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Chromatin Disassembly and Reassembly During DNA Repair

Jeffrey G. Linger and Jessica K. Tyler*

Department of Biochemistry and Molecular Genetics, School of Medicine, University of Colorado, Aurora, CO 80045.

Abstract

Current research is demonstrating that the packaging of the eukaryotic genome together with histone proteins into chromatin is playing a fundamental role in DNA repair and the maintenance of genomic integrity. As is well established to be the case for transcription, the chromatin structure dynamically changes during DNA repair. Recent studies indicate that the complete removal of histones from DNA and their subsequent reassembly onto DNA accompanies DNA repair. This review will present evidence indicating that chromatin disassembly and reassembly occur during DNA repair and that these are critical processes for cell survival after DNA repair. Concomitantly, candidate proteins utilized for these processes will be highlighted.

Keywords

histone chaperone; chromatin assembly; chromatin disassembly; ATP dependent remodellers

1. Overview of chromatin disassembly and assembly

Chromatin is comprised of a basic repeating unit called the nucleosome. The nucleosome core particle is made up of a histone octamer (two molecules each of histone proteins H2A, H2B, H3, and H4), which has approximately 147 base pairs of DNA wrapped around it [1]. The nucleosome core particles are regularly spaced along the DNA (separated by a DNA linker of 20–110 bp) and coat the entire nuclear genome of all eukaryotes to form the 11nm fiber of chromatin. This is further compacted into higher order chromatin structures resulting in the condensation of the human genome more than 10,000 fold during mitosis. It has been known for a long time that this packaging of the eukaryotic genome into chromatin is essential for the DNA to fit inside the nucleus. Additionally, the chromatin structure tightly regulates processes occurring on the DNA template, including DNA repair, replication and transcription. Interestingly, chromatin structure also carries critical epigenetic information that maintains transcription programs through cell divisions and this is a feature that is fundamentally important for development and differentiation.

By its physical nature, chromatin structure provides a formidable obstacle to the DNA repair machinery attempting to gain access to DNA lesions. Indeed, DNA repair *in vitro* is significantly reduced or prevented when a DNA lesion is contained within a nucleosome [2–9]. By contrast, DNA repair *in vivo* occurs efficiently throughout the entire genome, indicating

*Correspondence should be addressed to Jessica Tyler, Mail Stop 8101, PO Box 6511, Aurora, CO 80045 USA. Phone: 303 724 3224; Fax: 303 724 3221; Email: Jessica.tyler@uchsc.edu.

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that the cell has likely developed mechanisms to unwrap the chromatin during DNA repair and repackage the DNA into chromatin following DNA repair. Accordingly, a growing body of evidence indicates that histones are locally removed in the vicinity of the DNA lesion – a process termed “chromatin disassembly”, and that histones are then re-deposited onto the DNA – a process termed “chromatin assembly”, following DNA repair.

The process of chromatin assembly is best understood during DNA replication and is likely to occur in a similar manner during DNA repair. During DNA replication, histone proteins are removed from the parental chromatin strand ahead of the DNA replication machinery (Fig. 1A). The key evidence for chromatin disassembly during DNA replication is the observation that approximately 225–285 bp of naked DNA remains behind the replication fork (as demonstrated by electron microscopic analyses of psoralen crosslinked replicating SV40 DNA isolated from human cells [10]). However, it is not known whether this chromatin disassembly during DNA replication is the passive consequence of the DNA replication machinery physically displacing the histones as it progresses along the DNA or whether it is an active process mediated by specific chromatin disassembly factors.

Chromatin assembly onto the newly-replicated naked DNA occurs in a stepwise manner. Histones H3/H4 are first deposited to form the H3/H4 heterotetramer on DNA, followed by addition of two heterodimers of histones H2A/H2B to complete the nucleosome (Fig. 1A). This histone deposition during DNA replication is mediated by histone binding proteins termed “histone chaperones”. The purpose of histone chaperones is to shield the highly positively charged histone proteins from the negatively charged DNA, in order to slow down the interaction between histones and DNA enough to allow the ordered formation of the nucleosome structure. In the absence of histone chaperones *in vitro*, DNA and histones rapidly bind to each other to form highly disordered insoluble aggregates [11].

The establishment of an *in vitro* chromatin assembly system coupled to DNA replication [12] was critical for the subsequent identification of histone chaperones. Biochemical fractionation of the crude chromatin assembly extracts led to the discovery of Chromatin Assembly Factor 1 (CAF-1) and Anti-silencing function 1 (Asf1), which both mediate H3/H4 deposition during DNA replication [13,14]. Although both Asf1 and CAF-1 are by themselves histone H3/H4 chaperones, they work jointly together to deposit H3/H4 onto newly-replicated DNA *in vitro* [13] (Fig. 1A). The functional coordination between Asf1 and CAF-1 during chromatin assembly may be mediated via the direct physical interaction between these proteins [15–17] (Fig. 1A). Similarly, the recruitment of these histone chaperones to sites of DNA replication is likely mediated via a direct interaction between CAF-1 and a component of the replication machinery, Proliferating Cell Nuclear Antigen (PCNA) [18] (Fig. 1A). Asf1 binds to a heterodimer of histones H3/H4 rather than a heterotetramer of H3/H4 *in vitro* and *in vivo* [19,20], indicating that the H3/H4 heterotetramer on DNA is assembled from two H3/H4 heterodimers, although the mechanism for this is unclear. In addition to functioning together *in vitro*, Asf1 and CAF1 are likely to assemble chromatin onto newly-replicated DNA in cells. For example, Asf1 and CAF-1 localize to DNA replication forks [21,22] and their inactivation results in major defects in chromatin assembly of the newly-replicated DNA in cells [23,24]. H2A/H2B deposition following DNA replication is presumably also mediated by histone chaperones, where possible candidates include the NAP1/SET/TAFI [25] and FACT/SPN proteins [26].

Each nucleosome core particle is extremely stable, being held together by many interactions between the DNA and histones [1]. Presumably, the processes of chromatin assembly and disassembly require large amounts of energy expenditure. Indeed, chromatin assembly requires the hydrolysis of approximately 2–4 molecules of ATP per base pair of DNA [27]. In order to provide the necessary energy for chromatin assembly, histone chaperones are generally assisted

by ATP-dependent chromatin remodeling complexes [11] (Fig. 1A), which are discussed in much greater detail in Chapters X and X. In the present review, we focus on their role in chromatin assembly and disassembly. ATP-utilizing chromatin assembly and remodeling factor (ACF) and remodeling and spacing factor (RSF) are specific examples of ATP-dependent chromatin remodeling complexes that translocate along the DNA using energy supplied by ATP hydrolysis [27–29]. This activity results in the regular spacing of nucleosomes during chromatin assembly *in vitro* and also likely *in vivo* because ACF promotes chromatin formation during DNA replication in *Drosophila* [30].

Chromatin disassembly occurs by a stepwise process that appears to be the reverse of chromatin assembly. During chromatin disassembly, the H2A/H2B dimers are removed from the nucleosome, followed by removal of the H3/H4 heterotetramer [31]. Chromatin disassembly is also mediated by histone chaperones during processes other than DNA replication. For example, chromatin is disassembled from some yeast gene promoters during transcriptional activation by the H3/H4 chaperone Asf1 [32]. Another histone chaperone, FACT (facilitates chromatin transcription), mediates the transient disassembly and reassembly of histones H2A/H2B within open reading frames of genes to facilitate passage of RNA polymerase II [33]. Similarly, the histone H3/H4 chaperone Spt6 mediates chromatin reassembly within open reading frames following RNA polymerase II passage in yeast [34] and the reassembly of promoter nucleosomes during transcriptional repression [35]. The cooperation that exists between ATP-dependent chromatin remodelers and histone chaperones during chromatin assembly following DNA replication is likely to also be a feature of chromatin disassembly and assembly during DNA repair. Our understanding of chromatin disassembly and reassembly is more complete during DNA replication and transcription, but undoubtedly these processes also occur during DNA repair.

