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Journal
Annual Review of Biophysics, 45(1)

ISSN
1936-122X

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Publication Date
2016-07-05

DOI
10.1146/annurev-biophys-051013-022819

Peer reviewed

HHS Public Access

Author manuscript

Annu Rev Biophys. Author manuscript; available in PMC 2022 June 01.

Published in final edited form as:

Annu Rev Biophys. 2016 July 05; 45: 153–181. doi:10.1146/annurev-biophys-051013-022819.

Mechanisms of ATP-Dependent Chromatin Remodeling Motors

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Abstract

Chromatin remodeling motors play essential roles in all DNA-based processes. These motors catalyze diverse outcomes ranging from sliding the smallest units of chromatin, known as nucleosomes, to completely disassembling chromatin. The broad range of actions carried out by these motors on the complex template presented by chromatin raises many stimulating mechanistic questions. Other well-studied nucleic acid motors provide examples of the depth of mechanistic understanding that is achievable from detailed biophysical studies. We use these studies as a guiding framework to discuss the current state of knowledge of chromatin remodeling mechanisms and highlight exciting open questions that would continue to benefit from biophysical analyses.

Keywords

molecular motors; histones; nucleosome; ISWI; SWR; INO80; SWI/SNF; ATPase

INTRODUCTION

Packaging of DNA into chromatin provides the cell with a means of regulating all processes that require access to the genetic material. The basic building block of chromatin consists of a nucleosome, which contains ~150 base pairs (bp) of DNA wrapped around an octamer composed of two copies each of histones H2A, H2B, H3, and H4 (67). Multiple nucleosomes further interact with each other and other cellular factors to achieve higher-order compaction (83). Because the primary consequences of nucleosome formation are the compaction and occlusion of the wrapped DNA, in early years chromatin was thought of as mainly a packaging material that presented a barrier to DNA-based processes (68). However, the discovery over the last two decades of a variety of enzymatic complexes, which catalyze both covalent and conformational changes in chromatin, has helped shape current views that chromatin also plays sophisticated regulatory roles (19).

Noncovalent conformational changes of chromatin are catalyzed by chromatin remodeling motors that use the energy of ATP hydrolysis to alter nucleosome structure (10, 93). These motors carry out diverse transformations on chromatin and play central roles in all nuclear

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DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

processes including transcription, DNA replication and repair, and recombination. The recent identification of mutations in these motors as drivers of various cancers underscores the centrality of their biological roles (42, 63). In this review, we first summarize case studies of other well-studied nucleic acid motors to highlight general mechanistic principles and then use these principles to discuss the current state of understanding of chromatin remodeling motors. We address the unique challenges faced by chromatin remodeling motors and the regulatory principles these motors may use to overcome these challenges.

NUCLEIC ACID REMODELING ATPases

Molecular motors play an integral role in nucleic acid biology. These motors include polymerases, which catalyze the formation of covalent bonds during DNA replication and transcription, as well as motors that catalyze noncovalent rearrangements of their nucleic acid substrates. Here we focus on the second class of motors, which are classified under the SF1 and SF2 superfamilies of ATPases (37). Members of the SF1 superfamily are bona fide helicases that processively translocate along single-stranded DNA, whereas members of the SF2 family show a greater diversity of activities and include ATP-dependent chromatin remodeling motors (55). The core feature of both families is an ATPase domain containing two RecA-like lobes (see sidebar Structure and Function of RecA and Figure 1a). A closer look at the RecA-fold architecture provides structural clues to how these motors couple ATP hydrolysis to conformational changes.

The two copies of the RecA fold in the SF1 and SF2 superfamilies are asymmetric. The first copy retains the well-conserved ATP-binding (Walker A) and ATP-hydrolysis (Walker B) motifs found in the ancestral RecA protein, whereas the second copy has lost these motifs and instead supplies an arginine finger motif. These arginine fingers play a key role in ATP hydrolysis by stabilizing the transition state. A consequence of this split architecture is that closure of the two lobes is necessary for efficient ATP hydrolysis, providing a mechanism for these motors to couple ATP hydrolysis to movement (121). In current models of SF1 and SF2 motors, this movement of the RecA lobes relative to one another lies at the core of these motors' basic activities of translocation and rearrangement of nucleic acid structures. This general architecture of the ATPase domain is then further elaborated in different classes of motors depending on the type of reaction catalyzed (Figure 1b) (37). The elaborations include (*a*) a diverse set of N- and C-terminal domains that extend beyond the RecA-like lobes and (*b*) sequence insertions and variations within the RecA-like lobes. These extra domains, insertions, and variations regulate the ATPase domain of the motor and also allow for interactions with accessory subunits, thus providing an additional layer of specificity. The structural diversity of these ATPases and their respective accessory subunits is the basis for the functional diversity in the rearrangements they can catalyze.

Efforts to achieve a mechanistic understanding of nucleic acid motors raise three major questions:

1. 1. How is the ATPase cycle coordinated with distortion of the substrate?

2. 2. How is the activity of the core ATPase domain modulated either by accessory domains on the same protein or by accessory subunits as part of a larger complex?

3. 3. How does the motor achieve specificity for its biological substrate and directionality of output?

In this section, we illustrate each of these concepts through three case studies of well-studied nucleic acid motors. In the next section, we then discuss how some of the basic strategies used in these case studies help us understand the more complex molecular transformations catalyzed by chromatin remodeling motors.

Mss116

Mss116 provides an excellent example of how a motor couples ATP binding and hydrolysis to distortion of its substrate. Mss116 (Figure 1c) is an RNA chaperone that catalyzes folding of group I and II introns in vivo and is a member of the DEAD-box family of SF2 motors (57). The main activity of Mss116 is to unwind short (<15 bases) duplex RNAs without translocating (reviewed in 61). ATP and double-stranded RNA each bind to a single RecA lobe, which stabilizes an activated closed conformation of the enzyme (87). The structure of this closed conformation indicates that the ATPase physically bends the RNA duplex, destabilizing the interactions between the two single strands of RNA (32). After one strand of RNA is released, ATP is hydrolyzed, allowing the second strand of RNA to be released along with ADP and free phosphate. Thus, physical unwinding of the substrate is coupled to ATP binding, and ATP hydrolysis promotes substrate release and recycling of the enzyme (61).

RecBCD

The RecBCD system exemplifies how regulation of a motor can be achieved in a multisubunit complex. RecBCD (Figure 1d) is a three-subunit machine from bacteria that facilitates double-strand break repair by catalyzing the first steps in homologous recombination (140). The RecBCD complex binds to the end of a double-strand break, then unwinds and degrades the DNA as it translocates away from the break. Once the complex reaches a sequence-specific crossover hotspot instigator (Chi) site, the complex alters its activity to form a $3'$ overhang. This overhang serves as a platform for subsequent loading of RecA and the start of homologous recombination. RecB and RecD are both SF1-type helicases but have opposite polarities ($3'-5'$ and $5'-3'$, respectively), allowing them to translocate in the same direction along double-stranded DNA. Two types of subunit coordination are responsible for the biological activity of the RecBCD machine. The first is an enhancement of processivity of RecB by RecC (147). RecB itself is a weak helicase, but the crystal structure of RecBCD (Figure 1d) reveals that RecC contributes a critical pin motif, which prevents reannealing of the duplex as RecB separates the DNA strands (120). The second type of coordination relates to the regulation of the nuclease activity of RecB by the Chi recognition motif of RecC. The nuclease is connected to the rest of the motor via a long linker, allowing it the flexibility to cut either the $3'$ or the $5'$ end of the DNA. However, once the complex reaches a Chi site, RecC binds tightly to the $3'$ end of this site, protecting it from nuclease digestion. This protection allows preferential degradation of the

5′ end, creating a 3′ overhang that enables RecA to load onto the exposed single-stranded DNA (140).

Mot1

Modifier of transcription 1 (Mot1) exemplifies how a motor achieves specificity and drives directionality of the reaction output. Mot1 (Figure 1e) is an SF2 motor that facilitates eukaryotic transcriptional regulation by removing a transcriptional activator, TATA-binding protein (TBP), from DNA upstream of promoters, enabling its redistribution across the genome (7, 8, 148). TBP binds DNA in a high-affinity, long-lived complex, hence the necessity of a specialized ATPase to remove it (91, 100).

Mot1 consists of an ATPase domain, containing the two conserved RecA-like lobes, and a large N-terminal domain that binds TBP through a series of HEAT repeats (15, 141). Unlike the other nucleic acid motors discussed above, Mot1 has negligible DNA-binding affinity, and its ATPase activity is not stimulated by DNA but instead by TBP (2, 141). Interestingly, an N-terminal deletion that isolates the ATPase domain can weakly bind DNA, suggesting an autoinhibitory role for the N-terminal region (91). This autoinhibitory feature may contribute to Mot1's specificity by, for example, sequestering the ATPase domain until a TBP-DNA complex has been bound by the N-terminus. Although details of the mechanism by which Mot1 couples ATP hydrolysis to TBP removal remain unclear, the Mot1:TBP crystal structure sheds light on how Mot1 achieves directionality, namely through an alpha helix connected to the N-terminal TBP binding domain by a flexible linker called the latch. This latch binds the DNA-binding surface of TBP exposed by the action of Mot1. The latch thus prevents TBP-DNA rebinding much as the pin in RecC prevents the reassociation of the nucleic acid strands separated by RecB (15, 141).

A universal theme that emerges from these case studies is the ability of nucleic acid motors to act directionally. This directionality can be defined in terms of the direction of motor movement, such as the 3′−5′ directionality of RecB translocation versus the 5′−3′ directionality of RecD translocation on single-stranded DNA. Directionality of output, however, refers to the ability of nucleic acid motors to promote the forward reaction over the reverse reaction. This type of directionality requires specificity for the substrate over the product of the reaction, as well as the coupling of this specificity to the irreversibility of ATP hydrolysis. In the next section, we explore this theme both mechanistically and structurally in the context of chromatin remodeling motors. Unlike the motors just described, these chromatin remodeling motors must act on a vastly more complicated substrate, the nucleosome (Figure 2a).

