Noncovalent Modification of Chromatin: Different Remodeled Products with Different ATPase Domains

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The ability to maintain the same master regulatory gene in an "on" state in one cell lineage and in an "off" state in another cell lineage is fundamental to proper development. There is general agreement that modification of chromatin structure can contribute to this form of epigenetic regulation. Thus, a locus that must be repressed in a heritable fashion might have a specialized chromatin structure that is repressive to transcription, and the same locus in another cell lineage where it is heritably active might have a permissive chromatin structure.

THE DYNAMIC RANGE OF NUCLEOSOME STRUCTURE

To understand the roles that chromatin structure can play in epigenetic regulation, it is essential to understand the dynamic range of chromatin structure. The basic building block of chromatin, the nucleosome, is known to be dynamic: Covalent modification and noncovalent events that either move or lock the nucleosome in place can alter the stability of the nucleosome. An explosion of data generated using specific antisera and the technique of chromatin immunoprecipitation (ChIP) has shown that large regions of chromatin can be covalently modified in a manner that correlates with regulatory state and that therefore is likely to contribute to epigenetic regulatory events (Turner 2002; Fischle et al. 2003; Weissmann and Lyko 2003).

The role that noncovalent modification of the genome plays is not as well characterized. Noncovalent modification of chromatin has been strongly implicated in epigenetic events by genetic studies. Examples of this include the Polycomb and trithorax systems (Simon and Tamkun 2002). Polycomb-Group (PcG) and trithorax-Group (trxG) genes are required for maintaining expression patterns of master regulatory genes (Ringrose and Paro 2004). The expression pattern of these master regulators is established by one set of mechanisms early in embryogenesis, but must be maintained in spatially restricted patterns throughout the lifetime of any organism. Most PcG and trxG gene products that have been characterized are able to modify chromatin structure. Several of these products perform covalent modification events such as methylation or acetylation (Milne et al. 2002; Fischle et al. 2003; Schotta et al. 2004). Others modify chromatin structure without covalent modification, by creating chromatin structures refractory to transcription (e.g., PRC1) or by using the energy of ATP hydrolysis to create access to chromatin (e.g., SWI/SNF family complexes) (McCall and Bender 1996; Fitzgerald and Bender 2001; King et al. 2002; Simon and Tamkun 2002; Francis et al. 2004).

A key question concerns the dynamic range of these noncovalent modifications to the nucleosome: Do these modifications involve changes in nucleosome position, changes in nucleosome constitution, and/or changes in nucleosome conformation? It has long been appreciated that chromatin structure can be altered noncovalently. Theoretical considerations led to the hypothesis 30 years ago that transcription through the nucleosome might require or create alterations in position. Several subsequent studies have shown that transcribed genes have changes in histone content and nuclease sensitivity in vivo (Macleod and Bird 1982; Sweet et al. 1982; Weisbrod 1982). In vitro systems have shown that transcription of nucleosomal templates by RNA polymerase II can result in removal of a histone H2A/H2B dimer, providing one defined alteration in nucleosome constitution that can impact regulation (Kireeva et al. 2002).

The discovery of ATP-dependent nucleosome remodeling complexes 10 years ago demonstrated that there are complexes whose primary function is to alter nucleosome structure noncovalently (Cote et al. 1994; Imbalzano et al. 1994; Kwon et al. 1994). This class of complexes contains numerous families of complexes with distinct biological roles, prominently the SWI/SNF family and the ISWI family (Table 1). These complexes are abundant; for example, it is estimated that mammalian nuclei contain more than 100,000 copies of ISWI-family complexes and 25,000 copies of SWI/SNF-family complexes. Both of these families of complexes can create access to DNA-binding factors on nucleosomal templates (Cote et al. 1994; Imbalzano et al. 1994; Kwon et al. 1994; Tsukiyama and Wu 1995). One way that they can do this is by changing the position of nucleosomes, thereby moving a specific sequence in DNA from a position where it is inaccessible because of histone contacts to a position where it is accessible because it is in a region of free linker DNA between nucleosomes. Complexes in the ISWI family are known to be able to "slide" nucleosomes along a template, which allows them to alter spacing and also allows them

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Table 1. Functions of ATP-dependent Chromatin Remodeling Families

Family	Motor proteins	Functions
SWI/SNF	hBRG1, hBRM, dBRM, ySth1p, ySwi2p/Snf2p	Transcription DNA replication Recombination Higher-order chromatin structure?
ISWI	hSNF2h, hSNF21, dISWI, xISWI, yIsw1p, yIsw2p	Chromatin assembly Transcription Higher-order chromatin structure DNA replication Nucleotide excision repair
Mi-2	Mi2α/CHD3, Mi2β/CHD4, Chd1p, Hrp1, Hrp3	Transcription histone deacetylation
INO80	yIno80p, hINO80	Transcription DNA repair
SWR1	ySwr1p, hSCRAP, human p400, dDomino	Transcription Histone exchange DNA repair
CSB[a]	CSB/ERCC6, yRad26p	Transcription-coupled DNA repair
Rad54[a]	hRad54, hRad54B, dORK, yRad54p, hATRX, ARIP4, DRD1	Recombination Transcription DNA methylation
DDM1	DDM1, LSH1	DNA methylation

[a]Some members of these families demonstrate ATP-dependent chromatin remodeling activities in vitro; nonetheless, it is unclear if these activities represent their authentic biological functions.

to function in regulation by closing or opening regions of chromatin (Narlikar et al. 2002). In addition to regulating access and altering spacing, other ATP-dependent remodeling complexes (not the focus of this paper) are able to promote exchange of histone H2A/H2B dimers (Bruno et al. 2003; Mizuguchi et al. 2004).

