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 appears to utilize a combination of mechanisms.

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 disordered ways to fold chromatin into compact states.
References

Reviews on Histone Modifications


Histone Acetylation


**ATP-dependent Chromatin Remodeling Enzymes**


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 \textit{in vitro}
Lysine acetylation by GCN5

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.
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?
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(1) Does acetylation reduce histone-DNA interactions?

\[ K_{eq} = \frac{[\text{open}]}{[\text{closed}]} \]

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”

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
- Exchange and remove histone dimers
- Generate DNA “loops”

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:

1. **SWI/SNF family**
   - gene activation and some gene repression: multiple biochemical outcomes

2. **ISWI family**
   - some activation, mostly gene repression and heterochromatin formation: only moves nucleosomes

3. **CHD family**
   - gene activation and repression: only move nucleosomes

4. **INO80 family**
   - gene activation, DNA replication, DNA damage responses: move nucleosomes and exchange variant histones

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.
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

\[
\begin{align*}
\text{unmodified} & : & \text{H}^+ - & \text{H} - & \text{N} - & \text{C} - & \text{O} - & \text{CH}_3 \\
\text{acetylated} & : & \text{N} - & \text{C} - & \text{O} - & \text{CH}_3 \\
\text{methylated} & : & \text{N}^+ - & \text{CH}_3 - & \text{CH}_3 - & \text{CH}_3
\end{align*}
\]

Arginine

\[
\begin{align*}
\text{unmodified} & : & \text{H}^+ - & \text{H} - & \text{N} - & \text{C} - & \text{N} - & \text{H}_2 \\
\text{acetylated} & : & \text{N} - & \text{C} - & \text{N} - & \text{H}_2 \\
\text{methylated} & : & \text{N}^+ - & \text{CH}_3 - & \text{CH}_3 - & \text{N} - & \text{C} - & \text{H}_2
\end{align*}
\]
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
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?
HP1 Heterochromatin is a functionally versatile platform

- Repression of transcription
- Repression of recombination
- Chromosome segregation

Histone methyltransferases ➔ Histone deacetylases ➔ RNAi proteins ➔ Chromatin remodeling enzymes ➔ Spreading

H3K9 methylase can be recruited by:
(i) sequence specific DNA binding factors
(ii) RNAi based mechanisms

Model: Recognition of H3K9-methyl mark helps localize HP1 and oligomerization of HP1 promotes “spreading”
Cooperative assembly concentrates HP1α on chromatin and possibly induces compaction.

Phase separated droplets provides a means to sequester chromatin.

Phase-separation based mechanisms?
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

TAD: topologically associated domain

Current Opinion in Genetics & Development, Volume 37, 2016, 36–45
“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.