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Review

Template-switching during replication fork repair in bacteria

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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.

1. Introduction

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 an 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 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 consequences 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).

2. 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).

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

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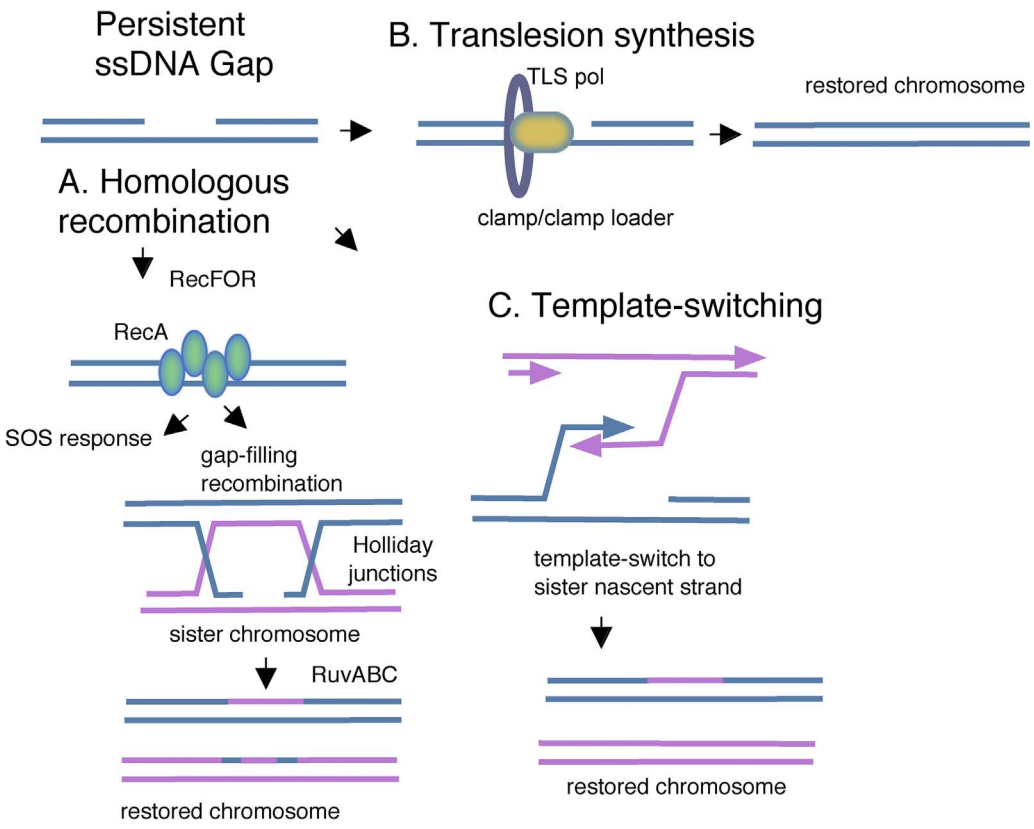

Fig. 1. Post-replication repair pathways that operate in gap-filling. A. Homologous recombination is initiated by RecFOR promoted RecA binding to ssDNA gaps. The RecA filament on ssDNA signals induction of the SOS DNA damage response and initiates strand invasion of the gap with the duplex DNA of the 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 the 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).

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 may 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 HR and template-switching. 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.

3. 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 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, 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].

4. 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].