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Interplay of yeast global transcriptional regulators Ssn6p–Tup1p and Swi–Snf and their effect on chromatin structure

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Transcriptional regulation in yeast involves a number of general *trans*-acting factors affecting chromatin structure. The Swi–Snf complex is required for expression of a large number of genes and has the ability to remodel chromatin *in vitro*. The Ssn6p–Tup1p repressor complex may be involved in chromatin organization through the interaction with pathway-specific DNA-binding proteins. To study the interplay of these factors and their effect on chromatin we have analyzed *SUC2* chromatin structure in wild-type cells and in strains bearing combinations of *ssn6/tup1* and *swi1* mutations. We have mapped nucleosome positioning of the repressed gene in wild-type cells using primer extension methodology, allowing base pair resolution, and have analyzed details of chromatin remodeling in the derepressed state. In *ssn6* or *tup1* mutants under repressing conditions the observed changes in *SUC2* chromatin structure may be suppressed by the *swi1* mutation, suggesting that Ssn6p–Tup1p is not required for the establishment of nucleosome positioning at the *SUC2* promoter. Our data indicate the involvement of chromatin remodeling factors distinct from the Swi–Snf complex in *SUC2* transcriptional regulation and suggest that Swi–Snf may antagonize Ssn6p–Tup1p by controlling remodeling activity. We also show that a relatively high level of *SUC2* transcription can coexist with positioned nucleosomes.

Keywords: chromatin/nucleosomes/Ssn6p(Cyc8p)-Tup1p/Swi–Snf/transcription

Introduction

Over the past decade the role of chromatin structure in transcriptional regulation has been extensively studied. In yeast, a number of transcription factors have been identified which may stabilize or alter chromatin structure (Winston and Carlson, 1992; Roth, 1995; Edmondson and Roth, 1996; Kingston *et al.*, 1996; Peterson, 1996). One of them, the Swi–Snf complex, is required for transcriptional activation of a number of genes (Winston and Carlson, 1992) and has the ability to alter nucleosome structure *in vitro* (Côté *et al.*, 1994; Owen-Hughes *et al.*, 1996; Wilson *et al.*, 1996). Histone mutations also suppress the transcriptional defects in *swi/snf* mutants (Hirschhorn *et al.*, 1992, 1995; Kruger *et al.*, 1995). These observations and studies of Swi–Snf homologs have led to its classification as a 'chromatin remodeling machine' (Peterson and Tamkun, 1995; Peterson, 1996). The fact that the components of the Swi–Snf complex are not required for basal transcription (Peterson and Tamkun, 1995) and the observation that Swi–Snf might be a part of RNA polymerase II holoenzyme complex (Wilson *et al.*, 1996) suggest that it is involved in transcriptional activation by pathway-specific activator proteins.

It has also been proposed that other factors, such as the Ssn6p–Tup1p complex, the Sir3–Sir4 complex and Spt6p, may stabilize chromatin by direct interaction with histones (Grunstein *et al.*, 1995; Roth, 1995; Bortvin and Winston, 1996; Edmondson and Roth, 1996). Ssn6p and Tup1p are associated in a large complex (Williams *et al.*, 1991; Varanasi *et al.*, 1996; Redd *et al.*, 1997) and are required for repression of a large number of yeast genes (Mukai *et al.*, 1991; Keleher *et al.*, 1992; Trumbly, 1992; Zitomer and Lowry, 1992; Elledge *et al.*, 1993; Teunissen *et al.*, 1995; Friesen *et al.*, 1997). Recently the involvement of Tup1p in donor preference during mating type switching has also been demonstrated (Szeto and Broach, 1997). Although neither Ssn6p nor Tup1p can bind DNA, they may be recruited to promoters by other DNA-binding proteins (Keleher *et al.*, 1992; Zitomer and Lowry, 1992; Balasubramanian *et al.*, 1993; Komachi *et al.*, 1994; Treitel and Carlson, 1995; Tzamarias and Struhl, 1995). Two models for Ssn6p–Tup1p-mediated repression have been proposed (Roth, 1995). One suggests that Tup1p may inhibit transcription by the organization of a repressive chromatin structure (Cooper *et al.*, 1994) through the direct interaction with histones (Edmondson *et al.*, 1996). The second model implies an interaction of Ssn6p–Tup1p with the general transcription machinery (Herschbach *et al.*, 1994; Redd *et al.*, 1996). The Ssn6p–Tup1p complex may also block the activation domain of pathway-specific DNA-binding proteins (Treitel and Carlson, 1995; Tzamarias and Struhl, 1995; Lutfiyya and Johnston, 1996).

A change in the micrococcal nuclease digestion pattern of the *SUC2* locus following the shift from repressing (high glucose) to derepressing (low glucose) conditions (Perez-Ortin *et al.*, 1987; Matallana *et al.*, 1992) requires the products of *SNF2* and *SNF5* genes, components of the Swi–Snf complex (Hirschhorn *et al.*, 1992, 1995). The lack of both *SUC2* derepression and chromatin alteration in *snf2* and *snf5* mutants at low glucose levels are partially suppressed by histone mutations suggesting the involvement of Swi–Snf in *SUC2* chromatin remodeling during activation. The inability to derepress *SUC2* in *snf* mutants is also suppressed by *ssn* mutations (Trumbly, 1992). Deletions in the *SSN6* gene result in high-level invertase expression even under repressing conditions (Vallier and Carlson, 1994). However, the genetic interaction of SNF and SSN class genes in *SUC2* transcriptional regulation may not be related to the chromatin structure.

To study the interplay of Swi–Snf and Ssn6p–Tup1p complexes in transcription regulation and their effect on chromatin we have analyzed *SUC2* chromatin structure in wild-type cells and in *ssn6/tup1* and *swi/snf* mutants using a primer extension technique. We show that the Ssn6p–Tup1p complex is not required for the establishment of nucleosome positioning at the *SUC2* promoter. Our data also suggest the involvement of chromatin remodeling factors distinct from the Swi–Snf complex in *SUC2* activation whose activity may be controlled by the antagonistic function of Swi–Snf and Ssn6p–Tup1p.

