INO80 subfamily of Chromatin Remodeling Complexes

Yunhe Bao and **Xuetong Shen**[*]
Department of Carcinogenesis, Science Park Research Division, University of Texas M.D. Anderson Cancer Center, Smithville, Texas 78957

Abstract

ATP-dependent chromatin remodeling complexes contain ATPases of the Swi/Snf superfamily and alter DNA accessibility of chromatin in an ATP-dependent manner. Recently characterized INO80 and SWR1 complexes belong to a subfamily of these chromatin remodelers and are characterized by a split ATPase domain in the core ATPase subunit and the presence of Rvb proteins. INO80 and SWR1 complexes are evolutionarily conserved from yeast to human and have been implicated in transcription regulation, as well as DNA repair. The individual components, assembly patterns, and molecular mechanisms of the INO80 class of chromatin remodeling complexes are discussed in this review.

Keywords

INO80; SWR1; ATP-dependent chromatin remodeling; DNA repair; histone variant Actin; Arps; Rvb1; Rvb2; H2AZ; H2AX

Introduction

Chromatin helps eukaryotic cells to compact and store DNA, which creates an impediment to the access of DNA during transcription, replication, repair and recombination. Therefore, modulation of chromatin structure plays an important role in the regulation of these nuclear processes in eukaryotes. To access the DNA within the chromatin context, the cell has developed dedicated chromatin modification mechanisms, which serve to alter the chromatin structure locally to facilitate or repress DNA access. These mechanisms include: histone covalent modifications; histone replacement by histone variants; and ATP-dependent chromatin remodeling. In many cases, all of these mechanisms may act in concert [1,2].

ATP-dependent chromatin remodeling is involved in all major reactions with chromatin substrates, such as the expression of genes, the duplication of the genome, the repair of DNA damage and the recombination of chromosomes [3]. ATP-dependent chromatin remodeling factors are multiprotein complexes that contain dedicated DNA-dependent ATPases of the Swi2/Snf2 subfamily [4,5]. These enzymes utilize ATP hydrolysis to facilitate histone octamer sliding, H2A-H2B dimer removal or exchange, alteration of histone-DNA interactions, which further increases the accessibility of DNA elements to the regulatory proteins [1]. ATP-dependent chromatin remodeling factors can be divided into several subfamilies on the basis of their ATPase domain structure. The four most prominent subfamilies are the SWI2, ISWI,

CHD and INO80 subfamilies. ATPases of the SWI2 subfamily are characterized by a bromodomain. The ISWI class contains SANT and SLIDE domains. The CHD subfamily is differentiated by an ATPase that contains a chromodomain and PHD fingers [6,7]. The INO80 subfamily includes the INO80 remodeling complex (INO80.com) and the SWR1 remodeling complex (SWR1.com) which are characterized by split ATPase domains (Ino80 and Swr1 respectively, see Figure 1) and the presence of two RuvB-like proteins, Rvb1 and Rvb2 [8,9]. The purpose of this review is to integrate and discuss the current knowledge of the biochemical composition and cellular functions of these two recently characterized ATP-dependent chromatin remodeling complexes.

1. Subunits of INO80.com

INO80.com was initially purified from *Saccharomyces cerevisiae* by immunoprecipitation. It was shown by gel-filtration chromatography that the bulk of INO80 is in a high molecular mass fraction between 1,000K and 1,500K. The purified INO80 complex contains 15 principal subunits with roughly equivalent stoichiometry except for Rvb1 and Rvb2, which show 6:1 stoichiometry with other polypeptides [8]. The components of the yeast INO80 complex are Ino80, Rvb1, Rvb2, Arp4 (Actin-related protein 4), Arp5, Arp8, actin, Nhp10 (Non-histone protein 10), Anc1/Taf14, Ies1 (Ino Eighty Subunit 1), Ies2, Ies3, Ies4, Ies5 and Ies6 [8,10] (Figure 1). INO80 complex is highly conserved and the purified human INO80 complex contains orthologs of Ino80, Rvb1, Rvb2, Arp4, Arp5, Arp8, Ies2 and Ies6, as well as five unique subunits [11]. Similar to its yeast counterpart, hINO80.com exhibits DNA- and nucleosome- activated activity and ATP-dependent nucleosome remodeling activity [8,11].

