Genomic Rescue:
Restarting failed replication forks

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RuvAB acts at arrested replication forks.

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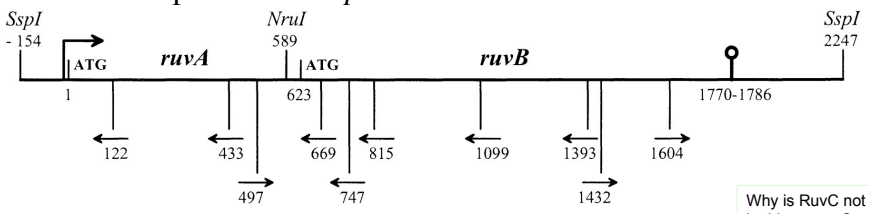
What does the title mean?

Why is this paper published in Cell?

CONNECT REPLICATION AND RECOMBINATION

Supressors of *rep recB*^{TS} *recC*^{TS} are in ruvAB



Why is RuvC not in this operon?

Figure 1. MudX Is Inserted in the *ruvAB* Operon in *rep recB* ^{TS} *recC* ^{TS} Thermoresistant Derivatives

Schematic representation of the *ruvAB* operon. The position of the *ruvAB* promoter is indicated by a bent arrow and that of the putative transcription terminator by a loop. The initiation codons of *ruvA* and *ruvB* are indicated (ATG). The vertical lines below show the position of the 10 MudX insertions that were determined by sequencing, the arrows pointing to the left end of Mu. The numbers indicate nucleotide positions relative to the A of the *ruvA* initiation codon, arbitrarily numbered 1. The SspI and NruI sites used for mapping by Southern hybridization are shown.

Observations:

Combination of *rep* and *recBC* is lethal due to chromosomal double-strand breaks

Inactivation of either ruvA or ruvB restores viability

Therefore:

The ruvAB complex is responsible for the lethality of the *rep recB*^{TS} *recC*^{TS} mutations.

But we already know:

The ruvAB complex is a Holliday junction branch migrator **so** we suspect Holliday junctions form and are processed by ruvAB when replication has difficulties

But also notice that no MudX insertions in ruvC were recovered.

RuvAB INVOLVED IN CONVERTING FORKS TO BREAKS

Table 1. *ruvAB* Mutations Suppress the Thermosensitive Phenotype of *rep recB*TS *recC*TS Cells

Strain	Genotype	Cfu 42°/30°	N[a]
JJC 505	Δ*rep*::*kan recB*TS *recC*TS	5.1×10^{-6}	4
JJC 706	Δ*rep*::*kan recB*TS *recC*TS *ruvA*::Tn*10*	0.9	8
Mu insertions in *ruvA*[b]	Δ*rep*::*kan recB*TS *recC*TS *ruvA*::MudX	0.8	3
Mu insertions in *ruvB*[c]	Δ*rep*::*kan recB*TS *recC*TS *ruvB*::MudX	0.8	8
JJC 821	Δ*rep*::*kan recB*TS *recC*TS *ruvA*::Tn*10* [pGB-ruvAB]	8×10^{-6} [d]	5
JJC 820	Δ*rep*::*kan recB*TS *recC*TS Δ*ruvABC*::*cam*	0.9	5

Isolated colonies were grown in minimal medium at 30° to saturation (OD 0.8 to 1 in 24 to 30 hr). These cultures were plated on minimal medium and plates were incubated at 30° or 42° for 2 to 3 days.
[a] N: number of independent determinations.
[b] Average of the three *ruvA*::MudX insertions obtained by mutagenesis.
[c] Average of the eight *ruvB*::MudX insertions obtained by mutagenesis.
[d] In most of these clones, the *ruvAB* genes present on the plasmid were, for unknown reasons, inactivated.

More proof that it really is the ruvAB complex responsible for the lethality of the *rep recBC*^{TS} mutant.

Genetics logic puzzle:

rep ruvC is alive
But *rep recBC ruvC* is dead

In the absence of ruvC cells require the action of recBC to survive…

Since *rep recBC ruvABC* is alive
And *rep recBC ruvAB* is alive

… only when ruvAB is present.

But, since recBC uses only DNA double-standed ends as a substrate, the action of ruvAB must result in the formation of DNA double-stranded ends.

RuvAB is a Holliday junction branch-migrator. How could Holliday juction branch-migration make DNA double-stranded ends?

