DNA Double Strand Break Repair and Its Control by Nucleosome Remodeling

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DNA double strand breaks (DSBs) are repaired in eukaryotes by one of several cellular mechanisms. The decision-making process controlling DSB repair takes place at the step of DNA end resection, the nucleolytic processing of DNA ends, which generates single-stranded DNA overhangs. Dependent on the length of the overhang, a corresponding DSB repair mechanism is engaged. Interestingly, nucleosomes—the fundamental unit of chromatin—influence the activity of resection nucleases and nucleosome remodelers have emerged as key regulators of DSB repair. Nucleosome remodelers share a common enzymatic mechanism, but for global genome organization specific remodelers have been shown to exert distinct activities. Specifically, different remodelers have been found to slide and evict, position or edit nucleosomes. It is an open question whether the same remodelers exert the same function also in the context of DSBs. Here, we will review recent advances in our understanding of nucleosome remodelers at DSBs: to what extent nucleosome sliding, eviction, positioning and editing can be observed at DSBs and how these activities affect the DSB repair decision.

Keywords: nucleosome remodeling, double strand break, DNA repair, DNA end resection, cell cycle, genome stability

INTRODUCTION

DNA double strand breaks (DSBs) are a highly toxic form of DNA damage, arising from intrinsic and extrinsic sources (Ciccia and Elledge, 2010). Eukaryotes are equipped with several mechanisms to repair DSBs, including non-homologous end joining (NHEJ), alternative end joining (alt-EJ), homologous recombination (HR) and single strand annealing (SSA) (Chang et al., 2017; Ranjha et al., 2018). Notably, these pathways do not only have different prerequisites (for example HR requiring a homologous donor sequence), but they also differ in the repair outcome and the potential to introduce genetic changes (such as mutations and chromosomal rearrangements). The cellular repair pathway decision is therefore critical for the survival of the affected cell or organism as well as for the stability of its genome (Symington and Gautier, 2011). Moreover, the fact that DSB repair is controlled by endogenous factors is a major limitation for genome editing strategies, which can nowadays involve efficient delivery of DSBs at the gene of interest, but often lead to a heterogenous outcome of the genome editing reaction across cell populations.

The cellular DSB repair pathway decision is made at the step of DNA end resection, the nucleolytic processing of DSB ends (Symington and Gautier, 2011; Cejka, 2015; Daley et al., 2015; Symington, 2016; Bonetti et al., 2018). Resection involves endo- and exonucleolytic cleavage of DNA ends that reveals 3' single-stranded DNA overhangs. Notably, resection destroys the substrate for repair by NHEJ and increasing amounts of 3' single-stranded DNA (ssDNA) predisposes for repair...
by different mechanisms (alt EJ < HR < SSA, Blier et al., 1993; Falzon et al., 1993; Ira et al., 2004). The enzymatic process of resection has been subject of excellent reviews in this issue and elsewhere (Symington and Gautier, 2011; Cejka and Symington, 2021; Elbakry and Löbrich, 2021; Sanchez et al., 2021). Here we focus on how resection and thereby the repair pathway decision is regulated by nucleosomes and nucleosome remodelers, enzymes that can evict, position and edit nucleosomes. For general reviews on how DNA damage triggers post-translational histone modifications, we refer to the following articles (Smeenk and van Attikum, 2013; Van and Santos, 2018).

Nucleosomes form obstacles to the resection nucleases (Figure 1). Initial short-range resection is carried out by the Mre11-complex (Mre11-C in the following, consisting of Mre11-Rad50-Xrs2 with the Sae2 activator in budding yeast, and analogously of MRE11-RAD50-NBS1 with CtIP in human) (Symington and Gautier, 2011; Cejka and Symington, 2021; Elbakry and Löbrich, 2021; Sanchez et al., 2021). Here we focus on how resection and thereby the repair pathway decision is regulated by nucleosomes and nucleosome remodelers, enzymes that can evict, position and edit nucleosomes. For general reviews on how DNA damage triggers post-translational histone modifications, we refer to the following articles (Smeenk and van Attikum, 2013; Van and Santos, 2018).

![Diagram of DNA end resection in the chromatin context](Image)

**FIGURE 1 |** Eukaryotic DNA end resection in the chromatin context. DNA end resection is a two step process that can be divided into short-range resection (orange) and long-range resection (yellow). Mre11-C initiates short-range resection by nicking the 5′-terminated strand in proximity to the DSB via its endonuclease activity. Then, Mre11-C generates a short 3′ ssDNA overhang close to the DSB using its 3′-5′ exonuclease function. Exo1 and STR-Dna2 carry out long-range resection and extend the length of the resected ssDNA tract through chromatin.

In many eukaryotes, DNA end resection is carried out by three resection enzymes, Mre11-C, STR-Dna2 and Exo1, which act specifically at one of the two stages of the resection process (short-range resection/resection initiation and long-range resection/resection elongation, Figure 1). Notably, all three act by distinct molecular mechanisms and it is therefore unsurprising that nucleosomes have distinct effects on each of them. Mre11-C recognizes the DSB end either directly or through a DSB end-binding protein (most likely the end-binding factor Ku) and, after activation by Sae2/CtIP, induces a single-strand break on the 5′-strand (Sartori et al., 2007; Cannavo and Cejka, 2014; Anand et al., 2016; Deshpande et al., 2016; Reginato et al., 2017; Wang et al., 2017). From this point, bidirectional resection occurs: Mre11-C catalyzes 3′-5′ exonucleolytic resection towards the break, while Dna2 and Exo1 exonucleases catalyze long-range resection with 5′-3′ polarity into undamaged chromatin (Mimitou and Symington, 2008; Zhu et al., 2008; Cejka et al., 2010; Niu et al., 2010; Garcia et al., 2011; Shibata et al., 2014).

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Preferential cleavage of linker DNA indicates that nucleosomal DNA may be refractory to endonucleolytic clipping by Mre11-C (Mimitou et al., 2017; Wang et al., 2017). However, nucleosomes per se are not a barrier to Mre11-C. Rather, it can slide or reach over nucleosomes (Myler et al., 2017; Wang et al., 2017). In cases where such bypass occurs, the nucleosome located between DSB and incision site could then potentially constitute a barrier to the 3′-5′ exonuclease activity of Mre11-C. Given the dual

**RESECTION IS AFFECTED BY NUCLEOSOMES**

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end- and exonucleolytic activities of Mre11-C, this question has so far been difficult to address.

Long-range resection enzymes are even more strongly affected by the presence of nucleosomes. For example, in vitro studies have shown that Exo1 cannot resect through nucleosomes (Adkins et al., 2013), suggesting that additional activities are needed to overcome the chromatin barrier. Interestingly, changing nucleosome composition may be sufficient to allow Exo1-mediated resection. Incorporation of the H2A-variant H2A.Z decreases nucleosome stability and increases accessibility of nucleosomal DNA (Abbott et al., 2001; Zhang et al., 2005; Jin and Felsenfeld, 2007; Adkins et al., 2013; Watanabe et al., 2013; Lewis et al., 2021), which may allow Exo1 to bypass the nucleosomal barrier (Adkins et al., 2013).

In contrast to Exo1, the other long-range resection enzyme STR-Dna2 is in principle able to bypass nucleosomes. This may be due to a different enzymatic mechanism. While during long-range resection STR-Dna2 has the net effect of an exonuclease, STR-Dna2 utilizes the combined action of the Sgs1 helicase that unwinds the DNA, followed by endonucleolytic cleavage of the emerging flap structure by Dna2 (Cejka et al., 2010; Niu et al., 2010). Apparently, the Sgs1 helicase motor is powerful enough to disrupt nucleosomes, allowing STR-Dna2 to resect nucleosomal DNA (Adkins et al., 2013). However, in order to carry out resection of nucleosomal DNA, STR-Dna2 will need as much as 300 bp of free DNA to be able to traverse through nucleosomes (Adkins et al., 2013). This distance is greater than the nucleosomal linker DNA-length and, consistently, STR-Dna2 is effectively inhibited by a nucleosomal array (Adkins et al., 2013).

Therefore, both long-range resection enzymes are blocked by chromatin and will require the activity of additional factors. One factor that could help to overcome the nucleosomal barrier is Mre11-C. Speculatively, Mre11-C could catalyze further endonucleolytic incisions downstream of the nucleosome from which long-range nucleases could (re-)initiate and thereby allow to bypass the nucleosome barrier. Currently, such an auxiliary role of Mre11-C in long-range resection lacks experimental support, but recent data suggest that short-range and long-range resection nucleases work in a coordinated fashion (Ceppi et al., 2020).

Alternatively, resection enzymes will need assistance by chromatin remodelers to get past nucleosomes and it is therefore important to consider how these enzymes may be able to modify the nucleosome barrier.

