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MOLECULAR MECHANISMS OF MUTAGENESIS AND CARCINOGENESIS

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ABSTRACT

The *recA* gene product mediates induction of a battery of cellular functions in *E. coli* collectively known as the SOS inducible functions. These include prophage induction, induction of filamentous cell growth, induction of DNA repair systems and induction of the *recA* gene itself. Prophage induction has been demonstrated to result from cleavage of the phage repressor protein by a specific protease function of the *recA* protein. Such cleavage destroys repressor function in lysogenic cells and allows the prophage to enter the vegetative growth cycle. Normally, the protease function of *recA* protein only becomes active in the cell in response to signals produced by agents (carcinogens) or treatments that specifically damage DNA or interfere with DNA replication. In the *tif*-1 mutant of *recA*, however, the protease can be made to be active simply by raising the temperature of the culture from 30° to 40° C; λ prophage is induced and the cell produces a normal burst of phage. Indeed, all known SOS functions are also induced under these conditions. Thus, it has been proposed that all SOS functions are similarly regulated by repressors that are sensitive to the *recA* protease function. We have sought to further characterize the *recA*-mediated cleavage reaction and its requirements for λ repressor *in vitro* and to extend our biochemical studies to a cellular SOS repressor, the *lexA* gene product which is proposed to negatively regulate several SOS inducible functions, including the *recA* gene, a gene controlling cell septation and the *lexA* gene itself.

ISOLATION OF NEW *tif* MUTANTS

The activation of the *recA* protease function toward λ repressor *in vitro* has been the object of studies in several laboratories. Because *recA* protease function in the *tif*-1 mutant is activated *in vivo* at 42° C in the absence of classical inducing treatments, we have shifted our emphasis in this area to attempts to understand the basis of such mutational activation. The goal has been to isolate new *tif*-like mutants in systems designed to provide opportunity for obtaining a variety of such mutants, especially with regard to strength and substrate specificity of the protease function. The general basis of the selections has been the spontaneous inactivation of λ repressor *in vivo*. The study utilized the λp*recA* specialized transducing phage in which the *recA* gene has been substituted for the non-essential *b* region in the phage (see Figure 1). The *recA* gene on the phage is regulated by its natural promoter and not by any phage promoter. The concept behind the selection was that if λp*recA* bore a *tif*-like mutation, then the phage should form plaques on a homoimmune lysogen since as it enters the cell, it should inactivate λ repressor and develop normally. The system was devised using the *tif*-1 derivative of λp*recA*c*I* which was found to exhibit the desired "virulent" phenotype only under a special set of conditions, namely, on a *spr*$^-$ *sfi*$^-$ *recA*Δ(λKB1) host. The *spr*$^-$ mutation inactivates the *lexA* gene product, the proposed repressor of the *recA* gene, and permits constitutive *recA* gene expression from the phage soon after infection; the *recA* deletion blocks synthesis of *recA* gene product from the host, thereby eliminating possible inhibitory co-dominant effects on the phage-encoded *recA* product; the *sfi*$^-$ (suppressor of filamentation) mutation permits survival of *spr*$^-$ cells which would otherwise filament and die. The phage itself must bear a mutation in the *c*I gene encoding phage repressor to prevent any contribution to repressor synthesis by the phage. λ repressor is supplied by the λKB1 prophage (see Figure 2) in which the *c*I gene of λ is under the control of the *lac* promoter. This enables the level of λ repressor in the lysogen to be experimentally regulated with IPTG (an inducer of the *lac* operon) independently of the elements normally controlling λ repressor synthesis. The level of λ repressor in such lysogens maximally stimulated with IPTG is about 40% of the monolysogen level (Backman and Ptashne, 1978). Under these conditions, λp*recA*$^+$*c*I$^-$ do not form plaques. However, within stocks of λp*recA*$^+$*c*I$^-$ (unmutagenized), plaque-forming mutant derivatives which resemble λp*tif*-1*c*I$^-$ can be detected at a frequency of about 1-2x10^{-6} (see Table).

Since *E. coli* mutants carrying certain other alleles of the *lexA* gene (e.g., *lexA*3) produce a *lexA* product whose function cannot normally be inactivated by *recA* protease, the efficacy of the above system was initially upheld by (a) failure of the mutant subpopulation within the λp*recA*$^+$ phage stock to form plaques at any detectable frequency on the *lexA*3 version of the λKB1 lysogen and (b) by the failure to detect a virulent subpopulation

in the λ*lexB*30*c*I^{-1} phage stock when plated either on the *spr*$^-$ or the *lexA*3(λKB1) lysogen (see Table). Four additional genetic tests confirmed the presence of *tif*-like mutations in the virulent derivatives of λ*precA*$^+$*c*I$^-$:

(a) In a genetic backcross system, the immunity region bearing the *c*I gene (*imm*λ) of each of 87 separate isolates was replaced first with the analogous immunity region from phage 21 (*imm*21) and then with the *imm*λ*c*I$^-$ region from the parent. Each backcrossed isolate was demonstrated to have retained the ability to form plaques on the λKB1 lysogen. This confirms that virulence does not result from mutations in the immunity region, e.g., operator or *c*I transdominant mutants. The backcrossed derivatives of the *tif*-1 and *recA*$^+$ control phage strains retained their respective phenotypes.

