Summer Course in Biophysics

June 5, 2014

How DNA Is Packed In The Cell: Chromosomes, Genes, Nucleosomes

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Outline

I. Chromatin organization
  - The DNA packaging problem
  - Histones and nucleosome core particle
  - Chromatin folding and nuclear organization
  - Euchromatin vs Heterochromatin

II. Factors that influence chromatin organization and gene function
  - Histone post-translational modifications (PTMs) and the ‘histone code’
  - Histone variants
  - DNA methylation

III. Tools and technologies leading the charge in chromatin research
  - Modification-specific antibodies and chromatin immunoprecipitation
  - High-throughput microarray/DNA sequencing technologies
  - Proteomics and mass spectrometric analyses

The DNA packaging problem

-E. Coli: (*Chlamydia trachomatis*)	1X	1 million base pairs
-Yeast genome:	12X	12 million base pairs
-Fruit fly genome:	122X	122 million base pairs
-Human genome:	3400X	3.4 billion base pairs

If our strands of DNA were stretched out in a line, the 46 chromosomes making up the human genome would extend more than six feet (~ 2 meters)

A Matter of Fitting In!

0.0043 meters

(0.17 inches)

8850 meters (~5.5 miles)

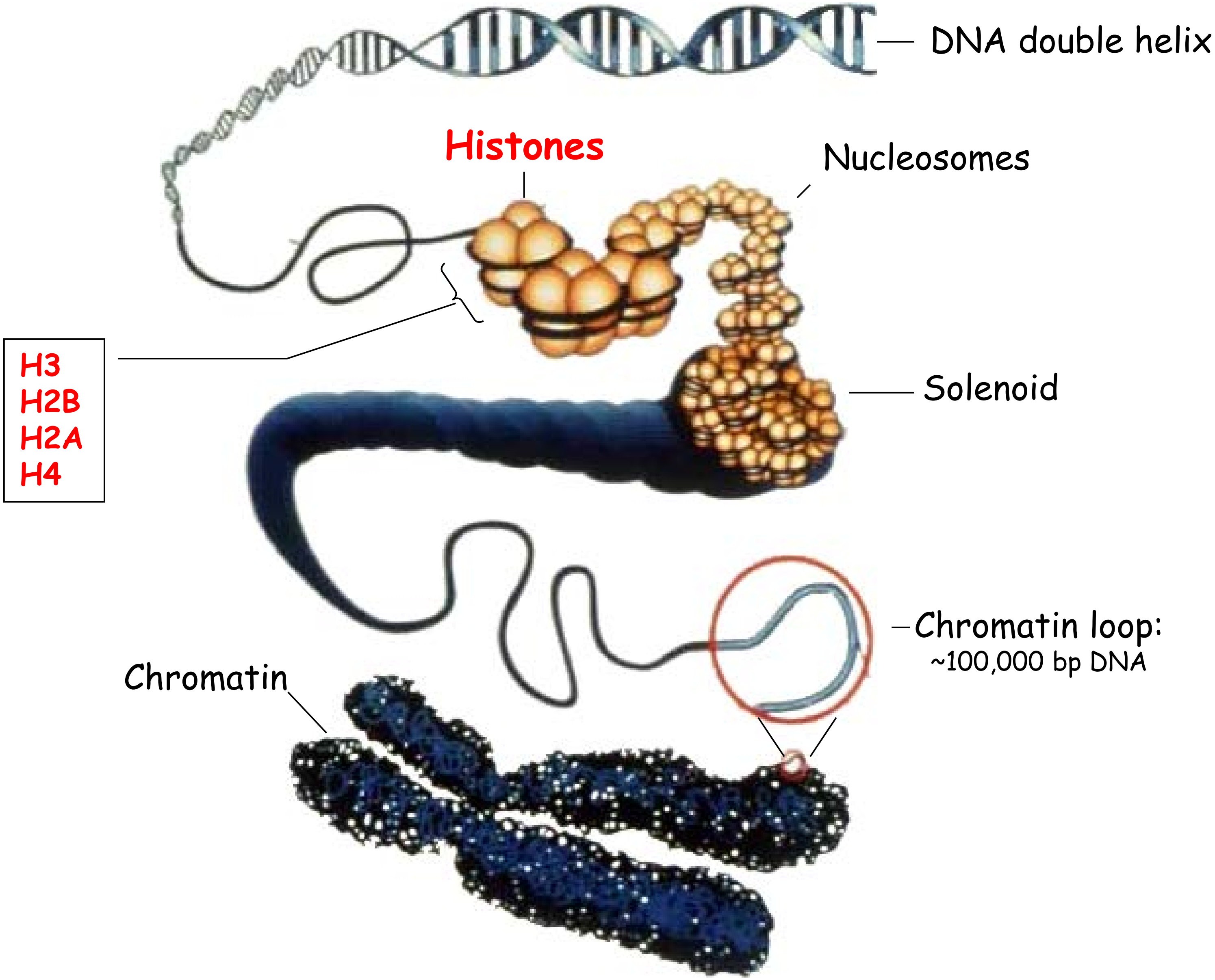
Mount Everest image:
http://www.unu.edu/mountains2002/photoexhibit/thehimalaya.htm

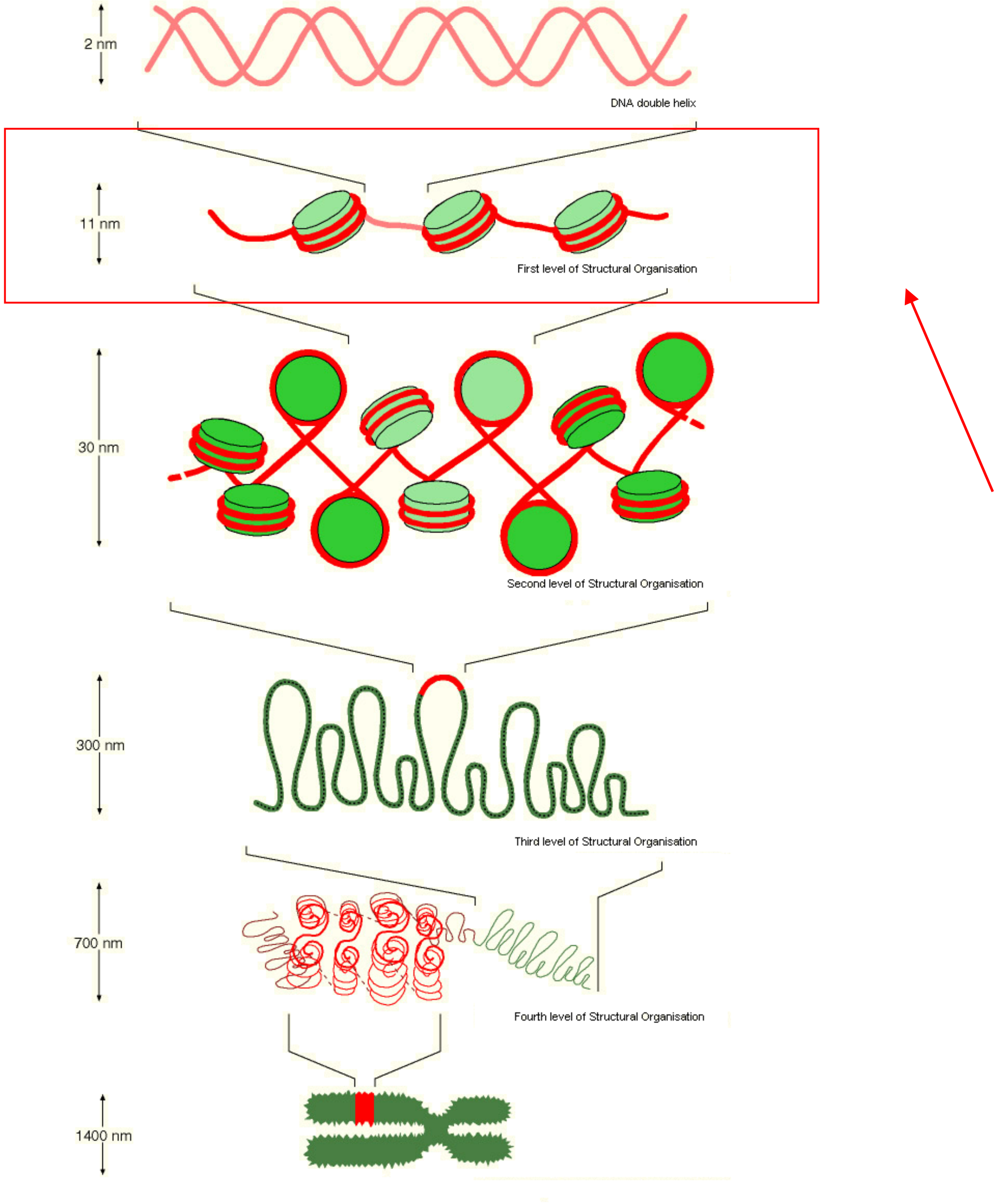
pipet tip image: Biologix Research

(slide provided by Raymond Reeves)

How is DNA packaging achieved?