RuvAB INVOLVED IN CONVERTING FORKS TO BREAKS

Physical measurement of chromosome breakage

Table 2. *ruvAB* Mutations Prevent the Formation of Linear DNA in *rep recB*TS *recC*TS Cells

Strain	Genotype	% of Linear DNA[a] 30°C	42°C	N
JJC 40	Wild Type	4.7 ± 0.4	4.4 ± 1.2	2/3
JJC 213	Δ*rep*::*kan*	2.4 ± 0.9	2.3 ± 0.9	2/3
JJC 330	*recB*TS *recC*TS	9.1 ± 3.4	19.1 ± 5.0	3
JJC 505	Δ*rep*::*kan recB*TS *recC*TS	15.3 ± 4.7	47.3 ± 4.5	3
JJC 706	Δ*rep*::*kan recB*TS *recC*TS *ruvA*::Tn*10*	4.8 ± 2.0	12.2 ± 1.2	3
JJC 821	Δ*rep*::*kan recB*TS *recC*TS *ruvA*::Tn*10* [pGB-ruvAB]	14.5 ± 1.6	49.5 ± 2.5	3
JJC 820	Δ*rep*::*kan recB*TS *recC*TS Δ*ruvABC*::*cam*	4.6 ± 0.9	8.7 ± 1.2	3

N, number of independent determinations at each temperature. JJC 40 and JJC 213 were tested twice at 30° and three times at 42°.
[a]Determined by PFGE analysis (see Experimental Procedures). In all strains, the linear DNA migrated as 3 to 5 megabase molecules.

In the absence of recBC, strains have trouble growing and suffer broken chromosomes (linear DNA). There is more breakage when rep is missing (which increases replication difficulties), and less breakage when ruvAB is missing.

Therefore in strains with replication problems, ruvAB proteins (Holliday junction branch migration) lead to broken chromosomes, which is likely the mechanism of the lethality established earlier.

REPLICATION PROBLEMS GENERALLY RATHER THAN REP MUTATION SPECIFICALLY INVOLVE RuvAB

Table 3. *ruvAB* and *ruvC* Mutations Prevent the Formation of Linear DNA in *dnaB*TS *recB*

Strain	Genotype	% of Linear DNA[a] 30°C	42°C	N
JJC 767	*dnaB*TS	4.2 ± 1.0	12.6 ± 1.5	4
JJC 774	*danB*TS *recB*::Tn*10*	29.3 ± 4.3	66.7 ± 3.4	3
JJC 800	*dnaB*TS *recB*::Tn*10* Δ*ruvC*::*cam*	15.4 ± 1.9	8.6 ± 2.0	3
JJC 824	*dnaB*TS *recB*::Tn*10* Δ*ruvC*::*cam* [pBR-ruvC]	17.6 ± 0.1	50.0 ± 2.2	2
JJC 775	*dnaB*TS *recB*::Tn*10* Δ*ruv ABC*::*cam*	18.3 ± 4.2	10.0 ± 3.0	4
JJC 823	*dnaB*TS *recB*::Tn*10* Δ*ruvABC*::*cam* [pBR-ruvC]	12.0 ± 5.8	11.3 ± 5.6	2
JJC 822	*dnaB*TS *recB*::Tn*10* Δ*ruvABC*::*cam* [pGB-ruvAB] [pBRruvC]	26.4 ± 3.7	61.4 ± 5.8	3

N, number of independent determinations at each temperature.
[a]In all strains, the linear DNA migrated as 3 to 5 megabase molecules.

Background: dnaB is the main replicative helicase. Inactivation of dnaB is lethal due to replication failure and chromosome breaks. So this experiment is performed on dying cells.

Using the *dnaB*^{TS} strain shows that the phenotypes being observed in *rep* strains are related to a general DNA replication problem, rather than due to some uncharacterized *rep* weirdness.

There is more linear DNA in the absence of recBCD (recall that recBCD eats linear DNA)

Observe: deletion of ruvC suppresses the linear DNA phenotype, just like deletion of ruvABC does.

Therefore: ruvC may be directly breaking the chromosome.

But note that *rep recBC*^{TS} *ruvC* is lethal while *rep recBC*^{TS} *ruvABC* is fine. So *ruvC* is lethal only when ruvAB are active.

