Investigating the Histone Binding Potential of the ZZ and SANT Domains of Ada2

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Background

- Histones play a pivotal role in condensing DNA into chomatin

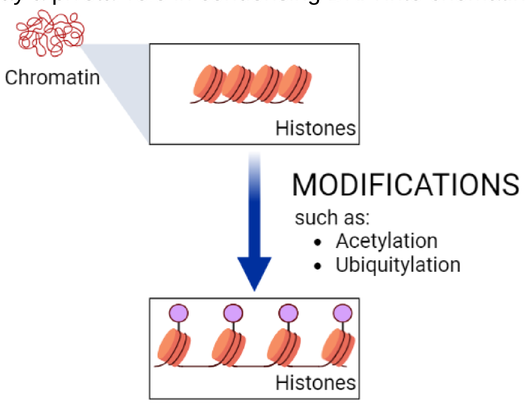
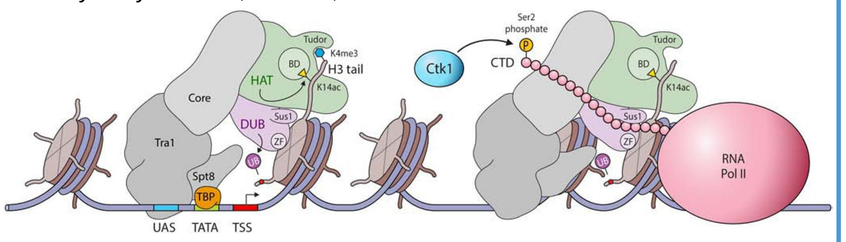


Fig. 1. Histone post-translation modifications modulate gene expression. Post-translational modifications (PTMs) are chemical alterations like acetylation and ubiquitylation. They cause remodeling of chromatin from their simple presence and recruitment of chromatin remodeling factors, changing access for polymerases, and ultimately modulating gene expression [1].

- PTMs are often associated with cancer progession.
- Many enzymes add, remove, or read PTMs



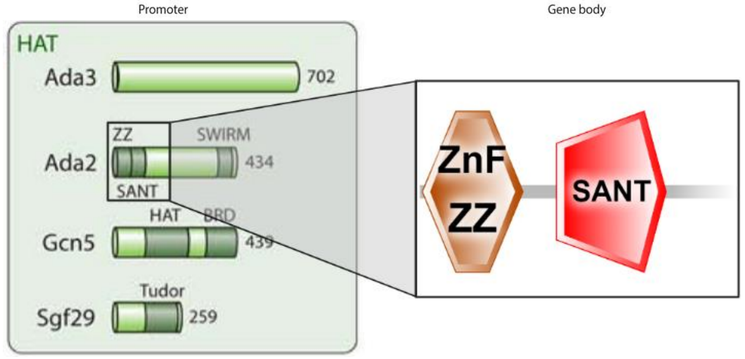


Fig. 2. SAGA Complex of *Saccharomyces cerevisiae* is involved in transcription and modifying & reading PTMs (Adapted from [2-3]). HAT module adds and reads PTMs. Gcn5 acetylates lysine and Ada2 reads histone modification. Ada2 consists of two major domains: ZZ and SANT.

Research Question

Do ZZ and SANT domains of Ada2 interactively and independently bind unmodified and modified H4 N-terminal tails?

In vitro Histone H4 N-terminal Interogation

METHOD: Peptide pulldowns paired with Western Blot (1° antibody – Anti-GST)

Unmodified and Modified H4 N-terminal tails bind ZZ and SANT

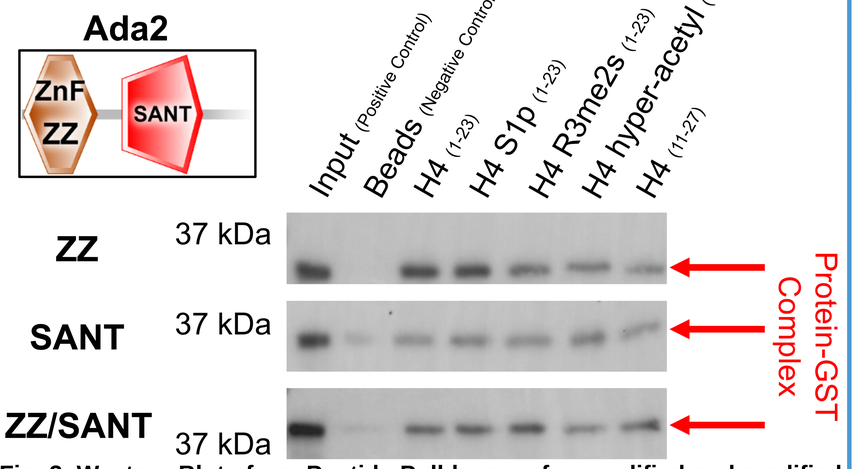


Fig. 3. Western Blots from Peptide Pulldowns of unmodified and modified H4 N-terminal tails with the isolated ZZ and SANT domains and a protein construct with both ZZ and SANT domains (ZZ/SANT) (Partly adapted from [3]). The input is a positive control with protein only (diluted to 0.8% of other lanes), while beads is a negative control with no peptides. H4 hyper-acetyl (1-23), which is acetylated at K5, K8, K12, & K16 and unmodified H4 (11-27) had slightly lower binding levels.

