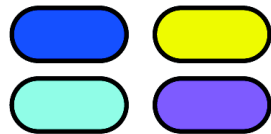
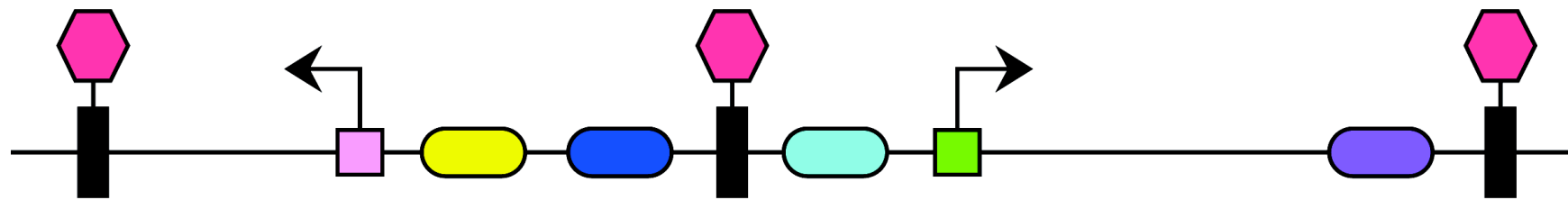


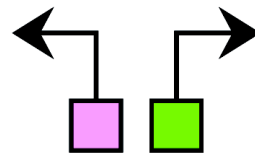
# Specific Topics

- 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

# How Do Enhancers Work?



Transcriptional  
Enhancers

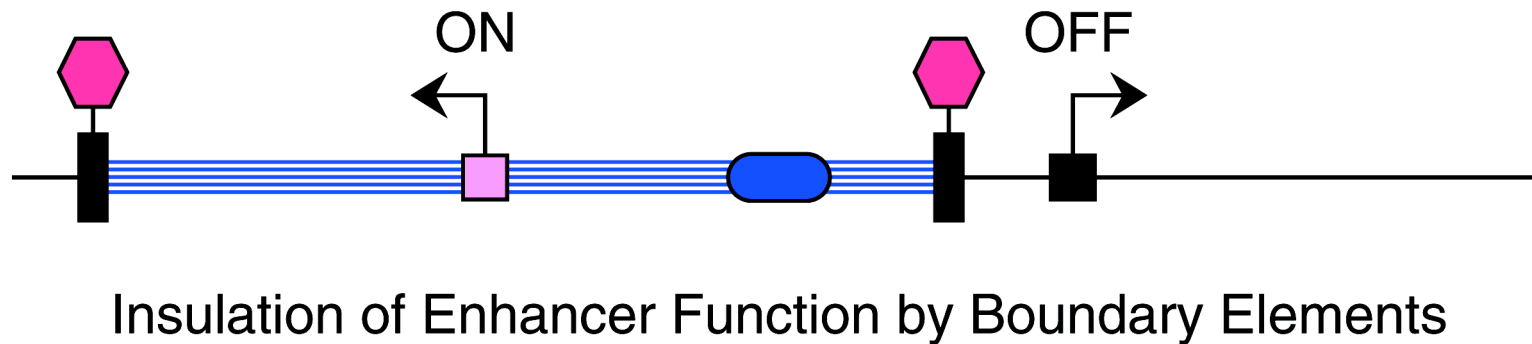
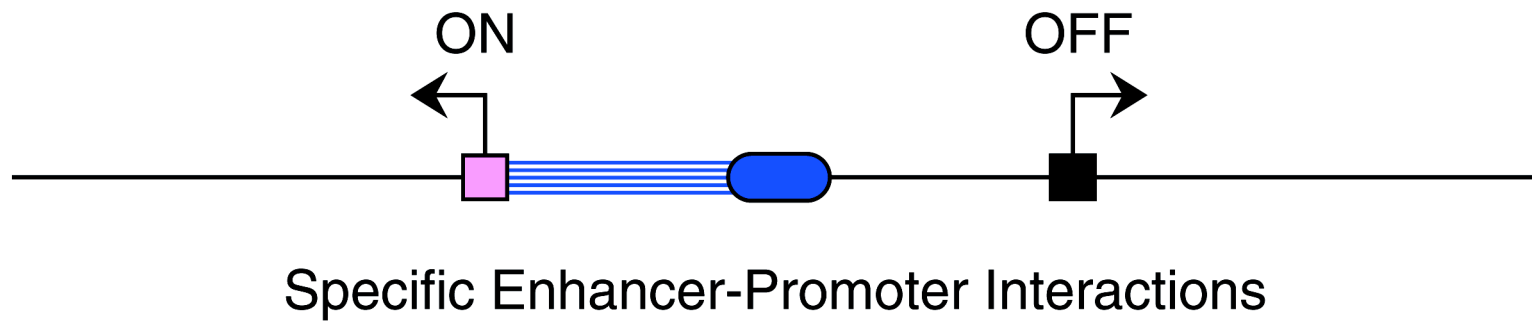


Promoters



Boundary or Insulator  
Elements

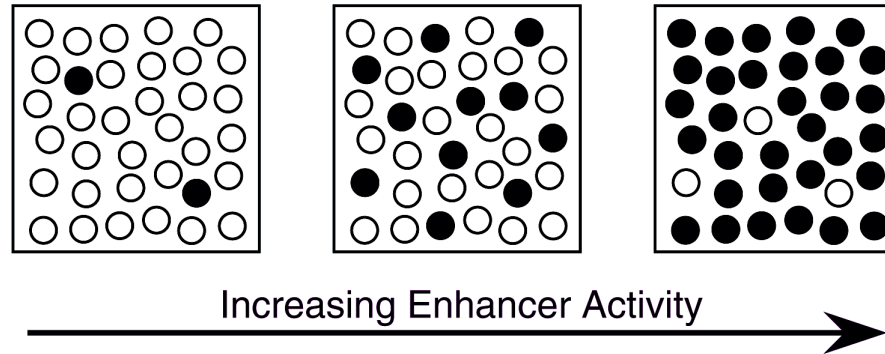
# Enhancer-Promoter Specificity vs. Insulator/Boundary Function



# What Happens When You See an Increase in Transcription in a Population of Cells?

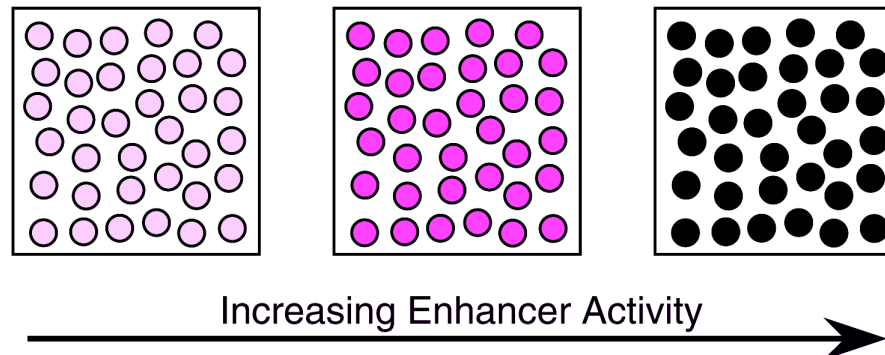
## Probability (On or Off) Model

Enhancers Increase the Probability of Transcription but not the Amount of Transcription in a Cell



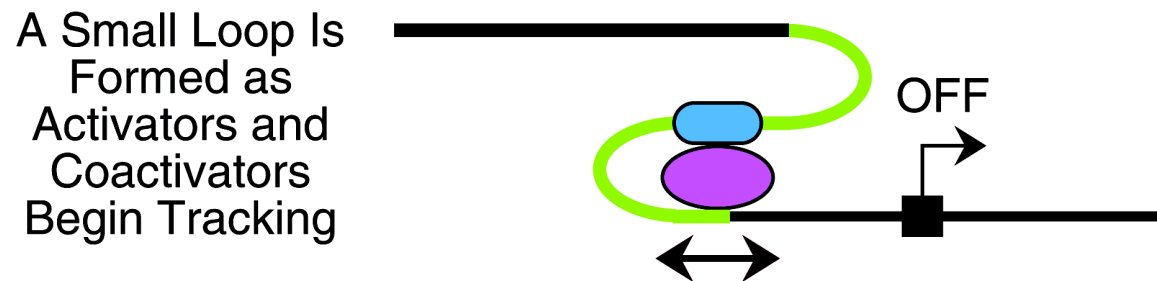
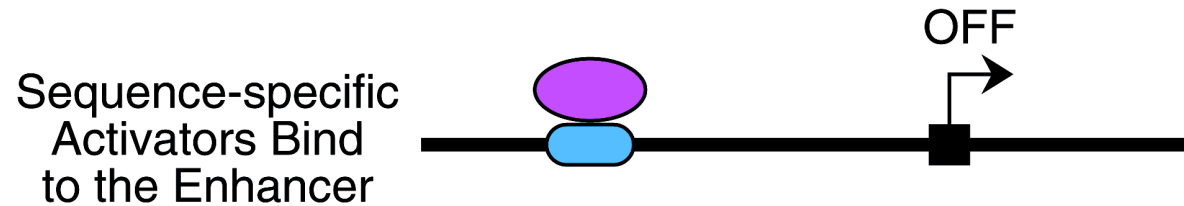
## Progressive Response Model

Enhancers Uniformly Increase the Amount of Transcription in a Cell in a Continuous Manner





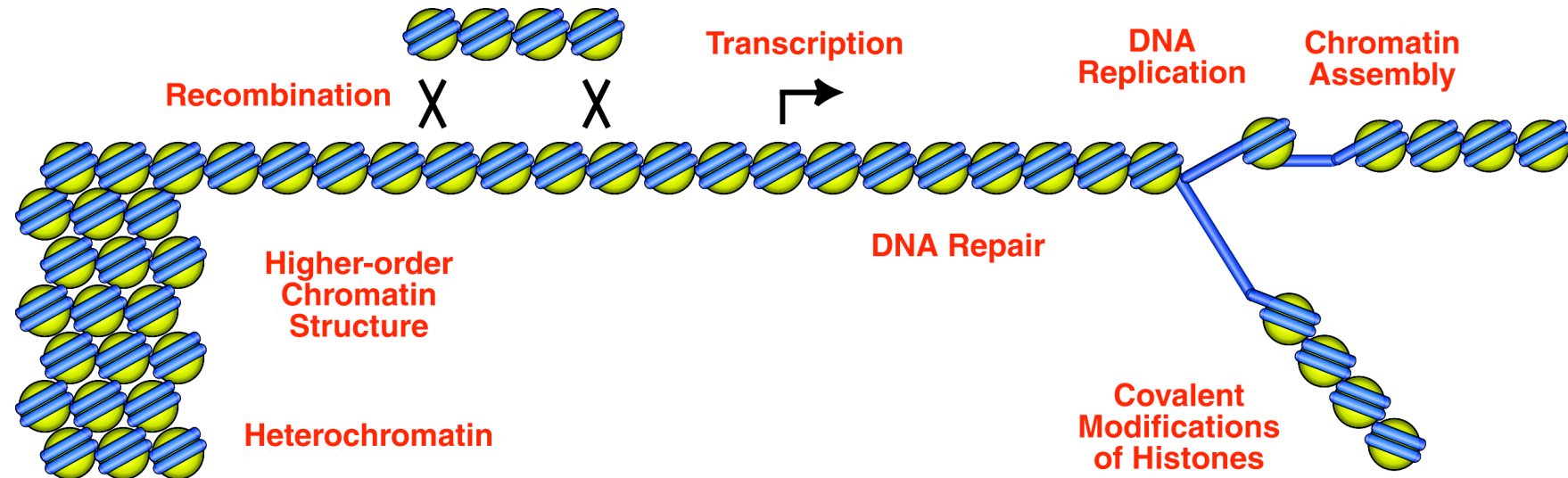
# A Facilitated Tracking Model for Enhancer Function



