

# Human ACF1 Alters the Remodeling Strategy of SNF2h<sup>\*S</sup>

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The human ACF chromatin-remodeling complex (hACF) contains the ATPase motor protein SNF2h and the non-catalytic hACF1 subunit. Here, we have compared the ability of SNF2h and a reconstituted hACF complex containing both SNF2h and hACF1 to remodel a series of nucleosomes containing different lengths of DNA overhang. Both SNF2h and hACF functioned in a manner consistent with sliding a canonical nucleosome. However, the non-catalytic subunit, hACF1, altered the remodeling properties of SNF2h by changing the nature of the requirement for a DNA overhang in the nucleosomal substrate and altering the DNA accessibility profile of the remodeled products. Surprisingly, addition of hACF1 to SNF2h increased the amount of DNA overhang needed to observe measurable amounts of DNA accessibility, but decreased the amount of overhang needed for a measurable binding interaction. We propose that these hACF1 functions might contribute to making the hACF complex more efficient at nucleosome spacing compared with SNF2h. In contrast, the SWI/SNF complex and its ATPase subunit BRG1 generated DNA accessibility profiles that were similar to each other, but different significantly from those of hACF and SNF2h. Thus, we observed divergent remodeling behaviors in these two remodeling families and found that the manner in which hACF1 alters the remodeling behavior of the ATPase is not shared by SWI/SNF subunits.

Regulation of chromatin structure is crucial to many fundamental cellular processes such as replication and transcription activation and repression (1, 2). The two classes of mechanisms that are believed to regulate structure are covalent and noncovalent modifications (1, 3–6). Covalent modifications to chromatin include acetylation, deacetylation, methylation, ubiquitylation, and phosphorylation (4–6). Noncovalent modifications encompass several mechanisms, the most prominent of which involves ATP hydrolysis-driven, multisubunit complexes whose central subunits are ATPases of

the SNF2 superfamily (1, 7). These complexes are divided into at least four subfamilies defined by the ATPase catalytic subunits Swi2/Snf2, ISWI, CHD, and Swr1.

The ISWI family has the most identified members of remodeling complexes, each of which contains a small number of non-catalytic subunits. In contrast, the members of the SWI/SNF family, the second most abundant remodeling family, contain a total of 10–15 subunits. Human complexes that contain the human ISWI homolog SNF2h include RSF (remodeling and spacing factor; contains SNF2h and RSF1), WICH (WSTF-ISWI chromatin remodeling; contains WSTF and SNF2h), hACF (ATP-utilizing chromatin assembly and remodeling factor; contains ACF1 and SNF2h), hCHRAC (chromatin accessibility complex; contains SNF2h, hACF1, p15, and p17), NoRC (nucleolar remodeling complex; contains SNF2h and TIP5), and the SNF2h/cohesin/NuRD complex (8–13). This diversity in SNF2h-based complexes raises the question of the mechanistic role played by these different subunits in the process of nucleosome remodeling.

The ISWI-based complexes have varied functions. For example, studies have suggested that ISWI complexes are involved in various aspects of transcription (14–19). In addition, mouse NoRC is involved in the formation of heterochromatin and silencing of rDNA (12, 20), and the human WICH complex is targeted to heterochromatic replication foci (9). Furthermore, ISWI has been implicated in the maintenance of higher order chromatin structure and the facilitation of DNA replication through heterochromatin (14, 21), consistent with structural data suggesting that regular spacing of nucleosomes facilitates formation of higher order nucleosomal structures (22). The ability of the *Drosophila* dACF and CHRAC complexes to create regular spacing of nucleosomes suggests that this is an important property of these complexes *in vivo* (10, 23–25). Although both ISWI and the dACF complex can space nucleosomes, dACF is more efficient (23, 24, 26). Other data suggest a more intricate interaction between ISWI and the dACF1 subunit in dACF than simple enhancement of ISWI activity. ISWI by itself prefers to move centrally located nucleosomes to the end of the template, whereas dACF prefers to move end-located nucleosomes to the central position (27–31). The underlying mechanisms that determine these differences in function have not yet been elucidated.

Differences in remodeling outcome catalyzed by the motor proteins in the ISWI and SWI/SNF complexes have been demonstrated previously (3, 7, 32–34). ISWI family complexes can efficiently slide nucleosomes, and their remodeling products have characteristics of canonical nucleosomes. In contrast,

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SWI/SNF family complexes create products with characteristics distinct from those of canonical nucleosomes and are able to efficiently create access to sites near the center of the nucleosome. These observations have led to the hypothesis that these remodeling complexes function by distinct mechanisms. Studies to date show that additional subunits in the SWI/SNF family of complexes can alter biological targeting of the complex and can increase the specific activity of the complex (35–38), but these studies have not shown significant changes caused by the non-catalytic subunits in the nature of the remodeling function of the complex. In contrast, initial data on directionality of movement have raised the possibility that partner proteins of ISWI might cause more substantive changes in remodeling outcome (27, 28, 30).

The major goal of this work was to measure the remodeling activity of SNF2h as an isolated protein and to compare its activity with that of the hACF complex. Previous work implied that ISWI family proteins require a nucleosomal DNA overhang for remodeling and that the length of overhang might affect the activity of the complex in binding to nucleosomes (31, 32, 39–41). Therefore, we set out to investigate the remodeling efficiencies of SNF2h and the hACF complex at a series of enzyme restriction sites throughout the nucleosome using an extensive set of templates with different lengths of DNA overhang. In addition, we also compared their activities in spacing nucleosomes. Our data suggest that, although SNF2h and hACF share a dependence on the presence of a nucleosomal DNA overhang, the nature of that dependence is changed in that hACF prefers substrates with longer overhangs. Furthermore, both SNF2h and hACF activities created regularly spaced nucleosomes on chromatin that had been assembled on supercoiled plasmid. In contrast, consistent with previous data, BRG1 and BRG1-based SWI/SNF complex remodeling did not require extranucleosomal DNA overhangs. Additional SWI/SNF subunits did not appear to significantly alter the characteristics of BRG1 function, revealing intriguing difference between the two families of complexes in the interaction between the motor protein and its binding partners.

## MATERIALS AND METHODS

**Construction of DNA Templates**—A PstI site was engineered into different positions of the 601 template using the Stratagene QuikChange kit.<sup>3</sup>

**Mononucleosome Assembly and Purification**—DNA fragments containing the 601 nucleosome positioning sequence (provided by the laboratory of J. Widom) were generated by PCR and body-labeled with [ $\alpha$ -<sup>32</sup>P]dATP as required. The templates were assembled into mononucleosomes with HeLa core histones by step gradient salt dialysis, followed by purification on a 10–30% glycerol gradient (32, 33, 42).

**Micrococcal Nuclease Digestion Mapping of Nucleosomes**—The nucleosome positions of assembled mononucleosomes were mapped using limited micrococcal nuclease digestion as described previously (29, 33, 43). 601 mononucleosomes with DNA linker lengths of 0, 20, 45, 91, and 120 bp have identical nucleosome positions (supplemental Fig. 1 and data not shown).

**Protein Purification**—C-terminally FLAG-tagged SNF2h and BRG1 were expressed in Sf9 cells using a baculovirus overexpression system and purified by M2 affinity chromatography (32, 38). C-terminally FLAG-tagged hACF1 (cDNA provided by P. Varga-Weisz) and untagged SNF2h were coexpressed in Sf9 cells and purified using the same system. Human SWI/SNF was affinity-purified from HeLa cells with INI1-FLAG stably integrated into the genome as described previously (44).

**ATP-dependent Remodeling Assays**—All remodeling reactions were performed in 12 mM HEPES (pH 7.9), 10 mM Tris-HCl (pH 7.5), 60 mM KCl, 8% glycerol, 4 mM MgCl<sub>2</sub>, 2 mM ATP·Mg, and 0.02% Nonidet P-40 at 30 °C. All reactions contained remodelers in excess of nucleosomal substrates (<1 nM) to drive reactions to completion (except for ATPase assays and specific activity determinations). When measuring the remodeling rate constants, PstI was continuously present at 0.4–2 units/ml (42).