Higher salt concentrations reduce binding

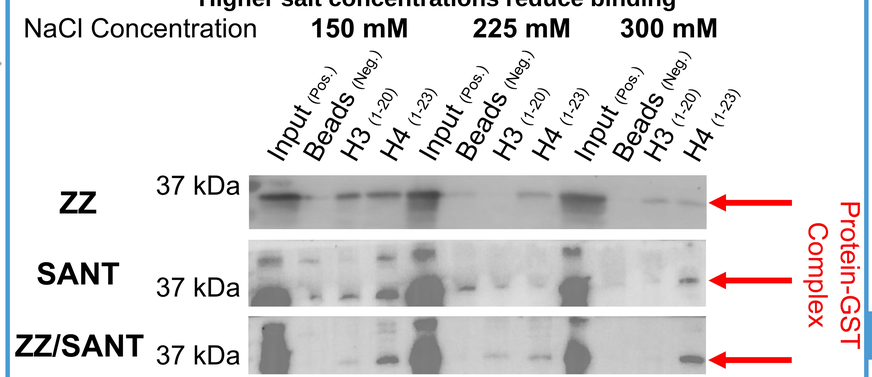


Fig. 4. Salt Concentration Test. Peptide Pulldowns were conducted for H3 and H4 N-terminal tails with ZZ, SANT, & ZZ/SAN at three salt concentrations (150 mM, 225 mM, 300 mM). The input is a positive control with protein only (diluted to 8% of other lanes), while beads is a negative control with no peptides. Higher salt concentration reduces binding with H3 & H4 tails.

In silico Histone N-terminal Interogation

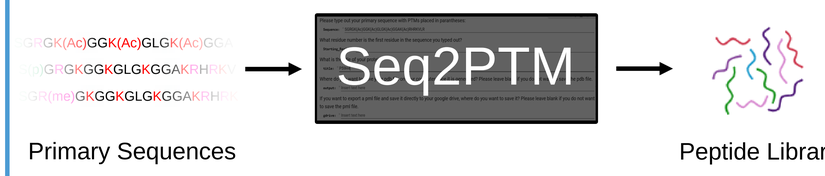


Fig. 5. Seq2PTM is a new useful tool to create peptide libraries.

- Developed novel algorithm, Seq2PTM, to generate library of post-translationally modified Histone H3 and H4 N-terminal tail pdb files
- Seq2PTM reads primary sequences with PTMs encoded in paretheses
- Seq2PTM generates code to fabricate the peptides with PTMs in PyMol

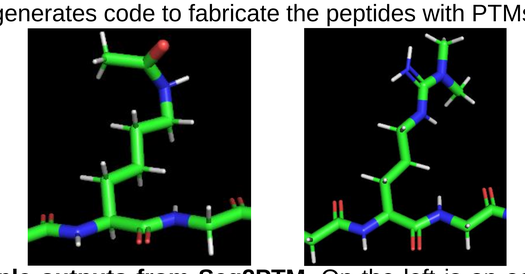


Fig. 6. Example outputs from Seq2PTM. On the left is an acteylated lysine and on the right is a symetrically dimethylated arginine.

- Conducting ongoing investigation to find appropriate program and methodology to simulate docking between peptides and Ada2 domains

Future Work

In silico

- Determine best program & methodology to dock N-terminal tails with Ada2
- Broaden functionality of Seq2PTM by adding support for more PTMs
- Expand use of Seq2PTM algorithm to other projects

In vitro

- Incorporate N-terminal tails from other histone proteins
- Expand analyses to mutated versions of Ada2

In vivo

- Compare levels of PTMs between wild type and mutated versions of Ada2
- Investigate effects of mutated versions of Ada2 on yeast growth

Acknolewdgements

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Conclusions

- H4 N-terminal tails, both unmodified and modified, bind with both ZZ and SANT domains in isolation and with constructs containing both domains
- H4 hyper-acetyl (1-23) and unmodified (11-27) N-terminal tails exhibit slightly lower levels of binding
- Higher salt concentrations reduce binding between H3 and H4 tails

References

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