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A Snapshot on the *Cis* Chromatin Response to DNA double-strand breaks

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Abstract

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.

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] (Box1). DSB can occur upon exposure to a variety of genotoxic agents (ionizing radiations, chemotherapeutic drugs...) and under physiological conditions including developmentally programmed DSBs in lymphocytes or during meiosis but also 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 instance, 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 (Box2). 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 **chromatin reader** proteins emphasizing the pivotal function of chromatin during transcription, replication and repair.

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 generation but 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 (Box3), 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, such as: 1) To what extent the chromatin response to DSB is universal, or in other words how does it change depending on the genomic position of the break, as well as 2) 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.

Repair histone code: Different scale, different functions

Elegant studies, following the recruitment kinetics for many repair factors upon DSB induction at localized microirradiation (for example [7, 8]) have started to accurately establish the spatio-temporal organization of the DDR. Additionally, the use of phospho-activatable GFP-tagged core histone identified that chromatin is rapidly relaxed following laser microirradiation in a manner that depends on PARP1 and chromatin remodelers (for instance [9–12]), followed by a recompaction phase, necessary for full DDR activation [12] (Fig. 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** (see Box3), 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 hours 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 local (<10kb) alterations [14] (Fig.1, Key Figure). Additionally, 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, with a focus on histone post-translational modifications occurring during either HR or NHEJ and thereby defining the “repair pathway histone code” (Fig. 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 γ H2AX [15]. This modification, mainly catalyzed by kinases such as ATM or ATR, displays the striking behavior to spread over incredibly large domains since it can cover up to 2 megabases of DSB-surrounding chromatin in mammals [16–18]. Interestingly, γ H2AX 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 γ H2AX [14]. Additionally, this study also found a reduction in linker histone H1 on large chromosomal domains (several megabase) 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 γ H2AX – and other associated megabase-scaled modifications - is still unclear. Indeed, H2AX-deleted mice are viable, although sterile, immune-deficient and cancer-prone [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 [27]) (Fig. 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 histone H2A [30, 31]. Additionally it was recently reported that the megabase-scaled modifications (γ H2AX, H1 removal and ubiquitin accumulation) are much more pronounced at DSB exhibiting movement, i.e. DSBs prone to form large clusters in G1 [14, 28]. Indeed, though the underlying mechanism remains unclear, this acute chromatin signaling and ability to move and cluster appears to be a key feature of persistent DSB such as DSBs in transcriptionally active genes which are not efficiently repaired in G1 but rather undergo HR repair in S/G2 [14, 28, 29]. Large-scale chromatin changes could contribute to DSB motion by a) regulating the stiffness (rigidity) of the chromatin fiber, b) altering its compaction and c) promoting **phase separation** mechanisms (Fig. 3). Interestingly evidence for all three mechanisms have been reported (see below). Computational models and experimental data suggest that DSB-driven increased chromatin stiffness could translate into enhanced chromatin mobility [31, 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. Additionally, the chromatin embedded in γ H2AX foci displays a decondensed-like appearance and increased short-range intra-chromosomal contacts [28, 34]. This is in line with H1 depletion, which increases DNA accessibility in other contexts (for example [35]). Finally, liquid-liquid phase separation occurs in irradiation-induced foci [36]. While such phase separation was shown to be seeded by Poly(ADP-Ribose) Polymerase (PARP) activity, it is tempting to envision that γ H2AX, ubiquitin or 53BP1, which decorates entire γ H2AX 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 γ H2AX-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 cyto-skeletal proteins [28, 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 factors accessibility and restricting resection

Beyond the large scale changes mentioned above, the local chromatin state (<10kb) also experiences severe changes around DSBs. A number of chromatin modifications are reported to foster NHEJ (Fig. 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, knock-down 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 associates

with an open chromatin structure (discussed in [47]). Additionally 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 in order 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 <2kb window around breaks (~8 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** (<500bp) 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 Histones Deacetylase (HDAC) (reviewed in [54]). Of interest, HDAC1/2 is recruited at sites of damage at very early stage (before ATM) [7] and promote NHEJ [55]. This raises the possibility that H3K36 trimethylation could contribute in HDAC recruitment at 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 whilst H3K36me3-mediated recruitment of HDAC would rather stabilize nucleosome further away to disfavor resection (Fig. 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 absence of damage, outside replication [67, 68]. Nevertheless DSB-induced accumulation of H4K20me1/2 (and in some instance me3) has been reported [69–74]. While no changes in H4K20me2 were detected in [14], NHEJ-repaired DSBs displayed a moderate increase of H4K20me1 on 2-3kb 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 (Fig. 4).

Local Chromatin changes promoting Homologous Recombination

Many histone modifications were shown to be involved in promoting HR (Fig. 2). ChIP-seq mapping revealed significant changes at 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 stimulates both H3K4/H3K79 methylation and H2A.Z stabilization onto chromatin (see for example [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 seen by others [83–86]. The reasons for such inconsistencies are yet unclear and call for further investigations, but may relate to the nature

and position of induced breaks and/or more likely to kinetics issues (see Box 3 and 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 a) controlling the initiation and extent of resection, b) regulating the assembly of RAD51 filament downstream of resection and c) regulating the chromatin reconstitution of newly synthesized DNA (see below).

- ***Chromatin as a pro-resection factor recruitment platform.***

Chromatin features have been shown to favor recruitment of pro-resection activities [56] (Fig.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 [29, 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 which will antagonize 53BP1 binding, promote BRCA1 recruitment and favor resection [93, 94].

- ***Relieving the nucleosomal barrier to resection***

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 end points around Spo11 DSB hotspots 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 in order to overcome this otherwise natural barrier. Indeed a plethora of 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 (Fig. 2) may allow to bypass 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 (Fig. 5a, left side of the break), as repeatedly documented in both yeast and mammalian cells [49, 50, 102–107].

- ***ssDNA nucleosomes during homologous recombination?***

Evidence suggests that nucleosomes could also reassemble onto ssDNA in order to favour 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 recognizes 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 DSB, 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, 29, 113, 114]. Furthermore, several chromatin-modifying activities promote RAD51 nucleofilament assembly downstream of resection. This includes INO80, Anp32E, p400, H2AZ removal and in yeast, Bre1-dependant H2BK123ub [106, 115, 116] suggesting that newly assembled nucleosomes on ssDNA requires remodeling to sustain efficient loading of RAD51. Taken together these data indicate that a specific nucleosomal state assembled onto ssDNA could regulates RAD51 filament formation (Fig. 5b). Yet, a dedicated methodology that would formally prove the existence of nucleosomes assembled in single-stranded DNA *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 if 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 PCNA, mediates the incorporation of H3.3 during repair-dependent DNA synthesis in G2 [117] highlighting a coupling between repair synthesis and chromatin assembly (Fig. 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 will be required to better understand

1) how chromatin assembly participates in these late stages of HR and 2) how accurate epigenetic information can be faithfully restored upon HR completion, since DNA damage have been linked with long term epigenetic defects [118].

Transient heterochromatin assembly *in cis* to DSB?

Several pieces of evidence also suggests that repressive histone marks and transient heterochromatinization could take place *in cis* to DSB. Both heterochromatin proteins (HP1, KAP1, Suv39h1 and Polycomb proteins) and histones 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 6-10kb 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 (see Box 3 and 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 re-condensation [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 (<10kb) 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 Pol II [119, 131–135]. This DSB-induced transcriptional silencing was also proposed to depend on Polycomb Group proteins-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, which recognizes acetylated H4, though 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, 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 apprehend in great details how and why chromatin is modified following DNA double-strand breaks 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 (for example [138]) at precisely annotated DSB, combined with advanced proteomics, 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 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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Competing interests

The authors have no competing interest to declare.

