Functional Differences between the Human ATP-dependent Nucleosome Remodeling Proteins BRG1 and SNF2H*

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ATP-dependent nucleosome remodeling complexes can be grouped into several classes that may differ in their biochemical remodeling activities and biological roles. Although there are a number of biochemical studies of each class of remodeler, there are very little data directly comparing the biochemical activities of remodelers from different classes. We have purified two ATP-hydrolyzing proteins, SNF2H and BRG1, which are members of complexes from two different classes of remodelers. Consistent with previous reports, these two homogeneous proteins can perform remodeling functions. We show significant functional differences between SNF2H and BRG1 in vitro; although both SNF2H and BRG1 hydrolyze ATP and remodel linear arrays of nucleosomes, only BRG1 can remodel mononucleosomes. Also, only BRG1 can alter the topology of nucleosomal plasmids. We propose that these functional differences reflect significant mechanistic differences between the two remodeler classes that will impact their biological roles.

The assembly of eukaryotic DNA into nucleosomes, necessary for packing the DNA into the nucleus, has a generally repressive effect on gene transcription. A number of multiprotein complexes have been purified that utilize ATP to alter the arrangement of eukaryotic chromatin (1–10). These complexes or the genes encoding their constituent proteins have been found in every eukaryote that has been studied, suggesting that they are necessary for normal cell function. Furthermore, genetic analyses have identified a number of promoters in yeast, Drosophila, and human cells that are dependent on specific remodeling complexes or proteins for their normal regulation (11–13).

The remodeling complexes identified to date can be roughly grouped into three classes, the SWI-SNF complexes, the ISWI complexes, and the CHD complexes. These three classes differ in size and subunit composition. The three classes of proteins also appear to differ in their biochemical activities; although all three complexes appear to alter chromatin structure to increase the accessibility of regulatory sequences within promoters, the physical changes underlying the increased accessibility appear to differ in each case. In some cases, chromatin remodeling appears to involve the re-positioning of otherwise intact histone octamers (14, 15); other remodeling events appear to change the conformation of specific nucleosomes without significantly displacing them (16, 17). Importantly, there is very little data directly comparing the biochemical functions of remodelers from different classes. This sort of information is critical in determining the products of each remodeling event, the mechanisms of the different remodeling complexes, and possibly the biological roles of different remodelers.

The SWI-SNF complexes contain between 8 and 16 distinct proteins and have molecular masses of ~2 MDa. Each member of the SWI-SNF complex family contains a member of the SWI2/SNF2 family of ATPase domain-containing genes. The SWI2/SNF2 proteins are all ~200 kDa in size and are all highly homologous. In addition to their ATPase domains, the SWI2/SNF2 genes contain a bromo-domain: the bromo-domain is found in a variety of chromatin-related genes and can bind acetylated histones (18). One member of the SWI2/SNF2 family, the human gene Brahma-related Gene One (BRG1) was expressed and purified as a homogeneous protein; when assayed in vitro, BRG1 could perform all of the functions of the human SWI-SNF complexes, albeit with a lower specific activity (19). These functions include the alteration of nuclease sensitivity on both mononucleosomes and linear arrays of nucleosomes (8, 9, 20, 21), the transfer of a histone octamer to a non-adjacent acceptor DNA (octamer transfer) (20, 22), changes in the linking number of a nucleosomal plasmid (6, 20, 22, 23), creation of a stable remodeled dinucleosome from two mononucleosomes (24, 25), and introduction of topological stress on naked templates (26). The addition of other SWI-SNF subunits greatly increased the specific activity of BRG1 in vitro; the other subunits are also likely to be involved in the regulation of SWI-SNF activity.

The ISWI complexes contain between 2 and 6 proteins and have molecular weights between 200 and 700 kDa. Each ISWI complex contains an ATPase protein from the ISWI gene family (27). The ISWI proteins (named for the first family member to be characterized, the Drosophila gene Imitation Switch or ISWI (28)) are ~130 kDa in size. The ISWI family ATPase domains are very similar to the ATPase domains of the SWI2/SNF2 proteins, but the two gene families are not significantly related outside of the ATPase domain. The ISWI gene product was, like BRG1, purified as a homogeneous protein and could perform many of the functions of ISWI-containing complexes in vitro (29). These functions include the assembly of nucleosomes (30), changes in nucleosome spacing (7), sliding of histone octamers along DNA (31, 32), and ATP hydrolysis, stimulated by nucleosomes but not by naked DNA (2, 33). As with BRG1, the specific activity of ISWI could be significantly increased by adding another subunit of an ISWI complex, the Drosophila protein ACF1 (30). The other subunits are also likely to have regulatory roles.

