SURVEY AND SUMMARY

Snf2 family ATPases and DExx box helicases: differences and unifying concepts from high-resolution crystal structures

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ABSTRACT

Proteins with sequence similarity to the yeast Snf2 protein form a large family of ATPases that act to alter the structure of a diverse range of DNA–protein structures including chromatin. Snf2 family enzymes are related in sequence to DExx box helicases, yet they do not possess helicase activity. Recent biochemical and structural studies suggest that the mechanism by which these enzymes act involves ATP-dependent translocation on DNA. Crystal structures suggest that these enzymes travel along the minor groove, a process that can generate the torque or energy in remodelling processes. We review the recent structural and biochemical findings which suggest a common mechanistic basis underlies the action of many of both Snf2 family and DExx box helicases.

PRIMARY SEQUENCE PROPERTIES OF THE SNF2 FAMILY

The Snf2 protein was originally identified as a result of genetic screens for genes involved in regulating mating type switching (SWI) and sucrose fermentation (Sucrose Non-Fermenting). It was subsequently found to be the catalytic subunit of the multi-subunit SWI/SNF complex that acts to alter chromatin structure (1). Since this time, many proteins have been identified that are related to Snf2p through sequence similarity. The common feature of all such Snf2 family proteins is a region of sequence similarity that includes seven helicase-related sequence motifs that are also found in DExx box helicases (2–5). Helicase-related proteins are classified in superfamilies (SF1, SF2, SF3 etc)

based from the sequence and spacing of these motifs. Snf2 family proteins fall into SF2 whereas DExx box helicases are classified variously into SF1, SF2 and SF3 (6). However, Snf2 family proteins are unusual compared to typical SF2 members, such as DEAD box helicases: the spacing between helicase-related motifs III and IV is significantly elongated, their helicase-related motifs Ia, III, IV, V and VI have a specific and conserved character, and they contain a number of other conserved sequence blocks (2,7). The rapid progress of genome sequencing has revealed a significant breadth and diversity within the Snf2 family. For example, there are 17 genes for Snf2 family proteins in *Saccharomyces cerevisiae* (Table 1) and at least 32 in humans (8).

It has been appreciated for some time that Snf2 family members fall into various groupings or 'subfamilies' (2), and a current survey shows they can be divided into some 24 different subfamilies on the basis of primary sequence of the common helicase-like region (8). For example, the 17 yeast Snf2 family proteins fall into at least 12 distinct subfamilies (Table 1). These subfamilies correlate well with known biological functions, suggesting that the helicase-like region is not a generic motor but is instead highly tuned for its biochemical role. This is supported by the recent observation that the swapping of the helicase-like region between SNF2h and BRG1 has a dominant effect on the activity of the resulting chimeric ATPases (9). For instance, a SNF2h–BRG1 chimera that contains the helicase-related domain of BRG1 and the N- and C-terminal domains of SNF2h exhibits BRG1 like remodelling properties, and vice versa.

Many Snf2 family proteins are part of larger complexes. For instance, the SWI/SNF, RSC, NURF, ACF and INO80 complexes each contain many polypeptides with combined molecular weights in the megadalton range (10–14). However, in several cases the isolated Snf2 family polypeptides alone have biochemical activity, but reduced efficiency

