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Original Research Article

Genetic analysis of transductional recombination in *Escherichia coli* reveals differences in the postsynaptic stages of RecBCD and RecFOR pathways

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Abstract

Background and purpose: *Homologous recombination in* Escherichia coli *proceeds via two pathways, RecBCD and RecFOR, which use different enzymes for DNA end resection and loading of RecA recombinase. The postsynaptic reactions following RecA-mediated homologous pairing have mostly been studied within the RecBCD pathway. They involve RuvABC helicase/resolvase complex, RecG and RadA helicases that process recombination intermediates to produce recombinant DNA molecules. Also, RecG functionally interacts with the PriA protein in initiation of recombination-dependent replication. Here, we studied the individual and combined effects of* ruvABC, recG *and* radA *null mutations on transductional recombination in both pathways. The effect of the* priA300 *mutation, which acts as a suppressor of the* recG *mutation, was also tested. The goal was to characterize the postsynaptic stage of transductional recombination in more details, especially in the RecFOR pathway, which is less well-studied.*

Materials and methods: *Phage P1*vir*-mediated transduction was used to measure recombination efficiency in a series of recombination mutants. The* proA$^+$ *marker was used for selection in transductional crosses with various Δ*proA *recipients.*

Results: *The* ruvABC *mutation moderately decreased recombination in both recombination pathways, while* radA *had no effect. The* recG *mutation reduced recombination in the RecBCD pathway but not in the RecFOR pathway. The strong recombination defect of* recG radA *double mutants in both pathways was completely suppressed by the* priA300 *mutation, and this suppression depended on the functional RuvABC complex.*

Conclusions: *RecG and RadA proteins have a redundant role in transductional recombination* via *RecFOR pathway. In both recombination pathways, RecG and RadA functionally interact with PriA, probably during initiation of recombination-dependent replication.*

INTRODUCTION

In the bacterium *Escherichia coli*, homologous recombination (HR) proceeds on two major recombinational pathways called RecBCD and RecFOR *(1,2)*. The RecBCD pathway mediates the recombinational events that involve double-strand DNA (dsDNA) ends. Such ends arise at sites of double-strand breaks (DSBs), at collapsed replication forks or may be exposed on foreign DNA that is introduced into cell by transduction and conjugation. Irrespectively of their origin, dsDNA

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ends are recognized and processed by RecBCD enzyme, a complex molecular machine with several enzymatic activities *(3,4)*. The RecBCD enzyme binds specifically blunt or nearly blunt dsDNA ends *(5)*, and then works as a powerful helicase-nuclease which simultaneously unwinds DNA duplex and degrades both separated DNA strands *(3,4)*. When the enzyme meets an octanucleotide sequence called Chi, its nuclease activity is modified so that its 3'–5' exonuclease activity is silenced while its 5'–3' exonuclease activity is increased *(6)*. This modification enables RecBCD to produce a long 3'-ending ssDNA tail. In addition, the modified RecBCD gains the ability to load the RecA protein onto nascent ssDNA tail *(7,8)*. Acting in that way, the RecBCD gives rise to the nucleoprotein (RecA) filament that subsequently searches for homology in adjacent DNA duplexes *(9)*. After homologous pairing, the invading RecA filament displaces one of the resident strands thus forming a three-strand recombination intermediate called D-loop *(9)*. In further reactions of branch migration and DNA strand exchange, the D-loop can be converted into a four-strand intermediate termed Holliday junction (HJ). Eventually, HJ is resolved by the RuvABC protein complex that possesses both helicase (branch migration) and endonuclease (resolvase) activities *(10)*. HJ might also be resolved by an alternative mechanism that involves branch migration by the RecG helicase and possibly, an unknown nuclease activity *(11–15)*. Finally, HJs may be acted upon by the RadA protein, which possesses branch migration activity *in vitro (16)*, and shows partial functional overlap with RuvABC and RecG proteins *in vivo (17,18)*.

Resolution of a Holliday junction in the final stage of dsDNA end-mediated recombination results in a structure resembling the replication fork. This structure is targeted by the PriA protein which in turn triggers the sequential recruitment of multiple proteins, including the replicative DnaB helicase. The cascade of events initiated by PriA leads to setting up of the replisome and initiation of recombination-dependent replication (RDR) *(2)*. Inactivation of the PriA activity by *priA* null mutations leads to the strong recombination and DNA repair deficiency which underlines the importance of RDR in recombination process *(19,20)*. Yet, the helicase activity of the PriA protein, which is not essential for the priming of RDR *(21)*, can be detrimental to the recombination process in the *recG* mutants *(22)*. Recent genetic evidence suggests that RecG helicase ensures proper binding of the PriA protein onto forks created by recombination and prevents nonproductive reverse replication initiated by PriA helicase activity *(23,24)*.

The RecFOR recombination pathway plays a major role in single-strand DNA gap (SSG) repair. SSGs are formed when replication forks proceed over non-coding DNA lesions, or when forks stall at different bulky obstacles that occasionally emerge on their path *(1,2)*. The RecFOR protein complex is required to promote loading of the RecA protein on SSGs, which is prerequisite for subsequent reactions of homologous pairing and DNA strand exchange.

