UNIVERSITY of York

This is a repository copy of *The balance between recombination enzymes and accessory replicative helicases in facilitating genome duplication*.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/102766/

Version: Accepted Version

**Article:**

McGlynn, Peter orcid.org/0000-0001-8629-4713 (2016) The balance between recombination enzymes and accessory replicative helicases in facilitating genome duplication. Genes. ISSN 2073-4425

https://doi.org/10.3390/genes7080042

**Reuse**
Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

**Takedown**
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

White Rose university consortium
Universities of Leeds, Sheffield & York

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

genes

MDPI

*Article*

# The balance between recombination enzymes and accessory replicative helicases in facilitating genome duplication

**Aisha H. Syeda[1], John Atkinson[2], Robert G. Lloyd[3] and Peter McGlynn[1,4]***

[1] Department of Biology, University of York, Wentworth Way, York, YO10 5DD, United Kingdom; aisha.syeda@york.ac.uk
[2] School of Medical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, United Kingdom; j.d.atkinson@gmx.com
[2] Centre for Genetics and Genomics, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, United Kingdom; bob.lloyd@nottingham.ac.uk
* Correspondence: peter.mcglynn@york.ac.uk; Tel.: +44-1904-328688

Academic Editor: name
Received: date; Accepted: date; Published: date

**Abstract:** Accessory replicative helicases aid the primary replicative helicase in duplicating protein-bound DNA, especially transcribed DNA. Recombination enzymes also aid genome duplication by facilitating the repair of DNA lesions via strand exchange and also processing of blocked fork DNA to generate structures onto which the replisome can be reloaded. There is significant interplay between accessory helicases and recombination enzymes in both bacteria and lower eukaryotes but how these replication repair systems interact to ensure efficient genome duplication remains unclear. Here we demonstrate that the DNA content defects of *Escherichia coli* cells lacking the strand exchange protein RecA are driven primarily by conflicts between replication and transcription, as is the case in cells lacking the accessory helicase Rep. However, in contrast to Rep, neither RecA nor RecBCD, the helicase/exonuclease that loads RecA onto dsDNA ends, is important for maintaining rapid chromosome duplication. Furthermore, RecA and RecBCD together can sustain viability in the absence of accessory replicative helicases but only when transcriptional barriers to replication are suppressed by an RNA polymerase mutation. Our data indicate that the minimisation of replisome pausing by accessory helicases has a more significant impact on successful completion of chromosome duplication than recombination-directed fork repair.

**Keywords:** genome stability/repair/replication/RNA polymerase

**PACS:** J0101

---

## 1. Introduction

The replication machineries of all organisms encounter many potential barriers whilst duplicating their genomes, presenting a major challenge to the maintenance of genetic stability [1,2]. These barriers include damage to the template, non-B form DNA structures, topological strain and proteins bound to the DNA. Transcription provides both a topological challenge to DNA replication due to the over- and underwinding ahead of and behind an advancing RNA polymerase [3,4] and substantial nucleoprotein barriers to fork movement due to their very high affinity [5]. Individual nucleoprotein complexes may have a low probability of halting a replication fork but the large number of barriers encountered creates a substantial risk of failure to complete

43 high fidelity genome duplication [6,7]. Replisomes paused at these barriers retain activity but this
44 activity is lost as a function of time [8-11]. There is thus a window of opportunity for removal or
45 bypass of the barrier and resumption of replication by the paused replisome. If clearance or bypass
46 of the barrier does not occur prior to loss of paused replisome function then the replication
47 machinery must be reloaded back onto the chromosome to faclitate completion of genome
48 duplication [1]. Given the importance of completing high fidelity genome duplication, all
49 organisms have evolved mechanisms to underpin replisome movement by facilitating the restart
50 of paused replisomes and by reconstituting an active replication fork after loss of paused
51 replisome activity.

52 Upon encountering a barrier, the replisome itself can clear or bypass certain types of obstacle.
53 Forks paused at single-stranded DNA lesions may bypass the lesion by repriming replication
54 downstream of the barrier, allowing resumption of replication at the cost of a gap in one of the
55 nascent DNA strands [12-16]. However, bypass does not approach 100% efficiency, implying that
56 replisomes encountering many lesions have a significant probability of losing activity [15].
57 Specialised translesion DNA polymerases can also replicate across such lesions under certain
58 circumstances but often at the cost of errors in base incorporation [17-19]. The replisome is also
59 capable of displacing proteins bound to the template DNA [20-22], a property that reflects the
60 ability of helicases to disrupt the non-covalent bonding between proteins and DNA [23,24]. Forks
61 do also pause stochastically at protein-DNA complexes but the paused replisome may resume
62 movement if the blocking protein dissociates from the DNA prior to loss of activity of the paused
63 replisome [20]. However, the barriers posed by the many protein-DNA complexes found within a
64 chromosome, especially those associated with transcription, appear to be too numerous and/or too
65 long-lived for the replisome itself to deal with during the course of genome duplication. The *S.*
66 *cerevisiae* RRM3 helicase minimises fork blockage at non-histone protein-DNA complexes and is
67 required for normal rates of fork movement [25-28]. Similarly, the *E. coli* Rep helicase promotes
68 fork movement through nucleoprotein complexes and its absence results in at least a twofold
69 increase in the time needed to replicate a chromosome [22,29-31]. This increase in the time needed
70 for genome duplication reflects the function of Rep in minimising the frequency and/or duration of
71 replisome pausing at protein-DNA complexes, the primary sources of replication pausing in *E. coli*
72 [6]. The *B. subtilis* helicase PcrA, a homologue of *E. coli* Rep, also facilitates replication of
73 transcribed DNA *in vivo* [32] indicating conservation of this function across evolutionarily very
74 divergent organisms. Both RRM3 and Rep also associate physically with components of their
75 respective replisomes [22,28,33]. In *E. coli* the physical association between Rep and the primary
76 replicative helicase DnaB promotes cooperative DNA unwinding and nucleoprotein complex
77 removal by the two helicases [22,34,35]. However, although *B. subtilis* PcrA is essential for viability
78 [36], neither *S. cerevisiae* RRM3 nor *E. coli* Rep are needed for viability [37,38]. These enzymes are
79 now considered to be accessory replicative helicases that minimise replisome pausing along
80 protein-bound DNA whilst the primary replicative helicase is responsible for template DNA
81 unwinding and acts as a hub for replisome organisation [39,40].

82 The above mechanisms reduce the probability of loss of function of replisomes encountering
83 barriers that can be either cleared or bypassed. These mechanisms therefore rely on retention of
84 function of paused replisomes. However, the large number of barriers encountered by replisomes
85 means that there is still a significant risk of a replisome pausing at a barrier and losing function

86 prior to bypass or clearance of the barrier [1,7]. This is a particular problem with arrays of
87 transcription complexes on highly transcribed genes [30,41-45]. Blockage of a fork and loss of
88 replisome function demands reloading of the replication machinery to complete genome
89 duplication, even when multiple origins exist on the same chromosome [46]. Generation of a DNA
90 structure onto which the replication machinery can be reloaded may require substantial
91 remodelling of the fork DNA by a combination of exonucleases, endonucleases and helicases to
92 facilitate replisome reloading [2,7]. Such processing may also require strand exchange proteins
93 either to reintegrate double-stranded DNA ends generated by fork processing, to repair
94 single-stranded DNA gaps or to catalyse replication fork regression [1,47,48]. Strand exchange
95 proteins might also promote blocked fork stabilisation, inhibiting extensive degradation of nascent
96 DNA via occlusion of nucleases [49-51]. The bacterial strand exchange protein RecA minimises
97 degradation of nascent DNA in *E. coli* cells exposed to UV light [52]. This minimisation also
98 requires RecFOR, factors that promote RecA loading onto ssDNA gaps rather than dsDNA ends,
99 together with RecJ exonuclease and RecQ helicase [52,53].

100 The general view now is that a major role of recombination enzymes, if not their primary
101 purpose, is to underpin replication fork movement [54]. The importance of such enzymes is
102 illustrated by the extensive DNA degradation in *recA* mutant cells [55]. This degradation is
103 catalysed by RecBCD, a helicase and exonuclease that unwinds and degrades dsDNA ends [55,56].
104 Degradation of both DNA strands by RecBCD is rapid and processive but recognition of a specific
105 DNA sequence, a $\chi$ site, within the DNA inhibits degradation of the 3' ended strand and promotes
106 loading of RecA onto this strand [56]. However, degradation continues in the absence of RecA,
107 with RecBCD being able to degrade an entire chromosome arm [55,57]. Some blocked forks may
108 also undergo regression and extrude a dsDNA arm which may be degraded by RecBCD in the
109 absence of RecA, effectively destroying the extruded arm of the fork and regenerating a fork
110 structure onto which the replisome can be reloaded [58].

111 Targeting of blocked forks by recombination enzymes comes at the cost of genome
112 rearrangements [59,60]. This genetic instability is a particular problem at highly transcribed genes
113 due to the density of transcribing RNA polymerases and the consequent high probability of fork
114 pausing, loss of replisome function and the need to process the DNA via recombination enzymes
115 to reload the replisome [2,61-63]. Moreover, loss of factors that minimise stalled and backtracked
116 transcription complexes increase the dependence of *E. coli* cells on recombination enzymes [42].
117 The absence of accessory replicative helicases that restart paused forks also exacerbates the
118 pathological effects of replication-associated recombination [64,65]. Thus *E. coli* Rep limits harmful
119 RecA loading at blocked forks [64]. Increasing the probability of fork pausing or of paused forks
120 losing function therefore results in an increased need for recombination enzymes to underpin
121 genome duplication.

122 Such is the potentially catastrophic effect of unregulated strand exchange that organisms have
123 also evolved other means of limiting binding of strand exchange proteins to ssDNA. Turnover of
124 strand exchange protein-ssDNA filaments by helicases is a key mechanism employed in both
125 bacteria and eukaryotes to limit homologous recombination [66] with UvrD helicase performing
126 this task in *E. coli* [67].

127 Accessory helicases target paused, active replisomes whereas recombination enzymes process
128 blocked forks that no longer retain an active replisome. The substrates for these classes of enzymes

129 are therefore very different. *S. cerevisiae rrm3* mutant cells are viable but require replication, repair
130 and checkpoint genes for normal growth [68-70]. Similarly, *E. coli* cells lacking either RecBCD or
131 Rep are viable but cells lacking both are inviable [71,72]. In contrast, the viability of *E. coli recA rep*
132 mutant cells indicates that processing of inactivated forks does not necessarily require strand
133 exchange [71]. This viability reflects the ability of RecBCD to degrade partly replicated
134 chromosomes when RecA is absent [55,58]. Indeed, RecBCD but not RecA is essential for viability
135 in the presence of an inverted and highly expressed ribosomal operon [73]. It should be borne in
136 mind, though, that RecBCD activity in the presence of RecA results in loading of RecA onto the
137 single-stranded DNA generated by RecBCD, strand exchange and priming of DNA replication via
138 a D-loop recombination intermediate [7,56,74].

