Published in final edited form as:

Curr Opin Struct Biol. 2012 April ; 22(2): 225–233. doi:10.1016/j.sbi.2012.02.007.

Swi2/Snf2 remodelers: hybrid views on hybrid molecular machines

Karl-Peter Hopfner^{1,2,*}, Christian Gerhold¹, Kristina Lakomek¹, and Petra Wollmann¹

¹Department of Biochemistry at the Gene Center, Ludwig-Maximilians-University Munich, Feodor-Lynen-Str. 25, 81377 Munich, Germany

²Center for Integrated Protein Sciences, Ludwig-Maximilians-University Munich, Feodor-Lynen-Str. 25, 81377 Munich, Germany

Summary

Swi2/Snf2 (switch / sucrose non-fermentable) enzymes form a large and diverse class of proteins and multiprotein assemblies that remodel nucleic acid:protein complexes, using the energy of ATP hydrolysis. The core Swi2/Snf2 type ATPase domain belongs to the “helicase and NTP driven nucleic acid translocase” superfamily 2 (SF2). It serves as a motor that functionally and structurally interacts with different targeting domains and functional modules to drive a plethora of different remodeling activities in chromatin structure and dynamics, transcription regulation and DNA repair. Recent progress on the interaction of Swi2/Snf2 enzymes and multiprotein assemblies with their substrate nucleic acids and proteins, using hybrid structural biology methods, sheds light onto mechanisms of the complex chemo-mechanical remodeling reactions. In the case of Mot1, a hybrid mechanism of remodeler and chaperone emerged.

The Swi2/Snf2 ATPase: A versatile motor

“Remodelers” are diverse molecular machines that use the energy of ATP to disrupt or remodel protein:nucleic acid complexes, most notably nucleosomes but also others such as transcription factors and RNA polymerase. The shared chemo-mechanical “engine” of remodelers is a Swi2/Snf2 ATPase, which possesses a Swi2/Snf2 ATPase domain along with other domains that target, regulate and/or mechanistically couple the ATPase domain with the substrate in the remodeling reaction (Fig. 1). Swi2/Snf2 domains form a subgroup among SF2 helicases/translocases, which also include DEAD box RNA helicases, DNA helicases, and innate immune sensors [1]. SF2 enzymes have at least 12 characteristic sequence motifs that are involved in ATP binding and hydrolysis or interaction with the nucleic acid substrates. ATP binds between and repositions the two RecA-like (sub)domains of the SF2 fold, leading to a conformational shift in nucleic acid binding motifs [2]. Depending on the SF2 family, the conformational switch can grip or bend nucleic acids without translocation, but often leads to an “inchworm” like movement along nucleic acids.

A variety of studies indicate that Swi2/Snf2 domains use ATP to track along the minor groove of dsDNA [3–5]. This tracking can go over considerable distances as in the case of Rad54 [6]. For RSC, a large nucleosome remodeling complex, optical tweezer experiments

© 2012 Elsevier Ltd. All rights reserved.

*Correspondence: hopfner@genzentrum.lmu.de, Tel: +49 (0) 89 2180 76953, Fax: +49 (0) 89 2180 76999.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

revealed for instance a translocation processivity of about 35 bp with a step size of approximately 2 bp and a rate of roughly 25 bp/s on dsDNA, applying substantial mechanical force (30 pN) that suffices to disrupt DNA-histone interactions after bulging the DNA during initiation [7].

While several structures of the Swi2/Snf2 domains alone, bound to DNA and in complex with accessory domains have been determined [3,8–10], it has not yet been worked out how ATP dependent conformational changes in the Swi2/Snf2 domain propel the enzyme in a directional manner along the minor groove. However, it is likely that the mechanism bears similarity to the single-base(pair) stepping mechanism of SF1 helicases [11].

Structural and functional versatility

Despite their conserved motor domain, Swi2/Snf2 enzymes execute a broad spectrum of remodeling reactions and a key question is how the force generated by the motor activity is used to disrupt substrate protein complexes. The most complex family of Swi2/Snf2 enzymes are protein assemblies that alter the position or composition of nucleosomes and are currently grouped into SWI/SNF, ISWI (imitation switch), CHD/Mi-2 and INO80 (inositol auxotroph mutant 80) families. SWI/SNF and the related RSC (Remodeling the structure of chromatin) complexes disrupt nucleosomes either through repositioning or dissociation [12,13], while equally complex INO80 and the related SWR1 exchange histone H2AZ and H2A variants [14,15]. Chd1 (Chromodomain-helicase-DNA-binding protein 1) and ACF/ISWI remodeler slide nucleosomes and catalyze nucleosome spacing [16,17]. Together, the combination of these activities ensures a dynamic nature of chromatin, and results from chromatin reconstitution procedures and analysis of DNA double-strand break repair hint at considerable collaboration of remodelers *in vivo* [18,19].

