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Original article

The Walker A motif mutation *recA4159* abolishes the SOS response and recombination in a *recA730* mutant of *Escherichia coli*

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Abstract

In bacteria, the RecA protein forms recombinogenic filaments required for the SOS response and DNA recombination. In order to form a recombinogenic filament, wild type RecA needs to bind ATP and to interact with mediator proteins. The RecA730 protein is a mutant version of RecA with superior catalytic abilities, allowing filament formation without the help of mediator proteins. The mechanism of RecA730 filament formation is not well understood, and the question remains as to whether the RecA730 protein requires ATP binding in order to become competent for filament formation. We examined two mutants, *recA730,4159* (presumed to be defective for ATP binding) and *recA730,2201* (defective for ATP hydrolysis), and show that they have different properties with respect to SOS induction, conjugational recombination and double-strand break repair. We show that ATP binding is essential for all RecA730 functions, while ATP hydrolysis is required only for double-strand break repair. Our results emphasize the similarity of the SOS response and conjugational recombination, neither of which requires ATP hydrolysis by RecA730.

Keywords: RecA730 protein; ATP binding; Filament formation

1. Introduction

The RecA protein, complexed with single-stranded DNA, forms a RecA filament which is the functional form of RecA involved in recombination, recombinational DNA repair and induction of the SOS response. The RecA filament is formed after processing of DNA damage and depends on three enzymatic activities, i.e., helicase, 5′-3′ exonuclease and RecA loading onto single-strand (ss) DNA [1]. The most important types of DNA damage are double-strand breaks (DSBs) and single-strand gaps (SSGs). A DSB is any DNA structure with a double-strand end, such as those found at collapsed replication forks. Collapsed replication forks are formed under normal physiological conditions when the replisome encounters a nick or gap on one DNA strand [2]. DSBs can also be formed after γ-irradiation or induced by restriction enzymes. In wild type (*wt*) bacteria, DSBs are processed by the RecBCD enzyme, which provides all three enzymatic activities required for production of a RecA filament [1,3]. DSBs can also be processed by RecF pathway gene products, i.e., RecQ (helicase), RecJ (5′-3′ exonuclease) and RecFOR (RecA loading onto ssDNA), if the RecBCD pathway is inactive, as is the case in the multiple mutant *recBC sbcBC(D)* [4]. In addition, the RecA filament can be formed by a hybrid recombination machinery which operates in the *recB1080* mutant [5–8]. The RecB1080CD protein has a mutation in the *recB* nuclease center, and is deficient for both nuclease activity and the ability to load RecA protein onto ssDNA [9–11]. In the *recB1080* mutant, DSBs are processed into a RecA filament by the concerted action of RecB1080CD (helicase), RecJ (5′-3′ exonuclease) and RecFOR (RecA loading onto ssDNA) [5].

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Single-strand gaps (SSGs) arise as the result of reinitiation of DNA replication downstream of primary non-coding lesions such as pyrimidine dimers. In *wt* bacteria, SSGs are processed into a RecA filament by the action of products of the RecF pathway recombination genes [1,12–17].

In order to promote RecA filament formation, the *wt* RecA protein needs to interact with ATP and mediator proteins (RecBCD or RecFOR) [1,3,4]. RecA-ATP binding is crucial for formation of an active RecA conformation. As a consequence of an allosteric change after ATP binding, the RecA-ATP complex shifts to a high affinity DNA binding state [18–22]. Following ATP hydrolysis, the high affinity DNA binding state, RecA-ATP (part of the extended filament) is converted into a low affinity DNA binding state, RecA-ADP (part of a compressed filament), whereupon RecA is subsequently released from DNA [23]. Binding of RecA protein to ssDNA occurs in two steps, i.e., nucleation and filament extension, wherein the SSB protein inhibits the nucleation step [24]. The *wt* RecA protein has a lower affinity for ssDNA than the SSB protein, and consequently cannot promote the nucleation step without the help of RecBCD or RecFOR proteins [25–27]. The RecA filament interacts with a second DNA molecule, promotes the search for homology and participates in strand exchange between two homologous DNA molecules [1]. Such activities are important for the process of homologous recombination and DNA repair in vivo. The RecA filament also acts as a co-protease in the process of autodigestion of the LexA repressor [28]. This activity of RecA causes induction of an SOS response in vivo, whereupon the expression of more than 50 genes is enhanced [29–31]. These genes are involved in multiple pathways including DNA repair, recombination, mutagenesis and inhibition of cell division.

