *recA* gene, but have not been called *recA* mutations, were also surveyed. An amber *recB* mutation, *recB156,* and an amber *recC* mutation, *recC155,* were isolated. One *recB* mutation, *recB95,* and four *recC* mutations, *recC22, recC38, recC82,* and *recC83,* were found to be suppressed by a UGA suppressor. In addition to the previously isolated amber *recA* mutation *recA99,* two other *recA* mutations, *recA52* and *recA123,* were found to be suppressed by amber suppressor *supD32* but not by *supE44.*

The first recombination-deficient mutants of *Escherichia coli* K-12 were isolated by Clark and Margulies (11) and were designated *recA*$^-$. These were found to be sensitive to UV irradiation (11). Subsequently, *recA* mutants were found to exhibit additional mutant phenotypes, including sensitivity to X-irradiation (26), noninducibility of lambda prophage by UV irradiation (17) or thymine starvation (13), and noninduction of mutations by UV irradiation (33, 46). The *recA* gene was originally mapped between *cysC* and *pheA* (44). Recently it has been found to be highly cotransducible with the *srl* (or *gut*) operon (32). Several mutations which are also highly cotransducible with *srl* (A. Templin and A. J. Clark, unpublished data) and exhibit some but not all of the *recA* phenotypes have been isolated. These mutations include *lexB* (4, 13), *recH* (39), *zab* (8), and *tif* (7, 21). Except for *tif,* all show an intermediate sensitivity to UV irradiation and X rays, and all lack the extreme recombination deficiency characteristic of *recA* mutants.

Other Rec$^-$ mutants with intermediate recombination deficiencies and UV sensitivities were isolated (14, 26) and found to map between *thyA* and *argA* (14, 45). These mutants were designated *recB* and *recC*. *recB* and *recC* have been shown to be the structural genes for exonuclease V (40), an ATP-dependent exonuclease which is active on both single- and double-stranded DNA (3, 5, 19, 20, 35).

We have surveyed many of the *recB, recC, recA,* and allied mutations to determine which are suppressible by nonsense suppressors. We have also isolated amber *recB* and *recC* mutations.

MATERIALS AND METHODS

Bacterial and phage strains. The basic bacterial strains used in these studies are described in Table 1. P1vira was used for transductions. Temperature induction studies were carried out with λ^+. Lambda phage strains JC1929 (previously called λ*N7N53cI26*) and JC1930 (previously called h^{80} i^{λ}*N7N53cI26*) were the gift of Noreen Murray. The T4 phage used to determine suppression patterns are listed in Table 2.

General methods and media. The procedures and media used for conjugational and transductional crosses were those described by Willetts et al. (44). LCTG top agar was Luria broth to which were added 2.5 ml of 40% glucose, 4 ml of 0.5 M $CaCl_2$, 4 ml of 0.25% thymidine, and 7 g of Difco agar per liter. Thymine-requiring mutants were isolated by the trimethoprim method of Stacy and Simpson (38). UV sensitivity was determined as described by Clark (10).

Nomenclature. Genotype designations are those used by Bachmann et al. (2); the recommendations of Demerec et al. (12) are followed for bacterial genetic nomenclature. Our supplementation involves the use of a minus-sign suffix to a gene symbol, e.g., *recA*$^-$, to stand for a set of mutant alleles at a locus. This practice supplements that recommended by Demerec et al. (12), in which the gene symbol without a minus-sign suffix is used as a modifier of the noun "mutant" or "mutation." Thus, "*recA*$^-$ allele" is synonymous with "*recA* mutant allele." Accordingly, the gene symbol without the minus-sign suffix or without the noun "mutant" or "mutation" does not specify whether the locus is wild type or mutant.

We have extended recommendations 9 and 10 of Demerec et al. (12) for the naming of bacterial strains to the naming of phage lambda and T4 strains.