Previous work had hinted at major functional differences between the SWI-SNF complexes and the ISWI complexes. In particular, the ATPase data showed a significant difference in
protein was loaded onto the gels and visualized by Coomassie staining. Approximately 400 ng of each protein was loaded onto the gels and visualized by Coomassie staining. Specifically, SNF2H was purified following the same protocol, Briefly, Sf9 cells were transfected with pFastBac1::SNF2H-A digested with HI/I1003 and HI/I1001, and XbaI and cloned into pFastBac1::SNF2H-A digested with HindIII and XbaI. Antiquities—BRG1 was detected with the anti-BRG1(unique) antibody, described previously (35). SNF2H was detected with the anti-SNF2H antibodies against high salt buffer (20 mM Tris pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 2 M KCl). The reaction was transferred into dialysis tubing (molecular mass = 12–14 kDa) and dialyzed at 4 °C against high salt buffer. Dialysis continued for 60 h, with the high salt buffer exchanged for low salt buffer (same as high salt buffer, with 0.25 mM KCl) at a rate of 12 to 13 ml/hr. After dialysis, nucleosomes were purified by glycerol gradient centrifugation over a 5–30% gradient, 16 h × 35,000 rpm (150,000 × g) in a SW-55 rotor (Beckman Instruments) for 16 h. Thirty 6-drop fractions were collected, and each fraction was assayed for protein concentration and activity. Mononucleosome Reconstitution—All mononucleosomes used here were made using the TTP Mu1-EcoRI fragment described previously (25). To make end-labeled nucleosomes, the Mu1 end was labeled with [α-32P]dCTP using Klenow fragment. Body-labeled TPT was made by polymerase chain reaction containing [α-32P]dCTP. End-labeled and body-labeled mononucleosomes were assembled by serial dilution from 2 μ to 200 mM NaCl or by octamer transfer. Unlabeled mononucleosomes were assembled by salt gradient dialysis. Unlabeled TPT was produced in a large scale polymerase chain reaction and assembled using unlabeld large scale dialysis technique. DNA and histones were mixed at a 1:1 ratio, each at a concentration of 0.7 mg/ml in high salt buffer (20 mM Tris pH 7.5, 1 mM EDTA, 1 mM dithiothreitol, 2 mM KCl). The reaction was assembled into aliquots and incubated at 4 °C until use. 300 μl reactions under the following conditions: 15.6 mM MgCl2, and the reactions were started by adding the reaction mixture to the rest of the reaction. At the indicated times after addition, 2-μl aliquots were removed from each reaction and quenched in 5 μl of stop buffer (3% SDS, 100 mM EDTA, 50 mM Tris, pH 7.5). Phosphate and ATP were separated by thin layer chromatography on cellulose plates (J. T. Baker Inc.) that were pre-run with Tris, pH 7.5. 4 mM HEPES, pH 7.9, 0.44 mM EDTA, 60 mM KCl, 4–10% glycerol, 4 mM MgCl2, and 2 μM to 1.5 mM ATP-Mg2+ (23). All components were pre-warmed to 30 °C, and the reactions were started by adding the equimolar ATP-Mg2+ mixture to the rest of the reaction. At the indicated times after addition, 2-μl aliquots were removed from each reaction and quenched in 5 μl of stop buffer (3% SDS, 100 mM EDTA, 50 mM Tris, Ph 7.5). Phosphate and ATP were separated by thin layer chromatography on cellulose plates (J. T. Baker Inc.) that were pre-run with water and dried. TLC was carried out in 1 st formic acid, 0.5 μL iCl. Plates were dried then visualized and quantitated using a Molecular Dynamics PhosphorImager. Reaction rates were determined using leucine- and arginine-32P-labeled substrates. The starting time for the reaction was taken as the time at which the activity was at its maximum. Kinetic data were analyzed by least-squares fits to the time points (Kaleidagraph). FE Accessibility Assays—The restriction enzyme assays were carried out in 25- or 50-μl reactions under the following conditions: 15.6 mM HEPES, pH 7.9, 10 mM Tris pH 7.5, 0.36 mM EDTA, 60 mM KCl, 6% glycerol, 0.02% Nonidet P-40, and 3.5 mM MgCl2. Where indicated, 0.5 mM ATP was added to reactions. The array was added at concentrations of 0–12 μM; the mononucleosomes were added at concentrations of 0.1 μM.
In all experiments, the restriction enzymes were added at 0.4–0.8 units/μl. Reactions were pre-warmed to 30 °C and started by adding the substrate to the other components. At specified time points, 6- or 12-μl aliquots were removed and quenched with stop buffer (1% SDS, 0.1 mM EDTA, 25% glycerol, 50 mM Tris, pH 7.5). Cut and uncut DNA were separated on 1% agarose gels run in 1× Tris acetate-EDTA buffer. Gels were visualized and quantitated on a Molecular Dynamics PhosphorImager. Reaction rates were determined by fitting the time points to an exponential function \( [S] = [S]_0 e^{-kt} \), where \([S]_0\) is the initial substrate concentration, and \(k\) is the rate of the reaction; the equation was corrected for the endpoint of each reaction.

