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Chromatin remodeling and spatial concerns in DNA double-strand break repair

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Abstract

The substrate for the repair of DNA damage in living cells is not DNA but chromatin. Chromatin bears a range of modifications, which in turn bind ligands that compact or open chromatin structure, and determine its spatial organization within the nucleus. In some cases, RNA in the form of RNA:DNA hybrids or R-loops modulates DNA accessibility. Each of these parameters can favor particular pathways of repair. Chromatin or nucleosome remodelers are key regulators of chromatin structure, and a number of remodeling complexes are implicated in DNA repair. We cover novel insights into the impact of chromatin structure, nuclear organization, R-loop formation, nuclear actin, and nucleosome remodelers in DNA double-strand break repair, focusing on factors that alter repair functional upon ablation.

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DNA repair in heterochromatin vs euchromatin

Both the positioning of DNA damage within the nucleus and the character of its flanking chromatin can influence the choice and efficacy of DNA repair mechanisms, particularly in higher eukaryotes (reviewed in Refs. [1,2]). Although an early study showed that, in flies, double-strand breaks (DSBs) could be repaired by either nonhomologous end joining (NHEJ) or homologous recombination (HR) with similar kinetics in both euchromatin and heterochromatin [3], it is now clear that repair mechanism choice between HR and NHEJ is not random. Pathway choice is biased first and foremost by phases of the cell cycle [4], something first shown in budding yeast: NHEJ dominates DSB repair in G1 phase, while HR, which requires S-phase-specific CDK1-dependent end resection, is favored in S and G2 phases [5]. Similar observations were made in vertebrates (*e.g.* Refs. [6,7]). Going beyond cell-cycle, recent research suggests that chromatin marks in mammals, and in particular those found in lamin-associated domains (LADs), which are transcriptionally repressed and enriched for H3K9me2/3, preferentially use error-prone mechanisms such as microhomology-mediated end-joining (MMEJ), rather than NHEJ [8]. This bias correlates strongly with local histone marks and their dynamics [8,9]: genomic domains bearing active marks (H3K4me2) were more often repaired by NHEJ, while repressive marks (H3K9me2/3) favored MMEJ. Unfortunately, HR was not monitored in this genome-wide study, yet earlier work had clearly implicated active chromatin marks in the choice of HR as a repair pathway [10]. Finally, it was shown that the loss of the polycomb mark, H3K27me3, reverted the MMEJ-NHEJ balance back toward NHEJ [8]. Altogether these results argue for a role for chromatin context in repair pathway choice.

Spatial positioning of damage within the nucleus may also influence outcome of repair. It was first shown in yeast that DSBs lacking an intact template for repair by recombination [11–13], behave like uncapped yeast telomeres [14,15], and shift to the nuclear periphery or to nuclear pores. The processing that occurs there eventually promotes alternative repair pathways, such as break-induced replication (BIR), which leads to a loss of distal chromosomal information, or imprecise end-joining through MMEJ, such as that measured in LADs [8,16,17].

