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Constitutive Expression of SOS Functions and Modulation of Mutagenesis Resulting from Resolution of Genetic Instability at or near the *recA* Locus of *Escherichia coli*

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Summary. Cellular activities normally inducible by DNA damage (SOS functions) are expressed, without DNA damage, in *recA441* (formerly *tif-1*) mutants of *Escherichia coli* at 42° C but not at 30° C. We describe a strain (SC30) that expresses SOS functions (including mutator activity, prophage induction and copious synthesis of recA protein) constitutively at both temperatures. SC30 is one of four stable subclones (SC strains) derived from an unstable recombinant obtained in a conjugation between a *recA441* K12 donor and a *recA*$^+$ B/r-derived recipient. SC30 does not owe its SOS-constitutive phenotype to a mutation in the *lexA* gene (which codes the repressor of *recA* and other DNA damage-inducible genes), since it is *lexA*$^+$. Each of the SC strains expresses SOS functions in a distinctively anomalous way. We show that the genetic basis for the differences in SOS expression among the SC strains is located at or very near the *recA* locus. We propose that resolution of genetic instability in this region, in the original recombinant, has altered the pattern of expression of SOS functions in the SC strains.

Introduction

The recA protein of *Escherichia coli* is necessary for homologous genetic recombination (Clark and Margulies 1965; Shibata et al. 1979; McEntee et al. 1979) and for the expression of cellular activities induced by DNA damage (SOS functions) (review, Witkin 1976). Some mutations in *recA* eliminate both of these activities, whereas others (Morand et al. 1977; Castellazzi et al. 1972b) spare the recombinogenic functions but inactivate the proteolytic capability of recA protein that is required for SOS induction (Roberts et al. 1978; Craig and Roberts 1980, 1981). Still other *recA* mutations (originally designated as *tif*) cause constitutive expression of SOS functions (including prophage induction, delayed cell division and error-prone DNA repair) at elevated temperatures (Castellazzi et al. 1972a; Witkin 1974).

A model of *recA* gene regulation (Gudas and Mount 1977; Emmerson and West 1977; Mount 1977; McEntee 1977) has recently been substantiated by biochemical studies (Little and Harper 1979; Little et al. 1980; Brent and Ptashne 1980; Little et al. 1981; Brent and Ptashne 1981). Synthesis of recA protein is controlled by lexA protein, functioning as a repressor, and by recA protein itself. In undamaged wild type cells, recA protein synthesis is maintained at a low level by lexA protein repression. Damage to DNA results in activation of the latent proteolytic activity of recA protein, possibly via its interaction with an oligonucleotide effector (Irbe et al. 1981) or with single-stranded DNA (Sussman et al. 1978; Craig and Roberts 1980). Activated recA protein then cleaves the lexA repressor, resulting in a greatly increased rate of synthesis of recA protein. Constitutively high levels of recA protein synthesis occur in strains carrying lexA mutations (*spr*) that code an inactive repressor (Pacelli et al. 1979), as well as in strains carrying an operator-constitutive mutation in the *recA* control region (Volkert et al. 1981). At least six other DNA damage-inducible (*din*) genes (Kenyon and Walker 1980) are also repressed by lexA protein (Kenyon and Walker 1981; Huisman and D'Ari 1981; Bagg et al. 1981), and are probably regulated in the same way as the *recA* gene.

In a series of conjugations designed to transfer the *recA441 (tif-1)* allele from *E. coli* K12 into a B/r derivative, we isolated a rare recombinant that expressed SOS functions constitutively at 30° C, unlike the Tif$^-$ parent, which is SOS-constitutive only at high temperatures. We later concluded that this recombinant, as originally isolated, must have been genetically unstable, since it had generated four stable subclones, each of which expressed SOS functions in a different anomalous way. In this report, we show that the genetic basis for the differences in SOS expression among these subclones is linked to *srl* and *alaS*, genes that flank *recA* closely (Bachmann and Low 1980). Our results suggest that SOS expression can be modulated in a variety of ways by changes in DNA sequence and/or conformation in or very near the *recA* gene.

Material and Methods

Bacterial Strains. Table 1 lists the strains used and their relevant genotypes.

Culture Media. Cultures were grown as previously described (Witkin 1974) in nutrient broth (NB) or in minimal E medium supplemented with casein hydrolysate (0.4%) and 20 μg/ml tryptophan (MCHT) or only with tryptophan (ET). Solid media were nutrient agar (NA), minimal E agar supplemented with 2.5% (v/v) NB (SEM agar), or unsupplemented minimal agar (E), prepared as described (Witkin 1974). Agar used to select transductants was appropriately supplemented E agar with 0.4% maltose, lactose or sorbitol replacing glucose for selection of Mal$^+$, Lac$^+$ or Srl$^+$ recombinants, or with 20 μg per ml tetracycline added for selection of tetracycline resistant (Tet^r) transductants. Thymine (50 μg per ml) was routinely added to all media for strains requiring thymine. Adenine (Ade) (75 μg per ml) or a combination of cytidine (Cyt) and guanosine (Guo) (100 μg per ml of each) were added, where indicated, to liquid or solid media.

