Published in final edited form as:

Nat Struct Mol Biol. 2007 November ; 14(11): 989–996. doi:10.1038/nsmb1333.

Chromatin remodeling: insights and intrigue from single-molecule studies

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Abstract

Chromatin remodelers are ATP-hydrolyzing machines specialized to restructure, mobilize or eject nucleosomes, allowing regulated exposure of DNA in chromatin. Recently, remodelers have been analyzed using single-molecule techniques in real time, revealing them to be complex DNA-pumping machines. The results both support and challenge aspects of current models of remodeling, supporting the idea that the remodeler translocates or pumps DNA loops into and around the nucleosome, while also challenging earlier concepts about loop formation, the character of the loop and how it propagates. Several complex behaviors were observed, such as reverse translocation and long translocation bursts of the remodeler, without appreciable DNA twist. This review presents and discusses revised models for nucleosome sliding and ejection that integrate this new information with the earlier biochemical studies.

Many processes involving chromosomes are guided by DNA-binding proteins, and the access of these proteins to DNA is regulated by chromatin. Nucleosomes, the primary repeating unit of chromatin, package DNA by wrapping 147 base pairs (bp) around an octamer of histone proteins in ~1.7 helical turns^{1–3}. This packaging provides topological order but occludes one face of the DNA, which slows or prevents the recognition of DNA sequences by DNA-binding proteins. To maintain topological order, and also to allow rapid and regulated access to the DNA, cells have evolved a set of chromatin-remodeling machines that alter nucleosome position, presence and structure.

Remodelers can be separated into several families on the basis of their composition and activities: SWI/SNF, ISWI, NURD/Mi-2/CHD and the ‘split ATPases’ INO80 and SWR1 (which bear an insertion within their ATPase domains). Rad54 may represent an additional family, as it perturbs chromatin *in vitro*, but it apparently lacks specific nucleosome-interacting domains^{4,5}. The participation of remodelers in various chromosomal processes has been the subject of many reviews^{6–9}. The present work focuses solely on remodeler mechanisms, and primarily on the function of their conserved catalytic subunit, a superfamily 2 (SF2) ATP-dependent DNA translocase. Recently, single-molecule approaches have been used to examine several remodelers, providing many new insights into their behavior. Here I discuss these recent studies, compare them with previous biochemical studies and suggest refinements to existing models to account for some of the observations.

Remodelers slide, eject and restructure nucleosomes

Remodelers can affect nucleosomes in at least four ways (Fig. 1): (i) sliding, or moving the histone octamer to a new position, which exposes DNA^{10–12}; (ii) ejection, or completely displacing the octamer to expose DNA^{13–16}; (iii) removal of H2A-H2B dimers, leaving only the central H3-H4 tetramer, which exposes DNA and destabilizes the nucleosome^{17,18}; and (iv) dimer replacement—for example, exchanging the resident H2A-H2B dimers for dimers

containing H2B and the histone H2A variant H2A.Z (termed Htz1 in *Saccharomyces cerevisiae*; ref. ¹⁹). In yeast, Htz1 nucleosomes are highly enriched at gene promoters^{20,21} and help promote gene activation and efficient DNA repair.

Remodelers share common properties, but they are also specialized for particular tasks. Most remodelers can slide nucleosomes, though with very different efficiencies and outcomes: most ISWI complexes promote the equal spacing of DNA between each nucleosome on the template^{22,23}, whereas SWI/SNF remodelers randomize the positions of nucleosomes on arrays that were initially evenly spaced²⁴. Furthermore, SWI/SNF-family remodelers can eject nucleosomes^{13,25}, whereas ISWI-family remodelers lack this activity. Specialization is further supported by the different roles and phenotypic effects of remodeler families *in vivo*⁹. Dimer ejection has been observed with all SWI/SNF remodelers tested, but with only a subset of ISWI remodelers^{17,18}. Perhaps the most specialized function of remodelers involves histone-variant exchange. Only SWR1 efficiently replaces H2A-H2B dimers with Htz1-H2B dimers *in vitro*¹⁹, and new *in vivo* evidence supports a role for the INO80 remodeler in the reverse reaction, which may be important for recovery from DNA repair²⁶.

Notably, both sliding and ejection require the breakage of all 14 histone-DNA contacts (though they are re-formed during sliding), which would require a total of $\sim 12\text{--}14$ kcal mol⁻¹ (~ 1 kcal mol⁻¹ per contact)²⁷. These thermodynamic properties underlie the very slow translational movement of nucleosomes *in vitro* by thermal diffusion²⁸ and their extremely slow rates of spontaneous disassembly and ejection. These properties also establish the energetic obstacles that remodelers must overcome and are important for interpreting the measurements obtained in single-molecule experiments (Fig. 2).

