Preface

Even before we completed Volumes I and II of *DNA Damage and Repair* in 1998, three facts made it very clear that a third volume would be necessary. First, despite our best attempts at providing comprehensive coverage of this rather large and rapidly expanding field, we were unable to identify authors for several important topics. Volume III: *Advances from Phage to Humans* thus fills some of the gaps in the previous volumes, including DNA repair in bacteriophage and *Drosophila*, and the role of DNA repair in the generation of immune diversity. Second, the DNA repair field continues to grow explosively, and several topics needed updating soon after the first volumes were published. Such topics include the role of homologous recombination in mammalian cells, and the new biochemistry and cell biology of DNA double-strand break repair, which has provided key information about protein function in this important biological process. Third, as might be expected from such an active field, there are several new areas of research that were not even imagined prior to 1998, including the finding that proteins involved in nonhomologous end-joining were also involved in gene silencing and telomere function, and the discovery that the breast cancer susceptibility genes, *BRCA1* and *BRCA2*, have important roles in several aspects of DNA repair.

The DNA repair field grew from basic studies in genetics and cell biology. These approaches are increasingly complemented by biochemical approaches that provide detailed descriptions of complex processes at the molecular level and identify functional interactions among the various proteins involved in each repair pathway. Although early work provided provocative hints that DNA repair processes were conserved from bacteria to higher eukaryotes, a full appreciation of this conservation was not possible until many more genes were isolated and sequenced, and their gene products characterized at the biochemical level. This area of research has in turn led to the understanding that functions carried out by a single protein in prokaryotes are often performed by several related proteins in eukaryotes, and that these protein family members are often found in multi-subunit complexes. Another new development in the field is that seemingly distinct DNA repair processes, such as mismatch repair and nucleotide excision repair, show functional overlap, particularly at the level of lesion recognition. Such overlap suggests that DNA repair processes form a complex network. It is likely that this network enables cells to respond appropriately to different quantities and qualities of DNA damage. As we stand on the threshold of the "New Age of Functional Genomics and Proteomics," it is clear that the next level of understanding will be a molecular description of DNA repair networks in various cell types, and how these networks produce the various cellular responses to different types of DNA damage. Of course, a discussion of the future of DNA repair research begs the question:

Will there there be a need for Volume IV? The answer of course is yes, but just when this task will be undertaken (and by whom) is not yet clear.

We thank all of the contributors for their considerable time and effort to produce the high-quality texts, and for their assistance in the development of the chapter titles and in reviewing draft manuscripts. We also thank our many colleagues, both within and outside our laboratories, for their continued support. And we again thank our families for their patience and understanding: Denise, Jake, Ben, Courtney, Debra, Brad, Lauren, Brielle, and Alexa.

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Post-Replication Repair

A New Perspective Focusing on the Coordination Between Recombination and DNA Replication

Steven J. Sandler

1. INTRODUCTION

The repair of DNA is crucial to the survival of every organism. Organisms have evolved many biochemical pathways for detecting and repairing DNA damage with high fidelity. Failure to repair DNA with high fidelity leads to a high mutation frequency. This in turn is correlated with a high risk of cancer in humans.

The type of DNA damage usually dictates the type of DNA repair pathway used by the cell. This article focuses on post-replication repair (PRR) of DNA. This process was first noted while using ultraviolet (UV)-irradiation as a source of DNA damage. While there are several types of UV-induced lesions (i.e., pyrimidine dimers), most are removed by the nucleotide-excision repair (NER) and photoreactivation repair (PR) pathways. However, when a replication fork encounters one of these noncoding lesions (not removed by NER or PR), a special type of recombinational repair pathway is available to repair the DNA damage. This type of repair has been referred to both daughter-strand gap repair (DSGR) and PRR. In recent years PRR has become synonymous with the RecF pathway of recombination in *Escherichia coli*. The RecF pathway of recombinational DNA repair operates on gapped DNA substrates that presumably arise after the replication fork has partially replicated past a noncoding lesion in the template DNA (leaving a gap opposite the noncoding lesion). The missing information (forming the gap) across from the noncoding lesion is then supplied by the other daughter duplex DNA in a process requiring RecA. In this process, information from the fully replicated daughter, complementary to the lesion, is placed across from the lesion. Thus the damage is now in a DNA duplex and can be repaired either NER or PR.

Recently PRR has been integrated into a broader paradigm that explains the coordination of DNA repair, DNA replication, and recombination in the cell. This paradigm, called CPR (*c*oordinated *p*rocessing of damaged *r*eplication forks) *(19,20,80),* combines many other aspects of DNA metabolism and cell division. CPR emphasizes that these processes are used by the cell in a housekeeping sense (without the occurrence of

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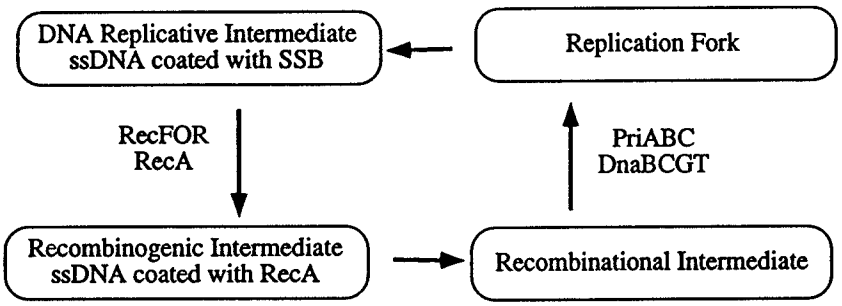

Fig. 1. Proposed relationship between DNA replication and recombination intermediates. The gene names along the vertical arrows indicate the proteins that perform multiple steps in the conversion between substrates. The reactions between boxes separated by the horizontal arrows are less well-defined. Once again they indicate conversion between one recombination or replication structure and another.

extra DNA damage). In a simple sense, CPR is the process by which collapsed or arrested replication forks are repaired by recombinational processes and then restarted. It is thought that restart is an essential process. It is important to emphasize that some of these repair and restart processes may be *recA*-independent since *recA* mutants are viable. Other aspects of CPR include chromosome partitioning (*dif*locus and *XerCD*) *(91)* and cell division (*ftsK*) *(91).*

Thus this review will explain PRR in terms of CPR and will focus on two groups of proteins thought to catalyze opposite reactions in the cell (Fig. 1). The first group includes the RecF, RecO, and RecR (RecFOR) proteins and are thought to catalyze presynaptic steps in PRR that convert a DNA replicative intermediate, ssDNA coated with SSB to a recombinogenic intermediate, ssDNA coated with RecA *(14,78).* The assembly of a RecA-ssDNA filament is critical for molecular healing of the damaged replication fork by recombination. The second group of *E. coli* proteins were initially characterized as elements required in vitro DNA replication of ΦX174 ssDNA phage. Their role in *E. coli* however, has only recently begun to be appreciated. These proteins, collectively called the primosome assembly proteins, include PriA, PriB, PriC, DnaT, DnaC, DnaB, and DnaG (reviewed in *34,57,58,81*). They are thought to restart DNA replication forks at recombinational intermediates and to be essential for normal vegetative growth. Thus they complete the transition in PRR, allowing a collapsed replication fork that has been repaired by the action of RecFOR, RecA, and the process of homologous recombination to be restarted.

The reader is also referred to other articles that review aspects of PRR *(37,38),* the homologous recombination machinery in general *(14,35,46,48), recFOR* specifically *(72),* CPR *(20,57,80),* and the primosome assembly proteins *(34,57,58,81).*

2. AN OVERVIEW OF HOMOLOGOUS RECOMBINATION

Recombination is often thought of as occurring in three distinct stages. The first stage is called pre-synapsis. In this stage, one of the two interacting duplexes of DNA is tailored so that it can be bound by the RecA protein. This tailoring often involves the generation of ssDNA through the action of helicases that unwind duplex DNA or sin-

gle-stranded exonulceases that selectively degrade one strand of DNA. Once the ssDNA is generated, it can be bound by RecA to create a protein-DNA helical filament. It is this filament that is thought to be the active agent that searches for a homologous region of duplex DNA. How RecA performs this search is still an unsolved aspect of RecA biochemistry. Several models have been proposed to explain RecA strand pairing, recognition of homologous regions, and strand exchange *(8,51,86)*. Once a homologous region is found, RecA can catalyze the invasion of the ssDNA into the duplex DNA displacing the identical strand. This DNA structure is often called a displacement loop or D-loop. This structure includes a crossover or Holliday junction *(90)* that can undergo branch migration in which the Holliday junction diffuses along the length of the DNA molecule. The Holliday structures may then be resolved enzymatically in *E. coli* by RuvC or Rus *(87)*. These enzymes can cleave two strands of the Holliday structure either vertically or horizontally. The position and way in which these structures are resolved determines the structure and phenotypes of the recombinants. Traditionally, the process of recombination has been thought to end after the post-synaptic steps described. However as will be discussed later, it is now necessary to invoke other post-synaptic steps that include the assembly of a DNA replication fork at a recombination intermediate in order to attain viable recombinants.