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Table 1. Bacterial strains

Strain	Relevant genotype	Source/derivation/references/comments
SC30	uvrA155 trpE65 (*recA*$_{30}$)	this study; (*recA*$_{30}$) = "recA allele of SC30"
SC11	uvrA155 trpE65 (*recA*$_{11}$)	this study; (*recA*$_{11}$) = "recA allele of SC11"
SC7	uvrA155 trpE65 (*recA*$_{7}$)	this study; (*recA*$_{7}$) = "recA allele of SC7"
SC18	uvrA155 trpE65 (*recA*$_{18}$)	this study; (*recA*$_{18}$) = "recA allele of SC18"
WP2$_s$	uvrA155 trpE65 *recA*$^-$	a derivative of *E. coli* B (Hill 1965)
WP44$_s$-NF	uvrA155 trpE65 *recA441* (*tif-1*)	this laboratory (Witkin 1975); a Tif$^-$ Sfi$^-$ K12/B hybrid
RT-4	uvrA155 trpE65 *recA441* (*tif-1*)	this laboratory; another Tif$^-$ Sfi$^-$ K12/B hybrid
SC30-RP	uvrA155 trpE65 *srlC300*::Tn*10 recA*$^+$ *alaS3*	this study; Tet^r Srl$^-$ AlaS$^-$ transductant of SC30[a]
SC11-RP	uvrA155 trpE65 *srlC300*::Tn*10 recA*$^+$ *alaS3*	this study; Tet^r Srl$^-$ AlaS$^-$ transductant of SC11[a]
SC18-RP	uvrA155 trpE65 *srlC300*::Tn*10 recA*$^+$ *alaS3*	this study; Tet^r Srl$^-$ AlaS$^-$ transductant of SC18[a]
SC30-LM	uvrA155 trpE65 (*recA*$_{30}$) *lexA102*	this study; *lamB*$^+$ *lexA102* transductant of SC30
SC30-30	like SC30	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of SC30-RP, donor SC30
SC30-11	like SC30, except (*recA*$_{11}$)	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of SC30-RP, donor SC11
SC30-18	like SC30, except (*recA*$_{18}$)	this study; Tet^s Srl$^+$ AlaS$^-$ transductant of SC30-RP, donor SC18
SC11-30	like SC11, except (*recA*$_{30}$)	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of SC11-RP, donor SC30
SC11-11	like SC11	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of SC11-RP, donor SC11
SC11-18	like SC11, except (*recA*$_{18}$)	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of SC11-RP, donor SC18
SC18-30	like SC18, except (*recA*$_{30}$)	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of SC18-RP, donor SC30
SC18-11	like SC18, except (*recA*$_{11}$)	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of SC18-RP, donor SC11
SC18-18	like SC18	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of SC18-RP, donor SC18
SC30-MP (λ)	like SC30, except *lamB*$^+$ (λ)	this study; λ lysogen of Mal$^+$ transductant of SC30 (SC30-MP)[b]
SC18-MP (λ)	like SC18, except *lamB*$^+$ (λ)	this study; λ lysogen of Mal$^+$ transductant of SC18 (SC18-MP)[b]
SC30-TF	like SC30, except *recA441*	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of SC30-RP, donor WP44$_s$-NF
SC30-TF-MP (λ)	like SC30-TF, except *lamB*$^+$ (λ)	this study; λ lysogen of Mal$^-$ transductant of SC30-TF (SC30-TF-MP)[b]
SC30-RP-MP (λ)	like SC30-RP, except *lamB*$^+$ (λ)	this study; λ lysogen of Mal$^-$ transductant of SC30-RP (SC30-RP-MP)[b]
WP44$_s$-MM	*uvrA155 trpE65 recA441 argE3 his217 leu81 ilv686 rpsL31 thyA713*	P. Kirschmeier, this laboratory, from WP44$_s$ (Witkin 1974)
WP44$_s$-MM-RP	like WP44$_s$-MM, except *srlC300*::Tn*10 recA*$^+$ *alaS3*	this study; Tet^r Srl$^-$ AlaS$^-$ transductant of WP44$_s$-MM
WP44$_s$-MM-30	like WP44$_s$-MM, except (*recA*$_{30}$)	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of WP44$_s$MM-RP, donor SC30
WP44$_s$-MM-18	like WP44$_s$-MM, except (*recA*$_{18}$)	this study; Tet^s Srl$^+$ AlaS$^+$ transductant of WP44$_s$MM-RP, donor SC18

[a] P1 grown on K12 strain JC10247 (*srlC300*::*Tn10 recA*$^+$ *alaS3*) was used in these transductions

[b] SC30 and SC18 are *lamB*$^-$ (Mal$^-$ and insensitive to λ phage), as is the wild type B strain and its derivatives; Mal$^+$ transductants are sensitive to λ

Transduction. P1 bacteriophage was used in transductions performed according to Miller (1972).

Scoring Spontaneous Mutability. Overnight cultures were screened to eliminate "jackpots" containing large numbers of Trp$^+$ revertants by plating samples on E agar and refrigerating the cultures. Log phase cultures were inoculated only from those refrigerated cultures showing few or no Trp$^+$ revertants on the E plates after 2 days of incubation. Undiluted aliquots (2–4 × 10^7 cells) of saline suspensions of these log cultures were spread on the surface of SEM plates, and on SEM containing adenine (Ade) or cytidine plus guanosine (Cyt+Guo). Plates were incubated for 3 days at 30° C or for 2 days at 42° C and Trp$^+$ colonies were counted.

Scoring UV Mutability. Saline suspensions of log phase cells were exposed to ultraviolet radiation (UV) as described (Witkin 1974), and undiluted samples of the irradiated suspensions were plated as described for spontaneous mutability above. Survival percentage was determined by plating appropriately diluted samples on SEM agar before and after UV irradiation. The frequency of UV-induced Trp$^+$ mutations was calculated after subtracting the number of Trp$^+$ colonies appearing on plates seeded with unirradiated controls.

