Chromatin Structure and Function in Transcription, Replication, Repair

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PHO5: "Classic Example" of Chromatin Remodeling in a Promoter (II)

1. Transcription activator Pho4 binds UASp1 in linker between nucleosomes
2. Pho4 recruits SAGA complex (containing Histone Acetyl Transferase Gcn5)
3. Gcn5p acetylates promoter region
4. Gcn5p (promodomain, binds acetylated histones) and recruits/stabilizes binding of SWI/SNF to newly hyperacetylated histones
5. SWI/SNF uses ATP hydrolysis to remodel promoter nucleosomes.
6. Histones are evicted (lost).
7. RNAPII and GTF bind promoter and initiate transcription


Epi-Genome Stability & Maintenance

Chromatin Dynamics

Transcription
Control Regions & Hypersensitive Sites
Promoters
Elongation
Nucleosome Positioning

Remodeling
Histone modifications & "Histone code"
ATP-dependent remodeling
Histone exchange
Nucleosome dynamics

Assembly
Replication
Recombination Repair

What determines DNA accessibility?
How can proteins access binding sites in nucleosomes?


Steric hindrance by histones & histone tails
Histone modifications might promote or prevent interactions

Nucleosome Structure
Steric hindrance by DNA
Histone modifications might promote or prevent interactions

Nucleosome Positions
(position of histone octamer on the DNA sequence)

Nucleosome Dynamics
(time dependent changes in structure and/or composition

5 bp

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Illustration of how nucleosome occupancy and positioning differ.

The upper panel shows a cross-section of a nucleosome, in which occupancy is distinguished from positioning. The lower panel shows how the two are measured. Occupancy is the area under the curve and reflects the local density of nucleosomes in a population, as illustrated by the column of spheres. Positioning or fuzziness is reflected in the standard deviation of the curve and is illustrated by how well the spheres are aligned in a column. The position of a nucleosome relative to some standard is indicated by how closely two peaks are separated. Comparing peaks of curves having high standard deviations is not likely to be meaningful because both peak locations have very high uncertainty.

Locus Specific Heterogeneity: nucleosome occupancy and positioning


High-Resolution Genome-wide Mapping of Nucleosomes

Chromatin Structure of the HIS3 Gene by MNase Digestion and Indirect Endlabeling

Suter et al. (1997) Embo J

1) Lee et al. (2007). 2) Yuan (2005)
Mechanisms of Nucleosome Positioning

**DNA sequence:**
- bendability
- flexibility

**Proteins:**
- direct contact
- indirect contact

**Boundaries:**
- exclusion by sequence
- exclusion by proteins

In its purest form, statistical positioning relies on a single positional barrier (left side), against which nucleosomes are ordered. A probabilistic density trace of where nucleosomes would reside in a population is shown.


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Nucleosome Positioning Mechanisms & DNA Accessibility

**Structural and dynamic properties are affected by**
- Histone variants
- Histone modifications
- Remodeling complexes
- NHCPs interacting with chromatin
- DNA-damage

Many amino acids of histones in the 'tails' and on the nucleosome surface are chemically modified.

Modification and demodification are done by enzymes included in (large) complexes

Histone modification is a dynamic and reversible process.

Histone modifications may alter the "intrinsic properties of nucleosomes and higher order structures

Histone modifications are binding sites for proteins (recruit NHCPs). Their presence on histones can dictate the higher-order chromatin structure in which DNA is packaged and can orchestrate the ordered recruitment of enzyme complexes to manipulate DNA

Crosstalk between histone modifications: a modification facilitates / inhibits modification at another site on the same or different histones /nucleosomes

Histone modifications constitute a set of markers (PTMs) of the local state of the genetic material, which has been called the 'histone code' (Strahl and Allis, 2000).
Posttranslational chemical modifications of histones (PTM)