**Nucleosome Mobility Assay**—All reactions were performed in 12 mM HEPES (pH 7.9), 10 mM Tris-HCl (pH 7.5), 60 mM KCl, 8% glycerol, 4 mM MgCl<sub>2</sub>, 2 mM ATP·Mg, and 0.02% Nonidet P-40. Reactions were incubated at 30 °C for 20 min. Time course experiments were performed to show that these reactions were complete within 5 min. Reactions were stopped by addition of 157 nM ADP and 1.5  $\mu$ g of salmon sperm DNA. The samples were run on 0.5 $\times$  Tris acetate/EDTA 5% gels (33).

**Determination of the Specific Activities of hACF and SNF2h**—The specific activities of SNF2h and hACF were determined under the conditions described previously (33). 1 unit is defined as the amount of enzyme required to generate 1 pmol of PstI-accessible mononucleosomes (substrate C91-25)/min at 30 °C. Rate constants were obtained from initial rates determined by linear fits of data for the first 15% of cut substrates.

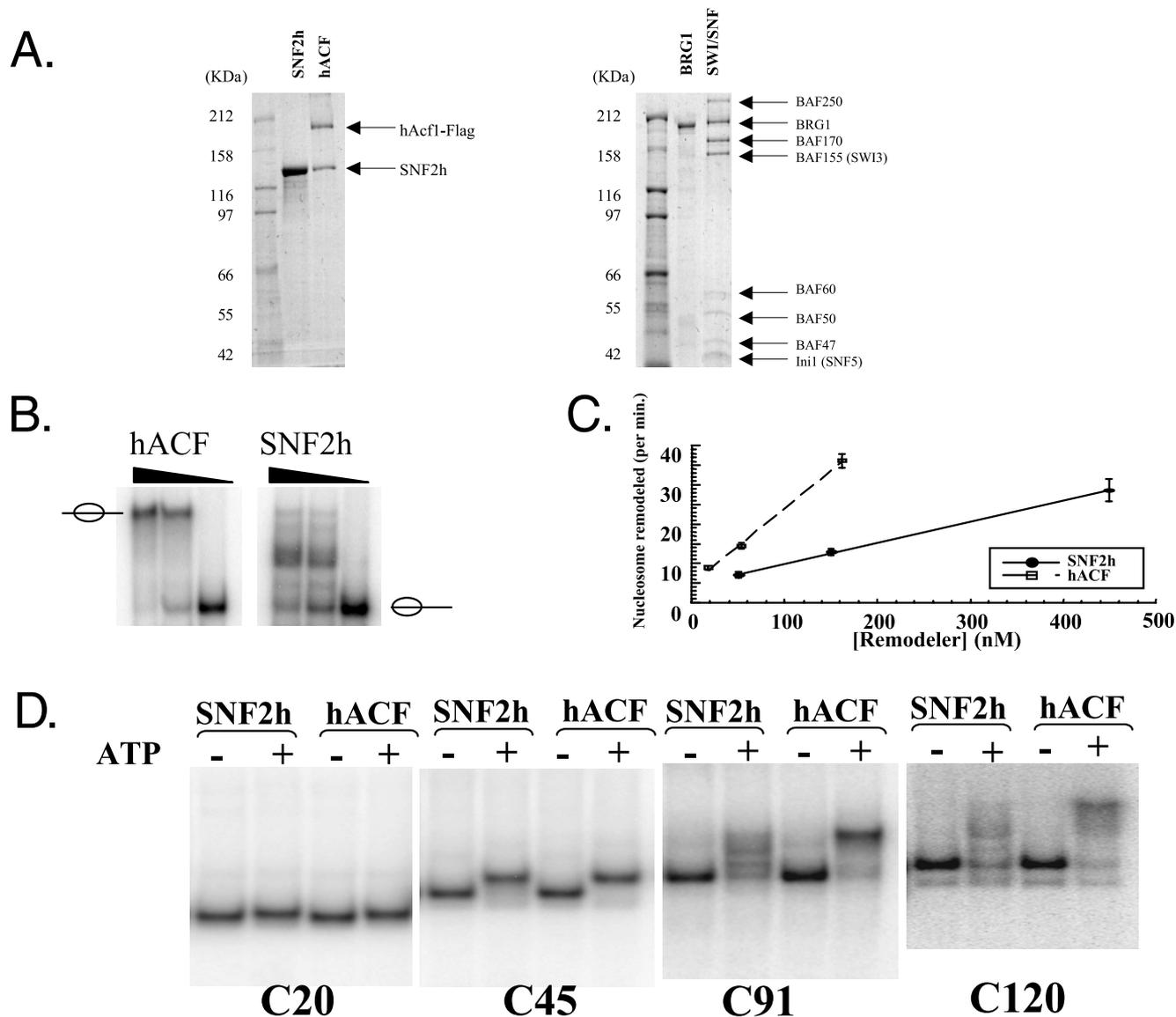
**ATPase Assay**—ATPase assays were performed using Michaelis-Menten conditions as described previously (32, 42). To determine the  $K_m$  of the remodelers for nucleosomal and naked DNA substrates, increasing amounts of substrates were titrated into reactions containing limited amounts of remodelers. To assay the turnover rate of ATP by the remodelers, saturating concentrations of remodelers and nucleosomes were used.

**Array Assembly**—2 kilobase pairs of supercoiled plasmid DNA G1E10 (10, 23) were assembled into arrays with HeLa core histone by salt dialysis (45). The ratio of DNA to histone was ~1:1, and the final concentration of the array was 0.12  $\mu$ g/ $\mu$ l.

**Spacing Assay**—All reactions were performed in 8 mM HEPES (pH 7.9), 8 mM KCl, 3 mM MgCl<sub>2</sub>, 3 mM ATP·Mg, 8% glycerol, 30 mM creatine phosphate, 6 ng/ $\mu$ l creatine kinase, and 0.4 mM EGTA at 30 °C for 3–4.5 h. The concentration of the array used was 5  $\mu$ g/ml, and the concentration of the remodelers used was 1  $\mu$ g/ml. After the array was incubated with each remodeler in the presence or absence of ATP, all the reactions were digested with micrococcal nuclease (Sigma) at two different concentrations (0.001  $\mu$ g/ $\mu$ l and 0.5 ng/ $\mu$ l) for 5 min in 25 °C. The concentration of SNF2h and hACF used was 0.001  $\mu$ g/ml each. After the reactions were stopped with 2% SDS, DNA was extracted from each reaction by proteinase K digestion (0.5  $\mu$ g/ $\mu$ l; Sigma), followed by phenol/chloroform purification. Ethanol-precipitated DNA samples were resus-

<sup>3</sup> Primer sequences are available upon request.

## Remodeling Activity of SNF2h Versus hACF



**FIGURE 1. SNF2h and hACF are comparably active, but differ in their remodeling behaviors.** *A*, shown is a Coomassie stain of purified SNF2h and hACF (*left panel*) and BRG1 and human SWI/SNF (*right panel*). *B*, both hACF and SNF2h remodeled the C120 nucleosome. In the mobility shift assay, we used a titration of 50, 25, and 10 nM SNF2h and a titration of 15, 7.5, and 3.8 nM hACF to remodel <1 nM labeled C120-25 nucleosome. *C*, SNF2h had slightly lower specific activity compared with hACF. In the restriction enzyme accessibility assay, 50 nM SNF2h and 15 nM hACF were used. Different amounts of unlabeled C91 mononucleosome were mixed with labeled C91-25 nucleosomes to achieve the intended nucleosome concentrations. *D*, SNF2h and hACF generated different distributions of products on longer templates. In the mobility shift assay, we used 50 nM SNF2h, 15 nM hACF, and <1 nM labeled substrate in all the reactions. The substrates had the following lengths of overhang: 20 bp (C20), 45 bp (C45), 91 bp (C91), and 120 bp (C120).

pendent in Tris/EDTA and resolved by 1.2% agarose gel electrophoresis in 1× Tris acetate/EDTA buffer against a 123-bp DNA ladder (Invitrogen).

