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GCN5, a Yeast Transcriptional Coactivator, Induces Chromatin Reconfiguration of *HIS3* Promoter *in Vivo*

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Gcn5p, the nuclear histone acetyltransferase (HAT A), is a component of the multiprotein adaptor complex, *ADA*. Its role as a transcriptional coactivator is required for full induction of most of the genes regulated by *GCN4*. In this study we present experimental evidence demonstrating that, during gene activation, the nuclease sensitive region of *HIS3* promoter, harbouring the poly (dA:dT) and the *GCN4* binding site, is invaded by nucleosomes in a *gcn5* disrupted strain. These data demonstrate, for the first time, that Gcn5p affects directly the chromatin organization of a chromosomal gene during its transcriptional activation.

INTRODUCTION

In yeast, the multimeric *ADA* complex has been described as a transcriptional coactivator (5). This complex includes the products of *ADA1-5* genes (1,2,6,7) associated with Gcn5p (8). It has been recently found (9) that Gcn5p is a HAT A yeast histone acetyltransferase, homologous to the *Tetrahymena* p55 protein. Gcn5p is a modular protein with four HAT catalytic domains, the binding domain for ADA2 which is required for full histone acetylase activity and a carboxyl terminal bromodomain (10) which is not found in the cytoplasmic HAT-B form (11). The presence of the C-terminal bromodomain led us to hypothesize a specific function of Gcn5p in rearranging the chromatin structure during gene expression, recalling the function initially demonstrated for *SNF2/SWI12* (12). To test this proposition, we chose *HIS3* promoter whose transcriptional activation is highly regulated by *GCN5* (2). At least two lines of experimental evidence indicate the importance of the chromatin structure for *HIS3* regulation. In the first place, depletion of histone H4 activates *HIS3* transcription to nearly fully induced levels (13). Secondly, it has been shown that the poly (dA:dT) stretch, upstream to the TATA box, is nucleosome free (14, 15) and is important for an efficient activation of the gene. In order to analyze the contribution of Gcn5p on the chromatin structure during gene activation we performed an *in vivo* study of the chromatin structure of *HIS3* in a wild type and in a *gcn5* disrupted strain. In this paper we show that, in Δ*gcn5*, the chromatin structure of *HIS3* promoter is altered during gene activation and that the promoter region (16,17), previously described as nuclease free (14, 15), is protected by a mispositioned nucleosome.

MATERIALS AND METHODS

Yeast strains and growth conditions. Strains were grown at 28°C in (SD) minimal medium containing 0.67% yeast nitrogen base (Difco) and 2% glucose plus the required supplements. The *gcn5* disrupted strains were obtained by subcloning the *GCN5* internal *AatI-BamHI* 751bp restriction fragment in the YIp5 integrative vector. 10μg of YIp5-Δ*gcn5* linearized with *EcoRI* were utilized to obtain *GCN5* disruption in a wild type, LA1: *MATa, ura3* strain. Yeast transformation was performed either by spheroplasting (18) or by electroporation (19) protocols. Disruption was confirmed by Southern blot analysis (20).

Northern (RNA) analysis. Northern analysis was carried out as previously described (21). A 224 bp and a 195 bp BamHI restriction fragments were used as probes for sequential hybridization of *HIS3* (22) or actin (23).

Chromatin analysis with micrococcal nuclease on nystatin-treated yeast cells Yeast spheroplasts from exponentially growing cells (0.3-0.5 OD) were essentially prepared as previously described. Chromatin preparations were performed following the nystatin method (24), this preparation was then subjected to micrococcal nuclease (MNase) digestion S7-nuclease (Boeringher). Permeabilized cells were digested at increasing nuclease concentration (0-100 U/ml) for 20 min. at 37°C. After extraction, all DNA samples were digested with *BamHI* for the end-labelling experiments, naked DNA was extracted, digested *in vitro* with MNase (0.2 U/ml) for 3 min. at 37°C. Gel-electrophoresis, Southern-blot and hybridizations were essentially carried out following the standard procedures. End-labelling experiments were probed with the PCR amplified actin 195 bp *BamHI* fragment and the 224 bp *BamHI HIS3* fragment (24). The DNA

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concentration was first calibrated by O.D. and by spot test and then loaded on 1.5% agarose-gel, electrophoresed and hybridized in formamide at 42°C.

Mononucleosome preparation. Extensive digestion with micrococcal nuclease was performed on nystatin treated spheroplasts ($\sim 1 \cdot 10^8$ cells/ml). Cells were grown under starvation conditions SD+(3AT 10mM) for 5.5 hrs. Mononucleosomes were digested with MNase (30-100 U/ml) and the reaction was controlled by ethidium bromide staining. After blotting, the mononucleosomes were sequentially hybridized with probes obtained by PCR amplifications of specific *HIS3* promoter regions: I°(-178/-83), II°(-5/+95), III°(+100/+178).