Table 1. Different Classes of Modifications Identified on Histones

<table>
<thead>
<tr>
<th>Chromatin Modifications</th>
<th>Residues Modified</th>
<th>Functions Regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylation</td>
<td>K-ac</td>
<td>Transcription, Repair, Replication, Condensation</td>
</tr>
<tr>
<td>Methyltransferase</td>
<td>K-met1 K-met2 K-met3</td>
<td>Transcription, Repair</td>
</tr>
<tr>
<td>Methyltransferase</td>
<td>R-met1 R-met2 R-met3</td>
<td>Transcription</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>S-pp T-pp</td>
<td>Transcription, Repair, Condensation</td>
</tr>
<tr>
<td>Ubiquitylation</td>
<td>K-ub</td>
<td>Transcription, Repair</td>
</tr>
<tr>
<td>Sumoylation</td>
<td>K-su</td>
<td>Transcription</td>
</tr>
<tr>
<td>ADP ribosylation</td>
<td>E-er</td>
<td>Transcription</td>
</tr>
<tr>
<td>Dimethylation</td>
<td>R&gt;K, C&gt;C</td>
<td>Transcription</td>
</tr>
<tr>
<td>Proline isomerization</td>
<td>P-cis&gt;P-trans</td>
<td>Transcription</td>
</tr>
</tbody>
</table>

Overview of different classes of modification identified on histones. The functions that have been associated with each modification are shown. Each modification is discussed in detail in the text under the heading of the function it regulates.

Modification

\[ M \rightarrow \] De-Modification

(Large) Enzyme-Complexes found for (almost) all reactions
Reversible, Transient, Dynamic


Histone Modifications: Acetylation

HAT (Histone Acetyl Transferase)

\[ \text{NH}_3^+ \rightarrow \text{Acetyl-CoA} \rightarrow \text{NH}_2-\text{CO}-\text{CH}_3 \]

HDAC (Histone Deacetylase)
Inhibitors: butyrate

Acetylation of lysine residues neutralizes positive charge
Loss of charges may destabilize nucleosomes or higher order chromatin structures

Histone Modifications (PTM): Crosstalk

Crosstalk between histone modifications: a modification facilitates / inhibits modification at an other site on the same or different histones /nucleosomes

Specialized chromatin structures containing heterochromatin specific sets of histone modifications and heterochromatin specific proteins (e.g. HP1)
Modes of multivalent chromatin engagement. To distinguish among several potential mechanisms of multivalent association, we propose the following nomenclature.

a | Intranucleosomal association can be subdivided into two distinct classes:
- cis-histone, when more than one discrete binding contact is made to a single histone, in particular the same tail; and
- trans-histone, whereby contacts are made to different histone protomers or attendant DNA within the same nucleosome.

b | By contrast, internucleosomal binding modes crosslink two nucleosomes that are either adjacent or discontinuous in DNA sequence. Most of these crucial interactions are envisioned as modification dependent; however, DNA interactions and modification-independent contacts may have a vital energetic role.

BPTF, bromodomain PHD finger transcription factor; HP1, heterochromatin protein-1; TAF1, TATA-binding protein-associated factor-1.

Crosstalk in Reading PTMs


Crosstalk in Chromatin "Writing & Reading"

Transcription is controlled by various distant and proximal control elements ("enhancers") that recruit factors for activation/repression

Chromatin folding brings distant DNA sites, nucleosomes, histones ... into close spatial proximity.

Chromatin structures might control modification ("writing"), recruitment and interactions ("reading") of NHCP (accessibility & crosstalk).

