Introduction to chromatin structure and gene regulation
Our genome is highly condensed
Chromatin tightly packages our DNA
A selection of fundamental questions in the field
Chromatin plasticity and biological regulation

Signaling to the nucleosome
Pathways that use metabolites to modify chromatin

Dealing with the nucleosome
Molecular machines and chaperones that reorganize chromatin

Encoding and decoding of epigenetics
How are histones modified post-translationally and how are these marks recognized and interpreted?

Histone dynamics in biology
Dynamic regulation of nucleosomes and biological function

The smallest, repeating unit of chromatin structure, the nucleosome

Luger et al. (1997) Nature
Gene transcription requires a multitude of machines and cofactors

Activators
Enhancer-binding proteins

Coactivators

Mediator

RNA Pol II
TFIID

General transcription factors

Tjian lab
General method for assessing changes in gene expression

Wild-type → total RNA → mRNA → cDNA → labelled cRNA

Altered condition → total RNA → mRNA → cDNA → labelled cRNA

Knockout

ts-mutant

+/- Drug

Environment

labeled cRNA → hybridize to array → wash → scan

compare → describe changes

labeled cRNA → hybridize to array → wash → scan
Yeast mutants scored by first-generation DNA microarrays

These early studies mapped the requirement of the core transcription machinery and cofactors for RNA Pol II-mediated gene expression

Holstege et al. (1998) Cell
<table>
<thead>
<tr>
<th>Complex and Subunit</th>
<th>Features</th>
<th>Fraction of Genes Dependent on Subunit Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA polymerase II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rpb1</td>
<td>Largest subunit, mRNA catalysis, contains CTD</td>
<td>100%</td>
</tr>
<tr>
<td>Srb/mediator (core)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Srb4</td>
<td>Target of Gal4 activator</td>
<td>93%*</td>
</tr>
<tr>
<td>Srb5</td>
<td>Unknown function</td>
<td>16%</td>
</tr>
<tr>
<td>Med6</td>
<td>Role in activation of some genes</td>
<td>10%</td>
</tr>
<tr>
<td>Srb CDK complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Srb10</td>
<td>CTD kinase, negative regulator</td>
<td>3%</td>
</tr>
<tr>
<td>Swi/Snf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swi2</td>
<td>ATP-dependent chromatin remodeling</td>
<td>6%</td>
</tr>
<tr>
<td>General transcription factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFIID (TAF\textsubscript{II,145})</td>
<td>Large TBP-associated factor, histone acetylase</td>
<td>16%</td>
</tr>
<tr>
<td>(TAF\textsubscript{II,17})</td>
<td>Component of both TFIID and SAGA</td>
<td>67%</td>
</tr>
<tr>
<td>TFIIE (Tfa1)</td>
<td>Promoter opening</td>
<td>54%</td>
</tr>
<tr>
<td>TFIIF (Kin28)</td>
<td>CTD kinase</td>
<td>87%*</td>
</tr>
<tr>
<td>SAGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gcn5</td>
<td>Histone acetylase</td>
<td>5%</td>
</tr>
<tr>
<td>TAF\textsubscript{II,17}</td>
<td>Component of both TFIID and SAGA</td>
<td>67%</td>
</tr>
</tbody>
</table>

* Srb4 and Kin28 results were essentially identical to Rpb1, but because of the stringency applied by the fit algorithm, a minimal estimate is produced.
How does RNA polymerase get access to nucleosome-wrapped DNA?

2x H2A/H2B dimers
1x H3/H4 tetramer

Cramer et al. (2000) Science
Luger et al. (1997) Nature
Chromatin tightly packages our DNA
Histone H4 depletion in yeast causes major changes in gene expression first-generation DNA microarrays

A large number of genes are upregulated upon H4 depletion
Wyrick et al. (2000) Nature
### Table 1 Summary of telomere-proximal genes affected by histone H4 depletion and SIR3 deletion