**DNase I Assay**—The DNase I accessibility assay was carried out by FIG. 2. SNF2H exhibits nucleosome-stimulated ATPase activity, which is inhibited by DNA. A, [ATP] curve. SNF2H and BRG1 were incubated with saturating amounts of TPT nucleosomes and the indicated concentrations of ATP. Reaction rates were determined by quantitating the fraction of ATP hydrolyzed at multiple time points. To normalize multiple experiments, all rates within a given trial were divided by the maximum rate in that trial; between trials, the maximum rates varied by less than 1.5-fold. The \(K_m (\text{ATP})\) for SNF2H was 37 μM ± 9 μM, whereas the maximum rate of hydrolysis was 0.35 μM/min; for BRG1, the \(K_m (\text{ATP})\) was 200 ± 50 μM, with a maximum rate of 1.2 μM/min. B, [nucleosome] curve. SNF2H and BRG1 were incubated at subsaturating ATP with the indicated concentrations of TPT mononucleosomes. Data from multiple trials were normalized by setting the maximum rates of hydrolysis in each trial to 1. The \(K_{(\text{nucleosomes})}\) of SNF2H is 25 ± 8 nM; for BRG1, it is 37 ± 10 nM. C, [DNA] curve. SNF2H and BRG1 were incubated with subsaturating ATP and DNA at the indicated concentrations. The \(K_{(\text{DNA})}\) of BRG1 is 20 ± 8 nM; the \(K_{(\text{DNA})}\) for SNF2H cannot be determined meaningfully. D, DNA inhibition. SNF2H was incubated in the absence of nucleosomes or DNA (open circles) or in the presence of saturating nucleosomes and TPT DNA at the indicated concentrations (black circles). \(K_i\) was determined by fitting to the equation: \(k = k_{\text{max}}/([S]_0 + 1/([K_i]))\); \(K_{(\text{DNA})}\) for SNF2H was 5 ± 2 nM. The \(R^2\) value for the curve was >0.98. E, DNA binding. SNF2H was incubated with body-labeled TPT fragment ([TPT] = 0.1 nM) at the concentrations shown for 20 min at 30 °C. The reactions were then blotted onto nitrocellulose (Top Filter) and Genescreen (Bottom Filter). Reactions were visualized and quantitated on a PhosphorImager. F, quantitation of filter binding. Data from two experiments were fitted to a binding \((K_d)\) curve. The \(K_d (\text{SNF2H})\) is 30 nM. Another experiment with an excess of TPT fragment (not shown) showed that 5% of the SNF2H molecules were active for binding. This gives an adjusted \(K_d (\text{SNF2H})\) of 1.5 nM. The \(R^2\) value is 0.86.
incubating nucleosomes containing end-labeled TPT at a concentration of ~3 mM, with remodelers at the indicated concentrations under the conditions listed in the restriction enzyme accessibility assays. The reactions were incubated for 30 min at 30 °C and then cooled to room temperature for 5 min. DNase I was added to each reaction; 0.4 units/reaction for nucleosomes, 0.04 units/reaction for bare DNA. Digestion proceeded for 2 min at room temperature and was stopped by adding 2 μl of 0.5 M EDTA with 73 μl of water. Samples were phenol-extracted, precipitated, and resuspended in 95% formamide loading buffer. Samples were separated on an 8% acrylamide-urea gel and visualized by autoradiography.

