Requirement of Hos2 Histone Deacetylase for Gene Activity in Yeast

Amy Wang,[1] Siavash K. Kurdistani,[1,2] Michael Grunstein[1]*

Histone deacetylases, typified by class I Rpd3 in the yeast *Saccharomyces cerevisiae*, have historically been associated with gene repression. We now demonstrate that Hos2, another member of the class I family, binds to the coding regions of genes primarily during gene activation, when it specifically deacetylates the lysines in H3 and H4 histone tails. Moreover, Hos2 is preferentially associated with genes of high activity genome-wide. We also show that Hos2 and an associated factor, Set3, are necessary for efficient transcription. Therefore, our data indicate that, in contrast to other class I histone deacetylases, Hos2 is directly required for gene activation.

Histone deacetylases such as Rpd3 and Hda1 can act locally at promoters or globally throughout the genome to repress gene activity (*1*–*5*). In yeast, Rpd3 is required for deacetylation of all lysines examined in the core histones H3, H4, H2A, and H2B except for lysine 16 (K16) in histone H4 (*4*). In contrast, Hda1 specifically deacetylates histones H2B and H3 (*5*). Because the histone sites deacetylated by Hos2 had not been determined, we screened for genes whose acetylation state is increased upon deletion of *HOS2* (*hos2*Δ) in yeast. This was done by chromatin immunoprecipitation (ChIP) with highly specific antibodies to sites of acetylation in all four core histones (*4*). Because Rpd3 also deacetylates chromatin globally, we anticipated that there might be redundancy between the enzymatic activities of Hos2 and Rpd3 and therefore screened for genes whose chromatin is hyperacetylated in *hos2*Δ, *rpd3*Δ, and *hos2*Δ*rpd3*Δ strains. We found that, in combination with Rpd3, Hos2 deacetylates the coding region of *ERG11*. Unlike *RPD3*, however, *HOS2* is required for the preferential deacetylation of histone H3 and H4 sites including H4-K16 and thus shows a unique histone specificity (fig. S1).

Because *ERG11* is activated in glucose-rich medium (*6*), the condition of the screen, we decided to investigate the effect of Hos2 on other genes under both repressive and activating conditions. We examined the effect of *hos2*Δ, *rpd3*Δ, and *hos2*Δ*rpd3*Δ on the acetylation state of *GAL1*, *GAL7*, and *GAL10*, a cluster of genes on chromosome II in yeast that is repressed in glucose and activated in galactose (*7*). As shown in Fig. 1A, when the cells are grown in glucose, *hos2*Δ has little or no effect on the acetylation state of H4-K12 at the *GAL* gene cluster compared with *WT*. Under the same repressive condition but in contrast to *hos2*Δ, *rpd3*Δ results in increased H4-K12 acetylation of not only the *GAL* cluster but the two adjacent genes *KAP104* and *FUR4*, consistent with the role of Rpd3 as a global deacetylase. *hos2*Δ*rpd3*Δ results in a similar increase in the acetylation state as *rpd3*Δ alone, arguing that Hos2 does not contribute substantially to the deacetylation of the *GAL* gene cluster in repressive conditions. However, as shown in Fig. 1B, under the activating galactose condition, only *hos2*Δ*rpd3*Δ results in a very large increase (four- to sixfold) in acetylation over *GAL7*, *GAL10*, and *GAL1* coding regions compared with *hos2*Δ and *rpd3*Δ. We conclude that, whereas Rpd3 deacetylates H4-K12 at the *GAL* genes and adjacent regions in both repressed and activated conditions, Hos2 deacetylates H4-K12 at the *GAL* genes in galactose only when the genes are being transcribed.

