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DNA damage tolerance

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Accurate chromosomal DNA replication is fundamental for optimal cellular function and genome integrity. Replication perturbations activate DNA damage tolerance pathways, which are crucial to complete genome duplication as well as to prevent formation of deleterious double strand breaks. Cells use two general strategies to tolerate lesions: recombination to a homologous template, and trans-lesion synthesis with specialized polymerases. While key players of these processes have been outlined, much less is known on their choreography and regulation. Recent advances have uncovered principles by which DNA damage tolerance is regulated locally and temporally — in relation to replication timing and cell cycle stage —, and are beginning to elucidate the DNA dynamics that mediate lesion tolerance and influence chromosome structure during replication.

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Introduction

Complete and accurate replication that happens precisely once in each cell cycle is essential to maintain genome integrity, support normal development and prevent disease. Many sources of replication stress can however challenge the replisome, causing its transient stalling and even inactivation [1]. Replication stress cues include difficult to replicate genomic regions, topological constrains, oncogene activation, and various types of DNA lesions. The challenge for the replisome is enormous, especially because DNA damage is extremely prevalent, with approximately 30,000 lesions being generated spontaneously in a mammalian cell per day due to aerobic metabolism and the inherent susceptibility of DNA to depurination and depyrimidination [2]. In addition, environmental factors and chemical agents cause modifications or breaks in the DNA. To complete replication and prevent formation of deleterious double strand breaks (DSBs) in the vicinity of the replication fork, it is crucial for replicating cells to swiftly tolerate the encountered DNA damage until dedicated DNA repair processes can remove it. This is primarily managed by DNA damage tolerance (DDT) mechanisms.

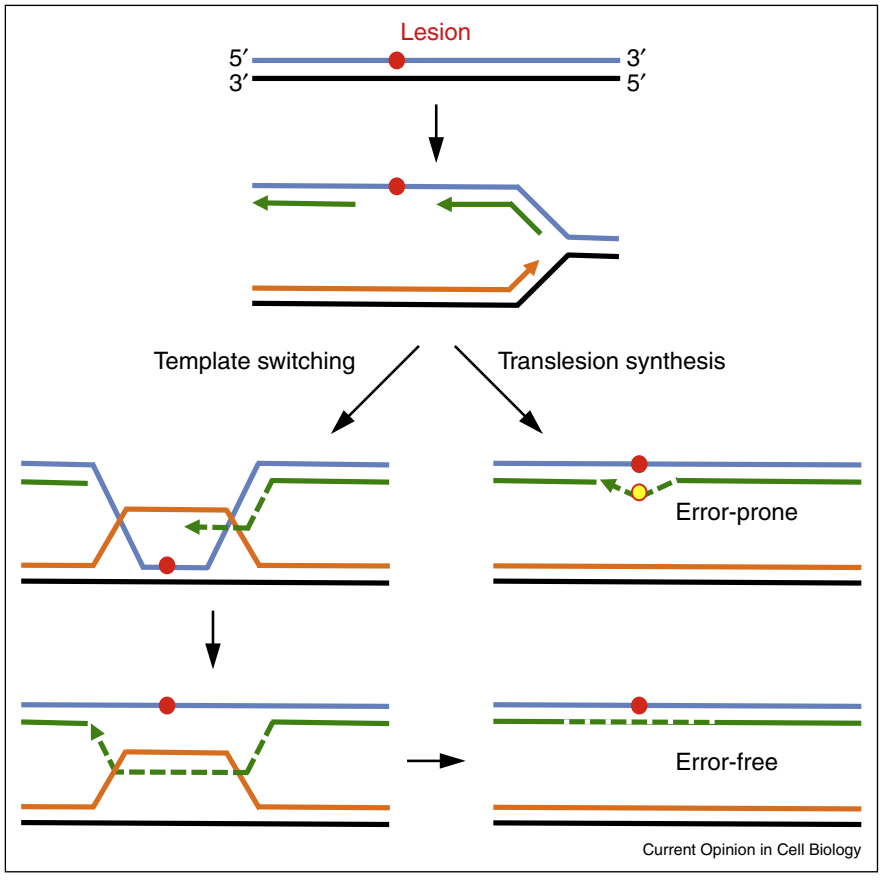
Here we start by summarizing basic concepts of DDT and mediating factors, highlighting points of controversies on their *modus operandi*. The review will focus on findings of the last two years related to the regulatory mechanisms and DNA transitions enabling DDT, and outline emerging concepts on the relationship between DDT and chromosome structure processes.

