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Evidence that Snf–Swi controls chromatin structure over both the TATA and UAS regions of the *SUC2* promoter in *Saccharomyces cerevisiae*

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ABSTRACT

The Snf–Swi complex of the yeast *Saccharomyces cerevisiae* has been shown to control gene expression by controlling chromatin structure. We have analyzed the promoter of the *SUC2* gene, a gene strongly controlled by Snf–Swi, by a high resolution analysis of micrococcal nuclease digests. This analysis suggests that there are at least four nucleosomes positioned over the *SUC2* TATA and UAS regions under conditions repressing *SUC2* transcription. Under derepressing conditions this entire promoter region is much more sensitive to MNase digestion. Analysis of an *snf2*Δ mutant demonstrates that even under derepressing conditions the *SUC2* promoter is resistant to MNase digestion. Thus, the Snf–Swi complex appears to control chromatin structure over both the *SUC2* TATA and UAS regions. The presence of nucleosomes over both promoter regions may explain the strong requirement of *SUC2* for Snf–Swi function.

INTRODUCTION

Genetic, biochemical and molecular experiments have strongly suggested that the Snf–Swi complex controls eukaryotic gene expression by antagonizing chromatin-mediated repression of transcription (1). This multiprotein complex is strongly required for transcription of *SUC2*, a *Saccharomyces cerevisiae* gene encoding the enzyme invertase, required for growth of yeast cells on sucrose. The *SUC2* gene is controlled by glucose repression: in the presence of high glucose *SUC2* is transcriptionally repressed; in low glucose *SUC2* is derepressed and transcription is increased >100-fold (2). Previous work has shown that under derepressing conditions *snf/swi* mutants have an ~10-fold lower level of *SUC2* mRNA. Analysis of *SUC2* chromatin structure in wild-type, *snf2* and *snf5* strains has shown that Snf–Swi functions by altering chromatin structure to a transcriptionally permissive conformation (3).

In order to gain a better understanding of the role Snf–Swi plays in regulation of chromatin structure at the *SUC2* gene, we analyzed the *SUC2* promoter chromatin structure by a high resolution analysis of digestion by micrococcal nuclease (MNase). Our results provide additional evidence for the presence of two nucleosomes previously identified over the TATA box and upstream of the TATA box. We have extended our knowledge of *SUC2* promoter chromatin structure by demonstrating the presence of two additional nucleosomes positioned over the UAS region. Alterations in chromatin structure occur over this entire region upon derepression in wild-type cells. In *snf2* mutants, in which Snf–Swi is not present, these changes in chromatin structure are not observed. Thus, Snf–Swi appears to be required for controlling at least four nucleosomes that cover the *SUC2* TATA and UAS regions.

MATERIALS AND METHODS

Yeast strains and genetic methods

The two *S.cerevisiae* strains used in these studies are congenic to S288C except that they are *GAL2*$^+$ (4). The two strains are FY120 (*MAT***a** *his4-912*δ *lys2-128*δ *leu2*Δ*1 ura3-52*) and FY458 (*MAT***a** *his4-912*δ *lys2-128*δ *snf2*Δ*1*::*HIS3 his3*Δ*200 ura3-52*). The *snf2*Δ*1*::*HIS3* null allele has been previously described (5).

All media were prepared as described (6). YEP + 0.05% glucose was identical to YPD except that it contained 0.05% glucose instead of 2% glucose.

Glucose-repressed cultures were grown in YPD at 30°C and harvested at 2×10^7 cells/ml. For derepressed cultures, cells were grown in YPD to 2×10^7 cells/ml and then washed twice with water, then grown in an equal volume of YEP + 0.05% for 165 min at 30°C.

Enzymes

MNase was purchased from Boehringer Mannheim Biochemicals and stored at 6000 U/ml at –20°C in 50% glycerol, 2 mM $CaCl_2$ and 50 mM Tris, pH 9.0. *Taq* polymerase was purchased from Promega.

MNase digestion and DNA isolation

From each culture, 4×10^9 cells were spun down and resuspended in 10 ml room temperature S buffer (1.1 M sorbitol, 20 mM KPO_4, pH 7.0, 0.5 mM $CaCl_2$) and 10 mM DTT (added fresh) and incubated for 15 min at 30°C. Cells were then centrifuged and all

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liquid was removed by aspiration. The cell pellet was resuspended in a total volume of 1 ml S buffer. To each 1 ml cells was added 1 ml S buffer with freshly added zymolyase T100 (1 mg/ml). Cells were shaken at 90 r.p.m. for 10–15 min at 30°C. Greater than 90% of the cells were converted to spheroplasts as monitored by lysis in a 50-fold excess of water. Spheroplasts were washed twice by adding 10 ml S buffer and centrifuged in a Sorvall H6000A rotor for 6 min at 1200 r.p.m. and resuspended gently.

