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Functional and Structural Dissection of the SWI/SNF Chromatin Remodeling Complex: A Dissertation

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FUNCTIONAL AND STRUCTURAL DISSECTION OF THE SWI/SNF CHROMATIN REMODELING COMPLEX

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ABSTRACT

The yeast SWI/SNF complex is the prototype of a subfamily of ATP-dependent chromatin remodeling complexes. It consists of eleven stoichiometric subunits including Swi2p/Snf2p, Swi1p, Snf5p, Swi3p, Swp82p, Swp73p, Arp7p, Arp9p, Snf6p, Snf11p, and Swp29p, with a molecular weight of 1.14 mega Daltons. Swi2p/Snf2p, the catalytic subunit of SWI/SNF, is evolutionally conserved from yeast to human cells. Genetic evidence suggests that SWI/SNF is required for the transcriptional regulation of a subset of genes, especially inducible genes. SWI/SNF can be recruited to target promotors by gene specific activators, and in some cases, SWI/SNF facilitates activator binding. Biochemical studies have demonstrated that purified SWI/SNF complex can hydrolyze ATP, and it can use the energy from ATP hydrolysis to generate superhelical torsion, mobilize mononucleosomes, enhance the accessibility of endonucleases to nucleosomal DNA, displace H2A/H2B dimers, induce dinucleosome and altosome formation, or evict nucleosomes. A human homolog of Swi2p/Snf2p, BRG1, is the catalytic subunit of the human SWI/SNF complex. Interestingly, isolated BRG1 alone is able to remodel a mononucleosome substrate. Importantly, mutations in mammalian SWI/SNF core subunits are implicated in tumorigenesis. Therefore, it remains interesting to characterize the role(s) of each subunit for SWI/SNF function. In this thesis project, I dissected SWI/SNF chromatin remodeling function by investigating the role of the SANT domain of the Swi3p subunit. Swi3p is one of the core components of SWI/SNF complex, and it contains an uncharacterized SANT domain that has been found in many chromatin regulatory proteins. Earlier studies suggested that the SANT domain of Ada2p

may serve as the histone tail recognition module. For Swi3p, a small deletion of eleven amino acids from the SANT domain caused a growth phenotype similar to that of other swi/snf mutants.

In chapter I, I have reviewed recent findings in the function of chromatin remodeling complexes and discuss the molecular mechanism of their action.

In chapter II, I characterized the role of the SANT domain of Swi3p. I found that deletion of the SANT domain caused a defect in a genome-wide transcriptional profile, SWI/SNF recruitment, and more interestingly impairment of the SANT domain caused the dissociation of SWI/SNF into several subcomplexes: 1) Swi2p/Arp7p/Arp9p, 2) Swi3p/Swp73p/Snf6p, 3) Snf5p, and 4) Swi1p. Artificial tethering of SWI/SNF onto a LacZ reporter promoter failed to activate the reporter gene in the absence of the SANT domain, although Swi2p can be recruited to the LacZ promoter. We thus demonstrated that the Swi3p SANT domain is critical for Swi3p function and serves as a protein scaffold to integrate these subcomplexes into an intact SWI/SNF complex.

In Chapter III, I first characterized the enzymatic activity of the subcomplexes, especially the minimal complex of Swi2p/Arp7p/Arp9p. We found that this minimal subcomplex is fully functional for chromatin remodeling in assays including cruciform formation, restriction enzyme accessibility in mononucleosomal and nucleosomal array substrates, and mononucleosome mobility shift. However, it is defective in ATP-dependent removal of H2A/H2B dimers. Moreover, we found that Swi3p and the N-terminal acidic domain of Swi3p strongly interact with GST-H2A and H2B but not GST-H3 or H4 tails. We purified a SWI/SNF mutant (SWI/SNF-Δ2N) that lacks 200 amino acids within the N-terminal acidic domain of Swi3p. Intriguingly, SWI/SNF-Δ2N failed to

catalyze ATP-dependent dimer loss, although this mutant SWI/SNF contains all the subunits and has intact ATP-dependent activity in enhancing restriction enzyme accessibility. These data help to further understand the molecular mechanism of SWI/SNF, and show that H2A/H2B dimer loss is not an obligatory consequence of ATP-dependent DNA translocation, but requires the histone chaperone function of the Swi3p subunit. Based on these findings, we proposed a new model of the structural and functional organization of the SWI/SNF chromatin remodeling machinery: SWI/SNF contains at least four distinct modules that function at distinct stages of the chromatin remodeling process. 1) Swi1p and Snf5p modules directly interact with gene specific activators and function as the recruiter; 2) Swi2p/Arp7p/Arp9p generates energy from ATP hydrolysis and disrupts histone/DNA interactions; and 3) Swi3p/Swp73p/Snf6p may play dual roles by integrating each module into a large remodeling complex, as well as functioning as a histone H2A/H2B chaperone to remove dimers from remodeled nucleosomes.

Chapter IV is a perspective from current work in this project. I first discuss the interest in further characterizing the essential role of Snf6p, based on its activation of LacZ reporter on its own. Using in vitro translated protein and co-IP studies, I tried to pinpoint the requirement of the SANT domain for SWI/SNF assembly. I found that Swi3p directly interacts with Swp73p, but not with other subunits. When Swi3p is first incubated with Swp73p, Swi3p also interacts with Snf6p, indicating that Swi3p indirectly interacts with Snf6p, therefore forming a subcomplex of Swi3p/Swp73p/Snf6p. This subcomplex can also be reconstituted using in vitro co-translation. Consistent with the TAP preparation of this subcomplex, partial deletion of the SANT domain of Swi3p does not

affect the assembly of Swi3p/Swp73p/Snf6p in vitro. However, the assembly of SWI/SNF complex was not detected in the presence of eight essential in vitro translated subunits or from co-translation of all the subunits. I have discussed the interest in further characterizing the histone chaperone role of the Swi3p N-terminal acidic domain and the role of other core subunits of SWI/SNF such as Snf6p for transcriptional regulation.

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LIST OF ABBREVIATIONS

a.a.	amino acid
ATP	adenosine 5'-triphosphate
bp	base pair
BRG1	Brahma-related gene 1
BRM	Brahma
BSA	Bovine serum albumin
ChIP	chromatin immunoprecipitation
C-terminal	carboxyl terminal
dCTP	deoxycytidine 5'-triphophate
DNA	deoxyribonucleic acid
DNAase I	deoxyribonuclease I
DTT	dithiothreitol
E buffer	extraction buffer
EDTA	ethylenediaminetetraacetic acid
EGTA	glycol ether diamine tetraacetic acid
Endo VII	T4 endonuclease VII
HA	influenza A virus haemagglutinin
HAT	histone acetyltransferase
hBRM	human Brahma
HDAC	histone deacetylase
HMT	histone methyltransferase
IgG	immunoglobin G
Kb	kilobase
kDa	kilodalton
MDa	megadalton
Min	minutes
MMTV	Mouse mammary Tumor Virus
Mono	mononucleosome
N-coR	nuclear receptor corepressor
nM	nanomoles

N-terminal	amino terminal
O.D.600	optical density at 600 nm of wavelength
P	phosphate
PAGE	polyacrylamide gel electrophoresis
PBAP	Polybromo Brahma-Associated Protein complex
PEI cellulose	polyethyleneimine cellulose
pN	piconewton
Pol II	RNA polymerase II
rDNA	ribosomal DNA
RNA	ribonucleic acid
RSC	remodels the structure of chromatin
SANT	Swi3, Ada2, N-CoR, and TFiiiB
S.C.	synthetic complete
SDS	sodium docecyl sulfate
SF2	helicase super family 2
SLIDE	SANT-Like domain
SMRT	silencing mediator for retinoid and thyroid hormone receptors
Snf	sucrose non-fermenting
ssDNA	Sonicated salmon sperm DNA
Swi	mating type switching
TAP	tandem affinity purification
TAE	Tris-Acetic-EDTA
TBE	Tris-Boric acid-EDTA
TE	Tris-EDTA buffer
TEV	Tobacco Etch Virus
TLC	thin layer chromatography
WB	western blot
WT	wild type
YEPD	Yeast Extract Peptone with 2% Dextrose

CHAPTER I

GENERAL INTRODUCTION

Eukaryotic genomes are organized into chromatin within nuclei. The fundamental repeating unit of chromatin known as the nucleosome consists of ~147 bp of DNA wrapped around a histone octamer. Conventionally, nucleosomal assembly is initiated by the association of histone $(H3/H4)_2$ tetramer with DNA after replication, followed by the association of one H2A/H2B dimer on each side of the tetramer (Arents, et al., 1991; Hansen, et al., 1991; Luger, et al., 1997a; Smith and Peterson, 2005a). High-resolution X-ray crystal structure of the canonical nucleosome indicates that histone-DNA interactions occur every 10 bp on each DNA strand and DNA-histone interaction clusters stabilize the structure of nucleosome. Importantly, all histone amino-termini, and in case of H2A, the carboxyl-terminus, protrude out of the globular region of histone octamer (Hayes, et al., 1991; Luger, et al., 1997a). These histone “tails” are critical for chromatin condensation since “tailless” histone octamers assembled on 5S nucleosomal array showed a chromatin condensation defect in vitro (Carruthers and Hansen, 2000). In the presence of linker histones, as well as through internucleosomal interactions or interactions with non-histone proteins, chromatin is further condensed into higher order chromatin fibers (Hansen, 2002) (Figure 1). Obviously, the condensation of chromatin creates a relatively inaccessible environment for the binding of regulatory proteins to the DNA template.

The dynamics of the chromatin structure plays an important role in regulating gene expression and other DNA-dependent nuclear processes such as DNA replication, homologous recombination, and DNA double strand break (DSB) repair (Horn and Peterson, 2002). In the past decade, compelling evidence supports that two major classes of chromatin modifying complexes are capable of manipulating chromatin configuration. The first class of remodeling enzymes catalyzes posttranslational modifications at the histone tails through acetylation, methylation, phosphorylation, ubiquitination, sumolation and ADP-ribosylation (Jenuwein and Allis, 2001; Strahl and Allis, 2000; van Attikum and Gasser, 2005). Among these modifications, histone acetylation catalyzed by histone acetyltransferases (HATs) and methylation catalyzed by histone methyltransferases have been extensively documented. Histone acetylation occurs at positively charged lysine residues, which not only neutralizes the overall charge of histones, but also creates signals and binding sites for other regulatory factors such as bromodomain-containing factors (Hassan, et al., 2001). The overall histone acetylation level maintained by the opposing effects of histone acetyltransferase and histone deacetylase (HDAC) enzymes has been implicated in transcriptional activation or repression for many genes. Lysine acetylation not only acts at a local chromatin, but also influences the global chromatin structure. Generally, histone hyperacetylation is linked to euchromatin formation and transcriptional activation. Using analytical centrifugation, Shogren-Knaak *et al* recently showed that uniformly acetylated nucleosome arrays at H4 lysine 16 (H4K16) inhibited the formation of 30-nanometer-like condensed chromatin fibers, reflecting a role of H4K16 acetylation in chromatin decondensation in vivo (Shogren-Knaak, et al., 2006). In contrast, histone

hypoacetylation is often associated with heterochromatin regions and generally reflects transcriptional repression. Histone methylation had been regarded as an irreversible posttranslational modification that regulates both chromatin structure and epigenetic memory until several histone demethylases were discovered recently (Wysocka, et al., 2005). Histone tails that are mono-, di- or tri-methylated at different lysine residues have been implicated in many biological processes such as heterochromatin formation, X chromosome inactivation, genomic imprinting and transcriptional regulation (Habu, et al., 2006; Imhof, 2006). Histone phosphorylation at a C-terminal serine residue of H2A is believed to signal DNA damage and regulate DNA repair after double strand breaks (Morrison, et al., 2004). Taken together, histone tail modifications have been linked to many cellular processes involved in physiological or pathological process including malignancy (Jenuwein and Allis, 2001). Histone modifications within the globular region of histones have emerged to be an important aspect in regulating both chromatin structure and cellular function (Feng, et al., 2002; Han, et al., 2007; van Leeuwen, et al., 2002). More importantly, HDAC inhibitors have been applied to develop new cancer and anti-parasitic chemotherapeutics (Marks and Dokmanovic, 2005; Ouaissi and Ouaissi, 2006).

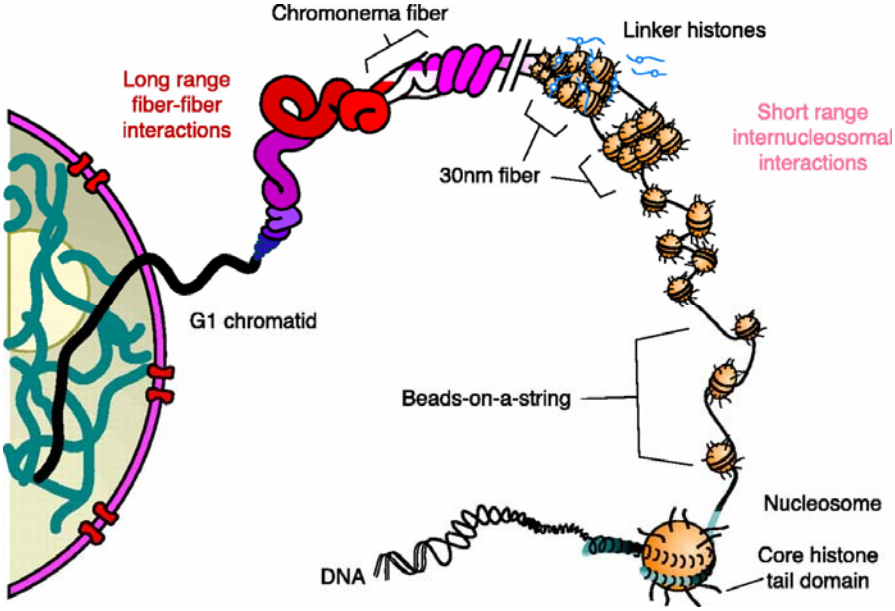

Figure 1. Organization of eukaryotic chromatin fibers. The fundamental unit of chromatin is defined as nucleosome that forms the “beads-on-a-string” chromatin structure. Internucleosomal interactions, linker histones and non-histone proteins mediate the further condensation of chromatin into 30nm fibers and higher order structures. Adapted from (Hansen, 2002).

The second class of enzymes is known as the ATP-dependent chromatin remodeling complexes, which use the energy from ATP hydrolysis to change chromatin structure (Smith and Peterson, 2005a). Yeast SWI/SNF is the first characterized enzyme, originally identified from genetic screens in the budding yeast *Saccharomyces cerevisiae* as a positive regulator for the *HO* gene responsible for mating type switching (*SWI*), and for the *SUC2* gene responsible for sucrose fermentation (*S*ucrose *N*on-*F*ermenter, *SNF*) (Neigeborn and Carlson, 1984; Stern, et al., 1984). Each of these multiprotein complexes contains a helicase-like ATPase subunit that belongs to the helicase superfamily 2 (SF2 helicase), and is evolutionarily conserved from yeast (Cairns, et al., 1996c; Cote, et al., 1994) to Drosophila (Papoulas, et al., 1998), mouse, and human cells (Imbalzano, et al., 1994; Kwon, et al., 1994). All the ATP-dependent chromatin remodeling complexes possess intrinsic DNA and/or nucleosome stimulated ATPase activity in vitro, although they do not exhibit DNA helicase activity that catalyzes the separation of DNA strands (Cote, et al., 1994). Interestingly, a single amino acid change in the helicase-like motifs is sufficient to abolish the ATPase activity and function of all ATP-dependent chromatin remodeling enzymes, indicating that the ATPase activity is essential for the enzymatic function of these proteins. Based on the homology of the ATPase domain of each enzyme, the ATP-dependent chromatin remodeling complexes are further divided into several subclasses including SWI2/SNF2, ISWI, Mi-2/CHD, and INO80/SWR1 (Smith and Peterson, 2005a).

In recent years, tremendous work has been done to understand the function(s) and molecular mechanisms of the ATP-dependent chromatin remodeling complexes in the context of transcription, DNA replication, recombination, DNA repair (Osley, et al.,

2007), and incorporation of histone variants (Henikoff, et al., 2004; McKittrick, et al., 2004). However, many fundamental questions remain. For instance, how do these enzymes recognize their targets in vivo? How do they make the nucleosomal DNA more accessible to other regulatory factors? How does each subunit contribute to the function of chromatin remodeling machinery in vivo? Due to the association of chromatin remodeling with tumorigenesis (Biegel, et al., 2002; Isakoff, et al., 2005; Roberts, et al., 2002; Sansam and Roberts, 2006), it becomes extremely important to investigate the function of individual genes that encode the subunits of each complex and the mechanism(s) of their action. In this chapter, I will outline recent findings on the function(s) of each subclass of the ATP-dependent chromatin remodeling enzymes in transcriptional regulation and discuss the molecular mechanisms, with an emphasis on the SWI/SNF subclass.

Functions of ATP-dependent chromatin remodeling complexes

SWI/SNF subclass

Swi2/Snf2 family members include yeast SWI/SNF (ySWI/SNF) (Cote, et al., 1994), RSC complexes (Cairns, et al., 1996c), Drosophila BRM-containing complexes (Dingwall, et al., 1995), and human SWI/SNF complexes (hSWI/SNF) that contain either BRG1 or hBRM as the catalytic subunit (Imbalzano, et al., 1994; Kwon, et al., 1994). Except for the highly homologous ATPase domain, each catalytic subunit also contains a C-terminal bromodomain that has been proposed to bind acetylated histone tails (Zeng and Zhou, 2002). Another feature of this subclass of ATP-dependent chromatin remodeling enzymes is that each enzyme is purified as a multiprotein complex that contains 8-15 stoichiometric subunits.

Yeast SWI/SNF complex is one of the most intensely studied ATP-dependent chromatin remodeling enzymes. It consists of eleven stoichiometric subunits including Swi2p/Snf2p, Swi1p, Snf5p, Swi3p, Swp82p, Swp73p, Arp7p, Arp9p, Snf6p, Snf11p, and Swp29p, with a molecular weight of 1.14 mega Dalton (Cairns, et al., 1996a; Cairns, et al., 1994; Cairns, et al., 1996b; Cote, et al., 1994; Peterson, et al., 1994; Smith, et al., 2003; Treich, et al., 1995). Although the catalytic subunit of ySWI/SNF, Swi2p/Snf2p, has low abundance and is nonessential for cell viability, yeast SWI/SNF is important for transcriptional activation or repression of ~5% of yeast genes, including some inducible genes (Holstege, et al., 1998a). Several core subunits such as Swi2p, Swi1p, Swi3p, Snf5p, Swp73p, Snf6p, and actin-related proteins (Arp7p and Arp9p) are essential for

the function of ySWI/SNF in vivo, and are highly conserved among different species except for Snf6p (Mohrmann and Verrijzer, 2005) (Table 1). In contrast, other members of this subfamily such as yeast Sth1p, the ATPase subunit of yeast RSC complexes (Cairns, et al., 1996c), Drosophila BRM and human BRG1 are more abundant and essential for cell viability or development. Mammalian SWI/SNF also plays an essential role in regulating nuclear receptor function and cell growth, and mutations in BRG1, hBRM and hSNF5/INI1 have been implicated in cancer development (Bochar, et al., 2000; Bultman, et al., 2000; Muchardt and Yaniv, 1999; Roberts, et al., 2002; Versteege, et al., 1998; Wong, et al., 2000).

Purified SWI/SNF complexes possess ATPase activity that is stimulated by both free DNA and nucleosomal DNA in vitro (Cote, et al., 1994; Kwon, et al., 1994). Although Swi2p/Snf2p forms a multiprotein complex in vivo, purified recombinant yeast Swi2p/Snf2p alone (Laurent, et al., 1993) and isolated human Snf2 (BRG1 or hBRM) (Phelan, et al., 1999) have ATPase activity in vitro. Interestingly, BRG1 and hBRM are capable of remodeling mononucleosome and nucleosomal arrays in a way similar to that of intact complex in vitro (Phelan, et al., 2000), although adding human Snf5 (INI1) and human Swi3 (BAF170/BAF155) can fully recover BRG1 activity to the level of the intact hSWI/SNF complex (Phelan, et al., 2000; Phelan, et al., 1999). Recombinant BRG1 and BAF155 (human Swi3 homolog) was also sufficient to activate EKLF activator-dependent transcription in vitro (Kadam, et al., 2000). Taken together, the fact that the ATPase subunit is competent for chromatin remodeling by itself further suggests that the ATPase subunit is fundamental for the function of ATP-dependent chromatin remodeling

complex. Meanwhile, it raised the question on the precise role(s) of other subunits for the function of chromatin remodeling machinery in vivo.

One favored explanation is that non-catalytic subunits of the complex may play a role at a different step during transcriptional regulation in vivo (Narlikar, et al., 2002; Peterson, 1998), since the chromatin remodeling complexes not only act on a chromatin substrate, but are also required in concert with other regulatory factors during transcriptional activation. For instance, some components may be involved in mediating the binding of additional factors upstream or downstream of the chromatin remodeling process. In fact, biochemical studies have shown that purified yeast SWI/SNF interacts with several acidic activators, such as Gal4-VP16, Swi5p, Gcn4p and Hap4p (Neely, et al., 2002). It is believed that these interactions are mediated by partially redundant activity of Swi1p and Snf5p subunits (Neely, et al., 2002; Prochasson, et al., 2003). Far-Western and GST-pull down analyses have detected the physical interaction between these activators and Swi1p, Swi2p, or Snf5p subunits in vitro. These observations have led to the recruiting model in which Swi1p and Snf5p subunits play a role in recruiting SWI/SNF to target promoters via direct interaction with gene-specific activators in vivo (Kadam, et al., 2000; Peterson and Workman, 2000; Yudkovsky, et al., 1999). In addition, acetylated histone tails may also recruit or stabilize SWI/SNF through the C-terminal bromodomain of Swi2p (Hassan, et al., 2002; Zeng and Zhou, 2002). This recruiting model is supported by in vivo chromatin immunoprecipitation (ChIP) data showing that the recruitment of yeast SWI/SNF to the *HO* promoter depends on the specific activator Swi5p (Krebs, et al., 2000). In contrast, Martens and colleagues have shown that transcriptional activation of yeast *SER3* regulatory gene 1 (*SRG1*) depends

largely on Swi2p, and deletion of other subunits of SWI/SNF does not affect *SRG1* transcriptional level (Martens and Winston, 2002; Martens, et al., 2005), probably due to the direct recruitment of Swi2p by an activator Cha4p. This result supports the notion that the recruitment of SWI/SNF by Swi1p or Snf5p may be redundant at some promoters when the activators directly interact with Swi2p. All these observations suggest that Swi2p/Snf2p may need different subunits that function at distinct steps in vivo, although the precise role of the majority of subunits are largely unknown.

Another interesting feature of the SWI/SNF subclass is that actin and actin-related proteins (Arp) are stoichiometric components of each of the family members (Cairns, et al., 1998), although their role for chromatin remodeling is not fully understood (Boyer and Peterson, 2000; Olave, et al., 2002). Arp7p and Arp9p are shared by both yeast SWI/SNF and RSC complexes (Cairns, et al., 1996c), and both are essential for cell survival. Human SWI/SNF complex has one β-actin and one ARP (BAF53). Drosophila BRM-containing complexes, BAP and PBAP, also contain a conventional actin (BAP47) and an ARP (BAP55). Strikingly, these actin and ARPs are tightly associated with the catalytic subunits of each complex. For example, the association of actin and BAF53 with BRG1 is so strong that they can only be separated under urea-denaturing conditions (Zhao, et al., 1998). A mutagenesis study in yeast suggested that actin and ARPs might directly interact with the N-terminus of the Swi2p ATPase subunit. Conditional viable ARP mutant analysis showed that Arp7p and Arp9p are not required for the assembly or enzymatic activity of RSC complex, although they might form a heterodimer in the complex (Szerlong, et al., 2003). In contrast, human BRG1 ATPase activity was inhibited by an actin-monomer inhibitor, Latrunculin B, and physical

association of actin and BAF53 with BRG1 appears to be required for optimal ATPase activity of BRG1 (Zhao, et al., 1998). Collectively, the direct interaction between actin and ARPs and the catalytic subunits reflects the importance of nuclear actin and ARPs for chromatin remodeling activity coupled with ATP hydrolysis, although further investigation is needed to understand the general role of actin and Arps for the function of this subclass of chromatin remodeling complex in vivo.

Table 1. Composition of SWI/SNF subclass of chromatin remodelers

Yeast		*Drosophila*		Human	
SWI/SNF	**RSC**	**BAP**	**PBAP**	**BAF**	**PBAF**
Swi2/Snf2	Sth1	Brahma	Brahma	BRG1 or hBRM	BRG1
Swi1/Adr6		OSA		BAF250	
	Rsc1, Rsc2, Rsc4		Polybromo		Polybromo/BAF180
	Rsc9*		BAP170*		
Swi3	Rsc8	Moira	Moira	BAF170 & BAF155	BAF170 & BAF155
		BAP111	BAP111	BAF57	BAF57
Swp73	Rsc6	BAP60	BAP60	BAF60a	BAF60a or BAF60b
Swp61/Arp7 Swp59/Arp9	Rsc11/Arp7 Rsc12/Arp9	BAP55	BAP55	BAF53	BAF53
		actin	actin	actin	actin
Snf5	Sfh1	Snr1	Snr1	hSNF5/INI1	hSNF5/INI1
	Rsc5, 7, 10, 13-15				
	Rsc3, Rsc30				
Swp82					
Swp29/Tfg3/TAF30/Anc1					
Snf6, 11					

Adapted from ref. (Mohrmann and Verrijzer, 2005). Conserved subunits are highlighted.

Yeast *SWI3* gene encodes one of the core subunits of SWI/SNF complex, although the precise role of Swi3p has not been fully characterized. It is known that Swi3p is essential for SWI/SNF function in vivo, since deletion of Swi3p caused severe swi/snf mutant phenotypes. Secondary structure prediction suggests that Swi3p contains several characteristic domains, including the N-terminal acidic domain, SWIRM domain, and a C-terminal SANT domain followed by a leucine zipper domain. Each SWI/SNF complex has two copies of Swi3p that may dimerize through its C-terminal leucine zipper domain (Smith, et al., 2003). The N-terminal acidic domain of Swi3p consists of 25% negatively charged glutamic acid and aspartic acid, a feature commonly found in histone chaperones. Recent study suggested that the SWIRM domain seems to bind both DNA and nucleosome in vitro, and may be important for the stability of Swi3p protein as well as the integrity of SWI/SNF complex (Aravind and Iyer, 2002; Da, et al., 2006).

The SANT domain of Swi3p is a module consisting of approximate 50 amino acids originally found in Swi3, Ada2, nuclear hormone receptor corepressor NcoR, and transcription factor TFIIIb, based on the sequence homology to the c-myb DNA binding domain (DBD) (Aasland, et al., 1996). However, the SANT domain does not seem to bind DNA. Instead, recent studies from the SANT domain of Ada2p, Swi3p and Rsc8p suggested that the SANT domain may serve as a histone tail recognition module that facilitates nucleosomal substrate binding (Boyer, et al., 2002; Boyer, et al., 2004). In *Saccharomyces cerevisiae,* the SANT domain of Ada2p is essential for the histone acetyltransferase (HAT) activity of Gcn5-containing SAGA complex and GST-H3 tail binding (Boyer, et al., 2002). Sterner *et al* also found that deletion of the C-terminal half or the SANT domain of yeast Ada2p resulted in the disassembly of SAGA complex,

probably due to abolishing Ada2p-Ada3p interaction (Sterner, et al., 2002). In contrast, the N-terminal half of Ada2p SANT domain is not required for SAGA complex integrity, but deletion significantly impaired the Gcn5p HAT activity (Sterner, et al., 2002). In mammalian cells, two tandem repeats of the SANT domain have been identified in nuclear hormone receptor corepressors NcoR, SMRT and CoREST. The N-terminal SANT domain of SMRT is required for histone deacetylase enzymatic activity, while the C-terminal SANT is important for unacetylated histone interaction (Yu, et al., 2003). In the case of Drosophila ISWI, a SANT and a SANT-like domain (SLIDE) are found at its C-terminal region. Recent X-ray crystallography showed that there is an overall negative charge on the surface of the helices of ISWI SANT domain, supporting the hypothesis that this SANT domain may be a candidate for interaction with positively charged histone tails (Grune, et al., 2003). In contrast, the SLIDE domain of ISWI seems to have DNA binding properties and is required for both the ATPase activity and nucleosome sliding activity of ISWI (Grune, et al., 2003). Collectively, SANT domains may in general mediate protein-protein interactions important for large protein complex assembly, and histone tail interactions to coordinate binding and the core enzymatic activity of chromatin remodeling complexes (Boyer, et al., 2004).

Yeast Swp82p, Snf6p, Swp29p and Snf11p subunits are not conserved in higher eukaryotes, and their function for chromatin remodeling is less clear. Genetic evidence supports that Swp73p and Snf6p also play critical roles for SWI/SNF function (Cairns, et al., 1996b; Laurent and Carlson, 1992). Although Swp82p, Swp29p and Snf11p are stoichiometric subunits of SWI/SNF complex, deletion of these subunits does not cause any swi/snf phenotypes (Cairns, et al., 1996a; Treich, et al., 1995; Wilson, et al., 2006).

Another interesting observation is that mutation in transcription elongation factor Spt6p suppressed the requirement of *SNF2* and *SNF5* but not *SNF6* (Laurent, et al., 1991), suggesting that Snf6p may have other functions beyond SWI/SNF chromatin remodeling.

One extremely interesting aspect of the chromatin remodeling field rises from recent observations that mutations of core components of mammalian SWI/SNF such as hSWI2/BRG1, hSNF5/INI1, hSWI3/BAF155, BAF180 or BAF250 are found in human cancer cell lines or various primary cancers (DeCristofaro, et al., 1999; Roberts and Orkin, 2004). SNF5 (INI1) is essential for the early embryonic development of mice, and heterozygosity of INI1 predisposed mice to aggressive rhabdoid cancers, indicating a critical role of hSNF5/INI1 involved in early development and tumor suppression in vivo (Klochendler-Yeivin, et al., 2002; Roberts, et al., 2000). Interestingly, human SWI/SNF also forms a repressor complex with the tumor suppressor Rb, SIN3, and HDAC during G1 phase, and represses genes that regulate G1-S transition (Kuzmichev, et al., 2002). In addition, the tumor suppressor BRCA1 is associated with the hSWI/SNF complex (Bochar, et al., 2000), and the c-Myc oncogene product physically interacts with hSNF5/Ini1 both in vitro and in vivo (Cheng, et al., 1999). Surprisingly, loss of heterozygosity of BRG1 is less common than that of INI1, and the tumors developed from mutations of BRG1 are different from those from hSNF5 mutation, indicating that hSNF5 might also play other roles independent of hSWI/SNF complex. Collectively, more investigation is required to fully understand the role(s) of each subunit for the function of SWI/SNF and how they coordinate with each other during transcriptional

regulation or other cellular processes. These findings may ultimately provide information to guide new strategies for designing cancer chemotherapeutics.

