



Genetic control of damage-inducible restriction alleviation in *Escherichia coli* K12: an SOS function not repressed by *lexA*

Brigitte Thoms and Wilfried Wackernagel

Universität Oldenburg, Fachbereich Biologie, AG Genetik, Ammerländer Heerstr. 67–99, D-2900 Oldenburg, Federal Republic of Germany

Summary. The alleviation of K-specific DNA restriction after treatment of cells by UV or nalidixic acid has been studied in mutants with various alleles of *recA* and *lexA* and combinations of these alleles and with *recB* and *recF* mutations. The studies show that induction of restriction alleviation by UV or nalidixic acid is abolished in mutants in which the *recA* protein is defective (*recA13*, *recA56*), its protease activity is altered (*recA430*) or in which it cannot be efficiently activated (*recA142*). Thermoinduction of restriction alleviation was observed in *tif* mutant (*recA441*). In *lexA* amber mutants restriction alleviation is not constitutive but is still inducible. In a *lexA3* mutant restriction alleviation is inducible by nalidixic acid provided that *recA* protein is overproduced as a result of a *recA* operator mutation. Induction by UV depends on the *recF* function and an unidentified function (Y) which is controlled by the *lexA* protein. The *recBC* enzyme is necessary for induction by UV or by nalidixic acid. Temperature shift experiments with a thermosensitive *recB* mutant indicate that the *recBC* enzyme functions in an early step during UV-induction. It is concluded that the damage-inducible function which alleviates restriction is similar to other damage inducible repair (SOS) functions in the dependence on activated *recA* protease for induction, but that it differs from these functions by the absence of a direct control through the *lexA* repressor.

Introduction

In *Escherichia coli* more than twelve cellular functions are switched on after the chromosome has been damaged (e.g. by UV-irradiation or other mutagens) or by treatments that block replication (e.g. antibiotics such as nalidixic acid) (Radman 1975; Witkin 1976; Little and Mount 1982). Since the damage-inducible functions are believed to aid in improving the survival of cells and of prophages, they have been called collectively SOS functions. They include increased and mutagenic DNA repair, inhibition of cell division, induction of prophages and partial alleviation of the K-specific DNA restriction (Little and Mount 1982). Genetic and biochemical evidence has accumulated which allows a description of the mechanism of SOS induction by a series of events following the halting of DNA replication (Gudas and Pardee 1977; Emmerson and West 1977; McPartland

et al. 1980; Little et al. 1980; Little et al. 1981; Horii et al. 1981; Brent and Ptashne 1981). According to the current model the major steps are the following: firstly a signal is generated from the blocked replication fork, the signal then activates the *recA* protein to make it a specific protease and this protease in turn cleaves the *lexA* protein which is the repressor of the *recA* gene (resulting in increased *recA* protein production) and of other SOS functions. Prophage repressors are also cleaved leading to prophage induction.

The phenomenon of restriction alleviation (RA) is the partial release of the K-specific DNA restriction in *E. coli* K12 following UV-irradiation of the cells (Day 1977). RA can be monitored most easily by measuring the increased survival of unmodified phage λ in treated cells. The absence of RA induction in *recA* and certain *lexA* mutants (Day 1977), the requirement for the novo protein synthesis and sufficient time for expression (Thoms and Wackernagel 1982, 1983) and the inducibility by nalidixic acid and mitomycin C (Thoms and Wackernagel, unpublished results) suggest that RA is an SOS function. Since UV-induction of RA requires functional *recBC* enzyme (Thoms and Wackernagel 1982) this process must be different from the UV-induction of *recA* protein synthesis which is independent of *recBC* (Bockrath and Hanawalt 1980; McPartland et al. 1980). For this reason and to establish the genetic determinants necessary for induction of RA we have examined the effects of various alleles of *recA*, *lexA*, *recB* and *recF* and of combinations of these on the induction of RA by UV and nalidixic acid. Different pathways for the generation of SOS signals have been proposed for these two agents (McPartland et al. 1980). Our results indicate that the genetic control of the RA function differs from the regulation of other SOS functions by the absence of a direct control by the *lexA* repressor.