While it is clear that noncovalent modification can involve changes in nucleosome position and changes in nucleosome constitution, it is not clear what structural alterations occur to the nucleosome upon ATP-dependent remodeling and whether these alterations lead to stable conformational changes in the nucleosome. In theory, there are several ways that the energy of ATP hydrolysis might alter histone–DNA contacts to create access to sites (see Fig. 7). These complexes might twist the DNA and that twisting might propagate through the nucleosome. DNA could be peeled off the edge of the nucleosome by movement of the complex into the edge of the nucleosome, or a writhe could be created that would form a bulge of DNA on the nucleosome that would propagate (see Fig. 7A). These events could transiently alter structure and lead to changes in position of a canonical nucleosome, they could transiently alter structure to create quasistable structures that are identical to canonical nucleosomes except for the inclusion of looped regions of DNA, or they could more fundamentally alter nucleosome conformation.

Based on the above considerations, two classes of hypotheses have been proposed for how noncovalent modification of nucleosome structure might occur. At one extreme, it is possible that noncovalent modification always creates remodeled products that are identical or highly related to the canonical nucleosome structure as defined crystallographically. At the other extreme, it is possible that noncovalent modification can create altered conformations of the nucleosome in which either histone–histone or histone–DNA interactions are demonstrably altered in nucleosomal structures that differ significantly from the canonical structure. Defining the dynamic range of noncovalently modified nucleosome structures is key to understanding the potential roles for the nucleosome in epigenetic regulation.

COMPARISON OF COMPLEXES IN THE SWI/SNF AND ISWI FAMILIES

There has been ongoing discussion concerning the ISWI and SWI/SNF families of ATP-dependent remodeling complexes around whether these complexes function by similar or distinct mechanisms. While it is too early to resolve this issue, the purpose here is to summarize recent data, with an emphasis on experiments done in our laboratory, concerning the characteristic behavior of these complexes in remodeling nucleosomes. We show that the ATPase domain itself plays a primary role in determining the outcome of the remodeling reaction (see Fig. 5). We use this observation and previous observations to argue in favor of the existence of nucleosomal structures that differ significantly from the canonical nucleosomal structure (Fan et al. 2003). Thus, the dynamic range of nucleosome architecture might be greater than is widely appreciated, which, if true, would significantly expand the regulatory capabilities of chromatin structural changes.

The premise of this article is that the nucleosome should not be assumed to have an unwavering stable conformation. There are a significant number of experiments whose results are difficult to reconcile with the hypothesis that dynamic changes to nucleosome structure consist solely of changes in position and/or changes in histone constitution. We begin with a summary of published experimental results that demonstrate that there are stable remodeled nucleosomal structures, and then we present data from our own laboratory that bear on the nature and genesis of these structures. The best studied example of a family of complexes that might create altered nucleosomal structures is the SWI/SNF family.

SWI/SNF-family remodeling complexes were originally identified in yeast, first via genetic studies using yeast strains that are *s*ucrose *n*on-*f*ermenters or that are defective in mating type *swi*tching (Carlson et al. 1981; Stern et al. 1984). Subsequent biochemical studies demonstrated that certain of the proteins encoded by the SWI and SNF genes form a large complex (Cote et al. 1994; Wang et al. 1996). There are several forms of this complex, most organisms having at leaast two versions; these complexes have a mass greater than 1 MD and are composed of at least 11–15 subunits (Fig. 1). The central subunit of these complexes, homologous to SWI2/SNF2,

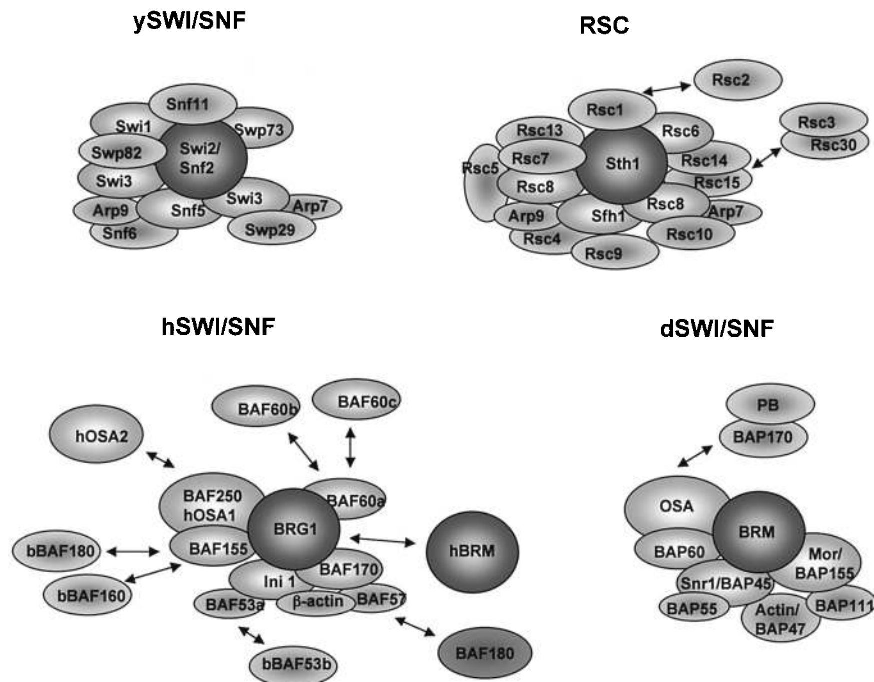

Figure 1. SWI/SNF complexes of yeast, fly, and human. Swi2/Snf2 and Sth1 are the motor proteins of the *Saccharomyces cerevisiae* SWI/SNF complexes. Human SWI/SNF complexes have either BRG1 or hBRM as the central ATPase subunit. *Drosophila* has only one SWI/SNF complex, and brama is the motor.

is the ATPase subunit, which has remodeling activity as an isolated protein (see Fig. 1). These complexes are involved in a wide variety of regulatory events on numerous genes and can be responsible for either activation or repression.

EVIDENCE FOR STABLY REMODELED STRUCTURES FORMED BY SWI/SNF

Two papers published in 1998 demonstrated that SWI/SNF-family complexes could create a stably remodeled product when a mononucleosome was used as substrate. This product had altered mobility on a native gel and could revert to a standard nucleosome with either incubation under specific conditions or through the ATP-dependent action of SWI/SNF (Lorch et al. 1998; Schnitzler et al. 1998). Both human and yeast members of the family were shown to create this structure; in subsequent work, it has been proposed that the structure consists of DNA strands that bridge two nucleosomes (Lorch et al. 1999). Similar structures have not been observed with other ATP-dependent remodeling complexes, suggesting that the ability to form this structure might represent a special aspect of SWI/SNF function.

