Chapter 12
Chromatin Remodeling in DNA Repair and Replication

Prabodh Kapoor and Xuetong Shen

12.1 Introduction

The genetic information encoded in enormous length of DNA is packaged and compartmentalized into the nucleus of eukaryotes as chromatin. Chromatin consists of nucleosomes as the fundamental unit, where ~146 bp of DNA is wrapped around an octamer of histones in nearly two superhelical turns. Within the histone octamer, two copies of H2A-H2B and H3-H4 dimer pairs form the core histones, whereas, histone H1, also called as linker histone, locks the DNA at the either end of the nucleosome and, along with other architectural proteins, folds the chromatin into more condensed and yet poorly defined higher order structures (see Chap. 1). In almost all nuclear processes involving DNA as a substrate, such as transcription, replication, recombination, and repair, the packaging of the genome in chromatin presents inherent barriers that restrict the access of DNA to processing enzymes. Therefore, to access DNA within a chromatin context, the chromatin is reversibly and locally unfolded by counteracting these chromatin constraints during the nuclear process and refolded back after the process is completed. In this regard, the eukaryotic cell has developed two fundamental chromatin modification strategies that includes: (1) Covalent modification of histones catalyzed by histone-modifying enzyme complexes and (2) ATP-dependent perturbations of histone–DNA interactions catalyzed by the SWI/SNF family of ATP-dependent chromatin remodeling complexes. The covalent modification of histone residues that primarily occurs at the N-terminal region of histones can disrupt histone interaction with DNA or alternatively serve as the binding sites for chromatin-associated factors (Jenuwein and Allis 2001). However, the mechanism employed by ATP-dependent chromatin remodeling complexes uses the energy of ATP hydrolysis to alter the positions or composition of nucleosomes in chromatin

P. Kapoor • X. Shen (✉)
Department of Molecular Carcinogenesis, Division of Basic Science Research, The University of Texas MD Anderson Cancer Center, Smithville, TX, USA
e-mail: xshen@mdanderson.org

J.L. Workman and S.M. Abmayr (eds.), *Fundamentals of Chromatin*,
DOI 10.1007/978-1-4614-8624-4_12, © Springer Science+Business Media New York 2014

(Eberharter and Becker 2004). Much of what we currently know about the biological roles of these two classes of chromatin-modifying factors has come from research on the transcriptional regulatory mechanisms that occur during gene activation, whereas studies from the past decade have also shown the link between chromatin modifications and other nuclear events such as DNA repair and replication. Both covalent modification of histones and ATP-dependent chromatin remodeling have been shown to maintain genome integrity and transmit the genetic and epigenetic information to the next generation. This chapter elaborates how the ATP-dependent chromatin remodeling complexes employ mechanisms that work in concert with the DNA repair and replication processes.

12.2 Chromatin Remodeling During DNA Double Strand Break Repair

Double Strand Breaks (DSBs) caused by genotoxic stress are particularly dangerous lesions that can result in mutations owing to error-prone repair or cell death if left unrepaired. The cell has evolved two highly conserved pathways to detect and repair DSBs, namely homologous recombination (HR) and nonhomologous end-joining (NHEJ) (Valerie and Povirk 2003). In HR, an undamaged homologous sequence is used as a template for repair, whereas in NHEJ, the broken ends are religated without the use of a template, resulting in a more error-prone repair mechanism. In yeast, HR is preferred over NHEJ whereas NHEJ is far more common in mammalian cells (Kim et al. 2005).

12.2.1 The Process of DNA Double Strand Break Repair

Upon DSB formation, the Mre11-Rad50-Xrs2 (MRX) complex, which contains exonuclease activity, collaborates with other factors to promote the production of single-stranded DNA, a process known as resection (Mimitou and Symington 2008). Repair and checkpoint factors then assemble to the break sites. During HR, *RAD52* epistasis group proteins (Rad50, Rad51, Rad52, Rad54, Rad55, and Rad57) play major roles in promoting the homology search, strand invasion, and synapsis between the invading recipient strand and donor DNA, leading to the formation of Holliday junctions. Briefly, the HR pathway is initiated by the binding of the trimeric ssDNA-binding factor RPA (replication protein A) to 5′–3′ resected DNA, followed by the replacement of RPA with a second ssDNA binding protein, Rad51, with the assistance of Rad52. Rad51, in turn, is followed by the recruitment of another set of HR proteins (Rad54, Rad55, Rad57), all of which participate in strand invasion and annealing steps to form the synaptic filament (Symington 2002). DNA repair is complete once DNA synthesis has finished and Holliday junctions have been resolved. Alternatively NHEJ, which is facilitated by the tethering and ligation of the broken DNA ends,

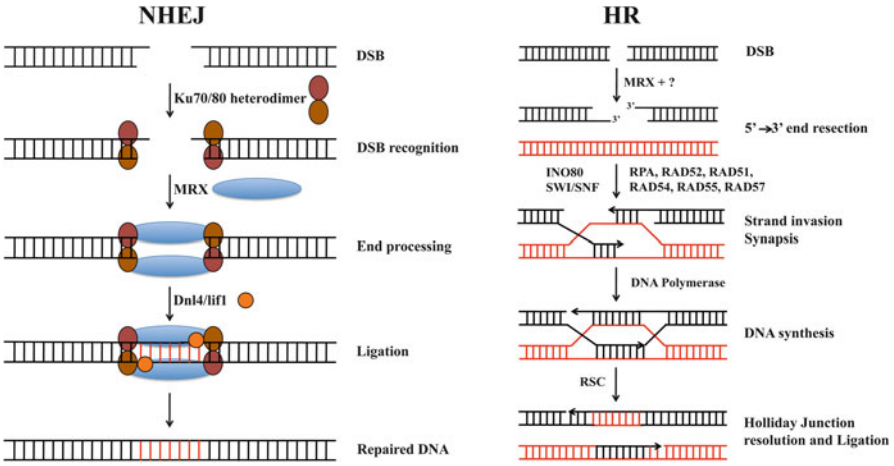

Fig. 12.1 NHEJ and HR pathways: A DSB can be repaired by either NHEJ or HR. In the NHEJ pathway the broken ends are directly rejoined under the control of the end-binding proteins Ku70 and Ku80, the MRX (Mre11–Rad50–Xrs2) complex, and DNA ligase IV and its associated cofactor Lif1. The HR pathway is initiated by the 5′–3′ resection of the broken ends of the recipient chromosome to form ssDNA. Resection is under control of the MRX complex and other factors

is initiated by the binding of the Ku70–Ku80 heterodimer to broken DNA ends, followed by MRX (Mre11– Rad50–Xrs2)-mediated "cleaning up" of ends, and then by Dnl4 (DNA ligase IV)-dependent ligation through an associated factor Lif1 (XRCC4) (Cahill et al. 2006; Daley et al. 2005; Lewis and Resnick 2000) (Fig. 12.1).

The pathway of factor recruitment during HR has been revealed through genetic and cytological analysis, and more recently by chromatin immunoprecipitation (ChIP) assays using antibodies against native or epitope-tagged HR proteins (Lisby et al. 2004; Shroff et al. 2004; Sugawara et al. 2003; Wolner et al. 2003). These assays have taken advantage of a genetic system generated by Haber's group in which a unique DSB can be created at the MAT locus of yeast at almost 100 % efficiency by the galactose-regulated induction of the homothallic switching (HO) endonuclease (Lee et al. 1998). HR can repair the MAT DSB from one of two silent copies of MAT DNA (HMRa or HMLa) present on the same chromosome and representing donor sequences. The system comes in two forms. When the HM loci are deleted, the DSB can only be repaired by NHEJ as in the haploid yeast there are no other copies of MAT DNA to copy. However, even in this instance HR factors involved in strand invasion and annealing are still recruited to the broken ends, and this particular version of the system thus offers a powerful way to monitor the kinetics, extent, and genetic dependency of factor recruitment to the recipient DSB during the initial stages of HR. When the donor loci are present, the system can be used to follow the assembly and distribution of the same HR factors at both donor and recipient loci, in addition to the completion of individual steps in the HR pathway. This system has also provided a mechanism to monitor the chromatin changes that occur at a DSB.

The MAT locus has a well-defined chromatin structure in which a series of positioned nucleosomes flank the promoter of the regulatory genes present at this locus (Weiss and Simpson 1998). Upon formation of the DSB, the first chromatin remodeling event that occurs is the rapid and extensive phosphorylation of the histone H2A C-terminus (phospho-H2A) (Rogakou et al. 1998). In higher eukaryotes, this phosphorylation motif occurs not on the core histone H2A but on the histone variant H2AX (Rogakou et al. 1998), which constitutes approximately 10 % of the total H2A (Redon et al. 2002). Yeast containing mutant H2A that is not able to be phosphorylated have a defect in NHEJ and are mildly sensitive to DNA-damaging agents (Downs et al. 2000), while mouse cells lacking H2AX are sensitive to IR and the animals are predisposed to cancer in the absence of p53 (Bassing et al. 2002, 2003; Celeste et al. 2002, 2003a). H2A phosphorylation is carried out by the damage-response phosphatidyl-3-OH-kinase-like kinases Tel1 and Mec1 in *S. cerevisiae* (Rogakou et al. 1998) (homologous to ataxia telangiectasia-mutated (ATM) and ATM- and Rad3-related (ATR), respectively, in mammals). These kinases also phosphorylate many other targets on SQ/TQ motifs in response to DNA damage (Chen et al. 2010; Matsuoka et al. 2007; Smolka et al. 2007). H2A phosphorylation spreads over a large region of ~50 kb in budding yeast (Shroff et al. 2004) or megabases in higher eukaryotes (Rogakou et al. 1999), with the levels of phosphorylation greatest at 3–5 kb from the break site, but low in the 1 kb immediately adjacent to the break (Chen et al. 2000). Tel1, in conjunction with the nuclease MRX (Mre11-Rad50-Xrs2), is primarily recruited to unprocessed DSBs. In contrast, Mec1 and its partner protein Ddc1 are recruited to tracts of RPA bound single-stranded DNA. Single-stranded DNA is generated via processing of DSBs through exonucleolytic resection by several nucleases including MRX and Exo1 to initiate HR (Shim et al. 2010).

Activation of Mec1 and Tel1 results in amplification of the DNA damage signal cascade and leads to recruitment and retention of many repair and checkpoint proteins near the site of the lesion. One of the consequences is arrest of the cell cycle by activation of checkpoints, permitting repair of DNA damage before cell division or DNA replication. In *S. cerevisiae*, the major checkpoint activated in response to DNA damage is at the G2/M boundary and involves the upregulation of the ribonucleotide reductase (RNR) genes and phosphorylation of Rad53 in a Mec1-dependent manner. Any of the steps of the DNA damage response including damage recognition, resection, H2A phosphorylation, checkpoint activation, or binding and retention of downstream effectors could conceivably be affected by chromatin structure and hence requires the action of chromatin remodeling complexes.

12.2.2 Chromatin Remodeling Complexes During DNA DSB Repair

Members of the SWI2/SNF2 (switching/sucrose non-fermenting) superfamily of conserved ATPases have initially been shown to play key roles in regulating transcription, where they function as multi-subunit complexes. Based on signature motifs/domains in the primary sequence of their core ATPase, they can be classified

Fig. 12.2 Classification of chromatin remodeling complexes: Chromatin remodeling complexes contain conserved core ATPase subunit and on the basis of signature domains/motifs in their core ATPase subunit, can be classified into four major categories: SANT domain containing ISWI complex, CHROMO domain containing Mi2, BROMO domain containing SWI/SNF, and split ATPase being the characteristic feature of INO80 family of chromatin remodeling complexes

into four distinct subfamilies (Fig. 12.2). The chromatin remodeling complexes that contain these ATPases, like INO80, SWR1, SWI/SNF and RSC, were also found to accumulate at MAT DSB in *S. cerevisiae* where they have been shown to have roles associated with both NHEJ and HR. However, each of these factors seems to have a different role at different stages of DSB repair. Moreover, ATP-dependent chromatin remodeling is postulated to have a mechanistically similar role in transcription and DNA damage repair by disrupting chromatin to give regulatory and repair factors direct access to DNA. Recent advances suggested that the activity of chromatin-modifying complexes in areas around DSBs occurs in a specific interdependent sequential order. Moreover, as discussed later in this chapter, ATP-dependent chromatin remodeling complexes like INO80 also regulate the cell cycle checkpoint by modulating the activity of effector proteins.

12.2.2.1 RSC and SWI/SNF Chromatin Remodeling Complexes

RSC

The RSC (remodels structure of chromatin) complex is a highly abundant ATP-dependent chromatin remodeling complex in budding yeast (~1,000–2,000 molecules per cell) (Cairns et al. 1996a) and is comprised of around 16 subunits (Table 12.1). Sth1 is the core catalytic subunit, which has been found to be essential for cell survival. Its ATPase domain is closely related to that of the Swi2/Snf2 subunit of the SWI/SNF complex. RSC complex shares three other subunits, Rtt102, Arp7, and Arp9, with SWI/SNF complex (Table 12.1). Two isoforms of the RSC complex exist based on the presence of Rsc1 or highly similar Rsc2 subunit. Rsc2 is approximately tenfold more abundant than Rsc1. However, they share similar domain organization that includes two bromodomains separated by a weak

Table 12.1 Subunit comparison of RSC and SWI/SNF chromatin remodeling complexes from *S. cerevisiae*

RSC	SWI/SNF
Sth1	Snf2
Arp7	Arp7
Arp9	Arp9
Rsc1/Rsc2	Swp73
Rsc3	Swp82
Rsc4	Swp29/Anc1/Taf30
Rsc6	Swi1
Rsc7	Swi3
Rsc8	Snf1
Rsc9	Snf5
Rsc30	Snf6
Rsc58	Rtt102
Rtt102	
Ldb7	
Htl1	

S. cerevisiae RSC and SWI/SNF complexes share Arp7, Arp9, and Rtt102 subunits

nonspecific DNA binding motif (AT hook), followed by a Bromo-Adjacent Homology (BAH) domain (important for nucleosome binding) (Cairns et al. 1999; Chambers et al. 2012). Simultaneous deletion of both rsc1 and rsc2 is lethal, whereas single deletion of either is viable, suggesting that there is functional redundancy between the two RSC isoforms.