For MNase digestion each sample was gently resuspended with a large bore pipet tip to a final volume of 600 µl in ice-cold buffer A (1 M sorbitol, 50 mM NaCl, 10 mM Tris–HCl, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM β-mercaptoethanol and 0.5 mM spermidine). To begin digestion, 200 µl aliquots of the cell slurry were added to 200 µl prewarmed (37°C) buffer A with 0.15% NP40 and MNase (0.5, 1.5 or 5 U/ml final concentration) and incubated for 5 min at 37°C. The reactions were terminated by addition of 40 µl stop buffer (250 mM EDTA, 5% SDS) and incubated for an additional 15 min at 37°C.

To isolate the DNA, 160 µl 5 M KOAc were added to each digest and incubated on ice for 30 min. After spinning down the precipitate for 15 min in a microfuge, the supernatant was transferred to a new 1.5 ml microcentrifuge tube and the DNA precipitated by adding an equal volume of isopropanol. The DNA was pelleted by spinning in a microfuge for 10 min. The DNA pellet was then resuspended in 500 µl TE buffer with 100 µg/ml RNase A and incubated for 30 min at 37°C. DNA was extracted with an equal volume of phenol/chloroform. The aqueous phase was transferred to a fresh tube and DNA was precipitated by adding 67 µl 7.5 M NH_4OAc and 500 µl isopropanol.

The naked DNA was prepared by starting with 8×10^9 cells. These cells were spheroplasted as above and resuspended in a final volume of 1200 µl buffer A and divided into 400 µl aliquots. Cells were lysed by adding 80 µl stop buffer to each tube. Then, 320 µl KOAc were added. RNase digests were then carried out as for the other samples. To digest with MNase, the DNA was resuspended in 300 µl buffer A containing 0.075% NP40. To start the digest, 100 µl buffer A + 0.075% NP40 containing MNase (0.01, 0.05 or 0.15 U/ml final concentration) were added. DNA was incubated for 1 min at 37°C. Digestions were stopped by addition of 10 µl 500 mM EDTA. Samples were then extracted with an equal volume of phenol/chloroform and precipitated with 3 M NaOAc.

All samples were resuspended in 100 µl water and 2 µl was run on a 1% agarose gel to determine the extent of digestion. For optimal analysis by primer extension methods the bulk of the DNA should run as a high molecular weight band. With increasing amounts of digestion a faint smear of DNA should be visible in the lane. If a nucleosome ladder is present, this indicates overdigestion.

In preparation for PCR analysis, DNA was digested with *Eco*RI (*Eco*RI cuts outside the *SUC2* region undergoing PCR analysis). Following digestion the DNA was phenol/chloroform extracted and precipitated and resuspended in 50 µl H_2O.

PCR primers

For primer extension analysis the following oligonucleotides were used. All oligonucleotides were purified by polyacrylamide gel electrophoresis before use. The annealing temperatures for each oligonucleotide are given in parentheses.

S13, 5′-GCACGGTGAGCTGTCGAAGG-3′ (63°C) [–755 to –736];
S11, 5′-GGTATGGTACGTTAGAAAGGC-3′ (57°C) [–587 to –567];
SUC2-2, 5′-GGTACGCCCGATGTTTGCCTATTACC-3′ (67°C) [–313 to –288];
SUC2-1, 5′-GGTAATAGGCAAACATCGGGCGTACC-3′ (67°C) [–288 to –313];
SUC2-3, 5′-GTGAAGTGGACCAAAGGTCTATCG-3′ (63°C) [+100 to +76].

Oligonucleotides (100 ng) were end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase (7).

Primer extension analysis by PCR

Primer extension analysis by PCR of DNA digested by MNase was carried out as described previously (8) except that 5 µg genomic DNA were used in each reaction and the following Taq buffer was substituted: 50 mM KCl, 10 mM Tris, pH 8.5, 2.5 mM $MgCl_2$, 170 µg/ml BSA.

Following PCR and precipitation, DNA fragments were separated on an 8% sequencing gel and visualized by autoradiography.

