

UvrD and UvrD252 Counteract RecQ, RecJ, and RecFOR in a *rep* Mutant of *Escherichia coli*[∇]

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Rep and UvrD are two related *Escherichia coli* helicases, and inactivating both is lethal. Based on the observation that the synthetic lethality of *rep* and *uvrD* inactivation is suppressed in the absence of the recombination presynaptic proteins RecF, RecO, or RecR, it was proposed that UvrD is essential in the *rep* mutant to counteract a deleterious RecFOR-dependent RecA binding. We show here that the synthetic lethality of *rep* and *uvrD* mutations is also suppressed by *recQ* and *recJ* inactivation but not by *rara* inactivation. Furthermore, it is independent of the action of UvrD in nucleotide excision repair and mismatch repair. These observations support the idea that UvrD counteracts a deleterious RecA binding to forks blocked in the *rep* mutant. An ATPase-deficient mutant of UvrD [*uvrD*(R284A)] is dominant negative in a *rep* mutant, but only in the presence of all RecQJFOR proteins, suggesting that the UvrD(R284A) mutant protein is deleterious when it counteracts one of these proteins. In contrast, the *uvrD252* mutant (G30D), which exhibits a strongly decreased ATPase activity, is viable in a *rep* mutant, where it allows replication fork reversal. We conclude that the residual ATPase activity of UvrD252 prevents a negative effect on the viability of the *rep* mutant and allows UvrD to counteract the action of RecQ, RecJ, and RecFOR at forks blocked in the *rep* mutant. Models for the action of UvrD at blocked forks are proposed.

Replication forks initiated at the chromosome replication origin can encounter obstacles that block their progression and lead to replication arrest. Replication restart is then essential to complete chromosome replication (32). In *Escherichia coli*, depending on the cause of arrest, the reassembly of a functional replication complex takes place directly on arrested replication forks or after DNA processing by recombination proteins (21). Defects in the processing of arrested replication forks can generate chromosome rearrangements, leading to genome instability (5). Replication impairment is an important source of instability in several organisms, and understanding the various pathways that process arrested replication forks to allow replication restart is an important issue (4, 34). For this purpose, we studied the fate of replication forks arrested in the absence of the Rep helicase in *Escherichia coli*.

In *E. coli*, chromosome replication is catalyzed by a replisome constituted of the replicative helicase (DnaB), the primase (DnaG), and the DNA polymerase III holoenzyme (Pol III_h), a 10-subunit complex (29). Rep is a nonessential replicative helicase; chromosome replication is twice as slow in *rep* mutants as in wild-type cells (17). Rep translocates on DNA in the 3'-5' direction and is proposed to play two roles in vivo. Based on the observation that in vitro Rep dislodges DNA-bound proteins, it was originally proposed that Rep acts by dislodging proteins in front of replication forks (38). More

recently, Rep was proposed to act in a Rep-PriC pathway of replication restart (31). In vitro experiments showed that Rep and PriC proteins act at replication forks with a gap on the leading strand: Rep helicase displaces the lagging-strand end, thus forming a DNA structure on which PriC can load the replicative helicase (12, 13). The role of the Rep-PriC pathway of replication restart in vivo has yet to be determined. *rep* inactivation (but not *priC* inactivation [our unpublished data]) is synthetic lethal with the inactivation of the recombination gene *recB* or *recC*, which inactivates RecBCD-dependent homologous recombination (33, 35). This indicated that replication arrest in the *rep* mutant causes the formation of RecBCD substrates. RecBCD is a highly processive helicase/exonuclease that acts at DNA double-strand ends; it unwinds and degrades double-stranded DNA (dsDNA) until it encounters a specific DNA sequence named Chi. At Chi sites, RecBCD shifts to a recombinase, forming a 3' single-stranded DNA (ssDNA) on which it directly loads RecA (14). Studies of the lethality of the *rep recBC* mutants showed that blocked replication forks undergo a specific reaction called replication fork reversal (RFR) (33). This reaction anneals the leading- and lagging-strand ends at blocked forks, creating a Holliday junction adjacent to a double-strand end, which renders RecB and RecC essential for viability (the RecD subunit is dispensable for homologous recombination). RecBCD processes reversed forks either by degrading the dsDNA end or by promoting homologous recombination. Both pathways lead to a structure on which PriA and other preprimosomal proteins load the replicative helicase DnaB, allowing the reassembly of a new replisome and replication restart. In addition to the *rep* mutant, the RFR reaction takes place in several other replication mutants, including Pol III(Ts) mutants at a high temperature (23).

The RecFOR recombination pathway is required for ssDNA

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gap repair. RecFOR proteins allow the formation of a RecA filament on ssDNA coated by the ssDNA binding (SSB) protein, by removing SSB from DNA and loading RecA (27). RecA filaments invade a dsDNA homologous molecule, leading to the formation of a Holliday junction recognized by RuvAB and RecG. The RuvAB complex and the RecG helicase catalyze branch migration of Holliday junctions, while RuvAB in complex with the endonuclease RuvC catalyzes resolution of Holliday junctions (16). RecFOR can act in conjunction with two other presynaptic proteins: the RecQ helicase, which translocates in the 3'-5' direction, and RecJ, a 5'-3' ssDNA exonuclease. These two proteins are needed for double-strand break repair by homologous recombination in a *recBC sbcB sbcCD* background, in which the absence of the SbcB and SbcCD nucleases allows DNA double-strand ends to be recombined by the successive action of RecQ, RecJ, RecFOR, RecA, and RuvABC (reviewed in reference 6). RecQ and RecJ are also required for RecFOR-promoted RecA binding at Pol III(Ts)-blocked forks (8). In *dnaE*(Ts) (Pol III polymerase) and *dnaN*(Ts) (Pol III clamp) mutants, RecQ, RecJ, and RecFOR promote RecA binding in a way that forms a toxic structure which prevents RFR and replication restart. This deleterious action of RecQ, RecJ, RecFOR, and RecA (called RecQJFORA hereafter) at Pol III(Ts)-blocked forks is counteracted by a helicase very similar to Rep, the UvrD helicase (8).

