DNA Repair xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

DNA damage tolerance by recombination: Molecular pathways and DNA structures

Dana Branzei*, Barnabas Szakal

IFOM, The FIRC Institute of Molecular Oncology, Via Adamello 16, 20139 Milan, Italy

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Chromosome replication
DNA damage tolerance
Replication stress
Homologous recombination
Fork reversal
PCNA
Ubiquitin/SUMO modifications

ABSTRACT

Replication perturbations activate DNA damage tolerance (DDT) pathways, which are crucial to promote replication completion and to prevent fork breakage, a leading cause of genome instability. One mode of DDT uses translesion synthesis polymerases, which however can also introduce mutations. The other DDT mode involves recombination-mediated mechanisms, which are generally accurate. DDT occurs prevalently postreplicatively, but in certain situations homologous recombination is needed to restart forks. Fork reversal can function to stabilize stalled forks, but may also promote error-prone outcome when used for fork restart. Recent years have witnessed important advances in our understanding of the mechanisms and DNA structures that mediate recombination-mediated damage-bypass and highlighted principles that regulate DDT pathway choice locally and temporally. In this review we summarize the current knowledge and paradoxes on recombination-mediated DDT pathways and their workings, discuss how the intermediate DNA structures may influence genome integrity, and outline key open questions for future research.

1. Introduction

Accurate genomic duplication is essential for genome integrity, normal development and disease prevention [1]. This tremendous undertaking is made possible by a task-force of highly conserved replication and DNA repair factors that generally work with astonishing rapidity and accuracy. Part of this success is attributed to intricate regulation of DNA replication and metabolism factors in response to replication stress, which is extremely prevalent. Replication stress comes in different flavors and generally associates with DNA damage or DNA structures that impede replication [1]. In response to replication stress, single stranded (ss) DNA is often exposed proximal to replication forks, leading to local activation of DNA damage response (DDR) pathways. DDR promotes faithful completion of replication by cooperating with and regulating DNA metabolism factors to ensure recognition, bypass and repair of lesions [1,2].

An important strategy to deal with replication-stalling lesions or DNA structures is to use specialized bypass mechanisms known as DNA damage tolerance (DDT) to replicate across the obstructing element, before attempting excision repair. Notably, if excision repair were to happen when the impediment for the replicative polymerase is encountered, while being present on ssDNA (the duplex DNA would have been already unwound by the replicative helicase), a double strand break (DSB) would be formed proximal to the fork. DSBs are extremely dangerous as their inappropriate repair is a leading cause for chromosomal rearrangements [3,4]. Notably, as ssDNA is fragile, persistent ssDNA may cause breakage in the discontinuity region [5]. Thus, an important function of DDT is to prevent replication-associated DSBs *via* its role in mediating replication bypass across lesions, which in turn serves also the important scope of completing replication [2,6].

Here we will summarize basic concepts of DDT, with a focus on recently emerged principles that govern the deployment, location and timing of DDT pathways, and discuss recent findings related to factors and structures that mediate damage bypass, which inform about the underlying mechanisms. We will highlight areas of future research and topics of debate, which will also bring into focus that DDT is at the nexus of various DNA metabolism pathways.

Abbreviations: DDT, DNA damage tolerance; DDR, DNA damage response; ss, single stranded; DSBs, Double strand breaks; TLS, Translesion Synthesis; PRR, Postreplication repair; HR, Homologous recombination; PCNA, proliferating cell nuclear antigen; SLDs, SUMO-like domains; STR, Sgs1-Top3-Rmi1; HJ, Holliday Junction; CFS, Common Fragile Sites; NPS, Natural Pausing Sites.

* Corresponding author.
E-mail address: dana.branzei@ifom.eu (D. Branzei).

http://dx.doi.org/10.1016/j.dnarep.2016.05.008

2. Two modes of DDT and three genetic ways for damage-bypass

Two modes or strategies of damage bypass or DNA damage tolerance (DDT) are highly conserved throughout the eukaryotic kingdom [1]. One mode involves usage of translesion synthesis (TLS) polymerases, which differently from replicative polymerases, can replicate directly across the lesions [7]. The trade-off for using TLS polymerases is increased risk to introducing mutations and, because of this, the TLS mode is considered to be error-prone. The other mode involves recombination to a homologous template, usually the sister chromatid, and is generally accurate in outcome [6].

Initially, DDT research revolved around genetic approaches that screened for mutations that impaired the ability of cells to tolerate exogenous damage. These endeavors revealed that a very important fraction of DDT is mediated by a so-called postreplication repair (PRR) pathway, in addition to the Rad51- and Rad52-mediated homologous recombination (HR) pathway (reviewed in Ref. [6]). The nomenclature of PRR was inspired by the crucial role of factors belonging to this pathway to promote filling of gaps left behind replication forks when cells were allowed to replicate in the presence of DNA damage [8]. PRR crucially depends on the conserved genes *RAD6* and *RAD18*, which encode for ubiquitin conjugating and ubiquitin ligase activities, respectively. TLS polymerases also belong to the *RAD6* pathway, but soon it became evident that the *RAD18* pathway contained activities different from TLS polymerases that were required to mediate damage bypass and gap filling *via* a recombination-like mechanism to the sister chromatid [9]. This recombination-like pathway, which was dependent on *RAD18-RAD6* genes, but genetically different from HR, was called template switching, although the mechanism and activities involved were puzzling. The Rad5 ubiquitin ligase and Mms2-Ubc13 ubiquitin-conjugating complex were later identified to belong to the template switch recombination branch of the *RAD18* pathway. Thus, three main genetic ways mediating DDT emerged: TLS, template switch, and HR, the latter also called the "salvage pathway" (Fig. 1 and see below).

