Inhibiting translation elongation can aid genome duplication in *Escherichia coli*

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Abstract

Conflicts between replication and transcription challenge chromosome duplication. *Escherichia coli* replisome movement along transcribed DNA is promoted by Rep and UvrD accessory helicases with Δ*rep* Δ*uvrD* cells being inviable under rapid growth conditions. We have discovered that mutations in a tRNA gene, *aspT*, in an aminoacyl tRNA synthetase, AspRS*,* and in a translation factor needed for efficient proline-proline bond formation, EF-P, suppress Δ*rep* Δ*uvrD* lethality. Thus replication-transcription conflicts can be alleviated by the partial sacrifice of a mechanism that reduces replicative barriers, namely translating ribosomes that reduce RNA polymerase backtracking. Suppression depends on RelA-directed synthesis of (p)ppGpp, a signalling molecule that reduces replication-transcription conflicts, with RelA activation requiring ribosomal pausing. Levels of (p)ppGpp in these suppressors also correlate inversely with the need for Rho activity, an RNA translocase that can bind to emerging transcripts and displace transcription complexes. These data illustrate the fine balance between different mechanisms in facilitating gene expression and genome duplication and demonstrate that accessory helicases are a major determinant of this balance. This balance is also critical for other aspects of bacterial survival: the mutations identified here increase persistence indicating that similar mutations could arise in naturally occurring bacterial populations facing antibiotic challenge.

Introduction

Cell survival from one generation to the next relies on efficient and faithful replication of the genome. However, the template for replication frequently harbors obstacles that have the potential to interfere with the progression of replisome complexes, the macromolecular machines responsible for driving genome duplication. Protein-DNA complexes are important sources of such obstacles and those associated with transcription are especially problematic, in part because of their abundance and in part because they present multiple different challenges to replisome movement (1-4). One major challenge is the very high free energy of binding of transcription complexes that creates a need to disrupt many RNA polymerase-nucleic acid interactions as replication proceeds. Transcribing RNA polymerases (RNAPs) also pause frequently either spontaneously or upon encountering DNA template damage (5). Paused RNAPs can also diffuse backwards along the template resulting in displacement of the transcript 3' end from the RNAP active site. This causes an inability to resume transcription, creating barriers to replication that threaten genome stability (6,7).

Mechanisms exist that reduce the probability of a replication fork encountering paused transcription complexes. Upstream RNAPs on the DNA and ribosomes on the emerging transcript may inhibit backtracking of a paused transcription complex in bacteria (7-9). RNA translocases such as the bacterial Rho hexamer can also translocate along transcripts not coated with ribosomes and displace paused and blocked RNAPs (7,10,11). Rho translocation also disrupts R-loops, structures in which an RNA transcript hybridises to the DNA template strand (11). Nucleases can also aid genome duplication by promoting the resumption of transcription by backtracked RNAP (7,12). GreA and GreB bind to *E. coli* RNAP and stimulate cleavage of backtracked transcripts, creating a new RNA 3' end that can be used to restart transcription (13). Conversely, the 3'-5' helicase UvrD can increase rather than decrease backtracking of RNAP and this may increase access of nucleotide excision repair enzymes UvrABC to DNA damage thus helping to clear barriers to RNAP and aid genome duplication (14).

Signalling molecules such as guanosine tetraphosphate and pentaphosphate in bacteria (referred to collectively as (p)ppGpp) can also reprogramme transcription. (p)ppGpp is central to the stringent response in bacteria, identified originally as a response to amino acid starvation in which the presence of uncharged tRNA within the ribosomal A site stimulates ribosome-bound RelA to synthesise (p)ppGpp (15-17). Control of the stringent response also requires a (p)ppGpp-specific pyrophosphohydrolase, SpoT, that also has low (p)ppGpp synthase activity (17). However, the synthase activity of SpoT is induced by other stresses such as depletion of fatty acids (18). Central to the stringent response is binding of (p)ppGpp to RNAP which leads to downregulation of stable RNA production and upregulation of stress response genes (17,19,20). Expression of ribosomal rRNA operons accounts for half of all transcription during rapid growth when (p)ppGpp levels are low and thus provides the majority of transcriptional obstacles to replisomes *in vivo* when cells are dividing quickly (21-24). Elevated (p)ppGpp therefore reduces conflicts between replication and transcription by decreasing the density of transcribing RNAPs on the genome. (p)ppGpp can also decrease the stability of transcription complexes blocked by template damage *in vitro* which may decrease the density of blocked RNAP on the genome (12). This destabilisation has been questioned, though, and a role for (p)ppGpp in increasing the fidelity of transcription and hence reducing RNAP pausing and backtracking has been proposed (25). Other work implicates (p)ppGpp acting synergistically with UvrD in promoting backtracking of paused RNAP, one function of which could be to facilitate transcription-coupled repair of any pause-inducing DNA damage (26). Thus how (p)ppGpp might act on transcription elongation complexes, as opposed to transcription initiation, is still far from clear. Transcription is not the only target of

(p)ppGpp, though, since (p)ppGpp also binds multiple other targets. Inhibited targets include DnaG primase (27-30) and the translation elongation factors EFG and EF-Tu (31), indicating the pleiotropic impact of elevated (p)ppGpp on replication, transcription and elongation. (p)ppGpp also affects other diverse aspects of metabolism such as phospholipid synthesis, oxidative metabolism and resistance to antibiotics (32). These signalling molecules also enhance the ability of *E. coli* to persist in the presence of antibiotics. Persistence is the non-heritable ability of a small fraction of a bacterial population to survive exposure to an otherwise lethal concentration of antibiotic (33). (p)ppGpp levels vary stochastically in a bacterial population and increased (p)ppGpp activates toxin-antitoxin systems that induce slow growth in a sub-population of cells, leading to antibiotic tolerance (34).

Mechanisms also exist to increase the probability of continued replisome movement in the event of a collision with an RNAP. Accessory replicative motors provide a supply of additional helicases at the fork to aid protein displacement ahead of the replisome (35,36). In *E. coli* Rep helicase promotes movement of replisomes along protein-bound DNA *in vitro* and *in vivo* (22,37). Rep appears to be the main replisome anti-pausing factor in *E. coli* (38) and absence of Rep results in at least a two-fold increase in genome duplication time (39,40) and higher dependence on recombination-directed repair of damaged forks (41-44). However, Δ*rep* cells remain viable since the homologous helicase UvrD can substitute for the absence of Rep at the replication fork (22,37). This substitution is only partial, though, and correlates with a physical and functional interaction between DnaB and Rep but not between DnaB and UvrD (37,45). This partial functional overlap is sufficient for single deletion mutants to be viable during rapid growth whereas Δ*rep* Δ*uvrD* cells are not (46). Δ*rep* Δ*uvrD* inviability can be suppressed by growth on minimal medium, conditions under which (p)ppGpp levels are high, and also by elevation of (p)ppGpp levels on rich medium via the *spoT1* mutation encoding a (p)ppGpp pyrophosphorylase-defective SpoT (22,37). Mutations within *rpo* genes can also suppress Δ*rep* Δ*uvrD* rich medium lethality (22,37,47). These mutant RNAPs display different phenotypes suggesting distinct mechanisms of suppression but some seem to phenocopy elevated (p)ppGpp and/or reduce RNAP backtracking (12,47,48).

The above mechanisms reduce conflicts between replication and transcription but under rapid growth conditions forks are still blocked sufficiently frequently to require replisome reloading enzymes to maintain viability (49,50). Replisome reloading also often requires remodelling of the DNA at the blocked fork by recombination enzymes in order to generate a DNA structure suitable for replisome reloading (51). However, recombinational processing can lead to genome instability and is thus tightly controlled. One control is exerted by UvrD as it can disrupt RecA-ssDNA filaments and this disruption prevents excessive RecFOR-dependent loading of RecA onto ssDNA at blocked forks (52). Such excessive RecA loading contributes to Δ*rep* Δ*uvrD* lethality, evinced by the weak suppression of Δ*rep* Δ*uvrD* rich medium lethality via mutations in *recF*, *recO* or *recR* (37,53,54).

We have searched for suppressors of Δ*rep* Δ*uvrD* rich medium lethality that are not within *rpo, spoT, recF, O* or *R*. We identified a spontaneous suppressor in a tRNA gene, *aspT[t8c]*, that mutated a highly conserved residue that is structurally important in other tRNA species. Probing the basis of this suppression revealed that defective tRNA aminoacylation or inefficient peptide bond formation within the ribosome also suppressed Δ*rep* Δ*uvrD* lethality. In all cases suppression required RelA-directed (p)ppGpp synthesis indicating that stalling of ribosome translocation by uncharged tRNA in the A site of ribosomes underpinned suppression. Thus although efficient translation elongation aids genome duplication (7,9), the partial inhibition of ribosome translocation is more effective at mitigating replication-transcription conflicts. These data illustrate the fine balance between the multiple mechanisms that promote simultaneous gene expression and genome duplication

and reveal the importance of accessory replicative helicase activity in determining this balance.

Materials and Methods
Plasmids and strains
pAM403 (55) and pAM407 (37) are pRC7 derivatives encoding *rep* and *uvrD*, respectively. p3LC-TL30-5P and p3LC-TL30-5D contain a *cadC-lacZ* gene fusion in which five tandem codons present within the linker encode proline or aspartate, respectively (56). Strains were constructed by P1 *vir* transduction and are listed in Supplementary Table 1.

Genome sequencing and analysis
DNA was extracted from 1 culture each of strain N7153 and N7182 grown in LB broth to stationary phase using Qiagen 100/G genomic tips from 5 ml cultures following the manufacturer's protocol. Genome sequencing was performed using an Illumina GAIIx instrument with 100 bp paired end reads. Paired reads were trimmed to remove adapters and mapped against the *E.coli* K12 strain MG1655 NC_000913 genome using bwa (57), duplicates marked using Picard (http://broadinstitute.github.io/picard) and variant analysis performed with SAMtools (58), followed by merging of variant tables using perl. Identified high quality synonymous and non-synonymous single nucleotide polymorphisms were annotated manually using the Integrative Genomics Viewer (59). The Illumina data were submitted in form of fastq files to the European Nucleotide Archive (ENA) and are available under accession number PRJEB14483 at http://www.ebi.ac.uk/ena/data/view/PRJEB14483.

Growth assays
All steps in plasmid loss assays were carried out at 37°C except those shown in Figures 2B and 3B in which all steps were conducted at 30°C or 25°C as indicated. Colonies were grown in LB broth and agar except the assays in Figure 2B and Supplementary Figures 3B and 4 which were performed with a defined rich medium broth and agar containing 0.2% glycerol (Figure 2B) or 0.2% glucose (Supplementary Figures 3B and 4) as a carbon source (60). The plates used in Supplementary Figures 3B and 4 also had decreasing concentrations of aspartate or phenylalanine, as indicated. Strains carrying derivatives of pRC7 were grown in LB broth or defined rich medium with 100 μg ml^{-1} of ampicillin overnight, diluted 100-fold into the same type of fresh liquid medium without ampicillin and grown to A_{650} 0.4. Dilutions were then plated onto LB or defined rich medium agar plates containing 120 μg ml^{-1} X-gal and 1 mM IPTG and incubated for 48 hours except those shown in Figure 2B. Plates were then photographed and scored for blue/white colony formation.

For assays to assess colony forming ability, strains were grown in LB broth overnight at 37°C or, when temperature sensitive strains were involved, at 30°C. Serial 10-fold dilutions were made with 56/2 salts (61) on ice and then 5 μl of each dilution was spotted onto LB agar plates. Plates were then incubated at 37°C for 16 hours. Ampicillin and bicyclomycin were included in LB agar plates where indicated at 100 and 25 μg ml^{-1}, respectively.

