Chapter 28

Chromatin Modifications in DNA Repair and Cancer

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Chapter Outline

1. INTRODUCTION

The DNA of eukaryotic organisms is bound and organized by numerous proteins. DNA and these proteins are collectively known as "chromatin." Gaining access to damaged DNA in the context is necessary for repair. Protein complexes can modify the structure of chromatin to alter the geometry of DNA and allow repair of damaged regions [2,3]. The pathway utilized for repair depends on the type of DNA lesion, the cell-cycle stage, and the chromatin environment in which the damage is detected [4]. It is generally accepted that all in vivo DNA transactions, including repair occur within the context of chromatin and its remodelers.

Cellular processes that are critical to survival and division are evolutionarily conserved, and the diverse pathways that modulate chromatin, to alter protein–DNA interactions during repair can be found in organisms ranging from humans to the budding yeast *Saccharomyces cerevisiae*. Furthermore, the factors that modulate chromatin in the inherently different processes of transcription, DNA replication, and repair have underlying similarities as each requires disruption of internucleosomal interactions, nucleosome disassembly to enable the process, and reassembly after it is complete. In this chapter, we review the hierarchical pathways of chromatin modifications in DNA replication, and repair, with emphasis on DNA-repair processes, focusing on the replication associated DNA-damage tolerance (DDT) pathway in the model system, *S. cerevisiae*.

2. INTERRELATIONSHIP OF DNA AND CHROMATIN

In eukaryotes, DNA is organized within the nucleus by octamers of histones that are assembled into nucleosomes, the basic units of chromatin [5]. Canonical nucleosomes contain two sets of core histones that preferentially form head-to-tail

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heterodimers of H2A–H2B and H3–H4 when they are not part of a nucleosome. The process of nucleosome construction occurs by stepwise assembly of H3–H4 dimers into $(H3–H4)_2$ tetramer. This half nucleosome is partially incorporated into DNA as two sets of H2A–H2B dimers are added to opposite sides of the tetramer (reviewed in Ref. [6]). Nucleosomes containing about 147 base pairs (bp) of DNA are spaced apart by between 10 and 70 bp of linker DNA [7]. The linker DNA can remain nucleosome free or become bound by the monomeric histone H1.

Normal expression of the core histones is highly regulated with respect to the DNA-replication cycle, and expression is maintained at stoichiometrically consistent levels for each dimer set, such that H2A and H2B are cotranscribed, and the same with H3 and H4 [6]. Misregulation causing an upset to the balance of histones can lead to cell-cycle arrest and cell death [8–10]. Histone chaperones are critical regulators of nucleosome-free histone pools, performing an essential role in nucleosome dynamics that permits transcription, replication, and repair, which require nucleosome disassembly, reassembly, and eviction [6,11].

Histones are bound by the chaperones Nap1 and Asf1 immediately after translation and these proteins facilitate transport into the nucleus. There are preferential associations of chaperones for different dimers: Nap1 binds H2A–H2B, while Asf1 binds H3–H4. After nuclear import, the chaperones may transfer the dimers to other chaperones. For example, H3–H4 dimers are transferred from Asf1 to the replication and DNA damage–specific chaperone CAF-1 (chromatin assembly factor-1), which incorporates nucleosomes into newly synthesized DNA [12]. Similarly, histone chaperones Spt6 and FACT permit RNA polymerase II (Pol II) passage during transcription. Nucleosomes are disassembled by sequential H2A–H2B removal by FACT and H3–H4 interaction with Spt6 ahead of RNA polymerase II (RNA Pol II) progression. Following the polymerase passage, FACT is instrumental to the incorporation of recycled H3–H4 into DNA [13].

Histone chaperones are critical to the dynamic placement of nucleosomes in chromatin and facilitate the changes that coincide with replication, transcription, and repair.

3. HISTONE MODIFICATIONS AND CHROMATIN REMODELERS

Some of the earliest biochemical characterizations of histones describe acetylation of H3 and H4 [14,15]. It was later found that histones are extensively modified, primarily by acetylation, methylation, citrullination, ubiquitination, sumoylation, and phosphorylation (reviewed in Refs. [16,17]) (Fig. 28.1). The posttranslational modifications (PTMs) serve several important functions. Many of the modifications occur on lysine (K) residues in the amino termini of histones, altering the net charge and topology, and changing the interactions of nucleosomes with each other. These modifications also serve as a signaling platform for chromatin modifiers, transcription factors, and polymerases. In addition to surface modification, nucleosomes can be translocated along the DNA by chromatin remodelers. For many DNA processes, the nucleosome presents a functional barrier that needs to be moved or evicted. In several cases, there are examples of hierarchical interactions, where a PTM facilitates a binding interaction with another complex leading to a downstream function [26,27]. Different combinations of posttranslational modifications can recruit downstream effectors that promote transcriptional activation or a type of DNA damage–repair pathway.

The combinatorial effect of histone PTMs on downstream function inspired the popular model known as the “histone code” [28]. Histone “code writers” add a modification to one or more histones of the complex and “code readers” recognize the PTM and initiate the subsequent signal propagation step or modification step. The enzymes that modify histones are usually components of multimeric protein complexes that are comprised of code readers, writers, and erasers [16]. As an example, the NuA3 complex has two methyl lysine recognizing subunits, Yng1 and Pdp3 [26,27], which allow the complex to recognize trimethylated H3–K4 and H3–K36 in promoter regions. Once bound, Sas3, another subunit and acetyltransferase, acetylates H3 at lysine 14. Additionally, there are cases where the same proteins can be found in complexes of different enzymatic function, such as Arp4 being a component of NuA4, SWR-C, and Ino80 [29].

Although there are several types of PTMs, not all histones are targeted equally for a type of modification, as shown in Fig. 28.1. For example, the majority of acetylations occur on histones H3 and H4. In *S. cerevisiae*, methylation is restricted to H3, and ubiquitination occurs on H2B. This may be a reflection of the asymmetry of histone placement within the nucleosome as well as binding constraints of the modifying complexes.

3.1 Histone Acetyltransferases and Deacetylases

Acetyltransferase proteins catalyze the addition of an acetyl group to the terminal amine of a lysine residue. This creates the effect of neutralizing the positively charged lysine (Fig. 28.2A), and has been proposed to weaken internucleosomal interactions to create more flexibility and access to chromatin [30,31] (Fig. 28.2B). Support for this model is the observation of an abundance of acetylated histones in highly transcribed euchromatic regions of the genome, as well as in the region

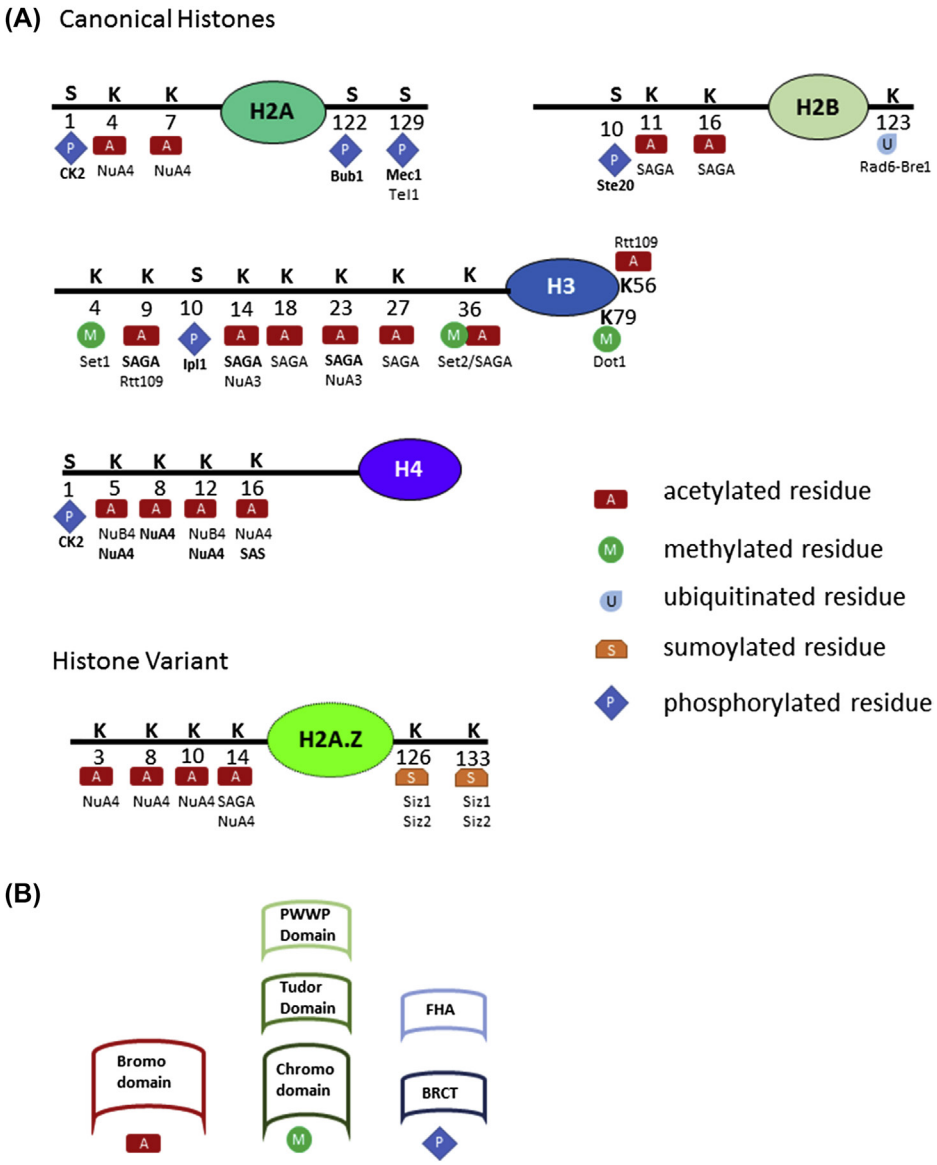

FIGURE 28.1 **Posttranslational modifications (PTMs) to histones in *Saccharomyces cerevisiae*.** (A) Histones are extensively posttranslationally modified by acetylation, methylation, ubiquitination, and sumoylation on lysine (K), and phosphorylation on serine (S) or threonine (T) [2,18–23]. While some modifications can only be made by a single complex, others are redundantly modified by several complexes (depicted beneath). With other modifications, such as sumoylation, all histones can be observed to be sumoylated at several K residues by Siz1 and Siz2 SUMO ligases [24]. There are many abundant PTMs observed in higher eukaryotes, which do not always occur on the exact residues that are observed with yeast. (B) PTMs often enable recognition by protein interaction motifs. The following are examples of common recognition motifs: bromodomains bind acetylated lysines; chromodomains, Tudor domains, and PWWP domains bind methylated lysines; BRCT repeats and FHA domains mediate phospho-serine and threonine interactions [25]. In many processes, the application of PTMs allows the histone tails to serve as signaling platforms for downstream processes.

directly adjacent to damaged DNA [32,33]. In addition to charge modulation, acetylation can also permit interactions with proteins that contain bromodomains. The RSC and SWR-C chromatin–remodeling complexes more strongly interact with acetylated histones through bromodomain-containing proteins [34–36].

