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DNA synthesis is an accurate and very processive phenomenon; nevertheless, replication fork progression on chromosomes can be impeded by DNA lesions, DNA secondary structures, or DNA-bound proteins. Elements interfering with the progression of replication forks have been reported to induce rearrangements and/or render homologous recombination essential for viability, in all organisms from bacteria to human. Arrested replication forks may be the target of nucleases, thereby providing a substrate for double-strand break repair enzyme. For example in bacteria, direct fork breakage was proposed to occur at replication forks blocked by a *bona fide* replication terminator sequence, a specific site that arrests bacterial chromosome replication. Alternatively, an arrested replication fork may be transformed into a recombination substrate by reversal of the forked structures. In reversed forks, the last duplicated portions of the template strands reanneal, allowing the newly synthesized strands to pair. In bacteria, this reaction was proposed to occur in replication mutants, in which fork arrest is caused by a defect in a replication protein, and in UV irradiated cells. Recent studies suggest that it may also occur in eukaryote organisms. We will review here observations that link replication hindrance with DNA rearrangements and the possible underlying molecular processes.

Large genome rearrangements, such as duplications, deletions, translocations and insertions, are mainly catalyzed by three classes of molecular processes that differ by length of homology of the joined sequences. Recombination events formed by joining of homologous sequences are mediated by specific enzymes. The key enzyme, RecA in prokaryotes and RecA-homologues in eukaryotes, catalyzes the strand exchange reaction and is highly conserved from bacteria to human (1, 2). A second type of recombination, called illegitimate, is characterized by the joining sequences whose length is below the minimal length required for recognition of homology by RecA (Minimal Efficient Processing Segment, or MEPS). In prokaryotes, illegitimate recombination can result from simple ligation of unrelated sequences (reviewed in ref. 3). In eukaryotes, this process is promoted by a battery of specialized enzymes and is called nonhomologous end-joining (NHEJ; reviewed in refs. 4 and 5). In addition, recombination between tandemly repeated sequences forms a distinct class of events that may be catalyzed by several specific pathways (reviewed in ref. 6). All classes of DNA rearrangements are important in human health, as they may cause cancers (reviewed in ref. 7) or hereditary disorders (reviewed in refs. 8 and 9) and are important in evolution (ref. 10; reviewed in ref. 11). All classes of rearrangements can result from the formation and repair of DNA double-strand breaks (DSBs) and have been shown to occur at an increased frequency in DNA regions difficult to replicate or when DNA replication is affected by a mutation. The correlation between replication hindrance and rearrangements suggests the existence of direct links between these two phenomena (reviewed in refs. 12–16). Indeed, recent studies confirmed that recombination proteins act during replication, particularly upon replication pause, and that, in turn, completion of certain recombination reactions requires the establishment of a *bona fide* replication fork. We will first review some examples of correlation between replication hindrance and the occurrence of DNA rearrangements, and then discuss the role of recombination proteins during DNA replication.

DNA Sequences, or Mutations that Cause Replication Arrest, Induce DNA Rearrangements

Increased Break and Join Recombination Frequency Caused by Replication Arrest. Stalling of replication forks has been proposed to induce homologous or illegitimate recombination in several organisms. In bacteria, chromosome replication is normally arrested in the terminus region at replication pause sites, named *Ter* sites, upon the binding of a specific protein, Tus. Insertion of a *Ter* site at an ectopic position in the chromosome stimulates homologous recombination in the vicinity. Furthermore, it renders RecA and RecBC (the *Escherichia coli* DSB repair enzymes) essential for viability, suggesting the occurrence of DSB upon replication blockage (Fig. 1*A*; ref. 17). The *Ter*/Tus complex can also induce illegitimate recombination events (18, 19). Genetic evidence indicated that illegitimate recombination associated with replication hindrance also results from the repair of DSBs (ref. 19; reviewed in ref. 15). Instability caused by replication arrest is not limited to the *Ter*/Tus complex because other DNA-bound proteins capable of arresting replication can also induce illegitimate recombination. A repressor bound to its cognate operator sequence or a highly transcribed region creates deletion hotspots in plasmids, provided that these elements are located downstream of the unidirectional plasmid replication origin and are oriented as to impair plasmid replication (20, 21).

Several replication arrest sites have been described in eukaryotes (reviewed in refs. 14 and 16). Among them, one of the best characterized is located in the rDNA. Every unit of the rRNA gene cluster contains a unique site, termed replication fork barrier (RFB), where progressing replication forks are stalled in a polar fashion (reviewed in refs. 14 and 16). These barriers prevent replication from entering into transcriptional units, avoiding collisions between transcription and replication apparatus. In yeast, the RFB colocalizes with a recombination hotspot, *HOT1*. Several studies indicate that homologous recombination at *HOT1* is directly linked to a DNA replication fork block at RFB (22, 23). Mutations that affect replication fork progression in rDNA and the efficiency of the RFB also modify the level of homologous recombination in rDNA (24). Increased levels of recombination in rDNA repeats are associated with DSBs and require Rad52, an enzyme essential for DSB repair by

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Abbreviations: DSB, double-strand break; RFR, replication fork reversal.