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 complex (MRE11- RAD50-NBS1) and CtIP and is further extended by EXO1, DNA2 and accessory factors such as the helicase BLM. RPA will bind newly generated single-stranded DNA 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 like HR deficiencies, alternative pathways such as Single-Strand Annealing (SSA) or MMEJ (Microhomology-Mediated End joining) can be mobilized, at a cost for genome integrity since these pathways tend to be highly mutagenic [140]. The initiation and extent of resection is a key aspect 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.

Box 2: Chromatin is organized at many different levels

The primary level of chromatin organization consists in DNA winding around octamers of core histones (H2A, H2B, H3 and H4) forming arrays of nucleosomes, 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, 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 or TADs [144].

Box 3: The Pros and Cons of prevalent DSB induction methods

Common DSB-induction methods include ionizing radiations (γ - or X-rays), cross-linking agents (mitomycin C, cisplatin), radiomimetic compounds (bleomycin, neocarzinostatin), topoisomerase inhibitors (camptothecin, etoposide), replication inhibitors (hydroxyurea, aphidicolin), chemicals (such as hydrogen peroxide) and localized laser microirradiation (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 “hotspots” (ie. respectively at sites of topoisomerase binding, or at sites where replication forks collapse). However even in the latter cases, where position of the DSBs are known, it is still unsure 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, single-strand DNA breaks) or may only effectively trigger DSBs in some cells in the population (for example 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 fashion 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 to induce multiple DSBs scattered across the genome allowing to

compare 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 DSB in heterochromatin (being inhibited by DNA methylation) and Cas9-induced breaks display an inherent propensity to be repaired by mutagenic pathways [146].

Glossary:

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

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.

DIvA system: DSB Induced via AsiSI, a system for controlled induction of about 100 DSB in the human genome based on the controlled nuclear translocation of the restriction enzyme AsiSI which will subsequently cleave endogenous recognition sites in the genome (GCGATCGC). DSB 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

Phase separation: A physicochemical process resulting in intracellular compartmentalization by 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.

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.

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

I-SceI break : A DNA double-strand break induced using the homing endonuclease I-SceI. Its large recognition site (18bp) is usually not found in the host genome and needs to be introduced by transgenic method, allowing for precise control of the position of the DSB.

AsiSI-induced DSB: DSBs induced using the restriction enzyme AsiSI, the key feature in the DIvA system (see Box3).

Figure legends

Figure 1: Chromatin during DSB repair

Upon 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 DSB induced in different chromatin context (thanks 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 elsewhere (see main text). In this review we rather focus on the chromatin changes induced by DSBs (Panel A, central grey box), with a strong emphasis on the spatial distribution of histone modifications around DSB 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 localised micro-irradiation (Panel B, also reviewed elsewhere, see main text). Describing the DDR 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).

Figure 2: A DSB repair histone code

Recent studies revealed that nucleosomes exhibit a specific composition and post-translational signature during repair by NHEJ (left) and 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

Figure 3: A function for large-scale chromatin modifications in DSB mobility?

Few large-scale, megabase-sized chromatin modifications occur in *cis* to DSBs, including the phosphorylation of the H2AX histone variant (γ H2AX), ubiquitin chains accumulation and

linker histone H1 removal. While their exact function still need 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, or/and 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 γ H2AX 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 γ H2AX foci structures in better details, 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 grey circles indicate γ H2AX modified and unmodified nucleosomes respectively.

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 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 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.

Figure 5: The various impacts of chromatin modifications upon HR repair

a) Several histone posttranslational modifications will facilitate resection via distinct mechanisms. On one hand (right side of the break), pre-existing or/and DSB-induced histone modifications will recruit pro-resection factors containing reader modules. For instance, 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 posttranslational signature could contribute to nucleosome destabilization, thereby favoring the progression of the resection machinery along the chromatin fiber.

b) Specific histones modifications could also favor RAD51 loading downstream of resection. Increasing evidence tend to suggest that nucleosome may be assembled on ssDNA (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 PCNA will stimulate the incorporation of H3.3 during repair-dependent DNA synthesis. Surprisingly, this process favors long gene conversion tracts and crossovers.

Bibliography

1. Mladenov, E., Magin, S., Soni, A., and Iliakis, G. (2016). DNA double-strand-break repair in higher eukaryotes and its role in genomic instability and cancer: Cell cycle and proliferation-dependent regulation. *Semin. Cancer Biol.* 37-38, 51–64.
2. Marnef, A., Cohen, S., and Legube, G. (2017). Transcription-Coupled DNA Double-Strand Break Repair: Active Genes Need Special Care. *J. Mol. Biol.* 429, 1277–1288.
3. Canela, A., Maman, Y., Jung, S., Wong, N., Callen, E., Day, A., Kieffer-Kwon, K.-R., Pekowska, A., Zhang, H., Rao, S. S. P., et al. (2017). Genome organization drives chromosome fragility. *Cell* 170, 507–521.e18.
4. Zhou, V. W., Goren, A., and Bernstein, B. E. (2011). Charting histone modifications and the functional organization of mammalian genomes. *Nat. Rev. Genet.* 12, 7–18.
5. Fortuny, A., and Polo, S. E. (2018). The response to DNA damage in heterochromatin domains. *Chromosoma* 127, 291–300.
6. van Sluis, M., and McStay, B. (2017). Nucleolar reorganization in response to rDNA damage. *Curr. Opin. Cell Biol.* 46, 81–86.
7. Aleksandrov, R., Dotchev, A., Poser, I., Krastev, D., Georgiev, G., Panova, G., Babukov, Y., Danovski, G., Dyankova, T., Hubatsch, L., et al. (2018). Protein dynamics in complex DNA lesions. *Mol. Cell* 69, 1046–1061.e5.
8. Kochan, J. A., Desclos, E. C. B., Bosch, R., Meister, L., Vriend, L. E. M., van Attikum, H., and Krawczyk, P. M. (2017). Meta-analysis of DNA double-strand break response kinetics. *Nucleic Acids Res.* 45, 12625–12637.
9. Sellou, H., Lebeaupin, T., Chapuis, C., Smith, R., Hegele, A., Singh, H. R., Kozlowski, M., Bultmann, S., Ladurner, A. G., Timinszky, G., et al. (2016). The poly(ADP-ribose)-dependent chromatin remodeler Alc1 induces local chromatin relaxation upon DNA damage. *Mol. Biol. Cell* 27, 3791–3799.
10. Smith, R., Sellou, H., Chapuis, C., Huet, S., and Timinszky, G. (2018). CHD3 and CHD4 recruitment and chromatin remodeling activity at DNA breaks is promoted by early poly(ADP-ribose)-dependent chromatin relaxation. *Nucleic Acids Res.* 46, 6087–6098.
11. Luijsterburg, M. S., de Krijger, I., Wiegant, W. W., Shah, R. G., Smeenk, G., de Groot, A. J. L., Pines, A., Vertegaal, A. C. O., Jacobs, J. J. L., Shah, G. M., et al. (2016). PARP1 Links CHD2-Mediated Chromatin Expansion and H3.3 Deposition to DNA Repair by Non-homologous End-Joining. *Mol. Cell* 61, 547–562.
12. Burgess, R. C., Burman, B., Kruhlak, M. J., and Misteli, T. (2014). Activation of DNA damage response signaling by condensed chromatin. *Cell Rep.* 9, 1703–1717.
13. Dantuma, N. P., and van Attikum, H. (2016). Spatiotemporal regulation of posttranslational modifications in the DNA damage response. *EMBO J.* 35, 6–23.