Gel Shifts—Gel shift assays were carried out in the same method as DNase I assays; however, after 30 min at 30 °C, 250 ng of cold DNA was added to compete the remodelers away from the nucleosomes. Samples were incubated for 4 min at 30 °C, then loaded onto a 5% acrylamide-urea gel. After electrophoresis for 3 h at 100 V, the gel was dried and visualized on a PhosphorImager.

Supercoiling Assay—PUC19 was linearized, de-phosphorylated, labeled with 32P, and re-ligated as described previously (23). Circular, labeled plasmid was assembled with HeLa histones at a mass:mass ratio of ~4:1 (histones:DNA) using Xenopus heat-treated egg extract for 90 min at 30 °C (39). Assembled plasmid was isolated by centrifugation on a 10–40% glycerol gradient, 16 h x 35,000 rpm (150,000 x g) in a SW-55 rotor (Beckman Instruments). The resulting chromatin was used at a concentration of ~0.1 mM in 25-μl reactions under the same conditions as the restriction enzyme assays, with the exception that topoisomerase I (Promega) was present at 0.08 units/μl. Reactions were incubated for 90 min at 30 °C, then stopped by adding 3 μl of stop buffer (130 mM Tris, pH 7.5, 217 mM EDTA, 4.3% SDS). 20 μg of proteinase K was added to each reaction and digested for 30 min at 55 °C. The reactions were then separated by 1× Tris borate-EDTA electrophoresis on a 1.1% agarose gel and visualized on film.

RESULTS

Purification of Homogeneous SNF2H Peptide—SNF2H has been purified as part of several nucleosome-remodeling complexes (40–42), and these complexes appear in both subunit composition and function to be homologous to the ISWI-containing complexes in Drosophila (2, 3, 7), and to the ISW-containing complexes in yeast (43). BRG1 is the core ATPase of several human SWI/SNF complexes (8, 44) that appear to be homologous both in composition and function to the yeast remodeler the structure of chromatin complex (10), the Drosophila brahma complex (1), and perhaps to the yeast SWI-SNF complex (45). Thus, in addition to their close sequence similarity to the ISWI and SWI2/SNF2 genes, SNF2H and BRG1 also appear to represent the central ATPases of two functionally distinct families of nucleosome remodeling activities. To begin to understand the mechanisms by which these two classes function, we purified and characterized SNF2H and BRG1.

We added a FLAG epitope tag to SNF2H and created a baculovirus to express the FLAG-tagged SNF2H in Sf9 insect cells. Nuclear extracts were made and fractionated by M2 (anti-FLAG antibody) affinity chromatography. This one-step purification yielded highly purified SNF2H (Fig. 1A) that we estimate our protein fractions to be >90% pure. BRG1 was expressed and purified by similar methods, as previously described (Fig. 1B; Phelan et al. (19)). The BRG1 was also highly purified; we estimate our fractions to be >70% pure, and many of the smaller proteins are breakdown products of the full-length protein as judged by Western analysis (data not shown). Pre-
In agreement with previous results, the BRG1 ATPase activity was also stimulated by naked DNA (Fig. 2C). The $K_m$(DNA) of BRG1 was $20 \pm 8$ nM. As expected, SNF2H ATPase activity was not detectably stimulated by naked DNA. To further examine the interaction of SNF2H with DNA, we mixed naked DNA with nucleosomes to look for inhibition (Fig. 2D). Under conditions where nucleosomes were saturating, relatively small amounts of naked DNA could inhibit the ATPase activity of SNF2H. The $K_m$(DNA) of SNF2H was calculated to be $5 \pm 2$ nM. This result is supported by direct measurement of protein-DNA binding using nitrocellulose filters to separate free DNA from protein-bound DNA (46); in this assay, we calculated the $K_m$(DNA) of SNF2H at 2 nM (Fig. 2E). We conclude that SNF2H can bind to DNA but that DNA binding does not detectably stimulate ATP hydrolysis.