Considering that the effect of *hos2*Δ on the *GAL* gene acetylation state is evident only during activation, we asked whether Hos2 also binds the *GAL* genes when they are activated. We integrated 13 tandem copies of the Myc tag onto the 3′-terminus of the chromosomal *HOS2* gene (*8*) and performed ChIP with a monoclonal antibody to Myc (fig. S2). As shown in Fig. 1C, Hos2-myc associates specifically with the coding regions of the *GAL* genes to a greater extent (about fivefold) in galactose than in glucose. In contrast, Rpd3 binding remains unchanged between growth in galactose and glucose compared with the telomere (*TEL*) (Fig. 1D) and *INO1* (*9*, *10*). Therefore, consistent

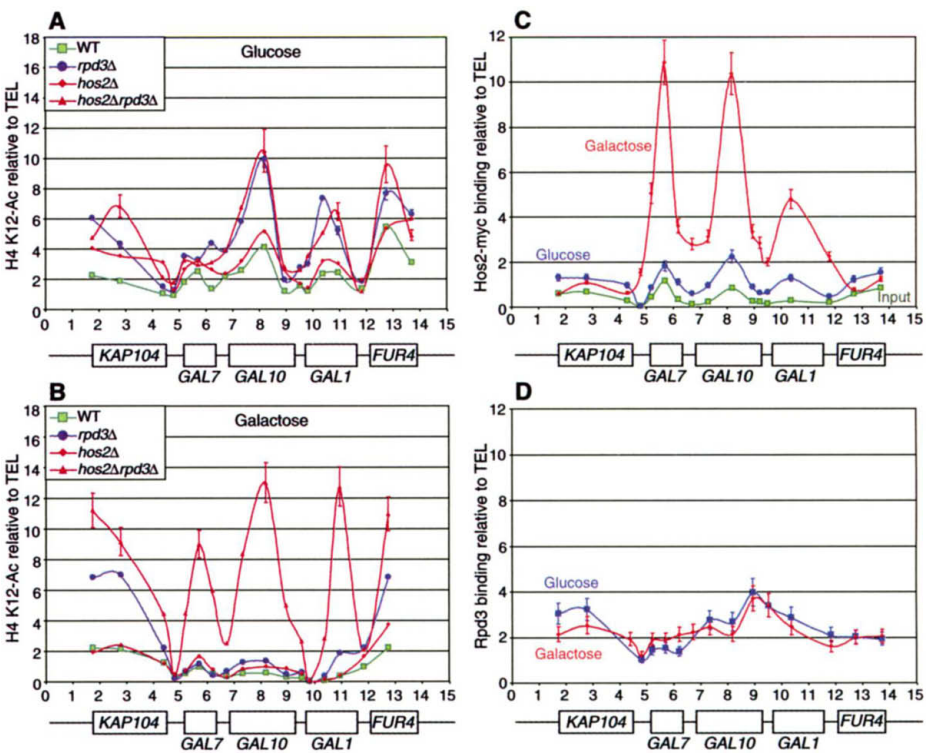

Fig. 1. Hos2 binds to and deacetylates the *GAL* genes primarily in activating conditions. Levels of H4-K12 acetylation at the *GAL* gene cluster in WT, *rpd3*Δ, *hos2*Δ, and *hos2*Δ*rpd3*Δ during growth in glucose-containing medium (YEPD) (**A**) and galactose-containing medium (YEPG) (**B**) are compared with a region near telomere VI-R (TEL). The location of the genes is relative to an arbitrary region upstream of the cluster. For clarity, most error bars are excluded. The level of Hos2-myc (**C**) and Rpd3 (**D**) binding to the *GAL* gene cluster under repressive (glucose, blue line) and activating (galactose, red line) conditions was determined by ChIP with antibodies to Myc and Rpd3, respectively, and normalized to TEL. Green line indicates input DNA. Results are averages of three independent experiments.

Departments of [1]Biological Chemistry and [2]Pathology and Laboratory Medicine, UCLA School of Medicine and the Molecular Biology Institute, Boyer Hall, University of California, Los Angeles, CA 90095, USA.

