The Swi/Snf family of nucleosome-remodeling complexes and transcriptional control

The Swi/Snf family of nucleosome-remodeling complexes has been shown to play important roles in gene expression throughout eukaryotes. Genetic and biochemical studies previously suggested that Swi/Snf activates transcription by remodeling nucleosomes, thereby permitting increased access of transcription factors for their binding sites. Recent studies have identified additional Swi/Snf biochemical activities and have suggested possible mechanisms by which Swi/Snf is targeted to specific promoters. Surprisingly, studies have also revealed that, besides being necessary for activation, Swi/Snf is required for transcriptional repression of some genes. These analyses have transformed our understanding of the function of the Swi/Snf family of complexes and suggest that they control transcription in diverse ways.

One can no longer accurately write that recent studies have demonstrated that chromatin structure plays a crucial role in eukaryotic transcription, for it has been over ten years since a combination of genetic and biochemical studies made this conclusion evident to most investigators in the transcription field. Although initial studies focused on nucleosomes, subsequent studies identified multi-protein complexes that controlled transcription by manipulating nucleosome structure. There are at least two main classes of such complexes: one class remolds nucleosomes in an ATP-dependent fashion and the other modifies histones by altering their acetylation patterns. This review discusses the Swi/Snf family of nucleosome-remodeling complexes. The Swi/Snf complex was first discovered in Saccharomyces cerevisiae, beginning with two genetic screens for altered gene expression. Mutations in SNF genes were identified as causing defects in expression of the SUC2 gene, which is required for growth on sucrose and raffinose as carbon sources (the name Snf is derived from sucrose nonfermenter). Similarly, mutations in SWI genes were identified as defective for expression of the gene, which is required for mating type switching (the name Swi is derived from switching defective). These genetic and biochemical analyses led to the model that Swi/Snf controls transcription \textit{in vivo} by disrupting nucleosome structure at a promoter and facilitating the binding of transcription factors to their cognate sites.

Swi/Snf function is conserved in eukaryotes, as related complexes have been discovered in Drosophila, Drosophila melanogaster and humans. Besides Swi/Snf, S. cerevisiae also contains a closely related complex called RSC (Ref. 13). In Drosophila, one Swi/Snf-like complex called RSC (Ref. 13).
complex has been discovered\textsuperscript{14}. Humans contain multiple Swi/Snf-like remodeling complexes\textsuperscript{5,6}. The division of labor between the different complexes is unclear. In addition to the Swi/Snf family of complexes, there is a related family of ATP-dependent nucleosome remodeling complexes, the ISWI family\textsuperscript{6,13,15}. This review focuses only on the Swi/Snf family.

Swi/Snf can remodel, slide and mobilize nucleosomes \textit{in vitro}\n
Recent biochemical studies show that Swi/Snf possesses an extensive repertoire of biochemical activities (Fig. 1). Swi/Snf can bind to either nucleosomes or DNA in an ATP-independent fashion\textsuperscript{16},\textsuperscript{18}, and electron spectroscopic imaging studies have shown that Swi/Snf binding creates loops in either nucleosomal arrays or naked DNA, bringing distant sites into close proximity\textsuperscript{17}. By contrast, the nucleosome-remodeling activity of Swi/Snf is ATP dependent and has been observed by two types of experiments. Initially, in assays containing purified Swi/Snf, ATP and nucleosomes, this activity was observed as increased DNaseI sensitivity of the nucleosomal DNA (Refs 6, 13). More recently, studies of human Swi/Snf (SWI/SNF)\textsuperscript{18} and \textit{S. cerevisiae} RSC (Ref. 19) have demonstrated that the remodeled nucleosome is stable in the absence of the remodeling complex and has the size of two nucleosomes. It is not yet clear if the remodeled nucleosomes observed in the earlier and later assays are the same as each other, as they display some differences in nuclease sensitivity. In addition, both SWI/SNF and RSC can act on the remodeled nucleosome to restore it to the original, inactive, nucleosomal state\textsuperscript{18,19}. While Swi/Snf acts catalytically on nucleosome arrays to form the active state\textsuperscript{10},\textsuperscript{18}, the properties of the reverse remodeling reaction remain to be characterized.