Remodelers can translocate DNA from a fixed internal site

To understand remodeler mechanisms, one must know how remodelers couple the hydrolysis of ATP to the breakage of histone-DNA contacts. Initial models proposed two ways to disrupt histone-DNA contacts: movement of the remodeler around the nucleosome or, alternatively, use of ATP hydrolysis by the remodeler to induce a conformational change in the octamer²⁹. The next generation of models were based on a series of elegant experiments showing that remodelers can impart torsion to DNA^{30,31} and that remodelers themselves undergo ATP-dependent conformational changes to expose DNA³²⁻³⁴. These studies initially proposed that the ATPase domain interacts with DNA just outside the nucleosome and that a twisting force or a conformational change pushes a DNA loop (or DNA torsion) into the nucleosome³⁰⁻³⁴. These models were intuitive and provided important initial concepts. However, all remodeler ATPases are classified as members of the SF2 family of DEAD/H-box helicase/translocases, and a significant advance was the demonstration that remodelers are indeed ATP-dependent DNA translocases^{4,5,35-38}. This indicated a possible motor force for breaking histone-DNA contacts and moving DNA on the octamer surface. Notably, remodeler ATPases lack the 'pin' motif required for DNA strand separation and are therefore DNA translocases but not helicases^{5,39}. One consistent feature of SF2 translocases is their tracking along the phosphate backbone of one of the two DNA strands, with limited interaction with the bases themselves^{39,40}. Consistent with the notion of tracking, the results of triplex-displacement assays suggest that SWI/SNF, ISWI and Rad54 track in a 3'-to-5' direction along one strand of the DNA duplex and are impeded if the tracking strand bears a gap in the phosphodiester backbone^{4,36,38,41}. Furthermore, a series of studies on chromatin assembly by the ACF remodeler (ISWI family) reveal that chromatin is assembled in a processive manner that involves DNA template commitment, consistent with the use of a DNA-tracking mode for the assembly process⁴².

An important mechanistic issue is how DNA translocation is applied—whether the remodeler translocates along the DNA by moving around the nucleosome, or whether the remodeler ATPase binds the histone octamer in a defined location and then engages and pumps DNA from that location around the octamer. Through a variety of approaches, several elegant studies have established the location on the nucleosome from which translocation is initiated. Cross-linking studies show that the ISWI ATPase binds the nucleosome at two locations, (i) on the linker DNA near the nucleosome entry/exit site, and (ii) at an internal site about two turns from the nucleosomal dyad, the central position of the nucleosome (see Fig. 3a). Moreover, the remodeling activity of both SWI/SNF and ISWI remodelers is greatly reduced if the nucleosome contains nicks or base gaps (in one strand of the DNA) at the internal position about two turns from the dyad^{38,41,43,44}, suggesting that this is a common location for ATPase interaction. Gaps also inhibit SWI/SNF and ISWI remodeling if they are located at this internal location or just to one side of it, between the binding site and the proximal entry/exit site³⁸ (Fig. 3a). Furthermore, during remodeling by either SWI/SNF or the SWI/SNF-family remodeler RSC, a small gap placed in various locations on the nucleosome will move from its initial position to a defined final position about two turns from the dyad^{38,41}. Finally, mononucleosomes remodeled by SWI/SNF or RSC move the free DNA end to a final position about two turns from the dyad^{28,38,41,45}. Together, these observations strongly suggest that the translocase domain pumps DNA from this internal position on the nucleosome and that the arrival of a DNA gap or end at this position stops translocation^{38,41,43,44}. This model has not been tested for Rad54; as Rad54 lacks specific nucleosome-interaction domains, current models favor the idea that Rad54 perturbs nucleosomes by exerting torsional stress when it encounters a nucleosome while translocating^{5,46}.

There are important similarities between the current models for SWI/SNF and ISWI mechanisms, as well as clear differences (Fig. 3). Certain models for RSC⁴¹ suggest that the translocase domain (Tr, green, Fig. 3c) initiates translocation near the dyad by pulling in small segments of DNA (~1 bp) and pumping them toward the dyad (Fig. 3c, arrow). This creates a small DNA loop near the dyad, which can then propagate around the distal part of nucleosome by one-dimensional diffusion^{9,41} (not depicted). The internal translocase has been proposed to function as an internal DNA ratchet as well as a DNA pump, enforcing the movement of DNA in one direction, toward the dyad⁴¹. In contrast, ISWI has been proposed to use the internal site to initially translocate DNA toward the proximal entry/exit site, a direction opposite to that of the net movement of DNA around the octamer³⁸ (Fig. 3a). To compensate for this initial motion, there is a counteracting larger conformational change in which a DNA-binding domain (DBD) pushes a DNA segment from the linker into the nucleosome, toward the dyad^{43,44,47} (Fig. 3a). Indeed, ATP-dependent conformational changes have been observed with ISWI³⁴. Together, these motions create a constrained DNA loop on the nucleosome surface between the dyad and the entry/exit site. The internal DNA-tracking domain is then proposed to disengage to allow the DNA loop to pass through to the opposite side of the nucleosome, enabling loop propagation. Below, I consider a synthesis of the ISWI and SWI/SNF models.

