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Rpd3p Relocation Mediates a Transcriptional Response to Rapamycin in Yeast

Brief Communication

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Summary

Treating yeast cells with rapamycin, a small molecule that inhibits the TOR proteins, leads to the repression of many genes [1, 2]. Consistent with prior studies, we find that *RPD3*, which encodes a histone deacetylase (HDAC), is required for repression upon rapamycin treatment. To elucidate the mechanism underlying *RPD3*-mediated repression, we screened all promoters in yeast for occupancy by Rpd3p before and after treatment with rapamycin. We find that Rpd3p binds to the promoters of rapamycin-repressible genes only following treatment. These data conflict with a previously proposed model suggesting that Rpd3p is constitutively bound to rapamycin-repressible genes and becomes active only after a stimulus such as treatment with rapamycin [3–5]. Rather, the comprehensive analysis presented here strongly supports a model in which recruitment of Rpd3p to gene promoters is a regulated step in the control of gene repression [6].

Results and Discussion

The TOR proteins regulate fundamental cellular processes in yeast such as transcription, translation, protein degradation, and differentiation in response to nutrient levels [7, 8]. The small molecule rapamycin, which forms a ternary complex with Fpr1p and TOR, has been a useful tool enabling the discovery and study of the TOR proteins [8]. Inhibition of the TOR proteins by rapamycin leads to a transcriptional response reminiscent of nutrient deprivation [1, 2, 9, 10]. The pathways leading to gene activation in response to rapamycin are known to involve changes in phosphorylation and localization of several transcription factors [1, 10–12]. However, the pathways leading to gene repression are less understood, and most studies have focused on the repression of one subset of repressed genes, those encoding ribosomal proteins [3, 9, 13, 14]. Our genome-wide study allows us to simultaneously examine the regulation of all genes repressed following treatment with rapamycin.

Deacetylation of histones, which correlates with gene repression, is catalyzed by enzymes called histone deacetylases (HDACs) [15–17]. To examine whether HDACs are required for the gene repression observed following treatment with rapamycin, we examined the transcriptional response to rapamycin in strains individually deleted for each known HDAC: ***RPD3***, ***SIR2***, ***HDA1***, ***HOS1***, ***HOS2***, and ***HOS3***. Deletion of ***RPD3***, but no other HDAC, blocks gene repression following treatment with rapamycin (Figure 1). This is consistent with previous studies that have shown that repression of ribosomal protein-encoding genes following treatment with rapamycin requires *RPD3* [3]. We further show that *RPD3* is required for the rapamycin-induced repression of almost all functional classes of genes (Table 1). We obtained similar results for a mutant allele of *RPD3* lacking deacetylase activity (see Figure S1 available online with this article) [18]. Specifically, the correlation between the transcription profiles of the catalytically inactive mutant and the deletion mutant is 77%. Lastly, *SIN3*, which encodes an Rpd3p-complex member, is also required for this gene repression, and the profile of *sin3* yeast treated with rapamycin correlates 90% with that of the *rpd3* deletion mutant (Figure S1). These data suggest that the Sin3p-Rpd3p complex acts downstream of the TOR proteins to mediate transcriptional repression.

Previous work has suggested that the promoters of rapamycin-repressible genes are occupied by Rpd3p under steady-state conditions [4]. Furthermore, it has been shown that the association of Rpd3p with the promoters of two rapamycin-repressible genes is unaffected by treatment with the small molecule [3]. These results have led to a model in which Rpd3p is constitutively associated with the promoters of genes it represses [5]. Transcriptional repression then requires the activity of Rpd3p to be modulated, such as by posttranslational modification. However, a mechanism that reconciles this model with the previous result that Rpd3p represses a set of genes under steady-state conditions would be very complex, requiring Rpd3p to be active at only a subset of the promoters to which it is bound under steady-state conditions. A simpler mechanism for how Rpd3p is regulated would be recruitment of the HDAC to the promoters of rapamycin-repressible genes following treatment with the small molecule. To determine whether the level of Rpd3p binding in gene promoters changes following treatment with rapamycin, we used chromatin immunoprecipitation (ChIP) to examine the promoters of all yeast genes for the presence of Rpd3p under steady-state conditions and following treatment with the small molecule. In contrast to previous results, we find that Rpd3p binds to the promoters of rapamycin-repressible genes following treatment with rapamycin, but we find no significant Rpd3p binding to these promoters under steady-state conditions (Figures 2A and 2B). We have confirmed our genome-wide result at several specific promoters, including those previously examined, by performing quantitative PCR on our ChIP samples (Figure 2C). These gene-specific studies and our genome-wide analysis both suggest that the TOR

