Whole-genome expression analysis of *snf/swi* mutants of *Saccharomyces cerevisiae*

Priya Sudarsanam*, Vishwanath R. Iyer†, Patrick O. Brown†‡, and Fred Winston*‡

*Department of Genetics, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115; and †Department of Biochemistry and Howard Hughes Medical Institute, Stanford University School of Medicine, 300 Pasteur Avenue, Stanford, CA 94305

Edited by Gerald R. Fink, Whitehead Institute for Biomedical Research, Cambridge, MA, and approved January 6, 2000 (received for review September 22, 1999)

The *Saccharomyces cerevisiae* Snf/Swi complex has been previously demonstrated to control transcription and chromatin structure of particular genes *in vivo* and to remodel nucleosomes *in vitro*. We have performed whole-genome expression analysis, using DNA microarrays, to study mutants deleted for a gene encoding one conserved (Snf2) or one unconserved (Swi1) Snf/Swi component. This analysis was performed on cells grown in both rich and minimal media. The microarray results, combined with Northern blot, computational, and genetic analyses, show that *snf2*Δ and *swi1*Δ mutations cause similar effects on mRNA levels, that Snf/Swi controls some genes differently in rich and minimal media, and that Snf/Swi control is exerted at the level of individual genes rather than over larger chromosomal domains. In addition, this work shows that Snf/Swi controls mRNA levels of *MAT*α-specific genes, likely via controlling transcription of the regulators *MAT*α*1* and *MCM1*. Finally, we provide evidence that Snf/Swi acts both as an activator and as a repressor of transcription, and that neither mode of control is an indirect effect of the other.

There is substantial evidence that the structure and position of nucleosomes can control transcription in eukaryotes. Both *in vivo* and *in vitro* experiments have shown that nucleosomes can cause repression of transcription by blocking transcription factor binding (1). In recent years, several studies have shown that the conserved protein complex, Snf/Swi, can relieve this repression by perturbing the structure of nucleosomes (2). This remodeling probably allows transcription factors to bind to their sites, facilitating transcription initiation. Since the discovery of the Snf/Swi complex, other classes of nucleosome remodeling complexes have been discovered throughout eukaryotes (3, 4), suggesting that nucleosome remodeling is a general mechanism for controlling transcription *in vivo*.

Several studies in *Saccharomyces cerevisiae* have demonstrated that Snf/Swi is required for the normal transcription of only a subset of genes. First, although *snf/swi* mutants exhibit many mutant phenotypes, including poor growth, the inability to use particular carbon sources, and a defect in sporulation, Snf/Swi is not required for viability (3). Second, Northern blot analyses have demonstrated transcriptional defects at merely a handful of genes (5). Finally, a recent study of the expression of all *S. cerevisiae* genes in a *snf2* mutant demonstrated that only a small percentage of mRNAs have altered levels when cells are grown in rich medium (6). The requirement for Snf/Swi in *S. cerevisiae* cells grown in less nutritious media has not been examined.

Snf/Swi is a multiprotein complex, and the roles of the different members of the complex are not well understood. Among the Snf/Swi complexes studied in *S. cerevisiae*, human, and Drosophila, only a subset of Snf/Swi proteins are conserved, including Snf2/Swi2, Snf5, Swi3, and Swp73 (1). All of the Snf/Swi complexes have been demonstrated to possess ATP-dependent nucleosome remodeling activity *in vitro* (7–9). Three of the conserved subunits in the human Snf/Swi complex have been demonstrated to constitute a "core" set of Snf/Swi factors (10). This core complex, which includes Snf2/BRG1, a DNA-dependent ATPase, Snf5/INI1, and Swi3/BAF155/BAF170, can carry out nucleosome remodeling activity in the absence any other Snf/Swi component. The other Snf/Swi subunits could be required for nucleosome remodeling activity *in vivo* or for other unknown aspects of Snf/Swi activity, such as response to signals or interactions with transcriptional regulators.

The factors that determine the dependence of a gene on Snf/Swi are not understood. Several studies have indicated that Snf/Swi may be targeted to particular promoters by physical interactions with specific transcriptional activators or repressors (11–18). However, it seems likely that such interactions cannot be the sole determinants of Snf/Swi-dependence. For example, Pho4 activates both *PHO5* and *PHO8*, yet *PHO5* transcription is Snf/Swi-independent whereas *PHO8* transcription is Snf/Swi-dependent (19). Other factors, such as a particular chromatin structure (3) or promoter strength (20), may also play a role in determining Snf/Swi action. In addition, Snf/Swi-control could be exerted at the level of chromosomal domains rather than specific genes. This model predicts that the genomic location of a gene would influence its dependence on Snf/Swi.

Recent studies have suggested that, in addition to its role in transcriptional activation, Snf/Swi may also play a role in transcriptional repression. This idea has come from a combination of gene expression studies (21–24), biochemical analysis of nucleosome remodeling by human Snf/Swi (25) and the Snf/Swi-related *S. cerevisiae* complex, RSC (26), and from whole-genome expression studies (6). It is unclear whether Snf/Swi-mediated repression is a direct or indirect effect of Snf/Swi activity and whether it involves nucleosome remodeling.

Therefore, several fundamental issues remain unresolved concerning Snf/Swi function. The introduction of methods to measure the mRNA levels for every *S. cerevisiae* gene (27) has provided the tools to answer some of these questions. We have used whole-genome DNA microarrays to identify the entire set of *S. cerevisiae* genes dependent on two subunits of the complex, Snf2 and Swi1. Using two different media conditions, we have identified several genes by microarray and subsequent Northern blot analyses that are good candidates for being directly controlled by Snf/Swi. One of these genes is the essential gene *MCM1*, the yeast homologue of human serum response factor (28). Our studies also show that Snf/Swi-dependent genes are scattered throughout the genome, indicating that Snf/Swi controls transcription at the level of individual genes and not at the level of chromosomal domains. Finally, we provide further evidence that Snf/Swi is required for transcriptional repression at some genes, and our genetic analysis strongly suggests that this repression is not an indirect effect of Snf/Swi activation.