Other Swi2/Snf2 enzymes have a much simpler architecture. For instance, the single subunit remodeler Mot1 (modifier of transcription 1, denoted BTAF1 in humans) acts on the transcription initiation factor TBP (TATA-box binding protein) to modulate TBP's association to promoter DNA [20]. The bacterial Swi2/Snf2 remodeler RapA, which recycles RNA polymerase, is functional as a single polypeptide chain and has even been structurally determined as whole (Fig. 2a) [10]. Other remodelers include DNA repair enzymes Rad54, Cockayne Syndrome protein B and Rad5, Rad16 and HLTF, but will not be discussed further. A remarkable Swi2/Snf2 “remodeler” is HARP (HepA-related protein), which acts as an annealing helicase [21].

A key feature of remodelers is the correct targeting of the Swi2/Snf2 motor to the substrate. An emerging targeting module is the SANT-SLIDE (comprised in switching-defective protein 3, adaptor 2, nuclear receptor co-repressor and transcription factor TFIIB / SANT-like ISWI) domain combination, which recognizes the nucleosome and internucleosomal DNA in ISWI type remodelers and is sufficient for nucleosomal DNA binding [22]. The SANT-SLIDE module targets nucleosomal substrates most likely based on a continuous sampling mechanism in a seconds-to-minutes-timescale [23]. This model is in accordance with the high abundance of ISWI complexes in the nucleus and their extreme mobility. They accumulate within tens of seconds at DNA damage sites upon UV exposure [24].

Additional recruitment to epigenetic marks is mediated for instance by tandem chromodomains of Chd1 or by human BPTF (bromodomain and PHD finger-containing transcription factor) - a subunit of the ISWI homologue NURF (nucleosome remodeling factor) complex. Here a PHD finger with an adjacent bromodomain recognizes nucleosomes that are trimethylated at H3K4 and also bear an acetyllysine in their H4 histone tail [25]. A notable set of subunits in SWI/SNF and INO80 type remodelers are actin related subunits. INO80 complexes that lack histone binding subunits Arp4 and Arp8 (actin-related proteins 4

and 8) fail to bind to DNA and to remodel nucleosomes and mammalian INO80 complexes require Arp8 for targeting to sites of DNA damage [26–28]. Together with other DNA-binding subunits such as human YY1, Arps could serve as nucleosome recognition modules targeting INO80 to the appropriate responsive elements in different contexts [29,30].

Targeting is not limited to the classic epigenetic marks and nucleosomes and more and more principles emerge. Recently, a functional poly(ADP-ribose) (PAR) binding macrodomain has been identified in the CHDL-type remodeler Alc1 (amplified in liver cancer 1), which could remodel chromatin after recruitment by ADP-ribosylation of DNA damage sites [31,32].

Towards a high resolution nucleosome remodeler structure and mechanism

A key question in the field is, how the ATP dependent DNA translocation activity is structurally coupled to substrate remodeling. Progress towards this goal has recently been achieved on the ISWI- and Chd1-type remodelers (Figs. 2b,c,d) although the molecular mechanism of chromatin remodeling applied by ISWI-family members is still under debate. In general, members of the ISWI/ACF-family generate regular nucleosomal arrays by shifting nucleosomes until the linker DNA becomes too short. Yeast Isw2 for instance binds around 20 bp of extranucleosomal DNA directly flanking the nucleosome on its entry site [33]. The remodeling process by Isw2 can be classified into distinct stages. Kinetic data uncovered a slow initiation process, which might be essential to make yIsw2 competent for DNA translocation upon binding. It could correspond to the second step of a two-step DNA binding mechanism [34], in which a first conformational switch of ISW2 upon ATP-binding enhances its association with extra- and nucleosomal DNA at the entry site and dyad axis. ATP hydrolysis and the resultant formation of a stable and processive ISW2:substrate nucleosome complex [35] leads to extended interactions with nucleosomal DNA. The dissociation of the catalytic subunit's translocase domain from the nucleosome is needed for effective nucleosome shift and goes along with a conformational rearrangement initiated upon the perception of the shortening DNA strand [33].

A combination of electron microscopy, biochemical and biophysical studies revealed the presence of two ACF complexes bound at nucleosomes and biochemically found that two ACF complexes cooperate and take turns to move a nucleosome [16]. An accompanying study using advanced single molecule FRET analysis showed that ACF translocates for approx. 7 bp, until it pauses. Nucleosomes were repositioned in steps closer to 3 bp [36]. Interestingly, all steps – binding, sliding, pausing – were ATP-dependent. This remarkably broad role of ATP indicates that the conformational cycle of the Swi2/Snf2 domain has functions apart from mere dsDNA groove tracking.