(b) λp*tif*-1*c*I$^-$ fails to form plaques on the *lexA*3(λKB1) lysogen (see Table) suggesting that the hyperactivity of the *tif* form of the *recA* product is controlled on the phage as it normally is in the host chromosome. Each new *tif* mutant isolate (87/87) behaves similarly (but see below).

(c) λ$^+$ and λ*precA*$^+$*c*I$^+$ (*imm*λ, *imm*21, or *imm*434) form normal turbid plaques on the *spr*$^-$ *sfi*$^-$ *recA*Δ strain. However, λp*tif*-1*c*I$^+$ of each immunity type forms clear plaques on this strain as do the *c*I$^+$ derivatives of each of the 87 new mutant isolates. Clear plaque formation by the *tif* mutants presumably occurs through continuous *tif*-mediated inactivation of repressor synthesized during phage development. The *cInd*$^-$ mutation of *imm*λ or *imm*434 block the clear plaque phenotypes of all the *tif* mutant isolates. Interestingly, only 10% of the new λp*tif* isolates form turbid plaques characteristic of λp*tif*-1*c*I$^+$ plaque formation on the *lexA*3 nonlysogen. The remainder which form clear plaques on the *lexA*3 host are considered to bear alterations which permit greater protease activity than *tif*-1 or the other new isolates.

(d) The *spr*$^-$*sfi*$^-$*tif*-1 allele combination in the *E. coli* chromosome causes infecting *c*I$^+$ phages (*imm*λ, *imm*21, *imm*434, *imm*22, etc.) to form clear plaques; the *cInd*$^-$ derivatives form turbid plaques (Mount, 1977). This host phenotype can be mimicked in the *spr*$^-$*sfi*$^-$*recA*Δ strain if made lysogenic with λp*tif*-1 (maintained by *cInd*$^-$ on the prophage or by a plasmid which overproduces homoimmune repressor). Nine new *tif* mutant phages screened in this manner each reproduce the phenotype whereas the *recA*$^+$ parental phage does not.

No direct test has been performed to demonstrate that the mutations specifically lie within the *recA* gene. However, the similarity of their phenotypes to the λp*tif*-1 phage (sensitivity to the *lexA*$^-$ control and absence of "virulent" mutations in the immunity region, together with their plating behavior) make it quite unlikely that the mutants comprise some unknown class of virulent phage types whose mutations are unrelated to *recA* gene

expression and which are also not observed in stocks of the λ*c*I$^-$ or λp*lexB*30*c*I$^-$ control phages.

In a second scheme, the *spr*$^-$ allele of the host has been substituted by the *lexA*3 allele to reduce the level of *recA* gene expression from the phage. Neither λp*recA*$^+$*c*I$^-$ nor λp*tif*-1*c*I$^-$ forms plaques on this host induced with IPTG (see Table). However, if each phage is first grown on the *mutD* strain of *E. coli* (which increases the level of mutants in the phage stock by about 10^4; see Degnen and Cox, 1974), plaque-forming derivatives of each phage were obtained (at frequencies of about 10^{-8} and 10^{-6}, respectively, for *recA*$^+$ and *tif*-1 forms of the phage). Analysis of eighteen such isolates from the *recA*$^+$ parent reveals the presence of strong *tif*-like mutations. These mutants, interestingly, all retain specificity for *ind*$^+$ repressor. Judging from the frequency of occurrence and accounting for the 10^4-fold increase in the level of mutagenesis imposed by the *mutD* strain, the derivatives of the *recA*$^+$ phage are almost certainly double mutants. They could be of three types: (a) mutants bearing multiple *tif*-like mutations that together greatly enhance the protease activity of *recA* in the absence of high level *recA* gene expression from the phage, (b) mutants bearing a single *tif* mutation plus a separate mutation in the putative operator region (see below) to allow the elevated level of *recA* gene expression from the phage, or (c) mutants bearing single or multiple *tif*-like mutations which confer upon the protease the capacity to inactivate the *lexA*$^-$ product which should also lead to increased expression of *recA* from the phage. The mutants deriving from the *tif*-1 parent could also be of the above general types. The three types are being distinguished genetically.

