**Raf60, a Novel Component of the Rpd3 Histone Deacetylase Complex Required for Rpd3 Activity in Saccharomyces cerevisiae**

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The Rpd3 histone deacetylase complex contains several previously characterized proteins, including Rpd3, Sin3, Sds3, Sap30, and Pho23. We purified the Rpd3 complex to near homogeneity using the tandem affinity purification method. Mass spectrometric analysis revealed the presence of a novel component, which we named Raf60. We showed that Myc-Raf60 co-fractionated with Rpd3-TAP by gel filtration chromatography, and both Myc-Rpd3 and Sin3 co-immunoprecipitated with HA-Raf60. In addition, HA-Raf60 immunoprecipitates displayed Rpd3-dependent histone deacetylase activity, and raf60 deletion resulted in loss of Rpd3 complex activity, as measured by *in vitro* assays. Furthermore, we found that raf60Δ cells exhibited phenotypes similar to those of rpd3Δ cells, including derepression of secreted acid phosphatase (Pho5), hypersensitivity to cycloheximide, and hypersensitivity to heat shock. Also, we found by reverse transcription-PCR that raf60Δ cells, similar to rpd3Δ cells, displayed elevated levels of *PHO5* and *INO1* mRNA. Our results demonstrate that Raf60 is a component of the Rpd3 histone deacetylase complex and that it is required for normal Rpd3 complex activity and repression of gene expression.

Histone acetylation and deacetylation play important roles in the regulation of gene expression and DNA repair in eukaryotic cells (1). These reversible processes are catalyzed by histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes (2, 3). Modification of histones by these complexes alter chromatin structure and can facilitate interactions of transcription and other factors with chromatin. In general, hyperacetylated histones are preferentially associated with transcriptionally active chromatin, whereas hypoacetylated histones are associated with transcriptionally repressed and silenced genes (4). In addition, HAT and HDAC complexes also acetylate and deacetylate transcription factors and other proteins (5). Considerable evidence suggests that inappropriate acetylation and deacetylation contribute to cancer and other human disease, and recent efforts have focused on examining inhibitors of class I/II HDACs for their potential value in the treatment of these diseases (6, 7).

Here we have examined the yeast Rpd3 HDAC complex as a model to understand the molecular mechanisms of HDAC complexes. At least six different HDACs are encoded by the Saccharomyces cerevisiae genome: Rpd3 (8, 9), Hda1, Hos1, Hos2, Hos3 (10–12), and Sir2 (13). Rpd3 is the enzymatic HDAC component of a large complex that primarily targets histones H3 and H4 (14, 15). Several subunits of this complex have been previously identified, including Rpd3, Sin3, Pho23, Sds3, and Sap30 (16–21). Mammalian homologues of these components (HDAC1/2, mSin3A/B, Ing1b, mSds3, mSap30) are present in the mSin3-HDAC1/2 co-repressor complex (18, 21–23). The high degree of conservation in subunit composition of the yeast Rpd3 and human Class I HDAC complexes suggests that their roles have been conserved throughout evolution. The mammalian mSin3-HDAC1/2 complex plays critical roles in cell differentiation, proliferation, and apoptosis and has been shown to be recruited to specific promoters by interaction with several transcriptional regulators, including Mad1, p53, unliganded hormone receptors (RAR and TR), and the retinoblastoma protein Rb (24–26). Similarly, the Rpd3 complex is recruited to promoters via the repressor Ume6 and by other mechanisms to regulate the expression of many genes (15, 27–30), including some involved in carbon, nitrogen, and iron metabolism, early meiosis, and replication timing (31–33). In addition, the Rpd3 complex is responsible for global histone deacetylation (29) and has been implicated in DNA damage repair pathways (34).

Despite recent progress in purifying the Rpd3 complex (17, 35), not all components of this complex have been identified and characterized. Here we report the initial characterization of Raf60 (Rpd3-associated factor) and demonstrate that Raf60 is a component of the Rpd3 HDAC complex that is essential for normal Rpd3 activity.