Results

Nucleosomes are positioned over the regions required for SUC2 expression

Indirect end-labeling experiments have provided suggestive evidence for organized chromatin at the repressed *SUC2* promoter (Perez-Ortin *et al.*, 1987; Matallana *et al.*, 1992; Hirschhorn *et al.*, 1995), but suffered from low resolution, which precluded detailed analysis of chromatin structure. In order to analyze the positions of nucleosomes relative to *SUC2* regulatory elements, we have examined chromatin structure using a primer extension methodology which allows base pair level resolution (Shimizu *et al.*, 1991). Figure 1 presents primer extension gels showing the distribution of micrococcal nuclease cleavage sites in the promoter region and in the beginning of the coding sequence. In the repressed state, the promoter and flanking sequences are organized as an array of regions protected from micrococcal nuclease cleavage which are 120–150 bp long and are flanked by hypersensitive sites. We interpret this motif to represent positioned nucleosomes as indicated by the ellipses. An array of positioned nucleosomes spans the whole region upstream of the promoter (Figure 1A, lanes 3 and 4). Nucleosome –1 occupies most of the UAS including the Sko1p binding site (Figure 1A, lanes 3 and 4; Figure 1B, lanes 6 and 7). The major binding sequence for Mig1p and Mig2p repressors, site I (Lutfiyya and Johnston, 1996), appears to be highly accessible to micrococcal nuclease with cutting that resembles the naked DNA pattern (Figure 1A, compare lanes 3 and 4 with lane 5; Figure 1B, compare lanes 6 and 7 with lane 8). The second site, for which both Mig1p and Mig2p have very low affinity, is located close to the end of the region protected by nucleosome +1. However, this region appears to be cut more by micrococcal nuclease consistent with indirect end-labeling data (Perez-Ortin *et al.*, 1987). Two closely packed nucleosomes, +1 and +2, are observed downstream of the second Mig1p/Mig2p site protecting about 290 bp and are separated by a minor cleavage site at position –346 at the end of nucleosome +2 (Figure 1B, lanes 6 and 7). The TATA box for secreted invertase (Sarokin and Carlson, 1984) and the alternative TATA-like element (Tillman *et al.*, 1995) are located close to

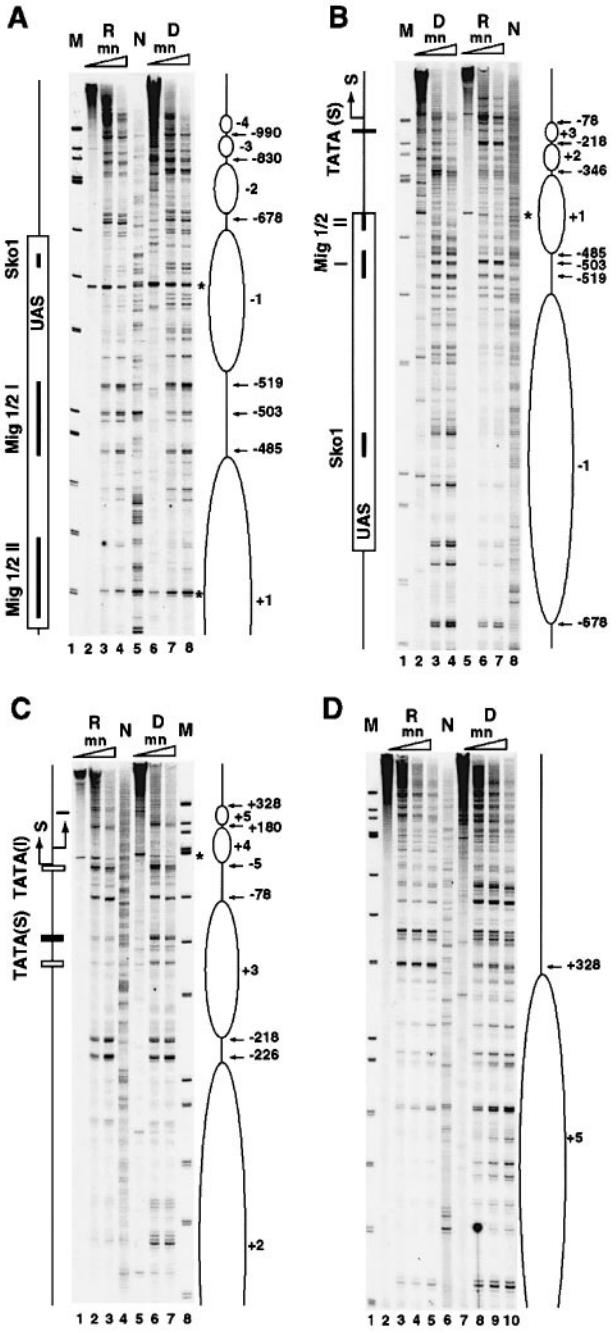

Fig. 1. Chromatin organization of the repressed *SUC2* locus and destabilization of nucleosome positions during derepression. Chromatin in nuclei isolated from yeast grown under repressing (R) or derepressing (D) conditions was digested with increasing amounts of micrococcal nuclease and subjected to primer extension using the primers R2 (**A**); F1 (**B**); F3 (**C**) and F5 (**D**). Location of the primers relative to *SUC2* sequences are shown schematically in Figure 2 and listed in Materials and methods. The first lane in chromatin samples corresponds to DNA from undigested chromatin as a control for Taq DNA polymerase pauses, marked by asterisks. N is naked DNA digested by micrococcal nuclease as a control for sequence specificity of the enzyme. M, marker DNA fragments corresponding to 726, 713, 553, 500, 427, 417, 413, 311, 249, 200, 151, 140, 118, 100 and 82 nucleotides from *Hin*fI digest of ϕX174 RF DNA. The UAS mapped at position from –650 to –418 relative to ATG for secreted invertase, the TATA element for secreted invertase (filled box) (Sarokin and Carlson, 1984), two binding sites for the Mig1p and Mig2p repressors (Nehlin and Ronne, 1990; Lutfiyya and Johnston, 1996), a binding site for the Sko1p repressor (Nehlin *et al.*, 1992), and the start of the coding sequence for intracellular (I) and secreted (S) invertase (Carlson and Botstein, 1982; Carlson *et al.*, 1983) are shown on the left of each gel. The putative TATA element for intracellular invertase (Sarokin and Carlson, 1984) and the alternative TATA-like sequence for secreted invertase (Tillman *et al.*, 1995) are shown by open boxes. Arrows indicate the location of major cleavage sites in the repressed *SUC2* locus and numbers correspond to their distance from the A residue of the initiation codon for secreted invertase. The inferred position of nucleosomes are shown by ellipses with assigned numbers.