ACTIVITIES OF REMODELING COMPLEXES

Chromatin remodeling motors are currently classified into four families on the basis of homology within the ATPase subunits: the ISWI family, CHD1 family, INO80 family, and SWI/SNF family (19). These complexes have overlapping roles in key nuclear processes such as transcriptional regulation and DNA repair, yet catalyze distinct transformations of nucleosomes, underscoring the complexity of regulating these processes in the context of chromatin. The activities catalyzed by these complexes include sliding an intact histone

octamer, disassembling the histone octamer, exchanging histone variants, and altering nucleosome conformation. Chromatin remodelers and their diverse activities have been the topic of a number of excellent reviews (10, 19, 93, 129). Here we build on these reviews to address how remodeling motors elaborate on the basic RecA architecture introduced in the previous section to achieve a diversity of outputs. We begin by introducing the biophysical properties of a nucleosome, focusing on uncatalyzed transitions between the canonical structure and other states that are accessible at physiological temperatures (Figure 2b). We then discuss how remodeling motors might alter the relative populations of accessible chromatin states or induce new chromatin states to generate a defined product.

INTRINSIC BIOPHYSICAL PROPERTIES OF A NUCLEOSOME

Under physiological conditions, nucleosome structure is stabilized by electrostatic interactions between the phosphate backbone of DNA and positively charged residues on histones (84). The histones make few specific interactions with the DNA bases. However, wrapping of the DNA around the octamer requires bending of the DNA and results in a different type of sequence specificity. For example, GC base pairs favor major groove compression, whereas AT base pairs favor minor groove compression. Consistent with these properties, DNA sequences that contain GC and AT base pairs spaced 5 bp apart show increased affinity for the histone octamer compared with sequences without such periodicity (118, 139).

The nucleosome structure allows for a diverse set of alternative states that are lowly populated under physiological conditions (Figure 2b). For example, the DNA wrapped around the histone octamer can transiently unwrap on the millisecond timescale, although the equilibrium lies in favor of the wrapped state (3, 72, 103). DNA sites closer to the dyad unwrap with a millionfold lower probability than sites closer to the entry/exit site of a nucleosome. In contrast to DNA unwrapping, sliding of the octamer with respect to the DNA is very slow under physiological conditions but can be detected by raising temperature (40). Finally, increasing salt has been shown to promote sequential loss of the H2A/H2B dimer followed by the H3/H4 tetramer or to promote a state in which the H2A/H2B dimer remains attached to the DNA but is detached from the H3/H4 tetramer (13). These intrinsic properties can be further regulated by posttranslational modifications of the canonical histone proteins and by replacement with histone variants (5). Such elaborations of the basic nucleosome structure play a major role in conferring biological specificity on the action of chromatin remodeling motors through recruitment and allosteric effects.

Uncatalyzed nucleosomal transformations are qualitatively similar to those achieved by chromatin remodeling motors and provide a conceptual framework to ask if and how remodeling motors exploit these lowly populated states to drive specific outcomes (103). This concept is best illustrated by a free-energy diagram (Figure 2d), which shows that a remodeling motor may either stabilize physiologically accessible transition states or create new states by lowering the barriers for alternate paths. Below, we focus on three distinct classes of remodeling motors that range from those that slide the histone octamer to those that can generate many of the lowly populated nucleosomal states described above.

ISWI FAMILY

The ISWI chromatin remodelers represent one of the best understood families in terms of their mechanism of action (Figure 3). ISWI complexes play broad and essential roles in almost every genetic process, including critical roles in transcriptional activation and repression, DNA repair, and DNA replication (25). In vitro, ISWI complexes have been shown to have two types of catalytic activities that can help explain their biological roles: nucleosome assembly and nucleosome sliding (49, 60, 70). The latter is the main focus of this section.

Basic In Vitro Action: Nucleosome Sliding

Nucleosome sliding entails the movement of a histone octamer with respect to DNA without dissociation of the histones. On mononucleosome templates assembled on short pieces of DNA, most ISWI complexes move the octamer away from DNA ends (1, 34, 124). Some ISWI family members can further equalize the DNA on either side of these mononucleosomes to generate centered nucleosomes (70). Although each of these complexes usually consists of one ATPase subunit and up to four accessory subunits, most of the nucleosome sliding activities of ISWI complexes can be recapitulated by the respective ATPase subunits (1, 24). ISWI motors can use this single basic activity—nucleosome sliding—to differentially regulate gene expression. For example, the nucleosome-centering activity of ACF (Figure 3a), a heterodimer of the ISWI ATPase and the accessory subunit Acf1, is thought to be related to its ability to generate evenly spaced nucleosomal arrays, which are associated with transcriptionally repressed heterochromatin in vivo (41, 60, 135). In contrast, the NURF complex, a multisubunit ISWI complex, helps activate transcription in vivo by sliding nucleosomes near promoters to make transcription factor binding sites accessible (9, 132).

Demands on the Motor

ISWI complexes face two challenges in sliding nucleosomes: the ability to regulate sliding to achieve a directionally biased outcome, and the physical barriers to sliding presented by the histone-DNA contacts in a nucleosome. Given that sliding can occur spontaneously as described in Figure 2, it is possible that ISWI motors simply accelerate the uncatalyzed sliding pathway by lowering the kinetic barrier. Consistent with this possibility, uncatalyzed nucleosome sliding is substantially slower than ISWI-driven sliding (40). However, whereas uncatalyzed nucleosome sliding results in nucleosomes equilibrating among the thermodynamically most favorable positions, ISWI-mediated sliding is dominated by directional movement (e.g., away from DNA ends). Recent single-molecule work suggests additional differences between ISWI-mediated and uncatalyzed sliding and is also beginning to address the question of how ISWI overcomes the physical barrier presented by histone-DNA contacts.

Similar to some SF2 motors, ISWI motors can translocate on duplex DNA (138). However, the ATPase domain binds near the SHL ± 2 location, where histones are tightly bound to the DNA (Figure 2b), making it difficult to imagine how translocation occurs from such a sterically restricted site in the context of a nucleosome (64, 114, 152). Single-molecule

studies of remodeling by the yeast ISW2 complex demonstrate that ISW2 first translocates 7 bp of DNA out of the nucleosome before any DNA enters the nucleosome from the other side, resulting in a strained intermediate (31). How this strain is accommodated by the nucleosome is unclear, but current hypotheses include underwinding of nucleosomal DNA or a conformational change within the histones. The implication of this result is that ISWI motors may actively distort the nucleosome, in an analogous fashion to the RNA distortion carried out by Mss116. An outstanding question is whether this strained nucleosome is an essential part of the mechanism of remodeling by ISWI motors or is a by-product of its action. If the former, it is possible that the generation of this strained intermediate enables ISWI motors to overcome the physical barriers to sliding presented by the histone-DNA contacts and/or enforce directional sliding in the direction that relieves the strain. The possibility of a strained nucleosome conformation during ISWI-catalyzed sliding further suggests that during catalysis, ISWI creates a new intermediate rather than simply accelerating uncatalyzed sliding.

Substrate Specificity: Regulation of Remodeling Activity by Substrate Cues

As described above, ISWI motors can slide nucleosomes to enable both gene repression and activation. Two features of the nucleosomal substrate play an important role in driving specific outcomes: a basic patch (residues 16–20) on the N-terminal tail of histone H4, and the length of DNA flanking the nucleosome (21, 22, 48, 53, 64, 124, 144). Both substrate cues stimulate the ATPase and nucleosome sliding activities of ISWI ATPase subunits. In vivo, the H4 basic patch is thought to promote chromatin compaction through interactions with an acidic patch on the H2A/H2B dimer of an adjacent nucleosome (33, 84, 151). Because one of the roles of ISWI motors is to generate chromatin structures that are conducive to compaction, occlusion of the H4 tail in compacted chromatin could help enforce directionality of output, as ATPase activity would be inhibited by the compacted product. Stimulation of ISWI motors by flanking DNA underlies the ability of ISWI motors such as ACF to move mononucleosomes away from DNA ends. The ATPase and sliding activities of ACF steadily increase as the flanking DNA length increases, such that nucleosomes are moved more quickly in the direction of the longer DNA (144). On short stretches of DNA, the result is the equalization of DNA on either side of the nucleosome. Thus, regulation by flanking DNA length may allow ISWI motors to “sense” the proximity of barriers, moving the target nucleosome away from these barriers (73). In the context of a nucleosomal array, this activity would lead to regular spacing between nucleosomes, whereas in the context of a neighboring transcription factor, it would expose a region of DNA adjacent to the transcription factor.

Regulation of Activity by Modules in the ISWI ATPase Subunit

Flanking DNA and the H4 tail are recognized via specific regulatory modules in ISWI ATPase subunits (Figure 3a). Importantly, the rate enhancements observed in the presence of these substrate cues are largely due to catalytic effects and not binding effects, suggesting that they are sensed at a step after substrate binding (21, 144).

Flanking DNA length is sensed by a C-terminal DNA binding module known as the HAND-SANT-SLIDE (HSS) motif (Figure 3a). The HSS motif contacts approximately 30 bp of

flanking DNA (27). Consistent with this physical interaction, the ATPase and nucleosome-sliding activities of the human ISWI ATPase subunit SNF2h increase with increasing flanking DNA from 0 to 40 bp and then remain constant (144). Studies with a highly related motor from a different family, Chd1, have shown that removal of the analogous module in the Chd1 motor (the SANT-SLIDE motif) changes the major outcome of sliding from a centered nucleosome to a distribution of positions (88, 99). These studies indicate that the SANT-SLIDE domain in Chd1 promotes directional nucleosome movement, and they have been used to infer a similar role for the HAND-SANT-SLIDE domain in ISWI motors. Although which module or modules recognize the H4 tail is still unclear, recent work has suggested that the H4 tail directly interacts with the ATP-binding pocket to promote closure of the RecA-like lobes (104).