REMODELERS HAVE DISTINCT ROLES IN CHROMATIN ORGANIZATION

Eukaryotes express several nucleosome remodelers (Flaus et al., 2006) and chromatin immunoprecipitation (ChIP) and related techniques have localized several of them to DSBs (Bantele et al., 2017; Bennett and Peterson, 2015; Bennett et al., 2013; Bird et al., 2002; Chai et al., 2005; Chen et al., 2012; Costelloe et al., 2012; Downs et al., 2004; Eapen et al., 2012; Gnugnoli et al., 2021; Lademann et al., 2017; Morrison et al., 2004; Shim et al., 2005; 2007; Tsukuda et al., 2005; van Attikum et al., 2004; 2007). This raises the question, whether these remodelers have distinct functions at DSBs or whether they act redundantly.

Nucleosome remodelers are found to be either single protein enzymes or multi-protein complexes. Historically, four major sub-families of remodelers have been proposed (Clapier and Cairns, 2009), but phylogenetic analysis based on sequence conservation of the catalytic ATPase subunits showed the existence of additional sub-families (Flaus et al., 2006). Five sub-families are found throughout eukaryotes - ISWI, SWI/SNF, CHD1, INO80 and Fan30/ETL. In contrast, ALC1, CHD7 and Mi2/NURD sub-families are not found throughout eukaryotes, with ALC1 and CHD7 orthologues specifically found in metazoans (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998; Ma et al., 2008; Bouazoune and Kingston, 2012). Table 1 summarizes the different remodeler sub-families with their putative catalytic activities and involvement in DSB repair.

It seems expedient to group remodelers not only by evolutionary conservation, but also by functional similarity (Figure 2, Table 1). In vitro and in vivo we can discriminate at least three activities of nucleosome remodelers: 1) sliding/eviction leads to movement of nucleosomes along DNA that can even result in the removal of the entire nucleosome (Figure 2A); 2) positioning involves movement of nucleosomes to form regularly spaced nucleosomal arrays (Figure 2B); 3) editing involves the exchange of histones (commonly H2A-H2B dimers) to alter the composition of nucleosomes (Clapier and Cairns, 2009; Clapier et al., 2017). Based on studies of genome-wide chromatin organization, we currently think that SWI/SNF sub-family complexes (SWI/SNF and RSC in yeast) act as major sliding/eviction enzymes, that ISWI and CHD1 sub-family remodelers as well as INO80-C act as positioning enzymes and that INO80 sub-family complexes (SWR1 and INO80 in yeast) catalyze editing (Table 1, Clapier and Cairns, 2009; Clapier et al., 2017). In the following, we will investigate whether nucleosome remodelers carry out the same activities at DSBs.

NUCLEOSOME EVICTION AND RESECTION ARE COUPLED

With nucleosomes forming a barrier to resection, nucleosome eviction is the most straight-forward solution to allow spreading of resection into chromatin (Figure 3). Indeed, nucleosomes are lost around DSBs in the region where resection occurs (Bantele and Pfander, 2019; Chen et al., 2008; Mimitou et al., 2017; Tsukuda et al., 2005; 2009; van Attikum et al., 2007). While it was proposed that nucleosomes may associate in some form with resected, single-stranded DNA to form single-stranded nucleosomes (Adkins et al., 2017; Huang et al., 2018), a dedicated study did not find evidence to support wide-spread association of nucleosomes with single-stranded DNA in vivo (Peritore et al., 2021). But how do nucleosomes become evicted and how do sliding/evicting nucleosome remodelers of the SWI/SNF sub-family facilitate this eviction (Figure 3A)? In budding yeast, the SWI/SNF and RSC complexes are specifically recruited to DSBs (Chai et al., 2005; Shim et al., 2005, 2007; Kent et al.,
RSC complexes playing a major role as nucleosome evictors also coupled. Altogether, these data are consistent with SWI/SNF and RSC are redundantly required for DNA end resection degradation of the ATPase subunits of both SWI/SNF and Snf2-like: The SWI/SNF (switch/sucrose non-fermentable) sub-family consists of two members in budding yeast - SWI/SNF and RSC (remodels the structure of chromatin) - as well as in human – BAF and PBAF. For human BAF variant complexes can be found harbouring ATPase subunit paralogues (Mittal and Roberts, 2020). The ISWI (innate switching with SWI/SNF) sub-family in yeast contains 3 active complexes - Islw1a, Islw1b, Islw2 - that combine 2 different catalytic subunits - Islw1 and Islw2 - with different sets of proteins. For humans the setup with 2 catalytic subunits is similar, but with a higher number of different complexes: ACF, CHRFC, NoRC, RSF, WICH, NURF, CERF (Aldrich et al., 2014). The CHD (chromodomain helicase DNA-binding) sub-family has a single member in yeast – Chd1 - and 3 subfamilies with in total 9 members in human: CHD1-2, CHD3-5 – forming NuRD/Me-2 complex and CHD6-9 (Martella and Imbattino, 2007). The ALC1 sub-family carries a macrodomain for poly(ADP-ribose)-binding instead of a chromodomain and is found in human (Ahel et al., 2009).

TABLE 1 | Overview of nucleosome remodeler sub-families and their members.

| Family | Sub-family | Putative activity | S. cerevisiae | H. sapiens orthologues | Function at DSBs |
|--------|------------|-------------------|---------------|------------------------|------------------|
| Snf2- | Snf2-like | Nucleosome sliding/eviction | SWI/SNF RSC | BAF PBAF | Delamarre et al. (2020), Hys et al. (2020), Hu et al. (2020), Kakaroukhas et al. (2014), Kent et al. (2007), Lee et al. (2010), Miesenbarg et al. (2019), Ogawa et al. (2011), Peng et al. (2009), Peritore et al. (2021), Qi et al. (2016), Shim et al. (2005), Shim et al. (2007), Uti et al. (2014), Watanabe et al. (2014), Wiest et al. (2017) |
| ISWI | Nucleosome positioning | Islw1a | ACF CHRFC | Delamarre et al. (2020), Casari et al. (2021), Delamarre et al. (2020), Heftie et al. (2013), Lan et al. (2010), Nakamura et al. (2011), Pessina and Lowndes, (2014), Sánchez-Molina et al. (2011), Sheu et al. (2010), Smeenk et al. (2012), Toiber et al. (2013), Vidi et al. (2014), Xiao et al. (2009) |
| ISWI | Nucleosome positioning | Islw1b | NoRC RSF | |
| ISWI | Nucleosome positioning | Islw2 | WICH NURF CERF | |
| CHD-I | Nucleosome positioning | Chd1 | CHD1, CHD2 | Chou et al. (2010), Goodarzi et al. (2011), Larsen et al. (2010), Lujisterburt et al. (2016), Pan et al. (2012), Polo et al. (2010), Qi et al. (2016), Smeenk et al. (2010), Smith et al. (2018), Spruijt et al. (2016) |
| CHD-II | Nucleosome positioning | Chd2 | CHD6, CHD7, CHD8, CHD9 | Rother et al. (2020) |
| CHD-III | Nucleosome positioning | Chd3 | - | |
| ALC1 | ALC1-like | Nucleosome editing | INO80 | ALC1 | Ahel et al. (2009), Blessing et al. (2020), Juhász et al. (2020), Selou et al. (2016) |
| Swr1 | INO80 | Nucleosome positioning | INO80 | INO80 | Adkins et al. (2013), Alatwi and Downs, (2015), Bennett et al. (2013), Brahma et al. (2017), Chen et al. (2012), Downs et al. (2004), Kálosca et al. (2008), Lademann et al. (2017), Marillo-Huesca et al. (2010), Morrison et al. (2004), Oberbeckmann et al. (2021b), Papamichos-Chronakis et al. (2006), Tsukuda et al. (2005), van Attikum et al. (2004), van Attikum et al. (2007) |
| Swr1 | INO80 | Nucleosome positioning | SWR1 | SRCAP TRAPP/Tip60 | |
| Fun30/ | ETL | Nucleosome editing | Fun30 | SMARCAD1 | Bantele et al. (2017), Chen et al. (2012), Costellio et al. (2012), Dernsham et al. (2016), Eapen et al. (2012) |

Nucleosome remodelers are grouped into two families based on conservation of the ATPase subunit. Snf2-like and Swr1-like. Both families have several sub-families.

Swr1-like: The INO80 (inositol requiring) sub-family has two members in yeast: INO80 and SWR1. In humans again there is additional complexity of this sub-family with INO80, SRCAP and Chd1 - and 3 subfamilies with in total 9 members in human: CHD1-2, CHD3-5 – forming NuRD/Me-2 complex and CHD6-9 (Martella and Imbattino, 2007). The ALC1 sub-family carries a macrodomain for poly(ADP-ribose)-binding instead of a chromodomain and is found in human (Ahel et al., 2009).

