The Roles of the Catalytic and Noncatalytic Activities of Rpd3L and Rpd3S in the Regulation of Gene Transcription in Yeast

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Abstract

In budding yeasts, the histone deacetylase Rpd3 resides in two different complexes called Rpd3L (large) and Rpd3S (small) that exert opposing effects on the transcription of meiosis-specific genes. By introducing mutations that disrupt the integrity and function of either Rpd3L or Rpd3S, we show here that Rpd3 function is determined by its association with either of these complexes. Specifically, the catalytic activity of Rpd3S activates the transcription of the two major positive regulators of meiosis, IME1 and IME2, under all growth conditions and activates the transcription of NDT80 only during vegetative growth. In contrast, the effects of Rpd3L depend on nutrients; it represses or activates transcription in the presence or absence of a nitrogen source, respectively. Further, we show that transcriptional activation does not correlate with histone H4 deacetylation, suggesting an effect on a nonhistone protein. Comparison of rpd3-null and catalytic-site point mutants revealed an inhibitory activity that is independent of either the catalytic activity of Rpd3 or the integrity of Rpd3L and Rpd3S.

Introduction

Histone deacetylation plays a pivotal role in the regulation of transcription. Deacetylation of lysine residues in the N-terminal tail domain of histones H3 and H4 correlates with transcriptional repression of most promoters, while transcriptional activation correlates with acetylation of these residues [1-4]. In some cases, histone deacetylation also correlates with transcriptional activation (for reviews and specific examples see 5,6). These activities were revealed using global chromatin immunoprecipitation (ChIP)-on-chip DNA microarray assays [7-9], as well as by direct gene-specific analysis. For example, in budding yeast, activation of osmoregulated genes depends on histone deacetylation through the recruitment of the histone deacetylase Rpd3 by the mitogen-activated protein kinase (MAPK) Hog1 [10]. Similarly, in mammals, deacetylation of cytokine-inducible genes such as interferon–β is essential for enhancing transcription [11]. All eukaryotic genomes contain genes encoding histone deacetylases (HDACs) that reside in large heterogeneous complexes [7,12-15]. In yeast, the HDAC Rpd3 is present in small (0.6 MDa) and large (1.2 MDA) complexes called Rpd3S and Rpd3L, respectively, that include unique and common subunits [7,14]. These complexes are recruited to specific sites on DNA as follows: Rpd3L is recruited to promoters in association with specific transcription factors such as Ume6, Ash1, Ime1, Whi5, and Stb1 [13,16-18]. At the promoter region, Rpd3 deacetylates specific lysine residues on histones H3 and H4 in a localized region spanning about two nucleosomes [19]. In contrast, Rpd3S is recruited to the open reading frame (ORF) by RNA polymerase II upon its phosphorylation by Cdk7/Kin28 on Ser5 within its carboxy-terminal domain [20]. Two of its specific components, Eaf3 and Rco1, recruits it to a methylated lysine residue of histone H3, a modification carried out by Set2 complexed with RNA polymerase II [21,22]. Nonetheless, Rpd3S is also recruited to the promoter of HSP82 [23].The specific subunits of these complexes may affect the stability of the complex or perform different catalytic functions such as histone methylation or chromatin remodeling [21,22,24]. Sin3 and Ume1 are shared by each complex [21,22]. Sin3 functions as a scaffold to assemble different proteins, and it is required to target the complex to specific promoters by its association with specific DNA-binding proteins [15,25,26]. For example, in S. cerevisiae,
Sin3 associates with the Zn-cluster protein Ume6 [19]. Further, HDAC function can lead to global repression via a nonspecific mechanism [27].

The molecular mechanism by which deacetylation activates transcription is still an enigma [5]. Transcriptional modulation may depend on dual roles of specific subunits present in the HDAC complexes [21,22,24]. For example, the Eaf3 subunit of the yeast Rpd3S complex is also a subunit of which is required for spreading of silenced chromatin, is facilitated by the acetylation of histone H4 lysine residue 12. Because this lysine residue is an Rpd3 substrate, it is possible that in cells deleted for Rpd3 the expected increase in acetylation leads to increased Sir3 binding and consequently reduced transcription. This is one example explaining how Rpd3 functions as a positive regulator [8].

In the budding yeast Saccharomyces cerevisiae, initiation of meiosis depends on nitrogen depletion in the absence of glucose and the presence of a nonfermentable carbon source such as acetate. Nitrogen depletion activates a transcriptional cascade, which is roughly divided into genes expressed at early (EMG), middle (MMG), and late (LMG) times during meiosis, which are all controlled by the master regulator Ime1. In S. cerevisiae, the histone deacetylase Rpd3 functions dynamically in the transcriptional repression and activation of IME1, EMGs, MMGs, and LMGs. For example, Rpd3 functions as a positive regulator of IME1 transcription early in meiosis and as a negative regulator that is required to inhibit IME1 transcription during late meiosis [16]. The effect is direct, because both Rpd3 and Sin3 are recruited by Ime1 to the IREu element in its promoter [16], to which Ime1 itself is recruited by the transcription factor Msn2/4 [28]. Rpd3 functions as a negative regulator of EMGs following recruitment to their promoters by Ume6 [19]; Ume6 binds to a specific sequence (URS1), which is present and active in all EMGs as well as in other genes not involved in meiosis [29-31]. Rpd3 functions as a positive, crucial regulator of the transcription of MMGs, LMGs, and the early-middle gene NDT80, which encodes the direct transcriptional activator of MMGs [32]. It is not known if Rpd3 binds to the promoters of these genes.

Our aim here was to determine how Rpd3 exhibits opposite functions when regulating genes encoding the components of different meiotic networks and how its affects on the transcription of a specific gene are modulated during different stages of meiosis. We postulated that the Rpd3L and Rpd3S might possess distinct functions. We show that Rpd3L and Rpd3S possess specific functions as follows: the catalytic activity of Rpd3 present in Rpd3S is required for the efficient transcription of IME1 and IME2 (representative EMGs) during vegetative growth with acetate as the sole carbon source (SA medium) or upon nitrogen depletion with acetate as the sole carbon source (SPM medium, meiotic conditions). This complex also activates the transcription of NDT80 but only in SA medium. In contrast, in SA medium, Rpd3L functions as a negative regulator for all genes, whereas upon nitrogen depletion it functions as a positive regulator early during meiosis. Moreover, we reveal a novel function of Rpd3, which is independent of its presence in intact Rpd3L or Rpd3S that repressed the transcription of all meiosis-specific genes during late meiosis; and in SA medium, it repressed transcription of only IME1 and IME2. Finally, we show that the essential positive role of Rpd3 on the transcription of NDT80 is not mediated through an effect on the pachytene checkpoint, a noncoding antisense RNA, or on the function of either Ndt80 or Sum1, which activate and repress the transcription of NDT80, respectively.

