

Deciphering HDAC1 Function In Vivo: A Structural Approach

BBSRC PhD Studentship in Cancer Research

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Project description:

Histone Deacetylases (HDAC) 1 and 2 are highly conserved enzymes (83% identical) present in the nucleus of all mammalian cells where they help regulate chromatin structure as the catalytic core of co-repressor complexes such as Sin3A, NuRD and CoREST. Each of these complexes, consisting of at least six core polypeptides, is recruited to target genes by specific transcription factors in order to regulate transcription. A combination of genetic ablation and chemical inhibition has linked the function of HDAC1/2 containing complexes to cell cycle progression, apoptosis, DNA repair, differentiation and cancer. Indeed, a number of HDAC inhibitors are currently in clinical trials for their anti-tumourigenic properties. However, little attention has been paid to the molecular assembly of individual HDAC1/2 complex components and the potential of more specific therapies that block this assembly. As yet, there are no structures of HDAC assemblies.

We aim to combine in vivo (Cowley lab) and structural (Schwabe lab) approaches to study the molecular detail of HDAC1/2 complex function. From a basic science perspective, how are these complexes put together..? And from a pharmacological point of view, how can we pull them apart..? Project Details:

1) HDAC1 and HDAC2 homo- and heterodimerize through an N-terminal HDAC Association Domain (HAD - approx. 53 aa). Dimerization is critical for HDAC1 activity and incorporation into the Sin3A complex.

- Purify recombinant HDAC1 'HAD' domain and determine key residues for dimerization by mutagenesis.
- Determine structure of HDAC1 'HAD' domain: combination of NMR / X-ray crystallography.
- Attempt to disrupt HDAC1 function in vivo using cell permeable 'HAD' mimetics / peptides.

2) HDAC1/2 interact with the co-repressor, Sin3A (central component of the Sin3A complex) via a region known as the HDAC Interaction Domain (HID). The HID has been loosely defined as a 350 amino acid region between Paired Amphipathic Helix (PAH) domains 3 and 4.

- Define a minimal region of the HID required for association with HDAC1 using Flag-tagged HID constructs to co-IP HDAC1 from 293T cells.
- Purify minimal recombinant HID – reconstitute HID/HDAC interaction in vitro.
- Determine structure of minimal 'HID' domain: combination of NMR / X-ray crystallography.
- Attempt to disrupt Sin3A/HDAC interaction in vivo using cell permeable 'HID' mimetics / peptides