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Review

Recs preventing wrecks

Justin Courcelle

Portland State University, Department of Biology, P.O. Box 751, Portland, OR 97207-0751, USA

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Abstract

The asexual cell cycle of *E. coli* produces two genetically identical clones of the parental cell through processive, semiconservative replication of the chromosome. When this process is prematurely disrupted by DNA damage, several *recF* pathway gene products play critical roles processing the arrested replication fork, allowing it to resume and complete its task. In contrast, when *E. coli* cultures are starved for thymine, these same gene products play a detrimental role, allowing replication to become unregulated and highly recombinagenic, resulting in lethality after prolonged starvation. Here, I briefly review the experimental observations that suggest how RecF maintains replication in the presence of DNA damage and discuss how this function may relate to the events that lead to a loss of viability during thymine starvation.

Keywords: Recs; Thymine; Wrecks

Contents

Prologue

I was a graduate student at Stanford working in immunology, when I found out my thesis professor was moving his lab to St. Louis. Although my project was going well, I had just met the person who would become my partner in life, I very much enjoyed the scientific atmosphere and energy at Stanford, and I did not want to move. I was roaming the campus, literally knocking on office doors, asking if other professors were accepting graduate students when I walked into Philip Hanawalt's office. Like many of the students

E-mail address: jcourcelle@pdx.edu.

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who walked into his office before me, Phil tried to interest me in a project that focused on determining why bacteria die when starved for thymine—a phenomenon termed "thymineless death." A large number of studies on thymineless death can be found in the literature, and several mutants are known that affect survival during thymine starvation [1]. However, despite all this information, the molecular pathway associated with thymineless death is made up of several large black boxes. The running joke among members of Phil's group is that the thymineless death project is a graveyard that contains, buried in it, the efforts of many students and colleagues. I am not sure that this is an entirely fair assessment. While no one would claim to have solved the thymineless death mystery outright, most of those who began work on this project have gotten side-tracked on one of the numerous black boxes that are found in this pathway and have helped to productively characterize a portion of this mystery. In doing so, they have often revealed new and fundamental aspects of cellular metabolism.

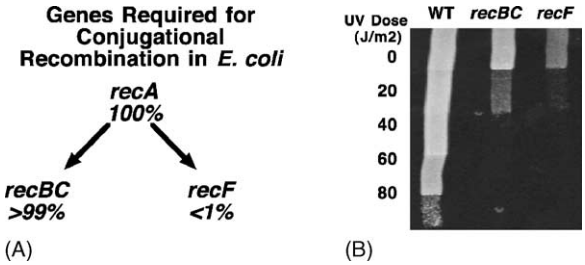

Fig. 1. The paradox of RecF function. (A) A classical (now outdated) view of the recombinational pathways in *E. coli*. *recA* is required for 100% of the recombinant genomes that form during conjugation in *E. coli*. Similarly in *recBC* mutants, the frequency of recombinant formation is reduced by more than 99%. *recF* was isolated as a gene that was required for recombination when the *recBC* pathway was inactivated, leading to the initial view that the *recF* pathway was a minor, or backup recombination pathway [6]. (B) Although the contribution of *recF* is minor during conjugational recombination, when compared to *recBC* mutants, *recF* mutants are as hypersensitive to UV-induced DNA damage as are *recBC* mutants [6]. The plate shown here was made by applying overnight cultures to an LB plate with a cotton swab. The plate was covered by a sheet of aluminum foil and placed under a 254 nm germicidal lamp. The foil was then progressively retracted following 20 J/m^2 exposures before the plate was incubated at 37 °C overnight.

