

Regulation by changes in chromatin structure

Active chromatin

Chromatin Structure

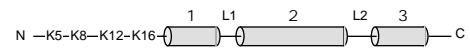
Principal proteins in chromatin are histones

H3 and H4 : Arg rich, mostly conserved sequence
H2A and H2B : Slightly Lys rich, fairly conserved

H1 : very Lys rich, most variable in sequence between species

Histone structure and function

"Minimal" structure for a core histone, e.g. H4. Others have one additional alpha helix.

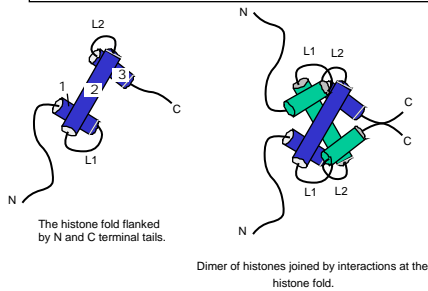


Highly charged N-terminal tail.

Globular, hydrophobic domain for histone-histone interactions and for histone-DNA interactions.

Histone interactions via the histone fold

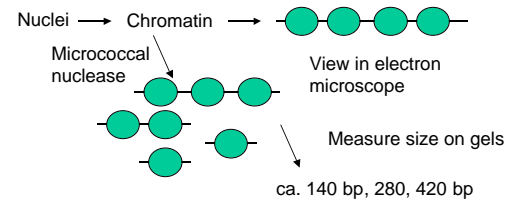
The alpha-helical regions of the core histones mediate dimerization.



Nucleosomes are the subunits of the chromatin fiber

• Experimental evidence:

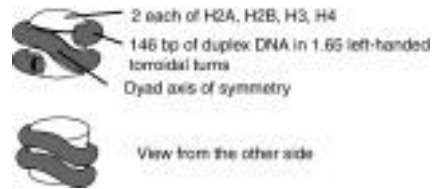
- Beads on a string in EM
- Micrococcal nuclease digestion



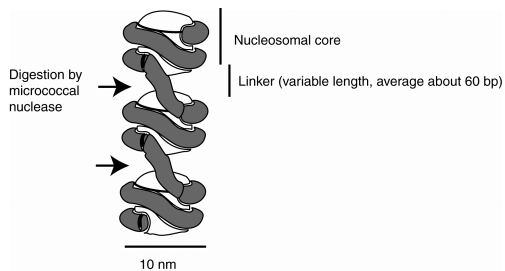
Nucleosome components

- Nucleosome core + histone H1 (in higher eukaryotes) + linker DNA (0-50bp)
- The **nucleosome core** contains
 - an octamer of 2 each of the **core histones (H2A, H2B, H3 and H4)** and
 - **146 bp of DNA** wrapped 1.75 turns.
- Core histones **dimerize** through their histone fold motifs generating H3/H4 dimers and H2A H2B dimers
- Each histone pair bends approximately 30bp of DNA around the histone octamer.

