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UvrD controls the access of recombination proteins to blocked replication forks

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Blocked replication forks often need to be processed by recombination proteins prior to replication restart. In *Escherichia coli*, the UvrD repair helicase was recently shown to act at inactivated replication forks, where it counteracts a deleterious action of RecA. Using two mutants affected for different subunits of the polymerase III holoenzyme (Pol IIIh), we show here that the anti-RecA action of UvrD at blocked forks reflects two different activities of this enzyme. A defective UvrD mutant is able to antagonize RecA in cells affected for the Pol IIIh catalytic subunit DnaE. In this mutant, RecA action at blocked forks specifically requires the protein RarA (MgsA). We propose that UvrD prevents RecA binding, possibly by counteracting RarA. In contrast, at forks affected for the Pol IIIh clamp (DnaN), RarA is not required for RecA binding and the ATPase function of UvrD is essential to counteract RecA, supporting the idea that UvrD removes RecA from DNA. UvrD action on RecA is conserved in evolution as it can be performed in *E. coli* by the UvrD homologue from *Bacillus subtilis*, PcrA.

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Introduction

During their progression, replication forks encounter obstacles that block their progression and eventually cause replisome disassembly. Replication impairment is now recognized as an important source of genetic instability in several organisms (Michel, 2000; Kolodner *et al*, 2002). Examples in mammalian systems include various disorders such as cancer (Shima *et al*, 2007). *Escherichia coli* chromosomal replication is catalyzed by a 10-subunit complex, the DNA polymerase III holoenzyme (Pol IIIh). Together with the replicative helicase (DnaB) and primase (DnaG), these complexes form the replisome (reviewed in Johnson and O'Donnell, 2005; O'Donnell, 2006). Replication restart requires PriA and other pre-primosomal proteins that reload the replicative helicase and, in turn, allow the re-assembly of a functional replication complex at inactivated replication forks (reviewed in Sandler and Marians, 2000). However, in several instances of replication arrest, replication does not restart directly and forks are first processed by recombination proteins (reviewed in Michel *et al*, 2004). Defects or errors in the processing of inactivated replication forks can have dramatic consequences as it can generate chromosome rearrangements. Our work aims at understanding the various pathways that process inactivated replication forks prior to replication restart. For this purpose, we used here mutants affected for two different subunits of Pol IIIh, the polymerase catalytic subunit DnaE and the clamp DnaN.

Any mutation or chemical that perturbs replication is likely to cause the formation of two types of abnormal structures: (i) single-strand gaps are formed when the lagging strand polymerase is impeded and are left behind the progressing replication fork and (ii) inactivated replication forks form when the leading strand polymerase is impeded (Langston and O'Donnell, 2006). These two types of structures co-exist in a cell population. Both contain single-strandeded DNA (ssDNA) and it is generally difficult to ascribe a deleterious phenotype caused by replication impairment to a defect in gap repair versus fork repair. In *E. coli* replication mutants, we can unambiguously determine when a protein acts at forks rather than gaps by studying a specific reaction, called replication fork reversal (RFR; Seigneur *et al*, 1998; Figure 1). The first step of the RFR reaction is the annealing of the leading- and lagging-strand ends at a replication fork to form a Holliday junction (HJ) with a DNA double-stranded end (dsDNA end). Recombination proteins reset the reversed fork. The dsDNA end is acted upon by RecBCD, a recombination complex specific for the repair of dsDNA breaks. RecBCD unwinds and degrades dsDNA up to a specific site named Chi, at which it shifts to a recombinase and loads the recombination enzyme RecA (Singleton *et al*, 2004, and references therein). We showed that RecBCD processes reversed forks either by degradation of the dsDNA end or by integration of this dsDNA end into the chromosome by homologous recombination, converting them into a structure from which replication restarts (Seigneur *et al*, 1998; pathways C and D in Figure 1). HJs made by homologous recombination or by fork reversal are branch-migrated by the RuvAB complex and resolved by the *E. coli* resolvase, the RuvABC complex (reviewed in West, 1997). Replication restarts from recombination intermediates or fork structures by the assembly of a new replisome via the action of PriA and its partner proteins (Figure 1). When RecBC is inactivated, the dsDNA end at reversed forks is not acted upon and resolution by RuvABC of the HJ made by fork reversal causes fork breakage (Figure 1E). Because resolution of HJs made by homologous recombination produces intact circular chromosomes, RuvABC only breaks chromosomes when it resolves an HJ made by fork reversal. Consequently, a modification of the level of RuvABC-dependent fork breakage by a mutation in a

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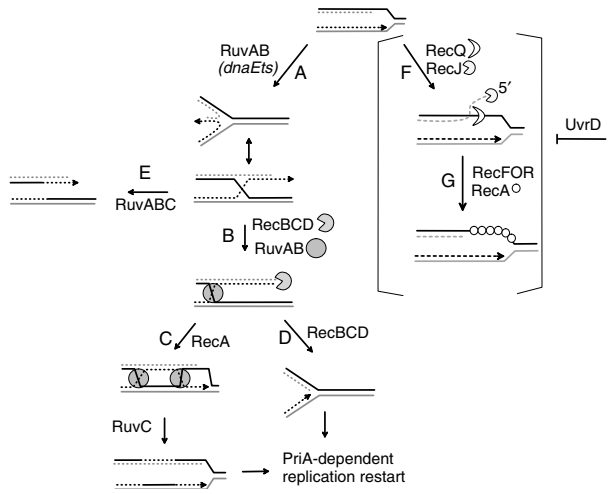

Figure 1 Replication fork reversal model (left, pathways A–E) and model of action of UvrD (right, pathways F–G) (adapted from Seigneur *et al*, 1998 and Flores *et al*, 2005). In the first step (A), the replication fork is arrested by impairment of a replication protein, causing fork reversal. In the *dnaEts* mutant, this step is catalyzed by RuvAB. In both *dnaEts* and *dnaNts* mutants, it is also antagonized by RecQJFORA, which are, in turn, counteracted by UvrD. Consequently, the anti-fork reversal action of RecQJFORA can only be detected in *uvrD* mutants. The reversed fork forms an HJ with an open dsDNA end (two alternative representations of this structure are shown, open X and parallel stacked X). In Rec^+ cells (pathways B–C), RecBCD initiates RecA-dependent homologous recombination at a Chi site present on the dsDNA end and the two HJs (one formed by reversal, one by homologous recombination) are resolved by RuvABC. Alternatively, if RecBCD encounters the HJ before a Chi site, or in the absence of RecA (B–D), the dsDNA end is degraded up to the HJ by the exonuclease V activity of RecBCD, restoring a fork structure. In both cases, replication restarts by a PriA-dependent process. In the absence of RecBC (E), resolution of the HJ by RuvABC causes fork breakage. In Δ*uvrD* mutants, RFR is prevented by the action of RecQJFORA; we proposed that RecA requires RecQ, RecJ and RecFOR to bind DNA at blocked forks (F, G), and that this binding prevents RFR but is counteracted by UvrD in $UvrD^+$ cells. Continuous lines represent parental chromosome. Dashed lines represent newly synthesized strands. Gray circle represents RuvAB. Gray incised circle represents RecBCD. White incised circle represents RecJ. White crescent represents RecQ. Small white circles represent RecA.

given gene indicates that the gene product affects RFR, hence acts at inactivated replication forks.

The first step of the RFR reaction, conversion of a blocked fork into an HJ, is catalyzed by different pathways depending on the cause of replication arrest, suggesting that the processing of inactivated replication forks depends on proteins that remain or become associated with the fork after inactivation. Two RFR pathways have been characterized. Forks are reversed by a peculiar RecA-dependent reaction, which requires no known presynaptic protein in mutants affected for the replicative helicase DnaB (Seigneur *et al*, 2000). An RuvAB-dependent pathway acts in the *rep* helicase mutant and in two Pol IIIh mutants, *dnaEts* affected for the catalytic subunit of Pol IIIh and *holD*, affected for one of the subunits of the clamp loader (Baharoglu *et al*, 2006). A third, still unknown pathway, is active in the *dnaNts* mutant affected for the Pol IIIh clamp, in which RFR is RecA- and RuvAB-independent.