While these studies provided insights on the action of the Swi2/Snf2 domain at the nucleosome, it remains unclear how nucleosomes are actually spaced. On the basis of a hybrid approach combining Isw1a-DNA complex crystal and Isw1a-nucleosome EM structures with site-specific photo-crosslinking experiments on Isw1a-nucleosome assemblies, Richmond and colleagues showed that the substrate for remodeling by Isw1a is in fact a dinucleosome and provided an intriguing model for nucleosome spacing (Figs. 2, 4b) [37]. The complex of HAND-SANT-SLIDE (HSS) domains with Ioc3 form a molecular ruler module that ensures correct nucleosome spacing by binding between two nucleosomes (Fig. 2). The Swi2/Snf2 domain binds the nucleosomal DNA, and its translocase activity helps to reel in linker DNA to move the mobile closer towards the static nucleosome until the HSS-Ioc3 module stops further translocation. A remarkable outcome of the study is also the implication of a potential sequence preference for the Ioc3 subunit that could be

important to reposition nucleosomes at promoter regions. In contrast to the dimeric motor ACF, Isw1a appears to work as single motor. Thus, the precise stoichiometry of ISWI factors on nucleosomal arrays needs to be studied further.

Recently, structures of Chd1's DNA-binding domain (DBD) have been determined alone [38] and bound to a dodecameric DNA duplex [39]. These structures revealed that the DBD is composed of a SANT and a SLIDE domain strikingly reminiscent of ISW1, a feature that was not obvious from the sequence. Hence, this module appears to be more broadly utilized by Swi2/Snf2-ATPases. However, based on the resemblance concerning SANT and SLIDE domains, the grouping of CHD1/Mi-2 and ISWI into different families rather than the same should perhaps be reconsidered.

Regulation of the Swi2/Snf2 ATPase

Numerous enzymes are regulated by internal regulatory or repressor domains. The crystal structure of yeast Chd1's tandem chromodomains together with the Swi2/Snf2 domain was determined and established this principle now within the Swi2/Snf2 family [9]. The most remarkable aspect of this study was the observation that the double chromodomains block DNA binding and activation of the Swi2/Snf2 ATPase in the absence of nucleosome substrates (Fig. 2). Indeed, removal of the chromodomains resulted in a greatly enhanced ATPase activity. Earlier studies showed that the human CHD1 double chromodomains target the lysine 4-methylated histone H3 tail (H3K4me), a hallmark of active chromatin [40]. However, the chromodomains of CHD1 in flies are not essential for chromatin localization of the enzyme but rather for enzymatic activity [41]. It is possible that CHD1 is targeted by other means, yet recognition of methylated lysine tails leads to activation of the Swi2/Snf2 ATPase at the target. Allosteric regulation may not be limited to chromodomains: a newly identified SnAC (Snf2 ATP coupling) domain has been shown to influence ATP hydrolysis of the Swi2/Snf2 domain as well [42], so we expect to see more of these internal regulatory mechanism of the ATPase activity. In any case, these results showed that internal repression or regulation of the ATPase could be an important feature to ensure that only the correct substrate DNA is bound by the Swi2/Snf2 motor, thereby enhancing specificity of the enzyme.

Mot1:TBP complex reveals hybrid remodeler-chaperone features

Given the prevailing scarcity of crystal structures of proteins bound to nucleosomes, progress towards the atomic structure of a remodeler:substrate complex has been obtained with Mot1 (Fig. 3a). Mot1 does not act on nucleosomes but on the transcription factor TBP. Besides the Swi2/Snf2 domain, Mot1 possesses a long N-terminal region that consists of HEAT (Huntingtin, elongation factor 3, protein phosphatase 2A, lipid kinase TOR) repeats and mediates interaction with TBP.

Mot1 was identified as repressor of transcription by removing TBP from TATA boxes, but new results suggest that it can also act as activator of transcription [43,44]. In this regard, Mot1 could dissolve "inactive" TBP bound to NC2 (negative cofactor 2), thus liberating active TBP [45]. However, one interesting finding is that Mot1 creates a dynamic TBP pool that helps TBP to diffuse from TATA promoters to TATA less promoters [46,47].

The Mot1-TBP system offers a unique simplicity for a hybrid approach to elucidate a complete remodeler architecture (Fig. 3a). In the recently solved crystal structure of the HEAT domain of Mot1 in complex with TBP, Mot1 wraps one half of the saddle shaped TBP [48]. Critical for initial recognition are highly conserved, mostly acidic residues located on loops of the HEAT domain that contact TBP's convex surface, a region that is also accessible in a DNA-bound form of TBP (Fig. 3b). This region can also be occupied by

higher assemblies of the transcription initiation complexes, suggesting a mode of restricting Mot1's activity to only a subset of TBP complexes.

An intriguing second binding site is located in the concave surface of TBP, evoking an interaction of a long Mot1 "latch" domain that directly competes with DNA binding and TBP self-dimerization (Fig. 3c). As a consequence, TBP does not form a dimer when bound to Mot1 and the observed complex is necessarily a "product" complex, i.e. after the remodeling reaction. These unexpected results identify Mot1 not only as remodeler but also as chaperone to prevent (re)formation of TBP:DNA or TBP:TBP complexes.

