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Gamma-irradiated RecD overproducers become permanent *recB*$^{-}$/*C*$^{-}$ phenocopies for extrachromosomal DNA processing due to prolonged titration of RecBCD enzyme on damaged *Escherichia coli* chromosome

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Abstract

The RecBCD enzyme of *Escherichia coli* consists of three subunits RecB, RecC and RecD. RecBCD enzyme activities are regulated by its interaction with recombination hotspot Chi. Biochemical and genetic evidence suggest that interaction with Chi affects RecD subunit, and that RecD polypeptide overproduction antagonizes this interaction, suggesting that intact RecD replaces a Chi-modified one. We used bacteria with fragmented chromosomes due to double-strand breaks inflicted by UV and γ-irradiation to explore in which way increased concentrations of RecBCD's individual subunits affect DNA metabolism. We confirmed that RecD overproduction alters RecBCD-dependent DNA repair and degradation in *E. coli*. Also, we found that RecB and RecC overproduction did not affect these processes. To determine the basis for the effects of RecD polypeptide overproduction, we monitored activities of RecBCD enzyme on γ-damaged chromosomal DNA and, in parallel, on λ and T4 *2* phage DNA duplexes provided at intervals. We found that γ-irradiated wild-type bacteria became transient, and RecD overproducers permanent *recB*$^{-}$/*C*$^{-}$ phenocopies for processing phage DNA that is provided in parallel. Since this inability of irradiated bacteria to process extrachromosomal DNA substrates coincided in both cases with ongoing degradation of chromosomal DNA, which lasted much longer in RecD overproducers, we were led to conclude that the RecB^{-}/C^{-} phenotype is acquired as a consequence of RecBCD enzyme titration on damaged chromosomal DNA. This conclusion was corroborated by our observation that no inhibition of RecBCD activity occurs in γ-irradiated RecBCD overproducers. Together, these results strongly indicate that RecD overproduction prevents dissociation of RecBCD enzyme from DNA substrate and thus increases its processivity.

Keywords: Double-strand break repair; λ *red gam* phage recombination; T4 *2* phage plating; RecBCD enzyme processivity

1. Introduction

A vast majority of homologous recombination and double-strand break (DSB) repair in wild-type *Escherichia coli* is initiated by RecBCD enzyme (for reviews, see [1,2]). That is why *recB* and *recC* null mutants, deprived of all RecBCD enzyme activities, are extremely sensitive to DNA damaging agents (such as UV and gamma (γ)-irradiation) [3], inefficiently recombine DNA molecules [4], and are poorly viable [5]. RecBCD enzyme is a powerful heterotrimeric helicase/nuclease (ExoV), which processes DNA duplexes containing blunt or nearly blunt dsDNA end(s). Upon binding to a dsDNA end, the enzyme unwinds the DNA molecule driven by helicase subunits RecB and RecD [6,7]. The unwinding is accompanied with vigorous degradation of a 3′-ended strand at the entry site, and with weaker degradation of a 5′-ending strand [8].

RecBCD enzyme activities are regulated by its interaction with a specific, properly oriented, 8-nt DNA sequence named Chi. When RecBCD encounters a Chi sequence, it pauses tem-

Abbreviations: DSB, double-strand break; EOP, efficiency of plating; γ, gamma rays; kbp, kilo base pair; MOI, multiplicity of infection; UV, ultraviolet light.