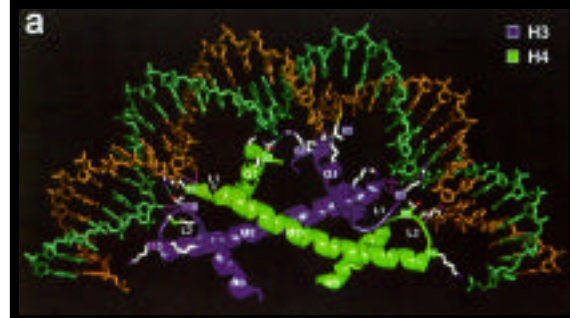
General model for the nucleosomal core



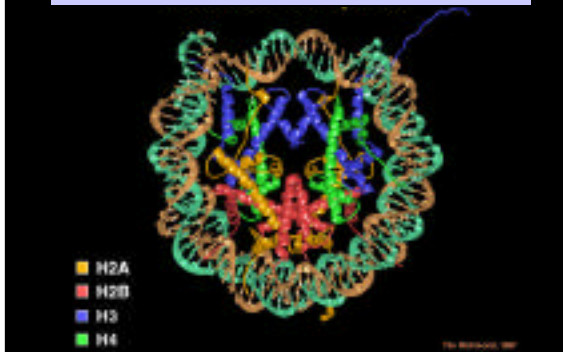
A string of nucleosomes



H3-H4 dimer bound to DNA



Nucleosome core particle



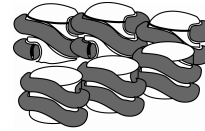
Side view of nucleosome



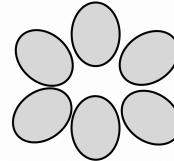
Chromatin higher order structure

- Arrays of nucleosomes condense into higher order chromatin fibers.
- Despite over 2 decades of investigation the structure of the “30nm” chromatin fiber is not known.
- This may be due to irregularity or instability of the structure.
- This level of structure has been implicated in mechanisms of chromatin repression, thus, the lack of structural information at this level is particularly troublesome.

Higher order chromatin structure



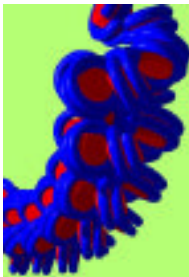
Side view



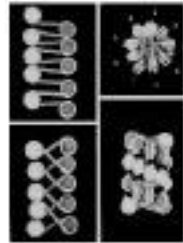
Top view

Histone H1 associates with the linker DNA, and may play a role in forming higher order structures.

Solenoid model for 30 nm chromatin fiber



Solenoid of nucleosomes



Path of DNA between nucleosomes is unknown

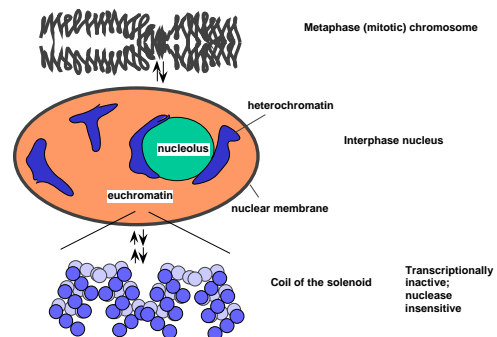
Transcriptionally active chromatin is more “open”

- Direct assays show that it is more accessible to DNases.
- We infer that it is more accessible to components of the transcriptional apparatus.
 - This inference is now being verified by *in vitro* experiments.

Classical evidence that chromatin structure can regulate genes

- Radiolabeled UTP is incorporated into RNA in regions of euchromatin, not heterochromatin
- Cells that are actively expressing their genes have larger nuclei than do quiescent cells.
- Activation of particular sets of genes in *Drosophila* generates visible **puffs** at defined loci on the polytene chromosomes.
- Lampbrush chromosomes show transcription in the more extended, open regions of the chromosomes.

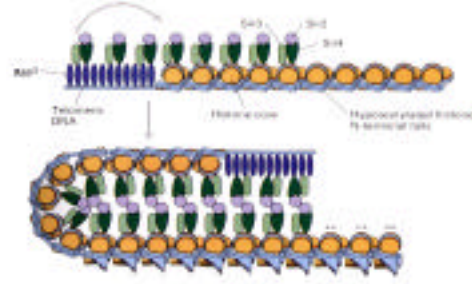
Condensed chromatin is transcriptionally inactive



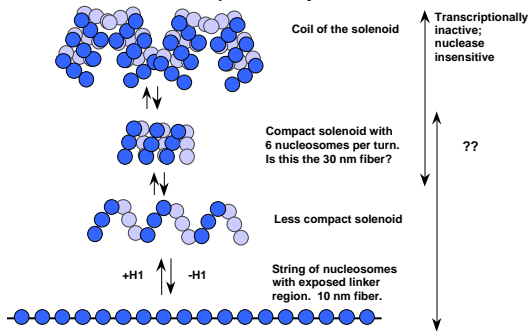
Heterochromatin is **not** transcribed

- **Position effect variegation**
- Wild-type *w+* gene produces red eyes in *Drosophila* when it is at its normal location.
- Movement of the *w+* gene close to the centromere causes it to not be expressed in some of the sections (ommatidia) of the eyes, generating white patches.
- This variegation in the pattern of expression is explained by whether the *w+* gene is in heterochromatin (OFF) or euchromatin (ON).

