

Enhanced monomer–monomer interactions can suppress the recombination deficiency of the *recA142* allele

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Summary

The RecA142 protein, in which valine is substituted for isoleucine-225, is defective for genetic recombination *in vivo* and for DNA strand exchange activity *in vitro* under conventional growth and reaction conditions respectively. However, we show that mildly acidic conditions restore both the *in vitro* DNA strand exchange activity and the *in vivo* function of RecA142 protein, suggesting that recombination function can be restored by a slight change in protein structure elicited by protonation. Indeed, we identified an intragenic suppressor of the recombination deficiency of the *recA142* allele. This suppressor mutation is a substitution of leucine for glutamine at position 124. Based on the three-dimensional structure, the Q-124L substitution is predicted to make a new monomer–monomer contact with residue phenylalanine-21 of the adjacent RecA monomer. The Q-124L mutation is not allele specific, because it also suppresses the recombination deficiency of a *recA* deletion ($\Delta 9$), lacking nine amino acids at the amino-terminus, presumably by reinforcing the monomer–monomer interactions that are attenuated by the $\Delta 9$ deletion. Expression of RecA(Q-124L) protein is toxic to *Escherichia coli*, presumably because of enhanced affinity for DNA. We speculate as to how enhanced monomer–monomer interactions and acidic pH conditions can restore the recombination activity of some defective *recA* alleles.

Introduction

The RecA protein of *Escherichia coli* plays a central role in genetic recombination and is essential for induction of the SOS pathway of DNA repair and mutagenesis. *In vitro*, RecA protein promotes an efficient DNA strand exchange reaction involving three or four strands of DNA (Cox, 1991;

West, 1992; Clark and Sandler, 1994; Kowalczykowski *et al.*, 1994; Stasiak and Egelman, 1994; Roca and Cox, 1997). The *recA142* point mutation is a substitution of Val for Ile-225 (Dutreix *et al.*, 1985). This allele is defective in both genetic recombination and damage-inducible λ prophage induction (Clark, 1973; Horii and Clark, 1973) but remains proficient in the spontaneous induction of bacteriophages λ and $\Phi 80$ (Roberts and Roberts, 1981; Dutreix *et al.*, 1985). Although defective for induction of wild-type λ phage, phage with a proteolysis-sensitive repressor, $\lambda cl Ind^s$, are induced in *recA142* lysogens (Dutreix *et al.*, 1985), demonstrating that it is not completely devoid of function.

In vitro, RecA142 protein is unable to promote DNA strand exchange in the presence of ATP (Kowalczykowski *et al.*, 1989; Kowalczykowski and Krupp, 1989), consistent with its phenotypic behaviour. Nevertheless, it is not completely devoid of function, because RecA142 protein can bind single-stranded (ss) DNA, hydrolyse ATP in a ssDNA-dependent manner and self-associate in a DNA-independent manner (Kowalczykowski *et al.*, 1989; Kowalczykowski and Krupp, 1989). The first two activities, however, are more sensitive to increased salt concentrations than those of wild-type RecA protein and are inhibited by SSB protein (Kowalczykowski *et al.*, 1989). Finally, RecA142 protein is also partially defective for double-stranded (ds) DNA-dependent ATP hydrolysis (Kowalczykowski *et al.*, 1989; Kowalczykowski and Krupp, 1989). All of these partial deficiencies are manifestations of the inability of RecA142 protein to develop fully characteristics of the high-affinity ssDNA-binding state that is necessary for DNA strand exchange (Kowalczykowski, 1991a). Despite its deficiencies when ATP is used as the nucleotide cofactor, either ATP γ S or dATP activates the co-protease activity of RecA142 protein (Roberts and Roberts, 1981; Roberts and Devoret, 1983). Similarly, in the presence of ATP γ S, RecA142 protein becomes proficient for DNA strand exchange (Kowalczykowski, 1991b). Moreover, decreasing the pH to 6.2 activates both the dsDNA-dependent ATPase activity and the ATP-dependent dsDNA-binding ability of RecA142 protein (Zaitsev and Kowalczykowski, 1998a), suggesting that RecA142 protein could form the ATP-induced, high-affinity DNA-binding state under slightly acidic conditions. Similar acidic pH-dependent activation *in vitro* was shown for the RecA(G-160N) and RecA(H-163A)

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proteins (Bryant, 1988; Muench and Bryant, 1990; 1991; Meah and Bryant, 1993).

The crystal structure of the *E. coli* RecA protein-ADP complex reveals that the residue Ile-225, which is altered in RecA142 protein (to a valine), is part of the hydrophobic core of RecA protein and makes van der Waals contacts with Leu-47 and Ile-64 (Story and Steitz, 1992; Logan and Knight, 1993). Interestingly, Ile-225 is invariant among the RecA proteins of proteobacteria, although it is replaced by valine among Gram-positive bacteria (Karlin *et al.*, 1995; Karlin and Brocchieri, 1996). The occurrence of a valine residue in the RecA proteins of Gram-positive bacteria suggests that this residue is tolerated at this position because of the existence of compensatory changes that are absent in the *E. coli* protein. This fact, together with the biochemical data described above, suggests that intragenic suppressors of the *recA142* mutation might readily be found.