In wild-type *E. coli* the RecFOR pathway plays only a minor role in recombinational reactions that involve dsDNA ends *(25,26)*. In the RecFOR pathway, dsDNA end processing is accomplished by a joint action of RecQ helicase and RecJ (5'–3') exonuclease, whereas the RecA loading activity is mediated by the RecFOR protein complex *(1,27)*. Normally, the RecFOR pathway is not efficient when working on dsDNA ends, and because of that, the *recBC(D)* mutants show strong defects in recombination and DNA repair. However, the recombination proficiency of *recBC(D)* mutants is strongly enhanced by suppressor mutations in *sbcB* and *sbcC(D)* genes that inactivate exonuclease I (ExoI) and SbcCD nuclease, respectively *(28,29)*. Inactivation of these 3'–5' exonucleases increases stability of recombinogenic 3'-ssDNA tails thus increasing the success of initiation of recombination in the RecFOR pathway *(28,30,31)*.

It is generally assumed that when working on dsDNA ends, the RecBCD and RecFOR pathways differ primarily in initiation phase of recombination; i.e., these pathways utilize different enzymes for dsDNA end processing and loading of RecA onto ssDNA. The reactions that follow the RecA-catalyzed synapsis are expected to be essentially the same in both pathways, thus involving the same enzymes such as RuvABC complex and RecG helicase *(1,2,32)*. Genetic studies of postsynaptic functions in *recBCD sbcB sbcC* mutants are relatively rare, and most of the data comes from early work on classical strains with partly characterized genetic backgrounds *(33–35)*. In addition, the *recBCD sbcB sbcC* mutants that had been used in these studies carried the *sbcB15* allele which was later shown to encode a defective ExoI with preserved DNA binding activity *(30)*. A series of studies reported specific recombination related phenotypes that are associated with *sbcB15* mutants but are absent in Δ*sbcB* (null) mutants *(30,36–40)*.

The transduction by P1 phage is a simple and useful tool in genetic analyses of recombination in *E. coli*. During transduction, a portion of the P1 phage particles transfer linear duplex DNA from donor to recipient *E. coli* cells. Upon entering into recipient cell, the transferred DNA can be integrated into resident chromosome by homologous recombination. During that process, both dsDNA ends of transferred DNA serve as entry points for the enzymes that commence the recombination process (reviewed in *(2)*). Thus, the transductional recombination belongs to the group of ends-out recombination events *(2)*.

In the present work, we have analyzed the individual and combined effects of *ruvABC, recG* and *radA* null mutations on transductional recombination in wild-type and *recB* Δ*sbcB sbcC* backgrounds. Also, we have studied genetic interactions of the abovementioned mutations with a *priA300* mutation (*priA* K230R), which selectively inactivates the helicase activity of the PriA protein but does not affect its RDR-priming activity *(21)*. Our results reveal significant difference in requirement for RecG function

