BIOCHEMISTRY
including biophysical chemistry & molecular biology
Article

Biochemistry **2010,** *49,* 6531–6540 6531
DOI: 10.1021/bi1006157

The *Saccharomyces cerevisiae* Swi/Snf Complex Can Catalyze Formation of Dimeric Nucleosome Structures in Vitro[†]

Wladyslaw A. Krajewski* and Oleg L. Vassiliev[‡]

Institute of Developmental Biology of the Russian Academy of Sciences, Moscow, Russia. [‡]Permanent address: Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences, Ul. Miklukho-Maklaya, 16/10, Moscow V-437, Russia.

Received April 22, 2010; Revised Manuscript Received July 5, 2010

ABSTRACT: The Swi/Snf chromatin-remodeling complexes, human BAF/PBAF and yeast RSC, can catalyze formation of stably altered dimeric forms of nucleosomes. However, the ability to create remodeled dimers has not yet been reported for the *Saccharomyces cerevisiae* Swi/Snf complex. Despite its similarity with the other Swi/Snf proteins, the yeast Swi/Snf complex features certain structural and functional differences. This raises the question of whether ySwi/Snf can in fact catalyze formation of dimeric nucleosomes. Dimer formation was proposed to have a specific impact on chromatin regulatory effects. Thus, the answer to the above question may be helpful in clarifying the ySwi/Snf functions in vivo and generalizing the notions of the regulatory principles of Swi/Snf family proteins. Here we describe ySwi/Snf-catalyzed formation of nucleosome dimers using mono- and dinucleosome templates assembled from purified histones and DNA of the high-affinity (601) nucleosome positioning sequence. We evaluated effects of nucleosome template geometry on the formation of altered dimers and assayed formation of altered nucleosome pairs on reconstituted dinucleosomes.

The basic unit of chromatin organization is the nucleosome, which consists of 146 bp of DNA wrapped around an octamer of histone proteins (*1*). Such dense DNA packaging, needed to fit DNA in nucleus, reduces DNA accessibility and dynamics, which are required for the expression and maintenance of the eukaryotic genome. A family of ATP-dependent chromatin remodeling complexes can alter the tight wrapping of nucleosomal DNA and facilitate mobilization of histone octamers. Remodeling complexes can be divided into Swi/Snf, Iswi, CHD, and INO80 protein families on the basis of the similarity of their ATPase subunits (*2*).

The Swi/Snf proteins, initially identified in *Saccharomyces cerevisiae* (*3*, *4*), in addition to the catalytic subunit contain 8−14 proteins, which determine the features and specialization of Swi/Snf complexes. Most eukaryotes utilize two related Swi/Snf remodelers: one subfamily comprises yeast Swi/Snf, fly BAP, and mammalian BAF, whereas the other subfamily includes yeast RSC, fly PBAP, and mammalian PBAF (*2*, *5*, *6*). All tested Swi/Snf complexes possess several common biochemical activities, such as the exposure of nucleosomal DNA, nucleosome sliding, and histone octamer transfer (*2*, *5*, *6*). Depending on the conditions, a particular effect (octamer transfer as in refs *7* and *8* or sliding as in refs (*9*−*13*)) may be dominating or an exclusive outcome of the remodeling reaction.

In addition to nucleosome mobilization activities, human BAF/PBAF complexes (purified via the affinity-tagged common subunit, Ini1) and related yeast RSC complex (*14*) can connect two mononucleosomes together to form an altered noncovalent dimer of mononucleosomes, in which ∼60 bp of DNA is more weakly bound than in normal nucleosomes (*8*, *15*, *16*). BAF/PBAF proteins can also generate structurally altered pairs of adjacent nucleosomes on polynucleosomes (termed "altosomes") as evidenced by altered MNase[1] digestion pattern, which shows an increase in the amount of internucleosomal fragments in the regular ladder of nucleosome-sized DNA multimers (*17*). These asymmetric structures appear to contain two intact histone octamers; however, instead of the single 292 bp protected fragment typical of two closely abutting nucleosomes, the altosomes protect one internucleosomal-sized fragment of 220 bp and one 70 bp subnucleosomal fragment (*17*). The formation of structurally altered dimers and dinucleosomes was supported by direct imaging of remodeled mono- and polynucleosomes by atomic force microscopy (*16*). Altered dimers and dinucleosomes possess an "inverted" accessibility to transcription factors, such as the accessibility of DNA in altered dimers for Gal4 binding being the opposite of that for normal nucleosome cores [highest in the middle of the template and lowest at the ends (*15*, *18*, *19*)], suggesting that dimers might function to facilitate factor access to nucleosomal DNA. The functional roles for altosomes in vivo were emphasized by studies showing that subnucleosomal MNase footprint products, associated with transcription, can result from BAF/PBAF action (*17*).

However, the ability to catalyze formation of altosomes or altered nucleosome dimers remained obscure for the *S. cerevisiae* Swi/Snf complex. It was mentioned that the altered dimers were not observed even under conditions that rule out possible dissociation of dimers upon gel electrophoresis (*11*); nucleosome dimers were also not observed in experiments, including activation domain-targeted ySwi/Snf remodeling (*20*). Despite the evident similarity

[†]The research was funded by the grants from the Russian Foundation for Basic Research (08-04-01010), the Association for International Cancer Research (06-466), and the Leukemia Research Foundation (Evanston, IL).

*To whom correspondence should be addressed: Institute of Developmental Biology, ul. Vavilova 26, Moscow 119334, Russia. Phone: (7-499) 1358047. Fax: (7-499) 1358012. E-mail: wkrajewski@hotmail.com.

[1]Abbreviations: BSA, bovine serum albumin; DNase I, deoxyribonuclease I; MNase, micrococcal nuclease; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

Published on Web 07/07/2010

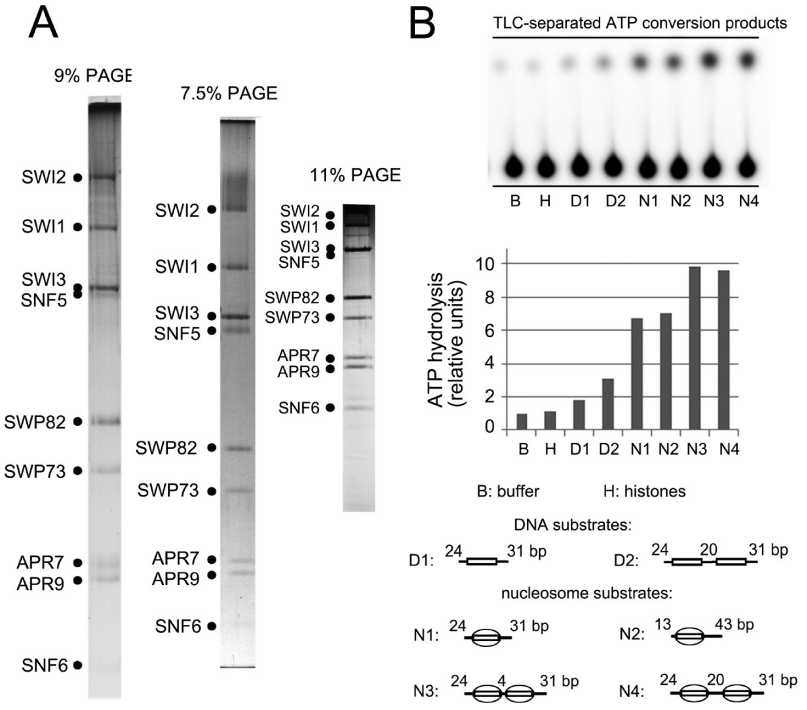

FIGURE 1: Purification and characterization of the *S. cerevisiae* Swi/Snf complex. (A) Purified ySwi/Snf, resolved on SDS−PAGE and stained with silver reagent. Three PAGE concentrations are given to show the separation of ySwi/Snf subunits. (B) ATPase activity of the isolated ySwi/Snf complex using various nucleosome and DNA substrates, depicted at the bottom. The rectangles and rectangles in ovals indicate the minimal 601 positioning sequence and the 601 positioned nucleosomes, respectively; the numbers give the sizes (base pairs) of extranucleosomal DNA.

with the other Swi/Snf family members and common major chromatin restructuring activities in vitro, ySwi/Snf possesses a number of structural and functional differences (see Discussion). This raised the question of whether yeast Swi/Snf can generate dimeric nucleosome structures. Altosome formation and nucleosome repositioning were proposed to have distinct, temporally staged regulatory effects in vivo (*17*, *19*); thus, an answer to the question posed above may help to clarify the ySwi/Snf functions in vivo and the gene regulatory principles of Swi/Snf chromatin modifiers in general.