ISWI subclass

Drosophila ISWI (Imitation SWItch) is the founding member of ISWI subclass identified based on the homology of its ATPase domain to that of BRM. In addition to the ATPase domain, each Iswi-like ATPase subunit contains two SANT domains that may play a role in histone tail binding discussed above. In contrast to SWI/SNF, the majority of known ISWI family members are relatively small complexes that contain only 2-4 subunits found in Drosophila, yeast, Xenopus, mice, and human cells (Dirscherl and Krebs, 2004). One exception is the human SNF2H/NuRD/cohesion complex, which has over ten subunits, including subunits of NURD and cohesion complexes (Hakimi, et al., 2002). None of the ISWI-containing complexes contain actin or ARPs.

Another characteristic of the ISWI subclass is the presence of multiple ISWI-containing complexes in all species (listed in Table 2). Drosophila ISWI is found in three complexes including ACF (ATP-dependent chromatin assembly and remodeling factor), NURF (Nucleosome remodeling factor), and CHRAC (Chromatin accessibility complex), which are differentiated by the existence of other subunits. Yeast has two ISWI-related genes, *ISWI1* and *ISWI2*, which encode the ATPase subunits of at least four different complexes, including ISW1a, ISW1b, ISW2, and yCHRAC. Xenopus ISWI is present in at least four ISWI complexes including xACF, xWICH, xCHRAC, and xISWI-A. Human ISWI-like ATPases, SNF2H and SNF2L share 86% sequence homology, and hSNF2H

protein has 73% of its amino acids identical to that of Drosophila ISWI (Aihara, et al., 1998). Human ISWI-like complexes include SNF2H-containing hACF, WICH, hCHRAC, RSF, and SNF2H/NURD/cohesion, and SNF2L-containg hNURF complex (Dirscherl and Krebs, 2004).

The various ISWI-containing complexes show functional diversity in vivo (summarized in Table 2). Drosophila ISWI-containing NURF complex was first purified from Drosophila embryo extracts, and functions as a positive transcription factor at the heat shock protein 70 (Hsp70) promoter. The largest subunit of NURF, named NURF301, is required for the expression of homeotic genes (Badenhorst, et al., 2002; Tsukiyama, et al., 1995; Tsukiyama and Wu, 1995). Deuring and colleagues found that deletion of Drosophila ISWI caused global decondensation of male X-chromosome, suggesting a role of ISWI in X-chromosome structural maintenance (Deuring, et al., 2000). A null mutation of Drosophila ISWI also caused the loss of germline stem cells, indicating that ISWI also plays a role in stem cell self-renewal (Xi and Xie, 2005).

Yeast ISWI-containing complexes are mainly involved in transcriptional repression. ISW1a complex appears to repress transcription at the initiation stage, whereas ISW1b may play a role in transcriptional elongation and termination by delaying RNA polymerase II release (Morillon, et al., 2005; Morillon, et al., 2003). Yeast ISW2 complex represses transcription of early meiotic genes that depend on Ume6, which recruits the complex to target promoters (Goldmark, et al., 2000). In addition, DNA microarray analysis suggests that a yeast isw2p mutation also caused transcriptional derepression of many Ume6-independent genes, and probably functions in parallel with Sin3-Rpd3 histone deacetylase complex (Fazzio, et al., 2001). Recently, the Tsukiyama

group used a catalytically inactive mutant of Isw2p (K215R) to identify genome-wide ISW2-targeting sites and found that Isw2p also regulates tRNA genes (tDNAs). Loss of Isw2p disrupted the periodic pattern of Ty1 integration upstream of tDNAs, but did not affect transcription of tDNAs or the associated Ty1 retrotransposons (Gelbart, et al., 2005).

A study by Collins and colleagues showed that depletion of human ACF1 by RNAi or an ACF1 mutation that interferes with the association of SNF2H impaired DNA replication of pericentric heterochromatin (Collins, et al., 2002). Human SNF2H and Williams syndrome transcription factor (WSTF) form human WICH complex that is recruited to the replication foci by the DNA clamp PCNA to maintain chromatin structure after DNA replication. RNAi-mediated depletion of WSTF or SNF2H caused abnormal heterochromatin formation on newly synthesized DNA (Poot, et al., 2004). Recent studies also showed that human SNF2H forms a nucleolar remodeling complex (NoRC) with bromodomain-containing protein Tip5 (TTF-I-interacting protein 5), and plays an important role in repressing the rDNA promoter through heterochromatin formation (Strohner, et al., 2001; Zhou and Grummt, 2005). Interestingly, hSNF2H co-purified and co-localized with cohesin and NuRD, a Mi-2/HDAC containing complex, which provides an example of interplay between chromatin remodeling complexes under certain circumstances. Human SNF2H physically interacts with human RAD21, a subunit of cohesin complex, suggesting a role of human ISWI in regulating sister chromatid cohesion and segregation (Hakimi, et al., 2002). Human NURF complex is the hSNF2L-containing human ortholog of Drosophila NURF. Like dNURF, human NURF is also involved in transcriptional activation of genes, especially those involved in neuronal

development (Barak, et al., 2003). Taken together, the presence of multiple forms of ISWI-containing complexes in each organism reveals the abundance and functional diversity of this subclass of ATP-dependent chromatin remodeling complexes.

Like Swi2p/Snf2p, ISWI alone has chromatin remodeling activity (Corona, et al., 1999) and is also able to catalyze nucleosome “sliding” in vitro (Clapier, et al., 2001). The role of other co-existing subunits is likely to coordinate the catalytic activity of ISWI during chromatin remodeling. For instance, the optimal ATPase activity and nucleosome sliding activity of Drosophila ISWI-containing NURF complex requires the presence of the largest subunit, NURF301 in vitro (Xiao, et al., 2001). Eberharter *et al.* also showed that the ACF1 subunit of the ACF complex binds the nucleosomal substrate and significantly stimulates the nucleosomal mobility activity of ISWI in vitro (Eberharter, et al., 2004). In addition, p14 and p16 subunits of CHRAC complex appear to form a heterodimer that transiently binds DNA to facilitate the nucleosomal sliding process (Eberharter, et al., 2001). As mentioned above, deletion or mutations of non-catalytic subunits that abolish the interaction with ISWI-like ATPase subunit also impair the in vivo function of the ISWI complex.

Table 2. Functional diversity of ISWI-subclass in different organisms

Organisms	Complex	No. of subunits	In vivo function(s)
Drosophila	dACF	2	nucleosome assembly, spacing
	dCHRAC	4	nucleosome assembly, spacing
	dNURF	4	transcription activation
Yeast	yISWI1a	2	transcription repression
	yISWI1b	3	transcription elongation, termination
	yISWI2	2	transcription repression, “sliding”
	yCHRAC	4	heterochromatin structure
Human	hACF	2	nucleosome assembly, spacing
	hCHRAC	4	nucleosome assembly, spacing
	hWICH	2	heterochromatin replication, transcription
	hNoRC	2	rDNA silencing
	hRSF	2	nucleosome assembly, spacing
	hSNF2H/NuRD/cohesion	10+	sister chromatid cohesion
	hNURF	4	transcription activation
Xenopus	xACF	3	nucleosome assembly, spacing
	xCHRAC	5	nucleosome assembly, spacing
	xWICH	2	heterochromatin replication, transcription

Modified from ref. (Dirscherl and Krebs, 2004)

Mi-2/CHD subclass

The Mi-2/CHD ATPase subunit contains one or two chromodomains, a helicase domain, and a DNA binding domain. Unlike the chromodomain of heterochromatin protein 1 (HP1), dMi-2 chromodomain seems to bind DNA rather than methylated histone tails, and is essential for Mi-2 ATPase activity (Bouazoune, et al., 2002). CHD family members are also conserved in different organisms. In general, this subclass of ATP-dependent chromatin remodeling enzymes possesses both ATPase and histone deacetylase enzymatic activities (Tong, et al., 1998). The Xenopus Mi-2 complex contains the key deacetylase subunits Rpd3 and RbAp48/p46, and a substoichiometric amount of Sin3 (Wade, et al., 1998), suggesting a role of this complex involved in transcriptional repression. The human Mi-2 complex NuRD contains Mi-2β, an autoantigen associated with dermatomyositis, and MTA-2 that may be involved in cancer metastasis. Histone deacetylases, HDAC1 and HDAC2, which are components of Sin3 corepressor complexes, are also components of human Mi-2 complex (Xue, et al., 1998; Zhang, et al., 1998). In addition, both human and Xenopus Mi-2 containing complexes contain putative methylated CpG-binding proteins (MBD family), which are also linked to transcriptional repression by recruiting HDACs (Wade, et al., 1999). Drosophila Mi-2 complex has similar composition to that of vertebrate NuRD complexes, and interacts with several transcriptional repressors. In addition, fission yeast *Saccharomyces pombe* Chd1 homolog, Hrp1, is required for transcriptional termination either alone or in redundancy with Iswi1 and Iswi2 (Alen, et al., 2002).

INO80/SWR1 subclass

The INO80 and SWR1 complexes are a recently characterized subclass of ATP-dependent chromatin remodeling enzymes in budding yeast (Mizuguchi, et al., 2004; Morrison, et al., 2004; Shen, et al., 2000). More recently, a similar multiprotein complex has been purified from mammalian cells that shares 8 subunits with the yeast INO80 complex (Jin, et al., 2005b). Like SWI/SNF, INO80 and SWR1 are also multiprotein complexes consisting of 11-14 subunits. Interestingly, the SWR1 complex shares six subunits with INO80 and with the NuA4 histone acetyltransferase complex, providing another link between different subclasses of chromatin remodeling complexes (Kobor, et al., 2004). Another similarity to SWI/SNF subclass is that both INO80 and SWR1 complexes contain Arps and actin. Yeast INO80 contains actin, Arp4p, Arp5p and Arp8p, while SWR1 has actin, Arp4p and Arp6p. Actin and Arp4p are required for yeast survival, whereas deletion of Arp5p and Arp8p are viable but lose INO80 function (Shen, et al., 2003). Both Arp5p and Arp8p are important for the ATPase activity and chromatin remodeling activity of the INO80 complex (Shen, et al., 2003; Stewart, et al., 2003). Interestingly, deletion of the N-terminus of Ino80p resulted in a mutant complex that is depleted of Arp8p, Arp4p and actin, suggesting that Arp and actin proteins may physically interact with the N-terminus of the catalytic subunit (Shen, et al., 2003). The precise role of Arps and actin for this subclass of chromatin remodeling is not fully understood. However, studies indicate that Arp4p interacts with core histones and co-immunoprecipitates with H2A (Harata, et al., 1999), and that Arp8p may function as a (H3/H4)2 tetramer chaperone (Shen, et al., 2003). In addition to actin and Arps, both

INO80 and SWR1 complexes contain RuvB helicase-like subunits, Rvb1 and Rvb2, which are essential for cell viability. Rvb1 is not required for the recruitment of Ino80 to target promoters, but seems to be critical for INO80 activity, probably due to loss of critical Arp5 from the complex in the absence of Rvb1. Rvb2 is also associated with Arp5 in vitro in an ATP-dependent manner (Jonsson, et al., 2004). Interestingly, purified INO80 and SWR1 complexes are associated with histones (Mizuguchi, et al., 2004), especially the histone variant H2A.Z.

Ino80p was first identified as a positive regulator of the structural genes of the phospholipid biosynthesis pathway (inositol/choline-responsive element-dependent genes) in vivo. The Ino80p C-terminal region shares more than 30% identity with the Swi2p ATPase domain. Like the catalytically inactive mutant of Swi2p (*swi2K798A*), mutation of the putative ATP-binding site at a lysine residue of Ino80 (*ino80*K737A) failed to complement *ino80* null phenotype (Ebbert, et al., 1999), but retained all the subunits of the INO80 complex (Shen, et al., 2003). In contrast to SWI/SNF, the purified INO80 complex has ATP-dependent 3' to 5' DNA helicase activity in vitro, probably due to the cooperation of RuvB helicase-like subunits (Shen, et al., 2000).

In addition to the role in transcriptional regulation, both the INO80 and SWR1 complexes have been implicated in double strand break (DSB) repair, although the precise role of both protein complexes remains to be investigated (Bao and Shen, 2007). Deletion of either INO80 or SWR1 caused hypersensitivity of yeast to exogenous DNA damaging agents such as UV, ionizing irradiation, methyl methane sulphonate (MMS) and hydroxyurea (Keogh, et al., 2006; Mizuguchi, et al., 2004; Shen, et al., 2000; van Attikum, et al., 2004). In mammalian cells, DNA double-strand breaks cause rapid

phosphorylation of the core histone variant H2AX at Ser139 (γ-H2AX) in megabase chromatin domains flanking the site of DNA damage (Rogakou, et al., 1999). Similarly in budding yeast, an inducible DSB causes rapid phosphorylation of H2A at the C-terminal Ser129, which can spread up to 50 kilobases flanking the break site (Downs, et al., 2000). H2A phosphorylation may signal a DSB and recruit INO80 or other regulatory factors to the break sites probably through interactions between the Arp4 subunit and phospho-H2A (Cairns, 2004; Morrison, et al., 2004; Redon, et al., 2003). Mutation of the H2A serine phosphorylation site has shown hypersensitivity to a wide-range of DNA-damaging agents (Bassing, et al., 2002; Nakamura, et al., 2004).

In contrast to INO80, SWR1 has higher affinity for yeast histone variant Htz1 than for phospo-H2A. The purified SWR1 complex is capable of exchanging nucleosomal H2A/H2B with H2AZ/H2B dimer in vitro (Jin, et al., 2005a; Mizuguchi, et al., 2004). Purified H2AZ is also associated with the SWR1 complex, supporting the notion that SWR1 plays a role in exchange of phospo-H2A/H2B for Htz1/H2B or vice versa during DSB repair in vivo (Mizuguchi, et al., 2004). DNA microarray analysis showed that less than half of the genes regulated by SWR1 are also regulated by H2AZ, suggesting that Swr1 and H2AZ regulate at least a subset of common genes in vivo (Kobor, et al., 2004; Mizuguchi, et al., 2004). H2AZ deposition at transcriptionally active regions proximal to telomeres and flanking the HMR mating-type locus required Swr1p. Zhang *et al.* recently found that the SWR1 complex largely co-localized with the yeast H2A variant H2AZ, and was required for H2AZ deposition into chromosomes (Zhang, et al., 2005). A more recent study showed that deletion of *INO80* caused increased incorporation of H2AZ into chromatin flanking the DSB site, while deletion of Swr1 eliminated the Htz1p

incorporation and restored H2AX-phos, suggesting that Ino80 and Swr1 may function antagonistically at DNA double strand break site to regulate cell cycle checkpoint adaptation (Papamichos-Chronakis, et al., 2006). The interest in the function of the INO80/SWR1 subclass in DSB repair is obviously growing.

Molecular mechanisms of the ATP-dependent chromatin remodeling enzymes

SWI/SNF complexes have been a model system to understand the molecular mechanisms by which ATP-dependent chromatin remodeling complexes alter chromatin conformation and regulate transcription. It is widely accepted that the energy from ATP hydrolysis is used to disrupt histone-DNA interactions within the nucleosome. Subsequently, SWI/SNF ATP-dependent chromatin remodeling complexes can change the DNase I digestion patterns, increase the sensitivity of restriction endonuclease digestion, and enhance activator binding to nucleosomal DNA both in vitro and in vivo. However, the molecular mechanism(s) by which each of the ATP-dependent chromatin remodeling complexes regulates chromatin structure is still under debate. For instance, how does each complex recognize its target properly? Is there any DNA sequence-dependent activity of different enzymes? What is the role of each subunit in concert with the core enzymatic activity during remodeling? How does the chromatin remodeling complex mobilize nucleosomes? Is there a general mechanism shared by different chromatin remodeling enzymes, since all enzymes contain a highly conserved Swi2p/Snf2p like ATPase/helicase-like domain?

Chromatin remodeling enzymes couple ATP hydrolysis to chromatin remodeling

The Swi2p/Snf2p ATPase domain contains seven motifs that are highly homologous to the helicase superfamily 2 (SF2), although purified ySWI/SNF has no detectable helicase activity (Cote, et al., 1994). In addition, the ATPase and chromatin

remodeling activity of different subclasses of the ATP-dependent chromatin remodeling enzymes requires distinct stimulatory factors for the catalytic activity in vitro. For instance, both naked DNA and nucleosomal DNA can stimulate the ATPase activity of the Swi2p/Snf2p and INO80 subclasses. However, Mi-2/CHD and ISWI ATPase activity is maximally stimulated by nucleosomal DNA rather than by naked DNA, and the histone H4 N-terminal domain is required for the ATPase activity and nucleosomal sliding activity of ISWI (Clapier, et al., 2001; Corona, et al., 1999). Interestingly, the ATPase activity of Swi2p/Snf2p can be completely abolished by a point mutation of a conserved lysine residue within the ATPase domain, therefore eliminating the function of the protein (Richmond and Peterson, 1996). Likewise, a recent mutagenesis study by Smith *et al.* showed that an eight-amino acid deletion within the conserved motif V of Swi2p ATPase domain caused severe *swi2* mutant phenotypes in vivo. However, purified SWI/SNF complex bearing this small deletion has WT ATPase activity and ATP hydrolysis kinetics (Smith and Peterson, 2005b). Surprisingly, this mutant SWI/SNF failed to remodel nucleosomes in vitro. This observation strongly suggests that the ATP hydrolysis activity of Swi2p is coupled to its chromatin remodeling activity for proper function (Smith and Peterson, 2005b). Disruption of this function by mutations within BRG1 motif V has been implicated in cancer formation (Wong, et al., 2000).

Molecular basis of ATP-dependent chromatin remodeling action

Chromatin remodeling refers to any change in the nucleosome structure by the action of SWI/SNF, RSC, ISWI and other chromatin remodeling enzymes. In general, these enzymes can cause nucleosome repositioning or “sliding”. The high resolution

crystal structure of the nucleosome suggests that there are over 100 histone-DNA interactions that stabilize the nucleosome position (Luger, et al., 1997b). Therefore, repositioning nucleosomes requires the disruption of many histone-DNA interactions. Two major models have been proposed to explain how the ATP-dependent chromatin remodeling complexes can mobilize nucleosomes or change the nucleosome configuration. In the first model, it has been argued that alteration in nucleosome position may be caused by the spontaneous torsional oscillation from the edge of nucleosomes (van Holde and Yager, 2003). Such a twist defect may be removed by a contrary oscillation and vanish from the nucleosome. When these distortions are propagated into the nucleosome, they may cause nucleosome migration one base pair at a time along the DNA (Langst and Becker, 2004) (Figure 2). Chromatin remodeling enzymes may act as a “molecular ratchet” or a “DNA twistase” that allows the twist defect to exit in one direction and results in DNA twist tension (van Holde and Yager, 2003). In support of this model, these “twist-defects” have been observed in nucleosome crystal structures (Edayathumangalam, et al., 2005). The spontaneous fluctuations of nucleosomal conformation may be sufficient for transient access of proteins to nucleosomal DNA, probably explaining the association of transcription activators or repressors with target promoters prior to chromatin remodeling activity (Li, et al., 2005). However, the “twist-diffusion” model was questioned by observations that a DNA nick or gap (loss of up to 10bp), which presumably dissipate the twist tension on DNA, had no effect on ISWI or RSC induced nucleosome remodeling (Langst and Becker, 2001; Lorch, et al., 2005; Strohner, et al., 2005). In addition, introduction of a DNA branch or hairpin as a barrier did not affect nucleosome remodeling by SWI/SNF and Mi-2 (Aoyagi

and Hayes, 2002; Aoyagi, et al., 2003). Moreover, ISWI-induced nucleosome sliding was facilitated by nicks in the linker DNA in front of the nucleosome (Langst and Becker, 2001).

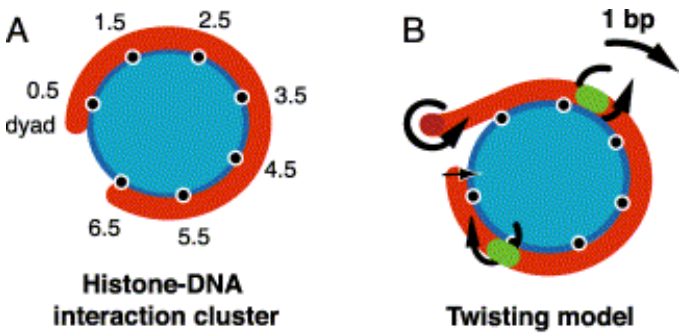

Figure 2. Nucleosome architecture and twist-diffusion mechanism of nucleosome remodeling. (A) Schematic illustration of the superhelical locations (SHL) that form DNA-histone interaction clusters. (B) The "twist-diffusion" model of nucleosome mobilization. The DNA distortion initiates from the edge of a nucleosome and can be propagated into the nucleosome in 1-bp increment. Adapted from (Langst and Becker, 2004)

Compelling evidence has favored a DNA translocation model (Figure 3), in which the DNA translocase activity of chromatin remodelers can translocate DNA into the nucleosome and disrupt histone-DNA interactions in a stepwise manner (Kassabov, et al., 2003; Shundrovsky, et al., 2006; Strick and Quessada-Vial, 2006; Strohner, et al., 2005; Zhang, et al., 2006; Zofall, et al., 2006). The step size of SWI/SNF and ISW2 are approximate 50 or 10 bp, respectively, probably through the formation of the DNA loop on nucleosome surface (Zofall, et al., 2006). Remarkably, the DNA translocation activity of the ATP-dependent chromatin remodeling enzymes is very similar to that of type I restriction enzymes, another helicase-like motor protein that does not have the helicase activity but is able to track along DNA (Stanley, et al., 2006). Using an optical tweezer, Zhang and colleagues recently observed that both SWI/SNF and RSC can translocate along DNA at a rate of 13 bp per second, and generate forces up to approximately 12 pN at single molecule level, producing an average of 100 bp DNA loop on the nucleosomal surface (Zhang, et al., 2006). Moreover, SWI/SNF can move nucleosomes up to 50 bp beyond DNA ends (Kassabov, et al., 2003; Ramachandran, et al., 2003). The DNA translocation activity of RSC has been detected on free DNA by AFM as well (Lia, et al., 2006).

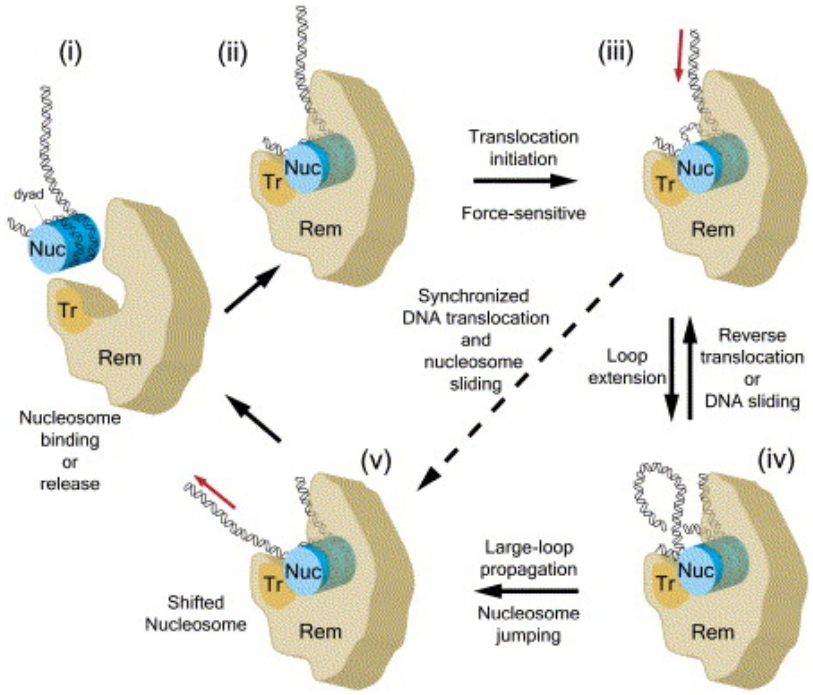

Figure 3. DNA translocation model of ATP-dependent chromatin remodeling. (i) Unbound state. (ii) The ATP-dependent chromatin remodeling complex (Rem) binds the nucleosome (Nuc) in a pocket. (iii) The ATPase/translocase subunit (Tr) engages nucleosomal DNA at a position flanking the dyad, forming a small bulge near the dyad. (iv) Subsequent processive translocation generates large intranucleosomal DNA loops that have three possible fates: forward propagation (resulting in nucleosome jumping), active reverse translocation, or DNA sliding (which may reflect the disengagement of the translocase subunit). Alternatively, the translocation can lead to immediate nucleosome sliding, as indicated by the dashed line, without large loops having been accumulated. (v) Following a remodeling cycle, the remodeler may release the nucleosome. Adapted from (Zhang, et al., 2006)

Altered nucleosomes are more accessible in different ways

The outcome of DNA translocation is believed to mobilize nucleosomes in cis (Narlikar, et al., 2002). This is consistent with the ability of chromatin remodeling complexes to increase the restriction enzyme accessibility of mononucleosomal and nucleosomal array substrates, and to generate fast-migrating nucleosomes in native gel electrophoresis. Consistently, after human SWI/SNF remodeling on a nucleosomal array, long stretches of free DNA and clusters of adjacent nucleosomes have been observed (Schnitzler, et al., 2001; Ulyanova and Schnitzler, 2005). All these observations support the nucleosome sliding model as a major consequence after chromatin remodeling by SWI/SNF, RSC and ISWI complexes in vitro (Narlikar, et al., 2002; Whitehouse, et al., 1999; Whitehouse, et al., 2003). Interestingly, a change in histone octamer composition is not necessary for nucleosome repositioning in most cases. This notion is further supported by a recent DNA unzipping study showing that nucleosomes assembled onto the “601” template, which has strong nucleosome positioning sequence (Anderson and Widom, 2000), had overall the same nucleosome unzipping force (therefore same histone-DNA contacts) as that of the unremodeled nucleosome (Shundrovsky, et al., 2006). Additionally, dinucleosome-like remodeled nucleosomes had the same histone-to-DNA composition as the standard nucleosome, although the sensitivity to restriction enzyme digestion had changed (Schnitzler, et al., 1998).

The re-association of the free DNA with the same histone octamer might be the molecular basis that causes the formation of DNA loop or bulge on the surface of nucleosome (Bazett-Jones, et al., 1999; Fan, et al., 2003). Likewise, the association of

the free DNA of a remodeled nucleosome with another remodeled species may explain the formation of slowing-migrating “dimers” after SWI/SNF chromatin remodeling (Lorch, et al., 1998; Schnitzler, et al., 1998; Ulyanova and Schnitzler, 2005; Ulyanova and Schnitzler, 2007) (Figure 4).

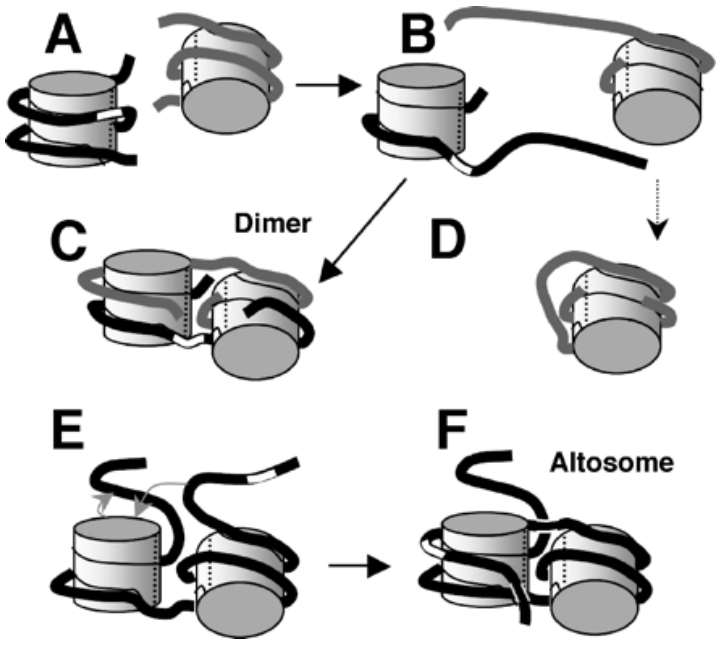

Figure 4. Dimer and altosome structure models. hSWI/SNF repositioning activity on normal nucleosome cores (*A*) can result in off the edge nucleosomes (*B*). Two off the edge nucleosomes might be stabilized as a dimer (*C*), or one off the edge nucleosome with sufficient DNA length might be stabilized as a loop mononucleosome (*D*). On polynucleosomal DNA, hSWI/SNF activity might promote release of ~70 bp from one nucleosome (*E*). If this DNA is replaced by linker DNA distal to an adjacent nucleosome, a stable altered dinucleosome, altosome, can be formed (*F*). Adapted from (Ulyanova and Schnitzler, 2007).

In other cases, yeast SWI/SNF (Gutierrez, et al., 2007; Hassan, et al., 2006; Owen-Hughes, et al., 1996; Whitehouse, et al., 1999) and RSC (Lorch, et al., 1999) complexes can induce histone octamer transfer to a free DNA accepter. The octamer transferring activity is conserved in human SWI/SNF as well, based on the observation that both the intact human SWI/SNF and isolated BRG1 subunit were able to induce histone octamer transfer in vitro (Phelan, et al., 2000). A recent study from the Workman group showed that ySWI/SNF can induce ATP-dependent nucleosome eviction and completely release the nucleosomal DNA. This nucleosome eviction activity seems to require the activation domain of an activator. When the activation domain was absent, SWI/SNF exclusively caused nucleosome sliding without loss of histones (Gutierrez, et al., 2007). Nucleosome eviction is thought to be important for SWI/SNF-dependent transcriptional activation at the yeast *PHO5* promoter in vivo (Boeger, et al., 2004; Korber, et al., 2004).

It is notable that yeast ISW2 can also catalyze nucleosome positional changes through a similar mechanism to that of SWI/SNF (Zofall, et al., 2006), although ISW2 slides nucleosomes away from DNA ends (Kassabov, et al., 2002). Interestingly, ISW2 slides nucleosomes closer to the promoter region of target genes in vivo, which led to the hypothesis that ISWI might counteract the action of SWI/SNF in vivo by sliding nucleosomes to positions that prevent activator binding (Fazzio and Tsukiyama, 2003; Kassabov, et al., 2002; Moreau, et al., 2003).

Dimer displacement activity of chromatin remodeling complexes can be assisted by histone chaperones

Early genetic evidence showed that defects caused by mutation of SWI/SNF subunits were suppressed by depletion of one of the histones, histone H2A and H2B in vivo, which has led to the hypothesis that H2A/H2B dimer loss might be one of the mechanisms of SWI/SNF action in vivo (Hirschhorn, et al., 1992). Conceivably, loss of DNA content from the nucleosomal entry/exit sites after chromatin remodeling action would abolish the histone-DNA contacts that are required to stabilize the histone H2A/H2B dimers (Aoyagi, et al., 2002; Kassabov, et al., 2003).