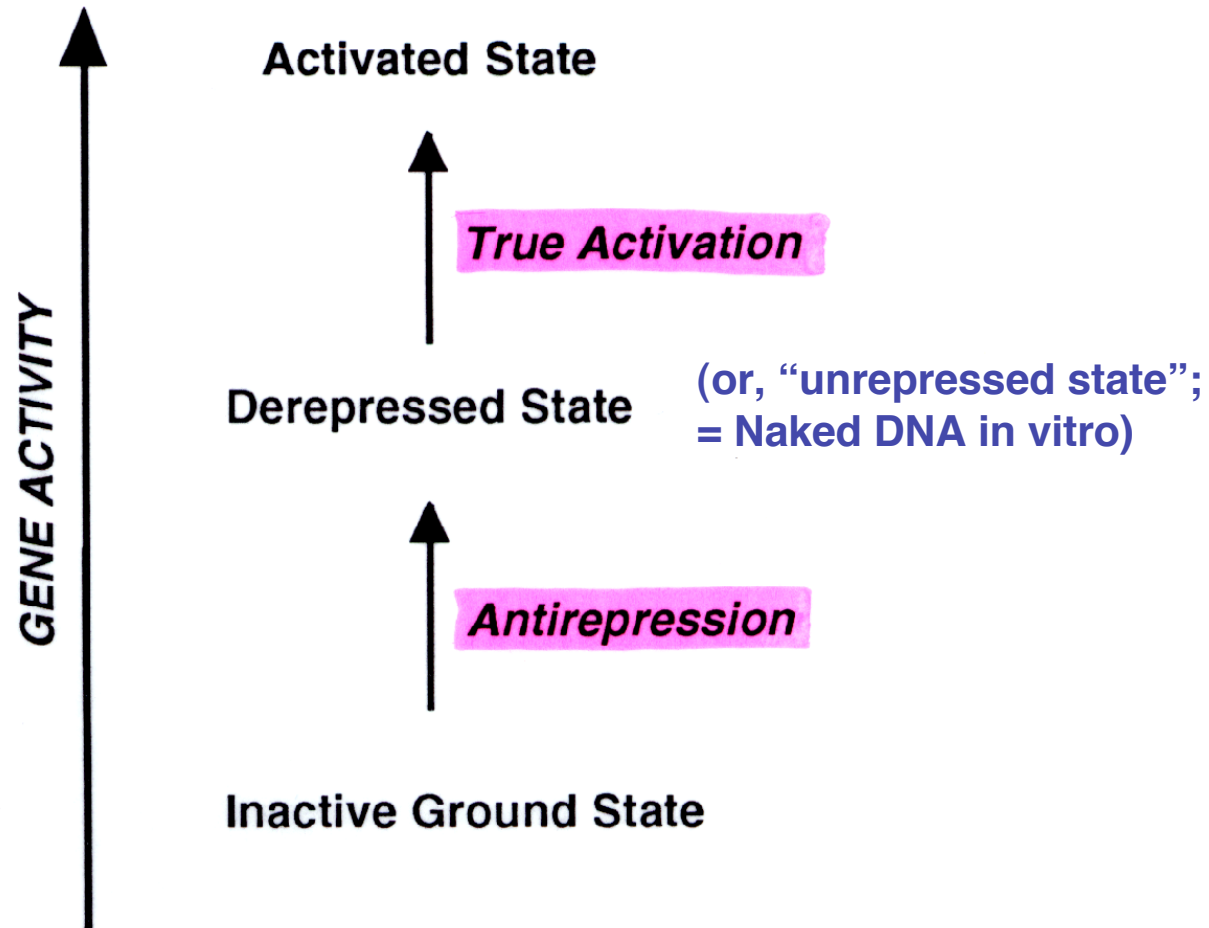
# Specific Topics

- Basal transcription by RNA polymerase II
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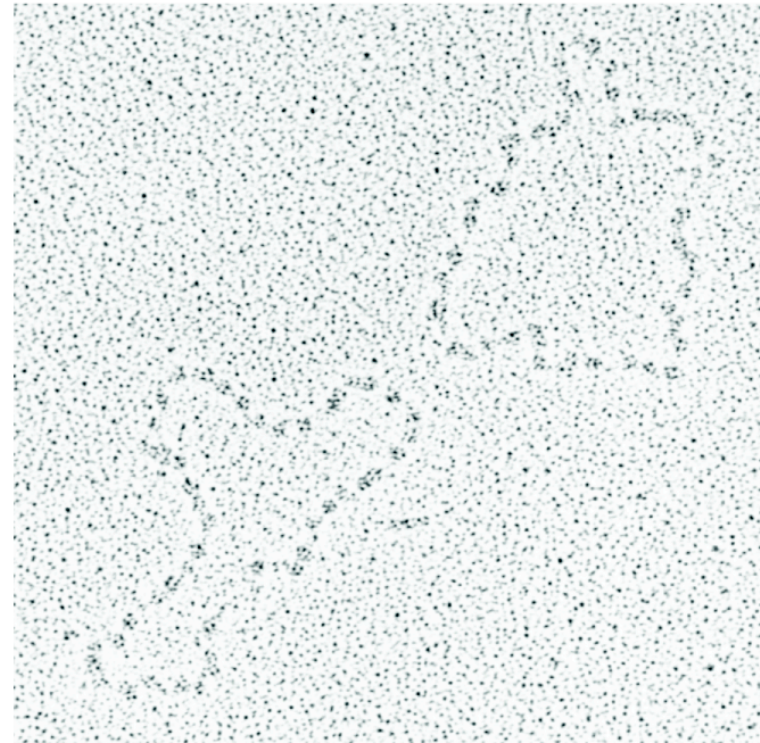
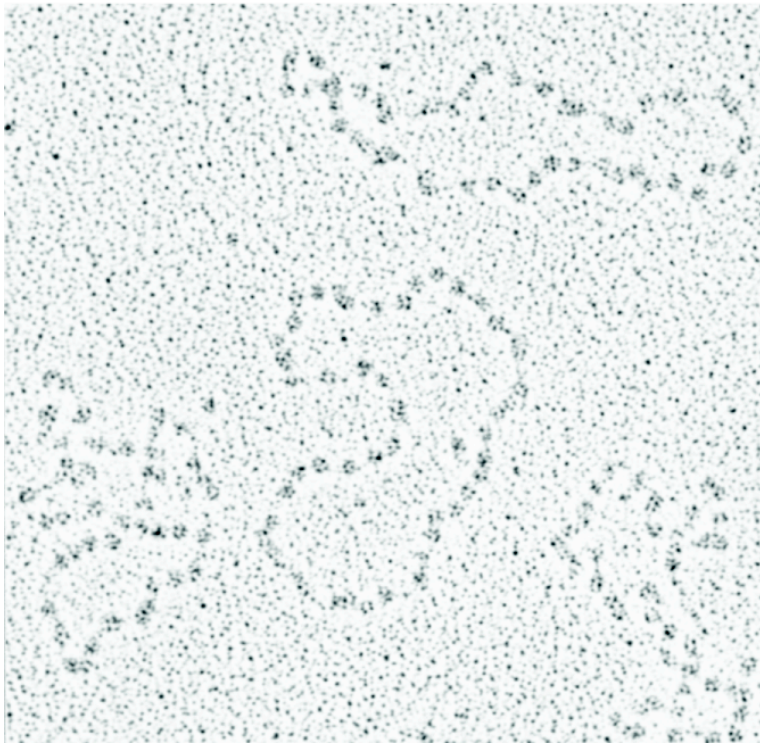
# A Variety of Biological Phenomena Involve Chromatin



## A TENTATIVE MODEL FOR GENE ACTIVATION



# Packaging of DNA into Chromatin



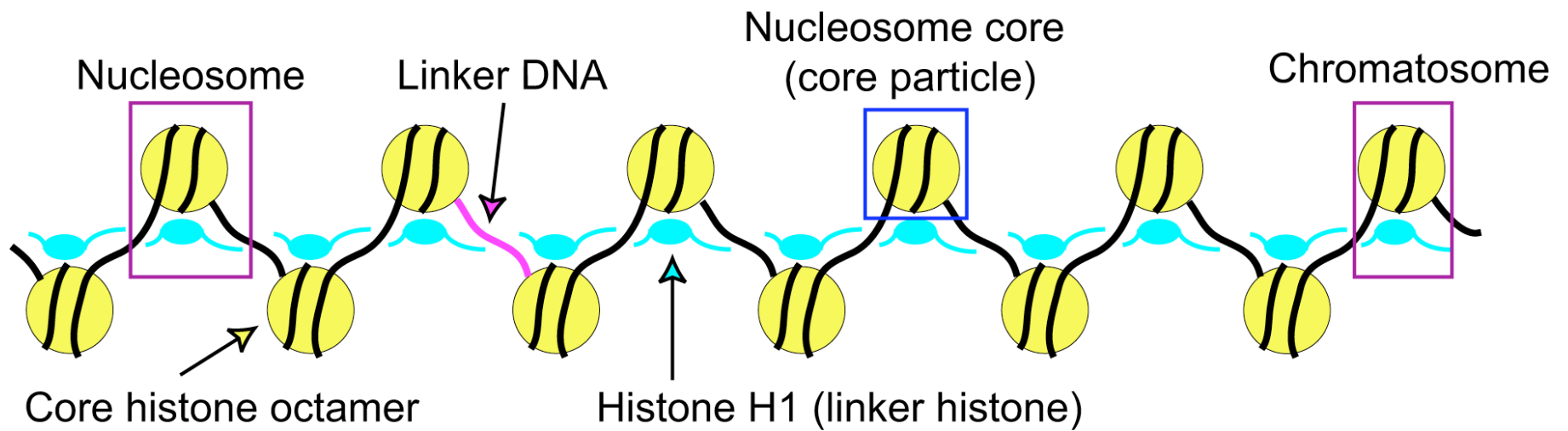
## Fun Facts about Chromatin

- An important function of chromatin is the packaging of DNA. In humans, approximately two meters of DNA is packaged into the nucleus, which has an average diameter of about 10  $\mu\text{m}$ .
- Chromatin has existed for several hundred million years. Over this time, DNA-utilizing processes have evolved to function optimally in chromatin rather than naked DNA.
- Therefore, the study of DNA in eukaryotes is best carried out in the context of chromatin – the natural state of DNA in the nucleus.