However, some structural and functional variability is to be expected among distinct Swi/Snf complexes as they come from different organisms and have different compositions and subspecialization. Given the degree of conservation among them (reviewed in ref *2*) and the evident separation of known chromatin remodeling complexes to the individual families, conserved from yeast to mammals (*2*), there is a reasonable expectation that members of the same family should share same general features, including basic biochemical activities, even though these activities may require different contexts to be clearly evinced in vitro. The in cis and in trans nucleosome dimerization is likely to be such a general feature of the Swi/Snf proteins, important for their specific functional roles in vivo.

Here we describe formation of altered nucleosome dimers in the ySwi/Snf-catalyzed reaction using a variety of mono- and dinucleosome templates assembled from purified HeLa histones and DNA of the high-affinity (601) nucleosome positioning sequence (*21*, *22*). We evaluated the effects of nucleosome template geometry on the formation of altered dimers and assayed formation of altered nucleosome pairs on reconstituted dinucleosome templates.

EXPERIMENTAL PROCEDURES

DNA Templates for Nucleosome Assembly. The NotI/NlaIII fragment of pGEM3Z601R (*23*), containing the minimal 601 nucleosome positioning sequence (*21*, *22*), was cloned between the NotI and XbaI sites of pBluescript II+ (the NlaIII and XbaI termini were preblunted with Klenow) to give plasmid pBS601. To prepare pBS601N, pBS601 was cleaved with NotI, and the termini were filled in with dNTPs/Klenow Exo- and religated. DNA templates for mononucleosomes were derived from pBS601 or pBS601N using pairs of restriction enzymes that leave a desirable amount of flanking DNA on the ends of the 601 sequence. To prepare DNA templates for dinucleosomes, an additional 601 fragment was cloned in the polylinker of pBS601 or pBS601N; the dimer of the 601 sequences was derived using appropriate pairs of restriction enzymes, usually SacI and SacII or EcoRI and HindIII (Figure 1B; see also the Supporting Information for the sequences and preparation of DNA templates and construction of plasmids). For MNase footprinting, DNA templates were end-labeled with [γ-^{32}P]dATP and Klenow Exo-.

ySwi/Snf Purification and Activity Assay. The yeast Swi/Snf complexes was purified from *S. cerevisiae* extracts through TAP-tagged Swi2 protein by the tandem-affinity (TAP) method as described elsewhere (*24*−*26*), except the complex was eluted from the Calmodulin Sepharose with a buffer containing 400 mM NaCl, concentrated, and brought to 100 mM NaCl by buffer exchange in Centricon 30 tubes (Millipore). Isolated proteins were examined by SDS−PAGE with subsequent staining with silver reagent (Bio-Rad). The ATPase activity of the remodeling complexes was assayed in a 5 μL reaction mixture, containing 50 ng of core histones or DNA or 100 ng of nucleosomes or buffer only, 5 ng of ySwi/Snf, 0.35 μCi of [γ-^{32}P]ATP, and 0.1 mM cold ATP. The reaction mixtures were incubated at room temperature for 40 min, and then 1 μL was spotted onto PEI Cellulose F plates (EMD Chemicals) and resolved in 0.15 M LiCl and 0.15 M formic acid. The plates were exposed to a Phosphorimage screen and quantified on a Typhoon Scanner (GE/Molecular Dynamics).

Nucleosome Reconstitution and Assays. Nucleosomes were reconstituted by step dilutions of a 2 M NaCl mixture of

purified histones and DNA (starting concentrations of 0.5 μg/μL) as described in ref *24*, except the initial dilution buffer contained 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 0.5 mM PMSF, and 50 μg/mL BSA (fraction V, Sigma), and the final dilution buffer contained 10 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 1 mM EDTA, 0.05% NP-40, 4 mM DTT, 0.5 mM PMSF, and 50 μg/mL BSA. Where indicated, nucleosomes were purified in native polyacrylamide gels. To evaluate reconstitutions, 5−7 μL nucleosome aliquots were mixed with $^1/_5$ volume of a 60% sucrose/0.01% xylene cyanol mixture in 1× TE (pH 7.6) and resolved via 5−5.5% native PAGE (29:1 or 50:1 acrylamide:bisacrylamide ratio) in 0.5E buffer (1×E = 40 mM Tris-OH, 20.6 mM acetic acid, 5 mM sodium acetate, and 2 mM EDTA) or a 0.5 TBE/0.5E mixture and stained with ethidium bromide.

Nucleosome remodeling was performed directly in the nucleosome assembly buffer [10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 100−110 mM NaCl, 2.5 mM $MgCl_2$, 2 mM DTT, 0.025% NP-40, 50 μg/mL BSA, and 0.5 mM PMSF], supplemented with 1 mM ATP and additional 2.5 mM $MgCl_2$. The 10 μL reaction mixtures contained 120 ng of assembled DNA templates and ∼7, ∼20, or ∼50 ng of ySwi/Snf complex (the molar ratios of ySwi/Snf to DNA templates were ∼1:15/45/120 and 1:30/100/250 for mono- and dinucleosome templates, respectively). After incubations at room temperature for 1.5 h, 5−7 μL reaction aliquots were mixed with $^1/_5$ volume of a 1×TE/60% sucrose/0.01% xylene cyanol mixture and resolved via 5−5.5% native PAGE as described above.

To examine nucleosome structure with micrococcal nuclease (Worthington Biochemical), remodeling reactions were performed in a total volume of 100 μL using nucleosomes assembled on [γ-^{32}P]DNA template. The position of the labeled nucleotide is indicated in the figures (the 3′-end of the DNA template). The ySwi/Snf to DNA molar ratios were 1:50 and 1:100 for mono- and dinucleosome templates, respectively. Remodeling was terminated by a 20 min incubation with 25−50 milliunits of apyrase (New England Biolabs). Nucleosome aliquots (15−20 μL) were digested for 2 min with increasing amounts of MNase. DNA was isolated and resolved via 7% native PAGE (19:1 acrylamide:bisacrylamide ratio). Gels were dried and exposed to a Phosphorimage screen.

The accessibility to restriction endonucleases (New England Biolabs) was assayed using unlabeled nucleosome templates in 20 μL remodeling reactions as described above. Remodeling was terminated by incubation with 10 milliunits of apyrase for 15 min at room temperature. Then nucleosomes were digested with 10 units of restriction enzyme for 30 min at room temperature. DNA was isolated and resolved on 7% native polyacrylamide gels (29:1 acrylamide:bisacrylamide ratio) and stained with ethidium bromide.

RESULTS

Justification of Experimental Conditions. The yeast Swi/Snf complex was isolated from *S. cerevisiae* cell extracts through the TAP-tagged catalytic subunit, Swi2p protein (Figure 1A). Nucleosomes were reconstituted using purified HeLa histones and DNA templates, containing monomers or dimers of the "minimal" 601 high-affinity nucleosome positioning sequence (Figure 1B). The 292 bp sequence "601", predicted from the alignment of a number of nonredundant high-affinity positioning sequences, features an inherent palindromic symmetry and several conserved stretches within a central 71 bp portion of the nucleosomal DNA (*21*, *22*). Its "minimal" 147 bp central fragment possesses affinity for histone octamers comparable to that of the full-length 601 sequence (*21*, *22*). One or two copies of the minimal 601 sequence were cloned in pBluescript polylinker; then DNA templates containing the required amount of the 601-flanking DNA were retrieved with appropriate pairs of restriction enzymes (see the Supporting Information for the sequences and preparation of DNA templates). The size of flanking DNA is indicated in the figures (see Figure 1B, for example). It has been proposed that formation of altered nucleosome dimers might be efficient only when the DNA template has sufficient DNA length to occupy normal histone/DNA contact points and also spans the gaps between octamers (*19*). Therefore, the used DNA templates contained at least 20 bp of the 601-flanking DNA.

Most of the previous ySwi/Snf studies utilized nucleosomes assembled on the moderate nucleosome affinity DNAs, like the *Xenopus* or sea urchin 5S rDNA positioning sequences (*27*, *28*), which do not show the high binding affinity or strong positioning properties of the 601 sequence (*21*, *22*). However, the action of Swi/Snf proteins can be differentially controlled by DNA sequence-specific properties, which influence both the distribution and nature of the remodeled product (*29*−*34*). More strong association of the histone octamer with the 601 DNA may reduce the level of formation of intramolecular intermediates, in which entering or exiting DNA ends are held by the same histone octamer, in favor of formation of the altered nucleosome dimers (see Discussion).