Induction of recA Protein Synthesis and Protein Labeling. In preparation for pulse-labeling of proteins, cultures were grown to log phase titers of 2–4 × 10^8 cells per ml in ET medium. Aliquots (2.5 ml) of log culture were or were not then given treatments capable of inducing recA protein synthesis in some strains. For UV induction, cells were exposed to 10 J/m^2 of UV. For thermal induction, cells were shaken for 35 min in a 42° C water bath. One ml of induced or uninduced log culture was added to 25 µg of a solution containing 15 tritiated amino acids (Amersham, Arlington Heights, Illinois). The mixtures were vortexed and incubated at 30° C or 42° C for exactly 5 min. Cold E medium (2.5 ml) was then added to stop label uptake, and the cultures placed on ice for 15 min, washed once in 2 ml of 0.01 M Tris HCl pH 6.8 and resuspended in 85 µl of the same buffer solution. Samples were then frozen at −20° C until needed.

Electrophoresis. Sodium dodecyl sulfate (SDS) gel electrophoresis was performed by the method of Laemmli and Favre (1973) with some modifications. Samples were thawed, mixed with an equal volume of "lysis buffer" (0.125 M Tris HCl pH 6.8, 20% glycerol, 2.5% SDS, 10% mercaptoethanol, 0.0025% bromophenol blue), and heated to 100° C for 3–3.5 min. Volumes of each sample to be applied to the gel were adjusted to equate the number of TCA-precipitable counts in each (determined by use of a liquid scintillation system). Electrophoresis was performed on a model 220 vertical slab gel apparatus (Hoeffer, San Francisco, CA). The gels contained 5.1% and 12.5% acrylamide for the stacking and running gels, respectively. Prior to addition of N,N,N′,N′-tetramethyl ethylene diamine (TEMED) and casting of the gels, the unpolymerized gel solutions were

passed through 0.45 μ type HA filters (Millipore Corp., Bedford MA) and gassed under vacuum. Electrophoresis was carried out at 20 mA per gel for the first 3 h and at 8 mA per gel for an additional 12 h, with the current held constant. Gels were fixed for one h in a solution composed of 45% methanol, 45% water and 10% acetic acid.

Autoradiography. Autoradiography was performed using the scintillator En^{3}Hance (New England Nuclear, Boston, MA). After drying on a standard slab gel dryer, the gels were used to expose X-Omat R X-ray film (Kodak, Rochester, NY) at −70° C.

Construction of Lysogenic Strains. Strains to be lysogenized were grown in NB supplemented with $MgSO_4$ (0.01 M) to log phase titers of $2–4 \times 10^8$ cells per ml. Aliquots of log cultures (0.2 ml) were mixed with 2 ml of soft agar containing 0.01 M $MgSO_4$ and 0.1 ml of an appropriately diluted stock of λBref. The mixture was poured onto NA plates, allowed to harden, and incubated overnight at 30° C. NB cultures were started with inocula taken from the center of placques, incubated overnight and streaked to obtain single colonies, which were then further purified by another round of growth in NB, streaking and single-colony isolation. Purified colonies were then tested for lysogenicity. Cytidine and guanosine were added to the NA and to the soft agar used in construction of lysogens of the SC strains.

Prophage Induction. Spontaneous inducibility of prophage in various lysogens was compared by assaying the number of free phage particles produced during incubation in MCHT for 4 h at 30° C. Cultures of the lysogenic strains were grown overnight in NB, with Cyt+Guo added, centrifuged, and the pellets washed and resuspended in the same volume of E salts. A 10^{-2} dilution was made into 10 ml of MCHT to give a titer of about 10^7 cells per ml. Adenine was added to the MCHT medium where indicated. The titer of free phage particles per ml in the medium was determined immediately, and again after 4 h of incubation with shaking at 30° C. For free phage assays, a few drops of chloroform were added to the supernatant after centrifugation of an aliquot of MCHT culture, to kill remaining cells. After overnight refrigeration, various dilutions of the chloroformed supernatant were mixed with about 10^7 indicator bacteria in 2 ml of soft agar containing 0.01 M $MgSO_4$ and the mixture poured over NA. Placques were counted after overnight incubation at 30° C.

Results

Isolation of SC Strains

In 1975 we repeated a conjugation described elsewhere (Witkin 1974) to transfer *recA441* (*tif-1*) from an F′ derivative of the K12 strain JM12 into a *thyA* variant of WP2$_s$ (Table 1). Two hundred *thyA*$^+$ recombinants, each presumably a different K12/B hybrid, were purified and tested for one facet of the Tif$^-$ phenotype: mutator activity at 42° C but not at 30° C. A reasonable proportion of the recombinants showed this property and were assumed to have received *recA441*. One recombinant, however, showed nearly as much mutator activity at 30° C as at 42° C. This anomalous recombinant later proved to express several other SOS functions constitutively, and its phenotype closely resembled that of the SOS-constitutive Spr$^-$ Tif$^-$ double mutants of K12 (Mount 1977; Pacelli et al. 1979). We considered the possibility that our SOS-constitutive (SC) recombinant had acquired a mutation in *lexA*, which, like *spr*, may have eliminated the ability of lexA protein to repress *recA*. This explanation (improbable, since both parents in the cross were *lexA*$^+$) was eliminated by transducing SC's *lexA* allele into a *lexA102 recA441* strain and obtaining typical LexA$^+$ Tif$^-$ transductants (data not shown).