2. Chromatin assembly factors and genetic links to DNA repair

The inactivation of particular histone chaperones and ATP-dependent chromatin remodelers leaves cells hypersensitive to DNA damaging agents. This observation has often been the initial trigger into further analyses of their role in DNA repair. These DNA damage sensitivities are summarized in Table 1 and are mentioned where relevant during the discussion of specific DNA repair pathways below. Any potential roles for essential chromatin assembly factors, such as Spt6 and FACT, during DNA repair can not be revealed by the analysis of sensitivity of mutants to damaging agents, because the assays depend on viability of mutant cells.

3. Chromatin assembly during excision repair

Nucleotide excision repair (NER) removes bulky DNA lesions from DNA, including those caused by UV light, such as cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts. Early analyses of nuclease accessibility during UV-induced DNA synthesis in mammalian cells provided the first observation that DNA becomes more accessible during DNA repair and less accessible after DNA repair is complete [48]. This led to the ‘access, repair, restore’ model which proposes that the nucleosome is unpackaged allowing the DNA repair machinery to access the DNA lesion, followed by restoration of the chromatin structure after DNA repair [49][50]. Only approximately 30 bases are excised and replaced during NER, suggesting that chromatin disassembly may not necessarily be required. Chromatin remodeling or the lateral movement of histones along the DNA would initially appear to be sufficient for NER. However, the available evidence indicates that not only do chromatin disassembly and reassembly occur during NER, but that they are also required for viability after NER. The major evidence for this assertion is provided by the links between the histone H3/H4 chaperones CAF-1 and Asf1 and NER *in vivo* and *in vitro*. The differential feature between chromatin assembly/disassembly and chromatin remodeling is that the former but not the latter requires histone chaperones.

The development of an *in vitro* chromatin assembly assay coupled to NER by the Almouzni lab has been particularly valuable in demonstrating that histone chaperone function during NER [51]. *In vitro*, CAF-1 mediates chromatin assembly coupled to NER on naked DNA templates [51] (Fig. 1B). This CAF-1-mediated chromatin assembly occurs in a bidirectional manner from the DNA lesion and is triggered by the initiation rather than the completion of NER [52]. PCNA is required for the repair synthesis step of NER [53]. In turn, PCNA recruits CAF-1 to reassemble chromatin following NER [17,54,55].

Although it will be technically difficult to directly demonstrate that chromatin is disassembled and reassembled during NER *in vivo* due to the sequence-independent nature of the DNA lesions, there is ample circumstantial evidence to indicate that this does occur. For example, the histone chaperone CAF-1 co-localizes with sites of NER *in vivo* [50,56]. Furthermore, budding yeast deleted for any of the three genes encoding the subunits of CAF-1, *CAC1/RLF2*, *CAC2* or *CAC3/MSH1*, are highly sensitive to UV irradiation [39]. The histone chaperone Asf1 also cooperates with CAF-1 to assemble nucleosomes following nucleotide excision repair *in vitro* [17] (Fig. 1B) as has been shown for chromatin assembly following DNA replication (Fig. 1A). Consistent with a role for Asf1 in chromatin assembly during NER, yeast *asf1* mutants are sensitive to UV irradiation [36].

The identity of the ATP-dependent chromatin remodeler that likely cooperates with histone chaperones to mediate chromatin disassembly and / or assembly during NER in the cell is unknown. *In vitro*, the ATP-dependent chromatin remodeler ACF can facilitate the dual incision step of NER within the linker DNA of a dinucleosomal template, although not within the nucleosome core particle [9]. Similarly, the ATP dependent chromatin remodeler SWI/SNF can facilitate repair within the nucleosome core particle by a reconstituted human NER system [57]. It is possible that the addition of histone chaperones may further increase the efficiency of NER *in vitro* by their ability to unpack chromatin, but this has not yet been experimentally demonstrated.

The base excision repair (BER) pathway is used to remove smaller alterations of bases than the NER pathway, such as 8-oxoguanine and uracil. Although it remains to be determined whether BER is accompanied by chromatin disassembly and reassembly, at least some steps of base excision repair can occur as efficiently on nucleosomal templates as on naked DNA *in vitro*. These include the FEN1-mediated DNA cleavage and DNA ligase I-mediated ligation [58,59]. However, replacement of the correct nucleotide by DNA polymerase beta is blocked by nucleosomes *in vitro* [60]. While it is possible that chromatin remodeling rather than gross chromatin alterations such as chromatin disassembly and reassembly may be sufficient for short patch BER, long patch BER is likely to require chromatin disassembly and reassembly. Accordingly, the histone chaperone CAF-1 colocalizes with the BER machinery in repair foci in cells undergoing base excision repair [61]. This study cleverly examined repair foci in quiescent cells rather than dividing cells in order to ensure that they were not observing foci of CAF-1 due to its role in DNA replication. This study indicates that chromatin assembly and therefore disassembly is likely to accompany at least some forms of BER.

4. Chromatin disassembly and assembly during double-strand DNA repair

The efficient and accurate repair of DNA double-strand breaks (DSBs) is vital for the maintenance of genomic integrity because failure to do so causes changes to the DNA sequence, potentially leading to cancer. DSBs are created by a wide variety of endogenous and exogenous agents including alkylating agents, radiomimetics and gamma irradiation. Cells employ two major pathways to repair a DSB, homologous recombination and non-homologous end joining (NHEJ). Homologous recombination can be thought of as ‘patching’ the DSB by copying homologous donor sequences [62,63]; this process involves resection of the DNA ends to yield

3' single-stranded DNA tails, which invade an intact homologous donor sequence (Fig. 1C) [64,65]. DNA strand exchange follows and the 3' ends are extended by DNA synthesis and the resulting interlinked DNA molecules are then resolved. On the other hand, repair of a DSB by NHEJ does not require sequence homology and can occur by simple religation [66]. Although the relative utilization of the different DSB pathways differs between yeast and vertebrates, they have been remarkably conserved through evolution [63].

An *in vitro* chromatin assembly system coupled to double-strand DNA repair has yet to be developed analogous to those used for DNA replication and NER. This has hindered the biochemical identification of factors that may be assembling chromatin during double-strand repair, which instead has occurred using cell biological and genetic approaches. A key indication that chromatin disassembly and reassembly are important during double-strand DNA repair was provided by the increased sensitivity of cells lacking the histone chaperones CAF-1 and Asf1 to double-strand DNA damaging agents (Table 1).