The mechanical principles of DNA tracking, pushing, pulling and loop formation are ripe for examination by single-molecule methods. At present, single-molecule studies with ISWI have not been reported, and these are eagerly anticipated. However, several recent studies have been performed with SWI/SNF remodelers and Rad54, which may reveal properties of the whole family.

Single-molecule analysis of remodeler mechanisms

Single-molecule techniques have transformed the analysis of molecular motors, and they can measure the physical parameters associated with DNA translocation, including translocation

speed, force, processivity and induced twist. These parameters are of central importance for deriving models of remodeling. For example, single-molecule experiments involving single nucleosomes have shown that a stretching force of 2–3 pN is sufficient to disrupt the outer wrap of DNA on the histone octamer, whereas disrupting both the outer and inner wrap requires a force >20 pN (ref. ⁴⁸). How much force can a remodeler impose, and how fast is the motor? In recent single-molecule studies, the DNA translocation and force parameters of the remodelers SWI/SNF, RSC and Rad54 have been measured directly. Each study used a different single-molecule technique: two studies monitored the ability of the remodeler to form DNA loops (via translocation) within a single DNA molecule (or single nucleosome) in real time^{49,50}, two studies imaged remodeler movement along DNA in real time^{46,51}, and one study analyzed single nucleosomes after SWI/SNF action⁵² (see Fig. 2 legend for an explanation of each method).

Magnetic tweezers

Lia *et al.*⁴⁹ used magnetic tweezers with a tethered single DNA molecule (Fig. 2a) to show that a single RSC complex creates a confined, negatively supercoiled DNA loop. In principle, a loop can be formed if a remodeler has two DNA-interaction domains: a static DNA-interaction domain and an ATPase/translocase domain. Movement of the translocase domain creates a confined DNA loop that shortens the apparent end-to-end length of the DNA molecule. The studies of Lia *et al.*⁴⁹ provided the first direct observations of DNA translocation by a remodeler in a single-molecule experiment. RSC translocated DNA at high speeds (200 bp per second) and for considerable distances (averaging ~420 bp) under conditions of very low tension (0.3 pN). Curiously, the processivity of RSC on free DNA in stopped-flow conditions (bulk measurements) was ~20 bp⁵³, and bulk length-dependent ATPase assays estimated the average translocation distance at ~20–25 bp (when the binding footprint and initial binding position were subtracted). Currently, it is not clear why the bulk processivity measurements are so remarkably different from the single-molecule processivity measurements. One possible explanation is the different experimental conditions in the bulk and single-molecule studies. Alternatively, the long distances observed in the magnetic-tweezers experiment might represent an infrequent but exceptionally processive mode of translocation, with the average distance translocated falling below the detection limit of the instrument (~50 bp).

The magnetic-tweezers method also can be used to monitor the amount of DNA twist that accompanies DNA translocation. As both strands of the DNA are tethered to both the slide and bead, bead rotation can be imposed by the instrument operator to generate a twisted DNA template upon which subsequent translocation and twist by the remodeler can be examined. Once a crucial number of turns are placed into the DNA, the imposition of additional twist causes the DNA to writhe; one twist causes one supercoiled loop, and additional twist rotations cause an equal number of additional writhes within the supercoiled extruded loop (also termed a plectoneme). Rotation can be imposed in either direction, to induce either negative or positive supercoiled loops in the DNA, which reduce the apparent end-to-end length of the DNA. This allows the examination of remodeler behavior on templates that initially contain either positive or negative plectonemes. For example, if the remodeler creates a constrained negatively supercoiled loop during translocation, this will impart positive supercoils to the flanking DNA, adding plectonemes to templates initially bearing positive plectonemes and reducing plectonemes on templates initially containing negative plectonemes.

This type of analysis demonstrated that loops confined by RSC contained negative supercoils⁴⁹, as RSC translocation imparted positive supercoils or twist to the flanking region (not depicted in Fig. 2a). Interestingly, RSC imparted only one superhelical twist for every ~100 bp translocated, suggesting that translocation by RSC does not involve the processive

tracking of the translocase domain along the phosphodiester backbone, a feature typical of other SF2-family translocases (discussed further below). Also surprising were events where translocation paused and then changed direction, causing the gradual retraction of the DNA loop. However, as translocation events were not observed at tensions above 0.3 pN, one might reasonably question whether the RSC motor truly has the power to disrupt histone-DNA contacts. The possibility remained that RSC simply needed anchoring to the nucleosome to apply sufficient force—an issue addressed in experiments described below.