SNF2H Can Remodel Nucleosomes in an Array—ISWI-containing complexes and the homogeneous ISWI protein catalyze movement of the histone octamer on DNA (31, 32). To determine whether purified SNF2H had remodeling activity, we tested its ability to increase the accessibility of restriction sites within a defined nucleosomal array. Restriction sites assembled into nucleosomes are refractory to digestion (47), and the movement or disruption of nucleosomes leads to increased cleavage of a restriction site within the array (21). We assembled the 2500-base pair 5 S (G5E4) DNA fragment (Fig. 3A; Steger et al. (38)) into nucleosomes. The fragment contained 10 repeats of the 200-base pair 5 S rDNA sequence from Lytechinus variegatus; each 5 S sequence stably positions 1 nucleosome. When the array was assembled with histones by salt gradient dialysis, a unique HhaI site in the central region of the array was covered by a nucleosome that inhibited cleavage (Fig. 3A). By measuring the rate of cutting at the HhaI site, we can detect the displacement or disruption of this nucleosome. SNF2H, when incubated with the 5 S array and ATP, enhanced the accessibility of the HhaI site as well as other restriction sites (Fig. 3B; data not shown). The activity of SNF2H was dependent on ATP (Fig. 3C).

BRG1 also increases the accessibility of nucleosomal restriction sites (21, 23). To compare the functions of SNF2H and BRG1, we measured HhaI cleavage rates within the linear range of remodeler concentrations (Fig. 3D). In these reactions, ATP is well above the $K_m$(ATP) of each remodeler; due to the difficulty in producing substantial amounts of defined arrays, nucleosome concentration is below the $K_m$(nucleosomes) of each remodeler. Tested under these reaction conditions, BRG1 had a specific activity of 9000 units/mg, whereas SNF2H had a specific activity of 4800 units/mg (1 unit = 1 pmol of template remodeled/min). We concluded that BRG1 and SNF2H increased the accessibility of restriction sites in linear nucleosomal arrays with similar specific activities and at similar rates.

One interesting observation from these experiments involved the extent of remodeling by SNF2H and BRG1. Although BRG1 routinely facilitated HhaI cutting of more than 90% of the template, the SNF2H reactions only showed 50–70% cutting of the same template preparations. Moreover, the extent of SNF2H-assisted cutting varied when templates from different assembly reactions were used; in contrast, BRG1 remodeled these different template assemblies to similar extents (not shown). These data suggest that SNF2H is only able to remodel a subset of templates within the total population and that the prevalence of this subset varies from assembly to assembly. BRG1, on the other hand, can remodel nearly all of the templates regardless of the assembly conditions. This difference in the accessibility of substrates suggests that the remodeling events catalyzed by BRG1 and SNF2H may be

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1 G. J. Narlikar, M. L. Phelan, and R. E. Kingston, submitted for publication.
biochemically distinct (see “Discussion”). It is important to point out that, although we know the total protein concentration and purity of each BRG1 and SNF2H fraction, we do not know the molar concentration of active remodeler in any fraction. There are two reasons for this. First, we do not know whether the peptides are active as monomers or multimers. Second, we do not have assays available to determine the fraction of molecules active for remodeling in either protein sample. The data above, however, define the specific activities of the BRG1 and SNF2H fractions for the ATP-dependent remodeling of a nucleosomal array. Thus, we were able to compare defined amounts of each protein in subsequent experiments to compare the activities of BRG1 and SNF2H in other remodeling reactions.