The DNA-dependent ATPase activity of the RSC complex is coupled to 3′–5′ translocase activity (Saha et al. 2002, 2005), and this activity of the RSC complex has been shown to be utilized for nucleosome remodeling, repositioning, disassembly, and histone octamer transfer. Nucleosomes containing tetra-acetylated H3 were remodeled ~16-fold faster than unmodified nucleosomes due to the preferential binding of RSC complex to acetylated nucleosomes (Ferreira et al. 2007). In addition, RSC has been shown to have increased affinity for nucleosomes acetylated by the NuA4 histone acetyl transferase complex (Ferreira et al. 2007), suggesting a role for the bromodomain containing subunits (Rsc1, Rsc2, Rsc4, and Sth1) in recruitment to chromatin and maximum remodeling activity. Mechanistically, it has recently been shown that nucleosome remodeling by RSC complex may occur by formation of a bulge of DNA on the surface of the nucleosome that can be extended by translocation to form a larger loop. Dissipation of the loop to the other side of the nucleosome can result in reverse translocation, a jump in the position of the nucleosome referred to as nucleosome sliding. ChIP on chip analysis of the genome-wide localization of the RSC complex in *S. cerevisiae* identified RSC binding at ~700 promoters (~11 % of genes). No difference was seen in the Rsc1 and Rsc2 profiles

(Ng et al. 2002). Furthermore, around 12 % of RNA polymerase II-transcribed genes were up- or downregulated at least twofold in a *rsc4* mutant strain (Soutourina et al. 2006). More recently, a putative Rsc3 sequence-specific binding site has been identified approximately 100 bp upstream of the transcription start site of 708 genes (169 of which are essential), and Rsc3 has been connected with nucleosome exclusion from the promoters of these genes (Badis et al. 2008). In vitro, RSC was also able to facilitate passage of RNA polymerase II through acetylated nucleosomes (Carey et al. 2006). Although, RSC has been observed in the transcriptional regulation of many essential and redundant genes, to date, none of the known DNA repair genes have been identified as being transcriptionally controlled by RSC.

SWI/SNF

The SWI/SNF complex is a highly conserved multi-subunit complex that plays a key role in the regulation of transcription. The components of the SWI/SNF chromatin-remodeling complex were initially identified in screens for genes that regulate mating-type switching (SWI) and sucrose non-fermenting (SNF) phenotypes in yeast (Abrams et al. 1986; Carlson and Laurent 1994; Carlson et al. 1981; Nasmyth and Shore 1987; Neigeborn and Carlson 1984, 1987; Stern et al. 1984). It was recognized that a subset of the SWI genes are identical to those identified in the SNF screen, and those genes that are involved both in mating-type switching and sucrose fermentation have come to be known as SWI/SNF genes (Peterson et al. 1994; Wolffe 1994). Although SWI/SNF is a relatively rare enzyme in yeast, present at only ~100–500 copies per nucleus (Cote et al. 1994), it has been estimated that 5–7 % of all yeast genes require SWI/SNF activity for expression (Monahan et al. 2008; Sudarsanam et al. 2000; Zraly et al. 2006). In yeast, the SWI/SNF complex can both promote and suppress gene expression and about a third of the yeast genes regulated by SWI/SNF are suppressed (Sudarsanam et al. 2000). The yeast SWI/SNF complex consists of ~11 different subunits (Table 12.1) and is required in vivo for the transcriptional induction of a large subset of yeast genes and for the functioning of a variety of sequence-specific transcriptional activators. Moreover, a *Drosophila* homologue of the SWI2/SNF2 subunit is required for activation of homeotic genes (Tamkun et al. 1992), and human SWI/SNF homologs facilitate the functioning of mammalian steroid receptors in cultured human cells (Chiba et al. 1994; Muchardt and Yaniv 1993). The mammalian SWI/SNF complexes are structurally and, perhaps functionally, more diverse than those of yeast or flies. The yeast SWI/SNF complex exhibits an apparent molecular mass of ~1.14 MDa (Smith et al. 2003), whereas the mammalian SWI/SNF complex has an apparent molecular mass of ~2 MDa. The stoichiometry of the SWI/SNF complexes has not been unambiguously resolved, but it is most likely that no single complex contains all of the subunits listed in Table 12.1.

In *S. cerevisiae* the purified SWI/SNF complex possesses similar biochemical activity as identified in RSC complex and contains several subunits homologous to

RSC subunits. A similar subunit composition is also present in SWI/SNF complexes in *Drosophila melanogaster* (BAP and PBAP) and multiple SWI/SNF complexes in mammals, including the BRG1-associated factor (BAF; also known as SWI/SNF-A) and the polybromo BRG1-associated factor (PBAF; also known as SWI/SNF-B) complexes (Cairns et al. 1996a; Imbalzano et al. 1994; Kwon et al. 1994; Mohrmann et al. 2004; Mohrmann and Verrijzer 2005; Papoulas et al. 1998). The mammalian complexes are comprised of one of two mutually exclusive catalytic ATPase subunits, either brahma homologue (BRM; also known as SMARCA2) or BRM/SWI2-related gene 1 (BRG1; also known as SMARCA4). These complexes also contain a set of highly conserved "core" subunits including SNF5 (also known as SMARCB1, INI1, and BAF47) and BAF155 and BAF170. In addition they contain variant subunits that are thought to contribute to the targeting, assembly, and the regulation of lineage-specific functions of the complexes (Phelan et al. 1999; Wang et al. 1996). The AT-rich DNA interactive domain-containing protein 1A,ARID1A (also known as BAF250A and SMARCF1) and ARID1B subunits are mutually exclusive and are present only in BAF complexes, whereas the BAF180 (also known as PBRM1), BAF200, and bromodomain-containing 7 (BRD7) subunits are exclusively present in PBAF complexes (Mohrmann and Verrijzer 2005; Wang et al. 1996, 2004; Kaeser et al. 2008). It has been suggested that BAF is similar to the yeast SWI/SNF complex and that PBAF is more likely similar to *S. cerevisiae* RSC (Xue et al. 2000). As several subunits that are common to both BAF and PBAF are encoded by gene families that often display differential lineage-restricted expression, a large number of variant SWI/SNF complexes probably exist in mammals and contribute to regulating lineage- and tissue-specific gene expression (Kaeser et al. 2008; Lessard et al. 2007; Lickert et al. 2004; Wu et al. 2009; Yan et al. 2008).

SWI/SNF complexes remodel nucleosome structure and are capable of mobilizing nucleosomes both by sliding and by catalyzing the ejection and insertion of histone octamers (Saha et al. 2006). Nucleosome sliding has been proposed to include the following steps: binding of SWI/SNF complexes to a fixed position on nucleosomal DNA, disruption of histone–DNA contacts, translocation of DNA that is initiated via the ATPase subunit and DNA loop formation that can then propagate around the nucleosome and generate sites that are more accessible to DNA binding factors (Saha et al. 2006; Lorch et al. 2010). The mechanism by which nucleosome ejection and insertion occurs is less well understood. However, it has been observed that histone chaperones may assist in this process and histone ejection may occur not at nucleosomes that are directly bound by SWI/SNF complexes but at adjacent nucleosomes following the repositioning of the bound nucleosome (Dechassa et al. 2010). Importantly, although nucleosome remodeling is the most studied effect of SWI/SNF activity, the complexes interact with numerous other chromatin proteins, and it is conceivable that they have additional effects on higher order chromatin structure.

Although *S. cerevisiae* SWI/SNF complexes were identified on the basis of their roles in the activation of transcription, evidence indicates that mammalian SWI/SNF complexes contribute to both repression and activation. During mammalian T lymphocyte development, BRG1 and BAF57 are required to both silence CD4 and

activate CD8 expression (Chi et al. 2002). In embryonic stem (ES) cells, BRG1 not only acts as a repressor to inhibit programs that are associated with differentiation, but it also facilitates the expression of core pluripotency programs (Ho et al. 2009). Similarly, deletion of Snf5 in murine fibroblasts results in more genes being activated than repressed (Isakoff et al. 2005). Contributing to the mechanism of repression, SWI/SNF complexes are capable of recruiting histone deacetylases (HDACs), which remove activating acetyl marks from histone tails. For example, SNF5 represses cyclin D1 (CCND1) in an HDAC1-dependent manner (Zhang et al. 2002). These seemingly opposing activities may actually be similarly achieved, by positioning nucleosomes away from binding sites to facilitate factor binding or by moving nucleosomes over sites to prevent binding. Collectively, dynamic activities of mammalian SWI/SNF complexes have essential roles in regulating both the activation and the repression of gene expression programs.

12.2.2.2 RSC and SWI/SNF in DNA DSB Repair

These two ATP-dependent chromatin remodeling factors have been intensely studied with respect to their roles in transcriptional regulation and chromosome transmission (Sudarsanam et al. 2000; Cao et al. 1997). Recent evidence indicates that these factors also directly participate in DSB repair. Both *swi/snf* and *rsc* mutants are hypersensitive to a broad spectrum of agents that cause DSBs, and several lines of evidence indicate that RSC in particular acts in DSB repair by NHEJ (Shim et al. 2005). Two *rsc* mutants were isolated in a genetic screen for mutants defective for NHEJ of an HO-endonuclease-induced DSB at MAT in a strain lacking donor template. These mutants showed defects in both imprecise and precise DNA end-joining (Shim et al. 2005). Genetic epistasis studies also revealed that *rsc* mutants exhibit enhanced hypersensitivity to DSB-inducing agents when combined with a *rad52Δ* mutation, which eliminates repair by HR (Shim et al. 2005; Chai et al. 2005). An important study has also linked RSC, and SWI/SNF, to DSB repair specifically by the HR pathway (Chai et al. 2005). Both *swi/snf* and *rsc* mutants are defective for HR repair by single-strand annealing in a plasmid-based assay. More importantly, analysis of these mutants shows that SWI/SNF and RSC act at distinct steps in HR repair of a MAT DSB in strains that contain homologous donor sequences. Both remodelers seem to regulate HR in the context of donor loci, after initial recruitment of strand invasion proteins to the recipient MAT locus (Chai et al. 2005).

SWI/SNF acts specifically during formation of the synaptic filament, and in its absence, the levels of Rad52 and Rad51 are reduced at the HML donor locus. By contrast, RSC regulates a postsynaptic step of HR following DNA synthesis primed from the invading 3′ strand, as revealed by the low level of donor-recipient DNA ligation products in a *rsc2Δ* mutant (Chai et al. 2005). Although SWI/SNF and RSC function as transcriptional regulators in yeast, they seem to have direct roles in DSB repair. Both complexes are physically present at the MAT DSB after HO induction, although they are recruited to MAT with different kinetics (Shim et al. 2005; Chai

et al. 2005). The RSC catalytic subunit, Sth1, is recruited to the DSB rapidly and reaches maximal levels within 20 min after the break is formed. By contrast, the Snf2 and Snf5 subunits of SWI/SNF are recruited to the DSB later, on a time scale similar to the recruitment of the strand invasion-annealing proteins Rad52 and Rad54, and they continue to accumulate at the break for 4 h.

Recently, it has been shown that Fun30, a Snf2 family member, ATP-dependent chromatin remodeling factor in *S. cerevisiae*, facilitates nucleosomal eviction near DSBs, which promotes both Exo1- and Sgs1-dependent activities, and thus enhances long range end resection for efficient HR at DSBs (Chen et al. 2012; Costelloe et al. 2012; Eapen et al. 2012). Both SWI/SNF and RSC also associate with the HML donor locus, consistent with a direct role for the remodeling factors in the interactions between recipient and donor DNA strands during repair by HR. Although the timing of SWI/SNF recruitment to MAT-HML is consistent with its postulated role in strand annealing, the much earlier recruitment of RSC to the MAT DSB is puzzling in light of its later role in the completion of HR. One possibility is that the remodeling activity of RSC is required early to set up a suitable chromatin environment for a later, postsynaptic step. A second is that the early recruitment of RSC, which coincides with the recruitment of NHEJ factors in a donor less strain (Shim et al. 2005), is related to the choice between NHEJ and HR upon DSB formation. Thus, in the presence of donor sequences, RSC could facilitate the HR pathway rather than the NHEJ pathway. One of the earliest events at a DSB is the phosphorylation of S129 of H2A by Mec1/Tel1. Recruitment of RSC and its remodeling of the surrounding chromatin occur on a similar timescale to H2A phosphorylation, raising the possibility that the two events are connected. Rsc1 enrichment to a DSB is unaffected in a mutant in which H2A S129 cannot be phosphorylated, but H2A S129 phosphorylation is defective in *rsc* mutants, placing RSC upstream of phosphorylation in the DNA damage response (Kent et al. 2007; Liang et al. 2007; Shim et al. 2007). Additionally, enrichment of Mec1 and Tel1 were decreased approximately twofold in *rsc2* strains, consistent with a defect in H2A phosphorylation (Liang et al. 2007).

Following DSB formation, a small amount of Mre11 or Ku rapidly binds to the ends of the break, which facilitates recruitment of RSC, either directly or indirectly, which in turn remodels the chromatin in the region of the break. The remodeled chromatin is more accessible and permissive for the accumulation of more Mre11 and Ku, acting in a positive feedback loop to recruit further RSC. The presence of Mre11 stimulates resection and consequently recruitment of Mec1- to RPA-coated single-stranded DNA and phosphorylation of H2A. This amplification cascade means that although resection and H2A phosphorylation still occur in the absence of RSC, they occur more efficiently in its presence.

Mre11 is a subunit of the evolutionarily conserved MRX and is itself rapidly recruited to DSBs, where it regulates strand resection during HR, cell cycle checkpoint activation, and interactions between broken ends (Lisby et al. 2004; Shroff et al. 2004; D'Amours and Jackson 2002; Petrini and Stracker 2003; Stracker et al. 2004). Importantly, this complex has been implicated in both NHEJ- and HR-mediated DSB repair (Symington 2002; Lewis and Resnick 2000; Haber 1998), and thus might

mediate the association of both SWI/SNF and RSC with a DSB. One of the key unanswered questions is why two factors with such similar chromatin remodeling activities should be required during repair by HR at MAT. Part of the answer could lie in the observation that the two factors act at distinct points in the HR pathway where different chromatin structures occur. The HML and HMR donor loci are assembled into a heterochromatin-like structure, and SWI/SNF remodeling might specifically disrupt this structure to reveal donor DNA to the factors involved in homology searching and synapsis. The remodeling activity of RSC, by contrast, has been suggested to regulate the postsynaptic dissociation of invading MAT DNA from donor DNA (Chai et al. 2005), and thus might reflect a role for RSC on DNA rather than chromatin.