ISWI motors also contain two autoinhibitory modules, NegC and AutoN (20). Both are small domains that flank the ATPase domain (Figure 3a). Replacement of NegC with a flexible linker abolishes DNA length sensitivity and nucleosome centering for SNF2h without compromising sliding rates, indicating that NegC regulates length sensing (71). Mutation of AutoN increases DNA-stimulated ATPase activity and nucleosome sliding and also partially relieves the dependence on the H4 tail for remodeling, suggesting that AutoN competes with the H4 tail for the active site of the ATPase (20). Much remains to be determined about the physical basis of autoinhibition by these two modules.

Regulation by Noncatalytic Subunits

The noncatalytic subunits of ISWI complexes influence ISWI localization in vivo, and in vitro evidence suggests that they also alter the remodeling reaction. For example, the Acf1 subunit of the ACF complex increases the affinity for the nucleosome, the overall rate of nucleosome sliding, and the maximal length of flanking DNA that the motor can sense, from 20 to 60 bp (144). Interestingly, in the context of ACF, the NegC module is not required for sensing flanking DNA length. Rather, length sensing seems to be accomplished solely through the N-terminal region of Acf1, which can bind either DNA or the H4 tail (Figure 3c) (58). Such regulation of fundamental steps by accessory subunits is conceptually similar to how the RecC subunit increases the processivity of RecB. This comparison raises the question of whether, as in RecBCD, accessory domains such as Acf1 simply enhance the activities of the ATPase subunit or whether they change how the modules of the ATPase subunit participate in the reaction.

Correlating Moving Parts with Reaction Steps

How is length sensing by the HSS communicated to the ATPase to enable sliding? Early models envisioned DNA length sensing to occur during translocation, with the HSS remaining bound to flanking DNA to promote DNA movement across the histone octamer (69, 111, 126). However, a series of recent studies paint a more complex picture of the overall reaction.

First, introducing a flexible linker between the HSS and ATPase domains does not compromise sliding activity, indicating that the HSS is not simply acting as a mechanical element that helps guide the DNA across the nucleosome (82, 95). Second, single-molecule

studies have revealed that remodeling by ISWI motors occurs in two phases: a rate-limiting pause phase in which the nucleosome is stationary but ATP is still hydrolyzed, and a translocation phase in which DNA is moved over the surface of the histone octamer (12). Interestingly, the flanking DNA and H4 tail cues are sensed in the pause phase and not in the translocation phase (58). Finally, recent ensemble biochemical work identified two distinct conformational states of the SNF2h ATPase: a state in which the HSS binds flanking DNA in the presence of ADP (HSS-out), and a state in which the HSS binds the nucleosome core in the presence of an activated ATP analog, ADP-BeFx (HSS-in) (71). These results raise the possibility that the two remodeling phases observed by single-molecule assays are carried out by two different conformations of the enzyme.

On the basis of these and related results, the following model has been proposed (Figure 3b): Binding of flanking DNA by the HSS stimulates ATP hydrolysis to promote the conformational change from HSS-out to HSS-in (71). Length sensing is thus established in the pause phase, such that increasing the length of flanking DNA increases the likelihood of the HSS entering the translocation-competent, HSS-in state. In this model, the autoinhibitory modules NegC and AutoN regulate the conformational change from HSS-out to HSS-in. Additional single-molecule studies directly correlating conformational transitions in SNF2h to the pause and translocation phases are needed to rigorously test this model.

Open Questions

Several fundamental mechanistic questions remain in our understanding of ISWI motors. Although nucleosome sliding by ISWI motors is directional, the directionality of sliding differs among ISWI family members. For example, *Drosophila* ISWI moves nucleosomes toward, rather than away from, DNA ends, yet can still space nucleosome arrays (24, 34). These data and recent work directly testing the relationship between centering and spacing raise the possibility that nucleosome spacing by ISWI motors involves more than simply equalizing linker lengths (74a). Furthermore, some ISWI complexes can dimerize on the nucleosome, which could increase the efficiency and processivity of sliding (105). However, how two ISWI protomers collaborate, rather than compete, to slide a nucleosome remains unclear (71). Finally, as noted above, in addition to sliding, ISWI motors also catalyze the seemingly unrelated activity of nucleosome assembly (60). In particular, ISWI complexes can “mature” prenucleosome particles assembled by histone chaperones, which contain a full complement of histones but adopt a noncanonical conformation (130). How one motor accomplishes both activities is unclear, but an intriguing speculative hypothesis is that sliding and maturation share a common strained intermediate with loosened histone-DNA contacts. Future biochemical, structural, and single-molecule work will be required to address these open questions.

INO80 FAMILY

SWR and INO80 are large, multisubunit complexes belonging to a family of remodeling enzymes called INO80 (19). In vivo, SWR and INO80 act at similar regions in the genome (double-strand breaks, replication forks, and transcription start sites), but their contributions at these regions can be distinct (43, 90). INO80 and SWR also differ significantly in their

activities in vitro. Whereas SWR's only known activity is to exchange H2A/H2B dimers for H2A.Z/H2B, INO80 centers mononucleosomes and can evenly space a tri-nucleosomal array (89, 117, 134). One study has suggested that INO80 can perform the opposite reaction of SWR, taking off H2A.Z/H2B dimers and replacing them with H2A/H2B (97). However another study did not see evidence for such an exchange activity by INO80 (86). In this section, we focus on the mechanism of SWR, as more is known about how SWR performs histone exchange. We then compare and contrast SWR and INO80 in terms of structure and mechanism.

Demands on the Motor

In addition to the four canonical histones that constitute the nucleosome, the incorporation of histone variants that can replace their canonical counterparts creates an additional layer of complexity in the regulation of chromatin structure. H2A.Z, a common variant of H2A, plays broad and essential roles in regulating transcription and maintaining genomic stability across all eukaryotes. H2A.Z is enriched at promoters of genes, where it is thought to interact with transcriptional machinery to regulate gene expression (also reviewed in 127). Although H2A.Z and H2A share only 60% identity in amino acid sequence, crystal structures of nucleosomes containing either H2A.Z or H2A reveal surprisingly similar architectures (128). SWR's ability to perform directional histone variant exchange requires that the complex distinguishes between substrate (H2A-containing nucleosomes) and product (H2A.Z-containing nucleosomes), which is a major challenge considering these two structures are highly similar. Specificity for H2A.Z- versus H2A-containing nucleosomes can in principle be achieved by binding modules that recognize small differences between the two histones. However, to achieve directional histone exchange, binding specificity needs to be coupled to the irreversibility of ATP hydrolysis. Below we explore the known modules of the SWR complex that contribute to its specificity for H2A.Z- versus H2A-containing nucleosomes and speculate as to how these modules coordinate with the ATPase subunit Swr1 to generate directionality.

Specificity for H2A.Z Is Encoded in Separate SWR Modules

Work from the last decade has revealed that the specificity of SWR's histone exchange activity relies on two separate modules of the complex: (*a*) Swc2, a subunit of the SWR complex, and (*b*) Swr1-Z, a small, 30–amino acid region immediately N-terminal to the RecA-like ATPase domain of the Swr1 ATPase. Both Swc2 and Swr1-Z interact specifically with H2A.Z/H2B but not H2A/H2B (54, 142). Deletion of either module impairs the ability of SWR to perform directional histone exchange in vitro (54, 137). The Swr1-Z region acts as a bona fide chaperone for H2A.Z/H2B dimers, as it can incorporate H2A.Z/H2B dimers onto preformed DNA-$(H3/H4)_2$ tetrasomes in the absence of the rest of the Swr1 ATPase domain (54).

Together, these data have led to a chaperone-like model of histone exchange by SWR (Figure 4a). In this model, Swc2 and Swr1-Z bind to H2A.Z/H2B, delivering the H2A.Z/H2B dimer close to the canonical nucleosome. The exchange reaction must require a handoff mechanism in which the interactions between SWR and H2A.Z/H2B are replaced by those between H2A.Z/H2B and H3/H4. At a macroscopic level, eviction of H2A/H2B

and deposition of H2A.Z/H2B are coupled as SWR cannot evict H2A.Z/H2B (86). It is not clear however if these processes are coupled at a microscopic level. Eviction of H2A/H2B may also require a separate module of SWR that has specificity for H2A/H2B over H2A.Z/H2B. This module could act analogously to the latch domain of Mot1 to help disrupt interactions between the H3/H4 tetramer and the H2A/H2B dimer, preventing reassociation of H2A/H2B (54). Although this module has not yet been identified, a subunit of SWR called Yaf9 has been shown to bind specifically to H3 and H4 and not to H2A or H2B (136). In fact, Yaf9 is required for histone exchange, raising the intriguing possibility that Yaf9 prevents reassociation of an evicted H2A/H2B dimer by blocking interactions with the tetramer (142).

Kinetic measurements of the exchange reaction show that histone exchange occurs in a stepwise manner, with a heterotypic nucleosome (containing one H2A/H2B dimer and one H2A.Z/H2B dimer) as an intermediate of the full exchange reaction (86). Consistent with stepwise exchange, H2A.Z-containing nucleosomes in vivo have either a single copy of H2A.Z/H2B (heterotypic) or two copies of H2A.Z/H2B (homotypic), raising the possibility that the number of copies of H2A.Z provides a source of biological regulation (86). Furthermore, because SWR has two separate modules that specifically recognize H2A.Z/H2B (Swc2 and Swr1-Z), it is also possible that a single SWR can processively catalyze two exchange reactions. However, the processivity would need to be regulated to generate the heterotypic nucleosomes seen in vivo.