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While its recruitment kinetics are therefore similar to those of Swr1-like, it becomes recruited to DSBs within 10 min of its occurrence Mre11-C as well as resection initiation. Whether SWI/SNF or RSC in influence Mre11-C activity, endonucleolytic clipping in particular, remains to be tested.

These findings raise the question of whether these nucleosome remodelers use the presence of a DSB and become recruited to DSB-proximal chromatin at such an early stage. Notably, RSC and SWI/SNF localize to the proximty of DSBs independently of each other and follow different recruitment kinetics, suggesting that they recognize DSBs by different mechanisms. RSC is recruited to a DSB within 10 min and thereby precedes resection initiation (Chai et al., 2005). While its recruitment kinetics are therefore similar to those of Mre11-C (Shim et al., 2007), we currently do not understand which signal is being recognized by RSC. SWI/SNF in contrast shows significantly slower recruitment (Chai et al., 2005) that depends on nucleosome modifications. Specificlly, histone...
Acetylation is recognized by SWI/SNF and appears to lead to its DSB recruitment, consistent with the presence of several acetylation-binding bromodomains in the SWI/SNF complex (Bennett and Peterson, 2015; Cheng et al., 2021). Notably, the histone acetyltransferase NuA4 is specifically recruited to DSBs and this recruitment was shown to depend on Mre11-C (Cheng et al., 2021). The fact that SWI/SNF was found to be required for recruitment of Mre11-C, but at the same time also dependent on Mre11-C activity (Shim et al., 2005, 2007; Wiest et al., 2017) is not necessarily a contradiction, but could suggest the presence of a positive feedback loop that promotes resection initiation.

Biochemical data suggest that long-range resection should be particularly dependent on nucleosome eviction (Adkins et al., 2013). Consistently, SWI/SNF appears to stimulate long range resection (Wiest et al., 2017), but this has yet to be correlated with nucleosome eviction. Altogether, these data show that RSC and SWI/SNF complexes promote DNA end resection in budding yeast and likely do so by acting as nucleosome evictors. Detailed biochemical and cell biological analysis will however be needed to

**FIGURE 2** Nucleosome remodeler activities and their effects on chromatin. The activity of different nucleosome remodelers (shades of green) can result in three principal effects on nucleosomes. **(A)**—Nucleosome sliding and eviction. While all remodelers have the propensity to slide nucleosomes, eviction of nucleosomes from double-stranded DNA is catalyzed mainly by the SWI/SNF sub-family of nucleosome remodelers. **(B)**—Nucleosome positioning. Some nucleosome remodelers have the ability to slide and position nucleosomes on DNA in a controlled fashion that leads to the formation of regularly spaced arrays. In the budding yeast system this activity is catalyzed mainly by ISW1a-, ISW1b-, Chd1 and INO80-complexes. **(C)**—Nucleosome editing. Nucleosome editing is defined as the exchange of canonical histones (grey) for non-canonical histone variants, like H2A.Z (purple), within the nucleosome and vice versa. In budding yeast H2A/H2A.Z exchange is performed by the INO80 sub-family of remodelers: the SWR1-complex catalyzes the incorporation of H2A.Z-H2B dimers, while the INO80-C is thought to catalyze the reverse reaction.

**FIGURE 3** Nucleosome eviction at DSBs. **(A)**—Resection nucleases (Exo1/Dna2) are inhibited by the presence of nucleosomes. Thus, eviction of nucleosomes from dsDNA is required to facilitate resection. This reaction may be catalyzed by nucleosome remodelers with evicting activity (light green). Moreover, binding of the Mre11-C to the DSB ends might be inhibited by nucleosomes (not shown). Therefore, eviction by nucleosome remodelers might be additionally required also for resection initiation. **(B)**—Incorporation of H2A.Z (purple) into nucleosomes by nucleosome remodelers with editing activity (dark green) leads to a reduced stability of nucleosomes. H2A.Z-containing nucleosomes may therefore be directly evicted by long-range resection nucleases, but nucleosome remodelers with evicting activity (light green) may be additionally involved (see “?”).
pinpoint exactly at which steps of DNA end resection these nucleosome remodelers act. These studies also need to account for the fact that RSC and SWI/SNF may also influence the long-range chromatin response to DSBs (on the 10 kb–1 Mb range). Indeed, γH2A - the long-range chromatin mark of DSBs - was found to be reduced in RSC mutants (Kent et al., 2007; Shim et al., 2007), but it is unclear whether this effect relates to nucleosome eviction.

Lastly, SWI/SNF is also required later during HR, as SWI/SNF mutants show defects in synopsis and strand invasion (Chai et al., 2005). It is currently unclear whether this is due to defects in resection, due to a second “late” role in HR or due to long-range chromatin changes on the broken chromosome.

Nucleosome eviction appears to be conserved in human remodeler complexes. Human BAF and PBAF complexes are recruits to sites of DSBs (Park et al., 2006; Hays et al., 2020). Moreover, they appear to promote resection, possibly by acting on the Mre11-C activator Ctp1 (Hays et al., 2020). This suggests an early role in resection and it will be interesting to investigate whether this function is linked to nucleosome eviction.

While SWI/SNF and RSC are the major players in nucleosome eviction, it could be possible that also other nucleosome remodelers evict nucleosomes during DSB repair and resection. In particular, the INO80 complex has been linked to the eviction of nucleosomes at sites of transcription and DSBs as well (Tsukuda et al., 2005; van Attikum et al., 2007; Qiu et al., 2020), but given several functions of INO80 during DSB repair (see below) this activity is particularly challenging to ascertain. Additionally, whatever this INO80 complex function is, it appears to act differently from SWI/SNF and RSC complexes (Peritore et al., 2021). In all, we therefore conclude that 1) histone eviction occurs at DSBs, that 2) it is critical for DSB resection and repair and that 3) it appears to be mediated by the major cellular eviction activities of the SWI/SNF sub-family complexes.

THE ROLE OF NUCLEOSOME POSITIONING AT DOUBLE STRAND BREAKS REMAINS TO BE DETERMINED

Nucleosomes are positioned in a non-random fashion throughout the genome. In particular, a specific organization is seen at sites of transcribed genes, where a nucleosome-free region marks or neighbors the transcription start site, followed by regularly spaced nucleosomal arrays (Yuan et al., 2005; Weiner et al., 2010; Baldi et al., 2020). Positioning remodelers are responsible for the characteristic spacing of nucleosomes within such nucleosome arrays (Baldi et al., 2020). To generate the specific spacing of nucleosomes within the array, positioning remodelers use intrinsic ruler mechanisms as well as sensing of DNA shapes (Yamada et al., 2011; Krietenstein et al., 2016; Oberbeckmann et al., 2021a; Oberbeckmann et al., 2021b). The generation of nucleosome arrays has been extensively studied in budding yeast, where a combination of in vitro and in vivo studies suggests that four remodelers – Chd1, ISW1a, ISW2 and INO80 – can specifically position nucleosomes to form nucleosome arrays (Gikopoulos et al., 2011; Krietenstein et al., 2016; Ocampo et al., 2016; Kubik et al., 2019; Oberbeckmann et al., 2021b). Importantly, these remodelers also sense the presence of barrier-factors bound at specific sites in the genome to which the array is aligned to or “phased” (Eaton et al., 2010; Li et al., 2015; Krietenstein et al., 2016; Kubik et al., 2018; Rossi et al., 2018). Typical barrier factors are DNA-binding factors, like the abundant general regulatory factors Abf1, Rap1 or Reb1 in budding yeast or genome organizing factors like CTCP in mammals or Phaser in flies (Fu et al., 2008; Wiechens et al., 2016; Baldi et al., 2018). Importantly, recent in vitro work suggests that also DSBs are sensed as a barrier-factor by nucleosome remodelers and guide the formation of nucleosome arrays (Oberbeckmann et al., 2021a).

The finding that regularly spaced nucleosome arrays can form around DSBs in in vitro systems raises two questions: do remodelers position nucleosomes to form arrays around DSBs also in vivo and would such arrays promote DNA end resection? Experimentally, nucleosome positioning is typically investigated using micrococcal nuclease (MNase), which cleaves preferentially non-nucleosomal DNA. Several studies that used MNase to investigate nucleosome localization around a single DSB showed eviction of DSB-proximal nucleosomes, but came to different conclusions as to whether DSB-distal nucleosomes would shift their position (Kent et al., 2007; Shim et al., 2007; Tsabar et al., 2016). While these results are seemingly contradictory, this may simply be due to the fact that results from a single DSB are difficult to interpret. For example the newly formed array can be indistinguishable from the initial nucleosome positions, if the DSB and initial barrier factor are located at the same position. To overcome these limitations, a recent study utilized the PHO5 gene, with its well characterized nucleosomal array and found evidence for eviction of the break-proximal nucleosome as well as repositioning of further distal nucleosomes (Tripuraneni et al., 2021). Further studies will need to show whether repositioned nucleosomes are indeed aligned to the DSB and whether the DSB itself or DSB-associated proteins serve as barrier. Furthermore, studies need to identify, if arrays are generated by positioning remodelers Chd1, ISW1a, ISW2 or INO80.