1. RecF in the recovery of replication

The black box that drew my attention in the thymineless death pathway was *recF*. At the time I joined Phil's group, it was known that *recF* mutants were more resistant to killing by thymine starvation than wild-type cells [2,3]. Yet, how RecF conferred resistance to thymineless death was not clear because the function of RecF was not well defined and somewhat paradoxical. Genetic recombination in *E. coli* was generally thought to occur by one of two distinct pathways. The rationale for this thinking grew out of the seminal studies of Clark and Margulies, who set out to identify the gene(s) responsible for recombination and the generation of genetic diversity during sexual cell cycles [4]. *recA* was isolated as a gene that was essential for the formation of recombinant genomes during bacterial sex or conjugation. *recB* and *recC* mutants were similarly shown to reduce the frequency of recombination during conjugation by more than 99% [5]. *recF* was then identified by screening mutagenized *recBC sbcB*(*C*) mutants to isolate the genes that were responsible for the 0.1%–1% of recombination occurring in the absence of the *recBC* pathway [6,7]. Although subsequent studies began to characterize conditions that differentiated these pathways, the manner in which *recF* was isolated led to its portrayal as a minor, or backup pathway of recombination that could be activated in the absence of *recBC* (Fig. 1A) (for reviews see [8–10]). Contrary to the view that RecF functioned as a backup recombination pathway, was the striking observation that *recF* mutants were hypersensitive to DNA damage [6,7]. In fact, they were approximately as sensitive as *recBC* mutants, which belong to the major pathway of recombination (Fig. 1B). In trying to understand the functional role of RecF, this seeming paradox led us to consider the possibility that recombination is not the primary function of RecF during the asexual cell cycle. Thus, rather than focus on the recombination phenotypes of RecF, which are relatively subtle, we began our studies by focusing on the comparatively dramatic UV-sensitive phenotype of RecF mutants. In essence, we asked the question, "Why do *recF* mutants die after UV-induced DNA damage?"

As an initial approach, we compared the survival of *recF* mutants to wild-type cells at various doses of UV irradiation and observed that the sensitivity of *recF* mutants depended on the replication state of

the culture [11]. When *recF* mutants were grown to stationary phase prior to irradiation, they were more resistant to UV than when they were irradiated as exponentially growing cultures. The survival of *recF* cultures increased in a similar fashion following pretreatment of the culture prior to UV irradiation with chloramphenicol, a protein synthesis inhibitor that prevents new rounds of DNA replication from initiating (Fig. 2A). This sensitivity to replication state is in contrast to nucleotide excision repair mutants, such as *uvrA*, whose sensitivity to UV does not vary following these treatments, a theme that had been observed by others using slightly different approaches [12,13]. Common to these approaches, is that ongoing replication is eliminated or greatly reduced. Thus, unlike the nucleotide excision repair mutant, the hypersensitivity of *recF* mutants is NOT due to the DNA damage per se. Instead, the hypersensitivity of *recF* mutants appears to arise when cells try to replicate in the presence of DNA damage. Consistent with this interpretation, we and others have shown that *recF* mutants remove UV-induced lesions from their genome with an efficiency that is comparable to wild-type cells [14,15].

The observation suggested that the hypersensitivity of *recF* correlates with replication in the presence of DNA damage rather than DNA damage itself. Therefore, to focus in on this aspect more directly, we monitored how DNA synthesis was affected by DNA damage in *recF* mutants. This can be done using a variety of experimental approaches. One of the more recent approaches we have used is shown in Fig. 2B. Essentially, [14C]thymine-prelabeled cultures are periodically pulsed with [3H]thymidine for 2 min after a moderate dose of UV irradiation so that the DNA accumulation and rate DNA synthesis is occurring can be followed during the recovery from DNA damage. A

