Chapter 1

Ubiquitylation and SUMOylation: An Orchestrated Regulation During DNA Damage Repair

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Abstract

DNA double-strand breaks (DSBs) are cytotoxic DNA lesions that must be repaired as soon as possible because it can cause chromosomal aberrations and cell death. Homologous recombination (HR) and nonhomologous end joining (NHEJ) are the pathways that mainly repair these ruptures. HR process is finely regulated by synchronized posttranslational modifications including phosphorylation, ubiquitylation, and SUMOylation. The ubiquitin (Ub) modifications at damaged chromatin serve as recruitment platforms for DSB repair complexes by facilitating binding sites or regulating the interaction between proteins. Thus, SUMOylation has been associated with protein interaction, enzymatic activity, and chromatin mobility. Several DNA damage factors have been found to be ubiquitylated and SUMOylated including histones (H2AX) and proteins such as Mre11, Rad51, NBS1, and BRCA1. Regarding ubiquitylation-mediated regulation of DNA repair, RNF168 and RNF8 E3 ligases have turned out to be a key step in DNA damage repair regulation. Interestingly, there is evidence that the Ub signaling mechanism is ancestral, and this emphasizes its importance.

Keywords: ubiquitylation, DSB, SUMOylation, DNA repair, chromatin architecture

1. Introduction

Genome integrity is compromised by the constant attack from exogenous and endogenous DNA-damaging factors such as radiation, carcinogens, reactive radicals, and errors in DNA replication. The most deleterious DNA lesion is the double-strand breaks (DSBs) because failure to repair them results in diverse changes in DNA such as mutations or chromosomal rearrangements. Thus, to maintain genomic stability, cells have developed an elaborate DNA damage response (DDR) system to detect, signal, and repair the DNA lesions [1–3].

DSBs are repaired by two main pathways: nonhomologous end joining (NHEJ) and homologous recombination (HR). NHEJ works with a fast kinetics throughout the cell cycle and joins broken DNA ends without the need of extended complementary sequences leading to an error-prone repair [4]. HR, on the other hand, takes longer and is restricted to the S and G2 phases of the cell cycle since an intact sister chromatid is required for repair based on a homologous template, and thus this process is carried out error-free [5].

HR is an evolutionary well-conserved mechanism, where nucleolytic degradation of the 5′ end in the DSB produces long 3′-single-stranded DNA (ssDNA) overhangs, and this is referred as DNA end resection [6, 7]. These dangling 3′ ends must be protected from nucleases, and the formation of tertiary structures is accomplished by **r**eplication **p**rotein **A (**RPA), which in turn is replaced by recombinase Rad51 to form the Rad51-ssDNA presynaptic filament to promote HR. Thus, DNA end resection is a key player for the Rad51-ssDNA filament formation, and it must be tightly controlled by diverse mechanisms; posttranslational modification to core components of resection machinery as well as antagonists is one of them (PMTs). These PMTs mainly phosphorylation [8] and recently ubiquitylation and SUMOylation have been shown to play an important control in many features of cellular responses to DNA damage, including the repair of DSBs [9, 10] as shown by high-throughput proteomics studies where it was observed that DSB repair is facilitated by global ubiquitylation and SUMOylation induced by DNA damage [11, 12]. This review will focus on ubiquitylation and SUMOylation participation in DSB response.

2. Ubiquitin in DSB response

Ubiquitin (Ub) is a 76 amino acid protein with seven lysine residues that can form polyubiquitin chains of eight different linkages (K6, K11, K27, K33, K48, K63, and Met1) as well as mixed and branched chains (**Figure 1**) [13]. The generation of different protein Ub chains provides structural diversity allowing proteins with specific Ub-binding domains (UBDs) to discriminate between these different structures. For example, Ub K48 and K63 polyubiquitin chains are structurally distinct and are differentially recognized by proteins containing different UBDs [14]. To date, over 200 proteins with at least 20 different types of UBDs have been identified that bind to different Ub structures in a noncovalent manner [15]. The ability of distinct protein Ub structures to specifically bind to proteins containing a particular UBD is important for generating specificity of protein-protein interactions and targeting proteins to different pathways and fates. For example, monoubiquitylation can regulate DNA repair, regulation of histone function, gene expression, and receptor endocytosis (**Figure 1**) [16].

