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# 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 ($UAS_{CHA}$) 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 $Cys_6$ 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 Δ*swi1*, Δ*swi3*, Δ*snf5* and Δ*snf6* 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 Δ*ada2*, Δ*ada3* and Δ*gcn5* 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.

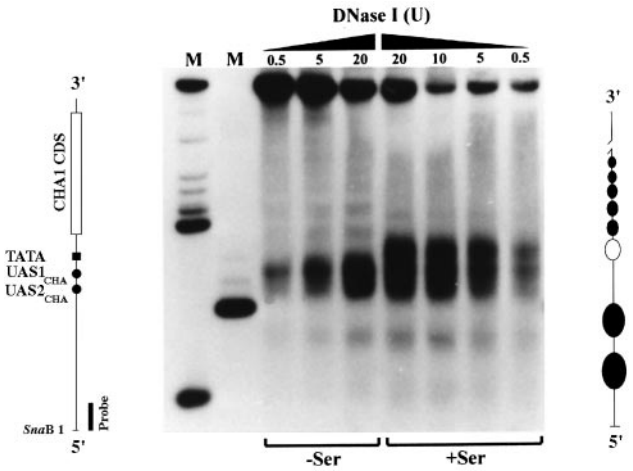


**Fig. 1.** Chromatin organization of the *CHA1* gene under non-induced (–Ser) and induced conditions (+Ser). Low-resolution analysis by digestion with DNase I. SG76 (*CHA4*) cells were grown in the absence or presence (1 g/l) of the inducer serine. Nuclei were digested for 10 min with 0.5, 5, 10 and 20 U/ml DNase I. DNA was isolated, digested with *Sna*BI, separated on a 1% agarose gel, blotted and hybridized with a labeled PCR amplificate obtained with the *CHA1* promoter set of primers. Nucleosomes (seen as protected areas) are pictured at the right as filled ellipses. The single open ellipse denotes a nucleosome remodeled upon induction. Lanes M contain restriction enzyme double digests of genomic DNA with *Sna*BI and *Eco*RI, *Cla*I or *Hpa*II, to generate position marker fragments. The vertical map at the left indicates the relative positions of the various *cis*-acting sequences and the *CHA1*-coding sequence.

## 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, $UAS1_{CHA}$ (positions –240 to –214) and $UAS2_{CHA}$ (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 repressed or derepressed SG76 (*CHA4*) cells. The results obtained with DNase 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 ($UAS1_{CHA}$ and $UAS2_{CHA}$) (Bornæs *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

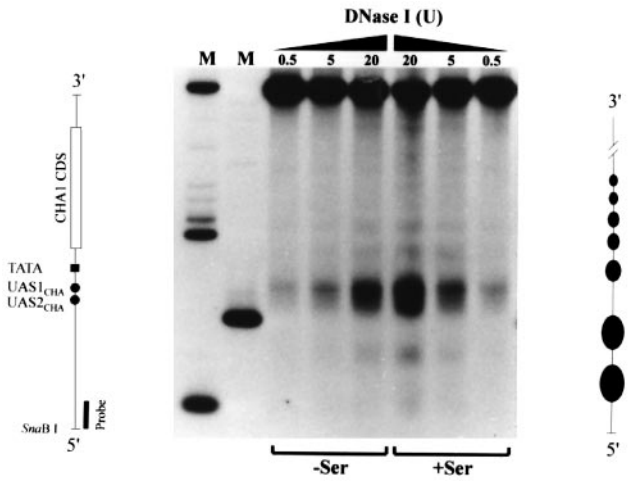


**Fig. 2.** DNase I analysis of the *CHA1* gene in a strain lacking the activator Cha4p. TG258 (Δ*cha4*) cells were grown in the absence (–Ser) or presence (+Ser) of serine (1 g/l). Chromatin analysis was performed as indicated in the legend to Figure 1.