12.2.2.3 INO80 and SWR1 Chromatin Remodeling Complexes

INO80

The INO80 chromatin remodeling complex was first identified as a coactivator of genes involved in inositol metabolism, and like SWI/SNF and RSC, it is also linked to DNA repair (Morrison et al. 2004; Shen et al. 2000, 2003a; Tsukuda et al. 2005; van Attikum et al. 2004). The INO80 complex was initially purified from *S. cerevisiae* and was shown to have a molecular mass of ~1.5 MDa. The purified INO80 complex contains 15 subunits with roughly equivalent stoichiometry except for Rvb1 and Rvb2, which shows 6:1 stoichiometry compared to rest of the subunits (Shen et al. 2000) (Fig. 12.3). The INO80 complex is highly conserved, and the purified human INO80 complex (hINO80) contains orthologs of Ino80, Rvb1,

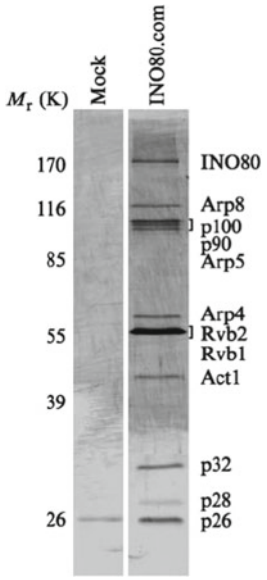

Fig. 12.3 The INO80 ATP-dependent chromatin remodeling complex: SDS-PAGE and *silver stain* showing the identity of subunits of the INO80 complex (INO80.com), purified by single step FLAG-immunoaffinity chromatography from yeast whole cell extract. Adapted from X. Shen, G. Mizuguchi, A. Hamiche, and C. Wu, *Nature* 406, 541 (2000) with permission

Rvb2, Arp4, Arp5, Arp8, Ies2, and Ies6, as well as five unique subunits (Jin et al. 2005). Similar to its yeast counterpart, hINO80 complex exhibits DNA- and nucleosome-activated ATPase activity and ATP-dependent nucleosome remodeling activity (Shen et al. 2000; Jin et al. 2005). The ATPase subunits of the INO80 subfamily are distinguished from other ATPases in the ISWI, SWI/SNF, and CHD subfamilies owing to the presence of a spacer region (1018–1299) that splits the conserved ATPase domain (698–1450) (Ebbert et al. 1999). Alteration of the lysine to arginine (K737R) in the conserved GXGKT motif for nucleotide binding results in a nonfunctional Ino80, which is unable to complement the corresponding null allele (Ebbert et al. 1999). Moreover, INO80 complex purified from a strain carrying a K737A substitution also failed to show ATPase activity, DNA helicase activity, and the ability to rescue the *ino80Δ* mutant phenotypes (Shen et al. 2000). These results indicate that ATP binding is essential for Ino80 function in vivo.

Other unique subunits of INO80 family of chromatin remodeling complexes (INO80 and SWR1) are Rvb1 and Rvb2, which are essential and highly conserved proteins from yeast to human (Tip49a and Tip49b in mammals) (Jonsson et al. 2004; Kanemaki et al. 1999; Qiu et al. 1998). Rvb proteins share limited homology to bacterial RuvB, the Holliday Junction DNA Helicase with a double hexamer composition (West 1996, 1997). Like bacterial RuvB, the yeast Rvb1 and Rvb2 each show 6:1 stoichiometry with other subunits in the complex (Shen et al. 2000). The eukaryotic counterparts of the bacteria RuvA/B have been elusive. Thus, INO80 and/or SWR1 may represent candidates for RuvB, together with a yet to be identified eukaryotic equivalent of RuvA, which may partially fulfill the function of eukaryotic Holliday Junction enzymes in the context of chromatin. This hypothesis is consistent with the observations that the INO80 complex is required for DNA repair. It has been shown that the yeast Rvb proteins are essential for the chromatin remodeling activity of INO80 complex (Jonsson et al. 2004), and the loss of Rvb proteins leads to the loss of Arp5, a functionally important subunit of the INO80 complex (Shen et al. 2003a). It is worth noting that Rvbs are subunits of the Swr1 complex as well, which does not contain Arp5, suggesting that one of the Swr1 subunits (such as Arp6) or another unknown subunit may associate with Rvbs and be required for the regulation of SWR1 complex.

Conventional actin and actin-related proteins (Arps) have been identified as subunits in many chromatin-modifying complexes (Boyer and Peterson 2000). The INO80 complex contains actin, Arp4, Arp5, and Arp8 (Shen et al. 2000). Arp5 and Arp8 have so far only been found in the INO80 complex. Arp5 appears to associate with the complex independently of any other subunit, while Arp8 is necessary for the inclusion of Arp4 and actin (Shen et al. 2003a). The functions of Arp5 and Arp8 appear to be important for the process of chromatin remodeling, since it has been shown that the phenotypes of *arp5Δ* and *arp8Δ* are similar to that of *ino80Δ*. In vitro DNA binding, nucleosome mobilization, and ATPase activities of the mutant INO80 complexes lacking Arp5 or Arp8 are compromised (Shen et al. 2003a). Like *rsc* mutants, an *arp8Δ* mutant show enhanced sensitivity to DSB agents when the HR pathway is eliminated by mutation, suggesting a role for INO80 in NHEJ (Morrison et al. 2004; Tsukuda et al. 2005). The *arp5Δ* mutant and to a lesser extent *arp8Δ*

mutants are also hypersensitive to an HO-induced DSB in a strain background lacking donors for HR repair (Tsukuda et al. 2005; van Attikum et al. 2004); whether this difference in sensitivity reflects a functional difference in the INO80 complex in the absence of the two subunits is not known.

Several lines of evidence indicate that INO80 is also associated with DSB repair by HR. Some of the strongest evidence comes from a study in Arabidopsis, which showed that the frequency of HR was reduced to 15 % of wild-type cells in a mutant defective for INO80 expression (Fritsch et al. 2004). Moreover, in budding yeast, HR frequencies are approximately fourfold lower in an *arp8Δ* mutant compared with a wild-type strain in both a mating type switching assay and in an allelic recombination assay in diploids. What is the role of INO80 in HR? Two opposing views have been put forward. First, it has been proposed that INO80 facilitates DNA end processing, which is a prerequisite for initiation of the HR pathway. This is based on the report that the formation of ssDNA at MAT is reduced in an *arp8Δ* mutant in a quantitative PCR-based amplification assay (van Attikum et al. 2004). However, a second report, using both a Southern blot assay to detect strand resection at MAT and a recruitment assay to measure the association and spreading of RPA with single-stranded MAT DNA concluded that strand resection occurs normally in an *arp8Δ* mutant. This study also revealed that recruitment of the Rad52 and Rad51 strand invasion proteins to the MAT DSB is delayed in an *arp8Δ* mutant, with RPA being displaced more slowly than in wild-type cells (Tsukuda et al. 2005). Nonetheless, the picture that has emerged is that INO80 enhances the recruitment of strand annealing factors to broken DNA ends either by regulating strand resection or by promoting the displacement of RPA from ssDNA.

As a major component of the cell, actin performs many important functions in the cytoplasm, through its ability to polymerize in a dynamic fashion, as well as to interact with other proteins and lipids (Cooper and Schafer 2000; Olave et al. 2002; Pollard et al. 1994; Sheterline and Sparrow 1994). Despite increasing evidence suggesting that actin is in the nucleus and may play roles in many nuclear functions, the research on nuclear actin was stalled by the lack of unambiguous demonstrations of an actin function in the nucleus both in vivo and in vitro. However, in a recent study, using temperature-sensitive actin mutants and employing biochemical approaches, it has been shown that actin participates as a monomer in INO80 chromatin remodeling by acting as an interacting surface/chaperone to facilitate the INO80 interaction with chromatin (Kapoor et al. 2013). Since actin and Arp4 are consistently present in several chromatin-modifying complexes, such as INO80, SWR1, and NuA4 (Shen et al. 2000; Galarneau et al. 2000; Mizuguchi et al. 2004), and the loss of Arp8 in the INO80 complex results in the loss of actin and Arp4 (Shen et al. 2003a), it can be argued that actin and Arp4 form a dimer and may represent a evolutionarily conserved and basic module involving nuclear actin. This actin/Arp4 module could be used repeatedly in combination with other Arps and proteins to suit specific functions in different chromatin modifying-complexes.

Nhp10, an HMG-1 like protein, has been revealed as a potential subunit of INO80 complex (Shen et al. 2003a; Gavin et al. 2002; Uetz et al. 2000) that binds to structured DNA or nucleosomes. Deletion of Nhp10 results in the loss of Ies3,

indicating that Nhp10 is important for recruitment of Ies3 into the complex. Moreover, an INO80 complex that lacks Nhp10 has reduced DNA binding activity but was able to mobilize nucleosome, suggesting that Nhp10 (and Ies3) has a less important role in chromatin remodeling compared to actin and Arps (Shen et al. 2003a). Recently, it was shown that Nhp10 (and/or Ies3) play an important role in recruitment of INO80 complex to DNA DSBs by mediating the interaction between the INO80 complex and the phosphorylated yeast H2A (γ-H2AX) (Morrison et al. 2004). Taken together, it seems that Nhp10, a unique subunit of the INO80 complex, mediates specific interactions with other factors, rather than playing an essential role in chromatin remodeling. Taf14 (also known as Swp29, Taf30, Tfg3, Anc1, and TafII30) is a subunit of Mediator, TFIID, TFIIF, SWI/SNF, NuA3, NuA4, and INO80 complexes (Shen et al. 2003a; Cairns et al. 1996b; Henry et al. 1994; John et al. 2000; Kim et al. 1994; Poon et al. 1995). Using yeast two-hybrid screening, it has been shown that Taf14 interacts with the key or catalytic subunits (such as Ino80 in the INO80 complex) of each complexes mentioned above, thereby suggesting that it plays a common regulatory role (Kabani et al. 2005). The Taf14 protein contains a conserved YEATS domain, which is also found in Yaf9, a component of NuA4 and SWR1 complexes (Bittner et al. 2004; Zhang et al. 2004), and in Sas5, a component of the SAS complex involved in chromatin silencing (Shia et al. 2005; Sutton et al. 2003). However, the function of this domain is still unclear. *taf14* null mutants display decreased transcription, defects in actin organization and hypersensitivity to heat, caffeine, hydroxyurea, UV irradiation, and methyl methanesulfonate (Henry et al. 1994; Zhang et al. 2004; Welch and Drubin 1994; Welch et al. 1993). Moreover, it has also been shown that Taf14 is involved in actin function and cell cycle arrest through Rad53 and Mec1, which play important roles in DNA damage responses (Welch and Drubin 1994; Li and Reese 2000).

Chromatin remodeling has also been linked to histone displacement in vivo during transcription initiation, suggesting that remodeling "primes" nucleosomes for removal by factors such as histone chaperones (Adkins et al. 2004; Boeger et al. 2003; Lorch et al. 2006; Reinke and Horz 2003). Nucleosome loss is the most dramatic way to expose factor-binding sites and has emerged as a general mechanism to increase chromatin accessibility during both the initiation and elongation phases of transcription and now seems to apply at DSB sites as well. Nucleosome positioning is disrupted and core histones are lost in an ~5–6-kb region around the MAT DSB within 2 h after the break is formed, and these events are dependent on the remodeling activity of INO80 (Tsukuda et al. 2005). Because the kinetics of Rad51 recruitment to the MAT DSB coincides with the kinetics of nucleosome loss, it has been proposed that a primary function of INO80 remodeling is removal of nucleosomes at the recipient broken chromosome to enable strand invasion proteins to bind to DNA (Tsukuda et al. 2005). A second consequence of INO80-mediated nucleosome loss is that it leads to removal of nucleosomes containing phospho-H2A. The dephosphorylation of phospho-H2A occurs before DNA repair is completed and is required for recovery from arrest at the DNA damage checkpoint (Keogh et al. 2006).

It was recently suggested that the protein phosphatase Pph3 dephosphorylates phospho-H2A that is no longer chromatin associated (Keogh et al. 2006). Thus, nucleosome loss at the MAT DSB site could serve at least two roles: promoting HR factor assembly on ssDNA and displacing phospho-H2A–H2B dimers that could serve as a substrate for Pph3-dependent dephosphorylation. Even though INO80 can move nucleosomes in *cis* along the chromatin fiber, it is unlikely on its own to be responsible for the loss of large numbers of nucleosomes at the DSB (estimated to be at least ten nucleosomes on each side of the break). It is more likely that a histone chaperone cooperates with INO80 to displace nucleosomes around the DSB. A good candidate for such a chaperone is the evolutionarily conserved Asf1 protein, which has global roles in chromatin disassembly in the cell and has also been implicated in DSB repair (Adkins and Tyler 2004; Prado et al. 2004). It is also possible that additional chromatin remodeling factors, working in concert with INO80, are able to displace nucleosomes around a DSB. Consistent with the idea that INO80, like SWI/SNF and RSC, directly participates in DSB repair. The Ino80 catalytic subunit is recruited to the MAT locus after DSB formation. Its recruitment depends on phospho-H2A and the INO80 Nhp10 and Arp4 subunits, both of which interact with chromatin (Morrison et al. 2004; Tsukuda et al. 2005; van Attikum et al. 2004; Downs et al. 2004). Phospho-H2A is formed normally in an *arp8Δ* mutant, indicating that the histone modification is at the head of a pathway that signals INO80 recruitment. However, histone loss at the MAT DSB occurs normally in an H2A mutant that cannot be phosphorylated, and thus histone displacement is independent of newly recruited INO80 (Tsukuda et al. 2005). This apparent contradiction is partly explained by the observation that there is a pool of INO80 at MAT even before the DSB is formed. INO80 is reported to have a role in transcription of the MAT regulatory genes (Shen et al. 2003b), and the DSB has been postulated to activate the nucleosome remodeling activity of this pool (Tsukuda et al. 2005). The additional recruitment of INO80 to the MAT locus after DSB formation could serve to regulate other aspects of NHEJ or HR. However, a preexisting pool of INO80 is unlikely to be present at every genomic location where a DSB is formed. Thus, at these locations, recruitment of INO80 by phospho-H2A is thought to be more important to provide nucleosome remodeling and/or eviction activities.