Although there are many histone acetyltransferases (HATs) that redundantly acetylate histones, two stand out as being the most important to acetylating bulk chromatin proteins: Gcn5 and Esa1. Gcn5 is part of the Spt-Ada-Gcn5-acetytransferase (SAGA) complex, SAGA-like (SLIK) complex and the smaller subcomplex ADA, and it acetylates H2B and H3 [37,38]. Similarly, Esa1 is a component of NuA4, the yeast homolog of Tip60, and the subcomplex piccolo NuA4. Esa1 acetylates H2A, H2A.Z, and H4 [18,39,40]. HAT activity is not restricted to histones or even other nuclear proteins, with new targets continually being discovered [41–43].

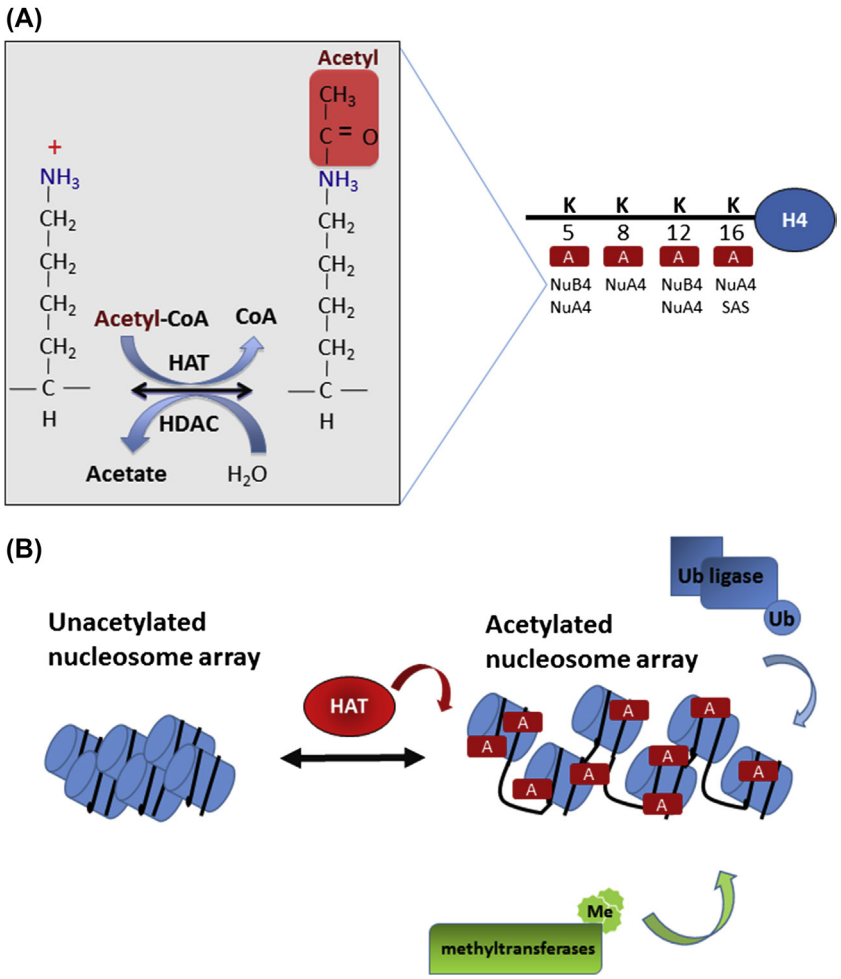

FIGURE 28.2 **Acetylation/deacetylation of lysine residues by histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes.** (A) HAT complex catalyzes the addition of acetyl groups to lysine residues, while HDACs catalyze the reverse reaction *(Adapted from Yang XJ, Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. Nat Rev Mol Cell Biol 2008;9:206–18.)*. Acetylation neutralizes the basicity of the lysine, which in the case of histones changes the chromatin environment, lessening the interactions of the nucleosomes with DNA. (B) Model depicting how abundant acetylation of histones creates more loosely associated nucleosomes. The "opening" of chromatin makes more downstream histone-modifying interactions more amenable.

Histone deacetylases (HDACs) catalyze the removal of the acetyl group from acetylated lysine residues and restore the lysine to its premodified state. There are four classes of HDACs, although the RPD3 and HDA complexes perform the vast majority of transcription-associated deacetylation [44–46]. An exception is Sir2 in budding yeast, together with Sir3 and Sir4, having a specialized role in deacetylating H4–K16 to augment the spread of silent chromatin [47]. As with the acetyltransferases, HDACs have targets that extend beyond histones and chromatin into every cellular system [42]. Much of RPD3 and HDA targeting is mediated by other proteins in the complex, which stabilize its localization to specific genomic sites including intergenic regions and certain promoters [46]. It appears that with regard to catalyzing deacetylation, RPD3 and HDA do not target specific lysines of histone tails but have a broad recognition of acetylated residues [44,47].

3.2 Histone Lysine Methyltransferases and Demethylases

Methylation of lysine residues differs from acetylation in that it functions primarily as a binding platform for signal transduction [25] (Fig. 28.1). Histone lysine methyltransferases (HKMT) have relatively fewer targets compared to acetyltransferases. In *S. cerevisiae*, the most described and biologically relevant HKMTs are Set1, Set2, and Dot1, which catalyze trimethylation of H3–K4, H3–K36, and H3–K79, respectively [48]. Generally, methylated lysines are binding targets for proteins that contain chromodomains, plant homeodomains (PHD), PWWP, and Tudor domains [49]. Unlike acetylation, where a single acetyl group can be added, up to three methyl groups can be added to lysine. HKMTs can be very precise as

to what modification they catalyze. An enzyme that adds a single methylation mark often will not di- or trimethylate that residue (reviewed in Ref. [50]). This variation creates a level of plasticity in which reader complexes will target a specific methylation mark, as they have a preference for certain lysine-modification signatures. An example of this targeted interaction is the H3–K4 trimethylation (H3–K4me3) mark, which recruits proteins with a PHD motif. Although these PHDs will bind dimethylated and monomethylated forms, it is to a much lesser degree than its preferential trimethyl target [26]. Downstream effects of methylation include: (1) regulating DNA repair, in the case of H3–K79me3; (2) distinguishing chromatin regions for transcriptional functions; and (3) marking boundaries between active chromatin and subtelomeric silent chromatin [51,52]. As with acetylation, methylation is a reversible modification, mediated by the Jumanji domain–containing histone lysine demethylases (HKDM).

3.3 Histone Ubiquitination and Sumoylation

Histone ubiquitinatination and sumoylation result from an enzymatic cascade where the small proteins ubiquitin or small ubiquitin-like modifier (SUMO) are covalently linked to a lysine residue [53]. Both ubiquitin and SUMO modifications produce bulkier alterations than acetylation or methylation, and may distort the nucleosomal conformation to make it more accessible for further modification [25], or compete with and block other modifications at the same, or nearby lysine residues. Ubiquitination of H2B at K123 is associated with the coding section of transcribed regions [19]. This modification plays a critical role in permitting downstream nucleosome methylation by Set1 (COMPASS complex) and Dot1 to methylate H3–K4 and K79, respectively [54,55]. Like acetylation and methylation, ubiquitin modifications can be removed by ubiquitin proteases [56].

Histone sumoylation is dependent on Siz1 and Siz2 E3 ligases, and generally corresponds to repression of transcription. Transcription induction produces a loss of SUMO and gain of acetylation. Therefore, sumoylation is proposed to compete with transcription-promoting modifications and help maintain transcriptional quiescence at inducible genes [24].

3.4 Histone Phosphorylation

Phosphorylation of histones is associated with signaling events that are extremely relevant to genome stability, such as chromatin condensation in mitosis and the response to DNA damage. Like acetylation, phosphorylation that is proximal to lysines can nullify the highly basic charge of histones. In damage and mitosis signaling, its primary function is to create interaction sites for phosphorylation-binding motifs of downstream targets. As part of the DNA damage and signaling cascade, H2A is phosphorylated on S129 in yeast (abbreviated to γH2A) [20] (Fig. 28.1). In this circumstance, it is bound by BRCT repeat or forkhead-associated domain (FHA)-containing proteins that are part of the signal cascade leading to activation of cell-cycle checkpoint. Coordination of mitotic processes is strongly regulated by histone phosphorylation/dephosphorylation events. Phosphorylation of H3–S10 by the kinase Ipl1 (homolog of Aurora B) [20] is important to establishment and maintenance of chromatin condensation. After mitosis, it is removed by PP1 phosphatase Glc7. Phosphorylation of H2A–S122 occurs as part of the spindle assembly checkpoint (SAC) in the event of DNA damage and misaligned spindles [57].