## More Facts about Chromatin

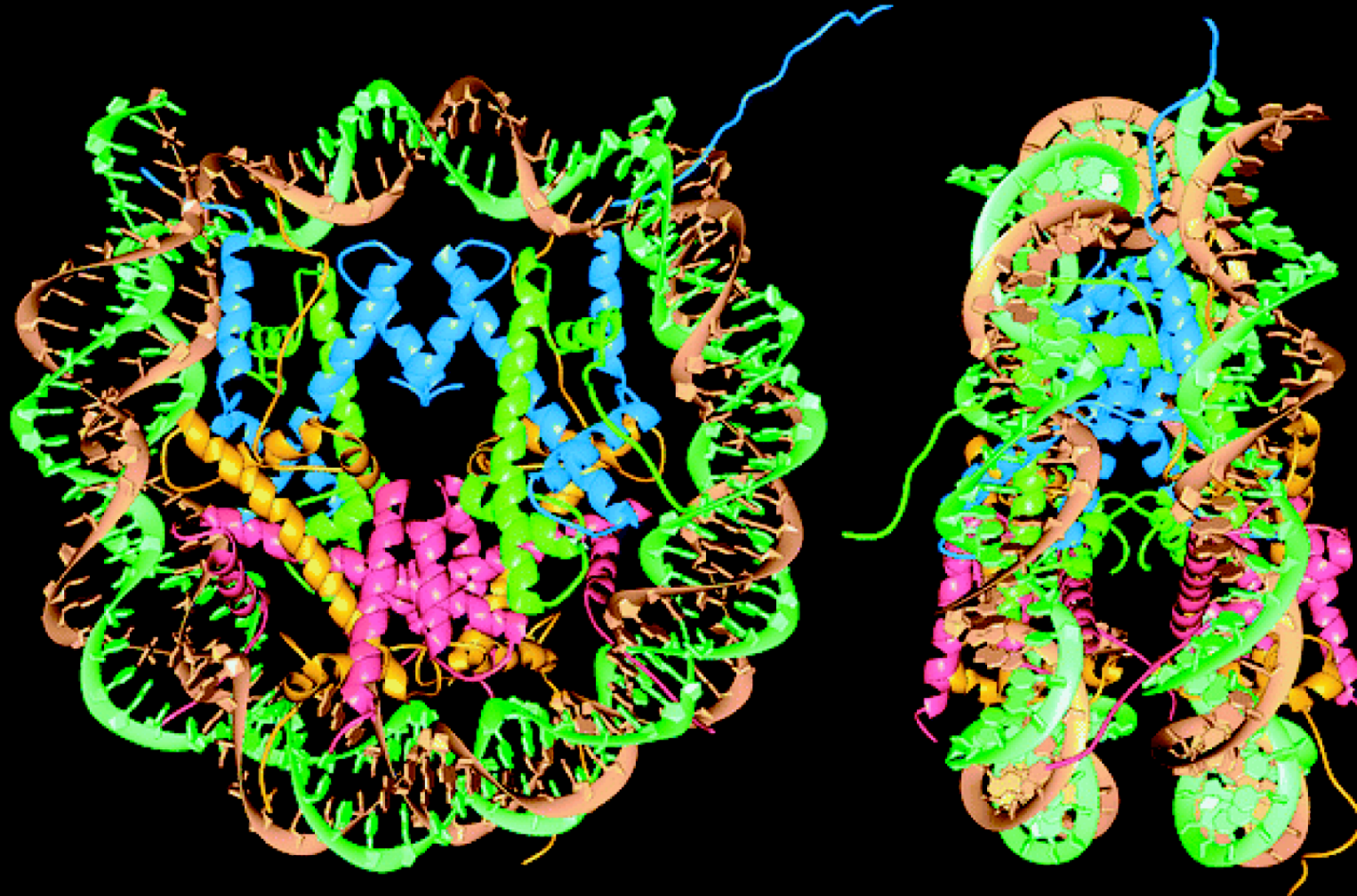
- The fundamental repeating unit of chromatin is the nucleosome, which consists of a core histone octamer, one molecule of histone H1 (linker histone), and about 180 to 200 bp DNA.
- The core histone octamer consists of two copies each of the core histones, H2A, H2B, H3, and H4.
- The nucleosome core consists of a central (H3-H4)<sub>2</sub> tetramer that is flanked by two H2A-H2B dimers.
- The core histones each have a highly structured central globular domain and unstructured N-terminal tails. H2A and H2B also have C-terminal tails.

# Schematic Diagram of Chromatin





# Crystal Structure of the Nucleosome Core



Luger *et al.*, *Nature* **389**, 251-260 (1997).

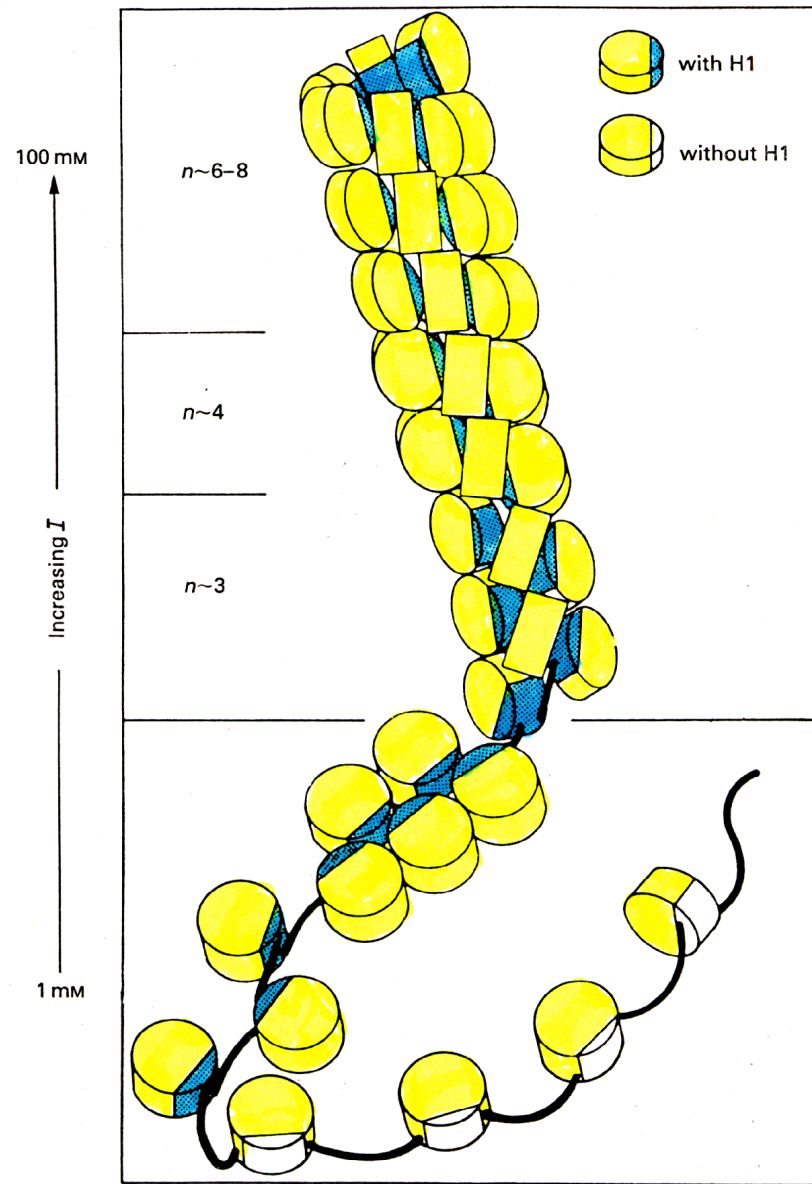
## **The 30 nm Diameter Chromatin Filament**

- The majority of chromatin at interphase in the metazoan nucleus exists as a 30 nm diameter chromatin filament.
- The extended 11 nm diameter filament that is typically seen in electron micrographs is observed only at low ionic strength (such as in water).
- At normal 'physiological' ionic strength, chromatin will fold into the 30 nm diameter fiber.
- Histone H1 promotes the formation of the 30 nm filament.

## Models for the 30 nm Chromatin Filament

- The structure of the 30 nm diameter chromatin filament is not known. Four models include:
- **Solenoid model** [Thoma et al., J. Cell Biol. 83, 403-427 (1979)]
- **Twisted ribbon model** [Worcel et al., PNAS 78, 1461-1465 (1981); Woodcock et al., J. Cell Biol. 99, 42-52 (1984)]
- **Supercoiled spacer model** [McGhee et al., Cell 33, 831-841 (1983)]
- **Crossed linker model** [Williams et al., Biophys. J. 49, 233-248 (1986)]

## Solenoid Model for Structure of the 30 nm Chromatin Fiber

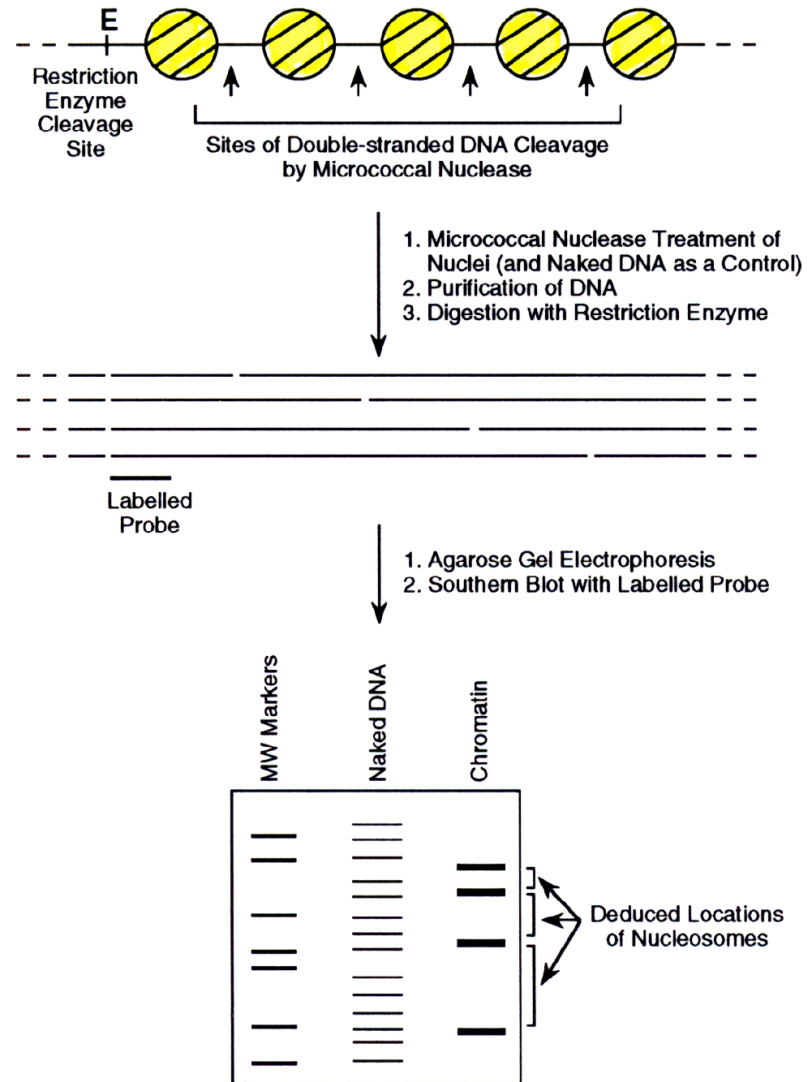


Thoma, F., Koller, Th., and Klug, A. (1979) *J. Cell. Biol.* **83**, 403-427.