### RESULTS

To examine how non-catalytic subunits in a remodeling complex might alter the function of the central ATP-dependent subunit, we compared the ATPase SNF2h in isolation with the hACF complex, which contains both SNF2h and the non-catalytic subunit hACF1. We used two protocols to examine the activity of these proteins following their purification from baculovirus-infected cells (Fig. 1*A*). The first protocol monitors the shift in nucleosome position upon remodeling using native gel electrophoresis. This protocol has been used previously to demonstrate that mammalian complexes containing SNF2h and *Drosophila*

complexes containing SNF2h homologs change the translational position of the nucleosome (also referred to as “sliding” of the nucleosome) (27–31). The preparations used here were able to move end-positioned nucleosomes that contained 120 bp of additional DNA to the 147 bp that formed the nucleosome. Both SNF2h and hACF moved the nucleosome away from the end position toward the center, thus slowing the mobility of the nucleosomal fragment. As anticipated from previous work, the hACF complex had higher activity compared with SNF2h alone in that hACF could move nucleosomes away from the starting position at lower concentrations than SNF2h alone (Fig. 1*B*).

The remodeling activities of SNF2h and hACF can be more readily quantified using a second protocol that measures restriction enzyme accessibility. Previously occluded restriction enzyme sites in nucleosomal DNA are made accessible when a produc-

tive remodeling event occurs (42, 46). Therefore, the rate of remodeling can be determined by measuring the rate of restriction enzyme cleavage in the presence of a remodeler and ATP. In describing these restriction enzyme accessibility experiments, we refer to the “short end” of the mononucleosome as the end of the template that lacks an extranucleosomal DNA overhang, whereas the “long end” has a DNA overhang. The mononucleosome used here had a 91-bp overhang at the long end and a PstI site that was 25 bp away from the short end of the nucleosomal DNA and thus was occluded for cleavage upon assembly into nucleosomes. Access to the PstI site is believed to be created by moving the nucleosome by at least 25 bp onto free DNA. Both the SNF2h and hACF preparations were able to efficiently create access to the PstI site on this template, with the hACF preparation displaying 3-fold higher activity compared with the SNF2h preparation (Fig. 1C).

**SNF2h and hACF Remodeling Requires Different Lengths of Nucleosomal DNA Overhang**—We compared the ability of SNF2h and hACF to remodel a series of defined substrates. SNF2h is unable to open a PstI site located at position 50 of mononucleosomes (158 bp) that lack a long DNA overhang (32), but it can open this site in substrates with a 55-bp overhang (33). In addition, ISWI-based remodelers from various organisms can reposition nucleosomes on substrates whose templates are longer than those of the core mononucleosome (11, 19, 40, 41, 47, 48). These previous results indicated that the nucleosomal DNA overhang might be essential for remodeling by SNF2h and hACF, prompting us to examine this issue using a wide variety of substrates. A crucial role for a DNA overhang in SNF2h and hACF remodeling might be due to any of the following mutually compatible possibilities. 1) The DNA overhang might be required as a place to reposition the remodeled nucleosome; 2) it might enhance the productive binding of remodelers to substrate; and 3) it might increase the efficiency of SNF2h and hACF in hydrolyzing ATP.

To test the above possibilities and to compare the impact of overhang length on SNF2h and hACF remodeling, we used templates based upon the 601 nucleosome positioning sequence (49). This template produces a defined nucleosome position, as measured by micrococcal nuclease mapping, with each of the different DNA overhang lengths and engineered restriction sites used in this study (supplemental Fig. 1). We initially characterized the impact of overhang length on remodeling using native gel electrophoresis. Templates with 20-, 45-, 91-, and 120-bp overhangs (referred to as C20, C45, C91, and C120, respectively) were all visibly remodeled by SNF2h and hACF in an ATP-dependent manner as measured using this protocol (Fig. 1D). The remodeled species created on the C45 template was similar for both preparations and had a mobility consistent with a centrally localized nucleosome. Despite the limited potential for change in mobility of the smallest C20 template, we observed a slightly slower mobility of this fragment following SNF2h and hACF remodeling that was also consistent with movement to a central position (Fig. 1D). Remodeling with both proteins on the C91 and C120 templates also moved the nucleosome away from an end position. Similar to previous studies comparing ISWI and *Drosophila* ISWI complexes (27, 28), the distribution of remodeled products differed

when SNF2h and hACF were compared. On these longer templates, the predominant remodeled product of the hACF reaction was the centrally positioned and therefore slowest moving nucleosome, whereas SNF2h created multiple species of products. We concluded that both SNF2h and hACF could move nucleosomes on each of the templates tested. Although movement on C20 and C45 overhangs appeared to be similar between the two, there were significant differences on templates with longer overhangs. One possible explanation for these observations, which is investigated further below, is that SNF2h and hACF differ in the way that overhang length affects their ability to remodel.

To determine the impact of overhang length on the rate of remodeling, we used a restriction enzyme accessibility assay to measure rates of remodeling on a series of defined substrates (Fig. 2). Experiments were done using excess remodeling enzyme over substrate, and remodeling rate constants were determined by computer-determined fit to the amount of cutting observed during a time course. These experiments were performed using an excess of restriction enzyme so that restriction enzyme cutting would not be the rate-limiting step of the reaction (42, 46, 49, 50). Control experiments demonstrated that both SNF2h and hACF released nucleosomes more rapidly than remodeling occurred (see “Materials and Methods” and supplemental Fig. 2), demonstrating that substrate release is not limiting.

We constructed four nucleosomal substrates that had an identically engineered PstI restriction site that was 18 bp from the short end of the nucleosomal DNA (“-18”). The respective lengths of the DNA overhang at the long end were 0 bp (with the substrate referred to as C-18), 20 bp (C20-18), 45 bp (C45-18), and 91 bp (C91-18). If the DNA overhang simply provides a place for nucleosomes to slide on to, then both SNF2h and hACF should be able to expose the PstI site at position 18 on all nucleosomes with overhangs of 20 bp or longer. Consistent with previous findings, SNF2h could not create access to the PstI site in a core mononucleosome; however, it could expose the PstI site in substrates C20-18, C45-18, and C91-18 (Fig. 2A). Similarly, hACF remodeling could not expose the PstI site in C-18, but surprisingly was also unable to create access to the PstI site in C20-18. The results above imply that hACF and SNF2h were able to remodel the C20 template as measured by a shift in mobility on native gels (Fig. 1D). The inability of hACF to expose the PstI site on C20 might be caused by the inability of hACF to slide the nucleosome sufficiently away from a central position to expose the site at position 18. The hACF complex could create access to the site in C45-18 and C91-18 (Fig. 2B).

To further probe the different overhang length requirements for hACF and SNF2h, we used the restriction enzyme accessibility protocol to measure the accessibility of additional sites as a function of overhang length. We constructed five sets of templates that were defined by the lengths of their DNA overhang at the long end of the template: 0, 20, 45, 91, and 120 bp. Within each set of C, C20, and C45, we made four distinct templates that contained unique PstI sites positioned 18, 25, 55, or 75 bp from the short end of the nucleosome (Fig. 2C). We also created six templates of C91 (with restriction sites at positions 18, 25, 40, 55, 64, and 75) and nine templates of C120 (with restriction



site at positions 18, 25, 40, 55, 64, 75, 94, 109, and 118) (Fig. 2C). In this manner, we created a panel of 27 templates with varying overhang lengths and distinctly positioned PstI sites.