A second unusual property of the SWI/SNF family is the ability to create significant topological changes in closed circular nucleosomal templates. These changes are also stable, reverting to standard topology with a half-life measured in hours (Guyon et al. 1999, 2001). The characteristics of these topologically altered products imply that there is a significant energy barrier between the stably remodeled state and a standard state. The effects of SWI/SNF-family complexes on topology and the ability to form stable remodeled products from mononucleosomes might be related to each other mechanistically. Both properties could be explained by an altered nucleosomal conformation induced by SWI/SNF that has distinct topology and that is prone to aggregation. Alternatively, both properties could be explained by modeling the standard nucleosome in a manner that has adjacent nucleosomes sharing DNA strands (Lorch et al. 1998; Schnitzler et al. 1998). These experiments indicated that SWI/SNF complexes can create distinct products from other remodeling complexes that might involve altered nucleosomal structures.

DIFFERENT REMODELED PRODUCTS PRODUCED BY BRG1 AND SNF2h

To follow up on these studies, we have performed a series of experiments that compare the remodeled products that are formed by a SWI/SNF-family complex and an ISWI-family complex. The goal of these ongoing studies is to understand the capabilities of these remodeling complexes that might relate to their biological activity and to determine characteristics of each complex that will facilitate a detailed mechanistic and structural understanding of the different remodeling reactions. We focused on the major remodeling ATPases in humans in each class: BRG1 (SWI/SNF family) and SNF2h (ISWI family). Each individual remodeling protein can perform a reaction that has similar characteristics to the reaction performed by the intact complexes that form around these proteins (Phelan et al. 1999; Aalfs et al. 2001).

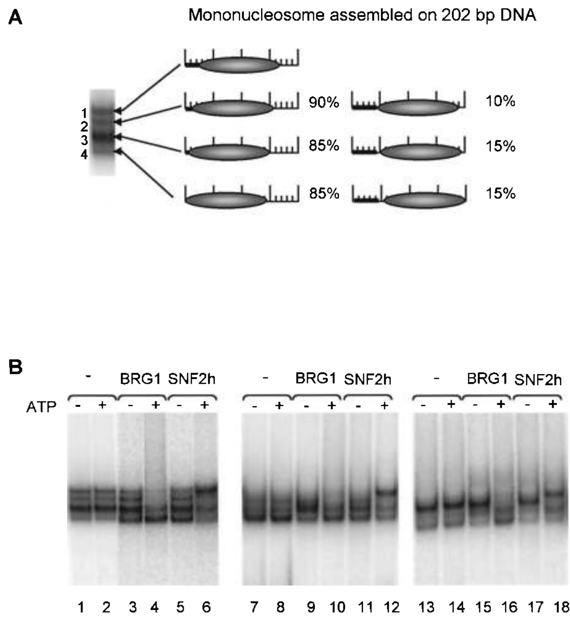

Figure 2. BRG1 and SNF2h generate different remodeled products. (*A*) 202-bp mononucleosomes were resolved as four bands on a 5% native polyacrylamide gel. The histone octamer positions in each band were mapped according to standard procedure and are indicated as *ovals* (Hamiche et al. 1999; Langst and Becker 2001). The *solid box* represents a 40-bp GT nucleosome phasing sequence. Distance between ticks is 10 bp. (*B*) Three different glycerol gradient nucleosome fractions were used in independent remodeling reactions.

Native gel electrophoresis can be used to analyze changes in nucleosome position. Mononucleosomes that have been formed on DNA that is longer than 146 base pairs (bp) (we have used a 202-bp DNA fragment in these studies) will run with different mobility during native gel electrophoresis depending upon whether the histone octamer is near the center of the DNA or near the end of the DNA fragment (Fig. 2A). Incubation of mononucleosomes with remodeling proteins results in ATP-dependent changes in mobility (Fig. 2B, lanes 4, 6, 10, 12, 16, and 18). SNF2h generates primarily slowly migrating species, while BRG1 generates more rapidly migrating species. Thus, these remodelers create qualitatively different products.

To understand how these products differ, the BRG1- and SNF2h-remodeled products were digested with MNase. The size of the resultant DNA fragments was determined by denaturing gel electrophoresis (Fig. 3A, lanes 3, 4, 6, and 7). Once again, there was a distinct difference between the SNF2h and BRG1 products: The SNF2h product ran with a discrete size appropriate to a 146-bp mononucleosome while the BRG1 product contained fragments across a spectrum of size. The location of the fragments that were protected from MNase cleavage was determined by excising the DNA fragment produced by MNase digestion and cleaving it with restriction enzymes. The primary SNF2h product mapped to a central position (Fig. 3B), while the BRG1 product had a large number of MNase fragments that mapped to a variety of positions (Fig. 3C).