The recruitment and activation of INO80 seem to be regulated by different factors, and interestingly, the MRX complex again seems to be involved. MRX also regulates 5′–3′ strand resection at DSBs (Bressan et al. 1999; Usui et al. 2001). Importantly, an *mre11Δ* mutant shows a defect in nucleosome displacement at the MAT DSB that is even more severe than that of an *arp8Δ* mutant, and Rad51 recruitment is also more delayed (Tsukuda et al. 2005). These results suggest that MRX has a novel role in nucleosome loss and that this role occurs through two pathways—one that activates the remodeling activity of INO80 and a second that regulates strand resection. This additionally suggests that INO80 cannot efficiently catalyze nucleosome displacement in the absence of substantial amounts of ssDNA. A key question is how MRX activates INO80; does it act directly or indirectly? So far there is no report for the direct activation of INO80 by MRX; however, INO80 has been shown to be phosphorylated on the Ies4 subunit in a Mec1- and

Tel1-dependent manner after DNA damage to regulate the cell cycle checkpoint. Tel1 is a known binding partner for MRX, suggesting that MRX may activate INO80 indirectly by Tel1. It may also be possible that some subunits other than Ies4 are posttranslationally modified to regulate the activity of INO80.

SWR1

The SWR1 complex, a histone exchange factor, has been found to specifically exchange histone H2A in nucleosomes for its variant H2AZ (Mizuguchi et al. 2004; Kobor et al. 2004; Krogan et al. 2003). Histone variants are distinct nonallelic forms of major-type core histones, and in contrast to the canonical histones, which are expressed and deposited into chromatin during DNA replication, histone variants are often expressed throughout the cell cycle, and their incorporation is often replication independent (Henikoff and Ahmad 2005). Recently, genome-wide studies demonstrated that yeast H2AZ (Htz1) is globally localized to most of the gene promoters in euchromatin and generally present in the single nucleosomes that flanks a nucleosome-free region that contains the transcription initiation site (Guillemette et al. 2005; Raisner et al. 2005; Zhang et al. 2005).

The purified yeast SWR1 complex contains 14 subunits (Fig. 12.4) (Mizuguchi et al. 2004; Wu et al. 2005), where actin, Arp4, Rvb1, and Rvb2 are shared subunits with INO80 complex, and actin, Arp4, Swc4, and Yaf9 are the shared subunits with NuA4 histone acetyltransferase complex (Shen et al. 2000; Doyon and Cote 2004). Interestingly, Htz1/H2AZ is associated with the purified SWR1 complex, and it has been observed that *swr1* mutants and the *htz1* mutant share similar phenotypes in

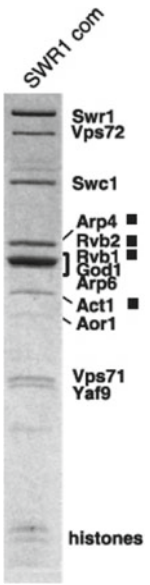

Fig. 12.4 The SWR1 chromatin remodeling complex: SDS-PAGE and Coomassie *Blue stain* showing the identity of subunits of the SWR1 (SWR1.com), purified by single step FLAG-immunoaffinity chromatography from yeast whole cell extract. Adapted from G. Mizuguchi, X. Shen, J. Landry, W. H. Wu, S. Sen, and C. Wu, *Science* 303, 344 (2004) with permission

budding yeast, thus suggesting a functional and genetic link between Htz1 and the SWR1 complex. Swr1 is a Swi2/Snf2-related ATPase and just like INO80 also contains the core ATPase with a split conserved ATPase domain. Similar to the INO80 complex, the SWR1 complex exhibits nucleosome-stimulated ATPase activity (Mizuguchi et al. 2004). Swr1 is the key catalytic subunit in the complex, which is crucial for its function since the catalytic site mutant (K727G) of Swr1 fails to rescue the *swr1* null phenotype, and the SWR1 complex containing the Swr1 K727G mutation fails to catalyze replacement of H2A with H2AZ in vitro (Mizuguchi et al. 2004). It was shown that an N-terminal region (N2) ending just before the ATPase domain of Swr1 is responsible for the binding of Arp4, Act1, Swc4, Swc5, and Yaf9; whereas the conserved ATPase domain, including the insert region, is crucial for the association of other components such as Swc2, Swc3, Rvb1, Rvb2, Arp6, and Swc6 (Wu et al. 2005). These results indicate that Swr1 is essential for the integrity of the complex and suggest that the INO80 and SWR1complexes share significant structural similarities. Swc2 is the second largest subunit in the SWR1 complex and is responsible for Swc3 association since removal of Swc2 results in the loss of Swc3 from the complex. However, Swc2 does not interact directly with Swr1, the scaffold of the complex, rather its association is bridged by Swc6 and Arp6 (Wu et al. 2005). The N-terminal region (1–281) of Swc2 displays strong binding affinity with Htz1 and was identified as the widely conserved H2AZ binding region because its metazoan counterpart, YL-1, is capable of binding to Htz1 selectively over H2A. The M6 region of Htz1 (the C-terminal α-helix), an essential region for Htz1 function, was found to be necessary for the association between Htz1 and the SWR1 complex. The acidic nature of Swc2 (1–281) and its ability to bind histones suggest that Swc2 is a histone chaperone-like subunit in the complex. The function of Swc3 remains unclear since the loss of Swc3 has no effect on association of other subunits including histones in the SWR1 complex, and in vitro histone exchange activity of SWR1 is unaffected in *swc3* mutants (Wu et al. 2005).

Swc5 is another subunit whose elimination does not influence the integrity of the SWR1 complex or Htz1 binding. However, it was found that Swc5 is necessary for functional replacement of Htz1. Interestingly, the purified SWR1 complex lacking Swc5 exhibits increased nucleosome-binding ability (Wu et al. 2005), suggesting that Swc5 may regulate the interaction between the SWR1 complex and chromatin during the Htz1 replacement process in vivo. Swc4 (also called God1, Eaf2) is encoded by an essential gene, and its mammalian homolog is DNA methyltransferase-associated protein 1 (DMAP1) (Rountree et al. 2000). Swc4 bears a SANT domain, which is present in several chromatin remodeling and HAT complexes and is crucial for their functions (Boyer et al. 2002, 2004). However, the function of Swc4 is still unknown. It was shown by yeast two-hybrid screening that Swc4 binds directly to Yaf9, another subunit of the SWR1 complex (Bittner et al. 2004), and that removal of Yaf9 results in the loss of Swc4 from the complex. Thus, the association of Swc4 is dependent on Yaf9 (Wu et al. 2005). Yaf9 is similar to, and was named after AF9, a human leukemogenic protein (Corral et al. 1996). Yaf9 is also similar to Taf14 (a component of INO80 complex) and also contains a conserved YEATS (Yaf9-ENL-AF9-Taf14-Sas5) domain. The YEATS protein family is essential in *S. cerevisiae* as

a strain lacking all three family members (Yaf9, Taf14, and Sas5) is nonviable, although none are essential individually (Zhang et al. 2004). In vitro studies revealed that Yaf9/Swc4 are required for Htz1 transfer, but not for Htz1 and nucleosome binding (Wu et al. 2005). *yaf9Δ* strains display reduced Htz1 deposition at telomere proximal genes and transcriptional profiles and phenotypes similar to *htz1Δ* mutants (Zhang et al. 2004). Taken together, these data suggest that Yaf9 and/or Swc4 plays an important role in Htz1 deposition.

Swc6 and Arp6 are mutually responsible for Swc2 and Swc3 association, since removal of either Swc6 or Arp6 results in the loss of all four subunits from the SWR1 complex. However, in the absence of Swr1, Swc2 does not associate with either Swc6 or Arp6, although Swc6 and Arp6 still tightly associate with each other.. As for their functional role in the SWR1 complex, it was revealed that Swc6 and Arp6 are required for Htz1 and nucleosome binding, as well as Htz1 exchange (Wu et al. 2005). Arp6 has been found in budding yeast, fission yeast, *Arabidopsis*, fruit fly, chicken, and humans (Kato et al. 2001; Martin-Trillo et al. 2006), indicating that it is important for conserved biological functions. Fission yeast Arp6 was found to be required for transcriptional silencing at telomeres (Ueno et al. 2004). Furthermore, both *Drosophila* and vertebrate Arp6 have been found to interact with heterochromatin protein 1 (HP1) and co-localize with HP1 in the pericentric heterochromatin (Frankel et al. 1997; Ohfuchi et al. 2006). Interestingly, proper interaction of mammalian HP1α with chromatin is disrupted in the absence of H2AZ (Rangasamy et al. 2004) In vitro studies showed that HP1α had an approximately 2.5-fold higher affinity for H2AZ-containing chromatin (Fan et al. 2004).

These findings suggest that metazoan HP1 and H2AZ, which are deposited by a SWR1 complex that contains Arp6, function together to play an important role in heterochromatin formation. However, it remains uncertain whether the Arp6 proteins in these studies function by themselves or as components of complexes similar to the SWR1 chromatin remodeling complex in budding yeast. Swc7 and Bdf1 (Bromodomain Factor 1) are the only two subunits whose assemblies have not been defined in the SWR1 complex. Bdf1 has two bromodomains (acetyl-lysine binding domains), a motif present in a number of proteins involved in transcription and chromatin modification, and associates substoichiometricly with yeast TFIID. Bdf1 and its homolog, Bdf2, are genetically redundant (Matangkasombut et al. 2000). However, only Bdf1 preferentially binds to acetylated histone H3 and H4 (Ladurner et al. 2003; Matangkasombut and Buratowski 2003) and associates with TFIID and SWR1 complex. Bdf1 was determined to be a subunit of Swr1 complex because it associates with several immuno-purified Swr1 complex components (Kobor et al. 2004). To date, the most attractive recruitment model of the SWR1 complex is that Bdf1 recognizes a specific H3 and H4 acetylation pattern and recruits the SWR1 complex, which deposits Htz1 at these chromatin loci (Raisner et al. 2005; Zhang et al. 2005). Interestingly, Bdf1 is known to be phosphorylated (Adkins et al. 2004). Therefore, the interaction between Bdf1 and acetylated histones and/or recruitment of TFIID and the SWR1 complex might be regulated by the phosphorylation status of Bdf1.

The SWR1 complex is conserved among eukaryotes. In *Drosophila*, histone variant H2Av is a bifunctional molecule since it harbors conserved sequences of both H2AZ and H2AX. It was demonstrated that phosphorylated H2Av in chromatin could be acetylated and replaced with an unmodified H2Av by the Tip60 complex, a *Drosophila* homolog of the SWR1 complex (Kusch et al. 2004). More interestingly, the Tip60 complex appears to be a fusion of yeast SWR1 and NuA4 since most subunits of the Tip60 complex have yeast homologues present in either SWR1 or NuA4 complexes in yeast (Doyon and Cote 2004; Raisner and Madhani 2006). Similarly, the human Tip60 complex is also a fusion of SWR1 and NuA4 complexes. The SRCAP (Snf2-related CREB-binding protein activator protein) complex is another SWR1 complex in human and was found to be able to replace preexisting nucleosomal H2A-H2B dimers with H2AZ-H2B dimers in an ATP-dependent manner (Raisner and Madhani 2006; Ruhl et al. 2006). Domino is the Swr1 ATPase homolog in *Drosophila*, and the human Swr1 orthologues are SRCAP and p400, primarily known for their roles in transcription (Cai et al. 2005). The Tip60 acetyltransferase, which is the homolog of yeast Esa1, is the HAT in these complexes. Although yeast SWR1 complex does not co-purify with the NuA4 complex (the major HAT for histones H2A and H4), growing evidence suggests that they work together to regulate H2AZ deposition. Furthermore, genome-wide studies revealed that both the NuA4 complex and the Gcn5 acetyltransferase (the HAT for histones H2B and H3) are required for efficient recruitment of Htz1, indicating that specific histone acetylation patterns play an important role in H2AZ deposition.

Similar to the INO80 complex, emerging evidence suggests that the SWR1 complex may also play a role in DNA repair. First of all, *swr1* mutants are hypersensitive to DNA damage-inducing agents (MMS and hydroxyurea) (Mizuguchi et al. 2004; Kobor et al. 2004). Second, it was demonstrated that the purified SWR1 complex specifically binds to H2A phosphoserine-129 peptides in vitro. The NuA4 complex and Rvb1-containing complexes, which may be INO80 and/or SWR1complexes, are recruited to DSB sites in vivo (Downs et al. 2004). The HAT activity of the human Tip60 complex, which is a fusion of NuA4 and SWR1 complexes, has been implicated in ATM signal pathway activation and DNA repair (Ikura et al. 2000; Sun et al. 2005). In addition, the *Drosophila* Tip60 complex is required for acetylation of phospho-H2Av at DNA lesions and subsequent replacement with unmodified H2Av (Kusch et al. 2004). As previously mentioned, the INO80 subfamily prominently and specifically associates with γ-H2AX and H2AZ, which have important roles in specifying the functions of the INO80 and SWR1 complexes in processes that maintain genome stability. The histone variant H2AX is phosphorylated on its C-terminus by ATM and ATR (or Tel1 and Mec1) in chromatin regions that surround damaged DNA (Burma et al. 2001; Ward et al. 2001) and is also a crucial component of DNA damage responses. Defects in the regulation of H2AX phosphorylation lead to alterations in the DNA damage checkpoint, genomic instability, and cancer predisposition in mice (Downs et al. 2000; Bassing et al. 2003; Celeste et al. 2003a; Keogh et al. 2006). γ-H2AX participates in the maintenance of genome integrity by serving as docking sites for several DNA

damage response components (Celeste et al. 2003b; Nakamura et al. 2004; Paull et al. 2000; Unal et al. 2004), including the INO80 and SWR1 complexes, thereby focusing the activity of these factors to regions that are proximal to the damage site.

12.2.2.4 INO80 and SWR1 in DNA DSB Repair

As previously mentioned, the *S. cerevisiae* INO80 and SWR1 complexes bind directly to sites of DNA DSBs through their association with γ-H2AX (Morrison et al. 2004; van Attikum et al. 2004, 2007). In particular, these complexes are required for proper processing of the DNA ends that are involved in the DSB (Fig. 12.5). Specifically, the *S. cerevisiae* INO80 complex influences the proximal eviction of nucleosomes surrounding DSBs, including nucleosomes that contain γ-H2AX and H2AZ. Deletion of *arp8*, which reduces the in vitro chromatin remodeling activity of the INO80 complex (Shen et al. 2003a), or deletion of *nhp10*, which decreases the recruitment of the INO80 complex to DSB, results in defective nucleosome eviction in regions proximal to the DSB (Tsukuda et al. 2005, 2009; van Attikum et al. 2007), and in chromatin of the homologous donor locus (Tsukuda et al. 2009). Impaired nucleosome eviction seems to alter the subsequent steps that facilitate the DNA damage response, owing to the reduced association of repair and checkpoint factors with the site of the DSB. Thus, this suggests that the presence of nucleosomes at repair sites impedes the association of proteins that facilitate these processes. For example, mutants of the INO80 complex in *S. cerevisiae* have defects in the association of the Mre11 nuclease with DSBs, and defects in the Mre11-mediated promotion of single-stranded DNA, which is a prerequisite for repair through HR (van Attikum et al. 2004, 2007; Morrison et al. 2007).

