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Composition and Function of Mutant Swi/Snf Complexes

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Article

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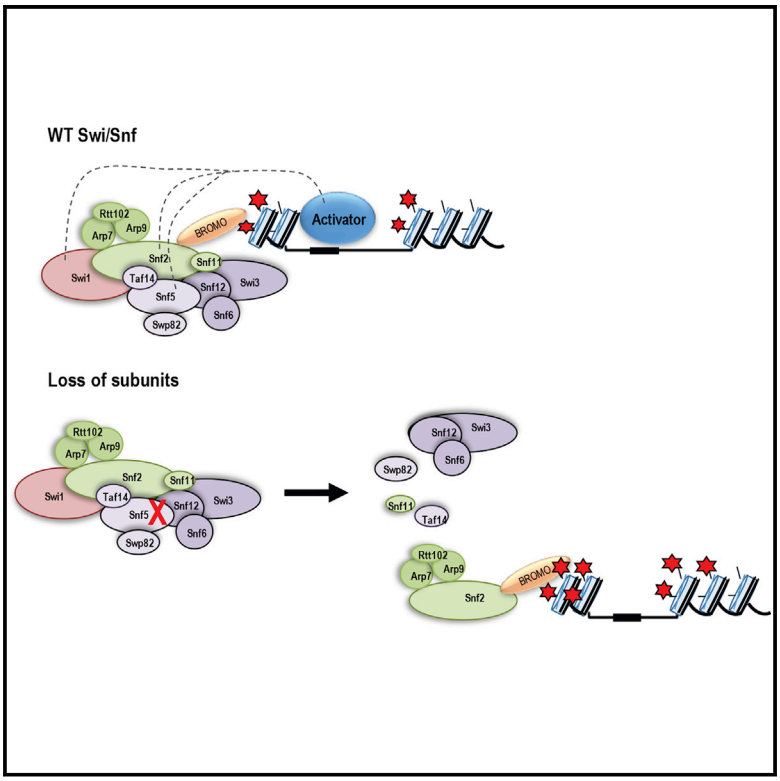
Graphical Abstract



Authors

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In Brief

Subunits of the Swi/Snf chromatin-remodeling complex are mutated in 20% of cancers. Dutta et al. report modularity within yeast Swi/Snf that regulates both architecture and genomic functions of the complex. These findings will help predict complex composition in diseased states where subunits of the complex are mutated.

Highlights

- Swi/Snf complex architecture reveals modules within the complex
- Regulation of gene expression reflects modularity within Swi/Snf
- Targeting of Snf2 to genes depends on Swi/Snf subunits and histone acetylation
- Modularity may explain functions of residual Swi/Snf complexes in human cancers

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Composition and Function of Mutant Swi/Snf Complexes

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SUMMARY

The 12-subunit Swi/Snf chromatin remodeling complex is conserved from yeast to humans. It functions to alter nucleosome positions by either sliding nucleosomes on DNA or evicting histones. Interestingly, 20% of all human cancers carry mutations in subunits of the Swi/Snf complex. Many of these mutations cause protein instability and loss, resulting in partial Swi/Snf complexes. Although several studies have shown that histone acetylation and activator-dependent recruitment of Swi/Snf regulate its function, it is less well understood how subunits regulate stability and function of the complex. Using functional proteomic and genomic approaches, we have assembled the network architecture of yeast Swi/Snf. In addition, we find that subunits of the Swi/Snf complex regulate occupancy of the catalytic subunit Snf2, thereby modulating gene transcription. Our findings have direct bearing on how cancer-causing mutations in orthologous subunits of human Swi/Snf may lead to aberrant regulation of gene expression by this complex.

INTRODUCTION

Eukaryotic cells tightly package DNA with histones into chromatin. Access to the underlying DNA sequence is critical for functioning of various cellular machineries involved in transcription of genes and DNA replication, recombination, and repair (Clapier and Cairns, 2009). Modulation of chromatin structure is dependent on chromatin-modifying enzymes, which work within large macromolecular complexes to either post-translationally modify histones or use ATP to slide or evict histone octamers (Narlikar et al., 2013). Recent genome-wide profiling in diseased states has highlighted that somatic mutations in genes encoding subunits of chromatin-modifying complexes are common drivers of tumorigenesis and disease (Morgan and Shilatifard, 2015; Ronan et al., 2013; Roy et al., 2014). Often these mutations lead to loss of proteins themselves. However, the mechanistic implications of these mutations on the overall structure of chromatin-modifying complexes and their bearing on altered transcriptional output remains poorly understood.

Among the ATP-dependent chromatin remodelers, the conserved Swi/Snf complex is the founding member. This 1.1-MDa complex, made up of 12 subunits in yeast and 11–15 subunits in humans, is targeted to genes by sequence-specific transcription factors and acetylation of histone tails (Becker and Workman, 2013; Chandy et al., 2006; Dutta et al., 2014; Hassan et al., 2006; Neely et al., 2002; Prochasson et al., 2003). Several hSwi/Snf subunits act as tumor suppressors, with frequency of mutations in Swi/Snf subunits estimated at 20% across all cancer types (Lawrence et al., 2014; Roberts and Orkin, 2004; Kadoch et al., 2013). Mutations in specific subunits (often non-ATPase subunits) that result in carcinogenesis in a tissue-specific manner, include loss-of-function mutations in Arid1a, Ini1, BAF180, Brg1, and BAF155, which are commonly associated with ovarian, rhabdoid, renal, lung, and breast cancers (Bultman et al., 2008; Girard et al., 2000; Jones et al., 2010; Kadoch et al., 2013; Varela et al., 2011). Although studies have highlighted the mechanism of action of the Swi/Snf complex, less is understood about how the different subunits contribute to the integrity of the complex (Helming et al., 2014a; Wang et al., 2014). Subunit loss that results from mutations in cancers and other diseases may alter the composition and in vivo function of Swi/Snf. Thus, it is essential to understand the architecture of this complex and consequences of changes in its composition.

Using ATP hydrolysis by the catalytic subunit (Snf2 in yeast; Brg1/Brahma in humans), Swi/Snf disrupts histone:DNA contacts, resulting in sliding of the histone octamer on DNA or the eviction of histones (Clapier and Cairns, 2009; Hirschhorn et al., 1992). Yeast Swi/Snf is recruited to ~10% of all genes during normal growth conditions but is required for transcription that occurs as a response to several stress conditions (Dutta et al., 2014; Shivaswamy and Iyer, 2008; Sudarsanam et al., 2000). Although yeast has one form of Swi/Snf, multiple human complexes often have unique components with specialized roles in development and tissue-specific gene regulation. For example, replacement of BAF53a with BAF53b is a crucial step in neuronal differentiation in mouse and flies (Lessard et al., 2007; Parrish

et al., 2006). Moreover, subunit paralogs in pluripotent embryonic stem cells and differentiated tissues determine the transition from pluripotency to multipotency during differentiation (Gao et al., 2008; Ho et al., 2009; Kidder et al., 2009; Lickert et al., 2004).