Due to the ability of the Ub molecule to be conjugated onto diverse substrate lysine(s), protein ubiquitylation is a multifunction-oriented process using its own lysines or via its N-terminal methionine residues, to generate a diverse range of structures and therefore modify activities in protein targets [17]. Each linkage kind promotes a different protein conformation providing a certain degree of diversity, thus exposing a specific Ub-binding domain (UBD) with a particular function like favoring or inhibiting protein-protein interactions, protein localization, and/or degradation. To illustrate this, polyubiquitin chains attached to a protein in its Ub K63 linkage could mostly apply to proteins mainly distributed in the lysosome/endocytosis, DNA repair, and signal transduction (**Figure 1**). The ubiquitylation process is a bit complex; it is carried out mainly by

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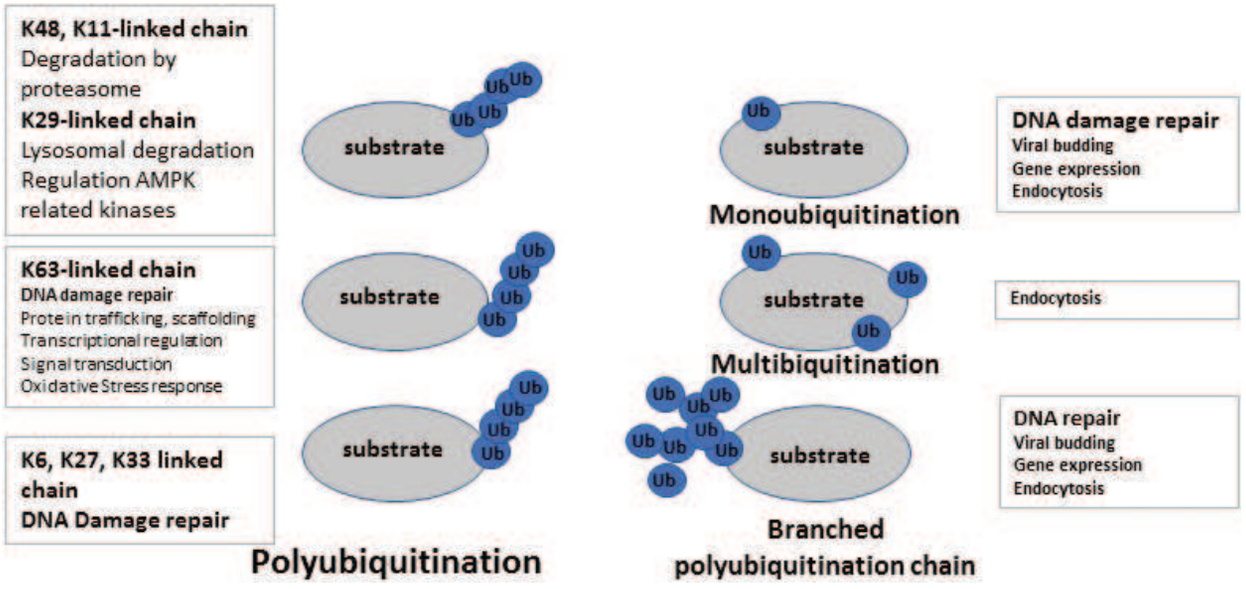

Figure 1. The ubiquitin linkage change the substrate at diverse range of structures and these modifications transform the affinity to other proteins, therefore the function is modified.

three proteins: E1 (activating enzyme), E2 (conjugating enzyme), and E3 (ligating enzymes). E1 activates ubiquitin (Ub) C-terminus by generating a thioester-linked E1~Ub conjugate which is dependent on adenosin-5′ triphosphate (ATP). Then, via a trans-thiolation reaction, the E2 active cysteine site receives the activated Ub from E1. E3 and E2 cooperate to facilitate the transfer of Ub onto a substrate lysine (K) of a protein target to form an isopeptide bond resulting in a ubiquitylated protein. E3 enzymes have been grouped in three E3 families: RING families (**r**eally **i**nteresting **n**ew **g**ene), HECT (**h**omologous to **E**6-AP **c**arboxyl **t**erminus), and hybrid RING/HECT E3 [18].

In order to promote the isopeptide formation between the lysine residue of the target protein and the glycine of the Ub C-terminus, the RING E3 ligase recruits both the E2-Ub conjugate and protein target. In contrast, HECT E3 ligases take Ub from E2-Ub conjugate on a catalytic cysteine and transfer the ubiquitin to a target lysine. On the other hand, the hybrid RING/HECT E3 ligase N-terminal RING1 domain works like the RING E3 ligases since they bind and recognize the E2-Ub conjugate, while the RING2 domain catalytic cysteine accepts a Ub molecule from E2-Ub conjugate before it is transferred to the target lysine [19]. Protein ubiquitylation is reversible through deubiquitylating enzymes (DUBs), which have the ability to cleave single Ub or polyubiquitin chains from targeted proteins.

