The nucleosome remodeling complex, Snf/Swi, is required for the maintenance of transcription *in vivo* and is partially redundant with the histone acetyltransferase, Gcn5

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Snf/Swi, a nucleosome remodeling complex, is important for overcoming nucleosome-mediated repression of transcription in *Saccharomyces cerevisiae*. We have addressed the mechanism by which Snf/Swi controls transcription *in vivo* of an Snf/Swi-dependent promoter, that of the *SUC2* gene. By single-cell analysis, our results show that Snf/Swi is required for activated levels of *SUC2* expression in every cell of a population. In addition, Snf/Swi is required for maintenance of *SUC2* transcription, suggesting that continuous chromatin remodeling is necessary to maintain an active transcriptional state. Finally, Snf/Swi and Gcn5, a histone acetyltransferase, have partially redundant roles in the control of *SUC2* transcription, suggesting a functional overlap between two different mechanisms believed to overcome repression by nucleosomes, nucleosome remodeling and histone acetylation.

Keywords: histone acetylation/nucleosome remodeling/*Saccharomyces cerevisiae*/Snf/Swi

Introduction

The mechanism by which transcriptional activators and enhancers facilitate transcription from their target promoters *in vivo* remains unclear. Two models have been proposed for how activators and enhancers control transcription—gradient and binary (Walters *et al.*, 1996). In the gradient model, activators control the level of transcription from each promoter template. In the binary model, activators increase the probability that a promoter will be active rather than controlling the level of transcription from each activated promoter. A large body of evidence has suggested that most activators affect transcription in a gradient manner (Lewin, 1990). These studies have used conventional techniques to study populations of cells. However, most single-cell assays have suggested that mammalian activators may control transcription in a binary manner (Weintraub, 1988; Fiering *et al.*, 1990; Walters *et al.*, 1995, 1996; Bagga and Emerson, 1997). For example, studies have shown that some mammalian enhancers increase the number of active promoters rather than the level of expression from each promoter. In

addition, these results have suggested that transcription can be maintained in a stable manner in the absence of an enhancer, albeit in a small number of cells (Weintraub, 1988; Fiering *et al.*, 1990; Walters *et al.*, 1995, 1996; Bagga and Emerson, 1997). In contrast, there has been a recent report of a graded transcriptional response in a eukaryotic system (Kringstein *et al.*, 1998). In this study, the expression of a reporter gene increased in a gradient manner, proportional to increasing concentrations of the transactivator, in every cell of the population. Taken together, these results suggest that neither a binary nor a gradient response is a universal phenomenon in transcriptional activation and different mechanisms may operate depending on the particular enhancers and activators.

In our work, we have established an *in vivo* system at the endogenous *SUC2* locus in *Saccharomyces cerevisiae* to distinguish between the binary and gradient models for transcriptional activation by the nucleosome remodeling complex Snf/Swi. Snf/Swi is conserved throughout eukaryotes (Kingston *et al.*, 1996) and, in *S. cerevisiae*, it is required for the transcription of a diverse set of genes, including *SUC2*, *HO*, *INO1* and Ty elements (Winston and Carlson, 1992). Considerable evidence strongly suggests that Snf/Swi controls transcription by its nucleosome remodeling activity (Peterson and Tamkun, 1995; Steger and Workman, 1996; Cairns, 1998). Snf/Swi in *S. cerevisiae* is an ~2 mDa complex containing eleven proteins, including Snf2, Snf5 and Swi1 (Cairns *et al.*, 1994; Côté *et al.*, 1994; Peterson *et al.*, 1994). Previous work has demonstrated that Snf/Swi is required for the *SUC2* promoter to have an active chromatin conformation (Hirschhorn *et al.*, 1992; Matallana *et al.*, 1992). *SUC2* encodes invertase, an enzyme required for growth on media containing the carbon sources sucrose and raffinose. *SUC2* transcription is repressed in high glucose (2%) and there is an ~100-fold derepression of *SUC2* transcription in media containing low glucose (0.05%), sucrose, or raffinose. In a *snf/swi* mutant such as *snf2Δ*, which lacks Snf/Swi nucleosome remodeling activity, derepression is defective and only 10% of the wild-type level of *SUC2* mRNA or invertase is expressed (Winston and Carlson, 1992).