**EXPERIMENTAL PROCEDURES**

Yeast Strains, Growth Conditions, and Genetic Methods—Yeast culture, transformation, and other genetic manipulations were performed as described previously (36). All strains used in this study were constructed in the S288c background. BY4742 (*Mata his3Δ leu2Δ0 lys2Δ0 ura3Δ0*), BY4728 (*Mata his3Δ200 trplΔ63 lys2Δ0 ura3Δ3*), BY4729 (*Mata his3Δ200 trplΔ63 lys2Δ0 ura3Δ3*), rpd3Δ (BY4742 rpd3Δ:kanMX4*), pho23Δ (Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 pho23Δ:kanMX4*), and raf60Δ (BY4742 raf60Δ:kanMX4) were obtained from Research Genetics/Invitrogen. The strains AC101 (BY4728 PHO23-TAP::TRP1) and AC102 (BY4729 RPD3-TAP::TRP1) were generated by integrating the TAP fusion cassette in-frame at the 3′ end of the genomic coding sequences of *PHO23* and *RDP3*, respectively, using the PCR-based genomic tagging method described previously (37). The TAP fusion cassette was amplified from pBS1479 using oligonucleotides with ~60 bp identity to sequences flanking the integration site. Appropriate genomic integration and protein expression was confirmed by PCR and Western blots, respectively.

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3 The abbreviations used are: HAT, histone acetyltransferase; HDAC, histone deacetylase; HA, hemagglutinin; TAP, tandem affinity purification.
Plasmids—Procedures for DNA manipulation and analysis were performed using standard protocols (38). pAD4H and pUAD6 contain the 2-μm origin of replication and the ADH1 promoter and encode the HA epitope and Myc epitope, respectively (39). pHA-Rpd3, pHA-Raf60, and pHA-Pho23 were generated by cloning the PCR-amplified open reading frames of RPD3, RAF6, and PHO23 into the SalI/Sacl sites of pAD4H. pMyc-Rpd3 and pMyc-Raf60 were generated by cloning the PCR-amplified open reading frames of RPD3 and RAF60 into the SalI sites of pUAD6.

Purification of Pho23- and Rpd3-associated Proteins—Pho23 and Rpd3 complexes were purified by the tandem affinity purification method (37, 40), with minor modifications. Whole cell extracts (15–20 mg/ml, −30 ml) derived from 3-liter cultures were incubated with 500 μl of IgG-Sepharose beads (Amersham Biosciences) for 2 h at 4 °C with rotation. Beads were then recovered in a Bio-spin column (Bio-Rad), washed with 30 ml of cleavage buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Nonidet P-40, 10 mM β-mercaptoethanol, 1 mM magnesium acetate, 1 mM imidazole, 2 mM CaCl2, 10% glycerol), resuspended in 500 μl of cleavage buffer, and incubated with 100 units of tobacco etch virus protease (Invitrogen) for 2 h at 16 °C. The beads were removed, and the supernatant was incubated overnight with 300 μl of calmodulin-agarose beads (Invitrogen), washed with 30 ml of calmodulin binding buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Nonidet P-40, 10 mM β-mercaptoethanol, 1 mM magnesium acetate, 1 mM imidazole, 2 mM CaCl2, 10% glycerol), resuspended in 500 μl of calmodulin elution buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Nonidet P-40, 10 mM β-mercaptoethanol, 1 mM magnesium acetate, 1 mM imidazole, 20 mM EGTA, 10% glycerol), and incubated for 30–60 min at 4 °C. Eluted protein complexes were concentrated using the Ultrafree Biomax 5 filter device (Millipore) or precipitated with 30% trichloroacetic acid. The proteins were resolved by SDS-PAGE, silver-stained, and bands were excised and analyzed by mass spectrometry.