Materials and Methods

Yeast Strains and Methods. *S. cerevisiae* strains are isogenic to S288C (29) and were constructed by standard methods (30).

This paper was submitted directly (Track II) to the PNAS office.

‡To whom reprint requests should be addressed. E-mail: winston@rascal.med.harvard.edu or pbrown@cmgm.stanford.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.050407197. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.050407197

They are FY2 (*MATα ura3-52*), FY20 (*MATα ura3-52*), FY31 (*MATα ura3-52 his3Δ200 snf2Δ1::HIS3*), FY32 (*MATα ura3-52 his3Δ200 snf2Δ1::HIS3*), FY57 (*MAT***a** *ura3-52 his4-912δ lys2-128δ*), FY458 (*MAT***a** *ura3-52 his4-912δ lys2-128δ his3Δ200 snf2Δ1::HIS3*), FY710 (*MAT***a** *ura3-52 leu2Δ1 his4-912δ lys2-128δ* (*hta1-htb1*)*Δ1::LEU2*), FY724 (*MAT***a** *ura3-52 leu2Δ1 his4-912δ lys2-128δ* (*hta1-htb1*)*Δ1::LEU2 snf2Δ1::HIS3*), FY1254 (*MATα ura3-52 his3Δ200 lys2-128δ leu2Δ1 swi1Δ1::LEU2*), FY1357 (*MAT***a** *ura3-52 arg4-12 lys2-173R2 leu2Δ1 snf2Δ::LEU2*), FY1882 (*MATα ura3-52 leu2Δ1 swi1Δ1::LEU2*), FY1884 (*MATα ura3-52 leu2Δ1* (*hta1-htb1*)*Δ1::LEU2*), FY1885 (*MATα ura3-52 leu2Δ1* (*hta1-htb1*)*Δ1::LEU2 snf2Δ1::HIS3*).

Microarray Analysis. FY2, FY31, and FY1882 were grown in rich medium (yeast extract/peptone/dextrose containing 2% glucose) or in synthetic minimal medium (yeast nitrogen base without amino acids plus ammonium sulfate), supplemented with uracil and 2% glucose, to a cell density of 1–2 $\times$ 10^7 cells/ml (30). Total RNA was prepared by using hot phenol extraction (31). poly(A)$^+$ RNA was prepared by using Oligotex resin from Qiagen (Chatsworth, CA). Microarray analysis of poly(A)$^+$ RNA was carried out as described (27). cDNA made from poly(A)$^+$ RNA from the wild-type strain was fluorescently labeled with Cy5 (represented as red), and that from the mutants was labeled with Cy3 (represented as green). The two labeled cDNA pools were mixed and hybridized simultaneously to a microarray. Each set of hybridizations was performed with an independent RNA preparation. Statistical analyses were carried out using the statistical toolpak software available with Microsoft EXCEL.

Northern Hybridization Analysis. Strains listed in the figure legends were grown in rich or minimal medium supplemented with appropriate amino acids to 1–2 $\times$ 10^7 cells/ml. RNA was prepared from 10-ml samples and was analyzed (32). The probes used for hybridization were ^{32}P-labeled using primers purchased from Research Genetics (Huntsville, AL) and PCR amplification from genomic DNA except *SPT15*. The *SPT15* probe was PCR amplified from pIP45, which contains the *Spe*I-*Hin*dIII fragment of *SPT15* cloned into pBluescript KS(+). RNA levels were quantitated by PhosphorImager analysis (Molecular Dynamics) normalizing to *SPT15*.

Computational Analyses. The computer programs Multiple EM for Motif Elicitation (MEME) (33) and Aligns Nucleic Acid Conserved Elements (ALIGNACE) (34) were used to search for putative sequence motifs, with 600 bp 5′ of each gene as input. Analyses of genome-wide distributions of promoter orientations and intergenic distances with respect to gene expression in *snf/swi* mutants were done with the assistance of B. A. Cohen and R. Mitra (personal communication).

Results

Measurement of mRNA Levels in *snf2*Δ and *swi1*Δ Mutants by Microarray Analysis. We have used whole-genome DNA microarrays with the goal of identifying all of the genes in *S. cerevisiae* that are controlled by the Snf/Swi complex. By this method, the mRNA levels in wild-type and *snf/swi* mutant strains were determined for each *S. cerevisiae* gene, and the results are expressed as ratios of mutant:wild-type mRNA levels. Our microarray analyses included two variables. First, to determine whether loss of two different Snf/Swi subunits causes the same transcriptional defects, we analyzed mRNA levels in both *snf2*Δ and *swi1*Δ mutants. Snf2 and Swi1 may have different roles *in vivo* because Snf2 is a conserved core member of Snf/Swi, capable of carrying out nucleosome remodeling *in vitro* (10), whereas Swi1 is not conserved and is not required for this core activity *in vitro*. Second, to determine the requirement for Snf/Swi under two different nutrient conditions, we analyzed expression of strains grown in rich and minimal media. In minimal medium, *S. cerevisiae* cells induce transcription of several sets of genes, such as those required for amino acid biosynthesis, whereas the expression of those genes is repressed in rich medium (35). Therefore, we reasoned that the requirement for Snf/Swi may differ between the two growth conditions.

