Colloquium

Effects of mutations involving cell division, recombination, and chromosome dimer resolution on a *priA2*::*kan* mutant

Jesse D. McCool and Steven J. Sandler*

Department of Microbiology, University of Massachusetts, 203 Morrill Science Center IVN, Amherst, MA 01003

Recombinational repair of replication forks can occur either to a crossover (XO) or noncrossover (non-XO) depending on Holliday junction resolution. Once the fork is repaired by recombination, PriA is important for restarting these forks in *Escherichia coli*. PriA mutants are Rec⁻ and UV sensitive and have poor viability and 10-fold elevated basal levels of SOS expression. *PriA sulB* mutant cells and their nucleoids were studied by differential interference contrast and fluorescence microscopy of 4′,6-diamidino-2-phenylindole-stained log phase cells. Two populations of cells were seen. Eighty four percent appeared like wild type, and 16% of the cells were filamented and had poorly partitioned chromosomes (Par⁻). To probe potential mechanisms leading to the two populations of cells, mutations were added to the *priA sulB* mutant. Mutating *sulA* or introducing *lexA3* decreased, but did not eliminate filamentation or defects in partitioning. Mutating either *recA* or *recB* virtually eliminated the Par⁻ phenotype. Filamentation in the *recB* mutant decreased to 3%, but increased to 28% in the *recA* mutant. The ability to resolve and/or branch migrate Holliday junctions also appeared crucial in the *priA* mutant because removing either *recG* or *ruvC* was lethal. Lastly, it was tested whether the ability to resolve chromosome dimers caused by XOs was important in a *priA* mutant by mutating *dif* and the C-terminal portion of *ftsK*. Mutation of *dif* showed no change in phenotype whereas *ftsK1*::*cat* was lethal with *priA2*::*kan*. A model is proposed where the PriA-independent pathway of replication restart functions at forks that have been repaired to non-XOs.

In *Escherichia coli*, accumulated evidence favors a model whereby replication forks routinely stop on their way from *oriC* to the terminus for a variety of reasons that include loss of helicase activity (arrest), encounter with a nick, or other type of DNA damage (collapse) (reviewed in refs. 1–3). Fork stoppage, repair, restart, and mitigation of the consequences of these events are therefore a common set of processes that occurs in most cells as they replicate their chromosomes. Repair of replication forks is accomplished by homologous recombination. Depending on the damage, different presynaptic enzymes such as RecBCD or RecFOR will aid RecA in the repair process and form a Holliday junction (reviewed in ref. 4). Postsynaptic enzymes for branch migration (RecG and RuvAB) and Holliday junction resolution (RuvC) will move and resolve the Holliday junction (5). Depending on resolution, exchange (crossing over) may occur between the sister chromosomes. If a crossover (XO) occurs then after replication has been restarted and completed, a chromosome dimer will be formed. The *dif*/XerCD/FtsK system then is needed to resolve these dimeric chromosomes to monomers (6, 7).

Replication restart proteins (RRPs) (3, 8) form multiple pathways (as defined genetically) to restart repaired forks as shown in Fig. 1 and ref. 9. Theoretically, the RRPs function equivalently to DnaA in that they form a protein-DNA complex
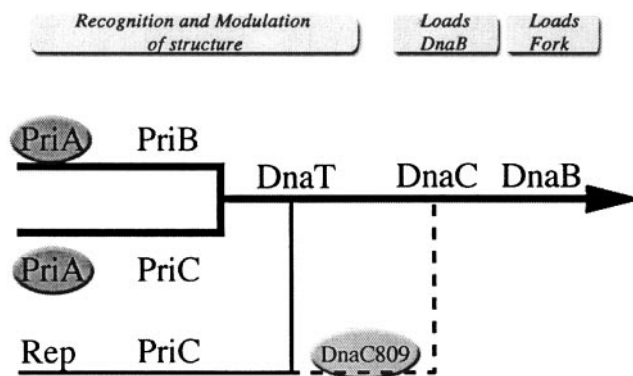

Fig. 1. Multiple biochemical pathways for replication restart from recombination intermediates. Three types of pathways: PriA-dependent (thick black lines), PriA-independent (thin black line), and PriA-independent using the suppressor *dnaC809* (dashed line). Possible function(s) of the genes are suggested by the headings at the top. Full explanation of genes and pathways can be found elsewhere (9).

so that the DnaB protein can be loaded onto the DNA (3, 10). However, unlike the loading of DnaB in a cell cycle, DNA sequence-dependent fashion at *oriC*, the RRPs can load DnaB anywhere on the chromosome by an *oriC*/*dnaA*-independent mechanism. Functionally, this is a key issue because the reasons for stopping can arise anywhere along the chromosome. Presumably, the RRPs load a single replication fork in the same direction the fork was traveling whereas DnaA loads two forks that replicate bidirectionally from *oriC*.

It is thought that PriA initiates the restart process by identifying and binding a recombinational intermediate (11, 12). In the absence of *priA*, cells are Rec⁻ and UV sensitive and have poor viability, high basal levels of SOS expression, and poor plating of *Mu* phage. Extragenic suppressors of *priA2*::*kan* have been found in *dnaC* (13). *In vitro*, DnaC810 can load DnaB onto single-stranded DNA in the absence of PriA or the other RRPs (*dnaC810* has a different nucleotide change as compared with *dnaC809* but produces the identical amino acid change) (14). *In vivo*, however, *dnaC809* requires *priC* and *rep* to suppress *priA2*::*kan* phenotypes (9).

To learn more about the PriA-independent pathway involving *priC* and *rep*, *priA2*::*kan* mutant cells and their nucleoids were studied by differential interference contrast (DIC) and fluores-

COLLOQUIUM

cence microscopy of 4′,6-diamidino-2-phenylindole-stained log phase cells. Unexpectedly two populations of cells were found: wild-type-appearing cells and filaments with abnormal nucleoids. Factors contributing to the formation of these two populations were studied by mutating genes involved with cell division, recombination, and resolution of chromosome dimers. A model is proposed where the PriA-independent pathway can restart only replication when repair processes have resolved the Holliday junctions to noncrossovers (non-XOs).

Materials and Methods

Bacterial Strains. All bacterial strains used in this work are derivatives of *E. coli* K-12 and are described in Table 1. Due to *priA1*::*kan* strain's sensitivity to rich media (15), cultures for all experiments described, unless otherwise indicated, were grown in 56/2 minimal media (16) at 37°C. The protocol for P1 transduction has been described (16).

