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Note

Exonuclease Requirements for Recombination of λ-Phage in *recD* Mutants of *Escherichia coli*

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ABSTRACT

Recombination of λ *red gam* phage in *recD* mutants is unaffected by inactivation of RecJ exonuclease. Since nucleases play redundant roles in *E. coli*, we inactivated several exonucleases in a *recD* mutant and discovered that 5′–3′ exonuclease activity of RecJ and exonuclease VII is essential for λ-recombination, whereas exonucleases of 3′–5′ polarity are dispensable. The implications of the presented data on current models for recombination initiation in *E. coli* are discussed.

MOST of homologous recombination in wild-type *Escherichia coli* is initiated by a heterotrimeric RecBCD enzyme, which binds to blunt-ended DNA duplex, unwinds it, and concomitantly degrades both unwound strands. *In vitro*, RecBCD enzyme activities are regulated by its interaction with a χ-sequence, upon which the modified enzyme loses most of its nuclease activities (preserving only a weak 5′–3′ exonuclease activity), but gains the ability to facilitate RecA protein loading onto a thus created 3′-ending tail (reviewed in Kuzminov 1999). In this way a nucleoprotein filament, the central recombination intermediate, is created.

When the enzyme lacks its RecD subunit, as in *recD* mutants, the resulting RecBC enzyme is devoid of any detectable nuclease activity (ExoV$^-$) and also of the ability to interact with χ sites, whereas its helicase and RecA loading activities are preserved (the latter being constitutive, focusing recombination exchanges to DNA ends) (reviewed in Kuzminov 1999). Although ExoV$^-$, *recD* mutants are recombination and DNA repair proficient (Chaudhury and Smith 1984; Lovett *et al.* 1988). This proficiency was shown to rely (to a varying extent) on a single-strand-specific 5′–3′ exonuclease activity of RecJ nuclease (Lovett and Kolodner 1989) in most recombination assays (*e.g.*, UV survival, Hfr conjugation, and P1 transduction) (Lloyd *et al.* 1988; Lovett *et al.* 1988). There is, however, a marked exception in RecJ requirement in *recD* mutants. Recombination of freely replicating λ *red gam* phages, which normally depends on host RecBC(D) functions (since λ's own recombination system is disabled), is unaffected by RecJ nuclease inactivation in *recD* mutants (Thaler *et al.* 1989). This finding [together with the one of the independence of λ-recombination on χ (Thaler *et al.* 1989)] had a great influence on the understanding of regulation of RecBCD enzyme functions in a cell as it led to a long-standing model according to which a χ-modified RecBCD is equivalent to the RecBC enzyme (suggesting that upon interaction with a χ site a RecD subunit is lost or inactivated) (Thaler *et al.* 1988, 1989). Yet, later biochemical studies showed that the 5′–3′ exonuclease activity of the χ-modified enzyme is absent in RecBC enzyme (Anderson *et al.* 1997). The paradoxical RecJ independence of λ *red gam* crosses in *recD* mutants has puzzled researchers for years, leading to several hypotheses. For instance, Amundsen and Smith (2003) proposed that recombination proficiency of a *recJ recD* mutant is due to single-stranded (ss) DNA substrates produced during λ-replication, *i.e.*, that 5′–3′ exonuclease activity is not required for λ-recombination.

However, a recent report (Đermić 2006) has shown that a phenotype of the *recJ recD* mutant does not reflect a need for a 5′–3′ exonuclease activity in a particular reaction; it rather represents a measure of the efficiency with which the lack of RecJ function is replaced by a function of exonuclease VII (ExoVII), another exonuclease with a processive 5′–3′ (and also 3′–5′) exonuclease activity on ssDNA (ssExo) (Chase and Richardson 1974). As that report also showed that the 5′–3′ exonuclease activity is essential for all events tested in *recD* mutants (UV and γ-survival, recombination in Hfr and P1 crosses, cell viability), it is conceivable that unaffected λ-recombination in the *recJ recD* mutant is due to activity of ExoVII. To test this hypothesis,