14. Clouaire, T., Rocher, V., Lashgari, A., Arnould, C., Aguirrebengoa, M., Biernacka, A., Skrzypczak, M., Aymard, F., Fongang, B., Dojer, N., et al. (2018). Comprehensive Mapping of Histone Modifications at DNA Double-Strand Breaks Deciphers Repair Pathway Chromatin Signatures. *Mol. Cell* 72, 250–262.e6.
15. Rogakou, E. P., Pilch, D. R., Orr, A. H., Ivanova, V. S., and Bonner, W. M. (1998). DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J. Biol. Chem.* 273, 5858–5868.
16. Iacovoni, J. S., Caron, P., Lassadi, I., Nicolas, E., Massip, L., Trouche, D., and Legube, G. (2010). High-resolution profiling of gammaH2AX around DNA double strand breaks in the mammalian genome. *EMBO J.* 29, 1446–1457.
17. Caron, P., Choudjaye, J., Clouaire, T., Bugler, B., Daburon, V., Aguirrebengoa, M., Mangeat, T., Iacovoni, J. S., Álvarez-Quilón, A., Cortés-Ledesma, F., et al. (2015). Non-redundant Functions of ATM and DNA-PKcs in Response to DNA Double-Strand Breaks. *Cell Rep.* 13, 1598–1609.
18. Savic, V., Yin, B., Maas, N. L., Bredemeyer, A. L., Carpenter, A. C., Helmink, B. A., Yang-Iott, K. S., Sleckman, B. P., and Bassing, C. H. (2009). Formation of dynamic gamma-H2AX domains along broken DNA strands is distinctly regulated by ATM and MDC1 and dependent upon H2AX densities in chromatin. *Mol. Cell* 34, 298–310.
19. Piquet, S., Le Parc, F., Bai, S.-K., Chevallier, O., Adam, S., and Polo, S. E. (2018). The Histone Chaperone FACT Coordinates H2A.X-Dependent Signaling and Repair of DNA Damage. *Mol. Cell* 72, 888–901.e7.
20. Morris, J. R., and Solomon, E. (2004). BRCA1 : BARD1 induces the formation of conjugated ubiquitin structures, dependent on K6 of ubiquitin, in cells during DNA replication and repair. *Hum. Mol. Genet.* 13, 807–817.
21. Li, Z., Li, Y., Tang, M., Peng, B., Lu, X., Yang, Q., Zhu, Q., Hou, T., Li, M., Liu, C., et al. (2018). Destabilization of linker histone H1.2 is essential for ATM activation and DNA damage repair. *Cell Res.* 28, 756–770.
22. Strickfaden, H., McDonald, D., Kruhlak, M. J., Haince, J.-F., Th'ng, J. P. H., Rouleau, M., Ishibashi, T., Corry, G. N., Ausio, J., Underhill, D. A., et al. (2016). Poly(ADP-ribosyl)ation-dependent Transient Chromatin Decondensation and Histone Displacement following Laser Microirradiation. *J. Biol. Chem.* 291, 1789–1802.
23. Li, Y., Li, Z., Dong, L., Tang, M., Zhang, P., Zhang, C., Cao, Z., Zhu, Q., Chen, Y., Wang, H., et al. (2018). Histone H1 acetylation at lysine 85 regulates chromatin condensation and genome stability upon DNA damage. *Nucleic Acids Res.* 46, 7716–7730.
24. Thorslund, T., Ripplinger, A., Hoffmann, S., Wild, T., Uckelmann, M., Villumsen, B., Narita, T., Sixma, T. K., Choudhary, C., Bekker-Jensen, S., et al. (2015). Histone H1

- couples initiation and amplification of ubiquitin signalling after DNA damage. *Nature* 527, 389–393.
25. Nowsheen, S., Aziz, K., Aziz, A., Deng, M., Qin, B., Luo, K., Jeganathan, K. B., Zhang, H., Liu, T., Yu, J., et al. (2018). L3MBTL2 orchestrates ubiquitin signalling by dictating the sequential recruitment of RNF8 and RNF168 after DNA damage. *Nat. Cell Biol.* 20, 455–464.
 26. Scully, R., and Xie, A. (2013). Double strand break repair functions of histone H2AX. *Mutat. Res.* 750, 5–14.
 27. Marnef, A., and Legube, G. (2017). Organizing DNA repair in the nucleus: DSBs hit the road. *Curr. Opin. Cell Biol.* 46, 1–8.
 28. Aymard, F., Aguirrebengoa, M., Guillou, E., Javierre, B. M., Bugler, B., Arnould, C., Rocher, V., Iacovoni, J. S., Biernacka, A., Skrzypczak, M., et al. (2017). Genome-wide mapping of long-range contacts unveils clustering of DNA double-strand breaks at damaged active genes. *Nat. Struct. Mol. Biol.* 24, 353–361.
 29. Aymard, F., Bugler, B., Schmidt, C. K., Guillou, E., Caron, P., Briois, S., Iacovoni, J. S., Daburon, V., Miller, K. M., Jackson, S. P., et al. (2014). Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks. *Nat. Struct. Mol. Biol.* 21, 366–374.
 30. Dion, V., Kalck, V., Horigome, C., Towbin, B. D., and Gasser, S. M. (2012). Increased mobility of double-strand breaks requires Mec1, Rad9 and the homologous recombination machinery. *Nat. Cell Biol.* 14, 502–509.
 31. Herbert, S., Brion, A., Arbona, J.-M., Lelek, M., Veillet, A., Lelandais, B., Parmar, J., Fernández, F. G., Almayrac, E., Khalil, Y., et al. (2017). Chromatin stiffening underlies enhanced locus mobility after DNA damage in budding yeast. *EMBO J.* 36, 2595–2608.
 32. Miné-Hattab, J., Recamier, V., Izeddin, I., Rothstein, R., and Darzacq, X. (2017). Multi-scale tracking reveals scale-dependent chromatin dynamics after DNA damage. *Mol. Biol. Cell.*
 33. Hauer, M. H., Seeber, A., Singh, V., Thierry, R., Sack, R., Amitai, A., Kryzhanovska, M., Eglinger, J., Holcman, D., Owen-Hughes, T., et al. (2017). Histone degradation in response to DNA damage enhances chromatin dynamics and recombination rates. *Nat. Struct. Mol. Biol.* 24, 99–107.
 34. Kruhlak, M. J., Celeste, A., Dellaire, G., Fernandez-Capetillo, O., Müller, W. G., McNally, J. G., Bazett-Jones, D. P., and Nussenzweig, A. (2006). Changes in chromatin structure and mobility in living cells at sites of DNA double-strand breaks. *J. Cell Biol.* 172, 823–834.
 35. Iwasaki, Y. W., Murano, K., Ishizu, H., Shibuya, A., Iyoda, Y., Siomi, M. C., Siomi, H., and Saito, K. (2016). Piwi modulates chromatin accessibility by regulating multiple factors including histone H1 to repress transposons. *Mol. Cell* 63, 408–419.

