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recA730-dependent suppression of recombination deficiency in RecA loading mutants of *Escherichia coli*

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Abstract

Homologous recombination is an essential process in double-strand break repair. The main requirement for recombination is formation of a RecA filament. Double-strand breaks can be processed into a RecA filament by the action of three enzymatic activities: helicase, 5′-3′ exonuclease and RecA loading onto ssDNA. These activities are provided by the RecBCD enzyme in wild type cells or by the RecF pathway gene products in *recBC sbcBC(D)* cells. In the *recBD1080A* mutant (*recB** mutant), the recombination machineries of RecBCD and RecF pathways are interchangeable and include RecB*CD enzyme (helicase), RecJ (5′-3′ exonuclease) and RecFOR (RecA loading). The mutant RecA730 protein is able to produce a RecA filament without the help of RecFOR mediators, since it more efficiently competes with SSB protein for ssDNA than the normal RecA protein. It was previously shown that the *recA730* mutation suppresses UV sensitivity in a *uvrA recFOR* genetic background. We tested whether the *recA730* mutation can suppress recombination and DNA repair deficiency in a *recB** mutant and its derivatives. We show that the *recA730* mutation suppresses recombination deficiency in a *recB* recFOR* background, where the defect is at the level of RecA loading, but not in the *recB* recJ* background where the defect is at the level of nuclease activity.

Keywords: RecA loading; RecA730; RecBCD; RecFOR; Recombination

1. Introduction

Homologous recombination is essential for DNA repair and the maintenance of genome integrity, as well as for producing new combinations of alleles during the process of conjugation in bacteria and gametogenesis in eukaryotes. A critical step in recombination is formation of a RecA-ssDNA filament (RecA filament) which searches for homology and catalyzes strand exchange between two DNA molecules (Dillingham and Kowalczykowski, 2008). Another role of the RecA filament is induction of an SOS response (Radman, 1975; Witkin, 1976), where it functions as a co-protease in autocleavage of the LexA repressor. This enables enhanced expression of more than 50 genes, since cleaved LexA has no affinity for SOS boxes (Michel, 2005). The RecA filament is formed after processing of DNA damage, i.e., double-strand breaks (DSBs) and single-strand gaps (SSGs). In wild type (wt) *Escherichia coli*, DSBs are processed by the RecBCD enzyme, whereas SSGs are processed by the proteins of the RecF recombination pathway (Kowalczykowski, 2000). Three essential biochemical activities are required for the processing of DSBs into RecA filaments: helicase, 5′-3′ exonuclease and RecA loading onto ssDNA. The RecBCD enzyme, after interaction with the Chi site (GCTGGTGG), manifests all of these activities and is responsible for the processing of DSBs in wt cells (Kowalczykowski, 2000; Dillingham and Kowalczykowski, 2008). However, when the RecBCD enzyme is inactive or absent, as is the case in *recBC sbcBC(D)* mutants, DSBs are processed into RecA filaments by the action of the proteins from the RecF recombination pathway, i.e., RecQ (helicase), RecJ (5′-3′ exonuclease) and RecFOR (RecA loading) (Kowalczykowski, 2000). There is a specific mutation in the *recB* nuclease center (when aspartic acid is replaced by alanine at position 1080, i.e., *recBD1080A*) which partially affects the RecBCD function preserving helicase

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but eliminating nuclease and RecA loading activities (Yu et al., 1998; Anderson et al., 1999; Wang et al., 2000). Hereafter, we will refer to the *recBD1080A* mutation as *recB**. In the *recB** mutant, the components of the two main recombination pathways (RecBCD and RecF) are interchangeable, and formation of a RecA filament is dependent on the RecB*CD enzyme (helicase), RecJ (5′-3′ exonuclease) and RecFOR (RecA loading) (Ivančić-Baće et al., 2003; Amundsen and Smith, 2003).

Binding of the RecA protein to ssDNA occurs in two phases: nucleation and filament extension (Shereda et al., 2008). If SSB is already bound to ssDNA, then the wt RecA protein cannot perform the nucleation reaction without the help of mediators, i.e., the RecFOR proteins. A specific mutant *recA730* (RecA E38K) encodes a form of RecA protein which is able to achieve the nucleation reaction without the help of RecA loading mediators RecFOR (Lavery and Kowalczykowski, 1990; Eggler et al., 2003). This is supported by suppression of UV sensitivity of *recFOR* mutants by the *recA730* allele in a *uvrA* genetic background (Wang et al., 1993), and the fact that the *recA730* mutant exhibits constitutive SOS expression (Witkin et al., 1982; Tessman and Peterson, 1985; Ennis et al., 1995). An additional SOS constitutive mutant *recA441* encodes a form of RecA protein with two point mutations. One is E38K (equivalent to *recA730*) and the second is I298V. The *recA441* allele suppresses the UV-sensitive phenotype of *recF* mutants in *sfiA* and *uvrA* genetic backgrounds (Thoms and Wackernagel, 1988). It was also shown that the RecA441 protein has an enhanced ability to compete with SSB protein for ssDNA in vitro (Lavery and Kowalczykowski, 1990). The E38K (*recA730*) mutation is responsible for efficient competition with the SSB protein and consequently constitutive SOS expression, whereas the I298V mutation is responsible for the suppression of this phenotype at permissive temperatures.