*To whom correspondence should be addressed. E-mail: mg@mbi.ucla.edu

with their effects on acetylation, Rpd3 is constitutively present throughout the *GAL* region under both repressive and activating conditions, whereas Hos2 associates with the coding regions of the *GAL* genes primarily during their activation. Similarly, Hos2 binds preferentially to the *INO1* coding region only in activating low inositol medium (fig. S3) (*7*). Taken together, these data indicate that Hos2 associates with independently regulated genes (the *GAL* cluster and *INO1*) only during gene activation.

To determine whether Hos2 is selectively associated with the coding regions of actively transcribed genes throughout the genome, we analyzed genome-wide binding of Hos2-myc with ChIP and DNA microarrays (*10*–*12*). Briefly, DNA from formaldehyde cross-linked chromatin was purified by immunoprecipitation with antibody to Myc, amplified by polymerase chain reaction (PCR), and labeled with either Cy3 or Cy5 fluorophores. The labeled DNA from Hos2-myc and isogenic untagged strains were combined and hybridized to a microarray glass slide containing >6200 open reading frames (University Health Networks, Toronto). The raw data were normalized by the ratio of total intensities across the entire array between the two fluorescent dyes. We then compared the binding data to a published whole-genome mRNA abundance database in which the absolute number of mRNA molecules per cell is calculated when cells are grown in glucose-rich medium at 30°C (*13*). As shown in Fig. 2, we find a positive correlation between levels of Hos2 enrichment and the number of mRNA copies per cell. This indicates that Hos2 preferentially associates with the coding regions of genes with high transcriptional activity genome-wide.

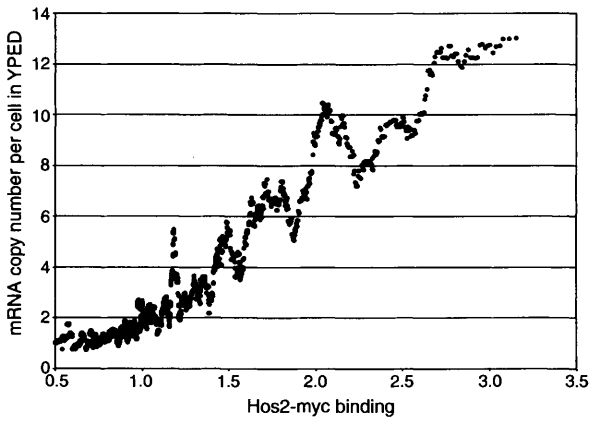

Fig. 2. Hos2-myc binds preferentially to open reading frames (ORFs) with high transcriptional activity. The moving average (window size = 100, step size = 1) of Hos2-myc enrichment on an ORF is plotted as a function of mRNA molecule copy number per cell.

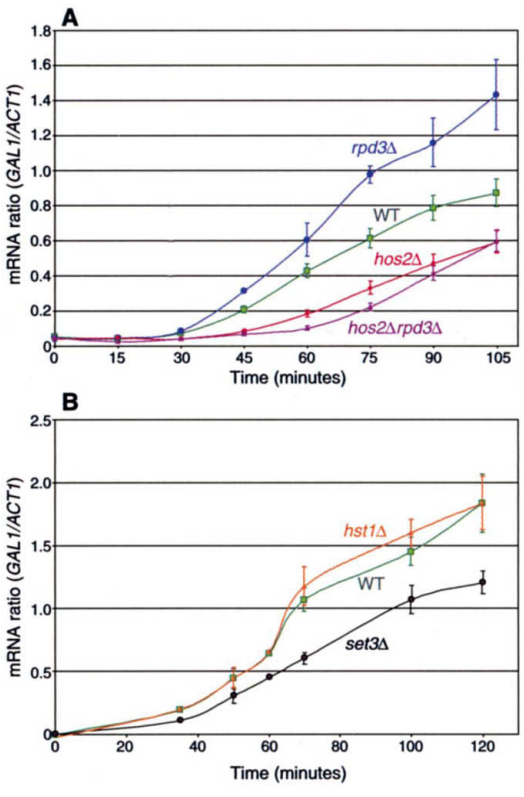

Fig. 3. Hos2 deletion causes inefficient activation of *GAL1*. Effects of *rpd3*Δ, *hos2*Δ, and *hos2*Δ*rpd3*Δ (**A**) and of *set3*Δ and *hst1*Δ (**B**) on *GAL1* gene activation. Aliquots of cells were harvested at the indicated intervals after addition of galactose at 16°C, and *GAL1* mRNA levels were quantitated by reverse transcription (RT) PCR (*8*) and normalized to *ACT1* transcript.