Two types of Swi/Snf nucleosome-mobilization activities have been discovered in recent biochemical studies. Swi/Snf can reposition nucleosomes in an ATP-dependent fashion by sliding histone octamers to other sites on the same DNA molecule (\textit{cis}-displacement; Fig. 1b)\textsuperscript{21}. Swi/Snf and RSC are also capable of transferring histone octamers to other DNA molecules (\textit{trans}-displacement; Fig. 1c)\textsuperscript{22,23}. The preference for \textit{cis}- or \textit{trans}-displacement of nucleosomes depends upon the ratio of Swi/Snf to octamer\textsuperscript{21}. Swi/Snf can catalyze \textit{cis}-displacement quite efficiently even at a low Swi/Snf:octamer ratio (one Swi/Snf complex per 200 nucleosomes), whereas \textit{trans}-displacement requires a tenfold greater Swi/Snf:octamer ratio\textsuperscript{21}.

The relationships between Swi/Snf-dependent remodeling, \textit{cis}-displacement and \textit{trans}-displacement, are not yet understood. For RSC activities, there is evidence that a nucleosome goes through an ‘activated’ intermediate for remodeling and \textit{trans}-displacement\textsuperscript{19}. However, the nucleosomal form(s) that are the substrates for, or products of, \textit{cis}- and \textit{trans}-displacement have not yet been identified for any Swi/Snf complex. Furthermore, biochemical assays of Swi/Snf activities have used a variety of substrates, including mononucleosomes and nucleosomal arrays\textsuperscript{6,11}. To understand the relationship between the different Swi/Snf activities, the preference for particular activities needs to be tested, where possible, using the different substrates.

The demonstration of different Swi/Snf activities \textit{in vitro} is intriguing because it suggests that the nature of Swi/Snf nucleosome remodeling \textit{in vivo} might vary among Swi/Snf-dependent promoters. Remodeling \textit{in vivo} might depend upon the local concentration of Swi/Snf, the chromatin structure at the particular genomic location, the presence of other DNA-bound regulatory factors, or the availability of other DNA to act as a histone octamer acceptor. However, the current methods for analyzing nucleosome structure and position \textit{in vivo} do not clearly distinguish between remodeled nucleosomes or nucleosome-free regions, nor can they distinguish the consequences of \textit{cis}- or \textit{trans}-displacement. \textit{In vivo} assays for different nucleosomal positions and high-resolution mapping of chromatin changes \textit{in vivo} will be important for understanding Swi/Snf activity.

\textbf{Why does Swi/Snf contain so many subunits?}\n
Swi/Snf complexes are large, multi-subunit complexes containing eight or more proteins (Table 1). All Swi/Snf complexes studied contain a core set of components conserved with \textit{S. cerevisiae} Swi/Snf members, including the conserved DNA-dependent ATPase Snf2/Swi2, Snf5 and Swi3 (Ref. 6). A ‘minimum catalytic core’ complex of three SWI/SNF components, BRG1, INI1 and BAF155/BAF170, can remodel both mononucleosomes and nucleosomal arrays\textsuperscript{3,4}. In addition, BRG1 alone can substitute for the core complex, albeit with less efficiency. Thus, the remaining SWI/SNF subunits are not required for these remodeling activities \textit{in vitro}.

The demonstration of different Swi/Snf activities \textit{in vitro} suggests that most of its subunits are required for common functions \textit{in vivo}. Null mutations in most of the SWI/SNF genes cause very similar phenotypes\textsuperscript{17}. Moreover, whole-genome expression analysis of \textit{snf2Δ} and \textit{swi1Δ} mutants indicate that the two mutations cause very similar effects on mRNA levels in cells grown in both rich and minimal media\textsuperscript{21}. Therefore, conserved Snf2 nonconserved Swi1 are devoted to all aspects of Swi/Snf function \textit{in vivo}. These studies imply that some of the non-‘core’ Swi/Snf members are required for nucleosome remodeling \textit{in vivo}, despite being unnecessary \textit{in vitro}.
There is, however, some evidence for distinct roles for certain Swi/Snf subunits. On the one hand, in S. cerevisiae, Snf6 can activate transcription independently of other Swi/Snf members. On the other hand, certain activators that require Snf2 do not require Swp73 and mutations in two other SNF/SWI genes, TFG3 and SNF11, do not cause any detectable Swi/Snf mutant phenotypes. Two subunits in RSC, Rsc1 and Rsc2, contain bromodomain motifs that are essential for their function. As bromodomains are believed to bind to acetyl-lysine, some of these subunits might facilitate RSC binding to acetylated histones or other factors. In addition, mammalian Swi/Snf complexes purified from different tissues display significant subunit heterogeneity, suggesting that some subunits play specialized roles in certain tissues or conditions. Finally, Swi/Snf complexes from S. cerevisiae, Drosophila and humans have been shown to contain either actin or actin-related proteins. Actin-related proteins recruit Swi/Snf to specific promoters. For example, chromatin immunoprecipitation (ChIP) experiments performed in S. cerevisiae at the HO promoter demonstrate that, in vivo, the presence of Swi/Snf at HO is dependent on the transcriptional activator, Swi5. Consistent with this result, in vitro studies show that purified Swi/Snf directly interacts with Swi5. S. cerevisiae Swi/Snf also associates with the activation domains of the activators Gcn4, Hap4, Gal4-AH and VP16. The physiological significance of these interactions is supported by tests of Gcn4 activation domain mutants: the degree of Swi/Snf-Gcn4 interaction correlates with the level of Gcn4 activation in vivo. In some cases, this interaction has been shown to be necessary for the recruitment of Swi/Snf to a promoter in vitro. The physiological significance of these interactions is supported by tests of Gcn4 activation domain mutants: the degree of Swi/Snf-Gcn4 interaction in vitro correlates with the level of Gcn4 activation in vivo. Interestingly, the S. cerevisiae repressors Hir1/Hir2 interact with Swi/Snf in vitro and are necessary for activation by Swi/Snf at the histone-encoding locus, HTA1-HTB1, in vivo. In addition to studies in S. cerevisiae, studies of activation and chromatin remodeling by mammalian Swi/Snf complexes strongly suggest a requirement for interactions with transcriptional activators. Thus, Swi/Snf recruitment by transcriptional activators is probably conserved throughout eukaryotes.