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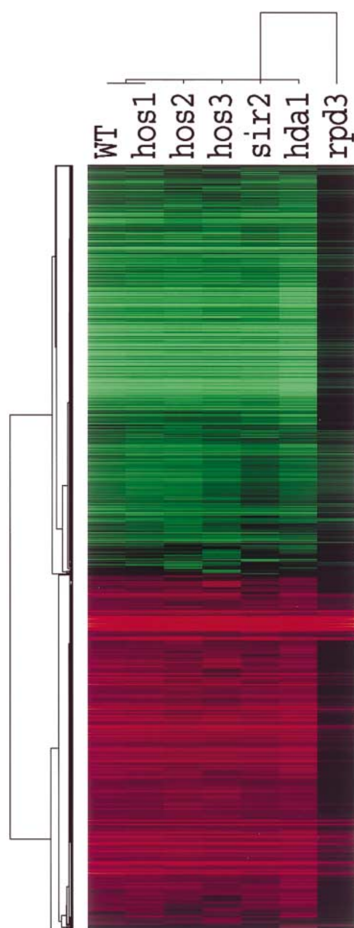

Figure 1. RPD3 but No Other HDAC Is Required for the Repression of Most Rapamycin-Repressible Genes Following Treatment with Rapamycin

Log-transformed, averaged transcription profile data sets were clustered using average linkage with the program Cluster [26]. The results are displayed with Treeview [26]. Red bars inidicate genes that were transcriptionally induced, and green bars indicate genes that were transcriptionally repressed. The data set for each strain compares yeast treated with vehicle to those treated with rapamycin.

proteins regulate the association of Rpd3p with the promoters of rapamycin-repressible genes to control gene repression. Although genes transcribed by RNA polymerase I can have distinct chromatin regulation [19], our results are consistent with the findings of Tsang et al. that Rpd3p is recruited to rDNA promoters to control repression following treatment with rapamycin [6]. These results suggest a new model in which TOR regulates gene activity by controlling the recruitment of Rpd3p to the promoters of genes (Figure 3).

Under steady-state conditions, we find that Rpd3p associates with promoters containing the Ume6p binding site (URS1), consistent with the known role for Ume6p in the recruitment of Rpd3p to repress these genes [4, 17, 20, 21]. Specifically, 17 of the 84 promoters bound by Rpd3p at least 2-fold under steady-state conditions contain URS1 sites ($p = 6.0 \times 10^{-8}$). This finding contrasts with another genome-wide study reporting the association of Rpd3p with additional promoters under steady-state conditions, including the promoters of

Table 1. All Rapamycin-Repressible Functional Classes of Genes Require RPD3 for Repression

Category	WT versus WT rap	*rpd3* versus *rpd3* rap	*rpd3*/WT
RNA polymerase I	0.11	0.68	6.16
ribosome biogenesis/rDNA transcription factor	0.21	0.69	3.34
rRNA processing	0.23	0.76	3.30
RNA polymerase III	0.27	0.79	2.97
mRNA decay	0.38	0.72	1.89
translation initiation factors	0.50	0.81	1.61
tRNA processing	0.57	0.92	1.61
mRNA export	0.61	0.89	1.45
cytoplasmic ribosomal proteins	0.45	0.64	1.43
purine biosynthesis	0.61	0.82	1.35

Genes were organized by functional category, and the geometric mean of fold-change for each category repressed at least 1.5-fold by rapamycin was computed. The ratio of the fold change in the *rpd3* deletion strain to that of the wild-type strain was calculated for each category.

many stress-responsive genes that we find to be rapamycin repressible [4]. We suspect that this discrepancy resulted from differences in our ChIP crosslinking conditions. Specifically, Kurdistani et al. crosslink the yeast by treating them with dimethyl adipimidate (DMA) in phosphate-buffered saline solution for 45 min before addition of formaldehyde [4], while we use only formaldehyde. It is possible that these conditions could lead to a stress response that, like treatment with rapamycin, would cause Rpd3p to be recruited to stress-responsive promoters. Alternatively, the binding observed by Kurdistani et al. may reflect low-level occupancy of these promoters by Rpd3p under steady-state conditions, which is greatly augmented following treatment with rapamycin.