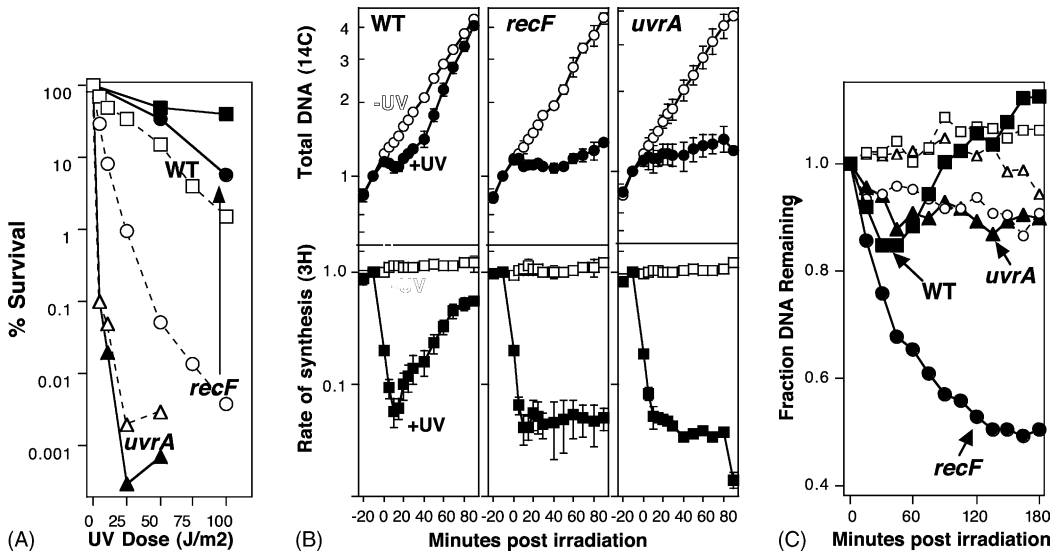

Fig. 2. Three assays, when taken together, have been interpreted to indicate that RecF is required to maintain replication forks that encounter DNA damage. (A) The UV hypersensitivity of *recF* mutants correlates with ongoing replication in the cell. The survival of isogenic WT, *recF* and *uvrA* mutants is shown following UV irradiation at the indicated doses in both exponentially growing cultures (open symbols) and cultures pretreated for 3 h with chloramphenicol to allow ongoing rounds of replication time to complete (filled symbols). In the absence of replication, the hypersensitivity of *recF* mutants is greatly reduced. In contrast, the UV sensitivity of *uvrA* mutants, which cannot remove DNA lesions, is not affected by the replication state of the cell [11]. (B) RecF fails to resume DNA synthesis following arrest by UV-induced damage. [3H]thymidine was added to [14C]thymine-prelabeled cultures for 2 min at the indicated times following either 27 J/m^2 UV irradiation (filled symbols) or mock irradiation (open symbols) at time 0. The relative amount of total DNA, 14C (circles), and DNA synthesis/2 min, 3H (squares), is plotted [16]. (C) Following irradiation, increased degradation occurs at the growing fork in *recF* mutants but not *uvr* mutants. [3H]thymidine was added to [14C]thymine prelabeled cells for 10–15 s immediately before the cells were filtered and irradiated with 25 J/m^2 in nonlabeled medium. The fraction of the radioactivity remaining in the DNA is plotted against time. Loss of 14C genomic DNA (open symbols) can be compared to the loss of the 3H DNA synthesized at the growing fork just prior to irradiation (filled symbols) [15].

dose was selected that arrested or blocked replication, but did not reduce survival in wild-type cells. Thus, the predominant molecular events that are observed should correlate with cellular mechanisms that occur in surviving cells. By this type of assay, the rate of DNA synthesis in UV-irradiated wild-type cultures initially decreased by more than 90% following the 27 J/m^2 dose, but began to recover 15 min post-UV and continued to increase until it approached unirradiated levels, approximately 80 min post-UV. At this time, the overall DNA accumulation also approached that of the unirradiated cultures. In *uvrA* mutants, which cannot remove the blocking lesions from the genome, no recovery in the rate of DNA synthesis occurred following inhibition. Similarly, in the absence of RecF, following the initial inhibition of DNA synthesis, replication failed to recover and no further DNA accumulation occurs. The failure to resume DNA synthesis in *recF* mutants was surprising since the blocking lesions are being removed from the genome. Thus, the downstream template has been cleared of roadblocks, but replication still fails to resume. This is not the case with other UV-hypersensitive mutants that have been examined, such as *ruvAB*, *recG*, or *recBC*, which appear to resume DNA synthesis at times comparable to wild-type cells [11,16]. Therefore, the defect in *recF* mutants is probably not simply due to elevated levels of cell death in these cultures but appears to be specifically associated with the inability to resume DNA synthesis once it is disrupted by DNA damage.