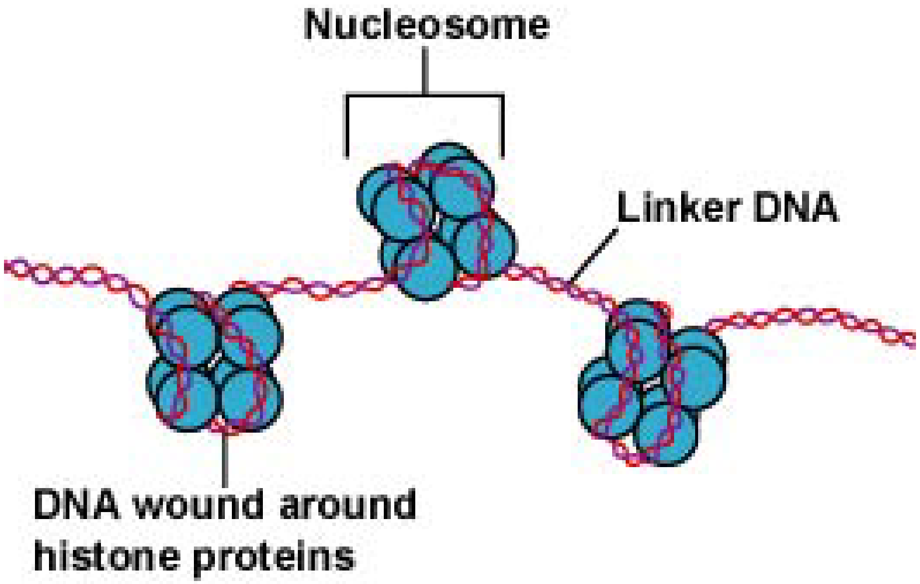
Organization of eukaryotic chromatin

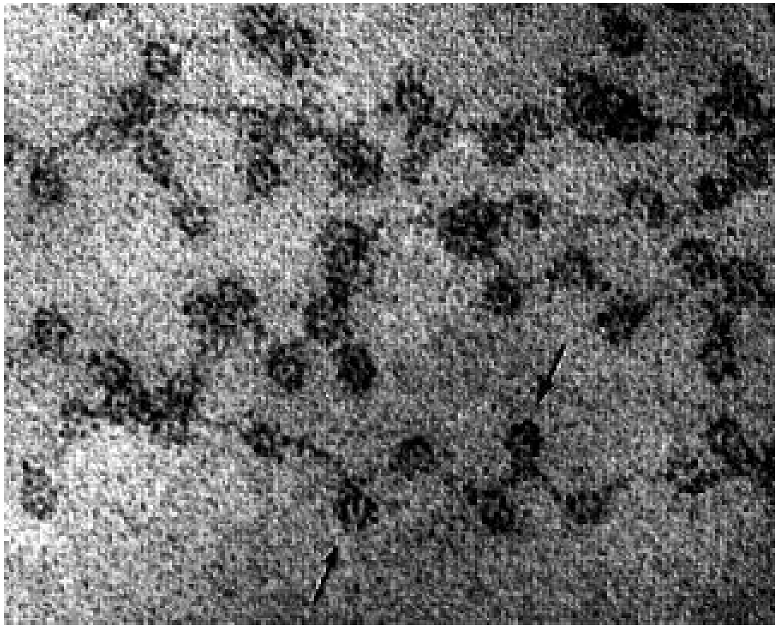




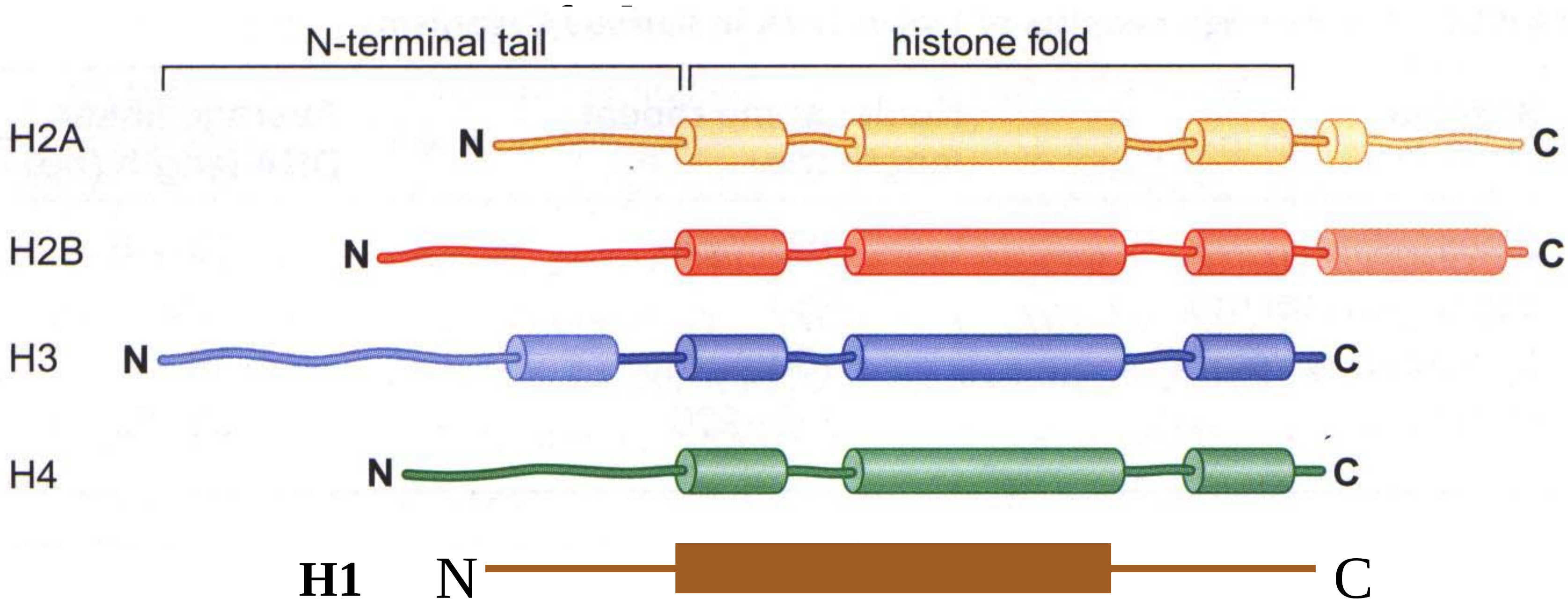
First order of DNA compaction

Nucleosomes are the building blocks of chromatin





Histone structure



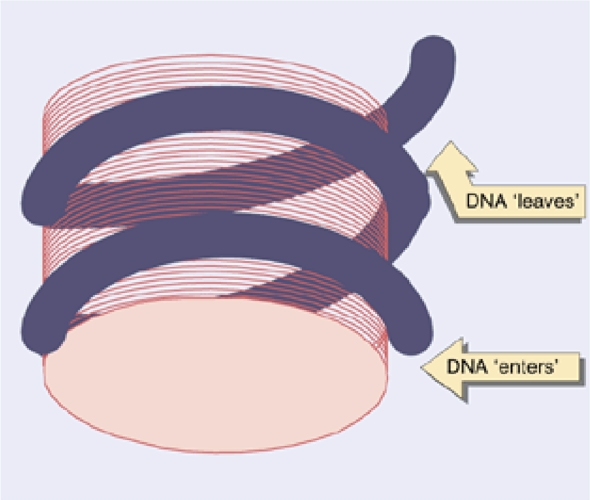
“Tail” domain

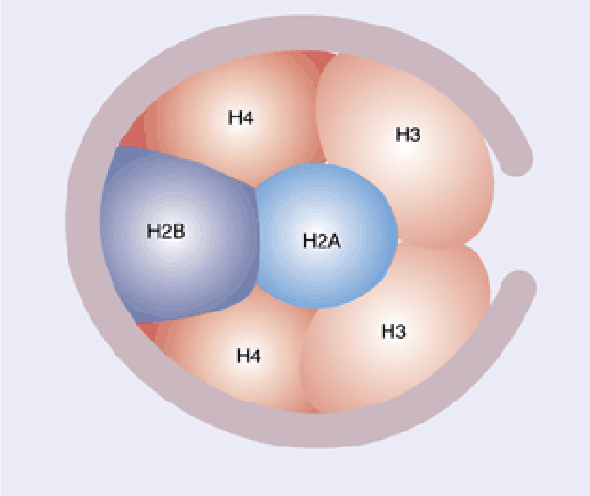
- Regulatory domain
- Involved in higher-order packing

“Globular” domain

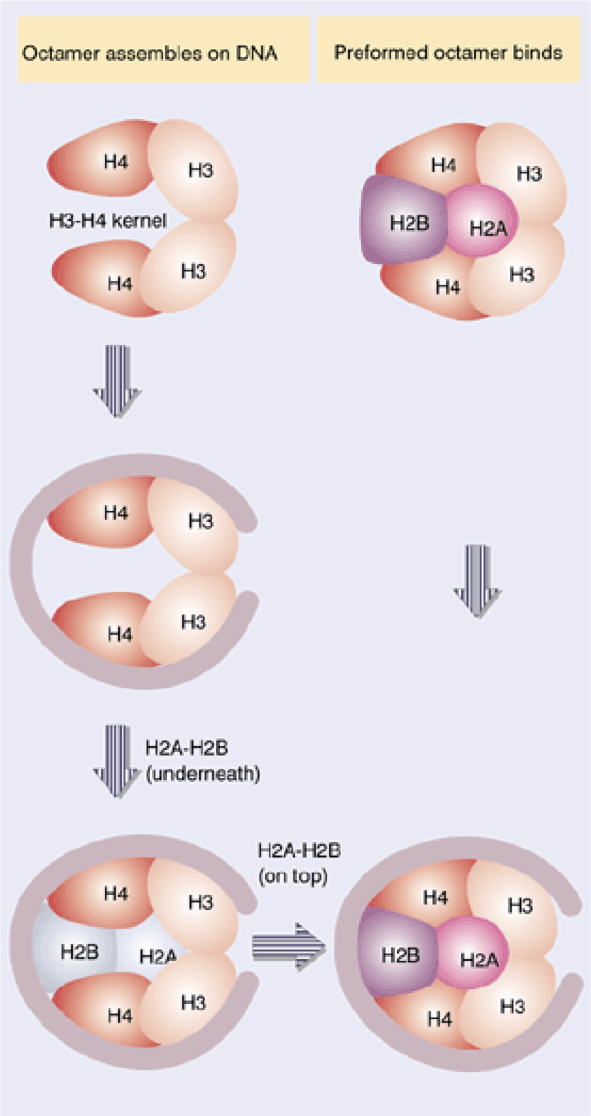
- Histone-histone interactions
- DNA wrapping

Nucleosome organization

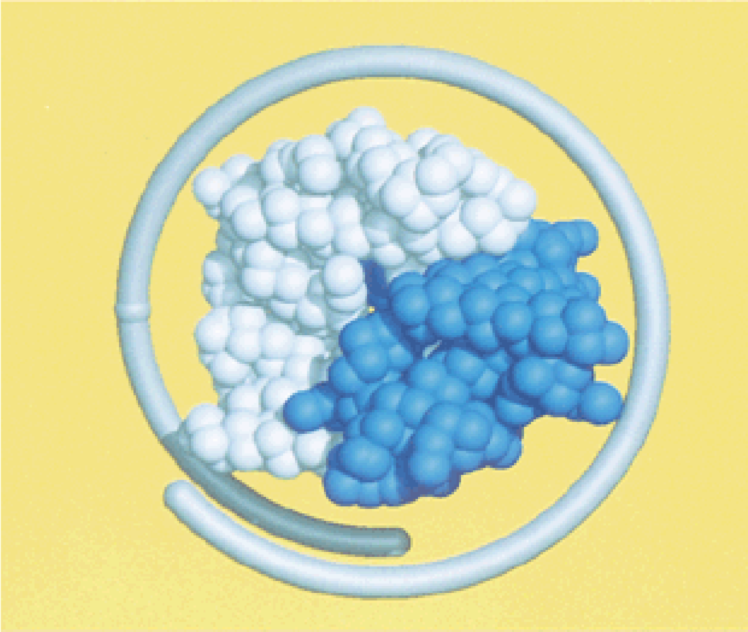




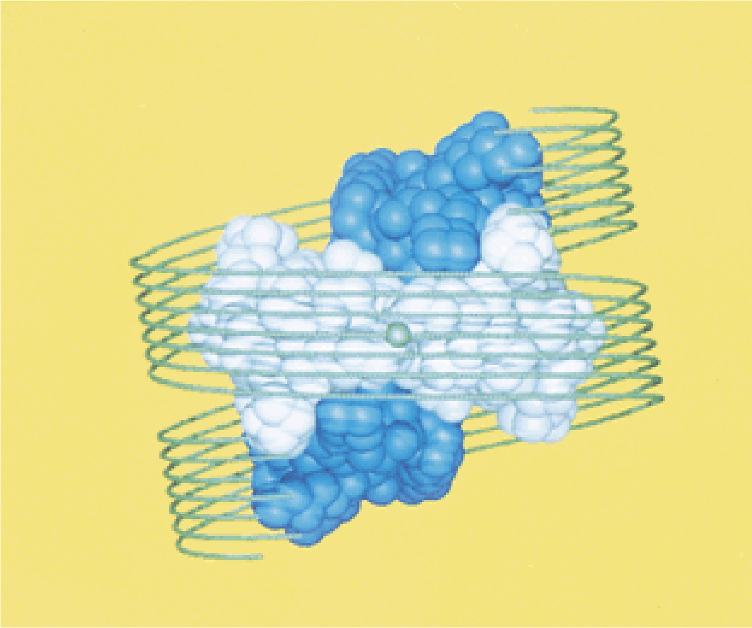
H3-H4 tetramers build a “wall” that is “capped” by H2A-H2B dimers



