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Solution Structure of the mSin3A PAH2–Pf1 SID1 Complex: A Mad1/Mxd1-Like Interaction Disrupted by MRG15 in the Rpd3S/Sin3S Complex

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Histone deacetylation constitutes an important mechanism for silencing genes. The histone-deacetylase-associated mammalian Rpd3S/Sin3S corepressor complex plays key roles in repressing aberrant gene transcription from cryptic transcription initiation sites and in mitigating RNA polymerase II progression in intragenic regions of actively transcribed genes. The Sin3 corepressor functions as a molecular adaptor linking histone deacetylases on the one hand, with the chromatin targeting subunits Pf1 and MRG15 on the other. Pf1 also functions as an adaptor by interacting with MRG15 and engaging in multivalent interactions with Sin3 targeting among other domains the two N-terminal paired amphipathic helix (PAH) domains that serve as sites of interaction with sequence-specific DNA-binding transcription factors. Here, we structurally and functionally evaluate the interaction between the PAH2 domain of mSin3A and the Sin3 interaction domain 1 (SID1) motif of Pf1 and find the structural aspects to be reminiscent of the interaction between the Mad1/Mxd1 transcription factor and Sin3. Pf1 residues within a highly conserved sequence motif immediately C-terminal to SID1 appear not to be important for the interaction with Sin3 PAH2. Unexpectedly, the MRG15 subunit competes, rather than collaborates, with Sin3 for the Pf1 segment encompassing the two conserved motifs, implying competition between two subunits for another subunit of the same chromatin-modifying complex.

Introduction

Posttranslational modification of histones is a facile yet powerful mechanism for epigenetic programming.[1,2] The reversible nature of these modifications affords reprogramming of any locus with concomitant alterations in gene expression levels. Histone acetylation and deacetylation constitute particularly well-characterized examples and are generally correlated with enhanced or diminished transcript levels, respectively. The enzymes that bring about these epigenetic changes are typically members of multi-protein complexes

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Abbreviations used: SID, Sin3 interaction domain; PAH, paired amphipathic helix; HDAC, histone deacetylase; MRG, mortality factor on chromosome 4-related gene; PHD, plant homeodomain; NOE, nuclear Overhauser enhancement; ITC, isothermal titration calorimetry; TCEP, tris(2-carboxyethyl)phosphine hydrochloride; 2D, two-dimensional; HSQC, heteronuclear single quantum coherence; 3D, three-dimensional; COSY, correlated spectroscopy; NOESY, NOE spectroscopy; PDB, Protein Data Bank.

whose targeting specificity often resides within the nonenzymatic subunits. Comparatively little is known about the precise molecular functions of many of the subunits of these complexes, and even less is known about how the subunits assemble to yield a functional complex.

The yeast histone deacetylase (HDAC) Rpd3 and its mammalian homologs HDAC1, HDAC2, and HDAC3 are nuclear proteins found in only a handful of complexes that have distinct functional properties and subunit compositions.[3,4] Two related, yet functionally distinct, forms of the Rpd3 complex in yeast and the orthologous Sin3 corepressor complex in mammals play fundamental roles in cellular physiology. The Rpd3L/Sin3L complex is recruited to the promoter regions and represses a broad range of genes involved in cell cycle regulation, differentiation, DNA replication and repair, apoptosis, and mitochondrial metabolism, whereas the Rpd3S/Sin3S complex is targeted to the intragenic regions of actively transcribed genes to suppress aberrant transcription initiation from cryptic sites and to mitigate RNA polymerase II progression.[4–13] The Rpd3/Sin3 complexes also play key roles in heterochromatin formation at centromeres, telomeres, and ribosomal DNA loci and in DNA replication timing and double-strand break repair.[5] Consistent with their fundamental roles, the majority of the subunits of the Rpd3/Sin3 complexes is evolutionarily conserved from yeast to human. The larger, 1.2- to 2-MDa Rpd3L/Sin3L complex comprises at least eight subunits, whereas the smaller, 0.6-MDa Rpd3S/Sin3S complex comprises at least five subunits, three of which, including the Sin3, Rpd3/HDAC1/HDAC2, and RbAp46/RbAp48 polypeptides, are shared by both complexes.[8,11,14–19]

The Pf1 and MRG15 subunits of the mammalian Rpd3S/Sin3S complex and their orthologs in budding yeast including Rco1p and Eaf3p, respectively, play critical roles in targeting the complex to the transcribed regions through direct interactions with nucleosomes.[9,11,20,21] Pf1 also functions as a molecular adaptor interacting with the mortality factor on chromosome 4-related gene (MRG) domain of MRG15 and linking it with the rest of the complex by engaging multiple domains of Sin3 in direct interactions including the three N-terminal paired amphipathic helix (PAH) domains and the HDAC interaction domain.[11,18,19] The Sin3 polypeptide—after which the mammalian complex is named—is thought to function as an organizing center or scaffold for the assembly of both Rpd3L/Sin3L and Rpd3S/Sin3S complexes. Sin3 is recruited by a variety of sequence-specific DNA-binding factors through direct protein–protein interactions involving its two N-terminal PAH domains.[22–34] The Sin3 PAH3 and HDAC interaction domains are targeted by various subunits unique to the Rpd3L/Sin3L complex.[15,17,35] Thus, the multivalent nature of the interactions between Pf1 and Sin3 implicates Pf1 in yet another role—that of limiting access to transcription factors and to Rpd3L/Sin3L subunits with disparate functions—thereby preserving the unique specificity of the smaller complex.

The interactions involving the Sin3 PAH1 and PAH2 domains have been structurally and functionally characterized.[24,36–40] These studies have shown that PAH domains bind to diverse targets, but do so with a high degree of specificity. The PAH1 and PAH2 domains recognize distinct sequence motifs (referred to as *S*in3 *i*nteraction *d*omains or SIDs), yet the directionality of the motifs appears to be unimportant, as motifs running both N-to-C and C-to-N are equally capable of interacting.[24,36] This was explained by the SIDs adopting helical conformations in respective complexes with the precise helical orientation determined by the chain direction of the underlying motif. Sequences analysis of Pf1 orthologs revealed a conserved motif that extended beyond the SID that was shown to be crucial for stable (and PAH2 dependent) association with Sin3. Here, we structurally and functionally characterize the interaction between Pf1 and mammalian Sin3A PAH2 and show that only residues within the SID are important for stable association with Sin3. We also show that, rather unexpectedly, MRG15 competes with Sin3 PAH2 for Pf1 SID1, disrupting a presumed important point of contact between Pf1 and Sin3.

Results

Low-resolution structural analysis of Pf1 SID1

Previous biochemical studies have identified at least three regions within Pf1 as being important for the association with Sin3, including the segments corresponding to SID1 and SID2 in the N- and C-terminal halves of the protein and the plant homeodomain 2 (PHD2) domain.[11,18] Comparative analysis of the linker segment connecting the PHD1 and PHD2 domains of various Pf1 orthologs revealed poor conservation of both length and sequence. MEME-based analysis of potential motifs in this region, on the other hand, indicated a conserved segment N-terminal to the PHD2 domain (Fig. 1a). The 23-residue motif spanning residues 209 to 231 overlaps with the 12-residue SID1 spanning residues 210 to 221, which was classified as a type I PAH2 interactor (i.e., Mad1/Mxd1-like) based on sequence analysis.[24] The high degree of sequence conservation immediately C-terminal to the SID1 prompted us to evaluate whether these residues might play a role in binding to Sin3 PAH2.