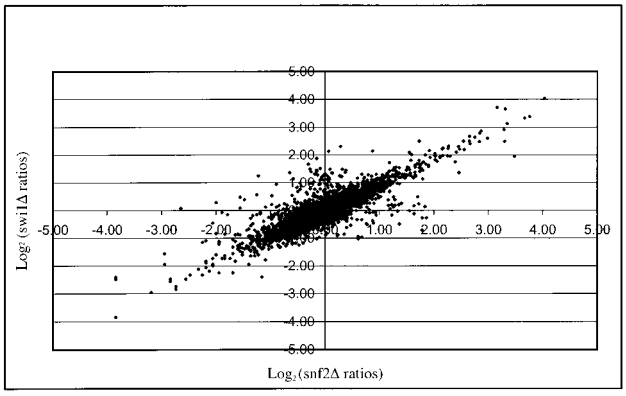

Fig. 1. Scatter plot of expression ratios of all genes in *snf2*Δ and *swi1*Δ in rich medium. The ratio of mutant to wild-type mRNA levels was calculated for every gene in both mutants. Only genes with at least 40 fluorescence units in the wild type were included. The ratios were transformed to Log_2 (ratio), such that a two-fold increase in gene expression in a mutant would equal 1 whereas a two-fold decrease would equal −1. To compare the expression of each gene in *snf2*Δ to that in *swi1*Δ, the transformed ratios for every gene in *snf2*Δ was plotted against those in *swi1*Δ.

Several independent measurements of mRNA levels were performed for cells grown in rich and in minimal medium. The data set and statistical analysis is available at http://genome-www.stanford.edu/swisnf. In addition, Northern hybridization analysis was carried out for several cases of particular interest as described below. Of the ≈6,014 genes tested, ≈1% show greater than a three-fold change in mRNA levels in both *snf2*Δ and *swi1*Δ mutants (Fig. 1). In both media conditions, some genes have decreased mRNA levels whereas others have increased mRNA. A similar effect was observed in a previous whole-genome expression study of an *snf2* mutant grown in rich media (6). We have analyzed the altered expression in *snf/swi* mutants by several criteria as described in the following sections.

We examined the list of genes affected in *snf/swi* mutants to learn of possible additional mutant phenotypes. We noticed that *HAP4*, which encodes a factor that controls the transcription of genes required for lactate utilization (36), has five-fold reduced mRNA levels in both *snf2*Δ and *swi1*Δ mutants in minimal medium. As expected, *snf2*Δ and *swi1*Δ mutants show a severe growth defect on medium containing lactate as the sole carbon source (data not shown). The decrease in *HAP4* mRNA levels may also explain the inability of *snf/swi* mutants to use glycerol as a carbon source (37).

Snf2 and Swi1 Are Likely Required for Transcription of the Same Genes *in Vivo*. Based on the microarray analysis, loss of Snf2 or Swi1 causes similar changes in mRNA levels (Fig. 1). The small number of differences observed could be caused either by variation in the microarray measurements or by real differences between *snf2*Δ and *swi1*Δ mutants. We studied one of these genes, *SER3*, in greater detail. By Northern analysis, *SER3* mRNA levels are increased 23-fold in *snf2*Δ and only 8-fold in *swi1*Δ (Fig. 2*A*). Thus, although a small number of genes may differ with respect to the magnitude of the effect in either *snf2*Δ or *swi1*Δ, most Snf/Swi-regulated genes seem to depend on both subunits of the complex. These results suggest that Swi1 plays as

GENETICS

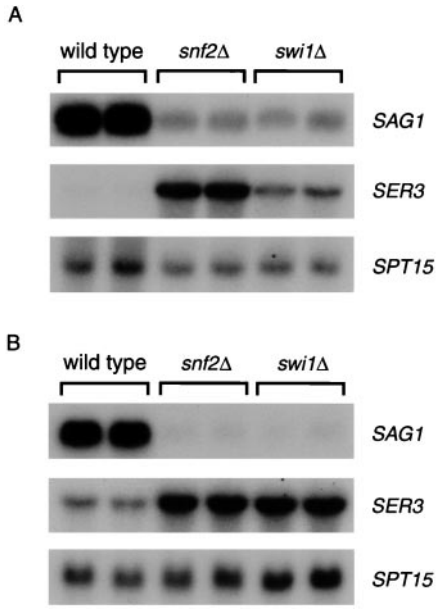

Fig. 2. Opposite effects of *snf/swi* mutations on mRNA levels. Northern blot analysis of *SAG1* and *SER3* mRNA levels in the strains FY2, FY20, FY31, FY32, FY1254, and FY1882 grown in rich medium (*A*) or minimal medium (*B*). mRNA levels of each pair of strains were averaged before normalization to the wild type. *SAG1* mRNA levels are 0.13 and 0.04 in rich and minimal media, respectively, in *snf2*Δ and 0.11 and 0.03 in rich and minimal media, respectively, in *swi1*Δ, compared with the wild-type strain (1.0). *SER3* mRNA levels are 21.4 and 3.19 in rich and minimal media, respectively, in *snf2*Δ and 6.94 and 2.36 in rich and minimal media, respectively, in *swi1*Δ compared with the wild-type strain (1.0).

important a role as Snf2 in *S. cerevisiae* under the conditions tested.

Snf/Swi Dependence Can Differ in Rich and Minimal Media. The microarray analysis revealed that some genes are more strongly Snf/Swi-dependent in one type of medium than the other. For example, genes encoding hexose transporters, *HXT1*, *HXT3*, *HXT6*, *HXT7*, have decreased mRNA levels only in minimal medium. In contrast, several amino acid biosynthesis genes, particularly several *MET* and *ARG* genes, have increased mRNA levels only in rich medium. We performed Northern blot analysis on two genes, *SAG1* and *SER3*, whose Snf/Swi-dependence is affected to different degrees in rich and minimal medium. These results (Fig. 2) verify that *SAG1* mRNA levels are more severely decreased in minimal medium compared with rich. *SER3* mRNA levels are increased in *snf/swi* mutants and are more significantly increased in rich medium compared with minimal. Another medium-specific effect is described in the next section.

