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**RNA polymerase mutations that facilitate replication progression in the rep uvrD recF mutant lacking two accessory replicative helicases**

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RNA polymerase mutations that facilitate replication progression in the *rep uvrD recF* mutant  
lacking two accessory replicative helicases

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Running title: Replication-transcription collisions

## 1 ABSTRACT

2 We observed that cells lacking Rep and UvrD, two replication accessory helicases, and the  
3 recombination protein RecF are cryo-sensitive on rich medium. We isolated five mutations  
4 that suppress this LB-cryo-sensitivity and show that they map in the genes encoding the RNA  
5 polymerase subunits RpoB and RpoC. These *rpoB* (D444G, H447R and N518D) and *rpoC*  
6 mutants (H113R and P451L) were characterized. *rpoB*<sup>H447R</sup> and *rpoB*<sup>D444G</sup> prevent activation  
7 of the *Prrn* core promoter in rich medium, but only *rpoB*<sup>H447R</sup> also suppresses the auxotrophy  
8 of a *relA spoT* mutant (stringent-like phenotype). *rpoC*<sup>H113R</sup> suppresses the thermo-sensitivity  
9 of a *greA greB* mutant, suggesting that it destabilizes stalled elongation complexes. All  
10 mutations but *rpoC*<sup>P451L</sup> prevent R-loop formation. We propose that these *rpo* mutations allow  
11 replication in the absence of Rep and UvrD by destabilizing RNA Pol upon replication-  
12 transcription collisions. In a RecF<sup>+</sup> context, they improve growth of *rep uvrD* cells only if  
13 DinG is present, supporting the hypothesis that Rep, UvrD and DinG facilitate progression of  
14 the replication fork across transcribed sequences. They rescue *rep uvrD dinG recF* cells,  
15 indicating that in a *recF* mutant replication forks arrested by unstable transcription complexes  
16 can restart without any of the three known replication accessory helicases Rep, UvrD and  
17 DinG.

18

19

20 Key words : replication restart, RNA polymerase, helicase, stringent response.

## 1 INTRODUCTION

2           Replication forks are susceptible to be arrested by a variety of obstacles, including  
3 DNA-bound proteins such as RNA polymerases (RNA Pol) (Mirkin and Mirkin, 2007;  
4 Rudolph *et al.*, 2007). DNA instability is associated with replication fork arrest (reviewed in  
5 Aguilera and Gomez-Gonzalez, 2008), and in order to limit the deleterious consequences of  
6 replication-transcription collisions, cells encode enzymes that facilitate replication through  
7 transcription units. In yeast, the Rrm3 helicase travels with the replication fork machinery and  
8 dislodges RNA Pols from tRNA and rRNA genes as well as tightly bound proteins from  
9 heterochromatin (Azvolinsky *et al.*, 2009 and references therein). In *E. coli*, this function was  
10 originally ascribed to the Rep helicase; firstly, based on the observations that in the *rep*  
11 mutant chromosome replication is slowed down and requires specific functions (Lane and  
12 Denhardt, 1975; Petit and Ehrlich, 2002; Seigneur *et al.*, 1998), and secondly, because the  
13 purified Rep helicase is specifically capable of dislodging DNA-bound proteins (Yancey-  
14 Wrona and Matson, 1992). More recently, evidence was provided that transcribed sequences  
15 are indeed a major obstacle to replication in rapidly growing cells, and that Rep is the main,  
16 though not the only helicase that assists replication progression across highly transcribed  
17 sequences (Boubakri *et al.*, 2010; Guy *et al.*, 2009).

18           Rep and UvrD are two 3' to 5' helicases that share 40% homology. The existence of a  
19 redundant function for these paralogues has been suspected since the original observation that  
20 the *rep uvrD* double mutant shows severe growth defects (Taucher-Scholtz *et al.*, 1983).  
21 However, the situation turned out to be more complex and UvrD is now known to play two  
22 different roles in *rep* mutants, revealed by the two classes of mutations that suppress the  
23 growth defects of the *rep uvrD* double mutant. The first class of mutations that was identified  
24 inactivates the RecFOR recombination pathway (Petit and Ehrlich, 2002). Combined with the  
25 observation that UvrD can remove RecA from ssDNA *in vitro*, the rescue of *rep uvrD* cells by

1 the inactivation of recombination proteins led to the proposal that UvrD is essential in *rep*  
2 mutants because it removes deleterious RecFOR-dependent RecA-filaments that assemble at  
3 blocked replication forks (Lestini and Michel, 2008; Veaute *et al.*, 2005). However, the nature  
4 of the obstacles arresting replication in the first place, thus allowing RecFOR-RecA binding  
5 to forks in *rep* mutants, remained unknown. Moreover, the suppression of *rep uvrD* co-  
6 lethality by *recFOR* inactivation was shown to be only partial, confirming the existence of an  
7 obstacle to replication restart other than RecFOR-RecA bound to DNA in *rep uvrD* cells  
8 (Boubakri *et al.*, 2010; Guy *et al.*, 2009). Two lines of evidence identified RNA polymerases  
9 as the original cause of replication arrest in *rep uvrD* cells: first, mutations that map in the  
10 RNA Pol genes *rpoB* and *rpoC* were shown to suppress the growth defects of this double  
11 mutant (Boubakri *et al.*, 2010; Guy *et al.*, 2009), second, replication arrest sites were directly  
12 visualized in *rep* and *rep uvrD recF* mutants at inverted ribosomal operons (*rrn*), provided  
13 that these operons were facing replication and were highly transcribed (Boubakri *et al.*, 2010).  
14 Rep was confirmed to be the major accessory helicase and was shown to be attracted to  
15 replication forks by a direct interaction with the replicative helicase DnaB (Guy *et al.*, 2009).  
16 In addition to UvrD, a third player was identified, the 5' to 3' helicase DinG (Boubakri *et al.*,  
17 2010). DinG is essential for the viability of the *rep uvrD recF* mutant and it was proposed  
18 that, provided that *recF* is inactivated, DinG can clear RNA Pols from blocked replication  
19 forks in the absence of both Rep and UvrD. In addition, DinG is essential for the viability of  
20 *rep* and *uvrD* single mutants when replication collides with RNA Pol at highly-expressed  
21 inverted *rrn*, indicating that the presence of two of these three helicases is required when  
22 replication-transcription collisions are increased. Finally, DinG has the specific function of  
23 unwinding RNA-DNA hybrids *in vivo*, since it is also essential for viability when replication  
24 forks are arrested by R-loops (Boubakri *et al.*, 2010).

1           The described RNA Pol mutations that suppress *rep uvrD* growth defects are  
2 mutations that constitutively confer phenotypes akin to the induction of the stringent response  
3 (Boubakri *et al.*, 2010; Guy *et al.*, 2009). The stringent response is an adaptation to amino  
4 acid starvation through the induction of the alarmone ppGpp (reviewed in Potrykus and  
5 Cashel, 2008). Binding of ppGpp to RNA Pol, as well as mutations that mimic this binding,  
6 affect transcription initiation from specific promoters, which decreases the expression of  
7 ribosomal operons (*rrn*) and activates the expression of amino acids biosynthetic genes in  
8 *relA spoT* double mutants, allowing their growth in minimal medium without amino acids  
9 (Barker *et al.*, 2001a; Barker *et al.*, 2001b; Bartlett *et al.*, 1998, 2000; Zhou and Jin, 1998).  
10 The so-called “stringent mutations” in RNA Pol mimic the presence of ppGpp in decreasing  
11 the half-life of open complexes. Although we have not measured the half-life of transcription  
12 open complexes, we will call here “stringent-like” the phenotype conferred by RNA Pol  
13 mutations that both decrease *rrn* expression in rich medium and allow growth of *relA spoT*  
14 double mutants on minimal medium. RNA Pol mutations that increase transcription of amino  
15 acid biosynthetic promoters also destabilize transcription elongation complexes (TEC)  
16 (Trautinger and Lloyd, 2002; Trautinger *et al.*, 2005). Such mutations were thought to  
17 suppress the lethality of *rep uvrD* double mutants owing to the destabilisation of RNA Pol-  
18 DNA complexes (Boubakri *et al.*, 2010; Guy *et al.*, 2009).

19           In this work, we report that *rep uvrD recF* cells grow poorly on rich medium at low  
20 temperature and we isolated five mutations suppressing this LB-cryo-sensitivity. Similarly to  
21 the mutations that suppress the growth defects of *rep uvrD* mutants at 37°C, the suppressor  
22 mutations isolated here in *rep uvrD recF* mutants at 30°C map in *rpoB* and *rpoC*. One of  
23 these mutations is close to the active site; the others are in, or close to, the primary DNA-  
24 RNA binding channel, suggesting that they affect the stability of transcription complexes on  
25 DNA. Only one of these mutations exhibits a stringent-like phenotype, showing that our assay

1 provides a new way of isolating RNA Pol mutants that are weakly bound to DNA, in  
2 transcription initiation or elongation complexes. Furthermore, these RNA Pol mutants allow  
3 us to extend our study of helicases that assist replication progression across transcription  
4 obstacles.

5

## 6 RESULTS

### 7 *rep uvrD recF mutants are cryo-sensitive*

8 We constructed *rep uvrD recF* triple mutants in the presence of a conditional plasmid  
9 that carries the *rep* wild-type gene (pAM-*rep*; Lestini and Michel, 2008). This plasmid only  
10 replicates in the presence of the Lac promoter inducer, isopropyl thio-galactoside (IPTG) and  
11 is cured upon cell propagation in the absence of IPTG. We analyzed the properties of *rep*  
12 *uvrD recF* cells cured of pAM-*rep*. For historical reasons the work was realized part in an  
13 AB1157 background (classically used for homologous recombination studies) and part in an  
14 MG1655 background (the more generally used, sequenced wild-type strain). In the AB1157  
15 background, colony formation was delayed on LB at 37°C and 30°C; at 25°C only a variable  
16 subpopulation of cells formed colonies (JJC4048, Table I). In the MG1655 background,  
17 results were similar except for a partial defect of plating efficiency on LB at 30°C (JJC5136  
18 and JJC5166 Table I). Finally, we constructed an Hfr strain which allowed the co-introduction  
19 of the three *rep uvrD recF* mutations by conjugation, and observed that in this Hfr-PK3-  
20 PO131 background the growth defect was more pronounced than in other backgrounds, as  
21 plasmid-less *rep uvrD recF* cells were not recovered with the expected efficiency even on  
22 minimal medium (MM) at 37°C (not shown). In order to understand the reasons for the cryo-  
23 sensitivity of *rep uvrD recF* mutants, we studied five AB1157 *rep uvrD recF* suppressed  
24 clones which are able to form large colonies on LB at 30°C in 2 days. The *rpoC*<sup>Δ215-220</sup>  
25 mutation, previously shown to restore the viability of *rep uvrD* cells at 37°C (Boubakri *et al.*,



1 2010), and a suppressor mutation identified in the Hfr *rep uvrD recF* context at 37°C were  
2 included in this study.

