Eukaryotic Gene Expression: Basics & Benefits

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Lecture 10

Eukaryotic gene regulation: chromatin remodelling

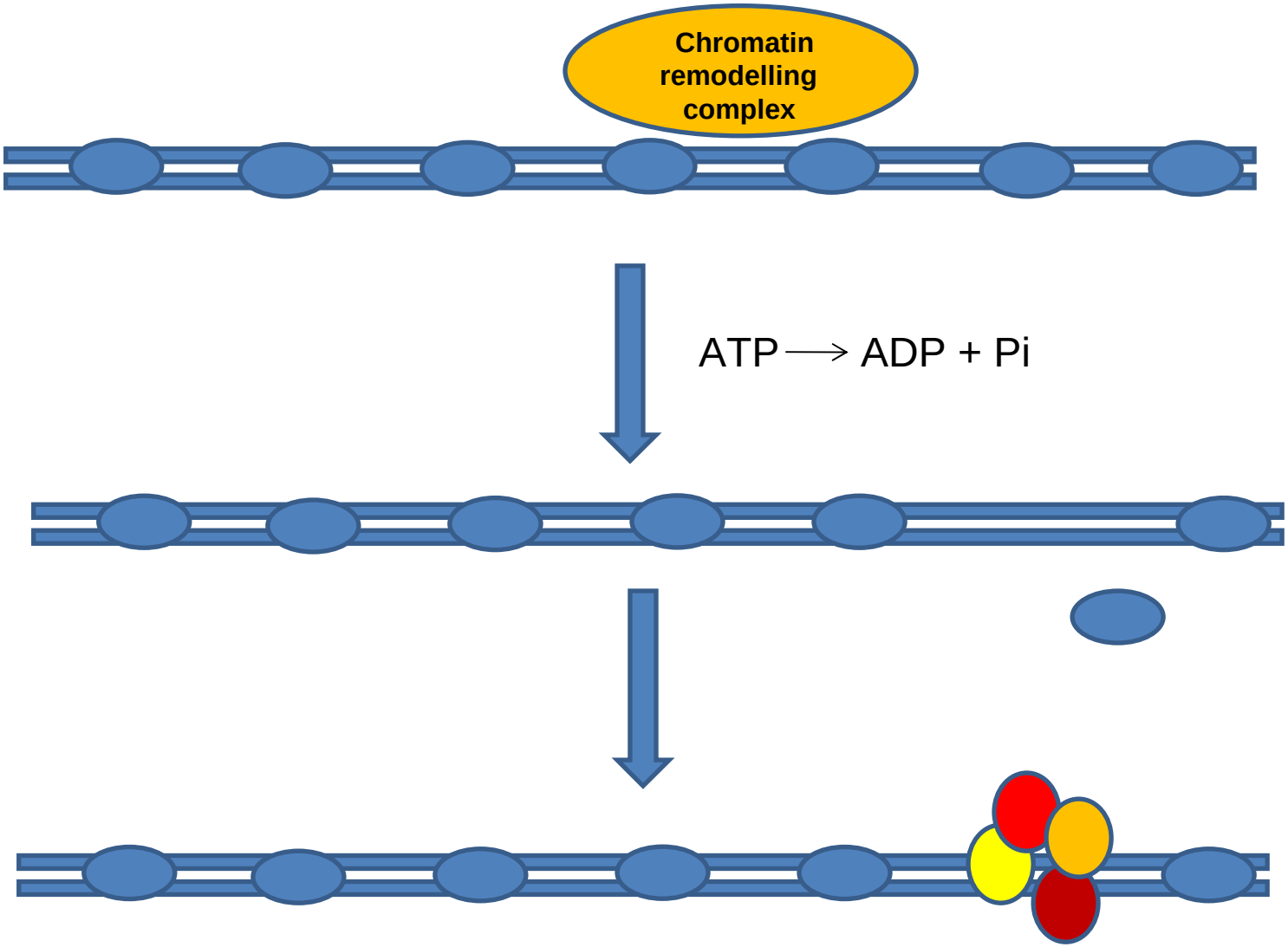
Recap……..