Monitoring RFR has recently allowed us to identify UvrD as a factor that participates in the resetting of inactivated replication forks (Flores *et al*, 2004, 2005). UvrD is a DNA helicase that belongs to the Super Family 1 of helicases (SF1; Gorbalenya and Koonin, 1993). It translocates on DNA in the 3′–5′ direction with a low processivity *in vitro*, and has been widely characterized for its role in nucleotide excision repair (NER) and mismatch repair (reviewed in Matson and Robertson, 2006). UvrD also acts during homologous recombination since *uvrD*-null mutants exhibit an increased frequency of homologous recombination (Zieg *et al*, 1978; Bierne *et al*, 1997), and a particular *uvrD* mutation (*uvrD252*, formerly *recL152*) inactivates homologous recombination in a *recBC sbcBC* background (Horii and Clark, 1973). Finally, UvrD is linked to replication: (i) it co-purifies with Pol III* (a Pol IIIh subcomplex that lacks the β-clamp DnaN) (Lahue *et al*, 1989), (ii) it is essential for the replication of rolling circle plasmids in *E. coli* (Bruand and Ehrlich, 2000), (iii) it is required for Tus removal from physiological replication arrest sites (Ter/Tus complexes) (Bidnenko *et al*, 2006) and (iv) it acts at inactivated replication forks in Pol III*ts* mutants (Flores *et al*, 2005).

In the two Pol IIIh mutants *dnaEts* and *dnaNts*, RFR and viability at semi-permissive temperature require the presence of UvrD. However, UvrD is only needed when RecA and all the pre-synaptic proteins of the RecFOR recombination pathway are present: RecQ (a helicase that translocates in the 3′–5′ direction), RecJ (a 5′–3′ ssDNA exonuclease) and RecFOR, which promotes RecA binding to the ssDNA gaps. The need for UvrD in Pol IIIts mutants only when RecQ, RecJ, RecFOR and RecA are all present, led us to propose that UvrD antagonizes a deleterious concerted action of all these proteins: a RecQ- RecJ- RecFOR-dependent RecA binding to forks, which prevents RFR (Figure 1F and G). Reports that *in vitro* UvrD can undo RecA-ssDNA filaments and RecA-made recombination intermediates (Morel *et al*, 1993; Veaute *et al*, 2005) suggested that UvrD acts at forks after RecA binding, and allows RFR by clearing RecA from DNA at forks (Flores *et al*, 2005).

The action of UvrD during recombination and replication seems to be conserved in other species. The inactivation of SRS2, the yeast UvrD homologue, increases the frequency of homologous recombination and causes cell lethality in the presence of chemicals that perturb replication or in combination with mutations that affect replication (Fabre *et al*, 2002; Ooi *et al*, 2003; Pfander *et al*, 2005; Schmidt and Kolodner, 2006). Therefore, like UvrD in *E. coli*, SRS2 acts in *Saccharomyces cerevisiae* and in *Schizosaccharomyces pombe* to limit recombination and for survival after replication impairment. In Gram-positive bacteria, PcrA, the UvrD homologue, also acts in recombination and replication and PcrA from the distantly related bacteria *Bacillus subtilis* suppresses the NER and Tus blockage defects of a *uvrD E. coli* mutant (Petit *et al*, 1998; Petit and Ehrlich, 2002; Bidnenko *et al*, 2006). In support of the idea that there is a conservation of function of helicases in the UvrD family, we show here that the expression of PcrA also restores RFR in *dnaEts* Δ*uvrD* and *dnaNts* Δ*uvrD* cells.

Some of the mutations that inactivate the ATPase activity of *uvrD in vitro* impair growth of wild-type *E. coli*. One that did not affect wild-type *E. coli* growth is the *uvrD*-R284A mutation, but it could not be combined with *dnaEts* and *dnaNts* mutations (our unpublished results). In order to test whether the ATPase function of UvrD is required for its anti-RecA action at *dnaEts* and *dnaNts*-blocked forks, we used the

uvrD252 mutant. The *uvrD252* mutation is just adjacent to the Walker A motif (Gly30Asp), and *in vitro* the ATPase activity of the UvrD252 protein is strongly reduced, as well as DNA unwinding (Washburn and Kushner, 1993). *UvrD252* mutant cells are defective for NER, but are capable of mismatch repair. Presumably this is due to MutL strongly activating the unwinding activity of UvrD (Hall *et al*, 1998; Yamaguchi *et al*, 1998; Guarne *et al*, 2004). We show here that, although UvrD252 is unable to dismantle RecA-ssDNA filaments or recombination intermediates *in vivo*, RFR occurs in a *dnaEts uvrD252* mutant, but not in a *dnaNts uvrD252* mutant. These results reveal the existence of two modes of action for UvrD at blocked forks, one that does not require the RecA removal activity of UvrD, and one that does, depending on the Pol IIIh subunit that is affected.

RarA (also called MgsA) has been proposed to act at replication forks (Barre *et al*, 2001). This protein is highly conserved in bacteria and eukaryotes. In yeast, Mgs1 plays a yet undetermined role in cell survival after replication impairment (Hishida *et al*, 2006; Vijeh Motlagh *et al*, 2006, and reference therein). In *E. coli*, RarA/MgsA is deleterious in the *dnaEts* mutant and its inactivation improves growth at semipermissive temperature (Shibata *et al*, 2005). We show here that the inactivation of *rarA* restores RFR in a *dnaEts uvrD* mutant, indicating that RarA is required for RecQ, RecJ, RecFOR and RecA (called thereafter RecQJFORA) binding and/or action at *dnaEts*-blocked forks. In contrast, *rarA* inactivation does not restore RFR in *dnaNts* Δ*uvrD* mutants, indicating that RarA/MgsA is not required for RecQJFOR-promoted RecA binding at *dnaNts*-blocked forks. Interestingly, the requirement for RarA correlates with a role of UvrD, independent of its ATPase activity.

Results

The helicase function of UvrD is not required for RFR in the dnaEts mutant

The occurrence of RFR upon replication impairment can be detected by measuring chromosome breakage, as resolution by RuvABC of the HJs formed at reversed forks leads to fork breakage in a *recBC* mutant context (Seigneur *et al*, 1998). In order to quantify fork breakage *in vivo*, we measure the proportion of broken chromosomes in a cell population by pulse field gel electrophoresis (PFGE). *dnaNts*- and *dnaEts*-dependent fork-breakage is maximum at 37 and 42°C, respectively, and is decreased to the level of replication proficient cells by *uvrD* inactivation (Figure 2; Flores *et al*, 2004, 2005; Supplementary Tables S2 and S3; Supplementary Figure S1). Therefore, UvrD is required for RFR to occur in these mutants.