Table 1. Snf2 family genes identified in *S.cerevisiae*

Gene name	Locus ID[a]	Subfamily[b]	Viability of deletion[c]	Proposed roles	Ref
Snf2	YOR290C	Snf2	+	Gene regulation of specific genes (e.g. Suc2, Pho5, mating type loci and mitotic genes)	(83)
Sth1	YIL126W	Snf2	−	Gene regulation, chromosome segregation	(4)
Isw1	YBR245C	Iswi	+	Transcription initiation and elongation	(84)
Isw2	YOR304W	Iswi	+	Repression of early meiotic genes	(85)
Chd1	YER164W	Chd1	+	Transcription elongation and termination	(86)
Unnamed	YFR038W	Lsh	+	Uncharacterized	—
Swr1	YDR334W	Swr1	+	Required to incorporate histone H2A.Z	(87)
Fun30	YAL019W	Etl1	+	Affects chromosome stability	—
Mot1	YPL082C	Mot1	−	Removes TBP from DNA may deliver TBP to non-TATA promoters	(88)
Ino80	YGL150C	Ino80	−	Double-stranded break repair. Affects gene expression.	(14)
Rad26	YJR035W	ERCC6	+	Transcription-coupled nucleotide excision repair, elongation through DNA damage	(36)
Rad54	YGL163C	Rad54	+	Homologous recombination, particularly in double-strand break repair	(47)
Rdh54	YBR073W	Rad54	+	Homologous recombination, particularly in meiotic recombination	(89)
Rad5	YLR032W	Rad5/16	+	Post replication repair	(90)
Rad16	YBR114W	Rad5/16	+	Nucleotide excision repair (excision step)	(91)
Ris1	YOR191W	Ris1	+	Mating type switching	(92)
Unnamed	YLR247C	SHPRH	+	Uncharacterized	—

[a]Official gene name and chromosomal locus as recorded in the *S.cerevisiae* Genome Database (SGD; http://www.yeastgenome.org/).
[b]Subfamily as assigned in (8) and proposed roles as summarized therein.
[c]Viability of deletion as assigned in SGD'.

relative to the intact complexes (15–17). Some Snf2 family polypeptides, such as Chd1p (18) and Rad54p (19) can be purified from yeast and show function without additional proteins. This suggests that in many cases the additional proteins of the large remodeller complexes may function to enhance specificity, processivity or targeting but are not central to the generation of mechanical force.

Snf2 family polypeptides also almost invariably contain one or more domains in addition to the helicase-like region. A number of these extra domains have been shown to interact with surfaces on the nucleosome, often involving post-translational modifications. For example, yeast Snf2p and homologues contain 'bromodomains' which bind acetyl lysine (20), whereas *Drosophila* ISWI and its homologues contain Myb-like 'SANT' domains (21–24). Although SANT domains are structurally related to the DNA-binding domain of Myb, they are functionally diverged to bind to unmodified histone tails (25). Mouse Chd1 and homologues contain chromodomains which bind methyl lysine (26,27) and also nucleic acids (28,29). It has been demonstrated that removing the Myb-like domains of ISWI (22) or the chromodomains of Mi-2 (29) compromises remodelling *in vitro*.

BIOCHEMICAL ACTIVITIES OF SNF2 PROTEINS

Historically, the involvement of the archetypal yeast Snf2 protein in chromatin remodelling has led to assumptions that all Snf2 family proteins are chromatin remodellers. This is unlikely to be true. Although Snf2 family proteins are ubiquitous in eukaryotes, they are also found in a significant number of the sequenced prokaryotic and archaeal genomes, which lack eukaryotic-type chromatin. These archaeal and prokaryotic relatives comprise at least two significant groups. The more distant of these, on the border

of the Snf2 family, includes *Escherichia coli* RapA (also known as HepA), which has been shown to be involved in polymerase recycling under high salt conditions when DNA is more highly supercoiled (30). Similarly, a number of eukaryotic Snf2 family members have functions which do not seem to be directly linked to chromatin. For example, yeast Mot1p acts to displace the transcription factor TBP from DNA (31,32), the transcription-coupled repair factor Cockayne syndrome protein B rescues RNA polymerase that is stalled at DNA lesions (33–36), and the Rad16 complex facilitates nucleotide excision repair (37).

