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## Article:

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1 Article

2 **The balance between recombination enzymes and**  
3 **accessory replicative helicases in facilitating genome**  
4 **duplication**5 **Aisha H. Syeda<sup>1</sup>, John Atkinson<sup>2</sup>, Robert G. Lloyd<sup>3</sup> and Peter McGlynn<sup>1,4\*</sup>**6 <sup>1</sup> Department of Biology, University of York, Wentworth Way, York, YO10 5DD, United Kingdom;  
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15 **Abstract:** Accessory replicative helicases aid the primary replicative helicase in duplicating  
16 protein-bound DNA, especially transcribed DNA. Recombination enzymes also aid genome  
17 duplication by facilitating the repair of DNA lesions via strand exchange and also processing of  
18 blocked fork DNA to generate structures onto which the replisome can be reloaded. There is  
19 significant interplay between accessory helicases and recombination enzymes in both bacteria and  
20 lower eukaryotes but how these replication repair systems interact to ensure efficient genome  
21 duplication remains unclear. Here we demonstrate that the DNA content defects of *Escherichia coli*  
22 cells lacking the strand exchange protein RecA are driven primarily by conflicts between  
23 replication and transcription, as is the case in cells lacking the accessory helicase Rep. However, in  
24 contrast to Rep, neither RecA nor RecBCD, the helicase/exonuclease that loads RecA onto dsDNA  
25 ends, is important for maintaining rapid chromosome duplication. Furthermore, RecA and  
26 RecBCD together can sustain viability in the absence of accessory replicative helicases but only  
27 when transcriptional barriers to replication are suppressed by an RNA polymerase mutation. Our  
28 data indicate that the minimisation of replisome pausing by accessory helicases has a more  
29 significant impact on successful completion of chromosome duplication than  
30 recombination-directed fork repair.

31 **Keywords:** genome stability/repair/replication/RNA polymerase32 **PACS:** J0101

33

34 **1. Introduction**

35 The replication machineries of all organisms encounter many potential barriers whilst  
36 duplicating their genomes, presenting a major challenge to the maintenance of genetic stability  
37 [1,2]. These barriers include damage to the template, non-B form DNA structures, topological  
38 strain and proteins bound to the DNA. Transcription provides both a topological challenge to  
39 DNA replication due to the over- and underwinding ahead of and behind an advancing RNA  
40 polymerase [3,4] and substantial nucleoprotein barriers to fork movement due to their very high  
41 affinity [5]. Individual nucleoprotein complexes may have a low probability of halting a replication  
42 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 facilitate 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 Δrep* 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 *Δrep ΔuvrD* 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 *Δrep* mutant cells but neither *ΔuvrD* nor  
147 *ΔdinG* 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 *Δrep ΔuvrD* double mutant inviability can be suppressed by growth of *Δrep ΔuvrD* mutant  
151 cells on minimal medium, conditions under which levels of transcription are reduced as compared  
152 with rich medium growth [22,30]. *Δrep ΔuvrD* 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 *Δrep ΔuvrD* 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 *Δrep ΔuvrD* 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

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

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

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

## 203 2. Materials and Methods

### 204 2.1. Plasmids and strains

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

212

### 213 2.2. Flow cytometry

214 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 cephalixin 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 cephalixin.  
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