The following phenotype abbreviations are used: Arg, arginine; His, histidine; Leu, leucine; Rec, recombination; Ser, serine; Sm, streptomycin; Srl, sorbitol (which is synonymous with glucitol); Tet, tetracycline; Thr, threonine; Thy, thymine; superscript s, sensitive;

TABLE 1. *Basic bacterial strains*

Strain no.	Sex	*sup* allele	*rec* allele	*arg*	*his*	*ilv*	*leu*	*pro*	*thr*	*thi*	Other markers	Source/reference
AB1157	F^-	*supE44*	*rec*$^+$	*E3*	*4*	+	*6*	*A2*	*1*	*1*		25
JC5422	F^-	*supE44*	*rec*$^+$	*E3*	*4*	+	*6*	*A2*	*1*	*1*	*thyA325*	43
JC5220	F^-	*sup*$^+$	*rec*$^+$	*E3*	*4*	+	*6*	*A2*	*1*	*1*	*met-323*	H. Nagaishi[a]
JC10236	F^-	*supE44*	*rec*$^+$	*E3*	*4*	+	*6*	*A2*	*1*	*1*	*srlA300*::Tn*10*	L. Csonka[b]
JC10258	F^-	*sup*$^+$	*rec*$^+$	*E3*	*4*	+	*6*	*A2*	*1*	*1*		L. Csonka
AB259	Hfr P01	*sup*$^+$	*rec*$^+$	+	+	+	+	+	+	*1*		1
JC158	Hfr P01	*sup*$^+$	*rec*$^+$	+	+	+	+	+	+	*1*	*serA6*	9
JC5029	Hfr P045	*sup*$^+$	*rec*$^+$	+	+	*318*	+	+	*300*	+		10
JC5412	Hfr P045	*sup*$^+$	*recB21*	+	+	*318*	+	+	*300*	+	*sbcA8*	45
JC5426	Hfr P045	*sup*$^+$	*recC22*	+	+	*318*	+	+	*300*	+		45
CAJ64	Hfr	*sup-71*	*rec*$^+$	+	+	+	+	+	+	+	*metB1* (λ^+)	37
S26R1e	Hfr P02A	*supD32*	*rec*$^+$	+	+	+	+	+	+	+	(λ^+)	18
WH-1	F196	*supD32*	*recA1*	+	+/*45*	+	+	+	+	+	*trp-37*	23

[a] Spontaneous mutant of AB1157.

[b] Mutant derived by transposition of Tn*10*.

TABLE 2. *Bacteriophage strains*

New name	Former name	Mutant codon	Gene affected	Source/reference
JC1911	$T4^+$			
JC1912	B22	UAG	43	16
JC1913	NG237	UAG	15	36
JC1914	N133	UAG	15	16
JC1915	NG319	UAG	15	36
JC1916	am882	UAG	*e*	36
JC1917	B17	UAG	23	16
JC1918	NG19	UAG	34	36
PS292	PS292	UAA	?	S. Person, personal communication
PS205	PS205	UAA	?	S. Person, personal communication
JC1921	oceL1	UAA	*e*	47
JC1922	oceL2	UAA	*e*	47
JC1923	oceL3	UAA	*e*	47
JC1924	oceL4	UAA	*e*	W. Wood, personal communication
JC1925	oceL5	UAA	*e*	47
JC1926	427	UAA	?	36
JC1927	opeL1P12	UGA	*e*	W. Wood, personal communication
JC1928	opeL1P41	UGA	*e*	W. Wood, personal communication

and superscript r, resistant. Superscript + and − signs each have three meanings when used with phenotypic abbreviations: independent and dependent, respectively, when used with abbreviations of amino acids or pyrimidines; utilizing and nonutilizing, respectively, when used with Srl; and proficient and deficient, respectively, when use with Rec. In addition, we use Su^+ to abbreviate "nonsense mutant permissive" and prefix it with the particular nonsense codon involved; e.g., UGA Su^+ would abbreviate "permissive for opal mutants." Likewise, Su^- abbreviates "nonpermissive." Note that the genotype of a Su^+ strain is *sup*$^-$; in other words, the permissive phenotype is produced by a mutant suppressor allele. Likewise, a Su^- (nonpermissive) strain is *sup*$^+$.