The histone H3/H4 chaperone CAF-1 is recruited to sites of double-strand DNA repair. Specifically, CAF-1 localizes to repair foci in response to the alkylating agent MNNG [67] and the radiomimetic agent bleomycin [61]. Data has also been published showing that CAF-1 is specifically recruited to immobilized DNA templates following gamma-irradiation [17]. However, the implication that CAF-1 may be recruited to double-strand DNA breaks was not specifically discussed in the text of the manuscript [17]. The available evidence indicates that CAF-1 localizes to sites of double-strand repair, but how is CAF-1 recruited to double-strand breaks? Given the manner in which CAF-1 is recruited to sites of NER and DNA replication (Fig. 1A and B), the obvious candidate to mediate CAF-1 recruitment is PCNA. Consistent with this prediction, point mutations in PCNA that specifically disrupt its interaction with CAF-1 lead to DNA damage sensitivities identical to that of CAF-1 mutants in yeast, and the further deletion of CAF1-encoding genes does not increase the DNA damage sensitivity of these specific PCNA mutants [41]. Perhaps also relevant to its role in double-strand DNA repair, CAF1 binds to the BLM protein, a RecQ family helicase, whose mutation leads to the genomic instability disorder known as Bloom's syndrome that is characterized by cancer susceptibility [67]. Although the functional consequence of the interaction between CAF-1 and BLM is not yet known, CAF-1 and BLM colocalize in cells during DNA repair [67].

CAF-1 has been implicated in not only homologous recombination, but also in NHEJ. The evidence for a role for CAF-1 in NHEJ comes from its colocalization with the NHEJ component XRCC4 in tissue culture cells [61]. Furthermore, the additional inactivation of CAF-1 in a yeast strain that is deleted for *RAD52* (disabling homologous recombination) leads to increased DNA damage sensitivity as compared to a strain deleted for *RAD52* only [41]. By eliminating the homologous recombination pathway in this manner, the cell is forced to use NHEJ and since the further inactivation of CAF-1 leads to even greater sensitivity to damage, this indicates genetically that CAF-1 is contributing to the NHEJ pathway. On the surface, a role for CAF-1 during NHEJ is somewhat surprising because CAF-1 is typically coupled to DNA synthesis and NHEJ does not involve significant DNA synthesis. However, NHEJ may involve nucleosome removal to facilitate access of the NHEJ machinery to the DNA lesion and therefore nucleosome replacement could be accomplished by CAF-1 after NHEJ is complete. It is noteworthy that the component of the replication machinery, PCNA, that is known to recruit CAF-1 to sites of DNA synthesis, has been shown to interact with the NHEJ protein Ku70/80 heterodimer and this interaction is enriched after DNA damage [68]. Furthermore, PCNA is actually required for NHEJ [69,70].

The sensitivity of yeast and Arabidopsis CAF-1 mutants to double-strand DNA damaging agents (Table 1) suggested that CAF-1 is important for double-strand DNA repair. Mutation of CAF-1 results in a globally under-assembled genome as a consequence of loss of its role in

chromatin assembly coupled to DNA replication [71]. Consequently, it was important to address the possibility that the less protected nature of the genome in cells lacking CAF-1 may be responsible for the increased sensitivity to DNA damage. Precedent exists for this scenario. Yeast lacking the HMG protein Nhp6A/B are hypersensitive to DNA damaging agents as a consequence of increased amounts of DNA lesions due to the more open nature of the chromatin [72]. This is not the case for yeast lacking CAF-1, because the rapid degradation of CAF-1 concomitant with double-strand DNA damage still leads to increased DNA damage sensitivity – indicating that CAF-1 function during or shortly after repair is important for cell viability following repair [41]. Conversely, when CAF-1 is induced only following the exposure to DNA damage, this is sufficient to restore DNA damage resistance [41]. As such, the histone chaperone CAF-1 is recruited to the sites of double-strand DNA damage and is important for cell survival due to its role following double-strand DNA repair. We now await a direct demonstration that CAF-1 is mediating chromatin assembly during double-strand DNA repair (Fig. 1C).

Other histone chaperones have been implicated in playing a role in double-strand DNA repair. For example, the Hif1 H3/H4 histone chaperone is recruited to a double-strand break in yeast [73]. Furthermore, yeast deleted for *HIF1* are sensitive to an induced double-strand break when combined with versions of histone H3 that cannot be acetylated [74]. However, due to the central role of Hif1 in the Hat1/Hat2/Hif1 histone acetyltransferase complex [74], the role of Hif1 in repair may be a consequence of its role in facilitating acetylation of histones H3/H4 rather than chromatin assembly/disassembly. Another histone H3/H4 chaperone, Asf1, has also been implicated in playing a role in double-strand DNA repair. The main evidence for this is the sensitivity of yeast lacking Asf1 to double-strand DNA damaging agents (Table 1). Interestingly, yeast lacking Asf1 are more sensitive to double-strand DNA damaging agents than yeast lacking CAF-1 mutants, while yeast lacking both Asf1 and CAF-1 are even more sensitive than either single mutant [41]. This suggests that Asf1 plays a more central role and at least a partially distinct role as compared to CAF-1 during double-strand DNA repair. Given that Asf1 can act as either a chromatin disassembly factor [32] or a chromatin assembly factor [13], we propose that Asf1 may be disassembling chromatin prior to DNA repair (Fig. 1C). Furthermore, given that chromatin assembly during NER and DNA replication requires both Asf1 and CAF-1 (Fig. 1A and B), we predict that Asf1 and CAF-1 assist each other during chromatin assembly following double-strand DNA repair (Fig. 1C). These predictions are supported by our studies (B. Tamburini and J. Tyler, unpublished data).

Chromatin assembly and disassembly is usually a coordinated effort between histone chaperones and an ATP-dependent chromatin remodeler. It is then relevant to ask which ATP-dependent chromatin remodeling factor assists the histone chaperones during chromatin assembly and disassembly during double-strand DNA repair? Several ATP-dependent chromatin assembly factors, including RSC, SWI/SNF, SWR1 and INO80, have been implicated in double-strand DNA repair. In fact, the most direct evidence for the role of any factor in chromatin disassembly during double-strand DNA repair exists for the ATP-dependent chromatin remodeler INO80 [75]. This study provides the first direct evidence for chromatin disassembly during double-strand DNA repair and these findings will be discussed in more detail below. However, we will first explain the nature of these studies because the molecular techniques that were used are extremely powerful for analyzing chromatin structure around a localized DSB *in vivo*.

The study of double-strand DNA repair in budding yeast has been greatly facilitated by the use of an inducible HO endonuclease which cleaves only once in the genome at the HO site within the *MAT* locus [76]. This HO lesion is normally repaired by homologous recombination with either the *HMR* or *HML* donor locus. Deletion of the *HMR* and *HML* donor loci in haploid yeast results in repair of the HO lesion by NHEJ [77], but the continued expression of the HO

endonuclease results in recleavage of the HO site. In fact, the only way that the yeast lacking the *HMR* and *HML* loci can permanently repair the HO lesion is by performing excessive DNA resection to identify small regions of homology that can mediate inaccurate NHEJ, such that the HO endonuclease recognition site is abolished. Consequently, the induction of the HO endonuclease in strains deleted for *HMR* and *HML* generates a double-strand lesion that is permanently repaired at a very low efficiency, providing the ideal template for performing analysis of recruitment of factors to a double-strand DNA break using the chromatin immunoprecipitation (ChIP) technique. This powerful system has been fundamentally important in the majority of studies in recent years revealing histone modifications, chromatin changes, and recruitment of repair machinery and chromatin modifying complexes around a DSB.