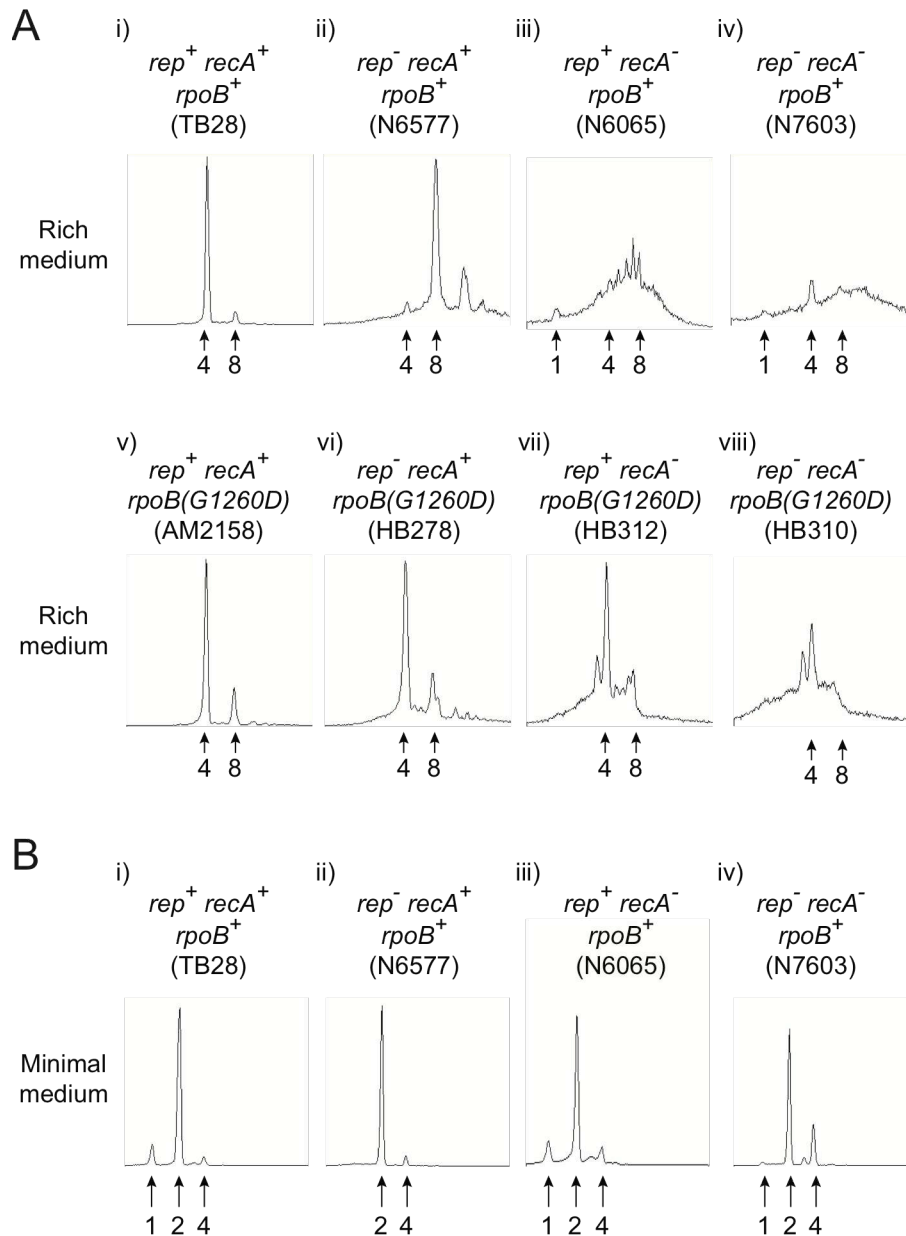
## 226 3. Results

### 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.

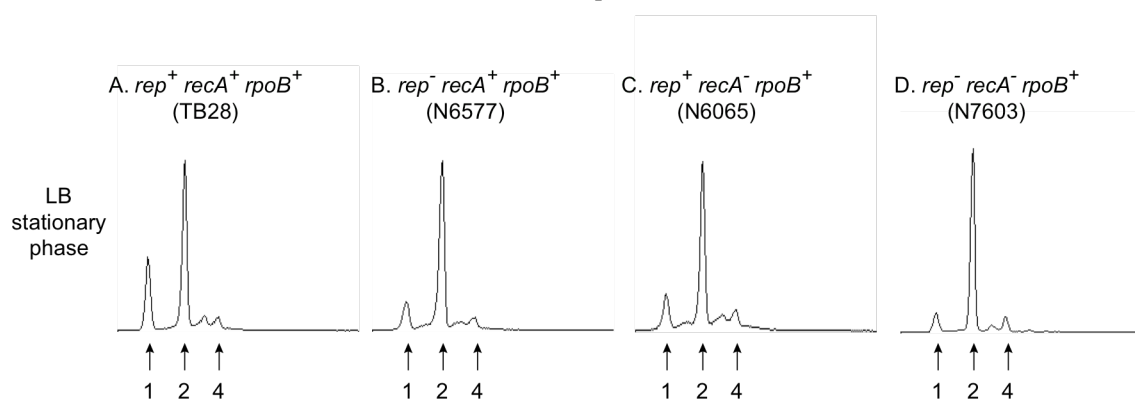
(A) DNA content of the indicated strains grown to mid-logarithmic phase in LB medium as monitored by flow cytometry under run out conditions. The number of chromosome equivalents is indicated below.