3.5 Nucleosome Exchangers and Remodelers

Histone modifiers often act cooperatively with histone exchangers and remodelers, to alter nucleosome composition, positioning, and occupancy. Chromatin remodelers can be grouped into six families: Snf2-like (Chd1, SWI/SNF, RSC, ISWI); Swr1-like (SWR-C, INO80); SMARCAL1-like (Fun30); Rad54-like; Rad5/16-like; and ERCC6/SSO1653-like [58–60]. Remodelers have different capabilities, some can slide histones relative to DNA and some can completely evict octamers; however, they all require ATP hydrolysis for functionality. Many remodelers contain histone code readers for targeting to the genomic regions that they alter. The Chd1 protein associates directly with the SAGA acetyltransferase complex, acting cooperatively with acetylation to promote transcription progression [61]. Additionally, Chd1 and other CHD family members contain tandem chromodomains at their N-termini that facilitate interaction with H3–K4 [62].

Remodelers can be grouped according to specific functional roles. Members of the Snf2-like and Swr1-like families tend to promote transcription and processes that open up the chromatin and make it more accessible, through both nucleosome movement, histone exchange, and eviction [63]. However, ISWI and CHD family members can behave in the opposite manner, altering internucleosomal spacing to remove nucleosome-free regions (NFRs) and shorten internucleosomal gaps to such a degree that further shortening is not possible [60,62,64].

3.6 Histone Variants

Histone variants share many of the structural features of their canonical nucleosomal counterparts but have variation in sequence and transcriptional regulation that is independent of core histones. Variants also associate with chaperones; however, unlike "bulk histones" their incorporation into chromatin is replication independent. A variant that is essential and conserved in all eukaryotes is a version of H3 that designates the chromatin region of the centromere (CenH3). CenH3 (also CENP-A or Cse4) is incorporated by its chaperone Scm3, and is critical for stable formation of the kinetochore in mitosis (reviewed in Choy [65]; and Henikoff, & Furuyama [66]).

The variant H2A.Z (Htz1) functions in transcription, boundary formation between silenced DNA and active genes, and the response to DNA damage [67]. H2A.Z shares only 60% sequence homology with canonical H2A, varying most in the linker regions within the histone fold and C-terminus [68,69]. The unique C-terminal region is required to allow the SWR–C complex to interact with it and insert it into the DNA. The difference in linker regions alters the interaction of H2A.Z with H3–H4, and is predicted to create a less stable nucleosome [70].

Like H2A, H2A.Z-H2B dimers are bound and imported into the nucleus by the chaperone Nap1. When in the nucleus, the H2A.Z–H2B-specific chaperone, Chz1, participates in correctly targeting H2A.Z to promoters and telomeres [71–73]. The incorporation of H2A.Z is mediated by the Swi2/Snf2-related chromatin-remodeling complex SWR-C [74–76], which exchanges canonical H2A–H2B in chromatin for the variant dimer H2A.Z–H2B. Biochemical experiments indicate that H2A.Z nucleosomes may be heterotypic, where there is one H2A.Z and one H2A, or homotypic, where there are two H2A.Zs [77]. Because of differences in linker region 1 (L1) length around the histone fold of the protein, it is predicted that a heterotypic H2A.Z nucleosome would be highly unstable [70]. This instability may account for observations of H2A.Z presence at transcription start sites reducing the barrier function that most nucleosomes present to RNA Pol II [78].

Although H2A.Z incorporation has diverse functions, this variant is often placed in regions that require dynamic regulation, and are proximal to NFRs. At transcription start sites, a 70–120 bp NFR precedes the coding region of the gene. The NFR is followed by the so-called "+1 nucleosome," which is the first nucleosome encountered by RNA Pol II. In most genes, regardless of activity, this region is bound by SWR-C and INO80, both of which are recruited by the NFR at that site (Swc2 of SWR-C and Nhp10, Arp8 and Ies5 of INO80) [79]. SWR-C localization is further augmented by NuA4-mediated acetylation of H4 nucleosomes (Bdf1 of SWR-C binds these) [36,79]. In a study measuring the processivity rate of RNA Pol II in transcription, it was observed that the nucleosomal barrier to transcription could be alleviated by H2A.Z occupying the +1 position [78,80]. The model of how the combined activity of these complexes cooperates with transcription is that SWR-C incorporates H2A.Z while INO80 removes it from the +1 nucleosome, in a dynamic fashion [79]. This disruption of the nucleosome eases progression of the polymerase. Along with affecting its incorporation, H2A.Z acetylation by NuA4 and Gcn5 is associated with both highly transcribed genes, and retention of the variant by inhibiting INO80-mediated H2A.Z to H2A exchange [81–83]. Additionally, Rtt109 enhances removal of H2A.Z through acetylation of H3–K56, permitting SWR-C to catalyze the reverse exchange of incorporation: H2A for H2A.Z [84].

H2A.Z nucleosomes are also localized in proximity to replication origins [85], and sites of DNA damage [86], which are also surrounded by NFRs [87]. Therefore, a general mechanism of H2A.Z incorporation may be to relieve the nucleosomal barrier to polymerases at transcription start sites and to replisomes of newly fired replication origins.

4. CHROMATIN MODIFIERS IN GENOME STABILITY

The activities of various chromatin modifiers and remodelers in the functions of replication and DNA-damage response are critical in the maintenance of genome stability. There are many instances of cooperative and antagonistic interactions that create or deny access to certain genomic regions.

4.1 DNA Replication

The process of DNA replication is a highly coordinated event where bidirectionally oriented replication complexes or replisomes unwind and duplicate DNA from several genomic loci (reviewed in Bell, & Dutta [88]). The intersection of unreplicated duplex DNA with a single replisome and the replicated daughter strands is referred to as the replication fork. The replication fork is comprised of a leading strand, which is polymerized continuously and the lagging strand, which is discontinuously synthesized as Okazaki fragments [88].

During S phase, chromatin is duplicated along with the DNA template and requires both histone recycling and the synthesis of new histones to have enough octamers for both the newly generated strands. The assembly of nucleosomes after fork passage is rapid (30 s), and it was found that nucleosomes were present on the leading and lagging strands 125–300 bp

from the replication complex (reviewed in Annunziato [89]). Therefore, addition of nucleosomes from recycled parental and newly synthesized histone pools progresses with similar timing to the synthesis of DNA. However, the presence of nucleosomes on nascent DNA did not follow the same kinetics as restoring all chromatin marks (chromatin maturity), which took about 15 min following replication [89]. This "maturity" time could be increased by the addition of sodium butyrate, an HDAC inhibitor, which provided some of the first evidence that newly translated H4 is acetylated [89].

It has since been found that newly translated histones H3 and H4 are acetylated in the cytoplasm by chaperone-associated acetyltransferase complexes. NuB4, which is comprised of the Hat1/Kat1, Hat2, and the chaperone Hif1, acetylates newly synthesized cytoplasmic H4 on K5, and K12 [90–92]. In a similar fashion, Rtt109 acetylates newly synthesized H3 on K9 and K56, associating with the chaperone Asf1 for H3–K56 and both Vps75 and Asf1 for H3–K9 [93]. Prior to nuclear import, H3 and H4 form dimers and are imported by Asf1 [92]. Experimental evidence suggests that H4 acetylation is important for nuclear import of these dimers, because this process is compromised in an H4–K5,12R allele, producing a defect of prolonged S phase [94]. Asf1 then transfers the H3–H4 dimers to the replication associated chaperones: CAF-1 and Rtt106. Although both are associated with the complex, CAF-1 incorporates the newly synthesized H3–H4, and this selectivity may be due to preferential association with the acetylated forms of the proteins [95,96]. CAF-1 and Rtt106 accept H3–H4 dimers from Asf-1 to form tetramers and place them into nascent DNA, while H2A–H2B dimers are added afterward.

The acetylation of newly translated histones is transient, and removal of these marks is evident in late S phase to G2 of the cell cycle [97]. As early studies of nascent chromatin indicate, this deacetylation event coincides with the appearance of compacted, nuclease-resistant chromatin [89]. Compaction is largely mediated by the remodeler Iswi2, which is associated with nucleosome assembly after replication [98]. Iswi2 preferentially interacts with unmodified H4 [99–101] and the Rpd3L HDAC complex [102] and is important for establishing correct spacing and nucleosome density after replication and transcription [64].

In replication, acetylation of nascent nucleosomes also provides continued access to underlying, newly replicated DNA. Open chromatin is more permissive to DNA-repair proteins, permitting access to damaged DNA and stalled replication forks. In support of this theory, H3–K56ac is associated with regions of DNA-damage and -replication stress that subsides only after repair has occurred [103]. This mark is positioned at the entry/exit point where DNA wraps around the nucleosome [104], and is proposed to create a loose interaction that inhibits compaction of nucleosome arrays and facilitates unwrapping. Therefore, the requirement of open chromatin may be the reason for H3–K56ac association with newly incorporated nucleosomes, highly transcribed genes, and sites of damage.