Table 1. Transcription of the acid phosphatase genes in rich medium is Snf/Swi-dependent

		Fold effect in rich medium*		Fold effect in minimal medium*	
ORF name	Gene name	*snf2*Δ	*swi1*Δ	*snf2*Δ	*swi1*Δ
YBR093C	*PHO5*	0.15	0.18	0.75	0.79
YAR071W	*PHO11*	0.08	0.18	0.58	0.78
YHR215W	*PHO12*	0.13	0.19	0.69	0.83
YML123C	*PHO84*	0.07	0.10	0.52	0.83
YFR034C	*PHO4*	1.08	1.11	1.11	1.30
YDL106C	*PHO2/GRF10*	0.85	0.85	0.87	1.03

*Ratio of mRNA levels of mutant to wild-type by microarray analysis. A typical result from a single experiment is shown. Results from other experiments are available at http://genome-www.stanford.edu/swisnf.

Table 2. Transcription of the *MATα*-specific genes in rich medium is Snf/Swi-dependent

		Microarray analysis*		Northern blot analysis†	
ORF name	Gene name	*snf2*Δ	*swi1*Δ	*snf2*Δ	*swi1*Δ
YKL178C	*STE3*	0.32	0.35	0.24	0.23
YPL187W	*MFα1*	0.70	0.63	0.50	0.62
YGL089C	*MFα2*	0.15	0.19	0.24	0.25
YJR004C	*SAG1*	0.11	0.09	0.13	0.11
YCR040W	*MATα1*	0.39	0.51	0.35	0.35
YMR043W	*MCM1*	0.57	0.70	0.42‡	0.32‡
				1.08§	1.09§
YHR084W	*STE12*	0.78	0.68	0.90	0.78

*Ratio of mRNA levels of mutant to wild-type, obtained by microarray analysis. A typical result from a single experiment is shown. Results from other experiments are available at http://genome-www.stanford.edu/swisnf.
†mRNA levels for each pair of wild-type and *swi1*Δ strains were averaged before fold difference was calculated. See Fig. 2.
‡mRNA level of the 1.6-kb *MCM1* transcript.
§mRNA level of the 1.2-kb *MCM1* transcript.

Effects on Two Gene Families: The Acid Phosphatase Genes and the *MATα*-Specific Genes Are Snf/Swi-Dependent. Members of a small number of gene families show changes in mRNA levels in *snf/swi* mutants. For example, mRNA levels of the inducible genes encoding acid phosphatase (*PHO5*, *PHO11*, *PHO12*) and the high-affinity phosphate permease (*PHO84*) were decreased 5- to 10-fold in rich medium but were unaffected in minimal medium in which their expression is significantly lower (Table 1; W. Hörz, personal communication). Transcription of the acid phosphatase genes is normally induced in medium with low inorganic phosphate (P_i) levels and is repressed by high P_i levels (38). It has been recently observed in an independent study that the basal transcription of *PHO5* that occurs in high P_i depends on Snf/Swi whereas the derepressed levels in low P_i are Snf/Swi-independent (W. Hörz, personal communication). The *snf/swi* effect on the transcription of these genes is unlikely to be an indirect effect of decreased levels of their transcriptional activators, Pho2 and Pho4, as *PHO2* and *PHO4* mRNA levels are not altered in *snf2*Δ and *swi1*Δ mutants (Table 1). Therefore, our microarray results suggest that Snf/Swi directly controls the promoters of the acid phosphatase genes in rich medium.

A second gene family observed to be Snf/Swi-dependent contains the four known *MATα*-specific genes, *STE3*, *MFα1*, *MFα2*, and *SAG1*. By microarray analysis, three of these genes have decreased mRNA levels in *snf/swi* mutants in rich and minimal media (Table 2). To confirm these results, we measured the mRNA levels for these four genes by Northern blot analyses. These results (Fig. 3*A*; Table 2) are close to those from the microarray analysis. Thus, three members of the *MATα*-specific gene family are affected by *snf2*Δ and *swi1*Δ mutations.

To address whether the Snf/Swi control of *MATα*-specific genes is direct or indirect, we investigated expression of their three known regulators, *MATα1*, *MCM1*, and *STE12* (39). These results (Fig. 3*B*; Table 2) showed that both *MATα1* and *MCM1* mRNA levels are reduced in *snf/swi* mutants whereas *STE12* mRNA levels are unaffected. *MATα1* mRNA levels were decreased approximately three-fold in both *snf2*Δ and *swi1*Δ strains compared with the wild type. The expression of *MCM1* in *snf2*Δ and *swi1*Δ was more complicated. *MCM1* has been previously reported to encode three transcripts, 1.0, 1.2, and 1.6 kb in length (40), although the 1.0-kb transcript was not detectable in our

analysis. Interestingly, the 1.2- and 1.6-kb *MCM1* transcripts show different Snf/Swi-dependence: the 1.6-kb *MCM1* mRNA level is reduced approximately three-fold in *snf/swi* mutants whereas the 1.2-kb *MCM1* mRNA level is unaffected. These results strongly suggest that the *MCM1* and the *MATα1* promoters are controlled by Snf/Swi and that the decrease in *MATα*-specific gene transcription in *snf/swi* mutants is caused by decreased expression of both *MATα1* and *MCM1*.

Promoter Analysis Reveals No Motifs Common to Snf/Swi-Dependent Genes. Snf/Swi has been shown to possess nonspecific DNA binding activity (41). However, no specific DNA sequence has been shown to confer Snf/Swi-dependence to a promoter. We wanted to determine whether Snf/Swi-dependent promoters had any common sequence motifs. Such motifs could target Snf/Swi to the promoter or could serve as binding sites for a common set of activators. We utilized two sequence analysis programs, MEME (33) and ALIGNACE (34) to analyze promoter sequences. Analysis of promoter sequences from all genes affected in rich medium revealed several motifs that are found upstream of the amino acid biosynthesis genes, like the *MET* gene family or upstream of some *PHO* genes (data not shown). However, neither program revealed any other motif that was specifically found in Snf/Swi-dependent genes. These negative results suggest that no single motif confers Snf/Swi-dependence to a promoter.