Materials and Methods

Strains and Plasmids

The relevant genotypes of the isogenic strains and plasmids used in this study are listed in Tables 1 and 2, respectively. Details on how these strains and plasmids were constructed are available upon request.

Media and Molecular Genetic Techniques

SA (PSP2) and SPM media were prepared as reported previously [33]. Meiosis was induced as follows: Cells grown to early exponential stage (0.8–1.23 × 10^7 cells/ml) in SA medium supplemented with the required amino acids were washed once with water and resuspended in SPM. β-Galactosidase assay: Proteins were extracted from 30 ml cells (1 × 10^7 cells/ml) as described [34], and assayed for β-Galactosidase activity as described [35]. β-gal in Miller units were calculated per mg protein. Protein was measured using Bio-Rad Bradford kit. Staining with 4’,6-diamidino-2-phenylindole (DAPI) [36], and repression assays [37] were performed as described previously.

Fluorescence-activated Cell Sorting (FACS)

Cellular DNA content was determined using FACS as described previously [38] using a FACS can analyzer (BD Biosciences, San Jose, CA). The percentage of cells with 4C DNA content was calculated using the WinMDI program (Joe Trotter, The Scripps Research Institute, La Jolla, CA, USA).

Quantitative Analysis of RNA Expression

RNA was extracted from 1 × 10^6 cells using the hot acidic-phenol method [39]. One microgram of total RNA was used as template for the reverse transcription reaction (total 20 µl) with random hexamer primers and SuperScript Reverse-iT transcriptase. Five nanograms of the cDNA product served as template for real-time polymerase chain reaction (qPCR) analysis according to the manufacturer’s instructions (ABGene, Surrey, UK). Primers used were: IME1: C4GCTGCGAGAATTGGTTCA and GTGGAAGCTAGATGCGGATT (199 to 438); IME2: TAGGCGCAAAGGAAGCAATTG and ATCGTGATCGTTGTTGCTGA (1181 to 1341; NDT80: CTCGTAATCCACACCAATGTG and CGGTTCTAGTTCCATTGTGCT (1182 to 1428); NDT80AS: AAATGGAGGGCAATTATAAGG and CTTTGAATATACATAGTGTTTC (356 to -85); SUM1: TCTACGACCTCCTGCACAAT and CGGTCATCAAGGAAGTCAAA (2981 to 3114); TAF10:
### Table 1. List of Strains.

| Relevant genotype | Remarks | Reference |
|-------------------|---------|-----------|
| **MATα haploids** |         |           |
| Y1064 ura3-52, leu2-3-112, trp1Δ, his3Δ::hisG, ade2-1, metX, gal80Δ::hisG, gal4Δ::hisG | Y1064 derivative using YIp1408 and Yip1875 | [66] |
| Y1043 ime1Δ::hisG, leu2,3-112::LEU2-IME1(+3.2 to +200)-lacZ | Y1064 derivative using Yip1408 |           |
| Y1075 ime1Δ::hisG | Y1064 derivative using Yip1408 |           |
| Y1179 leu2,3-112::LEU2-GAL1uas-HIS4uas-his4-lacZ | Y1064 derivative using Yip2218 |           |
| Y1214 leu2,3-112::LEU2-UASru-his4-lacZ | Y1064 derivative using Yip2102 |           |
| Y1332 ure3Δ::hisG | Y1064 derivative using Yip1583 |           |
| Y1381 leu2,3-112::LEU2-GAL1uas-HIS4uas-his4-lacZ, ure3Δ::hisG | Y1332 derivative using Yip2218 |           |
| Y1535 rpm3Δ::HIS3, ime1Δ::hisG, leu2,3-112::LEU2-IME1(+3.2 to +200)-lacZ | Y1304 derivative using Yip2566 |           |
| Y1762 ndt80ΔC::IME2p-NDT80-TRP1, ime1Δ::hisG | Y1075 derivative using Yip3078 |           |
| Y1765 rpm3::RPD3-13xmyc-URA3 | Y1064 derivative using Yip3081 |           |
| Y1813 rpm3::RPD3-13xmyc-URA3, leu2,3-112::LEU2-GAL1uas-HIS4uas-his4-lacZ | Y1179 derivative using p3109 |           |
| Y1827 rpm3::RPD3-13xmyc-URA3, leu2,3-112::LEU2-UASru-his4-lacZ | Y1214 derivative using P3153 |           |
| Y1843 sds3Δ::HIS3, leu2,3-112::LEU2-GAL1uas-HIS4uas-his4-lacZ | Y1179 derivative using P3144 |           |
| Y1846 rpm3Δ::HIS3, rad17Δ::URA3 | Y1535 derivative using Yip3158 |           |
| Y1879 rpm3Δ::HIS3 | Y1064 derivative using Yip2566 |           |
| Y1881 rpm3::HIS3, sds3Δ::HIS3, leu2,3-112::LEU2-GAL1uas-HIS4uas-his4-lacZ | Y1813 derivative using p3144 |           |
| Y1893 rpm3::HIS3, sds3Δ::HIS3, leu2,3-112::LEU2-UASru-his4-lacZ | Y1179 derivative using P3215 |           |
| Y1913 rpm3::HIS3, sds3Δ::HIS3, leu2,3-112::LEU2-UASru-his4-lacZ | Y1827 derivative using P2566 |           |
| Y1943 sds3::SDS3-13myc-TAD1-HIS3 | Y1064 derivative using Yip3182 |           |
| Y1948 sds3::SDS3-13myc-TAD1-HIS3, rpm3::RPD3-13xmyc-URA3, rco1::RCO1-6HA-K1TRP1 | Y1943 derivative using Yip3179 |           |
| Y2057 rpm3::HIS3, rpm3::TRP1::TRP1- rpm3H150AH151 | Y1879 derivative using Yip3316 |           |
| Y2060 rpm3Δ::HIS3, rpm3::TRP1- rpm3H150AH151 | Y1879 derivative using Yip3316 |           |
| **MATa/MATα diploids** | | |
| Y1065 ura3-52, trp1Δ, ade2-1, his3Δ::hisG, ade2-9, gal80Δ::hisG, gal4Δ::hisG | Y1065 derivative using Yip3081 |           |
| Y1328 ure3Δ::hisG-URA3-hisG | Y1065 derivative using Yip3081 |           |
| Y1536 rpm3Δ::HIS3 | Y1065 derivative using Yip2566 |           |
| Y1761 ndt80 ΔC::IME2p-NDT80-TRP1 | Y1065 derivative using Yip3078 |           |