Mechanistic insights regarding Swi/Snf have come from work in many model organisms like yeast. Given that the frequently mutated subunits Arid1a/BAF180, Ini1, Brg1, and BAF155 have orthologous yeast counterparts Swi1, Snf5, Snf2, and Swi3 (Kwon and Wagner, 2007; Wang et al., 1996), deciphering the architecture of yeast Swi/Snf is a valuable springboard to understanding its mammalian counterpart. Although three-dimensional reconstruction of cryo-electron microscopy has solved the structure of yeast Swi/Snf and human PBAF complexes, little is known about subunit organization (Dechassa et al., 2008; Leschziner et al., 2005; Smith et al., 2003). Also, the impact of altering the integrity of the Swi/Snf complex on Snf2 occupancy, nucleosome occupancy, and gene expression has not been reported genome-wide. We aimed to understand the organization of proteins within the yeast Swi/Snf complex and how changes in composition affect its functions in vivo. We used systematic biochemical purification of intact and partial Swi/Snf complexes for wild-type and Swi/Snf subunit deletion strains, quantitative proteomics, and genomic approaches. Our proteomic analysis revealed four distinct Swi/Snf modules: an Arp module, a catalytic module, a Snf5/Swi3 regulatory module, and a Swi1 module. Although some subunits are crucial for the integrity of the complex, others are peripheral. This modularity was also reflected in gene expression profiles, in which changes clustered similarly upon deletion of subunits in same module. Our results concerning the modularity of Swi/Snf are also consistent with the results in the accompanying paper in this issue of *Cell Reports* by Sen et al. (2017) where cross-linking mass spectrometry was used to analyze the architecture of the complex. Furthermore, we show that Snf2 occupancy and function are independent of complex integrity and levels of histone acetylation. Because the Swi/Snf complex is evolutionarily conserved, we hypothesize that human Swi/Snf will have a similar architecture to its yeast counterpart, that similar changes in composition and function will result from disease-associated mutations in hSwi/Snf subunits, and that these changes will alter critical transcriptional programs.

RESULTS

Mass-Spectrometric Analysis Shows Modularity within the Yeast Swi/Snf Complex

To determine the organization of subunits in the yeast Swi/Snf complex, we used quantitative mass spectrometry and systematic purification of TAP-tagged subunits from both wild-type (WT) and subunit deletion strains. The rationale was to determine how subunit mutations compromise the integrity of the complex. Using computational methods (Lee et al., 2011), the raw quantitative values were interpreted to determine the interrelationship between the subunits, allowing us to predict a macromolecular model for organization of Swi/Snf.

TAP-tag purifications were carried out for 11 of the 12 Swi/Snf subunits (Table S1) under stringent conditions. Silver staining of purified complexes is shown in Figure S1. Purified protein complexes were subjected to multidimensional protein identification technology (MudPIT) (Swanson et al., 2009) and protein abundance quantified using the distributed normalized spectral abundance factors (dNSAFs) (Zhang et al., 2010). Non-specific proteins (contaminants) were extracted by comparing the data from each purification to a mock control (untagged yeast strain). The dNSAF values were hierarchically clustered (Figure 1A). Purification through TAP-tags on the Swi/Snf specific subunits Swi1, Snf2, Snf5, Snf6, Swi3, and Swp82 co-purified the entire Swi/Snf complex minus Snf11, suggesting that each member is stably associated within Swi/Snf. Low dNSAF values for Snf11 were most likely due to its weak association with other Swi/Snf subunits, and not a reflection of low abundance, because purification through a TAP-tag on Snf11 pulled down all members of Swi/Snf. As anticipated, purification through TAP-tagged subunits Arp7, Arp9, Rtt102, and Taf14 showed lower dNSAF values for the remaining Swi/Snf subunits, due to association of Arp7, Arp9, and Rtt102 with the RSC chromatin-remodeling complex (Peterson et al., 1998; Szerlong et al., 2003) and for Taf14 due to its additional association with TFIID, TFIIF, Nua3, and Ino80 complexes (Cairns et al., 1996; Zhang et al., 2004).

To gain insights into the modularity and interrelationship of subunits in the Swi/Snf, complex, we used an unbiased comparative approach in which complexes were purified via TAP-tagged subunits from strains in which other individual subunits were deleted. We purified TAP-tag Swi1 from snf5Δ, snf11Δ, snf12Δ, swp82Δ, and taf14Δ deletion strains; TAP-tag Snf2 from snf5Δ, snf6Δ, snf11Δ, rtt102Δ, and taf14Δ deletion strains; and TAP-tag Snf6 from a snf5Δ deletion (Table S1 and Figure S1). Strains lacking *ARP7*, *ARP9*, and *SWI1* are not viable, and could not be used for purifications. To ensure the robustness of our results, replicates were included.

One method to analyze proteomics data is to cluster proteins hierarchically based on their relative abundance level. We subjected the 12 × 29 matrix to hierarchical clustering analysis to identify groups of proteins with similar abundance levels (Figure 1B). Loss of Snf5, Snf6, and Snf12 resulted in dissociation of the Swi/Snf complex, highlighting their roles in complex stability. Swp82 and Taf14 no longer associated with Swi1-, Snf2-, or Snf6-associated complexes in the absence of Snf5. Loss of Snf12 significantly decreased association of Snf6, Swp82, and Taf14 with Swi1. Loss of Snf6 decreased association of Swi3, Snf12, Snf5, Swp82, Taf14, and Swi1 with Snf2, Arp7, Arp9, and Rtt102. Interestingly, purification of TAP-Snf6 from a Snf5 deletion strain revealed an intact submodule comprised of Snf6, Snf12, and Swi3 that associated only weakly with the rest of the Swi/Snf complex. However, loss of Snf11, Swp82, Taf14, and Rtt102 did not result in dissociation of the complex or loss of other Swi/Snf subunits, suggesting that these subunits are peripheral members of the complex and not essential for its stability.

Based on hierarchical clustering analysis of the deletion dataset (Figure 1B), we propose that Swi/Snf is composed of four major groups of subunits (hereafter referred to as modules) (Figure 1C): (1) the Swi/Snf-RSC shared Arp module, composed of Arp7, Arp9, and Rtt102; (2) the catalytic module, consisting of

