Trends in Genetics

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Feature Review

A Snapshot on the *Cis* Chromatin Response to DNA Double-Strand Breaks

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In eukaryotes, detection and repair of DNA double-strand breaks (DSBs) operate within chromatin, an incredibly complex structure that tightly packages and regulates DNA metabolism. Chromatin participates in the repair of these lesions at multiple steps, from detection to genomic sequence recovery and chromatin is itself extensively modified during the repair process. In recent years, new methodologies and dedicated techniques have expanded the experimental toolbox, opening up a new era granting the high-resolution analysis of chromatin modifications at annotated DSBs in a genome-wide manner. A complex picture is starting to emerge whereby chromatin is altered at various scales around DSBs, in a manner that relates to the repair pathway used, hence defining a 'repair histone code'. Here, we review the recent advances regarding our knowledge of the chromatin landscape induced in *cis* around DSBs, with an emphasis on histone post-translational modifications and histone variants.

Highlights

The development of specialized tools has recently provided unprecedented insights regarding the role of chromatin structure in the response to DNA double-strand breaks.

High-resolution studies revealed that chromatin is modified on several different scales following DSB induction, ranging from a few kilobases to several megabases.

Chromatin modifications at the megabase level may alter the physical properties of the chromatin fiber and regulate long-range chromosomal mobility upon damage.

The nature and extent of local chromatin modifications are different upon repair by NHEJ or HR.

Repair pathway-specific modifications have the potential to regulate the access of repair factors and the physical properties of the nucleosome, thereby participating in downstream repair events.

DSB Repair Occurs within Chromatin

DNA double-strand breaks (DSBs) constitute very dangerous lesions having the potential to produce harmful genetic alterations such as local DNA sequence modifications (insertions/deletions) or more substantial chromosomal rearrangements (e.g., translocations). To faithfully maintain genomic integrity, cells deploy a DNA damage response (DDR) comprising specialized machineries able to sense, signal, and repair DSBs by homologous recombination (HR) or non-homologous end joining (NHEJ) [1] (Box 1). DSBs can occur upon exposure to a variety of genotoxic agents (e.g., ionizing radiation, chemotherapeutic drug) and under physiological conditions including developmentally programmed DSBs in lymphocytes or during meiosis but also during ubiquitous processes such as replication and transcription. Indeed, DSBs arise more frequently than previously anticipated, and their induction rate is not homogeneous along the genome. For example, transcribed genes (reviewed in [2]) or chromatin loop anchors [3] appear to represent regions of increased DSB frequency, and technological improvements will most likely identify other classes of endogenous DSB-prone regions in the future. It is therefore not surprising for DSB repair anomalies to be responsible for many disorders (premature aging, neurodegenerative syndromes) as well as the onset and progression of cancer.

Like any other DNA-related process, DSB repair occurs within a precisely organized and dynamic chromatin environment (Box 2). Histone post-translational modifications or variants can alter nucleosome structure and therefore control many properties of the chromatin fiber (such as DNA accessibility, secondary structures, topological constraints, stiffness, and mobility) but can also directly act as recruitment platforms for the **chromatin reader** (see Glossary) proteins, emphasizing the pivotal function of chromatin during transcription, replication, and repair.

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Box 1. DSB Repair Pathways

The two main DSB repair mechanisms are non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ functions by direct ligation of the two broken ends, requiring no or minimal processing of the DNA ends. This pathway operates throughout the cell cycle, and key NHEJ factors include Ku, DNA-PKcs, XRCC4, XLF, PAXX, and DNA ligase IV (recently reviewed in [140]). During HR, the undamaged copy of the broken chromosome will be used to initiate DNA synthesis and restore the nucleotide sequence (reviewed in [141]). For this, 5′ to 3′ nucleolytic degradation of the broken end, called resection, will be initiated by the MRN (MRE11–RAD50–NBS1) complex and CtIP and is further extended by EXO1, DNA2, and accessory factors such as the helicase BLM. RPA will bind newly generated ssDNA to stabilize it. Subsequently, BRCA2 will stimulate the loading of the RAD51 recombinase to resected DNA, which results in a nucleofilament that will find the homologous sequence to initiate DNA synthesis. Under particular circumstances, such as HR deficiencies, alternative pathways such as single-strand annealing (SSA) or microhomology-mediated end joining (MMEJ) can be mobilized, at a cost for genome integrity since these pathways tend to be highly mutagenic [140]. The initiation and extent of resection are key aspects governing DSB repair pathway choice since it is a requirement for HR (and alternative mutagenic pathways) and will inhibit the execution of NHEJ. Key DSB repair factors such as 53BP1 and BRCA1 play very important roles in controlling resection.

Chromatin in DSB Repair: New Tools, New Questions, New Answers

During DSB repair, chromatin modifications have the potential to contribute to the accurate execution of repair pathways by regulating DNA end accessibility, synapsis, or single-strand DNA (ssDNA) generation and also to participate in DSB signaling or the control of DSB mobility within the nucleus. Yet, while the genome-wide sequencing revolution strongly helped to understand the role of chromatin in transcription [4], its detailed contribution to DSB repair has long been impeded by the inability to induce DSBs at defined positions across entire cell populations. This precluded the use of dedicated methods such as **ChIP-seq** to analyze DSB-induced chromatin changes at high resolution or to compare DSB repair events occurring in different chromatin contexts. The recent development of tools to induce DSBs at annotated positions (Box 3), combined with high-throughput sequencing approaches and/or super-resolution microscopy, revolutionized our capability to study the nature and function of chromatin at DSBs. These new methods could make it possible to tackle new questions; (i) to what extent is the chromatin response to DSB universal, that is, how does it change depending on the genomic position of the break and (ii) what is the nature of DSB-induced chromatin and how does this chromatin landscape contribute to repair reactions and to the maintenance of genomic integrity? While it is now clearly established that the position of the break, and its associated chromatin features, plays a critical role in its subsequent repair (reviewed in [2,5,6]), the precise chromatin landscape induced around DSBs has only recently started to be deciphered.

Box 2. Chromatin Is Organized at Many Different Levels

The primary level of chromatin organization consists of DNA winding around octamers of core histones (H2A, H2B, H3, and H4), forming arrays of nucleosomes that is usually conceptualized as 'beads on a string'. In living cells, this basic chromatin fiber further folds and adopts a range of configurations and compaction states at many different scales, providing the foundation for the 3D organization of chromosomes in the nuclear space. Modifications at the nucleosomal level include DNA methylation, histone post-translational modifications (such as phosphorylation, acetylation, methylation, and ubiquitination), or incorporation of histone variants [142,143]. Nucleosomes themselves are also very dynamic in their positioning, occupancy, and turnover. Furthermore, linker histones such as H1 can directly interact with the nucleosome and participate in the folding of nucleosome arrays. These chromatin marks do not occur randomly on the genome but follow a stereotyped pattern depending on the function of the underlying locus [4]. Indeed, specific chromatin features associate and characterize promoters, active gene bodies, regulatory elements (enhancers, domain boundaries), Polycomb-repressed domains, and heterochromatin (pericentromeres, subtelomeric regions, lamina-associated domains, or nucleolus-associated domains). Furthermore, recent data suggest that genome folding in 3D is governed by both the segregation of active or inactive chromatin into compartmental domains as well as the formation of long-range chromatin loops by CTCF and cohesin, leading to the formation of topologically associating domains [144].