36. Altmeyer, M., Neelsen, K. J., Teloni, F., Pozdnyakova, I., Pellegrino, S., Grøfte, M., Rask, M.-B. D., Streicher, W., Jungmichel, S., Nielsen, M. L., et al. (2015). Liquid demixing of intrinsically disordered proteins is seeded by poly(ADP-ribose). *Nat. Commun.* 6, 8088.
37. Nuebler, J., Fudenberg, G., Imakaev, M., Abdennur, N., and Mirny, L. A. (2018). Chromatin organization by an interplay of loop extrusion and compartmental segregation. *Proc. Natl. Acad. Sci. USA* 115, E6697–E6706.
38. Caridi, C. P., D’Agostino, C., Ryu, T., Zapotoczny, G., Delabaere, L., Li, X., Khodaverdian, V. Y., Amaral, N., Lin, E., Rau, A. R., et al. (2018). Nuclear F-actin and myosins drive relocalization of heterochromatic breaks. *Nature* 559, 54–60.
39. Schrank, B. R., Aparicio, T., Li, Y., Chang, W., Chait, B. T., Gundersen, G. G., Gottesman, M. E., and Gautier, J. (2018). Nuclear ARP2/3 drives DNA break clustering for homology-directed repair. *Nature* 559, 61–66.
40. Lottersberger, F., Karssemeijer, R. A., Dimitrova, N., and de Lange, T. (2015). 53BP1 and the LINC Complex Promote Microtubule-Dependent DSB Mobility and DNA Repair. *Cell* 163, 880–893.
41. Oshidari, R., Strecker, J., Chung, D. K. C., Abraham, K. J., Chan, J. N. Y., Damaren, C. J., and Mekhail, K. (2018). Nuclear microtubule filaments mediate non-linear directional motion of chromatin and promote DNA repair. *Nat. Commun.* 9, 2567.
42. Xu, Y., Ayrapetov, M. K., Xu, C., Gursoy-Yuzugullu, O., Hu, Y., and Price, B. D. (2012). Histone H2A.Z controls a critical chromatin remodeling step required for DNA double-strand break repair. *Mol. Cell* 48, 723–733.
43. Gursoy-Yuzugullu, O., Ayrapetov, M. K., and Price, B. D. (2015). Histone chaperone Anp32e removes H2A.Z from DNA double-strand breaks and promotes nucleosome reorganization and DNA repair. *Proc. Natl. Acad. Sci. USA* 112, 7507–7512.
44. Hossain, M. B., Shifat, R., Johnson, D. G., Bedford, M. T., Gabrusiewicz, K. R., Cortes-Santiago, N., Luo, X., Lu, Z., Ezhilarasan, R., Sulman, E. P., et al. (2016). TIE2-mediated tyrosine phosphorylation of H4 regulates DNA damage response by recruiting ABL1. *Sci. Adv.* 2, e1501290.
45. Ramachandran, S., Haddad, D., Li, C., Le, M. X., Ling, A. K., So, C. C., Nepal, R. M., Gommerman, J. L., Yu, K., Ketela, T., et al. (2016). The SAGA Deubiquitination Module Promotes DNA Repair and Class Switch Recombination through ATM and DNAPK-Mediated γ H2AX Formation. *Cell Rep.* 15, 1554–1565.
46. Batta, K., Zhang, Z., Yen, K., Goffman, D. B., and Pugh, B. F. (2011). Genome-wide function of H2B ubiquitylation in promoter and genic regions. *Genes Dev.* 25, 2254–2265.
47. Billon, P., and Côté, J. (2013). Precise deposition of histone H2A.Z in chromatin for genome expression and maintenance. *Biochim. Biophys. Acta* 1819, 290–302.

48. Jin, C., and Felsenfeld, G. (2007). Nucleosome stability mediated by histone variants H3.3 and H2A.Z. *Genes Dev.* *21*, 1519–1529.
49. Li, X., and Tyler, J. K. (2016). Nucleosome disassembly during human non-homologous end joining followed by concerted HIRA- and CAF-1-dependent reassembly. *Elife* *5*.
50. Goldstein, M., Derheimer, F. A., Tait-Mulder, J., and Kastan, M. B. (2013). Nucleolin mediates nucleosome disruption critical for DNA double-strand break repair. *Proc. Natl. Acad. Sci. USA* *110*, 16874–16879.
51. Jha, D. K., and Strahl, B. D. (2014). An RNA polymerase II-coupled function for histone H3K36 methylation in checkpoint activation and DSB repair. *Nat. Commun.* *5*, 3965.
52. Pai, C.-C., Deegan, R. S., Subramanian, L., Gal, C., Sarkar, S., Blaikley, E. J., Walker, C., Hulme, L., Bernhard, E., Codlin, S., et al. (2014). A histone H3K36 chromatin switch coordinates DNA double-strand break repair pathway choice. *Nat. Commun.* *5*, 4091.
53. Fnu, S., Williamson, E. A., De Haro, L. P., Brenneman, M., Wray, J., Shaheen, M., Radhakrishnan, K., Lee, S.-H., Nickoloff, J. A., and Hromas, R. (2011). Methylation of histone H3 lysine 36 enhances DNA repair by nonhomologous end-joining. *Proc. Natl. Acad. Sci. USA* *108*, 540–545.
54. McDaniel, S. L., and Strahl, B. D. (2017). Shaping the cellular landscape with Set2/SETD2 methylation. *Cell Mol. Life Sci.* *74*, 3317–3334.
55. Miller, K. M., Tjeertes, J. V., Coates, J., Legube, G., Polo, S. E., Britton, S., and Jackson, S. P. (2010). Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. *Nat. Struct. Mol. Biol.* *17*, 1144–1151.
56. Wilson, M. D., and Durocher, D. (2017). Reading chromatin signatures after DNA double-strand breaks. *Philos. Trans. R. Soc. Lond. B, Biol. Sci.* *372*.
57. Panier, S., and Boulton, S. J. (2014). Double-strand break repair: 53BP1 comes into focus. *Nat. Rev. Mol. Cell Biol.* *15*, 7–18.
58. Gupta, R., Somyajit, K., Narita, T., Maskey, E., Stanlie, A., Kremer, M., Typas, D., Lammers, M., Mailand, N., Nussenzweig, A., et al. (2018). DNA repair network analysis reveals shieldin as a key regulator of NHEJ and PARP inhibitor sensitivity. *Cell* *173*, 972–988.e23.
59. Mirman, Z., Lottersberger, F., Takai, H., Kibe, T., Gong, Y., Takai, K., Bianchi, A., Zimmermann, M., Durocher, D., and de Lange, T. (2018). 53BP1-RIF1-shieldin counteracts DSB resection through CST- and Pol α -dependent fill-in. *Nature* *560*, 112–116.

60. Noordermeer, S. M., Adam, S., Setiাপutra, D., Barazas, M., Pettitt, S. J., Ling, A. K., Olivieri, M., Álvarez-Quilón, A., Moatti, N., Zimmermann, M., et al. (2018). The shieldin complex mediates 53BP1-dependent DNA repair. *Nature* 560, 117–121.
61. Ghezraoui, H., Oliveira, C., Becker, J. R., Bilham, K., Moralli, D., Anzilotti, C., Fischer, R., Deobagkar-Lele, M., Sanchiz-Calvo, M., Fueyo-Marcos, E., et al. (2018). 53BP1 cooperation with the REV7-shieldin complex underpins DNA structure-specific NHEJ. *Nature* 560, 122–127.
62. Gao, S., Feng, S., Ning, S., Liu, J., Zhao, H., Xu, Y., Shang, J., Li, K., Li, Q., Guo, R., et al. (2018). An OB-fold complex controls the repair pathways for DNA double-strand breaks. *Nat. Commun.* 9, 3925.
63. Dev, H., Chiang, T.-W. W., Lescale, C., de Krijger, I., Martin, A. G., Pilger, D., Coates, J., Sczaniecka-Clift, M., Wei, W., Ostermaier, M., et al. (2018). Shieldin complex promotes DNA end-joining and counters homologous recombination in BRCA1-null cells. *Nat. Cell Biol.* 20, 954–965.
64. Fradet-Turcotte, A., Canny, M. D., Escibano-Díaz, C., Orthwein, A., Leung, C. C. Y., Huang, H., Landry, M.-C., Kitevski-LeBlanc, J., Noordermeer, S. M., Sicheri, F., et al. (2013). 53BP1 is a reader of the DNA-damage-induced H2A Lys 15 ubiquitin mark. *Nature* 499, 50–54.
65. Wilson, M. D., Benlekbir, S., Fradet-Turcotte, A., Sherker, A., Julien, J.-P., McEwan, A., Noordermeer, S. M., Sicheri, F., Rubinstein, J. L., and Durocher, D. (2016). The structural basis of modified nucleosome recognition by 53BP1. *Nature* 536, 100–103.
66. Mattioli, F., Vissers, J. H. A., van Dijk, W. J., Ikpa, P., Citterio, E., Vermeulen, W., Marteijn, J. A., and Sixma, T. K. (2012). RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. *Cell* 150, 1182–1195.
67. Pesavento, J. J., Yang, H., Kelleher, N. L., and Mizzen, C. A. (2008). Certain and progressive methylation of histone H4 at lysine 20 during the cell cycle. *Mol. Cell. Biol.* 28, 468–486.
68. Saredi, G., Huang, H., Hammond, C. M., Alabert, C., Bekker-Jensen, S., Forne, I., Reverón-Gómez, N., Foster, B. M., Mlejnkova, L., Bartke, T., et al. (2016). H4K20me0 marks post-replicative chromatin and recruits the TONSL–MMS22L DNA repair complex. *Nature* 534, 714–718.
69. Hartlerode, A. J., Guan, Y., Rajendran, A., Ura, K., Schotta, G., Xie, A., Shah, J. V., and Scully, R. (2012). Impact of histone H4 lysine 20 methylation on 53BP1 responses to chromosomal double strand breaks. *PLoS One* 7, e49211.
70. Dulev, S., Tkach, J., Lin, S., and Batada, N. N. (2014). SET8 methyltransferase activity during the DNA double-strand break response is required for recruitment of 53BP1. *EMBO Rep.* 15, 1163–1174.

