Nucleosome structure of the yeast CHA1 promoter: analysis of activation-dependent chromatin remodeling of an RNA-polymerase-II-transcribed gene in TBP and RNA pol II mutants defective in vivo in response to acidic activators

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The Saccharomyces cerevisiae CHA1 gene encodes the catabolic l-serine (l-threonine) dehydratase. We have previously shown that the transcriptional activator protein Cha4p mediates serine/threonine induction of CHA1 expression. We used accessibility to micrococcal nuclease and DNase I to determine the in vivo chromatin structure of the CHA1 chromosomal locus, both in the non-induced state and upon induction. Upon activation, a precisely positioned nucleosome (nuc-1) occluding the TATA box and the transcription start site is removed. A strain devoid of Cha4p showed no chromatin alteration under inducing conditions. Five yeast TBP mutants defective in different steps in activated transcription abolished CHA1 expression, but failed to affect induction-dependent chromatin rearrangement of the promoter region. Progressive truncations of the RNA polymerase II C-terminal domain caused a progressive reduction in CHA1 transcription, but no difference in chromatin remodeling. Analysis of swi1, swi3, snf5 and snf6, as well as gcn5, ada2 and ada3 mutants, suggested that neither the SWI/SNF complex nor the ADA/GCN5 complex is involved in efficient activation and/or remodeling of the CHA1 promoter. Interestingly, in a sir4 deletion strain, repression of CHA1 is partly lost and activator-independent remodeling of nuc-1 is observed. We propose a model for CHA1 activation based on promoter remodeling through interactions of Cha4p with chromatin components other than basal factors and associated proteins.

Keywords: activation-dependent remodeling/chromatin structure/Saccharomyces cerevisiae/transcription activation

Introduction

In the yeast Saccharomyces cerevisiae, utilization of the hydroxy amino acids serine and threonine as the sole nitrogen source is dependent on the CHA1 gene. Expression of the CHA1 gene is induced 100-fold by serine/threonine, and in their absence no CHA1 transcript can be detected (Ramos and Wiame, 1982; Petersen et al., 1988). Cha4p is a regulatory protein that binds to serine/threonine response elements (UASCHA) in the CHA1 promoter and activates transcription (Bornæs et al., 1993; Holmberg and Schjerling, 1996). Cha4p is a 648-amino-acid, acidic transcriptional activator that belongs to the Cys6 Zn cluster class of yeast trans-acting factors (Holmberg and Schjerling, 1996; Schjerling and Holmberg, 1996).

Gene expression in eukaryotes is regulated at several levels. Assembly of the basal machinery and initiation of transcription requires many different proteins and interactions between transcriptional activators and basal factors, and is a major control point for gene expression (Orphanides et al., 1996, and references therein). In yeast, both UAS and TATA elements are required for high levels of transcription. The function of UASs is dependent on their recognition and binding by regulatory proteins, and these sequence-specific transcription factors interact with components of both the general transcriptional machinery and chromatin (Stringer et al., 1990; Lin et al., 1991; Xiao et al., 1994; Joliot et al., 1995; Kobayashi et al., 1995). Although acidic activation domains can interact in vitro directly with general transcription factors, co-activators and mediators, the in vivo interactions required for transcriptional activation are still unclear.

To understand how activators function, one must consider that eukaryotic DNA is packaged into chromatin. Assembly of DNA into nucleosomes often, but not always, imposes severe limitations on factor accessibility and recognition of the underlying DNA sequence, and in addition to a structural role, nucleosomes have been shown to work as repressors in vivo as well as in vitro (Lorch et al., 1987; Han and Grunstein, 1988; Roth et al., 1990; Straka and Hörz, 1991). In some cases, however, nucleosomes have a positive effect on transcription, presumably by creating a static loop to bring distal elements into close proximity (Thomas and Elgin, 1988; Schild et al., 1993; Lu et al., 1995; Pfaff and Taylor, 1998).

Genes that need to be active only under specific growth conditions or developmental states of the cell must otherwise be maintained repressed, and packaging of cis-acting sequences into nucleosomes is a simple inhibitory mechanism that affects all genes (Knezetic and Luse, 1986; Lorch et al., 1987; Workman et al., 1991). Activation of chromatin-assembled templates would thus require disruption of the repressive structure to allow accessibility of basal factors. The TATA-box-binding protein (TBP) and, presumably, transcription factor IID (TFIID) are essentially unable to bind the TATA element when the latter is complexed into nucleosomes (Workman and Roeder, 1987; Imbalzano et al., 1994; Godde et al., 1995; Li et al., 1998). It is noteworthy that nucleosome disruption occurs even if this regulatory response is tested when transcription is prevented, by deletion of the TATA element
both in an activator-dependent situation (Fascher et al., 1993) or by artificial recruitment of the holoenzyme (Gaudreau et al., 1997), arguing that chromatin is disrupted in an early step of the transcriptional activation process.