- **Eukaryotic RNA polymerases**
- **Core promoter elements**
- **General transcription factors**
- **Enhancers and upstream activation sequences**
- **Transcriptional activators: DNA binding, transactivation**
- **Role of chromatin: Acetylation & deacetylation of histones**
- **Histone methylation, demethylation, phosphorylation etc., Histone code**
- **DNA methylation, epigenetic code**

Regulation of gene expression
by
ATP-dependent chromatin remodelling

Thus far, we have studied chromatin remodelling involving post translational modifications of histones and DNA methylation leading to regulation of gene expression

ATP-dependent remodelling of chromatin



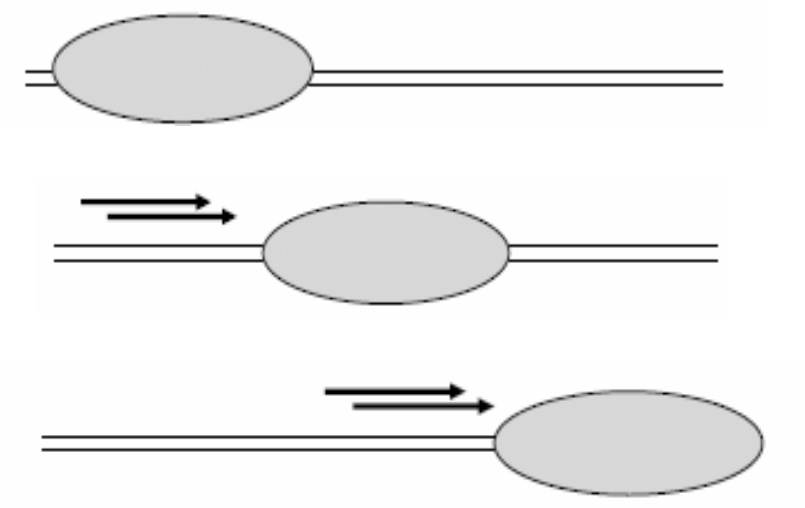
Displacement of nucleosomes during chromatin remodelling leading to the generation of a nucleosome-free region in the chromatin

In addition to nucleosome displacement,

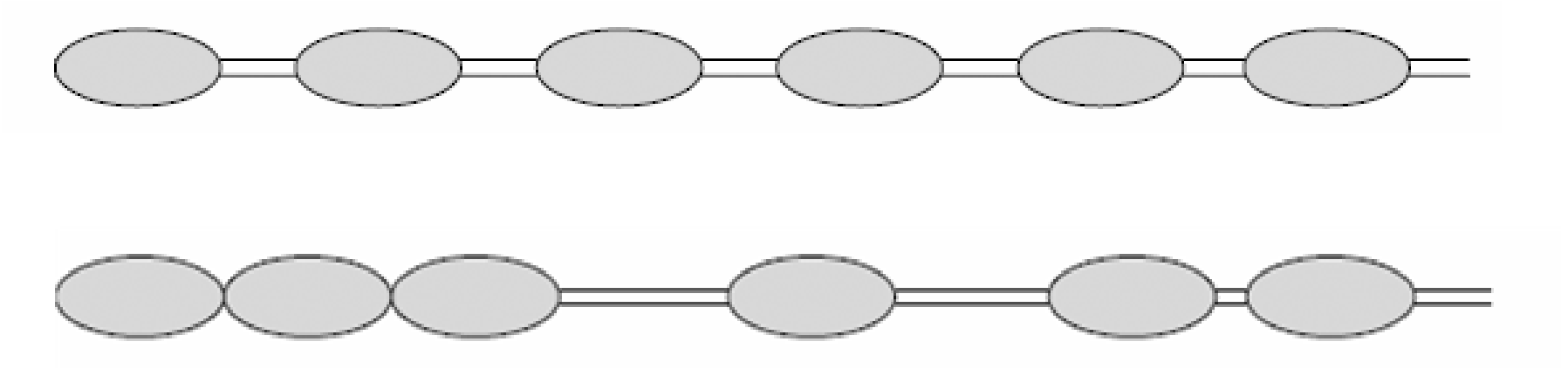
ATP-dependent chromatin remodelling may involve:

- **Nucleosome sliding**

  Histone actamers may slide along DNA, thereby exposing specific DNA sequences for interaction with other proteins



- **Adjusting spacing between histone octamers so that certain DNA sequences are exposed**