We used the *uvrD252* allele to test whether the helicase activity of UvrD is required for RFR at Pol III*ts*-blocked forks. The level of fork breakage in *dnaEts recBCts uvrD252* cells was not significantly different from that in *dnaEts recBCts* UvrD$^+$ cells ($P = 0.4$), and significantly higher than that in the *dnaEts recBCts* Δ*uvrD* mutant ($P < 0.001$; Figure 2A; Supplementary Table S2; Supplementary Figure S1). As a control, the Δ*uvrD* allele was introduced by P1 transduction into in the *dnaEts recBCts uvrD252* mutant; fork breakage decreased in this Δ*uvrD* construct, confirming that the presence of the UvrD252 protein allows fork breakage (JJC2545 Δ*uvrD*; Supplementary Table S2). Using cells that express the

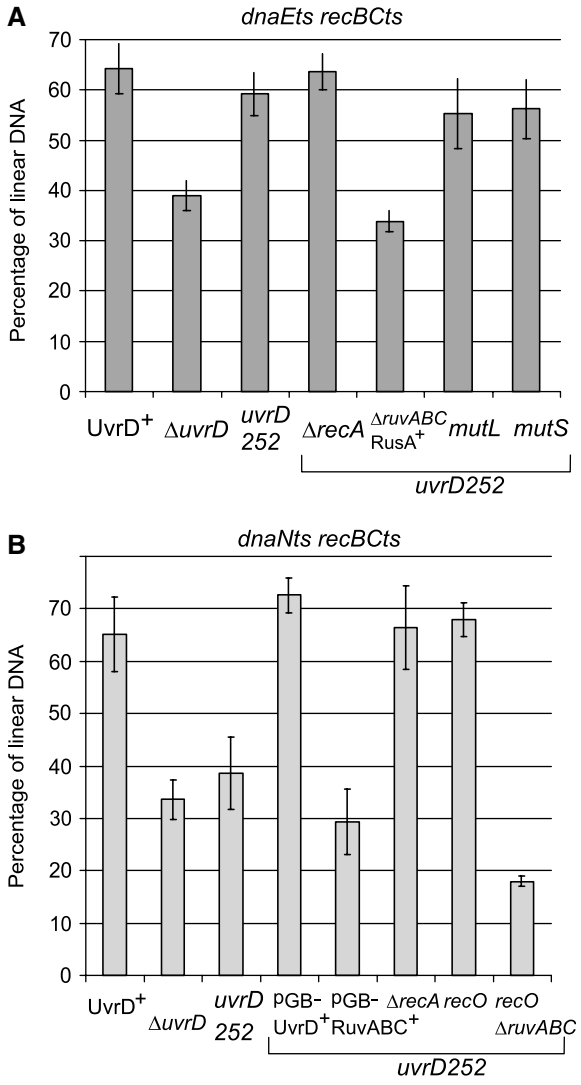

Figure 2 UvrD252 allows RFR in the presence of RecQJFORA at *dnaEts*-blocked forks, but not at *dnaNts*-blocked forks. The histograms indicate the percentage of linear DNA measured by PFGE in cultures propagated for three hours at restrictive temperature. Scale bars indicate the standard deviation. (**A**) *dnaEts* mutants at 42°C. From left to right: *dnaEts recBCts* (JJC1983), *dnaEts recBCts* Δ*uvrD* (JJC2007), *dnaEts recBCts uvrD252* (JJC2545 and JJC2566), *dnaEts uvrD252* Δ*recA* [pBR-Gam] (JJC2987 [pBR-Gam], *dnaEts recBCts uvrD252* Δ*ruvABC rusA* (JJC3911 and JJC3922, the *rusA* gene is inactive in wild-type *E. coli* and *rusA* designates here a mutation that activates the gene and consequently *rusA* mutant strains express the RusA resolvase), *dnaEts recBCts uvrD252 mutL* (JJC2567 and JJC3922), *dnaEts recBCts uvrD252 mutS* (JJC3141). (**B**) *dnaNts* mutants at 37°C. From left to right: *dnaNts recBCts* (JJC1982 and JJC2057), *dnaNts recBCts* Δ*uvrD* (JJC2006), *dnaNts recBCts uvrD252* (JJC2554 and JJC2656), JJC2554 or JJC2556 carrying pGB-UvrD$^+$, JJC2554 or JJC2556 carrying pGB-RuvABC$^+$, *dnaNts uvrD252* Δ*recA* [pBR-Gam] (JJC3038 [pBR-Gam]), *dnaNts recBCts uvrD252 recO* (JJC2577), *dnaNts recBCts uvrD252 recO* Δ*ruvABC* (JJC3809). Breakage in *dnaEts recBCts* (JJC1983), *dnaEts recBCts* Δ*uvrD* (JJC2007), *dnaNts recBCts* (JJC1982 and JJC2057), *dnaNts recBCts* Δ*uvrD* (JJC2006) were previously published (Grompone *et al*, 2002; Flores *et al*, 2004), and were reproduced here in parallel with the *uvrD252* strains.

RusA resolvase but lack RuvABC, we previously showed that, in a *dnaEts* mutant, RFR requires the HJ branch migration activity of the RuvAB complex (Baharoglu *et al*, 2006). The inactivation of DnaE did not cause fork breakage when RusA

was expressed in the *dnaEts uvrD252 recBCts* Δ*ruvABC* mutant, indicating that RFR requires the branch migration activity of RuvAB in *dnaEts uvrD252* mutant as in *dnaEts* UvrD$^+$ cells (Figure 2A; Supplementary Table S2). RFR is independent of RecA in the *dnaEts* mutant and, as expected, *recA* inactivation did not modify the high level of fork breakage in the *dnaEts recBCts uvrD252* mutant (Figure 2A; Supplementary Table S2). In conclusion, *dnaEts recBCts* cells behave similarly when they are UvrD$^+$ or combined with the *uvrD252* allele, namely they undergo a high level of fork breakage, which requires the branch migration activity of RuvAB. The high level of fork breakage in *uvrD252* cells that express all RecQJFORA proteins, indicates that, as UvrD wild-type protein, UvrD252 is able to allow RuvAB-dependent RFR in the presence of RecQJFORA. In other words, the *uvrD252* mutation does not inactivate the function of UvrD that allows it to counteract the deleterious action of RecQJFORA at *dnaEts*-blocked forks.

UvrD252 is active in mismatch repair presumably because it is activated by specific interactions with MutL; therefore we tested whether the activity of this mutant protein at *dnaEts*-blocked forks requires MutL. The inactivation of *mutL* or *mutS* did not modify the level of fork breakage in the *dnaEts recBCts uvrD252* mutant ($P = 0.43$; Figure 2A; Supplementary Table S2), indicating that MutL or MutS are not required for the action of UvrD252 at *dnaEts*-blocked forks.

The helicase function of UvrD is required for RFR in the dnaNts mutant

The *uvrD252* mutation was used to determine whether the ATPase function of UvrD is required for RFR at *dnaNts*-blocked forks. The level of fork breakage at 37°C was similar in *dnaNts recBCts uvrD252* to that of the *dnaNts recBCts* Δ*uvrD* ($P = 0.2$), however, significantly lower than in *dnaNts recBCts* UvrD$^+$ cells ($P < 0.001$; Figure 2B; Supplementary Table S3; Supplementary Figure S1). As expected, fork breakage was restored in *dnaNts recBCts uvrD252* mutant by the introduction of the wild-type *uvrD* gene in a plasmid. Moreover, the introduction of additional copies of RuvABC did not modify the level of breakage, indicating that the low level of fork breakage does not result from a limiting amount of the resolvase RuvABC in this strain (Figure 2B; Supplementary Table S3). A high level of fork breakage was restored by the inactivation of *recQ*, *recJ*, *recO* or *recA* (Figure 2B; Supplementary Table S3). We ensured that chromosome breakage in *dnaNts recBCts uvrD252 recO* cells results from RFR by testing that it required the resolvase activity of RuvABC (Figure 2B; Supplementary Table S3). These results indicate that, as previously observed in *dnaNts* Δ*uvrD* cells, RFR is prevented in the *dnaNts uvrD252* mutant by the presence of RecQJFORA. Therefore, the function of UvrD that allows RFR at *dnaNts*-blocked forks in the presence of RecQJFORA is inactivated by the *uvrD252* mutation, suggesting a requirement for the helicase or the translocase function of UvrD to counteract RecQJFORA in this replication mutant.