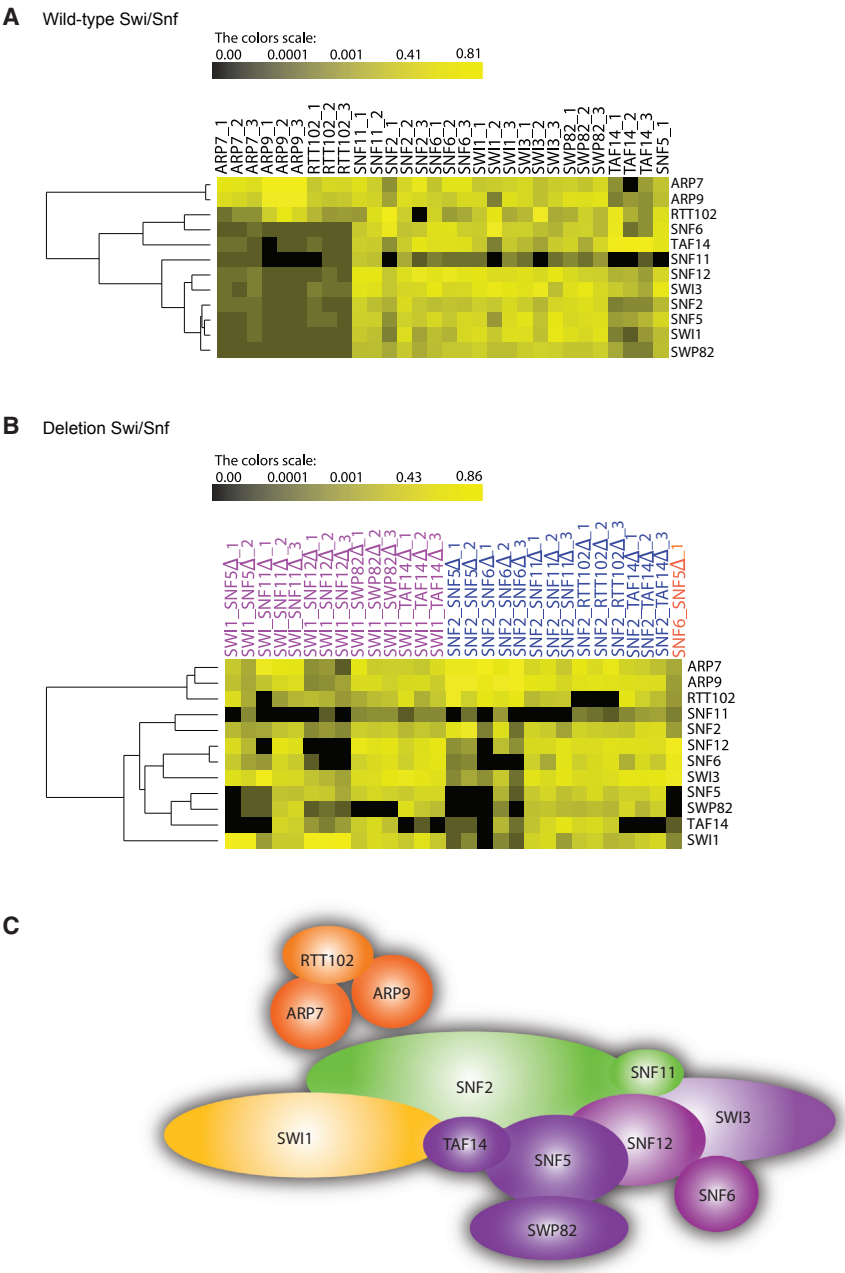

Figure 1. Architecture of Swi/Snf Displays Modularity within the Complex

(A) Hierarchical clustering from wild-type purifications. Hierarchical clustering using WARD algorithm and Pearson correlation as distance metric was performed on the relative protein abundance expressed as dNSAFs. Each column represents an isolated purification, and each row represents an individual protein. The color intensity depicts the protein abundance, with bright yellow indicating the highest abundance and black indicating when protein was not detected in a particular sample.

(B) Hierarchical clustering on different deletion strains. Each column represents an isolated TAP, and each row represent an individual protein, with a color code above.

(C) Macromolecular assembly of the SWI/SNF complex. Based on deletion purifications and hierarchical clustering, all SWI/SNF proteins were organized into modularity to assemble the macromolecular model. The clustering analysis revealed a clear dissociation of the Swi/Snf complex and four major modules: (1) Arp7-Arp9-Rtt102 (orange); (2) Snf11-Snf12 (green); (3) Snf12-Snf6-Swi3 submodule and Taf14-Snf5-Swp82 submodule (purple); (4) Swi1 (yellow).

Snf2 and Snf11; (3) the Snf5/Swi3 module, made up of two submodules: a Snf6-Snf12-Swi3 submodule and a Snf5-Swp82-Taf14 submodule; and (4) and the Swi1 module on its own. In addition, the Snf6-Snf12-Swi3 submodule requires the Snf5-Swp82-Taf14 submodule to interact with Snf2.

Modularity within Swi/Snf Is Reflected in Regulation of Gene Expression by Individual Subunits of Swi/Snf

We next set out to determine how this modularity affected regulation of gene expression. We isolated RNA from WT and deletion strains of all nonessential subunits of Swi/Snf. Ribosomal RNAs were removed using Ribo-deletion and RNA samples analyzed by strand-specific sequencing (RNA-seq). Genes that were either up or downregulated at least 2-fold ($p < 0.05$) in any mutant compared to WT were hierarchically clustered for both gene and sample ordering (Figure 2A). The most significant changes in gene expression were observed when *SNF2*, *SWI3*, and *TAF14* were deleted, whereas the least changes were observed in swp82Δ, snf11Δ, and rtt102Δ strains. Interestingly, the clustering of transcript levels reflected the modularity observed by proteomic analyses. Gene expression changes in deletions of *SWI3*, *SNF12*, *SNF5*, and *SNF6* clustered together and were separate from those observed in the snf2Δ strain. In addition, deletion of *SWP82*, *SNF11*, and *RTT102*, which did not affect complex stability, did not significantly affect gene expression and clustered with WT. Loss of *TAF14* resulted in a gene expression pattern separate from that seen in loss of other Swi/Snf-specific subunits and clustered separately. This pattern may be due to roles that Taf14 plays through its association with other complexes (Cairns et al., 1996; Kabani et al., 2005; Zhang et al., 2004). Together, these results suggest that the integrity of Swi/Snf is critical for gene regulation, and that structural modules within Swi/Snf may differentially regulate gene expression (Figure 2B).

To determine how some subunits regulate gene expression combinatorically and others disparately, we carried out K-means clustering of the RNA-seq data from Swi/Snf complex subunit

