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Inducibility of the SOS response in a *recA730* or *recA441* strain is restored by transformation with a new *recA* allele

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Abstract. *Escherichia coli* RecA protein plays an essential role in both genetic recombination and SOS repair; in vitro RecA needs to bind ATP to promote both activities. Residue 264 is involved in this interaction; we have therefore created two new *recA* alleles, *recA664* (Tyr264→Glu) and *recA665* (Tyr264→His) bearing mutations at this site. As expected both mutations affected all RecA activities in vivo. Complementation experiments between these new alleles and wild-type *recA* or *recA441* or *recA730* alleles, both of which lead to constitutively activated RecA protein, were performed to further investigate the modulatory effects of these mutants on the regulation of SOS repair/recombination pathways. Our results provide further insight into the process of polymerization of RecA protein and its regulatory functions.

Key words: RecA mutants – SOS response – Mutagenesis – Recombination

Introduction

Escherichia coli RecA protein plays an essential role in homologous recombination (Roca and Cox 1990; Kowalczykowski 1991; West 1992) and in the control of induction of the multifunctional SOS system, as well as in SOS mutagenesis (Walker 1984). In vitro, RecA protein requires the presence of ATP to catalyse the self-cleavage of lambda and the LexA repressors and of UmuD (Little et al. 1980; Craig and Roberts 1981; Burckhardt et al. 1988; Shinagawa et al. 1988) and for the various steps in the DNA strand exchange reaction (Menetski and Kowalczykowski 1985; Kowalczykowski 1991).

Biochemical and structural studies have provided some insight into the nature of the ATP binding site of RecA protein. Tyrosine 264 was identified as part of the nucleotide binding domain by photolabelling (Knight and McEntee 1985c). Modification of this residue by 5′-*p*-fluorosulphonylbenzoyladenosine was shown to prevent ATP hydrolysis, suggesting a key role of Tyr264 in the interaction of RecA with nucleotides (Knight and McEntee 1985a). Moreover alteration of Tyr264 to Gly, Ser or Phe completely inhibited in vivo RecA functions and led to a *recA* null phenotype (Freitag and McEntee 1991). In vitro, these proteins were able to drive efficient LexA protein self-cleavage but not the strand exchange reaction, suggesting that the RecA conformations required for recombination and SOS induction are distinct (Freitag and McEntee 1991). Recently Story and Steitz (1992) have located the nucleotide binding site of RecA by diffusing ADP into the RecA protein crystal. In that structure Tyr264 does not seem to make any specific contact with the nucleotide but the adjacent residue Gly265 directly interacts with the base.

In the present paper, we created two new point mutations at residue 264, introducing either a positive or a negative charge into this site by changing Tyr into His or Glu, respectively. The results show that each mutated protein is impaired in every in vivo RecA function. The results from complementation experiments between these new mutant genes and wild-type, *recA441* or *recA730* alleles support the suggestion that the formation of active RecA polymers that are effective in SOS induction precedes the formation of the presynaptic complexes required for recombination. In addition, our data are in agreement with the idea that an inactive RecA storage form exists in vivo composed of bundles of RecA polymers.

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Materials and methods

Construction of recA *mutants.* The *recA664* and *recA665* alleles were constructed by site-directed mutagenesis (Cazaux et al. 1991; Cazaux and Defais 1992) according to Eckstein's procedure (Taylor et al. 1985) by replacing the wild-type residue Tyr264 (TAC) by either Glu (GAG) or

His (CAT). In solution the pKs of free residues Tyr, Glu and His are approximately 7.0, 4.7 and 6.5, respectively (Alberts et al. 1989). At physiological pH, Glu is thus negatively, and His positively charged, whereas the original Tyr is neutral. In addition, histidine carries a planar ring structure similar to that of tyrosine whereas glutamic acid possesses a linear side chain. We thus expected that the two proteins RecA604 and RecA605 would modulate the multiple functions of RecA protein differently. Wild-type *recA*, *recA664* and *recA665* genes were completely sequenced in order to verify that no additional sequence changes had been introduced during the mutagenesis procedure, particularly in the promoter/operator sequences.