Given the regulated binding of Hos2 and constitutive binding of Rpd3 to *GAL* genes, we asked whether the deacetylases may play different roles in *GAL* gene transcription. Cells were grown to logarithmic phase in nutrient-rich medium containing 2% raffinose, washed in distilled water, and resuspended in medium containing 2% galactose. Aliquots were taken at 15-min intervals after the addition of galactose from each strain and analyzed for *GAL1* mRNA transcript levels (*7*). Because transcripts begin to appear within 20 min and full activation is reached 40 to 50 min after addition of galactose at 30°C (*14*), the cells were incubated at 16°C to slow the rate of activation in order to increase the resolution of our kinetic analysis. We find that *rpd3*Δ causes more rapid activation of *GAL1* than that occurring in WT cells (Fig. 3A and fig. S4), arguing that Rpd3 serves a repressive role in *GAL1* gene regulation. In contrast, *hos2*Δ results in slower accumulation of *GAL1* transcripts, indicating that Hos2 is required for normal *GAL1* gene activation. Interestingly, *hos2*Δ*rpd3*Δ results in activation kinetics similar to those of the *hos2*Δ strain, indicating that Hos2 can override the repressive effect of global Rpd3 deacetylation. Similarly, we find that Rpd3 acts as a repressor, but Hos2 is required for efficient activation of *INO1* transcription (fig. S4). These data indicate that Hos2 is important for activation of *GAL1* and *INO1* genes in vivo.

Recently, a Set3 complex was purified and found to contain Hos2 and Hst1 deacetylases (*15*). Expression analysis of deletion mutations of certain members of this complex revealed defective repression of genes (*IME1*, *IME2*, *NTD80*) that encode key regulators of middle sporulation genes. However, the direct involvement of the Set3 complex in regulation of these genes has not been determined. Pijnappel *et al.* (*15*) further showed that Set3 and Hos2 are both required for the integrity of the complex but Hst1 is dispensable. Thus, we asked whether deletion of *SET3* affects *GAL1* activation in the same manner as the *HOS2* deletion. As shown in Fig. 3B, we find that, compared with wild type, *set3*Δ prevents efficient *GAL1* expression whereas *hst1*Δ has little effect on *GAL1* activation. Therefore, our data suggest that the Set3 complex containing Hos2 functions as an activator of *GAL1* gene expression.

To determine whether the histone deacetylase activity of Hos2 is required for its activating function, we disrupted a pair of conserved histidine residues found in the putative catalytic domain of Hos2. These residues were previously shown to be required for Rpd3 deacetylase activity in vitro (*16*). We replaced genomic *HOS2* with an episomal *HOS2* construct in which the relevant histidines (H195, H196) were substituted