3

4 *Suppressors of rep uvrD recF LB-cryo-sensitivity map in rpoC and rpoB genes.*

5 Analysis of the subpopulation of Hfr plasmid-less colonies formed in two days at 37°C  
6 on MM supplemented with casamino acids revealed that one of them was resistant to  
7 rifampicin. Rif<sup>R</sup> mutations map in *rpoB*, the gene coding for the β subunit of RNA Pol, which  
8 suggested that this particular *rep uvrD recF* clone carries an *rpoB* mutation that suppresses  
9 the *rep uvrD recF* growth defect (JJC4100, Table S1). To ascertain that the Rif<sup>R</sup> mutation was  
10 responsible for the improved growth of the *rep uvrD recF* Rif<sup>R</sup> clone, pAM-*rep* was re-  
11 introduced and the suppressed clone was P1 transduced with *thiC::Tn10*, a locus close to the  
12 *rpoB rpoC* genes. As expected, the Rif resistance phenotype was 90% linked with *thiC::Tn10*  
13 (43/48 Tet<sup>R</sup> transductants were Rif<sup>S</sup>). Curing of pAM-*rep* showed that *rep uvrD recF*  
14 *thiC::Tn10* clones that had remained Rif<sup>R</sup> had kept the capacity to form colonies overnight  
15 (ON) at 37°C on LB whereas the *thiC::Tn10* clones that had lost the Rif<sup>R</sup> phenotype grew as  
16 poorly as the original Hfr *rep uvrD recF* mutant. This mutation is therefore necessary and  
17 sufficient for the improved viability of *rep uvrD recF* cells. Sequencing of the *rpoB* gene  
18 revealed the presence of a mutation, N518D, in the *rpoB* Rif<sup>R</sup> cluster 1.

19 Overnight cultures of AB1157 *rep uvrD recF* cells were plated on LB at 30°C. Five  
20 colonies isolated in three independent experiments were kept for further studies (called S1,  
21 S2, and S3a, S3b, S3m). Replacement in the five suppressed strains of the *rep uvrD recF*  
22 region of AB1157 by that of the Hfr JJC4100, or of the *rep::Ap<sup>R</sup>* allele by a *rep::cm<sup>R</sup>* allele,  
23 did not modify their growth properties (Table S1, data not shown). To determine whether the  
24 isolated mutations map in the *rpoBC* genes, a Tet<sup>S</sup> derivative of each suppressed clones was

1 P1 transduced with *thiC::Tn10*. For each mutant 7 to 11 out of 12 *thiC::Tn10* transductants  
2 became cryo-sensitive, indicating that these five suppressor mutations are linked to *thiC*.

3 A plasmid carrying an IPTG-inducible *rpoB*<sup>+</sup> gene was introduced in Ap<sup>S</sup> derivatives  
4 of the suppressed clones and viability was measured in the presence of IPTG (Table S2).  
5 Expression of RpoB rendered S3m cryo-sensitive on LB, suggesting that this mutant carries a  
6 recessive *rpoB* mutation; sequencing *rpoB* showed the presence of a H447R mutation. In S3a,  
7 the control vector pUC19 could not be introduced whereas the plasmid pUC-*rpoB*<sup>+</sup>  
8 transformed with a normal efficiency (not shown); nevertheless, transformants remained cryo-  
9 resistant (Table S2). Sequencing *rpoB* revealed the presence of a D444G mutation. These  
10 observations suggest that, in addition to suppressing the *rep uvrD recF* growth defect, the  
11 *rpoB*<sup>D444G</sup> mutation somehow prevents pUC propagation. This defect in plasmid propagation  
12 is recessive (as it is complemented in *cis* by the wild-type *rpoB*<sup>+</sup> allele) whereas the  
13 suppression of *rep uvrD recF* cryo-sensitivity is dominant over the wild-type allele. In S1 and  
14 S3b expression of *rpoB*<sup>+</sup> did not prevent growth at 25°C (Table S2), so we sequenced *rpoC* in  
15 these two mutants and found a P451L mutation (S1), and a H113R mutation (S3b). Finally,  
16 both *rpoB* and *rpoC* genes were sequenced in S2. *rpoB* was intact and *rpoC* carried the  
17 H113R mutation, which was thus obtained twice independently. To our knowledge, the three  
18 *rpoB*<sup>D444G</sup>, *rpoC*<sup>H113R</sup> and *rpoC*<sup>P451L</sup> mutations have not been described previously, whereas  
19 the *rpoB*<sup>N518D</sup> allele has been already isolated in a screen for Rif<sup>R</sup> clones (Garibyan *et al.*,  
20 2003), and the *rpoB*<sup>H447R</sup> allele in a screen for mutations that increase the expression of amino  
21 acid biosynthetic genes in a *relA spoT* context (Trautinger and Lloyd, 2002).

22

23 *The rpo<sup>sup</sup> mutations are necessary and sufficient for the suppressor phenotype*

24 To determine whether the *rpo* mutations isolated here (called *rpo*<sup>sup</sup> thereafter) are  
25 necessary and sufficient for the suppressor phenotype, these mutations were transferred to a

1 *rep uvrD recO* strain by P1 co-transduction with *thiC::Tn10* [RecF is known to act in  
2 conjunction with two other proteins, RecO and RecR (Kuzminov, 1999), and inactivation of  
3 either the RecF, RecO or RecR protein allows colony formation of *rep uvrD* cells at 37°C on  
4 MM with casamino acids (Petit and Ehrlich, 2002)]. *rep uvrD recO* cells harboring the  
5 plasmid pBGts-*rep* were used for strain construction; this plasmid carries the wild-type *rep*  
6 gene and can be cured by growing cells at 42°C (Petit and Ehrlich, 2002). The phenotype of  
7 plasmid-less cells obtained after propagation at 42°C was analyzed. The five *rep uvrD recO*  
8 *rpo<sup>sup</sup>* mutants formed about 100% colonies on LB at 37°C, 30°C and 25°C, as the original  
9 *rep uvrD recF rpo<sup>sup</sup>* cells (Table 1). We conclude that the *rpo<sup>sup</sup>* mutations are necessary and  
10 sufficient to restore full viability to *rep uvrD recF (recO)* cells at low temperatures.

11 RecQ acts in concert with RecFOR to promote RecA binding to blocked forks in the  
12 *rep uvrD* mutant (Lestini and Michel, 2008). We observed that the *rep uvrD recQ* mutant is  
13 more sensitive to LB than *rep uvrD recF (recO)* cells (Table I), suggesting that the RecFOR  
14 proteins still bind to arrested forks in a *rep uvrD recQ* mutant. The *rpo<sup>sup</sup>* alleles suppressed  
15 the LB-sensitivity of *rep uvrD recQ* cells, with the notable exception of the Rif<sup>R</sup> (*rpoB<sup>N518D</sup>*)  
16 mutation that suppressed only at 37°C (Table 1). The residual cryo-LB-sensitivity of the *rep*  
17 *uvrD recQ rpoB<sup>N518D</sup>* indicates that RecFOR bind blocked replication forks in the absence of  
18 RecQ in this particular RNA Pol mutant, and that this mutation is a less efficient suppressor  
19 than the others at low temperature, in agreement with its original isolation at 37°C.  
20 Nevertheless, all other *rpo<sup>sup</sup>* mutations and the *rpoC<sup>Δ215-220</sup>* mutation suppress the residual  
21 growth defects of *rep uvrD recQ* cells.

22

23 *Suppression of the LB-cryo-sensitivity of rep uvrD recF does not correlate with a stringent-*  
24 *like phenotype of these RNA Pol mutants.*

1           The *rpoC*<sup>Δ215-220</sup> mutation alters the kinetic properties of transcription complexes,  
2 reducing rRNA transcription and increasing transcription from some amino acid biosynthetic  
3 genes (Bartlett *et al.*, 1998). On the other hand, it behaves as most of the mutations isolated  
4 here since it suppresses the growth defects of *rep uvrD recF*, *recO* and *recQ* cells at all  
5 temperatures (Boubakri *et al.*, 2010) (Table 1). In addition, one of the mutations isolated here  
6 (*rpoB*<sup>H447R</sup>) was also previously isolated in a screen for mutations that confer a stringent-like  
7 phenotype (Trautinger and Lloyd, 2002). These observations prompted us to test whether the  
8 *rpo*<sup>sup</sup> mutations isolated here prevent the stimulation of the *rrnB* core promoter after a shift to  
9 rich medium. The *rpo*<sup>sup</sup> mutations were co-transduced with *thiC::Tn10* into a strain carrying  
10 a P1<sub>*rrnB*</sub>-*lacZ* fusion, which was used to compare the expression of the P1<sub>*rrnB*</sub> core promoter in  
11 MM and in LB (Bartlett *et al.*, 1998). As expected, in wild-type cells the expression of *lacZ*  
12 from P1<sub>*rrnB*</sub> promoter was higher in LB than in MM, whereas P1<sub>*rrnB*</sub> activity remained low in  
13 both media in the presence of the *rpoC*<sup>Δ215-220</sup> or *rpoB*<sup>H447R</sup> mutations (Fig. 1). This  
14 experiment revealed that *rpoB*<sup>D444G</sup> also reduces rRNA expression in LB (Fig. 1). In contrast,  
15 P1<sub>*rrnB*</sub> expression remained higher in LB than in minimal medium in the presence of the  
16 *rpoB*<sup>N518D</sup>, *rpoC*<sup>P451L</sup>, and *rpoC*<sup>H113R</sup> alleles as in wild-type cells, showing that none of these  
17 three mutations affects the activity of rRNA promoter.

18           RelA and SpoT proteins are implicated in ppGpp alarmone synthesis. *relA spoT*  
19 double mutants do not induce the stringent response and are thus unable to grow on MM  
20 unless amino acids are provided. RNA Pol mutations that mimic the stringent response are  
21 classically isolated as suppressing the auxotrophy of *relA spoT* mutants. Although this has not  
22 been actually demonstrated, it was suggested that the inability of *relA spoT* mutants to grow  
23 on MM and the ability of the *rpoBC* suppressor mutations to suppress this defect results from  
24 effects of the mutations on transcription of amino acid biosynthetic operons (Paul *et al.*, 2005;  
25 see Rutherford *et al.*, 2009, for discussion). In order to analyse the capacity of the *rpo*<sup>sup</sup>

1 mutations to suppress the auxotrophy of *relA spoT* double mutants, we constructed *rpo<sup>sup</sup>*  
2  $\Delta relA::Kan^R$  mutants (strains JJC 4553 to JJC4559 Table S1) and P1-transduced them with a  
3  $\Delta spoT::Cm^R$  null mutation, plating half of the transduction mixture on LB and half on MM.  
4 All *rpo<sup>sup</sup> relA::Kan<sup>R</sup>* mutants could be transduced by the  $\Delta spoT::Cm^R$  null mutation on LB  
5 while only one mutant, *rpoB<sup>H447R</sup> relA::Kan<sup>R</sup>*, provided transductants on MM (Table S3). As  
6 expected from this result, none of the *rpo<sup>sup</sup> relA::Kan<sup>R</sup> spoT::Cm<sup>R</sup>* mutants obtained on  
7 LB could grow on MM except for the *rpoB<sup>H447R</sup> relA::Kan<sup>R</sup> spoT::Cm<sup>R</sup>* strain (Table S3).  
8 The growth defect on MM was specific for a *relA spoT* context, since none of the RNA Pol  
9 mutations prevented growth on MM in *RelA<sup>+</sup> SpoT<sup>+</sup>* cells (see plating efficiencies on MM in  
10 Fig. 3 and Fig. 4). This result shows that only the *rpoB<sup>H447R</sup>* mutation prevents the increase of  
11 *rrn* expression in rich medium and allows growth of a *relA spoT* mutant on MM. The  
12 *rpoB<sup>D444G</sup>* mutation only affects *rrn* expression and the other *rpo<sup>sup</sup>* mutations exhibit none of  
13 these stringent-like phenotypes. This result shows that four of the five mutations isolated in  
14 this study would not be obtained in a classical screen for mutations that restore growth of a  
15 *relA spoT* mutant on MM. Although they do not confer a stringent-like phenotype, the *rpo<sup>sup</sup>*  
16 mutations bypass the need for accessory replication helicases, suggesting that they affect the  
17 stability of transcription complexes without affecting their kinetic properties on *rrn* and amino  
18 acid biosynthetic gene promoters. The possible instability of the mutant RNA Pol-DNA  
19 complexes was tested by two different genetic approaches.