The study of the ATP-dependent chromatin remodeling factor INO80 in chromatin disassembly around a DNA lesion used expression of the HO endonuclease in yeast lacking the *HMR* and *HML* donor loci [75]. This study showed that histone levels decrease in the vicinity of the DNA lesion by histone ChIP analyses [75], indicating localized chromatin disassembly at the DSB. Additionally, the authors demonstrate that the DNA resection machinery (the MRX complex) is required for chromatin disassembly around the DSB [75] (Fig. 1C). They showed that deletion of the gene encoding the Arp8 component of the INO80 ATP-dependent chromatin remodeling complex also results in reduced chromatin disassembly around the DSB. As such, this study demonstrated that DNA resection and the ATP-dependent chromatin remodeler INO80 contribute to chromatin disassembly during double-strand DNA repair. There are two possible scenarios for how INO80 contributes to chromatin disassembly around a DSB. INO80 may directly assist the histone chaperones in chromatin disassembly around a DSB via its ability to break histone-DNA contacts (Fig. 1C). Alternatively, because *arp8* mutants have a defect in DNA resection [78], INO80-dependent chromatin remodeling may help recruit the MRX complex to the DNA or may facilitate the ability of the MRX complex to mediate DNA resection on chromatin. In this scenario, chromatin disassembly may be the consequence of the resection machinery physically displacing histone proteins from the DNA – this is easy to imagine given the extensive contacts that are made between the DNA resection machinery and the DNA double-helix during the 5'-3' exonucleolytic attack. Further studies are required to establish whether chromatin disassembly around a DSB is in fact an active process mediated by histone chaperones and ATP-dependent chromatin remodelers or whether chromatin disassembly is a passive consequence of DNA resection physically displacing the histones. In either case, the clear demonstration that chromatin is disassembled during double-strand repair by the Osley lab infers that chromatin is also reassembled after DSB repair.

5. Regulation of histone chaperones by the DNA damage checkpoint

The DNA damage checkpoint is integral to DNA repair as it detects and signals the presence of DNA damage and arrests cell cycle progression until the damage is repaired [79]. The DNA damage checkpoint is initiated by the recruitment of multiple checkpoint components to the DSB by the DNA repair machinery, including the PI3family kinase ATR in mammals and its homolog Mec1 in yeast [80]. Once recruited to the DNA, ATR / Mec1 phosphorylates and activates downstream targets in the DNA damage checkpoint signaling cascade. A key downstream target of Mec1 is the effector kinase Rad53 [81]. Rad53 is important for maintaining nucleotide levels necessary for DNA synthesis and arresting the cell cycle at the metaphase to anaphase transition [82]. Once DSB repair is complete, the DNA damage checkpoint signals are reversed and the checkpoint is inactivated by a process that has been dubbed “checkpoint recovery”, so that cells can resume cell cycle progression. As one of the earliest responses to a DSB, Mec1 phosphorylates histone H2A on serine 129 (or serine 139 on the histone variant H2AX in mammals) in the chromatin flanking the lesion [83,84]. Intriguingly, dephosphorylation of H2A is essential for recovery from the DNA damage

checkpoint after DNA repair and this dephosphorylation can only happen after disassembly of phosphorylated H2A from chromatin [85].

The Asf1 and CAF-1 histone chaperones are activated in response to DNA damage, albeit in different ways. Quiescent cells normally only have trace amounts of the CAF-1 protein, but CAF-1 levels are greatly upregulated in response to the radiomimetic agent bleomycin and to ionizing radiation [61]. It is not known whether increased CAF-1 levels after damage are a result of transcriptional induction or stabilization of this normally very unstable protein complex. However, this work indicates that a novel response to DNA damage is the increase in the abundance of the CAF-1 histone chaperone that may be involved in chromatin assembly / disassembly during DNA repair (Fig. 2A). By contrast, the Asf1 histone chaperone is regulated directly by physical contact with a DNA damage checkpoint component Rad53. Seemingly all inactive Rad53 kinase in yeast is bound to Asf1 and this interaction prevents Asf1 from assembling chromatin [86,87]. Furthermore, there is evidence indicating that phosphorylation of Asf1 by Rad53 is required for this interaction [88]. Upon activation of the DNA damage checkpoint, Rad53 becomes phosphorylated and releases Asf1 so that Asf1 can then bind to histones and mediate chromatin assembly [86,87] (Fig. 2A). Asf1 is extremely abundant in the cell but only a small portion of the total Asf1 binds to Rad53. It is possible that this dynamic interaction with Rad53 represents a mechanism for delivering Asf1 to the vicinity of the DNA lesion.

Higher eukaryotic Asf1 is phosphorylated by the Tausled-like kinases (Tlks) which may regulate chromatin assembly and / or chromatin disassembly during DNA repair. Humans have two counterparts of Asf1, termed Asf1a and Asf1b [89]. By gene replacement studies in yeast, it has been shown that human Asf1a functions during double-strand DNA repair while human Asf1b is more centrally involved in DNA replication [90]. Both human Asf1a and Asf1b are major phosphorylation targets of Tlk1 and Tlk2 whose kinase activities peak in S-phase [89]. Similarly, *Drosophila* has only one Asf1 homolog and is the major phosphorylation target of their single Tlk [91]. Although the exact molecular role of Asf1 phosphorylation by the Tlks is not known, it may act to regulate Asf1 activity. One model that has been proposed for *Drosophila* is that inactive Tlk binds to Asf1, and upon activation of Tlk by the genomic integrity checkpoint in S-phase, Tlk phosphorylates Asf1 which is then released to mediate chromatin assembly (Fig. 2A) [91]. Somewhat surprisingly, treatment of mammalian cells with gamma irradiation leads to transient inactivation of Tlk1 and Tlk2 via the DNA damage checkpoint [92]. This downregulation of Tlk activity in response to DNA damage would presumably cause a delay in phosphorylation of Asf1, which may be critical for efficient repair of DNA lesions. Interestingly, overexpression of the splice variant Tlk1B protects against ionizing radiation and addition of Tlk1B to an *in vitro* chromatin assembly system stimulates the ability of Asf1 to assemble chromatin [93]. The regulation of Asf1 by Tlks is clearly complex and not fully understood, but is likely to be important for regulating chromatin assembly / disassembly during double-strand DNA repair.

6. The consequences of failure to assemble and disassemble chromatin during DNA repair

What is the outcome of failing to disassemble and reassemble chromatin during DNA repair? Does chromatin disassembly and reassembly just accompany DNA repair or are they in fact important for DNA repair? Despite the profound sensitivity of cells lacking the histone chaperones Asf1 and CAF-1 and the ATP-dependent chromatin remodeling factor INO80 to DNA damaging agents (Table 1), molecular analysis of the actual DNA repair events has revealed fairly subtle, if any, defect in DNA repair. For example, bulk chromosomal repair following gross double-strand DNA damage is not significantly different from wild type in yeast lacking Asf1 or CAF-1, as determined by pulse-field gel electrophoresis [41,94].

Similarly, no profound difference in the kinetics or extent of repair of the HO lesion at the *MAT* locus is seen in yeast lacking Asf1 or CAF-1 [41,94], although another study found a minor defect in repair of an HO lesion in yeast lacking Asf1 [37]. In yeast lacking the INO80 remodeler, an approximate 50% reduction in the rate of DNA resection at the HO site has been observed [78]. These defects in DNA repair are fairly subtle and indicate that chromatin disassembly by either Asf1, INO80 or CAF-1 is clearly not essential to enable the DNA repair machinery to access and repair the DNA lesion. It is still possible that even if these factors normally mediate chromatin disassembly prior to DNA repair, in their absence the resection of the DNA duplex into single strand DNA is sufficient to displace the histone proteins.