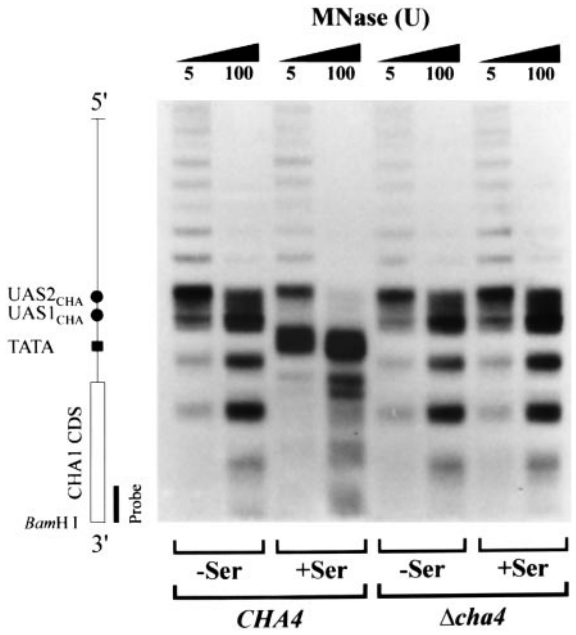


**Fig. 3.** MNase low-resolution analysis of the *CHA1* gene. SG76 (*CHA4*) and TG258 (Δ*cha4*) cells were grown in the absence (–Ser) or presence (+Ser) of serine as inducer (1 g/l). Nuclei were digested for 10 min with 5 and 100 U/ml MNase. DNA was isolated, digested with *Bam*HI, separated on a 1% agarose gel, blotted and hybridized with a labeled PCR amplificate obtained with the *CHA1* gene set of primers. The vertical map indicates the relative positions of the various *cis*-acting sequences and the *CHA1* coding sequence.

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 (Δ*cha4*) 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 (Δ*cha4*) 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, Δ*cha4* 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 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.*,

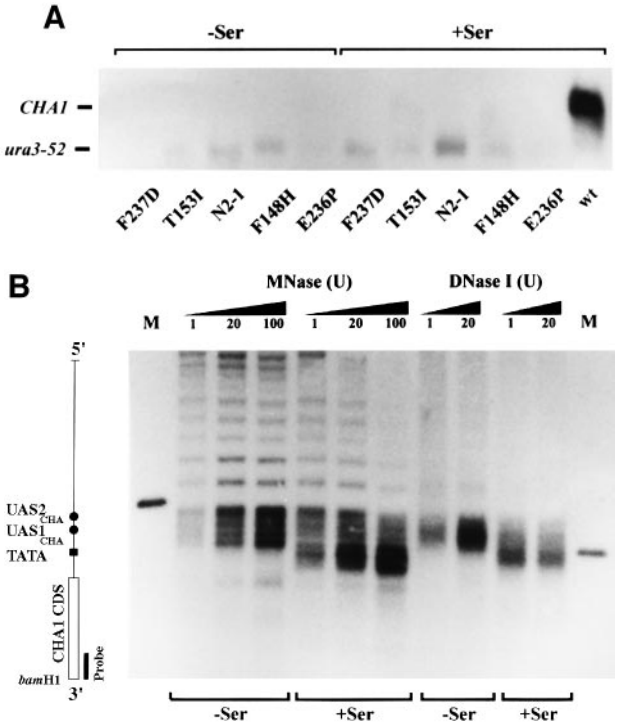


**Fig. 4.** Transriptional activity and chromatin remodeling of the *CHA1* gene in TBP derivatives. (**A)** Northern analysis of TBP mutant strains (F237D, T153I, N2-1, F148H and E236P) under non-induced (–Ser) and induced (+Ser) conditions. Ten micrograms of total RNA isolated from undigested nuclei was electrophoresed in a 1.5% formaldehyde agarose gel, blotted and hybridized with labeled PCR amplificates obtained with the *CHA1* gene set of primers and the *URA3* gene set of primers, respectively. RNA from a wild-type (wt) control sample (SG76) was included for comparison. (**B)** Chromatin analysis of the *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 *Bam*HI, separated on a 1% agarose gel, blotted and hybridized with a labeled PCR amplificate obtained with the *CHA1* gene set of primers. Lanes M contain restriction enzyme double digests of genomic DNA with *Bam*HI, and *Cla*I or *Hin*dIII, to generate position marker fragments. The vertical map indicates the relative positions of the various *cis*-acting sequences and the *CHA1*-coding sequence.