Histone H2A/H2B dimer exchange or displacement has been studied on MMTV nucleosomes by the chromatin remodeling complex alone or with histone chaperones, leading to another mechanism by which chromatin remodeling complexes modulate nucleosome configuration. Bruno and colleagues observed that both yeast RSC and SWI/SNF complexes are able to catalyze up to 33% dimer exchange. In contrast, ISWI subclass, ISW1a and ISW1b showed very limited efficiency in the dimer exchange assay (Bruno, et al., 2003). Interestingly, Vincent *et al* observed up to ~73% of dimer doss at nucleosomes assembled on MMTV-nucleosome B DNA but not on rDNA, claiming that dimer loss activity of SWI/SNF might be a DNA sequence-dependent event (Vicent, et al., 2004). More recently, Lorch and colleagues observed that the RSC complex requires histone chaperone Asf1 to remove dimers, whereas Nap1 can catalyze stepwise dimer loss and leads to the complete disassembly of the nucleosome in vitro, reflecting the collaboration of histone chaperones with chromatin remodeling enzymes in dimer displacement (Lorch, et al., 2006). Using an AFM imaging system, Bash and colleagues

found that the human SWI/SNF complex can also induce dimer dissociation from nucleosomes (Bash, et al., 2006). Interestingly, dimer dissociation and dimer transferring to tetramer acceptors by the human SWI/SNF complex seems to be stimulated by a nuclear histone chaperone protein, nucleolin (Angelov, et al., 2006).

Purified SWR1 complex is capable of exchanging H2A/H2B with H2AZ/H2B dimers both in vitro and in vivo (Mizuguchi, et al., 2004), although it is unclear whether this dimer exchange activity by SWR1 is related to the histone chaperone function of Arp proteins within the complex (Shen, et al., 2003). Based on these observations, we infer that DNA translocation catalyzed by chromatin remodeling enzymes may be a prerequisite for the dissociation of histone H2A/H2B dimers, since loss of DNA-histone contacts during DNA translocation destabilized the histone dimers (Aoyagi and Hayes, 2002; Kassabov, et al., 2003). Subsequently, histone chaperones may facilitate the chromatin remodeling process by removing the dimers from the remodeled nucleosomes (Loyola and Almouzni, 2004).

In summary, the ATP-dependent chromatin remodeling complexes can translocate nucleosomes, and cause the following changes in nucleosome configuration: (1) nucleosome sliding in cis; (2) DNA looping/bulging; (3) histone octamer transfer in trans; (4) nucleosome eviction; (5) remodeled dimer or altosome formation; (6) histone H2A/H2B displacement. It remains be investigated how these in vitro observations correlate with changes in chromatin structure *in vivo*. We propose that the diverse consequences after chromatin remodeling may reflect distinct mechanisms applied at different promoters in vivo. In addition, chromatin remodeling intermediates may exist and can be captured in biochemical studies. In this thesis, we will further investigate the

function(s) of core subunits of the SWI/SNF ATP-dependent chromatin remodeling complex, using yeast as the model system. These findings are expected to help us better understand the function and molecular mechanism(s) of the ATP-dependent chromatin remodeling enzymes.

Acknowledgements

Many thanks to the Peterson lab members, especially Manolis, Sharmistha, Pranav, and Manisha, for their comments on this review.

CHAPTER II

CHARACTERIZATION OF THE ROLE OF THE SANT DOMAIN FOR SWI/SNF FUNCTION

SUMMARY

SWI/SNF superfamily members of chromatin remodeling enzymes are multiprotein complexes. The fact that the isolated ATPase subunit alone is active for chromatin remodeling has led us to question the precise role(s) of other subunits for chromatin remodeling. In this chapter, we investigated the essential role of the SANT domain of the Swi3p subunit for ySWI/SNF function. We found that *swi3*Δ*SANT* caused a genome-wide transcriptional defect that is similar to that of Δ*swi3*. *Swi3*Δ*SANT* crippled the recruitment of SWI/SNF to target promoters in both asynchronous and nocodazole-synchronized cells. In the absence of the Swi3p SANT domain, tethering SWI/SNF via LexA-Swi2p was insufficient to activate a $LexA_{op}$-$GAL1_{TATA}$-*LacZ* reporter gene in vivo. Surprisingly, *swi3*Δ*SANT* caused dissociation of SWI/SNF into at least four stable subcomplexes: 1) Swi2p/Arp7p/Arp9p; 2) Swi3p/Swp73p/Snf6p; 3) Swi1p; and 4) Snf5p. These data indicate that the Swi3p SANT domain is critical for SWI/SNF assembly. Furthermore, a point mutant within the SANT domain, *swi3R564E*, or deletion of any of certain other core subunits of SWI/SNF also caused the dissociation of

SWI/SNF into the same subcomplexes. These observations help to dissect both the functional and architectural organization of SWI/SNF complex.

The data presented in this chapter are being prepared for publication. I would like to thank Dr. David Lambright for generating the structural image of the ISWI SANT domain shown in Figure 14.

INTRODUCTION

ATP-dependent chromatin remodeling enzymes were originally identified in budding yeast *Saccharomyces cerevisiae* from genetic screens for mutants defective in mating type switching (*SWI*) (Stern, et al., 1984), and mutants unable to ferment sucrose (*S*ucrose *N*on-*F*ermenter, *SNF*) (Neigeborn and Carlson, 1984). Yeast SWI/SNF is required for transcriptional regulation of many genes, especially a subset of inducible genes including *HO, SUC2, SIC1* and *INO1* (Holstege, et al., 1998b; Sudarsanam, et al., 2000; Winston and Carlson, 1992). SWI/SNF has been a paradigm to understand the function of ATP-dependent chromatin remodeling complexes in transcriptional regulation and the molecular mechanisms of their action.

SWI/SNF-like chromatin remodeling complexes have been identified in higher eukaryotes such as Drosophila, frog, mouse, and human (Imbalzano, et al., 1994; Peterson, 2000). All SWI/SNF family members consist of a helicase-like catalytic subunit that hydrolyzes ATP. The energy from ATP hydrolysis is believed to disrupt histone-DNA contacts, therefore enhancing the accessibility of transcription factors to nucleosomal DNA before, during, or after transcriptional initiation (Fry and Peterson, 2002). Recent biochemical evidence has suggested that several ATP-dependent chromatin remodeling complexes also catalyze histone H2A/H2B dimer loss (Bruno, et al., 2003; Vicent, et al., 2004) and nucleosome eviction (Gutierrez, et al., 2007). Both human SWI/SNF (Schnitzler, et al., 1998; Ulyanova and Schnitzler, 2005) and yeast RSC (Lorch, et al., 1998) can induce the formation of stable dinucleosome-like remodeled products or

altosomes, indicating that SWI/SNF may use different mechanisms to manipulate chromatin structure under different circumstances.

Yeast SWI/SNF is a multiprotein complex that consists of Swi2p/Snf2p, Swi1p, Snf5p, Swi3p, Swp82p, Swp73p, Arp7p, Arp9p, Snf6p, Snf11p, and Swp29p (Table 3) (Cairns, et al., 1994; Cairns, et al., 1996b; Peterson, et al., 1994; Peterson and Herskowitz, 1992; Smith, et al., 2003; Treich, et al., 1995; Wilson, et al., 2006). Among the ATP-dependent chromatin remodeling complexes, several core subunits, Swi2p, Swi1p, Swi3p, Snf5p, and Swp73p, are highly conserved from yeast to human (Aalfs and Kingston, 2000; Kingston and Narlikar, 1999). Surprisingly, the catalytic subunit of human SWI/SNF, BRG1 or hBRM, has ATPase activity by itself and is capable of remodeling a mononucleosome in vitro, although addition of hSnf5/INI1 and hSwi3 (BAF170 and BAF155) subunits can further stimulate BRG1 activity to that of the intact complex (Phelan, et al., 1999). Despite these studies, the function of majority of other subunits for ATP-dependent chromatin remodeling process remains largely unclear (Table 3). One possibility is that other subunits may coordinate the activity of core catalytic subunit with other transcription factors upstream or down stream of chromatin remodeling in vivo.

Table 3. Subunit composition of yeast SWI/SNF

Subunit	Gene Function	MW(Kd)	copy#
*Swi2	ATPase	194	1
*Swi1	activator binding	148	1
*Snf5	activator binding	103	1
*Swi3		93	2
Swp82		72	2
*Swp73		64	1
*Arp7	actin-related	54	1
*Arp9	actin-related	53	1
Snf6		38	2
Swp29		27	3
Snf11		18	2

* Subunits highly conserved in other organisms.

Since SWI/SNF does not show DNA-binding sequence specificity, it is believed that low abundant SWI/SNF is directed to target promoters through recruitment by specific activators. Biochemical studies have shown that Swi1p and Snf5p may play a role in recruiting SWI/SNF to the promoter through direct interaction with transcriptional activators (Neely, et al., 2002; Prochasson, et al., 2003). This notion is partially supported by in vivo studies that the recruitment of yeast SWI/SNF to the *HO* promoter depends on a specific activator Swi5p (Cosma, et al., 1999; Krebs, et al., 2000). However, recent studies by Martens JA *et al* have shown that the transcription of yeast *SER3* regulatory gene 1 (*SRG1*) depends largely on the catalytic subunit Swi2p in vivo, and deletion of other subunits such as Swi1p and Snf5p does not affect *SRG1* transcription significantly (Martens and Winston, 2002; Martens, et al., 2005). This discrepancy of requirement may reveal distinct mechanisms by which SWI/SNF

regulates transcription at different promoters, and that the recruitment of Swi2p/Snf2p is sufficient to activate certain promoters. Intriguingly, mutations of hSWI2/BRG1, hSNF5 (INI1), hSWI3 (BAF155), BAF180 or BAF250 have been found in human cancer cell lines or primary cancers (Decristofaro, et al., 2001; DeCristofaro, et al., 1999; Grand, et al., 1999; Klochendler-Yeivin, et al., 2002; Roberts and Orkin, 2004). Loss of heterozygosity of INI1 (Snf5p homolog) predisposed mice to aggressive cancers, indicating an important role of SWI/SNF genes involved in tumor suppression *in vivo* (Guidi, et al., 2001; Klochendler-Yeivin, et al., 2000).

The presence of conserved protein domains such as the bromodomain and chromodomain in ATP-dependent chromatin remodeling enzymes provides a connection between histone modification and ATP-dependent chromatin remodeling. Additionally, histone tail modifications may provide binding sites for ATP-dependent chromatin remodeling complexes during transcriptional activation or repression (de la Cruz, et al., 2005; Hassan, et al., 2001; Zeng and Zhou, 2002). Another conserved motif known as the SANT domain has stimulated some interest in understanding the function of chromatin remodeling complexes. The SANT domain is identified in many chromatin-regulatory proteins such as Swi3p of SWI/SNF, Ada2p of Gcn5p-containing SAGA, nuclear hormone receptor corepressor NcoR, and transcription factor TFIIIb, and has homology to the c-myb DNA binding domain (Rein Aasland, 1996) (Figure 5). The 50-amino acid motif is predicted to have three putative helices. Recent studies by several groups highlighted the important role of the SANT domains in histone tail binding (Boyer, et al., 2002; Boyer, et al., 2004; Grune, et al., 2003; Sterner, et al., 2002; Yu, et al., 2003). A deletion of eleven amino acids from the SANT domain of Swi3p (*swi3*Δ*SANT*)

failed to rescue the growth defect caused by *swi3* deletion (Boyer, et al., 2002) (Figure 6). In the case of Ada2p, the SANT domain is required for Gcn5p histone acetylation transferase (HAT) activity and histone H3 tail interaction (Boyer, et al., 2002; Sterner, et al., 2002). Therefore, the SANT domain may serve as a histone tail-binding module that is required for nucleosome recognition (Boyer, et al., 2004).

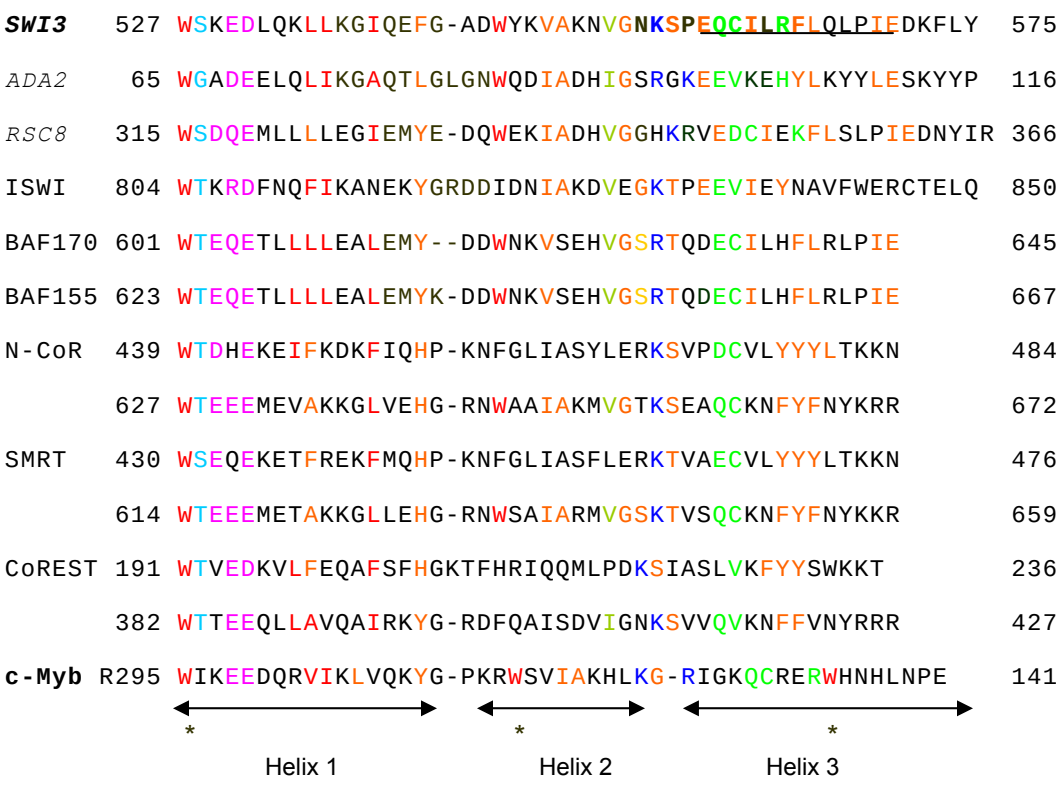

Figure 5. Sequence alignment of the SANT domains. Colored residues are highly conserved among SANT domains (42). Some domains were analyzed/identified with the Simple Modular Architecture Research Tool (SMART) program (http://smart.embl-heidelberg.de/smart). * Three conserved tryptophan residues of c-Myb DBD.

To further characterize the potential role of the SANT domain for SWI/SNF function, we first explored the genome-wide transcriptional profile in a *swi3*Δ*SANT* and Δ*swi3* backgrounds using an Affymetrix gene chip. Next, we investigated whether the SANT domain is generally required for the recruitment of SWI/SNF to target promoters such as *CDC6, SIC1,* and *HO* by chromatin immunoprecipitation analysis (ChIP). Interestingly, *swi3*Δ*SANT* crippled the recruitment of SWI/SNF to all target promoters. Furthermore, in the absence of an intact SANT domain, tethering SWI/SNF via LexA-Swi2p to a chromosome-integrated $LexA_{op}$-$GAL1_{TATA}$-*LacZ* reporter failed to activate the transcription of the LacZ reporter gene. Finally, I purified SWI/SNF complex from *swi3*Δ*SANT* strain, using the tandem affinity purification (TAP) technique. We found that deletion of the Swi3p SANT domain destabilized the SWI/SNF complex, and four distinct subcomplexes were purified from the *swi3*Δ*SANT* strain. Swi2p was associated with Arp7p and Arp9p. Snf6p was associated with Swi3p and Swp73p, while Swi1p and Snf5p were purified as monomers. I also purified SWI/SNF complexes from strains that lacked either Swi1p or Snf5p, and found that SWI/SNF also dissociates into the same subcomplexes in these mutant strains. These data suggest a new model for the role of the SANT domain as a protein scaffold for SWI/SNF assembly. In addition, these results indicate that SWI/SNF complex is an integration of at least four distinct functional modules: 1) Swi2p/Arp7p/Arp9p, 2) Swi3p/Swp73p/Snf6p, 3) Swi1p, and 4) Snf5p.

RESULTS

The SANT domain of Swi3p is required for SWI/SNF function and genome-wide gene expression in vivo

The role of the SANT domain remains largely unclear although it was originally identified as a module homologous to the cMyb DNA binding domain (Aasland, et al., 1996). Recent studies of the SANT-containing proteins, Ada2p (Boyer, et al., 2002; Sterner, et al., 2002), mammalian nuclear hormone receptor corepressor SMRT (Yu, et al., 2003), and Drosophila ISWI (Grune, et al., 2003), revealed that these SANT domains are involved in histone tail interactions and may coordinate core enzymatic activities (Boyer, et al., 2004). In the case of Swi3p (Figure 6), partial deletion or mutation of certain residues from the SANT domain (*swi3*Δ*SANT*) caused a severe growth phenotype (Boyer, et al., 2002). We hypothesized that the SANT domain of Swi3p may be required for the binding of SWI/SNF to nucleosomal substrates through direct interaction with histone tails (Boyer, et al., 2002).

To test this hypothesis, we initially investigated the genome-wide transcriptional profile in the absence of the SANT domain of Swi3p. Isogenic strains of wild type (WT), Δ*swi3*, and *swi3*Δ*SANT* were grown for total RNA extraction and for Affymetrix DNA microarray analysis. Original microarray signals from *Δswi3* and *swi3ΔSANT* strains were normalized to that of WT. From three independent experiments performed, only those genes whose expression changed 2-fold or more in all three experiments were selected for data analysis. Genes that changed only in one or two experiments were not included for initial data analysis. All signals were averaged from all three experiments for

comparison. Figure 7a,b represent original fluorescent signals from two independent chip analyses from wild type.

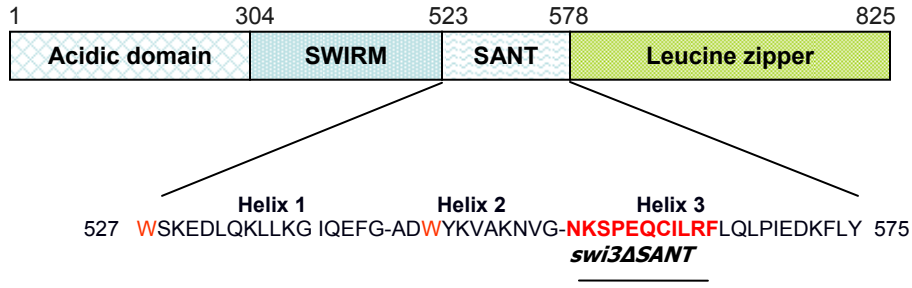

Figure 6. Domain organization of Swi3p. Swi3p is predicted to have several domains, including the N-terminal 300 amino acids acidic domain, SWIRM domain, C-terminal SANT domain and leucine zipper domain. The SANT domain consists of ~50 amino acids that contain three putative helices based on the homology to the c-Myb DNA binding domain. Deletion of the N-terminal 11 amino acids from the putative third helix of the SANT domain (swi3ΔSANT) causes a severe growth phenotype (Boyer, et al., 2002). The leucine zipper domain may mediate Swi3p dimerization.

Under these stringent criteria for data analysis described above, we found that Swi3p regulates approximate 3% of yeast genes in the whole genome. All the genes upregulated or downregulated more than 2-fold by deletion of Swi3p or the SANT domain of Swi3p were listed in Table 4 and Table 5, respectively. Diagrams in Figure 7c,d summarized the overlapping of genes affected more than 2-fold by deletion of *swi3* (empty oval) or deletion of the SANT domain of Swi3p (grey oval) in all three independent experiments. Among the 88 genes upregulated more than 2-fold by *swi3* deletion, 49 genes (56% overlap) were also upregulated by *swi3ΔSANT* (Figure 7c). From the 58 genes downregulated more than 2-fold by *Δswi3*, 40 genes (69% overlap) were also downregulated by *swi3ΔSANT* (Figure 7d). Only four genes downregulated in *swi3ΔSANT* were not affected in *Δswi3*. Consistent with the important role of the SANT domain (Boyer, et al., 2002), gene pools affected by swi3Δ*SANT* were almost indistinguishable from those affected by *Δswi3* when we compared genes changed 1.5-fold and up (not shown). Previously, it has been shown that functional Swi2p/Snf2p is required for the transcriptional regulation of ~5% genes (Holstege, et al., 1998a), when comparing genes affected from two independent experiments. Compared to genes whose expression was affected by Δ*swi2*, we found that ~40% of these genes are also affected by *Δswi3*, suggesting that these proteins function together in the same SWI/SNF complex. Genes that were not detected in our data set may be caused by different criteria of data analysis. In other words, we used a more stringent data analysis. Alternatively, Swi3p may be unnecessary for some of the ATP-dependent chromatin remodeling activities of Swi2p/Snf2p in vivo. This data strongly suggested that

swi3ΔSANT behaved like a weak null allele of *SWI3* and that the SANT domain is crucial for Swi3p function in vivo.

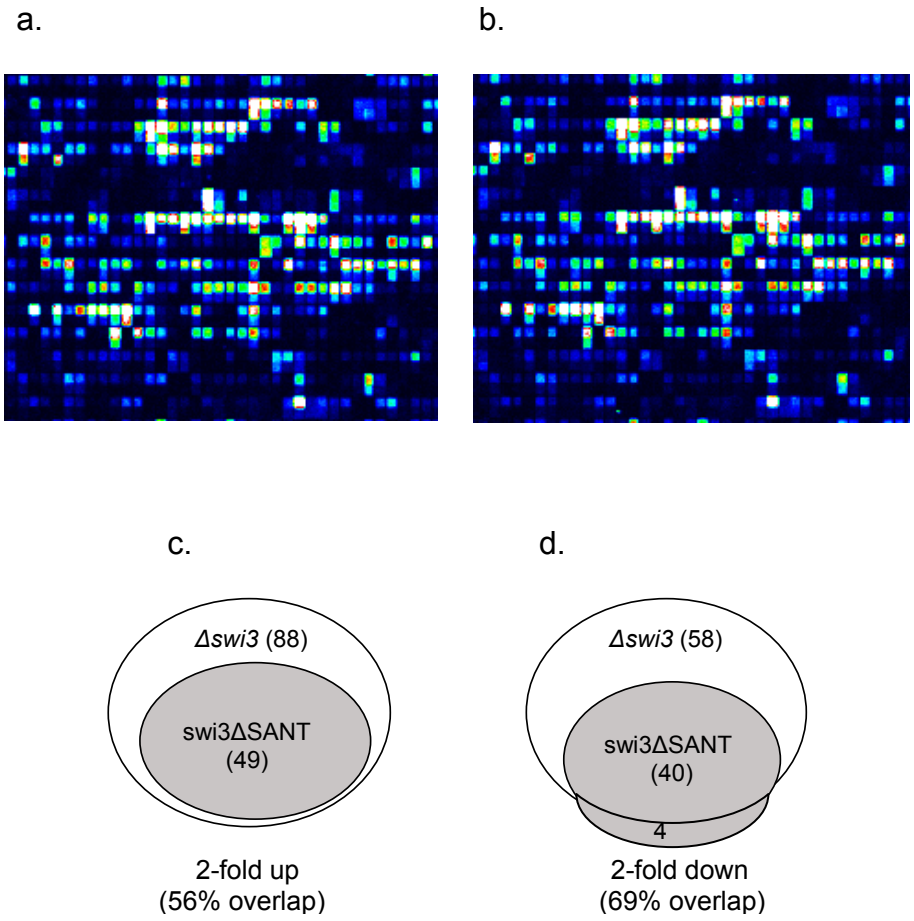

Figure 7. Affymetrix gene chip analysis. Total RNA was extracted from isogenic strains of WT, *Δswi3*, and *swi3ΔSANT*. Three independent experiments were performed for data analysis. Original signals from *Δswi3* or *swi3ΔSANT* were normalized to that for WT. **(a, b)** Reproducible DNA microarray signals from two independent experiments from WT. **(c, d)** Venn diagrams show total number of genes whose expression changed 2-fold up (c) or down (d) averaged from three independent data sets. The empty oval represents genes whose expression changed in *Δswi3*. The grey oval represents the genes whose expression changed in *swi3ΔSANT.*

Table 4. Common genes upregulated by *swi3* or *swi3*Δ*SANT*

common genes 2x UP in *swi3* and *SWI3*sant* strains (49)			
	avg. fold up	avg. fold up	
Gene name	*swi3*	*SWI3*sant*	Description of function
YOL161C	10.27811733	4.6119605	strong similarity to members of the Srp1p/Tip1p family
YOL101C	6.746812867	3.172161033	similarity to YOL002c and YDR492w
THI 11	5.7762994	2.608154467	Thiamine biosynthetic enzyme
YNL335W	5.274972	4.307694933	identical to hypothetical protein YFL061w
YLR312C	5.260503333	3.4313662	hypothetical protein
NER020W	5.030029267	4.412910133	SAGE orf. Characterization of the yeast transcriptome.
RTS 3	4.992031233	2.944705233	hypothetical protein
NMR044C	4.9131311	3.537257767	SAGE orf. Characterization of the yeast transcriptome.
JEN 1	4.7672838	4.491358033	carboxylic acid transporter protein homolog
YEL045C	3.94321	2.994443333	weak similarity to cytochrome c oxidase III of T.brucei kinetoplast
PAU2	3.93894	2.697716667	member of the seripauperin protein\/gene family (see Gene_class PAU)
NEL015C	3.74177	2.4575	SAGE orf
CAT8, DIL1	3.72261	2.22685	Zinc-cluster protein involved in activating gluconeogenic genes; related to Gal4p
ARG 3	3.523174733	2.825173667	Ornithine carbamoyltransferase
YBL065W	3.374273333	2.04787	questionable ORF
SUL2	3.373936667	2.05279	high affinity sulfate permease
ARS608	3.241896667	2.270603333	SAGE orf
MET2	3.225873333	2.013023333	homoserine O-trans-acetylase
YDR010C	3.2187418	2.7008143	hypothetical protein
NMR067W	3.202665333	2.547319867	SAGE orf. Characterization of the yeast transcriptome.
gGR12	3.191562133	2.394722867	complete chromosome sequence.
YLR338W	3.156951133	2.281434467	questionable ORF
YIL015C-A	3.048296667	2.01032	strong similarity to hypothetical protein YIL102c
GAT1, NIL1	3.040113333	2.17545	activator of transcription of nitrogen-regulated genes
STR3	3.031996667	2.413773333	strong similarity to Emericella nidulans cystathionine beta-lyase
BNA 5	3.015351467	2.3069476	strong similarity to rat kynureninase
ADH5	3.0029	2.08258	alcohol dehydrogenase isoenzyme V
NDR047C	3.000873333	2.333503333	SAGE orf
YOL114C	2.995036067	2.521193667	similarity to human DS-1 protein
PTI1	2.837843333	2.113536667	hypothetical protein
NIL005W	2.79426	2.589083333	SAGE orf
YHR095W	2.68148	2.81173	hypothetical protein
MMP1	2.640283333	2.115793333	strong similarity to amino acid transport protein Gap1p
NNL036W	2.633703333	2.01437	SAGE orf
MET10	2.623033333	2.0002	subunit of assimilatory sulfite reductase
gPL09	2.59815	2.040123333	complete chromosome sequence
YGL007W	2.58776	2.36344	questionable ORF
ICY2	2.547253333	2.03107	weak similarity to YMR195w
gPR12	2.543206667	3.174073333	complete chromosome sequence
YOL155C	2.5252241	2.1482835	similarity to glucan 1,4-alpha-glucosidase Sta1p and YAR066w
YOR318C	2.48528	2.38759	hypothetical protein
YOL155C	2.47221	2.017373333	similarity to glucan 1,4-alpha-glucosidase Sta1p and YAR066w
YDR542W	2.470247633	2.726105067	strong similarity to subtelomeric encoded proteins
LPE10	2.419726667	2.095543333	mitochondrial protein, strong similarity to Mrs2p, megnesium ion transporter
gKR07	2.336419733	2.223684167	complete chromosome sequence.
SHU2	2.324233333	1.998733333	Preferential Use of Neither donor locus during mating type switching.
PRR2	2.277776667	2.496296667	strong similarity to putative protein kinase NPR1
YPL261C	2.246913333	2.44691	questionable ORF
FRE 2	2.2383902	1.999410367	Ferric reductase, similar to Fre1p

Table 5. Common genes downregulated by *swi3* or *swi3*Δ*SANT*

common genes 2xdown in *swi3* and *SWI3*sant* strains (49)			
	avg. fold down	avg. fold down	
Gene name	*swi3*	*SWI3*sant*	Description of function
YOL161C	10.27811733	4.6119605	strong similarity to members of the Srp1p/Tip1p family
YOL101C	6.746812867	3.172161033	similarity to YOL002c and YDR492w
THI 11	5.7762994	2.608154467	Thiamine biosynthetic enzyme
YNL335W	5.274972	4.307694933	identical to hypothetical protein YFL061w
YLR312C	5.260503333	3.4313662	hypothetical protein
NER020W	5.030029267	4.412910133	SAGE orf. Characterization of the yeast transcriptome.
RTS 3	4.992031233	2.944705233	hypothetical protein
NMR044C	4.9131311	3.537257767	SAGE orf. Characterization of the yeast transcriptome.
JEN 1	4.7672838	4.491358033	carboxylic acid transporter protein homolog
YEL045C	3.94321	2.994443333	weak similarity to cytochrome c oxidase III of T.brucei kinetoplast
PAU2	3.93894	2.697716667	member of the seripauperin protein\/gene family (see Gene_class PAU)
NEL015C	3.74177	2.4575	SAGE orf
CAT8, DIL1	3.72261	2.22685	Zinc-cluster protein involved in activating gluconeogenic genes; related to Gal4p
ARG 3	3.523174733	2.825173667	Ornithine carbamoyltransferase
YBL065W	3.374273333	2.04787	questionable ORF
SUL2	3.373936667	2.05279	high affinity sulfate permease
ARS608	3.241896667	2.270603333	SAGE orf
MET2	3.225873333	2.013023333	homoserine O-trans-acetylase
YDR010C	3.2187418	2.7008143	hypothetical protein
NMR067W	3.202665333	2.547319867	SAGE orf. Characterization of the yeast transcriptome.
gGR12	3.191562133	2.394722867	complete chromosome sequence.
YLR338W	3.156951133	2.281434467	questionable ORF
YIL015C-A	3.048296667	2.01032	strong similarity to hypothetical protein YIL102c
GAT1, NIL1	3.040113333	2.17545	activator of transcription of nitrogen-regulated genes
STR3	3.031996667	2.413773333	strong similarity to Emericella nidulans cystathionine beta-lyase
BNA 5	3.015351467	2.3069476	strong similarity to rat kynureninase
ADH5	3.0029	2.08258	alcohol dehydrogenase isoenzyme V
NDR047C	3.000873333	2.333503333	SAGE orf
YOL114C	2.995036067	2.521193667	similarity to human DS-1 protein
PTI1	2.837843333	2.113536667	hypothetical protein
NIL005W	2.79426	2.589083333	SAGE orf
YHR095W	2.68148	2.81173	hypothetical protein
MMP1	2.640283333	2.115793333	strong similarity to amino acid transport protein Gap1p
NNL036W	2.633703333	2.01437	SAGE orf
MET10	2.623033333	2.0002	subunit of assimilatory sulfite reductase
gPL09	2.59815	2.040123333	complete chromosome sequence
YGL007W	2.58776	2.36344	questionable ORF
ICY2	2.547253333	2.03107	weak similarity to YMR195w
gPR12	2.543206667	3.174073333	complete chromosome sequence
YOL155C	2.5252241	2.1482835	similarity to glucan 1,4-alpha-glucosidase Sta1p and YAR066w
YOR318C	2.48528	2.38759	hypothetical protein
YOL155C	2.47221	2.017373333	similarity to glucan 1,4-alpha-glucosidase Sta1p and YAR066w
YDR542W	2.470247633	2.726105067	strong similarity to subtelomeric encoded proteins
LPE10	2.419726667	2.095543333	mitochondrial protein, strong similarity to Mrs2p, megnesium ion transporter
gKR07	2.336419733	2.223684167	complete chromosome sequence.
SHU2	2.324233333	1.998733333	Preferential Use of Neither donor locus during mating type switching.
PRR2	2.277776667	2.496296667	strong similarity to putative protein kinase NPR1
YPL261C	2.246913333	2.44691	questionable ORF
FRE 2	2.2383902	1.999410367	Ferric reductase, similar to Fre1p

An intact SANT domain of Swi3p is required for the recruitment of SWI/SNF to target promoters

Since partial deletion of the SANT domain of Swi3p causes growth defects and changes in the genome-wide transcriptional profile, we next investigated how the SANT domain contributes to the function of SWI/SNF *in vivo*. A study by Boyer *et al* showed that the SANT domain of Ada2p is required for the interaction of the Gcn5-containing SAGA complex with the histone H3 tails in a GST-pull down assay (Boyer, et al., 2002). In the case of the nuclear hormone corepressor SMRT, the N-terminal SANT domain of SMRT was required for histone deacetylase 3 (HDAC3) activity, whereas the C-terminal SANT domain interacted with non-acetylated histone tails (Yu, et al., 2003). Given that the SANT motif functions as a histone tail-binding module, deletion of the SANT domain might interfere with the binding or stability of SWI/SNF at target promoters.