Top view



Side view



Blue= H2A/H2B

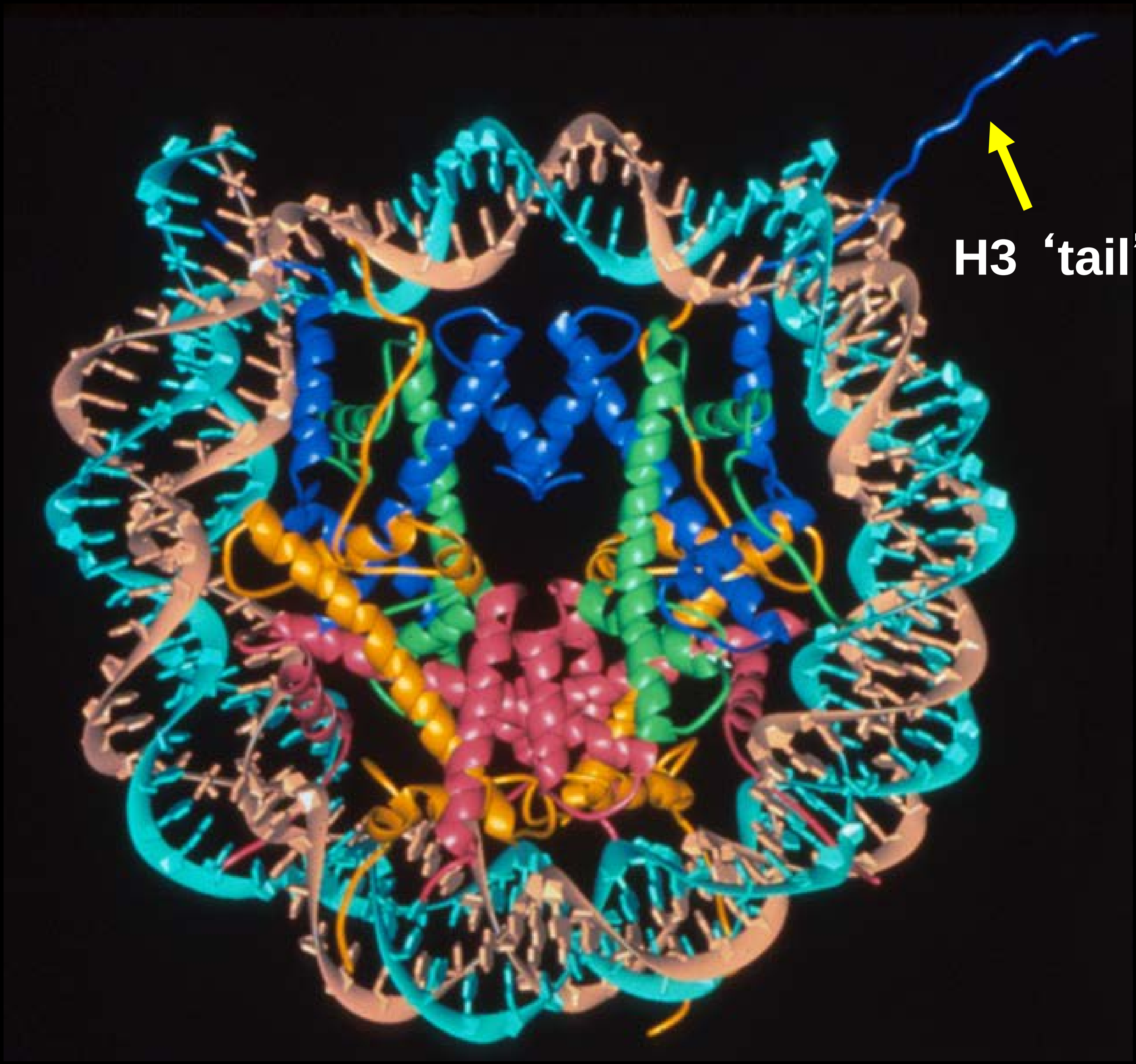
White= H3/H4

H3 🟦

H4 🟩

H2A 🟧

H2B 🟥



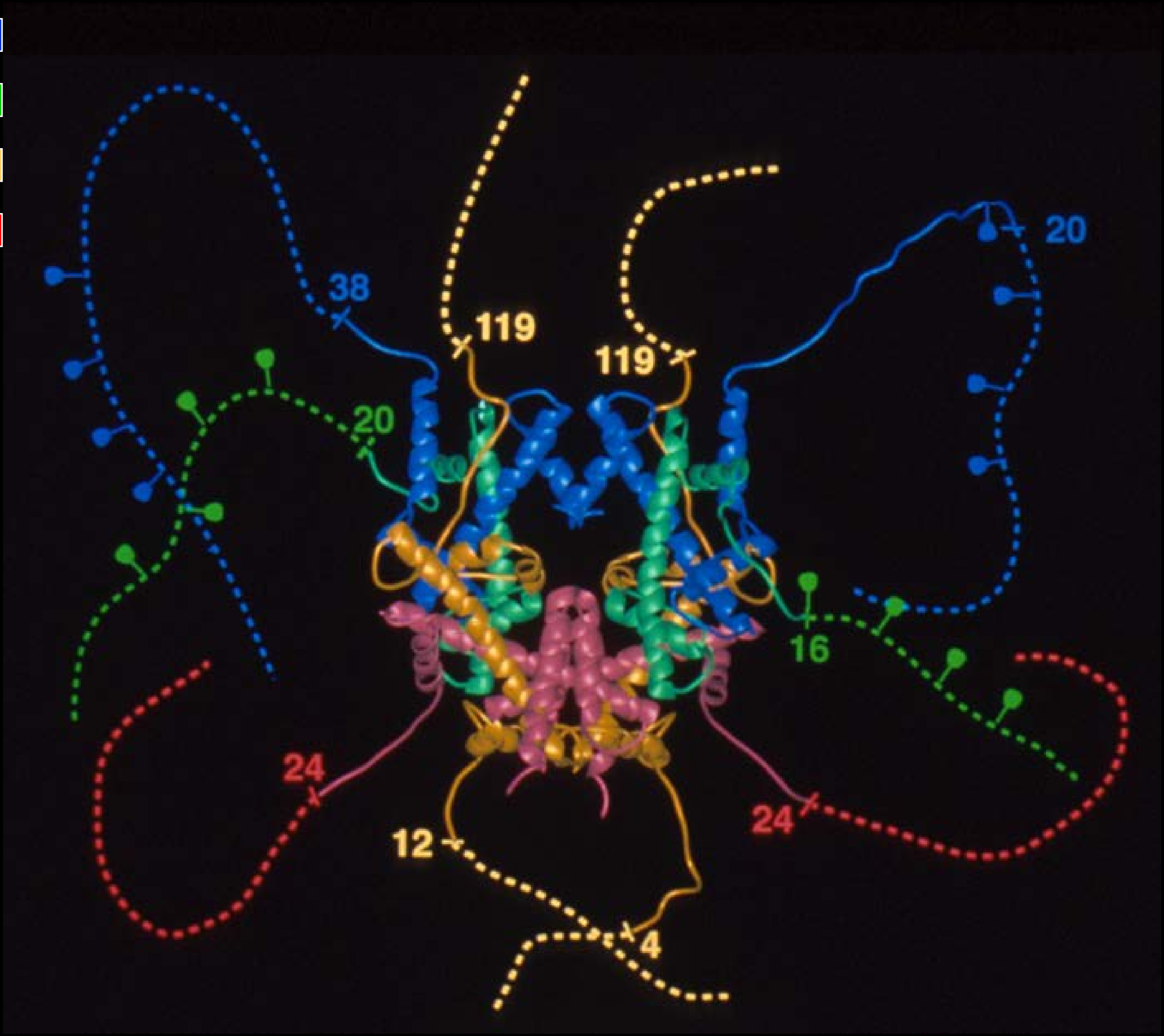
Luger et al, Nature 1997

H3 🟦

H4 🟩

H2A 🟧

H2B 🟥