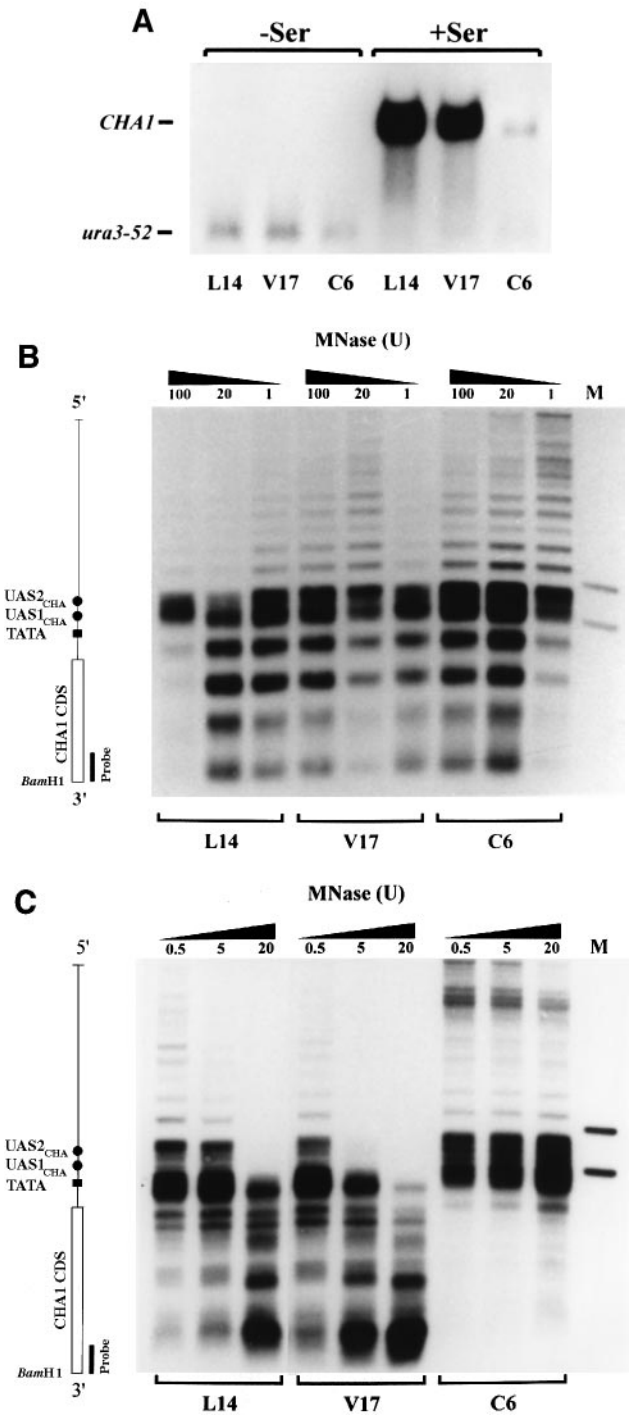


**Fig. 5.** Transcriptional activity and chromatin remodeling of the *CHA1* gene in RNA pol II CTD truncation mutants. (**A)** Northern analysis of the wild-type CTD (L14) and truncation mutants (V17 and C6) in non-induced (–Ser) and induced (+Ser) conditions. Northern analysis was performed as described in the legend to Figure 4A.
(**B** and **C)** Chromatin analysis of the *CHA1* gene in the CTD truncation mutants under non-induced (B) and induced (C) conditions. MNase analysis was performed as described in the legend to Figure 4B.

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 *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 *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), *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 *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 *CHA1* promoter are not affected to any noticeable degree in the CTD truncation mutants.