Following this genetic categorization of DDT, efforts were made to understand how the choice between these pathways took place and on solving the puzzle as to how protein ubiquitylation mediated by the *RAD6-RAD18* pathway was important for lesion tolerance. A groundbreaking discovery in both these respects was that the polymerase clamp, PCNA (proliferating cell nuclear antigen), is mono- and polyubiquitylated by factors belonging to the *RAD6* pathway [10]. These modifications of PCNA are important for DDT and could potentially explain the distribution of labor between TLS and template switch in the context of the *RAD6-RAD18* pathway. Specifically, replication stress leads to exposure of ssDNA and recruitment of the ssDNA-binding protein, Rad18 [11], which together with Rad6, mediates monoubiquitylation of PCNA at a conserved residue, K164 [10]. The additional recruitment of Rad5 to ssDNA, and with it, of Mms2-Ubc13 [12], causes extension of monoubiquitylation to K63-linked polyubiquitin chains [10]. Monoubiquitylation of PCNA facilitates the TLS mode [13,14], whereas polyubiquitylation mediates template switching, and inhibits the TLS mode [9,15–18] (Fig. 1). It is important to note, however, that PCNA functions as a trimer, and thus, more than one modification may occur on the same clamp (Fig. 1). Moreover, besides mono and polyubiquitylation, PCNA is also modified with SUMO at K164, and to a lesser extent at K127 in budding yeast [10,19]. SUMOylation of PCNA allows template switching, but prevents HR (Fig. 1 and see below) [15,16,20]. The identified DDT pathways, key factors in these pathways, and PCNA modification with ubiquitin and SUMO are conserved also in higher eukaryotes, indicating that the DDT orchestration occurs by similar mechanisms also in vertebrates.

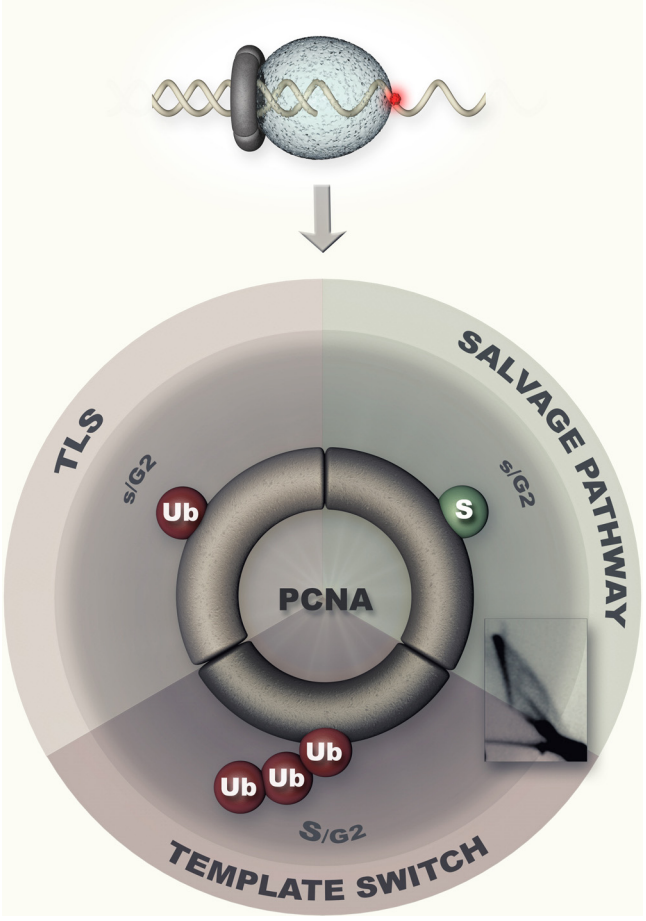

Fig. 1. Stalling of the replicative polymerase, depicted as a ball in light grey, upon encountering of a lesion (depicted in red), triggers DNA damage tolerance (DDT) pathways. Translesion synthesis (TLS), Template switch and the Salvage pathway are the three main DDT pathways, and they are facilitated, mediated, or inhibited, respectively, by PCNA modifications with ubiquitin (Ub), polyubiquitin and SUMO (S). The cell cycle phases in which these pathways are preferred are also indicated. The recombination structures arising *via* template switch and the salvage pathways can be visualized by 2D gel analysis of replication intermediates, and their migration properties are identical.

As PCNA modifications can both mediate and block specific DDT pathways (for instance PCNA polyubiquitylation mediates template switching, but counteracts TLS [17]), several important research areas for understanding DDT regulation will be to explore whether individual PCNA trimers carry more than one of these modifications *in vivo*, how dynamic these modifications are, if preference for an initial type of PCNA modification is induced by distinct replication stress cues, and whether the initial modification counteracts or rather supports subsequent modifications of the clamp with other types of ubiquitin or SUMO moieties. In other words, does the first modification on PCNA function to "lock" an individual clamp to serve in a specific DDT module (for instance, template switch or TLS), or do these modifications often cooperate or act sequentially to each other to allow fluidity and cooperation between different DDT pathways, as in the case of complex lesions?

A conundrum related to PCNA modifications and their roles in mediating specific DDT pathways comes out from recent observations that pinpoint cooperation between factors mediating PCNA polyubiquitylation and HR in the context of template switching ([20] and see Section 3). As HR is inhibited by PCNA SUMOylation [15,16], how PCNA modifications with polyubiquitin and

Please cite this article in press as: D. Branzei, B. Szakal, DNA damage tolerance by recombination: Molecular pathways and DNA structures, DNA Repair (2016), http://dx.doi.org/10.1016/j.dnarep.2016.05.008