In this paper, we studied the effect of the *recA730* mutation on recombination and DNA repair in the *recB** mutant and its derivatives. In order to avoid SfiA-dependent inhibition of cell division due to constitutive SOS expression caused by the *recA730* allele, all strains were in an *sfiA* genetic background. Since the *recB** mutation is not a null mutation (it affects nuclease and RecA loading activities but preserves helicase), this enabled us to specifically dissect enzymatic activities required for RecA filament formation. The *recB** mutant is resistent to UV and γ irradiation and is moderately proficient in conjugational recombination (Ivančić-Baće et al., 2003). The *recB* recO* double mutant has abolished RecA loading functions and exhibits recombination and DNA repair-deficient phenotypes. This recombination and DNA repair deficiency is very strong, and is comparable to that of *recB* null mutants (Ivančić-Baće et al., 2003). We show that the *recA730* mutation suppresses recombination and DNA repair deficiency in a *recB* recO* genetic background. The *recB* recJ* double mutant shows a similar deficiency in recombination and DNA repair. This phenotype is caused by different functional changes: the *recB* recJ* strain has abolished nuclease activities (RecB*CD and RecJ), but exhibits helicase activity (RecB*CD enzyme) and RecA loading (RecFOR proteins). The *recA730* mutation cannot suppress recombination and DNA repair deficiency in a *recB* recJ* background. These results are in agreement with the fact that the RecA730 protein can be loaded onto ssDNA without the help of RecFOR proteins. The results also emphasize the essential role of the nuclease in recombination.

2. Materials and methods

2.1. Bacterial strains and bacteriophages

The *E. coli* strains used in this study are derivatives of AB1157 and are listed in Table 1. All strains have an *sfiA* mutation to avoid inhibition of cell division. The N5208 strain was kindly provided by C. Buckman and R.G. Lloyd from the University of Nottingham, England. The LMM1934 was kindly provided by D. Zahradka from the Ruđer Bošković Institute, Croatia. New strains were constructed by P1 transduction, as described by Miller (Miller, 1992). In some bacterial strains, *recB** and *recA730* mutations were additionally confirmed by sequencing. The *recB268* and *recB270* mutations are the *recB* null alleles.

2.2. Media and growth conditions

Composition of LB broth and M9 medium was described previously (Ivančić-Baće et al., 2003, Ivančić-Baće et al., 2005). Bacteria were grown in LB medium at 37 °C with aeration to log phase (OD_{650} ∼ 0.4) and then used for determination of cell survival after γ or UV irradiation and for conjugational crosses.

2.3. Cell survival after γ- or UV irradiation

After reaching log phase, 0.01 ml aliquots of appropriate dilutions of the bacterial culture were spotted on LB plates. For γ irradiation, a ^{60}Co source with a dose rate of 4.3 Gy/s was used. For UV irradiation, a 30-W Philips low-pressure Hg germicidal lamp was used at the distance of 1 m. The incident dose was ∼ 0.25 mW/cm^2 as determined with a VLX-3 W UV dosimeter (Bioblock, Illkirch, France). Bacteria were irradiated at room temperature. Surviving cells formed visible colonies during overnight incubation at 37 °C and colonies were counted the next day. Cell survival is the ratio of the number of cells in a culture after an appropriate gamma dose or UV dose and the number of cells in control culture.

2.4. Conjugational crosses

Hfr crosses were performed as described previously (Miller, 1992). Strain V1306 was used as a donor and the selected marker was His$^+$. After reaching log phase, donor and recipient strains were mixed at a ratio of 1:10. Matings were performed in a water bath at 37 °C for 30 min with light agitation. The ex-conjugant mixture was interrupted by vigorous agitation, serially diluted and plated on M9 plates supplemented with 1 mg of thiamine, appropriate amino acids and 16 g of agar (Maršić et al., 1993). A total of 100 μg of streptomycin per ml was also added to M9 plates to counter-select donor cells.