REPLICATION FORKS CONVERTED TO HOLLIDAY JUNCTIONS BY RuvAB, THEN CUT BY RuvC

Table 4. Part of the *recB*-Dependent Linear DNA Is *ruvABC* Dependent in Strains Proficient for Replicative Helicases

Strain	Genotype	% of Linear DNA[a] 30°C	42°C	N
JJC 40	Wild Type	4.7 ± 0.4	4.4 ± 1.2	2/3
JJC 315[b]	*recB*::Tn*10*	25.3 ± 6.4	39.2 ± 6.9	5
JJC 806	*recB*::Tn*10* Δ*ruvC*::*cam*	11.7 ± 0.8	15.0 ± 4.5	3
JJC 813	*recB*::Tn*10* Δ*ruvABC*::*cam*	14.9 ± 2.6	20.1 ± 1.2	3

N, number of independent determinations at each temperature. JJC 40 was tested twice at 30° and three times at 42°.
[a] In all strains, the linear DNA migrated as 3 to 5 megabase molecules.
[b] At 42°C, the amount of linear DNA was higher in the *recB* null mutant than in the *recB*TS *recC*TS strain JJC330 (Table 2), probably because of residual RecBCD activity in the TS mutant.

Mutate recB to keep linear DNA from being degraded (so it can be quantified).

Observe that about half of the linear DNA arises from the action of ruvABC

Conclusion:
Holliday junctions are forming and being extended by RuvAB and cut by RuvC to form double-strand breaks even in cells wild-type for replication proteins so replication forks must fail spontaneously with reasonably high frequency.

DNA DEGREDATION REQUIRED, BUT NOT NEEDED TO BE EXTENSIVE
FORMATION OF HOLLIDAY JUNCTION DOES NOT REQUIRE RecA

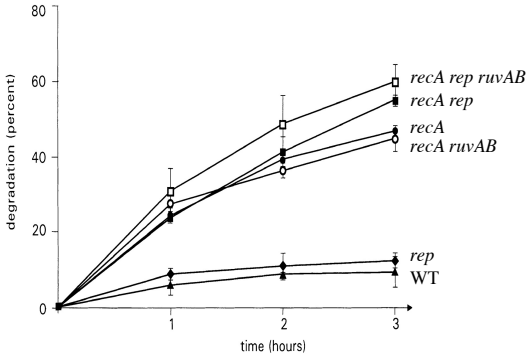


Figure 2. DNA Degradation in *recA* Strains Is Not Significantly Affected by *rep* or *ruvAB* Mutations

DNA degradation was determined as described in Experimental Procedures. Cells containing the plasmid pBRara-recA, carrying the *recA* gene under the control of the *araC* promoter were used. In these cells the *recA* gene is expressed in the presence of arabinose (RecA+) and repressed in the presence of glucose (*recA*). Results are the average of two or three experiments, standard deviations are shown.

JJC744 arabinose (wild-type) (closed triangle); JJC742 arabinose (*rep*) (closed diamond); JJC744 glucose (*recA*) (closed circle); JJC742 glucose (*recA rep*) (closed square); JJC745 glucose (*recA ruvAB*) (open circle); and JJC743 glucose (*recA rep ruvAB*) (open square).

DNA degradation was also measured in *recA* and *rep recA* strains cells with no plasmid; results were the same as in cells containing pBRara-recA grown in the presence of glucose (data not shown).

Background:
In a *recA* strain (most laboratory strains) there is a lot of DNA degradation because if recBCD starts eating DNA, it tends not to stop.

The *rep recA* strain is viable but the *rep recBC* strain and *rep recA recD* strains are not. Therefore, replication problems require the recBCD exonuclease activity to live, while the recBCD recombination activity is optional.

BUT: this required exonuclease activity must only be used to degrade small amounts of DNA in *rep* mutants, since there isn't a large increase in the amount of degradation observed between a *recA* strain, and a *recA rep* strain.