<table>
<thead>
<tr>
<th>Distance from telomere</th>
<th>Histone H4 depletion</th>
<th>SIR3 deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fraction of genes de-repressed</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>0–10 kb</td>
<td>56%</td>
<td>70</td>
</tr>
<tr>
<td>10–16 kb</td>
<td>52%</td>
<td>57</td>
</tr>
<tr>
<td>16–21 kb</td>
<td>28%</td>
<td>8</td>
</tr>
<tr>
<td>21–24 kb</td>
<td>26%</td>
<td>5</td>
</tr>
<tr>
<td>25–28 kb</td>
<td>26%</td>
<td>5</td>
</tr>
<tr>
<td>28–32 kb</td>
<td>22%</td>
<td>2</td>
</tr>
<tr>
<td>32–36 kb</td>
<td>28%</td>
<td>8</td>
</tr>
<tr>
<td>36–39 kb</td>
<td>8%</td>
<td>2</td>
</tr>
<tr>
<td>39–42 kb</td>
<td>16%</td>
<td>0.1</td>
</tr>
<tr>
<td>Genome</td>
<td>15%</td>
<td>–</td>
</tr>
</tbody>
</table>

Genes closest to a telomere were examined in consecutive intervals of 50 genes, and the fraction of genes de-repressed in each mutant was calculated for each interval. A $\chi^2$ value for each interval was calculated by comparing the fraction of genes de-repressed in the interval with the genome-wide average.
So how do you deal with chromatin?

* Regulating access to factors
* Transcribing through chromatin
* Maintaining chromatin states

- Posttranslational histone modifications
- ATP-dependent remodellers
- Histone variants
Gene transcription requires a multitude of machines and cofactors

Activators
- Enhancer-binding proteins
  - SREBP
  - CREB

Coactivators
- CBP
- MED/SRB proteins

Mediator
- NHR
- GRIP
- SRC
- DRIP/TRAP
- Sp1

General transcription factors
- TFIID
- RNA Pol II
- TAFs
- TBP
- TATA
- Inr
- DPE

Tjian lab
The general transcription factor TFIID localizes RNA polymerase II to the start site of transcription.

TFIID

- Core promoter recognition factors
- Activator Targets
- Enzymes that modify proteins
**TFIID structure resembles a horseshoe**

3D EM single-particle reconstruction

Andel, Ladurner, Tjian & Nogales (1999) *Science*
The double bromodomain module of TAF250

Two conserved surface binding pockets

Jacobson, Ladurner, King & Tjian (2000) Science
The four core histones are modified by acetylation.
The TAF250 bromodomains ‘translate’ the histone code

Jacobson, Ladurner, King & Tjian (2000) Science
Histone modifications are decoded by specific protein modules

Bromodomains of TFIID subunit Taf1

Jacobson, Ladurner, King & Tjian (2000) Science
Mutants in the acetyl-histone binding pocket of a yeast TFIID component reveal an anti-silencing function near telomeres.
Histone acetylation promotes the recruitment and stabilizes the binding of transcription cofactors

1: Activators recruits HATs
2: HATs acetylate histones
3: TAF250 binds acetylated histones
4: Stabilized TFIID leads to increased transcription
How does RNA polymerase deal with nucleosome-wrapped DNA?
RNA polymerase gets help from other proteins to displace histones and reorganize nucleosomes, so-called histone chaperones

**FACT** (Facilitates Chromatin Transcription)

(250 kDa complex of three proteins)
**In vivo assay:** The histone chaperone FACT is required for centromeric silencing and for accurate mitosis

- *pob3*+ deletion leads to loss of centromeric silencing
In vivo assay: The histone chaperone FACT is required for centromeric silencing and for accurate mitosis

- \textit{pob3}+ deletion leads to loss of centromeric silencing
- \textit{pob3}+ strain shows lagging chromosomes in mitosis

How does FACT promote histone exchange?

Lejeune et al. (2007) Current Biology
FACT promotes transcription and nucleosome reorganization by binding H2A-H2B

FACT complex

Nucleosome (octameric)  Altered nucleosome (hexanucleosome)
A ‘peptidase’ domain in FACT’s large subunit Spt16

50 kDa Spt16-N module

catalytic residues not conserved

PhD student Tobias Stuwe
Spt16-N binds histone H3/H4 tails and H3-H4 globular cores

Calorimetry binding assay with histone tails

GST-pulldown of histone cores

Where do the histones bind?