Mot1's ATPase contacts the DNA major groove upstream the TATA region [49]. Still under debate is whether ATP hydrolysis is used to move Mot1 towards or away from the TBP-DNA complex. However, in both scenarios the motor activity would generate force that leads to a conformational change in the HEAT repeats. Based on their notable flexibility and springiness these protein modules are in principle suited to store the energy of a few ATP driven groove tracking steps until a certain threshold leads to a rapid stroke that passes the energy towards TBP displacement.

At present, one draft is a multistep recognition - activation - remodeling - chaperone model for the action of Mot1 on TBP comparable with lessons from the research on ISWI and Chd1 (Fig. 4): After initial recognition of the surface of TBP and perhaps allosteric activation of the ATPase, torsional force generated by the Swi2/Snf2 domain on upstream DNA might be sufficient to weaken the TBP-DNA interface, allowing the latch to be inserted to inhibit TBP rebinding to the DNA. In this regard Mot1 could also act as an escort factor for TBP around the nucleus. Chaperone activities may also be part of e.g. SWI/SNF type remodelers, but more research needs to be done to understand this type of activity in a broader context. However, it is well known that histones are delivered by an intricate network of dedicated histone chaperones (reviewed in [50]), so partial nucleosomes and unincorporated histone dimers might require intrinsic chaperone activities of large multisubunit remodelers.

Outlook

In the past couple of years, substantial progress in both enzymatic and structural approaches led to insights in the functional and structural coupling of the ATPase motor with other functional modules of remodelers, resulting in mechanistically more detailed models of the intricate chemo-mechanical reactions. Single-molecule studies using FRET or optical tweezers shed light on steps of the reaction and properties of the enzymes that are difficult if not impossible to reveal with bulk biochemistry. From a structural point of view, hybrid methods approaches typically combining electron microscopy of remodelers alone or in complex with substrates together with crystal structures of substantial submodules emerge as one way to go (Fig. 3d). This has proven quite powerful for Mot1 and Isw1 remodelers, because a substantial part of protein could be crystallized, and structural information for the missing domains is available from homology models, to place into and interpret the EM density maps.

It is much more difficult to address the structure of the more complex SWI/SNF and INO80 type remodelers. Nevertheless, several of these assemblies have been studied with and without nucleosomes by electron microscopy (reviewed in [51]). Although the resulting 3D reconstructions give interesting insights into shape and nucleosome binding cavities, they do not yet allow the interpretation with atomic models or placement of domains. A promising line to complement the EM studies and to help placing domains is to provide additional structural information such as crosslinking and mass spectrometry [52]. Having these

powerful methods in place, we look forward to a more detailed understanding of the mechanistic architecture of the complex remodeler in the years to come.

Highlights

- Swi2/Snf2 proteins/multiprotein assemblies remodel nucleic acid-protein complexes.
- Core Swi2/Snf2 ATPase domain is a structurally and functionally versatile motor.
- Hybrid methods pave the way towards nucleosome:remodeler structures & mechanisms.
- The remodelers Mot1, Chd1 and Isw1 use different ATP-driven multistep mechanisms.
- Mot1 employs a hybrid mechanism of remodeler and chaperone.

Acknowledgments

Work on remodelers and related ATPases in K-P H's laboratory is supported by SFBs 646 and 684 of the German Research Council (DFG), by the research cluster CIPSM of the German Excellence Initiative and by NIH (U19AI083025) and by GRK1721.

References

1. Fairman-Williams ME, Guenther UP, Jankowsky E. SF1 and SF2 helicases: Family matters. *Curr Opin Struct Biol.* 2010; 20(3):313–324. [PubMed: 20456941]
2. Pyle AM. Translocation and unwinding mechanisms of RNA and DNA helicases. *Annu Rev Biophys.* 2008; 37:317–336. [PubMed: 18573084]
3. Dürr H, Korner C, Müller M, Hickmann V, Hopfner KP. X-ray structures of the *Sulfolobus solfataricus* Swi2/Snf2 ATPase core and its complex with DNA. *Cell.* 2005; 121(3):363–373. [PubMed: 15882619]
4. Zhang Y, Smith CL, Saha A, Grill SW, Mihardja S, Smith SB, Cairns BR, Peterson CL, Bustamante C. DNA translocation and loop formation mechanism of chromatin remodeling by SWI/SNF and RSC. *Mol Cell.* 2006; 24(4):559–568. [PubMed: 17188033]
5. Saha A, Wittmeyer J, Cairns BR. Chromatin remodeling by RSC involves ATP-dependent DNA translocation. *Genes Dev.* 2002; 16(16):2120–2134. [PubMed: 12183366]
6. Amitani I, Baskin RJ, Kowalczykowski SC. Visualization of Rad54, a chromatin remodeling protein, translocating on single DNA molecules. *Mol Cell.* 2006; 23(1):143–148. [PubMed: 16818238]
7. Sirinakis G, Clapier CR, Gao Y, Viswanathan R, Cairns BR, Zhang Y. The RSC chromatin remodelling ATPase translocates DNA with high force and small step size. *EMBO J.* 2011; 30(12):2364–2372. [PubMed: 21552204] *Although these types of remodelers are not discussed in detail, this work is of high technical and scientific interest because it directly demonstrates the translocation properties of a complex remodeling machine using advanced single molecule optical tweezer studies.*
8. Thoma NH, Czyzewski BK, Alexeev AA, Mazin AV, Kowalczykowski SC, Pavletich NP. Structure of the Swi2/Snf2 chromatin-remodeling domain of eukaryotic Rad54. *Nat Struct Mol Biol.* 2005; 12(4):350–356. [PubMed: 15806108]
9. Hauk G, McKnight JN, Nodelman IM, Bowman GD. The chromodomains of the Chd1 chromatin remodeler regulate DNA access to the ATPase motor. *Mol Cell.* 2010; 39(5):711–723. [PubMed: 20832723] *In this paper, a fragment of Chd1 comprising the double chromodomains and the Swi2/Snf2 ATPase domain is described along with functional studies. The remarkable insight was that the chromodomains block the DNA binding site of the motor and act as internal repressor. This*

