DNA Repair 9 (2010) 1273–1282

Contents lists available at ScienceDirect

DNA Repair

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

Mini-review

The influence of heterochromatin on DNA double strand break repair: Getting the strong, silent type to relax

Aaron A. Goodarzi[b], Penny Jeggo[b,*], Markus Lobrich[a,*]

[a] Darmstadt University of Technology, Radiation Biology and DNA Repair, 64287 Darmstadt, Germany
[b] Genome Damage and Stability Centre, University of Sussex, East Sussex BN1 9RQ, United Kingdom

ARTICLE INFO

Article history:
Accepted 9 July 2010
Available online 30 October 2010

Keywords:
DNA double strand break repair
ATM
heterochromatin
DNA non-homologous end-joining
Homologous recombination

ABSTRACT

DNA non-homologous end-joining (NHEJ) and homologous recombination (HR) represent the major DNA double strand break (DSB) pathways in mammalian cells, whilst ataxia telangiectasia mutated (ATM) lies at the core of the DSB signalling response. ATM signalling plays a major role in modifying chromatin structure in the vicinity of the DSB and increasing evidence suggests that this function influences the DSB rejoining process. DSBs have long been known to be repaired with two (or more) component kinetics. The majority (~85%) of DSBs are repaired with fast kinetics in a predominantly ATM-independent manner. In contrast, ~15% of radiation-induced DSBs are repaired with markedly slower kinetics via a process that requires ATM and those mediator proteins, such as MDC1 or 53BP1, that accumulate at ionising radiation induced foci (IRIF). DSBs repaired with slow kinetics predominantly localise to the periphery of genomic heterochromatin (HC). Indeed, there is mounting evidence that chromatin complexity and not damage complexity confers slow DSB repair kinetics. ATM's role in HC-DSB repair involves the direct phosphorylation of KAP-1, a key HC formation factor. KAP-1 phosphorylation (pKAP-1) arises in both a pan-nuclear and a focal manner after radiation and ATM-dependent pKAP-1 is essential for DSB repair within HC regions. Mediator proteins such as 53BP1, which are also essential for HC-DSB repair, are expendable for pan-nuclear pKAP-1 whilst being essential for pKAP-1 formation at IRIF. Data suggests that the essential function of the mediator proteins is to promote the retention of activated ATM at DSBs, concentrating the phosphorylation of KAP-1 at HC DSBs. DSBs arising in G2 phase are also repaired with fast and slow kinetics but, in contrast to G0/G1 where they all DSBs are repaired by NHEJ, the slow component of DSB repair in G2 phase represents an HR process involving the Artemis endonuclease. Results suggest that whilst NHEJ repairs the majority of DSBs in G2 phase, Artemis-dependent HR uniquely repairs HC DSBs. Collectively, these recent studies highlight not only how chromatin complexity influences the factors required for DSB repair but also the pathway choice.

Contents

* Corresponding authors at: Genome Damage and Stability Centre, University of Sussex, East Sussex BN1 9RQ, United Kingdom. Tel.: +44 1273 678482; fax: +44 1273 678121 and Darmstadt University of TEchnology, Radiation Biology and DNA Repair, 64287, Darmstadt, Germany. Tel: +49-6151-167460; fax: +49-6151-167461.
E-mail addresses: P.A.Jeggo@sussex.ac.uk (P. Jeggo), lobrich@bio.tu-darmstadt.de (M. Lobrich).

1568-7864/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2010.09.013

1. Introduction

Although DNA double strand breaks (DSBs) arise less frequently than DNA single strand breaks (SSBs) and many base alterations, they are, perhaps, the most critical DNA lesion since failure to repair a DSB has a high probability of causing cell death and, as importantly, erroneous DSB repair can lead to chromosomal rearrangements, a causal event in the aetiology of carcinogenesis. Consequently, understanding the factors that influence the efficacy and fidelity of DSB repair is important for assessing risks associated with exposure to DSB inducing agents, including an evaluation of the impact on overall human health as well as cancer avoidance [1]. Furthermore, now that we have at least a basic mechanistic understanding of most DNA repair processes, attention is shifting towards understanding how these pathways operate *in vivo* in the context of chromatin structure. To accommodate the enormous quantity of genetic material, which measures several metres when fully relaxed, into the dimensions of a nucleus, eukaryotes have evolved mechanisms to tightly compact their DNA. Genomic regions with specialist function or regions which are not undergoing active transcription are often further compressed into even more tightly compacted structures, termed heterochromatin (HC). There is a growing awareness that higher order chromatin architecture exerts just as profound an influence on DNA repair as it does on nuclear processes such as transcription and replication. It is becoming increasingly clear that a range of chromatin remodelling mechanisms function to facilitate these DNA transactions in their cellular context [2,3]. Such processes involve not only mechanisms that restore chromatin structure when DNA is damaged but also mechanisms that transiently or locally modify chromatin structure to promote DNA repair.

