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Conserved Features of Chromatin Remodeling Enzymes: A Dissertation

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A Dissertation Presented

By

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DEDICATION

This thesis is dedicated to my husband, David, who has always encouraged me to reach for the stars and has expected nothing less from me. You have made my dreams possible and I thank you with all of my heart for your limitless patience and understanding.

And to my son, Luke – I hope that my passion for science encourages you to find your own passion and enables you to seek out greatness in your life. You have been my true inspiration and my greatest joy.

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First, I would like to acknowledge my family and friends for their contributions to the making of a Ph.D. Of course, none of this would be possible without the support and encouragement I have received from my husband, David. Thank you so much for enduring the "ups" and "downs" and for understanding how important this is to me. Thanks to Grammy and Mimi for giving their love, time, and energy to Luke so that I could pursue my goals. Thanks to my parents for their genuine enthusiasm and support. I would especially like to acknowledge all of you who supported me during a very difficult illness.

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ABSTRACT

Chromatin structure plays an essential role in the regulation of many nuclear processes such as transcription, replication, recombination, and repair. It is generally accepted that chromatin remodeling is a prerequisite step in gene activation. Over recent years, large multisubunit enzymes that regulate the accessibility of nucleosomal DNA have emerged as key regulators of eukaryotic transcription. It seems likely that similar enzymes contribute to the efficiency of DNA replication, recombination, and repair. These chromatin remodeling complexes can be classified into two broad groups: (1) the ATP-dependent enzymes, which utilize the energy of ATP hydrolysis to increase the accessibility of nucleosomal DNA; and (2) histone modifying enzymes that phosphorylate, acetylate, methylate, ubiquitinate, or ADP-ribosylate the nucleosomal histones (for review see Kingston and Narlikar, 1999; Muchardt and Yaniv, 1999; Brown et al., 2000; Vignali et al., 2000; Strahl and Allis, 2000).

The mechanism by which these two groups of large, multi-subunit enzymes function to alter chromatin structure is enigmatic. Studies suggest that ATP-dependent and histone acetyltransferase chromatin remodeling enzymes have widespread roles in gene expression and perform both independent and overlapping functions. Interestingly, although both groups of enzymes appear to be distinct, several features of these enzymes have been conserved from yeast to man. Thus, understanding the role of these similar features will be essential in order to elucidate the function of remodeling enzymes, their functional interrelationships , and may uncover the fundamental principals of chromatin remodeling. In this study, we use a combination of yeast molecular genetics and

biochemistry to dissect out the function of individual parts of these chromatin remodeling machines and to understand how these large macromolecular assemblies are put together. In addition, we also investigate the mechanism by which the ATP-dependent enzymes exert their regulatory effects on chromatin structure.

Structure/function analysis of *Saccharomyces cerevisiae* Swi3p (conserved in SWI/SNF complexes across all eukaryotic phyla) reveals a unique scaffolding role for this protein as it is essential for assembly of SWI/SNF subunits. We have also characterized a novel motif that has homology to the Myb DNA binding domain, the SANT domain, and that is shared among transcriptional regulatory proteins implicated in chromatin remodeling. Mutational analysis of this domain in yeast Swi3p (SWI/SNF), Rsc8/Swh3p (RSC), and Ada2p (GCN5 HATs) reveals an essential function for the SANT domain in chromatin remodeling. Moreover, our studies suggest that this novel motif may be directly involved in mediating a functional interaction with chromatin components (i.e. histone amino terminal domains).

We have also directly compared the activities of several members of the ATP-dependent chromatin remodeling enzymes. Surprisingly, we find that these enzymes utilize similar amounts of ATP to increase nucleosomal DNA accessibility. In as much, we show that changes in histone octamer comformation or composition is not a requirement or consequence of chromatin remodeling by SWI/SNF. Taken together, these data suggest a similar mechanism for ATP-utilizing chromatin remodeling enzymes in which disruption of histone-DNA contacts occur without consequence to the structure

of the histone octamer. These data have striking implications for how we view the mechanism of chromatin remodeling.

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ABBREVIATIONS USED

A-absorbance

aa-amino acid

ACF-ATP-utilizing and chromatin remodeling and accessiblity factor

ADA-adaptor

AEDANS-acetylethylenediamine-(1,5)-napthol sulfonate

ATP-adenosine triphosphate

ADP-adenosine diphosphate

bp-base pair

CHRAC-chromatin remodeling and accessibility complex

cpm-counts per minute

DBD-DNA binding domain

DNA-deoxyribonucleic acid

DTT-dithiothreitol

EDTA-ethylenediaminetetraacetic acid

et al.-et alibi (and others)

FPLC-fast protein liquid chromatography

g-gram

GCN5-general control nonderepressible

GST-glutathione S-transferase

H2A-histone H2A

H2B-histone H2B

H3-histone H3

H4-histone H4

HAT-histone acetyltransferase

i.e.-id est (that is)

kb-kilo basepair

kDa-kilodalton

lacZ- β-galactosidase gene

L-liter

M-molar

Mda-megadalton

mg-milligram

μg-microgram

min-minute

ml-milliliter

μl-microliter

mmol-millimole

mM-millimolar

μM-micromolar

nm-nanometer

nM-nanomolar

NURD-nucleosome remodeling and deacetylase

NURF-nucleosome remodeling factor

OD-optical density

PAGE-polyacrylamide gel electrophoresis

PMSF-phenylmethylsulfonyl fluoride

RNA-ribonucleic acid

rpm-revolutions per minute

RSC-remodels the structure of chromatin

SAGA-Spt-Ada-Gcn5-Acteyltransferase

SANT-Swi3, Ada2, N-CoR, TFIIIB

SDS-sodium dodecyl sulfate

SNF-sucrose nonfermentable

SPT-suppressor of Ty

SWI-switch

TAE-tris acetate EDTA

TAF-TBP associated factor

TBE-tris borate EDTA

TBP-TATA box binding protein

TCA-trichloroacetic acid

WT-wild type

YEPD-yeast extract, peptone, dextrose media

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CHAPTER I

INTRODUCTION

Introduction

The compaction of DNA into higher order chromatin structure is essential in order for the full complement of genomic DNA to fit into the confines of the nuclear compartment. This packaging, however, poses a sizeable barrier to factors that must gain access to the DNA sequence such as the transcription machinery. In this regard, proper control of gene expression necessitates cellular mechanisms to contend with this chromatin-mediated repression of transcription. In recent years, several large, macromolecular protein 'machines' have been implicated as mediators of DNA accessibility and have emerged as key regulators of eukaryotic transcription. Investigations into how these 'machines' function will undoubtedly provide the fundamental tools for understanding how the cell manages two opposing forces –the compaction of DNA versus its functional utility, to carry out complex metabolic processes such as transcriptional regulation.

The research presented in this thesis extends our current knowledge of how these large, multi-subunit enzymes function to alter the dynamics of chromatin structure. The role of a highly conserved polypeptide in complex function as well as the functional properties of chromatin remodeling enzymes was investigated. These studies have been performed primarily in the yeast *Saccharomyces cerevisiae*. Early investigations determined that the chromatin structure of all eukaryotes (including yeast) is constructed in much the same way (Thomas and Furber, 1976). In addition, the transcriptional apparatus is surprisingly well conserved between yeast and higher eukaryotes. This fact

and the amenability of yeast to genetic and biochemical analyses makes *Saccharomyces cerevisiae* an ideal organism in which to study transcriptional regulation and allows for its application to higher eukaryotic systems as well. The relationship between chromatin structure, macromolecular enzymes, and transcription will be introduced and discussed in this chapter.

Chromatin Structure

Nearly fifty years ago, deoxyribonucleic acid (DNA) finally won its place in history as the genetic blueprint of the cell (Watson and Crick, 1953a; Watson and Crick, 1953b). Ten years later the first histones were purified by acid extraction (Johns, 1967). It was generally believed at this time that histones associated with DNA to form chromatin to allow its accommodation into the confines of the nuclear compartment. However, another decade passed before it was clear that the chromatin structure of higher eukaryotes existed as a repeating unit resembling a string of 'beads' (Hewish and Burgoyne, 1973; Kornberg and Thomas, 1974; Olins and Olins, 1974). It was determined that each 'bead' comprised approximately 200 base pairs of DNA wrapped around an octamer of histone proteins, coined the nucleosome (consisting of two copies each of histones H2A, H2B, H3, and H4) (Kornberg and Thomas, 1974). A fifth class of histone, histone H1 (linker histone), was also associated with nucleosomes and was thought to stabilize histone-DNA interactions by interacting with linker DNA (DNA between adjacent nucleosomes) (Kornberg and Thomas, 1974). This array of repeating

nucleosome units can be assembled into higher-order structures that further compact DNA (~10,000-fold over linear DNA).

Twenty-five years since the nucleosome was recognized as the basic building block of chromatin structure, the mechanism of higher order folding is still not well understood. The current view posits that all of the information for compaction resides within the nucleosomal arrays themselves (reviewed in Fletcher and Hansen, 1996). Recent biophysical studies indicate that formation of these chromatin fibers from nucleosomal arrays is an equilibrium process governed by charge shielding (e.g. by divalent ions - Mg^{2+}), nucleosome-nucleosome contacts, and the association of linker histones and non-histone chromatin associated proteins (reviewed in Fletcher and Hansen, 1996).

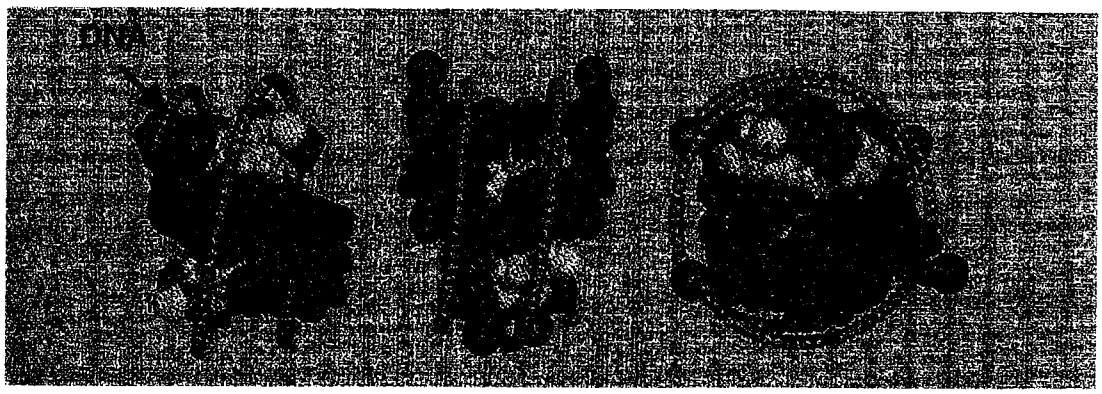
Histone proteins. Histone proteins are highly conserved across all eukaryotic phyla (for review see Fitzsimmons and Wolstenholme, 1976). These low molecular weight, highly basic proteins consist of a structured, three-helix bundle (histone fold motif), flanked by unstructured domains or 'tails' (Arents et al., 1991). The histone 'tails' were initially defined as regions of the histone proteins that were sensitive to proteases such as trypsin (Bohm and Crane-Robinson, 1984). The recent crystal structure of the nucleosome core particle was determined at 2.8 Å resolution and has provided confirmation of the previous low-resolution structures as well as some additional details (Richmond et al., 1984; Luger et al., 1997). Although the histone 'tails' comprise nearly 25-30% of the molecular mass of histone proteins, only approximately one third of their length is represented in the crystal structure (Luger et al., 1997). This is due to the lack

of secondary structure of these domains and their mobility in solution. Despite this, the remaining structure provides a clear picture of the nucleosome core particle. DNA is wrapped ~1.7 times around an octamer of histone proteins in a left-handed superhelix and adopts a specific rotational position where the minor groove faces the surface of the histone octamer (Arents et al., 1991; Arents and Moudrianakis, 1993; Luger et al., 1997); see also Figure I-1). The octamer of histone proteins is organized into a tripartite structure consisting of a tetramer of histones H3 and H4 (H3/H4 heterodimers interact to form a tetramer via dimerization of H3 molecules) bound on either side by histone H2A and H2B heterodimers (Arents et al., 1991; see Figure I-1). Formation of the above heterodimers is mediated by interaction between histone fold motifs and results in a very stable handshake-like arrangement between these proteins (Arents et al., 1991). Interestingly, the histone fold motif has emerged as an ubiquitous structural element used in protein dimerization and has additionally turned up in a number of eukaryotic transcriptional regulatory proteins suggesting a common ancestry among these factors (Arents and Moudrianakis, 1995; Corona et al., 2000; Luger and Richmond, 1998a).

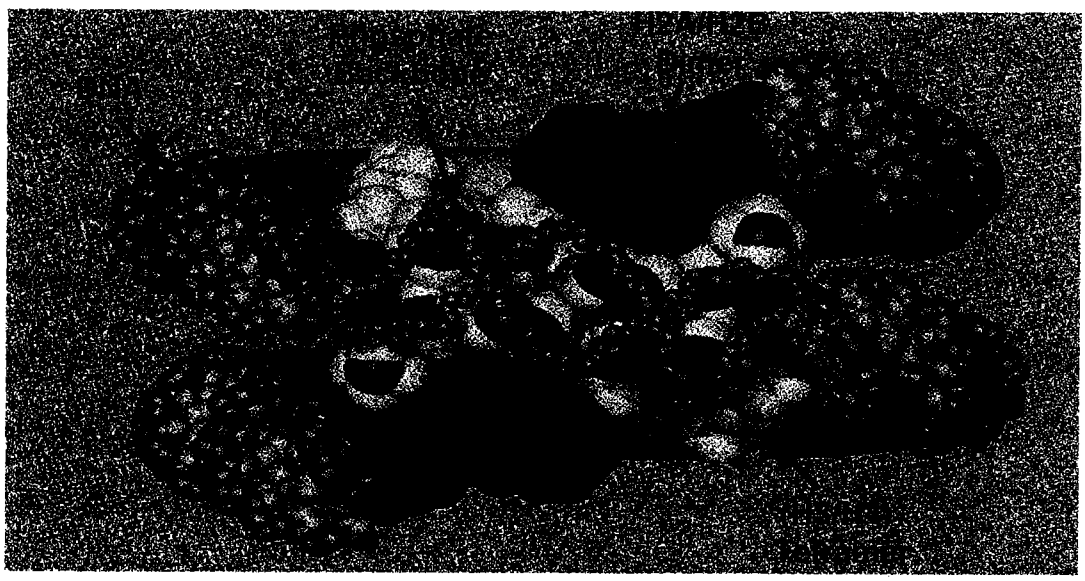
Histone Amino-Terminal Domains – 'Tails'. A great deal of attention has been given to dissecting the function of the histone 'tail' domains in chromatin structure and this remains a fruitful and exciting area of research. It is clear that these domains play a significant role in essential biological processes. This is evidenced by the fact that at least one of the two 'tails' for both the histone H2A/H2B and H3/H4 pairs must be intact to maintain viability in yeast suggesting an essential, but partially redundant role for these domains (Lenfant et al., 1996; Ling et al., 1996). The histone 'tails' are regions where a

Figure I-1. Representation of Nucleosome Structure. A. The nucleosome core particle is composed of 147 bp of DNA wrapped nearly two times around an octamer of histone proteins consisting of two copies each of histones H2A (light blue), H2B (dark blue), H3 (green), and H4 (white). **B.** The histone octamer is composed of a tripartite structure; a central tetramer of histone H3 and H4 (white) flanked on each side by histone H2A/H2B heterodimers (blue). The phosphate backbone of DNA contacts charged residues on the surface of the histone octamer (shown in red). Strategically placed arginine residues stabilize DNA on the surface of the histone octamer by interacting with the minor groove to create a left-handed superhelix. The central tetramer arranges ~120 base pairs of DNA and the remaining DNA is associated with the histone H2A/H2B dimers (Garcia-Ramirez et al., 1992; Luger et al., 1997). Not shown here are the amino terminal domains (common sites of posttranslational modifications) which exit the nucleosome through minor groove channels on all sides of the nucleosome. This figure was adapted from Chromatin Structure and Gene Expression (1995) Sarah C. R. Elgin, ed. Oxford University Press, NY.

A



B



number of post-translational modifications occur including acetylation, phosphorylation, methylation, ubiquitination and ADP-ribosylation (reviewed in Spencer and Davie, 1998; Strahl and Allis, 2000). These modifications have been linked to alterations in chromatin structure.

For many years, it was widely accepted that the primary role of the highly basic histone 'tails' was to interact with nucleosomal DNA to stabilize intra-nucleosomal histone-DNA interactions (reviewed in Fletcher and Hansen, 1996). Technical advances have allowed investigators to reconstruct the chromatin fiber *in vitro* allowing for biophysical analysis of a homogeneous population of nucleosomal arrays in solution (reviewed in Fletcher and Hansen, 1996). These data revealed an absolute requirement for the trypsin-sensitive histone 'tails' in formation of the folded structure (analogous to the 30 nm fiber which requires nucleosome-nucleosome contacts) and for self-association of these folded nucleosomal arrays into more compact structures (Fletcher and Hansen, 1995; Fletcher et al., 1994). This provided clear evidence that the histone amino-terminal 'tails' participate in protein-DNA as well as protein-protein interactions to mediate the different levels of compaction. The recent high resolution crystal structure provides details that confirm that the amino-terminal 'tails' can extend quite a distance from the nucleosome core and can interact with the neighboring nucleosome (Luger et al., 1997). Together, structural and biophysical studies of the nucleosome core particle and nucleosomal arrays have provided a means for understanding the relationship between modification of nucleosome structure and chromatin accessibility and have earned the histone 'tails' the reputation of key arbiters of chromatin structure.

Chromatin-associated Proteins. Numerous studies have shown that there is one copy of histone H1 per nucleosome (chromatosome) and that the presence of histone H1 is associated with highly condensed chromatin (reviewed in Simpson, 1978; Wolffe et al., 1997). However, histone H1 is not required for formation of higher order chromatin structure, but rather functions by stabilizing the folding of nucleosomal arrays into more condensed structures (discussed in Fletcher and Hansen, 1996). Consistent with this, rearrangement of the amino terminal 'tails' upon association of histone H1 with nucleosomes has been observed (discussed in Wolffe and Hayes, 1999). The implication from this would be that the histone 'tails' are now in a conformation that favors folding. Much less is known about histone H5, a tissue specific variant of H1 that accumulates during the process of terminal differentiation (Ramakrishnan et al., 1993). In any case, histone H5 is thought to be involved in the higher order compaction and inactivation that occurs in these cells.

The role of non-histone chromatin components in chromatin structure is less well characterized. The non-histone chromosomal proteins can be defined as those proteins other than the histones that are associated with chromatin *in vivo*. One such type is the high mobility group of proteins (HMG). HMG proteins are operationally defined as chromatin proteins extractable with 0.35 N NaCl and soluble in 2% trichloroacetic acid or 5% perchloric acid (Johns, 1982). HMG proteins possess a high proportion of charged amino acids, both positive and negative. These proteins can also be divided into a number of subclasses. HMG1 and HMG2 comprise the majority of these proteins in the cell (Ner, 1992). The DNA binding domain (HMG-1 box) is a characteristic feature of

these proteins. This unique motif binds to highly bent or distorted forms of DNA (Read et al., 1993; Weir et al., 1993). In chromatin, these proteins bind to linker DNA. Interestingly, HMG-1 like boxes have also been identified in a number of transcriptional regulatory proteins (Wolffe et al., 1997).

Two other subclasses of non-histone chromatin components appear to be specific to higher eukaryotes: the HMG-14 -17 and the HMG I/Y subclasses (Wolffe and Hayes, 1999). These proteins also bind DNA and have been implicated in transcriptional regulation. It seems likely that the histone and non-histone proteins will be targets of cellular mechanisms that deal with chromatin. In any case, identifying the role of these proteins will be essential to understanding the dynamics of chromatin structure and its relationship to biological processes such as the regulation of gene expression.

Chromatin Structure and Transcription

Cytological studies indicate that highly folded chromatin fibers (~200 nm) predominate in bulk chromatin even in the interphase nucleus – a time period where the DNA needs to be accessible to a variety of factors (Belmont and Bruce, 1994). It is obvious that the assembly of eukaryotic DNA into folded nucleosomal arrays has drastic consequences for many nuclear processes that require access to the DNA sequence, including transcription, replication, recombination, and repair. In fact, it is now generally recognized that disruption of chromatin structure is the rate-limiting step for most of these nuclear DNA transactions. Consistent with this idea, early studies indicated that positioning of a nucleosome over the adenovirus major late promoter inhibited

transcription (Knezetic and Luse, 1986; Lorch et al., 1987; Matsui, 1987). In a related study, investigators showed that binding of transcription factors to these templates prior to nucleosome assembly potentiated the subsequent initiation of transcription (Matsui, 1987; Workman and Roeder, 1987). The implication from these and other studies is that disruption or movement of positioned nucleosomes may be a prerequisite for transcriptional initiation (reviewed in Adams and Workman, 1993). Thus, even at the most basic level of compaction, the nucleosome provides a potent barrier to factor binding and appears to be the primary determinant of DNA accessibility.

Transcription-associated Conformational Changes in the Nucleosome. The correlation between altered chromatin structure and active transcription *in vivo* has been recognized for nearly thirty-five years and suggests that chromatin structure plays an active role in transcriptional regulation. Early investigations showed that histones remain present in actively transcribed regions, implying that chromatin in these regions must exist in an altered conformation or organization that was more permissive to transcription (Axel et al., 1974; Lacy and Axel, 1975). This is consistent with the fact that the nucleosome is not a static structure, but appears to be a dynamic and flexible assembly. This is evidenced by studies in which moderate changes in the concentration of NaCl can lead to several distinct changes in nucleosome conformation (Ausio et al., 1984a; Ausio et al., 1984b; Czarnota and Ottensmeyer, 1996; Dieterich et al., 1978; Dieterich et al., 1979; Yager et al., 1989; Yager and van Holde, 1984).

Subsequent groundbreaking studies made use of enzymatic probes (e.g. DNase I) to show increased DNA accessibility of active versus inactive chromatin (Weintraub and

Groudine, 1976). This generated an intense interest and enthusiasm for understanding the chromatin structure at active loci in more detail. Higher resolution mapping of chromatin structure followed and resulted in the identification of DNase I hypersensitive sites in the regulatory regions of genes in *Drosophila* (heat shock gene; Wu, 1980) and in higher eukaryotes (Groudine et al., 1983; Groudine and Weintraub, 1982). These DNase I hypersensitive sites were also identified in promoters of active and inducible genes in yeast (discussed in Krebs and Peterson, 2000). These discrete sites of exquisite nuclease accessibility became a hallmark of active genes and genes poised for tissue specific expression (discussed in Krebs and Peterson, 2000).

About the same time, other investigators took a different approach to study the structural phenomena of active versus inactive chromatin. Their studies indicated that nucleosomes isolated from transcriptionally active chromatin appear to be depleted of histone H2A/H2B dimers (Baer and Rhodes, 1983) and contain histone octamers whose interiors are more accessible to enzymatic and chemical modifications (Allegra et al., 1987; Chen et al., 1991; Prior et al., 1983). Recent studies have also shown that nucleosomes from transcriptionally active chromatin can be visualized microscopically as extended, largely unfolded structures (Bazett-Jones et al., 1996; Czarnota and Ottensmeyer, 1996). These and other studies have had a significant impact on the chromatin field and have led to the view that regulatory factors might antagonize the repressive effects of chromatin by disrupting the structure or conformation of the histone octamer (discussed in Workman and Kingston, 1998; see also below).

Post-translational Modification, Chromatin Structure, and Transcription. Post-translational modification (e.g. acetylation) of the histone amino-terminal domains has also been correlated with changes in chromatin structure (reviewed in Fletcher and Hansen, 1996; Workman and Kingston, 1998; Wolffe and Hayes, 1999; Strahl and Allis, 2000). A correlation between the reversible acetylation of histone 'tails' and transcriptional regulation was first proposed in the 1960's (Allfrey et al., 1964; Pogo et al., 1966). A number of subsequent studies pioneered by Grunstein and colleagues have shown that histone 'tails' are essential for the proper control of gene expression (Grunstein, 1992; 1997). The generation of antibodies that recognize specific acetylated states allowed investigators to examine patterns of acetylation *in vivo* by the method of chromatin immunoprecipitation (ChIP) (Braunstein et al., 1993; Hebbes et al., 1994; Hebbes et al., 1988). This was a major advance in understanding the role of acetylation of the individual histone 'tails' in biological processes such as transcriptional regulation. One of the first studies indicated that acetylation of histone H4 was associated with the transcriptionally active regions in the polytene chromosomes of *Drosophila* (Turner et al., 1992). On the other hand, a link between histone H4 hypoacetylation and X chromosome inactivation was seen (Belyaev et al., 1996; Jeppesen et al., 1992; Jeppesen and Turner, 1993). Additional observations made utilizing this approach showed the parallel between the presence of highly acetylated histones and the DNase I sensitive β-globin domain which includes transcriptionally active regions (Hebbes et al., 1994). Several other groups have exploited the utility of chromatin immunoprecipitation as a tool to fine-tune the map of highly acetylated histones H3 and H4. These investigators

found that promoter regions of active genes are enriched in the acetylated forms of histones H3 and H4, however upstream and coding regions are largely hypoacetylated (Cosma et al., 1999; Krebs et al., 1999). These and other related studies provided convincing evidence that histone acetylation is directly involved in transcriptional regulation.

How might the acetylation state of histone 'tails' contribute to gene activation? Initial insights came from studies that showed that DNA in hyperacetylated chromatin was more sensitive to nuclease digestion (Hebbes et al., 1994; Hebbes et al., 1988). In addition, two seminal studies revealed that acetylation of the histone amino-terminal 'tails' increased the accessibility of nucleosomal DNA to transcription factor binding (Lee et al., 1993; Vettese-Dadey et al., 1996; Vettese-Dadey et al., 1994). The correlation between histone 'tail' acetylation and chromatin structural changes can be understood in light of recent biophysical studies that implicate these domains in chromatin condensation. In this study, investigators showed that a critical level of acetylation (>12 acetates per octamer) was required for complete disruption of higher order folding and that this directly corresponds to gene activation (Tse et al., 1998a). This is entirely consistent with the fact that yeast have an overall high level of histone 'tail' acetylation (~13 acetates per octamer; 50% of available lysines) and that a large proportion of yeast genes are transcribed under normal growth conditions (Thomas and Furber, 1976; Waterborg, 2000).

Previous biophysical and structural studies indicate that histone 'tails' interact with DNA as well as with the histone proteins themselves to promote folding in part by

charge shielding (reviewed in Fletcher and Hansen, 1996). Thus, one could envision a scenario in which this neutralization of charge would lead to an overall decompaction of chromatin. However, the affects of acetylation may not simply be explained in these terms. For instance, the early studies showing an increase in factor binding to nucleosomal DNA upon acetylation of histone 'tails' were performed with mononucleosomes (168-220 bp DNA and histone octamer) which are incapable of folding (Lee et al., 1993; Vettese-Dadey et al., 1994). In addition, specific acetylation patterns are associated with gene regulation and would lead to the possibility of a number of charge distribution states (discussed in Workman and Kingston, 1998). Moreover, although acetylation is not generally associated with destabilization of the nucleosome core particle, modification of the histone 'tails' may lead to an altered conformation of the nucleosome structure (i.e. rearrangement of histone 'tails') to allow factor binding (reviewed in Fletcher and Hansen, 1996).

Alternatively, it is possible that different patterns of acetylation would serve as a histone code for the binding of other proteins that could serve to activate or repress transcription (discussed in Strahl and Allis, 2000). One example of this is that acetylation of histone H4 at lysine 16 is associated with its ability to interact with SIR3, a protein required to maintain the highly repressive chromatin structure at yeast telomeres (Hecht et al., 1995). In contrast, acetylation of lysines at sites 5 and 8 in histone H4 is associated with the transcriptionally active (euchromatic regions) polytene chromosomes (Turner et al., 1992). This idea of a histone code is also consistent with the notion that acetylation of histone 'tails' does not result in gross changes in nucleosome structure (reviewed in

Fletcher and Hansen, 1996; Strahl and Allis, 2000). The two scenarios presented above are not mutually exclusive and a combination of both may pertain *in vivo*. These and a number of subsequent studies have led to the current view that not only are the overall levels of histone acetylation important, but that specific patterns of acetylation are critical for transcriptional regulation (reviewed in Strahl and Allis, 2000; Wolffe and Hayes, 1999).

Protein Machines and the Alteration of Chromatin Structure

The last decade has witnessed a convergence of research in the fields of chromatin structure and transcription. The identification and characterization of numerous macromolecular enzymes that 'remodel' chromatin finally provided the missing link between chromatin structural alterations and transcriptional regulation. Importantly, this is also evidenced by the fact that many of these enzymes had previously been identified as transcriptional regulators. Two classes of highly conserved chromatin remodeling enzymes have been implicated as regulators of the repressive nature of chromatin structure. The first class is composed of multi-subunit complexes that use the energy of ATP hydrolysis to disrupt histone-DNA interactions (reviewed in Pollard and Peterson, 1998; Workman and Kingston, 1998; Kingston and Narlikar, 1999; Muchardt and Yaniv, 1999; Vignali et al., 2000). The second class includes enzymes that covalently modify the nucleosomal histones (acetylation, phosphorylation, methylation, ADP-ribosylation; reviewed by Strahl and Allis, 2000; Spencer and Davie, 1999; Wolffe and Hayes, 1999; Brown et al., 2000).

ATP-dependent Chromatin Remodeling Enzymes. The ySWI/SNF complex was the first member of the ATP-dependent class of chromatin remodeling enzymes to be discovered (Peterson et al., 1994; Cairns et al., 1994). Interestingly, mutations in *swi/snf* genes lead to a variety of transcriptional defects and genes encoding mutations in chromatin components bypass the need for SWI/SNF (reviewed in Winston and Carlson, 1992). This led to the initial hypothesis that SWI/SNF functioned to antagonize the repressive effects of chromatin structure on transcription (discussed in Winston and Carlson, 1992). Significant evidence supporting this hypothesis came from several complementary studies. First, SWI/SNF gene products are required for changes in chromatin structure associated with transcriptional initiation at the *SUC2* promoter (Hirschhorn et al., 1992). A second study showed that SWI/SNF facilitates the binding of activators to nucleosomal sites *in vivo* (Burns and Peterson, 1997b). Third, studies showed that direct recruitment of SWI/SNF to promoters could activate transcription from nucleosomal templates *in vitro* (reviewed in Vignali et al., 2000). The field has moved swiftly and persistently as a number of ATP-dependent chromatin remodeling enzymes have been and continue to be identified.

Each member of the ATP-dependent family of chromatin remodeling enzymes identified to date contains an ATPase subunit that is related to the SWI2/SNF2 subfamily of the DEAD/H superfamily of nucleic acid stimulated ATPases (Eisen et al., 1995). Seventeen members of the SWI2/SNF2 family have been identified in the yeast genome (reviewed in Pollard and Peterson, 1998), and to date, five of these ATPases have been purified as subunits of distinct chromatin remodeling complexes SWI/SNF (Cairns et al.,

1994; Cote et al., 1994), RSC (Cairns et al., 1996b), ISW1 and ISW2 (Tsukiyama et al., 1999), and INO80 (Shen et al., 2000). Additional ATP-dependent remodeling complexes that harbor SWI2/SNF2 family members have been identified in *Drosophila* [ACF (Ito et al., 1997), dNURF (Tsukiyama et al., 199; Tsukiyama and Wu, 1995), dCHRAC (Varga-Weisz et al., 1997), Brahma (Elfring et al., 1994; Papoulas et al., 1998)], human [hSWI/SNF (Kwon et al., 1994), huCHRAC (Poot et al., 2000), NURD (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998), RSF (LeRoy et al., 1998)], and frog [xMi-2 (Wade, 1998)].

The ATP-dependent class of chromatin remodeling complexes have been further divided into three groups based on whether the sequence of the ATPase subunit is more related to yeast SWI2 (ySWI/SNF, RSC, Brahma, hSWI/SNF), *Drosophila* ISWI (ISW1, ISW2, dNURF, dCHRAC, dACF, hRSF), or human Mi-2 (hNURD, xMi-2) (reviewed in (Muchardt and Yaniv, 1999; Vignali et al., 2000). Although each of these ATPases share a SWI2/SNF2-like ATPase domain, they harbor additional, unique sequence motifs adjacent to the ATPase domain that are characteristic of each group – the SWI2 group contains a bromodomain (Tamkun et al., 1992), the ISWI group contains a SANT domain (Aasland et al., 1996), and the Mi-2 group contains a chromodomain (Paro and Hogness, 1991). Differences among some groups are also apparent in the nucleic acid cofactor required for stimulation of ATPase activity. For enzymes that contain a SWI2-like ATPase (ySWI/SNF, yRSC, hSWI/SNF), ATPase activity is stimulated equally well by "free" DNA or nucleosomes (Cairns et al., 1996b; Cote et al., 1994; Phelan et al., 1999). In contrast, the ATPase activity of enzymes that contain an ISWI-like or Mi-2-like

ATPase is optimally stimulated by nucleosomes (Tong et al., 1998; Tsukiyama and Wu, 1995; Wade, 1998; Xue et al., 1998; Zhang et al., 1998). In the case of ISWI-like ATPases, this requirement for nucleosomal DNA may reflect obligatory interactions with the trypsin-sensitive, histone amino-terminal domains (Georgel et al., 1997).

Interestingly, the activities of ySWI/SNF, yRSC, dBrahma, dCHRAC, and hSWI/SNF complexes are required for transcriptional regulation of target genes *in vivo* suggesting an overall role for these enzymes in regulating gene expression through ATP-dependent chromatin disruption (Peterson and Herskowitz, 1992; Hirschhorn et al., 1992; Holstege et al., 1998; Moreira et al., 1999; de la Serna et al., 2000; Deuring et al., 2000; Biggar and Crabtree, 2000; Sudarsanam et al., 2000; Poot et al., 2000). What is ATP-dependent chromatin remodeling? A number of *in vitro* studies suggest that the ATP-dependent class of chromatin remodeling enzymes are functionally distinct, however, these enzymes have not been compared directly. In any case, each enzyme can apparently use the energy of ATP hydrolysis to alter chromatin structure and to enhance the binding of proteins to nucleosomal DNA binding sites (reviewed in Workman and Kingston, 1998; Kingston and Narlikar; 1999; Muchardt and Yaniv, 1999; Vignali et al., 2000). In many cases, these enzymes can disrupt the normal repeat pattern of DNase I cleavage of DNA on the surface of a nucleosome or are required to generate a DNase I hypersensitive site in promoter regions (Cote et al., 1994; Kwon et al., 1994; Imbalzano et al., 1994). These observations link the function of chromatin remodeling enzymes to previous studies showing a correlation between alteration of chromatin structure and transcription.

It has recently been shown that many of these enzymes can increase the mobility of the histone octamer in cis with respect to DNA in the presence of ATP (Hamiche et al., 1999; Jaskelioff et al., 2000; Langst et al., 1999; Whitehouse et al., 1999). This may account for the increased accessibility of binding factors to DNA sequences previously encompassed in nucleosomes. In fact, subtle differences in the ability of these enzymes to move histone octamers may explain their distinct behaviors in different remodeling assays (discussed in Peterson, 2000). Thus, it is possible that functional differences may ultimately reflect differences in the recruitment or regulation of these enzymes. However, there are still many unanswered questions and the exact nature of the remodeled nucleosome is unclear. Further analysis of the consequences of remodeling on nucleosome structure by these ATP-dependent enzymes and their comparison under the same assay conditions will be imperative to understanding the mechanism of chromatin remodeling and the functional relationship among this group of enzymes.

A hallmark of the SWI/SNF family of chromatin remodelers is that they are complex multi-subunit enzymes. The enormous size of these enzymes has led to their being labeled as chromatin remodeling 'machines'. Why do these enzymes have such complex subunit organization? The implication is that the chromatin remodeling reaction itself is complex, composed of multiple steps or subreactions that must be completed to achieve the 'remodeled' state (discussed in Peterson, 1998). Although studies have shown that both recombinant BRG1 and ISWI can remodel substrates *in vitro* (Corona et al., 1999; Phelan et al., 1999), additional subunits are required for function *in vivo*. Recent studies have shown that chromatin remodeling 'machines' are targeted by gene

specific transcriptional regulatory proteins to their site of action both *in vitro* and *in vivo* (Cosma et al., 1999; Deuring et al., 2000; Krebs et al., 1999; Natarajan et al., 1999; Neely et al., 1999; Yudkovsky et al., 1999). Thus, it is possible that some subunits may perform the primary catalytic function and additional subunits may play a regulatory role in these enzymes.

Interestingly, in addition to a DNA-dependent ATPase homologous to ySwi2/Snf2p, the *Drosophila* Brahma and human SWI/SNF complexes contain subunits that are homologues of yeast SWI3, SNF5, and SWP73 (Cairns et al., 1996a; Dingwall et al., 1995; Papoulas et al., 1998; Wang et al., 1996a; Wang et al., 1996b). RSC, another ATP-dependent chromatin remodeling complex in yeast, also contains a similar core of conserved subunits (Cairns et al., 1996b; Treich and Carlson, 1997). Recent studies suggest that these subunits define the functional core of the hSWI/SNF complex (Phelan et al., 1999). The SWI/SNF family of chromatin remodeling complexes, as well as yeast RSC, also contains proteins with homology to actin and/or actin-related proteins (Arps) (reviewed in Boyer and Peterson, 2000). It has been proposed that actin and Arps may act as conformational switches that govern the assembly or activity of these large macromolecular enzymes (discussed in Boyer and Peterson, 2000). Alternatively, actin and Arps may tether chromatin remodeling complexes to the nuclear scaffold (Zhao et al., 1998). Thus, it is important to determine how each of these conserved components function and to determine how these large multi-subunit machines are "put together" to form a functional complex in order to elucidate the precise biochemical mechanism of chromatin remodeling.

Histone Acetyltransferases. Histone Acetyltransferases (HATs) can be categorized into two broad groups. The first group consists of the Type B HATs, which are located in the cytoplasm and acetylate histones not associated with DNA. The second group, Type A HATs, is nuclear and can acetylate nucleosomal histones. These enzymes catalyze the transfer of an acetate moiety to the ε-amino group of lysine residues. A direct link between chromatin function and acetylation was established by the discovery that coactivators required for transcriptional activation function are Type A histone acetyltransferases. The ultimate breakthrough came from the identification of the first Type A HAT, p55, in *Tetrahymena* (Brownell and Allis, 1995). It was quickly realized that p55 had a homologue in yeast, Gcn5p, a previously identified 'adaptor' protein required for transcriptional regulation (Brownell et al., 1996). This has set the stage for the identification of numerous histone acetyltransferases, including a number with homology to the Gcn5p HAT domain, from yeast to human (reviewed in Berger, 1999; Brown et al., 2000).

Persuasive evidence has emerged that acetyltransferases function to acetylate histones *in vivo* to affect gene regulation. Investigators showed that mutations in the *GCN5* HAT domain directly correlated with the level of transcriptional activation *in vivo* (Kuo et al., 1998; Wang et al., 1998). Moreover, Allis and colleagues showed that Gcn5p HAT activity was directly correlated with acetylation of histone H3 and transcriptional activation at the *HIS3* promoter (Gcn5p-dependent gene) *in vivo* (Kuo et al., 1998). In addition, several *in vitro* studies showed that direct recruitment of GCN5 HATs to promoter regions by a variety of acidic activators led to increased acetylation of

neighboring nucleosomes and transcriptional activation of target genes (Ikeda et al., 1999; Steger et al., 1998; Utley et al., 1998).

Biochemical fractionation of yeast extracts has identified several nuclear histone acetyltransferase complexes (Type A HATs) which harbor Gcn5p as their catalytic subunit (Eberharter et al., 1999; Grant et al., 1997; Horiuchi et al., 1997; Pollard and Peterson, 1997; Saleh et al., 1997). SAGA (Spt-Ada-Gcn5-Acetyltransferase) is a nearly 2 MD multi-subunit complex which appears to be composed of modules that contribute to specific biochemical functions: HAT activity, activator interaction, complex integrity, and TBP interaction and regulation (Sterner et al., 1999). On the other hand, ADA is 0.8 MD complex and is functionally distinct from SAGA (Eberharter et al., 1999). The role of ADA is less clear as a number of subunits remain unidentified. Additional complexes that contain Gcn5p have also been identified, but are less well characterized (Pollard and Peterson, 1997; Saleh et al., 1997; Saleh et al., 1998; Sendra et al., 2000). In any case, GCN5-dependent HAT complexes are believed to facilitate transcriptional activation through acetylation of specific residues in the amino-terminal 'tails' of histone H3 in nucleosomes (reviewed in Berger, 1999; Brown et al., 2000; Luger and Richmond, 1998a). Previous studies have shown that although recombinant Gcn5p is capable of acetylating nucleosomal substrates *in vitro* (Tse et al., 1998b), Gcn5p HAT activity *in vivo* depends on its association with Ada2p and Ada3p (Candau et al., 1997; Marcus et al., 1994). In as much, *GCN5*, *ADA2*, and *ADA3* gene products appear to be conserved among all GCN5-related HAT complexes (reviewed in Brown et al., 2000). This

underscores the importance for understanding how these highly conserved proteins contribute to the acetylation function of these HATs *in vivo*.

In yeast, *GCN5* is not essential for cell growth suggesting that enzymes with redundant functions may exist. In this regard, several additional Type A HATs exist in yeast and a number of these have been identified as the catalytic subunit of multi-subunit complexes that are distinct from GCN5 HATs: Esa1 (NuA4; Allard et al., 1999; Grant et al., 1997), Sas3 (NuA3; Grant et al., 1997; John et al., 2000), and Elp3 (Elongator; Wittschieben et al., 1999). Each of these preferentially acetylate nucleosomal histones as part of a large, multi-subunit complex and have been implicated in transcriptional regulation and possibly DNA repair (reviewed in Brown et al., 2000). While NuA4 is capable of tetra-acetylating histone H4, NuA3 shows specificity to histone H3 (Grant et al., 1997). On the other hand, Elp3, an integral component of the RNA PolII elongation complex, has been shown to acetylate all four core histones *in vitro* (Wittschieben et al., 1999). It will be of great interest to determine the role of these enzymes in biological processes and their relationship to GCN5.

Although there is overwhelming evidence that histone 'tails' are relevant substrates *in vivo* for the HATs, recent reports suggest that this may be just a small piece of the puzzle in understanding how these enzymes affect gene expression. For example, Type A HATs in higher eukaryotes (P/CAF, CBP/p300) have been shown to acetylate and alter the activity of a variety of transcription factors (see Davie and Spencer, 1999; Berger, 1999; Brown et al., 2000). We are just beginning to uncover the multitude of affects that acetyltransferases have on gene regulation, thus designating these enzymes as

HATs may be a misnomer. It is likely that these enzymes will also play a significant role in other processes such as DNA repair and replication (discussed in Berger, 1999; Brown et al., 2000; Cheung et al., 2000).

Histone Deacetylases. Histone acetylation is a highly reversible process. Thus, it was not surprising that enzymes existed to catalyze the removal of these modifications. A number of corepressors of transcription have turned out to be histone deacetylases (reviewed in Knoepfler and Eisenman, 1999; Struhl et al., 1998). Keeping in theme with HATs, histone deacetylases (HDACs) are also housed in large multi-subunit complexes. The most well characterized histone deacetylases are members of a common family that includes the founding member from human, HDAC1 (Taunton et al., 1996). Homologues exist in a variety of eukaryotes including yeast (Rpd3). HDAC/Rpd3 complexes contain the Sin3 corepressor as well as a number of other proteins, some of which are also highly conserved among histone deacetylases (for review see Knoepfler and Eisenman, 1999; Struhl et al., 1998). The connection between histone deacetylase activity and transcriptional regulation stems from the observation that HDAC/Rpd3 complexes associate with DNA-binding repressors as well as with transcriptional corepressors for nuclear receptors (reviewed in Knoepfler and Eisenman, 1999; Struhl et al., 1998). Additional studies have suggested that histones are physiological substrates for yeast Rpd3 and that Rpd3 plays a role in transcriptional repression *in vivo* (Kadosh and Struhl, 1998a; Kadosh and Struhl, 1998b; Rundlett et al., 1996).

Recently, a number of histone deacetylase complexes have been identified that contain not only HDAC members, but also Mi-2 – a protein with homology to the

Swi2/Snf2 family of DNA-dependent enzymes (Tong et al., 1998; Wade, 1998; Xue et al., 1998; Zhang et al., 1998). These complexes, however, appear to lack the Sin3 counterpart. Interestingly, the combination of ATP-dependent remodeling (previously thought to activate genes) and histone deacetylase (HDAC) activities has been linked to transcriptional repression (reviewed in Knoepfler and Eisenman, 1999). This and the fact that hSWI/SNF has been associated with HDACs (Zhang et al., 2000) has led to a shift in paradigm. The current view of ATP-dependent enzymes in higher eukaryotes is that ATP-dependent remodeling may lead to activation or repression and appears to be context dependent.

Functional Relationship Among Chromatin Remodelers. Current studies suggest that ATP-dependent remodeling and HAT enzymes cooperate to regulate transcription by altering the equilibrium of DNA accessibility in chromatin (for review see Kingston and Narlikar, 1999). For example, SWI/SNF and GCN5 are required for the expression of a similar set of yeast genes (Pollard and Peterson, 1997). In addition, ySWI/SNF (a member of the ATP-dependent class) and SAGA (a yeast histone acetyltransferase) have recently been shown to play cooperative roles in transcriptional activation at the *HO* promoter in yeast (Cosma et al., 1999; Krebs et al., 1999). Other studies suggest that SWI/SNF and GCN5 have a pandemic role in gene expression and perform both independent and overlapping functions during transcriptional activation (Biggar and Crabtree, 1999; Pollard and Peterson, 1997; Roberts and Winston, 1997; Sudarsanam et al., 1999). ATP-dependent enzymes and deacetylases may also cooperate to regulate transcription. Recent studies have indicated a physical association between SWI/SNF

and HDACs although the functional relevance is currently under investigation (Zhang et al., 2000). However, the presence of a SWI/SNF-HDAC complex is consistent with the functional role of ATP-dependent remodeling in the Mi-2/NuRD histone deacetylase complex (Tong et al., 1998; Wade, 1998; Xue et al., 1998; Zhang et al., 1998).

The ability of these enzymes to be recruited to their sites of action has been the focus of a number of recent reports. One point of major interest is the fact that many members of both classes of chromatin remodeling enzymes can be targeted to promoters via interaction with the same activator or repressor (reviewed in Vignali et al., 2000; Brown et al., 2000; Peterson and Workman, 2000). This further supports the notion that the functions of the two classes of enzymes are cooperative and may overlap at a number of promoters. However, many of these studies have been performed *in vitro* and thus, the relevant physiological interactions *in vivo* need to be tested. These studies further support the role of chromatin remodelers in transcriptional regulation as well.

Although the two classes of chromatin remodeling enzymes appear to be functionally distinct, several motifs have been conserved between both classes of remodeling enzymes (e.g. bromodomain, SANT domain). The bromodomain which consists of a 4 helix bundle with a hydrophobic core is conserved in many subunits of chromatin remodeling complexes, has been recently implicated as a histone 'tail' binding domain (Dhalluin et al., 1999; Ornaghi et al., 1999). However, in many cases the bromodomain is not required for full activity *in vivo* suggesting that this domain may be redundant in function (Elfring et al., 1998; Laurent et al., 1993b; Marcus et al., 1994). The SANT domain (Swi3, Ada2, N-CoR, TFIIIB") is a novel motif found in a number of

transcriptional regulatory proteins, conserved from yeast to human, identified based on its homology to the DNA binding domain (DBD) of the cellular protooncogene, c-Myb (Aasland et al., 1996). Albeit its homology to Myb-DBD, the function of the SANT domain is not known. The SANT domain is predicted to be comprised of a three helix bundle that is maintained by a hydrophobic core (Aasland et al., 1996). Interestingly, many SANT-containing proteins have been identified as obligate members of chromatin remodeling complexes (Aasland et al., 1996). It is possible that elucidation of the role of the SANT domain may functionally link ATP-dependent and HAT chromatin remodeling complexes to chromatin substrates. Thus, understanding the role of conserved features of chromatin remodeling enzymes will be essential for dissecting their function and moreover, functional interrelationships.

Concluding Statement

A theme is emerging in which disruption of chromatin structure involves the orchestrated cooperation of large multi-subunit chromatin remodeling enzymes in order for the process of transcription to occur efficiently. A high degree of conservation exists among these enzymes at the domain, subunit, and functional level suggesting a similarity in the utilization of chromatin remodelers during gene regulation. It is appealing to speculate that understanding the conserved features of chromatin remodeling enzymes will uncover the fundamental principals of the 'remodeling' reaction and how it results in altered chromatin structure.

TABLE I-1. Conserved Features of Chromatin Remodeling Enzymes

ATP-dependent Remodeling Complexes

Complex	Catalytic Subunit	Organism	# Subunits	Comments	Bromo/SANT
Swi2/Snf2 Family					
SWI/SNF[a]	SWI2/SNF2	*S. cerevisiae*	11	Transcriptional Regulator	yes/yes
RSC[a]	STH1	*S. cerevisiae*	15	Required for Cell Cycle Progression; Transcriptional Regulator	yes/yes
Brahma[a]	BRM	*D. melanogaster*	>7	Transcriptional Regulator	yes/yes
hSWI/SNF[a]*	BRG1	*H. sapiens*	~10	Transcriptional Regulator	yes/yes
hSWI/SNF[a]*	hBRM	*H. sapiens*	~10	Transcriptional Regulator	yes/yes
ISWI Family					
ISW1	ISW1	*S.cerevisiae*	4	Nucleosome Disruption/Spacing Activity	no/yes
ISW2	ISW2	*S.cerevisiae*	2	Nucleosome Spacing Activity	no/yes
NURF	ISWI	*D. melanogaster*	4	Nucleosome Disruption/Spacing Activity	no/yes
CHRAC	ISWI	*D. melanogaster*	5	Nucleosome Disruption/Spacing Activity; Transcriptional Regulator	no/yes
ACF	ISWI	*D. melanogaster*	2	Nucleosome Assembly, Disruption, Spacing Activity	no/yes
RSF	hSNF2L	*H. Sapiens*	2	Transcriptional Regulator	no/yes

TABLE I-1. Conserved Features of Chromatin Remodeling Enzymes (cont'd)

ATP-dependent Remodeling Complexes

Complex	Catalytic Subunit	Organism	# Subunits	Comments	Bromo/SANT*
CHD Family					
Mi-2[c]	Mi-2/CHD	*X. laevis*	6	Remodeling/Deacetylase Activity	no/?
NRD[c]	Mi-2/CHD	*D. melanogaster*	?	Remodeling/Deacetylase Activity	no/?
NuRD[c]	Mi-2/CHD	*H. sapiens*	>7	Remodeling/Deacetylase Activity	no/?
Histone Acetyltransferases					
GCN5 Family					
ADA	GCN5	*S. cerevisiae*	>4	Acetylates Nucleosomal H3	yes/yes
SAGA	GCN5	*S. cerevisiae*	14	Acetylates Nucleosomal H3 Transcriptional Regulator	yes/yes
HATA2	GCN5	*S. cerevisiae*	~3	Acetylates Nucleosomal H3	yes/yes
STAGA/ TFTC	GCN5L	*H. sapiens*	11	Similarities to SAGA; Can substitute for TFIID	yes/?
PCAF[b]	P/CAF	*H. sapiens*	10+	Acetylates Nucleosomal H3 and transcription factors; Transcriptional Regulator	yes/yes

TABLE I-1. Conserved Features of Chromatin Remodeling Enzymes (cont'd)

Histone Acetyltransferases

Complex	Catalytic Subunit	Organism	# Subunits	Comments	Bromo/SANT*
Other					
?[b]	CBP/p300	*H. sapiens*	?	Acetylates All Nucleosomal Histones and many Transcription Factors; Subunit of RNA PolII Holo	yes/no
?[b]	SRC1/ACTR	*H. sapiens*	?	Acetylates Nucleosomal H3 and H4; Nuclear Receptor Transcriptional Regulator	no/no
NuA3	SAS3	*S. cerevisiae*	?	Acetylates Nucleosomal H3 MYST family member; Transcriptional Regulator	no/no
NuA4	ESA1/SAS2	*S. cerevisiae*	11	Acetylates Nucleosomal H4 Transcriptional Regulator	no/no
Elongin	ELP3	*S. cerevisiae*	?	Acetylates All Four Core Histones Transcriptional Regulator?	no/no
TFIID	$TAF_{II}130$	*S. cerevisiae*	++	Acetylates Free Histone H3 and H4 and Transcription Factors Subunit of RNA PolII Preinitiation Complex	yes/?
	$TAF_{II}230$	*D. melanogaster*	++		
	$TAF_{II}250$	*H. sapiens*	++		

TABLE I-1. Conserved Features of Chromatin Remodeling Enzymes (cont'd)

Histone Acetyltransferases

Complex	Catalytic Subunit	Organism	# Subunits	Comments	Bromo/SANT*
TFIIIC	p220, p110, P90	*H. sapiens*	++	Acetylates Nucleosomal Histones H2A, H3, and H4 Regulator of RNA PolIII Transcription	no/?

Listed here is a representation of several ATP-dependent and HAT chromatin remodeling complexes. The classification of complexes as Transcriptional Regulator is based on *in vitro* and/or *in vivo* studies. ?=the presence of this domain is questionable and may be present through interactions with other proteins (e.g. a SANT domain is in a subunit of RNA PolIII holo – TFIIIB"). [a] The SWI2/SNF2-family of complexes consists of a core of conserved subunits with homology to yeast Swi3p, Snf5p, Swp73p. [b] P/CAF and CBP/p300 have been physically associated with each other as well as individually in other complexes including RNA PolII Holoenzyme. [c] Mi-2-related complexes consist of homologous subunits some of which are shared among all HDAC-containing complexes. [*] The presence of a highly conserved bromo or SANT domain within the catalytic subunit or complex is indicated. [**] hSWI/SNF shows tissue specific heterogeneity.

Chapter II

Functional Analysis of Swi3p: A Conserved Subunit in ATP-Dependent Chromatin Remodeling Complexes

CHAPTER II

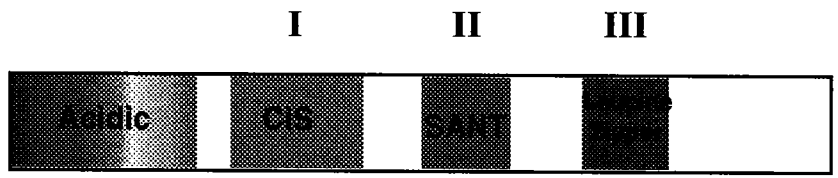
Functional Analysis of Swi3p: A Conserved Subunit in ATP-Dependent Chromatin Remodeling Complexes

Introduction

The prototype of ATP dependent chromatin remodeling complexes is the yeast SWI/SNF complex which is composed of 11 different polypeptide subunits with an apparent molecular mass of 2 MD (Cairns et al., 1994; Peterson et al., 1994; Treich et al., 1995). SWI/SNF is required for the transcriptional regulation of a number of yeast genes (Biggar and Crabtree, 1999; Holstege et al., 1998; Peterson and Herskowitz, 1992; Sudarsanam et al., 2000). SWI/SNF complexes are also found in higher eukaryotes as the *Drosophila* Brahma complex and human SWI/SNF complexes (Dingwall et al., 1995; Kwon et al., 1994; Papoulas et al., 1998; Wang et al., 1996a; Wang et al., 1996b). Each of these complexes contain multiple subunits including a DNA-dependent ATPase homologous to yeast Swi2/Snf2p (BRM in *Drosophila*, BRG1 or hBRM in human; for review see Pollard and Peterson, 1998; Vignali et al., 2000). In addition, the *Drosophila* Brahma and human SWI/SNF complexes contain subunits that are homologues of yeast SWI3, SNF5, and SWP73 (for review see Vignali et al., 2000). Furthermore, another ATP-dependent chromatin remodeling complex in yeast, RSC, also contains a similar core of conserved subunits (Cairns et al., 1996b; Treich and Carlson, 1997). In fact, recent studies suggest that these conserved subunits define the functional core of the

hSWI/SNF complex (Phelan et al., 1999). Investigations into the role of the individual subunits within these huge macromolecular complexes and dissecting how these large multi-subunit machines are "put together" to form a functional complex will provide important insights into the mechanism of chromatin remodeling.

In this study, we investigate the role of the highly conserved Swi3 protein in SWI/SNF function. Swi3p is an 825 amino acid protein that consists of multiple domains; a highly acidic amino terminus, a region rich in prolines, hydrophobic, and aromatic amino acids, a SANT domain (see Chapter III for extensive discussion), and a putative hydrophobic leucine zipper (Peterson and Herskowitz, 1992; Aasland et al., 1996; and see Figure II-1). Three of these motifs are conserved in all Swi3p family members (see Table II-1; see Vignali et al., 2000 for recent review). Mutational analysis indicates that the acid amino terminus (amino acids 1-306) is dispensable for Swi3p function. However, removal of an additional 100 amino acids (domain I) disrupts assembly of a functional complex suggesting that this region may contain a protein interaction motif. Mutation of the leucine zipper (domain III) reveals that it is required for self-association of Swi3p indicating that SWI/SNF may contain multiple copies of Swi3p. Taken together, these results suggest that Swi3p is a crucial subunit for assembly of the SWI/SNF complex and may serve as a scaffolding protein in SWI/SNF related complexes.



Motif	Amino Acids	Description	Function
Acidic N-terminus	1-304	Rich in aspartic and glutamic acids; net negative charge -60	Dispensable
CiS (Conserved in Swi3p Family members)	~300-500	Rich in hydrophobic and aromatic residues	Mediates interactions among components of SWI/SNF
SANT (Swi3, Ada2, N-CoR, TFIIIB")	523-578	Homology to Myb-DNA binding domain	Possible histone binding motif*
Leucine zipper	694-722	Predicted hydrophobic coiled-coil	Mediates self-association of Swi3p

Results

Conserved regions in Swi3p are required for SWI/SNF function. Swi3p is one of eleven polypeptides tightly associated with the 2 MD SWI/SNF complex and contains multiple highly conserved domains (Aasland et al., 1996; Cairns et al., 1994; Cairns et al., 1996b; Crosby et al., 1999; Papoulas et al., 1998; Peterson et al., 1994; Peterson and Herskowitz, 1992; Treich and Carlson, 1997; Wang et al., 1996b); see also Figure II-1). In order to understand the role of these domains in the function of Swi3p in chromatin remodeling we performed a structure/function analysis of Swi3p (See Chapter III of this thesis for extensive analysis of the SANT domain).

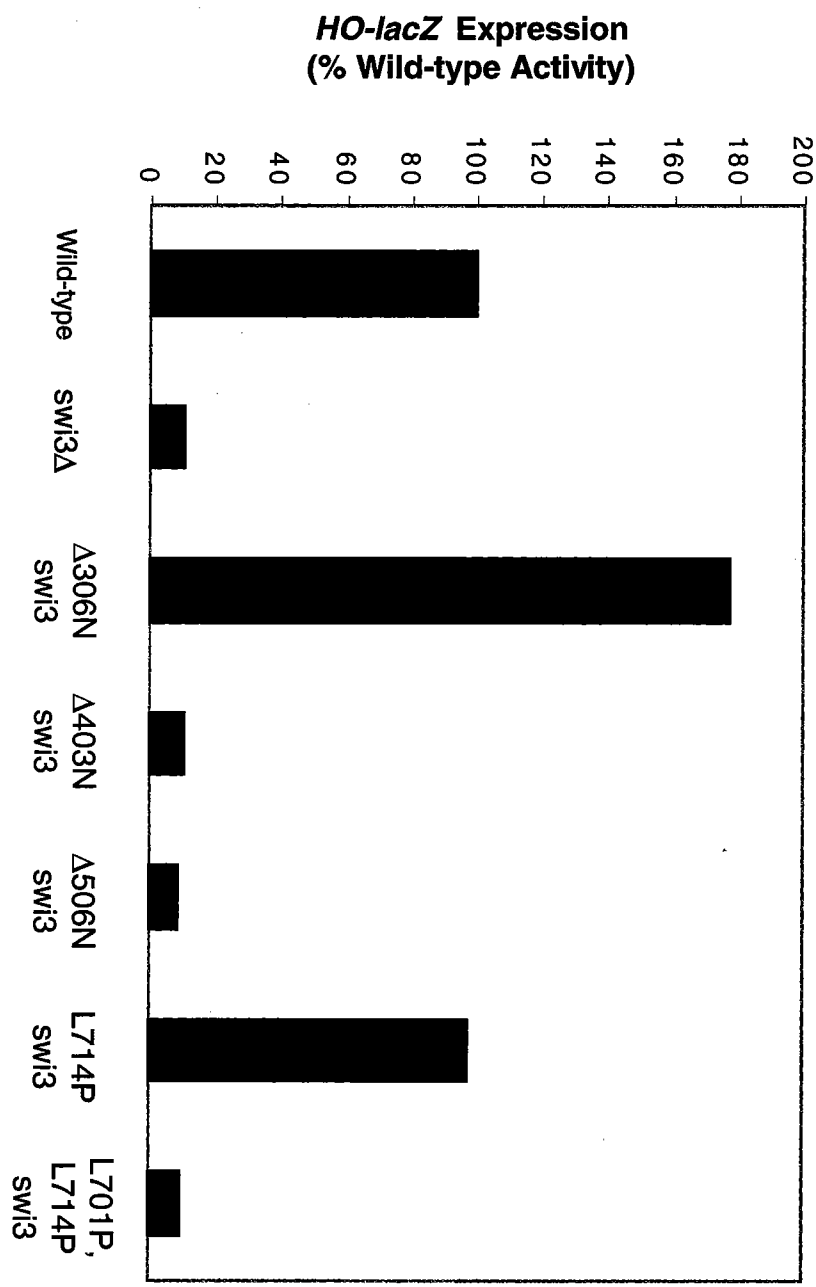
First, we constructed strains bearing successive 100 amino acid deletions from the amino terminus of Swi3p in order to define a role for the acidic and CiS domains. We then tested whether these mutations affected the regulation of the SWI/SNF-dependent gene, *HO* (which encodes for an endonuclease required for mating type switching), as a function of expression from an integrated *HO-lacZ* fusion gene (Figure II-2A). Consistent with previous studies, complete deletion of the chromosomal copy of *SWI3* resulted in an approximate 10-fold reduction in β-galactosidase activity as compared to wild-type (Figure II-2A; Peterson et al., 1992). Strikingly, the amino terminal 306 amino acids of Swi3p could be removed without any deleterious effect on SWI/SNF function. In fact, we consistently saw a nearly 2-fold increase in *HO-lacZ* expression as compared to wild-type (176% vs. 100%; Figure II-2A) suggesting that this domain may negatively regulate SWI/SNF activity. Interestingly, this region is specific for yeast Swi3p and is not conserved in any other Swi3p family member. Deletion of an additional 100 or 200

amino acids reduced activity 10-fold indicating that this region is critical for Swi3p function *in vivo* (Figure II-2A).

Swi3p also harbors a putative leucine zipper composed of multiple heptad repeats of leucine, however, it lacks the basic residues characteristic of bZip type domains suggesting that it is involved in protein-protein interactions (Peterson and Herskowitz, 1992). In order to gain insight into the role of this domain in Swi3p function we generated strains that harbored single and multiple amino acid substitutions in the putative leucine zipper. A single leucine to proline change in this motif (L714P) had no effect on β-galactosidase activity (96% as compared to wild-type; Figure II-2A) whereas substitution to proline at position 701 and 714 reduced activity 10-fold indicating an important role for this domain in Swi3p function (Figure II-2A).

Yeast strains bearing mutations in genes encoding components of the SWI/SNF complex display a slow growth phenotype on a variety of carbon sources (Neigeborn and Carlson, 1984; Peterson and Herskowitz, 1992; Sudarsanam et al., 2000). Like other *swi/snf* mutants, strains harboring mutations in the conserved regions of Swi3p also displayed growth defects on glucose media and were unable to form single colonies on media that contained sucrose, galactose, or glycerol (Figure II-2B and data not shown). The growth defect on sucrose medium is consistent with a defect in the expression of *SUC2* (Hirschhorn et al., 1992; Wu and Winston, 1997). Importantly, the phenotypes observed in the above analyses were not due to a decrease in expression of the various mutants as they were expressed at near wild-type levels (Figure II-3 and data not shown). In addition, overexpression of these mutants on a high copy plasmid did not affect the

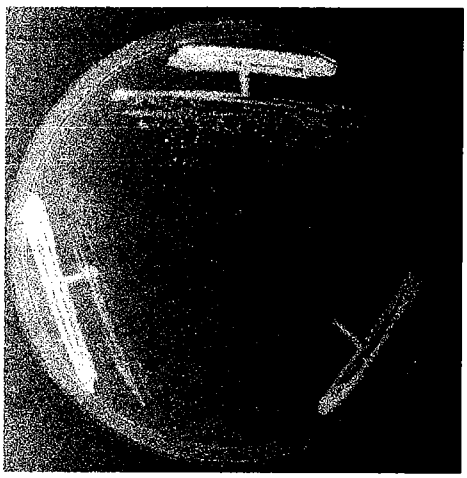
Figure II-2. CiS and Leucine Zipper domains of Swi3p are Required for Expression of SWI/SNF-dependent Genes. A. Analysis of the effects of mutations in the Swi3p CiS and Leucine Zipper domains on *HO-lacZ* expression. Strains were grown to mid log phage in YEPD and analyzed for β-galactosidase activity. The strains used are listed in Table II-4. Analyses were performed in triplicate and values were averaged. Values varied by <15%. Miller units were normalized to percentages of wild-type levels. **B.** Growth phenotypes of Swi3p mutants. Strains were streaked for single colonies on media containing either 2% sucrose or galactose and growth is compared to wild-type and swi3 deletion strains. Poor growth on media containing sucrose is consistent with a defect in the expression of the *SUC2* gene.


