Chromatin Structure and Its Regulation–2

Key Points

1. Histones provide a versatile regulatory platform through their many post-translational modifications. Specific modifications are bound by specialized protein domains that bring about distinct downstream events.

2. Acetylated histones strongly correlate with transcriptional activation and deacetylated histones with transcriptional repression. Acetylation can facilitate transcription initiation directly by disrupting higher order chromatin folding or indirectly by recruiting bromo-domain containing transcription factors.

3. A major pathway of altering chromatin structure is through the action of ATP-utilizing machines or ‘chromatin-remodeling complexes’. These ATPases have homology with DEAD box family DNA helicases. Different classes of chromatin-remodeling complexes generate different biochemical outputs. Not clear how the different biochemical outputs relate to their distinct biological roles.

4. Methylation of histones on lysines has context dependent effects. Trimethylation at Lysine 9 and 27 correlates with gene repression, whereas trimethylation at Lysine 4 often correlates with gene activation. Methylated histones are recognized by specific domains such as chromodomains or PHD fingers.

5. Heterochromatin allows for silencing of large domains of the genome and utilizes a combination of mechanisms to do so.

7. The presence of more than one type of binding domain within large regulatory complexes suggests mechanisms for specific recognition of different combinations of histone modifications within one nucleosome.

8. There seem to be both regular and more liquid like ways to fold chromatin into compact states.
References

Reviews on Histone Modifications


Histone Acetylation


### ATP-dependent Chromatin Remodeling Enzymes


3. Racki L.R. and Narlikar GJ. *ATP-dependent chromatin remodeling enzymes: two heads are not better, just different*. Curr Opin Genet Dev. 2008. **18:**137-44.


9. Zhang Y. et al., DNA translocation and loop formation mechanism of chromatin remodeling by SWI/SNF and RSC. Mol Cell. 2006. **24:**559-68


**Interplay between SWI/SNF and HATs**


**Chromodomains and PHD fingers**


**Heterochromatin formation and regulation**


**Higher Order Chromatin folding**


Outline

Functions of Chromatin

- Intrinsic properties
- Packing material
- Regulation of intrinsic properties
- Complex regulatory platform
- Coordination/coupling
- Replication
- Transcription
- RNA processing
Histone tails mediate inter-nucleosomal contacts through electrostatic interactions

- Highly basic histone tails interact with DNA of neighboring nucleosomes
- Histone H4 tail interacts with an acidic patch formed by H2A-H2B

Linker histones (Histone H1) promote chromatin folding
Histones contain many different post-translational modifications: concentrated on N-terminal tails but also found on internal regions.

**Implication:** more functions than just packing DNA
Two Case Studies

- **Histone H3 and H4 Acetylation** ➔ Euchromatin (active genes)
- **Histone H3 K9 Methylation** ➔ Heterochromatin (repressed genes)

*Drosophila* salivary glands polytene chromosomes stained to detect the DNA

Lighter stains = euchromatin
Darker stains = heterochromatin
**The role(s) of lysine acetylation in histone tails**

Hyperacetylation of histones was correlated with active genes over 30 years ago.

GCN5 was originally identified as a transcriptional co-activator of amino acid biosynthesis genes

1996: yeast GCN5 was shown to have acetyl transferase activity *in vitro*
Results in loss of one positive charge
GCN5 is part of SAGA complex

Other functions of SAGA: Several genes are SAGA dependent but GCN5 independent

- Interact with TBP
- Removes monoubiquitin from H2B (location in complex not known)
- Interacts with activators

from EM structure
Acetylation is reversible

Histone De-acetylases (HDACS or KDACs) remove acetyl groups
---most often correlate with gene repression

Histone acetyl transferases (HATs or KATs) add acetyl groups
---most often correlate with gene activation

Acetylation state is very dynamic
---turnover within minutes

How does histone acetylation enhance transcription?
How does histone acetylation enhance transcription?

Lys $\overset{+}{\text{H}} \text{N} - \text{H} \overset{\text{vs.}}{\text{vs.}} \text{Lys} \overset{\text{O}}{\text{C}} \text{N} - \text{H}$

(1) Does acetylation reduce histone-DNA interactions?

$K_{eq} = [\text{open}]/[\text{closed}]$

Hyperacetylation of all the histone tails increases $K_{eq}$ by ~2 fold
(2) Acetylation has larger effects (greater than 10-fold) on disrupting inter-nucleosomal contacts and on chromatin compaction.