In this paper, we demonstrate that recombination function can be restored to RecA142 protein by a reduction in pH. Furthermore, we describe an intragenic suppressor of the *recA142* allele and discuss how enhanced monomer-monomer interactions and acidic solution conditions can restore the recombination activity of some defective *recA* alleles.

Results

RecA142 protein can promote DNA strand exchange under slightly acidic reaction conditions in vitro

Recently, we have shown that RecA142 protein binds dsDNA efficiently in a pH-dependent manner, using both DAPI displacement and dsDNA-dependent ATPase assays (Zaitsev and Kowalczykowski, 1998a). Activation of dsDNA binding at pH 6.2 suggested that, under these conditions, RecA142 protein could adopt the high-affinity DNA-binding state that defines the active form of RecA protein (Kowalczykowski, 1991a). As activation of this state is an important prerequisite for DNA strand exchange activity, this finding suggested that RecA142 protein could manifest this activity at more acidic pH conditions. Figure 1 shows that, at reduced pH (pH 6.2), RecA142 protein can indeed promote DNA strand exchange whereas, at pH 7.5, it was already shown that this mutant protein is completely inactive and produces no paired DNA (see Kowalczykowski *et al.*, 1989). In contrast, extensive characterization by the Bryant laboratory revealed only subtle differences in DNA strand exchange activity for wild-type RecA protein at these different pH conditions (Bryant, 1988; Muench and Bryant, 1990; 1991; Meah and Bryant, 1993). The discovery that RecA142 protein could promote DNA strand exchange at slightly acidic pH demonstrated that it is not a fully deficient recombination protein and raised the possibility that the activity of RecA142 protein might also be restored *in vivo* by a reduction in the intracellular pH.

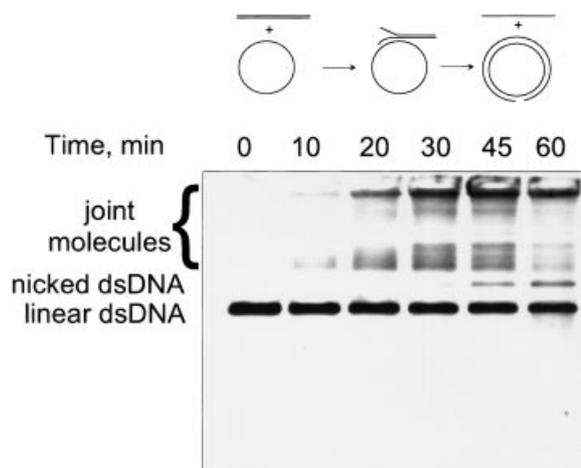


Fig. 1. DNA strand exchange activity of RecA142 protein at pH 6.2. The reaction was carried out as described in *Experimental procedures*.

Acidic pH reactivates RecA142 protein in vivo

To test the possibility that RecA142 protein can be activated by decreasing the intracellular pH, we added a particular weak acid, propionic acid, to LB medium. This acid, which is not metabolized, enters *E. coli* cells in the protonated form and then equilibrates inside the cell between its protonated and unprotonated forms, thereby releasing protons (Repaske and Adler, 1981). This process is accompanied by a stable decrease in the intracellular pH, which can be toxic for cells (see below) (Dri and Moreau, 1994).

We used this approach to decrease the intracellular pH in order to test the possibility that RecA142 protein can be reactivated *in vivo* to restore resistance to the DNA-damaging mutagen, nitrofurantoin (NF). The concentration of propionic acid (PA) used (40 mM) is quite toxic to *E. coli* and decreases cell viability by about three orders of magnitude (Dri and Moreau, 1994); although the intracellular pH value cannot be defined precisely because of the toxicity of PA and of some strain dependence, it is estimated to be ≈ 6.5 – 6.8 . As can be seen in Table 1, a *recA* deletion strain cannot grow in the presence of NF either in the presence or in the absence of PA. Introduction of the wild-type *recA* gene restores viability on the NF plates to $\approx 30\%$ of the cells. However, in the presence of PA, survival of this strain drops by three orders of magnitude as a result of the intrinsic toxicity of this agent. In comparison, for the *recA142* allele in the absence of PA, survival in the presence of NF is reduced by more than four orders of magnitude (compared with *recAwt*). This shows that *recA142* is severely impaired in its recombinational repair activity at neutral pH, confirming the original observations (Clark, 1973; Horii and Clark, 1973). The presence of PA, however, actually causes survival in the presence of NF to increase, reaching a level similar to that measured for the wild-type

Table 1. Activation of RecA142 protein by acidic pH conditions *in vivo*.

| <i>recA</i> allele | NF survival (fraction) | |
|--------------------|---------------------------|----------------------------|
| | Absence of propionic acid | Presence of propionic acid |
| None | $< 10^{-7}$ | $< 10^{-7}$ |
| <i>recAwt</i> | 3.4×10^{-1} | 4×10^{-4} |
| <i>recA142</i> | 1×10^{-5} | 1.7×10^{-4} |

DK1($\Delta recA_{306}$) cells bearing *recA*-encoding plasmids were plated on LB agar media supplemented with either ampicillin ($100 \mu\text{g ml}^{-1}$) and nitrofurantoin ($2 \mu\text{g ml}^{-1}$) or ampicillin, nitrofurantoin and propionic acid (40 mM). The values represent an average of at least two experiments and did not vary by more than 20%.

recA gene (in the presence of both PA and NF). Assuming that the toxic effect of both PA and NF is additive, we thus conclude that the decrease in the intracellular pH induced by PA does indeed activate the recombinational repair function of RecA142 protein. This finding is consistent with the view that *recA142* is not a fully deficient allele.