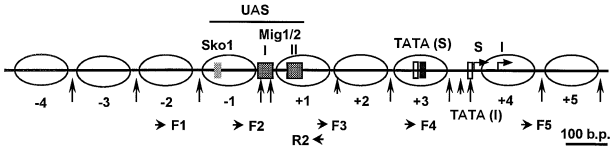

Fig. 2. Chromatin organization of the repressed *SUC2* locus. Summary of the primer extension data of Figure 1. The position of nucleosomes (ellipses) and micrococcal nuclease cleavage sites (vertical arrows) in the repressed *SUC2* locus are shown. Horizontal arrows correspond to the location of primers used in the assay.

the dyad of nucleosome +3 (Figure 1C, lanes 2 and 3) and thus are inaccessible in the repressed state. In contrast, the putative TATA box for intracellular invertase (Sarokin and Carlson, 1984) is highly accessible, consistent with constitutive low-level transcription from this promoter (Carlson and Botstein, 1982). Two positioned nucleosomes, +4 and +5, are observed in the coding region; beyond them the nucleosome pattern becomes less clear, disappearing in the middle of the gene (Figure 1D, lanes 3–5). Nucleosome positions in the promoter region of the repressed *SUC2* locus are shown schematically in Figure 2.

Destabilization of nucleosome positioning at the derepressed SUC2 promoter

SUC2 derepression by a shift from high to low glucose is accompanied by a dramatic change in the chromatin structure of the promoter and the 5′ end of the coding region (Figure 1). However, sequences upstream of the UAS, occupied by nucleosomes from –4 to –2, are not affected (Figure 1A, lanes 7 and 8). The most prominent change occurs in the region from –678 to –519, where the enhanced cleavage indicates the disruption of nucleosome –1 (Figure 1A, compare lanes 7 and 8 with lanes 3 and 4; Figure 1B, compare lanes 3 and 4 with lanes 6 and 7). There is also a significant decrease in accessibility of the Mig1p/Mig2p binding site I at position –503. The region at the edge of nucleosome +1 becomes more accessible and cleavage at position –346 increases. The region occupied by nucleosomes +2, +3 and +5, including the TATA box for secreted invertase, is also less protected (Figure 1C, compare lanes 6 and 7 with lanes 2 and 3; Figure 1D, compare lanes 8–10 with lanes 3–5). There is also a slight increase in the accessibility of the region occupied by nucleosome +4 which is resolved using primer F4 (data not shown). All these changes indicate the general destabilization of nucleosome positioning in the promoter region and in the 5′ end of the coding sequence following *SUC2* derepression.

Disruption of SUC2 chromatin structure in ssn6 and tup1 mutants

Tup1p is required for nucleosome positioning around the α2 operator in promoters of **a**-specific genes and in the recombination enhancer (Cooper *et al.*, 1994; Weiss and Simpson, 1997) and interacts with Matα2p (Komachi *et al.*, 1994) and the amino-terminal regions of histones H3 and H4 (Edmondson *et al.*, 1996) *in vitro*. The fact that the histone-binding domain of Tup1p coincides with the repression domain suggests that Tup1p may directly repress transcription by organizing chromatin in regulatory regions of Tup1p-dependent genes (Edmondson *et al.*, 1996). Ssn6p–Tup1p is also required for Mig1p-mediated repression and Ssn6p interaction with Mig1p was observed in a two-hybrid system (Treitel and Carlson, 1995). To answer the question of whether or not the Ssn6p–Tup1p complex is involved in chromatin organization at the *SUC2* locus, we have analyzed the chromatin structure at the *SUC2* promoter in *ssn6* and *tup1* deletion strains under repressing conditions. This regulatory protein mutant background normally leads to a high level of *SUC2* expression even in the presence of glucose (Trumbly, 1992; Vallier and Carlson, 1994). Unlike the destabilization of chromatin that was observed to be confined to the promoter region in wild-type cells in the derepressed state, nucleosome positioning in the entire *SUC2* locus, including the sequences upstream of the UAS, is disrupted in *ssn6* and *tup1* cells (Figure 3). We observe an increase in the accessibility of the sites marked with dots in Figure 3A within the regions previously protected by nucleosomes –2 to –4 and a relative decrease in the cleavage in the linker between nucleosomes –2 and –3 at position –830. The hypersensitivity of the Mig1p/Mig2p binding site I at positions –485 and –503 is completely lost and the cleavage in the UAS and flanking regions becomes more uniform (Figure 3D and E). The cleavage of chromatin does not resemble the naked DNA pattern, which is not surprising as DNA still interacts with histones in nuclei and its conformation would be expected to be different from naked DNA *in vitro*. Further derepression by glucose shift does not introduce any additional changes to micrococcal nuclease cleavage pattern in *ssn6/tup1* mutants (data not shown).