(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  $\Delta rep \Delta uvrD$  double mutant lethality and  
 240 suppression of chromosome replication defects in  $\Delta rep$  mutant 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<sup>+</sup>* and *rpoB(G1260D)* cells [6,55](also compare Figure 1Ai with v). In contrast,  
 243  $\Delta rep rpoB^+$  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  $\Delta rep$  mutant phenotype by reducing replisome  
 246 pausing, with most  $\Delta rep rpoB(G1260D)$  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.  $\Delta rep recA rpoB^+$  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<sup>+</sup>* strains in minimal  
 257 medium [22,30]. We tested therefore whether the major differences in DNA content in wild type  
 258 versus  $\Delta rep$ , *recA* or  $\Delta rep recA$  mutant cells seen in mid-logarithmic cells grown in rich medium  
 259 were recapitulated in minimal medium. We found that the majority of *rpoB<sup>+</sup>* cells either with or  
 260 without  $\Delta rep$  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  $\Delta rep recA$  mutant cells to remain viable even when so few

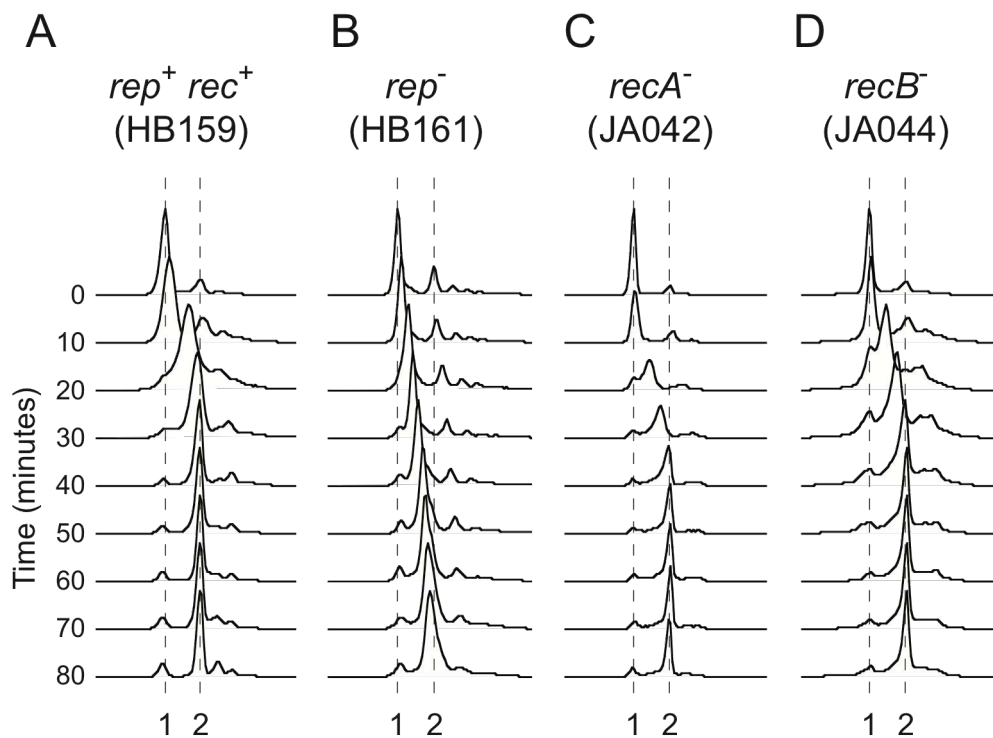
266 of the cells contain an integral number of chromosomes under run out conditions during  
 267 logarithmic growth in LB (Figure 1Aiv). Flow cytometric analyses of *rpoB*<sup>+</sup> strains grown to  
 268 stationary phase in LB revealed that the absence of functional Rep and/or RecA had little impact  
 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.