Like Rep, UvrD is a superfamily 1 helicase that translocates on DNA in a 3'-5' direction. UvrD is required for nucleotide excision repair (NER) and mismatch repair (MMR) (16, 19). In addition, *uvrD* deletion leads to an increase in homologous recombination, showing that UvrD counteracts homologous recombination *in vivo*. Actually, *in vitro*, UvrD is able to dismantle a RecA filament and RecA-made recombination intermediates, suggesting a RecA-removal activity of UvrD *in vivo* (26, 36). UvrD allows RFR at arrested replication forks in *dnaE*(Ts) and *dnaN*(Ts) mutants by counteracting RecQJFORA. This function corresponds to two different modes of action of UvrD: the *uvrD252* allele, largely impaired for its ATPase activity, still counteracts RecQJFORA at *dnaE*(Ts)-blocked forks, whereas it is inactive at *dnaN*(Ts)-blocked forks (18).

The inactivation of the helicases Rep and UvrD, which share 34% identity, is lethal. Because the viability of the $\Delta rep \Delta uvrD$ double mutant is restored by the inactivation of *recF*, *recO*, or *recR*, it was proposed that UvrD is required at *rep*-blocked forks for its anti-RecFOR action (30). The deleterious action of RecFOR in the *rep* mutant is likely to promote RecA binding, although RecA inactivation does not suppress the synthetic lethality of *rep uvrD* cells, presumably because RecA is needed in this double mutant for yet another unknown function. In the present work, we studied the role of UvrD in a *rep* mutant. First, we showed that the synthetic lethality of *rep* and *uvrD* inactivation is independent of the action of UvrD in NER and MMR and of the action of Rep in the Rep-PrIC replication restart pathway. Then, we observed that, similarly to the inactivation of *recFOR*, the inactivation of *recQ* or *recJ* also restores the viability of the $\Delta rep \Delta uvrD$ double mutant. Finally, we show that, as previously observed for *dnaE*(Ts)-blocked forks, the *uvrD252* mutation (Gly30Asp), which does not prevent RecFOR- and RecA-dependent SOS induction and hy-

perrecombination (18), nevertheless prevents RecQJFOR action at *rep*-blocked forks.

MATERIALS AND METHODS

Strains and plasmids. The strain background was JJC40, which is an *hsdR* Thr⁺ Pro⁺ derivative of AB1157 (*leu-6 thi-1 his-4 argE3 lacY1 galK2 ara-14 xyl-5 ml-1 tsx-33 rpsL31 supE44*). Most of the strains were constructed by P1 transduction. Details of strain constructions and strain genotypes are described in Table 1. *recBC*(Ts) stands for *recB270 recC271* mutations (15) (Table 1). All thermosensitive mutants were constructed and propagated at 25°C. Null mutants were checked by PCR with external oligonucleotides that amplify DNA fragments of different lengths for the wild-type and the interrupted alleles. *recBC* mutants were checked for the inactivation of exonuclease V (they are permissive for the growth of T4 gpII mutants). *uvrA* and *uvrD* mutants were checked for their UV-sensitive phenotypes. *mutL* and *uvrD* mutants were checked for their mutator phenotypes (increase in the proportion of Rif^r clones in overnight cultures). To ascertain that the *rep uvrD252 recBC*(Ts) mutant had not acquired a suppressor mutation that would allow *rep uvrD* viability, we compared the cotransduction efficiency of $\Delta uvrD$ and *met::Tn10* in JJC2540 [*uvrD252 recBC*(Ts)] and JJC4571 [*rep uvrD252 recBC*(Ts)]. As expected, 4 $\Delta uvrD$ mutants out of 11 *met::Tn10* transductants were obtained in JJC2540, while no $\Delta uvrD$ mutant cloned was obtained in JJC4571 out of 28 *met::Tn10* transductants; this shows that in the JJC4571 context, *rep* and *uvrD* inactivation is synthetic lethal, as expected. The minimum media (MM) was M9 supplied with thiamine (0.05%), CaCl₂ (100 μ M), MgSO₄ (2 mM), glucose (0.4%), and Casamino Acids (0.2%) (25). Plasmid pGBts-*rep* has been described previously (3); it carries the *rep* gene under the control of its own promoter. Plasmid pAM-*rep* was constructed by recloning the *rep* gene from pGBts-*rep* in the polylinker of the IPTG (isopropyl- β -D-thiogalactopyranoside)-dependent vector pAM34 (9).

Segregation of pGBts-*rep* and pAM-*rep*. Each segregation experiment was performed at least three times. Cells carrying the pGBts-*rep* plasmid were grown overnight in Luria-Bertani (LB) broth with 60 μ g ml⁻¹ of spectinomycin at 30°C. They were diluted 1,000-fold in MM and were grown for 6 h at 42°C. For segregation curves, after dilution in MM, cells were grown for 2 h at 30°C prior to the shift at 42°C, and aliquots were then plated on MM with or without spectinomycin every 2 h for 6 h. Plates were incubated at 37°C and counted every 1, 2, and 3 days. For pAM-*rep*, segregation experiments were performed at 37°C. Cells containing pAM-*rep* were grown overnight in LB broth in the presence of 500 μ M IPTG and 60 μ g \cdot ml⁻¹ of spectinomycin. Cells were diluted 1,000-fold in MM and grown for 8 h at 37°C. Segregation cultures were then treated as for pGBts-*rep*. For most of the strains, cells that kept the *rep*-carrying plasmid appeared overnight, whereas cells that lost it appeared in 2 days. Cells were picked on MM with or without spectinomycin (and IPTG for pAM-*rep*) to identify those that lost the plasmid. The plasmidless cells were checked systematically for resistance to antibiotics, UV resistance, and PCR amplification of relevant genes.

