# Modulation of RecFORQ- and RecA-mediated homologous recombination in *Escherichia coli* by isoforms of translation initiation factor IF2

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## Abstract

Homologous recombination (HR) is critically important for chromosomal replication as well as DNA damage repair in all life forms. In *Escherichia coli*, the process of HR is comprised of (i) two parallel pre-synaptic pathways that are mediated, respectively, by proteins RecB/C/D and RecF/O/R/Q; (ii) a synaptic step mediated by RecA that leads to generation of Holliday junctions (HJs); and (iii) post-synaptic steps mediated sequentially by HJ-acting proteins RuvA/B/C followed by proteins PriA/B/C of replication restart. Combined loss of RuvA/B/C and a DNA helicase UvrD is synthetically lethal, which is attributed to toxicity caused by accumulated HJs since viability in these double mutant strains is restored by removal of the pre-synaptic or synaptic proteins RecF/O/R/Q or RecA, respectively. Here we show that, as in Δ*uvrD* strains, *ruv* mutations confer synthetic lethality in cells deficient for transcription termination factor Rho, and that loss of RecFORQ pre-synaptic pathway proteins or of RecA suppresses this lethality. Furthermore, loss of IF2-1 (which is one of three isoforms [IF2-1, IF2-2, and IF2-3] of the essential translation initiation factor IF2 that are synthesized from three in-frame initiation codons in *infB*) also suppressed *uvrD-ruv* and *rho-ruv* lethalities, whereas deficiency of IF2-2 and IF2-3 exacerbated the synthetic defects. Our results suggest that Rho deficiency is associated with an increased frequency of HR that is mediated by the RecFORQ pathway along with RecA. They also lend support to earlier reports that IF2 isoforms participate in DNA transactions, and we propose that they do so by modulation of HR functions.

42 **Importance**

43 The process of homologous recombination (HR) is important for
44 maintenance of genome integrity in all cells. In *Escherichia coli*, the RecA protein
45 is a critical participant in HR, which acts at a step common to and downstream
46 of two HR pathways mediated by the RecBCD and RecFOR proteins, respectively.
47 In this study, an isoform (IF2-1) of the translation initiation factor IF2 has been
48 identified as a novel facilitator of RecA's function in vivo during HR.

## Introduction

Genetically first identified as a mechanism to mediate exchange of hereditary determinants between cells (1), the process of homologous recombination (HR) in bacteria has also been recognized to play a crucially important role in maintenance of genomic integrity both during chromosomal replication and following DNA damage, just as is the case in archaea and eukaryotes [reviewed in (2–11)]. In *Escherichia coli*, protein RecA is central to the synaptic step of HR: RecA binds a suitable single-strand (ss)-DNA substrate to form a nucleoprotein filament that performs a homology search to enable annealing to a second suitable DNA molecule. This synaptic step is flanked by pre-synaptic and post-synaptic reactions, as described below.

The pre-synaptic reactions are designed to generate the ss-DNA substrate for RecA's binding. Two alternative pre-synaptic pathways RecBCD and RecFOR (named after the principal proteins mediating them) exist to prepare substrates from, respectively, double-strand breaks (DSBs) and ss-gaps in DNA. Loss of either RecBCD or RecFOR pathway alone affects certain categories of DNA recombination and repair, whereas combined loss of both pathways confers a deficiency as severe as that with loss of RecA itself (3, 6).

The presence of RecA-bound nucleoprotein serves also as a trigger for an SOS response within the cell, by which several prophages are induced to enter lytic growth and genes of the LexA-repressed SOS regulon are transcriptionally activated. For the SOS response, RecA's co-protease activity is stimulated following nucleoprotein assembly to facilitate auto-cleavage of LexA and prophage repressors [reviewed in (12)].

The post-synaptic reactions act to generate discrete recombinant DNA molecules following annealing of the DNA molecules and formation of Holliday junctions (HJs). The RuvAB helicase and RuvC resolvase are primary mediators at this step, which catalyze branch migration and resolution of HJs, respectively (3). HJ resolution can also be achieved in absence of RuvABC, in *E. coli* cells harboring a mutation (*rus-1*) that activates expression of the protein Rus encoded by a cryptic prophage (13, 14); however, the physiological role if any of Rus is

80 unclear.

81 Included in the post-synaptic phase of HR are steps of replication restart,
82 by which products generated after resolution of HJs are assimilated into the
83 circular bacterial chromosome (or plasmid). Proteins mediating replication
84 restart include PriABC, DnaT, and Rep, which are proposed to act through
85 several alternative and redundant pathways (15–20).

86 Control of HR in *E. coli* is achieved by autoregulation (through the SOS
87 response) (12), as well as by several other factors (3, 21). One of the latter is
88 UvrD, which is a 3'-5' DNA helicase that also serves as an anti-recombinase to
89 disrupt RecA nucleoprotein filaments (22, 23). Loss of UvrD is associated with (i)
90 a hyper-recombination phenotype mediated by the RecFORQ pre-synaptic
91 pathway in HR assays, and (ii) synthetic lethality with *ruv* mutations, presumed
92 to be the consequence of accumulation of trapped HJs leading to impedance of
93 chromosomal replication (24–26). Both phenotypes are suppressed by loss of
94 RecA, which also indicates that hyper-recombination is not essential for viability
95 of *uvrD* mutants.

96 In this study we have identified that a deficiency of transcription
97 termination factor Rho also confers synthetic lethality with *ruv* mutations and
98 that, just as in the case with *uvrD-ruv* mutants, abrogation of the RecFORQ
99 pathway or of RecA rescues this lethality. Rho is an essential protein in *E. coli*
100 that mediates the termination of transcripts (other than rRNAs and tRNAs) which
101 are not being simultaneously translated (27–31). Rho's function has been
102 implicated in the maintenance of genomic integrity (32), and in avoidance of
103 RNA-DNA hybrids or R-loops from nascent untranslated transcripts including
104 from antisense RNAs (27, 28, 30, 33, 34).

105 Another finding being reported from this study is that both *rho-ruv* and
106 *uvrD-ruv* synthetic lethalities were suppressed by mutations in *infB*, that encodes
107 the translation initiation factor IF2 (35); we also show that these *infB* mutants
108 are down-regulated for HR functions. Studies of the past fifteen years from the
109 Nakai group had already identified an intriguing connection between IF2 on the

one hand and DNA transactions including DNA damage repair on the other (36–38). IF2 is essential for *E. coli* viability (35), and its function in translation initiation is conserved across bacteria, archaea, and eukaryotes (39, 40); a mammalian mitochondrial IF2 homolog can restore viability to an *E. coli* IF2 knockout strain (41). Three IF2 isoforms are synthesized in *E. coli* (see Supp. Fig. S3A), from in-frame initiation codons at positions 1,158, and 165 of the 890-codon-long *infB* ORF, that are herein designated as IF2-1, IF2-2 and IF2-3, respectively (42, 43). All isoforms are active for translation initiation and any one of them is sufficient for viability (44, 45). The Nakai lab had shown that isoforms IF2-1 and IF2-2,3 (the latter designation is used for the mixture of IF2-2 and IF2-3, since they are only marginally different from one another) behave differently in vitro in assays for phage Mu transposition, and that they confer differential tolerance in vivo to genotoxic agents. They had proposed that the isoforms exert varying influences on different pathways of replication restart (36–38).

In the accompanying paper (46), we show that *infB* mutants are also defective for two-ended DSB repair, which is mediated by RecA through the RecBCD-mediated pre-synaptic pathway. Based on these combined results, we propose that the IF2 isoforms differentially affect the efficiency of synapsis between DNA molecules during HR.

## Results

**The genetic assays to identify lethality of mutants and their suppression.** The genetic (blue-white) assay to demonstrate lethality has been described earlier (27, 47–50). This method makes use of a single-copy-number-shelter plasmid encoding trimethoprim (Tp)-resistance and carrying *lacZ*$^+$ as well as a functional copy of the gene(s) of interest, and whose partitioning into daughter cells during cell division is not stringently regulated. Consequently, when a strain with this plasmid, along with Δ*lac* and a mutation in the gene of interest on the chromosome, is grown in medium not supplemented with Tp, plasmid-free cells that arise spontaneously in the culture (at around 5 to 20%) will be

140 able to grow as white colonies on Xgal-supplemented plates if and only if the now
141 unsheltered chromosomal mutation is not lethal; on the other hand, control blue
142 colonies (formed from cells retaining the shelter plasmid) would be observed as
143 a majority in all situations. In the studies below, we have employed the blue-
144 white assay with a shelter plasmid carrying either *rho*$^+$ or both *rho*$^+$ and *infB*$^+$
145 genes to examine lethality or synthetic lethality of *rho*, *rho-ruv*, and *infB* genes.