To test whether the transcriptional defect caused by *swi3ΔSANT* is due to a recruitment defect of SWI/SNF at target promoters in vivo, I performed chromatin immunoprecipitation (ChIP) analysis on the promoters of *CDC6*, *SIC1* and *HO*, known SWI/SNF-dependent genes. To do this, Swi2p was tagged at its C-terminus with 13 tandem repeats of myc epitope in isogenic wild type (WT), swi3 deletion (Δ*swi3*), and *swi3ΔSANT* strains. The Myc tag does not interfere with the protein expression level (data not shown) or the function of Swi2p, as the myc-tagged allele did not show the growth defects associated with a swi2 mutant. Consistent with previous observations by Boyer et al (Boyer, et al., 2002), *swi3ΔSANT* does not affect the protein level of Swi2p (Figure 8). Anti-myc antibody was used to immunoprecipitate formaldehyde cross-linked chromatin from asynchronous cell cultures. We found that in the wild type (WT) strain,

Swi2-myc was associated with all the promoters tested (Figure 9, WT). In contrast, deletion of the Swi3p eliminated Swi2-myc recruitment, and swi3ΔSANT caused an approximately 60% decrease in Swi2-myc recruitment at all target promoters (Figure 9). Complete deletion of *swi3* seemed to impair the stability of Swi2p (Peterson, et al., 1994), which might explain the more severe recruitment defect in *Δswi3* background.

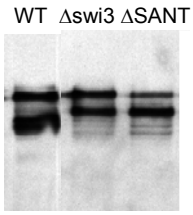

Figure 8. Deletion of the SANT domain of Swi3p does not affect Swi2p protein expression level. Western blot analysis of Swi2-13Myc from whole cell lysate of isogenic WT, ∆*swi3*, and *swi3*∆*SANT* strains, using monoclonal anti-Myc antibody.

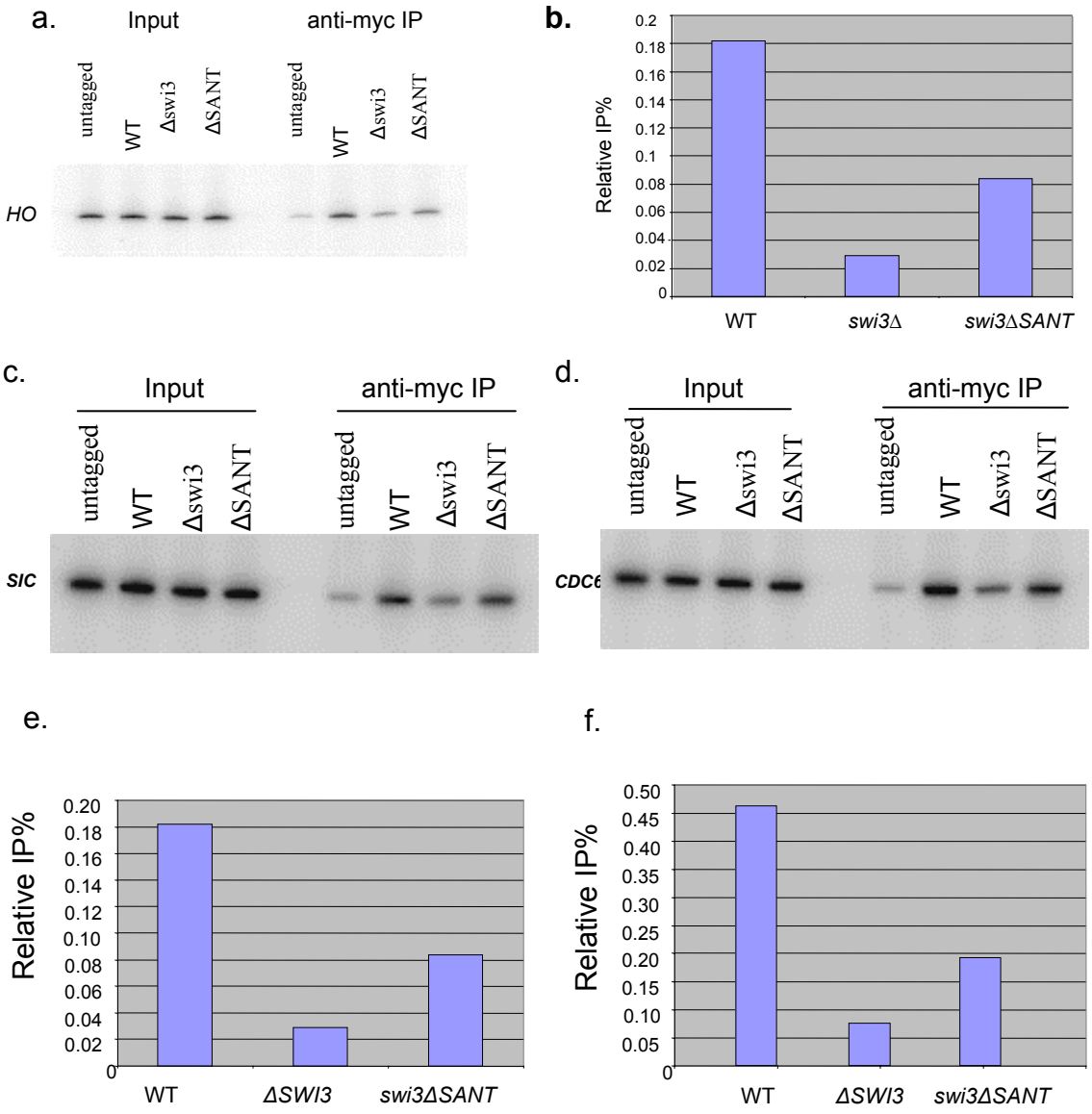

Figure 9. An intact SANT domain of Swi3p is required for recruitment of SWI/SNF to target promoters. (a, c, e) Representative gels of PCR products from chromatin immunoprecipitation (ChIP) analyses from asynchronized cells. The C-terminus of Swi2p was tagged by 13 tandem repeats of myc epitope tag in isogenic WT, *Δswi3* and *swi3ΔSANT* strains. A monoclonal antibody against myc was used for IP. Untagged WT strain served as background control. Primer sets from the *HO*, *CDC6* and *SIC1* promoter regions produced ~200bp DNA products. Primer sets from *ACT1* ORF region served as internal control (gel not shown). **(b, d, f)** Plot showing relative IP% quantified from (a, c, e) gels respectively, after subtracting the untagged signal from IP signal, then normalized by signal from ACT1 ORF.

Since several SWI/SNF dependent genes, including *HO* are cell cycle regulated, ChIP analyses were also performed in nocodazole-synchronized cells to enrich the recruitment signals. WT and *swi3*Δ*SANT* strains were grown in YEPD rich media to $O.D._{600}$ of 0.6, and were synchronized for two hours in the presence of 1 mM nocodazole at 30°C. G2/M arrest was indicated by the appearance of large buds in over 95% of the population. After synchronization, cells were washed and re-suspended in fresh YEPD media. Based on previous observations, WT cells began to exit G2/M phase 30-45 minutes after release, and most cells entered S phase 120 minutes after release (Krebs, et al., 1999). Therefore, a time course over 135 minutes was taken after cells were released from G2/M phase for ChIP assay. As shown in Figure 10, the maximal recruitment of SWI/SNF was significantly enriched at the *HO* promoter, compared with ChIP signals in asynchronous cells. In WT, SWI/SNF was recruited to the promoter ~60 minutes after released from G2/M phase, which is consistent with the timing of *HO* transcription (Krebs, et al., 1999). Maximal recruitment of SWI/SNF was observed between 60-120 minutes after release from G2/M (Figure 10b, right panel and Figure 10c). Dissociation of SWI/SNF from the promoter was observed ~120 minutes after release from G2/M phase in WT (Figure 10b right panel, and Figure 10c). In the absence of the SANT domain, recruitment of SWI/SNF to the promoter was severely impaired at each time point (Figure 10b left panel, and Figure 10c). The synchronized ChIP data supports the hypothesis that the SANT domain may be required for the binding or stability of SWI/SNF at the promoter region.

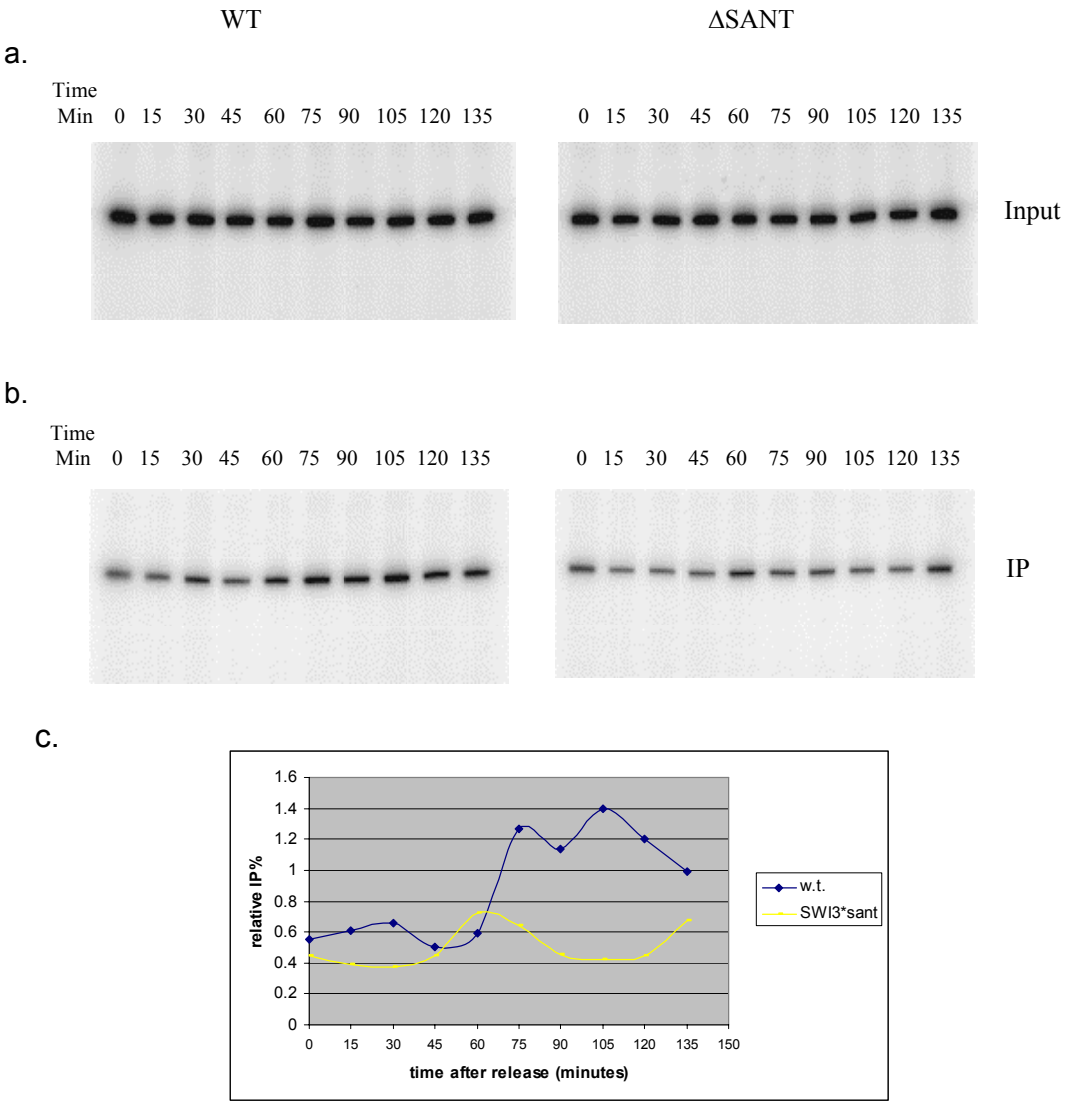

Figure 10. The SANT domain of Swi3p is required for SWI/SNF recruitment to the *HO* promoters in nocodazole-synchronized cells. (a) Gels represent PCR products from input DNA in synchronized ChIP analyses from the same WT (right panel) and *swi3ΔSANT* (left panel) strain described in Figure 8. When sells grew to $O.D._{600}$ of 0.6, 1 mM nocodazole (final concentration) was added directly into the media for 2 hours. After synchronization, cells were released into fresh YEPD media. An aliquot of cultures was taken from formaldehyde crosslink at times indicated at the top of each panel. **(b)** Gels showing PCR products from IP DNA of WT (right panel) and swi3ΔSANT strain (left panel). **(c)** Relative IP% quantified from gels in panel (a, b).

Tethering SWI/SNF to promoter via LexA-Swi2p is insufficient to activate LacZ reporter gene in vivo

Due to the strong affinity between bacterial repressor LexA DNA-binding domain (DBD) and its cognate DNA, fusion of LexA DBD to Swi2p, Snf6p and Swp73p activates the transcription a LacZ reporter gene that is under the control of a chimeric promoter containing LexA operator and *GAL1* TATA box (Cairns, et al., 1996b; Laurent and Carlson, 1992). Using LexA-Swi2p and LacZ reporter system, we expected that artificial tethering of SWI/SNF might rescue the recruitment defect caused by the Swi3p SANT domain, and that transcription in this assay would not require the SANT domain of Swi3p. To address this possibility, I integrated this ApaI linearized LacZ reporter gene into the chromosome at the *URA3* locus in a *swi3* deletion strain (Figure 11a). High copy expression plasmids of LexA-SWI2 (*HIS3*+), *SWI3* (*LEU2*+), or *swi3ΔSANT* (*LEU2*+) were co-transformed into the LacZ reporter strain. Beta-galactosidase (β-gal) activity assays repeatedly showed that an intact SWI/SNF complex is required for LacZ reporter gene activation. The ATPase activity of Swi2p is also required for beta-galactosidase expression since an ATPase dead mutant of Swi2p, swi2K798A, abolished transcriptional activation of LacZ (Figure 11b). Western blot analysis showed that swi2K798A protein expression level remains the same as WT Swi2p (Figure 11c). In the absence of Swi3p, LexA-Swi2p alone was insufficient for LacZ reporter gene activation (Figure 11b). Unexpectedly, tethering SWI/SNF by LexA-Swi2p in the absence of the SANT domain of Swi3p was not sufficient to activate the LacZ reporter. The inability of swi3ΔSANT to activate the LacZ reporter strongly suggests that the SANT domain of

Swi3p may play other roles for chromatin remodeling although its nucleosome binding property cannot be ruled out from this tethering result.

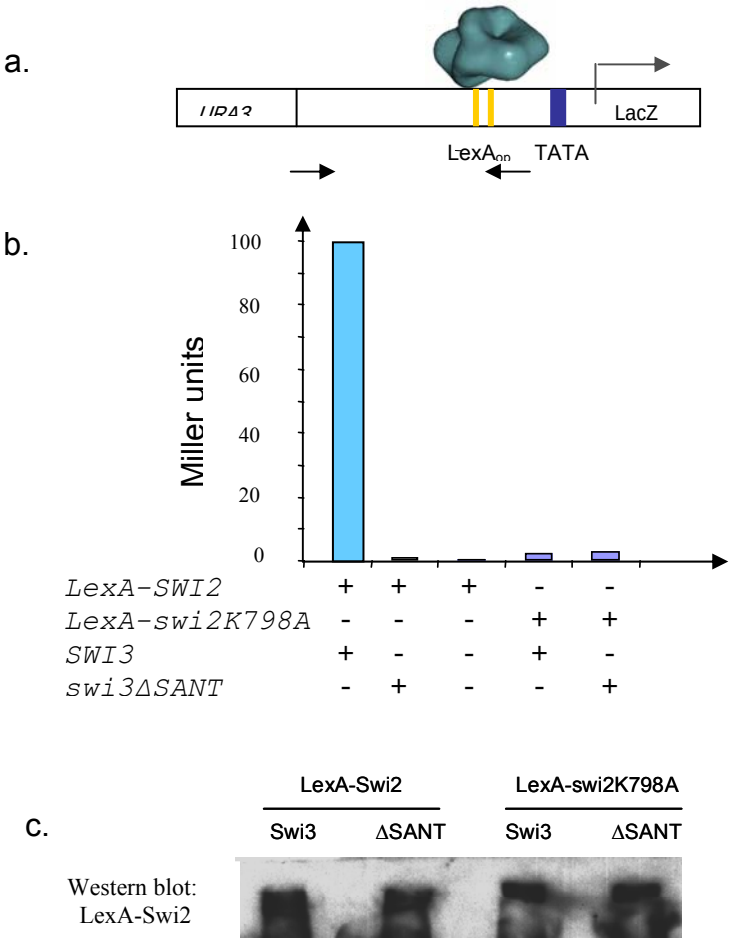

Figure 11. Transcriptional activation of LacZ reporter by artificial tethering of SWI/SNF to the promoter requires an intact SWI/SNF. (a) Schematic of ApaI-linearized LacZ reporter with a promoter containing 2 repeats of LexA binding sites and GAL1 TATA box integrated into chromosome at *URA3* locus in *Δswi3* strain. Arrows represent primer set used for PCR in ChIP analyses. **(b)** Representative beta-gal activity. High-copy expression plasmid of *LexA-SWI2* (*HIS*+) or LexA-swi2K798A was co-transformed with either *SWI3* (WT) or *swi3ΔSANT* (*LEU*+) into the *LacZ* reporter strain. Cells were selected on SC-HIS/-LEU media for plasmid selection. Beta-gal activity was measured from three individual cultures. **(c)** Western blot analysis of LexA-Swi2p showing equal expression of Swi2p and swi2K798A mutant from cells used for beta-gal analyses described above.

In order to confirm that SWI/SNF was tethered by LexA-Swi2p under different conditions, ChIP analysis was performed to check the physical association of SWI/SNF at the LacZ promoter. Polyclonal antibodies against Swi2p, Swi3p or Snf6p were used for parallel ChIP analyses. The results suggest that SWI/SNF can be recruited to the promoter in the wild type strain despite that different antibodies were used for immunoprecipitation (WT, Figure 12), indicating that SWI/SNF complex assembly does not cause epitope masking in these subunits, and each antibody has similar efficiency of immunoprecipitation. Consistent with previous experiments on endogenous promoters, the recruitment of SWI/SNF to Lac Z promoter decreased in all the ChIPs in the absence of an intact SANT domain (ΔSANT, Figure 12). Interestingly, the Swi2p signal only decreased slightly, whereas Swi3p and Snf6p ChIP signals decreased more dramatically in the swi3ΔSANT background (ΔSANT, Figure 12). The difference in recruitment levels between different subunits of SWI/SNF led us to suspect that the stability of the SWI/SNF complex may be impaired in the absence of an intact SANT domain. Taken together, these observations suggest that the SANT domain of Swi3p appears to play a role other than binding the nucleosome substrate.

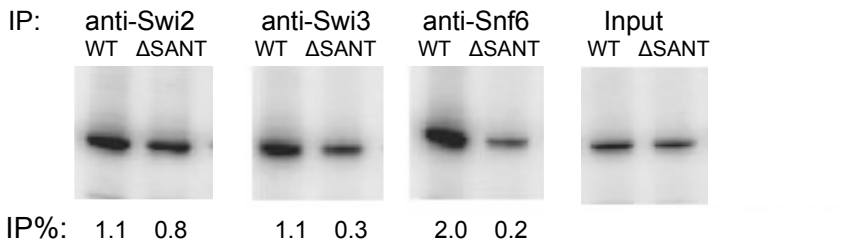

Figure 12. Recruitment of SWI/SNF subunits to LacZ promoter. Gels are representative PCR products of ChIP analyses. Cells used for ChIP are from the same cultures for beta-gal experiments described above. Polyclonal antibody against Swi2p, Swi3p or Snf6p were used for parallel ChIP analyses. Percentage IP was calculated at the bottom of each gel.

The Swi3p SANT domain is required for SWI/SNF assembly

To further characterize the essential role of the SANT domain of Swi3p, we purified the SWI/SNF complex from a *swi3ΔSANT* strain, then checked whether this mutant SWI/SNF impaired the chromatin remodeling activity. To do this, I TAP-tagged the Swi2p subunit at its C-terminus at the endogenous locus of the gene, and used a two-step affinity chromatography technique (Figure 13) to purify SWI/SNF complexes from wild type, *swi3*Δ, and the same *swi3*Δ*SANT* strain that harbors an eleven amino acid deletion within the SANT domain. Consistent with previous studies, purification of the TAP-tagged Swi2p subunit from a wild type strain yielded a SWI/SNF complex composed of 11 subunits, although the small subunits, Snf11p and Swp29p were not stained well by silver (Smith, et al., 2003; Treich, et al., 1995) (lane 1, Figure 14a). Surprisingly, lack of the Swi3p subunit (data not shown) or a small deletion within the

Swi3p SANT domain led to the disassembly of SWI/SNF complex, as only the Arp7p and Arp9p subunits were co-purified with Swi2p (Figure 14a, lane 2). The polypeptide at 150 kDa position was confirmed to be a proteolytic product of Swi2p by mass spectrometry (data not shown). Consistent with a role for the SANT domain in SWI/SNF assembly, the 11 amino acid deletion within the Swi3p SANT domain also altered the elution of Swi2p when whole cell extracts were fractionated on a Superose 6 gel filtration column (L. Boyer and C.L.P., unpublished results). Alterations within the SANT domain do not affect Swi3p expression (Boyer, et al., 2002), and thus Swi3p and an intact Swi3p SANT domain are required for assembly of an intact SWI/SNF complex.

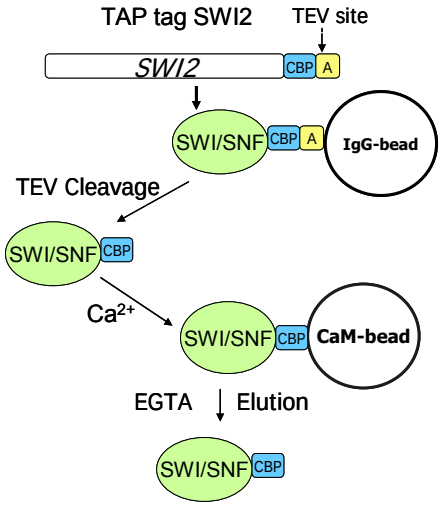

Figure 13. Scheme for TAP-tagged SWI/SNF protein purification. TAP tag containing a calmodulin binding peptide (CBP) and four tandem repeats of protein A (A) was integrated in frame at the C-terminus of Swi2p at chromosome locus. Using two-step affinity chromatography (see details in materials and methods), SWI/SNF complex was purified from different strain background described in the text.

To trace the missing components of SWI/SNF, I generated *TAP-SNF6*, *TAP-SWI1* or *TAP-SNF5* alleles in both wildtype and the same *swi3ΔSANT* strain background. Tandem affinity purifications of these tagged subunits from a wildtype strain repeatedly led to the purification of an intact SWI/SNF complex (Figure 14a, lane 4, 6). In contrast, when these subunits were purified from the *swi3ΔSANT* strain, Snf6p-TAP co-purified with only the Swi3p and Swp73p subunits (Figure 14a, lane 3), and Snf5p-TAP and Swi1p-TAP appeared to purify as single polypeptides (Figure 14a, lane 5, 7). Mass spectrometry analyses verified the subunit composition of each putative subcomplex, and Figure 14b represents a Western blot to confirm the composition of WT and a subcomplex from the TAP-Swi2p preparation from a *swi3ΔSANT* background (Figure 14b, and data not shown).

Since a point mutation of a conserved residue R564E from the putative helix 3 of Swi3p SANT domain also caused a similar growth defect to that of *swi3ΔSANT* (Boyer, et al., 2002), it is conceivable that this residue is critical for the function of the SANT domain, and therefore might also affect the stability of SWI/SNF complex. To test this idea, Swi2p or Snf6p was TAP-tagged in a strain bearing a *swi3R564E* mutation for SWI/SNF purification. As expected, *swi3R564E* point mutation also caused the dissociation of SWI/SNF in the same way as that of a *swi3* deletion or *swi3ΔSANT* (data not shown). The dissociation of SWI/SNF in the absence of an intact SANT domain or Swi3p further suggests that the SANT domain and Swi3p are critical for the assembly of SWI/SNF complex.

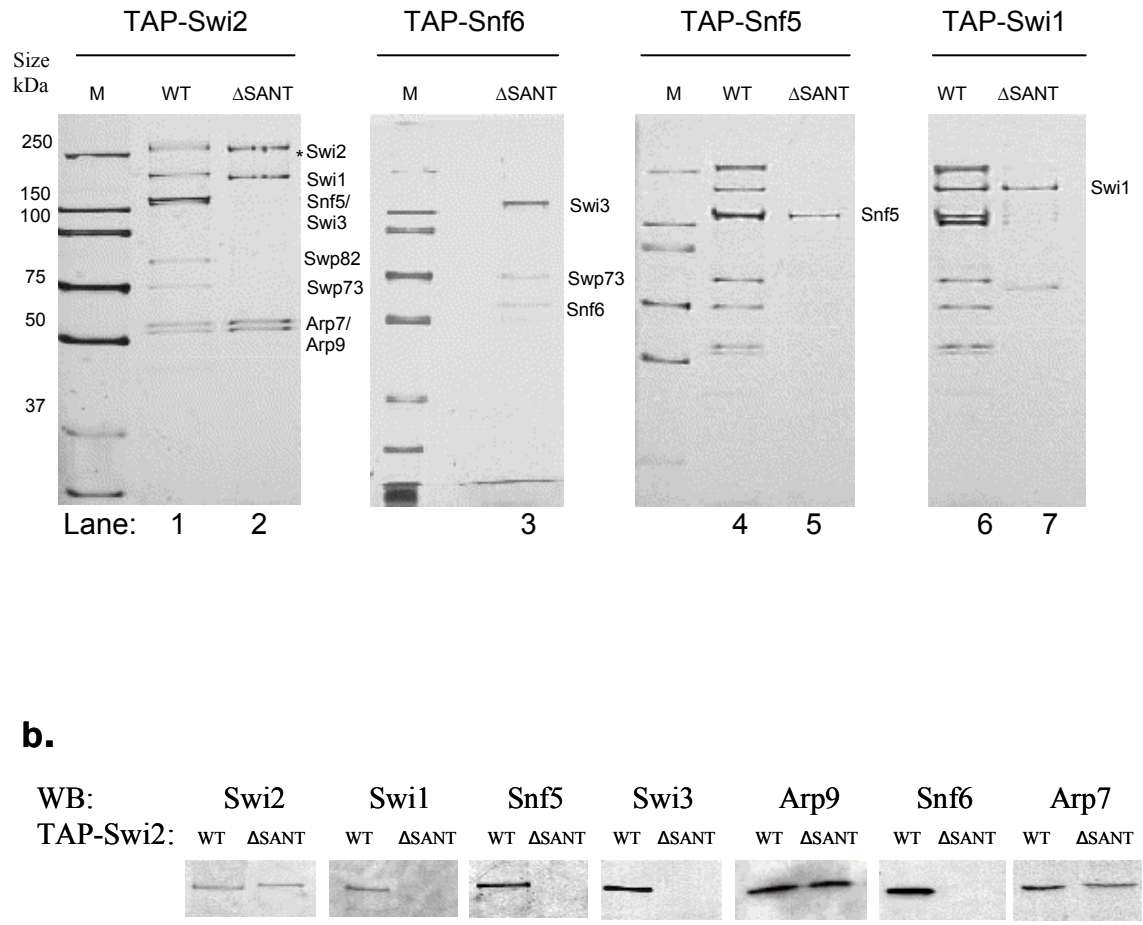

Figure 14. The SANT domain of Swi3p is required for SWI/SNF assembly. (a) TAP-Swi2p, TAP-Snf6p, TAP-Snf5p, or TAP-Swi1p proteins were purified from wild type (WT) or swi3ΔSANT strains (ΔSANT). Approximately one tenth of each preparation was resolved on 8-10% SDS PAGE for silver staining. M, molecular marker. Asterisk (*) represents Swi2p breakdown product. **(b)** Western blot analyses of TAP-Swi2p preparations purified from WT or *swi3ΔSANT* strains, using polyclonal antibodies specific for subunits indicated at the top of each panel.