Materials and methods

Bacterial and phage strains. The strain *E. coli* C (from G. Bertani) was used for preparation of unmodified λ CI857. Table 1 lists *E. coli* K12 strains, those constructed for this work were made by P1 transduction or conjugation following published procedures (Taylor and Trotter 1967). The *recA430* allele (from GY3448; Morand et al. 1977) transduced into AB1157 was identified in the transductants by their UV sensitivity and by the inability to induce λ^+ in a lysogenic derivative by UV. In a *lexA55* background

Table 1. *Escherichia coli* K12 strains (F⁻)

Strain designation	Relevant genotype ^a	Reference, derivation or source
AB1157	<i>rec</i> ⁺	Howard-Flanders and Boyce (1966)
AB2463	<i>recA13</i>	Howard-Flanders and Boyce (1966)
WA437	<i>recA430</i>	This work; as AB1157
JC4728	<i>recA142</i>	Clark (1973)
DM2211	<i>recAo98</i>	Ginsburg et al. (1982)
DM49	<i>lexA3</i>	Mount et al. (1972)
DM2001	<i>lexA55 recA441 sfiA11</i>	Pacelli et al. (1979)
AB2470	<i>recB21</i>	Howard-Flanders and Boyce (1966)
WA500	<i>recC22</i>	This work; from KL187 (B. Low)
JC7620	<i>recB21 recC22 sbcB12</i>	Kushner et al. (1971)
JC9239	<i>recF143</i>	Horii and Clark (1973)
JC11846	<i>recAo281 recF143</i>	A.J. Clark
WA457	<i>lexA55 recA430 sfiA11</i>	This work
WA449	<i>lexA55 recA56 sfiA11</i>	This work
WA444	<i>lexA55 recB21 sfiA11</i>	This work
WA510	<i>lexA55 recF143 sfiA11</i>	This work
DM2210	<i>lexA3 recAo98</i>	Ginsburg et al. (1982)
WA512	<i>recB21 recC22 recF143</i>	This work
SK119	<i>recB270</i>	Kushner (1974)

^a Details of the other genetic markers and the construction of strains are available on request

(DM2001; Pacelli et al. 1979) various Rec alleles were identified as follows: *recA56* by UV-sensitivity and recombination deficiency in a bacterial cross; *recA430* as noted above; *recB21* by UV sensitivity and the absence of recBC enzyme activity in crude extracts (Wackernagel and Hermanns 1974); *recF143* by slight UV sensitivity and the fact that the *recF143* allele could be transduced out of the double mutant by P1. Cotransductions were: *recA56 srlC300::Tn10* (Csonka and Clark 1980), *recA430 cysC43*, *recA430 srlC300::Tn10*, *recB21 thyA*, *recF143 pyrE*. Surprisingly the mutant *recB21 recC22 recF143* (strain JC3881 obtained from A.J. Clark) showed no K-specific phage restriction. We assumed that the reason for this might be an undiscovered mutation at the *hsd* locus. We crossed the strain with a prototrophic HfrC strain (transfer: *pro*, *leu*, *thr*) selecting for *pro*⁺ *leu*⁺ *thr*⁺ recombinants. One of the rare exconjugants had retained the arginine auxotrophy and high UV sensitivity of JC3881 but showed normal restriction of λ C. This strain (WA512) was used for studies on RA induction.

Media and plating conditions. Complete media (TBY) for cell growth and for the plating of phage λ were as described (Wackernagel 1972). Minimal medium (M⁺) was supplemented with 50 μ g/ml of histidine, proline, arginine, isoleucine, valine, threonine and leucine, 0.5 μ g/ml of thiamine and 10 μ g/ml of thymine. Growth of cultures, preadsorption of phage to bacteria (15 min), treatment of cells with nalidixic acid and incubation of plates were at 30° C except where indicated.

Treatment of cells with UV and nalidixic acid. Cells were irradiated with UV at room temperature as previously de-

scribed (Thoms and Wackernagel 1982). During post-irradiation incubation cells were aerated in complete medium at 30° C. Cells were treated with nalidixic acid by adding a sample from a concentrated stock solution (1 mg nalidixic acid/ml; Sigma Chemicals, Missouri) to a log-phase culture in complete medium to give a final concentration of 10 μ g/ml. The medium was buffered by 50 mM KPO₄, pH 7.0. The culture was aerated at 30° C during treatment. Samples from this culture were used directly to plate phage λ . Nalidixic acid was diluted during plating to less than 0.1 μ g/ml, a concentration that does not affect cellular or phage growth.

