Published in final edited form as:

DNA Repair (Amst). 2017 August ; 56: 118–128. doi:10.1016/j.dnarep.2017.06.014.

Template-switching during replication fork repair in bacteria

Susan T. Lovett

Department of Biology and Rosenstiel Basic Medical Sciences Research Center Brandeis University, Waltham, MA -2454-9110

Abstract

Replication forks frequently are challenged by lesions on the DNA template, replication-impeding DNA secondary structures, tightly bound proteins or nucleotide pool imbalance. Studies in bacteria have suggested that under these circumstances the fork may leave behind single-strand DNA gaps that are subsequently filled by homologous recombination, translesion DNA synthesis or template-switching repair synthesis. This review focuses on the template-switching pathways and how the mechanisms of these processes have been deduced from biochemical and genetic studies. I discuss how template-switching can contribute significantly to genetic instability, including mutational hotspots and frequent genetic rearrangements, and how template-switching may be elicited by replication fork damage.

Keywords

DNA Replication; Genetic Recombination; Mutagenesis; Copy Number Variation; Quasipalindrome; Postreplication Repair

Faithful DNA replication is vital to the survival of all organisms. However, replication problems can lead to arrest of DNA synthesis and the accumulation of ssDNA gaps. If these gaps are not repaired, convergence of another replication fork upon the gap inevitably causes the formation of a double-strand break in the chromosome, a potentially lethal form of DNA damage [1].

Studies in bacterial systems have implicated a number of DNA repair mechanisms that can fill such ssDNA gaps in DNA (“gDNA”) in a process originally termed “postreplication repair” [2, 3], but sometimes referred to as “daughter strand gap repair”. Genetic and biochemical studies suggest that gDNA repair plays an important role in tolerance of DNA damage. Single-strand gaps may be generated during replication of DNA containing unusual secondary structures, damage induced by UV irradiation or oxidation, interstrand crosslinks or templates with tightly bound proteins, such as transcription complexes [1, 4]. For lagging strand blocks, continued progression of the fork helicase and re-priming of successive

Corresponding author: Susan T. Lovett lovett@brandeis.edu; Ph: 781-736-2497; FAX 781-736-2405.

Conflicts of interest: The author declares no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Okazaki fragments leads to formation of gaps in the wake of the fork. Even for leading strand blocks, if the leading strand synthesis has been re-primed downstream, replication gaps may be left behind the moving fork, a scenario suggested by studies both *in vitro* [5] and *in vivo* [6, 7] in *E. coli*. However, gDNA repair is not without potential deleterious consequence and can lead to genetic rearrangements or mutations. This review focuses on how repair of gapped DNA promotes replication template-switching and how such mechanisms lead to genetic instability, including copy number variation of short direct repeats and genetic mutation hotspots at short inverted repeats (“quasipalindromes”). Although the discussion will feature mechanistic studies in *E. coli* there is evidence that similar processes occur in eukaryotic cells [8] (and see references below).

Post-replication repair

Physical analysis of DNA strands, resolved by centrifugation in alkaline sucrose gradients, showed that UV-irradiation of *E. coli* leads to the formation of ssDNA gaps that are subsequently repaired [9]. Some of this repair involves joining of parental DNA to nascent DNA [10, 11], implicating a homologous recombination (HR) mechanism (Fig. 1A). Later studies demonstrated that translesion DNA synthesis by specialized DNA polymerases (Fig. 1B) also contributes significantly to the repair of gaps (reviewed in [12]). A template-switching post-replication mechanism involving annealing of nascent DNA strands to overcome blocks to replication (Fig. 1C) was postulated from the properties of genetic rearrangements [13]. However, none of these post-replication “repair” mechanisms remove the replication-blocking lesion; rather, they constitute DNA damage tolerance mechanisms that can provide the opportunity for the cell to survive and the lesion to be subsequently removed by other means, such as excision-repair.

Repair of gDNA is somewhat difficult to ascertain by genetic approaches. UV-irradiation was the first agent used to infer gap repair through survival of the irradiated bacteria, although most lesions produced by UV do not lead to replication gaps and can be repaired by other means (such as simple excision repair or photoreactivation). Azidothymidine, a chain-terminating nucleoside, is a useful agent to study gap repair since it is incorporated during replication and causes gaps to accumulate downstream of the lesion or incomplete tracts of repair synthesis [14]. If gaps fail to be repaired, they may be converted to DSBs, which can be efficiently repaired. Therefore survival from gap-promoting lesions is not necessarily indicative of an efficient gap repair system, unless DSB repair is also disabled. Since TLS DNA polymerases are highly error-prone, utilization of TLS to repair gaps can be detected by increased point mutagenesis using mutation reporter assays. In contrast, repair of DNA gaps by HR or template-switching between sister chromosomes is more likely to be genetically silent. However, when repeated DNA sequences are present at the site of repair, genetic arrangements and crossing-over can result from both processes. Although crossing-over between sister bacterial chromosomes is difficult to ascertain, sister crossovers between small circular replicons (such as plasmids) lead to circular plasmid dimers, which can be easily detected by gel electrophoresis.

HR gap repair

The RecFOR pathway of homologous recombination is believed to be specialized as a gap-filling recombination mechanism (as opposed to the RecBCD pathway that is specialized for repair of DSBs). This pathway also requires the functions of the RecA, RecJ, RecN, RecQ, RuvABC proteins [15–17]. The RecA strand-transfer protein initiates recombination by binding to ssDNA; the RecA/ssDNA filament then promotes the search for homology and subsequent strand-exchange that underlies recombination. The RecA filament is also the signaling structure that induces the SOS response, a transcriptional response to DNA damage that up-regulates DNA repair factors and inhibits cell division [18, 19]. RecA binding to ssDNA, as would be found in replication gaps, is normally inhibited by the presence of single-strand DNA binding protein (SSB). The RecFOR proteins then act as so-called “mediator” proteins (reviewed in [20]), promoting the binding of RecA to SSB-coated DNA [21–23] and targeting RecA to DNA gaps [24–28]. In vivo, loss of RecF function blocks most gap-filling after UV-irradiation as detected by physical analysis [29]. However, it is interesting that a RecF- independent (and RecB-) gap-filling mechanism can play a minor role. The genetic basis for this latter pathway is currently unknown, but it also contributes to crossover recombination between plasmids [30]. Loss of RecFOR also blocks RecA filament formation and the induction of the SOS transcriptional response, either by UV-irradiation [31, 32] or by incorporation of the chain-terminating nucleotide azidothymidine [14]. Whereas recombination repair of broken forks via RecBCD requires reloading of the DnaB helicase via the PriA primosome replication complex to reestablish replication forks (reviewed in [33]), PriA function is not obligatory for RecFOR HR. This suggests that RecFOR recombination can proceed without a need to reload DnaB, either because DnaB is still present at the fork or because recombination occurs in gDNA left behind a replication fork. Yet, PriA mutants become dependent upon the RecFOR pathway for survival, presumably because of the excess gDNA formation in the absence of PriA-dependent replication restart [34].

TLS gap repair

E. coli has three DNA polymerase that are induced by DNA damage, Pol II (*polB*), Pol IV (*dinB*) and Pol V (*umuD' C*) that mediate translesion synthesis (reviewed in Fuchs 2013). Pol II is the founding member of the B-family of DNA polymerases and, because it contains a proofreading exonuclease domain, it is relatively error-free. Pol IV and Pol V are highly mutagenic Y-family polymerases. Major groove lesions are apparently preferentially bypassed by Pol V, whereas minor groove lesions are bypassed by Pol IV [12]. TLS operates in competition with HR and template-switching repair (see below), with TLS contributing to a lesser extent than the other DNA damage tolerance pathways in post-replication repair [35, 36].

What is template-switching?

Template-switching refers to the realignment of the nascent strand during replication, such that DNA synthesis is initiated from an alternative template: either to another place on the parental strand, to the other sister nascent strand or within the nascent strand itself (reviewed

in [37]). As such, it refers to a constellation of mechanisms (rather than a single mechanism), with the common feature of nascent strand realignment. Template-switching underlies several potential DNA damage tolerance mechanisms that allow replication blocks to be overcome. Although template-switching is usually genetically silent, misalignment of strands during the process or the use of ectopic templates can lead to frameshift mutations, copy number variation of repeats (insertion/deletion or “indels”), genetic rearrangements and mutational hotspots.