Coupling of Substrate Specificity to ATP Hydrolysis

Importantly, when both H2A.Z/H2B dimers have been incorporated in the nucleosome, SWR cannot catalyze the reverse reaction, even when provided with a large excess of H2A/H2B dimers (86). Similar to the motors discussed in the section titled Nucleic Acid Remodeling ATPases, this enforcement of directionality during histone exchange appears to be tightly coupled to its ATPase activity. Unlike remodelers that slide nucleosomes, SWR is not stimulated at all by naked DNA. In fact, SWR ATPase activity is stimulated only by canonical nucleosomes and not by H2A.Z-containing nucleosomes. There is a further 2.5-fold increase in ATPase rate when excess H2A.Z/H2B dimers, but not canonical H2A/H2B, are provided in addition to canonical nucleosomes. Moreover, both types of dimers, in the absence of a nucleosome, fail to stimulate SWR's ATPase activity, suggesting that a unique intermediate in which SWR binds both the nucleosome and a free H2A.Z/H2B dimer represents an activated state of the enzyme. Interestingly, in comparison with the structure of H2A.Z in a nucleosome, H2A.Z bound to Swr1-Z has an extended alpha helical structure, which could reflect the structural changes induced by the activated SWR-nucleosome-H2A.Z/H2B complex (54). This altered structure of H2A.Z/H2B could reflect a high-energy intermediate on the histone exchange pathway that is stabilized by SWR (Figure 2d).

Despite the tremendous progress in understanding how SWR is able to achieve specificity during histone exchange, we still do not understand how these H2A.Z-specific modules regulate the ATPase at different steps of the reaction. One hypothesis is that the Swc2 subunit and the Swr1-Z motif regulate the ATPase domain directly and potentially in concert

(142, 143). Swr1-Z is located directly adjacent to the ATPase domain, consistent with having a direct effect on ATPase activity. Deletion of the Swc2 subunit reduces all histone-specific ATPase stimulation to basal levels, suggestive of a direct regulatory function (137). However, whether or not Swc2 makes direct contacts with the ATPase domain is not clear (142). It would be informative to investigate how the Swc2 and Swr1-Z regulatory modules work together in regulating the activity of the complex and whether the two modules act in the same step or different steps along the histone exchange pathway.

Comparison with INO80: Similarities and Differences in Structure and Function

The INO80 family of remodeling enzymes are defined by a unique 300–amino acid insertion between the two RecA-like lobes of the ATPase subunit (Figure 4b) (39). In human cells, replacing the sequence of the insertion in the Ino80p ATPase with that of SRCAP, the human homolog of SWR, is sufficient for the replacement of several INO80-specific subunits with SRCAP-specific subunits (17). This result suggests that subunit composition is regulated by the identity of the insert and leads to the attractive hypothesis that the subunits recruited by the insert dictate the respective activities of the SWR and INO80 complexes. Indeed, unlike ISWI enzymes, Ino80 and Swr1 ATPases alone are not sufficient for remodeling activity (17, 142). This observation raises the question of how other subunits regulate the activity of the complex and how this regulation contributes to substrate and product specificity.

Biochemical and structural approaches have shown that both SWR and INO80 exhibit a modular architecture of subunit organization in which different regions of Swr1 and Ino80 ATPases act as a scaffold for association of different subcomplexes, or so-called modules (44, 94, 143). The term modules in this context refers to clusters of three to six subunits that form a functional unit of the larger complex. For example, the SWR complex is organized into two major modules—one module that associates with the N-terminus of the Swr1 ATPase and another module, which contains Swc2, that associates with the C-terminus of the ATPase (94). For INO80, further complementary functional studies have isolated specific roles for each module. For example, deleting the Arp8 module (subunits lost include Arp8, Arp4, Act1, Taf14, and Ies4) does not substantially affect nucleosome binding but instead greatly diminishes remodeling and ATPase activity (131). The differences in functional modules in SWR and INO80 may contribute to how these complexes use substrate cues such as flanking DNA. SWR preferentially binds to nucleosomes with longer flanking DNA (106). This property is thought to recruit the enzyme to the promoters of genes, which are immediately downstream of a nucleosome-free region. In contrast, INO80 preferentially remodels nucleosomes with longer flanking DNA length, whereas binding is unaffected (134). These and other structure/function studies are just beginning to provide an image of the moving parts involved in nucleosome remodeling by these large machines.

Open Questions

How do two highly related enzymes in the same family have such different activities in vitro? Given what we know about the dynamics of nucleosome structure, it is tempting to speculate that both motors take advantage of one type of uncatalyzed conformational change in the nucleosome to enable different outputs (Figure 4c): DNA unwrapping from one end of the nucleosome, which breaks DNA contacts with H2A/H2B. In the case of

INO80, this unwrapping may provide a precursor for propagating the unwrapped DNA across the histone octamer to enable sliding. In the case of SWR, unwrapping poises the H2A/H2B dimer for exchange, whereas the DNA propagation pathway may be blocked by an accessory subunit or unidentified domain of the Swr1 ATPase. Thus, both sliding by INO80 and histone exchange by SWR may share a common, physiologically accessible intermediate. Another major open question for the INO80 family is how movement of the RecA lobes of the ATPase subunit enables remodeling. Unlike ISWI, the INO80 family has not been shown to translocate along naked DNA, suggesting that the RecA-like lobes may have a completely different function, such as directly disrupting histone-histone contacts or regulating large-scale conformational changes within the complex during remodeling.

SWI/SNF FAMILY

Chromatin remodeling motors in the SWI/SNF family are large, multisubunit complexes implicated in nearly every nuclear process. SWI/SNF remodelers play roles in transcriptional activation and repression, transcriptional elongation, initiation of DNA replication, and homologous recombination (36). Not surprisingly, misregulation of these complexes is associated with many cancers (63).

Core Biochemical Activities

SWI/SNF family motors can catalyze a diverse set of changes that range from slightly disruptive to very disruptive to nucleosome structure. Developing a comprehensive model that can explain the mechanism by which these changes are catalyzed by SWI/SNF has been challenging, in part because of the wide variety of methods that are used to investigate the many reaction outcomes. Synthesizing the information gleaned from these experiments remains an outstanding issue for the field. A further challenge stems from the continuous interconversion among reaction products (discussed further below). We begin by enumerating some of the SWI/SNF products that persist after removal of the enzyme (see also Figure 5).

Similar to the ISWI family motors, SWI/SNF motors can slide mononucleosomes in vitro (14, 38, 96). However, unlike ISWI, SWI/SNF family members move mononucleosomes toward DNA ends and cannot evenly space nucleosomal arrays. On core nucleosomes, they can further make internal DNA sites accessible to transcription factors and restriction enzymes, even under conditions in which sliding is restricted (6, 59, 65, 75, 92). These regions of enhanced DNA accessibility created by SWI/SNF are often referred to as loops formed on the surface of the nucleosome. Interestingly, the human ISWI-family motor SNF2h is unable to slide these loop-containing nucleosomes, underscoring their noncanonical structure (38). Such surface loop formation helps explain early observations that SWI/SNF complexes introduce topological changes in the DNA of closed circular nucleosomal arrays. These changes are stable for several hours after removal of SWI/SNF, before relaxing to the original topology (47).

With some similarity to SWR, SWI/SNF remodelers can exchange H2A/H2B dimers between two mononucleosomes, but unlike SWR, they do so without apparent specificity for particular histone variants (89, 145). When given free DNA as an acceptor, SWI/SNF

motors can also transfer the entire histone octamer from a nucleosome to the acceptor DNA (79, 101, 108). However, efficient histone disassembly by SWI/SNF has been observed only in the presence of histone chaperones, which can bind the displaced histones (45, 77). For example, such transfer has been observed between the yeast SWI/SNF family member RSC and the histone chaperone Nap1 in vitro. Histone loss has also been observed in the absence of chaperones but in the context of dinucleosomal, rather than mononucleosomal, templates. This histone loss is proposed to arise from SWI/SNF sliding one nucleosome into another, causing disassembly of the second nucleosome (30). Continuous with this reasoning, recent single-molecule experiments suggest that yeast SWI/SNF can slide a nucleosome through a transcription factor that is bound to the flanking DNA, causing the dissociation of the transcription factor. However, dissociation of the transcription factor cannot occur without a nucleosome present (73).

Models for Generation of Diverse Products

SWI/SNF complexes tend to have upward of 10 noncatalytic subunits in addition to the ATPase subunit (36). Despite their large size, the ATPase subunits of at least some SWI/SNF complexes can generate by themselves many of the same products as those generated by the full complex. For example, the human ATPase subunit Brg1 has been shown to make all the products in Figure 5b except a transferred dimer, which has not been tested (101, 102). SWI/SNF remodelers therefore differ from the similarly large SWR-family remodelers, in which the ATPase subunit does not appear to function independently of the complex. To date, the additional noncatalytic subunits of SWI/SNF complexes have been shown primarily to increase the activity of the ATPase subunit and/or play a role in targeting the complex to specific genomic locations (36). An exception is the Swi3p subunit of the yeast SWI/SNF complex, which binds H2A/H2B dimers and has shown to be essential for dimer exchange (145).

How can a single ATPase catalyze so many diverse products? One possibility is that the various products are formed in a temporally ordered manner, such that one type of product is the precursor of another product. For example, the observation that SWI/SNF slides nucleosomes not simply toward DNA ends but even past them, exposing the H2A/H2B dimer, led to the proposal that such off-the-end sliding could enable dimer transfer (14, 38, 40, 65). However, dimer exchange was subsequently shown to occur in the absence of DNA ends, when nucleosomes are assembled on circular DNA templates, ruling out sliding off of DNA ends as a prerequisite (14). Similarly, dimer exchange could conceivably precede octamer transfer. However, SWI/SNF can transfer the octamer directly to a free acceptor DNA in the absence of histone chaperones (79, 101, 108). Because DNA in a nucleosome must wrap around the H3/H4 tetramer before wrapping around the H2A/H2B dimers, it is difficult to imagine how SWI/SNF could transfer a dimer to free DNA and then transfer the tetramer, strongly suggesting dimer exchange is not an essential precursor of octamer transfer.