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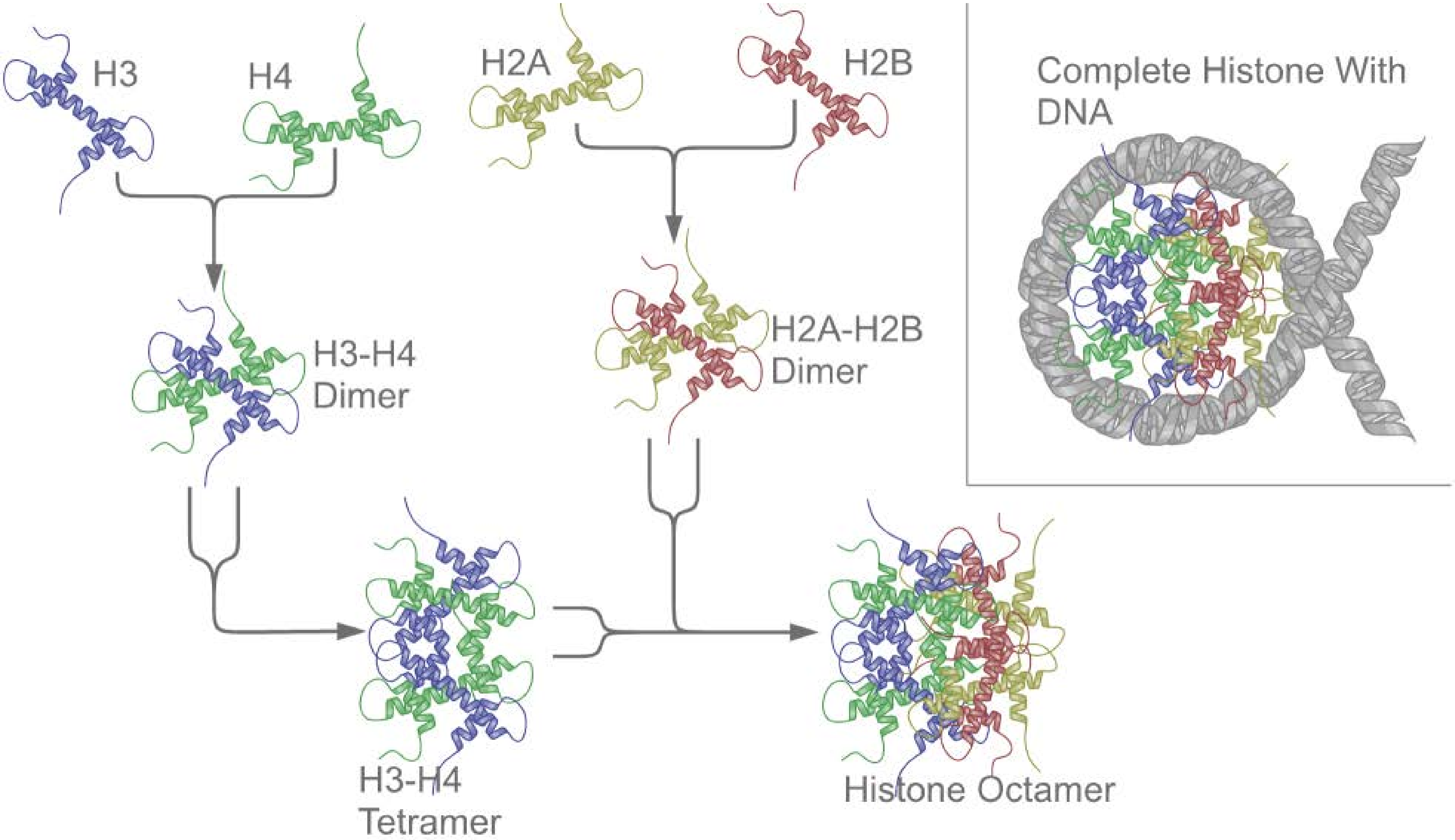
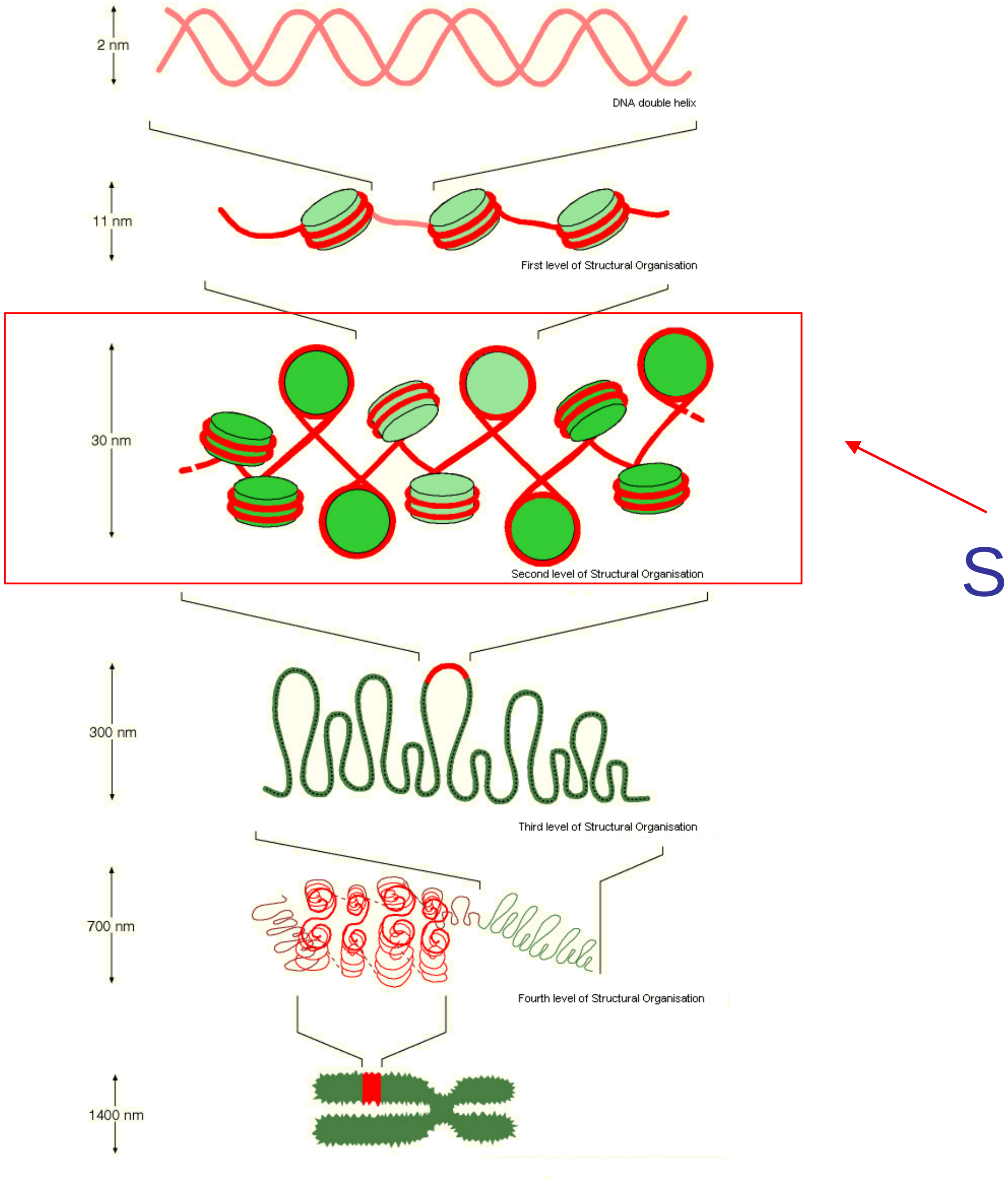
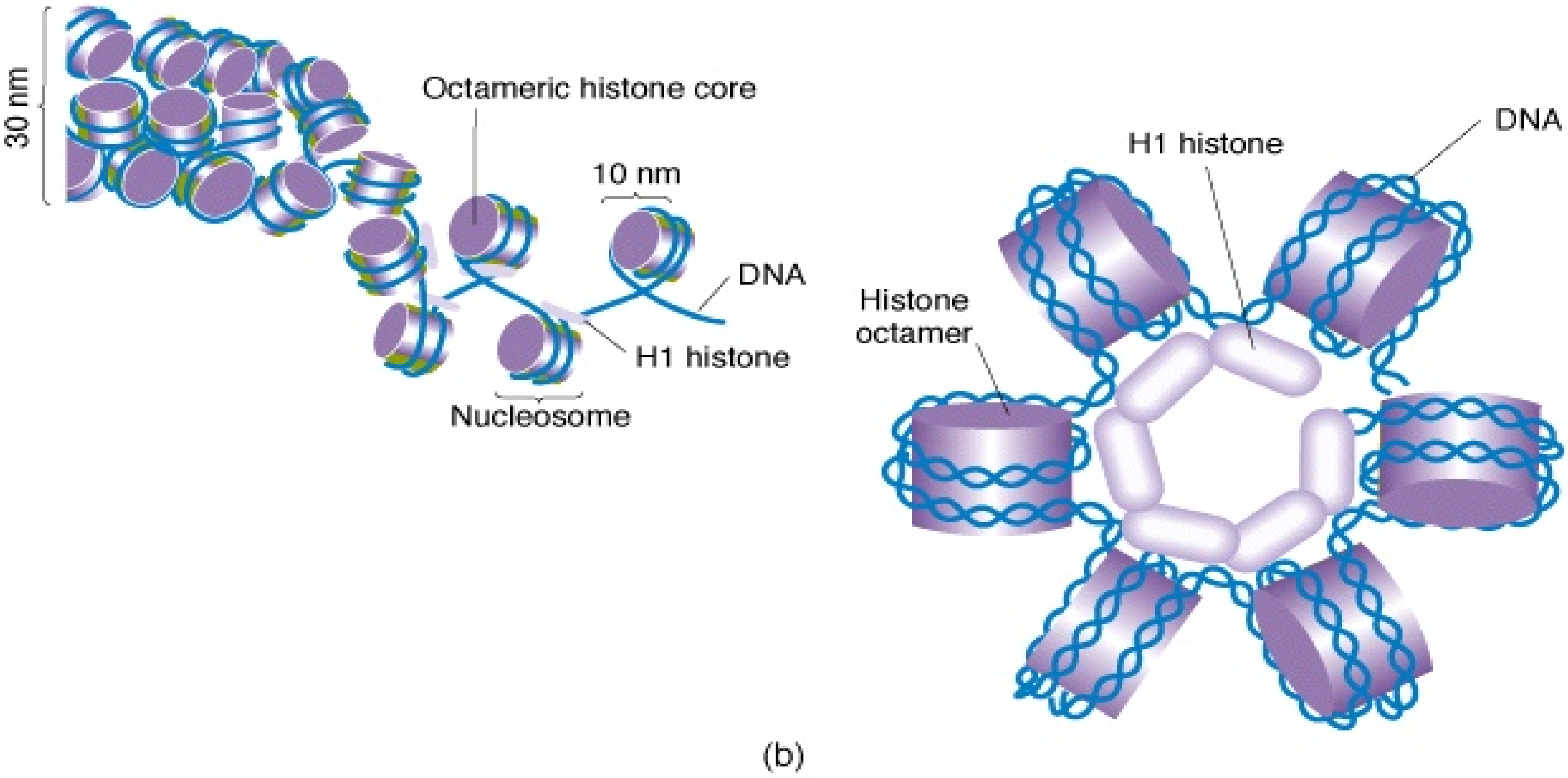

H3
H4
H2A
H2B
Complete Histone With DNA
H3-H4 Dimer
H2A-H2B Dimer
H3-H4 Tetramer
Histone Octamer

