ISWI Chromatin Remodeling: One primary actor or a coordinated effort?

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Abstract

The ISWI family of ATP-dependent chromatin remodelers regulates transcription of coding and noncoding RNA by mobilizing nucleosomes and controlling the length of linker DNA separating nucleosomes (spacing). Nucleosome movement is tightly coupled to the DNA translocation activity of the helicase domain in the catalytic subunit. There may be other domains besides the helicase domain needed to move DNA in and out of nucleosomes. The C terminus of the ISWI catalytic subunit with the conserved HAND, SANT, and SLIDE domains may be involved in nucleosome spacing. There are several models of how the C terminus may facilitate in ISWI remodeling such as regulating the activity of the helicase domain and causing the helicase domain to translocate more efficiently on DNA or to enhance its selectivity for nucleosomes. Another possibility is that domains like SLIDE promote linker DNA entering into nucleosomes in a coordinated manner with the helicase domain.

Introduction

The ISWI (Imitation SWI/SNF) subfamily of ATP-dependent chromatin remodelers is one of five major chromatin remodeler subfamilies (SWI/SNF, CHD, INO80 and ATRX). Remodelers contain a helicase domain conserved among members of the Snf2 family and SF2 superfamily of ATP-dependent DNA and RNA helicases. In *Saccharomyces cerevisiae* (Isw1 and Isw2) and humans (SNF2H and SNF2L) there are two versions of the ISWI catalytic subunit assembled with one to three accessory subunits to form 3–7 different complexes (Figure 1). Isw1 forms two complexes with distinct auxiliary subunits referred to as ISW1a with subunit Ioc3 and ISW1b with subunits Ioc2 and Ioc4 [1]. Isw2 forms primarily one complex with the accessory subunits Itc1, Dpb4 and Dls1; and is referred to as ISW2 [2]. Dpb4 and Dls1 are two histone-fold like subunits and similar histone-fold like subunits are found in ISWI complexes in Drosophila (CHRAC) and humans (hCHRAC) [3–5]. The ISWI remodelers are involved in transcription regulation of RNA polymerase I, II, and III; and DNA replication and repair[6]. ISW2 binds near promoter regions and moves

nucleosomes close to nucleosome free regions (NFR) to repress RNA pol II transcription [7–9]. ISW1a and ISW1b are recruited to the gene body regions and in particular ISW1b is recruited by trimethylation of lysine 36 of histone H3 (H3K36me3) [10–12]. ISW1b promotes the reassembly of chromatin over the coding regions after transcription, reducing cryptic transcription from these sites. ISW1b also has a role in proper termination of RNA Pol II and ISW1a in regulating the early phase of RNA Pol II elongation [13,14]. ISW2 along with the INO80 promotes the progression of DNA replication forks and attenuates S phase checkpoint activity [15,16]. Some of the ISWI complexes assemble nucleosomes onto DNA and can be involved in heterochromatin formation. ACF from Drosophila with its catalytic subunit ISWI and accessory subunit Acf1 was shown to assemble and space nucleosomes in an ATP-dependent manner requiring the histone chaperone Nap1 [17–19]. The complex of SNF2H and RSF-1 assembles nucleosomes without a histone chaperone and facilitates in the formation of centromeric chromatin containing the H3 variant CENP-A [20–22]. A nucleolar form of ISWI called NoRC also appears to be required for formation of heterochromatin over rRNA genes [23,24].