Interestingly, several studies in both yeast and human cells, point towards a function of these specific remodelers in promoting homologous recombination (Lan et al., 2010; Nakamura et al., 2011; Smeenk et al., 2012; Toiber et al., 2013; Kari et al., 2016; Zhou et al., 2018; Rother et al., 2020; Casari et al., 2021; Gnugnoli et al., 2021). In particular, remodelers of ISW1, CHD1 and CHD7 sub-families appear to be recruited to sites of DNA damage and to stimulate resection (Smeenk et al., 2012; Toiber et al., 2013; Kari et al., 2016; Delamarre et al., 2020; Rother et al., 2020; Gnugnoli et al., 2021). The precise mechanism by which these remodelers promote resection and HR is however uncertain. Moreover, even if these remodelers established nucleosome arrays around DSBs, it is at this point entirely unclear whether such arrays will have a positive function in DSB repair or whether they are simply a consequence of the enzymatic mechanism of positioning remodelers (Baldi et al.,
NUCLEOSOME EDITING AND H2A.Z EXCHANGE GUIDE DOUBLE STRAND BREAK REPAIR

Nucleosome editing describes the activity of exchanging canonical histone subunits with non-canonical histone variants and vice versa (Das and Tyler, 2013; Venkatesh and Workman, 2015). Nucleosome remodelers can facilitate editing by catalyzing the exchange of histone dimers. In eukaryotes, several histone variants exist primarily for H2A variants: H2A (which includes features of H2A.X) and H2A.Z (Sanstieban et al., 2000). The SWR1 complex catalyzes the incorporation of H2A.Z-H2B dimers (Krogan et al., 2003; Mizuguchi et al., 2004). Furthermore, the INO80 complex is thought to catalyze the reverse reaction, the exchange of H2A.Z-H2B with H2A-H2B dimers (Papamichos-Chronakis et al., 2011; Brahma et al., 2017). This model of INO80 function is based on the principal finding that deletion of the H2A.Z gene HTZ1 genetically suppresses many phenotypes of mutants deficient in INO80 function (Lademann et al., 2017; Papamichos-Chronakis et al., 2006; 2011).

The SWR1 complex is the prototypical nucleosome editing remodeler: mechanistically, it is able to translocate short stretches of DNA with no changes in nucleosome position, which then allows H2A-H2B dimers to be exchanged for H2A.Z-H2B dimers (Wu et al., 2009; Luk et al., 2010; Ranjan et al., 2015; Willhoft et al., 2018; Singh et al., 2019). In budding yeast, the SWR1 complex incorporates H2A.Z into chromatin around DSBs, as indicated by 1) the recruitment of SWR1 to DSB sites (van Attikum et al., 2007; Morillo-Huesca et al., 2010) and 2) a transient increase in H2A.Z occupancy in the DSB-surrounding chromatin shortly after DSB induction (Kalocsay et al., 2009). A transiently increased incorporation of H2A.Z into DSB-proximal chromatin was observed also in human cells (Xu et al., 2012; Nishibuchi et al., 2014; Alatwi and Downs, 2015; Gursoy-Yuzugullu et al., 2015). Compared to canonical nucleosomes, H2A.Z-containing nucleosomes are more labile (Abbot et al., 2001; Zhang et al., 2005; Jin and Felsenfeld, 2007) suggesting that their presence will promote DNA end resection. Consistently, yeast cells lacking H2A.Z show a pronounced resection defect (Kalocsay et al., 2009; Lademann et al., 2017). In contrast, the absence of SWR1 causes a much milder resection phenotype (van Attikum et al., 2007; Chen et al., 2012; Adkins et al., 2013). These data suggest that either 1) H2A.Z becomes incorporated at DSB sites by an SWR1-independent mechanism or that 2) H2A.Z-incorporation into DSB-surrounding chromatin is not a major regulator of resection and that H2A.Z regulates resection by means independent from its incorporation in DSB-surrounding chromatin. If H2A.Z-incorporation into DSB-proximal chromatin promotes resection, there are two putative mechanisms by which it could do so. First, the aforementioned reduction of nucleosome stability may allow remodelers or even resection nucleases to bypass and evict H2A.Z-containing nucleosomes (Adkins et al., 2013). Second, H2A.Z could serve as binding platform for associated factors (Xu et al., 2012) as has been shown for nucleotide excision repair (Yu et al., 2013). Binding of factors to H2A.Z or SUMO-modified H2A.Z is for example thought to lead to relocalization of DSBs to the nuclear periphery (Nagai et al., 2008; Kalocsay et al., 2009; Oza et al., 2009; Horigome et al., 2014). Relocalization of DSBs is also observed in Drosophila, where heterochromatic DSBs are first brought to the periphery of the heterochromatic domain (Chiolo et al., 2011) and then to the nuclear pore complex (Ryu et al., 2015). Similarly, in mammalian cells DSB relocation to discrete clusters in the periphery of heterochromatin has been observed (Jakob et al., 2011; Tsouroula et al., 2016; Schrank et al., 2018), but a connection between DSB relocation and H2A.Z has not been shown so far. Therefore, nucleosome editing and H2A.Z incorporation are used to regulate DSB repair, but the underlying molecular mechanisms warrant further investigation.

The importance of nucleosome editing for DSB repair raises the question whether H2A.Z incorporation becomes reversed at some point. Indeed, studies in budding yeast have shown that the INO80 complex is not only recruited to DSBs (Downs et al., 2004; Morrison et al., 2004; van Attikum et al., 2004; Bennett et al., 2013), but that it also counteracts H2A.Z incorporation (Papamichos-Chronakis et al., 2011). Also in human cells H2A.Z is removed from chromatin surrounding DSB sites (Xu et al., 2012; Nishibuchi et al., 2014; Alatwi and Downs, 2015; Gursoy-Yuzugullu et al., 2015; Clouaire et al., 2018). While INO80’s role as nucleosome editing and H2A.Z removal enzyme was initially controversial (Papamichos-Chronakis et al., 2011; Watanabe et al., 2013; Jeronimo et al., 2015; Tramantano et al., 2016; Wang et al., 2016; Watanabe and Peterson, 2016), recent structural work showed that besides its nucleosome positioning activity, the INO80 complex may be able to catalyze translocation of short stretches of DNA without nucleosome sliding, consistent with histone dimer exchange activity (Ayala et al., 2018; Eustermann et al., 2018). This suggests that at DSBs INO80 may have at least two activities: 1) a nucleosome positioning activity (see above) and 2) a nucleosome editing activity (Papamichos-Chronakis et al., 2006; Alatwi and Downs, 2015; Brahma et al., 2017; Lademann et al., 2017). Consistent with INO80 antagonizing the SWR1 complex and removing H2A.Z from chromatin, mutants deficient in INO80 complex function accumulate H2A.Z around DSBs (Papamichos-Chronakis et al., 2006; Alatwi and Downs, 2015; Lademann et al., 2017). The dual remodeling activity of the INO80 complex complicates the interpretation of ino80 mutant phenotypes. To overcome this issue, deletion of the H2A.Z gene HTZ1 has been used, because it suppresses phenotypes arising from an H2A.Z removal defect. Using this approach, an H2A.Z removal function of the INO80 complex was found to promote the formation of the Rad51 nucleo-protein filament downstream of resection.
In contrast, a resection-promoting function of the INO80 complex was found to be independent of H2A.Z (Lademann et al., 2017) and therefore unrelated to nucleosome editing. Moreover, also in human cells, nucleosome editing by the INO80 complex is important for DSB repair and acts after DNA end resection (Alatwi and Downs, 2015). Taken together, a picture emerges whereby nucleosome editing and H2A.Z incorporation by the SWR1 complex is involved in regulation of DNA end resection in yeast, while generally and throughout eukaryotes H2A.Z removal in DSB-surrounding chromatin is important for DSB repair, but likely acts only after resection.