Despite the diversity of biochemical activity, the presence of the conserved Snf2 family ATPase domain 'engine' suggests that shared mechanistic features may underlie the way by which these proteins act. In other DExx box enzymes the helicase-related motifs act to transduce the energy of ATP-hydrolysis into a conformational stress required for the remodelling of nucleic acid or protein–nucleic acid structure. Like many helicases, ATP-hydrolysis activity of Snf2 family enzymes is stimulated by DNA or DNA–protein substrates (15,31,38–41). However, a longstanding puzzle stems from the fact that Snf2 family enzymes do not show the DNA unwinding activity which defines helicases. Recently, a variety of biochemical and structural results indicate that instead of duplex unwinding, Snf2 family enzymes use the energy of ATP-hydrolysis to translocate on duplex DNA by a mechanism that does not require strand-separation (17,42–45). In this respect, they may act similarly to other members of SF2, such as the translocating subunits of type I restriction enzymes (46).

Significantly, the passage of a translocase along DNA provides a direct means of altering DNA–protein contacts. In addition, because the path of DNA is helical, translocation is also likely to be associated with rotation of DNA, which

could indirectly manipulate protein–DNA interactions. Consistent with this, several Snf2 family proteins have been shown to generate torsion in DNA (37,43,45,47–49).

REVIEW OF HELICASE STRUCTURE AND MECHANISM

Are there common mechanistic principles linking Snf2 family enzymes and DExx box helicases? Amongst the various groups of proteins within the SF1 and SF2 superfamilies of helicase-like proteins, a great deal of progress has recently been made in understanding the mechanism of DNA translocation and unwinding by DExx box helicases. In particular, detailed structural knowledge of the interaction of helicases with DNA has been obtained for the SF1 helicase *Bacillus stearothermophilus* PcrA in complex with a 3′-tail partial duplex DNA and in presence or absence of AMPNP (50,51), the SF2 helicase NS3 from Hepatitis C virus in complex with deoxyuridine octamer (dU$_8$) (52), the SF2 *Thermatoga maritima* RecG in complex with a three way junction (53), the SF2 DEAD box RNA helicase VASA in complex with ssRNA (54) and the SF1 related helicase complex RecBCD in complex with a partially unwound dsDNA substrate duplex (55). These crystal structures revealed an underlying common structural fold and a modular structural organization (6), and showed that these helicases typically consist of a DNA translocation module linked to a strand-separation module (56). A notable exception is VASA, where the DExx box ATPase module appears to bend DNA instead of translocating on it. This ATP induced bending is proposed to separate the two strands (54). The translocation module is highly conserved among helicases and consists of two RecA-like domains, plus their associated structural elements (domain 1 and 2) (50). Residues of the seven helicase-related motifs line the interdomain cleft separating the two RecA-like domains and are involved in ATP-binding/hydrolysis as well as DNA-binding (57,58). In addition, ATP-binding into the interface cleft has been shown to induce a conformational change that is linked to DNA translocation (51).

The available structures suggest that nucleic acids bind across the interface of the two RecA-like domains (51,52,54). Depending on the nature of the particular helicase, the translocation module can bind duplex DNA (e.g. RecG) or single-stranded nucleic acids (e.g. NS3). Biochemical analysis suggests that nucleic acid translocation and duplex DNA unwinding are separable processes in the reaction cycle of helicases (53,59,60). Consistent with earlier observations, recent structures of the SF1 helicase UvrD highlight the fact that the structural movements of SF1 helicase domains, which underlie the translocation and unwinding processes, are closely correlated (W. Yang, personal communication). Together, the structural and biochemical data are interpreted as an 'inchworm' mechanism for monomeric SF1 helicases (51,56,59). In this model, two DNA-binding sites alternate in high affinity for DNA and move the enzyme along the DNA in a process that resembles the movement of an inchworm. During translocation, ATP-driven conformational changes between the two RecA-like domains result in a closure of the cleft between them, advancing DNA by one base at a time, as judged from the PcrA–DNA complex crystal

structures (51). The closed arrangement of the domains is largely stabilized by interaction of a conserved arginine residue in helicase-related motif VI with the γ-phosphate of the bound ATP. This arginine is also necessary for ATP-hydrolysis (51,57). ATP-dependent closure and opening of the active site cleft could result in an alternating sliding of one domain, while the other domain tightly grips the DNA and serves as an anchor to generate inchworm-like progress. Recent biochemical observations indicate that within this cycle, single-strand binding provides a large part of the energy for unwinding, while ATP-binding weakens the interaction with DNA and allows the advance on the product strand (61). However, the detailed mechanism of helicases can be more complex, involving cycles of rapid advancement by many bases followed by pausing (62).