SNF2H Remodeling Activity Co-fractionates with the Homogeneous Peptide—Before testing the activity of SNF2H in other remodeling assays, we performed an experiment to verify that ATP-dependent remodeling activity and SNF2H co-fractionate. Although BRG1 and ISWI have previously been purified as homogeneous proteins that have remodeling activity, it remained a formal possibility that the observed activity of the SNF2H preparation was caused by a small amount of endogenous protein that might form a complex with SNF2H. When the ISWI protein is mixed with other subunits, its specific activity is increased dramatically (30), so a small amount of contaminating complex might make a disproportionate contribution to the activity of the fraction. To test this possibility, we fractionated SNF2H across a 5–30% glycerol gradient. Any minor contaminating complex would be expected to run at a larger size on this gradient than the bulk of the SNF2H protein. SNF2H eluted in a single broad peak from the gradient (Fig. 4A), and the nucleosome-stimulated ATPase activity as well as the remodeling activity co-fractionated with the immunoreactivity peak (Figs. 4, B and C). These data show that the activity of the SNF2H fraction can be attributed to the homogeneous peptide and is not caused by a minor contaminant complex.

SNF2H Has Minimal Activity in Mononucleosome-based Assays—Having demonstrated that SNF2H and BRG1 had comparable activities and that both could remodel arrays of nucleosomes, we wanted to further compare their remodeling activities. To do so, we moved from nucleosomal arrays to mononucleosomes reconstituted on short DNA fragments; the TPT fragment (Fig. 5A) is 155 base pairs in length, forming a nucleosome with 10 base pairs of free DNA. These TPT mononucleosomes were used in the ATPase assay, where they stimulated the ATPase activity of both SNF2H and BRG1.

We tested the ability of SNF2H and BRG1 to facilitate restriction enzyme cleavage on a mononucleosome. The TPT DNA sequence has two phasing sequences at one end; these phasing sequences position the PstI cleavage site in a specific translational and rotational location (48). In the absence of remodeling activity, the PstI site is only slightly (≤5%) accessible. Although BRG1 had a significant, ATP-dependent effect on PstI cutting, SNF2H had no detectable effect (Fig. 5B). As in the array restriction enzyme assay, these reactions were performed with saturating ATP and sub-saturating nucleosomes. Under these conditions, the specific activity of BRG1 for remodeling was below the limit of detection (≤200 units/mg). C, BRG1 and SNF2H were incubated with end-labeled TPT nucleosomes, then digested with DNase I and separated on an 8% acrylamide-urea gel. D, end-labeled TPT nucleosomes were incubated with SNF2H or BRG1, released by adding cold competitor DNA, and separated by 5% native acrylamide gel electrophoresis. The stable remodeled dinucleosome can be seen in lanes 4, 9, and 10.
eling this mononucleosome was 2500 units/mg (1 units = 1 pmol of mononucleosomes remodeled/min). The specific activity of SNF2H was less than 200 units/mg, based on the limits of detection in the assay. This difference in activity between SNF2H and BRG1 (10-fold) contrasts with the 2-fold difference seen using these same fractions in the array assay. Thus, the substitution of mononucleosomes for arrays in an otherwise identical reaction effectively eliminated SNF2H activity, whereas BRG1 was able to remodel both substrates.

_PetI_ cuts the DNA in the major groove at a specific position on the nucleosome. To make sure that the inactivity of SNF2H was not specific to a single site, we looked at the ability of BRG1 and SNF2H to enhance cutting by DNase I under identical conditions. DNase I cuts in the minor groove of the DNA and is much less sequence-specific. When incubated with end-labeled TPT mononucleosomes, BRG1 significantly altered the DNase I cutting pattern (Fig. 5C, lanes 3–6). SNF2H had no significant effect on the ability of DNase I to cut (7th–11th lanes). SNF2H was more than 10-fold less active than BRG1 in these reactions, since 400 ng of SNF2H displayed much less of an effect on DNase I cleavage than 50 ng of BRG1. These results corroborate the PetI result described above; using two enzymes with very different cutting patterns, we saw that BRG1 can alter the nuclease accessibility of DNA on a mononucleosome, whereas SNF2H has little or no such activity.