### Table 1 (continued).

| Relevant genotype | Remarks | Reference |
|-------------------|---------|-----------|
| Y1766 rpm3::RPD3-13xmyc-URA3 | Y1065 derivative using Yip3081 |           |
| Y1816 rpm3::URA3 | Y1065 derivative using Yip3109 |           |
| Y1844 rpm3::HIS3 | Y1065 derivative using Yip3144 |           |
| Y1847 rpm3::HIS3, rad17Δ::URA3 | Y1536 derivative using Yip3158 |           |
| Y1877 rpm3::HIS3, rad17Δ::URA3 | Y1065 derivative using Yip3153 |           |
| Y1880 rpm3::HIS3 | Y1065 derivative using Yip2566 |           |
| Y1882 rpm3::HIS3 | Y1065 derivative using Yip2566 |           |
| Y1892 rpm3::HIS3, sds3Δ::HIS3 | Y1816 derivative using P3144 |           |
| Y1944 sds3::SDS3-13myc-TAD1-HIS3 | Y1065 derivative using Yip3182 |           |
| Y1949 sds3::SDS3-13myc-TAD1-HIS3, rpm3::RPD3-13xmyc-URA3, rco1::RCO1-6HA-K1TRP1 | Y1944 derivative using Yip3179 |           |
| Y2058 rpm3::HIS3, rpm3::TRP1- rpm3H150AH151 | Y1880 derivative using Yip3315 |           |
| Y2061 rpm3::HIS3, rpm3::TRP1- rpm3H150AH151 | Y1880 derivative using Yip3315 |           |
ATATTCCAGGGATCAGGTCTTCGCTGAC and GTAGCTTCTCATTCTGTTGATGTTGTGTTTG (390 to 530). SUM1 and TAF10 RNAs were used as controls because the transcription of both genes does not fluctuate in meiosis (http://derisilab7.ucsf.edu:591/public_spo/FMPro? respectively).

**Table 1 (continued).**

| Relevant genotype | Remarks | Reference |
|-------------------|---------|-----------|
| dep1::HisG-dep1::HisG, leu2-3,112/leu2-3,112::LEU2::GAL1::His54::his4-lacZ | | Y1892 x Y1893 |
| sum1::URA3::sum1::URA3, rpd3::::HIS3 | | |
| Y1914 rpd3::::HIS3, leu2-3,112::LEU2::UASu::his4-lacZ/leu2-3,112 | Y1878 x Y1913 |
| Y1950 sds3::::SDS3-13myc-ADH1::HIS3/ sds3::::SDS3-13myc-ADH1::HIS3, rco1::::RCO1-6HA-k1TRP1/orc1::::RCO1-6HA-k1TRP1 | Y1948 x Y1949 |
| Y2059 rpd3::::HIS3/ rpd3::::HIS3, trp1::::TRP1-RPD3/trp1::::TRP1-RPD3 | Y2057 x Y2058 |
| Y2062 rpd3::::HIS3/ rpd3::::HIS3, trp1::::TRP1-RPD3/rpd3::::HIS3/ rpd3H150AH151/trp1::::TRP1-rpd3H150AH151 | Y2060 x Y2061 |

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**Table 2. List of plasmids.**

| Name | Details | Remarks | Reference |
|------|---------|---------|-----------|
| P1408 | B5, ime1::HisG-URA3::HisG | IME1 deletion is from −1118 to +946 |
| Ypl875 | pBR322, URA3, ime1::(−3.2 to +200)−lacZ/ lacZ | |
| P1583 | B5, um6::HisG-URA3::HisG | UME6 is disrupted at aa 158 |
| Ypl2192 | pBR322, LEU2, IME1::UASu::his4-LacZ | [28] |
| YEp2149 | pBR322, TRP1, 2µ, pADH1-ADH1::GAL4(dbd) | [37] |
| Ypl2218 | pBR322, LEU2, GAL1::His4::his4-lacZ | [37] |
| Ypl2566 | B5, LEU2, rpd3::::HIS3 | RPD3 deletion is from +84 to +744 |
| YEP2593 | pBR322, TRP1, 2µ, ADH1p::GAL4(dbd)-Rpd3-ADH1 | |
| P3190 | T-easy, rco1::::URA3 | RCO1 deletion from −146 to +2498 |
| P3144 | pUC18, sds3::::HIS3 | SDS3 complete ORF deletion |
| P3215 | T-easy, dep1::::HisG-URA3::HisG | DEP1 deletion from −216 to +1365 |
| P3153 | T-easy, sum1::::URA3 | SUM1 deletion is from −1 to +3189 |
| Ypl3158 | T easy, rad17::::URA3 | complete deletion of ORF |
| Ypl3179 | rco1::::(−876 to −2051)−6H-ott1TRP1 | |
| Ypl3182 | sds3::::(−471 to +963)−13myc-ADH1, HIS3 | |
| Ypl3315 | RPD3::::−350 to +100, TRP1 | |
| Ypl3316 | rpd3H150AH151::TRP1 | PEN153 (F. Posas) derivative |

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During Vegetative Growth with Acetate as the Sole Carbon Source, Rpd3L Represses Transcription,
assessed the effect(s) of Rpd3L and Rpd3S to induce specific effects. Therefore, we determined the role of deletion of his4-lacZ source (SA medium). Deletion of repression was mediated via Rpd3L (Table 3). Because were as follows: wild-type (Y1884), 10 cells/ml. The activity of β-galactosidase (Miller units) in cells expressing Gal4(dbd)-Rpd3 is relative to the level in the control cells (c) expressing only Gal4(dbd). Diploid strains used were as follows: wild-type (Y1884), rco1Δ/rco1Δ (Y1814), sds3Δ/sds3Δ (Y1845), dep1Δ/dep1Δ (Y1894), ume6Δ/ume6Δ (Y1388), and rco1Δ/rco1Δ sds3Δ/sds3Δ (Y1883). These strains carried either pADH1-gal4(dbd)-Rpd3 (YEp2593) or pADH1-gal4(dbd) (YEp1429) on a 2-µ vector. Proteins were extracted from at least three independent transformants.

whereas Rpd3S Activates the Transcription of Meiosis-Specific Genes

The requirement for both Rpd3L and Rpd3S to repress transcription of the artificial reporter gene (UASgal-UAShis4-lacZ) in SD medium implies that they regulate meiosis-specific genes using the same mechanism. However, it is also possible that under physiological conditions these complexes possess specific functions determined by interaction with specific proteins present on the promoters of target genes that induce specific effects. Therefore, we determined the role of Rpd3L and Rpd3S complexes in regulating the transcription of genes that encode components of meiotic networks.

