ABSTRACT

TITLE:	Quantification of Lysine Acetylation in Histones Isolated from Yeast Strains Lacking a Specific HAT or HDAC+B166
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ABSTRACT: Histone proteins provide the most basic form of structural organization for eukaryotic chromosomes. The dynamic nature of gene expression is dependent on the complex interaction of histones with both DNA and other proteins. Chromatin remodeling and post translational histone modification occurs via diverse mechanisms including acetylation, phosphorylation, methylation, ADP-ribosylation, and ubiquitination. Histones can be dually tailored by specific enzyme classes called histone acteylases (HATs) and histone deacetylases (HDACs). The addition and removal of acetyl groups (acetylation and deacetylation) to and from particular lysine (K) residues within histones provide a curious mechanism for the control of gene silencing and expression. Through in vitro assays and the use of antibodies, Gcn5, a particular HAT, has been shown to acetylate lysine residues K9, K14, K18, and K23 within the amine terminal tail region of histone H3; however, the in vivo histone acetylation targets of Gcn5 remain unknown. Using yeast as a model organism to study endogenous acetylation levels, we performed a multi-step histone purification process on wild-type (WT) and Gcn5 "knockout" strains. To determine in vivo acetylation levels, purified histone H3 was treated with deuterated acetic anhydride, which isotopically labels all unacetylated lysine residues. After protein digestion using the protease trypsin, histone samples were analyzed using electrospray ionization-collision induced dissociation-tandem mass spectrometry (ESI-CID-MS/MS). The ratio of protiated acetyls to deuterated acetyls was then determined. Our results suggest that there is a decrease in endogenous acetylation levels at K14 and K23 in Gcn5 "knockout" strains when compared to WT strains.