RA assay. RA was determined with unmodified phage λ as described (Thoms and Wackernagel 1982) except that all incubations were at 30° C. The restriction alleviation factor (RAF) is the efficiency of plating of λ C on induced cells relative to that on uninduced cells.

Determination of the recBC enzyme activity. The exonuclease activity on duplex DNA was determined in crude cell extracts. These extracts were prepared by a lysozyme-Brij 58 method at 30° C as described (Wackernagel and Hermanns 1974). The protein content was determined by staining with amido-black (Heil and Zillig 1970).

The reaction conditions for the recBC enzyme assay were 50 mM Tris-HCl, pH 7.5, 10 mM MgSO₄, 10 mM 2-mercaptoethanol, 10% glycerol, 0.5 mg/ml bovine serum albumin and 60 mM KCl. In a total volume of 0.5 ml, crude extract (0 to 20 μ l) and 10 nmole nucleotide of P22 DNA (labelled with [³H] thymidine; specific radioactivity 2.3 \times 10⁶ cpm/ μ mole nucleotide) were incubated for 30 min at 30° C in the presence and absence of 0.3 mM ATP. Acid-soluble products were quantified as described (Wackernagel and Hermanns 1974). One unit of recBC enzyme is defined by the ATP-dependent release of 1 nmole acid-soluble material from native P22 DNA in 30 min at 30° C.

Results

Induction by nalidixic acid

Nalidixic acid at 10 μ g/ml blocks replication in AB1157 efficiently and induces RA with maximum expression after 90 to 120 min. Figure 1 shows that the induction depends on the pH of the growth medium, a maximum being observed at pH 7.0. Induction by UV does not show such a pH profile. Furthermore, the final extent of RA was gradually reduced when the temperature was increased during the treatment of the cells with nalidixic acid (Fig. 2). The temperature sensitivity was also noted in other *E. coli* strains and after UV-induction (results not shown). Temperature-shift experiments showed that RA was blocked by incubation at 42° C after induction (by UV) had occurred at 30° C. This indicated that the expression of RA rather than the induction is a heat-sensitive process and so, essentially all experiments reported here were performed at 30° C and cultures for nalidixic acid induction were buffered by 50 mM KPO₄, pH 7.0. Temperature-sensitive expression of RA had previously escaped detection (Thoms and Wackernagel 1982) when the temperature of the post-induction incubation was not strictly controlled (indicator plates with a soft agar layer poured at room temperature were subsequently placed in a 42° C incubator). A spontaneous mu-

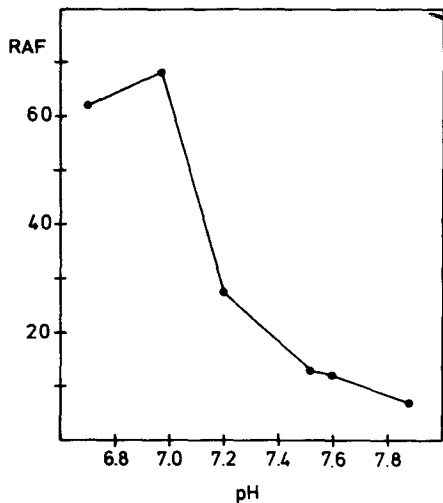


Fig. 1. Dependence of RA induction by nalidixic acid on the pH of the growth medium. Log-phase cells of AB1157 were induced in a complete medium buffered with 50 mM KPO_4 at various pH values