Optical tweezers

To address this and other questions, Zhang *et al.*⁵⁰ used optical tweezers (Fig. 2b) to monitor the action of SWI/SNF and RSC complexes on nucleosomes in real time. Although this method does not monitor DNA twist or supercoiling, it allows both monitoring and control of force conditions, and the particular instrument used could detect DNA-shortening events of ~25 bp. Remarkably, SWI/SNF and RSC both caused DNA shortening events, which were interpreted as resulting from the formation of DNA loops on the surface of the nucleosome (Fig. 2b). DNA was translocated at ~13 bp per second and for distances averaging ~105 bp under a moderately high tension range (3–7 pN). The ability of remodelers to provide a constant rate of DNA translocation under increasing and fairly great force suggests that remodelers function as motors rather than simply as ratchets that bias the thermal motion of nucleosomes. The slower rates of translocation on nucleosomes could be due to differences in conditions or, more likely, to the obstacle that the nucleosome presents to DNA movement. Translocation ceased at tensions >12 pN, the motor ‘stall force’. Interestingly, as in the magnetic-tweezers study⁴⁹, there was evidence of pausing and reverse translocation, the ATP-dependent retraction of the DNA loop. Thus, SWI/SNF and RSC translocate DNA loops onto nucleosomes, and they impart sufficient force to disrupt several histone-DNA contacts, but not a ‘rip force’ that can disrupt all the contacts at once (requiring >20 pN). This is consistent with most remodeling models^{41,44,45,54-56}, which propose that a DNA loop is created when an initial disruption of contacts on the outer wrap then propagates around the remaining portion of the nucleosome by diffusion. This requires only a small subset of histone-DNA contacts to be disrupted in propagation intermediates (see Fig. 3). Two notable limitations of the optical-tweezers study were that DNA loops <25 bp could not be clearly resolved from instrument noise and that the frequency of formation of large loops was lower than previous bulk measurements of DNA sliding frequencies collected in similar enzyme concentrations. Therefore, the possibility remains that DNA loops <25 bp could be generated.

Direct visualization

Two studies directly visualized the translocation of two different Rad54 derivatives along single or multiple DNA molecules. The studies used fluorescently tagged Rad54, an anchored and stretched DNA molecule or a ‘curtain’ of DNA molecules, and fluorescence microscopy to monitor movement of Rad54 along DNA in real time (Fig. 2c)^{46,51}. Both studies provide clear evidence of DNA translocation, namely rapid median translocation rates (80 or 300 bp per second) and high processivity (11 or 14 kbp), though force cannot be measured by this method. As with SWI/SNF remodelers, complex behaviors of pausing and reverse translocation were again observed, suggesting that these behaviors are shared by the related translocase motors.

Nucleosome unzipping

A fifth study analyzed single-nucleosome products after remodeling by SWI/SNF, using a complex but powerful single-nucleosome ‘unzipping’ technique⁵² (Fig. 2d). This technique can determine precisely (within 3 bp) the distance that the nucleosome moves after a short exposure to SWI/SNF, and it can also detect H2A-H2B dimer release. However, it provides a

precise product analysis of a remodeled nucleosome rather than a real-time measurement of remodeling. In this method, a single nucleosome is positioned on a strong nucleosome-positioning sequence (termed 601) within a larger DNA fragment. At one end of the nucleosome template, both DNA strands are tethered, one to a glass coverslip and the other to a bead held in an optical trap, which can monitor force. Movement of the coverslip ‘unzips’ the duplex DNA, and the force is measured as a function of the distance moved (in bp). The DNA unzips at a fairly constant force until a nucleosome is encountered, whereupon the nucleosome-DNA contacts increase the force required for DNA unzipping, with different positions on the nucleosome showing distinctive force signatures. A plot of force against base pairs unzipped indicates the location of the nucleosome on the DNA template either before or after remodeling. The plot also shows whether the remodeled nucleosome is physically similar to (that is, has the same force signature as) non-remodeled nucleosomes, which can reveal whether the H2A-H2B dimer(s) remain after remodeling.

After exposure to a molar equivalent of SWI/SNF for <1 minute, nucleosomes moved an average of 28 bp, with a very wide distribution of movement distances. One interpretation is that only a single remodeling event, involving a single DNA movement or loop, occurred with each nucleosome, and the lengths of these are distributed broadly around 28 bp. Another interpretation is that each nucleosome had a varying number of smaller movements or loops that summed up to 28 bp on average. A third possibility is that remodeling allowed the nucleosome to sample the DNA curvature in a very broad area around the initial position, with a location 28 bp away being slightly favored over other alternative positions. The latter possibility must be seriously considered, as the DNA sequence chosen for examination was a nucleosome-positioning sequence that strongly resists movement around itself, restricting the movement to units of ~10 bp, because of the sequence's intrinsic curvature. Regardless, the large movements of the nucleosome observed in the optical-tweezers experiments described above were not observed in this study, raising the possibility that formation of large DNA loops is not the most common remodeling event. Notably, no dimer loss was observed during these experiments (148 events), suggesting that dimer release is extremely infrequent with the reagents, conditions and template used.

Together, these data show that DNA translocation is a constant feature of this class of enzymes, although different methods and remodeler family members reveal different macroscopic behaviors. However, there are also consistent features that can be incorporated into new models for remodeling, once the relationship of these enzymes to known DNA translocases has been discussed.