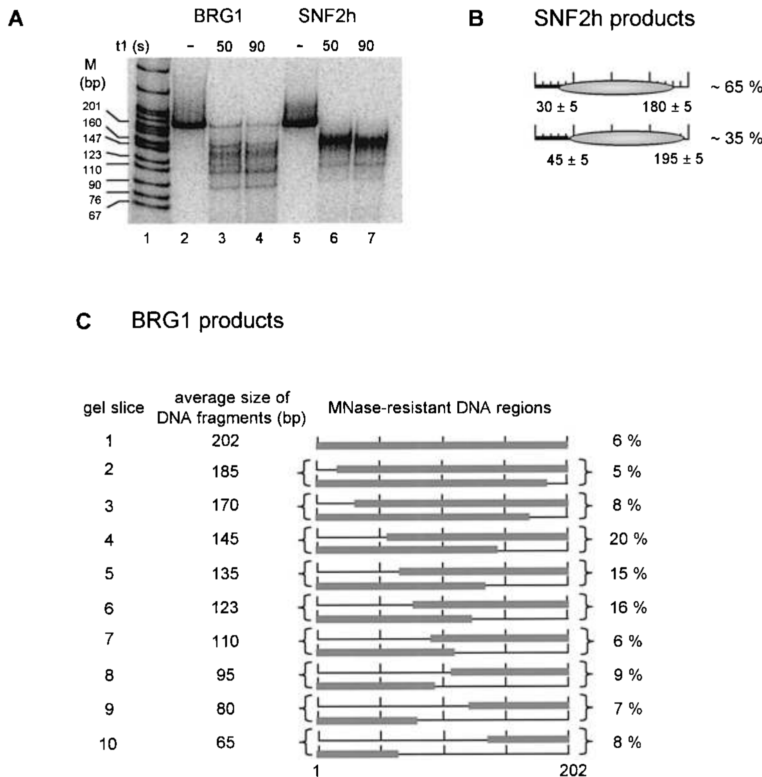

Figure 3. Mapping of the BRG1- and SNF2h-remodeled products. (*A*) BRG1- and SNF2h-remodeled products were treated with MNase, deproteinized, and resolved on an 8% polyacrylamide gel. (*B*) Mapping the major SNF2h products. (*C*) Mapping BRG1-remodeled mononucleosomes. *Bars* represent DNA regions protected by the histone octamer from MNase digestion. DNA fragments ranging from the average sizes (as shown) ± 10 bp were mapped. DNA fragments with an average size of 65, 95, 170, or 185 bp are more spread out and thus are less visible in *A*.

The surprising finding from these studies was that the major BRG1 product migrated as a discrete band when analyzed by native gel electrophoresis, but displayed a wide spectrum of differently sized and positioned MNase fragments. Why this happened is not clear. One possible explanation is that each BRG1 product had a similar amount of DNA that was associated with the histone octamer, but that there were MNase-sensitive regions where the DNA was looped away from the mononucleosome. By this hypothesis, the spectrum of MNase fragments formed would result from loops in different positions on the mononucleosome.

BRG1 CREATES MORE ACCESSIBLE DNA SITES THAN SNF2h

These studies demonstrate that there are measurable differences between the products that are created by BRG1 and SNF2h. We were interested in determining whether these differences also reflected differences in what is believed to be a key biological function of these remodeling proteins—the ability to create access of DNA-binding proteins to nucleosomal DNA. To study this problem, we constructed a series of mononucleosomal templates that were engineered to have restriction sites at a variety of different positions on the nucleosome. We then made use of protocols that have been shown to measure the rate of opening of a specific site by the rate of restriction enzyme digestion (Polach and Widom 1995; Logie and Peterson 1997; Narlikar et al. 2001). It has been shown previously that, under the conditions used here, the restriction enzyme is at high enough concentration to "report" whether a site has opened; the restriction enzyme will cleave every nucleosome that has been remodeled to have an open site and does not participate in the remodeling reaction.

Consistent with previous data on *Drosophila* ISWI-family complexes, we found that SNF2h opened sites near the edge of a nucleosome significantly faster than it opened sites near the center of the nucleosome (Fig. 4). This is consistent with a requirement for SNF2h to "slide" nucleosomes. If sliding is required, then it will prove dif-