These observations suggested that the problem in *recF* mutants might be associated directly with events at the DNA damage-arrested replication fork. Therefore, to focus on this event more closely, we designed a simple assay to focus specifically on the fate of the DNA that is made just prior to arrest at the DNA lesion. The assay utilized exponentially growing [14C]thymine labeled cultures that were pulse labeled with [3H]thymidine for 10 s to label the DNA at replication forks. The culture was then transferred to nonradioactive media and immediately irradiated. The 3H-label pulse allowed us to compare the degradation that occurred in the nascent strands near the arrested replication forks relative to that which occurred in the overall genome. Although simple in design, this assay provides an extensive amount of information. Using this approach, we demonstrated that some limited degradation occurred in the nascent DNA of the arrested replication fork at times prior to the resumption of replication in wild-type cells (Fig. 2C). We consistently observed an increase in precipitable 3H-label that occurs at times after replication has recovered, which appears to be due to the incorporation of the remaining intracellular pools of labeled nucleotides from the initial pulse [11,17]. In *uvr* mutants, the nascent strand degradation was limited to approximately the extent and duration seen in wild-type cells. In contrast, the DNA at replication forks in *recF* mutants underwent much more extensive degradation for a greater extent of time. The degradation was specific to growing fork regions and was limited to approximately half of the nascent DNA, an observation that would prove significant later. This result was interesting, because although neither *uvr* nor *recF* mutants recover replication, the *uvr* mutants did not display the extensive nascent strand degradation associated with *recF* deficiency. Thus, although replication does not recover in either *uvrA* or *recF* mutants, the failure to recognize the nascent strands and to protect them from degradation is specific to *recF* mutants.

If one were to momentarily suspend our knowledge that *recF* was initially isolated as a gene that affected recombination frequencies, what role would RecF appear to be required for following UV-induced DNA damage considering that (1) the UV hypersensitivity of *recF* correlates, not with DNA damage per se, but with replication occurring in the presence of DNA damage; (2) when replication does encounter DNA damage, RecF is required to resume DNA synthesis; and (3) in the absence of RecF, the failure to recover DNA synthesis is associated with the extensive degradation of the DNA at the replication fork?

Perhaps the simplest model one could imagine, is that following the disruption of replication at a DNA lesion, RecF is needed to maintain the replication fork until the lesion can be repaired and replication can resume (Fig. 3A). The arrest of replication progression and degradation of the nascent DNA suggests that replication is disrupted by UV-induced damage. That replication does not resume in *uvrA* mutants suggests that the removal of the lesions is an important step in the recovery process. Unlike the repair mutants however, *recF* mutants fail to resume replication despite the fact that the lesions are removed from the genome. In this case, the failure to recover is associated with extensive degradation occurring at the replication fork