Immunoprecipitations and Western Blots—Yeast cells harvested from 100-ml cultures (A600~1.0) were washed once with HDAC extraction buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 0.1% Tween 20, 10% glycerol) and resuspended in 1 ml of extraction buffer containing protease inhibitors (2 μg of leupeptin, 5 μg of aprotinin, 2 μg of pepstatin A, 1 μM phenylmethylsulfonyl fluoride, and 1 μl of yeast protease inhibitor mixture (Sigma)). Cell suspensions were mixed with ~1.5 g of glass beads (425–600 nm; Sigma) and shaken for 10 cycles (45 s/cycle followed by 45 s of cooling) in a Mini-BeadBuster (Biospec Products) at 4 °C; cell debris was removed by centrifugation at 20,800 × g for 15–20 min at 4 °C. Protein levels were determined using the Bradford assay (Bio-Rad). Samples of cell extract containing 5–10 mg of total protein were preclared by incubating with 50 μl of protein A-Sepharose beads for 30 min at 4 °C. After removal of the beads, the extracts were incubated overnight (~14 h) at 4 °C with 50 μl of protein A-Sepharose beads cross-linked to anti-HA (12CA5) or anti-Rpd3 antibodies. The beads were collected by centrifugation at 420 × g for 2 min at 4 °C and washed three times with 1 ml of extraction buffer. For Western blot analyses, the beads were resuspended in 20 μl of 4X SDS-polyacrylamide gel sample buffer, boiled, and proteins were resolved by SDS-PAGE. Proteins were transferred onto nitrocellulose membranes, blocked with 5% skim milk in TBS (25 mM Tris, pH 7.4, 137 mM NaCl, 2.7 mM KCl), and incubated for 2 h at room temperature or overnight at 4 °C with the appropriate antibody. After 1 h of incubation at room temperature with the appropriate secondary antibodies, the blots were developed using ECL reagents (Amersham Biosciences).

Histone Deacetylation Assays—HDAC activities were measured as reported previously (20) with minor modifications. Immunoprecipitated tates were incubated overnight at room temperature with 11 μg of [3H]acetyl-histones from HeLa cells in 200 μl of HDAC buffer.

Gel Filtration Chromatography—~30-ml cell extracts (15–20 mg protein/ml) were loaded onto nickel-nitrilotriacetic acid-agarose columns (Invitrogen) as previously described (19). The bound proteins were eluted with 5 ml of 300 μM imidazole buffer and concentrated five times by ultracentrifugation using a Centricon Plus-20 filter device (Millipore). Proteins (5 mg/0.5 ml) were fractionated on a Superose 6 10/300 GL gel filtration column using the AKTA fast protein liquid chromatography system (Amersham Biosciences) at a flow rate of 0.25 ml/min.

Reverse Transcription-PCR—Total RNA was extracted from 10–20 ml of cells (A600~1.5) grown in high phosphate medium (41) using the Hot Phenol method (42). Residual DNA was removed in 20-μl reactions containing 20 μg of total RNA, 20 units of DNase 1 (Amersham Biosciences), and 30 units of RNase (Amersham Biosciences) at 37 °C for 30 min. For reverse transcription, 5 μg of DNase-treated RNA was incubated in 20-μl reactions with 100 pmol of oligo(dT)15 (Promega), 200 units of Superscript I RT reverse transcriptase (Invitrogen), 30 units of RNase (Amersham Biosciences), 1 μM dNTP mix, and 10 μM dithiobitol. The reactions were stopped by incubation at 70 °C for 10 min. Regions of ACT1, PHOS, and INO1 cDNAs were amplified using the following primer sets: ACT1, 5′-AGGCCCGAAAGCTTGTGTC- and 5′-GGCATAACCTGGGAAACATGG; PHOS, 5′-GGGTCCAGTTACGATTA and 5′-GATTTCAGAAGCTTCGAC; INO1, 5′-GCTGTCACCAAGTACGTCAG- and 5′-GCCATTCCAGGGTGAATCCTGG.

