A Short-range Gradient of Histone H3 Acetylation and Tup1p Redistribution at the Promoter of the Saccharomyces cerevisiae SUC2 Gene*

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Chromatin immunoprecipitation assays are used to map H3 and H4 acetylation over the promoter nucleosomes and the coding region of the Saccharomyces cerevisiae SUC2 gene, under repressed and derepressed conditions, using wild type and mutant strains. In wild type cells, a high level of H3 acetylation at the distal end of the promoter drops sharply toward the proximal nucleosome that covers the TATA box, a gradient that becomes even steeper on derepression. In contrast, substantial H4 acetylation shows no such gradient and extends into the coding region. Overall levels of both H3 and H4 acetylation rise on derepression. Mutation of GCN5 or SNF2 lead to substantially reduced SUC2 expression; in gnc5 there is no reduction in basal H3 acetylation, but large reductions occur on derepression. SNF2 mutation has little effect on H3 acetylation, so SAGA and SWI/SNF recruitment seem to be independent events. H4 acetylation is little affected by either GCN5 or SNF2 mutation. In a double snf2/gcn5 mutant (very low SUC2 expression), H3 acetylation is at the minimal level, but H4 acetylation remains largely unaffected. Transcription is thus linked to H3 but not H4 acetylation. Chromatin immunoprecipitation assays show that Tup1p is evenly distributed over the four promoter nucleosomes in repressed wild type cells but redistributes upstream on derepression, a movement probably linked to its upconversion from a repressor to an activator.

The Saccharomyces cerevisiae SUC2 gene codes for invertase, an enzyme that catalyzes the hydrolysis of sucrose and raffinose to provide the cell with glucose in the absence of this essential fuel. It has been widely used to study the mechanisms underlying glucose regulation in yeast. Because these mechanisms result in changes in chromatin structure, this has been a long-lasting field of research. We first analyzed the DNase I sensitivity of the SUC2 gene (1) as well as nucleosome positioning under repressing and derepressing conditions, in both wild type cells (2) and in regulatory mutants (3). These initial data, obtained by indirect end labeling, showed that four nucleosomes are positioned on the promoter in such a way that certain crucial elements, including the TATA box, are occluded, whereas other regulatory sequences are nucleosome-free (2). These results were refined by other workers (4, 5) who mapped the four nucleosomes at high resolution by primer extension analysis.

The SUC2 gene is repressed in the presence of glucose by the Snf6-Tup1 corepressor complex (6, 7), which is tethered to the promoter by Mig1p (8). Tup1-mediated repression of SUC2 results in deacetylation of histone H3 at the promoter, as shown by an increase in H3 acetylation in tup1 mutants, but a lack of Tup1p does not change the acetylation level of H4 (9). This result contrasts with findings for other Tup1-regulated genes in which the repression also results in deacetylation of H4 (9).

The SUC2 gene is effectively derepressed by lowering the concentration of glucose (10), and several genes required for derepression were identified early through genetic screening (10, 11). These genes were collectively named SNF (sucrose-non-fermenting). Further studies revealed that the SNF genes play two clearly different roles. The first is accomplished by the SNF1 and SNF4 genes. The former encodes a protein kinase (12), which acts in the pathway leading to SUC2 derepression (13), whereas Snf4p regulates the kinase activity of Snf1p (14). The Snf1p kinase itself is rapidly phosphorylated in response to low glucose (15) and then, in turn, phosphorylates several proteins, including Mig1p (16). This seems to be a signal for the nucleus to cytoplasm translocation of Mig1p (17), and recent results seem to indicate that protein kinase C is also involved in this process (18). Importantly, translocation of Mig1p does not result in the release of Snf6-Tup1, which remains bound to the SUC2 promoter (19). The remaining SNF genes are involved in chromatin remodeling, as part of the SWI/SNF complex as revealed by the pioneering studies of Hirschhorn et al. (20).

Derepression of SUC2 also requires the action of the HAT

1 The abbreviations used are: SWI/SNF, mating-type switching/sucrose non-fermenting; SAGA, Spt-Ada-Gcn5-acetyltransferase; YPD, yeast extract-peptone-glucose; X-ChIP, cross-linking/chromatin immunoprecipitation; wt, wild type; HAT, histone acetyltransferase.
activity of Gcn5p, as well as that of Ada2p and Ada3p (21). These proteins are components of the SAGA complex, which functionally interacts with the remodeling SWI/SNF complex (22). The participation of SAGA, which is largely specific for H3 (23) in SUC2 derepression may explain observations that the level of H3 acetylation at its promoter increases in going from high to low glucose conditions (24).