An alternative model posits that SWI/SNF complexes generate a common intermediate that can collapse into different products depending on the context. In the language of the free-energy profile of Figure 2, SWI/SNF would stabilize a physiologically less accessible

intermediate, which lies on a pathway to multiple products. An attractive line of support for this model comes from the observation that SWI/SNF continuously interconverts reaction products in an ATP-dependent manner, providing multiple opportunities for the partitioning of these products (6, 76, 92, 113). Such interconversion between products suggests that they are actively recycled through at least one common intermediate. Evidence for such recycling was first proposed from studies of the dinucleosome-like species diagrammed in Figure 5 (76, 101, 113). These species (two octamers bridged by a single DNA strand) could be isolated by gel electrophoresis and subsequently reexposed to enzyme and ATP, and they would be recycled back to canonical nucleosomes. Furthermore, kinetic studies have suggested that even though stable transfer products are formed much more slowly (on a timescale of minutes) than other products such as repositioned nucleosomes, transfer products are initiated via an intermediate formed on a timescale of seconds, as detected by Förster resonance energy transfer (108). Thus, it is kinetically possible that transfer products do share an early intermediate with other products.

A model with a common intermediate and continuous recycling can in principle explain all the diverse outputs that can be generated by a single SWI/SNF ATPase (Figure 5). Additional noncatalytic subunits and/or other factors may bias the directionality of the reaction outcome. For example, in the presence of a DNA acceptor or a histone chaperone, the intermediate can transfer a histone octamer, whereas in the absence of an acceptor the intermediate could collapse to a slid nucleosome or a nucleosome with a stable DNA loop. However, these two models—linear ordering versus one common intermediate—represent a spectrum, and SWI/SNF remodeling likely occurs through a mixture of these models, perhaps with some products sharing intermediates but others not. If a common, early intermediate on the pathway to multiple products exists, what might it look like? Current evidence suggests that early intermediates probably involve multiple disruptions of histone-DNA contacts at sites throughout the nucleosome (6, 11, 65, 92, 152). In the absence of other factors such as histone acceptors, these large-scale disruptions may collapse into structures that resemble the nucleosomal surface loop structures that have been detected as stable products.

Demands on the Motor

Generating a globally disrupted intermediate requires breaking multiple internal histone-DNA contacts across the nucleosome without displacing histones. How might SWI/SNF ATPases accomplish such a task? Similar to ISWI motors, SWI/SNF also binds near the SHL ± 2 location of the nucleosome and can translocate on naked DNA (74, 109, 110, 152). However, SWI/SNF appears to use a mechanism different from that used by ISWI to solve the problem of translocation from a sterically restricted internal location (152). Cross-linking studies suggest that the ATPase domains of ISWI motors bind alongside the nucleosomal DNA, whereas the SWI/SNF ATPase domain is proposed to intercalate between the DNA gyre and the histone octamer to help displace DNA from histones (29). Some residues within the SWI/SNF ATPase may enable such intercalation by acting analogously to the pin of RecC in RecBCD. In addition, translocation by SWI/SNF complexes generates large looped structures both in naked DNA and in the presence of a nucleosome (11, 74, 75, 116, 149). This loop formation may be related to SWI/SNF's

ability to disrupt large swaths of DNA-histone contacts simultaneously. To form such loops, and in particular to form surface loops on nucleosomes as discussed above, SWI/SNF would need to bind the nucleosome in at least one location in addition to the binding site of the RecA-like lobes. Interestingly, when the DNA binding domain of Chd1 (see ISWI Family section above) is replaced with a monomeric streptavidin and presented with nucleosomes containing a biotinylated octamer, the resultant additional enzyme-octamer tether confers a number of SWI/SNF-like properties on the Chd1 chimera (99). For example, like SWI/SNF, the streptavidin-Chd1 chimera can change the topologies of nucleosome arrays in a reversible manner. Some evidence suggests that SWI/SNF does contain a motif that makes additional contacts with the nucleosome octamer. Deletion of a SWI/SNF-specific motif called the SnAC (Snf2 ATP coupling) domain, located C-terminal to the RecA-like lobes of the ATPase domain, appears to impair the ability of SWI/SNF to form loops in nucleosomal DNA, perhaps by removing interactions between SnAC and the histone octamer (116). These results underscore the potential of the N- and C-terminal elaborations of the basic RecA-like ATPase architecture to specialize the motor for particular tasks.

Conclusions and Outlook

Clearly fundamental questions remain, particularly about the nature and structure of the intermediate(s) of the SWI/SNF remodeling reaction. Single-molecule studies with either a minimal RSC complex or the full RSC complex from yeast suggest translocation by SWI/SNF motors occurs in 1–2-bp steps (50, 122). How is this local stepwise translocation converted into a large-scale disruption of DNA-octamer contacts? Does the proposed globally disrupted intermediate collapse into different products while remaining bound to SWI/SNF? Addressing such questions requires an ability to trap intermediates so they can be subjected to high-resolution structural studies. Furthermore, given the difficulty of following the asynchronous generation and recycling of the multiple SWI/SNF products in ensemble studies, recent applications of single-molecule biophysical techniques offer especially exciting avenues for addressing some of these questions.

ADDITIONAL COMPLEXITIES IMPOSED ON REMODELER ACTION IN VIVO

Our current mechanistic understanding of chromatin remodelers provides a starting point for asking how these motors may function in the complex environment of the nucleus (Figure 6). Below, we briefly discuss how additional features of the chromatin template as well other chromatin regulators may influence the activities of remodeling complexes.

DNA Sequence

DNA sequences that are more bendable in a manner favored for nucleosome formation, termed positioning sequences, have been shown to form more stable nucleosomes (23, 139). In vivo, such DNA sequences are correlated with well-positioned nucleosomes, raising the question of whether DNA sequence can influence the activity of remodelers by regulating the stability of the nucleosome (115).

In this context it is instructive to assess the dynamic range of stability that is achievable by varying DNA sequence. One of the highest-affinity sequences is a synthetic DNA sequence

obtained by in vitro selection assays, called 601. At 4°C, 601 has a ΔΔG of formation of ~ 3 kcal/mol compared with a natural nucleosome positioning sequence termed 5S (81, 98). This corresponds to an approximately hundredfold greater overall nucleosomal stability, suggesting in principle that DNA sequence could regulate remodeling rates over a hundredfold dynamic range. However, when measured at physiological temperatures, the hundredfold difference in stability diminishes to a fourfold range (ΔΔG ~ 0.8 kcal/mol) (4). In a manner consistent with this smaller range, nucleosomes that are assembled on either the 601 sequence or the 5S sequence are repositioned by ISWI and SWI/SNF family members at the same rate (98). Does this mean that DNA sequence cannot regulate remodeling activities? Not necessarily. Unlike overall nucleosome stability, DNA unwrapping is dramatically affected by DNA sequence at physiological temperatures (up to hundredfold greater for 5S compared with 601) (3). Thus, in principle, remodeling motors that use DNA unwrapping as part of their mechanism could be greatly influenced by DNA sequence. Sequence could also affect other aspects of nucleosome dynamics that remodelers could exploit. For example, certain DNA sequences promote octamer transfer by SWI/SNF, whereas others, specifically AT-rich sequences, promote octamer eviction by RSC in the presence of histone chaperones (78, 101).

Higher-Order Chromatin Structures and Histone Modifications

Most of the studies we have discussed so far were carried out with mononucleosomes or defined arrays of extended nucleosomes, and with recombinant histones that do not contain posttranslational modifications (PTMs) (85). Although these substrates are powerful tools for capturing core properties of remodeling motors, a large gap remains in the types of chromatin structures that we can study in vitro and the types of structures that exist in vivo. In vivo, internucleosomal interactions drive chromatin into higher-order states of folding (83). These states are thought to be driven by electrostatic interactions between the histone tails and DNA and between the H4 tail and a negatively charged patch between H2A and H2B called the acidic patch (33, 84, 151). Specific proteins also participate in stabilizing certain types of condensed chromatin, including the linker histone H1 as well as heterochromatin-associated proteins such as HP1 and Polycomb (16, 35, 51, 119). Furthermore, in vivo, histones are decorated with many different types of PTMs, which regulate remodelers either directly or through effects on chromatin structure (129). How different remodelers act on physiological nucleosomal substrates is unclear, though recent work has begun to shed light on this topic. For example, *Drosophila* ISWI remodeling activity is only minimally inhibited by nucleosome arrays containing H1, whereas SWI/SNF remodeling is more significantly inhibited on this more in vivo–like substrate (56, 66). Similarly, underscoring the importance of histone modifications, the CHD1 and SWI/SNF families each contain domains that recognize specific PTMs that affect their localization in vivo and their activity in vitro (129). Further progress toward addressing these questions, however, has been limited both by the difficulty of reconstituting these substrates homogeneously and by the limited range of assays that can directly report on the remodeling of these complex substrates.

Mechanisms of Collaboration with Other Nuclear Factors

In vivo, a remodeler acts in the context of many other factors that are competing for the opportunity to interact with the chromatin template. How do remodelers coordinate their actions with these other factors to achieve a defined outcome? Although much remains to be learned, some biochemical studies are beginning to shed light on how such coordination may be achieved.

Sequence-specific DNA binding factors.—In addition to passively taking advantage of DNA binding sites made accessible by chromatin remodeling motors, specific DNA binding factors can directly recruit chromatin remodeling complexes. For example, in Drosophila, the ISWI family member NURF is recruited by the GAGA factor to the Hsp70 promoter to promote transcriptional activation (9, 132). This role is particularly important, as no remodeling complex has been shown to possess high levels of DNA sequence specificity, yet many are known to be specifically recruited to particular DNA loci in vivo (62, 146). DNA binding factors can also act as barriers to the movement of nucleosomes. In the case of ISWI, in vitro single-molecule experiments have shown that a bound transcription factor can act as a barrier, such that the ISWI motors move nucleosomes away from the bound factor, in contrast to SWI/SNF, which uses the nucleosome to displace the bound factor (73).

Other chromatin remodeling motors.—Many sites of chromatin regulation involve action by multiple families of remodelers. For example, in yeast, promoters are defined by a nucleosome-free region at the transcriptional start site followed by a series of three to four well-positioned nucleosomes (150). Several remodelers have been shown to be necessary for generating this architecture in vivo (46, 52). How and why are these combinations of remodelers required? One possibility is their action is temporally controlled, so that only one remodeler is recruited and active at a time, paving the way for the next remodeler. Alternatively, all the remodelers act at the same time such that the steady state reflects the outcome seen in vivo.