FORMATION OF HOLLIDAY JUNCTION DOES NOT REQUIRE RecA

Table 5. *ruvAB* Mutations Suppress the Lethality of *rep recD recA* cells

Strain	Genotype	Cfu Glucose/ Arabinose	N	
JJC 748	Δ*rep*::*kan* Δ*recA*::*cam* *recD*1013 [pGBara-recA]	4.9 10^{-4}	10	*rep recA recD* is lethal
JJC 827	Δ*rep*::*kan* Δ*recA*::*cam* *recD*1013 *ruvA*::Tn*10* [pGBara-recA]	1.0	10	*rep recA recD ruvA* is viable
JJC 825	Δ*rep*::*kan* Δ*recA*::*cam* [pGBara-recA]	1.0	5	recA doesn't affect *rep* strain viability
JJC 826	Δ*rep*::*kan* Δ*recA*::*cam* *ruvA*::TN*10* [pGBara-recA]	1.2	5	recA doesn't affect *rep ruvA* viability

Isolated colonies were grown in LBT medium containing 0.2% arabinose at 37° to saturation, i.e., overnight for the *ruvA*$^+$ strains and up to 3 days for the *ruvA*$^-$ strains. These cultures were plated on rich medium containing either 0.2% arabinose or 1% glucose and plates were incubated at 37° for 24 hr to 3 days. N, number of independent determinations.

Note: recD is required only for the exonuclease V action of the recBCD complex. A *recD* mutant is proficient for recombination due to recBC.

Since *rep recA recD* is lethal but *rep recA recD ruvA* is viable, and since the recombination action of recBCD is not required but the exonuclease action is, we conclude that the double-stranded end which recBCD is required to eat is created by the action of ruvA on stalled replication forks. But since ruvA is a Holliday junction branch-migrator, we conclude that:

Stalled replication forks can be converted into Holliday junctions in the absence of recA-mediated recombination.

MODEL PULLING TOGETHER GENETICS AND BIOCHEMISTRY

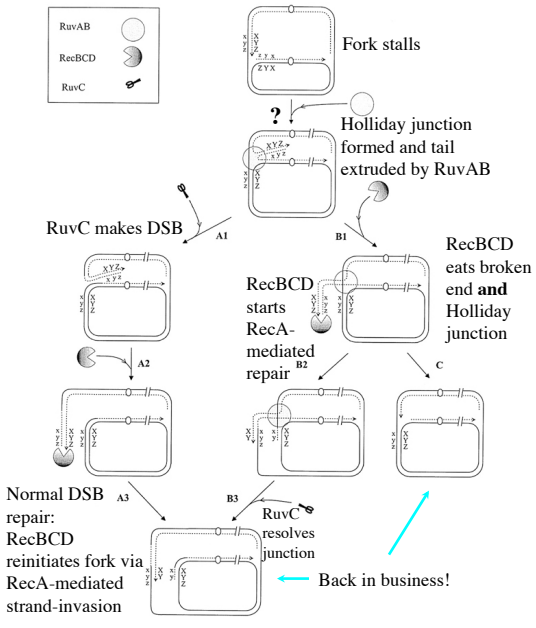


Figure 3. Model for RuvAB/RecBCD–Mediated Rescue of Blocked Replication ForksContinuous and discontinuous lines represent the template and the newly synthesized strand of the chromosome, respectively. The arrow indicates the 3' end of the growing strand.

In the first step the replication fork is blocked and the two newly synthesized strands anneal, forming a Holliday junction that is stabilized by RuvAB binding.

Pathway A:
(A1) RuvC resolves the RuvAB–bound junction.
(A2) RecBCD binds to the double–stranded end.
(A3) The double–stranded break is repaired by RecBCD/RecA–mediated homologous recombination. If the same strands are exchanged at both Holliday junctions, (patch type of event) a replication fork is reconstituted on a monomeric chromosome (shown here). Resolution using two strands at one junction and the two other strands at the other junction (splice type of event) leads to the reconstitution of a replication fork on a dimeric chromosome (not shown).

Pathway B:
(B1) RecBCD binds to the double–stranded tail.
(B2) Degradation has taken place up to the first CHI site (between locus yY and zZ) and is followed by a genetic exchange mediated by RecA (an exchange between the lagging strand and the leading strand template is shown).
(B3) RuvC resolves the first Holliday junction bound by RuvAB. As in pathway A, the outcome, monomeric or dimeric chromosome, depends on the strands used for the two resolution reactions.

Pathway C: RecBCD–mediated degradation of the tail progresses up to the RuvAB–bound Holliday junction. Replication can restart when RecBCD has displaced the RuvAB complex.

What we learned

- Even in normal cells, replication forks fail with regularity
- Failed forks are converted into Holliday junctions, then processed by recombination machinery

E. coli genetics can be really complicated