139 *E. coli* Δ*rep* mutant cells are viable in part because a homologous helicase, UvrD, can also
140 promote fork movement along protein-bound DNA and thus compensate partially for the absence
141 of Rep [22,30]. Single deletion mutants are therefore viable whereas Δ*rep* Δ*uvrD* mutants are not
142 [75]. The lack of full compensation may be because UvrD, unlike Rep, does not interact with the
143 replisome via DnaB [22]. DinG helicase has also been implicated in resolving conflicts between
144 replication and transcription in concert with Rep and/or UvrD [30]. However, the mechanistic
145 basis of this interplay remains unclear with no direct evidence that DinG displaces proteins ahead
146 of advancing replication forks [40]. It is clear, though, that Δ*rep* mutant cells but neither Δ*uvrD* nor
147 Δ*dinG* mutants exhibit a significant extension of the time needed to replicate the chromosome
148 [29,31]. This Rep-specific defect indicates that Rep rather than UvrD or DinG plays a key role in
149 maintaining rapid fork movement.

150 Δ*rep* Δ*uvrD* double mutant inviability can be suppressed by growth of Δ*rep* Δ*uvrD* mutant
151 cells on minimal medium, conditions under which levels of transcription are reduced as compared
152 with rich medium growth [22,30]. Δ*rep* Δ*uvrD* inviability on rich medium is also partially
153 suppressed by two classes of mutation. One class of mutants harbour mutations in *spoT* which
154 leads to elevated concentrations of the signalling molecule (p)ppGpp [22]. (p)ppGpp binds to RNA
155 polymerase and inhibits initiation of transcription of many genes including the *rrn* operons in *E.*
156 *coli*, the source of half of all transcription under rapid growth conditions, and also destabilises
157 stalled transcription complexes [42,76]. These effects may reduce the number of replicative barriers
158 presented by transcription. Elevated (p)ppGpp also reduces replication elongation rates which
159 might result in fewer collisions between transcription and replication, although the elongation rate
160 is only modestly affected in *E. coli* [77]. The second class of mutations reside in the structural genes
161 for RNA polymerase [22,30,78]. These mutations may suppress via different mechanisms
162 depending on the nature of the mutation but may act in a similar manner to elevated (p)ppGpp
163 [42,78,79] and/or reduce the extent of backtracking of paused RNA polymerases [80]. For example,
164 the Δ*rep* Δ*uvrD* double mutant suppressor *rpoB*35* allows cells unable to synthesise (p)ppGpp to
165 grow on minimal medium, a so-called stringent phenotype which indicates that *rpoB*35*
166 phenocopies elevated (p)ppGpp [22,79]. *rpoB*35* may also destabilise transcription complexes
167 stalled by nucleotide starvation or DNA lesions [42] although this has been questioned and data
168 presented indicating this mutant RNA polymerase has a reduced probability of backtracking [80].

169 Another class of mutations provide weaker suppression of Δ*rep* Δ*uvrD* double mutant
170 inviability. These suppressors have defects in the RecA loading factors RecF, RecO or RecR or in
171 RecJ exonuclease or RecQ helicase, all of which facilitate RecA loading onto single-stranded DNA

gaps [22,30,81,82]. This suppression may reflect the potential for toxic levels of RecFORQJ-dependent strand exchange by RecA at blocked forks [81,83,84]. In Δ*rep* Δ*uvrD* double mutant cells elevated fork pausing together with the lack of UvrD-catalysed disruption of RecA-ssDNA filaments may explain why ablation of RecFORQJ-dependent RecA loading partially suppresses Δ*rep* Δ*uvrD* double mutant inviability. However, UvrD cannot counter the adverse effects of RecAFORQJ in Δ*rep* mutant cells [64] implying that lack of RecA-ssDNA turnover is not the primary reason why RecAFORQJ is so toxic in Δ*rep* Δ*uvrD* mutant cells.

The relative importance of accessory helicases and recombination enzymes for genome duplication remains unclear. *E. coli* cells lacking RecA or RecBCD have reduced viability [85]. Cells bearing inverted *rrn* operons do not require Rep for viability but do require either RecBCD helicase/exonuclease or RecBCD helicase lacking exonuclease activity plus RecA [30,73]. These data argue that RecBCD and RecA have a more important role in replicating the chromosome than Rep. However, during normal growth without inverted highly expressed operons there is insufficient recombination to require Holliday junction resolution for viability [86]. Only in the absence of Rep does this resolution become important for viability [64], consistent with Rep having a primary role in sustaining completion of chromosome replication.

Here we show that the known chromosome content defects of *recA* cells is driven primarily by transcription, mirroring the importance of transcriptional barriers to replication in the chromosome content defects of Δ*rep* mutant cells [6]. Both RecA and Rep therefore have roles in mitigating the impact of transcription on genome duplication. However, in contrast to Rep [31], neither RecA nor RecBCD play important roles in sustaining wild type chromosome duplication times. These data indicate that accessory helicases play a more significant role than recombination enzymes in sustaining rapid chromosome duplication. This view is supported by RecA and RecBCD being able to sustain viability in the absence of Rep and UvrD but only in the presence of an RNA polymerase mutation that alleviates transcriptional barriers to replication. Furthermore, both RecA and RecBCD are needed for this viability, indicating that RecBCD-catalysed DNA degradation in the absence of RecA loading does not provide an efficient means of sustaining chromosome duplication. We conclude that accessory helicases are more important than recombination enzymes for replicating the *E. coli* chromosome but that replicative barriers normally dealt with by accessory helicases can be surmounted by less efficient mechanisms via recombination enzymes.

## 2. Materials and Methods

### *2.1. Plasmids and strains*

Strains are listed in Supplementary Table 1 and were constructed using P1 transduction. pAM375, pAM383, pAM403 [64], pAM406 and pAM407 [22] are derivatives of pRC7 [87] and encode *recB*$^{+}$, *recA*$^{+}$, *rep*$^{+}$, *recA*$^{+}$ *recB*$^{+}$ and *uvrD*$^{+}$, respectively. pAM406 was made by cloning an ApaI fragment carrying *recA*$^{+}$ from pAM383 [64] into the ApaI site of pAM375 [64]. N6618 is a derivative of MG1655 carrying a deletion of *recA* in which all but 42 bp at both the 5′ and 3′ end of the gene sequence has been replaced with a sequence encoding resistance to kanamycin. It was made using the protocols described [88].

### *2.2. Flow cytometry*

Flow cytometry to analyse DNA content in Figure 1 was performed on cells grown to mid-log

215 phase in either LB or 56/2 salts minimal medium after treatment with rifampicin and cephalexin as
216 described [6]. The DNA content of stationary phase cells (Figure 2) was performed in an identical
217 manner except that cells were grown overnight prior to treatment with rifampicin and cephalexin.
218 Flow cytometric analysis of chromosome duplication time (Figure 3) was performed as described
219 [31].
220

221 *2.3. Synthetic lethality assays*

222 The ability of strains to form colonies upon loss of pRC7 derivatives was assessed as
223 described [64]. After growth in the absence of ampicillin selection for pRC7 plasmids, cell were
224 plated onto LB agar containing 120 μg/ml Xgal and 1 mM IPTG and incubated at 37°C for 48 hours.
225

226 **3. Results**

227 *3.1. Transcription is a major cause of chromosome degradation in recA cells*
228

229 One key feature of *E. coli* cells lacking the strand exchange protein RecA in otherwise
230 unperturbed cells is elevated levels of RecBCD-dependent chromosome degradation [55]. This
231 degradation is manifest as formation of cells with a range of different numbers of chromosome
232 equivalents as detected by flow cytometry [55](see also Figure 1Ai and iii).

233 Thus cells require strand exchange for normal chromosomal duplication even in the absence
234 of elevated DNA damage or engineered nucleoprotein barriers. The trigger(s) for this enhanced
235 degradation are unclear and so we tested whether this degradation is attributable to transcription.
236 We employed flow cytometry under run-out conditions to monitor DNA content in cells

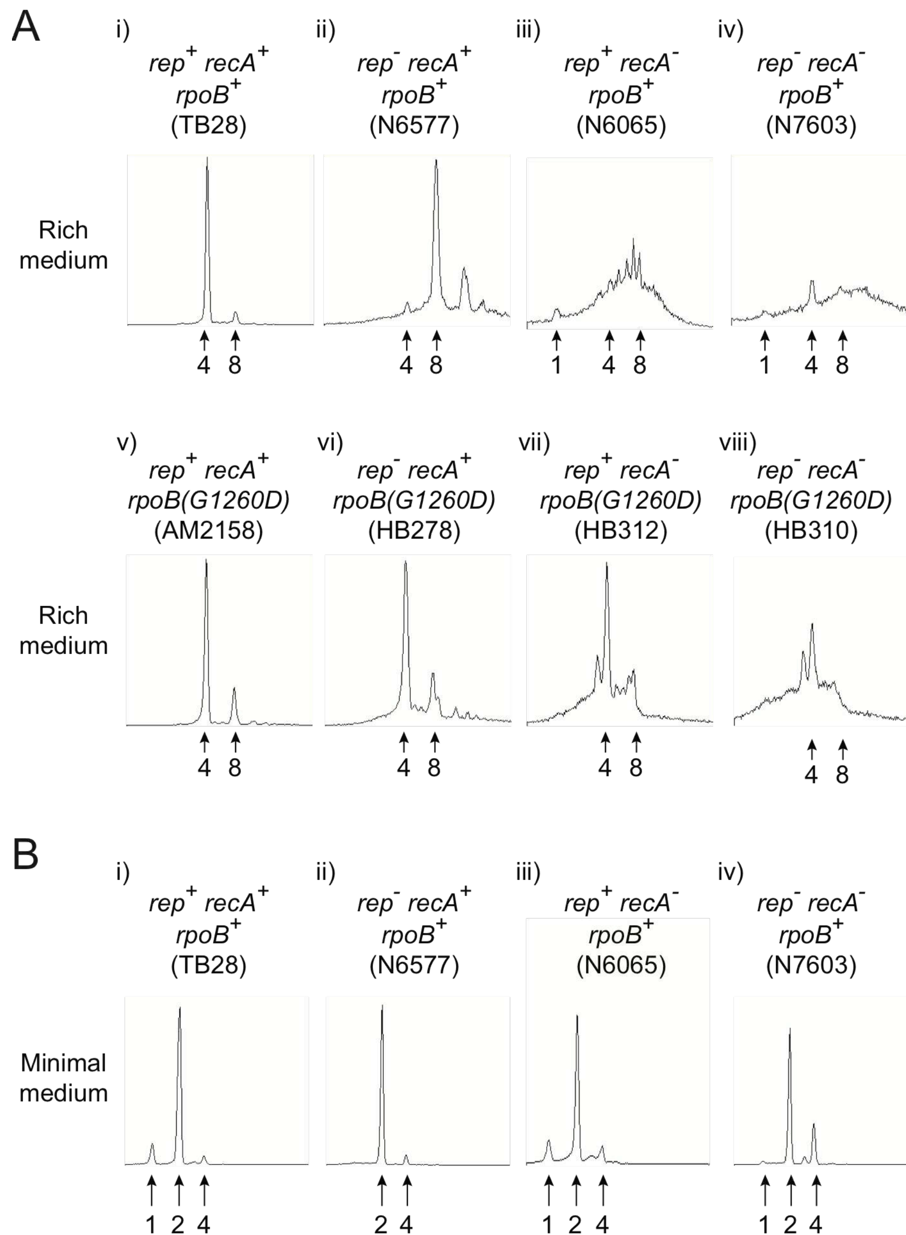



**Figure 1**. The chromosome content defects in the absence of Rep and RecA on rich medium are suppressed by an RNA polymerase mutation or by growth on minimal medium.
(A) DNA content of the indicated strains grown to mid-logarithmic phase in LB medium as monitored by flow cytometry under run out conditions. The number of chromosome equivalents is indicated below.
(B) DNA content of the strains used in Ai-iv grown to mid-logarithmic phase in minimal medium as monitored by flow cytometry under run out conditions.