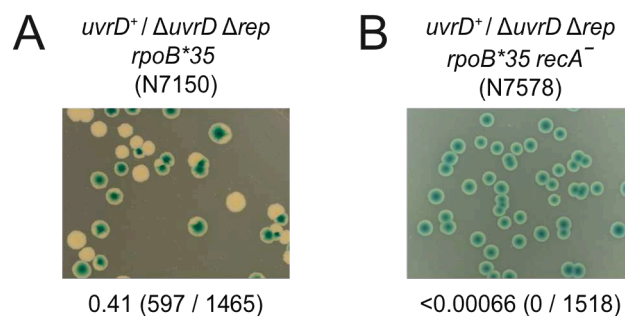
285 Upon synchronising replication initiation using the temperature-sensitive *dnaA46* allele, wild type  
 286 cells take 40-50 minutes for their DNA content to increase from 1 to 2 chromosome equivalents but  
 287  $\Delta rep$  cells take more than 80 minutes [31](see also Figure 3A and B). This extended duplication  
 288 time reflects the impact of nucleoprotein complexes on fork progression in the absence of Rep  
 289 [22,30]. In contrast to  $\Delta rep$  mutant cells, we found that the majority of *recA* mutant cells had  
 290 completed genome duplication after 40-50 minutes (Figure 3C). We also tested the time taken for  
 291 chromosome duplication in *recB* mutant cells. The requirement for either Rep or RecBCD for  
 292 survival implies that one or the other of these enzymes provides an essential means of  
 293 underpinning fork progression [71,72]. However, *recB* mutant cells had chromosome duplication  
 294 times similar to those found in wild type and *recA* mutant cells (Figure 3D).

295 These data demonstrate that absence of either RecA or RecBCD does not lead to significant  
 296 slowing of the mean time taken for replication forks to travel from *oriC* to the terminus region.  
 297 Processing of blocked replication forks by either RecA or RecBCD is therefore not critical for rapid  
 298 chromosome duplication.  
 299

### 300 3.3 Both RecA and RecBCD are needed in the absence of accessory helicase activity

301

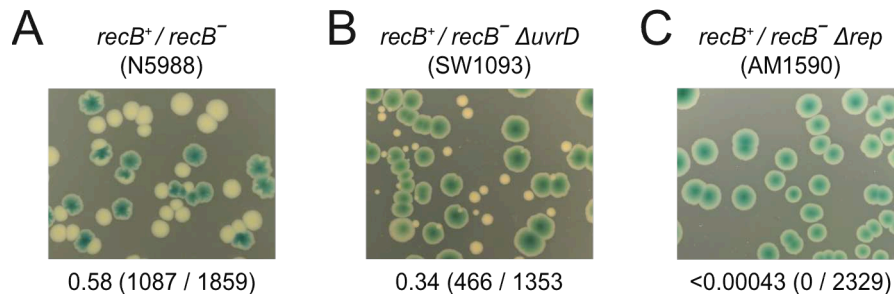
302 The above data suggest Rep rather than RecA plays the dominant role in ensuring rapid  
 303 genome duplication. It is clear, though, that transcription is a shared source of replicative defects  
 304 in cells deficient in either Rep or RecA (Figure 1). However, the viability of *rep recA* double mutant  
 305 cells [71] argues against a requirement for either Rep or RecA to overcome transcriptional barriers  
 306 to replication. Interpretation of *rep recA* double mutant viability is complicated, though, since  
 307 UvrD compensates partially for the absence of Rep accessory helicase function [22,30]. The  
 308 requirement for RecA was tested therefore in the absence of both Rep and UvrD by using a  $\Delta rep$   
 309  $\Delta uvrD$  double mutant strain rendered viable by the *rpoB\*35* allele via a reduction in  
 310 replication-transcription conflicts [22,42,79]. A plasmid loss assay was employed in which  
 311 retention of a highly unstable complementing plasmid can be monitored by blue/white screening  
 312 [87].  $\Delta rep \Delta uvrD rpoB*35$  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.