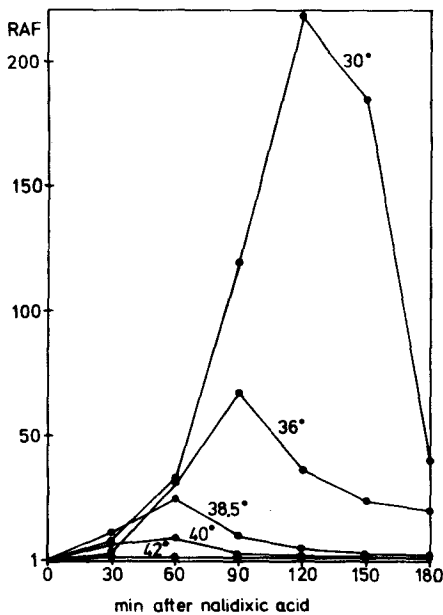


Fig. 2. Induction of RA at various temperatures. Log-phase cells of AB1157 grown at 30°C were induced with nalidixic acid and simultaneously shifted to the temperatures indicated. For the determination of RA factors preadsorption of λC and incubation of plates were at the respective temperatures

tant resistant to nalidixic acid was no longer inducible by this drug, whereas UV-induction of RA was unaffected.

RecA protease is required for induction

We have compared RA induction by nalidixic acid with UV-induction in mutants with various alleles of genes presumed to be involved in the regulation of SOS induction. Nalidixic acid was applied at 10 $\mu\text{g/ml}$ throughout. For each strain the dose dependency of UV-induction was determined as well as the optimal period of post-induction incubation for the dose giving a maximum of RA or for all doses

applied. Without induction all strains examined showed a normal high level of restriction (Table 2, last column).

The *recA13* mutation, which eliminates the recombination and protease function of *recA* protein (Roberts and Roberts 1975) abolished RA induction by UV and nalidixic acid (Table 2, lines 1 and 2). The *recA430* allele (formerly called *lexB30*) codes for a *recA* protein unable to cleave the λ repressor in vivo and in vitro because of an alteration of its protease activity (Morand et al. 1977; Roberts and Roberts 1981). In a *recA430* mutant some *lexA* protein cleavage has been inferred (Devoret et al. 1983), but efficient induction of RA by either UV or nalidixic acid was not observed (Table 2, line 3). A similar result was obtained in a *recA142* strain (Table 2, line 4) in which the UV-induction of λ is abolished (Clark 1973) presumably because activation of this mutant *recA* protein is ineffective in vivo (Roberts and Roberts 1981). In a strain with constitutive overproduction of *recA* protein due to an operator mutation (*recA0*; Clark 1982; Ginsburg et al. 1982) treatments with UV or nalidixic acid were still required for RA induction (Table 2, line 5), probably to provide the signal(s) for the activation of the *recA* protease. Finally, in a *tif* mutant (*recA441 lexA55 sfiA11*) RA was inducible by heating at 42°C (Fig. 3). The *recA441* allele allows constitutive expression of SOS functions at 42°C without a typical SOS-inducing treatment (Castellazzi et al. 1972; Phizicky and Roberts 1981). All these results suggest the involvement of the proteolytic function of the *recA* protein in the process of RA induction.

*The *lexA* protein is not the repressor of RA function*

Certain mutations in *lexA* (e.g. *lexA3*) make the *lexA* protein resistant to cleavage by *recA* protease in vitro and in vivo (Little et al. 1980) and provide a permanent block for the induction of *recA* protein synthesis (Gudas and Pardee 1975) and of SOS functions (Mount et al. 1972; Little and Mount 1982). In the *lexA3* mutant RA is uninducible by UV and nalidixic acid (Day 1977; Table 2, line 6). From these results, together with the data in the previous section, it would seem that the control mechanism of the RA function was the same as that assumed for other SOS functions, i.e. induction requires the protease function of activated *recA* protein and a cleavable *lexA* repressor controlling transcription of *recA* and the respective SOS gene. However, in an amber mutant of *lexA* (*lexA55*) in which the expression of several SOS genes including *recA*, *uvrA*, *uvrB*, *sfiA* and *umuC* is constitutive (Pacelli et al. 1979; Kenyon and Walker 1981; Fogliano and Schendel 1981; Huisman and D'Ari 1981; Bagg et al. 1981), RA was not constitutive but was still inducible by nalidixic acid or UV (Table 2, line 7). A similar result (not shown) was obtained with the missense mutant *lexA51* (Mount 1977). This is the first evidence that the *lexA* protein may not be the direct repressor of the RA function. The need for the protease activity of the *recA* protein for RA induction in a *lexA55* background (Table 2, lines 8 and 9) supports the interpretation that a repressor other than the *lexA* protein may be cleaved. Further evidence comes from the efficient induction of Ra by nalidixic acid in a *lexA3* mutant, in which *recA* protein is overproduced as a consequence of a *recA* operator-constitutive mutation (Table 2, line 10). Apparently, increased amounts of *recA* protein plus the nalidixic acid-provoked signal for *recA* activation suffice for derepression of the