In this work, we have addressed three issues concerning the mechanism of Snf/Swi function *in vivo* by studying the effect of *snf/swi* mutations on *SUC2* expression. First, we demonstrate that Snf/Swi is required for activated levels of *SUC2* expression in every cell of a population. Secondly, we provide evidence that Snf/Swi is continuously required for *SUC2* transcription. Finally, we show that Snf/Swi is partially redundant with the histone acetyltransferase Gcn5 in controlling *SUC2* transcription. Taken together, the continuous requirement for Snf/Swi in maintaining *SUC2* transcription provides new evidence for the dynamic nature of chromatin in every cell of a population.

Fig. 1. Northern analysis of *suc2Δ10::GFP* expression. *SNF2⁺ SUC2⁺* (FY86), *SNF2⁺ suc2Δ10::GFP* (FY1755) and *snf2Δ suc2Δ10::GFP* (FY1756) cells were grown in YPD (2% glucose) and shifted to YEP plus 0.05% glucose. RNA was isolated and analyzed from samples taken at the indicated times after the shift. *TUB2* mRNA was measured as a loading control. The top pair of panels shows the previously characterized derepression of wild-type *SUC2* RNA (Winston and Carlson, 1992). The middle and bottom pairs of panels compare the expression of the *suc2Δ10::GFP* allele in *SNF2⁺* and *snf2Δ* genetic backgrounds, respectively. The higher level of *suc2Δ10::GFP* mRNA compared with *SUC2* mRNA is probably caused by differential stability of the mRNAs and different specific activities of the ³²P-labeled probes used in these experiments.

Results

Snf/Swi controls the level of expression from each SUC2 template

We wanted to determine which model of transcriptional activation, binary or gradient, applies to Snf/Swi activation of *SUC2*. The gradient model predicts that every cell in a *snf2Δ* population would show the same reduced level of *SUC2* expression, that is 10% of the wild-type level. In contrast, the binary model predicts that there would be two populations in a *snf2Δ* mutant: 10% of the population with full activity and the remaining 90% of the population with no activity at all. Either model would be consistent with the observed 10% invertase activity in a population of *snf2Δ* cells.

To distinguish between these two models, we established an *in vivo* system in *S.cerevisiae* to analyze expression of the endogenous *SUC2* locus in individual cells of a population. *Saccharomyces cerevisiae* strains were constructed in which the *SUC2* open reading frame (ORF) is replaced by the ORF for green fluorescent protein (GFP) (Prasher, 1995; Tsien, 1998). Northern analyses of this allele (*suc2Δ10::GFP*) demonstrate that it derepresses at a similar rate as wild-type *SUC2* and that its expression is Snf/Swi dependent (Figure 1).

B

Strain	Median fluorescence
<i>SNF2⁺ repressed</i>	4.8
<i>SNF2⁺ derepressed</i>	34.6
<i>snf2Δ repressed</i>	7.3
<i>snf2Δ derepressed</i>	13.5

Fig. 2. Snf/Swi increases the level of *SUC2* expression in every cell of the population. (A) FACS profiles of GFP fluorescence in *SNF2⁺ suc2Δ10::GFP* (FY1755) and *snf2Δ suc2Δ10::GFP* (FY1756) repressed and derepressed cells. Cells were grown in YPD (repressed samples) and shifted to YEP plus 0.05% glucose (derepressed samples) for 2 h and 45 min before undergoing FACS analysis for green fluorescence. *SNF2⁺ SUC2⁺* cells gave similar levels of green fluorescence as *SNF2⁺ suc2Δ10::GFP* repressed cells in both repressing and derepressing conditions. The slight increase in green fluorescence of *snf2Δ* cells in repressing conditions is probably due to the larger cell size of the *snf2Δ* cells. (B) The median fluorescence of each sample in both repressing and derepressing conditions. The median fluorescence for the total population of cells is shown, as calculated by the CellQuest program.