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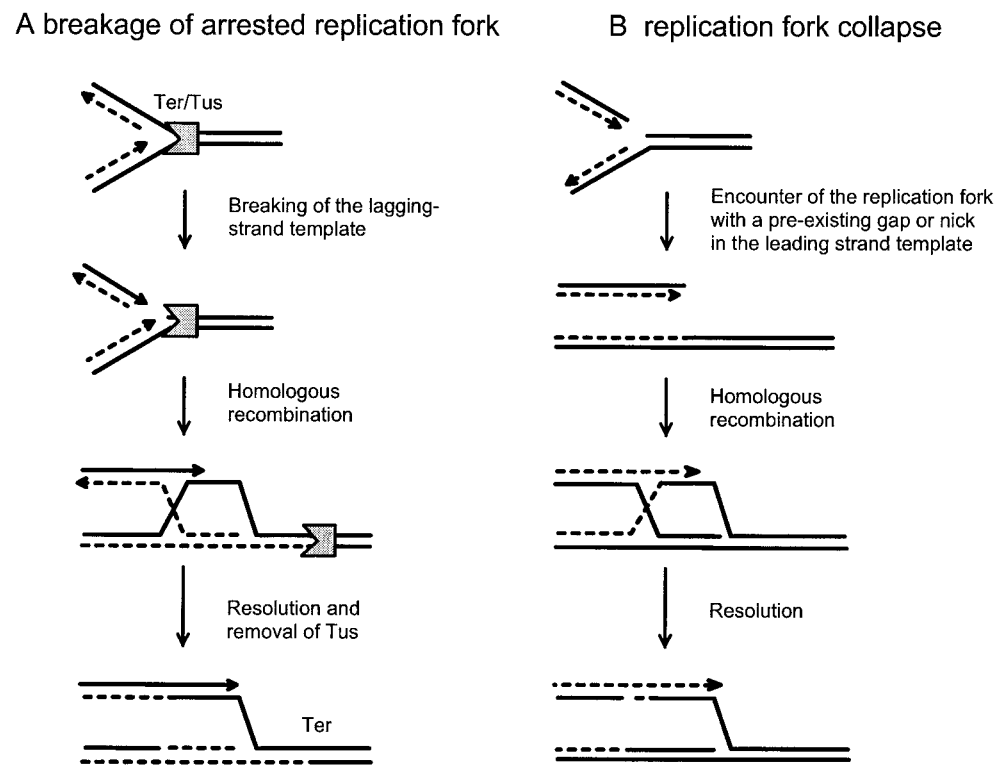

Fig. 1. Recombination repair of broken replication forks. (*A*) Rescue of blocked replication forks (adapted from ref. 17). The replication fork is blocked at the *Ter* site in the presence of Tus. A DSB occurs in the lagging-strand template. The RecBCD enzyme enters at the double-strand end and initiates homologous recombination catalyzed by RecA. Completion of the recombination reaction by resolution of the Holliday junction leads to restoration of a replication fork. Binding of the primosome allows loading of a new replisome to promote DNA replication restart. To account for the viability of a strain carrying an ectopic *Ter* site in a recombination-proficient background, one needs to assume that the newly reconstituted replication fork is not arrested again, and hence that Tus has been removed from *Ter* during the recombination reaction. DSB on the lagging strand is shown, but a similar model can apply to breakage and repair of the leading strand. (*B*) Replication fork collapse (adapted from ref. 31). The progressing fork encounters a single-strand interruption in the leading-strand template, because of a defect in closure of the lagging strand at the previous replication round. Reincorporation of the broken DNA strand by homologous recombination and replication restart are catalyzed by the same enzymes as on breakage of the fork. The full lines represent the two DNA strands, the dashed lines represents newly synthesized DNA strands. The arrowhead corresponds to DNA 3′ ends.

homologous recombination in yeast (24). However, *HOT1*-stimulated recombination could also occur in strains in which RFB is inactivated by a mutation (25). Although transcription is not essential for RFB, it is required for the *HOT1* activity (reviewed in ref. 13); possibly transcription is responsible for the remaining *HOT1* hyper-recombination activity in the absence of RFB.

Finally, DNA lesions are well known to impair replication and often require homologous recombination for repair. DNA lesions caused by UV irradiation, alkylating agents, cross-linking agents, or topoisomerase poisons all block the progression of replication forks and create a need for recombinational repair (refs. 26–28; reviewed in refs. 13 and 29).

Mutations in Replication Functions Stimulate DNA Rearrangements. In addition to DNA secondary structures or DNA modifications that cause replication arrest, mutations that affect replication progression also increase recombination frequency. A first class of mutations shown to stimulate homologous recombination in *E. coli* include mutations that impair the closing of Okazaki fragments (30). In ligase or polymerase I mutant strains, nicks or small gaps left in the lagging strand during replication progression were proposed to be transformed into DSBs by the arrival of a replication fork. The DSBs thus formed are then repaired by homologous recombination (reviewed in ref. 31; Fig. 1*B*).

Another class of replication mutants stimulates recombination events between long tandemly repeated sequences. In *E. coli*, the replisome is composed of the polymerase III holoenzyme, a DNA helicase called DnaB, and a primase. Mutations in genes encoding different holoenzyme polymerase III subunits, DnaB, or the primase could all affect, to a variable extent, the stability of long repeated sequences (32). Replication mutations also stimulate deletions of repeated sequences in an unrelated bacteria, *Bacillus subtilis* (33). In a screen for mutant strains in which recombination between long tandemly repeated sequences was stimulated, two replication mutants were isolated and found to be impaired for different polypeptides of the holoenzyme polymerase III. A mutant affected in the core subassembly of the DNA polymerase, the α subunit encoded by the gene *dnaE*, stimulated tandem repeat deletions in a RecA-independent way; this finding lead to the proposal of a replication slippage model (34). A strain bearing a point mutation in the *holD* gene, encoding ψ, one of the subunits of the clamp loader γ complex, stimulated tandem repeat recombination in a RecA-dependent way; this result lead to a model invoking the formation of dsDNA ends at arrested replication forks and the processing of these DNA ends by recombination proteins (35).