with alanines (H195A, H196A). By immunoblot analysis, the *hos2* H195A,H196A construct expresses a protein of similar size and level as wild-type *HOS2* (*9*). To assay the effect of this mutation on Hos2 histone deacetylase activity, we transformed the empty vector, *HOS2-myc*, and *hos2* H195A,H196A episomal constructs into the *hos2Δrpd3Δ* strain. We observe that in the background of *hos2Δrpd3Δ*, wild-type Hos2-myc results in deacetylation of H4-K12 at the *GAL1* coding region but not at the promoter compared with vector alone (Fig. 4A). In contrast, the *hos2* H195A,H196A mutant shows levels of hyperacetylation at the *GAL1* coding region similar to those obtained with the vector alone. Therefore, substituting histidine residues 195 and 196 with alanines strongly decreases the apparent histone deacetylase activity of Hos2. We also find that the H195A,H196A mutation causes inefficient binding of mutant Hos2 to chromatin at *GAL7*, *GAL10*, and *GAL1* during growth in galactose (Fig. 4B). Binding was decreased at *ERG11* as well (*9*). Therefore, the histone deacetylase activity of Hos2 is required for binding of Hos2 to chromatin at *ERG11* and the *GAL* genes. Next, we asked whether the deacetylase-defective Hos2 affects *GAL1* activation as assayed above (Fig. 3A). We find that *GAL1* activation is defective in H195A,H196A (Fig. 4C and fig. S5). The activation kinetics of the mutant are similar to those of the *hos2Δ* strain containing vector alone. This indicates that the deacetylase activity of Hos2 is required for binding to *GAL1*, which in turn is necessary for efficient gene activation.

Considering that Rpd3 and Hos2 are both histone deacetylases with overlapping specificities, it is unclear how they exert opposing effects on transcription. Differences in binding dynamics between Rpd3 and Hos2 as well as additional deacetylation of histones H2A and H2B by Rpd3 may be responsible for their dissimilar roles in gene regulation. Although histones are clearly deacetylated by Hos2, we cannot exclude the possibility that other nonhistone substrates may contribute to these different functions. However, given the role of Hos2 in histone deacetylation, we suggest the following working hypothesis. Before gene activity, basal transcription is repressed by Rpd3 but chromatin exists in a state that is poised or permissive for activated transcription. Once a gene is activated, the transcription complex and histone acetyltransferases (*17–20*) may disrupt the initial permissive state of chromatin so that further rounds of transcription are hindered. Hos2 would then be needed to reverse these disruptive effects by deacetylating the acetylated histones. Although Hos2 binds to coding regions, it is possible that it enters genes at promoters but, like RNA polymerase (*9*), is more easily detected over coding regions. In fact, at highly active genes, such as those coding for ribosomal proteins, deacetylation by Hos2 is found not only in coding regions but also at promoters to a lesser extent (*21*). Thus, Hos2 may be required for gene activity by following RNA polymerase to revert disrupted chromatin to the original permissive state that is required for efficient transcription.