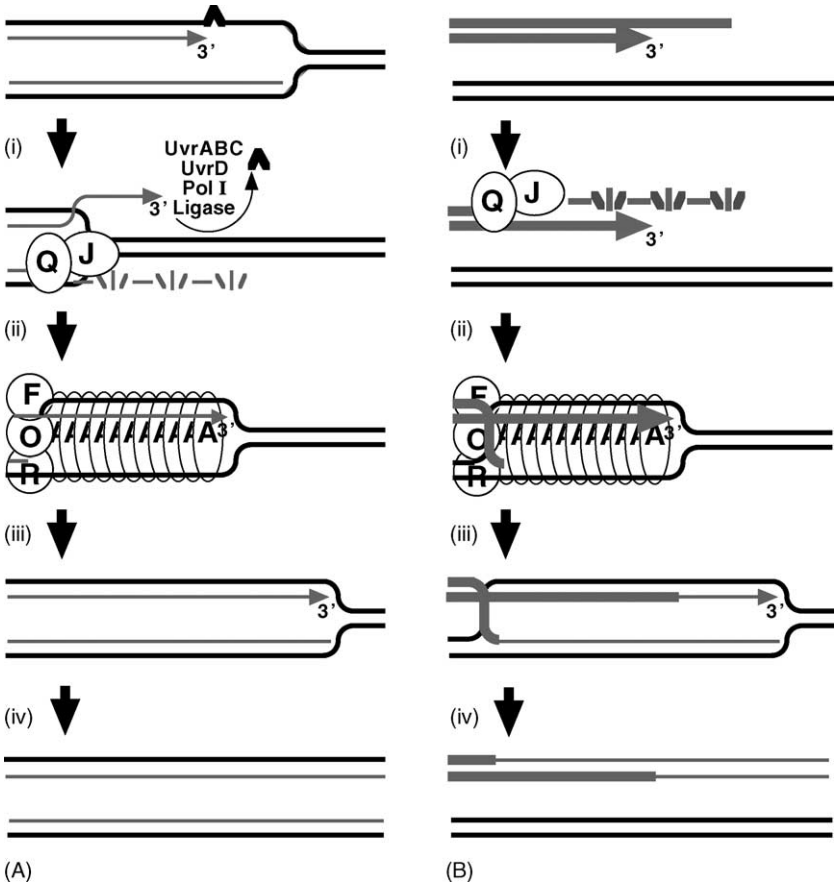

Fig. 3. Common substrates for RecF during the recovery of replication and recombination. (A): (i) Following the arrest of replication by UV-induced damage (ˆ), the fork is processed to allow repair enzymes to gain access to the DNA lesion and effect repair. (ii) RecF, RecO, and RecR recognize this substrate and promote RecA binding to maintain and protect the integrity of the replication fork (iii) until the lesion has been removed and replication can resume, (iv) thereby maintaining the processive replication of the genome. (B): (i) When exogenous DNA is introduced into the cell (thick lines), the ends are processed to generate a 3′ single-strand region. (ii) RecF, RecO, and RecR recognize this substrate and promote RecA binding, which pairs the DNA to a homologous region, generating a structure that mimics that of a disrupted replication fork. (iii) Initiation of replication from this structure, (iv) a recombinant molecule since the remaining portion of the invading DNA is replicated using the second molecule as a template.

DNA, indicating that RecF is needed to maintain and protect the fork when it is arrested. If this were all that RecF were doing, then once the offending lesion has been removed, replication could simply resume. Perhaps the most interesting part of this model, if true, is the implication that no strand exchanges, or recombinant products would be required during the *recF*-dependent reinitiation process once the offending lesion is removed. Consistent with our understanding of the products generated during asexual cell cycles, processive replication of the genome is maintained, and none of the genetic information on either genome would be obtained by copying or exchanging information from other molecules.

Other genes have now been shown to work with RecF in processing and maintaining arrested replication forks. In addition to RecF, both RecO and RecR are also required to maintain lesion-blocked replication forks and allow DNA synthesis to resume following arrest by DNA damage (Fig. 3A(ii)) [11,18]. Several lines of evidence, both in vivo and in vitro, suggest that these three gene products operate at a common step in

promoting the formation of a RecA filament that maintains the blocked replication fork structure. Mutants lacking any one, or all three, of these gene products are equally sensitive to DNA damage, and exhibit a delayed induction of SOS-regulated genes [6,19–22], suggesting that the activation of RecA occurs more slowly in the absence of RecF, RecO, or RecR. In vitro, RecF, RecO, and RecR promote the formation of RecA filaments on single-strand DNA and prevent their disassembly [23–25], consistent with the idea that these proteins play a role in upregulating the SOS response by enhancing RecA binding to single-strand DNA. This activation signal leads to the cleavage of the repressor protein, LexA, which prevents the expression of SOS-regulated genes in the absence of DNA damage [26,27]. In the absence of *recF*, *recO*, and *recR*, replication fails to recover following UV-induced DNA damage and the DNA at the replication fork is extensively degraded, consistent with a role for these genes in facilitating the protection of the replication fork by RecA [11,18,28].