For the colony formation assays in Supplementary Figure 3A, strains were grown in defined rich medium broth (60) containing 0.2% glucose, all amino acids and 100 μg ml^{-1} ampicillin overnight at 37°C. Tenfold serial dilutions were made in 56/2 salts and then 5 μl of each dilution spotted onto defined rich medium plates containing 0.2% glucose, 100 μg ml^{-1} ampicillin and with all amino acids or missing either aspartate or phenylalanine. These plates were incubated at 37°C for 16 hours. Minimal medium agar plates (61) were used in Supplementary Figure 3C without and with 100 μg ml^{-1} ampicillin as indicated and incubated at 37°C for 72 hours.

Colony-forming ability at increasing doses of UV light was assessed as

described (62). Mismatch repair capacity was measured as the fraction of cells in a culture that acquired spontaneous mutations leading to rifampicin resistance. Briefly, overnight LB cultures were washed once in 56/2 salts and serially diluted tenfold. 100 µl of the neat and the 10^{-1} dilution were spread on LB agar containing 15 µg ml^{-1} rifampicin. To estimate the total cfu ml^{-1} in the overnight culture, 10 µl of the 10^{-6} dilution was spotted in triplicate on LB agar without rifampicin. Plates were then incubated at 37°C for 16 hours and the colonies then counted.

The liquid growth assays in Figure 2A were performed using a Tecan Infinite M200 Pro plate reader. Overnight LB cultures were diluted to A_{600} 0.005 in LB and 150 µl of each culture was transferred into 20 wells of a 96-well flat bottom plate (Corning). The microplate was incubated at 37°C for 24 hours and A_{600} measurements were performed every 30 minutes preceded by plate shaking.

Persistence

Single colonies were inoculated into 5 ml LB broth and grown with shaking at 37°C overnight. 50 µl of overnight culture was then inoculated into 5 ml of LB broth and grown at 37°C with shaking until 1-2 x 10^8 colony forming units ml^{-1} reached. 500 µl was then transferred into two 1.5 ml microcentrifuge tubes. The first tube was centrifuged at 6000 rpm for 5 min at room temperature, resuspended in 500 µl 56/2 salts, serially diluted tenfold in 56/2 salts and 10 µl volumes of the 10^{-4} and 10^{-5} dilutions spotted onto LB agar plates containing 20 mM MgSO$_4$ in triplicate. To the second tube 5 µl of 10 µg ml^{-1} ciprofloxacin was added and the tube was then inserted into a 50 ml Falcon tube and placed in a shaking incubator at 37°C for 5 h. After the 5h incubation this tube was centrifuged at 6000 rpm for 5 min at room temperature, resuspended in 500 µl 56/2 salts, recentrifuged and resuspended in 500 µl 56/2 salts. Colony forming units were then assayed as for the first tube by serial dilution into 56/2 salts and plating in triplicate onto LB agar containing 20 mM MgSO$_4$. MgSO$_4$ was included to inhibit the activity of any traces of ciprofloxacin not removed by washing of the cells (63). All plates were incubated at 37°C overnight and then the fraction of colony forming units surviving ciprofloxacin exposure calculated.

Flow cytometry

Analysis of chromosome content after completion of all ongoing rounds of replication, so-called run-out conditions, was performed on mid-log phase cultures after treatment with rifampicin and cephalexin as described (64) using a Becton Coulter CyAn ADP cytometer with 488 nm excitation and a 530/540 nm bandpass filter. Analysis of (p)ppGpp-dependent formation of RpoS-mCherry by flow cytometry was performed by growing the indicated strains overnight in LB, transferring 100 µl into 10 ml LB in a 125 ml flask followed by incubation at 37°C for 2h. Then 1 ml of the culture was centrifuged, resuspended in 2 ml of 10% LB in M9 medium and analysed by flow cytometry on a Becton Dickinson LSRFortessa X-20 flow cytometer using 561 nm laser excitation and dection using a 610/620 nm bandpass filter.

Translation assays

β-galactosidase activity assays monitoring the relative levels of translation of *cadC-lacZ* fusions were performed in LB broth as described (56).

Results

A tRNA mutation suppresses the requirement for accessory replicative helicases

pRC7 is a very low copy plasmid which encodes β lactamase and can therefore be maintained in *E. coli* cells by the inclusion of ampicillin in the medium (65). However, the inefficient origin of replication within pRC7 results in rapid loss of the plasmid in the absence of ampicillin. This rapid loss can be detected if the host strain has a chromosomal deletion of *lacIZYA* since pRC7 encodes the *lac* operon and hence cells with and without the plasmid appear blue and white, respectively, on plates containing IPTG and X-gal (65). This retention or loss of pRC7 can be used to assess whether a gene is essential for colony formation by cloning of the test gene into pRC7, transformation of a Δ*lacIZYA* strain with the pRC7 derivative and subsequent deletion of the test gene from the chromosome. Plating of the strain onto medium containing IPTG and X-gal results in formation of only blue colonies if the test gene is essential or white and segregating colonies if the test gene is not essential (65). Rapid growth of *E. coli* requires accessory helicase activity and so pRC7 encoding *uvrD* can be lost rapidly from Δ*lacIZYA rep*$^+$ *uvrD*$^+$ cells on rich medium but pRC7*uvrD* cannot be lost from Δ*lacIZYA* Δ*rep* Δ*uvrD* cells as monitored by blue/white colony colour (37) (see also Figure 1A, compare i and ii). Spontaneous mutations that suppress this requirement for an accessory helicase can be isolated by exploiting the ability of Δ*rep* Δ*uvrD* cells to grow on minimal medium in the absence of a complementing pRC7 plasmid and subsequent plating of plasmid-less cells onto rich medium (37). Rare survivors on rich medium can then form colonies and the mutation(s) responsible for allowing Δ*rep* Δ*uvrD* cells to grow under rapid growth conditions can be analysed. We identified one such suppressor, the strain designation of which is N7182 (Supplementary Table 1). Potential linkage of the suppressor mutation to *rep* was tested by transducing Δ*rep*::*cat* from the suppressor strain into pRC7*uvrD/rep*$^+$ Δ*uvrD*::*dhfr* (N6639). Eleven chloramphenicol-resistant transductants were tested for loss of pRC7*uvrD* on rich medium. Four transductants could not lose pRC7*uvrD* but seven could, indicating close linkage of the suppressor mutation with Δ*rep*::*cat* (see also Figure 1A, compare ii and iii).

We sequenced the genome of this suppressor strain and compared it with the genome of N7153, a Δ*rep* Δ*uvrD* strain that contains a well-characterised suppressor mutation *rpoB*35* (7,12,37,66). Use of the Δ*rep* Δ*uvrD rpoB*35* strain as a reference genome avoided the need for the reference strain to retain a complementing plasmid for viability. Only one mutation in N7182 that was not present in N7153 was located sufficiently close to Δ*rep*::*cat* to explain the above linkage (see Supplementary File 1). This mutation resulted in replacement of T with C at position 8 within the *aspT* gene, one of three identical tRNA^{Asp} genes in *E. coli*. Sequencing of the *aspT* gene from the 11 transductants obtained in the above cross revealed that all seven strains able to lose pRC7*uvrD* contained *aspT[t8c]* whereas all four that could not lose pRC7*uvrD* retained a wild type copy of *aspT*. Suppression of the inviability of Δ*rep* Δ*uvrD* cells on rich medium was therefore associated with the *aspT[t8c]* allele, with suppression comparable to that of *rpoB*35* in a plasmid loss assay (Fig 1A, compare iii and iv).

Δ*rep uvrD*$^+$ cells have a growth defect defect since UvrD can compensate only partially for the absence of Rep accessory helicase activity, resulting in slower movement of replication forks in Δ*rep* cells (39,40). Thus the median number of copies of *oriC* in Δ*rep uvrD*$^+$ cells is twice that of *rep*$^+$ *uvrD*$^+$ cells in rich medium due to an extended cell cycle in Δ*rep* cells and hence more replication initiation events per cell cycle (38). This doubling in *oriC* numbers results in a doubling of chromosome content when cells are treated with cephalexin and rifampicin to inhibit cell division and reinitiation of replication, so-called run-out conditions (38) (see also Figure 1B, compare i and iii). *aspT[t8c]* suppressed the increased chromosome copy

number in Δ*rep* *uvrD*^{+} cells, reducing the median number of chromosomes from eight to four (Figure 1B, compare iii and iv). These data support the conclusion that this tRNA mutation reduces the need for accessory helicase activity. Furthermore, *aspT[t8c]* had no detectable impact on other UvrD-mediated processes. Defects in nucleotide excision repair, mismatch repair and control of recombination in *rep*[+] Δ*uvrD* cells (52,67,68) were not suppressed by *aspT[t8c]* (Supplementary Figure 1A, B and C, respectively), providing further support for the specific suppression of the accessory helicase defect in Δ*rep* Δ*uvrD* cells by *aspT[t8c]*.

The requirement for accessory helicase activity is driven primarily by transcriptional barriers to replication (22,37). Backtracking of paused RNA polymerases results in formation of particularly stable replicative barriers and multiple factors have evolved to reduce the numbers of backtracked complexes (7). Cells lacking two homologous anti-backtracking factors, GreA and GreB, display a temperature-sensitive growth defect that is a consequence of more frequent collisions between replisomes and backtracked transcription complexes (7,12,69). *aspT[t8c]* suppressed the temperature-sensitive phenotype of Δ*greA* Δ*greB* cells (Figure 1C), similar to the suppression seen with *rpoB*35* (48). We conclude that *aspT[t8c]* reduces the need for GreA/GreB-dependent rescue of backtracked RNA polymerase.

Taken together, these data indicate that *aspT[t8c]* reduces the need for Rep and UvrD to underpin replication and for anti-backtracking factors to resuscitate transcription complexes.

Suppression by *aspT[t8c]* does not occur via growth rate restriction

The uridine encoded at position 8 within the wild type *aspT* gene is post-transcriptionally modified to 4-thiouridine and this modified nucleotide is conserved across all kingdoms of life (70). This residue is involved in a triple non-Watson-Crick pairing interaction and is important in coordination of magnesium within tRNA (71,72). This central structural role is reflected in the temperature-dependent destabilisation of human mitochondrial tRNA[Met] structure by the same T to C mutation as found in *aspT[t8c]*. This transition mutation in tRNA[Met] inhibits aminoacylation and any mutated tRNA[Met] that is aminoacylated fails to form a stable ternary complex with elongation factor EF-Tu (70).

Given the conservation of tRNA structure, *aspT[t8c]* may result in similar structural destabilisation of the encoded tRNA[Asp] and consequent inhibition of interactions with aspartyl tRNA synthetase and EF-Tu. However, *aspT[t8c]* did not have a major impact on growth of *rep*[+] *uvrD*^{+} cells in liquid culture (Figure 2A, compare i and ii). This absence of a significant growth defect in *aspT[t8c]* strains may be due to the presence of two other identical tRNA[Asp] genes in *E. coli*, *aspU* and *aspV*.

Other means of growth restriction did not suppress Δ*rep* Δ*uvrD* inviability. Growth on defined rich medium containing all 20 amino acids but with a poor carbon source, glycerol, did not result in suppression of Δ*rep* Δ*uvrD* *aspT*[+] inviability at either 37°C or 25°C as determined using a plasmid loss assay (Figure 2B). Reduced growth rates therefore do not provide a general means of reducing the need for accessory helicase activity.