146 ***rho-ruv* is synthetically lethal.** As mentioned above, transcription termination
147 factor Rho is essential for *E. coli* viability (27, 32). In this study, a *rho* mutant
148 was obtained with an *opal* (TGA) chain-terminating missense substitution in
149 codon 136, which was shown to be viable in strains carrying the *E. coli* K-12
150 version of the *prfB* gene encoding release factor 2 (RF2) but not in those carrying
151 the *E. coli* B version (Supp. Fig. S1A i-ii); there is evidence that the former but
152 not the latter permits a low frequency of stop codon-readthrough (51). This was
153 validated by Western blot analysis, which showed, for the *rho*-136$^{opal}$ mutant,
154 bands corresponding to both full-length (faint) and truncated Rho polypeptides
155 (Supp. Fig. S1B). Another viable *opal* mutant in codon 157 of *rho* has been
156 reported recently in *E. coli* K-12 (52).

157 The *rho*-136$^{opal}$ mutation was synthetically lethal with disruptions of the
158 *ruv* genes (Δ*ruvA*, Δ*ruvC*, or Δ*ruvABC*) encoding the HJ enzymes RuvAB or RuvC,
159 and the phenotype was manifested on both rich and defined media (see, for
160 example, Fig. 1A ii-iii, Fig. 2i and Supp. Fig. S2 i). On the other hand, *rho*-136$^{opal}$
161 was not lethal with *recA* (Fig. 1A iv). These results are consistent with those from
162 an earlier report that *ruv*, but not *recA*, mutants are hyper-sensitive to the Rho
163 inhibitor bicyclomycin (32).

164 Synthetic *rho-ruv* lethality was suppressed by the *rus-1* mutation (Supp.
165 Fig. S2 ii), which activates expression of the Rus resolvase from a cryptic
166 prophage and thereby alleviates several *ruv* phenotypes (13, 14). The lethality
167 was likewise suppressed by the *rpoB***35* mutation (53–55) (Supp. Fig. S2 iii), as
168 also by ectopic expression of the (phage T4-encoded) R-loop helicase UvsW but

169 not its active site mutant version UvsW-K141R (56, 57) (Supp. Fig. S2 iv-v). Both
170 *rpoB*35* and UvsW alleviate the deleterious effects of Rho deficiency and can
171 rescue Δ*rho* lethality (27, 28, 32).

172 **Loss of RecA, or of RecFOR pathway components, suppress *rho-ruv***
173 **lethality.** Given the observations that the *rho*-136$^{opal}$ mutation was lethal with
174 *ruv* but not with *recA*, the possibility was considered that excessive (and
175 unnecessary) HR triggered in presence of the *rho* mutation was leading to
176 accumulation of HJ intermediates in absence of RuvABC, resulting in cell death.
177 Indeed, we could show that *rho-ruv* lethality is also rescued by Δ*recA* (Fig. 1A v,
178 and Supp. Fig. S2 vii). An alternative explanation, that death was on account of
179 excessive RecA-dependent SOS induction in *rho-ruv* mutants, was excluded
180 since lethality was not rescued by the *lexA3* mutation [which encodes a LexA
181 variant that is non-cleavable by RecA, and hence also abolishes SOS induction
182 (12)] (Supp. Fig. S2 viii).

183 We then tested which of the two pre-synaptic pathways putatively feeds
184 into the process of excessive HR in *rho-ruv* mutants. The synthetic lethality was
185 suppressed by mutations in *recO* or *recR* (of the RecFOR pathway) (Fig. 1A vi-
186 vii), but not by mutation in *recB* (of the RecBC pathway) (Fig. 1A viii). RecQ
187 helicase activity is implicated in RecFOR pathway function (3, 6), and Δ*recQ* also
188 was a suppressor of *rho-ruv* lethality (Fig. 1A ix).

189 **Suppression of *rho-ruv* lethality by loss of IF2-1.** A new suppressor of *rho-ruv*
190 lethality on both defined and rich media (Fig. 1A x, and Supp. Fig. S2 vi) was
191 obtained and characterized. It was mapped to the *infB-nusA* locus, and was
192 shown by DNA sequencing to be an *ochre* (TAA) nonsense codon mutation at
193 position 161 of the *infB* ORF. One would expect, from its location in the *infB*
194 ORF, that this mutation abrogates synthesis of the IF2-1 and IF2-2 isoforms,
195 which was confirmed by Western blotting (Supp. Fig. S3C, lane 6).

196 By itself, the *infB*-161$^{ochre}$ mutation was lethal (Supp. Fig. S3B i) but it
197 could be rescued by *rho* mutation (Supp. Fig. S3B ii). We interpret these findings

198 as indicative of the notions (i) that nonsense substitution at codon 161 in *infB*
199 induces Rho-mediated premature transcription termination within the gene, and
200 therefore (ii) that synthesis of the lone IF2-3 isoform to ensure viability is itself
201 contingent on relief of transcriptional polarity conferred by *rho* mutation.

202 Thus, viability of a triple mutant *rho ruv infB*-161$^{ochre}$ is based on mutual
203 suppression (i) of *infB*-161$^{ochre}$ lethality by *rho*, and (ii) of *rho ruv* lethality by *infB*-
204 161$^{ochre}$. This inference was supported by findings from experiments in which
205 expression of an ectopically located *rho*$^{+}$ gene was placed under control of an L-
206 arabinose (Ara)-inducible promoter (47) (Fig. 1B): that a *rho-ruv* derivative (row
207 3) is viable only on Ara-supplemented medium, whereas an *infB*-161$^{ochre}$-*rho*
208 mutant (row 2) as well as the triple mutant *infB*-161$^{ochre}$-*rho-ruv* (row 4) are viable
209 only on medium not supplemented with Ara. The *infB-rho* mutant was inhibited
210 for growth at 22° (Supp. Fig. S3D), consistent with the known requirement for
211 IF2 at low temperatures (58).

212 **Suppression of *rho-ruv* lethality by ectopic *infB* constructs.** Based on the
213 suppressor characterization results above, we surmised that it is the loss of
214 isoforms IF2-1 or IF2-2 (or both together), which confers suppression of *rho-ruv*
215 lethality. Accordingly, we then tested two other sets of ectopic chromosomal *infB*
216 constructs for their ability to rescue *rho-ruv* lethality, in derivatives carrying a
217 deletion of the native *infB* locus. (In the descriptions below, the designations
218 *infB*$^{+}$ and Δ*infB* are used for the wild-type and deletion alleles, respectively, at
219 the native chromosomal location).

220 In one set of three ectopic constructs, which has been described earlier by
221 Nakai and coworkers (37, 38), IF2 expression remains under control of the
222 natural *cis* regulatory elements for *infB* but not all isoforms are expressed from
223 the different constructs (designation used in this study for each of them given in
224 parentheses): that encodes only IF2-1, but not IF2-2 or IF2-3 (Δ*2,3*); that encodes
225 both IF2-2 and IF2-3, but not IF2-1 (Δ*1*); and that encodes all three isoforms
226 (Δ*Nil*). The constructs were validated for expression of the different IF2 isoforms

227 by Western blot analysis (Supp. Fig. S3C, lanes 2 to 5 and lane 9). Upon testing
228 in the *rho-ruv* strains, our results showed that the Δ*1* construct, but not Δ*2,3* or
229 Δ*Nil*, could suppress *rho-ruv* synthetic lethality (Fig. 2 and Supp. Fig. S4,
230 compare in each case iii with i and ii). These findings are consistent with that of
231 *rho-ruv* suppression by *infB*-161$^{ochre}$, since the latter also fails to express isoform
232 IF2-1.

233 The second set of ectopic chromosomal constructs were prepared in this
234 work and were designed for differential expression of IF2 isoforms from an
235 isopropyl-β-D-thiogalactoside (IPTG)-inducible heterologous $P_{trc}$ promoter
236 (designation used for each given in parentheses): that expresses both IF2-2 and
237 IF2-3, but not IF2-1 ($P_{trc}$-Δ*1*); and that expresses IF2-3 alone ($P_{trc}$-Δ*1,2*) (see Supp.
238 Fig. S3C, lanes 8 and 7, respectively, for Western blot confirmation). Both
239 constructs could suppress *rho-ruv* lethality (Fig. 2 iv and Supp. Fig. S4 iv-v),
240 indicating once again that lethality suppression occurs when isoform IF2-1 is
241 absent from the cells. Similar results were obtained irrespective of which *ruv*
242 mutation (Δ*ruvA*, Δ*ruvC*, or Δ*ruvABC*) was used in the experiments (Fig. 2 iv; and
243 Supp. Fig. S4 iv-v). With induced IF2-3 expression from the IPTG-regulated
244 construct, suppression was obtained even in derivatives carrying the native *infB*$^+$
245 locus, but the colony growth was less robust (Fig. 2 v).