Microscopy Techniques. Cultures were grown on a shaking water bath at 100 rpm to an optical density of 0.3 (A_{600}) at 37°C. A drop (10 μl) of culture was applied to each well of a poly-L-lysine-coated microscope slide (8-well multitest slides, ICN) and incubated at room temperature for 10 min. The wells were washed 10 times with standard PBS and allowed to dry. Cells were fixed with 80% methanol for 5 min and stained with 0.5 μg/ml solution of 4′,6-diamidino-2-phenylindoledihydrochloride. After 2 min, the wells were washed five times with PBS, and a coverslip was applied. A Nikon Eclipse 600 light microscope with a SPOT digital camera was used to collect DIC and fluorescence micrographs. NIH imaging software, SCION IMAGE (version 1.62a), was used to measure cell length. At least 500 cells from at least two individual trials were measured and used to calculate the percent filamentation.

Results

PriA2::*kan* Cultures Have Two Populations of Cells. We used DIC and fluorescence microscopy to visualize *priA* mutant cells and their nucleoids. Previous work revealed that *priA* mutant cells grown in rich media filament have poorly partitioned nucleoids and that *sulA* mutations suppressed the filamentation to a large degree (17, 18). Because it has since been shown that *priA* mutants are sensitive to rich media (15), a more in-depth study of *priA2*::*kan sulB103* mutant cells grown to midlog phase in minimal media at 37°C was done. *SulB103* is an allele of *ftsZ* that causes FtsZ to be insensitive to the action of the SulA cell division inhibitor (19).

Based on counting and measuring the lengths of 2,710 cells, two distinct populations of cells were seen in a *priA sulB* culture (Fig. 2). Eighty four percent of the cells examined appeared like wild type [less than three cell lengths (6 μm) in size and showing one or two nucleoids] (Table 2). The other 16% of the population show cells that are larger than 6 μm in size, and their nucleoids show a variety of conformations. Some nucleoids occupy the length of the cell and have a "knobby" appearance, whereas others show a single large nucleoid in the middle of the filament [filaments are defined as cells larger than three cell lengths (6 μm) in size regardless of the number of nucleoids]. Both nucleoid types suggest that these cells had difficulty in properly partitioning their chromosomes (Par⁻ is defined as cells containing multiple poorly condensed nucleoids). Some cells with no 4′,6-diamidino-2-phenylindole staining also are observed. These filaments were seen in early and late log phase growth (same proportion of the population) and with wet-mount preparations of live cells (data not shown).

We confirmed previous studies that cultures of *priA* cells contained two populations of cells: one that appears as wild type and one that appears as partitioning-defective filaments. For log phase cells grown in minimal media, the proportions of cells in these two groups were quantitated to be 84% and 16%. In the remainder of this report, the cellular processes that give rise to these two population cells will be investigated.

High Basal Levels of SOS Expression and *sulA* Contribute to the Filamentation Seen in a *priA sulB* Strain. It was interesting that both *sulB* (in minimal media) and *sulA* [in rich media (18)] mutations did not fully suppress the filamentation of a *priA* mutant as they do for *lexA*-defective mutants (20). Investigations as to why *sulA* and *sulB* mutations did not fully suppress the filamentation in a *priA* mutant (and did so differentially) were pursued. First, the level of filamentation of *sulA priA* strain grown in minimal media was determined and found to be 5% (Fig. 2, Table 2). The strain used, however, was of a different genetic background than the *priA sulB* strain (DM4000 vs. JC13509). Therefore a *sulA* mutation was introduced into the standard *sulB* strain. This *sulA sulB priA* mutant showed 7% filamentation. Previously it had been shown that the introduction of *lexA3* to a *priA sulA* mutant decreased the expression of the *sulA* promoter 10-fold to just below levels seen in the *priA*⁺ control (13). To test whether lowering levels of *sulA* (in addition to other *lexA*-regulated genes) was important for the filamentation occurring in *priA* mutants, *lexA3* was combined with the *priA sulB* mutations. Fig. 2 and Table 2 show that *lexA3* decreased filamentation from 16% to 7%. Finally, it was still unclear why *priA sulA* and *priA sulB* mutants had different levels of filamentation. To test whether *sulB* suppressed any *priA*-induced filamentation, a *priA sulB*⁺ was constructed. It showed 18% filamentation.

We conclude that *sulA* is an important determinant for filamentation in a *priA* mutant. Either removing *sulA* or lowering its concentration significantly reduces filamentation. The effect of a *sulB* mutation is much smaller. It remains unclear, however, why the two effects are not equal and why they do not fully suppress the *priA*-induced filamentation as they do for SOS-induced filamentation.

RecA and *recB* Contribute to the Partitioning Defect Observed in the Filamenting Subpopulation of *priA* Mutant Cells. Kuempel and colleagues (21) have shown that 15% of cells in a wild-type population undergo a XerCD-mediated recombination event at *dif* to produce hybrid *dif* sites. This number provides a minimum measure of the frequency of homologous recombination between sister chromosomes that result in XOs. The actual number could be greater if additional compensatory XOs took place after XO formation (6, 21). Because the percentage of cells showing a hybrid *dif* site was similar to the percentage of partitioning defective cells seen in a culture of a *priA* mutant, it was hypothesized that the partitioning defect was a result of recombinationally repaired replication forks where a XO had occurred. Then as in the *dif* system (6), removing presynaptic and synaptic recombination functions from a *priA* mutant should decrease the number of Par⁻ nucleoids. To test this, *del-(recA)306*, *recB258*::*Tn10*, and *recO1504*::*Tn5* mutations were combined with a *priA2*::*kan* mutation.

The *recA priA* and *recB priA* double mutant strains were viable and grew more slowly than *priA*, *recA*, or *recB* single mutants (data not shown). Several tests were performed to verify the genotypes of the double mutants and to check for suppressor mutations. These included reconstructing the strains, testing several different transductants, checking the alleles by PCR analysis, and sequencing the *dnaC* gene.

Removal of either *recA* or *recB* caused a dramatic change in the Par⁻ filaments seen in the *priA* mutant (Fig. 2 and Table 2). In both cases the Par⁻ phenotype nearly disappeared. This was more complete for the *recB priA* strain than for the *recA priA* strain. In some *recA priA* filaments, both Par⁺ and Par⁻ nucleoids were observed. The most dramatic differences, however, between the strains were in the length and number of filaments.