RESULTS

Raf60 is Associated with the Rpd3 HDAC Complex—We previously demonstrated that Pho23 is a stable component of the Rpd3 HDAC complex and that it is required for normal function of this complex (20). To further characterize the components of the Rpd3 complex, we purified it to near homogeneity by the TAP method (see “Experimental Procedures”). To do this, we first constructed strains in which the TAP tag coding sequence was integrated in-frame at the 3′-ends of the endogenous RPD3 and PHO23 coding sequences. Silver-stained gels of purified Rpd3-TAP and Pho23-TAP revealed the presence of 10–12 stably associated polypeptides ~30–200 kDa in size (Fig. 1A). Profiles of Pho23-TAP-associated proteins appeared to be similar in migration patterns, and stoichiometry to those present in the purified Rpd3-TAP preparation (not shown), indicating that Pho23 is associated exclusively with the Rpd3 complex. Several polypeptides detected in our purified preparations corresponded in size to known members of the Rpd3 complex, including Sin3 (~175 kDa), Rpd3 (~50), Pho23 (~40), Sds3 (~38), and Sap3 (~30 kDa) (Fig. 1A). The positions of these proteins in the profile were verified by Western blots (not shown). In addition, several other polypeptides were also detected. Two protein bands, ~175 (corresponding in size to Sin3) and ~60 kDa in size, were extracted from the gels, subjected to in-gel tryptic digestion, and analyzed by matrix-assisted laser desorption/ionization mass spectrometry. Peptides (FLDCAVGLR and HEYDFYIESNLR) derived from the 175-kDa band matched sequences from Sin3 as expected, whereas peptides (QLSQIALQR and LIYPEATGISSNR) obtained from the 60-kDa band corresponded to the sequence of a predicted protein encoded by the S. cerevisiae genome, which we have named Raf60 (Fig. 1B).

Raf60 is a non-essential 430-amino-acid residue protein that localizes in the nucleus (~43, 44); however its molecular functions are still unknown. The middle region (residues 133–228) of Raf60 has some.
Raf60, a Subunit of the Rpd3 HDAC Complex

sequence similarity with bromodomains (Fig. 1B), which are preferentially found in HAT-associated proteins and function as acetyl-lysine binding domains (45–47). In addition, the C-terminal region was predicted to form a coiled-coil domain, which is typically involved in protein–protein interactions (Fig. 1B).

To verify that Raf60 is a subunit of the Rpd3 complex, we performed co-immunoprecipitation studies using extracts from wild-type cells co-expressing HA epitope-tagged Raf60 and Myc epitope-tagged Rpd3 (Fig. 2A). Our results showed that Myc-Rpd3 (Fig. 2A, lane 4) and Sin3 (lanes 2 and 4) co-immunoprecipitated with HA-Raf60 but did not immunoprecipitate from control extracts.

Further evidence for the physical association of Raf60 and Rpd3 was obtained by gel filtration chromatography. Previous studies have reported the presence of two Rpd3 complexes, a large ~1.2-MDa complex and a smaller ~0.6-MDa complex (10, 17, 19), but only the larger complex binds to nickel-agarose (19). We partially purified HDAC complexes from crude extracts of cells co-expressing Rpd3-TAP and Myc-Raf60 on a nickel-agarose column and subsequently fractionated the bound material by gel filtration chromatography (see "Experimental Procedures"). Most of the endogenously expressed Rpd3-TAP co-fractionated with a ~1.2-MDa complex (Fig. 2B), consistent with previous reports (17, 19). Similarly, Myc-Raf60 co-fractionated with Rpd3-TAP (fraction 10). In contrast, the Hda1 HDAC eluted in fractions corresponding to ~0.5 MDa in size, consistent with previous reports (19, 48). Together these results demonstrate that Raf60 stably associates with the Rpd3 HDAC complex.