To test this model, we used several activation-defective TBP mutants originally isolated by Stargell and Struhl (1995, 1996a), and proposed by these authors to define a two-step mechanism for in vivo transcriptional activation. These mutant forms of TBP are defective in either recruitment to the promoter or post-recruitment interaction(s) (Stargell and Struhl, 1996a). Also, activator interactions with components of the SRB/mediator coactivator complex suffice for recruitment of the entire initiation machinery to a promoter (Barberis et al., 1995). Since the SRB/mediator complex interacts with the RNA polymerase II largest subunit C-terminal domain (pol II CTD), we also used various activation-defective pol II CTD truncations in our analysis.

We have characterized the nucleosomal structure of the CHA1 locus both in a basal and in an activated state. We have identified a nuclease-hypersensitive site in the promoter region, encompassing the cis-acting elements required for serine/threonine-dependent activation of the CHA1 gene. Upon induction, a single nucleosome occluding the TATA box undergoes an activator-dependent displacement. We show that in several activation-defective TBP mutants, as well as in RNA pol II CTD truncations, chromatin is efficiently remodeled under inducing conditions irrespective of the fact that transcription of the CHA1 gene is greatly decreased or abolished. We propose that disruption of chromatin is the initial step in the process of in vivo transcription initiation, preceding interactions with TBP and/or RNA polymerase II holoenzyme in the CHA1 promoter.

Regulation of transcription in eukaryotes requires that sequence-specific activators gain access to cognate sites present in DNA assembled into chromatin. Activator function may therefore be dependent on interactions that potentiate transcription. An ATP-dependent multiprotein subunit complex, the SWI/SNF complex, capable of altering chromatin structure and facilitating binding of TFIIA/TBP and activators to nucleosomal templates has been isolated and shown to be required for the activation of certain genes (reviewed in Winston and Carlson, 1992; Cairns, 1998). We investigated whether remodeling and activation of the CHA1 gene is SWI/SNF dependent, and verified that Δswi1, Δswi3, Δsnf5 and Δsnf6 mutant strains had wild-type levels of CHA1 expression and were able to remodel the CHA1 promoter. A second complex, the ADA/GCN5 complex, which has been implicated in activator function, was also investigated. We tested Δada2, Δada3 and Δgcn5 mutant strains and verified that all mutants displayed wild-type levels of CHA1 transcription and chromatin remodeling upon induction. Since CHA1 is located only 2 kb centromere-proximal from the HML, we also tested if activation of CHA1 had any dependency of efficient silencing. A disrupted sir4 strain showed CHA1 derepression as well as remodeling of the promoter even in the absence of serine/threonine in the growth medium, an effect also observed in a double cha4 sir4 mutant strain. We present a model of the interactions at the CHA1 promoter that are required for efficient activation.

Results

Serine induction causes Cha4p-dependent remodeling of the CHA1 promoter

Transcription of the S.cerevisiae CHA1 gene is induced by the presence of serine or threonine ~100-fold (Ramos and Wiame, 1982; Petersen et al., 1988). Transcriptional regulation is mediated by Cha4p through two binding sites, UAS1CHA (positions –240 to –214) and UAS2CHA (positions –214 to –161), present in the promoter region. Several loci in yeast show activation-dependent structural changes (Almer and Hörz, 1986; Fedor and Kornberg, 1989; del Olmo et al., 1993; Verdone et al., 1996). The nucleosomal organization of the CHA1 gene was investigated employing micrococcal nuclease (MNase) and DNase I digests of repressored or derepressed SG76 (CHA4) cells. The results obtained with MNase I show that the entire uninduced gene is assembled into an ordered nucleosomal array (Figure 1, –Ser). A typical nucleosomal ladder can be observed both in the promoter and in the coding region. A strong hypersensitive site with clearly defined boundaries is seen in the promoter region, overlapping the previously identified cis-acting elements required for serine/threonine inducibility (UAS1CHA and UAS2CHA) (Bormes et al., 1993), while the transcription start site (position –20) and the putative TATA box (–132 and –82) are included in a nucleosome. However, when transcription is induced by addition of serine to the growth medium, a striking change takes place (Figure 1, +Ser). In addition to the hypersensitive site, the TATA box and adjacent sequences also become hypersensitive to nuclease digestion in a region corresponding to a previously phased nucleosome. Furthermore, the coding region, which in the repressed state is assembled into regularly positioned
nucleosomes and displays a clear band pattern, becomes diffuse in the active state. These results show that the CHA1 gene undergoes a chromatin structure transition upon activation.