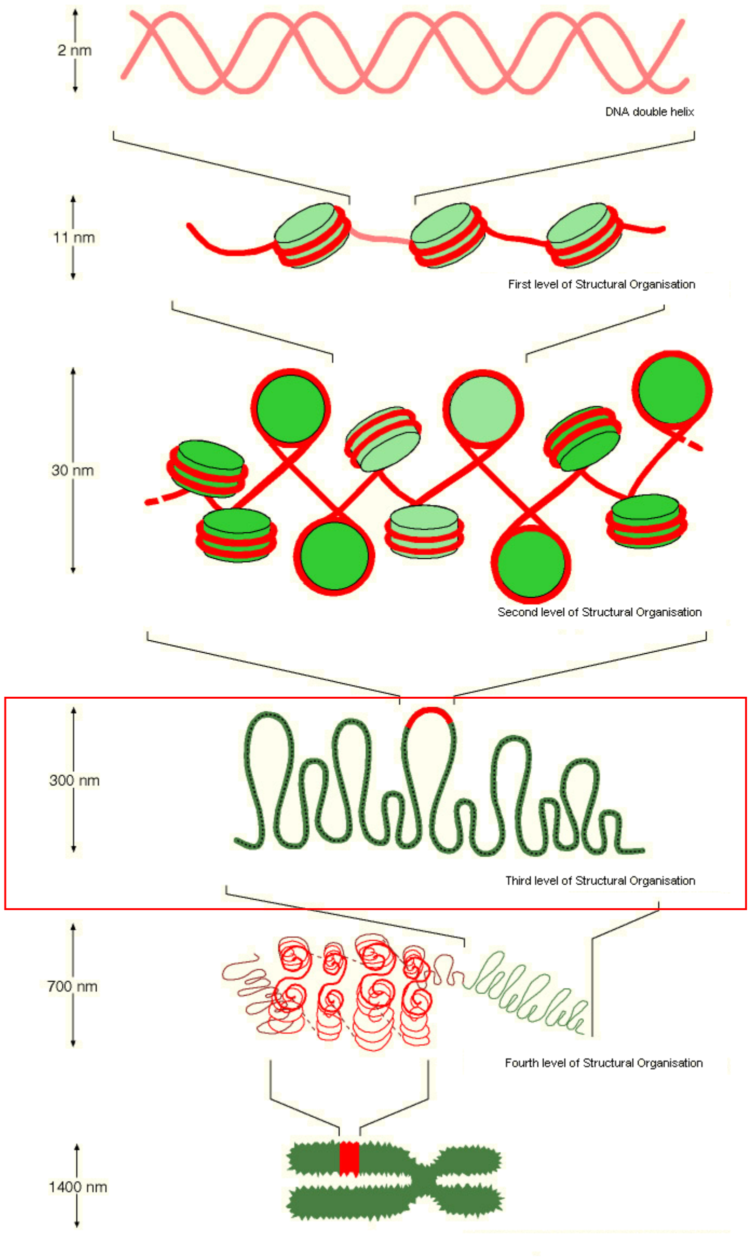


Second order of DNA compaction

Secondary Structure

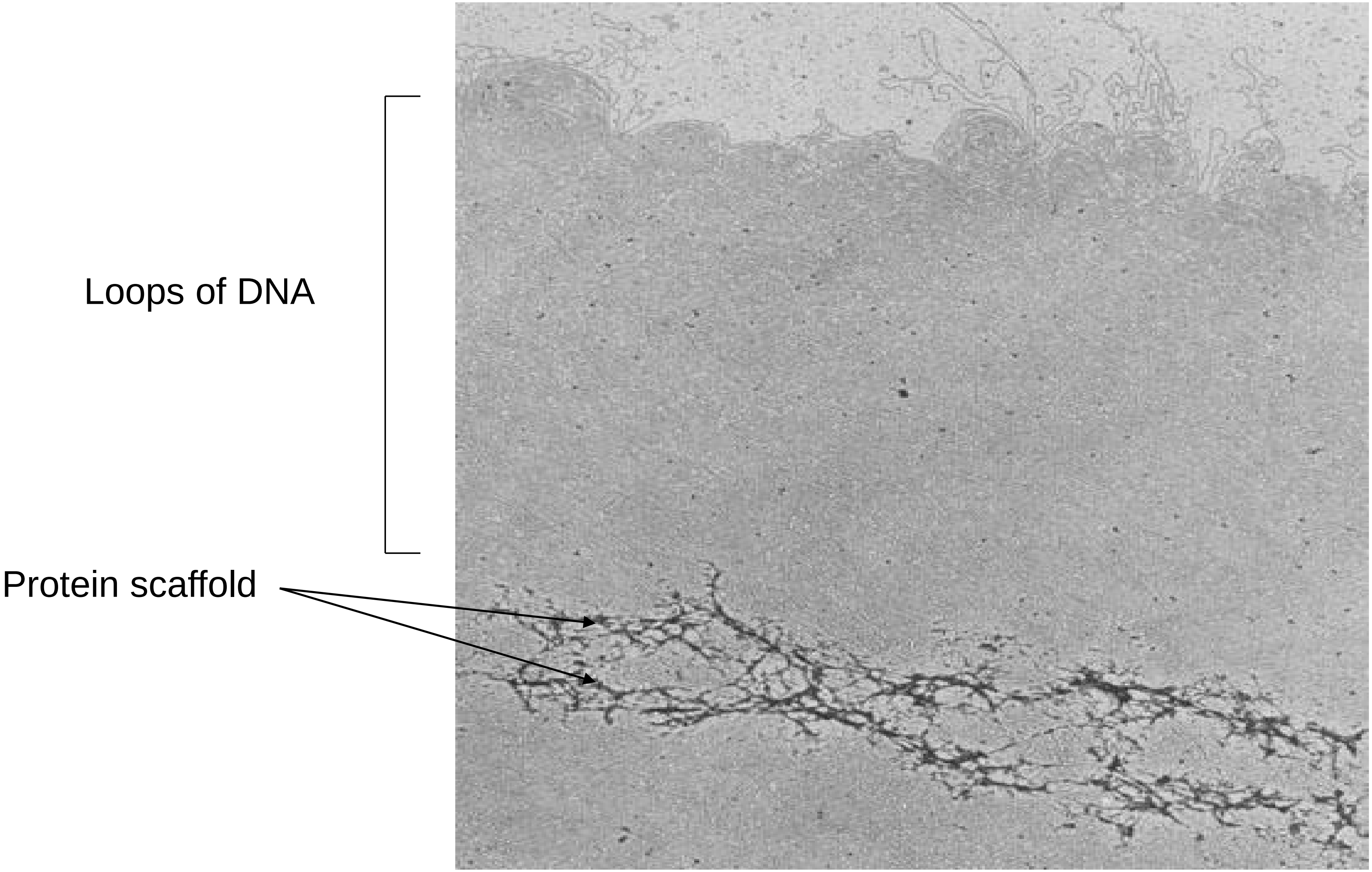
- H1 : essential for the solenoid structure