Glossary

AsiSI-induced DSB: DSBs induced using the restriction enzyme AsiSI, the key feature in the DIvA system (see Box 3).
ChIP-seq: chromatin immunoprecipitation followed by next-generation sequencing, a method to assess the localization of a protein, or its post-translationally modified form, over the entire genome.
Chromatin reader: a protein with the ability to recognize specific modification on a nucleosome, most often a post-translational modification state, usually using a dedicated protein domain. Specific reader domains include, for example, Bromo-, PHD-, SANT, Chromo-, or BRCT domains.
Chromatin remodelers: enzymes, usually part of large protein complexes, using the energy of ATP hydrolysis to alter the structure of nucleosomes or nucleosomal arrays. Remodelers can evict or slide entire octamers or promote the incorporation or eviction of histones from the nucleosome.
DIvA system: DSB induced via AsiSI, a system for controlled induction of about 100 DSBs in the human genome based on the controlled nuclear translocation of the restriction enzyme AsiSI that will subsequently cleave endogenous recognition sites in the genome (GCGATCGC). DSBs induced using AsiSI are restricted to the non-CpG-methylated fraction of the genome (the enzyme is inhibited by CpG methylation), so no cuts are induced in heterochromatin.
Histone variants: non-canonical variant of core histones, usually encoded by different genes and incorporated by specific complexes. Prominent histone variants include H2AZ or H2AX (for H2A) and H3.3 or CENP-A for H3.
I-SceI break: a DSB induced using the homing endonuclease I-SceI. Its large recognition site (18 bp) is usually not found in the host genome and needs to be introduced by a transgenic method, allowing for precise control of the position of the DSB.
Phase separation: a physicochemical process resulting in intracellular compartmentalization by

Box 3. Pros and Cons of Prevalent DSB Induction Methods

Common DSB induction methods include ionizing radiations (γ- or X-rays), crosslinking agents (mitomycin C, cisplatin), radiomimetic compounds (bleomycin, neocarzinostatin), topoisomerase inhibitors (camptothecin, etoposide), replication inhibitors (hydroxyurea, aphidicolin), chemicals (such as hydrogen peroxide), and localized laser micro-irradiation (damaging only a stripe in the nucleus). Since no new DSBs should occur after treatment, repair events are synchronous and can be kinetically resolved. Yet, while very potent and in many cases clinically relevant, these methods suffer drawbacks, limiting their use in high-resolution chromatin studies. First, most of these methods induce DSB randomly throughout the genome, with the exception of topoisomerase poisons and replicative stress inducers, which produce DSB within 'hot spots' (i.e., respectively at sites of topoisomerase binding, or at sites where replication forks collapse). However even in the latter cases, where the positions of the DSBs are known, it is still unclear how often breakage occurs across the cell population, which will effectively dilute any high-resolution signal obtained at the population level. Furthermore, most methods induce other types of damage (oxidized bases, abasic sites, ssDNA breaks) or may only effectively trigger DSBs in some cells in the population (e.g., in S phase). A variety of sequence-specific DSBs induction systems have been developed over the years. This includes the HO endonuclease in yeast, TALE nucleases, Zinc Finger nucleases (e.g., FokI), CRISPR/Cas9, homing endonucleases (I-SceI or I-PpoI), and restriction enzymes such as AsiSI (reviewed in [145]). These tools allow induction of DSBs at known positions on the genome, and importantly in a homogenous manner in the cell population, making them amenable for high-resolution studies such as ChIP-seq. Furthermore, some systems (including AsiSI, I-PpoI, or CRISPR/Cas9) allow one to induce multiple DSBs scattered across the genome, allowing the comparison of repair at various endogenous loci, while HO (unless used at its original Mat locus) or I-SceI require the enzyme recognition site to be artificially introduced in the genome. By design, the main disadvantage of these methods stems from the ability of nuclease to recut properly repaired sites, impairing efficient kinetic deconvolution of repair reactions, in sharp contrast with localized laser micro-irradiations, which can resolve events occurring a few seconds apart. Instead, the cell population represents a mixture of events ranging from early cleavage to late repair, which may impede the efficient detection of transient events. Lastly, it is important to stress that these annotated DSBs induction systems may not all be equivalent. For example, AsiSI is unable to create DSBs in heterochromatin (being inhibited by DNA methylation) and Cas9-induced breaks display an inherent propensity to be repaired by mutagenic pathways [146].

the formation of membrane-less compartments (or condensates). Liquid–liquid phase separation, or liquid demixing, leads to the formation of phase-separated liquid droplets with a denser liquid phase (with similarities to droplets of oil in water). Phase separation events can be seeded by multivalent interactions between DNA, RNA, or proteins. This process has been involved in the formation of heterochromatin, the nucleolus, or ionizing radiation-induced foci.

SAGA complex: Spt-Ada-Gcn5 acetyltransferase complex, a multiprotein chromatin-modifying complex with both histone acetyl transferase (HAT) and deubiquitinase (DUB) activities.

Repair Histone Code: Different Scale, Different Functions

Elegant studies, following the recruitment kinetics for many repair factors upon DSB induction at localized micro-irradiation (e.g., [7,8]) have started to accurately establish the spatio-temporal organization of the DDR. In addition, the use of phospho-activatable green fluorescent protein (GFP)-tagged core histone identified that chromatin is rapidly relaxed following laser micro-irradiation in a manner that depends on poly(ADP-ribose) polymerase 1 (PARP1) and chromatin remodelers (e.g., [9–12]), followed by a recompaction phase, necessary for full DDR activation [12] (Figure 1, Key Figure). Altogether, this revealed that chromatin structure is altered in an ordered manner following DSB (reviewed in [13]). Yet, the exact molecular nature of in *cis* chromatin modifications that accounts for this DSB-induced chromatin behavior visualized by microscopy is unknown. Since chromatin is organized at many different scales, it is important to precisely understand how DSBs can reshape this structure at each level, from the nucleosomes adjacent to the broken sites up to entire chromosomal domains, and this starts with the molecular characterization of the chromatin landscape induced at DSB. To this aim, a systematic and high-resolution ChIP-seq mapping of histone modifications induced at multiple DSBs was performed in human cells [14]. This study took advantage from the **DIvA system** (Box 3), which allows for the controlled induction of about 100 DSBs, in both transcriptionally active and inactive regions (with exception to heterochromatin), within a short time window of 4 h in cultured U2OS cells. This revealed a multiscale and complex pattern of induced histone modifications around DSBs, with few large-scale (megabase-wide) chromatin changes, as well as local (<10 kb) alterations [14] (Figure 1). In addition, mapping of repair proteins, such as those involved in HR or NHEJ, allowed assignment of a specific chromatin landscape to a particular repair pathway. These local alterations of the chromatin landscape are both involved in recruiting repair proteins (such as pro- or anti-resection factors) and in modulating the nucleosome stability onto DNA, thereby regulating DSB processing. Below, we provide a recent update on the nature and (potential) function of the chromatin-induced DSB landscape,

Key Figure

Chromatin during DSB Repair.