During vegetative growth with glucose as the sole carbon source, and independent of Rpd3, IME1 is not transcribed [16,42,43]. Therefore, we assessed the effect(s) of Rpd3L and Rpd3S on vegetative growth with acetate as the sole carbon source (SA medium). Deletion of RPD3 caused a significant increase in IME1 transcription (Table 3), suggesting that Rpd3 represses IME1 transcription. Deletion of either SDS3 or DEP1 caused a comparable increase in transcription, indicating that repression was mediated via Rpd3L (Table 3). Because deletion of RCO1 led to a significant reduction in the transcription of IME1 (Table 3), this suggests that Rpd3S functions as a positive regulator. Further, Rpd3 is recruited to the IME1 promoter [16], suggesting that Rpd3S and/or Rpd3L exert a direct effect on the transcription of IME1. When both complexes were disrupted (rco1Δ sds3Δ-double mutant) (Table 3), the transcription of IME1 was reduced, suggesting that the increase in the transcription of IME1 in the sds3Δ strain depended on Rco1. Moreover, this reduced transcription was unexpected, because an opposite effect, namely transcription was increased in the rpd3Δ strain (Table 3). This result suggests that Rpd3 represses the transcription of IME1 through an additional mechanism, which is independent of its presence and/or activity in Rpd3S or Rpd3L.

The effect on the level of transcription of IME2 was similar to that observed for IME1, namely, repression by Rpd3L and activation by Rpd3S. Thus, transcription was increased in the rpd3Δ, sds3Δ, and dep1Δ strains (Table 3), and was reduced in the rco1Δ strain (Table 3). Because the transcription of IME2 absolutely depends on Ime1 [44], these results indicate that the effect of Rpd3L and Rpd3S on IME2 is indirect and is mediated via their effect on IME1. However, the rco1Δ sds3Δ-double mutant exhibited a specific phenotype, that is, the transcription of IME2 increased whereas that of IME1 was reduced (Table 3), suggesting that the effect of Rpd3 on IME2 transcription is also direct. Moreover, the results of ChIP assays revealed that Rpd3 binds the IME2 promoter in SA medium (Figure 2, time 0).

The transcription of NDT80 in SA medium was increased in cells with deletions of either SDS3 or DEP1 (Table 3), suggesting that Rpd3L functions as a negative regulator of NDT80 transcription. In contrast, deletion of a gene encoding an Rpd3S component, RCO1, reduced transcription, suggesting that Rpd3S functions as a positive regulator. Deletion of SDS3 along with RCO1 reduced transcription...
similarly, suggesting that the increase in transcription of NDT80 by the sds3Δ mutant depends on Rco1. Deletion of RPD3 had no detectable effect (Table 3), suggesting that the negative and positive effects of Rpd3L and Rpd3S counteracted each other, leading to no net effect. Moreover, the data also suggest that Rpd3 functions independently of Rco1-Sds3.

Table 4 summarizes these results, which show during vegetative growth with acetate as the sole carbon source, Rpd3L inhibits transcription of IME1, IME2, and NDT80, whereas Rpd3S activates their transcription. These findings are consistent with our hypothesis stated above regarding the specific functions of these complexes.

Figure 2. The kinetics of Rpd3 binding, histone H4 acetylation, and transcription of meiosis-specific genes. MATa/MATα RPD3-13xmyc/RPD3-13xmyc (Y1767) cells were shifted to meiotic conditions (SPM media) for the times indicated and subjected to ChIP analysis to determine Rpd3 binding (white column) and acetylated H4 (gray column). Sequences of the IME2 (A), and NDT80 promoters (B), or the TEL1 locus were amplified using qPCR. Enrichment values represent the ratio between the relative levels of PCR amplicons recovered from the specific versus the non-specific probe, then the bound fraction was divided by input. Samples were taken simultaneously to isolate RNA for qPCR analysis (black line with triangles).

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Summary of results: A positive and negative role for the catalytic activity of Rpd3S was assigned when the deletion of RCO1 alone and together with SDS3 resulted in a decrease and increase, respectively, in transcription (Figures 2 and 4). A positive and negative role for the catalytic activity of Rpd3L was assigned when the deletion of SDS3 alone and together with RCO1 resulted in a decrease and increase, respectively, in transcription (Figures 2 and 4). A positive and negative roles for the non-catalytic activity of Rpd3 was assigned when the deletion of RPD3 resulted in the opposite result from rco1 sds3 and rpd3H150AH151A mutants (Table 3 and Figure 3).

The Role of Rpd3L and Rpd3S in the Expression of Meiosis-specific Genes during Meiosis

We examined the patterns of transcription of IME1, IME2 and NDT80 during meiosis of mutants with deletions of Rpd3L and Rpd3S components described above. Rpd3S and Rpd3L activate IME1 transcription throughout meiosis. Deletion of either RCO1 or SDS3 reduced IME1 transcription, albeit the effect of the absence of Rco1 was greater (Figure 3). The pattern of IME1 expression of the rco1Δ sds3Δ double mutant suggests that at early meiotic times the effect was mediated mainly by Rpd3S (Rco1) while at later times by Rpd3L (Sds3). Moreover, the epistatic relationship between RCO1 and SDS3 (no additive effect when both genes were deleted) suggests that these complexes deacetylate the same substrate. We expected that deleting DEP1, which is a specific component of Rpd3L, would exhibit the same phenotype as deleting SDS3, and that deletion of RPD3 will give the same phenotype as deletion of both RCO1 and SDS3, namely reduced transcription. However, this was not the case, because early in meiosis, Dep1 and Rpd3 functioned as positive regulators, but at later times as negative regulators (Figure 4), suggesting that Dep1 and Rpd3 may possess additional functions not in common with Sds3 and Rco1.

The transcription of IME2 was increased throughout meiosis in a strain deleted for RPD3 (Figure 3), suggesting that Rpd3 inhibits IME2 transcription. We assumed that Rpd3 mediates repression, because Rpd3 is recruited to IME2 by Ume6, which is a component of this complex [13,19]. However, to our
Fig. 3. The effect of Rpd3L and Rpd3S mutations on the transcription of meiosis-specific genes. RNA was purified from cells grown to a density of 1x10^7 cells/ml in SA and transferred to SPM for the indicated times. Isogenic strains were as follows: wt (Y1631), rpd3Δ/rpd3Δ (Y1888), rco1Δ/rco1Δ (Y1814), sds3Δ/sds3Δ (Y1845), dep1Δ/dep1Δ (Y1894), rco1Δ/ dep1Δ sds3Δ/sds3Δ (Y1883) rpd3Δ/ rpd3Δ (Y1888), rpd3Δ/ rpd3Δ trp1Δ::TRP1-RPD3/trp1Δ::TRP1-RPD3 (Y2059), and rpd3Δ/ rpd3Δ trp1Δ::TRP1-rpd3H150AH151A/trp1Δ::TRP1-rpd3H150AH151A (Y2062) diploids. The levels of expression of IME1 IME2 and NDT80 were determined using q-RT PCR and are expressed relative to those of either SUM1 or TAF10 (for strains Y2059 and Y2062). The levels shown here are relative to the level of wt at time 0. The results shown are from a representative experiment. Similar results were obtained for at least three independent experiments.