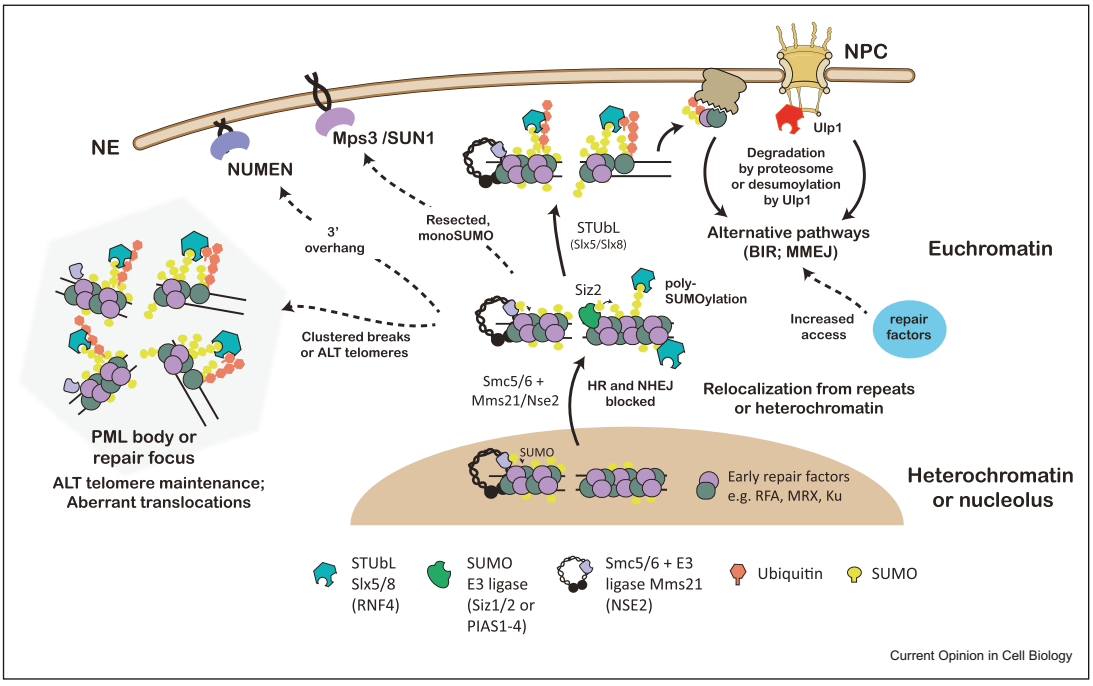
In mammals and flies, the relocation of DSBs was also observed, particularly those found in the repetitive heterochromatin of pericentric domains. By tracking irradiation-induced damage in *Drosophila*, DSBs were seen to shift away from centromeric satellite-enriched heterochromatin (or chromocenters) to the nuclear periphery, presumably as a prerequisite for repair by a mechanism that reduces chromosomal translocations [18,19]. In mouse cells, cell-cycle restriction was superimposed on this behavior: in G1 phase, DSBs in pericentric heterochromatin were repaired by NHEJ

and did not shift position, whereas in S or G2 phases they did shift to the edge of their respective repetitive domains, enabling repair by HR [4]. In human cells, however, breaks that occur in the SatIII centromeric repeat did not shift position and centromeric heterochromatin was surprisingly permissive to HR (to be discussed below) [20].

In yeast, the relocation of DSB away from repetitive rDNA required the Smc5/Smc6 complex and SUMOylation by its associated SUMO ligase, Mms21 [21]. Similar dependencies were observed for the relocation of breaks in unique sequence in both budding and fission yeast [11,12,16,22]. Finally, similar requirements were also found in *Drosophila* [18,19] and in mammalian cells as well, for the relocation of DSBs in the rDNA away from the nucleolus [23,24]. Importantly, the movement and repair of rDNA breaks required the action of the p97/Cdc48 ATPase, which recognizes ubiquitinated and sumoylated substrates to catalyze either protein unfolding or factor release [25]. One target of the p97 ATPase in yeast is a phosphorylated and sumoylated form of CLIP-cohibin, a specialized complex containing homodimers of Lrs4 and Csm1, which interact with components of the inner nuclear membrane to tether the rDNA at the nuclear envelope [26]. This suggests that release from tethering might actually increase repair.

Taken together, the available data support the following scenario (for reviews see Refs. [27,28]; Figure 1): first, sumoylation inhibits HR, permitting and perhaps promoting DSB movement away from zone of repetitive DNA. Thereafter, SUMO-dependent ubiquitination and/or proteasomal degradation triggers the release of factors that block the DNA end from appropriate processing. Since both degradation and desumoylation by Ulp1 are favored by nuclear pore complex proximity, this relocation will in turn enable either ectopic recombination, BIR, or if an appropriate template is found, HR, which will likely require release from the nuclear rim [25,26]. The question remains whether this pathway is functional for normal DSB repair or serves as a last-ditch effort to overcome persistent breaks or the dangers of broken replication forks, which were the experimental conditions used in most of these studies.

Figure 1



Simplified scheme of spatial relocation events at DNA breaks. When DNA DSBs occur in rDNA or repetitive DNA, they are not processed for HR immediately, but shift away in a manner dependent on sumoylation and ubiquitination. In yeast, these either shift to the peripheral SUN domain complex or to nuclear pores (thanks to mono- and poly-sumoylation, respectively). Relocation also requires INO80C in budding yeast [40–42]. Proteins blocking HR are either degraded or removed and processing occurs to enable recombination-mediated repair. In mammals there are two further options: DSBs with 3' overhangs can shift to NUMEN and be cleaved to favor NHEJ. Alternatively, ends cluster in repair foci or in PML bodies. This is particularly relevant for short telomeres in telomerase deficient cells. Ends are then repaired by recombination mechanisms leading in some cases to translocations. Details are in the text. Figure is modified from Stutz and Gasser [28]. Factor names are given for budding yeast, and in capitals are human homologs. NE: nuclear envelope; NPC: nuclear pore complex.