Fork reversal template-switching

After a series of transactions that leads to fork reversal, template-switching has been postulated to constitute a repair mechanism to overcome leading strand blocks to replication [38]. At a blocked replication fork, annealing of the 3′ end of the leading nascent strand to the 5′ end of the lagging nascent strand can produce a 4-stranded “chicken-foot” structure (Fig. 2, step 1). Such a reaction was first proposed as a mechanism to allow the fork to back away from a synthesis-blocking lesion so that the lesion may become accessible to excision repair and to facilitate synthesis from an alternative template [38]. Such 4-strand structures are observed in vitro, and are promoted by high positive supercoiling [39]. The short double-strand ended “nub” of the reversed forks (Fig. 2, step 5) would be subject to digestion by dsDNA exonuclease such as *E. coli* RecBCD nuclease; in essence these reactions lead to a “backing-up” or regression of the fork. In the absence of nub digestion, the fork may be subject to cleavage and linearization by Holliday junction cleavage enzymes such as RuvC (Fig. 2 step 4). Michel and colleagues provided evidence for replication fork reversal and cleavage, using a combination of pulsed-field gel electrophoresis and genetic analysis of viability. They found evidence for fork reversal in a number of mutants defective in replication fork progression or after replication fork collision with strongly transcribed regions [40–44]; the laboratory of Guzman implicated replication fork reversal likewise under conditions of depleted deoxyribonucleotide pools [45, 46]. Under these various conditions, if the strain is defective in RecBCD nuclease activity, chromosomal cleavage is observed, dependent on the RuvC resolvase function. McGlynn and Lloyd [47] provided genetic evidence for fork regression after collision of the replisome with transcription machinery, dependent on RecG. Based upon its biochemical properties, they proposed that RecG was required for the template-switch reaction, followed by resetting of the fork without cleavage (Fig. 2 steps 1–3).

Depending upon the source of the replication problem and the configuration of strands at the fork, different factors may drive branch migration during the initial step of replication fork reversal, including RecA, RuvAB or RecG. In some instances, the factor that drives fork reversal is unknown. For example, in a *dnaM*s clamp mutant [43] under conditions of fork collision with highly transcribed genes [44], there are additional unknown factors that can reverse forks. Fork reversal is observed in vitro, catalyzed by supercoiling, RecA, RuvAB, and/or RecG [39, 48–53]. If the 3′ nascent leading strand is recessed relative to the 5′ nascent lagging strand, synthesis from this switched template can occur which, in theory, can provide the information to bypass a blocking lesion, when the fork is reset by branch migration (Fig. 2, step 2) [38]. At least in wild-type *E. coli*, it is unknown whether this occurs in vivo, since the double-end end would be normally subject to digestion by the

voracious RecBCD nuclease (Fig. 2, step 5), rendering the 4-strand Holliday junction-like structure short-lived. Likewise, in vivo fork cleavage is only observed in *recBC* mutants and it is unclear whether it occurs at all in wild-type cells. The default pathway in wild-type cells may be simple fork regression (as in Fig. 2 step 5), in which the fork is reset, backwards from a blocking lesion. Based on the biochemical properties of RecG and PriA and supported by genetic data, Gregg et al. present a different view [54]: that RuvAB preferentially regresses forks with both nascent strands flush to the fork; this pathway obligatorily leads to fork cleavage and subsequent DSB repair. They present biochemical evidence that RecG is well-suited to regress forks with a gap on the leading strand; therefore RecG is required for the template-switch repair for leading strand blocks (Fig. 2, step 2). In their models, PriA is required to restart replication after either regression scenario.

It is unclear whether fork reversal is mutagenic, although one would imagine that the strand realignments during this process could produce mispaired structures in the context of repeated DNA sequences, leading to frameshifts or insertions/deletions (indels). Cleavage of the fork, followed by subsequent recombinational repair, could also produce RecA RecBCD-dependent rearrangements at repetitive sequences. This is seen for a mutant of the DnaB fork helicase [55, 56] that is prone to fork regression [41].

DnaK-dependent, sister-chromosome exchange (SCE)-associated template-switching

A second template-switch mechanism was deduced from the properties of RecA-independent rearrangements at tandem DNA repeats and proposed to constitute a post-replication repair pathway based on the properties of mutants that affect this pathway. Rearrangements between direct repeats, ranging in length of 10s to 100s of nucleotides, occur at high frequency in *E. coli* and are independent of all known recombination functions, such as RecA [57–60].

These RecA-independent events generate both expansions and contractions in the number of repeats and are about 25–30% as efficient as those mediated by RecA-dependent HR [58, 61]. Although RecA-independent copy number variation can be detected involving very short repeats at low frequency [59, 62, 63], these rearrangements are nonetheless homology-dependent [57, 59], reaching a frequency of 10^{-4} to 10^{-3} as the repeats approach 100 nucleotides or so in length. RecA-independent rearrangement rates are strongly dependent on the proximity of the repeats [59, 60, 64], presumably because the two repeats must interact within a single replication fork (see below and Fig. 3, Fig. 4).

RecA-independent rearrangements are elevated in mutants impaired in many aspects of DNA replication such as the DNA polymerase III core (*dnaE* polymerase subunit, *dnaQ* proofreading defective), accessory clamp loader complex (*holC*), single-strand DNA binding protein (*ssb*), or replication restart (*dnaC*, *priA*) [58, 65]. Most surprisingly, these rearrangements are often associated with crossovers between sister chromosomes, suggesting a fundamentally recombinational mechanism despite their independence from RecA, required for homologous recombination pathways.

A template-switch mechanism involving annealing of nascent strands across a blocked fork [58, 61] can explain the generation of RecA-independent rearrangements associated with crossovers (Fig. 3). This mechanism requires discontinuity on the lagging strand and invokes Holliday junction intermediates that can be processed to yield crossovers. In this model, strand exchange involves annealing of ssDNA generated from unwinding of nascent strands but does not involve true strand-invasion into duplex DNA, as does RecA-dependent recombination. Mutations in the Holliday junction branch migration complex, RuvAB, do not block product formation, but are observed to increase the proportion the crossover products that are reciprocal (Fig. 3, pathway A vs. B); this supports the notion that RecA-independent rearrangement intermediates do involve Holliday junction-like structures. The intermediates of this template-switch mechanism, with two branched structures, resemble those proposed for the RecFOR pathway of homologous recombination; however, unlike RecFOR recombination, evidence suggests that the template-switch mechanism occurs predominantly between replicating sister chromosomes and does not occur efficiently by intermolecular interaction with separate homologs [58].

In addition, RecA-independent rearrangements between tandem repeats may also occur by a misalignment of the nascent strand with its template strand [58, 61, 62], an intramolecular template-switch often referred to as “replication slippage” or “slipped mispairing”. (Fig. 4). This type of misalignment does not lead to sister chromosome crossing-over but may be initiated by the same types of events, such as nascent strand unwinding, that elicit the template-switching associated with sister-chromosome exchange (SCE). Effects of mismatch repair on deletion of homeologous (incompletely homologous) repeats argue that rearrangements (RecA-independent deletion, with and without SCE) occur during or very shortly after DNA replication, while the DNA remains hemi-methylated at adenine residues [66, 67]. Using silent genetic markers to define RecA-independent deletion endpoints and reversing the direction of replication through the repeats, slipped mispairing between 101 bp repeats appears to be more frequent on the leading strand [68]. A possible explanation for this bias is the preferential lagging-strand recruitment of ExoI (a 3′ exonuclease that aborts slipped mispairing [69]), through its interaction with SSB [70].