**FUN30/SMARCAD1 PROMOTE RESECTION BY ANTAGONIZING RESECTION-INHIBITORY FACTORS**

Fun30 (from budding yeast), ETL1 (from mouse) and SMARCAD1 (from human) are the prototypical members of a sub-family of nucleosome remodelers that is evolutionary conserved throughout eukaryotes (Clark et al., 1992; Adra et al., 2000; Hlau et al., 2006). Historically they have not been considered major nucleosome remodelers and their molecular mechanisms have not yet been entirely elucidated (Bantele and Pfander, 2019). Recently, a study by the Luger lab suggested that SMARCAD1 evicts and also assembles entire nucleosomes by a mechanism that involves unique contacts between remodeler and nucleosome (Markert et al., 2021). Work with yeast Fun30 suggests that it can slide nucleosomes and mediate histone dimer exchange (Awad et al., 2010).

A key function of yeast Fun30 and human SMARCAD1 appears to be the stimulation of long-range resection (Chen et al., 2012; Costelloe et al., 2012; Eapen et al., 2012). For example, in budding yeast cells lacking Fun30, long-range resection of a non-repairable DSB is 2-3-fold slower than in WT cells (Eapen et al., 2012; Bantele et al., 2017). Accordingly, fun30 mutants scored similarly to mutants deficient in the long-range resection nucleases, when they were initially found in screens for resection-dependent repair of DSBs (Chen et al., 2012; Costelloe et al., 2012). Moreover, an evolutionary conserved pathway facilitates recruitment of Fun30 to sites of DNA end resection. This pathway requires the 9-1-1 complex as recruitment platform at the ssDNA-dsDNA junction and is activated during cell cycle phases (S-M phase), when also resection is activated (Chen et al., 2016; Bantele et al., 2017).

In contrast, Fun30 did not stimulate Exo1’s ability to resect through a nucleosome in an in vitro system (Adkins et al., 2013). This finding raises the possibility that a crucial factor was missing from these reconstituted systems. Consistently, fun30 mutant phenotypes can be suppressed by the additional depletion of the resection inhibitor Rad9 from yeast cells (Chen et al., 2012; Bantele et al., 2017). These data indicate a functional antagonism between Fun30 and Rad9. Notably, also in human cells SMARCAD1 acts as resection activator, while the Rad9 orthologue 53BP1 is a resection inhibitor (Lazzaro et al., 2008; Bunting et al., 2010; Bothmer et al., 2011; Costelloe et al., 2012; Densham et al., 2016), suggesting that the antagonism of both factors is conserved throughout eukaryotic evolution (please see (Sanchez et al., 2021)) in this issue for a detailed review on the interaction between 53BP1 and BRCA1 in the DSB repair decision). Notably, Rad9, 53BP1, as well as the fission yeast orthologue Crb2 associate with chromatin and have all been shown to bind to nucleosomes, where they recognize specific histone modifications (Huyen et al., 2004; Nakamura et al., 2004; Sanders et al., 2004; Wysocki et al., 2005; Botuyan et al., 2006; Du et al., 2006; Toh et al., 2006; Grenon et al., 2007; Hammet et al., 2007; Fradet-Turcotte et al., 2013; Wilson et al., 2016; Hu et al., 2017; Kilic et al., 2019). Rad9 orthologues appear to recognize distinct histone marks, but in each case two or more histone marks are bound (reviewed in Marini et al., 2019; Panier and Boulton, 2014), suggesting that Rad9 orthologues are multivalent histone binders. We therefore hypothesize that both Fun30 and Rad9 influence DSB-surrounding chromatin in an antagonistic fashion and that Fun30 specifically acts on Rad9-bound nucleosomes (Bantele and Pfander, 2019).

In budding yeast cells lacking both Fun30 and Rad9, resection and nucleosome eviction are fully functional (Peritore et al., 2021), suggesting that Fun30 is not required to overcome the general nucleosome barrier and that it is not the essential nucleosome evictor at DSBs. Alternatively, Fun30 may rather catalyze the direct removal of Rad9 from nucleosomes (Figure 4A) or it may counteract Rad9 association with nucleosomes by catalyzing histone dimer exchange which may remove one or more binding site(s) for Rad9 (Figure 4B). Lastly, it is possible that Fun30 slides or even entirely evicts Rad9-bound nucleosomes (Figure 4C). Given that Rad9 and Fun30 antagonize each other on multiple levels, including also the competition for binding to the scaffold protein Dpb11 (Granata et al., 2010; Pfander and Diffley, 2011; Bantele et al., 2017), future biochemical and structural studies will be needed to reveal the mechanism by which Fun30 promotes DNA end resection.

Also human SMARCAD1 antagonizes 53BP1. Depletion of SMARCAD1 stabilizes 53BP1 around DSB sites (Densham et al., 2016). However, resection regulation in human cells is more complex compared to yeast as besides SMARCAD1 a second resection promoting factor exist, the BRCA1-BARD1 complex (reviewed in Densham and Morris, 2019; Sanchez et al., 2021). BRCA1-BARD1 form an E3 ubiquitin-ligase complex that mediates ubiquitylation of H2A (Kalb et al., 2014; Densham et al., 2016; Leung et al., 2017; Nakamura et al., 2019). BRCA1-BARD1 is likely to act upstream of SMARCAD1, as ubiquitin-modified H2A promotes SMARCAD1 binding to nucleosomes around DSBs (Densham et al., 2016). Therefore, SMARCAD1 function has to be seen in the context of post-translational histone modifications, which affect DSB-surrounding chromatin. DSB-localized SMARCAD1 may also become post-translationally modified itself, including phosphorylation by the ATM kinase and ubiquitylation by the RING1 ubiquitin ligase (Chakraborty et al., 2018), which appears to activate the pro-resection function of SMARCAD1. These factors need to be taken into consideration for biochemical studies that ultimately will allow to understand whether the Fun30/SMARCAD1 sub-family remodelers facilitate resection by nucleosomes sliding and eviction, positioning or editing and whether it acts on nucleosomes or rather on nucleosome-associated proteins.
CONCLUSION

In all, we think that previous studies collectively indicate that nucleosome remodelers may serve similar roles during DSB repair as during gene transcription with nucleosome eviction, editing and potentially even positioning taking place at DSBs. Knowledge of the specific activities of individual nucleosome remodelers and of their redundancies thereby offers the potential to get to grips with chromatin changes occurring at DSBs. Moreover, we think that studies of DSB resection and repair may be generally inspired by analogies to gene transcription. Both processes appear to be similarly affected by the presence of chromatin, with nucleosomes forming a dynamic barrier and nucleosome remodelers facilitating its bypass.

Importantly, while nucleosomes clearly form a barrier to the resection nucleases, nucleosome remodelers equip cells with multiple ways to overcome this barrier. In this review, we have outlined several putative mechanisms of how bypass may occur. These include eviction, sliding and editing of nucleosomes. While we are still only beginning to understand how the nucleosome barrier is overcome, a key future question will be which bypass mechanism is chosen in which cellular scenario. Importantly, the nucleosome barrier and its dynamic nature offers additional possibilities to regulate resection and DSB repair. Moreover, critical factors of the DSB repair decision, such as 53BP1 and BRCA1, are proteins that bind and modify nucleosomes. Therefore, we propose that convergence of resection-regulatory pathways on nucleosomes is a central part of the cellular DSB repair decision.

AUTHOR CONTRIBUTIONS

LAK, MP, and LG contributed equally to writing of the manuscript. LAK, MP, LG and BP developed concepts presented here and wrote the manuscript.

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Dynamic HP1a Domain to Complete Recombinational Repair. Cell 144, 732–744. doi:10.1016/j.cell.2011.02.012

Chou, D. M., Adamson, B., Dehoure, N. E., Tan, X., Nottke, A. C., Huvor, K. E., et al. (2010). A Chromatin Localization Screen Reveals Poly (ADP-Ribose)-Regulated Recruitment of the Repressive Polycrom and NuRD Complexes to Sites of DNA Damage. Proc. Natl. Acad. Sci. 107, 18475–18480. doi:10.1073/pnas.1012946107

Ciccia, A., and Elledge, S. J. (2010). The DNA Damage Response: Making it Safe to Die. Nat. Rev. Mol. Cell Biol. 11, 748–760. doi:10.1038/nrm2818

Clark, M. W., Zhong, W. W., Keng, T., Storms, R. K., Barton, A., Kaback, D. B., et al. (2012). Histone Exchange and Histone Modification by the Saccharomyces cerevisiae Chromatin Remodeler Fun30 Regulates DNA End Resection and Checkpoint Deactivation. Mol. Cell Biol. 32, 4727–4740. doi:10.1128/MCB.00566-12

Elbakry, A., and Lührick, M. (2021). Homologous Recombination Subpathways: A Tangle to Resolve. Front. Genet. 12, 723347. doi:10.3389/fgene.2021.723347