Measure of linear DNA by PFGE. Quantification of linear DNA by pulsed-field gel electrophoresis (PFGE) was performed as described previously (33).

RESULTS

***rep uvrD* lethality is independent of a role of UvrD in repair pathways.** UvrD is required for NER and MMR. We tested a possible role of the absence of NER and MMR in the $\Delta rep \Delta uvrD$ synthetic lethality. To inactivate NER, we introduced a *uvrA* deletion in a Δrep mutant by P1 transduction. The efficiency of $\Delta uvrA$ P1 transduction was similar in the Δrep mutant and in wild-type cells (10^{-6} transductant/viable cell), and inactivation of *uvrA* did not affect the viability of Δrep cells measured by the plating efficiency of overnight cultures (Table 2). Similarly *mutL*, which inactivates MMR, was transduced in Δrep cells as efficiently as in wild-type cells (10^{-6} transductant/viable cell), and *mutL* did not affect the viability of Δrep cells (Table 2). We inactivated both NER and MMR in Δrep cells by constructing a $\Delta rep \Delta uvrA mutL$ strain. The efficiency of transduction of $\Delta uvrA$ in the $\Delta rep mutL$ cells was the same as in wild-type cells, and $\Delta rep \Delta uvrA mutL$ cells showed the same

TABLE 1. Strains

Strain	Relevant genotype	Construction, source, or reference
CAG18491	<i>metE3079::Tn10</i>	28
JJC104	<i>recJ248::Tn10</i>	Susan Lovett, Brandeis University, MA
JJC330	<i>recB270(Ts) recC271(Ts)</i>	33
JJC1177	<i>rep::Ap</i>	Laboratory collection
JJC1702	<i>ΔrarA::Cm</i>	2
JJC1707	<i>Δrep::cam uvrD::Tn5 recR::Tn5 metE::Tn10</i> (pGBts-rep ⁺)	MAC 574 in reference 30
JJC1708	<i>Δrep::cam uvrD::Tn5</i> (pGBts-rep ⁺)	MAC 556 in reference 30
JJC1892	<i>rep::Ap</i> (pAM-rep ⁺)	Laboratory collection
JJC1945	<i>sfiA11</i>	10
JJC1960	<i>uvrD::Tn5 rep::Ap</i> (pAM-rep ⁺)	Laboratory collection
JJC2044	<i>ΔuvrA::Cm</i>	Laboratory collection
JJC2062	<i>recF400::Tn5 zid501::Tn10</i>	Laboratory collection
JJC213	<i>Δrep::kan</i>	35
JJC2230	<i>ΔruvABC::Cm</i> (pGB-ruvABC ⁺)	JJC754 transformed with pGB-ruvABC ⁺
JJC2386	<i>Δ(uvrD-yigB)::cam</i>	8
JJC2417	<i>rep::Ap uvrD::Tn5 recR::Tn5 metE::Tn10</i> (pGBts-rep ⁺)	JJC1707 *P1 JJC1177 ^a
JJC2457	<i>sfiA11 Δ(uvrD-yigB)::cam</i>	JJC1945 *P1 JJC2386
JJC2530	<i>sfiA11 uvrD252</i>	18
JJC2534	<i>sfiA11 uvrD252</i> (pGBts-rep ⁺)	JJC2530 transformed with pGBts-rep ⁺
JJC2540	<i>sfiA11 uvrD252 recB270(Ts) recC271(Ts)</i>	18
JJC2542	<i>sfiA11 uvrD252</i> (pGBts-rep ⁺) <i>rep::Ap</i>	JJC2534 *P1 JJC1177
JJC2632	<i>Δtus::kan pheA::Spc-TerB uvrD(R284A) yigB::Cm</i>	Laboratory collection
JJC2634	<i>ΔuvrD294::Kan</i>	18
JJC2643	<i>sfiA11 uvrD(R284A) yigB::Cm</i>	7
JJC3121	<i>Δrep::cam uvrD::Tn5</i> (pGBts-rep ⁺) <i>recJ248::Tn10</i>	JJC1708 *P1 JJC104
JJC3122	<i>Δrep::cam uvrD::Tn5</i> (pGBts-rep ⁺) <i>recQ1803::Tn3</i>	JJC1708 *P1 <i>recQ1803::Tn3 uvrD::Tn5</i>
JJC3236	<i>uvrD::Tn5 rep::Ap</i> (pAM-rep ⁺) <i>ΔrarA::Cm</i>	JJC1960 *P1 JJC1702
JJC3870	<i>Δrep::kan</i> (pAM-rep ⁺)	JJC213 transformed with pAM-rep ⁺
JJC3971	<i>Δrep::kan</i> (pAM-rep ⁺) <i>ΔuvrD294::Kan</i>	JJC3870 *P1 JJC2634
JJC405	<i>recQ1803::Tn3</i>	R. G. Lloyd, University of Nottingham, United Kingdom
JJC4090	<i>Δrep::kan mutL218::Tn10</i>	JJC213 *P1 <i>mutL218::Tn10</i>
JJC4092	<i>Δrep::kan ΔuvrA::Cm</i>	JJC213 *P1 JJC2044