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surprise, deletion of SDS3 decreased transcription rather than increasing it (Figure 3). This reduction in transcription might be indirect through reduced IME1 transcription, because the level of transcription of IME2 responds in a gradient mode to Ime1 levels [45]. A different picture emerged when we examined the effect of the Rpd3L component Dep1. Deletion of DEP1 reduced IME2 transcription similarly to that of the sds3Δ strain. However, during late meiosis, transcription increased, similar to that of the RPD3 deletion mutant (Figure 3).

IME2 transcription in the rco1Δ diploid did not resemble that of the rpd3Δ strain (Figure 3). Moreover, it was not as robust as wild-type, because complete induction took significantly longer (Figure 3). Evidence indicates that the reduction in the level of IME1 transcription immediately reduces that of IME2 [45], suggesting that the positive effect of Rco1 (Rpd3S) on IME2 transcription is direct. The transcription of IME2 in the rco1Δ sds3Δ-double mutant resembled the rco1Δ pattern but differed from that of rpd3Δ (Figure 3), suggesting that Rpd3 repressed the transcription of IME2 independent of Rpd3S and Rpd3L.

Next, we determined the effects of Rpd3L and Rpd3S on the transcription of NDT80. In diploid cells with deletions of SDS3, DEP1, or RPD3 the usual induction of NDT80 transcription was not detected (Figure 3). This result could not be attributed to the effect on IME1 and IME2 transcription, because this would only delay NDT80 [45]. Therefore, we conclude that Rpd3L mediates activation of NDT80 transcription. In contrast, in
diploid cells with \(R{C}O1\) deletion, the transcription of \(N{D}T80\) was initiated with a delay, but it reached the same level as that of the isogenic wild-type strain (Figure 3). Because the transcription of \(N{D}T80\) is absolutely dependent on \(I{M}E2\) [46], we suggest that the effect of Rco1 on \(N{D}T80\) is mediated through Ime2 either directly (Ime2 may recruit Rpd3S), or indirectly (the reduced level of Ime2 delayed the transcription of \(N{D}T80\)).

The level and pattern of expression of \(N{D}T80\) in the \(r{C}o1\Delta\) \(s{d}s3\Delta\)-double mutant was similar to that observed for the \(s{d}s3\Delta\) or the \(r{D}P3\Delta\) isogenic strains for 12 hours in SPM (Figure 3), suggesting that the positive effect of Rpd3 is mediated solely by Rpd3L. However, after 24 hours in SPM, transcription in the \(R{D}P3\Delta\) deletion mutant increased while in the \(r{C}o1\Delta\) \(s{d}s3\Delta\) mutant, the level of expression remained low (Figure 3). This result suggests that late in meiosis, the decline in the transcription of these genes requires Rpd3 but not Rco1 and Sds3.

In summary (Table 4), under meiotic conditions, both Rpd3S and Rpd3L function as positive regulators of \(I{M}E1\) and \(I{M}E2\) transcription. In contrast, \(N{D}T80\) transcription is positively regulated only by Rpd3L. Rpd3 also functions as a negative regulator independent of its presence in the Rpd3L and Rpd3S complexes.

**Rpd3L is required for Meiotic Nuclear Division whereas Rpd3S does not Affect Meiosis**

The above results indicate that Rpd3S functions as a nonessential positive regulator of \(I{M}E1\) and \(I{M}E2\) and plays no role in \(N{D}T80\) transcription (Figure 3). Because meiosis is robust and insensitive to the levels of its positive regulators (Ime1, Ime2, and Ndt80) [45], as expected, diploid \(R{C}O1\)-deletion mutants sporulated, producing 81.4% ascis after 48 h culture in SPM. In contrast, deletion of specific components of the Rpd3L complex, \(S{D}S3\) and \(D{E}P1\), reduced \(I{M}E1\) and \(I{M}E2\) transcription but \(N{D}T80\) was not transcribed (Figure 3). These results predict that diploid cells harboring mutations in Rpd3L components will arrest in the meiotic pathway following completion of premeiotic DNA replication but prior to nuclear division. Note that Ndt80 is required for the transcription of the middle meiosis-specific genes, which encode proteins required for nuclear division [47]. In agreement with this prediction, diploid cells with either \(D{E}P1\) or \(S{D}S3\) deletions along with \(R{C}O1\) deletions, completed premeiotic DNA replication and accumulated cells with a single nucleus (Figure 4).

**Transcriptional Repression and Catalysis by Rpd3 can Function Independently**

The results described above demonstrate that Rpd3 represses transcription during the late stages of meiosis independent of either Sds3 or Rco1 (Table 3 and Figure 3). Similarly, using a \(l{e}xA-U{m}e6\) reporter it was reported that Rpd3 may repress transcription independent of its histone deacetylase activity [48]. Moreover, the mechanism of repression apparently involves nucleosome stabilization by the Rpd3 core complex [49]. We postulated that diploid cells expressing catalytically inactive Rpd3 would exhibit a different phenotype than cells with a deleted \(R{D}P3\Delta\) allele and phenotype similar to that of \(s{d}s3\Delta\) \(r{C}o1\Delta\) diploids. A catalytic inactive Rpd3 mutant was constructed by mutating His150 and His151 to Ala [48, 49]. The mutant and wild-type (control) genes were each inserted into the \(T{R}P1\) loci of an \(R{D}P3\Delta\)-deletion mutant, and \(I{M}E1\), \(I{M}E2\), and \(N{D}T80\) transcription was measured during vegetative growth with acetate as the sole carbon source (Table 3) as well as during meiosis (Figure 3).

\(I{M}E1\) transcription in the \(r{p}d3\Delta H150A H151A\) and \(s{d}s3\Delta\) \(r{C}o1\Delta\) strains was reduced but increased in the \(r{p}d3\Delta\) diploid cultured in SA (Table 3), suggesting that as predicted, \(I{M}E1\) transcription was repressed by a noncatalytic function of Rpd3.

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**Figure 4.** Diploids with DEP1/RCO1 or SDS3/RCO1 deletions arrest in meiosis before nuclear division. Isogenic wt (Y1631, closed squares), dep1\Δ/dep1\Δ (Y1894, open circles), and rco1\Δ/rco1\Δ sds3\Δ/sds3\Δ (Y1883, open triangle) diploids were shifted to meiotic conditions (SPM medium). Samples were taken at the indicated times for FACS analysis to calculate the percentage of cells with 4C DNA content and to count the percentage of cells with more than 2 nuclei (DAPI stain).

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IME1 transcription was reduced during late meiosis in the wild-type as well as strains carrying the rpd3H150AH151A allele or sds3Δ rco1Δ double mutants. In contrast, IME1 expression increased in the rpd3Δ strain (Figure 3), demonstrating that its transcription was independent of the catalytic activity of Rpd3.

