Abstract 2338

A trans-HAT mechanism via HDAC3 acetylation allows GCN5 to regulate p300 sites

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While crosstalk between modifications of different histone lysine residues has been extensively studied, the impact of crosstalk between different histone-modifying enzymes, particularly HATs and HDACs, on the ability of specific HATs to regulate histone modifications remains poorly understood. Here, we report a trans-HAT mechanism that allows GCN5 to regulate p300-specific lysine sites by acetylation and inactivation of HDAC3. We show that GCN5 physically associates with HDAC3 and acetylates HDAC3 at K44 and K49 in the N-terminus of HDAC3, a region important for activation of HDAC3 by inositol tetraphosphate (IP4) and the deacetylase interaction domain (DAD) of nuclear receptor corepressors. Biochemical and functional studies show that HDAC3 acetylation by GCN5 prevents HDAC3 activation by preventing IP4-dependent interaction between acetyl-HDAC3 and DAD. The K44K49/QQ mutation recapitulates the effect of GCN5. We also show that HDAC3 can deacetylate not only histones but also p300, thereby inactivating p300. Although H3K27 is a p300-specific site, by inactivating HDAC3, GCN5 acquires the ability to regulate p300-dependent H3K27 acetylation by permitting and enhancing p300-mediated acetylation, representing a double-negative mechanism. *In vivo* analysis of ChIP-Seq and RNA-Seq results provided further evidence that GCN5 has the ability to control p300 sites (H3K27/K18) at genes colocalized with HDAC3 but not at genes bound by GCN5 alone. We propose that this trans-HAT mechanism is important for GCN5 to regulate transcription by controlling both canonical and non-canonical GCN5 sites.

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Abstract 2354

RNA Polymerase II transcription attenuation at multiple genes in yeast depends on the mRNA 3′-end processing factor Hrp1 and its RNA recognition motif

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Premature termination of transcription (i.e. attenuation) is an ancient and widespread form of gene regulation, spanning all three domains of life and viruses. The significance of premature termination is evidenced by attenuation defects linked to cancer, viral infection, developmental abnormalities, and neurodegeneration. Attenuation of eukaryotic RNA Polymerase II (Pol II) transcription is more prevalent than once thought, but its mechanism of action is poorly understood at most gene targets. Pol II attenuation is best studied in the yeast model eukaryote *Saccharomyces cerevisiae*, where it was originally discovered to occur via the Nrd1-Nab3-Sen1 non-coding RNA termination pathway. More recently our lab characterized a "hybrid" attenuation pathway involving Sen1 along with the Hrp1 cleavage factor from the mRNA 3′-end processing complex. How widespread is Hrp1 activity in Pol II attenuation, and how does Hrp1 function at different regulatory targets? In this study, we used a genetic selection to characterize the recognition elements of several Hrp1-dependent attenuators (MNR2, RAD3, and SNG1). Attenuator readthrough mutants clustered in conserved regions of the mRNA 5′-UTR, including putative Hrp1 consensus binding sites. We used site-directed mutagenesis to alter the Hrp1 RNA-recognition motif (RRM) at a highly conserved amino acid F162, comparing F162A, F162H, and F162W substitutions. The hrp1 RRM mutants in single copy exhibited slow-growing or lethal growth phenotypes, and in a heterozygous context we observed variable dominant negative attenuator readthrough defects. Our ongoing experiments are testing more direct interactions of the Hrp1 RRM with various attenuator RNAs using a yeast three-hybrid system, dissecting the sequence-dependency of attenuator recognition. Overall, these results expand the "hybrid" Pol II attenuation pathway, confirming it is Hrp1-dependent and likely involves direct Hrp1 recognition of attenuator elements near the 5′-end of mRNA targets.

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