The fold similarity suggests that SF2 helicases might in principle function in an analogous way. A mechanism similar to that of SF1 enzymes was postulated for the SF2 helicases NS3 and RecG (53,63). The crystal structure of NS3 revealed that both RecA-like domains contact the single-stranded DNA (52). ATP mediated weakening of the DNA-binding strength of domain 1, followed by a change of the relative orientation of domain 1 with respect to domain 2 that tightly grips DNA via Val432, would result in concomitant translocation of the DNA in NS3. This is consistent with ATP-dependent changes of the DNA-binding properties of NS3, which become weaker in the presence of ATP (64).

However, there are also important differences between SF1 and SF2 helicases. A closer inspection of the crystal structure of SF1 and SF2 helicases in complex with DNA reveals a divergence in the mode of DNA-binding. For instance, PcrA binds ssDNA mainly through hydrophobic contacts, which are formed by aromatic side chains that stack against the DNA base moieties. This mode of DNA-binding allows the enzyme grip tightly to ssDNA. In contrast, the SF2 helicases NS3 and VASA bind ssDNA predominantly by recognition of the DNA phosphate backbone (54,56,65). For high affinity ssDNA-binding, NS3 possesses a specialized domain that is attached to domain 1 and specifically interacts with the bases of single-strand nucleic acids. On the other hand, the SF2 helicase RecG lacks the ssDNA-binding domain of NS3 and translocates double-strand DNA (63). Unfortunately, the DNA in the RecG crystal structure does not extend as far as the RecA-like domains. Biochemical studies of nucleic acid recognition by the SF2 helicase NPH-II suggest that SF2 helicases maintain continuous contact with the phosphodiester linkage of one substrate strand (65). This suggests a 'molecular wire stripper' like mechanism, where one domain maintains contact with the substrate strand during the cycles of tight binding and sliding, while the other domain grabs and releases the nucleic acid strand. Overall, the generation of force to move the DNA phosphate backbone across the surface of SF2 helicases is much less understood than the pulling of DNA bases by PcrA.