Although the SOS-constitutive phenotype of the SC recombinant was reproducible, single-colony isolations made from the original SC slant yielded sublones having several different phenotypes. About 65% of the single colonies yielded stable SOS-constitutive subclones. This major class imposed the SOS-constitutive phenotype upon cultures inoculated from the slant, masking its heterogeneity. The remaining 35% of single-colony isolates yielded subclones that fell into three distinct phenotypic classes of approximately equal size. Thus, four stable subtypes were established, each breeding true and showing no further signs of instability. We assume that the original recombinant was unstable, and that the stable derivatives had segregated during the early divisions of the colony chosen to inoculate the stock slant. The four types were characterized initially according to their spontaneous mutability at 30° C and at 42° C, their UV mutability after exposure to a very low (virtually noninducing) dose of UV, and the effect on these responses of adding adenine or cytidine plus guanosine to the plating medium. (Adenine is known to promote Tif$^-$ expression, whereas cytidine and guanosine together inhibit Tif$^-$ expression (Kirby et al. 1972).) Table 2 shows typical results obtained with the four strains chosen to represent the four phenotypic classes, compared to typical *recA*$^+$ and *recA441* strains. A unique pattern of response is shown by each of the four SC strains. Most other single-colony isolates from the original SC stock slant closely resemble one or another of the four types shown in Table 2.

The Locus of SC Instability

To determine whether the genetic instability assumed to have been resolved by segregation of the four stable subclones could be localized at or near *recA*, we constructed *srlC300*::Tn*10 recA*$^+$ *alaS3* derivatives of each SC subclone, and used these as recipients in another round of transductions. Each of the recipients was infected with P1 bacteriophage grown on each of the SC subclones, and Srl$^+$ transductants were selected. Purified transductants that were also Tet^s and AlaS$^+$ were assumed to have received the *srl*$^+$ and *alaS*$^+$ markers of the donor, and therefore to have a high probability of having received, intact, the region between these closely linked genes, which includes *recA* (Bachmann and Low 1980). In each of the transductions, at least 20 Srl$^+$ AlaS$^+$ transductants were scored for mutator activity, UV mutability and response to adenine and to cytidine plus guanosine according to the protocol used in Table 2. Results obtained with a typical transductant from each of 12 of these crosses are shown in Table 3. These results show that, in each cross, the characteristic phenotype of the donor, with respect to the properties scored, are cotransducible with *srlC* and *alaS*. Of a total of 256 transductants scored in the crosses described in Table 3, only 5 retained the recipient's RecA$^+$ phenotype. All of the others resembled the donor, with minor quantitative variations that did not obscure the donor's unique pattern of response. We conclude that the genetic basis for the phenotypic differences among these SC donors is tightly linked to *srlC* and *alaS*, at or very near the *recA* locus. The "selfing" controls (Table 3) showed that transduction, per se, in this region, does not generate novel phenotypes, at least not as a frequent event. All of the "selfed" transductants had the phenotype of the donor strains.

Table 2. Spontaneous and UV-induced mutability of stable SC subclones compared to recA$^+$ and recA441 (tif-1) strains

Strain	Additions to medium	Trp$^+$ revertants per plate[a]				Comments
		No UV		UV (0.2J/m^2)		
		30° C	42° C	30° C	42° C	
SC30	–	218	432	460	444	Spontaneous and UV-induced mutability constitutively high; Tif$^-$-like
(*recA*$_{30}$)	Cyt+Guo	80	350	158	428	response to Ade and to Cyt+Guo
	Ade	486	420	532	480	
SC11	–	10	17	22	87	Relatively little enhancement of spontaneous or UV-induced mutability
(*recA*$_{11}$)	Cyt+Guo	8	12	17	20	by heat alone, but heat+Ade increase both
	Ade	12	75	28	240	
SC7	–	35	351	94	548	Strong Tif$^-$-like enhancement of spontaneous and UV-induced mutability
(*recA*$_7$)	Cyt+Guo	20	252	29	476	by heat, but Ade often *reduces* mutability at 30° C
	Ade	26	397	44	499	
SC18	–	22	15	180	106	No enhancement of spontaneous or UV-induced mutability by heat;
(*recA*$_{18}$)	Cyt+Guo	23	22	147	75	no significant effects of Ade or Cyt+Guo; UV-induced mutability
	Ade	19	24	143	101	unusually high
WP2$_s$	–	5	5	15	5	Typical *recA*$^+$ response: no enhancement of mutability by heat;
(*recA*$^+$)	Cyt+Guo	8	10	10	8	no significant effects of Ade or Cyt+Guo; UV mutability at this
	Ade	13	8	17	8	dose very low, especially at 42° C
WP44$_s$-NIT		2	55	12	190	Typical Tif$^-$ response: like *recA*$^+$ at 30° C; strong enhancement
(*recA441*)	Cyt+Guo	5	48	17	110	of spontaneous and UV-induced mutability by heat, decreased
	Ade	6	198	17	311	by Cyt+Guo, increased by Ade
RT-4	–	4	117	11	158	Another typical Tif$^-$ response
	Cyt−Guo	5	32	11	72	
	Ade	7	131	16	220	

[a] Each number is the average of four plates; data are representative of at least ten similar determinations

