Multiple pathways process stalled replication forks

Bénédicte Michel*, Gianfranco Grompone†, Maria-Jose Florès‡, and Vladimir Bidnenko

Laboratoire de Génétique Microbienne, Institut National de la Recherche Agronomique, 78352 Jouy en Josas Cedex, France

Edited by Nicholas R. Cozzarelli, University of California, Berkeley, CA, and approved June 22, 2004 (received for review March 30, 2004)

Impairment of replication fork progression is a serious threat to living organisms and a potential source of genome instability. Studies in prokaryotes have provided evidence that inactivated replication forks can restart by the reassembly of the replication machinery. Several strategies for the processing of inactivated replication forks before replisome reassembly have been described. Most of these require the action of recombination proteins, with different proteins being implicated, depending on the cause of fork arrest. The action of recombination proteins at blocked forks is not necessarily accompanied by a strand-exchange reaction and may prevent rather than repair fork breakage. These various restart pathways may reflect different structures at stalled forks. We review here the different strategies of fork processing elicited by different kinds of replication impairments in prokaryotes and the variety of roles played by recombination proteins in these processes.

Work from several laboratories has established that in bacteria, a recombination event can lead to the establishment of a unidirectional replication fork (reviewed in refs. 1–3). These observations extend the concept of a direct recombination–replication connection, originally proposed ≈ 30 years ago from studies of bacteriophage T4 and λ replication. Furthermore, the existence of recombination-dependent replication in yeast suggests that this connection may be a widely distributed phenomenon (4, 5).

Considering the tight control of replication initiation at chromosomal origins, the assembly of a complete replisome at recombination intermediates, independently of time and place, is paradoxical. One of the *raison d'être* of this potentially dangerous process was revealed by the finding that recombination intermediates form at inactivated replication forks and are used for replication restart. However, studies of the replication–recombination connection in *Escherichia coli* have indicated that recombination proteins do not necessarily catalyze strand exchange at blocked forks and rather act in a variety of reactions that depend on the origin of the arrest. We review here the diversity of fates of inactivated replication forks in bacteria, as well as our present knowledge of the roles played by recombination proteins during replication restart.

DNA Double-Strand End Repair in Bacteria

Almost 30 years ago, Higgins *et al.* (6) proposed that blocked replication forks could be isomerized into a four-way Holliday junction (HJ) with a DNA double-strand end, which could permit DNA repair and then continuation of replication (Fig. 1, step A). The test of the model, performed by treatment of mammalian cells with a DNA-damaging agent, was inconclusive (7). However, more recent studies in *E. coli* suggest that such replication fork reversal plays a crucial role in replication restart in bacteria (8, 9).

A key aspect of the replication fork reversal model is that it generates a DNA double-strand end at blocked forks without chromosome breakage. In *E. coli*, the enzyme that acts at DNA double-strand ends is a heterotrimer, RecBCD. RecBCD is a helicase that unwinds the linear double-stranded DNA and an exonuclease, ExoV, that simultaneously degrades it (reviewed in ref. 10) (Fig. 2). Upon encounter with a specific octameric sequence, named *chi*, the degradation activity of RecBCD is modified, and the enzyme acquires the ability to load the

homologous recombination protein RecA onto DNA (11–13). RecA forms a nucleoprotein filament that invades a homologous DNA molecule, and the strand-exchange reaction results in a four-arm junction named the HJ. Two helicases, RuvAB and RecG, are able to catalyze branch migration of the HJ (ref. 14; reviewed in ref. 15). RuvAB associates with a third polypeptide RuvC to resolve the HJ and form a recombinant molecule. HJ resolution in cells that lack RuvABC requires RecG helicase, but the resolution mechanism is unknown.

A direct link between replication and recombination was discovered when it appeared that DNA double-strand break repair results in a viable chromosome only when a replication fork is reassembled from a recombination intermediate (Fig. 2, last step, and refs. 16 and 17). The key enzyme for replication restart is PriA, which, in combination with other proteins, promotes the loading of the replicative helicase DnaB and in turn the assembly of a functional replisome (2). PriA allows replication to initiate from a recombination intermediate, possibly because, as described below, recombination intermediates can be generated from replication forks.