Isolation of Su^- from Su^+ strains. Su^- derivatives were isolated from Su^+ strains by selection of clones which were able to survive infection with double amber *N* mutant lambda phages of different host ranges. A mixture of 10^8 cells of a log-phase L broth culture, 10^6 plaque-forming units of JC1929, 10^6 plaque-forming units of JC1930, and 10^{-2} M $MgSO_4$ was allowed to incubate for 10 min at 37°C and plated with 2.5 ml of LCTG top agar onto L agar plates. The plates were incubated overnight at 37°C. Suspensions from single colonies were then streaked onto L plates, and the resulting colonies were cross-streaked with wild-type and suppressor phage.

Determination of suppressor patterns. The presence of amber, ochre, and opal suppressors in strains was confirmed by testing them against a series of T4 phage containing nonsense-suppressible mutations. A 0.5-ml volume of a suspension containing 10^6 of each T4 mutant per ml was added to separate wells of an autoclavable chamber containing 25 wells. Of an overnight L broth culture of the strain to be tested, 0.1 to 0.2 ml was plated with 2.5 ml of LCTG top agar onto a 2% L agar plate. Drops of the phage suspensions were transferred to the plate with a block containing 25 inoculating pegs, and the plate was incubated overnight at 37°C. A spot of lysis indicated suppression of the mutant in the bacterial strain. To determine suppression patterns, we used seven UAG mutants, eight UAA mutants, two UGA mutants, and the wild-type T4. One T4 amber mutant, NG1918, is suppressed by *supD* but not by *supE*, thus allowing discrimination of the two amber suppressors. The phage strains are listed in Table 2.

Derivation of nonsense-suppressing and -nonsuppressing strains. Strains used to test suppressibility of *rec* mutations were derived in this laboratory. For the determination of suppressibility of *recB* and *recC* mutations, strains were constructed which carried *thyA* mutations. JC9350, which carries two amber suppressors, *supD32* and *supE44,* was made by cotransduction of *supD32* with *his*$^{+}$ into JC5422. The transductional donor was S26R1e. JC9365, which carries an ochre suppressor, was derived from a His^{+} Arg^{+} Thr^{-} revertant of AB1157. Previous results (T. Kato and A. J. Clark, unpublished data) indicate that there are three kinds of UV-induced His^{+} revertants of AB1157: (i) His^{+} Arg^{-} Thr^{-}; (ii) His^{+} Arg^{+} Thr^{-}; and (iii) His^{+} Arg^{+} Thr^{+}. Kato and Clark (unpublished data) have shown that the first class contains backmutants at the *his-4* locus. The other two classes, which comprise the majority, carry UAA (ochre) suppressor mutations. Some of these UAA Su^{+} strains presumably arise by conversion of the UAG (amber)-suppressing allele *supE32* into an ochre-suppressing allele by secondary mutation. Kato and Clark inferred this from the loss of ability to suppress several UAG mutant T4 phage and the gain in ability to suppress UAA mutant T4 phage. One such UAA Su^{+} strain, JC9375, was treated with trimethoprim (38), and a spontaneous Thy^{-} derivative, JC9365, was isolated.

The *thyA*$^{-}$ UGA Su^{+} strain JC9802 is a derivative of AB1157 made through a series of steps. AB1157 was first treated with nitrosoguanidine to yield JC8403 *ilv-332.* A Su^{-} derivative of this UAG Su^{+} strain was then selected by infection with two double *N* mutant phage as previously described. The resulting Su^{-} Ilv^{-} strain, JC9385, was the recipient for the cotransduction of *sup-71* with *ilv*$^{+}$. The transductional donor of the UGA suppressor was CAJ64 (37). The UGA Su^{+} strain JC9399 was then made Thy^{-} by the trimethoprim method.

Strains which carried an Srl^{-} mutation were constructed to test the suppressibility of *recA* and allied mutations. An *srl* mutation was introduced into strains carrying the suppressors by use of the transposon Tn*10* which had been inserted into one of the *srl* genes. P1 lysates on the strain carrying the Tet insertion, JC10236, were used to transduce JC9350 (UAG Su^{+}), JC9375 (UAA Su^{+}), JC9399 (UGA Su^{+}), and a Su^{-} strain, JC10258. When Tet resistance was selected, the transductants were all Srl^{-}. The *srlA300*::Tn*10* strains produced were JC11,244 (UAG Su^{+}), JC11,245 (UAA Su^{+}), JC11,246 (UGA Su^{+}), and JC11,247 (Su^{-}).