To examine expression from the *SUC2* promoter in individual cells in a population, both *SNF2⁺* and *snf2Δ* cells containing *suc2Δ10::GFP* were subjected to fluorescence-activated cell sorting (FACS) analysis after growth in either repressing or derepressing conditions (Figure 2). In these analyses, the repressed/derepressed ratios of GFP fluorescence observed in *SNF2⁺* and *snf2Δ* are not identical to the ratios observed when measuring *SUC2* mRNA in the same genetic backgrounds. These differences probably reflect the inherent differences in measuring *SUC2* mRNA versus GFP protein. Nevertheless, the *suc2Δ10::GFP* fusion appears to be an accurate reporter of Snf/Swi control of the *SUC2* promoter. As expected, *SNF2⁺* cells did not show any green fluorescence in repressing

conditions but did show a high level under derepressing conditions (Figure 2). Also as expected, *snf2Δ* cells did not express any green fluorescence under repressing conditions. However, under derepressing conditions, *snf2Δ* cells showed a single, uniform population with only a small increase in GFP fluorescence (Figure 2). FACS analysis of a *swi1Δ suc2Δ10::GFP* mutant gave similar results (data not shown). In control experiments mixing *SNF2⁺* derepressed cells with *SNF2⁺* repressed cells, we detected the GFP-expressing cells as a distinct peak when they were as low as 2.5% of the total population (data not shown). Therefore, the presence of a single, uniform population of GFP-expressing *snf2Δ* cells suggests that Snf/Swi increases the level of *SUC2* expression in every cell and strongly supports the gradient model, and not the binary model, for Snf/Swi activity at *SUC2*. These results strongly suggest that Snf/Swi is required for maintenance of the activated state and do not rule out the possibility that it is also required for establishment. However, these data exclude the possibility that Snf/Swi is required solely for establishment of the activated state. Lastly, the small increase in green fluorescence in *snf2Δ* and *swi1Δ* cells under derepressing conditions suggests that other factors besides Snf/Swi may contribute towards *SUC2* transcription.

Snf/Swi is continuously required for *SUC2* transcription

To study the role of Snf/Swi in maintaining *SUC2* transcription, *SUC2* mRNA levels were measured in a *snf5* temperature sensitive mutant, *snf5-51*. *snf5-51* causes no growth defect at 30°C but prevents growth on raffinose media at 37°C. Analysis of a *snf5-51* mutant at 37°C has shown that *SUC2* chromatin is in a 'repressed' state, similar to that seen in *snf5Δ* and *snf2Δ* mutants (Y.Cao and B.C.Laurent, in preparation). To examine the need for Snf/Swi after *SUC2* derepression, the *snf5-51* mutant was grown at 25°C in raffinose to fully derepress *SUC2* and then shifted to 37°C. *SUC2* mRNA levels were measured over time by Northern analysis. Under the conditions of this experiment, the *snf5-51* cells did not divide following the shift to raffinose. *SUC2* mRNA levels significantly decreased in the *snf5-51* mutant after a shift to 37°C and were close to that of a *snf5Δ* mutant after 4 h (Figure 3). The kinetics of the decrease are consistent with the relatively long half-life of *SUC2* mRNA in media containing a poor carbon source such as raffinose or glycerol (Cereghino and Scheffler, 1996). Our studies indicate that the half-life of *SUC2* mRNA in the *snf5-51* mutant is ~3 h, consistent with *SUC2* transcription being significantly reduced after the shift to 37°C (see Figure 3 legend). Similar results have been obtained by Biggar and Crabtree (1999) by analyzing a *snf2Δ* mutant under conditions in which the half-life of the *SUC2* mRNA is much shorter. Both sets of results strongly suggest that Snf/Swi is continuously needed to maintain a normal level of *SUC2* transcription *in vivo*.

Gcn5 is partially redundant with Snf/Swi for *SUC2* transcription

The low level of *SUC2* expression observed in *snf/swi* mutants suggested that factors besides Snf/Swi could activate *SUC2* transcription to a limited degree. Recent

Fig. 3. Snf/Swi is required for maintenance of *SUC2* transcription. *SNF5⁺* (FY22), *snf5-51* (FY1757) and *snf5Δ2* (FY1658) cells were grown in YPD at 25°C and then shifted to YPRaffinose for 2 h and 45 min to derepress *SUC2*. After derepression, cells were shifted to 37°C. RNA was isolated and analyzed from samples taken at the time points indicated. Expression of *SUC2* and the loading control, *SPT15*, was measured by Northern analyses. The relative intensities of the *SUC2* and *SPT15* bands were quantitated using a PhosphorImager (Molecular Dynamics). The *SUC2*/*SPT15* ratio for each strain was normalized to the *SUC2*/*SPT15* ratio for the wild-type strain at 0 h, 37°C. The average values from two experiments are presented at the bottom.