Intragenic suppressors of the *recA142* allele

Cells bearing a defective *recA* gene are sensitive to both UV light and DNA-damaging agents (Quillardet *et al.*, 1982; Wang and Smith, 1986). In addition, they cannot support the growth of recombination-defective $\lambda\Delta(int red gam)$ bacteriophage (Zissler *et al.*, 1971; Enquist and Skalka, 1973; Furth, 1983). As cells carrying the *recA142* allele are severely impaired in their recombinational activity, intragenic suppressors of *recA142* were isolated as spontaneous phenotypic revertants of the plasmid-borne gene that acquired resistance to both NF and UV light. A recombination phenotype was then assigned to them based on the ability of the plasmid-borne *recA* alleles to complement cells for UV light and NF sensitivity, as well as for their ability to support the growth of $\lambda\Delta(int red gam)$ bacteriophage. The presence of the original *recA142* mutation in these revertants was confirmed by restriction mapping, as the *recA142* mutation creates a unique *AatII* restriction site. One of these phenotypic revertants, *recA142-6*, was investigated further.

Table 2. Ability of *recA* alleles to complement the recombination and repair deficiencies of JC10289 $\Delta recA_{306}$.

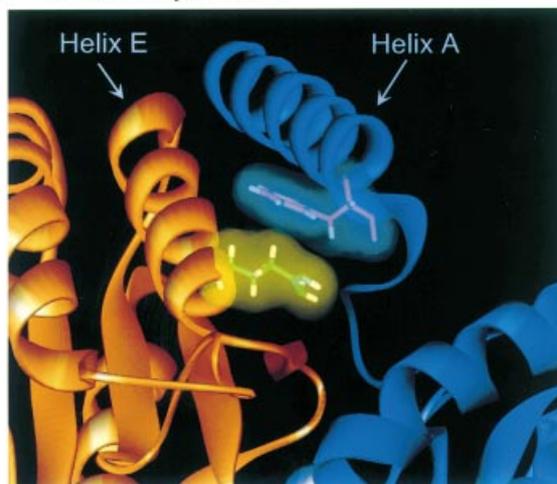
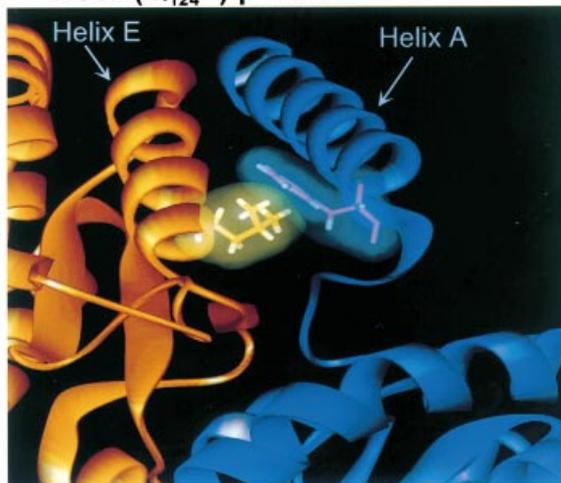
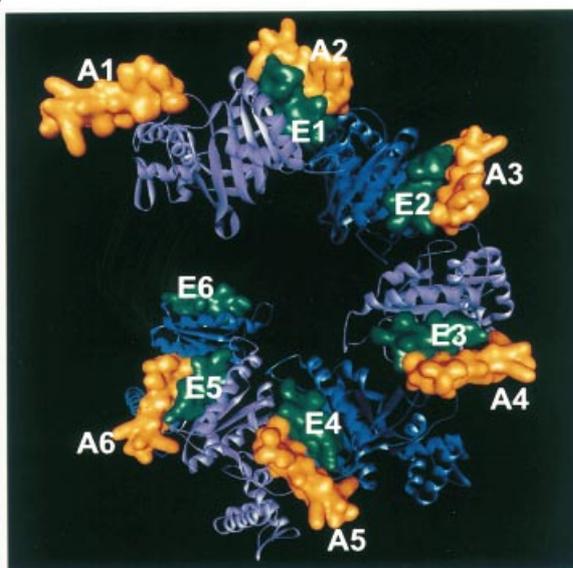
| <i>recA</i> allele | Relative plating efficiency of $\lambda\Delta(int red gam)$ | Fraction surviving (UV irradiation) | Fraction surviving (NF) |
|--|---|-------------------------------------|-------------------------|
| None | 1.4×10^{-9} | 1.0×10^{-4} | 1.6×10^{-7} |
| <i>RecAwt</i> | 1 | 1.0×10^{-1} | 8.7×10^{-1} |
| <i>recA142</i> | 2×10^{-1} | 3.7×10^{-4} | 9.0×10^{-7} |
| <i>recA142-6</i> | 1 | 1.0×10^{-1} | 9.1×10^{-1} |
| <i>recAΔ9</i> | $< 10^{-8}$ | 3.0×10^{-4} | 2.8×10^{-7} |
| <i>recAΔ9/Q-124L</i> | 1 | 8.0×10^{-2} | 7.8×10^{-1} |

