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Review

Ubiquitin family modifications and template switching

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ABSTRACT

Homologous recombination plays an important role in the maintenance of genome integrity. Arrested forks and DNA lesions trigger strand annealing events, called template switching, which can provide for accurate damage bypass, but can also lead to chromosome rearrangements. Advances have been made in understanding the underlying mechanisms for these events and in elucidating the factors involved. Ubiquitin- and SUMO-mediated modification pathways have emerged as key players in regulating damage-induced template switching. Here I review the biological significance of template switching at the nexus of DNA replication and recombination, and the role of ubiquitin-like modifications in mediating and controlling this process.

1. Introduction

Many endogenous and exogenous events, such as transcription, DNA packaging proteins, developmentally regulated genome rearrangements, as well as chemical and physical agents in the environment give rise to DNA lesions. Although DNA damage needs to be repaired or bypassed in order to ensure cell survival and replication completion, the repair and DNA damage tolerance (DDT) processes are often error-prone, leading to mutations and alterations in chromosome structure, such as chromosome translocations, partial deletions and amplifications. These chromosome alterations are associated with various disorders including developmental defects, neurodegeneration and cancer [1]. Chromosomal translocations are thought to arise mainly through inappropriate repair of DNA double strand breaks (DSBs) or by replication fork slippage events, some of which are called "template switching" [2]. In addition, single strand (ss) DNA gaps or breaks (SSBs) generated during replication or as intermediates in other repair processes can trigger recombination or lead to subsequent formation of DSBs. Cells have evolved two major pathways to repair DSBs: homologous recombination (HR), which requires the presence of a homologous template and generally provides for accurate repair in S and G2 phases of the cell cycle [3], and non-homologous end-joining (NHEJ), which is often mutagenic, does not require a template DNA and can operate throughout the cell cycle [4]. Although all HR reactions involve a switch to a homologous sequence that becomes template for repair, "template switching" is not generally used to refer to this step of HR.

Genetic studies have drawn the attention to the existence of damage tolerance mechanisms that promoted damage bypass and/or repeat-induced rearrangements. Based on the genetic requirements for these events, it was inferred that they were unlikely to initiate from DSBs but rather by strand annealing mechanisms that involved a switch of template strands during replication [5–10]. Therefore these events were said to arise through "template switching" [11].

This review focuses on replication fork responses to stresses such as DNA damage, replication fork barriers (RFB), and repetitive elements, which are likely to trigger template switching. After a general overview of template switching and how it is initiated under different replication stress contexts, I outline recent insights regarding the contribution of different factors and I highlight the role of ubiquitin-like modifications in mediating and controlling template switching. For a more general description of the role of ubiquitin modifications in HR-mediated DSB repair and/or replication, the readers are directed to other recent reviews addressing these topics [12–15].

2. Template switching in the context of HR and replication

HR plays a major role in promoting repair of DSBs using a homologous template (a sister chromatid or a homologous chromosome) and in promoting restart when progression of the replication fork is blocked (reviewed in [16,17]). The strand exchange

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step in HR is mediated by nucleation of Rad51 on RPA-coated ssDNA. The Rad51 nucleoprotein filament then catalyzes homologous pairing and invasion of a donor duplex to form a D-loop structure that initiates repair synthesis [3,18]. It has been shown that recombination between direct and inverted repeats can occur in the absence of Rad51, in a manner dependent on Rad59 [8,19,20]. Rad59, homologous with the DNA binding domain of Rad52, which is required for all HR reactions [3,18], promotes single strand annealing (SSA), but is unable to stimulate Rad51-mediated strand exchange [8,21–23]. These events, together with damage tolerance mechanisms mediated by *RAD18–RAD6* genes [5,6,11,24] (see below), were proposed to occur by template switching during replication.