237 harbouring either wild type RNA polymerase or a mutant form of the complex resulting from the
238 *rpoB(G1260D)* allele [6,89](Figure 1). *rpoB(G1260D)* displays the same phenotypes as *rpoB*35*
239 including a stringent phenotype, suppression of Δ*rep* Δ*uvrD* double mutant lethality and
240 suppression of chromosome replication defects in Δ*rep* muant cells (Traut 2002, Gupta 2013). Most
241 wild type cells contain 4 chromosome equivalents after run out during logarithmic growth in rich
242 medium in both *rpoB*+ and *rpoB(G1260D)* cells [6,55](also compare Figure 1Ai with v). In contrast,
243 Δ*rep rpoB*+ cells lacking the accessory replicative helicase Rep contain 8 chromosomes due to the
244 increased time needed to replicate the chromosome and hence more replication origin firings per
245 cell cycle [6,29,90]. *rpoB(G1260D)* suppresses this Δ*rep* mutant phenotype by reducing replisome
246 pausing, with most Δ*rep rpoB(G1260D)* cells having 4 rather than 8 chromosomes [6](see also
247 Figure 1Av-vi). We found that *rpoB(G1260D)* also substantially suppressed the broad spread of
248 chromosome equivalents seen in *recA* mutant cells (Figure 1A, compare iii with vii). We also tested
249 cells lacking both Rep and RecA. Δ*rep recA rpoB*+ cells had a more severe defect in chromosome
250 content as compared with the single mutants (Figure 1A, compare iv with ii and iii). There is
251 therefore significant synergy between Rep and RecA function in maintaining chromosome
252 duplication. However, *rpoB(G1260D)* still provided partial suppression of this severe defect (Figure
253 1A, compare iv and viii).

254 Suppression of chromosome replication defects by *rpoB(G1260D)* in cells lacking Rep, RecA or
255 both enzymes is consistent with transcription being the primary driver of these defects.
256 Replication-transcription conflicts can also be alleviated by growth of *rpoB*+ strains in minimal
257 medium [22,30]. We tested therefore whether the major differences in DNA content in wild type
258 versus Δ*rep, recA* or Δ*rep recA* mutant cells seen in mid-logarithmic cells grown in rich medium
259 were recapitulated in minimal medium. We found that the majority of *rpoB*+ cells either with or
260 without Δ*rep* and/or *recA* mutant alleles contained 2 chromosome equivalents when grown to
261 mid-logarithmic phase in minimal medium (Figure 1Bi-iv). Restricting growth rate reduces
262 therefore the chromosomal defects caused by the absence of Rep and/or RecA (compare Figure
263 1Ai-iv with 1Bi-iv) supporting our conclusion that transcription is a major cause of the perturbed
264 chromosome content observed in the absence of Rep and/or RecA.

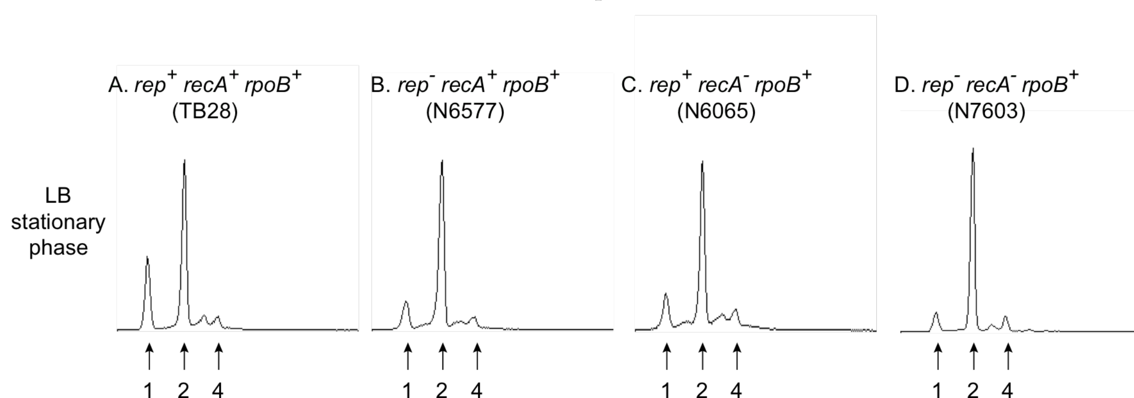



**Figure 2**. The chromosome content defects of *rep* and *recA* mutant cells at mid-logarithmic phase in rich medium are resolved by the time stationary phase is reached. Strains A-D are the same as those used in Figure 1Ai-iv.

265 We also investigated the ability of Δ*rep recA* mutant cells to remain viable even when so few

266 of the cells contain an integral number of chromosomes under run out conditions during
267 logarithmic growth in LB (Figure 1Aiv). Flow cytometric analyses of *rpoB*+ strains grown to
268 stationary phase in LB revealed that the absence of functional Rep and/or RecA had little impact
269 on chromosome content with the majority of cells in all cases containing two chromosomes (Figure
270 2A-D). Thus even cells lacking both Rep and RecA can eventually complete chromosome
271 duplication to allow formation of viable progeny. Any barriers to completion of chromosome
272 duplication in the absence of Rep and RecA must eventually be cleared therefore and must not
273 generate replication intermediates that cannot be resolved (compare Figure 2A and D). There is
274 much evidence that RecBCD helicase/exonuclease provides such a mechanism to degrade blocked
275 replication intermediates when RecA is not available to initiate strand exchange from
276 RecBCD-generated ssDNA [58,71,72]. However, the inviability of *rep recB* double mutant cells [71]
277 precludes direct analysis of absence of both Rep and RecBCD on chromosome content by flow
278 cytometry.
279
280 *3.2. Rapid chromosome duplication has a greater requirement for Rep than for RecA*
281
282 The above data do not address the relative importance of Rep and recombination enzymes in
283 underpinning efficient fork movement. The time taken to replicate chromosomes during a single
284 cell cycle was therefore estimated using flow cytometry in strains lacking either Rep or RecA.

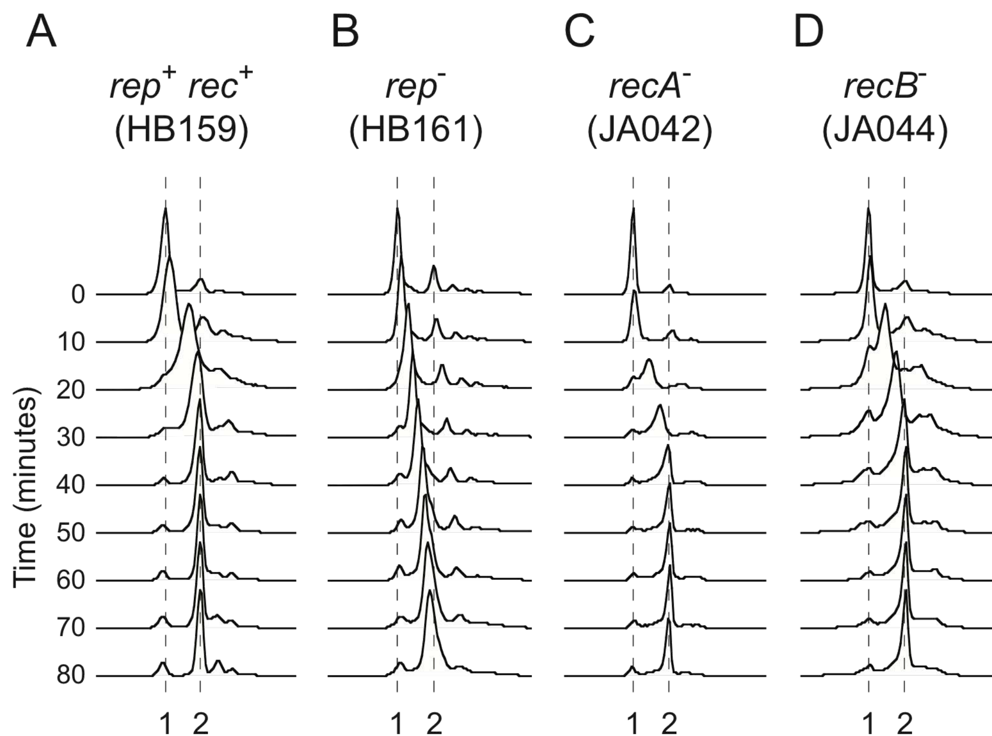



**Figure 3.** Chromosome duplication time is extended in *rep* but not *recA* or *recB* cells. (A-D) Flow cytometry profiles of the indicated strains in which initiation of chromosome duplication was synchronised at 42°C by exploiting the presence of the temperature-sensitive *dnaA46* allele. Samples were analysed immediately after shifting the temperature from 42°C to 30°C (time 0). Cultures were then returned to 42°C after 10 minutes. Samples were removed every 10 minutes after the temperature downshift. The number of chromosome equivalents is indicated below.

Upon synchronising replication initiation using the temperature-sensitive *dnaA46* allele, wild type cells take 40-50 minutes for their DNA content to increase from 1 to 2 chromosome equivalents but Δ*rep* cells take more than 80 minutes [31](see also Figure 3A and B). This extended duplication time reflects the impact of nucleoprotein complexes on fork progression in the absence of Rep [22,30]. In contrast to Δ*rep* mutant cells, we found that the majority of *recA* mutant cells had completed genome duplication after 40-50 minutes (Figure 3C). We also tested the time taken for chromosome duplication in *recB* mutant cells. The requirement for either Rep or RecBCD for survival implies that one or the other of these enzymes provides an essential means of underpinning fork progression [71,72]. However, *recB* mutant cells had chromosome duplication times similar to those found in wild type and *recA* mutant cells (Figure 3D).