Our results argue that a major function of Rpd3p in promoters is to mediate gene repression in response to stresses such as nutrient deprivation, which is mimicked by treatment with rapamycin. By comparing two different types of global data sets, transcription profiles and genome-wide binding data, we were able to show that *RPD3* is required for the repression of many genes with promoters that are bound by Rpd3p following treatment with rapamycin, suggesting direct regulation of these genes by Rpd3p. Some genes that are repressed following treatment with rapamycin in an RPD3-dependent manner are not significantly bound by Rpd3p following treatment with rapamycin. Additionally, deletion of *RPD3* also blunts the activation of many rapamycin-induced genes, but we do not observe significant association of Rpd3p with the promoters of these genes. This suggests that these are indirect effects of deletion of Rpd3p. Alternatively, Rpd3p may associate with the promoters of these genes transiently or weakly, such that our methods cannot detect the association. Thus, we suggest that a critical role of Rpd3p in the TOR pathway is to mediate gene repression.

Significance

We propose a model in which inhibition of the TOR pathway by rapamycin leads to the recruitment of

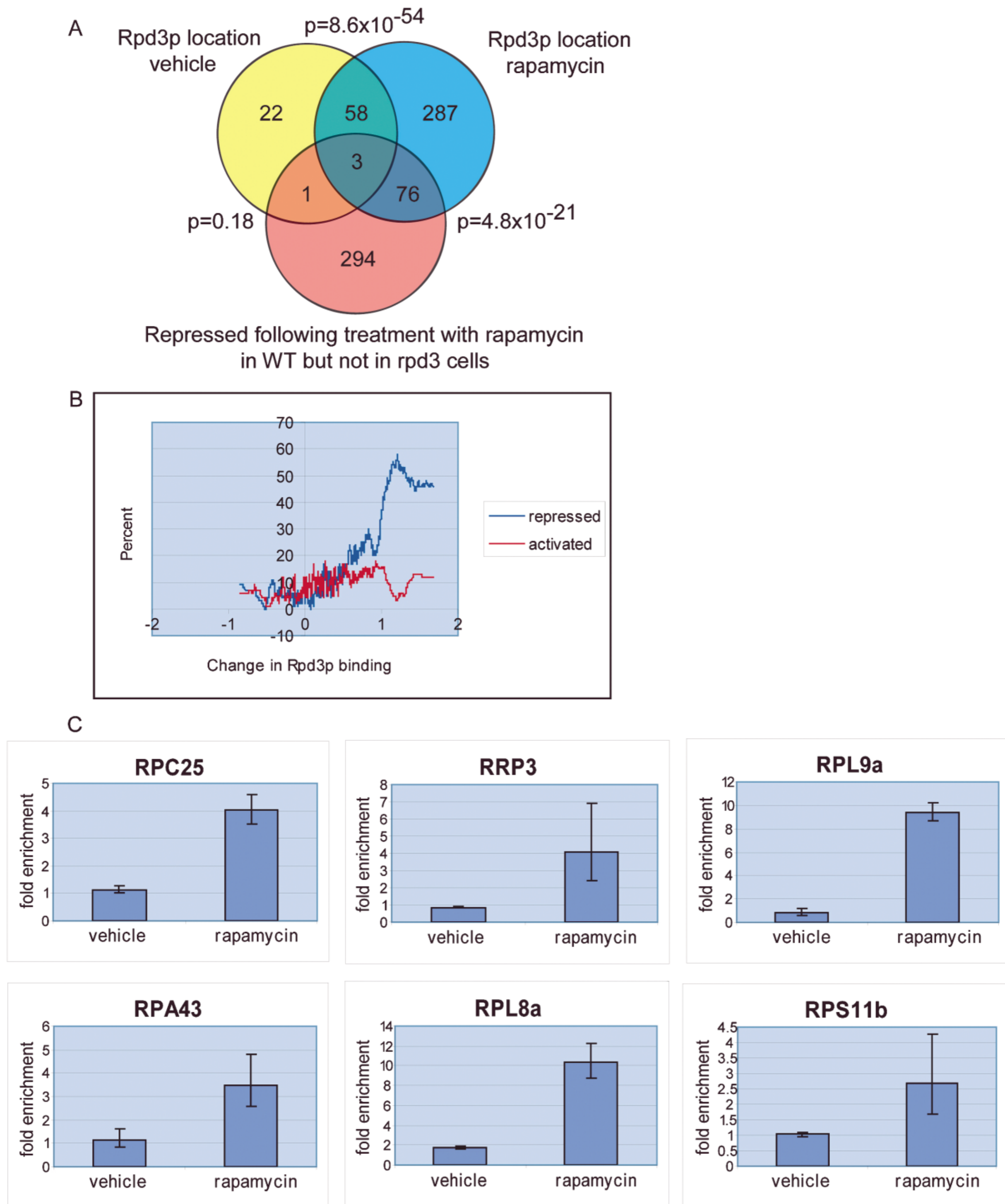

Figure 2. Treatment with Rapamycin Leads to the Recruitment of Rpd3p to the Promoters of Genes that Become Repressed

(A) Overlaps between genes repressed at least 2-fold following treatment with rapamycin but at least 2-fold less repressed in an *rpd3* mutant, genes bound at least 2-fold by Rpd3p under steady-state conditions, and genes bound at least 2-fold by Rpd3p following treatment with rapamycin.

(B) The moving average (window size, 50; step size, 1) of $\log_2$ fold change in binding of Rpd3p to gene promoters following treatment with rapamycin plotted against the percentage of corresponding genes that are activated or repressed.

(C) Quantitative PCR amplifying several promoters from Rpd3p ChIP samples from either vehicle- or rapamycin-treated cells. The fold-enrichment of the indicated promoter in Rpd3p ChIP samples relative to WCE samples is plotted.

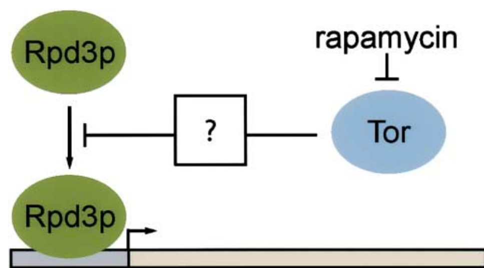

Figure 3. The TOR Signaling Pathway Regulates Gene Repression by Controlling the Recruitment of Rpd3p to Gene Promoters