THE STRUCTURE OF THE SNF2 ATPASE DOMAIN

Snf2 family proteins were placed within SF2 in the original studies of Gorbalenya and Koonin (66). In the last decade, biochemical characterization and recent crystal structures
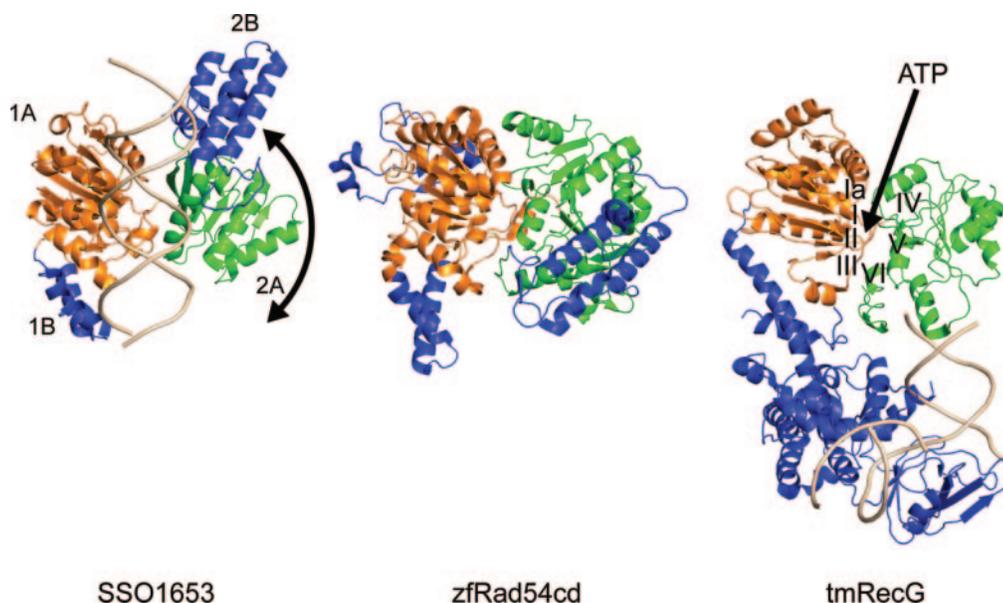
Figure 1. Comparison of Structures of Snf2 family enzymes and the RecG helicase. Structural comparison of the *S.solfataricus* (sso) SSO1653 catalytic domain [cd, (43), the zebrafish (zf) Rad54 cd (68) and *T.maritima* (tm) RecG (53). The three crystal structures are shown as ribbon models with highlighted secondary structures. DNA molecules bound to SSO1653 and tmRecG are shown as brown ribbon model. The two RecA-like domains (1A: orange, 2A: green) are shared across DExx box ATPases, and form the ATP-binding site in their interface cleft. The location of the ATP-binding site, as well as locations of the seven conserved helicase-related ATPase/DNA-binding motifs (Ia,I,II,III,IV,V,VI) are indicated in tmRecG. The two RecA-like domains interact with additional domains (1B and 2B: blue) that are suggested to convert ATP-driven rearrangements of 1A and 2A into the translocase or DNA unwinding function. For instance, these domains bind to the replication fork substrate in RecG, which is dragged like a plough through DNA by the action of the translocase module. The role of the helical domains of Snf2 family enzymes is not as well understood, but they could play a role in advancing the enzyme by ATP-driven conformational changes. Note that domain 2 of SSO1653 (2A and 2B) is flipped by 180° with respect to more typical conformations found in zfRad54cd and RecG (double arrow). This flip could represent an open conformation during substrate uptake.

have suggested that the catalytic core of the Snf2 family enzymes is a structure and sequence unspecific translocase for duplex DNA (17,42–44,67). The two new crystal structures of zebrafish Rad54 and the archaeal *Sulfolobus solfataricus* SSO1653 gene product in complex with a dsDNA substrate have revealed the first structural insights into this motor (43,68). The crystallized fragments are active ATPases that can translocate on DNA, introduce superhelical tension in DNA, and/or remodel chromatin. Additional high-resolution structural information is also available for the nucleosome binding domain of ISWI (23), although the nature of the interaction between this region and the catalytic motor is still unknown.

The crystal structures of the catalytic domains from the two Snf2 family members revealed that the enzymes possess two domains (domain 1 and 2), each containing a core RecA-like fold (1A and 2A), fused to Snf2 family specific helical domains (1B and 2B) (Figure 1). The two RecA-like domains are related to the equivalent region of the DExx box helicases and contain the seven characteristic helicase-related motifs. This similarity suggests that Snf2 family ATPases and DExx box helicases possess a related ATP-hydrolysis mechanism and probably exhibit related ATP-driven conformational changes.