Defective tRNA aminoacylation suppresses Δ*rep* Δ*uvrD* inviability

As reduced growth rate was not the cause of suppression by *aspT[t8c]*, we probed whether defective translation was responsible. We could not detect significant defects in translation *in vivo* at tandem aspartate codons in an *aspT[t8c]* strain (Supplementary Figure 2). Either this allele does not result in translational pausing or such pausing is below the limits of detection using this assay. We therefore used alternative approaches to probe the *aspT[t8c]* suppression mechanism. The same t8c mutation in human mitochondrial tRNA[Met] inhibits aminoacylation (70). Inhibition

of tRNA^{Asp} aminoacylation was therefore tested for suppression of Δ*rep* Δ*uvrD* lethality. The *E. coli tls-1* allele encodes a P555S mutation in aspartyl tRNA synthetase that reduces the thermal stability of the synthetase and causes a severe reduction in growth rate at 42°C under low salt conditions (73,74) (see also Figure 3A). Given that AspRS^{P555S} is less stable than wild type enzyme regardless of the temperature (74), we tested whether *aspS*^{P555S} suppressed Δ*rep* Δ*uvrD* lethality at 30°C on low salt medium, conditions under which viability is similar to wild type (73) (see also Figure 3A, compare i and ii). pRC7*uvrD* could be lost from Δ*rep* Δ*uvrD* *aspS*^{P555S} at 30°C on low salt medium but not on high salt medium (Figure 3Biii). Suppression of Δ*rep* Δ*uvrD* lethality correlates therefore with a defect in aspartyl tRNA synthetase.

We also tested whether an alternative means of inhibiting tRNA^{Asp} aminoacylation could suppress Δ*rep* Δ*uvrD* inviability. A pRC7*uvrD*/Δ*rep* Δ*uvrD* strain defective in aspartate biosynthesis could lose the complementing pRC7*uvrD* plasmid on defined rich medium upon restriction of aspartate availability (Supplementary Figure 3). Thus a defect in the enzyme needed to synthesise aspartyl tRNA, AspRS, or a limiting concentration of one of the substrates needed for formation of aspartyl tRNA, aspartate, can suppress the need for accessory replicative helicase activity. Moreover, suppression was not specific to aspartate starvation as restriction of availability of phenylalanine also allowed Δ*rep* Δ*uvrD* cells to survive in the absence of a complementing plasmid (Supplementary Figure 4). As expected, restricting amino acid availability also resulted in very poor growth (Supplementary Figures 3 and 4) but a restricted growth rate does not by itself provide suppression of Δ*rep* Δ*uvrD* lethality (Figure 2B). These data support the conclusion that inhibition of aminoacylation of tRNA^{Asp}, and of other tRNAs, reduces the need for accessory helicase activity.

Translational pausing suppresses Δ*rep* Δ*uvrD* inviability
Suppression of Δ*rep* Δ*uvrD* inviability via inhibition of tRNA^{Asp} or tRNA^{Phe} aminoacylation might occur directly via pausing of ribosomes but could also occur via decreased production of one or more specific proteins containing both aspartate and phenylalanine. We tested therefore whether increased translational pausing by a well-defined mechanism that does not rely on decreased tRNA aminoacylation also suppresses the need for accessory helicase activity. Peptide bond formation by ribosomes occurs with low efficiency within polyproline tracts as compared with other amino acids and this low efficiency is compensated for by interaction of elongation factor P (EF-P) with the ribosome (56,75). When EF-P is absent (Δ*efp*) ribosomes pause for extended periods at tandem proline codons (56,75) (see also Supplementary Figure 2) resulting in reduced growth rates (76) (see also Figure 2A). Despite its negative effect on growth rate, introduction of Δ*efp* clearly suppresses Δ*rep* Δ*uvrD* lethality (Figure 4, compare A and B). *yjeA* and *yjeK* encode enzymes needed for post-translational modification of EF-P to form fully functional enzyme (56,75-78) and deletion of either *yjeA* or *yjeK* also resulted in suppression (Figure 4C and D). Enhancement of ribosomal pausing at polyproline sequences can therefore reduce the need for accessory helicase activity.

Suppression by *aspT[t8c]* and Δ*efp* requires (p)ppGpp synthesis
One consequence of ribosomal pausing is increased synthesis of (p)ppGpp by RelA upon binding of RelA to a ribosome containing a non-acylated tRNA in the A site (15,16). Elevated (p)ppGpp is known to suppress Δ*rep* Δ*uvrD* inviability (37) and so *aspT[t8c]* and Δ*efp* might both therefore suppress via elevation of (p)ppGpp concentration.

Direct measurement of (p)ppGpp using ^{32}P cannot be performed on cells grown in rich media (79) which prevents direct assessment of (p)ppGpp levels in *aspT[t8c]* and Δ*efp* strains under conditions relevant to suppression of Δ*rep* Δ*uvrD* lethality. An

indirect assay was therefore used that employs an RpoS-mCherry translational fusion (34). This reporter provides a fluorescence signal that correlates with intracellular (p)ppGpp concentration due to (p)ppGpp both stimulating *rpoS* transcription and inhibiting RpoS degradation (34). Fluorescence was assayed by flow cytometry of cells grown to mid-logarithmic phase in rich medium. *relA$^+$ spoT$^+$* cells lacking the RpoS-mCherry fusion and *rpoS-mCherry ΔrelA ΔspoT* cells which are unable to synthesise (p)ppGpp gave similar levels of background fluorescence (Figure 5Ai and ii). An increase in fluorescence was observed in wild type cells carrying the fusion but this increase was modest (Figure 5A, compare iii with i and ii), consonant with low (p)ppGpp levels in wild type cells growing in nutrient-rich environments (34,80). mCherry fluorescence increased substantially in Δ*efp* cells but not in *aspT[t8c]* cells with respect to wild type (Figure 5A, compare iv and v with iii). We conclude that absence of EF-P results in elevation of (p)ppGpp concentration. Higher (p)ppGpp concentration in Δ*efp* cells as compared with wild type or *aspT[t8c]* cells is consistent with the significant retardation of growth of Δ*efp* cells (Figure 2A). In contrast, *aspT[t8c]* either does not increase (p)ppGpp levels as compared with wild type cells or any increase is below the limits of detection using this assay. To gauge the sensitivity of this assay we monitored fluorescence in *spoT1* cells. *spoT1* encodes a SpoT enzyme that retains (p)ppGpp synthase activity but lacks (p)ppGpp pyrophosphorylase activity, resulting in elevated (p)ppGpp (81) and the ability to suppress Δ*rep* Δ*uvrD* lethality (37). *spoT1 rpoS-mCherry* cells did not result in increased fluorescence as compared with *spoT$^+$ rpoS-mCherry* cells indicating that (p)ppGpp levels sufficient to reduce the need for accessory replicative helicases could go undetected using this assay (Figure 5Aiii and vi).

To address this potential sensitivity problem we employed a second assay to determine whether either Δ*efp* or *aspT[t8c]* results in elevated (p)ppGpp. Cells with elevated (p)ppGpp display elevated levels of persistence and thus *spoT1* enhances whereas Δ*relA* Δ*spoT* alleles reduce persistence (34,82) (see also Figure 5Bi-iii). *aspT[t8c]* and Δ*efp* both increased persistence with the increase being higher for Δ*efp* (Figure 5Biv and v). The large increase in persistence in Δ*efp* cells correlates with the enhancement of fluorescence in Δ*efp rpoS-mCherry* cells. The level of persistence in *aspT[t8c]* cells is consistent with a smaller increase in (p)ppGpp levels as compared with Δ*efp* that is below the limits of detection in the mCherry fluorescence assay.

We tested whether increased (p)ppGpp contributed to suppression of Δ*rep* Δ*uvrD* inviability by *aspT[t8c]* and Δ*efp* by deleting the gene encoding the primary (p)ppGpp synthase, *relA*. Deletion of *relA* prevented loss of pRC7*uvrD* from Δ*rep* Δ*uvrD aspT[t8c]* and Δ*rep* Δ*uvrD* Δ*efp* cells, indicating that RelA was required for suppression by both mutant alleles (Figure 5C, compare iii with iv and v with vi). In contrast, pRC7*uvrD* could be lost from Δ*rep* Δ*uvrD* Δ*relA rpoB*35* cells indicating that RelA was not required for the viability of suppressed Δ*rep* Δ*uvrD* strains under all circumstances (Figure 5C, compare vii and viii). This lack of dependence of Δ*rep* Δ*uvrD rpoB*35* on RelA likely reflects the recapitulation by *rpoB*35* of many phenotypes associated with elevated (p)ppGpp even in the absence of RelA (48,66).

Taken together, these data indicate that RelA-directed synthesis of (p)ppGpp is important for suppression of Δ*rep* Δ*uvrD* lethality by both *aspT[t8c]* and Δ*efp*.

aspT[t8c] and Δefp confer differing requirements for Rho activity

Translocation 5'-3' by Rho along untranslated and unstructured nascent transcripts can displace transcription complexes thus reducing both RNA polymerase occupancy on the chromosome and R-loop formation (11,83,84). Rep and Rho therefore provide two different mechanisms that reduce the impact of transcription on replication. Consequently, cells need either Rep or wild type levels of Rho activity to maintain genome duplication in the face of transcriptional barriers (10,85). One manifestation of this requirement is the hypersensitivity of Δ*rep uvrD$^+$* cells to low concentrations of

the Rho-specific inhibitor bicyclomycin (10) (see also Figure 6A and B, compare i and iii).

We assessed whether Δ*efp* or *aspT[t8c]* could suppress Δ*rep* bicyclomycin hypersensitivity. Neither Δ*efp* nor *aspT[t8c]* altered bicyclomycin sensitivity in a *rep*[+] background (Figure 6A and B, compare i and ii). In Δ*rep* cells hypersensitivity was suppressed by Δ*efp* but not by *aspT[t8c]* (Figure 6A and B, compare iii and iv). These data demonstrate that Δ*efp* but not *aspT[t8c]* can reduce the requirement for Rho activity in the absence of Rep.

We also tested whether this differential need for Rho activity was reflected in cells lacking both *rep* and *uvrD* by exploiting *rho[A243E]* which encodes a partial loss of function enzyme (86). Rho dependency was assessed by the ability of Δ*rep* Δ*uvrD* strains to lose a complementing pRC7*rep* rather than pRC7*uvrD* plasmid, allowing construction of pRC7*rep*/Δ*rep* Δ*uvrD rho[A243E]* strains regardless of *rep rho* double mutant lethality (85). The similar numbers and sizes of white plasmidless colonies formed by Δ*rep* Δ*uvrD* Δ*efp rho*[+] and Δ*rep* Δ*uvrD* Δ*efp rho[A243E]* cells indicate that wild type Rho function was not required for suppression by Δ*efp* (Figure 6C, compare v and vi). In contrast, Δ*rep* Δ*uvrD aspT[t8c] rho[A243E]* gave much smaller white plasmidless colonies as compared with the isogenic *rho*[+] strain indicating that wild type Rho function was important for growth of *aspT[t8c]* Δ*rep* Δ*uvrD* cells (Figure 6C, compare iii and iv).

The data in Figures 5 and 6 indicate that the viability of *aspT[t8c]* Δ*rep* Δ*uvrD* cells requires both the major (p)ppGpp synthase in the cell, RelA, and wild type Rho activity. In contrast, Δ*efp* Δ*rep* Δ*uvrD* cells require RelA but not wild type Rho activity. Δ*efp* cells have higher levels of (p)ppGpp as compared with *aspT[t8c]* (Figure 5A) and these elevated levels might explain the differential requirement for Rho, given the ability of (p)ppGpp to reduce replication/transcription conflicts (12,37,66). Such a model implies that (p)ppGpp synthesis is critical not only for the viability of Δ*efp* Δ*rep* Δ*uvrD* cells (Figure 5C) but also for suppression of bicyclomycin sensitivity of Δ*rep* cells (Figure 6A). Absence of the primary (p)ppGpp synthase RelA did not hypersensitise otherwise wild type cells to bicyclomycin (Figure 7Aiii). However, the suppression of Δ*rep* bicyclomycin hypersensitivity by Δ*efp* was abolished upon deletion of *relA* (Figure 7A, compare viii with v). Thus RelA-dependent (p)ppGpp synthesis in Δ*rep* Δ*efp* cells is essential for survival with lowered Rho activity, supporting the hypothesis that elevated (p)ppGpp can reduce the need for Rho.