Chromosome instability linked to replication mutations was also observed in eukaryotes. In yeast, several mutants, including those affected in replication functions, stimulate mitotic homologous recombination (reviewed in ref. 13). For example, recombination is enhanced in ligase mutants (36). The level of rDNA recombination is increased by mutations in Pol α and Pol δ polymerases (37) and in topoisomerases (38, 39), and is affected in strains lacking one of the two helicases (PIF and RMM) involved in the replication of this region (24). Mutation in RFC1,

the gene encoding the large subunit of the yeast clamp loader, results in instability of repeated sequences (40). Mutations in RFA1 (encoding a subunit of the single-stranded DNA binding protein) or RAD27 (encoding the flap endonuclease essential for the processing of Okazaki fragments) result in a stimulation of gross DNA rearrangements. These rearrangements may result from the mutagenic processing of DSBs that accumulate in such mutants (41, 42). Finally, in certain human syndromes resulting from a defect in a protein belonging to the family of RecQ helicases, a correlation is observed between a replication defect and a hyper-recombination phenotype (reviewed in ref. 43).

Recombination Functions Are Essential for the Viability of Some Replication Mutants. The correlation between DNA rearrangements and replication defects suggests that replication hindrance causes DNA lesions that are repaired by homologous recombination. In addition, such repair appears to be essential because homologous recombination is required for the viability of several replication mutants. In *E. coli*, two classes of replication mutants can be defined, depending on their requirement for recombination proteins. Mutants of the first class require RecBCD and RecA for viability, the recombination proteins essential for DSB repair. This class includes ligase and polymerase I mutants, supporting the idea that nicks or small gaps due to a defect in the closing of Okazaki fragments are transformed into DSBs (Fig. 1*B*; reviewed in ref. 31). Mutants of the second class require RecBCD for viability and not RecA. This class includes *rep* mutants, defective for an accessory replicative helicase, and a *holD* mutant impaired in a subunit of the *E. coli* polymerase III clamp loader, ψ (35, 44). As described below, mutants belonging to this second class suffer frequent replication fork arrest, supporting the idea that lesions induced by replication arrest lead to specific recombination protein requirements.

In yeast, replication mutants affected in the maturation of Okazaki fragments accumulate single-stranded DNA during replication. These mutants require the *RAD52* recombination pathway, hence a functional DSB repair system for viability (refs. 45–48; reviewed in ref. 13). This suggests that single-strand interruptions are also converted into DSBs in yeast, as in bacteria.

Finally, the homologous recombination enzymes Rad51 and DSB repair enzyme Mre11 are essential in vertebrate cells for cell proliferation in S phase (refs. 49 and 50; reviewed in refs. 51 and 52). This finding suggests that, in contrast to bacteria and yeast, DSB repair in higher eukaryotes may be essential for the normal progression of replication forks and not only when replication is impaired by a mutation. In hamster CHO cells, the Rad51-dependent recombination pathway can be substantially impaired without affecting cell viability; however, replication inhibition leads to the formation of DNA breaks and recombination is stimulated in a Rad51-dependent manner (Y. Saintigny and B. Lopez, personal communication). These observations suggest that links between replication hindrance and recombination may extend to mammalian cells.

The Replication Fork Reversal Reaction

The observation that replication defects are often associated with DNA rearrangements and render homologous recombination essential for viability implies that DNA substrates for homologous recombination are formed upon replication inhibition. The conversion of single-strand interruptions into DSBs after a replication fork passage appears to be one of the ways to generate recombination substrates during DNA replication. However, this cannot be the only way, because rearrangements also occur at sites of replication arrest. Recent studies in bacteria revealed the occurrence of a specific reaction at blocked forks, named replication fork reversal (RFR), that transforms blocked forks into a recombination substrate.

RFR in Helicase Mutants. *E. coli* mutants defective for a replication helicase were used to study the consequences of replication fork arrest. The *rep* mutant lacks an accessory replicative helicase required for replication fork progression at a normal rate. Consequently, the absence of Rep is thought to cause frequent replication pauses. Interestingly, the *rep* mutant requires RecBC, the enzyme essential for DSB repair, for viability. DSB formation is indeed observed *in vivo* in a *rep recBC* strain (53). In contrast, *rep* strains do not require RecA, which was surprising considering that both RecBC and RecA are essential for DSB repair in *E. coli* (54). Degradation of linear DNA in *rep recA* cells could have explained the viability of the strain, however, direct measurements showed that this did not occur. The identification of the enzymes responsible for the occurrence of DSBs in *rep recBC* mutants lead to a model that explains the genetic properties of the *rep* mutant. DSBs result from the action of the RuvABC proteins (44). RuvABC were originally known as proteins that act at the last step of homologous recombination (reviewed in ref. 55). RuvA and RuvB form a complex that catalyzes branch migration of Holliday junctions and RuvC resolves RuvAB-bound Holliday junctions by introducing nicks in strands of opposite polarities. The RFR model supposes the formation of a Holliday junction at blocked forks by reannealing of template strands and pairing of newly synthesized strands (Fig. 2). The key features of this intermediate structure formed by RFR are a double-strand end, substrate for RecBCD, and a Holliday junction, substrate for RuvABC. In recombination-proficient strains, reincorporation of the double-strand tail into the chromosome by RecBCD- and RecA-mediated homologous recombination allows replication restart from a recombination intermediate (Fig. 2*C*). DSBs occur only if both homologous recombination and DNA degradation are inactive and result from RuvABC-mediated resolution of the Holliday junction in the absence of any processing of the double-strand tail (Fig. 2*E*).