The models accounting for template switching emphasize the importance of genomic architectural elements such as repeats or DNA damage that may cause the initial stalling of the fork and then facilitate annealing to the sister chromatid or to the same template strand in case of direct repeats. This results in juxtaposition of non-contiguous DNA sequences, ranging from a few hundred base pairs to megabases (reviewed in [2]). Recently, a number of studies have proposed that such template switching events may occur not only on closely adjacent sites but also over long distances on other forks [25–27]. Furthermore, these fork arrest-induced events can be driven by as few as two to six nucleotides of microhomologies [26,28]. Template switching was thus proposed to account for complex genomic rearrangements in human cells [26,29–32].

The definition of template switching became even more complex due to distinct opinions of whether events classified as such involve a DSB intermediate [33–37] and if they occur at the stalled fork as means of rescuing the fork or rather behind the replication fork [15,38–41] (Fig. 1). Furthermore, although initially template switching was used to distinguish replication-induced strand-annealing events from Rad51-mediated DSB repair and fork rescue, this is not anymore the case. It is becoming clear that different template switching events show distinct requirements for Rad51 [7,34,35,42]. Furthermore, collapsed forks generate DSBs with only one free end that are repaired by break-induced replication (BIR) [43]; this process involves several rounds of "template switching", that is, multiple cycles of strand invasion, DNA synthesis and dissociation [31,32].

Notably, although "template switching" was used to explain the function of the Rad18–Rad5 pathway in damage tolerance in a manner that was thought to be independent of HR [5,39], recent findings suggest that Rad18–Rad5 may act in certain contexts coordinately with recombination factors to enable joining of sister chromatids and damage bypass by template switching [33,41,42,44]. Furthermore, evidence was provided that the Rad18–Rad5 pathway plays a role not only in replication completion and gap-filling by template switching [41,42,45–47], but also in DSB repair by HR [48–54].

With these historical considerations mentioned, it is important to note that only a subset of template switch events have been characterized for genetic requirements and/or DNA structures that may assist the transitions. Those are primarily damage- and replication fork block (RFB)-induced template switching and BIR, using budding and fission yeast as model organisms. Below follows an overview of these processes and the factors involved in regulating them.

3. Damage- and RFB-induced template switching

Bulky DNA lesions or RFBs can obstruct active replication forks leading to transient arrest. Based on evidence from *Escherichia coli* and in vitro studies using SV40 [55,56], it has been suggested that when DNA damage blocks the leading or the lagging strand, the normally coupled strand synthesis becomes uncoupled [57]. Prolonged stalling without restart can cause the replisome to dissociate, leading to replication fork collapse. The types of blocks that initially stalled the progression of the fork may influence the choice of damage-bypass events and of the polymerase carrying out the bypass [58,59], but details on the mechanisms underlying such events are missing. In addition to damage-bypass processes occurring to reactivate a stalled fork, recent evidence suggests that *RAD6*- and *RAD18*-mediated damage tolerance mechanisms in yeast cells operate largely on gaps behind replication forks [40,60]. Based on in vitro studies in *E. coli* it has been suggested