We tested this possible link between (p)ppGpp concentration and requirement for Rho by determining whether other means of increasing (p)ppGpp concentration can reduce the need for Rho activity in Δ*rep* cells. The *spoT1* allele suppressed Δ*rep* bicyclomycin hypersensitivity supporting the conclusion that elevated (p)ppGpp can reduce the need for Rho (Figure 7B, compare iv with ii).

The data in Figures 5-7 indicate that both *aspT[t8c]* and Δ*efp* require RelA-directed synthesis of (p)ppGpp to maintain the viability of Δ*rep* Δ*uvrD* cells. In contrast, Δ*efp* has a much lower dependence on Rho activity as compared with *aspT[t8c]*. This differential requirement for Rho activity correlates with higher (p)ppGpp levels in Δ*efp* cells as compared with *aspT[t8c]*. Resolving conflicts between replication and transcription involves therefore a fine balance between accessory replicative helicases, levels of (p)ppGpp and Rho activity.

Discussion

We have discovered three new types of suppressors of Δ*rep* Δ*uvrD* rich medium lethality: mutations in tRNA genes, in aminoacyl-tRNA synthetases and in translation elongation factors. Whilst the mechanistic consequences of the *aspT[t8c]* mutation are unclear, the inhibition of tRNA aminoacylation by *aspS*[P555S] and of translation elongation by Δ*efp* are well-characterised (56,74,75), indicating that partial inhibition of translation can suppress Δ*rep* Δ*uvrD* lethality. Previous studies have demonstrated that this lethality is caused primarily by the conflict between

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replication and transcription (22,37,54). Thus partial inhibition of translation can compensate for the impact of transcription on DNA replication in the absence of Rep and UvrD. Suppression requires RelA-directed synthesis of (p)ppGpp with (p)ppGpp concentration being higher with Δ*efp* than with *aspT[t8c]* (Figure 5). Wild type levels of Rho activity are also needed in *aspT[t8c]* Δ*rep* Δ*uvrD* but not Δ*efp* Δ*rep* Δ*uvrD* cells indicating that the relative importance of (p)ppGpp and Rho function depends upon the nature of the translational mutation (Figure 6).

Δ*rep* Δ*uvrD* cells are inviable on rich medium primarily because of lack of accessory replicative helicase function (22,37,54). Suppression of this growth defect by the above translation mutations (Figures 1A, 3B and 4) therefore suggest that it is the lack of accessory helicase activity in Δ*rep* Δ*uvrD* cells that is being suppressed. Suppression by *aspT[t8c]* of the chromosome content defect of Δ*rep* cells, a direct consequence of slower fork movement in the absence of Rep accessory helicase activity (37-39), supports this view (Figure 1B). UvrD also inhibits RecFOR-dependent loading of RecA at blocked replication forks (52,68) and absence of this function makes a minor contribution to Δ*rep* Δ*uvrD* lethality (22,37,53). However, the lack of suppression of this RecA displacement defect by *aspT[t8c]* (Supplementary Figure 1C) indicates that suppression of Δ*rep* Δ*uvrD* lethality by *aspT[t8c]* does not operate via an effect on RecA loading. UvrD might also aid replication of transcribed DNA by inducing backtracking of RNAP stalled at DNA lesions, one consequence of which may be to promote repair of the lesion and allow the RNAP to then continue transcription (14). However, Δ*rep* Δ*uvrA* cells, lacking nucleotide excision repair, are viable (54) indicating that lesion repair via UvrD-catalysed backtracking of RNAP cannot be a major contributor to Δ*rep* Δ*uvrD* lethality. *aspT[t8c]* also does not suppress the sensitivity of Δ*uvrD* cells to UV light indicating that *aspT[t8c]* does not suppress nucleotide excision repair defects (Supplementary Figure 1A). Furthermore, *aspT[t8c]* suppression of the Δ*greA* Δ*greB* growth defect indicates that *aspT[t8c]* can suppress the absence of an *anti*-backtracking activity, making it difficult to explain how *aspT[t8c]* could also suppress the absence of a UvrD *pro*-backtracking function (Figure 1C).

RelA-directed synthesis of (p)ppGpp plays a central role in maintaining Δ*rep* Δ*uvrD* viability by *aspT[t8c]* and by Δ*efp* (Figure 5C). (p)ppGpp dramatically inhibits transcription of *rrn* operons (19) which are the primary transcriptional barriers to genome duplication (21,24), together with other highly expressed operons (20). (p)ppGpp may also destabilise stalled RNAP (12) or increase the fidelity of transcription (25), both of which could decrease the impact of transcription on fork movement. *aspT[t8c]*- and Δ*efp*-directed suppression of Δ*rep* Δ*uvrD* lethality via (p)ppGpp is therefore likely to occur by impacting on transcription initiation, stalled RNAP stability and/or decreased pausing due to enhanced fidelity.

RelA is stimulated to synthesise (p)ppGpp when an uncharged cognate tRNA is bound to the ribosomal A site (15,16). *aspT[t8c]* and Δ*efp* mutations may therefore increase the probability of A site-bound uncharged tRNA either directly or indirectly. The uridine at position 8 is highly conserved in tRNA species and the equivalent *t8c* mutation within human mitochondrial tRNA[Met] results in inhibition of tRNA aminoacylation (70). If *aspT[t8c]* also resulted in inhibition of aminoacylation then the resulting increase in non-acylated tRNA[Asp] could lead to an increased probability of uncharged tRNA[Asp] occupying the ribosomal A site. However, whether the mutant tRNA[Asp] can still bind to the A site is unknown. Alternatively tRNA[*asp[t8c]*] might poison the aspartyl tRNA synthetase by binding to it and forming a dead end complex with respect to aminoacylation. Formation of such a dead end complex might titrate out aspartyl tRNA synthetase and increase levels of uncharged wild type tRNA[Asp] leading to increased non-acylated tRNA[Asp] occupying the ribosomal A site. For cells lacking EF-P, YjeA or YjeK it is difficult to conceive how a reduced rate of proline-proline bond formation within the ribosome could lead directly to increased

occupancy of the A site by uncharged tRNA. Absence of any one of these three factors, though, does impact on expression of many genes and so altered expression of one or more genes in Δ*efp*, Δ*yjeA* or Δ*yjeK* cells could result in an increased probability of A site-bound uncharged tRNA. For example, Δ*efp* cells have reduced expression of valyl tRNA synthetase (87) which could lead to accumulation of uncharged tRNA^{Val} and triggering of the stringent response. Suppression of Δ*rep* Δ*uvrD* lethality by *aspS*^{P555S} demonstrates that suppression via partial loss of tRNA synthetase function can occur (Figure 3).

In contrast to the requirement for RelA to sustain suppression of Δ*rep* Δ*uvrD* lethality by both *aspT[t8c]* and Δ*efp*, the requirement for Rho activity is reduced in Δ*efp* as compared with *aspT[t8c]* cells (Figures 6 and 7). This difference correlates with the higher concentration of (p)ppGpp in Δ*efp* as compared with *aspT[t8c]* cells (Figure 5). A role for (p)ppGpp in reducing the need for Rho activity is supported by the ability of *spoT1* to suppress Δ*rep* bicyclomycin hypersensitivity (Figure 7B). Suppression of Δ*rep* bicyclomycin hypersensitivity by Δ*efp* also depends on RelA which supports a critical balance between (p)ppGpp concentration and Rho activity in maintaining viability (Figure 7A). Elevated (p)ppGpp can therefore reduce the need for Rho.

The greater dependence of *aspT[t8c]*-directed suppression on Rho might reflect not just lower (p)ppGpp levels in *aspT[t8c]* versus Δ*efp* cells but possibly also enhancement of Rho binding on nascent transcripts by *aspT[t8c]*. In other words, suppression via *aspT[t8c]* might occur at least partly via the increased generation of Rho binding sites on emerging transcripts. Aspartate codons are more frequent than polyproline tracts and hence *aspT[t8c]* has the potential to impact on ribosome translocation more frequently than Δ*efp*. Enhancement of Rho binding would also be dependent only on ribosomal pausing and not specifically require ribosomal A site occupancy by a non-aminoacylated tRNA (88), in contrast to stimulation of RelA activity (15,16). However, any *aspT[t8c]*-directed increase of transcription complex displacement by Rho cannot be sufficient by itself to suppress Δ*rep* Δ*uvrD* lethality since RelA is also needed (Figure 5C).

Our data indicate that whilst translation helps prevent RNAP backtracking (7,9) the reduction of conflicts between replication and transcription can be achieved more effectively by partial inhibition of translation. Of course, the growth defect in Δ*efp* cells (Figure 2A) indicates why the balance between replication, transcription, translation and (p)ppGpp synthesis is poised as it is in wild type cells. Thus for the maintenance of rapid growth there is a very fine balance to be struck between gene expression and accurate, rapid genome duplication. Furthermore, accessory replicative helicases play a major role in determining this balance. In the absence of both Rep and UvrD the wild type balance between various other mechanisms that reduce the impact of transcription on replication is unable to effectively counter the adverse effects of transcription on completion of genome duplication. There may also be circumstances under which mutations such as those identified here confer a selective advantage even in *rep*$^+$ *uvrD*$^+$ cells. Elevated (p)ppGpp is a key factor in determining bacterial persistence in the face of antibiotic challenge (34) and both *aspT[t8c]* and Δ*efp* mutations enhance persistence (Figure 5B). It remains possible therefore that mutations such as *aspT[t8c]* that have only a modest inhibitory effect on growth could arise in bacterial populations continually exposed to antibiotics, especially given the many mutations known to affect translation (89). Increased persistence in strains lacking EF-P function also imply that EF-P and its unique post-translational modification pathway are poor potential targets for antibiotics.

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Figure Legends

Figure 1. A mutation in an aspartyl tRNA gene suppresses the need for accessory replicative helicases and for anti-backtracking factors.

(A) Retention or loss of pRC7*uvrD* (pAM407) from strains without or with Δ*rep* Δ*uvrD* deletions as judged by blue/white colony colour on LB plates containing X-gal and IPTG. Fractions of white colonies are indicated below each image with actual numbers of white versus total colonies counted in parentheses.

(B) DNA content of the indicated strains as monitored by flow cytometry under run out conditions in LB. The number of chromosome equivalents per cell is shown below.

(C) The viability of *greA*⁺ *greB*⁺ versus Δ*greA* Δ*greB* cells without and with *aspT[t8c]* as monitored by serial dilutions of liquid cultures grown at 30°C and plated subsequently on LB agar at 30°C and 42°C.

Figure 2. The impact of *aspT[t8c]* on growth and its relevance to Δ*rep* Δ*uvrD* inviability.

(A) Growth of (i) *aspT*⁺ *efp*⁺ (TB28), (ii) *aspT[t8c] efp*⁺ (KM231) and (iii) *aspT*⁺ Δ*efp* (MH299) in LB at 37°C as monitored by absorbance at 600 nm.

(B) Assessment of the ability of pRC7*uvrD* (pAM407) to be lost from Δ*rep* Δ*uvrD* cells by reducing growth rates via culturing at 37°C and 25°C for the indicated times on defined rich medium containing glycerol as a carbon source.

Figure 3. A mutation in aspartyl tRNA synthetase suppresses Δ*rep* Δ*uvrD* lethality.

(A) The indicated strains were grown overnight in high salt medium at 30°C and then serial dilutions plated onto low and high salt plates containing 100 μg ml⁻¹ ampicillin and incubated at either 30°C or 42°C.

(B) Suppression of Δ*rep* Δ*uvrD* lethality by *aspS*^P555S on rich medium was analysed by monitoring retention or loss of pRC7*uvrD* (pAM407) from the indicated strains at 30°C on either low or high salt LB medium.

Figure 4. Ribosomal pausing decreases the requirement for accessory helicase activity.

Retention or loss of pRC7*rep* (pAM403) was monitored at 37°C on LB X-gal IPTG plates for the indicated strains.

Figure 5. (p)ppGpp synthesis is needed for suppression of Δ*rep* Δ*uvrD* lethality by *aspT[t8c]* and Δ*efp*.