The finding that the INO80 complex directly influences single-stranded DNA resection is controversial—a separate study did not observe this defect in single-stranded DNA production (Tsukuda et al. 2005). Moreover, another study has questioned whether nucleosome eviction is a determinant or a consequence of single-stranded DNA production because these two events are tightly linked and are difficult to separate experimentally (Chen et al. 2008). Nevertheless, a downstream event of DNA resection, namely the invasion of the single stranded DNA into the homologous donor locus, is impaired in an *arp8* mutant (Tsukuda et al. 2009). The association of other DNA damage response factors, such as Mec1 and Rad51, is also decreased in this *arp8 Δ* mutant (Tsukuda et al. 2005, 2009; van Attikum et al. 2007). Conversely, the yeast

Fig. 12.5 (continued) INO80 production of single-stranded DNA. During HR, the single-stranded DNA-binding protein replication protein A (RPA) and the Mec1 checkpoint kinase bind to resected DNA. The cohesin complex assists in holding the sister chromatids together. The Rad52 epistasis group, which includes Rad51, Rad52, and Rad54, facilitate the search for and synapsis of homologous DNA sequences. A Holliday junction is formed between the two DNA strands, followed by DNA synthesis and resolution of the junction. During NHEJ, the SWR1 complex promotes the association of Ku80 to the DNA ends, a component of the Ku70–Ku80 complex that is required for NHEJ. Repair is then completed following ligation of the DNA ends. Mutants of the INO80 complex have defects in HR and NHEJ, whereas mutants of the SWR1 complex have defects in error-free NHEJ (From *Nature Reviews Molecular Cell Biology* 2009)

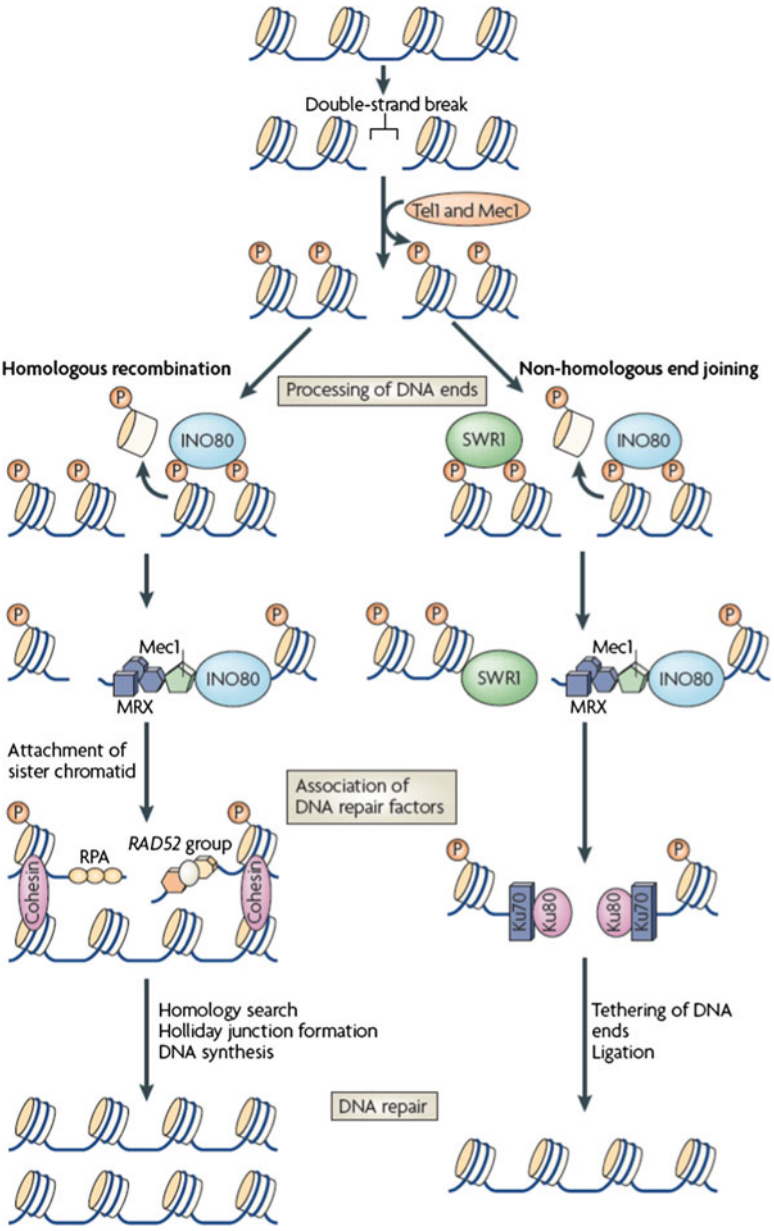

Fig. 12.5 INO80 and SWR1 complexes regulate double-strand break repair: The *S. cerevisiae* kinases Tel1 and Mec1 (ataxia telangiectasia (A-T) mutated (ATM) and A-T and RAD3-related (ATR) in mammals) phosphorylate H2AX after the creation of a double-strand break, which can be repaired by homologous recombination (HR) or nonhomologous end joining (NHEJ). During HR and NHEJ, the INO80 and SWR1 complexes bind to phosphorylated H2AX. The INO80 complex is involved in nucleosome eviction proximal to the break site. The DNA ends are then recognized by the Mre11–Rad50–Xrs2 (MRX) complex. The Mre11 nuclease is involved in the

SWR1 complex does not affect nucleosome eviction at DSBs (van Attikum et al. 2007). However, deletion of its chromatin substrate Htz1, which is transiently enriched at DSB sites, results in decreased production of single stranded DNA and reduced association of Rad51 to DSB proximal regions (Kalocsay et al. 2009). The SWR1 complex is also needed for efficient recruitment of Mec1 and Ku80 to DSBs, which are required for NHEJ (van Attikum et al. 2007). In addition, deletion of *htz1* results in the inability of a persistent DSB to localize to the nuclear periphery (Kalocsay et al. 2009), a rather enigmatic event that promotes DNA repair (Nagai et al. 2008).

Defects in the chromatin remodeling activity of INO80 subfamily complexes ultimately results in deficient DNA repair. For instance, mutants of the Arp subunits in the *S. cerevisiae* INO80 complex have defects in NHEJ (van Attikum et al. 2004, 2007) as well as the HR pathway (Kawashima et al. 2007). In *arp8* mutants, when HR repair does occur, gene conversion often consists of large and discontinuous DNA tracts that might result from unstable heteroduplex DNA that forms during strand invasion and branch migration (Tsukuda et al. 2009). Indeed, mutants of the INO80 complex in plants and mammals also display defects in DSB repair (Fritsch et al. 2004; Wu et al. 2007), suggesting conserved mechanisms for the INO80 complex in this pathway. By contrast, the *S. cerevisiae* Swr1 ATPase subunit does not seem to function in HR, but rather participates in the error-free NHEJ pathway (van Attikum et al. 2007; Kawashima et al. 2007). These results show that different complexes in the INO80 subfamily can contribute to distinct repair mechanisms, in part owing to the function of specialized subunits in each complex.

12.2.2.5 INO80 and SWR1 Influence Checkpoint Pathways

Checkpoint pathways function cooperatively with DNA repair pathways by altering cell cycle kinetics, which allows repair of damaged DNA and reentry into the cell cycle (Branzei and Foiani 2008; Harrison and Haber 2006). For example, the production of single-stranded DNA during DNA repair is required for the recruitment and activation of the checkpoint Mec1 kinase (Lisby et al. 2004; Nakada et al. 2004; Zou and Elledge 2003). Mec1 then activates downstream effector kinases that target proteins to arrest the cell cycle (Sweeney et al. 2005). Additional checkpoint proteins, such as *S. cerevisiae* Rad9 (53BP1 in mammals), bind to damage sites in a γ-H2AX-dependent manner and assist in the activation of downstream checkpoint signaling components (Ward et al. 2001; Celeste et al. 2003b; Nakamura et al. 2004; Gilbert et al. 2001). The Ies4 subunit of the INO80 complex is phosphorylated by the Tel1 and Mec1 kinases to modulate DNA replication checkpoint responses without altering DSB repair processes (Morrison et al. 2007). Activation of the S-phase checkpoint delays replication origin firing, and cells carrying mutations that mimic phosphorylated Ies4 display inappropriately elevated S-phase checkpoint activation. In cells with mutations that prevent Ies4 phosphorylation, deletion of the *tof1* checkpoint factor [which mediates the DNA damage checkpoint response during exposure to replication stress (Katou et al. 2003; Tourriere et al. 2005)] causes dramatic defects in the ability to resume the progression of the cell cycle when the replication stress is removed. These results suggest redundant or

compensating roles between Tof1 and phosphorylated Ies4 in the cell cycle checkpoint pathway. Thus, the INO80 complex is capable of many distinct activities in DNA damage response pathways, such as the repair of DSBs and the regulation of the replication checkpoint, in part by using specific subunits such as Nhp10 and Ies4.

Both INO80 and SWR1 complexes in *S. cerevisiae* regulate the abundance of H2A variants in chromatin following exposure to DNA-damaging agents (Papamichos-Chronakis et al. 2006). This influences checkpoint adaptation—a particularly rare occurrence in which the cell survives despite the presence of a persistently unrepaired DSB (Papamichos-Chronakis et al. 2006). Moreover, a recent report shows that deletion of *swr1* or the *htz1* histone variant in *S. cerevisiae* results in delayed checkpoint activation in response to a single persistent DSB (Kalocsay et al. 2009). Chromatin remodeling activities that alter the levels of γ-H2AX might indirectly regulate the abundance of DNA damage proteins that bind to γ-H2AX and activate cell cycle checkpoints at sites of DNA damage as well as the subsequent dissociation of these proteins to facilitate checkpoint recovery. Alternatively, the chromatin remodeling activity of INO80 subfamily complexes might produce DNA substrates that activate checkpoint factors. Indeed, mutants of the INO80 subfamily in *S. cerevisiae* that have reduced single-stranded DNA also have decreased recruitment of the checkpoint factor Mec1 to DSBs along with delayed checkpoint activation (van Attikum et al. 2007).

12.3 Chromatin Remodeling During DNA Replication

DNA replication is a highly complex nuclear process involving the interdependent activity of many factors that function in all phases of the cell cycle. Evidence suggests that, apart from high DNA sequence fidelity, the associated chromatin structure also has to transfer to the next generation to ensure that both the genetic and the epigenetic information remain unaltered over generations. Importantly, specific histone–DNA interactions need to be disrupted and reestablished during the cell cycle in order to allow faithful and rapid duplication of DNA, as well as associated chromatin structures. To achieve this, it is plausible that ATP-dependent chromatin remodeling may play important regulatory roles in facilitating many steps of the replication process. In this section, we summarize recent findings linking chromatin remodeling to DNA replication and address the potential roles of ATP-dependent chromatin remodeling in the key stages of DNA replication.

12.3.1 Before S-Phase

DNA replication starts before the S-phase transition with the ordered assembly of a multiprotein pre-replicative complex (pre-RC). Formation of the pre-RC begins with ORC (origin recognition complex) binding to replication origins.

Although the mechanism of ORC recruitment differs among eukaryotes, the assembly of pre-RC is conserved. ORC recruits the initiation factors Cdc6 and Cdt1 to origins. Cdc6 and Cdt1 are required for loading of the Mcm2-7 proteins that function as the replicative helicase during S-phase (Takeda and Dutta 2005). In *S. cerevisiae*, ORC recruitment depends on the recognition of an 11 base pair element in the autonomously replicating sequences (ARS) (Bell and Stillman 1992). In the fission yeast *S. pombe*, AT-rich elements appear to be sufficient for specifying a functional origin (Okuno et al. 1999; Segurado et al. 2003). However, in higher eukaryotes, the organization of origins is more complex and difficult to define, but it seems that epigenetic factors and chromatin structure might be important in defining origins in higher eukaryotes. Given that ORC binding may occur in the context of chromatin, the question arises whether chromatin remodeling has a role during DNA replication initiation. Based on nucleosome mapping, plasmid stability measurement, and 2D gel analyses, it was shown that a plasmid with an altered nucleosome structure next to the ORC binding site showed a reduction in DNA replication initiation efficiency, while the ORC binding pattern remained unaltered. Furthermore, alteration of the ORC-dependent nucleosome configuration of a yeast origin compromised origin function by disrupting pre-RC formation, supporting a positive role for nucleosomes at the origin (Lipford and Bell 2001).

It was shown that the SWI/SNF remodeling complex was required for replication initiation in a yeast minichromosome assay (Flanagan and Peterson 1999). Here, the stability of minichromosomes was assessed as a measurement of origin of replication function, and it was observed that minichromosomes containing ARS1, ARS307, or ARS309 were not significantly altered by inactivation of the SWI/SNF complex. In contrast, the stability of a minichromosome that contained ARS121 was dramatically reduced in the *swi/snf* mutant compared to the wild type. Together, these studies provide indirect evidence that chromatin remodeling may be required to move nucleosomes around the replication origin either to unmask the ORC binding site, or to configure the nucleosomes around the ORC-binding site to precise positions, allowing ORC to bind and function efficiently. ATP-dependent chromatin remodeling complexes are candidates for achieving such nucleosomal movements (Fig. 12.6).

If indeed chromatin remodeling complexes are needed to enhance ORC-binding or function, these complexes themselves need to be recruited to the replication origin. One mechanism could be through binding to ORC directly or by interacting with other replication initiating factors such as Cdc6 and Cdt1. Another potential mechanism could be the direct binding of chromatin remodeling complexes to replication origins, which could be mediated either through DNA binding or by recognition of a specific DNA replication histone code. The INO80 class of ATP-dependent chromatin remodeling complexes is of particular interest, since these complexes contain hexameric helicases, Rvb1 and Rvb2, which can potentially be used to unwind DNA during replication initiation. However, to date no such findings correlate the activity of INO80 chromatin remodeling complex to replication initiation.