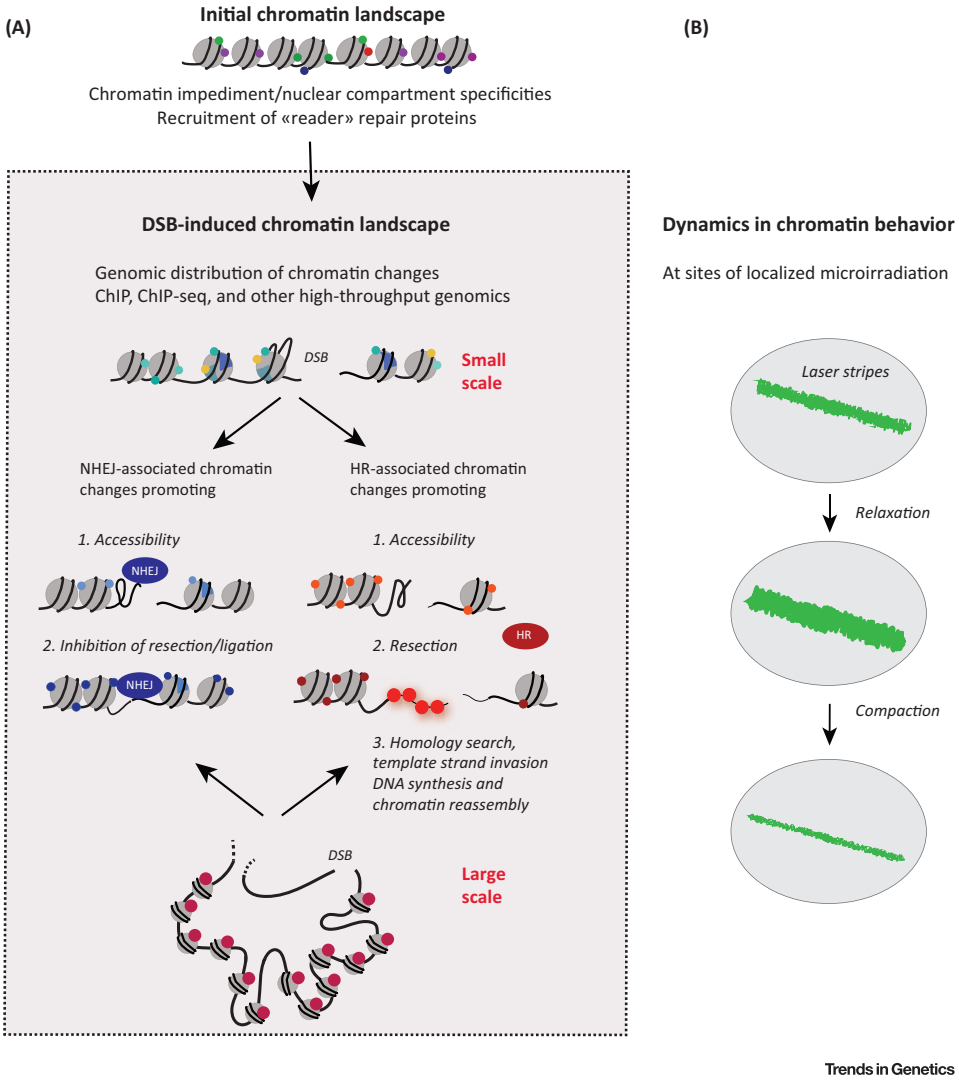

Figure 1. Upon double-strand break (DSB) detection, both the initial chromatin context and the induced chromatin landscape play a central role in the repair process. How DSB repair varies across the genome, to deal with DSBs induced in different chromatin contexts (owing to specific recruitment of reader proteins, due to specific chromatin impediments, or as a consequence of the particular physical position of the break in the nucleus) has been reviewed in the main text. Herein, we rather focus on the chromatin changes induced by DSBs (A, central gray box), with a strong emphasis on the spatial distribution of histone modifications around DSBs and their potential function during HR and NHEJ. However, since ChIP and ChIP-seq (allowing for a good characterization of the spatial distribution) only provide very low temporal resolution, it is unclear how these chromatin changes relate to the dynamic chromatin behavior observed by microscopy at sites of localized micro-irradiation (B; also reviewed in main text). Describing the DNA damage response and chromatin changes at a high spatio-temporal resolution remains one of the greatest challenge for future research in the field (see Outstanding Questions). HR, homologous recombination; NHEJ, non-homologous end joining.

with a focus on histone post-translational modifications occurring during either HR or NHEJ and thereby defining the 'repair pathway histone code' (Figure 2).

Large-Scale Modifications, Regulating the Mobility of the Fiber within the Nucleus?

The first histone modification shown to be specifically induced in *cis* to DSB was the phosphorylation of the H2AX **histone variant** on serine 139 also known as γH2AX [15]. This modification, mainly catalyzed by kinases such as ataxia telangiectasia mutated (ATM) or ataxia telangiectasia and Rad3-related (ATR), displays the striking behavior to spread over incredibly large domains since it can cover up to 2 Mb of DSB-surrounding chromatin in mammals [16–18]. Interestingly, γH2AX signaling may not only rely on modification of pre-existing H2AX but also on *de novo* deposition of this variant at damaged sites [19]. Remarkably, accumulation of ubiquitin conjugates on chromatin after damage [20] follows a near identical pattern to γH2AX [14]. In addition, this study also found a reduction in linker histone H1 on large chromosomal domains (several megabases) upon DSB induction [14], in agreement with previous findings [9,21,22]. This large-scale H1 eviction may depend on H1 degradation [21], or H1 post-translational modifications (such as acetylation [23] or ubiquitination [24], although controversial [25]) that may loosen its interaction with the nucleosomal fiber. Yet, while such extensive and seemingly disproportionate propagation around DSB triggers the formation of cytologically visible foci within the nuclei upon damage, the function of γH2AX, and other associated megabase-scaled modifications, is still unclear. Indeed, H2AX-deleted mice are viable, although sterile, immune deficient, and cancer prone