The usual in vitro studies of ySwi/Snf functions have used a total nucleosome concentration similar to, or only slightly greater than, the concentration of the complex, a situation that is unlikely to occur in vivo considering the low ratio of ySwi/Snf to nucleosomes in the nucleus (discussion and references in ref *20*). Under the in vitro conditions described above, a direct association between the complex and substrate may also take place, and even predominate over a catalytic ySwi/Snf action. In the experiments described below, the ratio of the ySwi/Snf complex to nucleosomes was ∼1:40−50 (plus 3-fold higher and lower if three ySwi/Snf concentrations were used).

To avoid possible interference with the carrier oligo- or mononucleosomes, the latter were omitted. Instead, the nucleosome probe of interest comprised the total chromatin content in the reaction (∼10−12 ng/μL of DNA). Higher concentrations of nucleosomes, smaller amounts of the Swi/Snf complex, and NaCl concentrations of 100−110 mM should emphasize the catalytic function of ySwi/Snf. This also allowed omission of the addition of an excess of competing nucleosomes or DNA to release the altered nucleosomes from the remodeling complex, avoiding associated uncontrolled effects (like the dissociation of altered dimers). Therefore, it was shown that altered species can be released from the RSC complex without the addition of competitors, if nucleosomes are assembled on ≥200 bp DNA (*14*). The liberation of altered dimers from the BAF/PBAF complex was also significantly facilitated by an increased (>100 mM) concentration of NaCl (*15*). In addition, elevated concentrations of nucleosomes were shown to facilitate formation and stabilization of the altered nucleosome dimers (*14*).

The ATPase activity of ySwi/Snf was assayed in the presence of free core histones, naked DNA, or mono- and dinucleosomes. Conditions were similar to the ones in the experiments described below (Figure 1B). To keep an equal amount of histone octamers in all reaction mixtures, dinucleosomes were added at a 2-fold lower molar ratio than mononucleosomes. The ATPase hydrolysis was not stimulated significantly by free histones but was stimulated by nucleosomes and, to some extent, by naked DNAs, which is consistent with the reported DNA-dependent ATPase activity of the SWI2 protein (*35*, *36*). The ySwi/Snf ATPase activity was higher on di- than on mononucleosomes. A similar effect was also

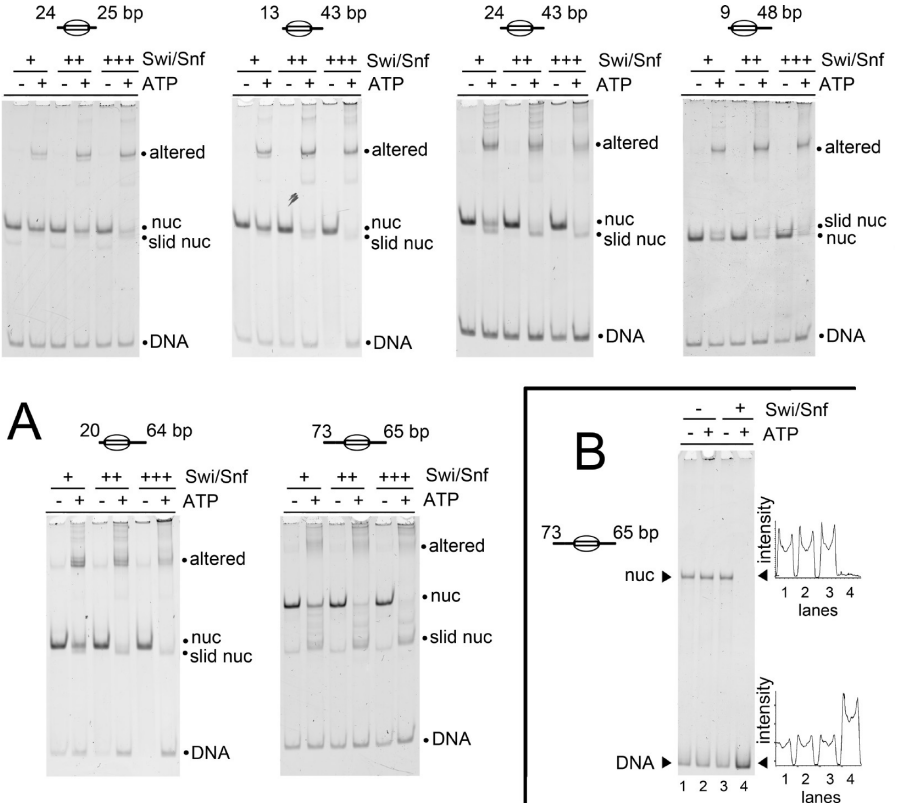

FIGURE 2: Yeast Swi/Snf catalyzes formation of noncovalent nucleosome dimers from mononucleosomes with various extranucleosomal DNA. The schemes of nucleosome templates are shown atop the figures. The rectangles in ovals indicate the 601-positioned nucleosomes; the numbers show the sizes (base pairs) of the extranucleosomal DNA. (A) Gel mobility shift assay of mononucleosomes (200 ng) remodeled with 7, 20, or 60 ng of the ySwi/Snf complex (the molar ratios of ySwi/Snf to the DNA template are ~1:120, ~1:45, or ~1:15). (B) ySwi/Snf remodeling products resolved via native PAGE. The ratio of ySwi/Snf to nucleosomes is ~1:5; the reaction was conducted at 30 °C. The densitometric tracing of gel segments is shown at the right.

seen upon comparison of equal (mass) quantities of di- or mononucleosomal bare DNA template. This may reflect a natural preference of ySwi/Snf for larger substrates, suggesting that nucleosome arrays may better mimic the native substrate of the ySwi/Snf complex. It may also be indicative of a certain cooperativity of Swi/Snf action: at the low ratio of the complex versus substrate, remodeling one nucleosome in a pair may target Swi/Snf activity to the other nucleosome. A higher ATPase activity on dinucleosomes also may be due to a continuous ATP hydrolysis required to maintain an altered state of a nucleosome in a dinucleosome, as arrayed nucleosomes, in contrast to mononucleosomes, reverse rapidly in the original state (*10*).

It has been found that the ySwi/Snf complex binds to nucleosome cores with an affinity only slightly higher than that for naked DNA; binding of the complex to DNA (*37*) and nucleosomes is independent of ATP (*37*, *38*).

The Yeast Swi/Snf Complex Can Catalyze Formation of Stable Noncovalent Nucleosome Dimers from Mononucleosomes Assembled on Various 601 DNA Templates. The formation of altered products was examined by the gel mobility shift assay after incubation of nucleosomes with ySwi/Snf and ATP. Remodeling was typically performed in a 10 μL reaction mixture containing 100 ng of DNA template assembled in nucleosomes at a NaCl concentration of 100−110 mM (at molar ratios of Swi/Snf to DNA template of ~1:15, ~1:45, or ~1:120). After a 1−1.5 h incubation at 25 °C, reaction mixtures were resolved via 5% native PAGE and stained with ethidium bromide (Figure 2A). Unremodeled nucleosomes formed a defined band indicative of strictly positioned histone octamers. In the presence of ATP, the nucleosomal pattern was resolved into two major bands, one that had the same mobility as original nucleosomes and a second, much slower migrating band, corresponding to the altered nucleosome species. Given the strong resemblance of the gel mobility shift pattern of this product and the gel shift patterns of RSC- and BAF/PBAF-generated altered nucleosome dimers (*8*, *14*, *15*, *18*), we have attributed this band to the noncovalent association of two nucleosomes in the altered form. Formation of such dimeric products was observed using mononucleosome templates with a variable amount of extranucleosomal DNA (Figure 2A). The band of altered species was well-defined for nucleosomes assembled on relatively short DNA templates but was fuzzier for nucleosomes containing more than 60−70 bp of extranucleosomal DNA. This might be expected due to the elevated conformational flexibility, caused by long extranucleosomal DNA. In addition, this effect may also derive from increased structural variability arising upon dimerization of nucleosomes with extended octamer-flanking DNA.

On particular templates, remodeled nucleosomes produced extra band(s), running closely below the original nucleosomes. This most likely reflects sliding of a portion of histone octamers to the end of the DNA template. It has been shown that electrophoretic mobility of a nucleosome is greater when a histone octamer is positioned close to the end than to the center of a DNA fragment, due to the kink that DNA forms at the entry−exit point of the nucleosome (*39*).