Transcriptional Control by Snf/Swi Is Gene-Specific. It is not clear whether Snf/Swi regulates transcription of individual genes or whether it exerts control over larger regions ranging from pairs of genes to extensive chromosomal domains. This issue can be addressed by examining the genomic positions of Snf/Swi-dependent genes. A clustering of the affected genes would support the possibility of Snf/Swi-controlled chromosomal domains of expression. Analysis of the positions of genes that show greater than a two-fold change in mRNA level in either *swi1*Δ or *snf2*Δ mutants shows that Snf/Swi-dependent genes are spread throughout the genome (http://genome-www.stanford.edu/swisnf) and demonstrate that there are no Snf/Swi-dependent chromosomal domains in the genome under the media conditions examined here.

Although Snf/Swi-dependence appears to be exerted at the level of individual genes, there are a small number of cases in which adjacent genes are both Snf/Swi-dependent. Therefore, we examined whether this co-dependence correlates with either the relative orientation of the two genes—for example, with divergently or convergently transcribed genes—or the distance between the genes. We found no correlation with either the orientation of the genes or the intergenic distance (data not shown). This analysis provides additional evidence that Snf/Swi control is specific to an individual gene and that the impact of its remodeling activity does not spread over the region to affect neighboring genes.

Evidence That Snf/Swi Repression and Activation Occur by Different Mechanisms. One surprising result from whole-genome expression analysis of *snf/swi* mutants, both from our studies and those previously described (6), is that the mRNA levels for many genes are elevated in *snf/swi* mutants, particularly in rich medium. In addition to these studies, there was some prior indication of a negative role for Snf/Swi in controlling transcription (21–24). The possibility of both positive and negative roles for Snf/Swi in transcription are consistent with recent biochemical results suggesting that Snf/Swi and related nucleosome-remodeling complexes can catalyze nucleosome remodeling from "repressive" to "remodeled" and vice versa (25, 26). However, the expression studies also raise the possibility that some of the effects observed may be indirect. For example, an apparent positive effect of a *snf/swi* mutation could result from decreased levels of a repressor whose expression is Snf/Swi-dependent.

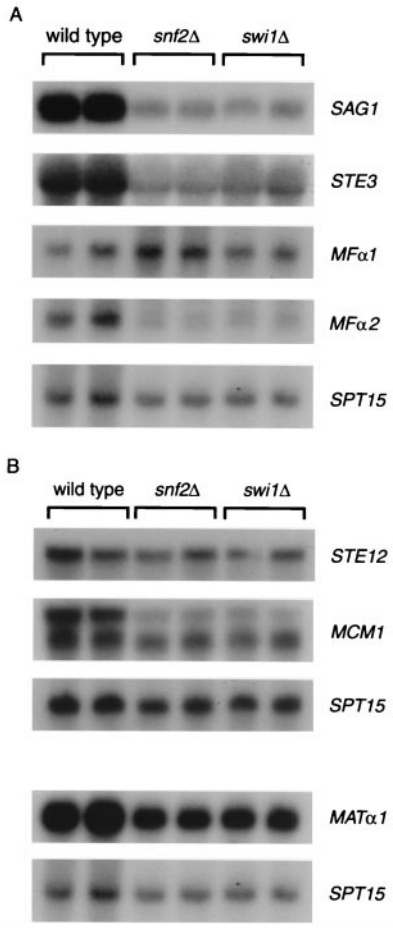

Fig. 3. (*A*) *snf2*Δ and *snf1*Δ mutations reduce the mRNA levels of *MATα*-specific genes. (*B*) *snf2*Δ and *snf1*Δ mutations reduce the mRNA levels of the *MATα* gene regulators *MATα1* and *MCM1*. Northern blot analysis was performed in the same strains described in the legend for Fig. 3, grown in rich medium. Quantitation, performed as described in the Fig. 3 legend, is shown in Table 2.

Previous studies have demonstrated that particular classes of mutations in histone genes—for example, a deletion of one copy of the two H2A-H2B-encoding loci in *S. cerevisiae*, (*hta1-htb1*)Δ—can partially suppress all of the phenotypes of *snf/swi* mutants, including the decrease in transcription observed at some genes (42). To understand whether the decreased expression of a repressor is responsible for the increased transcription of genes in both *snf2*Δ and *swi1*Δ, we analyzed mRNA levels of two genes, *SER3* and YOR222W, whose mRNA levels are greatly increased in a *snf2*Δ mutant. If transcription of these genes is increased indirectly, because of reduced transcription of a repressor in *snf/swi* mutants, then repressor levels would be restored in the (*hta1-htb1*)Δ *snf2*Δ double mutant and we should observe suppression of this *snf/swi* effect. However, Northern blot analysis (Fig. 4) demonstrates that the *snf/swi* effect on both *SER3* and YOR222W is not suppressed by the (*hta1-htb1*)Δ mutation as they have similar mRNA levels in both *snf2*Δ and (*hta1-htb1*)Δ *snf2*Δ strains. Because the (*hta1-htb1*)Δ mutation usually suppresses all of the known activation defects in *snf/swi* mutants, these results suggest that the increased transcription of these genes is not the result of decreased transcription of a Snf/Swi-dependent repressor but rather a direct effect of Snf/Swi action on *SER3* and YOR222W. In contrast, the decreased