71. Pei, H., Zhang, L., Luo, K., Qin, Y., Chesi, M., Fei, F., Bergsagel, P. L., Wang, L., You, Z., and Lou, Z. (2011). MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites. *Nature* 470, 124–128.
72. Tuzon, C. T., Spektor, T., Kong, X., Congdon, L. M., Wu, S., Schotta, G., Yokomori, K., and Rice, J. C. (2014). Concerted activities of distinct H4K20 methyltransferases at DNA double-strand breaks regulate 53BP1 nucleation and NHEJ-directed repair. *Cell Rep.* 8, 430–438.
73. Hajdu, I., Ciccica, A., Lewis, S. M., and Elledge, S. J. (2011). Wolf-Hirschhorn syndrome candidate 1 is involved in the cellular response to DNA damage. *Proc. Natl. Acad. Sci. USA* 108, 13130–13134.
74. Svobodová Kovaříková, A., Legartová, S., Krejčí, J., and Bártová, E. (2018). H3K9me3 and H4K20me3 represent the epigenetic landscape for 53BP1 binding to DNA lesions. *Aging (Albany, NY)* 10, 2585–2605.
75. Khurana, S., Kruhlak, M. J., Kim, J., Tran, A. D., Liu, J., Nyswaner, K., Shi, L., Jailwala, P., Sung, M.-H., Hakim, O., et al. (2014). A macrohistone variant links dynamic chromatin compaction to BRCA1-dependent genome maintenance. *Cell Rep.* 8, 1049–1062.
76. Xu, C., Xu, Y., Gursoy-Yuzugullu, O., and Price, B. D. (2012). The histone variant macroH2A1.1 is recruited to DSBs through a mechanism involving PARP1. *FEBS Lett.* 586, 3920–3925.
77. Gong, F., Clouaire, T., Aguirrebengoa, M., Legube, G., and Miller, K. M. (2017). Histone demethylase KDM5A regulates the ZMYND8-NuRD chromatin remodeler to promote DNA repair. *J. Cell Biol.* 216, 1959–1974.
78. Chen, H., Ruiz, P. D., Novikov, L., Casill, A. D., Park, J. W., and Gamble, M. J. (2014). MacroH2A1.1 and PARP-1 cooperate to regulate transcription by promoting CBP-mediated H2B acetylation. *Nat. Struct. Mol. Biol.* 21, 981–989.
79. McGinty, R. K., Kim, J., Chatterjee, C., Roeder, R. G., and Muir, T. W. (2008). Chemically ubiquitylated histone H2B stimulates hDot1L-mediated intranucleosomal methylation. *Nature* 453, 812–816.
80. Altaf, M., Utley, R. T., Lacoste, N., Tan, S., Briggs, S. D., and Côté, J. (2007). Interplay of chromatin modifiers on a short basic patch of histone H4 tail defines the boundary of telomeric heterochromatin. *Mol. Cell* 28, 1002–1014.
81. Segala, G., Benesch, M. A., Pandey, D. P., Hulo, N., and Picard, D. (2016). Monoubiquitination of Histone H2B Blocks Eviction of Histone Variant H2A.Z from Inducible Enhancers. *Mol. Cell* 64, 334–346.
82. Zhu, B., Zheng, Y., Pham, A.-D., Mandal, S. S., Erdjument-Bromage, H., Tempst, P., and Reinberg, D. (2005). Monoubiquitination of human histone H2B: the factors involved and their roles in HOX gene regulation. *Mol. Cell* 20, 601–611.

83. Evangelista, F. M., Maglott-Roth, A., Stierle, M., Brino, L., Soutoglou, E., and Tora, L. (2018). Transcription and mRNA export machineries SAGA and TREX-2 maintain monoubiquitinated H2B balance required for DNA repair. *J. Cell Biol.* 217, 3382–3397.
84. Zheng, S., Li, D., Lu, Z., Liu, G., Wang, M., Xing, P., Wang, M., Dong, Y., Wang, X., Li, J., et al. (2018). Bre1-dependent H2B ubiquitination promotes homologous recombination by stimulating histone eviction at DNA breaks. *Nucleic Acids Res.*
85. Moyal, L., Lerenthal, Y., Gana-Weisz, M., Mass, G., So, S., Wang, S.-Y., Eppink, B., Chung, Y. M., Shalev, G., Shema, E., et al. (2011). Requirement of ATM-dependent monoubiquitylation of histone H2B for timely repair of DNA double-strand breaks. *Mol. Cell* 41, 529–542.
86. Nakamura, K., Kato, A., Kobayashi, J., Yanagihara, H., Sakamoto, S., Oliveira, D. V. N. P., Shimada, M., Tauchi, H., Suzuki, H., Tashiro, S., et al. (2011). Regulation of homologous recombination by RNF20-dependent H2B ubiquitination. *Mol. Cell* 41, 515–528.
87. Pfister, S. X., Ahrabi, S., Zalmas, L.-P., Sarkar, S., Aymard, F., Bachrati, C. Z., Helleday, T., Legube, G., La Thangue, N. B., Porter, A. C. G., et al. (2014). SETD2-dependent histone H3K36 trimethylation is required for homologous recombination repair and genome stability. *Cell Rep.* 7, 2006–2018.
88. Carvalho, S., Vítor, A. C., Sridhara, S. C., Martins, F. B., Raposo, A. C., Desterro, J. M. P., Ferreira, J., and de Almeida, S. F. (2014). SETD2 is required for DNA double-strand break repair and activation of the p53-mediated checkpoint. *Elife* 3, e02482.
89. Daugaard, M., Baude, A., Fugger, K., Povlsen, L. K., Beck, H., Sørensen, C. S., Petersen, N. H. T., Sorensen, P. H. B., Lukas, C., Bartek, J., et al. (2012). LEDGF (p75) promotes DNA-end resection and homologous recombination. *Nat. Struct. Mol. Biol.* 19, 803–810.
90. Wu, W., Nishikawa, H., Fukuda, T., Vittal, V., Asano, M., Miyoshi, Y., Klevit, R. E., and Ohta, T. (2015). Interaction of BARD1 and HP1 is required for BRCA1 retention at sites of DNA damage. *Cancer Res.* 75, 1311–1321.
91. Leung, J. W. C., Makharashvili, N., Agarwal, P., Chiu, L.-Y., Pourpre, R., Cammarata, M. B., Cannon, J. R., Sherker, A., Durocher, D., Brodbelt, J. S., et al. (2017). ZMYM3 regulates BRCA1 localization at damaged chromatin to promote DNA repair. *Genes Dev.* 31, 260–274.
92. Pellegrino, S., Michelena, J., Teloni, F., Imhof, R., and Altmeyer, M. (2017). Replication-Coupled Dilution of H4K20me2 Guides 53BP1 to Pre-replicative Chromatin. *Cell Rep.* 19, 1819–1831.
93. Tang, J., Cho, N. W., Cui, G., Manion, E. M., Shanbhag, N. M., Botuyan, M. V., Mer, G., and Greenberg, R. A. (2013). Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination. *Nat. Struct. Mol. Biol.* 20, 317–325.