The evidence to date does not support a major requirement for chromatin disassembly or assembly for DNA repair *per se*, however they are critical for normal cell function after DNA repair. Yeast lacking Asf1, CAF-1 or INO80 activity have profound viability problems in response to DNA damaging agents [13,36,38,41,43]. A closer examination of the cell cycle stage where the CAF-1 and *asf1* mutants yeast die following double-strand DNA repair shows that they permanently arrest at metaphase due to activation of the DNA damage checkpoint leading to cell death [41,94] (our unpublished observations). This raises the intriguing possibility that the altered chromatin structure in the absence of chromatin assembly factors is sensed by the DNA damage checkpoint. This idea has been proposed previously in human cells where treatments that are thought to alter chromatin structure, such as high salt, the DNA intercalator chloroquine, or the histone deacetylase inhibitor TSA result in global activation of the DNA damage checkpoint [95]. Consistent with the idea that altered chromatin structure activates the DNA damage checkpoint, introduction of a dominant negative version of CAF-1 into tissue culture cells also leads to activation of the DNA damage checkpoint [96]. However, in this study it was not possible to distinguish whether activation of the DNA damage checkpoint was a consequence of DNA damage caused by replication problems or the altered chromatin structure *per se* in the absence of CAF-1 activity.

Perhaps related to a role in DNA damage repair, the CAF-1 and Asf1 histone chaperones do play an important role in maintaining genomic stability. Yeast *asf1* mutants have elevated rates of sister chromatid exchange, mutation, and spontaneous DNA damage foci [94,97]. Similarly, CAF-1 and *asf1* mutants have elevated rates of gross chromosomal rearrangements [98]. However, it has yet to be determined whether this genomic instability upon inactivation of CAF-1 and Asf1 is a consequence of failure to perform their role in chromatin disassembly / assembly during DNA repair or during DNA replication.

Resetting the chromatin structure after DNA repair may be important for turning off the DNA damage checkpoint, allowing the cell to proceed through the cell cycle. Indeed, one of the earliest responses to DNA damage is the phosphorylation of the mammalian histone variant H2AX (H2Av in *Drosophila* or H2A in yeast) in the chromatin around a DSB. Moreover, it has recently been shown that checkpoint recovery after DNA repair requires dephosphorylation of histone H2AX [85]. Quite unexpectedly, the dephosphorylation of histone H2A only occurs once phosphorylated H2A has been disassembled from the chromatin (Fig. 2B). The exchange of phosphorylated *Drosophila* H2Av around DSBs for unphosphorylated H2Av requires the Tip60 protein complex, which has both histone acetyl transferase and ATP-dependent chromatin remodeling activities [99]. Tip60 has also been linked to many earlier stages in DNA damage signaling and double-strand DNA repair, although this is mainly through its histone acetyl transferase activity (see Chapter X). However, the yeast counterpart of Tip60, NuA4, lacks an ATPase that would mediate ATP-dependent chromatin remodeling [100]. As such, it remains unknown how phosphorylated H2A is disassembled from chromatin to reset the DNA damage checkpoint in yeast. It will be interesting to learn whether other chromatin assembly / disassembly factors contribute to the removal of phosphorylated H2A via localized chromatin

disassembly / assembly around DSBs, in order to allow cell cycle progression after DNA repair (Fig. 2B).

7. Conclusions

The most profound way that chromatin can be altered is through the complete removal of histones from DNA, and the available evidence suggests that this does indeed occur during DNA repair processes. Accordingly, histones must be re-deposited onto DNA to restore the chromatin structure following DNA repair. This disassembly and reassembly of chromatin during repair appear to be critical for cell survival after DNA repair. These chromatin dynamics are likely to be orchestrated by the concerted effort of histone chaperones and ATP-dependent chromatin remodeling complexes. These factors are highly conserved in eukaryotes at both the sequence and functional level. For example, human and *Drosophila* Asf1 proteins can function in budding yeast [90]. The main limitation of our understanding of chromatin disassembly / reassembly during DNA repair is that it will be very difficult to ever observe the naked DNA intermediate during DNA repair *in vivo*, even if it does exist, because chromatin disassembly probably occurs concomitant with DNA processing and chromatin reassembly is usually coupled to DNA synthesis. As such, the future studies of chromatin assembly and disassembly during DNA repair will continue to rely on the identification and inactivation of the machinery that mediates these chromatin dynamics. Fundamental questions remain. How is chromatin disassembly and assembly influenced by the ever-growing crop of histone modifications that accompany DNA repair? What are the H2A/H2B chaperones that mediate chromatin disassembly and reassembly during DNA repair? How extensive is chromatin disassembly during DNA repair? What are the molecular mechanisms of these chromatin dynamics and how are they regulated? Why do cells die if they fail to reassemble their chromatin structure even after they have repaired their DNA? Undoubtedly, future studies will provide many exciting advances towards fully understanding the critical link between chromatin and DNA repair.

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References

1. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 1997;389:251–260. [PubMed: 9305837]
2. Liu X, Smerdon MJ. Nucleotide excision repair of the 5 S ribosomal RNA gene assembled into a nucleosome. *J Biol Chem* 2000;275:23729–23735. [PubMed: 10821833]
3. Hara R, Mo J, Sancar A. DNA damage in the nucleosome core is refractory to repair by human excision nuclease. *Mol Cell Biol* 2000;20:9173–9181. [PubMed: 11094069]
4. Nilsen H, Lindahl T, Verreault A. DNA base excision repair of uracil residues in reconstituted nucleosome core particles. *Embo J* 2002;21:5943–5952. [PubMed: 12411511]
5. Schieferstein U, Thoma F. Site-specific repair of cyclobutane pyrimidine dimers in a positioned nucleosome by photolyase and T4 endonuclease V *in vitro*. *Embo J* 1998;17:306–316. [PubMed: 9427764]
6. Wang ZG, Wu XH, Friedberg EC. Nucleotide excision repair of DNA by human cell extracts is suppressed in reconstituted nucleosomes. *J Biol Chem* 1991;266:22474–22478.
7. Araki M, Masutani C, Maekawa T, Watanabe Y, Yamada A, Kusumoto R, Sakai D, Sugawara K, Ohkuma Y, Hanaoka F. Reconstitution of damage DNA excision reaction from SV40 minichromosomes with purified nucleotide excision repair proteins. *Mutat Res* 2000;459:147–160. [PubMed: 10725665]