Table 2. Induction of restriction alleviation in *Escherichia coli* mutants^a

Genotype	Nalidixic acid for 90–120 min RAF	UV without postirradiation incubation ^b		UV with postirradiation incubation ^c		Efficiency of plating of λC
		RAF	(J/m ²)	RAF	(min)	
1. <i>rec</i> ⁺	90 ^d	50 ^d	(54)	240 ^d	(120)	2.5×10^{-4}
2. <i>recA13</i>	1.5	1		1		0.2×10^{-4}
3. <i>recA430</i>	8.5	1.5	(18)	4.5	(90)	1.9×10^{-4}
4. <i>recA142</i>	17	1		1		0.9×10^{-4}
5. <i>recAo98</i>	148	43	(54)	257	(120)	0.1×10^{-4}
6. <i>lexA3</i>	9	1		1		2.9×10^{-4}
7. <i>lexA55</i>	47	64	(54)	183	(90)	4.7×10^{-4}
8. <i>lexA55 recA430</i>	3	3.5	(18)	4	(90)	1.4×10^{-4}
9. <i>lexA55 recA56</i>	2.5	1		1		0.6×10^{-4}
10. <i>lexA3 recAo98</i>	150	2	(54)	1.5		0.2×10^{-4}
11. <i>recF143</i>	52	1	(18)	7	(90)	0.7×10^{-4}
12. <i>recF143 recAo281</i>	30	1		1		3.0×10^{-4}
13. <i>recF143 lexA55</i>	30	17	(27)	22	(30)	1.1×10^{-4}
14. <i>recB21</i>	2.5	5	(54)	1		1.1×10^{-4}
15. <i>recC22</i>	2	1.5	(54)	0.5		2.6×10^{-4}
16. <i>recB21 recC22 sbcB12</i>	1.5	1.5	(54)	1		1.7×10^{-4}
17. <i>recB21 recC22 recF143</i>	9	1		Not determined		1.8×10^{-4}
18. <i>recB21 lexA55</i>	1	1.5	(54)	1.5		3.3×10^{-4}

^a At full induction by nalidixic acid or UV (including postirradiation incubation) the capacity of the mutant strains to propagate λK was between 1.0 and 0.4 of uninduced cells

^b UV dose for maximum RA is given in brackets; omitted number indicates that a maximum was not observed between 9 and 144 J/m²

^c The number in brackets indicates the optimum duration of postirradiation incubation after applying a UV dose giving maximum RA; no number indicates that postirradiation incubation did not increase RA at any UV dose

^d Typical values

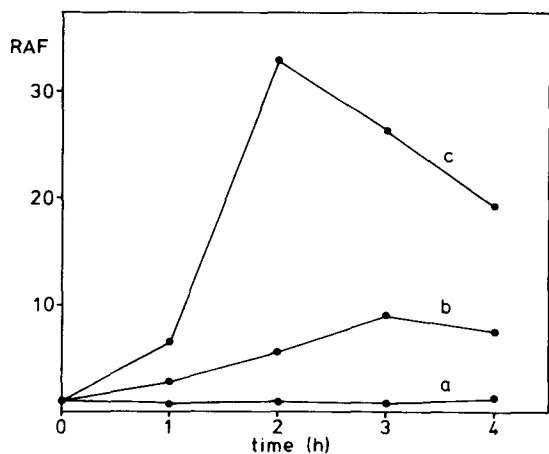


Fig. 3. Induction of restriction alleviation in the *tif* mutant DM2001 (*lexA55 recA441 sfiA11*; see line 7 in Table 2) by heat pulse. Cells were grown at 30° C in M9 minimal medium. At $t=0$ adenine was added (final concentration 75 μ g/ml) and the culture divided into three portions. One portion was further incubated at 30° C (a), the second was shifted for 20 min (b) and the third for 40 min (c) to 42° C, followed by 30° C. At the time intervals indicated samples of the culture were withdrawn and the RA factors determined at 30° C. In complete medium RA was not induced by 42° C in the *tif* mutant (not shown; Castellazzi et al. 1972)

RA function in a strain in which many other SOS functions are permanently repressed by the *recA* protease-resistant *lexA3* repressor. On the other hand, induction by UV in this *lexA3 recAo* double mutant was impaired (Table 2, line 10). This indicated the requirement for an additional *lexA*-

repressed function in the UV induction of RA and we designate this unidentified SOS gene Y.