Modifications and/or recruited NHCPs might affect stability of structures

Nucleosome Positioning Mechanisms & DNA Accessibility

Structural and dynamic properties are affected by
- Histone variants
- Histone modifications
- Remodeling complexes
- NHCPs interacting with chromatin
- DNA-damage

"Binding Site Protected"

"Binding Site Exposed"
**Epi-Genome Stability & Maintenance**

**Chromatin Dynamics**

**Transcription**
- Control Regions & Hypersensitive Sites
- Promoters
- Elongation
- Gene Mobility and Transcriptional Memory

**Remodeling**
- Histone modifications & "Histone code"
- ATP-dependent remodeling
- Histone exchange
- Nucleosome dynamics

**Assembly & Maintenance**
- Replication & Inheritance
- Recombination Repair

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**Replication**

**Genetics**
- Duplication and segregation of DNA
- Minimizing error rates

**Epigenetics**
- Disruption of existing chromatin structures
- Reassemby of chromatin structures with old and new components
- Regeneration of epigenetic modifications marks

**Initiation**
- ORIs (origins of replication)
- Controls, timing (early, late), once / cell cycle

**Elongation**
- Bidirectional
- Leading-/ lagging strands DNA synthesis
- Chromatin replication: "new & old" proteins, histones, NHCPs, specialized structures, modification patterns

**Termination**

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**Nucleosome Assembly At the Replication Fork In Vivo In Human Cells**

Structure of replicating SV 40 minichromosomes.

**Method**
- Non-acetylated nucleosomes
- Triplex-purification crosslinking
- DNA purification
- Electron microscopy under denaturation conditions

**Nucleosomes reassociate on the newly replicated DNA 225 to 285 (+/- 120) nucleotides behind the replication fork (< seconds).**

The ‘old’ and ‘new’ histones form nucleosomes within seconds on both chromatids

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**Synthesis of new histones and non histone chromosomal proteins in the cytoplasm**

Parental Histones
- H2A, H2B
- H4
- H3 (H3.1, H3.2)

Parental Chromatin
- Disruption
- Assembly old and new mixed on both strands

Maturation
- PTMs, NHCPs, Nucleosome Positioning

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**SECONDS**

**MINUTES - HOURS - G2/M**
Repair of Double Strand Breaks (DSB) by Homologous Recombination (I)

- DNA ends are bound by yKu and MRX complex (Mre11/Rad50/Xrs2)
- The checkpoint kinases (Mec1 and Tel1) are recruited and phosphorylate H2A
- A histone acetyltransferase (NuA4) acetylates histones of the region.
- Chromatin remodelers are recruited (INO80 and SWR1). SWR1 may cause exchange of histone H2A with the variant H2AZ (Htz1)

Take-home message (I):
The later steps of HR require chromatin assembly and over long distances.

The size of the disrupted and restored chromatin region as well as the impact on restoration of epigenetic marks is unknown.

Nucleotide excision repair in mammals and yeast

- Transcription Coupled Repair (TCR)
- Global Genome Repair (GGR)

Access
- Structural and dynamic properties of chromatin
- UV induced acetylation of histones by Histone Acetyl Transferase (Gcn5?) (random or targeted at the damage site is unknown)
- Recruitment of ATP-remodeling complex (Swi/Snf) may destabilize chromatin

Repair
- Early repair patches are nuclease sensitive and get slowly nuclease resistant due to incorporation in nucleosomes (Smerdon 1978) = chromatin rearrangement after repair.
- Repair patches can be labelled by incorporation of BrdU or 3H-T (= "UDS, Unscheduled DNA Synthesis")

Restore
- Early repair patches are nuclease sensitive and get slowly nuclease resistant due to incorporation in nucleosomes (Smerdon 1978) = chromatin rearrangement after repair.
- Repair patches can be labelled by incorporation of BrdU or 3H-T (= "UDS, Unscheduled DNA Synthesis")

Take-home message:
The size of the disrupted and restored chromatin region as well as the impact on restoration of epigenetic marks is unknown.
Epigenetics

Epigenetic processes play a critical role in creating stable patterns of gene expression during normal growth and differentiation.

Epigenetic processes regulate gene expression through heritable chromatin structures, creating distinctly different states of gene expression in genetically identical cells.

Creating epigenetic influences on gene expression has three requirements:

(i) the assembly of a specialized chromatin structure at a locus or loci,
(ii) the maintenance of that structure throughout the cell cycle, and
(iii) the ability of that structure to template its own replication, akin to the ability of complementary strands of DNA to template their replication.