To investigate whether the activation-dependent chromatin remodeling of the CHA1 promoter is mediated by the transcriptional activator Cha4p, DNase I digests of strain TG258 (Δcha4) cells were carried out (Figure 2). We observe the same band pattern in the absence (Figure 2, –Ser) or presence of serine (Figure 2, +Ser), namely a strong hypersensitive site in the 5′ flank, an ordered nucleosomal array covering the coding region and TATA box. Thus, remodeling of the CHA1 promoter is dependent on the Cha4p activator.

To complement the results obtained with DNase I, we digested nuclei from strains SG76 (CHA4) and TG258 (Δcha4) cells with MNase. The obtained band patterns support the DNase I results (Figure 3). A single strong hypersensitive site overlapping the UASs, a highly uniform nucleosome array covering the gene and 5′ flank in the repressed state (Figure 3, CHA4 –Ser) becomes diffuse in the coding region upon induction, and the nucleosome that occludes the TATA box is remodeled upon activation (Figure 3, CHA4 +Ser). In the absence of Cha4p, no remodeling is observed upon addition of serine to the growth medium (Figure 3, Δcha4 cf. –Ser and +Ser). These results complement our DNase I analysis of the CHA1 promoter and strengthen our interpretation of the organization of CHA1 chromatin structure, and activation and activator-dependent structural transitions.

**TBP mutants defective in activated transcription do not affect chromatin remodeling of the CHA1 promoter**

Increasing the accessibility of TATA-binding protein (TBP) to the promoter is possibly one of the mechanisms by which activators stimulate transcription (Meisterernst et al., 1990; Workman et al., 1991; Xiao et al., 1995). TBP mutants specifically defective in vivo in the response to acidic activators and proposed to define a two-step mechanism for transcription initiation have been described (Stargell and Struhl, 1995, 1996a). To address whether remodeling of the CHA1 promoter takes place at a step that occurs before or after the point at which these TBP mutant forms are defective, and whether the interactions that falter in these mutants are required for chromatin remodeling, we tested five such mutants (N2-1, F237D, E236P, T153I and T153I and F148H) for serine-induced activation of the CHA1 promoter (Figure 4). Northern analysis of total RNA showed that induced CHA1 mRNA levels were drastically reduced in all mutants (Figure 4A, +Ser), suggesting that Cha4p cannot activate transcription of CHA1 in these mutants. Nevertheless, MNase and DNase I analysis showed that in all cases, serine-dependent remodeling of the promoter took place (Figures 4B, and data not shown, cf. –Ser and +Ser). We conclude that, although the interaction defects of these TBP mutant forms affect activation, they do not influence the chromatin remodeling process.

**Effect of the C-terminal domain of RNA polymerase II on the CHA1 chromatin structure**

The C-terminal domain (CTD) of the largest subunit of yeast RNA polymerase II contains 26 or 27 tandem repeats of the consensus heptapeptide sequence TyrSerProThrSer-ProSer (Allison et al., 1985; Corden et al., 1985). The RNA polymerase II CTD is required for growth, but removal of a significant number of repeats is tolerated (Nonet et al., 1987; Scafe et al. 1990). In yeast, truncation mutations affect activated transcription of a subset of genes (Scafe et al., 1990). Transcriptional initiation, response to acidic activators and involvement in chromatin organization by association with the SRB/mediator complex are some of the functions ascribed to the CTD (Scafe et al.,
Fig. 4. Transcriptional activity and chromatin remodeling of the \textit{CHA1} gene in TBP derivatives. (A) Northern analysis of TBP mutant strains (F237D, T153I, N2-1, F148H and E236P) under non-induced (–Ser) and induced (+Ser) conditions. Ten micrograms of total RNA isolated from undigested nuclei was electrophoresed in a 1.5% formaldehyde agarose gel, blotted and hybridized with labeled PCR amplificates obtained with the \textit{CHA1} gene set of primers and the \textit{URA3} gene set of primers, respectively. RNA from a wild-type (wt) control sample (SG76) was included for comparison. (B) Chromatin analysis of the \textit{CHA1} gene in the N2-1 TBP derivative. DNase I and MNase low-resolution analyses. Cells were grown in the absence (–Ser) or presence (+Ser) of serine. Nuclei were digested for 10 min with 1 and 20 U/ml DNase I or 1, 20 and 100 U/ml MNase. DNA was isolated, digested with \textit{Bam}HI, separated on a 1% agarose gel, blotted and hybridized with a labeled PCR amplificate obtained with the \textit{CHA1} gene set of primers. Lanes M contain restriction enzyme double digests of genomic DNA with \textit{Bam}HI, and \textit{Cla}I or \textit{Hind}III, to generate position marker fragments. The vertical map indicates the relative positions of the various \textit{cis}-acting sequences and the \textit{CHA1}-coding sequence.