Source of *recA, recB,* and *recC* mutations. The *recB* and *recC* mutations which we surveyed are those listed by Willetts and Mount (45). All were induced by nitrosoguanidine mutagenesis. The *recA* and allied mutations which we studied are described in Table 3.

TABLE 3. *"Roots" of recA and allied mutations*

Strain no.	*rec* allele	Primary mutant phenotype	Mutagen	Reference
recA mutants				
JC2921	*recA1*	Conjugational Rec^{-}	MNNG[a]	11
JC1554	*recA2*	Conjugational Rec^{-}	MNNG	11
JC2923	*recA3*	Intragenic Rec^{-}	MNNG	42
GY3451	*recA11*	Resistance of lambda lysogen to thymine deprivation	5-BU[b]	17
JC2917	*recA12*	X-ray sensitivity	MNNG	26
JC2926	*recA13*	X-ray sensitivity	MNNG	26
KMBL239	*recA34*	UV sensitivity	MNNG	41
JC2922	*recA35*	UV sensitivity	MNNG	41
GY2661	*recA36*	UV sensitivity	MNNG	41
JC2925	*recA52*	Conjugational Rec^{-}	MNNG	10
JC2924	*recA56*	Conjugational Rec^{-}	MNNG	10
JC2932	*recA69*	Conjugational Rec^{-}	MNNG	10
JC4557	*recA74 tif-1*	42°C resistance	Spontaneous	27
JC2996	*recA75 tif-1*	42°C resistance	Spontaneous	27
DM455	*recA99*	Small colonies on MMS[c]	MNNG	34
JM12123	*recA123 tif-1*	42°C resistance	Spontaneous	7
JC7903	*recA140*	Conjugational Rec^{-}	MNNG	24
JC7940	*recA142*	Conjugational Rec^{-}	MNNG	24
KL399	*recA200*	42°C sensitive in PolA^{-} background	MNNG	30
Allied mutants				
PC0301	*recH166*	Conjugational Rec^{-}	MNNG	39
GY1163	*lexB30*	Resistance of lambda lysogen to thymine deprivation	2-AP[d]	13
JM1253	*zab-53 tif-1*	42°C resistance	Spontaneous	8
JM12	*tif-1*	40°C sensitivity in a lambda lysogen	MNNG	7, 21

[a] *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

[b] 5-Bromouracil.

[c] Methyl methane sulfonate.

[d] 2-Aminopurine.

Identification of transductants by recombination deficiency and thermal sensitivity. Transductants from each cross were streaked on the homologous minimal plates, and two colonies from each streak were patched onto the same medium. Replica plating was used to score the phenotypes. *recB*$^-$ and *recC*$^-$ transductants were identified by UV sensitivity, and *recA*$^-$ transductants were identified by both UV sensitivity and inability to produce recombinants. *lexB30*, *recH166*, and *zab-53 tif-1* transductants were identified by UV sensitivity, and *tif-1* transductants were identified by inability to grow at 42°C on minimal media supplemented with 75 μg of adenine per ml. Recombination ability was tested by crosses with JC158 and selection for Thr$^+$ Leu$^+$ (Ser$^+$ Sm^r) recombinants. A dose of 80 J/m^2 was used to determine UV sensitivity.

Detection of amber-suppressible *rec* mutants. A log-phase culture of JC5220 was centrifuged, and the pellet was suspended in water to the same volume. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (500 μg/ml in 1 M citrate buffer at pH 5.5) was added to a final concentration of 50 μg/ml. After 60 min of incubation without aeration at 37°C (50% survival), the culture was centrifuged and the cells were washed once with 56/2 buffer and suspended to the original volume in fresh L broth. The culture was then diluted 1:5 into fresh L broth, distributed into several tubes, and incubated with aeration at 37°C for 4 h. The cultures were then diluted in 56/2 buffer, and samples were spread on L plates plus methyl methane sulfonate (0.03%). After overnight incubation at 37°C, small clones (6.7% of the total number of colonies per plate) were purified by two single-colony isolations. Purified isolates were tested for their ability to survive 60 J of UV irradiation per m^2 and for their ability to produce Thr$^+$ Leu$^+$ [Sm^r] transconjugants in plate crosses with AB259. The clones that appeared defective by this plate mating method were tested in broth crosses with AB259 and F' donor WH-1, which carries *his*$^+$ and *supD32* on F196. Thr$^+$ Leu$^+$ [Sm^r] transconjugants were selected in the Hfr cross, and His$^+$ [Sm^r] transconjugants were selected in the F' cross. Of 643 clones tested, 11 proved to be Rec$^-$. Transconjugants of each of the 11 carrying F196 were tested for their recombination ability as phenocopy F$^-$ recipients in broth crosses with Hfr 3000. Two of the 11 were Rec$^+$. The *rec* mutations in these two strains were putative amber mutations and were characterized.