JC10289 $\Delta recA_{306}$ cells bearing *recA*-encoding plasmids were irradiated with UV light at a dose of 10 J m^{-2} or plated on LB agar containing nitrofurantoin at a concentration of $2 \mu\text{g ml}^{-1}$. The values represent an average of at least two experiments and did not vary by more than 20%.

The suppressor mutation was mapped to a 135 bp *AccI*–*ClaI* DNA fragment of the *recA* gene. This DNA fragment, when cloned back into the original *recA142* allele, conferred recombination proficiency, as expected (Table 2). The latter plasmid construct, which must be free from cryptic mutations outside of the 135 bp region, was used in all subsequent experiments. Sequencing of the 135 bp *AccI*–*ClaI* DNA region revealed a single T \rightarrow A substitution, resulting in a CAG to CTG triplet change that, in turn, corresponds to the amino acid substitution Gln-124 \rightarrow Leu. As shown in Table 2, the *recA142-6* allele fully complemented the sensitivity of JC10289($\Delta recA_{306}$) cells to both NF and UV light. Restoration of wild-type levels of bacteriophage $\lambda\Delta(int red gam)$ plating efficiency confirmed the recombination proficiency of the *recA142-6* allele. The high residual plating efficiency of bacteriophage $\lambda\Delta(int red gam)$ on *recA142* cells (only 0.2 instead of 10^{-9}) additionally demonstrates that the *recA142* allele retains some recombinational capacity, as reported initially (Roberts and Roberts, 1981; Dutreix *et al.*, 1985), and demonstrates the high sensitivity of the bacteriophage $\lambda\Delta(int red gam)$ plating assay for monitoring low levels of recombination.

Leucine-124 is likely to be involved in monomer–monomer interactions

Interestingly, the suppressor mutation occurred at a position, glutamine-124, that is conserved in both proteo- and Gram-positive RecA protein sequences. It is located in α -helix E, close to a RecA protein surface that is involved in monomer–monomer interactions and also includes amino acid residues 127–139 (Karlin and Brocchieri, 1996) (Fig. 2). This location for Gln-124 suggests the possible involvement of this amino acid residue in monomer–monomer interactions. In agreement, computer modelling of both RecAwt and RecA(Q-124L) (i.e. without the *recA142* mutation) proteins revealed that the amino acid closest to position 124 is from an adjacent RecA protein monomer. This residue, Phe-21, is in α -helix A (composed of residues 6–30) of the adjoining RecA protein and is involved in the complementary monomer–monomer interactions (Story and Steitz, 1992). The distances between Phe-21 and the

A RecAwt protein:**B RecA(Q₁₂₄L) protein:****C**

Gln-124 of RecAwt protein or the Leu-124 of RecA(Q-124L) protein are 2.9 Å and 1.9 Å respectively. As shown in Fig. 2A, Gln-124 of RecAwt protein does not contact the Phe-21 residue. In contrast, in the case of the mutant RecA(Q-124L) protein, the surfaces of Leu-124 and Phe-21 are overlapped, suggesting that a new hydrophobic contact at the monomer–monomer interface was created by the suppressor mutation (Fig. 2B).

We also used another genetic approach to verify that suppression by the *recA(Q-124L)* mutation was occurring via enhanced monomer–monomer interactions, as suggested by the molecular modelling. Earlier, we established that the *recAΔ9* gene, which encodes a truncated RecA protein missing eight amino acids from the N-terminus (RecAΔ9), is phenotypically RecA⁻, presumably because of inefficient RecA nucleoprotein filament formation (Zaitsev and Kowalczykowski, 1998b). One of the N-terminal amino acids that is deleted in RecAΔ9, K-6, is involved in monomer–monomer interactions (Story and Steitz, 1992; Karlin and Brocchieri, 1996). We suggested that the absence of only one monomer–monomer contact was sufficient to reduce the strength of monomer–monomer interactions below that required for stable RecA nucleoprotein filament formation and, as a consequence, to display a RecA⁻ phenotype (Zaitsev and Kowalczykowski, 1998b). Thus, if Leu-124 is indeed involved in monomer–monomer interactions and is able to enhance those interactions, then we expected that the double mutant *recA(Δ9/Q-124L)* would exhibit a RecA⁺ phenotype because the new monomer–monomer contact between L-124 and F-21 would provide an additional stabilization that compensates for the interactions lost in the Δ9 deletion. An analysis of the sensitivities to both UV light and NF and the ability to support growth of bacteriophage λΔ(*int red gam*) for cells (JC10289Δ*recA*₃₀₆) carrying the plasmid *precA(Δ9/Q-124L)*, showed that the *recA(Δ9/Q-124L)* gene displays a RecA⁺ phenotype (Table 2). This result supports our supposition that Leu-124 is directly involved in essential, presumably monomer–monomer, interactions and, furthermore, it demonstrates that the *recA142* suppressor mutation is not allele specific [the Q-124L mutation also suppressed the *recA2183* (K-183M) allele, but not the *recA1* allele (unpublished observations)].

