Distinct Functions of CHD1 and ACF in the ATP-dependent Assembly of Periodic Nucleosome Arrays
The Known ATP-utilizing Chromatin Assembly Factors Share an ISWI ATPase Subunit

<table>
<thead>
<tr>
<th>ACF</th>
<th>CHRAC</th>
<th>RSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acf1 ISWI</td>
<td>Acf1 ISWI</td>
<td>Rsf-1 hSNF2H</td>
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<tr>
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<td>CHRAC-16</td>
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<tr>
<td></td>
<td>CHRAC-14</td>
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</table>
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

- **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

ACF, an ATP-utilizing Chromatin Assembly and Remodeling Factor

- ACF catalyzes the ATP-dependent assembly of periodic nucleosome arrays. 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.

- We have generated a null allele of the Drosophila acf1 gene. Acf1-deficient flies exhibit about 25% viability.

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

- The absence of Acf1 results in suppression of position-effect variegation as well as an acceleration of progression through S phase. These and other data support a role of ACF in the assembly of repressive chromatin in vivo.
CHD1, a Member of the SNF2-like Family of ATPases

- CHD1 was originally found as a chromodomain-helicase-DNA-binding domain-containing protein.

- The analysis of CHD1 has suggested that it functions in the elongation of transcription by RNA polymerase II as well as in chromatin dynamics.

- CHD1 has been found to bind to DNA, to localize to regions of decondensed chromatin (interbands) and high transcriptional activity (puffs) in Drosophila polytene chromosomes, to participate in transcriptional termination, and to interact with the FACT, Rtf1, and Spt5 transcriptional elongation factors as well as with casein kinase II.

- Studies in S. cerevisiae have revealed synthetic genetic interactions between ISW1 and ISW2 with CHD1 as well as a partial loss of chromatin assembly in vitro by crude DEAE fractions derived from strains lacking either Asf1 or Chd1, but not Isw1, Isw2, Snf2, Swr1, NAP-1, or CAC-1 (large subunit of CAF-1).

- Purified yeast CHD1 has been shown to remodel mononucleosomes in vitro.
Purification of *Drosophila* CHD1

Coomassie Blue Staining
Native CHD1 Protein Appears to Exist Predominantly as a Monomer.
CHD1 Catalyzes the ATP-dependent Assembly of Periodic Nucleosome Arrays

<table>
<thead>
<tr>
<th></th>
<th>CHD1</th>
<th>NAP1</th>
<th>ATP</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>−</td>
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</table>

Micrococcal Nuclease Assay
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
CHD1 and ACF Can Rearrange Randomly-deposited Nucleosomes into Periodic Nucleosome Arrays

ATP-dependent Generation of Periodic Spacing by CHD1 or ACF

Nucleosome Spacing Reaction
Micrococcal Nuclease Digestion
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

2-D Agarose Gel Electrophoresis

Partially Supercoiled DNA
CHD1 catalyzes the ATP-dependent transfer of core histones from the NAP-1 chaperone to the DNA.

**CHD1 Catalyzes the ATP-dependent Transfer of Core Histones from the NAP-1 Chaperone to the DNA**

**Chromatin Assembly**

<table>
<thead>
<tr>
<th>Reaction Time</th>
<th>10 min</th>
<th>90 min</th>
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</thead>
<tbody>
<tr>
<td><strong>CHD1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NAP1</strong></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>ATP</strong></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**DNA Supercoiling Assay**

- N
- L
- SC

Relaxed
Supercoiled
DNA Supercoiling Analysis

1. Relaxation with Topoisomerase I
2. Deproteinization

1-D Agarose Gel Electrophoresis
- Relaxed
- Partially Supercoiled
- Supercoiled

N

2-D Agarose Gel Electrophoresis
- Partially Supercoiled DNA
- -6
- 0
- -14

Partially Supercoiled DNA
CHD1 assembles chromatin in a processive manner.
CHD1 and ACF Catalyze the Assembly of Chromatin with Different Nucleosome Repeat Lengths

Chromatin Assembly

Micrococcal Nuclease Assay
ACF, but not CHD1, Can Catalyze the Assembly of Histone H1-containing Chromatin

Histone H1

None  CHD1  ACF

-  +    -  +    -  +

Chromatin Assembly

Micrococcal Nuclease Assay
CHD1 Catalyzes the ATP-dependent Assembly of Periodic Nucleosome Arrays

• CHD1 mediates the ATP-dependent assembly of periodic nucleosome arrays. Thus, chromatin assembly is not restricted to ISWI-containing factors.

