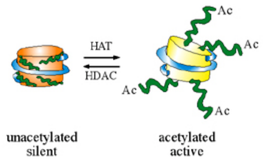
A proteomic approach to studying histone modifications using HDAC inhibitors



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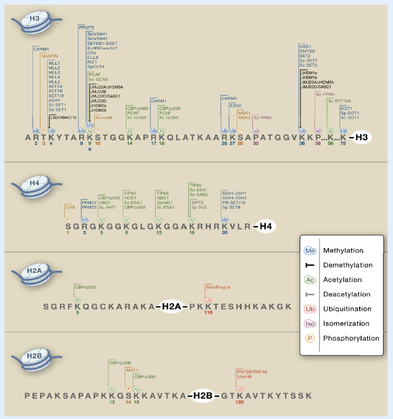
Introduction

Chromatin is made up of DNA coiled around proteins, the majority of which are histones. These histones have tails, which are usually positively charged and hold the negatively charged DNA closely coiled. This makes the DNA inaccessible to RNA polymerase which would otherwise transcribe it, preventing the genes from being expressed. When the amino acids that make up these histone tails are modified, this can change the charge and allow the DNA to coil more loosely, granting access to the RNA polymerase and allowing the genes to be expressed. This is one way in which gene expression is regulated.

One such modification of histones is acetylation, where acetyl groups are added to lysines on the histone tail. This neutralizes the charge of the histone tail, leaving the DNA to coil more loosely and up-regulating the relevant genes. Some processes of histone modification, like methylation and demethylation, have been explored, and it is known which enzymes cause which parts of the histone tails to be methylated and demethylated. Also, it is known which enzymes can make which lysines acetylated. What has not yet been explored is whether enzymes that cause histone deacetylation (called HDACs) have specificity, and whether this specificity could be mapped as with methylation. By using HDAC inhibitors, the hope of this project was to identify and map such specificity.



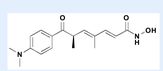
Picture from Kourarides, Tony. Chromatin Modifications and Their Function. *Cell* **128** 693-705 (2007)

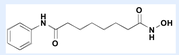
Materials

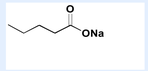
•HeLa cells, which are human cervical cancer cells, were used. Controls were grown in media with isotopically heavy arginine and samples were grown in isotopically light arginine.

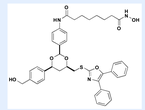
•Trypsin, which is a protease that "cuts" proteins after any arginines or unmodified lysines

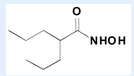
•15 known HDAC inhibitors (HDACi) chosen for their diverse structures (shown below)

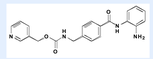

Trichostatin A (TSA)

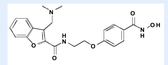

SAHA

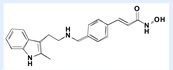

Sodium Butyrate

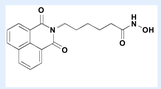

Tubacin


Valproic Acid


MS275


Benzoic hydroxymate (411711)


Cinnamyl Hydroxamate (40700)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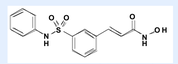
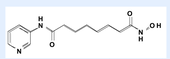
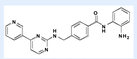

PXD101 (41437)