IME2 transcription was elevated in cells carrying the rpd3H150AH151A allele and grown in SA medium, similar to the increase observed in cells with RPD3, SDS3, DEP1 deletions or SDS3/RCO1-double deletion (Table 3), implying that only the catalytic activity of Rpd3L repressed IME2 transcription. Nonetheless, deletion of RPD3 increased transcription by a factor of 18, in contrast to the two-fold increase in cells expressing the catalytically active mutant. This result suggests that the catalytic and noncatalytic functions of Rpd3 repress IME2 transcription. At late meiotic times IME2 transcription was decreased in cells that expressed the catalytically inactive Rpd3 allele, although it was elevated in cells lacking Rpd3 (rpd3Δ) (Figure 3). The results suggest that under this condition Rpd3 activity was noncatalytic, reinforcing the conclusion that at this time, repression by Rpd3 was independent of its presence in Rpd3L or rpd3S.

Expression of a catalytically inactive Rpd3 mutants cultured in SA medium reduced NDT80 transcription similar to the level of the rco1Δ strain (Table 3), implying that the catalytic activity of Rpd3 in Rdp3S activates NDT80 transcription. During early meiosis, the phenotypes of the rpd3H150AH151A and rpd3Δ strains were similar (Figure 3), indicating that only the catalytic activity of Rpd3 was required for transcriptional activation of NDT80. However, transcription increased in the mutant carrying the point mutations in comparison to the wild-type strain (Figure 3), suggesting that transcriptional repression was mediated by the noncatalytic function of Rpd3.

Therefore, our results validate the prediction that Rpd3 functions catalytically as well as noncatalytically. The catalytic activity exerted either positive or negative effects, whereas the noncatalytic activity only repressed transcription (Table 4).

**NDT80 Transcription Correlates with Histone H4 Acetylation**

Deacetylation of lysine residues in the N-terminal tail domain of histones H3 and H4 is associated with either transcriptional repression or activation [1-4]. Therefore, using qChIP assays, we asked whether NDT80 transcription depends on deacetylation of histone H4. The mRNA level was determined simultaneously. We amplified NDT80 and IME2 as a control to represent early meiosis-specific genes.

IME2 transcription correlated with reduced Rpd3 binding to IME2 and a concomitant increase in histone H4 acetylation at the IME2 promoter region (Figure 2A) in agreement with previous findings [50]. Moreover, the decline in transcription correlated with increased Rpd3 occupancy and decreased acetylation (Figure 2A). Acetylation and transcriptional activation of NDT80 also correlated (Figure 2B). However, Rpd3 binding to the NDT80 promoter was detected only during late meiosis when NDT80 transcription was reduced (Figure 2B), suggesting that the effect of Rpd3 on the transcription of NDT80 was not mediated by deacetylation of histone H4.

**Possible Mechanisms of Transcriptional Activation by Rpd3**

The pachytene checkpoint. Cells impaired in meiotic recombination arrest in meiosis before NDT80 transcription commences [32], an arrest that depends on the pachytene checkpoint pathway (for review see 51). Because Rpd3 is required for the appropriate response of the ATM checkpoint to double-strand DNA breaks [52], we postulated that Rpd3 is required to relieve the inhibition mediated by the checkpoint during the recombination process, to promote the NDT80 transcription. This hypothesis predicts that deletion of RAD17 (a checkpoint component) would suppress rpd3Δ and promote NDT80 transcription. This hypothesis was discarded, because rpd3Δ/rpd3Δ rad17Δ/rad17Δ diploids remained sporulation deficient and did not express NDT80 or SPS1 (a mid-meiosis-specific gene) (Figure 5A).

Noncoding RNA. Numerous noncoding sense and antisense RNAs are transcribed from the yeast genome [53-55]. These RNAs might interfere with the transcription of coding RNA as reported, for example for the meiosis-specific genes IME4 [56] and IME1 [57]. Because the NDT80 promoter drives the transcription of an antisense RNA (~78 to ~390, http://yeast.ugtene.org/ and [53]), we postulated that this RNA inhibits transcription of coding sequences. Moreover, we suggested that Rpd3 is required to repress the transcription of this NDT80-antisense RNA and consequently promotes the transcription of NDT80. However, this hypothesis was disproved, because the levels of this antisense transcript were similar in wild-type and rpd3Δ diploids (Figure 5B). Moreover, the transcription of this antisense RNA was induced at the same time as the coding RNA (Figure 5B).

Deacetylation of nonhistone proteins. Proteomic analysis reveals that many, nonhistone proteins are subjected to acetylation, which affects their function (for review see 58). Because NDT80 transcription correlated with histone H4 acetylation rather than deacetylation (Figure 2B), and because it depended on the catalytic activity of Rpd3, we postulated that the positive effect of Rpd3 was mediated through a nonhistone protein. The transcription of NDT80 is repressed by Sum1 and activated by Ime1, Ime2, and Ndt80 [59]. Therefore, we first asked whether Sum1 acetylation is required for its ability to repress NDT80 transcription and whether its deacetylation by Rpd3 relieves this repression. This hypothesis predicts that deletion of SUM1 will suppress rpd3Δ; however, a sum1Δ rpd3Δ diploid strain was sporulation deficient, and NDT80 was not transcribed (Figure 6A). We conclude, therefore, that the effect of Rpd3 is not solely mediated by deacetylation of Sum1.

We next asked whether deacetylation of Ndt80 is required for its activity and consequently for transcription of mid-meiosis-specific genes and initiation of nuclear division. This hypothesis predicts that ectopic expression of Ndt80 will not suppress rpd3Δ; however, NDT80 expression controlled by the IME2 promoter led to a significant increase in the transcription of the mid-meiosis-specific gene SPS1 (Figure 6B) as well as the accumulation of cells with two nuclei (Figure 6B). The effect was only partial, likely because ectopic expression of NDT80 in the wild-type strain is deleterious; cells initiated premeiotic DNA replication and nuclear division simultaneously [45]. We
conclude, therefore, that the effect of Rpd3 on the transcription of \textit{NDT80} and mid-meiosis-specific genes is not mediated solely by an effect on the function of Ndt80.