The RFR model can account for all of the properties of the *rep* mutant. For example, *rep* mutants do not require the *E. coli* XerCD/dif system essential for chromosome dimer resolution. This finding was unexpected considering that the requirement for RecBC in *rep* mutants suggested frequent homologous recombination events. Such a high level of homologous recombination should theoretically render dimer resolution essential. The viability of *rep* mutants deficient for dimer resolution implied that reincorporation of the double-strand tail by homologous recombination does not lead to the rate of chromosome dimerization expected from random resolution of Holliday junctions (56). Indeed, two Holliday junctions are formed at the reversed fork, one by fork reversal and one by homologous recombination between the double-strand tail and the chromosome. Formation of a viable chromosome requires that these two junctions migrate in the same direction, away from the double-strand end. The finding of a bias in RuvABC-mediated resolution of Holliday junctions, imposed by the direction of migration of junctions (57, 58), implies that both junctions are mainly resolved with the use of the same strand, because they migrate in the same direction. This property of RuvABC activity explains the formation of predominantly monomers during reincorporation of the reversed forks (56).

Arresting replication forks by inactivation of the essential *E. coli* replicative helicase DnaB also lead to RuvABC-dependent DSBs in *recBC* context, suggesting that RFR is not limited to *rep* mutants (64). Furthermore, as described below, RFR seemed to occur also in *E. coli* upon inactivation of the main DNA polymerase, DNA polymerase III.

RFR Is Induced by Different Replication Mutations. *In vivo*, the *E. coli* polymerase III holoenzyme is composed of ten subunits assembled in two catalytic cores, two sliding clamps and a clamp loader (reviewed in ref. 59). The polymerase core contains three