The RFR defect of the uvrD mutant is suppressed by Bacillus subtilis PcrA

UvrD is largely conserved in bacteria and, in order to test whether its capacity to counteract RecA at blocked forks is conserved, we tested whether the expression of the PcrA protein from *B. subtilis* can complement the RFR defect of

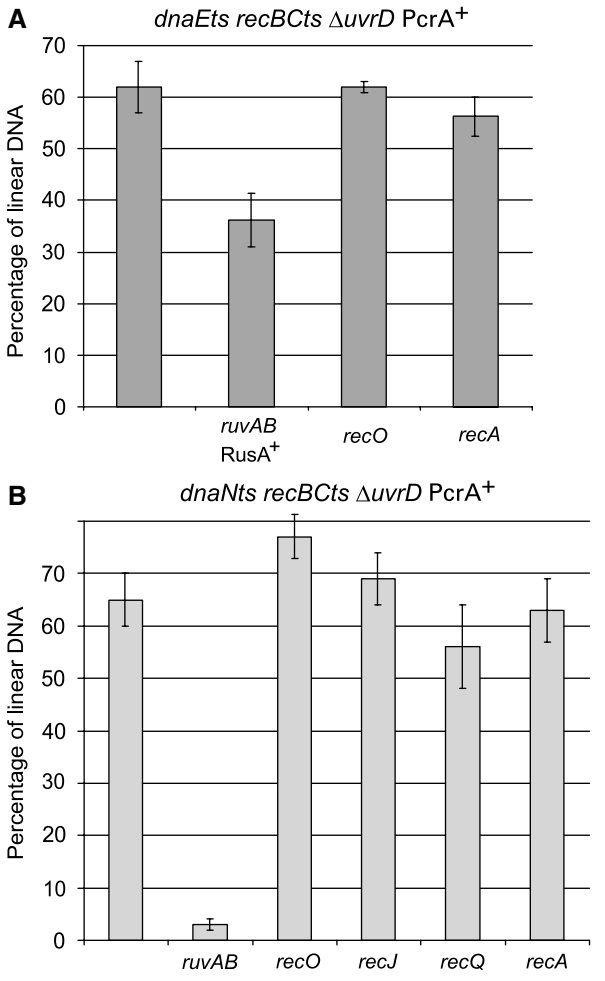

Figure 3 Expression of PcrA suppresses the RFR defect of *dnaEts* Δ*uvrD* and *dnaNts* Δ*uvrD* cells. (**A**) *dnaEts* mutants at 42°C. From left to right: *dnaEts recBCts* Δ*uvrD* PcrA$^+$ (JJC2692, fork breakage is not significantly different from *dnaEts recBCts* cells, $P = 0.9$), *dnaEts recBCts* Δ*uvrD* PcrA$^+$ *ruvAB* RusA$^+$ (JJC3156), *dnaEts recBCts* Δ*uvrD* PcrA$^+$ *recO* (JJC2719), *dnaEts* Δ*uvrD* PcrA$^+$ *recA* [pBR-Gam] (JJC3351 [pBR-Gam]). (**B**) *dnaNts* mutants at 37°C. From left to right: *dnaNts recBCts* Δ*uvrD* PcrA$^+$ (JJC3326 and JJC3502, fork breakage is not significantly different from *dnaNts recBCts* cells, $P = 0.8$), *dnaNts recBCts* Δ*uvrD* PcrA$^+$ *ruvAB* (JJC3724), *dnaNts recBCts* Δ*uvrD* PcrA$^+$ *recO* (JJC2720), *dnaNts recBCts* Δ*uvrD* PcrA$^+$ *recJ* (JJC3509), *dnaNts recBCts* Δ*uvrD* PcrA$^+$ *recQ* (JJC3339), *dnaNts recBCts* Δ*uvrD* PcrA$^+$ Δ*recA* (JJC3708).

dnaEts Δ*uvrD* and *dnaNts* Δ*uvrD* mutants. A *dnaEts recBCts* Δ*uvrD* PcrA$^+$ mutant was constructed. In this strain, the *pcrA* gene is inserted as a single copy in the chromosome and, although it is fused to the strong Pspac promoter, it is partially repressed by the adjacent LacI repressor. Experiments were performed in the absence of the IPTG inducer, so that PcrA is unlikely to be over-expressed, but complete induction of pSpac by IPTG did not affect results (Supplementary Tables S2 and S3, and data not shown).

Analysis of chromosomes by PFGE showed that the expression of PcrA restored fork breakage, hence fully suppressed the Δ*uvrD* defect (Figure 3; Supplementary Table S2). Fork breakage in *dnaEts recBCts* Δ*uvrD* PcrA$^+$ mutant was unaffected by the inactivation of *recO* or *recA* and required the HJ branch migration activity of RuvAB (no *dnaEts*-dependent fork breakage was observed in *ruvABC rusA1*

Table I RecFOR-dependent hyper-recombination in *uvrD* mutants make them incompatible with *ruvAB* inactivation

Receptor strain		Δ*ruvABC*::Cm^R clones among Tet^R *eda*::Tn*10* P1 transductants	
Strain	Genotype	Number of Cm^R/Tet^R transductants	Ratio
JJC40	*wild-type*	47/83	0.57
JJC1628	*recQ*::Tn3	47/69	0.68
JJC4174	Δ*recQ*::Cm	35/59	0.59
JJC2642	Δ*uvrD*	0/75	<0.01
JJC2821	Δ*uvrD recO*	32/51	0.63
JJC3850/JJC2603	Δ*uvrD recQ*::Tn3	0/131	<0.01
JJC4152	Δ*uvrD* Δ*recQ*::Cm	0/40	<0.02
JJC2530	*uvrD252*	0/133	<0.01
JJC2983	*uvrD252 recO*	48/63	0.76
JJC3129	*uvrD252 recQ*::Tn3	0/93	<0.02
JJC3686	*uvrD252* Δ*recQ*::Cm	0/40	<0.02
JJC2673	Δ*uvrD* PcrA$^+$	31/52	0.60

eda and *ruv* genes are about 12 kb apart. Δ*ruvABC* and Ruv$^+$ transductants colonies were of similar size. Δ*ruvABC*::Cm^R clones were identified by picking *eda*::Tn*10* Tet^R transductants clones on chloramphenicol-containing medium, and checked by PCR using oligonucleotides that flank the *ruvABC* region and by UV irradiation. For JJC2673, JJC4174, JJC4152 and JJC3686, which are Cm^R, Δ*ruvABC*::Cm^R clones, were identified by PCR. With the four *uvrD recQ* double mutants, some micro-colonies appeared besides the Ruv$^+$ Tet^R transductants. These micro-colonies might be abortive Δ*ruvABC* co-transductants, but this could not be checked because these micro-colonies could not be streaked or cultured.

mutants, in which the resolution of HJ is performed by the RusA resolvase; Figure 3; Supplementary Table S2). These results mimic those observed in *dnaEts recBCts* UvrD$^+$ cells (Flores *et al*, 2005; Baharoglu *et al*, 2006), indicating that at *dnaEts*-blocked forks PcrA is able to catalyze an anti-RecQJFORA action, thereby allowing RuvAB-dependent RFR.

The expression of PcrA also fully suppressed the Δ*uvrD* defect in *dnaNts recBCts* Δ*uvrD* PcrA$^+$ cells by restoring fork breakage (Figure 3; Supplementary Table S3). As expected, the inactivation of the resolvase RuvABC drastically reduced fork breakage, indicating that in a Δ*uvrD* PcrA$^+$, as in a UvrD$^+$ context, chromosome breakage upon *dnaN* inactivation is catalyzed by HJ resolution, hence results from RFR. RFR was independent of RecQ, RecJ, RecO and RecA in *dnaNts uvrD* PcrA$^+$, as in *dnaNts* UvrD$^+$ cells, suggesting that complementation of the *uvrD* defect by PcrA may not modify the mode of RFR (Figure 3; Supplementary Table S3). In addition to restoring RFR in both *dnaEts* Δ*uvrD* and *dnaNts* Δ*uvrD* mutants, PcrA also restored the viability of Pol III*ts* Δ*uvrD* cells at 37°C to the level of the Pol III*ts* single mutants (data not shown).