Eustermann, S., Schull, K., Kostrewa, D., Lakomek, K., Strauss, M., Moldt, M., et al. (2018). Structural Basis for ATP-dependent Chromatin Remodelling by the INO80 Complex. Nature 556, 386–390. doi:10.1038/s41586-018-0029-y

Falzon, M., Fewell, J. W., and Kuff, E. L. (1993). ERB-80, a Transcription Factor Closely Resembling the Human Autoantigen Ku, Recognizes Single- to Double-Strand Transitions in DNA. J. Biol. Chem. 268, 10546–10552. doi:10.1074/jbc.m102958182233-5

Flau, A., Martin, D. A. M., Barton, G. J., and Owen-Hughes, T. (2006). Identification of Multiple Distinct Snf2 Subfamilies with Conserved Structural Motifs. Nucleic Acids Res. 34, 2887–2905. doi:10.1093/nar/gkl295

Fradet-Turcotte, A., Canny, M. D., Escribano-Díaz, C., Orthiswein, A., Leung, C. C. Y., Huang, H., et al. (2013). 53BP1 Is a Reader of the DNA-Damage Induced H2A Lys 15 Ubiquitin Mark. Nature 499, 50–54. doi:10.1038/nature12318

Fu, Y., Sinha, M., Peterson, C. L., and Weng, Z. (2008). The Insulator Binding Protein CTCF Positions 20 Nucleosomes Around its Binding Sites across the Human Genome. Plos Genet. 4, e1000138. doi:10.1371/journal.pgen.1000138

Garcia, V., Phelps, S. E. L., Gray, S., and Neale, M. J. (2011). Bidirectional Resection of DNA Double-Strand Breaks by Mre11 and Exo1. Nature 479, 241–244. doi:10.1038/nature10151

Girikopoulos, T., Schofield, P., Singh, V., Pinskaya, M., Mellor, J., Smolle, M., et al. (2011). A Role for Snf2-Related Nucleosome-Spacing Enzymes in Genome-wide Nucleosome Organization. Science 333, 1758–1760. doi:10.1126/science.1206097

Gruenolli, M., Casari, E., and Longhese, M. P. (2021). The Chromatin Remodeler Chd1 Supports MRX and Exo1 Functions in Resection of DNA Double-Strand Breaks. Plos Genet. 17, e1009807. doi:10.1371/journal.pgen.1009807

Goodarzi, A. A., Karka, T., and Jeggo, P. A. (2011). KAP-1 Phosphorylation Regulates CHD3 Nucleosome Remodeling during the DNA Double-Strand Break Response. Nat. Struct. Mol. Biol. 18, 831–839. doi:10.1038/nsmb.2277

Granata, M., Lazzaro, F., Novarina, D., Panigada, D., Puodu, F., Abreu, C. M., et al. (2010). Dynamos of Rad9 Chromatin Binding and Checkpoint Function Are Mediated by its Dimerization and Are Cell Cycle-Regulated by CDK1 Activity. Plos Genet. 6, e1001047. doi:10.1371/journal.pgen.1001047

Grenon, M., Costelloe, J., Jimeno, S., O’Shaughnessy, A., FitzGerald, J., Zgheib, O., et al. (2007). Docking onto Chromatin via the Saccharomyces Cerevisiae Rad9 Tudor Domain. Yeast 24, 105–119. doi:10.1002/yea.1441

Gursoy-Yuzugullu, O., Ayrapetov, M. K., and Price, B. D. (2015). Histone chaperone Asp32 removes H2A.Z from DNA double-strand breaks and promotes nucleosome reorganization and DNA repair. Proc. Natl. Acad. Sci. USA 112, 7507–7512. doi:10.1073/pnas.1504868112

Hammet, A., Magill, C., Heiserhorst, J., and Jackson, S. P. (2007). Rad9 BRCT Domain Interaction with Phosphorylated H2AX Regulates the G1 Checkpoint in Budding Yeast. EMBO Rep. 8, 851–857. doi:10.1038/sj.embor.7401036

Hays, E., Nettleton, E., Carter, C., Morales, M., Vo, L., Passo, M., et al. (2020). The SWI/SNF ATPase BRG1 Stimulates DNA End Resection and Homologous Recombination by Reducing Nucleosome Density at DNA Double Strand Breaks and by Promoting the Recruitment of the CILP Nucleosome. Cell Cycle 19, 3096–3114. doi:10.1089/cc.2019.31256

Helfrich, A., Wiegant, W., Thijssen, P., Vertegaal, A., Luijsterburg, M., and van Attikum, H. (2013). Remodeling and Spacing Factor 1 (RSF1) Deposits Centromere Proteins at DNA Double-Strand Breaks to Promote Non-homologous End-Joining. Cell Cycle 12, 3070–3082. doi:10.4161/cc.26033

Horigome, C., Oma, Y., Konishi, T., Schmid, R., Marcomini, I., Hauer, M. H., et al. (2014). SWR1 and INO80 Chromatin Remodelers Contribute to DNA Double-Strand Break Perinuclear Anchorage Site Choice. Mol. Cell 55, 626–639. doi:10.1016/j.molcel.2014.06.027

Hu, K., Li, Y., Wu, W., Xie, L., Yan, H., Cai, Y., et al. (2020). ATM-Dependent Repair of DNA Double-Strand Breaks Is Required for Transcriptional Repression and DNA Repair at DNA Breaks Flanking Transcriptional Active Regions. Adv. Sci. 7, 1900157. doi:10.1002/advs.20190157

Hu, Q., Botuyan, M. V., Cui, G., Zhao, D., and Mer, G. (2017). Mechanisms of Ubiquitin-Nucleosome Recognition and Regulation of 53BP1 Chromatin Recruitment by RNf66/19 and RAD18. Mol. Cell 66, 473–487.e9. doi:10.1016/j.molcel.2017.04.009

