Many Factors Affect the Regulation of Transcription by RNA Polymerase II

- Ubiquitinylating Enzymes
- CpG Methylation
- HDAC Complexes
- p300/CBP
- ARC/TRAP/DRIP/SRCC/NAT/Mediator/SRB/CRSP complex
- Boundary Elements
- ATP-utilizing Chromatin Remodeling Factors
- Heterochromatin
- HMG Proteins
- Elongation Factors
- Kinases
- SRC/p160 Proteins
- Protein Methyltransferases
- Polycomb Group Proteins
- Protein Acetyltransferases
- Promoter- and Enhancer-binding Factors
- Basal/General Transcriptional Machinery
- Dr1-Drap1/NC2 Mot1 NOT proteins Srb10-Srb11
- Chromatin Assembly Factors
Specific Topics

• Basal transcription by RNA polymerase II
• Sequence-specific DNA-binding factors
• How might enhancers work?
• Chromatin structure – Introduction
• Covalent modification of histones
  • Chromatin remodeling factors
• Chromatin assembly
Chromatin Remodeling Factors

- Pure nucleosomes are effectively immobile on a biological time scale under 'physiological' conditions.

- The mobilization and/or disruption of nucleosomes is catalyzed by ATP-utilizing proteins termed 'chromatin remodeling factors'.

- All known chromatin remodeling factors possess a subunit that is a member of the SNF2-like family of DNA-stimulated ATPases.

- Many but not all chromatin remodeling factors are multiprotein complexes.

- Chromatin remodeling factors appear to function by different mechanisms. Some factors disrupt nucleosomes, whereas other factors translocate nucleosomes along the DNA.
Helicases and Related Proteins with Conserved NTP-binding Motifs

- **Superfamily 1** (includes RecB)
  - DEAD Box Family (includes eIF4A)
  - DEAH Box Family (includes PRP-16)
- **Superfamily 2**
  - SNF2-like Family
- **Superfamily 3** (includes SV40 T Antigen)
  - ERCC3 Family
  - Other Families

Subfamilies:
- **SNF2 Subfamily**
  - SWI2/SNF2
  - STH1
  - BRM
  - hSNF2L
  - et al.
- **SNF2L Subfamily**
  - ISWI
- **ERCC6 Subfamily**
  - ERCC6
  - RAD26
- **RAD54 Subfamily**
  - RAD54
  - ATR-X
  - et al.
- **CHD1 Subfamily**
  - CHD1
  - CHD3
  - CHD4
- **Other Subfamilies**
  - Includes: CSB
  - INO80
  - MOT1

Some Members of the SNF2-like Family of ATPases Are Subunits of Chromatin Remodeling Factors

ySWI/SNF
~ 2 MDa
~ 11 subunits

yRSC
~ 1 MDa
~ 15 subunits

dBRM
~ 2 MDa
~ 8 subunits

hBRM
> 2 MDa
10-14 subunits

hBRG
> 2 MDa
10-13 subunits

hBAF
~ 2 MDa
~ 9 subunits

hPBAF
~ 2 MDa
~ 9 subunits

SNF2 subfamily ATPase

dMi-2
~ 1 MDa
? subunits

xMi-2
~ 1 MDa
6 subunits

CHD1 subfamily ATPase

hNURD
~ 1.5 MDa
7 subunits

yISW1a
~ 400 kDa
2 subunits

yISW2
~ 300 kDa
2 subunits

dACF
~ 300 kDa
2 subunits

dCHRAC
~ 660 kDa
4 subunits
	ylno80.com
~ 1 MDa
12 subunits

yISW1b
~ 400 kDa
3 subunits

ISWI subfamily ATPase

dCHRAC
~ 550 kDa
4 subunits

dNURF
~ 550 kDa
4 subunits

hWRF
~ 600 kDa
2 subunits

hCHRAC
~ 800 kDa
4 subunits

hWICH
~ 700 kDa
~ 2 subunits

hRSF
~ 450 kDa
2 subunits

mNoRC
~ 800 kDa
~ 4 subunits

INO80 subfamily ATPase
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Includes:
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Shared and Unique Activities of SNF2 and ISWI Subfamily Remodeling Complexes

SNF2 subfamily-specific activities
- ATPase stimulated by DNA and nucleosomes
- DNase1 pattern of chromatin changed
- Transfer of histone octamer in trans
- Loss of supercoils from chromatin
- Dinucleosome formation
- Mediates translocation of nucleosomes by ~80 bp

ISWI subfamily-specific activities
- ATPase stimulated by nucleosomes
- ATPase stimulation H4-tail dependent
- Chromatin assembly
- Mediates modest (~10 bp) repositioning of nucleosomes

Common activities
- Repositioning of nucleosomes in cis
- Generation of superhelical torsion
- Triple helix displacement
A DNA-translocating Model for Chromatin Remodeling Factors
DNA Translocation Model for Chromatin Remodeling by ISWI Subfamily Complexes