Synthesis of recA Protein in the SC Derivatives

Figure 1 shows an autoradiograph of an SDS-PAGE gel visualizing pulse-labeled proteins in whole-cell extracts of strain SC30 and its *recA*$^+$ and *lexA102* transductants. SC30 synthesizes recA protein at high levels whether UV irradiated or not at both 30° C and 42° C. At 30° C, the *recA*$^+$ transductant of SC30 synthesizes recA protein at high levels only after UV irradiation, whereas the *lexA102* transductant fails to increase recA protein synthesis whether UV irradiated or not. These results show that SC30 expresses high-level synthesis of recA protein, as well as mutator activity, constitutively, and they confirm that the genetic basis for this SOS-constitutive phenotype is located at or near *recA*. Furthermore, the noninducibility of recA protein synthesis and its low constitutive level in the *lexA102* transductant of SC30 indicates that the lexA protein, as modified by *lexA102*, can still bind the *recA* operator of SC30 and repress recA protein synthesis irreversibly. The *lexA102* transductant of SC30, strain SC30-LM, has a typical LexA$^-$ phenotype in other respects, as well, i.e., it is UV-sensitive and UV-nonmutable (data not shown).

Figure 2 visualizes pulse-labeled proteins in whole-cell extracts of strain SC18, compared to a *recA*$^+$ strain. SC18 synthesizes recA protein at high levels only after UV irradiation, at both 30° C and 42° C, paralleling its lack of mutator activity at both temperatures (Table 2). However, SC18 differs from typical *recA*^{1} strains in other respects, as will be shown below.

Synthesis of recA protein in SC7 and SC11 (not shown) is similar to that previously shown for *recA441* strains (Gudas and Pardee 1975): relatively low at 30° C and inducible both by UV and by incubation at 42° C.

Prophage Induction in SC Strains

Strains lysogenic for λ phage were constructed from *lamB*$^+$ transductants of SC30, SC30-RP (*recA*$^+$), SC30-TF (*recA441*) and SC18. Table 4 shows the titers of free phage particles produced by each of these strains during 4 h of incubation in MCHT, with or without adenine, at 30° C. The lysogen of SC30-MP produces as much as 10^4 times more free phage than any of the other strains, indicating that SC30's SOS-constitutive phenotype at 30° C includes constitutive expression of λ inducer. Nevertheless, SC30-MP was readily lysogenized, and its lysogen stably maintained on NA supplemented with cytidine and guanosine. Many complete media, even without Cyt and Guo, suppress Tif$^-$ expression (Castellazzi et al. 1972b), and we have found that NA and NB suppress all aspects of the SOS-constitutive phenotype of SC30 and its lysogen. Thus, cultures of SC30-MP (λ) grown in NB plus Cyt and Guo release very little phage, but prophage is rapidly induced upon transfer to MCHT.

UV-Sensivity of SC Strains

Figure 3 shows UV survival curves for the SC strains and some of their transductants. SC30 is the most resistant of the four SC strains; SC7 and SC11 fall within an intermediate group, along with the *recA*$^+$ parent, strain WP2$_s$; SC18 is unusually sensitive to UV. Transductants of SC18 and SC11 carrying the *srl-recA-alaS* region derived from SC30 are as UV-resistant as SC30 itself, and transductants of SC11 carrying the *srl-recA-alaS* region of SC18 are as UV-sensitive as SC18. Transductants of SC30 carrying the *srl-recA-alaS* region of SC18 (not shown) are nearly as UV-sensitive as SC18. Transductants of SC18 carry-

Table 3. Cotransducibility of phenotypes of strains SC30, SC11 and SC18 with *srlC* and *alaS*

Donor	*srlC300*::Tn*10* *recA*$^+$ *alaS3* recipient	Srl$^+$ AlaS$^+$ transductant tested[a]	Additions to medium	Trp$^+$ revertants per plate[b]				Phenotype of transductant
				No UV		UV (0.02 J/m^2)		
				30° C	42° C	30° C	42° C	
SC30	SC30-RP	SC30-30 ("selfed")	–	197	354	331	404	Similar to SC30 (see Table 2)
			Cyt+Guo	208	329	139	365	
			Ade	424	444	476	496	
SC30	SC11-RP	SC11-30	–	102	322	118	254	Similar to SC30 (see Table 2)
			Cyt+Guo	70	204	138	199	
			Ade	213	199	276	243	
SC30	SC18-RP	SC18-30		70	270	132	472	Similar to SC30 (see Table 2)
			Cyt+Guo	36	145	70	416	
			Ade	224	524	465	560	
SC11	SC30-RP	SC30-11		17	17	42	73	Similar to SC11 (see Table 2)
			Cyt+Guo	29	9	9	32	
			Ade	47	97	144	287	
SC11	SC11-RP	SC11-11 ("selfed")	–	18	23	21	53	Similar to SC11 (see Table 2)
			Cyt+Guo	13	12	18	27	
			Ade	23	107	56	289	
SC11	SC18-RP	SC18-11	–	21	34	46	116	Similar to SC11 (see Table 2)
			Cyt+Guo	16	20	23	33	
			Ade	34	96	220	339	
SC18	SC30-RP	SC30-18	–	13	9	106	89	Similar to SC18 (see Table 2)
			Cyt+Guo	22	10	107	75	
			Ade	19	12	113	88	
SC18	SC11-RP	SC11-18	–	17	16	163	122	Similar to SC18 (see Table 2)
			Cyt+Guo	10	22	185	111	
			Ade	32	33	163	141	
SC18	SC18-RP	SC18-18 ("selfed")	–	22	14	165	110	Similar to SC18 (see Table 2)
			Cyt+Guo	17	23	172	101	
			Ade	15	19	144	88	