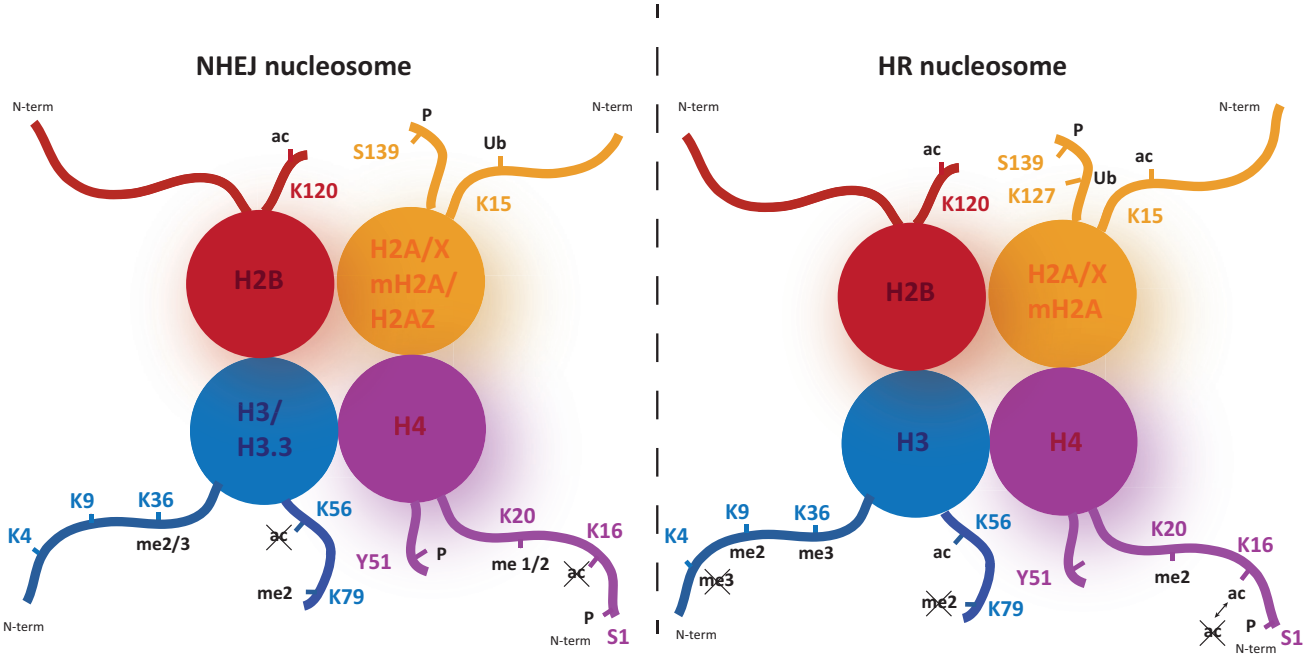

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Figure 2. A DSB Repair Histone Code. Recent studies revealed that nucleosomes exhibit a specific composition and post-translational signature during repair by non-homologous end joining (NHEJ; left) and homologous recombination (HR; right) repair. Yet, whether, how, and when these histone modifications, individually or collectively, are actually required for repair still remains to be determined. The cross on top of a histone modification indicates the disappearance of this particular modification upon damage. N-term, N terminus; Ac, acetyl-; P, phospho-; Me, methyl-; Ub, ubiquitin.

[26], indicating that H2AX phosphorylation upon damage is not absolutely required for DSB repair but may play a more subtle role. A tempting hypothesis is that chromatin modifications on such a large scale contribute to the motion of the damaged chromatin fiber in the nucleus, ensuring DSB mobilization, clustering, and/or homology search, thereby fine-tuning repair events (reviewed in [27]) (Figure 3). In support of this hypothesis, in yeast, DSB end mobility depends on the DDR kinase Mec1 and on the presence of its target phosphorylation sites on