Table 1. *E. coli strains used.*

Strain	Relevant genotype	Source or reference
Used for	**strain construction**	
BW13635	*proC677*(tetR)*::Tn5-132*	CGSC[a]
JJC889	Δ*sbcB::cam hisG4*	B. Michel
JW0233-2	Δ*proA761::kan*	CGSC[a]
JW2787-1	Δ*recD744::kan*	CGSC[a]
JW4352-1	Δ*radA785::kan*	CGSC[a]
LMM1672	*recB268::Tn10*	*(46)*
LMM1673	Δ*ruvABC::cam*	*(46)*
N5500	*priA300*	R.G. Lloyd
N6052	Δ*recG1919::apra*	R.G. Lloyd
MG1655[b]	**derivatives used for experiments**	
LMM2629	*proA::frt*	*(46)*
LMM2838	*proA::frt* Δ*sbcB::frt sbcC201*	Laboratory collection
LMM2869	*proA::frt* Δ*sbcB::frt sbcC201*Δ*recG1919::apra*	P1.N6052 × LMM2838 to Apra^r UV^s
LMM2880	*proA::frt* Δ*radA785::kan*	P1.JW4352-1 × LMM2629 to Km^r
LMM3008	*proA::frt* Δ*radA785::kan* Δ*ruvABC::cam*	P1.LMM1673 × LMM2880 to Cm^r UV^s
LMM3022	*proA::frt* Δ*radA785::kan* Δ*recG1919::apra*	P1.N6052 × LMM2880 to Apra^r UV^s
LMM3175	*proA::frt* Δ*radA785::kan* Δ*ruvABC::cam* Δ*recG1919::apra*	P1.LMM1673 × LMM3022 to Cm^r UV^s
LMM3188	*proA::frt* Δ*ruvABC::cam*	P1. LMM1673 × LMM2629 to Cm^r UV^s
LMM3196	*proA::frt* Δ*recG1919::apra*	P1.N6052 × LMM2629 to Apra^r UV^s
LMM3300	*priA300* Δ*proA761::kan*	P1.JW0233-2 × N5500 to Km^r
LMM3380	*proA::frt* Δ*recD744::kan*	P1.JW2787-1 × LMM2629 to Km^r
LMM3381	*proA::frt* Δ*recD744::kan* Δ*recG1919::apra*	P1.N6052 × LMM3380 to Apra^r UV^s
LMM3403	*proA::frt* Δ*sbcB::frt sbcC201 recB268::Tn10*	P1.LMM1672 × LMM2838 to Tc^r
LMM3414	*proA::frt* Δ*sbcB::frt sbcC201 recB268::Tn10* Δ*ruvABC::cam*	P1.LMM1673 × LMM3403 to Cm^r UV^s
LMM3425	*priA300 proC677*(tetR)*::Tn5-132*	P1.BW13635 × N5500 to Tc^r Pro$^-$
LMM3426	*priA300 proC*$^+$ *sbcC201*	P1.LMM2838 × LMM3425 to Pro$^+$ *λpal*^{S} Tc^s
LMM3429	*priA300 sbcC201* Δ*sbcB::cam hisG4*	P1.JJC889 × LMM3426 to Cm^r His$^-$
LMM3432	*priA300 sbcC201 hisG*$^+$ Δ*sbcB::frt*	P1.LMM2838 × LMM3429 to His$^+$ Cm^s
LMM3610	*proA::frt* Δ*ruvABC::cam* Δ*recG1919::apra*	P1.N6052 × LMM3188 to Apra^r UV^s
LMM3628	*proA::frt* Δ*sbcB::frt sbcC201 recB268::Tn10* Δ*recG1919::apra*	P1.N6052 × LMM3403 to Apra^r UV^s
LMM3629	*proA::frt* Δ*sbcB::frt sbcC201 recB268::Tn10* Δ*ruvABC::cam* Δ*recG1919::apra*	P1.N6052 × LMM3414 to Apra^r UV^s
LMM3632	*proA::frt* Δ*sbcB::frt sbcC201 recB268::Tn10* Δ*radA785::kan*	P1.JW4352-1 × LMM3403 to Km^r
LMM3633	*proA::frt* Δ*sbcB::frt sbcC201 recB268::Tn10* Δ*radA785::kan* Δ*ruvABC::cam*	P1.LMM1673 × LMM3632 to Cm^r UV^s
LMM3634	*proA::frt* Δ*sbcB::frt sbcC201 recB268::Tn10* Δ*radA785::kan* Δ*recG1919::apra*	P1.N6052 × LMM3632 to Apra^r UV^s
LMM3635	*proA::frt* Δ*sbcB::frt sbcC201 recB268::Tn10* Δ*radA785::kan* Δ*ruvABC::cam* Δ*recG1919::apra*	P1.N6052 × LMM3633 to Apra^r UV^s
LMM4095	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA761::kan*	P1.JW0233-2 × LMM3432 to Km^r
LMM4096	*priA300* Δ*proA::frt*	LMM3300 to Km^s
LMM4097	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA::frt*	LMM4095 to Km^s
LMM4101	*priA300* Δ*proA::frt* Δ*ruvABC::cam*	P1.LMM1673 × LMM4096 to Cm^r UV^s
LMM4102	*priA300* Δ*proA::frt* Δ*recG1919::apra*	P1.N6052 × LMM4096 to Apra^r UV^s
LMM4103	*priA300* Δ*proA::frt* Δ*radA785::kan*	P1.JW4352-1 × LMM4096 to Km^r
LMM4104	*priA300* Δ*proA::frt* Δ*ruvABC::cam* Δ*recG1919::apra*	P1.N6052 × LMM4101 to Apra^r UV^s
LMM4105	*priA300* Δ*proA::frt* Δ*radA785::kan* Δ*ruvABC::cam*	P1.LMM1673 × LMM4103 to Cm^r UV^s
LMM4106	*priA300* Δ*proA::frt* Δ*radA785::kan* Δ*recG1919::apra*	P1.N6052 × LMM4103 to Apra^r UV^s
LMM4107	*priA300* Δ*proA::frt* Δ*radA785::kan* Δ*ruvABC::cam* Δ*recG1919::apra*	P1.N6052 × LMM4105 to Apra^r UV^s
LMM4109	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA::frt recB268::Tn10*	P1.LMM1672 × LMM4097 to Tc^r
LMM4110	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA::frt recB268::Tn10* Δ*ruvABC::cam*	P1.LMM1673 × LMM4109 to Cm^r UV^s
LMM4111	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA::frt recB268::Tn10* Δ*recG1919::apra*	P1.N6052 × LMM4109 to Apra^r UV^s
LMM4112	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA::frt recB268::Tn10* Δ*radA785::kan*	P1.JW4352-1 × LMM4109 to Km^r
LMM4113	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA::frt recB268::Tn10* Δ*ruvABC::cam* Δ*recG1919::apra*	P1.N6052 × LMM4110 to Apra^r UV^s
LMM4114	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA::frt recB268::Tn10* Δ*radA785::kan* Δ*ruvABC::cam*	P1.LMM1673 × LMM4112 to Cm^r UV^s
LMM4115	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA::frt recB268::Tn10* Δ*radA785::kan* Δ*recG1919::apra*	P1.N6052 × LMM4112 to Apra^r UV^s
LMM4116	*priA300 sbcC201* Δ*sbcB::frt* Δ*proA::frt recB268::Tn10* Δ*radA785::kan* Δ*recG1919::apra* Δ*ruvABC::cam*	P1.LMM1673 × LMM4115 to Cm^r UV^s

[a] Strain obtained from the *Escherichia coli* Genetic Stock Center (CGSC), Yale, USA.
[b] MG1655 markers are F$^-$ *λ*$^-$ *ilvG rfb-50 rph-1*.

between the two recombination pathways. In addition, the results suggest overlapping roles of RecG and RadA in controlling PriA activity during transductional recombination.