94. Jacquet, K., Fradet-Turcotte, A., Avvakumov, N., Lambert, J.-P., Roques, C., Pandita, R. K., Paquet, E., Herst, P., Gingras, A.-C., Pandita, T. K., et al. (2016). The TIP60 Complex Regulates Bivalent Chromatin Recognition by 53BP1 through Direct H4K20me Binding and H2AK15 Acetylation. *Mol. Cell* 62, 409–421.
95. Adkins, N. L., Niu, H., Sung, P., and Peterson, C. L. (2013). Nucleosome dynamics regulates DNA processing. *Nat. Struct. Mol. Biol.* 20, 836–842.
96. Mimitou, E. P., Yamada, S., and Keeney, S. (2017). A global view of meiotic double-strand break end resection. *Science* 355, 40–45.
97. Rother, M. B., and van Attikum, H. (2017). DNA repair goes hip-hop: SMARCA and CHD chromatin remodellers join the break dance. *Philos. Trans. R. Soc. Lond. B, Biol. Sci.* 372.
98. Chiu, L.-Y., Gong, F., and Miller, K. M. (2017). Bromodomain proteins: repairing DNA damage within chromatin. *Philos. Trans. R. Soc. Lond. B, Biol. Sci.* 372.
99. Gursoy-Yuzugullu, O., House, N., and Price, B. D. (2016). Patching broken DNA: nucleosome dynamics and the repair of DNA breaks. *J. Mol. Biol.* 428, 1846–1860.
100. Densham, R. M., and Morris, J. R. (2017). The BRCA1 Ubiquitin ligase function sets a new trend for remodelling in DNA repair. *Nucleus* 8, 116–125.
101. Densham, R. M., Garvin, A. J., Stone, H. R., Strachan, J., Baldock, R. A., Daza-Martin, M., Fletcher, A., Blair-Reid, S., Beesley, J., Johal, B., et al. (2016). Human BRCA1-BARD1 ubiquitin ligase activity counteracts chromatin barriers to DNA resection. *Nat. Struct. Mol. Biol.* 23, 647–655.
102. Chen, C.-C., Carson, J. J., Feser, J., Tamburini, B., Zabaronick, S., Linger, J., and Tyler, J. K. (2008). Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. *Cell* 134, 231–243.
103. Tsukuda, T., Fleming, A. B., Nickoloff, J. A., and Osley, M. A. (2005). Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*. *Nature* 438, 379–383.
104. Chen, X., Cui, D., Papusha, A., Zhang, X., Chu, C.-D., Tang, J., Chen, K., Pan, X., and Ira, G. (2012). The Fun30 nucleosome remodeller promotes resection of DNA double-strand break ends. *Nature* 489, 576–580.
105. Costelloe, T., Louge, R., Tomimatsu, N., Mukherjee, B., Martini, E., Khadaroo, B., Dubois, K., Wiegant, W. W., Thierry, A., Burma, S., et al. (2012). The yeast Fun30 and human SMARCA1 chromatin remodellers promote DNA end resection. *Nature* 489, 581–584.
106. Courilleau, C., Chailleux, C., Jauneau, A., Grimal, F., Briois, S., Boutet-Robinet, E., Boudsocq, F., Trouche, D., and Canitrot, Y. (2012). The chromatin remodeler p400

- ATPase facilitates Rad51-mediated repair of DNA double-strand breaks. *J. Cell Biol.* **199**, 1067–1081.
107. Batenburg, N. L., Walker, J. R., Noordermeer, S. M., Moatti, N., Durocher, D., and Zhu, X.-D. (2017). ATM and CDK2 control chromatin remodeler CSB to inhibit RIF1 in DSB repair pathway choice. *Nat. Commun.* **8**, 1921.
 108. Huang, T.-H., Fowler, F., Chen, C.-C., Shen, Z.-J., Sleckman, B., and Tyler, J. K. (2018). The Histone Chaperones ASF1 and CAF-1 Promote MMS22L-TONSL-Mediated Rad51 Loading onto ssDNA during Homologous Recombination in Human Cells. *Mol. Cell* **69**, 879–892.e5.
 109. Piwko, W., Mlejnkova, L. J., Mutreja, K., Ranjha, L., Stafa, D., Smirnov, A., Brodersen, M. M., Zellweger, R., Sturzenegger, A., Janscak, P., et al. (2016). The MMS22L-TONSL heterodimer directly promotes RAD51-dependent recombination upon replication stress. *EMBO J.* **35**, 2584–2601.
 110. Duro, E., Lundin, C., Ask, K., Sanchez-Pulido, L., MacArtney, T. J., Toth, R., Ponting, C. P., Groth, A., Helleday, T., and Rouse, J. (2010). Identification of the MMS22L-TONSL complex that promotes homologous recombination. *Mol. Cell* **40**, 632–644.
 111. O'Donnell, L., Panier, S., Wildenhain, J., Tkach, J. M., Al-Hakim, A., Landry, M.-C., Escribano-Diaz, C., Szilard, R. K., Young, J. T. F., Munro, M., et al. (2010). The MMS22L-TONSL complex mediates recovery from replication stress and homologous recombination. *Mol. Cell* **40**, 619–631.
 112. Adkins, N. L., Swygert, S. G., Kaur, P., Niu, H., Grigoryev, S. A., Sung, P., Wang, H., and Peterson, C. L. (2017). Nucleosome-like, Single-stranded DNA (ssDNA)-Histone Octamer Complexes and the Implication for DNA Double Strand Break Repair. *J. Biol. Chem.* **292**, 5271–5281.
 113. Zhou, Y., Caron, P., Legube, G., and Paull, T. T. (2014). Quantitation of DNA double-strand break resection intermediates in human cells. *Nucleic Acids Res.* **42**, e19.
 114. Cohen, S., Puget, N., Lin, Y.-L., Clouaire, T., Aguirrebengoa, M., Rocher, V., Pasero, P., Canitrot, Y., and Legube, G. (2018). Senataxin resolves RNA:DNA hybrids forming at DNA double-strand breaks to prevent translocations. *Nat. Commun.* **9**, 533.
 115. Lademann, C. A., Renkawitz, J., Pfander, B., and Jentsch, S. (2017). The INO80 Complex Removes H2A.Z to Promote Presynaptic Filament Formation during Homologous Recombination. *Cell Rep.* **19**, 1294–1303.
 116. Alatwi, H. E., and Downs, J. A. (2015). Removal of H2A.Z by INO80 promotes homologous recombination. *EMBO Rep.* **16**, 986–994.
 117. Juhász, S., Elbakry, A., Mathes, A., and Löbrich, M. (2018). ATRX Promotes DNA Repair Synthesis and Sister Chromatid Exchange during Homologous Recombination. *Mol. Cell* **71**, 11–24.e7.