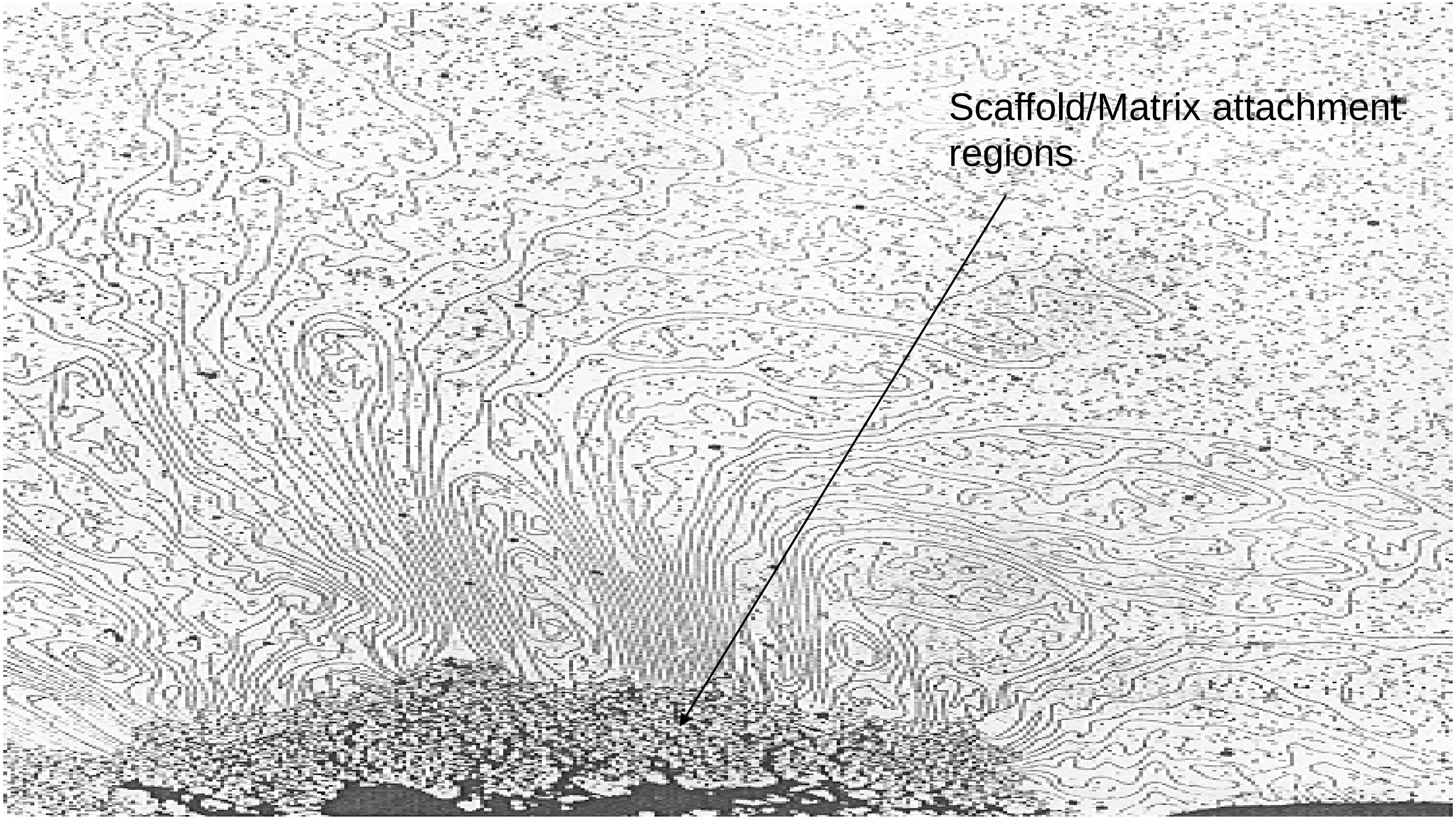


Third order of DNA compaction

Histone-depleted metaphase chromosome



Histone-depleted metaphase chromosome



A condensed metaphase human chromosome

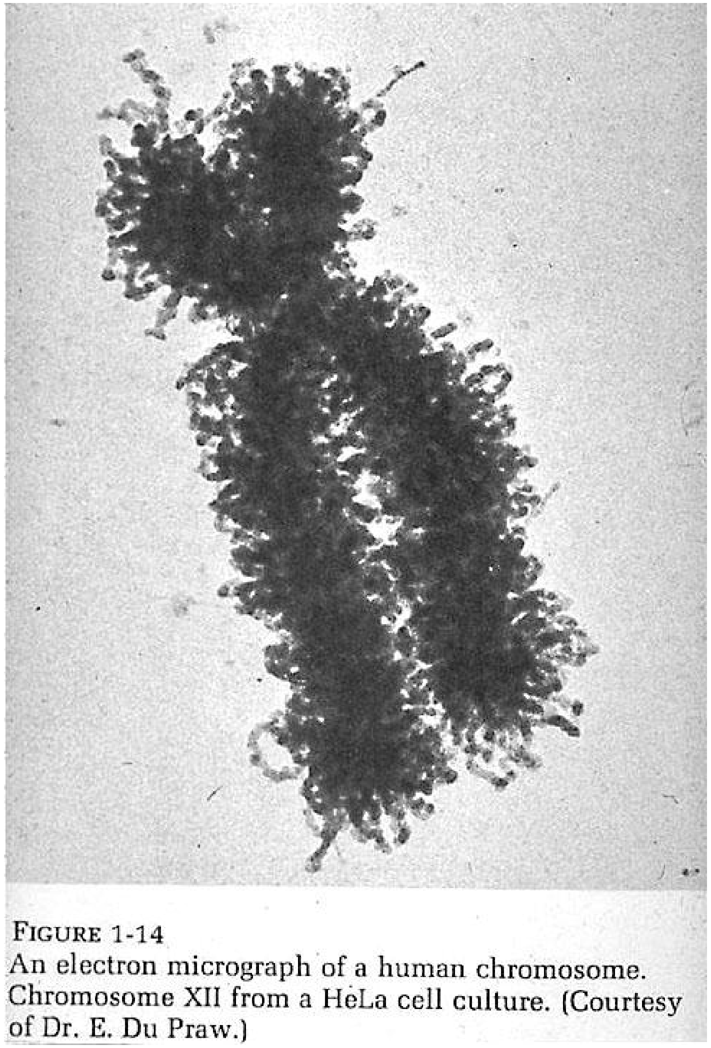
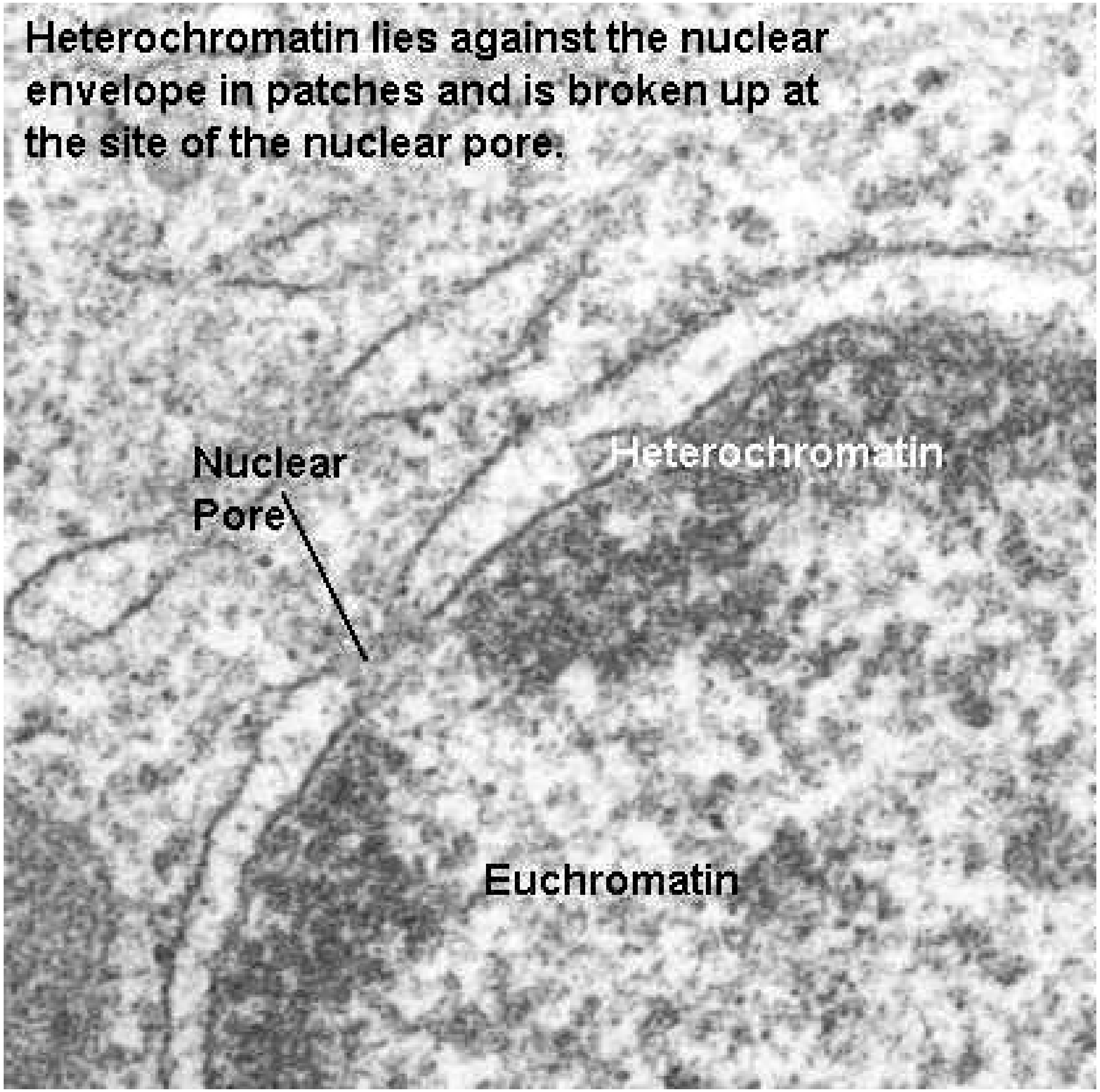


FIGURE 1-14
An electron micrograph of a human chromosome. Chromosome XII from a HeLa cell culture. (Courtesy of Dr. E. Du Praw.)

Genome architecture: chromatin domains



Heterochromatin vs. Euchromatin

• Highly condensed	• Decondensed
• Repetitive sequences	• Single copy sequences (genes)
• Replicates later in the cell cycle	• Replicates early in the cell cycle
• Transcriptionally OFF	• Transcriptionally ON

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Molecular mechanisms that influence chromatin structure and function

1. Chromatin remodeling complexes (e.g. Swi/Snf)
2. Histone modifications
3. Histone variants (e.g. H2A.Z, CENP-A, etc.)
4. DNA methylation

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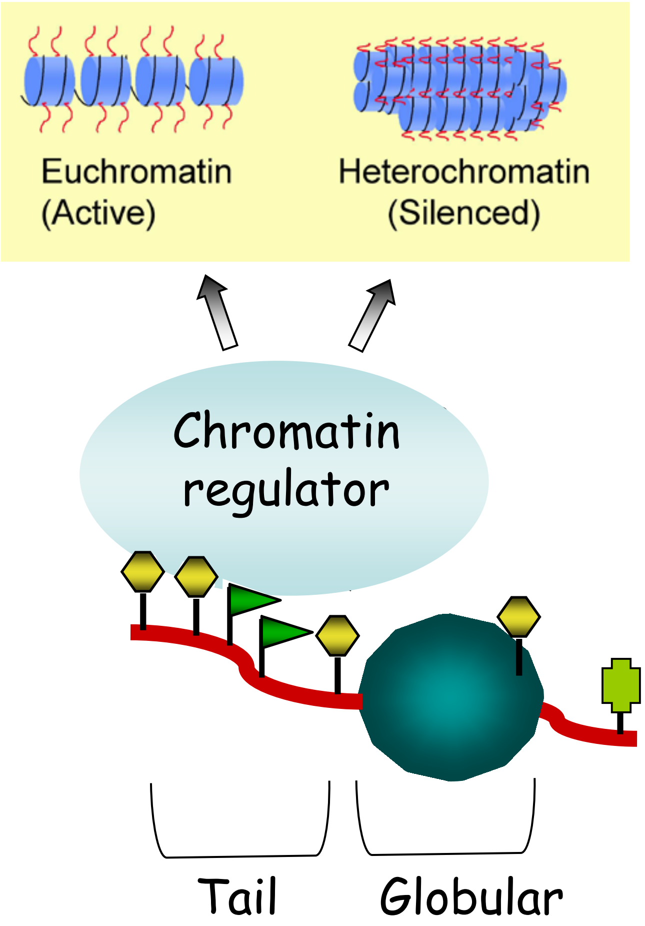
Histone Modifications

- Acetylation
- Phosphorylation
- Methylation
- ADP-ribosylation
- Ubiquitination
- Sumoylation



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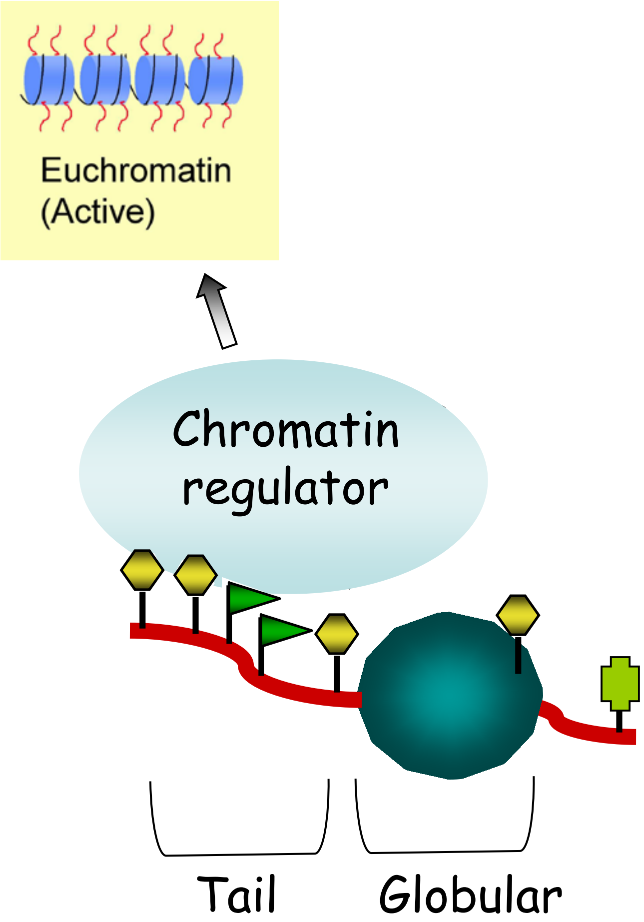
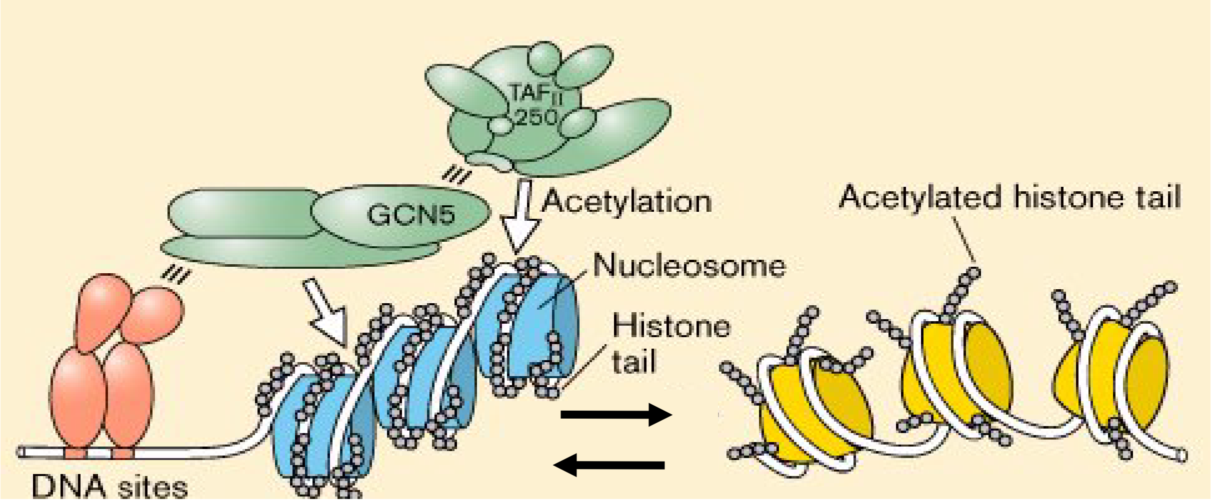