A new twist to the question of spatially localized repair arose recently with the discovery of NUMEN, an enzyme that generates short 5' overhangs through its endonuclease and 3' to 5' exonuclease activities, and which is an integral component of the inner nuclear membrane [29]. This enzyme is found in vertebrates at the nuclear periphery between pores. It cleaves overhangs to eliminate the 3′ overhang that is needed for HR, and thereby favours end-joining by NHEJ [29]. This provides a new rationale for the relocation of difficult to repair DSBs to the nuclear periphery, and may explain dichotomies between positioning damage at the nuclear envelope as opposed to nuclear pores [30].

The impact of damage clustering

When DSBs cluster near pores, which are enriched for both SUMO proteases and proteasomes [31], they juxtapose breaks from multiple chromosomes. This is reminiscent of the clustering of short telomeres in human ALT (alternative lengthening of telomeres) cancer cells, which assemble in PML bodies for repair [32]. This inter-chromosomal contact also occurs for centromeric repeats in mouse, which form chromocenters even in the absence of damage. The impact of such chromatin clustering on repair has been addressed in two recent papers. The first examined damage in the DAPI-bright chromocenters of mouse: they were found to block the recruitment of RAD51 and other HR repair factors to induced DNA DSBs, apparently due to the dense structure of the repetitive DNA [4,20]. Indeed, the barrier to resection and RAD51 binding can be generated artificially by tethering repeats together [20]. Based on this mouse data, it was proposed that extrusion of breaks from heterochromatic repeats was necessary to prevent translocations between chromosomes, as these would be favored if DSB repair occurred within a chromocenter. Intriguingly, DSBs found in the human pericentromeric repeat SatIII, do not shift out of the zone of repetitive DNA and can bind RAD51 and be efficiently repaired by HR within the heterochromatic domain (i.e. as defined by H3K9me2/3 modification) [20]. The fact that human centromere-proximal heterochromatin does not block access to the HR machinery as it does in mice, argues that the barrier is not simply a function of HP1 density – in fact, HP1 dimerization helps promote the resection of DSBs in repetitive domains in both mouse and human cells [33]. Rather, the authors suggest that another, unknown feature of mouse chromocenters blocks access to DSB processing and the HR machinery. Importantly, disruption of the barrier created by mouse centromeric heterochromatin, or artificial generation of centromere clustering in human cells, led to a 5- to 10-fold increase in RAD51-dependent chromosomal translocations during DSB repair [20].

In further support of the risks that might arise from DSB clustering, it was recently shown that the coalescence of breaks into foci promotes aberrant chromosomal translocations, rather than accurate repair by HR or other error-free recombination pathways [34]. As part of a multiscale reorganization of chromatin, Hi-C analysis was used to show that DSBs cluster independently of chromosomal linearity [34]. Clustering was sensitive to inhibitors of the actin filament chaperone, Arp2/Arp3, and potentially required the formation of short and transient nuclear actin filaments in the nucleus [35,36]. But rather than promoting or enabling repair by HR, this nuclear actin-dependent clustering led to aberrant NHEJ-mediated end-joining events, and higher translocation frequencies. Far from being beneficial, actin-mediated break clustering appears to be pathological, a fact that is particularly interesting given that the shift of DSBs out of the mouse chromocenters, which counteracts translocations, did not require nuclear actin polymerization nor Arp2/Arp3 [20]. Excess nuclear G-actin is also shown to be toxic in budding yeast after exposure to the base-oxidizing agent Zeocin (Hurst et al., personal communication; [37]).