Remodeler ATPases resemble ‘DNA inchworms’

To understand how remodelers translocate DNA on nucleosomes, one must understand the domain motions and interactions within the ATPase/translocase, how they engage and disengage DNA on the nucleosome surface and how domain dynamics are regulated by ATP hydrolysis. Crystal structures, biochemical studies and single-molecule analyses of both SF1- and SF2-family translocases (PcrA, RecG, UvrD, NS3 and others) have provided many important insights into these issues^{39,40,57-59}. Currently, there is no crystal structure of the ATPase domain of a nucleosome-binding remodeler (SWI/SNF or ISWI), but recent structures of the SWI/SNF-family member Rad54 (with DNA) show remarkable structural homology to the SF2 family of RNA and DNA translocases (see Fig. 3b)^{5,60}. I will next summarize mechanistic insights from the translocase field and then apply those concepts to derive models for the movement of DNA on nucleosomes.

Although there is some diversity in their mechanisms, most monomeric SF1 and SF2 translocases conform to a ‘DNA-inchworm’ model of movement, which involves the

coordinated movement of two domains: a DBD and a DNA-tracking domain^{39,40}. The DNA-tracking domain has two RecA-like motifs, and between them is a pocket for nucleotide binding as well as a platform for DNA interaction. Nucleotide binding and hydrolysis induce conformational changes between the two RecA-like motifs that result in the net movement of the tracking domain 1 bp in the 3'-to-5' direction along one of the DNA strands, termed the tracking strand^{58,61}. The DBD binds nucleic acid in front of the DNA-tracking domain and undergoes a conformational change that is coordinated with those of the tracking domain. For many translocases, the DBD releases from the DNA, steps forward and binds tightly again in an advanced position. This provides a new anchoring point on the DNA, to which the tracking domain then advances through ATP hydrolysis⁵⁹. After advancement, the tracking domain stops and the DBD again steps forward, yielding an inchworm-like movement. An alternative (but similar) mechanism starts with the DBD already bound at an advanced position, from which it then actively pulls DNA toward the tracking domain, essentially feeding nucleic acid substrate into the tracking domain for subsequent tracking. Once the tracking domain tracks through this DNA, the DBD then steps forward, completing the inchworm cycle⁵⁸. The key difference between these two similar models is whether the DBD in front of the tracking is a passive DNA anchor or whether it instead provides an active DNA-pulling force that feeds DNA into the tracking domain.

The DNA-twist conundrum

It is tempting to apply one of the simple versions of the DNA-inchworm model described above to explain DNA translocation by remodelers on nucleosomes. However, the histone octamer presents unique challenges. First, it presents the same steric challenge to a translocase that it does to a site-specific DNA-binding protein: the occlusion of one face of the DNA by the histone octamer. The SF2 ATPase/translocase domain is remarkably well suited for interaction with nucleosomes, as it binds only one face of the DNA (Fig. 3b) and can move DNA 1 or 2 bp at a time^{5,57,59}. However, the current understanding of translocation by SF2 translocases involves the directional tracking (typically 3'-to-5') of the translocase domain along the backbone of one strand of the DNA duplex (the tracking strand)^{59,62,63}, and this domain would encounter a steric clash with the octamer after tracking 1 or 2 bases. A simple application of the inchworm model using this tracking mode would require a full rotation of the DNA for every ~10 bp of translocation to maintain continuous association of the translocase domain with the tracking strand. However, several experimental results have now thrown into question whether this degree of DNA twist is generated during remodeling. As described above, RSC apparently imparts one helical twist for every ~100 bp translocated⁴⁹. Furthermore, biochemical data from Strohner *et al.*⁵⁴ show that ISWI remodelers can slide a nucleosome 40–50 bp that has a bead tethered to a biotin group located on a DNA base near the nucleosomal dyad. As Strohner *et al.*⁵⁴ suggest, this tethering to the bead should prevent a full DNA rotation during remodeling by ISWI. Furthermore, the ~100-bp DNA loops formed on nucleosomes in the optical-tweezers experiments⁵⁰ would bear about ten full negative turns if they were formed by processive backbone tracking, a twist force that should cause either DNA melting or release of the DNA from the nucleosome.

These observations have prompted remodeling models that enable DNA movement without large amounts of DNA twist. The proposed models focus on the use of conformational changes to push a segment of DNA into the nucleosome^{34,38,44,45,54-56}. Above, I mentioned a model for remodeling by ISWI that combines DNA translocation occurring from an internal position with a conformational change to push DNA into the nucleosome^{34,38,44,45,54-56} (Fig. 3a). There are many positive features of this model, and it accounts for many aspects of the current data. A related model has been proposed for the action of SWI/SNF³⁸. However, these models propose an initial movement of DNA in the direction opposite to that of net DNA movement around the octamer, and they do not account for the quantitative measurement of twist (with

RSC). Likewise, previous models proposing that SWI/SNF and RSC form a DNA loop by processively tracking along DNA from an internal site^{9,41} account for much of the existing data, but not for the imposition of one negative turn per 100 bp. Next, I present a speculative revision of the existing models that includes proper initial and net DNA movement, conforms to the general tracking properties of SF2 translocases, yields the observed single turn of twist per 100 bp and suggests a related basic mechanism for both SWI/SNF and ISWI enzymes.