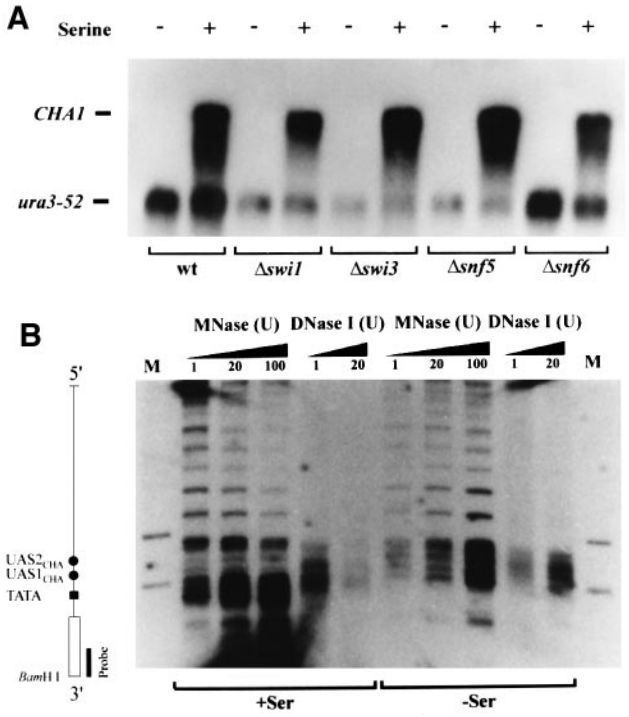


**Fig. 6.** Transcriptional activity and nucleosomal structure of the *CHA1* gene in SWI/SNF mutants. Northern blot analysis (**A)** of the various SWI/SNF mutants (Δ*swi1*, Δ*swi3*, Δ*snf5*, Δ*snf6* and an isogenic wild-type strain) was carried out as described in the legend to Figure 4A. (**B)** Chromatin structure of the *CHA1* gene in a Δ*snf5* strain was analyzed by DNase I and MNase digestion as in Figure 4B.

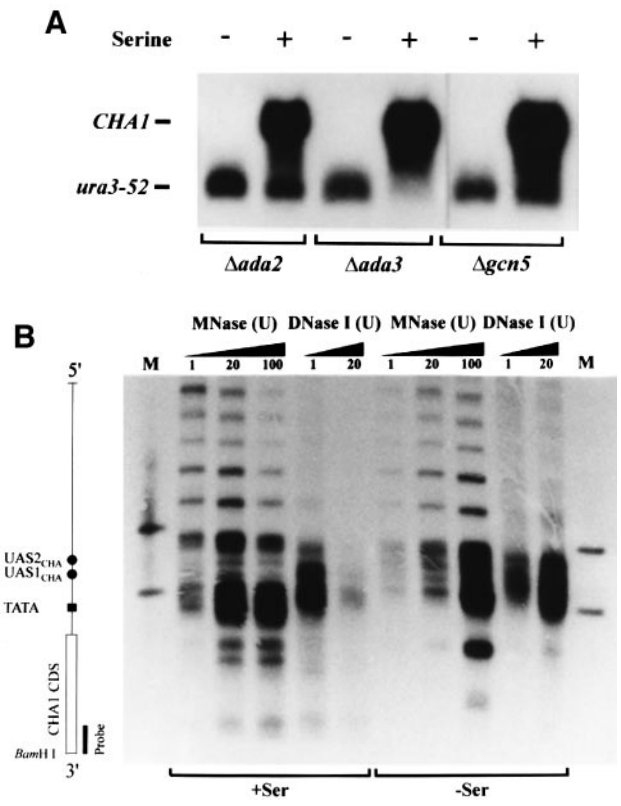


**Fig. 7.** Pattern of expression and chromatin structure of the *CHA1* gene in ADA/GCN5 mutants. (**A)** Northern analysis of Δ*ada2*, Δ*ada3* and Δ*gcn5* mutant strains as in Figure 4A. (**B)** The chromatin structure of the *CHA1* gene in the Δ*gcn5* strain was analyzed by DNase I and MNase digestion 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; Horiuchi *et al.*, 1997; Saleh *et al.*, 1997). Ada2p, Ada3p and Gcn5p have also been shown to be subunits of a heteromeric complex (Horiuchi *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 Δ*ada2*, Δ*ada3* and Δ*gcn5* 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.