**Discussion**

The choice between alternative developmental pathways is mainly controlled at the level of transcription. Frequently, genes
that are specific for one pathway are silenced in cells that are engaged in an alternative pathway. Gene inactivation can result from lack of specific activators and from active repression. Thus, during vegetative growth of budding yeasts, histone deacetylation mediated by Rpd3 silences EMGs [19,60]. During meiosis, the transcription of these genes requires two Ime1-dependent events, relief of repression, and transcriptional activation [37]. The effect of Rpd3 on meiosis is not mediated through its effect only on EMG, because it regulates the transcription of all meiosis-specific genes that

Figure 6. Possible molecular mechanisms of transcriptional activation by Rpd3. A. Deletion of SUM1 did not suppress rpd3Δ. Isogenic wild-type (Y1631, closed black squares), rpd3Δ/rpd3Δ (Y1888, empty black squares, dashed line) and rpd3Δ/rpd3Δ sum1Δ/sum1Δ (Y1914, gray circles, dashed lines). B. Ectopic transcription of NDT80 partially suppressed the effect of rpd3Δ on the transcription of SPS1 and nuclear division. Isogenic NDT80/NDT80 (Y1631, black squares), IME2p-6xHA-NDT80/pIME2p-6xHA-NDT80 (Y1763, gray triangles), rpd3Δ/rpd3Δ (Y1537, empty gray squares, dashed gray lines) and rpd3Δ/rpd3Δ IME2p-6xHA-NDT80/pIME2p-6xHA-NDT80 (Y1870, empty gray triangle, dashed gray lines) cells were shifted to meiotic conditions (SPM medium), and at the indicated times, samples were taken for RNA extraction and DAPI staining to determine the percentage of cells with more than 1 nucleus. NDT80 expression was measured using q-RT PCR. Levels of expression are relative to that of ACT1. The results of a representative experiment are shown. Similar results were obtained from three independent experiments.

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differ in magnitude and stage. For example, Rpd3 functions as a positive regulator of IME1 early in meiosis and as a negative regulator at later stages ([16] and Figure 3). NDT80 transcription is similarly influenced, notwithstanding that Rpd3 is indispensable for NDT80 transcription as well as mid- and late meiosis-specific genes ([32] and Figures 3, 5-6).

Our goal here was to determine the mechanisms underlying the dual and opposing functions of Rpd3. We reasoned that a likely explanation is the presence of Rpd3 in two distinct complexes that target specific genes. To study the functions of Rpd3 in either Rpd3L or Rpd3S, we genetically deleted SDS3 or DEP1 from Rpd3L and RCO1 from Rpd3S. We studied the effect of Rpd3 on the transcription of three major positive regulators of meiosis as follows: 1) IME1, the master transcriptional activator, is essential for the transcription of all meiosis-specific genes; 2) IME2, a representative of the network of early genes, is essential for the transcription of middle and late genes; and 3) NDT80, an early-middle gene, is essential for the transcription of middle and late genes (for review see 61). These genes respond in specific modes to Rpd3, and thus serve as a paradigm to study the requirements for Rpd3L and Rpd3S. The results show that Rpd3 has at least four distinct modes of action, depending on the gene(s) studied and growth conditions as follows: 1) Rpd3L and Rpd3S require Rpd3 to repress transcription. 2) Rpd3 functions only as a positive regulator in Rpd3S. 3) Rpd3 switches from a negative to a positive regulator depending on nitrogen depletion in Rpd3L. 4) The noncatalytic activity of Rpd3, which is independent of the integrity of Rpd3L and Rpd3S, represses transcription. Figure 7 summarizes the results for the control of the meiosis-specific genes IME1, IME2 and NDT80 by Rpd3.

The Integrity of both Rpd3S and Rpd3L is Required for Repression

Gal4(dbd)-Rpd3 repressed transcription of a synthetic gene, albeit dependent on the integrity and function of Rpd3S and Rpd3L (Figure 1). This indicates that for a specific gene, repression depends on the activity of Rpd3 when it interacts with promoter and ORF sequences as suggested previously [21,22]. In SA media repression by Rpd3 is relieved [37]. Furthermore, after six hours in SPM, the $\beta$-gal levels of the reporter in strains expressing Gal4(dbd-Rpd3) or Gal4(dbd) were 10.32 ± 1.89 and 10.37 ± 1.57 Miller units, respectively, suggesting relief of repression under this condition.

Rpd3S Functions Specifically as a Positive Transcriptional Regulator

Transcription of the meiosis-specific genes IME1, IME2, and NDT80 responded uniquely to Rpd3 present in Rpd3L and Rpd3S. Their expression was decreased in mutants that expressed only Rpd3L (Tables 3 and 4), whereas their expression was increased in mutants that expressed only Rpd3S (Tables 3 and 4). The ability of Rpd3S to activate transcription can be explained by the observation that the Eaf3 subunit of Rpd3S is also a subunit of the transcriptional activator NuA4-HAT [12]. Indeed, the opposing effects of Rpd3S and Rpd3L were revealed by specific genetic interactions with FACT or NuA4 [62]. In summary, our results support a model in which Rpd3L functions as a repressor, whereas Rpd3S activates transcription.

Nitrogen depletion did not change the positive effect of Rpd3S on the transcription of IME1 and IME2 (Table 4 and Figure 3). However, NDT80 transcription did not require Rpd3S, and Rpd3 activated transcription only as a component of Rpd3L (Table 4 and Figure 3). Accordingly, cells with RCO1 deleted sporulated, whereas cells with DEP1 or SDS3 deletions along with RCO1 arrested in meiosis and contained single nuclei after completing premeiotic DNA replication (Figure 4).

Rpd3L Switches its Activity from a Negative to a Positive Regulator Depending on Nitrogen Depletion

As described above, in cells cultured in SA medium, Rpd3L negatively regulates transcription of IME1, IME2, and NDT80, consistent with findings that deletion of UME6, which is an integral component of this complex [13] derepresses transcription of EMGs [63]. However, in the absence of a nitrogen source during early and mid-meiosis, Rpd3L acts specifically to induce IME1 and NDT80 transcription (Figure 3 and Table 4). The effect of Rpd3L on the transcription of NDT80 might be indirect, through its effect on the transcription of IME1, because Ime1 directly activates NDT80 transcription. We rejected this hypothesis, because the reduced Ime1 level is expected to delay and attenuate the transcription of NDT80, rather than inhibit it [45] as revealed in strains deleted for either SDS3 or DEP1 (Figure 3).