The importance of chromatin compartments in regulating repair was further demonstrated in an impressive study that combined Hi-C, 4-C, and extensive genetic analysis to identify a new A-type compartment which is formed in response to DNA damage [38]. This so-called D-compartment largely contains open chromatin, and it showed enhanced intra-TAD contacts upon damage induction, while contact with undamaged neighboring TADs was decreased. D-compartment formation was lost upon ATM kinase inhibition, but was enhanced by loss of DNA-PK, presumably because the HR pathway of repair was favored. NHEJ, on the other hand, increased when D-compartment clustering was reduced. These clusters are thought to be mediated by polymer–polymer interactions, and not the ever-popular liquid–liquid phase separation, and only rarely involved DSBs in heterochromatin (4 of 22 heterochromatic DSBs). Interestingly, the D-compartment also appeared to recruit a subset of genes that are induced by the DNA damage response, possibly through R-loop enrichment (see below). Importantly, and consistent with the findings in Zagelbaum et al. [34], illegitimate DSB rejoining events were enhanced by clustering of DNA damage in the D-compartment [38]. In conclusion, it is amply demonstrated that the clustering of DSBs is largely detrimental to genomic integrity.

Diverse roles attributed to nuclear actin in DSB and chromatin movement

In vertebrates and flies, several papers have argued that nuclear actin filaments play a role in DSB movement. This contrasts with yeast, where the increased chromatin expansion and mobility occurs at DSBs in response to the DNA damage checkpoint and does not require nuclear actin filament formation [39]. The

break-induced increase in chromatin mobility in yeast correlated with a drop in nucleosome density, both flanking the break and genome-wide, which is driven by a limited ubiquitin-medicated degradation of histones [40,41]. This transient drop in nucleosomal density also facilitated the ectopic homology search that is necessary for repair by recombination [42], either through BIR or the usual bidirectional strand invasion that occurs at resected breaks, and was independent of actin polymerization. In contrast to the situation in yeast, higher eukaryotic WASP and Arp2/Arp3 moonlight in the nucleus, and may actually nucleate short, transient actin filaments at sites of damage. These filaments were proposed either to provide "tracks" for the movement of breaks to the nuclear periphery [35] or to drive formation of damaged DNA clusters [36]. Given the conclusion of Zagelbaum et al. [34], however, one must question whether nuclear actin filament formation is a physiological repair pathway or a pathological event that leads to chromosomal translocations.

WASP, which serves both as an actin chaperone and cofactor for the Arp2/Arp3 nucleation of actin filaments, has also been implicated in the replication protein A complex (RPA) stabilization on ssDNA during replication stress in the nucleus of vertebrate cells [43,44]. These studies highlight the roles of actin filament formation at sites of replication fork stress and fork reversal. The appearance of RPA foci and RPA-bound ssDNA at replication forks stalled on HU is dependent on WASP, ARP2/3, DIAPH1, and N-WASP. Loss of these actin chaperones attenuates the checkpoint response, although resection proceeds normally in their absence. Nieminuszczy et al. [43,44] show that HU-arrested replication forks are degraded in the absence of actin nucleating factors. Another study on the same topic argued that nuclear actin polymerization at stalled forks served to prevent the binding of PrimPol, an error-prone polymerase that can initiate DNA synthesis on ssDNA by both priming and elongating, thereby thwarting the more robust events of fork reversal and restart [45]. This intriguing role for short transient actin filaments in the nucleus suggests that rather than forming a "track for movement" transient nuclear actin network buffer accessibility. This is reminiscent of the recruitment of H3K9 methylation activity at reversed forks, which also prevented alternative processing and enabled fork restart [46].

An alternative or parallel hypothesis for the role of nuclear actin, is that it regulates actin-dependent chromatin remodelers, such as BRG1/BAF, (*Sc* Snf2/Swi2) or INO80 (reviewed in Ref. [47]; see Table 1). The neglected role of nuclear actin as an integral component and key regulator of nucleosome remodelers provides alternative interpretations for the impact of genetic manipulations that lead ultimately to altered G-:F-actin ratios, driving a nuclear accumulation of globular actin, and indirectly influencing repair [48]. This is particularly relevant in yeast where the WASP homolog, Las17, does not enter the nucleus nor nucleate nuclear actin filaments, but instead appears to influence actin-dependent chromatin remodeler function indirectly by increasing nuclear actin concentration (Hurst et al., personal communication; [37]).

Chromatin remodeling and the interplay between transcription, R-loops, and DNA DSB repair

The recognition, signaling, and repair of DNA DSBs require access to the repair substrate and, in some cases,

Table 1

Chromatin remodelers, grouped by homology, involved in the repair (NHEJ or HR) or signaling (DDR) of DNA DSBs. Complexes containing actin and actin-related proteins (ARPs) are indicated with an asterisk.