An alternative mode of targeting Swi/Snf might be through interactions with the general transcription machinery, as suggested by studies that show an association of Swi/Snf with the RNA polymerase II (Pol II) holoenzyme in yeast and humans. However, other studies have reported no physical interaction between Swi/Snf and the holoenzyme. In addition, a recent study shows that Swi/Snf is recruited from yeast nuclear extracts by a DNA-bound activator, independently of Pol II holoenzyme or any other general transcription factor. These results complement in vivo studies at the S. cerevisiae SUC2 promoter that demonstrate the occurrence of Swi/Snf-mediated nucleosome remodeling in the absence of a TATA box and, presumably, TATA-binding protein (TBP) recruitment. Though, it seems unlikely that a Swi/Snf-h holoenzyme interaction constitutes a general

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**TABLE 1. Swi/Snf subunit composition in different species**

<table>
<thead>
<tr>
<th>Saccharomyces cerevisiae</th>
<th>Saccharomyces cerevisiae</th>
<th>Drosophila melanogaster</th>
<th>Homo sapiens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swi/Snf</td>
<td>RSC</td>
<td>Brahma</td>
<td>SWI/SNF</td>
</tr>
<tr>
<td>Snf2/Swi2</td>
<td>Sh1</td>
<td>Brahma</td>
<td>BRC1 or hBrm</td>
</tr>
<tr>
<td>Snf5</td>
<td>Sh1</td>
<td>SNR1</td>
<td>INI1/BAF47</td>
</tr>
<tr>
<td>Snf6</td>
<td>Nd</td>
<td>Nd</td>
<td>No</td>
</tr>
<tr>
<td>Snf11</td>
<td>Nd</td>
<td>Nd</td>
<td>No Swi/Snf phenotype in snf11A mutant</td>
</tr>
<tr>
<td>Swi1/Adr6</td>
<td>Rsc6/8/Swi3</td>
<td>BAP155</td>
<td>Core</td>
</tr>
<tr>
<td>Swi3</td>
<td>Rsc12/Arp9</td>
<td>BAP59</td>
<td>Core</td>
</tr>
<tr>
<td>Swp59/Arp9</td>
<td>Rsc11/Arp7</td>
<td>BAP55</td>
<td>Actin-related</td>
</tr>
<tr>
<td>Swp61/Arp7</td>
<td>Rsc6</td>
<td>BAP60</td>
<td>Actin-related</td>
</tr>
<tr>
<td>Swp73</td>
<td>Nd</td>
<td>Nd</td>
<td>No</td>
</tr>
<tr>
<td>Swp82</td>
<td>Rsc1 or Rsc2</td>
<td>BAP111</td>
<td>Also associated with TFIIID and TRIF</td>
</tr>
<tr>
<td>Tfg3/Asc/Taf30</td>
<td>Rsc3-5,7,9,10,13-15</td>
<td>BAP74</td>
<td>-actin/Bap47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β-actin/Bap47</td>
<td>β-actin</td>
</tr>
</tbody>
</table>

*Adapted from Ref. 4.
*Relationship to S. cerevisiae proteins needs to be resolved.