(A) Flow cytometric detection of *in vivo* levels of RpoS-mCherry fluorescence in the indicated strains. All strains contain the *rpoS-mCherry* fusion with the exception of (i) MG1655.

(B) Survival after challenge with ciprofloxacin. The strains are (i) TB28, (ii) KM241, (iii) N5777, (iv) KM231 and (v) MH372.

(C) Retention and loss of pRC7*uvrD* (pAM407) on LB X-gal IPTG agar in *relA*⁺ and Δ*relA* strains.

Figure 6. Δ*efp* but not *aspT[t8c]* can bypass the need for wild type Rho activity.

(A) and (B) The indicated strains were grown in liquid culture in the absence of bicyclomycin and their ability to continue to divide with reduced Rho activity was assessed after serial dilution onto plates without and with 25 μg ml⁻¹ bicyclomycin.

(C) Loss of pRC7*rep* (pAM403) on LB X-gal IPTG agar in *rho*⁺ (i, iii, v) and *rho[A243E]* (ii, iv, vi) strains.

Figure 7. The ability of Δ*efp* to bypass the requirement for wild type Rho activity is dependent on (p)ppGpp synthesis.

(A) and (B) Strains were grown in the absence of bicyclomycin and then serial

dilutions were plated onto LB agar without and with 25 µg ml^{-1} bicyclomycin.

Figure 1

Figure 2

A

LB +
0.5 g L^{-1} NaCl

LB +
5.0 g L^{-1} NaCl

10^{-1} ⟶ 10^{-6} 10^{-1} ⟶ 10^{-6}

i) N6644: *uvrD$^+$ / ΔuvrD Δrep aspS$^+$*

ii) KM025: *uvrD$^+$ / ΔuvrD Δrep aspS^{P555S}*

30°C

iii) N6644: *uvrD$^+$ / ΔuvrD Δrep aspS$^+$*

iv) KM025: *uvrD$^+$ / ΔuvrD Δrep aspS^{P555S}*

42°C

B

i) *uvrD$^+$ / uvrD$^+$ rep$^+$ aspS$^+$* (JA012)

ii) *uvrD$^+$ / ΔuvrD Δrep aspS$^+$* (N6644)

iii) *uvrD$^+$ / ΔuvrD Δrep aspS^{P555S}* (KM025)

30°C

LB +
0.5 g L^{-1} NaCl

0.66 (299/452)   0.009 (2/213)   0.58 (603/1044)

LB +
5.0 g L^{-1} NaCl

0.57 (184/322)   <0.005 (0/194)   0.001 (1/708)

Figure 3

A) *rep$^+$ / ΔuvrD Δrep*   B) *rep$^+$ / ΔuvrD Δrep*   C) *rep$^+$ / ΔuvrD Δrep*   D) *rep$^+$ / ΔuvrD Δrep*
                                        Δefp   *ΔyjeA*   *ΔyjeK*
      (JA033)   (MH301)   (MH184)   (MH298)

   0.05 (30/658)   0.78 (120/154)   0.52 (146/279)   0.58 (156/270)

Figure 4

A

Number of cells (y-axis)
Fluorescence intensity (610 nm) (x-axis)

- i) MG1655: no mCherry
- ii) KM285: ΔrelA ΔspoT
- iii) MG1655 RpoS-mCherry: wild type
- iv) KM257: Δefp
- v) KM071: aspT[t8c]
- vi) KM284: spoT1

B

Log percentage survival (y-axis)

- i) wild type
- ii) spoT1
- iii) ΔrelA ΔspoT
- iv) aspT[t8c]
- v) Δefp

C

i) uvrD⁺ / ΔuvrD Δrep relA⁺ (N6644)
0.00 (0/344)

iii) uvrD⁺ / ΔuvrD Δrep aspT[t8c] relA⁺ (KM154)
0.38 (131/212)

v) uvrD⁺ / ΔuvrD Δrep Δefp relA⁺ (KM260)
0.54 (126/274)

vii) uvrD⁺ / ΔuvrD Δrep rpoB*35 relA⁺ (N7150)
0.61 (118/194)

ii) uvrD⁺ / ΔuvrD Δrep ΔrelA (KM059)
0.03 (8/250)

iv) uvrD⁺ / ΔuvrD Δrep aspT[t8c] ΔrelA (KM155)
<0.003 (0/300)

vi) uvrD⁺ / ΔuvrD Δrep Δefp ΔrelA (KM344)
<0.003 (0/366)

viii) uvrD⁺ / ΔuvrD Δrep rpoB*35 ΔrelA (KM054)
0.60 (150/251)

Figure 5

A

	− bicyclomycin	+ bicyclomycin
	$10^0 \longrightarrow 10^{-6}$	$10^0 \longrightarrow 10^{-6}$

i) TB28: $efp^+ rep^+$

ii) MH299: Δefp

iii) N6577: Δrep

iv) MH374: $\Delta efp \Delta rep$

B

i) TB28: $aspT^+ rep^+$

ii) KM231: $aspT[t8c]$

iii) N6577: Δrep

iv) N8210: $aspT[t8c] \Delta rep$

C

i) $rep^+ / \Delta uvrD \Delta rep$

(KM359)

0.005 (2/410)

ii) $rep^+ / \Delta uvrD \Delta rep$
$rho[A243E]$
(KM361)

0.012 (2/154)

iii) $rep^+ / \Delta uvrD \Delta rep$
$aspT[t8c]$
(KM391)

0.54 (100/185)

iv) $rep^+ / \Delta uvrD \Delta rep$
$aspT[t8c] rho[A243E]$
(KM398)

0.39 (46/117)

v) $rep^+ / \Delta uvrD \Delta rep$
Δefp
(KM367)

0.59 (381/649)

vi) $rep^+ / \Delta uvrD \Delta rep$
$\Delta efp rho[A243E]$
(KM395)

0.76 (216/286)

Figure 6

A

- bicyclomycin   + bicyclomycin

$10^0 \longrightarrow 10^{-6}$ $10^0 \longrightarrow 10^{-6}$

i) TB28: $efp^+\ rep^+relA^+$

ii) MH299: Δefp

iii) KM225 $\Delta relA$

iv) N6577: Δrep

v) MH374: $\Delta efp\ \Delta rep$

vi) MH363: $\Delta relA\ \Delta rep$

vii) MH376: $\Delta relA\ \Delta efp$

viii) MH378: $\Delta relA\ \Delta efp\ \Delta rep$

B

i) TB28: $spoT^+\ rep^+$

ii) N6577: Δrep

iii) N7154: $spoT1$

iv) KM244: $spoT1\ \Delta rep$

Figure 7

Supplementary Information

Supplementary Figure 1. *aspT[t8c]* does not suppress defects in nucleotide excision repair, mismatch repair or control of recombination in Δ*uvrD* cells.

(A) *Nucleotide excision repair.* The ability of the indicated strains to survive exposure to DNA damage was assessed at increasing doses of 254 nm UV light. Δ*uvrD* cells were sensitive to UV light as compared with *uvrD*⁺ and this sensitivity was not suppressed by *aspT[t8c]* (compare KM235 and 237).