8. Allen MD, Buckle AM, Cordell SC, Lowe J, Bycroft M. The crystal structure of AF1521 a protein from *Archaeoglobus fulgidus* with homology to the non-histone domain of macroH2A. *J Mol Biol* 330;2003:503–511.
9. Ura K, Araki M, Saeki H, Masutani C, Ito T, Iwai S, Mizukoshi T, Kaneda Y, Hanaoka F. ATP-dependent chromatin remodeling facilitates nucleotide excision repair of UV-induced DNA lesions in synthetic dinucleosomes. *Embo J* 2001;20:2004–2014. [PubMed: 11296233]
10. Sogo JM, Stahl H, Koller T, Knippers R. Structure of replicating simian virus 40 minichromosomes. The replication fork, core histone segregation and terminal structures. *J Mol Biol* 1986;189:189–204. [PubMed: 3023620]
11. Tyler JK. Chromatin assembly. Cooperation between histone chaperones and ATP-dependent nucleosome remodeling machines. *Eur J Biochem* 2002;269:2268–2274. [PubMed: 11985607]
12. Stillman B. Chromatin assembly during SV40 DNA replication in vitro. *Cell* 1986;45:555–565. [PubMed: 3011272]
13. Tyler JK, Adams CR, Chen SR, Kobayashi R, Kamakaka RT, Kadonaga JT. The RCAF complex mediates chromatin assembly during DNA replication and repair. *Nature* 1999;402:555–560. [PubMed: 10591219]
14. Smith S, Stillman B. Purification and characterization of CAF-I, a human cell factor required for chromatin assembly during DNA replication in vitro. *Cell* 1989;58:15–25. [PubMed: 2546672]
15. Tyler JK, Collins KA, Prasad-Sinha J, Amiot E, Bulger M, Harte PJ, Kobayashi R, Kadonaga JT. Interaction between the *Drosophila* CAF-1 and ASF1 Chromatin Assembly Factors. *Mol Biol Cell* 2001;21:6574–6584.
16. Krawitz DC, Kama T, Kaufman PD. Chromatin assembly factor I mutants defective for PCNA binding require Asf1/Hir proteins for silencing. *Mol Cell Biol* 2002;22:614–625. [PubMed: 11756556]
17. Mello JA, Sillje HH, Roche DM, Kirschner DB, Nigg EA, Almouzni G. Human Asf1 and CAF-1 interact and synergize in a repair-coupled nucleosome assembly pathway. *EMBO Rep* 2002;3:329–334. [PubMed: 11897662]
18. Shibahara K, Stillman B. Replication-dependent marking of DNA by PCNA facilitates CAF-1-coupled inheritance of chromatin. *Cell* 1999;96:575–585. [PubMed: 10052459]
19. English CM, Maluf NK, Tripet B, Churchill ME, Tyler JK. ASF1 Binds to a Heterodimer of Histones H3 and H4: A Two-Step Mechanism for the Assembly of the H3-H4 Heterotetramer on DNA. *Biochemistry* 2005;44:13673–13682. [PubMed: 16229457]
20. Tagami H, Ray-Gallet D, Almouzni G, Nakatani Y. Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. *Cell* 2004;116:51–61. [PubMed: 14718166]
21. Krude T. Chromatin assembly factor 1 (CAF-1) colocalizes with replication foci in HeLa cell nuclei. *Exp Cell Res* 1995;220:304–311. [PubMed: 7556438]
22. Schulz LL, Tyler JK. The histone chaperone ASF1 localizes to active DNA replication forks to mediate efficient DNA replication. *Faseb J*. 2006
23. Nabatiyan A, Krude T. Silencing of chromatin assembly factor 1 in human cells leads to cell death and loss of chromatin assembly during DNA synthesis. *Mol Cell Biol* 2004;24:2853–2862. [PubMed: 15024074]
24. Sanematsu F, Takami Y, Barman HK, Fukagawa T, Ono T, Shibahara KI, Nakayama T. Asf1 is required for viability and chromatin assembly during DNA replication in vertebrate cells. *J Biol Chem*. 2006
25. Adams CR, Kamakaka RT. Chromatin assembly: biochemical identities and genetic redundancy. *Curr Opin Genet Dev* 1999;9:185–190. [PubMed: 10322140]
26. Formosa T. Changing the DNA landscape: putting a SPN on chromatin. *Curr Top Microbiol Immunol* 2003;274:171–201. [PubMed: 12596908]
27. Fyodorov DV, Kadonaga JT. Dynamics of ATP-dependent chromatin assembly by ACF. *Nature* 2002;418:897–900. [PubMed: 12192415]
28. Ito T, Bulger M, Pazin MJ, Kobayashi R, Kadonaga JT. ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* 1997;90:145–155. [PubMed: 9230310]

29. Loyola A, LeRoy G, Wang YH, Reinberg D. Reconstitution of recombinant chromatin establishes a requirement for histone-tail modifications during chromatin assembly and transcription. *Genes Dev* 2001;15:2837–2851. [PubMed: 11691835]
30. Fyodorov DV, Blower MD, Karpen GH, Kadonaga JT. Acf1 confers unique activities to ACF/CHRAC and promotes the formation rather than disruption of chromatin in vivo. *Genes Dev* 2004;18:170–183. [PubMed: 14752009]
31. Lorch Y, Maier-Davis B, Kornberg RD. Chromatin remodeling by nucleosome disassembly in vitro. *Proc Natl Acad Sci U S A* 2006;103:30903093
32. Adkins MW, Howar SR, Tyler JK. Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast PHO5 and PHO8 genes. *Mol Cell* 2004;14:657–666. [PubMed: 15175160]
33. Belotserkovskaya R, Oh S, Bondarenko VA, Orphanides G, Studitsky VM, Reinberg D. FACT facilitates transcription-dependent nucleosome alteration. *Science* 2003;301:1090–1093. [PubMed: 12934006]
34. Kaplan CD, Laprade L, Winston F. Transcription elongation factors repress transcription initiation from cryptic sites. *Science* 2003;301:1096–1099. [PubMed: 12934008]
35. Adkins MW, Tyler JK. Transcriptional activators are dispensable for transcription in the absence of Spt6-mediated chromatin reassembly of promoter regions. *Mol Cell* 2006;21:405–416. [PubMed: 16455495]
36. Le S, Davis C, Konopka JB, Sternglanz R. Two new S-phase-specific genes from *Saccharomyces cerevisiae*. *Yeast* 1997;13:1029–1042. [PubMed: 9290207]
37. Qin S, Parthun MR. Histone H3 and the histone acetyltransferase Hat1p contribute to DNA double-strand break repair. *Mol Cell Biol* 2002;22:8353–8365. [PubMed: 12417736]
38. Lewis LK, Karthikeyan G, Cassiano J, Resnick MA. Reduction of nucleosome assembly during new DNA synthesis impairs both major pathways of double-strand break repair. *Nucleic Acids Res* 2005;33:4928–4939. [PubMed: 16141196]
39. Kaufman PD, Kobayashi R, Stillman B. Ultraviolet radiation sensitivity and reduction of telomeric silencing in *Saccharomyces cerevisiae* cells lacking chromatin assembly factor-I. *Genes Dev* 1997;11:345–357. [PubMed: 9030687]
40. Bennett CB, Lewis LK, Karthikeyan G, Lobachev KS, Jin YH, Sterling JF, Snipe JR, Resnick MA. Genes required for ionizing radiation resistance in yeast. *Nat Genet* 2001;29:426–434. [PubMed: 11726929]
41. Linger J, Tyler JK. The Yeast Histone Chaperone Chromatin Assembly Factor 1 Protects Against Double-Strand DNA-Damaging Agents. *Genetics* 2005;171:1513–1522. [PubMed: 16143623]
42. Takeda S, Tadele Z, Hofmann I, Probst AV, Angelis KJ, Kaya H, Araki T, Mengiste T, Scheid OM, Shibahara K, Scheel D, Paszkowski J. BRU1, a novel link between responses to DNA damage and epigenetic gene silencing in *Arabidopsis*. *Genes Dev* 2004;18:782–793. [PubMed: 15082530]
43. Shen X, Mizuguchi G, Hamiche A, Wu C. A chromatin remodelling complex involved in transcription and DNA processing. *Nature* 2000;406:541–544. [PubMed: 10952318]
44. Koyama H, Itoh M, Miyahara K, Tsuchiya E. Abundance of the RSC nucleosome-remodeling complex is important for the cells to tolerate DNA damage in *Saccharomyces cerevisiae*. *FEBS Lett* 2002;531:215–221. [PubMed: 12417315]
45. Chai B, Huang J, Cairns BR, Laurent BC. Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. *Genes Dev* 2005;19:1656–1661. [PubMed: 16024655]
46. Klochendler-Yeivin A, Picarsky E, Yaniv M. Increased DNA damage sensitivity and apoptosis in cells lacking the Snf5/Ini1 subunit of the SWI/SNF chromatin remodeling complex. *Mol Cell Biol* 2006;26:2661–2674. [PubMed: 16537910]
47. Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C. ATP-Driven Exchange of Histone H2AZ Variant Catalyzed by SWR1 Chromatin Remodeling Complex. *Science*. 2003
48. Smerdon MJ, Lieberman MW. Nucleosome rearrangement in human chromatin during UV-induced DNA-repair synthesis. *Proc Natl Acad Sci U S A* 1978;75:4238–4241. [PubMed: 279912]
49. Smerdon MJ. DNA repair and the role of chromatin structure. *Curr Opin Cell Biol* 1991;3:422–428. [PubMed: 1892653]