2.1. Substrates for Homologous Recombination

When thinking about the molecular process of recombination, it is often instructive to focus on the different types of DNA substrates before introducing the gene products that operate on them. To a large degree, the type of DNA substrate dictates that set of gene products will be needed to perform the recombination event. In this sense recombination is thought to be substrate-limited. The field has largely divided the many different types of DNA substrates into two varieties: duplex molecules with double-strand ends and duplex molecules with regions of ssDNA (gaps). These substrates are operated on by the RecBCD (*see* Chapter 8 in Volume *1*) and RecF pathways of recombination respectively and the latter is described in more detail later. One way to think about these two pathways is that they describe sets of pre-synaptic enzymes that funnel the many different DNA substrates into substrates that can be bound by RecA (Fig. 2). Historically, the RecF pathway genes also included gene products involved in branch migration (*ruvA* and *ruvB*) and Holliday junction resolution *(ruvC)*. Now however, these enzyme are thought only to play a post-synaptic role. The roles of *recJ*, *recN*, and *recQ*, also commonly referred to as RecF pathway genes, in pre- and or post-synapsis are presently unclear.

2.2. Pathways of Homologous Recombination

Conceptually, a recombinational pathway for DNA is very much like a biochemical pathway for the biosynthesis of an amino acid. One has substrates that are acted on by enzymes that produce products. Genetically, if a mutation is introduced that blocks that pathway (when there is only one pathway), a phenotype will be seen. If the cell has multiple pathways that can provide the same end product, a phenotype will only be seen if genes in both pathways are mutated. For recombination, the phenotype seen is often a decrease in recombination frequency. This is often measured by the production of a physically or genetically scoreable recombinant normalized against experimental para-

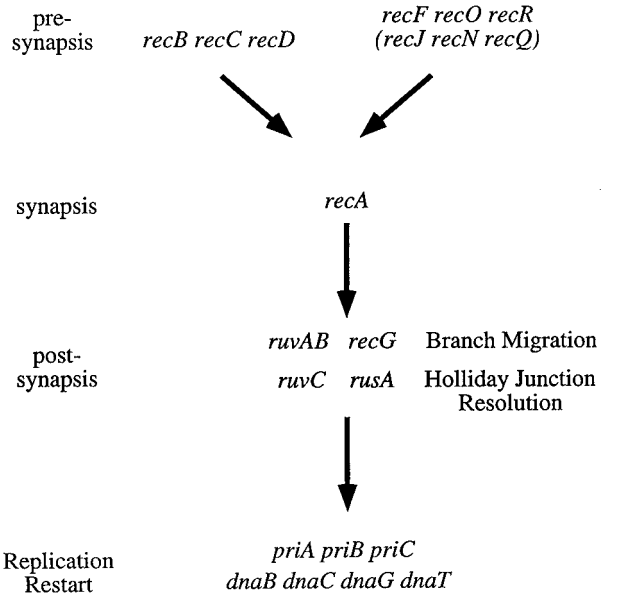

Fig. 2. Genes needed for RecBCD and RecF pathways of recombination in *E. coli*. The genes are grouped to correspond with their different roles in the stages of recombination. The genes in parentheses encode functions whose role is not yet clear. Although the genes involving replication restart have been placed after the post-synaptic steps of branch migration and Holliday junction resolution, this has not yet been demonstrated experimentally. These genes define a minimal set. Other genes know to have an affect on these processes include *ssb, lig,* mismatch repair, and genes encoding the different topoisomerases.

meters. For some years, the use of different recombinational substrates introduced by different protocols (i.e., conjugation, transduction, inter- and intra-plasmidic recombination, and substrates with direct or inverted repeats) caused some confusion. With the recognition that these substrates are physically different and need a different set of recombinases to be processed by the cell, illumination has come to the field.

In *E. coli,* there are commonly thought to be two main pathways for recombination. These have been alluded to earlier and are diagrammed in different ways in Figs. 2 and 3. The major differences between these pathways are in the pre-synaptic steps; the two pathways are named after the genes used in these initial steps. Figure 3 illustrates that the differences in recombination pathways are only in the pre-synaptic steps and that the substrates are processed to common intermediates. Although there is only one synaptic protein listed, the post-synaptic steps of branch migration and Holliday junction resolution require multiple enzymes. The reason for this redundancy is not clear.

The examples in Fig. 3 use replicative DNA substrates in which the DNA damage could result from housekeeping functions or additional insult to the cell. The left side of Fig. 2 shows a standard model for RecBCD mediated double-strand break repair *(88).* In the RecBCD pathway, a duplex DNA with a double-stranded end is produced when the replication fork encounters a nick. In short, the dsDNA end is tailored so that RecA can use it in strand invasion to produce a D-loop. Branch migration and Holliday junction resolution can leave a substrate that is then ready for restarting DNA replication. For additional details, *see* Chapter 8 in Volume 1.

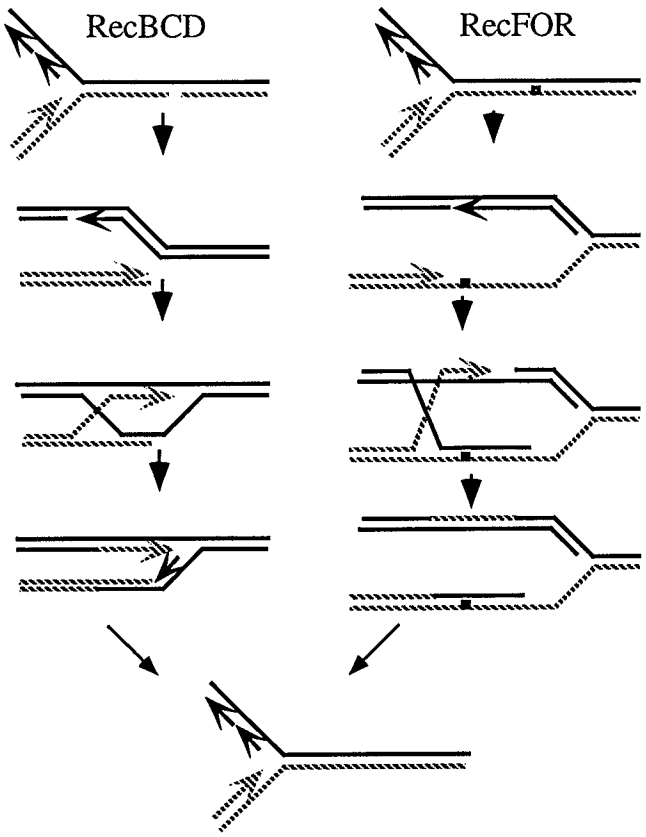

Fig. 3. How the RecBCD and RecFOR pathways of recombination may repair collapsed replication forks and then produce a structure that is suitable for restart of DNA replication. The structures shown include the starting substrate, replication-fork collapse, a synapsis/post-synapsis intermediate, Holliday junction intermediates, a substrate for replication fork restart and the restarted replication fork.

The right side of Fig. 3 shows PRR by the RecFOR pathway of a noncoding lesion. This process conjures up several theoretical problems and questions. How does the replication machinery replicate past the noncoding lesion on only one strand? How far past the lesion does it go? What is the molecular signal for this procedure? What are the required proteins? How large are the gaps produced? Is the process different if the damage is encountered by the lagging or leading strand polymerases? Although clear answers to none of these question are available, the events after production of the proposed gap have been addressed in some detail later.

To complement this broad overview of recombination, PRR and CPR, the remainder of the article will focus on *recF, recO,* and *recR (recFOR)* genes and proteins and then introduce the less well-known and -studied primosome assembly genes.

3. GENETICS OF RecFOR

3.1. Isolation and Characterization of the recFOR Genes

The first mutation found in *recF, recF143,* was identified by its ability to cause extreme recombination deficiency (Rec$^-$ as measured by conjugal recombination) and

UV sensitivity (UV^S) in a *recB recC sbcB sbcC* strain *(28)*. Unlike *recA, recB,* and *recC* single mutations that cause both Rec^- and UV^S phenotypes, *recF143* single mutations caused only UV^S. The ability to affect DNA repair and not recombination has been both the hallmark and the riddle of *recF* function in DNA metabolism. Mutations in *recO* and *recR* were subsequently shown to produce virtually identical phenotypes *(26,43,107).* It has been proposed that *recF, recO,* and *recR (recFOR),* act in a biochemical pathway that identifies and converts a DNA replicative intermediate (ssDNA coated with single-stranded DNA binding protein [SSB]) to a recombinogenic one (ssDNA coated with RecA) by helping RecA displace SSB on the proper DNA substrate *(14,78)* (Fig. 1). However, it is still unclear how RecFOR orchestrates the transition from DNA replication to recombination. Research suggests that subcomplexes of RecOR *(85,97),* RecFR *(105,106)* and RecF may also exist in addition to RecFOR *(27)* and may catalyze different parts of the complete reaction or they may have other roles in the cell. RecFOR homologs have only been found in bacteria. Nonetheless, their function appears to be maintained in other evolutionarily diverged systems. For example, yeast genes *RAD52, RAD55,* and *RAD57* have been proposed to have a function analogous to *E. coli* RecFOR *(92,93).*

3.1.1. Phenotypes of recFOR Mutants

Single mutations in the *recFOR* genes cause UV sensitivity *(28,33,55),* attenuation of UV-induction of the SOS response *(26,107),* and decreased plasmid recombination *(15,21,33).* For UV sensitivity, the three genes are epistatic *(13,44,45,47,55).* Other phenotypes of *recF* mutants include defects in mutagenesis of ssDNA phages *(12)* and induction of the adaptive response *(101).* These latter phenotypes have not been tested for *recO* and *recR* mutants. The effect of *recF, recO,* and *recR* mutations in other mutant backgrounds is of great interest. For example, it has been shown in *uvrA (96)* and *priA (73)* mutant strains, that *recF* mutations either decrease the UV resistance or viability of the strain. This argues that *RecF* function is needed in pathways that operate in the absence of these other gene products.