Abbreviation: Nd, not determined.
Repression by Swi/Snf – how might it happen?

Recent whole-genome mRNA expression studies suggest that Swi/Snf also represses transcription, as almost half of the genes affected in swi1Δ snf2Δ mutants have increased mRNA levels. This finding was unexpected because most early studies, with one exception, suggested that Swi/Snf activated transcription by overcoming nucleosomal repression. Repression is probably a general property of the Swi/Snf family, as examples of repression have also been observed with S. cerevisiae RSC and SWI/SNF complexes. Repression by nucleosome remodeling complexes could be caused indirectly by decreased expression of genes encoding repressors. However, mutations that suppress the activation defect of swi1Δ snf2Δ mutants do not suppress the repression defect of these mutants, suggesting that the repression of Swi/Snf activity is not an indirect effect of its activation activity.

There are several possible mechanisms by which Swi/Snf could repress transcription, three of which will be given. First, the ability of SWI/SNF and S. cerevisiae RSC to remodel nucleosomes between two states, the normal ‘inactive’ state and the ‘remodeled’ state, with equal proficiency, suggests that Swi/Snf could confer repression in vivo by creating ‘inactive’ nucleosomal states at a promoter. Second, Swi/Snf might facilitate the binding of transcriptional repressors by its nucleosome remodeling activity. Finally, Swi/Snf might cause repression by a mechanism independent of any of its known nucleosome remodeling activities, for example by histone modification. Future studies exploring the role of Swi/Snf in repression could include the isolation of mutants that only remodel in one direction and the detailed analysis of chromatin changes at Swi/Snf-repressed promoters.

The Swi/Snf family of nucleosome-remodeling complexes
as suppressors of swi/snf activation defects, provided the initial Swi/Snf-chromatin connection.

The discovery of Swi/Snf-related complexes that possess histone deacetylase activity, a function shown to repress transcription, has provided an important link between nucleosome remodeling and transcriptional repression. These complexes affect transcriptional repression in Drosophila, centromeric silencing and DNA methylation in humans. The in vivo requirements for the remodeling and deacetylase activities at repressed loci remain to be characterized. The discovery of these complexes shows that nucleosome-remodeling activities can be coupled with other chromatin modifying activities to manipulate transcription in vivo.

**Swi/Snf is required for maintenance of activated transcription in vivo**

An issue that has been of considerable interest is whether or not Swi/Snf is continuously required at Swi/Snf-dependent genes. Several studies have addressed the continued requirement for Swi/Snf in nucleosome remodeling in vitro and have obtained mixed results. Recent experiments have addressed the continued need for Swi/Snf-dependent activation in vivo by using either an snf2 (Ref. 69) or an snf5 (Ref. 70) conditional mutant of *S. cerevisiae* to inactivate Swi/Snf subsequent to transcriptional activation. These genetic studies conclude that Swi/Snf is continuously required during active transcription in vivo. The continuous need for Swi/Snf for active transcription suggests that its role is more complex than performing a one-time chromatin rearrangement at an early step in activation.

One model for the continuous requirement for Swi/Snf comes from the recent analysis of HO (Refs 41, 71). These studies show that Swi/Snf is present throughout HO transcription, although the continuous need for Swi/Snf has not been tested directly at this locus. The HO studies suggest that Swi/Snf works in concert with other activators, including the SAGA (Spt-Ada-Gcn5 acetyltransferase) complex, which contains histone acetylation activity, and in opposition to a repressor, the histone deacetylase complex Sin3/Rpd3 (Refs 41, 71). In fact, sin3 and rpd3 mutations suppress the requirement for Swi/Snf for HO activation. Thus, the continuous presence of Swi/Snf at HO is probably required to overcome repression via Sin3/Rpd3. This model suggests that during HO activation, an equilibrium is established in which the HO promoter is in the active state with remodeled, acetylated nucleosomes. When Swi/Snf is removed, the equilibrium shifts, Sin3/Rpd3 activity becomes more predominant, and the HO promoter becomes inactive. This possibility can be tested using conditional swi/snf mutations.

The continuous requirement for Swi/Snf might vary at different promoters, depending on the nature of the activator. First, the requirement for Swi/Snf is predicted to be greatest at promoters that might lack sequence-specific activators, such as the SUC2 promoter or that possess low-affinity activator binding, such as an attenuated GAL1 promoter. Such promoters would require help from Swi/Snf to establish and maintain an equilibrium in the active state. Second, for some genes, Swi/Snf might be required to maintain transcription elongation. Finally, Swi/Snf might be required for multiple rounds of initiation and elongation.