JJC4144	<i>rep::Ap</i> (pAM-rep ⁺) <i>uvrD(R284A) yigB::Cm</i>	JJC1892 *P1 JJC2632
JJC4201	<i>Δrep::kan mutL218::Tn10</i> (pAM-rep ⁺)	JJC4090 transformed with pAM-rep ⁺ *P1 JJC2457
JJC4207	<i>Δrep::kan ΔuvrA::Cm metE3079::Tn10</i>	JJC4092 *P1 CAG18491
JJC4263	<i>Δrep::kan ΔuvrA::Cm ΔuvrD294::Kan met⁺</i>	JJC4207 *P1 JJC2634
JJC4297	<i>Δrep::cam uvrD::Tn5 recJ248::Tn10</i>	JJC3121 cured of pGBts-rep ⁺
JJC4298	<i>Δrep::cam uvrD::Tn5 recQ1803::Tn3</i>	JJC3122 cured of pGBts-rep ⁺
JJC4326	<i>rep::Ap</i> (pAM-rep ⁺) <i>uvrD(R284A) yigB::Cm recF400::Tn5 zid501::Tn10</i>	JJC4144 *P1 JJC2062
JJC4362	<i>rep::Ap</i> (pAM-rep ⁺) <i>uvrD(R284A) yigB::Cm recJ248::Tn10</i>	JJC4144 *P1 JJC104
JJC4373	<i>sfiA11 uvrD252 rep::Ap</i>	JJC2542 cured of pGBts-rep ⁺
JJC4485	<i>rep::Ap uvrD(R284A) yigB::Cm recF400::Tn5 zid501::Tn10</i>	JJC4326 cured of pAM-rep ⁺
JJC4486	<i>rep::Ap uvrD(R284A) yigB::Cm recJ248::Tn10</i>	JJC4362 cured of pAM-rep ⁺
JJC4494	<i>Δrep::kan mutL218::Tn10 ΔuvrA::Cm</i>	JJC4090 *P1 JJC2044
JJC4509	<i>sfiA11 uvrD252</i> (pGBts-rep ⁺) <i>rep::Ap mutL218::Tn10</i>	JJC2542 *P1 <i>mutL218::Tn10</i>
JJC4526	<i>sfiA11 uvrD252 rep::Ap mutL218::Tn11</i>	JJC4509 cured of pGBts-rep ⁺
JJC4569	<i>uvrD::Tn5 rep::Ap</i> (pAM-rep ⁺) <i>ΔuvrA::Cm</i>	JJC1960 *P1 JJC2044
JJC4571	<i>sfiA11 uvrD252 recB270(Ts) recC271(Ts) Δrep::kan</i>	JJC2540 *P1 JJC213
JJC4572	<i>uvrD::Tn5 Δrep::Ap</i> (pAM-rep ⁺) <i>ΔuvrA::Cm mutL218::Tn10</i>	JJC4569 *P1 <i>mutL218::Tn10</i>
JJC4588	<i>sfiA11 uvrD252 recB270(Ts) recC271(Ts) Δrep::kan recF400::Tn5 zid501::Tn10</i>	JJC4571 *P1 JJC2062
JJC4616	<i>sfiA11 uvrD252 recB270(Ts) recC271(Ts) Δrep::kan mutL218::Tn10</i>	JJC4571 *P1 <i>mutL218::Tn10</i>
JJC4637	<i>sfiA11 uvrD252 recB270(Ts) recC271(Ts) Δrep::kan recF400::Tn5 zid501::Tn10</i>	JJC4588 *P1 JJC2230
JJC4702	<i>sfiA11 uvrD(R284A) yigB::Cm recQ1803::Tn3</i>	JJC2643 *P1 JJC405
JJC4704	<i>sfiA11 uvrD(R284A) yigB::Cm recQ1803::Tn3</i> (pGBts-rep ⁺)	JJC4702 transformed with pGBts-rep ⁺
JJC4763	<i>sfiA11 uvrD(R284A) yigB::Cm recQ1803::Tn3</i> (pGBts-rep ⁺) <i>Δrep::kan</i>	JJC4704 *P1 JJC213
JJC4799	<i>sfiA11 uvrD(R284A) yigB::Cm recQ1803::Tn3 Δrep::kan</i>	JJC4763 cured of pGBts-rep ⁺
JJC505	<i>Δrep::kan recB270(Ts) recC271(Ts)</i>	22
JJC754	<i>ΔruvABC::Cm</i>	33
JJC760	<i>Δrep::cam</i> (pGBts-rep ⁺)	24

^a *P1 indicates transduction by P1.

viability as the *Δrep* cells or the *Δrep mutL* cells (Table 2). These results indicate that NER and MMR are not essential in a *Δrep* mutant.

Since UvrD acts at the last step of NER and MMR, the

lethality of the *Δrep ΔuvrD* mutant could result from the formation of toxic intermediates by NER or MMR early proteins. To test this hypothesis, we analyzed the viability of the *Δrep ΔuvrD* mutant in cells where the genes acting upstream of *uvrD*

TABLE 2. Inactivation of NER or MMR does not affect the viability of the Δrep mutant

Strain	Relevant genotype	CFU/ml at 37°C ^a	Plating efficiency ^b
JJC213	Δrep	4.00E+09 ± 7.39E+08	1.00
JJC4092	$\Delta rep \Delta uvrA$	2.47E+09 ± 1.07E+09	0.62
JJC4090	$\Delta rep mutL::Tn10$	4.15E+09 ± 5.55E+08	1.04
JJC4494	$\Delta rep \Delta uvrA mutL::Tn10$	3.26E+09 ± 8.44E+08	0.81

^a Overnight cultures grown at 37°C were plated at 37°C to determine the CFU/ml.