[a] Data shown are from a typical transductant from each cross; at least 90% of Srl$^+$ AlaS$^+$ transductants in each transduction showed a pattern of response similar to the one described

[b] Each number is the average of four plates

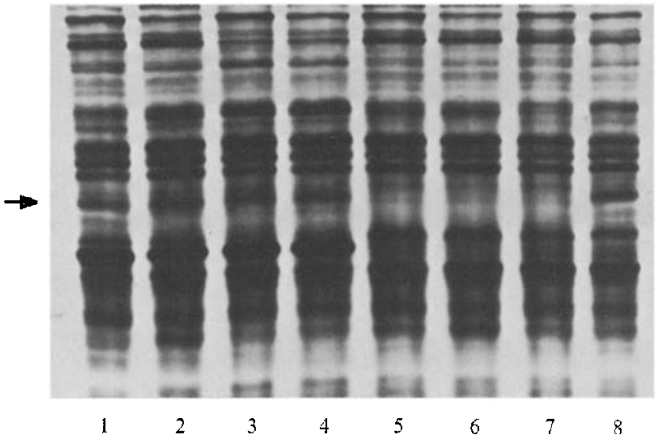


Fig. 1. Autoradiogram of proteins labeled with tritiated amino acids in extracts of strain SC30 and its *lexA102* and *recA*$^+$ transductants. Lanes 1–4, SC30; lanes 5,6, SC30-LM (*lexA102*); lanes 7,8, SC30-RP (*recA*$^+$). Lanes 3,4, 42° C, all other lanes, 30° C. Odd lanes, no UV; even lanes, labeled after UV irradiation. Arrow indicates position of recA protein

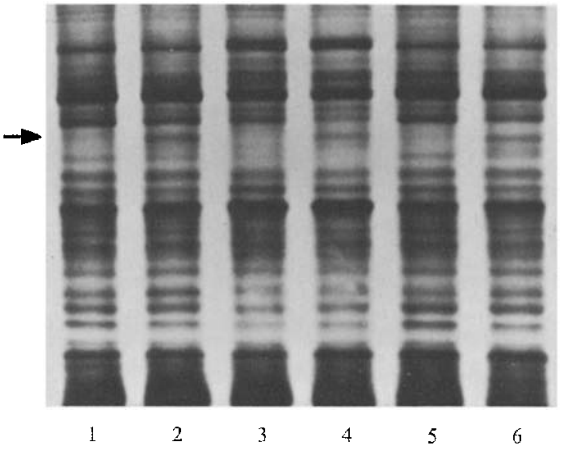
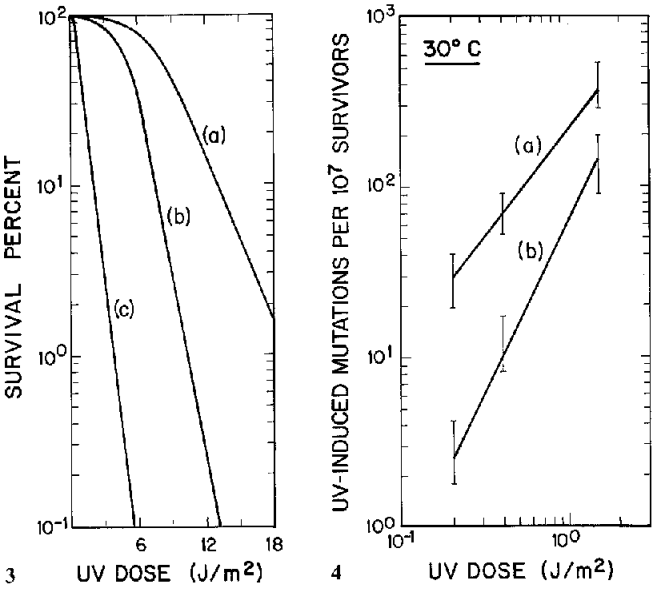


Fig. 2. Autoradiogram of proteins labeled with tritiated amino acids in extracts of strains SC18 and SC30-RP (*recA*$^+$). Lanes 1–4, SC 18; Lanes 5, 6, SC30-RP. Lanes 3, 4, 42° C, all other lanes, 30° C. Odd lanes, no UV; even lanes, labeled after UV irradiation. Arrow indicates position of recA protein

Table 4. Spontaneous release of λ bacteriophage by various lysogenic strains[a]

Strain	*recA* allele	Free phage particles per ml after incubation in MCHT for 4 h at 30° C	
		− Ade	+ Ade
SC30-MP (λ)	($recA_{30}$)	4.2×10^9	6.2×10^9
SC30-RP-MP (λ)	$recA^+$	7.2×10^4	9.8×10^4
SC30-TF-MP (λ)	*recA441*	7.1×10^4	1.0×10^6
SC18-MP (λ)	($recA_{18}$)	2.1×10^4	2.6×10^4

[a] See Material and Methods for details of procedure; Cultures had between 2.1 and 3.3×10^7 cells per ml at the start of the incubation in MCHT; Free phage titer of the MCHT cultures at the start was 3×10^5 particles per ml for SC30-MP (λ) and 2×10^3 per ml or lower for the other strains



3 4

Fig. 3a–c. UV survival curves of SC strains and some related strains at 30° C. **a** SC30, SC18-30 and SC11-30; **b** SC7, SC11, SC18-RP and $WP2_s$; **c** SC18, SC11-18

Fig. 4a, b. UV-induced Trp^+ mutation frequencies at 30° C in SC strains and some related strains. **a** SC30, SC18; **b** SC11, SC30-RP, SC18-RP, $WP2_s$, $WP44_s$-NF

ing $recA^+$ show the intermediate UV-sensitivity of group b. It is evident that the *srl-recA-alaS* regions of SC30, SC18 and SC11 differ from each other in one or more ways that influence UV sensitivity.