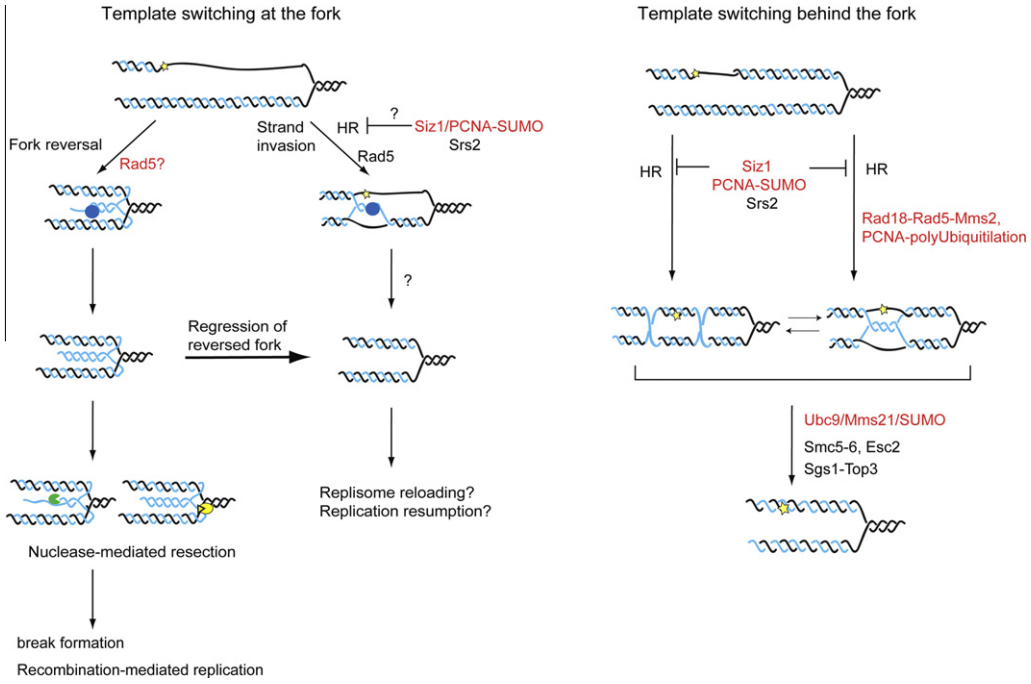

Fig. 1. Representation of mechanisms that promote template switch events at the fork or behind the fork through strand annealing or strand-invasion mediated mechanisms. The factors with Ubiquitin and SUMO-related activities are highlighted in red.

that such gaps are formed by repriming events that initiate replication downstream of the lesion [61], thus leaving the obstructing damage contained in an internal gap behind the fork (Fig. 1).

HR has been implicated as a major mechanism involved in the restart of blocked forks [28,34,62,63] as well as in the filling of the gaps induced by replication of UV- and MMS-damaged templates [42,64–67] (Fig. 1). Genetic studies conducted in *Saccharomyces cerevisiae* established that besides HR, DDT requires genes belonging to the *RAD6–RAD18* epistasis group, often referred to as post-replication repair (PRR) [24,45]. Proteins of the *RAD18* pathway are well conserved and have been implicated in DDT and gap-filling in both yeast and mammalian cells [42,47,68–73]. Rad6 and Rad18 control two distinct damage bypass pathways. One pathway involves DNA synthesis across the damaged template by translesion synthesis polymerases; this mechanism operates with high fidelity for UV lesions but is error-prone for most types of damage, and therefore is often mutagenic [74]. The second pathway is error-free bypass, is controlled by *RAD5–MMS2–UBC13* genes encoding ubiquitin ligases and ubiquitin conjugating enzymes that mediate PCNA polyubiquitylation [75] (see below), and is hypothesized to involve a switch of templates in which the blocked nascent strand uses the undamaged sister chromatid as a temporary replication template [24] (Fig. 1). The initial model proposed that this bypass occurs likely through a reversed fork intermediate, and without any requirement for recombination proteins [11]. Although Rad5, which is also an ATPase [76], can promote fork reversal in vitro [77], whether the reversed fork intermediate promotes error-free DDT in vivo remains a matter of debate (reviewed in [15,78]). Recent data suggested instead a recombination-like invasion mechanism in which the blocked nascent chain invades the opposite homologous duplex and uses the sister chromatid as a template [41,42,64,79] (Fig. 1). Whereas the reversed fork model restricts these events to the fork, the recombination-like invasion mechanism can theoretically occur either at the fork or behind the fork. Recent studies provided evidence that the Rad18 error-free pathway operates largely to promote gap-filling behind the replication fork [40,42,60]. Under conditions that induce site-specific stalling at replication origins, the Rad5-mediated pathway may promote fork restart [41]; whether this is specific for adozelesin-induced lesions and lesion density in the immediate proximity of origins is still not known. In both cases, compelling evidence suggests that the action of the error-free PRR pathway occurs through a recombinational mechanism and leads to the formation of X-shaped structures involving sister chromatid junctions (SCJs).