Our data show that unlike **a**-cell type-specific genes (Cooper *et al.*, 1994), the effect of *ssn6* and *tup1* mutations on *SUC2* chromatin structure is indistinguishable and *SUC2* is repressed more effectively by Ssn6p than Tup1p. Northern blot analysis reveals a 4- to 5-fold increase in *SUC2* mRNA levels in *ssn6* cells under repressing conditions relative to wild-type cells under derepressing conditions (Figure 4, compare lane 10 with lane 2), while expression in *tup1* cells is comparable with the wild-type derepressed level (Figure 4, compare lane 9 with lane 2). This suggests different mechanisms of transcriptional regulation by these factors, which is supported by the evidence that *SUC2* expression in *ssn6 tup1* double mutants is higher than in either of the single mutants (Williams and Trumbly, 1990).

The change in chromatin structure at the *SUC2* promoter following derepression after glucose shift does not require transcription (Hirschhorn *et al.*, 1992). However, the disruption of nucleosome positioning as well as loss of *SUC2* repression is higher in *ssn6* and *tup1* cells than in wild-type cells under derepressing conditions and the formal possibility remains that the chromatin disruption in these strains is the consequence of a high level of transcription. We therefore evaluated *SUC2* chromatin structure in *ssn6* and *tup1* cells where the transcription level was reduced by mutations in the TATA box. We replaced the TATAAA sequence at position from −133 to −128 by an *Nsi*I site (ATGCAT) which almost completely eliminates *SUC2* transcription under derepressing conditions (Figure 4, lane 3). This mutation reduces transcription 7-fold in *tup1* cells (Figure 4, compare lane 11 with lane 9) and 20-fold in *ssn6* cells (Figure 4, compare lane 12 with lane 10) to ~20% of the derepressed wild-type level (Figure 4, lane 2). However, the TATA box mutation has

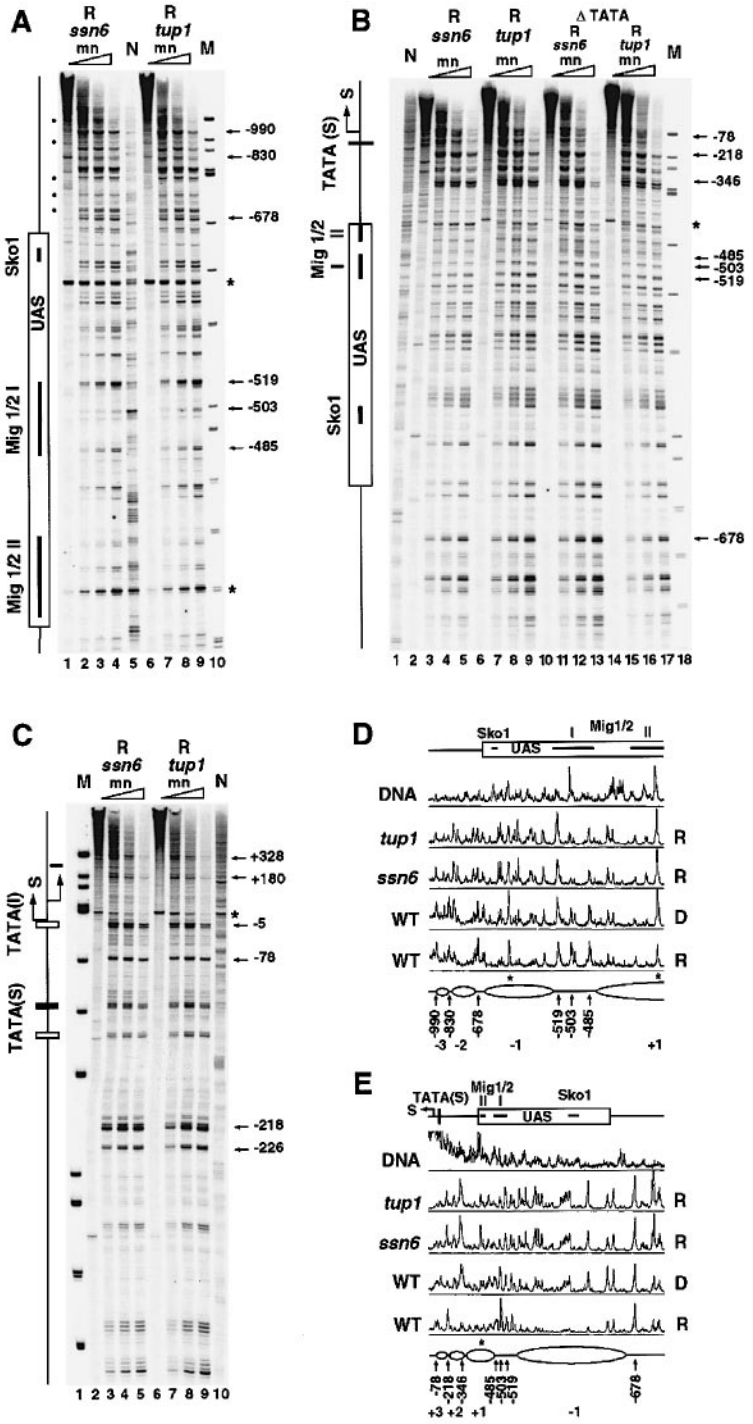

Fig. 3. Disruption of *SUC2* chromatin structure in *ssn6* and *tup1* deletion strains is independent of transcription. Primer extension analysis of chromatin structure of the wild-type *SUC2* locus and of the gene bearing a mutation of the TATA element for secreted invertase in *ssn6* and *tup1* cells grown under repressing conditions using the primers R2 (**A**), F1 (**B**) and F3 (**C**). Arrows and numbers at the side of each gel correspond to the micrococcal nuclease cleavage sites in the repressed *SUC2* locus for a wild-type strain and are the same as in Figure 1. Sites of increased cleavage in *ssn6* and *tup1* mutants that were unaffected during derepression by a shift to low glucose in the wild-type strain are shown by dots. For further details see legend to Figure 1. (**D** and **E**) Comparison of the chromatin structure at the *SUC2* promoter in *ssn6*, *tup1*, and in wild-type strains under repressing and derepressing conditions. Scanning profiles obtained from the gels shown in Figures 1 and 3 using primers R2 (D) and F1 (E) are shown using the same symbols as in Figure 1.

no effect on the change in *SUC2* chromatin structure in either *ssn6* or *tup1* cells (Figure 3B, lanes 11–13 and 15–17) indicating that the disruption of nucleosome positioning in these mutants is independent of transcription. The relatively high amount of *SUC2* mRNA in TATA mutants in *ssn6* and *tup1* cells is rather surprising, as this sequence is strongly required for *SUC2* expression in wild-type cells (Sarokin and Carlson, 1984; Hirschhorn *et al.*, 1992; Tillman *et al.*, 1995). The transcriptional machinery may use the alternative TATA-like sequence −160 to −155 which seems to be capable of binding TBP (Tillman *et al.*, 1995).