Fig. 2. Computer modelling of monomer–monomer interactions. The region of the RecA protein structure that shows the interactions between Q-124 and F-21 for wild-type RecA (A) and between L-124 and F-21 for RecA(Q-124L) protein (B) is illustrated. Shown are α-helix E of a RecA protein monomer containing either the Q-124 or the L-124 residue for RecAwt or RecA(Q-124L), respectively, and α-helix A of the adjacent RecA protein monomer containing the F-21 residue. The location of these helices in the context of a full turn of the RecA protein helical filament is shown in (C): the backbone of each monomer is shown in alternating purple and blue colour; α-helix A (orange) and α-helix E (green) are shown in space-filling form.

The expression of RecAQ-124L protein is toxic

To determine the genetic and physiological consequences of this enhanced monomer–monomer interaction for RecA protein both *in vivo* and *in vitro*, we constructed a plasmid for the controlled expression of RecA(Q-124L) protein using the plasmid vector pPROEX-1. In this construct, protein expression is under the control of a *tac* hybrid promoter, which is IPTG inducible; in the absence of IPTG, protein expression is minimal. Attempts to clone *recA(Q-124L)* into other controlled expression plasmids were unsuccessful, probably because of unacceptably high levels of constitutive expression (unpublished observations). When transformed into EZ1996 cells, the basal level of expression of RecA(Q-124L) protein was sufficient to confer both UV light and NF resistance in qualitative tests (data not shown). However, induction by 0.1 mM IPTG killed the cells (Fig. 3). Interestingly, death occurred even though the strain contains two mutations [*lexA3*, *sfia11* (a non-cleavable LexA SOS repressor and the A suppressor of the filamentation respectively)] that prevent SOS-related lethal filamentation. We did observe gross filamentation of EZ1996 cells that were induced for RecA(Q-124L) protein expression, in contrast to the uninduced cells (Fig. 4), but no filamentation occurred when either the wild-type *recA* or the *recA142* gene was expressed from the same plasmid in the same strain, either in the presence or in the absence of IPTG (unpublished observations). This behaviour is reminiscent of the SOS-independent lethality of toxic *recA* mutations reported previously (Weisemann and Weinstock, 1988; Campbell, 1997; Campbell and Davis, 1999a,b). When the host cells contained the

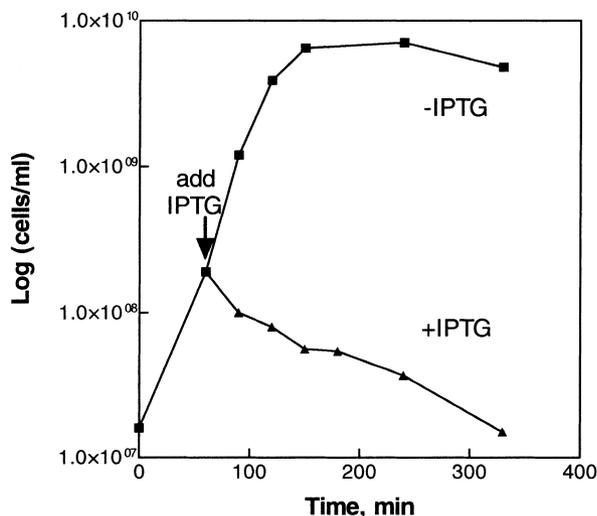


Fig. 3. Expression of RecA(Q-124L) protein is lethal. Growth curve for cells EZ1996 bearing the pPROEX*recA(Q-124L)* plasmid either with (▲) or without induction (■) by IPTG (1 mM) at the time indicated by the arrow.