Cloning of recA *mutants. recA664* and *recA665* alleles were cloned into the low-copy-number plasmid pGB2 (Churchward et al. 1984), generating plasmids pCC6641 and pCC6651. This enabled us to analyse and compare the properties of physiological levels of RecA proteins expressed from a plasmid (RecA664 or RecA665) and/or from the chromosome (wild-type RecA, RecA430, RecA441 or RecA730) with respect to both SOS repair and recombination pathways. Other procedures are detailed in the legends.

Results

Cell survival, SOS induction and genetic recombination in the presence of recA *mutants*

Table 1 shows that the presence of the *recA664* mutation restored only weak UV resistance to the Δ*recA* strain, whereas the *recA665* allele increased survival by two orders of magnitude. However *recA665* survival was still much lower than that of the wild type, although both proteins were normally expressed, as verified by polyacrylamide gel electrophoresis (data not shown). UV survival being the consequence of both SOS and recombinational repair, we checked the capacity of these mutants to perform SOS induction and genetic recombination. After UV irradiation neither *recA664* nor *recA665* was able to induce a *cea::lacZ* fusion controlled by LexA repressor (Table 1). In addition, no induced mutagenesis from His^- to His^+ could be detected in these strains. These results indicate that both mutants were deficient in promoting self-cleavage of LexA and UmuD proteins. Recombination measured either by genetic crosses or by intrachromosomal recombination of different duplicate *lacZ* regions was strongly affected in *recA664* and *recA665* mutants (Table 1). However the *recA665* mutant was able to promote formation of 100-fold more exconjugants than the *recA664* mutant. This capacity for recombination may explain the higher UV survival of the *recA665* strain.

Complementation experiments between recA664 *or* recA665 *and wild-type* recA, recA441 *or* recA730 *alleles*

The modifications introduced at position 264 may have consequences for the polymerization of RecA protein along the DNA and, as a result, affect the regulation of SOS repair and/or recombination pathways. To test this hypothesis we performed genetic complementation experiments by transforming strains having either a wild-type or a constitutively expressed chromosomal *recA* gene with pCC6641 or pCC6651 plasmids carrying the *recA664* or *recA665* mutation, respectively. Figure 1 shows that in the presence of plasmid pCC6641 or pCC6651, the UV survival of the wild-type strain was reduced but not to the levels seen with the *recA664* or *recA665* mutant. RecA665 protein together with wild-type (wt) RecA was able to induce the SOS response (Table 2), while RecA664 completely prevented RecA from catalysing LexA repressor cleavage (data not shown). In addition the $recA^+/recA665$ strain was proficient in SOS mutagenesis following UV-irradiation even though the number of mutants was lower than in a wild type (Table 2). Both $recA^+/recA664$ (data not shown) and $recA^+/recA665$ (Table 2) were deficient in recombination, with no significant differences between the two strains.

In order to investigate further the role of *recA664* and *recA665* mutations in the regulation of DNA repair, we transformed two mutants in which SOS functions are

Table 1. UV survival, SOS induction and mutagenesis after UV irradiation, and genetic recombination assays

recA allele	UV survival (%)[a] (15 J/m²)	SOS induction (β-gal units)[b] −UV/+UV (IF)	SOS mutagenesis (×10⁻⁹)[c] −UV/+UV	Recombination[d]	
				Intrachromosomal	Conjugational
Δ*recA*	<0.0001	01/01 (1.0)	01/ND	ND	ND
recA	80	11/82 (7.5)	127/1518	25	230
recA664	0.001	01/01 (1.0)	02/ND	ND	0.001
recA665	0.008	02/02 (1.0)	02/ND	ND	0.120

ND; Not detectable

All data are an average of at least three experiments

[a] Cell survival was measured by standard methods (Larminat et al. 1992)

[b] β-Galactosidase induction from a *cea::lacZ* fusion was measured following irradiaton with 10 J/m² after 60 min incubation (maximal induction) as described elsewhere (Miller 1972); IF, induction factor

[c] His^+ reversion was scored on M63 medium supplemented with 1 μg/ml histidine as described (Miller 1972). Mutation rate was determied relative to the number of surviving cells growing on medium supplemented with 40 μg histidine. UV fluence was 15 J/m²