HO-lacZ Expression
(% Wild-type Activity)
0
20
40
60
80
100
120
140
160
180
200
Wild-type
swi3Δ
Δ306N swi3
Δ403N swi3
Δ506N swi3
L714P swi3
L701P, L714P swi3

B

Sucrose

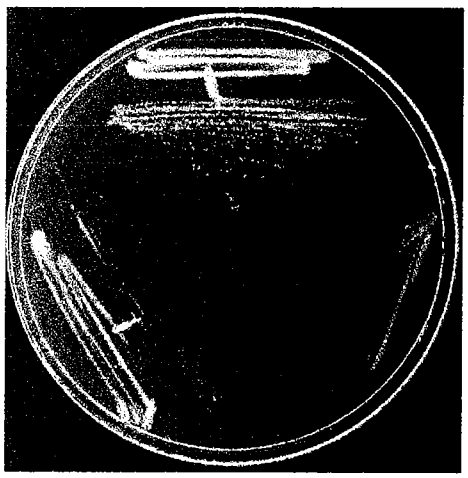
Wild-type



Swi3Δ

Swi3
L701P
L714P

Swi3Δ306

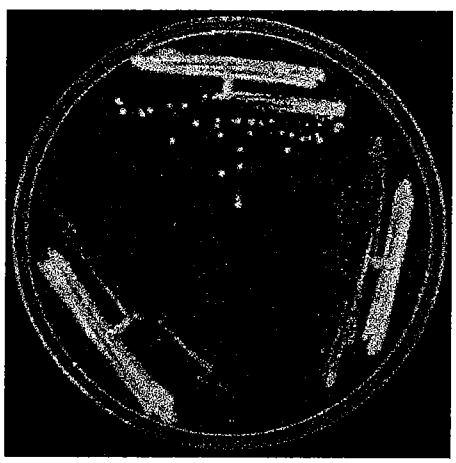


Swi3Δ403

Swi3Δ506

Galactose

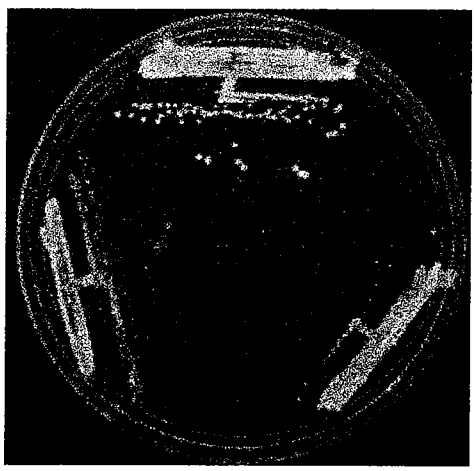
Wild-type



Swi3Δ

Swi3
L701P
L714P

Swi3Δ306



Swi3Δ403

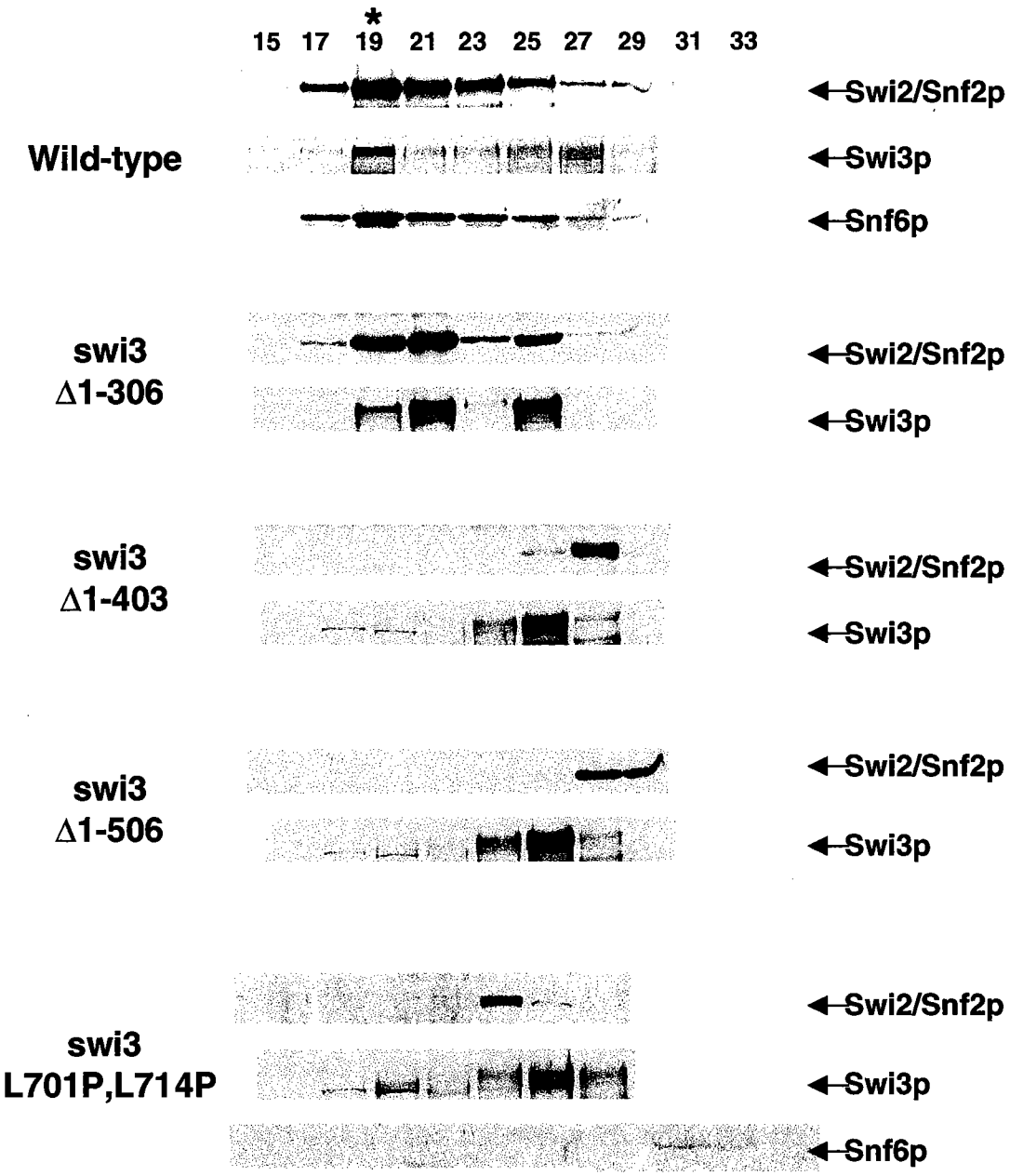
Swi3Δ506

observed phenotype (data not shown). Taken together, these data indicate that the acidic N-terminus is dispensable whereas the CiS (amino acids 306-506) and leucine zipper domains are required for SWI/SNF function *in vivo*.

Conserved domains in Swi3p are required for SWI/SNF stability *in vitro*. To investigate whether the conserved domains of Swi3p are required for SWI/SNF assembly, we fractionated whole cell extracts from a wild-type strain (CY664) and strains containing the amino terminal deletions of *SWI3* (CY677, CY678, and CY679) on an FPLC Superose 6 gel filtration column. Consistent with previous studies, fractionation of wild-type extracts showed that Swi3p and Swi2/Snf2p coelute from Superose 6 in a peak centered around fraction 19 as detected by immunoblotting (Peterson et al., 1994) and Figure II-3). This corresponds to an intact SWI/SNF complex with a molecular mass of ~2 MD. We have previously demonstrated that swi/snf deletion mutations alter the elution profile of the remaining subunits from a gel filtration column (Peterson et al., 1994). In extracts prepared from the strain harboring Swi3Δ306p, Swi3p (Fraction 21) and Swi2p (Fraction 21) cofractionate suggesting that the SWI/SNF complex is intact (Figure II-3), consistent with its ability to activate transcription *in vivo* (Figure II-2). However, in extracts prepared from strains harboring Swi3Δ403p or Swi3Δ506p, Swi3p and Swi2p do not cofractionate suggesting that this amino terminal region is required for complex assembly or stability (Figure II-3).

We also tested whether the double amino acid substitution in the leucine zipper region of Swi3p disrupted SWI/SNF assembly. In this case, Swi3p (Fraction 23) and

Figure II-3. A Role for Swi3p CiS and Leucine Zipper Domains in Mediating SWI/SNF Subunit Interactions. Gel filtration analysis of SWI/SNF harboring deletions/mutations in Swi3p. Crude whole cell extracts were prepared from CY664 (wild-type), CY677 (*swi3*Δ306), Cy678 (s*wi3*Δ403), CY679 (*swi3*Δ506), or CY668 (*swi3* L701P,L714P) and fractionated on an FPLC Superose 6 gel filtration column. Fractions were analyzed by SDS-PAGE and immunoblotting with antisera to Swi3p and HA (Swi2p). Swi3p containing amino terminal deletions migrated on SDS-PAGE consistent with the estimated size of the truncated protein. Molecular masses were estimated by extrapolation of the following calibration proteins: thyroglobulin (669,000 kD; fraction 25), apoferritin (443,000 kD; fraction 28), β-amylase (200,000 kD; fraction 30), and bovine serum albumin (66,000 kD; fraction 33). Similar results were seen with at least two independent whole cell extracts. The asterisk denotes the elution profile of wild-type SWI/SNF.

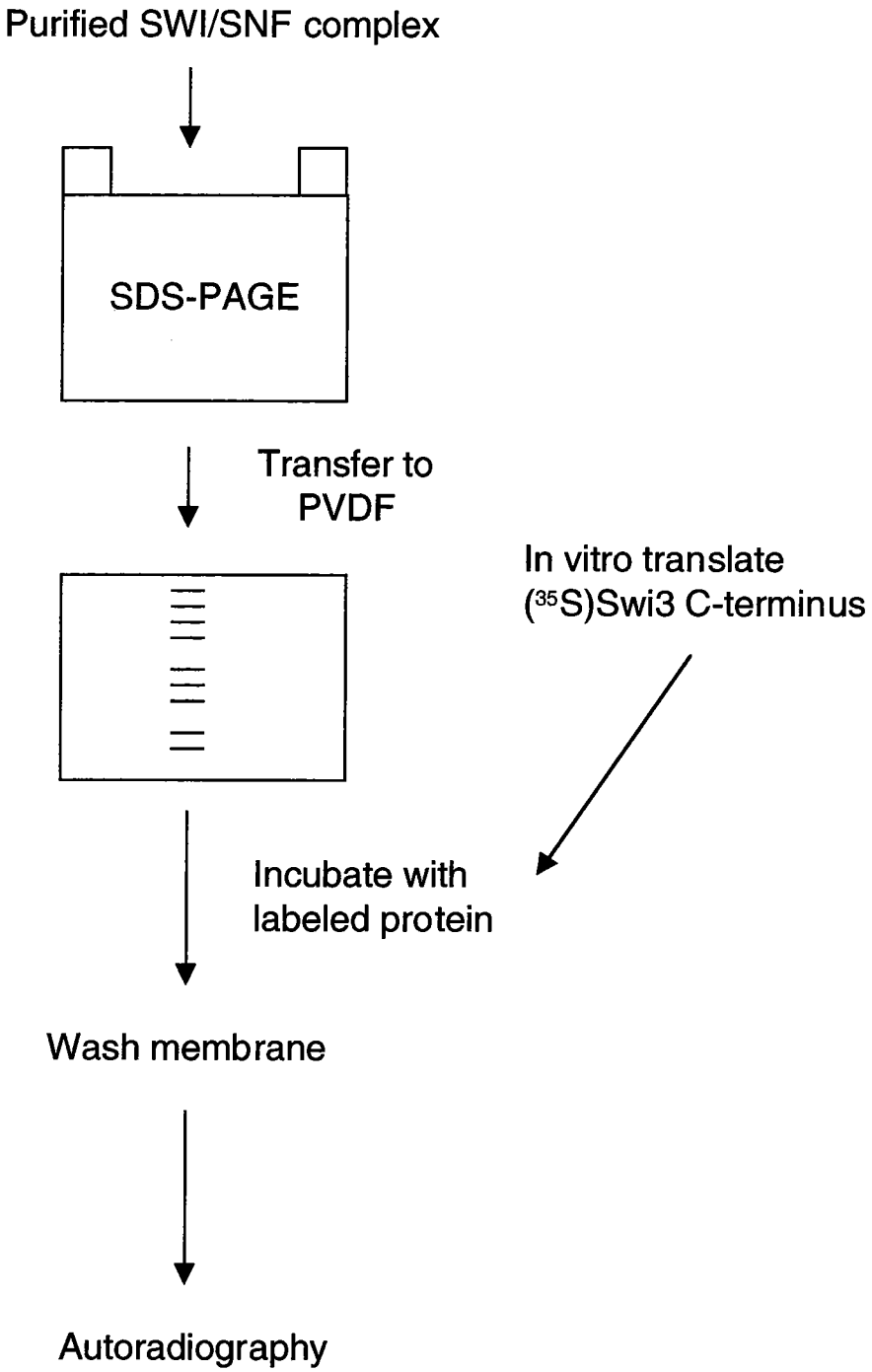

*
15 17 19 21 23 25 27 29 31 33
Wild-type
swi3 Δ1-306
swi3 Δ1-403
swi3 Δ1-506
swi3 L701P,L714P
Swi2/Snf2p
Swi3p
Snf6p

Swi2p (Fraction 25) do not cofractionate as compared to wild-type SWI/SNF complex (Figure II-3). The disruption of SWI/SNF complex is not as severe as that observed with the Δ403 or Δ506 alleles suggesting that some subunits remain associated with Swi3p in the absence of a functional leucine zipper. Other SWI/SNF subunits in wild-type strains were detected in a peak centered at fraction 19 from the gel filtration column (data not shown; see also Peterson et al., 1994). However, one of these components, Snf6p, elutes in fraction 31/33 and appears to be less stable in complexes containing the double amino acid substitution in the leucine zipper (Figure II-3). This suggests that association of Snf6p requires an intact leucine zipper. This also indicates that the transcriptional defects caused by mutations in the leucine zipper are likely due to the inability of SWI/SNF complexes to assemble properly *in vivo*. Taken together, these data suggest that the CiS (amino acids 306-506) and leucine zipper domains are involved in mediating functional interactions among SWI/SNF subunit(s) and suggest a role for the highly conserved Swi3 protein as a scaffolding subunit in SWI/SNF-related chromatin remodeling complexes.

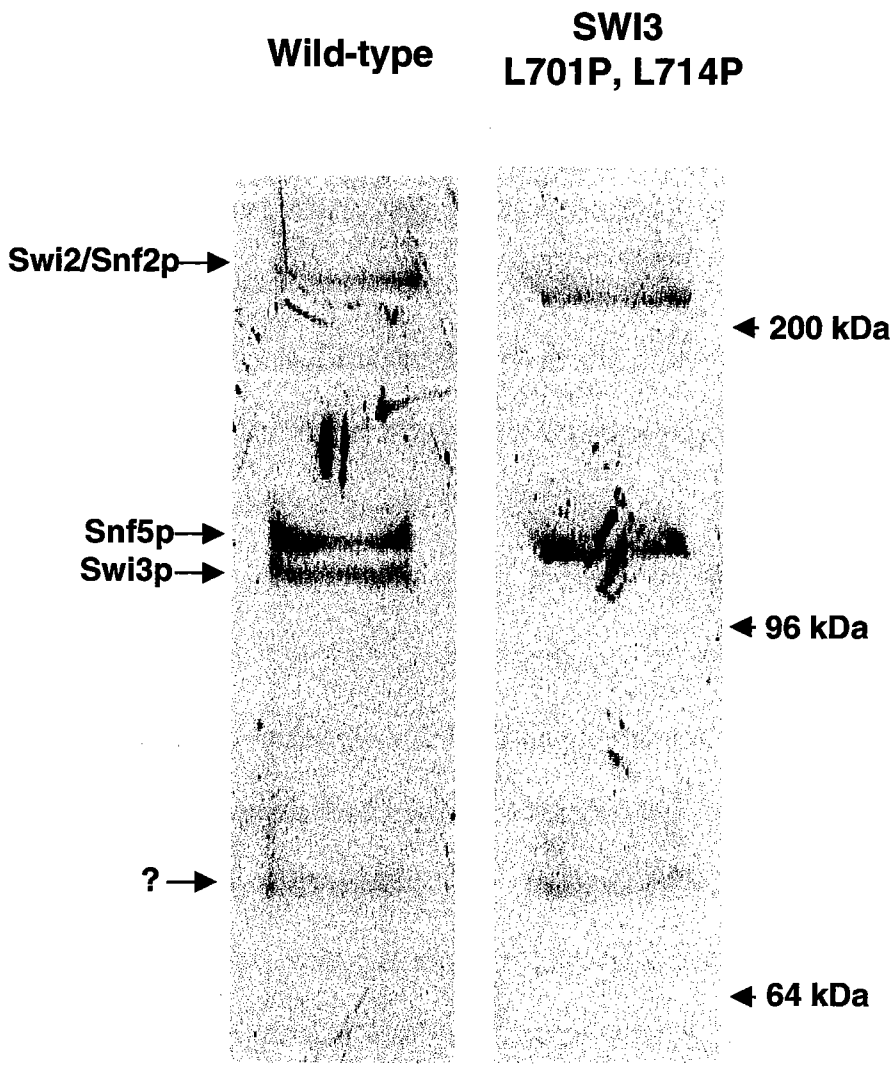
The leucine zipper mediates self-association of Swi3p. The leucine zipper of Swi3p harbors multiple heptad repeats of leucine characteristic of coiled coil regions, but lacks the basic residues characteristic of bZip type domain (Peterson and Herskowitz, 1992). This, combined with the fact that mutations in this domain interfere with the assembly of SWI/SNF, suggests that this domain mediates subunit-subunit interactions within SWI/SNF or more specifically is involved in self-association. In order to

Figure II-4. The Leucine Zipper Mediates Self-Association of Swi3p. A. Farwestern Protocol. SWI/SNF complex was separated by SDS-PAGE and transferred to PVDF membrane. Membranes were either subjected to immunoblotting to identify SWI/SNF subunits or hybridized with ^{35}S-methionine labeled in vitro translated Swi3 or swi3 L701PL714P protein fragments. Interacting subunits were visualized by autoradiography. **B.** Farwestern Analysis. Arrows indicate SWI/SNF subunits on the left and molecular weight markers on the right. Several subunits interacted with both the wild-type and mutant Swi3 polypeptides. This was confirmed by immunoblotting SWI/SNF complex run in parallel (data not shown). However, self-association was lost when membranes were hybridized with Swi3p containing mutations in the leucine zipper. Similar results were seen in three independent experiments.

A


Purified SWI/SNF complex
SDS-PAGE
Transfer to PVDF
In vitro translate (^{35}S)Swi3 C-terminus
Incubate with labeled protein
Wash membrane
Autoradiography

B


Wild-type
SWI3
L701P, L714P
Swi2/Snf2p
Snf5p
Swi3p
?
200 kDa
96 kDa
64 kDa

investigate this possibility, we separated SWI/SNF by SDS-PAGE, transferred the proteins to PVDF membrane and incubated the membrane with *in vitro* translated ^{35}S-methionine labeled wild-type or L701PL714P Swi3p polypeptides (amino acids 526-825) (Figure II-4A; see Experimental Procedures). In this assay ^{35}S-Swi3p bound to the immobilized Swi3p, Swi2/Snf2p, and Snf5p on the membrane (Figure II-4B). Binding to Swi2/Snf2p and Snf5p was insensitive to disruption of the leucine zipper, whereas interaction with Swi3p was disrupted (Figure II-4B). Taken together, these data suggest that the conserved leucine zipper in Swi3p mediates self-association and moreover, that Swi3p is present in multiple copies in SWI/SNF.

Discussion

In order to understand the individual contribution of the conserved subunits to SWI/SNF activity, we performed a structure/function analysis on the yeast Swi3 protein. A search of the NR databank at NCBI additionally revealed Swi3p homologues in *S. pombe*, *C. elegans*, and *A. thaliana* suggesting an important conserved function for this protein (Table II-1). All Swi3p family members consist of three highly conserved domains as well as additional unique sequences (Wang et al., 1996b; see Figure II-1). For example, Swi3p contains a unique highly acidic amino terminus (Figure II-1). It seemed possible that this acidic region in Swi3p may interact with highly basic proteins, such as histones. However, deletion of the entire region had no deleterious effect on SWI/SNF function. In fact, we consistently measured a nearly 2-fold increase in expression of the SWI/SNF-dependent gene, *HO* (Figure II-2A). Interestingly, this

suggested that the amino terminus might be involved in negatively regulating SWI/SNF. For instance, this domain may mask a site of interaction with an activator protein or may interact with a negative regulatory element to regulate the activity of SWI/SNF. Consistent with this idea, Swi2/Snf2p and Swi3Δ1-306p cofractionate in fraction 23 by gel filtration indicating that either the size or conformation of the complex is altered (Figure II-2 and II-3). However, it is unlikely that this is due to the loss of a subunit(s) from the complex as previous studies have shown that deletion of SWI/SNF subunits results in transcriptional defects and altered elution profiles by gel filtration in which Swi2p and Swi3p do not cofractionate (Laurent et al., 1993b; Peterson et al., 1994; Peterson and Herskowitz, 1992). In addition, SWI/SNF containing Swi3Δ306 is at least fully active *in vivo*.

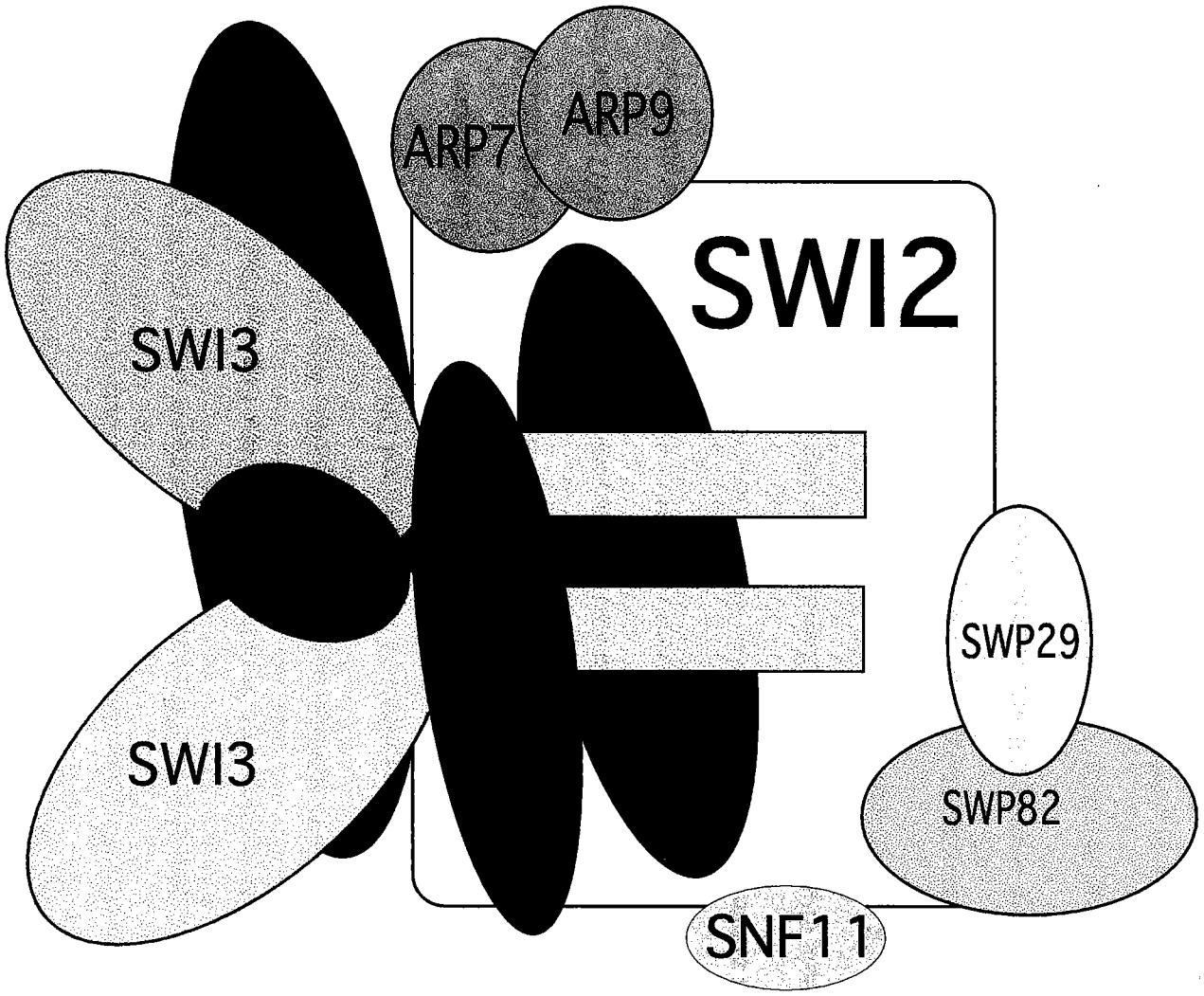
Domain I (CiS domain) is a region rich in prolines, aromatic, and hydrophobic amino acids suggesting that it may function as a protein interaction domain (Figure II-1). Consistent with this idea, partial deletion of this region (Swi3Δ403p) resulted in typical SWI/SNF transcriptional defects (Figure II-2) and gel filtration analysis indicated that this domain is required for SWI/SNF assembly or stability (Figure II-3). This also suggests that the region encompassing amino acid residues 306 to 403 mediates an interaction among components of SWI/SNF. Interestingly, deletion of an additional 100 amino acids encompassing the CiS domain resulted in a further shift of the elution profile of Swi3Δ506p to fraction 27/29 (Figure II-3). This difference (approximately 200 kD; see Figure II-3) cannot be accounted for by the removal of the additional amino acids (11

kD) suggesting that the CiS domain may mediate multiple contacts among SWI/SNF subunits.

We also performed mutational analysis of the conserved leucine zipper region (Domain III). Substitution of proline for leucine at two positions in this region abolished SWI/SNF activity *in vivo* (Figure II-2) and resulted in an altered elution profile by gel filtration (Figure II-3) indicating that this mutation interferes with the assembly or stability of a functional SWI/SNF complex. Further analyses revealed that the leucine zipper was required for self-association of Swi3p (Figure II-4). This is consistent with other studies that showed that the leucine zipper domains of Rsc8/Swh3p and moira (yeast and *Drosophila* homologues of Swi3p) are involved in self-association as well (Treich and Carlson, 1997; Crosby et al., 1999). In addition, our studies show an interaction between Swi2p/Swi3p and Snf5p/Swi3p that is independent of the ability of Swi3p to self-associate. Our studies also indicate that interaction of Swi2/Snf2p and Snf5p with Swi3p occurs between amino acids 526 and 825 (see Experimental Procedures). This data is consistent with studies showing interaction between Swi2/Snf2p and Swi3p by two hybrid analysis and with studies showing that an interaction between Sth1p (Swi2/Snf2 homologue) and Rsc8/Swh3p (Swi3p homologue) is independent of Rsc8/Swh3p self-association (Treich et al., 1995; Treich and Carlson, 1997). On the other hand, we see that Snf6p requires the leucine zipper for association with SWI/SNF (data not shown). In addition to the interactions observed in this study, Rsc8/Swh3p and Rsc6p (Swi3p and Swp73 homologues, respectively) have been shown to interact by two hybrid analysis (Treich et al., 1998).

Why multiple copies of Swi3p? Our analysis indicates that Swi3p makes multiple contacts with SWI/SNF subunits. Self-association of Swi3p would provide an extensive surface area for assembly of the large multi-subunit SWI/SNF complex. Self-association of Swi3p could also provide a unique site of interaction as is possibly the case with association of Snf6p with SWI/SNF. Swi2p and Snf5p do not require an intact leucine zipper for interaction with Swi3p suggesting that these subunits may be present in multiple copies in SWI/SNF. However this is unlikely, as studies have shown that recombinant BRG1 can function in the absence of additional subunits (Phelan et al., 1999). One explanation may be that self-association of Swi3p may sterically hinder the association of more that one copy of Swi2/Snf2p. On the other hand, previous studies have shown that Swi3p can contact Swi2p in multiple places (Treich et al., 1995). Thus, in a wild-type complex Swi2/Snf2p may contact its site on more than one copy of Swi3p. Taken together, Swi3p appears to be critical for interaction of numerous subunits in SWI/SNF. Thus, we propose a role for Swi3p and family members as a scaffolding protein within SWI/SNF-related complexes.

Figure II-5. Swi3p is the Scaffolding Subunit for the Assembly of SWI/SNF. SWI/SNF is comprised of 11 tightly associated polypeptides with an apparent molecular mass of 2 MD. Swi3p consists of multiple protein interaction domains. The leucine zipper of Swi3p is required for self-association. Swi3p is depicted as a dimer although it may be present in multiple copies. Assembly or stability of Snf6 requires an intact leucine zipper. Swi2p/Snf2p and Snf5 interact with Swi3p in Domain I. Swp73 also interacts with Swi3p (Treich et al., 1998).



ySWI/SNF -- 2 MDa

Experimental Procedures

PCR mutagenesis and plasmid construction. Oligonucleotides and plasmids used in this study are described in Table II-2 and II-3, respectively. The pRS series of plasmids used in this study has been described previously (Sikorski and Hieter, 1989). Site directed mutagenesis of the putative leucine zipper region of SWI3 was performed in a two step PCR reaction with Vent Polymerase (New England BioLabs) by standard methods. First, *SWI3* (CP596) sequence was amplified with LB1 and LB6 (see Table III-2) to create a TAG-Megaprimer. The mutagenized DNA fragment was generated in a second, subsequent PCR reaction using CP589, the mutagenic TAG-Megaprimer, TAG primer, and LB2. The resulting PCR product was then digested with EcoRI and ClaI and subcloned into the appropriate plasmid so as to replace the wild-type *SWI3* leucine zipper with the mutagenized version (CP435). CP630 (*swi3* L701PL714P) was generated by PCR as above using CP435 (*swi3* L714P) as the template and LB7 (L701P) as the mutagenic oligonucleotide. All plasmids were sequenced to ensure polymerase fidelity during PCR amplification.

Yeast strains and media. Standard genetic methods were followed to generate the appropriate strains. All strains used in this study are listed in Table III-4. Yeast cultures were grown at 30°C in yeast extract-peptone (YEP) media containing 2% glucose or selective synthetic complete (SC) media (Difco Laboratories) containing 2% glucose, galactose, or sucrose as specified (Rose et al., 1990) except where otherwise

noted. Yeast transformations were performed by the lithium acetate procedure as previously described (Geitz and Scheistl, 1991).

β-Galactosidase assays. β-Galactosidase activity was assayed quantitatively in permeabilized cells as previously described (Stern et al., 1984). Transformants were grown to mid-log phase in the appropriate media for plasmid selection. Activity was measured in Miller units followed by normalization to per cent wild-type activity.

Preparation of whole-cell extracts. Strains were grown in 100 ml of media at 30°C with agitation to an OD_{600} of 0.8-1.0. Cells were washed and resuspended in 0.8 ml of E Buffer (20 mM Hepes, 350mM NaCl, 10% glycerol, 0.1% Tween-20) with protease inhibitors. Cells were disrupted with glass beads using a mini bead beater (Biospec Products) for 5 pulses of 1 minute each separated by cooling on ice. Extracts were clarified with a 10 minute spin at 14K in a microfuge. Extracts were recovered and filtered through a 0.45μ membrane (Millipore) after a 45 min. spin at 48K (4°C) in a Beckman TL100 ultracentrifuge.

Gel filtration analysis. Crude whole cell extracts (for analysis of SWI/SNF) were fractionated on a fast protein liquid chromatography (FPLC) Superose 6 gel filtration column (Pharmacia) and proteins were precipitated by the addition of 15% trichloroacetic acid for subsequent immunoblot analysis.

Immunoblotting and antibodies. Typically TCA precipitated fractions were analyzed by SDS-PAGE and transferred to nitrocellulose membrane (Protran). Membranes were probed with antisera raised against either Swi3p (Peterson et al., 1994) or HA (Covance; for Swi2-HAp) followed by incubation with the appropriate secondary

antibody conjugated with horseradish peroxidase (rabbit-Amersham; mouse-Covance) and visualized by addition of chemiluminescent substrate (Amersham).

***In vitro* translations.** An NheI-BamHI fragment of *SWI3* and *swi3* L701PL714P was generated by PCR with oligos LB8 and LB9 and CP596 or CP630 as template (see Table II-2 and II-3). PCR products were digested with NheI and BamHI and cloned into the pET11A *in vitro* transcription vector (Novagen; see Table III-3). ^{35}S-methionine (NEN)-labeled Swi3p was obtained in each case using the TNT Coupled Wheat Germ Extract System (Promega) according to manufacturer's specifications and visualized by autoradiography. Autoradiography or immunoblotting confirmed the quality of all in vitro translation reactions.

Farwestern Analysis. ySWI/SNF complex was purified from yeast strain CY396 as described previously (Cote et al., 1994; Logie and Peterson, 1999). Purified SWI/SNF (10μl) was separated by SDS-PAGE and transferred to PVDF membrane (Immobilon). The membrane was allowed to dry and then it was wet in 100% methanol followed by dH_2O prior to hybridization with the appropriate probe. All subsequent steps were carried out at 4°C with shaking. The prewet membrane was soaked in HBB-A (25mM Hepes-KOH pH7.7, 25mM NaCl, 5mM $MgCl_2$, 1mM DTT) for 5 minutes followed by incubation with HBB-B (25mM Hepes-KOH pH7.7, 25mM NaCl, 5mM $MgCl_2$, 1mM DTT + 5% nonfat milk and 0.05% NP40) for 60 minutes and HBB-C (25mM Hepes-KOH pH7.7, 25mM NaCl, 5mM $MgCl_2$, 1mM DTT + 1% nonfat milk and 0.05% NP40) for 30 minutes. The membrane was then incubated overnight in Hyb(75) (20mM Hepes-KOH pH7.7, 75mM KCl, 0.1mM EDTA, 2.5 mM $MgCl_2$, 1mM DTT + 1% nonfat milk

and 0.05% NP40) and an appropriate dilution (1000x) of the ^{35}S-methionine labeled probe. Membranes were washed 3 x 10 minutes with Hyb(75) at 4°C while shaking. Membranes were dried and exposed to film for subsequent analysis.

Table II-2. Oligonucleotides

Name	Description	Sequence
LB1	(5')TAG@bp1621-SWI3	5'-GAGGATCCCCGGGTACGATGCCCCCAGAGGAC-3'
LB2	Adds ClaI to 3' end-SWI3	5'-GGCCATCGATGCCAAGTCAAGTGACGCACC-3'
LB3	5' BamHI site @ residue 306	5'-GGCCGGATCCCCAACTTATTCTAAATGGTTC-3'
LB4	5' BamHI site @ residue 403	5'-GGCCGGATCCCCACTAACGTCTCAATATTCC-3'
LB5	5' BamHI site @ residue 506	5'-GGCCGGATCCGGCGAAACTTCACGTCCTCT-3'
LB5	SWI3 L714P	5'-CCTGTTGTCTTTCCGGAGTTTTCC-3'
LB7	SWI3 L701P	5'-CTAGTTTCTTTGGATGATTTAATTTAGCGTCC-3'
LB8	5' NheI site @ bp1249-SWI3	5'-GGCCGCTAGCGAATTCGGCGCTGACTGG-3'
LB9	Adds BamHI to 3' end-SWI3	5'-GGCCGGATCCGCCAAGTCAAGTGACGCACC-3'
HHF2	TAG primer	5'-CTCCTAGGGGCCCATG-3'

Underscored sequence represents TAG sequence

Table II-3. Plasmids

Name	Relevant Description	Source
CP426	pET 11A-SWI3 (NheI-BamHI)	This study
CP427	pET 11A-SWI3 L701P,L714P (NheI-BamHI)	This study
CP430	pRS406-swi3 Δ1-306 aa	This study
CP435	pRS406-swi3 L714P	This study
CP596	pRS406-SWI3	This study
CP619	pRS405-SWI2-3HA-6His	This study
CP630	pRS406-swi3 L701P, L714P	This study
CP634	pRS406-swi3 Δ1-403 aa	This study
CP635	pRS406-swi3 Δ1-506 aa	This study

Note: All SWI3 constructs were also cloned into episomal low copy (ARS-CEN) and high copy (2μ) vectors.

Table II-4. Strains

Name	Relevant Genotype	Source
CY165	swi3Δ::trp1Δ1, leu2-Δ1, ura3-52, his3Δ200, ade2-101, lys2-801, Ho::LACZ	Peterson et al., 1992
CY664	SWI2::CP619 isogenic to CY165	This study
CY666	URA3::CP596, isogenic to CY664	This study
CY667	URA3::pRS406, isogenic to CY664	This study
CY668	URA3::CP630, isogenic to CY664	This study
CY677	URA3::CP430, isogenic to CY664	This study
CY678	URA3::CP634, isogenic to CY664	This study
CY679	URA3::CP635, isogenic to CY664	This study

Chapter III

The SANT Domain is Required for the Function of Two Classes of Chromatin Remodeling Enzymes

Chapter III

The SANT Domain is Required for the Function of Two Classes of Chromatin Remodeling Enzymes

Introduction

The compaction of genetic material in the nucleus into higher order chromatin structure has drastic consequences on processes that require transactions with DNA such as DNA replication, repair, recombination, and transcription. In fact, it is now generally recognized that disruption of chromatin structure is the rate-determining step for most of these nuclear DNA transactions. Two classes of highly conserved enzymes have been implicated as regulators of the accessibility of DNA in chromatin (reviewed in Kingston and Narlikar, 1999; Muchardt and Yaniv, 1999; Pollard and Peterson, 1998; Vignali et al., 2000; Workman and Kingston, 1998). The ATP-dependent class of chromatin remodeling enzymes utilizes the energy of ATP hydrolysis to disrupt histone-DNA interactions. The second class includes enzymes that covalently modify nucleosomal histones (e.g. acetylation, phosphorylation, methylation, ADP-ribosylation, ubiquitination; reviewed in Spencer and Davie, 1999; Strahl and Allis, 2000). However, the mechanism by which these complexes function on chromatin is unknown. Interestingly, although these enzymes appear to be functionally distinct, several motifs have been conserved between both classes of remodeling enzymes (e.g. bromodomain, SANT domain). Thus, understanding the role of these similar features will be essential in

order to elucidate the function of chromatin remodeling enzymes and moreover, their functional interrelationships.

The SANT domain (Swi3, Ada2, N-CoR, TFIIIB") is a novel motif found in a number of transcriptional regulatory proteins, conserved from yeast to human, identified based on its homology to the DNA binding domain (DBD) of the cellular protooncogene, c-Myb (Aasland et al., 1996). The Myb-DBD consists of tandem repeats (R1R2R3) of three α-helices in a helix-turn-helix motif distinct from that present in homeodomain proteins or bacterial repressors (Gonda et al., 1985; Klempnauer and Sippel, 1987; Ogata et al., 1994; Pabo and Sauer, 1992). Several amino acid residues that are critical to maintain the structural integrity or activity of the Myb-DBD are also conserved in the SANT domain (Aasland et al., 1996 and Figure III-1). In addition, secondary structure predictions suggest the presence of three α-helices in the SANT domain (Aasland et al., 1996 and Figure III-1). Taken together, this suggests that the tertiary structure of the SANT domain may be similar to that of the Myb-DBD as well (Figure III-2).

Despite its homology to the Myb-DBD, the function of the SANT domain in transcriptional regulatory factors is unknown. Interestingly, the presence of the SANT domain in subunits of both classes of chromatin remodeling complexes (Table III-1) suggests that this domain may play a specific role in transcription via regulation of the accessibility of chromatin structure. As such, it has been suggested that the SANT domain may be a DNA binding domain analogous to that of c-Myb (Aasland et al., 1996). Consistent with this idea, several members of the ATP-dependent class of chromatin remodeling enzymes have been shown to interact with DNA with high affinity

in vitro (Cairns et al., 1999; Quinn et al., 1996). However, studies indicate that it is unlikely that chromatin remodeling enzymes interact with DNA in a sequence specific manner (Cairns et al., 1999; Quinn et al., 1996; Sudarsanam et al., 2000). Furthermore, the primary target for chromatin remodelers is the nucleosome (which consists of DNA wrapped around an octamer of histone proteins) (Kingston and Narlikar, 1999; Workman and Kingston, 1998). Thus, the SANT domain may have diverged to contribute to the overall affinity for nucleosomal DNA or to mediate specific interactions with the histone portion of chromatin. Alternatively, this domain may have diverged to play a role in mediating protein interactions among components of these large, multi-subunit chromatin remodeling enzymes. Consistent with this idea, the *Drosophila* moira protein (homologue of ySwi3p and subunit of dBrahma ATP-dependent remodeling complex; see also Table III-1) appears to require an intact SANT domain for optimal binding to brahma (member of Swi2/Snf2 family of ATPases) (Crosby et al., 1999).

To investigate the role of the SANT domain in chromatin remodeling and transcription, we have exploited the c-Myb-DNA co-crystal structure as a model, to carry out an extensive structure/function analysis on several SANT-containing subunits of chromatin remodeling complexes in yeast: Swi3p (SWI/SNF), Rsc8p (RSC), and Ada2p (GCN5 histone acetyltransferases). These investigations indicate that the putative third α-helix of the SANT domain, which based on the c-Myb-DNA co-crystal structure is involved in base-specific DNA interactions (Ogata et al., 1992; Ogata et al., 1993; Ogata et al., 1994) (see also Figure III-1 and III-2), is crucial for the function of all three of

TABLE III-1. The SANT Domain is a Conserved Motif in Subunits of Chromatin Remodeling Enzymes

SUBUNIT	COMPLEX	REMODELING ACTIVITY
ySWI3	SWI/SNF	ATP-dependent chromatin Remodeling complex
yRSC8/ SWH3	RSC	ATP-dependent chromatin Remodeling complex
huBAF155 huBAF170	HuSWI/SNF hBrm and BRG1 (Complex A and B)	ATP-dependent chromatin Remodeling complex
dmoira	Brahma	ATP-dependent chromatin Remodeling complex
dISWI	NURF	ATP-dependent chromatin Remodeling complex
	CHRAC	ATP-dependent nucleosome Remodeling complex
	ACF	ATP-dependent nucleosome Assembly factor
yADA2	HAT A2	Nuclear histone acetyl-transferase (HAT); modifies Histone H3 N-terminal "tails"
	ADA	Nuclear HAT; modifies histone H3 "tails"
	SAGA	Nuclear HAT; modifies histone H3 "tails"
	ISAGA	Nuclear HAT; modifies histone H3 "tails"

Figure III-1. Sequence Comparison of SANT and Myb-DNA Binding Domains. The SANT domain is highly conserved from yeast to human in transcriptional regulatory proteins. The SANT domain shares extensive homology to the DNA-binding domain (DBD) of the c-Myb proto-oncogene (Aasland et al., 1996). The Myb-DBD consists of tandem repeats, R1R2R3, of an approximately 50 amino acid sequence. Here, we compare the amino acid sequence of the SANT domain of the yeast Swi3, Rsc8/Swh3, and Ada2 proteins with mouse c-Myb. Sequences were aligned with the Clustal W Program. Sequence-specific DNA binding requires two repeats and thus, the amino acid sequence of both repeats are represented here as R2 and R3. Both the Myb-DBD and the SANT domain are composed of three structured α-helices. The highly conserved tryptophans (asterisks) in Myb-DBD family members are also present in the SANT domain. Residues which are conserved among the SANT domain proteins or between the SANT domain proteins and Myb repeats are highlighted in color. Invariant residues are represented in red, highly conserved residues are green, similar amino acids are highlighted in blue, and residues that are not conserved are black.

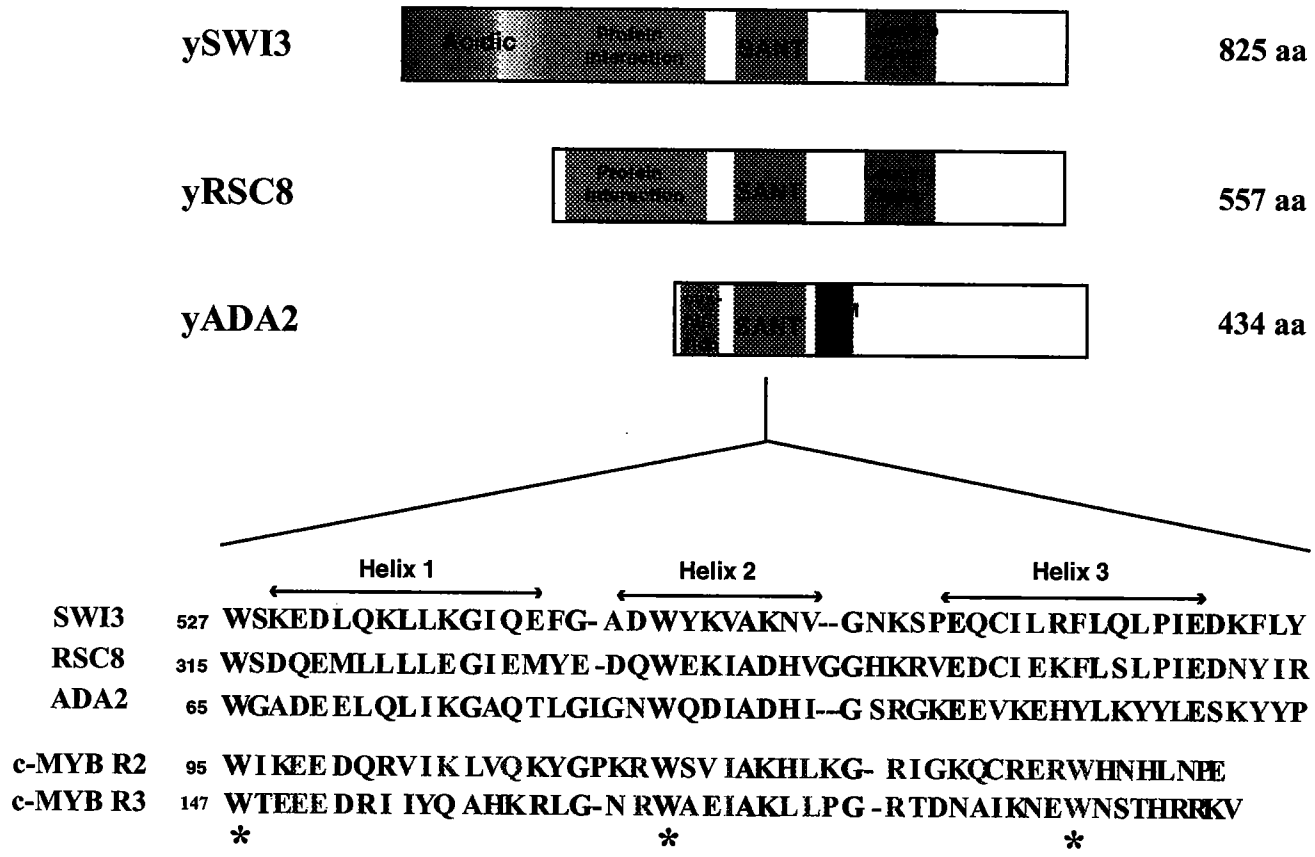

ySWI3
Acidic
Protein interaction
SANT
825 aa
yRSC8
Protein interaction
SANT
557 aa
yADA2
SANT
434 aa
Helix 1
Helix 2
Helix 3
SWI3 527 WSKEDLQKLLKGIQEFG-ADWYKVAKNV--GNKSPEQCILRFLQLPIEDKFLY 577
RSC8 315 WSDQEMLLLLEGIEMYE-DQWEKIADHVGGHKRVEDCIEKFLSLPIEDNYIR 366
ADA2 65 WGADEELQLIKGAQTLGIGNWQDIADHI--GSRGKEEVKEHYLKYYLESKYYP 116
c-MYB R2 95 WIKEEDQRVIKLVQKYGPKRWSVIAKHLKG-RIGKQCRERWHNHLNPE 141
c-MYB R3 147 WTEEEDRIIYQAHKRLG-NRWAEIAKLLPG-RTDNAIKNEWNSTHRRKV 193
*
*
*

Figure III-2: Cartoon of Myb DNA Binding Domain-DNA Co-Crystal Structure. Representation of a single Myb DNA-binding repeat bound to its cognate sequence. This figure was adapted with permission (Aasland et al., 1996). The Myb-DNA binding domain (DBD) consists of three α-helices in a helix-turn-helix variation motif (Ogata et al., 1992; Ogata et al., 1994). Two repeats are required for sequence specific DNA binding. Note that helix 3 is involved in base-specific interactions and contacts DNA in the major groove and thus, has been termed the "recognition" helix. It is predicted that the SANT domain may share a similar tertiary structure.

MYB-DNA Co-Crystal Structure

these proteins *in vivo*. Importantly, these studies also suggest a role for the SANT domain in mediating a functional interaction between remodeling enzymes and histones.

Results

The Swi3 SANT domain is required for SWI/SNF function. Swi3p is one of eleven polypeptides tightly associated with the 2 MD SWI/SNF complex and appears to harbor multiple domains (Aasland et al., 1996; Cairns et al., 1994; Peterson et al., 1994; Peterson and Herskowitz, 1992; see also Figure III-1). SWI/SNF is a member of the ATP-dependent class of chromatin remodeling enzymes and is required for the transcriptional regulation of a number of yeast genes (Biggar and Crabtree, 1999; Holstege et al., 1998; Peterson and Herskowitz, 1992; Sudarsanam et al., 2000). To investigate the role of the SANT domain in SWI/SNF function, we constructed strains bearing either a small deletion or specific amino acid changes in the Swi3p SANT domain, as guided by the c-Myb-DNA co-crystal structure (Figure III-1; see also Experimental Procedures). We then tested whether these mutations affected the regulation of the SWI/SNF-dependent gene, *HO*, as a function of expression of an *HO-lacZ* fusion gene (Figure III-3). Consistent with previous studies, complete deletion of the chromosomal copy of *SWI3* resulted in ~ 10-fold reduction in *HO-lacZ* expression as compared to wild-type (Figure III-3; Peterson and Herskowitz, 1992).

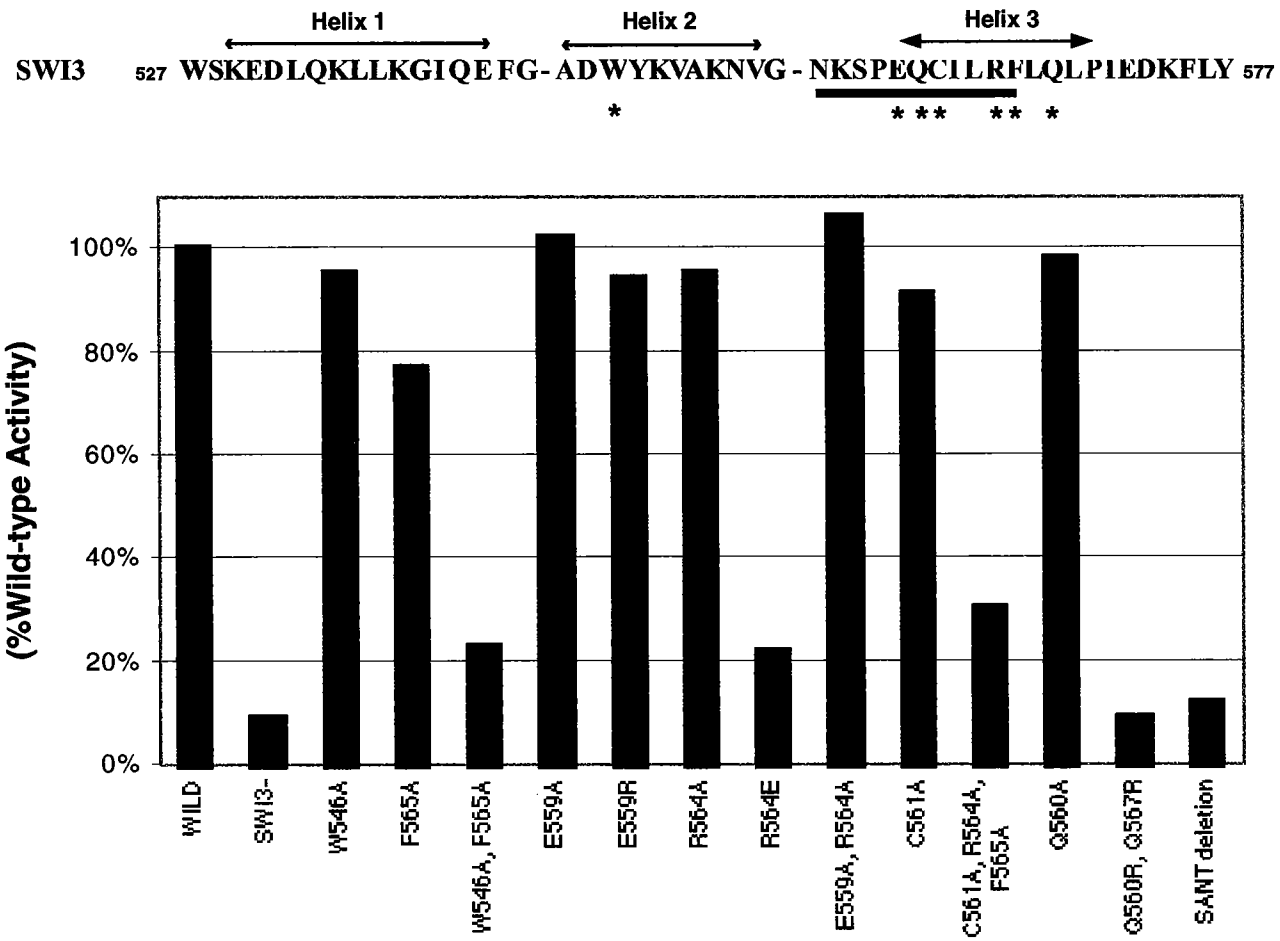
We generated an eleven amino acid deletion which removed nearly all of helix 3 in the Swi3p SANT domain. Expression of *HO-lacZ* was severely compromised in this mutant (12 % as compared to wild-type Swi3p) suggesting that this region of the SANT

domain is critical for the function of Swi3p and moreover, for the function of SWI/SNF *in vivo*. Next, in order to gain further insight into the role of the Swi3p SANT domain, we wished to identify specific amino acid residues that may be critical for its function. First, we generated both single and multiple amino acid substitutions in the aromatic residues that are highly conserved between SANT-containing proteins and Myb-DBD (Figure III-3). Single amino acid alanine substitutions in two of the three highly conserved aromatic residues important for the structural integrity of the Myb-DBD (W546 and F565 in the case of Swi3p) had little effect on SWI/SNF function (96%, and 78% expression, respectively; see Figure III-3). However, the double W546A F565A mutation resulted in ~5-fold reduction in *HO-lacZ* expression (Figure III-3) suggesting that these conserved residues contribute to the structure or function of the SANT domain.

Upon sequence comparison of the putative third "recognition" helix in the SANT domains of the Swi3, Rsc8/Swh3, and Ada2 proteins, we noticed the presence of several invariant residues. For instance, each of these proteins contains a glutamic acid at the same position in helix 3 (Figure III-1). Interestingly, the analogous residue conserved in the Myb-DBD is a lysine which is involved in making base-specific contacts with DNA (Ogata et al., 1994). We generated the mutant, E559A, in Swi3p. Substitution of this conserved, charged residue with alanine in the Myb-DBD obliterates its DNA binding activity (Ogata et al., 1994). Surprisingly, this mutation had virtually no effect on Swi3p activity (105% as compared to wild-type; Figure III-3). Even more surprising was the fact that mutation of this residue to one of opposite charge, E559R, also had no effect on the ability of SWI/SNF to activate transcription from the *HO-lacZ* fusion gene (94% of

Figure III-3: The Swi3p SANT Domain is Required for Expression of SWI/SNF-dependent Genes. The amino acid sequence of the SANT domain of Swi3p is shown in single amino acid code (see Figure III-1 for color code). The eleven amino acid deletion underlined by a thick black bar. All other residues targeted for mutagenesis are identified by asterisks. **A.** Analysis of the effects of mutations in the Swi3p SANT domain on *HO* expression. Strains were grown to mid log phage in YEPD and analyzed for β-galactosidase expression from an integrated *HO-lacZ* reporter construct. The strains used are listed in Table III-4. Analyses were performed in triplicate and values were averaged. Values varied by <15%. Activity was normalized to percentages of wild-type levels. **B.** Analysis of the effects of mutations in the Swi3p SANT domain on *SUC2* expression. Strains were streaked for single colonies on media containing 2% sucrose and compared to wild-type (+) and swi3 deletion (-) strains for growth. Poor growth on media containing sucrose is consistent with a defect in the expression of *SUC2*.

A


Helix 1
Helix 2
Helix 3
SWI3 527 WSKEDLQKLLKGIQEFG-ADWYKVAKNVG-NKSPEQCILRFLQLPIEDKFLY 577
*
* ** ** *
HO-lacZ Expression (%Wild-type Activity)
100%
80%
60%
40%
20%
0%
WILD
SWI3-
W546A
F565A
W546A, F565A
E559A
E559R
R564A
R564E
E559A, R564A
C561A
C561A, R564A, F565A
Q560A
Q560R, Q567R
SANT deletion

B

GROWTH:	+	-	+	+	-	+	+	+	-	+	+	-	+	-	-

wild-type activity; Figure III-3). This suggests that, at least in the case of Swi3p, neither the side chain nor charge of E559 is important for function.

Several residues are invariant between Swi3p and the Myb-DBD, suggesting that these residues may play a specific role in Swi3p function (Figure III-1). Thus, we wanted to characterize some of these residues in the Swi3p SANT domain. First, we generated a Q560A substitution in helix 3. The corresponding residue in the Myb-DBD is critical for interaction with the phosphate backbone and DNA binding activity (Gabrielsen et al., 1991; Ogata et al., 1994). Surprisingly, no effect of mutating this conserved residue was detected in this assay (98% of wild-type activity; see Figure III-3). Modeling of the SANT domain based upon the Myb-DBD structure suggested that Q560 might be positioned adjacent to Q567. These residues may be displayed on one face of the third helix of Swi3p and thus, may be poised to make specific interactions with substrate. The corresponding amino acids in the Myb-DBD are also two polar amino acids both of which have been implicated in base specific interactions and in phosphate backbone contacts (Gabrielsen et al., 1991; Ogata et al., 1994). This prediction prompted us to make a more drastic mutation in this region within Swi3p. The Q560R,Q567R mutation reduced expression nearly 10-fold to 9% as compared to wild-type, indicating that these residues are critical for SWI/SNF function (Figure III-3). One caveat, however, is that this double mutation in Swi3p appears to be expressed at less than wild-type levels *in vivo* (data not shown).

The Myb-DBD harbors a highly conserved cysteine residue in its third "recognition" helix. It has been suggested that this residue may function in DNA binding

by acting as a molecular sensor for a redox regulatory mechanism *in vivo* (Ogata et al., 1994). A cysteine-dependent redox regulation of DNA binding activity has been well characterized in the bZip-type DNA binding domains of the transcriptional activators, Jun and Fos (Abate et al., 1990). Consistent with this, replacement of Cys130 in Myb with serine resulted in the loss of DNA binding (Guehman et al., 1992). Interestingly, this residue is also conserved in the SANT domain of Swi3p and Rsc8p (Figure III-1). Thus, we generated a C561A mutation in Swi3p and analyzed its ability to activate transcription from the integrated *HO-lacZ* reporter construct. The C561A mutation had no effect on SWI/SNF activity suggesting that this residue may not play a role analogous to its conserved counterpart in the Myb-DBD (Figure III-3). However, Cys130 in the Myb DBD is also predicted to be buried in the hydrophobic core of the protein (Ogata et al., 1994) and the analogous cysteine residue in Swi3p is correspondingly predicted to be located on the hydrophobic face of the putative amphipathic third helix. Thus, it is possible that C561 in Swi3p participates in maintaining the hydrophobic core of the protein. This is also consistent with the fact that this nonpolar residue is conserved as a valine in Ada2p (Figure III-1).

Next, we investigated the role of R564 in Swi3p as this residue is also invariant in helix 3 between Swi3p and the Myb-DBD. Again, substitution of this amino acid for alanine, R564A, in Swi3p had no effect on SWI/SNF function (96% of wild-type activity; Figure III-3). However, *HO-lacZ* expression was reduced nearly 5-fold in an R564E mutant (22% of wild-type activity; Figure III-3). This indicates that although the amino acid side chain is not essential, a negative charge cannot be tolerated at this position.

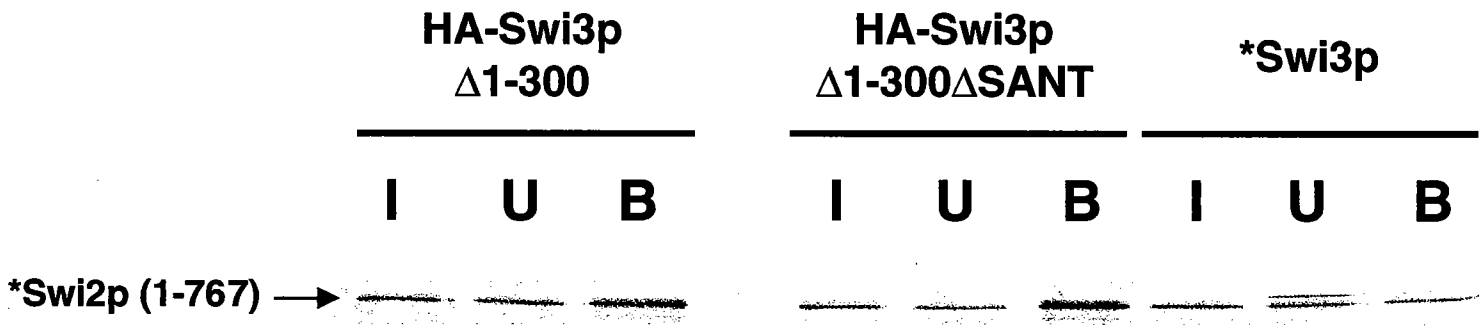
Importantly, this defines a critical residue for the function of Swi3p. The corresponding arginine in the Myb-DBD is not only involved in making contacts with the phosphate backbone of DNA as determined by the Myb-DNA co-crystal structure, but also participates in salt bridge formation within the structure and is essential for DNA binding activity (Gabrielsen et al., 1991; Ogata et al., 1994). Thus, mutation of this amino acid may interfere with SWI/SNF activity by disrupting protein structure or by directly affecting interactions with substrate. Interestingly, charged residues at this same position are also conserved in Rsc8p and Ada2p (Figure III-1).

Finally, since many of the single alanine substitutions in the Swi3p SANT domain did not yield a mutant phenotype, we generated several of the above mutations in combination with one another. For instance, although single substitutions of C561, R564, or F565 to alanine had little effect on activity, the triple mutation exhibited a ~4-fold reduction in *HO-lacZ* expression (Figure III-3). Combination of the E559A and R564A mutations still yielded no effect, suggesting that the side chains of these residues do not play a significant role in function (Figure III-3). Instead, it is possible that the location of these residues within the helix may provide backbone amides critical for the structure or function of this domain. Importantly, the phenotypes we observed in the above analysis were not due to a decrease in expression of the various mutants (with the possible exception of Q560RQ567R) as all were expressed at near wild-type levels (Figure III-10 and data not shown). In addition, overexpression of these mutants on a high copy plasmid did not affect the observed phenotype (data not shown).

Yeast strains bearing mutations in genes encoding components of the SWI/SNF complex display a slow growth phenotype on a variety of carbon sources presumably due to defects in the expression of metabolic genes (Neigeborn and Carlson, 1984; Peterson and Herskowitz, 1992; Sudarsanam et al., 2000). Like other *swi/snf* mutants, strains harboring mutations in the SANT domain also displayed growth defects on glucose media, and were unable to form single colonies on media that contained galactose or glycerol (data not shown). In addition, the slow growth of these strains on sucrose also closely paralleled *HO-lacZ* expression (Figure III-3). The strong growth defect of SANT mutants on sucrose media is consistent with a defect in expression of SUC2 as well (Hirschhorn et al., 1992; Wu and Winston, 1997).

Swi3 SANT domain is not required for interaction with Swi2/Snf2. It has been shown that a conserved region, D2, in the N-terminus of Swi2/Snf2p and Sth1p is sufficient for interaction with Swi3p and Rsc8/Swh3p, respectively (Treich et al., 1995; Treich and Carlson, 1997). In addition, recent studies show that an ~80 amino acid region (i.e. encompassing a portion of the SANT domain and a region N-terminal to the SANT domain) of the *Drosophila* moira protein (Swi3p homologue) was important for stable interaction *in vitro* between moira and possibly the D2 conserved region in brahma (*Drosophila* Swi2/Snf2p homologue) (Crosby et al., 1999). In order to determine if the Swi3p SANT domain mediates interactions with Swi2p, we performed a co-immunoprecipitation assay with the ^{35}S-methionine labeled N-terminus of Swi2p and HA-tagged Swi3p or HA-tagged Swi3ΔSANTp (see Experimental Procedures for

Figure III-4: Interaction between Swi3p and Swi2/Snf2 does not Require the SANT Domain. In vitro binding and immunoprecipitation reactions were performed with equivalent amounts of ^{35}S-methionine labeled Swi2p (amino acids 1-767) and HA-Swi3p or HA-Swi3pΔSANT. The HA-Swi3p constructs also lacked the dispensable acidic domain (Δ1-300) (see Chapter II of this thesis). ^{35}S-methionine labeled Swi3p was used as an immunoprecipitation specificity control in place of HA-Swi3p. Immunoprecipitations were carried out using HA antisera (see Experimental Procedures for details). Bound (B) proteins were eluted with SDS sample buffer and 50% of the eluted protein was analyzed. Results were analyzed by SDS-PAGE and autoradiography. Input (I) and Unbound (U) lanes are loaded at 1/40 of the reaction volume. Similar results were obtained in multiple independent experiments.


HA-Swi3p
Δ1-300
HA-Swi3p
Δ1-300ΔSANT
*Swi3p
I U B I U B I U B
*Swi2p (1-767)

details). Proteins were allowed to interact *in vitro* and immunoprecipitations were carried out with HA antisera conjugated to Sepharose beads. Consistent with previous studies, the N-terminus of Swi2p interacts with Swi3p in this assay (Figure III-4; Treich et al., 1995; Treich and Carlson, 1997). However, a small deletion in the SANT domain did not weaken the binding of Swi2p and Swi3p (Figure III-4). Similar results were also obtained with the Swi3p R564E SANT mutant (data not shown). This is also consistent with studies that indicate that the primary site of interaction between the D2 region of Sth1p and Rsc8/Swh3p maps outside of the SANT domain (Treich and Carlson, 1997). Together, these data suggest that the Swi3p SANT domain does not mediate an interaction with Swi2p and furthermore, suggests that mutations in the Swi3p SANT domain are still competent to interact with the catalytic subunit *in vivo*.

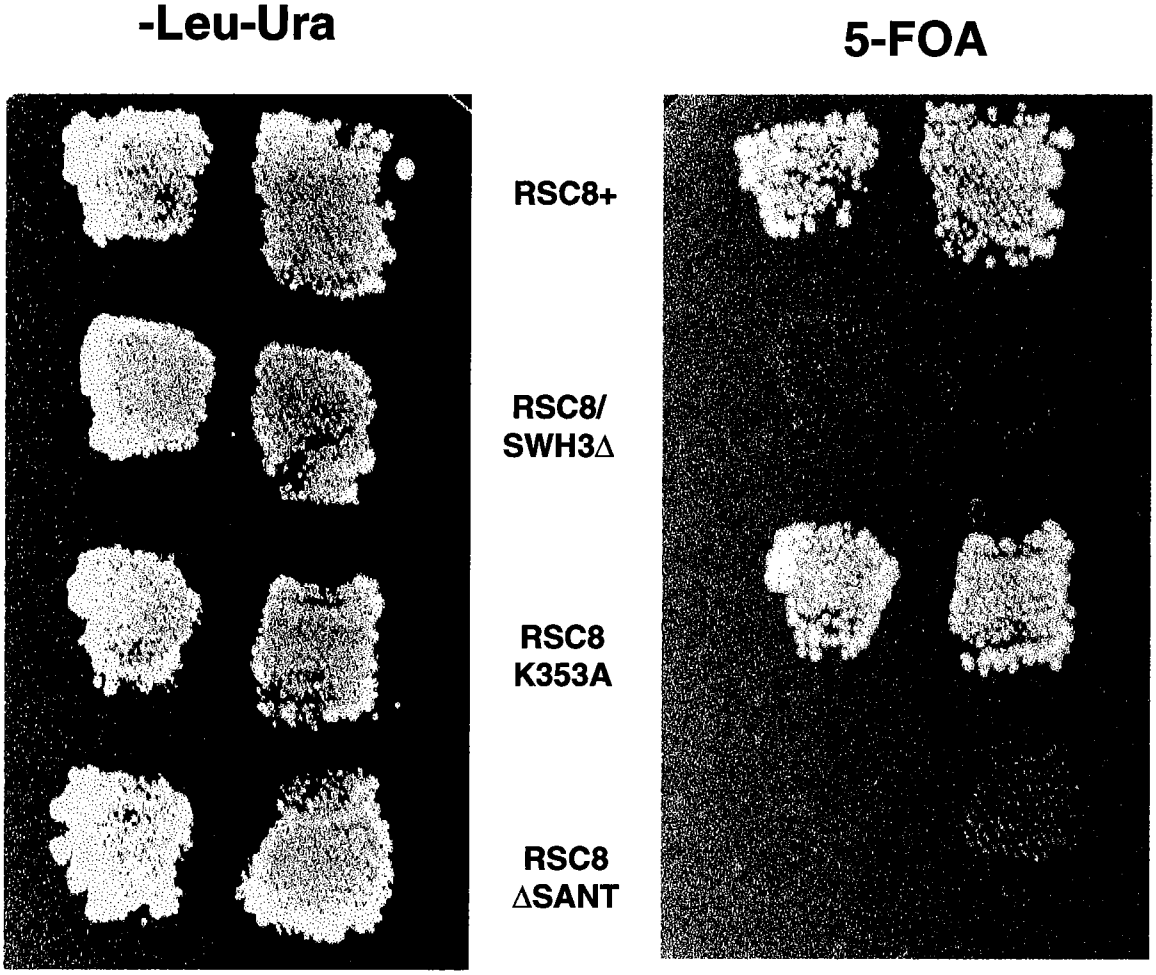
The SANT domain is required for essential functions of RSC complex. RSC is an essential, abundant, multi-subunit ATP-dependent chromatin remodeling enzyme (Cairns et al., 1996b) which has recently been implicated in transcriptional regulation (Cairns et al., 1998; Cairns et al., 1999; Moreira and Holmberg, 1999). In order to investigate a role for the SANT domain in RSC function, we generated mutations in Rsc8/Swh3p (Figure III-1; see also Experimental Procedures). Since it has previously been shown that Rsc8/Swh3p is essential for viability (Treich and Carlson, 1997), we initially constructed a strain that contained a deletion of the chromosomal copy of *RSC8/SWH3* and carried a *URA3*/CEN plasmid bearing a wild-type copy of *RSC8/SWH3*. Strains were then transformed with either a control *LEU2*-plasmid or with *LEU2*-

Figure III-5: The SANT Domain is Essential for RSC Function. RSC subunits are essential for growth and viability. Strains were generated which harbored a deletion in Rsc8/Swh3p and containing a wild-type copy of Rsc8/Swh3p on a *URA3*-CEN plasmid (CY885). CY885 was then transformed with a control *LEU2*-CEN plasmid or *LEU2*-CEN plasmid containing a copy of one of the following: wild-type *RSC8/SWH3*, *rsc8/swh3* K353A, or *rsc8/swh3* ΔSANT. Strains were initially grown on YEPD and replica plated onto either selective media or media containing 5-fluoroorotic acid (FOA) and grown for 5 days at 30°C. The Rsc8 SANT domain sequence is represented as single amino acid code (see Figure III-1 for color code). The five amino acids deleted from Rsc8/Swh3 are underlined by a thick black bar. K353 is identified with an asterisk.