The human and yeast SWI/SNF complexes as well as homologous BRG1 can convert mononucleosomes to a stable remodeled dimer in an ATP-dependent reaction (20, 24, 25). The dinucleosome is one of several products of SWI/SNF and BRG1 remodeling, and its nucleosome sensitivity is greater than that of native mononucleosomes. Under conditions where BRG1 was clearly active, SNF2H did not form detectable amounts of the dinucleosome (Fig. 5D, lanes 5–8). Also, SNF2H did not interfere with the activity of BRG1 (lanes 9 and 10), even when added in excess to BRG1.

These experiments suggest that SNF2H remodels mononucleosomes much less efficiently than BRG1, although the two are comparably active in remodeling arrays. In the ATPase assay, SNF2H activity was strongly stimulated by mononucleosomes, so it is clear that the inactivity of SNF2H in these assays is not due to a failure to recognize the substrate. It is possible that SNF2H has no effect on mononucleosomes, that SNF2H remodeling of mononucleosomes is simply not detectable by these assays, or that the absence of its normally associated proteins has a differential effect on the mononucleosome-remodeling ability of SNF2H. In any event, it is clear that SNF2H remodeling activity is much more efficient on arrays than on mononucleosomes, although SNF2H interacts biochemically with both substrates.

**SNF2H Does Not Alter the Topology of a Nucleosomal Plasmid**—The yeast and human SWI/SNF complexes and the homogenous BRG1 protein are able to change the topology of circular plasmids assembled into nucleosomes (6, 37). Recently, it has been suggested that the remodeling activity of the SWI/SNF complexes and BRG1 might result from ATP-dependent induction of topological stress (26, 49). ISWI also causes torsional stress, but only in the presence of nucleosomes; SWI/SNF can cause torsional stress on DNA in the presence or absence of nucleosomes. To compare the ability of BRG1 and SNF2H to introduce topological changes in closed circular arrays, we incubated these arrays together with remodeler and topoisomerase I. Topoisomerase I relaxes each array as it is remodeled; any change in topology of the array arising during the remodeling reaction is visualized by de-proteinization of the sample followed by non-denaturing gel electrophoresis. Under these conditions where BRG1 could clearly create a distribution of topoisomers (Fig. 6, compare lanes 3 and 4), SNF2H had no detectable activity, even when significantly more SNF2H activity (as measured in the array restriction enzyme assay) was added. SNF2H, however, was able to partially inhibit the activity of BRG1 when added in excess (lanes 8–10), possibly by competitive binding to the array.

### DISCUSSION

All of the ATP-dependent nucleosome remodeling complexes that have been purified perform some type of “chromatin remodeling function; that is, they increase the accessibility of nucleosomal DNA to nucleases or other DNA-binding proteins in vitro or in vivo. However, it is not clear that the actual biochemical products of the remodeling reactions are the same in every case; increased DNA accessibility could be the result of either the movement of histone octamers to create nucleosome-free regions or the disruption of nucleosome structure. If different remodelers alter chromatin structure through the same mechanisms, then one might expect them to have the same functions when assayed biochemically. Here, we have taken two remodeling proteins, representative of two of the major classes of remodeling complexes, and compared their activities quantitatively in a panel of nucleosome-remodeling assays. Both BRG1 and SNF2H could remodel nucleosomal arrays, but SNF2H had no detectable effect on the topology of nucleosomal plasmids or on the nuclease accessibility of mononucleosomes. These observations are most simply explained by differences in the mechanism by which these two remodeling proteins function.