118. Oberdoerffer, P., Michan, S., McVay, M., Mostoslavsky, R., Vann, J., Park, S.-K., Hartlerode, A., Stegmüller, J., Hafner, A., Loerch, P., et al. (2008). SIRT1 redistribution on chromatin promotes genomic stability but alters gene expression during aging. *Cell* *135*, 907–918.
119. Chou, D. M., Adamson, B., Dephoure, N. E., Tan, X., Nottke, A. C., Hurov, K. E., Gygi, S. P., Colaiácovo, M. P., and Elledge, S. J. (2010). A chromatin localization screen reveals poly (ADP ribose)-regulated recruitment of the repressive polycomb and NuRD complexes to sites of DNA damage. *Proc. Natl. Acad. Sci. USA* *107*, 18475–18480.
120. Baldeyron, C., Soria, G., Roche, D., Cook, A. J. L., and Almouzni, G. (2011). HP1 α recruitment to DNA damage by p150CAF-1 promotes homologous recombination repair. *J. Cell Biol.* *193*, 81–95.
121. Luijsterburg, M. S., Dinant, C., Lans, H., Stap, J., Wiernasz, E., Lagerwerf, S., Warmerdam, D. O., Lindh, M., Brink, M. C., Dobrucki, J. W., et al. (2009). Heterochromatin protein 1 is recruited to various types of DNA damage. *J. Cell Biol.* *185*, 577–586.
122. Ayrappetov, M. K., Gursoy-Yuzugullu, O., Xu, C., Xu, Y., and Price, B. D. (2014). DNA double-strand breaks promote methylation of histone H3 on lysine 9 and transient formation of repressive chromatin. *Proc. Natl. Acad. Sci. USA* *111*, 9169–9174.
123. Ayoub, N., Jeyasekharan, A. D., Bernal, J. A., and Venkitaraman, A. R. (2008). HP1- β mobilization promotes chromatin changes that initiate the DNA damage response. *Nature* *453*, 682–686.
124. Campbell, S., Ismail, I. H., Young, L. C., Poirier, G. G., and Hendzel, M. J. (2013). Polycomb repressive complex 2 contributes to DNA double-strand break repair. *Cell Cycle* *12*, 2675–2683.
125. Chandler, H., Patel, H., Palermo, R., Brookes, S., Matthews, N., and Peters, G. (2014). Role of polycomb group proteins in the DNA damage response--a reassessment. *PLoS One* *9*, e102968.
126. Young, L. C., McDonald, D. W., and Hendzel, M. J. (2013). Kdm4b histone demethylase is a DNA damage response protein and confers a survival advantage following γ -irradiation. *J. Biol. Chem.* *288*, 21376–21388.
127. Lee, Y.-H., Kuo, C.-Y., Stark, J. M., Shih, H.-M., and Ann, D. K. (2013). HP1 promotes tumor suppressor BRCA1 functions during the DNA damage response. *Nucleic Acids Res.* *41*, 5784–5798.
128. Strom, A. R., Emelyanov, A. V., Mir, M., Fyodorov, D. V., Darzacq, X., and Karpen, G. H. (2017). Phase separation drives heterochromatin domain formation. *Nature* *547*, 241–245.

129. Larson, A. G., Elnatan, D., Keenen, M. M., Trnka, M. J., Johnston, J. B., Burlingame, A. L., Agard, D. A., Redding, S., and Narlikar, G. J. (2017). Liquid droplet formation by HP1 α suggests a role for phase separation in heterochromatin. *Nature* 547, 236–240.
130. D'Alessandro, G., and d'Adda di Fagagna, F. (2017). Transcription and DNA damage: holding hands or crossing swords? *J. Mol. Biol.* 429, 3215–3229.
131. Shanbhag, N. M., Rafalska-Metcalf, I. U., Balane-Bolivar, C., Janicki, S. M., and Greenberg, R. A. (2010). ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. *Cell* 141, 970–981.
132. Kakarougkas, A., Ismail, A., Chambers, A. L., Riballo, E., Herbert, A. D., Künzel, J., Löbrich, M., Jeggo, P. A., and Downs, J. A. (2014). Requirement for PBAF in transcriptional repression and repair at DNA breaks in actively transcribed regions of chromatin. *Mol. Cell* 55, 723–732.
133. Ui, A., Nagaura, Y., and Yasui, A. (2015). Transcriptional elongation factor ENL phosphorylated by ATM recruits polycomb and switches off transcription for DSB repair. *Mol. Cell* 58, 468–482.
134. Awwad, S. W., Abu-Zhayia, E. R., Guttmann-Raviv, N., and Ayoub, N. (2017). NELF-E is recruited to DNA double-strand break sites to promote transcriptional repression and repair. *EMBO Rep.* 18, 745–764.
135. Pankotai, T., Bonhomme, C., Chen, D., and Soutoglou, E. (2012). DNAPKcs-dependent arrest of RNA polymerase II transcription in the presence of DNA breaks. *Nat. Struct. Mol. Biol.* 19, 276–282.
136. Abu-Zhayia, E. R., Awwad, S. W., Ben-Oz, B. M., Khoury-Haddad, H., and Ayoub, N. (2018). CDYL1 fosters double-strand break-induced transcription silencing and promotes homology-directed repair. *J. Mol. Cell Biol.* 10, 341–357.
137. Gong, F., Chiu, L.-Y., Cox, B., Aymard, F., Clouaire, T., Leung, J. W., Cammarata, M., Perez, M., Agarwal, P., Brodbelt, J. S., et al. (2015). Screen identifies bromodomain protein ZMYND8 in chromatin recognition of transcription-associated DNA damage that promotes homologous recombination. *Genes Dev.* 29, 197–211.
138. Wang, P., Byrum, S., Fowler, F. C., Pal, S., Tackett, A. J., and Tyler, J. K. (2017). Proteomic identification of histone post-translational modifications and proteins enriched at a DNA double-strand break. *Nucleic Acids Res.* 45, 10923–10940.
139. Szydlowski, N. A., Go, J. S., and Hu, Y. S. (2018). Chromatin imaging and new technologies for imaging the nucleome. *Wiley Interdiscip Rev Syst Biol Med*, e1442.
140. Chang, H. H. Y., Pannunzio, N. R., Adachi, N., and Lieber, M. R. (2017). Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat. Rev. Mol. Cell Biol.* 18, 495–506.

141. Wright, W. D., Shah, S. S., and Heyer, W.-D. (2018). Homologous recombination and the repair of DNA double-strand breaks. *J. Biol. Chem.* *293*, 10524–10535.
142. Talbert, P. B., and Henikoff, S. (2017). Histone variants on the move: substrates for chromatin dynamics. *Nat. Rev. Mol. Cell Biol.* *18*, 115–126.
143. Soshnev, A. A., Josefowicz, S. Z., and Allis, C. D. (2016). Greater than the sum of parts: complexity of the dynamic epigenome. *Mol. Cell* *62*, 681–694.
144. Rowley, M. J., and Corces, V. G. (2018). Organizational principles of 3D genome architecture. *Nat. Rev. Genet.* *19*, 789–800.
145. Mladenova, V., Mladenov, E., and Iliakis, G. (2016). Novel biological approaches for testing the contributions of single dsbs and DSB clusters to the biological effects of high LET radiation. *Front. Oncol.* *6*, 163.
146. Brinkman, E. K., Chen, T., de Haas, M., Holland, H. A., Akhtar, W., and van Steensel, B. (2018). Kinetics and Fidelity of the Repair of Cas9-Induced Double-Strand DNA Breaks. *Mol. Cell* *70*, 801–813.e6.