Occasionally, incubation of nucleosomes with ySwi/Snf and ATP resulted in some release of DNA from nucleosomes (Figure 2A). This effect was relatively small at a low concentration of the remodeling complex. However, at elevated concentrations, ySwi/Snf can completely displace histone octamers from

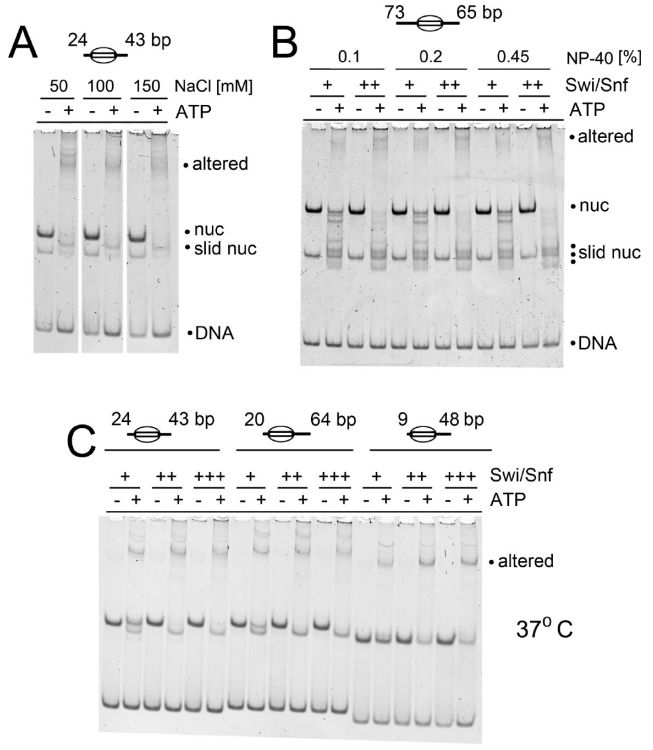

FIGURE 3: Yeast Swi/Snf can catalyze formation of nucleosome dimers under different reaction conditions. Remodeling reactions were performed at 50−150 mM NaCl (A), in the presence of 0.1−0.5% NP-50 (B), and at 37 °C (C). The molar ratios of ySwi/Snf to the DNA template are ~1:45 (A), ~1:120 or ~1:45 (B), and ~1:120, ~1:45, or ~1:15 (C). The schemes of the nucleosome templates showing the size of extranucleosomal DNA are depicted atop the figures.

DNA (Figure 2B). Such minor in-trans displacement of histone octamers (without an obvious histone acceptor) has occasionally been observed in experiments with the yeast Swi/Snf complex (*20*, *40*).

The dimeric nucleosome products were generated by ySwi/Snf under a variety of conditions, including the ionic strength of 50−150 mM of NaCl (Figure 3A) or the presence of 0.1−0.5% nonionic detergent NP-40 (Figure 3B); nucleosome dimers were also generated at 37 °C, a temperature that is higher than that normally used in yeast experiments (Figure 3C). Such ySwi/Snf activity was different from that of human BAF/PBAF, which did not generate altered dimers when 120 mM NaCl was present in the reaction mixture before the remodeling complex (*15*), supposedly due to the inhibition of the initial binding of BAF/PBAF to cores that would allow it to convert them to this product (*15*). This suggests that certain common biochemical activities of distinct Swi/Snf complexes may require different conditions to be evident in vitro. The lower sensitivity of ySwi/Snf to NaCl may be in part due to the more extensive contacts that ySwi/Snf makes with the nucleosome (*40*, *41*).

To further characterize the altered nucleosome species, their electrophoretic mobilities were compared to the mobilities of mononucleosomes bound with an extra histone octamer. It has been shown that such nucleosome/octamer dimers can be formed at an elevated (0.4 M) concentration of NaCl and elevated concentrations of bare histone octamers and nucleosomes (*42*−*44*). Therefore, these dimeric structures can accumulate upon reconstitution of nucleosomes by dilution of a histone/DNA mixture from high salt using higher histone to DNA ratios and higher concentrations of histones and DNA (Figure 4A). These assembly conditions also result in formation of self-associated nucleosomes caused by the presence of histone octamer dimers during the reconstitution (personal communication, S. Grigoryev, The Pennsylvania State University, Hershey, PA). Nucleosomes with an extra histone octamer and nucleosome dimers are expected to have similar mobility on a nondenaturing gel and not resolve well. For all tested nucleosome probes, including the ones with longer or shorter extranucleosomal DNA spacers (panels B and C of Figure 4, respectively), the gel mobility of Swi/Snf-generated species was the same as, or close to, the mobility of reconstituted nucleosome or nucleosome/octamer dimers, suggesting that the ySwi/Snf-produced species are the noncovalent nucleosome dimers. Although we cannot rule out the possibility that the comigrating species formed by ySwi/Snf are the octamer dimers on a monomeric DNA fragment rather than true "nucleosome dimers", the former possibility seems unlikely, as it has been shown that the hSWI/SNF proteins do not function as chaperones for free histone octamers and do not transfer histone octamers from one nucleosomal template to another nucleosomal template (*8*). In addition, ySwi/Snf-dependent removal of histones from one nucleosome template and their transfer to another nucleosome template (forming two histone octamers on one piece of DNA) must be accompanied by formation of an equivalent amount of bare DNA. The latter effect was not observed.

The Yeast Swi/Snf Complex Can Catalyze the Formation of Altered Nucleosome Pairs on Dinucleosomes with Various Spacing, Assembled on 601 Positioning Sequences. To assess whether ySwi/Snf can catalyze formation of altered dinucleosomes, histone octamers were assembled on a series of DNA templates containing two minimal 601 sequences, separated by 4, 10, or 20 bp of DNA. Such structures represent a simple model of a polynucleosome array. For the sake of consistency, all templates had the same orientation of positioning sequences (from 5′ to 3′), as nucleosomes on the 601 DNA exhibit certain asymmetry in the biological processes. For example, nucleosomes on the 601 DNA present a polar barrier to RNA polymerase II transcription in vitro (*45*, *46*).

DNA templates were assembled in the nucleosomes with empirically determined amounts of histone octamers, sufficient to fill both positioning sequences (Figure 5A, lanes 1−3). At higher concentrations of histones, one can observe formation of the dinucleosome dimers (lane 4). Remodeling of dinucleosomes with ySwi/Snf and the mobility shift assays of reaction products were performed essentially as described above for mononucleosomes. Similar to mononucleosomes, remodeled dinucleosomes were resolved on a native gel in two bands, corresponding to the original and more slowly migrating band of the structurally altered product (Figure 5B). The latter we attributed to a noncovalent dinucleosome dimer due to the strong parallel between the remodeling products formed from mono- and dinucleosomes. Remodeled dinucleosomes formed a fuzzier band than altered mononucleosome dimers that is likely due to higher flexibility and structural variability of dimerized dinucleosomes.

The slowly migrating product was generated by ySwi/Snf from dinucleosomes with various nucleosome spacing, including templates with virtually no octamer-free DNA. This suggests that ySwi/Snf can also act on nucleosomes with smaller extranucleosomal DNA regions (Figure 5C). The electrophoretic mobility of remodeled dinucleosome species was similar to the mobility of reconstituted dinucleosome dimers (Figure 5B,C), suggesting that the altered product is a noncovalent dimer of dinucleosomes.

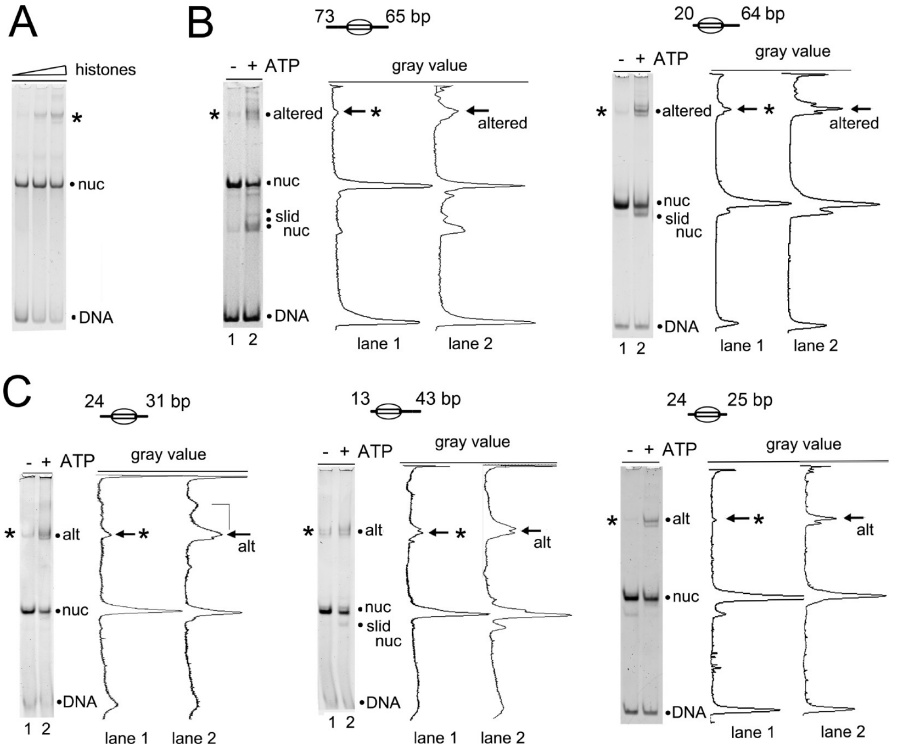

FIGURE 4: ySwi/Snf-converted products comigrate with reconstituted nucleosome/octamer dimers. (A) Products of nucleosome assembly at elevated histone:DNA ratios, resolved via native PAGE. (B and C) Nucleosomes, containing the indicated amounts of octamer dimers as internal markers (marked by an asterisk), were remodeled with ySwi/Snf and resolved via native PAGE. The gel scan profiles are shown at the right.