Rad6, a postreplication repair (PRR) protein [20], was the first enzyme involved in an ubiquitylation role. Also, a mutation in ubiquitin K63R caused sensitivity to UV and DNA damage in yeast [21]. Rap80 bears a tandem ubiquitin-interacting motif (UIM) that binds to K63 linkages in vitro and is attached to Ub through K63 linkages in vivo upon DNA damage. In humans Rap80 binds to BRCA1 (**br**east **ca**ncer type **1** susceptibility) protein that has an important role during HR repair [22]. BRCA1 and Bard1 form different complexes (BRCA1-A, BRCA1-B, and BRCA1-C) with Abraxas or Bach1/FancJ or CtIP [23]. BRCA1-A interacts with regions that flank DSBs after phosphorylation, and ubiquitylation reactions promoted by the MRN complex (Mre11-Rad50-NBS1) take place. MRN complex senses the DSB, recruits (through NBS1), and activates **a**taxia **t**elangiectasia **m**utated (ATM) to initiate the DNA repair signaling

response through histone H2AX phosphorylation on serine139 [24]. Thus, **Figure 1** illustrates the complex role of ubiquitin in both degradation and regulation of function on processes like DNA repair and endocytosis. The BRCA1/Rap80 complex contains other proteins such as MERIT40 and BRCC36. Interestingly, while MERIT40 facilitates BRCA1-A complex, assembly in response to DNA damage, BRCC36 is a deubiquitin enzyme with specificity on the Ub K63.

RING finger protein 8 (RNF8) is an E3 ligase that catalyzes Ub K63 linkages at DSBs in mammals. Once H2AX is phosphorylated by ATM in regions that flank DSBs, MDC1 (**m**ediator of **D**NA **d**amage **c**heckpoint 1) protein is also rapidly recruited by recognizing the phosphorylated H2AX through its BRTC domain. H2AX phosphorylation promotes RNF8 recruitment to the DSB regions by its interaction with the MDC1 terminal FHA domain. It has been proposed that RNF8 fast recruitment stimulates H1 type linker histone ubiquitylation (K63) mediated by UBC13 E2 ligase, which in turn recruits RNF168 through their UIMs, and this results in H2A ubiquitylation of H2A at K13 and K15 residues [25, 26]. These ubiquitylation modifications allow chromatin changes that facilitate the recruitment of other DSB response factors: RPA 80, 53BP1, and BRCA1 among them. Additionally, it is interesting that RNF168 extends the ubiquitylation degree on the flanking regions of DSBs, and this is required for DNA repair. This evidence highlights that ubiquitylation is a cornerstone of the DSB response, and its precise control is essential for genome stability [22].

3. SUMO in DSB response

In 1996, **s**mall **u**biquitin-related **mo**difier (SUMO) protein was discovered as a 100-amino acid-long protein. These proteins, though their sequence is not identical among them, share a common 3D structure and a C-terminal di-glycine motif that is required for its attachment to the lysine residue of the target protein via isopeptide bond [27]. SUMO could covalently be attached to target protein lysine residues by E1, E2, and E3 SUMO ligases in a similar manner to ubiquitin conjugation [28]. SUMO bears a long flexible N-terminal tail [29]. There are four different SUMO isoforms [1–4]; they are normally translated as longer precursors, consequently in order to obtain the mature forms, and they must be processed. As mentioned earlier, SUMO1 shares 48% sequence identity with SUMO2, while SUMO2 and SUMO3 present 90% sequence identity [30].

Because SUMO2 and SUMO3 isoforms are not distinguished by antibodies, they are usually referred as SUMO2/SUMO3. Further, recent data for SUMO4 indicates that this is processed to its mature form only under particular conditions [31]. *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* contain a single SUMO protein Smt3 [29]. In contrast, the SUMOylation modification is carried out by one heterodimeric E1, one E2 (UBC9), and approximately 10 E3s in humans [32–34]. As for ubiquitin-like modifiers, this process is also reversible by removal of SUMO from target proteins accomplished by SUMO/sentrin-specific proteases (SENPs) [9, 35]. Two E3 SUMO ligases involved in damage DNA repair (DDR) were identified: PIAS1 and PIAS4 E3 by immunofluorescence and biochemical assays. These ligases promote BRCA1 and 53BP1 protein SUMOylation [36]. When PIAS1 and PIAS4 are removed, there is a severe impairment in Ub K63 at damage sites, thus diminishing BRCA1 and 53BP1 recruitment and causing deficient DNA repair. Therefore, in addition to ubiquitin, SUMO modifications also occur at DSBs, and these modulate the DSB response [22]. Another protein that has an important role in DDR is Rad52, and this is also SUMOylated in

yeast and mammals; in *S. cerevisiae*, the RAD52 SUMOylation affects its stability and consequently the RAD52-dependent homologous recombination repair (HRR) [37]. As can be seen, SUMOylation and ubiquitylation are working together in DSB response.

