Histone H1 of *Saccharomyces cerevisiae* Inhibits Transcriptional Silencing

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**ABSTRACT**

Eukaryotic genomes contain euchromatic regions, which are transcriptionally active, and heterochromatic regions, which are repressed. These domains are separated by “barrier elements”: DNA sequences that protect euchromatic regions from encroachment by neighboring heterochromatin. To identify proteins that play a role in the function of barrier elements we have carried out a screen in *S. cerevisiae*. We recovered the gene *HHO1*, which encodes the yeast ortholog of histone H1, as a high-copy modifier of barrier activity. Histone H1 is a linker histone that binds the outside of nucleosomes and modifies chromatin dynamics. Here we show that Hho1p reinforces the action of several types of barrier elements, and also inhibits silencing on its own.

**MATERIALS AND METHODS**

**Plasmids:** The high-copy library we used contains yeast genomic DNA inserted into the LEU2-marked vector Yep13.
It was built in the Nasmyth lab and kindly made available to us by Etienne Schwob. Library plasmid p133 was digested with BamHI, filled in, and religated to generate plasmid p134, in which there is a frameshift in HHO1. Both reporter genes are truncated on plates lacking leucine and histidine. Individual transformants were picked and grown to saturation in 250 µl of selective medium in 96-well culture plates. Ten microliters of a 20-fold dilution of each culture was spotted in duplicate on SC-HL as well as on SC-HLW/FOA. Candidates with an increased or decreased proportion of TRP+ FOAr cells were picked on the SC-HL plate and retested. The plasmids were rescued from the candidate strains by smash recombination. Yeast strains used in this study are listed in Table 1.

**Yeast strains:** GF97 is a derivative of W303-1a that has already been described (FOUREL et al. 1999). This strain contains a silencing reporter construct integrated at telomere VIII by recombination with the ADH1 gene. The reporter construct contains, closest to ADH1, an 850-bp fragment containing the TRP1 gene, linked to a 150-bp fragment containing four Gal4p binding sequences (UASGal4), and finally a 1.3-kb promoter and terminator amplified by PCR and cloned into Yep13. This expression vector for Gal4p binding sequences (UASGal4) was amplified by PCR and used for Western blotting (dilution 1:5000) and chromatin immunoprecipitation. Sir4p was tagged with nine copies of the Myc epitope by PCR and homologous recombination. Yeast strains used in this study are listed in Table 2.

**Generation of anti-Hho1p serum:** We synthesized two 15-amino acid peptides matching the sequence of Hho1p: APKKSTTKTTSKGKK and CVENGELVQPKGPSG. They were amino acid peptides matching the sequence of Hho1p: APKKSTTKTTSKGKK and CVENGELVQPKGPSG. They were coupled to keyhole limpet hemocyanin (KLH) and used to immunize two rabbits (Eurogentec, Seraing, Belgium). Anti-Sir4p antibodies contained in the final bleed were affinity purified and used for Western blotting (dilution 1:5000) and chromatin immunoprecipitation.

**TABLE 2**

<table>
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<tr>
<th>Strain</th>
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<tr>
<td>W303-1a</td>
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<td>GF97</td>
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<td>FOUREL et al. (1999)</td>
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<td>H2</td>
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<td>H10</td>
<td>YXB476 HML::LexAOPp12::URA3</td>
<td>Bt et al. (2004)</td>
</tr>
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<td>H10'</td>
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<td>CUPERUS and SHORE (2002)</td>
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**TABLE 1**

<table>
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<th>Plasmid</th>
<th>Description</th>
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<tr>
<td>p8</td>
<td>Expression of Gal41-147 under ADH1 promoter; 2µ, HIS3</td>
<td>DEFOSSEZ et al. (2002)</td>
</tr>
<tr>
<td>p36</td>
<td>CTCF61-728. Fused to Gal41-147 in p8</td>
<td>DEFOSSEZ et al. (2002)</td>
</tr>
<tr>
<td>YEp13</td>
<td>2µ, LEU2</td>
<td>Kim Nasmyth</td>
</tr>
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<td>p13</td>
<td>3.2-kb genomic DNA fragment containing HHO1, in YEp13</td>
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<td>p134</td>
<td>p133 digested with BamHI, filled in, and religated.</td>
<td>This work</td>
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<tr>
<td>p172</td>
<td>HHO1 with natural promoter and terminator amplified by PCR and cloned into YEp13.</td>
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**RESULTS**