ATPase (yeast)	Complex(es)	ATPase (human)	Complex(es)	Repair activities
Isw1	ISW1A, ISW1B	SMARCA5/SNF2H	ACF, CHRAC and RSF	DDR, HR, NHEJ, repair in heterochromatin
Isw2	ISW2			HR
Snf2/Swi2	SWI/SNF*	SMARCA2/BRM	SWI/SNF* complexes: BAF (contains ARID1A), PBAF, or ncBAF	DDR, HR, NHEJ, HR in heterochromatin
Sth1	RSC*	SMARCA4/BRG1	SWI/SNF* complexes: BAF (contains ARID1A), PBAF, or ncBAF	DDR, HR, NHEJ
Chd1		CHD1		HR
		CHD2		NHEJ
		CHD3	NuRD	NHEJ in heterochromatin
		CHD4	NuRD	DDR, HR
		CHD7		NHEJ
		ALC1/CHDL1		NHEJ
Ino80	INO80C*	INO80	INO80*	DDR, NHEJ, HR
Swr1	SWR1C*	SRCAP	SRCAP*	NHEJ (yeast)
		P400	TIP60*	HR, NHEJ
Fun30		SMARCAD1		HR
		HELLS/SMARCA6		HR in heterochromatin

quite extensive manipulation of DNA. It is not surprising, therefore, that many nucleosome remodelers have been shown to play a direct role at the site of DNA breaks to promote these changes (reviewed in Refs. [49–52]). Remodelers have been implicated in promoting both NHEJ and HR, as well as promoting DNA damage response (DDR) signaling (Table 1). An emerging theme in the response to DNA DSBs is the interplay between transcription and RNA:DNA hybrids, and in particular, those in which an RNA displaces one strand of its complementary DNA forming a so-called R-loop, in chromatin flanking the break in the repair process (for review, see Refs. [53,54]). Here, we focus on how chromatin remodeling complexes contribute to repairing DNA breaks near actively transcribed genes and their connection with R-loop formation.

In mammalian cells, DNA breaks near ongoing transcription trigger rapid ATM-dependent transcriptional silencing, carried out in part through polycomb-mediated H2A K119 ubiquitination [55]. These breaks are preferentially repaired by HR [10], and failure to repress transcription leads to increased genome instability [56]. Early work established that the PBAF chromatin remodeling complex is required for break-induced silencing to take place [57], but more recently, the other two closely related SWI/SNF complexes, BAF and ncBAF, have also been implicated [58]. Multiple factors have since been shown to be involved in this pathway, including the CHD4-containing NuRD remodeling and histone deacetylase complex ([59]; for review see Ref. [54]).

It has become apparent that R-loops are formed in the vicinity of DNA DSBs [60]. While R-loops can be detrimental to genome stability and cause DNA damage under some circumstances, evidence suggests they play a positive role in during the repair of DSBs [60]. R-loops can be formed through stalled or paused transcription, and consequently, transcription inhibition in the vicinity of a DNA break is a likely source of R-loop formation. RNA molecules produced elsewhere could also be used, provided there is sufficient homology. Moreover, there is new transcription at the break that contributes to DNA damage signaling [53], which, when not processed into shorter noncoding species, also provides a source of RNA for R-loop formation. When DNA is resected, RNA molecules with homology to the single-stranded DNA overhang will form RNA:DNA hybrids rather than R-loops.

Negative supercoiling promotes the generation of R-loops [61], and SWI/SNF remodeling can alter torsional characteristics of chromatin and generate non–B form DNA structures [62–64]. This raises the intriguing possibility that SWI/SNF activity at DSBs in actively transcribed genes promotes R-loop formation (in addition to promoting their resolution, as described below). While potentially a by-product of remodeler activity during DSB-induced transcriptional repression, it could also serve as a deliberate strategy to facilitate the repair process (Figure 2).