UvrD252 does not dismantle recombination intermediates

UvrD is capable *in vitro* of two anti-RecA actions: unwinding of recombination intermediates to restore substrate molecules and dismantling of RecA filaments (Morel *et al*, 1993; Veaute *et al*, 2005). *In vivo*, the hyper-recombination phenotype of the Δ*uvrD* mutant presumably results from the loss of these activities (Zieg *et al*, 1978). We previously reported that Δ*uvrD ruvAB* cells are lethal, and this lethality is suppressed by the inactivation of *recFOR* or *recA* genes (Flores *et al*, 2005). Since RecFOR promotes RecA binding to gaps (Kuzminov, 1999; Morimatsu and Kowalczykowski, 2003), we proposed that the lethality of Δ*uvrD ruvAB* cells results from an increased formation of ssDNA gaps in the Δ*uvrD* mutant, which are repaired by RecFOR-dependent homologous recombination, increasing the need for HJ resolution by RuvABC. In the absence of RecFOR, HJ resolution is not needed because gaps are presumably repaired by gap filling. The Δ*uvrD ruvAB recQ* triple mutant could not be constructed, either by P1 transduction of *ruvABC* mutation in Δ*uvrD recQ* (Table I) or by P1 transduction of Δ*uvrD* in a *recQ ruvAB* mutant (JJC2776 Supplementary Table SI, data not shown). The lethality of the Δ*uvrD ruvAB recQ* triple mutant indicates that gap recombinational repair in Δ*uvrD* mutant is RecQ-independent. It should be noted that *uvrD ruv* (Bierne *et al*, 1997) and *uvrD ruv recQ* (Magner *et al*, 2007) mutants were observed to be viable when constructed in *E. coli* backgrounds other than the AB1157 context used in the present work.

Like Δ*uvrD*, the *uvrD252* mutation was co-lethal with *ruvAB* inactivation in our background. Viability was restored when gap recombinational repair was prevented by a *recO* mutation (Table I, and JJC3809 in Supplementary Table S3), when the RusA resolvase is activated (see JJC3911 in Supplementary Table S2), but not by *recQ* inactivation (Table I). The requirement for RuvABC in the *uvrD252* mutant suggests an increased level of gap recombinational repair. Furthermore, conjugational recombination was largely increased in the *uvrD252* mutant (Supplementary Table S4). Altogether, the observation that recombination is increased in *uvrD252* as in Δ*uvrD* mutant indicates that the ATPase function of UvrD is required for the dismantling of recombination intermediates. In contrast, the expression of PcrA suppressed the lethality of Δ*uvrD ruvAB* double mutant (Table I). Therefore PcrA suppresses the spontaneous hyper-recombination phenotype of Δ*uvrD* cells.

UvrD252 does not remove RecA from SOS-inducing ssDNA gaps

We tested the capacity of UvrD252 and PcrA to undo ssDNA-RecA filaments *in vivo* by measuring SOS induction using an *sfiA*::*lacZ* fusion (Table II). SOS induction and an increased formation of RecA-GFP foci were previously reported in *uvrD* mutants (Ossanna and Mount, 1989; SaiSree *et al*, 2000; Centore and Sandler, 2007). The SOS response was slightly less induced in the *uvrD252* mutant than in Δ*uvrD* cells, suggesting either the formation of less ssDNA substrates or a weak ability of UvrD252 to dismantle RecA filaments in a wild-type context (Table II). In both Δ*uvrD* and *uvrD252* mutants, SOS induction was RecQ-independent and partly

Table II SOS induction due to Pol III*ts* and *uvrD* mutations

Strain	Relevant genotype	30°C		37°C	
		Miller units	Ratio	Miller units	Ratio
JJC2255	Wild-type	44±1	1	37.6±9.6	1
JJC2988	Δ*uvrD*	284±3	6.5	297±52	7.8
JJC2989	*uvrD252*	216±12	4.9	183±35	4.9
JJC3066	*recO*	ND	ND	81±12	2.2
JJC3101	Δ*uvrD recF*	ND	ND	132±22	3.5
JJC3102	*uvrD252 recF*	ND	ND	114±7	3
JJC3114	*recQ*	ND	ND	52±20	1.4
JJC3108	Δ*uvrD recQ*	ND	ND	258±9	6.8
JJC3912	*uvrD252 recQ*	ND	ND	141±37	3.7
JJC2985	Δ*uvrD* PcrA$^+$	ND	ND	39±6	0.9
JJC3033	*rarA*	ND	ND	83±6	2.2
JJC2991	*dnaEts*	70±12	1	427±113	1
JJC2993	*dnaEts* Δ*uvrD*	1500±630	21	1518±551	3.5
JJC2995	*dnaEts uvrD252*	1653±370	24	1705±507	4
	dnaEts uvrD252 [pGB2]	1405±344	20	1922±6.4	4.5
	dnaEts uvrD252 [pGB-UvrD+]	76±17	1.1	200±23	0.5
JJC3069	*dnaEts recO*	98±21	1.4	127±19	0.3
JJC3075	*dnaEts* Δ*uvrD recO*	99±27	1.4	121±22	0.28
JJC3081	*dnaEts uvrD252 recO*	177±32	2.5	129±77	0.3
JJC3079	*dnaEts recQ*	94±27	1.3	473±70	1
JJC3068	*dnaEts* Δ*uvrD recQ*	646±134	9.2	1270±241	3
JJC3919	*dnaEts uvrD252 recQ*	750±132	10.7	1353±175	3.2
	dnaEts uvrD252 recQ [pGB2]	460±4	6.6	1448±47	3.4
	dnaEts uvrD252 recQ [pGB-UvrD+]	44±1	0.6	169±8	0.4
JJC2997	*dnaEts* Δ*uvrD* PcrA$^+$	74±32	1	548±139	1.3
JJC3034	*dnaEts rarA*	97±18	1.4	331±16	0.7
JJC2992	*dnaNts*	79±11	1	522±146	1
JJC2994	*dnaNts* Δ*uvrD*	1642±287	21	2361±537	4.5
JJC2996	*dnaNts uvrD252*	994±124	12.6	2113±550	4.0
	dnaNts uvrD252 [pGB2]	1101±230	14	1742±338	3.3
	dnaNts uvrD252 [pGB-UvrD+]	99±13	1.2	305±52	0.6
JJC3077	*dnaNts recO*	150±7.6	2	209±21	0.4
JJC3076	*dnaNts* Δ*uvrD recO*	149±8	1.9	207±23	0.4
JJC3078	*dnaNts uvrD252 recO*	238±21	3	349±23	0.7
JJC3080	*dnaNts recQ*	92±12	1.2	610±68	1.1
JJC3084	*dnaNts* Δ*uvrD recQ*	828±170	10.5	1602±591	3.1
JJC3083	*dnaNts uvrD252 recQ*	1214±77	15	2451±339	4.7
	dnaNts uvrD252 recQ [pGB2]	1310±49	16	2442±380	4.7
	dnaNts uvrD252 recQ [pGB-UvrD+]	104±8	1.3	381±11	0.7
JJC2998	*dnaNts* Δ*uvrD* PcrA$^+$	95±16	1.2	813±45	1.5
JJC3035	*dnaNts rarA*	96±15	1.2	567±39	1.1

RecF-dependent, indicating that it results, in part, from RecQ-independent RecA binding to ssDNA gaps. The remaining SOS induction is presumably RecBC-dependent, but *uvrD recB* mutants are too poorly viable to be tested. SOS induction was decreased to the wild-type level by the expression of PcrA in Δ*uvrD* PcrA$^+$ cells, indicating that the *B. subtilis* protein PcrA either dismantles ssDNA-RecA filaments in *E. coli* Δ*uvrD*, or complements the *uvrD* defects that originally cause their formation (Table II).