20

21 *rpo<sup>sup</sup>* mutations improve the resistance to UV irradiation of a *ruv* mutant and the *rpoC<sup>H113R</sup>*  
22 mutation rescues a *greA greB* double mutant.

23 RuvABC is a recombination complex that acts at the last step of homologous  
24 recombination by resolving recombination intermediates called Holliday junctions  
25 (Kuzminov, 1999). *ruv* mutants are hyper-sensitive to UV irradiation and *rpoB* or *rpoC*

1 mutations that exhibit a stringent-like phenotype partially relieve this hyper-sensitivity. It was  
2 proposed that they increase the intrinsic instability of RNA Pol-DNA complexes when RNA  
3 Pol is blocked by a DNA lesion (Trautinger and Lloyd, 2002). A *ruvABC* deletion was  
4 introduced in all *rpo<sup>sup</sup>* mutants and the UV resistance of the resulting strains was measured.  
5 With the exception of the *rpoC<sup>H113R</sup>* allele, which was poorly viable in a *ruv* mutant context  
6 and yielded variable results, all mutations improved the UV resistance of the *ruvABC* mutant  
7 (Fig. 2A). This result supports the idea that the mutations isolated here affect the stability of  
8 RNA Pol-DNA complexes, either at promoters or in transcription elongation complexes  
9 (TEC).

10 When RNA Pol encounters a block during elongation and backtracks, the transcription  
11 factors GreA and GreB suppress pausing by stimulating the intrinsic nucleolytic activity of  
12 RNA Pol (reviewed in Borukhov *et al.*, 2005). *greA greB* double mutants are non-viable at  
13 high temperature, presumably because prolonged RNA polymerase pausing prevents  
14 replication and/or transcription. A mutation isolated through its stringent-like phenotype was  
15 previously reported to suppress the thermo-sensitivity of *greA greB* mutants (Trautinger and  
16 Lloyd, 2002). *greA greB rpo<sup>sup</sup>* mutants were constructed and tested for growth at 42°C. Only  
17 the *rpoC<sup>H113R</sup>* mutation allowed the growth of *greA greB* mutant at 42°C (Fig. 2B). This result  
18 presumably reflects the destabilisation of backtracked RNA Pol by the *rpoC<sup>H113R</sup>*, but this  
19 level or type of destabilisation is not essential for the growth of *rep uvrD recF* cells at 30°C  
20 since it is observed for only one of the suppressor mutations. This result also indicates that  
21 mutations that do not affect *rrn* or amino acid biosynthetic gene expression can nevertheless  
22 decrease the stability of stalled TEC enough to rescue a *greA greB* mutant. Altogether, we  
23 infer from the phenotypes conferred by the RNA Pol mutations described here that these  
24 mutations compromise the stability of transcription complexes; the consequences of this

1 putative destabilisation were further investigated in backgrounds that lack different accessory  
2 helicases.

3

4 *Several  $rpo^{sup}$  mutants rescue  $rep\ uvrD$  mutants in a  $RecF^+$  context*

5         If the viability of  $rep\ uvrD\ recF\ rpo^{sup}$  mutants results from less replication arrest, or  
6 from facilitated replication restart, the inactivation of  $recF$  might not be needed for viability.  
7 Actually, certain RNA Pol mutations including the  $rpoC^{\Delta 215-220}$  allele were reported to  
8 suppress the growth defect of  $rep\ uvrD$  cells in a  $RecF^+$  context at 37°C (Boubakri *et al.*,  
9 2010; Guy *et al.*, 2009). To construct  $rep\ uvrD\ rpo^{sup}$  mutants,  $rep$  and  $uvrD$  were introduced  
10 in  $rpo^{sup}$  single mutants containing pGBts- $rep$ , and/or the  $rpo^{sup}$  mutation was co-transduced  
11 with  $thiC::Tn10$  to a  $rep\ uvrD$  [pAM- $rep$ ] mutant. The viability of plasmid-less clones was  
12 measured after curing cells by propagation at 42°C (pGBts- $rep$ ) or in the absence of IPTG  
13 (pAM- $rep$ ) (Fig. 3A). The  $rpoC^{H113R}$  mutation allowed formation of  $rep\ uvrD$  colonies on LB  
14 at all temperatures. The  $rpoC^{P451L}$  mutant yielded slow-growing colonies that were  
15 heterogeneous in size at low temperature. Two of the mutations that affect  $rrn$  expression  
16 ( $rpoC^{\Delta 215-220}$ ,  $rpoB^{H447R}$ ) restored  $rep\ uvrD$  cells viability on LB, but colony formation was  
17 delayed at low temperature and also slightly decreased for  $rpoC^{\Delta 215-220}$  at 25°C. In the  
18  $rpoB^{D444G}$  context, colony formation on LB was delayed at all temperatures and significantly  
19 decreased at 25°C. Finally, the  $rpoB^{N518D}$  mutation had only a partial effect at 37°C and did  
20 not allow colony formation at low temperatures. These observations indicate that in one  $rep$   
21  $uvrD\ rpo^{sup}$  mutant ( $rpoC^{H113R}$ ) RecFOR does not bind replication forks at any temperature. In  
22 the other  $rep\ uvrD\ rpo^{sup}$  mutants, although RecFOR is not lethal any more as in  $rep\ uvrD$   
23  $Rpo^+$  cells, it remains partly deleterious, slowing down and/or preventing growth, mainly at  
24 low temperature. Therefore, the modifications of RNA Pol activity caused by the different  
25 mutations determine the accessibility of replication forks to RecFOR recombination proteins.



1

2 *rpo<sup>sup</sup>* mutations rescue *rep uvrD recF dinG* mutants but not *rep uvrD dinG* mutants3 The viability of the *rep uvrD recF* mutant relies on the presence of a third helicase4 called DinG (Boubakri *et al.*, 2010). We previously reported that the *rpoC*<sup>Δ215-220</sup> mutation5 suppresses the lethality of *rep uvrD recF dinG* mutants at 37°C on MM and on LB, but not6 that of *rep uvrD dinG* mutants, indicating that it facilitates replication restart in the absence of7 all three accessory helicases only if RecF does not poison arrested forks (Boubakri *et al.*,8 2010). We tested whether the *rpo<sup>sup</sup>* mutations also suppress *rep uvrD recF dinG* lethality by9 constructing *rep uvrD recF dinG rpo<sup>sup</sup>* mutants in two ways, first a *dinG* deletion was10 introduced in the original *rep uvrD recF rpo<sup>sup</sup>* clones (JJC4043 to JJC4047, context AB1157,11 Table S1), and secondly the *rpo<sup>sup</sup>* mutations were introduced in a *rep uvrD recF dinG* mutant

12 (JJC5405 to JJC5426, context MG1655, Table S1). Results were similar in both backgrounds

13 (data not shown and Fig. 3B). *rpoC*<sup>P451L</sup> was the only mutation that did not suppress the14 lethality of *rep uvrD recF dinG* cells as no plasmid-less cells could be obtained (not shown).15 The best suppressor mutation was again the *rpoC*<sup>H113R</sup> which restored 100% *rep uvrD dinG*16 *recF* plating efficiency at 37°C (although colony formation was delayed) and at low17 temperature (Fig. 3B). The three mutations *rpoC*<sup>Δ215-220</sup>, *rpoB*<sup>H447R</sup> and *rpoB*<sup>D444G</sup> also restored18 100% plating efficiency but colony formation was delayed on LB at 37°C for *rpoB*<sup>D444G</sup>, on19 LB at all temperatures for *rpoC*<sup>Δ215-220</sup>, and in all growth conditions for *rpoB*<sup>H447R</sup>. Finally,20 *rpoB*<sup>N518D</sup> allowed normal colony formation on MM but formed only about 10% of21 heterogeneous slow-growing colonies on LB at 37°C and did not suppress the *rep uvrD recF*22 *dinG* lethality on LB at low temperature.23 It was previously reported that *rpoC*<sup>Δ215-220</sup> only rescues a *rep uvrD dinG* mutant that24 lacks RecF (Boubakri *et al.*, 2010). To address this question for the *rpo<sup>sup</sup>* mutants, *rep uvrD*25 *dinG rpo<sup>sup</sup>* RecF<sup>+</sup> mutants were constructed, firstly by introducing the *dinG* deletion in the



1 original *rep uvrD rpo<sup>sup</sup>* mutants previously made RecF<sup>+</sup> (JJC5253 to JJC5257 context  
2 AB1157 and JJC5258 context JJC4100, Table S1) and secondly by introducing successively  
3 all three helicase deletions in the *rpo<sup>sup</sup>* mutants (JJC4911 to JJC4918, JJC5310 and JJC5311,  
4 context AB1157, Table S1). All strains yielded similar results: the *rpo<sup>sup</sup>* mutations did not  
5 suppress the lethality of *rep uvrD dinG* mutants in a RecF<sup>+</sup> context, as either no plasmid-less  
6 colonies could be recovered after growth under non-permissive conditions for plasmid  
7 replication (*rpoB<sup>N518D</sup>*), or only a few plasmid-less colonies were obtained which could not be  
8 propagated and eventually acquired additional suppressive mutations, possibly because the  
9 *uvrD* context is mutator (data not shown). In conclusion, in the absence of all three helicases  
10 the *rpo<sup>sup</sup>* mutations restore cell viability only if *recF* is inactivated, as previously observed  
11 for the *rpoC<sup>Δ215-220</sup>* mutation in a MG1655 background at 37°C (Boubakri *et al.*, 2010). This  
12 result indicates that, at least in the absence of the three Rep, UvrD and DinG helicases,  
13 replication forks are still arrested by the encounter of the *rpo<sup>sup</sup>* mutated RNA polymerases,  
14 allowing RecFOR to gain access to DNA. This result also indicates that DinG is responsible  
15 of the viability of *rep uvrD rpo<sup>sup</sup> DinG<sup>+</sup>* mutants shown in Fig. 3A, since the inactivation of  
16 *dinG* in this context is lethal.

17 To further test the effects of the *rpo<sup>sup</sup>* mutations on replication-transcription  
18 collisions, we introduced these mutations in cells where such collisions are increased by a  
19 chromosome rearrangement.

20

21 *Rescue of helicase mutants that carry an inverted rrn operon depends on the rpo mutation.*

22 Inversion of an *rrn* operon creates a region of increased head-on collisions between  
23 replication and transcription. Such inversions render the *dinG* mutant sensitive to rich  
24 medium because of R-loop formation, and strongly impair growth of the *rep dinG* double  
25 mutant, even on MM, because of DNA Pol-RNA Pol collisions (Boubakri *et al.*, 2010) (Fig.

1 4A). In a strain that carries an inverted *rrnA* operon (InvA) the *rpoC*<sup>Δ215-220</sup> mutation  
2 suppresses the growth defects of both *dinG* and *dinG rep* mutants at 37°C, on MM and on LB,  
3 presumably by decreasing the transcription efficiency of *rrnA* (Boubakri *et al.*, 2010) (Fig.  
4 4A).