Model for the interactions of ISW2 with nucleosomes

The ISWI subfamily requires a minimal length of linker DNA for mobilizing nucleosomes, a property not shared by the SWI/SNF subfamily. At a mononucleosome level, ISW2 binds to at least 50–60 bp of extranucleosomal DNA shown by DNA footprinting [25]. ISW2 affinity for nucleosomes and its efficiency for mobilizing nucleosomes depend on the length of extranucleosomal DNA. More than 20–23 bp of extranucleosomal DNA is minimally required by ISW2 to mobilize nucleosomes when fully bound [26]. The accessory subunit Itc1 binds 53 bp of linker DNA spanning from the entry site of the nucleosome. In the catalytic subunit Isw2, the SLIDE domain binds DNA 19 bp from the entry site and the HAND domain ~11–13 bp from the entry site inside of nucleosomes [27]. Itc1 significantly contributes to the nucleosome affinity of ISW2 and Isw2 alone binds to nucleosomes with a 5 times lower affinity than native ISW2 [28]. DNA footprinting and site-directed crosslinking showed that the helicase domain of Isw2 was bound at Superhelical Location 2 (SHL2), 17 and 18 bp from the dyad axis. Structures of the helicase domain and C-terminus of Isw2 have not been determined, but models of these have been constructed based on their sequence homology with Rad54 [29,30] and Drosophila ISWI [31] whose structures have been obtained. DNA crosslinking and peptide mapping data were used to dock the structural models of the helicase domain and C-terminus of Isw2 to their nucleosome binding sites. Site-specific DNA crosslinking and structural models together pinpoint the most probable surfaces of the helicase, HAND and SLIDE domains which are in contact with DNA 17–18, 60–62, and 92 bp from the dyad axis, respectively (Figure 2a). Since the direction of translocation relative to the helicase structure is known for some helicases [32], the orientation of the helicase domain on nucleosomes was refined further by correlating it to the direction which ISW2 moves nucleosomal DNA (Figure 2b). One parameter that helps in proper docking of the C-terminus is the requirement of the distance between the HAND and SLIDE domains being consistent with the distance between the DNA sites shown to be crosslinked in nucleosomes. Another parameter that aids in the alignment of the C-terminus is the observation of an extended patch of conserved basic residues on its surface that could

bind DNA which aligns well with the binding surface predicted from DNA crosslinking ([31] and Figure 2c). These data together create a model from which structural and functional predictions can be made and tested further.

One aspect examined of this model is the manner in which the helicase domain functions deep inside of nucleosomes. Single nucleotide gaps in DNA at SHL2 interfered with nucleosomal movement through blocking DNA translocation or binding of the helicase domain of ISW2, and similar effects were seen with NURF and SWI/SNF [33,34]. DNA torsional strain in a 10 bp region outside of the helicase bound region appears to be important for ISW2 remodeling with nicks and gaps interfering with remodeling at these positions. Gaps and nicks at these positions are probably releasing DNA twist caused by translocation of the helicase domain since they are outside of the helicase binding site and nicks do not block DNA translocation. Gaps and nicks interfered only on the side of the helicase domain where DNA is pulled into nucleosomes. Torsional strain on the indicated side of the bound helicase domain was required to pull DNA inside nucleosomes as shown by single molecule Fluorescence Resonant Energy Transfer (smFRET) [35]. Initially ~7 bp of DNA exited out of nucleosomes in one base-pair increments before changes occurred at the entry of DNA into nucleosome (Figure 3). After this initial movement, ~3 bp of DNA is required to exit before any additional DNA can enter into nucleosomes and account for the observed sequential kinetic steps of ~7, ~3, and ~3 bp with ISW2. DNA torsional strain between the bound helicase domain and the site where linker DNA enters is required for pulling DNA into nucleosomes.

Nucleosome dynamics and ISWI

This uneven movement of DNA through nucleosomes is somewhat unexpected based on nucleosome energetics. There are 121 interactions between histones and DNA in the nucleosome that are mediated through hydrogen bonding with water molecules as well as 116 hydrogen bonds made directly between histones and DNA both of which should equally contribute to the stability of nucleosomes [36,37]. Many of the interactions are with the DNA phosphate backbone and there are 20 critical side-chain interactions that penetrate into the minor groove of DNA, most often mediated by the side-chains of arginine. All histone-DNA contacts are not energetically equivalent as shown by single molecule/optical trap type experiments in which DNA is unzipped from one end. DNA was reversibly peeled off nucleosomes by unzipping DNA and nucleosomes not unwrapped past the dyad axis could be rewrapped once the tension was released [38]. Refinements of this approach showed the energetic profile of DNA peeling off nucleosomes with nearly base pair resolution and found three regions having the strongest energetic barriers for pulling DNA from the histone octamer [39]. The strongest histone octamer binding site to DNA was comprised of contacts from the H3–H4 tetramer spanning 10–15 bp in either direction from the dyad axis (Figure 3). The two next strongest binding sites are ~40–50 bp from the dyad axis with the H2A-H2B dimers. They found a distinct ~5 bp periodicity of contacts, which suggests sequential breaking of a DNA contact first from one strand followed by that of the opposite strand.