A yeast screen for high-copy modifiers of barrier activity identifies histone H1: To identify yeast proteins involved in transcriptional insulation we designed a genetic screen. It involved a previously described yeast strain in which barrier activity can be monitored (FOUREL et al. 2001). This strain, GF97, has two reporter genes, TRP1 and URA3, integrated close to the left telomere on chromosome VII and separated by four upstream activation sequences (UAS) that can recruit Gal4p (Figure 1A). The two reporter genes are subject to telomeric position effect, and their expression is repressed in most cells in a population. However, repression is not absolute and the genes can be expressed in some cells. Cells in which TRP1 is expressed while URA3 is repressed can be detected by their ability to form colonies on medium lacking tryptophan and containing 5-fluoro-orotic acid (FOA), a drug toxic to cells expressing URA3.

Chromatin barriers can block the spreading of repressive chromatin (DHILLON and KAMAKAKA 2002). If a peptide with barrier activity is fused to the DNA-binding domain of Gal4p and expressed in strain GF97, TRP1 can be shielded from the telomeric repression that acts on URA3. This results in an increase in the number of TRP+/FOA− cells, which can then represent as much as 10–20% of the population (FOUREL et al. 2001). Using this assay we have found that the vertebrate insulating protein CTCF can also act as an insulator in S. cerevisiae (DEFOSSEZ and GILSON 2002). To identify yeast proteins involved in this phenomenon we undertook a screen for high-copy modifiers of insulation by CTCF. A C-terminal domain of chicken CTCF (amino acids 641–728) was fused to Gal41–147 to form the chimera Gal4-ID (insulation domain), which was expressed in strain GF97. Gal4-ID has relatively weak barrier activity and made only ~1% of the cells TRP+/FOA− (DEFOSSEZ and...
We then transformed this strain with a library of yeast genomic DNA fragments cloned in a multicopy vector. Individual transformants were picked, grown in 96-well plates, and spotted on selective plates to determine total cell number as well as the number of TRP+/FOAr cells. In the 2500 clones examined we found 11 plasmids that increased the fraction of TRP+/FOAr cells and 7 that decreased it. Plasmid p133 gave a strong increase in barrier activity and was selected for further work, which is reported here.

Plasmid p133 contains a 3.2-kb fragment of yeast chromosome XVI (coordinates 307660–310860). It encompasses HHO1, the gene encoding histone H1, as well as portions of the genes NAN1 and TBF1 (Figure 1B). We first tested whether overexpression of HHO1 was necessary to increase barrier activity. We modified p133 to create a frameshift after amino acid 62 in Hho1p, yielding a truncation that removes three-quarters of the protein. The resulting construct had no effect on barrier activity (Figure 1B), establishing that HHO1 was indeed necessary for the effect of plasmid p133. We then examined whether overexpression of HHO1 was in itself sufficient to increase insulation by the chimera. We cloned HHO1 with its promoter and terminator into the library plasmid. This construct fully recapitulated the effect of p133. From this we conclude that overexpression of yeast histone H1 is sufficient to enhance insulation by Gal4-ID. Next, we deleted the endogenous HHO1 gene in GF97. The barrier activity of Gal4-ID was weaker in Δhho1 than in a wild-type strain (Figure 1C). This shows that endogenous histone H1 contributes to insulation by Gal4-ID. Therefore, yeast histone H1 is a dose-dependent enhancer of insulation by Gal4-ID.