Ino80, the product of *INO80* (*YGL150C*) on *S. cerevisiae* chromosome VII is a protein of 1489 amino acids with significant similarity to the Snf2/Swi2 family of DNA-dependent ATPases. The defining feature of the Ino80 ATPase domain (698–1450) is the presence of a spacer (1018–1299), which splits the conserved ATPase domain [12]. GXGKT is a strictly conserved nucleotide binding motif within many ATPases, which contains a lysine that is involved in the interaction with the phosphate of NTP [13,14]. Alteration of the lysine to arginine (K737R) results in a non-functional Ino80, which is unable to complement the corresponding null allele [12]. Moreover, INO80.com purified from a strain carrying a K737A substitution also failed to show ATPase activity, DNA helicase activity, and the ability to rescue the *ino80* mutant phenotypes [8]. Taken together, these results indicate that ATP-binding is essential for Ino80 function *in vivo*. The N-terminal domain of Ino80 including the TELY motif is conserved in human, fly, and yeast Ino80 proteins and is believed to be an interaction domain for actin, Arp4, Arp8 and Taf14/Anc1 [8,10], while the function of the GTIE motif at the carboxyl terminus [8] is still unknown. In addition to being the core ATPase of the complex, Ino80 also appears to be the main scaffold for the assembly of the INO80 complex (Figure 1).

Rvb1 and Rvb2 are essential and highly conserved proteins from yeast to human (Tip49a and Tip49b in mammals)[15–17]. Rvb proteins share limited homology to bacterial RuvB, the Holliday Junction DNA Helicase with a double hexamer composition[18,19]. Like bacterial RuvB, the yeast Rvb1 and Rvb2 each show 6:1 stoichiometry with other polypeptides in the complex [8]. Since the eukaryotic counterparts of the bacteria RuvA/B have been elusive, INO80 and/or SWR1 may represent candidates for RuvB, together with an yet to be identified eukaryotic equivalent of RuvA, 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 (discussed later). The Rvb hexameric helicases belong to the large AAA+ (ATPases associated with various cellular activities) class of chaperone-like ATPases, in which the conserved ATPase domain assembles into oligomeric rings and undergoes conformational change upon nucleotide binding and hydrolysis [20]. Growing evidence suggests that AAA+ proteins all operate by promoting

conformational changes or remodeling, such as unfolding of the target proteins, and are mainly involved in reactions such as protein unfolding and degradation, the disassembly of protein aggregates, and protein-complex disassembly [20]. Recently, it was shown by the Dutta group that Rvb proteins are essential for the chromatin remodeling activity of INO80.com[17]. They further found that loss of Rvb proteins leads to the loss of Arp5, a functionally important subunit of INO80.com [10]. Their *in vitro* studies reveal that Rvbs and Arp5 associate in a complex and the interaction is Ino80 and ATP dependent[17]. Moreover, a recent proteomic study showed that Arp5 and Rvb1 can associate with each other and form a module [21]. Therefore, Arp5 may undergo conformational changes in connection with the Rvb double hexamers and play an important role in regulating INO80 chromatin remodeling activity. 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 polypeptide may associate with Rvbs and be required for the regulation of SWR1.com. Although it has been found that Sec53 (a phosphomannomutase) and Rvb2 can form a module *in vivo* [21], and that Pih1 (protein of unresolved function) associates with Rvb1 and Rvb2 to form a light Rvb.com [17], none of them has been related to chromatin remodeling functions.

Conventional actin and actin-related proteins (Arps), whose primary sequence display significant similarity to actin, have been identified as subunits in many chromatin modifying complexes [22]. The INO80 complex contains actin, Arp4, Arp5 and Arp8 [8]. Arp5 and Arp8 have so far only been found in INO80.com. Arp5 appears to associate with the complex independently of any other subunit, while Arp8 is necessary for the inclusion of Arp4 and actin [10]. The functions of Arp5 and Arp8 appears 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 [10]. Although all Arps contain the 'actin fold' domain, which includes putative ATP-binding sites, Arp4 is the only one has been shown to bind to ATP [22]. Moreover, ATP binding site mutants of Arp7, Arp9 (subunits of SWI/SNF and RSC), Arp5, and Arp8 do not show observable phenotypes [10, 23], suggesting that the ATP binding site of these Arps are dispensable for their functions in chromatin remodeling.