4.2 DNA-Damage Response and Repair

When studying DNA-damage processes, chromatin is typically viewed as an obstruction to repair. In the "access, repair, and restore" model [105], chromatin modifiers clear the way for repair processes and restore chromatin to the predamage state following repair completion. This model has evolved to acknowledge that the predamage chromatin state and chromatin modifiers that participate in the repair process can exert some control over the repair outcome [3]. Although there are many sources of DNA damage, the types of damage they create can be grouped into categories of DNA base lesions, mismatches, and breaks in the DNA backbone leading to single-strand breaks (SSBs) and double-strand breaks (DSBs) (Fig. 28.3). Base excision repair (BER) and nucleotide excision repair (NER) resolve base lesions, while mismatch repair (MMR) resolves incorrect base pairs. DNA DSBs are repaired by homologous recombination (HR) and nonhomologous end joining (NHEJ). These types of damage create a different type of lesion when encountered by replication complexes. Base lesions obstruct replication polymerases because they cannot incorporate bases across from damaged DNA. The DDT pathway permits replication to continue in spite of damage. In addition to pathways that mediate repair and damage tolerance during replication, checkpoint pathways coordinate repair with cell-cycle stage and prevent transitions until repair is complete. In this section we review how the chromatin environment and modifiers participate in these pathways with emphasis on replication and DDT.

4.2.1 Repair of DNA Base Damage

Chemical and environmental agents, including ultraviolet radiation (UV), reactive oxygen species (ROS), and chemotherapeutic agents such as alkylating agents cause base lesions. Repair of these requires excision of the damaged nucleotide, or a patch surrounding it, and use of the unaffected strand as a template to synthesize DNA to fill in the space [1] (Fig. 28.3). In the BER pathway, proteins that detect a lesion also initiate the repair process. DNA N-glycosylases translocate throughout the genome, providing a general surveillance of base damage. When a base lesion is recognized, the DNA glycosylase

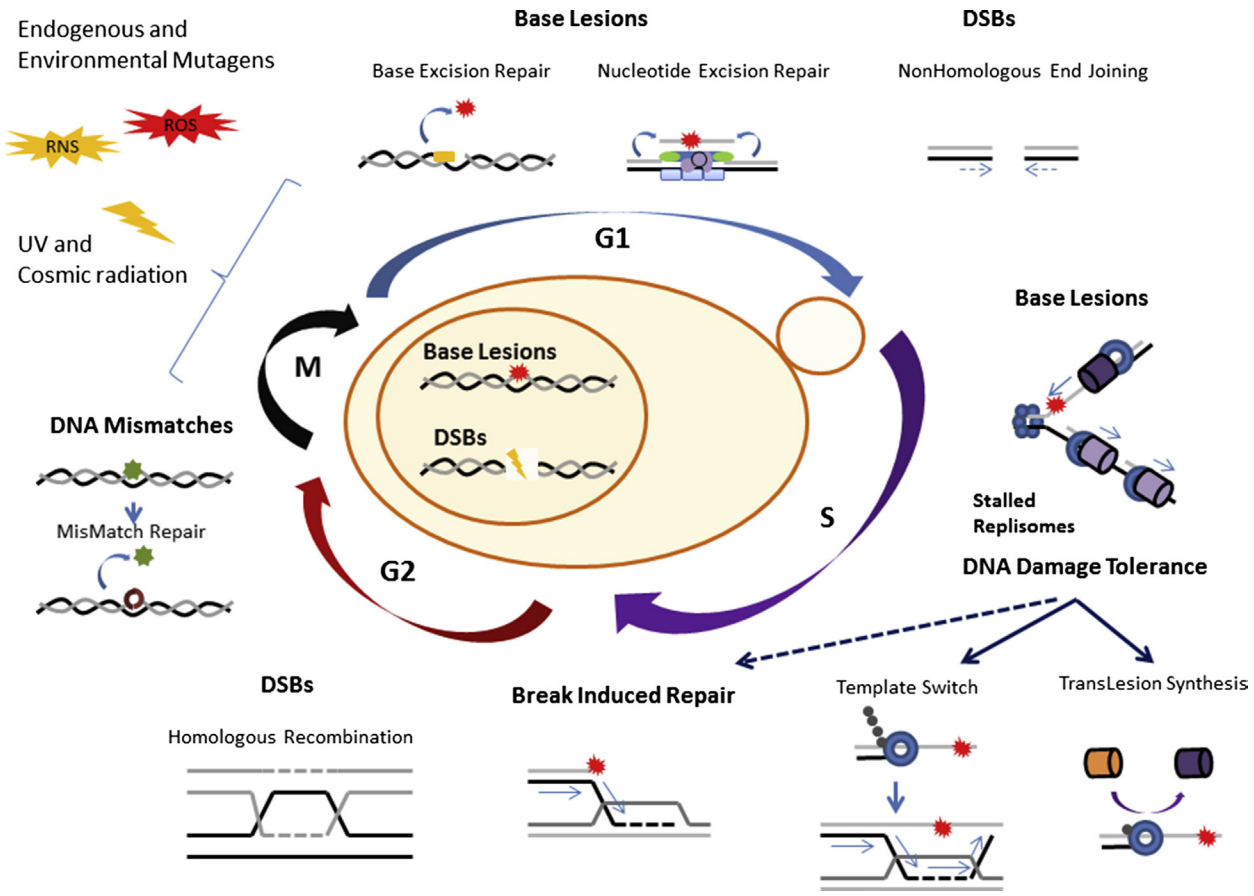

FIGURE 28.3 DNA-damage repair and damage bypass pathways in different phases of the cell cycle. The repair pathways that are utilized for different types of damage depend on the agent that caused the damage and the stage of the cell cycle [1,2,4]. Endogenous base lesions may be caused by reactive oxygen species (ROS) or reactive nitrogen species (RNS). Cosmic radiation or UV light cause base lesions and, more severely, DSBs. In G1, base lesions are repaired by BER and NER, while a DSB is repaired by NHEJ. In S phase, bypass of base lesions occurs via the DDT pathway, resulting in TLS or template switch. BIR occurs if the replisome encounters a nick in the DNA backbone or the replisome collapses. HR is the preferred repair pathway of DSBs during S and G2 of the cell cycle. Lastly, following replication, the newly replicated DNA is scanned for mismatches by the MMR pathway.

releases the damaged base from the deoxyribose sugar. This is followed by binding of an AP-endonuclease/lyase, which nicks the DNA backbone at the apyrimidinic/apurinic (AP) site. The nicked, AP deoxyribose is then recognized by a 3′- or 5′-phosphodiesterase, which removes the sugar leaving an empty space where the damage was. This gap is then filled by a DNA polymerase and the nick is sealed by DNA ligase I (reviewed in Boiteux, & Jinks-Robertson [4]). There is evidence, however, that BER can be inhibited at intermediate repair stages, after the DNA backbone is nicked, if the original DNA lesion resides is within a nucleosome. In an in vitro study of BER processes, it was found that repair of lesions near the center of the nucleosome was not completed because DNA Polβ could not access the abasic site [106]. Retention of repair intermediates at abasic sites can lead to genome instability as replication forks encountering nicked DNA can lead to DSBs in the DNA backbone [107]. Both in vitro and in vivo studies have identified a role for the RSC complex in supporting BER completion by moving the lesion to more accessible sites [108,109].

The NER pathway primarily repairs damage from UV and reactive oxygen-induced lesions. There are two types of NER: global genomic (GG-NER) and transcription coupled (TC-NER). GG-NER detects structural aberrations of the DNA helix resulting from a lesion that is often in untranscribed regions of the genome. Chromatin-modifying events in GG-NER are important to form the recognition platform and excision complex. The earliest steps in the pathway involve lesion recognition and localization of the heterodimers Rad4–Rad23 and Rad16–Rad7. Nucleosomes adjacent to the damage are repositioned by Rad4–Rad23-dependent recruitment of the chromatin remodeler SWI/SNF [110], and Rad16–Rad7 facilitates the recruitment of SAGA [111].

Histone acetylation by SAGA/Gcn5 and the recruitment of additional factors, Rad14 and Rad1–Rad10 (structure-specific endonuclease), assist in the subsequent incorporation of H2A.Z [112]. The initial GG-NER recognition factors eventually become targeted for Ub-mediated proteasomal degradation, while the NER pre-incision complex is formed.

This complex, which includes the essential RNA-Pol II–associated TFIIH, aggregates around the lesion and opens up the DNA, allowing binding of RPA to stabilize the undamaged ssDNA strand [4]. Following this, endonucleases are recruited to nick the DNA backbone 5′ and 3′ to the damaged region. The patch with the damage is then removed from the DNA and the gap is filled in by a DNA polymerase, Polε or Polδ, with DNA ligase I sealing the nick. TC-NER-damage removal from the pre-incision complex to completion of the repair is the same as with GG-NER, but the detection mechanism is different. The damage sensor is RNA-Pol II, which activates TC-NER when a lesion is encountered on the DNA template during transcription [4]. The chromatin remodeler RSC is required for efficient repair for both TC and GG-NER [113]. Although it is somewhat counterintuitive, there are some genes associated with TC-NER that are not a part of GG-NER. For example, Rad26 (ortholog of CSB), an Snf2-related DNA/RNA helicase, is proposed to function exclusively with TC-NER because it associates with RNA Pol II when it stalls during transcription elongation [114].

4.2.2 Repair of DNA Double-Stranded Breaks

DNA DSBs result from a variety of endogenous and exogenous sources. Hydroxyl radical attack and ionizing radiation can break the phosphodiester bond of the DNA backbone, replication stress can lead to fork collapse, and chemotherapeutic agents that poison topoisomerase II, can cause DSBs. DNA DSBs are considered to be the most dangerous DNA lesions, because they are the most likely to result in mutation or caused unresolvable lesions that may lead to cell death [115]. Repair of DSBs can be through two mechanisms: HR, in which newly replicated DNA serves as the template of repair, and NHEJ, in which broken DNA ends are directly annealed to each other (Fig. 28.3). It is generally felt that NHEJ is more error prone as it does not follow a template, and in the process of DSB formation and processing, some sequence between broken strands may be lost. Of these two repair pathways, HR is only selected between S phase and mitosis of the cell cycle, when there is a sister chromatid available to repair from. Because NHEJ does not use a template, it occurs during all cell-cycle phases though its function is most critical for cell survival in G1 and in mammalian cells, and G0 as well. As with base lesions, repair of DSBs involves several chromatin-modifying complexes, some of which are outlined in Fig. 28.4.