Comparison of the Zebrafish and *S.solfataricus* structures revealed a highly similar fold, but with large conformational difference in the orientation of the two RecA-like domains (43,68): the orientation of the second RecA-like domain with respect to the first differs by 180° between the crystal structures. The nature of this conformational difference is currently unclear. Biochemical analysis of mutant SSO1653 suggests that the second RecA-like domain can adopt a domain orientation that is similar to zebrafish Rad54 during the ATP-hydrolysis cycle. Only in the conventional conformation are all conserved helicase-related sequence motifs located in the ATP-binding cleft. Such a conformational change is also consistent with data for yeast Mot1p, where a mutation in the proposed pivot point of the two RecA-like domains abolishes ATP-hydrolysis activity (69). Whether this proposed large scale conformational change is part of the functional ATP-binding and hydrolysis cycle of SWI2/SNF2 enzymes or represents a DNA loading conformation, is not known. The recently determined structure of TRCF also has a SF2 helicase-like region related to RecG. In it, the second RecA-like domain is rotated by some 90° relative to the conventional orientation (70). This suggests a highly flexible domain connection between the two RecA-like domains of SF2 ATPases. In this respect, it might be interesting to probe conformational sub-strates of Snf2 family enzymes in the presence of various nucleotides by proteolysis or fluorescence resonance energy transfer experiments.

In any case, both crystallographic studies revealed the presence of the typical seven helicase-related motifs (43,68). Mutational analysis has revealed the significance of these motifs in remodeling processes or translocation (38,43,68). Interestingly, the structural and mutational analysis also suggested a variety of additional motifs that could be involved in dsDNA stimulated ATPase activity [IIa in Ref. (43)] or ATP-driven translocation [IVa in Ref. (43)].

Motif IVa, also dubbed the QxxR motif, has recently been identified as a nucleic acid phosphate-binding motif in the VASA DEAD box RNA helicase (54).

The structure of the SSO1653 region was determined bound to duplex DNA and provides a first detailed structural insight into how the Snf2 family ATPase domain interacts with DNA (43). Duplex DNA binds along the domain 1A:domain 2A interface in a position that is in principle well suited for DNA translocation by ATP-driven conformational changes between domains 1A and 2A. Comparison with the SF2 helicase RecG (53) suggests that the translocase module of RecG could interact with DNA in an analogous way (Figure 1). DNA binds to domain 1 with both phosphate backbone chains of the minor groove. The total footprint of both phosphate chains amounts to about 6–7 nt, comparable with footprint of ssDNA bound to helicases. The Snf2 family specific domains 1B and 2B might, by analogy with the accessory domains of the SF1 helicases, play a key role in DNA translocation. These domains do not directly contact DNA in the available crystal structure so their precise role is unclear. However, mutations in domain 2B interfere with catalytic activity (43).

Duplex DNA is bound to the *S.solfataricus* enzyme in B-form conformation without evidence for strand-separation. Strand-separating helicases have been found to contain 'wedge' or 'pin'-like features that force the duplex apart, or to bind the nucleic acids in a sharp bend, which is incompatible with base pairing. The lack of either type of duplex-destabilizing region in Snf2 family enzymes parallels the lack of helicase activity. Instead, the minor groove binding of B-form DNA suggests that Snf2 family enzymes probably track on DNA without strand-separation activity. This is similar to the non-helicase SF2 motor of a type I restriction enzyme, which can move on DNA with cross-linked strands (46).

UNIFIED MECHANISM FOR SNF2 ENZYMES AND DEXX BOX HELICASES

What can we infer about the mechanism of DNA translocation by Snf2 family enzymes from DExx box helicases? The remarkable structural and topological similarities of the core architecture in the two RecA-like domains suggest that Snf2 family ATPases and SF2 helicases share a fundamentally similar ATP-hydrolysis mechanism (Figure 2). In particular, the structure and arrangement of the ATP and Mg^{2+} binding motifs I (Walker A), II (Walker B) and VI correspond closely to equivalent motifs of other DExx box enzymes. Importantly, an invariant arginine residue of motif VI that conducts domain communication and senses ATP-hydrolysis is conserved in Snf2 family enzymes (39).