Helix 1 ⟷ Helix 2 ⟷ Helix 3 ⟷

RSC8 315 WSDQEMLLLLEGIEMYE-DQWEKIADHVGGHKRVEDCIEKFLSLPIEDNYIR 366

*



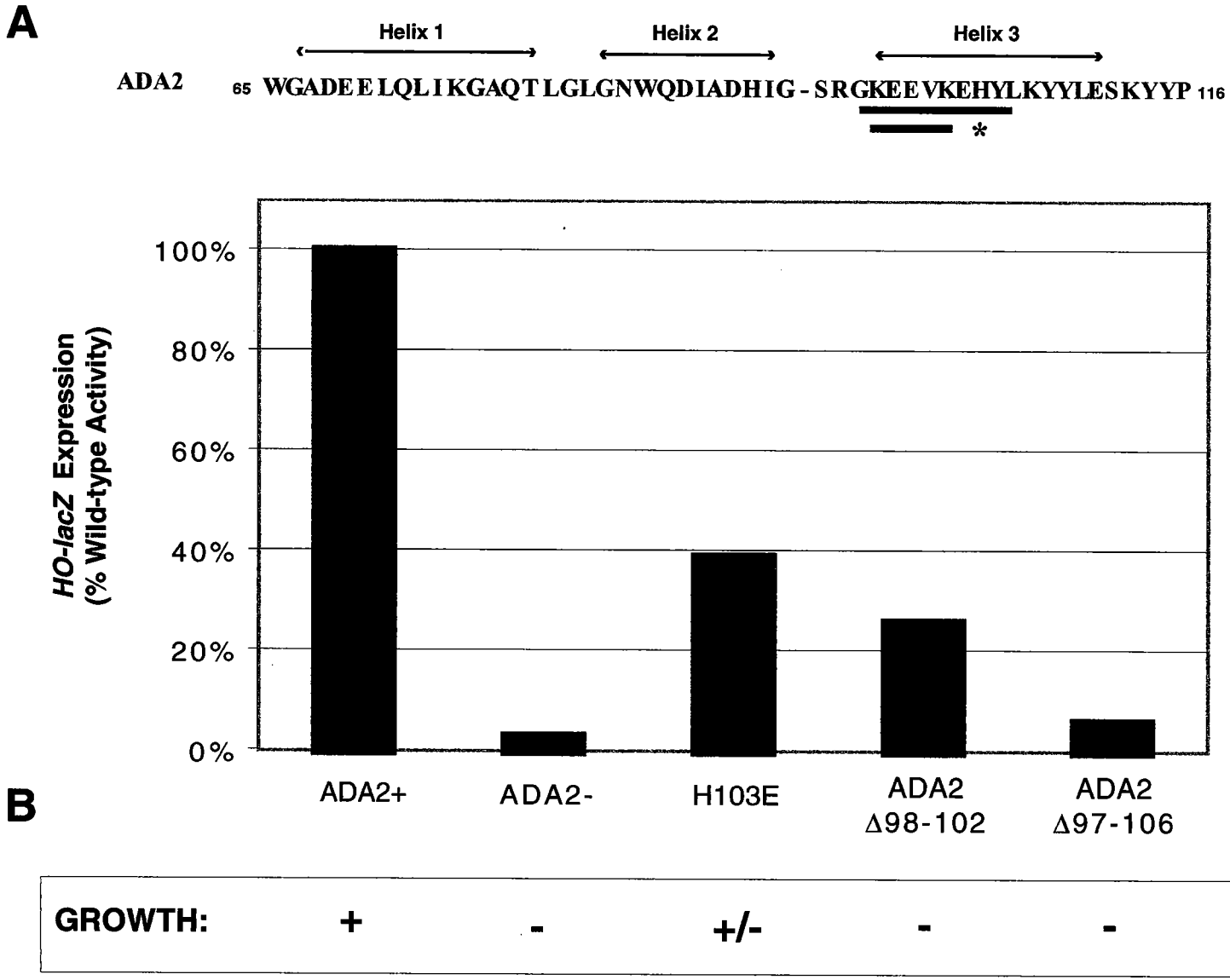
plasmids harboring a wild-type or mutant copy of *RSC8/SWH3*. Initially, each strain grew similarly on media that selected for the presence of both plasmids (Figure III-5; left panel). However, phenotypic differences became apparent when strains were replica plated onto media containing 5-fluoroorotic acid (FOA) which selects for cells that have lost the wild-type *URA3* plasmid. As expected, the strain harboring the *LEU2/RSC8* plasmid grew well on 5-FOA whereas the strain that contained the control *LEU2* plasmid was inviable (Figure III-5; right panel). Strains harboring a K353A amino acid substitution in Rsc8/Swh3p had no effect on growth, analogous to the effects observed for Swi3p R564A (also see above). Importantly, five amino acids in the N-terminal portion of helix 3 in the Rsc8/Swh3p SANT domain were absolutely required for viability (Figure III-5; right panel). We note that the observed phenotype is not due to a decrease in the protein levels of the K353A mutant as protein expression was equivalent to wild-type levels (Figure III-10 and data not shown). Importantly, this suggests that the Rsc8/Swh3p SANT domain plays an essential role in RSC function.

GCN5 HAT activity requires an intact SANT domain. Biochemical fractionation of yeast extracts has identified several nuclear histone acetyltransferase complexes (type A HATs) which harbor Gcn5p as their catalytic subunit (Eberharter, 1999; Grant et al., 1997; Horiuchi et al., 1997; Pollard and Peterson, 1997; Saleh et al., 1997). These complexes are believed to facilitate transcriptional activation through acetylation of specific residues in the N-terminal domains ('tails') of histone proteins (reviewed in Berger, 1999; Brown et al., 2000; Luger and Richmond, 1998a). Previous

studies have shown that although recombinant Gcn5p is capable of acetylating nucleosomal substrates *in vitro* (Tse et al., 1998b), GCN5 HAT activity *in vivo* depends on its association with other proteins such as Ada2p and Ada3p (Candau et al., 1997; Grant et al., 1997; Horiuchi et al., 1995; Pollard and Peterson, 1997). The role of these accessory factors in contributing to the HAT activity of Gcn5p *in vivo* remains unclear, although *ADA/GCN5* gene products are also required for the transcription of the same subset of genes in yeast (Marcus et al., 1994; Pollard and Peterson, 1997). Thus, we wanted to investigate a role of the highly conserved SANT domain in Ada2p in the function of GCN5 HAT complexes.

First, strains were generated which harbored mutations in the putative third "recognition" helix of the Ada2p SANT domain (see Experimental Procedures). We then analyzed the effects of Ada2p SANT mutants on the expression of the ADA/GCN5-dependent gene, *HO* as a function of their ability to activate an integrated *HO-lacZ* fusion gene. Consistent with previous studies, deletion of *ADA2* resulted in ~20-fold decrease in β-galactosidase activity as compared to wild-type (Figure III-6; see also Breeden and Nasmyth, 1987; Pollard and Peterson, 1997). Deletion of 5 amino acids (Δ98-102) from the N-terminal portion of helix 3 of the Ada2p SANT domain reduced expression nearly 5-fold (Figure III-6). Furthermore, deletion of an additional 5 amino acids (Δ97-106) from this domain abolished expression to levels similar to that of a complete deletion of *ADA2* (Figure III-6). We also generated a single amino acid change in the conserved H103 residue in Ada2p analogous to the critical R564 in Swi3p (see above). This H103E mutation reduced lacZ expression nearly 3-fold as compared to wild-type

Figure III-6: Expression of GCN5-dependent Genes Require a Functional SANT Domain. The Ada2p SANT domain sequence is listed in sinlge amino acid code (see Figure III-1 for color code). The five and ten amino acid deletion generated in the Ada2p SANT domain are underlined by thick black bars. H103 is identified by an asterisk. **A.** Analysis of the effects of Ada2p SANT mutants on expression of *HO-lacZ.* Strains were grown to mid log phase in YEPD and analyzed for β-galactosidase activity. All strains used are listed in Table III-4. Analyses were performed in triplicate and values were averaged. Values varied by <15%. Activity was normalized to percentages of wild-type levels. **B.** Growth phenotype of Ada2p SANT mutants. Strains were streaked for single colonies on minimal media and growth was compared to wild-type (+) and Ada2p deletion (-) strains.


A
Helix 1
Helix 2
Helix 3
ADA2
65 WGADEELQLIKGAQTLGLGNWQDIADHIG-SRGKEEVKEHYLKYYLESKYYP 116
*
HO-lacZ Expression
(% Wild-type Activity)
100%
80%
60%
40%
20%
0%
ADA2+
ADA2-
H103E
ADA2
Δ98-102
ADA2
Δ97-106
B
GROWTH:
+
-
+/-
-
-

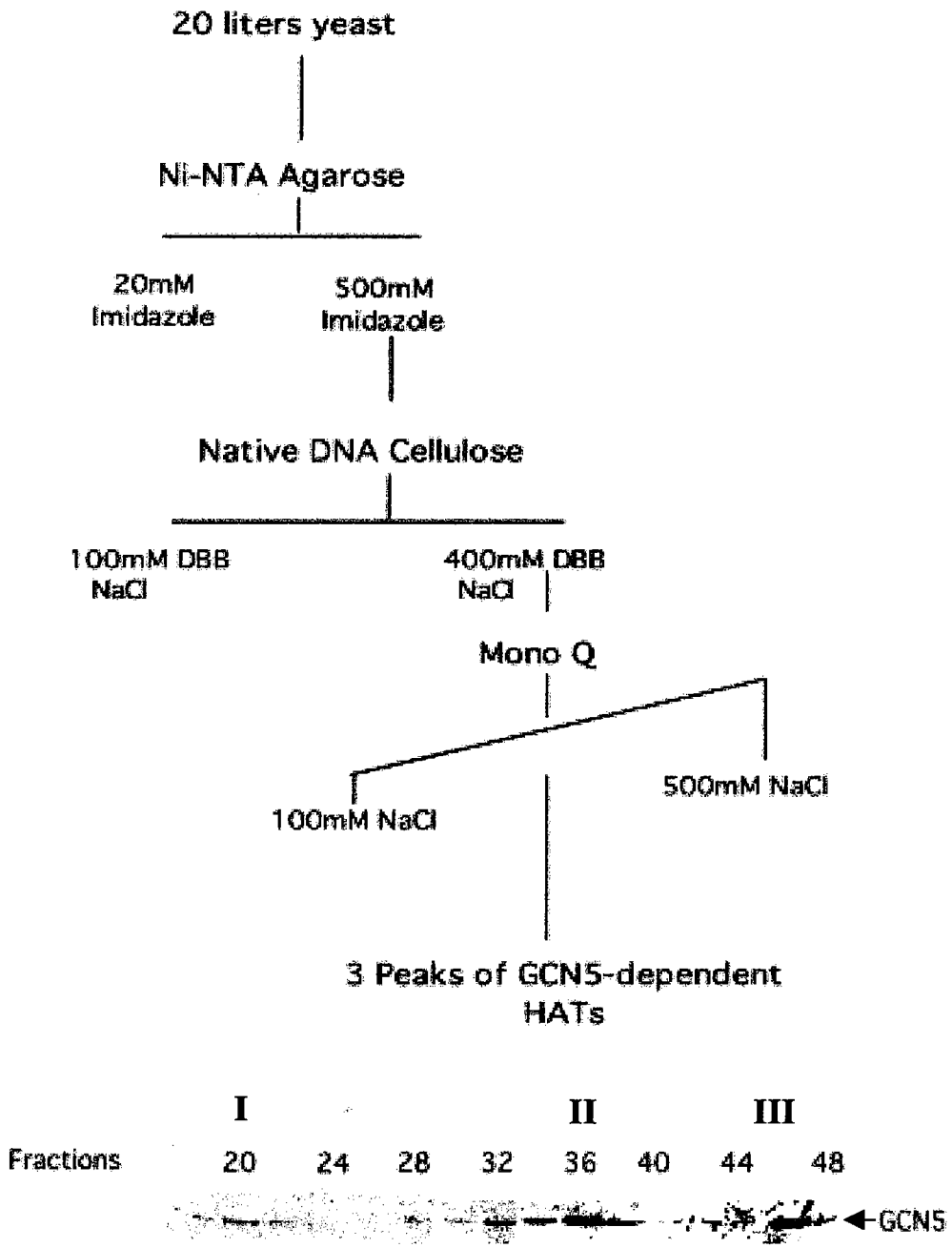
(Figure III-6) suggesting that this conserved residue is not only critical for SWI/SNF function *in vivo*, but is also important for the function of GCN5 HAT complexes as well.

A classic phenotype of ADA/GCN5 mutants is the inability to grow on minimal media due to defects in expression of amino acid biosynthetic genes (Berger et al., 1992). Thus, we analyzed each mutant described above for the ability to complement the growth defects caused by deletion of ADA2. Here, we note that growth of each strain closely paralleled lacZ expression; wild-type strains grew well whereas strains harboring deletions or a mutation in the SANT domain exhibited poor growth (Figure III-6). Importantly, the phenotypes observed are not due to reduced expression of these mutants as each is expressed at near wild-type levels (Figure III-10 and data not shown). In addition, overexpression of Ada2p SANT mutants on a high copy plasmid did not change the observed phenotypes (data not shown). Taken together, these data suggest that the function of the Ada2p SANT domain is not restricted to a role in transcriptional activation at *HO*, but that it appears to play a more global role in contributing to the activity of the GCN5 HAT complexes *in vivo*.

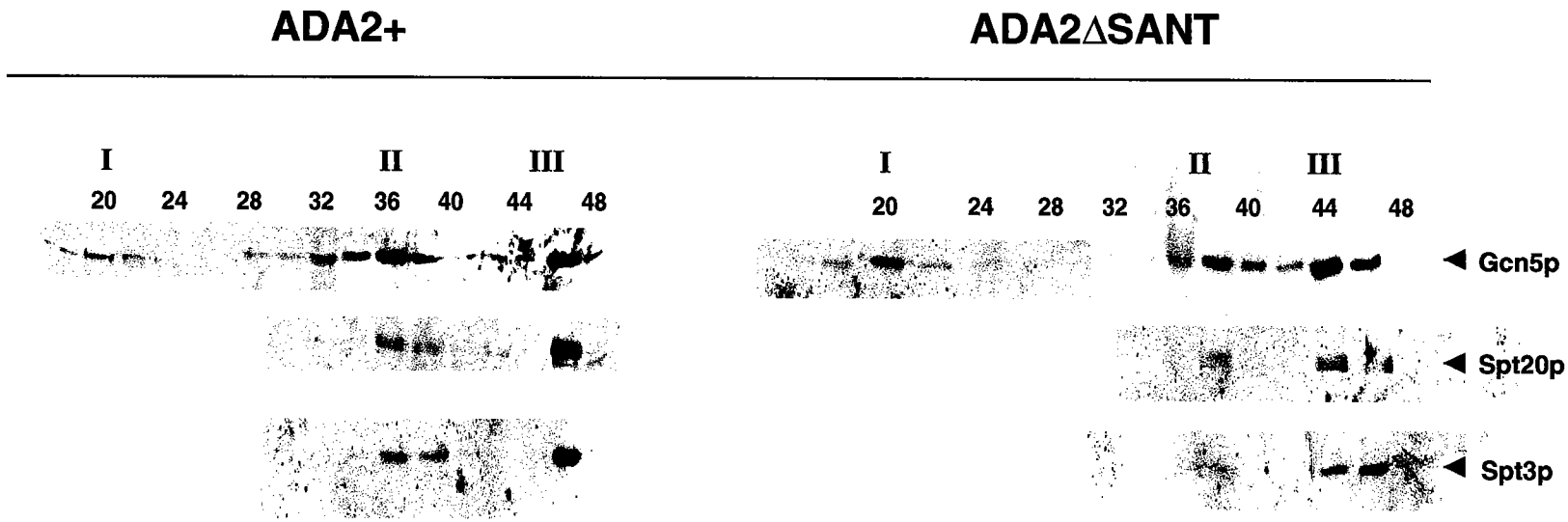
It has previously been shown that deletion of ADA2 results in loss of Gcn5p from native GCN5 HAT complexes (Grant et al., 1997). To test the possibility that SANT mutations alter the assembly of HAT complexes we biochemically fractionated crude extracts from wild-type (CY733) and ada2Δ97-106 (CY884) strains. We have previously shown that our fractionation procedure yields three distinct peaks of Gcn5p as visualized by immunoblotting (Figure III-7A, see also Experimental Procedures and Pollard and Peterson, 1997). The first peak (I) corresponds to the 0.8 MD ADA complex

Figure III-7: The SANT Domain is not Required for Assembly of GCN5 HATS *in vivo*. A. Biochemical fractionation of yeast cell extracts yields three GCN5 HAT complexes. Crude extracts were generated from 20 liters of either CY733 (Ada2p wild-type) or CY (Ada2pΔ97-106) and bound in batch to Ni^{2+} NTA agarose. Bound proteins were eluted with 300 mM Imidazole and dialyzed against DNA binding buffer (Cote et al., 1994). Proteins were further fractionated on a native DNA cellulose column. As a final purification step, proteins were separated on an FPLC MonoQ using a linear gradient of 0.1-0.5 M NaCl. Fractions were collected for subsequent analysis. **B.** Analysis of the elution characteristics and subunit composition of HAT complexes isolated from CY733 and CY884 . Odd fractions from the MonoQ column were analyzed by SDS-PAGE and immunoblotting. Shown are results using antisera to Gcn5p, Spt20p, and Spt3p. Three intact complexes (I=ADA/GCN5, II=SAGA, III=ISAGA) were isolated from both the wild-type strain and the strain harboring a 10 amino acid deletion in the Ada2p SANT domain. Similar results were seen in multiple experiments and with at least two independent fractionation procedures.

A


20 liters yeast
Ni-NTA Agarose
20mM Imidazole
500mM Imidazole
Native DNA Cellulose
100mM DBB NaCl
400mM DBB NaCl
Mono Q
100mM NaCl
500mM NaCl
3 Peaks of GCN5-dependent HATs
I
II
III
Fractions
20
24
28
32
36
40
44
48
GCN5

B

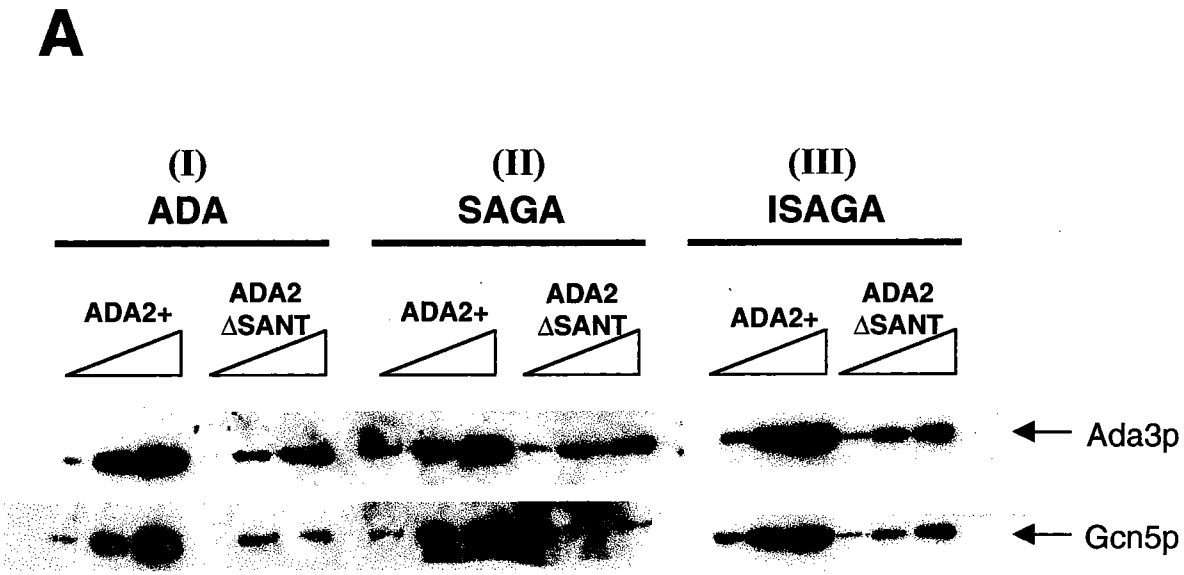

ADA2+
ADA2ΔSANT
I
II
III
20
24
28
32
36
40
44
48
Gcn5p
Spt20p
Spt3p

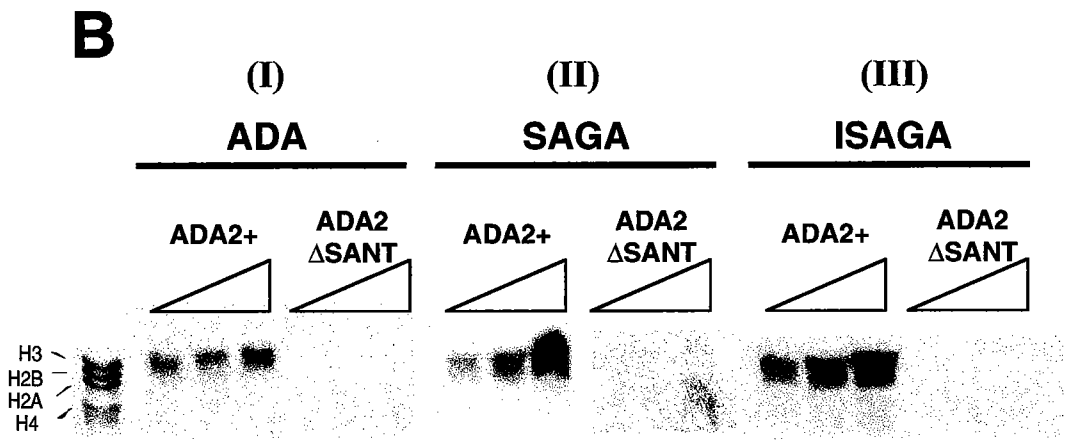
(Grant et al., 1997) whereas peak II is identical to the 1.8 MD SAGA complex (Grant et al., 1997). The third peak of GCN5 is novel to our purification scheme (Figure III-7A and Pollard and Peterson, 1997). Although, it has previously been shown that all three of these complexes contain a core of conserved subunits, Gcn5p, Ada2p, and Ada3p, the ADA and SAGA complexes are otherwise distinct in that they do not share any other subunits (Grant et al., 1997; Pollard and Peterson, 1997). Consistent with previous studies, fractionation of the wild-type strain showed that Gcn5p, Ada2p, and Ada3p associate in three chromatographically distinct peaks (Figure III-7B, III-8, III-10, and data not shown). Importantly, we note that complexes fractionated from the strain harboring a 10 amino acid deletion (Δ97-106) in the Ada2 SANT domain eluted in three distinct peaks containing Gcn5p, Ada2p, and Ada3p, similar to wild-type (Figure III-7B, III-8, III-10, and data not shown). To date, a total of fourteen subunits which include members of the Spt family of proteins, TFIID-related Tafs, and Tra1p, have been shown to co-purify with SAGA (Grant et al., 1997; Grant et al., 1998a; Grant et al., 1998b; Saleh et al., 1998). We compared SAGA fractions from wild-type and SANT mutant strains by immunoblotting with antisera against Spt3p, Spt7p, Spt20p, $TAF_{II}68$p, and Tra1p. Interestingly, these same components were present in SAGA fractions from both wild-type and Ada2ΔSANT-containing strains (Figure III-7B and data not shown). We were also interested in further characterizing and comparing peak III isolated from our purification scheme. Unexpectedly, peak III contained all of the same subunits as SAGA and again mutation in the Ada2p SANT domain does not appear to affect these interactions (Figure III-7B and data not shown).

Others and we have shown that each of these complexes preferentially acetylates the N-terminal domain of histone H3 in both free and nucleosomal substrates (Grant et al., 1997; Pollard and Peterson, 1997). Thus, we next wished to compare the ability of wild-type and SANT mutant complexes to acetylate its preferred substrate. First, levels of Gcn5p and Ada3p were titrated over a 6-fold range and visualized by immunoblotting in order to normalize for protein concentration (Figure III-8A). Next, we performed *in vitro* acetylation assays by titrating each complex in a reaction containing ^{3}H-acetyl CoA and oligonucleosomes (see Experimental Procedures for details). Reactions were analyzed by SDS-PAGE and incorporation of ^{3}H acetate was visualized by autoradiography. Strikingly, Figure III-8B shows that although all three wild-type complexes robustly acetylated histone H3, complexes that contained a deletion in the Ada2p SANT domain were incapable of acetylating substrate over the same 6-fold range of concentration. Similar results were also seen when free histones were utilized as substrates (data not shown). Taken together, this data strongly indicates that the SANT domain is absolutely required for the catalytic activity of GCN5 histone acetyltransferases *in vitro* and importantly this defect is not due to the loss of Gcn5p or other known subunits from the HAT complexes.

The SANT domain is required for functional interaction with histone H3 N-terminal domains. The conservation of a Myb-like, SANT domain in chromatin remodeling complexes is particularly interesting since it is not known how these

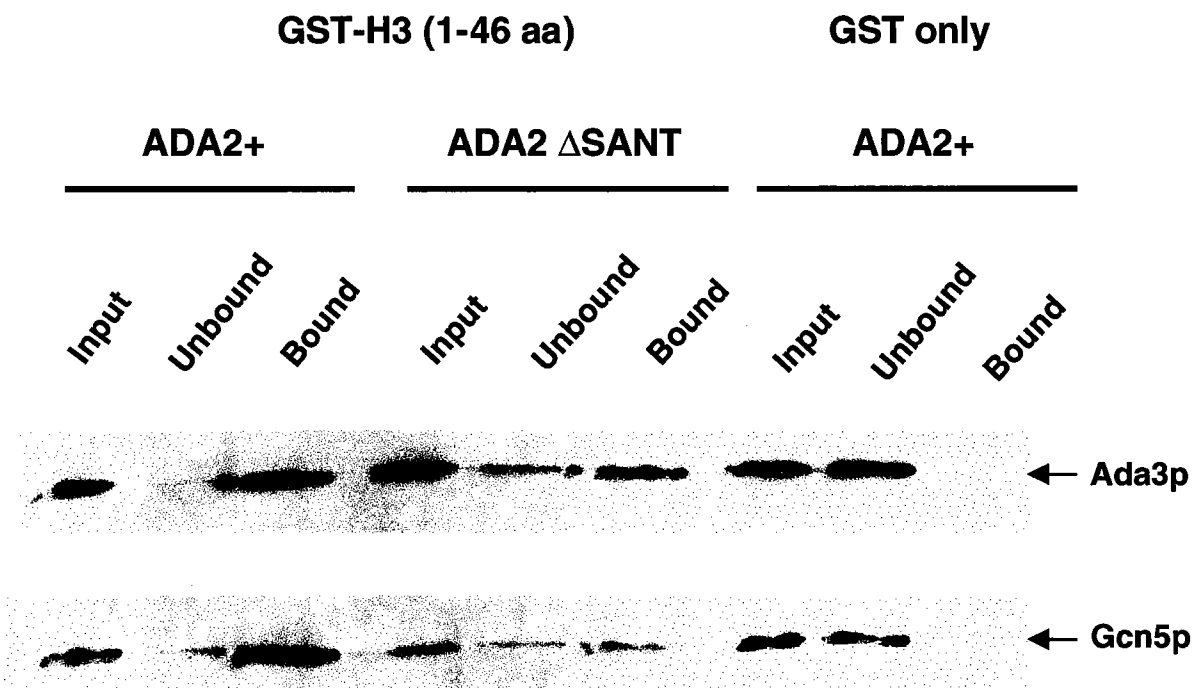
Figure III-8: The SANT Domain is Required for GCN5 HAT Activity. A. Titration of GCN5 HAT complexes. In order to compare relative protein levels between purified GCN5 HAT complexes, a six-fold range in concentration (2, 6, and 12 μl) of each peak Gcn5p fraction isolated from Cy733 and CY884 (as determined by immunoblotting; see Figure III-7B) was compared by SDS-PAGE and immunoblotting against Ada3p and Gcn5p. **B.** In vitro acetylation assays. Assays were performed using a six-fold range in concentration of GCN5 HAT complexes (0.5, 1.5, 3 μl). Each peak Gcn5p fraction (as above) was incubated with oligonucleosomes and ^{3}H-acetyl coA (see Experimental Procedures for details) and subjected to 18% SDS-PAGE. Gels were stained with Coomassie brilliant blue to visualize histones, destained, soaked in ENHANCE (NEN), and analyzed by autoradiography.


A
(I)
ADA
(II)
SAGA
(III)
ISAGA
ADA2+
ADA2 ΔSANT
ADA2+
ADA2 ΔSANT
ADA2+
ADA2 ΔSANT
Ada3p
Gcn5p


B
(I)
ADA
(II)
SAGA
(III)
ISAGA
ADA2+
ADA2 ΔSANT
ADA2+
ADA2 ΔSANT
ADA2+
ADA2 ΔSANT
H3
H2B
H2A
H4

regulatory complexes interact with their nucleosomal substrates. Given that the SANT domain is required for acetylation of histone H3 *in vitro* (see above), we wished to determine if the SANT domain plays a role in the ability of these enzymes to interact with their substrate. We performed GST binding experiments using GST-histone H3 'tail' fusions (amino acids 1-46) and either wild-type or Ada2p SANT mutant complexes (see Experimental Procedures for details). Figure III-9 shows results of a binding experiment using normalized amounts of peak III fractions from both strains. Addition of GST-H3 'tail' was able to deplete wild-type HAT complex from reactions as visualized by immunoblotting with antisera against Ada3p and Gcn5p (Figure III-9; see also Grant et al., 1997). This interaction was specific to the histone H3 'tail' as no binding was seen with the GST control (Figure III-9). However, we consistently observed a reduction in binding when the SANT mutant complex (peak III) was incubated with the GST-H3 "tail"(Figure III-9). Both the wild-type and SANT mutant complexes are stable under these experimental conditions as stoichiometric levels of Ada3p and Gcn5p are seen in both the unbound and bound fractions as compared to input (Figure III-9). Similar results were seen over a range of protein concentrations and with SAGA (peak II) fractions as well (data not shown). Importantly, the residual binding of the histone H3 'tail' by mutant SANT complexes does not result in acetylation of histone H3 (Figure III-8B and III-9). Together, these data suggest an important role for the SANT domain in mediating a physical and/or functional interaction with the histone H3 N-terminal domain.

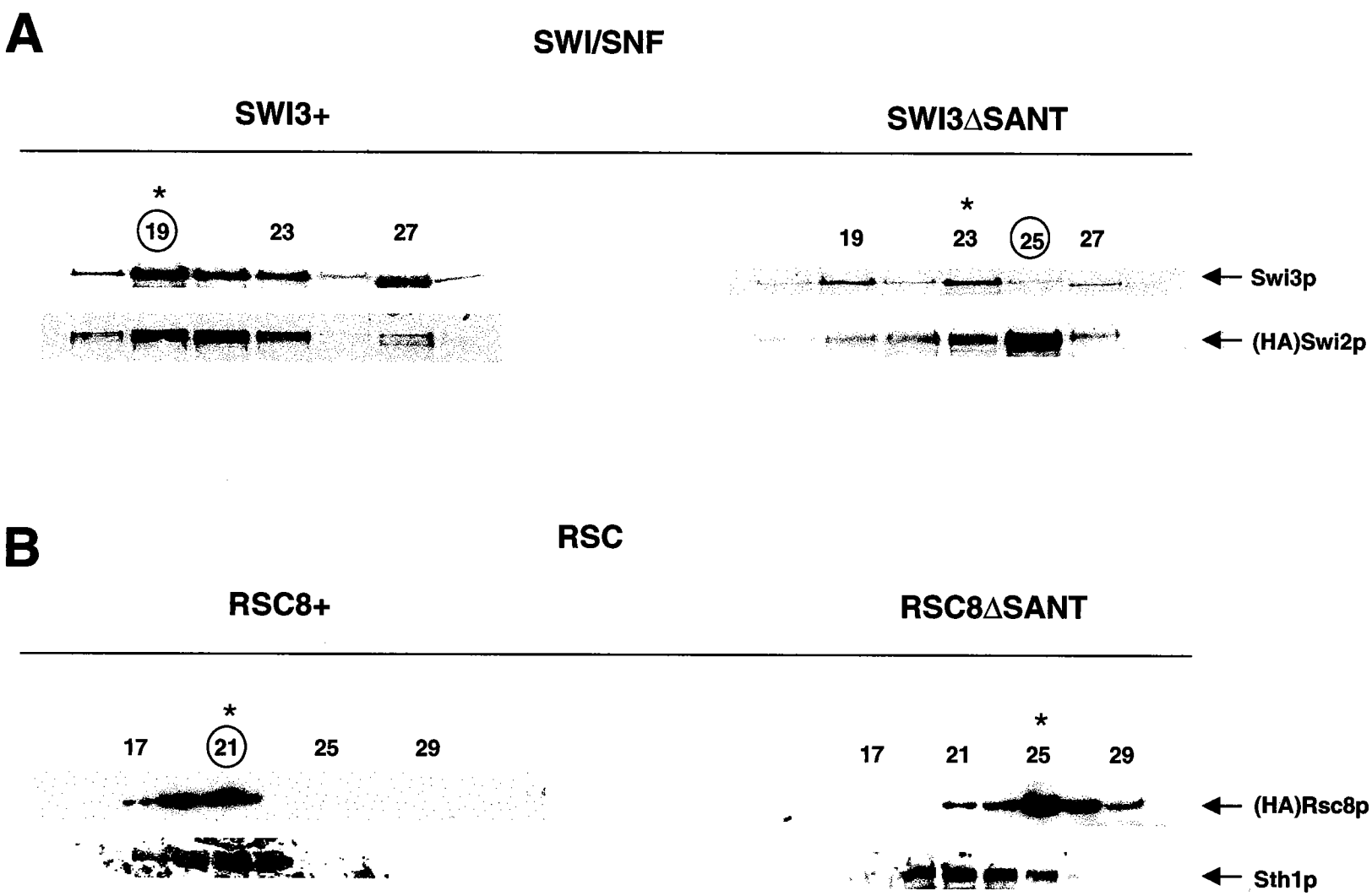
Figure III-9: Functional Interaction with the Histone H3 N-terminal Domain Requires an Intact SANT Domain. GST binding reactions were performed with peak III fractions isolated from CY733 (wild-type) and CY884 (Ada2Δ97-106). Equivalent amounts of peak III fractions were incubated with glutathione agarose beads with either bound GST-H3 (amino acids 1-46) or GST as control. Unbound proteins were recovered by TCA precipitation of the supernatants and bound proteins were eluted in SDS sample buffer. Binding reactions were analyzed by SDS-PAGE and immunoblotting with antisera to Ada3p or Gcn5p. Inputs represent 50% of the amount of peak III in the reactions. Similar results were seen in at least five independent experiments.


GST-H3 (1-46 aa)
GST only
ADA2+
ADA2 ΔSANT
ADA2+
Input
Unbound
Bound
Input
Unbound
Bound
Input
Unbound
Bound
Ada3p
Gcn5p

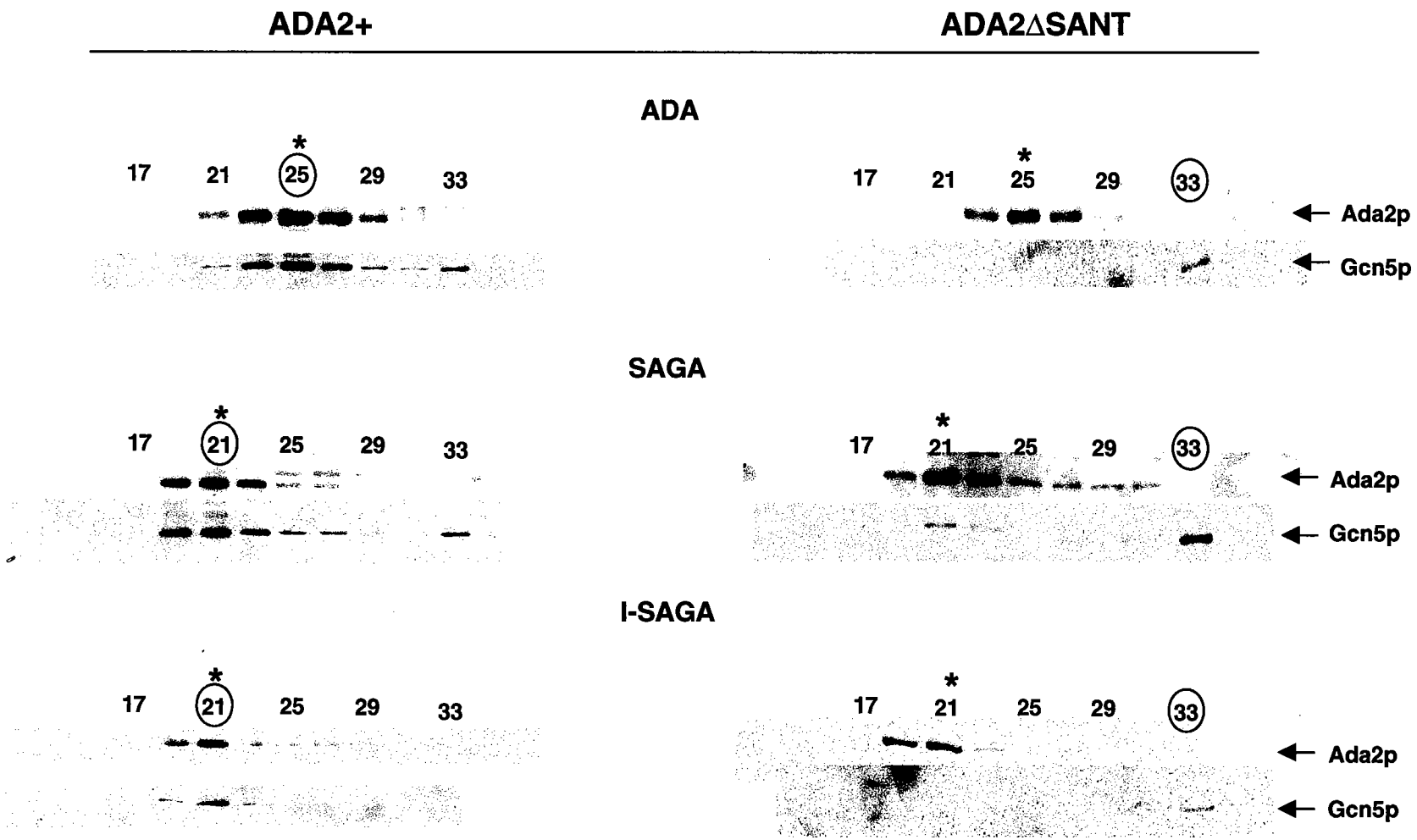
The SANT domain may be required for optimal association of multi-subunit complexes *in vitro*. Our analysis demonstrates that the SANT domain plays a crucial role in the function of SWI/SNF, RSC, and the GCN5 HATs. In addition, our studies suggest a role for the Ada2p SANT domain for substrate recognition in GCN5 HAT complexes (see above). However, one possibility is that this interaction may be indirect. Fractionation of GCN5 HATs from strains that harbored a small deletion in the Ada2p SANT domain suggests that this domain is not required for complex assembly. However, we could not rule out an effect of this mutation on loss of an unidentified subunit(s) or on the conformation of the complex. In that regard, we wanted to compare the elution properties of wild-type and SANT-mutant SWI/SNF, RSC and GCN5 HAT complexes by gel filtration analysis.

First, we fractionated crude whole extracts from wild-type (CY664) and swi3ΔSANTp (CY669) on an FPLC Superose 6 gel filtration column. Consistent with previous studies, fractionation of wild-type cells showed that Swi3p and Swi2/Snf2p coelute by gel filtration in a peak centered around fraction 19 as detected by immunoblotting (Peterson et al., 1994 and Figure III-10A, left panel). This corresponds to an intact SWI/SNF complex with a molecular mass of ~2 MD. We have previously demonstrated that *swi/snf* deletion mutations alter the elution profile of the remaining subunits from a gel filtration column (Peterson et al., 1994). In extracts prepared from strains harboring an 11 amino acid deletion of the Swi3p SANT domain, the majority of Swi3p (Fraction 23) and Swi2p (Fraction 25) do not cofractionate suggesting reduced stability of SWI/SNF complex *in vitro* (Figure III-10A, right panel). We note that similar

Figure III-10: The SANT Domain is Required for Optimal Association of Multi-subunit Chromatin Remodeling Complexes *in vitro*. **A.** Gel filtration analysis of SWI/SNF. Crude whole cell extracts were prepared from CY664 (wild-type) and CY669 (swi3pΔSANT) and fractionated on an FPLC Superose 6 column. Fractions were analyzed by SDS-PAGE and immunoblotting with antisera to Swi3p and HA (Swi2p). The asterisks denote the peak of Swi3p whereas circles denote the peak of Swi2p. Molecular masses were estimated by extrapolation of the following calibration proteins: thyroglobulin (669,000 kD; fraction 25), apoferritin (443,000 kD; fraction 28), β-amylase (200,000 kD; fraction 30), and bovine serum albumin (66,000 kD; fraction 33). **B.** Gel filtration analysis of RSC. Crude whole cell extracts were prepared from CY888 that additionally contained either wild-type Rsc8/Swh3p or HA-Rsc8/Swh3p plasmids and fractionated on an FPLC Superose 6 column. Fractions were analyzed by SDS-PAGE and immunoblotting with antisera to HA (Rsc8/Swh3p) or Sth1p. The asterisks denote the peak of Rsc8/Swh3p whereas circles denote the peak of Sth1p. Molecular mass was estimated by extrapolation of molecular weight standards. **C.** Approximately equivalent amounts of each partially purified GCN5 HAT isolated from CY733 and CY884 was fractionated on an FPLC Superose 6 column. Fractions were analyzed by SDS-PAGE and immunoblotting with antisera to Ada2p and Gcn5p. The asterisks denote the peak of Ada2p whereas circles denote the peak of Gcn5p. Molecular masses were estimated by extrapolation of molecular weight standards.


A
SWI/SNF
SWI3+
SWI3ΔSANT
*
19
23
27
*
19
23
25
27
Swi3p
(HA)Swi2p
B
RSC
RSC8+
RSC8ΔSANT
*
17
21
25
29
*
17
21
25
29
(HA)Rsc8p
Sth1p

C


ADA2+
ADA2ΔSANT
ADA
17
21
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33
Ada2p
Gcn5p
SAGA
17
21
25
29
33
Ada2p
Gcn5p
I-SAGA
17
21
25
29
33
Ada2p
Gcn5p

results were seen with all SANT mutants that exhibited defects in *HO-lacZ* expression and growth (data not shown).

The RSC complex has been estimated to have a mass of ~1 MD based on the molecular weight of the 15 tightly associated polypeptides (Cairns et al., 1996b). Given that the SANT domain of Rsc8/Swh3p was essential for viability (Figure III-5), we initially transformed a diploid strain (MCY3840) that contained a chromosomal deletion of one copy of *RSC8/SWH3* with a CEN-plasmid harboring a wild-type copy of *RSC8/SWH3*. Tetrad dissection was performed in order to generate a strain that harbored a deletion of RSC8/SWH3 and the wild-type *RSC8/SWH3* plasmid. The resulting strain was then transformed with an additional CEN plasmid containing either an HA-epitope tagged wild-type or *rsc8/swh3ΔSANT*. Thus, we could follow the elution of wild-type and SANT mutant RSC complexes by immunoblotting fractions from the gel filtration column with HA antisera. Superose 6 gel filtration of extracts from wild-type cells produced a Rsc8/Swh3p peak centered on fraction 21. This corresponds to a molecular mass of ~1-2 MD, consistent with previous estimates (Figure III-10B, left panel and Cairns et al., 1996b). In contrast, gel filtration of extracts prepared from CY888 (Table III-4) transformed with the HA-*rsc8/swh3ΔSANT* construct indicated that the elution profile shifts to fraction 25 as detected by immunoblotting (Figure III-10B, right panel).

Rsc8p has been shown to self-associate via a putative leucine zipper region independent of the SANT domain (Treich and Carlson, 1997). Thus, we note that in our analysis of the elution profile of SANT mutant RSC complex, we may detect complexes that contain a wild-type copy of Rsc8/Swh3p and a copy of HA-Rsc8/Swh3ΔSANTp and

complexes that contain only HA-Rsc8/Swh3ΔSANTp. The situation is less clear for Sth1p as we cannot distinguish between complexes containing two wild-type copies of Rsc8/Swh3p, wild-type/HA, or two HA-Rsc8/Swh3p polypeptides. Despite this complication, Sth1p (member of the Swi2/Snf2p family of ATPases) cofractionated with Rsc8/Swh3p from wild-type strains, consistent with the presence of an intact RSC complex. In contrast, a substantial amount of Sth1p is shifted to fraction 25 when gel filtration is performed with rsc8/swh3ΔSANT strains (Figure III-10B, both panels). This suggests that the SANT domain is required for stable association of RSC *in vitro*.

We then compared the elution profiles of wild-type (CY733) and GCN5 HAT complexes harboring a ten amino acid deletion in the SANT domain of Ada2p (CY884). An equivalent amount of each partially purified fraction (Figure III-7B) was analyzed on a Superose 6 gel filtration column. Consistent with previous results, wild-type ADA complex eluted as an intact complex with an apparent molecular mass of ~0.8 MD as determined by immunoblotting against Ada2p, Ada3p, and Gcn5p (Figure III-10C, left panel and data not shown, (Grant et al., 1997; Pollard and Peterson, 1997). In addition, SAGA complex eluted in fraction 21 by gel filtration consistent with the previously estimated molecular mass of ~1-2 MD (Figure III-10C, left panel and (Grant et al., 1997; Pollard and Peterson, 1997). Interestingly, analysis of the third peak from our purification scheme (Figure III-7B) demonstrated that Ada2p, Ada3p, Gcn5p, and Spt20 co-eluted in fraction 21 as well (Figure III-10C, left panel and data not shown). Thus, gel filtration analysis combined with the fact that SAGA and peak III share many of the same subunits has led us to name this complex ISAGA (imitation SAGA). This novel complex

may harbor additional subunits unrelated to SAGA components (J. Workman, personal communication).

When partially purified complexes from the Ada2ΔSANT strain were analyzed by gel filtration, followed by immunoblotting, we consistently noticed the loss of Gcn5p from these complexes (Figure III-10C, right panel). Interestingly, Gcn5p elutes in fraction 33 which is consistent with its molecular weight suggesting that *in vitro*, stable association of the catalytic subunit within these complexes requires an intact SANT domain. This contrasts to our finding that Gcn5p cofractionates with Ada and Spt subunits throughout several purification steps (Figure III-7B). Furthermore, Gcn5p does not appear to be released from ΔSANT HAT complexes in the histone acetylation reaction, since no free histone HAT activity is detectable in this assay (data not shown). Therefore, it is possible that the overall decrease in stability of SWI/SNF seen in this assay may be due to dilution of the sample by gel filtration analysis.

Discussion

The SANT (Swi3p, Ada2p, N-CoR, TFIIIB') domain has recently been identified as a novel motif in a variety of transcriptional regulatory proteins (Aasland et al., 1996). Interestingly, this motif is highly conserved in subunits of both the ATP-dependent and histone modifying class of chromatin remodeling complexes (Table III-1). Although the role of this domain is largely unknown, sequence analysis revealed a striking homology to the DNA binding domain (DBD) of the Myb family of proteins (Aasland et al., 1996). In order to gain insight into the role of the SANT domain in chromatin remodeling, we

exploited the Myb DBD-DNA co-crystal structure to carry out an extensive structure/function analysis on three subunits of chromatin remodeling complexes: Swi3p (SWI/SNF), Rsc8p (RSC), Ada2p (GCN5 histone acetyltransferases).

Comparison between the SANT and Myb-DBD domains. The Myb DBD-DNA co-crystal structure provides an important tool to analyze the function of the SANT domain. The Myb-DBD consists of tandem repeats (R1R2R3) of an approximately 50 amino acid sequence which is defined by the presence of three α-helices (Gonda et al., 1985; Klempnauer and Sippel, 1987; Ogata et al., 1994; see also Figure III-2). Interestingly, secondary structure predictions of the SANT domain suggest that this motif is also composed of three structured α-helices. In as much, the invariant tryptophan residues in the Myb-DBD protein family which are important for maintaining the tertiary structure of this domain are also conserved in the SANT domain (Figure III-1 and Ogata et al., 1993; Ogata et al., 1994). Mutational analysis of these hydrophobic amino acids in Swi3p confirms an important role for these residues in SANT domain function (Figure III-3). Another residue in the Myb-DBD that is critical for transcriptional activation is arginine 133 which appears to be important for formation of salt bridges with other residues within the structure and may be essential for maintaining the tertiary structure of this domain (Ogata et al., 1994). Interestingly, mutation of the corresponding residue in Swi3p (R564) and Ada2p (H103) to glutamic acid impairs function in both cases suggesting that these residues may also play a similar role (Figure III-3 and III-6). Thus, this analysis suggests that the overall structure may be similar between the Myb-DBD

and SANT domains and furthermore, defines a critical residue that is essential for the function of SWI/SNF and GCN5 HATs *in vivo*.

The structure also reveals that the third α-helix in the Myb-DBD is positioned to contact DNA directly and appears to be the DNA recognition helix (Ogata et al., 1994). Our mutational analysis shows that the corresponding α-helix in the SANT domain of Swi3p, Rsc8/Swh3p, and Ada2p is essential for the function of these proteins *in vivo* (Figure III-3, III-5, III-6). In addition, the Myb-DBD-DNA co-crystal structure identifies specific amino acids in the recognition helix involved in mediating base specific and phosphate backbone interactions with DNA (Ogata et al., 1994). In several cases we noticed that residues critical for DNA binding in Myb were neither conserved nor essential for the function of the SANT domain (Figure III-1). For instance, substitution of the conserved lysine at position 128 with alanine in the Myb-DBD caused a marked reduction in Myb transcriptional regulatory activity (Ogata et al., 1994), whereas mutation of the corresponding glutamic acid at position 559 in Swi3p (or the corresponding glutamic acid in Rsc8/Swh3p or Ada2p) to either alanine or arginine had no effect on function (Figure III-3). In addition, two residues, Lys182 and Asp183, in the Myb-DBD that are absolutely required for DNA binding activity are not conserved in the SANT domain and are in some cases hydrophobic residues (Gabrielsen et al., 1991; Saikumar et al., 1990; and Figure III-1). Moreover, arginine 190 in the Myb-DBD provides essential phosphate backbone contacts (Ogata et al., 1994) whereas the corresponding residue in Swi3p, Rsc8/Swh3p, and Ada2p is either an isoleucine or leucine (Figure III-1). We also note the presence of several invariant residues shared

among Swi3p, Rsc8/Swh3p, and Ada2p that are not conserved in the Myb-DBD (Figure III-1). This data suggests a possible diversification of function between the Myb DBD and SANT domain.

What is the function of the SANT domain? The presence of the SANT domain in subunits of multi-protein chromatin remodeling complexes as well as its requirement for the function of both classes of remodelers suggests a role for this motif in modulating chromatin structure (Table III-1 and see above). Although the SANT domain has sequence homology to Myb-DBD and may have maintained a similar tertiary structure, several lines of evidence argue against a role for the SANT domain in DNA binding. First, residues important for specific interactions with DNA and transcriptional activation in Myb are not conserved in the SANT domain of Swi3p, Rsc8/Swh3p, or Ada2p. Second, although SWI/SNF and RSC exhibit high affinity for DNA this appears to be sequence independent (Cairns et al., 1996b; Quinn et al., 1996). In as much, recombinant BRG1, the catalytic subunit of hSWI/SNF, which does not contain a SANT domain, has been shown to interact with DNA suggesting that DNA binding may be mediated by the ATPase domain (Phelan et al., 1999). Third, studies have shown that neither Swi3p nor BAF170 bind DNA *in vitro* (Quinn et al., 1996; Wang et al., 1996b). Fourth, DNA binding by Myb requires the presentation of two tandem repeats, R2R3, in a continuous fashion to DNA (Ogata et al., 1994), whereas in most cases the SANT domain is present in only one copy in chromatin remodeling complexes. Although Swi3p and Rsc8/Swh3p are likely present in multiple copies in SWI/SNF and RSC (via self-association through the leucine zipper - Treich and Carlson, 1997; see also Chapter II of this thesis), it is

unlikely that this dimer structurally mimics that of the Myb-DBD. And finally, GCN5 HATs are thought to exert their regulatory activities through interaction with the protein portion of chromatin, the histone 'tails' rather than DNA.

The SANT domain may have adapted to regulate intramolecular (between components of the multi-subunit complexes) or intermolecular (interaction with chromatin components) protein-protein interactions in order to modulate activity. For instance, recent *in vitro* studies show that the SANT domain may be required for optimal interaction between two components, moira and brahma, in the *Drosophila* homologue of SWI/SNF (Crosby et al., 1999). This suggests a role for the SANT domain in complex assembly. Several lines of evidence contradict this possibility for yeast complexes. First, our data indicate that an intact SANT domain in Swi3p is not required for interaction with Swi2p (Figure III-4). Second, we were able to isolate intact GCN5 HAT complexes that harbor mutations in the SANT domain of Ada2p over several successive purification steps (Figure III-7B). This is also consistent with previous studies showing that a large deletion encompassing the SANT domain of Ada2p does not affect its interaction with Gcn5p or Ada3p, further suggesting that the observed functional defects are not due to the inability of SANT mutants to assemble into complexes (Candau and Berger, 1996). Third, we see no evidence for decreased stability in other components of SWI/SNF, RSC, or GCN5 in our SANT mutant strains which is common when complexes are unstable *in vivo* (Peterson et al., 1994; Saleh et al., 1997).

There are several scenarios in which the SANT domain could mediate a functional interaction with chromatin substrates. One possibility is that the SANT

domain may interact indirectly with chromatin substrates. Recent studies indicate that SWI/SNF and SAGA (GCN5) activities may be targeted to chromatin through interaction with DNA binding transcriptional activator or repressor proteins (Ikeda et al., 1999; Natarajan et al., 1999; Neely et al., 1999; Dimova et al., 1999; Steger et al., 1998; Utley et al., 1998; Yudkovsky et al., 1999). Thus, one possibility is that the SANT domain may interact with transcriptional activator proteins. Although this model needs to be tested directly, it is unlikely as GCN5 HATs that contain a deletion in the Ada2p SANT domain are unable to acetylate histones whereas wild-type complexes are fully functional *in vitro* where activators are not required (Figure III-8). In another scenario, the SANT domain would functionally tether the catalytic subunit to other components of the remodeling complex. This interaction may serve to communicate structural changes from the catalytic subunit to other components of the complex upon substrate binding in order to regulate catalytic activity. Several reports indicate that the Myb-DBD can undergo a conformational change upon DNA binding (McIntosh et al., 1998; Zargarian et al., 1999). Thus, it is intriguing to speculate that the SANT domain may perform an analogous function, acting as a conformational sensor. This may explain the requirement for the SANT domain in optimal binding to brahma seen by Crosby et al. (1999) and may explain why *in vitro* complexes appear to be less stable (Figure III-10).

We favor a simple model in which the SANT domain mediates a direct interaction between remodeling complexes and chromatin. We find that a mutation in the Ada2p SANT domain impairs interaction with histone H3 N-terminal domains implicating the SANT domain in mediating a physical interaction between GCN5 HATs and histone

'tails' (Figure III-9). In addition, previous work has shown that histone 'tails' play an important role in regulating dissociation of SWI/SNF from nucleosomal arrays thereby regulating the remodeling cycle (Logie et al., 1999; Peterson, 1998). In fact, these studies also suggest that SWI/SNF might bind these domains directly as histone 'tails' compete for SWI/SNF activity in remodeling reactions *in vitro* (Logie et al., 1999). It is also interesting to note that ISWI complexes as well as recombinant ISWI (a member of the Swi2/Snf2p family of ATPases), which contain a SANT domain, absolutely require histone 'tails' for ATPase and remodeling activity (Corona et al., 1999; Georgel et al., 1997; see also Chapter V of this thesis).

Multiple histone binding domains? Modulation of the accessibility of chromatin structure and transcriptional activation through alteration of histone N-terminal domains has been well documented (reviewed in Fletcher and Hansen, 1996; Wolffe and Hayes, 1999). Thus, it is not surprising that histone 'tails' would be the target of chromatin remodeling enzymes. Several motifs associated with subunits of chromatin remodeling complexes have been implicated as histone binding domains. The bromodomain, which is conserved in many subunits of chromatin remodeling complexes, has been shown to be a histone 'tail' binding domain (Dhalluin et al., 1999; Ornaghi et al., 1999). However, in many cases the bromodomain is not required for full activity of chromatin remodelers *in vivo* suggesting that this domain may be redundant in function (Elfring et al., 1998; Laurent et al., 1993b; Marcus et al., 1994). In addition, a portion of the catalytic domain of Gcn5p may play a general role in recognition of histone 'tails'

(Trievel et al., 1999). Surprisingly, our data implicates a role for the SANT domain as a histone-binding motif as well. Unlike the case for bromodomains, an intact SANT domain is absolutely required for transcriptional regulation *in vivo* by SWI/SNF, RSC, and GCN5 HATs, suggesting an essential role for this domain in chromatin remodeling. Interestingly, SWI/SNF and GCN5 appear to play overlapping and redundant functions at some promoters to regulate chromatin structure (Biggar and Crabtree, 1999; Pollard and Peterson, 1997; Roberts and Winston, 1997; Sudarsanam et al., 1999). It is possible that these histone 'tail' binding domains function cooperatively and/or act coordinately to alter chromatin structure. Thus, we propose that the SANT domain may functionally link the activities of ATP-dependent chromatin remodeling enzymes and histone acetyltransferases to chromatin through interaction with histone proteins.

Experimental Procedures

PCR mutagenesis and plasmid construction. Oligonucleotides and plasmids used in this study are described in Table III-2 and III-3, respectively. The pRS series of plasmids used in this study has been described previously (Sikorski and Hieter, 1989). Mutations in the SANT domain of SWI3, RSC8, and ADA2 were generated in a two step PCR reaction by standard methods with Vent Polymerase (New England BioLabs). First, DNA sequences from SWI3 (CP596), RSC8 (CP682), and ADA2 (CP677) were amplified using a sequence specific oligo that also contained an unrelated TAG sequence and the appropriate mutagenic oligo (see Table III-2) to create a TAG-Megaprimer which harbors the mutation of interest. The mutagenized SANT domain DNA fragment was

generated in a second, subsequent PCR reaction using the appropriate plasmid as above, an oligo specific to the TAG sequence, the appropriate mutagenized TAG-Megaprimer, and a sequence specific oligo. The resulting PCR product was then digested with internal restriction enzymes and subcloned into the appropriate plasmid so as to replace the wild-type SANT domain with a specifically mutagenized version. All plasmids were sequenced to ensure polymerase fidelity during PCR amplification.

The pRS416-RSC8/SWH3 (CP883) construct was generated by excising the NotI fragment containing a triple hemaglutinin tag (3HA) from CP872 and religating the plasmid.

Yeast strains and media. Standard genetic methods were followed to generate the appropriate strains. All strains used in this study are listed in Table III-3. Yeast cultures were grown at 30°C in yeast extract-peptone (YEP) media containing 2% glucose or selective synthetic complete (SC) media (Difco Laboratories) containing 2% glucose as specified (Rose et al., 1990) except where otherwise noted.

Yeast transformations were performed by the lithium acetate procedure as previously described (Geitz and Scheistl, 1991). Standard procedures were used for sporulation and tetrad analysis.

Preparation of whole-cell extracts. Strains were grown in 100 ml of media at 30°C with agitation to an OD_{600} of 0.8-1.0. Cells were washed and then resuspended in 0.8 ml of E Buffer (20 mM Hepes, 350mM NaCl, 10% glycerol, 0.1% Tween-20) with protease inhibitors. Cells were disrupted with glass beads 5x 1 min. with intervening periods on ice, using a mini bead beater (Biospec Products). Extracts were clarified with

a 10 min spin at 14K in a microfuge. Extracts were recovered and filtered through a 0.45μ membrane (Millipore) after a 45 min. spin at 48K at 4°C in a Beckman TLA100 ultracentrifuge.

β-Galactosidase assays. β-Galactosidase activity was assayed quantitatively in permeabilized cells as previously described (Stern et al., 1984). Transformants were grown to mid-log phase in the appropriate medium for plasmid selection. Activity was measured in Miller units followed by normalization to per cent wild-type activity.

In vitro immunoprecipitation analysis. Either swi3Δ1-300 or swi3Δ1-300ΔSANT sequences were cloned into the HA in vitro translation vector (see Table II-3). SWI3 and SWI2 (1-767 amino acids) sequences were cloned into pET11A in vitro transcription vector (Novagen; see Table III-2). ^{35}S-methionine (NEN)-labeled Swi2p or Swi3p was obtained in each case using the TNT Coupled Wheat Germ Extract System (Promega) according to the manufacturer's specifications. Unlabeled HA-Swi3Δ306p or HA-Swi3Δ306ΔSANTp was generated by addition of unlabeled methionine to the above reaction in place of ^{35}S-methionine. Autoradiography or immunoblotting confirmed the quality of all in vitro translation reactions.

Binding reactions were performed by incubating approximately equal concentrations of the pre-cleared ^{35}S-met-labeled protein (as visualized by autoradiography) with similar concentrations of unlabeled HA-protein (as visualized by immunoblotting) in Buffer A (20 mM Tris- pH 8.0, 250 mM NaCl, 1 mM EDTA, 0.1% Triton X-100, 1 mM DTT, 0.1 mg/ml BSA) with rocking for 30 minutes at 4°C. Immunoprecipitations were carried out by addition of 10-μl anti-HA Sepharose bead

slurry (Covance) equilibrated in Buffer A to the binding reactions for 1 hr. at 4°C. Supernatants were recovered for subsequent analysis. Beads were washed five times with Buffer A and bound proteins were eluted with 15-µl of SDS-sample buffer. Binding was analyzed by SDS-PAGE and autoradiography.

Purification of HAT complexes. Whole cell yeast extracts were prepared from 20 liters of either CY561 (wild-type) or CY884 (ada2Δ97-106; see Table III-4) as described (Cote et al., 1994). Subsequent fractionation steps were carried out as described (Pollard and Peterson, 1997) with the exception that a 25-ml linear gradient of 100-500 mM NaCl was used to elute bound proteins from the MonoQ column (Pharmacia). Fractions were analyzed by immunoblotting and liquid acetylation assays.

HAT assays. In vitro liquid HAT assays were performed in a 30-µl reaction volume using 1µg of oligonucleosome cores, 1µl of each 0.5 ml fraction from the Mono Q elution (or appropriate dilutions for other studies), and ^{3}H-labeled acetyl-CoA (1µCi) (NEN) in HAT buffer (50 mM Tris-HCl pH 8.0, 50 mM KCl, 5% glycerol, 0.1 mM EDTA, 1 mM DTT, 1 mM PMSF, 10 mM sodium butyrate). Reactions proceeded at 30°C for 30 minutes (Grant et al., 1997; Pollard and Peterson, 1997). Reactions were subjected to 18% SDS-PAGE, stained with Coomassie brilliant blue to visualize the histones, destained and fluorographed with EN^{3}HANCE (NEN).

GST-Histone H3 tail binding assay. GST or GST-H3 (1-46 amino acids) was prepared from plasmids in E.coli (Hecht et al., 1995) and bound to Glutathione Sepharose 4B resin (Pharmacia) as described (Grant et al., 1997) equilibrated in Buffer B (10mM Tris, 8.0, 10% glycerol, 50 mM NaCl, 0.1% Tween, 0.5 mM DTT). Each HAT fraction

analyzed was normalized for Gcn5p levels (as visualized by immunoblotting) and bound in Buffer B to GST or GST-H3 Glutathione Sepharose beads at 4°C with rocking for 2 hrs. The supernatant was recovered from each binding reaction and proteins were precipitated with 15% TCA. The pellet was resuspended in 20-µl of SDS loading buffer (pH 11). Beads were washed three times with Buffer B and resuspended in 20-µl of SDS loading buffer. Binding was analyzed by SDS-PAGE followed by immunoblotting.

Gel filtration analysis. Either crude whole cell extracts (for analysis of SWI/SNF and RSC) or 100 µl of each partially purified GCN5-dependent HAT was fractionated on a fast protein liquid chromatography (FPLC) Superose 6 gel filtration column (Pharmacia). Fractions were precipitated with 15% trichloroacetic acid and analyzed by SDS-PAGE and immunoblotting.

Antibodies and immunoblotting. Typically 10-µl of each fraction was subjected to SDS-PAGE and transferred to nitrocellulose membrane (Protran). Membranes were probed with antisera raised against either Swi3p (Peterson et al., 1994), HA (Covance; for Swi2-HAp and HA-Rsc8p), Sth1p (Brehon Laurent, SUNY), Gcn5p (Santa Cruz Biotech), Ada3p (Santa Cruz Biotech), Ada2p (Shelley Berger, Wistar Institute, Pennsylvania), Tra1p (Jerry Workman, Penn State University), TAFII68p (Michael Green, UMass Medical School), Spt7p (Fred Winston, Harvard University), or Spt3p (Fred Winston, Harvard University); followed by incubation with the appropriate secondary antibody conjugated with horseradish peroxidase (rabbit-Amersham; goat-Santa Cruz). Immunoblots were visualized by addition of chemiluminescent substrate (Amersham).