Spontaneously-occurring deletions and duplications in *Escherichia coli* are often found adjacent to inverted repeat (IR) sequences (or palindromes) [71, 72]. As deduced by systematic analysis of such events in *E. coli*, inverted repeats strongly stimulate deletion at nearby direct repeat sequences [62, 72–78]. One study of deletion occurring between 101 bp direct repeats, separated by 100 bp inverted repeats [78], showed that inverted repeats with higher propensity to form cruciform structures in dsDNA were more mutagenic. In this study, the stimulatory effect of palindromic sequences on deletion was greatly enhanced by deficiency in RecA and in mutants of DNA polymerase III (*dnaE486*), presumably because secondary structure formation between the inverted repeats is favored in replication gaps formed under these conditions. Genetic analysis suggested that two pathways contribute to IR-stimulated deletion: one dependent on the SbcCD nuclease (orthologous to eukaryotic Mre11-Rad50-Xrs2/Nbs1) that cleaves secondary structures formed between inverted repeats [79] and one independent of SbcCD. The SbcCD-dependent pathway was deduced to involve cleavage and annealing of resected DNA ends (“single-strand annealing” or “SSA”). Properties of the SbcCD-independent pathway were consistent with template-switching by

slipped misalignment. The skew of deletion endpoints suggested that slipped misalignment promoted by IR was more frequent on the lagging strand, as had been suggested by previous studies [80, 81]. The lagging strand bias is likely explained by the stronger single-strand nature of the lagging-strand template, and a higher propensity to form hairpin structures at the inverted repeat, which then impede DNA replication and induce misalignment. The SbcCD-independent pathway is dependent on DnaK, which is also required for RecA-independent rearrangements between repeats without associated inverted repeats [82] (see below). This suggests that inverted repeats merely potentiate an existing template-switching pathway that can occur in their absence. In contrast, the SbcCD-dependent pathway of IR-stimulated deletion is DnaK-independent [82], consistent with it being mediated by a different, SSA-based mechanism.

Our understanding of the contribution of the template-switching mechanism to postreplication repair has been limited by the lack of genetic information about factors that influence this pathway. The first known factors required for template-switching associated with SCE were the replication processivity clamp and the clamp loader complex [65], that are essential functions for replication. Temperature-sensitive mutants in these functions (*dnaN159* and *dnaX2016*) are DNA replication-defective at restrictive temperatures, have depressed rates of RecA-independent rearrangements at their permissive temperatures and are sensitive to UV irradiation and the replication inhibitors HU and AZT. These results suggest that an interaction of DNA polymerases or other DNA repair factors with the processivity clamp underlies the template-switch mechanism. Later, the chaperone DnaK (Hsp70) was found to be required for RecA-independent rearrangements (with and without SCE) and for DNA damage survival, in a manner genetically epistatic with the the *dnaX2016* allele [82]. DnaK mutants are highly pleiotropic but the effect on template-switching is genetically separable from its function in the heat-shock response. Because DnaK mutants are defective in PolV-dependent UV-induced mutation [83], DnaK likely affects two postreplication pathways, template-switching and translesion synthesis. It is not known whether DnaK affects DNA synthesis mediated by *E. coli*'s other DNA damage-inducible DNA polymerases, DNA Pol II or Pol IV or whether its effect is restricted to Pol V.

What role does DnaK play in the promotion of template-switching? Although DnaK is nonessential for replication of the *E. coli* chromosome, it *is* required for replication of lambda bacteriophage [84] and for F and P1 plasmids [85]. DnaK is required to release lambda P protein from an inactive complex with lambda O and DnaB proteins [86]; its role in plasmid replication is to dissociate and/or refold inactive (RepE and RepA, respectively) [85, 87]. We have proposed that DnaK likewise is required to remodel the replisome complex in such a way to permit template-switch repair. One such interaction is of DNA polymerase III with the nascent strand and template DNA, an interaction which is modulated by interaction with the Tau protein of the clamp loader complex [88]. DnaK may free DNA pol III from its strong interaction with the nascent strand and template to allow unwinding of the 3' nascent strands, in the first step of the template-switch reaction. However, other roles for DnaK in template-switching are possible.

The models for DnaK-dependent template-switch repair invoke two steps that are possibly mediated by proteins, heretofore unidentified. The first involves the unwinding of both nascent 3' strands, a step that is likely mediated by a 5' to 3' DNA helicase. This step permits the annealing of complementary strands and may obviate the need for a strand-invasion protein such as RecA. The second step may involve an annealing protein, comparable to lambda beta protein [89] or the eukaryotic factor, Rad52 [90]. The known annealing factor in *E. coli*, RecO [91, 92], however, does not affect RecA-independent template-switching [58] (Bzymek and Lovett, unpublished results).

Phenotypes exhibited by *dnaK* mutants suggests that the template-switch pathway provides an alternative function to RecA-dependent homologous recombination in post-replication repair. When defective, RecA and DnaK appear to be synthetically lethal [82]. However, double mutants can be constructed with partial-loss-of-function alleles of DnaK (most commonly *dnaK306*, which is defective for template-switch rearrangements but proficient in the heat-shock response [82, 93]). DnaK mutants are sensitive to a broad range of DNA damaging agents, especially those that block DNA replication such as azidothymidine and hydroxyurea (HU) [82]. Indeed, for the replication inhibitors AZT and HU, *dnaK* mutants are more sensitive than those in *recA* (which blocks all HR pathways and TLS); furthermore, *dnaK* mutations show strong genetic synergy when combined with *recA*. DnaK mutants exhibit slow replication [94, 95] and defects in chromosome segregation [96], consistent with a replication defect. In mutants of the Rep helicase, which are also slow to complete replication [97, 98], both RecA and DnaK independently promote viability [99].

Among the spontaneous mutations that inactivate genes, intragenic deletions and duplications between short homologies are common [62, 71, 100] and therefore this mode of template-switching has profound effects on genetic stability. In addition, the first step of gene amplification in bacteria often involves an initial RecA-independent duplication between short homologous sequences [101] that is subsequently expanding to a high-copy, direct repeat array.

Cross-fork, SCE-associated template-switching vs. fork reversal

It is useful to distinguish elements and properties of the mechanisms proposed for cross-fork, DnaK-dependent, SCE-associated template-switching from that of fork reversal (Fig. 5). The cross-fork pathway requires a discontinuity on the lagging strand from which the nascent lagging strand is displaced; fork reversal strand displacement is at the fork junction and requires no such discontinuity on the lagging strand. Cross-fork template-switching allows the 3' end of the lagging strand to promote synthesis; fork reversal permits synthesis only from the 3' end of the nascent leading strand. For these reasons, cross-fork, DnaK-dependent template-switching may be invoked from lagging-strand replication blocks; fork reversal from leading-strand blocks. Resolution of the junctions generated during cross-fork template-switching can lead to RecA-independent crossovers but do not generate DSBs; resolution of the junction created by fork regression can cause DSBs, but does not lead to crossovers of sister chromosomes directly; note that subsequent RecA RecBCD-dependent DSB repair may lead to SCE. Another distinguishing feature is that fork reversal necessarily

occurs at the replication fork whereas cross-fork template-switching can occur at gaps left behind when the fork moves over or is re-primed past a lesion.

Template-switching vs. RecA RecFOR-dependent HR recombination

Using an in vivo daughter strand gap-repair, plasmid-based assay that could distinguish strand transfer from template-switch repair, Izhar et al. [35] estimated that 80% of repair occurred via strand transfer recombination and the remaining 20% via template-switching to a homologous plasmid. Similar results were obtained for gaps formed by both small and bulky replication-blocking lesions. In this assay, most gap-filling (including template-switching) was dependent on RecA (97–98%) and somewhat less so on RecF (72%), although a RecAF-independent component did contribute in a minor fashion. (This might be the DnaK-dependent template-switching pathway). This assay may underestimate the contribution of DnaK-dependent template-switching to repair since the assay demands interactions between homologs; DnaK-dependent template-switching to the sister chromosome, which is more efficient than that to a homolog [58], cannot contribute to the observed repair outcomes.

Quasipalindrome-associated template-switching and mutation hotspots

A different type of template-switch process also contributes to mutations, particular those at observed at mutational hotspots, and is associated with problems in replication. These mutations are found in imperfect inverted repeat sequences, known as “quasipalindromes” (“QPs”). The observed mutations convert the imperfections in the palindromes to form more perfect palindromes (Fig. 6), suggesting a mechanism where one of the inverted repeats templates the synthesis of the other. Mutation hotspots at QP sites are observed in mutational spectra of *rpsL* [102, 103] and *thyA* [104] (Fig. 7C) in *E. coli* and have been noted in bacteriophage [105, 106] (Fig 7B), yeast [107](Fig 7A) and humans [108, 109]. QP-associated mutations (“QPM”) can be base substitutions, frameshifts, short indels or sequence substitutions (complex mutations that involve changes at multiple bases) or even clusters of mutations [102, 104, 106]. Specific mutational reporters in chromosomal *lacZ* have been developed that revert to Lac⁺ specifically by a template-switch [110], which allows factors affecting QP-associated mutation to be more easily ascertained.