Raf60 Is Associated with Rpd3-dependent HDAC Activity—To further investigate the role of Raf60, we performed HDAC assays on immunoprecipitates of HA-Raf60, HA-Rpd3, and HA-Pho23 from wild-type cells expressing the HA epitope alone (Fig. 3A). Further, the HDAC activities associated with these proteins were reduced to background levels in the presence of trichostatin A, an HDAC inhibitor. To determine whether the HDAC activity associated with Raf60 was Rpd3-dependent, we performed similar assays on immunoprecipitates from rpd3Δ cells. As shown, no detectable HDAC activity was associated with HA-Raf60 or HA-Pho23 immunoprecipitates from rpd3Δ cells. Western blot analysis indicated that comparable amounts of HA-tagged proteins were present in the immunoprecipitates from wild-type and rpd3Δ cells (Fig. 3A, top panel). Together, these results demonstrate that Raf60 is associated with an Rpd3-dependent HDAC activity and is not stably associated with other HDAC complexes.

Raf60 Is Required for Normal Rpd3 HDAC Activity—To assess the role of Raf60 in the Rpd3 complex, we first examined whether Raf60 is required for Rpd3 HDAC activity. To do this, we performed HDAC assays on immunoprecipitated HA-Rpd3 and HA-Pho23 from raf60Δ cells. Our results show that the HDAC activities associated with HA-Rpd3 or HA-Pho23 from raf60Δ cells were not significantly above background levels, in contrast to the normal levels we observed with corresponding immunoprecipitates from wild-type cells (Fig. 3A). Immunoprecipitates of HA-Raf60 in raf60Δ or wild-type cells exhibited comparable levels of HDAC activity. We also measured the HDAC activities of endogenous Rpd3 immunoprecipitated from cell extracts using an anti-Rpd3 antibody. We found that the level of HDAC activity of anti-Rpd3 immunoprecipitates from wild-type cells was ~2.6-fold higher than from rpd3Δ cells (Fig. 3B). Furthermore, we found that the HDAC activities of anti-Rpd3 immunoprecipitates from raf60Δ and pho23Δ cells were reduced to background levels similar to that observed from rpd3Δ cells. Together, these observations suggest that Raf60 is required for HDAC activity of the Rpd3 complex in vitro.

Raf60 Deletion Results in Phenotypes Similar to Those of rpd3 Mutants—Deletion of RPD3 or other components of the Rpd3 complex have been previously shown to result in several phenotypes, including defects in the regulation of gene silencing and expression (including PHOS and INO1 expression) and hypersensitivity to cycloheximide and heat shock (20, 29, 49). To investigate whether Raf60 is required for Rpd3 function in vivo, we examined whether raf60Δ cells exhibit phenotypes similar to those of rpd3Δ cells. Our results show that raf60Δ cells were hypersensitive for growth on medium containing cycloheximide (0.32 μg/ml) or for heat shock treatment (55 °C, 10 min), similar to that observed for rpd3Δ and pho23Δ cells (Fig. 4). We also found that raf60Δ resulted in derepression of secreted acid phosphatase in high phosphate medium, which is indicative of increased PHOS expression. The level of secreted acid phosphatase activity appeared to be similar to the level of pho23Δ cells but not as much as rpd3Δ cells, suggesting that PHOS expression is only partially derepressed in raf60Δ cells.

Raf60 Is Required for Repression of PHOS and INO1—We next examined whether deletion of RAF60 had an effect on the level of PHOS and INO1 mRNA. By performing reverse transcription-PCR, we found that both PHOS and INO1 mRNA levels were detectable at a fewer number of cycles of amplification using mRNA purified from raf60Δ and rpd3Δ cells compared with wild-type cells (Fig. 5). These results indicate that PHOS and INO1 mRNA levels were similar in raf60Δ and rpd3Δ cells and were severalfold higher than in wild-type cells, suggesting that Raf60 is required for the proper repression of these genes. Together, our results suggest that Raf60 is required for normal functions of the Rpd3 complex in vivo.