The temporal switch in Rpd3L from a transcriptional repressor to an activator did not correlate with decreased histone acetylation. On the contrary, before either IME2 or NDT80 transcription was induced, the level of acetylated histone H4 increased (Figure 2), suggesting indirect activation by Rpd3L. Three possible mechanisms were examined: 1) Rpd3 is required to inhibit the pachytene checkpoint, which inhibits NDT80 transcription in the presence of nicks/breaks in the DNA strands [51]. Thus, the absence of Rpd3 at this checkpoint inhibits NDT80 transcription. We rejected this mechanism, because inactivation of the checkpoint by deleting RAD17 did not suppress rpd3Δ (Figure 5A). 2) High-throughput RNA sequence analysis revealed that IME1, IME2, and NDT80 express a noncoding RNA (http://yeast.utgenome.org). The noncoding RNA transcribed from the IME1 promoter represses, through the Set3 histone deacetylase, IME1 transcription in non-MATa/MATa strains [57]. This repression is independent of transcriptional activation by Rpd3, because it occurs in diploid cells that do not express this RNA. We did not examine the effect of antisense RNA on IME2 transcription, because recruitment of Rpd3 to the IME2 promoter by Ume6 induced deacetylation of histones H3 and H4, and consequently repression [17,48]. The NDT80 promoter expresses an antisense RNA [53]. The possibility that Rpd3 inhibits transcription of the NDT80 antisense RNA to induce transcription of the NDT80 ORF was rejected, because the induction of NDT80 transcription did not correlate with reduced levels of this RNA (Figure 5B). NDT80 antisense RNA expression first peaked simultaneously with that of the NDT80 ORF mRNA and was not affected by Rpd3. The presence of a
second antisense peak depended on Rpd3 and correlated with decreased NDT80 transcription (Figure 5B), indicating that the decline in NDT80 transcription may be mediated through this RNA (3). Acetylation regulates the activities of a wide array of nonhistone proteins as well as transcription factors [58,64]. These findings suggest that Rpd3 deacetylates transcription factors to modulate their activities. NDT80 transcription depends on four transcriptional activators, Ime1, Ime2, Ndt80, and Rpd3 as well as on the negative regulator Sum1 (reviewed in 61) that may be regulated by acetylation. We examined the possibility that the inhibitory activity of Sum1 depends on its acetylation, which is reversed by its deacetylation by Rpd3. This hypothesis predicts that deletion of SUM1 will suppress rpd3Δ. However, the rpd3Δ sum1Δ strain did not sporulate (Figure 6A), and the hypothesis was refuted. The second candidate examined was Ndt80. We postulated that acetylation of Ndt80 inhibits its ability to activate transcription. This model predicts that expression of Ndt80 from a heterologous promoter will not suppress rpd3Δ. However, NDT80 expression from the IME2 promoter in rpd3Δ cells promoted the transcription of the mid-meiosis-specific gene SPS1, and a fraction of cells initiated nuclear division (Figure 6B). Further work is therefore required to determine if Rpd3 deacetylates Ime1 and/or Ime2 or a different substrate(s).
Noncatalytic Transcriptional Repression by Rpd3

In budding yeast, following gametogenesis, an additional developmental pathway takes place, spore formation. At this time, the transcription of all meiosis-specific genes decline. We attribute this to histone deacetylation, because deletion of RPD3 caused persistent transcription (Figure 3) accompanied by reduced levels of histone H4 acetylation (Figure 2). We predicted therefore that deletion of ROC1 along with SDS3, would abolish Rpd3 activity and generate the rpd3Δ phenotype. Surprisingly, the phenotype exhibited by the rco1Δ sds3Δ-double mutant was unique, late in meiosis, the transcription of IME1, IME2, and NDT80 declined similarly to the wild-type strain (Figure 3), suggesting that Rpd3 possesses an additional function that is independent of Rco1 and Sds3, namely independent of the integrity of the Rpd3S and Rpd3L. This effect can be explained by two simple hypotheses as follows: 1) During late meiosis, Rpd3 is activated in a novel complex that does not include Sds3 or Rco1 and may include Dep1 that when absent led to increased transcription during late meiosis (Figure 3). We discounted this hypothesis, because mass spectrometry detected all of the integral components of Rpd3L and Rpd3S in cells cultured for 6 hours in SPM (Table S1). Interestingly, the only missing protein was Ume6, in agreement with a report that it is subject to ime1-dependent degradation [65]. In contrast, RNA polymerase II, which recruits Rpd3S [20], is present. 2) Rpd3 possesses a novel transcriptional repressor activity that is independent of Sds3 and Rco1, but requires Dep1. Because the histone deacetylase activity of Rpd3 requires Rco1 and Sds3, the latter hypothesis predicts that cells expressing catalytically inactive Rpd3 will exhibit the same phenotype as the sds3Δ rco1Δ-double mutant. This hypothesis was validated, because the transcription of IME1, IME2, and NDT80 during late meiosis declined in cells expressing the rpd3H150AH151A allele (Figure 3 and Table 4). During vegetative growth, repression of IME1 and IME2 was mediated by the catalytic activity of Rpd3L and the noncatalytic activity of Rpd3, whereas during late meiosis, the effect was mediated only by the latter. Hence, Rpd3 and H4 acetylation decreased during late meiosis (Figure 2), likely caused by the catalytic activity of Rpd3. This did not significantly affect transcription and possibly mediated by the counteracting effect of the noncatalytic activity of Rpd3. Our results support a recent in vitro observation demonstrating that Rpd3 possesses a catalytic function leading to deacetylated lysine residues and a noncatalytic activity that affects nucleosome stability [49]. Consistent with this scenario, mass spectrometry revealed that Rpd3 associated with proteins involved in chromatin remodeling (Table S1). Chen et al. reported that this activity requires only the core components of the Rpd3 complexes Rpd3, Sin3, and Ume1 [49]. Our results using the rco1Δ sds3Δ-double mutant agree, but the effect of deleting DEP1 suggests that Dep1 is also required for the noncatalytic activity of Rpd3.

In summary, our analysis of meiosis-specific genes demonstrates that 1) the histone deacetylase activity of Rpd3 that is mediated by Rpd3S activates transcription, 2) in Rpd3L the catalytic activity of Rpd3 switches from negative to positive, depending on the availability of a nitrogen source, and 3) a noncatalytic activity of Rpd3 represses transcription.

Supporting Information

Table S1. Mass spectroscopic analysis of Rpd3 isolated from cells cultured in SPM for six hours. Cells grown in SA to a density of 1 × 10^6 cell/ml were shifted to SPM. After six hours in SPM, proteins were crosslinked with 1% formaldehyde for 15 min. Rpd3 complexes isolated by IP were subjected to gel electrophoresis, excised from the gel, and analyzed using mass spectrometry. Strains: MATα/MAα RPD3-13xmyc-URA3/PRD3-13xmyc-URA3 (Y1767) and MATα/MAα rpd3Δ::HIS3/rpd3 Δ::HIS3 (Y1888, control). The results for strain Y1767 are shown. Sf - score for each peptide was calculated by a neural network algorithm that incorporates the Xcorr, DeltaCn, Sp, RSp, peptide mass, charge state, and the number of matched peptides for the search. P (pep) displays the probability of finding a match as good as or better than a random match.

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Author Contributions

Conceived and designed the experiments: DYH AK MS TH YK. Performed the experiments: DYH AK MS TH SS. Analyzed the data: DYH AK MS TH SS. Contributed reagents/materials/analysis tools: DYH AK MS TH SS. Wrote the manuscript: DYH MS YK.

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