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HISTONE DEACETYLATION: IP4 is an epigenetic coregulator:

Inositol tetrphosphate is required for both the incorporation of the histone deacetylase HDAC3 into a repressive complex and its enzymatic activity

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Acetylation and deacetylation of histones provide a fundamental epigenetic mechanism for regulating chromatin organization and gene expression programs. The acetylation balance is tightly controlled by histone acetyltransferases and histone deacetylases (HDACs), which catalyze the addition and removal of the acetyl group at lysine residues of histone proteins¹. Deacetylation causes lysine residues to become positively charged, thereby strengthening the interaction of histones with negatively charged DNA, restricting the DNA accessibility to gene-processing machinery and abolishing binding of acetyllysine-specific, bromodomain-containing coactivators. As a result, HDACs are often found in multisubunit repressive complexes and are associated with transcriptionally inactive heterochromatin. In a recent article in *Nature*, Watson *et al.*² demonstrate that recruitment of the histone deacetylase HDAC3 to a repressive complex and its deacetylase activity are regulated by inositol tetrphosphate (IP4) and provide mechanistic insight into this unexpected finding.

IP4 is a small signaling molecule in which inositol is phosphorylated at positions 1, 4, 5 and 6. It is an integral part of a large inositol signaling network—over a dozen phosphorylated inositol isoforms have been identified in eukaryotic cells, the majority of which remain ‘orphan’ molecules with unknown functions³. IP4 is produced through phosphorylation of the lipid-derived secondary messenger inositol 1,4,5-trisphosphate (IP3) by inositol 6-kinases and can undergo further phosphorylation to form IP5 and IP6. In budding yeast, IP4 has been shown to stimulate *in vitro* nucleosome remodeling by the SWI–SNF complex⁴, yet the details of this function remain unknown.

The enzymatic activity of HDAC3 in cells is dependent on formation of a stable SMRT–HDAC3 complex, which contains HDAC3, the nuclear hormone receptor co-repressor SMRT and two accessory proteins, TBL1 and GPS2. To understand the mechanism of repressive complex assembly, the Schwabe group and collaborators⁵ previously investigated interactions between SMRT, TBL1 and GPS2 and found that the three components are held together via multiple contacts (Fig. 1a).

In the current study, Watson *et al.*² examined the structural basis of recognition of the deacetylase activation domain (DAD) of SMRT by HDAC3 and uncovered the regulatory role of IP4 in HDAC3 binding and activation. The crystal structure of the DAD–HDAC3 complex reveals that, upon binding, the DAD undergoes conformational changes that allow for its N-terminal α -helix to interact to a greater extent with HDAC3. Surprisingly, the IP4

molecule was found sandwiched between DAD and HDAC3 (Fig. 1b). IP4 lies in a deep basic pocket, a distinguishing feature of phosphoinositol-binding proteins⁶, and creates multiple hydrogen bonds and salt bridges with basic and polar residues of both proteins. The presence of IP4 is necessary for the DAD-HDAC3 interaction, and replacement of the IP4 binding site residues impairs complex formation.

What is the role of IP4 in the activation of HDAC3? The data of Watson *et al.*² offer insight into the basis of the deacetylase activity. As HDAC3 is inactive on its own but becomes activated in the complex, the authors argue that conformational and dynamics changes, which occur upon its interaction with IP4 and DAD, facilitate substrate access to the HDAC3 active site (Fig. 1b). Particularly, Arg265, which is located in a flexible loop of HDAC3 and is in direct contact with the 4-phosphate group of IP4, may restrain this loop in the conformation most favorable for deacetylase activity. Using an *in vitro* reconstitution assay, Watson *et al.*² further demonstrated that neither IP3 nor IP6 could adequately substitute for IP4 in the formation of the DAD-HDAC3 complex, and thus IP4 is a specific ligand.

These comprehensive studies^{2,5} highlight a remarkably sophisticated mechanism of regulation of the SMRT-HDAC3 complex; however, several questions remain open. For example, it remains unclear how this complex associates with chromatin and whether the WD40 repeats of TBL1 contribute to this association. Both SMRT and TBL1 have been shown to have histone binding activity^{7,8}, but no mechanism has been identified. Is the SMRT-HDAC3 complex specific for a particular genetic region, and what are the determinants of specificity? Because the IP4 binding site residues of HDAC3 are conserved in HDAC1 and HDAC2, can their activity be modulated in a similar way? It will also be interesting to explore the effect of other regulatory mechanisms⁹, including post-translational modifications of HDAC3, in the context of IP4 binding. Addressing these questions in future research will further broaden our understanding of the molecular mechanisms underlying transcriptional repression and chromatin remodeling by HDACs.

Altogether, the study by Watson *et al.*² provides critical atomic-resolution information on SMRT-HDAC3 assembly and reveals a fascinating link between epigenetic regulation and inositol phosphate signaling. In recent years, the HDAC family has become a bona fide therapeutic target in the treatment of cancer and other human diseases, and therefore the findings by Watson *et al.*² could be imperative in the design of next-generation therapeutics for HDACs. The development of HDAC inhibitors has particularly been at the focus of pharmaceutical research, as aberrant expression and localization of HDACs are found in various human cancers. The discovery by Watson *et al.*² not only reveals a new strategy for the HDAC inhibition but also may lead to the selective targeting of a specific deacetylase or a subset of HDACs.

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a Assembly of SMRT, TBL1, GPS2 and HDAC3

Figure 1. The role of IP4 in the assembly and function of the SMRT-HDAC3 repression complex
(a) A model of the SMRT-HDAC3 complex. The major components of the complex, SMRT, TBL1, GPS2 and HDAC3, are in indigo, green, pink and wheat, respectively. SMRT associates with all other components: it forms an antiparallel coiled coil with GPS2, binds the TBL1 dimer (which tetramerizes) and interacts with HDAC3 through its DAD domain. IP4, shown in brick red, acts as a glue-like molecule between DAD and HDAC3. **(b)** The crystal structure of the DAD-IP4-HDAC3 complex. The IP4 molecule is shown as a stick model. An acetate molecule bound in the active site of HDAC3 and Arg265 are colored orange and brown, respectively.