RESULTS

Previous analysis of *SUC2* promoter chromatin structure by indirect end-labeling suggested that in either repressed cells or in *snf/swi* mutants the presence of two positioned nucleosomes, one centered over the TATA box and the second located in between the TATA box and UAS (3,9,10). This analysis further suggested that in wild-type derepressed cells these nucleosomes were either absent or altered in such a way as to allow digestion of the DNA by MNase. Given the nature of indirect end-labeling experiments, the previous analysis did not allow accurate mapping of the MNase cleavage sites. In addition, we were unable to determine if MNase digestion was affected over the UAS region. Therefore, we set out to analyze the entire *SUC2* promoter region from near the transcription initiation site (–40 relative to the ATG) upstream through the UAS region (to –650) by a high resolution analysis of MNase cleavage.

A positioned nucleosome covering the *SUC2* TATA box

To analyze the position of the nucleosome over the *SUC2* TATA box we used primer *SUC2*-2 and examined MNase cleavage under four sets of conditions: *SNF2*$^+$ repressed and derepressed and *snf2*Δ repressed and derepressed. In *SNF2*$^+$ repressed cells most of the DNA at the TATA box is not accessible to MNase digestion (Fig. 1). Two clusters of MNase cleavage sites are observed on the antisense strand; there is a doublet located at nucleotides –74 and –75 and a second cluster, composed of a triplet, located at nucleotides –215 to –217. The distance between the first cluster located at –74,–75 and the center of the second cluster is 146 bp, which corresponds to the length of DNA in a nucleosome. The TATA box, located at –133, is situated in the middle of the protected DNA region. When a *SNF2*$^+$ strain is grown under derepressing conditions, a significant increase in DNA cleavage by MNase is observed, indicating an increase in accessibility. The increase in DNA accessibility observed in derepressed cells indicates that this nucleosome is altered when *SUC2* is transcriptionally active. Similar results were obtained on the sense strand, although the observed cleavages were not as pronounced (data not shown). These results indicate that a nucleosome is positioned directly over the TATA box in repressed cells.

Interestingly, the cleavage pattern of derepressed chromatin differs from the cleavage pattern of naked DNA. For example, an AT-rich region located at nucleotides –184 to –174 that is strongly

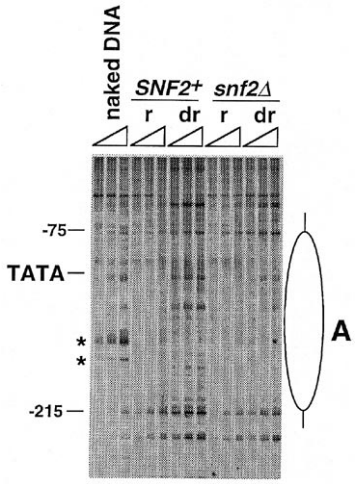

Figure 1. Micrococcal nuclease analysis of the *SUC2* TATA region. Strains were grown and chromatin was prepared and analyzed as described in Materials and Methods. The strains were grown under either repressing (r, 2% glucose) or derepressing (dr, 0.05% glucose) conditions. The position of the nucleosome indicated by MNase digestion is indicated to the right of the figure.

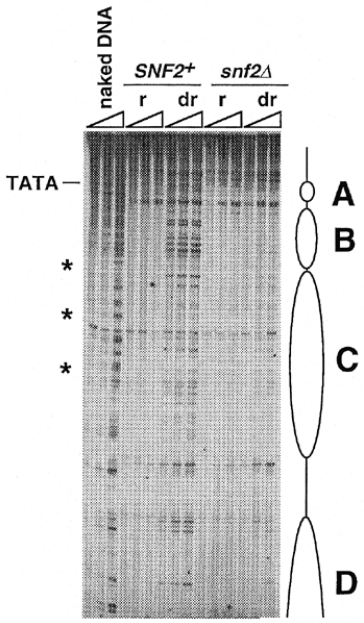

Figure 2. Micrococcal nuclease analysis of the *SUC2* UAS region. Strains were grown and chromatin was prepared and analyzed as described in Materials and Methods. The strains were grown under either repressing (r, 2% glucose) or derepressing (dr, 0.05% glucose) conditions. The position of the nucleosomes indicated by MNase digestion are indicated to the right of the figure.

digested by MNase in naked DNA is not cleaved by MNase in chromatin from derepressed cells, suggesting that another protein protects this region from MNase digestion (Fig. 1).