Replication Mutants and Replication Fork Reversal

To study the consequences of replication fork inactivation, our laboratory has used mutations that impair replication proteins. A mutation in the gene encoding the replicative helicase DnaB, a deletion of the *rep* gene (inactivating the Rep helicase thought to facilitate progression of replication forks), and mutations in genes encoding subunits of the *E. coli* replicative polymerase [(polymerase III holoenzyme (Pol III HE))] were used. They all render RecBC proteins essential for viability and cause chromosome linearization in a *recBC* mutant background (8, 18, 19). This observation indicated the formation of a DNA double-strand end at blocked forks. However, although RecBCD and RecA act in concert to promote double-strand break repair, *rep* and Pol III HE mutants do not require RecA for viability, leading us to propose the replication fork reversal model (Fig. 1; reviewed in ref. 9). According to this model, the substrate for RecBCD results from the annealing of the newly synthesized strands rather than being produced at blocked forks by chromosome breakage. The double-strand end made by fork reversal is blunted by one of the numerous *E. coli* single-strand exonucleases allowing RecBCD binding. In *RecA*⁺ cells, this DNA double-strand end is recombined into the chromosome when RecBCD encounters a *chi* site (Fig. 1, steps B and C, and ref. 18). *Chi* sites are not randomly distributed on the *E. coli* genome and are biased so that they are overrepresented on the DNA double-strand end formed by fork reversal (one *chi* site every 5 kb, on average), stimulating homologous recombination at reversed forks (9, 20). In *recA* mutant cells, RecBCD-mediated

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: HJ, Holliday junction; ssDNA, single-stranded DNA; SSB, ssDNA-binding protein; Pol III HE, polymerase III holoenzyme.

*To whom correspondence should be addressed. E-mail: bmichel@jouy.inra.fr.

†Present address: Unité de Pathogénie Microbienne Moléculaire Institut Pasteur, 25-28 Rue du Dr. Roux, 75015 Paris, France.

‡Present address: Unité d'Ecologie et de Physiologie du Système Digestif, Institut National de la Recherche Agronomique, 78352 Jouy en Josas Cedex, France.

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Fig. 1. Replication fork reversal model (adapted from ref. 9). In the first step (A), the replication fork is arrested, causing fork reversal. The reversed fork forms an HJ (two alternative representations of this structure are shown, open X and parallel stacked X). In *Rec⁺* cells (B and C), RecBCD initiates RecA-dependent homologous recombination at a *chi* site present on the DNA double-strand end, and the two HJ (one formed by reversal, one by homologous recombination) are resolved by RuvABC. Alternatively, if RecBCD encounters the HJ before encounter with *chi* or in the absence of RecA (B–D), the DNA double-strand end is degraded up to the HJ, restoring a fork structure. In both cases, replication restarts by a PriA-dependent process. In the absence of RecBCD (E), resolution of the HJ by RuvABC causes chromosome linearization. Continuous lines, parental chromosome; dashed lines, newly synthesized strands; green circle, RuvAB; pink incised circle, RecBCD.

DNA degradation progresses up to the HJ formed by fork reversal, generating a fork structure from which replication can restart (Fig. 1, step D; the same reaction can occur in *RecA⁺* cells if RecBCD encounters the HJ before a *chi* site). Because PriA recognizes both D loops formed by homologous recombination and fork structures (2), it promotes the reassembly of a functional replisome regardless of whether the DNA double-strand end is recombined into its homologous sister sequence or degraded (21). In the absence of RecBC, resolution of the HJ by RuvABC causes chromosome linearization (Fig. 1, step E), whereas in *RecBC⁺* cells, RecBC action on the double-strand DNA end before HJ resolution prevents chromosome arm linearization.

The DNA double-strand end formed by fork reversal needs to be processed. In *E. coli*, this processing does not necessarily occur by homologous recombination, because RecBCD can completely degrade this DNA end (Fig. 1, step D). In eukaryotes, it is likely to occur by either homologous recombination or non-homologous end-joining, because double-stranded DNA ends are not extensively degraded (reviewed in refs. 22 and 23). After fork reversal, enzymes that act at DNA double-strand ends will be targeted to blocked replication forks without chromosome breakage.