Swi/Snf might also be required continuously because its remodeling activity could be necessary in each cell cycle. In mammalian cells, there is a global inhibition of transcription during mitosis. Coincident with this inactivation, SWI/SNF is inactivated by phosphorylation and is excluded from condensed chromosomes. Therefore, Swi/Snf might play a role in the activation or deactivation of transcription during each cell division. Although *S. cerevisiae* cells do not exhibit inhibition of mitotic transcription, at least two components of RSC are required for normal cell cycle progression in this species. The reason for the cell-cycle requirement remains to be determined in each organism and for each complex.

**Partially redundant roles for Swi/Snf and other transcription complexes in vivo**

Substantial evidence exists that transcriptional control by *S. cerevisiae* Swi/Snf is partially redundant, with transcriptional control being exerted by other complexes, including SAGA and RSC. The first evidence for this possibility came from double mutant analysis: swi/snf mutations allow viability; however, in combination with mutations in certain RSC genes or the gene for SAGA, swi/snf mutations cause either inviability or extremely poor growth. More specifically, an overlapping role was found for Swi/Snf and the histone acetyltransferase, Gcn5 (a member of SAGA and other complexes) in transcription of particular genes. The nature of the requirement for both complexes has been examined for the HO and PHO8 genes where it has been shown that Swi/Snf and Gcn5 each act in a distinct fashion. The redundancy between Swi/Snf and other transcription complexes has probably obscured many genes at which Swi/Snf acts. Expression studies in double mutants will begin to clarify both the number of genes at which Swi/Snf acts as well as the nature of its overlap with other transcription complexes.
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NuRD and SIN3
histone deacetylase complexes in development

Transcription repression mediated through histone deacetylase (HDAC) complexes is widespread, and mechanisms by which HDAC complexes act have been revealed by extensive studies in vitro and in cell culture. However, until recently, little has been known about the developmental roles of histone deacetylation. Mutants now exist for a number of members of the two major HDAC complexes (NuRD and SIN3) and some associated proteins. The emerging picture is that these complexes have specific functions in development, rather than being required for most cellular processes.

During the development of an organism, a wide variety of cell fate decisions are taken based on specific inheritance or cell interactions. Ultimately, most of these decisions are carried out by sequence-specific transcription factors, through activation or repression of gene expression. Clearly, alterations of gene expression must occur in the context of chromatin, but until recently, only a few developmental roles for changes in chromatin structure were known. Over the past few years, a series of results has been helping to explain how chromatin regulation contributes to making developmental decisions.

It has long been known that activation and repression of gene expression correlate with the acetylation state of histones. In general, acetylated histones are correlated with more open chromatin and active gene expression, whereas deacetylated histones correlate with closed chromatin and repressed gene expression. Early work using histone deacetylase inhibitors showed that they disrupted normal development, indicating that the acetylation state of histones is developmentally important (e.g. see Ref. 1). Recently, enzymes that carry out histone acetylation (histone acetyltransferases, or HATs) and deacetylation (histone deacetylases, or HDACs) have been identified (reviewed in Ref. 2). These function in several different large multiprotein complexes that are associated with sequence-specific DNA-binding proteins, which are thought to target the complexes to specific genes, leading to local chromatin modification. Many studies have been concerned with the biochemical activities of these complexes and have been conducted in vitro or in cell culture. However, recent in vivo work in several systems has provided some functional information during development. A good review of developmental roles of HATs has been published recently3. Here, I review developmental functions of the two major histone deacetylation complexes, NuRD (for nucleosome remodelling and histone deacetylase) and SIN3 and their associated proteins, focusing primarily on cases where mutants have been studied.

Below, I briefly summarize the composition and biochemical properties of these complexes. The NuRD complex4–8 (also known as Mi-2) is approximately 2 MDa in size and in mammalian cells comprises at least seven polypeptides (Fig. 1 and Table 1; reviewed in Ref. 9). The histone deacetylases HDAC1 and HDAC2 and at least seven polypeptides (Fig. 1 and Table 1; reviewed in Ref. 9). The histone deacetylases HDAC1 and HDAC2 and two histone-binding proteins (RbAp46 and RbAp48) are also found in the SIN3 complex. In addition to histone-deacetylase activity, the NuRD complex has ATP-dependent nucleosome-remodelling activity because it contains Mi-2/CHD family proteins, which have a chromodomain, a DNA helicase/ATPase

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