Table 1. Strains of bacteria used in this work

Strain	$priA^c$	Relevant genotype and/or plasmid	Source or derivation
AD11	+	ftsK1::cat	(31)
CAG5052	+	metB1 btuB3191::Tn10	(46)
DM4000[a]	+		M. Volkert
GS1481	+	ruvC64::kan	B. Michel
JC10289	+	del(srl-recA) 306::Tn10	(47)
JC13503	+	del(srl-recA) 306::Tn10	JC10289→SK362[e]
JC13509[b]	+		(13)
JC15716	+	recO1504::Tn5	(48)
JC18983[a]	2		(13)
JC19021[b]	2		(13)
JC19008[a]	2	dnaC809	(13)
JC19018[a]	2	dnaC809 zjj-202::Tn10	(13)
JC19098[a]	+	lexA3 malE::Tn10	(13)
JC19238[a]	+	dnaC809 metB1 btuB3191::Tn10	(9)
JC19250[a]	2	metB1 btuB3191::Tn10	(9)
JC19328[b]	+	del(srl-recA) 306::Tn10	JC13503→JC13509[e]
JJC315	+	recB268::Tn10	B. Michel
N3695	+	recG258::kan	B. Lloyd
PK3881	+	dif::tet	P. Kuempel
PN105	2		(18)
SK362[b]	+	$sulB^+$	(49)
SS167[b]	+	del(srl-recA) 306::Tn10 pSJS1222 ($recA^+$)	pSJS1222→JC19328[e]
SS186[b]	2	del(srl-recA) 306::Tn10	JC19008→SS167[e]
SS324[b]	2	$sulB^+$	PN105→SK362[e]
SS328[b]	+	dif::tet	PK3881→JC13509[f]
SS329[b]	2	dif::tet	PN105→SS328[e]
SS380[b]	2	recO1504::Tn5	JC19008→SS481[i]
SS382[b]	+	sulA6209::tet	TP606→JC13509[e]
SS394[b]	2	sulA6209::tet	JC19008→SS382[e]
SS446[b]	+	recB268::Tn10	JJC315→JC13509[g]
SS447	2	$metB^+$ $argE^+$	Laboratory stock
SS459[b]	+	recB268::Tn10 pDSW2 ($recB^+$)	pDSW2→SS446[e]
SS460[b]	2	recB268::Tn10	PN105→SS459[e]
SS471[b]	+	recO1504::Tn5	JC15716→JC13509
SS481[b]	+	recO1504::Tn5 metB1 btuB3191::Tn10	CAG5052→SS471[d]
SS490[b]	+	metB1 btuB3191::Tn10	CAG5052→JC13509[d]
SS493[a]	+	recG258::kan metB1 btuB3191::Tn10	N3695→JC19250[i]
SS702[a]	+	recG258::kan dnaC809 metB1 btuB3191::Tn10	N3695→JC19238[i]
SS712[a]	2	recG258::kan dnaC809	JC19008→SS702[h]
SS725[a]	+	ruvC64::kan metB1 btuB3191::Tn10	GS1481→JC19250[i]
SS728[a]	+	ruvC64::kan dnaC809 metB1 btuB3191::Tn10	GS1481→JC19238[i]
SS735[a]	2	ruvC64::kan dnaC809	JC19018→SS728[h]
SS767[b]	+	lexA3 malE::Tn10	JC19098→JC13509[g]
SS768[b]	2	lexA3 malE::Tn10	SS447→SS767[e]
SS805[b]	+	ftsK1::cat metB1 btuB3191::Tn10	AD11→SS490[e]
SS818[b]	+	ftsK1::cat	AD11→JC13509[e]
SS822[b]	2	ftsK1::cat dnaC809 zjj-202::Tn10	SS447→SS823[e]
SS823[b]	+	ftsK1::cat dnaC809 zjj-202::Tn10	JC19018→SS818[f]
TP606	+	sulA6209::tet	(50)

[a]These strains have the following partial genotype: sulA::Mu-d(Ap, lac, B::Tn9) Δ(lac-pro)XIII hisG4 argE3 ara-14 xyl-5 mtl-1 rpsL31.

[b]These strains have the following partial genotype: $sulB103^-$ lacMS286 Φ80dIIlacBK1 argE3 his-4 thi-1 xyl-5 mtl-1 $Sm^R T6^R$.

[c]The only allele of priA used in this study is priA2::kan (18).

[d]Select for tetracycline resistance and screen Met^-.

[e]Select for either kanamycin, tetracycline, ampicillin, or chloramphenicol resistance.

[f]Select for tetracycline resistance and then screen for the presence of dnaC809 by PCR.

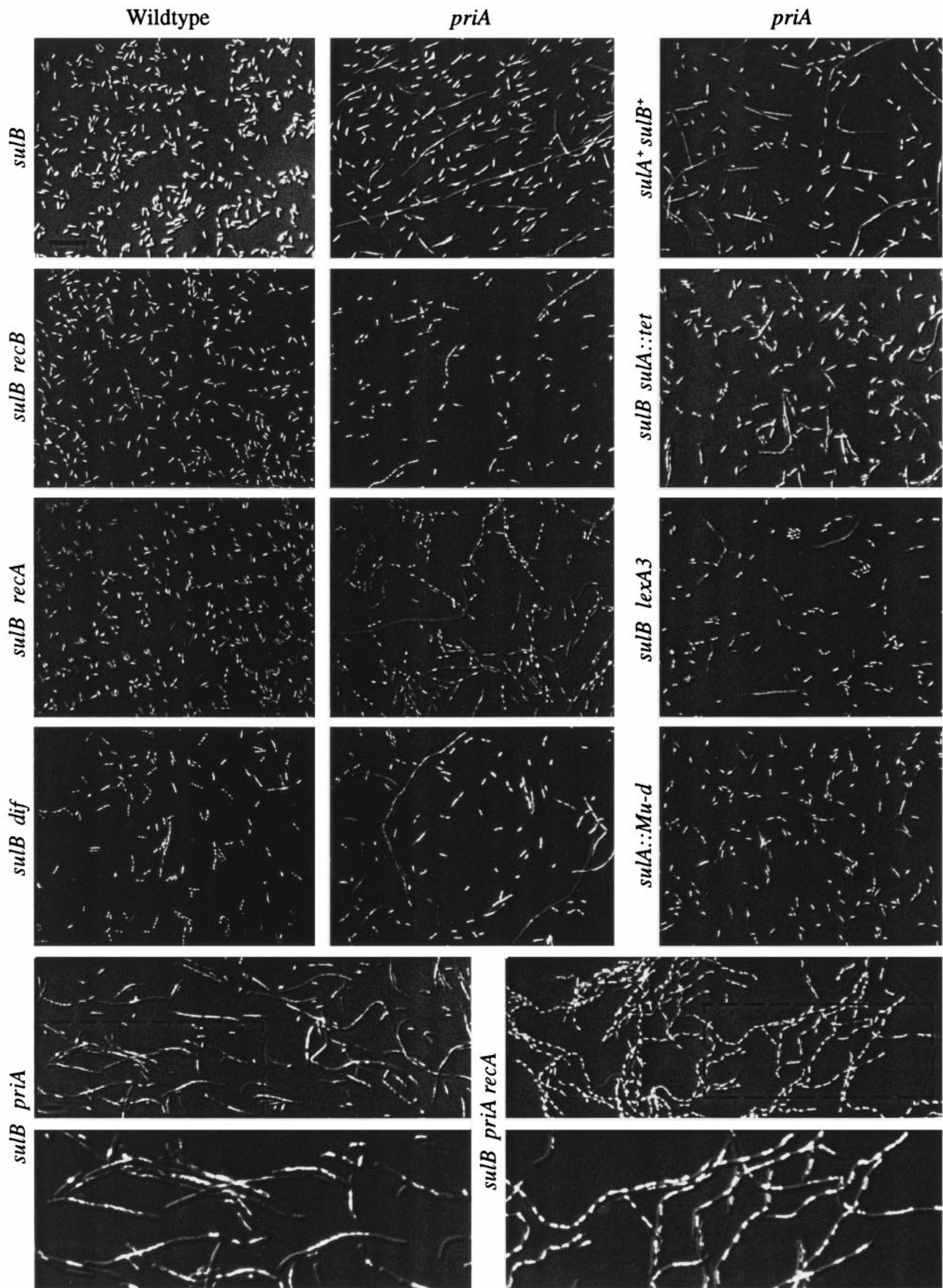[g]Select for tetracycline resistance and screen UV sensitive.