Effect of a *recF* mutation

In a *recF* mutant the induction of *recA* protein synthesis by nalidixic acid is normal (McPartland et al. 1980; Karu and Belk 1982) and so is RA induction (Table 2, line 11). Reports of the UV induction of *recA* expression in *recF* are controversial (Clark et al. 1978; McPartland et al. 1980; Salles and Paoletti 1983; Casaregola et al. 1982). In a *recF* mutant RA was not induced by UV (Table 2, line 11), even when the *recA* protein was overproduced due to a *recA* operator mutation (Table 2, line 12). This shows that *recF* has an important function only in the UV induction of RA. However, the lack of this function can be compensated by a *lexA55* mutation (Table 2, line 13).

The *recBC* enzyme is essential for RA

The *recBC* enzyme is required for the derepression of *recA* after nalidixic acid treatment (Gudas and Pardee 1975) but not after UV-irradiation of cells (Bockrath and Hanawalt 1980; McPartland et al. 1980). In *recB* and *recC* mutants RA was not obtained after treatment with UV (Thoms and Wackernagel 1982) or with nalidixic acid (Table 2, lines 14 and 15). Even in a strain, in which an extragenic suppressor of the *recBC* defect (*sbcB*; Kushner et al. 1971) restored the recombination and repair proficiencies and the inducibility by nalidixic acid of *recA* protein synthesis (Karu and Belk 1982), induction of RA by nalidixic acid and UV was

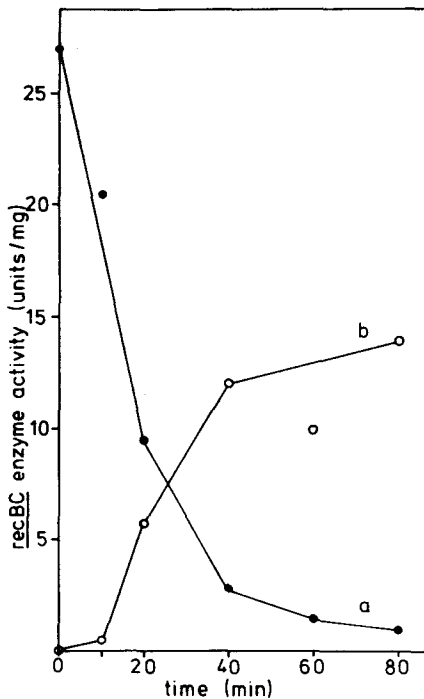


Fig. 4a, b. Specific activity of the recBC enzyme in cell extracts from the temperature-sensitive *recB* mutant (*recB270*). A log-phase culture was grown at 30°C and shifted to 42°C (6a). Another culture was grown at 42°C and shifted to 30°C (b). After the shift ($t=0$) samples were removed from each culture at the times indicated and rapidly chilled in ice. Extracts were prepared and assayed for recBC enzyme activity at 30°C as described in Materials and methods. For comparison, the specific activity of the recBC enzyme in wild type cells (strain AB1157) grown at 30°C was 110 units/mg and was not significantly affected at 42°C

blocked (Table 2, line 16). Another combination of *rec* alleles, *recB21 recC22 recF143*, which allows induction of recA protein synthesis (Karu and Belk 1982), also did not allow efficient RA (Table 2, line 17). The recBC enzyme was also required for RA in a *lexA55* strain (Table 2, line 18). These observations point to an essential role of the recBC enzyme in RA.