50. Green CM, Almouzni G. Local action of the chromatin assembly factor CAF1 at sites of nucleotide excision repair in vivo. *Embo J* 2003;22:5163–5174. [PubMed: 14517254]
51. Gaillard PH, Martini EM, Kaufman PD, Stillman B, Moustacchi E, Almouzni G. Chromatin assembly coupled to DNA repair: a new role for chromatin assembly factor I. *Cell* 1996;86:887–896. [PubMed: 8808624]
52. Gaillard PH, Moggs JG, Roche DM, Quivy JP, Becker PB, Wood RD, Almouzni G. Initiation and bidirectional propagation of chromatin assembly from a target site for nucleotide excision repair. *Embo J* 1997;16:6281–6289. [PubMed: 9321407]
53. Maga G, Hubscher U. Proliferating cell nuclear antigen (PCNA): a dancer with many partners. *J Cell Sci* 2003;116:3051–3060. [PubMed: 12829735]
54. Moggs JG, Grandi P, Quivy JP, Jonsson ZO, Hubscher U, Becker PB, Almouzni G. A CAF-1-PCNA-mediated chromatin assembly pathway triggered by sensing DNA damage. *Mol Cell Biol* 2000;20:1206–1218. [PubMed: 10648606]
55. Martini E, Roche DM, Marheineke K, Verreault A, Almouzni G. Recruitment of phosphorylated chromatin assembly factor 1 to chromatin after UV irradiation of human cells. *J Cell Biol* 1998;143:563–575. [PubMed: 9813080]
56. Okano S, Lan L, Caldecott KW, Mori T, Yasui A. Spatial and temporal cellular responses to single-strand breaks in human cells. *Mol Cell Biol* 2003;23:3974–3981. [PubMed: 12748298]
57. Hara R, Sancar A. The SWI/SNF chromatin-remodeling factor stimulates repair by human excision nuclease in the mononucleosome core particle. *Mol Cell Biol* 2002;22:6779–6787. [PubMed: 12215535]
58. Huggins CF, Chafin DR, Aoyagi S, Henricksen LA, Bambara RA, Hayes JJ. Flap endonuclease 1 efficiently cleaves base excision repair and DNA replication intermediates assembled into nucleosomes. *Mol Cell* 2002;10:1201–1211
59. Chafin DR, Vitolo JM, Henricksen LA, Bambara RA, Hayes JJ. Human DNA ligase I efficiently seals nicks in nucleosomes. *Embo J* 2000;19:5492–5501
60. Beard BC, Wilson SH, Smerdon MJ. Suppressed catalytic activity of base excision repair enzymes on rotationally positioned uracil in nucleosomes. *Proc Natl Acad Sci U S A* 2003;100:7465–7470. [PubMed: 12799467]
61. Nabatiyan A, Szuts D, Krude T. Induction of CAF-1 expression in response to DNA strand breaks in quiescent human cells. *Mol Cell Biol* 2006;26:1839–1849
62. Wyman C, Ristic D, Kanaar R. Homologous recombination-mediated double-strand break repair. *DNA Repair (Amst)* 2004;3:827–833. [PubMed: 15279767]
63. Aylon Y, Kupiec M. DSB repair: the yeast paradigm. *DNA Repair (Amst)* 2004;3:797–815. [PubMed: 15279765]
64. Ivanov EL, Sugawara N, White CI, Fabre F, Haber JE. Mutations in XRS2 and RAD50 delay but do not prevent mating-type switching in *Saccharomyces cerevisiae*. *Mol Cell Biol* 1994;14:3414–3425. [PubMed: 8164689]
65. Sugawara N, Haber JE. Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. *Mol Cell Biol* 1992;12:563–575. [PubMed: 1732731]
66. Dudasova Z, Dudas A, Chovanec M. Non-homologous end-joining factors of *Saccharomyces cerevisiae*. *FEMS Microbiol Rev* 2004;28:581–601. [PubMed: 15539075]
67. Jiao R, Bachrati CZ, Pedrazzi G, Kuster P, Petkovic M, Li JL, Egli D, Hickson ID, Stagljar I. Physical and functional interaction between the Bloom's syndrome gene product and the largest subunit of chromatin assembly factor 1. *Mol Cell Biol* 2004;24:4710–4719. [PubMed: 15143166]
68. Balajee AS, Geard CR. Chromatin-bound PCNA complex formation triggered by DNA damage occurs independent of the ATM gene product in human cells. *Nucleic Acids Res* 2001;29:1341–1351. [PubMed: 11239001]
69. Holmes AM, Haber JE. Double-strand break repair in yeast requires both leading and lagging strand DNA polymerases. *Cell* 1999;96:415–424. [PubMed: 10025407]
70. Pospiech H, Rytönen AK, Syvaoja JE. The role of DNA polymerase activity in human non-homologous end joining. *Nucleic Acids Res* 2001;29:3277–3288. [PubMed: 11470886]