These data demonstrate that absence of either RecA or RecBCD does not lead to significant slowing of the mean time taken for replication forks to travel from *oriC* to the terminus region. Processing of blocked replication forks by either RecA or RecBCD is therefore not critical for rapid chromosome duplication.

### *3.3 Both RecA and RecBCD are needed in the absence of accessory helicase activity*

The above data suggest Rep rather than RecA plays the dominant role in ensuring rapid genome duplication. It is clear, though, that transcription is a shared source of replicative defects in cells deficient in either Rep or RecA (Figure 1). However, the viability of *rep recA* double mutant cells [71] argues against a requirement for either Rep or RecA to overcome transcriptional barriers to replication. Interpretation of *rep recA* double mutant viability is complicated, though, since UvrD compensates partially for the absence of Rep accessory helicase function [22,30]. The requirement for RecA was tested therefore in the absence of both Rep and UvrD by using a Δ*rep* Δ*uvrD* double mutant strain rendered viable by the *rpoB*35* allele via a reduction in replication-transcription conflicts [22,42,79]. A plasmid loss assay was employed in which retention of a highly unstable complementing plasmid can be monitored by blue/white screening [87]. Δ*rep* Δ*uvrD rpoB*35* cells can lose pRC7*uvrD* on LB as indicated by the formation of white

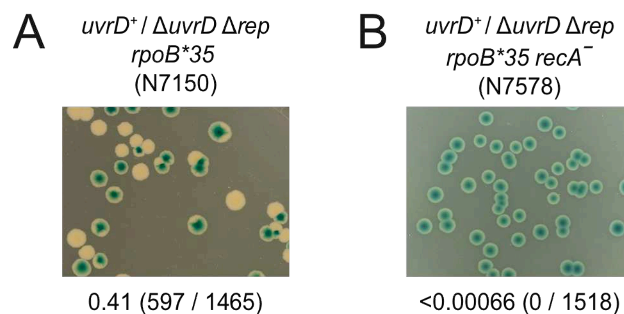



**Figure 4.** RecA is essential in the absence of Rep and UvrD on rich medium.
(A, B) The ability to form colonies in the absence of RecA was monitored in the indicated strains on LB plates containing Xgal and IPTG. The parental strains contain pAM407 (pRC7*uvrD*) bearing both the *uvrD* gene and the *lac* operon and plasmidless cells give rise to white or segregated colonies due to loss of the *lac* operon. Fractions of white colonies are indicated below each panel and the actual number of white colonies and of total colonies are shown in parentheses.

313 plasmidless colonies [22](see also Figure 4A). In contrast, Δ*rep* Δ*uvrD rpoB*35 recA* cells could not
314 lose pRC7*uvrD* on LB indicating that RecA is essential for viability in a Δ*rep* Δ*uvrD rpoB*35* strain
315 under rapid growth conditions (Figure 4, compare B with A). Thus even when transcriptional
316 barriers to replication are reduced by the *rpoB*35* allele there remains a requirement for either
317 accessory helicase function or RecA.

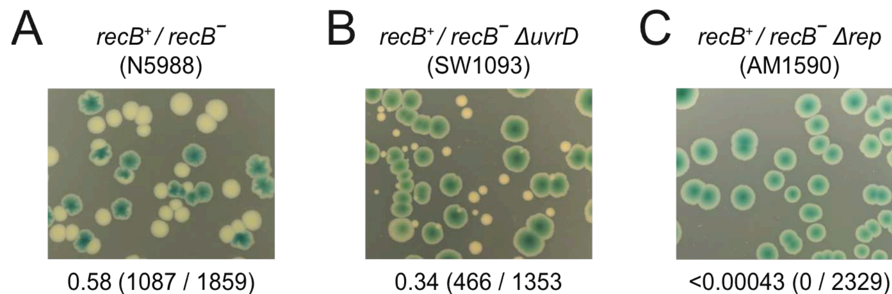



**Figure 5.** *uvrD recB* double mutant cells are viable but have a growth defect.
(A-C) The ability of the indicated strains to lose pAM375 (pRC7*recB*) was monitored on LB Xgal IPTG plates.

318 The corollary of Δ*rep* Δ*uvrD rpoB*35 recA* inviability is that RecBCD is unable to maintain
319 viability without RecA in this context. This requirement for RecA is in contrast to the viability of
320 *rep recA* double mutant cells versus the inviability of *rep recB* double mutants in a *rpoB*$^+$
321 background [71]. This differential requirement in *uvrD*$^+$ *rep*$^-$ cells reflects the generation of
322 double-stranded DNA ends by regression of blocked replication forks and the need for RecBCD to
323 process these ends [58]. Processing can occur either by loading of RecA followed by strand
324 exchange or, in the absence of RecA, RecBCD-catalysed degradation of the dsDNA end to
325 regenerate a fork structure [58]. The viability of *uvrD recB* double mutant strains is less certain.
326 Absence of UvrD-catalysed removal of RecFOR-loaded RecA from blocked forks may lead to an
327 increased need for RecBCD-dependent repair of dsDNA ends [84]. Some reports indicate reduced
328 viability of *uvrD recB* mutant strains [91] whereas others report inviability [92,93]. We assayed the
329 viability of Δ*uvrD recB*$^-$ *rpoB*$^+$ cells by analysing their ability to lose pRC7*recB*. Δ*uvrD recB*$^-$ *rpoB*$^+$
330 cells could generate white colonies on rich medium in contrast to Δ*rep recB*$^-$ *rpoB*$^+$ cells (Figure 5,
331 compare B with C). However, the frequency of Δ*uvrD recB*$^-$ *rpoB*$^+$ white colony formation was
332 lower and white colony sizes much smaller than with *uvrD*$^+$ *recB*$^-$ *rpoB*$^+$ cells (Figure 5, compare B
333 with A). Thus in this strain background cells can survive without both UvrD and RecBCD but

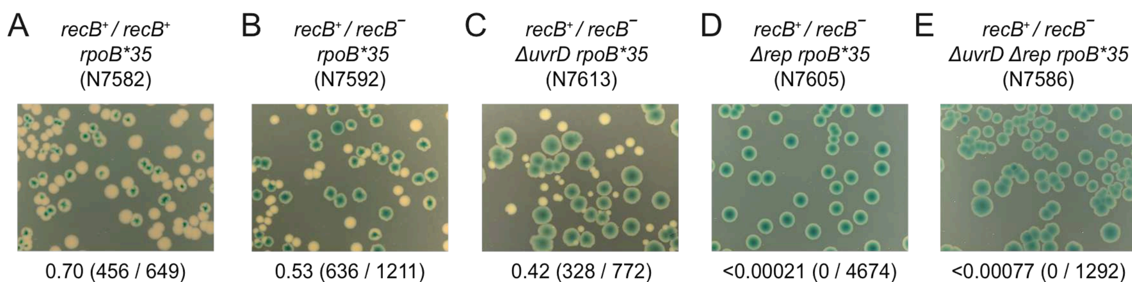



**Figure 6.** RecB is essential in *rep uvrD rpoB*35* cells on rich medium.
(A-E) Loss of pAM375 (pRC7*recB*) from the strains indicated was monitored on LB Xgal IPTG.

growth is impaired.

*rpo* mutations that suppress Δ*rep* Δ*uvrD* double mutant lethality are unable to suppress Δ*rep recB* double mutant lethality [78]. Similarly, reduction of transcription-driven replicative barriers using *rpoB*35* did not improve the viability of either Δ*rep recB* or Δ*uvrD recB* double mutant strains (compare Figure 6C with Figure 5B; Figure 6D with 5C). Δ*rep* Δ*uvrD rpoB*35* $recB^-$ was also inviable (Figure 6E), as expected given the growth defects of single *rep* and *uvrD* mutants [71](Figure 6C and D). It was possible that UvrD not being available to abort RecFOR-directed loading of RecA onto blocked replication forks [81,84] contributed to Δ*rep* Δ*uvrD rpoB*35 recB* inviability. However, Δ*rep* Δ*uvrD rpoB*35* $recF^-$ $recB^-$ remained inviable, indicating that countering RecFOR activity was not a major contributor to this inviability (Figure 7A-C). Δ*rep* Δ*uvrD rpoB*35* $recF^-$ $recA^-$ also remained inviable (Figure 7D), as expected given that RecFOR-dependent toxicity requires RecA [84].

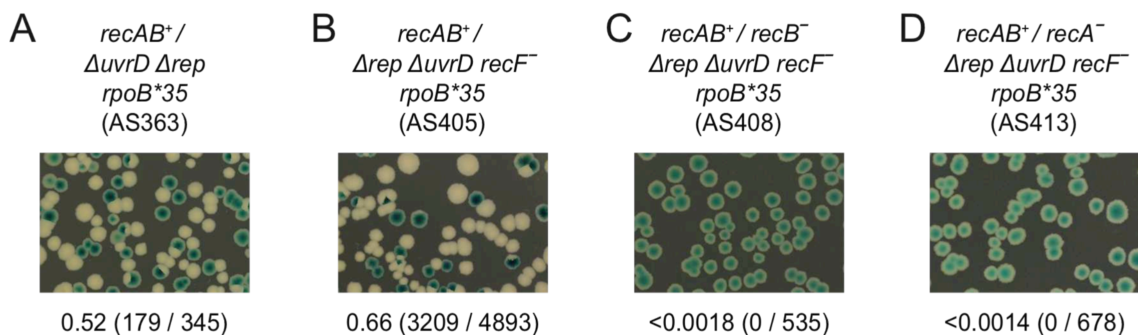



**Figure 7.** The requirement for RecBCD and RecA is not alleviated by mutation of *recF*. (A-D) Loss of pAM406 (pRC7*recA*,*recB*) from the strains indicated was monitored on LB Xgal IPTG.

These data indicate that RecA (Figures 4B and 7D) and RecBCD (Figures 6E and 7C) are both essential in Δ*rep* Δ*uvrD* mutant cells under rapid growth conditions even when replication-transcription conflicts are reduced by a mutation in RNA polymerase. Thus when accessory helicases are absent the degradation of double-stranded DNA ends by RecBCD is insufficient by itself to deal with blocked replication forks. Under such circumstances strand exchange is also needed, allowing D-loop formation from double-stranded DNA ends and subsequent replisome reloading [1,94].