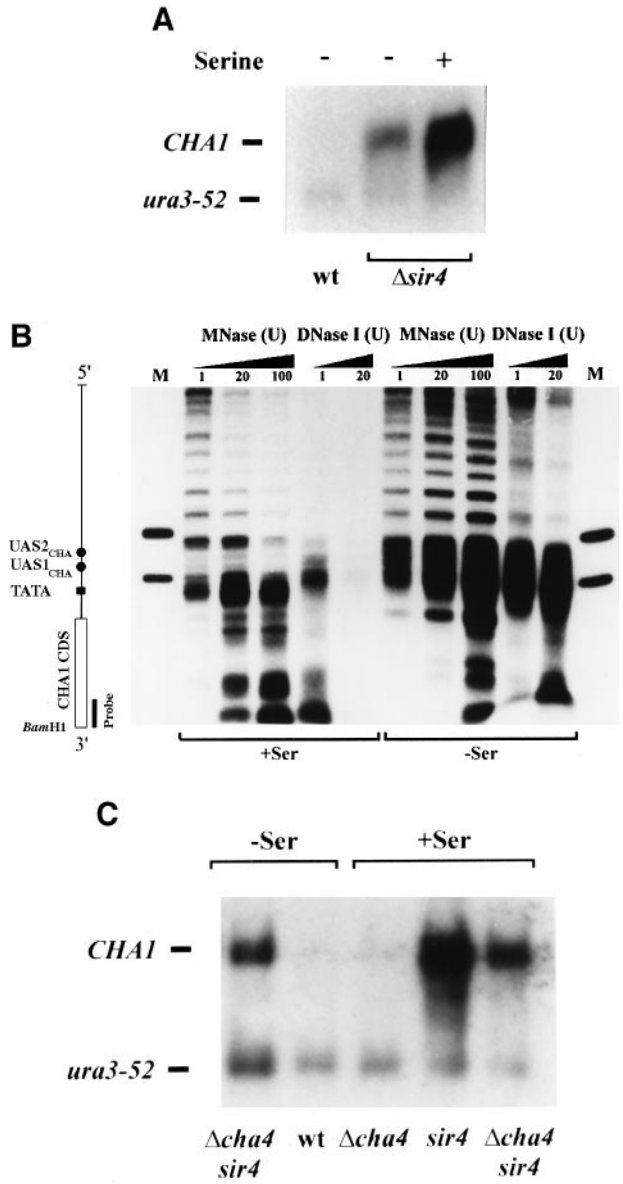


**Fig. 8.** Expression levels and chromatin structure of the *CHA1* gene in a *sir4* disruption strain. (**A)** Northern analysis of a *sir4::HIS3* and an isogenic wt strain was performed as described in Figure 4A. (**B)** The chromatin structure of the *CHA1* gene in this strain was analyzed by DNase I and MNase digestion as described in Figure 4B. (**C)** Northern analysis of Δ*cha4 sir4::HIS3*, *sir4::HIS3*, Δ*cha4* and wt strains was performed as described in Figure 4A.

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

## Discussion

### *Chromatin transitions at the CHA1 locus*

The results obtained with nuclease digestion of the *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 *cis*-acting sequences are located within this hypersensitive site (Bornæs *et al.*, 1993), suggesting that the *trans*-acting factors required for proper regulation of the *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, *CHA4* –Ser and +Ser), although it maintains its accessibility to DNase I (compare for example Figure 1, +Ser with Figure 3, *CHA4* +Ser). This peculiar change has been observed in other genes, namely in the UAS of the *GAL1-10* genes (Lohr and Hopper, 1985) and in the regulatory region of the *SUC2* gene (Perez-Ortin *et al.*, 1986, 1987) under derepressed conditions. In the *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 *CHA1* under repressed and derepressed conditions (Figure 9). In the repressed state a constitutive hypersensitive site exists, comprising the UASs with an ordered nucleosome array covering the coding region and a positioned nucleosome over the TATA box (nuc-1). Upon induction, the discrete band pattern observed for the coding region is lost and becomes diffuse and smeary, a change also observed in other genes in the active state and characteristic of active genes (Wu *et al.*, 1979; Lee and Garrard, 1991; Vincenz *et al.*, 1991; del Olmo *et al.*, 1993). Furthermore, upon activation the nucleosome