Since the *uvrD252* mutation allows RFR in *dnaEts* but not in *dnaNts* cells (Figure 2), we tested whether SOS induction is differentially affected by the *uvrD252* allele in these two replication mutants, by comparing SOS induction in *dnaEts* (or *dnaNts*) Δ*uvrD* and *dnaEts* (or *dnaNts*) *uvrD252* mutants. Because they behaved similarly, the *dnaEts* and *dnaNts* mutants are referred to as Pol III*ts* below.

As previously reported, Pol III*ts* single mutants are induced for SOS at 37°C (Table II; Flores *et al*, 2005). This SOS induction is shown here to depend entirely on RecO and not on RecQ, and therefore to result from RecQ-independent RecA filaments formed at ssDNA gaps. Since our previous results indicate that RecQ is required for prevention of RFR by RecFOR and RecA (Flores *et al*, 2005), we infer that SOS-inducing gaps are not at blocked forks but elsewhere on the chromosome. Altogether these results points to the formation of two types of RecFOR-dependent RecA filaments in Pol III*ts* cells: RecQ-dependent at forks, and RecQ-independent elsewhere on the chromosome.

SOS induction by Pol III*ts* and either Δ*uvrD* or *uvrD252* mutations was highly synergistic (Table II). SOS was induced even at the permissive temperature of 30°C in all Pol III*ts* *uvrD* double mutants, indicating that (as often observed with *ts* mutants) replication is not fully functional even in conditions permissive for growth. SOS induction was entirely RecO-dependent, showing that it results from RecA binding to ssDNA gaps. However, it was decreased at most two-fold, and only at 30°C, by *recQ* inactivation. Since RecQ act together with RecFOR at forks as deduced from fork breakage

measurements (Flores *et al*, 2005; Supplementary Table S3), the very weak effect of *recQ* inactivation compared to *recO* on SOS induction indicates that most of the SOS-inducing RecA filaments are not at forks, but are rather on gaps left behind progressing replication forks on daughter chromosomes. Importantly, the high level of SOS induction in the two Pol III*ts uvrD252* mutants clearly indicates that the UvrD252 mutant protein cannot remove RecA from ssDNA in Pol III*ts* contexts. Since UvrD252 is nevertheless able to prevent RecA deleterious action at *dnaEts*-blocked forks, this finding suggests that the UvrD252 (and possibly UvrD) target is not ssDNA-bound RecA filaments.

PcrA decreased the level of SOS induction in *dnaEts* Δ*uvrD* and *dnaNts* Δ*uvrD* nearly to the level of *dnaEts* and *dnaNts* single mutants. This result indicates that in both *dnaEts* Δ*uvrD* and in *dnaNts* Δ*uvrD* mutants, PcrA is able to undo or prevent the formation of the RecA filaments caused by the Δ*uvrD* mutation, but not those resulting from the Pol III defect (Table II). This result is compatible with the idea that PcrA allows RFR in a Δ*uvrD* context by removing RecA from DNA.

MgsA/RarA is needed for RecQJFORA action at dnaEts-blocked fork

dnaEts cells grow slowly at 37°C and are killed at 38°C. These growth defects at these semi-permissive temperatures result from the action of RecQJFORA proteins, as they are suppressed by the inactivation of any of *recQJFORA* genes (Hishida *et al*, 2004; Flores *et al*, 2005). The growth defect caused by the presence of RecQJFORA is strongly amplified in the absence of UvrD, as Pol III*ts* Δ*uvrD* mutants are lethal at 37°C and this lethality is suppressed by the inactivation of any of the *recQJFORA* genes (Flores *et al*, 2005). Like the inactivation of *recQ*, *recJ*, *recFOR* or *recA*, the inactivation of *rarA* (*mgsA*) allows growth of *dnaEts* single mutant at 38°C and improves its viability at 37°C (Hishida *et al*, 2006; Figure 4). Nevertheless, as with *recQ* inactivation, the *rarA* mutation did not suppress the SOS induction due to the *dnaEts* or *dnaNts* mutations (Table II). Furthermore, fork breakage was unaffected by *rarA* inactivation in *dnaEts recBCts* or in *dnaNts recBCts* cells and remained dependent on the branch migration activity of RuvAB in *dnaEts recBCts* (Figure 5; Supplementary Table S5), indicating that *rarA* does not affect RFR in these mutants, provided UvrD is active.

To gain more insight in the action of RarA (MgsA), we tested the effects of the *rarA* mutation in Pol III*ts uvrD* double mutants. The inactivation of *mgsA/rarA* improved the viability of *dnaEts uvrD* cells at 37°C to the same level as the inactivation of *recQ* (Figure 4A), but less than the inactivation of *recFOR*, which restored complete growth (Flores *et al*, 2005). Interestingly, *rarA* inactivation restored fork breakage in the *dnaEts recBCts uvrD* mutant (Figure 5; Supplementary Table S5). This result indicates that RarA (together with RecQJFORA) prevents RFR at *dnaEts*-blocked forks in the absence of UvrD, suggesting that RarA is required for RecQJFOR action at *dnaEts*-blocked forks.

The action of RarA in the *dnaNts uvrD* mutant was studied at 37°C by measuring viability of *dnaNts rarA uvrD*, and measuring fork breakage in *dnaNts rarA recBCts uvrD* cells. Interestingly, in contrast with results in the *dnaEts* context, *rarA* inactivation did not improve growth of *dnaNts* Δ*uvrD* cells (Figure 4B), and did not restore fork breakage in a *dnaNts recBCts uvrD* mutant (Figure 5). Therefore, the action of *rarA* is specific for *dnaEts*-blocked forks.

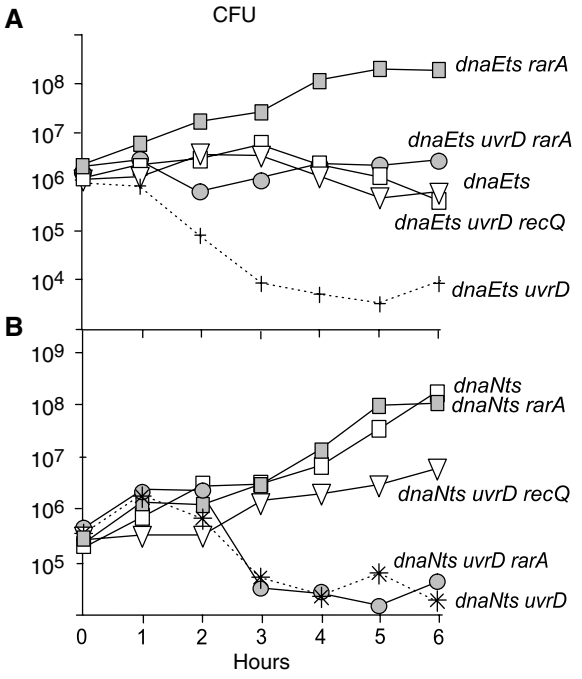

Figure 4 *rarA* inactivation specifically improves the viability of *dnaEts* and *dnaEts uvrD* cells. Cells propagated at 30°C for 2 h were shifted to 37°C (time 0), and appropriate dilutions were plated at the indicated times; plates were counted after 48 h incubation at 30°C. (**A**) *dnaEts rarA* (JJC2910, gray squares), *dnaEts* (JJC1954, white squares), *dnaEts uvrD rarA* (JJC2918, gray circles), *dnaEts uvrD recQ* (JJC2748, triangles), *dnaEts uvrD* (JJC2697, crosses and dashed line). (**B**) *dnaNts* (JJC2434, white squares); *dnaNts rarA* (JJC2911, gray squares), *dnaNts uvrD recQ* (JJC2759, triangles), *dnaNts uvrD rarA* (JJC2919, gray circles), *dnaNts uvrD* (JJC2698, stars and dashed line). Growth curves of *dnaEts, dnaEts uvrD, dnaEts uvrD recQ, dnaNts, dnaNts uvrD, dnaNts uvrD recQ* were previously published (Flores *et al*, 2005), and were reproduced here in parallel with the *rarA* strains.