1990; Liao et al., 1991; Seipel et al., 1994). We addressed whether CTD truncations, previously shown to decrease the ability of RNA polymerase II to respond to acidic activators (Liao et al., 1991), would affect \textit{CHA1} activation. Two truncation mutants containing 13 and 11 repeats (strains V17 and C6, respectively), and an isogenic wild-type counterpart with 27 repeats (L14), were analyzed with MNase or DNase I. Northern analysis of \textit{CHA1} transcription in the CTD truncation mutants showed, as expected, a progressive reduction of the serine-dependent activation potential (Figure 5A, +Ser). In the CTD mutant containing 13 repeats (Figure 5A, V17 +Ser), \textit{CHA1} transcript levels are reduced to ~50% those of the wild-type strain in the induced state (Figure 5A, L14 +Ser). The mutant containing 11 repeats displayed <5% of the levels of induced transcription in its wild-type counterpart strain (Figure 5A, C6 +Ser and L14 +Ser, respectively). However, MNase analysis of the \textit{CHA1} promoter in L14, V17 and C6 cells in the repressed state (Figure 5B) and derepressed state (Figure 5C) revealed a band pattern similar to that which we had previously observed in all strains. DNase I analysis confirmed these results (data not shown). This result suggests that interactions responsible for activation-dependent chromatin remodeling in the \textit{CHA1} promoter are not affected to any noticeable degree in the CTD truncation mutants.
Fig. 6. Transcriptional activity and nucleosomal structure of the CHA1 gene in SWI/SNF mutants. Northern blot analysis (A) of the various SWI/SNF mutants (Δswi1, Δswi3, Δsnf5, Δsnf6 and an isogenic wild-type strain) was carried out as described in the legend to Figure 4A. (B) Chromatin structure of the CHA1 gene in a Δsnf5 strain was analyzed by DNase I and MNase digestion as in Figure 4B.

**SWI/SNF requirement of the CHA1 promoter**

In yeast, a multiprotein complex termed the SWI/SNF complex is involved in chromatin destabilization to counteract a repressive chromatin structure and has been shown to be required for normal expression of various genes (reviewed in Winston and Carlson, 1992; Kingston et al., 1996). We therefore tested to what extent remodeling of the CHA1 promoter requires the presence of the SWI/SNF complex. To do so we measured serine-induced expression of CHA1 in four different mutants deleted for either swi1, swi3, snf5 or snf6 (Figure 6). Northern analyses of induced CHA1 transcript levels were indistinguishable from the wild-type strain in all mutants (Figure 6A, +Ser), suggesting that these mutants have no effect on CHA1 transcription. MNase and DNase I digests of strains grown in the presence or absence of serine were investigated for structural differences in the CHA1 promoter (Figure 6B, and data not shown). In all cases, serine-dependent remodeling of the promoter could be observed, suggesting that the SWI/SNF complex is dispensable for efficient activation and remodeling of the CHA1 gene.

**ADA/GCN5 requirement of the CHA1 promoter**

In addition to the SWI/SNF complex, histone acetyltransferase (HAT) activity may also function by destabilizing a repressive nucleosome structure. The yeast transcriptional adaptor protein Gcn5p has been shown to encode a histone acetyltransferase capable of acetylating several lysine residues in the N-terminal domains of histone H3 and H4 in vitro (Brownell et al., 1996; Kuo et al., 1996). The ADA2, ADA3 and GCN5 genes are required for full expression of a subset of genes, consistent with a model in which these adaptors bridge interactions between activators and basal factors (Barlev et al., 1995; Marcus et al., 1996; Horiiuchi et al., 1997; Saleh et al., 1997). Ada2p, Ada3p and Gcn5p have also been shown to be subunits of a heteromeric complex (Horiiuchi et al., 1995; Candau and Berger, 1996). We tested whether activation and/or remodeling of the CHA1 gene is dependent on the ADA/GCN5 coactivator complex. CHA1 transcript levels in Δada2, Δada3 and Δgcn5 mutant cells grown under induced conditions had a normal pattern of expression (Figure 7A). Also, MNase and DNase I analysis of the mutant strains revealed no difference in the nucleosomal band pattern compared with a wild-type strain (Figure 7B, and data not shown). No structural difference was observed at the CHA1 promoter, showing that the ADA/GCN5 complex is not essential for serine-mediated remodeling of the CHA1 gene.

