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Double strand break (DSB) repair in heterochromatin and heterochromatin proteins in DSB repair

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ABSTRACT

Chromosomal translocations are a hallmark of cancer cells and they represent a major cause of tumorigenesis. To avoid chromosomal translocations, faithful repair of DNA double strand breaks (DSBs) has to be ensured in the context of high ordered chromatin structure. However, chromatin compaction is proposed to represent a barrier for DSB repair. Here we review the different mechanisms cells use to alleviate the heterochromatic barrier for DNA repair. At the same time, we discuss the activating role of heterochromatin-associated proteins in this process, therefore proposing that chromatin structure, more than being a simple barrier, is a key modulator of DNA repair.

1. Introduction

DNA lesions and mutations although being the major motor of evolution and necessary for several cellular processes such as immune system diversification or meiosis, can threaten cell viability and genome stability because they can lead to chromosomal rearrangements [22]. DNA breaks can be induced by endogenous sources that are byproducts of our own metabolism or by exposure to damaging agents, UV light and irradiation [22].

Double strand breaks (DSBs) are among the most deleterious lesions since they affect both strands of DNA. To cope with damage, the appearance of a DSB activates the DNA Damage Response (DDR) – a complex network of processes that allows recognition of the break and the activation of checkpoints, allowing the coordination between cell cycle progression and DNA repair [36].

The early step of the signaling cascade involves sensing the break by the ATM kinase (Ataxia Telangiectasia Mutated), which initiates a megabase-wide spreading of a phosphorylated form of the histone variant H2AX at serine 139, around the lesion – γH2AX, which is considered the major transducer of the signaling cascade. These early events are in turn responsible for the subsequent recruitment of repair factors and the initiation of the repair mechanisms [36].

Two major mechanisms repair DSBs: Homologous Recombination (HR) and Non-homologous End Joining (NHEJ). HR takes place in the replicative and post-replicative stages of the cell cycle (S/G2), when sister chromatids are present and allow faithful repair [20]. However, the use of HR outside of the S or G2 phases of the cell cycle or between repetitive sequences can lead to major recombination events [20]. NHEJ on the other hand does not require the presence of an undamaged template in order to repair. It is a flexible but conservative mechanism that enables a direct rejoining of broken DNA ends and is thus active throughout the whole cell cycle. In few instances it involves processing of the DNA ends and thus can be an error-prone mechanism [17]. Recently, a third pathway has been described, called Alternative End Joining (A-EJ), which is highly mutagenic and can be revealed in the absence of key NHEJ factors [12].

Repair by these two pathways must be very tightly regulated in time and space to avoid deleterious chromosomal rearrangements. Increasing evidence suggests that chromatin and its compaction state plays a role in the regulation of DDR and DSB repair. Chromatin is the complex between DNA and its associated proteins. The fundamental unit of chromatin is the nucleosome which consists of ~200 bp of DNA wrapped around an octamer of core histones [25]. Interactions between individual nucleosomes mediated by numerous non-histone proteins lead to the formation of higher order chromatin structure that can have various compaction states.

Heterochromatin, in opposition to euchromatin, was originally described as densely stained regions of the nucleus and corresponds to a highly compacted form of chromatin [34]. Historically, it is considered transcriptionally inactive and rich in repetitive sequences whereas euchromatin is more gene-rich and transcriptionally active. Histone post-translational modifications (PTMs) are proposed to have a main role in defining a chromatin state, and

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specific histone marks are more enriched in heterochromatin such as the trimethylation of the histone H3 on lysine 9 (H3K9me3) whereas others are more enriched in euchromatin, such as histone acetylation that is generally depleted from heterochromatin [26]. The histone marks can be read and bound by specific non-histone proteins that can alter the overall structure of chromatin. Among these proteins, the heterochromatin protein 1 (HP1) proteins are key factors in the establishment and maintenance of heterochromatin. HP1 was initially discovered in drosophila as a protein involved in gene silencing [14]. It directly interacts with H3K9me3 and this binding is necessary for the maintenance of heterochromatin state [34]. It also interacts with the co-repressor KRAB-domain associated protein 1 (KAP1) which is also involved in the regulation of heterochromatin structure through its binding with the histone methyl-transferase SETDB1, the histone deacetylases HDAC1 and HDAC2 and the nucleosome remodeling factor CHD3 [21].

The high compaction of chromatin in heterochromatin is hypothesized to constitute a barrier for DNA repair and DSBs that are formed in heterochromatin are processed slower than in euchromatin [19,32]. On the other hand, recent findings show that heterochromatin-associated proteins play a positive role in DNA repair [45]. Here, we discuss this paradox and we review the recent literature that describes how DNA repair occurs within heterochromatin, and how certain proteins that have repressive roles in heterochromatin possess active roles in DNA repair, proposing thus that more than being a simple barrier, chromatin and its components are key regulators of DSB repair.

2. Ways to alleviate chromatin compaction for efficient DDR and DNA repair

Cells have evolved several mechanisms to allow DNA repair in the context of chromatin and especially in the highly condensed form of chromatin, the heterochromatin. In 2007, Cowell et al. described that cells exposed to ionizing irradiation (IR) depicted only a few number of foci of the early marker of DDR activation, γH2AX, within heterochromatin, suggesting that heterochromatin was less sensitive to IR [11].