## Translational vs. Rotational Nucleosome Positioning

- Most nucleosomes exhibit specific positioning both in vitro and in vivo.
- Nucleosome 'positioning' typically refers to translational positioning, which is the location of the core histone octamer relative to a specific DNA sequence. For example, there may be a core histone octamer translationally positioned from -300 bp to -144 bp relative to the transcription start site of a gene.
- Translational positioning of nucleosomes can be deduced by a method known as indirect end-labelling.
- Rotational positioning refers to the orientation of the DNA relative to the surface of the core histone octamer.
- The rotational positioning of nucleosomes is usually characterized by DNase I digestion of chromatin. When chromatin is treated with DNase I, the nuclease digests the DNA at 10 bp intervals (i.e., once every turn of the helix, where the minor groove of the DNA is facing away from the octamer and is accessible to the enzyme) and yields a repeated 10 bp ladder.

## Mapping of Nucleosome Positioning by Indirect End-Labeling

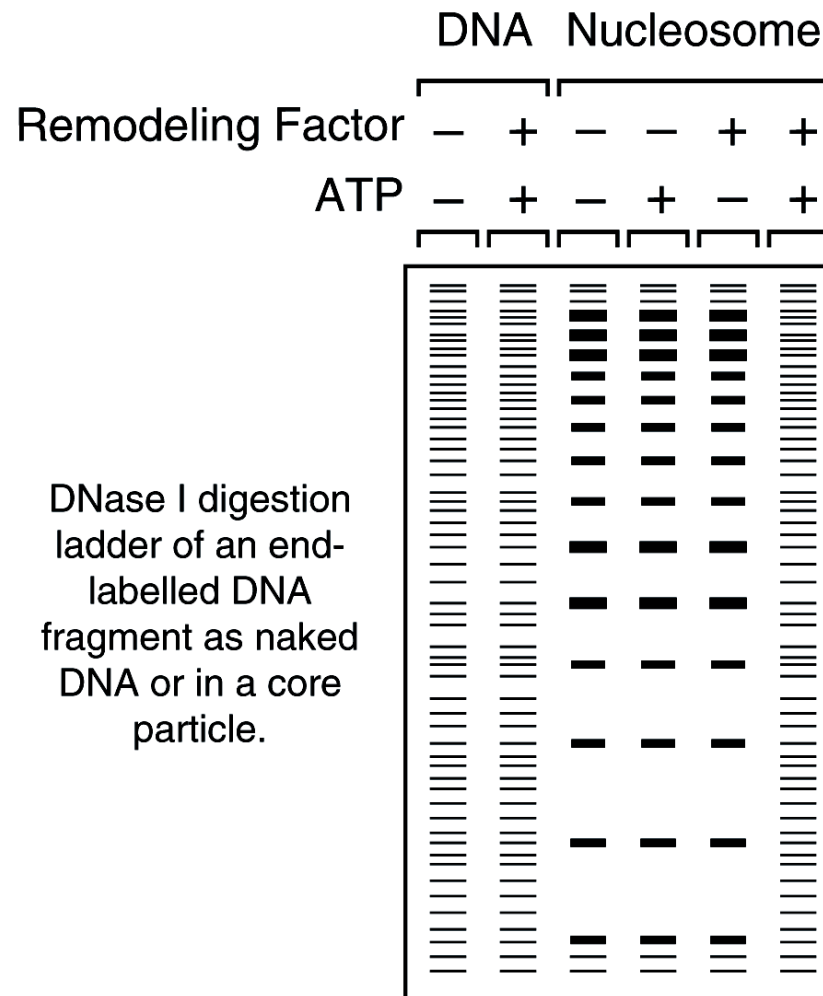




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# Remodeling of a Mononucleosome



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



## Histone H1 – the Linker Histone

- Histone H1 consists of a central globular region that is flanked by lysine-rich N- and C-terminal tails.
- There is approximately one molecule of H1 per nucleosome.
- The globular region of H1 interacts with the nucleosome core at the pseudo-dyad, whereas the lysine-rich tails interact with the linker DNA.
- H1 is present in metazoans. A canonical version of H1 (with a globular domain and tails) does not appear to exist in more primitive eukaryotes.
- The presence of H1 appears to correlate with transcriptional repression.
- In mammals, there are several H1 subtypes.
- H1 promotes the compaction of chromatin into the 30 nm fiber.
- In avian and amphibian erythrocytes, there is a special H1 variant that is termed H5. H5 is something like a super H1.
- H1 appears to exchange rapidly (relative to the core histones) in chromatin in vitro and in vivo.

## High Mobility Group (HMG) Proteins

- High mobility group (HMG) proteins are abundant non-histone chromosomal proteins.
- HMG proteins are traditionally defined to be those proteins (with the exception of ubiquitin) that can be extracted from nuclei at 0.35 M NaCl and are soluble in 2% trichloroacetic acid.
- There are three families of HMG proteins: HMG1/2 (= HMGB), HMG14/17 (= HMGN), and HMG-I/-Y (= HMGA; also known as  $\alpha$ -satellite protein).
- HMG proteins from different families are NOT RELATED. They share the 'HMG' name based only on their extraction and solubility properties.

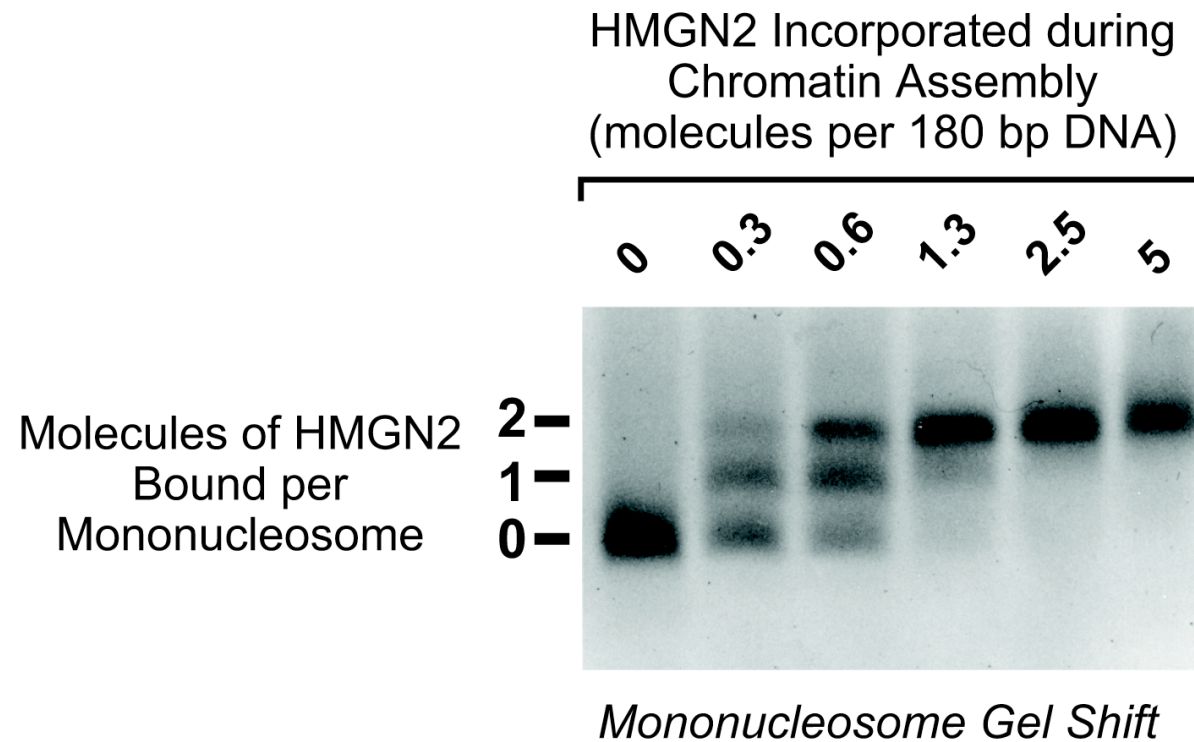
## HMGB (HMG1/2) Proteins

- HMGB (HMG1/2) proteins are about 25 to 30 kDa.
- They have a three domain structure: two related globular domains termed HMG-1 boxes and a C-terminal highly acidic tail.
- The HMG-1 boxes are DNA-binding regions that are present in both sequence-specific as well as nonspecific DNA-binding proteins.
- The abundance of the HMGB proteins is estimated to be about one molecule per two nucleosomes.
- HMGB proteins bind with high affinity to four-way junction DNA.
- HMGB proteins have also been found to stimulate the binding of some sequence-specific DNA-binding proteins to DNA.
- Their exact function is not known.

## HMGN (HMG14/17) Proteins

- HMGN (HMG14/17) proteins are small (~ 10 kDa), highly charged proteins with an overabundance of basic amino acid residues in the N-terminus and acidic amino acid residues in the C-terminus.
- There are four known HMGN proteins in humans: HMGN1 (HMG14), HMGN2 (HMG17), HMGN3 (Trip7), and HMGN4.
- HMGN proteins are present in vertebrates, and do not appear to be present in invertebrates.
- The abundance of the HMGN proteins is estimated to be about one molecule per two nucleosomes.
- HMGN proteins are specific nucleosome-binding proteins. Each nucleosome core has two high affinity binding sites for HMGN proteins.
- The available data suggest that HMGN proteins are involved in transcriptional activation.

## Binding of HMGN2 (HMG17) to Mononucleosomes



## HMGA (HMG-I/-Y) Proteins

- HMGA (HMG-I/-Y) proteins are small (~10 kDa) proteins that bind to A+T-rich DNA, such as that in satellite DNA. It appears to be a protein that binds to heterochromatin, and hence, was previously termed  $\alpha$ -satellite protein.
- They bind to the minor groove of DNA via a structural motif known as the AT hook.
- HMGA proteins bend DNA and have been suggested to be 'architectural' factors that are important for the formation of specific enhancer structures.