genetic studies have suggested that Gcn5, a histone acetyltransferase (Brownell *et al.*, 1996; Kuo *et al.*, 1998; Wang *et al.*, 1998), could be one of those other factors. Double mutant analysis demonstrated that a combination of a null mutation in *GCN5* with null mutations in different *SNF/SWI* genes caused double mutant sickness (Roberts and Winston, 1997; Recht and Osley, 1999). In addition, *gcn5Δ* and *snf2Δ* have shown double mutant lethality in other genetic backgrounds (Pollard and Peterson, 1997). One interpretation of these double mutant phenotypes is that Gcn5 is partially redundant with Snf/Swi for transcription.

To investigate whether Gcn5 was responsible for the low level of *SUC2* expression observed in a *snf2Δ* mutant, two tests were performed. First, a *gcn5Δ snf2Δ* double mutant was compared with *gcn5Δ* and *snf2Δ* single mutants for their ability to grow on media containing sucrose and raffinose as a carbon sources (Figure 4A). As sucrose is a less stringent carbon source than raffinose, the *SUC2* expression in either a *snf2Δ* mutant or a *gcn5Δ* mutant is sufficient to allow those strains to grow on sucrose plates but not on raffinose plates. However, a *gcn5Δ snf2Δ* double mutant was unable to grow even on sucrose plates, similar to a *suc2Δ*, suggesting that *SUC2* expression is decreased further in the *gcn5Δ snf2Δ* double mutant compared with the *snf2Δ* single mutant. Secondly, we measured *SUC2* mRNA levels by Northern analysis under repressing and derepressing conditions in *gcn5Δ*, *snf2Δ*, and *gcn5Δ snf2Δ* mutants (Figure 4B). *SUC2* mRNA levels were not significantly affected in a *gcn5Δ* mutant and were greatly reduced in a *snf2Δ* mutant under derepressing conditions (Figure 4B). In a *gcn5Δ snf2Δ* double mutant, the level of *SUC2* mRNA was reproducibly below that seen in a *snf2Δ* single mutant, close to repressed levels. The remaining signal detected in the *gcn5Δ snf2Δ* double mutant under derepressing conditions and under repressing conditions is probably the unregulated 1.8 kb constitutive, cytoplasmic *SUC2* RNA (Carlson *et al.*, 1981). Our analysis of the *gcn5Δ snf2Δ* effects agree with those obtained by Biggar and Crabtree (1999). These results strongly suggest that both Snf/Swi and Gcn5 contribute to transcriptional activation of *SUC2*.

Fig. 4. Snf/Swi and Gcn5 are partially redundant for transcriptional activation of *SUC2*. (A) *gcn5Δ snf2Δ* cells cannot grow on YEP plus 2% sucrose (YPSucrose). Wild-type (FY114), *gcn5Δ* (FY1354), *snf2Δ* (FY1360), and *gcn5Δ snf2Δ* (FY1352) cells were spotted onto YPD, YPSucrose and YPRaffinose plates and incubated for 3 days at 30°C. (B) The *gcn5Δ snf2Δ* double mutant has lower levels of *SUC2* RNA than the *gcn5Δ* or *snf2Δ* single mutants. Wild-type (FY114), *gcn5Δ* (FY1354), *snf2Δ* (FY1360) and *gcn5Δ snf2Δ* (FY1352) cells were grown in YPD (repressing, R) and shifted to YEP plus 0.05% glucose (derepressing, DR) for 2 h and 45 min. mRNA levels of *SUC2* and the loading control, *TUB2*, were measured by Northern analyses. The relative intensities of the *SUC2* and *TUB2* bands were quantitated using a PhosphorImager (Molecular Dynamics). The *SUC2/TUB2* ratio for each strain was normalized to the *SUC2/TUB2* ratio for the wild-type strain derepressed for *SUC2* expression. The average values from two experiments are presented at the bottom.