## 4. Discussion

We show here that transcription is a major cause of the chromosomal degradation seen in *recA* cells. The chromosome content defects of cells lacking either Rep [6] or RecA (Figure 1A, compare iii with vii) share the same primary cause therefore indicating that both Rep and RecA reduce the impact of gene expression on genome duplication. The synergistic increase in chromosome content defects in *rep recA* cells indicate that these enzymes provide alternative means of mitigating the impact of transcription on DNA replication (Figure 1Aiv). Furthermore, the significant suppression of DNA degradation in *recA* cells by an RNA polymerase mutation supports the view that protein-DNA complexes are the primary causes of replication defects in cells not exposed to elevated DNA damage [6]. However, the time taken to duplicate a chromosome is not extended in the absence of either RecA or RecB, in contrast to cells lacking Rep (Figure 3). Thus the

364 maintenance of rapid chromosome duplication has a greater dependency on Rep as opposed to
365 RecA or RecBCD. RecA and RecBCD do, though, have the ability to sustain chromosome
366 duplication in Δ*rep* Δ*uvrD* double mutant cells when transcriptional barriers to replication are
367 reduced (Figures 4 and 7). Both RecA and RecBCD are needed for this underpinning,
368 demonstrating that maintenance of chromosome duplication by recombination enzymes is most
369 efficient when RecBCD catalyses loading of RecA at dsDNA ends rather than large-scale
370 RecBCD-dependent degradation of such ends (Figure 7).

371 These data are apparently contradictory. RecA has little impact on chromosome duplication
372 times and, although the time resolution of the measurements in Figure 3 are relatively low, they
373 still imply infrequent engagement of RecA in genome duplication during a single cell cycle. In
374 contrast, the absence of RecA results in frequent chromosome degradation [55] and a reduction in
375 viability [85]. In considering this apparent contradiction, there are many factors that potentially
376 affect the time needed to copy a chromosome (Figure 8). The inherent speed and processivity of
377 the replisome is an important determinant of chromosome duplication time but the pausing
378 behaviour of replisomes, and what happens to these paused forks, will also impact on duplication
379 times (Figure 8i-v). Accessory helicases reduce the frequency and/or duration of replisome pauses
380 at nucleoprotein complexes and increase the probability of paused replisomes restarting
381 replication as opposed to losing function [6,22,27]. The extended chromosome duplication time in
382 Δ*rep* mutant cells [29,31] indicates that one or more of these pausing parameters are critical in
383 determining the speed of chromosome duplication. The more than twofold increase in
384 chromosome duplication times in Δ*rep* mutant cells probably also underrepresents the significance
385 of replisome pausing behaviour on these timings, given the ability of UvrD to compensate
386 partially for the absence of Rep [22,30].

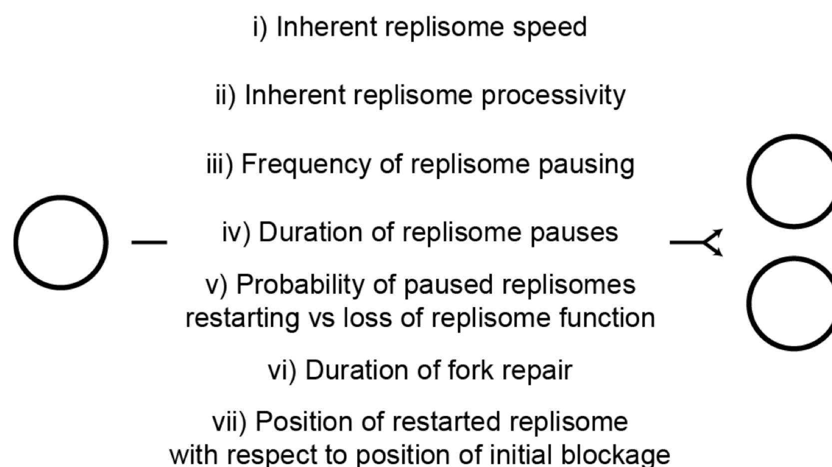



**Figure 8.** Summary of factors with potential influence on the probability of replisomes completing chromosome duplication and the time needed to do so.

387 Regardless of the cause of replisome pausing, there is no evidence that recombination
388 enzymes impact directly on paused replisomes but they can act after a replisome has lost function
389 to promote replication restart [7]. Loss of enzymes such as RecA or RecBCD might therefore

390 impact on the duration of any fork repair process since loss of an enzyme normally involved in
391 fork repair might lead to extension of repair times due to less efficient alternative pathways that
392 are not normally operative in wild type cells (Figure 8vi). A related consideration is the position of
393 replication re-initiation with respect to the position of the blocked initial replisome (Figure 8vii).
394 The extensive RecBCD-dependent degradation of DNA in *recA* mutant cells [55] argues for less
395 efficient replication repair for both of the above reasons. Firstly, the time taken to degrade
396 extensive sections of the chromosome is measured in minutes even with the high speed and
397 processivity of RecBCD-catalysed dsDNA end degradation [56]. Secondly, this extensive
398 degradation in effect means that replisome reloading must occur far upstream of the initially
399 blocked fork, possibly at *oriC* [95]. However, the absence of significant extension of chromosome
400 duplication time in *recA* or *recB* mutant cells (Figure 3) indicates that fork repair in the absence of
401 either activity does not impact significantly on the mean duplication time during a single cell cycle.
402 The reduced viability [85] and chromosomal degradation seen in *recA* mutant cells [55](and Figure
403 1Aiii) might therefore reflect the loss of replisome function when considering multiple cell cycles
404 rather than just one. These occasional repair events may be too infrequent to have a measurable
405 impact on mean chromosome duplication time during one cell cycle (Figure 3) but each event
406 might take significant time and result in accumulation of cells with different numbers of
407 chromosome equivalents over the course of multiple cell cycles. Given enough time, though, these
408 non-wild type repair events can resolve the majority of replicative problems, evinced by the
409 similar chromosome profiles of wild type and *recA* mutant cells in stationary phase (Figure 2).
410 Regarding reduced viability of *recA* mutant cells, such viability measurements involve comparing
411 the number of colony-forming units with the total number of cells as determined by microscopy
412 [85]. This measure of viability therefore indicates the relative frequency with which a population of
413 cells generates non-viable cells over the course of an extended period of time and cannot be
414 compared to a measure of chromosome duplication time during a single cell cycle as presented in
415 Figure 3. Infrequent engagement of RecA and RecBCD during chromosome duplication might
416 have an undetectable impact on the mean time taken to replicate a chromosome during a single
417 cell cycle. However, absence of RecA- and RecBCD-dependent processing of replication
418 intermediates could result in aberrant events in their absence which, over multiple cell cycles,
419 gives rise to cells that can no longer divide.

420 The inefficiency of non-wild type fork repair mechanisms might also relate to our finding that
421 Δ*rep* Δ*uvrD rpoB*35 recF*$^-$ cells require both RecA and RecBCD for survival (Figures 4 and 7). *rep*
422 *recA* double mutant cells are viable but *rep recB* double mutants are not, indicating that under some
423 circumstances RecBCD-catalysed degradation of dsDNA ends in the absence of RecA can
424 underpin genome duplication [58,71]. However, our data indicate that when both Rep and UvrD
425 are absent then RecBCD-dependent DNA degradation is not sufficient to sustain viability unless it
426 is coupled to loading of RecA. UvrD can act as an accessory helicase and compensate partially for
427 loss of Rep in Δ*rep uvrD*$^+$ cells [22,30]. This partial compensation may explain why DNA
428 degradation without RecA loading can maintain viability in Δ*rep uvrD*$^+$ cells but not in Δ*rep* Δ*uvrD*
429 *rpoB*35 recF*$^-$ cells: dsDNA end degradation alone provides an inefficient means of reinitiating
430 DNA replication if replisomes pause and lose function at an elevated rate, as in cells lacking both
431 Rep and UvrD.

432 It should also be borne in mind that, whilst transcription is a major source of replicative

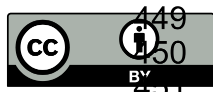
problems in unstressed cells [6,22,30,78](Figure 1), recombination enzymes have the ability to deal with replicative barriers other than protein-DNA complexes, unlike accessory replicative helicases [1,7]. Thus under conditions of elevated replicative stress such as exogenous DNA damaging agents then recombination enzymes may dominate replication repair. However, in otherwise unstressed cells our data are consistent with the accessory helicase-dependent minimisation of replisome pausing having a more significant impact on sustaining replisome movement than recombination-directed replisome reloading mechanisms.

**Supplementary Materials:** The following are available online at www.mdpi.com/link, Table S1: *Escherichia coli* K12 strains.

**Acknowledgments:** We thank Akeel Mahdi for help with strain constructions and Carol Buckman for excellent technical support. This work was funded by grant BB/J014826/1 provided by the UK Biotechnology and Biological Sciences Research Council (BBSRC) to PM.

**Author Contributions:** Peter McGlynn and Robert G. Lloyd conceived and designed the experiments. Aisha Syeda and John Atkinson performed experiments and analysed data.

**Conflicts of Interest: The authors declare no conflict of interest.**



© 2016 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).

## References

1. Yeeles, J.T.; Poli, J.; Marians, K.J.; Pasero, P. Rescuing stalled or damaged replication forks. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a012815.
2. Aguilera, A.; Garcia-Muse, T. Causes of Genome Instability. *Annu. Rev. Genet.* **2013**.
3. Wu, H.Y.; Shyy, S.H.; Wang, J.C.; Liu, L.F. Transcription generates positively and negatively supercoiled domains in the template. *Cell* **1988**, *53*, 433-440.
4. Olavarrieta, L.; Hernandez, P.; Krimer, D.B.; Schvartzman, J.B. DNA knotting caused by head-on collision of transcription and replication. *J. Mol. Biol.* **2002**, *322*, 1-6.
5. McGlynn, P.; Savery, N.J.; Dillingham, M.S. The conflict between DNA replication and transcription. *Mol. Microbiol.* **2012**, *85*, 12-20.
6. Gupta, M.K.; Guy, C.P.; Yeeles, J.T.; Atkinson, J.; Bell, H.; Lloyd, R.G.; Marians, K.J.; McGlynn, P. Protein-DNA complexes are the primary sources of replication fork pausing in *Escherichia coli*. *Proc. Natl. Acad. Sci. U S A* **2013**, *110*, 7252-7257.
7. Syeda, A.H.; Hawkins, M.; McGlynn, P. Recombination and replication. *Cold Spring Harb. Perspect. Biol.* **2014**, *6*, a016550.
8. Marians, K.J.; Hiasa, H.; Kim, D.R.; McHenry, C.S. Role of the core DNA polymerase III subunits at the replication fork. $\alpha$ is the only subunit required for processive replication. *J. Biol. Chem.* **1998**, *273*, 2452-2457.
9. McGlynn, P.; Guy, C.P. Replication forks blocked by protein-DNA complexes have limited stability *in vitro*. *J. Mol. Biol.* **2008**, *381*, 249-255.
10. Petermann, E.; Orta, M.L.; Issaeva, N.; Schultz, N.; Helleday, T. Hydroxyurea-stalled replication forks become progressively inactivated and require two different RAD51-mediated pathways for restart and repair. *Mol. Cell* **2010**, *37*, 492-502.