71. Adkins MW, Tyler JK. The histone chaperone Asf1p mediates global chromatin disassembly in vivo. *J Biol Chem* 2004;279:52069–52074. [PubMed: 15452122]
72. Giavara S, Kosmidou E, Hande MP, Bianchi ME, Morgan A, d'Adda di Fagagna F, Jackson SP. Yeast Nhp6A/B and mammalian Hmgb1 facilitate the maintenance of genome stability. *Curr Biol* 2005;15:68–72. [PubMed: 15649368]
73. Qin S, Parthun MR. Recruitment of the type B histone acetyltransferase hat1p to chromatin is linked to DNA double-strand breaks. *Mol Cell Biol* 2006;20:3649–3658. [PubMed: 16612003]
74. Ai X, Parthun MR. The nuclear Hat1p/Hat2p complex: a molecular link between type B histone acetyltransferases and chromatin assembly. *Mol Cell* 2004;14:195–205. [PubMed: 15099519]
75. Tsukuda T, Fleming AB, Nickoloff JA, Osley MA. Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*. *Nature* 2005;438:379–383. [PubMed: 16292314]
76. Haber JE. Mating-type gene switching in *Saccharomyces cerevisiae*. *Annu Rev Genet* 1998;32:561–599. [PubMed: 9928492]
77. Lee SE, Moore JK, Holmes A, Umez K, Kolodner RD, Haber JE. *Saccharomyces* Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* 1998;94:399–409. [PubMed: 9708741]
78. van Attikum H, Fritsch O, Hohn B, Gasser SM. Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. *Cell* 2004;119:777–788. [PubMed: 15607975]
79. Qin J, Li L. Molecular anatomy of the DNA damage and replication checkpoints. *Radiat Res* 2003;159:139–148. [PubMed: 12537518]
80. Melo J, Toczyski D. A unified view of the DNA-damage checkpoint. *Curr Opin Cell Biol* 2002;14:237–245. [PubMed: 11891124]
81. Gilbert CS, Green CM, Lowndes NF. Budding yeast Rad9 is an ATP-dependent Rad53 activating machine. *Mol Cell* 2001;8:129–136. [PubMed: 11511366]
82. Pasero P, Shimada K, Duncker BP. Multiple roles of replication forks in S phase checkpoints: sensors, effectors and targets. *Cell Cycle* 2003;2:568–572. [PubMed: 14512770]
83. Downs JA, Lowndes NF, Jackson SP. A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. *Nature* 2000;408:1001–1004. [PubMed: 11140636]
84. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* 1998;273:5858–5868. [PubMed: 9488723]
85. Keogh MC, Kim JA, Downey M, Fillingham J, Chowdhury D, Harrison JC, Onishi M, Datta N, Galicia S, Emili A, Lieberman J, Shen X, Buratowski S, Haber JE, Durocher D, Greenblatt JF, Krogan NJ. A phosphatase complex that dephosphorylates gammaH2AX regulates DNA damage checkpoint recovery. *Nature*. 2005
86. Hu F, Alcasabas AA, Elledge SJ. Asf1 links Rad53 to control of chromatin assembly. *Genes Dev* 2001;15:1061–1066. [PubMed: 11331602]
87. Emili A, Schieltz DM, Yates JR 3rd, Hartwell LH. Dynamic interaction of DNA damage checkpoint protein Rad53 with chromatin assembly factor Asf1. *Mol Cell* 2001;7:13–20. [PubMed: 11172707]
88. Schwartz MF, Lee SJ, Duong JK, Eminaga S, Stern DF. FHA domain-mediated DNA checkpoint regulation of Rad53. *Cell Cycle* 2003;2:384–396. [PubMed: 12851493]
89. Sillje HH, Nigg EA. Identification of human Asf1 chromatin assembly factors as substrates of Tousled-like kinases. *Curr Biol* 2001;11:1068–1073. [PubMed: 11470414]
90. Tamburini BA, Carson JJ, Adkins MW, Tyler JK. Functional conservation and specialization among Eukaryotic Anti-silencing function 1 histone chaperones. *Eukaryotic Cell* 2005;4:1583–1590. [PubMed: 16151251]
91. Carrera P, Moshkin YM, Gronke S, Sillje HH, Nigg EA, Jackle H, Karch F. Tousled-like kinase functions with the chromatin assembly pathway regulating nuclear divisions. *Genes Dev* 2003;17:2578–2590. [PubMed: 14561777]
92. Groth A, Lukas J, Nigg EA, Sillje HH, Wernstedt C, Bartek J, Hansen K. Human Tousled like kinases are targeted by an ATM- and Chk1-dependent DNA damage checkpoint. *Embo J* 2003;22:1676–1687. [PubMed: 12660173]

93. Sunavala-Dossabhoj G, Balakrishnan SK, Sen S, Nuthalapaty S, De Benedetti A. The radioresistance kinase TLK1B protects the cells by promoting repair of double strand breaks. *BMC Mol Biol* 2005;6:19. [PubMed: 16156902]
94. Ramey CJ, Howar S, Adkins M, Linger J, Spicer J, Tyler JK. Activation of the DNA damage checkpoint in yeast lacking the histone chaperone anti-silencing function 1. *Mol Cell Biol* 2004;24:10313–10327. [PubMed: 15542840]
95. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 2003;421:499–506. [PubMed: 12556884]
96. Ye X, Franco AA, Santos H, Nelson DM, Kaufman PD, Adams PD. Defective S phase chromatin assembly causes DNA damage, activation of the S phase checkpoint, and S phase arrest. *Mol Cell* 2003;11:341–351. [PubMed: 12620223]
97. Prado F, Cortes-Ledesma F, Aguilera A. The absence of the yeast chromatin assembly factor Asf1 increases genomic instability and sister chromatid exchange. *EMBO Rep* 2004;5:497–502. [PubMed: 15071494]
98. Myung K, Pennaneach V, Kats ES, Kolodner RD. *Saccharomyces cerevisiae* chromatin-assembly factors that act during DNA replication function in the maintenance of genome stability. *Proc Natl Acad Sci U S A* 2003;100:6640–6645
99. Kusch T, Florens L, Macdonald WH, Swanson SK, Glaser RL, Yates JR 3rd, Abmayr SM, Washburn MP, Workman JL. Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. *Science* 2004;306:2084–2087. [PubMed: 15528408]
100. Doyon Y, Cote J. The highly conserved and multifunctional NuA4 HAT complex. *Curr Opin Genet Dev* 2004;14:147–154. [PubMed: 15196461]

Figure 1. Models for chromatin disassembly and assembly during **A.** DNA replication, **B.** NER and **C.** Double-strand DNA repair (homologous recombination). Asf1 and CAF-1 are histone chaperones, while the yellow ovals represent ATP-dependent chromatin remodelers. A question mark indicates processes where the involvement of a histone chaperone or ATP-dependent chromatin remodeler has not yet been shown biochemically, but is inferred from genetic or cell biology studies. The arrows underneath the DNA repair and replication machinery indicate the direction of their movement along the DNA. Solid lines represent double-strand DNA, while the dashed lines indicate single-strand DNA. Red lines represent DNA from the homologous donor locus.

Figure 2. Links between chromatin assembly / disassembly and the DNA damage checkpoint. **A.** Alterations to the activities, modifications or levels of histone chaperones in response to DNA damage, as described in the text. **B.** Disassembly of phosphorylated H2A (H2AX in mammals or H2Av in *Drosophila*) from chromatin is essential for recovery from the DNA damage checkpoint. This requires Tip60 in *Drosophila* and although not yet tested, may be assisted by histone chaperones.

Table 1
Sensitivity to DNA damaging agents resulting from mutation of histone chaperones and ATP-dependent chromatin remodeling factors.

<u>Histone Chaperones:</u>	
Asf1	Inactivation in <i>Saccharomyces cerevisiae</i> causes sensitivity to UV, alkylating agents [36], radiomimetics [13], the HO endonuclease [37], and gamma irradiation [38].
CAF-1	Inactivation in <i>Saccharomyces cerevisiae</i> causes sensitivity to UV [39], gamma irradiation [40], HO endonuclease, alkylating agents, and radiomimetics [41]. Arabidopsis mutants of CAF-1 are sensitive to the alkylating agent MMS [42].
<u>Chromatin Remodelers:</u>	
INO80	Mutations in the <i>Saccharomyces cerevisiae</i> catalytic subunit cause sensitivity to UV, gamma irradiation and alkylating agents [43].
RSC	<i>rsc1</i> and <i>rsc2</i> <i>Saccharomyces cerevisiae</i> mutants are sensitive to gamma irradiation [40], and to radiomimetics, alkylating agents and UV when grown at their semipermissive temperatures [43–45].
SWI/SNF	<i>snf5</i> and <i>snf2</i> <i>Saccharomyces cerevisiae</i> mutants are sensitive to radiomimetics and are moderately sensitive to alkylating agents and UV irradiation [45].
SWR1	Human primary fibroblasts lacking Snf5 are hypersensitive to UV and doxorubicin [46]. Deletion of <i>Saccharomyces cerevisiae</i> <i>SWR1</i> causes sensitivity to MMS and moderate sensitivity to UV [47]. Interestingly, SWR1 also mediates the removal of histone H2A from chromatin and its replacement by the histone variant H2AZ, although it is not known if this occurs during DNA repair [47].