246 These results indicate that it is an imbalance between levels of isoforms
247 IF2-1 (low) and IF2-2,3 (high) that determines suppression of *rho-ruv* lethality.
248 This suppression is not because of a restoration (or bypass) of Rho or Ruv
249 function in these strains, since loss of IF2-1 did not reverse (i) the phenotypes
250 associated with *rho* mutation (33), such as a Gal$^+$ phenotype that follows relief of
251 premature transcription termination in the *gal* operon caused by *galEp3*
252 mutation (Supp. Fig. S1C, compare iii-iv with ii), or lethality caused by runaway
253 replication of plasmid pACYC184 (Supp. Fig. S1A iii-v); nor (ii) the UV-sensitivity
254 known for *ruv* mutants (3, 59), and indeed it aggravated the latter phenotype
255 (Supp. Fig. S5A).

256 **Loss of IF2-2,3 exacerbates *rho-ruv* defect.** The following experiments lent
257 additional support to the notion that *rho-ruv* lethality is modulated by the
258 balance between isoforms IF2-1 and IF2-2,3. Derivatives with the missense *rho-*
259 *4* mutation (encoding Rho-A243E (33), see Supp. Fig. S1B lane 2 for Western
260 blot) may be expected to be less compromised for Rho function than those with
261 *rho*-136$^{opal}$, and a test for intracellular R-loop prevalence with monoclonal
262 antibody S9.6 (28, 60) showed this to be so (Supp. Fig. S1D). Unlike *rho*-136$^{opal}$,
263 the *rho-4* mutation was not synthetically lethal with *ruv* in the Nakai Δ*Nil* strain
264 expressing all three IF2 isoforms (Fig. 2 vi). However, this combination (*rho-4 ruv*)
265 conferred lethality in the Δ*2,3* derivative (lacking IF2-2,3) (Fig. 2 vii), and this
266 lethality was rescued by IPTG-induced expression of IF2-2,3 (Fig. 2 viii). Thus,
267 absence of IF2-1 and of IF2-2,3 exert the apparently opposite phenotypes of,
268 respectively, alleviating and exacerbating the RecFORQ- and RecA-mediated
269 sickness of *rho-ruv* mutants.

270 **Synthetic lethality of *uvrD-ruv* is also suppressed by loss of IF2-1.** Several
271 groups have earlier reported synthetic *uvrD-ruv* lethality (24–26), which is
272 suppressed by *recA* and *recFORQ* but not *recBC* (that is, very similar to our
273 findings with *rho-ruv* lethality). The mechanism invoked has been that of
274 excessive and unnecessary HR in the *uvrD* mutant, leading to accumulation of
275 toxic recombination intermediates in absence of RuvABC.

276 To test whether IF2 isoforms affect *uvrD-ruv* lethality, we expressed (from
277 a doxycycline [Dox]-inducible promoter) a dominant-negative RuvC protein
278 designated as RDG, that binds and traps HJs (59); these experiments were done
279 in a set of Δ*uvrD* Δ*infB* derivatives each carrying one of the ectopically integrated
280 Nakai constructs for different IF2 isoforms. Viability of the Δ*Nil* and Δ*2,3* strain
281 derivatives was reduced $10^3$- and $10^4$-fold, respectively, upon Dox addition
282 whereas the Δ*1* derivative was only minimally affected (Fig. 3). A *recA* derivative
283 of the Δ*Nil* strain survived Dox, as too did the control *uvrD*$^+$ *recA*$^+$ strain (Fig. 3,
284 last and first rows, respectively). These results confirm that *uvrD-ruv* is
285 synthetically lethal (more severely so in absence of IF2-2,3), and that this

286 lethality is rescued upon loss of RecA or of IF2-1.

287 **HR frequency is oppositely affected by loss of IF2-1 and of IF2-2,3, and is**
288 **elevated in *rho* mutants.** The results above had indicated that loss of IF2-1
289 phenocopies the loss of RecA or of the pre-synaptic RecFORQ pathway to confer
290 suppression of *rho-ruv* and *uvrD-ruv* lethalities. We then tested the differential
291 effects, if any, of IF2 isoforms on recovery of recombinants following HR, for
292 which we employed several assays such as those of phage P1 transduction, inter-
293 plasmidic recombination (that leads to reconstitution of a tetracycline (Tet)-
294 resistance gene from two partially overlapping deletion alleles) (61–64), and the
295 Konrad assay (that similarly entails reconstitution of an intact *lacZ* gene from a
296 split pair of partially overlapping *lacZ* fragments located at distant sites on the
297 chromosome) (65, 66). Recombination events in each of the assays above are
298 RecA-dependent (3, 61, 65); inter-plasmidic recombination is mediated by the
299 RecFOR pre-synaptic pathway (61, 63, 64), whereas P1 transduction and
300 recombination in the Konrad assay are RecBCD-dependent (65).

301 In the HR assays, loss of IF2-1 and of IF2-2,3 were associated with
302 moderate reduction and elevation, respectively, in recovery of recombinants in
303 comparison with values for the Δ*Nil* strain (Fig. 4A-C). A moderate reduction in
304 P1 transduction frequency for the strain lacking IF2-1 has also been reported
305 earlier (37).

306 As expected (22, 65, 66), loss of UvrD conferred a hyper-recombination
307 phenotype (Fig. 4A-B), but the HR frequency in a derivative that had lost both
308 UvrD and IF2-1 was once again low and resembled that in a strain lacking IF2-
309 1 alone (Fig. 4A, compare columns 3 and 6). Thus, loss of IF2-1 is epistatic to
310 Δ*uvrD*, indicating that UvrD's function in HR precedes and is in the same
311 pathway as that of IF2-1, but that the two act oppositely.

312 Since the results above suggested that loss of IF2-1 is associated with
313 reduced RecA function in HR (see *Discussion*), we examined whether there is
314 reduction in SOS induction [which is mediated by RecA's coprotease activity that

315 is activated upon binding to ss-DNA (12)] as well in this situation. The results
316 indicate that, as measured by *sulA-lac* expression (67), the SOS response (both
317 basal as well as that following DNA damage with phleomycin) is not decreased
318 and may in fact be modestly elevated in the Δ*1* strain (Supp. Fig. S5B).

319 Our finding of *rho-ruv* synthetic lethality that is suppressed by *recA* also
320 suggests, based on its parallels with *uvrD-ruv* lethality, that non-essential HR
321 occurs at elevated frequency in the *rho*-136$^{opal}$ mutant. Measurements of HR
322 frequency, both by the Konrad assay and by conjugation, indicate that the *rho*
323 mutant does exhibit a moderate increase in HR frequency (Supp. Fig. S1E).

324 **Discussion**

325 The major findings of this study are (i) that Rho deficiency is synthetically
326 lethal with *ruv* mutations in a manner that is similar to *uvrD-ruv* lethality (with
327 trapped HJs, generated through the RecFORQ pre-synaptic pathway and RecA,
328 being responsible for inviability in both cases); and (ii) that an imbalance of
329 isoforms of the translation initiation factor IF2 affects HR functions in the cells.
330 Each of these is further discussed below.

331 **Why is *rho-ruv* lethal?** Several features are shared between synthetic lethalities
332 *rho-ruv* and *uvrD-ruv*, suggesting commonality of mechanisms in the two
333 instances. Thus, both lethalities require proteins RecA, RecFORQ, and
334 translation initiation factor isoform IF2-1.

335 In case of *uvrD-ruv*, the model proposed by Rosenberg and colleagues (24)
336 is that in absence of UvrD, there is excessive but unnecessary HR through the
337 RecFORQ pathway, which then renders RuvABC essential for resolving the
338 ensuing HJ intermediates. Likewise for the *rho* mutant, we suggest that on
339 account of an increased prevalence of R-loops with displaced ss-DNA, increased
340 HR is triggered through the RecFOR pathway thus necessitating RuvABC's
341 presence for viability (Fig. 5, panels a-c).