Based on the biochemical properties of these molecules, it has been suggested that template switching behind the fork leads to X-structures that contain ssDNA, and in which the sister chromatids are linked through base-pairing established by Polδ-mediated DNA synthesis [42,64,67,80]. These template switch intermediates may contain also Holliday Junctions (HJs) [81] (Fig. 1). On the other hand, template switching at the fork was proposed to lead to the formation of HJs [62,82]. Notably, the role of the Rad18–Rad5 error-free pathway in such processes requires ubiquitin conjugation and ligase activities [42,82] that are summarized below.

4. Ubiquitin- and SUMO- mediated regulation of template switching

Most factors of the error-free *RAD18* PRR pathway have ubiquitin conjugating or ligating activities [83–85] and their role in DNA damage bypass is associated with ubiquitin and SUMO modifications of PCNA [75]. In response to DNA-damage, PCNA is mono- or polyubiquitylated at the highly conserved lysine residue K164. Monoubiquitylation depends on the Rad6–Rad18 E2–E3 ubiquitin-conjugating complex [75] and promotes translesion synthesis [86,87]. The error-free template switch bypass is further controlled by the E3 ligase Rad5 that stimulates the E2 ubiquitin conjugating activity of the Mms2–Ubc13 complex to synthesize K63-linked polyubiquitin chains onto monoubiquitylated PCNA [75]. PCNA polyubiquitylation and error-free PRR factors (Rad18–Rad5–Mms2–Ubc13) are required for gap-filling damage tolerance [46,47,71,75,88] and for template switching-mediated SCJ formation behind replication forks [42,89] (Fig. 1). Furthermore, Rad5 (and its ligase activity) is required for formation of template switch structures containing HJs at forks stalled at replication origins by drugs such as adozelesin with specificity for AT-rich DNA sequences [82] (Fig. 1).

In addition, genetic studies have shown a role for Rad18 and Rad5 in fortuitous template switching during replication. Rad18–Rad5 promotes direct repeat instability and expansions of GAA trinucleotide repeats and the pentanucleotide repeat (ATTCT)n [6,90,91], whereas it restricts CAG/CTG expansions [92]. Anc1—a protein associated with multiple transcription and chromatin remodeling complexes and that together with Rad5 defines a new branch of error-free PRR, also suppresses the expansion of CAG repeats [93], although its role in template switching and PCNA modifications has not been yet analyzed. A modest role for Rad18 and PCNA monoubiquitylation in mediating BIR was also demonstrated [94].

In addition to being ubiquitylated, yeast PCNA is also sumoylated at two lysine residues, K164 and K127 [75]. Biochemically, sumoylation and ubiquitylation of PCNA are not dependent on one another [75,95,96]. However, in the absence of PCNA sumoylation, the ability of the error free PRR pathway to promote formation of damage-induced SCJs associated with template switching is impaired [42]. These results suggest that PCNA sumoylation favors damage-bypass of lesions by enabling the utilization of factors belonging to the error-free branch of PRR (Fig. 1). The mechanism underlying this phenomenon is unclear, but it may be mediated by interactions between SUMO-modified PCNA and Srs2—a helicase promoting channeling of lesions into the Rad18–Rad5 pathway [95,96], or between PCNA and other members of the error-free PRR pathway, such as Rad18, Rad5 and Elg1 [95,97]. Alternatively, or additionally, sumoylation may affect chromatin structure in a manner that impinges on the accessibility of PRR and/or HR factors to DNA lesions. Indeed, the chromatin remodeler INO80 was shown to affect template switch events and Rad18-recruitment to damaged replication forks [98].