The degradation that occurs at the replication fork following arrest has been shown to be dependant on two other RecF-pathway associated proteins, RecJ and RecQ, and is thought to be part of the normal recovery process (Fig. 3A(i)). RecQ is a 3′–5′ helicase, and RecJ is a 5′–3′ single-strand exonuclease [29,30]. The processing and degradation by RecJ and RecQ has been shown to preferentially occur on the nascent lagging strand. Although the partial degradation of the nascent DNA may appear counterproductive and inefficient, it is proposed that this degradation may serve to move the arrested replication fork back and allow repair enzymes to gain access to the blocking lesion and effect repair. Consistent with this interpretation, the recovery of DNA synthesis is significantly delayed in the absence of the nascent DNA processing and the recovery becomes dependent on translesion synthesis by Pol V, suggesting that repair enzymes can no longer gain access to the offending lesion (C. Courcelle, unpublished data). The nascent DNA processing may also serve to generate a more extensive substrate for RecA to bind and stabilize, thereby ensuring that replication resumes from the same site at which disruption occurred [17,31–33]. By analogy, RecQ homologs in yeast, Drosophila, and humans have been shown to play critical roles in maintaining processive replication and suppressing the frequency of DNA strand exchanges [34–38].

2. RecF-mediated recombination

The above model implies that the UV sensitivity of *recF* is not due to a recombination defect. However, *recF* was originally identified and characterized as a gene product that could promote recombination during conjugation or transduction. It is therefore, important to examine if the proposed function for RecF is consistent with when recombination is observed through the *recF* pathway. Unlike the asexual cell cycle, processes such as conjugation or transduction involve the introduction of foreign DNA into the cell. The formation of RecF-mediated recombinant molecules during these events appears to require that the invading DNA have a 3′ single-strand end (a review of this concept can be found in [8]). This requirement is inferred from the properties of the exonucleases that are associated with upregulating the frequency of *recF*-mediated recombination in vivo. Both RecJ and Exo VIII are 5′-3′ exonucleases that increase *recF*-mediated recombination [29,39]. Similarly, inactivation of ExoI, a 3′-5′ exonuclease that degrades these recombinagenic ends also increases the frequency of *recF*-mediated recombination [40].

recF recombination also requires the RecA protein. Biochemically, RecA binds single-strand DNA and pairs it with homologous duplex DNA [41,42]. In general, these biochemical and genetic characterizations suggest that *recF*-mediated recombination occurs when RecA utilizes a 3′ overhang to invade a homologous double strand target sequence as shown (Fig. 3B). When one compares this recombination intermediate to that of a disrupted replication fork, there is a striking structural similarity (Fig. 3A versus B). Just as RecA is thought to catalyze the strand invasion of a 3′ single-strand end into homologous duplex DNA, the replication machinery catalyzes the invasion of a 3′ single-strand end into homologous duplex DNA through polymerization of leading strand synthesis. If one postulates that RecF participates in and promotes the recognition of the recombination intermediate shown in Fig. 3B, then it is tempting to speculate that it should also promote the reassembly of the disrupted replication fork structure.

During asexual cell cycles, when no exogenous DNA ends are introduced or generated within the cell, the only time a structure such as that depicted in Fig. 3A should occur is when replication is disrupted before

completing its task. If so, this would represent a legitimate substrate from which to initiate replication, and still generate two daughter molecules that have been duplicated by continuous, semiconservative replication. However, in the case of recombinational processes when exogenous DNA ends are introduced into the cell, the initiation of replication would produce a recombinant molecule (Fig. 3B). In essence, the *recF*-mediated recombination may result when the DNA ends are processed to mimic those of a disrupted replication fork.

Thus, in trying to understand the biological role of these gene products in a given cellular context, it is important to consider the strategy and products of the reproductive cycle being studied. We believe that at the level of the chromosome, it is worthwhile to consider the possibility that during nonsexual cell cycles, many of the classically defined recombination proteins in both prokaryotes and eukaryotes may function to maintain the strands of the DNA rather than rearrange them.

3. Recs causing wrecks

The proposed model implies that the RecF pathway generally promotes the initiation of replication from 3′ DNA ends. Following DNA damage, this promotes cell survival by allowing replication to resume from the point where it has been prematurely disrupted. Paradoxically, during thymine starvation, the presence of RecF impairs, rather than promotes cell survival (Fig. 4). Could this proposed function of RecF provide any clues to the events leading to lethality during thymineless death?