5 We tested whether the *rpo*<sup>sup</sup> mutations decrease the formation of R-loops in inverted  
6 *rrn* by introducing these mutations in InvA *dinG* cells and measuring plating efficiencies of  
7 the resulting combination of mutations on LB. *rpoC*<sup>Δ215-220</sup>, previously tested at 37°C only,  
8 was tested here at 30°C. The *rpoC*<sup>H113R</sup> mutation and the three mutations that affect *rrn*  
9 expression (*rpoB*<sup>H447R</sup> *rpoB*<sup>D444G</sup> and *rpoC*<sup>Δ215-220</sup>) restored full viability of the InvA *dinG*  
10 mutant at 37°C and 30°C (Fig. 4A). Therefore, they decrease R-loop formation in this context  
11 at both temperatures. In contrast the Rif<sup>R</sup> mutation (*rpoB*<sup>N518D</sup>) allowed InvA *dinG* colony  
12 formation on LB at 37°C but not at 30°C, and the *rpoC*<sup>P451L</sup> mutations had no effect. The  
13 phenotype of this last mutant suggests that suppression of the *rep uvrD recF* LB-cryo-  
14 sensitivity by *rpo*<sup>sup</sup> mutations is independent of R-loop removal. To ascertain directly that the  
15 LB-cryo-sensitivity of the *rep uvrD recF* mutant does not result from R-loop formation, a  
16 plasmid that over-expresses RnaseH (pEM001) was introduced in this mutant. As shown in  
17 Table 1, this plasmid did not improve plating efficiency. In conclusion, most of the *rpo*<sup>sup</sup>  
18 mutations suppress or decrease R-loop formation in InvA cells, allowing InvA *dinG* viability,  
19 but conversely suppression of R-loops is neither necessary nor sufficient to improve growth  
20 of *rep uvrD recF* cells at 30°C on LB.

21 *rpo*<sup>sup</sup> mutations were also introduced in an InvA *dinG rep* mutant, where replication  
22 is arrested in the inverted *rrnA* by collisions with RNA Pol (Boubakri *et al.*, 2010). As  
23 previously reported the mutation *rpoC*<sup>Δ215-220</sup> restored a normal plating efficiency on MM and  
24 LB (Boubakri *et al.*, 2010), and as expected the *rpoB*<sup>D444G</sup> mutation, which decreases *Prrn*  
25 efficiency (Fig. 1), exhibited a similar phenotype (Fig. 4B). Surprisingly, the *rpoB*<sup>H447R</sup>

1 mutation improved colony formation only on MM. It is conceivable that the decrease of *rrn*  
2 expression measured in Fig. 1 with a promoter deprived of its FIS binding sites is  
3 compensated by the presence of these sites at inverted *rrnA* (Bartlett *et al.*, 2000). Finally,  
4 *rpoC*<sup>H113R</sup> also improved viability on MM whereas the *rpoC*<sup>P451L</sup> and *rpoB*<sup>N518D</sup> mutations had  
5 no effect. Altogether, these results indicate that in rich medium only two mutations, *rpoC*<sup>Δ215-</sup>  
6 <sup>220</sup> and *rpoB*<sup>D444G</sup> destabilize RNA Pol enough to allow replication across an inverted *rrn* in  
7 the absence of Rep and DinG, *i.e.* when only the UvrD helicase is active. Among the  
8 suppressor mutations that do not affect *rrn* expression, *rpoC*<sup>H113R</sup> is the only one that rescues  
9 an *InvA dinG rep* mutant, and only on MM.

10 A consequence of replication arrest in the *rep* mutant is a reaction called replication  
11 fork reversal, in which the two ends of the newly synthesised strands at a blocked replication  
12 fork anneal to form a DNA double-strand end and a Holliday junction. This reaction renders  
13 RecBC (the recombination enzyme specific for DNA double-strand ends) essential for  
14 viability (Michel *et al.*, 2007; Seigneur *et al.*, 1998). Interestingly, although this study and  
15 other recent studies suggest that in the *rep* mutant replication forks are arrested by transcribed  
16 sequences, none of the mutations isolated here rescued the viability of *rep recB* cells, as no  
17 plasmid-less colonies could be recovered after propagation of *rep recB rpo*<sup>sup</sup> [pAM-*rep*]  
18 mutants in the absence of IPTG (data not shown, strains JJC5532 to JJC5536 in Table S1).  
19 This indicates that the *rpoB* mutation does not prevent replication fork reversal in a *rep*  
20 mutant, and that, even when the RNA Pol is mutated and weakly bound to DNA, its removal  
21 from DNA by UvrD and/or DinG takes place within the context of restarting reverted  
22 replication forks.

23

24 DISCUSSION

1 We show here that transcription is a stronger obstacle to replication at lower  
2 temperatures than at 37°C and we isolated mutations that suppress the rich medium cryo-  
3 sensitivity of *rep uvrD recF* cells. The genetic properties of these mutants suggest that the *rep*  
4 *uvrD* and *rep uvrD recF* strains provide a new way of isolating mutations that decrease the  
5 stability of RNA Pol on DNA, at promoters and/or in TEC. Furthermore, the analysis of these  
6 RNA Pol mutants reinforces the notion that Rep, UvrD and DinG have redundant functions in  
7 *E. coli* to facilitate the progression of replication forks across transcribed sequences.

#### 8 *Isolation and properties of new RNA Pol alleles.*

9 The atomic structure of *Thermus thermophilus* and *Thermus aquaticus* RNA Pol have  
10 led to structural models of transcription elongation complexes (TEC) (Korzheva *et al.*, 2000;  
11 Vassylyev *et al.*, 2002; Vassylyev *et al.*, 2007a; Vassylyev *et al.*, 2007b; Zhang *et al.*, 1999).  
12 The similarity of these RNA Pols with the *E. coli* enzyme allows us to map the residues  
13 affected in our *rpoB* and *rpoC* mutants on these structures. The position of the mutated amino  
14 acids on a representation of a wild-type transcription complex is shown in Fig. 5. The three  
15 *rpoB* mutations, D444G, H447R and N518D (D324, H327 and T398 in *T. thermophilus* and  
16 *T. aquaticus*) lie in the major channel for the DNA/RNA hybrid, in, or close to the  $\beta$ -lobe 1,  
17 in a region previously described to be important for the stability of RNA Pol-DNA complexes  
18 (Rutherford *et al.*, 2009; Trautinger and Lloyd, 2002). The *rpoC*<sup>H113R</sup> mutation (H90 in *T.*  
19 *thermophilus* and H101 in *T. aquaticus*) is on the other side of the major channel and although  
20 the mutated residue is buried in the enzyme, it may affect interactions of RpoC with the  
21 hybrid DNA-RNA. *rpoC*<sup>P451L</sup> (P719 in *T. thermophilus* and P730 in *T. aquaticus*) is in a  
22 different location in the complex, in a highly conserved region around the active site. This  
23 mutation is adjacent to a mutation isolated as a  $\Delta dksA$  suppressor (H450R) and, since the  
24 RNA Pol cofactor DksA is also involved in the stability of transcription complexes, mutations  
25 in this region could affect the enzyme in another way to those which flank the major channel

1 (Rutherford *et al.*, 2009). In spite of the proximity of this mutation to the active site, we did  
2 not detect any deleterious effect of the *rpoC*<sup>P451L</sup> allele on *E. coli* growth (data not shown).  
3 Biochemical analysis of these mutant enzymes would tell whether the *in vivo* instability of  
4 transcription complexes is related to an increased propensity to pause and/or to terminate  
5 transcription.

6 The phenotypes of the isolated RNA Pol mutations do not allow their classification in  
7 specific groups but allow us to rank them according to the advantage that they confer to cells  
8 that lack Rep, UvrD and/or DinG helicases (summarised in Table 2). The best suppressor of  
9 growth defects in the absence of these helicases is the *rpoC*<sup>H113R</sup> mutation, which was isolated  
10 twice independently. This RNA Pol mutant is the only one that forms TEC unstable enough to  
11 suppress the temperature sensitivity of a *greA greB* double mutant. It confers LB resistance to  
12 *rep uvrD*, *rep uvrD dinG recF* and *InvA dinG* cells at all temperatures, and improves growth  
13 of *InvA dinG rep* cells at 37°C on MM. Of all mutants isolated here, the RpoC<sup>H113R</sup> RNA Pol  
14 is probably the one that forms the weakest complex on DNA. It is also the only one that slows  
15 down the growth of otherwise wild-type cells (data not shown), and could not be reliably  
16 combined with a *ruvABC* deletion. Slightly less efficient than *rpoC*<sup>H113R</sup>, the mutations  
17 *rpoB*<sup>H447R</sup> and *rpoB*<sup>D444G</sup> confer an intermediate phenotype, as the previously described  
18 *rpoC*<sup>Δ215-220</sup> allele. The destabilisation of transcription complexes caused by these mutations,  
19 which is deduced from their stringent-like phenotype and from the increased UV resistance  
20 conferred to *ruv* mutants, allows *rep uvrD dinG recF* and *rep uvrD* cells to grow on LB. They  
21 rescue *InvA dinG* and *InvA rep dinG* mutants, with the exception of the LB sensitivity of  
22 *InvA rep dinG rpoB*<sup>H447R</sup> cells (discussed below). Finally, the two less efficient suppressors  
23 are *rpoC*<sup>P451L</sup> and *rpoB*<sup>N518D</sup>. *rpoC*<sup>P451L</sup> is the only mutation that is close to the active site,  
24 while *rpoB*<sup>N518D</sup> was isolated at 37°C. The only sign that these mutated RNA Pols might be  
25 weakly bound to DNA, beside the rescue of *rep uvrD recF* cells, is the increase of UV

1 resistance in *ruv* mutants. In their presence, *rep uvrD* cells form slow growing colonies only  
2 at 37°C, and *InvA dinG rep* cells remain sick on MM. Furthermore, *rpoC*<sup>P451L</sup> fails to rescue  
3 *InvA dinG* cells on LB, while *rpoB*<sup>N518D</sup> only rescues it at 37°C and does not rescue *rep uvrD*  
4 *recQ* cells at 30°C.

5         Several mutations that mimic the effects of ppGpp have been isolated by different  
6 means, most often because they activate amino acid biosynthetic genes in a *relA spoT* double  
7 mutant, enabling it to grow on minimal medium (Bartlett *et al.*, 1998; Broccoli *et al.*, 2004;  
8 Szalewska-Palasz *et al.*, 2007; Trautinger and Lloyd, 2002; Zhou and Jin, 1998). Some of  
9 these mutations decrease expression from the *rrn* promoter regardless of the presence of the  
10 upstream FIS binding sites (Szalewska-Palasz *et al.*, 2007), while the FIS sites compensate for  
11 the *rpoC*<sup>Δ215-220</sup> mutation effects on the *rrnB* promoter (Bartlett *et al.*, 2000). One mutation  
12 conferring a stringent-like phenotype was shown to reduce R-loop formation in a non-  
13 translated sequence by causing premature transcription arrest *in vivo* (Broccoli *et al.*, 2004),  
14 and two others were shown to decrease the stability of TEC *in vitro* (Trautinger *et al.*, 2005).  
15 Therefore, the *rpoC*<sup>Δ215-220</sup>, *rpoB*<sup>D444G</sup> and *rpoB*<sup>H447R</sup> mutations may be affected for the  
16 stability of transcription initiation complexes, or for the stability of TEC, or for both. Because  
17 the FIS sites compensate for the *rpoC*<sup>Δ215-220</sup> mutation effects on the *rrnB* promoter, this  
18 mutation is likely to rescue helicase mutants carrying an inverted *rrn* by destabilizing TEC, as  
19 proposed in Boubakri *et al.* (2010). *rpoB*<sup>D444G</sup> may rescue *InvA dinG rep* cells because the  
20 promoter remains weakly expressed on LB in the presence of the FIS Sites, or because TEC  
21 are unstable. The RpoB<sup>H447R</sup> RNA Pol may fail to rescue *InvA dinG rep* cells because its  
22 defects are compensated for by the presence of the FIS sites and it forms more stable TEC  
23 than the RpoC<sup>Δ215-220</sup> RNA Pol during *rrn* transcription. This observation supports the idea  
24 that there is no necessary correlation between the instability of TEC and the instability of the  
25 open complexes on *rrn* promoters. Furthermore, we isolated here a mutant (*rpoB*<sup>D444G</sup>) that

1 affects transcription initiation at *rrn* promoters without affecting the expression of amino  
2 acids biosynthesis genes in a *relA spoT* context. To our knowledge, such a mutant was not  
3 reported before. Finally, rescue of a *greA greB* mutant at high temperature was previously  
4 described for a RNA Pol mutation isolated as increasing amino acids biosynthetic genes  
5 expression (Trautinger and Lloyd, 2002) whereas here it is observed for a RNA Pol mutant  
6 that does not affect these genes, nor *rrn* expression (*rpoC*<sup>H113R</sup>).