(B) *Mismatch repair.* The accumulation of rifampicin-resistant spontaneous mutations upon growth of a single colony to stationary phase in liquid culture was used as a readout of mismatch repair. The strains we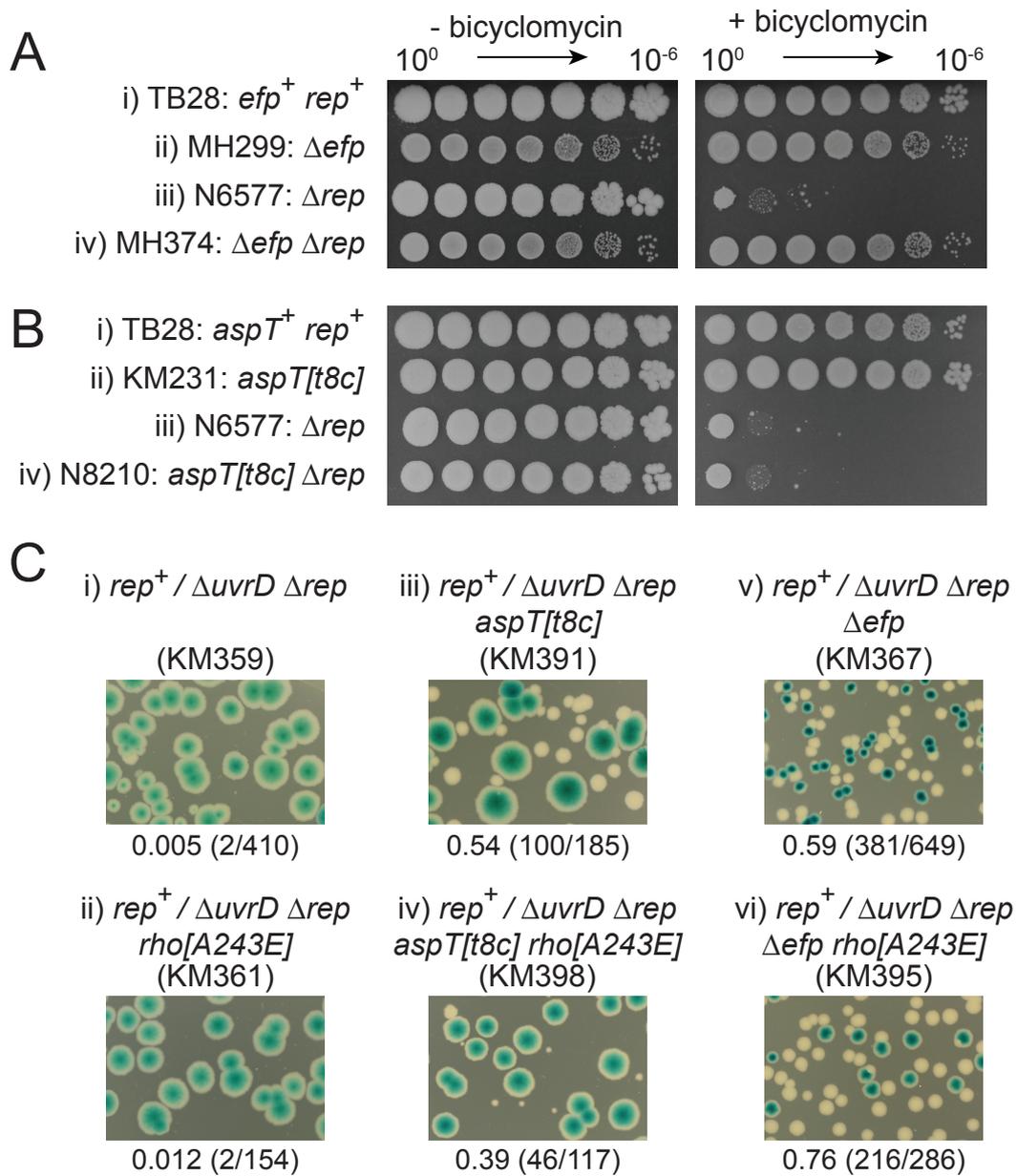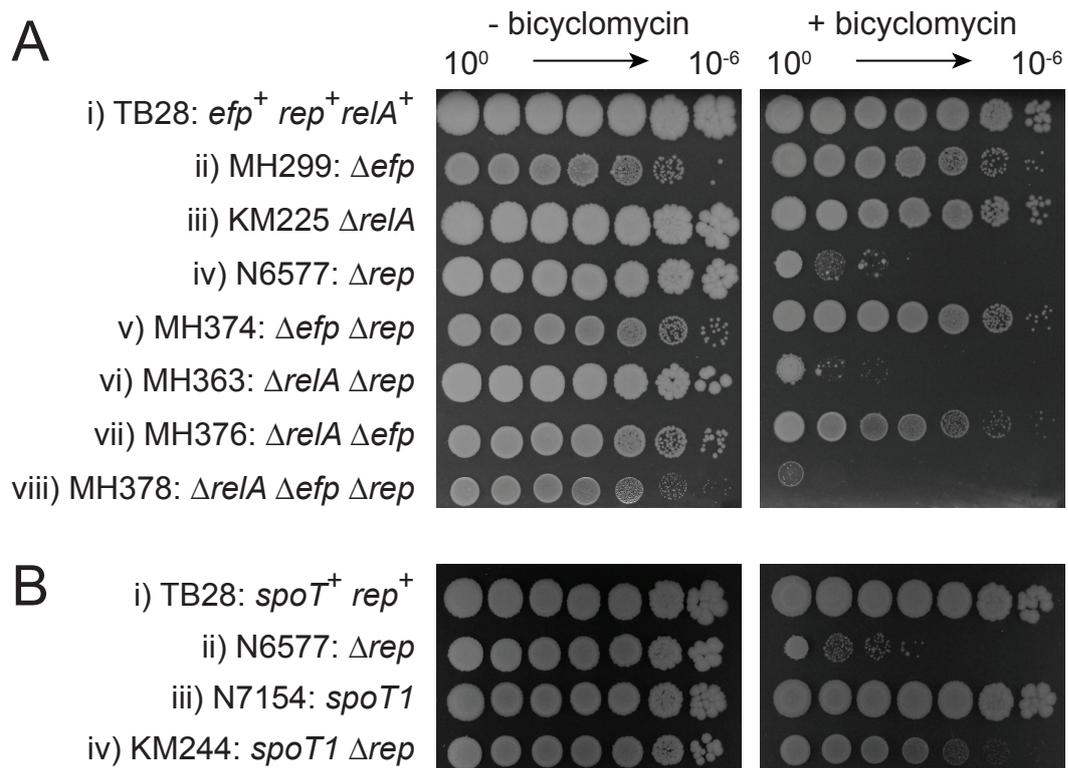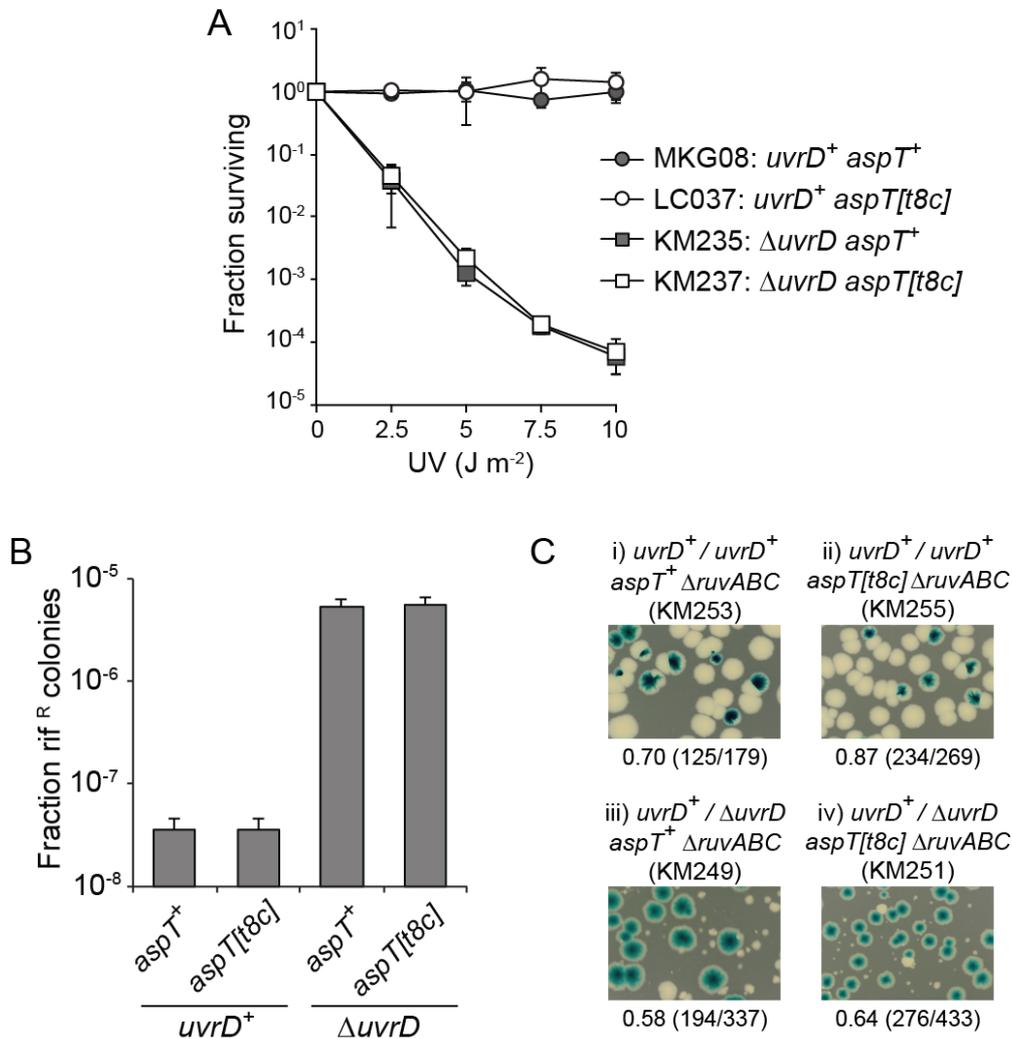re those used in panel (A). Δ*uvrD* cells gave rise to more rifampicin resistant colonies as compared with *uvrD*⁺ but this defect in mismatch repair was not suppressed by *aspT[t8c]*.

(C) *Control of recombination.* UvrD modulates levels of homologous recombination by displacing RecA from DNA (1). When RecA-directed strand exchange does proceed then RuvABC resolves any Holliday junctions formed thus facilitating chromosome segregation by removing interchromosomal Holliday junctions (2). Thus UvrD inhibits formation of interchromosomal Holliday junctions whilst RuvABC resolves such junctions when they do form. Efficient chromosome segregation requires either UvrD or RuvABC in *recA*⁺ cells, evinced by the very low viability of Δ*uvrD* Δ*ruvABC* cells (2). Thus pRC7*uvrD* can be lost from *uvrD*⁺ Δ*ruvABC* cells on LB agar at high frequency with no significant impact on plasmidless white colony size whereas plasmidless Δ*uvrD* Δ*ruvABC* colonies are very small, indicative of a severe viability problem (compare i with iii). This viability problem was not suppressed by *aspT[t8c]* (compare iii and iv).
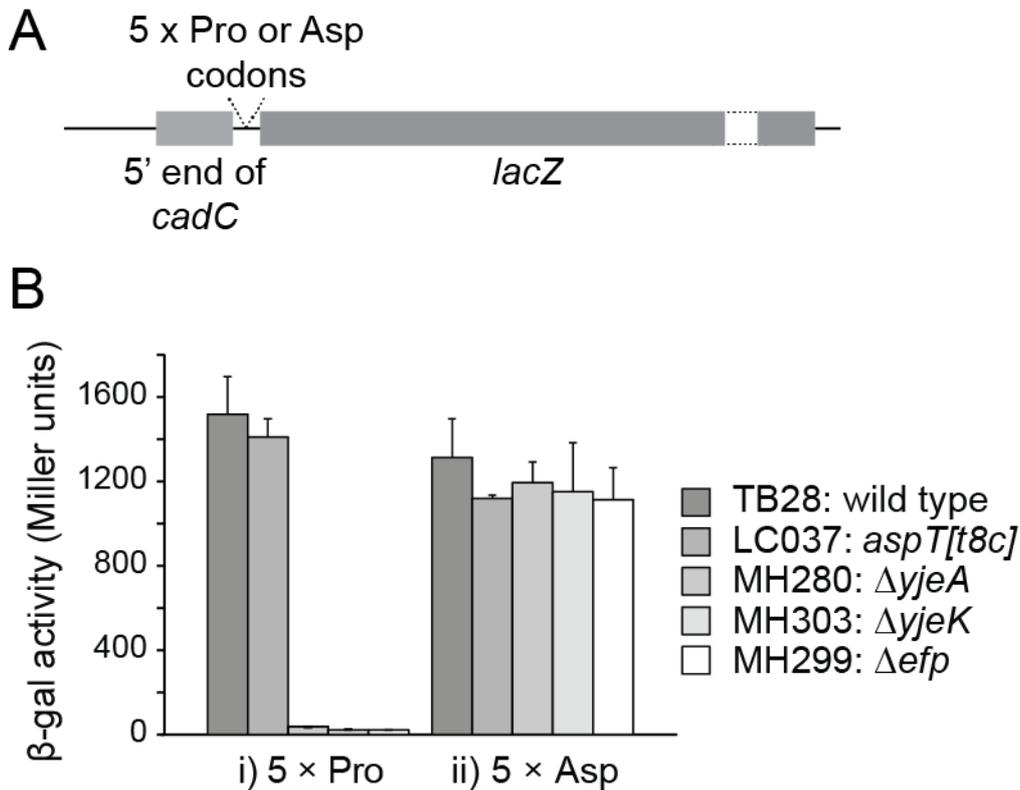
Supplementary Figure 2. *aspT[t8c]* does not cause significant pausing of ribosomes at aspartate codons.

(A) Schematic of the gene fusion cassette used to assess the relative impact of codons on efficiency of translation (3). The first 30 codons of *cadC* are fused in frame with *lacZ* via a linker sequence that encodes five consecutive aspartate or proline codons. β-galactosidase assays are then used with transformed *E. coli* cells to determine the impact of the repeated aspartate and proline codons on LacZ levels, providing a readout of translational efficiency.

(B) (i) YjeA, YjeK and EF-P are all needed for efficient translation of polyproline sequences and, as expected, levels of translation of the *lacZ* fusion protein from a plasmid containing five proline codons (p3LC-TL30-5P) (3) are inhibited when any one of these factors is absent as compared with a wild type strain. (ii) In contrast, the *aspT[t8c]* mutation does not result in significant inhibition of translation of *lacZ* from the plasmid encoding five tandem aspartate codons (p3LC-TL30-5D) as indicated by the similar levels of β-galactosidase activity in wild type and *aspT[t8c]* strains.

A

	+ all amino acids	- aspartate	- phenylalanine
	$10^{-1} \longrightarrow 10^{-6}$	$10^{-1} \longrightarrow 10^{-6}$	$10^{-1} \longrightarrow 10^{-6}$

i) N6644: *uvrD⁺ / ΔuvrD Δrep aspC⁺ tyrB⁺*

ii) KM011: *uvrD⁺ / ΔuvrD Δrep ΔaspC ΔtyrB*

B

	i) *uvrD⁺ / uvrD⁺ rep⁺* (JA012)	ii) *uvrD⁺ / ΔuvrD Δrep* (N6644)	iii) *uvrD⁺ / ΔuvrD Δrep ΔaspC ΔtyrB* (KM011)
400 µM Asp	0.42 (142/337)	0.015 (9/611)	<0.007 (0/148)
40 µM Asp	0.43 (130/300)	<0.001 (0/672)	0.007 (1/145)
4 µM Asp	0.42 (131/311)	<0/001 (0/854)	0.018 (3/166)
0 µM Asp	0.47 (154/325)	<0.001 (0/678)	0.16 (36/219)

C

EZ + glucose - Asp → MM + glucose + Asp

	- ampicillin	+ ampicillin

i) JA012: *uvrD⁺ / uvrD⁺ rep⁺ aspC⁺ tyrB⁺*
[W]
[W]
[B]
[B]

ii) KM011: *uvrD⁺ / ΔuvrD Δrep ΔaspC ΔtyrB*
[W]
[W]
[B]
[B]

[W] White colony from 0 µM Asp
[B] Blue colony from 0 µM Asp

Supplementary Figure 3. Restriction of aspartate availability suppresses Δ*rep* Δ*uvrD* lethality.