4. Ubiquitin and SUMOylation of DNA end resection machinery

In response to DNA double-strand break (DSB), various elements of DNA damage response are recruited to these injured sites. The gathering of these molecules at damage sites becomes visible as foci (or **i**onizing **r**adiation **i**nduced **f**oci (IRIF)) in the nucleus, which can be observed via immunofluorescence microscopy [38].

In the initial stage of HR, the DSB ends are resected in such a way that 3′-single-strand DNA (ssDNA) overhangs are generated. This process is started by the conserved MRX (comprises by Mre11-Rad50-Xrs2) nuclease complex, which in collaboration with Sae2 in yeast, and by the MRN (including Mre11-Rad50-NBS1) complex in conjunction with CtIP (**C**-**t**erminal-binding **i**nteracting **p**rotein) in human cells; MRN/MRX complex is able to eliminate oligonucleotides from the 5′ strand, resulting in an incomplete end processing [39–42]. Additionally, the MRN/MRX complex is necessary to recruit the kinase ATM (**a**taxia **t**elangiectasia **m**utated) kinase, Exo1, Sgs1, and Dna2, to the damage site [43]. Later, resection is prolonged by the 5′-3′ exonuclease, Exo1, or by the collective activities of the Sgs1-Top3-Rmi1 (STR) complex and Dna2 (**Figure 2**) [44].

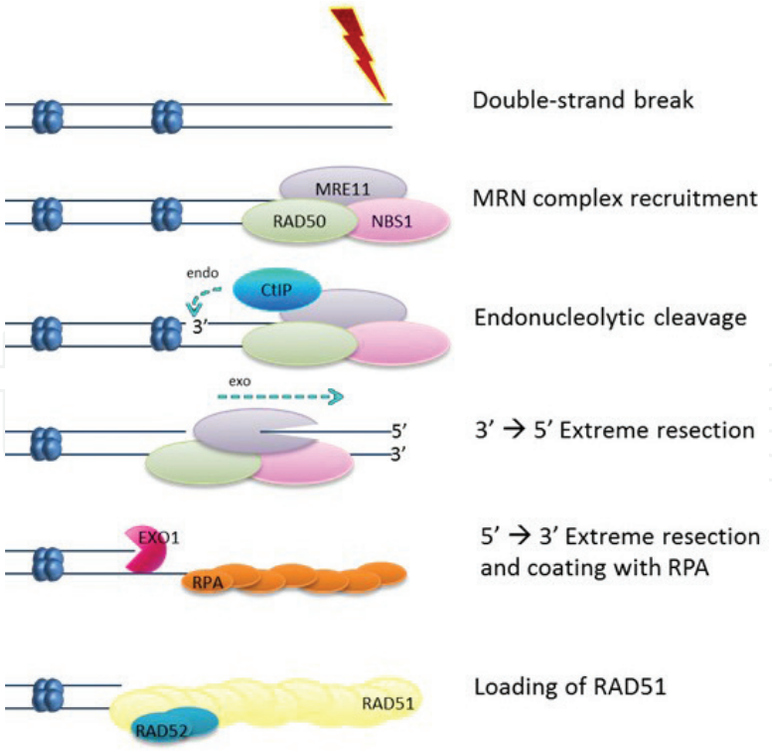

Figure 2. Scheme of DNA-end resection model. After DSB generation the MRN/X complex is recruited to the injurie site. In h. sapiens, Ctlp performs an endonucleolytic cleavage upstream from the DBS end on the 5′-terminated strand. Then, Mre11 exonuclease activity degrades DNA in a 3′-5′ direction, starting from the nick until the DSB end. The 3′ ssDNA is coated by RPA. Exonuclease EXO1 performs an extensive resection in a 5′-3′ direction. After resection, HR continues as a RAD52-mediated RPA replacement by RAD51 and further downstream steps.

After the resection process, the ssDNA overhangs are speedily coated by RPA (**r**eplication **p**rotein **A**), which is thought to eradicate secondary structures and at the same time protect the ssDNA from nuclease activities [45]. The BRCA1 protein recruits activated CtIP, PALB2, and BRCA2 (**br**east **ca**ncer **2**) to damage sites; PALB2 and BRCA2 enable RPA-RAD51 exchange on ssDNA [46, 47]. The subsequent RAD51-ssDNA filament searches for a sequence with homology, preferably on the identical sister chromatid. Afterward, the RAD51-ssDNA filaments invade the homologous sequence and anneal to the complementary ssDNA, allowing the DNA polymerases to synthesize DNA by using the undamaged DNA strand as a template. Thereby, HR repairs DSBs maintaining integrity and sequence, namely, without nucleotide deletion or alteration [38]. In response to DSBs, NBS1 interacts with components of the SCF (**S**kp1-**C**ullin1-**F**-box) E3 ligase complex and Skp2 (F-box protein) (**Figure 3**); this interaction conjugates K63-linked ubiquitin chains onto NBS1-K735 cells deficient in Skp2 which were defective in ATM activation and HR [48].