In the absence of rapamycin, the TOR signaling pathway prevents the recruitment of Rpd3p to the promoters of rapamycin-repressible genes through an unknown mechanism. Inhibition of the TOR pathway with rapamycin leads to the recruitment of Rpd3p to gene promoters, likely causing gene repression through local deacetylation of histones.

Rpd3p to gene promoters to cause gene repression. This model is reminiscent of the known role for the TOR pathway in regulating the association of Esa1p, a histone acetyltransferase, with its target promoters to cause gene activation [3, 22]. It is consistent with a dynamic and central role for chromatin-modifying enzymes in mediating the rapid changes in transcription required for adaptation to nutrient stress. Furthermore, this work demonstrates the utility of combining small molecule perturbations with genome-wide analysis to probe transcriptional regulatory pathways. Future work will be directed toward understanding the mechanism by which the TOR pathway regulates the recruitment of Rpd3p to gene promoters.

Experimental Procedures

Yeast Strains

The mutant strains *hda1*, *hos1*, *hos2*, *hos3*, *sir2*, and *sin3* and the otherwise isogenic wild-type strain BY4741 were obtained from Research Genetics. The *rpd3* mutant was previously described [23]. The catalytically inactive *rpd3* mutant and the myc-tagged *RPD3* strain were generous gifts from T. Tsukiyama [18] and M. Grunstein [4], respectively.

Transcription Profiling

Transcription profiling experiments were performed as previously described [23]. Briefly, 100 ml of yeast were grown to OD600 = 1 in yeast extract/peptone/dextrose and treated with either 50 nM rapamycin or vehicle control for 30 min. mRNA was purified and fluorescently-labeled cDNA probes were generated as previously described [23]. Vehicle-treated sample (Cy3) and rapamycin-treated sample (Cy5) were competitively hybridized to cDNA microarrays. Slides were scanned with an Axon 4000B scanner equipped with genepix pro software, and all microarray data sets were processed according to the Stanford microarray database protocol. Each data set represents the average of two independent experiments. DNA microarrays were constructed as described [23].