[h]Select for Met^+ and screen for the presence of priA2::kan by PCR.

[i]Select for kanamycin resistance and screen UV sensitive.

Removing recB from the priA mutant reduced filamentation from 16% to 3% and removing recA increased filamentation from 16% to 28%. These priA recA filaments observed were much longer as well (Table 2). Removal of recO from the priA mutant had little effect on the appearance or number of the Par⁻ cells (Table 2).

We conclude that under these conditions the recombination processes contributed by recB and recA, but not recO, help

Wildtype   *priA*   *priA*

sulB

sulB recB

sulB recA

sulB dif

sulB priA

sulA⁺ sulB⁺

sulB sulA::tet

sulB lexA3

sulA::Mu-d

sulB priA recA

(*Legend appears at the bottom of the opposite page.*)

Table 2. Percentage of cells showing filamentation

Strain	priA	recA	recB	recO	sulA	sulB	lexA	dif	Cells counted	Total filamentation	Distribution of cell sizes			
											6–10	10–20	20–50	>50
SK362	+	+	+	+	+	+	+	+	500	0	0	0	0	0
SS324	2	+	+	+	+	+	+	+	723	18.9	9.3	4.2	4.3	1.1
JC13509	+	+	+	+	+	103	+	+	500	0	0	0	0	0
JC19021	2	+	+	+	+	103	+	+	2,710	16.3	9.7	4.4	2.1	0.1
SS382	+	+	+	+	6209	103	+	+	500	0	0	0	0	0
SS394	2	+	+	+	6209	103	+	+	665	7.3	5.1	1.6	0.6	0
DM4000	+	+	+	+	Mu-d	+	+	+	500	0	0	0	0	0
JC18983	2	+	+	+	Mu-d	+	+	+	1,873	4.8	3.6	0.9	0.3	0
SS481	+	+	+	1504	+	103	+	+	500	1.4	1.4	0	0	0
SS380	2	+	+	1504	+	103	+	+	3,014	14.9	10.8	3.0	1.1	0
SS446	+	+	268	+	+	103	+	+	3,698	1.5	1.3	0.1	0.1	0
SS460	2	+	268	+	+	103	+	+	4,099	3.5	3.0	0.3	0.2	0
JC19328	+	306	+	+	+	103	+	+	973	2.1	2.0	0.1	0	0
SS186	2	306	+	+	+	103	+	+	2,200	28.1	17.6	6.6	3.0	0.9
SS767	+	+	+	+	+	103	3	+	500	0	0	0	0	0
SS768	2	+	+	+	+	103	3	+	2,020	7.0	3.8	1.9	1.0	0.3
SS328	+	+	+	+	+	103	+	tet	501	0.8	0.6	0.2	0	0
SS329	2	+	+	+	+	103	+	tet	1,683	18.7	11.5	4.0	3.0	0.2

The numbers for total filamentation and the distributions in the different size classes have been rounded off to the nearest tenth (0.1).

produce the Par⁻ phenotype seen in *priA* mutants. The difference in filamentation of the *recB priA* and *recA priA* mutant strains may be attributable to the removal of RecA-mediated SOS induction.

RecG258::*kan* and *ruvC64::kan* Are Synthetically Lethal with *priA2::kan*. Above, we hypothesized that the 16% of the *priA* mutant cells (the Par⁻ filaments) are the product of XOs. Two possibilities can explain the wild-type appearance of the remaining 84%: either they did not experience a repair event or the repair event was resolved to a non-XO. Michel *et al.* (22) have suggested that resolution of Holliday structures created during the recombinational repair of replication forks in *rep* mutants are biased to the production of non-XOs [other bias resolution observed in palindrome-induced DNA damage (23)]. If this bias also occurs in *priA* mutants, then removing functions that cause this biased resolution may result in fewer viable cells. This assumes that at least the same number of forks need repair and that *priA* cells in which forks are repaired to XOs are inviable. Candidate genes for providing this biased resolution include genes involved with branch migration (*recG* and *ruvAB*) and Holliday junction resolution (*ruvC*) (24). Therefore, *recG258::kan* and *ruvC64::kan* were added to a *priA* mutant. Because initial results indicated that both of these mutations were synthetically lethal with *priA2::kan*, this was tested definitively by using a previously described P1 transduction assay (9). Briefly, *priA2::kan* is introduced to a *metB1* recipient (also containing the other mutation to be tested) by selecting Met⁺ transductants. These are scored for the inheritance of *priA2::kan* by PCR analysis. Normal cotransduction frequencies can be monitored by testing for the conversion of the nearby *btuB* locus (from Tet^R to Tet^S). If no *priA2::kan* transductants are found in 32 independent events, then there is a 3 in 10⁷ chance this could have occurred by random chance. Table 3 shows that in a *recG* and *ruvC* mutant, respectively, none of 34 or 24 independent

Met⁺ transductants tested inherited *priA2::kan*. Conversion of the *btuB* locus occurred with a frequency similar to wild type.