Other nuclear machinery.—Chromatin remodeling is required for facilitating essentially all other nuclear processes. For example, the yeast SWI/SNF family member RSC is required for RNA polymerase elongation at coding regions of actively transcribed genes, possibly acting alongside histone chaperones to evict and reassemble histones (123). The INO80 and SWR complexes play essential roles in transcription as well as DNA repair, in which the action of these complexes are required for recruitment of repair factors to the site of DNA damage (90, 133). An exciting area of current research aims to understand the interplay between chromatin remodeling enzymes and the other nuclear machinery, including (*a*) which remodelers are directly recruited by other nuclear machines, versus those that act as the primary recruiting factors (e.g., at sites of DNA damage), (*b*) which types of nucleosome remodeling events are required for other processes, and (*c*) whether remodelers directly regulate the activity of other nuclear machines.

CONCLUSION

The field of chromatin remodeling motors is at an exciting juncture as the convergent applications of different biophysical methods are providing new mechanistic insights. These studies have uncovered how accessory domains and subunits regulate the activities of the core RecA-like ATPase domains and have also shed light on how these domains collaborate with substrate cues to achieve directionality of outcome. What remains to be explored is how the ATPase cycle of these motors is coordinated with distortion of the substrate and how these motors act in the more complex contexts encountered in vivo.

ACKNOWLEDGMENTS

We thank Lindsey Pack, Sophie Dumont, John Gross, César Díaz Celis, and Carl Wu for critical reading of the manuscript and helpful comments. This work was funded by a grant from the National Institutes of Health (R01 GM073767) to G.J.N., a Leukemia and Lymphoma Society Career Development Program Fellowship to S.L.J., a National Institutes of Health Ruth L. Kirschstein National Research Service Award (5F31CA180651–02) to C.Y.Z., and a Graduate Research Fellowship from the National Science Foundation to N.I.G.

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STRUCTURE AND FUNCTION OF RecA

Bacterial RecA plays a key role in catalyzing many of the DNA rearrangements that occur during homologous recombination, a major pathway cells use to repair damaged DNA. The main activity of RecA is ATP-dependent oligomerization along single-stranded DNA (reviewed in 26). The core domain of RecA contains the prototypical RecA fold, which binds ATP and DNA, and the N- and C-terminal domains mediate oligomerization (125). RecA oligomerizes along DNA such that ATP binds at the interface between two RecA monomers (18). The structure of the active site in the presence of an ATP analog ADP-AlF_4 provides an elegant physical explanation for how the ATPase cycle is coupled to RecA oligomerization. ATP-binding (Walker A) and hydrolysis (Walker B) motifs are provided by one monomer, whereas two lysine residues from the neighboring monomer stabilize the transition state mimicked by ADP-AlF_4. ATP hydrolysis causes disassembly of the RecA oligomer by weakening the monomer-monomer interface and DNA binding. Duplication of the RecA fold in SF1- and SF2-family proteins converts the ATP-driven intermolecular assembly cycle seen in the RecA oligomer into intramolecular conformational changes between the two RecA-containing lobes to catalyze diverse structural rearrangements in nucleic acid substrates.

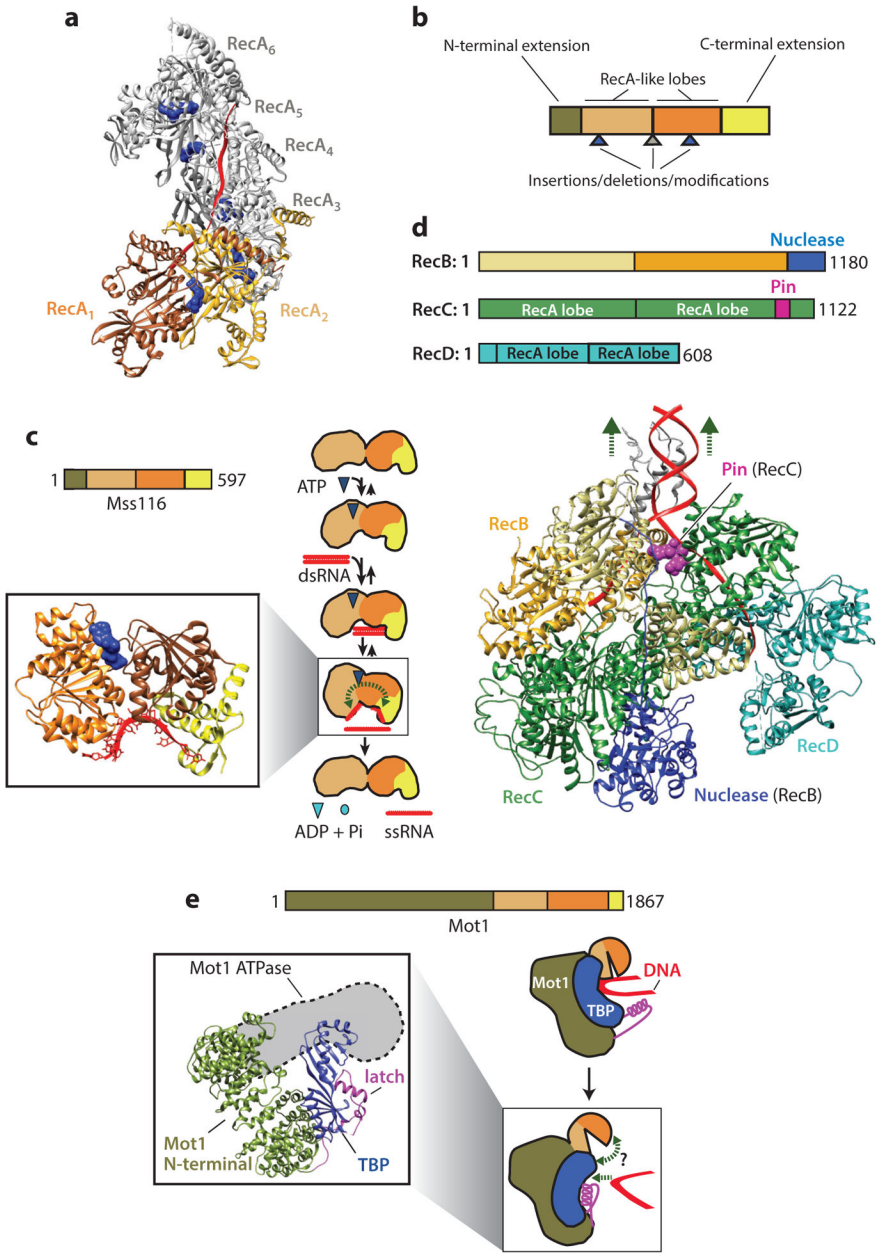

Figure 1.

Case studies of nucleic acid motors. (*a*) Crystal structure of a RecA filament bound to single-stranded DNA (*red ribbon*) with ADP-AlF_4 (*blue space-fill*) [Protein Data Bank ID (PDB ID): 3CMU] (18). (*b*) Domain architecture of SF1 and SF2 ATPases. The conserved RecA-like folds (*orange* and *tan*) can be elaborated by insertions or modifications between the RecA folds (*gray triangle*) or within either RecA-like fold (*blue triangles*), as well as by N- and C-terminal extensions (*green* and *yellow*, respectively). In panels *c* and *d*, for simplicity, schematics show only N- and C-terminal extensions without indication of insertions and modifications in the RecA folds. (*c*) Structure of Mss116 bound to duplex RNA and AMP-PNP (PDB ID: 3i5x) (32). The N-terminal extension is disordered in the crystal structure and is not shown. (*d*) Structure of the RecBCD complex and partially

unwound DNA (*red ribbons*) with the pin motif in magenta (PDB ID: 3K70) (112). (*e*) Crystal structure of the N-terminal domain of Modifier of transcription 1 (Mot1) bound to TATA-binding protein (TBP), with the approximate location of the ATPase domain, based on electron microscopy and cross-linking data, in gray (PDB ID: 3OC3) (141). Although Mot1's mechanism is not fully understood, movement of the RecA-fold-containing lobes of the ATPase is likely coupled to TBP removal. Crystal structure representations in all figures were made with UCSF Chimera (http://www.cgl.ucsf.edu/chimera). Abbreviations: dsRNA, double-stranded RNA; Pi, inorganic phosphate; ssRNA, single-stranded RNA.