RESULTS

Characterization of amber-suppressible *rec* mutations. Two Rec$^-$ strains were isolated which became Rec$^+$ upon introduction of an F-prime plasmid (F196) carrying an amber-suppressing allele. One of them, *rec-156*, was 45% cotransducible with *thyA*$^+$ and failed to complement *recB21* in the Hfr strain JC5412 when tested by the method of Willetts and Mount (45). The other, *rec-155*, was 65% cotransducible with *thyA*$^+$ and failed to complement *recC22* in JC5426 when tested similarly. F$^-$ and F196 strains carrying *recB156* or *recC155* were crossed with AB259, and Thr$^+$ Leu$^+$ [Sm^r] recombinants were selected. The F$^-$ strains yielded 0.9 and 0.3% as many recombinants as the wild-type strain, respectively. The F-prime derivatives (carrying the *supD32* amber suppressor) yielded 200 and 70% as many recombinants as the wild-type strain, respectively.

Survey of *recB* and *recC* mutations for suppressibility. The survey of preexisting *recB* and *recC* mutations was carried out by transducing the different alleles into strains containing UAG, UAA, and UGA suppressors. *recB* alleles are normally 40 to 50% cotransducible with *thyA*; *recC* alleles are normally 70 to 80% cotransducible with *thyA* (45). The mutant phenotype is not expressed in strains in which a nonsense mutation is introduced into a strain containing the corresponding suppressor. No amber or ochre mutations were discovered among the previously isolated mutants. However, one *recB* allele (*recB95*) and four *recC* alleles (*recC22*, *recC38*, *recC82*, and *recC83*) were found to be suppressed by a UGA suppressor. Table 4 shows representative data from the transductions into Thy$^-$ nonsense-suppressing strains. The amber *recB* mutant, *recB156*, was suppressed by the UAG Su$^+$ strain but not by the UAA Su$^+$ strain, whereas the amber *recC* mutant, *recC155*, was suppressed by both the UAG and UAA suppressors. Neither was suppressed in a UGA Su$^+$ background. The UGA mutations, *recB95* and *recC22*, conferred no UV sensitivity when transduced into the UGA Su$^+$ strain, but they conferred normal sensitivity in the UAG Su$^+$ and UAA Su$^+$ strains. The presence of the *rec* alleles in the suppressor strains

TABLE 4. *UV-sensitive Thy$^+$ transductants*[a]

Donor	*rec* allele	UAG Su$^+$ recipients[b] (%)	UAA Su$^+$ recipients[c] (%)	UGA Su$^+$ recipients[d] (%)
AB2470	*recB21*[e]	38	37	36
JC5723	*recB95*	53	43	≤1
JC4702	*recB156*	≤1	64	41
JC5489	*recC22*[f]	72	80	≤1
JC4456	recC73	76	86	61
JC4701	recC155	≤1	≤1	38

[a] *recB* and *recC* mutants were used as transductional donors, and Thy$^-$ nonsense-suppressing strains were used as recipients. A minimum of 100 transductants from each cross were screened.

[b] JC9350 carries *supE44* and *supD32*.

[c] JC9365 carries *sup-300*.

[d] JC9802 carries *sup-71*.

[e] Similar data were obtained with the following alleles: *recB22*, *recB58*, *recB60*, *recB61*, *recB85*, *recB88*, *recB89*, *recB90*, *recB91*, *recB92*, *recB93*, and *recB94*.