**SIR4 protein is required for maintenance of the repressed state of the CHA1 gene**

CHA1 is located only 2 kb centromere-proximal to HML. The yeast silent mating type loci, HML and HMR, provide a well-studied example of chromatin-mediated repression. Several factors are needed for establishment and maintenance of transcriptional silencing in yeast: among them are Sir2p, Sir3p and Sir4p, a group of proteins thought to play a structural role in silencing (Aparicio et al., 1991; Moazed et al., 1997). To determine whether the chromosomal location of CHA1 had any effect on its expression pattern, we constructed a sir4::HIS3 disruption strain.
performed as described in Figure 4A. Analysis of \( \Delta \)CHA1 under uninduced conditions also showed an increase in \( /H11001 \) (Figure 8B, \( –\text{Ser} \)). As expected, serine-dependent remodeling is the same as that in wild-type cells under non-induced growth conditions (Figure 8B, \( –\text{Ser} \)), as well as remodeling of the \( \Delta \)cha4 sir4::HIS3 strain. Activated expression of \( \Delta \)cha4 sir4::HIS3, sir4::HIS3, \( \Delta \)cha4 and wt strains was performed as described in Figure 4A.

Northern blot analysis of total RNA isolated from \( \text{SIR4} \) and \( \text{sir4} \) strains showed that \( \text{CHA1} \) transcript levels in cells grown under uninduced conditions were strongly increased in the \( \text{sir4} \) strain as compared with its isogenic wt strain (Figure 8A, \( \text{sir4} –\text{Ser} \) and wt \( –\text{Ser} \), respectively). Activated expression of \( \text{CHA1} \) was not affected (Figure 8A, \( \text{sir4} +\text{Ser} \)). Interestingly, MNase and DNase I digests of the \( \text{sir4} \) strain showed that remodeling of the promoter takes place under non-induced growth conditions (Figure 8B, \( –\text{Ser} \)). As expected, serine-dependent remodeling is the same as that in wild-type cells (Figure 8B, \( +\text{Ser} \)). Analysis of the \( \text{cha4 sir4} \) double mutant under uninduced conditions also showed an increase in \( \text{CHA1} \) expression (Figure 8C, cf. \( \Delta \)cha4 sir4 –\text{Ser} and wt \( –\text{Ser} \)), as well as remodeling of the \( \text{CHA1} \) promoter (data not shown). In \( \Delta \)cha4 cells, induction of \( \text{CHA1} \) transcription is abolished (Figure 8C, \( \Delta \)cha4 \( +\text{Ser} \) and \( \Delta \)cha4 sir4 +\text{Ser} ), demonstrating that Cha4p is responsible for serine-dependent activation of \( \text{CHA1} \) and that Sir4p affects basal but not activated transcription. Thus, Sir4p is required for full repression of the \( \text{CHA1} \) gene in a Cha4p-independent manner.

Discussion

Chromatin transitions at the \( \text{CHA1} \) locus

The results obtained with nuclease digestion of the \( \text{CHA1} \) gene clearly show a structural difference between the active and the repressed states of this gene. The entire locus possesses a very well-defined chromatin organization in the repressed state, with a regular nucleosomal band pattern over the promoter and coding region. One single strong nuclease-hypersensitive site is observed in the promoter region covering the UASs. All previously identified \( \text{cis} \)-acting sequences are located within this hypersensitive site (Bornæs et al., 1993), suggesting that the \( \text{trans} \)-acting factors required for proper regulation of the \( \text{CHA1} \) gene can gain access to their cognate sites constitutively. Another interesting feature of the promoter structure is the fact that the hypersensitive site becomes MNase resistant upon activation (compare for example in Figure 3, \( \text{CHA4} –\text{Ser} \) and \( +\text{Ser} \)), although it maintains its accessibility to DNase I (compare for example Figure 1, +\text{Ser} with Figure 3, \( \text{CHA4} +\text{Ser} \)). This peculiar change has been observed in other genes, namely in the UAS of the \( \text{GAL1-10} \) genes (Lohr and Hopper, 1985) and in the regulatory region of the \( \text{SUC2} \) gene (Perez-Ortin et al., 1986, 1987) under derepressed conditions. In the \( \text{CHA1} \) promoter, this change is specifically dependent on the transcriptional state of the gene, that is, even under derepressed conditions this MNase-specific protection is not seen unless the gene is actively being transcribed. Thus, if one compares the MNase digests of induced CTD truncation mutants (Figure 5C), in which progressive truncation of the CTD leads to progressive loss of transcriptional potential (Figure 5A), one can see that the L14 and V17 strains display a MNase protection of the UAS region, but that this protection is not present in the C6 strain (Figure 5C). This protection is probably caused by the binding of some protein(s) to the hypersensitive region during transcription that protects DNA from Mnase but allows DNase I (compare also Figures 1 and 3) to access the underlying sequences. Alternatively, the protection we see can reflect a conformational change of the DNA structure that prevents MNase but not DNase I from cutting in both strands.