Although the processing of reversed forks by recombination proteins is well understood, the molecular mechanism of the initial step of reversed fork formation, the conversion of a

Fig. 2. Recombination at a DNA double-strand end in bacteria. RecBCD binds to a DNA double-strand end and degrades both strands until it reaches a *chi* site. RecBCD loads RecA when it encounters *chi* and the RecA filament invades an homologous intact molecule. The HJ is resolved by RuvABC (or RecG). PriA-dependent primosome assembly promotes initiation of replication from the invading 3' end. Blue and red lines, two homologous DNA molecules; pink incised circle, RecBCD; small yellow circles, RecA.

three-arm fork structure into a four-arm HJ, remains unknown, except in the replicative helicase mutant *dnaBts* where RecA was shown to be involved.

RecA-Mediated Replication Fork Reversal in the *dnaBts* Mutant

Our studies of the *dnaBts* mutant indicated that in this mutant, reversed forks were not formed in the absence of RecA, suggesting that RecA may play a specific role at forks blocked by DnaB inactivation (24). This led us to propose that RecA binds to the single-stranded DNA (ssDNA) region on blocking the lagging strand, and that this RecA filament invades the homologous leading strand, thus forming a reversed fork (Fig. 3). A similar reaction was reconstituted *in vitro* with the use of an artificial fork structure in which strands had the opposite polarity to normal replication forks, as if the lagging-strand 5' end had progressed further than the blocked leading-strand 3' end (25). The inactivated forks that were not reversed in the *dnaBts recA* mutant were susceptible to DNA breakage (24).

In contrast to the *dnaBts* mutant, fork reversal occurred independently of RecA in *rep* and Pol III HE mutants. What is the molecular basis of this difference? RecA binding to ssDNA on the lagging strand template is likely to be prevented *in vivo* by ssDNA-binding protein (SSB). The SSB barrier is overcome during recombinational gap repair by RecFOR (26). Because RecA-dependent fork reversal in the *dnaBts* mutant did not require RecFOR, there has to be an alternative pathway for SSB removal (24). During replication restart, SSB is displaced from

Fig. 3. Model for RecA-dependent replication fork reversal (adapted from ref. 24). In the *dnaBts recB* strain, RuvABC-dependent chromosome linearization requires RecA. We propose that a RecA filament forms on the blocked lagging strand, which invades the homologous leading strand resulting in a reversed replication fork. The HJ is then bound by RuvAB and the double-strand end by RecBCD. The processing of the reversed fork is as in Fig. 1. Green circle, RuvAB; small yellow circles, RecA.

the lagging-strand template by PriA and its associated partners (the preprimosome proteins) in a reaction concerted with the loading of the DnaB helicase (2). We can speculate that in a *dnaBts* mutant, the preprimosome proteins displace SSB from the lagging-strand template without being able to replace it with a functional DnaB helicase and thereby provide SSB-free ssDNA, accessible to RecA. In other replication mutants, the coupling of SSB removal with DnaB loading may prevent RecA binding.

At present, the mechanism of replication fork reversal in *rep* and Pol III HE mutants is unknown. However, as an alternative model to RecA action, it was proposed that fork reversal could be driven by positive supercoiling.

Direct Replication Restart in Gyrase Mutant

In vitro studies of the fate of fork structures led to the observation of nonenzymatic fork reversal reaction. Incubation of fully circular plasmid molecules with a high concentration of intercalating agent renders them positively supercoiled. In contrast, partially replicated plasmids underwent a spontaneous reaction producing a HJ by fork reversal (the HJ was there called a “chicken foot”; refs. 27 and 28). This reaction suggested that the accumulation of positive supercoiling ahead of a replication fork could trigger a spontaneous replication fork reversal reaction. Because positive supercoils are removed in *E. coli* mainly by DNA gyrase, a type II topoisomerase, we tested this hypothesis with the use of gyrase point mutants in which impairment of gyrase activity was likely to provoke a stochastic accumulation of positive supercoils ahead of the progressing replication forks (29, 30). PriA was shown to be essential for the viability of the gyrase mutants, which implies that impairment of gyrase activity leads to replication arrest and, in turn, creates a need for restart (31). However, we found no indication of fork reversal, because RecBC was not required for cell viability in gyrase mutants, and there was no increase in the formation of linear chromosomes upon gyrase inactivation in a *recBC* mutant context. These results suggested that the replication forks arrested by positive supercoils could restart directly by a PriA-dependent pathway and were not converted into a HJ *in vivo*. The dissociation of the replisome from the replication fork allowed the free diffusion of positive supercoils across the fork and their conversion into precatenanes that would be removed later by Topoisomerase IV (Topo IV) (ref. 31; see refs. 32 and 33 for reviews on Topo IV and precatenanes). Studies of gyrase mutants thus indicated that if the replication block can freely diffuse upon disassembly of the