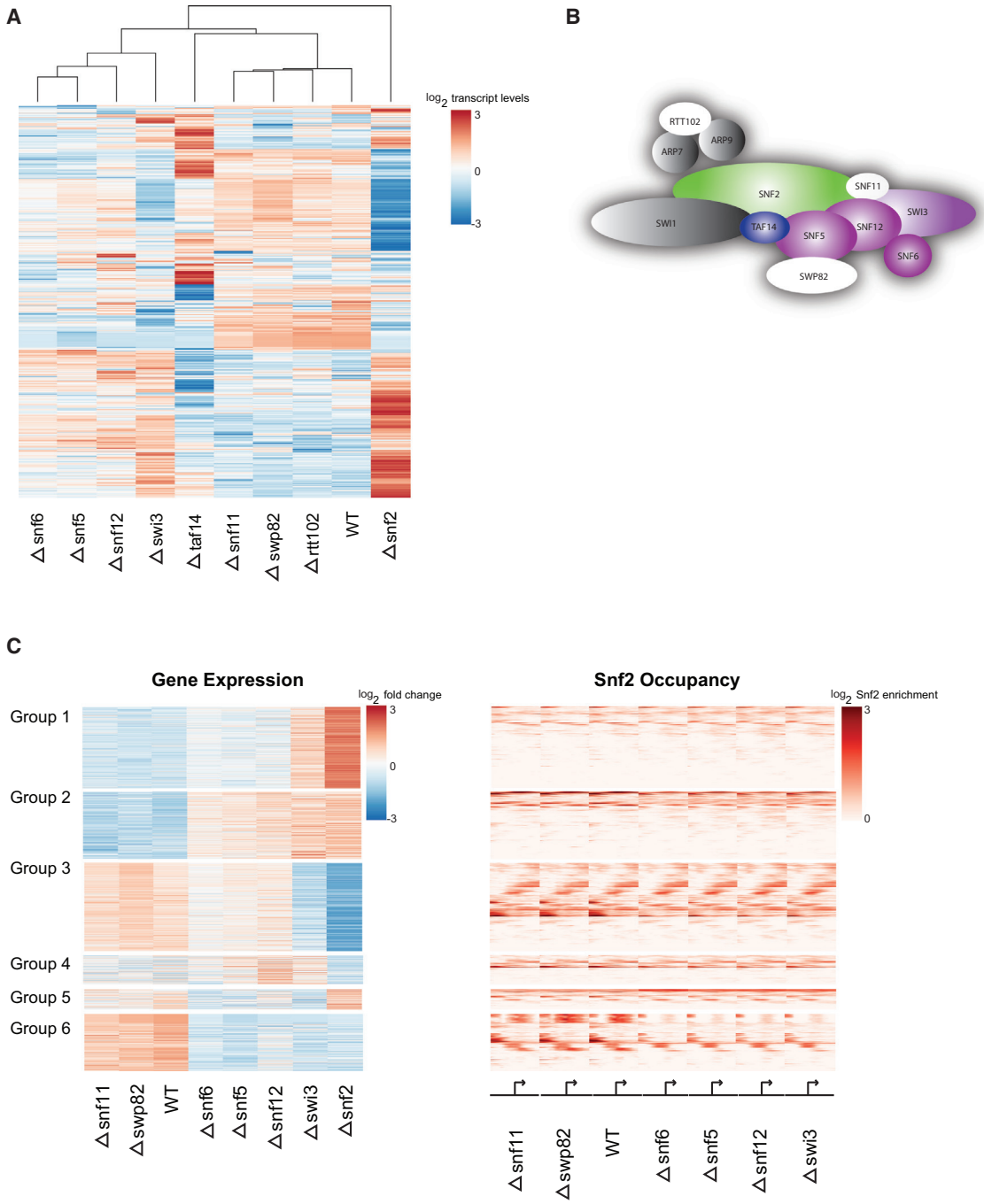

Figure 2. Modularity Reflects Regulation of Gene Expression by Subunits of the Complex

RNA-seq was carried out to determine changes in transcript levels in WT and Swi/Snf subunit deletion strains. Genes that changed at least 2-fold compared to WT in any mutant ($p < 0.05$) were used for further analysis.

(A) Heatmap of hierarchically clustered transcript levels in WT and snf6Δ, snf5Δ, snf12Δ, swi3Δ, snf11Δ, swp82Δ, rtt102Δ, taf14Δ, and snf2Δ strains. Each row represents a gene, and columns represent WT or Swi/Snf subunit deletion strains.

(B) Macromolecular assembly of the Swi/Snf complex based on the hierarchical clustering of changes in transcription in deletion strains. Snf12-Snf6-Swi3-Snf5 module (purple) is clustered together and away from Snf2 module (green). Taf14 (blue) shows a distinct pattern of gene expression that is different from other subunits. Loss of Rtt102, Snf11, and Swp82 did not result in significant changes compared to WT (white). Gray indicates essential genes that could not be included in this analysis (Swi1, Arp7, and Arp9).

(C) Heatmaps of $\log_2$ transcript levels of genes with 2-fold change in transcription compared to WT ($p < 0.05$) in snf6Δ, snf5Δ, snf12Δ, swi3Δ, snf11Δ, swp82Δ, and snf2Δ strains clustered by a k-means algorithm into six groups and plotted. The heatmap on the right shows Snf2 occupancy [$\log_2$ (immunoprecipitation/input) values] for regions −500/+700 around the TSS of genes. Genes in both heatmaps are ordered similarly to represent transcript levels and Snf2 occupancy at each gene.

deletion strains that include snf5Δ, snf6Δ, snf11Δ, snf2Δ, snf12Δ, swi3Δ, and swp82Δ. Clustering into six groups showed that changes in patterns of gene expression also reflect Swi/Snf modularity. Genes in groups 2 are upregulated compared to WT (gene ontology [GO] terms for sulfate assimilation, sulfur metabolism, methionine and cysteine biosynthesis, and glycolytic pathways), whereas genes in group 6 are downregulated (GO terms for genes involved in transposition, phosphate metabolism, and DNA recombination and replication) (Figure S2). These genes exhibited similar changes in expression in all deletion strains that affected Swi/Snf stability or function, and suggest that an intact Swi/Snf complex is essential for their regulation (Figure 2C). Genes in group 1 (GO terms for genes involved in arginine biosynthesis, mitochondrial degradation, and cellular amino acid synthesis) (Figure S2) were most significantly upregulated when *SNF2* and *SWI3* were deleted (Figure 2C). Genes in group 3 (GO terms for genes involved in regulation of translation, rRNA processing, and ribosomal assembly) (Figure S2) were downregulated in snf2Δ and swi3Δ strains, with lesser effects in snf5Δ, snf6Δ, and snf12Δ strains (Figure 2C). Expression of these groups (1 and 3) is more dependent on the catalytic functions of Snf2 and on the Swi3 subunit. Interesting changes in expression were observed for genes in groups 4 and 5 (Figure 2C). Although group 4 genes are involved in glucose import (Figure S2) and were downregulated in the snf2Δ strain, they showed modest upregulation in strains deleted for subunits in the Snf5/Swi3 regulatory module (snf12Δ, snf5Δ, snf6Δ, and swi3Δ strains). Following a similar trend, genes in group 5 (GO terms for genes involved in detoxification of copper and cadmium, sucrose catabolism, and rRNA pseudo-uridine synthesis) (Figure S2) were downregulated in strains deleted for Snf5/Swi3 regulatory module subunits (snf5Δ, snf6Δ, and swi3Δ strains) but increased in expression when *SNF2* was deleted (Figure 2C). Thus, although an intact Swi/Snf complex is required for transcription of a subset of yeast genes, loss of subunits can have different effects in the regulation of other genes.

Differential Requirement for Swi/Snf Subunits in Regulation of Snf2 Occupancy

Our RNA-seq analysis identified genes that are regulated by Swi/Snf. However, yeast Swi/Snf occupancy is observed at only 10% of genes, suggesting that many genes with changes in transcript level may not be direct targets (Dutta et al., 2014; Sudarsanam et al., 2000). To identify direct targets of the complex, we carried out chromatin immunoprecipitation (ChIP) using antibodies targeted against the catalytic subunit Snf2 in WT and Swi/Snf subunit deletion strains, followed by high-throughput sequencing (ChIP-seq). Snf2 binding peaks were called using a cutoff of 1.5-fold signal over input. Heatmaps of Snf2 occupancy (500 bp upstream and 700 bp downstream of the transcription start sites [TSSs]) revealed that only a subset of genes showing changes in expression were occupied by Snf2 (Figure 2C). Groups 3 and 6, where genes were downregulated compared to WT, included the highest number of genes occupied by Snf2. In addition, Snf2 occupancy was lost at a subset of genes in groups 3 and 6 when subunits in the Snf5/Swi3 regulatory module (*SNF5*, *SNF6*, *SNF12*, and *SWI3*) were deleted. This result suggests that Swi/Snf complex integrity plays an important role in regulating Snf2 occupancy at a subset of genes. Mirroring results observed in RNA-seq analysis, loss of Snf11 and Swp82, which do not contribute to Swi/Snf integrity, did not result in changes of Snf2 occupancy (Figure 2C).