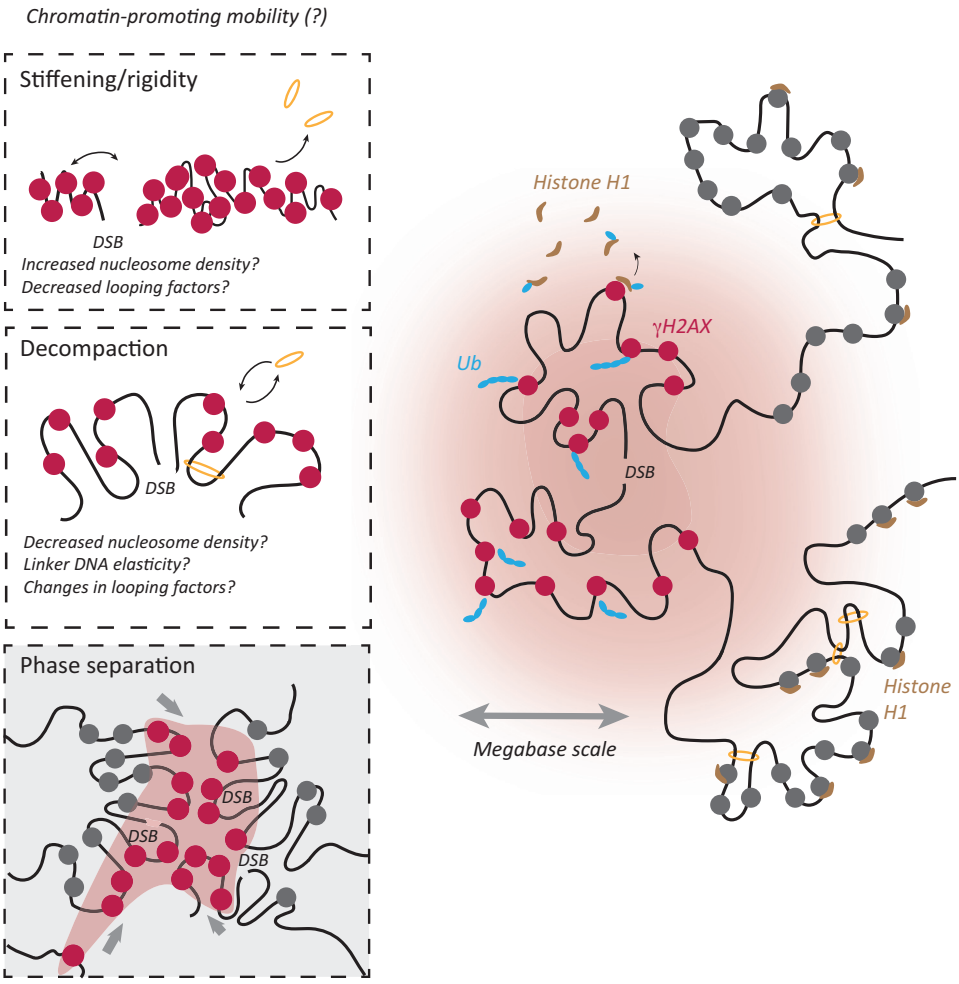

Figure 3. A Function for Large-Scale Chromatin Modifications in DSB Mobility? Few large-scale, megabase-sized chromatin modifications occur in *cis* to double-strand beaks (DSBs), including the phosphorylation of the H2AX histone variant (γH2AX), ubiquitin (Ub) chain accumulation, and linker histone H1 removal. While their exact function still needs to be deciphered, a tempting hypothesis is that they regulate the overall behavior of the damaged chromosomal domain, such as its mobility within the nuclear space. Widespread chromatin changes could alter physical properties of the chromatin fiber such as its rigidity (or stiffness), its compaction status, and/or its ability to phase separate from its local environment. These newly acquired physical properties would translate into a modified ability to move in the nucleus, hence fine-tuning downstream repair outputs. However, a detailed molecular and structural description of a γH2AX focus is still awaited. The use of super-resolution microscopy as well as sequencing-based technologies allowing the characterization of chromatin composition and conformation (ChIP-seq, 4C-seq, Hi-C) will surely help to characterize γH2AX foci structures in better detail, including the position of looping factors (such as cohesin, in yellow), nucleosomes density, or chromatin loops, which could alter the rigidity and compaction status of the fiber. Pink and gray circles indicate γH2AX-modified and unmodified nucleosomes, respectively.

histone H2A [28,29]. In addition, it was recently reported that the megabase-scaled modifications (γH2AX, H1 removal, and ubiquitin accumulation) are much more pronounced at DSBs exhibiting movement, that is, DSBs prone to form large clusters in G1 [14,30]. Indeed, although the underlying mechanism remains unclear, this acute chromatin signaling and ability to move and cluster appear to be key features of persistent DSB such as DSBs in transcriptionally active genes that are not efficiently repaired in G1 but rather undergo HR repair in S/G2 [14,30,31]. Large-scale chromatin changes could contribute to DSB motion by; (i) regulating the stiffness (rigidity) of the chromatin fiber, (ii) altering its compaction, and (iii) promoting **phase separation** mechanisms (Figure 3). Interestingly, evidence for all three mechanisms has been reported (see below). Computational models and experimental data suggest that DSB-driven increased chromatin stiffness could translate into enhanced chromatin mobility [29,32]. Yet, how these models fit with the finding that core histone eviction occurs genome-wide following DSB, leading to a generalized increase in chromatin flexibility [33], is unclear. In addition, the chromatin embedded in γH2AX foci displays a decondensed-like appearance and increased short-range intra-chromosomal contacts [30,34]. This is in line with H1 depletion, which increases DNA accessibility in other contexts (e.g., [35]). Finally, liquid-liquid phase separation occurs in irradiation-induced foci [36]. While such phase separation was shown to be seeded by PARP activity, it is tempting to envision that γH2AX, ubiquitin or 53BP1, which decorates entire γH2AX domains in G1 [10,34,35], could further contribute to phase separation. Like its role in the physical interaction of other chromatin compartments [37], phase separation could mediate physical interaction of multiple γH2AX-decorated chromatin domains (i.e., DSB clustering). Whether phase separation cooperates or acts in parallel to other processes promoting DSB mobility, for example, involving nucleo- and or cytoskeletal proteins [30,38–41] remains to be elucidated. Hence, large-scale modification of chromatin may not directly participate in the repair reaction itself but could still contribute to the specific dynamic properties of damaged genomic regions.

Local Chromatin Changes during NHEJ Repair: Promoting NHEJ Repair Factor Accessibility and Restricting Resection

Beyond the large-scale changes mentioned above, the local chromatin state (<10 kb) also experiences severe changes around DSBs. Several chromatin modifications are reported to foster NHEJ (Figure 2). Among them, the H3.3 histone variant was found deposited at sites of damage by the **chromatin remodeler** chromodomain helicase DNA-binding protein 2 (CHD2), where it contributes to efficiently recruit NHEJ core factors (Ku and XRCC4) [11]. Similarly, H2A.Z incorporation also potentiates Ku70 loading [42], although its further removal by ANP32e is required for completion of NHEJ repair [43]. H4Y51 was recently reported to be phosphorylated by the tyrosine kinase receptor TIE2 and contributes to NHEJ [44]. It was also reported that H2BK120 undergoes a monoubiquitin to acetyl transition on ~2–3 kb surrounding DSBs, a conversion that can be catalyzed by the deubiquitinase (DUB) and acetyl transferase (HAT) activities of the **SAGA complex** *in vitro* [14]. Importantly, knockdown of SAGA subunits impaired NHEJ [14], in agreement with a previous study [45]. Notably H2BK120ub removal reduces nucleosome occupancy genome wide [46], CHD2-mediated H3.3 deposition promotes chromatin expansion [11], and H2AZ-containing nucleosomes generally associate with an open chromatin structure (discussed in [47]). In addition, nucleosome core particles containing both H3.3 and H2AZ were described as particularly unstable [48]. Hence, several pathways seem to converge towards local nucleosome destabilization to promote access to repair machineries. In agreement, evidence suggest that in G1-arrested cells, where DSBs mainly undergo NHEJ, nucleosome removal occurs on a <2 kb window around breaks (approximately eight nucleosomes) [49], and histone displacement may facilitate the binding of NHEJ factors near the break [50].

The status of H3K36 also seems to play a critical role in NHEJ. In yeast, Set2-mediated H3K36 methylation promotes the use of NHEJ in G1 by antagonizing acetylation on H3K36 or H4 [51,52]. In mammalian cells, H3K36me2 increases at the immediate vicinity of a single **I-SceI break** (<500 bp) where it was shown to contribute to NHEJ [53]. Interestingly, genome-wide mapping data at **AsiSI-induced DSBs** could not recapitulate H3K36me2 accumulation; however, a modest increase of H3K36me3 at NHEJ-repaired DSB was found [14]. H3K36me3 accumulates on genes bodies during transcription elongation and prevents cryptic transcription initiation by recruiting of histone deacetylases (HDACs) (reviewed in [54]). Of interest, HDAC1/2 is recruited at sites of damage at a very early stage (before ATM) [7] and promotes NHEJ [55]. This raises the possibility that H3K36 trimethylation could contribute to HDAC recruitment in the immediate vicinity of the break during NHEJ repair. One could envision a mechanism whereby histone removal (via SAGA, CHD2, or H2AZ; see above) would promote access to NHEJ factors exactly at the break point, while H3K36me3-mediated recruitment of HDAC would rather stabilize the nucleosome further away to disfavor resection (Figure 4).