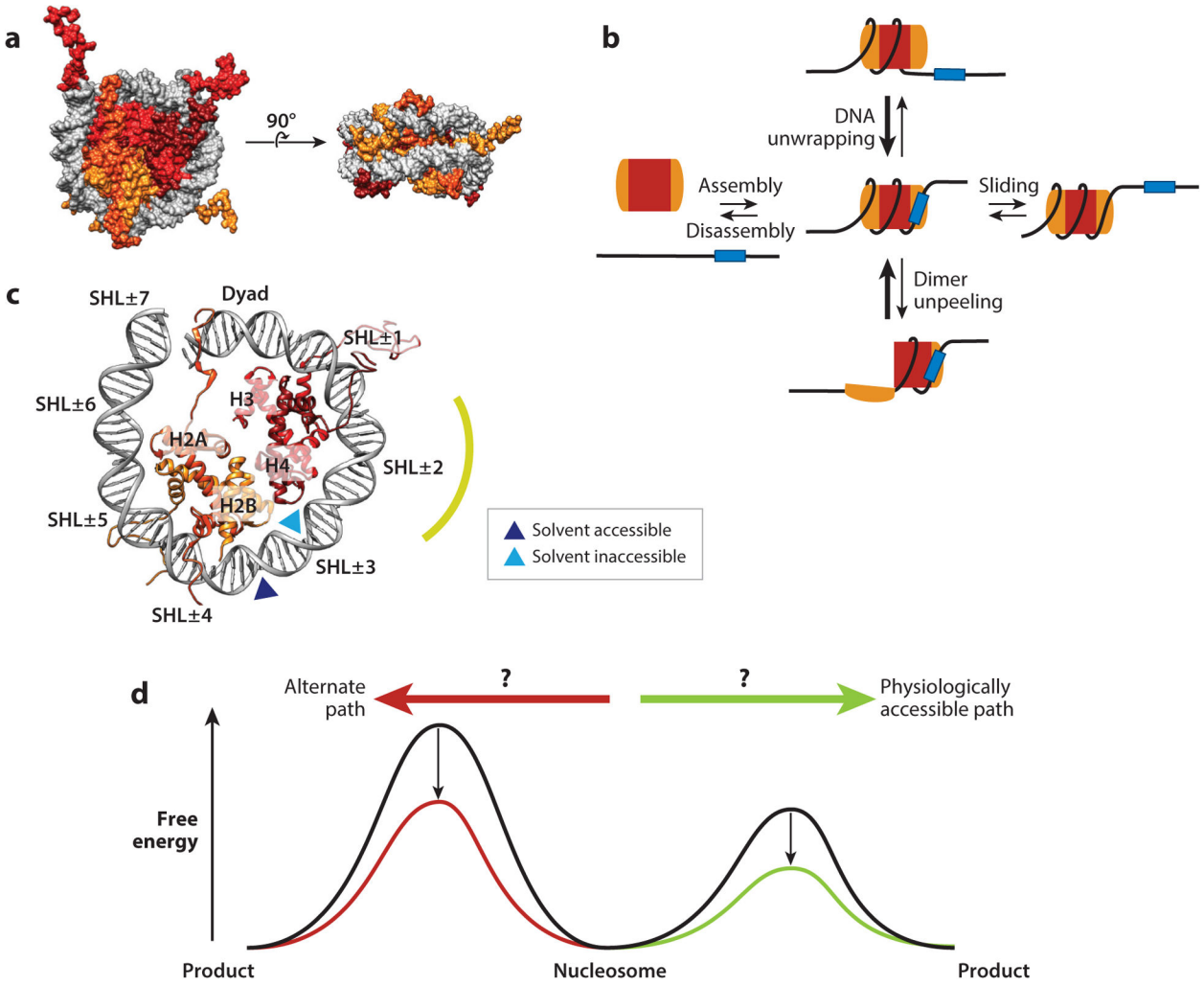

Figure 2.

General properties of nucleosomes. (*a*) Two space-filling views of a nucleosome core particle (PDB ID: 1KX5), with the DNA in gray and the histone proteins in red/orange (28). (*b*) Schematic of all physiologically accessible states for a nucleosome observed to date. H2A/H2B dimers are shown in orange and the H3/H4 tetramer is shown in red. (*c*) Top view of a nucleosome with histones and superhelical locations (SHL) on the DNA labeled. SHL ± 2, the binding location for several remodeler families, is highlighted in yellow. (*d*) Hypothetical free-energy profiles for a nucleosome remodeling reaction. The same remodeled product can be made through a physiologically accessible (*right*) or alternate (*left*) path.

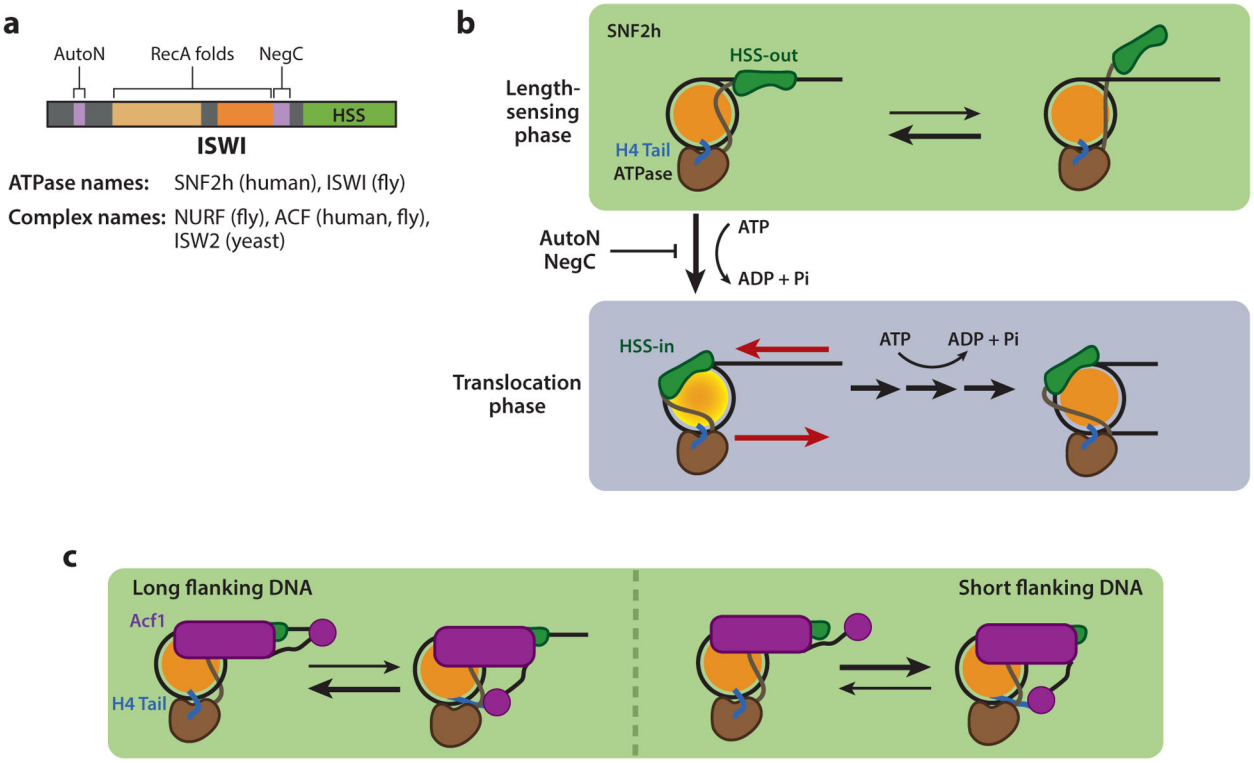

Figure 3.

Model for ISWI remodeling. (*a*) Domain architecture of ISWI-family ATPase subunits, with the names of ISWI ATPases and complexes that are referenced in this review. (*b*) A model for directional nucleosome sliding by the human ISWI motor SNF2h (adapted from Reference 71 with permission). The nucleosome is represented from a top-down perspective, and for simplicity, only one protomer of SNF2h is shown. The ATPase domain of SNF2h binds at SHL ± 2 and engages the H4 tail. Binding of the HSS domain to flanking DNA (HSS-out) stimulates ATP hydrolysis, driving SNF2h into a translocation competent state (HSS-in). Translocation (in the direction of red arrows) may be coupled to distortion of the nucleosome. Abbreviations: HSS, HAND-SANT-SLIDE; Pi, inorganic phosphate. (*c*) Model for additional regulation by Acf1 in the pause phase (58). When flanking DNA is short, Acf1 sequesters the H4 tail, inhibiting exit from the pause. When flanking DNA is long, the N-terminus of Acf1 instead binds to flanking DNA, allowing exit from the pause.

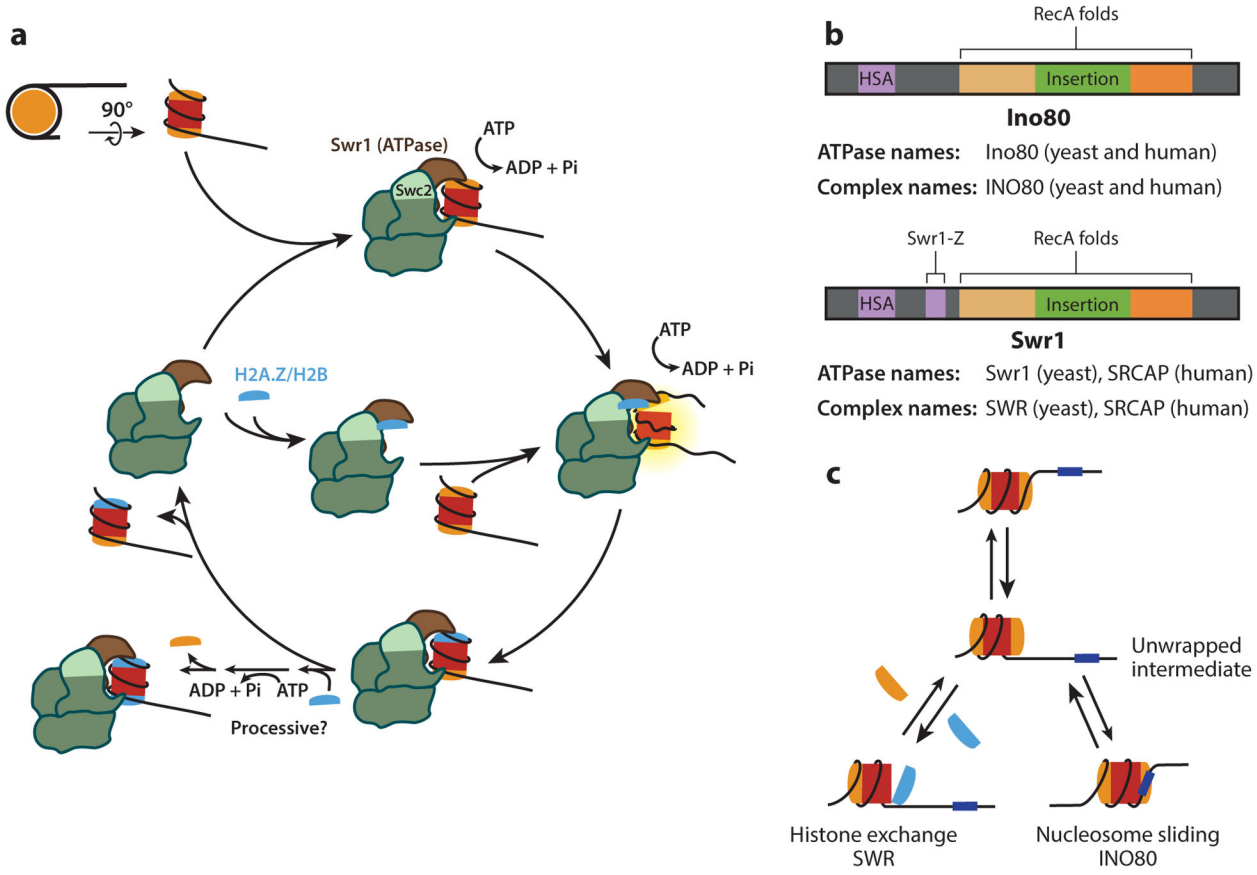

Figure 4.