DDT modes, activation mechanism, and mediating factors

Eukaryotic cells use two general strategies to tolerate lesions encountered during replication: a recombination-dependent mode that is generally error-free and uses a homologous template, usually the newly synthesized sister chromatid (template switching, TS) [3], and a trans-lesion synthesis (TLS) polymerases-dependent bypass mode that can occasionally introduce mutations and therefore is considered to be error-prone [4] (Figure 1). However, it is important to note that certain low-fidelity TLS polymerases such as Polη can replicate over particular DNA lesions in a relatively accurate manner [4], while restart of the forks by homologous recombination (HR) mechanisms has generally proved to be error-prone [5]. The error proneness of HR mechanisms is largely related to usage of templates other than the sister chromatid [5]. While this is generally not the case for TS, certain lesions or chromatin features that block fork movement predispose to strand invasion events that use limited homology and can result in genome rearrangements. Because of this aspect, and of its last-resort nature, this mode of recombination-mediated DDT is also known as the salvage pathway of recombination. In this review, we will focus primarily on TS. For opinions on the etiology of error-prone HR induced by replication stress and its place and regulation in the context of recombination-mediated DDT, we invite readers to recent reviews on these topics [5,6]. In the following, we will begin by discussing the DNA structural features and molecular switches contributing to DDT activation.

Single-stranded (ss) DNA, associated with replication and fork stalling, is crucial for DDT activation [7], although as we will discuss below, DDT does not need to operate prevalently at the site of stalling [8•]. In fact, lagging-strand template damage does not impede fork movement, since a new Okazaki fragment will allow replication

Figure 1



Representation of the two main modes of DNA damage tolerance. A lagging-strand template damage is depicted and its postreplicative tolerance via template switching or trans-lesion synthesis mediated damage-bypass.

downstream the lesion. Leading-strand template damage may cause uncoupling between the helicase and polymerase activities [1], although also in this case, helicase-coupled repriming that allows continued fork movement appears to be frequent and a crucial component of DDT in budding yeast [9••,10]. The repriming mechanism may be even further diversified in human cells due to the presence of specialized polymerases containing primase activities [11•,12•]. It is important to note that both stalling and repriming cause exposure of ssDNA proximal to the lesion (Figure 1), and that DNA gaps were observed on both strands of the newly replicated DNA following exposure to DNA damage [13]. When bound by RPA, ssDNA serves as a signal to activate DNA damage response via its damage checkpoint components [1]. In addition, ssDNA is a binding substrate for the Rad51 recombinase, as well as for Rad18 and Rad5 proteins, which contain ubiquitin ligase activities and are crucial components of DDT, due to their ability to induce (poly)ubiquitylation of the polymerase clamp, PCNA (Table 1).

PCNA is ubiquitylated in all eukaryotic species analyzed to date at a conserved lysine (K) residue, K164 [14,15]. Monoubiquitylation is mediated by the conserved Rad6 ubiquitin-conjugating enzyme that acts in complex with the ubiquitin ligase Rad18, a ssDNA-binding protein that recognizes DNA discontinuities. Another ubiquitin ligase and ssDNA-binding protein, Rad5, interacts with Rad18 and, together with the heterodimeric ubiquitin conjugation complex, Ubc13-Mms2, further extends the monoubiquitin modification to K63-linked polyubiquitin chains [16]. Monoubiquitylated PCNA facilitates interaction with TLS polymerases [17,18]. Conversely, polyubiquitylated PCNA inhibits mutagenic bypass [19] and mediates error-free DDT via TS [20–22]. TS is manifested by the formation of bypass recombination structures, preferentially between sister chromatids [23,24••,25].