Perhaps the study of *recFOR* has been given less emphasis historically than the RecBCD pathway because these genes were originally perceived to act in an alternate or secondary pathway of recombination in *E. coli.* This is certainly not the case; single mutations in *recFOR* have distinctive phenotypes (e.g., UV^S). The importance of the RecF pathway and its difference from the RecBCD pathway of recombination lies in the types of substrates that it handles. The RecBCD enzyme acts at the ends of linear DNA molecules, whereas the *recFOR* proteins have been associated with recombination in the middle of DNA molecules. Thus, *recB* or *recC* single mutations have major effects on conjugal recombination (linear DNA substrates with ends) and single mutations in *recFOR* affect recombination with plasmid substrates *(15,21,33),* P22 transduction and chromosomal recombination in *Salmonella typhimurium (24,60).* This may not be the only difference from the RecBCD pathway since UV-induced SOS expression by the RecF pathway requires DNA replication *(83,84).* It should also be noted that the RecF pathway is flexible and can be adopted to function on linear DNA substrates during conjugal recombination. Thus in a *recBC sbcBC* strain, *recFOR* mutations have dramatic effects on conjugal recombination *(28).*

Phenotypic studies on the overexpression of *recFOR* genes have revealed interesting and useful information about these proteins. Overexpression of the *recO* protein can

Table 1
Biochemistry of RecFOR proteins and Interactions DNA, SSB, and RecA

	RecO, 26 kDa	RecF, – 40 kDa	RecR, 22 kDa
O	1. Monomer in solution 2. Binds both ssDNA and ds DNA 3. Promotes renaturation of complementary ssDNA, requires Mg^{+2}, inhibited by 160 mM NaCl – ATP independent. *(50)* 4. Strand assimilates short oligo and supercoiled DNA, requires Mg^{+2}, ATP independent. *(49)*.		
F	1. RecF co-precipitates RecO. This is reversed in presence of ATP *(27)*. 2. RecF interacts with RecO even if SSB is present *(27)* .	1. Binds ssDNA, ATP independent *(25, 52)*. 2. Inhibits RecA joint molecule formation and RecA ATPase activity *(52)*. 3. Binds ATP, dsDNA binding is ATP or ATPγS dependent *(53)* Binding is stronger for ATPγS than ATP. 4. Binding ssDNA or dsDNA or dsDNA enhances ATP binding *(53)*. 5. ATPase activity with dsDNA (105). 6. Binding dsDNA with ATPγS – 1 monomer per 4–6 bp *(105)*.	
R	1. RecR and RecO overcome SSB inhibition of RecA catalyzed joint molecule formation *(97)* 2. RecOR-SSB ssDNA complex helps RecA to nulceate on DNA *(98)* 3. Stabilzes 5′ end-dependent dissociation of RecA filament *(85)*	1. RecR stablizes RecF on dsDNA in presence of ATP – 1:1 molar ratio of proteins coats dsDNA (105). 2. ATPase activity is stimulated by RecR *(105)*. 3. RecR-RecF complex limits extension of RecA filament from ssDNA in a gap to the dsDNA *(106)*.	1. *E. coli* protein – No reported activity by itself on dsDNA *(105)*. 2. *B. subtilis* protein – Binds ssDNA and ds DNA – binding enhanced by presence of damage in DNA, ATP and divalent metal ions *(3)*. Binding of RecR multimers associated with DNA loops in EM *(7)*.

partially suppress the UV^S caused by *recA* mutations *(49)*. This correlates with the finding that RecO can catalyze a similar reaction to RecA in vitro (see Subheading 4.2; Table 1; and *49*). Wild-type *recF* overexpression causes inhibition of UV-induced SOS expression, decreased UV^R and cell viability *(79)*. All known mutant *recF* genes show the same phenotypes as a *recF* null mutant when in single copy on the chromosome, but each displays only a subset of the overexpression phenotypes, indicating that the different mutations remove different subsets of RecF activities. Hence, analysis of overexpression phenotypes is a useful method to define and correlate in vivo and in vitro activities of RecF. The *recF4115* mutation is the only *recF* missense protein where all overexpression phenotypes have been eliminated *(74)*.

3.2. Evolutionary Conservation of recFOR Genes

Chromosomal mutations in either the *recF, recO,* and *recR* genes have been most intensively studied in *E. coli* (FOR) (for example, *see [78]*), *S. typhimurium* (F) *(24,60)* and *Bacillus subtilis* (FR) *(2–4)*. Several missense mutations have been isolated in *recF (recF143, recF4101, recF4104, recF4115) (74,79),* insertion mutations (*recF400::kan [94]* and *recF332::Tn3* [9]) and deletions *(recF349) (79)*. Only insertion mutations have been reported so far for *recR (55)* and *recO (33)*.

recF, recO, or *recR* homologs have been reported in 48 different types of bacteria spanning the kingdom of *Proteobacteria* to *Aquicales* (*see* Table 2). Many organisms have homologs of all three genes. In several of the completed genomic sequences only *recF* and *recR* homologs are reported. However, *Helicobacter pylori* and *Aquifex aeolicus* contain only a *recR* homolog and *Borrelia burgdorferi* and *Mycoplasma genitalium* lack *recFOR* homologs. It is worth equal note that while *Treponema pallidum* contains both *recF* and *recR* homologs, this organism has no *recBCD* homologs (and *Borrelia burgdorferi* has the converse:, only *recBCD* homologs and no *recFOR* homologs) *(22,23)*. These findings raise many questions. For example: Do organisms that only have *recF* and *recR* contain only a subset of RecFOR activities? If not, do they have an evolutionary nonhomologous (but functional analogous) *recO* gene? Are organisms without a full RecFOR complement more sensitive to DNA damage than ones with a full complement? It is also noteworthy that only two groups of proteobacteria contain *recO* homologs. From this observation and given the phylogeny of 16S rRNA, it is tempting to speculate that *recO* genes were a late acquisition in the evolution of bacterial-repair systems. Answers to these questions may take some time because little is known about how these diverse organisms repair, replicate, and recombine their DNA.

No *recFOR* evolutionary homologs have been reported in the *Archaea* or *Eucarya*. Rad52, Rad55, and Rad57 in yeast are reported to assist the yeast RecA homolog, Rad51 compete for ssDNA coated with RPA (SSB analog) *(92,93)*. Hence it is possible that while the *recFOR* homologs are not found in eucaryotes, their function is conserved.

3.3. Location and Regulation of E. coli recF, recO, and recR Genes

In bacteria, clues to biological functions of some genes can be suggested by the function of neighboring genes and patterns of transcriptional regulation. In *E. coli*, both *recF* and *recR* are in groups of genes that are needed for DNA replication. The *recO* gene is found downstream of the *era* gene. This gene of unknown function is essential

Table 2
Organisms Containing *recF, recO* and *recR* Homologs

Bacterial grouping	Organism	*recF*	*recR*	*recO*
α *Proteobacteria*	*Caulobacter crescentus*	U37793		
	Rickettsia prowazekii[a]		X[b]	
β *Proteobacteria*	*Neisseria gonorrhoeae*	X	X	X
	Neisseria meningitidis		X	X
	Bordetella pertussis		X	X
	Thiobacillus ferrooxidan	X	X	
γ *Proteobacteria*	*Escherichia coli*[a]	K02179	M38777	U36841
	Haemophilus influenzae Rd[a]	U32780	U32727	U32718
	Haemophilus ducreyi		AF017750	
	Pseudomonas aeruginosa	X	X	X
	Pseudomonas putida	X62504		
	Salmonella typhimurium	X62505	X	U48415
	Proteus mirabilis	M58352		
	Actinobacillus pleuropneumoniae	X63626		
	Actinobacillus actinomycetemcomitans	X	X	X
	Yersinia pestis	X	X	X
	Shewanella putrefaciens	X	X	X
	Pasteurella multocida	X	X	X
	Klebsiella pneumoniae	X	X	X
	Vibrio cholerae	X	X	X
	Azotobacter vinelandii	X86404		
	Coxiella burnetii			L27436
ε *Proteobacteria*	*Helicobacter pylori*[a]	AE000602		
	Campylobacter jejuni	X		
Firmicutes (Low GC gram positive)	*Bacillus subtilis*[a]	X02369	X17014	
	Lactococcus lactis	X89367		
	Staphylococcus aureus	X71437	X	
	Clostridium difficile	X	X	
	Clostridium acetobutylicum	X	X	
	Enterococcus faecalis	X	X	
	Streptococcus mutans		X	
	Streptococcus pneumoniae	X	X	
	Streptococcus thermophilus		P96053	
	Streptococcus pyogenes	U07342		
Actinomycetes (High GC gram positive)	*Mycobacterium smegmatis*	X92503		
	Mycobacterium tuberculosis[a]	Z80233	AL022121	
	Mycobacterium leprae[a]	Z70722	AL023596	
	Mycobacterium bovis	X	X	
	Streptomyces coelicolor	L27063		
Green sulfur	*Chlorobium tepidum*	X	X	

(Continues)

Table 2
Continued

Bacterial grouping	Organism	*recF*	*recR*	*recO*
Cyanobacteria	*Synechocystis sp. PCC6803*[a]	D90907	D90916	
Spirochaetales	*Treponema pallidum*[a]	AE001185	AE001268	
Cytophagales	*Porphyromonas gingivalis*	X	X	
Chlamydiales	*Chlamydia trachomatis*[a]	AE001282	AE001297	
	Chlamydia pneumoniae[a]	X	X	
Thermus/Deinococcus	*Deinococcus radiodurans*	X	X	
Aquificales	*Aquifex aeolicus*[a]		AE000742	

[a] Completed genome sequence.
"X" indicates either a partial or full sequence is available in the uncompleted genome database as of August 1999. Genbank accession numbers are given where known.

for growth. As will be expanded upon later, the association of *recF* and *recR* with DNA replication genes is suggestive that these two proteins (and possibly *recO* as well) may interact with the replication machinery.