By measuring the rates of SNF2h and hACF remodeling on these templates, we confirmed as well as expanded the analysis of their differential substrate requirements (Fig. 2, D and E). Neither SNF2h nor hACF could expose any PstI site on the core mononucleosomes. Although hACF could not open up any sites on the C20 series of templates, SNF2h could open up the sites at positions 18 and 25. When the C45 series was used, SNF2h facilitated more extensive access of DNA (positions 18, 25, and 55) compared with hACF (positions 18 and 25). Both hACF and SNF2h could open up all six sites on the C91 series, and both remodelers showed a similar spectrum of activities on the nine tested sites on the C120 series of templates (Fig. 2D). Consistent with the hypothesis that these proteins create access by sliding the nucleosome, both SNF2h and hACF could not open up the sites unless there was sufficient DNA for the repositioned octamer to form a canonical nucleosome; in addition, they both opened up sites closer to the entry point more quickly than the sites near the dyad. If we assume that the nucleosome slid toward the center of the fragment in these experiments, then we predict 0, 10, 23, 47, and 60 bp of flanking linker DNA on the remodeled products of the five substrates that were tested. This prediction is in agreement with the observed rate of PstI site exposure created by SNF2h and hACF on these substrates (Fig. 2, D and E). Their different patterns of site exposure on C20 and C45, relative to templates with longer DNA overhangs, suggest that hACF requires a longer DNA overhang compared with SNF2h for productive binding and/or remodeling.

**hACF1 Alters the Interaction Interface between SNF2h and Substrates**—It is possible that hACF remodels nucleosomes with a 20-bp (C20) or 45-bp (C45) overhang more slowly than nucleosomes with longer overhangs because it binds more weakly to nucleosomes with shorter overhangs. To test this possibility, we used the ATPase activities of SNF2h and hACF to examine how these enzymes compare in their ability to interact with different nucleosomal substrates. By varying the concentration of the nucleosomal substrate under Michaelis-Menten conditions, we were able to measure the apparent  $K_m$  of each enzyme for each substrate, which is likely to reflect the ability of the enzyme to bind to each substrate.

We found that SNF2h interacted significantly more strongly with nucleosomal substrates with an overhang of 45 bp or longer, as indicated by a much smaller  $K_m$  (Table 1). This dependence on longer DNA overhangs in SNF2h/substrate interaction

**TABLE 1**  
 $K_m$  of SNF2h versus hACF for various nucleosomal substrates determined by ATP hydrolysis analysis

Substrate	$K_m$	
	SNF2h	hACF
Core mononucleosome	>250	10 ± 6
C20 mononucleosome	>250	3 ± 2
C45 mononucleosome	30 ± 9	5 ± 3
C91 mononucleosome	10 ± 5	4 ± 3
10-bp DNA	>1400	ND <sup>a</sup>
45-bp DNA	150 ± 50	11 ± 7
60-bp DNA	10 ± 5	3 ± 2
80-bp DNA	9 ± 3	3 ± 2
100-bp DNA	4 ± 0.5	6 ± 3
12-Nucleosome array	0.4 ± 0.3	0.04 ± 0.03

<sup>a</sup> Not determined.

was not template-specific, as we saw similar results using nucleosomes assembled on a different nucleosome positioning template (data not shown). In comparison, we found that hACF interacted strongly with all substrates in a manner that was largely independent of the DNA overhang, as indicated by a  $K_m$  in each reaction that was lower than the  $K_m$  seen with SNF2h (Table 1). Finally, both SNF2h and hACF interacted strongly with assembled nucleosomal arrays.

The ability of SNF2h and hACF to interact with nucleosomes having different overhang lengths was mimicked by their interactions with naked DNA of similar lengths as the linkers (Table 1). Both SNF2h and hACF interacted weakly with 10-bp DNA. As expected from the nucleosomal data, hACF exhibited comparably strong affinity for all the other longer substrates, whereas the affinity of SNF2h for double-stranded DNA substrates correlated with their lengths (Table 1). This is also consistent with the previous finding of stronger association between ISWI and naked DNA in the presence of non-catalytic subunits (24, 41).

Taken together, it appears that hACF1 enhances the interaction between SNF2h and substrates in a way that largely abrogates the need of a DNA overhang for a stable interaction. On the basis of these results, we infer that the slower remodeling of C20 and C45 by hACF, relative to templates with longer overhangs, is not because of weak binding. This implies a role for the DNA overhang distinct from facilitating binding.

**The DNA Overhang Does Not Alter the Ability of SNF2h and hACF to Hydrolyze ATP**—We next determined whether the DNA overhang affected the ATPase activities of SNF2h and hACF by measuring the maximal rates of ATP hydrolysis in the presence of the different nucleosomal substrates. Consistent with previous findings (27, 28), hACF1 did not appear to signif-

**FIGURE 2. Subunit hACF1 of hACF changes the substrate requirement and remodeling profile of the motor protein SNF2h.** A, SNF2h required DNA linkers for productive remodeling. Mononucleosomes with no overhang or different overhang lengths (0, 20, 45, and 91 bp) were used in remodeling reactions. Each mononucleosome had only one PstI site located at position 18. To make position 18 accessible, SNF2h required an ~20-bp DNA overhang. B, hACF required longer DNA linker lengths for productive remodeling. The mononucleosomes used in this experiment were as described for A. Unlike SNF2h, a 20-bp DNA overhang was not sufficient for hACF to expose position 18. C, shown are schematic representations of the 27 distinct mononucleosome templates used. D and E, mononucleosomes with five different overhang lengths (0 bp (C), 20 bp (C20), 45 bp (C45), 91 bp (C91), and 120 bp (C120)) were used. Each mononucleosome of the C, C20, and C45 groups had only one PstI site located at position 18, 25, 55, or 75. The substrates in the C91 group had only one PstI site located at position 18, 25, 40, 55, 64, or 75. The substrates in the C120 group had only one PstI site located at position 18, 25, 40, 55, 64, 75, 94, 109, or 118. D, show is the remodeling profile of SNF2h. 50 nM SNF2h and <1 nM substrate were used in all the remodeling reactions. The positions at which there was no observed ATP-dependent increase in the remodeling rate constant are marked with asterisks. E, hACF showed lower function compared with SNF2h on templates with short overhangs. 15 nM hACF and <1 nM substrate were used in all the remodeling reactions. The positions at which there was no observed ATP-dependent increase in the remodeling rate constant are marked with asterisks.

## Remodeling Activity of SNF2h Versus hACF

**TABLE 2**  
ATP turnover rates of SNF2h versus hACF

Substrate	ATP turnover rate	
	SNF2h	hACF
	<i>min<sup>-1</sup></i>	
C45 mononucleosome	15	15
C91 mononucleosome	10	10
12-Nucleosome array	15	30

icantly increase the ATPase activity of SNF2h (Table 2). Furthermore, longer DNA overhangs did not appear to increase the maximal ATPase rates for hACF and SNF2h. We calculated that SNF2h hydrolyzed 200 ATP molecules for each successful exposure and cleavage of the previously occluded PstI site on the C91-18 template and that hACF hydrolyzed 100 ATP molecules for the same event. This is similar to the figures calculated for dACF (25, 28).

*hACF Exhibits More Acute Substrate Preference Compared with SNF2h*—To help verify the differences in SNF2h and hACF function described above and to further compare the ability of SNF2h and hACF to productively interact with templates with differing lengths of DNA overhang, we performed a series of experiments in which the two proteins were challenged with a mixture of templates. As described above, SNF2h and hACF differed in their relative abilities to create access to the PstI sites in C45-25 and C91-25. Although SNF2h remodeling opened up the two PstI sites with comparable efficiency, hACF exposed the PstI site in C91-25 about five times more efficiently than that in C45-25 (Fig. 2, D and E). To extend this analysis, we examined what would happen if, under conditions of substrate excess, we mixed equal amounts of templates with different lengths of overhang and measured the rate of remodeling of the mixed templates by a single remodeler in the same reaction. This assay was possible because the restriction enzyme cleavage of the different substrates created products of different and distinguishable sizes on a gel.

We first compared SNF2h and hACF discrimination between C45 and C120. When SNF2h was titrated into an equal mixture of C45-25 and C120-25, both templates were remodeled with similar efficiency (Fig. 3A, right panel). When hACF was titrated into the reaction, we observed very little remodeling of the C45 template relative to the C120 template (Fig. 3A, left panel).