In order to obtain information as to the time of action of the recBC enzyme in the process of RA we used a temperature-sensitive *recB* mutant. This mutant (*recB270*) is defective in the duplex-DNA exonuclease of the recBC enzyme (Kushner 1974), and we studied the intracellular heat inactivation of the enzyme and its effect on RA. Figure 4 shows that in this mutant 90% of the recBC enzyme activity (which at 30°C is only about 20% of that present in wild-type cells) disappears within 40 min after a temperature shift-up. After down-shift the mutant cells need about 60 min to regain 50% of the specific activity present in cells grown at 30°C. The induction of RA by UV in the *recB270* mutant at 30°C is similar to the induction in wild type cells (Fig. 5a) showing that the already low level of the recBC enzyme activity in the *recB270* mutant at 30°C suffices for production of the RA phenotype. However, RA was essentially eliminated in the *recB270* strain when the cells were incubated at 42°C for 40 min before and 20 min after UV-irradiation (Fig. 5b). This treatment destroyed the recBC enzyme activity before the irradiation and allowed the activity to reappear in significant amounts only after

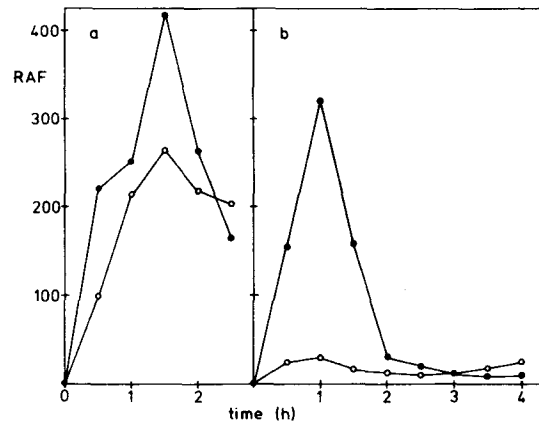


Fig. 5a, b. Effect of temperature shifts on the UV-induction of RA in wild type (AB1157) and *recB270* cells. a Growth and post-irradiation incubation of cells in complete medium at 30°C. b Cells were grown in complete medium at 30°C and shifted to 42°C for 40 min; they were then irradiated at 42°C and further incubated in complete medium at 42°C for 20 min followed by 30°C. Irradiation (54 J/m^2) was at $t=0$. At the times indicated RA factors were determined. Wild type: ●; *recB270*: ○

about 40 min after irradiation (compare Fig. 4). Under these conditions the expression of the RA function was not restored in the *recB270* strain (not even after prolonged incubation), although in the corresponding experiment with the *recB*⁺ strain the RA capacity at 40 min after induction was still building up. These observations are interpreted to indicate a need for the recBC enzyme at an early stage during development of the RA phenotype. The data are less compatible with an exclusive requirement for the recBC function directly in the RA process. It is possible, however, that the enzyme acts in both the induction and the RA process itself. Unfortunately, the experimental test (heat inactivation of the enzyme at later stages after induction) can not be done since expression of the RA function itself is thermosensitive (see first section).

Discussion

The observation that RA cannot be induced by UV or nalidixic acid in *recA* mutants, particularly in those with a defect in the protease activity of the *recA* protein (*recA340*) or in its activation (*recA142*) shows that this protease is required for RA induction. This notion is further supported by the thermo-inducibility of RA in the *tif* mutant *recA441*. In this respect RA is similar to the canonical SOS functions. A major point from our work is the conclusion that the RA function (or at least one of the functions required for the RA phenotype) is controlled by a *recA* protease-sensitive component other than the *lexA* repressor. This conclusion is based on the inducible expression of RA in strains having no active *lexA* repressor (*lexA55*; *lexA51*), on the need for *recA* protease to obtain induction in such strains and finally on the observation that RA is inducible (by nalidixic acid) in a strain with a mutant *lexA* repressor which is resistant to cleavage by *recA* protein. Thus, our analysis of the genetic control of RA provides evidence for the first example of a cellular damage-inducible function which is not directly controlled by the *lexA* repressor. The possibility that RA is not a bacterial function but resides on an unidentified defective prophage cannot be dis-

counted. However, as discussed in a previous paper, the only known defective prophage of *E. coli* carrying a gene for restriction alleviation is deleted in strain AB1157 and its derivatives employed in this study (Thoms and Wackernagel 1982).

It is possible that two damage-inducible functions in *E. coli* involved in the mutagenic response may also be controlled by components other than *lexA* protein. Recently it has been shown that activated *recA* protein was required in a *lexA51* mutant for increased spontaneous mutagenesis of phage fd (Froehlich 1981) and that a defect in the *recA* protease activity blocked UV-induced cellular mutagenesis in a *lexA51* strain (Blanco et al. 1982).

