Examination of Putative Histone Acetyltransferases in *Cryptococcus Neoformans*

LIBERTY UNIVERSITY

Felice Kho, Phoebe Bridy, Yuqi Wang and Gary D. Isaacs, Ph.D.

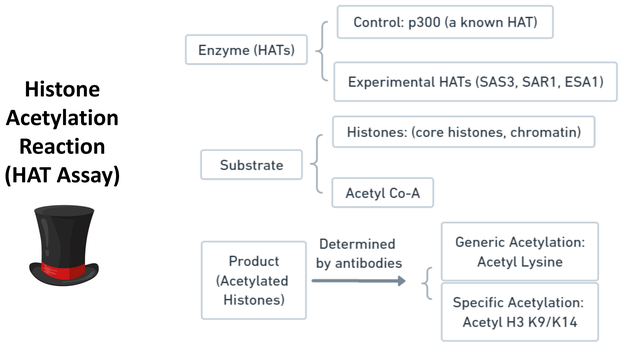
Introduction / Background

Histone Acetyl Transferases (HATs) are enzymes associated with the transcriptional activation of genes. p300, a known HAT enzyme in humans, acetylates histone tails at lysine and arginine residues, making DNA more prone to transcriptional activation. Therefore, identifying different HATs can aid in the understanding of gene regulation. A previous study by the Price lab demonstrated high expression levels of SAS3 in CSF-grown *C. neoformans*, suggesting a potential role in human pathogenesis. Based on protein sequence data, SAS3 has a putative HAT domain, although no definitive proof exists concerning its enzymatic activity in *C. neoformans*.

Similarly, two other genes in *C. neoformans* (SAR1 and ESA1) have homology to a known histone acetyltransferase in another organism, but little is known about the function of these genes in *C. neoformans*.

Therefore, our goal is to determine if SAS3, SAR1, and ESA1 are acetyltransferases.

Our research aims to first isolate these genes and then individually express them into a bacterial vector. Once expressed, the proteins can be assessed for potential HAT activity via our HAT assay. Our current working HAT assay protocol involves using core histones purified from HeLa cell nuclear extract as substrate. p300, containing a specific HAT domain region, is used as an enzymatic positive control.



Experimental Plan / Aims

Our plan is to assess novel protein activity on core histone substrates using our current working HAT assay protocol.

Aim #1 – Perform western-based HAT assay with purified core histones from HeLa cells and purified p300

Aim #2 – Clone putative HATs into bacterial expression vector.

Aim #3– Purify His-tagged proteins for use in HAT assay.

Lab Techniques Utilized

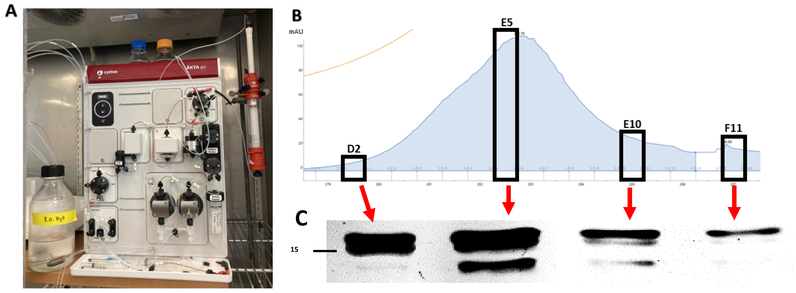
Nucleic acids protocols: E.coli transformation (GC5), Maxi prep of DNA plasmid without kits, DNA isolation from agarose gel with kits, Cloning primer design via ApE software, rtPCR/cDNA synthesis.

Protein purification: AKTA chromatography system (FPLC), E.coli transformation (BL21), His-tag protein isolation from E.coli, Dialysis.

Protein Analysis: SDS-PAGE (Coomassie staining and Western blot).

Histone Acetyl Transferase Assay : Polyclonal antibody system.

Core Histone Purification Using AKTA



*Gel image of literature C.H. was adapted from Doninck et al. (2009) Phylogenomics of Unusual Histone H2A Variants in Bdelloid Rotifers.

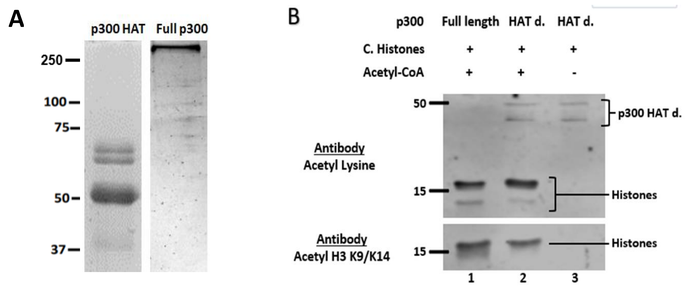
(A) This is an image of the major components of an AKTA-go fast protein liquid chromatography purification system with a column attached on the right.