Mutagenic bypass depends on TLS polymerases (Table 1), the diversity of which varies greatly depending on the species [4]. TS requires a task-force of activities

Table 1

Main factors implicated in DDT in *Saccharomyces cerevisiae*.

Protein/complex	Function in DDT
RFA (RPA) (Rfa1, Rfa2, Rfa3)	Recruiting platform for various DDT/DDR factors including Rad18 and Rad5, as well as Mec1/Ddc2 (ATR/ATRIP)
Pol30 (PCNA)	PCNA modifications with mono/poly-ubiquitin and SUMO influence DDT mode
Rad18/Rad6	Mediates monoubiquitylation of PCNA at K164
Rad5/Ubc13, Mms2	Mediates K63-linked polyubiquitylation of PCNA at K164
Siz1/Ubc9	Mediates SUMOylation of PCNA at K164
Srs2	Inhibits unscheduled homologous recombination during replication
Elg1	Facilitates unloading of PCNA from chromatin
DNA polymerase ζ (Rev3, Rev7; Pol31, Pol32)	TLS polymerase responsible for most of the observed mutagenesis
Rev1	Required for Pol ζ-mediated TLS
DNA polymerase η (Rad30)	TLS polymerase
DNA polymerase δ (Pol3, Pol31, Pol32)	Mediates DNA synthesis in DDT via template switching
Rad52, Rad51, Rad55/Rad57, Rad54	Required for homology search and strand invasion during template switching
Exo1	Interacts with 9-1-1 complex, promotes ssDNA gap resection and template switching
9-1-1 complex (Ddc1, Mec3, Rad17)	Required for template switch intermediate formation
Hmo1	Promotes template switching via DNA bending activities
Cohesin complex (Smc1, Smc3, Mcd1, Scc2)	Facilitates template switching by keeping the homologous DNA sequence in proximity
Ctf4-DNA polymerase α-Primase	Required for replicative helicase-coupled repriming during template switching
Smc5/6 complex	Facilitates processing of template switch intermediates
STR complex (Sgs1, Top3, Rmi1)	Mediates dissolution of template switch intermediates
Mus81-Mms4 Slx1-Slx4	Mediate crossover-prone resolution of template switch intermediates in G2/M
Esc2	Facilitates template switching by locally downregulating Srs2 and by promoting template switch intermediate resolution
Slx5/Slx8	Functions jointly with Esc2 in promoting Srs2 turnover

