

Perspectives

DNA Repair in the Context of Chromatin

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ABSTRACT

Modulation of chromatin is essential to nuclear processes that utilize DNA, such as transcription, replication, and repair. For example, transcription is assisted by histone post-translational modifications, as well as chromatin-remodeling complexes, which alter the structure of chromatin. Furthermore, recent advancements in the fields of DNA repair and chromatin reveal that both histone modifications and chromatin-remodeling complexes are essential for the repair of DNA lesions. In particular, chromatin-modifying complexes, such as the INO80 chromatin-remodeling complex and the Tip60 histone acetyltransferase complex, associate with the DNA damage-induced phosphorylated H2AX, which is often referred to as γ -H2AX. In *S. cerevisiae*, the association of INO80 with γ -H2AX is required for the recruitment of INO80 to sites of DNA double-strand breaks. Additionally, in *Drosophila*, Tip60 exchanges γ -H2AX for unmodified H2A in regions of DNA damage. This report reviews recent studies that emphasize the intimate relationship between evolutionarily-conserved chromatin-modifying complexes and histone post-translational modifications in the repair of DNA damage.

HISTONE MODIFICATIONS

Much like transcription, the process of DNA repair is influenced by histone post-translational modifications. For instance, histone acetylation by the NuA4 acetyltransferase complex in yeast, not only assists transcription, but is also required for DNA double-strand break (DSB) repair.¹⁻³ Histone acetylation is also involved in DNA repair in higher eukaryotes. Specifically, the human Tip60 histone acetyltransferase complex appears to be important for repairing DNA lesions because cells that express a mutant Tip60 that lacks acetyltransferase activity accumulate DSBs following exposure to γ -irradiation.⁴

A modification that occurs specifically at sites of DSBs is the rapid phosphorylation of histone H2AX on serine 139.⁵ This phosphorylated histone is often referred to as γ -H2AX, and for consistency will also be termed γ -H2AX in this report. Mammalian histone H2AX is a variant of H2A and accounts for approximately 10% of total histone H2A.⁵ In yeast, the only H2As, H2A1 and H2A2, are orthologous to the mammalian H2AX, and like H2AX, phosphorylation of yeast H2As are also implicated in the repair of DSBs.⁶ The kinases that phosphorylate yeast histone H2As are the phosphatidylinositol-3 kinase-related kinases Tel1 and Mec1, which are orthologues of the ATM (Ataxia telangiectasia-mutated) and ATR (Ataxia telangiectasia-related) proteins in mammals.^{2,7-9} H2AX is critical for the repair of DNA lesions in mice, as H2AX deficiency results in genomic instability and cancer predisposition.¹⁰⁻¹³

CHROMATIN-REMODELING

In addition to histone post-translational modifications, chromatin-remodeling complexes also assist many nuclear processes. These complexes use the energy of ATP hydrolysis to alter chromatin by such mechanisms as generating DNA superhelical torsion, disrupting DNA-histone contacts, and repositioning nucleosomes.¹⁴ All the ATP-dependent chromatin-remodeling complexes are classified in the SWI/SNF chromatin-remodeling superfamily by the presence of a SNF2-like DEAD/H(SF2) ATPase subunit in the complexes.¹⁵ The vast majority of research investigating the role of chromatin-remodeling complexes in nuclear processes has occurred in transcription, as all four subfamilies (SWI/SNF, ISWI, CHD, and INO80) in the chromatin-remodeling complex superfamily greatly influence this process.^{14,16}

However, one distant member of the SWI/SNF subfamily, Rad54, has a specialized function in DNA repair. Rad54 is a member of the *RAD52* epistasis group that interacts with and assists Rad51 during homologous recombination.^{15,17-19} Although the importance of Rad54 in the process of DNA repair is well-established, its function in chromatin-remodeling has not been clearly defined.²⁰⁻²² However, recent developments, which are described below, report that bona fide chromatin-remodeling complexes are indeed involved in DNA repair, thus exposing a novel function for ATP-dependent chromatin-remodeling complexes and revealing new research directions in the fields of DNA repair and chromatin.

CHROMATIN-REMODELING COMPLEXES UTILIZE MODIFIED HISTONES

Although the ATPase subunit in each chromatin-remodeling complex contains helicase motifs, only the INO80 complex has been shown to exhibit *in vitro* helicase activity.¹⁶ Unlike other subfamilies in the SWI/SNF superfamily, members of the INO80 subfamily contain Rvb1 and Rvb2, which are RuvB-like proteins.^{16,23} In prokaryotes, the RuvB helicase forms a complex that is involved in DNA Holliday Junction branch migration during homologous recombination.^{24,25} The presence of these helicases strongly suggests that the INO80 complex is directly involved in DNA repair and recombination. Accordingly, yeast strains that lack a functional INO80 complex are sensitive to DNA damaging agents, such as ultraviolet light (UV), ionizing radiation (IR), and alkylating agents (MMS).¹⁶ Therefore, the INO80 complex represents a unique class of chromatin-remodeling enzymes that is involved in DNA repair.