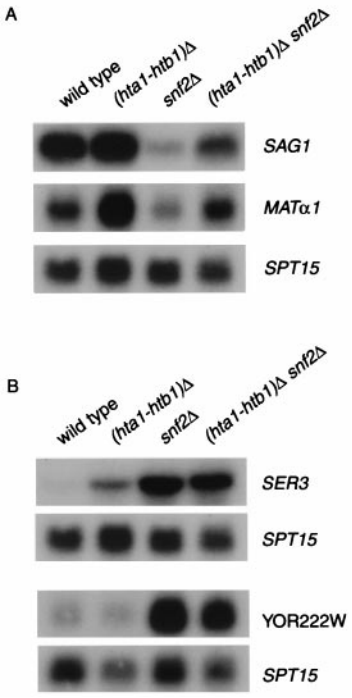

Fig. 4. The decreased mRNA levels of *SAG1* and *MATα1* in *snf2*Δ mutants are suppressed by (*hta1-htb1*)Δ, but the increased mRNA levels of *SER3* and YOR222W are not suppressed. (*A*) Northern blot analysis of *SAG1*, *MATα1*, and *SER3* was performed on RNA prepared from strains FY2, FY1884, FY31, and FY1885. *SAG1* mRNA levels in (*hta1-htb1*)Δ, *snf2*Δ, and (*hta1-htb1*)Δ *snf2*Δ are 0.82, 0.15, and 0.51, respectively. *MATα1* mRNA levels are 1.51, 0.39, and 1.20, respectively. (*B*) Analysis of YOR222W mRNA levels was done on strains FY57, FY710, FY458, and FY724. *SER3* mRNA levels in (*hta1-htb1*)Δ, *snf2*Δ, and (*hta1-htb1*)Δ *snf2*Δ are 5.28, 23.2, and 25.1, respectively. YOR222W mRNA levels are 1.79, 7.95, and 7.99, respectively. All mRNA levels are relative to those in the wild-type strain (1.0). All strains were grown in rich medium.

levels of the *SAG1* and *MATα1* mRNAs are suppressed by (*hta1-htb1*)Δ (Fig. 4). Thus, our results suggest that Snf/Swi may use different mechanisms for the activation and repression of transcription.

Discussion

We have combined the approach of whole-genome expression analysis with Northern blot, computational, and genetic analyses to understand more about the role of the Snf/Swi complex in controlling transcription in *S. cerevisiae*. These studies have provided information on Snf/Swi function *in vivo*. First, although only a small percentage of *S. cerevisiae* genes are strongly dependent on Snf/Swi, the set of Snf/Swi-controlled genes depends on growth conditions. Second, the loss of either of two different Snf/Swi components, Snf2 or Swi1, generally causes the same changes in mRNA levels. Third, Snf/Swi controls transcription at the level of individual genes, rather than targeting larger chromosomal domains. Fourth, Snf/Swi, which is nonessential for growth, contributes to the transcriptional control of at least one essential gene, *MCM1*. Finally, Snf/Swi appears to act as both a transcriptional activator and repressor *in vivo*, and our genetic evidence suggests that these opposite roles occur by distinct mechanisms. In these studies, the combination of whole genome expression analysis with more traditional methods has provided a productive approach to obtain a more complete picture of the *in vivo* roles of this transcription complex.

We have compared our results to those of a previously described whole-genome expression analysis of a *snf2* mutant grown in rich medium (6), and we find both similarities and differences. In both studies, it was observed that a *snf2* mutation causes increased and decreased mRNA levels. Both studies also concluded that a small subset of genes are strongly affected (greater than three-fold) in *snf/swi* mutants, although there is only partial overlap between the two studies (http://genome-www.stanford.edu/swisnf). The different findings can be attributed to the many differences between the two studies, including the experimental method, the genetic background of the *S. cerevisiae* strains used, and the particular *snf2* allele tested.

The limited requirement for Snf/Swi among all *S. cerevisiae* genes is likely caused by at least two different factors. First, most studies have concluded that Snf/Swi is not very abundant, existing at ≈100–200 complexes per haploid cell (7, 43, 44) (compared with an estimated 3,000 molecules of RNA polymerase II holoenzyme) (44). Snf/Swi is ≈10-fold less abundant than a closely related *S. cerevisiae* nucleosome remodeling complex, RSC (43). In addition, Snf/Swi has been shown to be partially redundant with other nucleosome modifying proteins, including Gcn5, a histone acetyltransferase required for transcriptional activation of some genes (45–48). Whole-genome expression studies (6) have shown that a *gcn5*Δ mutation, similar to *snf/swi* mutations, causes transcriptional effects on a small percentage of *S. cerevisiae* genes. A significantly greater number of genes would likely be affected in *gcn5*Δ *snf2*Δ double mutants.

Growth medium affects the set of Snf/Swi-dependent genes. There is a wealth of evidence that nutritional conditions alter gene expression (35, 38). A previous whole-genome expression study demonstrated that changing one environmental condition, the transition from glucose-rich to glucose-poor conditions, causes large-scale changes in the pattern of gene expression in *S. cerevisiae* (27). However, some of the differences that we found were surprising and may unveil previously unknown aspects of regulation. For example, the different control of the *PHO* genes in rich and minimal media, both containing high phosphate levels, is not understood. It seems likely that testing the requirement for Snf/Swi for cells grown under other conditions, such as low glucose or stress conditions, will uncover other sets of Snf/Swi-dependent genes.

A small number of gene families exhibit transcriptional changes in *snf/swi* mutants. Our results suggest that the Snf/Swi dependence of three *MATα*-specific genes is an indirect effect as Snf/Swi controls expression of the genes encoding two *MATα*-specific gene activators, Matα1 and Mcm1. Because Matα1 and Mcm1 assist each other's binding (39), the observed effects on their mRNA levels could cause a significant effect on activation. One of the *MATα*-specific genes, *MFα1*, does not appear to be Snf/Swi-dependent. The reasons for this difference are not currently understood. Similarly, it has recently been shown that, although *PHO5* and *PHO8* are both regulated by Pho4, the first is Snf/Swi-independent whereas the latter is Snf/Swi-dependent (19). The reduced mRNA levels for *MCM1* were not detected by the microarray analysis; the *snf/swi* defect was probably obscured by the fact that there are two detectable *MCM1* mRNAs and only one of the two is Snf/Swi-dependent. This effect on just one of the two *MCM1* mRNAs is reminiscent of the Snf/Swi control of *SUC2* mRNA (49).