342 An alternative model for *rho-ruv* lethality, that also invokes R-loops, is that

343 depicted in Figure 5, panels d to f. In this model, a chromosomal R-loop in the
344 *rho* mutant serves to inhibit replisome progression (68), which is then followed
345 by RecA- and RecFOR-mediated replication fork reversal (69–71). Since the
346 extruded duplex includes an RNA-DNA hybrid, the fork cannot be restored
347 through RecBCD action and hence the action of RuvABC at the HJ becomes
348 essential for viability. The two models are not mutually exclusive.

349 In both models, UvsW expression and *rpoB*35* are suppressors of *rho-ruv*
350 lethality because they presumably act to reduce R-loop prevalence in Rho-
351 deficient strains (27, 28, 32).

352 **Opposing effects of IF2-1 and IF2-2,3 isoforms in HR pathways.** Early studies
353 had established that isoforms IF2-1 and IF2-2,3 are together required for optimal
354 growth of *E. coli* (45). Nakai and coworkers (37, 38) have reported that loss of
355 IF2-1 or of IF2-2,3 is each associated with sensitivity to different kinds of DNA
356 damage.

357 In our study, loss of IF2-1 or of IF2-2,3 was associated with suppression
358 or aggravation, respectively, of *rho-ruv* and *uvrD-ruv* sickness. The model for
359 opposing effects of these isoforms is supported also by our findings in HR assays.
360 At a mechanistic level, however, it is unclear whether a particular phenotype is
361 caused by absence of one IF2 isoform or exclusive presence of another.

362 **How do IF2 isoforms influence HR?** The studies reported in this and in the
363 accompanying paper (46), taken together, have identified IF2 isoforms as novel
364 players in HR, but the mechanisms by which they act for this purpose are
365 unknown. The present study has shown that loss of IF2-1 (i) phenocopies loss of
366 the RecFORQ pre-synaptic pathway and of RecA in suppressing *rho-ruv* and
367 *uvrD-ruv* lethalities, and (ii) reduces the recovery of recombinants following HR.
368 In the accompanying paper (46), it is shown that loss of IF2-1 confers profound
369 sensitivity to two-ended DSBs, whose repair is mediated by the RecBCD pre-
370 synaptic pathway along with RecA.

371 The model we propose is that absence of IF2-1 leads directly or indirectly
372 to reduction in efficiency of a step in HR which is (i) downstream of (and common
373 to) the RecBCD and RecFOR pathways, and (ii) upstream of the post-synaptic
374 reactions mediated by RuvABC. Formation of the RecA nucleoprotein itself is
375 apparently unaffected, since the SOS response (12) is not perturbed by loss of
376 IF2-1. In a further elaboration of this model in the accompanying paper (46), it
377 is the strength of RecA-mediated annealing between a pair of homologous DNA
378 molecules that is postulated to be decreased in absence of IF2-1.

379 The proposed model is consistent with our finding that IF2-1's role in HR
380 is downstream to that of UvrD, which acts to modulate efficiency of RecA
381 nucleoprotein formation on DNA substrates generated by the two alternative pre-
382 synaptic pathways (22, 23). Deficiency of IF2-2,3 is proposed to have the opposite
383 effect, of enhancing the efficiency of the same step in HR as that diminished by
384 loss of IF2-1.

385 Our model that loss of IF2-1 compromises RecA function is different from
386 that of Nakai and colleagues (37, 38), who proposed that IF2 isoforms
387 differentially influence different replication restart pathways (which are post-
388 synaptic, and downstream of RuvABC action). As explained above, our finding
389 that loss of IF2-1 phenocopies the *recA* mutation in suppressing *rho-ruv* and
390 *uvrD-ruv* lethalities can best be explained only by invoking a role for IF2-1 prior
391 to the step of RuvABC action.

392 To test the models above, in vitro studies may be needed to examine
393 whether IF2 isoforms act directly to modulate HR, and to determine their precise
394 role(s) in the process. Regulation of HR and of recombinational repair functions
395 is important in both prokaryotes and eukaryotes (10, 72), and factors previously
396 identified for such regulation in *E. coli* include UvrD, mismatch repair proteins,
397 DinI, and RecX (3, 21, 22, 73, 74).

398 **Materials and Methods**

399 **Growth media, bacterial strains and plasmids**. The routine rich and defined

400 growth media were, respectively, LB and minimal A with 0.2% glucose (75) and,
401 unless otherwise indicated, the growth temperature was 37°. Supplementation
402 with Xgal and with antibiotics ampicillin (Amp), chloramphenicol (Cm),
403 kanamycin (Kan), spectinomycin (Sp), Tet, and Tp were at the concentrations
404 described earlier (47). Phleomycin supplementation was at 3 μg/ml. For
405 induction of gene expression from the appropriate regulated promoters, Ara,
406 Dox, and IPTG were added at 0.2%, 50 ng/ml, and 0.5 mM, respectively. *E. coli*
407 strains used are listed in Supplementary Table S1, with the following knockout
408 ($Kan^R$ insertion-deletion) alleles sourced from the collection of Baba et al. (76):
409 *dinF*, *ilvA*, *leuA*, *leuD*, *racC*, *recA*, *recB*, *recO*, *recQ*, *recR*, *ruvA*, *serA*, *thrA*, *uvrD*,
410 *ybfP*, and *yihF*; the Δ*infB* knockout mutation has also been described earlier (41).
411 Plasmids described earlier include pBR322 ($Tet^R$ $Amp^R$, ColE1 replicon)
412 (77); pACYC184 ($Tet^R$ $Cm^R$, p15A replicon) (78); pCL1920 ($Sp^R$, pSC101 replicon)
413 (79); pMU575 ($Tp^R$, single-copy-number vector with *lacZ*$^+$) (80); pHYD2411 ($Tp^R$,
414 pMU575 derivative with *rho*$^+$) (27); and pTrc99A ($Amp^R$, for IPTG-inducible
415 expression of gene of interest) (81). Plasmids pKD13 ($Kan^R$ $Amp^R$), pKD46 ($Amp^R$),
416 and pCP20 ($Cm^R$ $Amp^R$), for use in recombineering experiments and for Flp-
417 mediated site-specific excision of FRT-flanked DNA segments, have been
418 described by Datsenko and Wanner (82). Plasmids constructed in this study are
419 described in the *Supplementary Text*.
420 **Methods.** Procedures for P1 transduction (83), recombineering on the
421 chromosome or plasmids (82), determination of UV tolerance (75), and R-loop
422 detection with S9.6 monoclonal antibody (28) were as described. The Miller
423 protocol was followed for β-galactosidase assays (75), and enzyme specific
424 activity values are reported in the units defined therein. Protocols of Sambrook
425 and Russell (84) were followed for recombinant DNA manipulations, PCR, and
426 transformation. The Western blotting procedure, with rabbit polyclonal anti-IF2
427 or anti-Rho antisera (kind gifts from Umesh Varshney and Ranjan Sen,
428 respectively), was essentially as described (49). Chromosomal integration, at the
429 phage λ *att* site, of pTrc99A derivatives expressing IF2-2,3 or IF2-3, was achieved

430 by the method of Boyd et al. (85). HR assay methods are described in the
431 *Supplementary Text*, and include those based on conjugation (75), the Konrad
432 assay (65), and inter-plasmid recombination (61, 62).

433 **Supplemental material**

434 Supplemental material is provided as a PDF file "Supplemental File 1".

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446 **References**

447 1. Clark AJ. 1973. Recombination deficient mutants of *E. coli* and other
448 bacteria. Annu Rev Genet 7:67–86.

449 2. Kuzminov A. 1995. Collapse and repair of replication forks in *Escherichia*
450 *coli*. Mol Microbiol 16:373–384.

451 3. Kuzminov A. 1999. Recombinational Repair of DNA Damage in *Escherichia*
452 *coli* and Bacteriophage λ. Microbiol Mol Biol Rev 63:751–813.

453 4. Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Marians KJ.
454 2000. The importance of repairing stalled replication forks. Nature 404:37-
455 41.

456 5. Sinha AK, Possoz C, Leach DRF. 2020. The Roles of Bacterial DNA Double-
457 Strand Break Repair Proteins in Chromosomal DNA Replication. FEMS
458 Microbiol Rev 44:351-368.

459 6. Kowalczykowski SC. 2000. Initiation of genetic recombination and
460 recombination-dependent replication. Trends Biochem Sci 25:156–165.

461 7. Lenhart JS, Schroeder JW, Walsh BW, Simmons LA. 2012. DNA Repair and
462 Genome Maintenance in *Bacillus subtilis*. Microbiol Mol Biol Rev 76:530–
463 564.