A

initial chromatin landscape



Chromatin impediment/Nuclear compartment specificities
Recruitment of «Reader» Repair Proteins



DSB-induced chromatin landscape

Genomic distribution of chromatin changes
ChIP, ChIP-seq and other high throughput genomics



NHEJ-associated chromatin changes promoting:

1. accessibility



2. inhibition of resection/ligation



HR-associated chromatin changes promoting:

1. accessibility



2. Resection



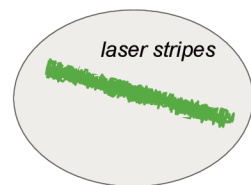
3. homology search,
template strand invasion
DNA synthesis and
chromatin reassembly



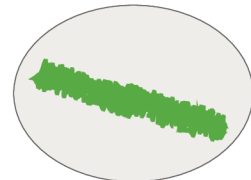
B

Dynamics in chromatin behaviour

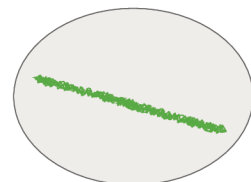
At sites of localised microirradiation



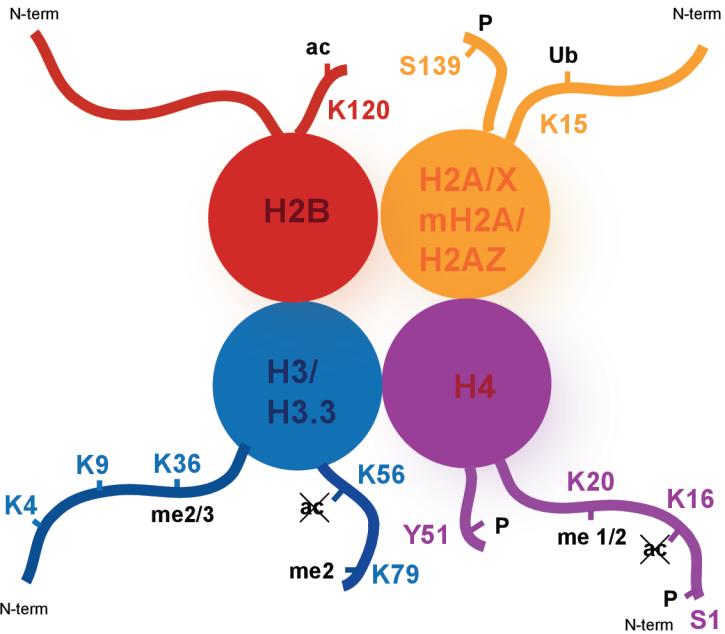
relaxation



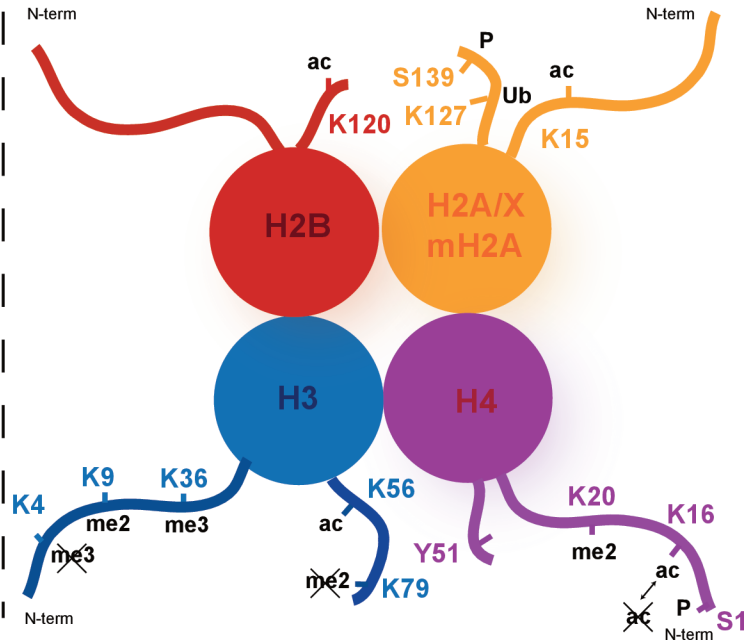
compaction



NHEJ Nucleosome

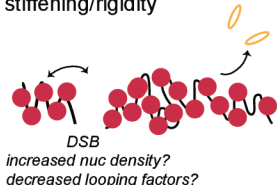


HR Nucleosome

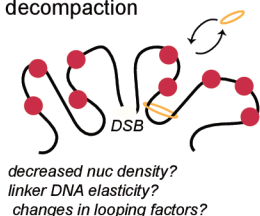


Chromatin Promoting Mobility (?)

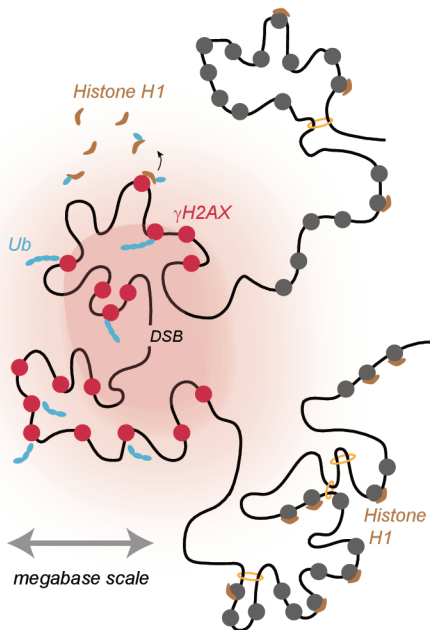
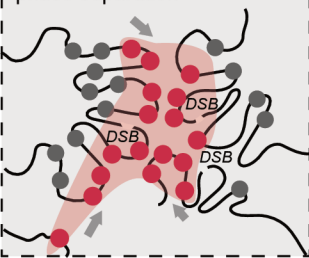
stiffening/rigidity

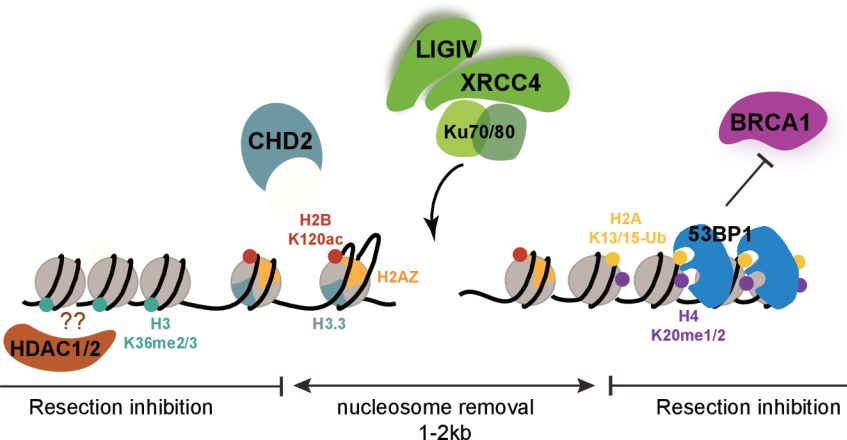


decompaction

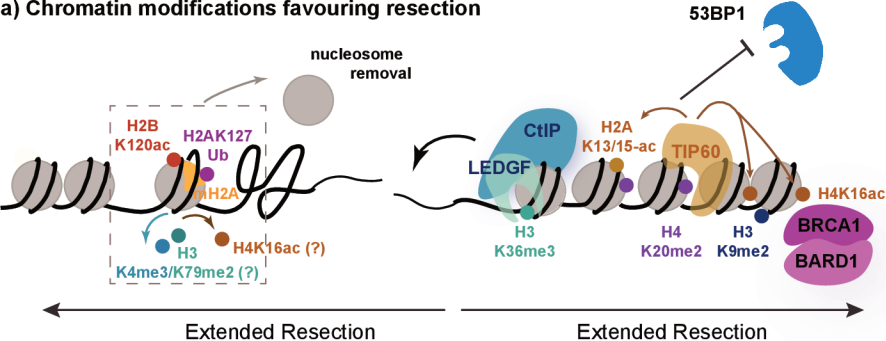


phase separation

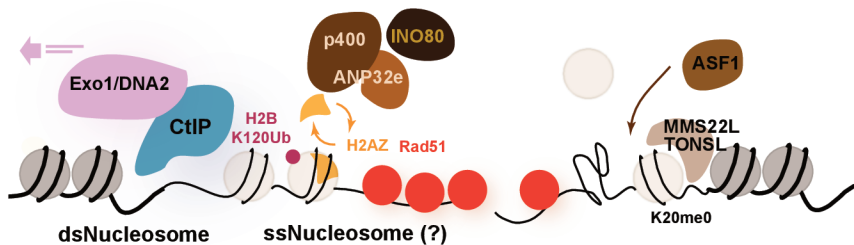




a) Chromatin modifications favouring resection



b) Single Strand nucleosome favouring Rad51 loading (??)



c) DNA synthesis coupled nucleosome reassembly