ATP  \rightarrow  ADP + P_i
Lateral Cross-transfer Model for Chromatin Remodeling by SNF2 Subfamily Complexes

ATP $\rightarrow$ ADP + $P_i$
Restriction Enzyme (Hae III) Accessibility Assay for Chromatin Remodeling

- **ACF**
  - Nucleosomes inhibit Hae III digestion of DNA
  - Large DNA Fragments
  - 1. Hae III
  - 2. Deproteinize
  - Hae III site =

+ **ACF**
  - Chromatin remodeling by ACF allows Hae III to cleave DNA by moving nucleosomes
  - ACF moves nucleosomes
  - Hae III cuts the DNA
  - Hae III + ACF + ATP
  - Deproteinize
  - Small DNA Fragments
Restriction Enzyme Accessibility Assay for Chromatin Remodeling

Hae III Digestion of Chromatin
Agarose Gel Electrophoresis
Gel Shift Analysis of Nucleosome Positioning on a Mononucleosome

The conversion from one species to the other is termed nucleosome 'sliding'. This process can be catalyzed by some ATP-dependent chromatin remodeling factors.
Micrococcal Nuclease Digestion Assay

1. Partial Digestion with Micrococcal Nuclease
2. Deproteinization
3. Agarose Gel Electrophoresis

7-mer
6-mer
5-mer
4-mer
3-mer
2-mer
1-mer
Assay for Disruption of Periodic Nucleosome Array

<table>
<thead>
<tr>
<th>Remodeling Factor</th>
<th>DNA-binding Protein</th>
<th>ATP</th>
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<tbody>
<tr>
<td>-</td>
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Southern blot analysis of chromatin that is partially digested with micrococcal nuclease

Loss of the Periodicity of a Nucleosome Array
Mapping of Nucleosome Positioning by Indirect End-Labelling

1. Micrococcal Nuclease Treatment of Nuclei (and Naked DNA as a Control)
2. Purification of DNA
3. Digestion with Restriction Enzyme

1. Agarose Gel Electrophoresis
2. Southern Blot with Labelled Probe

Deduced Locations of Nucleosomes
Analysis of Nucleosome Positioning by Micrococcal Nuclease Digestion and Indirect End Labelling

Micrococcal Nuclease Digestion
Indirect End Labelling Analysis
Remodeling of a Mononucleosome

DNase I digestion ladder of an end-labelled DNA fragment as naked DNA or in a core particle.

Loss of the Repeated 10 bp DNase I Digestion Pattern in a Rotationally-positioned Nucleosome
Chromatin Remodeling Factors

- Pure nucleosomes are effectively immobile on a biological time scale under 'physiological' conditions.

- The mobilization and/or disruption of nucleosomes is catalyzed by ATP-utilizing proteins termed 'chromatin remodeling factors'.

- All known chromatin remodeling factors possess a subunit that is a member of the SNF2-like family of DNA-stimulated ATPases.

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  - Chromatin assembly
Chromatin Assembly Is Important for Chromosome Structure

DNA Replication → Chromatin Assembly → Daughter Chromosomes

DNA Repair → Chromatin Assembly → Repaired Chromosomes
Chromatin Assembly Is at the Interface of the Cell Cycle and Gene Expression
Chromatin Assembly with the S-190 Extract
A Revised Working Model for Chromatin Assembly

ATP-utilizing Motor (ACF, CHD1, RSF)

CAF-1, NAP-1, ASF1

Core Histone Chaperones

et al.
Micrococcal Nuclease Digestion Assay

1. Partial Digestion with Micrococcal Nuclease
2. Deproteinization
3. Agarose Gel Electrophoresis

[Diagram showing DNA strands and fragments labeled as 1-mer to 7-mer]
Chromatin Assembly Activity of Native ACF + NAP-1 after POROS Heparin Chromatography
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  - ERCC6
  - RAD26

- RAD54 Subfamily
  - RAD54
  - ATR-X
  - et al.

Purified Chromatin Assembly Factors and Core Histones

ACF
- Acf1
- ISWI

dNAP-1
- dNAP1

Core Histones
- H3
- H2B
- H2A
- H4
Chromatin Assembly with Purified, Recombinant ACF

rACF  0  1  3  10  units

[1 unit corresponds to an ACF:octamer ratio of 1:150]
Two Models for the ATP-dependent Assembly of Periodic Nucleosome Arrays

Passive Histone Deposition

- ATP-independent, Random Deposition of Histones by Chaperone
- ATP-dependent Generation of Periodic Spacing by Remodeling Factor

Active Histone Deposition

- Direct Assembly of Periodic Nucleosome Arrays
DNA Supercoiling Analysis

1. Relaxation with Topoisomerase I
2. Deproteinization

1-D Agarose Gel Electrophoresis

Partial Supercoiled DNA

2-D Agarose Gel Electrophoresis
Purified, Recombinant ACF Facilitates the Deposition of Histones onto Relaxed DNA
Template Commitment by ACF during Chromatin Assembly

- 3K (●) and 8K (●)
- 3K (●), then 8K (●)
- 8K (●), then 3K (●)
ACF Remains Committed to the DNA Template for about 15 to 30 Minutes
A Processive Model for Chromatin Assembly
A New Working Model for Chromatin Assembly

1. Binding of NAP1-histone complexes

2. Template-committed complex

3. Nucleosome assembly by ACF

4. Processive assembly of nucleosomes by ACF

ACF
Core Histone Octamer
NAP1
NAP1-Histone Complex
ACF, an ATP-utilizing Chromatin Assembly Factor

• We have fractionated, purified, and cloned the factors that mediate the ATP-dependent assembly of chromatin. These studies led to the discovery of ACF.