Understanding of the transcriptional regulation of the three groups of genes encoding *recFOR* is at a rudimentary level. Most is known about the transcriptional regulation of the *recF* gene. Transcriptional studies using different fragments of the *dnaA-dnaN-recF* region fused to *lacZ* on both plasmids and chromosomes have identified several sequences that act as promoters and transcriptional terminator *(5,6,68).* These results suggest that this region of the chromosome is under complex regulation and that *recF* transcriptional regulation is part of a larger network involving *dnaA* and *dnaN (11,29).* It has been recently shown that *dnaN* and *recF* promoters are induced greater than 40-fold during entry into stationary phase *(100).* Studies on the transcriptional regulation of the *recO* and *recR* genes have not yet been reported.

The level of the *recFOR* proteins in the cell is thought to be quite low, although it has not been precisely determined. This speculation is based on several observations. First an upper limit of the amount of RecF has been estimated at less than 190 molecules per cell *(52).* Second, several processes that inhibit *recF* overexpression were identified when *recF* was overexpressed from a plasmid *(76,77).* Third, the codon usage of *recF* is similar to that of other poorly expressed *E. coli* genes *(9).* Although overexpression of *recR* is not problematic like *recF,* expression of *recR* in maxicells is quite low *(56).* Another interesting observation is that the *recF, recO,* and *recR* genes all overlap with the genes in upstream of them. This type of arrangement, called translational coupling *(67),* is used by the cell to ensure equal levels of expression of two proteins that interact. In the case of *recF* and *recR,* however, a mechanism opposite of translational coupling seems to operate because much less of the *recF* and *recR* gene products are seen relative to the *dnaN* and *orf12* gene products, respectively *(9,55).* A common mechanism acting either at the level of transcription or translation to regulate or coordinate levels of *recFOR* gene expression remains to be elucidated.

4. BIOCHEMICAL INTERACTIONS BETWEEN RecFOR AND OTHER PROTEINS

4.1. Interactions with SSB and RecA

The idea that *recFOR* modulates the binding of SSB and RecA to ssDNA derives from both genetic and biochemical data. Moureau *(61)* showed that overexpression of SSB yielded phenotypes similar to those of *recF* mutants. Volkert and colleagues *(102,104)* isolated several suppressors of *recF* mutations that mapped in *recA* (e.g., *recA803*). Others showed that these suppressors would also suppress *recO* and *recR* mutations *(52,103).* Biochemical analysis showed that RecA803 protein could make joint molecules under conditions where SSB was inhibitory to RecA-catalyzed reactions *(54).* Umezu et al. *(97)* showed that RecO and RecR were sufficient to overcome the SSB inhibition in RecA-catalyzed joint molecule reactions. The unresolved finding in this report was that RecF had no role in the in vitro reaction. This was in contradiction to the in vivo reaction, where *recF* has a definite role. At least one aspect of the reaction conditions in these experiments did not mimic the in vivo conditions as very high levels of RecO and RecR were used in vitro and only low amounts of RecOR are thought to present in vivo. This prompted further testing, which showed that overexpression of *recOR* could suppress *recF* mutations and provided a reason for why RecF was not needed in the in vitro reactions *(78).* These findings led to the molecular matchmaker model for RecFOR function in *E. coli (14,78).* This model proposes that RecF acts as a molecular matchmaker identifying and binding to a specific DNA structure: a gapped DNA intermediate left by DNA replication where SSB was bound to ssDNA. RecF would then help to load RecOR, which in turn helped to modify the SSB-ssDNA so that RecA could bind *(14,78).*

4.2. RecFOR Biochemistry

Biochemical analysis of the RecFOR proteins has been extremely difficult for at least three reasons: (1) There are no known enzymatic activities to follow during purification. Hence the proteins are purified on the criteria of solubility and electrophoretic purity. (2) The structure of the DNA substrate on which RecFOR operates is not known. It is, however, hypothesized to be a gapped DNA molecule produced by DNA replication. (3) Because DNA replication is required for RecF-dependent SOS induction *(84)*, it is likely that in vitro visualization of RecFOR activity will require components of the DNA replication machinery. In spite of these difficulties, significant achievements have been made in understanding the biochemical properties of these proteins. All three proteins have been overproduced and purified. Table 2 lists the known activities of the proteins both singly and in combination.

Singly, the *E. coli* RecO and RecF and *B. subtilis* RecR proteins display properties of proteins that are likely to be involved with DNA metabolism. They bind both ssDNA and dsDNA and this binding is modulated by divalent metal cations and nucleotide cofactors. RecO has two activities that could be specifically associated with recombination: renaturation of ssDNA and strand assimilation of an oligonucleotide with a homologous dsDNA circular supercoiled DNA substrate. Like RecO, the yeast *RAD52* protein also has an ATP-independent strand-transfer activity *(66).*

Early in its study, RecF activity was defined in terms of its ability to inhibit RecA catalyzed reactions *(52).* Although these are interesting reactions in their own right, one

needs to remember that in vivo there is usually a large excess of RecA over RecF. Therefore, these reactions may not be physiologically relevant. More recently, conditions for an ATPase activity have been identified for RecF in the presence of dsDNA *(105)*. It is likely that this activity is physiologically relevant and important. Evidence supporting this idea includes: (1) *RecF4101*, a mutant in the phosphate binding hole, is defective in RecF activity in vivo *(75)*. (2) RecF4101 does not display ATPase activity in vitro *(105)*. The RecF ATPase activity is also stimulated in the presence of RecR *(105)*.

RecOR and RecFR display activities that modulate the activity of RecA protein. RecOR help RecA load onto ssDNA coated with SSB, as previously mentioned. They also help to stabilize RecA filaments and prevent end-dependent dissociation *(85)*. These two mechanisms may be related. It is easy to visualize how these two activities would be useful in the early stages of RecA filament formation. The effect of RecFR on RecA protein filaments was discovered by first looking at the effect of RecR on RecF binding to dsDNA. RecF binds to dsDNA in a sequence-independent fashion. Binding is stronger in the presence of ATPγS than ATP. RecR stabilizes RecF binding to dsDNA in the presence of ATP *(105)*. When RecA binds to ssDNA on a gapped DNA substrate, eventually RecA filament formation will extend beyond the ssDNA into the dsDNA region. If RecFR is present, it will halt growth of the RecA filament *(106)*.

Madiraju and colleagues have defined complexes of RecFOR and SSB proteins in the absence of DNA using immunoprecipitation and different types of chromatography *(27)*. They find that RecO interacts with RecF, RecR, and SSB. In these assays, RecO can bind either RecR or SSB but not both. On the other hand, Umezu and Kolodner found using BIAcore sensor chips that RecO can bind both RecR and SSB and that SSB binds RecO with higher affinity than RecR *(98)*. The former group found that RecO can bind RecF and SSB at the same time and that the addition of ATP abolishes the RecFO interaction. They also see complexes between RecFOR and RecFOR-SSB depending on the order of addition.

In summary, it appears that the RecFOR proteins are capable of a variety of activities in vitro either singly or in combination that could be useful in recombination. Whether any of these activities is used by these proteins in vivo remains to be proven.

5. A MODEL FOR THE ROLE OF RecFOR IN THE CELL

It is arguable that the main function of recombination is to help restart stalled replication forks *(17,18,36,99)*. The process can be envisioned in the following steps: detection of DNA damage by the replication machinery, production of a gap in the DNA behind the replication fork, PRR substitution of DNA not replicated because of damage (e.g., RecA, RecFOR), and then restarting of the replication machinery. Several observations mentioned previously are consistent with RecFOR having an important role in the repair of collapsed and/or stalled replication forks by recombinational DNA repair. It is conceivable that RecF (OR) persists during the entire repair process and is not necessary only during the early pre-synaptic phase.