The E3 ligase RNF8 ubiquitylates NBS1 at Lys-435, mainly, and at Lys-6 that is promoted likely by E2 ligase UbcH5C. Ubiquitylation of NBS1 was detected before and after DNA damage. Studies with RNF8 mutants suggest that the interaction of RNF8 with NSB1 is mediated by the N-terminus of RNF8. RNF8 and certain RNF8 ubiquitylation activities are needed for efficient localization of NBS1 and MRN recruitment to DSB (**Figure 3**) [49].

S. cerevisiae Mre11 SUMOylation is required to interact with Ubc9 (E2) and Siz2 (an E3 related to mammalian PIAS proteins) (**Figure 3**) [11]. Also, SUMO-interacting motifs (SIMs) in Mre11 facilitate MRX complex assembly through poly-SUMO chains noncovalently recruitment [50].

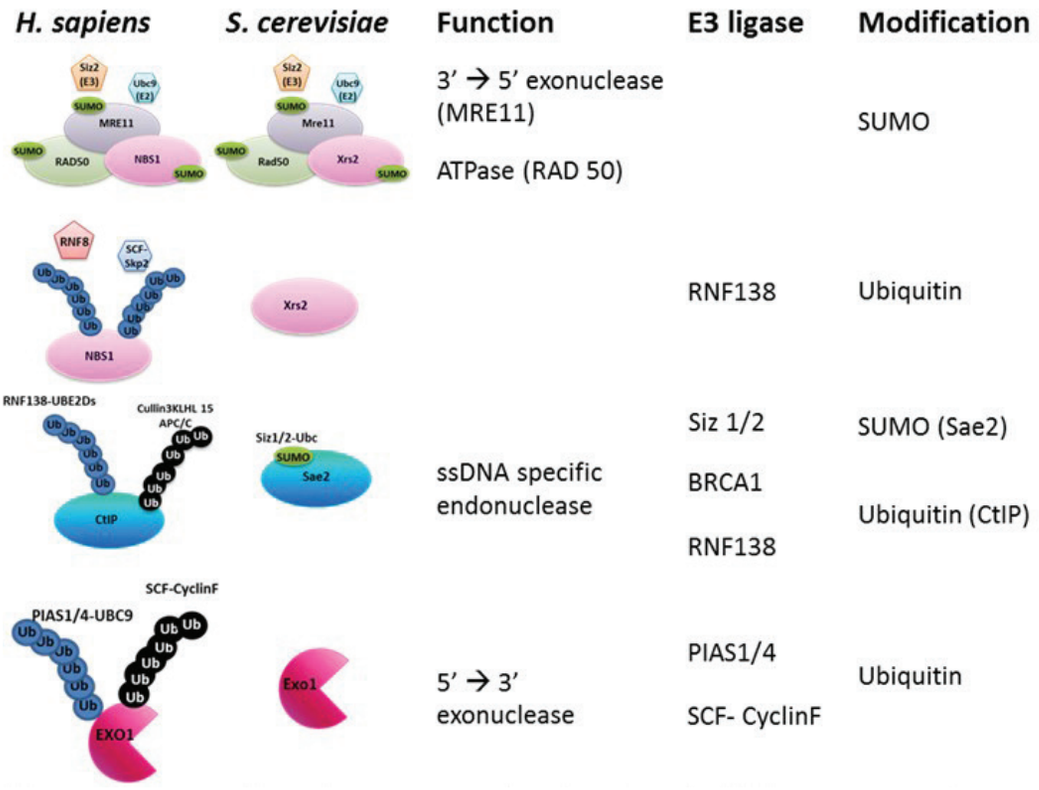

Figure 3. Illustration of some proteins involved in DNA-end resection process and their modifications. Blue dots, ubiquitin modifications involved in protein function regulation. Black dots, ubiquitin modifications involved in protein degradation. Green ovals, SUMO modification.

The heterodimeric RING-type E3 ligase BRCA1/BARD1 ubiquitylates CtIP to promote its stable retention at sites of DNA damage [51], although this physiological role still remains to be determined [52]. It has been proposed that ubiquitylation of CtIP by RNF138-UBE2D is a key event in promoting HR (**Figure 3**) [53].

During DNA end resection process, the participation of deubiquitinase (DUB) activity of USP4 (**u**biquitin-**s**pecific **p**eptidase 4) was observed. Two independent studies showed that CtIP recruitment to DSBs is regulated by interaction with USP4 and also USP4 binds to MRN complex [38, 39]. CtIP degradation via the ubiquitin-proteasome pathway is stimulated by its interaction with the CUL3 substrate adaptor Kelch-like protein 15 (KLHL15) (**Figure 3**) [42, 54].