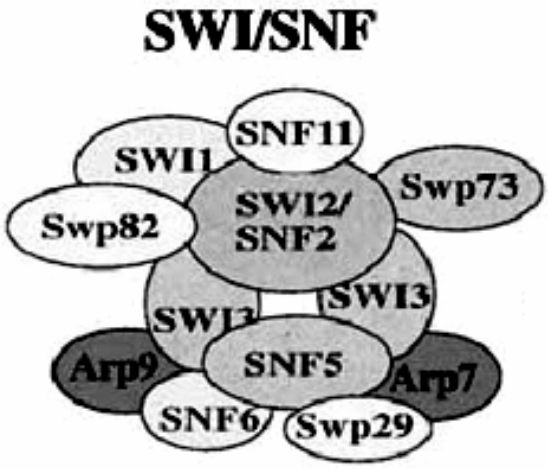
Why is ATP required for moving nucleosomes around?

- Bending DNA around nucleosomes depends on DNA sequence *to some extent (*Some sequences bend more easily than others)
- Nucleosomes *do* have strong affinity for DNA and even have sequence preferences
- This preference may be exploited in nature to organize a chromatin landscape energetically conducive or not conducive for gene expression
- But that landscape has to be changed to suit gene expression requirements
- …and this requires energy

The first ATP-dependent chromatin remodelling complex discovered:
yeast SWI/SNF complex
(SWItch/Sucrose NonFermentable)

SWI/SNF complex was originally identified genetically as a positive regulator of *HO and SUC2 genes:*

- SWI - *HO*-gene encodes an endonuclease that is involved in mating type switching in yeast
- SNF - Sucrose non-fermentor: *SUC2* involved in sucrose- ermentation



Several subunits:
SWI1, **SWI2, SWI3, SNF5, SNF6, SNF11, TFG3/ANC1,** SWP73 etc.

2 Mega daltons

100-500 molecules of Swi/Snf per cell)

The SWI/SNF complex is needed for transcriptional activation bytranscription factors such as GAL4.

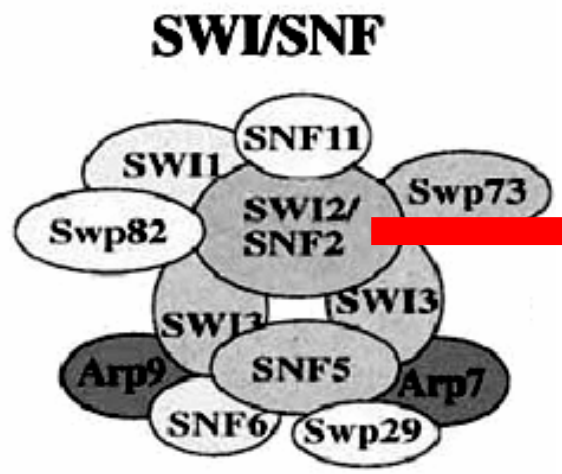
In *S. cerevisiae,* <5% of the total genes require Swi/Snf

Swi/Snf binds DNA and nucleosomes in an ATP-dependent manner with high affinity (Kd = nM range).

~1000 ATP molecules are consumed per minute.

After binding to DNA, it starts an ATP-dependent remodelling of chromating by displacement of nucleosomes

The key component of a chromatin remodelling complex is the ATPase, which provides energy for remodelling by ATP hydrolysis



SWI2 is a DNA-stimulated ATPase.

Chromatin remodeling complexes are classified based on protein motifs found *in addition* to the ATPase domain, or on how the ATPase domain itself is *structured*