It is concluded that *ruvC* and *recG* are essential genes in a *priA* mutant. This is consistent with a model where the *priA*-independent pathway can only restart recombinationally repaired replication forks to non-XOs that are produced through biased resolution of Holliday junctions.

Dif-Mediated Resolution of Dimers Is Not Essential in *priA* Mutants, but the Presence of *ftsK*⁺ Is Required. The above data are compatible with a model where recombinational repair of replication forks in a *priA* mutant are preferentially resolved to the non-XO state 84% of the time. Because *dif* and the machinery (*xerCD* and *ftsK*) used to resolve dimers are only needed when XOs occur, this locus and these gene products should not be needed in a *priA* mutant. To test this, *dif::tet* was first introduced into our test strain (JC13509). It was expected that this mutation by itself would cause a moderate amount of filamentation and defects in chromosome partitioning (25). We found, however, that *dif::tet* produced only a small amount (0.8%) of filamentation (Fig. 2 and Table 2). This smaller than expected amount may be due to the *sulB103* mutation and would make it unlikely that we should see any change in filamentation upon mutating *dif* in a *priA2::kan sulB103* mutant. This was tested and only a small difference was observed (Fig. 2 and Table 2). Similar results were seen with *xerC* and *xerC priA* mutants (data not shown). Although we cannot be sure whether there is an effect of *dif::tet* in a *priA* mutant, we can conclude that *dif* is not essential in a *priA* mutant. This is consistent with the model.

FtsK mutations are pleiotrophic, causing deficiencies in cell division (26), *dif*-mediated segregation of resolved dimers (7, 27–30), and regulation of *uspA* gene expression (31). *FtsK1::cat* is an insertion mutation that allows expression and function of the amino-terminal domain essential for cell division (32). Because *ftsK* also is required for *dif*-mediated recombination, we attempted to combine *ftsK1::cat* with *priA2::kan*. Table 3 shows

Fig. 2. (*On the opposite page.*) Pictures of overlays of individual DIC microscopy and fluorescence microscopy images using Abode PHOTOSHOP (version 5.5) are shown. Cells were prepared as noted in *Materials and Methods*. Genes mutated are indicated on the top and left sides. Wild type means *priA*⁺. See Table 1 for specific allele numbers and other genotypes of each strain. Strains are named starting from the top left and moving right. (*Upper*) Row 1: JC13509, JC19021, SS324. Row 2: SS446, SS460, SS392. Row 3: JC19328, SS186, SS768. Row 4: SS328, SS329, JC18983. (*Lower*) Row 1: JC19021, SS186. Row 2 shows enlargements of the regions in the dotted boxes of the pictures seen in the row above. The bars in the pictures indicate 10 μm.

COLLOQUIUM

Table 3. Number of cotransduction events of *priA2*∷*kan*, *btu*⁺ with *metB*⁺ into strains harboring mutations in either *ruvC*, *recG*, or *ftsK*

	Genotype of recipient			Met⁺ transductants			
Strain	*ruvC*	*recG*	*ftsK*	PriA⁺ TetR	PriA⁺ TetS	PriA⁻ TetR	PriA⁻ TetS
JC19250*	+	+	+	5/56	7/56	20/56	24/56
SS725	*64*	+	+	11/24	13/24	0/24	0/24
SS493	+	*258*	+	18/34	16/34	0/34	0/34
SS805	+	+	*1*	19/30	11/30	0/30	0/30

*The data from this control strain is taken from ref. 9 for the convenience of the reader.

that this was not possible and that *ftsK1*∷*cat* and *priA2*∷*kan* are synthetically lethal.

It is concluded that the absence of *dif*/*xerCD*-mediated resolution of dimers does not alter significantly the number or appearance of the Par⁻ filamentous *priA* cells. The *ftsK* gene product, however, is essential for the viability under these conditions. Roles of *ftsK* in cell division, segregation of chromosomes (other than XerCD/*dif*-mediated), and/or gene regulation may be responsible for the observed synthetic lethality between *ftsK1*∷*cat* and *priA2*∷*kan*.

Synthetic Lethality Between *priA* and *ftsK*, *recG*, and *ruvC* Is Rescued by *dnaC809*. The observations presented here that mutations in *ftsK*, *recG*, and *ruvC* are synthetically lethal with *priA2*∷*kan* mutations represent the third report of mutations that are synthetically lethal with *priA*. Two previous reports showed that *rep* and *priC* mutations also were synthetically lethal with a *priA* mutation (9, 33). The synthetic lethality between mutations in those two cases (*rep*/*priC* with *priA*) could not be rescued by *dnaC809*. This is noteworthy because in all other cases tested, *dnaC809* suppresses all other known *priA*-mutant phenotypes (9, 13). For this reason, it was hypothesized that *rep* and *priC* were required for *dnaC809* suppression of *priA2*∷*kan* and for the PriA-independent pathway (Fig. 1) (9). To test whether *ruvC*, *recG*, and *ftsK* were also part of this pathway, *dnaC809* was first combined with each of these mutations and then we attempted to introduce *priA2*∷*kan* into these double mutants. The ability to construct all three triple mutants is shown in Table 1 (see strains SS822, SS735, and SS712). Thus, in all three cases, *dnaC809* could rescue the synthetic lethality seen between *priA* and *ftsK*, *recG* and *ruvC* mutations. It is therefore concluded that although *recG*, *ruvC*, and *ftsK* are essential in a *priA* mutant, they are different from *priC* and *rep* and are not needed for the PriA-independent pathway.

Discussion

In this paper the appearance of log phase 4′,6-diamidino-2-phenylindole-stained *priA2*∷*kan* mutant cells were visualized by DIC and fluorescence microscopy. Two populations of cells were seen: 84% of the cells appeared like wild type whereas 16% were Par⁻ filaments. Mutations that affect cell division (*sulA*, *ftsK*), SOS regulation (*lexA*, *recA*), recombination (*recA*, *recB*, *recO*), the ability to resolve dimer chromosomes (*dif*, *ftsK*), and the ability to resolve and/or branch migrate Holliday junctions (*ruvC* and *recG*) were used to investigate how the two populations are produced. To explain these results, we formulated a model (see Fig. 3 and below for specifics) based on the consequences of forming XO products during the recombinational repair and the restart of forks in the absence of PriA. Aspects of SOS regulation and cell division (see below) also are invoked to explain the extent of filamentation seen in some of the double mutants.