• This reaction involves purified CHD1, NAP-1 chaperone, core histones, and relaxed DNA.

• CHD1 catalyzes the ATP-dependent transfer of histones from the chaperone to the DNA by a processive mechanism that yields regularly-spaced nucleosomes.

• The comparative analysis of CHD1 and ACF revealed that CHD1 assembles chromatin with a shorter nucleosome repeat length than ACF.

• ACF, but not CHD1, is able to assemble histone H1-containing chromatin.

• These results support a model in which ACF participates in the assembly of transcriptionally repressive chromatin, whereas CHD1 is involved in the assembly of active chromatin.
A Variety of Biological Phenomena Involve Chromatin
Helicases and Related Proteins with Conserved NTP-binding Motifs

Superfamily 1 (includes RecB)
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- RAD54 Subfamily
  - RAD54
  - ATR-X
  - et al.
- CHD1 Subfamily
  - CHD1
  - CHD3
  - CHD4
- Other Subfamilies
  - Includes: CSB
  - INO80
  - MOT1

Initial Steps of Homologous Recombination at a Double-strand Break
Catalysis of Strand Pairing by Rad51 and Rad54

Rad51
Rad54
ATP

D Loop
Synthesis and Purification of *Drosophila* Rad51 and Rad54

Coomassie Blue Staining
Formation of D Loops with Purified Rad51 and Rad54

- Complete
- No Rad51
- No ATP
- Non-homologous DNA

Naked DNA

D loop
Free oligo
Strand Pairing by Rad51 and Rad54 in Chromatin

Rad51
Rad54
ATP

D Loop
Rad51 and Rad54, but not RecA, Can Form D Loops with Chromatin

Salt Dialysis Chromatin

Rad51
Rad54

RecA

Naked DNA SD Chromatin

Naked DNA SD Chromatin

D loop

Micrococcal Nuclease Digestion Assay

Strand Pairing Assay
Rad51 and Rad54 Can Mediate Strand Exchange at the Site of a Specifically-positioned Nucleosome
Chromatin Facilitates D Loop Formation in the Absence of Superhelical Tension

Agarose Gel
+ 5 μM Chloroquine
Ethidium Fluorescence

Relative Activity
100 180 300 220

D loop
Free oligo

Relaxed SD Chromatin D Loop
The Assembly of Relaxed DNA into Chromatin Facilitates Strand Pairing by Rad54 and Rad51

Chromatin Assembly Reactions
ACF, NAP-1, DNA, ATP, and Topo I
- M iM iM

Strand Pairing Reactions
Rad51+Rad54
- + -
Core Histones during Assembly
- - +
Core Histones after Mock Assembly

D loop
Free oligo

Relative Activity ▼ 100 ▲

ACF Assembly Conditions ± Core Histones

Micrococcal Nuclease Digestion Assay
Helicases and Related Proteins with Conserved NTP-binding Motifs

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  - RAD54 Subfamily: RAD54, ATR-X, et al.
- Other Families
  - CHD1 Subfamily: CHD1, CHD3, CHD4
  - Other Subfamilies: Includes CSB, INO80, MOT1

Superfamily 2
- SNF2-like Family

Superfamily 3 (includes SV40 T Antigen)

Rad54 and Rad51 Function Cooperatively in the ATP-dependent Remodeling of Chromatin

<table>
<thead>
<tr>
<th>Factors</th>
<th>ATP</th>
<th>Naked DNA</th>
<th>Chromatin (salt dialysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hae III</td>
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<td>-Hae III</td>
<td>+Hae III</td>
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<tr>
<td>ACF</td>
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<td>None</td>
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</tr>
</tbody>
</table>

The figure shows a gel with lanes labeled with different combinations of factors and ATP conditions.
Cooperativity in Chromatin Remodeling by Rad54 and Rad51

Chromatin (salt dialysis) + Hae III

Rad51: 
- 1x - - - 1x 5x 10x
Rad54: 
- 1x 1x 5x 10x - - -
ATP: 
- + - + - + - + - + - + - +
Rad54 + Rad51 Do Not Catalyze Nucleosome Mobility in Minichromosomes

Salt Dialysis Chromatin

+ R3 Lac Repressor

| No R3 | None | Rad54 | Rad51 | Rad54 | Rad51 | ACF |

Micrococcal Nuclease Digestion Indirect End-Labelling
Rad51 and Rad54 Are Optimized for Strand Exchange with Chromatin
A Variety of Biological Phenomena Involve Chromatin