475 11. Mettrick, K.A.; Grainge, I. Stability of blocked replication forks *in vivo*. *Nucleic Acids Res.*
476 **2015**, *44*, 657-668.
477 12. Rupp, W.D.; Wilde, C.E., 3rd; Reno, D.L.; Howard-Flanders, P. Exchanges between DNA
478 strands in ultraviolet-irradiated *Escherichia coli*. *J. Mol. Biol.* **1971**, *61*, 25-44.
479 13. Lehmann, A.R. Postreplication repair of DNA in ultraviolet-irradiated mammalian cells. *J.*
480 *Mol. Biol.* **1972**, *66*, 319-337.
481 14. Smith, K.C. Recombinational DNA repair: the ignored repair systems. *Bioessays* **2004**, *26*,
482 1322-1326.
483 15. Yeeles, J.T.; Marians, K.J. The *Escherichia coli* replisome is inherently DNA damage tolerant.
484 *Science* **2011**, *334*, 235-238.
485 16. Yeeles, J.T.; Marians, K.J. Dynamics of leading-strand lesion skipping by the replisome. *Mol.*
486 *Cell* **2013**, *52*, 855-865.
487 17. Gabbai, C.B.; Yeeles, J.T.; Marians, K.J. Replisome-mediated Translesion Synthesis and
488 Leading Strand Template Lesion Skipping Are Competing Bypass Mechanisms. *J. Biol.*
489 *Chem.* **2014**, *289*, 32811-32823.
490 18. Fuchs, R.P.; Fujii, S. Translesion DNA synthesis and mutagenesis in prokaryotes. *Cold*
491 *Spring Harb. Perspect. Biol.* **2013**, *5*, a012682.
492 19. Goodman, M.F.; Woodgate, R. Translesion DNA polymerases. *Cold Spring Harb. Perspect.*
493 *Biol.* **2013**, *5*, a010363.
494 20. Payne, B.T.; van Knippenberg, I.C.; Bell, H.; Filipe, S.R.; Sherratt, D.J.; McGlynn, P.
495 Replication fork blockage by transcription factor-DNA complexes in *Escherichia coli*. *Nucleic*
496 *Acids Res.* **2006**, *34*, 5194-5202.
497 21. Pomerantz, R.T.; O'Donnell, M. The replisome uses mRNA as a primer after colliding with
498 RNA polymerase. *Nature* **2008**, *456*, 762-766.
499 22. Guy, C.P.; Atkinson, J.; Gupta, M.K.; Mahdi, A.A.; Gwynn, E.J.; Rudolph, C.J.; Moon, P.B.;
500 van Knippenberg, I.C.; Cadman, C.J.; Dillingham, M.S., *et al.* Rep Provides a Second Motor
501 at the Replisome to Promote Duplication of Protein-Bound DNA. *Mol. Cell* **2009**, *36*,
502 654-666.
503 23. Yancey-Wrona, J.E.; Matson, S.W. Bound Lac repressor protein differentially inhibits the
504 unwinding reactions catalyzed by DNA helicases. *Nucleic Acids Res.* **1992**, *20*, 6713-6721.
505 24. Byrd, A.K.; Raney, K.D. Protein displacement by an assembly of helicase molecules aligned
506 along single-stranded DNA. *Nat. Struct. Mol. Biol.* **2004**, *11*, 531-538.
507 25. Ivessa, A.S.; Zhou, J.Q.; Zakian, V.A. The *Saccharomyces* Pif1p DNA helicase and the highly
508 related Rrm3p have opposite effects on replication fork progression in ribosomal DNA. *Cell*
509 **2000**, *100*, 479-489.
510 26. Ivessa, A.S.; Zhou, J.Q.; Schulz, V.P.; Monson, E.K.; Zakian, V.A. *Saccharomyces* Rrm3p, a 5'
511 to 3' DNA helicase that promotes replication fork progression through telomeric and
512 subtelomeric DNA. *Genes Dev.* **2002**, *16*, 1383-1396.
513 27. Ivessa, A.S.; Lenzmeier, B.A.; Bessler, J.B.; Goudsouzian, L.K.; Schnakenberg, S.L.; Zakian,
514 V.A. The *Saccharomyces cerevisiae* helicase Rrm3p facilitates replication past nonhistone
515 protein-DNA complexes. *Mol. Cell* **2003**, *12*, 1525-1536.

516 28. Azvolinsky, A.; Dunaway, S.; Torres, J.Z.; Bessler, J.B.; Zakian, V.A. The *S. cerevisiae* Rrm3p
517 DNA helicase moves with the replication fork and affects replication of all yeast
518 chromosomes. *Genes Dev.* **2006**, *20*, 3104-3116.
519 29. Lane, H.E.; Denhardt, D.T. The rep mutation. IV. Slower movement of replication forks in
520 *Escherichia coli rep* strains. *J. Mol. Biol.* **1975**, *97*, 99-112.
521 30. Boubakri, H.; de Septenville, A.L.; Viguera, E.; Michel, B. The helicases DinG, Rep and
522 UvrD cooperate to promote replication across transcription units *in vivo*. *EMBO J.* **2010**, *29*.
523 31. Atkinson, J.; Gupta, M.K.; Rudolph, C.J.; Bell, H.; Lloyd, R.G.; McGlynn, P. Localization of
524 an accessory helicase at the replisome is critical in sustaining efficient genome duplication.
525 *Nucleic Acids Res.* **2011**, *39*, 949-957.
526 32. Merrikh, C.N.; Brewer, B.J.; Merrikh, H. The *B. subtilis* Accessory Helicase PcrA Facilitates
527 DNA Replication through Transcription Units. *PLoS Genet.* **2015**, *11*, e1005289.
528 33. Schmidt, K.H.; Derry, K.L.; Kolodner, R.D. *Saccharomyces cerevisiae* RRM3, a 5' to 3' DNA
529 helicase, physically interacts with proliferating cell nuclear antigen. *J. Biol. Chem.* **2002**, *277*,
530 45331-45337.
531 34. Atkinson, J.; Gupta, M.K.; McGlynn, P. Interaction of Rep and DnaB on DNA. *Nucleic Acids*
532 *Res.* **2011**, *39*, 1351-1359.
533 35. Bruning, J.G.; Howard, J.A.; McGlynn, P. Use of streptavidin bound to biotinylated DNA
534 structures as model substrates for analysis of nucleoprotein complex disruption by
535 helicases. *Methods* **2016**.
536 36. Petit, M.A.; Dervyn, E.; Rose, M.; Entian, K.D.; McGovern, S.; Ehrlich, S.D.; Bruand, C. PcrA
537 is an essential DNA helicase of *Bacillus subtilis* fulfilling functions both in repair and
538 rolling-circle replication. *Mol. Microbiol.* **1998**, *29*, 261-273.
539 37. Denhardt, D.T.; Dressler, D.H.; Hathaway, A. The abortive replication of fX174 DNA in a
540 recombination-deficient mutant of *Escherichia coli. Proc. Natl. Acad. Sci. U S A* **1967**, *57*,
541 813-820.
542 38. Keil, R.L.; McWilliams, A.D. A gene with specific and global effects on recombination of
543 sequences from tandemly repeated genes in *Saccharomyces cerevisiae*. *Genetics* **1993**, *135*,
544 711-718.
545 39. Bochman, M.L.; Sabouri, N.; Zakian, V.A. Unwinding the functions of the Pif1 family
546 helicases. *DNA Repair (Amst)* **2010**, *9*, 237-249.
547 40. Bruning, J.G.; Howard, J.L.; McGlynn, P. Accessory Replicative Helicases and the
548 Replication of Protein-Bound DNA. *J. Mol. Biol.* **2014**, *426*, 3917-3928.
549 41. Brewer, B.J. When polymerases collide: replication and the transcriptional organization of
550 the *E. coli* chromosome. *Cell* **1988**, *53*, 679-686.
551 42. Trautinger, B.W.; Jaktaji, R.P.; Rusakova, E.; Lloyd, R.G. RNA polymerase modulators and
552 DNA repair activities resolve conflicts between DNA replication and transcription. *Mol.*
553 *Cell* **2005**, *19*, 247-258.
554 43. Azvolinsky, A.; Giresi, P.G.; Lieb, J.D.; Zakian, V.A. Highly transcribed RNA polymerase II
555 genes are impediments to replication fork progression in *Saccharomyces cerevisiae*. *Mol. Cell*
556 **2009**, *34*, 722-734.
557 44. Merrikh, H.; Machon, C.; Grainger, W.H.; Grossman, A.D.; Soultanas, P. Co-directional
558 replication-transcription conflicts lead to replication restart. *Nature* **2011**, *470*, 554-557.