An alternative explanation for these results would be that HHO1 acts directly on the reporter genes to increase TRP1 expression and/or decrease URA3 expression. We undertook four control experiments to examine this possibility. First, we determined the effect of HHO1 overexpression in the absence of the Gal4-ID protein. No TRP+/FOA-resistant cells appeared under these conditions (see Figure 3A, left). Second, we used a strain in which the positions of TRP1 and URA3 were reversed (Figure 2A). TRP1 was partially silenced in this strain, and overexpression of HHO1 did not alleviate silencing. The proportion of FOA-resistant cells was also unaffected. As expected, no cells were simultaneously TRP+ and FOA-resistant in this strain, and no such cells appeared after HHO1 overexpression. Third, we eliminated telomeric silencing in strain GF97 by deleting SIR4. In this context, overexpression of HHO1 had no effect on the expression of TRP1 or URA3 (Figure 2B). Fourth, we asked whether the effect of HHO1 deletion observed in Figure 1C might be due to a general change in telomere position effect. To address this question, we deleted HHO1 in GF97 and monitored reporter gene expression (Figure 2C). The deletion did not affect TRP1 or URA3 expression. This result is consistent with earlier findings that failed to detect an effect of HHO1 deletion on the silencing of subtelomeric reporter genes (Escher and Schaffner 1997; Patterson et al. 1998). From this set of experiments we conclude that HHO1 does not act directly on the reporter genes, nor does it affect telomeric silencing. Rather, HHO1 stimulates the activity of the artificial barrier.

Overexpression of histone H1 increases insulation by Gal41–147: After finding that histone H1 increased the barrier activity of Gal4-ID, we carried out additional control experiments. First, we tested whether the effect of the histone required the presence of the Gal4-ID protein. There were no TRP+/FOA-resistant cells in GF97 in the absence of Gal4-ID, and overexpression of histone H1 in that context did not increase barrier activity (Figure 3A, top). Next, we tested whether Hho1p would have an effect on Gal41–147 in the absence of the fused insulation domain. We expressed Gal41–147 in GF97. This did not generate any detectable insulation (Figure 3A, bottom left). However, overexpression of histone H1 did not act directly on the reporter genes or affect telomeric silencing. (A) HHO1 overexpression has no effect on a reporter strain in which TRP1 and URA3 are swapped. Serial 10-fold dilutions of yeast cultures transformed with the indicated plasmid were spotted on selective plates as in Figure 1. (B) HHO1 overexpression has no effect in a sir4Δ strain that lacks telomeric silencing. (C) Deletion of HHO1 does not cause loss of telomeric silencing in strain GF97.
H1 in the presence of Gal41–147 gave rise to a large number of TRP1/FOAr cells. This suggests that Hho1p stimulates the barrier activity of Gal4-1D simply by acting on the Gal41–147 moiety. The DNA-binding domain of LexA has been shown to have barrier activity (Bi et al. 2004), but to our knowledge this is the first report that the DNA-binding domain of Gal4p also has barrier-forming potential.

We then tested three possible mechanisms by which histone H1 could increase the barrier activity of Gal41–147. First, we tested the possibility that HHO1 overexpression might increase the expression level of Gal41–147. This was ruled out; there was no difference in the level of Gal41–147 in cells overexpressing HHO1 relative to control cells, as judged by Western blotting (Figure 3B). A second possibility was that HHO1 might facilitate the binding of Gal41–147 to its target sites. We addressed this by chromatin immunoprecipitation (ChIP). Gal41–147 was crosslinked to DNA and immunoprecipitated. We then carried out quantitative PCR on the recovered DNA to examine the abundance of two sequences. The first one, located in the GAL1 promoter, contains a natural cluster of high affinity Gal4p binding sites (Ren et al. 2000). The second sequence contains the Gal4p binding sites located between the two reporter genes TRP1 and URA3 in GF97. This strain contains the endogenous Gal4p protein but it is present in very low amounts relative to Gal41–147, which is expressed from the ADH1 promoter on a high-copy vector. Overexpression of HHO1 in the presence of Gal41–147 generates an active barrier, as evidenced by growth on −HLW+FOA medium. (B) Histone H1 overexpression does not affect the expression of Gal41–147. Protein extracts were prepared from strains containing the indicated plasmid combinations, and used for Western blotting with an antibody directed against Gal4p. (C) Histone H1 does not recruit Gal41–147 to the barrier. Gal41–147 abundance at the indicated loci was measured by ChIP followed by quantitative PCR. Strains harbored an HHO1 overexpression plasmid (2µ HHO1) or a control plasmid (WT). (D) Characterization of an anti-Hho1p antibody. Total protein extracts from the indicated yeast cells were used for Western blotting. (E) Gal41–147 does not recruit Hho1p to the barrier. Strain GF97 with endogenous or elevated levels of HHO1 was transformed with a control vector or an expression vector for Gal41–147 (+Gal4p). Abundance of Hho1p at the indicated loci was measured by ChIP followed by quantitative PCR.
C-terminus with 3xHA or 3xMyc tags failed to reproduce the effect of the wild-type protein. We therefore raised Hho1p antisera in rabbits. The antibodies recognize only one band by Western blotting on a total yeast extract. The band matches the predicted mass of histone H1 and disappears in a \( \Delta hho1 \) strain (Figure 3D), validating the use of the serum for ChIP. Two loci were examined: the telomeric sequence containing Gal4p binding sites and the ORF of ACT1 to serve as a control (Figure 3E). First we examined the endogenous histone H1. The protein was more abundant at the subtelomeric site than at ACT1. The abundance of each site was not modified by the overexpression of Gal4-1-147. Then we overexpressed histone H1 in the same two strains. Binding to ACT1 and the subtelomeric locus was increased, in line with the idea that histone H1 is present in limiting amounts in the cell (Freidkin and Katcoff 2001). Overexpression of Gal4-1-147 did not enhance binding of histone H1 to either site.