4.2.2.1 Nonhomologous End Joining

One of the most important factors for the initiation of NHEJ is the stable association of DNA ends with the damage-recognition heterodimer yKu70/yKu80 [119]. These proteins serve two important functions: inhibiting 3′ to 5′ resection of DNA ends, and serving as the recruiting platform for downstream factors that will stabilize and form a protein bridge between broken DNA ends. In yeast cells, the MRX complex serves the cross-bridging function between ends, while the phosphatidyl inositol kinase-like kinase (PIKK) family member DNA-dependent protein kinase (DNA-PK) [120], is instrumental for this in mammalian cells. MRX is a highly conserved heterotrimeric complex comprised of the Mre11 exonuclease, the SMC family member Rad50 and scaffold protein Xrs2. Although it is not an essential complex in yeast, DNA-damage signaling and repair are severely compromised in MRX mutants [121]. In addition to cross-bridging, the ends need to be processed to become completely blunt ended, either through removal of overhangs or utilizing a polymerase, Pol4, to fill in the space. Ligation of the DNA ends in yeast is performed by Dnl4 ligase with the help of Nej1 and Lif1 (reviewed in Daley [119] and Mathiasen & Lisby [122]). In addition to the canonical NHEJ pathway, alternative NHEJ pathways, such as microhomology-mediated end joining (MMEJ), can anneal regions with short sections of conserved sequence; however, MMEJ is highly error prone [123–125].

4.2.2.2 Checkpoint Activation and DNA Resection at a DSB

There are extensive chromatin modifications in the region of a DSB and the phosphorylation of H2A at S129 is central for many downstream events. The cell-cycle checkpoint is a protein kinase-mediated signal transduction cascade, which is initiated in response to DNA damage, preventing transition to the next stage of the cell cycle until repair is completed. The damage checkpoint and the DSB-repair pathways are activated primarily through signal amplification from the binding of the MRX complex to DNA ends (Fig. 28.4). MRX binds at the DSB and acts as a recruiting platform and activator of the damage signal transduction PIKK kinase Tel1. PIKKs (reviewed in Jackson [126]) are central to the DNA-damage response in all eukaryotic organisms. Two proteins of this family that are critical to damage signal transduction in *S. cerevisiae* are Tel1 and Mec1, the orthologs of ATM and ATR, respectively. They are considered the main transducers of a damage signal because their activation is between proteins that directly sense damage and the amplification of kinase cascades to activate cell cycle–checkpoint proteins Chk1 and Rad53 and phosphorylate other targets proximal to the break.

During S and G2 of the cell cycle, when HR can occur, MRX recruits the Sae2 exonuclease to initiate resection of DNA from the break, creating a 3′ ssDNA overhang [127,128]. Resection is initiated by Sae2 (mammalian CtIP), and continued by the unwinding activities of the RecQ helicase, Sgs1, and the exonucleases, Exo1 and Dna2, which catalyze

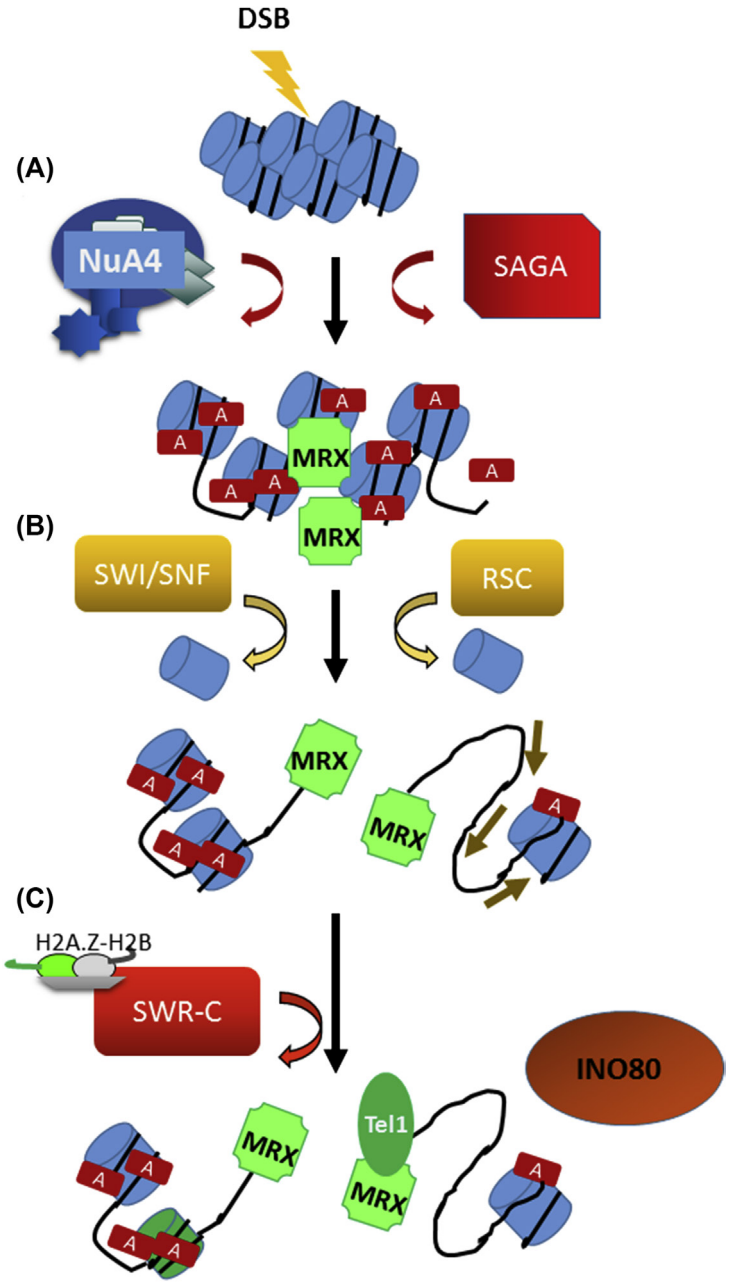

FIGURE 28.4 Activity of chromatin modifiers and remodelers at a DSB. An early event after damage induction is acetylation of histones H4 and H3 by NuA4 and SAGA, respectively [116,117]. Chromatin remodelers SWI/SNF and RSC recruit to the site of DSB, to slide nucleosomes and evict them from the region of the DSB [63]. SWR-C localizes to a DSB and incorporates H2A.Z into nucleosomes proximal to the break [86]. INO80 also recruits to a DSB, and is important in mediating downstream functions of break movement within the nucleus [118].

further resection. As the exonucleases resect DNA, the ssDNA-binding protein RPA accumulates and creates a platform for Mec1–Ddc2(ATRIP) binding. Mec1 kinase activity can be stimulated by interactions with Ddc1 (9-1-1 complex) and RPA interactions with both Ddc2 and Ddc1 function to tether Mec1–Ddc2 and 9-1-1 to the RPA-ssDNA [129], coupling Mec1 to its activator, the 9-1-1 complex.

Maintenance and amplification of checkpoint signaling is driven by a chromatin-based signaling platform that is initiated by Mec1- and Tel1-dependent γH2A formation. γH2A recruits Rad9 (homolog of 53BP1), a BRCT-containing scaffold protein that also binds the effector kinases Rad53 and Chk1 [122]. Once proximal to the break, Mec1/Tel1-dependent phosphorylation of Rad9 promotes its oligomerization via its BRCT repeats [130], and the further binding of Mec1 kinase–activating proteins, Ddc1 and Dpb11 [131]. Binding all of these proteins close to breaks serves to colocalize Mec1 with

its activators, amplifying the signal by recruiting more Mec1–Ddc2, and activating its effector kinases, Rad53 and Chk1. Once activated, effector kinases are then able to perform their functions, which include preventing cell-cycle progression and inducing transcription of repair factors.

4.2.2.3 Homology Search and Repair

Following resection, the ssDNA-binding protein Rad51 displaces RPA on the 3′ overhang, to align with regions of the sister-chromatid template in the search for a homologous region to copy for repair (Fig. 28.3). Sequence search and verification is mediated partially by the activities of Rad52, and other members of the HR complementation group (Rad54, Rad55, and the Shu complex) [132]. When a complementary region is found, the 3′ resected strand invades the undamaged duplex, displacing the other strand of the donor sequence. The donor sequence strand that is displaced is then used as a template for filling in the gap from the other strand of the break to form the Holliday junction. After the DNA polymerase has passed the region of the break, the junction is resolved by nucleases that separate the connections between the donor and invading strands [133].

4.2.2.4 Histone Modifications and Remodelers Associated With DSBs

Along with phosphorylation of histones [21], histone acetylation at H4 by NuA4, and H3 by SAGA occur as an early event in the DNA damage response [32,33,41] (Fig. 28.4). This initiating event is important to opening chromatin to facilitate remodeling [2,134]. Additionally, the acetylation marks are recognized by the bromodomain-containing chromatin remodeler SWI/SNF [116], which enhances γH2A signaling at the break [86]. The remodelers SWR-C, INO80, and RSC also recruit to the DSB [117], and H2A.Z has been reported to be incorporated in nucleosomes for several kilobases flanking the break. The localization of H2A.Z and remodelers at a DSB is associated with extensive resection at the break [86]. However, the trend of extensive processing of a DSB does not agree with events that would promote NHEJ. Therefore, it is unlikely that the very different repair processes of HR and NHEJ would utilize all the same mechanisms in nucleosome restructuring. HR would require extensive reorganization to facilitate DNA resection, while NHEJ would require inhibition of those modifications to prevent it.