7 We used strains carrying a chromosome inversion to measure the consequences of a  
8 weaker stability of transcription complexes when the rate of replication arrest is increased  
9 either by the formation of R-loops, or by encountering oppositely-oriented highly-active RNA  
10 Pol in inverted *rrn*. With the exception of *rpoC*<sup>P451L</sup>, all RNA Pol mutations isolated here  
11 prevent R-loop formation. This means that RNA Pols that form unstable transcription  
12 complexes are less prone than the wild-type enzyme to R-loop formation. Conversely, only  
13 one (*rpoB*<sup>D444G</sup>) could prevent the lethality associated with the encounter of a series of RNA  
14 Pol transcribing a ribosomal operon in the direction opposite to replication. This means that  
15 even RNA Pols weakly bound to DNA will arrest replication forks when the latter collide  
16 with an oppositely-oriented highly-transcribed operon.

17 *How does E. coli deal with replication-transcription collisions?*

18 The general scheme that emerges from the present study and from previous studies of  
19 *rep*, *dinG* and *uvrD* single and multiple mutants is that Rep is the first factor that facilitates  
20 replication through transcribed sequences *in vivo* (Boubakri *et al.*, 2010; Guy *et al.*, 2009;  
21 Lane and Denhardt, 1975; Petit and Ehrlich, 2002; Seigneur *et al.*, 1998). In the absence of  
22 Rep, UvrD becomes essential for *E. coli* viability and mutations in RNA Pol suppress this co-  
23 lethality, which points to UvrD as the main back up to Rep for RNA Pol removal (Boubakri *et*  
24 *al.*, 2010; Guy *et al.*, 2009; this work). Two lines of evidence point to DinG as the other  
25 helicase that acts as a back up. The first, is our previous report that even in the absence of



1 RecF, the combination of the *rep*, *uvrD*, and *dinG* mutations is lethal in *E. coli* expressing the  
2 wild-type RNA Pol (Boubakri *et al.*, 2010). This suggests that DinG removes wild-type RNA  
3 Pols from replication forks when Rep, UvrD and RecF are absent. The second, is our present  
4 observation that DinG is required for suppression of the *rep uvrD* co-lethality by most of the  
5 *rpo<sup>sup</sup>* mutations studied here, indicating that because they are unstable on DNA, the mutant  
6 RNA Pol can be removed from replication forks by DinG regardless of the presence of RecF.  
7 However, these mutated RNA Pols do not suppress the need for RecBC in a *rep* mutant,  
8 indicating that DinG and UvrD act in the context of restarting reversed replication forks.  
9 These helicases may recognize some features of reversed or of the restarting replication forks  
10 that are not shared by the original replication fork, whereas Rep may act within the context of  
11 the original replication fork that encounters the obstacle. It should be noted that the *rpo<sup>sup</sup>*  
12 mutations do not prevent spontaneous replication arrest in the *E. coli* chromosome since they  
13 did not relieve the growth defects of a *priA* mutant, which lacks the main replication restart  
14 protein PriA (strains JJC5540 and JJC5541 in Table S1, data not shown).

15 At low temperature, even in the presence of DinG, *rep uvrD recF* cells grow poorly on  
16 LB, indicating that DinG has a limited ability to remove the wild-type RNA Pol from  
17 replication forks under these growth conditions. Either transcription is a stronger obstacle to  
18 replication at low temperature, for example because transcriptional complexes are more  
19 stable, or for some reason DinG is less active. Provided that RecF is absent, several RNA Pol  
20 mutations isolated here bypass not only the need for Rep and UvrD but also the need for  
21 DinG (*rep uvrD dinG recF rpo<sup>sup</sup>* are viable, Fig. 3B). Removal of the mutant RNA Pol may  
22 then be catalyzed either by some yet unknown function or by the replisome itself. Actually in  
23 a purified system *in vitro* the wild-type RNA Pol initiation complex can be dislodged by the  
24 replisome (Pomerantz and O'Donnell, 2010). However, since RecF remains lethal in a *rep*  
25 *uvrD dinG rpo<sup>sup</sup>* mutant, replication forks are still arrested in these *rpo<sup>sup</sup>* cells that lack the



1 three helicases, which is another indication that the dislodging of RNA Pol takes place during  
2 replication restart.

3 Our study of RNA Pol mutants that form unstable complexes on DNA provided  
4 conditions where viability is dependent on DinG only when RecF is present (the *rep uvrD*  
5 *rpo<sup>sup</sup>* combinations of mutations become LB sensitive upon DinG inactivation only in a  
6 RecF<sup>+</sup> context). This observation suggests that DinG counteracts a deleterious action of RecF;  
7 either DinG could remove RecFOR / RecA from DNA, or it could prevent RecFOR binding.  
8 It should be noted that UvrD can act both ways, it removes RecA from DNA or prevents  
9 RecFOR binding, depending on the cause of replication arrest (Lestini and Michel, 2007). The  
10 hypothetical removal of RecFOR and/or RecA from DNA by DinG needs to be tested *in vitro*;  
11 nevertheless, we do not favour this hypothesis because it is difficult to explain why DinG  
12 would remove RecF only in certain RNA Pol mutants. We favour the hypothesis that DinG  
13 and RecFOR compete for blocked replication forks in *rep uvrD* cells. Because DinG is unable  
14 to remove the wild-type RNA Pol, *rep uvrD* cells are killed by RecFOR. Similarly, the *rep*  
15 *uvrD rpo<sup>N518D</sup>* mutant is non-viable on LB at low temperature because under these conditions  
16 DinG cannot dislodge this mutated RNA Pol from DNA, letting RecFOR bind to arrested  
17 replication forks. Conversely, DinG is capable of removing all other RNA Pol mutants  
18 isolated here before RecFOR binds, and consequently the viability of the *rep uvrD* mutant  
19 becomes independent of the *recF* context in these RNA Pol mutants.

20

## 21 **Experimental procedures**

### 22 *Strains and plasmids*

23 The strain backgrounds are MG1655, or JJC40, which is an *hsdR* Thr<sup>+</sup> Pro<sup>+</sup> derivative  
24 of AB1157 (*leu-6 thi-1, his-4, argE3, lacY1, galK2, ara-14, xyl-5, mtl-1, tsx-33, rpsL31,*  
25 *supE44, hsdR, hsdM*). JJC147 is HfrPK3-PO131 (*thr1, leuB6, azi-15, tonA1, lacY1, supE44,*

1 *argE86::Tn10*). Strains genotypes and plasmids are described in Table S1. *InvA* is described  
2 in (Boubakri *et al.*, 2010), it is an MG1655 derivative, deleted for *lacZ* and *attλ*, which carries  
3 a 18kb inversion (NT 4 025 300 to 4 043 723) encompassing the *rrnA* operon. Minimum  
4 medium is M9 supplied with thiamine 0.05%, CaCl<sub>2</sub> 100 μM, MgSO<sub>4</sub> 2 mM, glucose 0.4%  
5 (Miller, 1992). Casamino acids 0.2% were added for strain background other than MG1655.  
6 Most of the strains were constructed by P1 transduction (Miller, 1992). For Hfr conjugations,  
7 a mix of 10% donor cells / 90% recipient cells was incubated for 15 min with low agitation at  
8 37°C before plating on selective media. Null mutants were checked by PCR with external  
9 oligonucleotides that amplify a DNA fragment of different length for the wild-type and the  
10 interrupted alleles. Oligonucleotides used for checking mutations are shown in Table S4.  
11 *uvrD* mutants were checked for their UV sensitive phenotypes and for their mutator  
12 phenotype (about 100-fold increase in the proportion of Rif<sup>R</sup> clones in overnight cultures).  
13 The plasmids pGBts-*rep* (Petit and Ehrlich, 2002), pAM-*rep* (Lestini and Michel, 2008) and  
14 pAM-*priA* (Grompone *et al.*, 2004) were described; they carry the *rep* or the *priA* gene under  
15 the control of their own promoter, they were segregated as published.

16 *rpo* mutations are *rpoB* A1331G (*rpoB*<sup>D444G</sup>), A1340G (*rpoB*<sup>H447R</sup>), A1552G  
17 (*rpoB*<sup>N518D</sup>), *rpoCA*338G (*rpoC*<sup>H113R</sup>), C1352T (*rpoC*<sup>P451L</sup>). Following P1 transduction or  
18 conjugation, screening for A1552G (*rpoB*<sup>N518D</sup>) was based the Rif<sup>R</sup> phenotype it confers.  
19 Screening for A1331G (*rpoB*<sup>D444G</sup>) was done sequencing the *rpoB* region. Three mutations  
20 were screened after PCR amplification of the region either by sequencing or by looking for  
21 the presence of a restriction site that they create: A1340G (*rpoB*<sup>H447R</sup>) creates a *BsiEI* site,  
22 *rpoCA*338G (*rpoC*<sup>H113R</sup>) creates a *BssHII* site, C1352T (*rpoC*<sup>P451L</sup>) creates a *BspM1* site.  
23 Upon P1 co-transduction of *rpo*<sup>sup</sup> with *thiC::Tn10* in a context where the mutation confers a  
24 phenotype, the phenotype and the presence of the mutation were tested in four to eight  
25 different Tet<sup>R</sup> transductants and there was 100% correlation between the presence of the

1 mutation and the appearance of the new phenotype. 20 regularly spaced oligonucleotides in  
2 *rpoB* (10 on each strand) and 20 regularly spaced oligonucleotides in *rpoC* (10 on each  
3 strand) were used for sequencing of the entire genes.

4 *Measurement of plating efficiency.* Overnight cultures grown in MM (OD<sub>650</sub> 1 to 2) were  
5 diluted and plated on MM or LB plates, incubated at 37°C, 30°C or 25°. LB plates at 37°C  
6 were counted after 24 and 48 hours of incubation, MM plates at 37°C and LB plates at 30°C  
7 after 48 hours and 3 days of incubation, and MM plates at 30°C and LB plates at 25°C after 3  
8 and 4 days incubation.

9 *Measurement of UV sensitivity and of β-galactosidase activity.*

10 Survival to UV irradiation was performed as described (Baharoglu *et al.*, 2006). β-  
11 galactosidase assays for the measure of P<sub>*rmb*</sub> activity were performed as described (Miller,  
12 1992).

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## 17 **Legends to Figures**

18 Figure 1. Two of the five *rpo*<sup>sup</sup> RNA Pol mutations exhibit a stringent-like phenotype. β-  
19 galactosidase assays were performed on strains carrying a P<sub>*rmb*</sub>-*lacZ* fusion and an *rpo*<sup>sup</sup>  
20 mutation. The height of the histograms indicates β-galactosidase Miller Units, vertical bars  
21 indicate standard deviations. Wt stands for wild-type, Δ215-220 is the control *rpoC*<sup>Δ215-220</sup>  
22 mutation. P451L and H113R are *rpoC* mutations, D444G, H447R and N518D are *rpoB*  
23 mutations. Light grey: cells grown in MM; dark grey: cells grown in LB.