^b Ratio of the CFU/ml of the strain to the CFU/ml of the Δrep strain.

in NER or MMR are inactivated. We took advantage of a conditional plasmid carrying the wild-type *rep* gene, pAM-*rep*, which replicates only in the presence of IPTG. $\Delta rep \Delta uvrD \Delta uvrA$ (pAM-*rep*), $\Delta rep \Delta uvrD mutL$ (pAM-*rep*), and $\Delta rep \Delta uvrD \Delta uvrA mutL::Tn10$ (pAM-*rep*) mutants were constructed. No plasmidless segregant was recovered after propagation in the absence of IPTG (Table 3). These results indicate that the synthetic lethality of *rep* and *uvrD* genes is independent of UvrD action in DNA repair.

The restart pathway Rep-PriC is not essential in a $\Delta uvrD$ mutant. Since Rep and PriC act together in a replication restart pathway, the synthetic lethality of *rep* and *uvrD* could result from a requirement for this replication restart pathway in a $\Delta uvrD$ mutant. To test this hypothesis, the efficiency of cotransduction of $\Delta uvrD$ with the *Met*⁺ gene was compared in *priC* and *PriC*⁺ *met::Tn10* contexts (*met* is 15 kb downstream of *uvrD* and 30% linked in cotransduction). Transductants were selected for the loss of methionine auxotrophy (transduction of the wild-type *Met*⁺ gene), and the proportion of *uvrD::kan* cotransductants was measured. The percentage of cotransduction of $\Delta uvrD$ with *met* in *PriC*⁺ cells was 30% of that in *priC* mutant cells. The *priC uvrD* double mutant did not show any growth defect, indicating that the inactivation of *PriC* in $\Delta uvrD$ cells has no effect on viability. Thus, $\Delta rep \Delta uvrD$ lethality is independent of the action of Rep in the Rep-PriC pathway of replication restart.

TABLE 3. *rep uvrD* lethality is suppressed by the inactivation of *recJ* and *recQ*

Strain	Relevant genotype	Plasmidless colonies (CFU/ml) ^a
JJC3870	Δrep (pAM- <i>rep</i>)	2.01E+08 ± 1.88E+08
JJC760	Δrep (pGBts- <i>rep</i>)	1.65E+09 ± 1.31E+09
JJC3971	$\Delta rep \Delta uvrD$ (pAM- <i>rep</i>)	<1E+04
JJC1708	$\Delta rep uvrD::Tn5$ (pGBts- <i>rep</i>)	<1E+04
JJC4263	$\Delta rep \Delta uvrD \Delta uvrA$ (pAM- <i>rep</i>)	<1E+04
JJC4201	$\Delta rep \Delta uvrD mutL::Tn10$ (pAM- <i>rep</i>)	<1E+04
JJC4572	$\Delta rep uvrD::Tn5 \Delta uvrA mutL$ (pAM- <i>rep</i>)	<1E+04
JJC3122	$\Delta rep uvrD::Tn5 recQ::Tn3$ (pGBts- <i>rep</i>)	1.44E+09 ± 7.65E+08
JJC3121	$\Delta rep uvrD::Tn5 recJ::Tn10$ (pGBts- <i>rep</i>)	1.36E+09 ± 4.12E+08
JJC2417	$\Delta rep uvrD::Tn5 recR::Tn5$ (pGBts- <i>rep</i>)	1.78E+09 ± 1.06E+09
JJC3236	$\Delta rep uvrD::Tn5 \Delta rarA$ (pAM- <i>rep</i>)	<1E+04
JJC4144	$\Delta rep uvrD(R284A)$ (pAM- <i>rep</i>)	<1E+04
JJC4763	$\Delta rep uvrD(R284A) recQ::Tn3$ (pGBts- <i>rep</i>)	6.58E+08 ± 3.09E+08
JJC4362	$\Delta rep uvrD(R284A) recJ::Tn10$ (pAM- <i>rep</i>)	6.83E+08 ± 2.97E+08
JJC4326	$\Delta rep uvrD(R284A) recF::Tn5$ (pAM- <i>rep</i>)	1.16E+09 ± 5.92E+08
JJC2542	$\Delta rep uvrD252$ (pGBts- <i>rep</i>)	8.27E+08 ± 7.53E+08
JJC4509	$\Delta rep uvrD252 mutL::Tn10$ (pGBts- <i>rep</i>)	1.21E+09 ± 5.09E+08

^a Cells containing pGBts-*rep* or pAM-*rep* were cured by propagation at 42°C or in medium lacking IPTG, respectively, and colonies isolated on MM were checked for plasmid loss by picking on spectinomycin plates (see Materials and Methods).

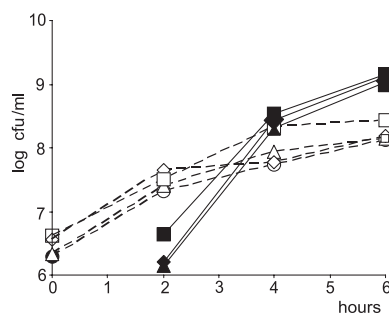


FIG. 1. *rep uvrD* lethality is suppressed by *recQ* or *recJ* inactivation. Segregation experiments were carried out as described in Materials and Methods. Briefly, plasmid-containing cells grown at 30°C were shifted to 42°C at time zero. After 2 to 4 h, they plateau due to the lack of plasmid replication at high temperature (open symbols), while plasmidless cells appear (closed symbols). No plasmidless cells appeared with the *rep uvrD* double mutant, while they did appear with similar kinetics with *rep uvrD recR*, *recJ*, or *recQ* triple mutants (and with the *rep* single mutant, JJC760; data not shown). Open symbols are CFU on spectinomycin plates, i.e., pGBts-*rep*-containing strains. Closed symbols are pGBts-*rep*-cured strains, measured as CFU on MM, identified as Spt^s. Circles, JJC1708 [*rep uvrD* (pGBts-*rep*)]; triangles, JJC2417 [*rep uvrD recR* (pGBts-*rep*)]; squares, JJC3121 [*rep uvrD recJ* (pGBts-*rep*)]; diamonds, JJC3122 [*rep uvrD recQ* (pGBts-*rep*)].