DNA binds to the SSO1653 Snf2 family structure along a similar surface path to both SF1 and SF2 helicases (43,51,52). Most intriguingly, the 3′–5′ strand of the DNA duplex bound to the SSO1653 structure overlays very well with the 3′–5′ oligo(dU) strand bound to the DNA helicase PcrA and the RNA helicases NS3 and VASA (43,52,54). Therefore, it is likely that ATP-driven opening and closing of the cleft between the RecA-like domains transports the 3′–5′ strand in helicases and Snf2 enzymes in an analogous manner. In support of such a mechanistic similarity, biochemical studies
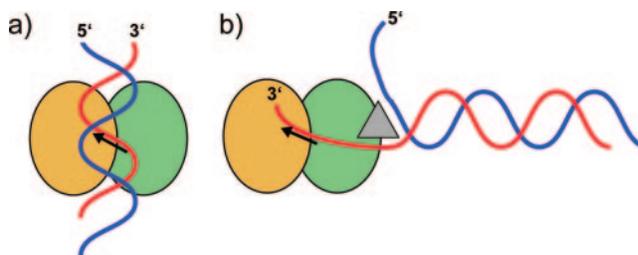

Figure 2. Unified mechanism of Snf2 family ATPases and DExx box helicases. Schematic comparison of (**A**) dsDNA translocases (e.g. Snf2) and (**B**) ssDNA translocases (e.g. PcrA and NS3 helicases). Both enzyme families contain a conserved RecA-like domain core (orange/green), but differ in other subunits (data not shown). Helicases transport product ssDNA and often contain an upstream DNA unwinding element (grey triangle). In contrast, Snf2 family enzymes also recognize the 5′–3′ strand (blue) and track along the minor groove. Despite many functional differences, however, both enzymes families bind the 3′–5′ strands at an equivalent site across the two RecA-like domains, indicating that ATP-driven conformational changes transport DNA substrates via the 3′–5′ strands in analogous ways (arrows).

indicated that the integrity of this 3′–5′ strand is more important for activity, while gaps in the 5′–3′ strand can be tolerated during translocation (17,71). Recent single molecule and bulk solution measurements on the dsDNA motor protein EcoR124I, an enzyme related to Snf2 ATPases, also indicate that an intact 3′–5′ strand is required for translocation, whereas the 5′–3′ strand only assists in processivity (46).

We do not currently know how the force is generated to propel DNA along its binding groove in Snf2 family enzymes. Based on present insights, several scenarios can be envisioned to guide experiments. For example, in analogy to the inchworm model, alternating high and low affinity binding sites in domain 1 and 2 could transport DNA along the DNA backbone. However, biochemical data show that domain 2 of SSO1653 has no significant DNA-binding affinity by itself, whereas domain 1 readily binds DNA (43). Alternatively, ATP-driven closure of the active site cleft might push on upstream DNA. Such a push could for instance propel the enzyme forward in analogy to the punting technique of fishermen (K. Theis, personal communication). Alternative mechanisms are also consistent with current information, such as the formation of small loops in the 3′–5′ strands (46). The situation is probably more complex. For instance, an ADP-driven conformational change has been identified in the Isw2 complex (72). Whether similar ADP dependent conformational changes exist in other Snf2 enzymes, needs to be clarified by further biochemical analysis. In addition, the crystallographic analysis is only a first snapshot, and structures of Snf2 enzymes in complex with DNA plus ADP or ATP are needed to provide further insights into the DNA transport mechanism.

HOW DO REMODELLING MACHINES WORK?

Although, all chromatin remodelling complexes have a Snf2 family polypeptide at their core, they contain divergent subunit compositions and exhibit many different functional roles *in vivo* and *in vitro*. For example, the action of different complexes can result in nucleosome sliding *in cis*, histone transfer *in trans* and histone variant exchange. Support for the idea that remodelling complexes use ATP-dependent translocation