SWI/SNF is an integration of several modules

Since SWI/SNF dissociated into small subcomplexes in the absence of Swi3p or the SANT domain, and none of the subcomplexes share any subunits, we wondered whether the deletion of other subunits would also cause the dissociation of SWI/SNF into the same subcomplexes. To test this, I purified Snf5p-TAP from a Δ*swi2* strain, Snf5p-TAP from a *swi1*Δ strain, Snf6p-TAP from a *snf5*Δ strain, and Snf6p-TAP from a Δ*swi3* strain (summarized in Table 6, data not shown). As expected from earlier studies (Peterson, et al., 1994), deletion of core subunit of SWI/SNF led to disassembly of SWI/SNF, and all of the data are fully consistent with an organization of SWI/SNF that involves the interdependent assembly of four distinct subcomplexes – (1) Swi2p/Arp7p/Arp9p, (2) Swi3p/Swp73p/Snf6p, (3) Snf5p, and (4) Swi1p.

Table 6. TAP-SWI/SNF complexes from different strain background.

Strain / TAP-	666 WT	667 Δswi3	669 ΔSANT	575 Δsnf5	119 Δswi1	678 swi3Δ4N	679 swi3Δ5N	694 R564E	407 Δswi2	1316 swi3Δ1N	1317 swi3Δ2N
Swi2	+ WT	+ mini	+ mini			+ mini	+ mini	+ mini			
Snf6	+ WT	+ No yield	+ Swi3 Swp73 Snf6	+ Swi3 Swp73 Snf6		+ Swi3 Swp73 Snf6	+ Swi3 Swp73 Snf6			+ intact	+ intact
Snf5	+ WT	+ Snf5	+ Snf5		No yield				+ Snf5		
Swi1	+ WT		+ Swi1								

DISCUSSION

In this part of study, I demonstrated that impairment of the SANT domain of Swi3p subunit changed the genome-wide transcriptional profile and crippled the recruitment of SWI/SNF to target promoters. However, tethering SWI/SNF via LexA-Swi2p was unable to rescue the recruitment defect caused by deletion of the SANT domain in *$LexA_{op}$-$GAL1_{TATA}$-LacZ* reporter assay, indicating that the SANT domain plays a role other than recruitment. From a series of SWI/SNF high quality purifications by TAP, we found the SANT domain of Swi3p is required for the integrity of SWI/SNF complex. More notably, SWI/SNF complex consists of at least four subcomplexes that dissociate in the absence of the SANT domain or any other core subunits: 1) Swi2p/Arp7p/Arp9p; 2) Swi3p/Swp73p/Snf6p; 3) Swi1p; and 4) Snf5p. This result strongly suggests that the SANT domain of Swi3p mediates protein-protein interactions that are required for SWI/SNF assembly, and the subcomplexes reveal the modular architecture of the SWI/SNF complex.

General role of SANT domains

Although the SANT domain was identified based on the homology to the c-myb DNA binding domain, in several cases it seems to bind histone tails rather than DNA (Boyer, et al., 2002; Boyer, et al., 2004; Ding, et al., 2004; Humphrey, et al., 2001). In *Saccharomyces cerevisiae,* the SANT domain of Ada2p is critical for histone H3 tail interaction (Boyer, et al., 2002). Interestingly, the C-terminal half or the whole SANT

domain of Ada2p is also required for the assembly of SAGA complex (Candau and Berger, 1996; Sterner, et al., 2002). In contrast, the N-terminal half of the SANT domain of Ada2p is not required for the assembly of SAGA complex, but is important for Gcn5p HAT activity (Sterner, et al., 2002). In mammalian cells, two tandem repeats of SANT domains of nuclear hormone receptor corepressors SMRT appear to have different functions as well. The N-terminal SANT domain of SMRT is required for histone deacetylase HDAC3 activity, whereas the C-terminal SANT is important for unacetylated histone tail interaction (Yu, et al., 2003). Drosophila ISWI also contains a SANT and a SANT-like domain (SLIDE) in its C-terminal region. The overall negative charge on the surface of the helices of ISWI SANT domain suggests that this SANT domain may be a candidate for interaction with positively charged histone tails (Grune, et al., 2003). However, the C-terminal SLIDE domain of ISWI seems to have DNA binding property, and is required for both DNA binding and ATPase activity of ISWI (Grune, et al., 2003). In this study, we found that deletion of 11 amino acids or R564E point mutation within the third putative helix of Swi3p SANT domain resulted in the disassembly of yeast SWI/SNF complex, supporting that the SANT domain of Swi3p is critical to mediate non-histone protein-protein interactions that are required for SWI/SNF assembly. The important role of Swi3R564 for Swi3p function led us to speculate that this arginine residue may be important for the folding of the putative helical structure of the SANT domain. When aligned with the sequence of ISWI SLIDE domain, the arginine residue (R955) within the third helix of ISWI SLIDE domain likely corresponds to Swi3R564. In X-ray crystal structure, ISWIR955 is located at the external region of the third helix. An intermolecular aspartic acid (ISWID907) is in close contact with R955 and may form an

ionic interaction that is critical for protein folding (Figure 15). These features might help us to understand the importance of the third helix of Swi3p SANT domain. Taken together, the SANT domains may in general mediate protein-protein interactions for large protein complex assembly and histone tail interactions to coordinate core enzymatic activity of chromatin modifying complexes on nucleosomal substrates.

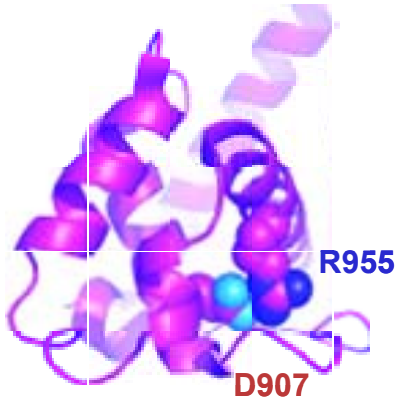

Figure 15. Crystal structure of ISWI SANT-like domain (SLIDE) (Grune, et al., 2003). Arginine residue (R955) within the third helix of SLIDE domain is in close contact with an aspartic acid (D907) that is buried under the helices. These opposite ionic residues seem to be critical for protein folding.

Structural and functional organization of SWI/SNF complex

SWI/SNF subcomplexes were purified from whole cell extract in the presence of high concentration of salt (350 mM NaCl), indicating the tight association of different subunits within these modules. Interestingly, extreme ionic strength did not cause the disassembly of WT SWI/SNF complex in vitro. Like some other large protein complexes, SWI/SNF chromatin remodeling machinery seems to be also constituted from distinct stable subcomplexes. The subcomplex composition may reveal the distinct function of each subunit.

Since SWI/SNF binds both DNA and nucleosome with low affinity and without sequence specificity, it has been proposed that Snf5p and Swi1p subunits may be partially redundant recruiters that recruit SWI/SNF to promoter via direct interaction with acidic activators (Narlikar, et al., 2002; Peterson and Workman, 2000; Prochasson, et al., 2003). It is interesting to find that native Swi1p or Snf5p proteins were purified as a monomer when SWI/SNF is disassembled. In the absence of the SANT domain of Swi3p, the dissociation of Swi1p and Snf5p from SWI/SNF complex might explain the recruitment defect of the SWI/SNF to most target promoters, therefore resulting in genome-wide transcriptional defect of target genes and growth phenotype. Due to the important role of mammalian SWI/SNF in tumor suppression (Roberts, et al., 2002) and in DNA double-strand break (DSB) repair (Chai, et al., 2005; Jaskelioff, et al., 2003; Morrison and Shen, 2006; Park, et al., 2006), it remains very important to characterize the role of each subunit for SWI/SNF functions.

Although the contribution of actin-related proteins (ARP) in chromatin remodeling is unclear, the ubiquitous existence of Arp proteins in chromatin remodeling complexes

and their tight association with the catalytic subunit suggests that they are structurally and/or functionally important for chromatin remodeling enzymes in vivo (Peterson, et al., 1998). It has been shown that purified INO80 complex in the absence of Arp5p or Arp8p is intact but has compromised ATPase activity and chromatin remodeling activity (Shen, et al., 2003). For yeast Swi2p, we found that it is physically associated with Arp7p and Arp9p when SWI/SNF falls apart in vivo, suggesting that the role of Arp proteins may be associated with the catalytic subunit of SWI/SNF. However, for yeast RSC complex, temperature sensitive mutant of Arp7p or Arp9p does not affect the assembly of RSC complex or the ATPase activity of RSC (Szerlong, et al., 2003). Therefore, the precise role of ARPs remains to be investigated.

Collectively, large protein complex of SWI/SNF integrates distinct functional and architectural modules of proteins, and each module seems to play a distinct role during multi-step chromatin remodeling process. The initial consequence of ATP hydrolysis by Swi2p is to disrupt histone-DNA contacts, which facilitates the change in nucleosome configuration through sliding histone octamer or nucleosome mobilization (Cote, et al., 1998; Phelan, et al., 2000). Depending on unknown properties of DNA sequence, histone H2A/2B dimer loss may occur following the disruption of DNA-histone interactions (Bruno, et al., 2003; Vicent, et al., 2004). Conceivably, SWI/SNF chromatin remodeling machinery may use different mechanism to make promoter more accessible for the binding of other regulatory factors. Different subcomplex may be required before, during, or after chromatin remodeling. Although the precise role of each subcomplex remains to be investigated, the dissociation of SWI/SNF complex in the absence of core subunits would disrupt the functional coordination between these modules and may

cause misregulation of target genes. All of these findings provide new insight into the molecular architecture of SWI/SNF complex and distinct function of each gene for ATP-dependent chromatin remodeling.

MATERIALS AND METHODS

Yeast strains and culture. The carboxyl-terminus of Snf2p was tagged with 13 tandem repeats of myc tag at endogenous locus in isogenic CY666 (wildtype *SWI3;* WT), CY667 (*⊿swi3*) and CY669 (*swi3⊿SANT*) strain background. For ChIP analysis, each strain was inoculated into yeast extract-peptone medium containing 2% glucose (YEPD), and grown to an $O.D._{600}$ of ~ 1.0 at 30ºC. Cells were collected by centrifugation at 4 ºC. LexA-*GAL1-LacZ* (*URA3*+) reporter strain (CY1053) was established by integrating ApaI linearized $LexA_{op}$-$GAL1_{TATA}$-*LacZ* reporter gene into chromosome at *URA3* locus in *swi3* deletion background. High copy expression plasmid of *LexA-SWI2* (*HIS3*+) was co-transformed with pRS415-*SWI3* or *swi3⊿SANT* (*LEU2*+) into CY1053 and grown in synthetic complete media lacking amino acids leucine and histidine (SC-HIS-LEU) to maintain the selection for plasmids.

Affymetrix Gene Chip analysis. Isogenic strains of WT, *Δswi3* and *swi3ΔSANT* were inoculated and grown in YEPD rich medium to $O.D._{600}$ of ~0.8. Cells were harvested for "hot phenol" total RNA extraction. Fifteen micrograms of total RNA purified from RNeasy column (QIAGEN) was used for cDNA synthesis (Invitrogen), and cDNA was cleaned up by Phase-lock heavy gel-Phenol/Chloroform Extraction (QIAGEN). Biotinated cRNA was synthesized using synthesized cDNA as template following manufacture's protocol (Enzo), and cleaned up by RNeasy column. cRNA (40 µg) was fragmented based on metal-induced hydrolysis in buffer containing 200 mM Tris-Acetic acid pH8.1, 500 mM

potassium acetate and 150 magnesium acetate in 94ºC water bath for 35 minutes. Hybridization and other steps were followed according to Affymetrix Gene Chip protocol. Three independent experiments were performed. Data analysis was based on fold change (up or down more than 2 fold or otherwise indicated) normalized to WT in all three independent experiments.

Chromatin immunoprecipitation (ChIP). ChIP assays were performed based on protocol described by Orlando *et al* with minor modifications (Orlando, et al., 1997). Briefly, asynchronized or nocodazole-synchronized cells were crosslinked with 1% of formaldehyde at room temperature for 20 minutes and quenched by glycine for 5 minutes. Equal amount of each whole cell extract was used for immunoprecipitation, using antibodies against myc (Santa Cruz), or polyclonal antibody against Swi2p, Swi3p or Snf6p (Gift from Reese lab). DNAs were sheared by sonication 10 seconds each time for a total of 6 times. Each sample was deproteinated in the presence of proteinase K at 50ºC and decrosslinked at 65ºC. Hot PCR reaction was performed in the presence of α-^{32}P-dCTP, using primers spanning target promoter. *ACT1* ORF region primers were used for internal control. Relative IP percentage was quantified by ImageQuant v1.2 (Amersham) after imaged with PhosphorImager (Molecular Dynamics).

Beta-galactosidase activity. Beta-galactosidase (β-gal) activity was detected and reported in Miller units as described everywhere. Three individual colonies were selected from each strain. Data presented was an average of the three independent results. Standard deviation of each group is less than 20%.

TAP-SWI/SNF purification. Isogenic strains of CY666 (WT), CY667 (*Δswi3*) and CY669 (*swi3ΔSANT*) was TAP tagged at the carboxyl-terminus of Snf2p, Swi1p, Snf5p, or Snf6p at endogenous locus of each gene. Each strain was typically inoculated into six liters of yeast extract-peptone medium containing 2% glucose (YEPD), and grown to $O.D._{600}$ approximate to 2.0~3.0 at 30ºC. Cells were harvested by centrifugation at 4ºC. TAP-SWI-SNF complexes were purified as described previously with minor modifications (Puig, et al., 2001). Briefly, yeast whole cell extracts from 6-liter culture were prepared by bead beater (Biospec Products, INC) in cold E buffer containing 20 mM Hepes (pH7.5), 350 mM NaCl, 10% glycerol, 0.1% Tween-20 and protease inhibitors (2 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mM PMSF). Cell lysate was clarified at 40,000 rpm at 4ºC for 1 hour (Beckman Ti45 rotor). Clear supernatant was incubated with rabbit IgG-agarose beads (Sigma) for 2 hours at 4ºC. SWI/SNF was cleaved off the IgG-beads by TEV protease (Invitrogen) overnight at 4ºC, and further incubated with calmodulin affinity beads (Stratagene) in E buffer plus 2 mM $CaCl_2$ for 2 hours at 4ºC. Finally, protein was eluted off the calmodulin beads in E buffer in the presence of 10 mM EGTA. Protein elute was dialyzed in E buffer in the presence of 50 μM $ZnCl_2$. The purity and components of each complex was confirmed by SDS-PAGE gel electrophoresis followed by silver staining or Western blot analysis. For mass spectrometry analysis, each band was sliced off the gel after silver staining.

Western blot analysis. TAP-purified SWI/SNF complexes were resolved into 8-10% SDS PAGE, transferred to cellulose membrane, then the membrane was blocked by 4% milk in TBST, and incubated with rabbit polyclonal IgG primary antibody against each

subunit of SWI/SNF (J. Reese lab gift) or Lex-A (Santa Crutz) overnight at 4ºC. On the second day, each membrane was washed 3 times with TBST and incubated with secondary antibody for 1 hr at 4ºC. Finally, proteins were visualized by developing the membrane in SuperSignal substrate (PIERCE).

Acknowledgement

I would like to thank Dr. Feng He from Dr. Allan Jacobson lab of UMASS Medical School for his help in Affymetrix microarray data analysis. We are also grateful to Dr. J Reese lab for their generous gift of polyclonal antibodies of Swi2p, Swi3p, and Snf6p.

CHAPTER III

MODULAR AND FUNCTIONAL ORGANIZATION OF YEAST SWI/SNF COMPLEX

SUMMARY

Yeast SWI/SNF is a multi-subunit, 1.14 MDa ATP-dependent chromatin remodeling enzyme required for transcription of a subset of inducible genes. Biochemical studies have demonstrated that SWI/SNF can use the energy from ATP hydrolysis to generate superhelical torsion, mobilize mononucleosomes, enhance the accessibility of nucleosomal DNA, and remove H2A/H2B dimers from mononucleosomes. In this chapter, we characterized the ATP-dependent chromatin remodeling activities of a SWI/SNF subcomplex that is composed of only three subunits, Swi2p, Arp7p, and Arp9p. Whereas this subcomplex is fully functional in most remodeling assays, Swi2p/Arp7p/Arp9p is defective for ATP-dependent removal of H2A/H2B dimers. We identified the acidic N-terminus of the Swi3p subunit as a novel H2A/H2B binding domain required for ATP-dependent dimer loss. Our data indicate that H2A/H2B dimer loss is not an obligatory consequence of ATP-dependent DNA translocation, and furthermore they suggest that SWI/SNF is composed of at least four interdependent modules.

Figure 19c,d in this chapter were provided by Roser Zaurin of Dr. Miguel Beato's group from the Centre for Genomic Regulation of Spain as a collaborator to confirm the dimer loss defect by in vitro chromatin immunoprecipitation.

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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. Two classes of highly conserved chromatin remodeling enzymes have been implicated in modulating the repressive nature of chromatin structure. The first class includes enzymes that covalently modify the nucleosomal histones (acetylation, phosphorylation, methylation, ubiquitylation, etc; reviewed by Strahl and Jenuwein) (Jenuwein and Allis, 2001; 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 (Narlikar, et al., 2002).

The *Saccharomyces cerevisiae* SWI/SNF complex is a prototype for ATP-dependent chromatin remodeling enzymes. Yeast SWI/SNF is a multiprotein complex that consists of eleven subunits with a molecular weight of 1.14 mega Dalton (Cairns, et al., 1996b; Peterson, et al., 1994; Smith, et al., 2003). This widely conserved assembly is required for the inducible expression of a number of diversely regulated yeast genes and for the full functioning of many transcriptional activators. SWI/SNF can be recruited to target genes via direct interactions with gene-specific activators, and in several cases SWI/SNF facilitates the binding of activators to nucleosomal sites in vivo (Cosma, et al., 1999; Neely, et al., 2002; Peterson and Workman, 2000; Prochasson, et al., 2003). 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 precise mechanism of SWI/SNF action is not clear, recent single molecule studies have suggested a model in which SWI/SNF uses a DNA “pumping” mechanism to generate transient DNA loops on the histone octamer surface (Mihardja, et al., 2006; Shundrovsky, et al., 2006). This ATP-dependent DNA translocation model is consistent with the ability of SWI/SNF to use the energy of ATP hydrolysis to generate superhelical torsion, mobilize nucleosomes, and enhance nucleosomal DNA accessibility. SWI/SNF remodeling can also lead to the removal of one or both histone H2A/H2B dimers from a mononucleosome substrate, and this reaction is sensitive to the underlying sequence of nucleosomal DNA (Bruno, et al., 2003; Vicent, et al., 2004). Whether this dimer loss reaction is simply an indirect consequence of the DNA translocation reaction is not known.

The catalytic subunit of yeast SWI/SNF is Swi2p/Snf2p which is the founding member of a subfamily of the SF2 superfamily of DNA-stimulated ATPases/helicases (Flaus, et al., 2006). A human homolog of Swi2p/Snf2p, BRG1, is the catalytic subunit of the human SWI/SNF complex, and the isolated BRG1 subunit is able to alter histone-DNA interactions on a mononucleosome substrate (Phelan, et al., 2000; Phelan, et al., 1999). If the ATPase subunit is sufficient for ATP-dependent remodeling, what role(s) do the other subunits play? Two subunits, Swi1p and Snf5p, interact with the acidic activation domains of gene-specific activators, and these interactions are essential for recruitment of SWI/SNF to target loci (Prochasson, et al., 2003). Early studies demonstrated that at least four subunits of SWI/SNF are required for assembly of the

Swi2p/Snf2p subunit into a high molecular weight complex (Swi1p, Swi3p, Snf5p, Snf6p), and inactivation of these same subunits leads to phenotypes identical to those of a *swi2* mutant (Peterson, et al., 1994; Peterson and Herskowitz, 1992). In contrast, the Swp82p (Wilson, et al., 2006), Swp29p (Cairns, et al., 1996a), and Snf11p (Treich, et al., 1995) subunits do not appear to play essential roles for SWI/SNF function in vivo.

The Swi3p subunit of SWI/SNF contains a conserved SANT domain which functions as a histone N-terminal tail interaction module for yeast Ada2p (Boyer, et al., 2002), a subunit of multiple histone acetyltransferase complexes (Grant, et al., 1997). Previously we demonstrated that an 11 amino acid deletion within the Swi3p SANT domain (*swi3*Δ*SANT*) yielded *swi/snf* mutant phenotypes, suggesting that this domain plays a key role in SWI/SNF function (Boyer, et al., 2002). In chapter II, I described the purification of SWI/SNF from this *swi3*Δ*SANT* strain, and showed that this small deletion leads to the dissociation of SWI/SNF into at least four subcomplexes: (1) Swi2p/Arp7p/Arp9p; (2) Swp73p/Swi3p/Snf6p; (3) Snf5p; and (4) Swi1p. Purification of SWI/SNF from strains that lack the entire Swi3p, Snf5p, or Swi1p subunits supports the view that these four subcomplexes define a modular organization of SWI/SNF. In this chapter, I characterized the function of Swi2p/Arp7p/Arp9p subcomplex, and demonstrated that it has robust ATPase and chromatin remodeling activities that are equivalent to that of intact SWI/SNF complex. The one exception, however, is that the Swi2p/Arp7p/Arp9p subcomplex is defective for catalyzing histone H2A/H2B dimer loss. We show that dimer loss requires an acidic N-terminal domain of the Swi3p subunit that binds specifically to histone H2A and H2B tails in vitro. These data indicate that the

Swi3p subunit provides a histone chaperone function that is essential for efficient removal of histone H2A/H2B dimers during ATP-dependent chromatin remodeling.

RESULTS

The Swi2p/Arp7p/Arp9p subcomplex has ATP-dependent chromatin remodeling activity

In chapter II, I found that SWI/SNF complex dissociates into 4 subcomplexes in the absence of the Swi3p SANT domain, and Swi2p is associated with Arp7p and Arp9p. Since isolated human BRG1 and hBRM has ATPase and chromatin remodeling activities, we expected that Swi2p/Arp7p/Arp9p subcomplex might be also having enzymatic activity. First, we tested both DNA and nucleosome-stimulated ATPase activity of each subcomplex that was purified from *swi∆SANT* or ∆*swi3* strains. Not too surprising, only the Swi2p/Arp7p/Arp9p subcomplex had detectable ATPase activity, and its DNA-stimulated (Figure 16) and nucleosome-stimulated ATPase activity (data not shown) were identical to that of intact SWI/SNF. We further investigated the ATP hydrolysis kinetics of the Swi2p/Arp7p/Arp9p subcomplex. The initial velocities of the ATPase reaction were determined at different ATP concentrations from the slope of linear ATP hydrolysis plots. Velocities were plotted as a function of ATP substrate concentration and fitted into the Michaelis-Menten equation (Figure 17a). The kinetic parameters averaged from three independent experiments showed that intact SWI/SNF exhibits a Km of 231.9±32.4 μM and a V_{max} of 3.1±0.2 μM/minute, and the Swi2p/Arp7p/Arp9p subcomplex shows a Km of 242.6±47.6 μM and V_{max} of 3.0±0.2 μM/minute (Figure 17b). These results further confirm that the ATPase activity of this "minimal" complex is identical to that of intact SWI/SNF.

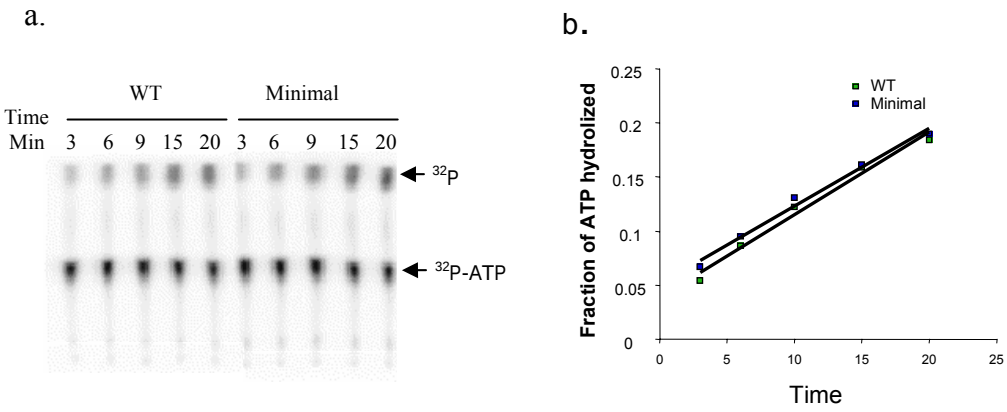
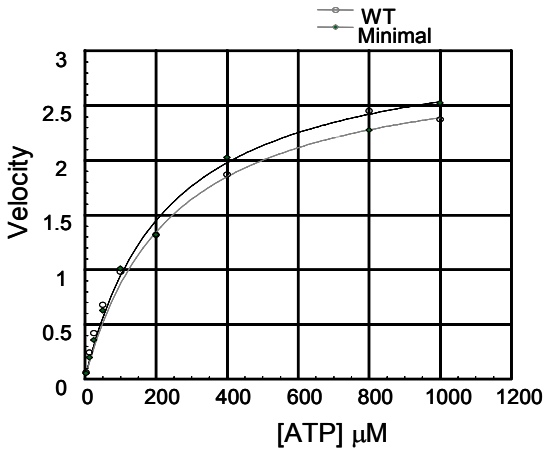


Figure 16. Swi2p/Arp7p/Arp9p subcomplex has ATPase activity. (a) DNA-stimulated ATPase activity of intact SWI/SNF (WT) and Swi2p/Arp7p/Arp9p minimal complex (Minimal) by Thin Layer Chromatography (TLC) as described in Methods. Each reaction contained 5 nM remodeling enzyme and 1µg plasmid DNA as nucleic acid cofactor. **(b)** Quantification of fraction of ATP hydrolyzed by WT and the minimal SWI/SNF complexes from left panel.

a.



b.

W.T.		
	Value	Error
Vmax	3.7156	0.19484
Km	214.62	32.957
Chisq	0.148	NA
R	0.99368	NA

Minimal		
	Value	Error
Vmax	4.0884	0.23281
Km	224.85	47.356
Chisq	0.10826	NA
R	0.99557	NA

Figure 17. ATP hydrolysis Kinetic analysis of WT and the minimal SWI/SNF complexes. (a) Initial velocities of each enzyme were calculated based on linear ATP hydrolysis at a fixed concentration of enzyme under a series of ATP concentrations from 3.125 μM to 1000 μM. Velocities were plotted as a function of ATP concentration and fitted to Michaelis-Menten equation by KaleidaGraph. Plots represent the average of three independent experiments, with standard deviation less than 15%. **(b)** Kinetic parameters of WT and the minimal SWI/SNF complexes.

Swi2p/Arp7p/Arp9p is an active chromatin remodeling enzyme

Since Swi2p/Arp7p/Arp9p has ATPase activity that is identical to that of intact SWI/SNF, we asked whether this subcomplex could couple ATP hydrolysis to chromatin remodeling. First, we tested whether the Swi2p/Arp7p/Arp9p subcomplex can generate superhelical torsion on a DNA substrate in an ATP-dependent reaction. Previous work from Owen-Hughes and colleagues indicated that the generation of superhelical torsion is a basic feature of ATP-dependent chromatin remodeling enzymes, and this activity is likely to reflect the ATP-dependent translocation of DNA (Havas, et al., 2000). In these assays, we used a linear, ^{32}P-labeled DNA template that contains an inverted AT-rich sequence that is extruded into a cruciform structure by ATP-dependent formation of superhelical torsion by remodeling enzymes (Smith and Peterson, 2005b) (Figure 18a). Formation of the cruciform can be detected by T4 endonuclease VII cleavage. Similar to previous results (Havas, et al., 2000; Smith and Peterson, 2005b), WT SWI/SNF was able to catalyze cruciform extrusion from naked DNA template as illustrated by the time-dependent T4 endonuclease VII cleavage of the DNA into two small fragments (Figure 18b, WT). Likewise, equal amounts of the Swi2p/Arp7p/Arp9p subcomplex provided equivalent rates of ATP-dependent cruciform formation (Figure 18b, Minimal), suggesting that this subcomplex is fully functional in this DNA-based remodeling reaction. Both WT and the minimal SWI/SNF complexes were capable of catalyzing cruciform protrusion on the same DNA assembled into nucleosome (Figure 18c).