24

25 Figure 2.A - Four *rpo*<sup>sup</sup> RNA Pol mutations improve growth of UV irradiated *ruvABC*  
26 mutants. Survival of UV irradiated cells, results are the average of 3 to 6 independent

1 determinations. Wild-type cells (JJC40) diamonds full line, *ruvABC* (JJC754) diamond  
 2 dashed line, *rpoC*<sup>Δ215-220</sup> *ruvABC* (JJC4886) crosses, *rpoC*<sup>P451L</sup> *ruvABC* (JJC4548) open  
 3 square, *rpoB*<sup>H447R</sup> *ruvABC* (JJC4832) closed circle, *rpoB*<sup>D444G</sup> *ruvABC* (JJC4549) open circles,  
 4 *rpoB*<sup>N518D</sup> *ruvABC* (JJC4547) triangles. B – The *rpoC*<sup>H113R</sup> mutation restores the viability of  
 5 the *ΔgreA::Cm<sup>R</sup> ΔgreB::Kan<sup>R</sup>* double mutant at 42°C. *ΔgreA::Cm<sup>R</sup> ΔgreB::Kan<sup>R</sup> rpo<sup>sup</sup>* strains  
 6 were streaked on LB Cm plates at 30°C (left) and at 42°C (right). Top sector, *greA greB*  
 7 *rpoC*<sup>H113R</sup> (JJC4923) the only thermoresistant *greA greB* double mutant. Turning in the  
 8 clockwise direction from this mutant: *greA greB rpoC*<sup>P451L</sup> (JJC5456), *greA greB Rpo wt*  
 9 (JJC5455), *greA greB rpoB*<sup>N518D</sup> (JJC4818), *greA greB rpoB*<sup>D444G</sup> (JJC4820), *greA greB*  
 10 *rpoB*<sup>H447R</sup> (JJC4821).

11  
 12 Figure 3. A. *rpo<sup>sup</sup>* RNA Pol mutations restore growth of *rep uvrD* cells. The height of the  
 13 histograms indicates the number of colony forming units (cfu)/ml, vertical bars indicate  
 14 standard deviations. Rpo wild-type *rep uvrD* cells are not shown because they are lethal in all  
 15 these conditions (plating on MM with casamino acids or on LB, at 37°C or 30°C). Mutants  
 16 are in the AB1157 context, similar results were previously published for the *rep uvrD*  
 17 *rpoC*<sup>Δ215-220</sup> mutant at 37°C in the MG1655 context (Boubakri *et al.*, 2010). Δ215-220 and  
 18 H113R are *rpoC* mutations, D444G, H447R and N518D are *rpoB* mutations. Light blue: MM  
 19 30°C (plating efficiencies on MM 37°C are not shown and were similar to those at 30°C);  
 20 purple: LB 25°C; yellow: LB 30°C; orange: LB 37°C. Full boxes: colonies formed in 24  
 21 hours (37°C LB), 48 hours (30°C LB), or 3 days (30°C MM, 25°C LB). Hatched boxes:  
 22 colonies appearing 24 hours later than these normal times. B. Three *rpo<sup>sup</sup>* RNA Pol mutations  
 23 restore growth of *rep uvrD dinG recF* cells at all temperatures. Rpo wild-type and *rpoC*<sup>P451L</sup>  
 24 cells are not shown because they are lethal under these conditions (plating on MM or on LB,  
 25 at 37°C or 30°C). Mutants are in the MG1655 context, similar results were obtained in the

1 AB1157 context (not shown). Results for the *rep uvrD dinG recF rpoC*<sup>Δ215-220</sup> mutant at 37°C  
2 were previously published (Boubakri *et al.*, 2010), and were reproduced here as a control.  
3 Plating efficiencies on MM 37°C are not shown and were similar to those at 30°C.

4  
5 Figure 4. A - Three *rpo*<sup>sup</sup> RNA Pol mutations restore growth of InvA *dinG* cells at all  
6 temperatures. The height of the histograms indicates the number of cfu/ml, vertical bars  
7 indicate standard deviations. Results for the InvA *dinG rpoC*<sup>Δ215-220</sup> mutant at 37°C were  
8 previously published (Boubakri *et al.*, 2010), and were reproduced here as a control. Δ215-  
9 220, P451L and H113R are *rpoC* mutations, D444G, H447R and N518D are *rpoB* mutations  
10 (MG1655 context). Symbols are as in Fig. 3, dark blue are plating efficiencies on MM at  
11 37°C. B – Three *rpo*<sup>sup</sup> mutations restores growth of InvA *dinG rep* cells at 37°C on MM,  
12 only *rpoB*<sup>D444G</sup> also restores viability on LB. Results for the InvA *rep dinG rpoC*<sup>Δ215-220</sup>  
13 mutant were previously published (Boubakri *et al.*, 2010), and were reproduced here as a  
14 control.

15  
16 Figure 5. Schematic representation of the RpoB and RpoC subunits of RNA Pol showing the  
17 position of the *rpo*<sup>sup</sup> mutations. In orange RpoB, in green RpoC. Blue and red lines represent  
18 the template and non-template DNA-strands respectively, the pink line represents the neo-  
19 synthesized RNA (the putative backtracked RNA is shown in dashed pink line). Positions of  
20 the *rpo*<sup>sup</sup> mutations are indicated (adapted with permission from Nudler, 2009).

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Table 1: *rpoB<sup>sup</sup>* and *rpoC<sup>sup</sup>* mutations suppress the LB-cryo-sensitivity of *rep uvrD recF*, *rep uvrD recO* and *rep uvrD recQ*..

Strain	Relevant genotype	37°C		30°C	25°C
		MM casa	LB	LB	LB
JJC4048	<i>rep uvrD recF</i>	$1.3 \cdot 10^9 \pm 3.7 \cdot 10^8$	$7.3 \cdot 10^8 \pm 5 \cdot 10^8$	$8 \cdot 10^8 \pm 2.2 \cdot 10^8$	$<10^7$
JJC4038 / JJC4162	<i>rep uvrD recF rpoC<sup>P451L</sup></i>	$1.1 \cdot 10^9 \pm 2.5 \cdot 10^8$	$2.2 \cdot 10^9 \pm 2 \cdot 10^9$	$9.8 \cdot 10^8 \pm 8.1 \cdot 10^8$	$6.4 \cdot 10^8 \pm 1.1 \cdot 10^8$
JJC4039 / JJC4170 JJC4053 / JJC4340	<i>rep uvrD recF rpoC<sup>H113R</sup></i>	$1.0 \cdot 10^9 \pm 1.7 \cdot 10^8$	$1.1 \cdot 10^9 \pm 3.9 \cdot 10^8$	$9.3 \cdot 10^8 \pm 4 \cdot 10^8$	$7.7 \cdot 10^8 \pm 3.6 \cdot 10^8$
JJC4040 / JJC4163	<i>rep uvrD recF rpoB<sup>H447R</sup></i>	$8.1 \cdot 10^8 \pm 1.2 \cdot 10^8$	$8.7 \cdot 10^8 \pm 4.5 \cdot 10^8$	$1.1 \cdot 10^9 \pm 5.6 \cdot 10^8$	$1.1 \cdot 10^9 \pm 6.2 \cdot 10^8$
JJC4041 / JJC4164	<i>rep uvrD recF rpoB<sup>D444G</sup></i>	$8.3 \cdot 10^8 \pm 2.1 \cdot 10^8$	$8.5 \cdot 10^8 \pm 3.1 \cdot 10^8$	$8.2 \cdot 10^8 \pm 6.7 \cdot 10^8$	$8.8 \cdot 10^8 \pm 7.6 \cdot 10^8$
JJC4186 / JJC4200	<i>rep uvrD recF rpoB<sup>N518D</sup></i>	$6.2 \cdot 10^8 \pm 1 \cdot 10^8$	$8.7 \cdot 10^8 \pm 2.3 \cdot 10^8$	$7.3 \cdot 10^8 \pm 2.5 \cdot 10^8$	$6.3 \cdot 10^8 \pm 2.1 \cdot 10^8$
JJC1706-S / JJC2488-S	<i>rep uvrD recO</i>	$1.1 \cdot 10^9 \pm 7.9 \cdot 10^8$	$6.7 \cdot 10^8 \pm 2.6 \cdot 10^8$	$5.4 \cdot 10^8 \pm 1.1 \cdot 10^8$	$<10^7$
JJC4283	<i>rep uvrD recO rpoC<sup>P451L</sup></i>	$9.8 \cdot 10^8 \pm 2.2 \cdot 10^8$	$8.6 \cdot 10^8 \pm 1.9 \cdot 10^8$	$5.3 \cdot 10^8 \pm 5.2 \cdot 10^8$	$6.1 \cdot 10^8 \pm 1.7 \cdot 10^8$
JJC4344	<i>rep uvrD recO rpoC<sup>H113R</sup></i>	$1.3 \cdot 10^9 \pm 5 \cdot 10^8$	$1.2 \cdot 10^9 \pm 2.9 \cdot 10^8$	$1.4 \cdot 10^9 \pm 1.1 \cdot 10^8$	$1.6 \cdot 10^9 \pm 6.6 \cdot 10^8$
JJC4342	<i>rep uvrD recO rpoB<sup>H447R</sup></i>	$7.7 \cdot 10^8 \pm 2.1 \cdot 10^8$	$7.3 \cdot 10^8 \pm 2.5 \cdot 10^8$	$5 \cdot 10^8 \pm 2 \cdot 10^8$	$5.1 \cdot 10^8 \pm 1.9 \cdot 10^8$
JJC4324	<i>rep uvrD recO rpoB<sup>D444G</sup></i>	$1.2 \cdot 10^9 \pm 8.3 \cdot 10^8$	$1.3 \cdot 10^9 \pm 9.8 \cdot 10^8$	$1.3 \cdot 10^9 \pm 1 \cdot 10^9$	$1.3 \cdot 10^9 \pm 1.1 \cdot 10^9$
JJC4257	<i>rep uvrD recO rpoB<sup>N518D</sup></i>	$9.1 \cdot 10^8 \pm 3.4 \cdot 10^8$	$6.8 \cdot 10^8 \pm 1.3 \cdot 10^8$	$6.6 \cdot 10^8 \pm 1.8 \cdot 10^8$	$5.6 \cdot 10^8 \pm 2.5 \cdot 10^8$
JJC5261	<i>rep uvrD recO rpoC<sup>A215-220</sup></i>	$3 \cdot 10^8 \pm 1 \cdot 10^8$	$7.5 \cdot 10^8 \pm 8.7 \cdot 10^7$	$6.1 \cdot 10^8 \pm 1.1 \cdot 10^7$	$6.8 \cdot 10^8 \pm 2.9 \cdot 10^7$
JJC3122-S	<i>rep uvrD recQ</i>	$5.8 \cdot 10^8 \pm 1.3 \cdot 10^8$	$3.2 \cdot 10^7 \pm 2.3 \cdot 10^7$	$3.1 \cdot 10^6 \pm 3.3 \cdot 10^6$	$<10^7$
JJC4902 / JJC5313	<i>rep uvrD recQ rpoC<sup>P451L</sup></i>	$1.1 \cdot 10^9 \pm 2.5 \cdot 10^8$	$8.2 \cdot 10^8 \pm 3.9 \cdot 10^8$	$6.6 \cdot 10^9 \pm 4.6 \cdot 10^8$	$9.6 \cdot 10^8 \pm 4.3 \cdot 10^8$
JJC4906	<i>rep uvrD recQ rpoC<sup>H113R</sup></i>	$1 \cdot 10^9 \pm 4.2 \cdot 10^8$	$1.1 \cdot 10^9 \pm 2.1 \cdot 10^8$	$1.2 \cdot 10^9 \pm 1.3 \cdot 10^8$	$1.1 \cdot 10^9 \pm 2.2 \cdot 10^8$
JJC4904	<i>rep uvrD recQ rpoB<sup>H447R</sup></i>	$8.9 \cdot 10^8 \pm 3.4 \cdot 10^8$	$8.6 \cdot 10^8 \pm 2.5 \cdot 10^8$	$7.6 \cdot 10^8 \pm 1.7 \cdot 10^8$	$8.3 \cdot 10^8 \pm 1.9 \cdot 10^8$
JJC4903	<i>rep uvrD recQ rpoB<sup>D444G</sup></i>	$6 \cdot 10^8 \pm 1.4 \cdot 10^8$	$7.5 \cdot 10^8 \pm 1.9 \cdot 10^8$	$7.2 \cdot 10^8 \pm 1.4 \cdot 10^8$	$6.8 \cdot 10^8 \pm 2.2 \cdot 10^8$