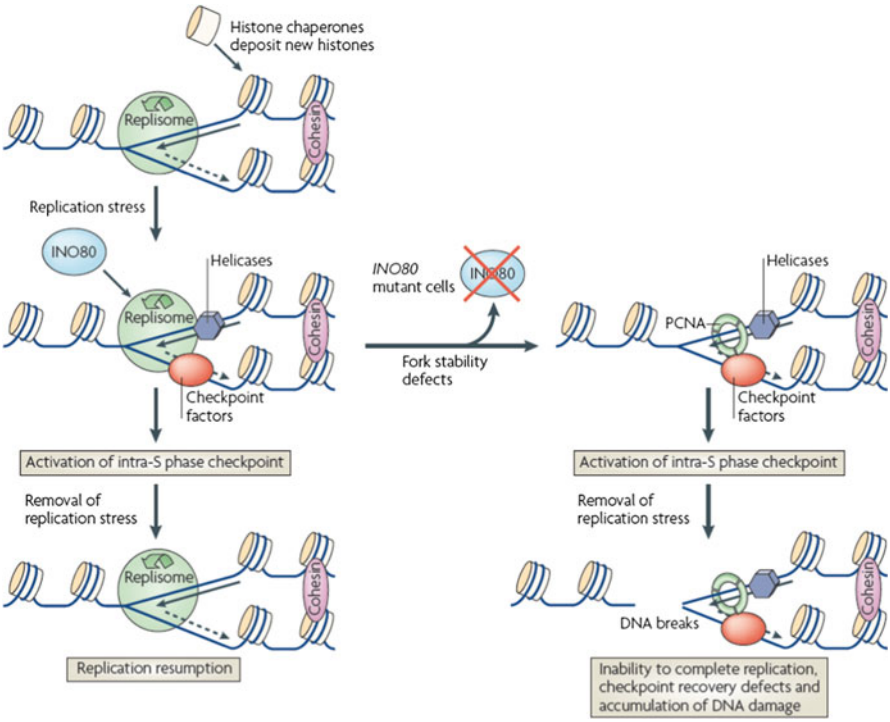

Fig. 12.6 The INO80 complex promotes recovery of stalled replication forks: During replication, DNA synthesis is catalyzed by the replisome, which contains polymerases, primases, and helicases. Histone chaperones deposit histones on to newly synthesized DNA. Replication forks stall when exposed to replication stress, such as depleted dNTP pools. When this happens, the replisome is stabilized by DNA damage response factors, such as the *S. cerevisiae* INO80 complex and the Tof1 and Mrc1 checkpoint factors, which activate the intra-S-phase checkpoint to prevent replication origin firing. On the removal of replication stress, the replication fork recovers and DNA synthesis resumes. In the absence of the INO80 complex, fork stability defects occur as the replisome is destabilized and some of its components dissociate, leaving others, such as proliferating cell nuclear antigen (PCNA), at the replication fork. In this case, replication does not restart following the removal of replication stress. Accordingly, checkpoint recovery is delayed and DNA damage accumulates (From *Nature Reviews Molecular Cell Biology* 2009)

12.3.2 The G1/S Transition

During S-phase, pre-RCs initiate replication by promoting origin unwinding and facilitating the recruitment of replicative DNA polymerases. This process is regulated by a set of replication factors, the activities of cyclin-dependent kinases (CDKs), and the Dbf-dependent kinase (DDK). Cell cycle regulation of DNA replication ensures that DNA replicates just once during S-phase of each cell cycle. Several replication factors must be loaded in order to pass through the G1/S transition. The MCM complex is thought to be the replicative helicase, and its loading correlates with the licensing and activation of a replication origin (Zhou et al. 2005).

As mentioned above, ATP-dependent chromatin remodeling is thought to be important for the repositioning of nucleosomes preceding the binding of DNA replication factors. Therefore, cell cycle changes in chromatin remodeling and histone modifications at eukaryotic origins might also be important regulatory features controlling replication and licensing factors access to DNA. Interestingly, in a recent study, it was found that the dyad symmetry (DS) region of origin of plasmid replication (OriP) was flanked by nucleosomes that undergo chromatin remodeling and histone deacetylation at the G1/S border of the cell cycle (Zhou et al. 2005). These changes correlated with MCM3 binding in the G1/S-phase of the cell cycle, suggesting that cell cycle changes in chromatin are coordinated with replication licensing at OriP. In this study, it was also found that SNF2h (a member of the Swi/Snf family) was enriched at DS in G1/S arrested cells. Moreover, depletion of SNF2h inhibited OriP replication and decreased G1/S associated binding of MCM3. These results are consistent with a role of SNF2h in the remodeling of nucleosomes, which facilitates the loading of MCM3.

ATP-dependent chromatin remodeling could potentially play several roles during the G1/S transition. As suggested by the SNF2h study, chromatin remodeling may be needed to reconfigure nucleosomes once the pre-RC is formed and to facilitate the loading of MCM proteins. Subsequently, the reconfigured chromatin structure may not be conducive to initiation events such as ORC binding. The reconfiguration of chromatin at the G1/S transition would therefore be an important way to ensure that initiation only happens once at a given origin. Similarly, the recruitment of ATP-dependent chromatin remodeling complexes at the G1/S transition can be achieved either by interactions with G1/S specific replication factors or by recognition of a particular pattern of histone modifications at the G1/S transition. Another potential mechanism to achieve tight regulation of chromatin remodeling during the cell cycle could be through cell cycle-dependent expression or posttranslational modifications of subunits of chromatin remodeling complexes.

12.3.3 Moving Along with the Replication Fork

The final step in replication initiation is the loading of the replicative polymerases. DNA polymerase alpha (Pol α) is recruited to origins and synthesizes short RNA primers for leading and lagging strand synthesis. DNA Pol α is the only polymerase that can initiate synthesis de novo on single stranded DNA. After primer synthesis, polymerase switching occurs, which replaces DNA Pol α with DNA polymerase delta (Pol δ) and/or DNA polymerase epsilon (Pol ε). Processive DNA synthesis requires DNA Pol δ and DNA Pol ε to associate with the ring-shaped processivity factor, proliferating cell nuclear antigen (PCNA), which encircles DNA and topologically links the polymerase to DNA. PCNA is loaded onto the DNA template by the clamp loader, replication factor C (RFC) (Takeda and Dutta 2005).

After loading of polymerase, the replication fork is established and it starts moving through euchromatin and heterochromatin. There are several ways in which ATP-dependent chromatin remodeling can potentially contribute at this stage. The loading of DNA polymerases and PCNA may be facilitated by local reconfiguration of nucleosomes. In this case, interactions between chromatin remodeling complexes and subunits of DNA polymerases, PCNA, or RFC may provide the necessary recruitment mechanism. Perhaps more importantly, in order for replication to proceed through chromatin, it might be necessary to pave the way for the replication fork to move without obstacles. In this regard, chromatin remodeling complexes might have an important role during fork movement. Interestingly, two remodeling complexes have been implicated in heterochromatin replication. RNAi mediated depletion of ACF1-ISWI (ATP utilizing chromatin assembly and remodeling factor 1) has been showed to impair the replication of heterochromatin in HeLa cells (Collins et al. 2002). It has been demonstrated that ACF1 in a complex with SNF2h was required for efficient DNA replication through highly condensed chromatin. It was proposed that ACF1-SNF2h complex might facilitate this process by remodeling chromatin structure to allow movement of the replication fork. Moreover, it has also been shown that WSTF (Williams syndrome transcription factor) interacts with PCNA directly to target chromatin remodeling by SNF2h to replication foci (Poot et al. 2004). RNAi depletion of WSTF or SNF2h caused a compaction of newly replicated chromatin and increased the amount of heterochromatin markers. Furthermore, it has been proposed that the WSTF-SNF2h complex could have a role in chromatin maturation and the maintenance of epigenetic patterns through DNA replication (Poot et al. 2005). Chromatin remodeling by WSTF-SNF2h might keep the chromatin open after the replication fork passes, thus creating a window of opportunity for the epigenetic machinery to copy all the epigenetic marks, passing them on to the next generation with high fidelity. Although it seems that the WSTF-SNF2h complex has a direct role in replication, its precise function during elongation remains to be investigated. Nonetheless, these studies suggest that ATP-dependent chromatin remodeling plays important roles during the progression of DNA replication, either by clearing the path for the replication fork, or by allowing efficient transmission of epigenetic memory.

Strikingly, in a recent study, it has been shown that the Okazaki fragments during replication elongation are sized according to the nucleosome repeat, thereby suggesting a role for nascent chromatin assembly immediately after the passage of replication fork (Smith and Whitehouse 2012). However, the precise mechanism and the regulated coordination of the factors with the replication fork governing this mechanism are still needed to be described. Since chromatin is quickly reassembled after DNA replication, mostly through replication-coupled chromatin assembly pathways such as the CAF1 and ASF1 pathways, it is also possible that ATP-dependent chromatin remodeling complexes facilitate replication-coupled chromatin assembly by enhancing the movements of histones in and out of the nucleosomes. The involvement of ATP-dependent chromatin remodeling in fork progression and its relationship with replication-coupled chromatin assembly should be further investigated.

12.3.4 Stalled Replication Forks

In general, a replication fork pauses after it encounters DNA lesions or when nucleotide pools are depleted. Replication forks appear to be able to sense these conditions since checkpoints are activated during S-phase. In this regard, it is of interest to highlight the involvement of chromatin remodeling activities in response to DNA damage. As discussed in a previous section, several recent studies have directly implicated chromatin remodeling activities in DNA repair (Morrison et al. 2004; van Attikum et al. 2004). It is thought that chromatin remodeling might affect DNA repair by providing the repair machinery with an exposed or open chromatin environment that might facilitate the recruitment of DNA repair proteins. However, it can also be argued that chromatin remodeling is needed to form a compact chromatin structure that would hold broken DNA ends close to each other (Fig. 12.5).

Chromatin remodeling might also assist in the restoration of the chromatin structure after DNA damage has been repaired. Interestingly, it was shown that the histone chaperone CAF1, important for replication-coupled chromatin assembly, deposits histones onto DNA after repair. CAF1 is recruited to sites of NER (nucleotide excision repair) and single-strand break repair, probably through interaction with PCNA, an essential molecule in the replication fork (Ehrenhofer-Murray 2004). Because of the intimate links between DNA replication and repair, chromatin remodeling complexes that assist DNA repair might also play a role in DNA replication, particularly at stalled replication forks. Regulation of DNA replication forks is tightly linked to DNA damage and DNA replication checkpoint controls. When replicative polymerases encounter a lesion during DNA replication, the replication fork stalls. Then, DNA polymerases capable of bypass synthesis have to be loaded. Recent observations have led to the conclusion that PCNA, due to its interaction with Pol δ, could be located at the point of polymerase stalling and play a role as a recruiting platform. This could promote the switch from replicative to trans-lesion polymerases required to re-start the replication fork. This observation suggests a window in which chromatin remodeling complexes may play a role during replication fork stalling. PCNA or other components, which already move with the replication fork, might be able to recruit chromatin remodeling complexes in order to assist with replication fork reestablishment after the stall.

The roles of chromatin remodeling complexes at the stalled replication forks might be similar to those proposed above for sites of DNA repair. Another mechanism by which ATP-dependent chromatin remodeling complexes may affect stalled replication forks is through the checkpoint responses. Recent studies have implicated several DNA replication factors in mediating the checkpoint response, such as PCNA, RFC, and RPA. It is possible that chromatin remodeling complexes may interact with these replication proteins at the stalled replication forks in order to efficiently activate checkpoints, either by facilitating the access of checkpoint proteins to stalled replication fork, or though direct activation of checkpoints. It is also possible that chromatin remodeling complexes exert their function after checkpoint activation by assisting the downstream DNA repair process or the loading of alternative polymerases. Finally, stability of the replication fork after the stall is important to avoid a collapse of the fork, and chromatin remodeling complexes might play an important role at stabilizing chromatin structure during the stall and the reestablishment of the fork. Given the

multiple potential roles of chromatin remodeling at stalled replication forks and in other steps of replication discussed above, it is important to begin investigating the involvement of a specific chromatin remodeling complex in a systematic way to reveal the contribution of ATP-dependent chromatin remodeling to DNA replication.

12.4 Concluding Remarks

Chromatin remodeling is the indispensable requirement of eukaryotic genome during almost all kinds of DNA transactions. The ATP-dependent chromatin remodeling complexes are the major players to remodel chromatin in context of DNA repair and replication. Mechanisms related to some chromatin remodeling complexes, which are required during these processes, have been thoroughly studied. However, mechanisms related to some conserved complexes are still vague. Our understanding of DNA repair and replication in context of chromatin suggest that chromatin has to be relaxed both temporally and spatially to get repair and replication machinery access to DNA. Importantly, during these processes, the eviction and repositioning of nucleosomes seems to be tightly regulated, which is essential for the maintenance of genome integrity over generations. Thus, ATP-dependent chromatin remodeling complexes are needed at both the initiation and completion of these processes. How might chromatin remodeling complexes work in concert with each other? Is there any mechanism, which keeps these remodeling complexes initially in check and further activates when they are required? Or is there any other factor, which helps to cross talk these complexes with each other during different stages of DNA repair and replication?

Further investigations are necessarily required to address these unanswered questions, which may help to clear the fuzzy picture of eukaryotic DNA repair and replication in context of chromatin remodeling.

Acknowledgments We would like to thank our laboratory colleagues for critical review of the manuscript. P.K. like to acknowledge Odyssey postdoctoral program and the Theodore N. Law Endowment for Scientific achievements at The UT MD Anderson Cancer Center, and X.S. want to acknowledge funds and grants in our laboratory from the US National Cancer Institute (K22CA100017), the US National Institute of General Medical Sciences (R01GM093104), and the Center for Cancer Epigenetics at MD Anderson Cancer Center.