We compared the MNase digestion pattern of nucleosome DNA in the presence of the ySwi/Snf complex with or without ATP. The 601 DNA contains several conserved regions with inherent palindromic symmetry and displays a distinct symmetrical pattern of nuclease accessibility. Without remodeling, nucleosomes strongly protect DNA from MNase cleavage (Figure 5D, left). After incubation of dinucleosomes with ySwi/Snf and ATP, the MNase protection pattern became more asymmetric. Now, a portion of the octamer-occupied DNA possesses MNase sensitivity similar to that of bare DNA, while the remaining nucleosome portion remains nuclease-resistant (Figure 5D, right). The more detailed picture of the MNase cutting pattern in the 5′-region of the DNA template (resolved on the lower-percentage gel) is shown in Figure S1B of the Supporting Information. The arising asymmetry in the MNase accessibility may indicate formation of altered asymmetric nucleosome structures. In polynucleosomes, such structures should produce an MNase cutting pattern with abundant internucleosome-size DNA, similar to the MNase cutting pattern observed on the BAF/PBAF-remodeled polynucleosomes (*17*, *19*).

This suggests that yeast Swi/Snf can catalyze formation of altered dinucleosomes like its human counterparts. On the ySwi/Snf-remodeled mononucleosomes, the asymmetric digestion pattern was still present although, for some reason, less pronounced.

Yeast Swi/Snf Generates an Altered Pattern of Restriction Endonuclease Accessibility on Mono- and Dinucleosomes Assembled on 601 DNA. The accessibility of DNA to restriction enzymes in ySwi/Snf-remodeled mono- and dinucleosomes was examined using DNA templates containing a monomer (Figure 6C) or a dimer of the minimal 601 sequence. DNA templates assembled in the nucleosomes as described above were incubated with ySwi/Snf with or without ATP for 1.5 h at 25 °C. Then, ATP was depleted by treatment with apyrase, and samples were digested for 30 min with the MseI, BstUI, or HaeIII restriction enzyme. Nucleosomal DNA was deproteinized, resolved on gel, and stained with ethidium bromide (Figure 6).

For all tested nucleosome templates, preincubation with ySwi/Snf and ATP significantly stimulated DNA cleavage by the MseI and, to a lesser extent, BstUI nucleases, which cut the DNA within ~10 or ~20 bp of the nucleosome dyad, , respectively (sites B2/B3 and B4/B5 and M1/M2 and M3/M4 in Figure 6, respectively). The increased sensitivity of nucleosomes to restriction endonucleases verifies the efficacy of the ySwi/Snf remodeling and indicates certain alterations in the DNA/histone contacts within nucleosome cores, as the observed changes in the MseI or BstUI cutting are not likely to be due to nucleosome sliding only, because the DNA templates are not sufficiently long to allow the nucleosomes to slide completely from the restriction enzyme recognition sites.

However, digestion of the ySwi/Snf-remodeled nucleosomes with the HaeIII enzyme, which cleaves the template within 20 bp of the nucleosome edge, resulted in a certain increase in the share of uncleaved template, when compared to digestion of unremodeled nucleosomes (Figure 6). The effect was more visible in the case of the ySwi/Snf-remodeled mononucleosomes (Figure 6C). The apparent explanation for this effect would be the sliding of histone octamers to the end of the DNA fragment as the relocated histone octamers will shield the HaeIII cutting sites located closer to the end of the DNA template (sites H1−H3 in Figure 6A−C). However, in dinucleosome templates (Figure 6A,B), the slid histone octamer will uncover the HaeIII sites located in the middle of the DNA template between the two minimal 601 sequences (sites H4 and H5). This should result in an increase in the share of the "monomeric" 601 DNA fragments due to enhanced HaeIII cleavage of the probes in the center of the dimer of the 601 sequences. However, this was not evident. In addition, the MNase

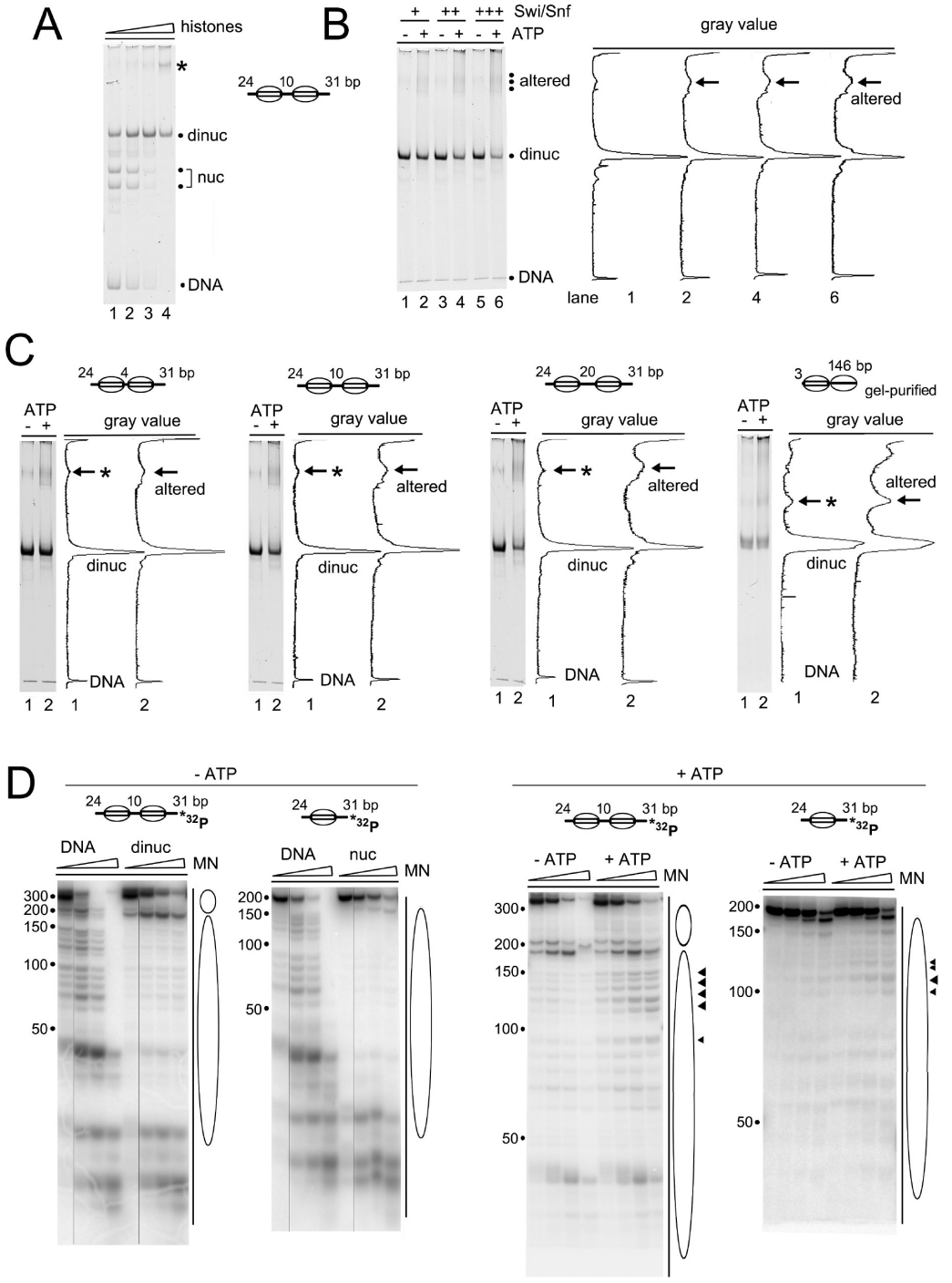

FIGURE 5: ySwi/Snf can catalyze formation of dinucleosome dimers and generate an altered micrococcal nuclease protection pattern on dinucleosomes. (A) Products of dinucleosome assembly at elevated histone:DNA ratios, resolved via native PAGE. (B and C) Dinucleosomes, containing the indicated amounts of octamer dimers (marked by an asterisk), were remodeled with ySwi/Snf and resolved via native PAGE. In the right-most panel, the reconstituted dinucleosomes were gel-purified after nucleosome assembly. The molar ratios of ySwi/Snf to DNA template are ~1:250, ~1:100, or ~1:30 (B) and 1:100 (C). (D) [γ-^{32}P]-labeled dinucleosomes or unassembled DNA templates (at left) or dinucleosomes and ySwi/Snf with or without ATP (at right) were digested with increasing amounts of MNase; isolated DNA was resolved via native PAGE and visualized by autoradiography in the Phosphorimage cassette. The ovals on the right of the gels indicate the positions of the minimal 601 DNA sequences, e.g., positions of nucleosomes on unremodeled templates.