MATERIALS AND METHODS

Strains and growth conditions

The *E. coli* strains used in this study are derivatives of MG1655 (Table 1). Bacterial cultures were grown in liquid LB medium at 37 °C with shaking or on LB agar plates kept at 37 °C *(41)*. Cell growth was monitored by measuring the optical density at 600 nm (OD_{600}). Various mutants were constructed by P1 transduction *(41)*. Transductants were isolated on LB plates supplemented with appropriate antibiotics: kanamycin (Km), 30 μg/ml; tetracycline (Tc), 10 μg/ml; apramycin (Apra), 50 μg/ml; chloramphenicol (Cm), 15 μg/ml. For some transductants, M9 plates *(41)* supplemented with glucose (0.4%), vitamin B1 (1 μg/ml), and required amino acids (100 μg/ml) were used. DNA repair-defective transductants were checked for their UV sensitivity phenotypes. The phenotype of the *sbcC201* transductants was confirmed by the increased efficiency of plating of λ phage carrying a 571-bp palindrome (*λpal571*) *(42)*.

The kanamycin-resistance gene was eliminated from the strains LMM3300 and LMM4095 carrying Δ*proA761::kan* mutation. These Δ*proA761::kan* mutants were transformed by pCP20, a plasmid with the gene coding for FLP recombinase. The expression of FLP leads to kanamycin-resistance gene excision, by recombination between two directly repeated FRT sequences flanking the gene *(43)*. The kanamycin sensitive derivatives free of pCP20 were isolated as described previously *(43)*.

Transductional recombination crosses

P1*vir* phage stocks necessary for transductional crosses were prepared on donor strain MG1655 (*proA*$^+$) *(41)*. The *proA*$^+$ marker was used for selection in crosses with various Δ*proA* recipients. Cultures of recipient strains were grown overnight in LB medium at 37 °C, diluted 200-fold in the same medium, and further grown until they reached an OD_{600} of 1. Six 1-ml aliquots of each culture were sedimented by centrifugation and resuspended in 200 μl of MC buffer composed of 100 mM $MgSO_4$ and 5 mM $CaCl_2$. Five samples were mixed with 100 μl of P1*vir* lysate (titer ~10^8 phage particles per ml) and incubated for 30 min at 37 °C to allow phage adsorption. One sample was incubated without P1 and used later for determination of colony-forming ability. After incubation, the mixtures containing *proA*$^+$ lysate were sedimented, resuspended in 200 μl of phosphate buffer, and plated on M9 plates *(41)* containing glucose (0.4%), vitamin B1 (1 μg/ml), and 5 mM sodium citrate. The phage-free sample was resuspended in 1 ml of phosphate buffer, serially diluted in the same buffer and plated on LB plates to determine colony-forming ability of the recipient strain.

Colonies of recipients were counted after 24 to 48 h of growth at 37 °C whereas colonies of transductants were incubated 48 to 72 h prior to counting. The number of colony-forming units (CFU) obtained for each strain was expressed relative to the CFU value of the wild-type strain and served as a measure of cell viability. The number of transductants was also expressed relative to the wild-type strain (which was used as Rec$^+$ control in each experiment). The relative number of transductants for each mutant was corrected for difference in CFU value by multiplying with the correction factor that was calculated as a ratio between CFU value of the wild-type strain and CFU value of the particular mutant.

Statistical analyses

Statistical significance of differences in the relative yield of recombinants between mutant strains was assessed with one-way ANOVA on log-transformed data. The normality of the residuals was confirmed with the Shapiro-Wilk test, and the homogeneity of variance with the Levene test. A significant ANOVA was followed by the Tukey HSD test which grouped the mutant strains into groups that differ significantly from each other. Additionally, we tested whether the relative yield of recombinants of mutant strains significantly differs from the wild-type value of 1 with one-sample t-tests on log-transformed data and adjusted the p-values for multiple testing using the FDR method. Statistical tests were carried out in R (version 4.2.0), with aov function for ANOVA (from the base package "stats"), leveneTest function for the Levene test (from the package "car" version 3.1-0), shapiro.test function for the Shapiro-Wilk test (from the base package "stats"), HSD.test function (from the package "agricolae", v.1.3-5) for Tukey HSD test with the grouping output, and t.test function (from the base package "stats") for one-sample t-test. All the statements comparing different strains in the Results section are in agreement with the results of the statistical analysis at alpha=0.05. Due to the large number of comparisons and to keep the text readable, we abstained from citing all the p-values in the text. Instead, the relevant test and p-values are cited only for the comparisons where there are small to moderate differences in the measured variable (i.e., up to around 4x change in the yield of recombinants) between two strains.

RESULTS

Effects of ΔruvABC, ΔrecG and ΔradA mutations on transductional recombination

It is known from previous studies that single *ruv*, *recG* and *radA* mutations have moderate effects on recombination and recombinational repair in otherwise wild-type background *(11,17,44)*. Our results show that individual

Table 2. *Transductional recombination via RecBCD and RecFOR pathways: effects of ruvABC, recG and radA mutations.*