A DNA inchworm on the nucleosome surface

Here I consider a modified inchworm model that would allow an SF2 translocase to contend with the steric constraints of the nucleosome surface and adhere to the general SF2 property of ATP-dependent backbone tracking using the RecA-like domains, but impart only ~1 bp of twist during the translocation of about one DNA helical repeat. For consistency, my presentation will adhere to the general inchworm model outlined above, but the modified model includes coordinated cycles of tracking and conformational change.

I begin by applying the model to a SWI/SNF-family remodeler, focusing on domain movements on nucleosomal DNA located in the region about two turns from the dyad (Fig. 3c,d), and then adapt the model to ISWI (Fig. 3a). First, SWI/SNF binds the histone octamer tightly. The tracking domain (Tr, green), postulated to be the conserved ATPase domain shown in Figure 3b, remains in a fixed position relative to the histone octamer. In contrast, the DBD (purple) alternates between one of two defined positions, rotating through a hypothetical hinge domain (H) affixed to the nucleosome (Fig. 3c,d). To begin, the tracking domain binds tightly to the DNA located about two turns from the dyad, whereas the DBD binds loosely at a location about one turn ahead (10 bp toward the entry/exit) (Fig. 3d, line 1). In this region of the nucleosome, the helical repeat is close to 10 bp per turn. Next, the tracking domain pulls or tracks 1 bp of DNA toward the dyad; the DNA moves and rotates 1 bp while the tracking domain remains in a fixed position relative to the histone octamer (Fig. 3d, line 2). The DBD then steps 10 bp (one DNA helical repeat) along the DNA, binding tightly to this new advanced position, which places the DBD 20 bp from the tracking domain (Fig. 3d, line 3). The translocase domain then loosens its grip on the DNA. However, the torsional strain (and the added base pair) remains constrained by the DBD, which serves as an internal DNA ratchet. The DBD then undergoes a conformational change, pulling in 10 bp (one helical repeat) during its return to its original position on the histone octamer (Fig. 3d, line 4). This requires that four histone-DNA contacts between this position and the entry/exit site be entirely broken during the pulling action. However, the amount of energy necessary to break four contacts is considerably less than that provided by a cycle of ATP hydrolysis. The tracking domain then allows one 10-bp helical repeat to pass through it, and it then reengages the next helical repeat, which has been delivered in an appropriate 10-bp register (Fig. 3d, line 5).

This can be termed the '1+10 ratchet': 1 bp of DNA with one-tenth of a turn of negative twist is contributed by tracking, and a twist-neutral 10 bp is then contributed by a conformational change, causing the injection of a total of 11 bp of DNA into the dyad region. At that location, the strained 11-bp DNA loop or wave then propagates around the nucleosome by one-dimensional diffusion and resolves in the distal linker, as proposed previously^{9,35,41}. This model yields about one negative turn for each 100 bp translocated. Moreover, the placement of the tracking domain near the dyad helps ensure directional movement of the DNA loop by providing an anchoring point to hold DNA while the DBD releases and moves to an alternative position; the DNA must always be held tightly at either the DBD or the tracking domain to constrain the loop. Thus, a simple conformational change that pushed 10 bp of DNA into the nucleosome would not suffice, nor would it impose the modest negative twist observed in the single-molecule study of RSC⁵⁰.

This model can easily be adapted to the current ISWI model, which already has several of these features (Fig. 3a). First, ISWI has a DBD that binds near the proximal linker^{47,64}, and particular ISWI-interacting proteins are known to assist ISWI in linker recognition and may help regulate the spacing between nucleosomes⁶⁵⁻⁶⁷. The DBD and the proposed conformational change that moves DNA 10 bp can, in principle, be placed at any helical-repeat position between the tracking domain and the proximal linker, because a net movement of 10 bp can be imparted from an upstream position (Fig. 3a) as easily as from a proximal position (Fig. 3c,d). For ISWI, this net movement may be a 10-bp movement of the DBD attached to the DNA linker (Fig. 3a)^{9,38,44}. Importantly, measurements of the kinetics of DNA movement by ISWI indicate an initial movement of DNA by ~10 bp, supporting a 10-bp step size for remodeling^{38,44}. Interestingly, most ISWI enzymes will not remodel nucleosomes that lack linker DNA⁴⁷. Indeed, ISWI enzymes seem to be regulated by both linker DNA and a portion of the histone H4 tail^{38,68,69}, whereas these epitopes are not crucial for remodeling by SWI/SNF enzymes.