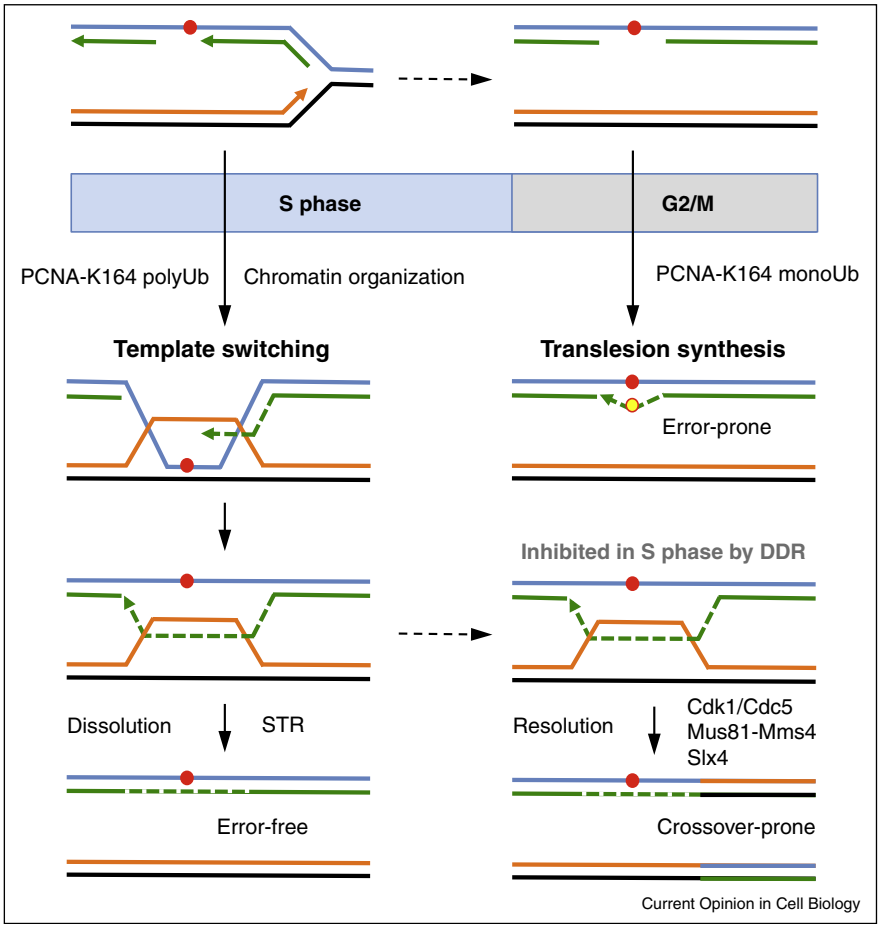
necessary for formation and/or resolution of the recombination intermediates (Table 1). The formation of TS intermediates in budding yeast relies on several factors associated with repriming, gap-processing, homologous recombination, PCNA polyubiquitylation and Polδ-mediated DNA synthesis [9••,23,26•,27] (Table 1). Rad51 is crucial for TS, but is globally repressed during replication by the anti-recombinase Srs2, which is present at replication forks via its interaction with SUMOylated PCNA [20,21], a PCNA modification induced during replication [16]. Thus, how Rad51 functions together with factors required for PCNA polyubiquitylation in TS [23] remained puzzling. An answer to this conundrum comes from recent findings showing that local assembly of Rad51 at damaged sites is facilitated by the conserved SUMO-like domains (SLDs)-containing factor, Esc2. Esc2 binds to replication-associated DNA structures and to SUMOylated PCNA readers, Elg1 and Srs2, facilitating local down-regulation and turnover of Srs2 [28••,29].

TS involves pseudo-double Holliday-Junctions as crucial intermediates [24••]. The replication checkpoint safeguards TS by protecting the intermediates against precocious processing by nucleases [30••], thereby facilitating their dissolution by the Sgs1-Top3-Rmi1 (STR) complex [23,31] (Figure 2). SUMO-dependent processes involving the Smc5/6 complex and Esc2 also facilitate the turnover/resolution of TS intermediates induced by genotoxic stress [32–34]. Recent work revealed that cooperation between STR and Smc5/6 is crucial also for resolving endogenous TS intermediates, which can block cell division and cause cell death if left unresolved [35••]. TS intermediates that persist till G2/M can be processed by crossover-prone Mus81-Mms4 and Slx4 nucleases [30••,36•], whose activity is upregulated by mitotic cell cycle kinases, Cdk1 and Plk1/Cdc5 [37] (Figure 2).

PCNA modifications and chromatin organization in DDT pathway choice

PCNA polyubiquitylation occurs after PCNA monoubiquitylation, but the concomitant presence of Rad18 and Rad5 on ssDNA may make PCNA polyubiquitylation more prevalent at the site of lesion in S phase (Figure 2). Substantiating this view, a deletion mutation in the Polδ non-catalytic subunit, Pol32, which renders cells defective in both DDT branches (Table 1), causes accumulation of polyubiquitylated PCNA [38]. The additional local coordination of Rad51 recruitment to damaged sites [28••,29] provides the right context for TS. This scenario explains why mutation rates are low early during replication in both

Figure 2



Hypothetical model of temporal regulation of DNA damage tolerance modes. Template switching is preferred over mutagenesis in S phase. PCNA polyubiquitylation and chromatin organization features established in S phase favor template switching, whereas other mutagenic pathways involving trans-lesion synthesis polymerases and error-prone nucleases are often potentiated in G2/M.

yeast and human cells [39,40], while damage-bypass by TS occurs even early in S-phase [26•,41••,42].