RESULTS AND DISCUSSION

Micrococcal Nuclease Analysis of His3 in Wild Type and in Gcn5 Disrupted Strains

A *gcn5* disrupted strain was prepared as described in Materials and Methods. Northern blot analysis and growth curves of the wild type strain and the *gcn5* disrupted strain showed that this strain displayed both a reduced transcriptional activation of *HIS3* and a significantly slower growth, as compared to the wild type parent strain (data not shown). Considering that it has been found that *GCN5* encodes a histone acetyltransferase (10), we decided to analyse whether Gcn5p played a role in chromatin organization during active transcriptional conditions (amino acids starvation). Chromatin preparations from wild type and Δ*gcn5* strains were digested *in vivo* with micrococcal nuclease. No differences in *HIS3* chromatin accessibility were found when strains were grown under conditions that did not induce transcription (data not shown). On the other hand, a different MNase sensitivity is shown in Δ*gcn5* strain when *HIS3* chromatin organization was analyzed under starvation conditions (growth on SD supplemented with 10mM 3-AT for 5.5 hrs) (Fig.1A). Actin was chosen as an internal standard of the degree of MNase digestion, since both *HIS3* and actin have a *Bam*HI restriction site located at the same distance from the transcriptional start site. In order to compare the wild type and mutant strains, the third lanes of both *HIS3* and actin hybridization patterns (Fig.1A) were compared by densitometric scanning. The hybridization signal over *HIS3* was weaker in the Δ*gcn5* strain; this effect was particularly pronounced around the hypersensitive sites. These data suggest that in Δ*gcn5* mispositioned nucleosomes would protect the promoter from MNase digestion. These results, together with the unmodified chromatin patterns observed in non-activated growing conditions (data not shown), suggest that the effect of Gcn5p on chromatin structure is not a prerequisite for basal transcription but is important for chromatin remodelling during transcriptional activation.

By this low resolution analysis we have observed a differential sensitivity of the *HIS3* locus to MNase digestion between the wild type and the disrupted Δ*gcn5* strains (Fig1A). This was particularly evident in the region containing the poly (dA:dT) and the *GCN4* binding site. In the same experiment, the analysis of the actin locus confirmed that this chromatin effect was directed, in particular, to Gcn5p regulated genes.

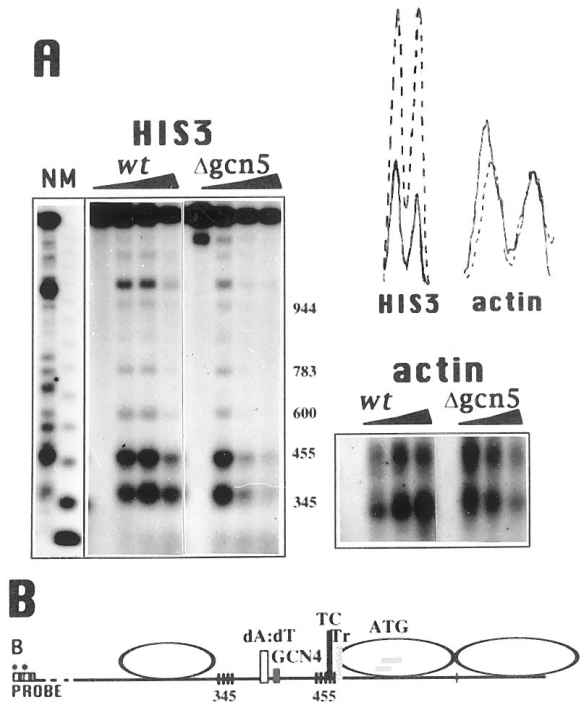

FIG. 1. Indirect end labelling analysis of *HIS3* promoter under amino acid starvation in wild type and Δ*gcn5* mutant strains. (**A**) Chromatin preparations of wild type and Δ*gcn5* strains were digested at increasing MNase concentrations, digested with *Bam*HI and sequentially hybridized with *HIS3* (224 bp *Bam*HI fragment) and actin (195 bp *Bam*HI fragment) probes. M, 123bp molecular size marker; N, naked DNA, *in vitro* digested with MNase. The actin panel is aligned with the *HIS3* hypersensitive region. Densitometric scanning of the wild type and the Δ*gcn5* third lane of MNase digestion: (---, LA1, —, Δgcn5). (**B**) Diagrammatic representation of HIS3 low resolution (+/-20bp) nucleosome (ellipsoid) mapping.

MONONUCLEOSOMAL DNA STRUCTURE

In order to better define the structure of the regulatory region we carried out the hybridization analysis over chromatin fully digested to mononucleosomal structure. This experimental approach excludes artifacts related to dishomogeneity in the degree of digestion and allows to distinguish whether a specific region is localized in a linker, in accessible regions or is wrapped in a nucleosome. Extensive MNase digestion converted chromatin from 3-AT treated cells into a homogeneous population of mononucleosomes. In order to avoid mononucleosome degradation the assay was carefully calibrated. The same filter was sequentially rehybridized with PCR amplified fragments selected on the basis of the low resolution nucleosome mapping (Fig.1B): probe I° hybridized with the regulatory sensitive region, probe II° with the inter-