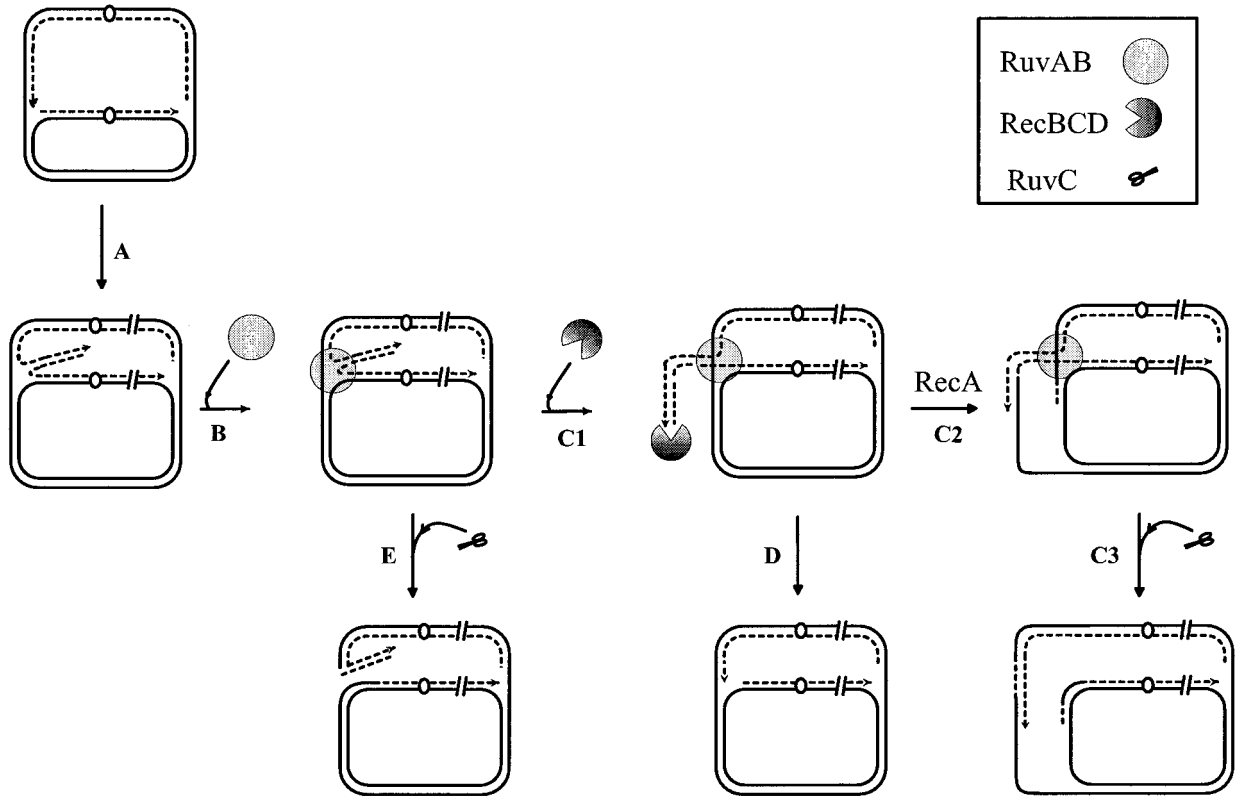

Fig. 2. RuvAB/RecBCD-mediated rescue of blocked replication forks (adapted from ref. 44). In the first step (*A*) the replication fork is blocked and the two newly synthesized strands anneal, forming a Holliday junction (see Fig. 3 for the different pathways proposed to promote this step). In a second step (*B*) the junction is stabilized by RuvAB binding. (*C*) In recombination proficient strains, RecBCD binds to the double-strand tail (*C1*); degradation takes place until the first recognized CHI site (CHI is an octameric sequence that switches RecBCD from an exonuclease to a recombinase enzyme) and is followed by a genetic exchange mediated by RecA (*C2*); RuvC resolves the first Holliday junction bound by RuvAB (*C3*). In *C2* and *C3*, the double-strand end is reincorporated into the circular chromosome by homologous recombination and the Holliday junction is resolved, which results in the reconstitution of a replication fork. This pathway is presumably used in recombination-proficient cells. (*D*) RecBCD-mediated degradation of the tail progresses up to the RuvAB-bound Holliday junction. Replication can restart when RecBCD has displaced the RuvAB complex. *D* can take place in recombination-proficient strains if RecBCD reaches RuvAB before encountering a CHI site; it is the only pathway that leads to a viable chromosome in *recA* and *ruvC* mutants. (*E*) RuvC resolves the RuvAB-bound Holliday junction. This pathway is used in the absence of RecBCD and leads to the RuvABC-dependent DSBs observed in *recBC* mutants. Continuous and discontinuous lines represent the template and the newly synthesized strand of the chromosome respectively; the arrowheads indicate the 3′end of the growing strands.

subunits including the catalytic subunit α encoded by *dnaE*. Each core polymerase is tethered to the DNA by a β clamp. The two cores are held together by a dimer of the τ subunit, which also binds the clamp loader (the γ complex) and the helicase DnaB, allowing the two core polymerases to function in a coordinated fashion, one on the leading strand and the other on the lagging strand.

To test whether RFR occurs on inactivation of the catalytic subunit α of *E. coli* Pol III, cells carrying a conditional mutation *dnaEts* were used. One consequence of the RFR reaction is the formation of RuvABC-dependent DSBs in cells deficient for RecBCD. Inactivation of *dnaE* and *recBC* lead to the formation of DSBs that occurred only in the presence of functional RuvABC proteins (G.G., M.S., and B.M., unpublished results). This observation suggests that replication forks blocked by Pol III inactivation form a Holliday junction by RFR, as previously observed for cells deficient for a replicative helicase.

In the *dnaEts* mutant, both leading and lagging-strand polymerases are inactivated upon shift to a high temperature, which implies that DNA synthesis is arrested on both strands in concert. As described below, we could show by genetic means and by direct measurements of DSB formation that inactivation of one of the clamp loader subunits also caused RFR, which suggests that RFR occurs in a strain deficient for lagging-strand synthesis. In a screen for mutations that increase the frequency of recombination between long tandemly repeated sequences, we isolated a point mutation in the *holD* gene, encoding the ψ subunit (35). ψ is a small component of the γ complex that acts as a bridge between two other polypeptides, γ and χ (59–62). The γ complex is thought to catalyze the loading and unloading the β clamp after completion of each Okazaki fragment, thus allowing rapid cycling of the lagging-strand polymerase (reviewed in refs. 59 and 63). Similarly to *rep* mutants, the *holD*^{Q10} mutation was lethal in combination with *recBC* and viable in combination with *recA* inactivation. Inactivation of *recBC* in the *holD*^{Q10} strain lead to the formation of DSBs that required RuvABC for their formation, leading us to conclude that RFR occurred in the *holD*^{Q10} strain. Importantly, because the *holD*^{Q10} mutation was originally isolated as increasing tandem repeat deletions, this study showed that reincorporation of the double-strand tail by homologous recombination could result in rearrangements in repeated sequences.

These results extend the RFR model to forks blocked by a defect in the DNA polymerase and to a strain deficient for the initiation of Okazaki fragment synthesis and indicate that RFR can be induced by different replication defects. The observation that the initial step of the reaction, fork reversal, could occur by different mechanisms brings further support to the idea that RFR may be widely distributed.

Several Pathways for RFR. Three means of initiating fork reversal have been proposed. Reannealing of the template strands could