Strain	Relevant genotype	37°C		30°C	25°C
		MM casa	LB	LB	LB
JJC4901 / JJC5309	<i>rep uvrD recQ rpoB</i> <sup>N518D</sup>	$7.2 \cdot 10^8 \pm 1.5 \cdot 10^8$	$7.1 \cdot 10^8 \pm 1.8 \cdot 10^8$	<i><math>5.1 \cdot 10^8 \pm 1.8 \cdot 10^8</math></i>	$4.2 \cdot 10^6 \pm 3.9 \cdot 10^6$
JJC4905	<i>rep uvrD recQ rpoC</i> <sup>Δ215-220</sup>	$7.3 \cdot 10^8 \pm 5.3 \cdot 10^7$	$7.8 \cdot 10^8 \pm 2.6 \cdot 10^7$	$8.5 \cdot 10^8 \pm 6.6 \cdot 10^7$	$9.8 \cdot 10^8 \pm 2.3 \cdot 10^8$
JJC5136 <sup>a</sup> / JJC5166 <sup>a</sup>	<i>rep uvrD recF</i>	$6.5 \cdot 10^8 \pm 1.1 \cdot 10^8$	$2.8 \cdot 10^8 \pm 1.7 \cdot 10^8$	<i><math>1.7 \cdot 10^7 \pm 5.8 \cdot 10^6</math></i>	$<10^7$
JJC5152 <sup>a</sup> / JJC5153 <sup>a</sup>	<i>rep uvrD recF rpoC</i> <sup>Δ215-220</sup>	$6.5 \cdot 10^8 \pm 1.9 \cdot 10^8$	$6.3 \cdot 10^8 \pm 1.5 \cdot 10^8$	$6.6 \cdot 10^8 \pm 1.8 \cdot 10^8$	$5.1 \cdot 10^8 \pm 8.4 \cdot 10^7$
JJC2451 [pEM001]-S	<i>rep uvrD recF</i> [pEM001]	$4.2 \cdot 10^8 \pm 9 \cdot 10^7$	$2.2 \cdot 10^8 \pm 1 \cdot 10^8$	$<10^7$	$<10^7$

Colonies were counted after 24 hours incubation (LB 37°C), 48 hours incubation (MM 37°C and LB 30°C), or 3 days incubation (MM 30°C and LB 25°C). Numbers in italics indicate the formation of small colonies appearing 24 hours later than wt.

JJCn-S indicates that the strain JJCn was used after segregation of the Rep encoding plasmid. A fresh plasmid-less colony was used for each experiment and cured clones were not kept.

a = context MG1655. All other strains are in an AB1157 context.

pEM001 is a plasmid that over-expresses RNase H.

Table 2. Summary of the phenotypes conferred by the different *rpoB/C* mutations.

	P1 <i>rrnB</i> in LB	<i>relA</i> <i>spoT</i> on MM	<i>greA</i> <i>greB</i> at 42°C	<i>rep uvrD</i> RecF <sup>+</sup> (LB)	<i>rep uvrD</i> <i>dinG recF</i> (LB)	InvA <i>dinG</i> (LB)	InvA <i>dinG</i> <i>rep</i> (LB)
<i>rpoBC</i> <sup>+</sup>	high	-	-	-	-	-	-
<i>rpoC</i> <sup>H113R</sup>	high	-	+	+	+	+	- (MM +)
<i>rpoC</i> <sup>Δ215-220</sup>	low	+	-	+	+	+	+
<i>rpoB</i> <sup>D444G</sup>	low	-	-	delayed and <i>cryoS</i>	+	+	+
<i>rpoB</i> <sup>H447R</sup>	low	+	-	+	+	+	- (MM +)
<i>rpoC</i> <sup>P451L</sup>	high	-	-	+	-	-	-
<i>rpoB</i> <sup>N518D</sup>	high	-	-	delayed and <i>cryoS</i>	<i>cryoS</i>	<i>cryoS</i>	-

In addition to the phenotypes indicated here, all mutations rescue a *rep uvrD recF (recO)* mutant on LB at all temperatures, all rescue a *rep uvrD recQ* mutant on LB (except *rpoB*<sup>N518D</sup> at low temperature), all improve the UV resistance of a *ruvABC* mutant (except *rpoC*<sup>H113R</sup> which could not be reliably combined with the *ruvABC* mutation) and none rescue the lethality of a *rep uvrD dinG* triple mutant.

(a) Bartlett et al, 1998

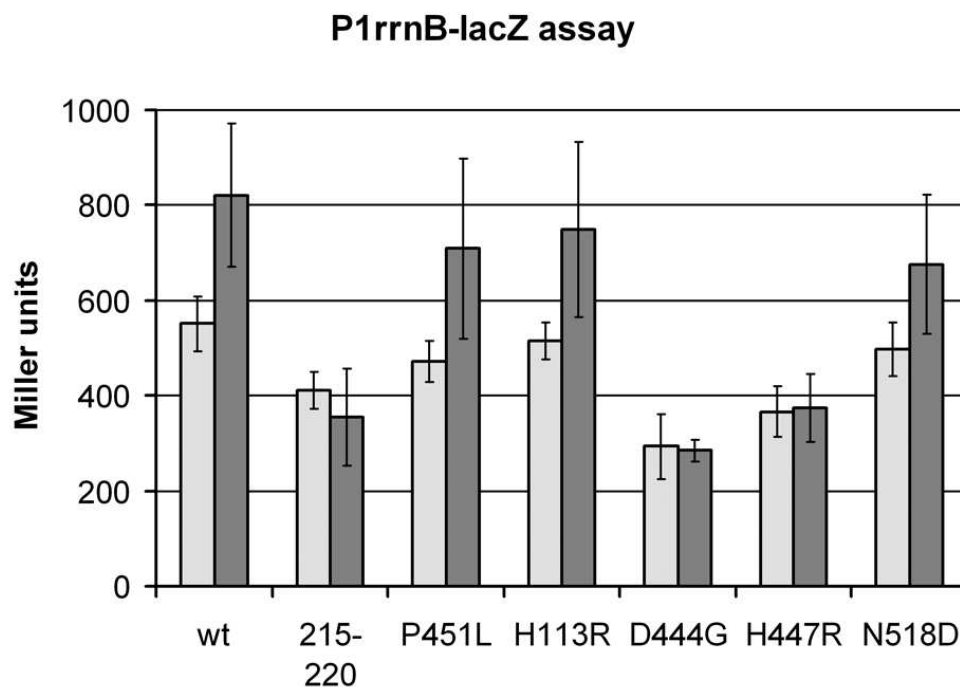


Figure 1. Two of the five *rposup* RNA Pol mutations exhibit a stringent-like phenotype.  $\beta$ -galactosidase assays were performed on strains carrying a P1rrnB-lacZ fusion and an *rposup* mutation. The height of the histograms indicates  $\beta$ -galactosidase Miller Units, vertical bars indicate standard deviations. Wt stands for wild-type,  $\Delta$ 215-220 is the control *rpoC* $\Delta$ 215-220 mutation. P451L and H113R are *rpoC* mutations, D444G, H447R and N518D are *rpoB* mutations. Light grey: cells grown in MM; dark grey: cells grown in LB.  
80x59mm (300 x 300 DPI)