464 8. Costes A, Lambert SAE. 2013. Homologous recombination as a replication
465 fork escort: Fork-protection and recovery. Biomolecules 3:39–71.

466 9. Bianco PR, Lu Y. 2021. Single-molecule insight into stalled replication fork
467 rescue in *Escherichia coli*. Nucleic Acids Res 49:4220–4238.

468 10. Kowalczykowski SC. 2015. An Overview of the Molecular Mechanisms of
469 Recombinational DNA Repair. Cold Spring Harb Perspect Biol 7:a016410.

470 11. Bell JC, Kowalczykowski SC. 2016. Mechanics and Single-Molecule
471 Interrogation of DNA Recombination. Annu Rev Biochem 85:193–226.

472 12. Simmons LA, Foti JJ, Cohen SE, Walker GC. 2008. The SOS Regulatory
473 Network. EcoSal Plus 3. doi:10.1128/ecosalplus.5.4.3.

474 13. Mandal TN, Mahdi AA, Sharples GJ, Lloyd RG. 1993. Resolution of Holliday
475 intermediates in recombination and DNA repair: Indirect suppression of
476 *ruvA*, *ruvB*, and *ruvC* mutations. J Bacteriol 175:4325–4334.

477 14. Sharples GJ, Chan SN, Mahdi AA, Whitby MC, Lloyd RG. 1994. Processing
478 of intermediates in recombination and DNA repair: Identification of a new
479 endonuclease that specifically cleaves Holliday junctions. EMBO J
480 13:6133–6142.

481 15. Michel B, Sinha AK, Leach DRF. 2018. Replication Fork Breakage and
482 Restart in *Escherichia coli*. Microbiol Mol Biol Rev 82:e00013-18.

483 16. Gabbai CB, Marians KJ. 2010. Recruitment to Stalled Replication Forks of
484 the PriA DNA Helicase and Replisome-loading Activities is Essential for
485 Survival. DNA Repair 9:202–209.

486 17. Michel B, Sandler SJ. 2017. Replication restart in bacteria. J Bacteriol
487 199:1–13.

488 18. Heller RC, Marians KJ. 2006. Replisome assembly and the direct restart of
489 stalled replication forks. Nat Rev Mol Cell Biol. 7:932-943.

490 19. Huang YH, Huang CY. 2014. Structural insight into the DNA-binding mode
491 of the primosomal proteins PriA, PriB, and AnaT. Biomed Res Int
492 http://dx.doi.org/10.1155/2014/195162.

493 20. Sandler SJ, Leroux M, Windgassen TA, Keck JL. 2021. *Escherichia coli* K-
494 12 has two distinguishable PriA-PriB replication restart pathways. Mol

495 Microbiol 116:1140–1150.

496 21. Cox MM. 2007. Regulation of bacterial RecA protein function. Crit Rev
497 Biochem Mol Biol. Crit Rev Biochem Mol Biol 42:41–63.

498 22. Veaute X, Delmas S, Selva M, Jeusset J, Le Cam E, Matic I, Fabre F, Petit
499 MA. 2005. UvrD helicase, unlike Rep helicase, dismantles RecA
500 nucleoprotein filaments in *Escherichia coli*. EMBO J 24:180–189.

501 23. Centore RC, Sandler SJ. 2007. UvrD limits the number and intensities of
502 RecA-green fluorescent protein structures in *Escherichia coli* K-12. J
503 Bacteriol 189:2915–2920.

504 24. Magner DB, Blankschien MD, Lee JA, Pennington JM, Lupski JRR,
505 Rosenberg SM. 2007. RecQ Promotes Toxic Recombination in Cells Lacking
506 Recombination Intermediate-Removal Proteins. Mol Cell 26:273–286.

507 25. Zhang J, Mahdi AA, Briggs GS, Lloyd RG. 2010. Promoting and avoiding
508 recombination: Contrasting activities of the *Escherichia coli* RuvABC
509 Holliday junction resolvase and RecG DNA translocase. Genetics 185:23–
510 37.

511 26. Florés MJ, Sanchez N, Michel B. 2005. A fork-clearing role for UvrD. Mol
512 Microbiol 57:1664–1675.

513 27. Leela JK, Syeda AH, Anupama K, Gowrishankar J. 2013. Rho-dependent
514 transcription termination is essential to prevent excessive genome-wide R-
515 loops in *Escherichia coli*. Proc Natl Acad Sci U S A 110:258–263.

516 28. Raghunathan N, Kapshikar RM, Leela JK, Mallikarjun J, Bouloc P,
517 Gowrishankar J. 2018. Genome-wide relationship between R-loop
518 formation and antisense transcription in *Escherichia coli*. Nucleic Acids Res
519 46:3400–3411.

520 29. Grylak-Mielnicka A, Bidnenko V, Bardowski J, Bidnenko E. Transcription
521 termination factor Rho: a hub linking diverse physiological processes in
522 bacteria. 10.1099/mic.0.000244.

523 30. Gowrishankar J, Harinarayanan R. 2004. Why is transcription coupled to
524 translation in bacteria? Mol Microbiol 54:598–603.

525 31. Peters JM, Mooney RA, Grass JA, Jessen ED, Tran F, Landick R. 2012. Rho
526 and NusG suppress pervasive antisense transcription in *Escherichia coli*.
527 Genes Dev 26:2621–2633.

528 32. Washburn RS, Gottesman ME. 2011. Transcription termination maintains
529 chromosome integrity. Proc Natl Acad Sci U S A 108:792–797.

530 33. Harinarayanan R, Gowrishankar J. 2003. Host factor titration by

531 chromosomal R-loops as a mechanism for runaway plasmid replication in
532 transcription termination-defective mutants of *Escherichia coli*. J Mol Biol
533 332:31–46.

534 34. Gowrishankar J, Krishna Leela J, Anupama K. 2013. R-loops in bacterial
535 transcription: Their causes and consequences. Transcription 4:153–157.

536 35. Mechulam Y, Blanquet S, Schmitt E. 2013. Translation Initiation. EcoSal
537 Plus 4:1–28.

538 36. North SH, Kirtland SE, Nakai H. 2007. Translation factor IF2 at the
539 interface of transposition and replication by the PriA-PriC pathway. Mol
540 Microbiol 66:1566–1578.

541 37. Madison KE, Abdelmeguid MR, Jones-Foster EN, Nakai H. 2012. A new role
542 for translation initiation factor 2 in maintaining genome integrity. PLoS
543 Genet 8(4): e1002648.

544 38. Madison KE, Jones-Foster EN, Vogt A, Kirtland Turner S, North SH, Nakai
545 H. 2014. Stringent response processes suppress DNA damage sensitivity
546 caused by deficiency in full-length translation initiation factor 2 or PriA
547 helicase. Mol Microbiol 92:28–46.

548 39. Lee JH, Choi SK, Roll-Mecak A, Burley SK, Dever TE. 1999. Universal
549 conservation in translation initiation revealed by human and archaeal
550 homologs of bacterial translation initiation factor IF2. Proc Natl Acad Sci U
551 S A 96:4342–4347.

552 40. Howe JG, Hershey JWB. 1984. The rate of evolutionary divergence of
553 initiation factors IF2 and IF3 in various bacterial species determined
554 quantitatively by immunoblotting 140:187-192.

555 41. Gaur R, Grasso D, Datta PP, Krishna PDV, Das G, Spencer A, Agrawal RK,
556 Spremulli L, Varshney U. 2008. A Single Mammalian Mitochondrial
557 Translation Initiation Factor Functionally Replaces Two Bacterial Factors.
558 Mol Cell 29:180–190.

559 42. Plumbridge JA, Deville F, Sacerdot C, Petersen HU, Cenatiempo Y, Cozzone
560 A, Grunberg-Manago M, Hershey JW. 1985. Two translational initiation
561 sites in the *infB* gene are used to express initiation factor IF2 alpha and IF2
562 beta in *Escherichia coli*. EMBO J 4:223–229.

563 43. Nyengaard NR, Mortensen KK, Lassen SF, Hershey JWB, Sperling-Petersen
564 HU. 1991. Tandem translation of *E.coli* initiation factor IF2β: Purification
565 and characterization in vitro of two active forms. Biochem Biophys Res
566 Commun 181:1572–1579.

567 44. Laalami S, Putzer H, Plumbridge JA, Grunberg-Manago M. 1991. A severely
568 truncated form of translational initiation factor 2 supports growth of

569 *Escherichia coli*. J Mol Biol 220:335–349.