Inactivation of RecQ and RecJ restores the viability of *rep uvrD* cells. To determine whether RecQ and RecJ are involved in the RecFOR-dependent formation of deleterious DNA structures in $\Delta rep \Delta uvrD$ cells, we tested whether *recQ* or *recJ* inactivation restores the viability of a $\Delta rep \Delta uvrD$ double mutant. pGBts-*rep*, a conditional plasmid that replicates only at 30°C, was used. We constructed $\Delta rep \Delta uvrD recQ::Tn3$ (pGBts-*rep*) and $\Delta rep \Delta uvrD recJ::Tn10$ (pGBts-*rep*) strains. In both cases, propagation of the strain at 42°C allowed the recovery of plasmidless colonies, as observed with $\Delta rep \Delta uvrD recF::Tn5$ (pGBts-*rep*) cells (30) (Fig. 1 and Table 3). The observation that the inactivation of RecQ, RecJ, or RecF restores the viability of $\Delta rep \Delta uvrD$ cells suggests that the lethality of $\Delta rep \Delta uvrD$ cells is caused by the concerted action of all these presynaptic proteins.

We have previously shown that in *dnaE*(Ts) cells (Pol III polymerase mutant), RarA is required with RecQ, RecJ, and RecFOR for the formation of deleterious RecA filaments at inactivated replication forks, whereas it is not required for the formation of RecA filaments in *dnaN*(Ts) cells (Pol III clamp mutant) (18). We tested whether RarA is required for the formation of deleterious RecA filaments in $\Delta rep \Delta uvrD$ cells by investigating whether the inactivation of *raraA* restores the viability of $\Delta rep \Delta uvrD$ cells. A $\Delta rep \Delta uvrD \Delta rarA$ (pGBts-*rep*) strain was constructed; no plasmidless colonies could be recovered after curing the plasmid by propagation at 42°C. Therefore, RarA is not required for the formation of deleterious RecA filaments by RecQJFOR in the Δrep mutant (Table 3).

Role of the *uvrD*(R284A) allele in Δrep cells. We tested the effect of the *uvrD*(R284A) allele, which totally inactivates the helicase function of UvrD by a mutation in the helicase domain IV (11, 39). The combination of the *uvrD*(R284A) allele with *rep* deletion in the presence of the pAM-*rep* plasmid does not allow the recovery of plasmidless colonies after cell propagation in the absence of IPTG (Table 3), indicating that the *rep uvrD*(R284A) double mutant is lethal. In order to test whether

TABLE 4. *uvrD252* does not suppress the *rep recBC(Ts)* lethality at high temperature

Strain	Mutant genotype	Plating efficiency at ^a :	
		37°C	42°C
JJC505	<i>recBC(Ts) Δrep</i>	<10 ⁻⁶	<10 ⁻⁶
JJC2540	<i>recBC(Ts) uvrD252</i>	1.24 ± 0.09	1.12 ± 0.46
JJC4571	<i>recBC(Ts) Δrep uvrD252</i>	<10 ⁻⁶	<10 ⁻⁶

^a Overnight cultures grown at 25°C were plated at 25°C and 37°C or 42°C. For each culture, the ratio of clones obtained at 37°C or 42°C to those obtained at 30°C was calculated, and the results are expressed as the averages of the ratios.

the lethality of *Δrep uvrD(R284A)* cells is due to a deleterious action of RecQ, RecJ, and RecFOR proteins, *Δrep uvrD(R284A) recJ(pAM-rep)*, *Δrep uvrD(R284A) recF(pAM-rep)*, and *Δrep uvrD(R284A) recQ(pGBts-rep)* strains were constructed. Plasmidless colonies were recovered with all three mutants (Table 3). In conclusion, the *uvrD(R284A)* mutation confers to *Δrep* the same phenotypes as the null *uvrD* mutation.

Role of the *uvrD252* allele in *Δrep* cells. UvrD252 is a partial mutant. As in *uvrD* null mutants, it is defective for NER and antirecombination action and is constitutively induced for the SOS response (18). However, it has retained the UvrD MMR function (37). We previously showed that UvrD252 retains the capacity to antagonize RecQJFORA-dependent prevention of RFR in a *dnaE(Ts)* mutant but not in a *dnaN(Ts)* mutant (18). We asked whether UvrD252 antagonizes RecQJFOR action at arrested replication forks in *Δrep* cells.

A *Δrep uvrD252* (pGBts-rep) mutant was constructed. Plasmidless colonies were obtained after cell propagation at 42°C to cure the plasmid (Table 3), showing that this combination of mutations is viable. This finding indicates that UvrD252 retains the capacity to antagonize RecQJFOR deleterious action in a *Δrep* mutant. The weak residual ATPase activity of the UvrD252 enzyme detected in vitro is only significant in vivo when the helicase activity of UvrD is activated by MutL during MMR. In order to test if the anti-RecQJFOR action of UvrD252 in *Δrep* cells is dependent on a stimulation of UvrD252 by MutL, we constructed a *Δrep uvrD252 mutL* (pGBts-rep) mutant. As with the *rep uvrD252* (pGBts-rep) mutant, plasmidless colonies were recovered after segregation (Table 3). Thus, UvrD252 does not require MutL to antagonize the deleterious action of RecQJFOR in *Δrep* cells.