[d] Intrachromosomal recombination between two *lac* alleles was assayed as described elsewhere (Dutreix et al. 1989). The number of papillae of identical colonies was averaged. Error bars were <15%. Conjugational crosses with Hfr *leu*::Tn*9* were measured by selecting recombinants on medium containing both 15 μg/ml chloramphenicol and 12.5 μg/ml tetracycline

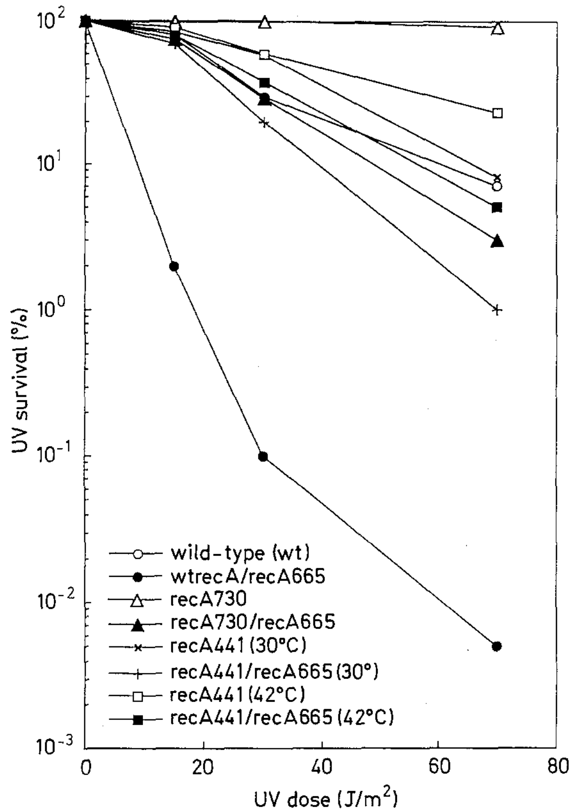

Fig. 1. Survival of UV-irradiated strains *recA441* (×, □) and *recA441/recA665* (+, ■) at 30° C *(crosses)* or 42° C *(squares)*, and wild-type (wt) *recA* (○), *recA730* (△), wt*recA/recA665* (●) and *recA730/recA665* (▲) at 37° C

constitutively expressed with plasmid pCC6641 or pCC6651. The *recA441* mutant (Kirby et al. 1967; Castellazzi et al. 1972; Knight et al. 1984) constitutively expresses SOS functions at 42° C in the presence of adenine but not at 30° C. The *recA730* mutant (Witkin et al. 1982; Roca and Cox 1990) has a similar phenotype except that the constitutive induction of SOS repair takes place at both temperatures, and the strain requires a *sfiA* mutation to grow. In addition *recA730* presents a stronger constitutive phenotype.

recA664 always showed about 10^2 to 10^3 fold higher sensitivity to UV irradiation than wild-type, *recA441* or *recA730* strains (data not shown). This result can be correlated with the drastic decrease provoked by this mutation in both recombination and SOS repair in wild-type and the constitutively induced strains *recA441* or *recA730* (data not shown). On the other hand, it is very interesting to note that, under conditions where SOS induction was constitutive, *recA665* affected UV survival of *recA441* and *recA730* to a much lower extent than that of the wild type (Fig. 1). This surprising effect of *recA665* on UV survival is corroborated by data presented in Table 2. After UV irradiation, the presence of the *recA665* allele in a *recA730* strain led to levels of SOS induction and mutagenesis that were comparable to these of wild type. The strain *recA730/recA665* did not express the SOS functions constitutively: it rather behaved like wild-type *recA*$^+$ and required a damaging agent to induce the SOS response. Moreover *recA730* recombination rates were only slightly decreased by the presence of *recA665* (Table 2). Similarly at 42° C the *recA441/recA665* strain induced the SOS functions as well as did the wild type. However, neither induced mutagenesis nor genetic recombination reached the wild-type level in this strain. No significant differences were observed on measuring recombination at 30 or 42° C (data not shown). Furthermore the *recA441/recA665* strain showed a high level of survival at 30° C, similar to that of a wild-type strain (Fig. 1). This ability of RecA441/RecA665 heteropolymers to promote survival at 30° C can be related to their capacity to induce the SOS response and mutagenesis (Table 2). These mixed multimers appeared less efficient in recombination (Table 2). Thus RecA665 protein seems to interact with RecA730 or RecA441 in heteropolymers better than with RecA.