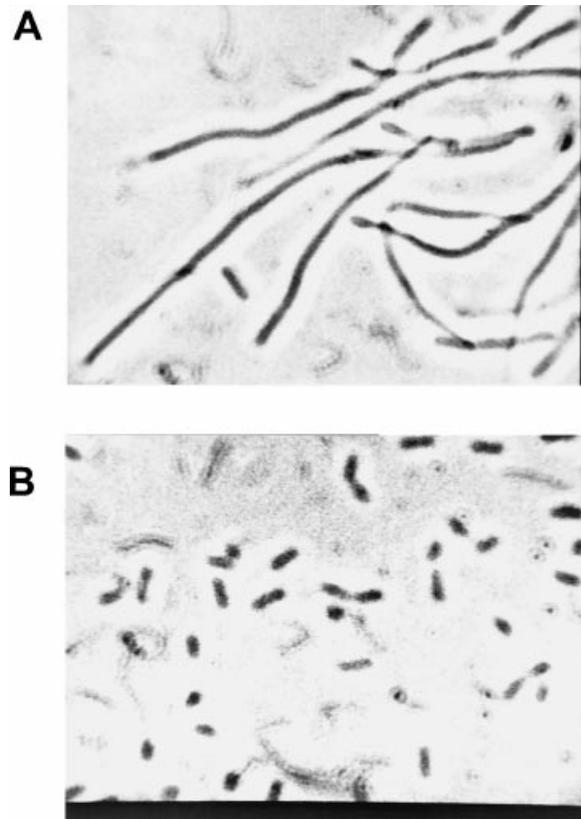


Fig. 4. Expression of RecA(Q-124L) protein results in SOS-independent filamentation. Cell morphology was observed by light microscopy after induction of the RecA(Q-124L) protein. A. Cells (EZ1996) bearing the pPROEX*recA(Q-124L)* plasmid after induction by IPTG (1 mM). B. Uninduced control.

recA56 allele, partial induction by IPTG of the plasmid-borne RecAQ-124L protein results in cells that are phenotypically nearly RecA⁺ (unpublished observations); however, it was difficult to quantify this behaviour, because of differing levels of protein expression and the toxicity of RecAQ-124L protein. Nevertheless, it appears that *recAQ-124L* shows at least partially dominant behaviour.

Discussion

In this study, we have established that functionality can be restored to the RecA142 protein by a modest reduction in pH both *in vitro* and *in vivo*. Furthermore, we isolated an intragenic suppressor mutation that also restored RecA142 protein function. This mutation, a change from glutamine-124 to leucine, suppresses the *in vivo* recombination deficiency of the *recA142* allele, apparently by enhancing monomer–monomer interactions. Expression of the RecA(Q-124L) protein was toxic in *E. coli*. This toxicity was independent of SOS induction and was

accompanied by gross filamentation. Collectively, these results are consistent with the participation of Q-124 in monomer–monomer interactions, and they demonstrate that certain enfeebled mutant RecA proteins can be rescued by what we infer to be enhanced monomer–monomer interactions. In agreement with our interpretations, of eight novel *recA* mutants displaying SOS-independent lethality, seven contained mutations located at the monomer–monomer interface (Campbell, 1997; Campbell and Davis, 1999a,b). Also in agreement, certain mutations at two residues (90 and 116) in the same interface are lethal when overproduced (Weisemann and Weinstock, 1988), and still another mutation that affects monomer–monomer interactions, *recA(H-97A)*, is apparently toxic because it could be overexpressed only in the presence of *recAwT* (Nguyen *et al.*, 1993).

Two mutant RecA proteins characterized by Bryant and colleagues, the RecA(G-160N) and RecA(H-163A) proteins, behave very similarly to RecA142 protein (Bryant, 1988; Muench and Bryant, 1990; 1991; Meah and Bryant, 1993; Pinsince *et al.*, 1993). These proteins are unable to promote ATP-dependent DNA strand exchange at neutral pH (pH 7.5), but can at pH values from 6.0 to 6.8, or in the presence of either ATP γ S or dATP. The ssDNA-dependent ATPase activity of these proteins is completely inhibited by SSB protein at neutral pH and above, but becomes SSB protein resistant at pH values lower than 6.8 (Bryant, 1988; Muench and Bryant, 1990; 1991; Meah and Bryant, 1993). A pH-dependent decrease in the K_m^{ATP} value for these mutant proteins correlates with the activation of DNA strand exchange activity at the lower pH values, but the structural basis for this pH-dependent isomerization has not been defined (Pinsince *et al.*, 1993).

Suppression of the recombinational deficiency of the RecA142 protein that occurred by a decrease in pH, both *in vitro* (at pH 6.2) and *in vivo* (in the presence of propionic acid), may have the same origin as suppression by the presumed enhanced monomer–monomer interactions afforded by the Q-124L mutation. As ATP γ S also ‘suppresses’ the *in vitro* defect in DNA strand exchange activity of RecA142 protein (Kowalczykowski, 1991b), this allows us to speculate about the mechanism of enhancement of RecA protein’s recombination function by low pH. We suggest that low pH conditions strengthen monomer–monomer interactions, perhaps through protonation of surface residues. These enhanced interactions would result in an increased apparent binding affinity for ssDNA and dsDNA, because the observed affinity of RecA protein for DNA derives from two thermodynamically distinct contributions: (i) the intrinsic affinity that a RecA monomer has for DNA; and (ii) the co-operative stabilization derived from protein–protein contacts (Kowalczykowski, 1991a). Thus, from the perspective of observed DNA binding affinity, a mutation can affect either the direct interactions

with DNA that affect the DNA-binding affinity of an individual monomer, or the monomer–monomer interactions that affect co-operative interactions between those monomers. Therefore, a mutational defect that reduces protein–DNA interactions can be compensated by a second mutation that sufficiently increases co-operative protein–protein interaction, and vice versa. Finally, DNA binding and nucleoside triphosphate binding are thermodynamically linked (an increased affinity for DNA results in an increased affinity for nucleoside triphosphate). Therefore, an increase in the apparent affinity for ssDNA would be reflected experimentally as a decrease in the apparent dissociation constant (K_m) for nucleotide cofactor (Kowalczykowski, 1991a). Indeed, UTP is hydrolysed by RecA protein at neutral pH, with a K_m^{UTP} of 147 μM at pH 8.0, and is reduced to 33 μM at pH 6.2 (Weinstock *et al.*, 1981a).