After DNA damage, Sae2 is SUMOylated at a single conserved lysine residue (K97) mediated by Ubc9-Siz1/Siz2, and the levels of soluble Sae2 were increased [55]. An indication of Sae2 SUMOylation critical role for DNA end resection was observed in Sae2-K97R mutant cells, in which the processing and repair of DSBs were decreased [42].

It has been shown that human EXO1 is targeted for degradation by the ubiquitin-proteasome pathway. Recently, it was demonstrated that PIAS1/PIAS4-UBC9-mediated EXO1 SUMOylation (**Figure 3**) is a prerequisite for EXO1 ubiquitylation [56]. Even though the interactions between EXO1 and SENP6 de-SUMOylating enzyme [57], EXO1 with SCF-cyclin F E3 ubiquitin ligase (**Figure 3**) [12], and EXO1 with UCHL5 [58] have been studied, their participation in DNA end resection process has not been determined. PIAS1 and/or PIAS4 SUMOylates BRCA1 when it is localized at DSB sites, enhancing its ubiquitin ligase activity [36]. The MRN, Ubc9, and Siz2 allows *S. cerevisiae* Rad52 SUMOylation. This SUMOylation protects Rad52 from degradation and excludes it from nucleoli [59].

5. Chromatin remodeling

In general, any process like transcription, replication, and DNA repair requires a certain degree of chromatin access; therefore, remodeling is an important prerequisite for factors related to such processes. The participation of ubiquitylation and SUMOylation role on DNA repair on chromosome topology are very important in chromatin structure and organization.

3C (**c**hromosome **c**onformation **c**apture) is a technique where loci that are spatially closed can be formaldehyde crosslinked and identified; it was designed to determine chromatin interactions at increasing scale and resolution [60]. An upgrade of 3C is Hi-C technique, in which the only difference is that a step of biotinylation on the enzyme-restricted ends before DNA ligation has been included; this is to ensure that only ligated junction between chromosomes are purified and sequenced.

Using 3C-based technology, it has been possible to determine intrachromosomal contacts within TADs (**t**opological **a**ssociated **d**omains) that can be measured in regions of hundreds of kilobases [61]. In general, these TADs comprise long-range interactions like those found between enhancers and promoters. There are also interchromosomal contacts that are defined within same chromosome boundaries and demonstrated by the technique FISH chromosome painting [62]. These findings provide support for a nucleus architecture with layers of organization that result in a chromatin particular orchestration. Recently, it has been suggested that

the chromatin organization dynamics can influence the DSB response as well as the outcome of DNA repair, which consequently will have effects on genetics stability and the production of genetic abnormalities.

These effects can be classified as bulky or large and localized.

5.1. Bulky effects

The bulky effects have been observed as long-range movement; for example, the case of localizing the VP16 activator to the nuclear periphery resulted in its relocalization to the nuclear interior, and also when RNA pol I transcription was inhibited, this caused movement of chromatin to the nucleolar periphery (**Figure 4**) [63].

Interestingly in *S. cerevisiae*, when DSBs were produced in the rDNA, these ruptures were moved to the exterior of the nucleolus [59]. Though it is not clear whether this is part of an ongoing movement or indeed due to the DSB-promoted process, nonetheless, this translocation depended on Rad52 SUMOylation which interestingly is also required for homology-directed repair (HDR). There have been other DSBs that produced chromatin mobility, and it has been shown that breaks elsewhere in the yeast genome also led to a greater mobility of chromatin that was dependent on RAD51, RAD54, MEC1, RAD9, and INO80 [64, 65].