(B) The UV trace of histone eluting from HTP column in action. HeLa cell nuclear extract was mixed with HTP powder and low salt buffer and self-poured into an empty column. The column was washed with low salt buffer. Histones were eluted with high salt buffer, showing a UV trace with a peak absorbance around 100 mAU and a similar peak shape with each CH purification. Fractions were collected in wells D4 to F10 (area shaded blue).

(C) Fraction D2, E5, E10 and F11 were run on SDS-PAGE to determine the range of fractions to collect. As indicated by the peak absorbance, fraction E5 indicated the successful isolation of core histones. The range of fractions E1 to E9 was collected as the purified histones and dialyzed.

In conclusion, core histones were successfully purified from HeLa cells with AKTA HTP column and used in further experimentation.

Purification of p300 HAT Domain and HAT Assay



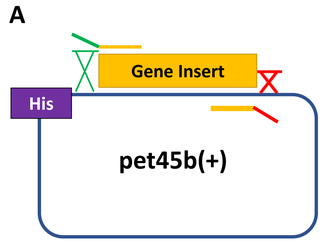
(A) This is an image of the purified p300 HAT domain construct, shown to be 50 kDa in size. The p300 HAT domain construct was obtained from Dr. Biswas at the Indian Institute of Chemical Biology. The construct was transformed into BL21 E.coli cells and grown in LB-ampicillin broth, after which a His-resin prep was performed to isolate the p300 HAT domain.

(B) Once purified, the p300 HAT domain was incorporated into a HAT assay as a positive control. The absence of acetyl-CoA, the acetyl group source for the p300 enzyme to catalyze the acetylation reaction, served as the negative control. Core histones purified from HeLa nuclei served as the substrate. The p300 HAT domain catalyzed acetylation (lane 2) but failed to do so in the absence of acetyl-CoA (lane 3).

p300 HAT domain was purified and used as a positive control within a HAT assay protocol that will be used to determine the potential HAT activity of SAS3 and SAR1.

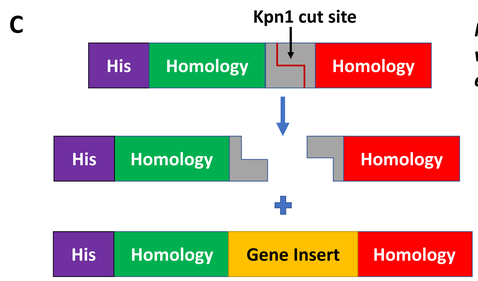
Cloning and Expression of Putative HATs

Before assessing HAT activity, SAS3, SAR1 and ESA1 must first be cloned using a homologous recombination system and purified using the same method as the p300 purification.



B

	Forward primers	Reverse primers
SAS3 Gene	CCACCATCACGTGGGTACCATGG CCTCGACAGTGCCATC	ACACGAGCTCCCAATTGGTCATA ACAAAACGCAATCCTC
SAR1 Gene	CCACCATCACGTGGGTACCATGT TCATCATCAACTGGTTC	TCGTCATCATTCGAACCGTCAGAT ATATTGACTCAGCCA
ESA1 Gene	CCACCATCACGTGGGTACCATGT CACCGTCAACACCGC	TCGTCATCATTCGAACCGTTAAAA ACCAAACGCCAATTGCG



For our procedure, the pet45b(+) vector was cut with restriction enzyme Kpn1.

Once the plasmid is cut with Kpn1, a homologous recombination kit will be used to insert the desired genes into the cut vector via overlapping homology regions.

(A) pet45b(+) vector with primer homology and annealing regions illustrated.

(B) Primers used to amplify putative HAT genes (homology regions highlighted green and red).

(C) Schematic of vector digestion and insertion by in vitro homologous assembly.

Current Trouble-shooting Plan

1. Due to low activity detection in a preliminary HAT assay, the SAR1 protein will be repurified and retested to optimize protocol parameters.
2. Optimize PCR conditions for ESA1 and SAS3 genes to obtain high PCR product yields.
3. Individually express ESA1 and SAS3 genes into E.coli, followed by purification and function assessment via HAT assay.

Future Goals/Acknowledgements

Future Goals:

1. Perform HAT Assay with purified putative HAT enzymes to determine if they are acetyltransferases.
2. Obtain a variety of histone substrates to incorporate into HAT Assay protocol:
   - Histones purified from *S. cerevisiae*
   - Histones obtained from *C. neoformans*
   - Histones in the context of chromatin (nucleosomes)

Acknowledgements:

We wish to thank Mariko Locke for providing core histones, Debabrata Biswas, Ph.D. for p300 HAT domain construct, the Price Lab for yeast cultures, and the Center of Research and Scholarship and Department of Biology and Chemistry for university support.