Chromatin Structure and Its Regulation-1

Key Points

1. Packaging of eukaryotic DNA into nucleosomes and higher order folded states provides a means to functionally compartmentalize genes. The DNA in nucleosomes is tightly bound but very dynamic, unpeeling and rebinding on the order of milliseconds.

2. The accessibility of DNA within a nucleosome is very sensitive to its location and can be reduced relative to free DNA from 10-fold to 500,000 fold.

3. The intrinsic preferences of DNA for histone octamers can play a role in positioning nucleosomes in vivo but there is more regulation of nucleosome positions than just sequence

References

BOOKS:

ARTICLES:

Nucleosome Structure and Dynamics


In vivo mapping of nucleosome positions and occupancy


**Higher Order Chromatin folding**


Regulating the DNA template
Organized nature of eukaryotic DNA observed more than a 100 years ago

1882: Salivary gland cell from Bloodworm(?) larvae observed by Walther Flemming

Lighter stains = euchromatin
Darker stains = heterochromatin
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A few decades ago: *Drosophila* salivary glands polytene chromosomes stained with similar dyes

Lighter stains = euchromatin
Darker stains = heterochromatin

‘puffs” associated with transcription suggested major changes in chromatin organization
Outline

Functions of Chromatin

Packing material → Complex regulatory platform → Replication, Transcription, RNA processing

coordination/coupling
Outline

Functions of Chromatin

Intrinsic properties  Regulation of intrinsic properties
Packing material  Complex regulatory platform

coordination/coupling

Replication  Transcription  RNA processing
On a simple level packaging into chromatin helps compartmentalize genes
A closer look at the nucleosome

- 4 histones: 2 copies of each form an octamer that wraps 147 bp of DNA.
- N-terminal tails of histones extend out (~1/4th the histone mass).
- DNA is highly bent. Histone-DNA interactions stabilize this unfavorable conformation.
- DNA becomes less accessible to DNA binding proteins
- Almost all the histone-DNA contacts are sequence independent. Most interactions are between the negatively charged phosphate backbone of DNA and positively charged histone residues.

Yet some DNA sequences can bind histone octamers 100-fold more strongly than others. Why?
DNA sequence can affect energetics of DNA bending

Proper placement of AT and GC base-pairs can give DNA sequences with high histone octamer binding affinities relative to bulk genomic DNA

This pattern is seen in naturally occurring “nucleosome positioning sequences”
Nucleosomal DNA is intrinsically dynamic

Above numbers are for a site that is ~10 bp in from one end

Overall $K_d$ for nucleosomal site = $10/(1/20) = 200$ nM
The specific placement of a histone octamer on the genome can matter for regulating the access of DNA to a transcription factor.

1. DNA accessibility is very sensitive to its location within a nucleosome.

2. Rotational placement of DNA sequence on nucleosome can affect factor affinity.
Cooperative binding through protein-protein interactions

1. A and B directly interact
The architecture of the nucleosome can give cooperative binding behavior for non-interacting proteins

1. A and B directly interact

2. A and B do not directly interact
Debate in the field: does the genome encode its own packaging?

- Placement of AT and GC base-pairs every 10 bp can increase histone octamer binding affinities relative to bulk genomic DNA.

- The specific translational and rotational position of nucleosomes can have a large impact on accessibility of regulatory sequences.
Do intrinsic properties of DNA sequences play a significant role in positioning nucleosomes \textit{in vivo}?

\textbf{In vivo}

- Cell culture
- Harvested cells
- Washed chromatin pellet
- Fragmented chromatin
- ChIP DNA
- Mononucleosomal DNA
- Library
- Deep sequencing
- Mapped nucleosome locations
- Genome-wide nucleosome patterns
- Interrelationship with other biological observations

\textbf{In vitro}

- Purified yeast DNA + purified histones
- Assemble into nucleosomes
- Map locations by MNase and deep sequencing or micro-array
- Compare to \textit{in vivo} positions
Comparison of *in vivo* and *in vitro* maps in yeast suggested *in vivo* role for DNA sequence preferences

Average occupancy at each base-pair = \[ \frac{\text{number of reads that cover the bp}}{\text{average number of reads per bp across genome}} \]

But, it can’t be that simple...

1. How can one nucleosome map explain multiple transcriptional states? If nucleosome placement affects transcription factor binding and transcription factor binding regulates expression of specific genes, then when gene expression patterns change upon changes in environment, differentiation etc., the nucleosome positions and occupancy should change as well.

2. What happens to nucleosome positions and occupancy in the coding region when gene expression levels change?
The devil is in the details and the differences may reveal more than the similarities

1. Correlation between *in vivo* and *in vitro* maps is greater in non-promoter intergenic regions (R=0.83) and lower in promoter and coding regions (0.69)

2. At coding regions depletion levels of nucleosomes *in vivo* relative to *in vitro* increases with the expression levels of the associated genes

3. Both maps show nucleosome depletion at transcription start sites, but level of depletion *in vivo* is greater

4. Level of nucleosome depletion around transcription factor binding sites generally correlates between the two maps but there are interesting differences.

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**Nucleosome occupancy near different transcription factor binding sites**

![Diagram showing nucleosome occupancy near different transcription factor binding sites](image)
Nucleosome Occupancy vs. Positioning

(Example: # reads) Signal:

Positioning: High → Low

Position (relative to a ‘gold standard’)

Good → Poor → Meaningless

Pugh BF. Nat Struct Mol Biol. 2010; 17:923
MNase Hyper-sensitive and Hyper-resistant Footprints

Agarose gel run after deproteinizing MNase digested chromatin

MNase based nucleosome mapping data from Maize

Vera D L et al. Plant Cell 2014;26:3883-3893
MNase-Hypersensitive Footprints Correlate with regulatory elements like transcription factor binding sites

MNase based nucleosome mapping data from Maize

Vera D L et al. Plant Cell 2014;26:3883-3893