A caveat to many of the studies that have characterized histone modifications and remodelers at a DSB is that they did not restrict their observations to discrete cell-cycle phases [135]. A 2013 study sought to observe if there were differences in levels of chromatin modifications and remodelers between G1 and G2/M of the cell cycle, which they were able to do by using mating pheromone to synchronize cells in G1. Their findings revealed that within a chromatin context, there are mechanisms in place to influence repair choice between NHEJ and HR [117].

There are discrete differences among cell-cycle phases in recruitment of NuA4, INO80, SWR-C, SWI/SNF, and RSC. Generally, all of these complexes abundantly localized to a DSB in G2/M, in support of previous publications (reviewed in Papamichos-Chronakis & Peterson [2]; Price & D'Andrea [134]), but were not present at a DSB in G1, in which γH2A and yKu70/yKu80 were strongly enriched [117]. This result strongly differed from previous publications where γH2A was previously identified as being a recruiting mark for complexes containing Arp4, which includes NuA4, SWR-C, and INO80 [32]. Furthermore, mutation of H2A, so that it cannot be phosphorylated at S129, had no negative influence on recruitment of NuA4, INO80, SWR-C, SWI/SNF, and RSC to a DSB.

Subsequent exploration of the inverse relationship between γH2A and chromatin-modifier recruitment revealed that inhibiting DNA resection and HR strongly reduced recruitment of NuA4, INO80, SWR-C, SWI/SNF, RSC, and RPD3L to DSBs, while it increased γH2A levels [117]. Therefore, end processing and resection of DNA at a break is required to initiate remodeler activity and inhibit γH2A. At the same time, they observed that yKu70/80 strongly influenced remodeler and γH2A enrichment. NuA4, INO80, SWR-C, SWI/SNF, and RSC localized to a DSB in an *yku70*Δ in G1, and additionally, γH2A signaling was lost [117]. This supports that extensive modifications and remodeling are required to create access to the damage to facilitate early HR steps of resection.

This study raises many questions about the regulation of factors that promotes or inhibits resection at the break. As previously mentioned, Rad9 interacts with H3–K79me3 and γH2A to provide a binding site for this scaffold at sites of damage in G1 and G2 [136,137]. Rad9 forms oligomers via its BRCT repeats interactions with phosphorylated residues from Mec1/Tel1, and that this ability is important for damage-signal propagation and checkpoint signaling [130]. However, there is a model that suggests that Rad9 and associated proteins form an obstacle to nucleases, helicases, and chromatin modifiers that is proposed to inhibit resection [138,139]. To circumvent this obstacle, the SMARCAL1-like family chromatin remodeler, Fun30, is necessary to promote resection in the presence of Rad9 [138]. Fun30 localization at a DSB depends on proteins that facilitate resection (Sgs1, Exo1, and MRX). Additionally, Fun30 coimmunoprecipitates with RPA, Exo1, and Dna2 in damaged cells.

The Rad9 obstacle is likely to vary with the cell cycle, as it forms a complex at damage with Dpb11, 9-1-1, and Mec1 that is CDK dependent and uniquely occurring in G2 of the cell cycle [131]. CDK activity also has an impact on the capacity of Rad9 to regulate resection in G2 [140]. Therefore, the dynamic assembly of Rad9 at damage likely creates a different kind of relationship to downstream factors that promote resection and modify chromatin such as Fun30, causing Rad9 to be a barrier to resection that is dependent on the cell-cycle phase.

Another set of papers that supports different chromatin events happening in HR vs. NHEJ are from studies in *Schizosaccaromyes pombe* (fission yeast) and human cells [141,142]. In these studies, an intriguing relationship was identified where acetylation and methylation at H3–K36 compete to either enable or block resection of a DSB. Methylation of H3–K36 by Set2 occurs primarily in G1, in association with transcription, preventing resection and thus HR. However, acetylation of H3–K36 by Gcn5 in S and G2 allows HR to occur.

The observation that different chromatin modifiers and remodelers are present for G1 and S/G2 phases are intuitive for what would be expected at a DSB, based on the extreme difference in HR- and NHEJ-pathway mechanisms that are required for repair.

5. REPLICATION STRESS, ACTIVATION OF THE S-PHASE CHECKPOINT AND DNA-DAMAGE TOLERANCE

The S-phase checkpoint integrates the DNA-damage response with the replication process, enabling the cell to proceed through replication in the presence of damage and replication fork obstruction. Obstruction to replisome progression causes RPA-ssDNA accumulation, which results in a stressful configuration that can cause the replisome to disassemble, the replication fork to collapse, and the formation of a DSB (reviewed in Friedel et al. [143]; Yekezare et al. [144]; Yoshida et al. [145]). As with activation of checkpoint due to a DSB, RPA-ssDNA is again the recruiting platform for proteins that sense replication stress, most critical of which are Ddc2 (Mec1–Ddc2) and Ddc1 (9-1-1 complex). Binding of Ddc1 and Dpb11 to Mec1 activates Mec1 kinase activity, leading to phosphorylation of many components of the replisome to slow fork progression in a controlled manner and directly recruit and activate the checkpoint-kinase Rad53 [143].

The main repair pathways active during replication are MMR, when a base is misincorporated in the daughter strand, DDT pathway, when replication past a damaged lesion must occur, and HR when there is a DNA-DSB [4]. MMR is similar to BER and NER in that one half of the helix is the repair template for the mismatched base. MMR occurs only after DNA replication, proofreading the newly synthesized DNA for mismatches, insertions, and deletions using the MutS homologs (MSHs). When MSH proteins identify mismatches, the MutL homologs (MLH) proteins interact and begin to mediate the excision of the misincorporated daughter nucleotide and replacement with a correct one [4]. Much of what is known about MMR was derived from in vitro studies with "naked" DNA. Purified MMR proteins, however, are unable to perform the repair in nucleosome-DNA arrays, implying that like BER and NER, access to the lesion is important.

A current model is that MMR complex components are recruited to nascent DNA by association to modified histones, either by localizing to marks that are placed prior to replication and nucleosome disassembly, or to newly incorporated nucleosomes following the replication fork [146,147]. There is evidence in *S. cerevisiae* that H3–K56Ac plays a role in MMR [148]. As previously mentioned, H3–K56Ac is a mark of newly synthesized H3 and replication associated incorporation of new nucleosomes [93]. Association with H3–K56Ac would also localize MMR proteins to nascent DNA and the replication fork. Additionally, H3–K56Ac creates more flexibility in nucleosome/DNA interactions because the modification is at the entry/exit point of where DNA interacts with the nucleosome [104]. Association with modifications that are indicative of immature chromatin may indicate that MMR proteins interact with DNA while nucleosomal positioning is flexible, creating the ability to scan newly synthesized DNA for mismatches.

5.1 DNA-Damage Tolerance

The base lesion repair pathways of BER and NER are not usually sufficient to repair all damage while DNA is being replicated in S phase [4]. A replication fork will stall at a base lesion because it is unable to incorporate a base opposite the one that is damaged [149]. In this instance, the replication bypass pathway known as DDT or post-replication repair (PRR) is utilized (Fig. 28.5). This allows DNA replication to occur through the damage and permit NER or BER to do the actual repair after replication has occurred. DDT occurs by two mechanisms considered error free (EF) and translesion synthesis (TLS). When EF is selected, the replication template is switched to the undamaged strand in a process that utilizes many of the proteins and mechanisms of HR [150,151]. The template is switched back once replication has bypassed the region that was damaged. In TLS, the high fidelity–replication polymerase is switched to a lower-fidelity

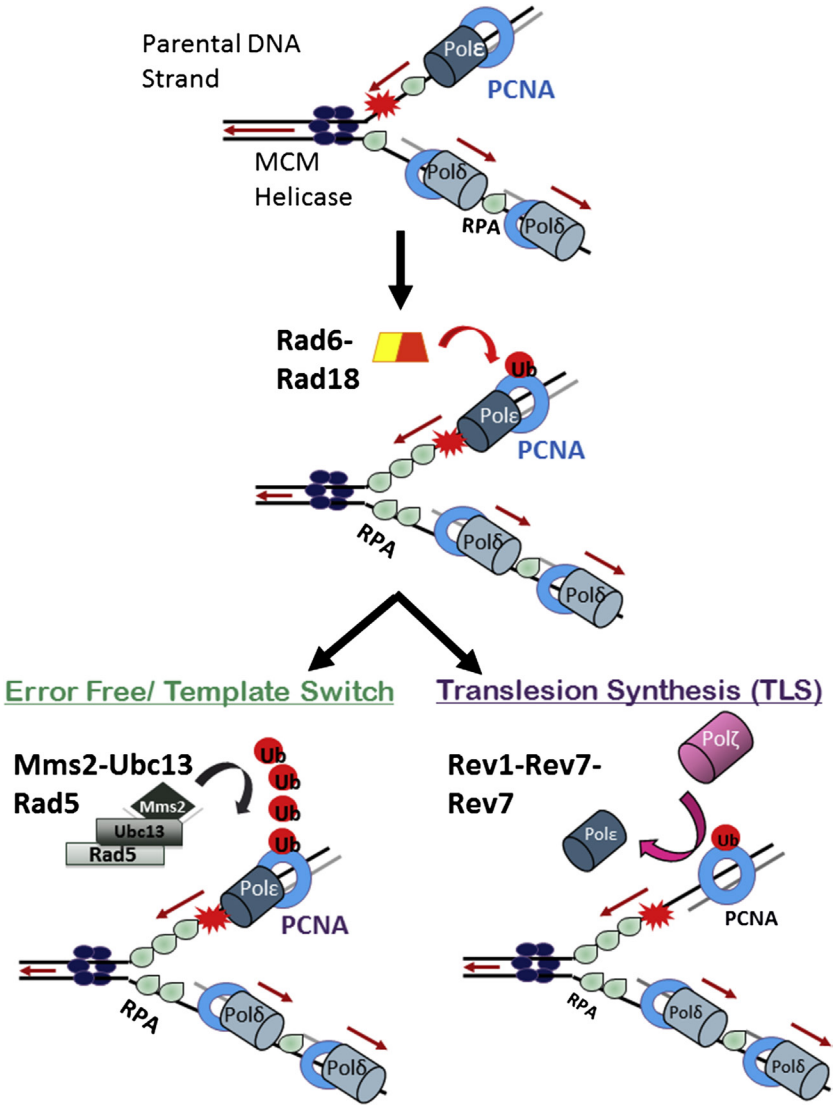

FIGURE 28.5 **Activation of the DNA-damage tolerance (DDT) pathway.** DDT is a means of bypassing base damage that is encountered by a replisome [4]. When a progressing replisome encounters base damage, the complex stalls because the high-fidelity polymerase cannot incorporate a nucleotide across from a damaged base. PCNA becomes ubiquitinated on K164 by Rad6–Rad18. If PCNA remains monoubiquitinated, bypass will proceed by TLS, where a low-fidelity polymerase (Polζ: Rev1, Rev3, and Rev7) replicates past the damaged base. If PCNA is polyubiquitinated by Mms2–Ubc13 and Rad5, bypass will be mediated by EF-DDT.

polymerase that is able to incorporate a base across from the damage, though it may be mutagenic. Once damage is bypassed, the high fidelity–replication polymerase continues DNA replication (reviewed in Sale et al. [149]; Moldovan, et al. [152]; Fu et al. [153]).