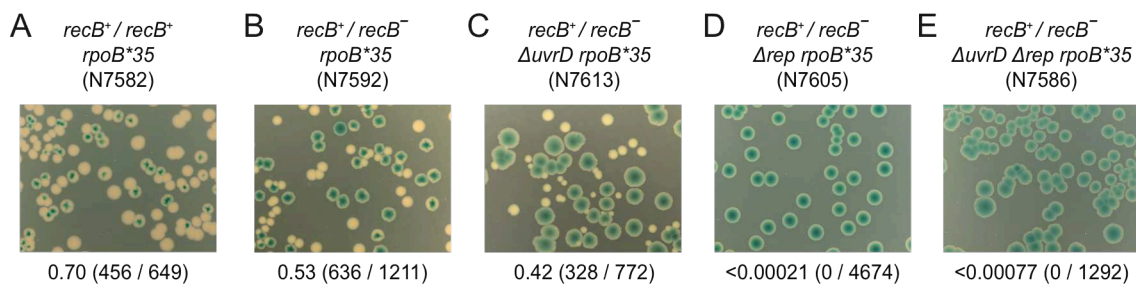
(A, B) The ability to form colonies in the absence of RecA was monitored in the indicated strains on LB plates containing Xgal and IPTG. The parental strains contain pAM407 (pRC7*uvrD*) bearing both the *uvrD* gene and the *lac* operon and plasmidless cells give rise to white or segregated colonies due 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,  $\Delta rep \Delta uvrD rpoB^*35 recA$  cells could not  
 314 lose pRC7uvrD on LB indicating that RecA is essential for viability in a  $\Delta rep \Delta uvrD rpoB^*35$  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.

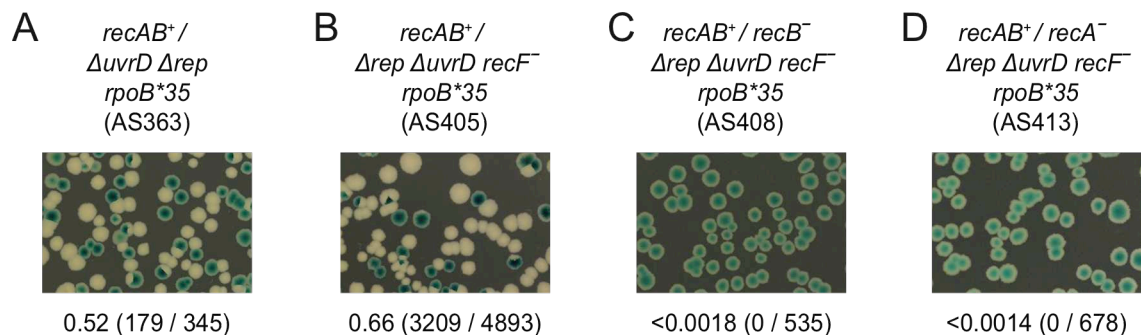
318 The corollary of  $\Delta rep \Delta uvrD rpoB^*35 recA$  inviability is that RecBCD is unable to maintain  
 319 viability without RecA in this context. This requirement for RecA is in contrast to the viability of  
 320 *rep recA* double mutant cells versus the inviability of *rep recB* double mutants in a  $rpoB^+$   
 321 background [71]. This differential requirement in *uvrD*<sup>+</sup> *rep*<sup>-</sup> 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  $\Delta uvrD recB^- rpoB^+$  cells by analysing their ability to lose pRC7*recB*.  $\Delta uvrD recB^- rpoB^+$   
 330 cells could generate white colonies on rich medium in contrast to  $\Delta rep recB^- rpoB^+$  cells (Figure 5,  
 331 compare B with C). However, the frequency of  $\Delta uvrD recB^- rpoB^+$  white colony formation was  
 332 lower and white colony sizes much smaller than with *uvrD*<sup>+</sup> *recB*<sup>-</sup>  $rpoB^+$  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.

334 growth is impaired.

335 *rpo* mutations that suppress  $\Delta rep \Delta uvrD$  double mutant lethality are unable to suppress  $\Delta rep$   
 336 *recB* double mutant lethality [78]. Similarly, reduction of transcription-driven replicative barriers  
 337 using *rpoB\*35* did not improve the viability of either  $\Delta rep recB$  or  $\Delta uvrD recB$  double mutant strains  
 338 (compare Figure 6C with Figure 5B; Figure 6D with 5C).  $\Delta rep \Delta uvrD rpoB*35 recB^-$  was also  
 339 inviable (Figure 6E), as expected given the growth defects of single *rep* and *uvrD* mutants  
 340 [71](Figure 6C and D). It was possible that UvrD not being available to abort RecFOR-directed  
 341 loading of RecA onto blocked replication forks [81,84] contributed to  $\Delta rep \Delta uvrD rpoB*35 recB^-$   
 342 inviability. However,  $\Delta rep \Delta uvrD rpoB*35 recF^- recB^-$  remained inviable, indicating that countering  
 343 RecFOR activity was not a major contributor to this inviability (Figure 7A-C).  $\Delta rep \Delta uvrD rpoB*35$   
 344 *recF^- recA^-* also remained inviable (Figure 7D), as expected given that RecFOR-dependent toxicity  
 345 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.