The helicase domain binds a region in nucleosomes where energetically it has more pliable histone-DNA contacts due to a low energy valley and is bordered on either side by stronger

histone-DNA contacts (Figure 3). Based on energetic constraints, the accumulation of torsional strain on only one side of the helicase domain is not likely to be due to the histone contacts being stronger on that side compared to the other thus requiring extra force to disrupt histone-DNA contacts. It might be that the interactions of the remodeler near the entry site are the "brake" that causes the torsional strain to be required before releasing the DNA into nucleosomes and could be mediated by the C-terminus of ISWI.

Role of the SANT, SLIDE, and HAND domains

The binding of the C-terminus of Isw2 to linker DNA and the entry side of nucleosomes could be important for proper recruitment of the complex to nucleosomes as well as for controlling remodeling. The SLIDE domain from *Drosophila* ISWI has high affinity for DNA[31] and, based on DNA crosslinking, the HAND and SLIDE domains of ISW2 also bind DNA when ISW2 docks onto nucleosomes [27]. The role of the C-terminus of the catalytic subunit of ISWI has been examined with the catalytic subunit alone and truncation of the entire C-terminus or in the native complex by mutagenesis of the individual domains. In one study loss of the C-terminus of the catalytic subunit of *Drosophila* ISWI had no effect on the ATPase activity of the helicase domain with free DNA substrate, with or without the addition of H4 peptide, or with nucleosomes [40]. If nucleosomes were subsaturating an order of magnitude difference was observed between full length and truncated ISWI, suggesting the C-terminus increased the selectivity or affinity of the helicase domain. In a different study of the same protein, the C-terminus had the role of stimulating DNA translocation of the helicase domain [41]. Importantly these studies come to two different conclusions of the role of the C-terminus of the ISWI catalytic subunit; namely as a selectivity factor or regulation of helicase activity. In terms of selectivity this might be easiest to explain by the C-terminus of ISWI recruiting the complex to linker DNA and thereby to the core nucleosome.

A potential problem with these studies is the activity of the free catalytic subunit being less than the native complex and the accessory subunit(s) being important for the overall activity of the enzyme. Deletion of the entire C-terminus can also have a combination of effects on the ATPase and remodeling activities, and doesn't address well the separate activities of the HAND, SANT and SLIDE domains. A small subset of the conserved residues was changed to alanine in the SLIDE domain that was predicted to bind DNA based on the model in Figure 2. These changes reduced ISW2 binding ~19 bp from the edge of the nucleosome without disturbing the other interactions of ISW2 with nucleosomal or extranucleosomal DNA [28]. These mutations in SLIDE severely affected the ATPase and nucleosome mobilizing activities of ISW2 under nucleosome saturating conditions and pointed to recruitment not being its sole function. In the absence of SLIDE domain binding DNA, the helicase domain moved only ~10 bp of DNA out of nucleosomes before stalling due to the lack of DNA coming in. The Isw2 subunit alone without the SLIDE domain is unable to remodel nucleosomes even though it still binds nucleosomes [28] and thus has an important role in mobilizing nucleosomes in coordination with the helicase domain (Figure 4).

Nucleosome movement: Coordinated action versus solely the helicase domain

ISWI remodeling has been suggested to be dependent exclusively on the helicase domain and other parts of the complex having supporting roles such as facilitating in nucleosome recruitment and regulating the helicase domain activities ([40] and Figure 4). Other suggestions are that domains like the SLIDE domain can have additional roles in remodeling and work in a coordinated manner with the helicase domain to mobilize nucleosomes [28,42]. The interactions of ISW2 with linker DNA promote the stable binding of the helicase domain to DNA at SHL2 as shown in DNA footprinting experiments, supporting a role in recruitment [43], and consequently can regulate the kinetic activity of the helicase domain [44]. There is good evidence that other domains can also be involved in regulating the ATPase and DNA translocation properties of the helicase domain [41]. It is also possible for domains such as those in the C-terminus to facilitate the passage of DNA into nucleosomes and regulate its flow through the nucleosome.