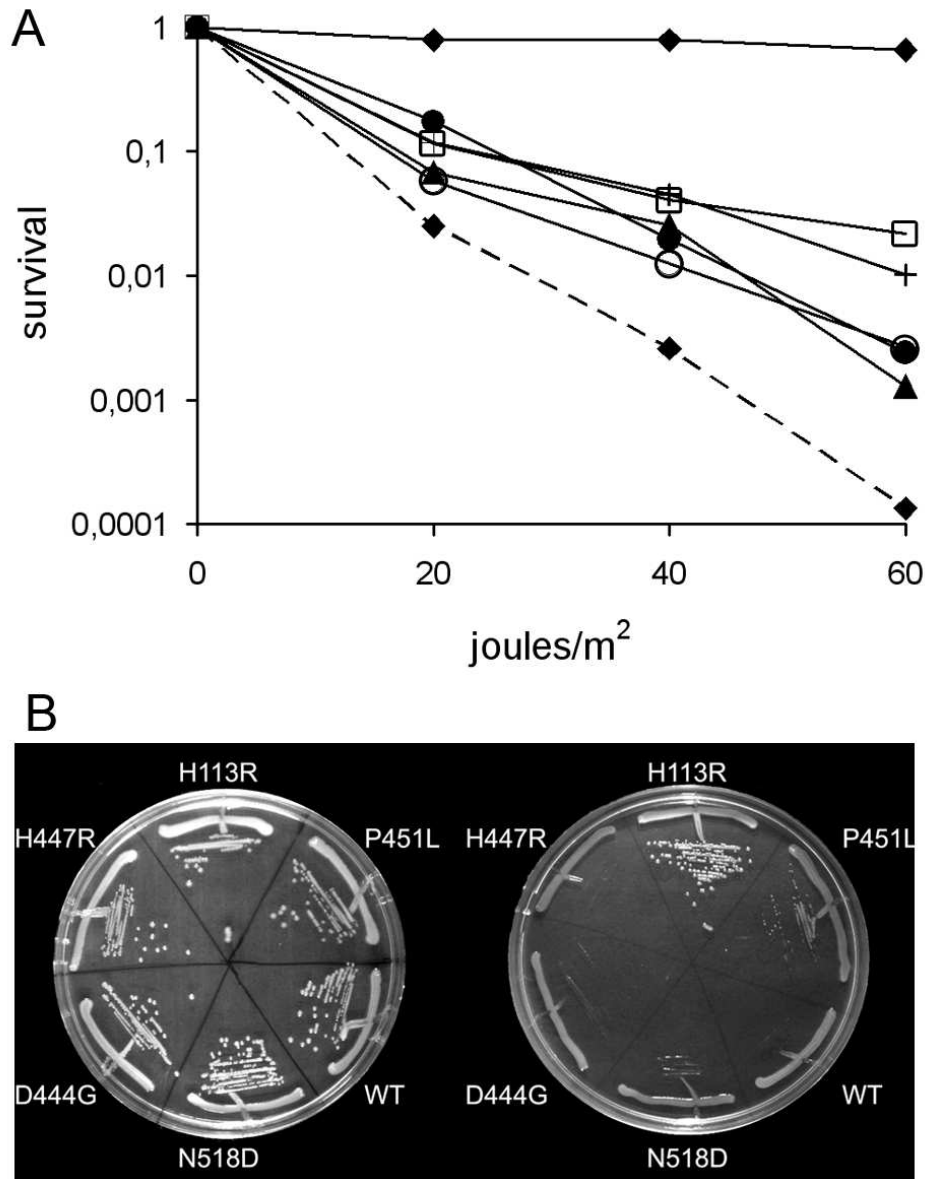


Figure 2.A - Four rposup RNA Pol mutations improve growth of UV irradiated *ruvABC* mutants. Survival of UV irradiated cells, results are the average of 3 to 6 independent determinations. Wild-type cells (JJC40) diamonds full line, *ruvABC* (JJC754) diamond dashed line, *rpoCA* $\Delta$ 215-220 *ruvABC* (JJC4886) crosses, *rpoCP451L ruvABC* (JJC4548) open square, *rpoBH447R ruvABC* (JJC4832) closed circle, *rpoBD444G ruvABC* (JJC4549) open circles, *rpoBN518D ruvABC* (JJC4547) triangles. B - The *rpoCH113R* mutation restores the viability of the  $\Delta$ *greA*::CmR  $\Delta$ *greB*::KanR double mutant at 42°C.  $\Delta$ *greA*::CmR  $\Delta$ *greB*::KanR rposup strains were streaked on LB Cm plates at 30°C (left) and at 42°C (right). Top sector, *greA greB rpoCH113R* (JJC4923) the only thermoresistant *greA greB* double mutant. Turning in the clockwise direction from this mutant: *greA greB rpoCP451L* (JJC5456), *greA greB Rpo wt* (JJC5455), *greA greB rpoBN518D* (JJC4818), *greA greB rpoBD444G* (JJC4820), *greA greB rpoBH447R* (JJC4821).  
80x99mm (300 x 300 DPI)



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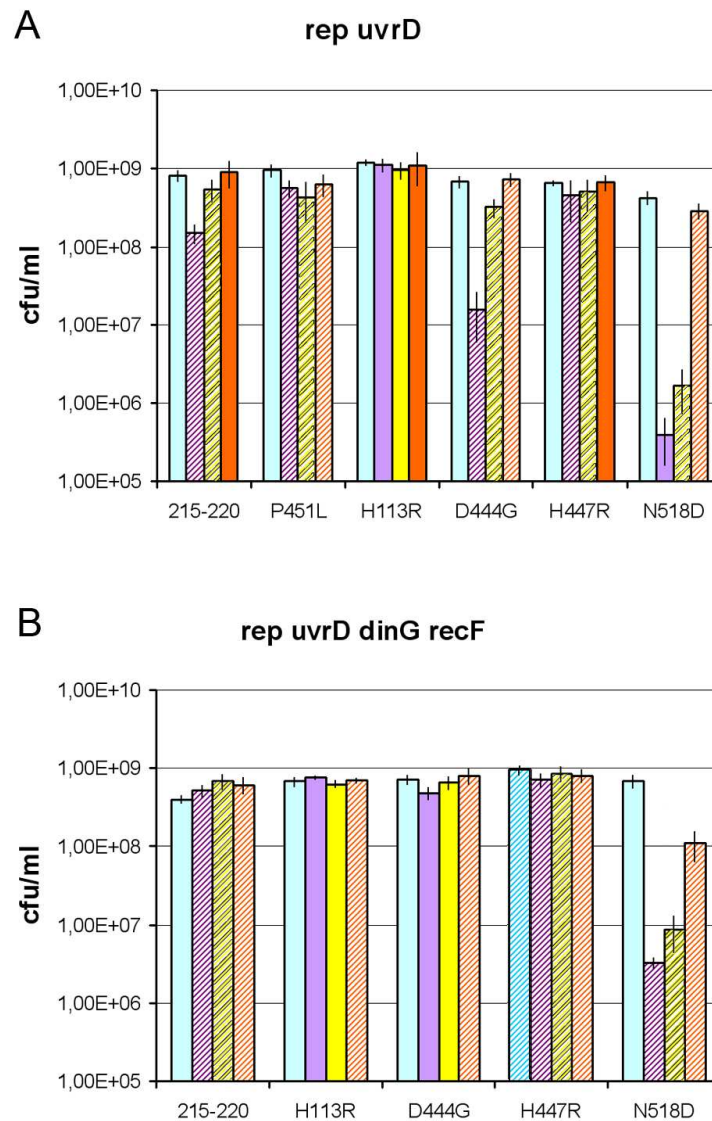


Figure 3. A. *rposup* RNA Pol mutations restore growth of *rep uvrD* cells. The height of the histograms indicates the number of colony forming units (cfu)/ml, vertical bars indicate standard deviations. *Rpo* wild-type *rep uvrD* cells are not shown because they are lethal in all these conditions (plating on MM with casamino acids or on LB, at 37°C or 30°C). Mutants are in the AB1157 context, similar results were previously published for the *rep uvrD rpoC* $\Delta$ 215-220 mutant at 37°C in the MG1655 context (Boubakri et al., 2010).  $\Delta$ 215-220 and H113R are *rpoC* mutations, D444G, H447R and N518D are *rpoB* mutations. Light blue: MM 30°C (plating efficiencies on MM 37°C are not shown and were similar to those at 30°C); purple: LB 25°C; yellow: LB 30°C; orange: LB 37°C. Full boxes: colonies formed in 24 hours (37°C LB), 48 hours (30°C LB), or 3 days (30°C MM, 25°C LB). Hatched boxes: colonies appearing 24 hours later than these normal times. B. Three *rposup* RNA Pol mutations restore growth of *rep uvrD dinG recF* cells at all temperatures. *Rpo* wild-type and *rpoCP451L* cells are not shown because they are lethal under these conditions (plating on

MM or on LB, at 37°C or 30°C). Mutants are in the MG1655 context, similar results were obtained in the AB1157 context (not shown). Results for the rep uvrD dinG recF rpoC $\Delta$ 215-220 mutant at 37°C were previously published (Boubakri et al., 2010), and were reproduced here as a control. Plating efficiencies on MM 37°C are not shown and were similar to those at 30°C.  
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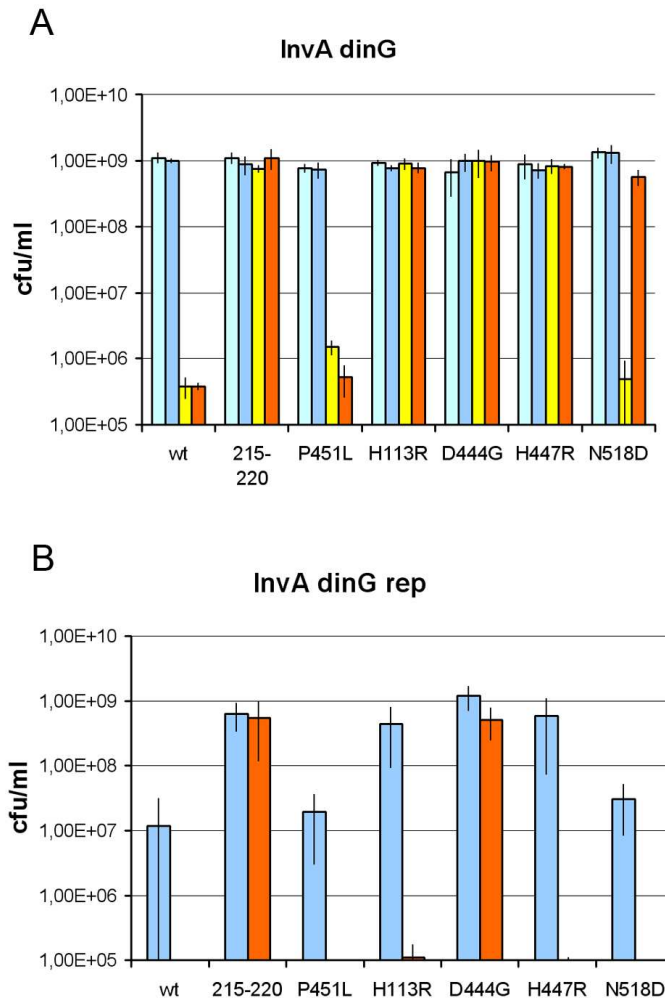


Figure 4. A - Three rposup RNA Pol mutations restore growth of InvA dinG cells at all temperatures.

The height of the histograms indicates the number of cfu/ml, vertical bars indicate standard deviations. Results for the InvA dinG rpoC $\Delta$ 215-220 mutant at 37°C were previously published (Boubakri et al., 2010), and were reproduced here as a control.  $\Delta$ 215-220, P451L and H113R are rpoC mutations, D444G, H447R and N518D are rpoB mutations (MG1655 context). Symbols are as in Fig. 3, dark blue are plating efficiencies on MM at 37°C. B - Three rposup mutations restores growth of InvA dinG rep cells at 37°C on MM, only rpoBD444G also restores viability on LB. Results for the InvA rep dinG rpoC $\Delta$ 215-220 mutant were previously published (Boubakri et al., 2010), and were reproduced here as a control.

80x140mm (300 x 300 DPI)

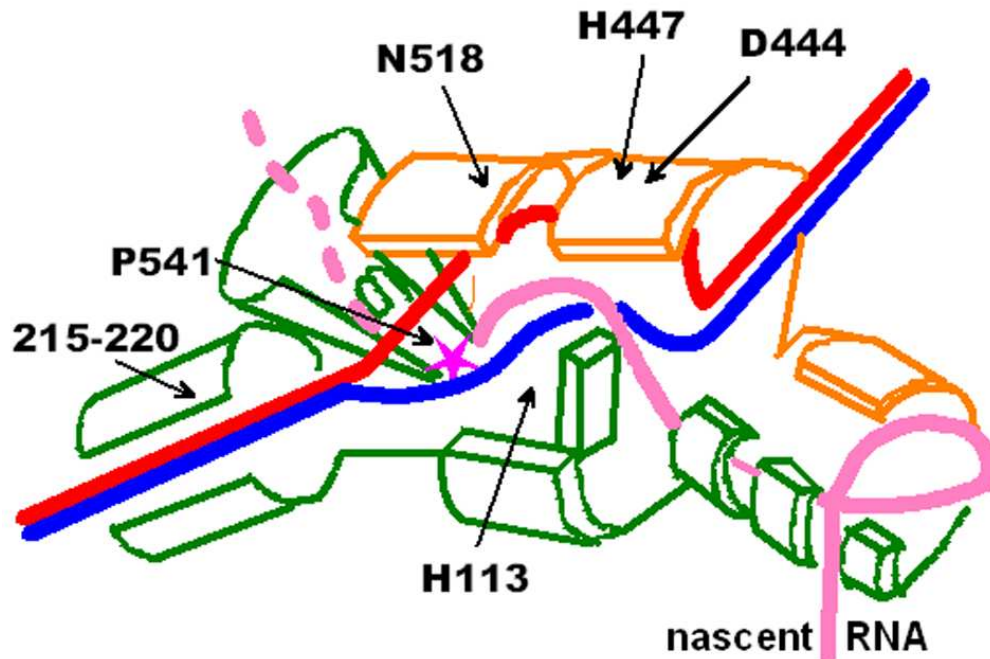


Figure 5. Schematic representation of the RpoB and RpoC subunits of RNA Pol showing the position of the rposup mutations. In orange RpoB, in green RpoC. Blue and red lines represent the template and non-template DNA-strands respectively, the pink line represents the neo-synthesized RNA (the putative backtracked RNA is shown in dashed pink line). Positions of the rposup mutations are indicated (adapted with permission from Nudler, 2009).

80x57mm (300 x 300 DPI)