References

Abrams E, Neigeborn L, Carlson M (1986) Molecular analysis of SNF2 and SNF5, genes required for expression of glucose-repressible genes in Saccharomyces cerevisiae. Mol Cell Biol 6:3643–3651

Adkins MW, Tyler JK (2004) The histone chaperone Asf1p mediates global chromatin disassembly in vivo. J Biol Chem 279:52069–52074

Adkins MW, Howar SR, Tyler JK (2004) Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast PHO5 and PHO8 genes. Mol Cell 14:657–666

Badis G et al (2008) A library of yeast transcription factor motifs reveals a widespread function for Rsc3 in targeting nucleosome exclusion at promoters. Mol Cell 32:878–887
Bassing CH et al (2002) Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. Proc Natl Acad Sci USA 99:8173–8178
Bassing CH et al (2003) Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumors. Cell 114:359–370
Bell SP, Stillman B (1992) ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. Nature 357:128–134
Bittner CB, Zeisig DT, Zeisig BB, Slany RK (2004) Direct physical and functional interaction of the NuA4 complex components Yaf9p and Swc4p. Eukaryot Cell 3:976–983
Boeger H, Griesenbeck J, Strattan JS, Kornberg RD (2003) Nucleosomes unfold completely at a transcriptionally active promoter. Mol Cell 11:1587–1598
Boyer LA, Peterson CL (2000) Actin-related proteins (Arps): conformational switches for chromatin-remodeling machines? Bioessays 22:666–672
Boyer LA et al (2002) Essential role for the SANT domain in the functioning of multiple chromatin remodeling enzymes. Mol Cell 10:935–942
Boyer LA, Latek RR, Peterson CL (2004) The SANT domain: a unique histone-tail-binding module? Nat Rev Mol Cell Biol 5:158–163
Branzei D, Foiani M (2008) Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol 9:297–308
Bressan DA, Baxter BK, Petrini JH (1999) The Mre11-Rad50-Xrs2 protein complex facilitates homologous recombination-based double-strand break repair in *Saccharomyces cerevisiae*. Mol Cell Biol 19:7681–7687
Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ (2001) ATM phosphorylates histone H2AX in response to DNA double-strand breaks. J Biol Chem 276:42462–42467
Cahill D, Connor B, Carney JP (2006) Mechanisms of eukaryotic DNA double strand break repair. Front Biosci 11:1958–1976
Cai Y et al (2005) The mammalian YL1 protein is a shared subunit of the TRRAP/TIP60 histone acetyltransferase and SRCAP complexes. J Biol Chem 280:13665–13670
Cairns BR et al (1996a) RSC, an essential, abundant chromatin-remodeling complex. Cell 87:1249–1260
Cairns BR, Henry NL, Kornberg RD (1996b) TFG/TAF30/ANC1, a component of the yeast SWI/SNF complex that is similar to the leukemogenic proteins ENL and AF-9. Mol Cell Biol 16:3308–3316
Cairns BR et al (1999) Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains. Mol Cell 4:715–723
Cao Y, Cairns BR, Kornberg RD, Laurent BC (1997) Sfh1p, a component of a novel chromatin-remodeling complex, is required for cell cycle progression. Mol Cell Biol 17:3323–3334
Carey M, Li B, Workman JL (2006) RSC exploits histone acetylation to abrogate the nucleosomal block to RNA polymerase II elongation. Mol Cell 24:481–487
Carlson M, Laurent BC (1994) The SNF/SWI family of global transcriptional activators. Curr Opin Cell Biol 6:396–402
Carlson M, Osmond BC, Botstein D (1981) Mutants of yeast defective in sucrose utilization. Genetics 98:25–40
Celeste A et al (2002) Genomic instability in mice lacking histone H2AX. Science 296:922–927
Celeste A et al (2003a) H2AX haploinsufficiency modifies genomic stability and tumor susceptibility. Cell 114:371–383
Celeste A et al (2003b) Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nat Cell Biol 5:675–679
Chai B, Huang J, Cairns BR, Laurent BC (2005) Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. Genes Dev 19:1656–1661
Chambers AL et al (2012) The two different isoforms of the RSC chromatin remodeling complex play distinct roles in DNA damage responses. PLoS One 7:e32016

Chen HT et al (2000) Response to RAG-mediated VDJ cleavage by NBS1 and gamma-H2AX. Science 290:1962–1965
Chen CC et al (2008) Acetylated lysine 56 on histone H3 drives chromatin assembly after repair and signals for the completion of repair. Cell 134:231–243
Chen SH, Albuquerque CP, Liang J, Suhandynata RT, Zhou H (2010) A proteome-wide analysis of kinase-substrate network in the DNA damage response. J Biol Chem 285:12803–12812
Chen X et al (2012) The Fun30 nucleosome remodeller promotes resection of DNA double-strand break ends. Nature 489:576–580
Chi TH et al (2002) Reciprocal regulation of CD4/CD8 expression by SWI/SNF-like BAF complexes. Nature 418:195–199
Chiba H, Muramatsu M, Nomoto A, Kato H (1994) Two human homologues of Saccharomyces cerevisiae SWI2/SNF2 and Drosophila brahma are transcriptional coactivators cooperating with the estrogen receptor and the retinoic acid receptor. Nucleic Acids Res 22:1815–1820
Collins N et al (2002) An ACF1-ISWI chromatin-remodeling complex is required for DNA replication through heterochromatin. Nat Genet 32:627–632
Cooper JA, Schafer DA (2000) Control of actin assembly and disassembly at filament ends. Curr Opin Cell Biol 12:97–103
Corral J et al (1996) An Mll-AF9 fusion gene made by homologous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. Cell 85:853–861
Costelloe T et al (2012) The yeast Fun30 and human SMARCAD1 chromatin remodellers promote DNA end resection. Nature 489:581–584
Cote J, Quinn J, Workman JL, Peterson CL (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science 265:53–60
D'Amours D, Jackson SP (2002) The Mre11 complex: at the crossroads of dna repair and checkpoint signalling. Nat Rev Mol Cell Biol 3:317–327
Daley JM, Palmbos PL, Wu D, Wilson TE (2005) Nonhomologous end joining in yeast. Annu Rev Genet 39:431–451
Dechassa ML et al (2010) SWI/SNF has intrinsic nucleosome disassembly activity that is dependent on adjacent nucleosomes. Mol Cell 38:590–602
Downs JA, Lowndes NF, Jackson SP (2000) A role for *Saccharomyces cerevisiae* histone H2A in DNA repair. Nature 408:1001–1004
Downs JA et al (2004) Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. Mol Cell 16:979–990
Doyon Y, Cote J (2004) The highly conserved and multifunctional NuA4 HAT complex. Curr Opin Genet Dev 14:147–154
Eapen VV, Sugawara N, Tsabar M, Wu WH, Haber JE (2012) The *Saccharomyces cerevisiae* chromatin remodeler Fun30 regulates DNA end-resection and checkpoint deactivation. Mol Cell Biol 32(22):4727–4740
Ebbert R, Birkmann A, Schuller HJ (1999) The product of the SNF2/SWI2 paralogue INO80 of *Saccharomyces cerevisiae* required for efficient expression of various yeast structural genes is part of a high-molecular-weight protein complex. Mol Microbiol 32:741–751
Eberharter A, Becker PB (2004) ATP-dependent nucleosome remodelling: factors and functions. J Cell Sci 117:3707–3711
Ehrenhofer-Murray AE (2004) Chromatin dynamics at DNA replication, transcription and repair. Eur J Biochem 271:2335–2349
Fan JY, Rangasamy D, Luger K, Tremethick DJ (2004) H2A.Z alters the nucleosome surface to promote HP1alpha-mediated chromatin fiber folding. Mol Cell 16:655–661
Ferreira H, Flaus A, Owen-Hughes T (2007) Histone modifications influence the action of Snf2 family remodelling enzymes by different mechanisms. J Mol Biol 374:563–579
Flanagan JF, Peterson CL (1999) A role for the yeast SWI/SNF complex in DNA replication. Nucleic Acids Res 27:2022–2028
Frankel S et al (1997) An actin-related protein in Drosophila colocalizes with heterochromatin protein 1 in pericentric heterochromatin. J Cell Sci 110(Pt 17):1999–2012

Fritsch O, Benvenuto G, Bowler C, Molinier J, Hohn B (2004) The INO80 protein controls homologous recombination in *Arabidopsis thaliana*. Mol Cell 16:479–485
Galarneau L et al (2000) Multiple links between the NuA4 histone acetyltransferase complex and epigenetic control of transcription. Mol Cell 5:927–937
Gavin AC et al (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 415:141–147
Gilbert CS, Green CM, Lowndes NF (2001) Budding yeast Rad9 is an ATP-dependent Rad53 activating machine. Mol Cell 8:129–136
Guillemette B et al (2005) Variant histone H2A.Z is globally localized to the promoters of inactive yeast genes and regulates nucleosome positioning. PLoS Biol 3:e384
Haber JE (1998) The many interfaces of Mre11. Cell 95:583–586
Harrison JC, Haber JE (2006) Surviving the breakup: the DNA damage checkpoint. Annu Rev Genet 40:209–235
Henikoff S, Ahmad K (2005) Assembly of variant histones into chromatin. Annu Rev Cell Dev Biol 21:133–153
Henry NL et al (1994) TFIIF-TAF-RNA polymerase II connection. Genes Dev 8:2868–2878
Ho L et al (2009) An embryonic stem cell chromatin remodeling complex, esBAF, is essential for embryonic stem cell self-renewal and pluripotency. Proc Natl Acad Sci USA 106:5181–5186
Ikura T et al (2000) Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. Cell 102:463–473
Imbalzano AN, Kwon H, Green MR, Kingston RE (1994) Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature 370:481–485
Isakoff MS et al (2005) Inactivation of the Snf5 tumor suppressor stimulates cell cycle progression and cooperates with p53 loss in oncogenic transformation. Proc Natl Acad Sci USA 102:17745–17750
Jenuwein T, Allis CD (2001) Translating the histone code. Science 293:1074–1080
Jin J et al (2005) A mammalian chromatin remodeling complex with similarities to the yeast INO80 complex. J Biol Chem 280:41207–41212
John S et al (2000) The something about silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF(II)30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3)-FACT complex. Genes Dev 14:1196–1208
Jonsson ZO, Jha S, Wohlschlegel JA, Dutta A (2004) Rvb1p/Rvb2p recruit Arp5p and assemble a functional Ino80 chromatin remodeling complex. Mol Cell 16:465–477
Kabani M, Michot K, Boschiero C, Werner M (2005) Anc1 interacts with the catalytic subunits of the general transcription factors TFIID and TFIIF, the chromatin remodeling complexes RSC and INO80, and the histone acetyltransferase complex NuA3. Biochem Biophys Res Commun 332:398–403
Kaeser MD, Aslanian A, Dong MQ, Yates JR 3rd, Emerson BM (2008) BRD7, a novel PBAF-specific SWI/SNF subunit, is required for target gene activation and repression in embryonic stem cells. J Biol Chem 283:32254–32263
Kalocsay M, Hiller NJ, Jentsch S (2009) Chromosome-wide Rad51 spreading and SUMO-H2A.Z--dependent chromosome fixation in response to a persistent DNA double-strand break. Mol Cell 33:335–343
Kanemaki M et al (1999) TIP49b, a new RuvB-like DNA helicase, is included in a complex together with another RuvB-like DNA helicase, TIP49a. J Biol Chem 274:22437–22444
Kapoor P, Chen M, Winkler DD, Luger K, Shen X (2013) Evidence for monomeric actin function in INO80 chromatin remodeling. Nat Struct Mol Biol 20(4):426–432
Kato M, Sasaki M, Mizuno S, Harata M (2001) Novel actin-related proteins in vertebrates: similarities of structure and expression pattern to Arp6 localized on Drosophila heterochromatin. Gene 268:133–140
Katou Y et al (2003) S-phase checkpoint proteins Tof1 and Mrc1 form a stable replication-pausing complex. Nature 424:1078–1083
Kawashima S et al (2007) The INO80 complex is required for damage-induced recombination. Biochem Biophys Res Commun 355:835–841

Kent NA, Chambers AL, Downs JA (2007) Dual chromatin remodeling roles for RSC during DNA double strand break induction and repair at the yeast MAT locus. J Biol Chem 282:27693–27701
Keogh MC et al (2006) A phosphatase complex that dephosphorylates gammaH2AX regulates DNA damage checkpoint recovery. Nature 439:497–501
Kim YJ, Bjorklund S, Li Y, Sayre MH, Kornberg RD (1994) A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell 77:599–608
Kim JS et al (2005) Independent and sequential recruitment of NHEJ and HR factors to DNA damage sites in mammalian cells. J Cell Biol 170:341–347
Kobor MS et al (2004) A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. PLoS Biol 2:E131
Krogan NJ et al (2003) A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Mol Cell 12:1565–1576
Kusch T et al (2004) Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions. Science 306:2084–2087
Kwon H, Imbalzano AN, Khavari PA, Kingston RE, Green MR (1994) Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. Nature 370:477–481
Ladurner AG, Inouye C, Jain R, Tjian R (2003) Bromodomains mediate an acetyl-histone encoded antisilencing function at heterochromatin boundaries. Mol Cell 11:365–376
Lee SE et al (1998) Saccharomyces Ku70, mre11/rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. Cell 94:399–409
Lessard J et al (2007) An essential switch in subunit composition of a chromatin remodeling complex during neural development. Neuron 55:201–215
Lewis LK, Resnick MA (2000) Tying up loose ends: nonhomologous end-joining in *Saccharomyces cerevisiae*. Mutat Res 451:71–89
Li B, Reese JC (2000) Derepression of DNA damage-regulated genes requires yeast TAF(II)s. EMBO J 19:4091–4100
Liang B, Qiu J, Ratnakumar K, Laurent BC (2007) RSC functions as an early double-strand-break sensor in the cell's response to DNA damage. Curr Biol 17:1432–1437
Lickert H et al (2004) Baf60c is essential for function of BAF chromatin remodelling complexes in heart development. Nature 432:107–112
Lipford JR, Bell SP (2001) Nucleosomes positioned by ORC facilitate the initiation of DNA replication. Mol Cell 7:21–30
Lisby M, Barlow JH, Burgess RC, Rothstein R (2004) Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. Cell 118:699–713
Lorch Y, Maier-Davis B, Kornberg RD (2006) Chromatin remodeling by nucleosome disassembly in vitro. Proc Natl Acad Sci USA 103:3090–3093
Lorch Y, Maier-Davis B, Kornberg RD (2010) Mechanism of chromatin remodeling. Proc Natl Acad Sci USA 107:3458–3462
Martin-Trillo M et al (2006) EARLY IN SHORT DAYS 1 (ESD1) encodes ACTIN-RELATED PROTEIN 6 (AtARP6), a putative component of chromatin remodelling complexes that positively regulates FLC accumulation in Arabidopsis. Development 133:1241–1252
Matangkasombut O, Buratowski S (2003) Different sensitivities of bromodomain factors 1 and 2 to histone H4 acetylation. Mol Cell 11:353–363
Matangkasombut O, Buratowski RM, Swilling NW, Buratowski S (2000) Bromodomain factor 1 corresponds to a missing piece of yeast TFIID. Genes Dev 14:951–962
Matsuoka S et al (2007) ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. Science 316:1160–1166
Mimitou EP, Symington LS (2008) Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature 455:770–774
Mizuguchi G et al (2004) ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science 303:343–348