Table III-2. Oligonucleotides

Name	Description	Sequence
LB1	(5')TAG@bp1249-SWI3	5'-GAGGATCCCCGGGTACGATGCCCCCAGAGGAC-3'
LB2	(3')ClaI-SWI3	5'-GGCCATCGATGCCAAGTCAAGTGACGCACC-3'
LB10	SWI3Δ555-565(ΔSANT)	5'-CTTCAATGGGCAATTGAAGGCCAACGTTTTTGGCCAC-3'
LB11	SWI3 W546A	5'-GGCCACTTTGTACGCGTCAGCGCCCGAATTC-3'
LB12	SWI3 E559A	5'-GGGCAATTGAAGGAAACGTAAGATACACTGCGCAGGCG-3'
LB13	SWI3 E559R	5'-GGGCAATTGAAGGAAACGTAAGATACACTGGCGAGGCG-3'
LB14	SWI3 Q560A	5'-GGGCAATTGAAGGAAACGTAAGATACACTGCGCAGGCG-3'
LB15	SWI3 Q560R, Q567R	5'-GGGCAAGCGAAGGAAACGTAAGATACAGCGTTCAGGCG-3'
LB16	SWI3 C561A	5'-GGGCAATTGAAGGAAACGTAAGATCGCCTGTTCAGGCG-3'
LB17	SWI3 R564A	5'-GGGCAATTGAAGGAACGCTAAGATACACTGTTCAGGCG-3'
LB18	SWI3 R564E	5'-GGGCAATTGAAGGAATTCTAAGATACACTGTTCAGGCG-3'
LB19	SWI3 E559A, R564A	5'-GGGCAATTGAAGGAACGCTAAGATACACTGCGCAGGCG-3'
LB20	SWI3 C561A, R564A, F565A	5'-GGGCAATTGAAGCGCCGCTAAGATCGCCTGTTCAGGCG-3'
LB21	SWI3 F565A	5'-GGGCAATTGAAGCGCACGTAACATACACTGTTCAGGCG-3'
LB22	(5')NsiI-RSC8	5'-GGCCGCACAAGACTTCAATGCATTACAAGACG-3'
LB23	(3')TAG-SalI-RSC8	5'-GAGGATCCCCGGGTACGTCGACGCAAACGACAACCAC AACCCC-3'
LB24	RSC8Δ348-352aa	5'-GGGCACAAGCGTGTAAAATTCCTAAGCTTACCG-3'
LB25	RSC8 K353A	5'-GCATTGAAGCGTTCCTAAGCTTACCG-3'
LB26	(5')TAG-XhoI-ADA2	5'-GAGGATCCCCGGGTACCTCGAGGGGTGTAATTTTAACCG-3'
LB27	(3')BglII-ADA2	5'-GGAGATCTCAGCAAACTTTAAATGCGGTACTG-3'
LB28	ADA2Δ98-102aa	5'-CCAGATAATATTTTAGGTAATGTTTGCCTCTGCTGCC-3'
LB29	ADA2Δ97-106aa	5'-GCTTTCCAGATAATAGCCTCTGCTGCC-3'
LB30	ADA2 H103E	5'-GGTACTCTTCCTTAACTTCTTCTTTGCC-3'
HHF2	TAG primer	5'-CTCCTAGGGGCCCATG-3'

Underscored sequence represents TAG sequence

Table III-3. Plasmids

Name	Relevant Description	Source
CP555	pRS406-SWI3Δ555-565(ΔSANT)	This study
CP596	pRS406-SWI3	This study
CP597	pRS406-swi3 W546A	This study
CP617	pRS406-swi3 C561A, R564A, F656A	This study
CP619	pRS405-SWI2-3HA-6His	This study
CP626	pRS406-swi3 C561A	This study
CP628	pRS406-swi3 F565A	This study
CP631	pRS406-swi3 R564A	This study
CP651	pRS406-swi3 E559R	This study
CP652	pRS406-swi3 Q560R, Q567R	This study
CP653	pRS406-swi3 W546A, F565A	This study
CP654	pRS406-swi3 E559A	This study
CP655	pRS406-swi3 E559A, R564A	This study
CP656	pRS406-swi3 R564E	This study
CP658	pRS406-swi3 Q560A	This study
CP671	pRS406-ada2 H103E	This study
CP672	pRS406-ada2Δ98-102aa	This study
CP677	pRS406-ADA2	This study
CP682	pRS315-3HA-SWH3(RSC8)	Treich and Carlson, 1997
CP712	peT11A-SWI2(1-767aa)	Joan Flanagan
CP746	HA**-SWI3	Joan Flanagan
CP748	HA**-swi3Δ1-306aa	Joan Flanagan
CP805	HA**-swi3Δ1-306aaΔSANT	This study
CP816	pRS315-HA-rsc8/swh3Δ348-352(ΔSANT)	This study
CP817	pRS315-HA-rsc8/swh3 K353A	This study
CP872	pRS416-HA-RSC8/SWH3	This study
CP873	pRS406-ada2Δ97-106(ΔSANT)	This study
CP883	pRS416-SWH3 (without 3HA-tag)	This study

Note: All SWI3 and ADA2 constructs were also cloned into episomal low copy (ARS-CEN) and high copy (2μ) vectors

Table III-4. Strains

Name	Relevant Genotype	Source
CY165	swi3Δ::trp1Δ1, leu2-Δ1, ura3-52, his3Δ200, ade2-101, lys2-801, ho::LACZ	Peterson and Herskowitz, 1992
CY432	**a** 1107- ura3-52, ade2-1, met-, his3-, leu2-3,112, trp1-1 ho::LACZ	Breden and Nasmyth, 1987
CY561	ada2Δ::hisG-URA3-hisG, isogenic to CY432	Pollard and Peterson, 1997
CY664	SWI2::CP619 isogenic to CY165	This study
CY666	URA3::CP596, isogenic to CY664	This study
CY667	URA3::pRS406, isogenic to CY664	This study
CY669	URA3::CP555, isogenic to CY664	This study
CY671	URA3::CP597, isogenic to CY664	This study
CY673	URA3::CP617, isogenic to CY664	This study
CY674	URA3::CP626, isogenic to CY664	This study
CY675	URA3::CP631, isogenic to CY664	This study
CY676	URA3::CP628, isogenic to CY664	This study
CY691	URA3::CP654, isogenic to CY664	This study
CY692	URA3::CP655, isogenic to CY664	This study
CY693	URA3::CP651, isogenic to CY664	This study
CY694	URA3::CP656, isogenic to CY664	This study
CY695	URA3::CP658, isogenic to CY664	This study
CY696	URA3::CP652, isogenic to CY664	This study
CY697	URA3::CP653, isogenic to CY664	This study
CY716	Ada2Δ without URA3, isogenic to CY561	This study
CY718	URA3::CP671, isogenic to CY716	This study
CY719	URA3::CP672, isogenic to CY716	This study
CY720	URA3::pRS406, isogenic to CY716	This study
CY733	URA3::CP677, isogenic to CY716	This study
CY884	URA3::CP873, isogenic to CY716	This study
CY885	rsc8/swh3::HIS3, CP682, leu2-3,112, ura3-52 "segregant" from MCY3840	This study
CY888	rsc8/swh3::HIS3, CP883 , leu2-3,112, ura3-52 "segregant" from MCY3840	This study
MCY3840	a/α swh3Δ1::HIS3/+, +/leu2-3,112, his3Δ200/his3Δ200, ura3-52/ura3-52, lys2-301/+	Treich and Carlson, 1997

Chapter IV

Roles Of The Histone H2A/H2B Dimers And $(H3/H4)_2$ Tetramer In Nucleosome Remodeling By The SWI/SNF Complex

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Chapter IV

Roles Of The Histone H2A/H2B Dimers And $(H3/H4)_2$Tetramer In Nucleosome Remodeling By The SWI/SNF Complex

Introduction

The assembly of eukaryotic DNA into folded nucleosomal arrays has drastic consequences for many nuclear processes that require access to the DNA sequence, including RNA transcription, DNA replication, recombination, and repair. The nucleosome, which consists of 147 bp of DNA wrapped nearly twice around an octamer of histones H2A, H2B, H3 and H4, can occlude DNA sequences both in vivo and in vitro. The nucleosome is not a static structure, but appears to be a dynamic and flexible assembly. For instance, moderate concentrations of NaCl can lead to several distinct changes in nucleosome conformation (Czarnota and Ottensmeyer, 1996; Dieterich et al., 1978; Dieterich et al., 1979; Yager et al., 1989; Yager and van Holde, 1984). In addition, nucleosomes isolated from transcriptionally active chromatin appear to be depleted of histone H2A/H2B dimers (discussed in Hansen and Ausio, 1992) and contain histone octamers whose interiors are more accessible to enzymatic and chemical modifications (Allegra et al., 1987; Chen et al., 1991; Prior et al., 1983). Nucleosomes from transcriptionally active chromatin can also be visualized microscopically as extended, largely unfolded structures (Bazett-Jones et al., 1996; Czarnota and Ottensmeyer, 1996). These and other studies have led to the view that regulatory factors might antagonize the

repressive effects of chromatin by disrupting the structure or conformation of the histone octamer (discussed in Workman and Kingston, 1998).

Two types of highly conserved chromatin remodeling enzymes have been implicated as regulators of the repressive nature of chromatin structure (Kingston and Narlikar, 1999; Pollard and Peterson, 1998). Several members of the SWI2/SNF2 family of DNA-stimulated ATPases use the energy of ATP-hydrolysis to disrupt nucleosome structure which can lead to an enhanced mobility of nucleosomes (Hamiche et al., 1999; Jaskelioff et al., 2000; Langst et al., 1999; Whitehouse et al., 1999). The second type consists of the nuclear histone acetyltransferases that covalently modify lysine residues within the flexible N-terminal domains of the histone proteins. The *Saccharomyces cerevisiae* SWI/SNF complex is the prototype ATP-dependent chromatin remodeling complex. This widely conserved 2 MDa multisubunit assembly is required for the inducible expression of a number of diversely regulated yeast genes and for the full functioning of many transcriptional activators (reviewed in Burns and Peterson, 1997a; Winston and Carlson, 1992). SWI/SNF can be recruited to target genes via direct interactions with gene-specific activators (Natarajan et al., 1999; Neely et al., 1999; Yudkovsky et al., 1999), and in several cases SWI/SNF facilitates the binding of activators to nucleosomal sites *in vivo* (Burns and Peterson, 1997b; Cosma et al., 1999). In vitro, the purified SWI/SNF complex is a DNA-stimulated ATPase that can use the energy of ATP hydrolysis to disrupt histone-DNA interactions. Although the mechanism by which SWI/SNF disrupts nucleosome structure is not known, this "remodeling" reaction leads to an enhanced accessibility of nucleosomal DNA to Dnase I (Cote et al.,

1998; Cote et al., 1994; Owen-Hughes et al., 1996), restriction enzymes (Logie and Peterson, 1997; Logie et al., 1999), and sequence-specific DNA binding proteins (Cote et al., 1998; Cote et al., 1994; Utley et al., 1997). More recently, SWI/SNF has been shown to increase DNA accessibility of nucleosomal arrays in a catalytic manner that is dependent on the presence of histone N-terminal domains (Logie and Peterson, 1997; Logie et al., 1999).

Four different models have been proposed to explain the mechanism by which ATP-dependent remodeling by the SWI/SNF complex increases nucleosomal DNA accessibility. (1) Several studies have suggested that SWI/SNF might remove or rearrange the H2A/H2B dimers (Cote et al., 1994; Hirschhorn et al., 1992; Lee et al., 1999; Luger et al., 1997; Peterson and Tamkun, 1995; Spangenberg et al., 1998). (2) SWI/SNF remodeling may induce a novel conformation of the histone octamer (Lorch et al., 1998; Schnitzler et al., 1998; discussed in Workman and Kingston, 1998) which might involve conformational changes in the $(H3/H4)_2$ tetramer analogous to the transcription-associated transitions described above. (3) SWI/SNF may use the energy of ATP hydrolysis to translocate along DNA and destabilize histone-DNA interactions (discussed in Pazin et al., 1997). This model is similar to the octamer spooling mechanism described by Studitsky et al. for passage of polymerases through nucleosomes (Studitsky et al., 1994). (4) And finally, SWI/SNF might bind directly to nucleosomes and use the energy of ATP hydrolysis to change the path of nucleosomal DNA (Cote et al., 1998) or to peel DNA off the surface of the histone octamer without changing octamer structure (Bazett-Jones et al., 1999).

In this study, we have directly tested whether ATP-dependent chromatin remodeling by the SWI/SNF complex alters the composition or conformation of the histone octamer. We use a nucleosomal array remodeling assay (Logie and Peterson, 1997; Logie and Peterson, 1999) to quantify SWI/SNF activity on arrays of histone $(H3/H4)_2$ tetramers and on nucleosomal arrays reconstituted with histone octamers containing internally-crosslinked tetramers. In order to monitor more subtle or transient changes in octamer structure, we also measured the effects of SWI/SNF remodeling on the steady state fluorescence of nucleosomal and tetramer arrays harboring a histone H3 derivative site specifically modified at residue 110 with the fluorescent probe acetylethylenediamine-(1,5)-naphthol sulfonate (AEDANS) (Daban and Cantor, 1989; Dieterich et al., 1978; Dieterich et al., 1979). Taken together, our data are consistent with a model in which substrate recognition by SWI/SNF requires an intact histone octamer, and subsequent ATP-dependent "remodeling" disrupts histone-DNA contacts without a concomitant loss of histone proteins or perturbation of the histone octamer.

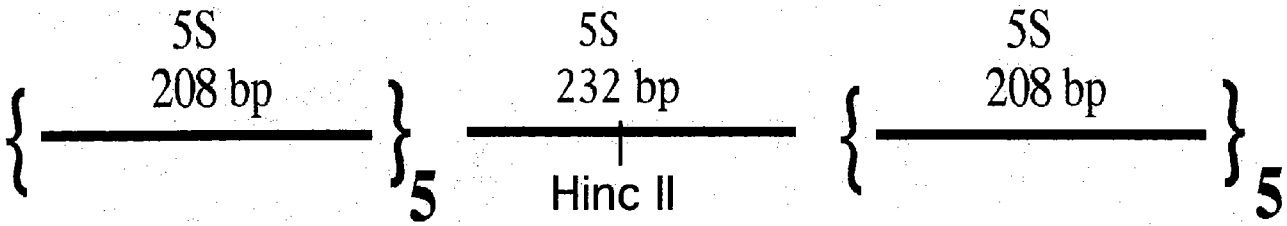
Results

SWI/SNF remodels arrays of histone $(H3/H4)_2$ tetramers. It has been proposed that SWI/SNF increases the accessibility of nucleosomal DNA by depletion or rearrangement of the histone H2A/H2B dimers (Cote et al., 1994; Lee et al., 1999; Luger et al., 1997; Spangenberg et al., 1998; discussed in Peterson and Tamkun, 1995). In order to investigate whether loss of the dimers is equivalent to the SWI/SNF remodeled state, we used purified chicken erythrocyte histone octamers or $(H3/H4)_2$ tetramers

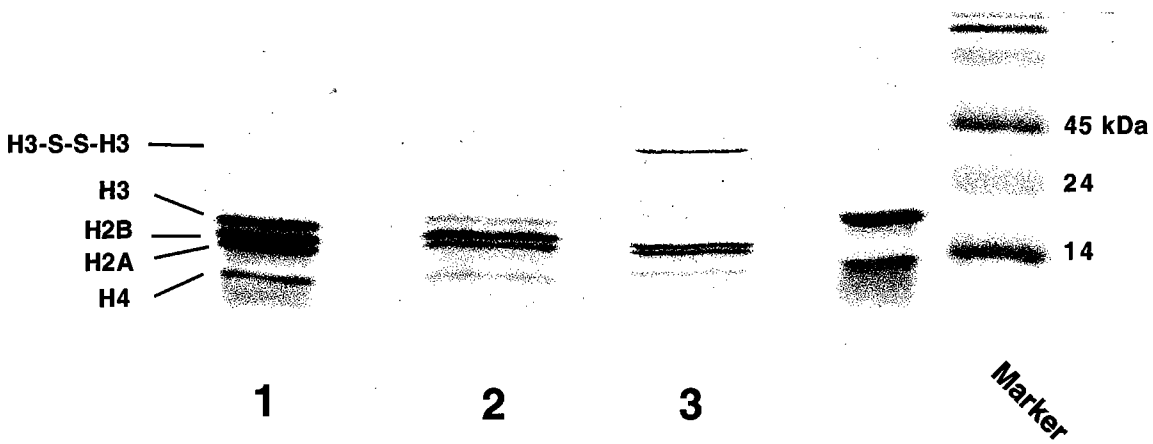
(Figure IV-1B, lanes 1 and 4) to reconstitute nucleosomal or tetramer arrays. The DNA template used for these reconstitutions is composed of 11 head-to-tail repeats of a nucleosome positioning sequence from the *L. variegatus* 5S rRNA gene, each of which is flanked by EcoRI restriction sites (Figure IV-1A). Nucleosomes that are reconstituted onto each of these 5S repeats assume a major translational position that is present in at least 50% of the population (Dong et al., 1990; Pennings et al., 1991). Minor translational positions also exist and differ from the major frame by multiples of 10 bp. The central repeat of our array template also bears a unique SalI/HincII restriction site close to the dyad axis of symmetry of a nucleosome positioned at the major frame (Logie and Peterson, 1997; Polach and Widom, 1995). Array reconstitutions were analyzed for extent of DNA repeat saturation and for correct positioning by restriction enzyme cleavage (Figure IV-1C; see also Experimental Procedures) (Hansen et al., 1989; Logie and Peterson, 1997; Logie and Peterson, 1999). EcoRI digestion of nucleosomal or tetramer arrays releases primarily mononucleosome-sized particles and few of the high molecular weight partial digestion products that would be indicative of alternative positioning or oversaturation (Figure IV-1C). Digestion of nucleosomal or tetramer arrays with MspI (whose site is located ~30 bp from the predicted dyad axis of a nucleosome positioned at the major frame) demonstrates that these sites are fully protected by nucleosomal arrays, but accessible in tetramer arrays (Figure IV-1C). These data are consistent with the fact that an $(H3/H4)_2$ tetramer assumes the same translational positions as an intact octamer (Dong et al., 1990; Hayes et al., 1991), but that the tetramer assembles less DNA (Luger et al., 1997).

Figure IV-1. Analysis of Array Reconstitutions. **A.** Schematic representation of the 208-11S template DNA used to reconstitute nucleosomal and tetramer arrays. It consists of 11 head-to-tail repeats of a 5S rRNA nucleosome positioning sequence, the central repeat bearing an unique SalI/HincII restriction enzyme site. Each repeat is flanked by EcoRI restriction enzyme sites. In addition, an MspI site is located ~30 bp from the predicted dyad axis of symmetry of each positioned nucleosome. **B**. Analysis of histone proteins used for array reconstitutions by 18% SDS-PAGE and Coomassie staining. Lane 1, chicken histone octamers; Lane 2, chicken histone octamers contained 1,5-IAEDANS conjugated to histone H3; Lane 3, oxidized chicken histone octamers electorphoresed under non-reducing conditions; Lane 4, chicken $(H3/H4)_2$ tetramers. **C.** Analysis of saturation and positioning of reconstituted arrays by restriction enzyme digestion and native polyacrylamide gel electrophoresis. Digestion of arrays with EcoRI releases primarily mononucleosome- or monotetramer-sized particles (★) as well as some free 208 bp 5S repeats (•). The ratio of free 5S repeats to monnucleosome/mono-tetramer particles provides a qualitative measurement of array saturation (60-90% saturation for the arrays shown here). Higher molecular weight species represent partial EcoRI digestion products. Nucleosome assembly on the 5S repeats results in occlusion of the MspI sites, whereas these sites are accessible for the tetramer arrays and yield a mono-tetramer size particle (★), indicating that the $(H3/H4)_2$ tetramers protect less DNA as expected.

A


5S
208 bp
5
5S
232 bp
Hinc II
5S
208 bp
5

B


H3-S-S-H3
H3
H2B
H2A
H4
45 kDa
24
14
1
2
3
Marker

C

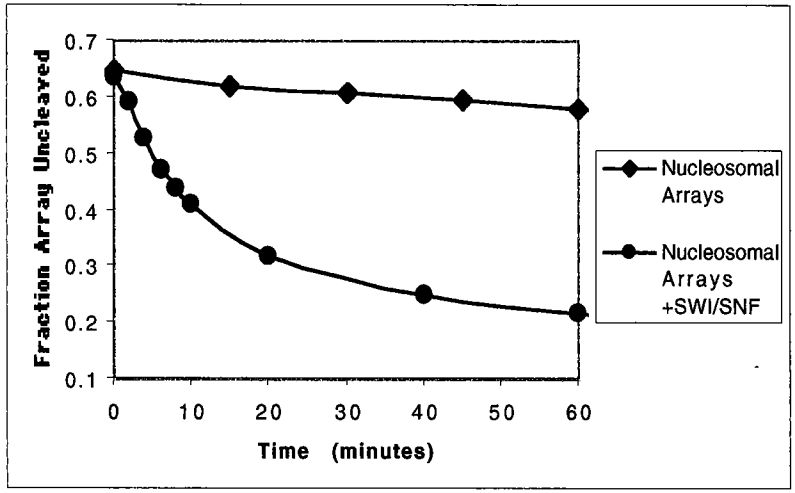
EcoRI
MspI
Tetramer Arrays
Disulfide-linked Nuc. Arrays
Nucleosomal Arrays
Naked Template
100bp Ladder

We then exploited a sensitive nucleosomal array remodeling assay in which SWI/SNF remodeling activity is coupled to restriction enzyme activity (Logie and Peterson, 1997; Logie et al., 1999). Previously we used this assay to determine the kinetic parameters of ATP-dependent nucleosomal array remodeling by the SWI/SNF and RSC complexes (Logie and Peterson, 1997; Logie and Peterson, 1999; Logie et al., 1999). To quantify the remodeling capacity of SWI/SNF complex, 3 nM of nucleosomal or tetramer array was exposed to 500 U/ml HincII, either in the presence or absence of 3 nM SWI/SNF complex and 1mM ATP (Figure IV-2A). As described previously, HincII digestion of nucleosomal arrays is biphasic (Logie and Peterson, 1997); the first, rapid phase of the reaction represents digestion of arrays harboring HincII sites positioned between nucleosomes, and the second, slow phase represents digestion of the nucleosomal HincII sites. By limiting our analysis to the second phase of HincII digestion, the first order rate of HincII cleavage yields a quantitative measurement of nucleosomal DNA accessibility (Logie and Peterson, 1997; Logie et al., 1999; Polach and Widom, 1995).

In the absence of SWI/SNF, the first order rate of HincII cleavage was 1.3×10^{-5} for tetramer arrays and 2.4×10^{-6} for nucleosomal arrays (Figure IV-2; see also Table IV-1). The 5-fold higher rate for the tetramer arrays is comparable to the rate observed previously for nucleosomal arrays reconstituted with histones that lack their trypsin-sensitive N-terminal domains (Logie et al., 1999) and is consistent with nucleosomal DNA being more accessible in the absence of the histone H2A/H2B dimers (Dong and van Holde, 1991; Hayes and Wolffe, 1992; Spangenberg et al., 1998; Tse et al., 1998c).

Figure IV-2. Tetramer arrays are not optimal substrates for SWI/SNF. A. Representative time course for HincII digestion of 3 nM nucleosomal (left panel) or $(H3/H4)_2$ tetramer (right panel) arrays in the presence or absence of 3 nM SWI/SNF. The "0" time point reflects a 20 minute preincubation with HincII in the absence of SWI/SNF. The high percentage of tetramer array cleaved in the first phase of the digestion is due in part to slight undersaturation of these arrays. We note that similar levels of undersaturation of nucleosomal arrays has no effect on SWI/SNF remodeling rates (data not shown; see also 41). **B.** Quantification of the data shown in Panel A. Data is presented as the concentration of nucleosomal and tetramer array cleaved by HincII in the presence of SWI/SNF during the initial 10 minutes of the reaction. Similar results were obtained in at least three different experiments using both different SWI/SNF preparations and independent nucleosomal and tetramer array reconstitutions.

A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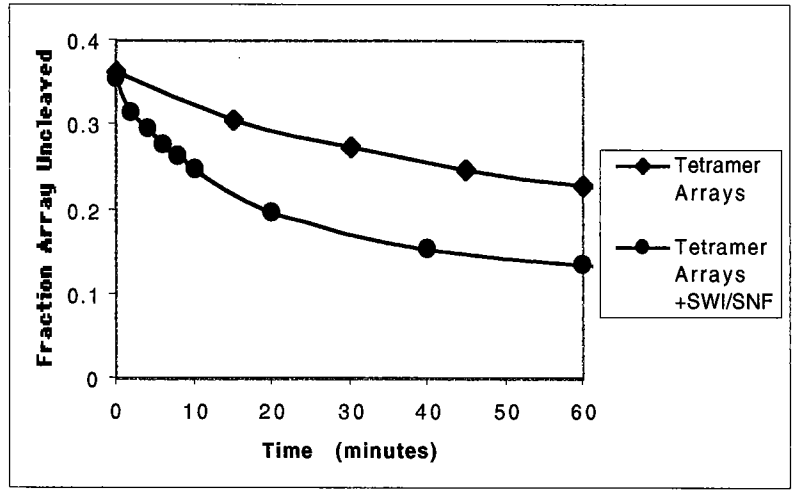
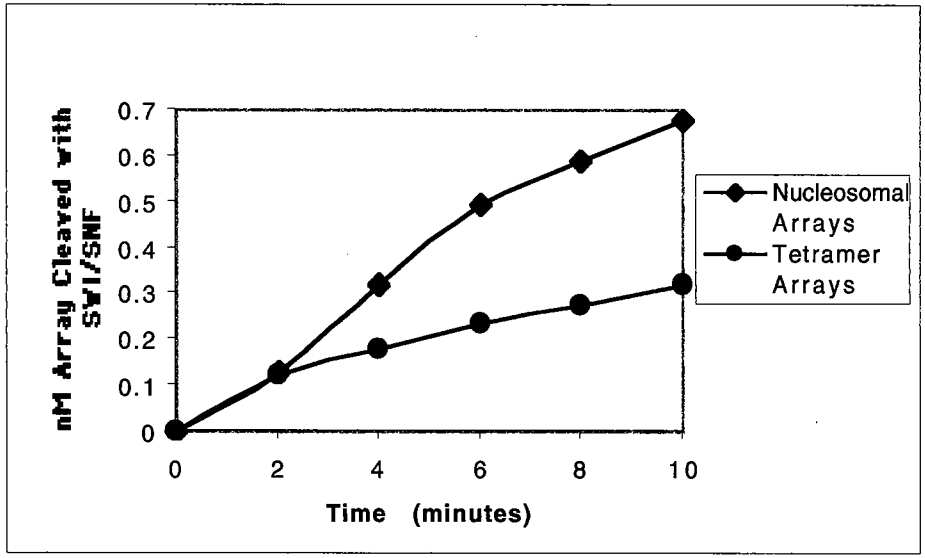
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
Time (minutes)
Nucleosomal Arrays
Nucleosomal Arrays +SWI/SNF


Fraction Array Uncleaved
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
Time (minutes)
Tetramer Arrays
Tetramer Arrays +SWI/SNF

B


nM Array Cleaved with SWI/SNF
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
Time (minutes)
Nucleosomal Arrays
Tetramer Arrays

In the presence of SWI/SNF the first order rate of HincII cleavage was 6.4 x 10^{-5} for tetramer arrays and 9.2 x 10^{-5} for nucleosomal arrays (Figure IV-2; Table IV-1). The fact that the rate of cleavage of nucleosomal arrays in the presence of SWI/SNF significantly exceeded the rate of cleavage of tetramer arrays in the absence of SWI/SNF (see Table IV-1) indicates that remodeling is not equivalent simply to loss of histone H2A/H2B dimers. The fact that the rate of cleavage of tetramer arrays in the presence of SWI/SNF is 5-fold higher than the rate in the absence of SWI/SNF indicates further that SWI/SNF can remodel tetramer arrays. However, SWI/SNF remodeling of tetramer arrays appears to be quantitatively less efficient than SWI/SNF remodeling of nucleosomal arrays, as the rate of cleavage of tetramer arrays in the presence of SWI/SNF was 30% slower than that of nucleosomal arrays in the presence of SWI/SNF (Figure IV-2, see also Table IV-1).

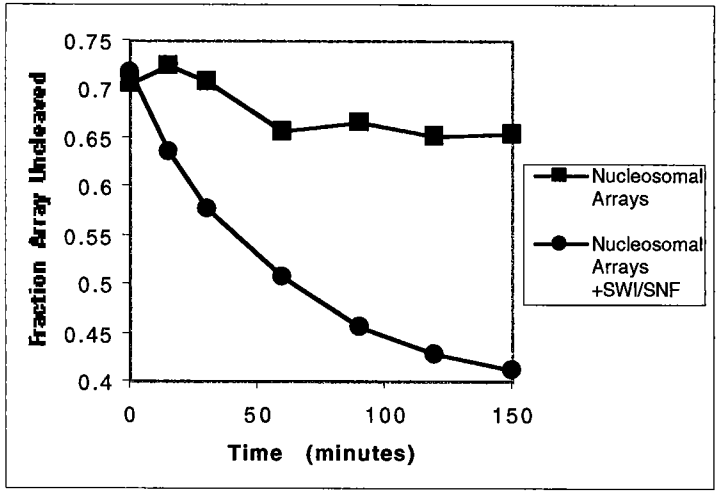
SWI/SNF activity is not catalytic on arrays of $(H3/H4)_2$ tetramers. Previously we showed that SWI/SNF is able to catalytically remodel multiple nucleosomal arrays and that histone N-terminal domains are required for this reaction (Logie and Peterson, 1997; Logie et al., 1999). As remodeling of tetramer arrays appears to be quantitatively less effective than remodeling of nucleosomal arrays (see above), we investigated whether SWI/SNF was able to function catalytically, or only stoichiometrically, on tetramer arrays. Remodeling assays were carried out in which there was a 10-fold molar excess of array to remodeling complex (12 nM array to 1.2 nM SWI/SNF). Consistent with previous data, SWI/SNF was able to stimulate HincII cleavage of nucleosomal arrays throughout the 150 minute time course (Figure IV-3A, left panel; see also Logie

and Peterson, 1997; Logie et al., 1999). However, there was only minor stimulation of HincII cleavage by SWI/SNF on tetramer arrays (Figure IV -3A; right panel). In fact, the rate of HincII cleavage on tetramer arrays in the presence of SWI/SNF approximated the rate determined in the absence of remodeling complex (Figure IV-2A, right panel; and Table IV-1). Figure IV-3B shows the quantification of the amount of SWI/SNF-dependent array cleavage during the 150 minute time course. As expected, SWI/SNF was able to perform approximately 2.5 rounds (3 nM of array cleaved by 1.2 nM SWI/SNF) of nucleosomal array remodeling in 150 minutes (~50 minutes per round; see also (Logie and Peterson, 1997). In contrast, SWI/SNF was unable to complete even one round of remodeling on tetramer arrays (0.3 nM array cleaved due to 1.2 nM SWI/SNF) during the time course. Therefore, SWI/SNF is unable to catalytically remodel multiple arrays of $(H3/H4)_2$ tetramers.

One possible explanation for the lack of catalytic remodeling of tetramer arrays may be that SWI/SNF has a higher affinity for tetramer arrays and is thus defective for product release (analogous to the explanation for the lack of catalytic remodeling of trypsinized nucleosomal arrays discussed in Logie et al., 1999). In order to investigate this possibility, SWI/SNF remodeling reactions were assembled which contained labeled nucleosomal array and a 3-fold or 12-fold molar excess of free DNA, nucleosomal, trypsinized nucleosomal, or tetramer competitor array. Under these conditions, tetramer arrays competed for SWI/SNF activity to the same extent as nucleosomal arrays or free DNA. In contrast, trypsinized nucleosomal arrays were markedly more potent in competing for SWI/SNF activity (data not shown; see also Logie et al., 1999). This

Figure IV-3. SWI/SNF activity is not catalytic on arrays of $(H3/H4)_2$ tetramers. **A.** Representative time course for HincII digestion of 12 nM nucleosomal (left panel) or $(H3/H4)_2$ tetramer (right panel) arrays in the presence or absence of 1.2 nM SWI/SNF complex. The "0" time point reflects a 30 minute preincubation with HincII in the absence of SWI/SNF. **B.** Quantification of the data shown in Panel A. Data is presented as the remodeler-dependent HincII cleavage of either nucleosomal or tetramer arrays versus time. Remodeler-dependent HincII cleavage events were obtained by subtracting the fraction of cleaved arrays in the absence of SWI/SNF (squares in part A) from the fraction of cleaved arrays in the presence of SWI/SNF (circles in part A). Data are presented as nanomolar remodeled nucleosomal arrays versus time.

A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0.75
0.7
0.65
0.6
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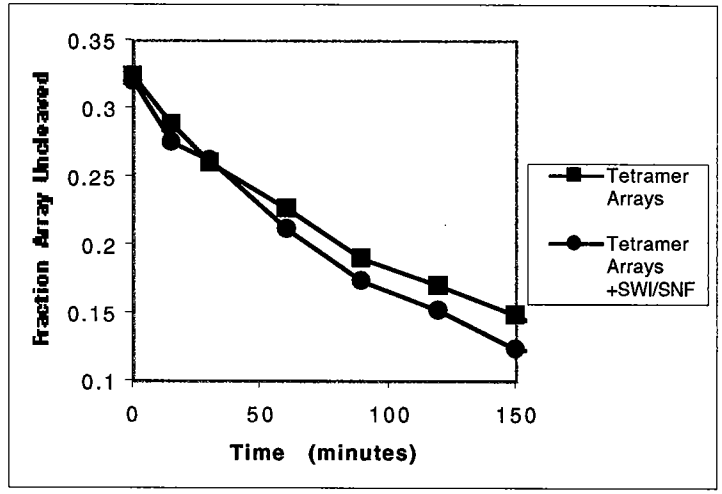
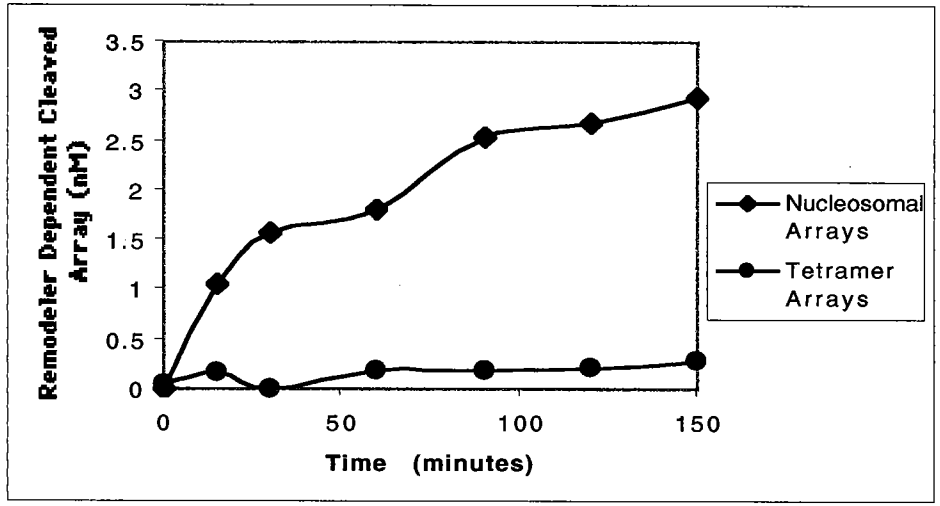
0.45
0.4
0
50
100
150
Time (minutes)
Nucleosomal Arrays
Nucleosomal Arrays +SWI/SNF


Fraction Array Uncleaved
0.35
0.3
0.25
0.2
0.15
0.1
0
50
100
150
Time (minutes)
Tetramer Arrays
Tetramer Arrays +SWI/SNF

B


Remodeler Dependent Cleaved Array (nM)
3.5
3
2.5
2
1.5
1
0.5
0
0
50
100
150
Time (minutes)
Nucleosomal Arrays
Tetramer Arrays

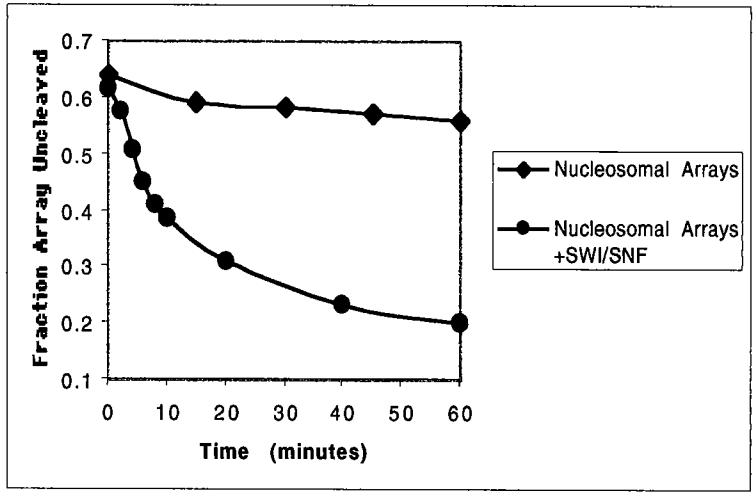
suggests that the defect in catalytic remodeling of tetramer arrays, unlike the defect in catalytic remodeling of trypsinized nucleosomes, was not due to a higher affinity of SWI/SNF for these arrays.

SWI/SNF remodeling does not require disruption of the $(H3/H4)_2$ tetramer. Chicken histone octamers contain only two cysteine residues (Cys110 of each of the two copies of histone H3), and these cysteine residues are in close apposition within the interior of the histone octamer near the dyad axis of symmetry (Camerini-Otero and Felsenfeld, 1977; Luger et al., 1997). The cysteine residues normally are inaccessible to chemical modification, although denatured histone H3 can be disulfide-linked or chemically modified in vitro with minimal perturbation to the subsequently reconstituted histone octamer (Camerini-Otero and Felsenfeld, 1977; Ausio et al., 1984b; Protacio and Widom, 1996). In contrast, these cysteine residues appear to be more exposed to solvent in nucleosomes isolated from transcriptionally active chromatin (Allegra et al., 1987; Chen et al., 1991; Prior et al., 1983) or when nucleosomes are exposed to higher salt concentrations (Dieterich et al., 1978; Dieterich et al., 1979).

To investigate whether SWI/SNF action might require a structural transition of the $(H3/H4)_2$ tetramer, we reconstituted nucleosomal arrays with histone octamers that contain disulfide-linked histone H3 (Figure IV-1B, lane 3; see Experimental procedures for details). As described above, 3 nM of nucleosomal or disulfide-linked nucleosomal array was incubated in the presence or absence of SWI/SNF and 1 mM ATP. In the absence of SWI/SNF, the first order rate of cleavage by HincII was 3.8×10^{-6} for the

Figure IV-4. SWI/SNF can remodel nucleosomal arrays reconstituted with disulfide-linked histone octamers. A. Representative time course for HincII digestion of 3 nM nucleosomal (left panel) or disulfide-linked nucleosomal (right panel) array in the presence or absence of 3 nM SWI/SNF. The "0" time point reflects a 20 minute preincubation with HincII in the absence of SWI/SNF. **B.** Quantification of data shown in Panel A. Results are presented as the concentration of nucleosomal and tetramer arrays cleaved by HincII in the presence of SWI/SNF during the initial 10 minutes of the reaction. Similar results were obtained in at least three different experiments using independent nucleosomal and disulfide-linked nucleosomal array reconstitutions and SWI/SNF preparations.

A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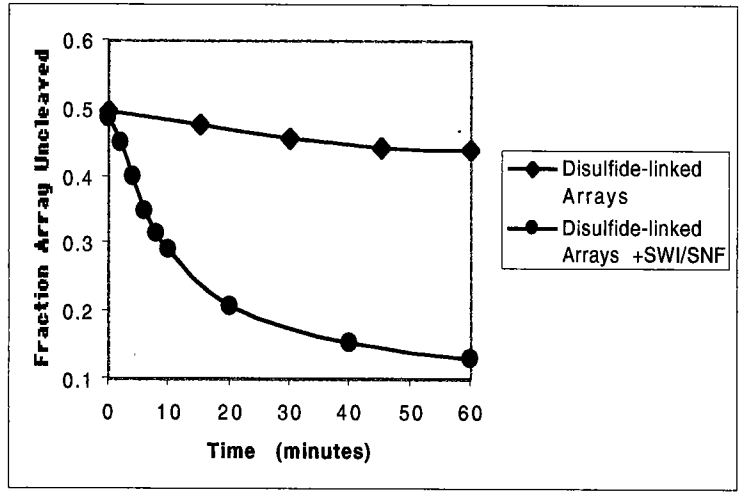
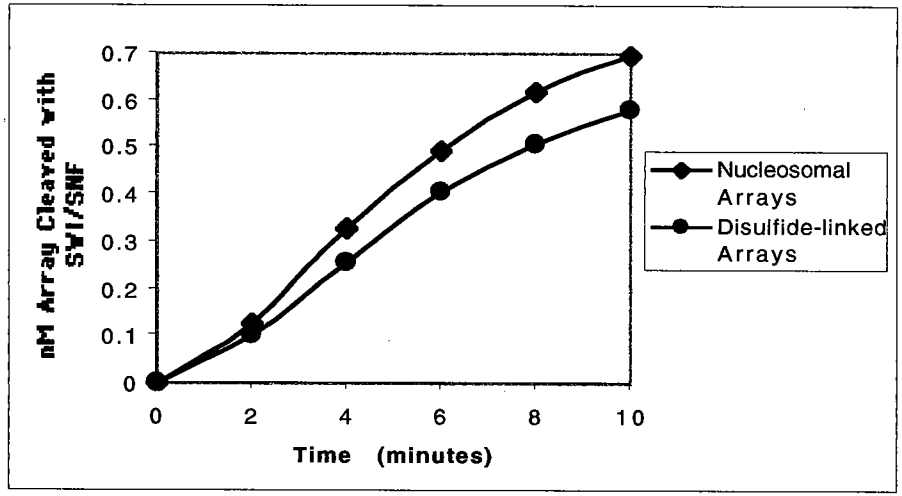
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
Time (minutes)
Nucleosomal Arrays
Nucleosomal Arrays +SWI/SNF


Fraction Array Uncleaved
0.6
0.5
0.4
0.3
0.2
0.1
0
10
20
30
40
50
60
Time (minutes)
Disulfide-linked Arrays
Disulfide-linked Arrays +SWI/SNF

B


nM Array Cleaved with SWI/SNF
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
Time (minutes)
Nucleosomal Arrays
Disulfide-linked Arrays

disulfide-linked nucleosomal array compared to 2.4 x 10^{-6} for nucleosomal arrays (Figure IV-4A; Table IV-1). This is consistent with previous observations that disulfide-linked histone octamers can be reconstituted into nucleosomes that are not grossly different from cannonical nucleosomes (Camerini-Otero and Felsenfeld, 1977; Ausio et al., 1984b; Protacio and Widom, 1996; see also Figure IV-1C). We then quantified the ability of SWI/SNF to increase the accessibility of nucleosomal DNA to restriction enzyme cleavage on the disulfide-linked and nucleosomal arrays. In the presence of SWI/SNF complex, HincII cleavage was stimulated 25-fold on the disulfide-linked arrays and 37-fold on the control nucleosomal arrays (Figure IV-4A and Table IV-1). Furthermore, the first order rate of HincII cleavage in the presence of SWI/SNF was nearly identical for the disulfide-linked and control nucleosomal arrays (Figure IV-4B, Table IV-1). These data strongly suggest that structural perturbation of the histone $(H3/H4)_2$ tetramer is not an obligatory intermediate or product of ATP-dependent nucleosome remodeling.

SWI/SNF action does not alter the steady state fluorescence of AEDANS-modified arrays. The cysteine residue of histone H3 can also be modified with sulfhydryl-specific fluorescent groups (Cantor et al., 1981; Daban and Cantor, 1989; Dieterich et al., 1978; Dieterich et al., 1979; Eshaghpour et al., 1980; Feinstein and Moudrianakis, 1986). For instance, reconstitution of nucleosomes with AEDANS-labeled H3 does not cause a significant perturbation of nucleosome structure (Dieterich et al., 1978; Dieterich et al., 1979; Protacio and Widom, 1996), and steady state AEDANS fluorescence has been used to detect changes in octamer conformation as a function of monovalent cation

concentration (Dieterich et al., 1978; Dieterich et al., 1979). We used this method in an attempt to detect more subtle or transient changes in histone octamer integrity and conformation as a result of ATP-dependent SWI/SNF activity.

Nucleosomal and tetramer arrays were reconstituted after modification of histone H3 with the sulfhydryl-specific fluorescent probe AEDANS (Dieterich et al., 1978; Dieterich et al., 1979); see Experimental procedures for details). To confirm that the modification did not disrupt the structure of the arrays or affect the activity of SWI/SNF, we quantified the kinetics of HincII cleavage of the AEDANS-modified nucleosomal arrays in the presence or absence of SWI/SNF activity. In the absence of SWI/SNF the first order rate of cleavage by HincII was 6.6×10^{-6} for the AEDANS-modified nucleosomal arrays and 2.4×10^{-6} for the nucleosomal arrays (Figure IV-5A; Table IV-1). The higher rate of cleavage observed for the AEDANS nucleosomal arrays indicates that this histone H3 modification may cause a slight perturbation in the structure or stability of the arrays. In the presence of SWI/SNF complex, however, the rate of HincII cleavage was nearly identical for the AEDANS-modified and nucleosomal arrays (Figure IV-5A; Table IV-1). Thus the data indicates that SWI/SNF is fully functional on arrays reconstituted with AEDANS-H3.

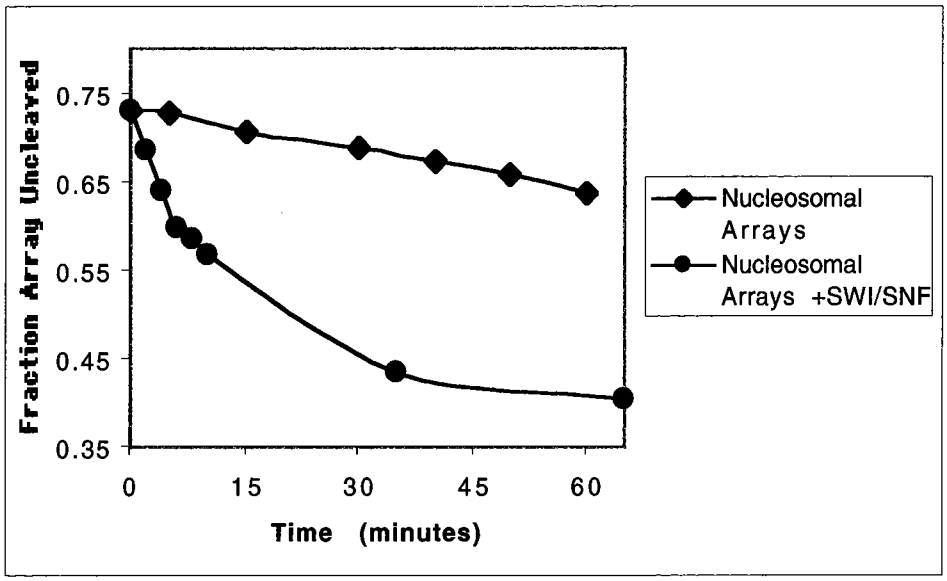
AEDANS-modified nucleosomal and tetramer arrays were titrated with increasing NaCl concentrations in the absence of SWI/SNF and fluorescence emission intensities were recorded 30 seconds after each adjustment of NaCl concentration. Consistent with previous studies (Dieterich et al., 1978; Dieterich et al., 1979), a dramatic decrease in fluorescence emission intensity was detected with increasing NaCl concentration,

Figure IV-5. Effects of SWI/SNF on steady state fluorescence emission intensity of nucleosomal and tetramer arrays reconstituted with AEDANS-H3.

Panel A. SWI/SNF can remodel AEDANS-labeled nucleosomal arrays (see also Table I). Representative time course for HincII digestion of 3 nM nucleosomal (left panel) or AEDANS-modified nucleosomal (right panel) arrays in the presence or absence of 3 nM SWI/SNF. The "0" time point reflects a 20 minute preincubation with HincII in the absence of SWI/SNF. Similar results were obtained in at least three different experiments using independent nucleosomal and AEDANS-modified nucleosomal array reconstitutions.

Panel B. Fluorescence emission spectra of AEDANS-modified nucleosomal and tetramer arrays. The upper and lower left panels illustrate the effect of increasing concentrations of NaCl on the steady state fluorescence of the modified nucleosomal arrays and tetramer arrays, respectively. Fluorescence emission intensities are shown in arbitrary units for AEDANS-modified arrays at equilibrium in 50 mM NaCl; 30 seconds after adjustment to 75, 150, 300, and 600 mM NaCl, and 1, 2, 4, 8, and 16 hours after adjustment to 600 mM NaCl. The upper and lower right panels illustrate the effect of SWI/SNF and ATP on the fluorescent-labeled nucleosomal and tetramer arrays, respectively. Fluorescence emission intensities are shown in arbitrary units for AEDANS-modified arrays at equilibrium in 5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), and 1 mM DTT (line); 5 minutes after addition of SWI/SNF to 10 nM (long dashes), and 5, 10, and 60 minutes after subsequent addition of ATP to 1 mM (medium dashes, short dashes, and dots, respectively). The experiment yielded identical results in the presence of 2 nM or 20 nM SWI/SNF (data not shown).

A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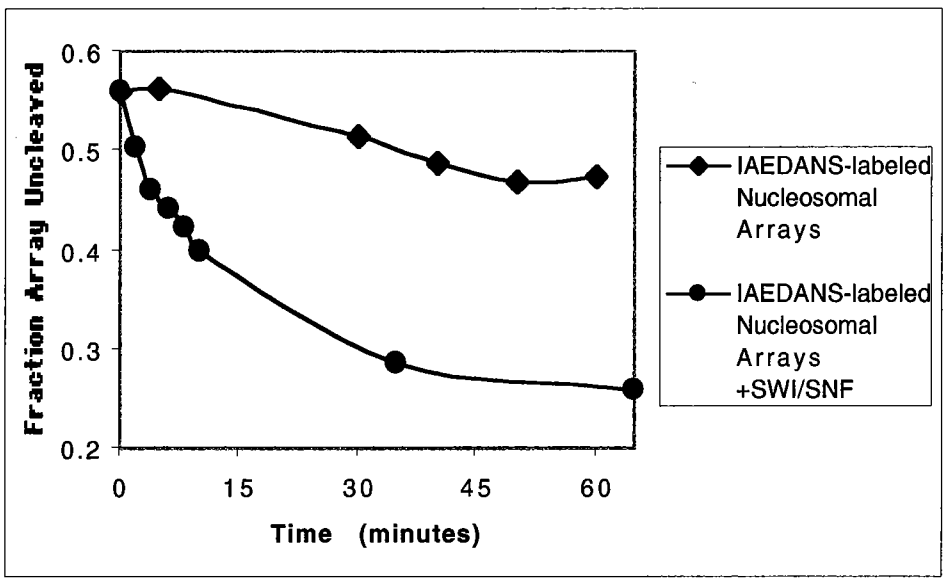
0.75
0.65
0.55
0.45
0.35
0
15
30
45
60
Time (minutes)
Nucleosomal Arrays
Nucleosomal Arrays +SWI/SNF


Fraction Array Uncleaved
0.6
0.5
0.4
0.3
0.2
0
15
30
45
60
Time (minutes)
IAEDANS-labeled Nucleosomal Arrays
IAEDANS-labeled Nucleosomal Arrays +SWI/SNF

B

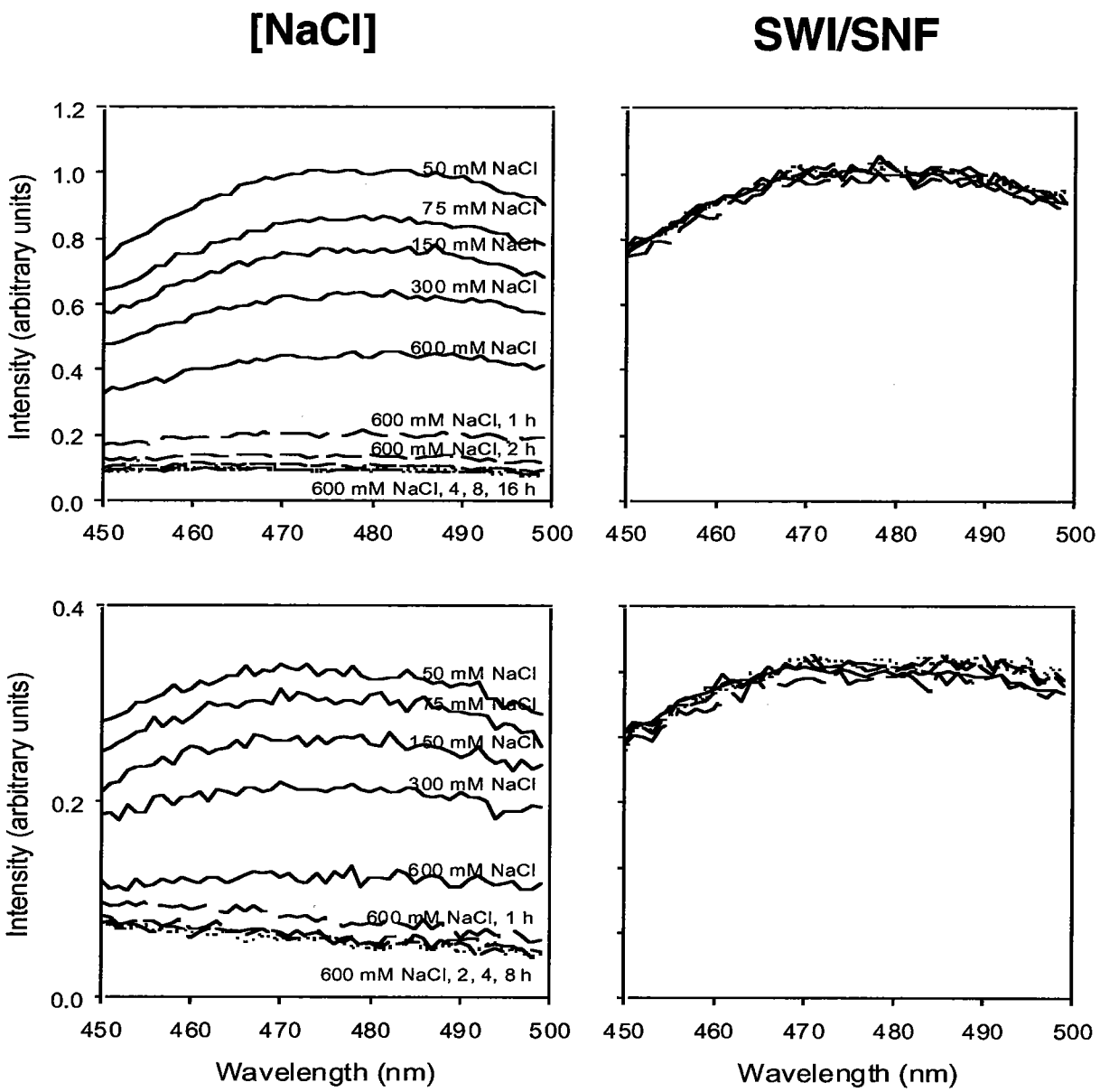

[NaCl]
SWI/SNF
Intensity (arbitrary units)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
50 mM NaCl
75 mM NaCl
150 mM NaCl
300 mM NaCl
600 mM NaCl
600 mM NaCl, 1 h
600 mM NaCl, 2 h
600 mM NaCl, 4, 8, 16 h
450
460
470
480
490
500
0.4
0.2
0.0
50 mM NaCl
75 mM NaCl
150 mM NaCl
300 mM NaCl
600 mM NaCl
600 mM NaCl, 1 h
600 mM NaCl, 2, 4, 8 h
Wavelength (nm)

Table IV-1. Comparative Analysis of Array Substrates

Array Substrate	*K_{conf}	Rates of HincII Cleavage (min^{-1})		SWI/SNF-dependent
		-SWI/SNF	+SWI/SNF	Rate stimulation
Nucleosomal	4.3×10^{-4}	2.4×10^{-6} ($+/-2.0 \times 10^{-7}$)	9.2×10^{-5} ($+/-1.0 \times 10^{-5}$)	37x (+/-14%)
Tetramer	2.3×10^{-3}	1.3×10^{-5} ($+/-1.7 \times 10^{-6}$)	6.4×10^{-5} ($+/-6.6 \times 10^{-6}$)	5x (+/-17%)
Disulfide-linked Nucleosomal	6.8×10^{-4}	3.8×10^{-6} ($+/-2.8 \times 10^{-7}$)	9.6×10^{-5} ($+/-1.7 \times 10^{-5}$)	25x (+/-19%)
AEDANS-labeled Nucleosomal	1.2×10^{-3}	6.6×10^{-6} ($+/-8.0 \times 10^{-7}$)	1.1×10^{-4} ($+/-1.5 \times 10^{-5}$)	17x (+/-18%)

*Kconf is the Conformational Equilibrium Constant and is defined as the ratio of the rate of digestion of substrate array to the rate of digestion of naked DNA per unit enzyme (Polach and Widom, 1995). The rate of digestion of naked DNA per unit enzyme used for the Kconf determination is 5.6×10^{-3} min^{-1}.

suggesting increased solvent accessibility of the internal structure of the histone octamer (Figure IV-5B, left panels). After adjustment of NaCl concentration to 600 mM, fluorescence emission intensities of nucleosomal and tetramer arrays were monitored as a function of time (Figure IV-5B, left panels). Consistent with previous results (Dieterich et al., 1978; Dieterich et al., 1979), a biphasic decrease in fluorescence emission intensity was observed, with a fast component on the second time scale (and complete within 30 seconds) and a slow component on the hour timescale (and complete within 4 hours). These salt-dependent decreases in fluorescence emission intensity were observed for both nucleosomal and tetramer arrays, indicating that the observed decreases are not due to disruption of the interface between the $(H3/H4)_2$ tetramer and the H2A/H2B dimers. However, we note that AEDANS-modified tetramer arrays exhibit a ~3-fold lower fluorescence emission intensity than AEDANS-modified nucleosomal arrays (see Figure IV-5B, left panels), suggesting that the presence of the H2A/H2B dimers decreases solvent accessibility of the $(H3/H4)_2$ tetramer.

We next measured the effect of SWI/SNF remodeling activity on the steady state fluorescence of AEDANS-modified nucleosomal or tetramer arrays. SWI/SNF (2, 10, 20 nM) was added to arrays (4 nM) equilibrated in remodeling buffer (see Experimental Procedures for details) in the absence of ATP and emission intensities were recorded (Figure IV-5B; right panel, and data not shown). Subsequently, ATP was added to a final concentration of 1 mM, and emission intensities were recorded 5, 10, and 60 minutes after its addition (Figure 5B; right panel). In striking contrast to NaCl titration (Figure IV-5B, left panels), neither the binding of SWI/SNF (which occurs in the absence of ATP) nor

ATP-dependent remodeling altered the fluorescence emission intensities of arrays (Figure IV-5B, right panels). Addition of HincII directly to the reaction cuvettes confirmed that SWI/SNF and ATP stimulated HincII cleavage of the arrays, and thus, was active under these conditions (data not shown). Thus, these data indicate that SWI/SNF remodeling is not accompanied by the loss of the H2A/H2B dimers or by disruption or changes in the internal accessibility of the $(H3/H4)_2$ tetramer.

Discussion

The *Saccharomyces cerevisiae* SWI/SNF complex is the paradigm of a family of eukaryotic protein assemblies that function in an ATP-dependent manner to alter chromatin structure. Although it is evident that chromatin remodeling by SWI/SNF and other related complexes results in enhanced accessibility of nucleosomal DNA and an increased mobility of nucleosomes, the mechanism by which this reaction occurs remains controversial. This study was designed to directly test simple predictions for several models that have been proposed for ATP-dependent chromatin remodeling.

The "dimer disruption" model. In the extreme case, the "dimer disruption" model predicts that ATP-dependent remodeling by SWI/SNF will generate a $(H3/H4)_2$ tetramer. Although assembly of a $(H3/H4)_2$ tetramer is sufficient to position at least 90 bp of DNA (Luger et al., 1997), several studies have shown that DNA-tetramer particles are more accessible to DNA binding proteins (Hayes and Wolffe, 1992; Spangenberg et al., 1998; Tse et al., 1998c), and removal of the histone H2A/H2B dimers facilitates

transcription in vitro (Hansen and Wolffe, 1994). A simple prediction of such a model is that the accessibility of DNA within $(H3/H4)_2$ tetramer arrays should be equivalent to that of nucleosomal DNA after SWI/SNF remodeling. However, we find that DNA wrapped around a $(H3/H4)_2$ tetramer is only about 5-fold more accessible to HincII digestion compared to DNA assembled onto a complete histone octamer. SWI/SNF action, on the other hand, enhances nucleosomal DNA accessibility by ~35-fold (see Table IV-1). Thus, the remodeled state of a nucleosome is not equivalent to a $(H3/H4)_2$ tetramer. The "dimer disruption" model also predicts that SWI/SNF will not be able to enhance the accessibility of $(H3/H4)_2$ tetramers. We found, however, that SWI/SNF can remodel arrays of $(H3/H4)_2$ tetramers, which leads to an additional 5-fold enhancement of HincII cleavage rates. Finally, we show that fluorescence emission intensity of AEDANS-modified tetramer arrays is significantly lower than that of AEDANS-modified nucleosomal arrays (presumably due to increased solvent accessibility of the internal structure of the tetramer) (Figure IV-5B). The fact that SWI/SNF remodeling of AEDANS-modified nucleosomal arrays does not decrease fluorescence emission intensity from the level characteristic of nucleosomal arrays to the level of tetramer arrays (Figure IV-5B, right panels) also indicates that remodeling does not involve dimer disruption. Thus, SWI/SNF action does not convert a nucleosome into a tetramer.

The "nucleosome spooling" model. The "nucleosome spooling" model for SWI/SNF action is based on the mechanism for passage of some RNA polymerases through a nucleosome (Studitsky et al., 1994). This model proposes that the energy of

ATP hydrolysis might be used to translocate SWI/SNF along DNA and around a nucleosome in a "wave-like" fashion (discussed in Pazin et al., 1997). Such ATP-driven translocation of SWI/SNF along the DNA would disrupt histone-DNA contacts and may also lead to movement of the histone octamer. Such a reaction mechanism might also result in transfer of intact histone octamers onto an acceptor DNA, which has been observed during nucleosome remodeling by the yeast RSC complex (Lorch et al., 1999). The nucleosome "spooling" model predicts that SWI/SNF will not discriminate between a tetramer and nucleosomal substrate. In fact, one might predict that the absence of H2A/H2B dimers might facilitate the ability of SWI/SNF to translocate through the residual histone-DNA interactions of the $(H3/H4)_2$ tetramer. Although SWI/SNF does remodel the $(H3/H4)_2$ tetramer arrays, the apparent rate of remodeling was approximately 30% slower than the rate for nucleosomal array remodeling. Furthermore, SWI/SNF was inactive on the $(H3/H4)_2$ tetramers in remodeling reactions where the concentration of tetramer array was in excess over SWI/SNF. These results demonstrate that arrays of $(H3/H4)_2$ tetramers are poor substrates for ATP-dependent remodeling by SWI/SNF, a result which is not predicted by the "nucleosome spooling" model. Furthermore, SWI/SNF does not show ATP-dependent tracking activity in a DNA supercoiling assay (Quinn et al., 1996), nor do other SWI2/SNF2 family members (e.g. Mot1p) demonstrate DNA tracking activity (Auble and Steggerda, 1999). Together, these data suggest that ATP-dependent remodeling by SWI/SNF does not involve DNA tracking nor is it equivalent to the loss of the H2A/H2B dimers. Our data does indicate that efficient

remodeling activity requires a cannonical histone octamer that contains both an $(H3/H4)_2$ tetramer and one or more H2A/H2B dimers.

SWI/SNF action does not perturb octamer structure. Several groups have recently suggested the alternative possibility that SWI/SNF activity might induce a novel conformation of the nucleosome which may involve rearrangement of the histone octamer without loss of histone proteins (Lee et al., 1999; Lorch et al., 1998; Schnitzler et al., 1998). This model is consistent with recent electron microscopy studies which indicated that ATP-dependent remodeling by SWI/SNF does not change the protein mass of a nucleosome and that remodeling is relatively insensitive to addition of an external crosslinking reagent, dimethyl suberimidate to mononucleosomes or nucleosomal arrays (Bazett-Jones et al., 1999). What is this alternate conformation? Lee et al. (1999) proposed that human SWI/SNF might use the energy of ATP hydrolysis to rearrange one or both H2A/H2B dimers such that only the flexible N-terminal domain is contacting DNA close to the nucleosomal dyad. This novel octamer conformation might then have a propensity to form the dinucleosome-like particle that was previously observed (Lorch et al., 1998; Schnitzler et al., 1998). Alternatively, SWI/SNF action might lead to a conformational change in the $(H3/H4)_2$ tetramer which might mimic the "split nucleosome" or "lexosome" structure that that has been proposed for the structure of transcriptionally active chromatin (Bazett-Jones et al., 1996; Czarnota et al., 1997; Prior et al., 1983).

To test these possibilities we took advantage of the single cysteine residue found within each copy of chicken histone H3. These two cysteines are buried within the histone octamer and are located very close to each other at nucleosomal dyad axis (Camerini-Otero and Felsenfeld, 1977; Luger et al., 1997). To test for gross changes in the structure of the $(H3/H4)_2$ tetramer, we monitored the apparent rates of remodeling of nucleosomal arrays that contain disulfide-linked $(H3/H4)_2$ tetramers. We found that SWI/SNF remodeled these substrates with rates equivalent to nucleosomal arrays. These data indicate that SWI/SNF action does not require a significant rearrangement of the tetramer.

To probe for more subtle changes in the structure of the histone octamer, we also monitored the effects of ATP-dependent remodeling on the steady state fluorescence of AEDANS-labeled nucleosomal and tetramer arrays. Steady state fluorescence of AEDANS-H3 has been used to detect at least three distinct conformational states of the nucleosome as a function of salt concentration (Dieterich et al., 1978; Dieterich et al., 1979). Furthermore, the solvent accessibility of the AEDANS group, and thus its fluorescence emission intensity, is predicted from previous fluorescence studies to be highly dependent on the presence of one or both histone H2A/H2B dimers (Feinstein and Moudrianakis, 1986; see also Figure IV-5B, left panels).

We found that, in contrast to increased salt concentrations, which have large effects on fluorescence emission intensities of nucleosomal or tetramer arrays (Dieterich et al., 1978; Dieterich et al., 1979); see also Figure IV-5B, left panels), addition of SWI/SNF (one SWI/SNF per two nucleosomes), with or without ATP, had no measurable

effect on fluorescence emission intensities (Figure IV-5B, right panels). These data suggest that SWI/SNF does not change the overall structure or the solvent accessibility of the histone octamer or tetramer.

What is ATP-dependent chromatin remodeling? Our data are consistent with previous suggestions that ATP-dependent nucleosome remodeling by SWI/SNF disrupts histone-DNA contacts without a structural change in the histone octamer (Bazett-Jones et al., 1999; Cote et al., 1998); discussed in Peterson, 1998). How does SWI/SNF accomplish this feat? The remodeling reaction randomizes the rotational setting of both wraps of nucleosomal DNA without removing most of the DNA from the octamer surface (Cote et al., 1998). Furthermore, SWI/SNF action does not enhance the reactivity of nucleosomal DNA to potassium permanganate indicating that remodeling does not involve an unwinding of the DNA double helix (Cote et al., 1998). We favor models in which the energy of ATP hydrolysis is used to rotate the DNA helix along its long axis relative to the histone octamer. In one such model, SWI/SNF would remain at a fixed position relative to the histone octamer, and both SWI/SNF and the octamer would remain in a fixed translational position relative to DNA. In this model, SWI/SNF would rotate the DNA helix back and forth with each round of ATP hydrolysis, changing rotational phasing and disrupting histone-DNA contacts throughout both wraps of nucleosomal DNA. In another similar model, SWI/SNF would remain in a fixed position relative to the octamer, but SWI/SNF and the octamer would not remain in a fixed translational position relative to the DNA. In this model ATP-driven DNA helix rotation

would "screw" the octamer along the DNA helix, changing the translational position of the octamer (analogous to a model proposed in (Varga-Weisz and Becker, 1995). Either of these models would be consistent with previous data (see above, Cote et al., 1998) as well as with recent studies indicating that several members of the SWI/SNF family of chromatin remodeling complexes can enhance nucleosome mobility (Hamiche et al., 1999; Jaskelioff et al., 2000; Langst et al., 1999; Whitehouse et al., 1999). Studies designed to test and distinguish between these models are in progress.