570 45. Sacerdot C, Vachon G, Laalami S, Morel-Deville F, Cenatiempo Y,
571 Grunberg-Manago M. 1992. Both forms of translational initiation factor IF2
572 (α and β) are required for maximal growth of *Escherichia coli*. Evidence for
573 two translational initiation codons for IF2β. J Mol Biol 225:67–80.

574 46. Mallikarjun J, Gowrishankar J. 2021. Essential role for an isoform of
575 *Escherichia coli* translation initiation factor IF2 in repair of two-ended DNA
576 double-strand breaks. Accompanying article.

577 47. Anupama K, Leela JK, Gowrishankar J. 2011. Two pathways for RNase E
578 action in *Escherichia coli* in vivo and bypass of its essentiality in mutants
579 defective for Rho-dependent transcription termination. Mol Microbiol
580 82:1330–1348.

581 48. Raghunathan N, Goswami S, Leela JK, Pandiyan A, Gowrishankar J. 2019.
582 A new role for *Escherichia coli* Dam DNA methylase in prevention of
583 aberrant chromosomal replication. Nucleic Acids Res 47:5698–5711.

584 49. Ali N, Gowrishankar J. 2020. Cross-subunit catalysis and a new
585 phenomenon of recessive resurrection in *Escherichia coli* RNase E. Nucleic
586 Acids Res 48:847–861.

587 50. Leela JK, Raghunathan N, Gowrishankar J. 2021. Topoisomerase I
588 Essentiality, DnaA-Independent Chromosomal Replication, and
589 Transcription-Replication Conflict in *Escherichia coli*. J Bacteriol 203:
590 e00195-21

591 51. Johnson DBF, Wang C, Xu J, Schultz MD, Schmitz RJ, Ecker JR, Wang L.
592 2012. Release factor one is nonessential in *Escherichia coli*. ACS Chem Biol
593 7:1337–1344.

594 52. Hu K, Artsimovitch I. 2017. A screen for *rfaH* suppressors reveals a key
595 role for a connector region of termination factor *rho*. mBio 8(3): e00753-17.

596 53. McGlynn P, Lloyd RG. 2000. Modulation of RNA polymerase by (p)ppGpp
597 reveals a RecG-dependent mechanism for replication fork progression. Cell
598 101:35–45.

599 54. Trautinger BW, Jaktaji RP. 2005. RNA Polymerase Modulators and DNA
600 Repair Activities Resolve Conflicts between DNA Replication and
601 Transcription. Mol Cell 19:247–258.

602 55. Kamarthapu V, Epshtein V, Benjamin B, Proshkin S, Mironov A, Cashel M,
603 Nudler E. 2016. ppGpp couples transcription to DNA repair in *E. coli*.
604 Science 352:993–996.

605 56. Carles-Kinch K, George JW, Kreuzer KN. 1997. Bacteriophage T4 UvsW

606 protein is a helicase involved in recombination, repair and the regulation
607 of DNA replication origins. EMBO J 16:4142–4151.

608 57. Dudas KC, Kreuzer KN. 2001. UvsW Protein Regulates Bacteriophage T4
609 Origin-Dependent Replication by Unwinding R-Loops. Mol Cell Biol
610 21:2706–2715.

611 58. Brandi A, Piersimoni L, Feto NA, Spurio R, Alix JH, Schmidt F, Gualerzi
612 CO. 2019. Translation initiation factor IF2 contributes to ribosome
613 assembly and maturation during cold adaptation. Nucleic Acids Res
614 47:4652–4662.

615 59. Xia J, Chen LT, Mei Q, Ma CH, Halliday JA, Lin HY, Magnan D, Pribis JP,
616 Fitzgerald DM, Hamilton HM, Richters M, Nehring RB, Shen X, Li L, Bates
617 D, Hastings PJ, Herman C, Jayaram M, Rosenberg SM. 2016. Holliday
618 junction trap shows how cells use recombination and a junction-guardian
619 role of RecQ helicase. Sci Adv 2: e1601605.

620 60. Boguslawski SJ, Smith DE, Michalak MA, Mickelson KE, Yehle CO,
621 Patterson WL, Carrico RJ. 1986. Characterization of monoclonal antibody
622 to DNA · RNA and its application to immunodetection of hybrids. J Immunol
623 Methods 89:123–130.

624 61. Lovett ST, Hurley RL, Sutera VA, Aubuchon RH, Lebedeva MA. 2002.
625 Crossing over between regions of limited homology in *Escherichia coli*:
626 RecA-dependent and RecA-independent pathways. Genetics 160:851–859.

627 62. Dutra BE, Sutera VA, Lovett ST. 2007. RecA-independent recombination is
628 efficient but limited by exonucleases. Proc Natl Acad Sci U S A 104:216–
629 221.

630 63. Laban A, Cohen A. 1981. Interplasmidic and intraplasmidic recombination
631 in *Escherichia coli* K-12. MGG Mol Gen Genet 184:200–207.

632 64. Cohen A, Laban A. 1983. Plasmidic recombination in *Escherichia coli* K-12:
633 The role of *recF* gene function. MGG Mol Gen Genet 189:471–474.

634 65. Konrad EB. 1977. Method for the isolation of *Escherichia coli* mutants with
635 enhanced recombination between chromosomal duplications. J Bacteriol
636 130:167–172.

637 66. Zhang G, Deng E, Baugh L, Kushner SR. 1998. Identification and
638 Characterization of *Escherichia coli* DNA Helicase II Mutants That Exhibit
639 Increased Unwinding Efficiency. J Bacteriol 180:377-387.

640 67. Nazir A, Harinarayanan R. 2016. Inactivation of cell division protein FtsZ
641 by SulA makes Lon indispensable for the viability of a $ppGpp^0$ strain of
642 *Escherichia coli*. J Bacteriol 198:688–700.

643 68. Brüning JG, Marians KJ. 2021. Bypass of complex co-directional
644 replication-transcription collisions by replisome skipping. Nucleic Acids
645 Res 49:9870–9885.

646 69. Seigneur M, Ehrlich SD, Michel B. 2000. RuvABC-dependent double-
647 strand breaks in dnaB$^{ts}$ mutants require RecA. Mol Microbiol 38:565–574.

648 70. Mahdi AA, Buckman C, Harris L, Lloyd RG. 2006. Rep and PriA helicase
649 activities prevent RecA from provoking unnecessary recombination during
650 replication fork repair. Genes Dev 20:2135–2147.

651 71. Khan SR, Kuzminov A. 2012. Replication forks stalled at ultraviolet lesions
652 are rescued via RecA and RuvABC protein-catalyzed disintegration in
653 *Escherichia coli*. J Biol Chem 287:6250–6265.

654 72. Krejci L, Altmannova V, Spirek M, Zhao X. 2012. Homologous
655 recombination and its regulation. Nucleic Acids Res 40:5795–5818.

656 73. Matic I, Rayssiguier C, Radman M. 1995. Interspecies Gene Exchange in
657 Bacteria: The Role of SOS and Mismatch Repair Systems in Evolution of
658 Species. Cell 80:507-515.

659 74. Renzette N, Gumlaw N, Sandler SJ. 2007. DinI and RecX modulate RecA-
660 DNA structures in *Escherichia coli* K-12. Mol Microbiol 63(1):103–115.

661 75. Miller JH. 1992. A Short Course in Bacterial Genetics: A Laboratory
662 Manual and Handbook for *Escherichia coli* and Related Bacteria. Cold
663 Spring Harb Lab Press NY.

664 76. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA,
665 Tomita M, Wanner BL, Mori H. 2006. Construction of *Escherichia coli* K-12
666 in-frame, single-gene knockout mutants: The Keio collection. Mol Syst Biol
667 2:2006.0008.

668 77. Bolivar F, Rodriguez RL, Greene PJ, Betlach MC, Heyneker HL, Boyer HW,
669 Crosa JH, Falkow S. 1977. Construction and characterization of new
670 cloning vehicle. II. A multipurpose cloning system. Gene 2:95–113.

671 78. Chang ACY, Cohen SN. 1978. Construction and characterization of
672 amplifiable multicopy DNA cloning vehicles derived from the p15A cryptic
673 miniplasmid. J Bacteriol 134:1141–1156.

674 79. Lerner CG, Inouye M. 1990. Low copy number plasmids for regulated low-
675 level expression of cloned genes in *Escherichia coli* with blue-white insert
676 screening capability. Nucleic Acids Res. Nucleic Acids Res 18:4631.

677 80. Andrews AE, Lawley B, Pittard AJ. 1991. Mutational analysis of repression
678 and activation of the *tyrP* gene in *Escherichia coli*. J Bacteriol 173:5068–
679 5078.