Model for SWR remodeling. (*a*) Model for directional histone exchange by SWR (adapted from Reference 86 with permission). The Swr1 ATPase (*brown*) engages a nucleosome containing a canonical H2A/H2B dimer (*orange*) at SHL ± 2 on the distal side relative to flanking DNA (107). The Swc2 subunit and SWR1 both engage an H2A.Z/H2B dimer (*blue*). When both the nucleosome and H2A.Z are bound, Swr1's ATPase activity is maximally stimulated and a single H2A/H2B dimer is replaced by an H2A.Z/H2B dimer. Swr1 may also act processively until both canonical dimers have been replaced by H2A.Z/H2B dimers. (*b*) Domain architectures of the Ino80 and Swr1 ATPases. (*c*) The INO80 and SWR complexes may share a common intermediate in which DNA is unwrapped from one side of the nucleosome. SWR may use this intermediate to facilitate histone exchange with H2A.Z/H2B, whereas INO80 may use it to slide the histone octamer toward the site of unwrapping. Abbreviations: HSA, helicase-SANT–associated; Pi, inorganic phosphate.

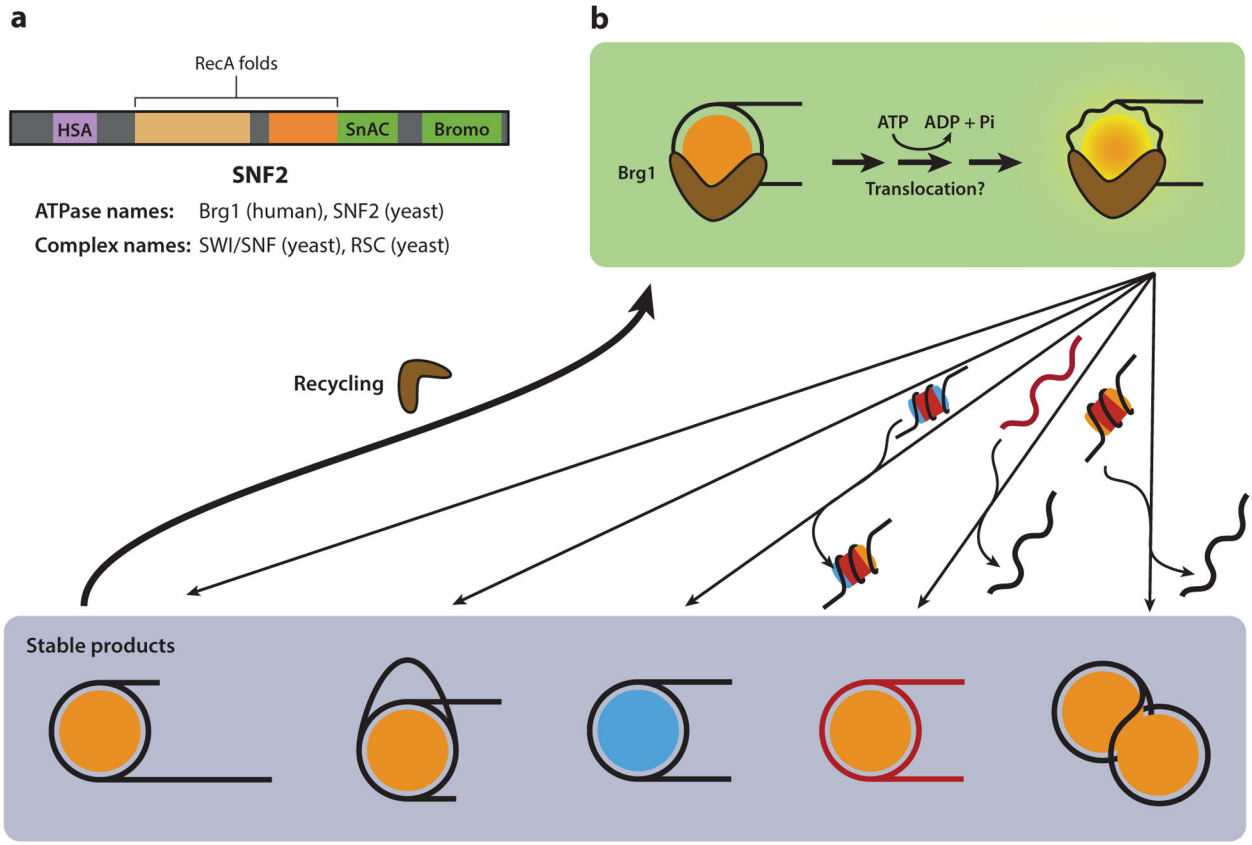

Figure 5.

Model for SWI/SNF remodeling. (*a*) Domain architecture of SWI/SNF-family ATPase subunits, with the names of SWI/SNF ATPases and complexes that are referenced in this review. (*b*) The SWI/SNF ATPase domain engages the nucleosome at SHL ± 2 (110, 152). ATP-dependent translocation by SWI/SNF disrupts histone-DNA contacts across the nucleosome. From this disrupted intermediate, the nucleosome may collapse into several stable products, including (from *left* to *right*) repositioned nucleosomes, nucleosomes with stable surface loops, nucleosomes with exchanged dimers, and octamers transferred to free DNA. SWI/SNF can also generate a dinucleosome-like species (*far right*) between two nucleosomes formed on separate DNA templates, in which one DNA strand bridges two octamers (76, 80, 101, 113). A key aspect of this model is that most or all of these products can be recycled back to the disrupted intermediate through the action of the motor. Abbreviations: HSA, helicase-SANT–associated; Pi, inorganic phosphate.

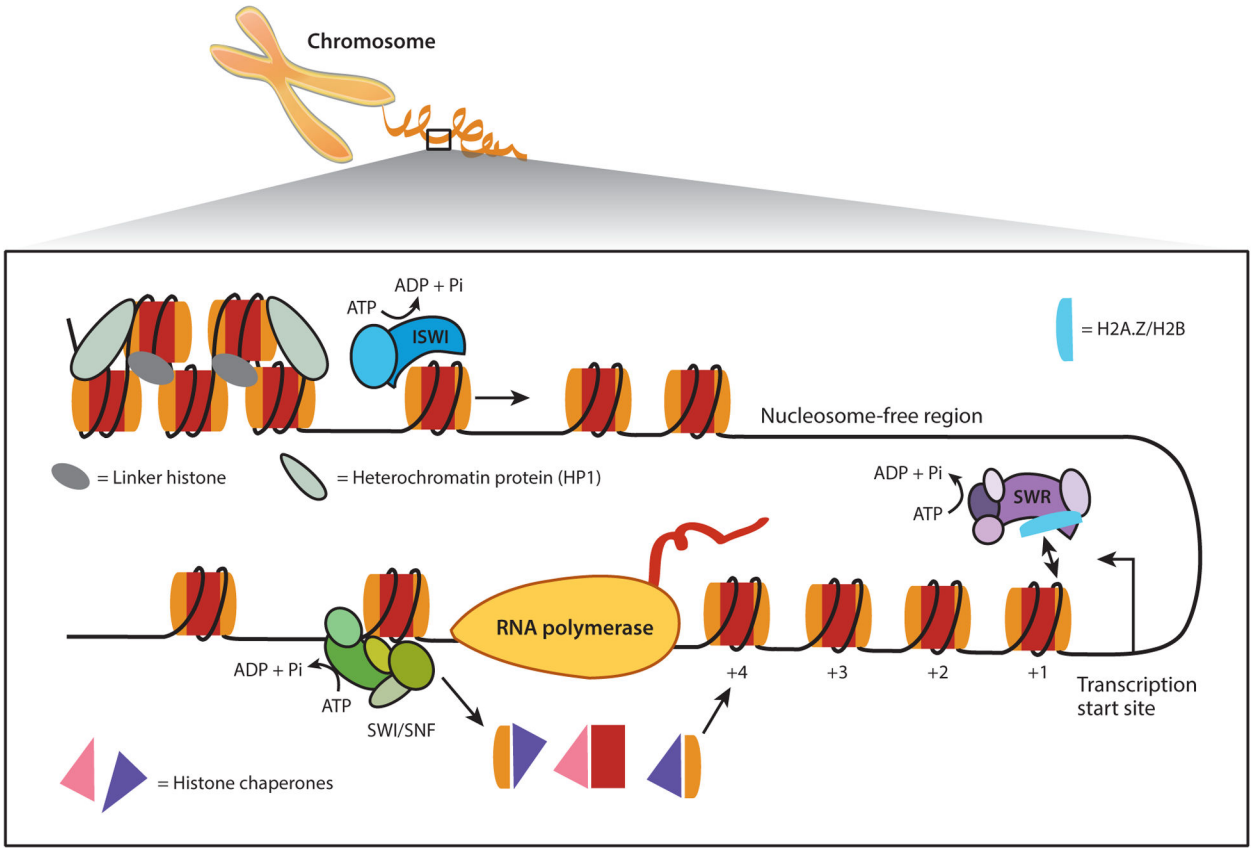

Figure 6.

In vivo, different families of remodeling enzymes collaborate with one another and with other nuclear factors to regulate gene expression. The mechanisms behind this collaboration are not well understood and are an area of continuing interest in the field. Abbreviation: Pi, inorganic phosphate.