We propose a model for the chromatin organization and remodeling of \( \text{CHA1} \) under repressed and derepressed conditions (Figure 9). In the repressed state a constitutive hypersensitive site exists, comprising the UASs with an\( \text{cis} \)-acting sequences located within this hypersensitive region (Bornæs et al., 1993). Furthermore, upon activation the nucleosome
occluding the TATA box (nuc-1) is remodeled, thereby broadening the hypersensitive site in the promoter region. A stretch of DNA (about 250 bp) is permanently nucleosome-free under all growth conditions in the presence or absence of the transcriptional activator Cha4p. This means that Cha4p can bind all UASCHA without disrupting nucleosomes. In addition, in vivo DMS footprinting has shown that Cha4p is poised to the promoter also under repressed conditions, enabling the cells a quick switch to begin utilizing serine/threonine as the sole nitrogen source (Schjerling, 1997). We conclude that Cha4p binding and nucleosome disruption are two separate events in the CHA1 promoter. This is in contrast to the remodeling of the PHO5 promoter by Pho4p, in which these two functions seem to be linked (Svaren and Hoër Z, 1997). Presently, we do not know the mechanism by which nucleosomes are precisely positioned to create the nucleosome-free region in the CHA1 promoter. Incorporating the TATA box into a nucleosome severely inhibits binding of TBP (Imbalzano et al., 1994; Godde et al., 1995), and has been shown to reduce greatly transcription initiation in vitro (Knezetic and Luse, 1986; Workman and Roeder, 1987; Laybourn and Kadonaga, 1991) as well as in vivo (Li et al., 1998). A precisely positioned nucleosome has been implicated in regulating expression of several promoters. A nucleosome positioned over the TATA region of the β-phaseolin (phas) promoter in transgenic tobacco is responsible for the lack of phas expression in vegetative tissues (Li et al., 1998). The yeast PHO5 and Drosophila Krüppel are further examples of promoters in which a positioned nucleosome occluding the transcription start site is involved in regulating gene expression (Laybourn and Kadonaga, 1991; Straka and Hörz, 1991). Thus, nucleosome displacement in the CHA1 promoter to allow TBP binding to its cognate site can be one mode of action of the Cha4 protein, showing this gene to be a good model for studying transcription-associated chromatin remodeling.

**Analysis of protein interactions in the CHA1 gene required for the process of chromatin remodeling and transcription initiation**

Transcription initiation of RNA polymerase II-transcribed genes involves the assembly of a pre-initiation complex (PIC), composed of the polymerase and associated factors, and a number of general transcription factors (TFIIA, B, D, E, F, G/J and H) (reviewed in Buratowski, 1994; Tjian and Maniatis, 1994; Sheldon and Reinberg, 1995). Formation of a functional PIC requires the stepwise assembly of interacting factors in an ordered sequential manner on the promoter. The pathway of interactions leading to enhancement of transcription by acidic activators was suggested to be a multistep process. The first step in the assembly of the PIC is thought to be the recruitment of TBP to the promoter. Acidic activators enhance the rate of recruitment of TBP to the promoter by interacting, directly or indirectly, with TBP and/or by remodeling the chromatin structure of the promoter, increasing the accessibility of the TATA sequence (Klein and Struhl, 1994; Klages and Strubin, 1995; Xiao et al., 1995).