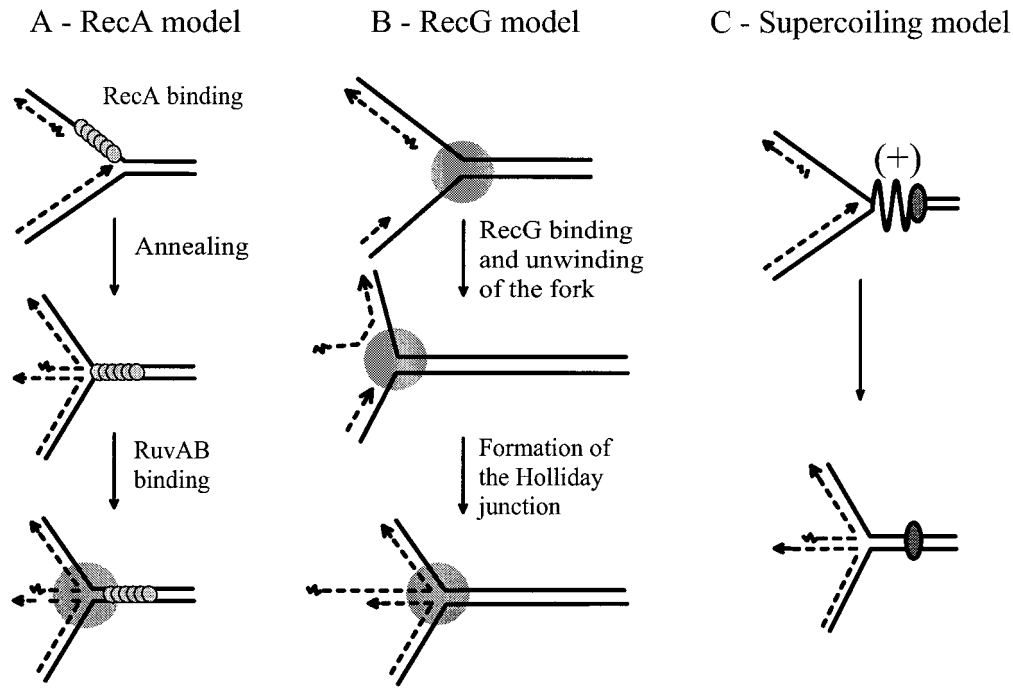

Fig. 3. Models for formation of Holliday junctions at arrested replication forks by RFR. (*A*) RecA binds to the single-stranded region of the lagging-strand template, polymerizing in the 5′ to 3′ direction. Pairing of the lagging-strand template with the leading-strand template renders the leading strand free to anneal with the 5′ end of the lagging strand. This results in the formation of a Holliday junction that can be bound directly by RuvAB. (Adapted from ref. 64.) (*B*) RecG binds to the replication fork and migrates toward the chromosomal replication origins, displacing the 5′ end of the lagging strand. RecG activity ultimately creates a four-stranded junction. (Adapted with modifications from ref. 76.) (*C*) (+) Topological stress that accumulates downstream of the fork on arrest is relaxed by unwinding of the two newly synthesized strands from the template strands and their annealing. (Schematic representation based on results in ref. 78.) Full and dashed lines represent the template and the newly synthesized DNA, respectively; the arrowheads indicate the 3′ end of the growing strands.

be the initial event; this reaction could be catalyzed by RecA (Fig. 3*A*). Alternatively, the newly synthesized strands could be peeled off, allowing the template strands to reanneal. Such a reaction could be promoted by a DNA helicase activity (Fig. 3*B*). Finally, the reaction could be independent of enzymatic activities, and be driven by supercoiling forces (Fig. 3*C*). Experimental support for each of these proposed models has been presented, *in vitro* or *in vivo*.

The involvement of RecA in RFR was investigated *in vivo* in replication mutants. Inactivation of RecA suppressed essentially all RuvABC-dependent breakage in the *dnaBts recBC* mutant (64). This finding suggests that the initial event in the reaction is the binding of RecA to the single-stranded region present on the lagging-strand template, followed by reannealing to the complementary leading strand, resulting in the formation of a Holliday junction (Fig. 3*A*). A similar homologous recombination reaction could be reconstituted *in vitro* (M. Robu, R. Inman, and M. Cox, personal communication). However, *in vivo* the single-stranded binding protein, SSB, covers single-stranded DNA, especially at the replication fork. During homologous recombination, RecA binding to SSB-coated DNA is facilitated by the action of either of the two presynaptic complexes, RecBCD or RecFOR (65, 66). Surprisingly, RuvABC-dependent DSBs, indicative of Holliday junction formation by RecA, did not appear to require any of the presynaptic enzymes, as it still occurred in *recBC recF* or *recBC recO* double mutants (64). How SSB is displaced from the lagging-strand template for RecA binding is still unknown. The multiple role of RecA at blocked replication forks, both for the formation of the reversed fork and for its reincorporation in the chromosome, underlines the existence of important specific functions for this recombination protein on replication arrest. However, RecA was not required for the formation of RuvABC-dependent DSBs, hence for the initial step of RFR, in either *rep*, *dnaEts*, or *holD*^{Q10} strains (refs. 35 and 64; G.G., M.S., and B.M., unpublished results). It remains to be tested whether one of the other proposed mechanisms is involved in RFR in these strains.

A second mechanism of RFR implies the role of helicases that would recognize forked structure to anneal the two growing strands (Fig. 3*B*). Two proteins that bind and unwind forked structures have been described in *E. coli*, the helicases PriA and RecG (67). PriA is an essential component of the primosome, the complex that reloads a replisome at nonorigin sequences (reviewed in refs. 68–70). PriA also binds to forked DNA and promotes unwinding of the 5′ end of the lagging strand at Mu transposition intermediates (71, 72). However, no experimental evidence has been provided so far that supports the hypothesis that PriA may play a role in RFR in *E. coli* replication mutants. *recG* inactivation confers a mild sensitivity to DNA damaging agents in *E. coli*, and because the RecG protein has a high affinity for Holliday junctions *in vitro*, it was proposed to participate in the resolution of recombination intermediates (73–75). *In vitro* experiments showed that RecG could transform a forked structure into a Holliday junction (76). RecG ability to bind both forked DNA and Holliday junctions, hence both the substrate and the product of the fork reversal reaction support a role for RecG in RFR. Furthermore, RecG is able to unwind RNA–DNA hybrids (77), which might facilitate the reaction because the 5′ end of the lagging strand at an arrested replication fork is the RNA primer of the last Okazaki fragment. Genetic experiments suggested that RecG might reverse forks blocked by the encounter of an RNA polymerase stalled at a DNA lesion in UV irradiated cells (76). Surprisingly, under these specific conditions RecBCD does not seem to have access to the double-strand tail made by RFR. The reasons for the difference between the crucial role of RecBCD at reversed forks in replication mutants and the lack of effects of *recBCD* mutations at forks reversed in UV irradiated cells remain to be elucidated.

In *rep* and *dnaBts* strains, inactivation of *recG* had little effect on RuvABC-dependent DSBs if any (M.S. and B.M., unpublished results), indicating that RecG is not essential for RFR in these strains.