The continuing study of the new *tif*-like mutants will attempt to further classify the isolates based on other genetic parameters such as temperature sensitivity, genetic map location, recombinational groups, dominance and activity toward λ repressor and other SOS repressors as well as toward their mutant forms. The subsequent biochemical study of the isolates will involve DNA sequence analysis of the mutants and analysis of the possible differences in protease activity of the mutant *recA* proteins and their requirements for such activity in the *in vitro* cleavage reaction.

ISOLATION OF *recA* OPERATOR-CONSTITUTIVE MUTANTS

Inactivation of the *lexA* product leads to constitutive high level expression of the *recA* gene. The *lexA*3 form of the *lexA* protein prevents normal inactivation of *lexA* repressor function and therefore induction of SOS functions following exposure to classical inducing agents. Such cells are extremely sensitive to agents that damage DNA (e.g., UV). We have attempted in a variety of systems to isolate *recA* mutants affected in negative control by the *lexA*3 mutant protein hypothesizing that *recA* operator constitutive mutants should be one subclass of isolates obtained. The method that has provided mutants that exhibit "induced" phenotypes in the presence of the *lexA*3 protein is the second selection scheme described for isolation of new *tif*-like mutants (see above). In addition to exhibiting substantial repressor inactivating capacity, the mutants, unlike their parents, confer enhanced UV resistance upon a *lexA*3 host (lysogenic for the noninducible λind^- prophage to prevent vegetative growth of the isolates) suggesting that the elevated level of *recA* protein thought to be required for DNA repair can be exhibited by the mutants. Mutants identified genetically as bearing alterations in the site of *lexA* binding (operator mutants) will be analyzed by DNA sequencing techniques and studied in a purified *in vitro* system containing purified *lexA* protein to demonstrate the effect of the mutations on the function of the putative *recA* operator.

FOOTNOTE

[1]The *lexB*30 allele of *recA* has been demonstrated to inactivate *recA* protease function or some function of the protein required for activation of the protease.

TABLE

Plating character of λ*precA* and derivatives.

Phage Strains	Efficiency of Plating on Lysogens of λKBl + IPTG	
	spr$^-$	*lexA*3
λp*tif*-1*c*I$^-$	1.0	$<10^{-9}$
λp*recA*$^+$*c*I$^-$	$1\text{-}2\times10^{-6}$	$<10^{-9}$
λp*lexB*30*c*I$^-$	$<10^{-9}$	$<10^{-9}$
λ*c*I$^-$	$<10^{-9}$	$<10^{-9}$
λ*imm*434*c*I$^-$	1.0	1.0

FIGURE 1

λp*recA* (see McEntee and Epstein, 1977).

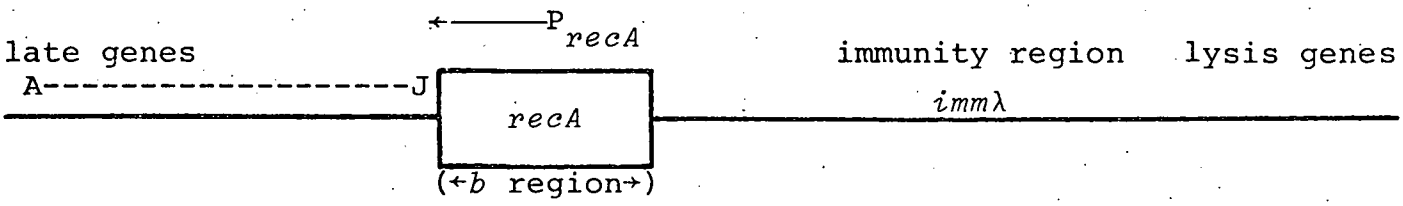
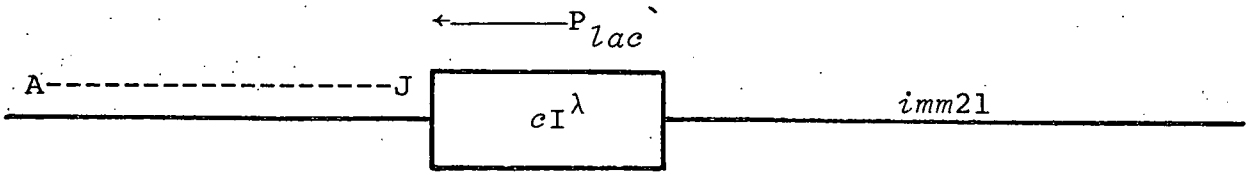


FIGURE 2

λKB1 (see Backman and Ptashne, 1978).



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