In addition, sumoylation as well as PCNA- and Srs2-related activities have been implicated in replication fork restart at specific RFBs [99]. In fission yeast, the *RTS1* element is a mating type locus-specific RFB that ensures efficient mating type switching by regulating the direction of replication [100]. The Rtf1 protein binds to a single *RTS1* DNA sequence and the Rtf1–RTS1 protein–DNA complex blocks DNA replication and ensures unidirectional replication through the mating type locus. Inagawa et al. have identified that Rtf2, a PCNA-interacting protein, works together with SUMO to promote termination at *RTS1*; in the absence of Rtf2 and SUMO stabilizing activities of the stalled fork, replication forks are re-established in an Srs2-dependent manner [99]. In line with this result, it has also been shown that when replication forks are efficiently stalled by inserting a pair of *RTS1* sequences in inverted orientation, Srs2 promotes fork restart and template switching [62].

5. Interplay between ubiquitin-mediated activities and HR in template switching

Several studies have connected the functionality of the *RAD6–RAD18* pathway to HR in the context of DSB repair [51,52].

Although the *RAD18–RAD5* pathway described above plays an important role in DSB repair, the Rad6 ubiquitin conjugating enzyme has Rad18-independent roles that affect DDR and HR (reviewed in [101]). In *S. cerevisiae*, Rad6 interacts with three separate ubiquitin ligases, Ubr1, Rad18 and Bre1. Mutations in Rad18, Bre1 and Ubr1 lead to sensitivity to ionizing radiation (IR), but each single mutant (*rad18*, *bre1*, and *ubr1*) shows less sensitivity than *rad6* [102]. However, the X-ray sensitivity of *rad18 ubr1 bre1* strain is equal to that of *rad6*, suggesting that the function of Rad6 in DSB repair is accounted by processes involving all its three known E3 ligase partners. Genetic studies have shown that of these three pathways the main ones contributing to X-ray resistance are the *RAD18*- and *BRE1*-mediated ones [102]. Bre1 and its associated protein Lge1 act together with Rad6 to promote histone H2B ubiquitylation on lysine 123 [103–105]. This H2B ubiquitylation is then required for H3–K4 and H3–K79 di- and tri-methylation mediated by Set1 and Dot1, respectively [106]. The IR resistance conferred by Bre1 and Dot1 is mediated through HR repair and not by *RAD18*-dependent PRR. Although these pathways of chromatin modification mediated by Rad6 may affect other HR-mediated processes such as template switching, this has not been formally addressed.

Regarding template switching, both Rad18–Rad5 and Rad51-like recombination factors were shown to be required [42,64,82,107] (Fig. 1). Although the traditional view based on epistasis tests holds that Rad18- and Rad51-mediated pathways are parallel and independent, evidence has started to accumulate for a coordinate action of these factors in template switching and for a role of error-free PRR factors and PCNA polyubiquitylation in regulating the activity of HR factors [33,42,51,52,82,108]. In addition, recent evidence suggested a role for Rad18 and Rad5 in the *RAD51*-dependent pathway of recombination triggered by inverted repeats [33].

It must be noted that Rad18 and Rad5 have also been documented to act in template switch events independently of Rad51. For instance, Rad18 and Rad5 play a role in both *RAD51*-dependent and *RAD51*-independent pathways of recombination at inverted repeats, with a more prominent role in the *RAD51*-dependent pathway [33]. Replication of plasmids containing thymine–thymine photoadducts in excision-defective yeast strains was shown to occur largely by recombination, with 60–70% of these events being dependent on the error-free component of the *RAD18* pathway and the remaining events on the *RAD52* pathway [5]; although the mechanism through which the error-free *RAD18* pathway promotes template switching under these circumstances remains unknown, based on the genetic data provided it can be concluded that it operates independently of HR factors. Furthermore, Mms2 and Rad51 paralogues, such as Shu1, promote damage-induced template switch events [42,107], but they affect the damage-sensitivity and accumulation of template switch intermediates in *smc6* mutants by different mechanisms [89]. In what regards the role of Rad5 in fortuitous template switching associated with trinucleotide repeat expansion, preliminary genetic data suggested this function is manifested independently of HR [91,92].