# Specific Topics

- Basal transcription by RNA polymerase II
- Sequence-specific DNA-binding factors
- How might enhancers work?
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## **Core Histones Are Subjected to a Variety of Covalent Modifications**

- Acetylation of Lysine residues
- Methylation of Lysine and Arginine residues
- Phosphorylation of Serine residues
- Ubiquitylation of Lysine residues
- Poly(ADP)ribosylation at Glutamate (Aspartate) residues

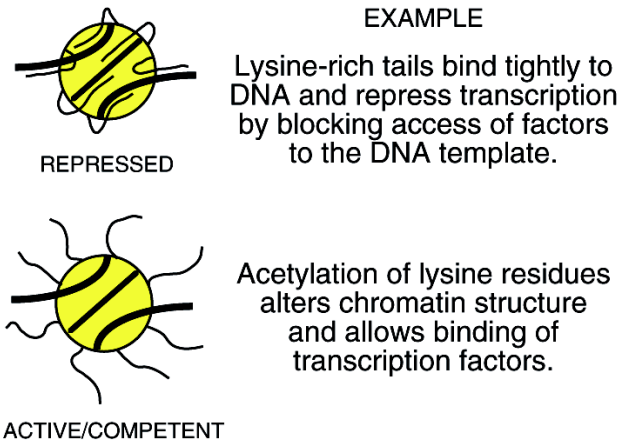
### **General Trends**

- Histone acetylation generally correlates with gene activation.
- Methylation of histone H3 Lysine 4 correlates with activation.
- Methylation of histone H3 Lysine 9 correlates with repression.

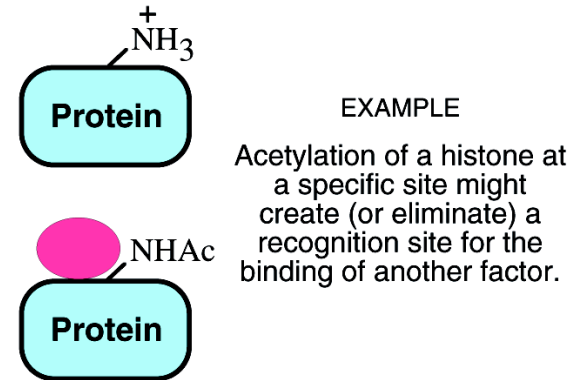


# Models for Transcriptional Regulation by Protein Acetylation

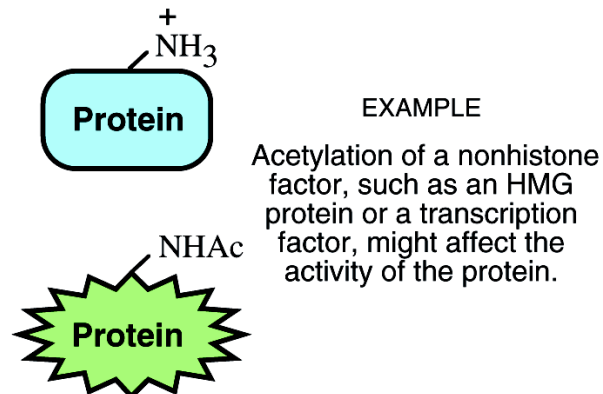
## Acetylation Induces a Conformational Change in the Core Histones



## Acetylation Is a Transducing Signal

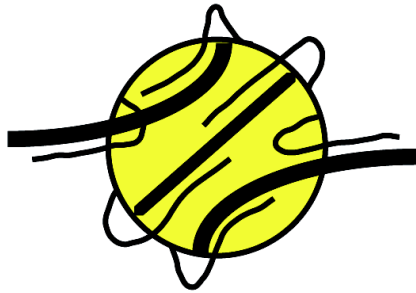


## Acetylation Affects the Activity of a Nonhistone Protein



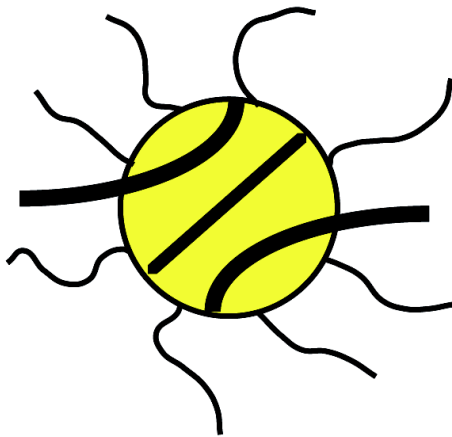
# Acetylation Induces a Conformational Change in the Core Histones

## EXAMPLE



REPPRESSED

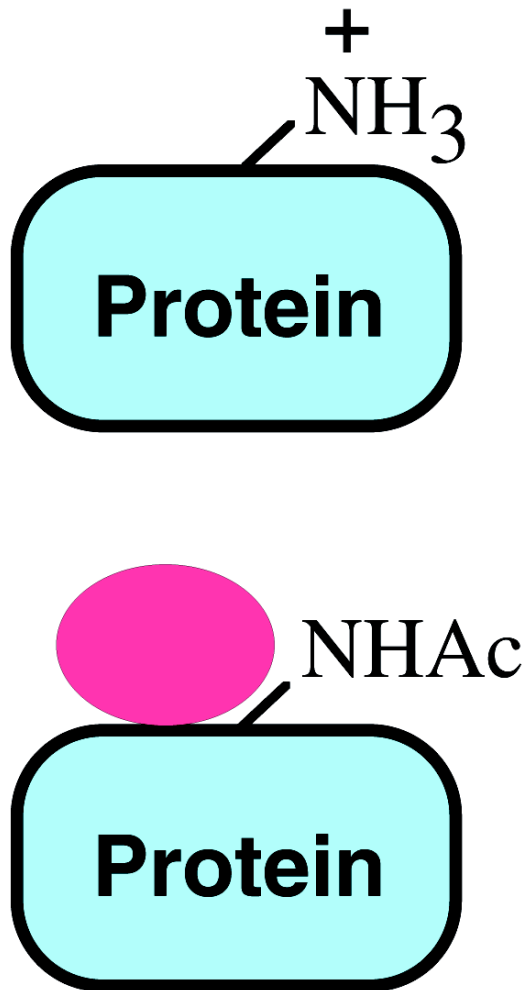
Lysine-rich tails bind tightly to DNA and repress transcription by blocking access of factors to the DNA template.



ACTIVE/COMPETENT

Acetylation of lysine residues alters chromatin structure and allows binding of transcription factors.

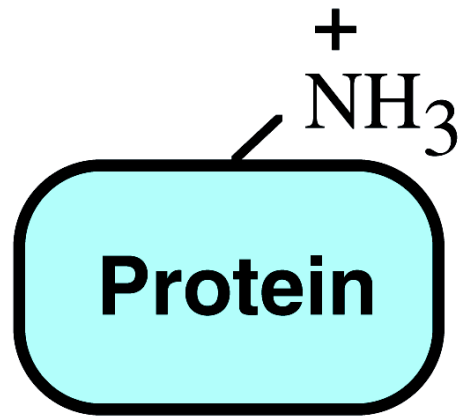
# Acetylation Is a Transducing Signal



## EXAMPLE

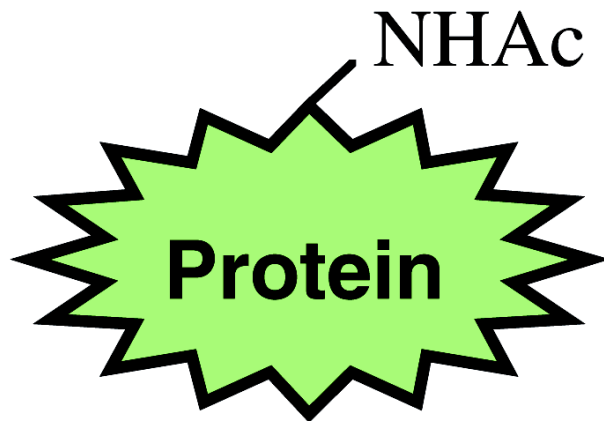
Acetylation of a histone at a specific site might create (or eliminate) a recognition site for the binding of another factor.

# Acetylation Affects the Activity of a Nonhistone Protein



## EXAMPLE

Acetylation of a nonhistone factor, such as an HMG protein or a transcription factor, might affect the activity of the protein.



## Covalent Modifications of the Histones

- There are many covalent modifications of the core histones – a variety of different modifications at a variety of different amino acid residues.
- Histone acetylation generally correlates well with transcriptional activation.
- Methylation of histone H3 at lysine 4 correlates with activation, whereas methylation of histone H3 at lysine 9 correlates with repression. Note that lysines can be mono-, di-, or trimethylated.
- How universal are the histone modification patterns at different genes and in different organisms? Is there a histone code?
- Are the histone modification patterns the cause or the effect of gene activation?
- How much does each histone modification contribute to the total activation or repression that is observed?
- It is important to note that histone-modifying enzymes have also been observed to modify non-histone proteins, such as transcription factors.

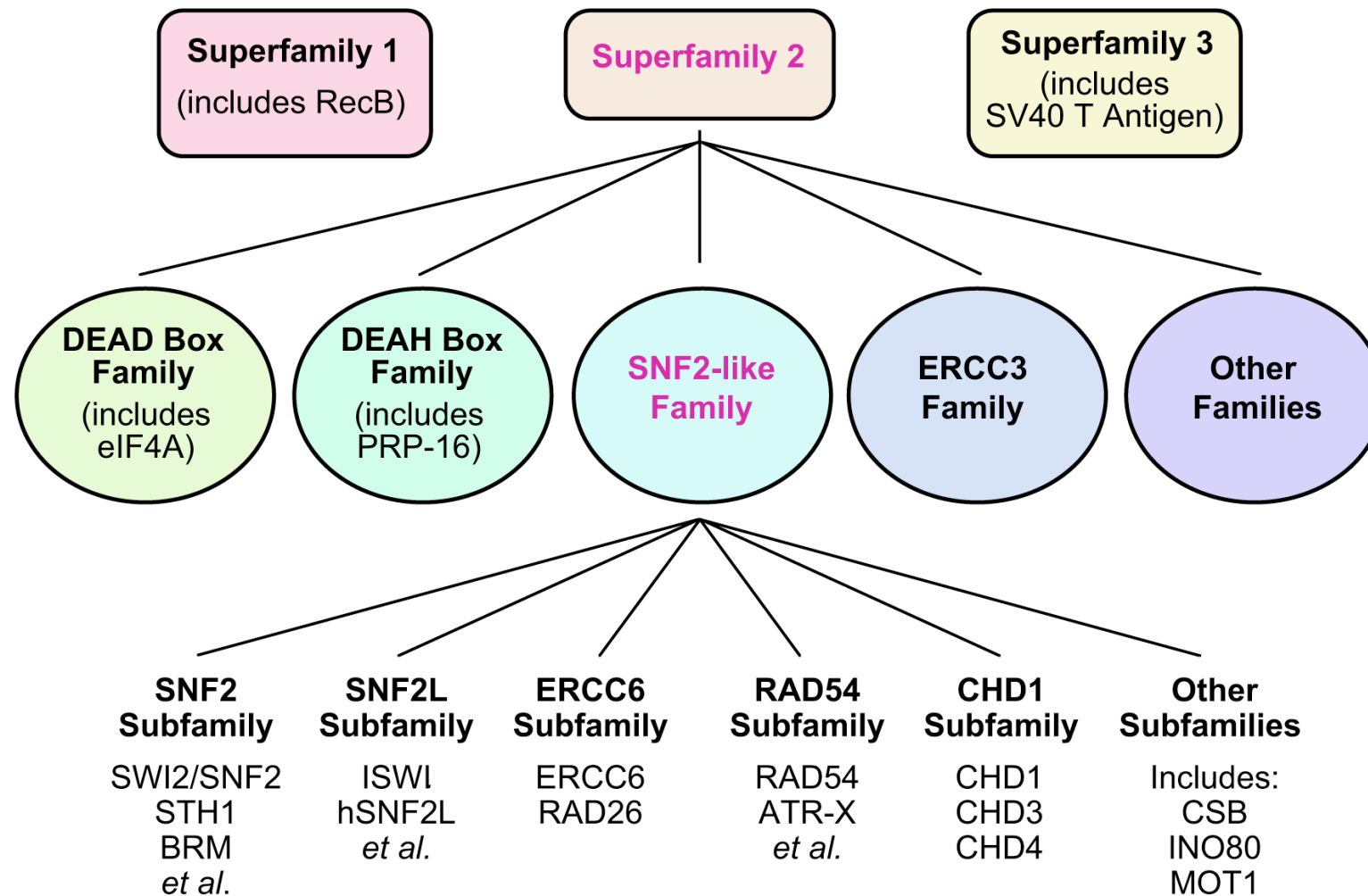
# Specific Topics

- Basal transcription by RNA polymerase II
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- 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

