Multiply Acetylated Histone H3 Generated by Nonsense Suppression and Preliminary Investigations of its Function

Isaac Young, Chitvan Mittal, Melissa J. Blacketer, Ying Lai, Yeon-Kyun Shin, and Michael Shogren-Knaak
Outline

• Background
  • Lysine Acetylation Affects Chromatin

• Research
  • Making Acetylated H3
  • Nucleosome Stability
  • Acetylation Affects Protein Recruitment
Chromatin Structure

[Diagram showing the structure of chromatin, with DNA and histone tails highlighted.]
Acetylation Promotes Transcription

Enzyme

Lysine → Acetyl-Lysine

Tetra-Acetylated H3

H3

K9 K14 K18 K23

Acetylation
Outline

• Chromatin and Lysine Acetylation
• Making Tetra-Acetylated H3
• Nucleosome Stability
• Acetylation and Protein Recruitment
Making Acetylated H3

- Isolation
- Enzymatic Treatment
- Native Chemical Ligation
- Non-Sense Suppression
Nonsense Suppression

K14 to “Stop”

+Acetyl Lysine tRNA With “Stop” Anticodon

Acetylation
Nonsense Suppression
Expression Acetylated H3

Tetra Acetylated H3

Differentially Acetylated H3
Tetra-Acetylated His-6 Histone Purification Strategy

Typical Strategy

- Induced Lysate
- Insoluble Protein
- Ni-NTA Capture
- Protease Release
- Cation Exchange

Uncleaved

- Non-Induced Lysate

Cleaved
Mass Spec Tetra-Acetylated H3
Producing Mono-Nucleosomes In-Vitro

Recombinant Histones

2x H2A + 2x H2B + 2x H4 + 2x H3

• Unfold
• Dialysis, High Salt

Gel Filtration

Histone Dimer

Fraction

DNA

Histone Octamer

Mononucleosome Assembly Gel

Octamer:DNA Ratio

Mononucleosomes

Free DNA
Outline

• Chromatin and Lysine Acetylation
• Making Acetylated H3
• Nucleosome Stability
• Acetylation and Protein Recruitment
Acetylation Destabilization Hypothesis

Lysine → Enzyme → Acetyl-Lysine

H3: Acetylation sites 9, 14, 18, 23
Stability Assay

Hypothesis: $k_1 > k_2$
Following Nucleosome Dissociation - Single Molecule Approach

\[ k_2 \]

[Diagram showing nucleosome dissociation with +NaCl and laser emissions for Cy3 and Cy5 fluorescence.]

Collaboration Shin Lab
Preliminary Dissociation Results For Unacetylated Nucleosomes

\[ y = 1.1187e^{-0.064x} \]

\[ R^2 = 0.9747 \]
Single Step Photobleaching
Proof of Single Molecules

Fluorescence Intensity (au)

molecule 18

Cy3 Fluorescence
Cy5 Fluorescence
Outline

• Chromatin and Lysine Acetylation
• Making Acetylated H3
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Acetylation Spreading Hypothesis

Acetylation — SAGA Recruitment — Neighboring Nucleosome Acetylation?
Acetylation Spreading Assay

Low Recruitment \[\rightarrow\] Low Acetylation?

High Recruitment \[\rightarrow\] High Acetylation?

Radioactive Acetylation= • Chitvan Mittal
Dr. Michael Shogren-Knaak

Everyone in my lab:
Chitvan Mittal
Melissa J. Blacketer
Sannie Jane Olson
Maggie Gannon

Previous members in my lab:
Divya Sinha
Abdelhamid Azzaz

The Shin Lab
Ying Lai
Xiao Chou
Jaeil Shin
Yeon-Kyun Shin
Chromatin Post Translational Modifications (PTMs)
1. **Clone**

   Lysine Codons:
   
<p>| | | | |</p>
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<tbody>
<tr>
<td>9</td>
<td>14</td>
<td>18</td>
<td>23</td>
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   Wild Type
   
   AMBER STOP
   
   Pet Vector

2. **Mutagenesis**

   AAA
   
   AAG
   
   AAG
   
   AAG

   Ochre Stop

   pCDF Duet vector

3. **Mutagenesis**

   AAA
   
   AAG
   
   AAG
   
   AAG

   Single Amber Stop

4. **Mutagenesis**

   AAA
   
   TAG
   
   AAG
   
   AAG

   Double Amber Stop

5. **Mutagenesis**

   TAG
   
   TAG
   
   TAG
   
   AAG

   Triple Amber Stop

6. **Mutagenesis**

   TAG
   
   TAG
   
   TAG
   
   TAG

   Quadruple Amber Stop
Steps in Expression
① charge TRNA's
② Induce transcription
③ 3 hour expression time
Acetylated histone purification

Typical Purification

1. Inclusion Body Isolation
2. Solvation/Denaturation
3. Size Exclusion Chromatography
   - Denaturing Conditions
4. Ion Exchange Chromatography
   - Denaturing Conditions

Resulting Product is impure:

Revised Purification Strategy

1. Inclusion Body Isolation
2. Solvation/Denaturation
3. Nickel-Histidine Affinity Capture
   - Denaturing Conditions
4. TEV Protease Digestion
   - Non-Denaturing Conditions
5. Elution of cleaved product
   - Denaturing conditions
6. Ion Exchange Chromatography
   - Denaturing Conditions
7. C8 Reverse Phase Sep-Pak Waters™
   - Concentrates the Protein Sample
   - Prepares it for Lyophylization
Generating Acetylated and Non-Acetylated In-Vitro Chromatin Model Systems

Non-acetylated Nucleosomal Array

Acetylated Nucleosomal Array
Nonsense Suppression Data

[Acetyl-lysine] (mM) | Tetra-Acetylated H3 | Mono-Acetylated H3
10 | 20 | 40 | 40 | 10 | 40

Western Anti-H3

Pre post

pre post pre post
Red+Green Laser Excitation

Cy3 and Cy5 dye excitation
Three Control Experiments

- Photobleaching Events
  - Falsely Read as Dissociation Events
- Other Contaminants Detected as Dots
- We may detect many mono-nucleosomes in a single dot.
  - Clouded Data = Harder to Interpretable
Data of Control Experiments using Cy3 and Cy5 Labeled DNA

**cy3 and cy5 photobleaching events in image buffer**

- **Cy3 Dye photobleaching events**
- **cy5 dye photobleaching events**

**Cy3 and Cy5 photobleaching events in the presence of Tris buffer**

- **cy3 photobleaching events**
- **cy5 photobleaching events**

**Cy3 and cy5 photobleaching events in the presence of 1.5 M NaCl image buffer**

- **Cy3 photobleaching events**
- **cy5 photobleaching events**
Preliminary Data Shows Wild-Type Nucleosomes Dissassemble When Salt Buffer is Added

1 Molar NaCl Induced Nucleosome Dissassembly