Mohrmann L, Verrijzer CP (2005) Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. Biochim Biophys Acta 1681:59–73
Mohrmann L et al (2004) Differential targeting of two distinct SWI/SNF-related Drosophila chromatin-remodeling complexes. Mol Cell Biol 24:3077–3088
Monahan BJ et al (2008) Fission yeast SWI/SNF and RSC complexes show compositional and functional differences from budding yeast. Nat Struct Mol Biol 15:873–880
Morrison AJ et al (2004) INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. Cell 119:767–775
Morrison AJ et al (2007) Mec1/Tel1 phosphorylation of the INO80 chromatin remodeling complex influences DNA damage checkpoint responses. Cell 130:499–511
Muchardt C, Yaniv M (1993) A human homologue of Saccharomyces cerevisiae SNF2/SWI2 and Drosophila brm genes potentiates transcriptional activation by the glucocorticoid receptor. EMBO J 12:4279–4290
Nagai S et al (2008) Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science 322:597–602
Nakada D, Hirano Y, Sugimoto K (2004) Requirement of the Mre11 complex and exonuclease 1 for activation of the Mec1 signaling pathway. Mol Cell Biol 24:10016–10025
Nakamura TM, Du LL, Redon C, Russell P (2004) Histone H2A phosphorylation controls Crb2 recruitment at DNA breaks, maintains checkpoint arrest, and influences DNA repair in fission yeast. Mol Cell Biol 24:6215–6230
Nasmyth K, Shore D (1987) Transcriptional regulation in the yeast life cycle. Science 237:1162–1170
Neigeborn L, Carlson M (1984) Genes affecting the regulation of SUC2 gene expression by glucose repression in Saccharomyces cerevisiae. Genetics 108:845–858
Neigeborn L, Carlson M (1987) Mutations causing constitutive invertase synthesis in yeast: genetic interactions with snf mutations. Genetics 115:247–253
Ng HH, Robert F, Young RA, Struhl K (2002) Genome-wide location and regulated recruitment of the RSC nucleosome-remodeling complex. Genes Dev 16:806–819
Ohfuchi E et al (2006) Vertebrate Arp6, a novel nuclear actin-related protein, interacts with heterochromatin protein 1. Eur J Cell Biol 85:411–421
Okuno Y, Satoh H, Sekiguchi M, Masukata H (1999) Clustered adenine/thymine stretches are essential for function of a fission yeast replication origin. Mol Cell Biol 19:6699–6709
Olave IA, Reck-Peterson SL, Crabtree GR (2002) Nuclear actin and actin-related proteins in chromatin remodeling. Annu Rev Biochem 71:755–781
Papamichos-Chronakis M, Krebs JE, Peterson CL (2006) Interplay between Ino80 and Swr1 chromatin remodeling enzymes regulates cell cycle checkpoint adaptation in response to DNA damage. Genes Dev 20:2437–2449
Papoulas O et al (1998) The Drosophila trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. Development 125:3955–3966
Paull TT et al (2000) A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr Biol 10:886–895
Peterson CL, Dingwall A, Scott MP (1994) Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc Natl Acad Sci USA 91:2905–2908
Petrini JH, Stracker TH (2003) The cellular response to DNA double-strand breaks: defining the sensors and mediators. Trends Cell Biol 13:458–462
Phelan ML, Sif S, Narlikar GJ, Kingston RE (1999) Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. Mol Cell 3:247–253
Pollard TD, Almo S, Quirk S, Vinson V, Lattman EE (1994) Structure of actin binding proteins: insights about function at atomic resolution. Annu Rev Cell Biol 10:207–249
Poon D et al (1995) Identification and characterization of a TFIID-like multiprotein complex from Saccharomyces cerevisiae. Proc Natl Acad Sci USA 92:8224–8228

Poot RA et al (2004) The Williams syndrome transcription factor interacts with PCNA to target chromatin remodelling by ISWI to replication foci. Nat Cell Biol 6:1236–1244
Poot RA, Bozhenok L, van den Berg DL, Hawkes N, Varga-Weisz PD (2005) Chromatin remodeling by WSTF-ISWI at the replication site: opening a window of opportunity for epigenetic inheritance? Cell Cycle 4:543–546
Prado F, Cortes-Ledesma F, Aguilera A (2004) The absence of the yeast chromatin assembly factor Asf1 increases genomic instability and sister chromatid exchange. EMBO Rep 5:497–502
Qiu XB et al (1998) An eukaryotic RuvB-like protein (RUVBL1) essential for growth. J Biol Chem 273:27786–27793
Raisner RM, Madhani HD (2006) Patterning chromatin: form and function for H2A.Z variant nucleosomes. Curr Opin Genet Dev 16:119–124
Raisner RM et al (2005) Histone variant H2A.Z marks the 5' ends of both active and inactive genes in euchromatin. Cell 123:233–248
Rangasamy D, Greaves I, Tremethick DJ (2004) RNA interference demonstrates a novel role for H2A.Z in chromosome segregation. Nat Struct Mol Biol 11:650–655
Redon C et al (2002) Histone H2A variants H2AX and H2AZ. Curr Opin Genet Dev 12:162–169
Reinke H, Horz W (2003) Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter. Mol Cell 11:1599–1607
Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 273:5858–5868
Rogakou EP, Boon C, Redon C, Bonner WM (1999) Megabase chromatin domains involved in DNA double-strand breaks in vivo. J Cell Biol 146:905–916
Rountree MR, Bachman KE, Baylin SB (2000) DNMT1 binds HDAC2 and a new co-repressor, DMAP1, to form a complex at replication foci. Nat Genet 25:269–277
Ruhl DD et al (2006) Purification of a human SRCAP complex that remodels chromatin by incorporating the histone variant H2A.Z into nucleosomes. Biochemistry 45:5671–5677
Saha A, Wittmeyer J, Cairns BR (2002) Chromatin remodeling by RSC involves ATP-dependent DNA translocation. Genes Dev 16:2120–2134
Saha A, Wittmeyer J, Cairns BR (2005) Chromatin remodeling through directional DNA translocation from an internal nucleosomal site. Nat Struct Mol Biol 12:747–755
Saha A, Wittmeyer J, Cairns BR (2006) Chromatin remodelling: the industrial revolution of DNA around histones. Nat Rev Mol Cell Biol 7:437–447
Segurado M, de Luis A, Antequera F (2003) Genome-wide distribution of DNA replication origins at A+T-rich islands in Schizosaccharomyces pombe. EMBO Rep 4:1048–1053
Shen X, Mizuguchi G, Hamiche A, Wu C (2000) A chromatin remodelling complex involved in transcription and DNA processing. Nature 406:541–544
Shen X, Ranallo R, Choi E, Wu C (2003a) Involvement of actin-related proteins in ATP-dependent chromatin remodeling. Mol Cell 12:147–155
Shen X, Xiao H, Ranallo R, Wu WH, Wu C (2003b) Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. Science 299:112–114
Sheterline P, Sparrow JC (1994) Actin. Protein Profile 1:1–121
Shia WJ et al (2005) Characterization of the yeast trimeric-SAS acetyltransferase complex. J Biol Chem 280:11987–11994
Shim EY, Ma JL, Oum JH, Yanez Y, Lee SE (2005) The yeast chromatin remodeler RSC complex facilitates end joining repair of DNA double-strand breaks. Mol Cell Biol 25:3934–3944
Shim EY et al (2007) RSC mobilizes nucleosomes to improve accessibility of repair machinery to the damaged chromatin. Mol Cell Biol 27:1602–1613
Shim EY et al (2010) Saccharomyces cerevisiae Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. EMBO J 29:3370–3380
Shroff R et al (2004) Distribution and dynamics of chromatin modification induced by a defined DNA double-strand break. Curr Biol 14:1703–1711
Smith DJ, Whitehouse I (2012) Intrinsic coupling of lagging-strand synthesis to chromatin assembly. Nature 483:434–438

Smith CL, Horowitz-Scherer R, Flanagan JF, Woodcock CL, Peterson CL (2003) Structural analysis of the yeast SWI/SNF chromatin remodeling complex. Nat Struct Biol 10:141–145
Smolka MB, Albuquerque CP, Chen SH, Zhou H (2007) Proteome-wide identification of in vivo targets of DNA damage checkpoint kinases. Proc Natl Acad Sci USA 104:10364–10369
Soutourina J et al (2006) Rsc4 connects the chromatin remodeler RSC to RNA polymerases. Mol Cell Biol 26:4920–4933
Stern M, Jensen R, Herskowitz I (1984) Five SWI genes are required for expression of the HO gene in yeast. J Mol Biol 178:853–868
Stracker TH, Theunissen JW, Morales M, Petrini JH (2004) The Mre11 complex and the metabolism of chromosome breaks: the importance of communicating and holding things together. DNA Repair (Amst) 3:845–854
Sudarsanam P, Iyer VR, Brown PO, Winston F (2000) Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 97:3364–3369
Sugawara N, Wang X, Haber JE (2003) In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. Mol Cell 12:209–219
Sun Y, Jiang X, Chen S, Fernandes N, Price BD (2005) A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. Proc Natl Acad Sci USA 102:13182–13187
Sutton A et al (2003) Sas4 and Sas5 are required for the histone acetyltransferase activity of Sas2 in the SAS complex. J Biol Chem 278:16887–16892
Sweeney FD et al (2005) Saccharomyces cerevisiae Rad9 acts as a Mec1 adaptor to allow Rad53 activation. Curr Biol 15:1364–1375
Symington LS (2002) Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. Microbiol Mol Biol Rev 66:630–670, table of contents
Takeda DY, Dutta A (2005) DNA replication and progression through S phase. Oncogene 24:2827–2843
Tamkun JW et al (1992) brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell 68:561–572
Tourriere H, Versini G, Cordon-Preciado V, Alabert C, Pasero P (2005) Mrc1 and Tof1 promote replication fork progression and recovery independently of Rad53. Mol Cell 19:699–706
Tsukuda T, Fleming AB, Nickoloff JA, Osley MA (2005) Chromatin remodelling at a DNA double-strand break site in Saccharomyces cerevisiae. Nature 438:379–383
Tsukuda T et al (2009) INO80-dependent chromatin remodeling regulates early and late stages of mitotic homologous recombination. DNA Repair (Amst) 8:360–369
Ueno M et al (2004) Fission yeast Arp6 is required for telomere silencing, but functions independently of Swi6. Nucleic Acids Res 32:736–741
Uetz P et al (2000) A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. Nature 403:623–627
Unal E et al (2004) DNA damage response pathway uses histone modification to assemble a double-strand break-specific cohesin domain. Mol Cell 16:991–1002
Usui T, Ogawa H, Petrini JH (2001) A DNA damage response pathway controlled by Tel1 and the Mre11 complex. Mol Cell 7:1255–1266
Valerie K, Povirk LF (2003) Regulation and mechanisms of mammalian double-strand break repair. Oncogene 22:5792–5812
van Attikum H, Fritsch O, Hohn B, Gasser SM (2004) Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. Cell 119:777–788
van Attikum H, Fritsch O, Gasser SM (2007) Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. EMBO J 26:4113–4125
Wang W et al (1996) Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. EMBO J 15:5370–5382
Wang X et al (2004) Two related ARID family proteins are alternative subunits of human SWI/SNF complexes. Biochem J 383:319–325

Ward IM, Wu X, Chen J (2001) Threonine 68 of Chk2 is phosphorylated at sites of DNA strand breaks. J Biol Chem 276:47755–47758
Weiss K, Simpson RT (1998) High-resolution structural analysis of chromatin at specific loci: *Saccharomyces cerevisiae* silent mating type locus HMLalpha. Mol Cell Biol 18:5392–5403
Welch MD, Drubin DG (1994) A nuclear protein with sequence similarity to proteins implicated in human acute leukemias is important for cellular morphogenesis and actin cytoskeletal function in *Saccharomyces cerevisiae*. Mol Biol Cell 5:617–632
Welch MD, Vinh DB, Okamura HH, Drubin DG (1993) Screens for extragenic mutations that fail to complement act1 alleles identify genes that are important for actin function in *Saccharomyces cerevisiae*. Genetics 135:265–274
West SC (1996) The RuvABC proteins and Holliday junction processing in *Escherichia coli*. J Bacteriol 178:1237–1241
West SC (1997) Processing of recombination intermediates by the RuvABC proteins. Annu Rev Genet 31:213–244
Wolffe AP (1994) Transcriptional activation. Switched-on chromatin. Curr Biol 4:525–528
Wolner B, van Komen S, Sung P, Peterson CL (2003) Recruitment of the recombinational repair machinery to a DNA double-strand break in yeast. Mol Cell 12:221–232
Wu WH et al (2005) Swc2 is a widely conserved H2AZ-binding module essential for ATP-dependent histone exchange. Nat Struct Mol Biol 12:1064–1071
Wu S et al (2007) A YY1-INO80 complex regulates genomic stability through homologous recombination-based repair. Nat Struct Mol Biol 14:1165–1172
Wu JI, Lessard J, Crabtree GR (2009) Understanding the words of chromatin regulation. Cell 136:200–206
Xue Y et al (2000) The human SWI/SNF-B chromatin-remodeling complex is related to yeast rsc and localizes at kinetochores of mitotic chromosomes. Proc Natl Acad Sci USA 97:13015–13020
Yan Z et al (2008) BAF250B-associated SWI/SNF chromatin-remodeling complex is required to maintain undifferentiated mouse embryonic stem cells. Stem Cells 26:1155–1165
Zhang ZK et al (2002) Cell cycle arrest and repression of cyclin D1 transcription by INI1/hSNF5. Mol Cell Biol 22:5975–5988
Zhang H et al (2004) The Yaf9 component of the SWR1 and NuA4 complexes is required for proper gene expression, histone H4 acetylation, and Htz1 replacement near telomeres. Mol Cell Biol 24:9424–9436
Zhang H, Roberts DN, Cairns BR (2005) Genome-wide dynamics of Htz1, a histone H2A variant that poises repressed/basal promoters for activation through histone loss. Cell 123:219–231
Zhou J et al (2005) Cell cycle regulation of chromatin at an origin of DNA replication. EMBO J 24:1406–1417
Zou L, Elledge SJ (2003) Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300:1542–1548
Zraly CB, Middleton FA, Dingwall AK (2006) Hormone-response genes are direct in vivo regulatory targets of Brahma (SWI/SNF) complex function. J Biol Chem 281:35305–35315