An effect on *MCM1* expression may account for several *snf/swi* mutant phenotypes. Mcm1 acts as either an activator or a repressor at several loci, including the *MATα*-specific, *MAT***a**-specific (39), arginine anabolic, and arginine catabolic genes (50, 51). Previous studies have shown that several members of the *ARG* gene family exhibit defective repression in *mcm1* mutants (50, 51), suggesting that the increased mRNA levels of several *ARG* genes, observed in our studies (see http://genome-www.stanford.edu/swisnf), may be a result of decreased *MCM1* expression. It is also intriguing that both *snf/swi* and *mcm1* mutants impair the mitotic stability of minichromosomes con-

taining particular DNA replication origins (ARS) (40, 52). In addition, the slow growth of *snf/swi* mutants could be caused by reduced expression of *MCM1*, an essential gene.

Analysis of promoter sequences of Snf/Swi-dependent genes did not identify any common sequence motifs. This negative result fits with recent evidence that suggests that Snf/Swi may be targeted to the *HO* promoter by the Swi5 transcription factor rather than by particular promoter sequences (11, 53). Although it is likely, then, that Snf/Swi is targeted to promoters at least in part by interactions with transcriptional activators (or repressors) (12–18), it is still not understood what aspect of those activators governs Snf/Swi-dependence. In addition, a previous study of Gal4 activation suggested that promoter strength also plays an important role in Snf/Swi dependence of an activator (20).

Analysis of the genomic positions of Snf/Swi-dependent genes has shown that Snf/Swi control is limited to individual genes. Genes adjacent to a Snf/Swi-dependent genes are largely unaffected in *snf/swi* mutants. These results suggest that Snf/Swi-mediated nucleosome perturbations are highly localized and raises the possibility that there may be a mechanism to control the spread of a particular type of chromatin structure or of Snf/Swi control of that chromatin structure. In addition, these results suggest that Snf/Swi is not physically restricted to certain genomic locations and is recruited to each individual target gene.

Finally, the genome-wide transcription profiling studies described here and by Holstege *et al.* (6) have provided evidence that Snf/Swi acts as both an activator and as a repressor. These results fit well with other recent expression studies that have suggested that nucleosome remodeling complexes such as Snf/Swi and RSC are involved in transcriptional repression (21–24). In addition, they agree with biochemical studies that have demonstrated the bi-directional nature of the Snf/Swi and RSC remodeling activities *in vitro* (25, 26). Our genetic tests have provided strong evidence that, between activation and repression by Snf/Swi, one is not likely to be an indirect effect of the other. Taken together, these results support the emerging view that nucleosome remodeling complexes modulate nucleosome structure *in vivo* to help facilitate a dynamic and reversible transcriptional state (2). Further genetic and biochemical analysis of Snf/Swi repression will help us understand the mechanisms by which it controls transcription, as well as its roles in controlling transcription *in vivo*.

We thank Joe DeRisi for software and advice. We thank Jason Hughes, Barak Cohen, Rob Mitra, and George Church for help with computational analysis. This work was supported by National Institutes of Health Grants GM32967 to F.W. and HG00983 to P.O.B. and by the Howard Hughes Medical Institute. P.O.B. is an associate investigator of the Howard Hughes Medical Institute.