Finally, a model has been proposed for the reversal of arrested replication forks that implies a nonenzymatic reaction (Fig. 3*C*). *E. coli* plasmid replication intermediates that had been stalled at a *Ter* termination site were used as a model to study the conformation of partially replicated molecules with a (−) or a (+) ΔLk (ΔLk is the difference between the linking number of a molecule and the linking number of the same molecule in a relaxed state; ref. 78). Replication intermediates produced in the presence of gyrase have a (−) ΔLk that equilibrates between two forms (−), supercoils, and (−) precatenates. When deproteinized replication intermediates are treated with intercalating agents to generate (+) ΔLk, no (+) supercoils or (+) precatenates are generated. Instead, the (+) topological stress is relieved by the formation of a reversed replication fork (78). The observation that RFR could be promoted by chemicals that generate (+) ΔLk in purified partially replicated molecules suggests that *in vivo*, the (+) ΔLk generated ahead of the progressing replication fork may also promote RFR upon replication blockage, without the need for any enzymatic activity (Fig. 3*C*). RFR due to spontaneous branch migration *in vitro* has also been seen in replication intermediates without a (+) ΔLk (79).

Resetting of Reversed Forks. The conversion of a Holliday junction formed by RFR into a forked structure on which DNA replication can be reinitiated is essential to prevent RuvABC-mediated breakage of the chromosome. According to the model proposed in Fig. 2, this conversion can occur by two pathways, degradation of the double-strand tail (Fig. 2*D*) or reincorporation of this double-strand end into the chromosome by homologous recombination (Fig. 2*C*). In addition, branch migration of the Holliday junction would restore the initial structure (reversal of *A* in Fig. 2). Resetting of reversed replication fork by branch migration would render RecBCD dispensable by providing an alternative way of restoring a viable chromosome. Therefore, this reaction does not seem to occur in *E. coli rep* or *holD*^{Q10} strains, because RecBC is essential for the viability of these mutants. However, in UV-irradiated cells, RecG was indeed proposed to catalyze two opposite reactions: (*i*) formation of a Holliday junction by reversal of the replication fork and (*ii*) migration of this Holliday junction back to the original configuration (76). Furthermore, resetting of a forked structure by branch migration of reversed forks has also been proposed in organisms other than bacteria.

In eukaryotes, proteins belonging to the RecQ subfamily of DNA helicases were proposed to play a role during replication through their action on Holliday junctions. Named after the RecQ protein of *E. coli*, this family is widespread and comprises Sgs1 from *Saccharomyces cerevisiae*, Rqh from *Schizosaccharomyces pombe*, and several human homologues including the Bloom (BLM) and Werner syndrome proteins (WRN). *In vivo*, both Sgs1 and Rqh were shown to remove Holliday junctions and the defects of *sgs1*mutants were alleviated by inactivation of proteins required for the initial steps of homologous recombination (80, 81). Expression of a bacterial Holliday junction resolvase (Rus) in the nucleus of Rqh mutants suppressed some of the defects of the mutant while promoting rearrangements. It was proposed that Rqh would remove Holliday junctions formed during replication in a nonrecombinogenic way, by branch migration of reversed forks back to the original configuration (Fig. 4; ref. 81). In the absence of Rqh, the reversed fork would be processed either by resolution of the Holliday junction or by reincorporation of the double-strand tail by homologous recombination (as shown on Fig. 2 *C* or *E*), leading to DNA rearrangements. Similarly, the human BLM protein was shown to bind Holliday junctions *in vitro*. Because the major defects of BLM cells are observed during S phase, BLM protein was proposed to act at Holliday junctions formed by RFR on replication blockage (82).

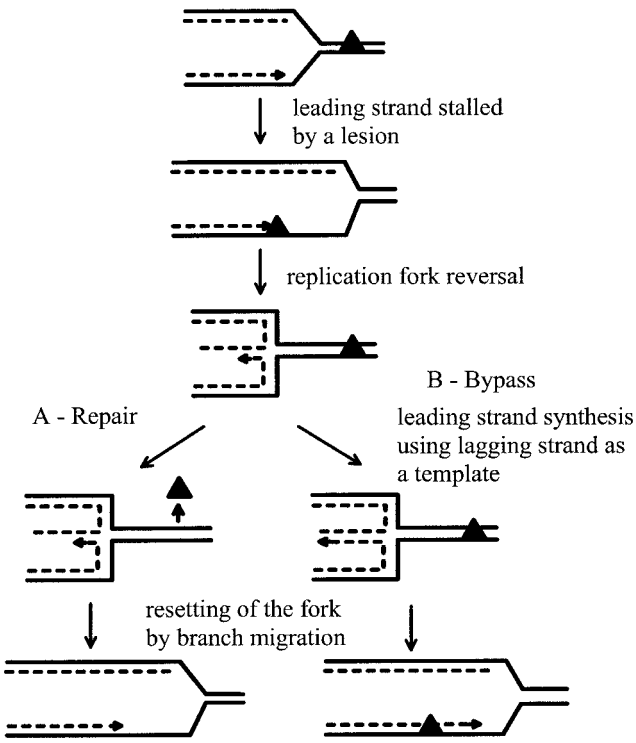

Fig. 4. RFR in UV-irradiated cells (adapted from refs. 76, 80, and 92). The replication fork is blocked by a UV photo-product (black triangle) in the leading-strand template. RFR, proposed to be catalyzed by RecG in *E. coli* (76), or by Rad51 (the yeast RecA homologue) in *S. pombe* (80), renders the damaged DNA double stranded and thereby allows direct repair by nucleotide excision repair enzymes (*A*). If the lagging-strand polymerase has continued synthesis past the lesion, leading-strand DNA synthesis using the lagging strand as template followed by reverse branch migration [proposed to be catalyzed by RecG in *E. coli* (76) and by Rqh in *S. pombe* (80)] reconstitutes a fork on which the lesion has been bypassed (*B*; ref. 94). Full and dashed lines represent the template and the newly synthesized DNA, respectively; the arrowhead indicates the 3′ end of the growing strand.