559 45. Petryk, N.; Kahli, M.; d'Aubenton-Carafa, Y.; Jaszczyszyn, Y.; Shen, Y.; Silvain, M.;
560 Thermes, C.; Chen, C.L.; Hyrien, O. Replication landscape of the human genome. *Nature*
561 *communications* **2016**, *7*, 10208.
562 46. Helmrich, A.; Ballarino, M.; Nudler, E.; Tora, L. Transcription-replication encounters,
563 consequences and genomic instability. *Nat. Struct. Mol. Biol.* **2013**, *20*, 412-418.
564 47. Seigneur, M.; Ehrlich, S.D.; Michel, B. RuvABC-dependent double-strand breaks in *dnaBts*
565 mutants require *recA*. *Mol. Microbiol.* **2000**, *38*, 565-574.
566 48. Petermann, E.; Helleday, T. Pathways of mammalian replication fork restart. *Nat. Rev. Mol.*
567 *Cell Biol.* **2010**, *11*, 683-687.
568 49. Courcelle, J.; Hanawalt, P.C. RecA-dependent recovery of arrested DNA replication forks.
569 *Annu. Rev. Genet.* **2003**, *37*, 611-646.
570 50. Hashimoto, Y.; Ray Chaudhuri, A.; Lopes, M.; Costanzo, V. Rad51 protects nascent DNA
571 from Mre11-dependent degradation and promotes continuous DNA synthesis. *Nat. Struct.*
572 *Mol. Biol.* **2010**, *17*, 1305-1311.
573 51. Schlacher, K.; Christ, N.; Siaud, N.; Egashira, A.; Wu, H.; Jasin, M. Double-strand break
574 repair-independent role for BRCA2 in blocking stalled replication fork degradation by
575 MRE11. *Cell* **2011**, *145*, 529-542.
576 52. Courcelle, J.; Donaldson, J.R.; Chow, K.H.; Courcelle, C.T. DNA damage-induced
577 replication fork regression and processing in *Escherichia coli*. *Science* **2003**, *299*, 1064-1067.
578 53. Courcelle, C.T.; Chow, K.H.; Casey, A.; Courcelle, J. Nascent DNA processing by RecJ
579 favors lesion repair over translesion synthesis at arrested replication forks in *Escherichia coli*.
580 *Proc. Natl. Acad. Sci. U S A* **2006**, *103*, 9154-9159.
581 54. Cox, M.M.; Goodman, M.F.; Kreuzer, K.N.; Sherratt, D.J.; Sandler, S.J.; Marians, K.J. The
582 importance of repairing stalled replication forks. *Nature* **2000**, *404*, 37-41.
583 55. Skarstad, K.; Boye, E. Degradation of individual chromosomes in *recA* mutants of
584 *Escherichia coli*. *J. Bacteriol.* **1993**, *175*, 5505-5509.
585 56. Dillingham, M.S.; Kowalczykowski, S.C. RecBCD enzyme and the repair of
586 double-stranded DNA breaks. *Microbiol. Mol. Biol. Rev.* **2008**, *72*, 642-671.
587 57. Kuzminov, A.; Stahl, F.W. Stability of linear DNA in *recA* mutant *Escherichia coli* cells
588 reflects ongoing chromosomal DNA degradation. *J. Bacteriol.* **1997**, *179*, 880-888.
589 58. Seigneur, M.; Bidnenko, V.; Ehrlich, S.D.; Michel, B. RuvAB acts at arrested replication
590 forks. *Cell* **1998**, *95*, 419-430.
591 59. Lambert, S.; Watson, A.; Sheedy, D.M.; Martin, B.; Carr, A.M. Gross chromosomal
592 rearrangements and elevated recombination at an inducible site-specific replication fork
593 barrier. *Cell* **2005**, *121*, 689-702.
594 60. Saveson, C.J.; Lovett, S.T. Enhanced deletion formation by aberrant DNA replication in
595 *Escherichia coli*. *Genetics* **1997**, *146*, 457-470.
596 61. Vilette, D.; Uzest, M.; Ehrlich, S.D.; Michel, B. DNA transcription and repressor binding
597 affect deletion formation in *Escherichia coli* plasmids. *EMBO J.* **1992**, *11*, 3629-3634.
598 62. Prado, F.; Aguilera, A. Impairment of replication fork progression mediates RNA polII
599 transcription-associated recombination. *EMBO J.* **2005**, *24*, 1267-1276.

600 63. Duch, A.; Felipe-Abrio, I.; Barroso, S.; Yaakov, G.; Garcia-Rubio, M.; Aguilera, A.; de Nadal,
601 E.; Posas, F. Coordinated control of replication and transcription by a SAPK protects
602 genomic integrity. *Nature* **2012**.
603 64. Mahdi, A.A.; Buckman, C.; Harris, L.; Lloyd, R.G. Rep and PriA helicase activities prevent
604 RecA from provoking unnecessary recombination during replication fork repair. *Genes Dev.*
605 **2006**, *20*, 2135-2147.
606 65. Schmidt, K.H.; Kolodner, R.D. Suppression of spontaneous genome rearrangements in
607 yeast DNA helicase mutants. *Proc. Natl. Acad. Sci. U S A* **2006**, *103*, 18196-18201.
608 66. Krejci, L.; Altmannova, V.; Spirek, M.; Zhao, X. Homologous recombination and its
609 regulation. *Nucleic Acids Res.* **2012**, *40*, 5795-5818.
610 67. Veaute, X.; Delmas, S.; Selva, M.; Jeusset, J.; Le Cam, E.; Matic, I.; Fabre, F.; Petit, M.A. UvrD
611 helicase, unlike Rep helicase, dismantles RecA nucleoprotein filaments in *Escherichia coli.*
612 *EMBO J.* **2005**, *24*, 180-189.
613 68. Tong, A.H.; Lesage, G.; Bader, G.D.; Ding, H.; Xu, H.; Xin, X.; Young, J.; Berriz, G.F.; Brost,
614 R.L.; Chang, M., *et al.* Global mapping of the yeast genetic interaction network. *Science* **2004**,
615 *303*, 808-813.
616 69. Schmidt, K.H.; Kolodner, R.D. Requirement of Rrm3 helicase for repair of spontaneous
617 DNA lesions in cells lacking Srs2 or Sgs1 helicase. *Mol. Cell. Biol.* **2004**, *24*, 3213-3226.
618 70. Torres, J.Z.; Schnakenberg, S.L.; Zakian, V.A. *Saccharomyces cerevisiae* Rrm3p DNA helicase
619 promotes genome integrity by preventing replication fork stalling: viability of rrm3 cells
620 requires the intra-S-phase checkpoint and fork restart activities. *Mol. Cell. Biol.* **2004**, *24*,
621 3198-3212.
622 71. Uzest, M.; Ehrlich, S.D.; Michel, B. Lethality of *rep recB* and *rep recC* double mutants of
623 *Escherichia coli*. *Mol. Microbiol.* **1995**, *17*, 1177-1188.
624 72. Michel, B.; Ehrlich, S.D.; Uzest, M. DNA double-strand breaks caused by replication arrest.
625 *EMBO J.* **1997**, *16*, 430-438.
626 73. De Septenville, A.L.; Duigou, S.; Boubakri, H.; Michel, B. Replication fork reversal after
627 replication-transcription collision. *PLoS Genet.* **2012**, *8*, e1002622.
628 74. Kuzminov, A.; Stahl, F.W. Double-strand end repair via the RecBC pathway in *Escherichia*
629 *coli* primes DNA replication. *Genes Dev.* **1999**, *13*, 345-356.
630 75. Taucher-Scholtz, G.; Abdel-Monem, M.; Hoffmann-Berling, H. Functions of helicases in *E.*
631 *coli*. In *Mechanisms of DNA replication and recombination*, Cozzarelli, N.R., Ed. Alan R. Liss
632 Inc.: New York, 1983; pp 65-76.
633 76. Potrykus, K.; Cashel, M. (p)ppGpp: still magical? *Annu. Rev. Microbiol.* **2008**, *62*, 35-51.
634 77. Denapoli, J.; Tehranchi, A.K.; Wang, J.D. Dose-dependent reduction of replication
635 elongation rate by (p)ppGpp in *Escherichia coli* and *Bacillus subtilis*. *Mol. Microbiol.* **2013**, *88*,
636 93-104.
637 78. Baharoglu, Z.; Lestini, R.; Duigou, S.; Michel, B. RNA polymerase mutations that facilitate
638 replication progression in the *rep uvrD recF* mutant lacking two accessory replicative
639 helicases. *Mol. Microbiol.* **2010**, *77*, 324-336.
640 79. McGlynn, P.; Lloyd, R.G. Modulation of RNA polymerase by (p)ppGpp reveals a
641 RecG-dependent mechanism for replication fork progression. *Cell* **2000**, *101*, 35-45.

642 80. Dutta, D.; Shatalin, K.; Epshtein, V.; Gottesman, M.E.; Nudler, E. Linking RNA polymerase
643 backtracking to genome instability in *E. coli*. *Cell* **2011**, *146*, 533-543.
644 81. Petit, M.A.; Ehrlich, D. Essential bacterial helicases that counteract the toxicity of
645 recombination proteins. *EMBO J.* **2002**, *21*, 3137-3147.
646 82. Lestini, R.; Michel, B. UvrD and UvrD252 counteract RecQ, RecJ, and RecFOR in a *rep*
647 mutant of *Escherichia coli*. *J. Bacteriol.* **2008**, *190*, 5995-6001.
648 83. Moore, T.; McGlynn, P.; Ngo, H.P.; Sharples, G.J.; Lloyd, R.G. The RdgC protein of
649 *Escherichia coli* binds DNA and counters a toxic effect of RecFOR in strains lacking the
650 replication restart protein PriA. *EMBO J.* **2003**, *22*, 735-745.
651 84. Magner, D.B.; Blankschien, M.D.; Lee, J.A.; Pennington, J.M.; Lupski, J.R.; Rosenberg, S.M.
652 RecQ promotes toxic recombination in cells lacking recombination intermediate-removal
653 proteins. *Mol. Cell* **2007**, *26*, 273-286.
654 85. Capaldo-Kimball, F.; Barbour, S.D. Involvement of recombination genes in growth and
655 viability of *Escherichia coli* K-12. *J. Bacteriol.* **1971**, *106*, 204-212.
656 86. Lloyd, R.G. Conjugational recombination in resolvase-deficient *ruvC* mutants of *Escherichia*
657 *coli* K-12 depends on *recG*. *J. Bacteriol.* **1991**, *173*, 5414-5418.
658 87. Bernhardt, T.G.; de Boer, P.A. Screening for synthetic lethal mutants in *Escherichia coli* and
659 identification of EnvC (YibP) as a periplasmic septal ring factor with murein hydrolase
660 activity. *Mol. Microbiol.* **2004**, *52*, 1255-1269.
661 88. Datsenko, K.A.; Wanner, B.L. One-step inactivation of chromosomal genes in *Escherichia coli*
662 K-12 using PCR products. *Proc. Natl. Acad. Sci. U S A* **2000**, *97*, 6640-6645.
663 89. Trautinger, B.W.; Lloyd, R.G. Modulation of DNA repair by mutations flanking the DNA
664 channel through RNA polymerase. *EMBO J.* **2002**, *21*, 6944-6953.
665 90. Lane, H.E.; Denhardt, D.T. The *rep* mutation. III. Altered structure of the replicating
666 *Escherichia coli* chromosome. *J. Bacteriol.* **1974**, *120*, 805-814.
667 91. Howard-Flanders, P.; Bardwell, E. Effects of *recB21*, *recF143*, and *uvrD152* on recombination
668 in lambda bacteriophage-prophage and Hfr by F- crosses. *J. Bacteriol.* **1981**, *148*, 739-743.
669 92. Bidnenko, V.; Lestini, R.; Michel, B. The *Escherichia coli* UvrD helicase is essential for Tus
670 removal during recombination-dependent replication restart from Ter sites. *Mol. Microbiol.*
671 **2006**, *62*, 382-396.
672 93. Centore, R.C.; Sandler, S.J. UvrD limits the number and intensities of RecA-green
673 fluorescent protein structures in *Escherichia coli* K-12. *J. Bacteriol.* **2007**, *189*, 2915-2920.
674 94. Liu, J.; Marians, K.J. PriA-directed assembly of a primosome on D loop DNA. *J. Biol. Chem.*
675 **1999**, *274*, 25033-25041.
676 95. Kuzminov, A. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage
677 lambda. *Microbiol. Mol. Biol. Rev.* **1999**, *63*, 751-813.

