Chromatin Structure and Its Regulation–2

Key Points

1. There are at least two ways in which arrays of chromatin can fold into the 30 nm fiber.

2. 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.

3. 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.

4. 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.

5. 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.

6. Heterochromatin allows for silencing of large domains of the genome and appears to utilize 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.
References

Higher Order Chromatin folding


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.


10. 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**


Outline

Functions of Chromatin

Intrinsic properties → Regulation of intrinsic properties
Packing material → Complex regulatory platform → coordination/coupling

Replication → Transcription → RNA processing
Higher order compaction of chromatin

Electron micrographs

One start

Two start

30 nm
Regulation of higher order chromatin folding

Short inter-nucleosomal spacing ($\leq 20$ bp) may favor two-start structure while longer spacing may favor one start structure.

In vivo folding may be regulated by other proteins. Perhaps the chromatin fiber adopts additional packing conformations *in vivo*?
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

H2A-H2B acidic patch

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
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

from EM structure

Interact with TBP

Removes monoubiquitin from H2B (location in complex not known)

Interacts with activators

Ubp8

Spt3

Spt20

Ada1

6

Taf5

Dna1

12

10

Gcn5
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?

\[
\text{Lys} \quad \text{H}^+ \quad \text{vs.} \quad \text{Lys} \quad \text{CH}_3
\]

(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

SWI/SNF

Snf11
Swp82
Swi2/Snf2
Swi3
Snf5
Swp73
Swi6
Arp9
Arp7
Swp29

1 bromodomain

RSC

Sth1
Rsc1
Rsc2
Rsc3
Rsc4
Rsc5
Rsc6
Rsc7
Rsc8
Rsc9
Rsc10
Rsc13
Rsc14
Rsc15

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

- **SWI/SNF family**
  - HSA
  - Bromo
  - DExx
  - Short insertion
  - HELICc
  - Gene activation and some gene repression: multiple biochemical outcomes

- **ISWI family**
  - SANT
  - SLIDE
  - Tandem chromo
  - Some activation, mostly gene repression and heterochromatin formation: only moves nucleosomes

- **CHD family**
  - Tandem chromo
  - Gene activation and repression: only move nucleosomes

- **INO80 family**
  - HSA
  - Long insertion
  - DExx
  - HELICc
  - 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

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mutually exclusive

mutually exclusive
Lysine

unmodified

acetylated

methylated

Arginine

unmodified

acetylated

methylated
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.
A new role for histone modifications: allosteric regulation of enzyme activity

Binding of H3K27 methyl mark by EED C-term domain stimulates methylase activity of EZH2, the H3K27methylase
Histone Variants - more Diversity and more Regulation

Variants are deposited by specific histone chaperones or ATP-dependent remodeling enzymes.