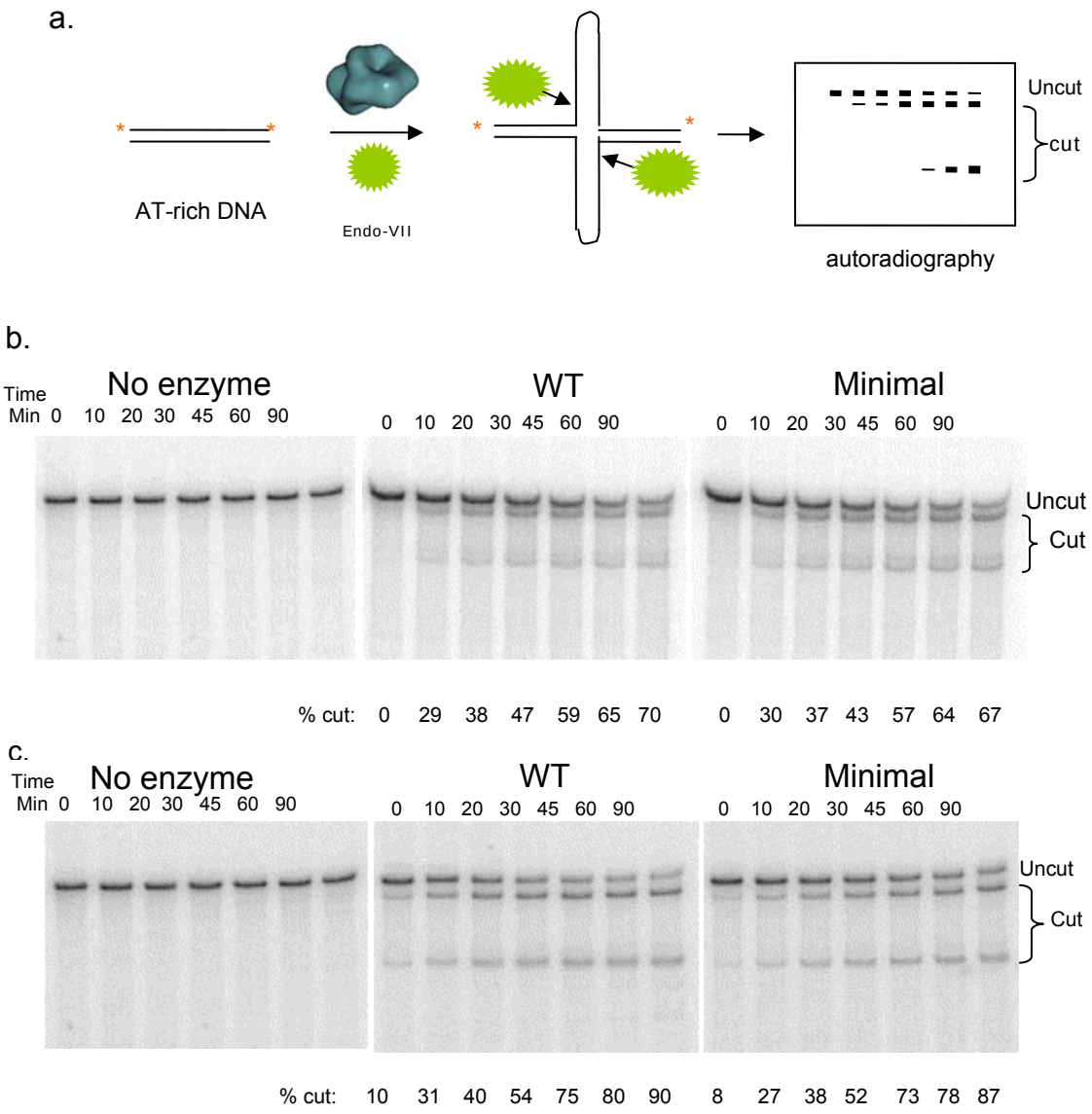
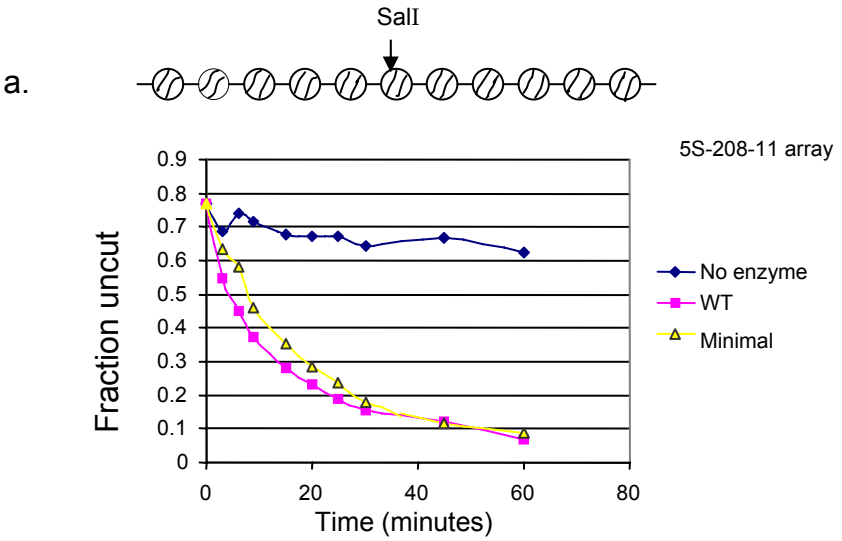


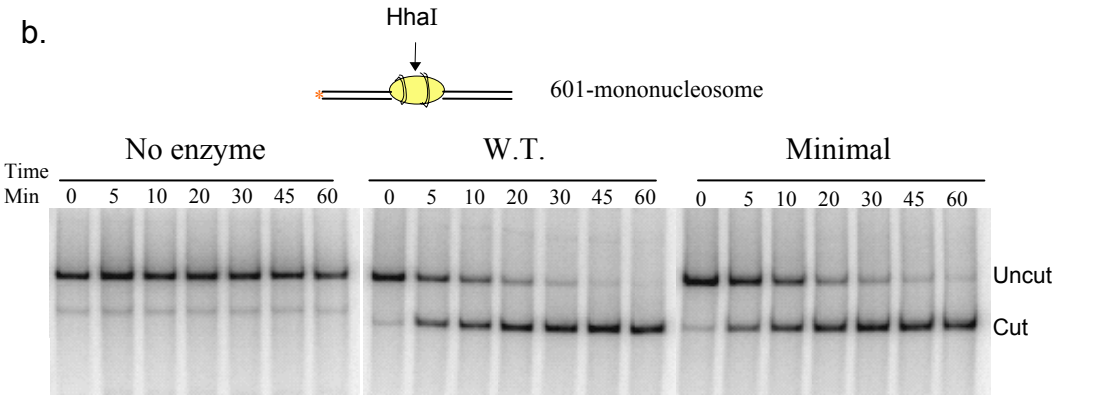
Figure 18. Swi2p/Arp7p/Arp9p subcomplex is capable of generating superhelical torsion. (a) Schematic of DNA cruciform formation assay. **(b)** The ability to generate DNA superhelical torsion was tested on AvaI-linearized pXG540 template that contains an inverted (AT)34 repeat. Reactions containing 1.5 nM WT or the minimal SWI/SNF, 0.1 nM ^{32}P-labeled linearized pXG540, 3 mM ATP, 0.15 mg/ml Endo VII in a 30-µl reaction were incubated at 30°C for 90 minutes. An aliquot of each sample was taken at time points denoted, quenched, deproteinated and electrophoresed on 4% native gel. Data shown is representative of multiple experiments. **(c)** Similar cruciform formation analysis by WT and the minimal SWI/SNF using nucleosome assembled at linearized pXG540 template.

A hallmark of ATP-dependent remodeling enzymes is the ability to use the energy from ATP hydrolysis to increase restriction enzyme accessibility on mononucleosome or nucleosomal array substrates. To investigate the functioning of the Swi2p/Arp7p/Arp9p subcomplex in this type of assay, we used stepwise salt dialysis to reconstitute ^{32}P-labeled nucleosomal arrays with recombinant Xenopus laevis histone octamers and a DNA template that consists of 11 tandem repeats of a 5S rDNA nucleosomal positioning (Logie and Peterson, 1999). In these arrays, the central nucleosome positioning sequence contains a unique SalI restriction enzyme recognition site that is occluded by nucleosome assembly (Logie and Peterson, 1997) (Figure 19a, top scheme). In the presence of intact SWI/SNF and ATP, SalI digestion kinetics was dramatically enhanced (WT, Figure 19a). Surprisingly, the Swi2p/Arp7p/Arp9p subcomplex showed equivalent activity in this assay (Minimal, Figure 19a). Similar results were obtained when the remodeling enzymes were present at stoichiometric or substoichiometric levels with respected to nucleosomes.

Restriction enzyme accessibility assays were also performed for mononucleosome substrates. Mononucleosomes were reconstituted onto a ^{32}P-labeled 343 bp DNA fragment that harbored the “601” nucleosome positioning sequence (Thastrom, et al., 1999). A unique HhaI restriction enzyme site is located close to the nucleosomal dyad and is occluded by nucleosome assembly. In the absence of SWI/SNF, there was little HhaI digestion of the mononucleosome substrate, whereas addition of SWI/SNF and ATP led to more than 90% cleavage of nucleosomal DNA by 60 minutes (Figure 19b). Likewise, the Swi2p/Arp7p/Arp9p subcomplex showed equivalent activity in this assay (Figure 19b).

One of the mechanisms by which SWI/SNF catalyzes chromatin remodeling is by mobilizing a histone octamer bidirectionally towards the ends of a DNA template (Kassabov, et al., 2003; Shundrovsky, et al., 2006). These end-positioned nucleosomes migrate faster than the original, centrally positioned species after native gel electrophoresis (Flaus and Owen-Hughes, 2003). The “601” mononucleosome substrates were also used for mobilization assays. In this case, mononucleosomes were remodeled by SWI/SNF or the Swi2p/Arp7p/Arp9p subcomplex, the remodeling enzyme was then removed by competition with unlabeled DNA, and then the nucleosome products were electrophoresed on a native polyacrylamide gel. As shown in Figure 18c, both the intact and Swi2p/Arp7p/Arp9p subcomplexes were able to generate a faster migrating mononucleosome species with similar kinetics. Taken together, these different approaches demonstrate that the Swi2p/Arp7p/Arp9p subcomplex is a robust chromatin remodeling enzyme, and that the other eight SWI/SNF subunits do not contribute significantly to these remodeling activities.


a.
SalI
5S-208-11 array
Fraction uncut
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
Time (minutes)
No enzyme
WT
Minimal


b.
HhaI
601-mononucleosome
No enzyme
W.T.
Minimal
Time
Min
0 5 10 20 30 45 60
0 5 10 20 30 45 60
0 5 10 20 30 45 60
Uncut
Cut

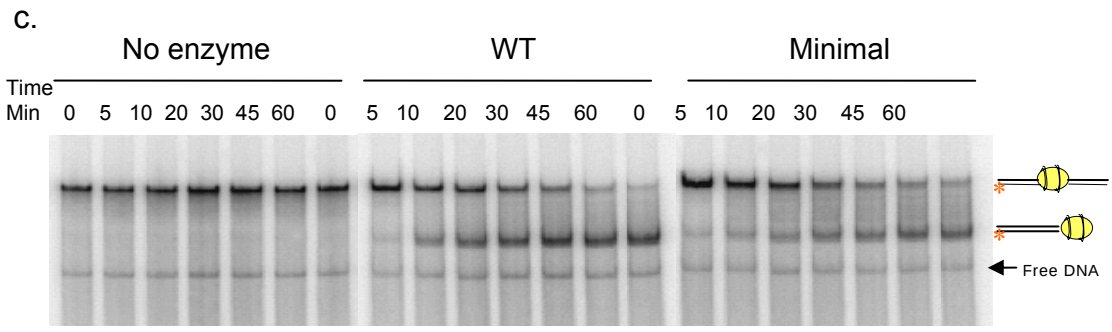

c.
No enzyme
WT
Minimal
Time
Min
0 5 10 20 30 45 60 0
5 10 20 30 45 60 0
5 10 20 30 45 60
Free DNA

Figure 19. Chromatin remodeling activity of the minimal SWI/SNF complex. (a) Schematic of 5S-208-11 nucleosomal array substrate on top. Plot showed fraction of nucleosomal DNA remains uncut over 60-minute time course in the presence of 1 nM of WT or the minimal subcomplexes, 2 nM nucleosomal array, 1 mM ATP, and 10 Units of SalI restriction enzyme. Closed diamonds, no remodeling enzyme; closed squares, WT; open triangles, the minimal complex. **(b)** Schematic of 343 bp 601-mononucleosome substrate on top. Reactions containing 1 nM WT or the Minimal SWI/SNF, 2 nM radiolabeled 601-mononucleosome, 1 mM ATP and 10 Units of HhaI restriction enzyme. Samples were taken at given times indicated, quenched, deproteinated, and resolved on 8% TBE native gels. Gel shown is representative of multiple experiments. Hha I accessibility catalyzed by WT and the minimal SWI/SNF. **(c)** 601-mononucleosomal mobility assay. Similar reaction as in (b). Samples were taken at times indicated, incubated with excess dsDNA and glycerol to remove SWI/SNF, and electrophoresed on 4% native TBE gels. Schematics to the right denote predicted nucleosome positions (positions have not been experimentally determined).

Histone H2A/H2B dimer displacement requires an intact SWI/SNF complex

Recent studies of Bruno and colleagues showed that SWI/SNF can catalyze the displacement of histone H2A/H2B dimers from a nucleosomal substrate (Bruno, et al., 2003). Subsequently, it was shown that this dimer displacement activity is sensitive to the underlying sequence of the nucleosomal substrate; SWI/SNF shows potent dimer displacement activity with a MMTV mononucleosome, but no appreciable dimer displacement activity is detected when a rDNA (Vicent, et al., 2004) or "601" mononucleosome (Shundrovsky, et al., 2006) is used as a substrate. Interestingly, SWI/SNF-dependent H2A/H2B dimer displacement appears to play a key role in the activation of the MMTV promoter in vivo by progesterone receptor (Vicent, et al., 2004).

To quantify the efficiency of dimer displacement by intact and minimal SWI/SNF complexes, we used a strategy developed by Owen-Hughes group (Bruno, et al., 2003) in which we reconstituted MMTV mononucleosomes with recombinant histone octamers that harbored an Oregon Green fluorescent group covalently coupled to a unique cysteine residue on the C-terminal tail of histone H2A (H2A-S113C). After electrophoresis on a 4% native polyacrylamide gel, reconstituted MMTV-mononucleosomes migrate to a single predominant position as detected by Oregon Green fluorescence (Figure 20a, left panel) and ethidium bromide staining of DNA (Figure 20a, right panel). The fluorescence signal remained unchanged following incubation for 60 minutes at 30^{o}C in the absence of SWI/SNF (Figure 20a, no enzyme) but the addition of SWI/SNF and ATP led to the loss of ~60% of the Oregon Green signal (Figure 20a, WT). Loss of Oregon green signal was also associated with the appearance of faster migrating, non-fluorescent MMTV-DNA species. These data are

consistent with the ATP-dependent removal of both histone H2A/H2B dimers, resulting in formation of the tetrasome particles. Strikingly, the Swi2p/Arp7p/Arp9p subcomplex was unable to catalyze loss of the Oregon Green fluorescence, suggesting a defect in dimer displacement (Figure 20a, minimal). Likewise, a time course experiment illustrated that intact SWI/SNF displaces significant levels of H2A within 10 minutes of incubation (Figure 20b, WT), but the Swi2p/Arp7p/Arp9p subcomplex showed no activity at any time point in this assay (Figure 20b, minimal).

To further investigate the ability of the Swi2p/Arp7p/Arp9p subcomplex to displace H2A/H2B dimers, we collaborated with Beato group who performed an in vitro chromatin immunoprecipitation (ChIP) analysis, using antibodies against H2A and H4 (Vicent, et al., 2004). In this assay, MMTV mononucleosomes were incubated with remodeling enzymes, proteins were crosslinked with formaldehyde, histones H2A or H4 were immunoprecipitated, and the recovery of MMTV DNA was analyzed by PCR. The data in Figure 20c shows that incubation with intact SWI/SNF led to the loss of ~60% of histone H2A in 30 minutes. As expected, SWI/SNF action had no effect on the levels of histone H4 associated with MMTV sequences. In contrast, only ~27% of H2A was displaced by the Swi2p/Arp7p/Arp9p subcomplex. Furthermore, addition of 2-fold higher levels of the Swi2p/Arp7p/Arp9p subcomplex led to only ~43% loss of H2A (Figure 20d). Thus, in contrast to the equivalent activity of SWI/SNF and the Swi2p/Arp7p/Arpp9 subcomplex in a variety of other remodeling assays, the minimal subcomplex is specifically defective in catalyzing the ATP-dependent displacement of histone H2A/H2B dimers.

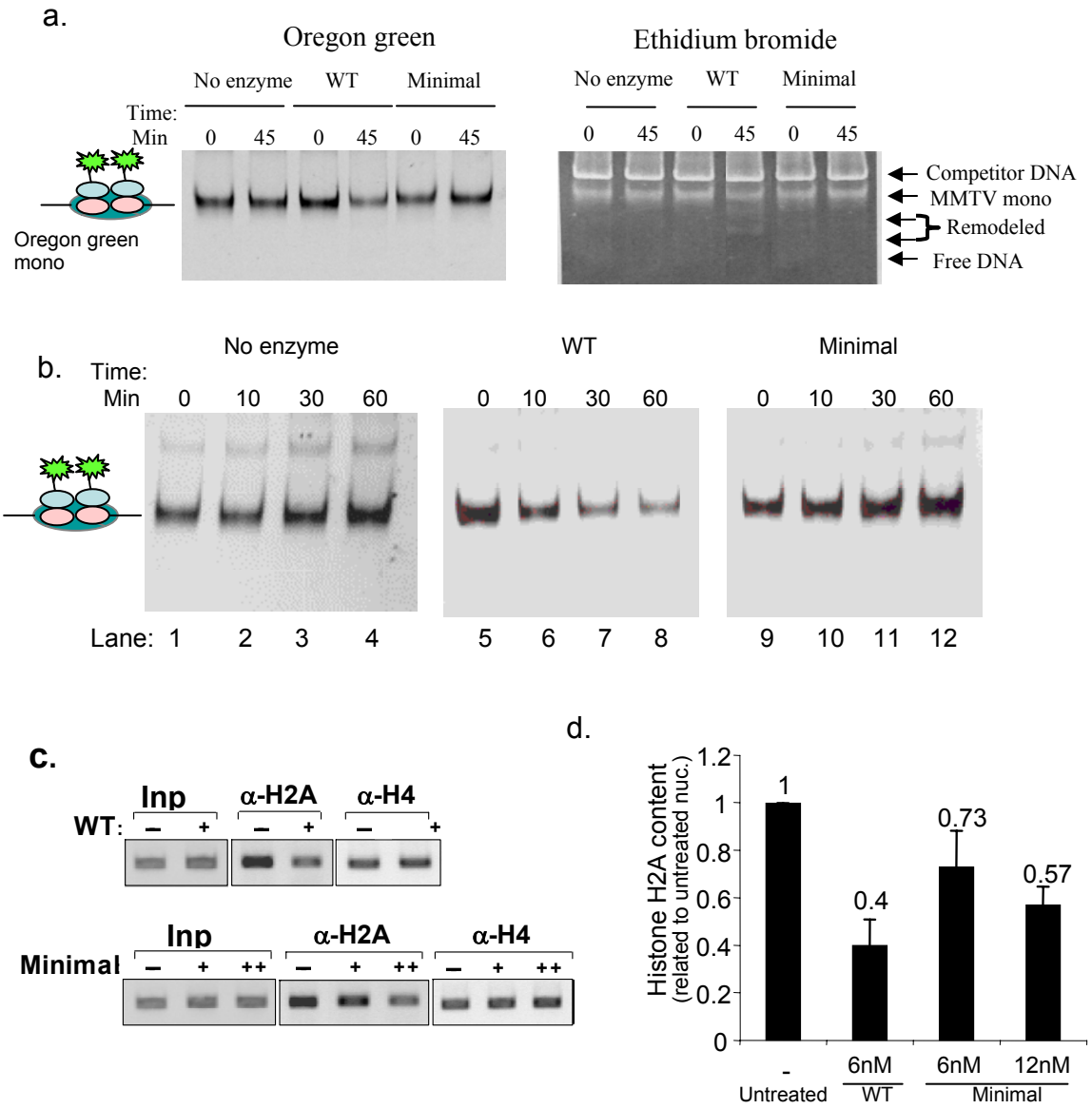


Figure 20. Swi2p/Arp7p/Arp9p subcomplex is defective to catalyze histone H2A/H2B dimer displacement. (a) Native PAGE analysis of MMTV mononucleosome assembled with H2A that is labeled by Oregon green fluorescent dye at a unique cysteine introduced at the C-terminus. Left panel represents Oregon green signal of MMTV-mononucleosome on 4% native gel PAGE at time 0 and 45 minutes after incubation with ATP and WT or the Minimal SWI/SNF enzymes. Loss of dimers was indicated by the decreased fluorescent signal at mononucleosome position (schematic on left). Right panel shows ethidium bromide staining of the same gel on left (details in Methods). **(b)** Time course of dimer loss assay under similar condition to that described above. **(c)** Representative *In vitro* ChIP assay to test dimer loss. **(d)** Real Time-PCR to quantify DNA from two independent *in vitro* ChIPs.

Swi3p contains a novel histone tail binding domain

The inability of the Swi2p/Arp7p/Arp9p subcomplex to function efficiently in dimer displacement assays suggested that dimer loss is not a simple consequence of ATP-dependent changes in histone-DNA interactions. In contrast, these data suggest that dimer loss might involve interactions between histones H2A/H2B and SWI/SNF subunits other than Swi2p, Arp7p, and Arp9p. One of the subunits missing from the minimal SWI/SNF complex is Swi3p, which has a 300-residue N-terminal domain that contains 25% glutamic and aspartic acid residues. A similar enrichment for acidic residues is a common feature of histone binding proteins. To test whether Swi3p binds to histones in vitro, we monitored the binding of ^{35}S-labeled Swi3p to GST fusion proteins that harbor the N-terminal domains of each of the core histones. Strikingly, ^{35}S-labeled Swi3p was quantitatively retained on resins that harbored either GST-H2A or GST-H2B but no binding was observed to the GST-H3 or GST-H4 resins (Figure 21a). Likewise, the 300 residue of the N-terminal domain of Swi3p (3N) was sufficient for GST-H2A tail binding, and the C-terminal ~600 residues of Swi3p also retained weaker binding activity with both GST-H2A and H2B (Figure 21b). Thus, the N-terminus of Swi3p appears to harbor a histone H2A/H2B binding domain.

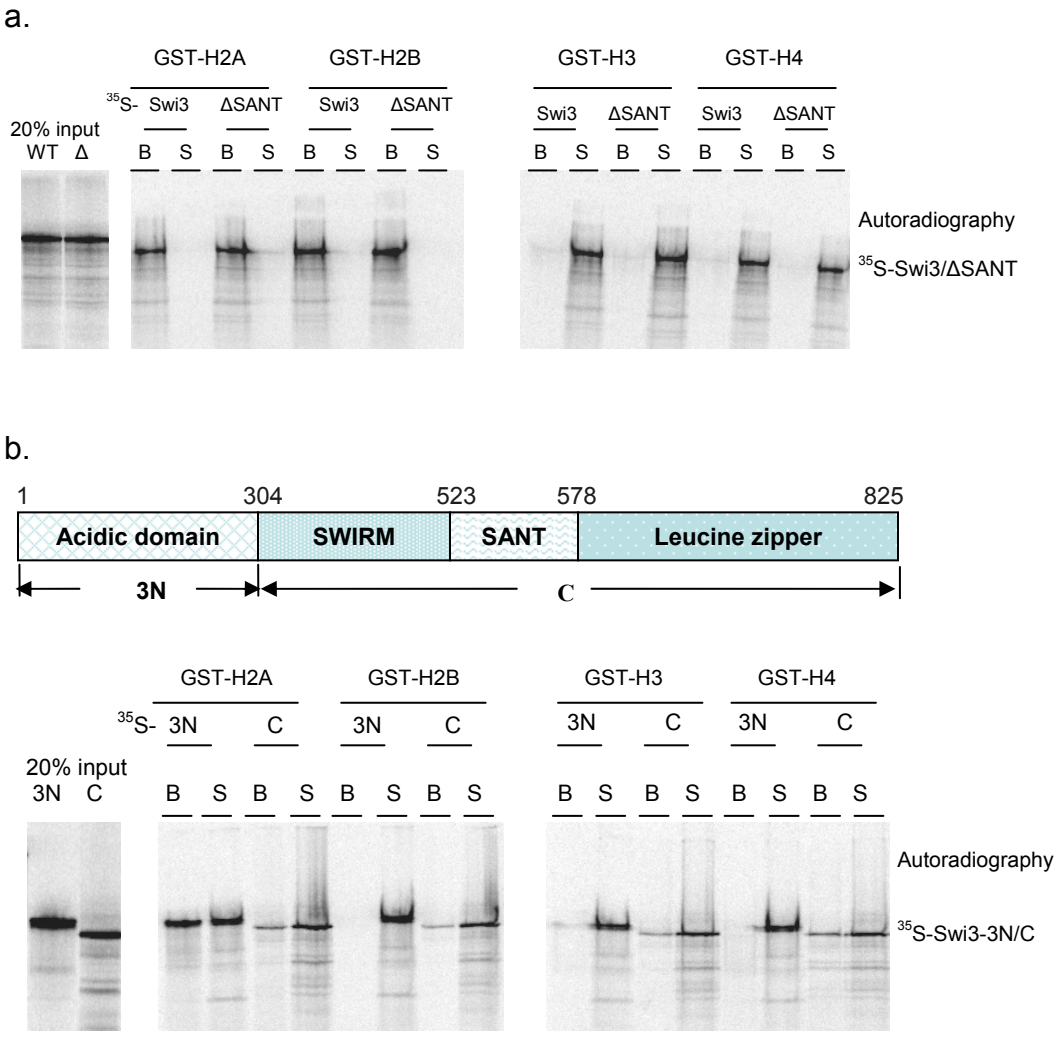


Figure 21. Swi3p has a novel histone tail binding domain. GST-histone tail fusion proteins were used in GST-pulldown assays with in vitro translated ^{35}S-labeled Swi3p polypeptides. **(a**) Full length ^{35}S-Swi3p was incubated with GST-H2A, GST-H2B, GST-H3 or GST-H4 tails. B, bound (100%); S, unbound supernatant (15%). **(b)** Top panel is the schematic illustration of predicted domains within Swi3p. Bottom panel shows GST-histone tails pulldown with ^{35}S-labeled N-terminal domain (3N) or the C-terminal domain(C).

Swi3 N-terminal acidic domain is not required for SWI/SNF assembly but is required for dimer displacement in vitro

To test whether the N-terminal acidic domain of Swi3p is functionally relevant to SWI/SNF remodeling activities, we TAP-purified SWI/SNF from a *swi3* mutant that encodes a Swi3p harboring a deletion of the N-terminal 200 amino acids (*swi3*Δ*2N*). Surprisingly, and unlike the case of the *swi3*Δ*SANT* allele, removal of the first 200 residues of Swi3p did not affect SWI/SNF assembly (Figure 22a). The ATPase activity of the SWI/SNF-Δ2N complex was equivalent to intact SWI/SNF (Figure 22b), and both complexes functioned equivalently in "601" mononucleosome restriction enzyme accessibility assay (Figure 23a). Thus, as expected from the analysis of the Swi2p/Arp7p/Arp9p subcomplex, the Swi3p N-terminal acidic domain is not required for ATPase or nucleosome remodeling activities of SWI/SNF. We then tested the activity of SWI/SNF-Δ2N for H2A/H2B dimer displacement, using the Oregon Green-H2A fluorescence assay. Strikingly, the 200 amino acid deletion within the Swi3p acidic domain eliminated the ability of SWI/SNF to displace histone H2A as monitored by loss of Oregon Green fluorescence (Figure 23b, Δ2N). Addition of purified GST fusion protein that harbors the 300 amino acids from Swi3p acidic domain was unable to rescue the defect in dimer loss in trans (data not shown). Likewise, addition of the Swi3p/Swp73p/Snf6p subcomplex to the Swi2p/Arp7p/Arp9p subcomplex did not reconstitute the dimer displacement activity (data not shown). These two subcomplexes did not stably interact with each other in vitro, suggesting that Swi3p acidic domain functions most effectively when stably associated with SWI/SNF complex. These data

indicate that the N-terminus of Swi3p functions as a novel histone interaction domain that plays an essential role for in vitro histone H2A/H2B displacement.

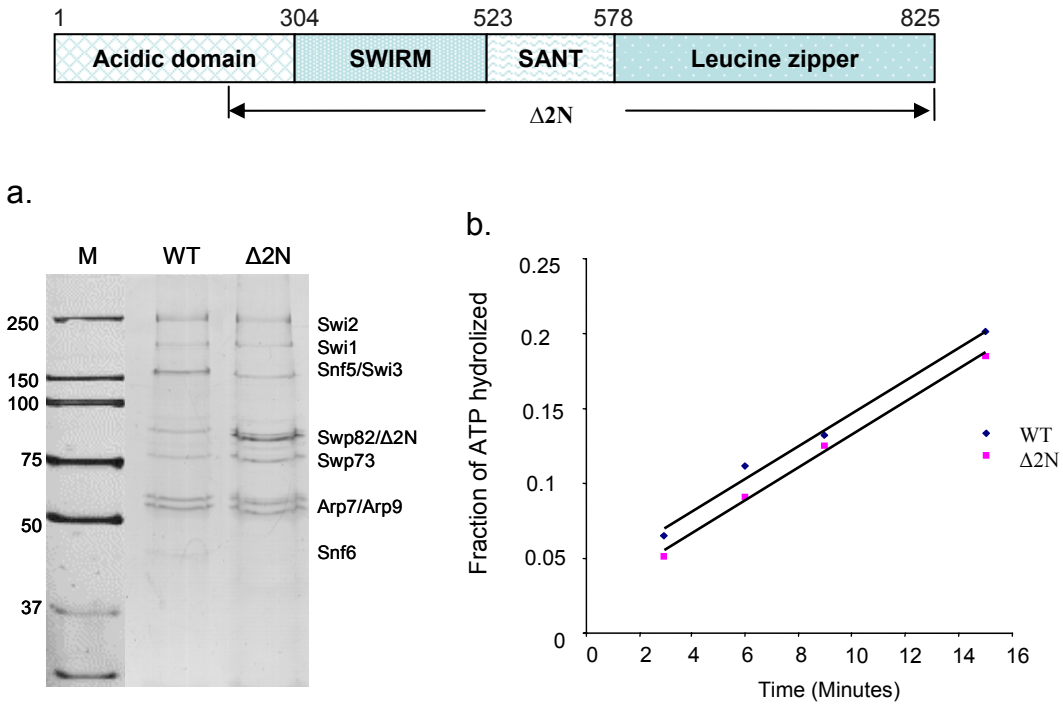


Figure 22. The N-terminal acidic domain of Swi3p is not required for SWI/SNF assembly or the ATPase activity. (a) Top panel is the schematic illustration of Swi3Δ2N on top panel. Bottom panel shows the silver stained gel of TAP preparation of SWI/SNF purified from TAP-Swi2p from WT or TAP-Snf6p from swi3Δ2N strain that encodes a Swi3p mutant that lacks 200-amino acids of the N-terminus (Δ 2N). M, molecular weight marker. **(b)** SWI/SNF-Δ2N complex shows DNA-stimulated ATPase activity similar to that of intact SWI/SNF.

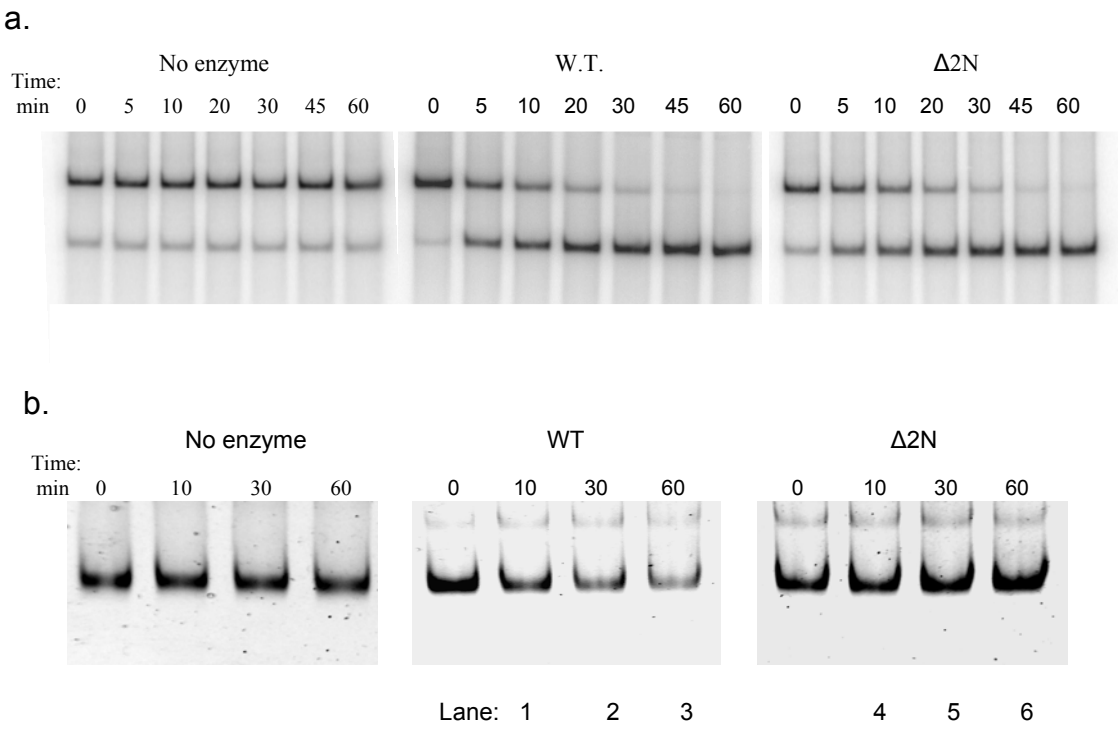


Figure 23. The N-terminal acidic domain of Swi3p is required for displacement of histone dimer. (a) SWI/SNF-Δ2N complex increases restriction enzyme Hha I accessibility on "601" mononucleosome substrate described in Figure 18b. **(b)** SWI/SNF-Δ2N complex is defective for displacement of Oregon green-labeled H2A from MMTV mononucleosomes. Reactions contain 30 nM fluorescent MMTV mononucleosome substrate and 5 nM of WT or SWI/SNF-Δ2N complex.