680 81. Amann E, Ochs B, Abel KJ. 1988. Tightly regulated *tac* promoter vectors
681 useful for the expression of unfused and fused proteins in *Escherichia coli*.
682 Gene 69:301–315.

683 82. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal
684 genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S
685 A 97:6640–6645.

686 83. Gowrishankar J. 1985. Identification of osmoresponsive genes in
687 *Escherichia coli*: Evidence for participation of potassium and proline
688 transport systems in osmoregulation. J Bacteriol 164:434–445.

689 84. Sambrook D, Russell J. 2001. Molecular Cloning: A Laboratory Manual.
690 3rd edn. Cold Spring Harb Lab Press NY.

691 85. Boyd D, Weiss DS, Chen JC, Beckwith J. 2000. Towards single-copy gene
692 expression systems making gene cloning physiologically relevant: Lambda
693 InCh, a simple *Escherichia coli* plasmid-chromosome shuttle system. J
694 Bacteriol 182:842–847.

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696

697 **Legends to Figures**

698 **Figure 1**: Synthetic lethality of *rho ruv* mutants, and their suppression by *infB*-
699 161$^{ochre}$ or *rec* mutations. Mutant designations *rho* and *ruv* refer to alleles *rho*-
700 136$^{opal}$ and Δ*ruvABC*::Cm, respectively. All strain numbers mentioned are
701 prefixed with GJ. (A) Blue-white screening assays, on defined medium at 30°, for
702 strains carrying *rho*$^{+}$ *infB*$^{+}$ shelter plasmid pHYD5212. Representative images are
703 shown; for each of the sub-panels, relevant chromosomal genotypes/features are
704 given on top while the numbers beneath indicate the percentage of white colonies
705 to total (minimum of 500 colonies counted). Examples of white colonies are
706 marked by yellow arrows. Strains employed for the different sub-panels were
707 pHYD5212 derivatives of: i, 15441; ii, 15447; iii, 19150; iv, 19845; v, 15460; vi,
708 15471; vii, 15491; viii, 15487; ix, 15497; and x, 15446. (B) Dilution-spotting
709 assay, on minimal A supplemented with D-glucose (Glu) or Ara as indicated, of
710 strains whose relevant genotypes/features are shown at left. Strains for different
711 rows were (from top): 19379, 19380, 19381 (this strain was grown in Ara-
712 supplemented medium before dilutions were spotted), and 19382.

713 **Figure 2**: Modulation of *rho ruv* synthetic lethality by differential expression of
714 IF2 isoforms. Blue-white screening assays were performed on defined medium
715 at 30°, with strains carrying *rho*$^{+}$ *infB*$^{+}$ shelter plasmid pHYD5212. General
716 notations are as described in legend to Figure 1A. All strains were Δ*infB*, with
717 the exception of that for sub-panel v. Growth medium for sub-panels iv, v, and
718 viii was supplemented with IPTG. Strains employed for the different sub-panels
719 were pHYD5212 derivatives of (all strain numbers are prefixed with GJ): i, 15498;
720 ii, 15500; iii, 15499; iv, 15458; v, 15457; vi, 19134; vii, 19131; and viii, 19132.

721 **Figure 3**: Synthetic lethality conferred by dominant negative *ruvC* mutation
722 (RDG) in Δ*uvrD* mutant, and its suppression by loss of IF2-1. Dilution-spotting
723 assay was performed on LB medium without (−) and with (+) Dox
724 supplementation, of RDG-bearing derivatives whose relevant genotypes/features

725 are indicated at left; strains on all but the top two rows were also Δ*infB*. Strains
726 employed for different rows were (from top, all strain numbers are prefixed with
727 GJ): 19127, 19161, 19801, 19802, 19803, 19843, and 19835.

728 **Figure 4**: Effect of IF2 isoforms on HR. Recombination frequency data are given
729 for strains with the indicated genotypes/features in the Konrad (A), inter-plasmid
730 recombination (B), and P1 transduction (C) assays, after normalization to the
731 value for the cognate control strain (taken as 1, and shown at extreme left for
732 each panel); the actual control strain values were $2.8*10^{-7}$/viable cell,
733 $6*10^{-4}$/viable cell, and $1.5*10^{-5}$/phage, respectively. In panels A and B, values
734 from every individual experiment is shown, and median values are given beside
735 the denoted horizontal lines. Panel C depicts the mean value (given alongside
736 each bar) and standard error for each strain. In all panels, strains whose
737 designations include Δ*Nil*, Δ*1*, or Δ*2,3* were also Δ*infB*. Strains used were (from
738 left, all strain numbers mentioned are prefixed with GJ unless otherwise
739 indicated): in panel A, SK707, 19171, 19186, 19162, 19184, and 19165; in panel
740 B, 19197, 19196, 19195, and 19847; and in panel C, 19193, 19194, and 15494.

741 **Figure 5**: Two models to explain HJ formation (and hence need for RuvABC) in
742 *rho* mutants. **(a-b)** Rho deficiency provokes R-loop generation from nascent
743 untranslated transcripts, associated with RNA polymerase (RNAP) backtracking
744 and arrest. **(c)** HR is initiated by invasion of ss-DNA of R-loop into a sister
745 chromosome, mediated by RecFOR and RecA. **(d-e)** Co-directional replisome
746 traverses R-looped region before it is arrested by backtracked RNAP. **(f)**
747 Replisome disassembly followed by RecA- and RecFOR-mediated replication fork
748 reversal generates HJ, with the RNA-DNA hybrid now as part of the extruded
749 tail.

750

**Fig. 1**

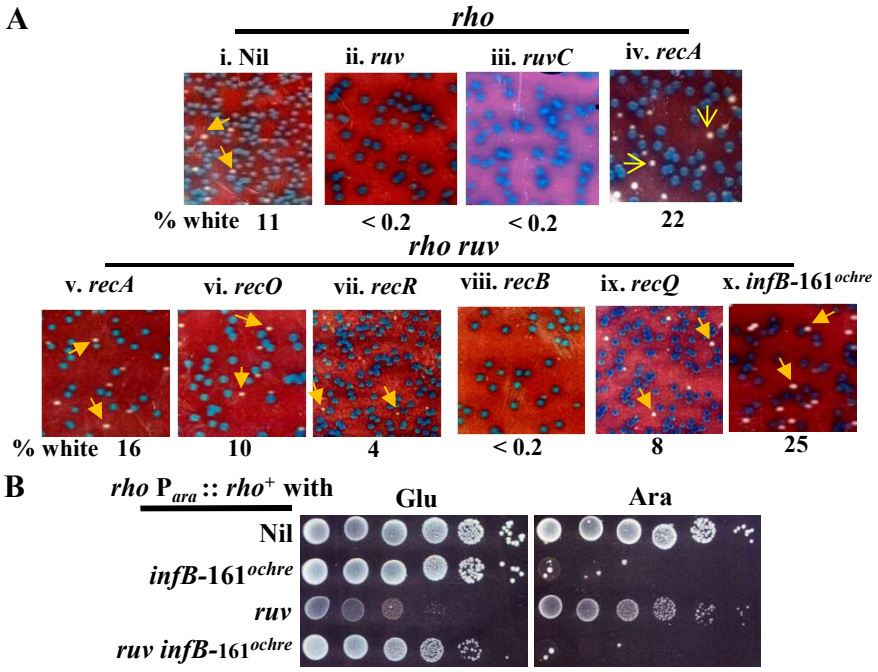


**Figure 1**: Synthetic lethality of *rho ruv* mutants, and their suppression by *infB*-161$^{ochre}$ or *rec* mutations. Mutant designations *rho* and *ruv* refer to alleles *rho*-136$^{opal}$ and Δ*ruvABC*::Cm, respectively. All strain numbers mentioned are prefixed with GJ. (A) Blue-white screening assays, on defined medium at 30°, for strains carrying *rho*$^{+}$ *infB*$^{+}$ shelter plasmid pHYD5212. Representative images are shown; for each of the sub-panels, relevant chromosomal genotypes/features are given on top while the numbers beneath indicate the percentage of white colonies to total (minimum of 500 colonies counted). Examples of white colonies are marked by yellow arrows. Strains employed for the different sub-panels were pHYD5212 derivatives of: i, 15441; ii, 15447; iii, 19150; iv, 19845; v, 15460; vi, 15471; vii, 15491; viii, 15487; ix, 15497; and x, 15446. (B) Dilution-spotting assay, on minimal A supplemented with D-glucose (Glu) or Ara as indicated, of strains whose relevant genotypes/features are shown at left. Strains for different rows were (from top): 19379, 19380, 19381 (this strain was grown in Ara-supplemented medium before dilutions were spotted), and 19382.