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 [24–27]. Despite increasing evidence suggesting that actin is in the nucleus and may play roles in many nuclear functions, the research on nuclear actin has been stalled by the lack of unambiguous demonstrations of an actin function in the nucleus both *in vivo* and *in vitro*. Previous studies appear to suggest that the INO80 complex contains a sub-complex consisting of actin, Arp4, Arp8 and Taf14, that associating with the ΔN domain [10] of the Ino80 ATPase. Interestingly, all subunits of this actin/Arp sub-complex, including the ΔN domain of the Ino80 ATPase are evolutionarily conserved, suggesting that this sub-complex represents a unique and evolutionarily conserved module used throughout the yeast INO80 complex and orthologous INO80 complexes in higher organisms. Since actin and Arp4 are consistently present in several chromatin modifying complexes, such as INO80, SWR1 and NuA4 [8,9, 28], and the loss of Arp8 in the INO80 complex results in the loss of actin and Arp4 [10], 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 in chromatin modifying complexes. In yeast, this actin/Arp4 module probably has also evolved into a less conserved Arp7/Arp9 dimer found in the SWI/SNF and RSC chromatin remodeling complexes [29]. It has been shown that Arp4 binds to all four histones *in vitro* and *in vivo* [30] and Arp8 binds to H3 and H4 with some preference *in vitro* [10], suggesting that the actin/Arp modules in chromatin modifying complexes might provide chaperone functions for the histone octamer and mediate the

rearrangement of histone-histone, and/or histone-DNA contacts during chromatin remodeling. The defined actin-containing module in the INO80 complex provides a unique opportunity to understand the mechanism of nuclear actin.

Nhp10 has been revealed as a subunit of INO80.com by peptide sequencing, as well as global protein-protein interaction maps [10,31,32]. It is an HMG-1 like protein that can potentially bind to structured DNA or nucleosome. Deletion of Nhp10 results in the loss of Ies3, indicating that Nhp10 is important for recruitment of Ies3 into the complex [10] (Figure 1). Moreover, the 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 compare to the Arps [10]. Recently, it was shown by our lab that Nhp10 (and/or Ies3) plays an important role in recruitment of INO80.com to DNA double strand breaks (DSB) via mediating the interaction between the INO80 complex and the phosphorylated yeast H2A (γ-H2AX) [33] (see below). Taken together, it seems that Nhp10, a unique subunit of INO80.com, mediates specific interaction with other factors, rather than plays an essential role in chromatin remodeling.

Taf14 (also known as Swp29, Taf30, Tfg3, Anc1, TafII30) is a subunit of Mediator, TFIID, TFIIF, SWI/SNF, NuA3, and INO80 complexes [10,34–38]. Using yeast two-hybrid screening, Kabani and colleagues found that Taf14 interacts with the key or catalytic subunits, such as Ino80 in INO80.com, of each complexes mentioned above, suggesting that it plays a common regulatory role [39]. The Taf14 protein contains a conserved YEATS domain, which is also found in Yaf9, a component of NuA4 and SWR1 complexes [40,41] (see below); and in Sas5, a component of the SAS complex involved in chromatin silencing [42,43]. However, the function of this domain is still unclear. *taf14* null mutants display decreased transcription, defects in actin organization, hypersensitivity to heat, caffeine, hydroxyurea, UV irradiation, and methyl methanesulfonate [36,41,44,45]. Moreover, it has also been shown that Taf14 is involved in actin function and cell cycle arrest functions of Rad53 and Mec1, which play important roles in DNA damage responses [44,46].

The molecular functions of **Ies1, 3, 4** and **5** are still unknown, since they are not evolutionarily conserved, they may provide regulatory functions to the INO80 complex in yeast. It also worth to note that orthologs of Ies2 and Ies6 have been found in human INO80.com [11], suggesting that these proteins are important for the conserved INO80.com functions.

2. INO80.com functions

The *INO80* gene (*YGL150C*) was identified in a genetic screen for mutants affecting inositol biosynthesis [12]. The product of this gene is highly related to the DNA-dependent ATPases in the SNF2/SWI2 superfamily of chromatin remodeling complexes. The Wu group purified and characterized the INO80 complex. Their *in vitro* biochemical studies showed that the INO80 complex has DNA-dependent ATPase activity, as well as 3′ to 5′ helicase activity [8]. INO80.com was also found to be able to bind to free DNA with an apparent binding constant (~10 nM), which is comparable to that of SWI/SNF [10]. Moreover, the chromatin remodeling activity of INO80.com was investigated by monitoring the alteration of DNA accessibility on a model chromatin template reconstituted from fly embryo extracts. The INO80.com exhibited similar remodeling activity as that of *Drosophila* NURF complex, an ISWI-class remodeller [8]. The chromatin remodeling activity of INO80.com was further characterized using reconstituted mononucleosomes. It was observed that INO80.com could mobilize mononucleosomes in an ATP-dependent manner, while the NURF complex caused a different nucleosomal redistribution pattern, suggesting that different remodellers alter DNA-histone interactions by different mechanisms [10]. However, the actual mechanism for INO80.com remodeling activity remains obscure. It has been shown that *ino80* null mutants exhibited