[f] Similar data were obtained with the following alleles: *recC38*, *recC82*, and *recC83*.

was confirmed by transducing them back into a UGA Su$^-$ strain in which the RecB$^-$ or RecC$^-$ phenotype was again expressed (Table 5).

Quantitative determinations of recombination proficiency and UV survival were performed on the suppressed strains (Table 5). The presence of the UGA suppressor restored the Rec$^+$ phenotype although not to wild-type levels. The suppressed *rec*$^-$ strains showed deficiency indexes of 4 to 8 as compared with 1 for the wild type and 40 to 300 for the unsuppressed *rec*$^-$ strains. Similarly, survival after 10 J of UV irradiation per m^2 was 13 to 45% in the suppressed *rec*$^-$ strains as compared with 93% for the wild type and 2 to 5% for the unsuppressed *rec*$^-$ strains.

Survey of *recA* mutations for suppressibility. The survey of the *recA* mutations was performed by cotransduction of the alleles with *srl*$^+$ into *srlA300*::Tn*10* strains carrying the nonsense suppressors. Most were found to be nonsuppressible; i.e., they were Rec$^-$ and UV sensitive in all suppressor backgrounds, presumably deletion, missense, or frameshift mutations. Table 6 shows some representative data from transductions. *recA99,* which was originally isolated as an amber mutation, was suppressed in the UAG Su$^+$ strain, as were two other *recA* mutations. One was *recA52,* which was isolated from a strain which contains the amber suppressor *supE44* (AB1157). The other, *recA123,* was detected in JM12123, which is a revertant of *tif-1* strain JM12 that no longer expressed the thermal induction of filamentation and of lambda prophage characteristic of *tif-1.* Our isolates of JM12 and JM12123 contained an amber suppressor which showed the phage-suppressing pattern conferred by *supE44.* Nevertheless, JM12123 was Rec$^-$, indicating that *supE44* did not suppress *recA123.* Our UAG Su$^+$ strain JC11,244 carried *supD32* in addition to *supE44,* however. Tests which will be reported elsewhere (L. Csonka, A. Templin, and A. J. Clark, unpublished data) show that it is *supD32* which suppresses *recA123.* The presence of *recA52* and *recA123* in the UAG Su$^+$ background was confirmed by transducing them back into a Su$^-$ strain in which the Rec$^-$ UV^s phenotype was again expressed.

TABLE 5. *Phenotypes of suppressed and nonsuppressed recB and recC alleles in UGA Su*$^+$ *strains*

UGA Su$^+$ strain	% Survival (10 J of UV/m^2)	Recombination deficiency[a] index Thr$^+$ Leu$^+$ [Ser$^+$ Sm^r]	% Cotransduction[b] of *rec*$^-$ with *thyA*$^+$	Confirmed *rec* genotype
JC9823	93	1.0	<1	*rec*$^+$
JC9829	2.2	2.7×10^2	37	*recB21*
JC9830	13	6.9	33	*recB95*
JC9835[c]	27	4.1	64	*recC22*
JC9824	4.6	2.4×10^2	81	*recC73*

[a] Deficiency index is a ratio of the number of progeny obtained from a Rec$^+$ recipient to the number of progeny obtained from a Rec$^-$ recipient. Recombinants were selected in crosses with JC158 at 37°C; after 60 min the matings were interrupted with a Vortex mixer.

[b] The transductional recipient was JC5422, which is UGA Su$^-$ *thyA325 rec*$^+$. UV sensitivity was used to detect *rec*$^-$ transductants.

[c] Similar data were obtained for UGA Su$^+$ strains carrying *recC38, recC82,* and *recC83.* The ranges mentioned in the text include the data for these strains.