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. doi:10.1016/j.molcel.2018.01.031
Huyen, V., Zgheib, O., DiTullio Jr, R. A., Jr, Grizou, V. G., Zacheratos, P., Petty, T. J., et al. (2014). Methylation of Lysine 79 of Histone H3 Targets 53BP1 to DNA Double-Strand Breaks. Nature 432, 406–411. doi:10.1038/nature13114
Ira, G., Pellicoli, A., Bajila, A., Wang, X., Fiorani, S., Carotenuto, W., et al. (2004). DNA End Resection, Homologous Recombination and DNA Damage Checkpoint Activation Require CDK1. Nature 431, 1011–1017. doi:10.1038/ nature02964
Jakob, B., Splinter, J., Conrad, S., Voss, O.-O., Zink, D., Durante, M., et al. (2011). DNA Double-Strand Breaks in Heterochromatin Elicit Fast Repair Protein Recruitment, Histone H2AX Phosphorylation and Relocation to Euchromatin. Nucleic Acids Res. 39, 6489–6499. doi:10.1093/nar/gkr230
Jeromino, C., Watanabe, S., Kaplan, C. D., Peterson, C. L., and Robert, F. (2015). The Histone Chaperones FACT and Sp6 Restrict H2AZ from Intragenic Locations. Mol. Cell 58, 1113–1123. doi:10.1016/j.molcel.2015.03.030
Jin, C., and Felsenfeld, G. (2007). Nucleosome Stability Mediated by Histone Variants H3.3 and H2AZ. Genes Dev. 21, 1519–1529. doi:10.1101/ gad.1547707
Juhász, S., Smith, R., Spekhardt, T., Mamar, H., Zentout, S., et al. (2009). Chromosome-wide Rad51 Spreading and SUMO-H2A.Z-dependent Chromosome Fixation in Response to a Persistent DNA Double-Strand Break. Mol. Cell 33, 335–343. doi:10.1016/j.molcel.2009.01.016
Kari, V., Mansour, W. Y., Raul, S. K., Baumgart, S. J., Mund, A., Grade, M., et al. (2010). The Chromatin Remodeling Factor CHD4 Coordinates Signaling and Repair after DNA Damage. J. Cell Biol 190, 731–740. doi:10.1083/jcb.200921235
Kilic, S., Lezaja, A., Gatti, M., Pellicoli, A., Vaze, M., Haber, J. E., et al. (2008). Histone Methyltransferase Dot1 and Rad9 Inhibit Single-Stranded DNA Accumulation at DSBs and Uncapped Telomeres. Embo J. 27, 1502–1512. doi:10.1038/embj.2008.81
Lee, H.-S., Park, J.-H., Kim, S.-J., Kwon, S.-I., and Kwon, J. (2010). A Cooperative Activation Loop Among SWI/SNF, γ-H2AX and H3 Acetylation for DNA Double-Strand Break Repair. Embo J. 29, 1434–1445. doi:10.1038/embj.2010.27
Leung, J. W. C., Makharashvili, N., Agarwal, P., Chiu, L.-Y., Pourre, R., Cammarata, M. B., et al. (2017). ZMYM3 Regulates BRCA1 Localization at Damaged Chromatin to Promote DNA Repair. Genes Dev. 31, 260–274. doi:10.1101/gad.292516.116
Lewis, T. S., Sokolova, V., Jung, H., Ng, H., and Tan, D. (2021). Structural Basis of Chromatin Regulation by Histone Variant H2AZ. Nucleic Acids Res. 49, 11379–11391. doi:10.1093/nar/gka8907
Li, M., Hada, A., Sen, P., Olufemi, L., Hall, M. A., Smith, B. Y., et al. (2015). Dynamic Regulation of Transcription Factors by Nucleosome Remodeling. eLife Sci. 6, e6249. doi:10.7554/elife.06249
Liang, B., Qu, J., Ratnakumar, K., and Laurent, B. C. (2007). RSC Functions as an Early Double-Strand-Break Sensor in the Cell’s Response to DNA Damage. Genes Dev. 21, 1432–1437. doi:10.1101/gad.6596310.1016
Luger, K., Dechassa, M. L., and Tremethick, D. J. (2012). New Insights into Nucleosome and Chromatin Structure: an Ordered State or a Disordered Array. Nat. Rev. Mol. Cell Biol. 13, 436–447. doi:10.1038/nrm3388
Luijsterburg, M. S., Acs, K., Ackermann, L., Wiegant, W. W., Bekker-Jensen, S., Larsen, D. H., et al. (2012). A New Non-catalytic Role for Ubiquitin Ligase RNF8 in Unfolding Higher-Order Chromatin Structure. EMBO J. 31, 2511–2527. doi:10.1038/emboj.2012.104
Luijsterburg, M. S., de Kruijf, L. I., Wiegant, W. W., Shah, R. G., Smeenk, G., de Groot, A. J. L., 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. doi:10.1016/j.molcel.2016.01.019
Luk, E., Ranjan, A., FitzGerald, P. C., Mizuguchi, G., Huang, Y., Wei, Y., et al. (2010). Stepwise Histone Replacement by SWR1 Requires Dual Activation with Histone H2AZ and Canonical Nucleosome. Cell 143, 725–736. doi:10.1016/j.cell.2010.01.019
Ma, N. F., Hu, L., Fung, J. M., Xie, D., Zheng, B. J., Chen, L., et al. (2008). Isolation and Characterization of a Novel Oncogene, Amplified in Liver Cancer 1, within a Commonly Amplified Region at 1q21 in Hepatocellular Carcinoma. Hepatology 47, 503–510. doi:10.1002/hep.22072
Marfella, C. G. A., and Imbalzano, A. N. (2007). The Chd Family of Chromatin Remodelers. Mutat. Research/Fundamental Mol. Mech. Mutagenesis 618, 30–40. doi:10.1016/j.mrfmmm.2006.07.012
Marini, F., Rawal, C. C., Liberi, G., and Pellicoli, A. (2019). Regulation of DNA Double Strand Breaks Processing: Focus on Barriers. Front. Mol. Biosci. 6. doi:10.3389/fmbio.2019.00055
Markert, J., Zhou, K., and Luger, K. (2021). SMARCAD1 is an ATP-Dependent Histone Oxidase Exchange Factor with De Novo Nucleosome Assembly Function in Liver Cancer 1, within a Commonly Amplified Region at 1q21 in Hepatocellular Carcinoma. Front. Mol. Biosci. 6. doi:10.3389/fmbio.2019.00055
Meisenberg, C., Pinder, S. I., Hopkins, S. R., Wooller, S. K., Benstead-Flume, G., Pear, F. M. G., et al. (2019). Repression of Transcription at a Commonly Amplified Region at 1q21 in Hepatocellular Carcinoma. Nature 578, 212–223.e7. doi:10.1016/j.nature.2018.11.001
Mimitou, E. P., and Symington, L. S. (2008). Sae2, Exo1 and Sgs1 Collaborate in DNA Double-Strand Break Processing. Nature 455, 770–774. doi:10.1038/ nature07312
Mimitou, E. P., Yamada, S., and Keener, S. (2017). A Global View of Meiotic Double-Strand Break End Resection. Science 355, 40–45. doi:10.1126/science.aab7044
Mittal, P., and Roberts, C. W. M. (2020). The SWI/SNF Complex in Cancer - Biology, Biomarkers and Therapy. Nat. Rev. Clin. Oncol. 17, 435–448. doi:10.1038/s41571-020-0357-3
Mizuguchi, G., Shen, X., Landry, J., Wu, W.-H., Sen, S., and Wu, C. (2004). ATP-driven Exchange of Histone H2AZ Variant Catalyzed by SWR1 Chromatin Remodeling Complex. Science 303, 343–348. doi:10.1126/science.1090701
Sartori, A. A., Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., et al. (2007). Human ChlP Promotes DNA End Resection. Nature 450, 509–514. doi:10.1038/nature06337

Schrank, B. R., Aparicio, T., Li, Y., Chang, W., Chait, B. T., Gundersen, G. G., et al. (2018). Nuclear ARP2/3 Drives DNA Break Clustering for Homology-Directed Repair. Nature 559, 61–66. doi:10.1038/s41586-018-0237-5

Sellou, H., Lebeaufin, T., Chapsis, C., Smith, R., Hegele, A., Singh, H. R., et al. (2016). The poly(ADP-ribose)-dependent Chromatin Remodeler Alc1 Induces Local Chromatin Relaxation upon DNA Damage. MboC 27, 3791–3799. doi:10.1091/mbc.E15-05-0269

Shu, J. J.-C., Guan, B., Zheng, C., Lin, A., Lee, C.-H., Hsiao, T.-Y., et al. (2010). Rsf1, a Chromatin Remodeling Protein, Induces DNA Damage and Promotes Genome Instability. J. Biol. Chem. 285, 38260–38269. doi:10.1074/jbc.M110.138735

Shibata, A., Moiani, D., Arvai, A. S., Perry, J., Harding, S. M., Genois, M.-M., et al. (2010). Histone Variants on the Move: Substrates for Histone Acetylation Switch Regulates H2A.Z Deposition by the SWR-C Chromatin Remodeling Enzyme. Nature 467, 703–706. doi:10.1038/nature09341

Symington, L. S. (2016). Mechanism and Regulation of DNA End Resection in Eukaryotes. Crit. Rev. Biochem. Mol. Biol. 51, 195–212. doi:10.3109/10409238.2016.1172552

Symington, L. S., and Gautier, J. (2011). Double-strand Break End Resection and Repair Pathway Choice. Annu. Rev. Genet. 45, 247–271. doi:10.1146/annurev-genet-110410-132435

Symington, L. S. (2016). Mechanism and Regulation of DNA End Resection in Eukaryotes. Crit. Rev. Biochem. Mol. Biol. 51, 195–212. doi:10.3109/10409238.2016.1172552

Toiber, D., Erdel, F., Bouzourene, K., Silberman, D. M., Zhong, L., Mulligan, P., et al. (2013). SIR16 Recruits SNF2H to DNA Break Sites, Preventing Genomic Instability through Chromatin Remodeling. Mol. Cell 51, 454–468. doi:10.1016/j.molcel.2013.06.018

Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E., and Schreiber, S. L. (1998). Chromatin Deacetylation by an ATP-dependent Nucleosome Remodeling Complex. Nature 395, 917–921. doi:10.1038/27699

Tramantano, M., Sun, L., Au, C., Labuz, D., Liu, Z., Chou, M., et al. (2016). Constitutive Turnover of Histone H2A.Z by Yeast Promoters Requires the Preinitiation Complex. eLife 5, e14243. doi:10.7554/eLife.14243

Triprapani, V., Memisoglu, G., MacAlpine, H. K., Tran, T. Q., Zhu, W., Hartemink, A. J., et al. (2021). Local Nucleosome Dynamics and Evolution Following a Double-Strand Break Are Reversible by NHEJ-Mediated Repair in the Absence of DNA Replication. Genome Res. 31, 775–788. doi:10.1101/gr.271155.120

Tsabari, M., Hicks, W. M., Tsapounina, O., and Haber, J. E. (2016). Re-establishment of Nucleosome Occupancy during Double-Strand Break Repair in Budding Yeast. DNA Repair 47, 21–29. doi:10.1016/j.dnarep.2016.09.005

Tsuoura, K., Furst, A., Bogier, M., Heyer, V., Maglott-Roth, A., Ferrand, A., et al. (2016). Temporal and Spatial Uncoupling of DNA Double Strand Break Repair Pathways within Mammalian Heterochromatin. Mol. Cell 63, 293–305. doi:10.1016/j.molcel.2016.06.002