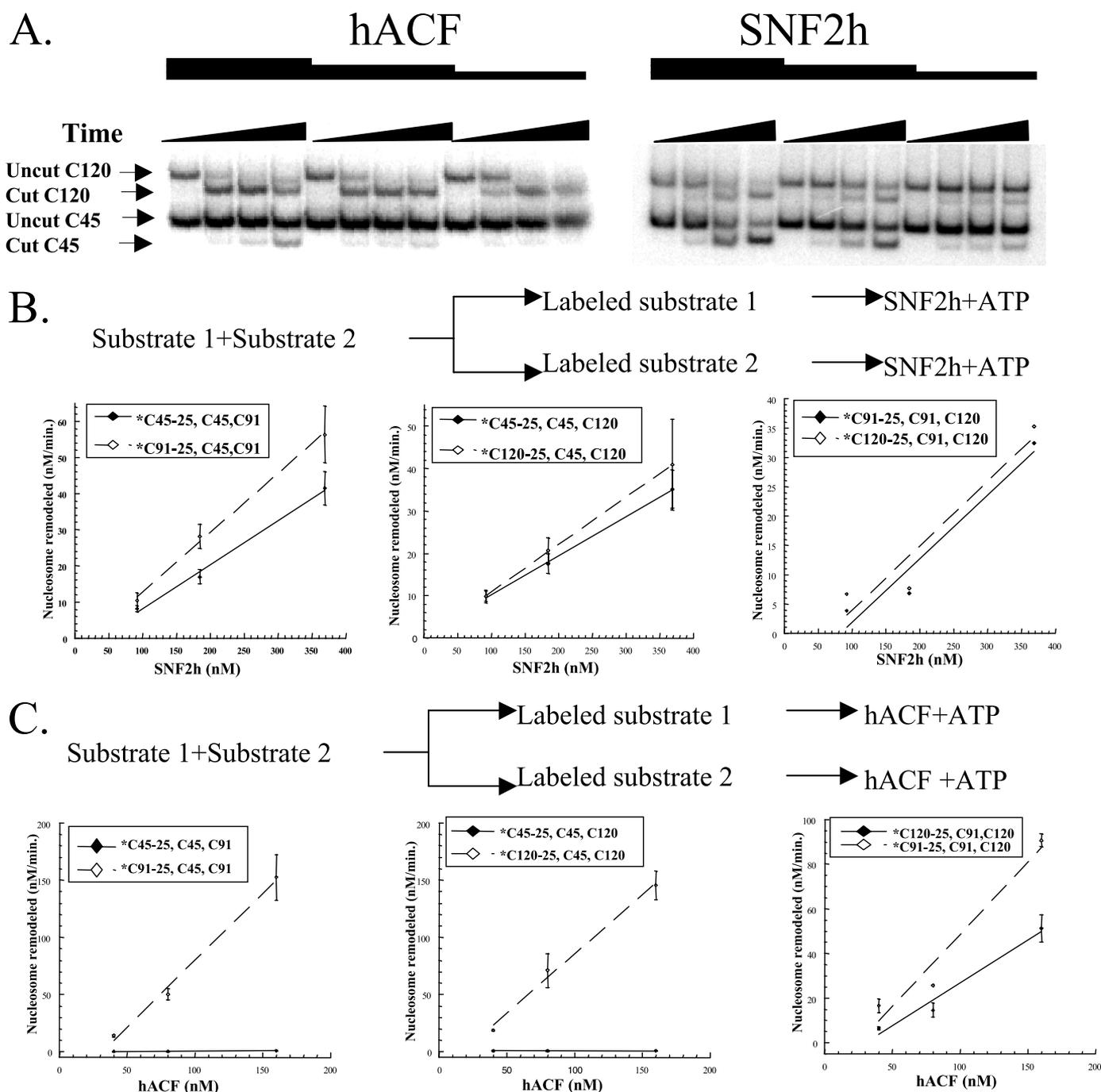
To provide a quantifiable measure of the ability of SNF2h and hACF to discriminate between two mixed substrates, we performed a series of experiments in which equal amounts of unlabeled templates with two different overhang lengths were mixed and then added to a small amount of one labeled template with a restriction site at position 25. For example, when we mixed unlabeled C45 and C91, the labeled C45-25 that was added to the reaction was remodeled by SNF2h at a similar rate as when, in a parallel reaction, labeled C91-25 was added to the reaction (Fig. 3B, left panel). Thus, SNF2h did not discriminate between these substrates. Similar results were seen when SNF2h was tested using mixtures of C45 and C120 and of C91 and C120 (Fig. 3B, middle and right panels). hACF also remodeled C91 and C120 comparably in a mixture of C91 and C120

(Fig. 3C, right panel). However, hACF remodeled the C45 substrate ~100-fold less efficiently than either C91 or C120 in mixed reactions (Fig. 3C, left and middle panels). These experiments extend the previous results by demonstrating that hACF can discriminate between templates with different overhang lengths, favoring the template with a 91-bp overhang. This behavior differs from that of SNF2h.

*SNF2h and hACF Exhibit Different Activities in Nucleosome Spacing*—Creating regularly spaced nucleosomes has been proposed to be an important function for the ISWI-based remodeling family. The ability of hACF to discriminate between substrates with short overhangs and those with longer overhangs might allow hACF to create longer regularly spaced arrays. The DNA overhang might mimic the linker DNA in nucleosome spacing, causing hACF to favor remodeling near long stretches of linker DNA and to disfavor remodeling near short stretches, thereby promoting regular spacing.

We tested this hypothesis using salt dialysis to assemble a given amount of nucleosomes on a supercoiled array, therefore obtaining a population of randomly assembled nucleosomal arrays that had various linker DNA lengths. We then investigated whether SNF2h and/or hACF activities could make the linker DNA lengths more uniform, therefore making the arrays more evenly spaced. After incubation of the remodeling proteins with the nucleosomes, we used micrococcal nuclease digestion to ascertain the regularity of spacing in the products. We saw that, in an ATP-dependent manner, the hACF reaction yielded a ladder of cleanly cut DNA that contained higher molecular weight bands than that of the starting product, whereas the SNF2h reaction yielded a ladder of cut DNA that was similar to that of the starting product and that had a less distinct banding pattern than that obtained in the reaction performed with hACF (Fig. 4, \*). Thus, SNF2h was less able than hACF to create uniformly spaced arrays.

*DNA Overhang Lengths Do Not Significantly Affect BRG1-based Remodeling*—The data presented above suggest that the length of DNA overhang is critical for the ability of SNF2h and hACF to function on mononucleosomal templates and further indicate that the hACF1 subunit alters the requirement for an overhang. Two considerations prompted us to use the same set of templates to profile the pattern of remodeling of BRG1 and the BRG1-containing SWI/SNF complex. First, we wished to determine whether the ability of a remodeling subunit to alter the interaction of the core remodeling protein with DNA overhangs is a shared phenomenon between ISWI and SWI/SNF remodelers. Second, we were interested in determining whether the amount of DNA overhang had similar effects on BRG1 and SWI/SNF as were seen with SNF2h and hACF. Several previous studies have demonstrated differences in function between BRG1- and SNF2h-containing complexes. It has been proposed that sliding of the histone octamer is the main outcome of SNF2h-based remodeling, whereas sliding is one of many outcomes of BRG1-based remodeling (3, 27, 32, 33). If BRG1-based complexes use sliding as a primary mechanism, then a simple prediction is that the extent of DNA overhang will significantly alter the rate of remodeling of centrally positioned sites, as was observed with SNF2h and hACF. This is because it is energeti-