DISCUSSION

Fifteen *recB* mutations were surveyed, and two were found to be suppressible by any of the four nonsense suppressors used, whereas five of the six *recC* mutants surveyed were suppressible. It is unclear whether or not these disparate ratios are significant. On one hand, the role of the *recB* and *recC* genes in determining exonuclease V is not clear. It is tempting to consider that each determines one of the two very large subunits of the enzyme described by Goldmark and Linn (20). Lieberman and Oishi (28), however, have indicated that exonuclease V consists of a very large and a moderate-sized subunit; and the very large subunit complements both *recB* and *recC* mutant extracts in in vitro reconstitution of enzyme activity. To add to the complexity of the *recB* and *recC* loci, mutations in them produce a high degree of cell inviability (6) as well as absence of exonuclease V activity. Recently, M. R. Fortson and S. R. Kushner (personal communication) have detected three temperature-conditional mutations that map between known *recB* and *recC* mutations. These mutations affect only the viability of cells; they do not affect exonuclease V activity at a high temperature. It is possible that they occur in another gene on which *recB* nonsense mutations would be polar. Alternatively, they might affect a portion of the *recB* gene product necessary for viability but unnecessary for nuclease activity. In this case, the one UGA and the one UAG mutation discovered in *recB* would be predicted either not to be polar on this gene or not to truncate the protein short of the vital portion. Nonsense mutations in *recC,* on the other hand, might in general have a lesser effect on the vital activity.

Of the 18 *recA* alleles tested, two were found to be suppressible by UAG suppressors and none was suppressible by UAA and UGA suppressors.

TABLE 6. *Fraction of Srl$^+$ transductants showing a Rec$^-$, UV^s, or 42°C^s phenotype*[a]

Donor	Donor mutant allele	Fraction with recipient phenotype[b]:			
		UAG Su$^+$	UAA Su$^+$	UGA Su$^+$	Su$^-$
JC2921	*recA1*[c]	8/10	10/10	9/10	8/10
JC2926	*recA13*	8/10	10/10	9/10	10/10
JC2922	*recA35*	10/10	10/10	9/10	9/10
JC2925	*recA52*	0/10	6/10	8/9	8/10
JC2924	*recA56*	10/10	10/10	9/10	7/9
DM455	*recA99*	0/10	9/10	8/10	8/10
PC0301	*recH166*	10/10	6/10	8/10	8/10
GY1163	*lexB30*	4/10	8/10	10/10	9/10
JM12	*tif-1*	6/10	6/9	5/7	6/7
JM12123	*recA123 tif-1*	0/10	9/10	8/10	10/10
JM1253	*zab-53 tif-1*	8/10	6/6	8/9	8/8

[a] Srl$^-$ Su$^+$ and Srl$^-$ Su$^-$ strains were used as transductional recipients, and *recA* or allied mutant strains were used as transductional donors. Inheritance of unsuppressed *recA* alleles was detected by both recombination deficiency and UV sensitivity. Inheritance of unsuppressed *recH166, lexB30,* and *zab-53 tif-1* alleles was detected by UV sensitivity. Inheritance of unsuppressed *tif-1* was detected by the absence of growth at 42°C on minimal medium plus 75 μg of adenine per ml.

[b] The UAG Su$^+$ strain was JC11,244; the UAA Su$^+$ strain was JC11,245; the UGA Su$^+$ strain was JC11,246; and the Su$^-$ strain was JC11,247.

[c] Similar data were obtained for the following alleles: *recA2, recA3, recA11, recA12, recA34, recA36, recA69, recA74 tif-1, recA75 tif-1, recA140, recA142,* and *recA200*. The results for *recA13, recA35,* and *recA56*, although similar to those for *recA1*, are included in the table because these alleles were used for complementation experiments to be reported elsewhere (L. Csonka, A. Templin, and A. J. Clark, in preparation).

An additional *recA* allele known to be UAG suppressible was tested as a standard. The inability to suppress *recA1* is consistent with its identification as a missense mutant allele producing a protein of altered isoelectric point (22). The inability to suppress *recA12* is consistent with its identification as a deletion mutant allele producing a protein of altered molecular weight (31). Of the four *recA*-allied mutations (*tif-1, recH166, lexB30,* and *zab-53 tif-1*), none was found suppressible. The relationship between the amber-suppressible *recA123* and *tif-1* is discussed elsewhere (L. Csonka, A. J. Clark, and A. Templin, in preparation). It was found that the amber-suppressed *recA123 tif-1* strain was fully wild type in phenotype. This means that suppression of *recA123* also phenotypically suppresses the *tif-1* phenotype, thus allowing us to add a bit of evidence consistent with the recent identification of the *tif* gene product as that produced by *recA* (15, 22, 29, 31).

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