Besides post-translational modifications of PCNA, chromatin organization emerged as an important regulator of DDT. Three examples are discussed here: chromatin status, sister chromatid cohesion and chromosome architectural changes mediated by DNA bending. First, the closed or open state of chromatin was shown to influence local rates of mutations [43,44]; this can be explained by the fact that the chromatin status affects the replication timing of the genomic locus considered, with the closed chromatin being replicated preferentially late. However, it is also possible that the nucleotide sequence of DNA and the DDT pathway employed for endogenous lesion tolerance influence its dynamic packaging into chromatin. Indeed, impediments to DNA replication and global replication stress were shown to favor gene silencing and cause changes in gene expression [45–47], suggesting a two-way relationship between DDT and chromatin structure. Second, sister chromatid cohesion, which is established during replication, is negatively influenced by replication stress situations associated with mismanagement of ssDNA, defective TS and altered fork topology [9••,10,48]. On its turn, sister chromatid cohesion, largely mediated by acetylated cohesin rings, facilitates error-free DDT by keeping the recombination template in proximity [9••,10]. Third, genome architectural transitions involving intra-chromosomal DNA bending events mediated by a conserved High-Mobility Group B (HMGB) protein, Hmo1, favor TS over mutagenic bypass early during replication in budding yeast, probably by facilitating engagement of the lesion-containing ssDNA filament in recombination with the homologous duplex [41••].

Thus, the picture that emerges is that DDT modes are temporally regulated, with a preference for TS over TLS

early during replication (Figure 2). The temporal regulation of DDT pathways is largely compounded by chromatin organization events and post-translational modifications of PCNA (Figure 2). Additionally, it may involve cell-cycle dependent regulation of DDT factors' levels or activity, as reported for upregulation of TLS factors' gene expression in G2/M [49] and for structure-specific endonucleases' activity by mitotic kinases [37]. The above-mentioned DDT events potentiated in mitosis are more prone to induce mutations and cause genome rearrangements. Thus, they may represent the last-resort options employed by the cells when faced with persistent DNA lesions, enabling cell division, but often at the price of genome integrity.

DDT at the fork and behind the fork

Whether DDT occurs primarily at the fork or behind the fork is controversial. This notion is important, because the two scenarios have different implications for genome stability. If DDT occurs predominantly at the replication fork, cells can only detect the damage locally, due to exposure of ssDNA at individual forks, but are not able to assess the lesions present on unduplicated DNA ahead of the forks. This would be required to estimate the total amount of inflicted damage before shifting to induction of potentially deleterious mutagenesis. Moreover, fork restart by HR pathways is generally error-prone [5,6]. On the other hand, the uncovering of fork remodeling activities that can stabilize stalled forks by promoting fork regression and of various specialized TLS polymerases, may have led to the hypothesis that DDT at the fork would provide quick solutions for lesions-bypass. Several factors have fork-remodeling activities in vitro and contribute to DDT. These include Rad5 and its human ortholog, HLTF [50,51], and DNA translocases with strand annealing activities, SMARCAL1 and ZRANB3 [52–55], which do not have clear orthologs in yeast. Thus, fork regression provides means of DDT at stalled replication forks when fork progression per se is perturbed.