Because of the lethality of the *rep uvrD* double mutant, the effects of *uvrD* inactivation on RFR in a *rep* mutant cannot be tested. However, the viability of the *Δrep uvrD252* cells offered the possibility to study the effect of the *uvrD252* allele on RFR in *Δrep* cells. As presented in the introduction, the first step of RFR is the formation of a Holliday junction with a dsDNA end. In the absence of RecBC, the dsDNA end is not processed and the reversed fork is recognized only by RuvABC, which resolves the Holliday junction, causing chromosome linearization. Linear chromosome fragments can be detected by PFGE, as intact chromosomes or σ molecules are entrapped in the wells while linear fragments enter gels. Thus, the amount of linear DNA detected in a *recBC(Ts)* context at the restrictive temperature of 42°C is a measure of RFR. A *recBC(Ts) Δrep uvrD252* strain was constructed at 25°C by transducing a

TABLE 5. *uvrD252* does not decrease the percentage of linear chromosome in the *rep recBC(Ts)* mutant

Strain	Relevant genotype	Percentage of linear DNA ^a at:		<i>n</i> ^b
		30°C	42°C	
JJC330	<i>recBC(Ts)</i>	9.1 ± 3.4	19.1 ± 5	3/3
JJC505/790	<i>recBC(Ts) rep</i>	16.8 ± 4.9	71.1 ± 8.6	3/7
JJC2540	<i>recBC(Ts) uvrD252</i>	20	39 ± 1	1/3
JJC4571	<i>recBC(Ts) rep uvrD252</i>	14.5 ± 3.5	71.5 ± 3.5	2/6
JJC4616	<i>recBC(Ts) rep uvrD252 mutL</i>	11 ± 1	73 ± 3	2/3
JJC4588	<i>recBC(Ts) rep uvrD252 recF</i>	12 ± 4.2	58.8 ± 6	2/4
JJC4637	<i>recBC(Ts) rep uvrD252 recF ruvABC</i>	5.5 ± 0.7	26.1 ± 1	2/7

^a The percentage of linear DNA was quantified by PFGE performed with ³H-Thy-labeled chromosomes, by measuring the proportion of linear chromosome that enters the pulsed-field gels (33). RecBC⁺ cells always contain less than 10% linear DNA at all temperatures by this technique, as shown here by the low level of linear DNA in the *recBC(Ts)* mutant at 30°C (33; also data not shown). Results for JJC330 and JJC505/790 (33) and for JJC2540 (18) were previously published and reproduced here as controls.

^b Number of independent experiments (30°C/42°C).

Δrep::Kan deletion in a *recBC(Ts) uvrD252* strain. The *rep* gene is 37 kb distant from the *uvrD* gene, and these two genes are 25% to 30% linked in P1 transduction. Accordingly, more than half of *Δrep::Kan* transductants remained *uvrD252* based on their UV sensitivity, showing that a *recBC(Ts) Δrep uvrD252* triple mutant is fully viable at 25°C. The thermosensitivity of *recBC(Ts) Δrep uvrD252* cells was determined by measuring the plating efficiency at 37°C and 42°C of overnight cultures grown at 25°C (Table 4). The results showed that *recBC(Ts) Δrep uvrD252* cells are as thermosensitive at 37°C and 42°C as *recBC(Ts) Δrep* cells. Thus, the *uvrD252* allele can be combined with *rep* deletion but does not suppress the requirement for RecBC for viability.

We measured the proportion of broken chromosomes at 42°C in *recBC(Ts) Δrep uvrD252* cells. The high level of fork breakage in *recBC(Ts) Δrep uvrD252* was not significantly different from that in *recBC(Ts) Δrep* cells (about 70%; Table 5). To ascertain that chromosome breakage results from RFR, we tested that it requires the Holliday junction resolving complex RuvABC. However, owing to its hyperrecombination phenotype, the *uvrD252 ruvABC* double mutant is viable only if gap repair is prevented by *recFOR* inactivation (18). *recFOR* inactivation has no effect on fork breakage in *rep recBC(Ts) uvrD252* cells, as previously reported for *rep recBC(Ts)* cells (3) (see JJC4588 in Table 5). The level of linear DNA decreases significantly upon inactivation of RuvABC in a *recBC(Ts) Δrep uvrD252 recF* mutant (26%; Table 5). Thus, fork breakage in the *rep uvrD252 recBC(Ts)* context is RuvABC dependent, indicating that it results from RFR. The inactivation of *mutL* did not modify the level of fork breakage in the *recBC(Ts) Δrep uvrD252* mutant (Table 5), indicating that MutL is not required for the action of UvrD252 at *rep*-blocked forks. In conclusion, the UvrD252 protein allows RFR in the presence of all RecQJFORA proteins. As for the UvrD wild-type protein, it can prevent or counteract the deleterious action of these proteins.

DISCUSSION

In this work, we characterized *in vivo* the action of UvrD in a *rep* mutant. Our results support the proposal that UvrD acts at *rep*-blocked forks to counteract a deleterious RecA filament and show that the strongly impaired *uvrD252* mutant has retained this function.