To further understand how loss of Swi/Snf integrity impacts Snf2 occupancy and associated changes in gene expression, we plotted changes in Snf2 occupancy compared to WT as a function of changes in gene expression for each Swi/Snf deletion strain (Figure S3). Genes that were occupied by Snf2, showed a significant change in expression ($p < 0.05$), and were either upregulated or downregulated at least 2-fold in any mutant, were chosen for further analyses. This analysis revealed three distinct classes: genes with at least a 1.3-fold decrease in Snf2 occupancy (Figure 3A, groups A, B1, and B2), genes with at least a 1.3-fold increase in Snf2 occupancy (Figure 3A, group C), and genes in which Snf2 occupancy was unaffected (Figure 4A, groups D and E). Loss of Snf5/Swi3 regulatory module subunits (Snf5, Snf6, Snf12, and Swi3) affected Snf2 occupancy at a subgroup of 206 genes (groups A, B1, and B2), but no change in Snf2 occupancy was observed when Snf11 and Swp82 were deleted, suggesting that integrity of the Swi/Snf complex is essential for Snf2 occupancy at these genes (Figure 3A). Intriguingly, loss of Snf2 occupancy resulted in genes being both upregulated (group A) and downregulated (groups B1 and B2) (Figure 3A). Genes that were downregulated overlapped with genes in group 6 (Figure 2C), whereas genes showing an increase in transcription overlapped with genes in group 2 (Figure 2C).

We also observed an increase in Snf2 occupancy at 41 genes in Snf5/Swi3 regulatory module subunit deletion strains, but not in snf11Δ and swp82Δ strains (group C) (Figure 3A). For most genes in this group, the increased Snf2 occupancy was associated with their upregulation, and they overlapped well with genes in group 2 (Figure 2C). Although loss of Snf2 can be explained by effects on complex integrity when Swi/Snf components were deleted, we wondered why we observed an increase in Snf2 occupancy at some genes upon loss of Swi/Snf subunits. Analysis of these genes (group C) revealed their involvement in sulfur amino acid synthesis pathways (Ljungdahl and Daignan-Fornier, 2012). Important regulators of this pathway include Met28, Met31, Met32, and Met4 (Blaiseau et al., 1997; Kuras et al., 1996). To determine whether expression of genes encoding transcriptional activators was regulated by Swi/Snf, we clustered changes in transcript levels of 249 known yeast transcription factors in the different subunit deletion strains (Figure S4). Interestingly, we found that loss of subunits in the Snf5/Swi3 regulatory module (Snf5, Snf6, Snf12, and Swi3) resulted in upregulation of *MET28* and *MET32*, as is normally observed in response to growth under amino acid starvation conditions. Thus, increased levels of Met28 could be responsible for recruiting Snf2 to these genes, resulting in their upregulation.

Although Swi/Snf integrity was expected to play a major role in determining Snf2 occupancy at most genes, our analysis show that this is not the case. We observed that loss of Swi/Snf subunits did not affect Snf2 occupancy at many genes that exhibited changes in gene expression (401), suggesting that Snf2 can be targeted to genes when the complex is not intact. Such a situation would occur in the absence of Snf5/Swi3 regulatory module subunits (Figure 4A). However, the presence of Snf2 alone might

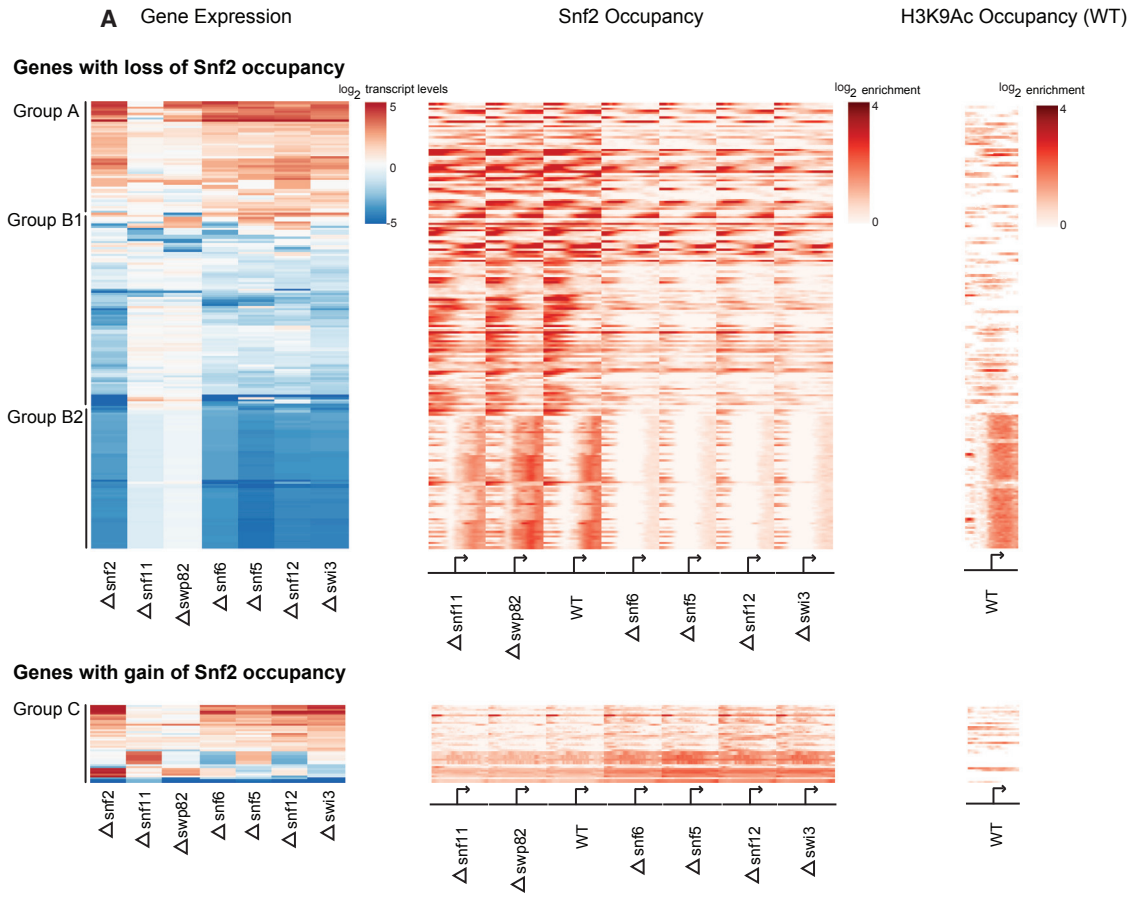

Figure 3. Swi/Snf Integrity Is Essential for Snf2 Occupancy at a Subset of Genes

(A) Genes with changes of both Snf2 occupancy (either 1.3-fold increase or decrease in occupancy) and expression (2-fold) in deletion strains compared to WT ($p < 0.05$) as determined by plots in Figure S3, were separated into groups of genes with loss of Snf2 occupancy and genes with increased Snf2 occupancy. For each group, genes were ordered on the basis of changes in transcript levels in mutants compared to WT and heatmaps for Snf2 occupancy −500/+700 around the TSS of genes and with changes in gene expression were plotted. Genes with decreased Snf2 occupancy were subdivided into those with an increase in gene expression (group A) and those with decreased gene expression (groups B1 and B2). Genes with increased Snf2 occupancy were treated as a single cluster (group C). Heatmaps for H3K9ac levels in WT were plotted for each group and are shown to the right.