PhD student Tobias Stuwe
A conserved pocket in FACT mediates histone H4 tail binding

2.0 Å resolution, MAD phasing, R_free: 25%

<table>
<thead>
<tr>
<th></th>
<th>KD (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spt16-N, wild-type</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>D262A/K264A/S266A</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>F364L/R365A</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>S83A/K86A</td>
<td>31 ± 4</td>
</tr>
</tbody>
</table>

FACT helps to promote histone exchange during transcription

Where do histones localize in the genome?
General method for detecting where proteins bind chromatin

**Wild-type**
1. crosslink
2. fragm.
3. extract
4. IP
5. label DNA

**Altered condition**
1. Crosslink
2. fragm.
3. extract
4. IP
5. label DNA
6. Different/unmodified protein
   - Knockout/ts-mutant
   - +/- Drug, changed environment

**Input DNA**
1. labeled DNA
   - hybridize to array
   - wash
   - scan
   - compare
   - describe/quantify

**Input DNA**
1. labeled DNA
   - hybridize to array
   - wash
   - scan
General method for detecting where proteins bind chromatin

Wild-type → crosslink → fragm. → extract → IP → label DNA

Altered condition → crosslink → fragm. → extract → IP → label DNA

Different/unmodified protein
Knockout/ts-mutant
+/- Drug, changed environment

labeled DNA
input DNA → HT sequence → process data...

compare → describe/quantify

labeled DNA
input DNA → HT sequence → process data...
Mapping histone H2AZ nucleosomes in Drosophila

HT-sequencing based analysis of H2AZ-enrichment across the genome

Mavrich et al. (2008) Nature
H2AZ nucleosomes define the 3' end of ORFs
HT-sequencing based analysis of H2AZ-enrichment across the genome

Mavrich et al. (2008) Nature
H2AZ nucleosomes define the location of ‘paused’ RNA polymerase II in vivo

Mavrich et al. (2008) Nature
Chromatin plasticity
How do you read, copy, fix and/or silence our histone-wrapped genome?

Post-translational histone modifications
acetylation, methylation, Ub, phosphorylation, poly-ADP-ribosylation

Nucleosome reorganization
(ATP-remodellers, histone chaperones)

Non-canonical replacement histones
(H2AZ, CenpA, H3.3)

Non-coding RNAs and transcriptional gene silencing
(RoX, Xist, siRNAs-RITS complex)
MacroH2A - an unusual heterochromatric histone

* **Vertebrate-only histone**

* **MacroH2A represses transcription and marks heterochromatin**

* **Macro domains occur in enzymes that metabolize NAD**

* **These enzymes produce:**
  (i) free metabolite O-acetyl-ADP-ribose
  (ii) poly-ADP-ribose (post-translational modification)
**MacroH2A - an unusual heterochromatic histone**

* Vertebrate-only histone  

* MacroH2A represses transcription and marks heterochromatin

* Macro domains occur in enzymes that metabolize NAD

* These enzymes produce:  
  (i) free metabolite O-acetyl-ADP-ribose  
  (ii) poly-ADP-ribose (post-translational modification)  
  (iii) mono-ADP-ribosylated (nuclear) proteins

Does macroH2A recognize NAD metabolites?
The Sir2 NAD metabolites 2′- and 3′-O-acetyl-ADP-ribose

Ac-Ac-Ac-Ac

Ac-SGRGKGGKGLGKGGAKRHRKVL

Deacetylated H4

Nicotinamide

β-NAD⁺

O-Acetyl-ADP-ribose

2′-O-Acetyl-ADP-ribose
The Sir2 metabolite binds macroH2A1.1

Affinity of the mH2A1.1 macro domain for the Sir2 metabolite:

\[ K_D = 1.6 \pm 0.1 \, \mu M \]
Chromatin may act as a ‘receptor’ for metabolites

NAD metabolism may affect chromatin through macroH2A

Alternative splicing changes all this......
An unusual form of splicing in the MACROH2A1 gene
Mutually exclusive splicing generates two distinct histone isoforms.
The alternative splicing closes the adenine-binding pocket.

Structural plasticity leads to functional plasticity.

The alternative splicing closes the adenine-binding pocket

structural plasticity leads to functional plasticity

The alternative splicing closes the adenine-binding pocket

structural plasticity leads to functional plasticity

The alternative splicing closes the adenine-binding pocket

structural plasticity leads to functional plasticity