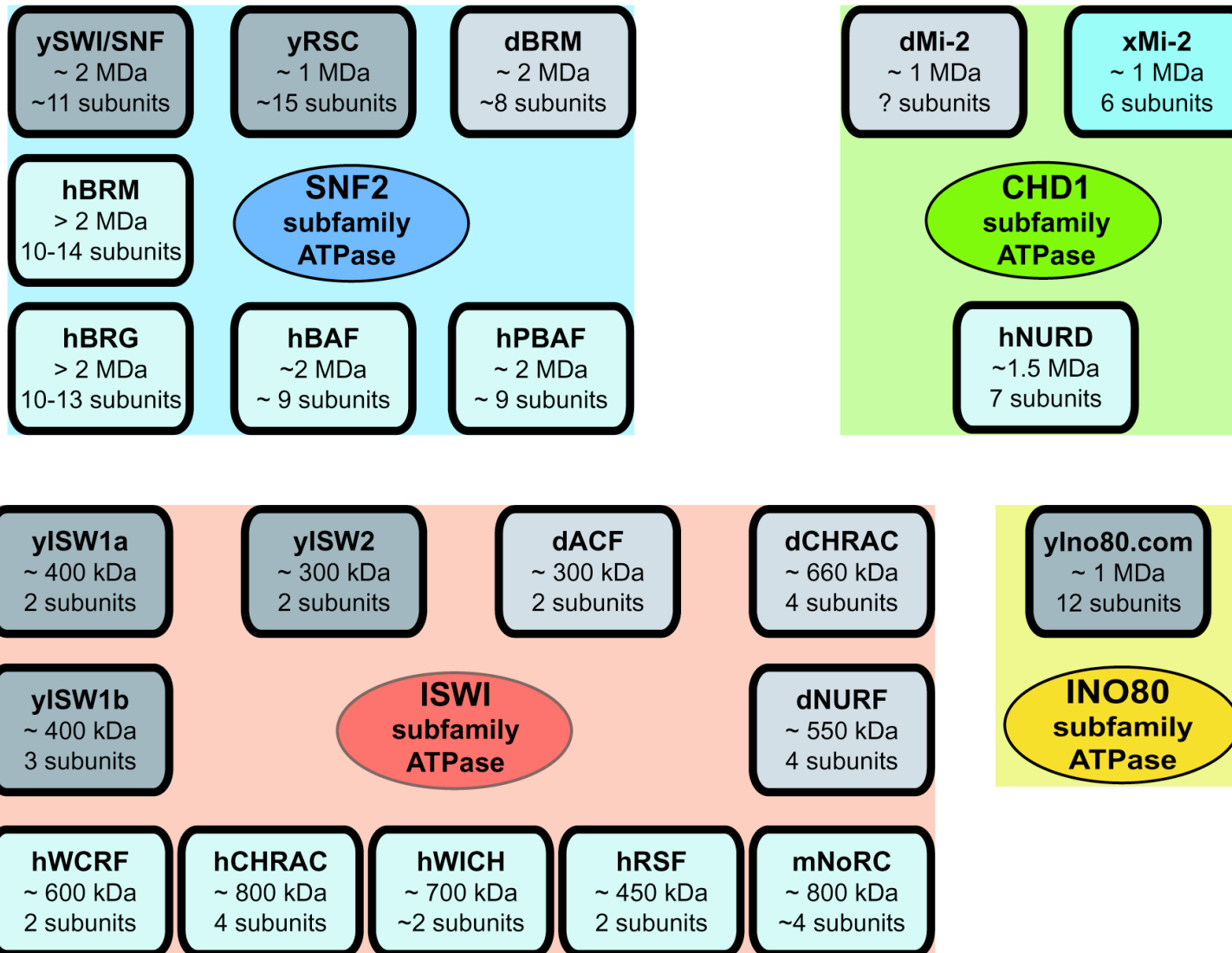


Gorbalenya and Koonin, *Curr. Opin. Structural Biol.* **3**, 419-429 (1993).

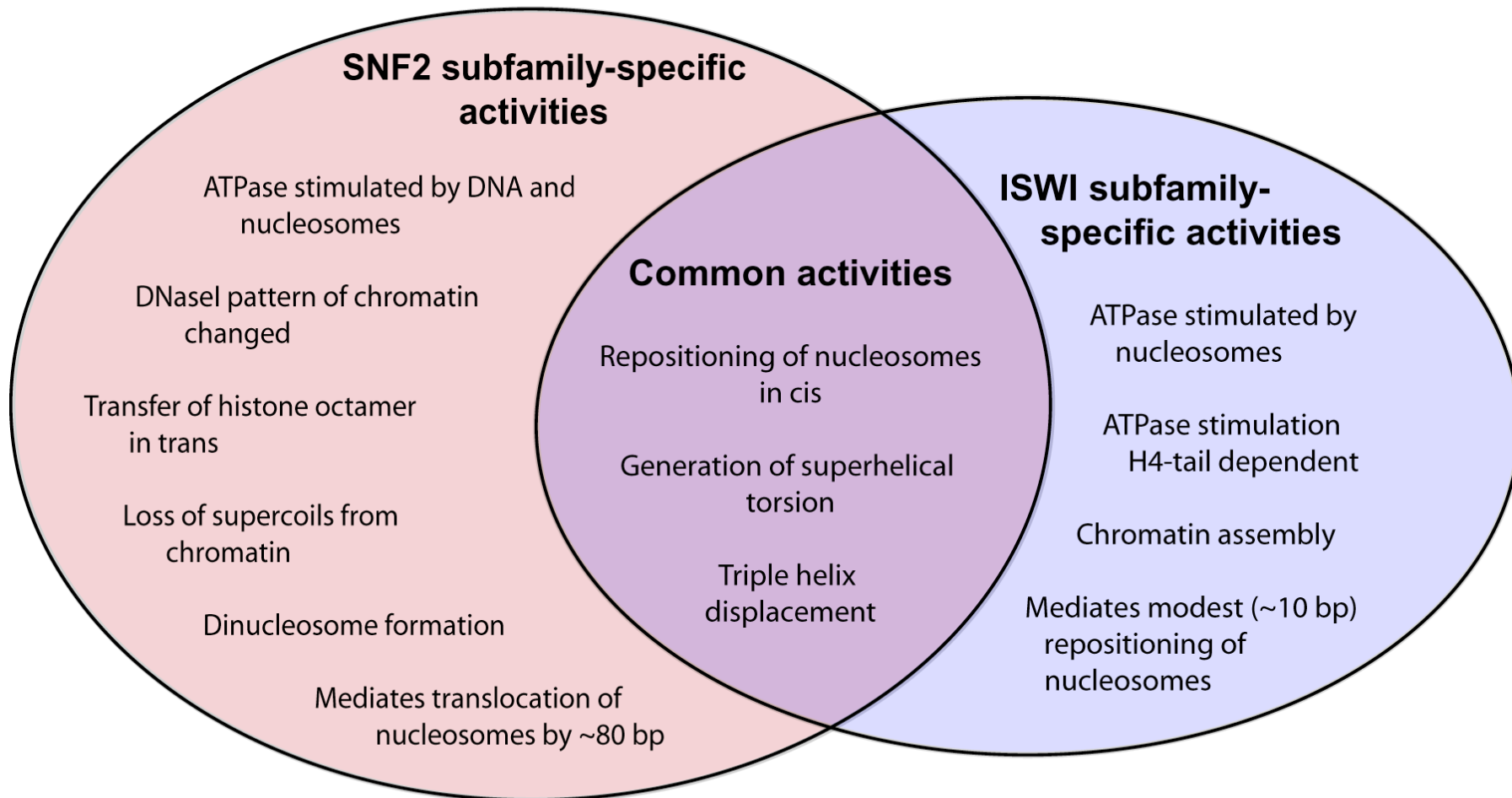
Eisen *et al.*, *Nucleic Acids Res.* **23**, 2715-2723 (1995).