decreased gene activation mediated by a UAS element, ICRE (inositol/choline-responsive element), and pleiotropic expression defects [12]. In a microarray assay, it was found that 150 out of 5602 yeast genes showed at least 2 fold change in mRNA level upon acute removal of INO80, with roughly equal numbers of up regulated and down regulated genes [17]. Several other global gene expression profiling experiments also showed that the INO80 complex regulates a specific set of gene both negatively and positively [9,47]. Two phosphate regulated genes, *PHO5* and *PHO84* were used to study the promoter regulation mechanism of INO80, and it was found that INO80 had a repressive effect on the *PHO5* promoter and a stimulatory effect on the *PHO84* promoter, suggesting that INO80 is required for proper regulation of these promoters [17]. Furthermore, *in vitro* transcription assays show a 10 fold increase in transcriptional activation of chromatin remodeled by INO80.com [8]. Together, these data suggest that the INO80 complex regulates the expression of the subset of genes via its ATP-dependent chromatin remodeling activity.

In addition to its function in transcriptional regulation, the INO80 complex has also been shown to be involved in DNA damage responses. Recent advances in the fields of DNA repair and chromatin reveal that both histone modifications and chromatin remodeling are important for the repair of DNA lesions, such as DSBs (see [48–51] for reviews). The mammalian H2AX histone variant, which is orthologous to the major H2A histones in yeast, is rapidly phosphorylated on the SQ motif located in the carboxyl-terminal tail of the histone in the chromatin regions surrounding a DSB [52,53]. Phosphorylation of H2AX is critical for the accurate repair of DNA lesions, as H2AX deficiency results in genomic instability and cancer predisposition in mice [54–57]. Phosphorylated H2AX, referred to as γ-H2AX, is needed for the recruitment and/or retention of several DNA repair proteins [58]. Recent studies indicate that yeast γ-H2AX is also required for the recruitment of the chromatin remodeling complex INO80 to DSB sites [33,47,59], thus established a link between chromatin remodeling and DNA repair. Given that the *ino80* mutant is hypersensitive to DNA damage agents [8,10], Morrison *et al* and van Attikum *et al* asked whether Ino80 is involved directly in the DNA damage processing. They found that transcriptional and checkpoint responses to DNA damage were normal in *ino80* and *arp8* mutants by comparing specific genes and global expression patterns, suggesting that INO80.com participates in the DNA damage response independent of transcription [33,47]. Moreover, an HO endonuclease-induced DSB system and ChIP assays were used to show that Ino80, Arp5, Arp8, and Rvbs are recruited directly to the HO-induced DSB in yeast [33,47,59]. γ-H2AX was shown to be necessary for the recruitment of INO80.com by *in vitro* and *in vivo* assays [33,47,59]. To gain insights into the interaction between INO80.com and γ-H2AX, Morrison and coworkers used deletion strains of different INO80 subunits and found that Nhp10 (and Ies3) is required for this specific interaction [33]. Interestingly, it was shown that Arp4 is responsible for the interaction between γ-H2AX and the NuA4 HAT complex, suggesting that Arp4 of INO80.com may possess a similar function [59]. However, in the absence of actin, Arp4 and Arp8, the INO80 (*arp8Δ*) mutant complex displayed significantly higher levels of interaction with γ-H2AX than that of the INO80 (*nhp10Δ*) mutant complex, in which Nhp10 and Ies3 are lost, suggesting that Nhp10 and Ies3 play a more significant role in the interaction between INO80.com and γ-H2AX than the Arps. To address the function of INO80 recruitment at DSBs, the Gasser group monitored the formation of single-stranded DNA (ssDNA) at DSBs and found that both *arp8* and nonphosphorylatable H2A mutants had reduced ssDNA production, suggesting that INO80.com facilitates ssDNA formation, a critical step in the homologous recombination (HR) DNA repair pathway, by its chromatin remodeling activity [47]. Furthermore, it was shown recently by the Osley group that the histone eviction near DSBs that is mediated by INO80 remodeling activity is dependent on MRX (Mre11-Rad50-Xrs2) complex, a DNA damage sensor, and that a delayed recruitment of the Rad51 repair protein results from defects in histone loss [60]. Interestingly, INO80 has also been shown to affect HR pathway in plants [61]. It is worth noting that INO80 is also implicated in the non-homologous end joining (NHEJ) pathway

[47]. Taken together, these data suggest that INO80.com participates in multiple DNA repair pathways by its nucleosome remodeling ability and by regulating the accessibility of DNA repair proteins around the DSB site. Though the INO80 complex has been previously shown to play an important role in transcription, the recent finding on the roles of INO80 complex during the DNA damage response emphasizes the notion that chromatin remodeling complexes can be involved in distinctly different cellular processes.