To characterize interactions required for transition from a non-activated state to an active or potentially active state in a RNA polymerase II-transcribed gene, we examined the nucleosomal structure of the CHA1 gene in several activation-defective TBP mutants. A TBP mutant (N2-1) specifically defective in the interaction with TFIIA has been isolated (Stargell and Struhl, 1995). This TBP mutant form does not support activation by acidic activators in vivo, suggesting that the TBP–TFIIA interaction is required for transcriptional activation in vivo. Transcriptional activation of the CHA1 gene is abolished in this mutant (Figure 4A, N2-1 + Ser). However, lack of an efficient TBP–TFIIA interaction does not affect chromatin remodeling of the CHA1 promoter (Figure 4B). Recently, four additional activation-defective TBP mutants (F237D,
T153I, F148H and E236P) were described (Stargell and Struhl, 1996a) that have some unusual biochemical defects. The F237D mutant form is defective for all tested protein–protein interactions in vitro, namely interaction with TFIIA, TFIIB and acidic activator VP16, but binds the TATA element with wild-type affinity. Accordingly, artificial recruitment of the mutant protein does not lead to transcriptional activation (Stargell and Struhl, 1996a). Nevertheless, remodeling of the CHA1 promoter is still observed upon induction with serine. Another TBP mutant form, T153I, is defective in its recruitment to the TATA element, presumably due to an impairment in an activation-specific protein interaction (Stargell and Struhl, 1996a). Again, remodeling of the promoter was seen upon induction, although activation of the CHA1 gene was impaired. The same was observed for the two mutants F148H and E236P: severe impairment of activation potential but remodeling of the promoter upon induction. These data suggest that: first, all the interactions in which the studied TBP mutants are defective are not essential for chromatin remodeling of the CHA1 promoter in vivo; secondly, since the various mutants block the process of activation at distinct stages, chromatin remodeling must occur prior to the blocked steps. The defects observed in these TBP mutants led to the proposal of a two-step mechanism in the response to acidic activators in vivo by TBP (Stargell and Struhl, 1996a,b). According to this model, the activator protein recruits TFIIID and RNA polymerase II holoenzyme in sequential order. Thus, remodeling of the nucleosome covering the TATA sequence would precede the formation of a stable PIC. RNA polymerase II holoenzyme might be able, under TATA sequence would precede the formation of a stable TFIID and RNA polymerase II holoenzyme in sequential order. According to this model, the activator protein recruits TFIIID and RNA polymerase II holoenzyme in a TFIID-independent manner, and, as the CTD is recruited to a promoter, it activates transcription (Stargell and Struhl, 1996a). Again, remodeling of the promoter was seen upon induction, although activation of the CHA1 gene was impaired. The same was observed for the two mutants F148H and E236P: severe impairment of activation potential but remodeling of the promoter upon induction. These data suggest that: first, all the interactions in which the studied TBP mutants are defective are not essential for chromatin remodeling of the CHA1 promoter in vivo; secondly, since the various mutants block the process of activation at distinct stages, chromatin remodeling must occur prior to the blocked steps. The defects observed in these TBP mutants led to the proposal of a two-step mechanism in the response to acidic activators in vivo by TBP (Stargell and Struhl, 1996a,b). According to this model, the activator protein recruits TFIIID and RNA polymerase II holoenzyme in sequential order. Thus, remodeling of the nucleosome covering the TATA sequence would precede the formation of a stable PIC. RNA polymerase II holoenzyme might be able, under certain conditions, to disrupt chromatin in an SWI/SNF–PIC. RNA polymerase II holoenzyme might be able to recruit TFIID. We addressed this question by analyzing RNA polymerase II CTD truncation mutants containing 13 and 11 consensus heptapeptide repeats out of the 27 in a wild-type situation (strains V17, C6 and L14, respectively). It was expected that the progressive reduction in the length of the CTD would drastically reduce the activation potential of RNA polymerase II of the CHA1 gene (Figure 5A), since partial truncations of the CTD had been shown to cause defects in activated transcription (Scafe et al., 1990; Liao et al., 1991). However, in the CTD mutants, remodeling of the CHA1 promoter was not affected (Figure 5C), suggesting that CTD interactions are necessary for efficient activated CHA1 expression but not for chromatin remodeling.

Chromatin remodeling complexes such as the yeast SWI/SNF complex (reviewed in Pazin and Kadonaga, 1997) and histone acetyltransferases such as Gcn5p (reviewed in Struhl, 1998) play key roles in countering chromatin-mediated repression. However, our observation that deletion of SWI1, SWI3, SNF5 or SNF6 has no effect, either on expression (Figure 6A) or on chromatin transition in CHA1 (Figure 6B, and data not shown) suggests that the SWI/SNF complex is not required for the remodeling of the CHA1 promoter. Another multimeric complex, the ADA/GCN5 complex, implicated in activator function, has been proposed to facilitate transcription by targeting disruption of chromatin structure and to act concertedly with the SWI/SNF complex to facilitate activator function (Pollard and Peterson, 1997). We found that deletion of ADA2, ADA3 or GCN5, however, had no effect on induced or non-induced expression of CHA1 (Figure 7A). Furthermore, these mutant strains showed no defect in chromatin transitions in the CHA1 promoter upon serine-mediated induction (Figure 7B and data not shown). Thus, regulation of the CHA1 gene seems to be independent of the SWI/SNF and ADA/GCN5 complexes.