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Highlights

- The C terminus of the catalytic subunit of ISWI complexes is evolutionarily conserved and required for efficient nucleosome mobilization

- The SANT, SLIDE and HAND domains are in the C-terminus of ISWI

- The SLIDE domain binds to linker DNA in the ISW2 complex and facilitates linker DNA movement into nucleosomes

- The C-terminus of ISWI may also be required for helicase domain selectivity and efficient DNA translocation.

- DNA twist between the helicase domain and the entry site of nucleosomes is required for nucleosome movement
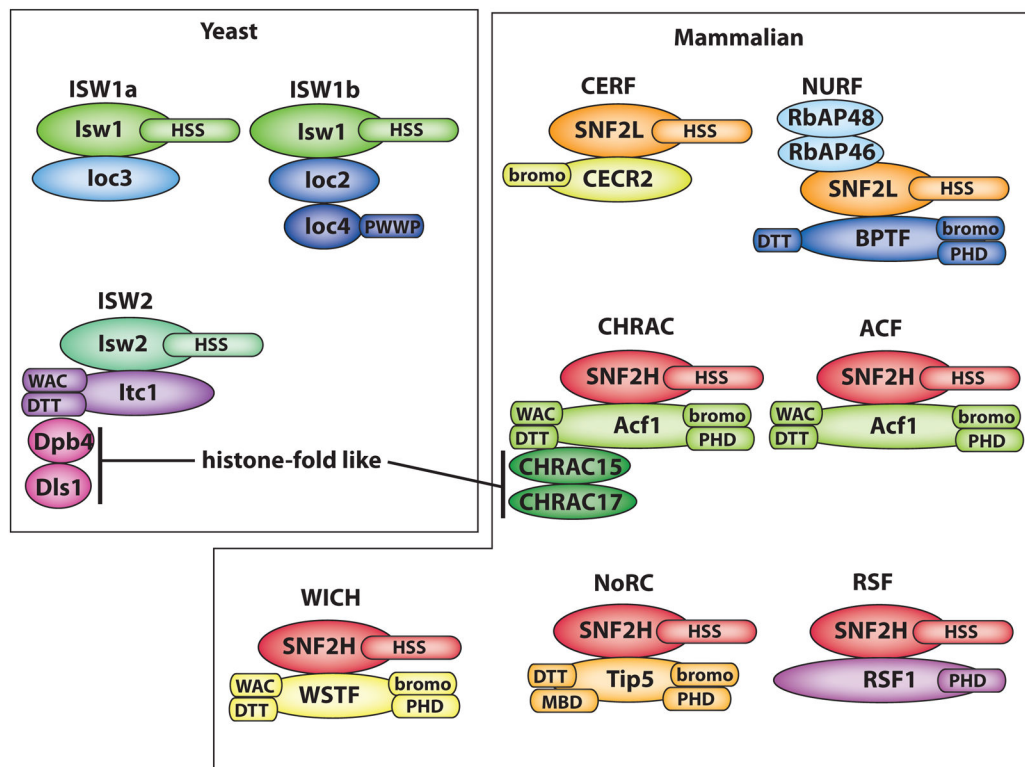
Figure 1. ISWI family of chromatin remodelers in Saccharomyces cerevisiae and mammals
The subunits are represented as ellipses and conserved protein motifs as rectangles. HSS is the HAND, SANT and SLIDE domains. Some of the conserved proteins domains bind to modified sites in histones such as the bromo, PHD and PWWP domain; and others are involved in subunit-subunit interactions like the WAC and DTT domains.
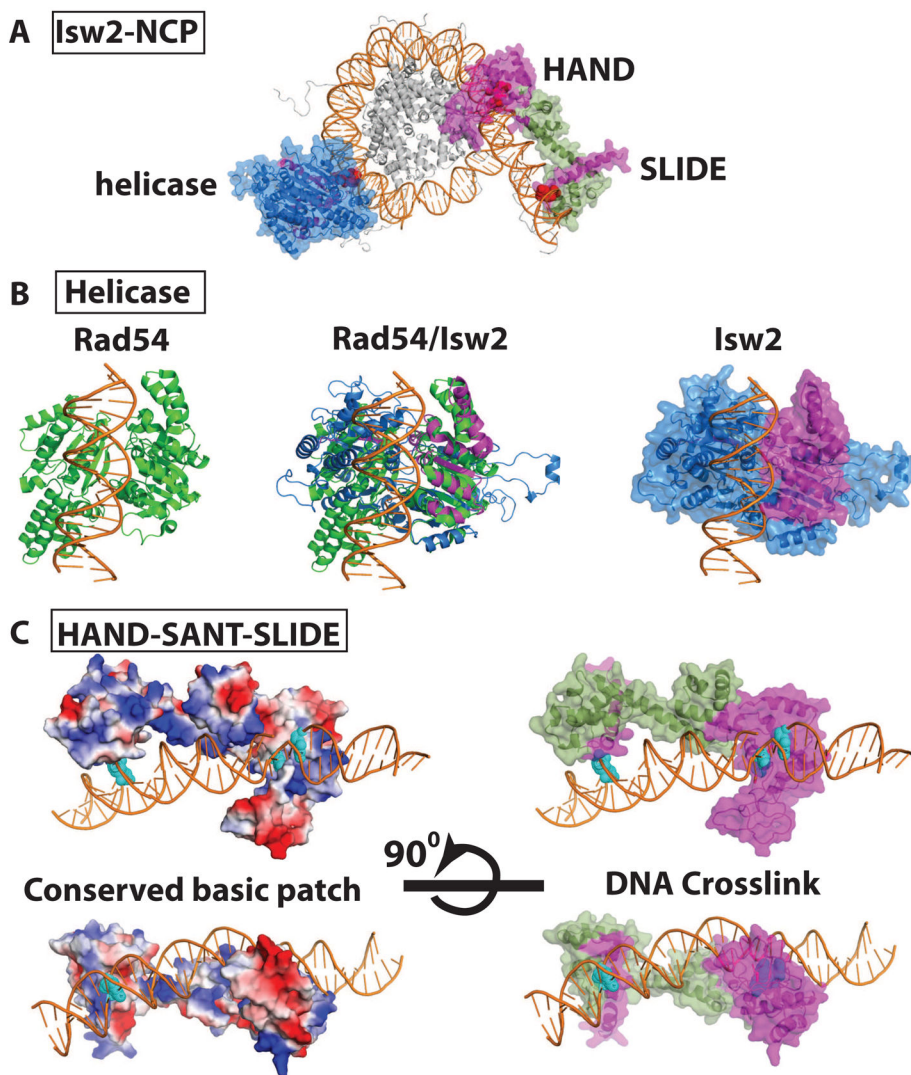
Figure 2. Interactions of Isw2 with nucleosomes