3. Subunits of SWR1 complex

The SWR1 complex was found to be able to specifically exchange histone H2A in nucleosomes for its variant H2AZ by three laboratories using different experimental approaches [9,62,63]. The purified yeast SWR1 complex contains fourteen polypeptides: Swr1, Swc2/Vp372, Swc3, Swc4/Eaf2/God1, Swc5/Aor1, Swc6/Vps71, Swc7, Yaf9, Bdf1, Act1/actin, Arp4, Arp6, Rvb1 and Rvb2 (Figure 1) [9,64]. Interestingly, actin, Arp4, Rvb1 and Rvb2 are shared subunits with INO80.com, whereas actin, Arp4, Swc4 and Yaf9 are also present in the NuA4 histone acetyltransferase complex [8,65]. The fact that Htz1/H2AZ is associated with the purified SWR1 complex raised the question whether they are functionally and genetically linked. Subsequently, it was shown that the *swr1* mutants and the *htz1* mutant share similar phenotypes, such as hypersensitivities to caffeine, methyl methanesulfonate (MMS), and UV irradiation [9]. Moreover, genome-wide transcription profiles of *swr1•* cells and *htz1•* cells reveal that there is a ~40% overlap between genes regulated by Swr1 and Htz1, while only ~10% overlap between genes regulated by Ino80 and Htz1 [9]. These data suggest that Swr1 and H2AZ share a common function in transcription regulation with some degrees of functional independence.

Swr1 is a Swi2/Snf2-related ATPase with a split conserved ATPase domain, characteristic of the core ATPases of the INO80 subfamily. Similar to the INO80 complex, the SWR1 complex exhibits nucleosome stimulated ATPase activity [9]. Swr1 is the key catalytic subunit in the complex and 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* [9]. Recently, it was revealed by Wu et al. 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 [64] (Figure 1). These results indicate that Swr1 is essential for the integrity of the enzyme complex and suggest that the INO80 and SWR1 complexes share significant structural similarities (Figure 1).

Swc2 is the second largest subunit in the SWR1 complex with an overall acidic property (pI = 4.9). It 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 (Figure 1) [64]. 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 [64]. 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 [64]. 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 effects on association of other subunits, including histones, in the SWR1 complex, In addition, *in vitro* histone exchange activity of SWR1 is unaffected in *swc3* mutants [64]. **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 [64]. Interestingly, the

purified SWR1 complex lacking Swc5 exhibits increased nucleosome binding ability [64], 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) [66]. Swc4 bears a SANT domain, which is present in several chromatin remodeling and HAT complexes and is crucial for their functions [67,68]. However, the function of Swc4 is still unknown. It was shown by yeast two-hybrid system that Swc4 binds directly to Yaf9, another subunit of the SWR1 complex [40]. The removal of Yaf9 results in the loss of Swc4 from the complex, indicating that the association of Swc4 is dependent on Yaf9 [64].

Yaf9 is similar to, and was named after, AF9, a human leukemogenic protein [69]. Similar to Taf14 (a component of INO80 complex), Yaf9 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 [41]. Zhang and colleagues found that the *yaf9* null mutant is sensitive to MMS, cold and caffeine, and that the YEATS domain is important for Yaf9 function [41]. Furthermore, it was found that *yaf9Δ* mutants are hypersensitive to microtubule depolymerizing agents, suggesting that Yaf9 is important for the cellular response to spindle stress [70]. *In vitro* studies revealed that Yaf9/Swc4 are required for Htz1 transfer, but not for Htz1 and nucleosome binding [64]. *yaf9Δ* strains display reduced Htz1 deposition at telomere-proximal genes and a significant similarity with *htz1Δ* mutants in transcriptional profiles and phenotypes [41]. Taken together, these data suggest that Yaf9 and/or Swc4 play an important role in Htz1 deposition. Strikingly, Yaf9 and Swc4 are also present in the NuA4 histone acetyltransferase (HAT) complex, which acetylates nucleosomal H4/H2A (for review see [65]). Although a NuA4 complex lacking Yaf9 exhibits normal acetyltransferase activity and H4 specificity, the loss of Yaf9 correlates with a significant reduction in the H4 acetylation at specific loci, such as telomeres [41]. The functional correlation between SWR1 complex and NuA4 complex will be discussed below.