Finally, the histone modification landscape also controls NHEJ by attracting anti-resection factors (reviewed in [56]) such as 53BP1 (reviewed in [57]), which exerts its activity by recruiting downstream effectors including the recently characterized Shieldin complex [58–63] and the CST/DNA polymerase α that reconstitutes processed DNA ends [59]. 53BP1 displays a strong binding affinity for H4K20me1/2 and for H2A ubiquitinated at lysine 15 (H2AK15ub), via a tandem Tudor-UDR (ubiquitin-dependent interacting region) domain [64,65]. While RNF168 ensures H2AK15ub accumulation in response to DSB [66], the majority of nucleosomes are methylated on H4K20, even in the absence of damage, outside replication [67,68]. Nevertheless, DSB-induced accumulation of H4K20me1/2 (and in some instances me3) has been reported [69–74]. While no changes in H4K20me2 were detected in [14], NHEJ-repaired DSBs

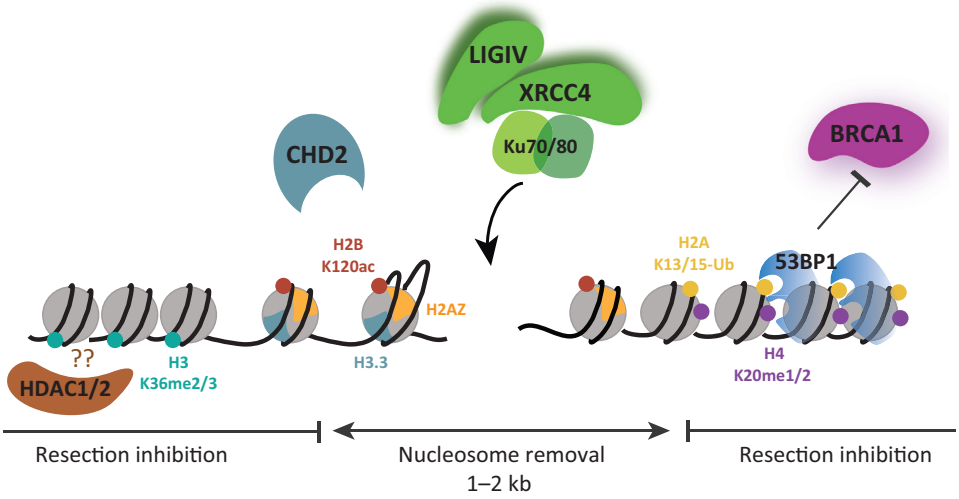

Figure 4. Proposed Roles for Chromatin Modifications in NHEJ Repair. H3.3 incorporation, H2BK120 deubiquitination, and the complex cycle of H2AZ exchange will tend to locally destabilize nucleosomes, contributing to recruitment of non-homologous end joining (NHEJ) factors near the break. Other modifications will tend to create an environment that is not favorable for resection. For example, H3K36 methylation may reduce accessibility away from the break by potentially recruiting histone deacetylases (HDACs), generally associated with chromatin condensation. Local methylation of H4K20, ubiquitination of H2AK15, and the absence of H4K16ac will favor the recruitment of 53BP1, a factor able to actively inhibit resection.

displayed a moderate increase of H4K20me1 on 2–3 kb in these ChIP-seq assays. Hence, pre-existing or *de novo*-deposited H4K20me1/2 may allow, in G1, initial recruitment of 53BP1, which would be further stabilized by DSB-induced H2AK15 ubiquitination (Figure 4).

Local Chromatin Changes Promoting Homologous Recombination

Many histone modifications were shown to be involved in promoting HR (Figure 2). ChIP-seq mapping revealed significant changes in the vicinity of DSB repaired by HR. This includes macroH2A deposition, in agreement with previous reports [75,76], and a decrease in H2BK120ub, H2A.Z, H3K4me3, H3K79me2, and H4K12ac [14,77]. Importantly, most of these modifications were previously shown to be interdependent since macroH2A regulates H2BK120 acetylation, while H2BK120ub and H4 acetylation stimulate both H3K4/H3K79 methylation and H2A.Z stabilization onto chromatin (e.g., [78–82]). Of note, this work [14] could neither recapitulate the increase of H2B ubiquitination nor the requirement of the SAGA H2B DUB activity in counteracting HR as seen by others [83–86]. The reasons for such inconsistencies are unclear and call for further investigations, but they may relate to the nature and position of induced breaks and/or more likely to kinetics issues (Box 3; see Outstanding Questions). The contribution of this specific nucleosome composition to HR remains to be elucidated.

Indeed, a better characterization of these DSB-induced chromatin modifications across the cell cycle together with functional investigation of their involvement in the execution of accurate HR repair still need to be performed (see Outstanding Questions). Yet evidence suggests that the chromatin landscape can regulate HR by; (i) controlling the initiation and extent of resection, (ii) regulating the assembly of RAD51 filament downstream of resection, and (iii) regulating the chromatin reconstitution of newly synthetized DNA (see below).

Chromatin as a Pro-resection Factor Recruitment Platform

Chromatin features have been shown to favor recruitment of pro-resection activities [56] (Figure 5, right side of the break). Pre-existing SETD2-dependent H3K36me3 can be recognized by the PWWP domain of LEDGF, resulting in CtIP recruitment and stimulation of resection at DSBs in active genes, where H3K36me3 is usually present [31,87–89]. Furthermore, DSB-induced H3K9me2 favors recruitment of BRCA1 and its partner BARD1 [75,90]. BRCA1 recruitment to damaged sites is also enhanced by the DNA- and H2A/H2AX-binding protein ZMYM3 [91]. Finally, recruitment of the anti-resection factor 53BP1 is also downregulated as cells progress to S and G2 phases. Dilution of H4K20me2 on newly replicated chromatin triggers eviction of 53BP1 [92] and in G2, the histone acetyltransferase complex TIP60 can compete with 53BP1 for H4K20me2 binding, promoting H2AK15 and H4K16 acetylation that will antagonize 53BP1 binding, promote BRCA1 recruitment, and favor resection [93,94].

Relieving the Nucleosomal Barrier to Resection

The pre-existing and DSB-established chromatin state can also directly control the generation of ssDNA. The presence of nucleosomes inhibits resection *in vitro* [95]. Moreover, a recent genome-wide analysis of resection endpoints around Spo11 DSB hot spots during meiosis clearly demonstrated that, in yeast, resection frequently terminates at nucleosomes [96], providing the first experimental proof that nucleosomes somehow represent barriers to exonucleases processivity *in vivo*. Yet, Exo1 can efficiently progress through nucleosomes *in vivo*, since the resection rate fits with the one observed on naked DNA [96]. This suggests that histone modifications and ATP-dependent chromatin remodelers ensure nucleosome destabilization/remodeling to overcome this otherwise natural barrier. Indeed a plethora of