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TABLE 1

Bacterial and phage strains used

Strain	Relevant genotype	Source or derivation
	AB1157 and derivatives	
AB1157	F⁻ *thr-1 ara-14 leuB6* Δ*(gpt-proA)62 lacY1 tsx-33 supE44 galK2* λ⁻ Rac⁻ *hisG4 rfbD1 mgl-5 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1 qsr⁻*	Bachmann (1996)
DE101	*recB268*::Tn*10*	Đermić *et al.* (2005)
DE302	*recJ2052*::Tn*10kan*	P1 STL113 × AB1157 (Km^r)
DE303	*recJ2052*::Tn*10kan* Δ*xseA18*::*amp*	P1 STL4537 × DE302 (Ap^r)
DE100	*recD1903*::Tn*10d(tet)*	Đermić (2006)
DE1061	*recD1903*::Tn*10d(tet) recJ2052*::Tn*10kan*	Đermić (2006)
DE1052	*recD1903*::Tn*10d(tet)* Δ*xseA18*::*amp*	Đermić (2006)
DE1050	*recD1903*::Tn*10d(tet)* Δ*xonA300*::*cat*	Đermić (2006)
DE1063	*recD1903*::Tn*10d(tet) recJ2052*::Tn*10kan* Δ*xseA18*::*amp*	Đermić (2006)
DE1062	*recD1903*::Tn*10d(tet) recJ2052*::Tn*10kan* Δ*xonA300*::*cat*	Đermić (2006)
DE1054	*recD1903*::Tn*10d(tet)* Δ*xonA300*::*cat* Δ*xseA18*::*amp*	Đermić (2006)
DE1055	*recD1903*::Tn*10d(tet)* Δ*xonA300*::*cat* Δ*exoX1*::*npt*	Đermić (2006)
DE1059	*recD1903*::Tn*10d(tet)* Δ*xonA300*::*cat* Δ*exoX1*::*npt* Δ*xseA18*::*amp*	Đermić (2006)
DE1060	*recD1903*::Tn*10d(tet)* Δ*xonA300*::*cat* Δ*xseA18*::*amp* Δ*sbcD300*::*kan*	Đermić (2006)
	Other strains	
STL4537	Δ*xseA18*::*amp*	S. T. Lovett
STL113	*recJ2052*::Tn*10kan*	Viswanathan and Lovett (1998)
V371	*recA56 recC1010 srl300*::Tn*10* Su°	R. S. Myers
	λ-phage strains	
MMS555	*Jam6 b1453 cI857* χ	R. S. Myers
MMS754	*b1453 cI857* χ*D Rts129*	R. S. Myers

we performed λ *red gam* χ⁺-lytic crosses in *recD* mutants devoid of activities of various exonucleases (Table 1).

As shown in Table 2, RecJ inactivation left λ-recombination in the *recD* mutant background mostly unaffected, confirming earlier reports (Thaler *et al.* 1989; Jockovich and Myers 2001). This proficiency was indeed dependent on ExoVII since the triple RecD⁻ RecJ⁻ ExoVII⁻ mutant was recombination deficient, even more so than the *recB* null mutant. As the RecJ⁻ ExoVII⁻ derivative of the wild-type strain exhibited unaffected recombination proficiency, we may conclude that λ-recombination requires a 5′–3′ exonuclease activity either of χ-modified RecBCD in wild-type cells or of RecJ and ExoVII nucleases in *recD* mutants. This process therefore does not differ from the other DNA end-dependent recombination processes in *E. coli* and other organisms, which rely on resection of a 5′-ending tail (discussed in Đermić 2006). The indispensability of the 5′–3′ exonuclease activity argues against models that posit that a helicase activity is sufficient for initiation of recombination [*i.e.*, "split-end" models (Rosenberg and Hastings 1991), proposing that either of the unwound strands (Razavy *et al.* 1996) or both of them (reviewed in Myers and Stahl 1994; Smith 2001) invade the homologous DNA duplex; also a concept of RecBCD enzyme unwinding and rewinding DNA duplex, with just a nick at a χ site being required for recombination initiation (reviewed in Smith 2001)].

In contrast, inactivation of exonucleases of the opposite, 3′–5′ polarity [exonuclease I (ExoI), ExoVII, exonuclease X (ExoX), and SbcCD] did not reduce λ-recombination markedly, suggesting that it is largely independent of that activity. This is in accord with earlier studies that found that 5′–3′ exonucleases are more important than 3′–5′ ones in DNA repair reactions in both the wild-type (Viswanathan and Lovett 1998) and the *recD* backgrounds (Đermić 2006). The strongest requirement for a 3′–5′ exonuclease was for ExoI (the strongest ssExo acting on a 3′-ending strand; Lehman and Nussbaum 1964) in the *recJ recD* mutant. Since the main role of exonucleases in *recD* mutants is apparently in preventing reannealing of the strands unwound by the RecBC enzyme (Đermić 2006), it seems, therefore, that ExoVII resects a 5′-ending tail less efficiently than RecJ does, thereby increasing the requirement for degradation of the complementary strand. Moreover, inactivation of ExoI in the *recJ recD* mutant might further reduce the efficiency of ExoVII-catalyzed resection of the 5′-ending strand as it might enable the 3′-ending strand to compete for the ExoVII binding. Finally, the observed synergism between ExoI and RecJ ssExos (this study; Đermić 2006) should not come as a surprise, considering that these two proteins interact (form a complex) in a cell (Butland *et al.* 2005), which may provide an additional function for both of them.