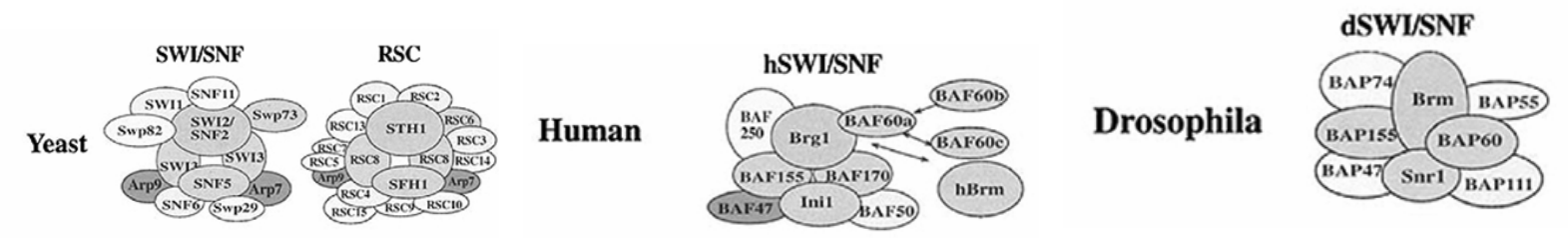
This classification is purely *structural*, designed to make it easier for us to sort them all out

The classificationi s not necessarily based on *functional* criteria

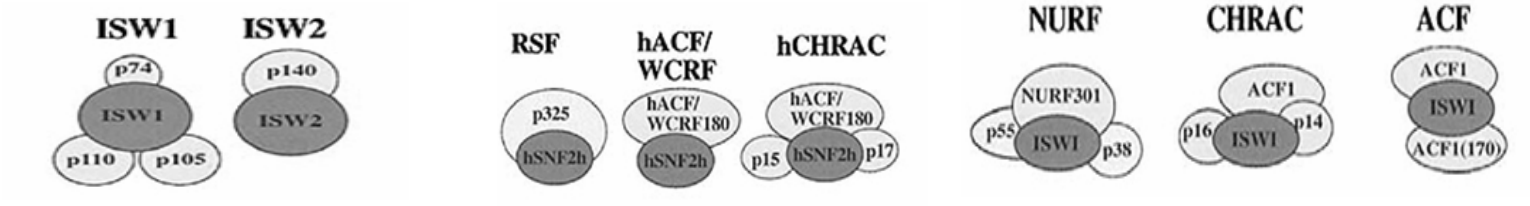
SWI2/SNF2 ATPase SUPERFAMILY

Originally isolated genetically in *Saccharomyces cerevisiae* as mutants in *mating type switching* and *sucrose non-fermenting* functions

SWI2/SNF2 subfamily



ISWI subfamily



CHD/Mi2 subfamily

Ino80 subfamily

NURF- Nucleosome remodeling factor

CHRAC - Chromatin accessibility complex

ACF - ATP-dependent chromatin assembly and remodeling factor

NURF- Nucleosome remodeling factor is invovled in Transcriptional activation of homeotic genes

Different requirements of various nucleosome remodeling complexes

SWI/SNF
Maximal activity with DNA alone

Mi-2
Requires nucleosomal structure
Does not require histone tails

ISWI
Partially activated by DNA
Further stimulated by nucleosomes
Requires histone H4 tails

Nucleosome repositioning

ISWI moves nucleosome from central to terminal position

CHRAC (contains ISWI) facilitates movement from terminal to central position

BRAHMA

ATPase

Associates with acetylated histones

Mechanisms of ATP remodelling

Twist defect diffusion model

In this model, small local alterations from mean DNA twist (‘defects’) propagate around the nucleosome

Bulge propagation model

In the bulge diffusion model, unwinding of DNA from the histone octamer at the entry/exit of the nucleosome and subsequent rebinding of more distal sequences to the same histone contact points would establish nucleosomal particles harbouring excess DNA.

Subsequent migration of the bulge around the nucleosomal superhelix would result in the nucleosome apparently increasing 40-60 bp

Cis vs trans

Langst & Becker 2001 Journal of Cell Science 114 2561-8

Eviction- displacement of histones

Dimers of H2A and H2B can be rather easily exchanged in and out of nucleosome

Entire histone octamers, including H3 and H4, can also be displaced or exchanged under certain circumstances

Variant histone incorporation

Many variant forms of histones exist, different from the classical core histones, are often expressed in a cell cycle-specific manner and these are incorporated into chromatin in a DNA replication-independent manner.