Is the Rad18 pathway activated by similar lesions as the ones inducing HR? How could Rad18-mediated ubiquitylation events promote or enhance the efficiency of HR repair in certain conditions? Rad18 interacts with RPA-coated ssDNA present at gaps and stalled replication forks, and Rad5 interacts with ssDNA and Rad18 [76,85,109]. After being loaded on the ssDNA regions formed at stalled forks or on the gaps left behind replication forks, Rad18 and Rad5 could promote PCNA polyubiquitylation and facilitate the recombinational, Rad51-dependent template switch. In mammalian cells, the ability of Rad18 to stimulate HR-mediated repair of DSBs involves a direct interaction between Rad18 and a Rad51 paralogue, Rad51C [49]. In this context, Rad18 functions as an adaptor protein, as it is recruited to sites of damage through its interaction with ubiquitin chains and then, by interacting with Rad51C, it allows the accumulation of HR proteins at sites of damage [49].

An alternative mechanism could be that Rad18 facilitates template switch steps occurring downstream of the Rad51-mediated strand invasion. In support of this view, previous studies conducted in chicken DT40 cells have found that deletion of the *RAD51* paralogue, *XRCC3*, suppresses the genome instability and UV damage sensitivity of *rad18* cells [48]. In *S. cerevisiae*, a role for the *RAD51* paralogues in error-free PRR involving homologous recombination has been suggested [44]. Furthermore, in budding yeast, the gross chromosomal rearrangements associated with *rad18* and *rad5* depend on Rad51 [110]. Taken together, these results indicate that homologous recombination may be toxic in the absence of a functional Rad18–Rad5 pathway.

One documented step in which the Rad18-mediated PCNA polyubiquitylation works downstream of the Rad51-mediated strand invasion is the DNA synthesis step required to extend the 3′ of the invading end. Recent studies have found that this step is mediated specifically by polymerase δ [67]. Polδ is also required for the DNA synthesis step of BIR [111]. Interestingly, genetic data have pointed out that the functionality of Polδ in DNA repair is modulated by error-free factors of PRR and by PCNA polyubiquitylation, which perhaps act to modulate the processivity of Polδ during DNA repair synthesis [40,47,112–114]. It was shown that during BIR, cells carrying mutation in Polδ have limited homology-dependent DNA synthesis and the repair intermediates are cleaved in a manner that is partly dependent on the Mus81 nuclease [115]. Thus, the template switch defect observed in *rad18*, *rad5*, *pcna* and Polδ mutants during replication under damaging conditions may be due not only to defective or abortive DNA synthesis, but also to cleavage or processing of such incomplete template switch intermediates by nucleases.

6. SUMO-mediated resolution of damage-induced template switch intermediates

The template switch intermediates need to be resolved in order to restore a normal replication fork and chromosomal structure. Several resolvases have been identified in eukaryotic cells: little is known about their regulation and coordination with one another, but it is becoming clear that their impact on genome stability and effect on crossover formation is different (reviewed in [17]). In what regards the resolution of damage-induced template switch intermediates, it has been shown that in cells with an unfunctional RecQ helicase Sgs1 (BLM in humans) or topoisomerase III, Top3, SCJ-containing intermediates accumulate in the proximity of replication forks [64,116]. Considering the genetic evidence suggesting that Sgs1 works downstream of Rad18–Rad5- and PCNA polyubiquitylation- [40,42,44], as well as of Rad51-mediated steps [117,118], and the physical evidence providing for Sgs1 resolving Rad18, Mms2, PCNA polyubiquitylation, and Rad51-dependent structures [42], it is reasonable to assume that Sgs1–Top3 represents the major activity involved in template switch intermediate resolution. The resolution of these intermediates in S-phase is controlled and requires sumoylation events mediated by the E2 conjugating enzyme, Ubc9, and the SUMO E3 ligase Mms21 [119] (Fig. 1). Recently, mutations in the SUMO deconjugating enzyme, Ulp2, were also shown to accumulate SCJ intermediates in response to MMS treatment as well as persistent recombination foci, suggesting that Ulp2 is also required to suppress or facilitate the resolution of the damage-induced template switch intermediates [120]. The SUMO substrates involved in template switch intermediate resolution are not known, but they may include structural maintenance of chromosomes (SMC),