not be sufficient for proper gene regulation and may require additional subunits, because defects in transcription were observed at these genes.

Variable Requirement of an Intact Swi/Snf Complex for Snf2 Occupancy May Be Determined by Levels of Histone Acetylation

Our results thus far suggest that presence of the Snf5/Swi3 regulatory module of the Swi/Snf complex only partially regulates Snf2 targeting and occupancy, and that Snf2 can be targeted at many genes even when the Snf5/Swi3 regulatory module is disrupted (Figures 3 and 4). Studies in our laboratory and others have shown that both levels of histone acetylation and the presence of transcriptional activators affect recruitment of Swi/Snf (Chandy et al., 2006; Gutiérrez et al., 2007; Neely et al., 2002; Prochasson et al., 2003). To further understand the disparate requirement of an intact Swi/Snf complex in regulating Snf2 occupancy, we evaluated the levels of H3K9Ac in the WT strain for all groups of genes. Although the average occupancy of H3K9Ac was higher in groups B2, D, and E, less acetylation was observed for groups A, B1, and C (Figure S5). We then generated heatmaps of H3K9Ac to determine whether genes within each group displayed varied levels of histone acetylation. Most genes in groups D and E, where Snf2 occupancy was not dependent on the Snf5/Swi3 regulatory module, showed high levels of histone acetylation (Figures 4A and S5). Furthermore, although most genes in groups A and C had low levels of histone acetylation, genes in group B were clearly divided into two classes. Group B1 genes showed low levels of histone acetylation, whereas B2 genes showed higher levels of acetylation around the TSS (Figures 3A and S5). Closer analysis of these two clusters showed that genes within yeast transposable elements (group B2) required both an intact Swi/Snf complex and high levels of acetylation for Snf2 occupancy and expression. By comparison, protein-coding genes in group B1 had lower levels of histone acetylation and were more dependent on the Snf5/Swi3 regulatory module subunits (Snf5, Snf6, Snf12, and Swi3) for Snf2 occupancy and expression. These observations suggest that high

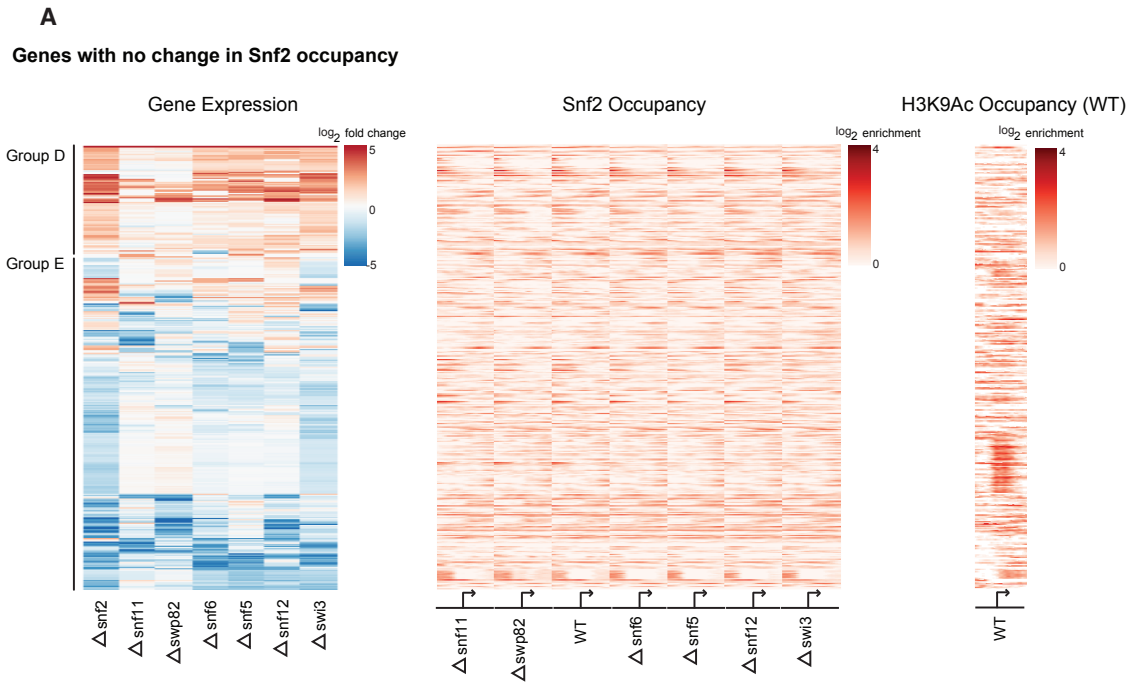

Figure 4. Snf2 Is Targeted to Genes in the Absence of an Intact Swi/Snf Complex

(A) Genes with no change in Snf2 occupancy and 2-fold in expression in subunit deletion strains compared to WT ($p < 0.05$), as determined by plots in Figure S3, were clustered based on changes in transcript level in the deletion strains. Heatmaps showing Snf2 occupancy −500/+700 around the TSS of genes and changes in gene expression were plotted. Genes were subdivided based on changes in transcription levels in mutants to those with increased expression (group D) and decreased expression (group E). Corresponding H3K9ac level in WT was plotted as a separate heatmap shown to the right.

levels of histone acetylation (e.g., group D and E genes) may be sufficient to recruit Snf2 via interaction with its bromodomain.