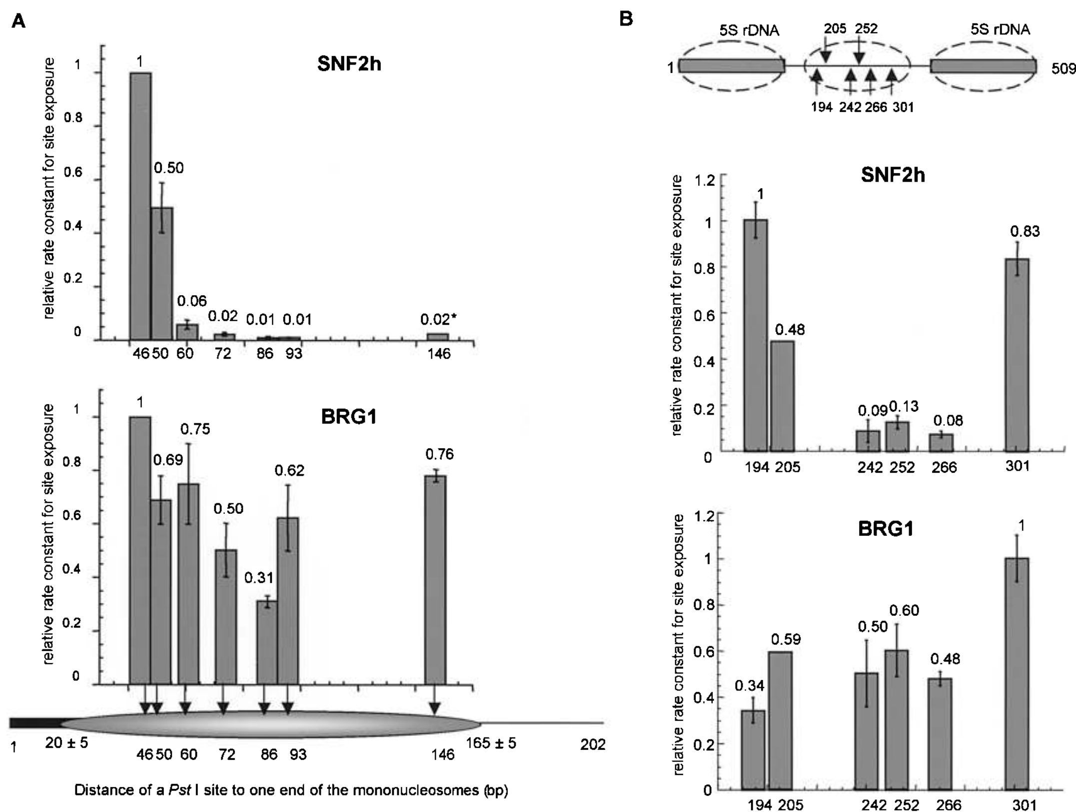

Figure 4. SNF2h and BRG1 create different profiles of accessible DNA sites on mononucleosomes and trinucleosomes. (*A*) Mononucleosome remodeling profiles of SNF2h and BRG1 monitored by continuous restriction enzyme accessibility assays. The rate constants for cutting each *Pst* I position were normalized to that of position 46 for BRG1 (0.2–0.3 min^{-1}) and SNF2h (0.2–0.4 min^{-1}): The normalized values are shown above the bars. At positions 46 and 50, SNF2h increased the rate of *Pst* I exposure by at least 30-fold relative to reactions without ATP. BRG1 increased the rate of *Pst* I exposure at all positions by at least 30-fold relative to reactions without ATP. (*, No increase in DNA exposure relative to the reaction with no ATP.) (*B*) A schematic illustration of the 509-bp DNA templates used to assemble trinucleosomes. The *Pst* I sites in the different templates are indicated by *arrows*. The rate constants for opening each *Pst* I position were measured relative to that for opening up position 205 for SNF2h (0.1–0.3 min^{-1}) and BRG1 (0.1–0.2 min^{-1}); the relative values were then normalized with respect to the highest remodeling rate constants (positions 194, 301, and 252, for SNF2h and BRG1, respectively). Normalized values are shown above the bars. SNF2h opened up positions 194, 205, and 301 at least 30-fold faster than reactions without ATP. BRG1 opened up the different positions at least tenfold faster than reactions without ATP.

ficult to open sites at the center of a fragment that has insufficient room for a nucleosome to slide. In contrast, BRG1 was able to open sites throughout the fragment at roughly similar rates. This could be explained by an ability of BRG1 to promote a sliding reaction where the histone octamer slides off of the end of the DNA fragment. Alternatively, this might result from the trapping of loops of DNA on the surface of the histone octamer.

These marked differences in the ability to open sites throughout a nucleosome provided a straightforward way to compare mutant remodeling proteins to see whether they had characteristics of SWI/SNF-family proteins or ISWI-family proteins. In the experiments described below, we focused on access to sites at positions 50 and 93, as ISWI-family proteins show a significantly lower ability to open site 93 than site 50, while SWI/SNF-family proteins open both sites efficiently.

THE IDENTITY OF THE ATPase DOMAIN DEFINES REMODELING CAPABILITY

The experiments described above demonstrate that the SNF2h and BRG1 proteins create products with different characteristics and have different abilities to create access to nucleosomal DNA sites. To understand these differences, we designed chimeric proteins that contained mixtures of BRG1 and SNF2h in order to map which region determined the characteristic activity of each remodeling protein (Fig. 5). The first set of constructs that were examined swapped ATPase domains. (The boundaries of the ATPase domain in these experiments were defined by homology between BRG1, SNF2h, and their orthologs; deletion analysis; and published genetic studies [Khavari et al. 1993; Elfring et al. 1994].) Surprisingly, we found that the ATPase domain itself determined both the nature of the remodeled products that were formed and the ability of the remodeling protein to create access to a series of sites.

When restriction enzyme access was used to characterize these chimeric proteins, the construct containing the BRG1 ATPase domains with SNF2h flanking regions (called "S-B-S"; Fig. 5A) showed significant ability to create access to site 93 (Fig. 5B). In contrast, the construct containing the SNF2h ATPase domain surrounded by BRG1 flanking regions ("B-S-B"; Fig. 5A) was not able to create efficient access to site 93 (Fig. 5B). Both of these chimeric remodeling proteins showed activity at