DISCUSSION

Function of a minimal Swi2p/Arp7p/Arp9p remodeling subcomplex

Previous studies have indicated that the isolated ATPase subunits of chromatin remodeling enzymes are often sufficient to carry out much of the remodeling activities of the native, intact complex (Phelan, et al., 2000; Phelan, et al., 1999; Saha, et al., 2005). In the case of the human SWI/SNF complex, the BRG1 ATPase alone has ATPase activity and mononucleosome remodeling activity that is ~20% of the intact complex. Addition of the hSnf5p and hSwi3p (BAF155/BAF170) subunits is sufficient to restore full levels of activity (Phelan, et al., 2000; Phelan, et al., 1999). Likewise, for the yeast RSC complex, the isolated Sth1p ATPase is sufficient for ATP hydrolysis activity and DNA translocation (Saha, et al., 2005). In contrast, the catalytic subunit of the INO80 complex, Ino80p, is inactive by itself and requires the Arp5 and Arp8 subunits for ATPase activity and ATP-dependent nucleosome mobilization (Shen, et al., 2003). Likewise, the activities of the Drosophila and yeast ISWI ATPases are enhanced by the Acf1 (Eberharter, et al., 2004; Ito, et al., 1999) and Itc1 subunit (Gelbart, et al., 2001), respectively. For yeast SWI/SNF, we found that a Swi2p/Arp7p/Arp9p subcomplex is sufficient for ATP hydrolysis and for the majority of ATP-dependent chromatin remodeling activities. Since *ARP7* and *ARP9* are essential genes in yeast (Cairns, et al., 1998), and we have not been successful at expressing full-length Swi2p in bacteria or yeast cells, we have not been able to test whether Swi2p by itself is sufficient.

The Swi3p N-terminus contains a novel histone tail binding domain

The ATP-dependent depletion of histone H2A/H2B dimers by chromatin remodeling enzymes is a relatively new activity ascribed to these enzymes, and consequently this activity has not previously been tested with isolated ATPase subunits. Our results indicate that ATP-dependent dimer loss is not efficiently catalyzed by the Swi2p/Arp7p/Arp9p ATPase subcomplex, but that the Swi3p subunit must contribute an acidic histone binding surface. It has been known for some time that the acidic N-terminus of Swi3p is not a conserved feature of human Swi3p homologs, BAF155 and BAF170, and thus the functional importance of this domain had not been tested. However, recently it was reported that the ATP-dependent dimer displacement activity of the human SWI/SNF complex is stimulated by the acidic protein nucleolin (Angelov, et al., 2006). These data suggest that human Swi3p homologs may not have conserved their acidic domains because SWI/SNF was able to take advantage of a functional interaction with other histone chaperones, such as nucleolin.

Our results indicate that the acidic N-terminus of Swi3p is essential for ATP-dependent dimer loss in vitro; however, deletion of this acidic domain does not lead to obvious *swi/snf* mutant phenotypes in vivo. For instance, strains that harbor a *swi3* allele that encodes a 300 amino acid N-terminal truncation of Swi3p grow at wild-type rates, and this alteration does not lead to defects in expression of two SWI/SNF-dependent target genes, *SUC2* and *HO-lacZ* (unpublished data). While these results might suggest that ATP-dependent dimer loss does not contribute significantly to transcriptional control in vivo, we believe that it is more likely that this activity may control only a small subset of SWI/SNF-dependent genes. Consistent with this view, it is known that ATP-dependent

dimer loss is highly DNA sequence-dependent, and thus it is expected that only a subset of remodeling events in vivo will give rise to dimer loss events. Alternatively, it is possible that one or more abundant histone chaperones that are present within cells may compensate for the lack of the Swi3p N-terminus.

Dimer loss vs DNA translocation

Recent data from both our lab and others support a DNA translocation model for ATP-dependent remodeling by the SWI/SNF family of enzymes (Jaskelioff, et al., 2000; Saha, et al., 2005; Zhang YL, 2006). In this model, SWI/SNF binds to a single nucleosome within a substrate binding pocket and interacts with nucleosomal DNA near the dyad axis (Saha, et al., 2005; Smith, et al., 2003). ATP-dependent DNA translocation generates a dynamic loop of DNA on the nucleosomal surface which has an average size of ~100 bp and which rapidly dissipates by a continuous or discontinuous process (Zhang, et al., 2006). The formation of an intranucleosomal DNA loop is likely to reflect a transient intermediate in the process of nucleosome mobilization. In addition, it seems likely that DNA loop formation may also be a prerequisite for displacement of histone H2A/H2B dimers, as loss of DNA-histone contacts is predicted to destabilize the histone dimers, making them more prone for capture by histone binding proteins such as the Swi3p acidic domain. If this is the case, then this may provide an explanation for why the Swi2p/Arp7p/Arp9p subcomplex retained some ability to “displace” H2A when an in vitro ChIP assay was used, but this same subcomplex was inactive for H2A displacement using the Oregon green assay. The ChIP assay measures the ATP-dependent decrease in the ability to crosslink H2A to MMTV DNA, and destabilization of the dimers due to DNA

translocation may lead to decreased cross linking. In contrast, the Oregon green assay requires that the dimers be completely displaced from the MMTV mononucleosome. Thus, dimer displacement may involve at least two distinct steps – (1) destabilization of the H2A/H2B dimers due to ATP-dependent intranucleosomal DNA loop formation and (2) H2A/H2B dimer capture by the Swi3p subunit.

MATERIALS AND METHODS

Histone protein purification and octamer reconstitution. Xenopus histone H2A-S113C and H3-C110A mutants were generated by site-directed mutagenesis (Stratagene). All the histone proteins were expressed and induced in BL21 (pLys) (Invitrogen). The histone inclusion bodies were prepared as described previously (Luger, et al., 1999). Briefly, each histone inclusion body was dissolved in unfolding buffer (7M Guanidine-HCl, 20 mM Tris-HCl pH7.5, 10 mM DTT) for at least 1 hour at room temperature. After centrifugation, the supernatant containing unfolded histones was subjected to Superdex-200 HR gel filtration column. Fractions containing histones were pooled and further purified through a cation-exchange column HiTrap SP HP (Pharmacia). Histone aliquots were lyophilized and stored at -80ºC.

For histone octamer reconstitution, equal moles of each histone was mixed in unfolding buffer at room temperature for 1-2 hours and subjected to dialysis against 2 liters refolding buffer (2 M NaCl, 10 mM Tris-HCl pH7.5, 1 mM EDTA and 5 mM beta-mercaptoethanol) with 3 changes of refolding buffer at 4ºC. Refolded histone octamer was purified through Superdex-200 HR gel filtration column. Fractions containing histone octamer were confirmed by Coomassie blue staining, then pooled, concentrated and stored at 4ºC until use.

Reconstitution of nucleosomal arrays and mononucleosomes. DNA template of 5S-208-11 consists of 11 tandem repeats of *L. variegates* 5S rDNA nucleosome positioning sequence with a Sal I restriction enzyme recognition site at the central region. 208-11

DNA templates were released from plasmids (CP589) by Not I and Hind III restriction enzyme digestion, and purified from agarose gel. The “601”-DNA templates contain 343 base pairs (bp) of DNA released from plasmids (CP1024) by EcoR I and Hind III restriction enzyme digestion, and further purified from agarose gel. Mouse mammary tumor virus (MMTV) DNA template containing 350 bp of DNA with centrally located B nucleosome positioning sequence was purified from PCR amplification for Oregon Green assay. 5S-208-11 and “601” templates were end-labeled with α-^{32}P-dCTP by Klenow fill-in at 37°C and purified through a Sephadex G-25 column after brief phenol/chloroform extraction. Nucleosomal arrays and mononucleosomes were assembled by stepwise dialysis in a series of NaCl in TE buffer at 4°C, with histone octamer-to-DNA ratio of 0.9-1.0 (Logie and Peterson, 1999).

ATPase activity and kinetics study. DNA-stimulated ATPase activity of each purified SWI/SNF complex was carried out in a 10-µl reaction mixture containing 20 mM Tris (pH8.0), 5 mM MgCl2, 0.2 mM DTT, 5% glycerol, 0.1% Tween, and 100 ug/ml bovine serum albumin (BSA), 1 µg plasmid DNA, 100 µM cold ATP, 0.01 µCi γ-^{32}P-ATP, and 5 nM SWI/SNF (Logie and Peterson, 1999). Each reaction was incubated at 30ºC and 0.6 µl of each reaction was taken at 3, 6, 10, 15 and 20 minutes time point and spotted onto PEI-cellulose F plate (EMD Chemicals, INC) for thin-layer chromatography (TLC) in 0.75 M KH_2PO4 (pH 3.5). The TLC plate was air-dried and imaged by PhosphoImager (Molecular Dynamics). The fraction of ATP hydrolyzed at each time point was quantified by ImageQuant v1.2 (Amersham). For ATP hydrolysis kinetics, similar reactions were performed in the presence of a series of different ATP concentrations varying from 3.125

μM to 1000 μM. Initial velocities were calculated from the slope of each linear plot of ATP hydrolysis by Excel (Microsoft). Velocity was plotted as a function of ATP substrate concentration, using KaleidaGraph v3.6 software (Synergy Software). Kinetic parameters were retrieved from non-linear fitting to Michaelis-Menten equation. Data from three independent experiments were averaged for plot with standard deviation less than 15%.

Cruciform formation assay. The cruciform formation assay was performed in a 30-μl reaction containing 1X 5-50 buffer (10 mM Tris pH 8.0, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml BSA), 1.5 nM SWI/SNF, 3 mM ATP, 0.15 μg/ml endonuclease VII and 0.1 nM ^{32}P-labeled 3.8 kb DNA (AvaI linearized pXG540) containing an inverted $(AT)_{34}$ sequence described by Smith CL *et al* (Smith and Peterson, 2005b). At each time point, 4 μl of reaction cocktail was terminated by 4 μl 2X stop solution containing 10 mM Tris pH8.0, 0.6% SDS, 40 mM EDTA, 10% glycerol and 0.1 mg/ml proteinase K. Each quenched sample was deproteinated at 50ºC for 20 minutes, and finally resolved on 4% native polyacrylamide gel in 0.5XTBE. Gel was dried and imaged by PhosphoImager. Percentage of DNA fragment cut was quantified by ImageQuant v1.2 (Amersham).

Restriction enzyme accessibility analysis. Restriction enzyme accessibility of SWI/SNF complexes on 5S-208-11 array or 601-mononucleosome substrate was performed described by Logie and Peterson (Logie and Peterson, 1997). Briefly, 30μl reaction cocktail containing 2 nM 5S-208-11 nucleosome arrays or 601-mononucleosomes, 10 units of restriction enzymes (Sal I or Hha I) and 1-3 nM SWI/SNF

in 5-50 buffer was incubated at 30°C. At each time point, 4 µl of each reaction was taken and terminated by 4 µl of 2X stopping solution consisting of 1.2% SDS, 20 mM Tris (pH 8.0), 80 mM EDTA, 10% glycerol, and 1 mg/ml proteinase K, and incubated at 50°C for 20 minutes. DNA was extracted by phenol/chloroform. Samples were resolved on 8% naive polyacrylamide gel for 601 and 1% agarose gel for 5S-208-11. Gels were dried and imaged by PhosphorImager (Molecular Dynamics).

601-mononucleosome gel mobility assay. 601-mononucleosome gel mobility was performed in 30-µl reaction cocktail similar to that of HhaI restriction enzyme accessibility assay described above. At each time point, 4 µl of each reaction was terminated by 4 µl of 2X quenching solution consisting 10% glycerol and 200 ng competitor DNA to remove SWI/SNF, quenched on ice for at least 30 min. Samples were resolved on a 4% native polyacrylamide gel and running in 0.5X TBE buffer. Gels were dried and imaged by PhosphorImager (Molecular Dynamics).

Histone H2A/H2B dimer loss assay. Reconstituted Xenopus histone octamers containing H2A-S113C and H3-C110A were covalently labeled with Oregon Green (Molecular Probes) fluorescent dye as described by Bruno et al (Bruno, et al., 2003). Free Oregon Green dye was removed by 3 times dilution and concentration with 10,000MW CO Centricon (VIVA SCIENCE) in labeling buffer (10 mM Tris pH7.5, 2 M NaCl, 0.1 mM EDTA). MMTV-mononucleosomes were reconstituted with Oregon Green labeled octamer and MMTV DNA templates by stepwise dialysis. H2A/H2B dimer loss analysis was performed in a 10 µl reaction containing 30 nM MMTV-mononucleosomes

and 5 nM SWI/SNF in 5-50 buffer described earlier. Reactions of the time-course experiments aliquots were terminated at each time point by adding 2X termination buffer containing 10% glycerol and 200 ng competitor DNA to remove SWI/SNF, and quenched on ice for 30 minutes. Samples were resolved on 4% native polyacrylamide gel run in 0.5X TBE buffer. Oregon Green signal was detected by Kodak Imaging scanner (Kodak), with excitation filter of 465 nm, and emission filter of 535 nm.

MMTV mononucleosomes reconstitution for in vitro ChIP assay. Mononucleosome templates for the *in vitro* Chromatin Immunoprecipitation (ChIP) assay were generated with a 232 bp EcoRI-BamHI fragment containing the MMTV promoter sequence (from –221 to +1). The fragment was radiolabeled at the 5' ends with the Klenow fragment of DNA polymerase and α-^{32}P-dCTP. Mononucleosomes were reconstituted by the salt dialysis method as described (Vicent, et al., 2002), using recombinant *X.laevis* histones expressed in *E.Coli* (Luger, et al., 1999). Purification of the reconstituted material was subjected to glycerol gradient ultracentrifugation using a linear gradient from 10-30% (v/v) glycerol in 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA. Centrifugation was performed in an SW60 rotor for 9h at 55,000 rpm and 4ºC. Fractions of 100 μl were collected from the bottom of the gradient.

***In vitro* chromatin immunoprecipitation (ChIP) assays.** Nucleosome remodeling reactions (10 μl) were done in 10 mM HEPES (pH 7.9), 60 mM KCl, 6 mM MgCl2, 60 μM EDTA, 2 mM DTT, 13% glycerol containing 20 nM of MMTV nucleosomes and 6 or 12 nM of WT-SWI/SNF or Minimal SWI/SNF complex, in the presence of 1 mM ATP.

Nucleosomes were incubated for 30 minutes at 30 ºC followed by additional 30 minutes incubation with 250 ng of poly-dIdC as competitor. Remodeled nucleosomes were cross-linked with 2.5% HCHO for 10 minutes at 37ºC, and the reaction was stopped by 0.1 M glycine (pH 7.5) for 5 minutes at room temperature. 1 ml of ChIP Immunoprecipitation (IP) buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-pH8.1, 167 mM NaCl, 1 mM PMSF, 1 μg/ml aprotinin and 1 μg/ml pepstatinA) was added and, after removal of an aliquot for input control (10% total volume), mononucleosomes were subjected to immunoprecipitation with antibodies against histones H2A or H4 (Angelov, et al., 2000). Before extraction with phenol/chloroform and ethanol precipitation, the samples were decross-linked at 65ºC. The PCRs were carried out with Taq DNA polymerase under standard conditions. The specific primers generate a 232 bp fragment of the nucleosome B of the MMTV promoter. PCR products were resolved on 1% agarose gels and stained with ethidium bromide.

Acknowledgement

We are grateful to Dr. Owen-Hughes' lab for providing Endonuclease VII used in cruciform assay. Thank Dr. Migeul Beato's lab for providing MMTV construct, from which the MMTV mononucleosome template was made.

CHAPTER IV

ASSEMBLY OF THE SWI/SNF COMPLEX IN VITRO

SUMMARY

SWI/SNF consists of at least four functionally distinct modules that are integrated through the SANT domain of Swi3p. In this chapter, we further investigated the important role of the SANT domain for SWI/SNF assembly in vitro. Using in vitro transcription coupled translation system, we expressed eight essential subunits of SWI/SNF in vitro, including Swi2p, Swi1p, Snf5p, HA-Swi3p, Swp73p, Arp7p, Arp9p, and Snf6p. A series of anti-HA co-immunoprecipitation analyses suggested that Swi3p strongly interacts with Swp73p, but not with Snf6p. Interestingly, Swi3p indirectly interacts with Snf6p when Swp73p was present. This result is consistent with the formation of Swi3p/Swp73p/Snf6p subcomplex, which is independent of the SANT domain of Swi3p. We tried to use Swi3p/Swp73p/Snf6p and Swi3pΔSANT/Swp73p/Snf6p subcomplexes to pinpoint the requirement of the SANT domain for SWI/SNF assembly, and found none of other subunits of SWI/SNF seemed to interact with Swi3p/Swp73p/Snf6p subcomplex. Moreover, SWI/SNF complex cannot be assembled from in vitro translated proteins, suggesting that SWI/SNF assembly may require all the eleven subunits or it is assembled only under physiological conditions.

RESULT

Swi3p physically interacts with Swp73p but not Snf6p

For a better understanding of the intermolecular organization of SWI/SNF complex and the role of the SANT domain as a protein scaffold for SWI/SNF assembly, we have tried different strategies to reconstitute SWI/SNF complexes in vitro. Initially, I cloned SWI3 and swi3ΔSANT into an in vitro expression vector that contains an HA tag under T7 promoter. I also cloned SNF5, SWP73, ARP7 and ARP9 into a pCMX expression vector. Swi2p and Swi1p expression constructs are gift from Jerry Workman lab. Using in vitro transcription/translation (IVT) system, Swi2p, Swi1p, Snf5p, Swi3p, Swi3ΔSANT, Swp73p, Arp7p, Arp9p, and Snf6p proteins were translated and can be easily detected by ^{35}S-methionine labeling and autoradiography (Figure 24a). Swi2p and Swi1p were not expressed well under the standard salt condition used for other subunits, but optimized translation required higher KCl concentration of 60mM and 40mM, respectively (Figure 24b).

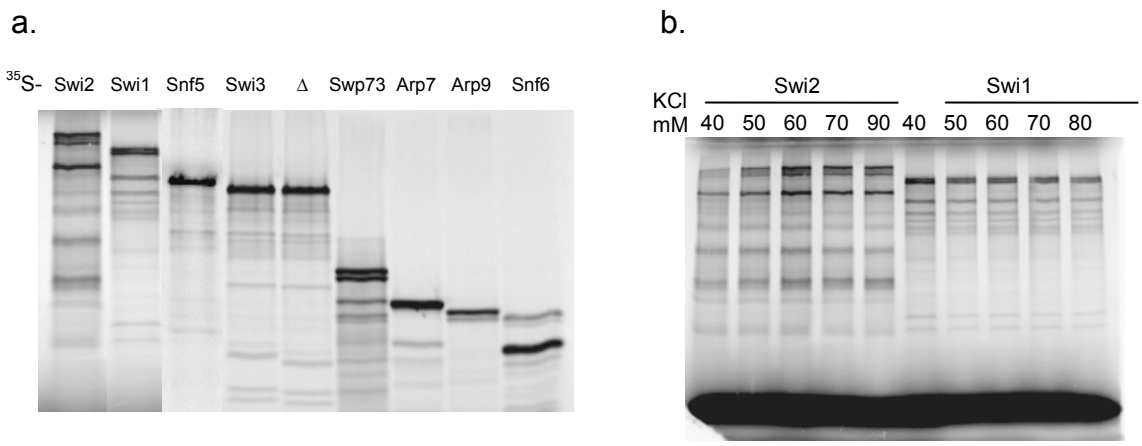


Figure 24. In vitro translation of ^{35}S-labeled SWI/SNF subunits. (a) Autoradiography showing 1 µl of ^{35}S-labeled, in vitro translated SWI/SNF subunits resolved on 8% SDS-PAGE. Δ represents swi3ΔSANT. **(b)** Optimized translation of Swi2p and Swi1p at higher KCl concentration of 60 mM and 40 mM, respectively**.**

Using in vitro translated HA-Swi3, HA-Swi3ΔSANT, and ^{35}S-methionine labeled other subunits, a series of anti-HA co-IPs were performed to examine whether or not the SANT domain of Swi3p is required for subunit-subunit interactions. Results repeatedly show that Swi3p only strongly interacts with Swp73p (Figure 25a, lane 7) and weakly interacts with Snf5p (Figure 25a, lane 5), but no interaction was detected with most of the other subunits in vitro. Figure 25b shows both positive and negative controls for co-IPs. Due to initial difficulty in expression of large proteins such as Swi2p and Swi1p, the N-terminus and C-terminus of Swi2p and Swi1p were also made for co-IP studies, but no interaction with these truncated proteins was detected (Figure 25c). Using ^{35}S-HA-Swi3p alone, anti-HA antibody was able to immunoprecipitate significant amount of ^{35}S-HA-

Swi3p (positive control, Figure 25b, lane 13). Protein A beads alone showed no non-specific affinity with ^{35}S-HA-Swi3p (beads alone, Figure 25b, lane 15).

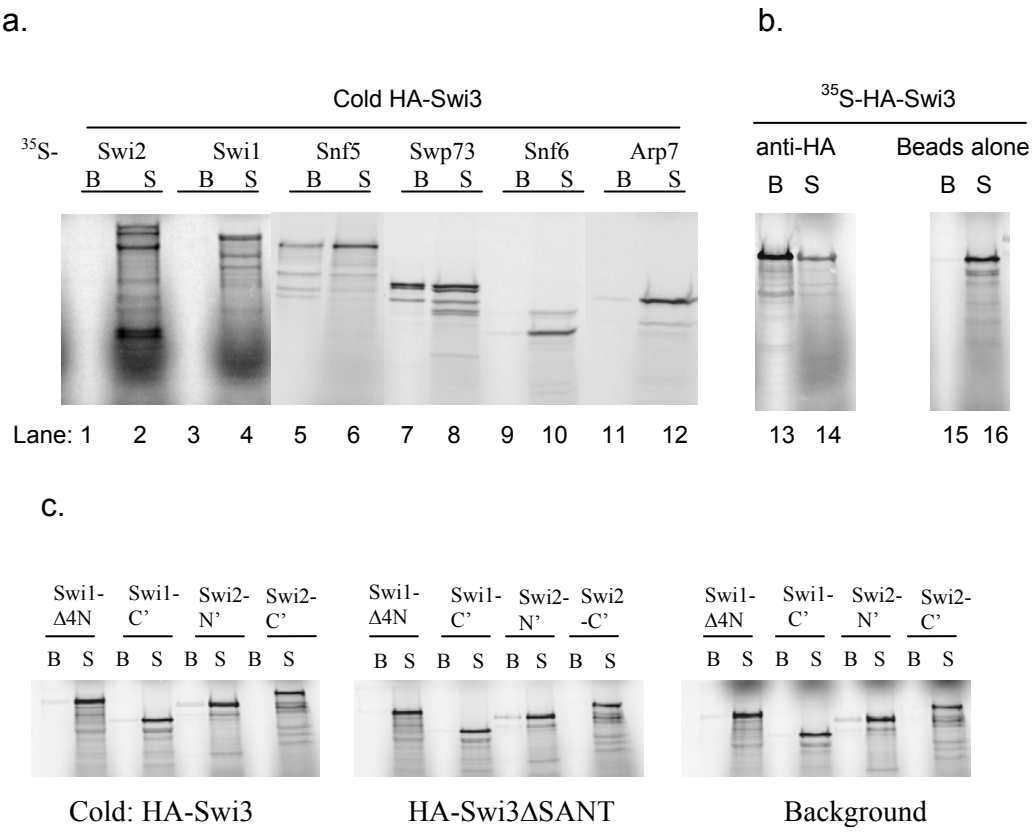


Figure 25. Swi3p directly interacts with Swp73p. (a) Anti-HA Co-IP analysis. In vitro translated cold HA-Swi3 (5 µl) was incubated with individual ^{35}S-labeled in vitro translated SWI/SNF subunits (5 µl each) indicated in a total of 200 µl binding buffer described in Methods. Proteins retain on protein A beads (100%, B) or 15% of the unbound supernatant (S) were resolved on 8-10% SDS-PAGE. Gels are dried for autoradiography. Swi3p physically interacts with Swp73p (lane 7) and weakly with Snf5p (lane 5). **(b)** Positive and negative controls of Co-IP. ^{35}S-HA-Swi3p was incubated with anti-HA and protein A beads as positive control (Anti-HA, lane 13) or with protein A beads only as negative control (background, lane 15). **(c)** cold HA-Swi3 or HA-Swi3ΔSANT was incubated with individual ^{35}S-labeled truncated Swi2p or Swi1p for anti-HA co-IP. Background binding mixture excluded anti-HA antibody.

Swi3p interacts with Snf6p when Swp73p is present

Next, I added Swp73p with Swi3p or Swi3ΔSANT, and incubated for 1 hour before ^{35}S-Snf6p, ^{35}S-Snf5p or ^{35}S-Arp7p were added for co-IP. Interestingly, both Swi3p (Figure 26, lane 4) and Swi3ΔSANT (Figure 26, lane 8) interacted with Snf6p when pre-incubated with Swp73p, suggesting that Swi3p may indirectly interact with Snf6p through Swp73p. No significant interaction was observed for Snf5p or Arp7p even in the presence of Swp73p (Figure 26). This data is fully consistent with the formation subcomplex of Swi3p/Swp73p/Snf6p purified from cell lysate that lacks the intact SANT domain of Swi3p in vivo.

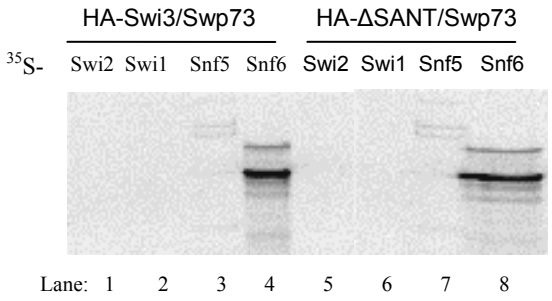


Figure 26. Swi3p indirectly interacts with Snf6p through Swp73p. Co-IP assay under conditions similar to described above. Autoradiography showing indirect interaction of ^{35}S-labeled Snf6p with cold Swi3p in the presence of cold Swp73p. Cold HA-Swi3p was pre-incubated with cold Swp73p before adding ^{35}S-Snf6p and other ^{35}S-labeled subunits indicated on top of gel. Both Swi3p (lane 4) and swi3ΔSANT (lane 8) indirectly interact with Snf6p when Swp73p was present.

Since Swi3p/Swp73p/Snf6p and Swi3ΔSANT/Swp73p/Snf6p subcomplexes can be assembled in vitro, therefore we expected to use these subcomplexes to pinpoint the requirement of the SANT domain for SWI/SNF assembly. In similar anti-HA coIP studies, we used co-translated ^{35}S-HA-Swi3p, ^{35}S-Swp73p, and ^{35}S-Snf6p incubated with ^{35}S-labeled other subunits indicated in lane 2-10 of Figure 27. Unfortunately, no interaction was observed between Swi3p subcomplexes and other subunits (Figure 27, Bound). One possible explanation is that other subunits such as Swp82p, Swp29p and Snf11p may be required for SWI/SNF assembly in vitro, although they are not essential for SWI/SNF function in vivo. Alternatively, some epitope tag may interfere with the intermolecular interactions that are required for SWI/SNF assembly. It is less likely that the SWI/SNF complex masks the HA tag on Swi3p, or decreases the epitope recognition by antibody, since the HA-Swi3p/Swp73p/Snf6p subcomplexes can be immunoprecipitated from all the co-translated reactions (Figure 27, Bound).

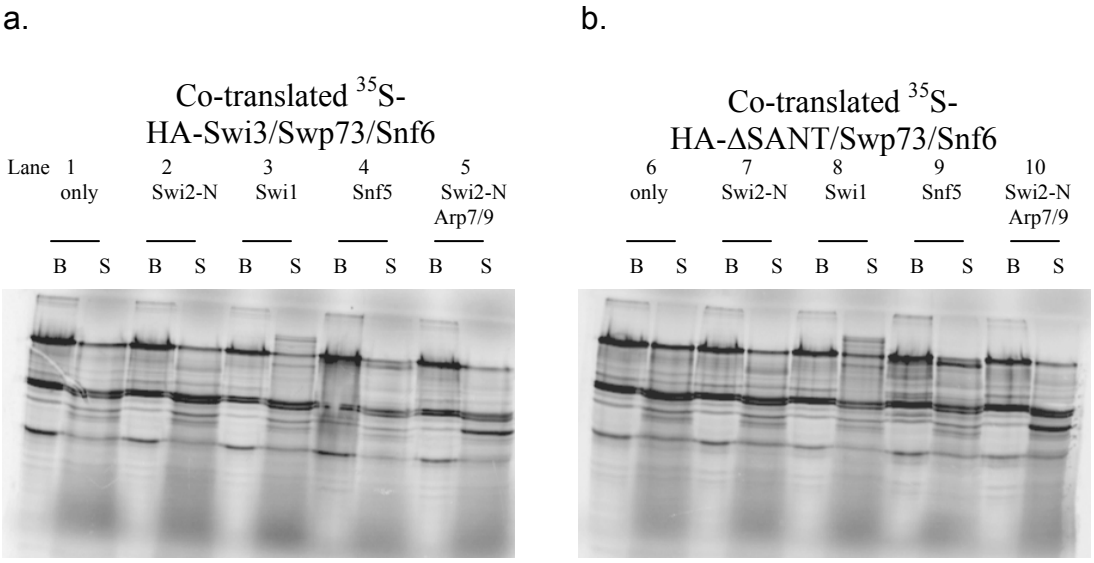


Figure 27. Swi3p/Swp73p/Snf6p subcomplexes can be assembled from co-translation. Anti-HA Co-IP. ^{35}S-HA-Swi3p/Swp73p/Snf6p **(a)** or HA-Swi3ΔSANT/Swp73p/Snf6p **(b)** were co-translated, and incubated with individually ^{35}S-labeled protein under the same condition mentioned in Lane 2-10 above. No significant interaction was observed between individual subunits and WT or HA-Swi3pΔSANT/Swp73p/Snf6p subcomplex. However, each IP predominantly pulls down three subunits that represent the formation of subcomplex of Swi3p/Swp73p/Snf6p from co-translation. B, 100% bound. S, 15% unbound supernatant.