One notable difference remains between the 1+10 model proposed here (Fig. 3c,d) and previous models: the direction of initial DNA movement previously proposed for ISWI near the dyad (Fig. 3a)³⁸ is opposite to that proposed in the present model (Fig. 3c,d). However, for the ISWI model presented in Figure 3a, if one assumes that DNA moves through the anchored tracking domain, rather than having the ATPase move along the DNA toward the entry/exit, then the initial movement of the DNA through the tracking domain is indeed toward the dyad. This interpretation reverses the direction of the 'DNA translocation' arrow in Figure 3a, restricting DNA movement to the direction depicted in Figure 3c,d.

In summary, the revised 1+10 ratchet model maintains the tracking feature of SF2 translocases while accounting for the observed limited twist, and it also posits the same direction for both initial and net DNA movement. The model is an attempt at synthesis; it is based on current data as well as the notion that SWI/SNF and ISWI probably share certain mechanistic features, as suggested by the similarity of their ATPases. However, models with somewhat less mechanistic overlap can also be derived to fit most of the existing data. Furthermore, certain aspects of the model are speculative and await experimental validation. Indeed, high-resolution (<5 bp) analysis of remodeling by SWI/SNF and ISWI in single-molecule experiments will be important for testing these and other models.

Remodeler structure-mechanism relationships

Three separate studies have provided structures of the RSC complex that can be examined in light of the mechanistic studies. Each structure was solved by electron microscopy, though with different methods of image reconstruction⁷⁰⁻⁷². Although the structures are moderately different, each reveals a large pocket of approximately the dimensions of a nucleosome, though the placement and orientation of the nucleosome can only be inferred by modeling. One structure shows a deep central pocket that is only slightly larger than a nucleosome and has a geometry in which only one nucleosome orientation could avoid steric clashes with the entry/exit DNA⁷¹. In this model, RSC envelops most of the nucleosome, except for the face bearing the dyad and entry/exit sites. In two other structures, the pocket resembles a claw or pincer that also accommodates a nucleosome well^{70,72}. However, in all three structures, the propagation of a large loop around the entire nucleosome would require a large change in RSC structure (or release of the nucleosome), as the putative RSC-nucleosome contacts constitute a steric block at one or more locations to the propagation of a large extruded loop. One solution could be conformational flexibility that would allow a portion of the remodeler to release from a portion of the nucleosome, allowing loop passage, and then to reengage. Notably, conformational flexibility is a feature of the remodeling models described above and previously. Another solution to the steric challenge to loop propagation would be for the remodeler to typically use small loops (<20 bp) for sliding, with large loops representing the

occasional build-up of small loops that fail to propagate. As described above, these larger loops might resolve by partial or complete remodeler disengagement; however, the single-molecule experiments suggest that an additional mechanism might be used, as described below.

Reverse translocation: switching direction or switching tracks?

A particularly intriguing observation from the single-molecule studies was the occasional retraction of extruded DNA loops at almost the same rate as their creation^{49,50}. This raises several interesting questions. Perhaps remodeler motors also have a transmission function—a ‘reverse gear’ that allows them to run the motor backwards to conduct 5'-to-3' tracking. Another possibility is that the translocase domain switches strands—that is, it disengages, flips around, engages the other strand in the same DNA duplex and then translocates the second strand 3'-to-5'. Although formally possible, this would require remarkable conformational flexibility in the remodeler translocase domain. A further possibility is that the translocase simply switches DNA gyres on the nucleosome: moving from the upper to the lower DNA gyre would cause the extruded loop to be pumped out of the dyad region and into the proximal linker by precisely the same mechanism that created it. However, the frequency of reverse translocation was actually more common in the experiments involving naked DNA than with nucleosomes, suggesting that the gyre proximity provided by the nucleosome is not required for reverse translocation.

Nucleosome ejection: pulling the rug from under your neighbor?

Remarkably, SWI/SNF remodelers are able to eject a nucleosome and also to transfer the histone octamer to another DNA molecule *in vitro*^{13,25}. Studies of the yeast *PHO5* gene suggest that nucleosomes are removed from the promoter upon activation^{15,16}. Interestingly, genome-wide analyses show that promoters are intrinsically nucleosome deficient and become more deficient during activation^{73,74}. Furthermore, promoter remodeling is considerably slower in strains bearing mutations in components of the SWI/SNF complex⁷⁵.

A key question is how remodelers perform ejection. One possibility is that the DNA loops created on the nucleosome surface partially destabilize the nucleosome, lowering the energy barrier for histone chaperones to remove either the dimers or the whole octamer¹⁸. Loops of larger size may also provide the opportunity for another DNA molecule to invade the open histone-DNA contacts and spool onto the nucleosome while the resident DNA spools off, yielding octamer transfer^{13,25}.