To explain a mutational hotspot in bacteriophage T4 (Fig. 7B), Ripley [105] proposed two template-switch models involving strand dissociation and mispairing, either intramolecularly to form a hairpin structure or intermolecularly, across the replication fork (examples diagrammed in Fig. 8). Both of these can explain the known properties of QPM. These mechanisms involve two template-switch reactions: the first switch is to a complementary inverted repeat, within a hairpin (intramolecular, Fig. 8AB) or across the fork (intermolecular, Fig. 8CD) after which the mutation is templated by subsequent DNA synthesis in the misaligned configuration. The second template-switch returns the nascent strand to its normal template. A third model involving mismatch repair excision and synthesis (not shown) can be ruled out by the fact that, for at least the *thyA* mutational hotspot, QPM is not dependent on mismatch repair [104]. Rather, mutation rate at *thyA* QP hotspot was *reduced* by a functional MMR system, in a manner dependent on adenine hemi-

methylation, a state of DNA that exists only for very short time (on the order of minutes) after DNA replication [111]. This suggests that QPM arises during replication, consistent with either intra- or inter-molecular template-switching, and can be corrected by subsequent MMR. Exonucleases I (ExoI) and VII (ExoVII), the major 3' ssDNA exonucleases in *E. coli*, appear to redundantly abort QPM at the *thyA* hotspot and at the *lacZ* QPM reporters; the double ExoI⁻ ExoVII⁻ mutants shows rates of QPM 5–40 fold higher than wild-type strains [104, 110, 112]. This observation is consistent with the template-switching models (Fig. 8), which predict transient dissociation of 3' ended nascent strands, expected to be vulnerable in this state to exonuclease digestion. The SbcCD nuclease has no effect on QPM at *thyA* [104], presumably because the secondary structures formed at the hotspot are too small to be recognized by the enzyme.

Supporting the template-switch model, a base substitution at a position predicted to template a QPM site in *rpsL* did not reduce the strength of the hotspot but changed the mutated residue to that which was complementary to the substitution [103]. Removing potential base pairs before or after the *thyA* QPM hotspot reduced the mutation rate, whereas increasing the number of base pairs elevated mutation rate [112], also supporting a template-switch mechanism. In addition, adjacent silent (for protein coding) sequence changes were always co-mutated with the *thyA* QPM hotspot. One type of intermolecular template-switch, shown in Fig. 8D, involving pairing of the first replicated repeat to the second repeat across the fork, predicts an inversion of the center of the quasipalindrome concomitant with QPM, because synthesis proceeds in a retrograde fashion across the two inverted repeats. Rosche et al. devised a genetic selection for such events (QPM + center inversion), which indeed do occur [113]. Nevertheless, it remains possible that both events occur, with intramolecular template-switching more frequent than intermolecular template-switching, as is suggested by our work with *lacZ* QPM reporters, with and without selection for center inversion (Zilberberg and Lovett, unpublished results).

QPM is influenced by the direction of replication [103, 110, 113–115], consistent with more efficient template-switching on the leading strand. This observation has been used to support an intermolecular template-switching model for QPM [103, 113, 114] because the leading nascent strands pairs with the lagging template strand, which is expected to be more single-stranded (and hence available for pairing) because of discontinuous replication (Fig. 8CD). However, the leading-strand bias of QPM, as evident in the *lacZ* QPM reporters, is lost in strains lacking the major 3' ssDNA exonucleases, ExoI and ExoVII; if anything, there may be a slight bias to lagging-strand events [110]. This suggests that template-switching occurs at roughly equal frequencies on both strands but is more likely to be aborted by exonuclease digestion when it occurs on the lagging strand. Unpublished work shows that Exonuclease I alone is responsible for the leading strand bias in wild-type cells and that its interaction with SSB is necessary for this effect (L. Laranjo and S. T. Lovett, unpublished results). Our other unpublished work suggests that the size of the non-palindromic center of the QP has a strong influence on mutation rate, an observation more consistent with a predominant intramolecular template-switch mechanism (G. Zilberberg and S. T. Lovett, unpublished results).

How do replication fork problems influence QPM? In the *rpsL* gene, some QPM sites are strongly induced by a mutation affecting the DNA Pol III polymerase subunit (*dnaE173*) of DNA pol III [102]. The *dnaE173* mutant exhibits slow replication elongation rates in vivo [116] and the purified core polymerase exhibits a low Km for nucleotide and a proofreading deficit [117]. Mutagenesis at *thyA* QPM hotspot is elevated 11-fold by the loss of all three of *E. coli*'s DNA damage induced polymerases, Pol II, Pol IV and PolV; no single or double mutant shows an effect, suggesting that all three polymerases function in a redundant fashion for QPM avoidance [112]. One possible scenario to explain this effect is that polymerase-switching from Pol III to the SOS polymerases may allow replication through the palindrome that would otherwise stall, leading to QPM; the TLS polymerases may also fill gaps or overcome replication problems that would otherwise promote QPM. Although Pol II and Pol IV have significant levels in the cell in the absence of DNA damage [118, 119], levels of Pol V are very low, under tight multilevel regulation, rising only after DNA damage treatment [120]. An effect of PolV likely means that some cells undergoing QPM are induced for SOS and have hence experienced spontaneous DNA damage.

Certain DNA damaging agents have been shown to be mutagens for QPM using the *lacZ* reporter systems; AZT is a particularly strong QPM mutagen, with no significant effect on base substitutions or frameshift mutations [121]. This is consistent with the idea that persistent nascent strand 3' ends, promoted by AZT incorporation, are more likely to be displaced and to template-switch. Other agents that affect nucleotide pools, such as hydroxyurea, zebularine and an *ndk* mutant, also elevate QPM as detected with the *lacZ* reporters.

QPM, as assayed with the *lacZ* reporters, is not dependent on DnaK (Seier, Pearl-Waserman, Lovett, unpublished) and it is not clear whether there is any connection between the template-switching at quasipalindromes (that yields QPM) and that at direct repeats (that yields DnaK-dependent and RecA-independent rearrangements). It is possible that the spontaneous replication problems that promote QPM differ from those that promote RecA-independent genetic rearrangements and/or the two pathways have different genetic determinants.

Future directions and outstanding questions

The relative contribution of template-switching to replication gap repair has been difficult to establish, both from the lack of specific genes affecting the pathway and from the lack of physical methods to differentiate between it and homologous recombination. Although DnaK is required for template-switching, what molecular role it plays is unknown, nor is it known how the clamp and clamp-loader participate in the process. There is a missing DNA helicase activity to promote nascent-strand unwinding that is proposed to initiate template-switching and, potentially, other missing factors as well. Physical validation of the cross-fork mechanism (Fig. 3) is lacking.

As mentioned above, it remains unknown how much template-switching associated with fork regression (Fig. 2) contributes to DNA damage tolerance and whether it is mutation- or

rearrangement-prone. We are also uncertain how often regressed forks are cleaved in wild-type cells by Holliday junction resolution.

It is not clear how QPM (Fig. 6, Fig. 8) is associated with repair processes. It is highly elevated during DSB repair in yeast [122] but it remains unknown whether QPM is associated with DSB repair or postreplication repair in bacteria. The molecular mechanism by which TLS is antimutagenic for QPM is also not understood. Despite our ignorance of these issues, it is clear that template-switching associated with replication problems contributes significantly to genetic instability, both spontaneously and after DNA damage. Studies in the bacterial systems have greatly aided our mechanistic understanding of these processes and we look forward to studies that will expand our knowledge about these important mechanisms.

Acknowledgments

This work was supported by grants R01 GM51753 and P01 GM105473.