In the *snf2*Δ mutant grown under conditions repressing for *SUC2* transcription we observed the same pattern of MNase digestion revealed under repressive conditions in the wild-type strain, indicating the presence of a nucleosome in the same position over the TATA box (Fig. 2). However, under derepressing conditions the *snf2*Δ mutant differed from the wild-type *SNF2*$^+$ strain: an increase in DNA cleavage by MNase was not observed in the *snf2*Δ mutant. Instead, the DNA region over the TATA box in the *snf2*Δ mutant appears to be mostly inaccessible to MNase digestion even under derepressing conditions, suggesting that most templates still have a nucleosome positioned over the TATA box. This result correlates with the transcriptionally defective status of the *snf2*Δ mutant.

Evidence for positioned nucleosomes over the *SUC2* UAS

To determine if nucleosomes might also be positioned over the *SUC2* UAS we examined the MNase cleavage pattern of the region upstream of the transcription initiation site to ~650 bp upstream of the initiation site using two different primers. This analysis was also done under the same four different conditions. First, using primer S11, we analyzed the antisense strand of the UAS. In chromatin from wild-type cells grown under repressing conditions we observed a large protected region of DNA extending from nucleotide –226 to a doublet located at nucleotides –490/–500 (Fig. 2). The length of the protected DNA corresponds to the amount of DNA in two nucleosomes and thus indicates the presence of two more nucleosomes upstream of the one over the TATA box. The chromatin from wild-type cells grown under derepressing conditions shows much more cleavage by MNase, again suggesting the absence or alteration of nucleosomes. Similar results were obtained with our analysis of the sense strand over the UAS region (data not shown). Using primer *SUC2*-1 to look further upstream we detected protection of a region ~150 bp in size (as determined by DNA size markers) that begins at the doublet located at –490/–500 and extends upstream to approximately –650 (Fig. 3), indicating the presence of a fourth nucleosome. These results suggest that under glucose repressing conditions at least four nucleosomes are positioned over the *SUC2* promoter, extending from the transcription initiation site back over the UAS. Furthermore, under derepressing conditions these nucleosomes become altered or removed to allow cleavage by MNase.

As for the region over the TATA box, the pattern of MNase digestion of derepressed chromatin is similar but not identical to the pattern observed in naked DNA. Some sites cleaved in naked DNA by MNase are resistant to digestion in derepressed cells. These sites occur at various regions throughout the UAS, with prominent examples at –246 to –257, –266 to –296 and –311 to –323 (Fig. 2).

To determine if Snf–Swi affects the upstream sensitivity to MNase, we also analyzed the chromatin structure of this region in *snf2*Δ mutants in both repressed and derepressed cells (Figs 2 and 3). Similar to the results we obtained for the TATA box, we found that the pattern of MNase cleavage in repressed *snf2*Δ cells resembled that of repressed wild-type cells. In addition, for chromatin from derepressed cells the chromatin was still mainly resistant to MNase cleavage, resembling the repressed state and indicating that the nucleosomes were still present.

DISCUSSION

In this work we have examined the chromatin structure of the *SUC2* promoter region by MNase digestion under both repressing and derepressing conditions and in both *SNF2*$^+$ and *snf2*Δ genetic backgrounds. This analysis extends previous work in that it has

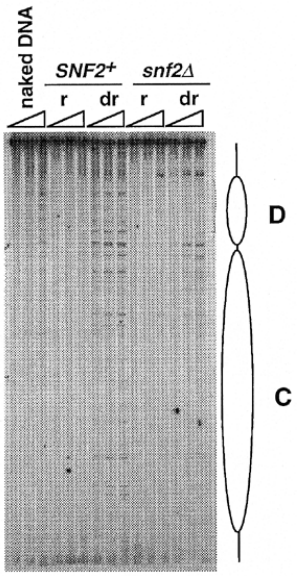

Figure 3. Strains were grown and chromatin was prepared and analyzed as described in Materials and Methods. The strains were grown under either repressing (r, 2% glucose) or derepressing (dr, 0.05% glucose) conditions. The position of the nucleosome indicated by MNase digestion is indicated to the right of the figure.