The Model. Several lines of evidence lead to the hypothesis that recombinational repair of replication forks results in either a XO or non-XO (reviewed in ref. 1). It is presumed that depending on the reason for the fork stoppage, the cell will use appropriate recombination functions to repair the fork. Many models call for production and resolution of a Holliday junction that leads to either the XO or non-XO structure. When replication has been completed, the XO will produce a dimeric chromosome and non-XO will produce two monomers. If a XO has occurred and a chromosome dimer is formed, this must be resolved to monomers before cell division can take place. Resolution of the dimer depends on at least three factors: *dif*, XerCD, and FtsK (7, 28, 30).

Our working model hypothesizes that the formation of partitioning-defective filaments in a *priA* mutant is a consequence of the inability to restart DNA replication at XOs produced by recombinational repair of replication forks (Fig. 3). This idea was adapted from models proposed for *dif*-mediated recombination (i.e. refs. 21 and 23). Several assumptions were made to more fully consider the role of replication restart in a *priA* mutant cell. The first assumption is that commitment to Holliday junction resolution in a particular orientation and/or resolution itself occurs before restart of the replication forks by the PriA-independent pathway. This is necessary because of the second assumption: the PriA-independent pathway can only restart replication forks when repair has or will lead to the non-XO state. Presumably *priA*⁺ cells do not have this limitation because restart and resolution of the dimers occurs without incident. The third assumption is that the Par⁻ filaments lead to inviability. Fourth, all rounds of chromosome replication experience at least one event where repair/restart of the fork is necessary.

Other models also may be possible. For instance, in a *priA* mutant, failure to correctly restart any repaired fork, regardless of XO formation, could lead to the Par⁻ filaments. This model, however, does not explain the effects of the *rec* mutants on the Par⁻ filaments or the synthetic lethality of *priA* and *recG*/*ruvC* mutations.

Comparisons with Existing *dif*/*xerCD* Data. Although the percentage of Par⁻ filaments seen in *priA* mutants is very similar to the percentage of XerCD-mediated recombination at *dif* seen in a wild-type strain (6, 21), this could be purely coincidental. The observation that *recA* mutant cells show hardly any XerCD-mediated recombination at *dif* sites (6) and that the *priA recA* double mutants show very little of the Par⁻ nucleoids suggests, however, that this comparison may not be purely coincidental. This becomes less general, however, when the *recB* data are reviewed. *RecB* mutants show only a 50% decrease in *dif*-mediated recombination in *priA*⁺ cells whereas they show almost no Par⁻ nucleoids in a *priA* mutant. It is possible that the differences observed between the sets of experiments are due to the types of media used (rich vs. minimal) or the pleiotropic effects of having a *priA* mutation (high basal levels of SOS expression in the *recB* mutant and not the *recA* mutant). No measurement for XerCD-mediated recombination event at *dif* sites has been published for a *recO* mutant.

Can Bias in Holliday Junction Resolution Explain the Data? It is noteworthy that the *priA recB* mutant showed essentially no Par⁻
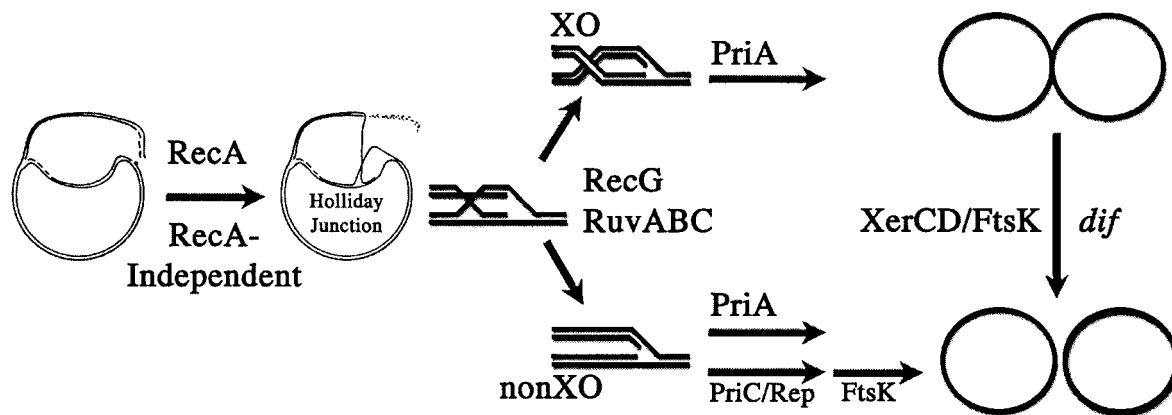
McCool and Sandler

Fig. 3. A model for the reactivation of collapsed replication forks emphasizing a differentiation in the usage of replication restart pathways specified by Holliday junction resolution products. The flow of the diagram is from left to right. A broken chromosome is depicted. This is converted by RecA-dependent pathway to a Holliday junction. In the absence of RecA, RecA-independent pathways become available. See text for explanation. Other proteins such as RecBCD and RecFOR also may aid in these reactions. Resolution of the Holliday junction to either a XO or non-XOs is catalyzed by RecG and RuvABC. If available, PriA can restart replication forks from both resolution products. The PriA-independent pathway (PriC and Rep), however, can only restart from non-XO products. *dif*-mediated *xerCD* site-specific recombination can resolve chromosome dimers formed by XOs to monomeric circles. FtsK is important for partitioning chromosome dimers resolved at *dif*. It also has an additional role in the viability of *priA2*::*kan* strains. This role could be in either chromosome segregation or cell division.

nucleoids (even though there was still 3% filamentation). This suggests that the processing of Holliday junctions in this mutant was almost entirely to the non-XO state. Because *recB* is mutated, it is plausible that the presynaptic recombination functions used to repair these forks are accomplished by Rec-FOR (4). This suggests that repair by RecFOR (in the absence of RecBCD) favors non-XO. This agrees with Cromie and Leach (23) who observed that repair of a gap produced by DNA replication of a palindrome (a RecFOR substrate) leads to non-XOs.