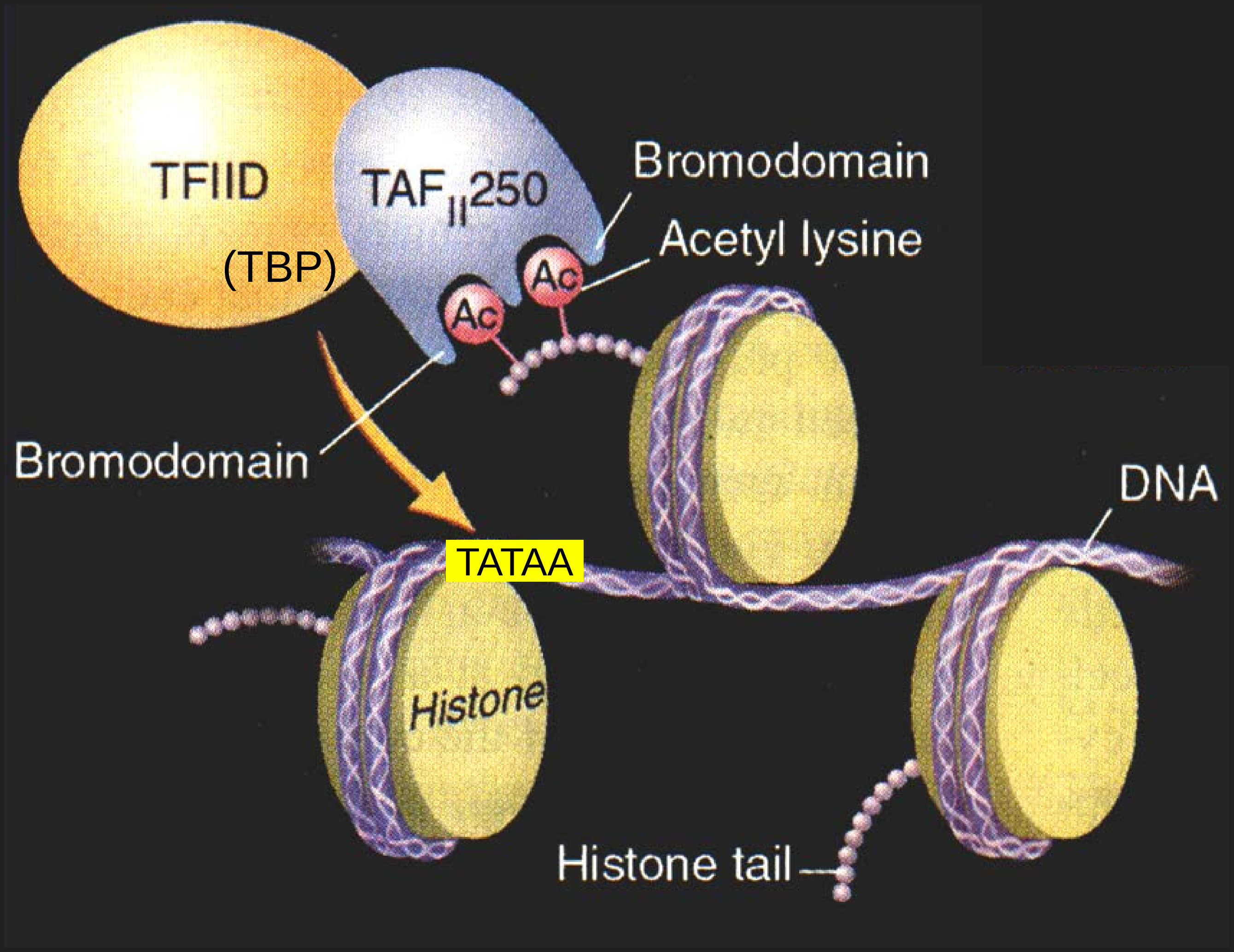
Histone acetylation and chromatin structure



"Off" **"On"**

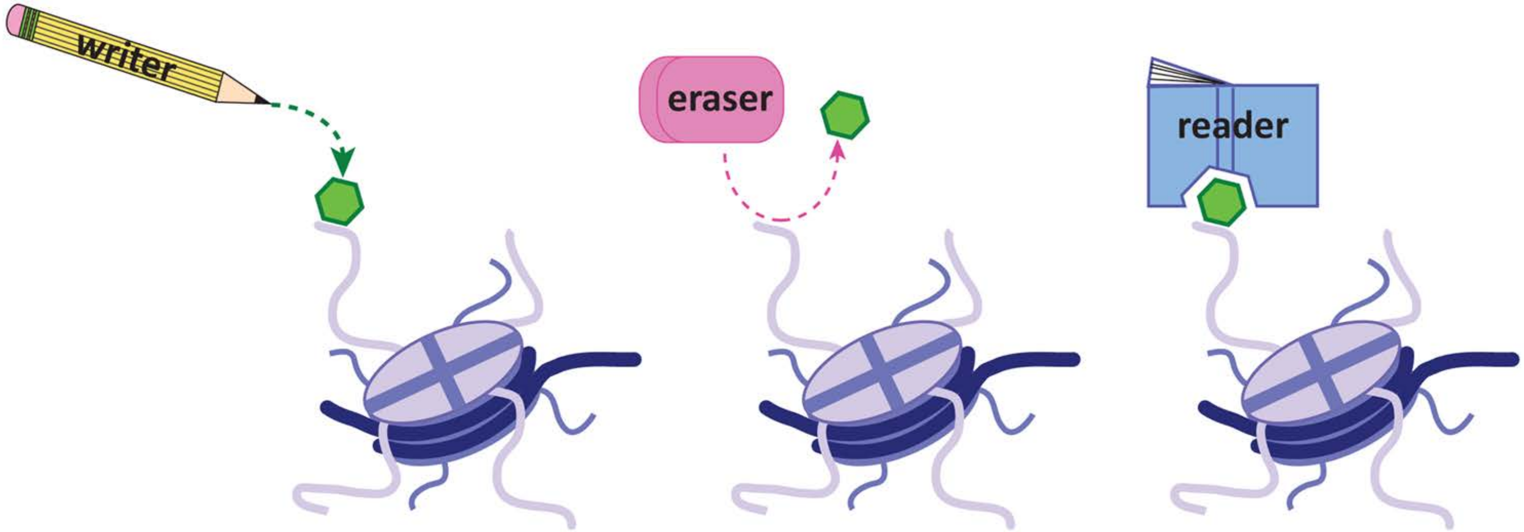
(Adapted from Wade & Wolffe - Current Biology, 1997)

Bromodomain-containing proteins can bind to acetylated histones



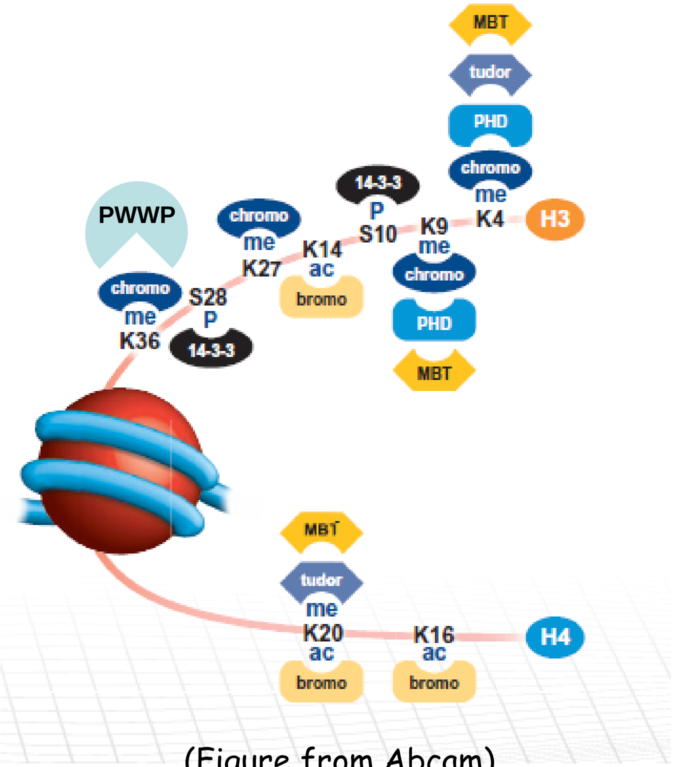
(Taken from E. Pennisi - Science, 2000)

Epigenetic 'Toolkit'



Gardner, Allis & Strahl (2011) OPERating ON chromatin, a colorful language where context matters. *J. Mol. Biol*. **409**:36-46.