Table 2. SOS induction and mutagenesis after UV irradiation, and genetic recombination assays

recA allele	SOS induction (β-gal units) −UV/+UV (IR)	SOS mutagenesis ($\times 10^{-9}$) −UV/+UV (IMF)	Recombination	
			Intra-chromosomal	Conjugational
recA	11/82 (7.5)	127/1518 (12.0)	25	230
recA/recA665	10/35 (3.5)	38/125	ND	0.5
recA730	194/241 (1.2)*	$9\times10^{-6}/2\times10^{-6}$*	42	360
recA730/recA665	07/31 (4.4)	10/300 (30.0)	12	40
recA441	11/83 (7.5)[a], * 28/34 (1.2)[b]	24/2800 (116.7)[a] 130/92[b], *	26	160
recA441/recA665	12/33 (2.8)[a] 04/26 (6.5)[b]	04/39 (9.8)[a] 10/41 (4.1)[b]	ND	06

ND, Not detectable; IMF, induced mutagenesis factor: the ratio of His reversion frequencies with and without UV irradiation
All data are an average of at least three experiments
[a] Strains were grown at 30° C
[b] Strains were grown at 42° C in the presence of 100 μg/ml adenine
The procedures were as described in Table 1
* Constitutive induction

Discussion

A primary role of ATP in the biochemical reactions catalysed by RecA protein, such as DNA strand exchange (DasGupta et al. 1980; Cox and Lehman 1981) and stimulation of LexA, λcI or UmuD autoproteolysis (Craig and Roberts 1980; Little et al. 1980; Burckhardt et al. 1988; Nohmi et al. 1988; Shinagawa et al. 1988) seems to be the promotion of structural modifications. It also serves as a substrate for the DNA-dependent RecA ATPase activity (Ogawa et al. 1979; Roberts et al. 1979). Neither repressor cleavage activity nor DNA strand exchange involving three strands requires ATP hydrolysis (Phizicky and Roberts 1981; Menetski et al. 1990). However the ATPase activity allows RecA irreversibly to bypass heterologous DNA regions during recombination processes (Livneh and Lehman 1982; Bianchi and Radding 1983; Rosselli and Stasiak 1991; Kim et al. 1992a) and is necessary in four-strand exchanges (Kim et al. 1992b). This reaction could also play a role in SOS induction (DiCapua et al. 1992).

There is a single nucleotide binding site per RecA monomer (Cotterill et al. 1982) but several residues may be involved in the RecA/ATP interaction. Biochemical evidence has shown that Tyr264 is involved in the ATP binding to RecA (Knight and McEntee 1985a, b, c). However the crystal structure of RecA protein indicates that the base should interact with Asp100 and Gly265 (Story et al. 1992). This structure was based on interaction with ADP and it is well known that the conformation of RecA changes following binding to either ADP or ATP (Kobayashi et al. 1987; Egelman and Stasiak 1988).

The substitution of Tyr264 by His, mutation *recA665* described here, does not produce a *recA* null phenotype, since it allows a 100-fold increase in the survival of a Δ*recA* strain. However, the *recA665* mutation is associated with a significant degree of UV sensitivity. On the other hand, *recA664* (Tyr264→Glu) only restores a low level of UV resistance to a Δ*recA* strain. These results may be explained by the fact that both residues Tyr and His have a planar ring structure, while in *recA664* the cyclic structure has been lost. A change from Tyr264 to Phe, retaining the same planar ring structure, has been reported to allow RecA protein to direct efficient LexA cleavage in vitro and catalyse some ATP hydrolysis (Freitag and McEntee 1991). However the presence of His264 in RecA665 protein prevented in vivo SOS induction. In this context it should be noted that the in vitro cleavage of LexA catalysed by the Tyr264→Phe mutant was measured in non-physiological conditions.