Despite these data, many questions still remain concerning the mechanisms of SUC2 derepression at a chromatin level. The precise role of the Ssn6-Tup1 complex in the activation of the gene and the relative roles of H3 and H4 acetylation in the process need clarification. Finally, the nature of the interactions between the SAGA and SWI/SNF complexes in the SUC2 promoter are not yet clear. Because SUC2 stands as a paradigm among the glucose-repressed genes in yeast, the answers to these questions are particularly important.

MATERIALS AND METHODS

**Yeast Strains, Plasmids, and Media**—The gen5 and ado2 deletion mutants were obtained by appropriate gene disruptions of a parental strain with the genotype MATA leu2Δ1 trp1Δ63 uro3–52, further referred to as the wild type strain. All of these strains were kindly provided by G. Thireos. They are isogenic to the FY50, FY1291, FY1312, and FY1352 strains (spt3, spt20, snf2, and gen5snf2, respectively), which were a gift from F. Winston. To construct the gen5ΔBr mutant, lacking the bromodomain of Gen5p, the gen5 deletion mutant was transformed according to the protocol of Ito et al. (25) with a centromeric plasmid bearing a truncated version of GCN5 (26), which was also provided by G. Thireos.

Yeast cells were grown in a standard YPD medium (1% yeast extract, 2% bactopeptone, 2% glucose). To derepress SUC2, cells were transferred to a YPD medium containing low glucose (0.05%). For the growth of the mutant yeast cells, the YPD medium was supplemented with the required amino acids at the following concentrations (μg/ml): lysine, 40; histidine, 20; leucine, 60; tryptophan, 40; uracil, 20.

**Cross-linking and Immunoprecipitation of Yeast Chromatin**—Cross-linking and immunoprecipitation of yeast chromatin was carried out by a modification of a previously published procedure (27). Cells harvested at mid-log phase (A600 = 0.5) were transferred to a conical flask, and formaldehyde (1% final concentration) was added. Cells were fixed at room temperature for 15 min under shaking, and the reaction was stopped by adding glycine to a final concentration of 0.125 M. The cross-linked yeast cells were collected by centrifugation and washed twice in ice-cold buffer containing 200 mM NaCl, 20 mM Tris-HCl, pH 7.5. The pellets were then resuspended in 500 μl of ice-cold lysis buffer (150 mM NaCl, 1 mM EDTA, 1% Triton X 100, 0.1% SDS, 0.1% sodium deoxycholate, 50 μM Tris-HCl, pH 8.0) supplemented with a mixture of yeast inhibitors of proteolytic enzymes (Sigma) and transferred to screw-top tubes. To homogenize the yeast cells, an equal mixture of yeast inhibitors of proteolytic enzymes (Sigma) and trans-1 min between two consecutive vortexings. The homogenates were freed from each tube, and the cell suspensions were vortexed eight times at 600 of 0.5 and collected by centrifugation, washed twice with cold distilled water, and resuspended to a final concentration of 0.125 M. The DNA probes for hybridization corresponded to positions 156 to 878 for ACT1 and from 1856 to 1974 of the SUC2 gene.

**RNA Isolation and Northern Analysis**—The hot acid-phenol method (32) was used to extract total RNA from 25 ml cell cultures. The RNA obtained was dissolved in sterile distilled water and its concentration was determined by measuring A260. Total RNA (25 μg) from each sample was fractionated on a 1.2% formaldehyde agarose gel and blotted into a Hybond membrane (Amersham Biosciences) by capillary transfer. DNA was cross-linked by UV irradiation (254 nm) in a Bio-Rad apparatus. Hybridization was performed using Rapid-Hyb solution (Amersham Biosciences) for 1 h at 70°C. All washes were done at 65°C. The DNA probes for hybridization corresponded to positions +85 to +882 for SUC2 and from +124 to +878 for ACT1. They were generated by PCR and labeled by random priming with [α-32P]dCTP. Quantitation was performed with a FLA3000 Phosphorimager (FujiFilm) using the Image Gauge version 3.12 software.

**Invertase Activity Analysis**—Whole yeast cell preparations were grown to an A600 of 0.5 and collected by centrifugation, washed twice with cold distilled water, and resuspended to a concentration of 100 μg of cells/ml. Invertase activity was determined as described by Goldstein and Lampen (33) with the modifications of Celenza and Carlson (34). The invertase activity was expressed as μmol of sucrose hydrolyzed by the enzyme per min under standard assay conditions.