Although SNF2H and BRG1 are nearly equal in their activity on an array of nucleosomes, the specific activity of SNF2H on mononucleosomes is much lower, falling below the limits of detection in our assays. One possible explanation for this result is that SNF2H needs a longer stretch of free DNA to bind to the nucleosomes than BRG1 needs. This seems unlikely; in the ATPase assay, SNF2H can recognize the same mononucleosomes used in the remodeling assays and does so with a _K_m that is comparable with the _K_m of BRG1. An alternative explanation is that SNF2H does not cause the kind of conformational changes in mononucleosomes that can open up the _PstI_ or DNase I sites without removing the histones. If SNF2H operated by sliding the histone octamer, for example, it might simply need a longer piece of flanking DNA in order to move the _PstI_ or DNase sites off of the histone octamer. This is consistent with previous data regarding ISWI-based complexes, which suggest that the ISWI-based activities operate primarily by sliding histone octamers over DNA.
The use of sliding to remodel chromatin would explain why SNF2H does not alter the topology of circular chromatin even though the same preparations can remodel linear arrays, since sliding of nucleosomes does not cause changes in linking number. It is possible that SNF2H induces transient torsional stress to generate sliding, as suggested by recent results. However, this work also revealed a difference between BRG1 and ISWI, the Drosophila homolog of SNF2H: BRG1 and SWI-SNF could cause torsional stress on both nucleosomal and free DNA templates, whereas ISWI could cause torsional stress only on nucleosomal templates (26). This study and other recent results show that changes in topology are likely to be closely related to the mechanisms of nucleosome remodeling as measured in other assays such as restriction site accessibility (49); however, there is currently no data regarding the chain of causation between topological changes and chromatin remodeling. If the induction of persistent changes in topology in the causation between topological changes and chromatin remodeling, however, there is currently no data regarding the chain of events catalyzed by SNF-SNF complexes and ISWI complexes.

A mechanistic difference between SNF2H and BRG1 might explain the differences in the extent of remodeling in the restriction site accessibility assay on arrays. Assembly of the 5 S array into nucleosomes is likely to result in a heterogeneous population of nucleosomes, where the central region containing the unique restriction sites can be covered by one or two nucleosomes in different translational frames. If SNF2H requires unoccupied DNA to slide nucleosomes away from a restriction site and facilitate cutting, it would be unable to increase the accessibility of sites on tightly packed arrays. In contrast, BRG1 appears to be able to remodel nucleosomes without displacing them (49), it should be relatively insensitive to the positions or density of nucleosomes over the unique sequence of the array. This model can explain why BRG1 was able to facilitate cutting of >90% of arrays from each assembly, whereas SNF2H could only facilitate the cutting of 50–70% of the arrays, depending on the assembly.

Previous work shows that the ATPase activity of ISWI was stimulated by nucleosomes but not by bare DNA, whereas BRG1 ATPase activity could be stimulated by both (6, 19, 33). We observed similar behavior; surprisingly, however, we saw that bare DNA could significantly inhibit the nucleosome-stimulated ATPase activity of SNF2H. Parallel experiments that directly examined the DNA binding capability of SNF2H gave a $K_d$ of ~2 nM, in agreement with the $K_d$ obtained from the ATPase experiments. The simplest explanation for this result is competitive inhibition, although we cannot rule out non-competitive inhibition at this point. SNF2H recognizes the DNA portion of the mononucleosome, but DNA by itself is not sufficient to trigger ATP hydrolysis. Other studies show that the histone tails, particularly the H4 tails, are necessary for stimulated ATPase activity but not sufficient (33). It may be that DNA binding is a component of nucleosome recognition and movement but that the histone octamer induces a conformational change required to activate the ATPase and movement activities.

Given the diversity of subunit composition, size, and apparent function among nucleosome remodeling complexes, one of the main challenges of the field is to integrate all of these complexes into a unified model of gene regulation. To understand their biological roles, it is critical that we gain an understanding of the nature of remodeling events catalyzed by each complex. By studying the biochemical functions of different remodelers in vitro, we can better predict their likely roles in vivo. For example, one prediction based on the known biochemistry is that ISWI-based complexes might use their octamer-sliding activity to remodel promoters where short, nucleosome-free regions are required in the course of gene regulation. The biochemical association of the ISWI-containing remodeler NURF (nucleosome remodeling factor) with the HP1 protein is consistent with this prediction (2). On the other hand, we might expect SWI-SNF to be required at promoters where multiple nucleosomes are disrupted but not necessarily removed from the promoter region. The involvement of SWI-SNF complexes in the regulation of the yeast HO promoter (16) and the mouse mammary tumor virus promoter in mammalian cells (50), two promoters where multiple nucleosomes appear to be disrupted but not removed, is also consistent with biochemical functions of BRG1 and the SWI-SNF complexes. Further study of the biochemistry of purified remodelers combined with more detailed studies of remodeling events in vivo will greatly increase our understanding of the role of chromatin remodeling in gene regulation.

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