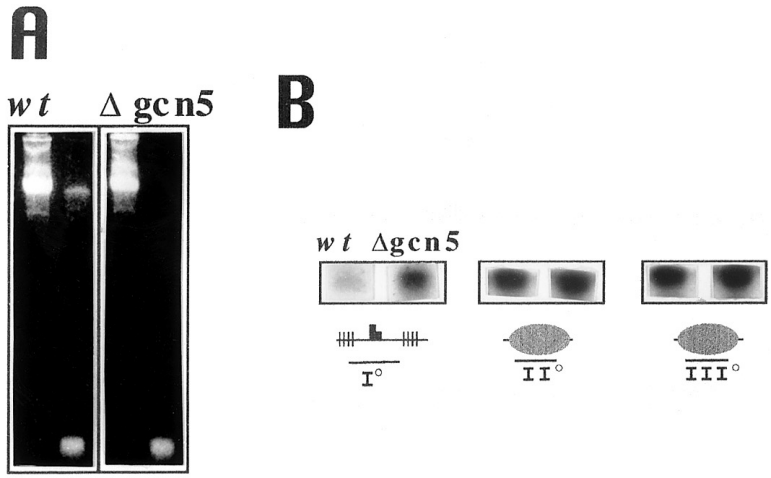

FIG. 2. Wild type and Δ*gcn5* mononucleosome preparation. Equal amounts of monomer preparation were electrophoresed and sequentially hybridized with PCR amplified probes: I°(−178/ −83), II°(−5/+95), III°(+100 / +178). (A) Ethidium bromide staining. (B) Sequential rehybridization of the mononucleosomal bands with the diagrammed probes (ellipsoids: nucleosome).

nal region of the nucleosome at the transcriptional start site, and probe III° with a nucleosome covering a coding region. The ethidium bromide staining of the overall pattern of MNase digestion and a summary panel showing the comparative analysis of sequential hybridizations over the mononucleosomal bands are presented in Figure 2, respectively A and B. This experiment shows that, in the wild type strain, the sensitive promoter region described by Losa et al., (1990) (15) was accessible to MNase degradation (Fig. 2B, probe I°), while in Δ*gcn5*, nucleosomal sized protection was detected. The residual signal in the wild type strain was probably due to the fact that, although MNase digestion was intended to be complete, a small amount of partial digests should be expected in order to prevent degradation of mononucleosomes. Hybridization with the internal nucleosome probes II° and III° was similar in both strains, and provided an optimal internal standard of the degree of chromatin digestion. Considering these results, we suggest that Gcn5p plays a direct role in keeping promoter regions in an uncondensed structure during *HIS3* activation. We might hypothesize that this effect is correlated to acetylation of specific nucleosomes in the promoter.

Histone acetylation has become a central clue in the understanding of gene expression. The increased interest in histone acetylation and deacetylation has focused also on the role of these processes in transcription (25). In this study we provide the first, direct evidence of the *in vivo* effect of the yeast Gcn5p histone acetyltransferase on chromatin structure.

We propose that when *GCN5* is impaired, nucleosomes can be positioned over the open sensitive region (15) producing a chromatin barrier which hinders the access of activator molecules, and thus lowers *HIS3* transcriptional activation upon starvation (Fig.3). Our study focuses the attention on the role exerted by Gcn5p directly on the chromatin structure over *HIS3* regulatory regions, facilitating or establishing a transcriptional competent state through the maintenance of an open chromatin conformation. In a GCN5 disrupted strain, hypoacetylated nucleosomes might stall on regulatory regions producing a structural barrier for the transcriptional machinery. Gcn5p seems to be therefore a fundamental link between acetylation and chromatin during gene activation.

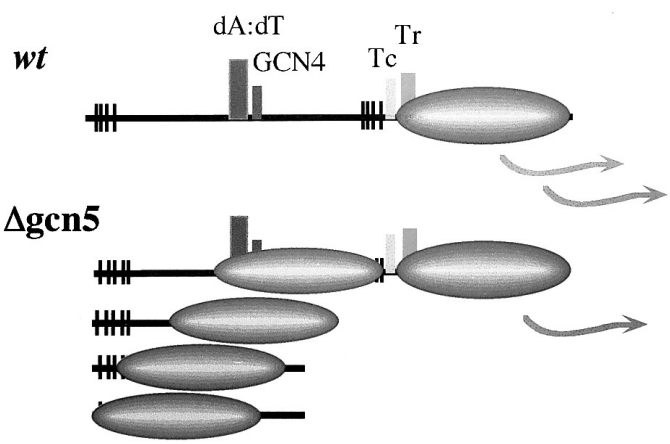

FIG. 3. Our model proposes that the effect of Gcn5p is exerted over a specific *HIS3* promoter region during transcriptional activation. In the wild type strain this region spanning the poly (dA:dT) stretch and the *GCN4* binding site is nuclease sensitive and nucleosome free. On the contrary, in Δ*gcn5* mutant strain, nucleosomes attain an altered positioning, giving rise to a population of MNase protected molecules (dark ellipsoids: nucleosome).

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