digestion (Figure 5D and Figure S1 of the Supporting Information) and gel mobility shift data (Figures 2−4 and 5B,C) show little evidence of significant nucleosome sliding, compared to octamer sliding that was observed by a gel shift assay on the same mono- and, especially, dinucleosome templates remodeled with purified Isw1 and Isw2 complexes (*47*). Therefore, in addition to nucleosome sliding, the HaeIII cutting pattern could also be due to the elevated nuclease resistance of DNA at the ends of the ySwi/Snf-remodeled nucleosomes [as described previously for the BAF/PBAF-generated altered nucleosome, dimers, and altered dinucleosomes (*8*, *15*, *17*−*19*)].

DISCUSSION

We have found evidence that the *S. cerevisiae* Swi/Snf complex can convert nucleosomes to stable dimeric structures in vitro. This was suggested by the gel mobility shift data and alterations

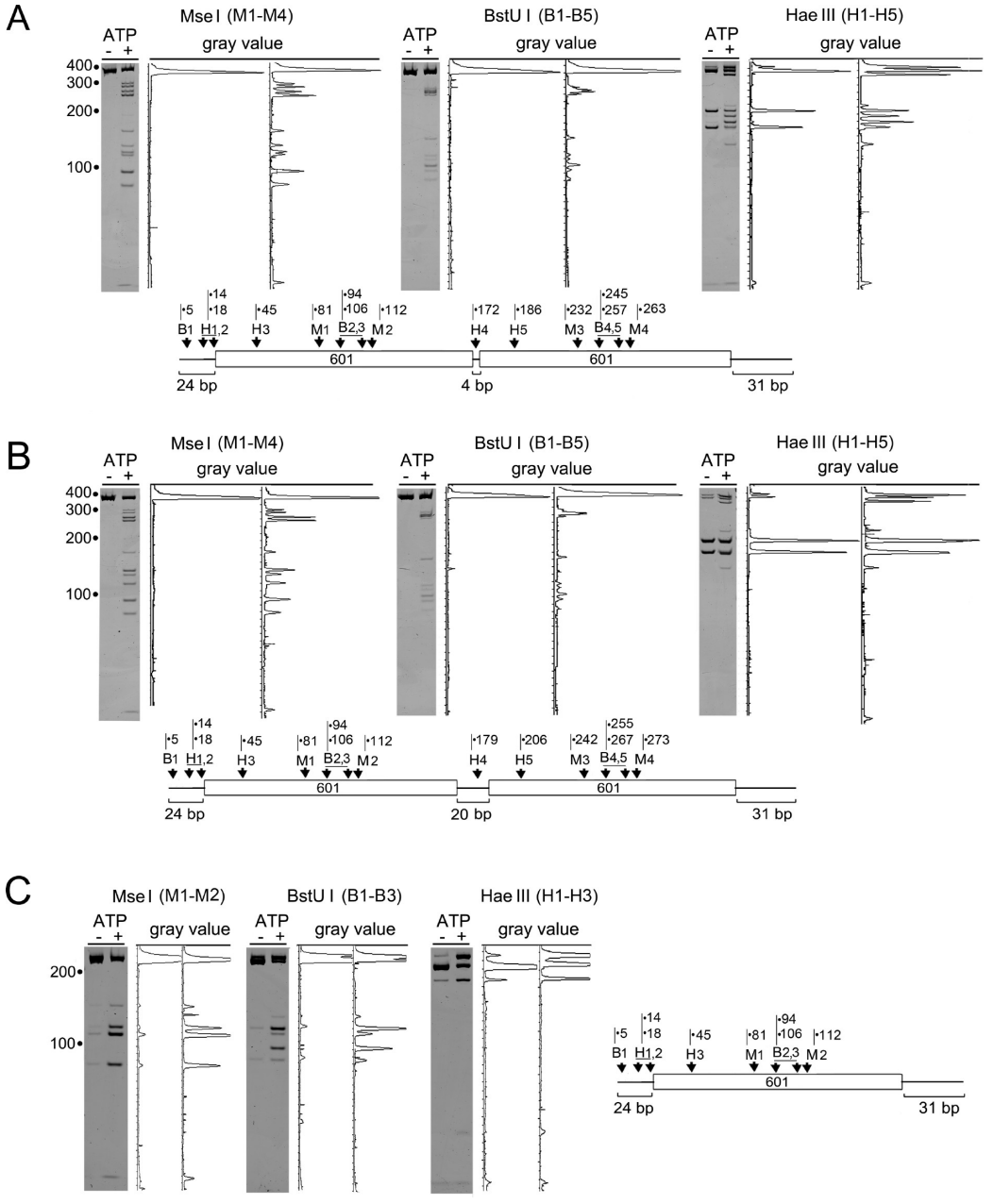

FIGURE 6: ySwi/Snf generates the elevated accessibility of nucleosomal DNA to restriction enzymes. (A and B) Dinucleosome or (C) mononucleosome templates were incubated with ySwi/Snf and ATP and digested with the MseI, BstUI, or HaeIII restriction endonuclease. DNA was isolated and resolved via native PAGE. Gel scan profiles are shown at the right. The schemes of the nucleosome templates and the restriction cleavage site are depicted.

in the nuclease cutting patterns of the ySwi/Snf-remodeled products, which are similar to those seen in reactions of human BAF/PBAF and yeast RSC complexes with nucleosomes. This function of the ySwi/Snf complex was not reported in previous studies.

Despite the overall similarity between the ySwi/Snf and other Swi/Snf family proteins, they have certain genetic and structural differences. ySwi/Snf is not required for mitotic growth (*48*), although it participates in most chromatin-related processes in *S. cerevisiae*, including transcriptional activation (*49*, *50*), telomeric and rDNA silencing (*51*), and DNA repair (*52*). The Swi/Snf activity is only required for 5% constitutively expressed yeast genes, including an important subset of highly inducible genes (*49*, *50*, *53*, *54*); however, gene expression at the end of mitosis generally requires Swi/Snf activity (*53*), consistent with the observation that ySwi/Snf might regulate high-order chromatin folding in vivo (*55*, *56*). The ySwi/Snf complex is present in cells in small amounts, so it is 10-fold less abundant than RSC, the other yeast Swi/Snf family complex (*5*, *48*). The low copy number of ySwi/Snf in the nucleus implies that this complex needs to be recruited to target genes by transcription factors (*57*). This was supported by in vitro and in vivo data demonstrating direct interaction between ySwi/Snf and transcription activators or repressors (*49*, *58*−*61*).

Cryo-electron microscopy and three-dimensional reconstruction (*41*, *62*) indicated that the structure of the nucleosome binding region of ySwi/Snf is different from that of yeast RSC and human PBAF, which both feature a large central cavity to accommodate nucleosomes (*63*−*66*). Although ySwi/Snf shows a surface depression, proposed as the nucleosome binding site (*41*, *62*), it shows no evidence of a cavity analogous to the one present in RSC and PBAF or of any other common features (*41*, *63*−*66*).

However, photochemical mapping of histone/DNA contacts in the nucleosome before and after remodeling demonstrated that ySwi/Snf action involves displacement of 30−50 bp of DNA from the nucleosome surface, resulting in the formation of partially unwrapped nucleosomal species (*11*, *13*, *67*), which are similar to the proposed intermediates in stable dimer formation catalyzed by RSC and human BAF/PBAF on mononucleosomes (*7*−*9*, *14*, *57*, *67*). In the case of ySwi/Snf, however, the rebinding of the spooled-out DNA with the histone octamer was primarily intramolecular rather than intermolecular and results in the formation of a relatively stable entry−exit site loop altered nucleosome conformation (*11*). It was proposed (*11*) that the ySwi/Snf-generated intranucleosomal DNA loop was formed with a much higher efficiency and rate versus what is needed to allow a slower concurrent formation of nucleosome dimers or altered dinucleosomes.