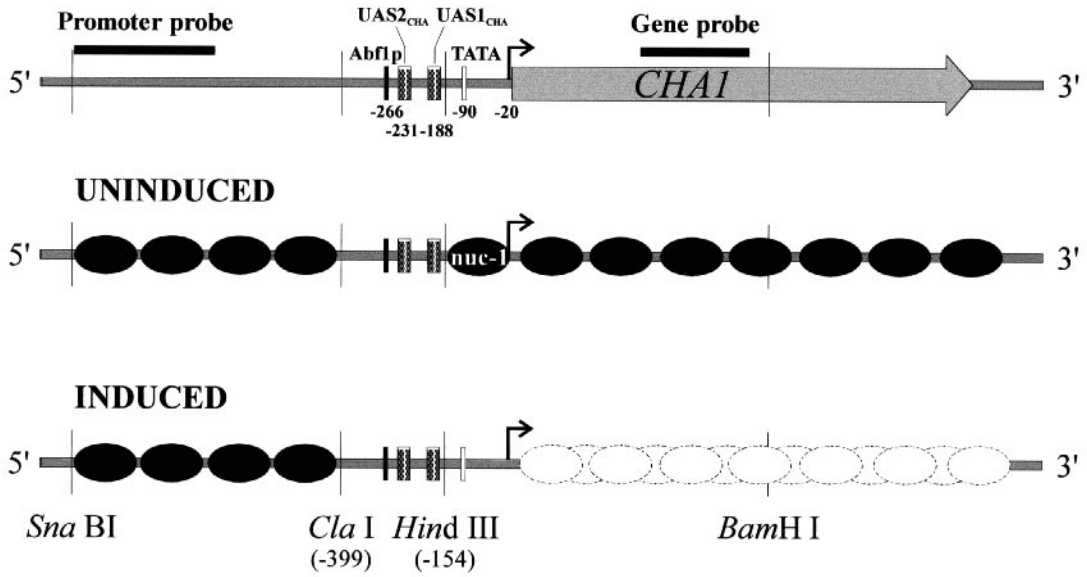


**Fig. 9.** A model for the chromatin structure of the yeast gene *CHA1* in the induced and non-induced states. Positioned nucleosomes are depicted by closed ellipses. The overlapping open ellipses depict the situation found in the active gene, where DNA is nucleosomal without clear positioning. Fragments used as probes and positions of relevant restriction sites and *cis*-acting sequences are shown. Thus, the Abf1p-binding site is represented by a filled rectangle, $UAS_{CHA}$ by half-filled rectangles and the TATA element by an open rectangle.

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 $UAS_{CHA}$ 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 Hörz, 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(s), but when artificially 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 TFIID 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–independent manner (Gaudreau *et al.*, 1997). Thus, one could envision an alternative mechanism whereby alteration of chromatin structure in the *CHA1* promoter might reflect the action of an one-step recruitment of the holoenzyme. Such a process would entail recruitment of the holoenzyme in a TFIID-independent manner, and, as the CTD can bind to TBP (Usheva *et al.*, 1992), the holoenzyme would 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 counteracting 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, Δ*cha4 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 Δ*cha4 sir4* double mutant strain (Figure 8C, cf. Δ*cha4 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 (*MAT*α *trp1 ura3-52* Δ*ilv1*), TG258 (*MAT*α *trp1 ura3-52* Δ*ilv1* Δ*cha4*)

(Holmberg and Schjerling, 1996), SG115 (*MAT*α *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*), TG325 (*MAT*α *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sir4::HIS3*) and TG 325-C {*MAT*α *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sir4::HIS3* Δ*cha4*). Strains L14 {*MAT*α *his3-Δ200 leu2-3 rpb1Δ187::HIS3 ura3-52* [*pL14*(*LEU2 rpb1Δ100*)]}, V17 {*MAT*α *his3-Δ200 leu2-3 rpb1Δ187::HIS3 ura3-52* [*pV17*(*LEU2 rpb1Δ115*)]} and C6 {*MAT*α *his3-Δ200 leu2-3 rpb1Δ187::HIS3 ura3-52* [*pL14*(*LEU2 rpb1Δ104*)]} were a gift of R.A.Young (Liao *et al.*, 1991). TBP mutant strains N2-1, F237D, E236P, T153I and F148H were kindly provided by K.Struhl (Stargell and Struhl, 1995, 1996a). Strains CY26 (*MAT*α *his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ1 lys2-801 ade2-101*), CY58 (*MAT*α *his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ1 lys2-801 ade2-101 swi1Δ::LEU2*), CY72 (*MAT*α *his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ1 lys2-801 ade2-101 swi3*Δ), CY332 (*MAT*α *his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ1 lys2-801 ade2-101 snf6*Δ) and CY335 (*MAT***a** *his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ1 snf5*Δ) were a gift of C.L.Peterson. Strains GMY27 (*MAT***a** *ade2 ura3 leu2 lys2* Δ*gcn5::hisF*), PSY316 (*MAT*α *ade2-101 leu2-3,112 his3-Δ200 ura3-52 lys2 GAL*$^+$), PSY316Δada2 (*MAT*α *ade2-101 leu2-3,112 his3-Δ200 ura3-52 lys2 GAL*$^+$ Δ*ada2::hisG*) and PSY315Δada3 (*MAT*α *ade2-101 leu2-3,112 his3-Δ200 ura3-52 lys2 GAL*$^+$ Δ*ada3::hisG*) were kindly provided by L.Guarente. The *sir4::HIS3* derivative of strain SG115—strain TG325—was made by targeted disruption of *SIR4* using *Pvu*II-digested plasmid pJR276 as described by Kimmerly and Rine (1987). The integration was verified by Southern blotting. That derepression of the *HM* loci in strain TG325 takes place was verified by a halo assay for pheromone production using the *bar1* α-factor tester strain SG225 (*MAT***a** *leu2-3,112 gal2 bar1*). The Δ*cha4* derivative of strain TG325—strain TG325-C—was made by loop-in loop-out based deletion of *CHA4* using *Nhe*I-digested plasmid pTK329 as described by Holmberg and Schjerling (1996).