The structure of the RecA/ADP complex shows that the backbone NH group of Gly265 interacts directly with the N3 of the adenine ring of the nucleotide (Story and Steitz 1992). By analysing the peptide domain surrounding residue Tyr264 with the FRODO program giving the three-dimensional coordinates of the X-ray structure of the RecA molecule, we observed that the minimal distance between ADP and either residue 264 or 265 is about 4 Å (data not shown). Moreover, the residue Val238 located in the proximity of the phenyl group of Tyr264 could stabilize the conformation of this residue. It is thus possible that Tyr264 plays a role as crucial as Gly265 in ATP binding and that a modification of tyrosine is directly responsible for the loss of RecA activities.

Genetic analysis of interactions of products of the wild-type and various *recA* alleles with the *recA665* gene product indicates that the RecA665 protein is able to interact with wild-type and other mutated proteins. Additionally, in the presence of RecA665, constitutive expression of SOS functions directed by some RecA proteins, e.g. RecA730 and RecA441, becomes inducible as in wild-type. This suggests that the mixed multimers have lost their constitutively active form, but are still able to take on an active conformation under inducing conditions. Story et al. (1992) have recently proposed a regulatory model for RecA activities, in which they propose that a storage form of RecA protein, constituted of "bundles" of RecA polymers inactive in both strand exchange and coprotease functions preexists in the cell. This form would be in thermodynamic equilibrium with active protein polymers able to promote recombination and to induce SOS response by binding single-stranded (ss)DNA. *recA* mutations that induce SOS functions constitutively e.g. *recA441* or *recA730*, and which are situated at the interpolymer interface, could disrupt the storage form and thus lead to a permanent overproduction of active filaments (Story and Steitz 1992).

The results presented here lend support to this model. When active mixed polymers are formed by *recA665* and either *recA730* or *recA441* at the permissive temperature, they behaved like wild-type polymers. In other words, the SOS response and, to a lesser extent, SOS mutagenesis became inducible and lost their constitutivity. Since RecA665 is not mutated at the interpolymer interface, we propose that the storage form present in *recA665/recA730(recA441)* strains is not totally disrupted. The active filaments liberated would thus be constituted of both RecA665 and RecA730 or RecA441 monomers. RecA665 would be deficient in the binding of ATP and consequently also in the fixation of DNA, since the binding of ATP to RecA induces an allosteric transition to a conformation referred to as the high-affinity DNA binding state (Kowalczykowski 1991). On the other hand, RecA730 or RecA441 are very proficient in this function (Lavery and Kowalczykowski 1988, 1992). It is not difficult to imagine that in the aggregate these hybrid filaments would be as active as the wild type for both SOS induction and recombination.

However recombination catalysed by these heteromultimers was less efficient than SOS induction, indicating that functions that do not seem to require long polymers, such as repressor cleavage (Moreau 1987), can be promoted by such mixed multimers. The presence of RecA665 in the polymer may disturb its structure, leading to small heterologous multimers and consequently to a low amount of recombination. Comparable results were obtained when mixed polymers were formed by mixing RecA and RecA665. These findings can be integrated with the work of Freitag and McEntee (1991), who substituted Tyr264 by Phe or Ser, and produced RecA proteins able to cleave LexA in vitro but unable to

promote DNA strand exchange. They suggested that the RecA-DNA complexes that are required for LexA cleavage and DNA strand exchange are distinct and proposed that formation of the nucleoprotein complex involved in SOS induction precedes that implicated in recombination. The inability of mixed multimers to catalyse recombination could also be explained by an impairment of ATP hydrolysis. This event seems to occur cooperatively along the RecA filament (Roca and Cox 1990). If one monomer such as RecA665 is inefficient in catalysing ATP hydrolysis in the polymer it could block the reaction along the filament, leading to an impairment of recombination.

When RecA664 participated in the formation of the mixed filament, all RecA functions were impaired. It is conceivable that the structural modification created by this mutation prevents the polymerization of RecA monomers. Tyr264 is a highly conserved residue among at least 26 prokaryotic analogs of RecA proteins (Roca and Cox 1991; P. Duwat, personal communication). Five different mutants have been created at this residue (Freitag and McEntee 1991; this work) and all of them have dramatic effects on all RecA functions. This suggests that Tyr264 participates directly in ATP binding to RecA protein and could thus modulate all the RecA protein activities.

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