• ACF consists of Acf1 and the ISWI ATPase.

• We have achieved a purified, recombinant chromatin assembly system with ACF, NAP-1, core histones, histone H1, ATP, and DNA.

• ACF appears to function as a processive, ATP-driven DNA motor that translocates along DNA and mediates chromatin assembly.
Generation of a Deletion in the \textit{acf1} Gene by Imprecise Excision of a P Element

[Diagram showing the generation of a deletion in the acf1 gene by imprecise excision of a P element.]
acf1[1] is a Null Mutant Allele

Western Blot
Loss of Acf1 causes reduced nucleosome periodicity and a shorter repeat length in *Drosophila* embryos in vivo.
Acf1-deficient Extracts Contain Residual ATP-dependent Chromatin Assembly Activity

S-190 extract  \textit{acf1[1]}
ATP [+ -]

Micrococcal Nuclease Digestion Assay
Suppression of Position-effect Variegation upon Mutation of \textit{acf1}

\begin{align*}
\text{\textit{w}\text{[\textit{m4}]h}; +/+} & \quad \text{\textit{w}\text{[\textit{m4}]h}; \textit{acf1}/\textit{acf1}}
\end{align*}
Real Time Microscopy Reveals Faster S Phase in Acf1-deficient *Drosophila* Embryos

H2AvD-GFP Fluorescence in Nuclear Cycle 13

![Image of fluorescence stages](image)

**wt**

- Beginning of Cycle 13: 6.9±0.8 min
- Initiation of Chromosome Condensation: 4.8±0.7 min
- Prophase: 6.8±0.9 min
- Initiation of Anaphase: 3.6±0.8 min

**acf1**

- Beginning of Cycle 13: 4.6±1.0 min
- Initiation of Chromosome Condensation: 3.9±1.5 min
- Prophase: 5.8±1.0 min
- Initiation of Anaphase: 3.8±0.8 min
Studies of Acf1 In Vivo in *Drosophila*

• We have generated a null allele of the *Drosophila acf1* gene.

• Acf1-deficient flies exhibit about 25% viability.

• Mutation of *acf1* results in a decline in nucleosome periodicity as well as a decrease in the nucleosome repeat length.

• There are additional ATP-utilizing chromatin assembly factors in the Acf1-deficient flies.

• Several lines of evidence support a role of Acf1 in the assembly of repressive chromatin in vivo.
How to Reconstitute Chromatin In Vitro

- Minimal Process requires core histones, a chaperone, and DNA.
- Periodic nucleosome arrays require an ATP-utilizing factor.
- Mononucleosomes or Extended Nucleosome Arrays?
- Randomly-distributed Nucleosomes or Periodic Arrays?
- Crude extracts or purified factors?
- Recommend salt (NaCl) dialysis method for minimal process.
- Recommend ACF and NAP-1 for ATP-dependent process.

Different Types of Chromatin Can Be Reconstituted In Vitro

- Salt dialysis with nonrepetitive DNA: Irregularly-spaced nucleosomes
- Salt dialysis with tandemly-repeated positioning sequences: Nucleosomes located at specific positions
- ATP-dependent assembly with ACF, RSF or crude extracts: Periodic arrays of nucleosomes that are not specifically positioned
- ATP-dependent assembly (ACF) with sequence-specific DNA-binding factors: Periodic arrays of locally-positioned nucleosomes
Many Factors Affect the Regulation of Transcription by RNA Polymerase II
Some Current Topics in Eukaryotic Transcriptional Regulation

- Protein/Histone-Modifying Enzymes
  - Protein/Histone Acetyltransferases (HATs)
  - Protein/Histone Deacetylases (HDACs)
  - Protein/Histone Methyltransferases (HMTs)
  - Ubiquitylation, Phosphorylation, ADP-ribosylation
- ATP-dependent Chromatin Remodeling Factors
- Mediator/SRB/ARC/TRAP/DRIP/SMCC/NAT Complex
- Coactivators and Corepressors
- Heterochromatin and Epigenetic Phenomena
- Regulation of Transcriptional Elongation
- Boundary/Insulator Elements
- CpG Methylation of DNA
- Regulation via the Basal Machinery and Core Promoter Motifs
- Factors That Regulate the Activities of the Transcriptional Machinery
- Coupling of Transcription with Other Processes, such as Splicing
- Nuclear Localization and Dynamics
- Long Distance Regulation of Transcription