Discussion

Knowledge of the reactions that occur upon replication impairment is crucial for a complete understanding of the links between replication and genome stability. The natural causes of spontaneous replication arrests are unknown and are likely to be multiple, as DNA and replication proteins are susceptible to be affected. In the present work, we analyzed the action of UvrD and RarA (MgsA) at replication forks inactivated by a Pol III*ts* mutation. When Pol III*ts* mutants are propagated at the semi-permissive temperature of 37°C they undergo stochastic replication fork arrest (Grompone *et al*, 2002; Flores *et al*, 2004, 2005). In addition, this defective replication also forms of ssDNA gaps that can be distinguished from blocked forks because RecA binds them in a RecQ-independent manner (Table II). Presumably, depending on whether the lagging or the leading strand polymerase is inactivated first, a gap on the lagging strand template or a blocked fork will form, and both are present in a cell population. *uvrD* mutations also cause the formation of ssDNA gaps, which are revealed by SOS induction and recombination increase. Regardless of whether a *uvrD*-null

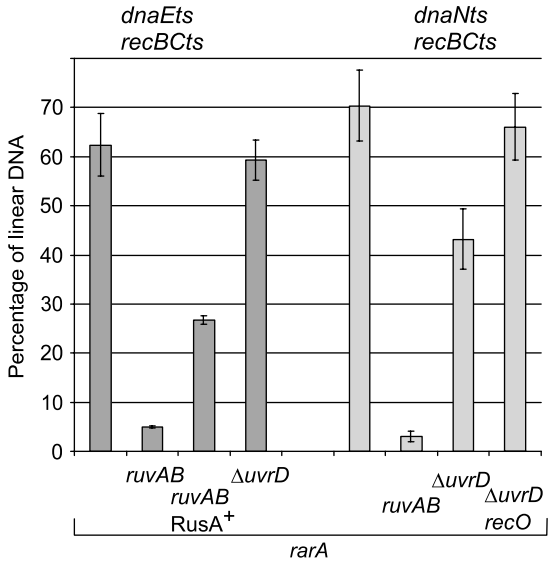

Figure 5 *rarA* inactivation suppresses the RFR defect in *dnaEts* Δ*uvrD* but not *dnaNts* Δ*uvrD* cells. Dark bars, *dnaEts recBCts rarA*, gray bars, *dnaNts recBCts rarA*. From left to right: *dnaEts recBCts rarA* (JJC1744 and JJC2912), *dnaEts recBCts rarA ruvAB* (JJC1752), *dnaEts recBCts rarA ruvAB* RusA$^+$ (JJC3055), *dnaEts recBCts rarA uvrD* (JJC2932), *dnaNts recBCts rarA* (JJC1743, JJC2913 and JJC3729), *dnaNts recBCts rarA ruvAB* (JJC3781), *dnaNts recBCts rarA uvrD* (JJC2920), *dnaNts recBCts rarA uvrD recO* (JJC3057). *dnaEts recBCts rarA* and *dnaEts recBCts rarA* Δ*uvrD* are not significantly different ($P = 0.6$), whereas *dnaNts recBCts rarA* and *dnaNts recBCts rarA* Δ*uvrD* are ($P < 0.001$).

or a *uvrD252* mutation is used, the SOS response is highly induced in Pol III*ts uvrD* double mutants. Therefore, in agreement with its strong ATPase defect *in vitro*, the UvrD252 protein cannot remove RecA from ssDNA *in vivo*. The use of this mutant allows us to demonstrate the existence of two different modes of action of the helicase UvrD that antagonize two different pathways for RecQJFORA access to blocked forks. When replication forks are arrested by the inactivation of the catalytic subunit of Pol IIIh DnaE, RarA participates in the blockage of RFR in the absence of UvrD (together with RecQJFORA; Flores *et al*, 2005), and the ATPase-deficient UvrD mutant is able to overcome this blockage. In cells deficient for the β-clamp DnaN, RecQJFORA does not require RarA for blocking RFR, and the ATPase activity of UvrD is required to counteract their deleterious action.

RarA (MgsA) is required for RecQ action at certain blocked forks

rarA is coexpressed in operon with *ftsK*, which encodes a multifunctional septum protein required for chromosome segregation and chromosome dimer resolution. It shares homology with the *dnaX*-encoded subunits of Pol IIIh and with RuvB (Barre *et al*, 2001). RarA is a highly conserved protein; the *S. cerevisiae* and *E. coli* proteins share 40% identity (Barre *et al*, 2001). This high level of conservation suggests an important role for the protein. Using GFP-RarA and GFP-RecQ fusion proteins, it was observed that RarA and RecQ are both located in the region of the replication factory in growing cells (Sherratt *et al*, 2004). Nevertheless, the function of RarA in *E. coli* and of its counterpart Mgs1 in yeast has remained elusive. Both the *rarA* mutation in *E. coli* and the Mgs1 mutation in *S. cerevisiae* improve the growth of mutants affected for the replicative polymerase (Hishida *et al*, 2002; Vijeh Motlagh *et al*, 2006). Mgs1 protein interacts with the Pol 31, one of the subunits of the yeast replicative polymerase and with the PCNA sliding clamp (the DnaN eukaryote homologue) (Hishida *et al*, 2006; Vijeh Motlagh *et al*, 2006). Mgs1 from human cells (termed WHIP) interacts with the RecQ human homologue, the Werner helicase (Kawabe *et al*, 2001). The similarity of phenotypes conferred by *rarA* or *recQJFORA* inactivation in the *dnaEts* mutant suggests that RarA is required for RecQJFOR-dependent RecA binding to blocked forks. RarA might interact with RecQ and load it onto replication fork. However, this hypothesis is difficult to reconcile with the observation that RarA is not required for RecQ loading at *dnaNts*-blocked forks and for RecQ-dependent homologous recombination in *recBC sbcBC* cells (Nakayama *et al*, 1985, our unpublished results). We instead favor a model in which RarA binds to *dnaEts*-blocked forks and, in the absence of UvrD, renders forks accessible to RecQ. Clearly, the structure of DNA and/or the proteins present are different after replication arrest in *dnaEts* and *dnaNts* mutants. We can speculate that RarA is required for RecQ binding only in the *dnaEts uvrD* mutant, because the disassembly of Pol IIIh is partial in this mutant, preventing RecQ access while still allowing RuvAB to revert forks. In this scenario, DnaN could be among the Pol IIIh subunits that remain bound to DNA in a *dnaEts* mutant, and its absence from *dnaNts*-blocked forks would render RarA dispensable for RecQ binding.