Following a single initiation event during the asexual cell cycle, the parental genome is processively replicated to produce two genetically identical clones. However, during prolonged thymine starvation, several studies have demonstrated that the normally tight regulation of replication breaks down. Early studies by Hanawalt showed that each new round of replication initiation required new proteins to be synthesized [43,44]. By inhibiting protein synthesis with antibiotics or amino acid deprivation, he showed that ongoing rounds of replication continued to completion but new initiation events were prevented from occurring. Subsequent work by Tokio Kogoma demonstrated that a period of thymine starvation activated the SOS response and could circumvent this tight regulation of replication, inducing a stable form of replication that continued for several hours, independent of any de novo protein synthesis [45]. Unlike normal genomic replication, the DNA replication induced following thymine starvation initiated aberrantly at multiple sites around the genome and generated large numbers of nonviable cells [46–51]. Work by Hiroaki Nakayama showed

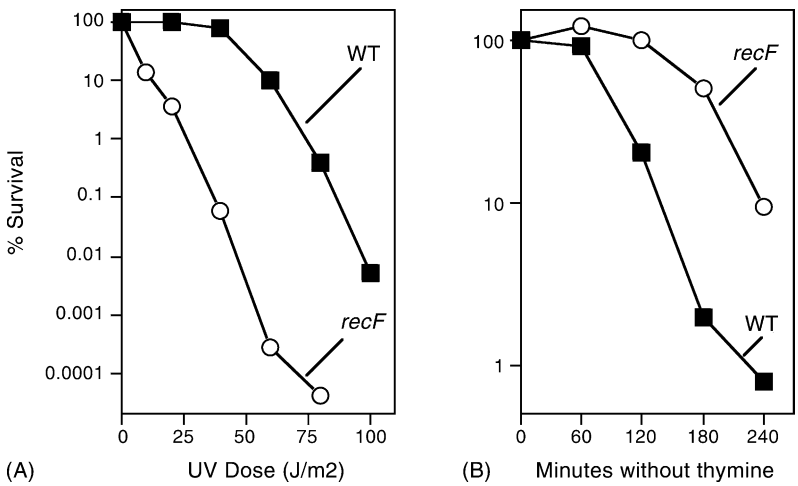

Fig. 4. RecF promotes survival following UV-induced DNA damage but promotes lethality in the absence of thymine. (A) The surviving fraction of WT and *recF* cultures is plotted at increasing doses of UV-irradiation. (B) The surviving fraction of WT and recF cultures is plotted following increasing periods of thymine starvation [2].

that during the period of thymine starvation, high frequencies of strand exchanges, which could be visualized by pulsed field gel electrophoresis and electron microscopy, accumulated in the replicated portions of the chromosome [52,53]. In mutants lacking *recF* or other *recF* pathway genes, the DNA exchanges accumulated with much slower kinetics. Based on these observations, it is reasonable to speculate that replication initiating from these strand exchanges after thymine is restored to the media would not generate intact chromosomes. Instead, replication from these substrates would be expected to produce a meshwork of partially replicated genomes. In cells lacking RecF, the inability to form these illegitimate recombination substrates and re-initiate replication may perhaps "save" these individuals. Consistent with this view, cells which are not replicating at the time thymine is removed from the media are resistant to thymineless death, irrespective of whether *recF* is present or not [53–56].

During the normal asexual cell cycle, the sites where *recF* pathway genes are proposed to promote the resumption of replication are found at the 3′ ends of replication forks, sites that should represent legitimate substrates to resume replication from without inducing aberrant copy numbers or partially replicated chromosomes. However, following thymine starvation, it appears that *recF* is involved in initiating replication from alternative sites around the genome. How might these be created? In the absence of thymine, some studies have indicated that elevated levels of uracil are incorporated into the DNA [57]. Uracil incorporated into DNA is subject to excision by the uracil-DNA glycosylase and subsequent incision by AP endonucleases leaving a nick in the DNA backbone that contains a free 3′ DNA end (reviewed in [58]). In the absence of thymine, it is proposed that large numbers of nicks are generated by incorporation and subsequent excision of uracil near ongoing replication and possibly at other sites that are subject to elevated levels of DNA turnover in the genome [57,59]. Speculatively, the excessive number of 3′ ends generated by these events may overwhelm the *recF* system and begin to compete with the bona fide 3′ end present at the stalled replication fork. The improper utilization of 3′ ends generated in duplicated regions of the chromosome could allow RecF to initiate replication by pairing free ends with the homologous sister chromosome leading to either re-duplication of the replicated region, extensive crossovers, or aberrant replication on the chromosome that could continue in the absence of additional initiation proteins for several hours. Consistent with the work of Kogoma and Nakayama, this form of replication would be expected to generate large quantities of DNA such as that observed during thymineless-induced stable DNA replication, but yield few molecules that constitute complete chromosomes [45,46,53]. *E. coli* do contain enzymatic activities capable of resolving these crossover events, and perhaps degrading DNA generated by the mis-initiation of replication [8,9]. However, the exchange intermediates that are observed to accumulate during prolonged thymine starvation, suggest that the cell reaches a point at which the copy number imbalances and exchanges exceed the cell's ability to productively resolve these structures into intact chromosomes.