Abbreviations

AZT	Azidothymidine
DSB	Double-strand break
dsDNA	Double-strand DNA
gDNA	Gapped DNA
HR	Homologous recombination
HU	Hydroxyurea
indel	Insertion/deletion
IR	Inverted repeats
QP	Quasipalindrome
QPM	Quasipalindrome-associated mutagenesis
SCE	Sister-chromosome exchange
ssDNA	Single-strand DNA
SSA	Single-strand annealing
TLS	Translesion synthesis

Bibliography

1. Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Marians KJ. The importance of repairing stalled replication forks. *Nature*. 2000; 404:37–41. [PubMed: 10716434]

2. Lehmann AR, Fuchs RP. Gaps and forks in DNA replication: Rediscovering old models. *DNA Repair*. 2006; 5:1495–1498. [PubMed: 16956796]

3. Bichara M, Meier M, Wagner J, Cordonnier A, Lambert IB. Postreplication repair mechanisms in the presence of DNA adducts in *Escherichia coli*. *Mutation Res.* 2011; 727:104–122. [PubMed: 21558018]
4. Mirkin EV, Mirkin SM. Replication fork stalling at natural impediments. *Microbiol Mol Biol Rev.* 2007; 71:13–35. [PubMed: 17347517]
5. Heller RC, Marians KJ. The disposition of nascent strands at stalled replication forks dictates the pathway of replisome loading during restart. *Mol Cell.* 2005; 17:733–743. [PubMed: 15749022]
6. Pagès V, Fuchs RP. Uncoupling of leading- and lagging-strand DNA replication during lesion bypass in vivo. *Science.* 2003; 300:1300–1303. [PubMed: 12764199]
7. Amado L, Kuzminov A. Low-molecular-weight DNA replication intermediates in *Escherichia coli*: mechanism of formation and strand specificity. *J Mol Biol.* 2013; 425:4177–4191. [PubMed: 23876705]
8. Branzei D, Foiani M. Template switching: from replication fork repair to genome rearrangements. *Cell.* 2007; 131:1228–1230. [PubMed: 18160033]
9. Rupp WD, Howard-Flanders P. Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation. *J Mol Biol.* 1968; 31:291–304. [PubMed: 4865486]
10. Rupp WD, Wilde CE, Reno DL, Howard-Flanders P. Exchanges between DNA strands in ultraviolet-irradiated *Escherichia coli*. *J Mol Biol.* 1971; 61:25–44. [PubMed: 4947693]
11. Ganesan AK. Persistence of pyrimidine dimers during post-replication repair in ultraviolet light-irradiated *Escherichia coli* K12. *J Mol Biol.* 1974; 87:103–119. [PubMed: 4610149]
12. Fuchs RP, Fujii S. Translesion DNA synthesis and mutagenesis in prokaryotes. *Cold Spring Harb Perspect Biol.* 2013; 5:a012682. [PubMed: 24296168]
13. Lovett ST. Encoded errors: mutations and rearrangements mediated by misalignment at repetitive DNA sequences. *Mol Microbiol.* 2004; 52:1243–1253. [PubMed: 15165229]
14. Cooper DL, Lovett ST. Toxicity and tolerance mechanisms for azidothymidine, a replication gap-promoting agent, in *Escherichia coli*. *DNA Repair (Amst).* 2011; 10:260–270. [PubMed: 21145792]
15. Kowalczykowski SC, Dixon DA, Eggleston AK, Lauder SD, Rehrauer WM. Biochemistry of homologous recombination in *Escherichia coli*. *Microbiol Rev.* 1994; 58:401–465. [PubMed: 7968921]
16. Persky NS, Lovett ST. Mechanisms of recombination: lessons from *E. coli*. *Crit Rev Biochem Mol Biol.* 2008; 43:347–370. [PubMed: 19016098]
17. Michel B, Leach D. Homologous Recombination-Enzymes and Pathways. *EcoSal Plus.* 2012; 5
18. Simmons LA, Foti JJ, Cohen SE, Walker GC. The SOS regulatory network. *EcoSal Plus.* 2009; 1–60.
19. Kreuzer KN. DNA Damage Responses in Prokaryotes: Regulating Gene Expression, Modulating Growth Patterns, and Manipulating Replication Forks. *Cold Spring Harbor perspectives in biology.* 2013; 5:a012674–a012674. [PubMed: 24097899]
20. Korolev S. Advances in structural studies of recombination mediator proteins. *Biophysical Chemistry.* 2016
21. Umez K, Chi NW, Kolodner RD. Biochemical interaction of the *Escherichia coli* RecF, RecO, and RecR proteins with RecA protein and single-stranded DNA binding protein. *Proc Natl Acad Sci USA.* 1993; 90:3875–3879. [PubMed: 8483906]
22. Umez K, Kolodner RD. Protein interactions in genetic recombination in *Escherichia coli*. Interactions involving RecO and RecR overcome the inhibition of RecA by single-stranded DNA-binding protein. *J Biol Chem.* 1994; 269:30005–30013. [PubMed: 7962001]
23. Shan Q, Bork JM, Webb BL, Inman RB, Cox MM. RecA protein filaments: end-dependent dissociation from ssDNA and stabilization by RecO and RecR proteins. *J Mol Biol.* 1997; 265:519–540. [PubMed: 9048946]
24. Hegde SP, Qin MH, Li XH, Atkinson MA, Clark AJ, Rajagopalan M, Madiraju MV. Interactions of RecF protein with RecO, RecR, and single-stranded DNA binding proteins reveal roles for the RecF-RecO-RecR complex in DNA repair and recombination. *Proc Natl Acad Sci USA.* 1996; 93:14468–14473. [PubMed: 8962075]

25. Webb BL, Cox MM, Inman RB. Recombinational DNA repair: the RecF and RecR proteins limit the extension of RecA filaments beyond single-strand DNA gaps. *Cell*. 1997; 91:347–356. [PubMed: 9363943]
26. Morimatsu K, Kowalczykowski SC. RecFOR proteins load RecA protein onto gapped DNA to accelerate DNA strand exchange: a universal step of recombinational repair. *Mol Cell*. 2003; 11:1337–1347. [PubMed: 12769856]
27. Makharashvili N, Mi T, Koroleva O, Korolev S. RecR-mediated modulation of RecF dimer specificity for single- and double-stranded DNA. *The J Biol Chem*. 2009; 284:1425–1434. [PubMed: 19017635]
28. Morimatsu K, Wu Y, Kowalczykowski SC. RecFOR proteins target RecA protein to a DNA gap with either DNA or RNA at the 5' terminus: implication for repair of stalled replication forks. *J Biol Chem*. 2012; 287:35621–35630. [PubMed: 22902627]
29. Wang TV, Smith KC. *recF*-dependent and *recF recB*-independent DNA gap-filling repair processes transfer dimer-containing parental strands to daughter strands in *Escherichia coli* K-12 uvrB. *J Bacteriol*. 1984; 158:727–729. [PubMed: 6373727]
30. Lovett ST, Hurley RL, Sutura VA, Aubuchon RH, Lebedeva MA. Crossing over between regions of limited homology in *Escherichia coli*. RecA-dependent and RecA-independent pathways. *Genetics*. 2002; 160:851–859. [PubMed: 11901106]
31. Thoms B, Wackernagel W. Regulatory role of *recF* in the SOS response of *Escherichia coli*: impaired induction of SOS genes by UV irradiation and nalidixic acid in a *recF* mutant. *J Bacteriol*. 1987; 169:1731–1736. [PubMed: 3031020]
32. Hegde S, Sandler SJ, Clark AJ, Madiraju MV. *recO* and *recR* mutations delay induction of the SOS response in *Escherichia coli*. *Mol Gen Genet*. 1995; 246:254–258. [PubMed: 7862097]
33. Gabbai CB, Marians KJ. Recruitment to stalled replication forks of the PriA DNA helicase and replisome-loading activities is essential for survival. *DNA Repair*. 2010; 9:202–209. [PubMed: 20097140]
34. Grompone G, Sanchez N, Dusko Ehrlich S, Michel B. Requirement for RecFOR-mediated recombination in *priA* mutant. 2004; 52:551–562.
35. Izhar L, Goldsmith M, Dahan R, Geacintov N, Lloyd RG, Livneh Z. Analysis of strand transfer and template switching mechanisms of DNA gap repair by homologous recombination in *Escherichia coli*: predominance of strand transfer. *J Mol Biol*. 2008; 381:803–809. [PubMed: 18585391]
36. Fuchs RP. Tolerance of lesions in *E. coli*: chronological competition between translesion synthesis and damage avoidance. *DNA Repair*. 2016; 44:51–58. [PubMed: 27321147]
37. Lovett ST. Encoded errors: mutations and rearrangements mediated by misalignment at repetitive DNA sequences. *Mol Microbiol*. 2004; 52:1243–1253. [PubMed: 15165229]
38. Higgins NP, Kato K, Strauss B. A model for replication repair in mammalian cells. *J Mol Biol*. 1976; 101:417–425. [PubMed: 1255724]
39. Postow L, Ullsperger C, Keller RW, Bustamante C, Vologodskii AV, Cozzarelli NR. Positive torsional strain causes the formation of a four-way junction at replication forks. *J Biol Chem*. 2001; 276:2790–2796. [PubMed: 11056156]
40. Seigneur M, Bidnenko V, Ehrlich SD, Michel B. RuvAB acts at arrested replication forks. *Cell*. 1998; 95:419–430. [PubMed: 9814711]
41. Seigneur M, Ehrlich SD, Michel B. RuvABC-dependent double-strand breaks in *dnaBts* mutants require *recA*. *Mol Microbiol*. 2000; 38:565–574. [PubMed: 11069680]
42. Flores MJ, Bierne H, Ehrlich SD, Michel B. Impairment of lagging strand synthesis triggers the formation of a RuvABC substrate at replication forks. *EMBO J*. 2001; 20:619–629. [PubMed: 11157768]
43. Grompone G, Seigneur M, Ehrlich SD, Michel B. Replication fork reversal in DNA polymerase III mutants of *Escherichia coli*: a role for the beta clamp. *Mol Microbiol*. 2002; 44:1331–1339. [PubMed: 12028381]
44. De Septenville AL, Duigou S, Boubakri H, Michel B. Replication fork reversal after replication-transcription collision. *PLoS Genetics*. 2012; 8:e1002622. [PubMed: 22496668]