Figure 4 shows a model that combines several of the ideas represented in the literature *(14,17,27,78)*. The model focuses on the role of the RecFOR, RecA, and SSB proteins in PRR. The model assumes that the replication fork encounters a noncoding lesion on the template for the leading strand. The replication fork leaves a gap and pauses or stalls at least one Okazaki fragment upstream. The ssDNA is first covered

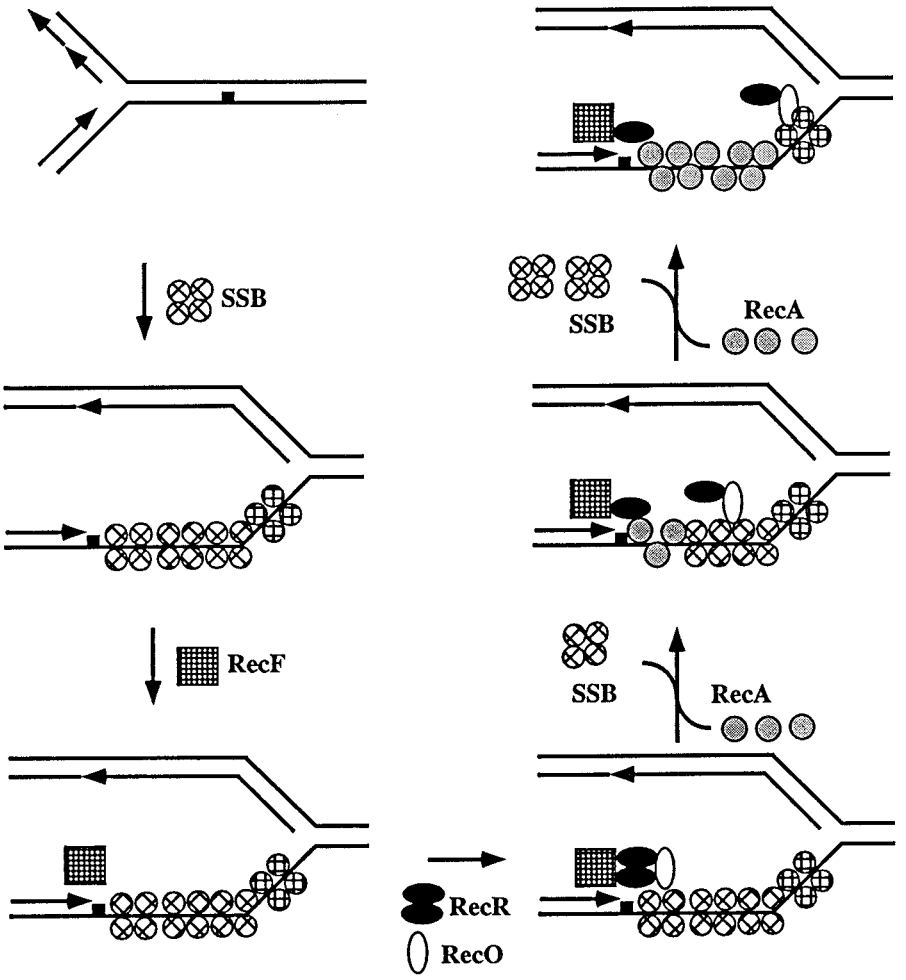

Fig. 4. Model of RecFOR in PRR. In the first panel, a replication fork approaching a noncoding lesion (small black box on the DNA) is diagrammed. In the second panel, the replication fork has translocated one Okazaki fragment past the noncoding lesion. No replication has occurred on the template strand containing the noncoding lesion. The remainder of the diagram is explained in the text.

with SSB. RecF then recognizes the DNA-protein structure (assumed in this diagram to be at the left edge of the gap) and then helps to load RecO and RecR. RecR is shown as a dimer at this point. After RecFOR assembly, the complex splits. One subunit of RecR goes with each RecO and the other RecR subunit remains with RecF. The RecOR complex is free to interact with SSB and help load RecA. When it reaches a dsDNA section, it stops and anchors the 5′ end of the RecA filament preventing dissociation. The RecFR complex left behind prevents the RecA filament from extending in to the dsDNA and focuses the RecA filament in the region of ssDNA. The RecA filament then searches the other daughter duplex for a region of homology and places the noncoding lesion across from its complement. The DNA lesion is now ready for removal by an excision repair reaction.

This model incorporates many of the observations mentioned in this article. However, it does not indicate how RecOR moves along the DNA removing the SSB, helping RecA to bind to the ssDNA or define when the RecF ATPase activity is needed. It also has RecA loading in a discontinuous fashion (3′ to 5′) *(69)*. An alternative scheme (not shown) that does not require RecOR to migrate along the DNA suggests RecR protein stabilizes a loop of ssDNA (as has been shown for the *B. subtilis* RecR protein) such that ends of the ssDNA region are close together. The complex can then split into RecFR and RecOR subcomplexes attached to each end of the gap and function as explained earlier. This scheme also has the advantage that RecA can load continuously in the 5′ to 3′ direction from the RecOR nucleating point.

5.1. A Molecular Connection Between RecF(OR) and DNA Replication

The position of *recF* and *recR* on the chromosome in the middle of operons encoding DNA-replication proteins and the observation that UV-induction of the SOS response by *recF* requires DNA replication suggests a connection between *recF, recR,* and DNA replication. Rothman and Clark *(70)* showed that after UV irradiation, DNA synthesis in a *recF143* mutant was greatly reduced relative to wild-type. Courcelle et al. *(16)* have shown that both *recF* and *recR* mutations lead to much greater amounts of degradation of newly synthesized DNA after UV irradiation and a decreased ability to complete ongoing rounds of DNA replication than wild-type.

A role for *recF* in DNA replication has been suggested by overlapping activity with *priA* in UV-induction of the SOS response (pre-synaptic role) and cell viability (post-synaptic role) *(73)*. PriA, originally isolated as a component of the ΦX174 in vitro DNA replication system (a model for primosome assembly and synthesis of RNA primers during lagging strand DNA synthesis at a replication fork [58]) has been shown to be essential for DNA repair and homologous recombination *(32,82)*. Interestingly, the overlapping roles of *priA* and *recF* are not shared by *recR* and *recO*. This is the only example of a phenotype where *recF* is different from that of *recO* and *recR* (where all three have been tested). It should be noted that the proposed post-synaptic role for *recF* is highly speculative.

Three additional observations support a role for *recF* in DNA replication and or cell viability. The first is that *recF143* causes a decrease in UV-mutagenesis with ssDNA phages *(12)*. This function may be overlapping with *priA*. The second is that *recF* is necessary for stable DNA replication and cell viability in a *rnh-102 recA200 rin-15* and *rnh-102 recA200 rin-15 dnaA508* strains, respectively *(95)*. The third situation in which *recF* may have a role in DNA replication is that it is needed for viability in a *recA200 polA25::spc lexA71::Tn5* strain *(10)*. Although a common thread between the latter three mutant strains is not apparent, all have a defect in DNA replication (and or cell viability) that is suppressed by some mutation (either *rin-15* or *lexA71::Tn5*) and this new situation is then dependent on the *recF* gene product. It is not clear, however, if *recF* participates in a pathway that is active to a small degree in wild-type cells and this becomes the major pathway in the mutant cells, or if the pathway only becomes active in these "suppressed" states. The dependence of *recO* and *recR* in these strains has not been addressed.

6. THE ROLE OF THE PRIMOSOME ASSEMBLY PROTEINS IN RESTARTING REPLICATION FORKS

The role of the primosome assembly proteins (PriA, PriB, PriC, DnaB, DnaC, DnaG, DnaT) in the cell is beginning to become clear (reviewed in *[57,58,80,81]*). These *E.*

coli proteins were originally discovered as host proteins required in the ΦX174 in vitro DNA replication system. The biochemical properties of these proteins suggested that they were involved in the synthesis of RNA primers on the lagging strand at a replication fork. Although this is still possible, it now appears that these proteins have different or additional roles. It is now believed that these proteins help to restart replication forks that have stalled or collapsed and were repaired by recombinational and RecA-independent processes.

6.1. Primosome Assembly Proteins in DNA Replication In Vitro

Although the ΦX174 in vitro DNA replication system may not be the best model of the in vivo function of the primosome assembly proteins, this system provides the clearest picture at the biochemical level and provides a platform for discussing their in vivo functions. A key feature of the assembly process is the DNA substrate to which the proteins are loaded. This is the primosome assembly site (PAS) of the ΦX174 chromosome. Although other ssDNA phages and some plasmids have these sites, they have never been found on the *E. coli* chromosome. It is thought that both sequence and secondary structure are important for PAS *(1,89).* It is suspected that the PAS is a structure that some phages and plasmids have evolved to take advantage of the *E. coli* host system for their replication. Figure 5 shows the order of assembly of the primosomal proteins onto the PAS *(62,63).* The PriA protein binds to PAS and serves as a platform for the loading of the other proteins. PriA is a multifunctional protein with helicase, ATPase, and translocase activities that are genetically separate from its ability to assemble primosomes *(109).* The PriB protein binds to PriA-PAS. It is thought that PriB stabilizes PriA at PAS *(41).* DnaT then loads onto the PriA-PriB-PAS complex. These three proteins form a protein-nucleic acid complex that serves as an entry point for DnaC to load DnaB, the replicative helicase. DnaC is the only primosome assembly protein not part of the final primosome. Using some DNA substrates with PAS, there is an optimizing requirement for PriC before this step. However, the role of PriC is unknown. The PriABC-DnaTB complex is called the pre-primosome and DnaG (Primase) can interact with it in a distributive *(108)* fashion to synthesize RNA primers that are competent to be extended by Pol III holoenzyme.

6.2. Primosome Assembly Proteins and E. coli Replication Restart

Many questions were raised that eventually helped to clarify the role of the primosome assembly proteins in *E. coli.* The first is based on the observation that no PAS sites have been found on the *E. coli* chromosome. If true, then what is the natural substrate for PriA? Also, no slow or fast stop mutants of *priA*, *priB*, or *priC* have ever been isolated and none of these proteins are needed in an in vitro system that mimic initiation of DNA replication at *oriC.* This raised questions about how and when PriA PriB, DnaT, and PriC become associated with a replication fork. Surprisingly, a *priA* null mutant was unexpectedly found to be viable, deficient in recombination and DNA repair, and had high basal levels of SOS expression *(39,65).* Hence PriA was a DNA replication protein that also had roles in recombination. Tokio Kogoma *(30–32)* proposed that the pathway of primosome assembly might be used by *E. coli* to load replication forks at recombinational intermediates. This hypothesis has led to many advances in understanding the biochemistry of PriA. Several studies *(40,59,64)* have now shown that PriA binds to D-loops, a key recombinational intermediate.