Pyroxamide (40529)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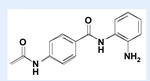
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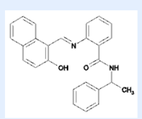
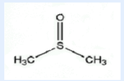
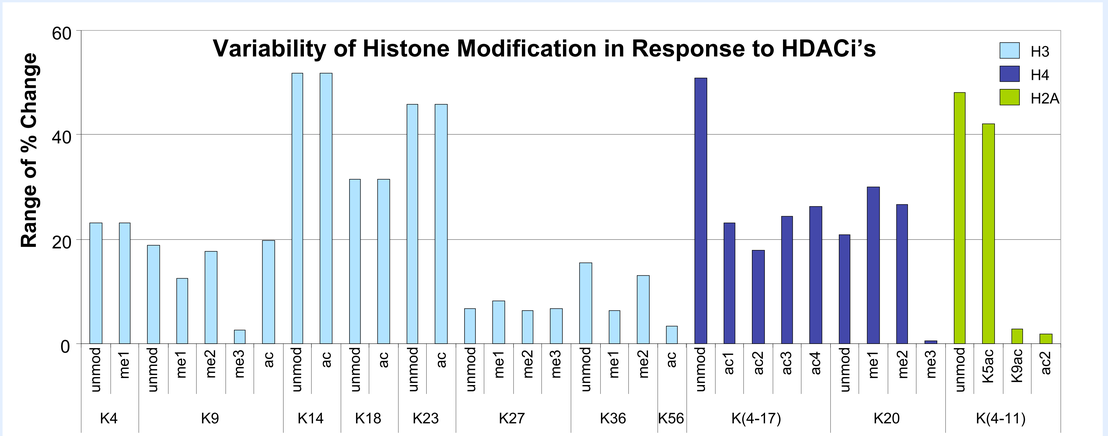
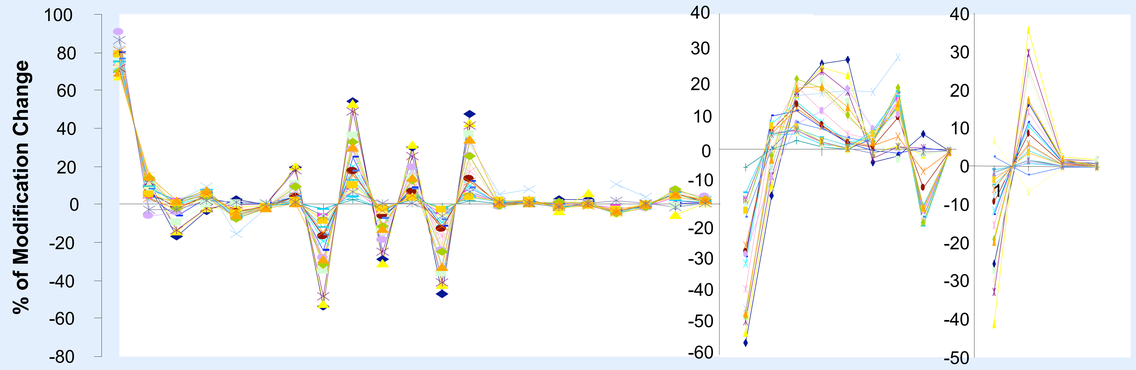

Sirtinol


DMSO

Results





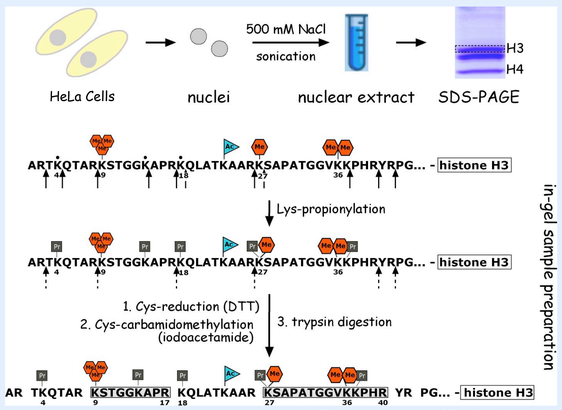
The upper graph shows the variability for modification of each lysine site. The variability is the difference between the change generated by the HDACi inducing the greatest amount of modification and the HDACi inducing the least amount of modification.

The lower graph plots the amount of modification at each lysine site separately for each HDACi, showing the effect of each HDACi on each site.

TSA_H
TSA_L
SAHA_H
SAHA_L
Sodium butyrate_H
Sodium butyrate_L
Tubacin_L
Valproic acid_H
Valproic acid_L
MS275_L
41171_H
41171_L
40700_H
40700_L
Scriptaid_H
Scriptaid_L
41437_H
41437_L
40529_H

Methods

1. HeLa cells were grown and treated with small molecules
2. Cells were lysed
3. Electrophoresis gel was run to isolate histones
4. Gel was stained to see bands
5. Appropriate band was cut out, and each sample's piece of gel was cut into 1 mm cubes
6. Histones were extracted from gel
7. Unmodified lysines were propionylated
8. Trypsin was used to cut after each arginine
9. Samples were run through the mass spectrometer



Picture courtesy of Stefan Kubicek

Conclusions

•Different sites for modification demonstrate differing levels of modifiability.

•It seems HDACi's have no specificity on histone H3, but are specific for H4 and H2A.

Acknowledgments

Thank you to Jianping Cui, Henry Jung, Stuart Schreiber, Stefan Kubicek, Megan Rokop, Julie Boehm, and Kate MacSwain.