45. Guarino E, Salguero I, Jiménez-Sánchez A, Guzmán EC. Double-strand break generation under deoxyribonucleotide starvation in *Escherichia coli*. *J Bacteriol.* 2007; 189:5782–5786. [PubMed: 17526701]
46. Salguero I, Guarino E, Guzmán EC. RecA-dependent replication in the *nrdA101*(Ts) mutant of *Escherichia coli* under restrictive conditions. 2011; 193:2851–2860.
47. McGlynn P, Lloyd RG. Modulation of RNA polymerase by (p)ppGpp reveals a RecG-dependent mechanism for replication fork progression. *Cell.* 2000; 101:35–45. [PubMed: 10778854]
48. McGlynn P, Lloyd RG, Marians KJ. Formation of Holliday junctions by regression of nascent DNA in intermediates containing stalled replication forks: RecG stimulates regression even when the DNA is negatively supercoiled. *Proc Natl Acad Sci USA.* 2001; 98:8235–8240. [PubMed: 11459958]
49. Robu ME, Inman RB, Cox MM. RecA protein promotes the regression of stalled replication forks in vitro. *Proc Natl Acad Sci USA.* 2001; 98:8211–8218. [PubMed: 11459955]
50. Robu ME, Inman RB, Cox MM. Situational repair of replication forks: roles of RecG and RecA proteins. *J Biol Chem.* 2004; 279:10973–10981. [PubMed: 14701860]
51. Gupta S, Yeeles JTP, Marians KJ. Regression of replication forks stalled by leading-strand template damage: I. Both RecG and RuvAB catalyze regression, but RuvC cleaves the holliday junctions formed by RecG preferentially. *J Biol Chem.* 2014; 289:28376–28387. [PubMed: 25138216]
52. Gupta S, Yeeles JTP, Marians KJ. Regression of replication forks stalled by leading-strand template damage: II. Regression by RecA is inhibited by SSB. *J Biol Chem.* 2014; 289:28388–28398. [PubMed: 25138217]
53. Bianco PR, Lyubchenko YL. SSB and the RecG DNA helicase: An intimate association to rescue a stalled replication fork. *Protein Sci.* 2017
54. Gregg AV, McGlynn P, Jaktaji RP, Lloyd RG. Direct rescue of stalled DNA replication forks via the combined action of PriA and RecG helicase activities. *Mol Cell.* 2002; 9:241–251. [PubMed: 11864599]
55. Saveson CJ, Lovett ST. Tandem repeat recombination induced by replication fork defects in *Escherichia coli* requires a novel factor, RadC. *Genetics.* 1999; 152:5–13. [PubMed: 10224240]
56. Lovett ST. Replication arrest-stimulated recombination: Dependence on the RecA paralog, Rada/Sms and translesion polymerase, DinB. *DNA Repair (Amst).* 2006; 5:1421–1427. [PubMed: 16904387]
57. Dianov GL, Kuzminov AV, Mazin AV, Salganik RI. Molecular mechanisms of deletion formation in *Escherichia coli* plasmids. I. Deletion formation mediated by long direct repeats. *Mol Gen Genet.* 1991; 228:153–159. [PubMed: 1679524]
58. Lovett ST, Drapkin PT, Sutera VA Jr, Gluckman-Peskind TJ. A sister-strand exchange mechanism for *recA*-independent deletion of repeated DNA sequences in *Escherichia coli*. *Genetics.* 1993; 135:631–642. [PubMed: 8293969]
59. Bi X, Liu LF. *recA*-independent and *recA*-dependent intramolecular plasmid recombination: differential homology requirement and distance effect. *J Mol Biol.* 1994; 235:414–423. [PubMed: 8289271]
60. Lovett ST, Gluckman TJ, Simon PJ, Sutera VA, Drapkin PT. Recombination between repeats in *Escherichia coli* by a *recA*-independent, proximity-sensitive mechanism. *Mol Gen Genet.* 1994; 245:294–300. [PubMed: 7816039]
61. Morag AS, Saveson CJ, Lovett ST. Expansion of DNA repeats in *Escherichia coli*: effects of recombination and replication functions. *J Mol Biol.* 1999; 289:21–27. [PubMed: 10339402]
62. Albertini AM, Hofer M, Calos MP, MJH. On the formation of spontaneous deletion: the importance of short sequence homologies in the generation of large deletions. *Cell.* 1982; 29:319–328. [PubMed: 6288254]
63. Mazin AV, Kuzminov AV, Dianov GL, Salganik RI. Mechanisms of deletion formation in *Escherichia coli* plasmids. II. Deletions mediated by short direct repeats. *Mol Gen Genet.* 1991; 228:209–214. [PubMed: 1679526]
64. Chedin F, Dervyn E, Dervyn R, Ehrlich SD, Noirot P. Frequency of deletion formation decreases exponentially with distance between short direct repeats. *Mol Microbiol.* 1994; 12:561–569. [PubMed: 7934879]