The single-molecule studies described above show that remodelers can use a highly processive translocation mode to move hundreds of base pairs. *In vivo*, nucleosomes are flanked by other nucleosomes, typically separated by internucleosomal (linker) distances of 10–50 bp. This suggests another possible mechanism for promoting either dimer release or full octamer ejection: processive DNA translocation by a remodeler–nucleosome complex would spool DNA from the surface of the neighboring nucleosome, causing either dimer release or full ejection of the neighboring octamer.

Summary

Examination of remodeler behavior in single-molecule experiments has verified that remodelers are DNA translocases and has also suggested that DNA translocation is a highly complex process. The structure of the nucleosome presents certain challenges for DNA movement that require these complex behaviors. Existing models have provided an excellent foundation for understanding remodeler action, and the revisions suggested here add features that would help ensure processive directional movement of DNA around the octamer, and that would also increase the conceptual similarity of the mechanisms of ISWI and SWI/SNF

remodelers. However, as each remodeler alters nucleosomes in a particular manner, each probably tailors DNA translocation for its specific task and has a unique mode of regulation, including the recognition of particular epitopes on the nucleosome. Furthermore, processive DNA translocation leading to nucleosome sliding or ejection is not the goal for all remodelers: SWR1 may need only limited DNA movement to effect histone dimer exchange. Future work that applies single-molecule techniques at high resolution to other remodeler families (such as ISWI, INO80 and SWR1) will probably reveal additional surprises and regulatory insights.

Acknowledgments

I thank R. Viswanathan for considerable help in making figures, and J. Wittmeyer for helpful discussions and comments on the manuscript.

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Figure 1.

Modes of nucleosome remodeling. Remodelers enable access to nucleosomal DNA through sliding, ejection or H2A-H2B dimer removal. The SWR1 complex is unique in its ability to efficiently replace H2A-H2B dimers with dimers bearing a histone H2A variant (Htz1 or H2A.Z). Act denotes an activator with a binding site on the nucleosome.

Figure 2.

Single-molecule methods for observing remodeler translocation. **(a)** Magnetic tweezers tether a single DNA molecule between a glass slide and a magnetic bead. Magnets placed above the slide provide a calibrated stretching force (F_{mag}), and they can be rotated to cause bead rotation and the formation of plectonemes outside of the region bound by RSC (the formation of external plectonemes is not shown, but is discussed in the text). Translocation by RSC forms a constrained loop (which may include a plectoneme) and reduces the apparent end-to-end length of the DNA, as measured by the position of the bead (detected by video microscopy). See text for additional details. **(b)** Optical tweezers tether a single molecule of DNA (torsionally unconstrained) between two polystyrene beads⁵⁰. One bead is connected to a pipet, which is calibrated for movement along the axis parallel to the stretching force. The other bead is held by a laser (an optical trap), with the force needed to keep the bead in the center of the trap calibrated by laser power. After DNA has been stretched to a defined tension, remodeler action generates a DNA loop constrained to the nucleosome surface (Nuc), which shortens the distance between the beads, and the change in the pipet's position is directly measured. **(c)** Direct visualization of translocation. Two methods have been used^{46,51}, only one of which is depicted⁴⁶. In this method, a single DNA molecule is tethered at one end to a polystyrene bead by a biotin/streptavidin linkage, anchored by an optical trap and stretched by laminar flow. Yeast Rad54 was labeled with fluorescein isothiocyanate (FITC, via an antibody) and movement (+ATP) was visualized with an epifluorescence microscope. See ref. ⁵¹ for a description of a related and highly elegant technique involving stretched 'DNA curtains'. **(d)** Analysis of nucleosome remodeling products by DNA unzipping. This technique reports the

position of a nucleosome on a DNA fragment, and its structure, by measuring the force required to separate the two DNA strands⁵². See text for details. ss, single-stranded; ds, double-stranded; Dig, digoxigenin; Bio, biotin.

Figure 3.

Models for DNA movement around nucleosomes. **(a)** Model for remodeling by ISWI, with contributions from many studies^{34,38,44,45,54-56}. See text for details. A DNA loop is formed on the nucleosome surface by the concerted action of a DNA translocase (Tr) domain located near the dyad and a DBD located in the linker, near the nucleosome entry/exit site. DNA release by the Tr domain enables loop passage, and the complex then resets in the original position. **(b)** Structure of Rad54 ATPase domain⁵ with the two RecA-like regions in red and green. Interaction is limited to one face of the DNA, enabling interaction with nucleosomal DNA. **(c)** The 1+10 ratchet model, depicting the relative orientations of key domains on the nucleosome. The Tr domain remains in a fixed position relative to the histone octamer, whereas the DBD alternates between one of two conformations, through a flexible hinge (H). **(d)** The 1+10 ratchet model (see text). Triangle and square mark fixed positions as a reference for DNA movement relative to the stationary tracking domain and mobile DBD. **c** and **d** illustrate the

same process: **c** shows top view and nucleosome context; **d** shows the movement of the DNA in the translocation cycle.