Our proposal that low-pH conditions enhance monomer–monomer interactions which, in turn, increase the apparent affinity for DNA is consistent with the following facts: (i) a decrease in the pH increases the nucleation-limited association of RecA protein with ssDNA (Chabbert *et al.*, 1987); (ii) RecA protein displaces SSB protein from ssDNA faster at acidic pH (unpublished observations); and (iii) RecA protein assembles on dsDNA with a shorter lag time at lower pH values. In addition, the DNA-independent ATPase activity of RecA protein has an optimum at pH 6.2 (Weinstock *et al.*, 1981b). This feature can be explained by enhanced monomer–monomer interactions that allow limited assembly of monomers into filaments in the absence of DNA, a behaviour that would be analogous to the salt-stimulated formation of RecA protein filaments and the resultant activation of ATPase activity (Pugh and Cox, 1988). Whether the RecA(Q-124L) protein displays higher DNA-independent ATPase remains to be elucidated, but preliminary studies show that RecA(Q-124L) protein possesses enhanced dsDNA binding affinity at neutral pH (unpublished observations).

The expression in *E. coli* of many heterologous RecA analogues is toxic: examples include the RecA proteins from *P. aeruginosa*, *B. subtilis* and *D. radiodurans*, and the Rad51 protein from *S. cerevisiae*. Although the reason for toxicity could be different in each case, it is reasonable to imagine that an enhanced affinity for DNA is responsible. This is true for RecA protein from *P. aeruginosa* (Namsaraev *et al.*, 1998), RecA(E-96D) protein (Campbell, 1997; Campbell and Davis, 1999a,b), Rad51 protein from *S. cerevisiae* (Sung, 1994; Zaitseva *et al.*, 1999) and probably RecA(Q-124L) (unpublished observations). It is tempting to speculate that modulation of monomer–monomer interaction strength is used by the family of RecA-like proteins to accommodate the different growth and intracellular conditions of these widely disparate organisms. Given the ubiquitous presence of RecA-like proteins, modulation of protein–protein interactions would afford an additional

Table 3. Bacterial and bacteriophage strains used in this study.

| Strain | Relevant genotype | Source/reference |
|----------------|--|--------------------------|
| JC10289 | $\Delta recA_{306}::Tn10$ | A. J. Clark's collection |
| DM1623 | <i>lexA3 sfiA11</i> | A. J. Clark's collection |
| DK1 | $\Delta recA_{306}$ | Kurnit (1989) |
| EZ1996 | <i>lexA3 sfiA11</i> $\Delta recA_{306}::Tn10$ | This work |
| $\lambda\#873$ | $\lambda b1453 \Delta(int red gam) Cl857 \chi76$ | G. Smith's collection |

means of tailoring the function of each RecA protein to the specific needs of a given organism.

Experimental procedures

Bacteria, media and growth conditions

All strains used in this study are listed in Table 3. EZ1996 (*lexA3 sfiA11* $\Delta recA_{306}::Tn10$) was constructed from DM1623 in two steps. First, DM1623 was cured of *Tn10* using a standard curing protocol (Maloy and Nunn, 1981). Then, the deletion, $\Delta recA_{306}::Tn10$, was transferred to DM1623 by P1 transduction from JC10289.

The toxic effect of RecAQ-124L protein expression was observed by monitoring cell growth as follows. Cells [EZ1996 pPROEX*recA(Q-124L)*] were grown at 37°C in Luria–Bertani (LB) media supplemented with ampicillin (100 µg ml⁻¹) and divided into two portions at a cell density of 2×10^8 . One portion was induced with IPTG (1 mM), and both portions were incubated for an additional 5 h. Aliquots were taken at the indicated times, and cells were plated on LB agar media supplemented with ampicillin at a concentration of 100 µg ml⁻¹. Nitrofurantoin and propionic acid were from Sigma and were used at concentrations of 2 µg ml⁻¹ and 40 mM respectively.