The helicase activity of UvrD is not required when RarA and RecQ act in concert

The existence of two pathways, RarA-dependent and RarA-independent, for RecQJFORA binding correlates with two different modes of action of UvrD. The existence of these two modes of action are revealed by the use of the *uvrD252* allele, which affects RFR differently in the two Pol III*ts* mutants tested. 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 mismatch repair. We observe here that the *uvrD252* mutant is completely defective for three anti-RecA actions of UvrD, which are its capacity to limit homologous recombination and SOS induction and its capacity to antagonize RecA-dependent blockage of RFR in the *dnaNts* mutant. Nevertheless, UvrD252 fully allows RFR in the presence of all RecQJFORA proteins in a *dnaEts* mutant. It is possible that the UvrD252 ATPase function is specifically activated by an unknown protein other than MutLS, after RecA binding to inactivated forks in a *dnaEts* mutant. Although we cannot formally exclude this possibility, it implies the presence of different factors after RecQJFOR-dependent RecA binding to inactivated replication forks. Instead, we favor an alternative model (Figure 6), which suggests that UvrD does not need its helicase activity to allow RFR at *dnaEts*-blocked fork, because it acts before RecA binding. The correlation between UvrD252 and RarA action leads us to speculate that UvrD (or UvrD252) could prevent RecA binding by antagonizing RarA. In contrast, in the *dnaNts* mutant, the structure of the forks and the associated proteins are such that RecQ gains access to forks and promotes RecA binding regardless of the presence of UvrD and RarA. Nevertheless, UvrD can then

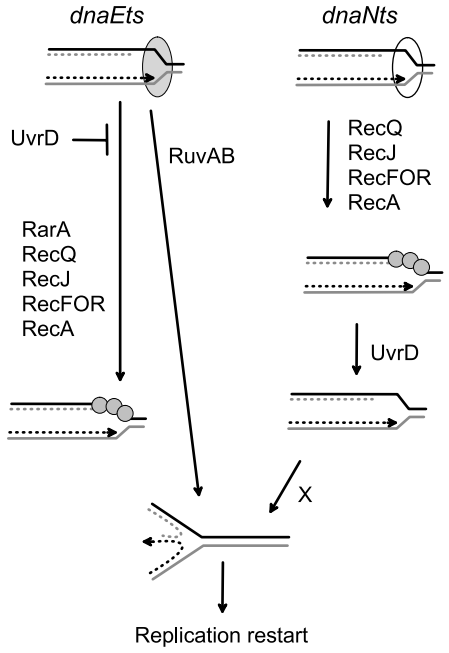

Figure 6 Model of action of UvrD in *dnaEts* and *dnaNts*. In the *dnaEts* mutant (left part of the figure) inactivated replication forks can be channeled to two different pathways depending on the presence (but not on the activity) of UvrD. UvrD wild-type or ATPase-deficient proteins prevent RarA and the presynaptic proteins RecQJFOR from promoting RecA binding, allowing RuvAB-dependent RFR. In the absence of UvrD, RarA and RecQJFOR promote RecA binding, preventing RFR. In the *dnaNts* mutant, formation of a RecA filament at blocked forks does not require RarA, and is not prevented by UvrD252. UvrD act only after RecA binding, presumably by removing RecA from DNA. The enzymes that catalyze RFR in this mutant are unknown. We propose that the different processing of forks in the two mutants results from differences in the disassembly of the replisome, symbolized by ovals of different colors. Small circles represent RecA.

dislodge RecA from blocked forks DNA, provided its ATPase is functional (Figure 6).

The anti-RecA action of UvrD is conserved in B. subtilis

UvrD shares 42% sequence identity with PcrA and 37% with Rep. The three proteins also share very similar structures, even if they may use different modes of action (Korolev *et al*, 1997; Velankar *et al*, 1999; Lee and Yang, 2006). Rep is expressed in all mutants used here and is clearly unable to perform UvrD functions. This does not result from a lower level of proteins, since overexpression of Rep from a high-copy number plasmid did not restore the viability of Pol III*ts* Δ*uvrD* mutants at 37°C (our unpublished data). It was previously reported that PcrA suppresses the lethality of *rep uvrD* double mutants, and the NER defect but not the mismatch repair defect of *uvrD* single mutants (Petit and Ehrlich, 2002). Furthermore, PcrA suppresses the lethality caused by *uvrD* inactivation in cells that carry additional Ter sites. Hence, PcrA shares with UvrD the capacity to remove the Tus protein from DNA *in vivo* (Bidnenko *et al*, 2006). Despite the homology between Rep, UvrD and PcrA, the Δ*uvrD* mutant defects are suppressed by the expression of PcrA and not Rep. This emphasizes the separation of function between Rep and UvrD and the conservation of UvrD functions in PcrA. We show here that PcrA also suppresses all defects of Δ*uvrD* mutants that are linked to RecA action: SOS induction, RecFOR-dependent hyper-recombination, lethality and lack of RFR in Pol III*ts* contexts. These results are compatible with the idea that PcrA can remove *E. coli* RecA from DNA at gaps and at replication forks whereas Rep does not, in agreement with the observation that Rep is unable to undo *E. coli* RecA filaments *in vitro* (Veaute *et al*, 2005). If RecA removal requires an interaction between RecA and UvrD, this interaction is conserved in PcrA. RecA is highly conserved between *E. coli* and *B. subtilis*, and the conservation of the anti-RecA action of UvrD in a distant bacterial species underlines the importance of this function.

In conclusion, our results show that UvrD has multiple functions at inactivated replication forks, which are all linked to the action of recombination proteins but by different means. We previously reported that to remove DNA-bound Tus protein, UvrD acts in concert with RecBCD-dependent homologous recombination (Bidnenko *et al*, 2006). We show here that to rescue *dnaNts*-blocked forks, UvrD needs its ATPase activity and may remove RecA from DNA. In contrast, to allow replication restart from *dnaEts*-blocked forks, UvrD does not require either DNA translocation or unwinding activities. Therefore, UvrD is likely to act by preventing the access of RarA and RecQ either directly by steric hindrance, or indirectly by controlling the presence of other proteins at forks. This is the first indication that a protein of the UvrD family plays a role at replication forks that does not require any ATPase-dependent activity.

Materials and methods

Strains and plasmids

The strain background is JJC40, which is an *hsdR* Thr^+ Pro^+ derivative of AB1157 (*leu-6 thi-1, his-4, argE3, lacY1, galK2, ara-14, xyl-5, mtl-1, tsx-33, rpsL31, supE44*). Most of the strains were constructed by P1 transduction. Details of strain constructions and strains genotypes are described in in Supplementary Table S1. All thermosensitive mutants were constructed and propagated at 30°C, except for *dnaEts* Δ*uvrD* and *dnaEts uvrD252* mutants and their derivatives, which were constructed and propagated at 25°C. *recBCts* stands for *recB270 recC271* mutations (Supplementary Table S1). Because the viability of *dnaEts* (or *dnaNts*) *recA recBCts uvrD252* mutants was very low, to measure chromosome breakage in a *recA* mutant context, RecBC was inactivated in the *dnaEts* (or *dnaNts*) *recA uvrD252* mutants using a plasmid expressing the λ Gam protein under the control of the λ P_L promoter, induced at 42 or 37°C. In previous works, where *recBC* could be inactivated either by a *recBCts* mutation or by Gam expression in a *recA* mutant context, we observed no difference between these two ways of inactivating *recBC*, as expected from the properties of the λ Gam protein (Grompone *et al*, 2002; Flores *et al*, 2005; Baharoglu *et al*, 2006). Null mutants were checked by PCR with external oligonucleotides that amplify a DNA fragment of different length for the wild-type and the interrupted alleles. Recombination mutations were checked as conferring UV sensitivity. In addition, *recBC* and *recD* mutants were checked for the inactivation of exonuclease V (they are permissive for the growth of T4 gpII mutants). UvrD mutants were checked for their UV sensitive and mutator phenotypes (increase in the proportion of Rif^R clones in overnight cultures). In $PcrA^+$ strains, the *B. subiltis pcrA* gene is integrated in the *lacZ* gene of the *E. coli* chromosome under the control of a P*spac* promoter. P*spac* repression by LacI is weakly efficient and PcrA is expressed in our $LacI^+$ background regardless of the presence of IPTG, as measured by the UV resistance conferred by the *lacZ*::PcrA insertion in Δ*uvrD* mutants.

Growth curves and measures of SOS induction

Overnight cultures grown at 30°C were diluted to OD 0.001 or 0.002 in Luria Broth medium (LB), grown at 30°C for two hours and then

shifted to 37°C. Aliquots were taken and dilutions were plated on LB every hour. Plates were incubated for 48 h at 30°C. β-Galactosidase assays for the measure of SOS induction were performed as described (Miller, 1992).

Measure of linear DNA by PFGE

Quantification of pulsed field gels was performed as previously described (Seigneur *et al*, 1998). Values are considered as highly significantly different when $P < 0.001$.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (http://www.embojournal.org).

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