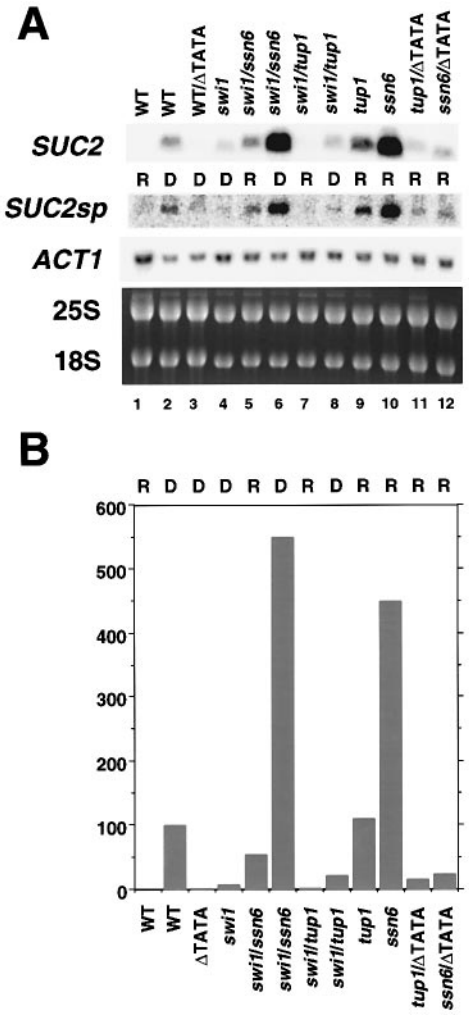

Fig. 4. High transcription level can coexist with positioned nucleosomes at the *SUC2* promoter under repressing conditions. (**A**) Northern blot of *SUC2* mRNA from the indicated strains grown under repressing (R) or derepressing (D) conditions. Total RNA from the cells was fractionated in a formaldehyde–agarose gel, transferred to a nylon membrane and hybridized to the ^{32}P-labeled DNA probes specific to the *SUC2* coding sequence common for both types of invertase or to the sequence encoding signal peptide (sp) for the secreted form of invertase as described in Materials and methods. The lower panels show the same blot hybridized to the *ACT1* probe and rRNAs stained by ethidium bromide as a control for equal loading. (**B**) Comparison of the relative amount of *SUC2* mRNA in different strains grown under repressing or derepressing conditions. The quantitative analysis was performed by scanning the Northern blot images obtained in several series of experiments with a Molecular Dynamics PhosphorImager and normalized to levels of *ACT1* mRNA. Numbers represent the amount of *SUC2* mRNA relative to the wild-type strain under derepressing conditions as a percentage.

The Ssn6p–Tup1p complex is not required for the establishment of nucleosome positioning at the SUC2 promoter in a swi1 background

The evidence that the disruption of *SUC2* chromatin structure in *ssn6* and *tup1* mutants is independent of transcription suggests two possible models for the role of Ssn6p–Tup1p in chromatin organization. This complex might be directly involved in the establishment of nucleosome positioning or stabilize the organized chromatin structure by direct interaction with histones (Edmondson *et al.*, 1996). Alternatively, it may block the binding or activity of chromatin remodeling factors in the repressed state and partially inhibit them under derepressing conditions. The latter possibility arises from the fact that the chromatin structural alteration at the *SUC2* promoter under derepressing conditions requires the products of the *SWI2/SNF2* and *SNF5* genes, members of the Swi–Snf family (Hirschhorn *et al.*, 1992). To investigate the role of Ssn6p–Tup1p and Swi–Snf in controlling the chromatin organization at the *SUC2* promoter we have analyzed *SUC2* chromatin structure and transcription in *swi1* as well as in *swi1 ssn6* and *swi1 tup1* double mutants under repressing and derepressing conditions (Figure 5).

Mutations in various components of the Swi–Snf complex have a similar effect on transcription of Swi–Snf-dependent genes (Peterson and Herskowitz, 1992). A *swi1* null mutation reduces transcription of *SUC2* gene 12-fold under derepressing conditions as assayed by Northern blotting (Figure 4, compare lane 4 with lane 2). The analysis of the chromatin structure at the *SUC2* promoter in *swi1* mutants is shown in Figure 5. Like mutations in the *SWI2/SNF2* and *SNF5* genes, the *swi1* mutation abolishes the destabilization of nucleosome positioning in wild-type cells under derepressing conditions (Figure 5A and B, lanes 6 and 7). The chromatin structure of the promoter region in *swi1* cells under derepressing conditions is identical to that of repressed wild-type cells (Figure 5D and E). The primer extension gels showing chromatin structure at the *SUC2* promoter in *swi1 ssn6* and *swi1 tup1* double mutants under repressing conditions are shown in Figure 5A (lanes 3, 4 and 9, 10) for the UAS and in Figure 5B (lanes 3, 4 and 9, 10) for the TATA region. We observe the same arrangement of nucleosomes over the UAS and the TATA box as in wild-type cells under repressing conditions (Figure 5D and E), suggesting that chromatin remodeling in the absence of Ssn6p–Tup1p depends on Swi–Snf and that the Ssn6p–Tup1p complex is not required for the establishment of nucleosome positioning at the *SUC2* promoter. However, the possibility remains that the Ssn6p–Tup1p complex may stabilize the chromatin structure dictated by DNA sequence or by other factors.