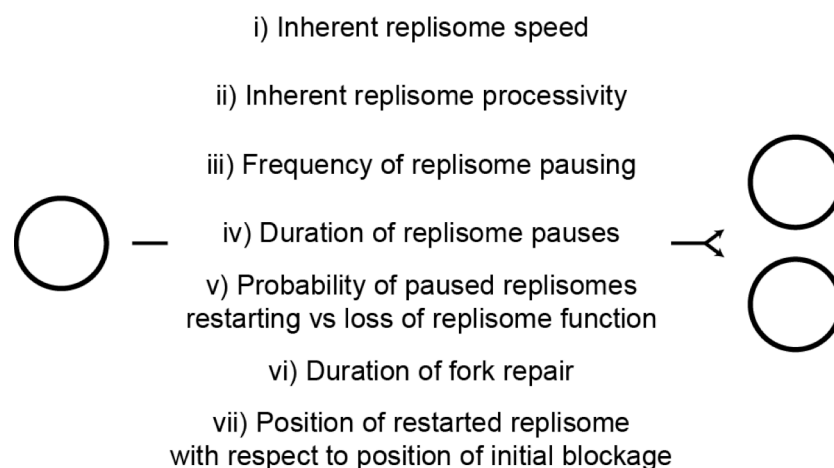
346 These data indicate that RecA (Figures 4B and 7D) and RecBCD (Figures 6E and 7C) are both  
 347 essential in  $\Delta rep \Delta uvrD$  mutant cells under rapid growth conditions even when  
 348 replication-transcription conflicts are reduced by a mutation in RNA polymerase. Thus when  
 349 accessory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

#### 353 4. Discussion

354 We show here that transcription is a major cause of the chromosomal degradation seen in *recA*  
 355 cells. The chromosome content defects of cells lacking either Rep [6] or RecA (Figure 1A, compare  
 356 iii with vii) share the same primary cause therefore indicating that both Rep and RecA reduce the  
 357 impact of gene expression on genome duplication. The synergistic increase in chromosome content  
 358 defects in *rep recA* cells indicate that these enzymes provide alternative means of mitigating the  
 359 impact of transcription on DNA replication (Figure 1Aiv). Furthermore, the significant  
 360 suppression of DNA degradation in *recA* cells by an RNA polymerase mutation supports the view  
 361 that protein-DNA complexes are the primary causes of replication defects in cells not exposed to  
 362 elevated DNA damage [6]. However, the time taken to duplicate a chromosome is not extended in  
 363 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  $\Delta rep \Delta uvrD$  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  $\Delta rep$  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  $\Delta rep$  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  $\Delta rep \Delta uvrD rpoB^{*35} recF^{-}$  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  $\Delta rep uvrD^{+}$  cells [22,30]. This partial compensation may explain why DNA  
428 degradation without RecA loading can maintain viability in  $\Delta rep uvrD^{+}$  cells but not in  $\Delta rep \Delta uvrD$   
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

433 problems in unstressed cells [6,22,30,78](Figure 1), recombination enzymes have the ability to deal  
 434 with replicative barriers other than protein-DNA complexes, unlike accessory replicative helicases  
 435 [1,7]. Thus under conditions of elevated replicative stress such as exogenous DNA damaging  
 436 agents then recombination enzymes may dominate replication repair. However, in otherwise  
 437 unstressed cells our data are consistent with the accessory helicase-dependent minimisation of  
 438 replisome pausing having a more significant impact on sustaining replisome movement than  
 439 recombination-directed replisome reloading mechanisms.

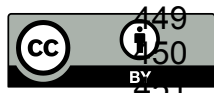
440 **Supplementary Materials:** The following are available online at [www.mdpi.com/link](http://www.mdpi.com/link), Table S1: *Escherichia coli*  
 441 K12 strains.