We show here that a strain deficient for Sir4p has a modified pattern of CHA1 expression. In the absence of inducer, a CHA1-specific transcript is observed in this strain (Figure 8A, sir4–Ser), whereas an isogenic wild-type strain has no detectable expression under the same growth conditions (Figure 8A, wt). Furthermore, the increase in expression is accompanied by remodeling of the CHA1 promoter. Interestingly, we could not detect any difference between the two strains in the CHA1 gene under inducing conditions, either at the transcriptional level (Figure 8A, sir4 +Ser) or at the structural level (Figure 8B, +Ser). This effect on CHA1 expression and chromatin structure is Cha4p independent, since a strain disrupted for both cha4 and sir4 showed the same increase in non-induced expression and promoter remodeling (Figures 8C, Δcha4 sir4–Ser and data not shown, respectively). As expected, addition of serine to the growth medium had no detectable effect on CHA1 expression in the Δcha4 sir4 double mutant strain (Figure 8C, cf. Δcha4 sir4, +Ser and –Ser). We therefore propose that efficient repression of the CHA1 promoter is, directly or indirectly, dependent on the presence of SIR4. In a wild-type strain, only upon induction is nuc-1 remodeled, and the Cha4p activator is able actively to recruit TBP to an exposed TATA sequence for binding, thus starting the process of assembly of an active PIC. However, in the uninduced sir4 strain, nuc-1 is not positioned over the TATA element, thus allowing TBP binding and transcription of the CHA1 promoter at a high level even under non-induced growth conditions.

In conclusion, using low-resolution analyses we have mapped the nucleosomal structure of the yeast CHA1 gene. This gene is quickly and strongly induced in the presence of serine/threonine in the growth medium. Nucleosomal structure of the CHA1 gene is markedly ordered and undergoes a clearly detectable activation-dependent rearrangement, making this gene a good model to study transcription-associated nucleosomal remodeling. We find that chromatin transition at the CHA1 promoter can take place without transcription and propose that remodeling is caused by direct or indirect interactions of Cha4p with chromatin-influencing factor(s) as a first step in the process of gene activation.

Materials and methods

Strains and media

The following S. cerevisiae strains have been used in this study: SG76 (MATα trp1 ura3-52 Δlilv1), TG258 (MATα trp1 ura3-52 Δlilv1 Δcha4)
Chromatin analysis

Micrococcal nucleases and DNase I-based mapping of nucleosome organization was carried out essentially as described (Savare et al., 1985). Cells from 11 yeast cultures (5×10^6 cells) were pelleted, washed in cold water and 1 M Sorbitol, and resuspended in 5 ml lysis solution (1 M Sorbitol, 5 mM EDTA, 0.25 M EGTA, 0.25 M EDTA) per 1 g of cells (wet weight). Incubation was carried out with slight agitation for 20 min at 30°C. The supernatants thus obtained were washed in ice-cold 1 M Sorbitol and resuspended in 7 ml Ficoll solution (1 M Sorbitol, 5 mM 2-mercaptoethanol) containing 2 mg of Zymolyase 39010. Cells were incubated at 55°C for 2 h. DNA was purified by two rounds of phenol-chloroform extraction and ethanol precipitation.

Indirect end-labeling analysis

After secondary digestion with the appropriate restriction enzyme, the treated samples were electrophoresed in 1.5% agarose gels in 1× TBE, transferred onto Positive™ nylon membranes (Oncor, Gaithersburg, MD) and hybridized following standard protocols.

Northern analysis

Total RNA was isolated from untreated nuclei using Qiagen RNeasy Total RNA Kit according to the manufacturer’s instructions (Qiagen, Germany). Ten micrograms of RNA per sample were loaded onto a 1.4% agarose formaldehyde gel and electrophoresed in 1× MOPS, transferred onto Positive™ nylon membranes and hybridized following standard protocols.

Radiolabeling of probes

Oligonucleotides were synthesized at Synmobil (Copenhagen, Denmark). The following primer sets were used: URA3 gene set, URA3BIO 5′-CCTGCGAAGAAAGAGATAA-3′ and URA3 5′-TTTTTGAGCACTATATGTTCA-3′; CHAI1 gene set, CHAI1BIO 5′-AGGAACCCCCGTCACGAAAGGATGTG-3′ and CHAICS 5′-TAAAACCACCTCCCAACACGGTCG-3′; and CHAI1 promoter set, CHAIUP-BIO 5′-CCGAGAAAGACCGGTTTTCTTCA-3′ and CHAIUP 5′-CCGAGATATCACTCTTAGGTTG-3′.

Labeling was carried out according to a modification of a previously described procedure (Espelund et al., 1993). Biotinylated DNA was made by PCR using 50 ng yeast genomic DNA as template and one of the above-described primer sets with 30 cycles of 1 min 94°C, 30 s 55°C, 1 min 72°C (Robocycler Gradient96, Stratagene). The biotinylated PCR product was bound to streptavidin magnetic beads (Dynabeads M-280 Streptavidin, Dynal, Norway), washed and the non-biotinylated strand was removed by two cycles of alkali denaturation. After washing twice in H₂O, the template was labeled with [α-³²P]-dCTP by a standard primer extension reaction with the non-biotinylated primer of the set. The bound double-stranded probe was then washed and alkali denatured. The supernatant was recovered and used in subsequent hybridizations after neutralization.

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