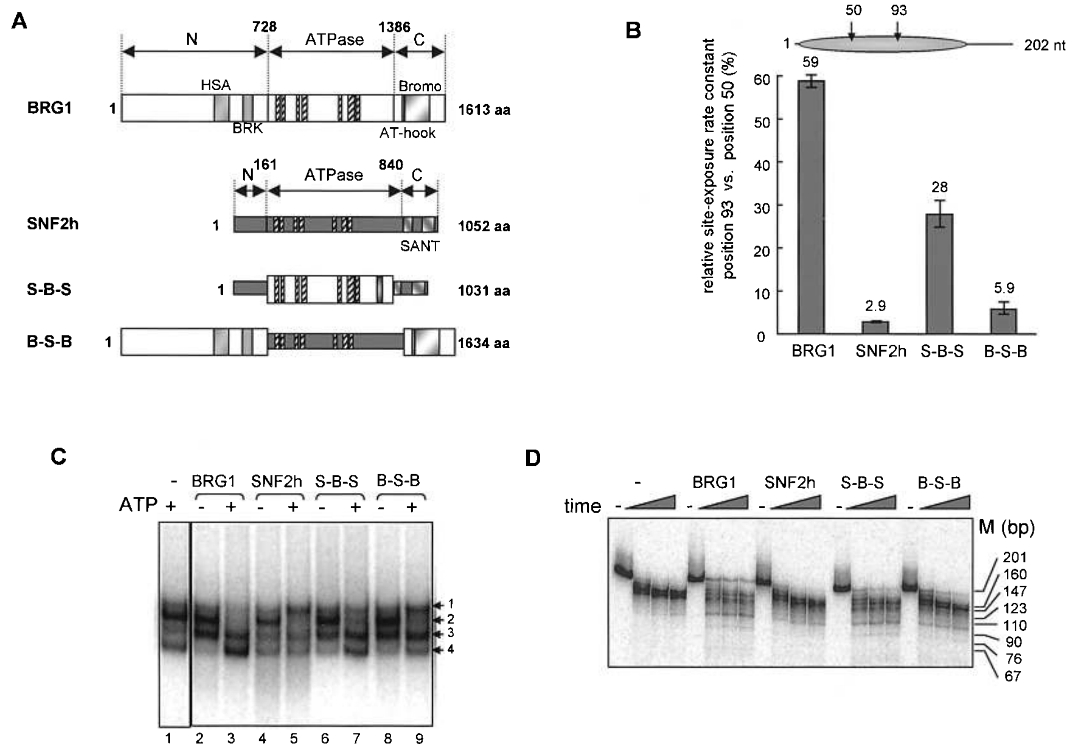

Figure 5. Remodeling activities of BRG1/SNF2h chimeric proteins. (*A*) Schematic representation of the proteins used in this study. BRG1 and SNF2h are divided into three regions: a homologous central ATPase domain (ATPase) and nonhomologous amino-terminal (N) and carboxy-terminal (C) regions. BRG1 and SNF2h contain seven conserved helicase motifs (*striped boxes*). BRG1 also contains HSA, BRK, AT-hook, and bromodomains, while SNF2h contains two SANT domains. Exchanging the central ATPase domains (BRG1 residues 728–1386 and SNF2h residues 161–840) forms the chimeric proteins B-S-B and S-B-S. The detailed breakpoints for construction of chimeras are B(1-727L)-S(161A-840Q)-B(1387G-end) and S(1-160K)-B(728Q-1386A)-S(841G-end). (*B*) Restriction enzyme accessibility assays using 202-bp mononucleosomes containing a *Pst* I site at either position 50 or 93. Site-exposure rate constants were determined. Results are expressed as the ratio of the site-exposure rate constants of positions 93 vs. 50. Relative rate constants are averages of at least three independent experiments. (*C*) Nucleosome mobility assays. Remodeled products as well as unremodeled nucleosomes were resolved on a 5% native polyacrylamide gel. (*D*) MNase sensitivity assays. Mononucleosomes remodeled by BRG1, SNF2h, S-B-S, and B-S-B were treated with 0.03 units of MNase for 0, 1, 2, and 3 min, deproteinized, and resolved on an 8% PAGE.

opening site 50 that was similar to intact BRG1 and intact SNF2h, demonstrating that swapping the ATPase domains does not generally impair remodeling activity.

These rate experiments suggest that the characteristic remodeling behavior of BRG1 and SNF2h is largely determined by the ATPase domain. To buttress these rate studies, we also performed qualitative measurements of remodeling function. We used native gel electrophoresis to show that B-S-B protein created remodeled products with similar migration to the products of the SNF2h reaction and S-B-S protein created products with similar migration to those of the BRG1 reaction (Fig. 5C). We characterized the products of these remodeling reactions using MNase digestion, and found that the B-S-B protein created a primarily a single protected band centered at 150, while the S-B-S protein created a spectrum of bands of varying lengths. We conclude from these studies that the characteristic differences in outcome of the remodeling reactions that are catalyzed by BRG1 and SNF2h are determined by the BRG1 and SNF2h ATPase domains.

To determine whether swapping the ATPase domain could alter function of a remodeling complex, we assembled a minimal SWI/SNF remodeling complex using both BRG1 and B-S-B. In addition to the ATPase subunit, these minimal complexes contain the human homologs of yeast SWI3 (BAF170 and BAF155) and SNF5 (INI1) proteins. Both BRG1 and the B-S-B chimeric protein were able to form complexes with these subunits with similar stoichiometries (Fig. 6A). Both complexes were active for remodeling site 50 of a mononucleosome. As was seen with the isolated ATPase subunits, the complex that contained BRG1 was able to efficiently open site 93, while the complex that contained B-S-B was not (Fig. 6B). Thus, swapping the SNF2h ATPase domain into a minimal SWI/SNF complex created remodeling activity that mirrored that of SNF2h. As above, we used native gel electrophoresis and MNase cleavage analysis to analyze the products of the remodeling reactions. The minimal complex containing BRG1 created products that ran on a native gel with different characteristics than the products created by the minimal complex containing B-S-B (Fig. 6C). Similarly, swapping the ATPase domain into the minimal complex caused a change in the MNase pattern of the remodeled products (Fig. 6D; compare “BRG1 minimal complex” to “B-S-B minimal complex”).

Similar results were obtained when we isolated intact SWI/SNF complexes from SW13 cells that had been transfected with expression constructs for BRG1 and B-