examined a larger region of the promoter and at higher resolution than in past studies. Our results have produced two main findings. First, in a wild-type background *SUC2* promoter chromatin is generally inaccessible to MNase when cells are grown under repressing conditions (high glucose) and it is very accessible to MNase when cells are grown under derepressing conditions. Second, in an *snf2*Δ background *SUC2* promoter chromatin is inaccessible to MNase under both repressing and derepressing conditions. The MNase digestion pattern under repressing conditions suggests the presence of at least four nucleosomes over the *SUC2* promoter, including the TATA box and the UAS (summarized in Fig 4). Because changes in chromatin structure were not observed in *snf2*Δ mutants, we conclude that Snf2 and the Snf–Swi complex are required for the alteration in chromatin structure at all four nucleosomes. While this work was in progress Gavin *et al.* (11) also described studies of the chromatin structure at *SUC2*. Their experiments demonstrated positioned nucleosomes over a larger region of *SUC2* than we examined in our studies. In addition, their studies demonstrate that a *swi1* mutation, like a *snf2* mutation, causes a repressing chromatin state, even under derepressing conditions.

Upon derepression it is clear that the UAS becomes more accessible to MNase digestion compared with the repressed state. However, several sites within the UAS are protected when compared with naked DNA. Most of these regions of differential accessibility do not correspond to binding sites for known transcription factors. One plausible explanation is that although the nucleosome undergoes a structural alteration that results in increased accessibility to MNase, this alteration represents a 'loosening' of the DNA from the histone octomer rather than a complete loss of the octomer from the DNA (9,12).

As can be seen in the schematic in Figure 4, the TATA box falls within the center of one of the nucleosomes. The specific positioning of this nucleosome is likely to play a critical role in repression of *SUC2* transcription. TBP binding is blocked by the presence of a nucleosome *in vitro* (13). Furthermore, transcription initiation is severely reduced from such nucleosomal templates (14). In addition, the presence of the three additional nucleosomes over the UAS may contribute further to the severity of the transcriptional defect in *snf2*Δ mutants. Such a nucleosomal template is likely to be more dependent upon the chromatin remodeling function of the Snf–Swi complex than a nucleosome-free template.

In addition to the TATA box, several other known transcription factor binding sites are located within the *SUC2* UAS. Binding sites for Mig1, a zinc finger protein that mediates glucose repression in yeast, can be found at –449 and –506. Mig1 binds to a sequence that contains a GC-rich core flanked by a 5′-AT-rich region. The AT box has been shown to be necessary for binding of Mig1 and upon binding bending of the DNA occurs in this region (15). The AT box of the proximal Mig1 site appears to be protected in the derepressed cell when compared with naked DNA. This proximal site lies within the center of a predicted nucleosome, explaining why evidence for occupancy is only seen in the derepressed cell. In the repressed cell the presence of an octomer protects DNA from digestion, preventing our determining if this site is occupied in the repressed state.

In contrast, the distal Mig1 site, located at –506, does not appear to be occupied even in derepressed cells. This apparent lack of binding at the distal Mig1 site may be due to the binding of Gcr1, a positive transcriptional regulator, whose putative site overlaps with the Mig1 site. The Gcr1 site, located at –517, is situated at the juncture of two predicted nucleosomes. It is interesting to speculate whether this intranucleosomal position of the Gcr1 site implies a role for Gcr1 in initiating disruption of the chromatin structure at *SUC2*. Gcr1 has been implicated in chromatin remodeling at other yeast promoters (16). Furthermore, deletion analysis of the *SUC2* UAS (17) revealed a 2-fold decrease in transcription when the Gcr1/Mig1 site was removed, which supports the idea that Gcr1 contributes to transcriptional activation of *SUC2*.

One current model of Snf–Swi action proposes that the complex does not itself bind directly to DNA but that it functions together with a DNA binding transcriptional activator to regulate chromatin

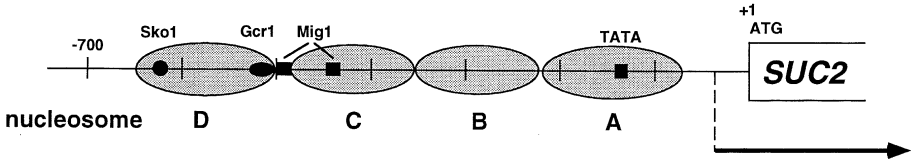

Figure 4. Diagram of the *SUC2* promoter region. Shown is the *SUC2* promoter region with the positions of the known protein binding sites. The positions of the nucleosomes, based on the MNase analysis, are shown as the shaded ellipses.

structure and transcription. Perhaps the Snf–Swi complex at *SUC2* functions in some capacity through Gcr1 to remodel chromatin structure. Further analysis of the function of Snf2 in *SUC2* transcription is likely to provide valuable insight into the mechanism of Snf–Swi action in the regulation of chromatin and transcription.

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