UV Mutability of SC Strains

Figure 4 shows frequencies of UV-induced Trp^+ mutations at 30° C in some SC strains and their $recA^+$ and *recA441* transductants. The data fall within two distinct classes. The more UV-mutable group (a) includes SC30 and SC18, the most UV-resistant and the most UV-sensitive, respectively, of the SC subclones (Fig. 3). The less UV-mutable group includes SC11 and several $recA^+$ and *recA441* strains, including transductants of SC30. We conclude that the *srl-recA-alaS* regions of SC30, SC11 and SC18 differ from each other, and that those of SC30 and SC18 differ also from the corresponding regions of $recA^+$ and *recA441* strains, in one or more ways that influence UV mutability.

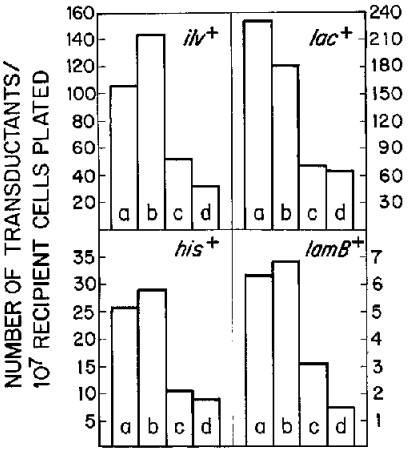


Fig. 5a–d. Frequency of P1 transductants selected for four markers at 30° C in strain $WP44_s$-MM (*recA441*) and its transductants carrying different *recA* alleles. **a** $WP44_s$-MM-30 ($recA_{30}$); **b** $WP44_s$-MM (*recA441*); **c** $WP44_s$-18 ($recA_{18}$); **d** $WP44_s$-MM-RP ($recA^+$). P1 was grown on strain $WP2_s$

Recombination Proficiency Promoted by the recA Alleles of SC30 and SC18

The multiauxotrophic strain $WP44_s$-MM-RP was used as a recipient in transductions with P1 phage grown on SC30 and SC18, with selection for Srl^+. A Tet^s $AlaS^+$ transductant from each cross, showing the SOS phenotype of the donor, was chosen for use in these experiments, along with otherwise isogenic $recA^+$ and *recA441* strains. The four recipients were infected with the same P1 stock, and in each cross four widely spaced donor markers were selected. Figure 5 shows that the yield of transductants was 2–5 times greater, for each marker selected, in recipients carrying ($recA_{30}$) or *recA441* than in recipients carrying ($recA_{18}$) or $recA^+$. The four recipient strains were equally capable of adsorbing P1 phage, and showed differences even greater than those shown in Fig. 5 in the yield of recombinants selected for two of the same markers in conjugation with an Hfr donor (data not shown). The results suggest that *recA441* and ($recA_{30}$) promote genetic recombination more actively than $recA^+$ or ($recA_{18}$).

Discussion

A rare unstable recombinant in a cross designed to transfer *recA441* from K12 to a B/r derivative gave rise to four stable subclones, each of which expresses SOS functions anomalously in a distinctive way. When the chromosomal region that includes *recA* was transferred by P1 transduction from each of these strains into each of the others, the essential features of the donor's unique SOS phenotype were also transferred to most of the transductants. We conclude that the genetic basis for the differences in SOS expression within this group of strains is located in or very near *recA*. Since three of the four SC subclones share some properties with the Tif^- parent (e.g., SOS expression enhanced by temperature elevation and/or adenine, and diminished by cytidine plus guanosine), it is likely that the original unstable recombinant received the *recA441* allele from the K12 parent, albeit with some sort of alteration in or near the gene that caused local genetic instability.

The nature of the perturbation that generated the instability of the original recombinant is unknown. In conjugation, tandem

duplications occur rather frequently, often yielding unstable recombinants that segregate stable subclones (Anderson and Roth 1977). The nucleotide sequence of the *recA* gene in K12 (Sancar et al. 1980) contains several direct and inverted repeats, which might increase the probablity of unequal exchanges. Furthermore, the cross in question mated a K12 donor with a B/r recipient, and it is possible that interstrain differences in the *recA* sequence may have enhanced the probability of generating an unstable recombinant. However, any gross difference in the sequence of the *recA* structural gene between K12 and B/r is unlikely, since their respective recA$^+$ proteins comigrate in 2-dimensional gel electrophoresis (Volkert et al. 1981).