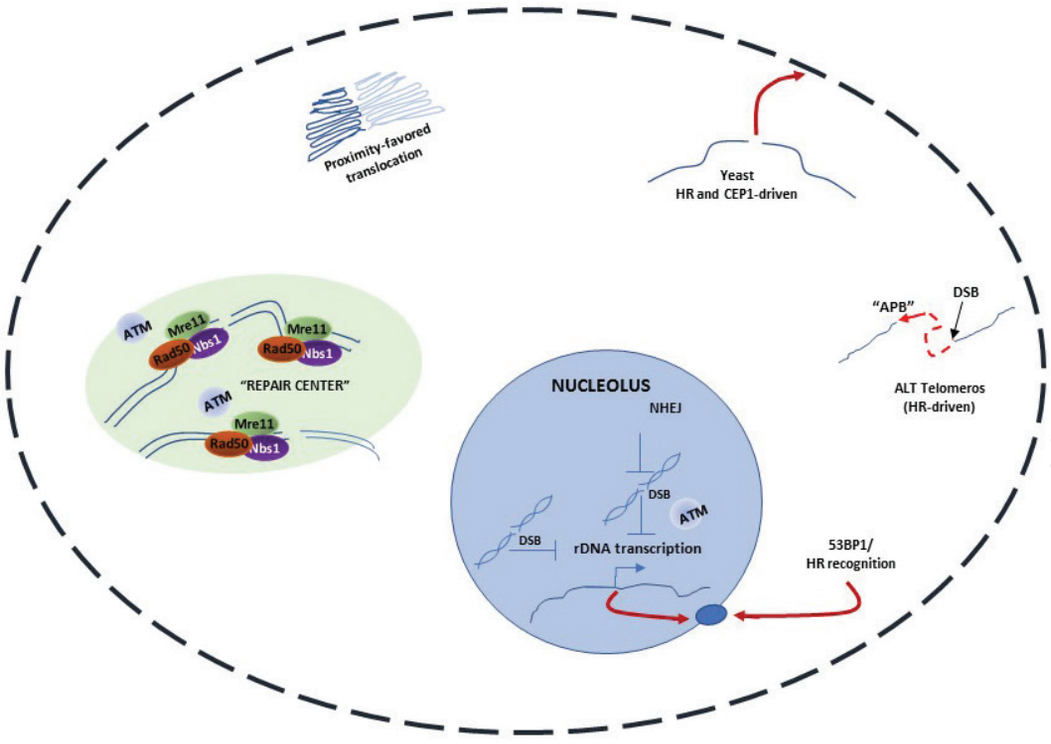

Figure 4. Chromatin remodeling during DSB response. As observed at the top, DSBs induce chromatin remodeling, as failure in DNA repair may result in translocation that occur in chromosomes in spatial proximity (TMPRSS/ETV1). Following to the right, unrepaired DSBs in yeast are recruited to SUMO mediated-nuclear periphery to be repaired. Homology repair on telomeres promotes their clustering in ALT-promyelocytic bodies (APBs). Next in same direction, unrepaired DSBs in rDNA genes in nucleoli silence transcription and this favors their relocalization to the nucleolar periphery. Often, multiple DSBs localize in repair centers and as well there are DSBs that remain in one position and depend on Ku80. Mainly where HR machinery is recruited.

It has recently been shown that INO80 also promotes chromatin movement due to DSB in telomeres, and this depends on actin polymerization [66]. It has been proposed that these movements contribute at least in part to homology searches during HR [67]. In the same line, a recent finding showed that MEC1-driven phosphorylation of the kinetochore component Cep1 induced by DSB caused centromere release from the spindle pole body explaining chromatin movement [68]. Further, it was observed that fixing telomeres to the nuclear periphery limits chromatin movement and that its physical rupture allows additional mobility. In this study, it was proposed that HR was defective and the mobility increase somehow facilitated activation of cell cycle checkpoints. Nonetheless, a wide body of evidence shows that DSBs are mobile in *S. cerevisiae* and that SUMOylation and DSB response leads to this movement, and it suggests that this movement has a positive effect on the ability to survive DNA ruptures. One of the first studies that contributed to these notions was obtained by using α-radiation to generate DSBs along a determined linear track of the nucleus [69], and the results indicated that DSBs were redistributed into clusters and that they were "repair centers" dependent on Mre11 observed in G1 phase (**Figure 4**) [70]. In the same manner, DSBs created by etoposide or gamma rays induced relocalization of damaged chromatin [71]. Consistently, repair center as observed in GFP-53BP1 foci that were induced by ionizing radiation were seen to be 1 and 2 mm apart gathered in large cluster very rapidly [72]. On the other hand, ATM loss reduced chromatin relocalization GFP-53BP1 foci when induced either by gamma rays or by charged nuclei [73] as well as with DSB generated by a nuclease [74]. In contrast, DSBs induced by UV or gamma rays were found to induce restricted mobility, however leading to a somewhat degree of chromatin decondensation [75].

In an early study, it was observed that chromatin constrain was dependent on Ku80, which suggested that NHEJ machinery rejoins fast the broken ends to limit chromatin mobility (**Figure 4**) [76].

Further, a report where transgenes were analyzed revealed long-distance movement that was dependent on MRE11 and was also associated with chromosome translocations [77]. *S. cerevisiae* generally prefers HR over NHEJ, the pathway with less error when repairing DSB since it uses a template to resolve the break. In contrast mammalian cell uses NHEJ over HR, being the most error-prone mechanism because it relies on the direct joining of broken ends [78].