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 [64]. However, in the absence of Swr1, Swc2 does not associate with either Swc6 or Arp6, although these two subunits associate tightly in the absence of Swr1 [64]. 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 [64]. Arp6 has been found in budding yeast, fission yeast, *Arabidopsis*, fruit fly, chicken, and humans [71,72], indicating that it is important for conserved biological functions. Fission yeast Arp6 was found to be required for transcriptional silencing at telomeres [73]. Furthermore, both *Drosophila* and vertebrate Arp6 have been found to interact with heterochromatin protein 1 (HP1) and colocalize with HP1 in the pericentric heterochromatin [74,75]. Interestingly, the Tremethick group found that proper mammalian HP1α-chromatin interaction is disrupted in the absence of H2AZ [76], and their *in vitro* studies showed that HP1α had a ~2.5 fold higher affinity for H2AZ-containing chromatin [77]. Taken together, these findings suggest that HP1 and H2AZ, which is deposited by a SWR1 complex that contains Arp6, function together to play an important role in heterochromatin formation. *Arabidopsis arp6* null mutants display numerous defects, including altered development of the leaf, inflorescence, and reduced female fertility [78]. Subsequently, it was found that *Arabidopsis arp6* mutants have reduced expression of the central floral repressor gene Flowering Locus C (FLC) [78], and that Arp6 is required for histone H3 acetylation and methylation in FLC chromatin locus [72]. 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 are the only two subunits whose assemblies have not been defined in the SWR1 complex. Bdf1 (Bromodoman Factor 1) has two bromodomans (acetyl-lysine binding domains), a motif present in a number of proteins involved in transcription and chromatin modification, and associates with yeast TFIID substoichiometricly [79]. Bdf1 and its homolog, Bdf2, are genetically redundant [79]. However, only Bdf1 preferentially binds to acetylated histone H3 and H4 [80,81] and associates with TFIID and SWR1 complex. Bdf1 was determined to be a subunit of Swr1 complex because it associates with several immunopurified Swr1 complex components [63]. 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 [82,83]. Interestingly, Bdf1 is known to be phosphorylated [79]. Therefore, the interaction between Bdf1 and acetylated histones, and/ or recruitment of TFIID and the SWR1 complex might be regulated by Bdf1 phosphorylation status.

4. The functions of SWR1 complex

It is clear that the major function of the SWR1 complex is to deposit the histone variant dimer H2AZ-H2B into nucleosomes by replacing the pre-existing H2A-H2B dimer. Histone variants are distinct nonallelic forms of major-type core histones. 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 the incorporation of histone variants is often replication independent (reviewed in [84]). The substitution of core histones with the corresponding histone variants can generate a structurally and functionally distinct region in the chromatin [85,86]. The histone H2A variant, H2AZ, is highly conserved from yeast to human and has been shown to be essential in *Drosophila*, mouse and *Tetrahymena* [87–89]. Most interestingly, the results from different structural and functional studies of H2AZ-containing nucleosomes from different organisms are apparently controversial (for a review, see [90,91]). For example, *in vitro* biophysical studies reveal that mouse H2AZ stabilizes histone octamers [92], while the yeast H2AZ (Htz1) can be released from purified chromatin at a lower salt concentration compare to the salt concentration needed for conventional H2A release [82]. Furthermore, mouse H2AZ was found to be enriched in pericentric heterochromatin in early embryos [93], whereas yeast H2AZ is critical for preventing the spread of silent chromatin into adjacent euchromatic regions [94]. As for the function of H2AZ, this histone variant has been demonstrated to play important roles in transcriptional activation, antagonization of gene-silencing, and chromosome stability (reviewed in [91]). Recently, genome-wide studies demonstrated that yeast Htz1 is globally localized to most of the gene promoters in euchromatin, and generally present in the single nucleosomes flanked a nucleosome-free region that contains the transcription initiation site [82,83,95]. Consistent with previous results, these studies further confirm that the SWR1 complex deposits H2AZ into the genome, probably in a replication independent manner.