TABLE 2

λ *red gam* lytic crosses in *recD* mutants lacking activities of various exonucleases

Strain	Description	Total yield ($\times 10^7$)	Relative J^+R^+ recombinants
DE100	$RecD^-$	94.0	1.0[a]
AB1157	Wild type	9.2	0.28 ± 0.138
DE1061	$RecD^-$ $RecJ^-$	16.2	0.79 ± 0.099
DE302	$RecJ^-$	5.3	0.34 ± 0.230
DE1063	$RecD^-$ $RecJ^-$ $ExoVII^-$	0.25	0.0064 ± 0.0039
DE303	$RecJ^-$ $ExoVII^-$	2.5	0.23 ± 0.092
DE1062	$RecD^-$ $RecJ^-$ $ExoI^-$	8.5	0.13 ± 0.081
DE1050	$RecD^-$ $ExoI^-$	53.0	0.51 ± 0.198
DE1052	$RecD^-$ $ExoVII^-$	97.5	1.03 ± 0.225
DE1055	$RecD^-$ $ExoI^-$ $ExoX^-$	83.0	0.55 ± 0.101
DE1054	$RecD^-$ $ExoI^-$ $ExoVII^-$	14.1	1.22 ± 0.295
DE1059	$RecD^-$ $ExoI^-$ $ExoVII^-$ $ExoX^-$	4.67	1.10 ± 0.302
DE1060	$RecD^-$ $ExoI^-$ $ExoVII^-$ $SbcD^-$	1.85	1.34 ± 0.354
DE101	$RecB^-$	5.1	0.022 ± 0.005

The λ-lytic crosses were performed essentially as described (Đermić *et al.* 2006). Bacteria were grown in tryptone broth with 0.3% maltose at 34° to a density of 10^8 cells/ml (an OD_{600} of 0.25). A total of 0.3 ml of each culture was mixed with 0.1 ml of phage mixture. The multiplicity of infection was 10 for MMS555 (*Jam*) and 0.2 for MMS754 (*Rts*). After 15 min of incubation at 34°, unadsorbed phages (on average, ~1% of total infecting phages) were removed by centrifugation. Infected cells were resuspended in 1 ml of tryptone broth with Nozu supplements (Arber *et al.* 1983) and gently aerated for 90 min at 34°. The remaining cells were then lysed with 0.2 ml of chloroform. The phage titers were determined on V371 host strain at 34° for total Am^+ phage and at 42° for Am^+Ts^+ recombinants. Plaques were counted after 18–24 hr of growth on tryptone plates. The frequency of recombinants was calculated as the recombinant titer divided by the total (Am^+) titer.

[a] Recombination frequency of 1.0 corresponds to 13 J^+R^+ recombinants per 100 J^+ phages. All values are averages of at least three independent experiments ± standard deviations.

Overall exonuclease requirements for λ-phage recombination in *recD* mutants resemble those for P1 transduction in that background (Đermić 2006), which is likely due to the similarity of DNA substrates delivered by the two phages [*i.e.*, linear DNA duplex lacking substantial heterologous regions (discussed in Đermić 2006)].

Total yield of λ-phage (MMS754) was in correlation with the recombination efficiency in most crosses (Table 2), which was expected since recombination is thought to be required for an efficient replication and packaging of λ-progeny (reviewed in Kuzminov 1999). An exception was a low total yield of crosses done in recombination-proficient mutants defective in multiple exonucleases of a 3′–5′ polarity ($RecD^-$ $ExoI^-$ $ExoVII^-$ $ExoX^-$ and $RecD^-$ $ExoI^-$ $ExoVII^-$ $SbcD^-$), which suggested that these exonucleases have a role in the λ-phage life cycle other than the recombinational one. They may participate in replication, as shown for some exonucleases in yeast (Tishkoff *et al.* 1997). A very low total yield in crosses involving the $RecD^-$ $RecJ^-$ $ExoVII^-$ mutant may be due to its recombination deficiency (Table 2). But, as this mutant has an extremely low viability (<1%, Đermić 2006), a low yield may also result from titration of infecting phage on cells that are unable to support its growth. Thus, a simple method was used to determine a fraction of $RecD^-$ $RecJ^-$ $ExoVII^-$ mutant cells that enable phage-λ growth (Đermić and Trgovčević 1999). Bacteria were lysogenized with a thermoinducible λ *cI857* phage and its recombination-deficient *red3* derivative (Arber *et al.* 1983). Cells were grown as for λ-crosses (except for a lower temperature: 31.5°) and, upon reaching an OD_{600} of 0.25, they were serially diluted and mixed with indicator bacteria in soft tryptone agar, poured on tryptone plates, and incubated at 42° (to determine their plaque-forming ability). Compared to the $RecD^-$ mutant, $RecD^-$ $RecJ^-$ $ExoVII^-$ mutant cells produced just 31% less infective centers of λ *cI857* phage, suggesting that although mostly incapable of giving rise to a colony, a majority of them were metabolically active and thus supported phage growth. However, this growth required recombination activity since only 15% of $RecD^-$ $RecJ^-$ $ExoVII^-$ mutant cells developed λ *cI857 red3* infective centers. This showed that recombination deficiency (of both bacteria and phages) is indeed the main reason for a low yield of λ-progeny in that mutant.

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