Although the recombinagenic replication induced by thymine starvation is clearly not a productive mechanism for generating precise copies of the cellular chromosome, some organisms with reproductive cycles that produce large quantities of progeny do replicate through mechanisms that appear similar to those induced by thymine starvation.

In Herpes simplex virus (HSV), the progeny are largely comprised of recombinant genomes [60–62]. Here, the recombinational exchanges are thought to generate substrates to initiate replication at the onset of lytic cyle, allowing the virus to rapidly produce more of its genetic material than it might if it relied strictly on a unique origin of replication. Similar to the replication that is observed following thymine starvation, this form of viral replication produces a large, intricate meshwork of branched, genomic concatamers rather than discrete viral chromosomes [60–62]. Although much of the genetic material that is produced will neither form complete genomes nor be packaged into viral particles, the strategy is effective for this organism which depends more on mass production than precision in reproducing its genetic message. Interestingly, many eukaryotic viruses, including Herpes simplex family, encode several genes to regulate thymine metabolism and often encode their own uracil-DNA glycosylase, which plays a critical role in allowing the lytic amplification to occur [63,64].

Similar reproductive strategies can be seen in the viruses of *E. coli*. Bacteriophage such as T4 use recombination as a mechanism to amplify their own

genetic material during lytic replication. Interestingly, although T4 does not encode a uracil DNA glycosylase, it does encode a glycosylase/AP endonuclease that is specific for UV-induced DNA damage [65,66]. In vitro, T4s Endonuclease V associates with UV-irradiated T4 DNA and forms recombinational branches [67]. In addition, the T4 phage is much more resistant to inactivation by UV irradiation than the related T2 phage which lacks this endonuclease, making it tempting to speculate that in the presence of UV-induced damage, the virus may be utilize these lesions as a mechanism for the recombinational initiation of replication [68,69]. Other phage, such as lambda, encodes a homolog of RecF that appears to promote a similar type of recombinagenic replication to amplify their genetic material during lytic viral replication [70].

In trying to understand the biological role of any gene product in a given cellular context, it is useful to consider the strategy and products of the process that is being studied. Many *rec* gene products, originally isolated and characterized because they affect recombination frequencies, were subsequently found to have roles during the normal asexual cell cycle. During an asexual reproductive cycle, RecF is intimately associated with the ability of cells to resume replication when it is prematurely disrupted. During conjugation or lytic viral replication, RecF may act on a substrate formed by two separate DNA molecules that mimic a replication fork, leading to the generation of recombinant products or elevated copy numbers that are very different from those observed during chromosomal replication. We speculate that the substrates generated by thymine stavation lead to a *recF*-mediated recombinational initiation of replication similar to that which is observed during lytic phage replication. This form of replication may then lead to a loss of viability when the recombinational events exceed the cell's ability to resolve and extend these intermediates into complete chromosomes.

The role of RecF in the recovery from DNA damage and the conceptual idea that many of the classically defined recombination proteins may function to maintain the chromosome without DNA strand exchange during recombinational processes were initiated because of Phil's intuitive belief that understanding something as trivial as why bacteria die without thymine would reveal new fundamental aspects about the inner workings of a cell. Given the number of black boxes that remain in this pathway, it is likely that many more interesting stories remain to be told.

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