SWI/SNF cannot be assembled from in vitro translated proteins

Next, we used a different strategy to assemble the SWI/SNF complex in vitro. TAP tagged Swi2p/Arp7p/Arp9p minimal complex that remained bound to the CAM-resin was used for in vitro assembly and pull down assays, in which IVT HA-Swi3p, Swp73p, and Snf5p were mixed with ^{35}S-Snf6p as a probe. If SWI/SNF was assembled, the CAM-resin should be able to pull down the entire complex and ^{35}S-Snf6p would be detected by autoradiography. As a negative (or background) control, BSA-preblocked CAM-resins were incubated with ^{35}S-Snf6p alone. Unfortunately, Snf6p has very high affinity with CAM-resin (Figure 28a), which made this strategy unrealistic (Figure 28b). At this point, eight (Figure 28c, lane 1-4) or six (Figure 28c, lane 5-8) subunits of SWI/SNF were co-translated for SWI/SNF assembly. Unfortunately, none of the strategies showed the assembly of SWI/SNF complex in vitro. Several possibilities may explain the negative result of SWI/SNF assembly from IVT proteins: (1) the presence of epitope tag such as HA of Swi3p and Flag on Snf5p may interfere with intermolecular contacts. (2) Swp82p, Snf29p and Snf11p subunits may be required for the formation of intact SWI/SNF complex in these assays. (3) SWI/SNF assembly might be coupled with protein translation and protein folding under physiological conditions.

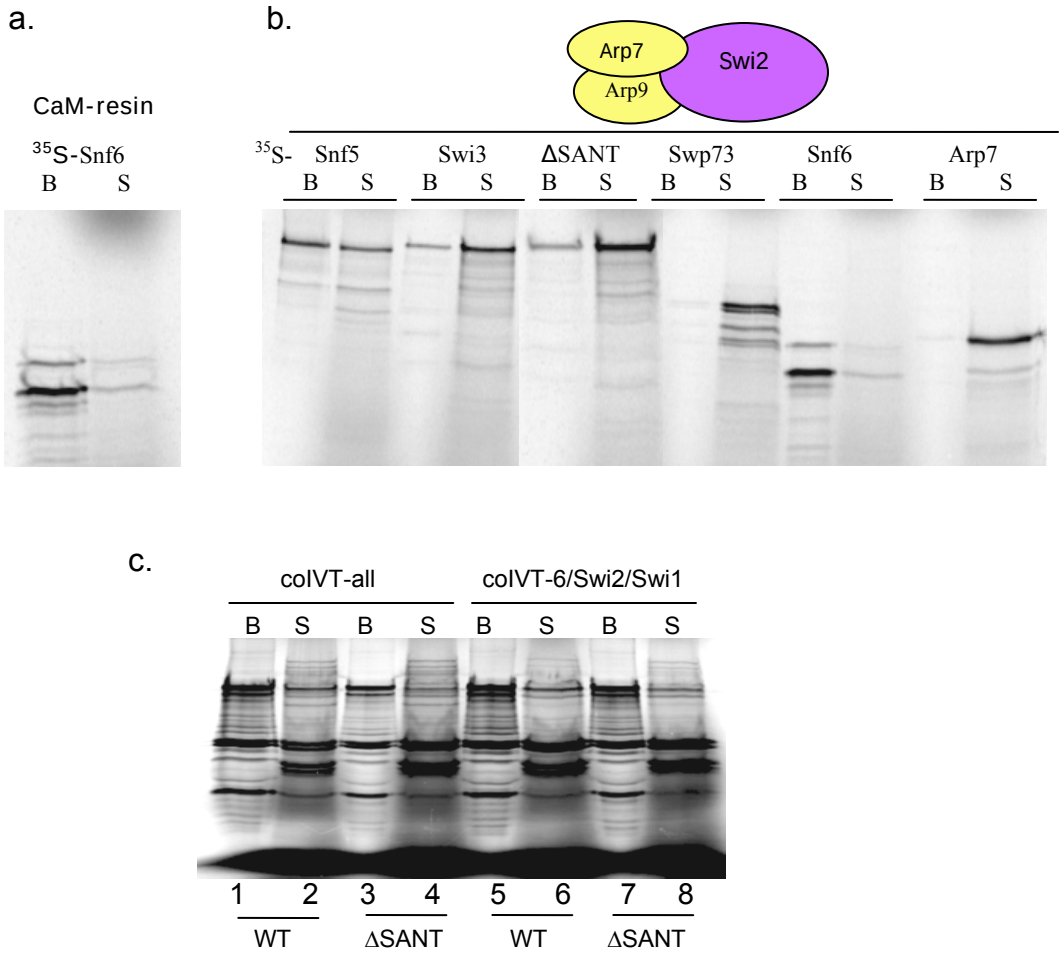


Figure 28. SWI/SNF cannot be assembled from in vitro translated proteins. (a) Nonspecific background binding of ^{35}S-Snf6p to CAM resin. **(b)** Minimal subcomplex pulldown assay. TAP preparation of Swi2p/Arp7p/Arp9p was remained on CAM resin and was used to pulldown ^{35}S-labeled SWI/SNF subunit indicated above the gel. Due to high background binding of CAM resin with ^{35}S-Snf6p shown in (a), interaction between Snf6p and Swi2p/Arp7p/Arp9p minimal complex is most likely an artifact. **(c)** Anti-HA co-IP to test SWI/SNF assambly from co-IVT of 8 subunits (coIVT-all), or co-IVT 6 subunits (coIVT-6) plus individually translated Swi2p and Swi1p. Neither strategy detected SWI/SNF assembly.

MATERIALS AND METHODS

In vitro translation and co-IP. SNF5, HA-SWI3, HA-swi3ΔSANT, swi3-3N, SWP73, SNF6, ARP7, SWI1Δ4N, SWI1Δ5N, and SWI1-C' (652 a.a) constructs were made by cloning PCR-amplified and restriction enzyme digested DNA fragments into T7 promoter vector pCMX with or without tag. All constructs were confirmed by sequencing analysis. All proteins were made by TNT T7 in vitro coupled transcription/translation kit following instructions provided by Promega. For Swi2p and Swi1p, KCl was added to adjust the final KCl concentration to 40-90 mM to maximize protein expression. For ^{35}S-labeled proteins, ^{35}S-methionine was used. For unlabeled proteins (cold), cold methionine was added into the mixture. For co-translation, DNA constructs were mixed to a total amount of 2 µg DNA in 50 µl reaction.

For co-IP, five microliter of cold HA-Swi3p or HA-Swi3ΔSANT was mixed with ^{35}S-labeled IVT proteins in binding buffer (20 mM Tris pH8.0, 150 mM NaCl, 0.25 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 0.1 mg/ml BSA, 1 mM PMSF) to a final volume of 200 µl. After incubation for 1 hour at 4ºC, 5 µl anti-HA monoclonal antibody (Convace) was added into the mixture and continued incubation for 2 hours at 4ºC. Then 20 µl of 50% protein A slurry was added into each sample for another 1 hour. Finally, protein A beads were spun down and 15% of supernatant was taken for unbound fraction. The beads were washed three times with 1 ml binding buffer and resuspended into 2X SDS sample buffer as fraction bound. Samples were resolved into 8-10% SDS PAGE and gels were dried and autoradiographed.

Acknowledgement

We thank Dr. Jerry Workman lab for providing pRS-SWI2 and pRS-SWI1 constructs used for in vitro translation.

CHAPTER V

CONCLUSIONS AND FUTURE DIRECTIONS

In this thesis work, we dissected the structural and functional organization of the SWI/SNF chromatin remodeling complex via the SANT domain of Swi3p. In several cases, the small, ~50 amino acid SANT domain that is found within many chromatin regulatory proteins plays a key role in functional interactions with the histone N-terminal tail domains (Boyer, et al., 2004). However, in the case of Swi3p, our data indicates that the SANT domain is crucial for SWI/SNF assembly. Likewise, Guenther and colleagues found that one of the two SANT domains within the SMRT co-repressor is also required for HDAC complex assembly (Guenther, et al., 2001). Structural studies of the ISWI SANT domain confirm earlier predictions that this domain is composed of a three alpha helical bundle similar to the c-myb DNA binding domain (Grune, et al., 2003). A single amino acid substitution or a small deletion within helix 3 of the Swi3p SANT domain yields *swi/snf* mutant phenotypes in vivo (Boyer, et al., 2002), and both alterations lead to SWI/SNF disassembly in vitro. Consistent with the disassembly of SWI/SNF, the 11 amino acid deletion within the Swi3p SANT domain leads to defects in the genome-wide gene expression profile similar to a complete deletion of *SWI3*.

The purification of SWI/SNF subcomplexes from multiple *swi/snf* deletion strains or from the *swi3*Δ*SANT* strain strongly supports a model in which the SWI/SNF complex is composed of at least four interdependent modules (Figure 29) – (1) Swi2p/Arp7p/Arp9p, (2) Swi3p/Swp73p/Snf6p, (3) Snf5p, and (4) Swi1p. Since inactivation of the Swp82p,

Swp29p, and Snf11p subunits do not lead to a loss of SWI/SNF function in vivo, the organization of these nonessential subunits have not been evaluated. The interdependent nature of these SWI/SNF subcomplexes is consistent with our previous gel filtration analyses of crude yeast whole cell extracts where we found that loss of any one SWI/SNF subunit led to similar changes in the elution of the other subunits (Peterson, et al., 1994). Furthermore, loss of the Swi3p subunit caused Snf6p to elute at an apparent monomer position (Peterson, et al., 1994), consistent with our proposed Swi3p/Swp73p/Snf6p subcomplex. Remarkably, each of the four subcomplexes provide distinct functions for SWI/SNF complex: 1) the Swi2p/Arp7p/Arp9p subcomplex provides ATP-dependent DNA translocation activity coupled to chromatin remodeling (Figure 29b), since it is competent for the majority of the chromatin remodeling activities that WT SWI/SNF has. 2) The Swi3p/Swp73p/Snf6p subcomplex provides histone binding activity coupled to dimer loss (Figure 29c), and 3) the Snf5p and Swi1p subunits provide gene targeting functions by interaction with acidic activation domains of gene-specific activators (Prochasson, et al., 2003) (Figure 29a). Table 7 summarizes the ATP-dependent chromatin remodeling activities of the minimal complex and SWI/SNF- Δ2N.

Table 7. Comparison of chromatin remodeling activities of SWI/SNF complexes.

	WT	Minimal	SWI/SNF-Δ2N
ATPase activity	+	+	+
Kinetics	+	+	
DNA superhelical torsion	+	+	
R.E. accessibility on 208-11 array	+	+	
R.E. accessibility on 601-mono	+	+	+
601-mononucleosome mobility	+	+	
MMTV-mono dimer loss	+	–	–

Minimal, Swi2p/Arp7p/Arp9p subcomplex; SWI/SNF-Δ2N, SWI/SNF complex harboring a 200 a.a. deletion of the N-terminal acidic domain of Swi3p.

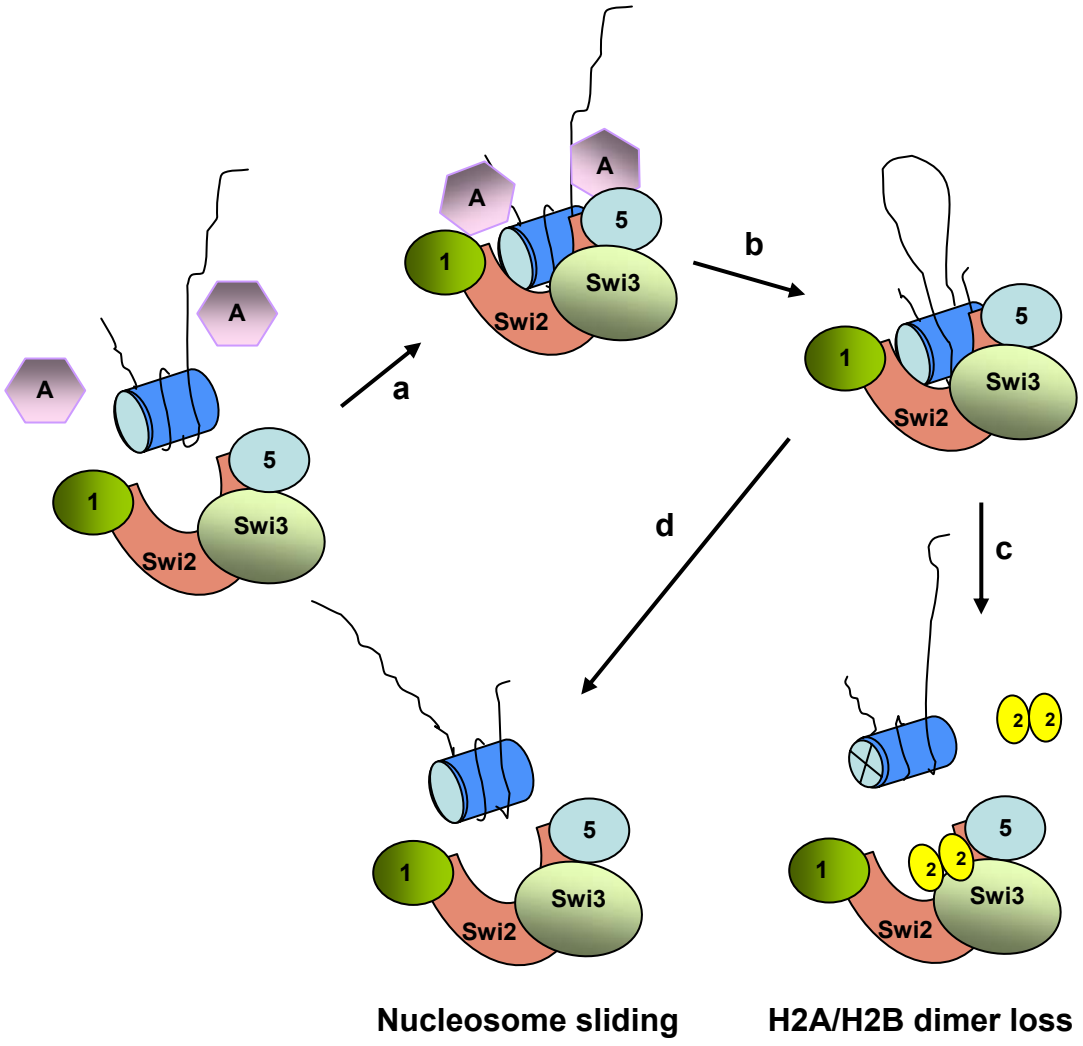


Figure 29. A model for the intermolecular and functional organization of SWI/SNF. **(a)** Swi1p and Snf5p may recruit SWI/SNF to target promoters through direct interaction with activators (Act). **(b)** SWI/SNF translocates DNA and induces the formation of DNA loop on nucleosome surface. **(c)** Destabilized nucleosome may lose H2A/H2B dimer with the help of Swi3p, or reposition the nucleosome (sliding) **(d).**

Future directions

Although both genetic and biochemical studies have revealed the important function of the ATP-dependent chromatin remodeling complexes in modifying chromatin structure, regulating transcription, DNA damage repair, and other cellular processes, there are still many fundamental questions need to be answered. What is the precise role of individual subunit for chromatin remodeling? Do they have other functions independent of SWI/SNF complex? How does each subunit contribute to chromatin remodeling and transcription beyond chromatin remodeling in vivo? What is the molecular mechanism(s) by which SWI/SNF alters the chromatin structure in vivo? The observations that mutations of human SWI2/SNF2 (BRG1), hSNF5 (INI1) and human SWI3 homologs cause cancer emphasize the importance to completely understand the functions of these genes and the molecular mechanisms of the ATP-dependent chromatin remodeling complexes. I will discuss some interesting aspects in this project.

Transcription activation of the LacZ reporter by mutant SWI/SNF complexes

During my thesis research, one of the interesting observations is about the role of different SWI/SNF mutants in transcription activation of LacZ reporter gene when SWI/SNF is tethered by LexA-Swi2p and LexA-Snf6p. In chapter II, I have demonstrated that both Swi2p catalytic activity and SWI/SNF integrity are required for LacZ reporter gene activation in vivo, since LexA-Swi2p alone, the catalytic mutant of Swi2p (swi2K798A), and swi3ΔSANT are unable to activate the LacZ reporter gene. In chapter III, I have demonstrated that Swi3p N-terminal 200 amino acids are not required for SWI/SNF assembly, and purified SWI/SNF-Δ2N complex has ATP-dependent chromatin

remodeling activity except for that it is defective for dimer loss. Therefore, we wonder if dimer displacement is required for LacZ gene activation. To test this, I co-transformed LexA-SWI2 with SWI3 or swi3Δ2N into the same LacZ reporter strain described in chapter II, and detected the beta-gal activity from these strains. Result showed that SWI/SNF-Δ2N is fully competent to activate the lacZ reporter gene, indicated by WT level of beta-gal activity (Figure 31). This data suggests H2A/H2B dimer displacement is not required for the LacZ promoter.

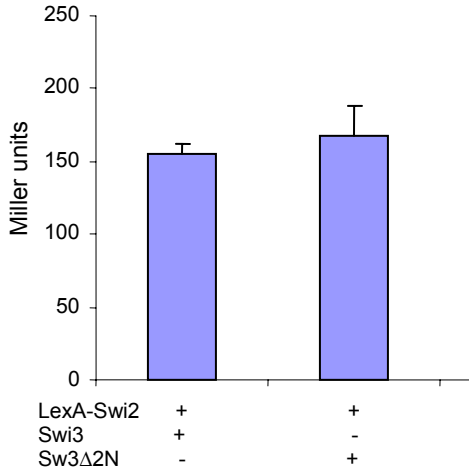


Figure 30. Swi3p N-terminal acidic domain is not required for LacZ reporter gene activation. Beta-gal assay. LexA-Swi2 (HIS3+) and SWI3 (LEU2+) or swi3Δ2N were co-transformed into the same LacZ reporter strain described in Figure 11 in chapter II. All transformants grew on SC-HIS-LEU plate for plasmid selection. Plots are averaged from three independent experiments.

Genetic evidence has shown that Snf6p is also essential for yeast SWI/SNF function (Estruch and Carlson, 1990; Laurent, et al., 1991), although Snf6p is not conserved in higher eukaryotes. Interestingly, LexA-Snf6p seems to be a better activator than LexA-Swi2 and LexA-Snf5 to activate LacZ reporter gene (Laurent, et al., 1991). As showed in Figure 32, in the ∆swi3 reporter strain, LexA-Snf6p alone is sufficient to induce the expression of beta-galactosidase in repeated experiments. In contrast to the inability of LexA-Swi2 alone or LexA-swi2K798A to activate the LacZ promoter, this result suggests that LexA-Snf6p might play other role that is independent of Swi2p at least at this promoter. Alternatively, Snf6p may play a role at a step after chromatin remodeling by Swi2p, and may directly recruit TBP or other down-stream factors for transcriptional initiation. In addition, Snf6p might play a role in transcriptional elongation, since early genetic study showed that mutation of a Pol II transcription elongation factor Spt6p suppressed the mutant phenotype of Swi2p and Snf5p, but not Snf6p (Laurent, et al., 1991). It would be interesting to perform DNA microarray in the future to identify Snf6p target genes that are both Swi2p-dependent and Swi2p-independent.

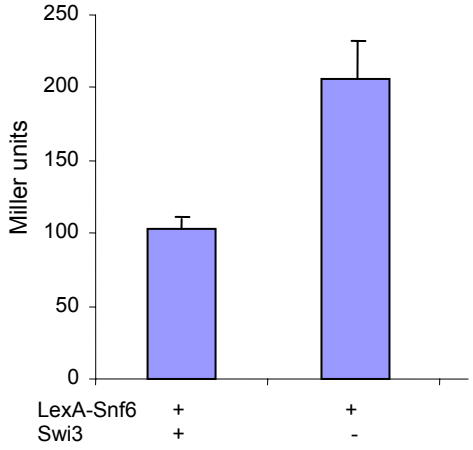


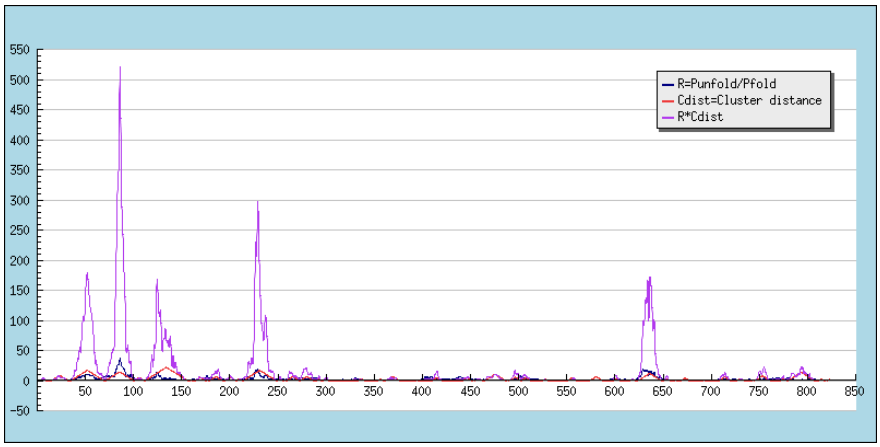
Figure 31. Tethering LexA-Snf6 alone is sufficient to activate LacZ reporter. Beta-gal activity assay. LexA-SNF6 (HIS3+) alone or with SWI3 (LEU2+) was transformed into the same LacZ reporter strain described above. All transformants grew on SC-HIS (for LexA-SNF alone) of SC-HIS-LEU (for LexA-SNF6 and SWI3) plate for plasmid selection. Three independent colonies were used to calculate the average beta-galactosidase activity.

The N-terminal acidic domain of Swi3p is predicted to be intrinsically disordered

In chapter III, I have shown that the N-terminal acidic domain of the Swi3p subunit functions as a novel histone H2A/H2B binding module that is required for dimer displacement in vitro. Although the Swi3p N-terminal acidic domain is not conserved in human Swi3 homologs, the histone chaperone function of this domain may help us to further understand dimer displacement as a mechanism for SWI/SNF to manipulate chromatin structure in vivo. The fact that Swi3p N-terminus deletion allele does not cause a growth phenotype in YEPD rich media suggests that its function may be redundant with other histone chaperones. It remains interesting to test whether the histone chaperone role of the Swi3p N-terminal acidic domain is required under other growth conditions, especially under specific gene induction conditions, since dimer loss was observed after progesterone induced activation of MMTV promoter in human cells (Vicent, et al., 2004). In budding yeast, it would be interesting to check whether dimer loss occurs at the *SUC2* promoter since deletion of either H2A or H2B suppressed the mutant phenotype of swi/snf (Hirschhorn, et al., 1992).

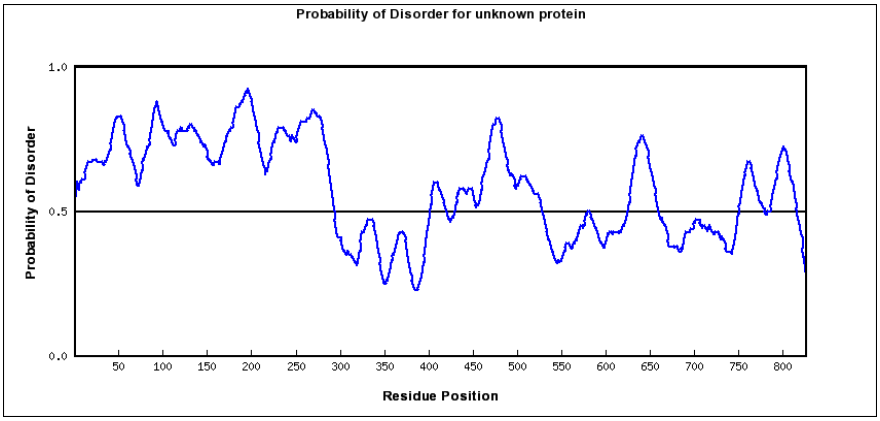
Formation of a proper 3-dimensional (3D) structure is critical for protein function. However, many proteins have large intrinsically disordered domains that are known to be functionally important. It has been hypothesized that the disordered regions may only acquire a 3D ordered structure after protein-protein interactions (Hansen, et al., 2006). Using bioinformatics tools, we found that the N-terminal acidic domain of Swi3p is predicted to be intrinsically disordered compared to other regions (Figure 32). Therefore, the Swi3p acidic domain may provide an example to understand the function of disordered structures during ATP-dependent chromatin remodeling.

a.



http://genomics.eu.org/prelink/

b.



http://www.strubi.ox.ac.uk/RONN

Figure 32. Prediction of intrinsic disordered region within Swi3p. (a) Intrinsic disorder of Swi3p is predicted by http://genomics.eu.org/prelink/. The N-terminal 300 amino acids are predicted to be predominantly disordered. **(b)** High probability of the intrinsic disorder of Swi3p N-terminal 300 amino acids predicted by http://www.strubi.ox.ac.uk/RONN.

APPENDIX

List of Yeast Strains Used in Thesis Research

CY165	*MATα swi3Δ :: URA3*
CY437	*MATα swi3Δ :: TRP1*
CY666	*MATα swi3Δ URA3 :: SWI3*
CY667	*MATα swi3Δ URA3*
CY669	*MATα swi3Δ URA3 :: swi3ΔSANT*
CY675	*MATα swi3Δ URA3 :: swi3R564A*
CY678	*MATα swi3Δ URA3 :: swi3Δ4N'* (deletion of 400 a.a. from N-terminus)
CY679	*MATα swi3Δ URA3 :: swi3Δ5N'* (deletion of 500 a.a. from N-terminus)
*CY1004	*MATα swi3Δ URA3 :: SWI3 SWI2-13XMyc^{kan}*
*CY1005	*MATα swi3Δ URA3 SWI2-13XMyc^{kan}*
*CY1006	*MATα swi3Δ URA3 :: swi3ΔSANT SWI2-13XMyc^{kan}*
*CY1053	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ* integrated into CY165
*CY1054	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ LexA-SWI2 HIS3 SWI3 LEU2*
*CY1055	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ LexA-SWI2 HIS3 swi3ΔSANT LEU2*
*CY1063	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ LexA-SWI2 HIS3*
*CY1064	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ SWI3 LEU2*
*CY1078	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ LexA-SWI2K978A HIS3 SWI3 LEU2*
*CY1079	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ LexA-SWI2K978A HIS3 swi3ΔSANT LEU2*
*CY1063	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ LexA-SNF6 HIS3*
*CY1080	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ LexA-SNF6 HIS3 SWI3 LEU2*
*CY1081	*MATα swi3Δ::URA3 LexA$_{op}$-GAL1$_{TATA}$-LacZ LaexA-SNF6 HIS3 swi3ΔSANT LEU2*
*CY1103	*MATα swi3Δ URA3 :: SWI3 SWI2-TAP^{kan}*
*CY1104	*MATα swi3Δ URA3 :: swi3ΔSANT SWI2-TAP^{kan}*
*CY1105	*MATα swi3Δ URA3 SWI2-TAP^{kan}*
*CY1106	*MATα swi3Δ URA3 :: SWI3 SNF6-TAP^{kan}*
*CY1107	*MATα swi3Δ URA3 :: swi3ΔSANT SNF6-TAP^{kan}*
*CY1108	*MATα swi3Δ URA3 SNF6-TAP^{kan}*
*CY1161	*MATα swi3Δ URA3 :: swi3Δ4N' SWI2-TAP^{kan}*
*CY1162	*MATα swi3Δ URA3 :: swi3Δ5N' SWI2-TAP^{kan}*

*CY1163 *MATα swi3Δ URA3 ::swi3Δ4N' SNF6-TAP^{kan}*
*CY1164 *MATα swi3Δ URA3 :: swi3Δ5N' SNF6-TAP^{kan}*
*CY1316 *SWI3Δ1N :: URA3* integrated into CY437 (SWI3 delta N'-terminal 100 a.a.)
*CY1317 *SWI3Δ2N :: URA3* integrated into CY437 (SWI3 delta N'-terminal 200 a.a.)
*CY1318 *SWI3Δ3N :: URA3* integrated into CY437 (SWI3 delta N'-terminal 300 a.a.)
*CY1319 TAP-SWI2 in CY1316
*CY1320 TAP-SNF6 in CY1317 (SWI3 delta N'-terminal 200 a.a.)
*CY1321 TAP-SWI1 IN CY666
*CY1322 TAP-SWI1 in CY669
*CY1323 TAP-SNF5 in CY666
*CY1324 TAP-SNF5 in CY669
*CY1325 TAP-SNF5 in CY119 (Δswi1)
*CY1326 TAP-SNF5 in CY407
*CY1327 TAP-SNF6 in CY575 (Δsnf5)
*CY1328 TAP-SWI2 in CY694 (swi3R564E)
*CY1340 CY1053+LexA-Swi2+pRS415-swi3Δ2N)

*These strains are made by myself during thesis research.

List of Plasmids Used in This Thesis Research

CP310	$LexA_{op}$-$GAL1_{TATA}$-LacZ
CP430	SWI3Δ1N in RS406 (SWI3 delta N'-terminal 100 a.a.)
CP431	SWI3Δ2N in RS406 (SWI3 delta N'-terminal 200 a.a.)
CP432	SWI3Δ3N in RS406 (SWI3 delta N'-terminal 300 a.a.)
CP488	pLexA-SWI2
CP645	pLexA-SNF6
CP717	xenopus histone H2A
CP718	xenopus histone H2B
CP719	xenopus histone H3
CP720	xenopus histone H4
CP740	xenopus histone H2A-GST fusion
CP741	xenopus histone H2B-GST fusion
CP742	xenopus histone H3-GST fusion
CP743	xenopus histone H4-GST fusion
*CP988	pRS415-SWI3
*CP989	pRS415-swi3ΔSANT
CP999	pFA6a-TAP (CaM-protein A):Kan
CP1024	"601" nucleosome positioning sequence
CP1035	xenopus H3-C110A
*CP1052	pCMX-Flag-SNF5 (T7, IVT)
*CP1058	pCMX-HA-SWI3 (T7, IVT)
*CP1059	pCMX-HA-swi3ΔSANT (T7, IVT)
*CP1063	pCMX-SWP73 (T7, IVT)
*CP1064	pCMX-ARP7 (T7, IVT)
*CP1065	pCMX-ARP9 (T7, IVT)
CP1071	MMTV nucleosome B positioning sequence (Beato group gift)
*CP1101	pGEX-2TA-GST-SWI3-3N
*CP1102	pGEX-2TA-GST-SANT (GST-SANT)
*CP1103	pET-3A-SWI2-N' (1-834 a.a., T7, IVT)

*CP1104	pCMX-SWI2-C' (after 834a.a., T7, IVT)
*CP1105	pCMX-SWI3-3N (N'-terminal 300a.a., T7, IVT)
*CP1106	pCMX-SWI3-C' (C-terminus after 3N, T7, IVT)
*CP1107	pGEX-2TA-GST (GST expression only)
*CP1108	pCMX-SNF6 (T7, IVT)
*CP1109	xenopus H2A-C113A
*CP1110	pRS415-swi3Δ2N (LEU2+)
*CP1113	pCMX-swi1Δ4N (deletion N'-terminal 400a.a)
*CP1120	pCMX-Flag-Arp9 (T7, IVT)

*These plasmids are made by myself during thesis research.

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