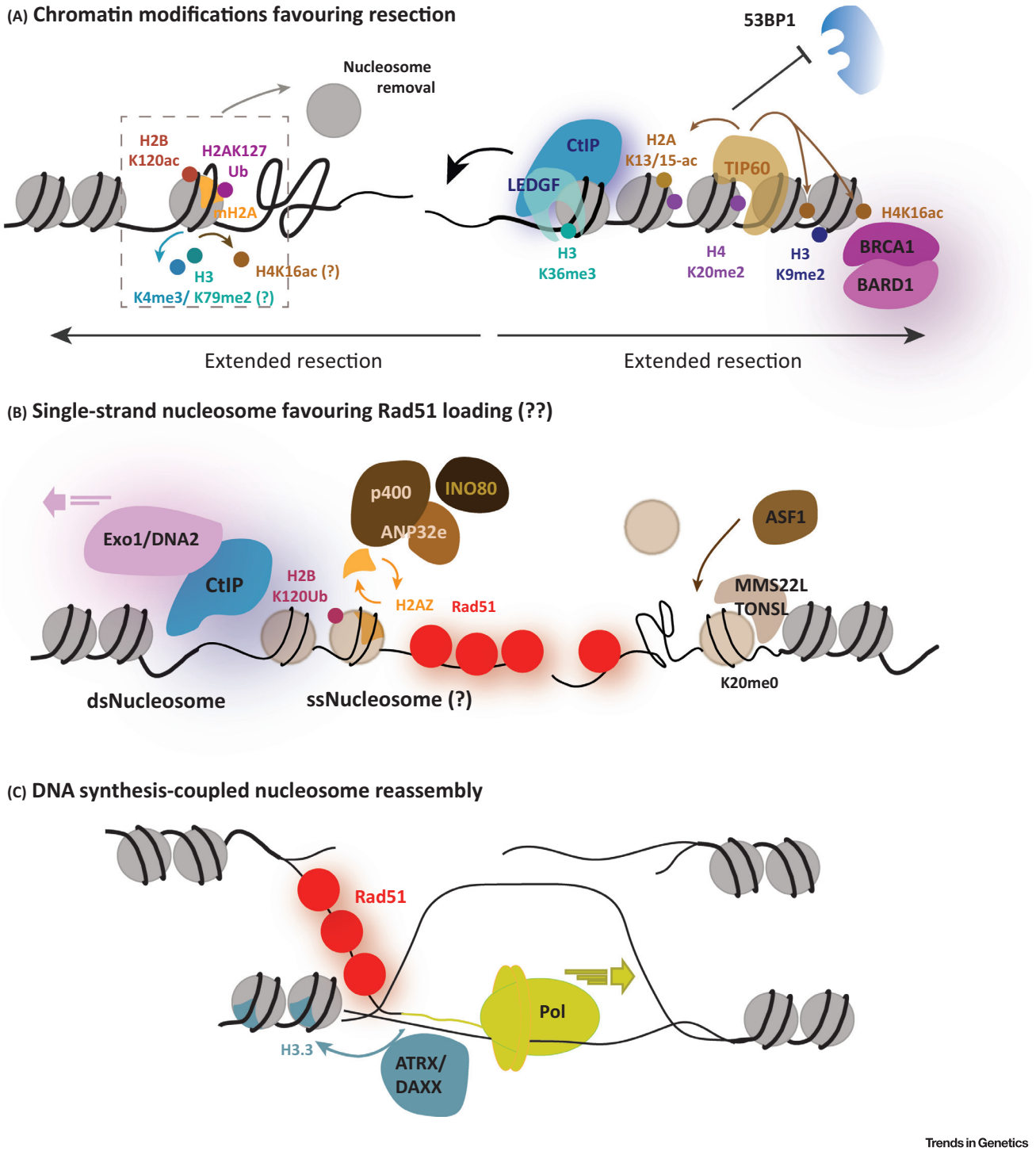

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Figure 5. Various Impacts of Chromatin Modifications upon HR Repair. (A) Several histone post-translational modifications will facilitate resection via distinct mechanisms. On one hand (right side of the break), pre-existing and/or double-strand break (DSB)-induced histone modifications will recruit pro-resection factors containing reader modules. For example, H3K36me3 favors the recruitment of CtIP via the adaptor protein LEDGF, H3K9me2 can be recognized by BRCA1/BARD1, and Tip60-mediated acetylation of H2AK15 and H4K16 reduce 53BP1 binding near DSBs and favors BRCA1 recruitment. On the other hand (left side of the break), a specific nucleosome composition and post-translational signature could contribute to nucleosome destabilization, thereby favoring the progression of the resection

(Figure legend continued on the bottom of the next page.)

chromatin-modifying activities were found to control resection, both in yeast and mammals, including chromatin remodelers (SWI/SNF, INO80, SMARCAD1), H2A.Z exchange factors, and histone-modifying enzymes (extensively and recently reviewed in [97–100]). In that context, a specific HR nucleosome post-translational signature (Figure 2) may allow the bypass of the inherent anti-resection properties of nucleosomes by decreasing its stability onto DNA or/and by acting as a recruitment platform for ATP-dependent remodelers, as shown, for example, for BRCA1/BARD1-mediated H2AK125/127/129 ubiquitination [101]. This would translate into partial or total nucleosome disassembly around DSBs (Figure 5A, left side of the break), as repeatedly documented in both yeast and mammalian cells [49,50,102–107].

ssDNA Nucleosomes during HR?

Evidence suggests that nucleosomes could also reassemble onto ssDNA to favor downstream recombinational events. A recent study suggested that histone chaperones ASF1 and CAF-1 can assemble nucleosomes on resected DNA, promoting the recruitment of MMS22L-TONSL [108], previously shown to specifically recognize newly incorporated histone H4 unmethylated on lysine 20 [68] and to promote RAD51 nucleofilament assembly [109–111]. Remarkably, nucleosomes can form *in vitro* on ssDNA [112] and ChIP analyses at DSBs, especially in yeast, show rather moderate histone loss on efficiently resected regions [84,108]. Similarly, H3 was only mildly depleted in human cells at HR-repaired, AsiSI-induced DSBs, despite strong RAD51 binding and efficient resection [14,31,113,114]. Furthermore, several chromatin-modifying activities promote RAD51 nucleofilament assembly downstream of resection. This includes INO80, Anp32E, p400, and H2AZ removal and in yeast, Bre1-dependent H2BK123ub [106,115,116] suggesting that newly assembled nucleosomes on ssDNA require remodeling to sustain efficient loading of RAD51. Taken together, these data indicate that a specific nucleosomal state assembled onto ssDNA could regulate RAD51 filament formation (Figure 5B). Yet, a dedicated methodology that would formally prove the existence of nucleosomes assembled in ssDNA *in vivo* is still awaited.