suggests that Chd1 and perhaps other Swi2/Snf2 enzymes are activity-regulated by correct substrate targeting.

10. Shaw G, Gan J, Zhou YN, Zhi H, Subburaman P, Zhang R, Joachimiak A, Jin DJ, Ji X. Structure of Rapa, a Swi2/Snf2 protein that recycles RNA polymerase during transcription. *Structure*. 2008; 16(9):1417–1427. [PubMed: 18786404]
11. Myong S, Bruno MM, Pyle AM, Ha T. Spring-loaded mechanism of DNA unwinding by hepatitis C virus NS3 helicase. *Science*. 2007; 317(5837):513–516. [PubMed: 17656723]
12. Liu N, Balliano A, Hayes JJ. Mechanism(s) of Swi/Snf-induced nucleosome mobilization. *Chembiochem*. 2011; 12(2):196–204. [PubMed: 21243709]
13. Rowe CE, Narlikar GJ. The ATP-dependent remodeler RSC transfers histone dimers and octamers through the rapid formation of an unstable encounter intermediate. *Biochemistry*. 2010; 49(45): 9882–9890. [PubMed: 20853842]
14. Mizuguchi G, Shen X, Landry J, Wu WH, Sen S, Wu C. ATP-driven exchange of histone H2az variant catalyzed by Swr1 chromatin remodeling complex. *Science*. 2004; 303(5656):343–348. [PubMed: 14645854]
15. Papamichos-Chronakis M, Watanabe S, Rando OJ, Peterson CL. Global regulation of H2a.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity. *Cell*. 2011; 144(2):200–213. [PubMed: 21241891]
16. Racki LR, Yang JG, Naber N, Partensky PD, Acevedo A, Purcell TJ, Cooke R, Cheng Y, Narlikar GJ. The chromatin remodeller ACF acts as a dimeric motor to space nucleosomes. *Nature*. 2009; 462(7276):1016–1021. [PubMed: 20033039] *In this work, an ACF type remodeler is visualized on the nucleosome using electron microscopy. Along with biophysical and biochemical approaches, the authors show that the motor works as a dimer, taking turns to slide the nucleosome unidirectionally.*
17. Strohner R, Wachsmuth M, Dachauer K, Mazurkiewicz J, Hochstatter J, Rippe K, Langst G. A 'loop recapture' mechanism for ACF-dependent nucleosome remodeling. *Nat Struct Mol Biol*. 2005; 12(8):683–690. [PubMed: 16025127]
18. Wippo CJ, Israel L, Watanabe S, Hochheimer A, Peterson CL, Korber P. The RSC chromatin remodelling enzyme has a unique role in directing the accurate positioning of nucleosomes. *The EMBO journal*. 2011; 30(7):1277–1288. [PubMed: 21343911]
19. Bao Y, Shen X. Chromatin remodeling in DNA double-strand break repair. *Curr Opin Genet Dev*. 2007; 17(2):126–131. [PubMed: 17320375]
20. Viswanathan R, Auble DT. One small step for Mot1; one giant leap for other Swi2/Snf2 enzymes? *Biochim Biophys Acta*. 2011; 1809(9):488–496. [PubMed: 21658482]
21. Yusufzai T, Kadonaga JT. HARP is an ATP-driven annealing helicase. *Science*. 2008; 322(5902): 748–750. [PubMed: 18974355]
22. Grüne T, Brzeski J, Eberharder A, Clapier CR, Corona DF, Becker PB, Muller CW. Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. *Mol Cell*. 2003; 12(2):449–460. [PubMed: 14536084]
23. Erdel F, Schubert T, Marth C, Langst G, Rippe K. Human ISWI chromatin-remodeling complexes sample nucleosomes via transient binding reactions and become immobilized at active sites. *Proc Natl Acad Sci U S A*. 2010; 107(46):19873–19878. [PubMed: 20974961]
24. Erdel F, Rippe K. Binding kinetics of human ISWI chromatin-remodelers to DNA repair sites elucidate their target location mechanism. *Nucleus*. 2011; 2(2):105–112. [PubMed: 21738833]
25. Ruthenburg AJ, Li H, Milne TA, Dewell S, McGinty RK, Yuen M, Ueberheide B, Dou Y, Muir TW, Patel DJ, Allis CD. Recognition of a mononucleosomal histone modification pattern by BPTF via multivalent interactions. *Cell*. 2011; 145(5):692–706. [PubMed: 21596426]
26. Shen X, Ranallo R, Choi E, Wu C. Involvement of actin-related proteins in ATP-dependent chromatin remodeling. *Mol Cell*. 2003; 12(1):147–155. [PubMed: 12887900]
27. Harata M, Oma Y, Mizuno S, Jiang YW, Stillman DJ, Wintersberger U. The nuclear actin-related protein of *Saccharomyces cerevisiae*, Act3p/Arp4, interacts with core histones. *Mol Biol Cell*. 1999; 10(8):2595–2605. [PubMed: 10436015]