ChIP

DNA associated with Rpd3p was immunoprecipitated from 90 ml of yeast cells containing endogenously myc-tagged RPD3 grown to an OD600 of ∼1.0, then treated for 30 min with either 50 nM rapamycin or a vehicle control. Cells were crosslinked and lysed as described [23]. The immunoprecipitation was carried out using 30 μg of 9E10 anti-myc antibody (Upstate Biotechnology) as described [23]. Approximately 1/50 of the preimmunoprecipitation material, referred to as whole-cell extract (WCE), was reserved and treated exactly as the immunoprecipitated DNA.

Quantitative PCR

The promoters of selected genes were each amplified from approximately 1/30 of the immunoprecipitated material using Qiagen SYBR green PCR mix in an MJ Research real-time PCR machine according to the manufacturers' instructions. The following primer pairs were used for quantitative PCR: *RPC25 promoter*, 5′-TTCGTGCTTTTTATATTCCCCTTA-3′ and 5′-ATAACGCTTCTTGCGTCTATTTCT-3′; *RPA43 promoter*, 5′-GCCCCGTAATAGATGTATTTCAGG-3′ and 5′-CCACGTTTGTCTCTTATCTTTCTG-3′; *RRP3 promoter*, 5′-AAAGAAGTTTGACCCACTCTGATT-3′ and 5′-TCTCATCGCGATATTAGTTCGATA-3′; *RPL8a promoter*, 5′-GAAAACACCCAAACATATCTAGGC-3′ and 5′-AGCAATGTAAACTGACAACTGGAA-3′; *RPL9a promoter*, 5′-TGTCCTGCGTATAGAGGAGAAAAA-3′ and 5′-ATGTTGGAAATTTCATCGCTTCTT-3′; *RPS11b promoter*, 5′-GAAGAAATATTTCCTTGCTGCACC-3′ and 5′-CTGGCTTGATACGTTTCCTCTAAG-3′; *TUB2 promoter*, 5′-GGCCTAACAGTAAAGATATCCTCC-3′ and 5′-GTTGTAGTAGCTGCTATGTCACTC-3′.

Fold binding of Rpd3p to gene promoters was determined using the $2^{-\Delta\Delta C_T}$ method described in the Applied Biosystems User Bulletin No. 2 (P/N 4303859). For each promoter examined, the promoter of *TUB2* was used as the normalizer, and the WCE sample was used as the calibrator. Each fold binding reported represents the average of three independent experiments, each done in duplicate.

DNA Amplification, Labeling of ChIP Samples, and Hybridizations

DNA immunoprecipitated from 90 ml of cells and DNA isolated from approximately half of the reserved WCE were amplified as described [24]. WCE DNA and immunoprecipitated DNA were fluorescently labeled by incubating them with monofunctional reactive Cy3 and Cy5 dyes, respectively, as described [25]. Samples were hybridized to microarrays, and data were analyzed as for transcription profiling experiments. Each data set represents the average of three independent experiments.

Complete genome-wide data sets are available at http://www.schreiber.chem.harvard.edu.

Supplemental Data

Supplemental data including a figure, genome-wide transcription profiling data, and Rpd3p binding data are available at http://www.chembiol.com/cgi/content/full/11/3/295/DC1.

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References

1. Hardwick, J.S., Kuruvilla, F.G., Tong, J.K., Shamji, A.F., and Schreiber, S.L. (1999). Rapamycin-modulated transcription defines the subset of nutrient-sensitive signaling pathways directly controlled by the TOR proteins. Proc. Natl. Acad. Sci. USA *96*, 14866–14870.
2. Cardenas, M.E., Cutler, N.S., Lorenz, M.C., Di Como, C.J., and Heitman, J. (1999). The TOR signaling cascade regulates gene expression in response to nutrients. Genes Dev. *13*, 3271–3279.
3. Rohde, J.R., and Cardenas, M.E. (2003). The tor pathway regulates gene expression by linking nutrient sensing to histone acetylation. Mol. Cell. Biol. *23*, 629–635.