678

**Supplementary Table 1**. *Escherichia coli* K12 strains.

**A)** MG1655 derivatives

| MG1655 | F$^-$ *rph-1* | [1] |
|---|---|---|
| AM1573 | *ΔlacIZYA recB270::*kan | P1.RJ1003 x TB28 to Km$^r$ |
| AM1590 | pAM375 *(lac$^+$ recB$^+$)* / *ΔlacIZYA Δrep::cat recB268::*kan | [2] |
| AM1657 | *ΔuvrD::dhfr* | [3] |
| AM2158 | *ΔlacIZYA rpoB[G1260D]* | [4] |
| AS301 | *ΔlacIZYA rpoB*35 ΔuvrD::dhfr Δrep::cat* | Plasmid-free segregant of N7150 |
| AS351 | pAM383 *(lac$^+$ recA$^+$)* / *ΔrecA::spec* | pAM383 x N7358 to Ap$^r$ |
| AS363 | pAM406 *(lac$^+$ recA$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 ΔuvrD::dhfr Δrep::cat* | pAM406 x AS301 to Ap$^r$ |
| AS370 | pAM406 *(lac$^+$ recA$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 ΔuvrD::dhfr Δrep::cat recB268::*Tn*10* | P1.BP45 x AS363 to Tc$^r$ |
| AS371 | pAM406 *(lac$^+$ recA$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 ΔuvrD::dhfr Δrep::cat ΔrecA::spec* | P1.AS351 x AS363 to Spec$^r$ |
| AS405 | pAM406 *(lac$^+$ recA$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 ΔuvrD::dhfr Δrep::cat ΔrecF735::<kan>* | P1.JW3677 x AS363 to Km$^r$ |
| AS408 | pAM406 *(lac$^+$ recA$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 ΔuvrD::dhfr Δrep::cat ΔrecF735::<kan> recB268::*Tn*10* | P1.JW3677 x AS370 to Km$^r$ |
| AS413 | pAM406 *(lac$^+$ recA$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 ΔuvrD::dhfr Δrep::cat ΔrecF735::<kan> ΔrecA::spec* | P1.JW3677 x AS371 to Km$^r$ |
| BP45 | *Δara714 argEC::[apra$^r$ lacO$_{34}$] recB268::*Tn*10* | [5] |
| HB159 | *ΔlacIZYA dnaA46 tna300::*Tn*10* | [6] |
| HB161 | *ΔlacIZYA dnaA46 tna300::*Tn*10 Δrep::cat* | [6] |
| HB278 | *ΔlacIZYA rpoB[G1260D] Δrep::cat* | [7] |
| HB310 | *ΔlacIZYA rpoB[G1260D] Δrep::cat recA269::*Tn*10* | P1.N4279 x HB278 toTc$^r$ |
| HB312 | *ΔlacIZYA rpoB[G1260D] recA269::*Tn*10* | P1.N4279 x AM2158 toTc$^r$ |
| JA042 | *ΔlacIZYA dnaA46 tna300::*Tn*10 ΔrecA::kan* | P1.N6618 x HB159 to Km$^r$ |
| JA044 | *ΔlacIZYA dnaA46 tna300::*Tn*10 recB270::kan* | P1.N4600 x HB159 to Km$^r$ |
| N4279 | *recA269::*Tn*10* | [8] |
| N4600 | *recB270::kan* | P1.RJ1003 x MG1655 |
| N5925 | *ΔlacIZYA rpoB*35* | [4] |
| N5988 | pAM375 *(lac$^+$ recB$^+$)* / *ΔlacIZYA recB270::*kan | pAM375 x AM1573 to Ap$^r$ |
| N6065 | *ΔlacIZYA recA269::*Tn*10* | [2] |
| N6524 | pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA* | [4] |
| N6540 | pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA Δrep::cat* | P1.JJC735 x N6524 to Cm$^r$ |
| N6577 | *ΔlacIZYA Δrep::cat* | [4] |
| N6618 | *ΔrecA::kan* | This work |
| N7150 | pAM407 *(lac$^+$ uvrD$^+$)* / *ΔlacIZYA rpoB*35 ΔuvrD::dhfr Δrep::cat* | [4] |
| N7153 | *ΔlacIZYA rpoB*35 ΔuvrD::dhfr Δrep::cat* | Plasmid-free segregant of N7150 |
| N7358 | *ΔrecA::spec* | [9] |

| N7578 | pAM407 *(lac$^+$ uvrD$^+$)* / *ΔlacIZYA rpoB*35 ΔuvrD*::*dhfr Δrep*::*cat recA269::*Tn*10* | P1.N3072 x N7150 to Tc$^r$ |
|---|---|---|
| N7581 | pAM375 *(lac$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 ΔuvrD*::*dhfr Δrep*::*cat* | pAM375 x N7153 to Ap$^r$ |
| N7582 | pAM375 *(lac$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35* | pAM375 x N5925 to Ap$^r$ |
| N7586 | pAM375 *(lac$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 recB268::*Tn*10 ΔuvrD*::*dhfr Δrep*::*cat* | P1.TRM308 x N7581 to Tc$^r$ |
| N7592 | pAM375 *(lac$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 recB268::*Tn*10* | P1.TRM308 x N7582 to Tc$^r$ |
| N7602 | pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA Δrep::cat recA269::*Tn*10* | P1.N3072 x N6540 to Tc$^r$ |
| N7603 | *ΔlacIZYA Δrep::cat recA269::*Tn*10* | Plasmid-free segregant of N7602 |
| N7605 | pAM375 *(lac$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 recB268::*Tn*10 Δrep*::*cat* | P1.JJC735 x N7592 to Cm$^r$ |
| N7613 | pAM375 *(lac$^+$ recB$^+$)* / *ΔlacIZYA rpoB*35 recB268::*Tn*10 ΔuvrD*::*dhfr* | P1.AM1657 x N7592 to Tm$^r$ |
| RJ1003 | *relA1 ΔspoT207::cat rpoB*35 ΔruvAC65 eda-51::*Tn*10 recB270::kan* | [10] |
| SW1093 | pAM375 *(lac$^+$ recB$^+$)* / *ΔlacIZYA recB270::kan ΔuvrD*::*dhfr* | P1.AM1657 x N5988 to Tm$^r$ |
| TB28 | *ΔlacIZYA* | [11] |
| TRM308 | *recB268::*Tn*10 sbcA* | [2] |

**B)** Other derivatives

| JW3677 | BW25113 *rrnB3 ΔlacZ4787 hsdR514 Δ(araBAD)567 Δ(rhaBAD)568 rph-1 ΔrecF735*::*<kan>* | [12] |
|---|---|---|
| JJC735 | AB1157 *hsdR Δrep*::*cat* | [13] |
| N3072 | W3110 *rph-1 IN(rrnD-rrnE)1 recA269::*Tn*10* | [14] |

**References**


1. Bachmann, B.J. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12. In *Escherichia coli and Salmonella cellular and molecular biology*, Second ed.; Neidhardt, F.C.; Curtiss III, R.; Ingraham, J.L.; Lin, E.C.C.; Low, K.B.; Magasanik, B.; Reznikoff, W.S.; Riley, M.; Schaechter, M.; Umbarger, H.E., Eds. ASM Press: Washington, DC, 1996; pp 2460-2488.
2. Mahdi, A.A.; Buckman, C.; Harris, L.; Lloyd, R.G. Rep and PriA helicase activities prevent RecA from provoking unnecessary recombination during replication fork repair. *Genes Dev.* **2006**, *20*, 2135-2147.
3. Zhang, J.; Mahdi, A.A.; Briggs, G.S.; Lloyd, R.G. Promoting and avoiding recombination: contrasting activities of the *Escherichia coli* RuvABC Holliday junction resolvase and RecG DNA translocase. *Genetics* **2010**, *185*, 23-37.
4. Guy, C.P.; Atkinson, J.; Gupta, M.K.; Mahdi, A.A.; Gwynn, E.J.; Rudolph, C.J.; Moon, P.B.; van Knippenberg, I.C.; Cadman, C.J.; Dillingham, M.S., *et al.* Rep Provides a Second Motor at the Replisome to Promote Duplication of Protein-Bound DNA. *Mol. Cell* **2009**, *36*, 654-666.

5. Payne, B.T.; van Knippenberg, I.C.; Bell, H.; Filipe, S.R.; Sherratt, D.J.; McGlynn, P. Replication fork blockage by transcription factor-DNA complexes in *Escherichia coli*. *Nucleic Acids Res.* **2006**, *34*, 5194-5202.
6. Atkinson, J.; Gupta, M.K.; Rudolph, C.J.; Bell, H.; Lloyd, R.G.; McGlynn, P. Localization of an accessory helicase at the replisome is critical in sustaining efficient genome duplication. *Nucleic Acids Res.* **2011**, *39*, 949-957.
7. Gupta, M.K.; Guy, C.P.; Yeeles, J.T.; Atkinson, J.; Bell, H.; Lloyd, R.G.; Marians, K.J.; McGlynn, P. Protein-DNA complexes are the primary sources of replication fork pausing in *Escherichia coli*. *Proc. Natl. Acad. Sci. U S A* **2013**, *110*, 7252-7257.
8. McGlynn, P.; Lloyd, R.G. Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. *Cell* **2000**, *101*, 35-45.
9. Briggs, G.S.; Yu, J.; Mahdi, A.A.; Lloyd, R.G. The RdgC protein employs a novel mechanism involving a finger domain to bind to circular DNA. *Nucleic Acids Res.* **2010**, *38*, 6433-6446.
10. Trautinger, B.W.; Jaktaji, R.P.; Rusakova, E.; Lloyd, R.G. RNA polymerase modulators and DNA repair activities resolve conflicts between DNA replication and transcription. *Mol. Cell* **2005**, *19*, 247-258.
11. Bernhardt, T.G.; de Boer, P.A. Screening for synthetic lethal mutants in *Escherichia coli* and identification of EnvC (YibP) as a periplasmic septal ring factor with murein hydrolase activity. *Mol. Microbiol.* **2004**, *52*, 1255-1269.
12. Baba, T.; Ara, T.; Hasegawa, M.; Takai, Y.; Okumura, Y.; Baba, M.; Datsenko, K.A.; Tomita, M.; Wanner, B.L.; Mori, H. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* **2006**, *2*, 2006 0008.
13. Bidnenko, V.; Seigneur, M.; Penel-Colin, M.; Bouton, M.F.; Dusko Ehrlich, S.; Michel, B. *sbcB sbcC* null mutations allow RecF-mediated repair of arrested replication forks in *rep recBC* mutants. *Mol. Microbiol.* **1999**, *33*, 846-857.
14. Picksley, S.M.; Lloyd, R.G.; Buckman, C. Genetic analysis and regulation of inducible recombination in *Escherichia coli* K-12. *Cold Spring Harb. Symp. Quant. Biol.* **1984**, *49*, 469-474.