Discussion

Our studies have investigated the mechanism of Snf/Swi activity *in vivo* at the *SUC2* promoter and have provided three new results. First, we have conducted the first study of transcriptional activation at the level of single cells in *S.cerevisiae*. Using a GFP-based reporter integrated at the endogenous *SUC2* locus and FACS analysis, we have shown that Snf/Swi is required for activated levels of *SUC2* expression in every cell in the population. Secondly, our results strongly suggest that Snf/Swi is continuously required to maintain *SUC2* transcription *in vivo* and not merely required for establishing an active chromatin structure at the locus. Thirdly, we have demonstrated a functional redundancy between Snf/Swi and the histone acetyltransferase, Gcn5.

The use of a GFP reporter has allowed us to analyze the role of Snf/Swi in transcriptional control at the level of individual cells. In the case that we have studied, all

cells in the population had a similar level of transcription and did not show any bimodal expression patterns. Among single-cell studies using mammalian cells, both gradient and binary responses have been obtained (Weintraub, 1988; Fiering *et al.*, 1990; Walters *et al.*, 1995, 1996; Bagga and Emerson, 1997; Kringstein *et al.*, 1998) suggesting that mechanisms for transcription will differ among various activators and loci. Given the relative ease of this type of analysis in yeast, it should be straightforward to understand the role of any transcriptional activator at this level in *S.cerevisiae*. In addition, each promoter can be studied at its endogenous genomic location avoiding artifacts resulting from changes in position of the reporter.

The role of Snf/Swi in maintaining transcription has been an open issue. Several *in vitro* studies have examined the need for continuous Snf/Swi activity for nucleosome remodeling, both on mononucleosomes and on nucleosomal arrays. Studies performed on mononucleosomes and some plasmid templates have suggested that Snf/Swi, or some aspect of Snf/Swi activity, may not be continuously required *in vitro* to maintain an altered nucleosomal structure (Imbalzano *et al.*, 1996; Côté *et al.*, 1998). Other studies, with nucleosomal arrays, have suggested that Snf/Swi-dependent remodeling is reversed upon removal of Snf/Swi, implying a need for continuous chromatin remodeling (Owen-Hughes *et al.*, 1996; Logie and Peterson, 1997). Our results suggest that, *in vivo*, continuous remodeling by Snf/Swi is necessary to maintain the transcriptionally active state of promoters like *SUC2*.

There are several possible reasons why Snf/Swi might be continuously needed for *SUC2* transcription. Snf/Swi may facilitate increased rates of RNA polymerase II reinitiation, as has been observed for other activators (Ho *et al.*, 1996). Alternatively, Snf/Swi may play a role in transcription elongation at *SUC2*. In support of this possibility, Snf/Swi has been shown to help overcome the inhibitory effect of nucleosomes on elongation (Brown *et al.*, 1996). Finally, Snf/Swi may be continuously required because, *in vivo*, other factors may remodel chromatin back to an inactive state. By this model, chromatin structure at Snf/Swi-dependent genes would be in a dynamic equilibrium between a remodeled 'open' state and a non-remodeled 'closed' state, constantly requiring Snf/Swi activity for transcription. This picture of transcription is further complicated by recent studies showing that human Snf/Swi and the related yeast complex, Rsc, can cycle nucleosomes between different conformations *in vitro*. These work suggest that, in addition to other factors, Snf/Swi itself may also contribute to modulating this dynamic equilibrium *in vivo* (Lorch *et al.*, 1998; Schnitzler *et al.*, 1998).

Lastly, we have shown that the remaining *SUC2* transcription in *snf/swi* mutants is dependent on the action of Gcn5. The genetic interactions between Gcn5 and Snf/Swi and their effects on *SUC2* expression suggest that their roles in transcriptional control overlap. The comparison of the phenotypes of the *snf2Δ* and *gcn5Δ* single mutants to those of the *gcn5Δ snf2Δ* double mutant, taken in combination with their effects on *SUC2* expression and other genes (Biggar and Crabtree, 1999), suggests that both Snf/Swi and Gcn5 contribute to the activation of a common set of genes or to an overlapping but non-identical set of genes. Measurement of all mRNA levels

in these mutants (Schena *et al.*, 1995; Chee *et al.*, 1996; Velculescu *et al.*, 1997) should help to distinguish between these possibilities. Based on this type of mRNA analysis for cells grown under one set of conditions, Snf/Swi and Gcn5 clearly do not control an identical set of genes (Holstege *et al.*, 1998). However, mRNA analysis in the *gcn5Δ snf2Δ* double mutant would be required to truly clarify these possibilities.