Silenced chromatin at telomeres



More open chromatin can be transcriptionally active



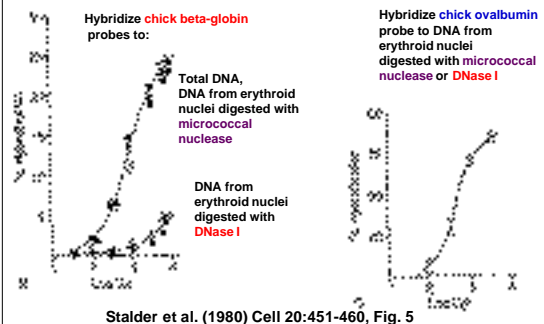
Direct measurement of accessibility of chromatin

Nuclease sensitivity

Nuclease sensitivity assays

- The overall sensitivity of a gene to DNase I is increased about 3 to 10 fold when it is expressed.
- Can measure this by
 - Isolating nuclei from cells expressing or not expressing the gene.
 - Digest nuclei (chromatin) with DNase I
 - Measure how much DNA from that gene survives nuclease treatment.

DNase I digestion of nuclei reduces the concentration of actively transcribed DNA



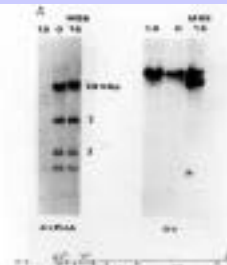
Map the extent of the region around a gene that is accessible to nucleases

- Combine nuclease treatment of chromatin with restriction digestion
- Assay by blot-hybridization

DNase I digestion of nuclei preferentially cuts restriction endonuclease fragments containing actively transcribed DNA

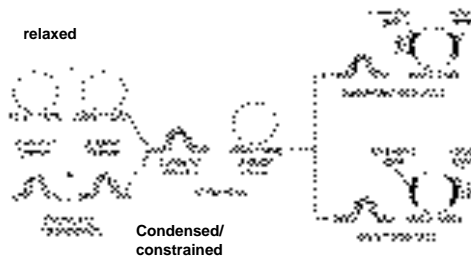
Lanes 1 and 3: nuclei digested with DNase I
 Lane 2: not digested
 Lanes 1 and 2: nuclei from erythroid cells
 Lane 3: nuclei from lymphoid cell line.

DNA from nuclei was digested with BamHI, run on gel and hybridized with chick alpha-globin (left) or ovalbumin (right) probes.



Stalder et al. (1980) Cell 20:451-460, Fig. 2

Domains as loops



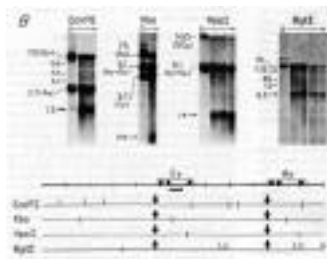
"Interpretation of moderate sensitivity to DNase I in terms of "lampbrush" chromosome-like loops or domains."
 Stalder et al., 1980, Cell 20:451

Map DNase hypersensitive sites = HSs

- Use "indirect end-labeling" to find the sites of discrete, **double-strand breaks** caused by nuclease digestion of chromatin.
- These correspond to **discrete** regions of substantially altered chromatin structure
 - In some cases they lack nucleosomes
- **Landmarks** to functional sites on the DNA
 - Transcriptional activators at enhancers
 - Replication proteins at origins

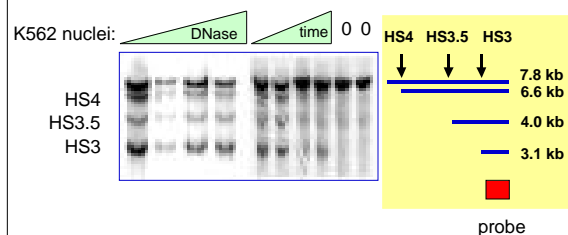
Indirect end-labeling to see DNase HSs in gamma globin genes

Nuclei from human fetal erythroblasts were digested with DNase I. DNA was purified, digested with the indicated restriction endonuclease, run on a gel and blotted. A fragment from the gamma-globin gene was used as a hybridization probe. **DNase HSs** are revealed as new fragments smaller than the parental bands.



Groudine et al. (1983) PNAS 80:7551-7555.