In the above discussion, it is inferred that the key enzyme complex to catalyze the production of a XO or non-XO is RuvABC. It has been shown *in vitro* that the way in which RuvAB binds to a Holliday junction dictates how RuvC will resolve the junction (34). Because Holliday junctions are symmetric, one can reasonably ask how the asymmetry is initiated so that resolution can be bias in a particular situation. One idea is that the replication fork itself confers the asymmetry for loading of RuvAB. This could be determined by either the DNA directly [structure of the DNA at the fork or combination of new and parental strand (methylation)] or the presence of replication proteins or both. Depending on the type of damage, a different set of proteins or DNA structures could exist to dictate RuvAB loading.

A New Relationship Between RuvC and RecG? The synthetically lethal combinations of *ruvC priA* and *recG priA* mutations could be a consequence of that both of these genes must conspire to help resolve Holliday junctions to the non-XO state when *priA* is not available. Alternately, it is possible, that in a *priA* mutant, it is the balance of RuvABC and RecG activities that is important for viability. Currently we favor the former explanation, but we have no reason to reject the latter. If in a *priA* mutant, *ruvC* and *recG* do conspire to yield viability, then this would indicate a different relationship between *ruvC* and *recG* than has been seen for *Hfr*-mediated recombination. In this process, these mutants show additive effects (35). One reason a different relationship between *ruvC* and *recG* may exist could be that the DNA substrates produced by conjugation are different from those generated during repair of replication forks.

McGlynn and Lloyd (36) reported construction of a Δ*ruvAC65 priA2*::*kan* strain and referred to unpublished data of a *recG priA*

double mutant. One possible explanation for why McGlynn and Lloyd may be able to construct the Δ*ruvAC65 priA2*::*kan* double mutant and we could not construct a *ruvC64*::*kan priA2*::*kan* strain is that the Δ*ruvAC65* mutant is more viable. It has been shown that in a *recBC sbcBC* background, a *ruvA60 ruvC64*::*kan* double mutant has a 15-fold greater viability than the *ruvC64*::*kan* single mutant (37). Another possibility is that Δ*ruvAC65* deletes *ruvA*, *yebB* (a gene of unknown function), and *ruvC* (38) whereas *ruvC64*::*kan* affects only *ruvC*. It is possible that in a *priA* mutant either the absence of yebB allows viability or RuvAB without RuvC causes inviability. Because McGlynn and Lloyd give no details as to the genotype, construction, or characterization of their *recG priA* double mutant, we are unable to comment on the differences between their results and ours.

***DnaC809* Provides a Test for Different Types of Synthetic Lethality that Mutations May Have with *priA2*::*kan*.** From the accumulated data presented in this paper and others (9, 33), it is clear that mutations in many genes are synthetically lethal with *priA2*::*kan*. This is most easily understood in that these genes do something essential in a *priA* mutant. One, however, can distinguish these synthetic lethalities into at least two groups by an experimental criterion: whether or not the synthetic lethality is suppressed by *dnaC809*. Combinations of mutations in *priA* and *recG*, *ruvC*, *ftsK*, or *recF* (39) are suppressed with *dnaC809*. *PriA* with *priC* or *rep* mutations are not. One therefore can hypothesize that *priC* and *rep* have an essential role in the *dnaC809* suppression of *priA2*::*kan* where the other genes do not.

Repair of Replication Forks in a *recA* Mutant. Currently, the major proposed mechanisms for repair of replication forks necessitate the process of healing the forks using homologous recombination. If so, then how can *recA* mutants survive? Most models also hypothesize that formation and resolution of the Holliday junction is essential for the healing process. The question then becomes how can one make Holliday junctions in a *recA* mutant? At least three *recA*-independent pathways for Holliday junction formation at a replication fork can be hypothesized. All three are presumably very inefficient and only occur to an appreciable degree in a *recA* mutant cell. In any given *recA* mutant cell, any combination of these could occur. The first is replication fork reversal (33). This is where the replication fork "backpedals," the

two newly synthesized strands are allowed to hybridize, and this in turn forms a Holliday junction. Second, *in vivo* overproduction of RecO protein can indirectly suppress the UV sensitivity of a *recA* mutant and *in vitro* RecO protein can catalyze D-loop formation (40). If RecO activity is concentrated in a *recA* mutant and if the two arms of a broken fork are in close proximity, then RecO could catalyze the strand invasion that could lead to a Holliday junction. Such a mechanism is compatible with a factory model for DNA replication (41). Lastly, Cao and Kogoma (42) showed that strains defective in *recA lexA polA* have significantly higher levels of UV resistance and recombination proficiency than a *recA* single mutant and this depended on *recF*.

Unusual Effects on Cell Division. Analysis of *priA* and *recA* mutant cells is difficult because these mutations are pleiotrophic, affecting replication restart, recombination, and regulation of SOS- or LexA-regulated genes. The LexA regulon comprises at least 31 known genes involved in DNA repair, mutagenesis, and cell division through the SOS response (43). The absence of each of these genes affects SOS expression in important ways. In *priA* mutants, SOS levels are increased (18); whereas in the absence of *recA*, SOS expression cannot be induced and remain at basal levels (44). Thus to explain the combined effects of the two mutants together one has to consider not only the absence of recombination and poorer replication restart in a cell, but also changes in SOS expression. It is interesting that *sulA* is SOS-regulated and induced by *priA2::kan*. In the *recA priA* double mutant, SOS induction should not occur and yet levels and sizes of the filaments actually increase. Therefore we propose that the reason filaments are more abundant and longer in a culture of

the *priA recA* strain is that FtsK, a critical cell division protein that is induced by the SOS response, may be rate limiting (31, 45). Consistent with this proposal is that the appearance of these *priA recA* mutant cells are similar to the appearance of *ftsK* mutant cells at the nonpermissive temperature (26). Other explanations also may be possible.

Other Implications of the Data. In this work it is postulated that the PriA-independent pathway can only restart replication forks at structures where the repair process has made non-XO. One question that arises from this way of thinking is what is the difference in structure (or what the structures are) at XOs and non-XOs that limits the PriA-independent pathway to function at the latter? A related question is what is the mechanism of PriA function that allows it to work at both structures? These remain important issues to address. Another issue raised is whether the 8- to 10-fold higher basal SOS expression in culture of *priA* mutant cells is due to all of the cells in culture equally having 8- to 10-fold higher levels of expression, or whether only a portion of the cells, in this case the Par$^-$ filaments, are fully induced for SOS expression and the remaining 84% are not induced at all. Lastly, this work suggests that the reason *priA* mutants are Rec$^-$ is that these cells cannot restart replication forks at XOs needed to form recombinants.