(A) Aspartate auxotrophy requires mutations in both aspartate aminotransferase and aromatic amino acid aminotransferase, encoded by *aspC* and *tyrB* respectively, since each can catalyse the transamination of oxaloacetate from glutamate to form aspartate (4,5). Both these aminotransferases can also catalyse the final step in phenylalanine biosynthesis but *aspC tyrB* strains still synthesise phenylalanine since the branched chain amino acid aminotransferase encoded by *ilvE* can catalyse this reaction to a limited extent (4,6). We introduced both *aspC* and *tyrB* deletions into pRC7*uvrD*/Δ*rep* Δ*uvrD*. This strain (ii) could grow on defined rich medium when all amino acids were provided, although colony sizes were reduced. Absence of aspartate or phenylalanine exacerbated this growth defect severely but weak growth was still observable. Restricted growth was expected in the absence of phenylalanine via the *ilvE*-encoded branched chain amino acid aminotransferase (4,6). However, the

3

limited growth in the absence of added aspartate suggests that trace amounts of aspartate were present in preparations of the other amino acids present in the defined medium.

(B) Retention or loss of pRC7*uvrD* was monitored for the indicated strains on defined rich medium with decreasing concentrations of added aspartate. Absence of added aspartate had no significant impact on colony sizes or on frequency of loss of pRC7*uvrD* for either (i) JA012 or (ii) N6644, as expected since both strains retain the *aspC* and *tyrB* genes but colony sizes for (iii) KM011 decreased with decreasing aspartate concentration. Very small KM011 colonies could still form in the absence of added aspartate, consonant with the very restricted growth seen in (Aii), and some of these colonies were white indicating loss of pRC7*uvrD*. Limiting the availability of aspartate therefore suppresses Δ*rep* Δ*uvrD* lethality.

(C) Loss of pRC7*uvrD* from KM011 in the absence of added aspartate (see Biii) was confirmed by using ampicillin resistance as a marker for the presence or absence of pRC7*uvrD* which encodes β lactamase. Two white [W] and two blue [B] colonies of KM011 and JA012 from the 0 µM aspartate plates in (B) were resuspended in 100 µL 56/2 salts solution, serially diluted in the same salts solution and 5 µL of the 10^0-10^{-5} dilutions spotted onto minimal medium plates containing glucose and all amino acids without and with ampicillin. All dilutions of resuspended blue colonies gave colonies on plates regardless of the presence of ampicillin indicating that these colonies retained pRC7*uvrD* as expected. In contrast, all dilutions of resuspended white colonies gave colonies only in the absence of ampicillin. We conclude that the small white colonies isolated from KM011 on 0 µM aspartate plates in (B) had indeed lost pRC7*uvrD*, confirming suppression of Δ*rep* Δ*uvrD* lethality by restricting aspartate availability.
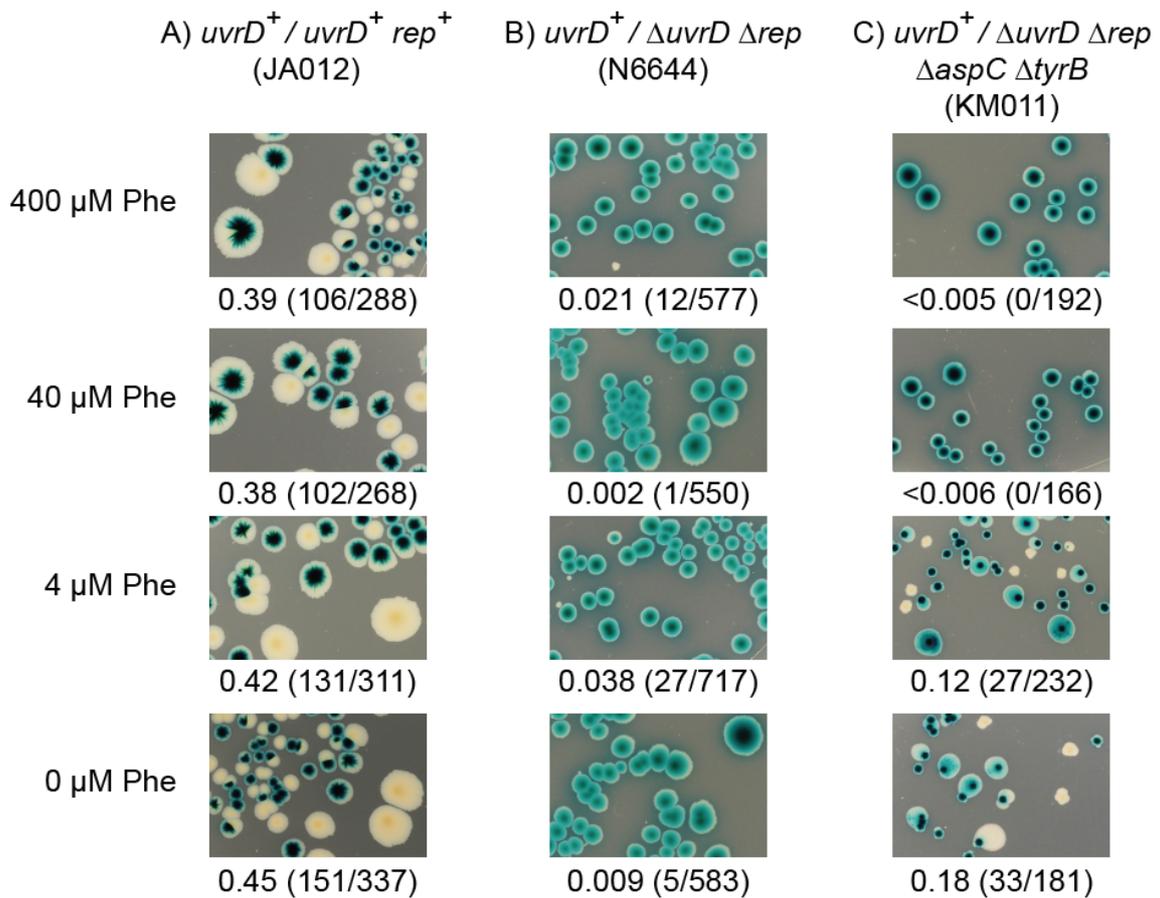
Supplementary Figure 4. Suppression of Δ*rep* Δ*uvrD* lethality by restriction of amino acid availability is not specific to aspartate metabolism.

The ability of a Δ*rep* Δ*uvrD* Δ*aspC* Δ*tyrB* strain, KM011, to lose pRC7*uvrD* at decreasing phenylalanine concentrations on defined rich medium was assessed. Colony morphology was altered at lower added phenylalanine concentrations but it was also clear that some of these colonies were white, indicating loss of pRC7*uvrD* (compare B and C at 4 and 0 μM phenylalanine). Restriction of phenylalanine availability therefore reduced the need for accessory replicative helicase activity in a manner similar to the suppression seen upon aspartate restriction (Supplementary Figure 3).

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Supplementary Table 1. *Escherichia coli* K12 strains.

A) MG1655 derivatives

MG1655	F⁻ *rph-1*	(1)
GJ6520	*lacI lacZU₁₁₈ trpR55 trpE9777 rho[A243E] Δ(yefM-yoeB)::Cm*	(2)
JA012	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<>*	TB28 x pAM407 to Ampʳ
JA016	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr*	N6632 x pAM407 to Ampʳ
JA033	pAM403 *(lac⁺ rep⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat*	Plasmid-free segregant of N6644 formed on minimal medium subsequently transformed with pAM403 to Ampʳ
KM001	*ΔlacIZYA::<> ΔuvrD::dhfr ΔaspC745::<Kan>*	P1.JW0911 x N6632 to Kmʳ
KM003	*ΔlacIZYA::<> ΔuvrD::dhfr ΔaspC745::<>*	KM001 x pCP20 (3) to Ampʳ then plasmid-free Kmˢ segregant identified after growth on LB at 42°C
KM005	*ΔlacIZYA::<> ΔuvrD::dhfr ΔaspC745::<> ΔtyrB747::<kan>*	P1.JW4014 x KM003 to Kmʳ
KM007	*ΔlacIZYA::<> ΔuvrD::dhfr ΔaspC745::<> ΔtyrB747::<>*	KM005 x pCP20 (3) to Ampʳ then plasmid-free Kmˢ segregant identified after growth on LB at 42°C
KM009	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr ΔaspC745::<> ΔtyrB747::<>*	pAM407 x KM007 to Ampʳ
KM011	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat ΔaspC745::<> ΔtyrB747::<>*	P1.N6577x KM009 to Cmʳ
KM021	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr tls-1 eda-51::Tn10*	P1.CS89 x N6632 to Tmʳ
KM025	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat tls-1 eda-51::Tn10*	P1.N6577 x KM021 to Cmʳ
KM050	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr ΔrelA782::kan*	JA016 x P1.JW2755 to Kmʳ
KM054	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> rpoB*35 ΔuvrD::dhfr Δrep::cat ΔrelA782::kan*	N7150 x P1.JW2755 to Kmʳ
KM059	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat ΔrelA782::kan*	N6644 x P1.JW2755 to Kmʳ
KM071	*rpoS-mCherry::<> rep⁺<kan> aspT[t8c]*	MG1655 RpoS-Mcherry x P1.LC037 to Kmʳ
KM154	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat aspT[t8c]*	JA016 x P1.PM647 to Cmʳ. *aspT[t8c]* allele confirmed by sequencing
KM155	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat aspT[t8c] ΔrelA782::kan*	KM050 x P1.N7182 to Cmʳ
KM189	pAM407 *(lac⁺ uvrD⁺)* / *ΔlacIZYA::<> ΔuvrD::dhfr tls-1 Δeda775::kan*	KM021 x P1.JW1839 to Kmʳ and temperature-

		sensitive growth.
KM225	*ΔlacIZYA::<> ΔrelA782::kan*	TB28 x P1.JW2755 to Km[r]
KM230	*lacI lacZU₁₁₈ trpR55 trpE9777 rho[A243E] Δ(yefM-yoeB)::Cm Δrep729::kan*	*Δrep729::kan* integration into GJ6520 using pKD46 (4)
KM231	*ΔlacIZYA::<> rep⁺ <> aspT[t8c]*	LC037 x pCP20 (3) to Amp[r] then plasmid-free Km[s] segregant identified after growth on LB at 42°C
KM235	*ΔlacIZYA::<> ΔuvrD::dhfr rep⁺ <kan>*	N6632 x P1.MKG08 to Km[r]
KM237	*ΔlacIZYA::<> ΔuvrD::dhfr rep⁺<kan> aspT[t8c]*	N6632 x P1.LC037 to Km[r]
KM239	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> ΔuvrD::dhfr rep⁺ <kan>*	KM235 x pAM407 to Amp[r]
KM240	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> ΔuvrD::dhfr rep⁺<kan> aspT[t8c]*	KM237 x pAM407 to Amp[r]
KM241	*ΔlacIZYA::<> spoT1*	N7154 x pCP20 (3) to Amp[r] then plasmid-free Km[s] segregant identified after growth on LB at 42°C
KM244	*ΔlacIZYA::<> Δrep::cat spoT1*	PM567 x pCP20 (3) to Amp[r] then plasmid-free Km[s] segregant identified after growth on LB at 42°C
KM245	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> rep⁺ <kan>*	MKG08 x pAM407 to Amp[r]
KM246	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> rep⁺<kan> aspT[t8c]*	LC037 x pAM407 to Amp[r]
KM247	*ΔlacIZYA::<> spoT1 rpoS-mCherry::<kan>*	KM241 x P1. MG1655 RpoS-Mcherry Kan to Km[r]
KM249	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> ΔuvrD::dhfr rep⁺ <kan> ΔruvABC::cat*	KM239 x P1.N6268 to Cm[r]
KM251	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> ΔuvrD::dhfr rep⁺<kan> aspT[t8c] ΔruvABC::cat*	KM240 x P1.N6268 to Cm[r]
KM253	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> rep⁺ <kan> ΔruvABC::cat*	KM245 x P1.N6268 to Cm[r]
KM255	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> rep⁺<kan> aspT[t8c] ΔruvABC::cat*	KM246 x P1.N6268 to Cm[r]
KM257	*rpoS-mCherry::<> Δefp772::kan*	MG1655 RpoS-Mcherry x P1.JW4107 to Km[r]
KM260	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat Δefp772::<>*	KM280 x P1.N6644 to Km[r]
KM268	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> ΔuvrD::dhfr Δefp772::kan*	JA016 x P1.JW4107 to Km[r]
KM269	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> rpoB*35 ΔuvrD::dhfr Δrep729::kan*	N7150 x P1.JW5604 to Km[r]
KM271	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<> rpoB*35 ΔuvrD::dhfr Δrep729::kan rho[A243E]*	N7150 x P1.KM230 to Km[r] and then screening for the mutant *rho* allele by sequencing
KM273	pAM407 *(lac⁺ uvrD⁺) / ΔlacIZYA::<>*	N6644 x P1.KM269 to Km[r]