The comparison of the induction of RA by UV and by nalidixic acid in the various mutants shows that these two SOS-inducing agents depend, in addition to the *recA*⁺ and *recB*⁺*C*⁺ alleles, on somewhat different genes. It has already been proposed that UV and nalidixic acid require different pathways for the generation of the signal that triggers the SOS response (McPartland et al. 1980). Nalidixic acid induces RA independently of the functions of *lexA* (as long as the *recA* protein is present in increased amounts; compare line 6 and 10 in Table 2) and *recF*. The independence from *recF* parallels the genetic requirements for derepression of the *recA* gene by nalidixic acid (Karu and Belk 1982). In contrast, RA induction by UV was seen neither in *lexA3 recAo* nor in *recF* and *recF recAo* mutants, suggesting that *recF* functions only in the sequence of events following UV-damage of the chromosome (such as generation, transport or action of a signal). The observation that a defect in the *recF* gene is compensated for by the absence of an active *lexA* repressor (in a *recF lexA55* mutant) indicates that *recF* might act in the derepression of a *lexA*-controlled function. Since derepression of the *recA* gene after UV or nalidixic acid treatment is independent of *recF* (McPartland et al. 1980; Karu and Belk 1982; Casaregola et al. 1982; Salles and Paoletti 1983) the role of *recF* in RA induction may be confined to the regulation of a function required for UV-induction of RA. The notion that *recF* is a gene with a regulatory function may also apply to its role in the so called "*recF* pathway" of recombination and repair (Armengod 1982; Lovett and Clark 1983; Lloyd et al. 1983). In this context it is interesting to note that induction of RA by UV (but not by nalidixic acid) is impaired also in a *lexA3 recAo* mutant. This may result from the permanent repression of a *lexA*-controlled function (Y) necessary for RA induction by UV. It is possible that the unknown function Y and the hypothetical *recF*-regulated function are identical. All the observations and interpretations regarding *recF* highlight the following unsolved discrepancy: why in a *recF* mutant is the *recA* gene fully derepressed after UV but not the additional *lexA*-controlled function(s) required for UV-induction of RA? This inconsistency cannot easily be explained on the basis of a strict SOS model which postulates that only one common type of SOS signal exists which has *recA* protein as the sole target and activates it to become a protease with a fixed specificity. It is too early to speculate on the type of control exerted by *recF*. Studies on the effect of the *recF* mutation on the induction of other SOS functions are in progress.

Damage-induced derepression of the *recA* gene after treatment with nalidixic acid but not with UV depends on the *recBC* enzyme (Gudas and Pardee 1975; Bockrath and Hanawalt 1980; McPartland et al. 1980) and is restored

in *recBC* mutants by an extragenic suppressor like *sbcB* or by a *recF* mutation (Karu and Belk 1982). In contrast to the *recA* protein the induction of RA by UV or nalidixic acid is, in all combinations of mutations, strictly dependent on the *recBC* enzyme. Our temperature-shift experiments show that the enzyme functions immediately after UV-irradiation, possibly during signal generation, but they do not allow one to decide whether or not the enzyme might in addition be directly involved in the RA process.

The mechanism by which restriction is alleviated is not yet understood. The determination of RA by observing increased survival of unmodified phage λ is the simplest test, but RA can also be demonstrated by transformation with unmodified plasmid DNA (Bujanowski and Wackernagel, unpublished results). This latter observation and the fact that RA is independent of the multiplicity of λ infection (from less than 0.001 to greater than 3) argues against a recombinational repair involving several damaged genomes. Since both strands of duplex DNA are relatively rapidly cleaved during restriction by *EcoK* (Endlich and Linn 1981) a recombinational repair of DNA gaps also appears unlikely as a mechanism for RA. On the other hand, a direct effect of a damage-inducible component on the activity of *EcoK* has not been demonstrated so far. Nevertheless, it is conceivable that it is advantageous for cellular survival if the balance between DNA modification and DNA restriction could be temporarily changed towards modification at times of extensive DNA repair, i.e. under conditions which provoke the SOS response.

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