Fig. 4. Model for replication-dependent formation of linear DNA at Ter-blocked forks (adapted from ref. 34). Replication forks are arrested by ectopic *Ter* sites. Progression of a second round of replication forks up to the first blocked forks generates DNA double-strand ends. The linear DNA formed by overreplication will be repaired by homologous recombination (not shown). Black lines, parental chromosome; blue lines, first round of replication, blocked at *Ter*; red lines, strands of the second round of replication; O, chromosome origin; pink incised rectangle, *Ter* sites; yellow ovals, replisome.

replication proteins and if the replication proteins are still intact, the fork is simply and directly reused for restart.

Overreplication of Ter Blocked Forks

The range of reactions that was known to occur at blocked forks was extended by the observation that forks arrested by a physiological arrest site behave differently from forks arrested by the inactivation of a replication protein (34). In *E. coli*, forks are naturally arrested in the terminus region upon reaching *Ter* sites bound by termination protein Tus (reviewed in ref. 35). Blocking of forks halfway between the origin and the terminus by introducing two ectopic *Ter* sites did not cause replication fork reversal or chromosome breakage. In contrast, the *Ter*/Tus blocked forks were stable until they were reached by a subsequent round of replication, which converted the ends of the blocked leading and lagging strands into DNA double-strand ends (Fig. 4). Blockage of replication at ectopic *Ter* sites thus causes the formation of replication-dependent linear DNA that needs to be repaired, rendering the RecBC, RecA, RuvABC, and PriA proteins essential for viability. The formation and repair of DNA double-strand ends at *Ter* sites are thus akin to the formation and repair of the DNA end formed by encounter of a replication fork with a single-strand interruption, a reaction originally called “collapse” (36, 37). In a wild-type chromosome, such a reaction is unlikely to occur at natural *Ter* sites, because only a converging fork should approach a fork blocked at *Ter*-Tus in the terminus region of the chromosome. This study revealed the differences between the passive stability of naturally blocked forks, regardless of their position on the chromosome, and the processing of accidentally inactivated forks (Table 1).

Replication Fork Arrest by DNA Damage

The operation of different replication fork reversal pathways that act after impairment of replication underlines the importance of processing arrested forks on intact nondamaged DNA. However, DNA damage encountered during chromosomal replication can also disrupt the replication machinery. The consequences of DNA damage have been studied in bacteria for >40

Table 1. The outcome of replication fork blockage depends on the origin of arrest

Cause of replication arrest	Recombination proteins essential for restart*	Chromosome breakage	Model
Replisome mutation: Pol III HE, <i>dnaBts</i> , <i>rep</i> (8, 9, 18, 19)	RecBC	RuvABC-dependent linear DNA in <i>recBC</i> mutant only	Replication fork reversal (Figs. 1 and 3)
Supercoiling mutant <i>gyrase</i> (31)	None	None	Direct restart from stalled forks
Ectopic Ter sites (34)	RecBC, RecA, and RuvABC	Replication-dependent linear DNA	Overreplication (Fig. 4)
UV irradiation (38–53)	RecFOR and RecA	Replication-dependent linear DNA	Several models

*In replication mutants or strains with ectopic Ter sites, because replication blockage is the only damage, the recombination proteins essential for viability are presumably essential for restart. In UV-irradiated cells, several types of damage occur, and all recombination proteins are required for full viability. To date, only RecFOR and RecA have been shown to be essential for replication restart.

years, mainly with the use of UV irradiation. It was observed that DNA synthesis was transiently halted after UV irradiation, and that RecA was essential for the recovery of DNA replication, a process originally called “induced replisome reactivation” (refs. 38 and 39; reviewed in ref. 40). As in other RecA-dependent reactions, a presynaptic activity was later shown to be required for induced replisome reactivation, provided by RecFOR, which promote RecA binding to SSB-bound gapped DNA (41–43). In contrast, RecBCD, the presynaptic complex that acts at DNA double-strand ends, was not required (refs. 39 and 44 and Table 1).