Histone Acetylation and Tup1p Binding in Yeast SUC2 Gene
RESULTS

RNA Expression Analysis of the SUC2 Gene in Wild Type and Mutant Strains—On shifting to low glucose concentration, SUC2 is readily induced (derepressed). This system was used to examine the time course of expression levels in wild type yeast cells and in the mutant strains gcn5, snf2, gcn5/snf2, ada2, spt3, spt20, and gcn5ΔBr.

Fig. 1 (upper panel) shows the profile of SUC2 mRNA expression. Set arbitrarily to 1 under basal conditions (wt 0 h), SUC2 expression rises to 8 after 1 h in low glucose and to 20 at full derepression (2 h in low glucose). Under the same conditions, the gcn5 mutant displays a severe phenotype; there is little change in the level after 1 h in low glucose, and it increases only 5-fold at 2 h. The snf2 mutant shows a similar phenotype, which is in accordance with the involvement of SNF2 in the expression of SUC2. The transcription rate of gcn5ΔBr mutant is roughly similar to that of the gcn5 and snf2 mutants, but the double mutant gcn5/snf2 shows a very severe phenotype, and the expression of SUC2 gene is almost negligible before induction and after 2 h of induction is similar to that of the repressed wt state. To evaluate the transcriptional rate in the double mutant, a correction for the expression level of the repressed wt state. To evaluate the transcriptional rate in the double mutant, a correction for the expression level of the repressed wt state.

The acetylation state of H3 and H4 at the SUC2 gene in low glucose occurs to a very low extent in the gcn5, snf2, gcn5/snf2, ada2, spt3, spt20, and gcn5ΔBr mutants, and therefore, a repressing chromatin state may be expected in these mutants even under derepressing conditions. This conclusion agrees with our previous results with the snf2 mutant (3).

High Resolution Mapping of Histone H3 and H4 Acetylation at the SUC2 Gene in Wild Type Cells—The acetylation state of histones H3 and H4 at the SUC2 promoter was determined using the ChIP methodology combined with quantitative PCR as described under “Materials and Methods.” Chromatin from cross-linked yeast cells grown under repressing (0 h in low glucose) and derepressing conditions (1 and 2 h in low glucose) was sonicated and analyzed as described under “Materials and Methods.”

Fig. 2 gives enrichments for the four promoter and one coding amplicon. The upstream amplicons are short and correspond to the positions of the four promoter nucleosomes. Because the actual resolution is limited by the size of the fragments obtained after sonication, the SUC2 coding sequences are probed with an amplicon located well downstream from the initiation site, at about the middle of the transcribed sequence, to avoid overlapping either with the SUC2 promoter or with the gene that is of unknown function but constitutively expressed, which lies immediately downstream of SUC2 (36). In the repressed gene, the level of H4 acetylation is roughly constant along the promoter and coding region, but the acetylation of H3 steadily decreases from the region of the distal nucleosome 1 to the coding region. Within the promoter itself, a 6-fold decrease is ongoing from nucleosome 1 to nucleosome 4. Upon derepression there is a general increase in H3 and H4 acetylation in the promoter. In the coding region the acetylation of H3 remains very low, but the substantial acetylation of H4 shows a 2-fold increase after 2 h in low glucose. The enrichment of H4 acetylation is roughly similar for nucleosomes 1 to 3 and in the coding region, but upon full derepression (2 h) a more pronounced hyperacetylation (more than a 3-fold increase over basal level) is observed centered on nucleosome 4, which lies over the TATA box.

High Resolution Mapping of Histone H3 Acetylation in the SUC2 Gene in the Mutant Strains—Fig. 3 shows the levels of H3 acetylation in the gcn5, snf2, gcn5/snf2 and gcn5ΔBr mutants, as well as in the wild type strain. Results, given as histograms, are relative to the amount of acetylated H3 (taken as unity) in the repressed wild type cells. This normalization is to facilitate the comparison between wt and mutant strains at
each individual amplicon. In the \textit{gcn5} mutant, the basal level of histone H3 acetylation is similar to that of the wild type strain and even somewhat higher in nucleosome 1. On the other hand, under derepressing conditions, a substantial decrease in histone H3 acetylation is observed along the entire promoter. These results indicate that the rise in histone H3 acetylation observed upon \textit{SUC2} derepression in the wild type strain is dependent on Gcn5p, whereas the basal level observed in repressed cells is not. These comments do not apply to the coding region, where, after 2 h low glucose, the level of histone H3 acetylation is similar to that in wild type cells.