28. Kashiwaba S, Kitahashi K, Watanabe T, Onoda F, Ohtsu M, Murakami Y. The mammalian INO80 complex is recruited to DNA damage sites in an Arp8 dependent manner. *Biochem Biophys Res Commun.* 2010; 402(4):619–625. [PubMed: 20971067]
29. Cai Y, Jin J, Yao T, Gottschalk AJ, Swanson SK, Wu S, Shi Y, Washburn MP, Florens L, Conaway RC, Conaway JW. YY1 functions with INO80 to activate transcription. *Nat Struct Mol Biol.* 2007; 14(9):872–874. [PubMed: 17721549]
30. Hogan CJ, Aligianni S, Durand-Dubief M, Persson J, Will WR, Webster J, Wheeler L, Mathews CK, Elderkin S, Oxley D, Ekwall K, et al. Fission yeast Iec1-Ino80-mediated nucleosome eviction regulates nucleotide and phosphate metabolism. *Mol Cell Biol.* 2010; 30(3):657–674. [PubMed: 19933844]
31. Gottschalk AJ, Timinszky G, Kong SE, Jin J, Cai Y, Swanson SK, Washburn MP, Florens L, Ladurner AG, Conaway JW, Conaway RC. Poly(adp-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler. *Proc Natl Acad Sci USA.* 2009; 106(33):13770–13774. [PubMed: 19666485]
32. Ahel D, Horejsi Z, Wiechens N, Polo SE, Garcia-Wilson E, Ahel I, Flynn H, Skehel M, West SC, Jackson SP, Owen-Hughes T, et al. Poly(adp-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme Alc1. *Science.* 2009; 325(5945):1240–1243. [PubMed: 19661379]
33. Prasad P, Bartholomew B. Control of nucleosome movement: To space or not to space nucleosomes? *Epigenetics.* 2010; 5(4):282–286. [PubMed: 20421732]
34. Fischer CJ, Yamada K, Fitzgerald DJ. Kinetic mechanism for single-stranded DNA binding and translocation by *Saccharomyces cerevisiae* Isw2. *Biochemistry.* 2009; 48(13):2960–2968. [PubMed: 19203228]
35. Gangaraju VK, Prasad P, Srour A, Kagalwala MN, Bartholomew B. Conformational changes associated with template commitment in ATP-dependent chromatin remodeling by Isw2. *Mol Cell.* 2009; 35(1):58–69. [PubMed: 19595716]
36. Blosser TR, Yang JG, Stone MD, Narlikar GJ, Zhuang X. Dynamics of nucleosome remodelling by individual ACF complexes. *Nature.* 2009; 462(7276):1022–1027. [PubMed: 20033040] Single-molecule studies on an ACF type remodeler reveal multiple roles of ATP in different functional states of the remodeler and give insights into the processivity and stalling properties of the Swi2/Snf2 domain on nucleosomes during the sliding process.
37. Yamada K, Frouws TD, Angst B, Fitzgerald DJ, DeLuca C, Schimmele K, Sargent DF, Richmond TJ. Structure and mechanism of the chromatin remodelling factor Isw1a. *Nature.* 2011; 472(7344):448–453. [PubMed: 21525927] In this work, a hybrid structural biology approach is used to visualize the interaction of the Isw1a remodeler with a dinucleosome and provides a model for how Isw1 catalyzes nucleosomal spacing. The structures and biochemical data reveal that an Isw1a's motor reels in linker DNA between two nucleosomes until a molecular rule domain between the nucleosomes prevents further movement.
38. Ryan DP, Sundaramoorthy R, Martin D, Singh V, Owen-Hughes T. The DNA-binding domain of the Chd1 chromatin-remodelling enzyme contains sant and slide domains. *EMBO J.* 2011; 30(13):2596–2609. [PubMed: 21623345] This paper addresses the structure and function of the DNA binding domain of CHD type remodelers and reveals that this family contains a SANT-SLIDE domain module similar to ISWI type remodelers. Thus, the two families are mechanistically presumably closely related and could be considered as a single family from this point of view.
39. Sharma A, Jenkins KR, Heroux A, Bowman GD. Crystal structure of the chromo-helicase-DNA-binding protein 1 (Chd1) DNA-binding domain in complex with DNA. *J Biol Chem.* 2011; 286:42099–42104. [PubMed: 22033927] See comments to 38. This work also reveals how DNA interacts with the SANT-SLIDE domains of Chd1, revealing remarkable similarities to DNA recognition by ISWI type remodelers.
40. Flanagan JF, Mi LZ, Chruszcz M, Cymborowski M, Clines KL, Kim Y, Minor W, Rastinejad F, Khorasanizadeh S. Double chromodomains cooperate to recognize the methylated histone h3 tail. *Nature.* 2005; 438(7071):1181–1185. [PubMed: 16372014]
41. Morettini S, Tribus M, Zeilner A, Sebald J, Campo-Fernandez B, Scheran G, Worle H, Podhraski V, Fyodorov DV, Lusser A. The chromodomains of chd1 are critical for enzymatic activity but less important for chromatin localization. *Nucleic Acids Res.* 2010; 39(8):3103–3115. [PubMed: 21177652]