Experimental Procedures

Reagent preparation. Array DNA template was isolated by digestion of plasmid pCL7c with NotI, HindIII, and HhaI (New England Biolabs) followed by FPLC purification on Sephacryl-500 (Pharmacia) essentially as described (Logie and Peterson, 1997; Logie and Peterson, 1999). Array DNA template was end-labeled as described (Logie and Peterson, 1997; Logie and Peterson, 1999).

Chicken erythrocyte histone octamers were purified from chicken whole blood (Pel-Freez) as described previously (Hansen et al., 1989). Tetramers were provided as a kind gift from Jeff Hansen and were purified by stepwise elution from hydroxylapatite columns as described (Hansen et al., 1989). Tetramers were dialyzed against Buffer T (1M NaCl, 10 mM Tris-HCl, pH 8.0, 0.25 mM EDTA, 0.1 mM DTT) prior to array reconstitution. Disulfide-linked histone octamers were generated by first diluting histone octamers 2-fold with Buffer D1 (10M urea, 2 M NaCl, 20 mM Tris-HCl, pH 8.0) followed by dialysis against Buffer D2 (5M urea, 2 M NaCl, 10 mM Tris-HCl, pH 8.0) at 4° C with constant

agitation for 4 days. Histone octamers were then reconstituted by dialysis against Buffer D3 (2 M NaCl, 10 mM Tris-HCl (pH 8.0), 0.25 mM EDTA). Histone H3 oxidation efficiency was analyzed by SDS-PAGE in the absence of reducing agent (see Figure IV -B, lane 3). AEDANS-modified histone H3 was generated by first diluting histone octamers 2-fold with Buffer D1, followed by addition of iodoacetylethylenediamine-(1,5) napthol sulfonate (1,5-IAEDANS; Molecular Probes, Inc.) at a molar ratio of 20:1 (1,5-IAEDANS:H3) and incubation for 2 hours at room temperature on a nutator. Reactions were quenched with an excess of ß-mercaptoethanol (Sigma) and dialyzed against Buffer D2 to remove unreacted 1,5-IAEDANS. Samples were then dialyzed against Buffer D3 to reconstitute histone octamers. Labeling specificity was verified by visualization of the corresponding fluorescent histone band upon illumination with longwave ultraviolet light.

SWI/SNF complex was purified from yeast strains CY396 or CY743 (*sin3Δ*) as described in Logie and Peterson (1999). The concentration of complex was determined to be approximately 300 nM by comparative Western Blot and by ATPase assays (Logie and Peterson, 1997; Logie and Peterson, 1999).

Reconstitution and analysis of nucleosomal arrays. Arrays were reconstituted onto the 208-11S DNA template (Figure IV-1A) in a slide-a-lyzer dialysis cassette (Pierce) using the salt dialysis protocol of Hansen and Lohr (Hansen and Lohr, 1993). Octamer concentrations were determined by A_{230} (Stein, 1979). Array saturation and nucleosome/tetramer positioning was determined by EcoRI or MspI digestions using approximately 20 nM array in Remodeling Buffer (5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCL (pH 8.0), 1 mM DTT) as previously described (Hansen et al., 1989; Logie and

Peterson, 1997; Logie and Peterson, 1999; Logie et al., 1999). Arrays were digested for 30 minutes at 37° C and the reactions were electrophoresed on 4% native polyacrylamide gels (See Figure IV-1C). The gel was briefly soaked in 2 μg/ml ethidium bromide and photographed under ultraviolet illumination.

Assay conditions. Coupled array remodeling-restriction reactions were performed in a final concentration of 5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCL (pH 8.0), 1 mM DTT, 0.1 mg/ml BSA, 1 mM ATP, and 500 U/ml HincII (New England Biolabs) as previously described (Logie and Peterson, 1997; Logie and Peterson, 1999; Logie et al., 1999).

Fluorescence spectroscopic studies were carried out using a PTI QM1/SE901/SE910Q fluorescence spectrophotometer. Samples were excited at 344 nm (slit width = 2.5 nm), and emission intensities were recorded from 450 nm to 500 nm (slit width = 4 nm). Samples (80 μl in quartz microcuvettes) contained 4 nM AEDANS-labeled octamer or tetramer array in Remodeling Buffer (described above) at room temperature. In experiments assessing effects of monovalent salt, emission intensities were recorded initially 0.5 minutes after adjustment to 50, 75, 150, and 300 mM NaCl; and 0.5, 60, 120, 240, 480, and 960 minutes after subsequent adjustment to 600 mM NaCl. In experiments assessing effects of SWI/SNF, emission intensities were recorded initially at 5 minutes after addition of a final concentration of 2, 10, or 20 nM purified SWI/SNF and 5, 10, and 60 minutes after subsequent addition of a 1 mM final concentration of ATP. For samples in which SWI/SNF, ATP, or salt was added to the arrays, data were corrected for a buffer addition control. All data were corrected for dilution.

Chapter V

Functional Delineation of Three Groups of The ATP-Dependent Family of Chromatin Remodeling Enzymes

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Chapter V

Functional Delineation of Three Groups of the ATP-dependent Family of Chromatin Remodeling Enzymes

Introduction

The assembly of eukaryotic DNA into folded nucleosomal arrays is likely to have a major impact on the efficiency or regulation of nuclear processes that require access to the DNA sequence, including RNA transcription, DNA replication, recombination, and repair. In fact, it is now generally recognized that disruption or remodeling of chromatin structure may be a prerequisite step for most of these nuclear DNA transactions (Burns and Peterson, 1997a; Cairns, 1998; Muchardt and Yaniv, 1999). Two classes of highly conserved chromatin remodeling enzymes have been implicated as regulators of the repressive nature of chromatin structure – the first class includes enzymes that covalently modify the nucleosomal histones (acetylation, phosphorylation, methylation, ADP-ribosylation, ubiquitination; reviewed by Spencer and Davie, 1999; Strahl and Allis, 2000), and the second class is composed of multi-subunit complexes that use the energy of ATP hydrolysis to disrupt histone-DNA interactions (reviewed in Pollard and Peterson, 1998; Kingston and Narlikar, 1999).

Each member of the ATP-dependent family of chromatin remodeling enzymes contains an ATPase subunit that is related to the SWI2/SNF2 subfamily of the DEAD/H superfamily of nucleic acid stimulated ATPases (Eisen et al., 1995). Seventeen members

of the SWI2/SNF2 family have been identified in the yeast genome (Pollard and Peterson, 1998), and to date, four of these ATPases have been purified as subunits of distinct chromatin remodeling complexes SWI/SNF (Cairns et al., 1994; Cote et al., 1994), RSC (Cairns et al., 1996b), ISW1 and ISW2 (Tsukiyama et al., 1999). Additional ATP-dependent remodeling complexes that harbor SWI2/SNF2 family members have been identified in *Drosophila* [ACF (Ito et al., 1997), dNURF (Tsukiyama et al., 1995),dCHRAC (Varga-Weisz et al., 1997), Brahma (Elfring et al., 1994; Papoulas et al., 1998)] , human [hSWI/SNF (Kwon et al., 1994), NURD (Tong et al., 1998; Xue et al., 1998; Zhang et al., 1998), RSF (LeRoy et al., 1998)], and frog [xMi-2 (Wade, 1998)]. Although these complexes have a variable number of subunits (i.e. 2-15), and many different types of assays have been used to monitor the activity of individual complexes, each enzyme can apparently use the energy of ATP hydrolysis to alter chromatin structure and to enhance the binding of proteins to nucleosomal DNA binding sites (reviewed in Kingston and Narlikar, 1999; Muchardt and Yaniv, 1999). Furthermore, in the case of the ySWI/SNF, *Drosophila* Brahma, and hSWI/SNF complexes, remodeling is required for transcriptional regulation of target genes *in vivo* (de la Serna et al., 2000; Deuring et al., 2000) for review see Kingston and Narlikar, 1999).

ATP-dependent chromatin remodeling complexes have been further divided into three groups based on whether the sequence of the ATPase subunit is more related to yeast SWI2 (ySWI/SNF, RSC, Brahma, hSWI/SNF), *Drosophila* ISWI (ISW1, ISW2, dNURF, dCHRAC, dACF, hRSF), or human Mi-2 (hNURD, xMi-2) (reviewed in Muchardt and Yaniv, 1999). Although each of these ATPases share a SWI2/SNF2-like

ATPase domain, they harbor additional, unique sequence motifs adjacent to the ATPase domain that are characteristic of each group – the SWI2 group contains a bromodomain (Tamkun et al., 1992), the ISWI group contains a SANT domain (Aasland et al., 1996), and the Mi-2 group contains a chromodomain (Paro and Hogness, 1991). Differences among some groups are also apparent in the nucleic acid cofactor required for stimulation of ATPase activity. For enzymes that contain a SWI2-like ATPase (ySWI/SNF, yRSC, hSWI/SNF), ATPase activity is stimulated equally well by "free" DNA or nucleosomes (Cairns et al., 1996b; Cote et al., 1994; Phelan et al., 1999). In contrast, the ATPase activity of enzymes that contain an ISWI-like or Mi-2-like ATPase is optimally stimulated by nucleosomes (Tong et al., 1998; Tsukiyama and Wu, 1995; Wade, 1998; Xue et al., 1998; Zhang et al., 1998). In the case of ISWI-like ATPases, this requirement for nucleosomal DNA may reflect obligatory interactions with the trypsin-sensitive, histone N-terminal domains (Georgel et al., 1997).

Here we report the first direct comparison of the biochemical properties of six different chromatin remodeling enzymes (ySWI/SNF, yRSC, dCHRAC, dNURF, hSWI/SNF, xMi-2) which encompass all three previously suggested groups. Surprisingly, each complex shows nearly identical ATPase activity on nucleosomal array substrates, and they are each able to facilitate nucleosome mobilization within an array at nearly equivalent rates. We have also investigated the nucleosome substrate requirements for each enzyme by using reconstituted arrays with hyperacetylated or trypsinized histone octamers, as well as histone $(H3/H4)_2$ tetramers. ATPase and remodeling assays with these different substrates identify new common features, as well

as new distinctions among enzymes. In addition, we test the ability of the GAL4-VP16 chimeric transcriptional activator to recruit these remodeling complexes to a nucleosomal array substrate. We report that yeast SWI/SNF is uniquely potent for recruitment by GAL4-VP16 in this assay. Our data are consistent with the differential regulation of ATP-dependent enzymes that each share a similar mechanism of nucleosome remodeling.

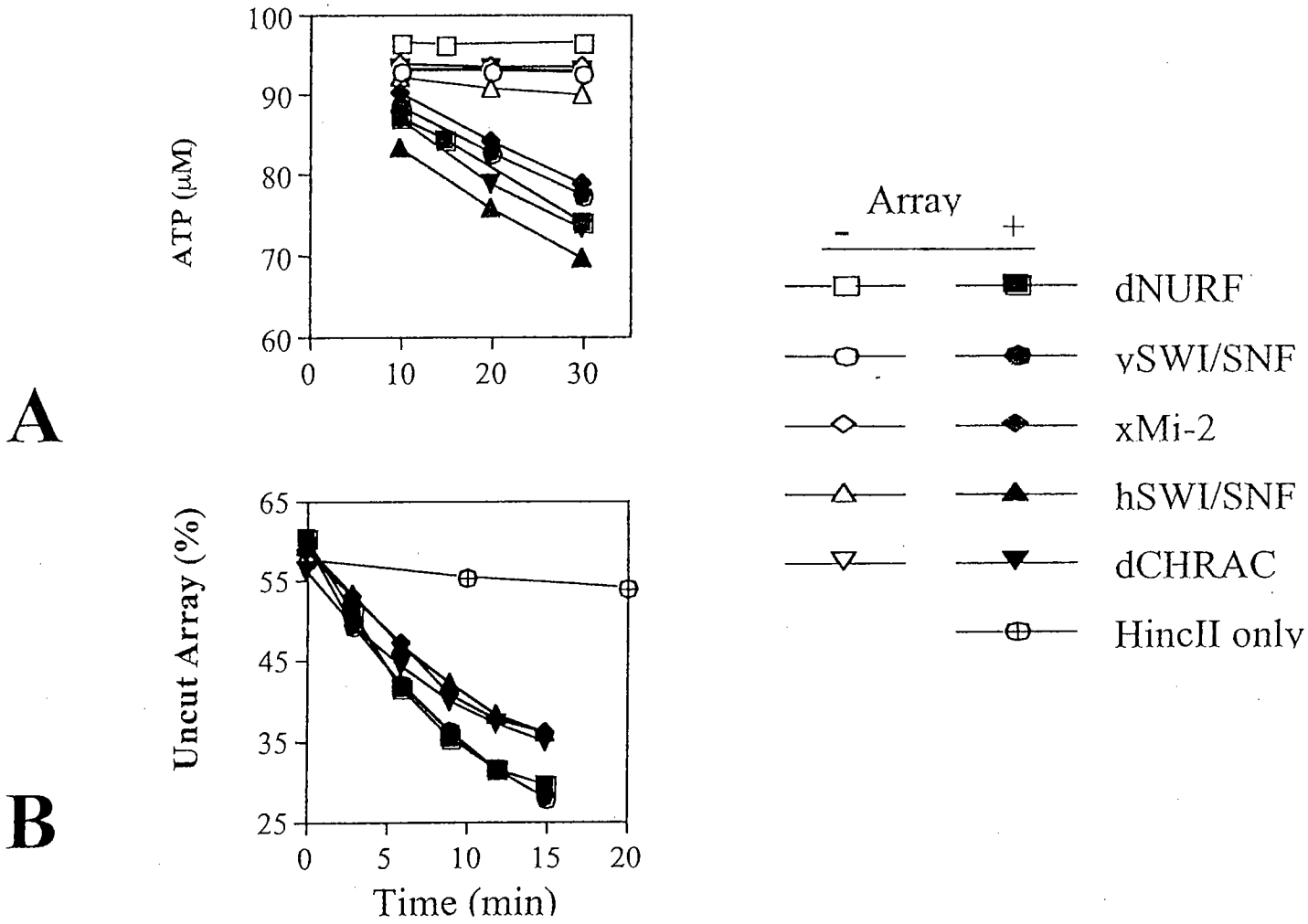
Results

ATPase and remodeling activities of chromatin remodeling enzymes. In order to quantify the nucleosome remodeling activity of ATP-dependent remodeling enzymes, we have developed a biochemical assay where nucleosome remodeling activity is coupled to restriction enzyme activity such that remodeling is revealed as an enhancement of restriction enzyme cleavage rates (Logie and Peterson, 1997; Logie and Peterson, 1999). This assay uses a novel nucleosomal array substrate in which the central nucleosome of an 11-mer nucleosomal array contains a unique SalI/HincII site located at the predicted dyad axis of symmetry (Logie and Peterson, 1997; Logie and Peterson, 1999). Restriction enzyme kinetics are bi-phasic in this system; the first phase is rapid and reflects the fraction of SalI/HincII restriction sites that are not occluded by a nucleosome (due primarily in our assays to nucleosomes that occupy minor translational positions; see Dong et al., 1990; Logie and Peterson, 1997; Pennings et al., 1991). The second phase is slow and reflects a dynamic equilibrium between the occluded and "open" nucleosomal DNA states (Polach and Widom, 1995; Polach and Widom, 1996). In previous studies, addition of yeast SWI/SNF and ATP stimulated the second phase of

SalI/HincII digestion 20- to 30-fold (Logie and Peterson, 1997; Logie et al., 1999). Recently we have found that SWI/SNF remodeling leads to a rapid redistribution of nucleosome positions within these arrays and that the apparent rate of remodeling determined in this assay provides an estimate of the rate of nucleosome mobilization (Jaskelioff et al., 2000).

Purified preparations of ySWI/SNF, yRSC, hSWI/SNF, dCHRAC, dNURF, and xMi-2 were analyzed in parallel for nucleosome-stimulated ATPase activity (see Experimental Procedures). Each complex was titrated in an ATPase reaction that contained 100 μM ATP and12 nM of a reconstituted, 11-mer nucleosomal array. Surprisingly, the approximate concentration of each remodeling complex that was required to achieve equivalent velocities of ATP hydrolysis were similar; for ySWI/SNF (2 nM), yRSC (2 nM), hSWI/SNF (5 nM), dCHRAC (2 nM), and dNURF (4 nM); each complex catalyzed the hydrolysis of 450-600 nmol of ATP per minute (Figure V-1A, see also Logie et al., 1999). xMi-2 was slightly less active in this assay as ~15 nM was required to achieve this level of ATPase activity (Figure V-1A). Given that our estimates of active enzyme concentrations are only approximate (see Experimental Procedures), the data shown in Figure V-1A indicate that each of these enzymes have similar nucleosome-stimulated ATPase activities within an order of magnitude. The similar levels of ATPase activity among complexes was unexpected given that each complex has different associated subunits which, at least in the case of the hSWI/SNF complex, can have a large impact on the ATPase activity of the catalytic subunit (i.e. BRG1; Phelan et al., 1999).

Figure V-1. Comparison of ATPase and remodeling activities of ATP-dependent chromatin remodeling complexes. A. ATPase assays. The indicated remodeling complexes (~1-5 nM ySWI/SNF, hSWI/SNF, dNURF, dCHRAC or ~15 nM xMi-2) were analyzed in ATPase reactions that contained (closed symbols) or lacked (open symbols) 12 nM of nucleosomal array, and ATP hydrolysis was monitored with time. Velocities of ATP hydrolysis were calculated from at least three reaction timepoints. **B.** Nucleosomal array remodeling assays. HincII digestion of nucleosomal arrays incubated in the presence (closed symbols) or absence (open symbols) of the indicated remodeling complexes. HincII digestion rates were calculated from the slopes of plots of the natural logarithm of the fraction of uncut array vs time. These results are representative of multiple, independent experiments. Similar results were also obtained with at least two independent enzyme preparations for each complex except yRSC.


A
B
ATP (μM)
100
90
80
70
60
0
10
20
30
Uncut Array (%)
65
55
45
35
25
0
5
10
15
20
Time (min)
Array
-
+
dNURF
ySWI/SNF
xMi-2
hSWI/SNF
dCHRAC
HincII only

To assess the capacity of the six different complexes to remodel an 11-mer nucleosomal array, each remodeling enzyme (1-5 nM ySWI/SNF, yRSC, hSWI/SNF, dCHRAC, dNURF or 15 nM xMi-2) was added to 1.5 nM nucleosomal array and the initial rates of HincII digestion were measured in parallel reaction time courses in the presence of ATP. We found that all six complexes enhanced the rate of HincII digestion essentially equivalently (Figure V-1B; see also Logie et al., 1999 for a detailed comparison of ySWI/SNF and yRSC). The dCHRAC complex reproducibly yielded an approximately 2-fold lower rate of HincII digestion than all other complexes which probably reflects the fact that a significant amount of the ATPase activity of dCHRAC appears to be contributed by topoisomerase II (see below). Since the initial rate of HincII digestion provides an indirect measurement of the rate of remodeling, these data indicate that all six enzymes use similar amounts of ATP to remodel nucleosomal arrays at similar rates. Furthermore, since it appears that this coupled restriction enzyme-remodeling assay monitors the rate of nucleosome mobilization (Jaskelioff et al., 2000), all six enzymes can apparently redistribute nucleosomes within an array at comparable rates.

A hallmark of our nucleosomal array assay is that the SWI/SNF- dependent enhancement of restriction enzyme accessibility requires continuous ATP hydrolysis (Jaskelioff et al., 2000; Logie and Peterson, 1997). This requirement reflects a state of constant redistribution of nucleosome positions in the presence of ATP, and the subsequent inactivation of SWI/SNF "freezes" a random positioning of nucleosomes which is characterized by a general occlusion of restriction enzyme sites (Jaskelioff et al., 2000). We carried out similar remodeling/"reversal" assays with hSWI/SNF, dCHRAC,

dNURF, or xMi-2 and in all cases the enhanced rates of HincII digestion were lost after ATP was enzymatically removed with apyrase (data not shown; for analysis of yRSC see Logie et al., 1999). Thus, these results indicate that all six complexes use the energy of ATP hydrolysis to create a dynamic, reversible state of nucleosome mobilization. Our results are consistent with previous demonstrations of mononucleosome mobilization catalyzed by ySWI/SNF (Jaskelioff et al., 2000; Whitehouse et al., 1999), dCHRAC (Langst et al., 1999), and dNURF (Hamiche et al., 1999).

Nucleosome moiety requirements of the chromatin remodeling complexes. Previous studies have demonstrated that optimal ATPase activity of dNURF (Tsukiyama and Wu, 1995), dCHRAC (Varga-Weisz et al., 1997) and xMi-2/NURD (Tong et al., 1998; Wade, 1998; Xue et al., 1998; Zhang et al., 1998) complexes requires nucleosomal DNA, whereas the ATPase activities of hSWI/SNF(Kwon et al., 1994), ySWI/SNF (Cote et al., 1994)and yRSC (Cairns et al., 1996; Logie et al., 1999) complexes are stimulated equally well by "free" DNA. Furthermore, in the case of the dNURF complex, the nucleosome stimulation of ATPase activity requires one or more trypsin-sensitive histone N-terminal domain(s) (Georgel et al., 1997). To further define the nucleosome moiety requirements for all six complexes, we reconstituted nucleosomal arrays with hyperacetylated or trypsinized histone octamers, as well as with histone $(H3/H4)_2$ tetramers. To ensure that each type of array reconstitution was of similar quality, all reconstitutions were analyzed for extent of DNA repeat saturation and for correct positioning by multiple restriction enzyme mapping and native polyacrylamide gel

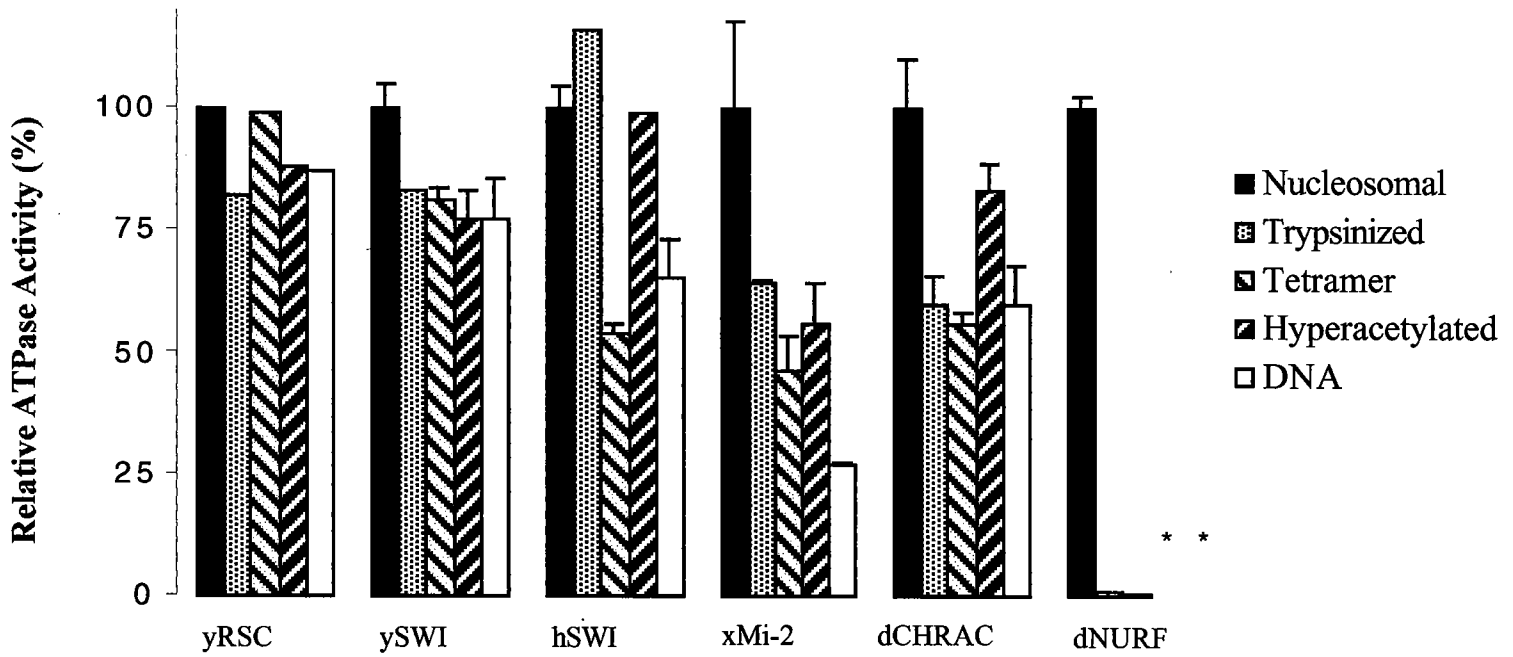
electrophoresis (see Experimental Procedures). We then measured the ability of these arrays to stimulate the ATPase activity of each complex (Figure V-2A). As expected, ySWI/SNF and yRSC complex hydrolyzed ATP with similar kinetics on all substrates, including "free" DNA (Figure V-2A; see also Logie et al., 1999). Likewise, the ATPase activity of hSWI/SNF complex was stimulated by all substrates, with the exception that activity was consistently 40-50% less in the presence of arrays reconstituted with histone $(H3/H4)_2$ tetramers (Figure V-2A).

In agreement with previous studies, we also found that the ATPase activity of dNURF complex was maximally stimulated only by nucleosomal DNA (for analysis of ATPase activity with DNA or hyperacetylated substrates, see Georgel et al., 1997); little ATPase activity was detected with arrays reconstituted with trypsinized histone octamers or histone $(H3/H4)_2$ tetramers. Given that the ATPase activity of dNURF requires one or more histone N-terminal domains (Figure V-2A; see also Georgel et al., 1997), the lack of ATPase activity in the presence of the histone $(H3\text{-}H4)_2$ tetramer arrays suggested that the N-terminal domains of the histone H2A/H2B dimers might play a key role. However, nucleosomal arrays reconstituted with hybrid histone octamers composed of intact histone $(H3\text{-}H4)_2$ tetramers and tailless histone H2A-H2B dimers yielded maximal stimulation of dNURF ATPase activity (P. Horn and C.L.Peterson., unpublished observation). Thus, the inability of $(H3\text{-}H4)_2$ tetramer arrays to stimulate the ATPase activity of dNURF does not reflect a key for the N-terminal domains of histone H2A/H2B dimers.

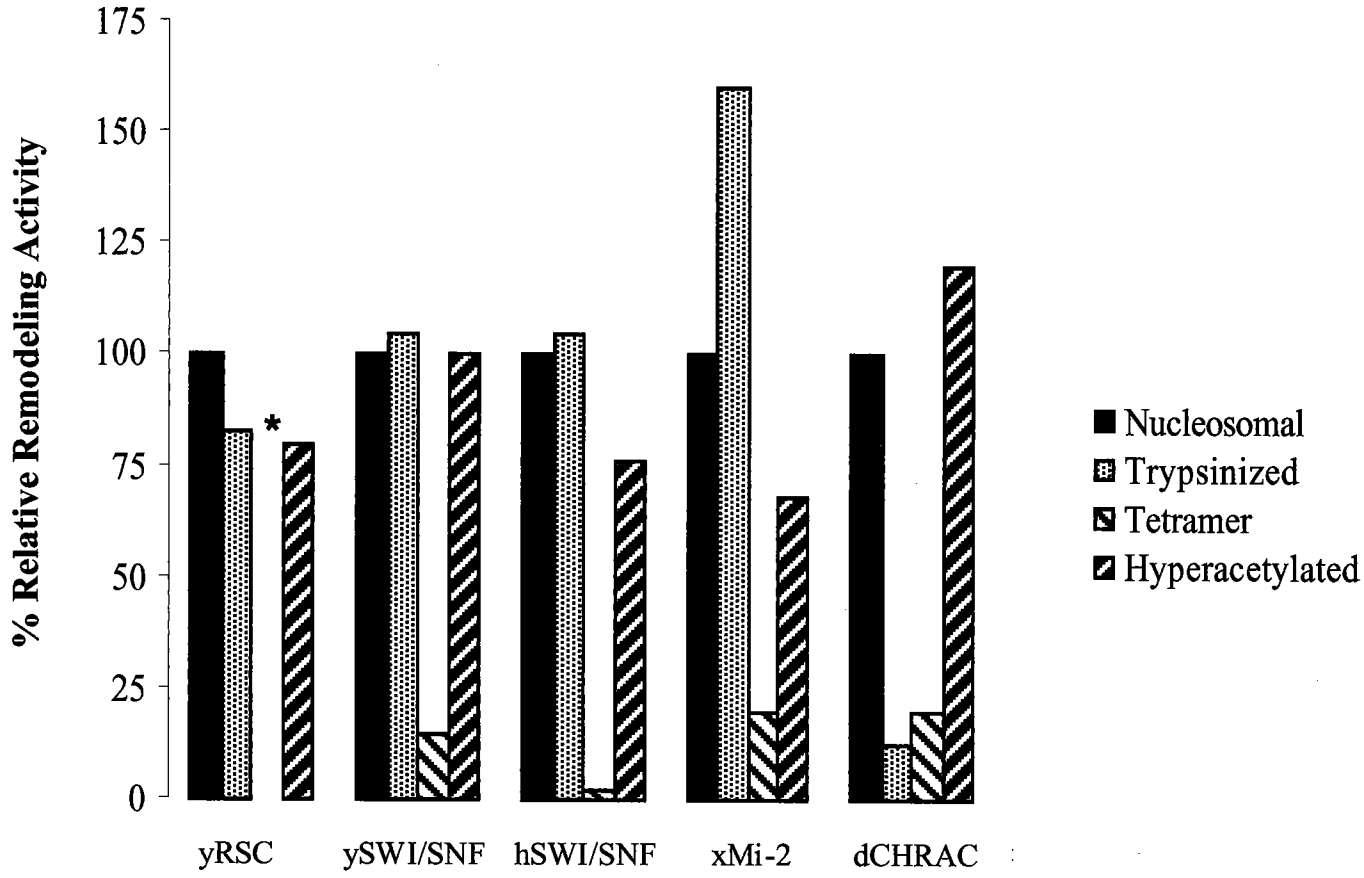
The dCHRAC complex, like dNURF, contains ISWI, which is a nucleosome-stimulated ATPase. In addition, dCHRAC also contains topoisomerase II which is a

Figure V-2. Nucleosome moiety requirements for ATP-dependent chromatin remodeling enzymes. A. ATPase assays. The indicated remodeling enzymes were added to ATPase assays that contained either 208-11S DNA template (DNA) or 208-11S arrays reconstituted with histone octamers, hyperacetylated histone octamers, trypsinized histone octamers, or isolated $(H3\text{-}H4)_2$ tetramers. Each reaction represented an ATPase timecourse, and ATP hydrolysis velocities were calculated for each substrate. Data is presented as a percentage of the ATPase velocity exhibited with the nucleosomal array substrate. Data shown for yRSC is the result of a single experiment which essentially repeated our prior study (Logie et al., 1999). * Denotes that ATPase assays with DNA and hyperacetylated array substrates were not performed with dNURF (see Geogel et al., 1997 for detailed analysis). **B.** Nucleosomal array remodeling assays. HincII digestion rates were determined for each enzyme on each array substrate. For each substrate, rates were calculated from the slopes of plots of the natural logarithm of the fraction of uncut array versus time. Data is presented as a percentage of the remodeler-dependent HincII digestion rate of the nucleosomal array. With the exception of yRSC, the results shown include experiments with at least two independent enzyme preparations for each complex. * Denotes that remodeling of tetramer arrays was not performed with yRSC.

A


Relative ATPase Activity (%)
100
75
50
25
0
yRSC
ySWI
hSWI
xMi-2
dCHRAC
dNURF
* *
Nucleosomal
Trypsinized
Tetramer
Hyperacetylated
DNA

B


% Relative Remodeling Activity
175
150
125
100
75
50
25
0
*
yRSC
ySWI/SNF
hSWI/SNF
xMi-2
dCHRAC
Nucleosomal
Trypsinized
Tetramer
Hyperacetylated

DNA-stimulated ATPase (Varga-Weisz et al., 1997). Thus, the ATPase activity associated with dCHRAC is a composite of ISWI and topoisomerase II which complicates the analysis of the substrate preferences of this complex (Figure V-2A). The ATPase activity of dCHRAC was stimulated by all substrates, although ATPase activity is reproducibly higher in the presence of nucleosomal or hyperacetylated arrays. Since the ATPase activity of NURF is only stimulated by a nucleosomal or hyperacetylated substrate (Figure V-2A; see also Georgel et al., 1997), our data suggest that only 30-40% of the overall ATPase activity of CHRAC is due to the ISWI subunit, and the remaining, DNA-stimulated ATPase activity, is due to topoisomerase II.

The ATPase activity of the xMi-2 complex was distinct from both the SWI/SNF (ySWI/SNF, yRSC, hSWI/SNF) and ISWI groups (dNURF, dCHRAC) of ATPases (Figure V-2A). Like the ISWI group, the ATPase activity of xMi-2 was maximally stimulated by nucleosomal arrays, although "free" DNA did stimulate a significant amount of ATPase activity (27% of the nucleosomal level). In contrast to the ISWI group, arrays reconstituted with trypsinized histones were still able to stimulate the ATPase activity of xMi-2 to nearly 70% the level of intact nucleosomal arrays. Likewise, arrays reconstituted with hyperacetylated histones or histone $(H3\text{-}H4)_2$ tetramers were more similar to the nucleosomal arrays. Thus, the observed preference for nucleosomal arrays does not reflect an obligatory interaction with the histone N-terminal domains. Thus, based on a preference for a nucleosomal substrate and a lack of histone tail dependence, xMi-2 appears to define a third group of the ATP-dependent chromatin remodeling family.

Table V-I: Substrate Specificity of ATP-Dependent Chromatin Remodeling Complexes

Remodeling Complex	Array Substrates									
	DNA		Nucleosomal		Hyperacetylated		Trypsinized		Tetramer	
	ATPase[1]	HincII[2]	ATPase	HincII	ATPase	HincII	ATPase	HincII	ATPase	HincII
ySWI/SNF	+	NA	+	+	+	+	+	+	+	+/-
yRSC	+	NA	+	+[3]	+	+[3]	+	+[3]	+	ND
hSWI/SNF	+/-	NA	+	+	+	+	+	+	+/-	-
xMi-2	+/-	NA	+	+	+/-	+	+/-	+	+/-	-
dCHRAC	+/-	NA	+	+	+	+	+/-	-	+/-	-
dNURF	-	NA	+	+	+[4]	+	-	-	-	ND

[1] Relative ATPase activity; +/- was defined as 20 -75% of nucleosomal array-stimulated ATPase activity
[2] Defined as remodeler-dependent HincII cleavage of substrate arrays
[3] Logie et al. (1999)
[4] Georgel et al. (1997)

We also performed coupled restriction enzyme-remodeling assays for each of the different array substrates and each remodeling complex. As shown in Figure V-2B, remodeling of the different substrate arrays paralleled the ATPase activity of the complexes except in three cases. First, although arrays reconstituted with histone $(H3\text{-}H4)_2$ tetramers were able to stimulate the ATPase activity of ySWI/SNF and hSWI/SNF, the apparent rate of remodeling of these tetramer arrays was reduced 10- to 50-fold compared to remodeling of nucleosomal arrays (Figure V-2B; Boyer et al., 2000; see also Chapter IV of this thesis for an extensive discussion). Second, although dCHRAC showed high levels of ATPase activity with all substrates, it was not able to remodel arrays reconstituted with either trypsinized histones or with the histone $(H3/H4)_2$ tetramers (Figure V-2B). These results suggest that the ATPase activity that is presumably contributed by the topoisomerase II subunit of dCHRAC is not sufficient to enhance restriction enzyme accessibility in these array assays. Furthermore, the results from this remodeling analysis indicate that dCHRAC and dNURF, which each contain the ISWI ATPase, have indistinguishable histone moiety requirements. And finally, although the ATPase activity of xMi-2 was stimulated well by arrays reconstituted with hyperacetylated histone octamers and (H3-H4)2 tetramers (Figure V-2A), these arrays were only poorly remodeled by xMi-2 (Figure V-2B). The results from Figure V-2 are summarized in Table V-1.

Targeting of the chromatin remodeling complexes by transcriptional activators. Recently we have shown that the remodeling activity of ySWI/SNF can be

targeted to reconstituted nucleosomal arrays by GAL4 derivatives that contain an acidic transcriptional activation domain (Yudkovsky et al., 1999). For these targeting assays we used a modified array DNA template which contains five high affinity GAL4 binding sites adjacent to the 5S repeat that harbors the HincII/SalI site (208-11S-GAL4; Yudkovsky et al., 1999). Reconstitution of nucleosomal arrays with this DNA template positions the GAL4 binding sites in the linker region between two positioned nucleosomes (Yudkovsky et al., 1999). Targeting of remodeling activity is then assayed in HincII reactions which contain a ^{32}P-labeled 208-11S-GAL4 array and 15-fold molar excess of an unlabeled 208-11S array (which lacks GAL4 sites). In the absence of targeting, the remodeling enzyme is sequestered by the excess unlabelled, competitor array and there is little stimulation of HincII digestion kinetics. Targeting of remodeling activity is scored by any stimulation of HincII digestion kinetics due to a functional GAL4 derivative (Yudkovsky et al., 1999). Note in this assay that a GAL4 derivative does not affect HincII digestion kinetics in the absence of remodeling complex or when the labeled and unlabeled arrays lack GAL4 binding sites. Previously, using this assay we were able to detect targeting of ySWI/SNF remodeling activity by GAL4-VP16 and GAL4-AH acidic activators (Yudkovsky et al., 1999).

We wished to investigate whether other members of the ATP-dependent family of remodeling enzymes could also be recruited by acidic activators in our purified system. Each of the six remodeling complexes were added in parallel to HincII targeting assays which contained 0.2 nM of ^{32}P-labeled 208-11S-GAL4 nucleosomal array, 3 nM unlabeled 208-11S nucleosomal array, and 10 nM of a GAL4 derivative (Figure V-3).

Figure V-3. Recruitment of ATP-dependent enzymes by GAL4-VP16.

HincII digestion kinetics of reactions containing 0.2 nM labeled 208-11S-GAL4 array, 3 nM unlabeled 208-11S competitor array in the presence or absence of 10 nM GAL4-VP16, and in the presence or absence of the indicated chromatin remodeling complexes. Note that addition of GAL4-VP16 had no effect on HincII digestion kinetics in the absence of remodeling complex. Experiment shown is representative of multiple, independent experiments.

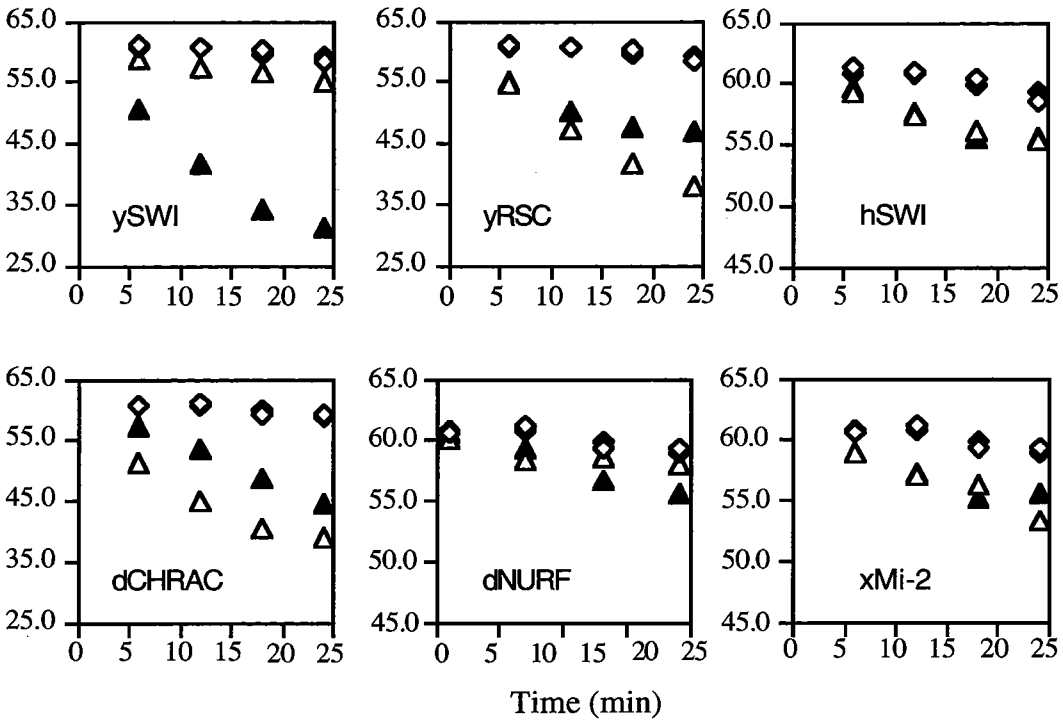

ySWI
yRSC
hSWI
dCHRAC
dNURF
xMi-2
Time (min)
HincII
HincII + Gal4-VP16
HincII + Remodeler
HincII + Remodeler + Gal4-VP16

Table V-2. Recruitment of Chromatin Remodeling Complexes by GAL4-VP16

Complex	-GAL4-VP16 (rate*)	+GAL4-VP16 (rate*)	fold rate stimulation
ySWI/SNF	$2 \times 1\ 0^{-3}$	12×10^{-3}	6
yRSC	$9 \times 1\ 0^{-3}$	$4 \times 1\ 0^{-3}$	-2.5
hSWI/SNF	$2 \times 1\ 0^{-3}$	$2 \times 1\ 0^{-3}$	0
dCHRAC	$8 \times 1\ 0^{-3}$	$6 \times 1\ 0^{-3}$	-1.3
dNURF	$1 \times 1\ 0^{-3}$	$2 \times 1\ 0^{-3}$	2
xMi-2	$2 \times 1\ 0^{-3}$	$2 \times 1\ 0^{-3}$	0

*First order rates of HincII digestion of 208-11S-GAL4 nucleosomal arrays

Under these reaction conditions, little remodeling of the labelled 208-11S-GAL4 array was observed in the absence of activator-dependent targeting. Likewise, addition of the isolated GAL4 DNA binding domain did not enhance HincII digestion kinetics in the presence or absence of remodeling enzyme (data not shown). Furthermore, similar to our previous studies (Yudkovsky et al., 1999), the remodeling activity of ySWI/SNF was effectively targeted to the 208-11S-GAL4 array by both the GAL4-VP16 and GAL4-AH acidic activators, as visualized by an activator- and ySWI/SNF-dependent stimulation of HincII digestion kinetics (Figure V-3 and data not shown; see also Table V-2). In contrast, the remodeling activity of yRSC, hSWI/SNF, dNURF, dCHRAC, or xMi-2 was not significantly targeted by either GAL4-VP16 or GAL4-AH activators (Figure V-3A and data not shown; see also Table V-1). In fact, we reproducibly observed some activator-dependent inhibition of remodeling by dCHRAC and yRSC (Figure V-3). Similar results were obtained in several independent experiments and with a range of remodeler concentrations. In the case of hSWI/SNF we also failed to observe targeting in this assay using an immunoaffinity purified form of this enzyme (Sif et al., 1998; P. Horn, R.E. Kingston, and C.L.Peterson, unpublished observations). Thus, for the six purified remodeling complexes tested here, only the remodeling activity of ySWI/SNF is effectively targeted by prototype acidic activators.

Discussion

Our results indicate that six different members of the ATP-dependent family of chromatin remodeling enzymes use similar levels of ATP hydrolysis to rapidly establish a

dynamic state of enhanced nucleosome mobilization. This "fluid" chromatin state is characterized by an enhanced accessibility of restriction enzymes and DNA binding transcription factors. Furthermore, the nearly identical rates of nucleosomal array remodeling (Figure V-1B) and the common requirement for histone H2A/H2B dimers (Figure V-2) are consistent with a similar remodeling mechanism for all members of this ATP-dependent family. Although the mechanistic details of "remodeling" are not clear, all of these enzymes can apparently transduce the energy of ATP hydrolysis into an enhanced mobilization of nucleosomes within linear arrays (as suggested in Wolffe, 1994).

In contrast to our studies with nucleosomal arrays, some differences between remodeling complexes have been observed with mononucleosome substrates. For example, ySWI/SNF, dNURF, and recombinant ISWI have been shown to a move histone octamer from a central position to an end position , whereas dCHRAC and ySWI/SNF can also move histone octamers in the opposite direction. dCHRAC, however, also contains the ISWI ATPase, and thus these differences are not intrinsic to the catalytic subunit or to the basic mechanism of remodeling. Alternatively, the differences in the direction of histone octamer movement may reflect the propensity of some complexes (such as dCHRAC) to bind to DNA ends. In this scenario, protection of the DNA ends may block end-directed movements and favor movements from the ends to more central locations. In contrast, on nucleosomal arrays, where free DNA ends do not flank individual nucleosomes, we propose that the direction of histone octamer movement is random for all remodeling complexes. This situation is consistent with our

observation that the rates of nucleosome remodeling in the coupled array assay are similar for all complexes.

We were surprised to discover that arrays reconstituted with histone $(H3\text{-}H4)_2$ tetramers are not efficiently remodeled by any of the complexes tested. In the absence of remodeling enzyme, arrays of $(H3\text{-}H4)_2$ are digested at rates only 3-5 fold faster than nucleosomal arrays (see Chapter IV of this thesis), whereas ATP-dependent remodeling of nucleosomal arrays typically yields 20-30 fold increases in restriction enzyme rates. Thus, arrays of $(H3\text{-}H4)_2$ tetramers still provide a potent barrier to factor access and, furthermore, the inability to score remodeling of tetramer arrays is not due to a high level of restriction enzyme cleavage in the absence of remodeling enzyme. Interestingly, this requirement for the histone H2A/H2B dimers also does not reflect an obligatory need for the N-terminal domains of these two histones, since xMi-2, ySWI/SNF, and hSWI/SNF are insensitive to removal of all the N-terminal domains (Figure V-2A). Instead, we favor a model in which all of these enzymes require a cannonical nucleosome structure either for substrate recognition or for the mechanism of remodeling. For instance, these enzymes may need to interact with two adjacent gyres of DNA in order to induce nucleosome mobilization (see also Chapter IV of this thesis).

Our data also suggest the dileneation of three groups within the ATP-dependent family: 1) a SWI/SNF group (ySWI/SNF, yRSC, hSWI/SNF) whose ATPase activity does not require an intact nucleosome and whose remodeling function is independent of the histone tails; 2) an ISWI group (dNURF, dCHRAC) whose ATPase activity requires an intact nucleosome and whose remodeling function is histone tail dependent; and 3) a

Mi-2 group (xMi-2) whose optimal ATPase activity requires an intact nucleosome and whose remodeling function is histone tail independent. In fact, we found that the remodelng activity of xMi-2 was actually enhanced by removal of the histone N-terminal domains (Figure V-2B). Similar results have been obtained previously using a subset of the complexes tested here as well as with recombinant BRG1 (ATPase subunit of hSWI/SNF) and ISWI. We note, however, that recombinant ISWI also shows significant stimulation of ATPase activity by free DNA. Furthermore, like dNURF and dCHRAC, the histone N-terminal domains promote efficient remodeling by ySWI/SNF and yRSC complexes under different reaction conditions where these enzymes must be catalytic (Logie et al., 1999). Thus, although the different nucleosome moiety requirements are important for defining distinctions among enzymes, these distinctions are likely to reflect subtle differences in nucleosome recognition or in regulation of the remodeling cycle (Logie et al., 1999; see also Peterson, 1998), rather than key differences in the basic remodeling mechanism.

Molecular phylogenetic analysis has been used to organize the SWI2/SNF2 family of DNA-stimulated ATPases into multiple subfamilies. These studies included sequence comparisons among different SWI2/SNF2 ATPase domains as well as among sequences N-terminal or C-terminal to the ATPase domain. Interestingly, ATPases from three of the subfamilies defined by phylogenetic analysis are the catalytic subunits associated with the three groups of ATP-dependent remodeling enzymes delineated by our biochemical analyses (e.g. SWI2, ISWI, Mi-2). This correspondence between such completely different experimental approaches was not expected, since the homology

among the ATPase domains of SWI2, ISWI, and Mi-2/CHD proteins is very high. One possibility is that a small number of amino acid changes can lead to large differences in nucleic acid substrate requirements (i.e. nucleosomes versus free DNA). Consistent with this view, previous studies have found that ATPase domain swaps between two members of the same subfamily (i.e. brahma and SWI2/SNF2) yield a SWI2 protein that retains function *in vivo* in yeast, whereas, swaps between members of different families (i.e. ISWI and SWI2/SNF2) are not functional. Alternatively, sequence elements that are unique to each subfamily that lie outside the ATPase domain (i.e. bromodomains, SANT domains, chromodomains) might also contribute to the interactions with the histone N-terminal domains or other nucleosomal components.

Although our comparative analysis delineates three groups of ATP-dependent remodeling enzymes, our data also suggests that individual enzymes within a single group are likely to be subject to differential modes of regulation. For instance, we found that ySWI/SNF was recruited by an acidic activator in our nucleosomal array system, whereas other members of the SWI/SNF group (e.g. yRSC, hSWI/SNF) were not. We anticipate that yRSC and hSWI/SNF can be recruited by other types of activators in this assay. Likewise, members of the ISWI or Mi-2 groups are likely to be recruited by nonacidic activators or by transcriptional repressors. These ideas are consistent with several previous studies. First, acidic activators are unable to recruit yRSC complex to an immobilized DNA template from a yeast nuclear transcription extract (Yudkovsky et al., 1999). Second, Mi-2 complexes are believed to function in transcriptional repression (Kehle et al., 1998; Wade et al., 1999) and thus it is not surprising that an acidic activator

is unable to recruit xMi-2. And finally, hSWI/SNF has recently been demonstrated to be targeted *in vivo* by the glucocorticoid receptor (Fryer, 1998), an isoform of C/EBP- β (Kowenz-Leutz and Leutz, 1999) and erythroid kruppel-like factor (EKLF) (Lee et al., 1999).

Clearly, acidic activators are likely to recruit ATP-dependent remodeling complexes in *Drosophila* and mammalian cells. One possibility is that there exists additional, uncharacterized members of the ATP-dependent remodeling family that can be recruited by acidic activators and which might play a key role in acidic activator function. It is also possible that regulatory subunits, which might facilitate interactions with acidic activators, have been lost during purification of one or more of the remodeling complexes that we have tested here. Alternatively, several of the more abundant complexes (e.g. dCHRAC and yRSC) may establish more global domains of "fluid" chromatin, and thus they may not rely on gene-specific targeting proteins.

Experimental Procedures

Reagent preparation. The array DNA template contains eleven tandem, head-to-tail repeats of a 208 bp Sea Urchin 5S rRNA gene (Logie and Peterson, 1997; Logie and Peterson, 1999). Template was isolated by digestion of plasmid pCL7c (208-11S) or pCL8b (208-11S-Gal4) with NotI, HindIII, and HhaI (New England Biolabs) followed by FPLC purification on Sephacryl-500 (Pharmacia) essentially as described (Logie and

Peterson, 1997; Logie and Peterson, 1999). Array DNA template was end-labeled by Klenow fill-in reaction with [α-^{32}P]dATP as described (Logie and Peterson, 1997; Logie and Peterson, 1999).

Chicken erythrocyte histone octamers were purified from chicken whole blood (Pel-Freez Biologicals) as described previously (Hansen et al., 1989). Hyperacetylated histone octamers were purified from butyrate-treated HeLa cells as described (Workman et al., 1991). Trypsinized histone octamers and $(H3/H4)_2$ tetramers were provided as a kind gift from Jeff Hansen and were purified as described (Hansen et al., 1989; Tse and Hansen, 1997). $(H3/H4)_2$ tetramers were dialyzed against Buffer T (1M NaCl, 10 mM Tris-HCl, pH 8.0, 0.25 mM EDTA, 0.1 mM DTT) prior to array reconstitution.

ySWI/SNF complex was purified from yeast strains CY396 or CY743 (*sin3Δ*) as described in Logie and Peterson (1999). The concentration of complex was determined to be approximately 300 nM by comparative Western Blot and by ATPase assays (Logie and Peterson, 1997; Logie and Peterson, 1999). yRSC (Cairns et al., 1996b), xMi-2 (Wade et al., 1999), dCHRAC (Varga-Weisz et al., 1997), dNURF (Tsukiyama and Wu, 1995), and hSWI/SNF "A" (Kwon et al., 1994) were purified as previously described. Approximate concentrations were estimated from total protein concentration in the purified fractions and complexes were assumed to be 100% active. Thus, our concentration estimates are likely to be an overestimate. Most complexes had a high degree of purity, but in the case of hSWI/SNF "A", purity was estimated to be ~10%. We confirmed that the activity monitored was in fact due to hSWI/SNF by antibody inhibition -- addition of antisera directed to the BRG1 subunit of hSWI/SNF eliminated

remodeling activity, whereas addition of preimmune sera had no effect (D. Hill and A.N.Imbalzano, unpublished observations). For all the studies described here, each assay was performed with two independent preparations of each remodeling complex, with the exception of yRSC.

Reconstitution and analysis of substrate arrays. Histone proteins used for array reconstitutions were analyzed by 18% SDS-PAGE and Coomassie staining. Octamer concentrations were determined by A_{230} (Stein, 1979). Histone octamers were reconstituted onto the 208-11S DNA templates (or 208-11S-Gal4 for recruitment assay) in a slide-a-lyzer dialysis cassette (Pierce) by salt gradient dialysis as previously described (Hansen et al., 1991). Each repeat of the 208-11S template (or 208-11S-Gal4 for recruitment assay) is flanked by EcoRI restriction enzyme sites. In addition, a novel MspI site is located 30 bp from the predicted dyad axis of symmetry of each positioned nucleosome. Array quality, saturation and positioning was determined by EcoRI or MspI digestion using approximately 20 nM array in Remodeling Buffer (5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCL (pH 8.0), 1 mM DTT) as previously described (Chapter IV; Hansen et al., 1991; Logie and Peterson, 1997; Logie and Peterson, 1999). Arrays were digested for 30 minutes at 37° C and electrophoresed on a 4% native polyacrylamide gel. The gel was briefly soaked in 2 μg/ml ethidium bromide and photographed under ultraviolet illumination. Saturation of arrays was analyzed by digestion with EcoRI and comparison of the ratio of nucleosome bound repeat to uncomplexed 208 bp 5S repeat. Positioning was analyzed by digestion with MspI (Chapter IV of this thesis; Logie and Peterson, 1997). Whereas, nucleosomal, trypsinized, and hyperacetylated arrays were

inaccessible to digestion with MspI, $(H3/H4)_2$ tetramer arrays digested with MspI released a mononucleosome size fragment indicating that the $(H3/H4)_2$ tetramers protect less DNA as expected (Luger et al., 1997).

Assay conditions. ATPase reactions were performed with respect to the optimal temperature for remodeling complex activity: 27° C for xMi-2, dCHRAC, and dNURF, 30°C for yRSC and ySWI/SNF, and 37°C for hSWI/SNF using 100 μM ATP and 0.2 μCi of [γ-^{32}P]-ATP (Amersham) in 0.1% Tween, 20 mM Tris, pH8, 5% glycerol, 0.2 mM DTT, 5 mM $MgCl_2$, 1 mM PMSF, and 0.1 mg/ml BSA as described (Logie and Peterson, 1997; Logie and Peterson, 1999; Logie et al., 1999). Released phosphate was monitored with time by resolution of P_i from ATP on plastic plates coated with PEI cellulose (EM Science) with 0.75 M KPO_4 (pH3.5) as solvent and quantified by Phosphorimager analysis.

Coupled array remodeling-restriction reactions were performed in a final concentration of 5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCL (pH 8.0), 1 mM DTT, 0.1 mg/ml BSA, 1 mM ATP, and 500 U/ml *Hinc* II (New England Biolabs) as previously described (Logie and Peterson, 1997; Logie and Peterson, 1999; Logie et al., 1999). Assays were performed with respect to the optimal temperature for remodeling complex activity (see above). *Hinc* II cleavage was quantified by PhosphorImager analysis, and first order rates were determined by curve fitting. In multiple independent experiments, the first-order rates of restriction enzyme cleavage for each particular combination of array and remodeler varied by less than 20%.

CHAPTER VI

CONCLUSIONS AND SUMMARY

CONCLUSIONS

The role of chromatin in the regulation of DNA-mediated biological processes such as replication, repair, recombination, and transcription has only been fully appreciated in the last decade. Recent years have been witness to the identification of an astonishing number of chromatin remodeling enzymes that have emerged as key regulatory elements of DNA accessibility. These enzymes are functionally distinct and can be categorized into two broad groups: ATP-dependent enzymes which use the energy of ATP hydrolysis to disrupt histone-DNA contacts and enzymes which covalently modify the histone proteins (for review see Workman and Kingston, 1998; Kingston and Narlikar, 1999).

Recent studies have only begun to shed light on the functional role of the two classes of multi-subunit enzymes in managing the equilibrium between inaccessible and accessible chromatin states. Interestingly, many previously characterized coactivators and corepressors of transcription have turned out to belong to one or the other group of chromatin remodeling enzymes. Nature has devised a system in which several aspects of these enzymes are conserved across eukaryotic phyla. The research presented in this thesis has focussed on understanding some of the conserved features of chromatin remodeling enzymes in order to obtain a more detailed understanding of how these machines function to regulate transcription.

Dissecting the Function of Individual Parts of the Machine

ATP-dependent chromatin remodeling enzymes are typically large, macromolecular assemblies (reviewed in Muchardt and Yaniv, 1999; Vignali et al., 2000). The purification and characterization of several complexes has led to the surprising finding that the SWI/SNF family of ATPases consist of a core of conserved subunits with homology to yeast *SWI3*, *SNF5*, and *SWP73* (see Chapter II). In as much, Swi2/Snf2p, Swi3p, and Snf5p homologs appear to define the functional core of these enzymes *in vitro* (Phelan et al., 1999). We chose to study Swi3p as it consists of a number of distinct motifs (Domains I, II, and III) which are also highly conserved in other Swi3p-family members (see Chapter II). Swi3p also contains a highly acidic amino terminal region which is not conserved. At the time, it had been suggested that SWI/SNF might function to remove histone H2A/H2B dimers to facilitate transcription factor binding to nucleosomes. Thus, we proposed that this region of high acidic content of Swi3p may serve an analogous function to that of histone chaperones to remove the dimers. We were surprised to find that deletion of the entire region did not have a negative effect on SWI/SNF activity, but rather promoted its activity nearly 2-fold. This suggested that this domain might define a novel regulatory domain in SWI/SNF by interacting with a negative regulator or by masking an active site. Further studies to distinguish between these possibilities should prove interesting, as it is unclear how these macromolecular assemblies are regulated.

Our studies show that Domain I (rich in hydrophobic and aromatic residues) was required for the assembly of a functional SWI/SNF complex. This suggested that

Domain I is important for interaction among SWI/SNF subunits. In addition, Domain III, which is composed of a putative leucine zipper, was also required for complex assembly. Further studies indicated that this region mediated self-association of Swi3p. This indicated that Swi3p was present in multiple copies in SWI/SNF. These data were consistent with other studies in which the leucine zipper of Rsc8/Swh3p (Swi3p homolog in RSC) was also required for self-association (Treich and Carlson, 1997). Interestingly, self-association of Swi3p provided a binding surface for the Snf6p subunit of the complex. Several studies also indicate that Swi3p family members can interact with both Swi2/Snf2p and Swp73p family members (Crosby et al., 1999; Phelan et al., 1999; Treich et al., 1995; Treich and Carlson, 1997; Treich et al., 1998). Together, these data suggest that Swi3p (and family members) may function as a scaffold for the assembly of SWI/SNF subunits. In this regard, self-association of Swi3p could provide an extensive surface in which to nucleate the assembly of a functional complex.

Although it appears that only a small number of subunits are required for full activity of these enzymes *in vitro*, most of the subunits (in addition to the conserved core) in the 11 polypeptide 2 MD SWI/SNF complex are required for its function *in vivo* (Cairns et al., 1996a; Laurent et al., 1993b; Peterson and Herskowitz, 1992). In as much, subunits of RSC are essential for viability (Cairns et al., 1998; Cairns et al., 1996b; Cao et al., 1997; Treich and Carlson, 1997). This suggests that the unique subunits among these enzymes perform additional essential functions. Future experiments will undoubtedly uncover the role of each subunit as it relates to the activity of the complex as a whole.

Swi3p also contains another highly conserved region, the SANT (*S*wi3, *A*da2, *N*-CoR, *T*FIIIB") domain (Domain II). Interestingly, this domain is contained within a number of transcriptional regulatory proteins that have turned out to be subunits of both groups of chromatin remodeling enzymes (see Chapter III; see also Table I-1.). The SANT domain exhibits homology to the DNA binding domain of the c-Myb proto-oncogene (Aasland, 1996; Ogata et al., 1992; Ogata et al., 1994). The presence of a c-Myb-DNA binding domain in chromatin remodelers was particularly interesting since it was not known how these enzymes interacted with chromatin. In as much, SWI/SNF and RSC complexes have been shown to possess high affinity for DNA (Quinn et al., 1996; Cairns et al., 1996b). Thus, it seemed possible that the SANT domain could mediate an interaction with nucleosomal DNA. We performed a structure/function analysis of Swi3p (SWI/SNF), Rsc8/Swh3p (RSC), and Ada2p (ADA, SAGA) in yeast to ascribe a function to the SANT domain in chromatin remodeling. Strikingly, this analysis showed that the SANT domain was absolutely required for the function of these proteins *in vivo*. Our studies, however, indicated that the SANT domain was unlikely to be involved in direct interaction with DNA, but was more likely involved in mediating protein-protein interactions.

Further analysis revealed the unexpected finding that the SANT domain may be required for physical and/or functional interactions with histone 'tails'. This was surprising in that additional histone 'tail' binding domains (bromodomain) already existed in the ATP-dependent and Histone Acetyltransferase (HAT) chromatin remodeling complexes. However, the bromodomain is not required for activity in many

cases (Elfring et al., 1998; Laurent et al., 1993b; Marcus et al., 1994), whereas the SANT domain is absolutely required in all cases tested (Chapter III). The implication of these findings are striking in that the SANT domain may be the major interacting surface that functionally links chromatin remodeling enzymes to their substrate. In any case, it will be interesting to determine the relationship between the bromodomain and the SANT domain.

It is also possible that the SANT domain may be more indirectly involved in histone 'tail' interactions. For instance, the SANT domain may be tethered to the catalytic subunit and serve to report changes in conformation upon substrate binding to the other subunits in the complex. This may be consistent with findings that a conformational change in the Myb-DBD occurs upon substrate binding (McIntosh et al., 1998; Zargarian et al., 1999). This scenario would also fit with our data implicating Swi3p as a scaffolding subunit thereby allowing the SANT domain to directly communicate with the other SWI/SNF subunits. It is intriguing to speculate that the SANT domain may undergo a conformational switching mechanism as a means for chromatin remodeling enzymes to function catalytically. Such an allosteric mechanism may be required to 'reset' the complex for additional rounds of 'remodeling'. This would be consistent with studies in which the histone 'tails' are required for catalytic remodeling by SWI/SNF (Logie and Peterson, 1999) and with the notion that the remodeling reaction is composed of a number of subreactions, each of which have to be regulated (discussed in Peterson, 1998). A similar mode of action may be envisioned for the activity of histone acetyltransferases as well. Obviously, more work needs to be done

to dissect the role of this highly conserved domain in chromatin remodeling. For instance, it would be predicted from these studies that SWI/SNF complexes that contain a small deletion in the *SWI3* SANT domain would be unable to support catalytic remodeling on nuclesomal arrays *in vitro*. In addition, it would be predicted that mutations in the SANT domain of the ATP-dependent remodeler ISWI (which requires histone 'tails' even for a single round of remodeling and lacks a bromodomain) would affect its chromatin remodeling activity on nucleosomal arrays *in vitro* as well.

The Whole is More than the Sum of its Parts

SWI/SNF was the first member of the ATP-dependent family of chromatin remodeling enzymes to be identified and has been shown to be fundamentally important for the regulation of eukaryotic transcription. It has been shown that purified SWI/SNF can disrupt nucleosome structure which leads to an enhanced accessibility of nucleosomal DNA to DNase I (Cote et al., 1998; Cote et al., 1994; Owen-Hughes and Workman, 1996), restriction enzymes (Logie and Peterson, 1997; Logie et al., 1999), and sequence specific DNA-binding proteins (Cote et al., 1998; Cote et al., 1994; Utley et al., 1997). However, the mechanistic basis for how SWI/SNF uses the energy of ATP hydrolysis to alter nucleosome structure has remained controversial. Several groups proposed that SWI/SNF disruption of chromatin leads to rearrangements of the histone octamer or displacement of the histone H2A/H2B dimers (Cote et al., 1994; Hirschhorn et al., 1992; Lee et al., 1999; Lorch et al., 1998; Luger et al., 1997; Schnitzler et al., 1998; Spangenberg et al., 1998). However, studies from other groups indicated that SWI/SNF

action does not dislodge the dimers, but alters the path of DNA around the histone octamer (Bazett-Jones et al., 1999; Cote et al., 1998). In order to distinguish between these possibilities, we took advantage of the amenability of the nucleosome to modification and the sensitive nucleosome array remodeling assay (Chapter IV).

In this study, we were able to show that SWI/SNF required a canonical nucleosome structure (which contains the full complement of histone proteins) for efficient and catalytic remodeling. In as much, SWI/SNF could efficiently remodel nucleosomes which contained a disulfide-linked $(H3/H4)_2$ tetramer suggesting that rearrangement of the tetramer interface is neither a requirement nor consequence of remodeling as was alluded to in previous studies. We also exploited the functional utility of an internal fluorescent probe within the histone octamer (covalently attached to a single cysteine in histone H3) to analyze the effects of SWI/SNF on nucleosomal arrays. Previous analysis with this type of fluorescently labeled nucleosome showed that the histone octamer undergoes salt-dependent conformational transitions (Dieterich et al., 1978; Dieterich et al., 1979). However, our study clearly indicated that neither dimer loss nor rearrangement of the $(H3/H4)_2$ tetramer was a result of SWI/SNF activity. Our data is consistent with a model in which SWI/SNF disrupts histone-DNA contacts without structural changes in the histone octamer. This has striking consequences for how we view the mechanism of remodeling (discussed in more detail below).

One rather interesting side point of these studies was that an array of $(H3/H4)_2$ tetramers was sufficiently refractory to DNA binding factors and that accessibility was enhanced by SWI/SNF although less efficiently as compared to nucleosomal arrays. This

suggests that $(H3/H4)_2$ may be a physiological target of remodeling enzymes and has important implications for how we may view the regulation of chromatin structure during periods of rapid histone turnover in chromatin. For instance, a tetramer of histones H3 and H4 are deposited on DNA and may remain this way for some time before a canonical nucleosome is formed by addition of the histone H2A/H2B dimers during replication. It is certain that binding factors still require access to DNA sequences during this time. Thus, it is possible that factors with low affinity for DNA may require additional help from chromatin remodeling enzymes as a result of tetramer deposition. This may provide a low level of regulation of the intrinsic competition between DNA binding factors and histones for DNA.

A growing number of ATP-dependent enzymes have been isolated from yeast to human. Each member of the ATP-dependent family of chromatin remodeling enzymes contains an ATPase subunit that is related to the SWI2/SNF2 subfamily of the DEAD/H superfamily of nucleic acid-stimulated ATPases (Eisen et al., 1995). Several studies have indicated that these enzymes are functionally distinct based largely on their activity in different assays. Although these enzymes fall into three subgroups based on sequences that lie outside of the ATPase domain (comparison included sequences within ATPase domain as well), they are strikingly similar with regard to the ATPase domain (Eisen et al., 1995). Thus, we wanted to compare these enzymes under a similar set of reaction conditions in order to elucidate any functional similarities or differences (Chapter V).