This set of experiments shows that histone H1 does not recruit Gal4-1-147, and that Gal4-1-147 does not recruit histone H1. Therefore, the two proteins seem to act independently to form barriers, and we believe their synergistic action is due to a threshold effect.

**Histone H1 synergizes with different types of barrier elements and can also act alone:** Because HHO1 seemed to act independently of Gal4-1-147 we asked whether it would also synergize with barrier elements that did not involve Gal4-1-147. First we tested whether it might also potentiate the effect of LexA, a DNA-binding protein that has barrier activity (Bt et al. 2004) and is unrelated to Gal4p. In the strain we used, URA3 is separated from an inverted HML-I by two target sites for LexA. Binding of LexA to these sites has been shown to generate a barrier (Bt et al. 2004). We observed that overexpression of HHO1 increased the activity of the barrier in this context as well (Figure 4A). We then examined a strain in which URA3 is separated from the inverted HML-I by four CGGNN repeats (Figure 4B). These sequences have a low propensity to form nucleosomes and interfere with the spread of silencing (Bt et al. 2004). Overexpression of HHO1 increased barrier activity in that strain (Figure 4B), while deletion of HHO1 decreased barrier activity (Figure 4C). The observations made on these two strains reinforce the fact that the effect we first noted on Gal4-1-147 was not specific, and that HHO1 might more generally contribute to barrier activity.

These experiments yielded another important result. Indeed, we observed in all three strains (H0, H2, and H10) that histone H1 overexpression decreased the silencing of URA3 even in the absence of the barrier element (Figure 4A and B). Conversely, deletion of HHO1 increased the silencing of URA3 even in the absence of a barrier (Figure 4C, bottom).

**Overexpression of Histone H1 decreases the abundance of SIR proteins at a silenced locus:** Next we asked whether the effect of HHO1 was indeed a direct effect on silencing. For this we measured the abundance of Sir4p at different positions in a silenced locus, in the presence or absence of overexpressed histone H1 (Figure 5). The experiment was performed in a derivative of strain H10, in which a 9xMyc epitope tag was added to Sir4p. We started by verifying that URA3 silencing was not affected by the modification of Sir4p and also that HHO1 overexpression decreased URA3 silencing as it did in the parent strain (Figure 5A). The amount of Sir4p was measured by ChIP at three loci: HML-I, and sites a and b, which are increasingly distant from HML-I. In addition we used a nonsilenced locus, ACT1, as a negative control for Sir4p binding. In the absence of overexpressed HHO1 we detected binding of Sir4p to HML-I and to sites a and b (Figure 5B). This is consistent with the silencing of URA3 that is observed under these conditions. When HHO1 was overexpressed, the binding of Sir4p to all three test sites was decreased. The decrease followed a gradient and was
least pronounced at HML-I and most pronounced at the greatest distance from HML (Figure 5B). This supports the notion that histone H1 overexpression inhibits silencing directly, by impeding the propagation of the SIR complex away from silencers.