The *swi1* mutation reduces *SUC2* transcription in *tup1* cells under repressing conditions to the level observed in *swi1* mutants under derepressing conditions (Figure 4, compare lanes 7 and 4). However, the *SUC2* mRNA level in *ssn6 swi1* cells at high glucose is half that in derepressed wild-type cells (Figure 4, compare lanes 5 and 2) in spite of the organized chromatin structure. The stability of *SUC2* mRNA is much higher in the absence of glucose and *ssn6* mutations have no effect on the rate of *SUC2* mRNA turnover (Cereghino and Scheffler, 1996). The difference in *SUC2* mRNA degradation rates in high versus low glucose media may contribute to the total mRNA level; therefore the ratio of transcription rates in *ssn6 swi1* mutants under repressing conditions to wild-type cells under derepressing conditions may be even higher. The protection of the TATA box in *swi1 ssn6* mutants under repressing conditions (Figure 5B) is also surprising considering the high level of *SUC2* expression. One possible explanation is that mutations in these genes may also affect intracellular invertase expression; the putative intracellular invertase TATA box (Sarokin and Carlson, 1984) is located in a hypersensitive region (Figure 1C). To address this possibility, we have hybridized the

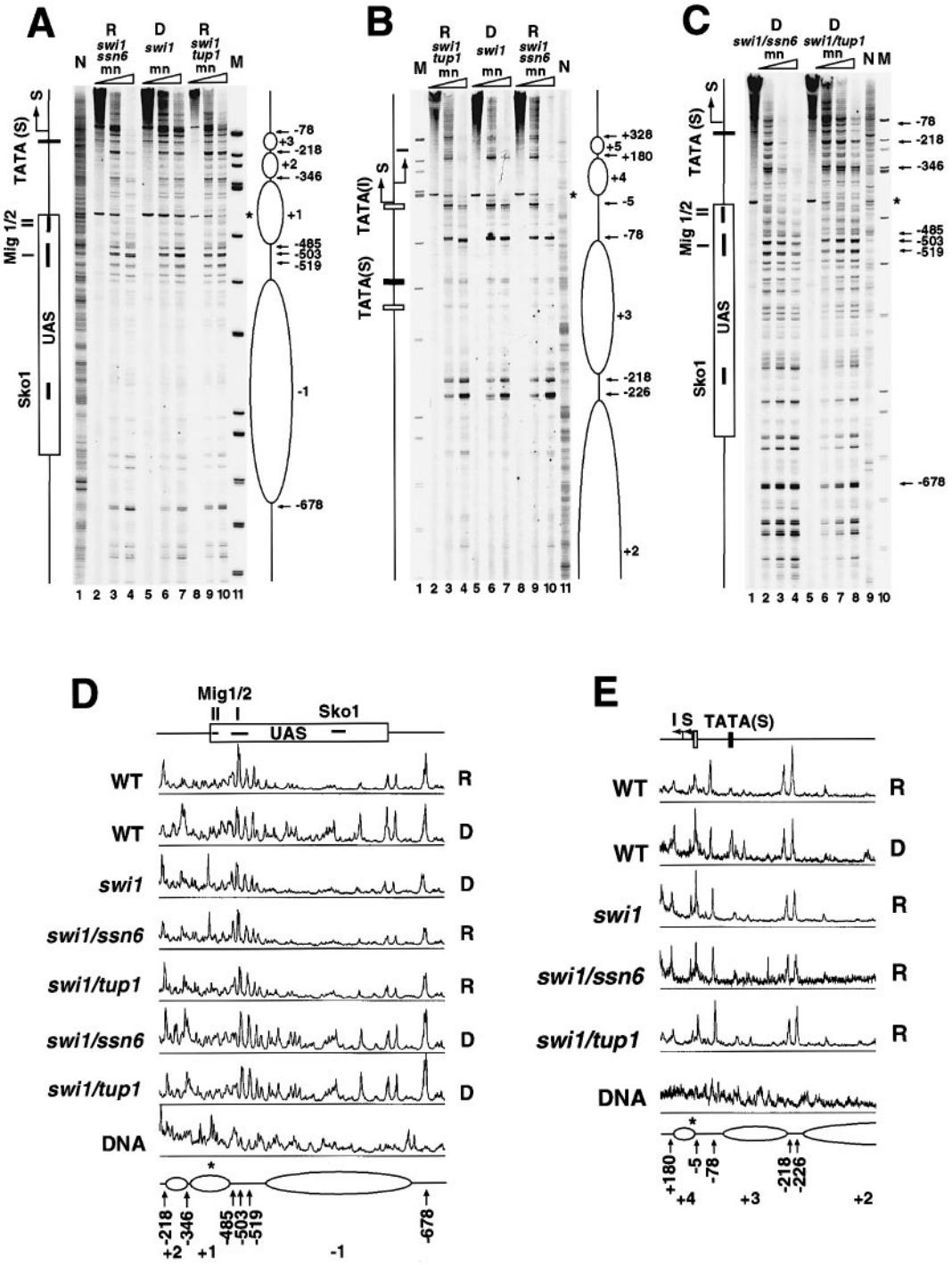

Fig. 5. Ssn6p–Tup1p and Swi–Snf control chromatin remodeling activity at the *SUC2* promoter. Primer extension analysis of *SUC2* chromatin structure in *swi1*, *swi1 ssn6* and *swi1 tup1* mutants under repressing (R) or derepressing (D) conditions using the primers F1 (**A** and **C**) or F3 (**B**). (**D** and **E**) Comparison of the chromatin structure at the UAS (D) and at the TATA box region (E) in these mutants with wild-type cells. For other details see legends to Figures 1 and 3.

blot using a probe to the sequence encoding the signal peptide for secreted invertase (Carlson *et al.*, 1983). The hybridization pattern for this probe and for the probe common for both types of invertase is identical (Figure 4A).