Although these findings support the hypothesis of the resetting of reversed replication forks by branch migration, it is also theoretically possible that in eukaryotes, reversed forks are also reset by homologous recombination (as schematized in Fig. 2*C*). Indeed, DSB repair by enzymes belonging to the Rad52 pathway of recombination can lead to extensive DNA replication in eukaryotes (refs. 83 and 85; reviewed in refs. 7 and 84).

The Advantages of RFR. Initiation of DNA replication at forked templates created by homologous recombination or on replication arrest have been recognized in the past decade as important for the maintenance of cell viability (reviewed in ref. 86). The complex essential for the loading of the replicative helicase, and consequently for the loading of the entire replication machinery, is called the primosome assembly complex (reviewed in refs. 68–70). DNA structures recognized by PriA, the key protein of the primosome assembly complex, have been characterized *in vitro*. The primosome can assemble a replication complex at a D-loop formed by homologous recombination (87) or at a forked structure (72). *In vivo*, PriA-dependent assembly of the replisome occurs at recombination intermediates (88–90). *priA* mutants have poor viability that is not significantly improved by the *recA* mutation (ref. 91; J. McCool and S. Sandler, personal communication), suggesting that PriA has an important repair function in a process other than homologous recombination. In

particular, PriA may restart replication directly from an arrested replication fork (70). If the PriA-dependent primosome is able to assemble and to restart replication directly at blocked forks, why should arrested forks be transformed into recombination substrates? This raises the question of the advantage of RFR for growth. Although there are no specific answers yet, several hypothesis can be proposed.

RFR may facilitate replication restart by allowing the removal of the blocking element. RFR was originally proposed 25 years ago as a possible step in the repair of DNA lesions (ref. 92; Fig. 4) and has remained a common model for DNA damage avoidance in all organisms. Lesions that block replication are recognized by nucleotide excision repair enzymes on double-strand DNA only. Therefore, models of damage avoidance are generally based on the need to render these lesions accessible to nucleotide excision repair enzymes, by presenting them on double-stranded DNA. Reannealing of lagging and leading strands is a simple way to achieve this goal (Fig. 4*A*; ref. 76 and references therein; ref. 81). RFR might thereby provide a nonmutagenic, nonrecombinogenic means of rendering lesions that block replication accessible to the DNA repair enzymes (Fig. 4*A*). An alternative model was also proposed in which the sequence complementary to the lesion is synthesized by using the neo-synthesized lagging strand as template, so that the lesion is on double-stranded DNA after return to the forked structure (Fig. 4*B*; ref. 92). This latter model specifically assumes that an uncoupling occurs between leading and lagging-strand synthesis and that the lagging strand progresses beyond the leading strand stalled at a lesion. There is no direct evidence that such an uncoupling may occur, although some *in vitro* data support this idea (93).

Observations that support the occurrence of RFR in different replication mutants (35, 44) suggest that RFR may also facilitate the removal of blocking elements other than DNA lesions. In *rep* mutants, replication arrest is thought to result from the encounter of replication forks with proteins involved in other DNA transactions (e.g., RNA polymerases or other DNA bound proteins). RFR may stimulate the release of these proteins.

Fork reversal may confer a further advantage to the cell by protecting the DNA at blocked forks. If the single-stranded DNA region on the lagging-strand template is exposed on replication arrest, it may be the target of single-strand nucleases. By making the fork region double-stranded, RFR may protect the fork against nuclease attack. Indeed, in *dnaBts recA* mutants, RuvABC-dependent DSBs, and hence RFR, do not occur. However in this case, DSBs do occur that are independent of the presence of RuvABC, and are presumably formed by the action of single-strand nucleases on stalled replication forks (64). Breakage of arrested replication forks has been shown to promote illegitimate recombination (19). Concerted processing of the fork by homologous recombination and replication enzymes should prevent such deleterious reactions.

In eukaryotes, RFR might provide a signal for checkpoint proteins. Surveillance mechanisms, called checkpoints, detect damaged DNA or replication arrest and block cell cycle progression to allow adequate time to repair the damaged DNA (reviewed in ref. 94). RFR provides a double-stranded end, without actual breakage of the DNA, and a Holliday junction. Double-stranded ends are thought to signal DNA damage to checkpoint proteins. In *S. pombe*, most of the genes that have been identified in the checkpoint cascade are required both for DNA damage repair and for the replication checkpoints (reviewed in ref. 94). We can speculate that formation of a double-stranded end on exposure to DNA damaging agents or on replication blockage would provide a common signal for cell-cycle arrest. In *S. cerevisiae*, the Sgs1 protein, which is a RecQ-helicase homologue, has been shown to act at recombination intermediates, presumably Holliday junctions (80) and to be directly implicated in S-phase-specific checkpoints (ref. 95; reviewed in ref. 96). Sgs1 colocalizes with the signal-transducing kinase Rad53p, and is required for Rad53p phosphorylation in response to replication fork arrest (95). We can speculate that the formation of a Holliday junction on replication arrest would allow the targeting of Sgs1 to blocked forks, similarly to its binding to recombination intermediates formed during the repair of DNA damage, again providing a unique signal for checkpoint proteins.

These works suggest a role for individual proteins like RecBCD, RecG, or RuvABC—whose role was always restricted to RecA-assisted recombination—during replication progression. The existence of a coordinated backward migration and restart of the fork suggests a "proofreading" function for "recombination" proteins, that would take place at the level of the entire replication fork, on encounter with obstacles on the DNA.

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