on duplex DNA to generate the force in remodelling processes comes from recent single molecule biophysical analysis of the RSC complex (45). In principle, translocation along DNA by a motor domain could disrupt DNA–protein complexes or slide nucleosomes simply by collision. Such a sliding effect has been found to occur when polymerases collide with nucleosomes (73). However, the action of specialized remodellers is probably much more complex and involves the specific positioning of the motor domain relative to the remodelled substrate. For example, some of the larger chromatin remodeling complexes are big enough to encapsulate nucleosomes or bind to substrates via multiple binding sites (74–76). In these cases, differences in the location and orientation with which the translocating motor engages DNA may influence the outcome of remodeling reactions. For example, recent studies point to an important role for DNA contacts within the nucleosome for action of the SWI/SNF and RSC complexes (71,77) whereas nucleosome spacing enzymes, such as Isw2 make contact both with nucleosomes and the adjacent DNA (77,78).

Once engaged, translocation of the motor domain along the minor groove will necessarily lead to a pulling or pushing force due to the translocation component, and also to a twisting force due to the rotation component of minor groove tracking. This would be expected to result in the application of a force between the ATPase motor and other contacts restraining the enzyme relative to the DNA or remodelled substrate. Such restraints could be provided by additional substrate binding domains of the remodeller. For instance, the SANT and SLIDE domains could anchor ISWI to nucleosomes (23). Likewise, the N-terminal region of Mot1 binds the TATA box binding (TBP) protein (31). In the case of nucleosome sliding, the force created by the motor domains could for example result in the generation of DNA loops that peel DNA away from the surface of the histone octamer. Alternatively, the rotation of DNA could lead to an altered twist that disrupts the histone–DNA complex. Diffusion of these types of distortion around the histone octamer provides an attractive means by which these enzymes might alter chromatin structure (1). In this way, transient or non-processive alterations may be sufficient to cause persistent changes to chromatin structure. While it is possible to conceive how directed DNA translocation may underlie the mechanism by which many ATP-dependent chromatin remodelling enzymes act, this need not necessarily be the case for all Snf2 family proteins. For example, Mot1p has no detectable DNA translocase activity, but disrupts TBP–DNA complexes (79). The CSB protein has been observed to wrap DNA around itself in an ATP-dependent reaction (80). Further investigation will be required to determine how the mechanisms by which these proteins act are related to their chromatin remodeling siblings!

CONCLUSIONS AND OUTLOOK

Structural homology of the two RecA-like domains suggest that Snf2 family dsDNA translocases and SF2 helicases use related mechanisms for ATP-driven transport of their nucleic acid substrates across the active site cleft. Based on these structures, a model of dsDNA translocation by Snf2 family

enzymes and possibly other dsDNA translocases can be envisioned that is similar to the ssDNA translocation by DExx box helicases. However, detailed insights how ATP-driven conformational changes propel duplex DNA along the active site of the Snf2 ATPase domain are still missing and need to be addressed in future studies. In this regard, it will be interesting to probe conformational changes of Snf2 enzymes at the single molecule level, or to probe the precise structure of DNA during the translocation process. Recent breakthroughs in the application of this technique to remodelling complexes and helicases can reveal a wealth of mechanistic insights how these enzymes move on DNA (45,81,82). With structures of Snf2 enzymes in complex with ATP or ADP, and analysis of conformational changes using tools like fluorescence energy resonance transfer or small angle solution scattering, we should be able to dissect the conformational substates of remodellers and the mechanistic coupling of ATP-binding with DNA transport.

In addition, we are only at the beginning of our understanding of how the force generated by the Snf2 translocase module is used by complex multidomain remodelling factors. For instance, more detailed studies on the reaction cycle of Snf2 family enzymes, how they engage with their substrates and the nature and role of conformational changes within the RecA-like domains are needed. In this regard, what is the role of the various domains that flank the translocase module of Snf2 family enzymes? Do they target the enzyme to particular places on the genome, do they grip the substrate to provide a handle for the action of the ATPase module, or do they do both? Ultimately, we need to understand how DNA tracking by the translocase domains generates the diverse range of macromolecular changes in substrate DNA–protein complexes during the course of remodelling reactions.

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