Recently published studies have further characterized the role of *S. cerevisiae* INO80 in DSB repair. These studies demonstrate that INO80 is directly involved in DNA repair by binding to the HO endonuclease-induced DSB at the *MAT* locus, as assayed by chromatin immunoprecipitation.^{2,26,27} Of particular interest is the finding that the recruitment of the INO80 complex to the DSB is dependent on its association with the DNA damage-induced γ -H2AX.^{26,27} Accordingly, the recruitment of INO80 to the DSB is greatly reduced in strains that lack the Mec1 and Tel1 kinases, as well as strains expressing a mutant H2A that cannot be phosphorylated.^{26,27} The association between INO80 and γ -H2AX, and also the recruitment of INO80 to the DSB, is greatly diminished in strains that lack the Nhp10 and the Ies3 (INO Eighty Subunit 3) subunits of the INO80 complex.²⁷ The assembly of Ies3 into the INO80 complex is dependent on the presence of Nhp10, an HMG-like protein.^{27,28} Therefore, these results indicate that Nhp10, or both Nhp10 and Ies3, are responsible for establishing the interaction between INO80 and γ -H2AX at sites of DSBs.²⁷ This is a somewhat surprising result because it has been previously reported that the Arps (Arp8 and Arp4), which are actin-related proteins found in several chromatin-modifying complexes, including INO80, interact with histones.²⁷⁻²⁹ Interestingly, another recent report indicates that the Arp4 subunit of both the INO80 complex and the NuA4 acetyltransferase complex can bind to γ -H2AX peptides.² The source of this discrepancy, concerning the investigation of the interaction between γ -H2AX and chromatin-modifying complexes, remains unclear. However, it is possible that both Nhp10 and Arp4 are involved in the interaction between these complexes and γ -H2AX but that this association varies under different experimental conditions. Nevertheless, unlike the Arp4 subunit, Nhp10 is uniquely present in the INO80 complex, which further emphasizes the specialized function of INO80 in DNA repair.²⁷

Another chromatin-modifying complex that has recently been implicated in DNA repair is Tip60. As mentioned, the histone acetyltransferase activity of the mammalian Tip60 complex is involved in DNA damage repair.⁴ The Tip60 complex also contains an ATPase subunit, and like INO80, Tip60 contains RuvB-like proteins.³⁰ Therefore, the Tip60 complex represents a unique complex that combines acetyltransferase activity and chromatin-remodeling activity. A recent report by Kusch et al. demonstrates that the *Drosophila melanogaster* homologue of Tip60 (dTip60) preferentially binds to and acetylates nucleosomal phosphorylated H2Av, the *Drosophila* homologue of γ -H2AX.³¹ The dTip60 complex also catalyzes the exchange of phosphorylated H2Av with unmodified H2Av in chromatin.³¹ Consequently, cells lacking a functional dTip60 complex lose the transient acetylation of H2Av that normally occurs after exposure to γ -irradiation.³¹ Also, an accumulation of phosphorylated H2Av persists in these mutant cells following exposure to DNA damaging agents.³¹

These studies firmly establish the role of chromatin-remodeling complexes in DNA repair and demonstrate that this process utilizes histone modifications to recruit complexes that can manipulate chromatin in order to assist repair.

OUTSTANDING ISSUES

Another chromatin-remodeling complex that may have a role in DNA repair is SWR1. SWR1 is another member of the Rvb1/2-containing INO80 chromatin-remodeling subfamily.²³ In *S. cerevisiae*, SWR1 has been found to catalyze the exchange of the histone variant H2AZ into chromatin in order to regulate gene expression and control the spread of heterochromatin.²³ Like INO80, yeast strains lacking a functional SWR1 complex also display increased sensitivities to DNA damaging agents, such as MMS and UV light.²³ Interestingly, the SWR1 complex also associates with γ -H2AX, although this interaction is not as robust as that of INO80 for γ -H2AX.²⁷ Nevertheless, these results suggest that the SWR1 complex may also be involved in the repair of DNA lesions. Furthermore, because both INO80 and SWR1 share similarities with Tip60 it can be postulated that INO80 and/or SWR1 may serve a similar function in yeast as Tip60 does in *Drosophila*, which is the exchange of γ -H2AX with unmodified H2A during the repair of DNA. It remains to be seen whether additional remodeling complexes and/or histone modifications are involved in DNA repair.