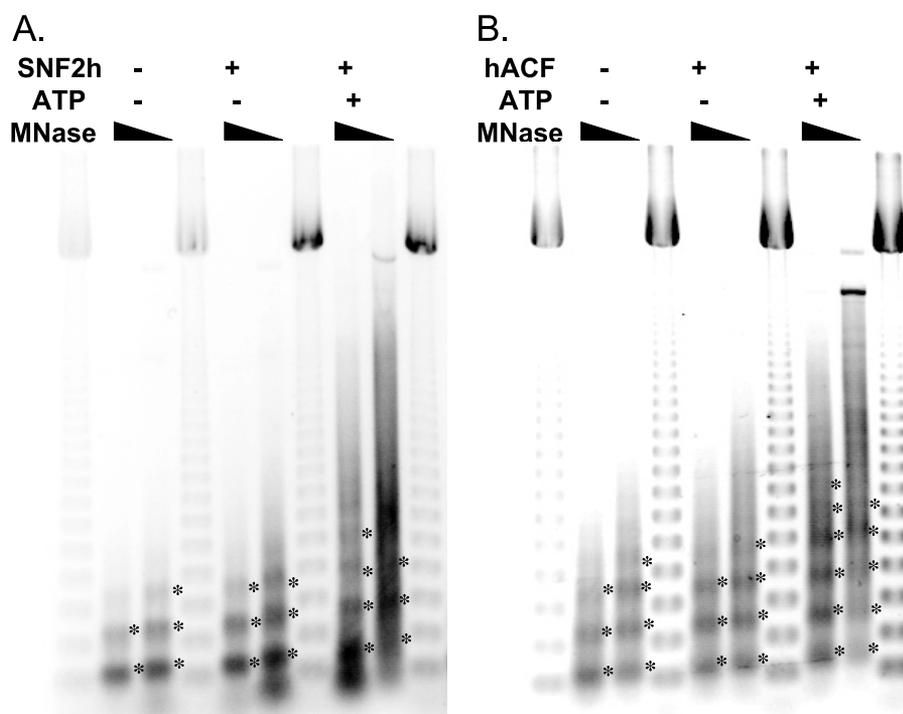
**FIGURE 3. hACF, but not SNF2h, can discriminate between mononucleosomes with shorter DNA overhangs and those with longer overhangs.** *A*, in the restriction enzyme accessibility assay, equal amounts of C120-25 and C45-25 were used with PstI continuously present. Three different concentrations of hACF and SNF2h were used. Fractions of reactions were terminated at different times, deproteinized, and resolved by 8% PAGE in 1 × Tris borate/EDTA. *B*, in the restriction enzyme accessibility assay, equal amounts of C45 and C91 (*left panel*), C45 and C120 (*middle panel*), and C91 and C120 (*right panel*) were used with three concentrations of SNF2h. Radiolabeled C45-25 (indicated with *asterisks*) was added to the C45/C91 mixture to monitor remodeling of C45 templates. In parallel, radiolabeled C91-25 (denoted with *asterisks*) was added to the C45/C91 mixture to monitor remodeling of C91 templates (*left panel*). In the next set, radiolabeled C45-25 and radiolabeled C120-25 were each added to different mixtures of C45 and C120 to monitor remodeling of C45 and C120 templates, respectively (*middle panel*). In the final set, radiolabeled C91-25 and C120-25 were each added to a different mixture of C91 and C120 to monitor remodeling of C91 and C120 templates, respectively (*right panel*). *C*, in the restriction enzyme accessibility assay, equal amounts of C45 and C91 (*left panel*), C45 and C120 (*middle panel*), and C91 and C120 (*right panel*) were used with three concentrations of hACF. The scheme used to add radiolabeled substrates to different mixtures of templates was as described for *B*.

cally favorable for the repositioned histone octamer to have contact with DNA.

Using a different positioning sequence, we had previously shown that BRG1 and SWI/SNF can open sites on nucleosomes

with a 55-bp overhang in a position-independent manner (33). This raised the possibility that the activity of these enzymes was not significantly affected by the length of DNA overhang. To test this hypothesis, we measured the rates of site opening as a

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**FIGURE 4. SNF2h and hACF space nucleosomes to different extent.** Chromatin assembled on supercoiled plasmid was incubated with 1  $\mu\text{g/ml}$  SNF2h (A) or hACF (B) with or without ATP. SNF2h and hACF were both used at 0.001  $\mu\text{g/ml}$ . Micrococcal nuclease (MNase) was used at 0.001  $\mu\text{g}/\mu\text{l}$  and 0.5  $\text{ng}/\mu\text{l}$ . The resulting nucleosomal arrays were deproteinized and resolved on 1.2% agarose against a 123-bp DNA ladder. The DNA bands are marked with asterisks.

function of overhang length. We saw that BRG1 and SWI/SNF could expose all the PstI sites in C-18, C20-18, C45-18, and C91-18 at comparable rates (Fig. 5, A and B). We next proceeded to examine BRG1 and SWI/SNF remodeling at positions 18, 25, 55, and 75 on C, C20, C45, and C91 substrates (Fig. 5, C–E). Both BRG1 and SWI/SNF were able to create access to centrally located sites on a core nucleosome based upon the 601 nucleosome positioning sequence (the C series) (Fig. 5, D and E).

When we investigated remodeling rates using the full series of templates, we found that BRG1 and SWI/SNF had similar remodeling profiles (Fig. 5, D and E). These profiles differed from those observed with SNF2h and hACF (Fig. 5, D and E). For example, both BRG1 and SWI/SNF opened up the PstI site at position 55 in C, C20, C45, and C91 nucleosomes at comparable rates that varied within 3-fold (Fig. 5, D and E). This was in marked contrast to the significant increase in the rate of remodeling at position 55 seen with SNF2h and hACF as the overhang length increased (compare Fig. 5 (D and E) with Fig. 2 (D and E)). As seen previously, BRG1 and SWI/SNF could expose sites on a core mononucleosome, demonstrating a characteristic different from that of SNF2h-based remodeling. This further stresses the difference in substrate requirement and possibly in remodeling strategies between these two families of remodelers (32, 33). The lack of a distinct difference between the remodeling profiles of BRG1 and SWI/SNF suggested that the other subunits did not appear to fundamentally alter the outcome of BRG1 remodeling. Taken together, the ISWI family of remodelers appears to differ from the SWI/SNF family of remodelers not only in substrate requirement, but

also in the manner in which additional subunit(s) alter remodeling function.