We were surprised to find that ySWI/SNF, yRSC, x-Mi-2, dCHRAC, dNURF, and hSWI/SNF, which encompass all three previously proposed subgroups, utilize similar

amounts of energy to remodel nucleosomal arrays in the coupled array remodeling assay (the remodeling assay is discussed in Chapters IV and V and in Logie et al., 1997). This suggested that the basic remodeling reaction may be the same among all of these enzymes. The studies shown in Chapter IV, together with this analysis, suggests that all ATP-dependent enzymes work similarly to disrupt histone-DNA interactions without consequence to the structure of the histone octamer. Comparative analysis also showed a common requirement for histone dimers for efficient remodeling by all of the ATP-dependent chromatin remodeling enzymes. This is consistent with the idea presented in Chapter IV that a canonical nucleosome is required for substrate recognition or for the mechanism of remodeling.

Our data also suggest the delineation of three groups within the ATP-dependent family: 1) a SWI/SNF group (ySWI/SNF, yRSC, and hSWI/SNF) whose ATPase activity does not require an intact nucleosome and whose remodeling function is independent of the histone 'tails'; 2) an ISWI group (dCHRAC, dNURF) whose ATPase activity requires an intact nucleosome and whose remodeling function is histone 'tail' dependent; and 3) a Mi-2 group whose optimal ATPase activity requires an intact nucleosome and whose remodeling function is histone 'tail' independent. Unexpectedly, ATPases from three of the subfamilies defined by phylogenetic analysis are the catalytic subunits associated with the three groups of ATP-dependent remodeling enzymes delineated by our biochemical analyses (e.g. SWI2, ISWI, Mi-2).

The ATPase domains among the three subgroups may have evolved subtle differences in nucleosome cofactor requirements or sequences outside the ATPase

domain specific to each subgroup might contribute to interactions with the histone N-terminal domains or other nucleosomal components. This may be the basis for these enzymes being categorized as functionally distinct. However, our data would suggest that although the different nucleosome moiety requirements are important for defining distinctions among enzymes, these distinctions are likely to reflect subtle differences in nucleosome recognition or in the regulation of the remodeling cycle (discussed in Peterson, 1998), rather than key differences in the basic remodeling mechanism. How might the ATP-dependent enzymes be regulated differently? One possibility is through their differential recruitment to chromatin. This would be consistent with our data in which only ySWI/SNF was recruited by an acidic activator in our nuclesomal array assay system. This is also consistent with recent studies that recruitment of ySWI/SNF and hSWI/SNF occur via different types of activators (Kim, 1999; Lee et al., 1999; Natarajan et al., 1999; Neely et al., 1999; Yudkovsky et al., 1999; see below for further discussion).

How might these enzymes disrupt histone-DNA interactions without loss or rearrangement of the histone octamer? This reaction may be directly related to the mechanism by which another helicase superfamily member, PcrA, destabilizes duplex DNA. This bacterial DNA helicase couples ATP binding and hydrolysis to localized DNA helix distortion (Soultanas et al., 2000; Velankar et al., 1999). PcrA contains the seven DNA helicase motifs that are also found in SWI2/SNF2 family members (Eisen et al., 1995). Recent functional and structural studies of PcrA have suggested that this monomeric enzyme utilizes ATP to carry out two distinct functions and that these functions can be uncoupled (Soultanas et al., 2000). We propose that SWI/SNF may

harbor only one of these two activities – ATP-dependent DNA duplex destabilization. This would likely cause a local disruption of histone-DNA contacts that could be propagated throughout the nucleosome. This hypothesis is entirely consistent with the data presented in Chapters IV and V. It is also consistent with recent studies showing that several ATP-dependent remodeling enzymes can apparently transduce the energy of ATP-hydrolysis into an enhanced mobilization of nucleosomes within linear arrays (Hamiche et al., 1999; Jaskelioff et al., 2000; Langst et al., 1999; Whitehouse et al., 1999). It has been estimated that SWI/SNF can hydrolyze approximately 1000 ATP's per minute. Thus, one could envision a scenario in which DNA duplex destabilization by these extremely active enzymes could ultimately lead to large changes in nucleosome positioning in a random fashion. Obviously, studies will need to be designed to test this prediction.

Summary and the Big Picture

The studies presented in this thesis address a basic fundamental question. What is the role of conserved features of chromatin remodeling enzymes? Our data support a notion that there is an underlying functional similarity among ATP-dependent chromatin remodeling enzymes and that functional similarities probably exist between the two classes of chromatin remodelers as well. Although initially this may seem somewhat surprising, it is important to note that these enzymes ultimately act on the same target – chromatin. Of course, this is an overly simplistic view and it must be recognized that ATP-dependent chromatin remodeling enzymes and histone acetyltransferases employ an

entirely different mechanism to alter the accessibility of DNA in the context of chromatin. The point of the matter is that there are conserved themes that underlay the basic fundamentals of chromatin remodeling (e.g. both classes of enzymes exploit the histone 'tails').

Why so many chromatin remodeling complexes? A number of studies suggest that the actions of ySWI/SNF and GCN5 are gene specific. Thus, each of these enzymes may perform some specific function or may be regulated in such a way so that they are available only for specific time periods. Alternatively, the activity of these enzymes may be targeted to promoters by virtue of their interaction with DNA-binding proteins. This interaction would provide the specificity and temporal regulation for these complexes. This idea has recently received a great deal of attention (discussed in Brown et al., 2000; Peterson and Workman, 2000; Vignali et al., 2000). It is interesting to note that the same activators and repressors are able to recruit different remodeling complexes. Moreover, these same activators and repressors can target members of the same class of remodeling enzymes. Specificity would be governed by external cues such as growth factor or stress signals or other chromatin modifications (see Strahl and Allis, 2000 for discussion). Thus, it is interesting to speculate that the activity of chromatin remodeling enzymes may be regulated by cellular cues. This would allow for combinatorial control of gene regulation and would allow for the integration of multiple cellular signals to achieve the proper transcriptional state and serve as a dynamic mechanism for regulation of higher order chromatin structure. On the other hand, it is also possible that the function of chromatin remodeling enzymes is context dependent. Consistent with this idea, it has

recently come to light that SWI/SNF and SAGA are largely required for expression of mitotic-specific genes (Krebs et al., 2000). Thus, the local chromatin structure may play a role in determining which enzymes are needed. This may be consistent with the idea of a histone code as presented by Strahl and Allis (Strahl and Allis, 2000).

How do chromatin remodeling enzymes work *in vivo*? The short answer is that we don't know. Although a great deal of genetic analyses and *in vitro* biochemistry suggests that these enzymes modify chromatin structure as a means to regulate DNA-mediated processes, it may not be that simple. What if our current tools are not targeted at asking the appropriate questions? Only limited evidence links the *in vitro* studies to the *in vivo* situation. For instance, SWI/SNF is required for the disruption of Mnase I digestion patterns and presumably chromatin structure in the *SUC2* promoter (Hirschhorn et al., 1992; Wu and Winston, 1997). Histones are clearly a target for acetyltransferases *in vivo*, however, it is not clear if this is the limit of what these enzymes can do. Studies showing that HATs can also acetylate a variety of other proteins including transcription factors and nonhistone components suggest that gene activation by HATs may be more complex than we think. In the heart of transcriptional regulation is RNA polymerase II. Is RNA PolII the ultimate target for chromatin remodeling enzymes? SAGA contains many of the TafIIs found in TFIID as well as subunits thought to interact with TBP suggesting that it may ultimately communicate with RNA PolII (reviewed in Brown et al., 2000). In some cases, HATs (e.g. CBP/p300) have been shown to be part of RNA PolII holoenzyme (reviewed in Berger, 1999; Brown et al., 2000). However, in most cases, it is unclear. It will also be interesting to determine how chromatin remodeling

enzymes contribute to other DNA-mediated processes such as DNA replication, recombination, and repair. Significantly, enzymes that have been implicated in these processes are also members of the helicase superfamily in which the catalytic subunits of ATP-dependent chromatin remodelers belong. Further studies await the generation of tools that allow for dissection of the activities of chromatin remodeling enzymes *in vivo*.

Why is it important to understand how chromatin remodeling enzymes function as transcriptional regulators? The manifestation of disease in many cases is a result of aberrant or untimely gene expression. Given the fact that many of the chromatin remodeling enzymes have been directly connected to transcriptional regulation, it is not surprising that recent reports link the activity of these enzymes to cancer (for review see Archer and Hodin, 1999; Jacobson and Pillus, 1999). An entire volume could be written to discuss the evidence that chromatin remodeling enzymes are causative agents for disease. The following will just provide a general overview. For instance, somatic mutations truncating the hSNF5 gene have been identified in several aggressive pediatric malignant rhabdoid tumors (Versteege et al., 1998). Thus, SWI/SNF in higher eukaryotes may contribute to cellular proliferation. Consistent with this idea, SWI/SNF can enhance the transcriptional activation of the estrogen, retinoic acid, and glucocorticoid receptors (Fryer, 1998; Ichinose et al., 1997; Trouche et al., 1997). On the other hand, SWI/SNF has recently been found in a complex with both Rb (retinoblastoma protein) and HDACs to promote E2F-mediated G1 arrest (Zhang et al., 2000). Interestingly, recent work also demonstrated the association between BRCA1 and a human SWI/SNF-related complex providing a link between chromatin remodeling and

breast cancer (Bochar et al., 2000). Thus, mutations in components of SWI/SNF appear to contribute to cancer progression. Histone acetyltransferases have also been broadly implicated in cancer. One example is provided by numerous studies that support a role for CBP and p300 as transcriptional integrators for physiological cues to coordinate cell-cycle regulation, differentiation, DNA repair and apoptosis (discussed in Jacobson and Pillus, 1999). It is obvious that disruption of important interactions mediated by or functional abrogation of CBP/p300 could lead to alteration of one or more of these processes and thereby lead to cancer or a variety of other diseases. There is little doubt that continued investigations to further define the physiological role of chromatin remodeling enzymes will provide a molecular basis for understanding disease. It is intriguing to speculate that chromatin remodeling enzymes may become targets for gene therapy or for novel drug therapies in the future.

REFERENCES

Aasland, R., Stewart, A. F., and Gibson, T. (1996). The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. Trends Biochem Sci *21*, 87-8.

Abate, C., Patel, L., III, F. J. R., and Curran, T. (1990). Redox regulation of Fos and Jun DNA-binding activity in vitro. Science *249*, 1157-1161.

Adams, C. C., and Workman, J. L. (1993). Nucleosome displacement in transcription. Cell *72*, 305-8.

Allard, S., Utley, R. T., Savard, J., Clarke, A., Grant, P., Brandl, C. J., Pillus, L., Workman, J. L., and Cote, J. (1999). NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATM-related cofactor Tra1p. Embo J *18*, 5108-19.

Allegra, P., Sterner, R., Clayton, D. F., and Allfrey, V. G. (1987). Affinity chromatographic purification of nucleosomes containing transcriptionally active DNA sequences. J Mol Biol *196*, 379-88.

Allfrey, V. G., Faulkner, R., and Mirsky, A. E. (1964). Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. Proc Natl Acad Sci USA *51*, 786-794.

Archer, S. Y., and Hodin, R. A. (1999). Histone acetylation and cancer. Curr Opin Genet Dev *9*, 171-4.

Arents, G., Burlingame, R. W., Wang, B. C., Love, W. E., and Moudrianakis, E. N. (1991). The nucleosomal core histone octamer at 3.1 A resolution: a tripartite protein assembly and a left-handed superhelix. Proc Natl Acad Sci U S A *88*, 10148-52.

Arents, G., and Moudrianakis, E. N. (1995). The histone fold: a ubiquitous architectural motif utilized in DNA compaction and protein dimerization. Proc Natl Acad Sci U S A *92*, 11170-4.

Arents, G., and Moudrianakis, E. N. (1993). Topography of the histone octamer surface: repeating structural motifs utilized in the docking of nucleosomal DNA. Proc Natl Acad Sci U S A *90*, 10489-93.

Auble, D. T., and Steggerda, S. M. (1999). Testing for DNA tracking by MOT1, a SNF2/SWI2 protein family member. Mol Cell Biol *19*, 412-23.

Ausio, J., Borochov, N., Seger, D., and Eisenberg, H. (1984a). Interaction of chromatin with NaCl and MgCl2. Solubility and binding studies, transition to and characterization of the higher-order structure. J Mol Biol *177*, 373-98.

Ausio, J., Seger, D., and Eisenberg, H. (1984b). 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.

Axel, R., Cedar, H., and Felsenfeld, G. (1974). Chromatin template activity and chromatin structure. Cold Spring Harb Symp Quant Biol *38*, 773-83.

Baer, B. W., and Rhodes, D. (1983). Eukaryotic RNA polymerase II binds to nucleosome cores from transcribed genes. Nature *301*, 482-8.

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*, 1470-8.

Bazett-Jones, D. P., Mendez, E., Czarnota, G. J., Ottensmeyer, F. P., and Allfrey, V. G. (1996). Visualization and analysis of unfolded nucleosomes associated with transcribing chromatin. Nucleic Acids Res *24*, 321-9.

Belmont, A. S., and Bruce, K. (1994). Visualization of G1 chromosomes: a folded, twisted, supercoiled chromonema model of interphase chromatid structure. J Cell Biol *127*, 287-302.

Belyaev, N., Keohane, A. M., and Turner, B. M. (1996). Differential underacetylation of histones H2A, H3 and H4 on the inactive X chromosome in human female cells. Hum Genet *97*, 573-8.

Berger, S. L. (1999). Gene activation by histone and factor acetyltransferases. Curr Opin Cell Biol *11*, 336-341.

Berger, S. L., Pina, B., Silverman, N., Marcus, G. A., Agapite, J., Regier, J. L., Triezenberg, S. J., and Guarente, L. (1992). Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic acid domains. Cell *70*, 251-265.

Biggar, S. R., and Crabtree, G. R. (1999). Continuous and widespread roles for the Swi-Snf complex in transcription. EMBO J *18*, 2254-2264.

Bochar, D. A., Wang, L., Beniya, H., Kinev, A., Xue, Y., Lane, W. S., Wang, W., Kashanchi, F., and Shiekhattar, R. (2000). BRCA1 is Associated with a Human

SWI/SNF-Related Complex: Linking Chromatin Remodeling to Breast Cancer. Cell *102*, 257-265.

Bohm, L., and Crane-Robinson, C. (1984). Proteases as structural probes for chromatin: the domain structure of histones. Bioscience Rep *4*, 365-386.

Boyer, L. A., Shao, X., Ebright, R. H., and Peterson, C. L. (2000). Roles of the histone H2A-H2B dimers and the $(H3/H4)_2$ tetramer in nucleosome remodeling by the SWI-SNF complex. J Biol Chem *275*, 11545-11552.

Boyer, L. A., and Peterson, C. L. (2000). Actin-related proteins (Arps): conformational switches for chromatin-remodeling machines? BioEssays *22*, 666-672.

Braunstein, M., Rose, A. B., Holmes, S. G., Allis, C. D., and Broach, J. R. (1993). Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. Genes Dev *7*, 592-604.

Breeden, L., and Nasmyth, K. (1987). Cell cycle control of the yeast HO gene: cis- and trans-acting regulators. Cell *48*, 389-397.

Brown, C. E., Lechner, T., Howe, L., and Workman, J. L. (2000). The many HATs of transcription coactivators. Trends Biochem Sci *25*, 15-9.

Brownell, J. E., and Allis, C. D. (1995). An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in Tetrahymena macronuclei. Proc Natl Acad Sci U S A *92*, 6364-8.

Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y., and Allis, C. D. (1996). Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell *84*, 843-51.

Burns, L. G., and Peterson, C. L. (1997a). Protein complexes for remodeling chromatin. Biochim Biophys Acta *1350*, 159-68.

Burns, L. G., and Peterson, C. L. (1997b). The yeast SWI-SNF complex facilitates binding of a transcriptional activator to nucleosomal sites in vivo. Mol Cell Biol *17*, 4811-9.

Cairns, B. R. (1998). Chromatin remodeling machines: similar motors, ulterior motives. Trends Biochem Sci *23*, 20-5.

Cairns, B. R., Erdjument-Bromage, H., Tempst, P., Winston, F., and Kornberg, R. D. (1998). Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF. Mol Cell *2*, 639-51.

Cairns, B. R., Kim, Y.-J., Sayre, M. H., Laurent, B. C., and Kornber, 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 USA *91*, 1950-1954.

Cairns, B. R., Levinson, R. S., Yamamoto, K. R., and Kornberg, R. D. (1996a). Essential role of Swp73p in the function of yeast Swi/Snf complex. Genes Dev *10*, 2131-44.

Cairns, B. R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B., and Kornberg, R. D. (1996b). RSC, an essential, abundant chromatin-remodeling complex. Cell *87*, 1249-60.

Cairns, B. R., Schlichter, A., Erdjument-Bromage, H., Tempst, P., Kornberg, R. D., and Winston, F. (1999). Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains. Mol Cell *4*, 715-23.

Camerini-Otero, R. D., and Felsenfeld, G. (1977). Histone H3 disulfide dimers and nucleosome structure. Proc Natl Acad Sci U S A *74*, 5519-5523.

Candau, R., and Berger, S. L. (1996). Structural and functional analysis of yeast putative adaptors. J Biol Chem *271*, 5237-5245.

Candau, R., Zhou, J. X., Allis, C. D., and Berger, S. L. (1997). Histone acetyltransferase activity and interaction with ADA2 are critical for GCN5 function in vivo. Embo J *16*, 555-65.

Cantor, C. R., Dieterich, A. E., and Prior, C. P. (1981). Fluorescence studies on chromatin structure. Ann N Y Acad Sci *366*, 246-52.

Cao, Y., Cairns, B. R., Kornberg, R. D., and Laurent, B. C. (1997). Sfh1p, a component of a novel chromatin-remodeling complex, is required for cell cycle progression. Mol Cell Biol *17*, 3323-34.

Chen, T. A., Smith, M. M., Le, S. Y., Sternglanz, R., and Allfrey, V. G. (1991). Nucleosome fractionation by mercury affinity chromatography. Contrasting distribution of transcriptionally active DNA sequences and acetylated histones in nucleosome fractions of wild-type yeast cells and cells expressing a histone H3 gene altered to encode a cysteine 110 residue. J Biol Chem *266*, 6489-98.

Cheung, W. L., Briggs, S. D., and Allis, C. D. (2000). Acetylation and chromosomal functions. Curr Opin Cell Biol *12*, 326-333.

Corona, D. F., Eberharter, A., Budde, A., Deuring, R., Ferrari, S., Varga-Weisz, P., Wilm, M., Tamkun, J., and Becker, P. B. (2000). Two histone fold proteins, CHRAC-14

and CHRAC-16, are developmentally regulated subunits of chromatin accessibility complex (CHRAC). Embo J *19*, 3049-59.

Corona, D. F., Langst, G., Clapier, C. R., Bonte, E. J., Ferrari, S., Tamkun, J. W., and Becker, P. B. (1999). ISWI is an ATP-dependent nucleosome remodeling factor. Mol Cell *3*, 239-45.

Cosma, M. P., Tanaka, T., and Nasmyth, K. (1999). Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. Cell *97*, 299-311.

Cote, J., Peterson, C. L., and Workman, J. L. (1998). Perturbation of nucleosome core structure by the SWI/SNF complex persists after its detachment, enhancing subsequent transcription factor binding. Proc Natl Acad Sci U S A *95*, 4947-52.

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.

Crosby, M. A., Miller, C., Alon, T., Watson, K. L., Verrijzer, C. P., Goldman-Levi, R., and Zak, N. B. (1999). The trithorax group gene moira encodes a brahma-associated

putative chromatin-remodeling factor in Drosophila melanogaster. Mol Cell Biol *19*, 1159-70.

Czarnota, G. J., Bazett-Jones, D. P., Mendez, E., Allfrey, V. G., and Ottensmeyer, F. P. (1997). High resolution microanalysis and three-dimensional nucleosome structure associated with transcribing chromatin. Micron *28*, 419-31.

Czarnota, G. J., and Ottensmeyer, F. P. (1996). Structural states of the nucleosome. J Biol Chem *271*, 3677-83.

Daban, J. R., and Cantor, C. R. (1989). Methods Enzymol. *170*, 192-214.

Davie, J. R. and Spencer, V. A. (1999). Control of histone modifications. J Cell Biochem *32-33*, 141-148.

de la Serna, I. L., A., C. K., Hill, D. A., Guidi, C. J., Stephenson, R. O., Sif, S., Kingston, R. E., and Imbalzano, A. N. (2000). Mammalian SWI/SNF complexes contribute to activation of the hsp70 gene. Mol Cell Biol *20*, 2839-2851.

Deuring, R., Fanti, L., Armstrong, J. A., Sarte, M., Papoulas, O., Prestel, M., Daubresse, G., Verardo, M., Moseley, S. L., Berloco, M., Tsukiyama, T., Wu, C., Pimpinelli, S., and Tamkun, J. W. (2000). The ISWI chromatin-remodeling protein is required for gene

expression and the maintenance of higher order chromatin structure in vivo. Molec Cell *5*, 355-365.

Dhalluin, C., Carlson, J. E., Zeng, L., He, C., Aggarwal, A. K., and Zhou, M. (1999). Structure and ligand of a histone acetyltransferase bromodomain. Nature *399*, 491-496.

Dieterich, A. E., Axel, R., and Cantor, C. R. (1978). Dynamics of nucleosome structure studied by fluorescence. Cold Spring Harb Symp Quant Biol *42*, 199-206.

Dieterich, A. E., Axel, R., and Cantor, C. R. (1979). Salt-induced structural changes of nucleosome core particles. J Mol Biol *129*, 587-602.

Dimova, D., Nackerdien, Z., Furgeson, S., Eguchi, S., and Osley, M. A. (1999). A role for transcriptional repressors in targeting the yeast SWI/SNF complex. Mol Cell *4*, 75-83.

Dingwall, A. K., Beek, S. J., McCallum, C. M., Tamkun, J. W., Kalpana, G. V., Goff, S. P., and Scott, M. P. (1995). The Drosophila snr1 and brm proteins are related to yeast SWI/SNF proteins and are components of a large protein complex. Mol Biol Cell *6*, 777-91.

Dong, F., Hansen, J. C., and van Holde, K. E. (1990). DNA and protein determinants of nucleosome positioning on sea urchin 5S rRNA gene sequences in vitro. Proc Natl Acad Sci U S A *87*, 5724-8.

Dong, F., and van Holde, K. E. (1991). Nucleosome positioning is determined by the (H3-H4)2 tetramer. Proc Natl Acad Sci U S A *88*, 10596-600.

Eberharter, A., Sterner, D. E., Schieltz, D., Hassan, A., Yates, J. R. 3rd, Berger, S. L., Workman, J. L. (1999). The ADA complex is a distinct histone acetyltransferase complex in Saccharomyces cerevisiae. Mol Cell Biol *19*, 6621-6631.

Eisen, J. A., Sweder, K. S., and Hanawalt, P. C. (1995). Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. Nucleic Acids Res *23*, 2715-23.

Elfring, L. K., Daniel, C., Papoulas, O., Deuring, R., Sarte, M., Moseley, S., Beek, S. J., Waldrip, W. R., Daubresse, G., DePace, A., Kennison, J. A., and Tamkun, J. W. (1998). Genetic analysis of brahma: the Drosophila homolog of the yeast chromatin remodeling factor SWI2/SNF2. Genetics *148*, 251-65.

Elfring, L. K., Deuring, R., McCallum, C. M., Peterson, C. L., and Tamkun, J. W. (1994). Identification and characterization of Drosophila relatives of the yeast transcriptional activator SNF2/SWI2. Mol Cell Biol *14*, 2225-34.

Eshaghpour, H., Dieterich, A. E., Cantor, C. R., and Crothers, D. M. (1980). Singlet-singlet energy transfer studies of the internal organization of nucleosomes. Biochemistry *19*, 1797-805.

Feinstein, D. L., and Moudrianakis, E. N. (1986). Thiol reactivity of histone H3 in soluble and DNA-associated histone complexes: evidence for allosteric and torsional regulation. Biochemistry *25*, 8409-18.

Fitzsimmons, D. W., and Wolstenholme, G. E. W. (1976). The structure and function of chromatin. In Ciba Foundation Symposium, 368.

Fletcher, T. M., and Hansen, J. C. (1995). Core histone tail domains mediate oligonucleosome folding and nucleosomal DNA organization through distinct molecular mechanisms. J Biol Chem *270*, 25359-62.

Fletcher, T. M., and Hansen, J. C. (1996). The nucleosomal array: structure/function relationships. Crit Rev Eukaryot Gene Expr *6*, 149-88.

Fletcher, T. M., Krishnan, U., Serwer, P., and Hansen, J. C. (1994). Quantitative agarose gel electrophoresis of chromatin: nucleosome- dependent changes in charge, sharp, and deformability at low ionic strength. Biochemistry *33*, 2226-33.

Fryer, C. J., Archer, Trevor K. (1998). Chromatin remodeling by the glucocorticoid receptor requires the BRG1 complex. Nature *393*, 88-91.

Gabrielsen, O. S., Sentenac, A., and Fromageot, P. (1991). Specific DNA binding by c-Myb: evidence for a double helix-turn-helix-related motif. Science *253*, 1140-1143.

Garcia-Ramirez, M., Dong, F., and Ausio, J. (1992). Role of the histone "tails" in the folding of oligonucleosomes depleted of histone H1. J Biol Chem *267*, 19587-95.

Geitz, R. D., and Scheistl, R. H. (1991). Applications of high efficiency lithium acetate transformation of intact yeast cells using single stranded nucleic acids as carrier. Yeast *7*, 253-263.

Georgel, P. T., Tsukiyama, T., and Wu, C. (1997). Role of histone tails in nucleosome remodeling by Drosophila NURF. Embo J *16*, 4717-26.

Gonda, T. J., Gough, N. M., Dunn, A. R., and de Blaquiere, J. (1985). Nucleotide sequence of cDNA clones of the murine myb protooncogene. EMBO J *4*, 2003-2008.

Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones:

characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev *11*, 1640-50.

Grant, P. A., Schieltz, D., Pray-Grant, M. G., Steger, D. J., Reese, J. C., Yates, J. R., 3rd, and Workman, J. L. (1998a). A subset of TAF(II)s are integral components of the SAGA complex required for nucleosome acetylation and transcriptional stimulation. Cell *94*, 45-53.

Grant, P. A., Schieltz, D., Pray-Grant, M. G., Yates, J. R., 3rd, and Workman, J. L. (1998b). The ATM-related cofactor Tra1 is a component of the purified SAGA complex. Mol Cell *2*, 863-7.

Groudine, M., Kohwi-Shigematsu, T., Gelinas, R., Stamatoyannopoulos, G., and Papayannopoulou, T. (1983). Human fetal to adult hemoglobin switching: changes in chromatin structure of the beta-globin gene locus. Proc Natl Acad Sci U S A *80*, 7551-5.

Groudine, M., and Weintraub, H. (1982). Propagation of globin DNAase I-hypersensitive sites in absence of factors required for induction: a possible mechanism for determination. Cell *30*, 131-9.

Grunstein, M. (1992). Histones as regulators of genes. Sci Am *267*, 68-74.

Grunstein, M. (1997). Histone acetylation in chromatin structure and transcription. Nature *389*, 349-52.

Guehman, S., Vorbrueggen, G., Kalkbrenner, F., and Moelling, K. (1992). Reduction of a conserved Cys is essential for Myb DNA-binding. Nucl Acids Res *20*, 2279-2286.

Hamiche, A., Sandaltzopoulos, R., Gdula, D. A., and Wu, C. (1999). ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. Cell *97*, 833-42.

Hansen, J. C., and Ausio, J. (1992). Chromatin dynamics and the modulation of genetic activity. Trends Biochem Sci *17*, 187-91.

Hansen, J. C., Ausio, J., Stanik, V. H., and van Holde, K. E. (1989). Homogeneous reconstituted oligonucleosomes, evidence for salt-dependent folding in the absence of histone H1. Biochemistry *28*, 9129-36.

Hansen, J. C., and Lohr, D. (1993). Assembly and structural properties of subsaturated chromatin arrays. J Biol Chem *268*, 5840-8.

Hansen, J. C., van Holde, K. E., and Lohr, D. (1991). The mechanism of nucleosome assembly onto oligomers of the sea urchin 5 S DNA positioning sequence. J Biol Chem *266*, 4276-82.

Hansen, J. C., and Wolffe, A. P. (1994). A role for histones H2A/H2B in chromatin folding and transcriptional repression. Proc Natl Acad Sci U S A *91*, 2339-43.

Hayes, J. J., Clark, D. J., and Wolffe, A. P. (1991). Histone contributions to the structure of DNA in the nucleosome. Proc Natl Acad Sci U S A *88*, 6829-33.

Hayes, J. J., and Wolffe, A. P. (1992). Histones H2A/H2B inhibit the interaction of transcription factor IIIA with the Xenopus borealis somatic 5S RNA gene in a nucleosome. Proc Natl Acad Sci U S A *89*, 1229-33.

Hebbes, T. R., Clayton, A. L., Thorne, A. W., and Crane-Robinson, C. (1994). Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken beta-globin chromosomal domain. Embo J *13*, 1823-30.

Hebbes, T. R., Thorne, A. W., and Crane-Robinson, C. (1988). A direct link between core histone acetylation and transcriptionally active chromatin. Embo J *7*, 1395-402.

Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S. M., and Grunstein, M. (1995). Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: A molecular model for the formation of heterochromatin in yeast. Cell *80*, 583-592.

Hewish, D. R., and Burgoyne, L. A. (1973). Chromatin sub-structure. The digestion of chromatin DNA at regularly spaced sites by a nuclear deoxyribonuclease. Biochem Biophys Res Commun *52*, 504-10.

Hirschhorn, J. N., Brown, S. A., Clark, C. D., and Winston, F. (1992). Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. Genes Dev *6*, 2288-98.

Holstege, F. C. P., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengarner, 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.

Horiuchi, J., Silverman, N., Marcus, G. A., and Guarente, L. (1995). ADA3, a putative transcriptional adaptor, consists of two separable domains and interacts with ADA2 and GCN5 in a trimeric complex. Mol Cell Biol *15*, 1203-1209.

Horiuchi, J., Silverman, N., Pina, B., Marcus, G. A., and Guarente, L. (1997). ADA1, a novel component of the ADA/GCN5 complex, has broader effects than GCN5, ADA2, or ADA3. Molec Cell Biol *17*, 3220-3228.

Ichinose, H., Garnier, J. M., Chambon, P., and Losson, R. (1997). Ligand-dependent interaction between the estrogen receptor and the human homologues of SWI2/SNF2. Gene *188*, 95-100.

Ikeda, K., Steger, D. J., Eberharter, A., and Workman, J. L. (1999). Activation domain-specific and general transcription stimulation by native histone acetyltransferase complexes. Mol Cell Biol *19*, 855-63.

Imbalzano, A. N., Kwon, H., Green, M. R., and Kingston, R. E. (1994). Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature *370*, 481-5.

Ito, T., Bulger, M., Pazin, M. J., Kobayashi, R., and Kadonaga, J. T. (1997). ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. Cell *90*, 145-55.

Jacobson, S., and Pillus, L. (1999). Modifying chromatin and concepts of cancer. Curr Opin Genet Dev *9*, 175-84.

Jaskelioff, M., Gavin, I., 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.

Jeppesen, P., Mitchell, A., Turner, B., and Perry, P. (1992). Antibodies to defined histone epitopes reveal variations in chromatin conformation and underacetylation of centric heterochromatin in human metaphase chromosomes. Chromosoma *101*, 322-32.

Jeppesen, P., and Turner, B. M. (1993). The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. Cell *74*, 281-9.

John, S., Howe, L., Tafrov, S. T., Grant, P. A., Sternglanz, R., and Workman, J. L. (2000). The something about silencing protein, Sas3, is the catalytic subunit of NuA3, a yTAF(II)30-containing HAT complex that interacts with the Spt16 subunit of the yeast CP (Cdc68/Pob3)-FACT complex. Genes Dev *14*, 1196-208.

Johns, E. W. (1982). The HMG chromosomal proteins., E. W. Johns, ed. (London: Academic Press).

Johns, E. W. (1967). A method for the selective extraction of histone fractions f2(a)1 and f2(a)2 from calf thymus deoxyribonucleoprotein at pH7. Biochem J *105*, 611-4.

Kadosh, D., and Struhl, K. (1998a). Histone deacetylase activity of Rpd3 is important for transcriptional repression in vivo. Genes Dev *12*, 797-805.

Kadosh, D., and Struhl, K. (1998b). Targeted recruitment of the Sin3-Rpd3 histone deacetylase complex generates a highly localized domain of repressed chromatin in vivo. Mol Cell Biol *18*, 5121-7.

Kehle, J., Beuchle, D., Treuheit, S., Christen, B., Kennison, J. A., Bienz, M., and Muller, J. (1998). dMi-2, a hunchback-interacting protein that functions in polycomb repression. Science *282*, 1897-900.

Kim, J., Sif, S., Jones, B., Jackson, A., Koipally, J., Heller, E., Winandy, S., Viel, A., Sawyer, A., Ikeda, T., Kingston, R. E., Georgopoulos, K. (1999). Ikaros DNA-binding proteins direct formation of chromatin remodeling complexes in lymphocytes. Immunity *10*, 345-355.

Kingston, R. E., and Narlikar, G. J. (1999). ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. Genes Dev *13*, 2339-52.

Klempnauer, K. H., and Sippel, A. E. (1987). The highly conserved amino-terminal region of the protein encoded by the v-myb oncogene functions as a DNA binding domain. EMBO J *6*, 2719-2725.

Knezetic, J. A., and Luse, D. S. (1986). The presence of nucleosomes on a DNA template prevents initiation by RNA polymerase II in vitro. Cell *45*, 95-104.

Knoepfler, P. S., and Eisenman, R. N. (1999). Sin meets NuRD and other tails of repression. Cell *99*, 447-50.

Kornberg, R. D., and Thomas, J. O. (1974). Chromatin structure; oligomers of the histones. Science *184*, 865-8.

Kowenz-Leutz, E., and Leutz, A. (1999). A C/EBP beta isoform recruits the SWI/SNF complex to activate myeloid genes. Mol Cell *4*, 735-43.

Krebs, J. E., Fry, C. J., Samuels, M., and Peterson, C. L. (2000). Global role for chromatin remodeling enzymes in mitotic gene expression. Cell *102*, 587-598.

Krebs, J. E., Kuo, M. H., Allis, C. D., and Peterson, C. L. (1999). Cell cycle-regulated histone acetylation required for expression of the yeast HO gene. Genes Dev *13*, 1412-21.

Krebs, J. E., and Peterson, C. L. (2000). Understanding "active" chromatin: a historical perspective of chromatin remodeling. Crit Rev Eukaryot Gene Expr *10*, 1-12.

Kuo, M. H., Zhou, J., Jambeck, P., Churchill, M. E., and Allis, C. D. (1998). Histone acetyltransferase activity of yeast Gcn5p is required for the activation of target genes in vivo. Genes Dev *12*, 627-39.

Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E., and Green, M. R. (1994). Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. Nature *370*, 477-81.

Lacy, E., and Axel, R. (1975). Analysis of DNA of isolated chromatin subunits. Proc Natl Acad Sci U S A *72*, 3978-82.

Langst, G., Bonte, E. J., Corona, D. F., and Becker, P. B. (1999). Nucleosome movement by CHRAC and ISWI without disruption or trans- displacement of the histone octamer. Cell *97*, 843-52.

Laurent, B. C., Treich, I., and Carlson, M. (1993). Role of yeast SNF and SWI proteins in transcriptional activation. Cold Spring Harb Symp Quant Biol *58*, 257-63.

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*, 583-91.

Lee, C. H., Murphy, M. R., Lee, J. S., and Chung, J. H. (1999). Targeting a SWI/SNF-related chromatin remodeling complex to the beta- globin promoter in erythroid cells. Proc Natl Acad Sci U S A *96*, 12311-5.

Lee, D. Y., Hayes, J. J., Pruss, D., and Wolffe, A. P. (1993). A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell *72*, 73-84.

Lee, K. M., Sif, S., Kingston, R. E., and Hayes, J. J. (1999). hSWI/SNF disrupts interactions between the H2A N-terminal tail and nucleosomal DNA. Biochemistry *38*, 8423-9.

Lenfant, F., Mann, R. K., Thomsen, B., Ling, X., and Grunstein, M. (1996). All four core histone N-termini contain sequences required for the repression of basal transcription in yeast. Embo J *15*, 3974-85.

LeRoy, G., Orphanides, G., Lane, W. S., and Reinberg, D. (1998). Requirement of RSF and FACT for transcription of chromatin templates in vitro. Science *282*, 1900-4.

Ling, X., Harkness, T. A., Schultz, M. C., Fisher-Adams, G., and Grunstein, M. (1996). Yeast histone H3 and H4 amino termini are important for nucleosome assembly in vivo and in vitro: redundant and position-independent functions in assembly but not in gene regulation. Genes Dev *10*, 686-99.

Logie, C., and Peterson, C. L. (1997). Catalytic activity of the yeast SWI/SNF complex on reconstituted nucleosome arrays. Embo J *16*, 6772-82.

Logie, C., and Peterson, C. L. (1999). Purification and biochemical properties of yeast SWI/SNF complex. Methods Enzymol *304*, 726-41.

Logie, C., Tse, C., Hansen, J. C., and Peterson, C. L. (1999). The core histone N-terminal domains are required for multiple rounds of catalytic chromatin remodeling by the SWI/SNF and RSC complexes. Biochemistry *38*, 2514-22.

Lorch, Y., Cairns, B. R., Zhang, M., and Kornberg, R. D. (1998). Activated RSC-nucleosome complex and persistently altered form of the nucleosome. Cell *94*, 29-34.

Lorch, Y., LaPointe, J. W., and Kornberg, R. D. (1987). Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. Cell *49*, 203-10.

Lorch, Y., Zhang, M., and Kornberg, R. D. (1999). Histone octamer transfer by a chromatin-remodeling complex. Cell *96*, 389-92.

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 A resolution. Nature *389*, 251-260.

Luger, K., and Richmond, T. J. (1998a). The histone tails of the nucleosome. Curr Opin Genet Dev *8*, 140-6.

Luger, K., and Richmond, T. J. (1998b). DNA binding within the nucleosome core. Curr Opin Struct Biol *8*, 33-40.

Marcus, G. A., Silverman, N., Berger, S. L., Horiuchi, J., and Guarente, L. (1994). Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adaptors. EMBO J *13*, 4807-4815.

Matsui, T. (1987). Transcription of adenovirus 2 major late and peptide IX genes under conditions of in vitro nucleosome assembly. Mol Cell Biol *7*, 1401-8.

McIntosh, P. B., Frenkiel, T. A., Wollborn, U., McCormick, J. E., Klempnauer, K. H., Feeney, J., and Carr, M. D. (1998). Solution structure of the B-Myb DNA-binding

domain: a possible role for conformational instability of the protein in DNA binding and control of gene expression. Biochemistry *37*, 9619-29.

Moreira, J. M. A., and Holmberg, S. (1999). Transcriptional repression of the yeast CHA1 gene requires the chromatin-remodeling complex RSC. EMBO J *18*, 2836-2844.

Muchardt, C., and Yaniv, M. (1999). ATP-dependent chromatin remodelling: SWI/SNF and Co. are on the job. J Mol Biol *293*, 187-98.

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*, 657-64.

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*, 649-55.

Neigeborn, L., and Carlson, M. (1984). Genes affecting the regulation of SUC2 gene expression by glucose repression in *Saccharomyces cerevisiae*. Genetics *108*, 845-858.

Ner, S. S. (1992). HMGs Everywhere. Curr Biol *2*, 208.

Ogata, K., Hojo, H., Aimoto, S., Nakai, T., Nakamura, H., Sarai, A., Ishii, S., and Nishimura, Y. (1992). Solution structure of a DNA-binding unit of Myb: a helix-turn-helix-related motif with conserved tryptophans forming a hydrophobic core. Proc Natl Acad Sci U S A *89*, 6428-32.

Ogata, K., Kanai, H., Inoue, T., Sekikawa, A., Sasaki, M., Nagadoi, A., Sarai, A., Ishii, S., and Nishimura, Y. (1993). Solution structures of Myb DNA-binding domain and its complex with DNA. Nucleic Acids Symp Ser *29*, 201-2.

Ogata, K., Morikawa, S., Nakamura, H., Sekikawa, A., Inoue, T., Kanai, H., Sarai, A., Ishii, S., and Nishimura, Y. (1994). Solution structure of a specific DNA complex of the Myb DNA-binding domain with cooperative recognition helices. Cell *79*, 639-48.

Olins, A. L., and Olins, D. E. (1974). Spheroid chromatin units (v bodies). Science *183*, 330-2.

Ornaghi, P., Ballario, P., Lena, A. M., Gonzalez, A., Filetici, P. (1999). The bromodomain of Gcn5p interacts in vitro with specific residues in the N terminus of histone H4. J Mol Biol *287*, 1-7.

Owen-Hughes, T., Utley, R. T., Cote, J., Peterson, C. L., and Workman, J. L. (1996). Persistent site-specific remodeling of a nucleosome array by transient action of the SWI/SNF complex. Science *273*, 513-6.

Owen-Hughes, T., and Workman, J. L. (1996). Remodeling the chromatin structure of a nucleosome array by transcription factor-targeted trans-displacement of histones. Embo J *15*, 4702-12.

Pabo, C. O., and Sauer, R. T. (1992). Transcription factors: structural families and principles of DNA recognition. Annu Rev Biochem *61*, 1053-1095.

Papoulas, O., Beek, S. J., Moseley, S. L., McCallum, C. M., Sarte, M., Shearn, A., and Tamkun, J. W. (1998). The Drosophila trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. Development *125*, 3955-66.

Paro, R., and Hogness, D. S. (1991). The Polycomb protein shares a homologous domain with a heterochromatin- associated protein of Drosophila. Proc Natl Acad Sci U S A *88*, 263-7.

Pazin, M. J., Bhargava, P., Geiduschek, E. P., and Kadonaga, J. T. (1997). Nucleosome mobility and the maintenance of nucleosome positioning. Science *276*, 809-12.

Pennings, S., Meersseman, G., and Bradbury, E. M. (1991). Mobility of positioned nucleosomes on 5 S rDNA. J Mol Biol *220*, 101-10.

Peterson, C. L. (2000). ATP-dependent chromatin: going mobile. FEBS Lett *476*, 68-72.

Peterson, C. L. (1998). SWI/SNF Complex: Dissection of a Chromatin Remodeling Cycle. Cold Spring Harbor Symposia on Quantitative Biology *LXIII*, 545-552.

Peterson, C. L., Dingwall, A., and Scott, M. P. (1994). Five SWI/SNF gene products are components of a large multi-subunit complex required for transcriptional enhancement. Proc. Natl. Acad. Sci. USA *91*, 2905-2908.

Peterson, C. L., and Herskowitz, I. (1992). Characterization of the yeast SWI1, SWI2, and SWI3 genes, which encode a global activator of transcription. Cell *68*, 573-83.

Peterson, C. L., and Tamkun, J. W. (1995). The SWI-SNF complex: a chromatin remodeling machine? Trends Biochem Sci *20*, 143-6.

Phelan, M. L., Sif, S., Narlikar, G. J., and Kingston, R. E. (1999). Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. Mol Cell *3*, 247-53.

Pogo, B. G., Allfrey, V. G., and Mirsky, A. E. (1966). RNA synthesis and histone acetylation during the course of gene activation in lymphocytes. Proc Natl Acad Sci U S A *55*, 805-12.

Polach, K. J., and Widom, J. (1995). Mechanism of protein access to specific DNA sequences in chromatin: a dynamic equilibrium model for gene regulation. J Mol Biol *254*, 130-49.

Polach, K. J., and Widom, J. (1996). A model for the cooperative binding of eukaryotic regulatory proteins to nucleosomal target sites. J Mol Biol *258*, 800-12.

Pollard, K. J., and Peterson, C. L. (1998). Chromatin remodeling: a marriage between two families? Bioessays *20*, 771-80.

Pollard, K. J., and Peterson, C. L. (1997). Role for ADA/GCN5 products in antagonizing chromatin-mediated transcriptional repression. Mol Cell Biol *17*, 6212-22.

Poot, R. A., Dellaire, G., Hulsmann, B. B., Grimaldi, M. A., Corona, D. F., Becker, P. B., Bickmore, W. A., and Varga-Weisz, P. D. (2000). HuCHRAC, a human ISWI chromatin remodelling complex contains hACF1 and two novel histone-fold proteins. Embo J *19*, 3377-3387.

Prior, C. P., Cantor, C. R., Johnson, E. M., Littau, V. C., and Allfrey, V. G. (1983). Reversible changes in nucleosome structure and histone H3 accessibility in transcriptionally active and inactive states of rDNA chromatin. Cell *34*, 1033-42.

Protacio, R. U., and Widom, J. (1996). Nucleosome transcription studied in a real-time synchronous system: test of the lexosome model and direct measurement of effects due to histone octamer. J Mol Biol *256*, 458-72.

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*, 844-7.

Ramakrishnan, V., Finch, J. T., Graziano, V., Lee, P. L., and Sweet, R. M. (1993). Crystal structure of globular domain of histone H5 and its implications for nuclesome binding. Nature *362*, 219-223.

Read, C. M., Cary, P. D., Crane-Robinson, C., Driscoll, P. C., and Norman, D. G. (1993). Solution structure of a DNA-binding domain from HMG1. Nucleic Acids Res *21*, 3427-36

Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D., and Klug, A. (1984). Structure of the nucleosome core particle at 7 Å resolution. Nature *311*, 532-7.

Roberts, S. M., and Winston, F. (1997). Essential functional interactions of SAGA, a Saccharomyces cerevisiae comples of Spt, Ada, and Gcn5 proteins, with the Snf/Swi and Srb/Mediator complex. Genetics *147*, 451-465.

Rose, M. D., Winston, F., and Hieter, P. (1990). Methods in yeast genetics, a laboratory course manual. (Cold Spring Harbor, NY: Cold Spring Harbor).

Rundlett, S. E., Carmen, A. A., Kobayashi, R., Bavykin, S., Turner, B. M., and Grunstein, M. (1996). HDA1 and RPD3 are members of distinct yeast histone deacetylase complexes that regulate silencing and transcription. Proc Natl Acad Sci U S A *93*, 14503-8.

Saikumar, P., Murali, R., and Reddy, E. P. (1990). Role of tryptophan repeats and flanking amino acids in Myb-DNA interactions. Proc Natl Acad Sci USA *87*, 8452-8456.

Saleh, A., Lang, V., Cook, R., and Brandl, C. J. (1997). Identification of native complexes containing the yeast coactivator/repressor proteins NGG1/ADA3 and ADA2. J Biol Chem *272*, 5571-5578.

Saleh, A., Schieltz, D., Ting, N., McMahon, S. B., Litchfield, D. W., Yates III, J. R., Lees-Miller, S. P., Cole, M. D., and Brandl, C. J. (1998). Tra1p is a component fo the yeast Ada-Spt transcriptional regulatory complexes. J Biol Chem *273*, 26559-26565.

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.

Sendra, R., Tse, C., and Hansen, J. C. (2000). The yeast histone acetyltransferase A2 complex, but not free Gcn5p, binds stably to nucleosomal arrays. J Biol Chem *275*, 24928-24934.

Shen, X., Mizuguchi, G., Hamiche, A., and Wu, C. (2000). A chromatin remodelling complex involved in transcription and DNA processing. Nature *406*, 541-4.

Sif, S., Stukenberg, P. T., Kirschner, M. W., and Kingston, R. E. (1998). Mitotic inactivation of a human SWI/SNF chromatin remodeling complex. Genes Dev *12*, 2842-51.

Sikorski, R. S., and Hieter, P. (1989). A system of vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics *122*, 19-27.

Simpson, R. T. (1978). Structure of the chromatosome, a chromatin particle containing 160 base pairs of DNA and all the histones. Biochemistry *17*, 5524-31.

Soultanas, P., Dillingham, M. S., Wiley, P., Webb, M. R., and Wigley, D. B. (2000). Uncoupling DNA translocation and helicase activity in PcrA: direct evidence for an active mechanism. Embo J *19*, 3799-3810.

Spangenberg, C., Eisfeld, K., Stunkel, W., Luger, K., Flaus, A., Richmond, T. J., Truss, M., and Beato, M. (1998). The mouse mammary tumour virus promoter positioned on a tetramer of histones H3 and H4 binds nuclear factor 1 and OTF1. J Mol Biol *278*, 725-39.

Spencer, V. A., and Davie, J. R. (1999). Role of covalent modifications of histones in regulating gene expression. Gene *240*, 1-12.

Steger, D. J., Eberharter, A., John, S., Grant, P. A., and Workman, J. L. (1998). Purified histone acetyltransferase complexes stimulate HIV-1 transcription from preassembled nucleosomal arrays. Proc Natl Acad Sci U S A *95*, 12924-9.

Stein, A. (1979). DNA folding by histones: the kinetics of chromatin core particle reassembly and the interaction of nucleosomes with histones. J Mol Biol *130*, 103-134.

Stern, M., Jensen, R., and Herskowitz, I. (1984). Five SWI/SNF genes are required for expression of the HO gene in yeast. J Mol Biol *178*, 853-868.

Sterner, D. E., Grant, P. A., Roberts, S. M., Duggan, L. J., Belotserkovskaya, R., Pacella, L. A., Winston, F., Workman, J. L., and Berger, S. L. (1999). Functional organization of

the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA- binding protein interaction. Mol Cell Biol *19*, 86-98.

Strahl, B. D., and Allis, C. D. (2000). The language of covalent histone modifications. Nature *403*, 41-5.

Struhl, K., Kadosh, D., Keaveney, M., Kuras, L., and Moqtaderi, Z. (1998). Activation and repression mechanisms in yeast. Cold Spring Harb Symp Quant Biol *63*, 413-21.

Studitsky, V. M., Clark, D. J., and Felsenfeld, G. (1994). A histone octamer can step around a transcribing polymerase without leaving the template. Cell *76*, 371-382.

Sudarsanam, P., Cao, Y., Wu, L., Laurent, B. C., and Winston, F. (1999). The nucleosome remodeling complex, Snf/Swi, is required for the maintenance of transcription in vivo and is partially redundant with the histone acetyltransferase, Gcn5. EMBO J *18*, 3101-3106.

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 USA *97*, 3364-3369.

Tamkun, J. W., Deuring, R., Scott, M. P., Kissinger, M., Pattatucci, A. M., Kaufman, T. C., and Kennison, J. A. (1992). brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell *68*, 561-72.

Taunton, J., Hassig, C. A., and Schreiber, S. L. (1996). A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science *272*, 408-11.

Thomas, J. O., and Furber, V. (1976). Yeast chromatin structure. FEBS Lett *66*, 274-80.

Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E., and Schreiber, S. L. (1998). Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. Nature *395*, 917-21.

Treich, I., Cairns, B. R., de los Santos, T., Brewster, E., and Carlson, M. (1995). SNF11, a new component of the yeast SNF-SWI complex that interacts with a conserved region of SNF2. Mol Cell Biol *15*, 4240-8.

Treich, I., and Carlson, M. (1997). Interaction of a Swi3 homolog with Sth1 provides evidence for a Swi/Snf-related complex with an essential function in Saccharomyces cerevisiae. Mol Cell Biol *17*, 1768-75.

Treich, I., Ho, L., and Carlson, M. (1998). Direct interaction between Rsc6 and Rsc8/Swh3,two proteins that are conserved in SWI/SNF-related complexes. Nucleic Acids Res *26*, 3739-45.

Trievel, R. C., Rojas, J. R., Sterner, D. E., Venkataramani, R. N., Wang, L., Zhou, J., Allis, C. D., Berger, S. L., and Marmorstein, R. (1999). Crystal structure and mechanism of histone acetylation of the yeast GCN5 transcriptional coactivator. Proc Natl Acad Sci USA *96*, 8931-8936.

Trouche, D., Le Chalony, C., Muchardt, C., Yaniv, M., and Kouzarides, T. (1997). RB and hbrm cooperate to repress the activation functions of E2F1. Proc Natl Acad Sci U S A *94*, 11268-73.

Tse, C., and Hansen, J. C. (1997). Hybrid trypsinized nucleosomal arrays: identification of multiple functional roles of the H2A/H2B and H3/H4 N-termini in chromatin fiber compaction. Biochemistry *36*, 11381-8.

Tse, C., Sera, T., Wolffe, A. P., and Hansen, J. C. (1998a). Disruption of higher-order folding by core histone acetylation dramatically enhances transcription of nucleosomal arrays by RNA polymerase III. Mol Cell Biol *18*, 4629-38.

Tse, C., Georgieva, E. I., Ruiz-Garcia, A. B., Sendra, R., and Hansen, J. C. (1998b). Gcn5p, a transcription-related histone acetyltransferase, acetylates nucleosomes and folded nucleosomal arrays in the absence of other protein subunits. J Biol Chem *273*, 32388-92.

Tse, C., Fletcher, T. M., and Hansen, J. C. (1998c). Enhanced transcription factor access to arrays of histone H3/H4 tetramer.DNA complexes in vitro: implications for replication and transcription. Proc Natl Acad Sci U S A *95*, 12169-73.

Tsukiyama, T., Daniel, C., Tamkun, J., and Wu, C. (1995). ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. Cell *83*, 1021-6.

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*, 686-97.

Tsukiyama, T., and Wu, C. (1995). Purification and properties of an ATP-dependent nucleosome remodeling factor. Cell *83*, 1011-20.

Turner, B. M., Birley, A. J., and Lavender, J. (1992). Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in Drosophila polytene nuclei. Cell *69*, 375-384.

Utley, R. T., Cote, J., Owen-Hughes, T., and Workman, J. L. (1997). SWI/SNF stimulates the formation of disparate activator-nucleosome complexes but is partially redundant with cooperative binding. J Biol Chem *272*, 12642-9.

Utley, R. T., Ikeda, K., Grant, P. A., Cote, J., Steger, D. J., Eberharter, A., John, S., and Workman, J. L. (1998). Transcriptional activators direct histone acetyltransferase complexes to nucleosomes. Nature *394*, 498-502.

Varga-Weisz, P. D., and Becker, P. B. (1995). Transcription factor-mediated chromatin remodelling: mechanisms and models. FEBS Lett *369*, 118-21.

Varga-Weisz, P. D., Wilm, M., Bonte, E., Dumas, K., Mann, M., and Becker, P. B. (1997). Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II [published erratum appears in Nature 1997 Oct 30;389(6654):1003]. Nature *388*, 598-602.

Velankar, S. S., Soultanas, P., Dillingham, M. S., Subramanya, H. S., and Wigley, D. B. (1999). Crystal structures of complexes of PcrA DNA helicase with a DNA substrate indicate an inchworm mechanism. Cell *97*, 75-84.

Versteege, I., Sevenet, N., Lange, J., Rousseau-Merck, M. F., Ambros, P., Handgretinger, R., Aurias, A., and Delattre, O. (1998). Truncating mutations of hSNF5/Ini1 in aggressive paediatric cancer. Nature *394*, 203-206.

Vettese-Dadey, M., Grant, P. A., Hebbes, T. R., Crane- Robinson, C., Allis, C. D., and Workman, J. L. (1996). Acetylation of histone H4 plays a primary role in enhancing transcription factor binding to nucleosomal DNA in vitro. Embo J *15*, 2508-18.

Vettese-Dadey, M., Walter, P., Chen, H., Juan, L. J., and Workman, J. L. (1994). Role of the histone amino termini in facilitated binding of a transcription factor, GAL4-AH, to nucleosome cores. Mol Cell Biol *14*, 970-81.

Vignali, M., Hassan, A. H., Neely, K. E., and Workman, J. L. (2000). ATP-dependent chromatin-remodeling complexes. Mol Cell Biol *20*, 1899-910.

Wade, P. A., Jones, Peter L., Vermaak, Danielle, Wolffe, Alan P. (1998). A multiple subunit Mi-2 histone deactylase from Xenopus laevis cofractionates with an associated Snf2 superfamily ATPase. Current Biology *8*, 843-846.

Wade, P. A., Gegonne, A., Jones, P. L., Ballestar, E., Aubry, F., and Wolffe, A. P. (1999). Mi-2 complex couples DNA methylation to chromatin remodelling and histone deacetylation [see comments]. Nat Genet *23*, 62-6.

Wang, L., Liu, L., and Berger, S. L. (1998). Critical residues for histone acetylation by Gcn5, functioning in Ada and SAGA complexes, are also required for transcriptional function in vivo. Genes Dev *12*, 640-53.

Wang, W., Cote, J., Xue, Y., Zhou, S., Khavari, P. A., Biggar, S. R., Muchardt, C., Kalpana, G. V., Goff, S. P., Yaniv, M., Workman, J. L., and Crabtree, G. R. (1996a). Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. Embo J *15*, 5370-82.

Wang, W., Xue, Y., Zhou, S., Kuo, A., Cairns, B. R., and Crabtree, G. R. (1996b). Diversity and specialization of mammalian SWI/SNF complexes. Genes Dev *10*, 2117-30.

Waterborg, J. H. (2000). Steady-state levels of histone acetylation in Saccharomyces cerevisiae. J Biol Chem *275*, 13007-11.

Watson, J. D., and Crick, F. H. C. (1953a). Molecular structure of nucleic acid. Nature *171*, 737-738.

Watson, J. D., and Crick, F. H. C. (1953b). Genetic implications of the structure of deoxyribonucleic acid. Nature *171*, 964-967.

Weintraub, H., and Groudine, M. (1976). Chromosomal subunits in active genes have an altered conformation. Science *193*, 848-56.

Weir, H. M., Kraulis, P. J., Hill, C. S., Raine, A. R., Laue, E. D., and Thomas, J. O. (1993). Structure of the HMG box motif in the B-domain of HMG1. Embo J *12*, 1311-9.

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*, 784-7.

Winston, F., and Carlson, M. (1992). Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet *8*, 387-91.

Wittschieben, B. O., Otero, G., de Bizemont, T., Fellows, J., Erdjument-Bromage, H., Ohba, R., Li, Y., Allis, C. D., Tempst, P., and Svejstrup, J. Q. (1999). A novel histone

acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. Mol Cell *4*, 123-8.

Wolffe, A. P. (1994). Transcriptional activation. Switched-on chromatin. Curr Biol *4*, 525-8.

Wolffe, A. P., and Hayes, J. J. (1999). Chromatin disruption and modification. Nucleic Acids Res *27*, 711-20.

Wolffe, A. P., Khochbin, S., and Dimitrov, S. (1997). What do linker histones do in chromatin? Bioessays *19*, 249-55.

Workman, J. L., and Kingston, R. E. (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu Rev Biochem *67*, 545-79.

Workman, J. L., and Roeder, R. G. (1987). Binding of transcription factor TFIID to the major late promoter during in vitro nucleosome assembly potentiates subsequent initiation by RNA polymerase II. Cell *51*, 613-22.

Workman, J. L., Taylor, I. C., Kingston, R. E., and Roeder, R. G. (1991). Control of class II gene transcription during in vitro nucleosome assembly. Methods Cell Biol *35*, 419-47.

Wu, C. (1980). The 5' ends of Drosophila heat shock genes in chromatin are hypersensitive to DNase I. Nature *286*, 854-60.

Wu, L., and Winston, F. (1997). Evidence that Snf-Swi controls chromatin structure over both the TATA and UAS regions of the SUC2 promoter in Saccharomyces cerevisiae. Nucleic Acids Research *25*, 4230-4234.

Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Cote, J., and Wang, W. (1998). NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Mol Cell *2*, 851-61.

Yager, T. D., McMurray, C. T., and van Holde, K. E. (1989). Salt-induced release of DNA from nucleosome core particles. Biochemistry *28*, 2271-81.

Yager, T. D., and van Holde, K. E. (1984). Dynamics and equilibria of nucleosomes at elevated ionic strength. J Biol Chem *259*, 4212-22.

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*, 2369-74.

Zargarian, L., Le Tilly, V., Jamin, N., Chaffotte, A., Gabrielsen, O. S., Toma, F., and Alpert, B. (1999). Myb-DNA recognition: role of tryptophan residues and structural changes of the minimal DNA binding domain of c-Myb. Biochemistry *38*, 1921-9.

Zhang, H. S., Gavin, M., Dahiya, A., Postigo, A. A., Ma, D., Luo, R. X., Harbour, J. W., and Dean, D. C. (2000). Exit from G1 and S phase of the cell cycle is regulated by repressor complexes containing HDAC-Rb-hSWI/SNF and Rb-hSWI/SNF. Cell *101*, 79-89.

Zhang, Y., LeRoy, G., Seelig, H. P., Lane, W. S., and Reinberg, D. (1998). The dermatomyositis-specific autoantigen Mi2 is a component of a complex containing histone deacetylase and nucleosome remodeling activities. Cell *95*, 279-89.

Zhao, K., Wang, W., Rando, O. J., Xue, Y., Swiderek, K., Kuo, A., and Crabtree, G. R. (1998). Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. Cell *95*, 625-36.

APPENDIX

Actin-related Proteins (Arps): Conformational Switches for Chromatin Remodeling Machines?

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Actin-related proteins (Arps): conformational switches for chromatin-remodeling machines?

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Summary
The actin superfamily of ATPases includes cytoskeletal actins, the stress 70 proteins (e.g. hsc70), sugar kinases, glycerol kinase, and several prokaryotic cell cycle proteins. Although these proteins share limited sequence identity, they all appear to maintain a similar tertiary structure, the "actin fold", which may serve to couple ATP hydrolysis to protein conformational changes. Recently, an actin-related protein (Arp) subfamily has been identified based on sequence homology to conventional actin. Although some Arps are clearly involved in cytoskeletal functions, both actin and/or Arps have been found as stoichiometric subunits of several nuclear chromatin-remodeling enzymes. Here we present two related models in which actin and/or Arps function as conformational switches that control either the activity or the assembly of chromatin-remodeling machines. ***BioEssays*** **22:666–672, 2000.**

Introduction

Over recent years, large multisubunit enzymes that regulate the accessibility of nucleosomal DNA have emerged as key regulators of eukaryotic transcription. It seems likely that similar enzymes contribute to the efficiency of DNA replication, recombination and repair. These chromatin-remodeling "machines" can be classified into two broad groups: (1) the ATP-dependent enzymes, which use the energy of ATP hydrolysis to increase the accessibility of nucleosomal DNA; and (2) histone-modifying enzymes that phosphorylate, acetylate, or methylate the N-terminal or C-terminal "tail" domains of the nucleosomal histones.[1–3] Recently, conventional actin and several members of an actin-related protein (Arp) family have been identified as stoichiometric subunits of ATP-dependent remodeling enzymes and nuclear histone acetyltransferases (Refs. 4–7 and J. Cote, Y. Nakatani, and C. Wu, pers. comm.). In the yeast *Saccharomyces cerevisiae*, the Arp family is composed of ten family members originally identified by virtue of sequence homology to conventional actin[8] (Table 1). Arps are functionally distinct from cytoplasmic actin and are highly conserved across many eukaryotic phyla, suggesting an evolutionarily important role for these proteins in many cellular processes.[9–13] Arps are most similar to the "actin fold" region of conventional actin suggesting that these proteins are likely to possess both structural and functional similarities to the actin superfamily of ATPases.[8] By analogy to the functioning of other members of the actin superfamily, here we explore the possibility that Arps may function as conformational switches that control the assembly or activity of chromatin-remodeling enzymes.

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Structural and mechanistic similarities among actin superfamily members

The crystal structures for five members of the actin superfamily have been determined at atomic resolution (two actin isoforms, hexokinase, glycerol kinase, and the 44-kDa ATPase fragment of heat shock cognate 70 [hsc70]).[14–20] Although sequence comparisons do not indicate strong resemblances, the three-dimensional structures display similarity in domain organization.[21,22] This has led to the common classification of these proteins as members of an extended protein superfamily. Despite the limited sequence identity, a conserved "sequence fingerprint" has been identified to describe the ATP-binding pocket of these actin superfamily members[21,22] (Fig. 1). This highly conserved domain is termed the "actin fold".

The "actin fold" motif of hsc70 and actin is organized into two domains, I and II, which are further divided into the subdomains Ia, Ib, IIa, and IIb[23–25] (Fig. 1). Domains Ia and IIa have a similar topology which consists of a common core of five stranded β-sheets and three α-helices (Fig. 1). The duplication of this common core leads to the appearance of a lobed structure with bilateral symmetry. A single ATP molecule is situated in the interdomain cleft that bridges the symmetrically disposed halves of the protein structure. Although the adenosine group is bound in a highly conserved hydrophobic pocket between subdomains IIa and IIb, no specific side-chain contacts are made.[23–25] This provides one explanation for the enormous sequence diversity that is tolerated by this structure. The hydrophilic nature of the

Table 1. *Saccharomyces cerevisiae* actin-related protein family

Actin-related protein	% identity to actin[a]	Cellular localization	Function	Refs.
Arp1p (Act3/Act5)	45%	Cytoplasm	Major component of dynactin; involved in a wide range of motile processes	9–11
Arp2p (Act2)	45%	Cytoplasm	Subunit of Arp2p–Arp3p complex; multifunctional organizer of actin filaments	11–13
Arp3p (Act4)	39%	Cytoplasm	Subunit of Arp2p–Arp3p complex; multifunctional organizer of actin filaments	11–13
Arp4p (Act3)	30%	Nucleus	Subunit of NuA4 histone acetyl-transferase; transcription defects; interacts with core histones; ATP-binding protein	J. Cote pers. comm. 28,43,44
Arp5p	26%	Nucleus	Unknown	28
Arp6p	24%	Unknown	Unknown	
Arp7p	22%	Nucleus	Subunit of SWI/SNF and RSC chromatin-remodeling complexes	4,5
Arp8p	21%	Nucleus	Unknown	
Arp9p	17%	Nucleus	Subunit of SWI/SNF and RSC chromatin-remodeling complexes	4,5
Arp10p	17%	Unknown	Unknown	

[a]As identified in Ref. 8.

phosphate moieties from the bound nucleotide, along with contributions from several other conserved residues, serve to coordinate the position of the catalytically essential metal ion cofactor (Mg^{2+} or Ca^{2+}).[23–25] Thus, the common core includes the ATP-binding pocket and a large part of the interface between subdomains Ia and IIa. Importantly, the structure appears to be rather flexible as the four subdomains are held together mainly by ATP and salt bridges.[23–25]

Outside of the conserved core, the structures of actin superfamily members are quite different. For instance, regions of subdomains Ia and IIa that are not conserved among superfamily members are found within solvent-exposed loops, which in some cases mediate protein-protein interactions (e.g. actin-actin interactions or sugar binding in hexokinase).[26,27] In addition, subdomains Ib and IIb, which are the least conserved among family members, appear to have evolved even more specialized functions such as mediating interactions with histones in the case of Arp4p.[28]

A convergence of biochemical and genetic data for actin, hsc70 (as well as its prokaryotic analogue, dnaK) and hexokinase[23–25] has led to the common view that binding and hydrolysis of ATP within the highly conserved "actin fold" regulates distinct conformational states of the protein.[29–37] Specifically, ATP hydrolysis and the subsequent release of inorganic phosphate appears to destabilize interdomain bridging interactions which leads to the characteristic "open" conformation in which there is a reorientation of the two domains around a central point or hinge.[23–25] The turnover rate for ATP is generally slow (0.02 min^{-1} for hsc70), although this slow rate of ATP hydrolysis can be modulated by the binding of other proteins.[37] The coupling of ATP binding and hydrolysis to distinct conformational states provides a means to "switch" the functional properties of an actin superfamily member. For instance, the ATP hydrolysis cycle appears to control the polymerization and stability of actin filaments, the binding and release of peptide substrates by hsc70, and the phosphorylation of glucose by hexokinase.[23–25]

The Arps

The complete sequence of the *Saccharomyces cerevisiae* genome facilitated the identification and classification of an Arp family that currently comprises ten members; these are designated Arp1p to Arp10p in descending order of homology to actin[8] (Table 1). The Arps share significant homology to 29 conventional actins from many different organisms over their entire sequence, and it is predicted that these proteins have conserved the overall tertiary structure common to actin superfamily members (i.e. the "actin fold"). In particular, Arps appear to have conserved many of the residues important for binding ATP, and thus, by analogy to other actin superfamily members, Arps may undergo ATP-dependent conformational changes that regulate the function of these proteins.[8,11] However, so far, Arp1p and Arp4p are the only family members that have been shown to bind ATP;[28,38,39] the ATP-binding properties of other family members have not yet been tested directly.