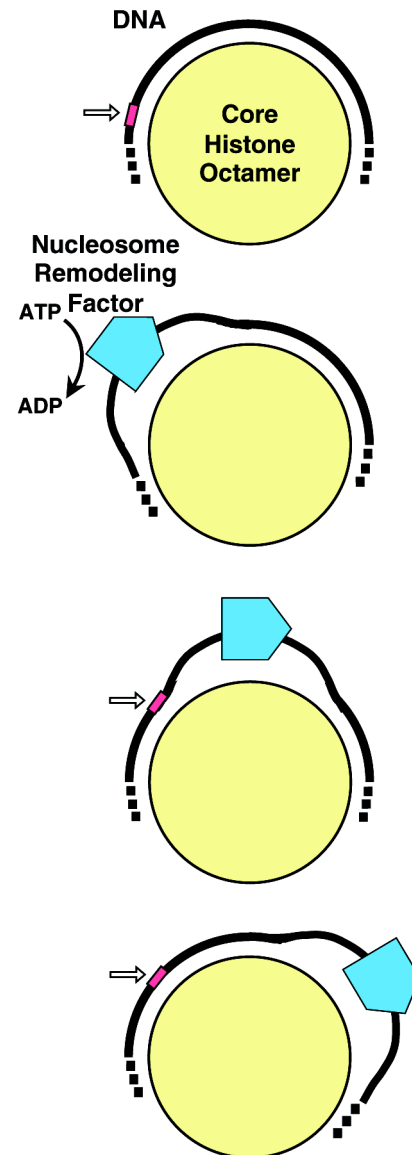
# Some Members of the SNF2-like Family of ATPases Are Subunits of Chromatin Remodeling Factors



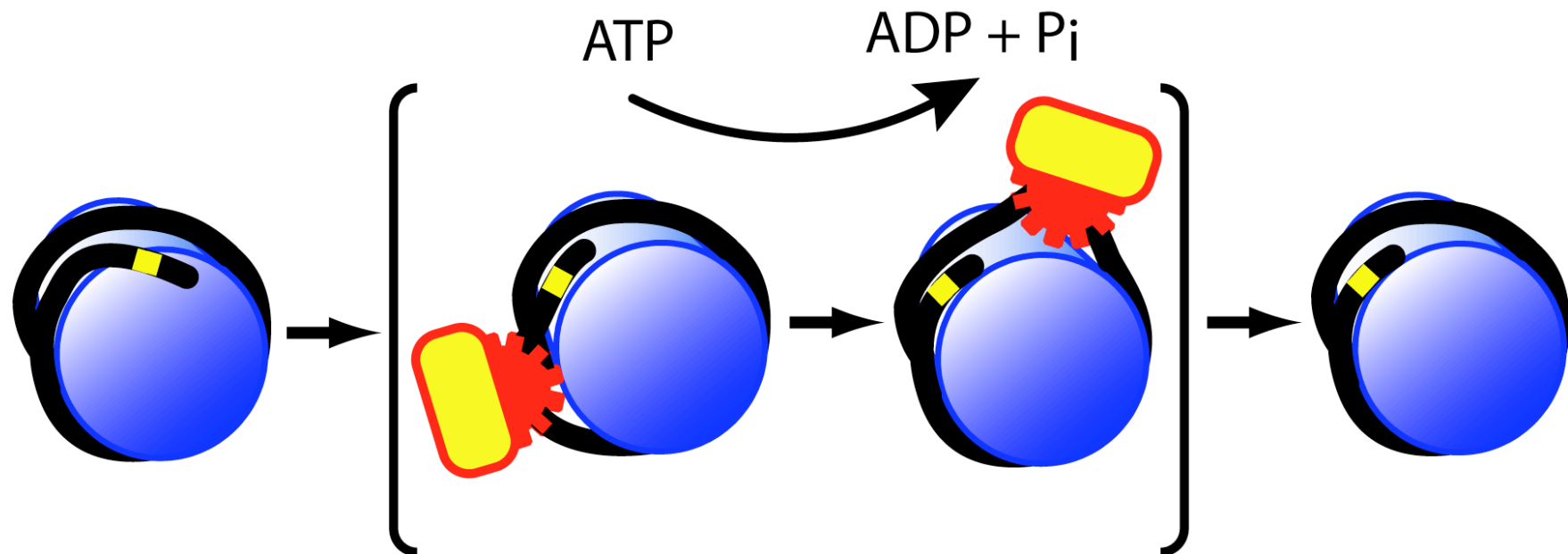
# Shared and Unique Activities of SNF2 and ISWI Subfamily Remodeling Complexes



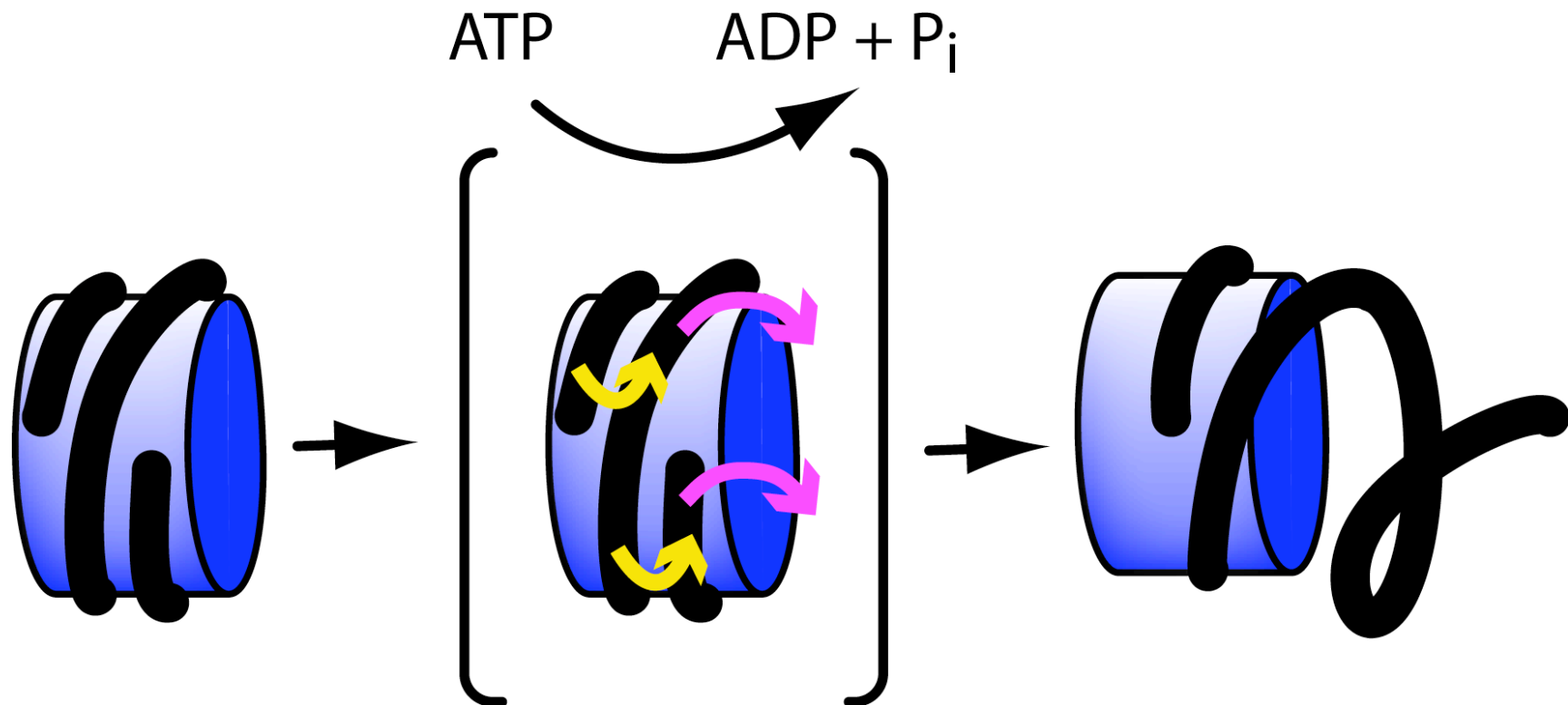
## A DNA-translocating Model for Chromatin Remodeling Factors



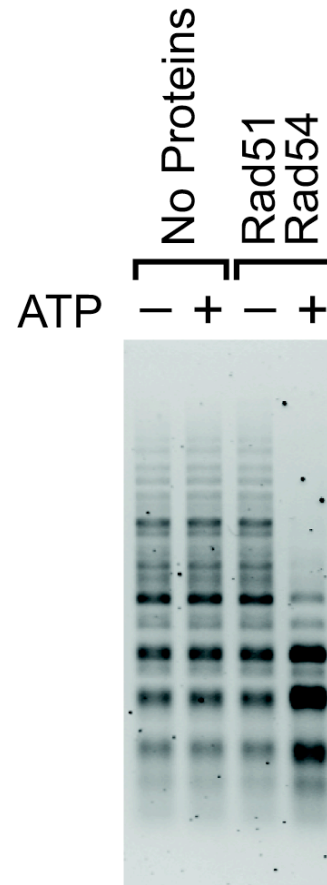
## DNA Translocation Model for Chromatin Remodeling by ISWI Subfamily Complexes



# Lateral Cross-transfer Model for Chromatin Remodeling by SNF2 Subfamily Complexes

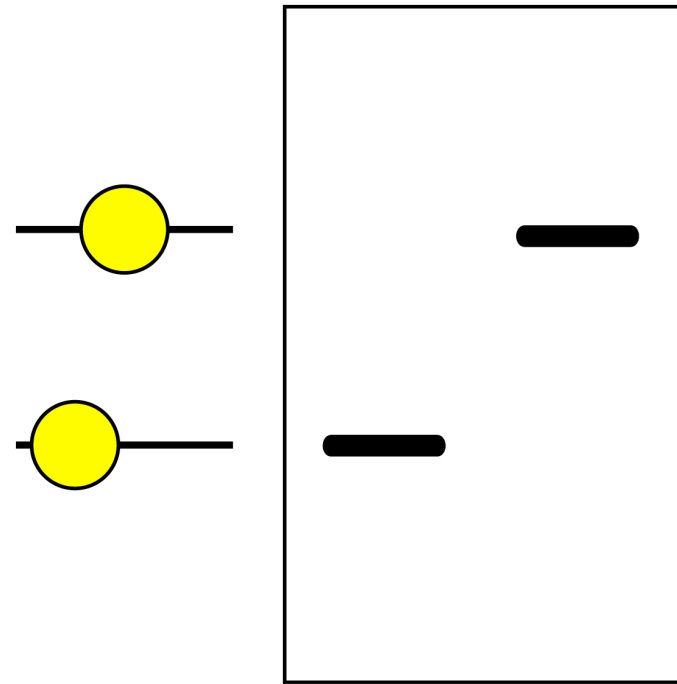


## Restriction Enzyme Accessibility Assay for Chromatin Remodeling



*Hae III Digestion of Chromatin*  
*Agarose Gel Electrophoresis*

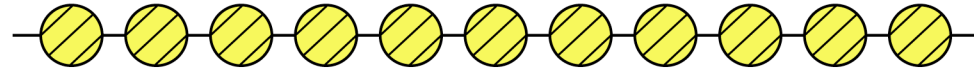
## Gel Shift Analysis of Nucleosome Positioning on a Mononucleosome