(A) Three DNA sites probed by crosslinking are in red and the regions of Isw2 crosslinked to them in magenta. The helicase, HAND and SLIDE domains are labeled. The region in the helicase domain crosslinked to DNA cannot be seen in this orientation. (B) The crystal structure of the Sulfulobus Rad54 helicase domain (green) with DNA (orange) bound is shown on the left. In the middle is the overlay of the model of Isw2 with the known crystal structure of Rad54. On the right is Isw2 (blue) with DNA bound similar to that shown for Rad54 and the region crosslinked to DNA in magenta. (C) On the left is shown the predicted electrostatic surface potential for the HAND-SANT-SLIDE domains and on the right the regions shown to crosslink DNA in magenta. These structures are rotated by 90° to more fully visualize the conserved basic patch in blue.
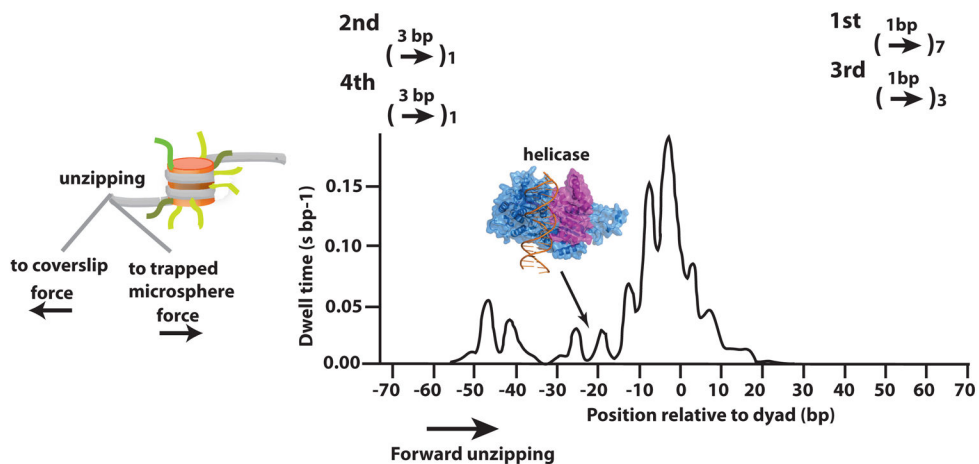
Figure 3. Nucleosome dynamics and ISW2
Nucleosome energetics were measured using the single molecule approach shown on the left in which the two ends of DNA are fixed and then progressively unzipped. A typical profile of these experiments is shown on the right and the position where the helicase domain is bound. Above the plot is shown the progressive movement of DNA in (left) and out (right) of nucleosomes by ISW2 using smFRET.
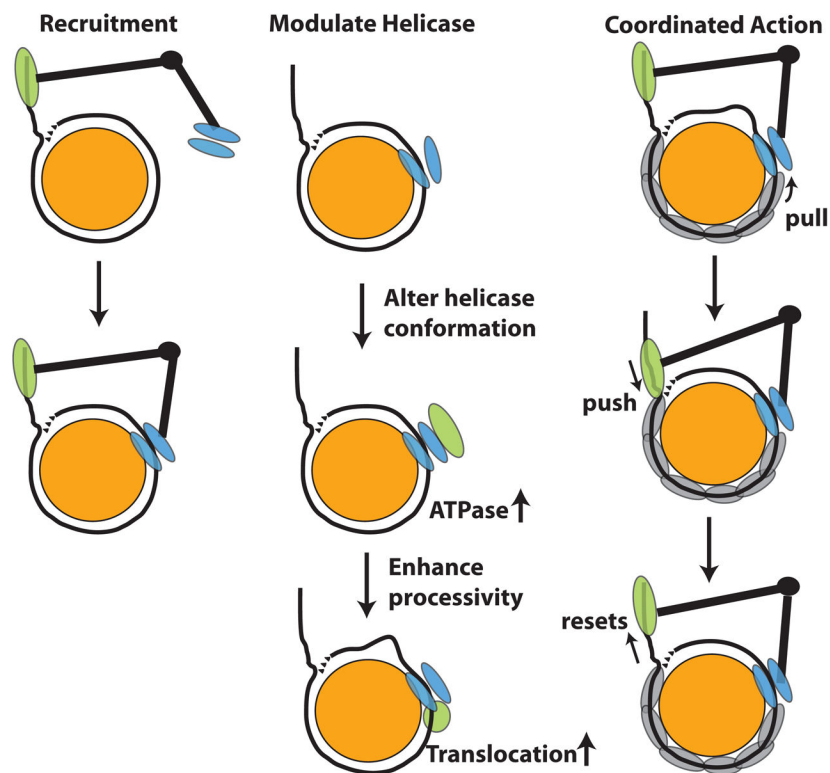
Figure 4. Roles for the HAND-SANT-SLIDE domains in ISWI remodeling
The three ways that these domains might be involved in mobilizing nucleosomes are recruitment, modulating the helicase domain activity, and coordinating the movement of DNA in nucleosomes. The helicase domain is represented in blue, histone octamer in orange, and HSS or SLIDE domains in green.