This observation suggests that Snf2 recruitment to promoters of these genes may be more dependent on histone acetylation than on Snf5/Swi3 regulatory module subunits. If this were indeed the case, recruitment of Snf2 to genes with high levels of histone acetylation (groups B2, D, and E) would require the Snf2 bromodomain for recruitment. To test whether this were the case, we plotted Snf2 occupancy in WT and Snf2-delta bromodomain strains in each of the groups (Figure 5) (Dutta et al., 2014). We found that genes in groups B2 and E, which had high levels of histone acetylation, the loss of the Snf2 bomodomain resulted in a significant decrease (p value < 0.05) in Snf2 occupancy compared to WT. This suggests that indeed at these genes Snf2 can be recruited via its bromodomain and may not require the Snf5/Swi3 regulatory module. However, although genes in group D had higher levels of H3K9ac (Figure S5), loss of the Snf2 bromodomain did not significantly alter occupancy of Snf2 (Figure 5). Interestingly, loss of Swi/Snf subunits showed a decrease in expression of genes in groups B2 and E, whereas genes in group D showed an increase (Figures 3 and 4). Our results are consistent with previous results where loss of the Snf2 bromodomain decreased gene expression. We speculate that, at genes that are suppressed by Swi/Snf (group D), additional factors may be required for Snf2 occupancy at these genes. Thus, our results clearly show that failure of the Snf2 bromodomain to bind acetylated histones necessitates additional activator contacts for recruitment of Snf2 at genes with low levels of histone acetylation. These interactions are provided by the Snf5/Swi3 regulatory module, including Snf5, which can directly interact with activators (Neely et al., 2002).

DISCUSSION

To understand organization of yeast Swi/Snf and the impact of subunit loss on complex integrity, we conducted a systematic proteomics analysis of Swi/Snf purified from subunit deletion strains. The stable subcomplexes revealed in this analysis suggest structural modularity (Figure 1). We propose that Swi/Snf is composed of at least four distant functional modules: (1) the Arp module, which is shared with the RSC complex (Peterson et al., 1998; Szerlong et al., 2003) and contains Arp 7, Arp9, and Rtt102; (2) the Snf2 and Snf11-containing ATPase module; (3) a Snf5/Swi3 regulatory module of Snf5, Swp82, Taf14, Snf6, Snf12, and Swi3; and (4) Swi1 on its own (Figure 1C). Our model validates previous studies showing direct binding of Snf11 with Snf2 (Treich et al., 1995) and association of the Arp module (Arp7, Arp9, and Rtt102) with the Snf2 HSA domain (Yang et al., 2007). Our suggested organization of Swi/Snf also helps explain results of nucleosome cross-linking studies where Snf5, Taf14, and Swp82 cross-linked with nucleosomes at sites in close proximity and overlapping with Snf2 (Dechassa et al., 2008). Cross-links of Snf11 and Rtt102 with the nucleosome are also found proximal to Snf2 (Dechassa et al., 2008), in

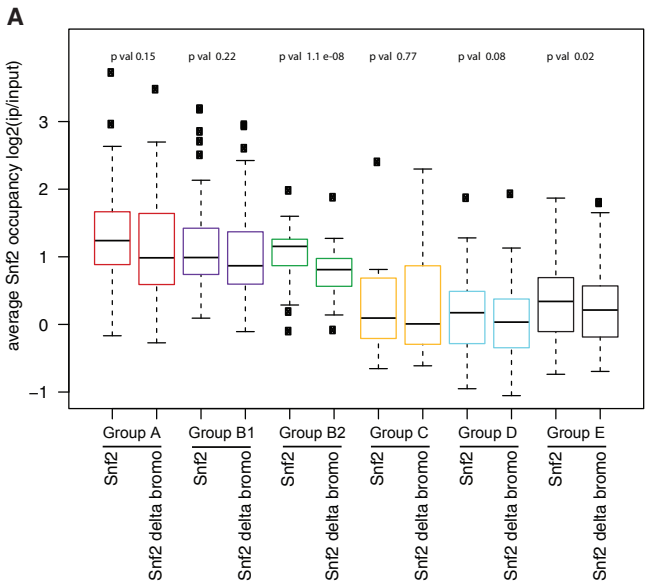

Figure 5. Levels of Histone Acetylation May Determine Snf2 Occupancy

(A) Snf2 occupancy at genes in WT and Snf2-delta bromodomain strains grown in YPD were obtained from previously published datasets (Dutta et al., 2014). Boxplots showing average occupancy of Snf2 in WT and Snf2-delta bromodomain strains (−500/+700 around the TSS) for each group of genes (groups A, B1, B2, C, D, and E in Figures 3 and 4) were plotted. The p values represent a significant change in Snf2 occupancy between WT and Snf2-delta bromodomain for each group.

agreement with their position in our model. We found that the small submodule of Snf6-Snf12-Swi3 requires Snf5 for association with Snf2 and Swi1. The Swi3-SANT domain has been shown to play a critical role in this interaction (Yang et al., 2007), raising the possibility that the SANT domain interacts directly with Snf5. Our results are also consistent with those observed earlier showing that human Brg1 (yeast Snf2) requires the hSnf5, Baf155, and Baf170 (human orthologs of yeast Swi3) for complete activity in vitro (Phelan et al., 1999). Furthermore, our observations reveal the importance of Snf12 in Swi/Snf integrity and suggest extensive interactions within the Snf5/Swi3 regulatory module. Snf6 requires Snf12 to associate with this module (data not shown), and cross-links with extra-nucleosomal DNA are located away from Snf2-nucleosome cross-linking sites (Dechassa et al., 2008; Sengupta et al., 2001), placing it at the periphery of the complex.

Many of the core subunits of Swi/Snf that are frequently mutated in human diseases are evolutionarily conserved (Kwon and Wagner, 2007; Wang et al., 1996). These subunits include hBAF155/BAF170 (ySwi3), hIni1 (ySnf5), hBAF60, hArid1a and hArid1b (ySwi1), hBAF60 (ySnf12), and the catalytic subunits hBrg1 and hBRM (ySnf2). Although loss of Swi/Snf subunits in cancers is well documented, less is known about the potential roles of residual complexes (Helming et al., 2014a). If the modularity of Swi/Snf is conserved, we predict the presence of intact subcomplexes of hSwi/Snf in tumor cells when subunits critical to module integrity are lost. Indeed, co-immunoprecipitation experiments from Ini1 (ySnf5)-deficient tumor cells show that Brg1 (ySnf2) interacts with BAF180 (ySwi1) and Act6La (yArp7/yArp9), and loses its association with other members of the complex (e.g., our Snf5/Swi3 regulatory module) (Wei et al., 2014). By analogy, we purified an intact Snf2-Arp7-Apr9 complex with ySwi1 in the absence of Snf5. Loss of both Brg1 and BRM (orthologs of ySnf2), which disrupts the catalytic module, keeps the interaction of Baf155 (ySwi3) with Baf60a (ySnf12), Ini1 (ySnf5), Baf170 (ySwi3), and Baf57 intact, suggesting that homologous factors also form an intact Snf5/Swi3 regulatory module in humans (Wilson et al., 2014). As predicted by our model, the Baf155-associated complex loses interaction with human Act6La (yArp7/yArp9), which is part of the remaining Arp and catalytic modules (Wilson et al., 2014). In the absence of Arid1a and Arid1b (human orthologs of ySwi1), the interaction of Brg1 and Act6La with the remainder of the complex is lost (Helming et al., 2014b). This result suggests that Arid1a and Arid1b are more closely associated with the catalytic module and may be required for its association with the rest of the complex. Consistent with this hypothesis, we found that the yeast catalytic module co-purified with Swi1 when the Snf5/Swi3 regulatory module was destabilized by the loss of Snf5 or Snf12. Together, these results provide evidence that Swi/Snf complex architecture is conserved and provide a road map to predict residual complexes in human disease that can differentially affect gene expression.