DISCUSSION

The Rpd3 HDAC complex plays a key role in the modification of chromatin structure and consequently impinges on mechanisms that regulate gene expression and DNA repair. The identification and characterization of all subunits of the Rpd3 HDAC complex is essential to fully understanding the mechanisms involving this complex. In this
Raf60, a Subunit of the Rpd3 HDAC Complex

FIGURE 2. Raf60 co-immunoprecipitates and co-fractionates with Rpd3. A, extracts from BY4742 cells co-expressing Myc-Rpd3 (lanes 3 and 4) and either HA-Raf60 (lanes 2 and 4) or HA-epitope alone (lanes 1 and 3) were assayed for expression of Myc-Rpd3 by Western blot analysis using anti-Myc (9E10) antibody (top panel). Extracts were immunoprecipitated (I.P.) with anti-HA (12CAS) antibody (see “Experimental Procedures”). The immunoprecipitates were probed with anti-HA (1:2000) antibody (second panel), anti-Myc antibody (1:2000) (third panel), and anti-Sin3 antibody (1:1000; bottom panel) kindly provided by D. Stillman. Plasmids used to express proteins were pAD4H (HA alone), pUAD6 (Myc alone), pHA-Raf60, and pMyc-Rpd3. B, extracts from AC102 (Rpd3-TAP) cells overexpressing Myc-Raf60 were loaded onto a nickel-nitrilotriacetic acid-agarose column to partially purify Rpd3-associated proteins. The bound proteins were eluted with 300 μM imidazole and fractionated on a Superose 6 10/300 GL column (see “Experimental Procedures”). 0.5-ml fractions were collected and aliquots (15 μl) of even-numbered fractions were analyzed by Western blots using peroxidase-anti-peroxidase-soluble complex (1:2000; Sigma) to detect Rpd3-TAP, anti-Myc (9E10, 1:2000) to detect Myc-Raf60, and anti-Hda1 (γ-N-19, 1:100; Santa Cruz Biotechnology). The positions of molecular mass markers and fraction numbers are shown (top).

FIGURE 3. Raf60 is associated with Rpd3-dependent HDAC activity. A, extracts from BY4742 (wild type, WT), rpd3Δ, or raf60Δ cells expressing HA epitope alone, HA-Rpd3, HA-Raf60, or HA-Raf60 were immunoprecipitated with anti-HA (12CAS) antibody. Immunoprecipitates were examined by Western blots using anti-HA antibody (top panel) and assayed for HDAC activity (bottom) (see “Experimental Procedures”). Immunoprecipitates from WT cells were assayed in the presence or absence of 1 μM trichostatin A (TSA), an HDAC inhibitor. Graphs indicate the average counts/min (CPM) released from [3H]acetate-histones in samples from three independent experiments. Plasmids used to express HA-tagged proteins were pAD4H (HA alone), pHA-Rpd3, pHA-Raf60, and pHA-Pho23. B, extracts from BY4742 (WT), rpd3Δ, raf60Δ, and pho23Δ cells were immunoprecipitated with anti-Rpd3 antibody (Upstate Biotechnology). Immunoprecipitates were examined by Western blots using anti-Rpd3 antibody (top panel) and assayed for HDAC activity (bottom).

FIGURE 4. raf60Δ cells display phenotypes similar to rpd3Δ cells. BY4742 (WT), rpd3Δ, pho23Δ, and raf60Δ cells were grown in liquid yeast/peptide/dextrose (YPD) medium and then spotted onto solid medium plates without (YPD) or with (+CYH) cycloheximide (0.32 μg/ml) at 30 °C for several days. To test for heat shock sensitivity, the strains were incubated at 55 °C for 10 min in liquid YPD medium, and then 5 μl aliquots were spotted onto YPD plates (+HS) and incubated at 30 °C for several days. Strains were also tested for deregulation of PHO5 by an acid phosphatase plate assay in high phosphate medium (+PO4) as described previously (41). Strains that expressed PHOS stained a darker color by this assay.

with any other HDAC complex, as the HDAC activity associated with Raf60 is dependent on Rpd3.

The composition of the yeast Rpd3 and mammalian Class I HDAC complexes have been highly conserved during evolution. However, the primary sequence of Raf60 does not appear to have been highly conserved among eukaryotes. It remains possible that there are functional homologues in mammals and other organisms that play similar roles.