5.2 PCNA Modification and DNA-Damage Tolerance

The upstream mechanism that initiates utilization of EF vs. TLS bypass is through the posttranslational modification of the replication sliding–clamp PCNA [154,155] (Fig. 28.5). PCNA is a homotrimeric complex that encircles the DNA duplex and is essential to the function of DNA polymerases. Replication stalling occurs when the polymerase encounters a base lesion, resulting in an accumulation of ssDNA. Rad18, an E3 ubiquitin ligase, is recruited to ssDNA and sumoylated PCNA [156,157], and together with the E2 ubiquitin ligase Rad6, can ubiquitinate PCNA at K164 [154,155,158,159]. If PCNA remains monoubiquitinated, then bypass will proceed by the TLS pathway. However, if PCNA is polyubiquitinated on K164, then the EF pathway will be selected.

5.3 EF-Damage Tolerance

EF-DDT is selected by polyubiquitination of PCNA on K164 [160]. A K63 polyubiquitination linkage is added to PCNA by the E2 ligases Mms2–Ubc13, and E3 ligase Rad5 [158,161–164] (Fig. 28.5). Because of the similarity between EF-DDT and HR, many of the proteins utilized in homology search and replication using the sister template are also critical for template switch. Among the proteins needed for both pathways are Rad51, Rad52, Exo1, the Shu complex, Sgs1, and the 9-1-1 complex [151,165–167].

5.4 Translesion Synthesis–Damage Tolerance

TLS is selected when PCNA remains monoubiquitinated on K164 (Fig. 28.5) [155]. An error-prone polymerase assumes replication for sufficient number of bases to permit the damage to be bypassed and is then displaced by the higher fidelity–replication polymerase (reviewed in Makarova & Burgers [168]). Rev3 is from the same protein family as Pol3 of the Polδ lagging–strand polymerase, and like Pol3, Rev3–Rev7 interacts with the accessory subunits Pol31 and Pol32 [169,170]. The in vivo complex of Polζ also includes Rev1 [171]. Rev1's function when associated with Polζ is independent of its catalytic function, and it appears that acting as a scaffold via association with PCNA and DNA is the critical function of the protein for TLS [172–174]. Because it operates as a trimer with Rev1, Polζ is sometimes dubbed "the mutasome" [168]. Mutasome activity is generally restricted to G2 of the cell cycle, with Rev1 levels being regulated by proteolytic targeting [173,175]. Additionally, the activity of Rev1 has been shown to be regulated by Mec1 phosphorylation [176].

5.5 Checkpoint Signaling, Chromatin, and DDT

Mec1 and Rad53 function in the DDT pathway by different mechanisms, in that Mec1 promotes the TLS pathway, while Rad53 promotes template switch [177,178]. A potential mechanism for this is that Mec1 modifies targets that are distinct from the Rad53 checkpoint kinase that is downstream of it. To differentiate the function of Mec1 and one of its most important kinase targets, Rad53, it is intuitive that Mec1 is affecting processes that are independent of checkpoint activation. This has been demonstrated in its role in the localization and activity of Rev1 in DDT, at a DSB and potentially also with NER [176,179–181]. Genetic analysis of the interaction of the Mec1 target γH2A with the DDT pathway suggests that γH2A also supports TLS [182]. Also, given that Rad9 association with chromatin functions to activate the checkpoint and influence resection, it is possible that a role for Mec1 and Rad9, independent of Rad53 activity, is to restrict resection and favor TLS over EF [136,140]. In budding yeast, EF is preferentially used during S phase of the cell cycle [183]. The mechanisms mediating this bias appear to be chromatin based and could be dependent on the DNA damage–associated scaffold protein Rad9.

The signaling cascade involving ubiquitination of H2B–K123 and methylation of H3–K79 biases DDT away from TLS [184,185]. Histone H2B is ubiquitinated by the E2 and E3 ligases Rad6 and Bre1, respectively. If H2B–K123ub is present on nucleosomes bound by Dot1, its methyltransferase activity is directed toward H3–K79 [186,187]. Genetic interactions of *DOT1* support a role for it as promoting template switch DDT [184,185]. Consistent with a function in EF-DDT, loss of *DOT1* produces an increase in the rate of spontaneous mutagenesis, which is used as an indirect measurement of TLS polymerase activity. H3–K79me3 may be bound by Rad9 in the event of DNA damage via its Tudor domains [188] (Fig. 28.6). The interaction of *DOT1* with *RAD9* is dependent on the type of DNA damage, in that they are additively sensitive when treated with IR, but epistatic when treated with the base lesion causing agent methyl methanesulfonate (MMS). This indicates that Rad9 is associated with H3–K79me3 to mediate the survival promoting response to base lesion–induced damage. Support for Rad9 cooperating with EF is that a deletion mutant has a high rate of spontaneous mutagenesis which is dependent on functional TLS polymerases [189]. Therefore, a model for H3–K79me3 promoting EF could be that *dot1*Δ mutants combined with repair defects do not accumulate damage intermediates due to their mutated repair pathways because they undergo unregulated TLS bypass of the damage due to the inability of Rad9 to block TLS (Fig. 28.6). Dot1/H3–K79me3-mediated recruitment of Rad9 is an important activator of Rad53, and initiation of resection, which, by genetic interactions, is shown to support EF-DDT. Mec1 and γH2A promote TLS. It may be that Mec1 and the γH2A alter the chromatin platform of DDR to create an environment more conducive to TLS by inhibition of Rad9.

Other chromatin components have been found to have a role in DDT using the trinucleotide repeat screen. The DDT pathway is important for accurate replication of genomic regions that are difficult to replicate due to repeats of CAG/CTG sequences. Trinucleotide repeats are fragile sites within the genome that are vulnerable to expansion and contraction due to their tendency to spontaneously form DNA hairpins when unwound [190]. Errors in DNA repair, namely BER [191–194], lead to replication fork stalling at repeat expansions [195]. Defects in genes associated with HR and EF have a higher level

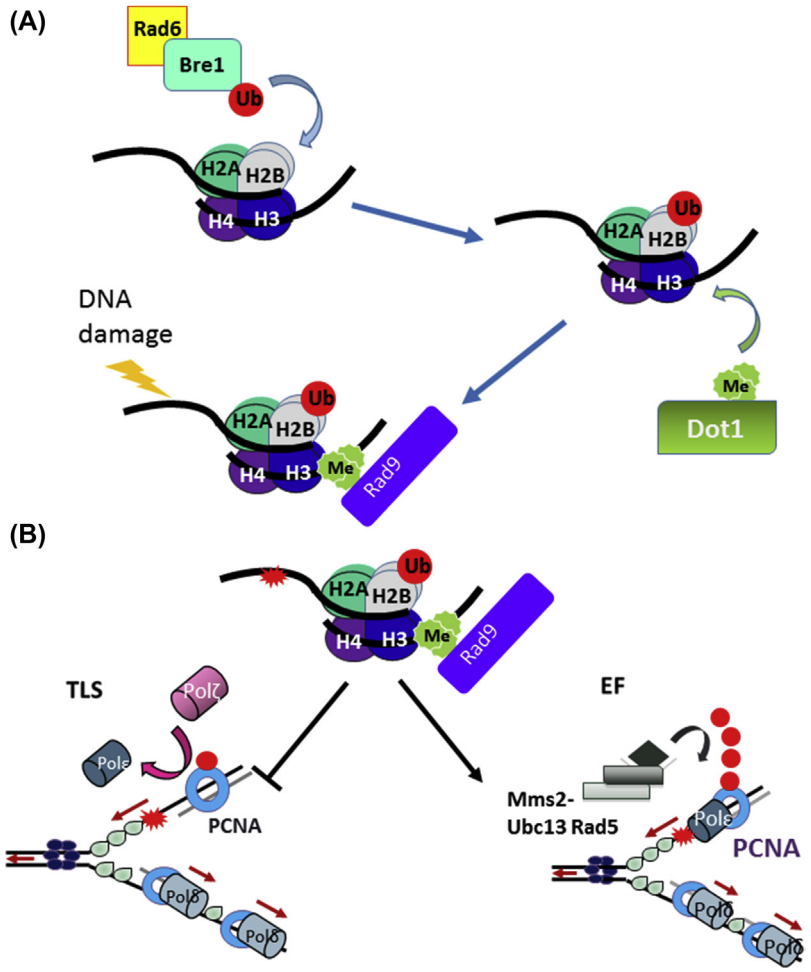

FIGURE 28.6 **The histone H3–K79me3 modification biases DDT toward EF-mediated bypass.** Rad9 is able to interact with the H3–K79me3 chromatin modification via its Tudor domains [186,187] (A). This signal cascade biases DDT toward the EF pathway by unknown mechanisms (as of 2016). Loss of DOT1 rescues mutants of the EF pathway and other damage-repair pathways by permitting unregulated TLS to occur [184,185] (B).

of repeat instability [195]. Contrary to EF-associated genes, TLS genes can lead to a greater rate of errors when other genes important for repeat stability are mutated, such as the *MPH1* helicase [196] or the replicative polymerases, Pol2 (ε) and Pol3 (δ) [197]. Additionally, TLS polymerases and template switch genes may cooperatively introduce repeat expansions at stalled replication structures [198].