In the \textit{snf2} mutant, the basal level of histone H3 acetylation is higher than in the wild type along the entire \textit{SUC2} locus. Moreover, H3 acetylation remains roughly constant over the 2 h of induction, in sharp contrast to the \textit{gcn5} cells. The double mutant \textit{gcn5\textsc{snf2}} is characterized by a low level of H3 acetylation in both the promoter and the coding region, and this does not depend on the glucose concentration in the culture medium.

In the mutant strain \textit{gcn5\textsc{Br}}, there is little change in H3 acetylation ongoing from repressing to fully derepressing conditions. For nucleosome 1 the basal H3 acetylation is somewhat higher than in the \textit{gcn5} mutant and drops slightly upon transfer to low glucose.

High Resolution Mapping of Histone H4 Acetylation in the \textit{SUC2} Gene in the Mutant Strains—Fig. 4 shows changes in H4 acetylation in \textit{SUC2} promoter and coding region in the wt and four mutant strains. In the \textit{gcn5} mutant the basal level of histone H4 acetylation in the promoter as well as in the coding region is almost identical to that in the wild type cells. Upon \textit{SUC2} induction, the increase in H4 acetylation is similar to that in the wild type strain over the entire locus. The increase in H4 acetylation on the TATA box nucleosome, which becomes heavily acetylated in wild type cells (see Fig. 2), takes place even faster in the \textit{gcn5} mutant (compare the wt and \textit{gcn5} data at 1 h of derepression).

In the \textit{snf2} mutant strain, the basal level of H4 acetylation over the entire \textit{SUC2} locus is somewhat below that found in the repressed wild type. Nevertheless, significant increases are found after 2 h in low glucose, and the final levels generally match those found in wild type cells after 2 h induction (except, perhaps, for nucleosome 4). In contrast, the level of H4 acetylation in the coding region, although increasing 2-fold over its basal value on induction, still does not rise above the basal level found in wt and \textit{gcn5} strains.

The \textit{gcn5\textsc{snf2}} double mutant shows H4 acetylation levels similar to wt under repressed conditions and on induction.
increases 2–2.5-fold, rather as seen for the snf2 strain and not so differently from wt cells. It is interesting to note that for this double mutant in which SUC2 RNA levels are very much lower than in wt cells, the time-dependent pattern of H4 acetylation at the promoter is nevertheless similar to that of the wt strain, except in the region of the TATA box (nucleosome 4), where H4 acetylation remains approximately unchanged.

In the gen5ΔBr mutant the H4 acetylation levels are similar to those of the gen5 mutant at nucleosome 1. At the other three nucleosomes and in the coding region, H4 acetylation levels are less in gen5 and do not increase much on derepression, although mRNA levels and invertase activity in gen5ΔBr are similar to those in the gen5 (Fig. 1).

Tup1p Mapping in the SUC2 Locus in the Repressed and Derepressed States—High resolution mapping of Tup1p occupancy in the SUC2 gene (Fig. 5) revealed that when the SUC2 gene is repressed, the Tup1p signal in the regions of nucleosomes 2, 3, and 4 is roughly constant, while somewhat smaller than in gen5 and do not increase much on derepression, although mRNA levels and invertase activity in gen5ΔBr are similar to those in the gen5 (Fig. 1).

DISCUSSION

The sizes of the amplicons selected for the PCR analyses range from 97 to 127 bp and were selected to match the positioned nucleosomes of the promoter. The small size of the amplicons allowed acetylation and Tup1p mapping at a resolution limited only by the size of the chromatin fragments after sonication, which is on average 350 bp. This represents resolution at the dinucleosome level. Because in many cases the signal from amplicons in adjacent nucleosomes differs substantially, the implied differences in acetylation or Tup1p occupancy are presumably underestimated.

SUC2 Expression Levels—The present results show that the full expression of the SUC2 gene depends upon the presence of intact SAGA and SWI/SNF complexes. In fact, the gene is incompletely derepressed (induced) upon shifting to low glucose in all of the studied strains bearing mutations in components of either the SAGA HAT complex (gen5, ada2, spi3, and spi20) or the SWI/SNF remodeling complex (snf2). These results agree with previous reports (21, 22, 37), and the present experiment extends the study to other components of the above complexes. It is interesting to note that the bromodomain of Gcn5p is required for full induction of the SUC2 gene, because the increase in transcriptional rate under derepressing conditions in the gen5ΔBr mutant is roughly similar to that in the strain lacking the whole GCN5 gene (Fig. 1). On the other hand, a double mutation removing both the Gen5p acetyltransferase activity and the remodeling activity of Snf2p results in the total unresponsiveness of SUC2 to derepression by low glucose (Fig. 1).