Example of indirect end-labeling to see multiple HSs

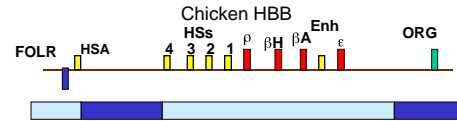


H. Petrykowska

Features of active chromatin

- Accessible to nucleases
- DNA is less methylated
- Less histone H1
- Core histones are acetylated at discrete sites
- Presence of nonhistone proteins HMG14 and HMG17
- Nucleosome phasing

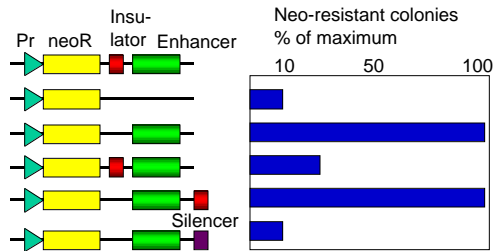
Biochemically defined domain can correspond to a set of coordinately expressed genes



DNase Sensitive	+	-	+	-
Histone Ac'n	-	-	+	-
Histone H1	-	+	-	-
DNA methylation	-	+	-	+
Expressed:	Progenitors		Maturing erythroblasts	

HS4 from chick *HBB* complex

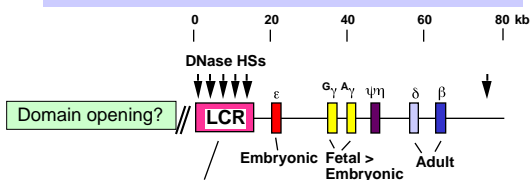
- Marks a **boundary** in chromatin: open to closed
- Acts as an **insulator**: Blocks activation of promoter by an enhancer



Cis-regulatory elements that act in chromatin

- **Generate an open, accessible chromatin structure**
 - Can extend over about hundreds of kb
 - Can be tissue specific
- **Enhance** expression of individual genes
 - Can be tissue specific
 - Can function at specific stages of development.
- **Insulate** genes from position effects.
 - Enhancer blocking assay

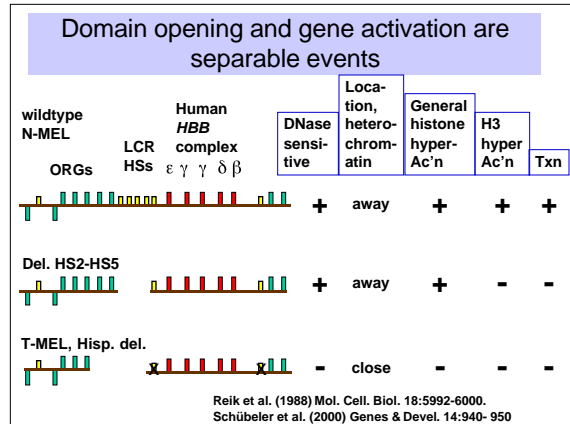
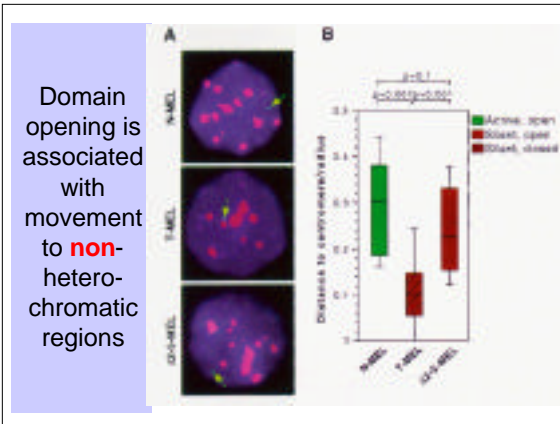
Human γ -globin gene cluster



Locus control region:
 Activate linked globin gene expression in erythroid cells.
 Overcome position effects at many integration sites in transgenic mice.
 Role in switching expression?

HBB LCR will activate expression at many chromosomal locations

DNase HSs	Expressed in red cells	Developmental Regulation	Position Effects	Erythroid Chromatin
LCR	Yes	Yes	No	Open
Hispanic () thalassemia	No	No	Yes?	Closed
In transgenic mice:				
	Sometimes	Yes	Yes	Sometimes open
LCR	Yes	Precocious expression	No	Open



- Proposed sequence for activation
1. Open a chromatin domain
 - Relocate away from pericentromeric heterochromatin
 - Establish a locus-wide open chromatin configuration
 - General histone hyperacetylation
 - DNase I sensitivity
 2. Activate transcription
 - Local hyperacetylation of histone H3
 - Promoter activation to initiate and elongate transcription