Hmo1 (high mobility group protein 1) localizes to repeat tracts and reduces instability [199], a function consistent with its endogenous role of binding to the gene bodies of rDNA to prevent DNA-hairpin formation during transcription [200]. It was later shown that Hmo1 binding with DNA predisposes the pathway choice toward the EF pathway with a predicted model in which Hmo1 bends ssDNA to favor the initiation of strand invasion into the duplicated sister strand for template switch [201].

The acetyltransferase Rtt109, which acetylates H3K56 and strongly contributes to nucleosome–DNA interaction flexibility has been shown to have a role in suppressing repeat contractions [202]. In a synthetic genetic analysis screen, Rtt109 has been shown to favor TLS DDT [203]. A 2014 publication demonstrates that the acetyltransferase complex NuA4 and chromatin remodeler RSC suppress Rad5-dependent trinucleotide repeat expansions [204]. Without NuA4-acetylating histones which then recruit RSC to the region, EF bypass is highly error prone. Together, these reports indicate the need for manipulation of nucleosome–DNA interactions to either prevent hairpins from forming or cooperatively resolve them when they do occur.

A study that more comprehensively explored the role of NuA4 in DDT revealed that the primary role of the complex was toward the process of TLS [205]. Mutants of NuA4 reduced the rate of spontaneous mutagenesis in addition to increasing the sensitivity of mutants of EF to MMS. In addition to NuA4, its downstream effectors H4 and H2A.Z demonstrated

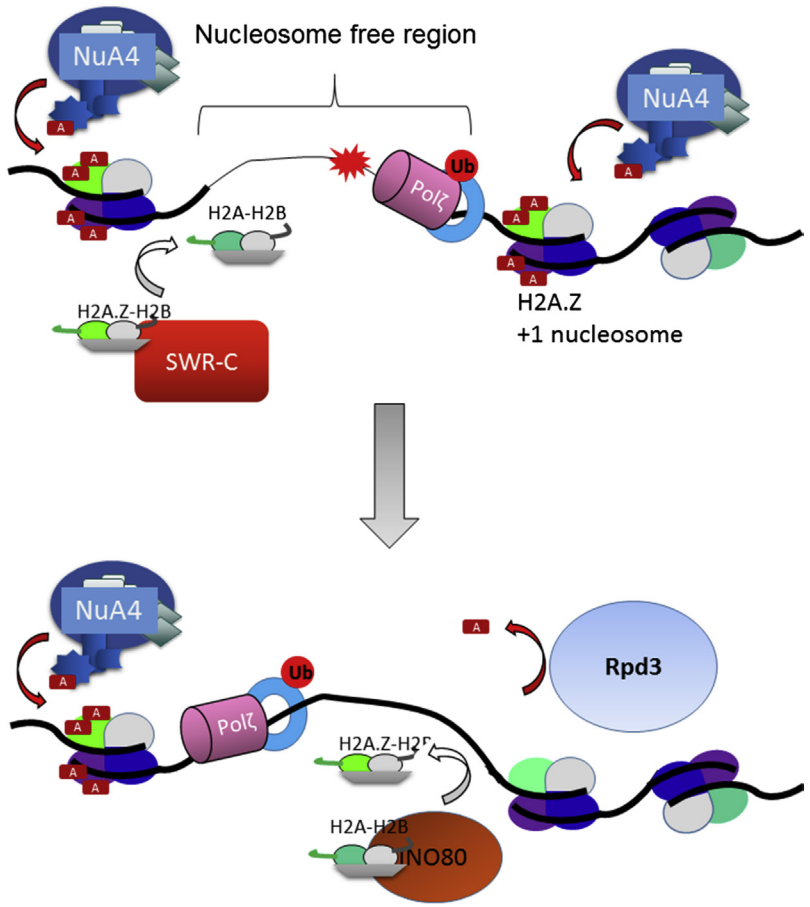

FIGURE 28.7 Model of NuA4 and other regulators of H2A.Z in promoting function of TLS polymerases. Regulators of H2A.Z function to promote TLS. TLS preferentially occurs in G2 after the bulk of replication completion, where remaining lesions in the genome are gaps of ssDNA [183]. NuA4 acetylates H4 proximal to damaged DNA, stabilizing SWR-C at the region, and leading to H2A.Z incorporation at regions proximal to damage. Acetylation of H2A.Z may stabilize its retention further by protecting it from INO80-mediated removal [83]. The presence of H2A.Z creates a boundary to an NFR surrounding the lesion and ssDNA gap [79,206]. Following TLS of the lesion, INO80 as well as SWR-C and Rtt109 facilitate removal of H2A.Z from the damage site to restore the chromatin environment after damage [84].

genetic interactions with the DDT pathway. An interpretation of this is that complexes that control H2A.Z incorporation and removal perform a function that is important to allow TLS (Fig. 28.7). NuA4 acetylates H4 to create binding sites for the bromodomains of SWR-C. NuA4 further stabilizes H2A.Z at specific loci by acetylation. Rtt109 facilitates removal of H2A.Z by acetylation of H3–K56, permitting SWR-C to remove this variant.

A function for H2A.Z may be to keep the chromatin environment accessible until replication over the damaged region has occurred. H2A.Z nucleosomes are considered to be less stable than canonical nucleosomes where a dynamic instability exists at transcription start sites, yet they can form stable barriers to the spread of heterochromatin. Perhaps the most critical function of H2A.Z behind the fork is to maintain a flexible chromatin environment that is accessible to TLS polymerases. When damage is present, NuA4 mediated acetylation of the N-terminus of H2A.Z may be needed to protect it from removal by INO80 and following TLS, H2A.Z would be deacetylated, allowing its removal by INO80 or SWR-C.

6. OVERVIEW: THE RELATIONSHIP BETWEEN CHROMATIN AND REPAIR CHOICE

The modification of chromatin is essential for optimal transcription, replication, and DNA repair. In spite of the inherent differences in these DNA processes, there are consistent steps in histone-modification pathways to enable these functions.

Chromatin opening is facilitated by posttranslational modifications of nucleosomes. Acetylation is often the first modification step to create open chromatin at transcription start sites, newly translated nucleosomes, and regions of damage. The hierarchical modifications of acetylation are often followed by the activity of remodelers such as SWR-C, RSC, SWI/SNF, and INO80, many of which have bromodomains that allow interactions with acetylated nucleosomes. NuA4- and

SAGA-mediated nucleosome acetylation followed by SWR-C-mediated histone exchange of H2A for H2A.Z, is associated with transcriptional start sites, heterochromatin boundaries, and TLS-DDT. Common pathways are also involved in chromatin condensation following replication, transcription, and repair. HDAC complexes such as RPD3 deacetylate histones and ISWI alters spacing between nucleosomes.

The hierarchy of interactions is also dependent on what the process requires and the phase of the cell cycle. Throughout the cell cycle, histone modifiers and remodelers participate in repair processes of BER and NER to shift nucleosomes and allow repair machinery to access DNA. In the case of the DSB-repair processes of NHEJ and HR, very different modifications around the break occur, in a cell cycle–dependent manner. In the case of HR, which occurs in S and G2, and requires resection to create access to large tracts of DNA, the acetyltransferases SAGA and NuA4, as well as the chromatin remodelers RSC, SWI/SNF, SWR-C, and INO80 localize to DSBs. However, for NHEJ which is restricted mostly to G1, resection is inhibited, and therefore there is a low level of recruitment of HATs and remodelers, but an abundance of γH2A and yKu70/yK80 [117]. The differences in chromatin modifications required for these two processes strongly illustrates the interplay between chromatin-modification complexes and repair pathways.

The use of the budding yeast model has been critical in gaining understanding of the involvement of chromatin modifications in processes associated with DNA transcription, replication, and repair. The goal of this chapter was to highlight the consistencies of chromatin-modifying activities in these processes, so as to illustrate cooperative behaviors among complexes and enable a better understanding of how they function in repair and genome stability. Indeed, some chromatin modifications play a role in repair that is so extensive as to determine repair-pathway outcome, such as with the replication-damage pathway of DDT. A full understanding of the many chromatin modifications needed for efficient DNA repair are clearly relevant to understanding human disease. This is particularly evident given that many human cancers likely originate from errors in DNA replication accumulated during the many cycles that stem cells need to undergo throughout the lifetime, explaining, in part, the strong linkage between age and the incidence of all forms of cancer [207].

GLOSSARY

Mutasome Protein complex allowing error-prone translesion synthesis.

LIST OF ABBREVIATIONS

BER Base excision repair
DDT DNA-damage tolerance
DSB Double-strand break
EF Error free
FHA Forkhead-associated domain
GG Global genome
HDAC Histone deacetylase
HKDM Histone lysine demethylases
HKMT Histone lysine methyltransferases
HR Homologous recombination
MMEJ Microhomology-mediated end joining
MMR Mismatch repair
NER Nucleotide excision repair
NHEJ nonhomologous end joining
NRFs Nucleosome-free regions
PHD Plant homeodomains
PRR Postreplication repair
TC Transcription coupled
TLS Translesion synthesis

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