Interestingly, changes in transcript levels in various Swi/Snf subunit deletion strains recapitulates the modularity suggested by our proteomic studies. Loss of Snf5/Swi3 regulatory module subunits (Snf5, Snf6, Snf12, and Swi3) causes similar changes in gene expression, but different from those that occur upon loss of Snf2, in the catalytic module (Figure 2). No significant changes in gene expression are observed upon loss of Snf11, Swp82, and Rtt102, which do not disrupt Swi/Snf stability (Figure 2). Our ChIP-seq analysis of Snf2 occupancy in WT and Swi/Snf subunit deletion strains showed that Swi/Snf directly regulated only a subset of genes (Figure 2C). Surprisingly, only a subset of Snf2 occupied genes showed a decrease in this occupancy and concomitant change in gene expression when *SNF5*, *SNF6*, *SNF12*, and *SWI3* were deleted (Figure 3), whereas many genes were affected at the level of transcription but did not show defects in Snf2 recruitment (Figure 4). These results suggest that the Snf2-containing catalytic module may be sufficient to target Snf2 to many gene promoters but that the Snf5/Swi3 regulatory module is required for efficient nucleosomes remodeling and control of gene expression (Figure 4).

To address why some genes need an intact Swi/Snf for Snf2 recruitment whereas others do not, we analyzed levels of H3K9 acetylation at genes regulated by Swi/Snf (Figures 3, 4, and S5). Our analysis showed that high levels of histone acetylation were sufficient for Snf2 recruitment even in the absence of the Snf5/Swi3 regulatory module, whereas an intact complex was crucial for recruitment of Snf2 when histone acetylation was low (Figures 3, 4, and S5). This is further supported by our results that show that, in fact, loss of the Snf2 bromodomain decreases Snf2 occupancy at genes with high levels of histone acetylation and that require Swi/Snf for their expression (Figure 5). The notion that individual modules of Swi/Snf can be targeted on

their own is also supported by studies in which Swi1 and members of the Snf5/Swi3 regulatory module (Snf5, Swp82, Snf6, Snf12, and Swi3) could be targeted to the yeast *ARG1* promoter in the absence of Snf2 (Yoon et al., 2003). Additionally, studies in HeLa S3 cells showed that human Ini1 (ySnf5) and Baf155/BAF170 (ySwi3) were targeted to distinct loci not bound by Brg1 (ySnf2) and vice versa (Euskirchen et al., 2011). These observations raise interesting questions about the roles other modules might play in the absence of the catalytic module, how they are recruited to genes, and how they control gene expression. The ability of Swi1 and Snf5 to interact with transcriptional activators provides one possible mechanism for recruitment of Swi/Snf components in the absence of Snf2 (Neely et al., 2002). These subunits may then recruit other modules of Swi/Snf with or without the catalytic enzyme. A more provocative idea is that preformed partial complexes are recruited to genes, and then recruit the catalytic module and/or tissue-specific Swi/Snf subunits to control spatial and temporal patterns of transcription.

The existence of distinct modules within the Swi/Snf complex raises important questions about whether they exist as separate entities in vivo. Interestingly, Dastidar et al. (2012) cataloged changes in cellular localization of several yeast nuclear proteins under conditions of hypoxia, and showed that Snf11 and members of the Snf5/Swi3 regulatory module (Snf5, Swp82, Snf6, Snf12, and Swi3) migrated to the cytoplasm, whereas Snf2, Swi1, Taf14, and the Arp module (Arp7, Arp9, and Rtt102) remained in the nucleus. Thus, separation of Swi/Snf modules has been observed in vivo. The findings that the human Swi/Snf complex regulates genes in response to hypoxia (Kenneth et al., 2009), and that a small subcomplex of Snf2, Arp7, and Arp9 is sufficient for ATP hydrolysis and sliding of nucleosomes on DNA (Yang et al., 2007), raises the intriguing possibility that separate modules might regulate gene expression by permitting unique interactions that are not favored in the presence of the Snf5/Swi3 regulatory module.

EXPERIMENTAL PROCEDURES

Strains and Media

S. cerevisiae strains used for TAP-tag purification were obtained from Open Biosystems. Deletion strains were generated by homologous recombination (Table S1). Cells were grown at 30°C in YPD (1% yeast extract, 2% bactopeptone, and 2% dextrose) medium.

Purification of Complexes

Swi/Snf complexes were purified using strains carrying TAP-tag on complex subunits as described in Supplemental Experimental Procedures.

Mass-Spectrometric Analysis

TCA-precipitated Swi/Snf complexes purified from WT and mutant cells were urea-denatured, reduced, alkylated, and digested with endoproteinase Lys-C (Roche) followed by modified trypsin (Promega). MudPIT analyses are described in Supplemental Experimental Procedures. Relative protein abundance expressed as dNSAFs were hierarchically clustered using WARD algorithm and Pearson correlation as distance metric.

Chromatin Immunoprecipitation, Library Generation, and Sequencing

Yeast was grown in YPD and cross-linked with formaldehyde. Chromatin immunoprecipitation was carried out using antibody to Snf2.

ChIP-Seq Analysis

ChIP-seq reads were aligned to yeast genome sacCer2 using bowtie2 (2.1.0) with −k 1. Analysis was done in R (3.0.2) using the packages GenomicRanges (1.14.4), Rsamtools (1.14.2), and rtracklayer (1.22.0). Peaks were called using a custom Perl script run on read count-normalized immunoprecipitation/input ratio tracks for each replicate, requiring a peak of 1.5-fold with a minimum run of 50 bases and a maximum gap of 400 bases. Peaks were intersected with gene transcript start sites (−500/+700) using BEDTools (2.17.0). The heatmap was created by hierarchically row-clustering normalized $\log_2$ (immunoprecipitation/input) values for regions −500/+700 around the TSSs of genes and combining them into a heatmap. Heatmap values outside the range of the scale were set to the maximum or minimum value of the scale. The heatmap was generated in R using custom image plotting. Average of two replicates is shown.

RNA-Seq

Yeast strains were grown in YPD medium at 30°C to A_{600nm} of 0.8. Total RNA was isolated using acid phenol extraction. RNA-seq data were aligned to the University of California, Santa Cruz (UCSC), sacCer2 genome using Tophat version 2.0.10 with gene annotations from Ensembl 63. Tophat options were -x 1 -g 1–segment-mismatches 1–no-coverage-search. Downstream analysis and visualization was done in R (3.2.2). Differentially expressed genes were called using edgeR (3.12.0). Heatmaps were in general clustered in R using the heatmap function, which uses hierarchical clustering based on Euclidean distance between rows or using k-means function in R with six centers.

ACCESSION NUMBERS

The accession number for the genomic-wide analyses in this study is GEO: GSE81722.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, five figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.01.058.

AUTHOR CONTRIBUTIONS

A.D. and D.Z. purified complexes. M.S., J.G., L.F., and M.P.W. carried out mass-spectrometric analysis. A.D. carried out all genomic experiments. A.D. and M.G. analyzed genome-wide data. A.D., S.M.A., and J.L.W. wrote the manuscript.

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