It is unclear how the the Snf/Swi and Gcn5 activities might be coordinated to facilitate transcription from the *SUC2* promoter. The relative effects of *snf2Δ* and *gcn5Δ* mutations suggest that chromatin remodeling by Snf/Swi is very important for *SUC2* transcription whereas histone acetylation by Gcn5 plays a more minor role. Since the most striking effect of a *gcn5Δ* mutation is observed in a *snf2Δ* background, Gcn5 may prefer to acetylate histones at *SUC2* when they have not been remodeled by Snf/Swi. Analyzing acetylation states at *SUC2* in both wild-type and *snf/swi* mutant strains will be important to understand the functional relationships between these factors. These studies should enhance our understanding of how different chromatin modifying factors control and maintain a dynamic chromatin structure at a particular locus.

Materials and methods

Yeast strains and methods

The *S.cerevisiae* strains used in these studies were constructed by standard methods (Rose and Winston, 1990). They are FY22 (*MATa his3Δ200 ura3-52*), FY86 (*MATα his3Δ200 ura3-52 leu2Δ1*), FY114 (*MATa ura3-52 lys2-173R2*), FY1352 (*MATa his3Δ200 ura3-52 leu2Δ1 lys2-173R2 gcn5Δ::HIS3 snf2Δ::LEU2*), FY1354 (*MATα his3Δ200 ura3-52 leu2Δ1 lys2-173R2 gcn5Δ::HIS3*), FY1360 (*MATa his3Δ200 ura3-52 leu2Δ1 lys2-173R2 snf2Δ::LEU2*), FY1658 (*MATa his3Δ200 ura3-52 lys2-1288 snf5Δ2*), FY1755 (*MATα his3Δ200 ura3-52 leu2Δ1 suc2Δ10::GFP*), FY1756 (*MATα his3Δ200 ura3-52 snf2Δ::HIS3 suc2Δ10::GFP*), and FY1757 (*MATa his3Δ200 ura3-52 snf5-51*). Media was prepared according to standard methods (Rose and Winston, 1990).

In the *suc2Δ10::GFP* allele, the coding region of *SUC2* is replaced with the ORF of yeast-enhanced GFP (yEGFP3) (Cormack *et al.*, 1997). This allele was constructed in two steps. First, the *URA3* ORF was inserted into the *Bam*HI site within the *SUC2* coding region. Then, a PCR fragment containing yEGFP3 flanked by 41 bp of 5' and 3' sequence immediately adjacent to the *SUC2* ORF was used to transform the *suc2::URA3* strain. Selecting for 5-fluoroorotic acid resistance enriched for cells in which the GFP-encoding sequence had integrated at *SUC2*, precisely replacing the *SUC2* ORF.

FACS analysis

Cells were grown in yeast extract/peptone/2% glucose (YPD) to a density of $1-2 \times 10^7$ cells/ml. They were washed in water, and either resuspended in YPD (repressed sample) or shifted to yeast extract/peptone (YEP) plus 0.05% glucose (derepressed sample). Cells were then grown at 30°C for 2 h and 45 min, washed once and resuspended in water before FACS analysis. Thirty thousand cells were scanned for green fluorescence in a Becton Dickinson FACScan and results were analyzed using the CellQuest 3.1 program (Becton Dickinson).

Northern hybridization analysis

Strains were grown in YPD (2% glucose) to $\sim 8 \times 10^6$ cells/ml. They were washed in water and resuspended in either YPD (repressed sample), YEP plus 0.05% glucose (derepressed sample), or YEP plus 2% raffinose (YPRaffinose; derepressed sample). Ten milliliter samples were taken at the times indicated in the figures. RNA was prepared and analyzed as described previously (Swanson *et al.*, 1991). RNA levels were quantitated by PhosphorImager analysis (Molecular Dynamics).

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