Recipient strain	Relevant genotype	Relative CFU[a]	Relative yield of recombinants[b]
LMM2629	Wild type	1	1
LMM3188	Δ*ruvABC*	0.57 ± 0.047	0.48 ± 0.13
LMM3196	Δ*recG*	0.85 ± 0.032	0.23 ± 0.005
LMM3610	Δ*ruvABC* Δ*recG*	0.26 ± 0.074	0.013 ± 0.001
LMM2880	Δ*radA*	0.98 ± 0.086	0.98 ± 0.03
LMM3008	Δ*radA* Δ*ruvABC*	0.53 ± 0.092	0.20 ± 0.05
LMM3022	Δ*radA* Δ*recG*	0.20 ± 0.035	0.058 ± 0.022
LMM3175	Δ*radA* Δ*ruvABC* Δ*recG*	0.035 ± 0.021	0.002 ± 0.0007
LMM3403	*recB* Δ*sbcB sbcC*	0.56 ± 0.053	0.45 ± 0.055
LMM3414	*recB* Δ*sbcB sbcC* Δ*ruvABC*	0.34 ± 0.068	0.32 ± 0.06
LMM3628	*recB* Δ*sbcB sbcC* Δ*recG*	0.63 ± 0.009	0.51 ± 0.05
LMM3629	*recB* Δ*sbcB sbcC* Δ*ruvABC* Δ*recG*	0.25 ± 0.038	0.013 ± 0.005
LMM3632	*recB* Δ*sbcB sbcC* Δ*radA*	0.54 ± 0.052	0.40 ± 0.03
LMM3633	*recB* Δ*sbcB sbcC* Δ*radA* Δ*ruvABC*	0.34 ± 0.040	0.16 ± 0.02
LMM3634	*recB* Δ*sbcB sbcC* Δ*radA* Δ*recG*	0.28 ± 0.039	0.007 ± 0.002
LMM3635	*recB* Δ*sbcB sbcC* Δ*radA* Δ*ruvABC* Δ*recG*	0.062 ± 0.026	<0.0019[c]
LMM2838	Δ*sbcB sbcC*	1.03 ± 0.059	0.59 ± 0.09
LMM2869	Δ*sbcB sbcC* Δ*recG*	0.79 ± 0.028	0.13 ± 0.025
LMM3380	Δ*recD*	1.13 ± 0.05	1.56 ± 0.10
LMM3381	Δ*recD* Δ*recG*	0.66 ± 0.029	0.26 ± 0.075

[a] The number of colony-forming units (CFU) for each strain was measured at an OD_{600} of 1, and expressed relative to the CFU value of the control recipient strain MG1655, which averaged 9×10^8 CFU/ml. The values are averages ± standard deviations of results of at least three independent experiments.

[b] The yields of recombinants are relative to the yield of control strain MG1655 and were corrected for any deficiency in the CFU value of the recipient strain. The average yield for control strain MG1655 was 5×10^2 CFU per ml of the transduction mixture. The values are averages ± standard deviations of results of at least three independent experiments.

[c] No transductants were obtained in four independent experiments performed with this strain.

Δ*ruvABC* and Δ*recG* mutations cause a modest (two- and four-fold, respectively) decrease of transductional recombination in comparison to the wild-type strain (Table 2), that is essentially in accord with earlier reports *(11,44)*. These changes from the wild type frequency are, however, statistically significant (one-sample t-test, p_{adj}=0.00010 and 0.000067, respectively). In our hands, the Δ*radA* mutation did not produce any significant change to transductional recombination (Table 2 and one-sample t-test, p_{adj}=0.41), although the *radA1::kan* mutation was previously shown to cause a moderate (two-fold) reduction in transduction efficiency *(44)*. This slight discrepancy may be due to different genetic backgrounds used in the two studies, i.e., MG1655 background used in this study *vs.* AB1157 background used in Lovett *(44)*. Interestingly, it was previously observed that in some recombination assays, *ruv* and *recG* mutations also have a less severe effect in the MG1655 background than in AB1157 *(45)*.

It has been shown previously that *ruv*, *recG* and *radA* mutations act synergistically in decreasing conjugational recombination and recombinational DNA repair *(17)*. This synergy was also observed in our transductional crosses; the double Δ*ruvABC* Δ*radA*, Δ*recG* Δ*radA* and Δ*ruvABC* Δ*recG* mutants showed significant (five-, 17- and 77-fold) decrease of recombination, respectively, in comparison to the wild-type strain (Table 2). As expected, the strongest (500-fold) decrease of recombination was observed with the triple Δ*ruvABC* Δ*recG* Δ*radA* mutant (Table 2).

The *recB* Δ*sbcB sbcC* strain that recombines via the RecFOR pathway showed 45% the recombination efficiency of the wild-type strain (Table 2, one-sample t-test, p_{adj}=0.0000027). In the *recB* Δ*sbcB sbcC* background the Δ*ruvABC* mutation caused further 30% decrease of recombination, statistically not a significant change (Tukey HSD test). The Δ*recG* mutation had no adverse effect on recombination in this background (Table 2 and Tukey HSD test). However, the Δ*ruvABC* Δ*recG* combination resulted in a significant, 35-fold decrease recombination relative to the *recB* Δ*sbcB sbcC* parent. These results confirm our previously published data *(46)*. As in the wild-type background, the Δ*radA* mutation alone had no significant effect on recombination in the *recB* Δ*sbcB sbcC* background (Tukey HSD test). It had synergistic effects with Δ*ruvABC*, Δ*recG* mutations, and Δ*ruvABC* Δ*recG* mutations, causing significant three-, 64-, and more than 237-fold decrease in recombination, respectively. Taken together, these results show that RuvABC, RecG and RadA proteins have partly redundant roles in both recombination pathways. However, the redundancy of these

proteins is generally more pronounced in the RecFOR pathway. This is particularly evident for RecG and RadA proteins, which are individually dispensable for recombination in the *recB* Δ*sbcB sbcC* background, but whose simultaneous inactivation severely reduces recombination in this background.