Despite these advances, it is not known precisely what happens to the chromatin structure around DSBs during repair. It is possible that chromatin-remodeling activities are required to "slide" nucleosomes at specific sites to allow repair machinery to bind or function. It is also possible that the histones around the DSB are being actively exchanged during repair. The turnover of γ -H2AX around the DSB can be achieved by such a histone exchange mechanism. As previously discussed, this exchange may be catalyzed by dTip60 or equivalent complexes in other organisms.³¹ However, dephosphorylation of γ -H2AX may also lead to the same end result. It is likely that multiple mechanisms are involved, much like the complex regulation of chromatin modifications during promoter activation, where histone modification and chromatin-remodeling events are precisely choreographed depending on the specific promoter. Such potential sequences of events should be investigated for the repair of a DSB.

Additionally, it has not yet been clearly demonstrated whether chromatin-remodeling complexes are involved in homologous recombination (HR) or non-homologous end joining (NHEJ) or both. Synthetic genetic analyses (SGA) have revealed that components

of the INO80 complex genetically interact with several members of the homologous recombination *RAD52* epistasis group.²⁷ This further supports the role for INO80 in DNA repair but does not definitively demonstrate whether INO80 is involved in HR or NHEJ. However, a recent report by Fritsch et al. implicates INO80 in HR but not NHEJ in plants.³² A mechanism for the involvement of INO80 in HR is supported by data presented in the publication by van Attikum et. al., which shows that mutant yeast strains of the INO80 complex are defective in the single-stranded DNA resection that occurs prior to strand invasion in HR.²⁶ In addition, this same report also presented data demonstrating that mutant yeast strains of the INO80 complex are defective in NHEJ.²⁶ Therefore, these studies suggest that INO80 is involved in both HR and NHEJ in yeast but not in plants. Clearly, there is still much to be discovered regarding the involvement of various chromatin-modifying complexes in different organisms and whether these complexes are specialized in the repair of specific DNA lesions.

One potential way to direct the activity of chromatin-modifying complexes to specific sites of different DNA lesions is for these complexes to recognize and bind various histone post-translational modifications that occur in response to DNA damage in the chromatin regions surrounding DNA lesions. In such situations, the chromatin that is involved in the DNA damage would become modified in order to recruit a complex or complexes that modulate the chromatin environment to facilitate efficient DNA repair. The binding of proteins to specifically modified histones, often referred to as a 'histone code', has been previously suggested for the process of transcription.^{33,34} The recent studies that have been discussed in this report suggest that a histone code also exists for DNA repair.

For instance, not only histone phosphorylation, but also histone acetylation, is involved in recruiting chromatin-remodeling complexes to sites of DSBs. Specifically, it was determined that loss of acetylation by the NuA4 complex results in reduced localization of Rvb-containing complexes, such as INO80 and/or SWR1, to DSBs.² As previously noted, not only is the binding of INO80 to DSBs dependent on the association of the complex with the DNA damage-induced γ -H2AX,^{26,27} but it was also recently discovered that components of the NuA4 acetyltransferase complex also bind to γ -H2AX.² Because NuA4 has previously been implicated in DNA repair³ it logical to postulate that this chromatin-modifying complex is recruited to sites of DNA damage through this interaction. Additionally, not only has histone acetylation and phosphorylation proven to be important for DNA repair, but also histone methylation, which is intimately involved in transcriptional regulation, has recently been implicated in DNA repair.³⁵

However, it should be noted that alternatives or complementary mechanisms to the DNA repair histone code hypothesis might exist. For example, some chromatin-modifying complexes may also be recruited through direct interaction with the DNA repair machinery or damaged DNA itself. While these potential mechanisms remain to be investigated, recent reports clearly demonstrate that histone modifications are able to direct the recruitment of various chromatin-modifying complexes involved in repair of DNA.

In conclusion, the significance of chromatin-modifying complexes in DNA repair is now beginning to emerge. The importance of histone modifications in coordinating the recruitment of these complexes to sites of DNA damage is becoming evident. It remains to be determined whether histone post-translational modifications are the dominating factor for the association of chromatin-modifying complexes to areas that contain DNA lesions. Additionally, the precise in vivo chromatin-remodeling activities, such as nucleosome

sliding and histone exchange, that occur during repair also remain to be determined. It is tempting to hypothesize that the activity of these complexes may actually be stimulated by specific histone modifications. Nevertheless, recent advancements in this research field demonstrate that a dependence exists between chromatin-modifying complexes and histone modifications.

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