**Overexpression of histone H1 decreases silencing at some loci but not all:** After observing an effect for histone H1 overexpression outside of HML-I, we tested whether this situation also occurred at other silenced loci. First we examined a strain containing two reporter genes embedded within HML (Ishii et al. 2002). Histone H1 overexpression did not protect URA3 or ADE2 against silencing (Figure 6A), suggesting that it can only act outside, and not within, the silenced domain. Because silencing at HMR often behaves differently from silencing at HML, we also tested GCY317, a reporter strain in which ADE2 is inserted within HMR. In this strain, increasing degrees of HMR silencing translate into increasing frequency and color intensity of pink sectors in the yeast colonies (Cupeur and Shore 2002). We could not detect any difference between colonies of GCY317 containing an empty vector and colonies containing an HHO1 overexpression vector (data not shown). Therefore, as for HML, HHO1 does not affect silencing within the silenced HMR domain.

Next, we asked whether HHO1 overexpression could affect telomeric silencing. We tested a strain in which URA3 is located in close proximity to a telomere (Figure 6B). Overexpression of histone H1 did not relieve the silencing of URA3 in this strain. To test whether HHO1 might decrease silencing of a reporter gene placed farther away from the telomere, we used the strain GF97 again. Overexpression of HHO1 in GF97 had a modest inhibitory effect on the silencing of both on URA3 and TRP1 (Figure 6C). These results suggest that histone H1 can oppose telomeric silencing, but only weakly and in certain contexts.

Last, we asked whether HHO1 could affect silencing at the rDNA by using a strain in which URA3 integrated in the rDNA array (Figure 6D). We failed to detect an effect of HHO1 overexpression in that strain. We controlled the behavior of the strain by overexpressing SIR2 and observed, as expected, an increase in silencing (not shown). We can therefore conclude that HHO1 overexpression does not affect rDNA silencing.

**Interaction with Histone H2A.Z:** The variant histone H2A.Z can protect genes against the spread of silencing (Meneghini et al. 2003). We asked whether the effect of
HHO1 required the presence of H2A.Z. For this we deleted HTZ1, the gene encoding H2A.Z, in the reporter strain H0. We found that HHO1 overexpression in this mutant strain still decreased the silencing of URA3, but to a lesser extent than in the wild-type strain, suggesting that HHO1 is partially dependent on HTZ1 for function (Figure 7).

**DISCUSSION**

Here we report that histone H1 of *S. cerevisiae* influences the activity of barrier elements; elevated expression of Hho1p increases the function of Gal4p-generated barriers, while loss of Hho1p decreases their activity. In addition, Hho1p directly inhibits silencing, at least outside of HML. However, this effect is not general, as overexpression of Hho1p had no effect on silencing within HML and HMR, at telomere-proximal positions, or at the rDNA. It had a subtle effect on genes placed at a distance from a telomere, and a stronger effect on genes located outside of HMR-I.

We do not know the reason for that sensitivity of Hho1p effect to context. It could be quantitative: the silencing may be too strong to be opposed in certain regions, such as the telomere ends. Alternatively, it could be qualitative, and the determinants of silencing at the different loci may respond differently to histone H1. Along similar lines, histone acetyltransferases have different effects when targeted to different silenced loci (Jacobson and Pillus 2004).

These results contrast with earlier reports that failed to detect any effect of Hho1p on silencing (Escher and Schaffner 1997; Patterson et al. 1998). We believe there are two reasons for these discrepancies. First, histone H1 is not abundant: the most recent estimate is 6500 molecules/cell (Ghaemmaghami et al. 2003), well under the estimated number of nucleosomes, which is in the order of 50,000. In addition, histone H1 is spread over different sites in the genome; in agreement with previous reports (Friedkin and Katcoff 2001; Downs et al. 2003), we were able to detect some histone H1 bound within the rDNA (not shown) as well as in the coding sequence of an expressed gene, ACT1. Therefore, only a fraction of the total histone H1 resides around silenced regions, probably explaining why the deletion has only a subtle effect, while the overexpression has a larger effect. The second reason may be technical: some of the tagged versions of Hho1p that have been used (C-terminally tagged with HA or Myc) do not reproduce the effect of the wild-type protein, at least in our assays.

Finally, how might Hho1p impede silencing? There are two appealing possibilities. One would be that the linker histone influences nucleosomal histone modification, for instance by activating histone acetylation by Sas2p or impeding histone deacetylation by the SIR complex. Another possibility would be that Hho1p competes with the SIR complex for binding to nucleosomes. We have observed decreased binding of the SIR proteins to a silenced locus upon overexpression of Hho1p, suggesting that the second model may be true, but the first possibility cannot be ruled out at this point.

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**LITERATURE CITED**


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