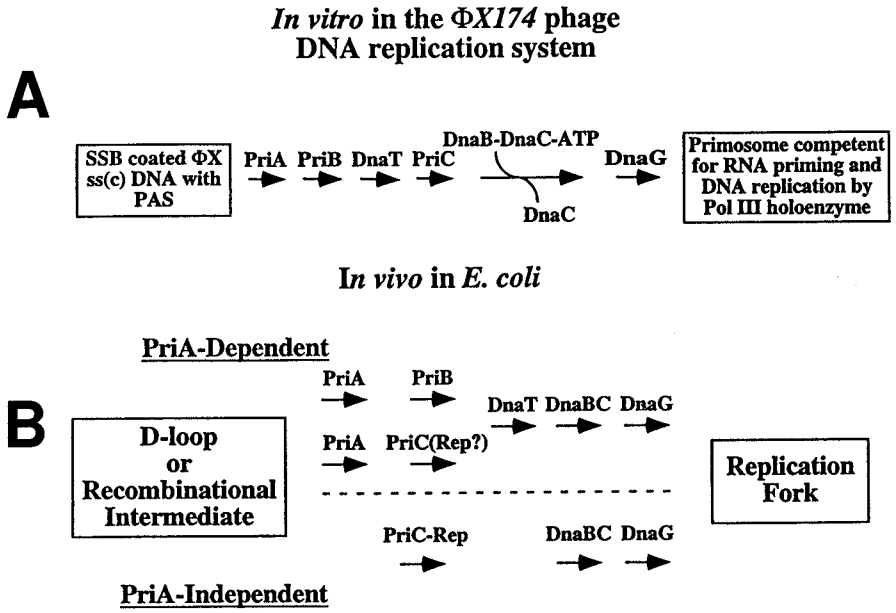

Fig. 5. Two models for the action of the primosome assembly proteins. In **A,** the in vitro assembly of the primosome assembly proteins on the ssDNA phage is shown. In the **B,** is a model for two pathways of primosome assembly as a prelude to the loading of a DNA replication fork. One should note that in the bottom half, although the substrate is referred to as a recombinational intermediate like a D-loop, in actuality this substrate has yet to be identified in vivo. The Rep protein in the PriA-dependent pathway is listed with a question mark because its role is currently not clear.

Understanding the genetics of the primosome assembly genes have been of intense interest to our lab. Almost every simple prediction of ΦX174 model so far has not proved true for *E. coli.* For instance, one would predict that *priB* and to lesser extent, *priC,* should have the same mutant phenotypes as *priA* mutants, but this was not the case. Null mutants of *priB* and *priC* are not readily distinguishable from wild-type *(81).* Yet the *priB priC* double mutant is inviable, suggesting that these proteins have a redundant and essential role in *E. coli.* Extragenic suppressors of *priA* mutations have been found and mapped in *dnaC (82).* These are thought to load DnaB at the correct DNA substrate in the absence of PriA, PriB, PriC and DnaT. While these *dnaC* suppressors fully suppress *priA* mutant phenotypes, they only partially suppress *priB priC* mutant phenotypes *(81).* In vitro, the PriA suppressor DnaC810 can load Pol III holoenzyme (via DnaB) at a D-loop *(42).* Hence the biochemistry is beginning to agree with the in vivo data.

Finally, a lingering question has been why *priA* mutations are not lethal, as it would seem that restarting replication forks at recombinational intermediates is essential. One idea is that there are multiple pathways for restarting replication forks. Evidence for this has come from synthetic lethality studies of pairs of primosome assembly mutants *(71).* Figure 5 shows a diagram of one model that explains how some gene products may be involved in these multiple pathways. It is noteworthy that the *priC* protein, which has not found a secure home in the ΦX174 model, is essential for the PriA-independent path-

way. Also, another orphan DNA replication gene, *rep*, is essential for this pathway. Additional experiments show that the *dnaC809* suppression pathway of *priA* mutant phenotypes occurs by the elevation or modulation of the PriC-Rep pathway *(71,80).*

Why would *E. coli* two pathways to restart replication? Although there are several possible reasons, the most appealing is that there are different DNA substrates that need to be processed into replication forks. These could arise by different mechanisms such as replication fork arrest or collapse. One might expect that repair of these two situations would lead to different DNA structures with different complements of proteins and thus may be optimally restarted by two different systems. This idea is analogous to the RecBCD and RecF pathways of recombination, which act preferentially on different types of substrates (double-strand ends or gaps) to repair and recombine DNA.

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REFERENCES

1. Abarzua, P., W. Soeller, and K. J. Marians. 1984. Mutational analysis of primosome assembly sites. I. Distinct classes of mutants in the pBR322 *Escherichia coli* factor Y DNA effector sequences. *J. Biol. Chem.* **259:** 14,286–14,292.
2. Alonso, J. C., G. Luder, and R. H. Tailor. 1991. Characterization of *Bacillus subtilis* recombinational pathways. *J. Bacteriol.* **173:** 3977–3980.
3. Alonso, J. C., A. C. Stiege, B. Bobrinski, and R. Lurz. 1993. Purification and properties of the RecR protein from *Bacillus subtillis* 168. *J. Biol. Chem.* **268:** 1424–1429.
4. Alonso, J. C., R. H. Tailor, and G. Luder. 1988. Characterization of recombination-deficient mutants of *Bacillus subtilis. J. Bacteriol.* **170:** 3001–3007.
5. Armengod, M.-E., M. Garcia-Sogo, and E. Lambies. 1988. Transcriptional organization of the *dnaN* and *recF* genes of *Escherichia coli* K-12. *J. Biol. Chem.* **263:** 12,109–12,114.
6. Armengod, M.-E., and E. Lambies. 1986. Overlapping arrangement of the *recF* and *dnaN* operons of *Escherichia coli;* positive and negative control sequences. *Gene* **43:** 183–196.
7. Ayora, S., A. C. Stiege, R. Lurz, and J. C. Alonso. 1997. *Bacillus subtilis* 168 RecR protein-DNA complexes visualized as looped structures. *Mol. Gen. Genet.* **254:** 54–62.
8. Bazemore, L. R., M. Takahashi, and C. M. Radding. 1997. Kinetic analysis of pairing and strand exchange catalyzed by RecA. Detection by fluorescence energy transfer. *J. Biol. Chem.* **272:** 14,672–14,682.
9. Blanar, M. A., S. J. Sandler, M.-E. Armengod, L. W. Ream, and A. J. Clark. 1984. Molecular analysis of the *recF* gene of *Escherichia coli. Proc. Natl. Acad. Sci. USA.* **81:** 4622–4626.
10. Cao, Y., and T. Kogoma. 1995. The mechanism of *recA polA* lethality: suppression by RecA-independent recombination repair activated by the *lexA*(def) mutation in *Escherichia coli. Genetics* **139:** 1483–1494.
11. Chiaramello, A. E., and J. W. Zyskind. 1990. Coupling of DNA replication to growth rate in *Escherichia coli:* a possible role for guanosine tetraphosphate. *J. Bacteriol.* **172:** 2013–2019.
12. Ciesla, Z., P. O'Brian, and A. J. Clark. 1987. Genetic analysis of UV mutagenesis of the *Escherichia coli glyU* gene. *Mol. Gen. Genet.* **207:** 1–8.
13. Clark, A. J. 1991. *rec* genes and homologous recombination on *Escherichia coli. Biochimie* **73:** 523–632.
14. Clark, A. J., and S. J. Sandler. 1994. Homologous genetic recombination. *Crit. Rev. Microbiol.* **20:** 125–142.