The critical domains of RecA protein relevant to ATP binding and hydrolysis are two well defined motifs known as Walker A and Walker B motifs. These motifs are also present in many other proteins that have ATP hydrolytic activity [32]. The mutation *recA2201* represents a change in the Walker A motif at amino acid site 72, where the conserved lysine residue is replaced by an arginine (K72R). The resulting RecA K72R (RecA2201) protein is able to bind ATP, but is no longer able to hydrolyze it [33]. In vitro, the RecA2201 protein has the ability to form only short filaments in the presence of ATP, whereas the *wt* RecA protein forms long extended filaments [34]. The ability of the RecA2201 protein to produce filaments is enhanced when dATP is present instead of ATP [34]. In addition, the RecA2201 protein does not facilitate LexA cleavage when ATP is present [34]. However, when dATP is present, the RecA2201 protein facilitates LexA cleavage almost as well as the *wt* RecA protein [34]. It is important to emphasize that reactions with ATP are biologically more relevant since the in vivo concentration of ATP is 10 times higher than the concentration of dATP [35]. In addition, a *recA2201-gfp* strain shows a 40% reduction in the ability to form filaments (foci) in vivo compared to the *wt recA-gfp* strain, but is completely deficient in the ability to form new foci after UV irradiation [36]. In genetic assays, the *recA2201* mutant exhibits a *recA* null phenotype (high sensitivity to UV irradiation, low conjugational recombination frequency and no SOS induction after UV irradiation) [36]. A second mutation in the Walker A motif at amino acid position 72 is *recA4159*, where the lysine is replaced by an alanine (K72A). In many other proteins involved in ATP binding and hydrolysis, this particular mutation causes a defect in ATP binding [37–41]. Therefore, it is predicted that the RecA K72A (RecA4159) protein is deficient in ATP binding and consequently, all RecA catalytic activities. The fact that the *recA4159-gfp* strain is unable to form filaments (foci) in vivo, as well as the observation that *recA4159* exhibits a *recA* null phenotype [36], support this prediction. The experiments with *recA4159* and *recA2201* single mutants provide a framework for understanding the *wt* RecA function in the process of filament formation.

Another important mutant in the study of RecA function is the *recA730* mutant (E38K). The RecA730 protein has the extraordinary property of being better able to compete with SSB for ssDNA than is the *wt* RecA protein [25,26]. Consequently, the RecA730 protein is able to promote the nucleation step in filament formation without the help of mediator proteins, and is able to produce extended filaments. Consistent with this, the *recA730* strain shows a constitutive SOS (cSOS) response, resistance to UV irradiation and *wt* levels of conjugational recombination [42–44]. In addition, the *recA730* mutation is a suppressor of recombination deficiency in *recFOR* mutants [45–47]. An important strain for the study of RecA730-mediated ATP hydrolysis is the double mutant *recA730,2201* (a combination of E38K and K72R point mutations). The double mutant RecA730,2201 protein is able to bind ATP, but is deficient in ATP hydrolysis [34]. In addition, the RecA730,2201 protein produces filaments on circular ssDNA and facilitates cleavage of the LexA repressor similar to *wt* RecA in vitro [34]. In vivo, the *recA730,2201* double mutant shows cSOS expression and a UV-induced SOS response (both are weaker than in the *recA730* single mutant) [34]. In contrast, the sensitivity to UV irradiation of the *recA730,2201* double mutant is very strong (only slightly less sensitive than the *recA* null mutant) [34]. These results lead to the conclusion that SOS induction mediated by the RecA730 protein does not require ATP hydrolysis for formation of an extended filament [34]. However, the question that remains is whether the RecA730 protein requires ATP binding in order to become competent for filament formation.

The mechanism of filament formation by the RecA730 protein is poorly understood. In this paper, we address the question of how ATP binding affects both the SOS response and recombination in a *recA730* mutant background. We found that the *recA4159* mutation eliminates the cSOS response, as well as the SOS response, after introduction of a DSB (DSB-SOS) in the *recA730* and *recB1080 recA730* genetic backgrounds. We also found that introduction of the *recA4159* allele into *wt*, *recB1080*, *recA730* and *recB1080 recA730* backgrounds makes the resulting strains sensitive to γ-irradiation and deficient for conjugational recombination, similar to that of a *recA* null mutant. These results strongly support the idea that ATP binding is essential for all functions of the RecA730 protein. The *recA730,2201* double mutant is able to

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