42. Sen P, Ghosh S, Pugh BF, Bartholomew B. A new, highly conserved domain in swi2/snf2 is required for swi/snf remodeling. *Nucleic Acids Res.* 2011; 39(21):9155–9166. [PubMed: 21835776]
43. Dasgupta A, Darst RP, Martin KJ, Afshari CA, Auble DT. Mot1 activates and represses transcription by direct, atpase-dependent mechanisms. *Proc Natl Acad Sci U S A.* 2002; 99(5): 2666–2671. [PubMed: 11880621]
44. Hsu JY, Juven-Gershon T, Marr MT 2nd, Wright KJ, Tjian R, Kadonaga JT. Tbp, mot1, and nc2 establish a regulatory circuit that controls dpe-dependent versus tata-dependent transcription. *Genes Dev.* 2008; 22(17):2353–2358. [PubMed: 18703680]
45. van Werven FJ, van Bakel H, van Teeffelen HA, Altelaar AF, Koerkamp MG, Heck AJ, Holstege FC, Timmers HT. Cooperative action of nc2 and mot1p to regulate tata-binding protein function across the genome. *Genes Dev.* 2008; 22(17):2359–2369. [PubMed: 18703679]
46. Sprouse RO, Karpova TS, Mueller F, Dasgupta A, McNally JG, Auble DT. Regulation of tata-binding protein dynamics in living yeast cells. *Proc Natl Acad Sci U S A.* 2008; 105(36):13304–13308. [PubMed: 18765812]
47. de Graaf P, Mousson F, Geverts B, Scheer E, Tora L, Houtsmuller AB, Timmers HT. Chromatin interaction of tata-binding protein is dynamically regulated in human cells. *J Cell Sci.* 2010; 123(Pt 15):2663–2671. [PubMed: 20627952]
48. Wollmann P, Cui S, Viswanathan R, Berninghausen O, Wells MN, Moldt M, Witte G, Butryn A, Wendler P, Beckmann R, Auble DT, et al. Structure and mechanism of the swi2/snf2 remodeler mot1 in complex with its substrate tbp. *Nature.* 2011; 475(7356):403–407. [PubMed: 21734658]
This paper uses hybrid structural biology methods to derive the first high resolution complex structure of a remodeler with its substrate protein. A combination of X-ray crystallography and electron microscopy reveals the architecture of the Mot1:TBP complex, indicating how ATP dependent groove tracking is structurally coupled to substrate remodeling. An interesting outcome was that Mot1 combines motor and chaperone function to disrupt TBP:DNA complexes.
49. Sprouse RO, Brenowitz M, Auble DT. Snf2/swi2-related atpase mot1 drives displacement of tata-binding protein by gripping DNA. *EMBO J.* 2006; 25(7):1492–1504. [PubMed: 16541100]
50. Avvakumov N, Nourani A, Cote J. Histone chaperones: Modulators of chromatin marks. *Mol Cell.* 2011; 41(5):502–514. [PubMed: 21362547]
51. Leschziner AE. Electron microscopy studies of nucleosome remodelers. *Curr Opin Struct Biol.* 2011; 21:1–10. [PubMed: 21196111]
52. Leitner A, Walzthoeni T, Kahraman A, Herzog F, Rinner O, Beck M, Aebersold R. Probing native protein structures by chemical cross-linking, mass spectrometry, and bioinformatics. *Molecular & cellular proteomics : MCP.* 2010; 9(8):1634–1649.