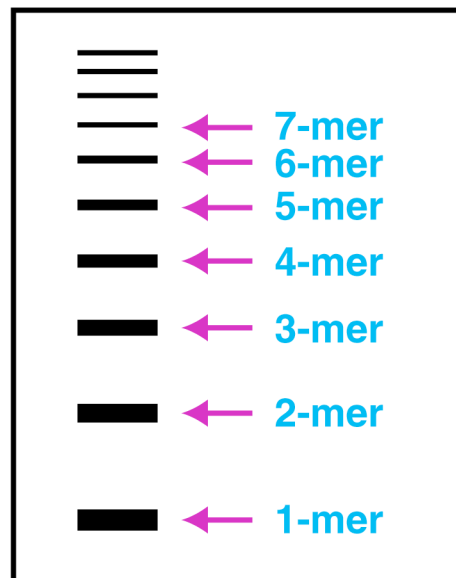
*Gel Mobility Shift Assay*

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

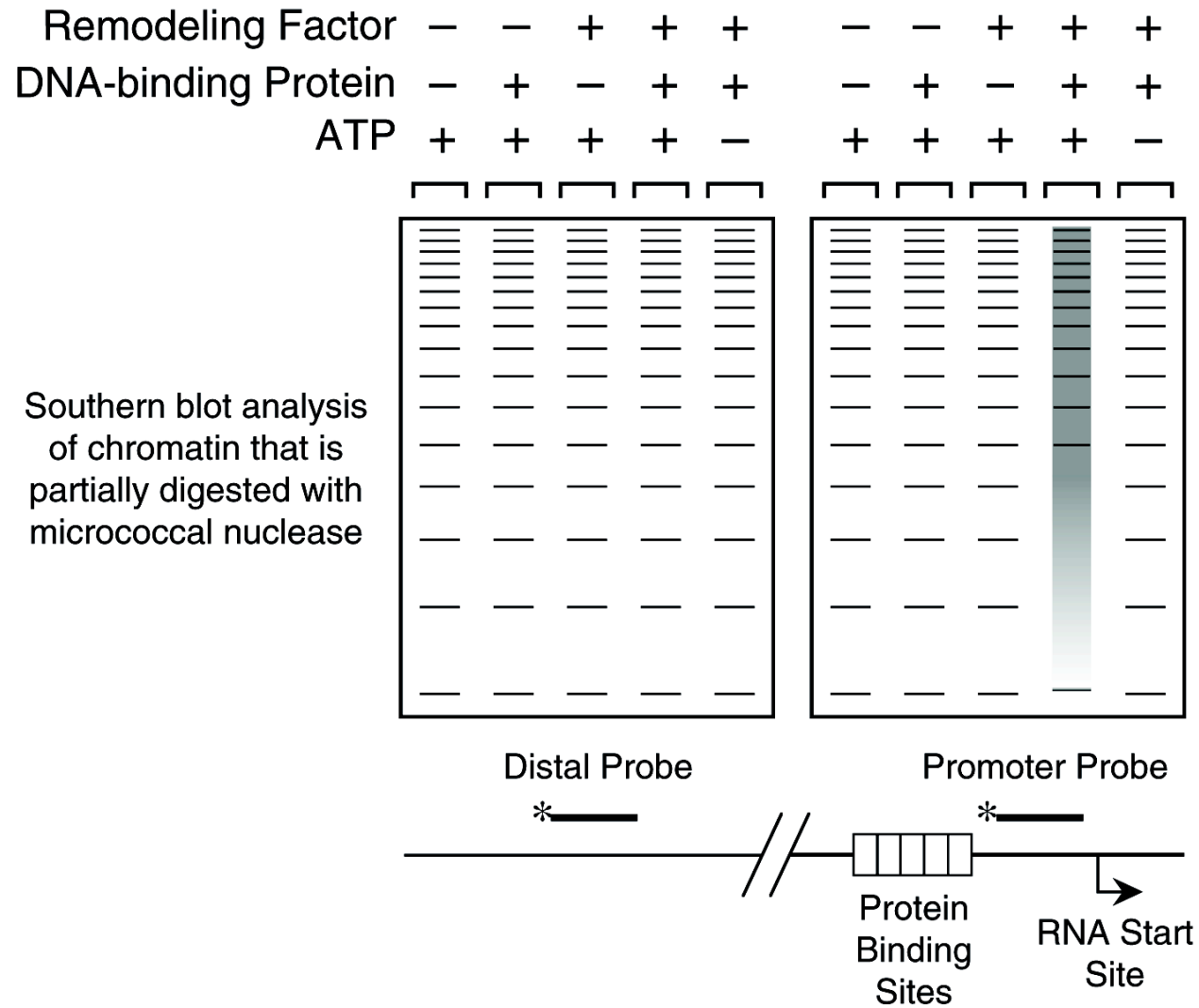


1. Partial Digestion with Micrococcal Nuclease
2. Deproteinization
3. Agarose Gel Electrophoresis



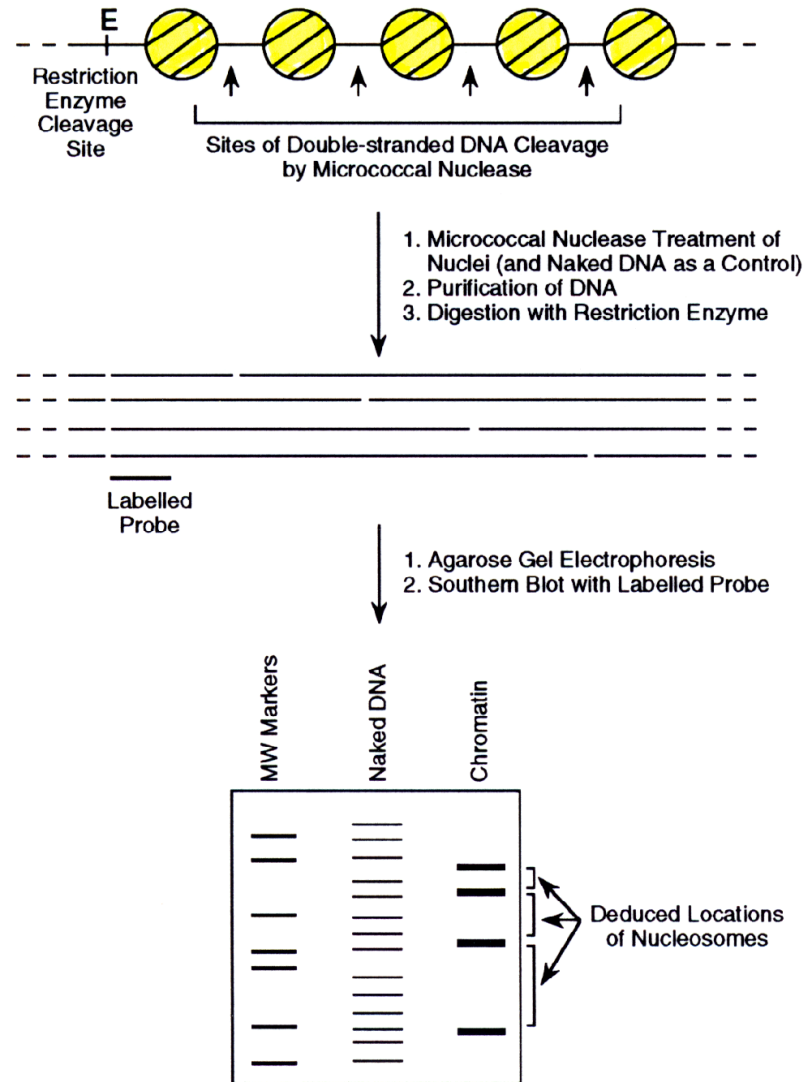


## Assay for Disruption of Periodic Nucleosome Array

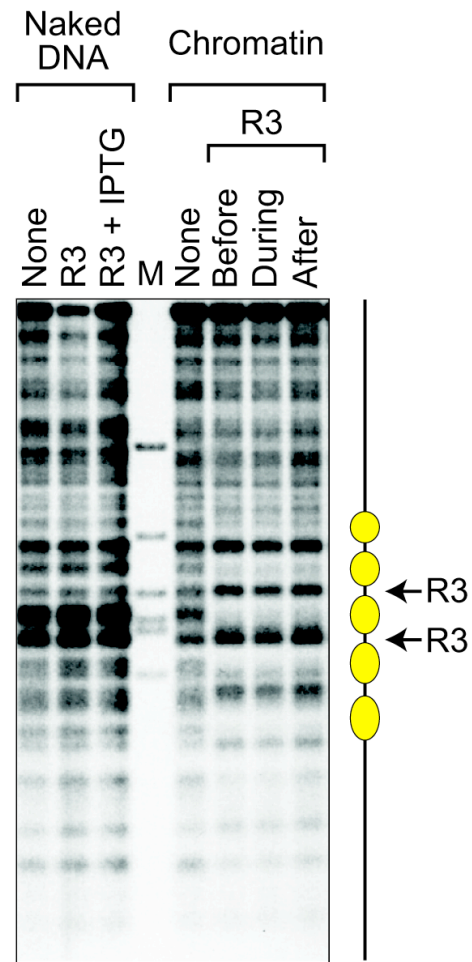


## Loss of the Periodicity of a Nucleosome Array

## Mapping of Nucleosome Positioning by Indirect End-Labeling

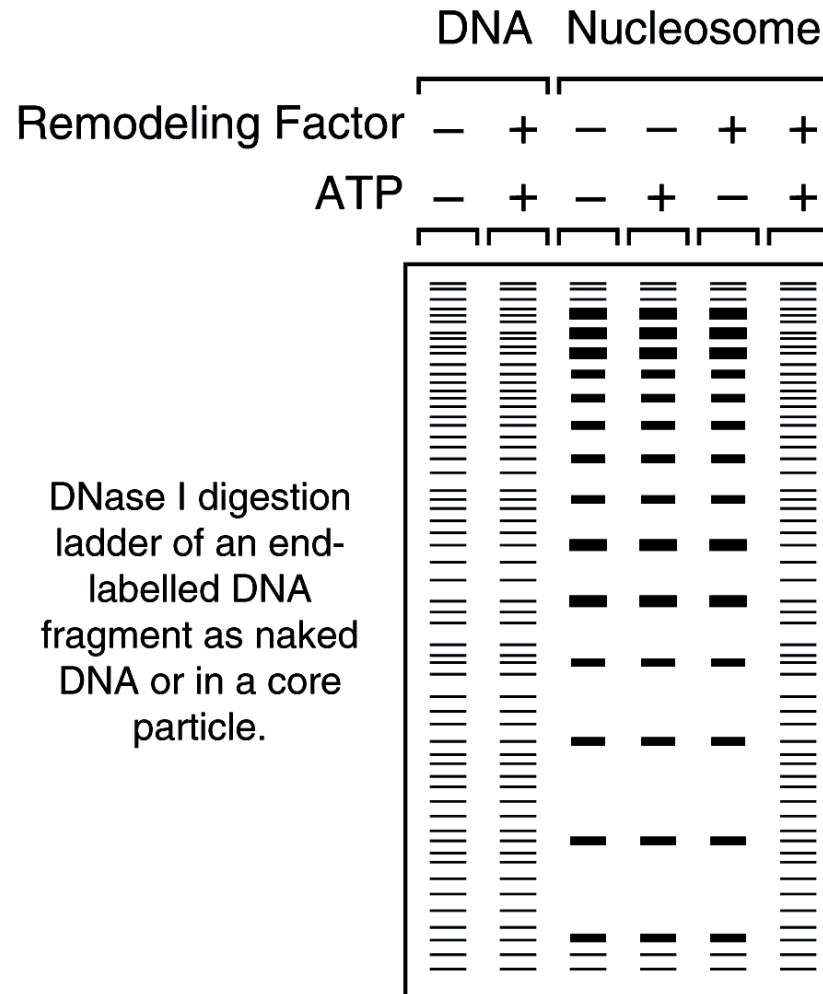


## Analysis of Nucleosome Positioning by Micrococcal Nuclease Digestion and Indirect End Labelling



*Micrococcal Nuclease Digestion  
Indirect End Labelling Analysis*

# Remodeling of a Mononucleosome



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