Alteration in SUC2 chromatin structure in ssn6 and tup1 cells under derepressing conditions occurs in the absence of the Swi–Snf complex

Histone mutations and mutations in the *SPT6* gene, whose product interacts with histones *in vitro*, partially restore both *SUC2* expression and the change in the chromatin structure in a *snf2/snf5* background in the derepressed state (Hirschhorn *et al.*, 1992, 1995; Bortvin and Winston, 1996). The Ssn6p–Tup1p complex may also stabilize the chromatin structure at the *SUC2* locus. To determine whether or not the lack of chromatin remodeling in *swi/snf* mutants under derepressing conditions is Ssn6p–Tup1p-dependent, we have analyzed the chromatin structure at the *SUC2* promoter in *swi1 ssn6* and *swi1 tup1* double mutants under derepressing conditions. The results

are shown in Figure 5C. The micrococcal nuclease digestion pattern in these cells is identical to that in wild-type cells under derepressing conditions (Figure 5D) but differs from that in *ssn6* or *tup1* mutants under repressing conditions (Figure 3D). The Mig1p/Mig2p binding site I is still hypersensitive and there is a slight residual protection of the UAS by nucleosome –1. The gene is still induced 11-fold by the shift to low glucose in *ssn6 swi1* and *tup1 swi1* cells (Figure 4, compare lane 5 with 6, and lane 7 with 8). These observations suggest that the chromatin remodeling and the transcriptional activation of the *SUC2* locus under derepressing conditions in these mutants depend on the binding or activity of factors distinct from the Swi–Snf complex and that Swi–Snf is required to antagonize the Ssn6p–Tup1p-mediated repression.

Discussion

In this paper we present a detailed analysis of the chromatin structure of the *SUC2* locus in the repressed state, the derepressed state and in regulatory mutants. The repressed *SUC2* locus is organized as a set of well-positioned nucleosomes protecting the sequences required for gene expression including the UAS and the TATA box for the extracellular form of invertase (Figure 2). The major binding site of Mig1p/Mig2p repressor/activator proteins is located in a linker region between nucleosomes. Similar chromatin organization where positioned nucleosomes flank activator or repressor binding sites and protect the TATA box and other *cis*-acting elements was found in *PHO5* (Svaren and Hörz, 1997), *ADH2* (Verdone *et al.*, 1996) and in **a**-type cell-specific genes (Simpson *et al.*, 1993) in the repressed state. The chromatin structure at these loci may be programmed for an initial step of transcriptional activation where activator binding to a nucleosome-free hypersensitive region disrupts nucleosome positioning followed by pre-initiation complex formation (Lu *et al.*, 1994; Kingston *et al.*, 1996). On the other hand, the location of repressor binding sites, such as the α2 operator, in a linker region may be indicative of the involvement of these factors in chromatin organization (Roth, 1995).

Ssn6p and Tup1p have distinct roles in SUC2 repression

A shift from high to low glucose concentration results in the destabilization of nucleosome positions in the promoter region and the 5′ end of the coding sequence independent of transcription. This observation suggests the existence of chromatin remodeling factors which can alter *SUC2* chromatin structure during activation. Alternatively, this change may be due to a disruption of histone interactions with factors which may stabilize the chromatin at the promoter region. The fact that the nucleosome array is present in *ssn6 swi1* and *tup1 swi1* double mutants under repressing conditions rules out the possibility that the Ssn6p–Tup1p complex directly organizes nucleosomes at the *SUC2* promoter. However, Ssn6p–Tup1p might stabilize nucleosome positioning which could be dictated by DNA sequence itself or by other factors.

The dominant role of Tup1p in Ssn6p–Tup1p-mediated repression was demonstrated by the observations that the LexA–Tup1p fusion protein represses transcription in the absence of Ssn6p (Tzamarias and Struhl, 1994) while repression by LexA–Ssn6p requires Tup1p (Keleher *et al.*, 1992; Tzamarias and Struhl, 1994). Moreover, the Tup1p binding domain of Ssn6p is necessary and sufficient for LexA–Ssn6p-mediated repression, suggesting that Ssn6p represses transcription by recruiting Tup1p (Tzamarias and Struhl, 1995). Tup1p plays the major role in α2-mediated repression of **a**-type specific genes and in the stability of chromatin structure around the α2 operator (Cooper *et al.*, 1994; Tzamarias and Struhl, 1995; Weiss and Simpson, 1997). On the contrary, the level of *SUC2* expression in *ssn6* cells is much higher than in *tup1* cells and the loss of nucleosome positioning at the *SUC2* locus is about the same in *tup1* and in *ssn6* mutants, indicating the existence of an additional mechanism of *SUC2* repression by Ssn6p. This is supported by the evidence that the effect of *ssn6 tup1* double mutations on *SUC2* derepression is higher than the effect of single mutations (Williams and Trumbly, 1990).

Although positioned nucleosomes protect the UAS and the TATA element, the amount of *SUC2* mRNA in *swi1 ssn6* mutants under repressing conditions is comparable with the wild-type level in the derepressed state, suggesting that the organized chromatin structure at the *SUC2* promoter allows a relatively high transcription level. However, organized chromatin might have a repressive effect on *SUC2* transcription, a suggestion which is supported by the observation that histone mutations partially derepress *SUC2* (Hirschhorn *et al.*, 1992, 1995; Kruger *et al.*, 1995). This implies two different mechanisms of *SUC2* repression. For example, the increased level of *SUC2* transcription in *swi1 ssn6* cells at high glucose might be explained by activation by Mig1p/Mig2p (Treitel and Carlson, 1995; Tzamarias and Struhl, 1995; Lutfiyya and Johnston, 1996) or by other factors which are activated in the absence of Ssn6p, while the combination of Ssn6p-mediated repression and organized chromatin structure completely eliminates transcription in wild-type cells under repressing conditions.