However, lagging-strand templates do not pose any problem for fork movement, and coordinated repriming events on the leading strand can often relieve the problem, allowing potentially time-consuming polymerase swapping and recombination-mediated processes to take place in defined regions behind replication forks. Several converging lines of evidence obtained in the last years bring support for DDT behind the fork as a prevailing mode of damage-bypass when lesions allow replicative-helicase advance: (i) replicative-helicase coupled repriming is important for DDT, providing in vivo evidence for postreplicative DDT [9••]; (ii) the structures of TS intermediates, visualized by a combination of 2D gel and electron microscopy (EM) provide evidence for the post-replicative gap-filling model [24••], although they do not rule out that reversed forks might have formed earlier in the process before being resolved or converted into other recombination intermediates [56]; (iii) factors that contribute to gap-processing during Okazaki fragment processing, such as 9-1-1 and Exo1 play an important role in TS beyond what is expected if their contribution were limited to lagging-strand lesions [26•,27]; (iv) limiting critical error-free and error-prone DDT factors to G2/M allows normal tolerance of lesions [38] and reveals productive DDT synthesis tracts [57].

The above notions should not be interpreted as to mean that DDT at the fork is exceedingly rare, nor that it must be associated with an error-prone outcome. The existence of factors manifesting in vitro fork-remodeling activity, present in both yeast and mammalian cells, but the more frequent visualization of reversed forks in mammalian cells, but not yeast, following different DNA damaging conditions [8•,9••,13], may cause a false dichotomy in the field with regard to concepts of DDT regulation and prevalence of fork remodeling in different model systems. Various lines of evidence suggest that also in mammalian cells exposed to DNA damage, such as UV, discontinuities are created in the newly synthesized strands and are subsequently filled in using the newly synthesized strand as template [58], likely in a manner analogous to the TS mechanism identified in yeast models [24••]. Moreover, as mentioned above, many DDT factors are highly conserved from yeast to humans, and the ones belonging to the error-free branch often function as tumor suppressors.

It is important to note, however, that certain lesions, such as inter-strand crosslinks and replication fork barriers that block replicative-helicase advance are more likely to induce fork remodeling. In this vein, recent work in budding yeast identified that genomic sequences containing intrinsic replication fork barriers or natural pausing sites trigger a replication stress response accompanied by formation of recombination structures that are distinct in terms of genetic dependency, and potentially fine structure, from those arising at stalled replication forks [35••]. If those recombination structures are substantially composed of reversed forks will remain to be addressed however by future studies.

Repetitive sequences or regions that block fork progression are an example of genomic locations where fork-remodeling events are likely beneficial. Such sequences are much more prevalent in the human genome than in yeast. This may explain also why some fork remodeling activities, such as provided by, for example, ZRANB3 and SMARCAL1, were not found in yeast. Interestingly, these factors are related to chromatin remodelers. We propose that they may prevalently function in specific chromatin contexts, such as those that predispose to silencing and cause increased fork pausing. The fragility of repetitive regions is also enhanced by various genotoxic

treatments. Increased percentage of reversed forks observed in mammalian cells using genome-wide enrichments of replication intermediates [8•] may be a reflection of these sequences differences and explain why different drug treatments cause a similar increase in fork reversal. In addition, it is possible that in mammalian cells, endogenous metabolites that mimic the effect of inter-strand crosslinkers [59] are more abundant compared to yeast systems, providing an explanation both for the increase in overall fork reversal events associated with replication, and a molecular rationale for the significantly more developed and diversified Fanconi Anemia (FA) repair pathways in mammalian cells compared to yeasts.

Conclusion and perspective

Sources of DNA damage are many and varied. To deal with these problems, cells are equipped with a versatile kit of DDT factors. Their temporal and local activity is coordinated by additional regulatory checkpoint and cell cycle kinases, as well as by chromatin organizational features. The underlying factors mediating DDT transactions are generally highly conserved, and accumulating evidence speaks for commonality in principles of regulation, rather than mechanistic divergence between yeast and mammalian cellular systems. However, inherent genome sequences differences between yeast and mammalian cells are likely to cause distinct outcomes with respect to the prevalence of fork remodeling events mediating DDT and the DDT location relative to the fork. Recent studies suggest that DDT and replication fork topology may be intricately related with epigenetic stability and sister chromatid cohesion. An important topic for the future will be to reveal, beyond DDT mechanisms and regulators, the principles that integrate DDT within other replication-associated genome integrity processes, including chromatin state and chromosome structure.

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