Smc5–6—associated with the Mms21 SUMO ligase, as well as Esc2, an Smc5–6 functionally associated factor with regulatory functions in sumoylation. This inference is based on the observation that also mutations in Esc2 and Smc5–6 lead to accumulation of template switch structures in the proximity of replication forks [121,122].

Considering that out of the factors that affect damage-induced template switch intermediate accumulation in S-phase, Sgs1 is the only protein with potential resolvase activity [81,121,123], it is perhaps reasonable to assume that Ubc9- and Mms21-, Smc5–6-mediated sumoylation affects the functionality of Sgs1–Top3 in this process [119,121] (Fig. 1). Although Sgs1, like BLM [124], is sumoylated, this sumoylation does not depend on the Mms21 SUMO ligase [119]. Recently, the lysine K621 of Sgs1 has been identified as a prominent sumoylation site of Sgs1 [125], but the role of Sgs1 sumoylation in template switching remains unknown. The effects of SUMO-mediated events in controlling template switch intermediate resolution and/or Sgs1 functionality may be indirect and involve control of chromatin structure, protein localization, stability and recruitment. Further studies addressing how genome architecture, topology and chromatin structure affect replication-associated repair events should improve our understanding on the underlying mechanism of template switching.

7. Crosstalk between DDR kinases and ubiquitin-mediated pathways in template switching

The replication and damage checkpoint kinases are known to affect HR-mediated events and to some extent the error-free PRR (reviewed in [15,126]), and a role for the replication checkpoint in promoting the formation of template switch intermediates has been reported [64]. The potential substrates of the replication and damage checkpoint relevant for template switching are numerous. For instance, Rad55 is required to stabilize the Rad51 filament and for strand-invasion activity (reviewed in [18]) and is phosphorylated by the Rad53 checkpoint kinase [127]; however, deficiency in Rad55 phosphorylation has no obvious effect in facilitating template switching [67]. A factor promoting efficient damage-induced template switching as well as faulty template switching at inverted repeats is the Exo1 nuclease [67,128]. Checkpoint-mediated phosphorylation of Exo1 [129] inhibits Exo1-mediated degradation of replication fork associated intermediates [130]. The high level of inverted repeat fusions occurring in *rad53* cells, but not in *mec1*, appeared to be reversed by introduction of an *exo1* mutation, suggesting that failure to restrict Exo1 activity may facilitate faulty template switching between nearby inverted repeats [128].

In fission yeast, phosphorylation of Rad9 of the 9-1-1 checkpoint complex by the ATM and ATR-related checkpoint kinase, Rad3, at Thr225, is enhanced by DNA damage and functions to direct repair through a Pli1 (Siz1)-mediated sumoylation pathway into the error-free branch of PRR; the mechanism likely involves a physical interaction between Rad9 and Mms2 [131]. Although a direct role for 9-1-1 and the Rad9–T225 phosphorylation in template switching has not been yet addressed, this is a likely possibility considering that in budding yeast Siz1-dependent sumoylation is required for Rad18–Mms2 pathway to promote gap-filling by means of SCJs [42]. Furthermore, similar to Srs2 and PCNA–SUMO in budding yeast [95,96], the Thr225 phosphorylation of Rad9 was shown to prevent inappropriate Rad51-dependent recombination [131]. The nature of these inappropriate recombination events prevented by Siz1/Pli1 SUMO ligase, PCNA–SUMO and Srs2 are not known, but they were speculated to be related to the ones promoting SCJ formation in budding yeast but occurring independently of Rad18–Rad5–Mms2 activities [42]. Whether similar to the fission yeast situation, the Rad18 error-free pathway in other eukaryotes is controlled by checkpoint-mediated phosphorylation events is not known. Considering that human Rad18 was reported as a potential ATR-ATM target [132], and that MMS-induced accumulation of human Rad18 at stalled/damaged forks is affected by wortmannin treatment, an inhibitor of S-phase checkpoint kinases [133], this becomes an intriguing possibility. Human Rad18 was recently found to be phosphorylated by the Dbf4–Cdc7 kinase (DDK) in response to DNA damage; this modification promotes recruitment of a TLS polymerase, Polη, to sites of fork stalling but its impact on other DDR events is not yet known [134].