**Fig. 2**

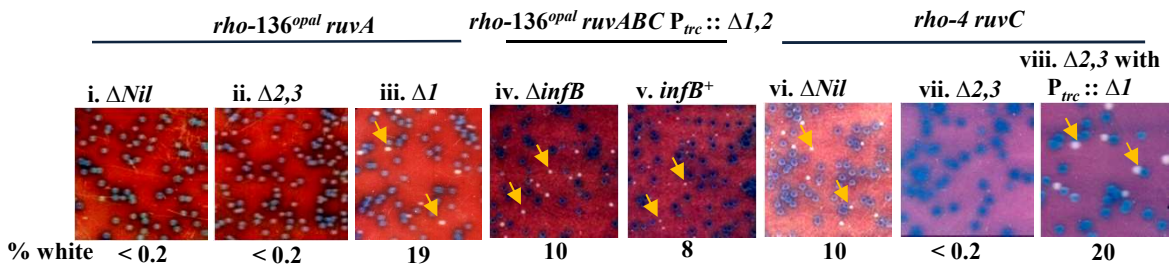


**Figure 2**: Modulation of *rho ruv* synthetic lethality by differential expression of IF2 isoforms. Blue-white screening assays were performed on defined medium at 30°, with strains carrying *rho*$^{+}$ *infB*$^{+}$ shelter plasmid pHYD5212. General notations are as described in legend to Figure 1A. All strains were Δ*infB*, with the exception of that for sub-panel v. Growth medium for sub-panels iv, v, and viii was supplemented with IPTG. Strains employed for the different sub-panels were pHYD5212 derivatives of (all strain numbers are prefixed with GJ): i, 15498; ii, 15500; iii, 15499; iv, 15458;v, 15457; vi, 19134; vii, 19131; and viii, 19132.

**Fig. 3**

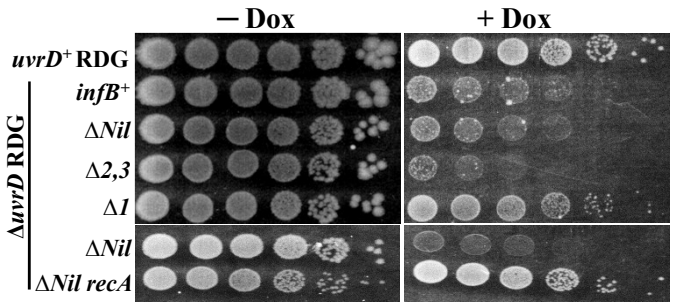


**Figure 3**: Synthetic lethality conferred by dominant negative *ruvC* mutation (RDG) in Δ*uvrD* mutant, and its suppression by loss of IF2-1. Dilution-spotting assay was performed on LB medium without (−) and with (+) Dox supplementation, of RDG-bearing derivatives whose relevant genotypes/features are indicated at left; strains on all but the top two rows were also Δ*infB*. Strains employed for different rows were (from top, all strain numbers are prefixed with GJ): 19127, 19161, 19801, 19802, 19803, 19843, and 19835.

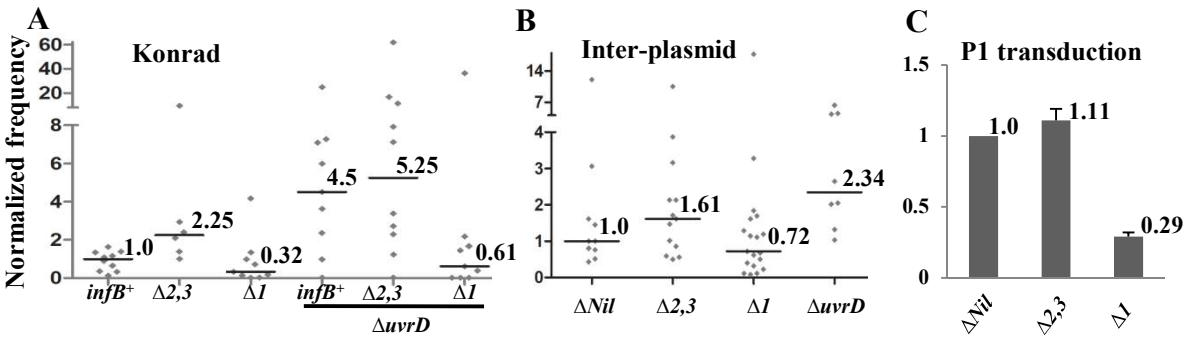


**Figure 4**: Effect of IF2 isoforms on HR. Recombination frequency data are given for strains with the indicated genotypes/features in the Konrad (A), inter-plasmid recombination (B), and P1 transduction (C) assays, after normalization to the value for the cognate control strain (taken as 1, and shown at extreme left for each panel); the actual control strain values were $2.8*10^{-7}$/viable cell, $6*10^{-4}$/viable cell, and $1.5*10^{-5}$/phage, respectively. In panels A and B, values from every individual experiment is shown, and median values are given beside the denoted horizontal lines. Panel C depicts the mean value (given alongside each bar) and standard error for each strain. In all panels, strains whose designations include Δ*Nil*, Δ*1*, or Δ*2,3* were also Δ*infB*. Strains used were (from left, all strain numbers mentioned are prefixed with GJ unless otherwise indicated): in panel A, SK707, 19171, 19186, 19162, 19184, and 19165; in panel B, 19197, 19196, 19195, and 19847; and in panel C, 19193, 19194, and 15494.

Fig. 5

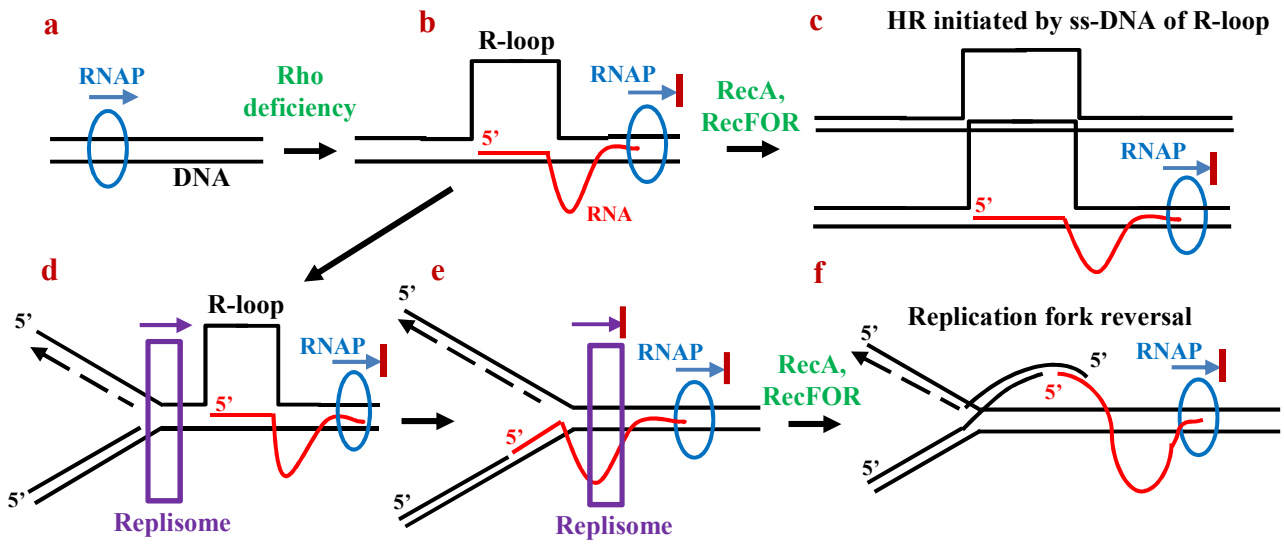


**Figure 5**: Two models to explain HJ formation (and hence need for RuvABC) in *rho* mutants. **(a-b)** Rho deficiency provokes R-loop generation from nascent untranslated transcripts, associated with RNA polymerase (RNAP) backtracking and arrest. **(c)** HR is initiated by invasion of ss-DNA of R-loop into a sister chromosome, mediated by RecFOR and RecA. **(d-e)** Co-directional replisome traverses R-looped region before it is arrested by backtracked RNAP. **(f)** Replisome disassembly followed by RecA- and RecFOR-mediated replication fork reversal generates HJ, with the RNA-DNA hybrid now as part of the extruded tail.