Understanding the processing of replication forks blocked by UV lesions is complicated because of the multiple potential roles played by RecFOR and RecA, the enzymes required for the recovery of replication after irradiation. These enzymes are essential (i) for the recombinational repair of gaps formed in the chromosomes of UV irradiated cells (45–47); (ii) for the induction of the SOS response after UV irradiation, with SOS induction being an essential component of induced replisome reactivation (39, 48); and (iii) for lesion bypass by the mutagenic polymerase UmuCD (Pol V; refs. 49, 50). Recent studies have suggested that RecFOR and RecA are needed for replication recovery for reasons other than gap repair or SOS induction. First, the recent report that efficient replisome reactivation occurs in UV-irradiated cells that lack RuvABC or RecG or both supports the idea that gap repair is not a prerequisite for induced replisome reactivation (51), as recombinational gap repair is likely to require the enzymes involved in HJ resolution, RuvABC, or RecG (reviewed in ref. 10). Second, RecFOR were shown to be still needed in cells that constitutively expressed the SOS response, indicating that SOS induction was not the only reason for the RecFOR requirement during recovery of DNA replication (42).

Taken together, the published data support a model in which the binding of RecA directly to UV-blocked forks is needed for replication restart, a process that requires the assistance of RecFOR for displacing SSB (Fig. 5A; adapted with modification from ref. 44). In an alternative model, because UV irradiation induces one-strand lesions that theoretically block only one DNA polymerase, it has been proposed that a lesion on the leading strand template does not preclude progression of the lagging strand polymerase, resulting in a gap on the leading strand (refs. 52 and 53 and Fig. 5B, first step). In the template strand switching model, it was proposed that reversal of such a lagging strand protruding fork allows leading strand extension using the intact newly synthesized lagging strand as a template (ref. 6 and Fig. 5B). The sequence carrying the lesion is thus rendered double stranded, which allows repair (see Fig. 5B). It should be noted that the template strand-switching model implies the protection of the linear DNA end from the action of nucleases, while it remains accessible to a DNA polymerase. This model recently

received increased attention with the observation that *in vitro* the *E. coli* RecG helicase could catalyze both the reversal and the resetting of a fork with a 5' protruding end, the first and last steps of the template strand-switching model (54–56). However, genetic support for a RecG-catalyzed strand switching reaction *in vivo* was only indirect (survival after UV irradiation), and direct measurements of DNA synthesis show that RecG is not required for replication restart in exponentially growing *E. coli* cells after UV irradiation (51). A model in which linearization of UV-induced reversed forks by RuvABC would be followed by RecBCD–RecA-mediated recombinational repair has also been proposed (55, 57). Such a model appears to contradict published observations that replication restart in UV-treated cells is unaffected by inactivation of either RecBC or RuvABC or both RuvABC and RecG (39, 41, 51). How replication fork reversal is compatible with the known requirement for replication restart at UV-blocked forks is unclear.

As summarized in Table 1, the recombination proteins required for the restart of inactivated replication forks after UV irradiation (RecFOR and RecA) are different from those required in replication mutants (RecBC), in a gyrase mutant (which requires no recombination function), or at *Ter*/Tus blocked forks (which requires RecBCD, RecA, and RuvABC-mediated double-strand end repair). The only proteins that are required for all these replication restart reactions are PriA and its partners, indicating that in all cases a replisome needs to be reassembled at inactivated forks by a mechanism that can be distinguished from initiation by DnaA at origins.