What is the function of the Arps? In almost every case where an *ARP* deletion allele has been constructed (*ARP2,3,4,7,9*), *ARPs* have been found to be essential for yeast cell viability.[5,40,42] As described in more detail below,

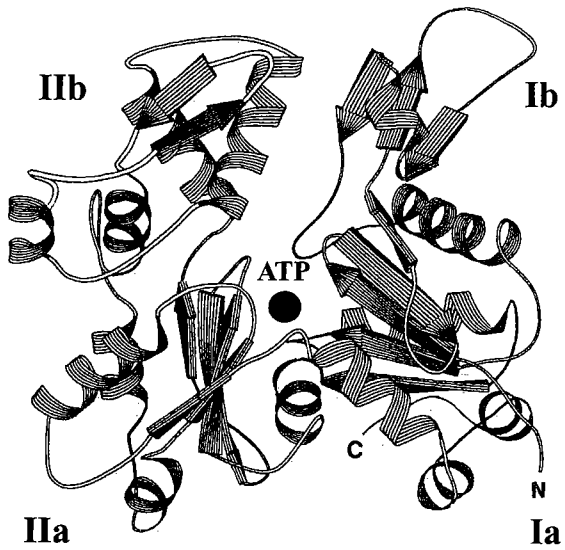

Figure 1. Domain organization of actin. Analysis of actin, hsc70, hexokinase and glycerol kinase by X-ray crystallography has revealed similar three-dimensional structures, as represented here by the actin structure (adapted from ref. 14). The overall domain organization consists of two flexible globular domains—I and II—which are further subdivided into subdomains a and b. Domain Ia and IIa are the most highly conserved among actin superfamily members and have nearly identical topology. Recently, a conserved amino acid sequence has been identified for the adenine nucleotide β-phosphate-binding pocket in subdomain Ia, which may be diagnostic of actin superfamily members: [Ile/Leu/Val]-X-[Ile/Leu/Val/Cys]-Asp-X-Gly-[Thr/Ser/Gly]-[Thr/Ser/Gly]-X-X-[Arg/Lys/Cys], where X = any amino acid.[21] Domains Ib and IIb are less conserved and may provide the specificity for these functionally divergent proteins. ATP and Mg^{2+} (represented by the black ball) are situated in the interdomain cleft and are essential to the function of these proteins.

ARP1, *ARP2*, and *ARP3* encode cytoplasmic Arp proteins involved in cytoskeletal functions,[9–13] whereas the majority of the *ARP 4–10* group appear to encode nuclear Arps that are subunits of chromatin-remodeling complexes([4,5,28,43,44] and J. Cote, Y. Nakatani, and C. Wu, pers. comm.).

Arp1p, Arp2p, and Arp3p: cytoskeletal regulators

Arp1p, Arp2p, and Arp3p appear to be common to all eukaryotes and are probably the best-characterized family members.[9–13] These three Arps are the most closely related in sequence to actin (39–45% identity; see also Table 1), and their cellular localization closely mimics conventional actin.[9–13] Arp1p is most similar to actin (45% identity) and appears to be the only Arp to have maintained the ability to polymerize into filaments, which contain multiple Arp1p subunits, although these filaments are shorter.[45] This is not surprising considering that Arp1p, in contrast to all other Arps, has maintained many of the actin-actin interaction surfaces.[9,10] Recent studies have shown that Arp1p can bind and exchange ATP for ADP and AMP,[38,39] and like conventional actin, Arp1p filament formation and stability is probably controlled by an ATP-dependent mechanism. Arp1p filaments are part of a large multisubunit complex, called dynactin, that mediates dynein-based motility along microtubules.[46] Arp1p, along with other dynactin subunits, has also been shown to play a role in a wide range of motile processes.[47–50] Interestingly, in addition to Arp1p multimers, dynactin also contains one subunit of conventional actin. Recent work has identified a protein complex that contains a novel Arp and binds to the pointed ends of Arp1 filaments, akin to the Arp2p–Arp3p complex, which binds to the pointed ends of conventional actin filaments (also see below).[51]

Arp2p and Arp3p were found to copurify as stoichiometric subunits of a multiprotein complex required for polymerization of actin filaments in *Acanthomoeba*.[52] The Arp2p–Arp3p complex is now thought to play a central role in controlling actin dynamics in all eukaryotes by virtue of its ability to nucleate assembly of actin filaments, to cap the pointed ends of filaments, and to crosslink filaments into a network.[53] Like all members of the Arp family, Arp2p and Arp3p are predicted to have maintained the "actin fold", but other domains have diverged such that Arp2p and Arp3p are incapable of forming filaments and are likely instead to form heterodimers.[54] It is currently unclear whether Arp2p and Arp3p are capable of binding or hydrolyzing ATP.

Arp4p to Arp10p: chromatin-remodeling proteins?

Whereas Arp1p, Arp2p and Arp3p are localized in the cytoplasm and play essential roles in cytoskeletal function, much less is known about the function of the more divergent Arps. Arps 4–10 share significant homology to actin (17–30% identity; see also Table 1) and they are still predicted to maintain the overall tertiary structure of actin superfamily members.[8] Interestingly, many members of this subgroup (Arp4p, Arp5p, Arp7p and Arp9p) are localized to the nucleus suggesting that a further subdivision exists among the Arp classes[4,5,28] (see also Table 1). In the case of Arp4p, the *Drosophila* homolog colocalizes with heterochromatin and the heterochromatin-specific protein, HP1.[55]

What role do Arps play in the nucleus? Recently, nuclear Arps have been identified as stoichiometric subunits of many different chromatin-remodeling complexes. Arp7p and Arp9p are essential subunits of the related ATP-dependent chromatin-remodeling complexes, SWI/SNF and RSC, which are required for transcriptional regulation and cell cycle control, respectively.[4,5] Arp4p and conventional actin are subunits of the NuA4 histone acetyltransferase complex([56] and J. Cote, pers. comm.), and a novel ATP-dependent remodeling complex, called ARI1, also contains multiple Arp subunits as well as a copy of conventional actin (C. Wu, pers. comm.).

The presence of Arps in chromatin-remodeling enzymes is not peculiar to yeast, as conventional actin and Arps have been discovered as subunits of both the mammalian and *Drosophila* SWI/SNF complexes as well as theTIP60 histone acetyltransferase complex([6,7] and Y. Nakatani, pers. comm.).

Both genetic and biochemical evidence point to a functionally relevant role for Arps in chromatin-remodeling enzymes. Mutations in *ARP7* or *ARP9* result in typical *swi/snf* transcriptional defects, and null alleles share the lethal phenotype of mutations in other RSC complex subunits.[4,5] Likewise, mutant alleles of *ARP4* exhibit phenotypes that are similar to those due to mutations in *ESA1* which encodes the catalytic acetylase subunit of the NuA4 histone acetyltransferase complex (J. Cote, pers. comm.). In addition, conditional alleles of *ARP4* affect the DNA-linking number of episomal plasmids in vivo, and they lead to epigenetic suppression of transcriptional defects caused by certain transposon insertions (an Spt^- phenotype), further linking Arp4p function to chromatin structure.[28,44,57] Interestingly, *arp7* and *arp9* mutants also exhibit an Spt^- phenotype.[5] More recently, Arp4p has also been shown to bind to all four core histones, and this interaction is mediated via a novel domain that is not conserved in other Arps but is inserted between subdomains Ia and Ib.[28] It seems likely that this histone-binding domain of Arp4p influences the ability of the NuA4 histone acetyltransferase complex to interact with its chromatin substrate. Other Arps are also distinguished by distinct sequence insertions relative to actin which may account for additional functional diversity.[9]

Role of Arps in chromatin remodeling

Why are Arps essential subunits of highly conserved chromatin-remodeling complexes? It has been proposed that the presence of Arps in chromatin-remodeling complexes may target these complexes to a cytoskeletal-like nuclear architecture.[6] Although this may be plausible for complexes that contain a copy of conventional actin, it is unlikely to be a general feature of Arps since protein surfaces known to mediate interactions with cytoskeletal components are not conserved in the nuclear Arps. In contrast, all Arps have maintained the overall "actin fold" motif that is shared by actin superfamily members. As described above, the "actin fold" represents a flexible, hinged domain that is characterized by its ability to convert between conformational states in an ATP-dependent manner.[23–25] In the following sections we use this conserved feature of the "actin fold" as a framework for two related models that explain how Arps might control the function of chromatin-remodeling enzymes.

Arps regulate the catalytic activity of chromatin-remodeling enzymes

In many complex biochemical reactions, ATP hydrolysis is used to drive conformational changes in proteins that irreversibly move reaction cycles forward or into abortive reactions. Thus, one possibility is that Arps might use ATP binding and hydrolysis as molecular switches that regulate the activity of chromatin-remodeling complexes (Fig. 2A). For example, we previously proposed that Arp7p and Arp9p may regulate the dissociation of SWI/SNF (and by analogy RSC) from its nucleosomal substrate.[58] Specifically, the ATP-bound form of Arp7p/Arp9p (conformation 1) may yield a SWI/SNF complex that is unable to bind one or more histone N-terminal domains. Previous work has shown that the histone N-terminal domains play an important role in regulating the dissociation of SWI/SNF from nucleosomal arrays.[59] The slow hydrolysis of ATP by Arp7p/Arp9p might function as an internal "clock" that couples a conformational change (conformation 2) to subsequent binding of histone N-terminal domain(s), which then promote dissociation of SWI/SNF from the nucleosomal array. In essence, a similar model has been proposed for how ATP governs binding and release of peptide substrates by Hsc70.[37] It is interesting to note that recent studies have also implicated the Arp4p subunit of the NuA4 histone acetyltransferase complex in binding histones.[28]

Several chromatin-remodeling complexes (human and *Drosophila* SWI/SNF, NuA4, ARI1) contain at least one copy of conventional actin, which would be able to provide the ATPase activity required for this model. However, one potential limitation of this model is that chromatin-remodeling enzymes that lack actin (e.g. yeast SWI/SNF and RSC) may contain Arps that do not bind ATP. To date, only Arp4p is known to bind ATP, although the ATP-binding properties of other nuclear Arps have not been investigated directly. Furthermore, several single and multiple amino acid changes in the putative ATP-binding pocket of Arp7p or Arp9p (subunits of SWI/SNF and RSC) did not yield observable phenotypes, suggesting that these proteins may not bind ATP.[5] One possibility is that some Arps may rely on protein-protein interactions to govern conformational changes that might control chromatin remodeling activity. In such a model, the transient binding of a regulatory protein (in place of ATP) to the "actin fold" domain would drive the conformational change and thereby govern the activity of the chromatin-remodeling complex (Fig. 2A). In the case of the yeast SWI/SNF complex, the histone N-terminal domains would represent an excellent candidate for this type of interaction.

Arps regulate the assembly of chromatin-remodeling enzymes

One common feature of all Arps is that they appear to be obligate members of large multisubunit protein assemblies. In the case of chromatin-remodeling enzymes, the NuA4, SWI/SNF and RSC complexes each contain at least 11 different polypeptide subunits and range in size from 1–2 million Da.[56,60,61] Thus, a second model posits that Arps

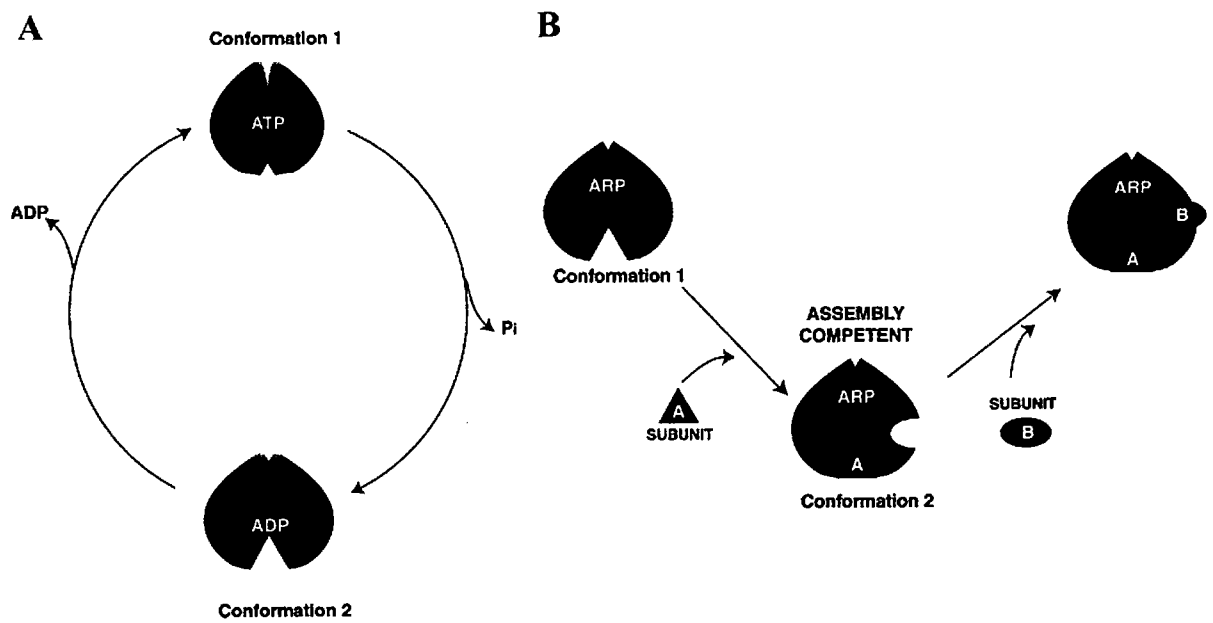

Figure 2. The role of actin-related proteins (Arps) in chromatin remodeling. Two general models are proposed in which Arps serve as conformational switches for regulating the activity or assembly of chromatin-remodeling complexes. In the first model **(A)**, the energy of ATP hydrolysis is used to drive a conformational change in the ARP, which regulates the activity of the chromatin-remodeling complex. The slow rate of ATP hydrolysis generally associated with actin superfamily members may serve as an internal clock governing a particular step in the remodeling reaction. The transient binding of a regulatory protein instead of ATP may also influence the conformational state of the Arp and hence may also control the activity of the remodeling reaction. In Model **(B)**, Arps may regulate the assembly of multisubunit complexes. This model predicts that the binding of one subunit to the Arp drives a conformational change of the protein such that it becomes competent to bind one or more additional subunits.

regulate the stepwise assembly of these chromatin remodeling enzymes (Figure 2B). For example, an actin–Arp or Arp–Arp heterodimer may exist initially in a conformation that is only competent for binding of a hypothetical subunit "A". Binding of subunit "A", however, would trigger a conformational change in the "actin fold" that promotes binding of subunit "B". In essence, this model predicts that the Arps may provide a protein surface for nucleating the directional assembly of large multisubunit complexes. This is consistent with previous evidence that Arp1p filaments may act as a protein scaffold for assembly of dynactin, and that the Arp2p–Arp3p complex may provide a binding surface for the nucleation of actin filaments.[9,10] This assembly model may also provide an explanation for why different chromatin-remodeling complexes harbor members of different Arp classes. For instance, the NuA4 and SWI/SNF complexes do not share common subunits, and each complex contains different Arps. In contrast, the SWI/SNF and RSC complexes contain a similar core of four highly related subunits, and both complexes contain Arp7p and Arp9p.[4,5] One obvious prediction of this model is that mutations in Arp subunits should compromise complex assembly. Specifically, SWI/SNF or RSC complexes could be isolated from strains harboring temperature-sensitive alleles of *ARP7* or *ARP9*.[5] Model A predicts that these *arp* mutations will alter the activity of these complexes, whereas Model B suggests that complexes isolated from cells grown at the nonpermissive temperature would be incompletely assembled. Furthermore, Model B predicts that extragenic suppressors of *arp7* or *arp9* mutants might yield mutations in other SWI/SNF or RSC subunits.

Conclusions

The recent identification of Arps as a common feature of chromatin-remodeling complexes has generated much surprise and enthusiasm in both the cytoskeleton and transcription fields. The actin superfamily of proteins is characterized by a common tertiary structure that functions by coupling

ATP hydrolysis to large protein conformational changes. As such, it is predicted that the Arps might share this ATP-dependent function. However, the question remains as to whether all Arps bind nucleotides and, if not, whether they bind other protein effectors that can also induce protein conformational changes. In this paper, we have presented two testable models to explain how Arps might control the function of chromatin-remodeling enzymes. Studies are under way to define the biochemical roles of Arps in chromatin-remodeling reactions, and such studies will undoubtedly yield new insights into how these large macromolecular enzymes operate and how they are regulated.

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References

1. Workman JL, Kingston RE. Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annu Rev Biochem 1998;67: 545–579.
2. Pollard KJ, Peterson CL. Chromatin remodeling: a marriage between two families? Bioessays 1998;20:771–780.
3. Kingston RE, Narlikar G. ATP-dependent remodeling and acetylation as regulators of chromatin fluidity. Genes Dev 1999;13:2339–2352.
4. Peterson CL, Zhao Y, Chait BT. Subunits of the yeast SWI/SNF complex are members of the actin-related protein (ARP) family. J Biol Chem 1998; 273:23631–23644.
5. Cairns BR, Erdjument-Bromage H, Tempst P, Winston F, and Kornberg RD. Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF. Mol Cell 1998;2: 639–651.
6. Zhao K, Wang W, Rando OJ, Xue Y, Swiderek K, Kuo A, Crabtree GR. Rapid and phosphoinositol-dependent binding of the SWI/SNF-like BAF complex to chromatin after T lymphocyte receptor signaling. Cell 1998; 5:625–636.
7. Papoulas O, Beek SJ, Moseley SL, McCallum CM, Sarte M, Shearn A, Tamkun JW The *Drosophila* trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. Development 1998; 125:3955–3966.
8. Poch O, Winsor B. Who's who among the Saccharomyces cerevisiae actin-related proteins? A classification and nomenclature proposal for a large family. Yeast 1997;13:1053–1058.
9. Mullins RD, Kelleher JF, Pollard TD. Actin' like actin? Trends in Cell Biology 1996;6:208-211.
10. Schroer TA, Bingham JB, Gill SR. Actin-related protein 1 and cytoplasmic dynein-based motility—what's the connection? Trends in Cell Biol 1996;6:211–215.
11. Frankel S, Mooseker MS. The actin-related proteins. Curr Opin Cell Biol 1996;8:30–37.
12. Machesky LM, Gould KL. The Arp2/3 complex: a multifunctional actin organizer. Curr Opin Cell Biol 1999;11:117–121.
13. Mullins RD, Pollard TD. Structure and function of the Arp2/3 complex. Curr Opin Struct Biol 1999;9:244–249.
14. Kabsh W, Mannherz HG, Suck D, Pai EF, Holmes KC. Atomic structure of the actin:DNase I complex. Nature 1990;347:37–44.
15. Schutt CE, Myslik JC, Rozycki MD, Goonesekere NCW, Lindberg U. The structure of crystallin profilin-β-actin. Nature 1993;365:810–816.
16. Chik JK, Lindberg U, Schutt CE. The structure of an open state of β-actin at 2.65 A resolution. J Mol Biol 1996;263:607–623.
17. Flaherty KM, DeLuca-Flaherty C, McKay DB. Three dimensional structure of the ATPase fragment of a 70K heat shock cognate protein. Nature 1990;346:623–628.
18. Anderson CM, Stenkamp RE, Steitz TA. A refined model of the sugar binding site of yeast hexokinase B. J Mol Biol 1978;123:207–219.
19. Anderson CM, Stenkamp RE, McDonald RC, Steitz TA. Sequencing a protein by x-ray crystallography. II Refinement of yeast hexokinase B co-ordinates and sequence at 2.1 A resolution. J Mol Biol 1978;123: 15–33.
20. Hurley JH, Faber HR, Worthylake D, Meadow ND, Roseman S, Pettigrew DW, Remington SJ. Structure of the regulatory complex of Escherichia coli III^{Glk} with glycerol kinase. Science 1993;259:673–677.
21. Flaherty KM, McKay DB, Kabsch W, Holmes KC. Similarity of the three-dimensional structures of actin and the ATPase fragment of a 70-kDa heat shock cognate protein. Proc Natl Acad Sci USA 1991;88:5041–5045.
22. Bork P, Sander C, Valencia A. An ATPase domain common to prokaryotic cell proteins, sugar kinases, actin, and hsp70 heat shock proteins. Proc Natl Acad Sci USA 1992;89:7290–7294.
23. Holmes KC, Sander C, Valencia, A. A new ATP-binding fold in actin, hexokinase, and Hsc70. Trends in Cell Biol 1993;3:53–59.
24. Kabsch W, Holmes KC. The actin fold. FASEB J 1995;9:167–174.
25. Hurley J. The Sugar Kinase/Heat Shock Protein Family: Implications of conserved structure for mechanism. Annu Rev Biophys Biomol Struct 1996;25:137–162.
26. Holmes KC, Popp D, Gebhard W, Kabsch W. Atomic model of actin filaments. Nature 1990;347:44–49.
27. Arora KK, Filburn CR, Pedersen PL. Glucose phosphorylation. Site-directed mutations which impair the catalytic function of hexokinase. J Biol Chem 1991;266:5359–5362.
28. Harata M, Oma Y, Mizuno S, Jiang YW, Stillman DJ, Wintersberger U. The nuclear actin-related protein of Saccharomyces cerevisiae, Act3p/Arp4, interacts with core histones. Mol Biol Cell 1999;10:2595–2605.
29. Drewes G, Faulstich H. A reversible conformational transition in muscle actin is caused by nucleotide exchange and uncovers cysteine in position 10. J Biol Chem 1991;266:5508–5513.
30. Miki M, Kouyama T. Domain motion in actin observed by fluorescence resonance energy transfer. Biochemistry 1994;33:10171–10177.
31. Belmont LD, Orlova A, Drubin DG. A change in actin conformation associated with filament instability after P_i release. Proc Natl Acad Sci USA 1999;96:29–34.
32. Liberek K, Skowyra D, Zylicz M, Johnson C, Georgopoulos C. The Escherichia coli DnaK chaperone, the 70-kDa heat shock protein eukaryotic equivalent, changes conformation upon ATP hydrolysis, thus triggering its dissociation from a bound target protein. J Biol Chem 1991;266:14491–14496.
33. Ha J, and McKay DB. ATPase kinetics of recombinant bovine 70 kDa heat shock cognate protein and its amino-terminal ATPase domain. Biochemistry 1994;33:14625–14635.
34. Ha J, McKay DB. Kinetics of nucleotide-induced changes in the tryptophan fluorescence of the molecular chaperone Hsc70 and its subfragments suggest the ATP-induced conformational change follows initial ATP binding. Biochemistry 1995; 34:11635–11644.
35. Wilbanks SM, DeLuca-Flaherty C, McKay DB. Structural basis of the 70-kilodalton heat shock cognate protein ATP hydrolytic activity. I. Kinetic analyses of active site mutants.J Biol Chem 1994;269: 12893–12898.
36. Flaherty KM, Wilbanks SM, DeLuca-Flaherty C, McKay DB. Structural basis of the 70-kilodalton heat shock cognate protein ATP hydrolytic activity. II. Structure of the active site with ADP or ATP bound to wild type and mutant ATPase fragment. J Biol Chem 1994;269:12899–12907.
37. Beissinger M, Buchner J. How chaperones fold proteins. Biol Chem 1998;379:245–259.
38. Melki R, Vainberg IE, Chow RL, Cowan NJ. Chaperonin-mediated folding of vertebrate actin-related protein and γ-tubulin. J Cell Biol 1993;122: 1301–1310.
39. Bingham JB, Schroer TA. Self-regulated polymerization of the actin-related protein Arp1. Curr Biol 1998;9:223–226.
40. Schwob E, Martin RP. New yeast actin-like gene required late in the cell cycle. Nature 1992;355:179–182.

41. Huang ME, Souciet JL, Chuat JC, Galibert F. Identification of ACT4, a novel essential actin-related gene in the yeast Saccharomyces cerevisiae. Yeast 1996;12:839–848.
42. Harata M, Karwan A, Wintersberger U. An essential gene of Saccharomyces cerevisiae coding for an actin-related protein. Proc Natl Acad Sci USA 1994;91:8258–8262.
43. Weber V, Harata M, Hauser H, Wintersberger U. The actin-related protein Act3p of Saccharomyces cerevisiae is located in the nucleus. Mol Biol Cell 1995;6:1263–1270.
44. Jiang YW, Stillman DJ. Epigenetic effects on yeast transcription caused by mutations in an actin-related protein present in the nucleus. Genes Dev 1996;10:604–619.
45. Schaefer DA, Gill SR, Cooper JA, Heuser JE, Schroer TA. Ultrastructural analysis of the dynactin complex: an actin-related protein is a component of a filament that resembles F-actin. J Cell Biol 1994;126: 403–412.
46. Schroer TA, Sheetz MP. Two activators of microtubule-based vesicle transport. J Cell Biol 1991;115:1309–1318.
47. Muhua L, Karpova TS, Cooper JA. A yeast actin-related protein homologous to that in vertebrate dynactin complex is important for spindle orientation and nuclear migration. Cell 1994;78:669–679.
48. Clark SW, Meyer DI. ACT3: a putative centractin homologue in *S. cerevisiae* is required for proper orientation of the mitotic spindle. J Cell Biol 1994;127:129–138.
49. Echeverri CJ, Paschal BM, Vaughan KT, Vallee RB. Molecular characterization of the 50kD subunit of dyanctin reveals function for the complex in chromosome alignment and spindle organization during mitosis. J Cell Biol 1996;132:617–633.
50. Vallee RB, Sheetz MP. Targeting of motor proteins. Science 1996;27: 1539-1544
51. Garces JA, Clark IB, Meyer DI, Vallee RB. Interaction of the p62 subunit of dynactin with Arp1 and the cortical actin cytoskeleton. Curr Biol 1999;9:1497–1500.
52. Machesky LM, Atkinson SJ, Ampe C, Vandekerckhove J, Pollard TD. Purification of a cortical complex containing two unconventional actins from *Acanthamoeba* by affinity chromatography on profilin-agarose. J Cell Biol 1994;127:107–115.
53. Mullins RD, Heuser JA, Pollard TD. The interaction of Arp2/3 complex with actin: nucleation, high affinity pointed end capping, and formation of branching networks of filaments. Proc Natl Acad Sci USA 1998;95: 6181–6186.
54. Kelleher JF, Atkinson SJ, Pollard TD. Sequences, structural models, and cellular localization of the actin-related proteins Arp2 and Arp3 from Acanthamoeba. J Cell Biol 1995;131:385–397.
55. Frankel S, Sigel EA, Craig C, Elgin SC, Mooseker MS, Artavanis-Tsakonas S. An actin-related protein in Drosophila colocalizes with heterochromatin protein 1 in pericentric heterochromatin. J Cell Sci 1997;110:1999–2012.
56. Allard S, Utley RA, Savard J, Clarke A, Grant P, Brandl CJ, Pillus L, Workman JL, Cote J. NuA4, an essential transcription adaptor/histone H4 acetyltransferase complex containing Esa1p and the ATP-related cofactor Tra1p. EMBO J 1999;18:5108–5119.
57. Winston F, Carlson M. Yeast SNF/SWI transcriptional activators and the SPT/SIN connection. Trends Genet 1992;8:38–41.
58. Peterson CL. SWI/SNF complex: dissection of a chromatin remodeling cycle. Cold Spring Harbor Symp Quan Biol 1998;LXIII:545–552.
59. Logie C, Tse C, Hansen JC, Peterson CL. The core histone N-terminal domains are required for multiple rounds of catalytic chromatin remodeling by the yeast SWI/SNF and RSC complexes. Biochemistry 1999;38:2514–2522.
60. Côté J, Quinn J, Workman J, Peterson CL. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF protein complex. Science 1994;265:53–60.
61. Cairns BR, Lorch Y, Li Y, Zhang M, Lacomis L, Erdjument-Bromage H, Tempst P, Du J, Laurent B, Kornberg RD. RSC, an essential, abundant chromatin-remodeling complex. Cell 1996;87:1249–1260.

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Roles of the Histone H2A-H2B Dimers and the $(H3\text{-}H4)_2$ Tetramer in Nucleosome Remodeling by the SWI-SNF Complex*

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SWI-SNF is an ATP-dependent chromatin remodeling complex required for expression of a number of yeast genes. Previous studies have suggested that SWI-SNF action may remove or rearrange the histone H2A-H2B dimers or induce a novel alteration in the histone octamer. Here, we have directly tested these and other models by quantifying the remodeling activity of SWI-SNF on arrays of $(H3\text{-}H4)_2$ tetramers, on nucleosomal arrays reconstituted with disulfide-linked histone H3, and on arrays reconstituted with histone H3 derivatives site-specifically modified at residue 110 with the fluorescent probe acetylethylenediamine-(1,5)-naphthol sulfonate. We find that SWI-SNF can remodel $(H3\text{-}H4)_2$ tetramers, although tetramers are poor substrates for SWI-SNF remodeling compared with nucleosomal arrays. SWI-SNF can also remodel nucleosomal arrays that harbor disulfide-linked $(H3\text{-}H4)_2$ tetramers, indicating that SWI-SNF action does not involve an obligatory disruption of the tetramer. Finally, we find that although the fluorescence emission intensity of acetylethylenediamine-(1,5)-naphthol sulfonate-modified histone H3 is sensitive to octamer structure, SWI-SNF action does not alter fluorescence emission intensity. These data suggest that perturbation of the histone octamer is not a requirement or a consequence of ATP-dependent nucleosome remodeling by SWI-SNF.

The assembly of eukaryotic DNA into folded nucleosomal arrays has drastic consequences for many nuclear processes that require access to the DNA sequence, including RNA transcription, DNA replication, recombination, and repair. The nucleosome, which consists of 147 bp[1] of DNA wrapped nearly twice around an octamer of histones H2A, H2B, H3, and H4, can occlude DNA sequences both *in vivo* and *in vitro*. The nucleosome is not a static structure but appears to be a dynamic and flexible assembly. For instance, moderate concentrations of NaCl can lead to several distinct changes in nucleosome conformation (1–4). In addition, nucleosomes isolated from transcriptionally active chromatin appear to be depleted of histone H2A-H2B dimers (discussed in Ref. 5) and contain histone octamers the interiors of which are more accessible to enzymatic and chemical modifications (6–8). Nucleosomes from transcriptionally active chromatin can also be visualized microscopically as extended, largely unfolded structures (9, 10). These and other studies have led to the view that regulatory factors might antagonize the repressive effects of chromatin by disrupting the structure or conformation of the histone octamer (discussed in Ref. 11).

Two types of highly conserved chromatin remodeling enzymes have been implicated as regulators of the repressive nature of chromatin structure (12, 13). Several members of the SWI2/SNF2 family of DNA-stimulated ATPases use the energy of ATP hydrolysis to disrupt nucleosome structure, which can lead to an enhanced mobility of nucleosomes (14–16, 64). The second type consists of the nuclear histone acetyltransferases that covalently modify lysine residues within the flexible N-terminal domains of the histone proteins. The *Saccharomyces cerevisiae* SWI-SNF complex is the prototype ATP-dependent chromatin remodeling complex. This widely conserved 2-MDa multisubunit assembly is required for the inducible expression of a number of diversely regulated yeast genes and for the full functioning of many transcriptional activators (reviewed in Refs. 17 and 18). SWI-SNF can be recruited to target genes via direct interactions with gene-specific activators (19–21), and in several cases, SWI-SNF facilitates the binding of activators to nucleosomal sites *in vivo* (22, 23). *In vitro*, the purified SWI-SNF complex is a DNA-stimulated ATPase that can use the energy of ATP hydrolysis to disrupt histone-DNA interactions. Although the mechanism by which SWI-SNF disrupts nucleosome structure is not known, this "remodeling" reaction leads to an enhanced accessibility of nucleosomal DNA to DNase I (24–26), restriction enzymes (27, 28), and sequence-specific DNA-binding proteins (24, 25, 29). More recently, SWI-SNF has been shown to increase DNA accessibility of nucleosomal arrays in a catalytic manner that is dependent on the presence of histone N-terminal domains (27, 28).

Four different models have been proposed to explain the mechanism by which ATP-dependent remodeling by the SWI-SNF complex increases nucleosomal DNA accessibility. 1) Several studies have suggested that SWI-SNF might remove or rearrange the H2A-H2B dimers (24, 30–34). 2) SWI-SNF remodeling may induce a novel conformation of the histone octamer (Refs. 35 and 36; discussed in Ref. 11) that might involve conformational changes in the $(H3\text{-}H4)_2$ tetramer analogous to the transcription-associated transitions described above. 3) SWI-SNF may use the energy of ATP hydrolysis to translocate along DNA and destabilize histone-DNA interactions (discussed in Ref. 37). This model is similar to the octamer spooling mechanism described by Studitsky *et al.* (38) for passage of polymerases through nucleosomes. 4) Finally, SWI-SNF might bind directly to nucleosomes and use the energy of ATP hydrolysis to change the path of nucleosomal DNA (25) or to peel DNA

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[1] The abbreviations used are: bp, base pair(s); AEDANS, acetylethylenediamine-(1,5)-naphthol sulfonate; 1,5-IAEDANS, iodoacetylethylenediamine-(1,5)-naphthol sulfonate.

off the surface of the histone octamer without changing octamer structure (39).

In this study, we have directly tested whether ATP-dependent chromatin remodeling by the SWI-SNF complex alters the composition or conformation of the histone octamer. We use a nucleosomal array remodeling assay (27, 28) to quantify SWI-SNF activity on arrays of histone $(H3\text{-}H4)_2$ tetramers and on nucleosomal arrays reconstituted with histone octamers containing internally cross-linked tetramers. In order to monitor more subtle or transient changes in octamer structure, we also measured the effects of SWI-SNF remodeling on the steady state fluorescence of nucleosomal and tetramer arrays harboring a histone H3 derivative site specifically modified at residue 110 with the fluorescent probe acetylethylenediamine-(1,5)-naphthol sulfonate (AEDANS) (1, 2, 40). Taken together, our data are consistent with a model in which substrate recognition by SWI-SNF requires an intact histone octamer, and subsequent ATP-dependent remodeling disrupts histone-DNA contacts without a concomitant loss of histone proteins or perturbation of the histone octamer.

EXPERIMENTAL PROCEDURES

Reagent Preparation—Array DNA template was isolated by digestion of plasmid pCL7c with *Not*I, *Hin*dIII, and *Hha*I (New England Biolabs) followed by FPLC purification on Sephacryl-500 (Amersham Pharmacia Biotech) essentially as described (27, 41). Array DNA template was end-labeled as described (27, 41).

Chicken erythrocyte histone octamers were purified from chicken whole blood (Pel-Freez) as described previously (42). Tetramers were provided as a kind gift from Jeff Hansen and were purified by stepwise elution from hydroxylapatite columns as described (42). Tetramers were dialyzed against Buffer T (1 M NaCl, 10 mM Tris-HCl, pH 8.0, 0.25 mM EDTA, 0.1 mM dithiothreitol) prior to array reconstitution. Disulfide-linked histone octamers were generated by first diluting histone octamers 2-fold with Buffer D1 (10 M urea, 2 M NaCl, 20 mM Tris-HCl, pH 8.0) followed by dialysis against Buffer D2 (5 M urea, 2 M NaCl, 10 mM Tris-HCl, pH 8.0) at 4 °C with constant agitation for 4 days. Histone octamers were then reconstituted by dialysis against Buffer D3 (2 M NaCl, 10 mM Tris-HCl (pH 8.0), 0.25 mM EDTA). Histone H3 oxidation efficiency was analyzed by SDS-polyacrylamide gel electrophoresis in the absence of reducing agent (see Fig. 1*B, lane 3*). AEDANS-modified histone H3 was generated by first diluting histone octamers 2-fold with Buffer D1, followed by addition of iodoacetylethylenediamine-(1,5)-naphthol sulfonate (1,5-IAEDANS) (Molecular Probes, Inc.) at a molar ratio of 20:1 (1,5-IAEDANS:H3) and incubation for 2 h at room temperature on a nutator (Adams). Reactions were quenched with an excess of β-mercaptoethanol (Sigma) and dialyzed against Buffer D2 to remove unreacted 1,5-IAEDANS. Samples were then dialyzed against Buffer D3 to reconstitute histone octamers. Labeling specificity was verified by visualization of the corresponding fluorescent histone band upon illumination with long wave ultraviolet light.

SWI-SNF complex was purified from yeast strain CY396 or CY743 (*sin3*Δ) as described (41). The concentration of complex was determined to be approximately 300 nM by comparative Western blot and by ATPase assays (27, 41).

Reconstitution and Analysis of Nucleosomal Arrays—Arrays were reconstituted onto the 208-11 S DNA template (Fig. 1*A*) in a Slide-a-lyzer dialysis cassette (Pierce) using the salt dialysis protocol of Hansen and Lohr (43). Octamer concentrations were determined by A_{230} (44). Array saturation and nucleosome/tetramer positioning was determined by *Eco*RI or *Msp*I digestions using approximately 20 nM array in remodeling buffer (5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol) as described previously (27, 28, 41, 42). Arrays were digested for 30 min at 37 °C, and the reactions were electrophoresed on 4% native polyacrylamide gels (see Fig. 1*C*). The gel was briefly soaked in 2 μg/ml ethidium bromide and photographed under ultraviolet illumination.

Assay Conditions—Coupled array remodeling-restriction reactions were performed in a final concentration of 5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 1 mM ATP, and 500 units/ml *Hin*cII (New England Biolabs) as described previously (27, 28, 41).

Fluorescence spectroscopic studies were carried out using a PTI QM1/S.E.901/S.E.910Q fluorescence spectrophotometer. Samples were excited at 344 nm (slit width, 2.5 nm), and emission intensities were recorded from 450 to 500 nm (slit width, 4 nm). Samples (80 μl in quartz microcuvettes) contained 4 nM AEDANS-labeled octamer or tetramer array in remodeling buffer (described above) at room temperature. In experiments assessing effects of monovalent salt, emission intensities were recorded initially 0.5 min after adjustment to 50, 75, 150, and 300 mM NaCl and 0.5, 60, 120, 240, 480, and 960 min after subsequent adjustment to 600 mM NaCl. In experiments assessing effects of SWI-SNF, emission intensities were recorded initially at 5 min after addition of a final concentration of 2, 10, or 20 nM purified SWI-SNF and 5, 10, and 60 min after subsequent addition of a 1 mM final concentration of ATP. For samples in which SWI-SNF, ATP, or salt was added to the arrays, data were corrected for a buffer addition control. All data were corrected for dilution.

RESULTS

SWI-SNF Remodels Arrays of Histone $(H3\text{-}H4)_2$ Tetramers—It has been proposed that SWI-SNF increases the accessibility of nucleosomal DNA by depletion or rearrangement of the histone H2A-H2B dimers (Refs. 24, 30, 31, 32, and 34; discussed in Ref. 33). In order to investigate whether loss of the dimers is equivalent to the SWI-SNF remodeled state, we used purified chicken erythrocyte histone octamers or $(H3\text{-}H4)_2$ tetramers (Fig. 1*B, lanes 1* and *4*) to reconstitute nucleosomal or tetramer arrays. The DNA template used for these reconstitutions is composed of 11 head-to-tail repeats of a nucleosome positioning sequence from the *Lytechinus variegatus* 5 S rRNA gene, each of which is flanked by *Eco*RI restriction sites. Nucleosomes that are reconstituted onto each of these 5 S repeats assume a major translational position that is present in at least 50% of the population (45, 46). Minor translational positions also exist and differ from the major frame by multiples of 10 bp. The central repeat of our array template also bears a unique *SalI*/*Hin*cII restriction site close to the dyad axis of symmetry of a nucleosome positioned at the major frame (27, 47). Array reconstitutions were analyzed for extent of DNA repeat saturation and for correct positioning by restriction enzyme cleavage (Fig. 1*C*; see also under "Experimental Procedures") (27, 41, 42). *Eco*RI digestion of nucleosomal or tetramer arrays releases primarily mononucleosome-sized particles and few of the high molecular weight partial digestion products that would be indicative of alternative positioning or oversaturation (Fig. 1*C*). Digestion of nucleosomal or tetramer arrays with *Msp*I (the site of which is located ~30 bp from the predicted dyad axis of a nucleosome positioned at the major frame) demonstrates that these sites are fully protected by nucleosomal arrays but accessible in tetramer arrays (Fig. 1*C*). These data are consistent with the fact that an $(H3\text{-}H4)_2$ tetramer assumes the same translational positions as an intact octamer (48, 49) but that the tetramer assembles less DNA (34).

We then exploited a sensitive nucleosomal array remodeling assay in which SWI-SNF remodeling activity is coupled to restriction enzyme activity (27, 28). Previously we used this assay to determine the kinetic parameters of ATP-dependent nucleosomal array remodeling by the SWI-SNF and RSC (remodels the structure of chromatin) complexes (27, 28, 41). To quantify the remodeling capacity of SWI-SNF complex, 3 nM of nucleosomal or tetramer array was exposed to 500 units/ml *Hin*cII, either in the presence or absence of 3 nM SWI-SNF complex and 1 mM ATP (Fig. 2*A*). As described previously, *Hin*cII digestion of nucleosomal arrays is biphasic (27); the first, rapid phase of the reaction represents digestion of arrays harboring *Hin*cII sites positioned between nucleosomes, and the second, slow phase represents digestion of the nucleosomal *Hin*cII sites. By limiting our analysis to the second phase of *Hin*cII digestion, the first order rate of *Hin*cII cleavage yields a quantitative measurement of nucleosomal DNA accessibility (27, 28, 47).

In the absence of SWI-SNF, the first order rate of *Hin*cII

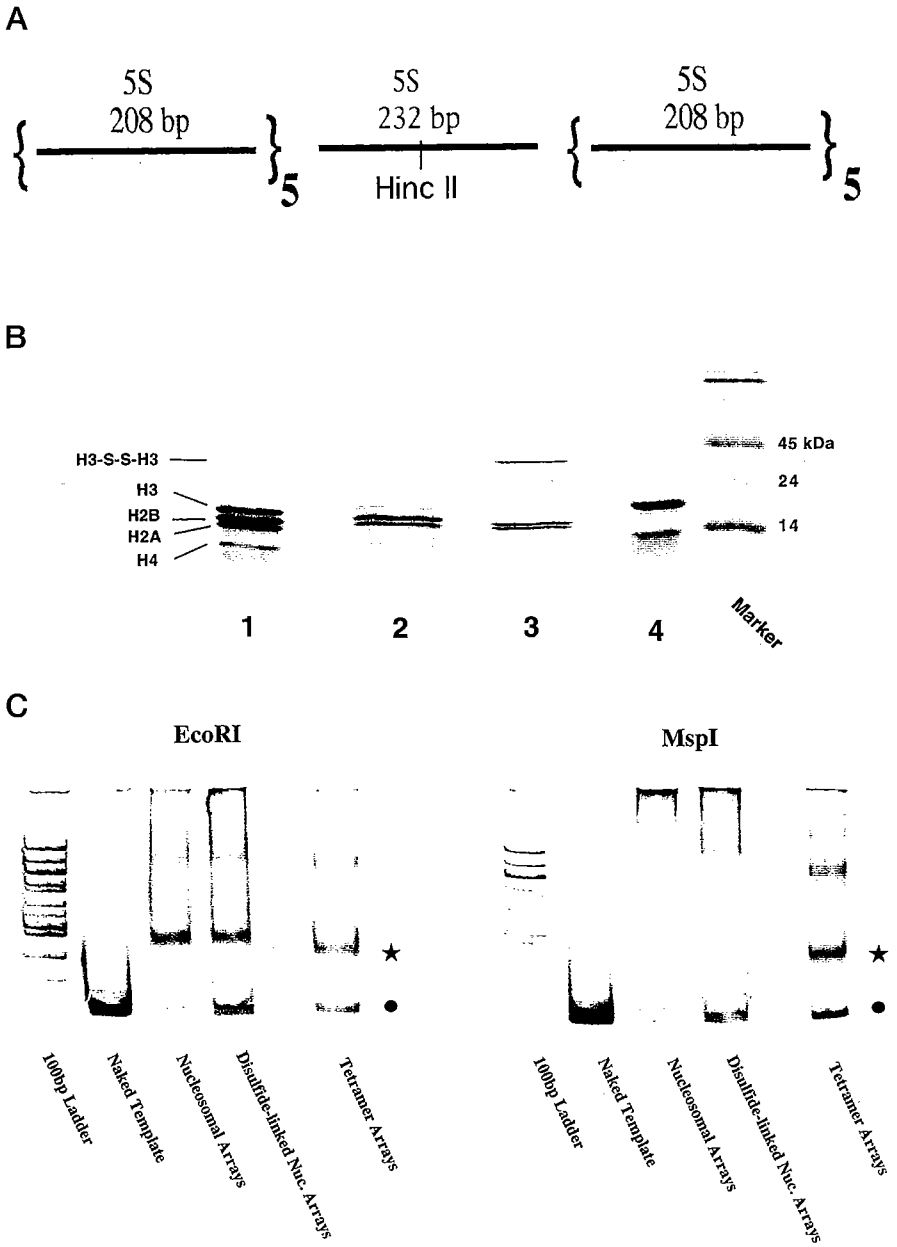

FIG. 1. **Array reconstitutions.** *A,* schematic representation of the 208-11 S template DNA used to reconstitute nucleosomal and tetramer arrays. It consists of 11 head-to-tail repeats of a 5 S rRNA nucleosome positioning sequence, the central repeat bearing an unique *SalI*/*Hinc*II restriction enzyme site. Each repeat is flanked by *Eco*RI restriction enzyme sites. In addition, an *Msp*I site is located ~30 bp from the predicted dyad axis of symmetry of each positioned nucleosome. *B,* analysis of histone proteins used for array reconstitutions by 18% SDS-polyacrylamide gel electrophoresis and Coomassie staining. *Lane 1,* chicken histone octamers; *lane 2,* chicken histone octamers contained 1,5-IAEDANS conjugated to histone H3; *lane 3,* oxidized chicken histone octamers electrophoresed under nonreducing conditions; *lane 4,* chicken $(H3\text{-}H4)_2$ tetramers. *C,* analysis of saturation and positioning of reconstituted arrays by restriction enzyme digestion and native polyacrylamide gel electrophoresis. Digestion of arrays with *Eco*RI releases primarily mononucleosome- or monotetramer-sized particles (★), as well as some free 208-bp 5 S repeats (●). The ratio of free 5 S repeats to mononucleosome/monotetramer particles provides a qualitative measurement of array saturation (60–90% saturation for the arrays shown here). Higher molecular weight species represent partial *Eco*RI digestion products. Nucleosome assembly on the 5 S repeats results in occlusion of the *Msp*I sites, whereas these sites are accessible for the tetramer arrays and yield a monotetramer-sized particle (★), indicating that the $(H3\text{-}H4)_2$ tetramers protect less DNA as expected.

cleavage was 1.3×10^{-5} for tetramer arrays and 2.4×10^{-6} for nucleosomal arrays (Fig. 2; see also Table I). The 5-fold higher rate for the tetramer arrays is comparable to the rate observed previously for nucleosomal arrays reconstituted with histones that lack their trypsin-sensitive N-terminal domains (28) and is consistent with nucleosomal DNA being more accessible in the absence of the histone H2A-H2B dimers (31, 48, 50, 51). In the presence of SWI-SNF, the first order rate of *Hinc*II cleavage was 6.4×10^{-5} for tetramer arrays and 9.2×10^{-5} for nucleosomal arrays (Fig. 2; Table I). The fact that the rate of cleavage of nucleosomal arrays in the presence of SWI-SNF significantly exceeded the rate of cleavage of tetramer arrays in the absence of SWI-SNF (see Table I) indicates that remodeling is not equivalent simply to loss of histone H2A-H2B dimers. The fact that the rate of cleavage of tetramer arrays in the presence of SWI-SNF is 5-fold higher than the rate in the absence of SWI-SNF indicates further that SWI-SNF can remodel tetramer arrays. However, SWI-SNF remodeling of tetramer arrays appears to be quantitatively less efficient than SWI-SNF remodeling of nucleosomal arrays, as the rate of cleavage of tetramer arrays in the presence of SWI-SNF was 30% slower than that of nucleosomal arrays in the presence of SWI-SNF (Fig. 2*C*; see also Table I).

SWI-SNF Activity Is Not Catalytic on Arrays of $(H3\text{-}H4)_2$ Tetramers—Previously, we showed that SWI-SNF is able to catalytically remodel multiple nucleosomal arrays and that histone N-terminal domains are required for this reaction (27, 28). As remodeling of tetramer arrays appears to be quantitatively less effective than remodeling of nucleosomal arrays (see above), we investigated whether SWI-SNF was able to function catalytically, or only stoichiometrically, on tetramer arrays. Remodeling assays were carried out in which there was a 10-fold molar excess of array to remodeling complex (12 nM array to 1.2 nM SWI-SNF). Consistent with previous data, SWI-SNF was able to stimulate *Hinc*II cleavage of nucleosomal arrays throughout the 150-min time course (Fig. 3*A, left panel*; see also Refs. 27 and 28). However, there was only minor stimulation of *Hinc*II cleavage by SWI-SNF on tetramer arrays (Fig. 3*A, right panel*). In fact, the rate of *Hinc*II cleavage on tetramer arrays in the presence of SWI-SNF approximated the rate determined in the absence of remodeling complex (Fig. 2*A, right panel*; and Table I). Fig. 3*B* shows the quantification of

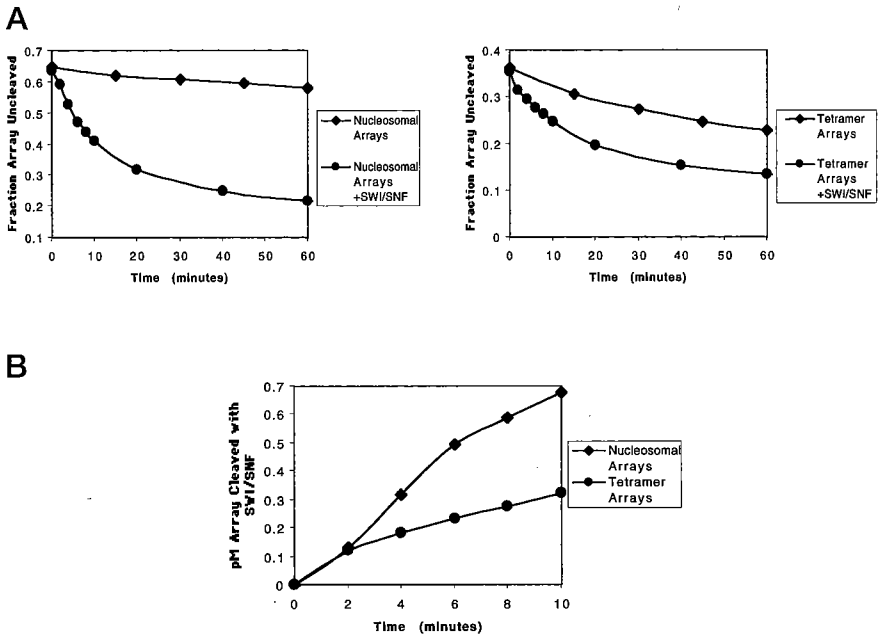

FIG. 2. **Tetramer arrays are not optimal substrates for SWI-SNF.** *A*, representative time course for *Hin*cII digestion of 3 nM nucleosomal (*left panel*) or $(H3\text{-}H4)_2$ tetramer (*right panel*) arrays in the presence (♦) or absence (●) of 3 nM SWI-SNF. *Time point 0* reflects a 20-min preincubation with *Hin*cII in the absence of SWI-SNF. The high percentage of tetramer array cleaved in the first phase of the digestion is due in part to slight undersaturation of these arrays. We note that similar levels of undersaturation of nucleosomal arrays have no effect on SWI-SNF remodeling rates (data not shown; see also Ref. 41). *B*, quantification of the data shown in *A*. Data are presented as the concentration of nucleosomal (♦) and tetramer (●) arrays cleaved by *Hin*cII in the presence of SWI-SNF during the initial 10 min of the reaction. Similar results were obtained in at least three different experiments using both different SWI-SNF preparations and independent nucleosomal and tetramer array reconstitutions.

TABLE I
Comparative analysis of array substrates

Array substrate	K_{conf}[a]	Rates of *Hin*cII cleavage		SWI/SNF-dependent rate stimulation
		Without SWI/SNF	With SWI/SNF	
		min^{-1}		
Nucleosomal	4.3×10^{-4}	2.4×10^{-6} ($\pm 2.0 \times 10^{-7}$)	9.2×10^{-5} ($\pm 1.0 \times 10^{-5}$)	37× (±14%)
Tetramer	2.3×10^{-3}	1.3×10^{-5} ($\pm 1.7 \times 10^{-6}$)	6.4×10^{-5} ($\pm 6.6 \times 10^{-6}$)	5× (±17%)
Disulfide-linked nucleosomal	6.8×10^{-4}	3.8×10^{-6} ($\pm 2.8 \times 10^{-7}$)	9.6×10^{-5} ($\pm 1.7 \times 10^{-5}$)	25× (±19%)
AEDANS-labeled nucleosomal	1.2×10^{-3}	6.6×10^{-6} ($\pm 8.0 \times 10^{-7}$)	1.1×10^{-4} ($\pm 1.5 \times 10^{-5}$)	17× (±18%)

[a] K_{conf} is the conformational equilibrium constant and is defined as the ratio of the rate of digestion of substrate array to the rate of digestion of naked DNA/unit of enzyme (47). The rate of digestion of naked DNA/unit of enzyme used for the K_{conf} determination is 5.6×10^{-3} min^{-1}.

the amount of SWI-SNF-dependent array cleavage during the 150-min time course. As expected, SWI-SNF was able to perform approximately 2.5 rounds (3 nM of array cleaved by 1.2 nM SWI-SNF) of nucleosomal array remodeling in 150 min (~50 min per round; see also Ref. 27). In contrast, SWI-SNF was unable to complete even one round of remodeling on tetramer arrays (0.3 nM array cleaved due to 1.2 nM SWI-SNF) during the time course. Therefore, SWI-SNF is unable to catalytically remodel multiple arrays of $(H3\text{-}H4)_2$ tetramers.

One possible explanation for the lack of catalytic remodeling of tetramer arrays may be that SWI-SNF has a higher affinity for tetramer arrays and is thus defective for product release (analogous to the explanation for the lack of catalytic remodeling of trypsinized nucleosomal arrays (28)). In order to investigate this possibility, SWI-SNF remodeling reactions were assembled that contained labeled nucleosomal array and a 3- or 12-fold molar excess of free DNA, nucleosomal, trypsinized nucleosomal, or tetramer competitor array. Under these conditions, tetramer arrays competed for SWI-SNF activity to the same extent as nucleosomal arrays or free DNA. In contrast, trypsinized nucleosomal arrays were markedly more potent in competing for SWI-SNF activity (data not shown; see also Ref. 28). This suggests that the defect in catalytic remodeling of tetramer arrays, unlike the defect in catalytic remodeling of trypsinized nucleosomes, is not due to a higher affinity of SWI-SNF for these arrays.

SWI-SNF Remodeling Does Not Require Disruption of the $(H3\text{-}H4)_2$ Tetramer—Chicken histone octamers contain only two cysteine residues (Cys-110 of each of the two copies of histone H3), and these cysteine residues are in close apposition within the interior of the histone octamer near the dyad axis of symmetry (34, 52). The cysteine residues normally are inaccessible to chemical modification, although denatured histone H3 can be disulfide-linked or chemically modified *in vitro* with minimal perturbation to the subsequently reconstituted histone octamer (52–54). In contrast, these cysteine residues appear to be more exposed to solvent in nucleosomes isolated from transcriptionally active chromatin (6–8) or when nucleosomes are exposed to higher salt concentrations (1, 2).

To investigate whether SWI-SNF action might require a structural transition of the $(H3\text{-}H4)_2$ tetramer, we reconstituted nucleosomal arrays with histone octamers that contain disulfide-linked histone H3 (Fig. 1*B*, *lane 3*; see under "Experimental Procedures" for details). As described above, 3 nM of nucleosomal or disulfide-linked nucleosomal array was incubated in the presence or absence of SWI-SNF and 1 mM ATP. In the absence of SWI-SNF, the first order rate of cleavage by *Hin*cII was 3.8×10^{-6} for the disulfide-linked nucleosomal array compared with 2.4×10^{-6} for nucleosomal arrays (Fig. 4*A*; Table I). This is consistent with previous observations that disulfide-linked histone octamers can be reconstituted into nucleosomes that are not grossly different from canonical nucleosomes (Refs. 52–54; see also Fig. 1*C*). We then quantified the ability of SWI-SNF to increase the accessibility of nucleosomal DNA to restriction enzyme cleavage on the disulfide-linked and nucleosomal arrays. In the presence of SWI-SNF complex, *Hin*cII cleavage was stimulated 25-fold on the disulfide-linked arrays and 37-fold on the control nucleosomal arrays (Fig. 4*A*; Table I). Furthermore, the first order rate of *Hin*cII cleavage in the presence of SWI-SNF was nearly identical for the disulfide-linked and control nucleosomal arrays (Fig. 4*B*; Table I). These data strongly suggest that structural perturbation of the his-

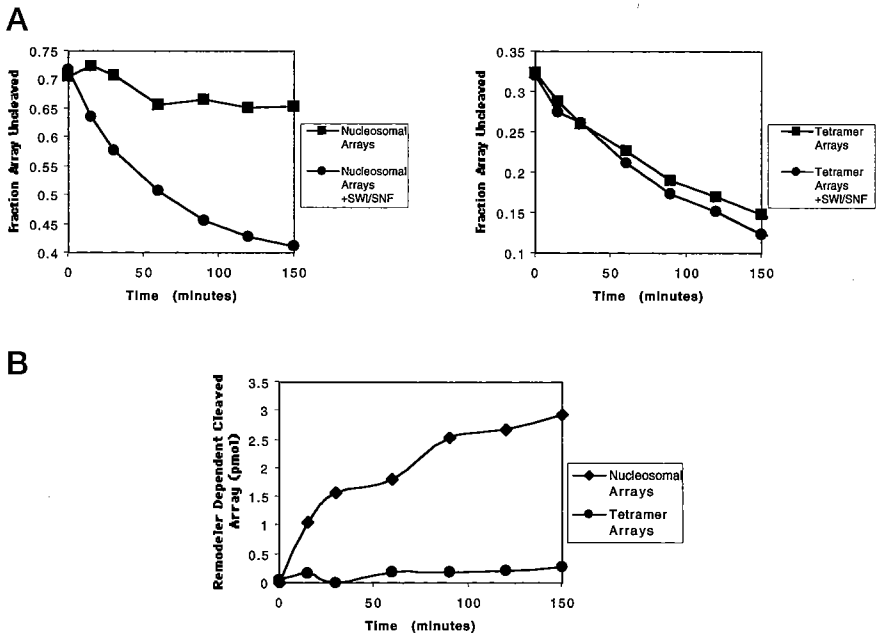

FIG. 3. **SWI-SNF activity is not catalytic on arrays of (H3-H4)$_2$ tetramers.** *A,* representative time course for *Hin*cII digestion of 12 nM nucleosomal (*left panel*) or (H3-H4)$_2$ tetramer (*right panel*) arrays in the presence (●) or absence (■) of 1.2 nM SWI-SNF complex. *Time point 0* reflects a 30-min preincubation with *Hin*cII in the absence of SWI-SNF. *B,* quantification of the data shown in *A*. Data are presented as the remodeler-dependent *Hin*cII cleavage of either nucleosomal (♦) or tetramer (●) arrays *versus* time. Remodeler-dependent *Hin*cII cleavage events were obtained by subtracting the fraction of cleaved arrays in the absence of SWI-SNF (*A,* ■) from the fraction of cleaved arrays in the presence of SWI-SNF (*A,* ●). Data are presented as nanomolar remodeled nucleosomal arrays *versus* time.

tone (H3-H4)$_2$ tetramer is not an obligatory intermediate or product of ATP-dependent nucleosome remodeling.

SWI-SNF Action Does Not Alter the Steady State Fluorescence of AEDANS-modified Arrays—The cysteine residue of histone H3 can also be modified with sulfhydryl-specific fluorescent groups (1, 2, 40, 55–57). For instance, reconstitution of nucleosomes with AEDANS-labeled H3 does not cause a significant perturbation of nucleosome structure (1, 2, 54), and steady state AEDANS fluorescence has been used to detect changes in octamer conformation as a function of monovalent cation concentration (1, 2). We used this method in an attempt to detect more subtle or transient changes in histone octamer integrity and conformation as a result of ATP-dependent SWI-SNF activity.

Nucleosomal and tetramer arrays were reconstituted after modification of histone H3 with the sulfhydryl-specific fluorescent probe AEDANS (1–2; see under "Experimental Procedures" for details). To confirm that the modification did not disrupt the structure of the arrays or affect the activity of SWI-SNF, we quantified the kinetics of *Hin*cII cleavage of the AEDANS-modified nucleosomal arrays in the presence or absence of SWI-SNF activity. In the absence of SWI-SNF, the first order rate of cleavage by *Hin*cII was 6.6×10^{-6} for the AEDANS-modified nucleosomal arrays and 2.4×10^{-6} for the nucleosomal arrays (Fig. 5*A*; Table I). The higher rate of cleavage observed for the AEDANS nucleosomal arrays indicates that this histone H3 modification may cause a slight perturbation in the structure or stability of the arrays. In the presence of SWI-SNF complex, however, the rate of *Hin*cII cleavage was nearly identical for the AEDANS-modified and nucleosomal arrays (Fig. 5*A*; Table I). Thus, the data indicate that SWI-SNF is fully functional on arrays reconstituted with AEDANS-H3.

AEDANS-modified nucleosomal and tetramer arrays were titrated with increasing NaCl concentrations in the absence of SWI-SNF, and fluorescence emission intensities were recorded 30 s after each adjustment of NaCl concentration. Consistent with previous studies (1, 2), a dramatic decrease in fluorescence emission intensity was detected with increasing NaCl concentration, suggesting increased solvent accessibility of the internal structure of the histone octamer (Fig. 5*B, left panels*). After adjustment of NaCl concentration to 600 mM, fluorescence emission intensities of nucleosomal and tetramer arrays were monitored as a function of time (Fig. 5*B, left panels*). Consistent with previous results (1, 2), a biphasic decrease in fluorescence emission intensity was observed, with a fast component on the second time scale (complete within 30 s) and a slow component on the hour time scale (complete within 4 h). These salt-dependent decreases in fluorescence emission intensity were observed for both nucleosomal and tetramer arrays, indicating that the observed decreases are not due to disruption of the interface between the (H3-H4)$_2$ tetramer and the H2A-H2B dimers. However, we note that AEDANS-modified tetramer arrays exhibit a ~3-fold lower fluorescence emission intensity than AEDANS-modified nucleosomal arrays (see Fig. 5*B, left panels*), suggesting that the presence of the H2A-H2B dimers decreases solvent accessibility of the (H3-H4)$_2$ tetramer.

We next measured the effect of SWI-SNF remodeling activity on the steady state fluorescence of AEDANS-modified nucleosomal or tetramer arrays. SWI-SNF (2, 10, 20 nM) was added to arrays (4 nM) equilibrated in remodeling buffer (see under "Experimental Procedures" for details) in the absence of ATP, and emission intensities were recorded (Fig. 5*B, right panel,* and data not shown). Subsequently, ATP was added to a final concentration of 1 mM, and emission intensities were recorded 5, 10, and 60 min after its addition (Fig. 5*B; right panel*). In striking contrast to NaCl titration (Fig. 5*B, left panels*), neither the binding of SWI-SNF (which occurs in the absence of ATP) nor ATP-dependent remodeling altered the fluorescence emission intensities of arrays (Fig. 5*B, right panels*). Addition of *Hin*cII directly to the reaction cuvettes confirmed that SWI-SNF and ATP stimulated *Hin*cII cleavage of the arrays and thus was active under these conditions (data not shown). Thus, these data indicate that SWI-SNF remodeling is not accompanied by the loss of the H2A-H2B dimers or by disruption or changes in the internal accessibility of the (H3-H4)$_2$ tetramer.

DISCUSSION

The *S. cerevisiae* SWI-SNF complex provides a paradigm for a family of eukaryotic protein assemblies that function in an ATP-dependent manner to alter chromatin structure. Although it is evident that chromatin remodeling by SWI-SNF and other related complexes results in enhanced accessibility of nucleosomal DNA and an increased mobility of nucleosomes, the mechanism by which this reaction occurs remains controversial. This study was designed to directly test simple predictions

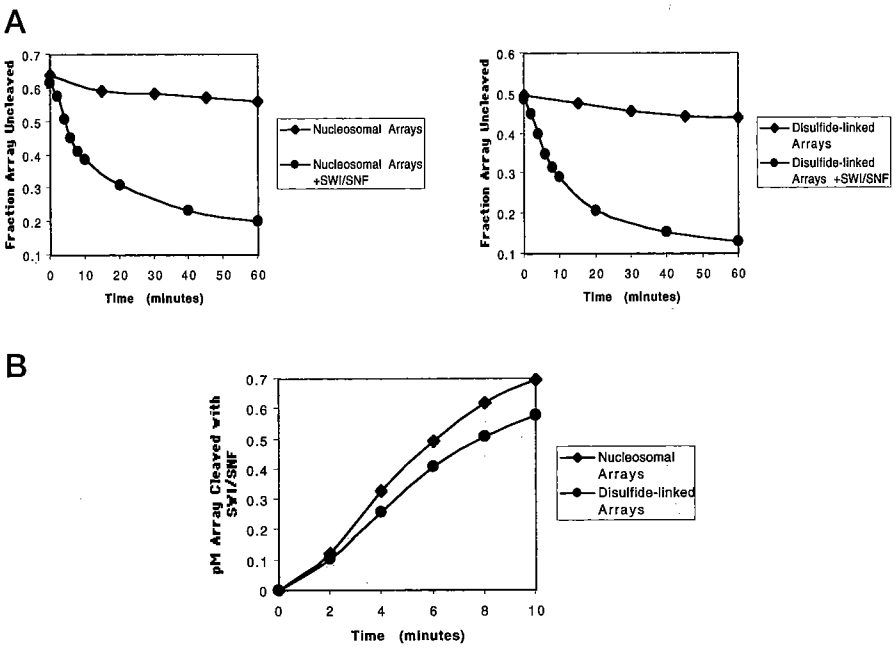

FIG. 4. **SWI-SNF can remodel nucleosomal arrays reconstituted with disulfide-linked histone octamers.** *A*, representative time course for *Hin*cII digestion of 3 nM nucleosomal (*left panel*) or disulfide-linked nucleosomal (*right panel*) array in the presence (●) or absence (♦) of 3 nM SWI-SNF. *Time point 0* reflects a 20-min preincubation with *Hin*cII in the absence of SWI-SNF. *B*, quantification of data shown in *A*. Results are presented as the concentration of nucleosomal and tetramer arrays cleaved by *Hin*cII in the presence of SWI-SNF during the initial 10 min of the reaction. Similar results were obtained in at least three different experiments using independent nucleosomal (♦) and disulfide-linked (●) nucleosomal array reconstitutions and SWI-SNF preparations.

for several models that have been proposed for ATP-dependent chromatin remodeling.