Single acetylation mark on H4 lysine 16 has similar impact on chromatin compaction as deleting H4 tail.
(3) Acetylated lysine provides a recognition motif for an “effector protein”

Bromodomain

Bromodomains specifically recognize acetylated lysines
GCN5 bromodomain with H4 tail acetylated at K16
Budding yeast has 15 bromodomains

1 bromodomain

8 bromodomains

9 of them are distributed between two ATP-dependent chromatin remodeling complexes
SWI/SNF and RSC can enable many different outcomes

transfer histone octamers

move histone octamers

Generate DNA “loops”

exchange and remove histone dimers

Many open mechanistic questions
So far four major families of ATP-dependent chromatin remodeling complexes:

**High degree of conservation from yeast to humans**

Classified based on their ATPase subunits, which are shown below:

<table>
<thead>
<tr>
<th>Family</th>
<th>Gene Activation and Some Gene Repression</th>
<th>Multiple Biochemical Outcomes</th>
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</thead>
<tbody>
<tr>
<td>SWI/SNF</td>
<td>Some Activation, Mostly Gene Repression</td>
<td>With Heterochromatin Formation: Only Moves Nucleosomes</td>
</tr>
<tr>
<td>ISWI</td>
<td>Gene Activation and Repression</td>
<td>Only Move Nucleosomes</td>
</tr>
<tr>
<td>CHD</td>
<td>Gene Activation, DNA Replication, DNA Damage Responses</td>
<td>Move Nucleosomes and Exchange Variant Histones</td>
</tr>
<tr>
<td>INO80</td>
<td>Not Clear (i) If the Different Families Work by Similar or Distinct Mechanisms and (ii) How Their Different Biochemical Outputs Relate to Their Different Biological Roles</td>
<td></td>
</tr>
</tbody>
</table>
A cascade of events at the promoter of the human interferon-b gene

Human TFIID contains TAFII250, which is a HAT and also contains a double bromodomain.
Several methylation marks with different readouts

- Methyl marks are bound by Chromodomains and PHD fingers
- Methylases put on the mark and De-methylases remove the marks
Lysine

\[ \begin{align*}
\text{unmodified} & : H_N^+H \\
\text{acetylated} & : H_N^+C(CH_3)_2O \\
\text{methylated} & : H_N^+CH(CH_3)_3
\end{align*} \]

Arginine

\[ \begin{align*}
\text{unmodified} & : H_N^+H \overset{\text{+}}{\longrightarrow} N-C\overset{\text{+}}{\longrightarrow} NH_2 \\
\text{acetylated} & : H_N^+C\overset{\text{+}}{\longrightarrow} NH_2 \\
\text{methylated} & : H_N^+CH(CH_3)_3 \overset{\text{+}}{\longrightarrow} N-C\overset{\text{+}}{\longrightarrow} NH_2
\end{align*} \]
Chromodomain and PHD fingers have independently evolved to use cation-π Interactions to stabilize methylated lysines.

Hydrophobic cage gives mark specificity

Crystal structure of chromodomain from Drosophila HP1 protein

Interactions with residues surrounding the ARKS motif give position specificity

(mutually exclusive)
Position Effect Variegation reveals ability of heterochromatin to spread

Heterochromatin can spread over more than 1000 kb of previously euchromatic chromatin and heritably silences genes

Effects linked to the HP1 protein and methylation of Histone H3 at K9

HP1 binds methyl mark using a chromodomain
Some open questions

1) How does heterochromatin spread?

2) How is silencing achieved?

- Is the silencing achieved by heterochromatin qualitatively different than repressors binding at gene promoters?
- Does chromatin condensation also contribute to gene silencing?
- Any additional mechanisms?
Paradoxically, in some well-studied model systems like fission low levels of transcription are needed to enable heterochromatin formation and function........

RNAi based mechanisms provide a second path to recruiting methylase
“Histone Code” Hypothesis

“Histone modifications, on one or more tails, act sequentially or in combination to form a 'histone code' that is, read by other proteins to bring about distinct downstream events”


PHD fingers and chromo- and bromodomains are present in large complexes

ATP-dependent chromatin remodeling complex - opens up chromatin

Nucleosome as a template to integrate signals
Histone Variants - more Diversity and more Regulation

Variants are deposited by specific histone chaperones or ATP-dependent remodeling enzymes.
What do we know about higher-order chromatin folding?

30 nm fibers
- seen in some terminally differentiated cells
- seen with reconstituted chromatin
- not seen in proliferating cells
- not seen in mitotic chromosomes
Some newer models for packing of Interphase chromatin
Methods to look for long-range DNA interactions

a Chromosome conformation capture: converting chromatin interactions into ligation products

Cross-linking of interacting loci → Fragmentation → Ligation → DNA purification

b Ligation product detection methods

3C
One-by-one
all-by-all

4C
One-by-All

5C
Many-by-Many

ChIA-PET
Many-by-Many
• DNA shearing
• Immunoprecipitation

Hi-C
All-by-All
• Biotin labeling of ends
• DNA shearing

PCR or sequencing
Inverse PCR sequencing
Multiplexed LMA sequencing
Sequencing
Sequencing
Chromosomes seem to occupy distinct territories in three-dimensional (3D) space.

Job Dekker, and Tom Misteli Cold Spring Harb Perspect Biol 2015;7:a019356