DSB movement has been observed at unprotected and damaged telomeres. These DSBs are protected as they are part of the shelterin complex, thus impeding access to the DSB machinery [79]. The shelterin role has been revealed by showing that its depletion causes DSB response activation, and then telomeres are joined by NHEJ, thus inducing telomere fusions [80]. Further, 53BP1 loss reduced telomere end mobility and promoted almost complete absence of telomeric fusions [81]. Consistently with the previous data, it has been shown that this mobility is dependent on the LINC complex, which is known by connecting dynamic microtubules to the interior of the nucleus [82]. ALT (**a**lternative **l**engthening of **t**elomeres) cells employ a homology-driven mechanism to promote lengthening of telomeres [83]. When DSBs are induced in telomeres in ALT cells [84], these DSBs promoted mobility of telomere ends into clusters referred as ALT-associated PML bodies (APBs) (**Figure 4**) [85]. These movements were dependent at least partly on the HR machinery (e.g. RAD51) and also on protein involved in meiotic interhomolog recombination. Consistently, ALT telomere replication stress due to SMARCAL1 deficiency resulted in Rad51 telomere-telomere clustering and a significant

telomere enlargement [86]. These findings highlight the first example of HR-mediated DSB mobility in mammalian cells and reveal that this dynamic chromatin mobility contributes to genomic stability as well as cellular immortality through telomere maintenance.

5.2. Localized movement

3C methodologies have facilitated the chromosome contacts that occur within and between chromosomes. In *S. cerevisiae*, 3C studies indicate that DSBs reduce the general frequency of local (<100 kb) interactions [87]. This reduction seems to be related to the HR-dependent DSB mobility to the nuclear periphery (**Figure 4**), as in G1-arrested cells where HR is not active. This data led to the proposal that damaged DNA is taken from the local chromatin environment to facilitate accurate DSB repair. Thus, data obtained in mouse B cells is consistent with this. Arresting cells in G1 phase to remove HR-driven repair mechanisms, DSBs within a given chromosome most frequently promoted translocation with genomic loci present in cis to these DSBs [60]. In the same line of thought, in prostate cancer cells when TMPRSS2 gene expression is stimulated by dihydrotestosterone in a TOP2-dependent manner, TOP2 catalyzed DSBs that release torsional stress which in turn inhibits transcription [88]. These ruptures have been identified, mapped, and found to be present in TMPRSS2 clinical fusions with ETS transcription factors (**Figure 4**) (e.g. ERG) [89]. Regarding nuclear organization, both TMPRSS2 and ETS transcription factor loci are often associated within the nuclear extent [90]. Therefore, chromosomal proximity can explain some translocations that are typical of genomic instability related to cancer. The relationship between transcription and DNA repair is known to modulate local chromatin structure.

6. Effect of DSB response on transcription

Many studies have described posttranslational modifications in histones that can regulate the transcription process near a DSB as part of DDR. Among them, ubiquitylation and SUMOylation modifications have been shown to silence transcription in the vicinity of DSB regions, thus allowing an efficient repair process and preventing RNA polymerase from producing aberrant transcripts. This phenomenon has been characterized in cells whose DSBs have been produced by either exogenous agents or as a part of a programed cell mechanism, like meiosis.

6.1. DSBs, transcription, and ubiquitination in somatic cells

Kruhlak et al. [91] showed for the first time a correlation between DSBs and transcription in somatic mammalian cells. In this study, they observed a decrease in transcription in nucleoli (RNA pol I) after irradiation in an ATM, Nbs1-, and DMC1-dependent manner, and consequently a prolonged and deficient repair. Later, using a reporter system that allows in single cells the visualization of repair factors recruitment, as well as local transcription, an ATM-dependent transcriptional silencing program in cis to DSBs was described. In this study, ATM prevents chromatin decondensation, thus affecting RNA polymerase II elongation at regions distal to DSBs. It was also observed that silencing, at least partially depends on RNF8 and RNF168 (E3 ubiquitin ligases), while its reversal relies on the uH2A USP16 (deubiquitylating enzyme) [92]. This study suggested that H2A ubiquitylation on areas near DSBs is important for efficient recruitment of

repair factors. In contrast, deficiency of E3 ligases like RNF8 or RNF168 does not deeply impact in silencing in the context of DSB, suggesting that even though these specific ubiquitylation modifications contribute to DSB silencing, other ATM-dependent events surely cooperate in suppressing transcription [22]. TDP2 is a phosphodiesterase needed for the accurate repair of DSB caused by topoisomerase II (TOP2) abortive activity [93]. TOP2 removes hurdles on the way for efficient transcription and replication such as torsional stress from DNA, by generating intermediate cleavages and binding to the DSB 5′ terminus [94]. Normally, the cleavage and rejoining of DNA strand are transitory processes; however, this may be halted by DNA or RNA polymerases that could convert complexes into abortive DSBs which could activate the DNA repair response [95]. As shown, TDP2 ensures gene transcription from endogenous abortive TOP2 activity. Further, TDP2 has one ubiquitin-associated (UBA) domain, which is able to bind several forms of ubiquitin, thus providing potential multiple biological functions of TDP2 [96].

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