However, a recent study performed in drosophila cells showed that the initial formation of γH2Av (the drosophila homologue of H2AX) was equivalent in heterochromatin and euchromatin but that the number of γH2Av foci remaining in heterochromatin 1 h after IR was lower than in euchromatin. By following cells expressing fluorescently tagged versions of HP1a and DSB repair proteins with live cell imaging, Chiolo et al. further showed that the heterochromatic DSB foci relocate at the periphery of the heterochromatin domains [9]. Similar relocation was also observed in mammalian cells upon single ion microirradiation [23].

The increased motion of heterochromatic DSBs is quite unique since repair foci were shown to exert very limited mobility in mammalian cells [46]. On the other hand, unprotected telomeres, which resemble DSBs, were also shown to be mobilized in mouse cells [13], suggesting that motion of DSBs might occur specifically in heterochromatinized nuclear domains. It was proposed that relocation is a mechanism to avoid recombination between repetitive sequences. Although this relocation is dispensable for the first steps of DDR or DNA repair and the recruitment of early factors happens within the heterochromatin [9,23], the late steps of HR are only effective outside the heterochromatin domains. Indeed, RAD51 was shown to be recruited only after the relocation and showed mutual exclusivity with HP1a [9]. Interestingly, the relocation of repair foci outside of heterochromatin requires the activity of the ATR kinase and functional resection [9].

Additional heterochromatin associated factors were shown to be important for this mechanism. Particularly, the SUMO ligase complex Smc5/6 is necessary for the relocalization of heterochromatic DSBs and for the inhibition of Rad51 recruitment within heterochromatin [9]. Nevertheless, the exact mechanism by which DSBs are mobilized remains to be elucidated. Strikingly, an expansion of the heterochromatin domain was observed in parallel to the relocation of the break, suggesting a local decompaction of the compartment [9]. A possible hypothesis is that this alteration of the heterochromatin domain allows the increase of DSB mobility.

Chromatin structure alterations are thought to influence the strength of DDR. In response to DNA damage, chromatin undergoes global decondensation, a process that has been proposed to facilitate genome surveillance by enhancing access of DDR proteins to sites of damage [27]. In line with this idea, when DNA lesions occur at embryonic stem cells (ESCs) from transgenic mice with reduced amounts of the linker histone H1, and thus less compacted chromatin, the strength of the DDR signal that is generated at each break site is enhanced, suggesting that DDR is amplified in the context of open chromatin [37]. The enhanced DDR upon chromatin decondensation is achieved by over activation of the major driver of DDR, ATM [4]. Indeed, ATM becomes rapidly activated in response to changes in chromatin structure, upon exposure of cells to mild hypotonic buffers, treatment with the histone deacetylase (HDAC) inhibitor TSA, depletion of HP1, all conditions that lead to chromatin decondensation [4,24].

On the other hand, the mechanisms by which chromatin decompaction occurs after DSB induction seem to involve the activity of ATM. Indeed, ATM phosphorylates KAP1 on its serine 824 and this phosphorylation is necessary for a proper DNA repair in heterochromatin [19,40]. The phosphorylation of KAP1 induces chromatin decompaction by several mechanisms, including the release of the histone deacetylase CHD3 [18].

Furthermore, the sumoylation of KAP1 is regulated upon DNA damage, leading to chromatin decompaction. Indeed, the corepressive activity of KAP1 is dependent on its sumoylation that allows the binding of CHD3 and the histone methyl-transferase SETDB1. Upon DSB induction, the desumoylase SENP7 desumoylates KAP1, leading to CHD3 release from chromatin [15]. SENP7 was subsequently shown to be necessary for a proper DSB repair by homologous recombination [15]. Collectively, this data show a tight regulation of the balance between phosphorylation and sumoylation of KAP1 during DDR, allowing a modification of chromatin state that is necessary for subsequent DNA repair. Interestingly, different phosphatases like PP4C and PP1alpha and beta (PP1a and b) have been reported to interact and dephosphorylate KAP1 [30,31,28]. Dephosphorylation of KAP1 by PP1a and PP1b was also reported to stimulate KAP1 sumoylation [30]. These phosphatases could then be involved in the restoration of chromatin state after repair since prolonged KAP1 phosphorylation at S843 was shown to delay chromatin restoration after DSB repair [31,28].

Furthermore, KAP1 mediates chromatin decompaction upon DNA damage through the disruption of its interaction with HP1β. In fact, HP1β was shown to be rapidly mobilized and released from heterochromatin upon DNA damage [2] and this mobilization is mediated by the phosphorylation of HP1β by the casein kinase 2 (CK2) and by the phosphorylation of KAP1 by the checkpoint kinase Chk2 [7]. Indeed, additionally to its phosphorylation by ATM, KAP1 is phosphorylated by Chk2 on serine 473 that is located in the HP1 binding motif of KAP1 [7,6,8,50]. This phosphorylation is necessary for the release of HP1β from chromatin and for subsequent DNA repair within heterochromatin [7].

Another important player for chromatin decompaction upon DSB induction is the histone acetyl-transferase Tip60. Tip60 is recruited to DSBs by the MRN complex, leading to the acetylation of histone H4, which induces a subsequent chromatin decompaction.

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