4. Kurdistani, S.K., Robyr, D., Tavazoie, S., and Grunstein, M. (2002). Genome-wide binding map of the histone deacetylase Rpd3 in yeast. Nat. Genet. *31*, 248–254.
5. Rando, O.J., Chi, T.H., and Crabtree, G.R. (2003). Second messenger control of chromatin remodeling. Nat. Struct. Biol. *10*, 81–83.
6. Tsang, C.K., Bertram, P.G., Ai, W., Drenan, R., and Zheng, X.F. (2003). Chromatin-mediated regulation of nucleolar structure and RNA Pol I localization by TOR. EMBO J. *22*, 6045–6056.
7. Cutler, N.S., Pan, X., Heitman, J., and Cardenas, M.E. (2001). The TOR signal transduction cascade controls cellular differentiation in response to nutrients. Mol. Biol. Cell *12*, 4103–4113.
8. Schmelzle, T., and Hall, M.N. (2000). TOR, a central controller of cell growth. Cell *103*, 253–262.
9. Shamji, A.F., Kuruvilla, F.G., and Schreiber, S.L. (2000). Partitioning the transcriptional program induced by rapamycin among the effectors of the TOR proteins. Curr. Biol. *10*, 1574–1581.
10. Beck, T., and Hall, M.N. (1999). The TOR signalling pathway controls nuclear localization of nutrient-regulated transcription factors. Nature *402*, 689–692.
11. Saxena, D., Kannan, K.B., and Brandriss, M.C. (2003). Rapamycin treatment results in GATA factor-independent hyperphosphorylation of the proline utilization pathway activator in Saccharomyces cerevisiae. Eukaryot. Cell *2*, 552–559.
12. Komeili, A., Wedaman, K.P., O'Shea, E.K., and Powers, T. (2000). Mechanism of metabolic control. Target of rapamycin signaling links nitrogen quality to the activity of the Rtg1 and Rtg3 transcription factors. J. Cell Biol. *151*, 863–878.
13. Powers, T., and Walter, P. (1999). Regulation of ribosome biogenesis by the rapamycin-sensitive TOR-signaling pathway in Saccharomyces cerevisiae. Mol. Biol. Cell *10*, 987–1000.
14. Duvel, K., Santhanam, A., Garrett, S., Schneper, L., and Broach, J.R. (2003). Multiple roles of Tap42 in mediating rapamycin-induced transcriptional changes in yeast. Mol. Cell *11*, 1467–1478.
15. Robyr, D., Suka, Y., Xenarios, I., Kurdistani, S.K., Wang, A., Suka, N., and Grunstein, M. (2002). Microarray deacetylation maps determine genome-wide functions for yeast histone deacetylases. Cell *109*, 437–446.
16. Kurdistani, S.K., and Grunstein, M. (2003). Histone acetylation and deacetylation in yeast. Nat. Rev. Mol. Cell Biol. *4*, 276–284.
17. Kadosh, D., and Struhl, K. (1997). Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. Cell *89*, 365–371.
18. Goldmark, J.P., Fazzio, T.G., Estep, P.W., Church, G.M., and Tsukiyama, T. (2000). The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. Cell *103*, 423–433.
19. Cioci, F., Vu, L., Eliason, K., Oakes, M., Siddiqi, I.N., and Nomura, M. (2003). Silencing in yeast rDNA chromatin: reciprocal relationship in gene expression between RNA polymerase I and II. Mol. Cell *12*, 135–145.
20. Bernstein, B.E., Tong, J.K., and Schreiber, S.L. (2000). Genomewide studies of histone deacetylase function in yeast. Proc. Natl. Acad. Sci. USA *97*, 13708–13713.
21. Rundlett, S.E., Carmen, A.A., Suka, N., Turner, B.M., and Grunstein, M. (1998). Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3. Nature *392*, 831–835.
22. Reid, J.L., Iyer, V.R., Brown, P.O., and Struhl, K. (2000). Coordinate regulation of yeast ribosomal protein genes is associated with targeted recruitment of Esa1 histone acetylase. Mol. Cell *6*, 1297–1307.
23. Bernstein, B.E., Humphrey, E.L., Erlich, R.L., Schneider, R., Bouman, P., Liu, J.S., Kouzarides, T., and Schreiber, S.L. (2002). Methylation of histone H3 Lys 4 in coding regions of active genes. Proc. Natl. Acad. Sci. USA *99*, 8695–8700.
24. Liu, C.L., Schreiber, S.L., and Bernstein, B.E. (2003). Development and validation of a T7 based linear amplification for genomic DNA. BMC Genomics *4*, 19.
25. Carroll, A.S., Bishop, A.C., DeRisi, J.L., Shokat, K.M., and O'Shea, E.K. (2001). Chemical inhibition of the Pho85 cyclin-dependent kinase reveals a role in the environmental stress response. Proc. Natl. Acad. Sci. USA *98*, 12578–12583.
26. Eisen, M.B., Spellman, P.T., Brown, P.O., and Botstein, D. (1998). Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA *95*, 14863–14868.