Chromatin Re-assembly during Recombination-Dependent DNA Synthesis

Following resection and homology search, the canonical HR reaction proceeds with templated DNA synthesis restoring an undamaged sequence at the broken locus. Yet, it is unclear whether chromatin is reassembled during repair synthesis, similarly to normal DNA replication, or only after the resolution of recombination intermediates. It was recently shown that the chromatin remodeler ATRX, together with the histone chaperone DAXX and proliferating cell nuclear antigen (PCNA), mediates the incorporation of H3.3 during repair-dependent DNA synthesis in G2 [117], highlighting a coupling between repair synthesis and chromatin assembly (Figure 5C). Maybe more surprisingly, ATRX-dependent chromatin assembly can stimulate long-range DNA synthesis and sister chromatid exchanges, thereby favoring a particular form of HR leading to longer gene conversion tracts and potential crossovers. Further studies are required to better understand; (i) how chromatin assembly participates in these late stages of HR and (ii) how accurate epigenetic information can be faithfully restored upon HR completion, since DNA damage have been linked with long-term epigenetic defects [118].

machinery along the chromatin fiber. (B) Specific histone modifications could also favor RAD51 loading downstream of resection. Increasing evidence tends to suggest that nucleosomes may be assembled on single-strand DNA (ssNucleosomes). ssNucleosome-driven protein recruitment (MMS22L–TONSL) or remodeling (p400, INO80, or ANP32e) would further promote RAD51 nucleofilament assembly. (C) At later stages of HR, ATRX, DAXX, and proliferating cell nuclear antigen (PCNA) will stimulate the incorporation of H3.3 during repair-dependent DNA synthesis. Surprisingly, this process favors long gene conversion tracts and crossovers.

Transient Heterochromatin Assembly in *Cis* to DSB?

Several pieces of evidence also suggest that repressive histone marks and transient heterochromatinization could take place in *cis* to DSB. Both heterochromatin proteins (HP1, KAP1, Suv39h1, and Polycomb proteins) and histone marks associated with heterochromatin (H3K9me2, H3K9me3, H3K27me3, and macroH2A) accumulate around DSBs [53,75,76,119–122], but this was not consistent across all studies [123–126]. While a recent genome-wide ChIP-seq study confirmed macroH2A deposition on a 6–10 kb window around DSBs, no changes in H3K9me2/3 were reported [14], suggesting that some of these events may only occur at specific subsets of DSBs or that such events are too transient to be captured by ChIP-seq (Box 3; see Outstanding Questions).

The function of this transient heterochromatin state is not fully established but H3K9 methylation, HP1, and macroH2A may help to promote resection and assemble BRCA1/RAD51 at sites of breaks [75,90,120,122,127]. In agreement, HP1 recruitment precedes those of BARD1, RPA, and RAD51 at sites of micro-irradiation [7]. Importantly, after an initial chromatin decondensation, macroH2A promotes recondensation [75], suggesting that heterochromatin marks deposition indeed modifies chromatin structure around DSBs. H3K9me3, via HP1, plays a pivotal role in heterochromatin domain formation through phase separation [128,129]. Hence, local (<10 kb) and transient heterochromatinization could participate in DSB mobility and facilitate homology search, in agreement with the reported function of HP1/macroH2A in HDR. In addition, this heterochromatic state could help to turn off transcription at damaged genes (see below).

Transcriptional Repression-Related Changes

It has been convincingly demonstrated that the induction of a lesion in or near a transcribed unit will result in transcriptional silencing locally, in *cis* to the DSB (reviewed in [2,130]). This does not solely arise from the mere presence of the lesion itself and likely involves several different mechanisms, including DNAPK-, ATM-, and PARP1-mediated control of RNA polymerase II (pol II) [119,131–135]. This DSB-induced transcriptional silencing was also proposed to depend on Polycomb group protein-mediated H2AK119 ubiquitination and H3K27 methylation [131–134,136] and on the NURD (nucleosome remodeling and histone deacetylase) complex [77,137]. NURD is recruited to DSB at transcribed genes by the bromodomain protein ZMYND8 that recognizes acetylated H4, although the precise mechanism is not fully resolved [137]. Furthermore, NURD-mediated transcriptional silencing was shown to be necessary for the completion of HR [77,137], suggesting that it may represent a crucial step in DSB repair. Transcription inhibition may also depend on a reduction in activating histone marks. Indeed, it was found that DSB induction leads to a decrease in many histone modifications associated with active transcription (H3K4me3, H3K79me2, H2AZ, and H4ac [14,77]), and H3K4me3 drop was shown to be required to mediate NURD recruitment and subsequent transcription silencing [77]. Further experiments are required to distinguish modifications solely required to ensure accurate transcriptional silencing from DSB-induced chromatin features directly participating in the execution of HR at transcriptionally active genes (see Outstanding Questions). Of note, several studies have reported that DSB can also lead to RNA pol II-dependent *de novo* RNA synthesis (reviewed in [130]), but the contribution of chromatin structure in this process remains to be elucidated.

Concluding Remarks

Recent methodological developments have provided us with a unique opportunity to appreciate in great detail how and why chromatin is modified following DNA DSBs and to understand how specialized chromatin pathways are set up depending on where the lesion occurs. Systematic mapping of the expanding number of chromatin features potentially involved in DNA repair (e.g., [138]) at precisely annotated DSBs, combined with advanced proteomics,

Outstanding Questions

What are the kinetics of the various chromatin modifications in *cis* to DSBs, and how does this relate to the different repair steps? Indeed, while sequence-specific DSB induction tools provide the most precise spatial resolution, they are powerful enough for kinetics studies (Box 3). Experimental systems need to be developed or improved to achieve the best spatio-temporal resolution when analyzing chromatin changes at DSBs.

What are the functions of each histone modification, either individually or in combination, in each repair step (during either NHEJ or HR)? How are they cell cycle regulated? Functional assays to measure, for example, resection, RAD51 assembly, histone modification patterns following loss of function of histones modifiers will be required to better understand their contribution during repair (as performed over the past 20 years to understand their role during transcription).

What is the structure of a γH2AX focus, and how does this contribute to the physical properties of the damaged chromatin? Using super-resolution microscopy combined with locus labeling, sequencing-based approaches such as Hi-C, mapping of looping factors, and computational modelling will surely improve our knowledge of DSB-induced chromatin behavior.

How robust is the chromatin response in *cis* to DSB, and how does this change from cell to cell? While ChIP-seq and generally most genomic studies provide averaged snapshots of the entire population, single-cell approaches (imaging and genomic) should allow assessment of cell-to-cell heterogeneity within the population.

What is the contribution of newly identified or yet unknown histone modifications? Can we identify specific histone codes associated with alternative repair pathways?

How and when are the initial epigenomic landscapes recovered following repair to maintain cell fate?

genome editing, and super-resolution microscopy, will undoubtedly provide new and exciting results. Yet, key challenges will need to be tackled in the near future (see Outstanding Questions). For example, a kinetically resolved map of DSB-induced chromatin modifications, integrated with orderly recruitment of DSB repair proteins, will significantly increase our understanding of the spatio-temporal organization of DSB repair. Furthermore, the advent of single-cell methods will allow us to characterize the extent of cell-to-cell variability in the response to DSBs. In this regard, the integration of advanced imaging using state-of-the-art locus labeling technologies [139] with the extent of details provided from single-cell next-generation sequencing methods will undeniably continue to revolutionize our understanding of DSB repair in the context of organized chromosomes.

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