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porarily and then continues moving along the DNA, although with reduced rate [9] and exhibiting weaker nuclease activity of switched polarity (from predominant 3′–5′ to 5′–3′) [10]. This change in extent and polarity of RecBCD nuclease activity enables a Chi-modified enzyme to produce a 3′-terminating single strand overhang, onto which it starts loading RecA protein, thereby creating a nucleoprotein filament [11]. As a consequence, most of the recombinational exchanges in wild-type *E. coli* are focused at Chi [12], which is therefore called a recombination hot spot. Genetic and biochemical evidence [9,13–16] have suggested that Chi imposes its influence on RecBCD enzyme via the RecD subunit, which becomes modified upon interaction with Chi. This modification is probably not ejection (since in vitro studies failed to detect RecD subunit detaching from Chi-modified enzyme [17,18]) but rather inactivation. This conclusion is based on remarkable similarities in behavior of RecBC enzyme, which is devoid of the RecD subunit, and Chi-modified RecBC(D). RecBC does not recognize Chi (resembling Chi-modified enzyme, which is unable to recognize another Chi site provided *in cis* or *in trans* [19]), but catalyzes homologous recombination with efficiency similar to that of Chi-modified RecBC(D) [20]. Recombinational exchanges in *recD* mutants are focused at dsDNA ends instead at a Chi site [21], apparently due to constitutive loading of RecA protein by RecBC onto the 3′-terminating overhang that it produces [22]. RecBC enzyme also lacks detectable nuclease activity and unwinds DNA with reduced speed [23] and processivity [24], compared to RecBCD. Furthermore, when in wild-type *E. coli* RecBCD enzyme is saturated with Chi sites (provided on plasmid or on fragmented chromosomal DNA), these cells become *recD*$^-$ phenocopies, i.e. the Chi-modified enzyme looses its nuclease activity (enabling an efficient T4 *2* mutant phage plating) and ability to interact with another Chi site while catalyzing recombination exchanges focused at dsDNA ends in λ crosses [15,16]. Some of these phenotypic changes can be antagonized by a RecD polypeptide overexpression. An excess of RecD polypeptide reverses the Chi effect, causing recombination exchanges in λ crosses not to be focused at dsDNA ends any more [15]. These results suggest that an intact RecD polypeptide in excess can replace a Chi-modified RecD subunit by mass action. The importance of RecD inactivation upon RecBCD interaction with Chi, and subsequent reversal of that reaction by RecD polypeptide overproduction is outlined by distinct phenotype of RecD overproducing bacteria. RecD overproducers have impaired DSB repair, increased DNA degradation [25], lower viability [26] and reduced recombination efficiency [15]. The RecBCD enzyme activities are apparently deregulated in RecD overproducers (resulting in "reckless" degradation of γ-irradiated chromosomes [25]), suggesting that overexpression of RecD antagonizes the Chi effect by mass action and thus changes the behavior of Chi-modified RecBCD, which affects cellular physiology. A crystal structure of RecBCD-DNA complex revealed a structural basis for exchange of RecD subunits in Chi-modified enzyme since tight contacts between RecB and RecC subunits were observed, while RecD was much more loosely connected to RecC subunit only [27]. However, the means by which an excess of RecD polypeptide imposes its effect on *E. coli* cell is still unclear.

The aim of this study was to elucidate the mechanism by which an excess of RecD polypeptide affects RecBCD enzyme behavior in *E. coli*. Furthermore, since increase in concentration of RecBCD and RecBC enzymes and also of RecD polypeptide in *E. coli* results in deterioration of DNA repair and homologous recombination [28,15,25], we wanted to examine the effect of increased concentrations of RecB or RecC polypeptide on some DNA metabolic processes, namely DNA repair and degradation.

2. Materials and methods

2.1. Bacterial strains, plasmids and phages

We used AB1157, a standard recombination- and repair-proficient strain (F$^-$ *thr-1 ara-14 leuB6 Δ[gpt-proA]62 lacY1 tsx-33 supE44 galK2* λ$^-$ Rac$^-$ *hisG4* [Oc] *rfbD1 mgl-5 rpsL31 kdgK51 xyl-5 mtl-1 argE3* [Oc] *thi-1 qsr*$^-$ [29]), and its *recB268*::Tn*10* derivative DE101 [30]. They were transformed as previously described [31] with the following plasmids: pKI13 is a pUC18 *recD*$^+$ derivative, which increases the cellular concentration of RecD polypeptide about 60-fold [25]; pDW11 (kindly provided by Dr. Wilfried Wackernagel) and pDWS2 are pBR322 derivatives that carry *recC*$^+$ and *recBCD*$^+$ alleles, respectively [32]. The expression of *recB*, *recC* and *recD* genes in these plasmids is controlled by their natural (noninducible) promoters, meaning that the amount of RecC or RecBCD proteins the plasmids produce depends entirely on their copy-number and is therefore expected to be elevated about 25-fold [32]. The gene expression from these plasmids was confirmed by complementation tests: pDW11 rendered *recC* mutants resistant to UV radiation, capable of degrading T4 *2* phage DNA and fully viable, while no effect was observed on *recB* mutants (not shown). Plasmid pDWS2 reduced the efficiency of T4 *2* phage plating on both *recC* and *recB* mutants to the level of wild-type, but only partially restored their resistance to UV irradiation, which is typical for bacteria with increased concentration of RecBCD enzyme [28]. Plasmid pPB700 contains *recB*$^+$ gene under control of *Ptac* inducible promoter [33]. Wild-type cells harboring pPB700 plasmid plated λ *red* mutant phage with reduced efficiency when induced by IPTG (not shown). This effect is due to titration of λ Gam protein by an overexpressed RecB polypeptide [30].

Phage λ crosses were made with *red gam* mutant phages MMS555 (*Jam6 b1453 c*I*857* χ) and MMS754 (*b1453 c*I*857* χ*D Rts129*), kindly provided by Dr. Richard S. Myers. A gene *2* amber mutant of phage T4 was from our laboratory collection.

2.2. Media and growth conditions

Bacteria were grown in LB broth and on LB plates [34] at 37 or 34 °C. The strains containing plasmids were grown in