65. Saveson CJ, Lovett ST. Enhanced deletion formation by aberrant DNA replication in *Escherichia coli*. Genetics. 1997; 146:457–470. [PubMed: 9177997]
66. Lovett ST, Feschenko VV. Stabilization of diverged tandem repeats by mismatch repair: evidence for deletion formation via a misaligned replication intermediate. Proc Natl Acad Sci USA. 1996; 93:7120–7124. [PubMed: 8692955]
67. Bzymek M, Saveson CJ, Feschenko VV, Lovett ST. Slipped misalignment mechanisms of deletion formation: in vivo susceptibility to nucleases. J Bacteriol. 1999; 181:477–482. [PubMed: 9882661]
68. Bzymek M, Lovett ST. Evidence for two mechanisms of palindrome-stimulated deletion in *Escherichia coli*: single-strand annealing and replication slipped mispairing. Genetics. 2001; 158:527–540. [PubMed: 11404319]
69. Feschenko VV, Rajman LA, Lovett ST. Stabilization of perfect and imperfect tandem repeats by single-strand DNA exonucleases. Proc Natl Acad Sci USA. 2003; 100:1134–1139. [PubMed: 12538867]
70. Lu D, Windsor MA, Gellman SH, Keck JL. Peptide inhibitors identify roles for SSB C-terminal residues in SSB/exonuclease I complex formation. Biochem. 2009; 48:6764–6771. [PubMed: 19527069]
71. Galas DJ. An analysis of sequence repeats in the lacI gene of *Escherichia coli*. J Mol Biol. 1978; 126:858–863. [PubMed: 370409]
72. Glickman BW, Ripley LS. Structural intermediates of deletion mutagenesis: a role for palindromic DNA. Proc Natl Acad Sci USA. 1984; 81:512–516. [PubMed: 6582506]
73. Foster TJ, Lundblad V, Hanley-Way S, Halling SM, Kleckner N. Three Tn 10-associated excision events: relationship to transposition and role of direct and inverted repeats. Cell. 1981; 23:215–227. [PubMed: 6260376]
74. Singer BS, Westlye J. Deletion formation in bacteriophage T4. J Mol Biol. 1988; 202:233–243. [PubMed: 3172217]
75. Weston-Hafer D, Berg DE. Palindromy and the location of deletion endpoints in *Escherichia coli*. Genetics. 1989; 121:651–658. [PubMed: 2656400]
76. Pierce JC, Kong D, Masker W. The effect of the length of direct repeats and the presence of palindromes on deletion between directly repeated DNA sequences in bacteriophage T7. Nucleic Acids Res. 1991; 14:3901–3905.
77. Sinden RR, Zheng G, Brankamp RG, Allen KN. On the deletion of inverted repeated DNA in *Escherichia coli*: effects of length thermal stability, and cruciform formation *in vivo*. Genetics. 1991; 129:991–1005. [PubMed: 1783300]
78. Bzymek M, Lovett ST. Evidence for two mechanisms of palindrome-stimulated deletion in *Escherichia coli*: single-strand annealing and replication slipped mispairing. Genetics. 2001; 158:527–540. [PubMed: 11404319]
79. Connelly JC, Kirkham LA, Leach DR. The SbcCD nuclease of *Escherichia coli* is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. Proc Natl Acad Sci USA. 1998; 95:7969–7974. [PubMed: 9653124]
80. Trinh TQ, Sinden RR. Preferential DNA secondary structure mutagenesis in the lagging strand of replication in *E. coli*. Nature. 1991; 352:544–547. [PubMed: 1865910]
81. Pinder DJ, Blake CE, Lindsey JC, Leach DR. Replication strand preference for deletions associated with DNA palindromes. Mol Microbiol. 1998; 28:719–727. [PubMed: 9643540]
82. Goldfless SJ, Morag AS, Belisle KA, Suter VA Jr, Lovett ST. DNA repeat rearrangements mediated by DnaK-dependent replication fork repair. Mol Cell. 2006; 21:595–604. [PubMed: 16507358]
83. Petit MA, Bedale W, Osipiuk J, Lu C, Rajagopalan M, McInerney P, Goodman MF, Echols H. Sequential folding of UmuC by the Hsp70 and Hsp60 chaperone complexes of *Escherichia coli*. J Biol Chem. 1994; 269:23824–23829. [PubMed: 7916347]
84. Liberek K, Georgopoulos C, Zylicz M. Role of the *Escherichia coli* DnaK and DnaJ heat shock proteins in the initiation of bacteriophage lambda DNA replication. Proc Natl Acad Sci USA. 1988; 85:6632–6636. [PubMed: 2970643]
85. Chattoraj DK. Role of molecular chaperones in the initiation of plasmid DNA replication. Genet Eng (N Y). 1995; 17:81–98. [PubMed: 7779516]

86. Hoffmann HJ, Lyman SK, Lu C, Petit MA, Echols H. Activity of the Hsp70 chaperone complex--DnaK, DnaJ, and GrpE--in initiating phage lambda DNA replication by sequestering and releasing lambda P protein. *Proc Natl Acad Sci USA*. 1992; 89:12108–12111. [PubMed: 1361234]
87. Matsunaga F, Ishiai M, Kobayashi G, Uga H, Yura T, Wada C. The central region of RepE initiator protein of mini-F plasmid plays a crucial role in dimerization required for negative replication control. *J Mol Biol*. 1997; 274:27–38. [PubMed: 9398513]
88. Leu FP, Georgescu R, O'Donnell M. Mechanism of the *E. coli* tau processivity switch during lagging-strand synthesis. *Mol Cell*. 2003; 11:315–327. [PubMed: 12620221]
89. Muniyappa K, Radding CM. The homologous recombination system of phage lambda. Pairing activities of beta protein. *J Biol Chem*. 1986; 261:7472–7478. [PubMed: 2940241]
90. Mortensen UH, Bendixen C, Sunjevaric I, Rothstein R. DNA strand annealing is promoted by the yeast Rad52 protein. *Proc Natl Acad Sci USA*. 1996; 93:10729–10734. [PubMed: 8855248]
91. Luisi-DeLuca C, Kolodner R. Purification and characterization of the *Escherichia coli* RecO protein. Renaturation of complementary single-stranded DNA molecules catalyzed by the RecO protein. *J Mol Biol*. 1994; 236:124–138. [PubMed: 8107098]
92. Kantake N, Madiraju MVVM, Sugiyama T, Kowalczykowski SC. *Escherichia coli* RecO protein anneals ssDNA complexed with its cognate ssDNA-binding protein: A common step in genetic recombination. *Proc Natl Acad Sci USA*. 2002; 99:15327–15332. [PubMed: 12438681]
93. Wild J, Kamath-Loeb A, Ziegelhoffer E, Lonetto M, Kawasaki Y, Gross CA. Partial loss of function mutations in DnaK, the *Escherichia coli* homologue of the 70-kDa heat shock proteins, affect highly conserved amino acids implicated in ATP binding and hydrolysis. *Proc Natl Acad Sci USA*. 1992; 89:7139–7143. [PubMed: 1386674]
94. Bukau B, Walker GC. Cellular defects caused by deletion of the *Escherichia coli* *dnaK* gene indicate roles for heat shock protein in normal metabolism. *J Bacteriol*. 1989; 171:2337–2346. [PubMed: 2651398]
95. Ohki M, Smith CL. Tracking bacterial DNA replication forks in vivo by pulsed field gel electrophoresis. *Nucleic Acids Res*. 1989; 17:3479–3490. [PubMed: 2657661]
96. Bukau B, Walker GC. Delta *dnaK52* mutants of *Escherichia coli* have defects in chromosome segregation and plasmid maintenance at normal growth temperatures. *J Bacteriol*. 1989; 171:6030–6038. [PubMed: 2681151]
97. Lane HE, Denhardt DT. The rep mutation. IV. Slower movement of replication forks in *Escherichia coli* rep strains. *J Mol Biol*. 1975; 97:99–112. [PubMed: 1100854]
98. Atkinson J, Gupta MK, McGlynn P. Interaction of Rep and DnaB on DNA. *Nucleic Acids Res*. 2011; 39:1351–1359. [PubMed: 20959294]
99. Bredeche MF, Ehrlich SD, Michel B. Viability of *rep recA* mutants depends on their capacity to cope with spontaneous oxidative damage and on the DnaK chaperone protein. *J Bacteriol*. 2001; 183:2165–2171. [PubMed: 11244053]
100. Farabaugh PJ, Schmeissner U, Hofer M, Miller JH. Genetic studies of the *lac* repressor. VII. On the molecular nature of spontaneous hotspots in the *lacI* gene of *Escherichia coli*. *J Mol Biol*. 1978; 126:847–857. [PubMed: 370408]
101. Reams AB, Roth JR. Mechanisms of gene duplication and amplification. *Cold Spring Harb Perspect Biol*. 2015; 7:a016592. [PubMed: 25646380]
102. Mo JY, Maki H, Sekiguchi M. Mutational specificity of the dnaE173 mutator associated with a defect in the catalytic subunit of DNA polymerase III of *Escherichia coli*. *J Mol Biol*. 1991; 222:925–936. [PubMed: 1762158]
103. Yoshizawa K, Higuchi K, Matsumura H, Maki H. Directionality of DNA replication fork movement strongly affects the generation of spontaneous mutations in *Escherichia coli*. *J Mol Biol*. 2001; 307:1195–1206. [PubMed: 11292335]
104. Viswanathan M, Lacirignola JJ, Hurley RL, Lovett ST. A novel mutational hotspot in a natural quasipalindrome in *Escherichia coli*. *J Mol Biol*. 2000; 302:553–564. [PubMed: 10986118]
105. Ripley LS. Model for the participation of quasi-palindromic DNA sequences in frameshift mutation. *Proc Natl Acad Sci USA*. 1982; 79:4128–4132. [PubMed: 7051004]