The SWR1 complex is conserved in eukaryotes. In *Drosophila*, histone variant H2Av is a bi-functional molecule since it harbors conserved sequences of both H2AZ and H2AX. It was demonstrated by Kusch et al. that the phosphorylated H2Av in chromatin can be acetylated and replaced with an unmodified H2Av by the Tip60 complex, which is a *Drosophila* homolog of SWR1 complex [96]. More interestingly, the Tip60 complex appears to be a fusion of yeast SWR1 and NuA4 complexes since most subunits of the Tip60 complex have yeast homologues present in either SWR1 or NuA4 complexes (reviewed in [65,91]). Similarly, the human Tip60 complex is also a fusion of SWR1 and NuA4 complexes. SRCAP (Snf2-related CREB-binding protein activator protein) complex is another SWR1 complex in human and was found to be able to replace pre-existing nucleosomal H2A-H2B dimers with H2AZ-H2B dimers in an ATP-dependent manner [91,97]. Domino is the Swr1 homolog in *Drosophila*, and human Swr1

orthologues are SRCAP and p400, primarily known as transcription factors [98]. Tip 60, the homolog of yeast Esa1, is the HAT in these complexes.

Although yeast SWR1 complex does not co-purified with the NuA4 complex, the major HAT for histones H2A and H4, growing evidence suggests that they work together to regulate H2AZ deposition. Yaf9 is the shared component of SWR1 and NuA4 complexes. Removal of Yaf9 results in a defect in antagonizing gene-silencing near certain telomeres, which correlates with reduced H4 acetylation and reduced Htz1 occupancy [41]. In another report, it was found that the NuA4 and SWR1 complexes shared similar phenotypes and interact genetically, suggesting important functional links between the two complexes [63]. Furthermore, genome-wide studies revealed that both NuA4 complex and Gcn5 (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. Who is the reader for this "histone code"? Bdf1, the shared component of SWR1 and TFIID complexes bearing two bromodomans, is a good candidate, because it selectively binds acetylated forms of histone H4 [99]. More importantly, Bdf1 occupancy was positively correlated with Htz1 occupancy in the genome [82], as deletion of either Bdf1 alone or Bdf1 and its redundant homolog, Bdf2, resulted in significant reductions in Htz1 recruitment [82,83], indicating that Bdf1 is required for efficient H2AZ deposition.

The similarities between Htz1, the SWR1 and NuA4 complexes raise some interesting questions. For example, does NuA4 also acetylate Htz1? If so, does the acetylation occur before or after Htz1 recruitment? And what is the physiological role of Htz1 acetylation? Three research groups answered these questions recently. The Rine group found that multiple sites of H2AZ were acetylated by NuA4 complex *in vivo* when H2AZ was deposited in chromatin by the SWR1 complex. In addition, H2AZ mutants that can not be acetylated display reduced ability to antagonize gene-silencing, even when enriched in the heterochromatin boundaries by SWR1 complex, suggesting that acetylated H2AZ plays an important role in blocking the spreading of heterochromatin into euchromatin [100]. Similarly, the Buratowski laboratory revealed that Lys 14 of Htz1 was acetylated by NuA4 complex once Htz1 was deposited into chromatin by the SWR1 complex [101]. The Grunstein group also found that Htz1 can be acetylated by the NuA4 complex [102]. Taken together, the model for H2AZ deposition is emerging: Bdf1 recognizes a specific histone acetylation pattern and recruits the SWR1 complex to specific loci in the genome; H2AZ-H2B dimers associate with Swc2 and other subunits of SWR1 complex and is exchanged into chromatin by the remodeling activity of the SWR1 complex; interactions between the SWR1 and NuA4 complexes (potentially mediated by shared subunits) may recruit the NuA4 complex, which further acetylates the deposited H2AZ; properly modified H2AZ functions in transcriptional activation, antagonization of gene-silencing, and chromosome stability.