1. Workman, J. L. & Kingston, R. E. (1998) *Annu. Rev. Biochem.* **67,** 545–579.
2. Kingston, R. E. & Narlikar, G. J. (1999) *Genes Dev.* **13,** 2339–2352.
3. Cairns, B. R. (1998) *Trends Biochem. Sci.* **23,** 20–25.
4. Kornberg, R. D. & Lorch, Y. (1999) *Curr. Opin. Genet. Dev.* **9,** 148–151.
5. Kingston, R. E., Bunker, C. A. & Imbalzano, A. N. (1996) *Genes Dev.* **10,** 905–920.
6. Holstege, F. C., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S. & Young, R. A. (1998) *Cell* **95,** 717–728.
7. Côté, J., Quinn, J., Workman, J. L. & Peterson, C. L. (1994) *Science* **265,** 53–60.
8. Imbalzano, A. N., Kwon, H., Green, M. R. & Kingston, R. E. (1994) *Nature (London)* **370,** 481–485.
9. Kwon, H., Imbalzano, A. N., Khavari, P. A., Kingston, R. E. & Green, M. R. (1994) *Nature (London)* **370,** 477–481.
10. Phelan, M. L., Sif, S., Narlikar, G. J. & Kingston, R. E. (1999) *Mol. Cell* **3,** 247–253.
11. Cosma, M. P., Tanaka, T. & Nasmyth, K. (1999) *Cell* **97,** 299–311.
12. Dimova, D., Nackerdien, Z., Furgeson, S., Eguchi, S. & Osley, M. A. (1999) *Mol. Cell* **4,** 75–83.
13. Natarajan, K., Jackson, B. M., Zhou, H., Winston, F. & Hinnebusch, A. G. (1999) *Mol. Cell* **4,** 657–664.
14. Kowenz-Leutz, E. & Leutz, A. (1999) *Mol. Cell* **4,** 735–743.
15. Yoshinaga, S. K., Peterson, C. L., Herskowitz, I. & Yamamoto, K. R. (1992) *Science* **258,** 1598–1604.
16. Yudkovsky, N., Logie, C., Hahn, C. & Peterson, C. L. (1999) *Genes Dev.* **13,** 2369–2374.
17. Neely, K. E., Hassan, A. H., Wallberg, A. E., Steger, D. J., Cairns, B. R., Wright, A. P. & Workman, J. L. (1999) *Mol. Cell* **4,** 649–655.
18. Fryer, C. J. & Archer, T. K. (1998) *Nature (London)* **393,** 88–91.
19. Gregory, P. D., Schmid, A., Zavari, M., Munsterkotter, M. & Horz, W. (1999) *EMBO J.* **18,** 6407–6414.
20. Burns, L. G. & Peterson, C. L. (1997) *Mol. Cell. Biol.* **17,** 4811–4819.
21. Moehle, C. M. & Jones, E. W. (1990) *Genetics* **124,** 39–55.
22. Moreira, J. M. & Holmberg, S. (1999) *EMBO J.* **18,** 2836–2844.
23. Murphy, D. J., Hardy, S. & Engel, D. A. (1999) *Mol. Cell. Biol.* **19,** 2724–2733.
24. Trouche, D., Le Chalony, C., Muchardt, C., Yaniv, M. & Kouzarides, T. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 11268–11273.
25. Schnitzler, G., Sif, S. & Kingston, R. E. (1998) *Cell* **94,** 17–27.
26. Lorch, Y., Cairns, B. R., Zhang, M. & Kornberg, R. D. (1998) *Cell* **94,** 29–34.
27. DeRisi, J. L., Iyer, V. R. & Brown, P. O. (1997) *Science* **278,** 680–686.
28. Shore, P. & Sharrocks, A. D. (1995) *Eur. J. Biochem.* **229,** 1–13.
29. Winston, F., Dollard, C. & Ricupero-Hovasse, S. L. (1995) *Yeast* **11,** 53–55.
30. Rose, M. D., Winston, F. & Hieter, P. (1990) *Methods in Yeast Genetics: A Laboratory Course Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
31. Guthrie, C. & Fink, G. R. (1991) *Methods Enzymol.* **194,** 398–401.
32. Swanson, M. S., Malone, E. A. & Winston, F. (1991) *Mol. Cell. Biol.* **11,** 3009–3019.
33. Chu, S., DeRisi, J., Eisen, M., Mulholland, J., Botstein, D., Brown, P. O. & Herskowitz, I. (1998) *Science* **282,** 699–705.
34. Roth, F. P., Hughes, J. D., Estep, P. W. & Church, G. M. (1998) *Nat. Biotechnol.* **16,** 939–945.
35. Hinnebusch, A. G. (1992) in *General and Pathway-Specific Regulatory Mechanisms Controlling the Synthesis of Amino Acid Biosynthetic Enzymes in Saccharomyces cerevisiae*, eds. Jones, E. W., Pringle, J. R. & Broach, J. R. (Cold Spring Harbor Lab. Press, Plainview, NY), Vol. 2, pp. 319–414.
36. Forsburg, S. L. & Guarente, L. (1989) *Genes Dev.* **3,** 1166–1178.
37. Neigeborn, L. & Carlson, M. (1984) *Genetics* **108,** 845–858.
38. Johnston, M. & Carlson, M. (1992) in *Regulation of Carbon and Phosphate Utilization*, eds. Jones, E. W., Pringle, J. R. & Broach, J. R. (Cold Spring Harbor Lab. Press, Plainview, NY), Vol. 2, pp. 193–281.
39. Herskowitz, I., Rine, J. & Strathern, J. (1992) in *Mating-Type Determination and Mating-Type Interconversion in Saccharomyces Cerevisiae*, eds. Jones, E. W., Pringle, J. R. & Broach, J. R. (Cold Spring Harbor Lab. Press, Plainview, NY), Vol. 2, pp. 583–656.
40. Passmore, S., Maine, G. T., Elble, R., Christ, C. & Tye, B. K. (1988) *J. Mol. Biol.* **204,** 593–606.
41. Quinn, J., Fyrberg, A. M., Ganster, R. W., Schmidt, M. C. & Peterson, C. L. (1996) *Nature (London)* **379,** 844–847.
42. Hirschhorn, J. N., Brown, S. A., Clark, C. D. & Winston, F. (1992) *Genes Dev.* **6,** 2288–2298.
43. Cairns, B. R., Lorch, Y., Li, Y., Zhang, M., Lacomis, L., Erdjument-Bromage, H., Tempst, P., Du, J., Laurent, B. & Kornberg, R. D. (1996) *Cell* **87,** 1249–1260.
44. Wilson, C. J., Chao, D. M., Imbalzano, A. N., Schnitzler, G. R., Kingston, R. E. & Young, R. A. (1996) *Cell* **84,** 235–244.
45. Biggar, S. R. & Crabtree, G. R. (1999) *EMBO J.* **18,** 2254–2264.
46. Pollard, K. J. & Peterson, C. L. (1997) *Mol. Cell. Biol.* **17,** 6212–6222.
47. Roberts, S. M. & Winston, F. (1997) *Genetics* **147,** 451–465.
48. Sudarsanam, P., Cao, Y., Wu, L., Laurent, B. C. & Winston, F. (1999) *EMBO J.* **18,** 3101–3106.
49. Carlson, M. & Botstein, D. (1982) *Cell* **28,** 145–154.
50. Dubois, E. & Messenguy, F. (1994) *Mol. Gen. Genet.* **243,** 315–324.
51. Messenguy, F. & Dubois, E. (1993) *Mol. Cell. Biol.* **13,** 2586–2592.
52. Flanagan, J. F. & Peterson, C. L. (1999) *Nucleic Acids Res.* **27,** 2022–2028.
53. Krebs, J. E., Kuo, M. H., Allis, C. D. & Peterson, C. L. (1999) *Genes Dev.* **13,** 1412–1421.