For now, we cannot conclude with certainty why the altered dimers were not observed in ySwi/Snf experiments before. It is possible that the particular characteristics of the ySwi/Snf complex may result in specific requirements for some of its biochemical activities to be evident in vitro. These specific requirements may include the underlying DNA sequence, a certain affinity of the DNA template for histone octamers, the length of the DNA template, and concentrations of the remodeler and nucleosome probes (see the justification for the experimental conditions in Results). However, we observed formation of altered dimers (Figure S1 of the Supporting Information) also using the relatively low-octamer affinity 220 bp positioning sequence containing the nucleosome "B" DNA from the promoter of the mouse mammary tumor virus (*34*) [although under the conditions that were employed we did not observe the reported (*34*) ySwi/Snf-catalyzed displacement of H2A/H2B dimers from a mononucleosome assembled on the B nucleosome sequence (not show; see also ref *47*)]. The ability of yeast Swi/Snf to catalyze formation of both altered dimers and altered dinucleosomes would not be unexpected, as the principles of their formation are not mutually exclusive. Prior studies of these structures suggest that these two products may have very similar structures; the proposed models for the altered dimer and altosome structures are essentially analogous, with the major difference being the break between the two DNA fragments in nucleosomes in the dimer (ref *19* and references cited therein).

Our observation of dimer formation by the yeast Swi/Snf complex may be helpful for the clarification of multiple mechanisms of ySwi/Snf function in vivo, since the formation of nucleosome dimeric structures by the human Swi/Snf counterparts has been proposed to be a distinct regulatory scheme of chromatin accessibility (*17*−*19*). Together with translational repositioning of nucleosomes and formation of stably altered mononucleosomes, the creation of remodeled dimeric products, which allow greater access of the factor to a site originally near the dyad of the unremodeled nucleosome, may allow remodeling complexes to create highly dynamic and accessible chromatin (*8*, *19*).

ACKNOWLEDGMENT

We thank Dr. Sergei Grigoryev (The Pennsylvania State University) for the helpful comments.

SUPPORTING INFORMATION AVAILABLE

Sequences of the DNA templates and construction of plasmids, gel mobility shift assay of ySwi/Snf-remodeled mononucleosomes, assembled on a DNA template, containing the MMTV nucleosome B positioning sequence (Figure S1A), and the MNase footprint of the [γ-^{32}P]-labeled dinucleosomes (24-Nuc-10-NPS-31) remodeled with ySwi/Snf and resolved via native PAGE (Figure S1B). This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature 389*, 251–260.
2. Clapier, C. R., and Cairns, B. R. (2009) The biology of chromatin remodeling complexes. *Annu. Rev. Biochem. 78*, 273–304.
3. Laurent, B. C., Treitel, M. A., and Carlson, M. (1991) Functional interdependence of the yeast SNF2, SNF5, and SNF6 proteins in transcriptional activation. *Proc. Natl. Acad. Sci. U.S.A. 88*, 2687–2691.
4. Peterson, C. L., Dingwall, A., and Scott, M. P. (1994) Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. *Proc. Natl. Acad. Sci. U.S.A. 91*, 2905–2908.
5. Martens, J. A., and Winston, F. (2003) Recent advances in understanding chromatin remodeling by Swi/Snf complexes. *Curr. Opin. Genet. Dev. 13*, 136–142.
6. Mohrmann, L., and Verrijzer, C. P. (2005) Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes. *Biochim. Biophys. Acta 1681*, 59–73.
7. Lorch, Y., Zhang, M., and Kornberg, R. D. (1999) Histone octamer transfer by a chromatin-remodeling complex. *Cell 96*, 389–392.
8. Phelan, M. L., Schnitzler, G. R., and Kingston, R. E. (2000) Octamer transfer and creation of stably remodeled nucleosomes by human SWI-SNF and its isolated ATPases. *Mol. Cell. Biol. 20*, 6380–6389.
9. Whitehouse, I., Flaus, A., Cairns, B. R., White, M. F., Workman, J. L., and Owen-Hughes, T. (1999) Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature 400*, 784–787.
10. Jaskelioff, M., Gavin, I. M., Peterson, C. L., and Logie, C. (2000) SWI-SNF-mediated nucleosome remodeling: Role of histone octamer mobility in the persistence of the remodeled state. *Mol. Cell. Biol. 20*, 3058–3068.
11. Kassabov, S. R., Zhang, B., Persinger, J., and Bartholomew, B. (2003) SWI/SNF unwraps, slides, and rewraps the nucleosome. *Mol. Cell 11*, 391–403.
12. Saha, A., Wittmeyer, J., and Cairns, B. R. (2006) Mechanisms for nucleosome movement by ATP-dependent chromatin remodeling complexes. *Results Probl. Cell Differ. 41*, 127–148.
13. Zofall, M., Persinger, J., Kassabov, S. R., and Bartholomew, B. (2006) Chromatin remodeling by ISW2 and SWI/SNF requires DNA translocation inside the nucleosome. *Nat. Struct. Mol. Biol. 13*, 339–346.
14. Lorch, Y., Zhang, M., and Kornberg, R. D. (2001) RSC unravels the nucleosome. *Mol. Cell 7*, 89–95.
15. Schnitzler, G., Sif, S., and Kingston, R. E. (1998) Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. *Cell 94*, 17–27.
16. Schnitzler, G. R., Cheung, C. L., Hafner, J. H., Saurin, A. J., Kingston, R. E., and Lieber, C. M. (2001) Direct imaging of human SWI/SNF-remodeled mono- and polynucleosomes by atomic force microscopy employing carbon nanotube tips. *Mol. Cell. Biol. 21*, 8504–8511.
17. Ulyanova, N. P., and Schnitzler, G. R. (2005) Human SWI/SNF generates abundant, structurally altered dinucleosomes on polynucleosomal templates. *Mol. Cell. Biol. 25*, 11156–11170.
18. Schnitzler, G. R., Sif, S., and Kingston, R. E. (1998) A model for chromatin remodeling by the SWI/SNF family. *Cold Spring Harbor Symp. Quant. Biol. 63*, 535–543.
19. Ulyanova, N. P., and Schnitzler, G. R. (2007) Inverted factor access and slow reversion characterize SWI/SNF-altered nucleosome dimers. *J. Biol. Chem. 282*, 1018–1028.
20. Gutierrez, J. L., Chandy, M., Carrozza, M. J., and Workman, J. L. (2007) Activation domains drive nucleosome eviction by SWI/SNF. *EMBO J. 26*, 730–740.
21. Lowary, P. T., and Widom, J. (1998) New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J. Mol. Biol. 276*, 19–42.
22. Thastrom, A., Bingham, L. M., and Widom, J. (2004) Nucleosomal locations of dominant DNA sequence motifs for histone-DNA interactions and nucleosome positioning. *J. Mol. Biol. 338*, 695–709.
23. Carey, M., Li, B., and Workman, J. L. (2006) RSC exploits histone acetylation to abrogate the nucleosomal block to RNA polymerase II elongation. *Mol. Cell 24*, 481–487.