The absence of any effect of Δ*recG* mutation in *recB* Δ*sbcB sbcC* background is quite interesting given the significant (four-fold) decrease of recombination caused by this mutation in wild-type background (see above). To further check if the effects of the Δ*recG* mutation are solely pathway dependent, we have introduced the Δ*recG* mutation into the Δ*sbcB sbcC* background and measured transduction proficiency of the resulting strain. The Δ*sbcB sbcC* mutant itself showed similar recombination frequency as *recB* Δ*sbcB sbcC* mutant (Table 2 and Tukey HSD test). However, the Δ*sbcB sbcC* Δ*recG* derivative showed a significant, three-fold lower recombination frequency than its *recB* counterpart (Table 2 and Tukey HSD test). These results indicate that the presence of functional RecBCD enzyme leads to an increased requirement for RecG in transductional recombination.

The RecBCD enzyme has two activities that influence DNA metabolism and that might formally account for modulation of the Δ*recG* effects. Apart from its recombinase activity that is crucial for recombination on dsDNA ends (reviewed in *(3)*), RecBCD possesses a nuclease activity (called ExoV) that is suggested to participate in the recovery of stalled and regressed replication forks in a fashion that does not involve recombination (reviewed in *(2)*). In the latter case, the ExoV degrades an extruded dsDNA arm of the regressed fork, thus allowing the fork to adopt structure that is suitable for replisome (re)assembly. The ExoV activity is selectively inactivated by *recD* null mutations, which do not affect the recombination capacity of the RecBC(D) enzyme *(3)*. We have tested how a Δ*recD* mutation influences the *recG* phenotype in transductional recombination. The *recD* mutation itself produced a moderate hyper-rec phenotype (one-sample t-test, p_{adj}=0.018), while introduction of the Δ*recG* mutation into the Δ*recD* strain led to five-fold decrease of recombination, a significant difference (Table 2 and Tukey HSD test). In other words, the Δ*recD* Δ*recG* strain showed similar recombination level as the single Δ*recG* mutant (Tukey HSD). These data show that recombinase activity of the RecBCD enzyme is associated with an increased requirement for RecG function during transductional recombination.

Inactivation of the PriA helicase reveals specific functional relations between RecG and RadA proteins

The *priA300* mutation belongs to the class of mutations that selectively inactivate the PriA helicase activity. Genetic studies have revealed that inactivation of the PriA helicase suppresses recombination defects in the *recG* mutants leading to the idea that RecG helicase curbs a potentially harmful action of PriA helicase during recombination *(22, 23)*.

We have analyzed how *priA300* mutation, alone or in combination with *ruvABC*, *recG* and *radA* mutations, influences transductional recombination in wild-type and *recB* Δ*sbcB sbcC* backgrounds. When tested alone in the wild-type background, the *priA300* mutation caused a

Table 3. *Transductional recombination via RecBCD and RecFOR pathways: effects of the priA300 mutation.*

Recipient strain	Relevant genotype	Relative CFU[a]	Relative yield of recombinants[b]
LMM2629	Wild type	1	1
LMM4096	priA300	0.99 ± 0.05	0.79 ± 0.07
LMM4101	*priA300 ΔruvABC*	0.51 ± 0.05	0.25 ± 0.03
LMM4102	*priA300 ΔrecG*	0.93 ± 0.10	0.92 ± 0.07
LMM4103	*priA300 ΔradA*	0.97 ± 0.08	0.73 ± 0.04
LMM4104	*priA300 ΔruvABC ΔrecG*	0.23 ± 0.02	0.027 ± 0.006
LMM4105	*priA300 ΔruvABC ΔradA*	0.25 ± 0.03	0.038 ± 0.017
LMM4106	*priA300 ΔrecG ΔradA*	0.91 ± 0.09	0.78 ± 0.09
LMM4107	*priA300 ΔruvABC ΔrecG ΔradA*	0.053 ± 0.003	0.0068 ± 0.0034
LMM4109	*recB ΔsbcB sbcC priA300*	0.44 ± 0.05	0.36 ± 0.04
LMM4110	*recB ΔsbcB sbcC priA300 ΔruvABC*	0.34 ± 0.011	0.27 ± 0.03
LMM4111	*recB ΔsbcB sbcC priA300 ΔrecG*	0.62 ± 0.04	0.87 ± 0.14
LMM4112	*recB ΔsbcB sbcC priA300 ΔradA*	0.47 ± 0.05	0.30 ± 0.07
LMM4113	*recB ΔsbcB sbcC priA300 ΔruvABC ΔrecG*	0.22 ± 0.04	0.023 ± 0.007
LMM4114	*recB ΔsbcB sbcC priA300 ΔruvABC ΔradA*	0.27 ± 0.07	0.064 ± 0.019
LMM4115	*recB ΔsbcB sbcC priA300 ΔrecG ΔradA*	0.54 ± 0.04	1.02 ± 0.10
LMM4116	*recB ΔsbcB sbcC priA300 ΔruvABC ΔrecG ΔradA*	0.10 ± 0.01	0.0074 ± 0.0015

[a] The number of colony-forming units (CFU) for each strain was measured at an OD_{600} of 1, and expressed relative to the CFU value of the control recipient strain MG1655, which averaged 9×10^8 CFU/ml. The values are averages ± standard deviations of results of at least three independent experiments.