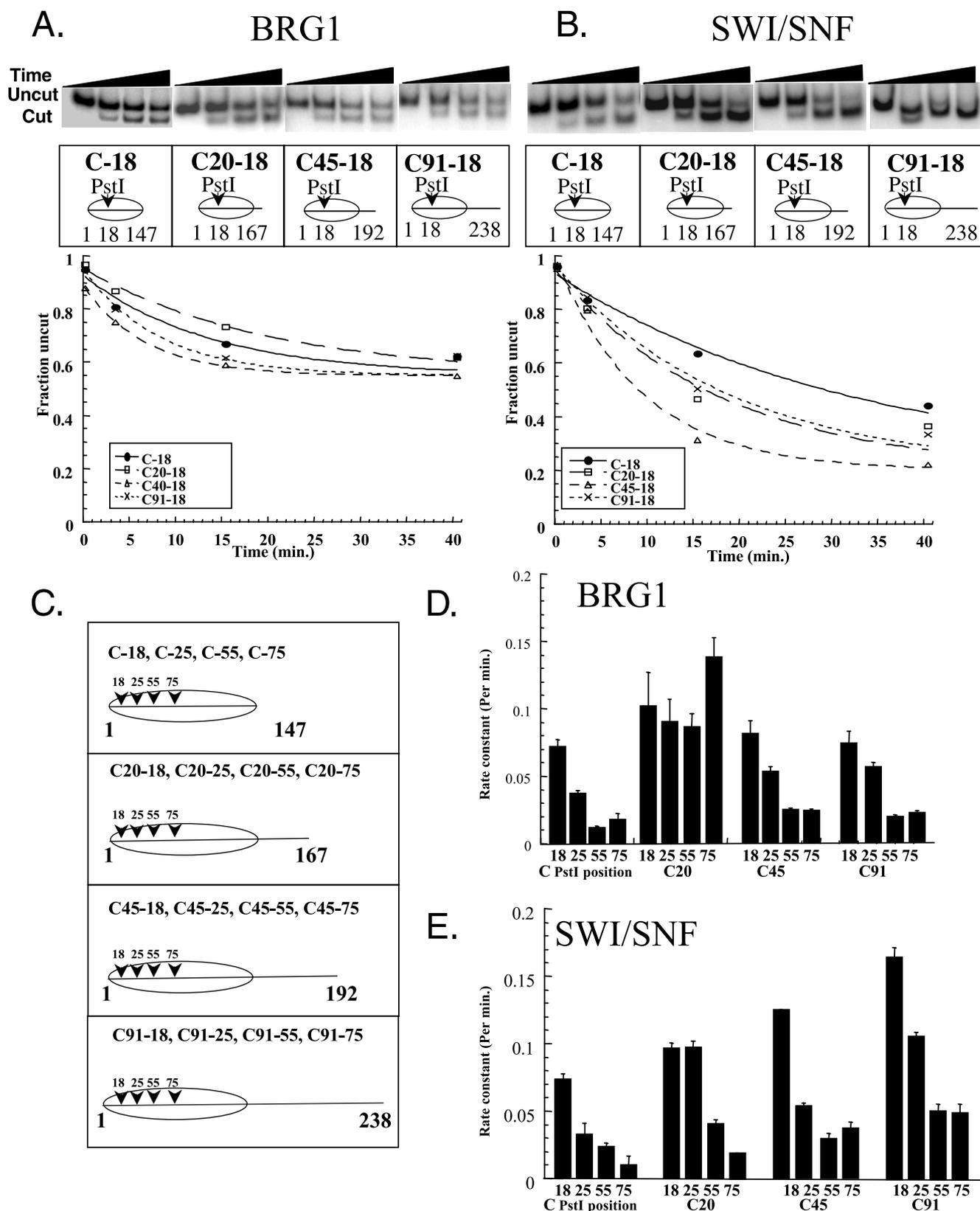
## DISCUSSION

This study has demonstrated two distinguishing characteristics of SNF2h that might pertain to the *in vivo* function of the ISWI family of remodeling complexes. First, SNF2h requires a DNA overhang to function, as anticipated from previous work (see below); surprisingly, addition of hACF1 changes this requirement such that a longer overhang is needed for optimal activity of the complex (Figs. 2–5). The DNA overhang is expected to be functionally related to the linker DNA between histone octamers in an array, so this enhanced sensitivity toward DNA linker length might be germane to the ability of this particular SNF2h-based complex, hACF, to space nucleosomes. Second, both SNF2h and the hACF complex have significantly different requirements for a DNA overhang

compared with BRG1 and the SWI/SNF complex (Figs. 2 and 4). In addition, hACF1 appeared to alter the remodeling pattern of SNF2h more significantly than subunits in the SWI/SNF complex altered the remodeling pattern of BRG1. The latter findings further highlight the functional differences between these two families of remodeling complexes.

The non-catalytic hACF1 protein interacts with the catalytic subunit SNF2h to change the requirement for a DNA overhang. On templates with 20- or 45-bp overhangs, SNF2h is more active than hACF as measured by the restriction enzyme cleavage assay (Fig. 2), and both remodelers show similar behavior as judged by changes in mobility as measured by native gel electrophoresis (Fig. 1D). On templates with longer overhang lengths, hACF displays more activity in both assays. It is interesting that hACF is able to remodel the C20 template as measured by native gel electrophoresis, but not as measured by restriction enzyme access. One explanation for this is that hACF moves the C20 template sufficiently to result in changes in mobility, but not extensively enough to expose the site at position 18. This hypothesis is consistent with the pronounced ability of hACF to move nucleosomes to the center of fragments with 91- and 120-bp overhangs (Fig. 1).

It is apparent from our measurement of binding affinities that a longer nucleosomal DNA overhang is essential for SNF2h to form stable interaction with the substrates, whereas hACF1 abrogates such dependence on the DNA overhang for hACF. This suggests that the inability of hACF to remodel templates with no overhang or a short overhang is not a defect in binding substrate, but would appear to be a defect in forming a productive interaction with substrate. This observation raises the pos-



**FIGURE 5. Subunits of SWI/SNF do not change the substrate requirement or remodeling profile of its motor protein BRG1.** *A* and *B*, neither BRG1 nor SWI/SNF required extranucleosomal DNA linker for effective remodeling. In the restriction enzyme accessibility assay, mononucleosomes with four different overhang lengths (0, 20, 45, and 91 bp) were used in remodeling reactions. Each mononucleosome had only one PstI site located at position 18. 50 nM BRG1 or 10 nM SWI/SNF was used to remodel <1 nm substrate. *C*, shown are schematic representations of the mononucleosomes used in *D* and *E*. Mononucleosomes with four different overhang lengths (0, 20, 45, and 91 bp) were used. Each mononucleosome had only one PstI site located at position 18, 25, 55, or 75. *D* and *E*, shown are the remodeling profiles of BRG1 and SWI/SNF, respectively. In the restriction enzyme accessibility assay, 50 nM BRG1 (*D*) or 10 nM human SWI/SNF (*E*) was used in all the reactions to remodel <1 nm substrate.

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sibility that hACF1 changes the interaction interface between SNF2h and the substrates.

These experiments are consistent with and expand previous work on the ISWI family of remodelers done mainly with the *Drosophila* and yeast homologs. The requirement for a DNA overhang is consistent with the finding that the yeast Isw2 complex is more likely to slide a nucleosome on a template that has one or two DNA overhangs (41). The differential requirement of hACF and SNF2h for an overhang might be functionally related to the predominance of end-located nucleosomal products resulting from ISWI remodeling as opposed to centrally located nucleosomal products resulting from dACF (and hACF, as seen here) remodeling (27, 28). The finding that SNF2h moves nucleosomes away from the ends of the templates used in the present study might indicate a functional difference between ISWI and SNF2h or might instead reflect the different templates used in this study and those used in the previous work investigating ISWI function (25, 27, 28). Finally, these findings are consistent with the observation that dACF increases spacer lengths in closely packed chromatin (10, 23). It is possible that the increased ability of hACF to space nucleosomes (Fig. 4) is related to the preference of this complex for longer stretches of adjacent DNA. This latter observation is consistent with previous experiments performed with ISWI family members in different organisms (32, 33, 41).

Both SNF2h and hACF display low template commitment, suggesting that their respective releases of substrate are quick upon binding. This implies that several rounds of sampling of targets would occur before a successful remodeling event takes place. Because only hACF can discriminate between substrates of different lengths of overhang, the quick release of substrates may reflect multiple rounds of target sampling before a successful event.

Both SNF2h and the hACF complex show a dramatic dependence upon overhang length that is not seen with either BRG1 or the SWI/SNF complex. It has been known for over a decade that SWI/SNF family complexes are able to remodel nucleosomal substrates with no overhang (32, 51–53). The data presented here extend these studies by showing that both SWI/SNF and BRG1 do not display a dramatic change in activity at either internal sites or sites near the entry/exit point as the length of overhang changes. This is consistent with previous hypotheses that the SWI/SNF family of remodeling proteins does not use a sliding mechanism as a primary means to open a site. These data extend the differences in behavior between the SWI/SNF and ISWI families of remodeling proteins and are consistent with the hypothesis that these two families use distinct strategies to make nucleosomal DNA accessible. In addition, we observed that SWI/SNF and BRG1 remodeling had very similar activity profiles on substrates with different lengths of DNA, suggesting that other subunits in SWI/SNF do not appear to fundamentally alter the remodeling outcome produced by BRG1. Our data further buttress the proposal that the SWI/SNF and ISWI families of remodelers work in fundamentally different ways, not only in the level of motor protein activities, but also in the higher level of interplay between subunits and the motor protein.