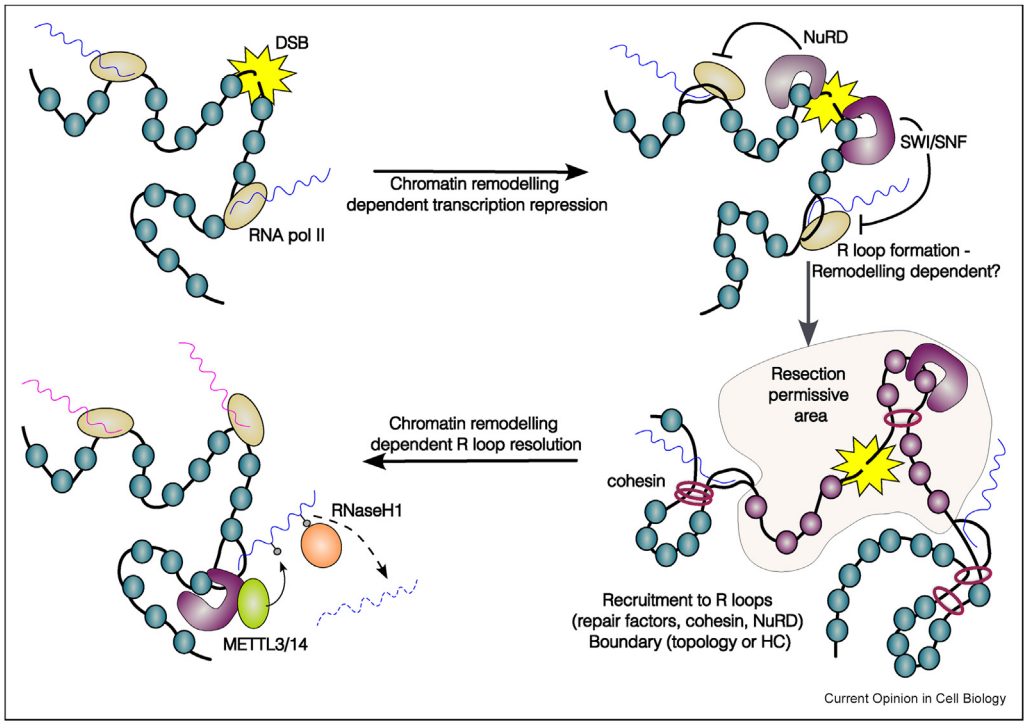
Once stabilized at DSBs, R-loops can act as a recruitment platform. Repair factors such as RPA, PARP1, BRCA1, and RAD52 can bind to R-loops, and evidence suggests their recruitment or retention at DNA DSBs is influenced by both transcription and RNA:DNA hybrids [60]. Consistent with their occurrence and role at DSBs in actively transcribed genes, R-loops influence pathway choice by favoring HR over NHEJ [65].

How does this work? There is evidence that R-loops can both impede long-range resection and promote extensive non-canonical resection [60]. Notably, a recent paper demonstrated that the NuRD complex is recruited to DNA breaks in an R-loop dependent manner and that NuRD-dependent deacetylation creates a heterochromatin barrier that prevents hyper-resection [66]. When R-loops have been mapped in relation to DSBs, they appear to be enriched in chromatin several kb from the break [65,66], consistent with the idea that they form a restrictive boundary (Figure 1). Whether and how these species differ from (or indeed, interact with) R-loops or RNA:DNA hybrids formed at DNA break ends is not yet clear (for review, see Ref. [60] and discussed below).

In addition to delineating a boundary between euchromatin and heterochromatin, R-loops could orchestrate three-dimensional chromatin organization around DNA DSBs by interacting with cohesin (Figure 2). Cohesin dynamics at DSBs influence the architecture of DNA damage foci formed intra-chromosomally (in *cis*) and regulate the spread of γH2AX [67,68]. Notably, the STAG1 and STAG2 subunits of cohesin can bind R-loops and promote cohesin loading at these sites [69]. R-loops also act as a barrier to cohesin translocation [70], which defines γH2AX spreading [68]. Consequently, by modulating cohesin dynamics, R-loops could regulate the topology and organization of DNA damage foci, thereby facilitating synapsis or repair, regulating resection, or influencing strand invasion.