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

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

448



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

**A)** MG1655 derivatives

MG1655	F <sup>-</sup> <i>rph-1</i>	[1]
AM1573	$\Delta$ <i>lacIZYA recB270::kan</i>	P1.RJ1003 x TB28 to Km <sup>r</sup>
AM1590	pAM375 ( <i>lac<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA</i> $\Delta$ <i>rep::cat</i> <i>recB268::kan</i>	[2]
AM1657	$\Delta$ <i>uvrD::dhfr</i>	[3]
AM2158	$\Delta$ <i>lacIZYA rpoB</i> [G1260D]	[4]
AS301	$\Delta$ <i>lacIZYA rpoB*35</i> $\Delta$ <i>uvrD::dhfr</i> $\Delta$ <i>rep::cat</i>	Plasmid-free segregant of N7150
AS351	pAM383 ( <i>lac<sup>+</sup> recA<sup>+</sup></i> ) / $\Delta$ <i>recA::spec</i>	pAM383 x N7358 to Ap <sup>r</sup>
AS363	pAM406 ( <i>lac<sup>+</sup> recA<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> $\Delta$ <i>uvrD::dhfr</i> $\Delta$ <i>rep::cat</i>	pAM406 x AS301 to Ap <sup>r</sup>
AS370	pAM406 ( <i>lac<sup>+</sup> recA<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> $\Delta$ <i>uvrD::dhfr</i> $\Delta$ <i>rep::cat</i> <i>recB268::Tn10</i>	P1.BP45 x AS363 to Tc <sup>r</sup>
AS371	pAM406 ( <i>lac<sup>+</sup> recA<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> $\Delta$ <i>uvrD::dhfr</i> $\Delta$ <i>rep::cat</i> $\Delta$ <i>recA::spec</i>	P1.AS351 x AS363 to Spec <sup>r</sup>
AS405	pAM406 ( <i>lac<sup>+</sup> recA<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> $\Delta$ <i>uvrD::dhfr</i> $\Delta$ <i>rep::cat</i> $\Delta$ <i>recF735::&lt;kan&gt;</i>	P1.JW3677 x AS363 to Km <sup>r</sup>
AS408	pAM406 ( <i>lac<sup>+</sup> recA<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> $\Delta$ <i>uvrD::dhfr</i> $\Delta$ <i>rep::cat</i> $\Delta$ <i>recF735::&lt;kan&gt;</i> <i>recB268::Tn10</i>	P1.JW3677 x AS370 to Km <sup>r</sup>
AS413	pAM406 ( <i>lac<sup>+</sup> recA<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> $\Delta$ <i>uvrD::dhfr</i> $\Delta$ <i>rep::cat</i> $\Delta$ <i>recF735::&lt;kan&gt;</i> $\Delta$ <i>recA::spec</i>	P1.JW3677 x AS371 to Km <sup>r</sup>
BP45	$\Delta$ <i>ara714 argEC::[apra<sup>r</sup> lacO<sub>34</sub>] recB268::Tn10</i>	[5]
HB159	$\Delta$ <i>lacIZYA dnaA46 tna300::Tn10</i>	[6]
HB161	$\Delta$ <i>lacIZYA dnaA46 tna300::Tn10</i> $\Delta$ <i>rep::cat</i>	[6]
HB278	$\Delta$ <i>lacIZYA rpoB</i> [G1260D] $\Delta$ <i>rep::cat</i>	[7]
HB310	$\Delta$ <i>lacIZYA rpoB</i> [G1260D] $\Delta$ <i>rep::cat</i> <i>recA269::Tn10</i>	P1.N4279 x HB278 to Tc <sup>r</sup>
HB312	$\Delta$ <i>lacIZYA rpoB</i> [G1260D] <i>recA269::Tn10</i>	P1.N4279 x AM2158 to Tc <sup>r</sup>
JA042	$\Delta$ <i>lacIZYA dnaA46 tna300::Tn10</i> $\Delta$ <i>recA::kan</i>	P1.N6618 x HB159 to Km <sup>r</sup>
JA044	$\Delta$ <i>lacIZYA dnaA46 tna300::Tn10</i> <i>recB270::kan</i>	P1.N4600 x HB159 to Km <sup>r</sup>
N4279	<i>recA269::Tn10</i>	[8]
N4600	<i>recB270::kan</i>	P1.RJ1003 x MG1655
N5925	$\Delta$ <i>lacIZYA rpoB*35</i>	[4]
N5988	pAM375 ( <i>lac<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA recB270::kan</i>	pAM375 x AM1573 to Ap <sup>r</sup>
N6065	$\Delta$ <i>lacIZYA recA269::Tn10</i>	[2]
N6524	pAM403 ( <i>lac<sup>+</sup> rep<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA</i>	[4]
N6540	pAM403 ( <i>lac<sup>+</sup> rep<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA</i> $\Delta$ <i>rep::cat</i>	P1.JJC735 x N6524 to Cm <sup>r</sup>
N6577	$\Delta$ <i>lacIZYA</i> $\Delta$ <i>rep::cat</i>	[4]
N6618	$\Delta$ <i>recA::kan</i>	This work
N7150	pAM407 ( <i>lac<sup>+</sup> uvrD<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> $\Delta$ <i>uvrD::dhfr</i> $\Delta$ <i>rep::cat</i>	[4]
N7153	$\Delta$ <i>lacIZYA rpoB*35</i> $\Delta$ <i>uvrD::dhfr</i> $\Delta$ <i>rep::cat</i>	Plasmid-free segregant of N7150
N7358	$\Delta$ <i>recA::spec</i>	[9]