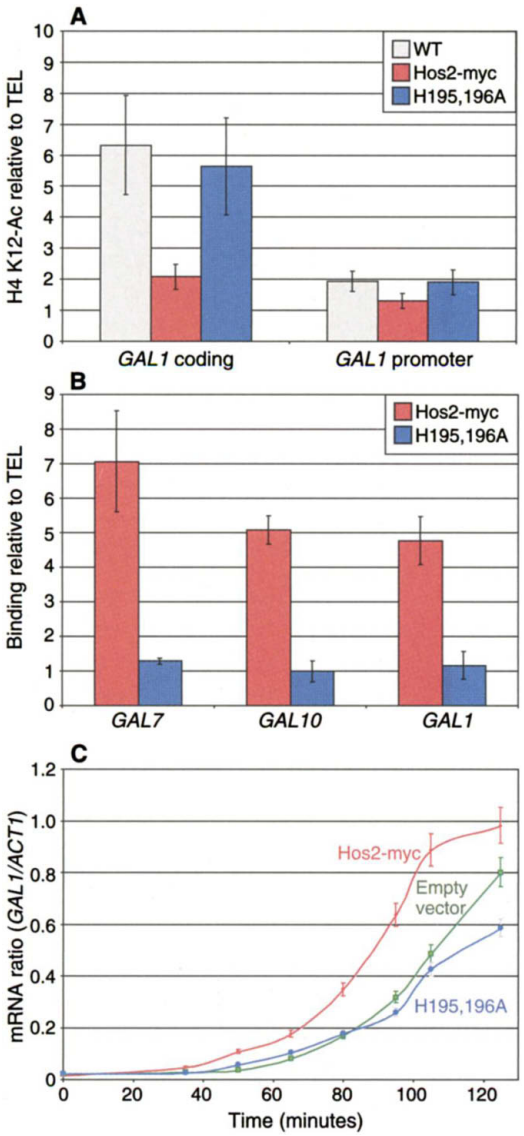

Fig. 4. Histone deacetylase activity of Hos2 is required for binding and activation of *GAL1*. (**A**) The *hos2Δrpd3Δ* strain was transformed with either the empty vector, the *Hos2-myc*, or the *hos2-myc H195A,H196A* episomal constructs. ChIP was performed with antibody to H4-K12Ac and normalized to TEL. (**B**) The episomal construct *Hos2-myc* or *hos2-myc H195A,H196A* was transformed into the *hos2Δ* strain. ChIP was performed with monoclonal antibody to Myc and normalized to TEL. (**C**) Aliquots of the *hos2Δ* strain containing the episomal constructs were taken at the indicated intervals at 16°C. *GAL1* mRNA levels were determined by RT-PCR and normalized to the *ACT1* transcript. All experiments were done in galactose-containing medium.

References and Notes

1. D. Kadosh, K. Struhl, *Cell* **89**, 365 (1997).
2. S. E. Rundlett, A. A. Carmen, N. Suka, B. M. Turner, M. Grunstein, *Nature* **392**, 831 (1998).
3. M. Vogelauer, J. Wu, N. Suka, M. Grunstein, *Nature* **408**, 495 (2000).
4. N. Suka, Y. Suka, A. A. Carmen, J. Wu, M. Grunstein, *Mol. Cell* **8**, 473 (2001).
5. J. Wu, N. Suka, M. Carlson, M. Grunstein, *Mol. Cell* **7**, 117 (2001).
6. T. G. Turi, J. C. Loper, *J. Biol. Chem.* **267**, 2046 (1992).
7. Materials and methods are available as supporting material on *Science* Online.
8. M. S. Longtine *et al.*, *Yeast* **14**, 953 (1998).
9. A. Wang, S. K. Kurdistani, M. Grunstein, unpublished observations.
10. S. K. Kurdistani, D. Robyr, S. Tavazoie, M. Grunstein, *Nature Genet.* **31**, 248 (2002).
11. B. Ren *et al.*, *Science* **290**, 2306 (2000).
12. V. R. Iyer *et al.*, *Nature* **409**, 533 (2001).
13. F. C. Holstege *et al.*, *Cell* **95**, 717 (1998).
14. J. G. Yarger, H. O. Halvorson, J. E. Hopper, *Mol. Cell. Biochem.* **61**, 173 (1984).
15. W. W. Pijnappel *et al.*, *Genes Dev.* **15**, 2991 (2001).
16. D. Kadosh, K. Struhl, *Genes Dev.* **12**, 797 (1998).
17. N. A. Hawkes *et al.*, *J. Biol. Chem.* **277**, 3047 (2002).
18. B. O. Wittschieben *et al.*, *Mol. Cell* **4**, 123 (1999).
19. G. S. Winkler, A. Kristjuhan, H. Erdjument-Bromage, P. Tempst, J. Q. Svejstrup, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 3517 (2002).
20. B. O. Wittschieben, J. Fellows, W. Du, D. J. Stillman, J. Q. Svejstrup, *EMBO J.* **19**, 3060 (2000).
21. D. Robyr *et al.*, *Cell* **109**, 437 (2002).

22. We thank M. Longtine for his gift of plasmid pFA6a-13Myc-His3MX6. We are grateful to the members of M.G.'s laboratory, especially L. Milne, for their critical comments and discussion throughout this work. S.K.K. is a Howard Hughes Medical Institute Physician Postdoctoral Fellow. Supported by Public Health Service grant GM23674 of the NIH to M.G.

Supporting Online Material
www.sciencemag.org/cgi/content/full/298/5597/1412/DC1
Materials and Methods
Figs. S1 to S5

27 August 2002; accepted 11 September 2002