Acetylation and Tup1p Occupancy in the wt Strain—A gradient of H3 acetylation is observed from the distal elements of the promoter to the coding region of the repressed SUC2 gene in the wild type strain (Fig. 2), the acetylation of H3 being very low within the coding region. Upon derepression, the level of H3 acetylation in the coding region remains very low, but the gradient along the promoter becomes even sharper. This is in strong contrast to the distribution of acetylated H4, which spreads uniformly along the promoter and coding sequences in the repressed state but adopts a somewhat bimodal distribution upon derepression. In fact, the overall level of H4 acetylation increases along the whole SUC2 locus upon shifting to low glucose, but the increase is particularly noticeable over the TATA box (nucleosome 4) and in the distal regions of the promoter (nucleosome 1).

There is a clear relationship between the level of histone acetylation and Tup1p occupancy. In the repressed gene, the Tup1p signal is spread over the four promoter nucleosomes (Fig. 5). The Sn6-Tup1 complex is targeted to the promoter by the DNA-binding factor, Mig1p (8). There are two Mig1 sites in the SUC2 promoter (38); the major one is at a GC-rich box located at −499, lying between nucleosomes 1 and 2, whereas the second site, of low affinity, is located at −442 within nucleosome 2 (Fig. 2). It is likely that the first site acts as an anchorage for the Ssn6-Tup1 complex via interactions with Mig1p in the repressed state. Although this site is almost 400 bp away from the TATA box, the Ssn6-Tup1 complex (a tetramer of Tup1 and a single Ssn6 subunit) has a very elongated shape as concluded from its hydrodynamic behavior (39), so it can readily extend toward nucleosomes 3 and 4. The physical interaction of Tup1p with Hse2p and Rpd3p histone deacetylases was described by Watson et al. (40) and a recent report from the same laboratory has established that multiple class I histone deacetylases, but not Hda1p, a class II enzyme, interact in vivo with both components of the corepressor complex (41). Tup1p shows a preferred interaction with hypoacetylated histone tails in vitro (42). If it behaves in vivo in the same manner, this would then contribute to generating a fixed repressed state, as suggested by Davie et al. (43).

Upon changing the medium to low glucose, Tup1p remains bound to the SUC2 promoter (Fig. 5), in agreement with the recent data of Papamichos-Chronakis et al. (19). The high resolution analysis carried out in the present work allowed us to demonstrate that under derepressing conditions there is a shift of the complex toward the distal regions of the promoter, because the maximal Tup1p signal is now found at nucleosome 2 (Fig. 5). In fact, the amplicon used by Papamichos-Chronakis et al. (19) in their Tup1p mapping corresponds to nucleosome 2 and some of nucleosome 1.

We propose the following mechanism to explain Tup1p displacement. First, under derepressing conditions, Mig1p is translocated to the cytoplasm (17) after being phosphorylated by the protein kinase Snf1p (16, 44). Then the corepressor complex Ssn6-Tup1 is converted into a coactivator; the shift between these two opposite functions of Ssn6-Tup1 has been described recently (19, 45). In stress-regulated promoters, this conversion of function involves the phosphorylation of Sko1p, the factor that tethers the corepressor complex to DNA, by the
mitogen-activated protein kinase Hog1p (45). It is interesting to note that in the SUC2 promoter there is an Sko1 site upstream of the Mig1 high affinity site (46), which is shown in Fig. 2. If a mechanism similar to that acting on stress-regulated promoters is operative in the SUC2 promoter, then the Snf6-Tup1 complex would be retained in the derepressed state by interacting with phosphorylated Sko1p. This would explain the upstream displacement observed in the Tup1p signal upon derepression (Fig. 5). It has also been shown that Tup1p inhibits the binding of TBP at the SUC2 promoter (47), and this could be why Tup1p moves away from nucleosome 4 on derepression.

It is worth noting that the movement of Tup1p relative to nucleosomes 1–4 might also result from a reorganization of chromatin rather than from actual Tup1-Ssn6 displacement. Therefore, it could be why Tup1p moves away from nucleosome 4 on derepression. This finding correlates with the observation that induced SUC2 transcription is greater in gcn5Δsnf2 mutant than in gcn5.

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