Plasmids

pT7-7*recA* plasmid was generated by polymerase chain reaction (PCR) amplification of the entire *recA* gene from pUC21*recA* (Zaitsev *et al.*, 1994), using two synthetic oligonucleotide primers: 5'-AACATATGGCTATCGACGAAAACAAA-3', as the forward primer; and 5'-GATAAGCTTCTGTCATGGCATATCCCTA-3', as the reverse one. These primers contain a unique *NdeI* restriction site immediately upstream of the ATG initiation codon and a unique *HindIII* restriction site downstream of the *recA* gene terminator respectively. The *recA* gene was cloned into the similarly digested pT7-7 plasmid, kindly provided by S. Tabor. The plasmid, *precA142*, was constructed using the same PCR strategy, using chromosomal DNA from JWR259 as a template for PCR (Kowalczykowski *et al.*, 1989). The plasmid, *precA*Δ9, encoding RecA protein truncated at the N-terminus (RecAΔ9), was generated using the same PCR approach, except that the forward primer was 5'-ATCATATGGCGCTGGCGGCCGCACTTGGCCAGATTGA-3' (Zaitsev and Kowalczykowski, 1998b). For all of the plasmid constructs, RecA protein expression was from a constitutive promoter that is independent of *lexA* repression. This ensured a constant level of RecA protein expression and therefore permitted an appropriate phenotypic comparison among mutants that possess different levels of LexA repressor cleavage activity (e.g. RecA142 protein). The plasmid pPROEX*recA(Q-124L)* was constructed by substituting the *Clal*–*AccI* fragment

of the *recAwt* gene with the same DNA fragment bearing the *recA(Q-124L)* mutation. All of the aforementioned cloned *recA* constructs were confirmed by sequencing of the cloned regions.

Functional analyses

Complementation by the plasmid-borne *recA* alleles was tested in *E. coli* JC10289 ($\Delta recA_{306}::Tn10$). For a qualitative survey of UV light sensitivity, cells were irradiated in a streak on the surface of an LB plate as described previously (Nastri *et al.*, 1997). For a quantitative test, strains containing plasmid were grown in liquid L broth with ampicillin (100 µg ml⁻¹) to a density of 10^8 cells ml⁻¹, sedimented and then resuspended at the original density in medium M9. A 5 ml portion in glass Petri dishes was irradiated with a UV dose of 10 J m⁻², with gentle swirling, under a Sylvania 15 W germicidal lamp. Aliquots (200 µl) were removed, and serial dilutions were plated onto LB agar plates with ampicillin (100 µg ml⁻¹) to determine survival. For NF sensitivity, cells bearing *recA*-encoding plasmids were plated on LB agar plates containing both ampicillin (100 µg ml⁻¹) and NF (2 µg ml⁻¹); where indicated, propionic acid (40 mM) was present in these plates, onto which the cells (200 µl) from serial dilutions were plated. For bacteriophage plating, $\lambda\#873$ (*b1453* $\Delta(int red gam)$ *Cl857* $\chi76$) was used.

DNA strand exchange reaction

RecA142 protein was purified as published (Kowalczykowski *et al.*, 1989). DNA strand exchange was performed using DNA purified from M13mp7 bacteriophage as published (Lauder and Kowalczykowski, 1993), except that 25 mM Na-Mes buffer (pH 6.2) was used instead of Tris acetate buffer. The concentrations of RecA protein, SSB protein, ssDNA and dsDNA were 3 µM, 0.45 µM, 5 µM (nucleotides) and 10 µM (nucleotides) respectively. RecA protein and ssDNA were incubated together at 37°C for 2 min in reaction buffer [25 mM Na-Mes, pH 6.2, 10 mM Mg acetate, 0.1 mM dithiothreitol (DTT), 1 mM ATP, 3 mM phosphoenolpyruvate (PEP), 15 units ml⁻¹ pyruvate kinase] to form the RecA protein–ssDNA complex. SSB protein was then added, and DNA strand exchange was started by the addition of dsDNA. Aliquots were taken at the times indicated, and the reaction was stopped by the addition of 0.1 volume of 0.5% SDS and 0.25 M EDTA. The samples were analysed by electrophoresis through a 0.75% agarose gel for 6 h at 1.6 V cm⁻¹. After staining the gel in ethidium bromide (1 µg ml⁻¹) and destaining in distilled water, a digital photoimage was taken using the Gel Printer 2000I system (Bio Photonics).

DNA cycling sequencing

DNA cycling sequencing was carried out either manually using a CircumVent Thermal Cycle DNA sequencing kit (New

England Biolabs) or automatically using an ABI Prizm 377 DNA sequencer. Analysis of the DNA sequencing electrophoretograms was carried out using the computer program CHROMAS (version 1.41). The primers used for sequencing were: 5'-GATGTCGACGCCAGTTTACGTGCGTAGA-3' (#3EcoL), 5'-CCGGTAAAACACGCTGACGCTGC-3' (#12PstR) and 5'-CTGCGCCGTTTAAACAGGCTGAA-3' (#2EcoR) to read *recA* gene sequence upstream of the *EcoRI* restriction site and downstream of the *PstI* and of *EcoRI* restriction sites respectively.

Protein modelling

Protein modelling was carried out using molecular visualization programs WEPLAB VIEWER version 1.1 (Molecular Simulation) and RASMOL version 2.5 (Glaxo Research and Development). The substitution Q-124L was made using SWISS-PDBVIEWER version 2.6 (Geneva Biomedical Research Institute).

Light microscopy

Wet-mounted living cells were observed under a Leitz Orthoplan light microscope equipped with a Leitz Vario Orthomat 2 automatic microscope camera and a Leitz MPV compact microscope photometer. Cells [EZ1996 pPROEX*recA(Q-124L)*] were grown at 37°C for 3 h after induction by IPTG (1 mM).

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