Role of Ssn6p–Tup1p and Swi–Snf complexes in controlling SUC2 chromatin structure

In *ssn6 swi1* and *tup1 swi1* double mutants, *SUC2* transcription can still be induced by a glucose shift, resulting in a change in the chromatin structure, which suggests the involvement of chromatin remodeling factors distinct from the Swi–Snf complex in *SUC2* transcriptional activation. This chromatin remodeling activity cannot be attributed to the formation of RNA polymerase II preinitiation complex since *SUC2* expression in *ssn6 swi1* mutants under repressing conditions, where organized chromatin is present, is much higher than in *tup1 swi1* cells under derepressing conditions, where the disruption of nucleosome positioning was observed. This is also supported by the evidence that *SUC2* chromatin remodeling in wild-type as well as in *ssn6* and *tup1* cells does not require canonical TATA element. If Ssn6p–Tup1p does stabilize chromatin structure, the requirement for the Swi–Snf complex may be to help an activator to bind to the promoter and disrupt the nucleosome array during activation.

Our observation that *swi1* mutations restore the chro-

matin organization disrupted in either *ssn6* or *tup1* mutants under repressing conditions indicates that the role of Ssn6p–Tup1p in *SUC2* transcriptional regulation is, at least in part, to block the activity of the Swi–Snf complex. This may be achieved by blocking the Swi/Snf interaction with chromatin, a suggestion supported by the evidence that hSwi/Snf complexes are excluded from heterochromatin (Reyes *et al.*, 1997). This functional relationship is reversed when cells are shifted to derepressing conditions. The lack of both *SUC2* expression and chromatin remodeling in *swi1* cells at low glucose is Ssn6p–Tup1p-dependent, suggesting that Swi–Snf antagonizes repression by Ssn6p–Tup1p at *SUC2*. In this context, one may speculate that the Swi–Snf and Ssn6p–Tup1p complexes control the chromatin remodeling activity which is not available or active when cells are grown under repressing conditions but becomes activated in the derepressed state. Ssn6p–Tup1p may block this activity under derepressing conditions when Swi–Snf is not present so that *SUC2* transcription and the change in the chromatin structure cannot be induced. On the other hand, it becomes hyperactivated by Swi–Snf in the absence of Ssn6–Tup1p, resulting in high transcription level and disruption of nucleosome positioning even under repressing conditions. The derepressing signal may either change the balance between the Swi–Snf-dependent activation and Ssn6p–Tup1p-dependent repression or activate a chromatin remodeling factor by an independent mechanism so that it may overcome the Swi–Snf inhibition by Ssn6p–Tup1p observed in the repressed state.

Materials and methods

Yeast strains, plasmids and media

Yeast strain FY24 (MATα, *ura3-52*, *trp1-Δ63*, *leu2-Δ1*) and its derivatives FY24 *ssn6* (MATα, *ura3-52*, *trp1-Δ63*, *leu2Δ-1*, *Δssn6::URA3*) and FY24 *tup1* (MATα, *ura3-52*, *trp1-Δ63*, *leu2-Δ1*, *Δtup1::URA3*) were grown either in rich media (YPD) or in selective media (CSM) supplemented with the appropriate drop-out mix (Sherman, 1991). Plasmid pRS406 (Sikorski and Heiter, 1989); BD39 for *SWI1* replacement (Peterson and Herskowitz, 1992) and *SSN6* and *TUP1* disruption plasmids (Cooper *et al.*, 1994) were described previously. Yeast were transformed according to Hill *et al.* (1991).

Genetic manipulations

The TATA box for secreted invertase at position from –133 to –128 (the position +1 denotes the A residue of ATG for secreted invertase) was replaced by a *Nsi*I site using the pop-in/pop-out allele replacement technique (Rothstein, 1991). Two DNA fragments corresponding to *SUC2* sequences at positions from –559 to –134 and from –127 to +258 were produced by polymerase chain reaction (PCR) of genomic DNA using oligonucleotides: 5′-CGGAATTCTTCTACCAAAGGCGTGCC; 5′-GAAGAATAATACATATCTAATGCATTGTTTCTTTTCAGGAGG and 5′-CCTCCTGAAAAGAAACAATGCATTAGATATGTATTATTC-TTC; 5′-CGGAATTCCCAATTAGTCAAATCATCGGA, thus creating novel *Eco*RI and *Nsi*I sites flanking *SUC2* sequences. These fragments were tandemly cloned into the *Eco*RI site of pRS406. The plasmid was linearized by *Mlu*I and used to transform FY24 to uracil prototrophy. Cells were streaked out on 5-fluoroorotic acid plates to select for the pop-out event. Both pop-in and pop-out events were confirmed by PCR of genomic DNA and by checking for the presence of the *Nsi*I site in amplified products. The null alleles of *ssn6*, *tup1* and *swi1* were constructed by one-step gene disruption (Rothstein, 1991).

Micrococcal nuclease digestion of nuclei and primer extension analysis

Yeast nuclei were isolated according to Roth and Simpson (1991) from cells grown in YPD (2% dextrose, repressing conditions) or in YP (0.05% dextrose, derepressing conditions) for 1 h before harvesting as described in Carlson and Botstein (1982). Micrococcal nuclease digestion and isolation of DNA from nuclei were carried out as described previously (Roth *et al.*, 1992). The location of micrococcal nuclease cleavage sites was determined by primer extension according to Shimizu *et al.* (1991) using a set of primers corresponding to base pairs: –784 to –755 (F1); –593 to –564 (F2); –386 to –357 (F3); –173 to –144 (F4); +130 to +159 (F5); +2068 to +2039 (R1) and –353 to –382 (R2) of the *SUC2* sequence.

Isolation of total mRNA and Northern blot analysis

Total RNA was isolated from cells grown under repressing or derepressing conditions by a LiCl method (Rose *et al.*, 1990), fractionated in a 1.5% formaldehyde agarose gel, transferred to a nylon membrane and hybridized according to Church and Gilbert (1984). The probes were generated by PCR and corresponded to positions –40 to +44 and +130 to +771 of *SUC2*, or to position +60 to +404 of *ACT1*.

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