Tsukuda, T., Fleming, A. R., Nickoloff, J. A., and Osley, M. A. (2005). Chromatin Remodeling at a DNA Double-Strand Break Site in Saccharomyces cerevisiae. Nature 438, 379–383. doi:10.1038/nature04148

Tsukuda, T., Lo, Y.-C., Krishna, S., Sterk, R., Osley, M. A., and Nickoloff, J. A. (2009). INO80-dependent Chromatin Remodeling Regulates Early and Late Stages of Mitotic Homologous Recombination. DNA Repair 8, 360–369. doi:10.1016/j.dnarep.2008.11.014

Ui, A., Ogwara, H., Nakajima, S., Kanno, S., Watanabe, R., Harata, M., et al. (2014). Possible Involvement of LKB1-AMPK Signaling in Non-homologous End Joining. Oncogene 33, 1640–1648. doi:10.1038/onc.2013.125

van Attikum, H., Fritsch, O., and Gasser, S. M. (2007). Distinct Roles for SWR1 and INO80 Chromatin Remodeling Complexes at Chromosomal Double-Strand Breaks. Embo J. 26, 4113–4125. doi:10.1038/sj.emboj.6101835

van Attikum, H., Fritsch, O., Hohn, B., and Gasser, S. M. (2004). Recruitment of the INO80 Complex by H2A Phosphorylation Links ATP-dependent Chromatin Remodeling with DNA Double-Strand Break Repair. Cell 119, 777–788. doi:10.1016/j.cell.2004.11.033

Van, H. T., and Santos, M. A. (2018). Histone Modifications and the DNA Double-Strand Break Repair Response. Cell Cycle 17, 2399–2410. doi:10.1080/15384101.2018.1542899

Venkatesh, S., and Workman, J. L. (2015). Histone Exchange, Chromatin Structure and the Regulation of Transcription. Nat. Rev. Mol. Cell Biol 16, 178–189. doi:10.1038/nrm3941

Vidic, P.-A., Liu, J., Salles, D., Jayaraman, S., Dorfman, G., Gray, M., et al. (2014). NudM Promotes Homologous Recombination Repair by Regulating the Accumulation of the ISWI ATPase SNF2h at DNA Breaks. Nucleic Acids Res. 42, 6365–6379. doi:10.1093/nar/gku296

Wang, F., Ranjan, A., Wei, D., and Wu, C. (2016). Comment on "A Histone Acetylation Switch Regulates H2A.Z Deposition by the SWR-C Remodeling Enzyme". Science 353, 358. doi:10.1126/science.aad3921

Wang, W., Daley, J. M., Kwon, Y., Krasner, D. S., and Sung, P. (2017). Plasticity of the Mre11-Rad50-Xrs2-Sae2 Nucleosome Ensemble in the Processing of DNA-Bound Obstacles. Genes Dev. 31, 2331–2336. doi:10.1101/gad.307900.117

Watanabe, R., Utsu, A., Kanno, S.-I., Ogwara, H., Nagase, T., Kohno, T., et al. (2014). SWI/SNF Factors Required for Cellular Resistance to DNA Damage Include ARID1A and ARID1B and Show Interdependent Protein Stability. Cancer Res. 74, 2465–2475. doi:10.1158/0008-5472.CAN-13-3608

Watanabe, S., and Peterson, C. L. (2016). Response to Comment on "A Histone Acetylation Switch Regulates H2A.Z Deposition by the SWR-C Remodeling Enzyme". Science 353, 358. doi:10.1126/science.aad6398

Watanabe, S., Radman-Livaja, M., Rando, O. J., and Peterson, C. L. (2013). A Histone Acetylation Switch Regulates H2A.Z Deposition by the SWR-C Remodeling Enzyme. Science 340, 195–199. doi:10.1126/science.1229758
Weiner, A., Hughes, A., Yassour, M., Rando, O. J., and Friedman, N. (2010). High-resolution Nucleosome Mapping Reveals Transcription-dependent Promoter Packaging. Genome Res. 20, 90–100. doi:10.1101/gr.098509.109

Wichens, N., Singh, V., Gkikopoulos, T., Schofield, P., Rocha, S., and Owen-Hughes, T. (2016). The Chromatin Remodelling Enzymes SNF2H and SNF2L Position Nucleosomes Adjacent to CTCF and Other Transcription Factors. Plos Genet. 12, e1005940. doi:10.1371/journal.pgen.1005940

Wiest, N. E., Houghtaling, S., Sanchez, J. C., Tomkinson, A. E., and Osley, M. A. (2017). The SWI/SNF ATP-dependent Nucleosome Remodeler Promotes Resection Initiation at a DNA Double-Strand Break in Yeast. Nucleic Acids Res. 45, 5887–5900. doi:10.1093/nar/gkx221

Willhoft, O., Ghoneim, M., Lin, C.-L., Chua, E. Y. D., Wilkinson, M., Chaban, Y., et al. (2018). Structure and Dynamics of the Yeast SWR1-Nucleosome Complex. Science 362, eaat7716. doi:10.1126/science.aat7716

Willhoft, O., and Wigley, D. B. (2020). INO80 and SWR1 Complexes: the Non-dependent Promoter Packaging. High-resolution Nucleosome Mapping Reveals Transcription-Dependent Promoter Packaging. Genome Res. 20, 90–100. doi:10.1101/gr.098509.109

Willhoft, O., and Wigley, D. B. (2020). INO80 and SWR1 Complexes: the Non-dependent Promoter Packaging. High-resolution Nucleosome Mapping Reveals Transcription-Dependent Promoter Packaging. Genome Res. 20, 90–100. doi:10.1101/gr.098509.109

Wysocki, R., Javaheri, A., Allard, S., Sha, F., Cote’, J., and Kron, S. J. (2005). Role of Dot1-dependent Histone H3 Methylation in G1 and S Phase DNA Damage Checkpoint Functions of Rad9. Mol. Cel Biol 25, 8430–8443. doi:10.1128/MCB.25.19.8430-8443.2005

Xiao, A., Li, H., Shechter, D., Ahn, S. H., Fabrizio, L. A., Erdjument-Bromage, H., et al. (2009). WSTF Regulates the H2A.X DNA Damage Response via a Novel Tyrosine Kinase Activity. Nature 457, 57–62. doi:10.1038/nature07668

Xu, Y., Ayyarpetov, 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. Cel 48, 723–733. doi:10.1016/j.molcel.2012.09.026

Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Côté, J., and Wang, W. (1998). NURD, a Novel Complex with Both ATP-dependent Chromatin Remodeling and Histone Deacetylase Activities. Mol. Cel 2, 851–861. doi:10.1016/s1097-2765(00)80299-3

Yamada, K., Frouws, T. D., Angst, B., Fitzgerald, D. J., DeLuca, C., Schimmele, K., et al. (2011). Structure and Mechanism of the Chromatin Remodelling Factor ISW1a. Nature 472, 448–453. doi:10.1038/nature09947

Yu, Y., Deng, Y., Reed, S. H., Millar, C. B., and Osley, M. A. (2013). Histone Variant Htz1 Promotes Histone H3 Acetylation to Enhance Nucleotide Excision Repair in Htz1 Nucleosomes. Nucleic Acids Res. 41, 9006–9019. doi:10.1093/nar/gkt688

Yuan, G.-C., Liu, Y.-J., Dion, M. F., Slack, M. D., Wu, L. F., Altschuler, S. J., et al. (2005). Genome-Scale Identification of Nucleosome Positions in S. cerevisiae. Science 309, 626–630. doi:10.1126/science.1112178

Zhang, H., Roberts, D. N., and Cairns, B. R. (2005). Genome-Wide Dynamics of Histone H2A Variant that Poises Repressed/Basal Promoters for Activation through Histone Loss. Cell 123, 219–231. doi:10.1016/j.cell.2005.08.036

Zhang, Y., LeRoy, G., Seelig, H.-P., Lane, W. S., and Reinberg, D. (1998). The Dermatomyositis-specific Autoantigen Mi2 Is a Component of a Complex Containing Histone Deacetylase and Nucleosome Remodeling Activities. Cell 95, 279–289. doi:10.1016/s0092-8674(00)81758-4

Zhou, J., Li, J., Serafin, R. B., Ketchum, S., Ferreira, C. G., Liu, J. C., et al. (2018). Human CHD1 Is Required for Early DNA-Damage Signaling and Is Uniquely Regulated by its N Terminus. Nucleic Acids Res. 46, 3891–3905. doi:10.1093/nar/gky128

Zhu, Z., Chang, W.-H., Shim, E. Y., Lee, S. E., and Ira, G. (2008). Sgs1 Helicase and Two Nucleases Dna2 and Exo1 Reset DNA Double-Strand Break Ends. Cell 134, 981–994. doi:10.1016/j.cell.2008.08.037

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