[b] The yields of recombinants are relative to the yield of control strain MG1655 and were corrected for any deficiency in the CFU value of the recipient strain. The average yield for control strain MG1655 was 5×10^2 CFU per ml of the transduction mixture. The values are averages ± standard deviations of results of at least three independent experiments.

slight (~20%) decrease of recombination (Table 3 and one-sample t-test, p_{adj}=0.015), that resembles the effect of this mutation in conjugational recombination *(47)*. In a combination, the *priA300* and Δ*ruvABC* mutations had a moderate synergistic effect, conferring a four-fold decrease of recombination, a significant difference from the wild type (one-sample t-test, p_{adj}=0.000091, and compare results in Tables 2 and 3). In contrast, the *priA300* Δ*recG* double mutant recombined similar to *priA300* and significantly better than Δ*recG* single mutant, thus showing strong suppression of *recG*-associated recombination defect (Tables 2 and 3). However, the triple *priA300* Δ*ruvABC* Δ*recG* mutant was similarly recombination deficient as Δ*ruvABC* Δ*recG* mutant (Tukey HSD test and Tables 2 and 3), showing that inactivation of RuvABC complex eliminates Δ*recG* suppression by *priA300*. Quite similar joint effects of *priA300*, Δ*ruvABC*, and Δ*recG* mutations have been described previously for conjugational crosses *(47)*.

The *priA300* Δ*radA* double mutant recombined with similar efficiency as the *priA300* single mutant (Table 3 and Tukey HSD test). However, introducing Δ*ruvABC* mutation into the *priA300* Δ*radA* double mutant led to the significant (~26-fold) decrease of recombination, indicating an increased importance of RuvABC function in the absence of PriA helicase and RadA protein. In contrast, when the Δ*recG* mutation was introduced into the *priA300* Δ*radA* background, it had no effect at all (Table 3 and Tukey HSD test). Given the strong recombination defect of Δ*radA* Δ*recG* double mutant (Table 2), the latter result shows that the *priA300* mutation efficiently suppresses not only the effects of Δ*recG* mutation alone but also the joint effects of Δ*radA* and Δ*recG* mutations. The *priA300* Δ*radA* Δ*recG* Δ*ruvABC* quadruple mutant displayed more than 100-fold lower recombination than the *priA300* single mutant (Table 3), as did the *priA*$^+$ Δ*radA* Δ*recG* Δ*ruvABC* mutant when compared to the wild type (Table 2). This result shows that *priA300*-associated suppression of recombination defects in Δ*radA* Δ*recG* double mutants depends on active RuvABC complex.

In the *recB* Δ*sbcB sbcC* background the *priA300* mutation caused a slight (~20%) decrease of recombination similar to the effect of this mutation in the wild-type background (Tables 2 and 3). Adding the Δ*ruvABC* mutation to the *recB* Δ*sbcB sbcC priA300* background caused further ~25% decrease of recombination, not a significant difference (Tukey HSD test). Interestingly, introduction of the Δ*recG* mutation into the *recB* Δ*sbcB sbcC priA300* background significantly increased recombination by more than two-fold (Table 3 and Tukey HSD test). Moreover, the *recB* Δ*sbcB sbcC priA300* Δ*recG* mutant recombined almost two-fold better than its parental *recB* Δ*sbcB sbcC priA*$^+$ *recG*$^+$ strain, although this change proved to be statistically non-significant (Tukey HSD test and Tables 2 and 3). The Δ*radA* mutation did not significantly change recombination in the *recB* Δ*sbcB sbcC priA300* background (Table 3 and Tukey HSD test).

The joint Δ*ruvABC* Δ*recG* mutations conferred a significant, 16-fold drop of recombination to the *recB* Δ*sbcB sbcC priA300* strain (Table 3). This is about the same recombination defect as observed with *recB* Δ*sbcB sbcC* Δ*ruvABC* Δ*recG* (Tukey HSD and Tables 2 and 3). Combining Δ*ruvABC* and Δ*radA* mutations led to a significant, ~six-fold decrease of recombination in the *recB* Δ*sbcB sbcC priA300* background. A striking result was obtained with Δ*recG* Δ*radA* pair of mutations; when introduced into the *recB* Δ*sbcB sbcC priA300* background, these mutations improved recombination to the level of the wild-type strain (one-sample t-test, p_{adj}=0.84, and Table 3). However, this improvement of recombination was abolished by addition of Δ*ruvABC* mutation which reduced recombination almost to the level of the *recB* Δ*sbcB sbcC* Δ*ruvABC* Δ*recG* Δ*radA* strain (Tables 2 and 3).

DISCUSSION

RuvABC, RecG and RadA proteins are implicated in the postsynaptic stage of recombination in *E. coli*. Among these proteins, RadA is the least studied. It is involved in conjugational recombination, recombinational repair of DNA lesions inflicted by different agents *(17,18)*, and restoration of broken replication forks *(44)*. *In vitro*, RadA displays a branch migration activity that is in accord with envisaged role of this protein in stabilization of homologous joints and processing of recombination intermediates such as D-loops and HJs *(16,18)*.