In Pol III(Ts) mutants, replication perturbation at high temperature (37°C or 42°C) leads to the formation of (i) gaps left behind replication forks and (ii) inactivated forks. We previously showed that, while the formation of RecA filaments at gaps is independent of RecQ, the formation of RecA filaments at arrested replication forks requires RecQ and renders UvrD anti-RecA action essential for RFR and for viability (18). We show here that the RecQ helicase and the RecJ exonuclease are also needed for RecFOR lethal action in *rep uvrD* cells, which suggests that RecQ and RecJ participate in the formation of RecA filaments counteracted by the UvrD helicase at *rep*-blocked forks. As previously observed in the *dnaN*(Ts) mutant, RarA is not required for the fixation of RecA at *rep*-blocked forks. Therefore, the action of RarA does not correlate with that of UvrD252, arguing against a role for RarA in UvrD252 activity.

To address the role of the ATPase activity of UvrD for its anti-RecA action, we took advantage of two *uvrD* mutants, *uvrD252* and *uvrD(R284A)*. *In vitro*, both mutant proteins bind ssDNA; the G30D mutation in *uvrD252* is adjacent to the first helicase motif and the ATPase activity of UvrD252 is largely decreased, while the R284A mutation is within the helicase motif IV and the ATPase activity of the UvrD(R284A) mutant is fully abolished (11, 39). In agreement with its lack of ATPase activity *in vitro*, the *uvrD(R284A)* mutant is defective in NER and MMR *in vivo*. In contrast, the *uvrD252* mutant is defective for NER but supports MMR, probably through a stimulation of its residual ATPase activity by MutL. In addition, UvrD252 is fully deficient for the prevention of SOS induction and for the prevention of homologous recombination, indicating that it cannot remove RecA from DNA gaps, in the wild type as well as in Pol III(Ts) mutants (18).

We previously reported that the *uvrD252* allele can be combined with *dnaE*(Ts) and *dnaN*(Ts) mutations at low temperature, while *uvrD(R284A)* is lethal in these two Pol III(Ts) backgrounds even at permissive temperatures (30°C or 25°C). We propose that the dominant-negative phenotype conferred by the *uvrD(R284A)* mutation in Pol III(Ts) mutants results from a poisoning effect of stable UvrD(R284A)-DNA complexes, owing to a total absence of ATPase activity (18). Here, we found that the *uvrD(R284A)* mutation is also synthetic lethal with *rep* inactivation, whereas *uvrD252* is not, suggesting that the residual ATPase activity of UvrD252 is responsible for the lack of the dominant-negative effect of this mutation. Our study of Pol III(Ts) *uvrD252* mutants indicated that UvrD counteracts RecA filaments at blocked replication forks by two different modes of action. In the *dnaN*(Ts) mutant, UvrD252 is unable to antagonize RecA binding to forks, suggesting that UvrD acts by dismantling RecA filaments from *dnaN*(Ts)-blocked forks (Fig. 2). In contrast, in the *dnaE*(Ts) mutant, UvrD252 is able to prevent RFR inhibition by RecA, and we proposed that it acts prior to RecA binding to DNA (Fig. 2). The occurrence of RFR in the *rep uvrD252* double mutant

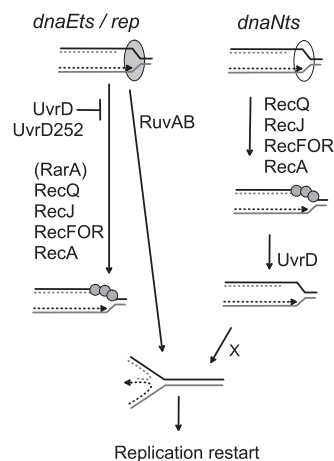


FIG. 2. Model of action of UvrD/*uvrD252* in replication mutants (adapted from reference 18). At *dnaN*(Ts)-blocked forks (right portion), UvrD252 is unable to counteract RecQJFORA, and we propose that UvrD acts by removing RecA from DNA. At *dnaE*(Ts)-blocked (13) and *rep*-blocked (this work) forks, UvrD252 can counteract RecQJFORA, and we propose that it acts prior to RecA binding, by preventing it. RarA is shown in parentheses because it is only essential for RecA binding in the *dnaE*(Ts) mutant and not in the *rep* mutant. RuvAB is the only way of RFR in the *dnaE*(Ts) mutant and one of two pathways in the *rep* mutant. In the *dnaN*(Ts) mutant, RFR is RuvAB independent and the enzymes that reverse forks are unknown (1).

suggests that UvrD also acts prior to RecA in the *rep* mutant and that this action is unaffected by the *uvrD252* mutation (Fig. 2). We show here that the UvrD(R284A) mutant protein does not poison *rep*-blocked forks when RecQJFOR are absent, suggesting that UvrD(R284A) does not access *rep*-blocked forks in the absence of these proteins and that the actions of RecQJFOR and UvrD are concerted. *uvrD252* was originally isolated as a recombination-deficient mutant in a *recBC sbcB sbcCD* background. This property does not simply reflect a loss of function of the *uvrD252* mutant allele, since *uvrD* null mutations do not inactivate homologous recombination in this background (20). It is interesting to note that *recBC sbcB sbcCD* is the only background where homologous recombination is RecQ dependent and the only background where *uvrD252* confers a recombination-deficient phenotype, and hence counteracts recombination proteins. We can propose two models that account for these observations. In the first model, although UvrD252 has lost most of the UvrD *in vivo* activities, it has acquired a particular property of counteracting RecQ, observed at dsDNA ends in the *recBC sbcB sbcCD* mutant and at blocked forks in *dnaE*(Ts) and *rep* mutants but not at *dnaN*(Ts)-blocked forks. In the second model, by blocking several RecQ activities, UvrD252 reveals that UvrD and RecQ proteins share common targets, suggesting that certain RecQ functions, performed with RecJ and RecFOR, are controlled by UvrD. The “raison d’être” of this control could be a need to prevent inappropriate RecQ (and RecJFOR) action at certain inactivated replication forks.

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