1. Cox, M. M., Goodman, M. F., Kreuzer, K. N., Sherratt, D. J., Sandler, S. J. & Marians, K. J. (2000) *Nature (London)* **404,** 37–41.
2. Kowalczykowski, S. C. (2000) *Trends Biochem. Sci.* **25,** 156–165.
3. Marians, K. J. (2000) *Trends Biochem. Sci.* **25,** 185–189.
4. Clark, A. J. & Sandler, S. J. (1994) *Crit. Rev. Microbiol.* **20,** 125–142.
5. Stahl, F. W. (1994) *Genetics* **138,** 241–246.
6. Steiner, W. W. & Kuempel, P. L. (1998) *J. Bacteriol.* **180,** 6269–6275.
7. Steiner, W., Liu, G., Donachie, W. D. & Kuempel, P. (1999) *Mol. Microbiol.* **31,** 579–583.
8. Sandler, S. J. & Marians, K. J. (2000) *J. Bacteriol.* **182,** 9–13.
9. Sandler, S. J. (2000) *Genetics* **155,** 487–497.
10. Bramhill, D. & Kornberg, A. (1988) *Cell* **52,** 743–755.
11. Liu, J. & Marians, K. J. (1999) *J. Biol. Chem.* **274,** 25033–25041.
12. McGlynn, P., Al-Deib, A., Liu, J., Marians, K. & Lloyd, R. (1997) *J. Mol. Biol.* **270,** 212–221.
13. Sandler, S. J., Samra, H. S. & Clark, A. J. (1996) *Genetics* **143,** 5–13.
14. Liu, J., Xu, L., Sandler, S. J. & Marians, K. J. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 3552–3555.
15. Asai, T. & Kogoma, T. (1994) *J. Bacteriol.* **176,** 1807–1812.
16. Willetts, N. S., Clark, A. J. & Low, B. (1969) *J. Bacteriol.* **97,** 244–249.
17. Lee, E. H. & Kornberg, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3029–3032.
18. Nurse, P., Zavitz, K. H. & Marians, K. J. (1991) *J. Bacteriol.* **173,** 6686–6693.
19. Bi, E. & Lutkenhaus, J. (1990) *J. Bacteriol.* **172,** 5602–5609.
20. Huisman, O., D'Ari, R. & George, J. (1980) *J. Bacteriol.* **144,** 185–191.
21. Steiner, W. W. & Kuempel, P. L. (1998) *Mol. Microbiol.* **27,** 257–268.
22. Michel, B., Recchia, G. D., Penel-Colin, M., Ehrlich, S. D. & Sherratt, D. J. (2000) *Mol. Microbiol.* **37,** 180–191.
23. Cromie, G. A. & Leach, D. R. F. (2000) *Mol. Cell* **6,** 815–826.
24. Lloyd, R. G. & Low, K. B. (1996) in *Escherichia coli and Salmonella*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington, DC), Vol. 2, pp. 2236–2255.
25. Kuempel, P. L., Henson, J. M., Dircks, L., Tecklenburg, M. & Lim, D. F. (1991) *New Biol.* **3,** 799–811.
26. Begg, K. J., Dewar, S. J. & Donachie, W. D. (1995) *J. Bacteriol.* **177,** 6211–6222.
27. Yu, X. C., Weihe, E. K. & Margolin, W. (1998) *J. Bacteriol.* **180,** 6424–6428.
28. Recchia, G. D., Aroyo, M., Wolf, D., Blakely, G. & Sherratt, D. J. (1999) *EMBO J.* **18,** 5724–5734.
29. Boyle, D. S., Grant, D., Draper, G. C. & Donachie, W. D. (2000) *J. Bacteriol.* **182,** 4124–4127.
30. Liu, G., Draper, G. C. & Donachie, W. D. (1998) *Mol. Microbiol.* **29,** 893–903.
31. Diez, A., Gustavsson, N. & Nystrom, T. (2000) *Mol. Microbiol.* **36,** 1494–1503.
32. Diez, A. A., Farewell, A., Nannmark, U. & Nystrom, T. (1997) *J. Bacteriol.* **179,** 5878–5883.
33. Seigneur, M., Bidnenko, V., Ehrlich, S. D. & Michel, B. (1998) *Cell* **95,** 419–430.
34. van Gool, A. J., Hajibagheri, N. M., Stasiak, A. & West, S. C. (1999) *Genes Dev.* **13,** 1861–1870.
35. Lloyd, R. G. (1991) *J. Bacteriol.* **173,** 5414–5418.
36. McGlynn, P. & Lloyd, R. G. (2000) *Cell* **101,** 35–45.
37. Mandal, T. N., Mahdi, A. A., Sharples, G. J. & Lloyd, R. G. (1993) *J. Bacteriol.* **175,** 4325–4334.
38. Rudd, K. E. (1998) *Microbiol. Mol. Biol. Rev.* **62,** 985–1019.
39. Sandler, S. J. (1996) *Mol. Microbiol.* **19,** 871–880.
40. Luisi-DeLuca, C. (1995) *J. Bacteriol.* **177,** 566–572.
41. Lemon, K. P. & Grossman, A. D. (1998) *Science* **282,** 1516–1519.
42. Cao, Y. & Kogoma, T. (1995) *Genetics* **139,** 1483–1494.
43. Fernandez De Henestrosa, A. R., Ogi, T., Aoyagi, S., Chafin, D., Hayes, J. J., Ohmori, H. & Woodgate, R. (2000) *Mol. Microbiol.* **35,** 1560–1572.
44. Walker, G. (1996) in *Escherichia coli and Salmonella*, ed. Neidhardt, F. C. (Am. Soc. Microbiol., Washington, DC), Vol. 1, pp. 1400–1416.
45. Wang, L. & Lutkenhaus, J. (1998) *Mol. Microbiol.* **29,** 731–740.
46. Singer, M., Baker, T. A., Schnitzler, G., Deischel, S. M., Goel, M., Dove, W., Jaacks, K. J., Grossman, A. D., Erickson, J. W. & Gross, C. A. (1989) *Microbiol. Rev.* **53,** 1–24.
47. Csonka, L. N. & Clark, A. J. (1979) *Genetics* **93,** 321–343.
48. Sandler, S. J. & Clark, A. J. (1994) *J. Bacteriol.* **176,** 3661–3672.
49. Zieg, J. & Kushner, S. R. (1977) *J. Bacteriol.* **131,** 123–132.
50. Murphy, K. C., Campellone, K. G. & Poteete, A. R. (2000) *Gene* **246,** 321–330.