24. Owen-Hughes, T., Utley, R. T., Steger, D. J., West, J. M., John, S., Cote, J., Havas, K. M., and Workman, J. L. (1999) Analysis of nucleosome disruption by ATP-driven chromatin remodeling complexes. *Methods Mol. Biol. 119*, 319–331.
25. Tsukiyama, T., Palmer, J., Landel, C. C., Shiloach, J., and Wu, C. (1999) Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. *Genes Dev. 13*, 686–697.
26. Vary, J. C., Jr., Gangaraju, V. K., Qin, J., Landel, C. C., Kooperberg, C., Bartholomew, B., and Tsukiyama, T. (2003) Yeast Isw1p forms two separable complexes in vivo. *Mol. Cell. Biol. 23*, 80–91.
27. Gottesfeld, J. M. (1987) DNA sequence-directed nucleosome reconstitution on 5S RNA genes of *Xenopus laevis*. *Mol. Cell. Biol. 7*, 1612–1622.
28. Simpson, R. T., Thoma, F., and Brubaker, J. M. (1985) Chromatin reconstituted from tandemly repeated cloned DNA fragments and core histones: A model system for study of higher order structure. *Cell 42*, 799–808.
29. Sims, H. I., Lane, J. M., Ulyanova, N. P., and Schnitzler, G. R. (2007) Human SWI/SNF drives sequence-directed repositioning of nucleosomes on C-myc promoter DNA minicircles. *Biochemistry 46*, 11377–11388.
30. Sims, H. I., Baughman, C. B., and Schnitzler, G. R. (2008) Human SWI/SNF directs sequence-specific chromatin changes on promoter polynucleosomes. *Nucleic Acids Res. 36*, 6118–6131.
31. van Vugt, J. J., de, J. M., Murawska, M., Brehm, A., van, N. J., and Logie, C. (2009) Multiple aspects of ATP-dependent nucleosome translocation by RSC and Mi-2 are directed by the underlying DNA sequence. *PLoS One 4*, e6345.
32. Ramachandran, A., Omar, M., Cheslock, P., and Schnitzler, G. R. (2003) Linker histone H1 modulates nucleosome remodeling by human SWI/SNF. *J. Biol. Chem. 278*, 48590–48601.
33. Pham, C. D., He, X., and Schnitzler, G. R. (2010) Divergent human remodeling complexes remove nucleosomes from strong positioning sequences. *Nucleic Acids Res. 38*, 400–413.
34. Vicent, G. P., Nacht, A. S., Smith, C. L., Peterson, C. L., Dimitrov, S., and Beato, M. (2004) DNA instructed displacement of histones H2A and H2B at an inducible promoter. *Mol. Cell 16*, 439–452.
35. Cairns, B. R., Kim, Y. J., Sayre, M. H., Laurent, B. C., and Kornberg, R. D. (1994) A multisubunit complex containing the SWI1/ADR6, SWI2/SNF2, SWI3, SNF5, and SNF6 gene products isolated from yeast. *Proc. Natl. Acad. Sci. U.S.A. 91*, 1950–1954.
36. Laurent, B. C., Treich, I., and Carlson, M. (1993) The yeast SNF2/SWI2 protein has DNA-stimulated ATPase activity required for transcriptional activation. *Genes Dev. 7*, 583–591.
37. Quinn, J., Fyrberg, A. M., Ganster, R. W., Schmidt, M. C., and Peterson, C. L. (1996) DNA-binding properties of the yeast SWI/SNF complex. *Nature 379*, 844–847.
38. Cote, J., Quinn, J., Workman, J. L., and Peterson, C. L. (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science 265*, 53–60.
39. Meersseman, G., Pennings, S., and Bradbury, E. M. (1992) Mobile nucleosomes: A general behavior. *EMBO J. 11*, 2951–2959.
40. Sengupta, S. M., VanKanegan, M., Persinger, J., Logie, C., Cairns, B. R., Peterson, C. L., and Bartholomew, B. (2001) The interactions of yeast SWI/SNF and RSC with the nucleosome before and after chromatin remodeling. *J. Biol. Chem. 276*, 12636–12644.
41. Dechassa, M. L., Zhang, B., Horowitz-Scherer, R., Persinger, J., Woodcock, C. L., Peterson, C. L., and Bartholomew, B. (2008) Architecture of the SWI/SNF-nucleosome complex. *Mol. Cell. Biol. 28*, 6010–6021.
42. Eisenberg, H., and Felsenfeld, G. (1981) Hydrodynamic studies of the interaction between nucleosome core particles and core histones. *J. Mol. Biol. 150*, 537–555.
43. Daban, J. R., and Cantor, C. R. (1982) Structural and kinetic study of the self-assembly of nucleosome core particles. *J. Mol. Biol. 156*, 749–769.
44. Ausio, J., Seger, D., and Eisenberg, H. (1984) Nucleosome core particle stability and conformational change. Effect of temperature, particle and NaCl concentrations, and crosslinking of histone H3 sulfhydryl groups. *J. Mol. Biol. 176*, 77–104.
45. Bondarenko, V. A., Steele, L. M., Ujvari, A., Gaykalova, D. A., Kulaeva, O. I., Polikanov, Y. S., Luse, D. S., and Studitsky, V. M. (2006) Nucleosomes can form a polar barrier to transcript elongation by RNA polymerase II. *Mol. Cell 24*, 469–479.
46. Kulaeva, O. I., Gaykalova, D. A., and Studitsky, V. M. (2007) Transcription through chromatin by RNA polymerase II: Histone displacement and exchange. *Mutat. Res. 618*, 116–129.
47. Krajewski, W. A., and Reese, J. C. (2010) SET domains of histone methyltransferases recognize ISWI-remodeled nucleosomal species. *Mol. Cell. Biol. 30*, 552–564.
48. Cairns, B. R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B., and Kornberg, R. D. (1996) RSC, an essential, abundant chromatin-remodeling complex. *Cell 87*, 1249–1260.
49. Holstege, F. C., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S., and Young, R. A. (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell 95*, 717–728.
50. Sudarsanam, P., Iyer, V. R., Brown, P. O., and Winston, F. (2000) Whole-genome expression analysis of snf/swi mutants of *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U.S.A. 97*, 3364–3369.
51. Dror, V., and Winston, F. (2004) The Swi/Snf chromatin remodeling complex is required for ribosomal DNA and telomeric silencing in *Saccharomyces cerevisiae*. *Mol. Cell. Biol. 24*, 8227–8235.
52. Chai, B., Huang, J., Cairns, B. R., and Laurent, B. C. (2005) Distinct roles for the RSC and Swi/Snf ATP-dependent chromatin remodelers in DNA double-strand break repair. *Genes Dev. 19*, 1656–1661.
53. Krebs, J. E., Fry, C. J., Samuels, M. L., and Peterson, C. L. (2000) Global role for chromatin remodeling enzymes in mitotic gene expression. *Cell 102*, 587–598.
54. Winston, F., and Carlson, M. (1992) Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet. 8*, 387–391.
55. Krebs, J. E., and Peterson, C. L. (2000) Understanding "active" chromatin: A historical perspective of chromatin remodeling. *Crit. Rev. Eukaryotic Gene Expression 10*, 1–12.
56. Horn, P. J., Crowley, K. A., Carruthers, L. M., Hansen, J. C., and Peterson, C. L. (2002) The SIN domain of the histone octamer is essential for intramolecular folding of nucleosomal arrays. *Nat. Struct. Biol. 9*, 167–171.
57. Peterson, C. L., and Workman, J. L. (2000) Promoter targeting and chromatin remodeling by the SWI/SNF complex. *Curr. Opin. Genet. Dev. 10*, 187–192.
58. Becker, P. B., and Horz, W. (2002) ATP-dependent nucleosome remodeling. *Annu. Rev. Biochem. 71*, 247–273.
59. Natarajan, K., Jackson, B. M., Zhou, H., Winston, F., and Hinnebusch, A. G. (1999) Transcriptional activation by Gcn4p involves independent interactions with the SWI/SNF complex and the SRB/mediator. *Mol. Cell 4*, 657–664.
60. Neely, K. E., Hassan, A. H., Wallberg, A. E., Steger, D. J., Cairns, B. R., Wright, A. P., and Workman, J. L. (1999) Activation domain-mediated targeting of the SWI/SNF complex to promoters stimulates transcription from nucleosome arrays. *Mol. Cell 4*, 649–655.
61. Yudkovsky, N., Logie, C., Hahn, S., and Peterson, C. L. (1999) Recruitment of the SWI/SNF chromatin remodeling complex by transcriptional activators. *Genes Dev. 13*, 2369–2374.
62. Smith, C. L., Horowitz-Scherer, R., Flanagan, J. F., Woodcock, C. L., and Peterson, C. L. (2003) Structural analysis of the yeast SWI/SNF chromatin remodeling complex. *Nat. Struct. Biol. 10*, 141–145.
63. Asturias, F. J., Chung, W. H., Kornberg, R. D., and Lorch, Y. (2002) Structural analysis of the RSC chromatin-remodeling complex. *Proc. Natl. Acad. Sci. U.S.A. 99*, 13477–13480.
64. Chaban, Y., Ezeokonkwo, C., Chung, W. H., Zhang, F., Kornberg, R. D., Maier-Davis, B., Lorch, Y., and Asturias, F. J. (2008) Structure of a RSC-nucleosome complex and insights into chromatin remodeling. *Nat. Struct. Mol. Biol. 15*, 1272–1277.
65. Leschziner, A. E., Lemon, B., Tjian, R., and Nogales, E. (2005) Structural studies of the human PBAF chromatin-remodeling complex. *Structure 13*, 267–275.
66. Leschziner, A. E., Saha, A., Wittmeyer, J., Zhang, Y., Bustamante, C., Cairns, B. R., and Nogales, E. (2007) Conformational flexibility in the chromatin remodeler RSC observed by electron microscopy and the orthogonal tilt reconstruction method. *Proc. Natl. Acad. Sci. U.S.A. 104*, 4913–4918.
67. Bazett-Jones, D. P., Cote, J., Landel, C. C., Peterson, C. L., and Workman, J. L. (1999) The SWI/SNF complex creates loop domains in DNA and polynucleosome arrays and can disrupt DNA-histone contacts within these domains. *Mol. Cell. Biol. 19*, 1470–1478.