N7578	pAM407 ( <i>lac<sup>+</sup> uvrD<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> <i><math>\Delta</math>uvrD::dhfr <math>\Delta</math>rep::cat recA269::Tn10</i>	P1.N3072 x N7150 to Tc <sup>r</sup>
N7581	pAM375 ( <i>lac<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> <i><math>\Delta</math>uvrD::dhfr <math>\Delta</math>rep::cat</i>	pAM375 x N7153 to Ap <sup>r</sup>
N7582	pAM375 ( <i>lac<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i>	pAM375 x N5925 to Ap <sup>r</sup>
N7586	pAM375 ( <i>lac<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> <i>recB268::Tn10 <math>\Delta</math>uvrD::dhfr <math>\Delta</math>rep::cat</i>	P1.TRM308 x N7581 to Tc <sup>r</sup>
N7592	pAM375 ( <i>lac<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> <i>recB268::Tn10</i>	P1.TRM308 x N7582 to Tc <sup>r</sup>
N7602	pAM403 ( <i>lac<sup>+</sup> rep<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA <math>\Delta</math>rep::cat</i> <i>recA269::Tn10</i>	P1.N3072 x N6540 to Tc <sup>r</sup>
N7603	$\Delta$ <i>lacIZYA <math>\Delta</math>rep::cat recA269::Tn10</i>	Plasmid-free segregant of N7602
N7605	pAM375 ( <i>lac<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> <i>recB268::Tn10 <math>\Delta</math>rep::cat</i>	P1.JJC735 x N7592 to Cm <sup>r</sup>
N7613	pAM375 ( <i>lac<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA rpoB*35</i> <i>recB268::Tn10 <math>\Delta</math>uvrD::dhfr</i>	P1.AM1657 x N7592 to Tm <sup>r</sup>
RJ1003	<i>relA1 <math>\Delta</math>spoT207::cat rpoB*35 <math>\Delta</math>ruvAC65 eda-51::Tn10 recB270::kan</i>	[10]
SW1093	pAM375 ( <i>lac<sup>+</sup> recB<sup>+</sup></i> ) / $\Delta$ <i>lacIZYA recB270::kan</i> <i><math>\Delta</math>uvrD::dhfr</i>	P1.AM1657 x N5988 to Tm <sup>r</sup>
TB28	$\Delta$ <i>lacIZYA</i>	[11]
TRM308	<i>recB268::Tn10 sbcA</i>	[2]

## B) Other derivatives

JW3677	BW25113 <i>rrnB3 <math>\Delta</math>lacZ4787 hsdR514</i> <i><math>\Delta</math>(araBAD)567 <math>\Delta</math>(rhaBAD)568 rph-1</i> <i><math>\Delta</math>recF735::&lt;kan&gt;</i>	[12]
JJC735	AB1157 <i>hsdR <math>\Delta</math>rep::cat</i>	[13]
N3072	W3110 <i>rph-1 IN(rrnD-rrnE)1 recA269::Tn10</i>	[14]

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