106. de Boer JG, Ripley LS. Demonstration of the production of frameshift and base-substitution mutations by quasipalindromic DNA sequences. *Proc Natl Acad Sci USA*. 1984; 81:5528–5531. [PubMed: 6089210]
107. Hampsey DM, Ernst JF, Stewart JW, Sherman F. Multiple base-pair mutations in yeast. *J Mol Biol*. 1988; 201:471–486. [PubMed: 2843649]
108. Greenblatt MS, Grollman AP, Harris CC. Deletions and insertions in the p53 tumor suppressor gene in human cancers: confirmation of the DNA polymerase slippage/misalignment model. *Cancer Res*. 1996; 56:2130–2136. [PubMed: 8616861]
109. Bissler JJ. DNA inverted repeats and human disease. *Front Biosci*. 1998; 3:d408–418. [PubMed: 9516381]
110. Seier T, Padgett DR, Zilberberg G, Sutera VA Jr, Toha N, Lovett ST. Insights into mutagenesis using *Escherichia coli* chromosomal *lacZ* Strains that enable detection of a wide spectrum of mutational events. *Genetics*. 2011:247–262. [PubMed: 21441210]
111. Campbell JL, Kleckner N. *E. coli oriC* and the *dnaA* gene promoter are sequestered from dam methyltransferase following the passage of the chromosomal replication fork. *Cell*. 1990; 62:967–979. [PubMed: 1697508]
112. Dutra BE, Lovett ST. Cis and trans-acting effects on a mutational hotspot involving a replication template switch. *J Mol Biol*. 2006; 356:300–311. [PubMed: 16376936]
113. Rosche WA, Trinh TQ, Sinden RR. Leading strand specific spontaneous mutation corrects a quasipalindrome by an intermolecular strand switch mechanism. *J Mol Biol*. 1997; 269:176–187. [PubMed: 9191063]
114. Rosche WA, Ripley LS, Sinden RR. Primer-template misalignments during leading strand DNA synthesis account for the most frequent spontaneous mutations in a quasipalindromic region in *Escherichia coli*. *J Mol Biol*. 1998; 284:633–646. [PubMed: 9826504]
115. Yoshiyama K, Maki H. Spontaneous hotspot mutations resistant to mismatch correction in *Escherichia coli*: transcription-dependent mutagenesis involving template-switching mechanisms. *J Mol Biol*. 2003; 327:7–18. [PubMed: 12614604]
116. Pham TM, Tan KW, Sakumura Y, Okumura K, Maki H, Akiyama MT. A single-molecule approach to DNA replication in *Escherichia coli* cells demonstrated that DNA polymerase III is a major determinant of fork speed. *Mol Microbiol*. 2013; 90:584–596. [PubMed: 23998701]
117. Maki H, Mo JY, Sekiguchi M. A strong mutator effect caused by an amino acid change in the alpha subunit of DNA polymerase III of *Escherichia coli*. *J Biol Chem*. 1991; 266:5055–5061. [PubMed: 2002048]
118. Qiu Z, Goodman MF. The *Escherichia coli polB* locus is identical to *dinA*, the structural gene for DNA polymerase II. Characterization of Pol II purified from a *polB* mutant. *J Biol Chem*. 1997; 272:8611–8617. [PubMed: 9079692]
119. Kim SR, Matsui K, Yamada M, Gruz P, Nohmi T. Roles of chromosomal and episomal *dinB* genes encoding DNA pol IV in targeted and untargeted mutagenesis in *Escherichia coli*. *Mol Genet Genomics*. 2001; 266:207–215. [PubMed: 11683261]
120. Woodgate R, Ennis DG. Levels of chromosomally encoded Umu proteins and requirements for in vivo UmuD cleavage. *Mol Gen Genetics*. 1991; 229:10–16.
121. Seier T, Zilberberg G, Zeiger DM, Lovett ST. Azidothymidine and other chain terminators are mutagenic for template-switch-generated genetic mutations. *Proc Natl Acad Sci USA*. 2012; 109:6171–6174. [PubMed: 22474374]
122. Hicks WM, Kim M, Haber JE. Increased mutagenesis and unique mutation signature associated with mitotic gene conversion. *Science*. 2010; 329:82–85. [PubMed: 20595613]

Figure 1.

Post-replication repair pathways that operate in gap-filling. A. Homologous recombination is initiated by RecFOR promoted RecA binding to ssDNA gaps. The RecA filament on ssDNA signals induction of the SOS DNA damage response and initiates strand invasion of the gap with the duplex DNA of the sister chromosome. Resolution of a double Holliday junction by RuvABC restores an intact chromosome. B. Translesion synthesis involving the exchange of Pol II, IV or V for the replicative Pol III polymerase can fill gaps, especially those caused by template lesions. C. A cross-fork template switch pathway can provide an alternative template for nascent strands. Due to mispairing of nascent strands in the annealing step, this pathway can lead to RecA-independent rearrangements between tandem direct repeats. This mechanism can also lead to crossovers between sister chromosome (see Fig. 3).

Figure 2.

Fork reversal and template-switching. Step 1: A replication fork is blocked by a leading strand lesion (blue triangle). Branch migration factors drive reversal of the fork, allowing the nascent strands to pair. This reversal generates a Holliday junction, with one arm as a double-strand ended "nub". Step 2: If the 3' nascent strand is recessed relative to the 5' nascent strand, synthesis can occur from this alternative template. Step 3: Reversed branch migration regenerates a fork structure, with the newly synthesized DNA bypassing the blocking lesion. Step 4: Alternatively, cleavage of the Holliday junction can break the fork, generating a double-strand end that can be repaired by subsequent RecABCD-mediated DSB repair. Step 5: From the 4-stranded reversed fork, the nub may be degraded by RecBCD exonuclease. The fork has backed away from the blocking lesion and, because it is now in a dsDNA, can be repaired by excision repair (or other repair processes).

Fig. 3. SCE-associated template-switching mechanism for deletion formation between repeated sequences. In a replication fork blocked on the lagging strand, nascent strands are unwound. Mispairing of the nascent strands at direct repeats (highlighted in blue) and Holliday junction resolution (indicated by scissors) generates: A. a reciprocal crossover product between sister chromosomes, with one deletion and a triplication; or B. a non-reciprocal crossover product with a deletion and the original duplication. Similar structures with different mispairing at the repeats can generate a triplication associated with SCE (see [61]).

Fig. 4.

Simple replication “slippage” (“slipped mispairing”) mechanism for RecA-independent deletion between direct repeats. Replication stalls during replication of a tandem direct repeat. The nascent strand is unwound, which allows it to mispair with the downstream repeat. This mispaired structure will generate a deletion. Similar events with synthesis of both repeats, unwinding and mispairing to the template at the first repeat can generate a triplication (see [61]).

Fig. 5.

Comparison of template-switching mediated by: A. Cross-fork pairing; and B. Fork reversal. A. Unwinding is initiated from the 3' ends of the leading nascent strand and 3' end of a blocked lagging strand. RecA-independent pairing of nascent strands generates two Holliday junctions that can be resolved to yield RecA-independent crossover products. B. Unwinding is initiated from the 3' end of the nascent leading strand and 5' end of the nascent lagging strand. This produces a Holliday junction whose cleavage generates a double-strand break. Subsequent repair of this break could yield crossovers, although unlike cross-fork template switching, these crossovers would be RecA-dependent.

Fig. 6.

Quasipalindromes (imperfect palindromes). These are inverted repeats with “imperfections” shown in pink and blue. Such sequences can form hairpin and cruciform structures. An intramolecular template switch from the nascent strand to itself (A) can template a mutational change, that upon a second, restoration template switch (B) generates the sequence change (boxed). Note that depending on the direction of replication, different sides of the quasipalindrome are mutated. A leading strand template switch generates a mutation in the rightward repeat, as shown; a lagging strand template switch generates a mutation in the leftward repeat.

Fig. 7.

Quasipalindrome-associated mutation (QPM) hotspots. A. The first recognized QPM site, in *Saccharomyces cerevisiae CYC1*, original sequence at left and mutated product at right. B. QPM site in bacteriophage T4 *rII* gene identified by Ripley et al. [106] C. The *E. coli thyA* QPM hotspot that accounts for 60% of mutations that inactivate the gene. Shown are the three types of mutational products recovered. The most common product mutates *thyA*131 alone (the middle imperfect site). In ExoI⁻ ExoVII⁻ strains, a complex, co-mutation at *thyA*124-127 (the bottom imperfect site) occurs frequently. More rarely, a single mutation at *thyA*124-127 is recovered.