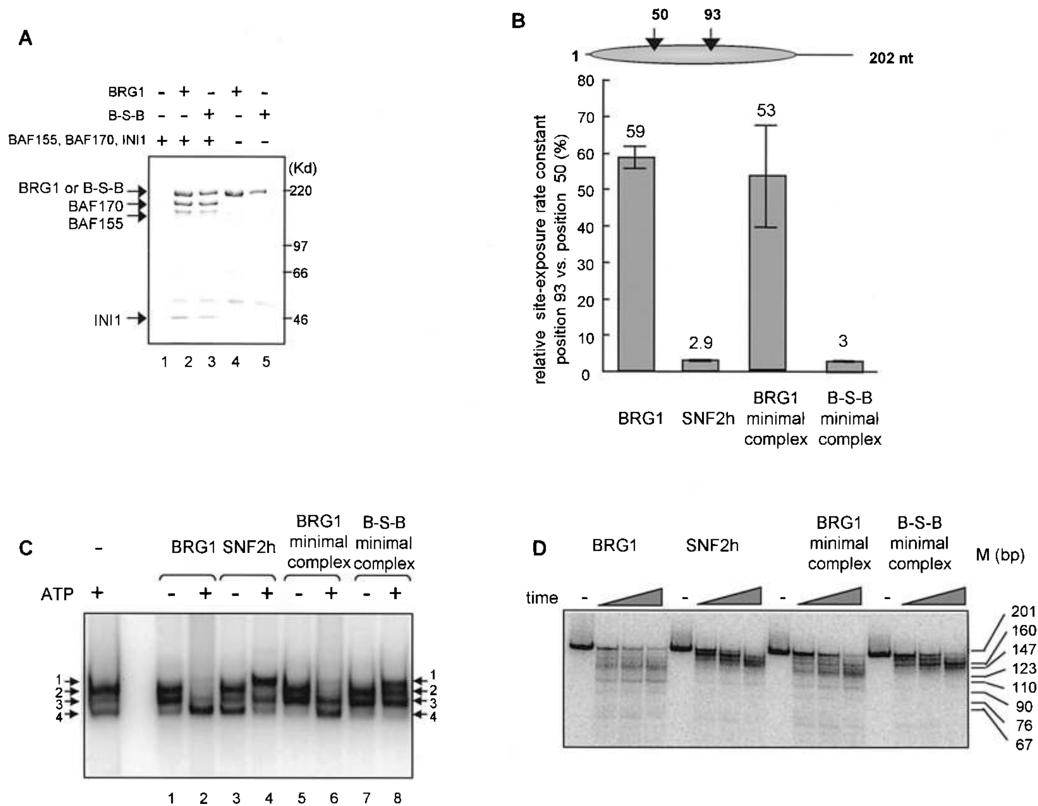

Figure 6. In vitro characterization of the reconstituted BRG1 and B-S-B minimal complex. (*A*) SF9 cell nuclear extracts containing Flag-tagged BRG1 or B-S-B were mixed with an SF9 cell nuclear extract containing BAF170, BAF155, and INI1, and anti-Flag M2 beads were used to purify BRG1 or B-S-B complexes. The purified complexes were eluted with the Flag peptide, resolved by 8% SDS-PAGE. (*B*) Comparison of the relative site-exposure rate constants at position 93 vs. position 50 of mononucleosomes remodeled by the BRG1 complex, the B-S-B complex, BRG1, or SNF2h. Relative rate constants are averages of at least three independent experiments. (*C*) Gel mobility assays to compare mononucleosomes remodeled by the BRG1 complex, the B-S-B complex, BRG1, and SNF2h. (*D*) The remodeled products from *C* were treated with 0.03 units of MNase for 0, 0.75, 1.5, and 3 min, deproteinized, and resolved on an 8% PAGE.

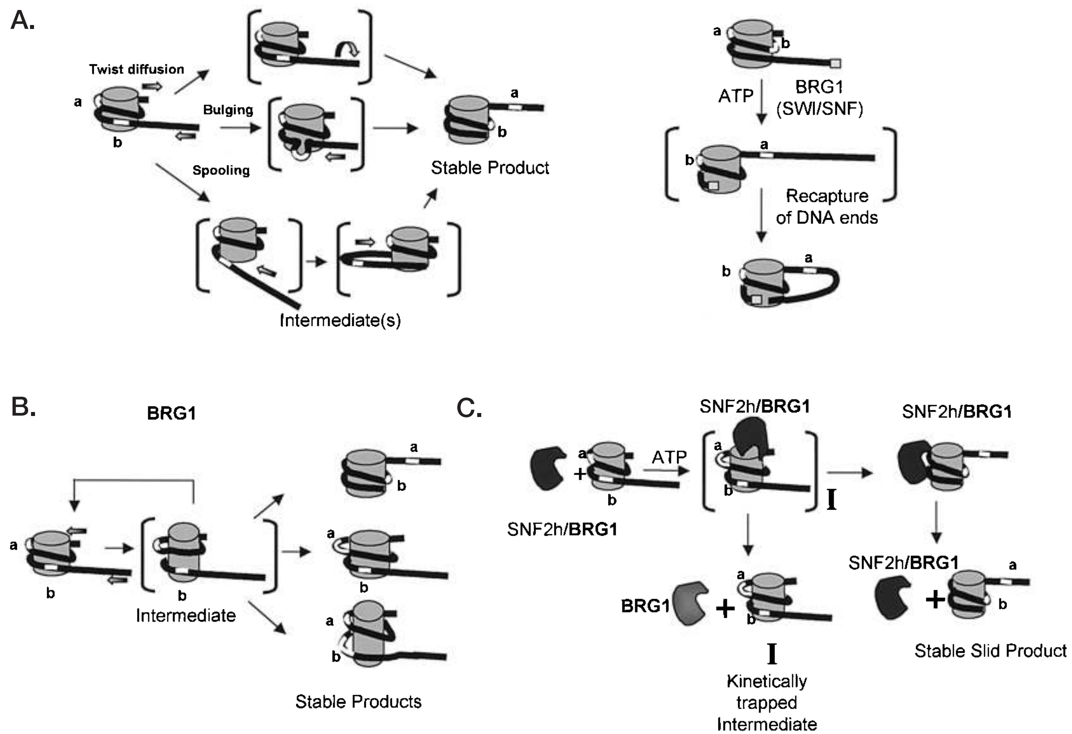

Figure 7. Models accounting for different products generated by SNF2h and BRG1. (*A*) Both SNF2h and BRG1 slide histone octamers. SNF2h may slide histone octamers via twist diffusion, bulging, or spooling to expose a DNA site such as "**a**." BRG1 and SWI/SNF may expose site "**a**" by sliding the histone octamer off the DNA ends. The exposed DNA end subsequently rebinds the histone octamer to form a stable loop (Kassabov et al. 2003). (*B*) SNF2h and BRG1 use different mechanisms to remodel nucleosomes. While SNF2h slides histone octamer to remodel nucleosome (*A*), BRG1 may create an altered nucleosome conformation; in this model, repositioning of a histone octamer is not a necessary outcome for exposure of site "**a**" or "**b**." (*C*) BRG1 (and hSWI/SNF) and SNF2h remodel nucleosomes by the same mechanism and the two reactions proceed though similar intermediates such as "**I**." In this model, BRG1 and hSWI/SNF release "**I**" more often than SNF2h does and thus create a kinetically trapped intermediate (**I′**) with site "**a**" exposed within the histone bounds. The structures depicted for the intermediate and final products in *A* and *B* are hypothetical and could involve changes in the conformation of DNA, histones, or both.

S-B (data not shown). Therefore, we conclude that the ATPase domain itself plays a critical role in determining the outcome of the remodeling reaction; in fact, simply swapping this domain changes the measured characteristics of the remodeling reaction even in the context of a full remodeling complex.