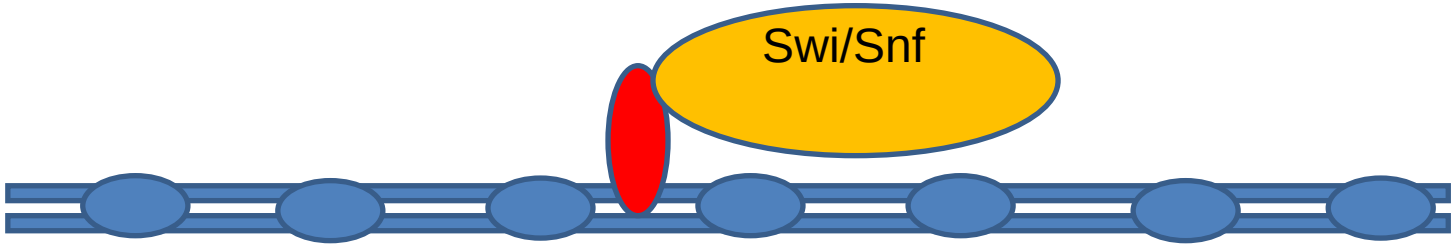
For example, a chromatin remodelling complex known as SWR1 promotes the exchange of H2A from the nucleosome with a histone variant called H2AZ

DNA Repair

Certain Chromatin remodelling complexes such as the INO80 complex is involved in DNA repair

Swi/Snf recruitment by transcription factors

Just as certain transcription factors recruit histone modifying enzymes such as HATs, HDACS, HMTs, DNMTs etc.,
certain gene-specific activators (GCN4, GAL4-VP16 etc.) recruit Swi/Snf-complex directly



Purified Swi/Snf complex stimulates binding of GAL4-AH to nucleosomal DNA 10-30-fold

Altering chromatin structure and gene expression by a combination of histone modifications and ATP-dependent chromatin remodelling

Targeted acetylation of histone H3 by SAGA stabilizes its binding and that of a targeted SWI/ SNF chromatin-remodeling complex. This requires the bromodomains of Gcn5 and Swi2, respectively, which bind to acetyl-lysine.

It appears that different promoters may use different combinations of histone modifiers and chromatin remodellers.

There is no general rule that is common to all promoters.

Certain activators/co-activator complexes contain components that recognize a specific histone modification as well as those that can remodel chromatin

For example, the HURF complex contains BPTF, which can recognize methylated lysine 4 of Histone H3 as well as SNF2L ATPase

Chromatin remodelling may depend on nucleotide sequence of promoters

Certain promoters are more susceptible or sensitive to chromatin remodelling than others.

For ex., AT-rich regions are often nucleosome-free and therefore promoters containing such regions are better primed for transcription activation

Transcription factor binding sites are often present in nucleosome-free regions and therefore does not require nucleosome displacement.
It is often found that nucleosome density at promoter regions is typically lower than that in the coding region

Rotational positioning of nucleosomes

A 5 bp sliding of nucleosome can expose a transcription factor binding site

In case of the MMTV LTR, 6 partly palindromic sites, each bound by one dimer of glucocorticoid receptor (GR) is present. It also has two adjascent sirtes for binding of NFI and OCTI.

Both GR and another TF called NFI cannot bind to MMTV LTR simultaneously on a linear DNA template but they can bind together on a chromatin template.

In the absence of hormone, the GR can only bind to two of its four binding sites. In order to bind to the remaining two sites, a change in rotational positioning on the nucleosome is required.

When hormone bound GR bind to MMTV LTR chromatin template, it results in recruitment of SWI-SNF complex, leading to dissociation of histone H1 and exposure of binding sites for NF1 + OCTI leading to recruitment of TFIID and ultimately transcriptional activation.

Infact, NFI binding to chromatin template can be footprinted after hormone addition but not before.

Interactions between transcription factors and remodelling complexes provides important insights into their mechanism of action.

For ex., in case of the TF Swi5p, an activator of the HO locus in yeast,
It enters the nucleus at the end of mitosis and bind to the HO promoter
And recruits SWI/SNF.

Swi5p is then released, leaving SMI/SNF at the promoter.