At some point during the repair process, DSB proximal R-loops must be dissolved, and SWI/SNF has been implicated in this step [58,71] (Figure 2). The ARID1A subunit of the BAF SWI/SNF complex was shown to recruit METTL3 and METTL14 to R-loops at DNA DSBs to promote m6A modification of the RNA, one of several RNA modifications that have been implicated in DNA repair (for review, see Ref. [72]). In this study, m6A incorporation into RNA at DNA break-associated R-loops was shown to facilitate RNase H1 binding, thus promoting resolution [71]. However, a previous study found that m6A incorporation by METTL3 at DNA breaks led to increased RNA:DNA hybrid stability

Figure 2



Simplified scheme of events at DNA breaks in actively transcribed genes. Nearby transcription is repressed, and this is dependent on SWI/SNF and NuRD chromatin remodeling complexes. It is possible that their activity promotes R-loop formation distal to the DNA break (top right). R-loops recruit not only repair factors, but cohesin and additional NuRD, which could establish topological or heterochromatic boundaries, respectively (bottom right). Cohesin regulates the spread of γH2AX (purple nucleosomes) and the HDAC activity of NuRD restricts resection. Following repair, SWI/SNF helps resolve R-loops by recruiting METTL3/14, leading to m6A modification of the RNA (lollipops on RNA) and RNaseH1-mediated degradation (bottom left, see text for details).

through interaction with the m6A reader protein YTHDC1 [73]. It is possible that the timing or location of the modified RNA determines the impact on downstream binding factors. One attractive possibility is that DNA end-proximal RNA:DNA hybrids are responsible for signaling and HR factor recruitment, while R-loops produced more distally to the break function as boundary elements.

Since ARID1A is exclusive to BAF, the finding that ARID1A recruits METTL3 and METTL14 to R-loops suggests that perhaps the other two SWI/SNF complexes, PBAF and ncBAF, are not required for dissolution of R-loops at DSBs. In support of dedicated functions, Lans et al. found that the eviction of RNA pol II from chromatin flanking DSBs is dependent only on BAF and PBAF, whereas all three SWI/SNF complexes were required for maintaining transcriptional repression at later time points [58]. How these activities are coordinated with R-loop establishment and resolution remains to be determined.

The complexity of remodeling complexes in damage responses

As described above, SWI/SNF and NuRD complexes mediate repair, R-loop resolution, and transcriptional silencing at DSBs in actively transcribed chromatin. Yet these complexes have also been implicated in repairing DNA breaks in other contexts. SWI/SNF was first implicated in heterochromatic repair in budding yeast [74], but more recently, SWI/SNF and NuRD complexes were found to work with PHF6 to mediate repair in heterochromatin in mammalian cells as well [75]. Adding to the challenge is the fact that remodelers can contribute to more than one repair pathway. HR is preferentially used at actively transcribed genes where SWI/SNF is working (Figure 2). Notably, however, SWI/SNF complexes also promote NHEJ [49]. NHEJ activity at DSBs undergoing HR could be deleterious or even toxic, suggesting that pathway-specific functions of SWI/SNF are subject to regulation in a way that can be toggled on or off, allowing SWI/SNF to promote either HR or NHEJ depending on the specific context.

In addition to working at DSBs, these complexes regulate the expression of genes involved in DSB repair and signaling. For instance, SWI/SNF maintains the G2/M checkpoint in response to DNA damage through remodeling activity at p53-dependent promoters [76]. Likewise, BRD4, which works together with SWI/SNF at DNA breaks to promote HR [77], regulates transcription of genes involved in controlling R-loop dynamics and DNA damage responses [78]. Importantly, BRD4 also prevents R-loop formation through promoting RNA pol II elongation globally [79–81], and therefore BRD4 might function antagonistically with SWI/SNF at DNA breaks to modulate the timing or extent of R-loop formation. Distinguishing the contribution of gene expression regulation from activities that directly regulate DSB repair is an ongoing challenge.

Finally, the relationship between reorganization of chromatin immediately flanking a break and larger scale changes in nuclear organization and chromosome mobilization is starting to come to light. For example, as described above, DSBs are subject to clustering in an Arp2/3-dependent manner [34], and the broken chromosomes are organized into a new chromatin compartment, referred to as the D compartment [34,38]. This involves larger scale changes than those occurring in chromatin flanking breaks, but interestingly, is also regulated by R-loops and leads to upregulation of DNA damage-responsive genes [38], a subset of which are also dependent on SWI/SNF for upregulation [76], raising the possibility that the activities are coordinated or interdependent. These new insights deepen our understanding of cellular responses to DNA damage, but also underscore the complexity of the biological processes at play.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data were used for the research described in the article.

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