The role of Raf60 in the Rpd3 complex remains unclear; however our results indicate that it is essential for Rpd3 activity in vitro, and our genetic analysis suggests that Raf60 is required for functions that are similar to Rpd3 in vivo. Our observation that Raf60 is required for normal repression of PHOS and INO1 suggests that it is essential for the ability of the Rpd3 complex to properly regulate the expression of these genes. Other laboratories have shown that the Rpd3 complex binds and deacetylates the PHOS and INO1 promoters (30, 50). Thus, the dere-

study, we report the initial characterization of a novel Rpd3 subunit, Raf60. The co-purification of Raf60 with Rpd3-TAP and Pho23-TAP suggests that Raf60 is a component of the Rpd3 complex. Recent genome-wide proteomic studies have also identified Raf60 and several other proteins in purified Rpd3-TAP and Sin3-TAP preparations (35). Our observations that Raf60 co-immunoprecipitates and co-fractionates by gel filtration with Rpd3 further demonstrates that Raf60 stably associates with this complex. Raf60 does not appear to be associated
expression of these genes in rad60Δ cells is likely a consequence of the inability of the Rpd3 complex to deacetylate histones at these promoters. Although Raf60 lacks a recognizable DNA binding domain, it is possible that it is involved in nucleosome and/or histone recognition. Indeed, the potential bromodomain present in Raf60 could be involved in histone recognition, as it has been shown for other bromodomains (47).

The Rpd3 HDAC complex is composed of several subunits, yet the precise roles of many of these subunits remain unclear. Rpd3 appears to be enzymatically active only in association with other components of the complex, and mutations in several of these components result in phenotypes similar to rpd3 mutants, indicating that they are required for the normal function of the Rpd3 complex in vivo. Further studies will help decipher the roles of these subunits and reveal mechanisms that regulate Rpd3 HDAC activity and target specificity and may provide insights into similar mechanisms that regulate mammalian cell growth and apoptosis and contribute to human cancer and other disease.

REFERENCES

1. Iizuka, M., and Smith, M. M. (2003) Curr. Opin. Genet. Dev. 13, 154–160
2. Roth, S. Y., Denu, J. M., and Allis, C. D. (2001) Annu. Rev. Biochem. 70, 81–120
3. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
4. Eberharter, A. (2002) BioEssays 24, 1057–1067
5. Marks, P. A., Richon, V. M., Miller, T., and Kelly, W. K. (2004) Adv. Cancer Res. 91, 137–168
6. Drummond, D. C., Noble, C. O., Kirpotin, D. B., Guo, Z., Scott, G. K., and Benz, C. C. (2004) Annu. Rev. Pharmacol. Toxicol. 45, 495–528
7. Vidal, M., Buckley, A. M., Hilger, F., and Gaber, R. F. (1990) Genetics 125, 313–320
8. Vidal, M., Buckley, A. M., Hilger, F., and Gaber, R. F. (1990) Genes Dev. 4, 491–502
9. Rundlett, S. E., Carmen, A. A., Rundlett, S. E., and Grunstein, M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14405–14508
10. Wang, A., Kurdistani, S. K., and Grunstein, M. (2002) Science 298, 1412–1414
11. Sabet, N., Volo, S., Yu, C., Madigan, J. P., and Morse, R. H. (2004) Mol. Cell. Biol. 24, 4769–4780
12. Rigaut, G., Schwenkenko, A., Rutz, B., Wilm, M., and Seraphin, B. (1999) Nature 395, 141–147
13. Adams, A., Gottschling, D. E., Kaisers, K., and Stearns, T. (1998) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Plainview, NY
14. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
15. Jackson, P. K., and Reinberg, D. (1998) Nature 395, 141–147
16. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
17. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
18. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
19. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
20. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
21. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
22. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
23. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
24. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
25. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
26. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
27. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
28. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
29. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
30. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
31. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
32. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
33. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
34. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
35. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
36. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
37. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
38. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
39. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
40. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
41. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
42. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
43. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
44. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
45. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
46. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37
47. Gu, W., Gudkov, A. V., and Qin, J. (2001) Annu. Rev. Biochem. 70, 1–37