One of the stable subclones described, SC30, expresses SOS functions constitutively at 30° C, including mutator activity, elevated UV mutability, spontaneous induction of prophage and synthesis of recA protein at high levels. A similar SOS-constitutive phenotype is expressed by the K12 Spr$^-$ Tif$^-$ double mutant (Mount 1977; Pacelli et al. 1979). SC30 and the Spr$^-$ Tif$^-$ k12 strain also show the same response in tests for constitutive expression of another SOS function, stable DNA replication (Kogoma et al. 1979). Despite their phenotypic similarity, the two strains clearly differ in the genetic basis for their SOS-constitutivity. The *spr* mutation, which maps in *lexA* (Mount 1977; Pacelli et al. 1979), eliminates the ability of lexA protein to repress *din* genes, even in *recA*$^-$ strains (Bagg et al. 1981), obviating the need for recA protease activity to derepress these genes. However, *spr* strains express all SOS functions constitutively only if they also carry *recA441*, suggesting that proteolytically active recA protein promotes SOS expression not only by cleaving lexA protein, but perhaps by cleaving other repressors (as in λ prophage induction) or by processing proteins necessary for some facets of SOS expression (Bagg et al. 1981). SC30 is *lexA*$^+$, and therefore does not code a defective *din* repressor. The SOS-constitutivity of SC30 depends upon a genetic singularity in or near *recA* that causes constitutive high-level synthesis of recA protein. Since overproduction of recA$^+$ protein does not cause SOS expression, we assume that SC30 codes a proteolytically active recA protein, identical or similar to the one coded by *recA441*, and that copious synthesis of this protein is a sufficient condition for derepression of other SOS genes and for constitutive expression of SOS functions.

Three types of changes at the *recA* locus could account for SC30's consitutive synthesis of recA protein: (1) an operator alteration that reduces binding by lexA$^+$ protein (but not by lexA$^-$ protein, since *lexA102* transductants of SC30 have the LexA$-$ phenotype, including noninducible low-level synthesis of recA protein); (2) an "up-promoter" alteration that increases the baseline rate of *recA* transcription, or (3) a change in the structural gene that increases the proteolytic activity of SC30's recA protein above that of recA441 protein at 30° C. Our present information does not distinguish among these possibilities, which we hope to resolve by additional genetic and biochemical studies. Another possibility, of course, is a rearrangement outside *recA* but very close to it that increases the rate of *recA* transcription indirectly, e.g., via a local change in DNA helicity.

Strain SC18 shows no Tif$^-$-like response to temperature elevation, and therefore probably codes a recA protein that is not proteolytically active at 42° C, unlike that produced by the other three SC subclones. However, SC18 does not have a RecA$^-$ phenotype, since it is UV sensitive (Fig. 3) and yields a much higher frequency of UV-induced mutations than *recA*$^+$ strains, including its own *recA*$^+$ transductants (Fig. 4 and data not shown). In Uvr$^-$ strains such as SC18, unusual UV sensitivity is usually associated with defective postreplication repair, which requires recA protein function. Although SC18 increase recA protein synthesis in response to UV (Fig. 2) and shows about the same recombination proficiency in transduction as *recA*$^+$ strains (Fig. 5), it could be partially deficient in postreplication repair, if the quantity of recA protein induced by UV is suboptimal, or if its structure is modified so as to reduce its efficiency in recombinational repair. The elevated UV mutability of SC18 could indicate that error-prone repair of DNA is more active, relative to recombinational repair, than in normal *recA*$^+$ strains. The phenotype of SC18 is contransducible with *srl* not only to other SC subclones (Table 3), but also to other more distantly related strains (data not shown). Analysis of the structure of the recA protein and of the *recA* gene sequence of SC18 may reveal the molecular basis for its unusual phenotype.

We have shown (Table 3) that a given SC donor's phenotype is inherited by most of the transductants regardless of the SC strains used to construct the Srl$^-$ AlaS$^-$ recipient. This implies that the SC subclones share a common genetic background except in the *recA* region, or at least that they do not differ elsewhere in ways that significantly influence the pattern of mutability. This is as expected if the SC subclones are segregants from a single recombinant in which genetic instability was limited to the *recA* region.

The four SC subclones are not only genetically different from each other in the *recA* region, but at least three of them (SC30, SC7 and SC18) are also genetically distinguishable, in this region, from several Tif$^-$ strains presumably carrying *recA441*. This follows from a series of transductions (to be described elsewhere) in which *recA441* was transduced into SC30-RP from several Tif$^-$ donors, including K12 strain JM12. Most of the transductants had a distinctive phenotype, different from that of the Tif$^-$ donor, as well as from SC30, SC7 and SC18, in that SOS functions were not expressed at 42° C unless adenine was present. The *recA441* allele itself had probably not been changed, since transductional backcrosses restored the typical Tif$^-$ phenotype of the donor. Tif$^-$ expression has been found to be highly variable among recombinants obtained in conjugation between K12 and B/r (Witkin and Kirschmeier 1978). Such variation may evidently be due either to differences in or near *recA* or to the interaction of different hybrid genotypes with an unaltered *recA441* allele, or both. Among those genetic differences likely to modify Tif$^-$ expression could be any affecting availability of the single-stranded polynucleotide and nucleoside triphosphate cofactors required for proteolytic activity of recA protein (Craig and Roberts 1981).

Our finding (Fig. 5) that genetic recombination in one-point transductions is significantly more active in *recA441* and ($recA_{30}$) strains than in those carrying *recA*$^+$ or ($recA_{18}$) is not in accord with the results of Lloyd (1978), who found enhanced frequencies of multiple exchanges in *tif*, *spr* or *tif spr* strains but no elevation of recombination frequency in one-point crosses. In contrast, Castellazzi et al. (1972a) found no evidence for Tif$^-$ enhancement of genetic recombination in Red$^-$ λ phage. Genetic background differences in the strains used may account for these varied results.

Although the main features of the regulation of recA protein synthesis and of some other DNA damage-inducible functions are now reasonably well understood, little is known of the processes whereby their quantitative expression is modulated. Further study of the SC strains may suggest some ways in which such fine-tuning can be accomplished.

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