Strains were grown in minimal medium (0.67% Bacto Yeast Nitrogen Base without amino acids, 2% glucose, buffered with 10 g succinic acid and 6 g NaOH per liter) supplemented with the required amino acids at appropriate concentrations. *CHA1* induction was achieved with the addition of serine to the medium at a concentration of 1 g/l.

Restriction endonucleases and DNA-modifying enzymes were purchased from Boehringer (Mannheim, Germany). *Taq* polymerase was from Pharmacia (Amersham Pharmacia Biotech). Zymolyase 100T was from Seikagaku America, Inc. (USA). Radiolabeled nucleotides were from ICN Pharmaceuticals, Inc. (Costa Mesa, CA).

### DNA methodology

All nucleic acid manipulation was performed according to established protocols (Sambrook *et al.*, 1982). Polymerase Chain Reaction (PCR) was used under standard conditions (0.2 mM of each of dATP, dCTP, dTTP, dGTP; 20 mM Tris–HCl pH 8.4; 50 mM KCl; 5 mM $MgCl_2$; 0.5 μM of each primer; 2.5 U *Taq* DNA polymerase per reaction).

### Chromatin analysis

Micrococcal nuclease and DNase-I-based mapping of nucleosome organization was carried out essentially as described (Svaren *et al.*, 1995). Cells from 1 l yeast culture (5–10×$10^9$ cells) were pelleted, washed in cold water and 1 M Sorbitol, and resuspended in 5 ml lysis solution (1 M Sorbitol, 5 mM 2-mercaptoethanol) containing 2 mg of Zymolyase 100T per 1 g of cells (wet weight). Incubation was carried out with slight agitation for 20 min at 30°C. The spheroplasts thus obtained were washed in ice-cold 1 M Sorbitol and resuspended in 7 ml Ficoll solution (18% w/v Ficoll, 20 mM $KH_2PO_4$ pH 6.8, 1 mM $MgCl_2$, 0.25 mM EGTA, 0.25 mM EDTA) per 1 g cells (wet weight) and divided into 3 ml aliquots. Aliquots were centrifuged for 30 min at 30 000 *g* and 5°C. The nuclear pellet was washed in 5 ml digestion buffer (15 mM Tris–HCl pH 7.5, 75 mM NaCl, 3 mM $MgCl_2$, 1.5 mM $CaCl_2$, 1 mM 2-mercaptoethanol) and resuspended in 1.2 ml digestion buffer. Aliquots (200 μl) were transferred to eppendorf tubes. One aliquot was kept on ice without nuclease for subsequent RNA isolation, and MNase (0.5–100 U/ml) or DNase I (0.5–20 U/ml) was added to the remaining aliquots. Incubation was carried out at 37°C for 10 min. The reaction was terminated with 1% SDS, 5 mM EDTA (final concentration). Twenty microliters of Proteinase K per sample were added, and the samples 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$^{TM}$ 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$^{TM}$ nylon membranes and hybridized following standard protocols.

### Radiolabeling of probes

Oligonucleotides were synthesized at Symbion (Copenhagen, Denmark). The following primer sets were used: URA3 gene set, URA3BIO 5′Biotin-CCTGCAGGAAACGAAGATAA-3′ and URA3 5′-TTTTGG-GACCTAATGCTTCA-3′; CHA1 gene set, CHA1BIO 5′Biotin-ATG-AGGAACACCGGTGCCCAGGT-3′ and CHA1CDS 5′-TAAAC-CACCTCCACCAACGCTGC-3′; and CHA1 promoter set, CHA1UP-BIO 5′Biotin-CCGAGGAAGACGGTTTCTTAC-3′ and CHA1UP 5′-CCAGGATATCACTTTGAGGTTG-3′.

Labeling was carried out according to a modification of a previously described procedure (Espelund *et al.*, 1990). 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 with $H_2O$, the template was labeled with [$\alpha^{32}$P]-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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