SPECULATIONS ON THE ROLE OF THE ATPase DOMAIN IN DEFINING THE REMODELING REACTION

These studies demonstrate that there is a direct link between the nature of the ATPase domain and the outcome of the remodeling reaction. The surprising finding that the ATPase domain is the primary determinant for outcome, even in the context of intact complexes, raises the possibility that the differences in function between complexes are determined by differences in the manner in which ATP hydrolysis is coupled to remodeling. This finding is emphasized by the observation that the isolated BRG1 ATPase domain is functional for remodeling (data not shown). Thus, the ATPase domain serves as the central component of the engine that drives remodeling.

Do the engines of BRG1 and SNF2h differ solely in efficiency, or do they differ in the mechanism by which they harness the energy of ATP hydrolysis? One possible explanation for the data above is that both BRG1 and SNF2h perform precisely the same function (e.g., sliding the nucleosome) (Fig. 7A), and that BRG1 opens up a greater spectrum of sites because it is more potent at promoting the sliding reaction. A greater potency might allow BRG1 to slide the nucleosome off of the ends of the DNA or into adjacent nucleosomes. This ability could lead to the increased restriction enzyme access that is observed, and folding back of the DNA onto the nucleosome could result in the formation of loops of DNA (Fig. 7A). A second possibility is that BRG1 uses a distinct mechanism that creates access in the middle of the nucleosome; for example, BRG1 might use energy to push DNA toward the nucleosome dyad from both the entry and exit points, thereby inducing a strain that creates an altered conformation (Fig. 7B).

There is no data that allows one to rule out either of the above hypotheses for the differences in BRG1 and SNF2h function. We argue, however, that the characteristics of the SWI/SNF remodeling reaction might be most simply explained by the hypothesis that BRG1 differs fundamentally from SNF2h in mechanism.

To frame these arguments, it is necessary to define the types of mechanisms that might be involved (Fig. 7). One

prominent hypothesis for sliding a nucleosome by ISWI-family complexes involves the creation of a segment of DNA that dissociates from the nucleosome, creating a "bulge." This bulge might then propagate through the entirety of the nucleosome to cause histone displacement in the size of the bulge (thus sliding the nucleosome by an amount determined by the amount of DNA in the bulge) (Fig. 7A). It has been proposed that all ATP-dependent remodeling enzymes share this common mechanism, and that the differences in outcome of the remodeling reaction such as those highlighted above are caused either by sliding the octamer off of the edge of the histone, followed by strand recapture to create loops of DNA with altered topology, or by having the bulge arrest in the center of the nucleosome to create loops of DNA that are accessible to restriction enzyme access. The alternative hypothesis considered here is that the energy of ATP hydrolysis is used differently for different classes of complexes, in that some such as ISWI might induce a bulge that propagates, and others such as SWI/SNF-family complexes might either push DNA into the structure from both the entry and exit points or otherwise induce a strain on the canonical structure that favors the formation of quasistable nucleosome structures that have exposed sites near the center of the nucleosome (Fig. 7B).

Two considerations argue against a model in which the mechanism that induces sliding is also used to create access to sites that are centrally located. The first concerns energetics. If SWI/SNF were doing something more energetically unfavorable like moving the octamer off the end of the DNA, we might expect it to use more ATP per remodeling event than ISWI complexes. Instead it uses similar or less ATP suggesting SWI/SNF action is not a simple extension of ISWI action (Narlikar et al. 2001; Fyodorov and Kadonaga 2002). In addition, this simplest of notions of sliding as a mechanism of creating access would necessitate an energy gradient, where, for example, sites near the entry/exit point required less energy to open than sites near the dyad. Instead, with SWI/SNF-family enzymes, similar amounts of ATP hydrolysis are needed to open sites near or at the nucleosome dyad as are needed at sites away from the dyad.

A variant on this hypothesis is that SWI/SNF-family enzymes create access by arresting a propagating bulge as it traverses the nucleosome (Fig. 7C). By this model, both ISWI complexes and SWI/SNF complexes function by creating propagating bulges, but these bulges arrest during SWI/SNF remodeling. This would require that the components of the complex that maintain contact with the nucleosome to either cause dissociation from the nucleosomes partway through the remodeling reaction, or actively arrest the bulge. The finding that ATPase domains determine the outcome of the remodeling reaction, combined with this and previous studies that show that many of the domains that interact with the nucleosome are outside the ATPase domain, argues against this model.

Thus, we argue that current data do not support the hypothesis that ISWI and SWI/SNF remodeling complexes function by fundamentally similar mechanisms. While these data by no means disprove these hypotheses, they favor the consideration of the alternative hypothesis that fundamentally distinct mechanisms are used by the SWI/SNF family of remodeling complexes to create access to nucleosomal sites. Previous data has shown that SWI/SNF complexes create stable remodeled structures with long half-lives (Guyon et al. 1999, 2001). In addition, SWI/SNF complexes create dramatic changes in topology of nucleosomal arrays (Guyon et al. 1999; Gavin et al. 2001). The nature of these stable remodeled structures and these topological shifts is not known. The finding that the ATPase domain is central to function of SWI/SNF complexes implies that the remodeling parameters are tightly connected to ATP hydrolysis. These considerations are all consistent with the possibility that a specialized reaction is performed by SWI/SNF in which ATP hydrolysis creates stably altered nucleosomal structures. To test this hypothesis, it will be essential to identify the nature of these putative structures and the relationship of these structures to that of the canonical nucleosome.

SUMMARY AND PERSPECTIVES ON THE ROLE OF REMODELING IN EPIGENETIC REGULATION

It is generally agreed that different ATP-dependent remodeling complexes create different products. We have argued here that these differences might reflect a diverse range of dynamic changes in nucleosome structure. Identifying the nature of the products of these different remodeling reactions is a matter that is central to determining the range of possible chromatin structures that might contribute to epigenetic regulation. If the nucleosome is more dynamic than currently demonstrated, and if complexes have evolved to regulate that dynamic state, then the pallet of changes that can occur to chromatin structure to create stable epigenetic states is increased. Any changes in the nucleosomal structure would also be anticipated to impact the range of potential higher-order structures. An important and interesting frontier is the elucidation of the full spectrum of structures that can be formed in chromatin.

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