The "Dimer Disruption" Model—In the extreme case, the dimer disruption model predicts that ATP-dependent remodeling by SWI-SNF will generate a $(H3\text{-}H4)_2$ tetramer. Although assembly of a $(H3\text{-}H4)_2$ tetramer is sufficient to position at least 90 bp of DNA (34), several studies have shown that DNA-tetramer particles are more accessible to DNA-binding proteins (31, 50, 51), and removal of the histone H2A-H2B dimers facilitates transcription *in vitro* (58). A simple prediction of such a model is that the accessibility of DNA within $(H3\text{-}H4)_2$ tetramer arrays should be equivalent to that of nucleosomal DNA after SWI-SNF remodeling. However, we find that DNA wrapped around a $(H3\text{-}H4)_2$ tetramer is only about 5-fold more accessible to *Hin*cII digestion compared with DNA assembled onto a complete histone octamer. SWI-SNF action, on the other hand, enhances nucleosomal DNA accessibility by ~35-fold (see Table I). Thus, the remodeled state of a nucleosome is not equivalent to a $(H3\text{-}H4)_2$ tetramer. The dimer disruption model also predicts that SWI-SNF will not be able to enhance the accessibility of $(H3\text{-}H4)_2$ tetramers. We found, however, that SWI-SNF can remodel arrays of $(H3\text{-}H4)_2$ tetramers, which leads to an additional 5-fold enhancement of *Hin*cII cleavage rates. Finally, we show that fluorescence emission intensity of AEDANS-modified tetramer arrays is significantly lower than that of AEDANS-modified nucleosomal arrays (presumably due to increased solvent accessibility of the internal structure of the tetramer) (Fig. 5*B*). The fact that SWI-SNF remodeling of AEDANS-modified nucleosomal arrays does not decrease fluorescence emission intensity from the level characteristic of nucleosomal arrays to the level of tetramer arrays (Fig. 5*B*, *right panels*) also indicates that remodeling does not involve dimer disruption. Thus, SWI-SNF action does not convert a nucleosome into a tetramer.

The "Nucleosome Spooling" Model—The nucleosome spooling model for SWI-SNF action is based on the mechanism for passage of some RNA polymerases through a nucleosome (38). This model proposes that the energy of ATP hydrolysis might be used to translocate SWI-SNF along DNA and around a nucleosome in a "wave-like" fashion (discussed in Ref. 37). Such ATP-driven translocation of SWI-SNF along the DNA would disrupt histone-DNA contacts and may also lead to movement of the histone octamer. Such a reaction mechanism might also result in transfer of intact histone octamers onto an acceptor DNA, which has been observed during nucleosome remodeling by the yeast RSC complex (59). The nucleosome spooling model predicts that SWI-SNF will not discriminate between a tetramer and nucleosomal substrate. In fact, one might predict that the absence of H2A-H2B dimers might facilitate the ability of SWI-SNF to translocate through the residual histone-DNA interactions of the $(H3\text{-}H4)_2$ tetramer. Although SWI-SNF does remodel the $(H3\text{-}H4)_2$ tetramer arrays, the apparent rate of remodeling was approximately 30% slower than the rate for nucleosomal array remodeling. Furthermore, SWI-SNF was inactive on the $(H3\text{-}H4)_2$ tetramers in remodeling reactions in which the concentration of tetramer array was in excess over SWI-SNF. These results demonstrate that arrays of $(H3\text{-}H4)_2$ tetramers are poor substrates for ATP-dependent remodeling by SWI-SNF, a result that is not predicted by the nucleosome spooling model. Furthermore, SWI-SNF does not show ATP-dependent tracking activity in a DNA supercoiling assay (60), nor do other SWI2/SNF2 family members (*e.g.* Mot1p) demonstrate DNA tracking activity (61). Together, these data suggest that ATP-dependent remodeling by SWI-SNF does not involve DNA tracking, nor is it equivalent to the loss of the H2A-H2B dimers. Our data do indicate that efficient remodeling activity requires a canonical histone octamer that contains both an $(H3\text{-}H4)_2$ tetramer and one or more H2A-H2B dimers.

SWI-SNF Action Does Not Perturb Octamer Structure—Several groups have recently suggested the alternative possibility that SWI-SNF activity might induce a novel conformation of the nucleosome that may involve rearrangement of the histone octamer without loss of histone proteins (32, 35, 36). This model is consistent with recent electron microscopy studies that indicated that ATP-dependent remodeling by SWI-SNF does not change the protein mass of a nucleosome and that remodeling is relatively insensitive to addition of an external cross-linking reagent, dimethyl suberimidate to mononucleosomes or nucleosomal arrays (39). What is this alternate conformation? Lee *et al.* (32) proposed that human SWI-SNF might use the energy of ATP hydrolysis to rearrange one or both H2A-H2B dimers such that only the flexible N-terminal domain contacts DNA close to the nucleosomal dyad. This novel octamer conformation might then have a propensity to form the dinucleosome-like particle

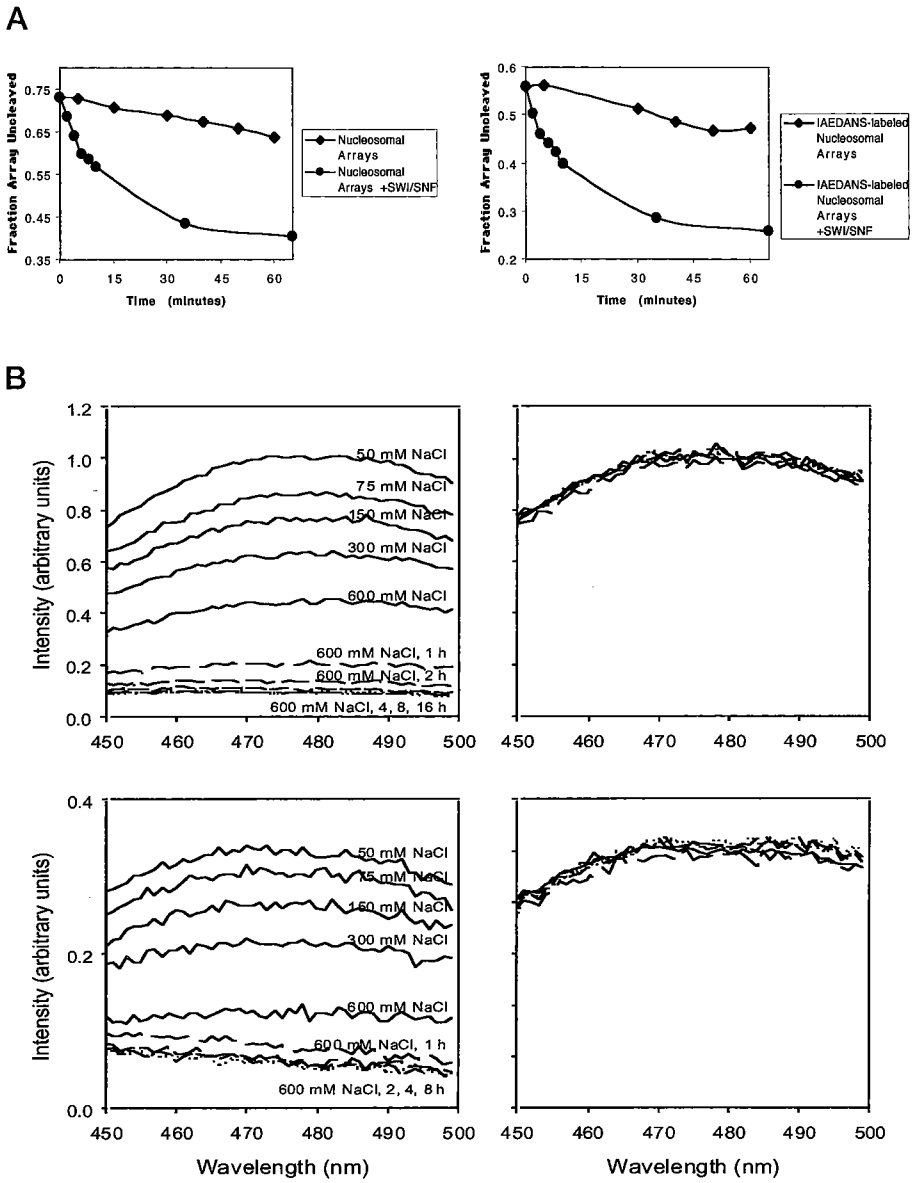

FIG. 5. **Effects of SWI-SNF on steady state fluorescence emission intensity of nucleosomal and tetramer arrays reconstituted with AEDANS-H3.** *A,* SWI-SNF can remodel AEDANS-labeled nucleosomal arrays (see also Table I). Representative time course for *Hin*cII digestion of 3 nM nucleosomal (*left panel*) or AEDANS-modified nucleosomal (*right panel*) arrays in the presence (●) or absence (♦) of 3 nM SWI-SNF. *Time point 0* reflects a 20-min preincubation with *Hin*cII in the absence of SWI-SNF. Similar results were obtained in at least three different experiments using independent nucleosomal and AEDANS-modified nucleosomal array reconstitutions. *B,* fluorescence emission spectra of AEDANS-modified nucleosomal and tetramer arrays. The *upper* and *lower left panels* illustrate the effect of increasing concentrations of NaCl on the steady state fluorescence of the modified nucleosomal arrays and tetramer arrays, respectively. Fluorescence emission intensities are shown in arbitrary units for AEDANS-modified arrays at equilibrium in 50 mM NaCl, 30 s after adjustment to 75, 150, 300, and 600 mM NaCl and 1, 2, 4, 8, and 16 h after adjustment to 600 mM NaCl. The *upper* and *lower right panels* illustrate the effect of SWI-SNF and ATP on the fluorescent-labeled nucleosomal and tetramer arrays, respectively. Fluorescence emission intensities are shown in arbitrary units for AEDANS-modified arrays at equilibrium in 5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), and 1 mM dithiothreitol (*solid line*); 5 min after addition of SWI-SNF to 10 nM (*long dashes*); and 5, 10, and 60 min after subsequent addition of ATP to 1 mM (*medium dashes, short dashes,* and *dots,* respectively). The experiment yielded identical results in the presence of 2 or 20 nM SWI-SNF (data not shown).

that was previously observed (35, 36). Alternatively, SWI-SNF action might lead to a conformational change in the $(H3\text{-}H4)_2$ tetramer that might mimic the "split nucleosome" or "lexosome" structure that that has been proposed for the structure of transcriptionally active chromatin (6, 9, 10).

To test these possibilities, we took advantage of the single cysteine residue found within each copy of chicken histone H3. These two cysteines are buried within the histone octamer and are located very close to each other at the nucleosomal dyad axis (34, 52). To test for gross changes in the structure of the $(H3\text{-}H4)_2$ tetramer, we monitored the apparent rates of remodeling of nucleosomal arrays that contain disulfide-linked $(H3\text{-}H4)_2$ tetramers. We found that SWI-SNF remodeled these substrates with rates equivalent to nucleosomal arrays. These data indicate that SWI-SNF action does not require a significant rearrangement of the tetramer.

To probe for more subtle changes in the structure of the histone octamer, we also monitored the effects of ATP-dependent remodeling on the steady state fluorescence of AEDANS-labeled nucleosomal and tetramer arrays. Steady state fluorescence of AEDANS-H3 has been used to detect at least three distinct conformational states of the nucleosome as a function of salt concentration (1, 2). Furthermore, the solvent accessibility of the AEDANS group, and thus its fluorescence emission intensity, is predicted from previous fluorescence studies to be highly dependent on the presence of one or both histone H2A-H2B dimers (Ref. 57; see also Fig. 5*B, left panels*).

We found that in contrast to increased salt concentrations, which have large effects on fluorescence emission intensities of nucleosomal or tetramer arrays (Refs. 1 and 2; see also Fig. 5*B, left panels*), addition of SWI-SNF (one SWI-SNF per two nucleosomes), with or without ATP, had no measurable effect on fluorescence emission intensities (Fig. 5*B, right panels*). These data suggest that SWI-SNF does not change the overall structure or the solvent accessibility of the histone octamer or tetramer.

What Is ATP-dependent Chromatin Remodeling?—Our data are consistent with previous suggestions that ATP-dependent nucleosome remodeling by SWI-SNF disrupts histone-DNA contacts without a structural change in the histone octamer (Refs. 25 and 39; discussed in Ref. 62). How does SWI-SNF accomplish this feat? The remodeling reaction randomizes the rotational setting of both wraps of nucleosomal DNA without removing most of the DNA from the octamer surface (25). Furthermore, SWI-SNF action does not enhance the reactivity of nucleosomal DNA to potassium permanganate, indicating

that remodeling does not involve an unwinding of the DNA double helix (25). We favor models in which the energy of ATP hydrolysis is used to rotate the DNA helix along its long axis relative to the histone octamer. In one such model, SWI-SNF would remain at a fixed position relative to the histone octamer, and both SWI-SNF and the octamer would remain in a fixed translational position relative to DNA. In this model, SWI-SNF would rotate the DNA helix back and forth with each round of ATP hydrolysis, changing rotational phasing and disrupting histone-DNA contacts throughout both wraps of nucleosomal DNA. In another, similar model, SWI-SNF would remain in a fixed position relative to the octamer, but SWI-SNF and the octamer would not remain in a fixed translational position relative to the DNA. In this model ATP-driven DNA helix rotation would "screw" the octamer along the DNA helix, changing the translational position of the octamer (analogous to a model proposed in Ref. 63). Either of these models would be consistent with previous data (see above and Ref. 25), as well as with recent studies indicating that several members of the SWI-SNF family of chromatin remodeling complexes can enhance nucleosome mobility (14–16, 64). Studies designed to test and distinguish between these models are in progress.

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REFERENCES

1. Dieterich, A. E., Axel, R., and Cantor, C. R. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **42,** 199–206
2. Dieterich, A. E., Axel, R., and Cantor, C. R. (1979) *J. Mol. Biol.* **129,** 587–602
3. Yager, T. D., and van Holde, K. E. (1984) *J. Biol. Chem.* **259,** 4212–4222
4. Czarnota G. J., and Ottensmeyer, F. P. (1996) *J. Biol. Chem.* **271,** 3677–3683
5. Hansen, J. C., and Ausio, J. (1992) *Trends Biochem. Sci.* **17,** 187–191
6. Prior, C. P., Cantor, C. R., Johnson, E. M., Littau, V. C., and Allfrey, V. G. (1983) *Cell* **34,** 1033–1042
7. Allegra, P., Sterner, R., Clayton, D. F., and Allfrey, V. G. (1987) *J. Mol. Biol.* **196,** 379–388
8. Chen, T. A., Smith M. M., Le, S. Y., Sternglanz, R., and Allfrey, V. G. (1991) *J. Biol. Chem.* **266,** 6489–6498
9. Bazett-Jones, D. P., Mendez, E., Czarnota, G. J., Ottensmeyer, F. P., and Allfrey, V. G. (1996) *Nucleic Acids Res.* **24,** 321–329
10. Czarnota, G. J., Bazett-Jones, D. P., Mendez, E., Allfrey, V. G., and Ottensmeyer, F. P. (1997) *Micron* **28,** 419–431
11. Workman, J. L., and Kingston, R. E. (1998) *Annu. Rev. Biochem.* **67,** 545–579
12. Pollard, K. J., and Peterson, C. L. (1998) *BioEssays* **20,** 771–780
13. Kingston, R. E., and Narlikar, G. J. (1999) *Genes Dev.* **13,** 2339–2352
14. Hamiche, A., Sandaltzopoulos, R., Gdula, D. A., and Wu, C. (1999) *Cell* **97,** 833–842
15. Langst G., Bonte, E. J., Corona, D. F., and Becker, P. B. (1999) *Cell* **97,** 843–852
16. Whitehouse I., Flaus, A., Cairns, B. R., White, M. F., Workman, J. L., and Owen-Hughes, T. (1999) *Nature* **400,** 784–787
17. Winston, F., and Carlson, M. (1992) *Trends Genet.* **8,** 387–391
18. Burns, L., and Peterson, C. L. (1997) *Biochim. Biophys. Acta* **1350,** 159–168
19. Natarajan, K., Jackson, B. M., Zhou, H., Winston, F., and Hinnebusch, A. G. (1999) *Mol. Cell* **4,** 657–664
20. Neely, K. E., Hassan, A. H., Wallberg, A. E., Steger, D. J., Cairns, B. R., Wright, A. P. H., and Workman, J. L. (1999) *Mol. Cell* **4,** 649–655
21. Yudkovky, N., Logie, C., Hahn, S., and Peterson, C. L. (1999) *Genes Dev.* **13,** 2369–2374
22. Burns, L., and Peterson, C. L. (1997) *Mol. Cell. Biol.* **17,** 4811–4819
23. Cosma, M. P., Tanaka, T., and Nasmyth, K. (1999) *Cell* **97,** 299–311
24. Côté, J., Quinn, J., Workman, J. L., and Peterson, C. L. (1994) *Science* **265,** 53–60
25. Côté, J., Peterson, C. L., and Workman, J. L. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 4947–4952
26. Owen-Hughes, T. A., Utley, R. T., Côté, J., Peterson, C. L., and Workman, J. L. (1996) *Science* **273,** 513–516
27. Logie, C., and Peterson, C. L. (1997) *EMBO J.* **16,** 6772–6782
28. Logie, C., Tse, C., Hansen, J., and Peterson, C. L. (1999) *Biochemistry* **38,** 2514–2522
29. Utley, R. T., Côté, J., Owen-Hughes, T., and Workman, J. L. (1997) *J. Biol. Chem.* **272,** 12642–12649
30. Hirschhorn, J. N., Brown, S. A., Clark, C. D., and Winston, F. (1992) *Genes Dev.* **6,** 2288–2298
31. Spangenberg, C., Eisfeld, K., Stunkel, W., Luger, K., Flaus, A., Richmond, T. J., Truss, M., and Beato, M. (1998) *J. Mol. Biol.* **278,** 725–739
32. Lee K. M., Sif, S., Kingston, R. E., and Hayes, J. J. (1999) *Biochemistry* **38,** 8423–8429
33. Peterson, C. L., and Tamkun, J. W. (1995) *Trends Biochem. Sci.* **20,** 143–146
34. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) *Nature* **389,** 251–260
35. Lorch, Y., Cairns, B. R., Zhang, M., and Kornberg, R. D. (1998) *Cell* **94,** 29–34
36. Schnitzler, G., Sif, S., and Kingston, R. E. (1998) *Cell* **94,** 17–27
37. Pazin, M. J., and Kadonaga, J. T. (1997) *Cell* **88,** 737–740
38. Studitsky, V. M., Clark, D. J., and Felsenfeld, G. (1994) *Cell* **76,** 371–382
39. Bazett-Jones, D. P., Cote, J., Landel, C. C., Peterson, C. L., and Workman, J. L. (1999) *Mol. Cell. Biol.* **19,** 1470–1478
40. Daban, J. R., and Cantor, C. R. (1989) *Methods Enzymol.* **170,** 192–214
41. Logie, C., and Peterson, C. L. (1999) *Methods Enzymol.* **304,** 726–741
42. Hansen, J. C., Ausio, J., Stanik, V. H., and van Holde, K. E. (1989) *Biochemistry* **28,** 9129–9136
43. Hansen, J. C., and Lohr, D. (1993) *J. Biol. Chem.* **268,** 5840–5848
44. Stein, A. (1979) *J. Mol. Biol.* **130,** 103–134
45. Dong, F., Hansen, J. C., and van Holde, K. E. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 5724–5728
46. Pennings, S., Meersseman, G., and Bradbury, E. M. (1991) *J. Mol. Biol.* **220,** 101–110
47. Polach, K. J., and Widom, J. (1995) *J. Mol. Biol.* **254,** 130–149
48. Dong, F., and van Holde, K. E. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 10596–10600
49. Hayes, J. J., Clark, D. J., and Wolffe, A. P. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 6829–6833
50. Hayes, J. J., and Wolffe, A. P. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 1229–1233
51. Tse, C., Fletcher, T. M., and Hansen, J. C. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 12169–12173
52. Camerini-Otero R. D., and Felsenfeld G. (1977) *Proc. Natl. Acad. Sci. U. S. A.* **74,** 5519–5523
53. Ausio, J., Seger, D., and Eisenberg, H. (1984) *J. Mol. Biol.* **176,** 77–104
54. Protacio, R. U., and Widom, J. (1996) *J. Mol. Biol.* **256,** 458–472
55. Eshaghpour, H., Dieterich, A. E., Cantor, C. R., and Crothers, D. M. (1980) *Biochemistry* **19,** 1797–1805
56. Cantor, C. R., Dieterich, A. E., and Prior, C. P. (1981) *Ann. N Y Acad. Sci.* **366,** 246–252
57. Feinstein, D. L., and Moudrianakis, E. N. (1986) *Biochemistry* **25,** 8409–8418
58. Hansen, J. C., and Wolffe, A. P. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 2339–2343
59. Lorch, Y., Zhang, M., and Kornberg, R. D. (1999) *Cell* **96,** 389–392
60. Quinn, J., Fyrberg, A., Ganster, R., Schmidt, M., and Peterson, C. L. (1996) *Nature* **379,** 844–847
61. Auble, D. T., and Stefferda, S. M. (1999) *Mol. Cell. Bio.*. **19,** 412–423
62. Peterson, C. L. (1998) *Cold Spring Harbor Symp. Quant. Biol.* **63,** 545–552
63. Varga-Weisz P. D., and Becker P. B. (1995) *FEBS Lett.* **369,** 118–121
64. Jaskelioff, M., Gavin, I., Peterson, C. L., and Logie, C. (2000) *Mol. Cell. Biol.*, in press

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Functional Delineation of Three Groups of the ATP-dependent Family of Chromatin Remodeling Enzymes*

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ATP-dependent chromatin remodeling enzymes antagonize the inhibitory effects of chromatin. We compare six different remodeling complexes: ySWI/SNF, yRSC, hSWI/SNF, xMi-2, dCHRAC, and dNURF. We find that each complex uses similar amounts of ATP to remodel nucleosomal arrays at nearly identical rates. We also perform assays with arrays reconstituted with hyperacetylated or trypsinized histones and isolated histone $(H3/H4)_2$ tetramers. The results define three groups of the ATP-dependent family of remodeling enzymes. In addition we investigate the ability of an acidic activator to recruit remodeling complexes to nucleosomal arrays. We propose that ATP-dependent chromatin remodeling enzymes share a common reaction mechanism and that a key distinction between complexes is in their mode of regulation or recruitment.

The assembly of eukaryotic DNA into folded nucleosomal arrays is likely to have a major impact on the efficiency or regulation of nuclear processes that require access to the DNA sequence, including RNA transcription, DNA replication, recombination, and repair. In fact, it is now generally recognized that disruption or remodeling of chromatin structure is a rate-determining step for most of these nuclear DNA transactions (1–3). Two classes of highly conserved chromatin remodeling enzymes have been implicated as regulators of the repressive nature of chromatin structure, the first class includes enzymes that covalently modify the nucleosomal histones (*e.g.* acetylation, phosphorylation, methylation, ADP-ribosylation; reviewed in Ref. 4), and the second class is composed of multisubunit complexes that use the energy of ATP hydrolysis to disrupt histone-DNA interactions (reviewed in Refs. 5 and 6).

Each member of the ATP-dependent family of chromatin remodeling enzymes contains an ATPase subunit that is related to the SWI2/SNF2 subfamily of the DEAD/H superfamily of nucleic acid-stimulated ATPases (7). Seventeen members of the SWI2/SNF2 family have been identified in the yeast genome (6), and to date, four of these ATPases have been purified as subunits of distinct chromatin remodeling complexes ySWI/SNF (8, 9), yRSC (10), ISW1 and ISW2 (11). Additional ATP-dependent remodeling complexes that harbor SWI2/SNF2 family members have been identified in *Drosophila* (dACF (12), dNURF (13), dCHRAC (14), Brahma (15, 16)), human (hSWI/SNF (17), hNURD (18–20), hRSF (21)), and frog (xMi-2 (22)). Although these complexes have a variable number of subunits (*i.e.* 3–15), and many different types of assays have been used to monitor the activity of individual complexes, each enzyme can apparently use the energy of ATP hydrolysis to alter chromatin structure and enhance the binding of proteins to nucleosomal DNA-binding sites (3, 5). Furthermore, in the case of the ySWI/SNF, *Drosophila* Brahma, and hSWI/SNF complexes, remodeling is required for transcriptional regulation of target genes *in vivo* (Refs. 23 and 24, for review, see Ref. 5).

ATP-dependent chromatin remodeling complexes have been further divided into three groups based on whether the sequence of the ATPase subunit is more related to yeast SWI2 (ySWI/SNF, yRSC, Brahma, and hSWI/SNF), *Drosophila* ISWI (ISW1, ISW2, dNURF, dCHRAC, dACF, and hRSF), or human Mi-2 (hNURD, xMi-2) (reviewed in Ref. 3). Although each of these ATPases share a SWI2/SNF2-like ATPase domain, they harbor additional, unique sequence motifs adjacent to the ATPase domain that are characteristic of each group, the SWI2 group contains a bromodomain (25), the ISWI group contains a SANT domain (26), and the Mi-2 group contains a chromodomain (27). Differences among some groups are also apparent in the nucleic acid cofactor required for stimulation of ATPase activity. For enzymes that contain a SWI2-like ATPase (ySWI/SNF, yRSC, and hSWI/SNF), ATPase activity is stimulated equally well by "free" DNA or nucleosomes (8, 10, 28). In contrast, the ATPase activity of enzymes that contain an ISWI-like or Mi-2-like ATPase is optimally stimulated by nucleosomes (18–20, 22, 29). In the case of ISWI-like ATPases, this requirement for nucleosomes may reflect obligatory interactions with the trypsin-sensitive, histone N-terminal domains (30).

Here we report the first direct comparison of the biochemical properties of six different chromatin remodeling enzymes (ySWI/SNF, yRSC, dCHRAC, dNURF, hSWI/SNF, and xMi-2) which encompass all three previously suggested groups. Surprisingly, each complex shows similar ATPase activity on nucleosomal array substrates, and they are each able to facilitate

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nucleosome mobilization within an array at nearly equivalent rates. We have also investigated the nucleosome substrate requirements for each enzyme by using arrays reconstituted with hyperacetylated or trypsinized histone octamers, as well as histone $(H3/H4)_2$ tetramers. ATPase and remodeling assays with these different substrates identify new common features, as well as new distinctions among enzymes. In addition, we test the ability of the GAL4-VP16 chimeric transcriptional activator to recruit these remodeling complexes to a nucleosomal array substrate. We report that ySWI/SNF is uniquely potent for recruitment by GAL4-VP16 in this assay. Our data are consistent with the differential regulation of ATP-dependent enzymes that each share a similar mechanism of nucleosome remodeling.

EXPERIMENTAL PROCEDURES

Reagent Preparation—The array DNA template contains 11 tandem, head-to-tail repeats of a 208-base pair sea urchin 5 S rRNA gene (31, 32). Template was isolated by digestion of plasmid pCL7c (208-11S) or pCL8b (208-11S-Gal4) with *Not*I, *Hin*dIII, and *Hha*I (New England Biolabs) followed by fast protein liquid chromatography purification on Sephacryl-500 (Amersham Pharmacia Biotech) essentially as described (31, 32). Array DNA template was end-labeled by Klenow fill-in reaction with [α-^{32}P]dATP as described (31, 32).

Chicken erythrocyte histone octamers were purified from chicken whole blood (Pel-Freez Biologicals) as described previously (33). Hyperacetylated histone octamers were purified from butyrate-treated HeLa cells as described (34). Trypsinized histone octamers and $(H3/H4)_2$ tetramers were purified as described (33, 35). $(H3/H4)_2$ tetramers were dialyzed against Buffer T (1 M NaCl, 10 mM Tris-HCl, pH 8.0, 0.25 mM EDTA, 0.1 mM dithiothreitol) prior to array reconstitution.

ySWI/SNF complex was purified from yeast strains CY396 or CY743(*sin3*Δ) as described in Logie and Peterson (31). The concentration of complex was determined to be approximately 300 nM by comparative Western blot and ATPase assays (31, 32). yRSC (10), xMi-2 (36), dCHRAC (14), dNURF (29), and hSWI/SNF "A" (17) were purified as described previously. Approximate concentrations were estimated from total protein concentration in the purified fractions and complexes were assumed to be 100% active. Thus, our concentration estimates are likely to be an overestimate. Most complexes had a high degree of purity, but in the case of hSWI/SNF A, purity was estimated to be ~10%. We confirmed that the activity monitored was in fact due to hSWI/SNF by antibody inhibition, addition of antisera directed to the BRG1 subunit of hSWI/SNF eliminated remodeling activity, whereas addition of preimmune sera had no effect.[1] For all the studies described here, each assay was performed with two independent preparations of each remodeling complex, with the exception of yRSC.

Reconstitution and Analysis of Substrate Arrays—Histone proteins used for array reconstitutions were analyzed by 18% SDS-polyacrylamide gel electrophoresis and Coomassie staining. Octamer concentrations were determined by A_{230} (37). Histone octamers were reconstituted onto the 208-11S DNA templates (or 208-11S-Gal4 for recruitment assay) in a slide-a-lyzer dialysis cassette (Pierce) by salt gradient dialysis as described previously (38). Each repeat of the 208-11S template (or 208-11S-Gal4 for recruitment assay) is flanked by *Eco*RI restriction enzyme sites. In addition, a unique *Msp*I site is located 30 base pairs from the predicted dyad axis of symmetry of each positioned nucleosome. Array quality, saturation, and positioning was determined by *Eco*RI or *Msp*I digestion using approximately 20 nM array in Remodeling Buffer (5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol) as described previously (31, 32, 38, 39). Arrays were digested for 30 min at 37 °C and electrophoresed on a 4% native polyacrylamide gel. The gel was briefly soaked in 2 μg/ml ethidium bromide and photographed under ultraviolet illumination. Saturation of arrays was analyzed by digestion with *Eco*RI and comparison of the ratio of nucleosome bound repeat to uncomplexed 208-base pair 5 S repeat. Positioning was analyzed by digestion with *Msp*I (32, 39). Whereas, nucleosomal, trypsinized, and hyperacetylated arrays were inaccessible to digestion with *Msp*I, $(H3/H4)_2$ tetramer arrays digested with *Msp*I released a mononucleosome size fragment indicating that the $(H3/H4)_2$ tetramers protect less DNA as expected (40).

Assay Conditions—ATPase reactions were performed with respect to the optimal temperature for remodeling complex activity: 27 °C for xMi-2, dCHRAC, and dNURF, 30 °C for yRSC and ySWI/SNF, and 37 °C for hSWI/SNF using 100 μM ATP and 0.2 μCi of [γ-^{32}P]ATP (Amersham Pharmacia Biotech) in 0.1% Tween, 20 mM Tris, pH 8, 5% glycerol, 0.2 mM dithiothreitol, 5 mM $MgCl_2$, 1 mM phenylmethylsulfonyl fluoride, and 0.1 mg/ml bovine serum albumin as described (31, 32, 41). Released phosphate was monitored with time by resolution of P_i from ATP on plastic plates coated with PEI cellulose (EM Science) with 0.75 M KPO_4 (pH 3.5) as solvent and quantified by PhosphorImager analysis.

Coupled array remodeling-restriction reactions were performed in a final concentration of 5 mM $MgCl_2$, 50 mM NaCl, 10 mM Tris-HCl (pH 8.0), 1 mM dithiothreitol, 0.1 mg/ml bovine serum albumin, 1 mM ATP, and 500 units/ml *Hin*cII (New England Biolabs) as described previously (31, 32, 41). Assays were performed with respect to the optimal temperature for remodeling complex activity (see above). *Hin*cII cleavage was quantified by PhosphorImager analysis, and first-order rates were determined by curve fitting. In multiple independent experiments, the first-order rates of restriction enzyme cleavage for each particular combination of array and remodeler varied by less than 20%.

RESULTS

ATPase and Remodeling Activities of Chromatin Remodeling Enzymes—In order to quantify the nucleosome remodeling activity of ATP-dependent remodeling enzymes, we have developed a biochemical assay where nucleosome remodeling activity is coupled to restriction enzyme activity such that remodeling is revealed as an enhancement of restriction enzyme cleavage rates (31, 32). This assay uses a nucleosomal array substrate in which the central nucleosome of an 11-mer nucleosomal array contains a unique *Sal*I/*Hin*cII site located at the predicted dyad axis of symmetry (31, 32). Restriction enzyme kinetics are biphasic in this system; the first phase is rapid and reflects the fraction of *Sal*I/*Hin*cII restriction sites that are not occluded by a nucleosome (due primarily in our assays to nucleosomes that occupy minor translational positions; see Refs. 32, 42, and 43). The second phase is slow and reflects a dynamic equilibrium between the occluded and "open" nucleosomal DNA states (44, 45). In previous studies, addition of yeast SWI/SNF and ATP stimulated the second phase of *Sal*I/*Hin*cII digestion 20–30-fold (32, 41). Recently we have found that SWI/SNF remodeling leads to a rapid redistribution of nucleosome positions within these arrays and that the apparent rate of remodeling determined in this assay provides an estimate of the rate of nucleosome mobilization (46).

Purified preparations of ySWI/SNF, yRSC, hSWI/SNF, dCHRAC, dNURF, and xMi-2 were analyzed in parallel for nucleosome-stimulated ATPase activity (see "Experimental Procedures"). Each complex was titrated in an ATPase reaction which contained 100 μM ATP and 12 nM of a reconstituted, 11-mer nucleosomal array. Surprisingly, the approximate concentration of each remodeling complex that was required to achieve equivalent velocities of ATP hydrolysis were similar; for ySWI/SNF (2 nM), yRSC (2 nM), hSWI/SNF (5 nM), dCHRAC (2 nM), and dNURF(4 nM), each complex catalyzed the hydrolysis of 450–600 nmol of ATP/min (Fig. 1*A*, see also Ref. 41). xMi-2 was slightly less active in this assay as ~15 nM was required to achieve this level of ATPase activity (Fig. 1*A*). Given that our estimates of active enzyme concentrations are only approximate (see "Experimental Procedures"), the data shown in Fig. 1*A* indicate that each of these enzymes have nucleosome-stimulated ATPase activities that are similar within an order of magnitude. The similar levels of ATPase activity among complexes was unexpected given that each complex has different associated subunits which, at least in the case of the hSWI/SNF complex, can have a large impact on the ATPase activity of the catalytic subunit (*i.e.* BRG1; Ref. 28).

To assess the capacity of the six different complexes to remodel an 11-mer nucleosomal array, each remodeling enzyme (1–5 nM ySWI/SNF, yRSC, hSWI/SNF, dCHRAC, dNURF, or 15 nM xMi-2) was added to 1.5 nM nucleosomal array and the

[1] D. Hill and A. N. Imbalzano, unpublished observations.

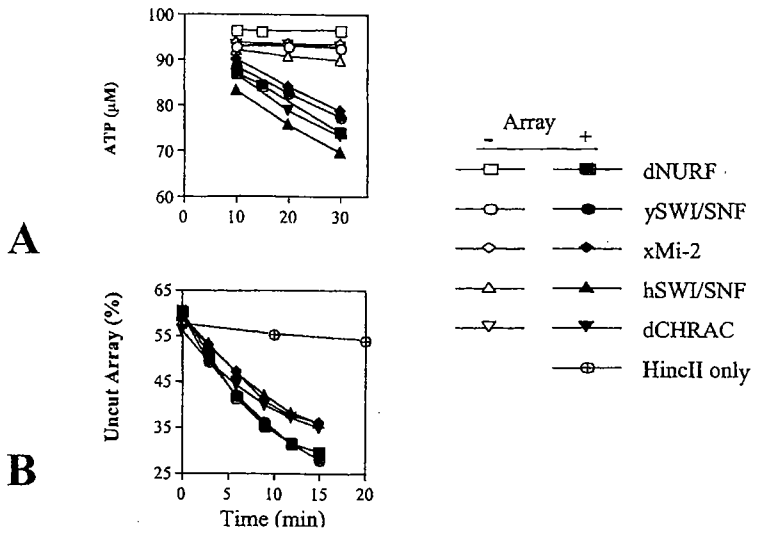

FIG. 1. **Comparison of ATPase and remodeling activities of ATP-dependent chromatin remodeling complexes.** *A*, ATPase assays. The indicated remodeling complexes (~1–5 nM ySWI/SNF, hSWI/SNF, dNURF, dCHRAC, or ~15 nM xMi-2) were analyzed in ATPase reactions that contained (*closed symbols*) or lacked (*open symbols*) 12 nM nucleosomal array, and ATP hydrolysis was monitored with time. Velocities of ATP hydrolysis were calculated from at least three reaction time points. *B*, nucleosomal array remodeling assays. *Hin*cII digestion of nucleosomal arrays incubated in the presence (*closed symbols*) or absence (*open symbols*) of the indicated remodeling complexes. *Hin*cII digestion rates were calculated from the slopes of plots of the natural logarithm of the fraction of uncut array *versus* time. These results are representative of multiple, independent experiments. Similar results were also obtained with at least two independent enzyme preparations for each complex except yRSC.

initial rates of *Hin*cII digestion were measured in parallel reaction time courses in the presence of ATP. We found that all six complexes enhanced the rate of *Hin*cII digestion essentially equivalently (Fig. 1*B*, see also Ref. 41 for a detailed comparison of ySWI/SNF and yRSC). The dCHRAC complex reproducibly yielded an approximately 2-fold lower rate of *Hin*cII digestion than all other complexes which probably reflects the fact that a significant amount of the ATPase activity of dCHRAC appears to be contributed by topoisomerase II (see below). Since the initial rate of *Hin*cII digestion provides an indirect measurement of the rate of remodeling, these data indicate that all six enzymes use similar amounts of ATP to remodel nucleosomal arrays at similar rates. Furthermore, since it appears that this coupled restriction enzyme-remodeling assay monitors the rate of nucleosome mobilization (46), all six enzymes can apparently redistribute nucleosomes within an array at comparable rates.

A hallmark of our nucleosomal array assay is that the SWI/SNF-dependent enhancement of restriction enzyme accessibility requires continuous ATP hydrolysis (32, 46). This requirement reflects a state of constant redistribution of nucleosome positions in the presence of ATP, and the subsequent inactivation of SWI/SNF "freezes" a random positioning of nucleosomes which is characterized by a general occlusion of restriction enzyme sites (46). We carried out similar remodeling/"reversal" assays with hSWI/SNF, dCHRAC, dNURF, or xMi-2 and in all cases the enhanced rates of *Hin*cII digestion were lost after ATP was enzymatically removed with apyrase (data not shown; for analysis of yRSC, see Ref. 41). Thus, these results indicate that all six complexes use the energy of ATP hydrolysis to create a dynamic, reversible state of nucleosome mobilization. Our results are consistent with previous demonstrations of mononucleosome mobilization catalyzed by ySWI/SNF (46, 49), dCHRAC (47) or dNURF (48).

Nucleosome Moiety Requirements of the Chromatin Remodeling Complexes—Previous studies have demonstrated that optimal ATPase activity of dNURF (29), dCHRAC (14), and xMi-2/NURD (18–20, 22) complexes requires nucleosomes, whereas the ATPase activities of hSWI/SNF (17), ySWI/SNF (8), and yRSC (10, 41) complexes are stimulated equally well by free DNA. Furthermore, in the case of the dNURF complex, the nucleosome stimulation of ATPase activity requires one or more trypsin-sensitive histone N-terminal domain(s) (30). To further define the nucleosome moiety requirements for all six complexes, we reconstituted nucleosomal arrays with hyperacetylated or trypsinized histone octamers, as well as with histone $(H3/H4)_2$ tetramers. To ensure that each type of array reconstitution was of similar quality, all reconstitutions were analyzed for extent of DNA repeat saturation and correct positioning by multiple restriction enzyme mapping and native polyacrylamide gel electrophoresis (see "Experimental Procedures"). We then measured the ability of these arrays to stimulate the ATPase activity of each complex (Fig. 2*A*). As expected, ySWI/SNF and yRSC complex hydrolyzed ATP with similar kinetics on all substrates, including free DNA (Fig. 2*A*; see also, Ref. 41). Likewise, the ATPase activity of the hSWI/SNF complex was stimulated by all substrates, with the exception that activity was consistently 40–50% less in the presence of arrays reconstituted with histone $(H3/H4)_2$ tetramers (Fig. 2*A*).

In agreement with previous studies, we also found that the ATPase activity of the dNURF complex was maximally stimulated only by nucleosomal arrays (for analysis of ATPase activity with DNA or hyperacetylated substrates, see Ref. 30); little ATPase activity was detected with arrays reconstituted with trypsinized histone octamers or histone $(H3/H4)_2$ tetramers. Given that the ATPase activity of dNURF requires one or more histone N-terminal domain(s) (Fig. 2*A*; see also, Ref. 30), the lack of ATPase activity in the presence of the histone $(H3\text{-}H4)_2$ tetramer arrays suggested that the N-terminal domains of the histone H2A/H2B dimers might play a key role. However, nucleosomal arrays reconstituted with hybrid histone octamers composed of intact histone $(H3\text{-}H4)_2$ tetramers and tail-less histone H2A-H2B dimers yielded maximal stimulation of dNURF ATPase activity.[2] Thus, the inability of $(H3\text{-}H4)_2$ tetramer arrays to stimulate the ATPase activity of dNURF does

[2] P. Horn and C. L. Peterson, unpublished observation.

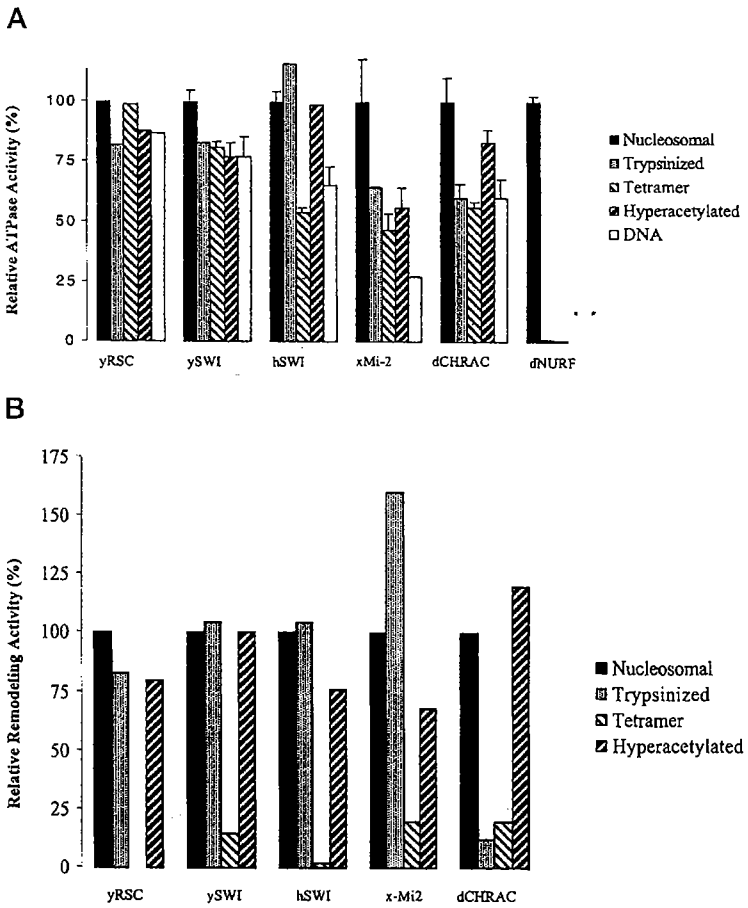

FIG. 2. **Nucleosome moiety requirements for ATP-dependent chromatin remodeling enzymes.** *A*, ATPase assays. The indicated remodeling enzymes were added to ATPase assays that contained either 208-11S DNA template (DNA) or 208-11S arrays reconstituted with histone octamers, hyperacetylated histone octamers, trypsinized histone octamers, or isolated $(H3\text{-}H4)_2$ tetramers. Each reaction represented an ATPase time course, and ATP hydrolysis velocities were calculated for each substrate. Data is presented as a percentage of the ATPase velocity exhibited with the nucleosomal array substrate. Data shown for yRSC is the result of a single experiment which essentially repeated our prior study (41). *, denotes that ATPase assays with DNA and hyperacetylated array substrates were not performed with dNURF (see Ref. 30 for detailed analysis). *B*, nucleosomal array remodeling assays. *Hin*cII digestion rates were determined for each enzyme on each array substrate. For each substrate, rates were calculated from the slopes of plots of the natural logarithm of the fraction of uncut array *versus* time. Data is presented as a percentage of the remodeler-dependent *Hin*cII digestion rate of the nucleosomal array. With the exception of yRSC, the results shown include experiments with at least two independent enzyme preparations for each complex. *, denotes that remodeling of tetramer arrays was not performed with yRSC. In addition, remodeling data shown for yRSC with hyperacetylated and trypsinized nucleosomal arrays is from Logie *et al.* (41) and is shown for comparison purposes only.

not reflect a key role for the N-terminal domains of histones H2A/H2B dimers.

The dCHRAC complex, like dNURF, contains ISWI, which is a nucleosome-stimulated ATPase. In addition, dCHRAC also contains topoisomerase II which is a DNA-stimulated ATPase (14). Thus, the ATPase activity associated with dCHRAC is a composite of ISWI and topoisomerase II which complicates the analysis of the substrate preferences of this complex (Fig. 2*A*). The ATPase activity of dCHRAC was stimulated by all substrates, although ATPase activity is reproducibly higher in the presence of nucleosomal or hyperacetylated arrays. Since the ATPase activity of dNURF is only stimulated by a nucleosomal or hyperacetylated substrate (Fig. 2*A*, see also Ref. 30), our data suggest that only 30–40% of the overall ATPase activity of CHRAC is due to the ISWI subunit, and the remaining DNA-stimulated ATPase activity is due to topoisomerase II.

The ATPase activity of the xMi-2 complex was distinct from both the SWI/SNF (ySWI/SNF, yRSC, hSWI/SNF) and ISWI groups (dNURF, dCHRAC) of ATPases (Fig. 2*A*). Like the ISWI group, the ATPase activity of xMi-2 was maximally stimulated by nucleosomal arrays, although free DNA did stimulate a significant amount of ATPase activity (27% of the nucleosomal level). In contrast to the ISWI group, arrays reconstituted with trypsinized histones were still able to stimulate the ATPase activity of xMi-2 to nearly 70% the level of intact nucleosomal arrays. Likewise, arrays reconstituted with hyperacetylated histones or histone $(H3\text{-}H4)_2$ tetramers were more similar to the nucleosomal arrays. Thus the observed preference for nucleosomal arrays does not reflect an obligatory interaction with the histone N-terminal domains. Thus, based on a preference for a nucleosomal substrate and a lack of histone tail dependence, xMi-2 appears to define a third group of the ATP-dependent chromatin remodeling family.

We also performed coupled restriction enzyme-remodeling assays for most of the different array substrates and each remodeling complex. As shown in Fig. 2*B*, remodeling of the different substrate arrays paralleled the ATPase activity of the complexes except in three cases. First, although arrays reconstituted with histone $(H3\text{-}H4)_2$ tetramers were able to stimulate the ATPase activity of ySWI/SNF and hSWI/SNF, the

apparent rate of remodeling of these tetramer arrays was reduced 10–50-fold compared with remodeling of nucleosomal arrays (Fig. 2*B*; see also Ref. 39 for an extensive discussion). Second, although dCHRAC showed high levels of ATPase activity with all substrates, it was not able to remodel arrays reconstituted with either trypsinized histones or with the histone $(H3/H4)_2$ tetramers (Fig. 2*B*). These results suggest that the ATPase activity that is presumably contributed by the topoisomerase II subunit of dCHRAC is not sufficient to enhance restriction enzyme accessibility in these array assays. Furthermore, the results from this remodeling analysis indicate that dCHRAC and dNURF, which each contain the ISWI ATPase, have indistinguishable histone moiety requirements. And finally, although the ATPase activity of xMi-2 was stimulated well by arrays reconstituted with $(H3\text{-}H4)_2$ tetramers (Fig. 2*A*), these arrays were only poorly remodeled by xMi-2 (Fig. 2*B*).

Targeting of the Chromatin Remodeling Complexes by Transcriptional Activators—Recently we have shown that the remodeling activity of ySWI/SNF can be targeted to reconstituted nucleosomal arrays by GAL4 derivatives that contain an acidic transcriptional activation domain (50). For these targeting assays we used a modified array DNA template which contains five high affinity GAL4-binding sites adjacent to the 5 S repeat that harbors the *Hin*cII/*Sal*I site (208-11S-GAL4; see Ref. 50). Reconstitution of nucleosomal arrays with this DNA template positions the GAL4-binding sites in the linker region between two positioned nucleosomes (50). Targeting of remodeling activity is then assayed in *Hin*cII reactions which contain a ^{32}P-labeled 208-11S-GAL4 array and 15-fold molar excess of an unlabeled 208-11S array (which lacks GAL4 sites). In the absence of targeting, the remodeling enzyme is sequestered by the excess unlabeled, competitor array and there is little stimulation of *Hin*cII digestion kinetics. Targeting of remodeling activity is scored by any stimulation of *Hin*cII digestion kinetics due to a functional GAL4 derivative (50). Note in this assay that a GAL4 derivative does not affect *Hin*cII digestion kinetics in the absence of remodeling complex or when the labeled and unlabeled arrays lack GAL4-binding sites. Previously, using this assay we were able to detect targeting of ySWI/SNF remodeling activity by GAL4-VP16 and GAL4-AH acidic activators (50).

We wished to investigate whether other members of the ATP-dependent family of remodeling enzymes could also be recruited by acidic activators in our reconstituted nucleosomal array system. Each of the six remodeling complexes were added in parallel to *Hin*cII targeting assays which contained 0.2 nM ^{32}P-labeled 208-11S-GAL4 nucleosomal array, 3 nM unlabeled 208-11S nucleosomal array, and 10 nM of a GAL4 derivative (Fig. 3). Under these reaction conditions, little remodeling of the labeled 208-11S-GAL4 array was observed in the absence of activator-dependent targeting. Likewise, addition of the isolated GAL4 DNA-binding domain did not enhance *Hin*cII digestion kinetics in the presence or absence of remodeling enzyme (data not shown). Furthermore, similar to our previous studies (50), the remodeling activity of ySWI/SNF was effectively targeted to the 208-11S-GAL4 array by both the GAL4-VP16 and GAL4-AH acidic activators, as visualized by an activator- and ySWI/SNF-dependent stimulation of *Hin*cII digestion kinetics (Fig. 3 and data not shown; see also Table I). In contrast, the remodeling activity of yRSC, hSWI/SNF, dNURF, dCHRAC, or xMi-2 was not significantly targeted by either GAL4-VP16 or GAL4-AH activators (Fig. 3*A* and data not shown; see also Table I). In fact, we reproducibly observed some activator-dependent inhibition of remodeling by dCHRAC and yRSC (Fig. 3). Similar results were obtained in several independent experiments and with a range of remodeler concentrations. In the case of hSWI/SNF we also failed to observe targeting in this assay using an immunoaffinity purified form of this enzyme (51).[3] Thus, for the six purified remodeling complexes tested here, only the remodeling activity of ySWI/SNF is effectively targeted by prototype acidic activators.

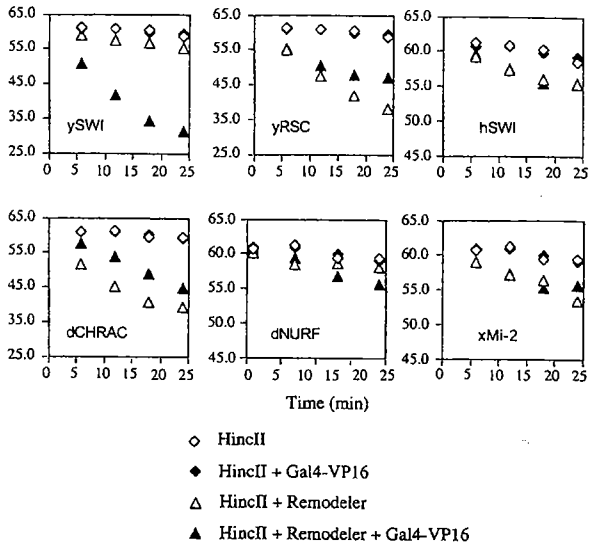

FIG. 3. **Recruitment of ATP-dependent enzymes by GAL4-VP16.** *Hin*cII digestion kinetics of reactions containing 0.2 nM labeled 208-11S-GAL4 array, 3 nM unlabeled 208-11S competitor array in the presence or absence of 10 nM GAL4-VP16, and in the presence or absence of the indicated chromatin remodeling complexes. *y* axis shows percent uncut nucleosomal array. Note that addition of GAL4-VP16 had no effect on *Hin*cII digestion kinetics in the absence of remodeling complex. Experiment shown is representative of multiple, independent experiments.

TABLE I
Recruitment of chromatin remodeling complexes by GAL4-VP16

Complex	−GAL4-VP16 (rate)[a]	+GAL4-VP16 (rate)[a]	Rate stimulation
			-fold
ySWI/SNF	2×10^{-3}	12×10^{-3}	6
yRSC	9×10^{-3}	4×10^{-3}	−2.5
hSWI/SNF	2×10^{-3}	2×10^{-3}	0
dCHRAC	8×10^{-3}	6×10^{-3}	−1.3
dNURF	1×10^{-3}	2×10^{-3}	2
xMi-2	2×10^{-3}	2×10^{-3}	0

[a] First order rates of *Hin*cII digestion of 208–11S-GAL4 nucleosomal arrays.

DISCUSSION

Our results indicate that six different members of the ATP-dependent family of chromatin remodeling enzymes use similar levels of ATP hydrolysis to rapidly establish a dynamic state of enhanced nucleosome mobilization. This "fluid" chromatin state is characterized by an enhanced accessibility of restriction enzymes and DNA binding transcription factors. Furthermore, the nearly identical rates of nucleosomal array remodeling (Fig. 1*B*) and the common requirement for histone H2A/H2B dimers (Fig. 2) are consistent with a similar remodeling mechanism for all members of this ATP-dependent family. Although the mechanistic details of "remodeling" are not clear, all of these enzymes can apparently transduce the energy of ATP hydrolysis into an enhanced mobilization of nucleosomes

[3] P. Horn, R. E. Kingston, and C. L. Peterson, unpublished observations.

within linear arrays (as suggested in Ref. 52).

In contrast to our studies with nucleosomal arrays, some differences between remodeling complexes have been observed with mononucleosome substrates. For example, ySWI/SNF, NURF, and recombinant ISWI have been shown to move a histone octamer from a central position to an end position (46–48), whereas dCHRAC and ySWI/SNF can also move histone octamers in the opposite direction (47, 49). dCHRAC, however, also contains the ISWI ATPase, and thus these differences are not intrinsic to the catalytic subunit or to the basic mechanism of remodeling. Alternatively, the differences in the direction of histone octamer movement may reflect the propensity of some complexes (such as dCHRAC) to bind to DNA ends. In this scenario, protection of the DNA ends may block end-directed movements and favor movements from the ends to more central locations. In contrast, on nucleosomal arrays, where free DNA ends do not flank individual nucleosomes, we propose that the direction of histone octamer movement is random for all remodeling complexes. This situation is consistent with our observation that the rates of nucleosome remodeling in the coupled array assay are similar for all complexes.

We were surprised to discover that arrays reconstituted with histone $(H3\text{-}H4)_2$ tetramers are not efficiently remodeled by any of the complexes tested. In the absence of remodeling enzyme, arrays of $(H3\text{-}H4)_2$ tetramers are digested at rates only 3–5-fold faster than nucleosomal arrays (data not shown; see also Ref. 39), whereas ATP-dependent remodeling of nucleosomal arrays typically yields 20–30-fold increases in restriction enzyme rates. Thus, arrays of $(H3\text{-}H4)_2$ tetramers still provide a potent barrier to factor access and, furthermore, the inability to score remodeling of tetramer arrays is not due to a high level of restriction enzyme cleavage in the absence of remodeling enzyme. Interestingly this requirement for the histone H2A-H2B dimers also does not reflect an obligatory need for the N-terminal domains of these two histones, since xMi-2, ySWI/SNF, and hSWI/SNF are insensitive to removal of all the N-terminal domains (Fig. 2*A*) (41, 53). Instead, we favor a model in which all of these enzymes require a canonical nucleosome structure either for substrate recognition or for the mechanism of remodeling. For instance, these enzymes may need to interact with two adjacent gyres of DNA in order to induce nucleosome mobilization (see also, Ref. 39).

Our data also suggest the delineation of three groups within the ATP-dependent family: 1) a SWI/SNF group (ySWI/SNF, yRSC, and hSWI/SNF) whose ATPase activity does not require an intact nucleosome and whose remodeling function is independent of the histone tails; 2) an ISWI group (dCHRAC, dNURF) whose ATPase activity requires an intact nucleosome and whose remodeling function is histone tail dependent; and 3) a Mi-2 group (xMi-2) whose optimal ATPase activity requires an intact nucleosome and whose remodeling function is histone tail independent. In fact, we found that the remodeling activity of xMi-2 was actually enhanced by removal of the histone N-terminal domains (Fig. 2*B*). Similar results have been obtained previously using a subset of the complexes tested here as well as recombinant BRG1 (ATPase subunit of hSWI/SNF) and ISWI (8, 14, 17, 28, 30, 41, 53). We note, however, that recombinant ISWI also shows significant stimulation of ATPase activity by free DNA (54). Furthermore, like dNURF and dCHRAC, the histone N-terminal domains promote efficient remodeling by ySWI/SNF and yRSC complexes under different reaction conditions where these enzymes must be catalytic (41). Thus, although the different nucleosome moiety requirements are important for defining distinctions among enzymes, these distinctions are likely to reflect subtle differences in nucleosome recognition or in regulation of the remodeling cycle (41, 56), rather than key differences in the basic remodeling mechanism.

Molecular phylogenetic analysis has been used to organize the SWI2/SNF2 family of DNA-stimulated ATPases into multiple subfamilies (7). These studies included sequence comparisons among different SWI2/SNF2 ATPase domains as well as among sequences N-terminal or C-terminal to the ATPase domain. Interestingly, ATPases from three of the subfamilies defined by phylogenetic analysis are the catalytic subunits associated with the three groups of ATP-dependent remodeling enzymes delineated by our biochemical analyses (*e.g.* SWI2, ISWI, Mi-2). This correspondence between such completely different experimental approaches was not expected, since the homology among the ATPase domains of SWI2, ISWI, and Mi-2/CHD proteins is very high (7). One possibility is that a small number of amino acid changes can lead to large differences in nucleic acid substrate requirements (*i.e.* nucleosomes *versus* free DNA). Consistent with this view, previous studies have found that ATPase domain swaps between two members of the same subfamily (*i.e.* brahma and SWI2/SNF2) yield a SWI2 protein that retains function *in vivo* in yeast, whereas swaps between members of different families (*i.e.* ISWI and SWI2/SNF2) are not functional (15). Alternatively, sequence elements that are unique to each subfamily that lie outside of the ATPase domain (*i.e.* bromodomains, SANT domains, chromodomains) might also contribute to interactions with the histone N-terminal domains or other nucleosomal components.

Although our comparative analysis delineates three groups of ATP-dependent remodeling enzymes, our data also suggests that individual enzymes within a single group are likely to be subject to differential modes of regulation. For instance, we found that ySWI/SNF was recruited by an acidic activator in our nucleosomal array system, whereas other members of the SWI/SNF group (*e.g.* yRSC, hSWI/SNF) were not. We anticipate that yRSC and hSWI/SNF can be recruited by other types of activators in this assay. Likewise, members of the ISWI or Mi-2 groups are likely to be recruited by nonacidic activators or by transcriptional repressors. These ideas are consistent with several previous studies. First, acidic activators are unable to recruit yRSC complex to an immobilized DNA template from a yeast nuclear transcription extract (50). Second, xMi-2 complexes are believed to function in transcriptional repression (36, 57) and thus it is not surprising that an acidic activator is unable to recruit xMi-2. And finally, hSWI/SNF has recently been demonstrated to be targeted *in vivo* by the glucocorticoid receptor (58), an isoform of C/EBP-β (59) and erythroid kruppel-like factor (60).

Clearly, acidic activators are likely to recruit ATP-dependent remodeling complexes in *Drosophila* and mammalian cells. One possibility is that there exists additional, uncharacterized members of the ATP-dependent remodeling family that can be recruited by acidic activators and which might play a key role in acidic activator function. It is also possible that regulatory subunits, which might facilitate interactions with acidic activators, have been lost during purification of one or more of the remodeling complexes that we have tested here. Alternatively, several of the more abundant complexes (*e.g.* dCHRAC and yRSC) may establish more global domains of fluid chromatin, and thus they may not rely on gene-specific targeting proteins.

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REFERENCES

1. Cairns, B. R. (1998) *Trends Biochem. Sci.* **23,** 20–25
2. Burns, L. G., and Peterson, C. L. (1997) *Biochim. Biophys. Acta* **1350,** 159–168
3. Muchardt, C., and Yaniv, M. (1999) *J. Mol. Biol.* **293,** 187–198

4. Strahl, B. D., and Allis, C. D. (2000) *Nature* **403,** 41–45
5. Kingston, R. E., and Narlikar, G. J. (1999) *Genes Dev.* **13,** 2339–2352
6. Pollard, K. J., and Peterson, C. L. (1998) *Bioessays* **20,** 771–780
7. Eisen, J. A., Sweder, K. S., and Hanawalt, P. C. (1995) *Nucleic Acids Res.* **23,** 2715–2723
8. Cote, J., Quinn, J., Workman, J. L., and Peterson, C. L. (1994) *Science* **265,** 53–60
9. Cairns, B. R., Kim, Y.-J., Sayre, M. H., Laurent, B. C., and Kornber, R. D. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 1950–1954
10. 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) *Cell* **87,** 1249–1260
11. Tsukiyama, T., Palmer, J., Landel, C. C., Shiloach, J., and Wu, C. (1999) *Genes Dev.* **13,** 686–697
12. Ito, T., Bulger, M., Pazin, M. J., Kobayashi, R., and Kadonaga, J. T. (1997) *Cell* **90,** 145–155
13. Tsukiyama, T., Daniel, C., Tamkun, J., and Wu, C. (1995) *Cell* **83,** 1021–1026
14. Varga-Weisz, P. D., Wilm, M., Bonte, E., Dumas, K., Mann, M., and Becker, P. B. (1997) *Nature* **388,** 598–602
15. Elfring, L. K., Deuring, R., McCallum, C. M., Peterson, C. L., and Tamkun, J. W. (1994) *Mol. Cell. Biol.* **14,** 2225–2234
16. Papoulas, O., Beek, S. J., Moseley, S. L., McCallum, C. M., Sarte, M., Shearn, A., and Tamkun, J. W. (1998) *Development* **125,** 3955–3966
17. Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E., and Green, M. R. (1994) *Nature* **370,** 477–481
18. Tong, J. K., Hassig, C. A., Schnitzler, G. R., Kingston, R. E., and Schreiber, S. L. (1998) *Nature* **395,** 917–921
19. Xue, Y., Wong, J., Moreno, G. T., Young, M. K., Cote, J., and Wang, W. (1998) *Mol. Cell* **2,** 851–861
20. Zhang, Y., LeRoy, G., Seelig, H. P., Lane, W. S., and Reinberg, D. (1998) *Cell* **95,** 279–289
21. LeRoy, G., Orphanides, G., Lane, W. S., and Reinberg, D. (1998) *Science* **282,** 1900–1904
22. Wade, P. A., Jones, P. L., Vermaak, D., and Wolffe, A. P. (1998) *Curr. Biol.* **8,** 843–846
23. de la Serna, I. L., Carlson, K. A., Hill, D. A., Guidi, C. J., Stephenson, R. O., Sif, S., Kingston, R. E., and Imbalzano, A. N. (2000) *Mol. Cell. Biol.* **20,** 2839–2851
24. Deuring, R., Fanti, L., Armstrong, J. A., Sarte, M., Papoulas, O., Prestel, M., Daubresse, G., Verardo, M., Moseley, S. L., Berloco, M., Tsukiyama, T., Wu, C., Pimpinelli, S., and Tamkun, J. W. (2000) *Mol. Cell* **5,** 355–365
25. Tamkun, J. W., Deuring, R., Scott, M. P., Kissinger, M., Pattatucci, A. M., Kaufman, T. C., and Kennison, J. A. (1992) *Cell* **68,** 561–572
26. Aasland, R., Stewart, A. F., and Gibson, T. (1996) *Trends Biochem. Sci.* **21,** 87–88
27. Paro, R., and Hogness, D. S. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 263–267
28. Phelan, M. L., Sif, S., Narlikar, G. J., and Kingston, R. E. (1999) *Mol. Cell* **3,** 247–253
29. Tsukiyama, T., and Wu, C. (1995) *Cell* **83,** 1011–1020
30. Georgel, P. T., Tsukiyama, T., and Wu, C. (1997) *EMBO J.* **16,** 4717–4726
31. Logie, C., and Peterson, C. L. (1999) *Methods Enzymol.* **304,** 726–741
32. Logie, C., and Peterson, C. L. (1997) *EMBO J.* **16,** 6772–6782
33. Hansen, J. C., Ausio, J., Stanik, V. H., and van Holde, K. E. (1989) *Biochemistry* **28,** 9129–9136
34. Workman, J. L., Taylor, I. C., Kingston, R. E., and Roeder, R. G. (1991) *Methods Cell Biol.* **35,** 419–447
35. Tse, C., and Hansen, J. C. (1997) *Biochemistry* **36,** 11381–11388
36. Wade, P. A., Gegonne, A., Jones, P. L., Ballestar, E., Aubry, F., and Wolffe, A. P. (1999) *Nat. Genet.* **23,** 62–66
37. Stein, A. (1979) *J. Mol. Biol.* **130,** 103–134
38. Hansen, J. C., van Holde, K. E., and Lohr, D. (1991) *J. Biol. Chem.* **266,** 4276–4282
39. Boyer, L. A., Shao, X., Ebright, R. H., and Peterson, C. L. (2000) *J. Biol. Chem.* **275,** 11545–11552
40. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) *Nature* **389,** 251–260
41. Logie, C., Tse, C., Hansen, J. C., and Peterson, C. L. (1999) *Biochemistry* **38,** 2514–2522
42. Dong, F., Hansen, J. C., and van Holde, K. E. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 5724–5728
43. Pennings, S., Meersseman, G., and Bradbury, E. M. (1991) *J. Mol. Biol.* **220,** 101–110
44. Polach, K. J., and Widom, J. (1995) *J. Mol. Biol.* **254,** 130–149
45. Polach, K. J., and Widom, J. (1996) *J. Mol. Biol.* **258,** 800–812
46. Jaskelioff, M., Gavin, I., Peterson, C. L., and Logie, C. (2000) *Mol. Cell. Biol.* **20,** 3058–3068
47. Langst, G., Bonte, E. J., Corona, D. F., and Becker, P. B. (1999) *Cell* **97,** 843–852
48. Hamiche, A., Sandaltzopoulos, R., Gdula, D. A., and Wu, C. (1999) *Cell* **97,** 833–842
49. Whitehouse, I., Flaus, A., Cairns, B. R., White, M. F., Workman, J. L., and Owen-Hughes, T. (1999) *Nature* **400,** 784–787
50. Yudkovsky, N., Logie, C., Hahn, S., and Peterson, C. L. (1999) *Genes Dev.* **13,** 2369–2374
51. Sif, S., Stukenberg, P. T., Kirschner, M. W., and Kingston, R. E. (1998) *Genes Dev.* **12,** 2842–2851
52. Wolffe, A. P. (1994) *Curr. Biol.* **4,** 525–528
53. Guyon, J. R., Narlikar, G. J., Sif, S., and Kingston, R. E. (1999) *Mol. Cell. Biol.* **19,** 2088–2097
54. Corona, D. F., Langst, G., Clapier, C. R., Bonte, E. J., Ferrari, S., Tamkun, J. W., and Becker, P. B. (1999) *Mol. Cell* **3,** 239–245
55. Deleted in proof
56. Peterson, C. L. (1998) *Cold Spring Harbor Symp. Quant. Biol.* **63,** 545–552
57. Kehle, J., Beuchle, D., Treuheit, S., Christen, B., Kennison, J. A., Bienz, M., and Muller, J. (1998) *Science* **282,** 1897–1900
58. Fryer, C. J., and Archer, T. K. (1998) *Nature* **393,** 88–91
59. Kowenz-Leutz, E., and Leutz, A. (1999) *Mol. Cell* **4,** 735–743
60. Lee, C. H., Murphy, M. R., Lee, J. S., and Chung, J. H. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 12311–12315