Given the high degree of compaction of HC DNA and its efficacy at blocking transcription, it is perhaps not surprising that it has been shown to act as a barrier for some DNA repair processes. Intriguingly, it appears that in mammals ataxia telangiectasia mutated (ATM) signalling plays a critical role in relieving the constraints on DSB repair posed by the highly compacted HC. In contrast, Tel1, the yeast homologue of ATM, has a less significant role in DSB repair, raising the possibility that ATM signalling has, at least in part, evolved to help overcome the increased chromatin compaction arising in more complex eukaryotic genomes [4]. Here, we review how HC impacts upon DSB repair, focusing on the role of ATM and its damage response mediator proteins in overcoming the constraints posed by the HC superstructure. Further, we discuss how higher order chromatin structure influences DSB repair pathway choice in mammalian cells.

2. DSB repair and DNA damage response signalling

DNA non-homologous end-joining (NHEJ) represents the major DSB repair process in mammalian cells and functions throughout the cell cycle [5]. This process has been reviewed in detail previously and will only be outlined here. In brief, the Ku70/80 heterodimer (Ku) is a basket shaped structure with a central pore, whose structure confers an avid ability to bind double stranded DNA ends [6]. The central pore allows Ku to thread onto double stranded DNA ends and a ratchet mechanism facilitates its internal translocation. DNA bound Ku recruits the large DNA-dependent protein kinase catalytic subunit (DNA-PKcs) forming the DNA-PK holoenzyme and activating its kinase activity [5]. DNA-PK phosphorylates components of the NHEJ machinery and undergoes autophosphorylation. Although the precise function of DNA-PK activity is still under investigation, it appears to play a central role in DSB processing, potentially recruiting and activating end-modifying proteins. DNA-PK autophosphorylation appears to be important in co-ordinating rejoining with end-processing [7]. An example of this is the role of DNA-PK in promoting the activity of Artemis, an endonuclease which, although not a core NHEJ protein, functions in a subset of NHEJ events [8,9]. DNA-PK assembly at the DSB also recruits a complex encompassing DNA ligase IV, XRCC4 and XLF/Cernunnos, which carries out the rejoining step of the process. The significant role that NHEJ plays in the rejoining of non-replication associated DSBs is evident from the dramatic ionizing radiation (IR) sensitivity displayed by NHEJ deficient mutants, which can be observed in all cell cycle phases [10].

In addition to NHEJ, homologous recombination (HR) also functions to repair IR-induced DSBs in late S and G2 phase [11]. The process of HR will be covered elsewhere in this issue and will not be discussed in detail here. It is important to appreciate, however, that HR in mammalian cells demands a sister chromatid as a template for repair and hence functions uniquely in late S and G2 phase [12]. As one step to ensure that HR is restricted to these cell cycle phases, DSB end resection, an important step initiating HR, is regulated by CDK1 and hence HR does not occur in G0/G1 phase. Whilst HR makes a contribution to the repair of the two-ended DSBs in G2 phase, which will be discussed in Section 8 below, more substantial roles occur at the replication fork [11]. One such function is to repair one ended DSBs that arise during replication fork collapse. However, there is increasing evidence that an additional role of HR is to promote recovery of stalled replication forks via a process that does not involve DSB formation [13].

DSBs also trigger a signal transduction response that regulates cell cycle checkpoint arrest and/or apoptosis. ATM, a phosphoinositol 3-kinase like kinase (PIKK), plays the key role in activating the DSB signalling response [14]. Although most NHEJ occurs independently of ATM signalling, recent studies have shown that ATM deficient cells fail to repair a subcomponent of DSBs, demonstrating that ATM also influences DSB repair [15]. This aspect of ATM-dependent signalling will be discussed in greater detail below. The response to DSBs involves the orchestrated assembly of damage response signalling proteins at the site of the DSB, often referred to as ionising radiation-induced foci (IRIF). Although the elegant choreography of IRIF formation will be described elsewhere in this issue, here we will briefly overview steps of relevance to the consideration of HC DSB repair. It is important to appreciate the delicate interplay between ATM and the assembly of the damage response signalling proteins. Perhaps surprisingly, IRIF assembly is largely dispensable for ATM activation and distinct end points of ATM signalling are impacted differently when the signalling proteins fail to assemble into IRIF normally. Likewise, ATM signalling is not essential for IRIF formation, although most of the signalling proteins are ATM substrates and their phosphorylation impacts the damage response in diverse ways, some of which are significant, others less so [16,17].

The Mre11-Rad50-NBS1 (MRN) complex functions as the initial DSB sensor which, via an interaction between the C-terminus of NBS1 and ATM, recruits ATM to the DSB [17,18]. This causes initial ATM activation, autophosphorylation and monomerisation of ATM multimers, a step leading to sustained activation [19]. One of the first substrates of ATM to be phosphorylated is histone H2AX, generating γH2AX. This early step in the signalling cascade is redundantly carried out by DNA-PK, a significant factor contributing to the ATM-independence of the IRIF assembly process [20]. γH2AX interacts with the mediator protein, MDC1, which in turn interacts with MRN and provides a second 'wave' of ATM tethering at the DSB site [21–23]. MDC1 accumulation at the DSB serves to recruit two ubiquitin ligases and associated proteins required for ubiquitylation, including RNF8, RNF168 and HERC2. The main ubiquitylation target is histone H2A (and potentially additional substrates) in the vicinity of the DSB ([24] and see Ref. [25] for a review). It is believed that these steps result in the exposure of spe-