Thus, the function of the TF is only to recruit a remodelling complex
To the promoter

Several genetic diseases are associated with chromatin remodeling

Huang et al. 2003 Curr Opin Genet Dev 13 246-52

Fry & Peterson 2001 Curr Biol 11 R185-97

Kingston & Narlikar 1999 G&D 18 2339-92

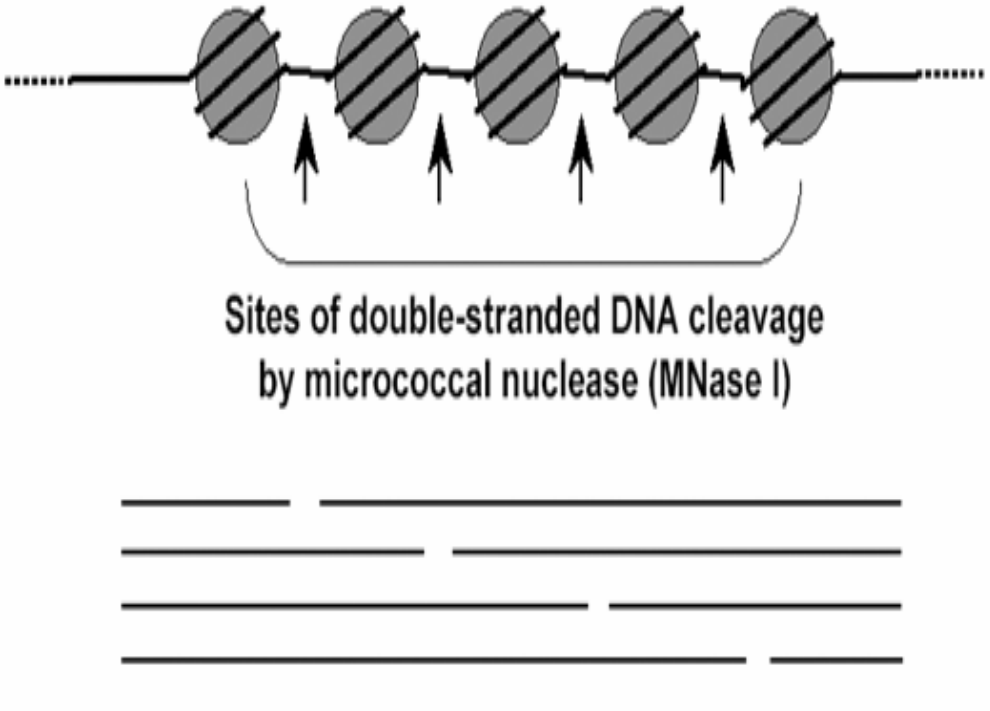
Wagner 2003 Curr Opin Plant Biol 6 20-8

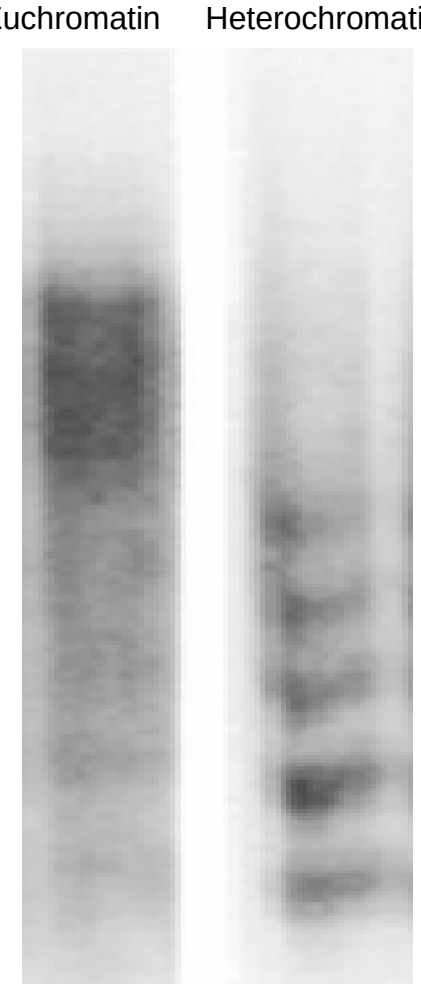
Narlikar et al. 2002 Cell 108 475-87

Langst & Becker 2001 JCS 114 2561-8

SUMMARY

- Chromatin remodelling complexes are ATPases that form large multi-subunit chromatin remodeling complexes.
- These complexes use the energy of ATP to remodel nucleosomal DNA.
- They are associated with activation of chromatin but they can silence as well





Cryderman et al. (1999) NAR 27(16): 3364