	$\Delta uvrD::dhfr$ $\Delta rep729::kan$	
KM275	pAM407 (lac$^+$ uvrD$^+$) / $\Delta lacIZYA::<>$ $\Delta efp772::<>$	MH372 x pAM407 to Amp^r
KM277	pAM407 (lac$^+$ uvrD$^+$) / $\Delta lacIZYA::<>$ $\Delta uvrD::dhfr$ $\Delta rep729::kan$ $\Delta efp772::<>$	KM275 x P1.KM269 to Km^r then screened for Tm^r
KM280	pAM407 (lac$^+$ uvrD$^+$) / $\Delta lacIZYA::<>$ $\Delta uvrD::dhfr$ $\Delta efp772::<>$	Plasmid-free segregant of KM268 formed on LB subsequently transformed with pCP20 (3) to Amp^r. Plasmid-free Km^s segregant then identified after growth on LB at 42°C and then retransformed with pAM407 to Amp^r.
KM281	pAM407 (lac$^+$ uvrD$^+$) / $\Delta lacIZYA::<>$ $\Delta uvrD::dhfr$ $\Delta rep729::kan$ rho[A243E]	N6644 x P1.KM271 to Km^r
KM284	$\Delta lacIZYA::<>$ spoT1 rpoS-mCherry::<>	KM247 x pCP20 (3) to Amp^r then plasmid-free Km^s segregant identified after growth on LB at 42°C
KM285	rpoS-mCherry::<> $\Delta relA251::kan$ $\Delta spoT207::cat$	KM287 x P1.N5777 to Cm^r
KM287	rpoS-mCherry::<> $\Delta relA251::kan$	MG1655 RpoS-Mcherry x P1.N5777 to Km^r
KM301	pAM407 (lac$^+$ uvrD$^+$) / $\Delta lacIZYA::<>$ $\Delta uvrD::dhfr$ $\Delta efp772::<>$ $\Delta relA782::<>$	MH376 x pCP20 (3) to Amp^r then plasmid-free Km^s segregant identified after growth on LB at 42°C. Km^s strain then transformed with pAM407 to Amp^r and transduced with P1.KM189 to Tm^r
KM307	pAM407 (lac$^+$ uvrD$^+$) / $\Delta lacIZYA::<>$ rep$^+$<> aspT[t8c]	KM231 x pAM407 to Amp^r
KM327	MG1655 $\Delta lacIZYA::<>$ $\Delta greA788::<kan>$	TB28 x P1.JW3148 to Km^r
KM331	$\Delta lacIZYA::<>$ rep$^+$<> aspT[t8c] $\Delta greA788::<kan>$	KM231 x P1.JW3148 to Km^r
KM335	MG1655 $\Delta lacIZYA::<>$ $\Delta greA::<>$ $\Delta greB740::<kan>$	KM338 x P1.JW3369 to Km^r
KM336	$\Delta lacIZYA::<>$ rep$^+$<> aspT[t8c] $\Delta greA788::<>$ $\Delta greB740::<kan>$	KM339 x P1.JW3369 to Km^r
KM337	pAM403 (lac$^+$ rep$^+$) / $\Delta lacIZYA::<>$ rep$^+$ <> aspT[t8c]	KM231 xpAM403 to Amp^r
KM338	$\Delta lacIZYA::<>$ $\Delta greA788::<>$	KM327 x pCP20 (3) to Amp^r then plasmid-free Km^s segregant identified after growth on LB at 42°C
KM339	$\Delta lacIZYA::<>$ rep$^+$<> aspT[t8c] $\Delta greA788::<>$	KM331 x pCP20 (3) to Amp^r then plasmid-free Km^s segregant identified after growth on LB at 42°C
KM344	pAM407 (lac$^+$ uvrD$^+$) / $\Delta lacIZYA::<>$ $\Delta uvrD::dhfr$ $\Delta rep::cat$ $\Delta efp772::<>$	KM301 x P1.N6644 to Cm^r

	ΔrelA782::<>	
KM346	pAM407 *(lac$^+$ uvrD$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep729::kan aspT[t8c] rho[A243E]*	KM307 x P1.KM271
KM359	pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep729::kan*	Plasmid-free segregant of KM273 formed on minimal medium subsequently transformed with pAM403 to Amp^r
KM361	pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep729::kan rho[A243E]*	Plasmid-free segregant of KM281 formed on minimal medium subsequently transformed with pAM403 to Amp^r
KM367	pAM403 *(lac$^+$ uvrD$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep729::kan Δefp772::<>*	Plasmid-free segregant of KM277 formed on minimal medium subsequently transformed with pAM403 to Amp^r
KM389	pAM407 *(lac$^+$ uvrD$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep729::kan aspT[t8c]*	KM154 x P1.KM346 to Km^r
KM391	pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep729::kan aspT[t8c]*	Plasmid-free segregant of KM389 formed on minimal medium subsequently transformed with pAM403 to Amp^r
KM393	pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA::<>* *Δrep729::kan Δefp772::<> rho[A243E]*	MH388 x pAM403 to Amp^r
KM395	pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep729::kan Δefp772::<> rho[A243E]*	KM393 x P1.KM271 to Tm^r
KM398	pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep729::kan aspT[t8c] rho[A243E]*	KM337 x P1.KM346
LC037	*ΔlacIZYA::<> rep$^+$<kan> aspT[t8c]*	TB28 x P1.MKG121
MG1655 RpoS-Mcherry	*rpoS-mCherry::<>*	(5)
MG1655 RpoS-Mcherry Kan	*rpoS-mCherry::<kan>*	Kenn Gerdes
MH184	pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep::cat ΔyjeA782::kan*	JA033 × P1.JW4116 to Km^r
MH280	*ΔlacIZYA::<> ΔyjeA782::kan*	TB28 × P1.JW4116 to Km^r
MH298	pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep::cat ΔyjeK771::kan*	JA033 × P1.JW4106 to Km^r
MH299	*ΔlacIZYA::<> Δefp772::kan*	TB28 × P1.JW4107 to Km^r
MH300	*ΔlacIZYA::<> Δefp772::kan*	TB28 × P1.JW4107 to Km^r
MH301	pAM403 *(lac$^+$ rep$^+$)* / *ΔlacIZYA::<>* *ΔuvrD::dhfr Δrep::cat Δefp772::kan*	JA033 × P1.JW4107 to Km^r
MH303	*ΔlacIZYA::<> ΔyjeK771::kan*	TB28 × P1.JW4106 to Km^r
MH363	*ΔlacIZYA::<> Δrep::cat ΔrelA782::kan*	N6577 × P1. JW2755 to

Km^r

MH364	*ΔlacIZYA::<> Δefp772::kan Δrep::cat*	MH300 × P1.PM412 to Cm^r
MH372	*ΔlacIZYA::<> Δefp772::<>*	MH299 x pCP20 (3) to Amp^r then plasmid-free Km^s segregant identified after growth on LB at 42°C
MH374	*ΔlacIZYA::<> Δefp772::<> Δrep::cat*	MH364 x pCP20 (3) to Amp^r then plasmid-free Km^s segregant identified after growth on LB at 42°C
MH376	*ΔlacIZYA::<> Δefp772::<> ΔrelA782::kan*	MH372 × P1. JW2755 to Km^r
MH378	*ΔlacIZYA::<> Δefp772::<> Δrep::cat ΔrelA782::kan*	MH374 × P1. JW2755 to Km^r
MH388	*ΔlacIZYA::<> Δrep729::kan Δefp772::<> rho[A243E]*	MH372 × P1.KM230 to Km^r then *rho* allele screened by sequencing
MKG08	*ΔlacIZYA::<> rep$^+$ <kan>*	(6)
MKG121	*ΔlacIZYA::<> rep$^+$<kan> aspT[t8c]*	*rep$^+$ <kan>* integration into N8210 using pKD46 (4)
N4304	*ΔrelA251::kan ΔspoT207::cat*	(7)
N5771	*ΔlacIZYA::<> ΔrelA251::kan*	TB28 x P1.N4304 to Km^r
N5777	*ΔlacIZYA::<> ΔrelA251::kan ΔspoT207::cat*	N5771 x P1.N4304 to Cm^r
N6268	*ΔlacIZYA::<> ΔruvABC::cat*	(8)
N6577	*ΔlacIZYA::<> Δrep::cat*	(9)
N6632	*ΔlacIZYA::<> ΔuvrD::dhfr*	(9)
N6639	pAM407 *(lac$^+$ uvrD$^+$)* / *ΔlacIZYA::<> ΔuvrD::dhfr*	(9)
N6644	pAM407 *(lac$^+$ uvrD$^+$)* / *ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat*	(9)
N7120	*ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat*	(9)
N7150	pAM407 *(lac$^+$ uvrD$^+$)* / *ΔlacIZYA::<> rpoB*35 ΔuvrD::dhfr Δrep::cat*	(9)
N7153	*ΔlacIZYA::<> rpoB*35 ΔuvrD::dhfr Δrep::cat*	Plasmid-free segregant of N7150
N7154	*ΔlacIZYA::<kan> spoT1*	(9)
N7182	*ΔlacIZYA::<> ΔuvrD::dhfr Δrep::cat aspT[t8c]*	Isolated as a larger colony variant from a culture of N7120 grown in 56/2 glucose medium and spread on LB agar
N7187	pAM407 *(lac$^+$ uvrD$^+$)* / *ΔlacIZYA::<kan> Δrep::cat spoT1*	(9)
N8210	*ΔlacIZYA::<> Δrep::cat aspT[t8c]*	P1.N7182 x TB28 to Cm^r
PM567	*ΔlacIZYA::<kan> Δrep::cat spoT1*	Plasmid-free segregant of N7187
TB28	*ΔlacIZYA::<>*	(10)

B) Other strains

BW25113	*rrnB3 ΔlacZ4787 hsdR514 Δ(araBAD)567 Δ(rhaBAD)568 rph-1*	(11)
JW0911	BW25113 *ΔaspC745::kan*	(11)
JW1839	BW25113 *Δeda775::kan*	(11)
JW2755	BW25113 *ΔrelA782::kan*	(11)
JW3148	BW25113 *ΔgreA788::kan*	(11)
JW3369	BW25113 *ΔgreB740::kan*	(11)
JW4014	BW25113 *ΔtyrB747747::kan*	(11)
JW4106	BW25113 *ΔyjeK771::kan*	(11)
JW4107	BW25113 *Δefp772::kan*	(11)
JW4116	BW25113 *ΔyjeA782::kan*	(11)
JW5604	BW25113 *Δrep729::kan*	(11)
AB1157	*araC14 thi-1 hisG4 Δ(gpt-proA)62 argE3 thr-1 leuB6 kdg51 rfbD1 lacY1 galK2 xyl-5 mtl-1 tsx-33 supE44 rac⁻ mgl-51 rpsL31 qsr⁻*	(1)
CS89	AB1157 *tls-1 eda-51::Tn10*	(12)

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