Our results suggest a high level of functional overlap between RadA and RecG proteins during transductional recombination that is essentially in line with previous results obtained in other recombination assays *(17,18)*. Moreover, our results reveal almost complete redundancy of RadA and RecG proteins during transductional recombination *via* RecFOR recombination pathway. Another interesting finding of this work is that the *priA300* mutation completely suppresses a strong recombinational defect of Δ*radA* Δ*recG* double mutants in both wild-type and *recB* Δ*sbcB sbcC* backgrounds. The level of suppression by *priA300* is particularly intriguing in the latter background; the *recB* Δ*sbcB sbcC priA300* Δ*radA* Δ*recG* mutant recombines even better than its *priA*$^+$ *radA*$^+$ *recG*$^+$ parent.

The RecG protein is a multifunctional DNA helicase that was initially associated primarily with the processing of recombination intermediates such as D-loops and HJs, thus being considered an alternative for the RuvABC branch migration/resolution complex *(11,48)*. However, more recent research has offered additional roles of RecG, some of which are only indirectly associated with recombination. Several studies suggest that RecG activity prevents pathological DNA synthesis that might occur either at D-loops formed by recombination *(23,24)* or at 3' flaps that result from head-on collision of the two converging replication forks that regularly happens at replication ter-

minus *(47,49)*. In both cases, a potential source of the problem is the PriA helicase which, if not constrained by RecG, remodels aforementioned forked structures to initiate an unproductive DNA replication, usually referred to as over-replication. It was suggested that such over-replication gives rise to dsDNA arms that initiate the excessive recombination reactions thus leading to further disorder in structure and function of the chromosome. The results of our study suggest that in *recG* mutants, the RadA protein provides an alternative control against the harmful effects of "reckless" PriA helicase activity. The redundancy of RecG and RadA functions indicates how important is for the cell to ensure a tight coordination of replication and recombination in time and space.

Transductional recombination is intimately linked with DNA replication. In fact, transduced DNA that has established homologous joints on both of its ends may be considered an alternative origin of replication, with two replication forks progressing in opposite direction until they eventually meet in terminus region *(2,50)*. Thus, RecG and RadA and other recombination functions could be involved in at least two instances during transductional recombination: (i) in a set of reactions that lead to proper establishment of RDR and (ii) in reactions that ensure proper termination of RDR.

Our results show that in the RecBCD pathway the role of the RecG protein cannot be entirely replaced by the RadA function, while in the RecFOR pathway RecG and RadA are completely interchangeable. The reasons for this difference are not obvious based on existing recombination models. It is possible that the structure of the recombination intermediates formed in the two recombination pathways is somewhat different, which consequently changes the enzymology of the postsynapsis. Another possibility is that the proteins participating in the earlier stages of the recombination process have an influence on the recruitment of postsynaptic proteins. Additional experiments are needed to resolve these questions.

In both recombinational pathways tested, the Δ*radA* Δ*recG* Δ*ruvABC* combination of mutations resulted in severe recombination deficiency. The presence of an additional *priA300* mutation only slightly improved recombination in the triple mutants. These findings show that RadA and RecG proteins have additional roles apart from regulating PriA helicase activity. However, these roles are not critical until RuvABC function is maintained, which is compatible with notion that RecG and RadA participate in processing of Holliday junctions.

The RecBCD enzyme is thought to be indispensable for all recombination events that occur at dsDNA ends. In the absence of RecBCD recombinase, transductional recombination is reduced to approximately 1% of the wild-type value *(39,40)*. As mentioned previously, the combination of *sbcB* and *sbcCD* mutations increases recombination proficiency of the *recB* mutants to 40–50% of the wild-type level *(40,46)* (this paper). Interestingly, our results have revealed that adding the *priA300 recG radA* combination of mutations to the *recBCD sbcB sbcC* background lead to a further increase of recombination, up to the level of the wild-type strain. Given the current recombination models, our finding suggests that the efficiency of the RecFOR recombination pathway on dsDNA ends can be improved in two ways: (i) by protection of recombinogenic 3' ends from degradation thus ensuring the substrate for RecA filament formation, and (ii) by modulation of the postsynaptic stage in order to ensure optimal stability of homologous joints and proper initiation of RDR.

Recently, a lot of the research has been focused on DNA transactions that occur during replication termination. The picture of exactly how two converging replication forks merge in the *ter* region and how they give rise to intact and faithfully replicated sister chromosomes is still far from being complete. However, a growing list of enzymes involved in these reactions is emerging, along with data indicating their possible role in replication termination. This list includes RecBCD enzyme, RecG and PriA helicases, exonuclease I and SbcCD nuclease; all of them being involved in recombination processes studied in our work. Different models have been proposed to explain the intricate interplay of these enzymes during termination of replication. The RecG, ExoI, and SbcCD are suggested to prevent over-replication in the *ter* region, either by removing DNA structures (3' flaps) that could serve as origins of over-replication *(14,47,49)*, or by remodeling and degrading the transient over-replicated intermediates *(51–53)*. The RecBCD nuclease (ExoV) is suggested to be involved in the latter scenario as a major degradase that acts after degradation was initiated by ExoI and SbcCD *(53)*. Given all these facts, there is a possibility that some of the phenotypes observed in our study are not related solely to recombination reactions at sites of initial homologous joints, but also to the reactions that occur later, when RDR reaches its terminus. Our results obtained with the *recB* Δ*sbcB sbcC priA300* Δ*recG* mutant show that despite the lack of many terminus-processing functions, it still exhibits relatively high cell viability and recombination level close to that of the wild-type (Table 3). This finding suggests that there are additional functions that enable the successful completion of chromosome replication, regardless of whether replication is initiated from its normal origin or from a recombination site.

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