Spontaneous Replication Fork Arrest: The *priA* Mutant

Because the cause of fork arrest directs how a fork will be reactivated, we reasoned that the origin of spontaneous replication arrest in bacteria could be inferred from the study of chromosomes when spontaneously arrested forks persist. Spontaneously arrested forks presumably persist in a *priA* null mutant, which is defective for replication restart (58). We observed RuvABC-dependent linearization of chromosomes in a *priA* null mutant in the absence of RecBC, but not in its presence, indicating that the spontaneously arrested replication forks undergo replication fork reversal when replication restart is impaired (59). As in the *rep* and Pol III HE mutants, replication fork reversal in a *priA* mutant was RecA-independent. However, in contrast with other replication mutants, preventing the processing of reversed forks by *recBC* inactivation did not further decrease the low viability of the *priA* mutant, presumably because replication restart is inefficient in the absence of PriA, regardless of the fork processing pathway (reviewed in refs. 2 and 58). The observation that the *priA* mutant undergoes replication fork reversal led us to conclude that the types of replication inactivation that cause replication fork reversal, such as a

Fig. 5. Possible models for replication restart after UV irradiation. When a UV lesion forms downstream of a replication fork on the leading strand template, it may either block the entire replisome (A) or arrest the leading strand, whereas the lagging strand synthesis progresses further (B). In model A (adapted with modifications from ref. 44), RecFOR and RecA bind to the lagging strand template and promote the invasion of the leading strand, which renders the lesion double stranded and thus accessible to nucleotide excision repair (NER). We propose that the displaced leading strand end is degraded by a 3' exonuclease (such as Exo I, Exo VII, or Exo X). In the absence of a functional NER system, or at very high UV doses saturating NER, bypass polymerases would be needed to incorporate a nucleotide opposite the lesion site. In model B (adapted with modifications from ref. 6), the lagging strand progresses further than the leading strand. In contrast with model A, both lagging and leading strand ends need to be displaced to render the lesion double-stranded, hence accessible to NER, resulting in a reversed fork. The lesion could be repaired either before or after (drawn here) fork resetting. The question marks indicate that, considering the lack of requirement for RecBC, RuvABC, and/or RecG for replication restart after UV irradiation, how the reversed fork would be formed and reset is presently unknown. Continuous lines, parental chromosome; dashed lines, newly synthesized strands; small yellow circles, RecA; pink rectangle, RecFOR; black triangle, DNA lesion.

double-stranded DNA roadblock or a replication protein defect, occur spontaneously.

Replication Impairment by Nucleotide Depletion

Drugs that inhibit ribonucleotide reductase, such as hydroxyurea (HU), impair replication by decreasing the nucleotide pool. In bacteria, HU induces a need for all enzymes of the RecBCD pathway of homologous recombination, RecBC, RecA, RuvABC, and RecG, suggesting the formation of DNA double-strand ends upon HU treatment (60). The need for RecBC and RecA in HU-treated cells was suppressed by the inactivation of RadA, a protein of unknown function, consistent with RadA being able to convert damaged or stalled forks into double-strand breaks, which *recA* and *recB* mutants are unable to process (60).

Conclusion

To date, we know that in bacteria, inactivated replication forks can be reversed or directly restarted (Table 1). However, it is still

unclear what decides whether a fork will be reversed, what enzymatic activities might be involved in reversal, and whether reversed forks might be sometimes simply converted back to forks, in contrast with replication mutants in which RecBC is essential for viability. Of the three different mechanisms of fork reversal that have been observed *in vitro* (RecA-mediated strand annealing, positive supercoiling constraint, and helicase unwinding), one appears to take place *in vivo* (RecA-dependent fork reversal in the *dnaBts* mutant), and one is controversial (RecG-catalyzed fork reversal in UV-irradiated cells). Nevertheless, our understanding of the ways in which stalled forks can be processed in the "model" situations described here may help us comprehend how other processes that impair normal replication lead to eventual faithful genome replication. For example, the observation of low viability in cells that massively overproduce DnaA and consequently overinitiate *E. coli* chromosome replication, a situation aggravated by RecA or RecBC inactivation, suggested the "collapse" of secondarily initiated forks when they run into the preceding forks (61). In less dramatic conditions of DnaA overproduction, which did not cause a decrease in cell viability, overinitiated replication forks stopped preferentially in a defined origin domain of the chromosome. A requirement for RecBC and not for RecA for viability was observed, suggesting the occurrence of replication fork reversal at the point of arrest (62). Another example of replication fork reversal in bacteria was observed in *Salmonella enterica* serovar Typhimurium, a close relative of *E. coli*. A *recB* derivative of *S. enterica* is highly attenuated for virulence (63). The nitric oxide produced by macrophages when they ingest *S. enterica* causes bacterial replication inhibition. In the presence of nitric oxide, *S. enterica* requires RecBC but not RecA for viability and undergoes RuvABC-dependent chromosome breakage in the *recB* background. These observations led to the proposal that upon infection, the inhibition of *S. enterica* replication by macrophage-produced nitric oxide causes replication fork reversal, which is lethal for the *S. enterica recB* mutant and thus accounts for its decreased virulence (63).