(a) Domain Structure of SWI/SNF ATPases in their respective complexes

(b)

(c) Selected structures of adjacent domains

(d) Motifs accompanying Swi/Snf ATPases

Motif/Domain	Function
QLQ Gln-Leu-Gln	protein-protein interaction
SnAC Snf ATP Coupling	positively regulates catalytic activity of the ATPase domain
HAND	HAND, SANT and SLIDE bind to chromatin and function as a nucleosome spacing module
SANT SWI3, ADA2, N-Cor, TFIIIB	
SLIDE SANT-like ISWI domain	
AT-Hook	binds AT-rich DNA
CHROMO Chromatin Organisation Modifier	binds methylated lysines in histones and negatively regulates ATPase activity
BROMO	recognizes acetylated residues in histone tails
HSA Helicase SANT associated	recruits actin-related proteins to the remodeler
HEAT Repeats Huntingtin, Elongation factor 3, Protein phosphatase 2A, Lipid Kinase TOR	protein-protein interaction
Macrodomein	PAR (poly(ADP-ribose)) binding
RING finger Really interesting new gene	ubiquitin ligase
HARP HepA related protein	ATP-dependent annealing helicase activity
HIRAN Hip116, Rad5 N-terminal domain	binds DNA

Fig. 1.

Swi2/Snf2 ATPases. (a) Domain organization of selected Swi2/Snf2 ATPases. (b) The Swi2/Snf2 ATPase domain consists of a DExx (RecA like 1) and a HELICc (RecA like 2) domain that together have at least 12 characteristic sequence motifs with roles in nucleic acid and/or nucleotide binding or hydrolysis. (c) Selected structures of adjunct domains in Swi2/Snf2 ATPases in complex with their substrates. (d) Functions of respective domains present in various remodelers.

Fig. 2.

Swi2/Snf2 enzyme architecture and model for nucleosome spacing by ISWI. **(a)** Crystal structure of *E. coli* RapA, the only full length Swi2/Snf2 enzyme structure [10]. The positions of the ATPase lobes 1A and 1B (DEXx domain) as well as 2A and 2B (HELICc) are indicated. **(b)** Crystal structures of *S. cerevisiae* Chd1 domains. Left: SANT (dark green) and SLIDE (light green) domains bound to DNA (brown) [39]. Right: The tandem chromodomains (pink) block the DNA binding site on the Swi2/Snf2 ATPase domain. **(c)** Crystal structure of yeast Isw1a(Δ ATPase) in complex with DNA (brown) [37]. According to the proposed dinucleosome model of remodeling (cf. (d)) the two DNA duplexes bound by the yIoc3 subunit (yellow) and the SANT (dark green) and SLIDE (light green) domains of yIsw1, represent external- and internal-linker DNA, respectively. **(d)** Dinucleosome model of remodeling by yeast Isw1a [37]. Nucleosome spacing by Isw1a is proposed to occur by one of the two schematically represented alternative mechanisms with either flexible linker DNA or flexible protein linker between Isw1's ATPase domain and the HSS (HAND-SAND-SLIDE) assembly. In both scenarios, the Swi2/Snf2 domain slides the mobile nucleosome and pulls together the dinucleosome until the HSS-HL (HSS – helical linker) module between the two nucleosomes prevents further movements.

Fig. 3. Hybrid remodeler - chaperone. **(a)** Crystal structure of the HEAT domain of *Encephalitozoon cuniculi* Mot1 (yellow) in complex with its substrate TBP (grey). Loops of the HEAT domain and the latch are highlighted in pink. **(b)** Highly conserved acidic loops of Mot1 are important for recognition of TBP's convex surface. **(c)** Chaperone activities by the Mot1 latch, which blocks the hydrophobic site on TBP that binds DNA or another TBP molecule in forming dimers. **(d)** A hybrid methods approach. The principle is to combine information from different structural methods, typically X-ray crystallography, homology modeling and electron microscopy. Interactions constraints provided by e.g. crosslinking

studies and mass spectrometry can help put together the individual pieces of high resolution structures in the EM 3D reconstruction.

Fig. 4.

Comparative multistep remodeling models. **(a)** Mot1. (I) TBP surface recognition of Mot1 by HEAT repeat loops (I) activates the ATPase activity of the enzyme and DNA binding by TBP (II). Groove tracking of the ATPase domain generates torque that weakens TBP:DNA interactions, leading to TBP displacement from the DNA (III). The Mot1 latch acts as a chaperone to prevent TBP from binding to DNA and from dimer formation (IV). **(b)** Isw1a and Chd1. (I) Nucleosomal linker-DNA duplexes are bound by the yIoc3 subunit and by the SANT domain of yIsw1. (II) Yeast Chd1's chromodomains regulate the DNA binding site [9]. (III) Remodeling by yeast Isw1a by the dinucleosome spacing model (see Fig. 2d for details). (IV) Nucleosome remodelers may have intrinsic chaperone functions similar to Mot1, however there is a dedicated chaperone network for histones to maintain correct delivery to sites of remodeling and chromatin formation.