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**Human ACF1 Alters the Remodeling Strategy of SNF2h**  
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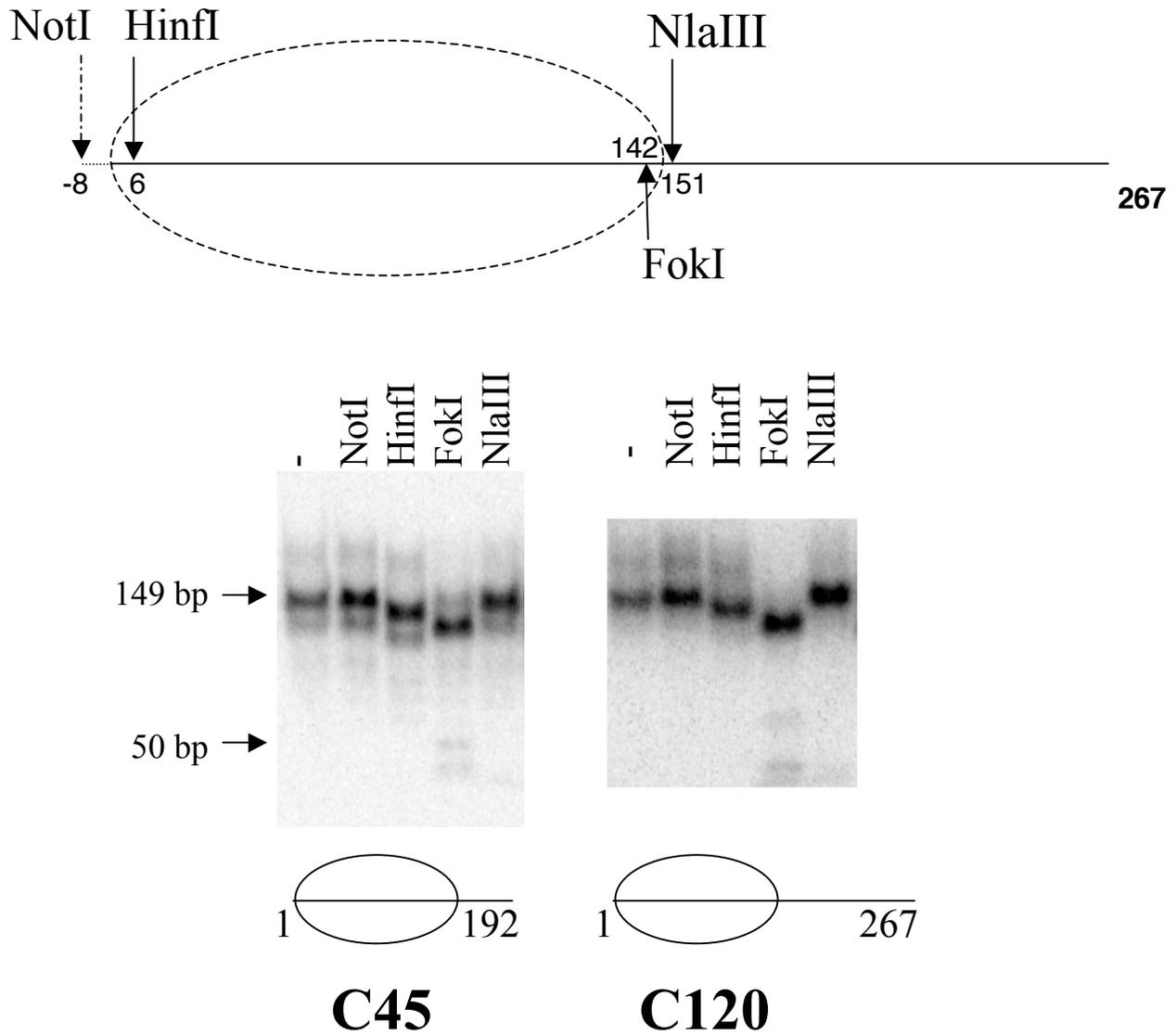
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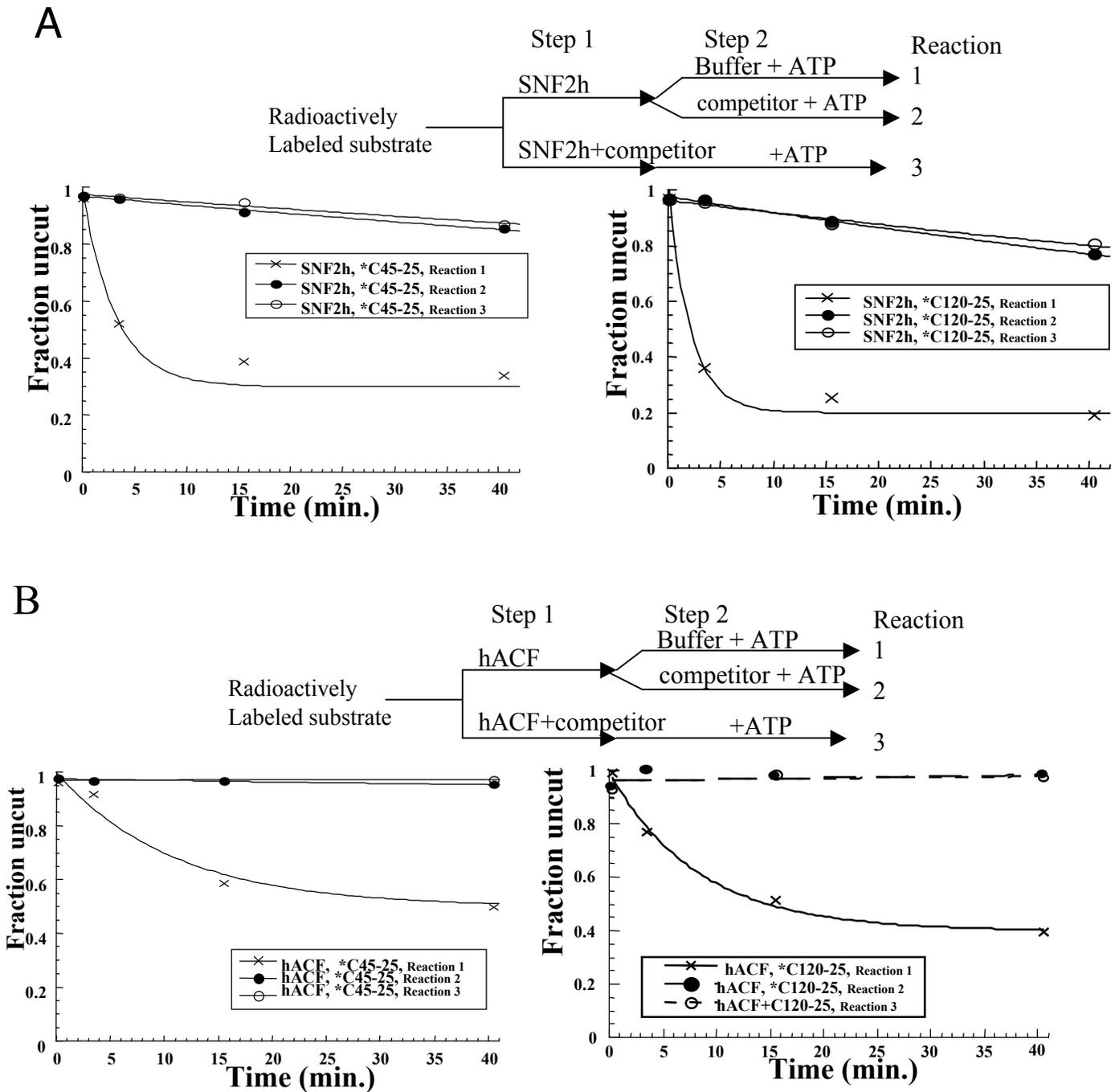
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Supplementary Figure 1. Mapping nucleosome positions of C45 and C120.

C45 and C120 were treated with MNase, deproteinized, digested with restriction enzymes as indicated and resolved on an 8% polyacrylamide gel. Ovals represent the nucleosomal region. Both C45 and C120 have identical nucleosome positions.



Supplementary Figure 2. Template commitment of SNF2h vs. hACF.

SNF2h (A) or hACF (B) were pre-incubated with labeled C45 (\*C45, left) for five minutes at 30°C, remodeling reactions were started by adding ATP (reaction 1) or ATP together with an excess amount of the cold C45 competitor (reaction 2). Reaction 3, SNF2h was pre-incubated with both labeled C45 and excess cold competitor C45 for 5 minutes at 30°C. ATP was then added to start the remodeling reaction. Right panel, identical conditions, but C120 was used instead of C45. Adding an excess amount of cold mononucleosomes (reaction 2) decreased the ability of SNF2h and hACF to expose the PstI site on the radio-labeled mononucleosomes, suggesting these proteins have low commitment to both C45 and C120 templates.