Despite a high conservation of structure and function of replication and recombination proteins, replication arrests in eukaryotes will clearly have consequences different from those in prokaryotes. For example, the existence of multiple origins per chromosome ensures that the blockage of one replication fork has no dramatic consequences, because this blocked fork can be rescued by the arrival of a fork initiated nearby and progressing in the opposite direction. Multiple origins also increase the need for the coordination of replication with cell division, and conditions that impair DNA replication will induce checkpoint proteins (reviewed in refs. 64–66). Given the obvious numerous differences, how can studies of replication arrest and restart in bacteria help us understand that in other organisms?

The first message, deduced from the studies of blocked forks in prokaryotes, is one of diversity (Table 1). The replication forks arrested by a bona fide replication terminator are not processed like accidentally arrested forks, indicating that there is no single unifying model for replication impaired conditions. Furthermore, forks blocked by DNA damage that arrests DNA synthesis on one strand are processed differently from those blocked by inactivation of the entire replisome, where DNA synthesis is simultaneously arrested on both strands. Finally, inactivation of the entire replisome by impairment of different replication proteins has different consequences. Similarly, in eukaryotes, different checkpoint proteins are induced by a stalled replication fork, by a ssDNA region, or by a DNA double-strand end (64). Consequently, the nature and level of the cellular outcome will depend on the origin and extent of replication fork inhibition. Therefore, caution is needed when extrapolating from one replication arrest condition to another.

A second message from studies in prokaryotes is that the involvement of “recombination” proteins does not necessarily imply a “recombination” reaction. Blocked replication forks are a target for recombination proteins, and replication mutations stimulate homologous recombination in eukaryotes as in prokaryotes (reviewed in refs. 67 and 68). However, the action of recombination proteins at blocked forks may be more often to prevent the occurrence of DNA damage than to repair actual damage. At UV-blocked forks, RecFOR and RecA may simply protect DNA, induce the SOS response, and facilitate lesion repair. In replication mutants, replication fork reversal causes the formation of a HJ without homologous recombination and formation of a DNA double-strand end without actual DNA breakage. To date, replication inhibition has been shown to induce chromosome breakage only when the replication defect is accompanied by a recombination or repair defect. Indeed, our initial report of fork breakage in a replication mutant was deduced from studies performed in the absence of RecBC (69). We now know that such arrested replication forks are not broken but reversed, thereby allowing them to be reset without DNA damage when the RecBC complex is present (8,

9). Evidence for fork reversal has been reported in yeast, and similarly the observation of reversed forks required the combination of a replication defect (nucleotide pool depletion) and a DNA repair/checkpoint defect (Rad53 inactivation; ref. 70). Several “recombination” proteins, such as the three- and four-way junction-specific enzymes Mus81-Eme1, the RecQ homologues (Rqh, Sgs1), and the double-strand end specific complex Mre11/Rad50/Xrs2, are part of the S phase checkpoint response (reviewed in refs. 65 and 66). Understanding whether in eukaryotes the action of recombination proteins after replication inactivation is aimed at preventing chromosome breakage or repairing it is certainly an important future issue. A further question is how these actions relate to genome instability and DNA mutagenesis.

We are very grateful to Andrei Kuzminov, David Leach, and David Sherratt for very generous help in reading the manuscript and discussing its content with us and for insightful exchanges of ideas over several years. We thank Justin Courcelle and Mike Cox for communicating results before publication and Jeffrey Schapiro and Ferric Fang for communicating unpublished results. B.M. is on the staff of the Centre National de la Recherche Scientifique.

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