15. Cohen, A., and A. Laban. 1983. Plasmidic recombination in *Escherichia coli* K-12: the role of *recF* gene function. *Mol. Gen. Genet.* **189:** 471–474.
16. Courcelle, J., C. Carswell-Crumpton, and P. C. Hanawalt. 1997. *recF* and *recR* are required for resumption of replication at DNA replication forks in *Escherchia coli. Proc. Natl. Acad. Sci. USA.* **94:** 3714–3719.
17. Cox, M. M. 1998. A broadening view of recombinational DNA repair in bacteria. *Genes Cells* **3:** 65–78.
18. Cox, M. M. 1991. The RecA protein as a recombinational repair system. *Mol. Microbiol.* **5:** 1295–1299.
19. Cox, M. M. 1999. Recombinational DNA repair in bacteria and the RecA protein. *Prog. Nucleic Acids Res. Mol. Biol.* **63:** 311–366.
20. Cox, M. M., M. F. Goodman, K. N. Kreuzer, D. J. Sherratt, S. J. Sandler, and K. J. Marians. 1999. Importance of repairing stalled replication forks. *Nature* **404:** 37–41.
21. Fishel, R. A., A. A. James, and R. Kolodner. 1981. *recA*-independent general genetic recombination of plasmids. *Nature* **294:** 184.
22. Fraser, C. M., S. Casjens, W. M. Huang, and et al. 1997. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi. Nature* **390:** 580–586.
23. Fraser, C. M., S. J. Norris, G. M. Weinstock, O. White, and G. G. Sutton. 1998. Complete genome sequence of *Treponema pallidum*, the Syphilis spirochete. *Science* **281:** 375–388.
24. Galitski, T., and J. R. Roth. 1997. Pathways for homologous recombination between chromosomal direct repeats in *Salmonella typhimurium. Genetics* **146:** 751–767.
25. Griffin, T. J., and R. D. Kolodner. 1990. Purification and preliminary characterization of the *Escherichia coli* K-12 RecF protein. *J. Bacteriol.* **172:** 6291–6299.
26. Hedge, S., S. J. Sandler, A. J. Clark, and M. V. V. S. Mardiraju. 1995. *recO* and *recR* mutations delay induction of SOS response in *Escherichia coli. Mol. Gen. Genet.* **246:** 254–258.
27. Hegde, S. P., M. H. Qin, X. H. Li, M. A. Atkinson, A. J. Clark, M. Rajagopalan, and M. V. Madiraju. 1996. Interactions of RecF protein with RecO, RecR, and single-stranded DNA binding proteins reveal roles for the RecF-RecO-RecR complex in DNA repair and recombination. *Proc. Natl. Acad. Sci. USA.* **93:** 14,468–14,473.
28. Horii, Z.-I., and A. J. Clark. 1973. Genetic analysis of the RecF Pathway to genetic recombination in *Escherichia coli* K-12: isolation and characterization of mutants. *J. Mol. Biol.* **80:** 327–344.
29. Katayama, T., T. Kubota, K. Kurokawa, E. Crooke, and K. Sekimizu. 1998. The initiator function of DnaA protein is negatively regulated by the sliding clamp of the *E. coli* chromosomal replicase. *Cell* **94:** 61–71.
30. Kogoma, T. 1996. Recombination by replication. *Cell* **85:** 625–627.
31. Kogoma, T. 1997. Stable DNA replication: interplay between DNA replication, homologous recombination and transcription. *Micro. Mol. Biol. Rev.* **61:** 212–238.
32. Kogoma, T., G. W. Cadwell, K. G. Barnard, and T. Asai. 1996. The DNA replication priming protein, PriA, is required for homologous recombination and double-strand break repair. *J. Bacteriol.* **178:** 1258–1264.
33. Kolodner, R., R. A. Fishel, and M. Howard. 1985. Genetic recombination of bacterial plasmid DNA: effect of RecF pathway mutations on plasmid recombination in *Escherichia coli. J. Bacteriol.* **163:** 1060–1066.
34. Kornberg, A., and T. Baker. 1992. *DNA Replication*, 2nd. W. H. Freeman and Company, New York.
35. Kowalczykowski, S. C., D. A. Dixon, A. K. Eggleston, S. D. Lauder, and W. M. Rehrauer. 1994. Biochemistry of homologous recombination in *Escherichia coli. Microbiol. Rev.* **58:** 401–465.
36. Kuzminov, A. 1995. Collapse and repair of replication forks in *Escherichia coli. Mol. Microbiol.* **16:** 373–384.

37. Kuzminov, A. 1996. *Recombinational Repair of DNA Damage.* R. G. Landes Company, Austin, TX.
38. Kuzminov, A. 1999. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage λ. *MMBR* **63:** 751–813.
39. Lee, E. H., and A. Kornberg. 1991. Replication deficiencies in *priA* mutants of *Escherichia coli* lacking the primosomal replication n′ protein. *Proc. Natl. Acad. Sci. USA* **88:** 3029–3032.
40. Liu, J., and K. J. Marians. 1999. PriA-directed assembly of a primosome on D loop DNA. *J. Biol. Chem.* **274:** 25,033–25,041.
41. Liu, J., P. Nurse, and K. J. Marians. 1996. The ordered Assembly of the ΦX174-type primosome III. PriB facilitates complex formation between PriA and DnaT. *J. Biol. Chem.* **271:** 15656–15661.
42. Liu, J., L. Xu, S. J. Sandler, and K. J. Marians. 1999. Replication fork assembly at recombination intermediates is required for bacterial growth. *Proc. Natl. Acad. Sci. USA* **96:** 3552–3555.
43. Liu, Y. H., A. J. Cheng, and T. C. Wang. 1998. Involvement of *recF, recO,* and *recR* genes in UV-radiation mutagenesis of *Escherichia coli. J. Bacteriol.* **180:** 1766–1770.
44. Lloyd, R. G., and C. Buckman. 1991. Overlapping functions of *recD, recJ,* and *recN* provide evidence of three epistatic groups of genes in *Escherichia coli* recombination and DNA repair. *Biochimie* **73:** 313–320.
45. Lloyd, R. G., N. P. Evans, and C. Buckman. 1987. Formation of recombinant *lacZ*$^+$ DNA in conjugational crosses with a *recB* mutant of *Escherichia coli* K12 depends on *recF, recJ,* and *recO. Mol. Gen. Genet.* **209:** 135–141.
46. Lloyd, R. G., and K. B. Low. 1996. Homologous recombination, in *Escherichia coli* and *Salmonella,* vol. 2 (Neidhardt F. C., ed.), ASM Press, Washington, DC, pp. 2236–2255.
47. Lloyd, R. G., M. C. Porton, and C. Buckman. 1988. Effect of *recF, recJ, recN, recO* and *ruv* mutations on ultraviolet survival and genetic recombination in a *recD* strain of *Escherichia coli* K-12. *Mol. Gen. Genet.* **212:** 317–324.
48. Lloyd, R. G., and G. J. Sharples. 1992. Genetic analysis of recombination in prokaryotes. *Curr. Opin. Genet. Dev.* **2:** 683–690.
49. Luisi-DeLuca, C. 1995. Homologous pairing of single-stranded DNA and superhelical double-stranded DNA catalyzed by RecO protein from *Escherichia coli. J. Bacteriol.* **177:** 566–572.
50. Luisi-DeLuca, C., and R. Kolodner. 1994. Purification and characterization of the Escherichia coli RecO protein. *J. Mol. Biol.* **236:** 124–138.
51. MacFarland, K. J., Q. Shan, R. B. Inman, and M. M. Cox. 1997. RecA as a motor protein. Testing models for the role of ATP hydrolysis in DNA strand exchange. *J. Biol. Chem.* **272:** 17,675–17,685.
52. Madiraju, M. V. V. S., and A. J. Clark. 1991. Effect of RecF protein on reactions catalyzed by RecA protein. *Nucleic Acids Res.* **19:** 6295–6300.
53. Madiraju, M. V. V. S., and A. J. Clark. 1992. Evidence for ATP binding and double-stranded DNA binding by *Escherichia coli* RecF protein. *J. Bacteriol.* **174:** 7705–7710.
54. Madiraju, M. V. V. S., A. Templin, and A. J. Clark. 1988. Properties of a mutant *recA*-encoded protein which reveal a possible role for *Escherichia coli recF*-encoded protein in genetic recombination. *Proc. Natl. Acad. Sci. USA* **85:** 6592–6569.
55. Mahdi, A. A., and R. G. Lloyd. 1989. Identification of the *recR* locus of *Escherichia coli* K-12 and analysis of its role in recombination and DNA repair. *Mol. Gen. Genet.* **216:** 503–510.
56. Mahdi, A. A., and R. G. Lloyd. 1989. The *recR* locus of *Escherichia coli* K-12: Molecular cloning, DNA sequencing and identification of the gene product. *Nucleic Acids Res.* **17:** 6781–6794.
57. Marians, K. J. 1999. PriA: at the crossroads of DNA replication and recombination. *Prog. Nucleic Acids Res. Mol. Biol.* **63:** 39–67.