Recently the Rad18–Rad5 error free pathway was elegantly demonstrated to provide for error-free lesion tolerance when the expression of such key factors is restricted to G2/M [40]. This finding compellingly suggests that Rad18–Rad5 primarily promotes damage tolerance behind replication forks rather than acting at the fork (Fig. 1). The time has now come ripe to ask new questions. Is template switching showing cell-cycle-related characteristics? Are there differences between S and G2 phases of the cell cycle in what regards factors availability and DDT efficiency? These characteristics may relate to the expression or stability of certain proteins, competition for factors in coincident processes (such as Polδ in template switching behind the fork and bulk DNA replication [67]) as well as to chromatin features that may affect the accessibility of DDR factors. It is expected that the answer to these questions will prove very informative for understanding the regulation of this intricate process.

Considering that ssDNA is a substrate for different types of DDT mechanisms and DNA metabolism processes, such as checkpoint activation, Rad51 presynaptic filament formation, specialized polymerases whose activity largely depends on PCNA modifications, and Rad18–Rad5 mediated template switching, it is likely that a complex interplay of regulatory pathways and their substrate modifications act to modulate these choices. Rad18 was shown to interact with RPA bound to ssDNA [109]. In mammalian cells RPA is kept in a hypoSUMOylated state during S phase through an interaction with a SUMO-specific protease, SENP6; however following DNA damage induced by camptothecin (CPT), which induces topological, replicational stress and DSB formation, RPA becomes sumoylated and this facilitates Rad51-mediated repair through homologous recombination [135]. Is this modification of RPA specific to HR-repair of DSBs? How would this modification affect the Rad18–Rad5–Ubc13 role in DSB repair? And if it is not specific to DSBs, how would sumoylated RPA affect the functionality of the Rad18-mediated error-free damage tolerance? The interplay between SUMO and ubiquitin-mediated responses with checkpoint and cyclin dependent kinase-mediated events begins to be appreciated in the DSB repair field (reviewed in [13]), but much remains to be learnt about the potential cross-talk of these regulatory pathways in template switching.

8. Concluding remarks

Template switching has evolved as an important mechanism to promote fork restart, gap-filling and damage bypass. In what regards damage tolerance mechanisms, template switching contributes largely to error-free bypass and opposes the undesirable effects associated with mutagenesis. However, genomic architectural features such as repeat elements induce fork stalling and facilitate faulty template switching that results in genomic rearrangements. Although these events are often associated with genomic instability and cancer development, they also play an important role in long-term evolutionary changes in the genome [2,30]. Furthermore, template-switching events associated with rearrangements may be relevant for physiological processes, such

as generating antibody diversity, and in development [136]. Thus, it will be exciting to learn more about the mechanisms controlling template switching events in distinct cellular settings, such as germ line versus somatic cells, stem and cancer cells versus differentiated cells. Finally, understanding how different post-translational modifications such as those involving ubiquitin chains facilitate recruitment of DDR factors and modulate the organization and landscape of the genome, are fascinating topics not only in the mutagenesis and cancer fields but also for chromosome biology in general.

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