Similar to the INO80 complex, emerging evidence suggests that the SWR1 complex may also play roles in DNA repair. First of all, *swr1* mutants are hypersensitive to DNA damage-inducing agents (MMS and hydroxyurea) [9,63]. Second, Downs et al demonstrated that purified SWR1 complex specifically binds to H2A phosphoserine-129 peptides *in vitro* and that NuA4 complex and Rvb1-containing complexes, which may be INO80 and/or SWR1 complexes, are recruited to double-strand break (DSB) sites *in vivo* [59]. Finally, the HAT activity of human Tip60 complex, which is a fusion of NuA4 and SWR1 complexes, has been implicated in ATM, an important kinase in the DNA damage response signal pathway, activation and DNA repair [103,104]. In addition, *Drosophila* Tip60 complex is required for acetylation of phospho-H2Av at DNA lesions and subsequent replacement with unmodified H2Av [96]. The SWR1 complex, and its associated H2AZ deposition activity, may be recruited to DSBs, in the concert with NuA4 and/or INO80 complexes, to exchange γ-H2AX with H2AZ, which may further alter the local chromatin structure and facilitate the process of DNA repair.

Concluding remarks

Emerging evidence indicates that INO80 and SWR1 remodeling complexes are evolutionarily conserved and are key players in basic biological processes, such as transcription and DNA repair. The detailed mechanisms of these multiprotein complexes remain uncertain. Nonetheless, research investigating the INO80 subfamily of ATP-dependent chromatin remodeling complexes provides unique opportunities to reveal the complex connections between chromatin remodeling and multiple cellular processes. The correlations among histone H2AZ, NuA4, SWR1 and INO80 complexes further support the emerging theme that histone variants, histone modifications and chromatin remodeling work together to regulate chromatin dynamics. Future studies will shed light on the detailed mechanisms of how the INO80 subfamily of complexes remodels chromatin, as well as provide additional links to other cellular processes, such as DNA replication and recombination.

Acknowledgments

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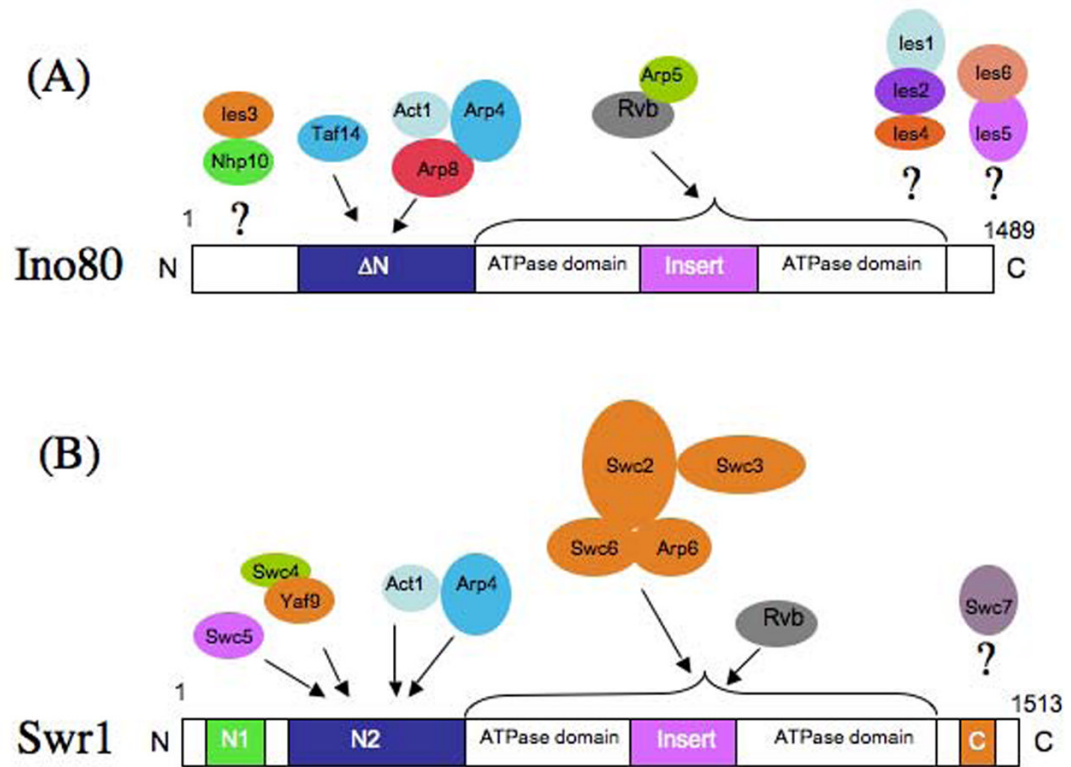
Figure 1.
Schematic model of subunit interactions of the INO80 and SWR1 complexes. A) INO80
subunits and their interactions. B) SWR1 subunits and their interactions. The known
interactions between subunits and ATPases are shown by arrows, while the question marks
indicate unknown interactions. Figure 1B is adapted from Wu et al. [64].