58. Marians, K. J. 1992. Prokaryotic DNA replication. *Ann. Rev. Biochem.* **61:** 673–719.
59. McGlynn, P., A. Al-Deib, J. Liu, K. Marians, and R. Lloyd. 1997. The DNA replication protein PriA and the recombination protein RecG bind D-loops. *J. Mol. Biol.* **270:** 212–221.
60. Miesel, L., and J. R. Roth. 1996. Evidence that SbcB and RecF pathway functions contribute to RecBCD-dependent transductional recombination. *J. Bacteriol.* **178:** 3146–3155.
61. Moreau, P. L. 1988. Overproduction of single-stranded-DNA-binding protein specifically inhibits recombination of UV-irradiated bacteriophage DNA in *Escherichia coli. J. Bacteriol.* **170:** 2493–2500.
62. Ng, J. Y., and K. J. Marians. 1996. The ordered assembly of the ΦX174-type primosome I. Isolation and identification of intermediate protein-DNA complexes. *J. Biol. Chem.* **271:** 15642–15648.
63. Ng, J. Y., and K. J. Marians. 1996. The ordered assembly of the ΦX174-type primosome II. Preservation of primosome composition from assembly through replication. *J. Biol. Chem.* **271:** 15649–15655.
64. Nurse, P., J. Liu, and K. J. Marians. 1999. Two modes of PriA binding to DNA. *J. Biol. Chem.* **274:** 25,026–25,032.
65. Nurse, P., K. H. Zavitz, and K. J. Marians. 1991. Inactivation of the *Escherichia coli* PriA DNA replication protein induces the SOS response. *J. Bacteriol.* **173:** 6686–6693.
66. Ogawa, T., A. Shinohara, A. Nabetani, T. Ikeya, X. Yu, E. H. Egelman, and H. Ogawa. 1993. RecA-like recombination proteins in eukaryotes: functions and structures of RAD51 genes. *Cold Spring Harbor Sym. Quant. Biol.* **58:** 567–576.
67. Oppenheim, D. S., and C. Yanofsky. 1980. Translational coupling during expression of the tryptophan operon of *Escherichia coli. Genetics* **95:** 785–795.
68. Perez-Roger, I., M. Garcia-Sogo, J. P. Navarro-Avino, C. Lopez-Acedo, F. Macian, and M. E. Armengod. 1991. Positive and negative regulatory elements in the *dnaA-dnaN-recF* operon of *Escherichia coli. Biochimie* **73:** 329–334.
69. Register, J. C. d., and J. Griffith. 1985. The direction of RecA protein assembly onto single strand DNA is the same as the direction of strand assimilation during strand exchange. *J. Biol. Chem.* **260:** 12,308–12,312.
70. Rothman, R. H., and A. J. Clark. 1977. The dependence of postreplication repair on *uvrB* in a *recF* mutant of Escherichia coli K-12. *Mol. Gen. Genet.* **155:** 279–286.
71. Sandler, S. J. Multiple genetic pathways of restarting replication forks in *Escherichia coli. K-12 Genetics* **155:** 487–497.
72. Sandler, S. J. 1999. *On the Role of the RecF, RecO and RecR Proteins in Escherichia coli,* Encyclopedia of Life. Macmillian Reference Limited.
73. Sandler, S. J. 1996. Overlapping functions for *recF* and *priA* in cell viability and UV-inducible SOS expression are distinguished by *dnaC809* in *E. coli* K-12. *Mol. Microbiol.* **19:** 871–880.
74. Sandler, S. J. 1994. Studies on the mechanism of reduction of UV-inducible *sulAp* expression by *recF* overexpression in *E. coli* K-12. *Mol. Gen. Genet.* **245:** 741–749.
75. Sandler, S. J., B. Chackerian, J. T. Li, and A. J. Clark. 1992. Sequence and complementation analysis of *recF* genes from *Escherichia coli, Salmonella typhimurium, Pseudomonas putida* and *Bacillus subtilis:* evidence for an essential nucleotide binding fold. *Nucleic Acids Res.* **20:** 839–845.
76. Sandler, S. J., and A. J. Clark. 1990. Factors affecting expression of the *recF* gene of *E. coli* K-12. *Gene* **86:** 35–43.
77. Sandler, S. J., and A. J. Clark. 1994. Mutational analysis of sequences in the *recF* gene of *Escherichia coli* K-12 that affect expression. *J. Bacteriol.* **176:** 4011–4016.
78. Sandler, S. J., and A. J. Clark. 1994. RecOR suppression of *recF* mutant phenotypes in *E. coli* K-12. *J. Bacteriol.* **176:** 3661–3672.
79. Sandler, S. J., and A. J. Clark. 1993. Use of high and low level overexpression plasmids to test mutant alleles of the *recF* gene of *E. coli* K-12 for partial activity. *Genetics* **135:** 643–654.

80. Sandler, S. J., and K. J. Marians. 2000. Role of PriA replication fork reactivation in *Escherichia coli. J. Bacteriol.* **182:** 9–13.
81. Sandler, S. J., K. J. Marians, K. H. Zavitz, J. Coutu, M. A. Parent, and A. J. Clark. 1999. *DnaC* mutations suppress defects in DNA replication and recombination associated functions in *priB* and *priC* double mutants in *E. coli* K-12. *Mol. Microbiol.* In press.
82. Sandler, S. J., H. S. Samra, and A. J. Clark. 1996. Differential suppression of *priA2::kan* phenotypes in *Escherichia coli* K-12 by mutations in *priA, lexA,* and *dnaC. Genetics* **143:** 5–13.
83. Sassanfar, M., and J. Roberts. 1991. Constitutive and UV-mediated activation of RecA protein: combined effects of *recA441* and *recF143* mutations and of addition of nucleotides and adenine. *J. Bacteriol.* **173:** 5869–5875.
84. Sassanfar, M., and J. W. Roberts. 1990. Nature of the SOS-inducing signal in *Escherichia coli:* the involvement of DNA replication. *J. Mol. Biol.* **212:** 79–96.
85. Shan, Q., J. M. Bork, B. L. Webb, R. B. Inman, and M. M. Cox. 1997. RecA protein filaments: end-dependent dissociation from ssDNA and stabilization by RecO and RecR proteins. *J. Mol. Biol.* **265:** 519–540.
86. Shan, Q., and M. M. Cox. 1997. RecA filament dynamics during DNA strand exchange reactions. *J. Biol. Chem.* **272:** 11,063–11,073.
87. Sharples, G. J., S. M. Ingleston, and R. G. Lloyd. 1999. Holliday junction processing in bacteria: insights from the evolutionary conservation of RuvABC, RecG, and RusA. *J. Bacteriol.* **181:** 5543–5550.
88. Smith, G. R. 1989. Homologous recombination in *E. coli:* multiple pathways for multiple reasons. *Cell* **58:** 807–809.
89. Soeller, W., P. Abarzua, and K. J. Marians. 1984. Mutational analysis of primosome assembly sites. II. Role of secondary structure in the formation of active sites. *J. Biol. Chem.* **259:** 14,293–14,300.
90. Stahl, F. W. 1994. The Holliday junction on its thirtieth anniversary. *Genetics* **138:** 241–246.
91. Steiner, W., G. Liu, W. D. Donachie, and P. Kuempel. 1999. The cytoplasmic domain of FtsK protein is required for resolution of chromosome dimers. *Mol. Microbiol.* **31:** 579–83.
92. Sung, P. 1997. Function of the yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombinase. *J. Biol. Chem.* **272:** 28,194–28,197.
93. Sung, P. 1997. Yeast Rad55 and Rad57 proteins form a heterodimer that functions with replication protein A to promote DNA strand exchange by Rad51 recombination. *Genes Dev.* **11:** 1111–1121.
94. Thoms, B., and W. Wackernagel. 1988. Suppression of the UV-sensitive phenotype of *Escherichia coli recF* mutants by *recA*(Srf) and *recA*(Tif) mutations requires *recJ*$^+$. *J. Bacteriol.* **170:** 3675–3681.
95. Torrey, T. A., and T. Kogoma. 1987. Genetic analysis of constitutive stable DNA replication in *rnh* mutants of *Escherichia coli* K12. *Mol. Gen. Genet.* **208:** 420–427.
96. Tseng, Y. C., J. L. Hung, and T. C. Wang. 1995. Involvement of RecF pathway recombination genes in postreplication repair in UV-irradiated *Escherichia coli* cells. *Mutation Res.* **315:** 1–9.
97. Umezu, K., N.-W. Chi, and R. D. Kolodner. 1993. Biochemical interaction of the *Escherichia coli* RecF, RecO and RecR proteins with RecA and single-stranded DNA binding protein. *Proc. Natl. Acad. Sci. USA* **90:** 3875–3879.
98. Umezu, K., and R. D. Kolodner. 1994. Protein interactions in genetic recombination in *Escherichia coli:* Interactions involving RecO and RecR overcome the inhibition of RecA by Single-stranded DNA-binding protein. *J. Biol. Chem.* **269:** 30,005–30,013.
99. Uzest, M., S. D. Ehrlich, and B. Michel. 1995. Lethality of *rep recB* and *rep recC* double mutants of *Escherichia coli. Mol. Microbiol.* **17:** 1177–1188.
100. Villarroya, M., I. Perez-Roger, F. Macian, and M. E. Armengod. 1998. Stationary phase induction of *dnaN* and *recF,* two genes of *Escherichia coli* involved in DNA replication and repair. *EMBO J.* **17:** 1829–1837.

101. Volkert, M. R. 1989. Altered induction of the adaptive response to alkylation damage in *Escherichia coli recF* mutants. *J. Bacteriol.* **171:** 99–103.
102. Volkert, M. R., and M. A. Hartke. 1984. Suppression of *Escherichia coli recF* mutations by *recA*-linked *srfA* mutations. *J. Bacteriol.* **169:** 498–506.
103. Wang, T.-C. V., H.-Y. Chang, and J.-L. Hung. 1993. Co-suppression of *recF, recR* and *recO* mutations by mutant *recA* alleles in *Escherichia coli* cells. *Mutation Res.* **294:** 157–166.
104. Wang, T. C. V., M. V. V. S. Madiraju, A. Templin, and A. J. Clark. 1991. Cloning and preliminary characterization of *srf-2020* and *srf-801,* the *recF* partial suppressor mutations which map in *recA* of *Escherichia coli* K-12. *Biochimie* **73:** 335–340.
105. Webb, B. L., M. M. Cox, and R. B. Inman. 1995. An interaction between the *Escherichia coli* RecF and RecR proteins dependent on ATP and double-stranded DNA. *J. Biol. Chem.* **270:** 31,397–31,404.
106. Webb, B. L., M. M. Cox, and R. B. Inman. 1997. Recombinational DNA repair: the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. *Cell* **91:** 347–356.
107. Whitby, M. C., and R. G. Lloyd. 1995. Altered SOS induction associated with mutations in *recF, recO* and *recR. Mol. Gen. Genet.* **246:** 174–179.
108. Wu, C. A., E. L. Zechner, J. A. Reems, C. S. McHenry, and K. J. Marians. 1992. Coordinated leading- and lagging-strand synthesis at the *Escherichia coli* DNA replication fork. V. Primase action regulates the cycle of Okazaki fragment synthesis. *J. Biol. Chem.* **267:** 4074–4083.
109. Zavitz, K. H., and K. J. Marians. 1992. ATPase-deficient mutants of the *Escherichia coli* DNA replication protein PriA are capable of catalyzing the assembly of active primosomes. *J. Biol. Chem.* **267:** 6933–6940.