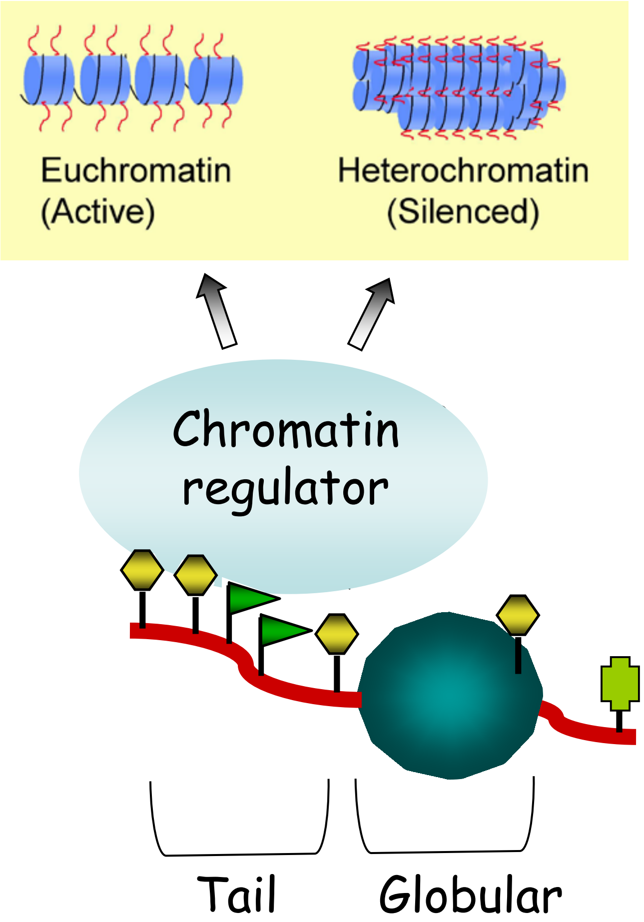
Histone Code ‘readers’



(Figure from Abcam)

Histone Modifications

- Acetylation
- Phosphorylation
- **Methylation**
- ADP-ribosylation
- Ubiquitination
- Sumoylation



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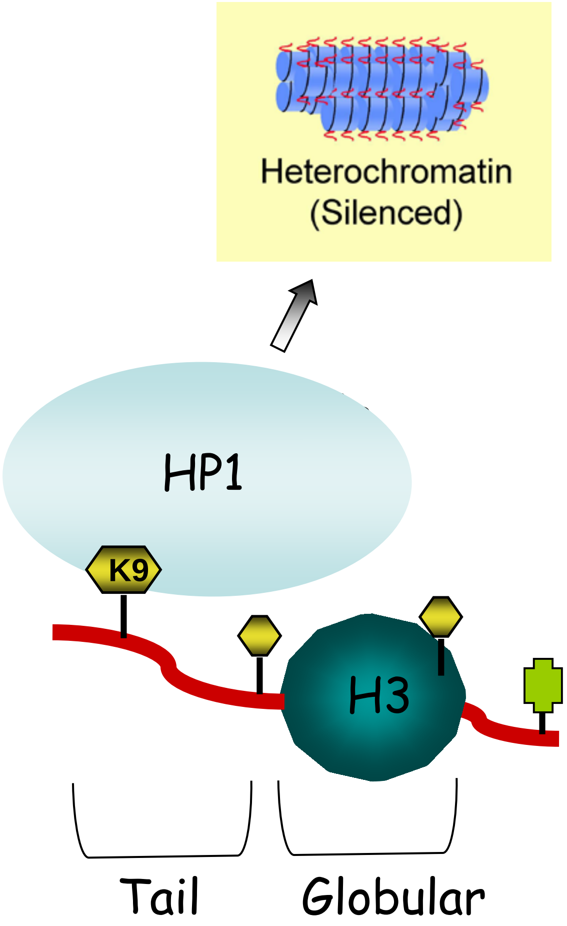
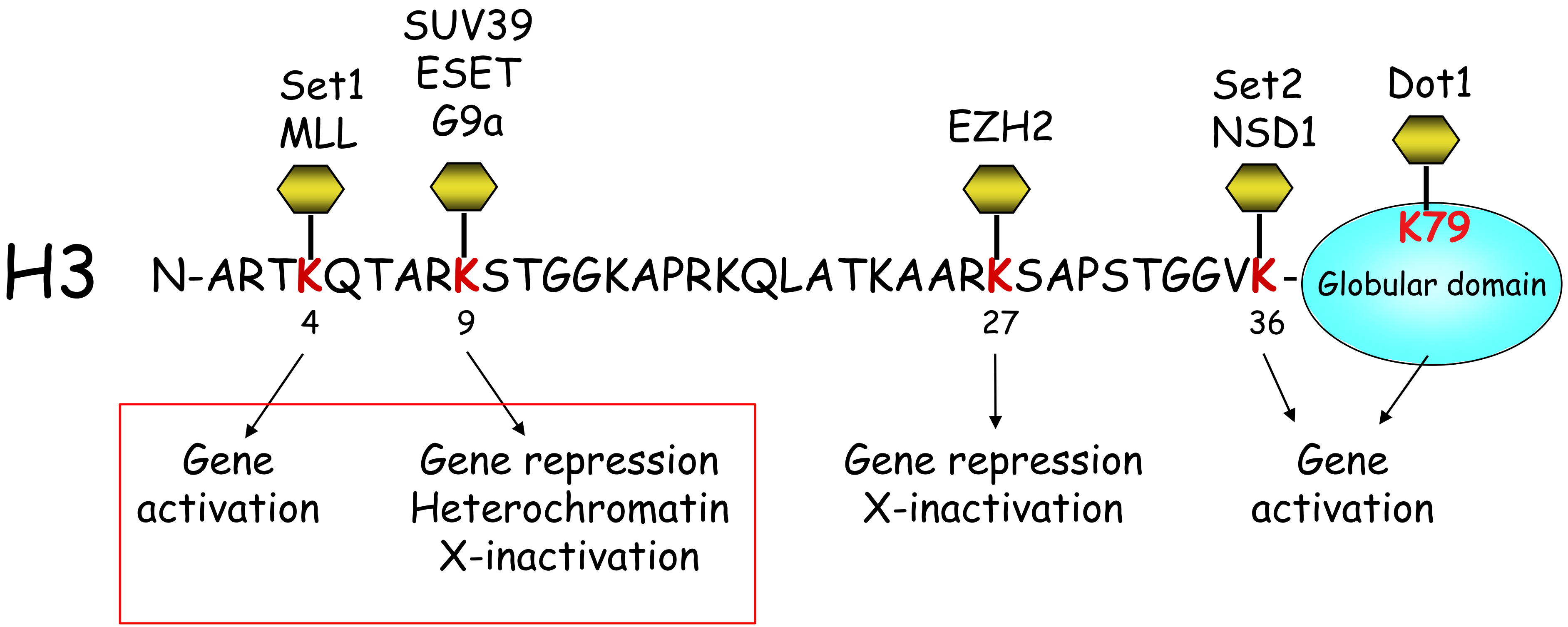


Histone H3 methylation



Set1
MLL

SUV39
ESET
G9a

EZH2

Set2
NSD1

Dot1

H3 N-ART**K**QTAR**K**STGGKAPRKQLATKAAR**K**SAPSTGGV**K**- Globular domain **K79**

4 9 27 36

Gene
activation

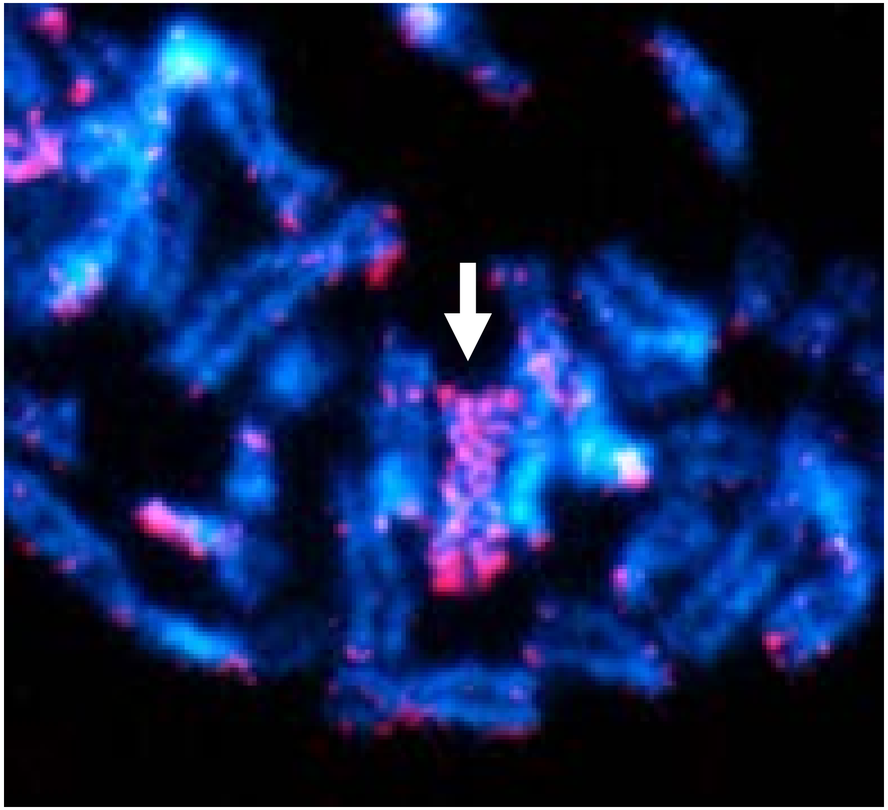
Gene repression
Heterochromatin
X-inactivation

Gene repression
X-inactivation

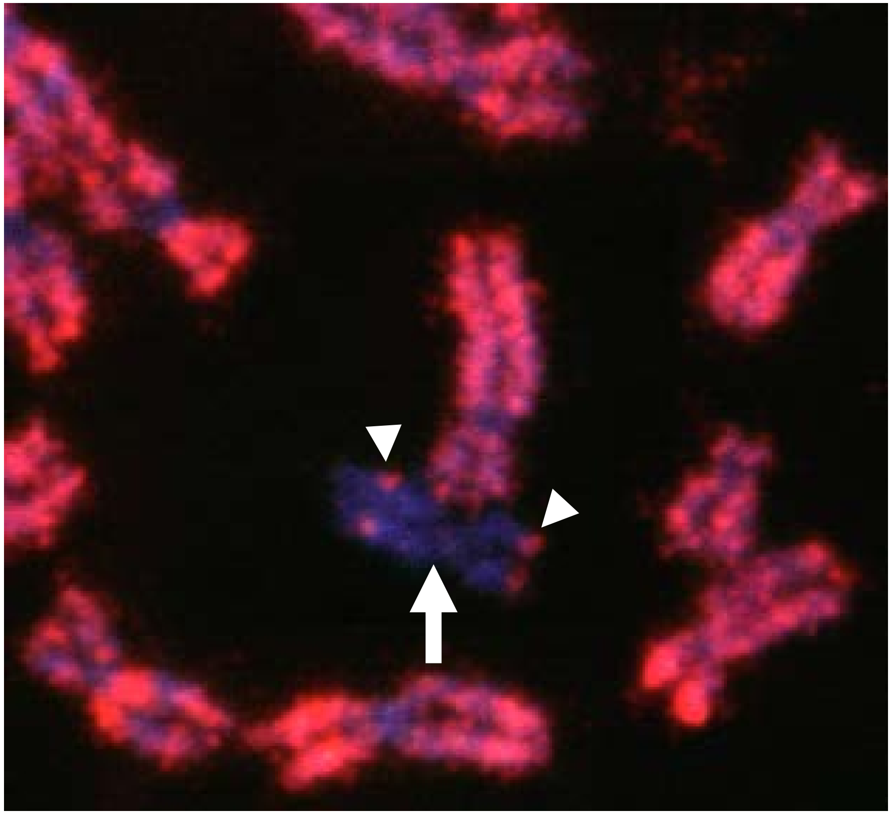
Gene
activation

Staining of female metaphase chromosomes with site-specific methyl H3 antibodies

methyl (**Lys 9**) H3



methyl (**Lys 4**) H3

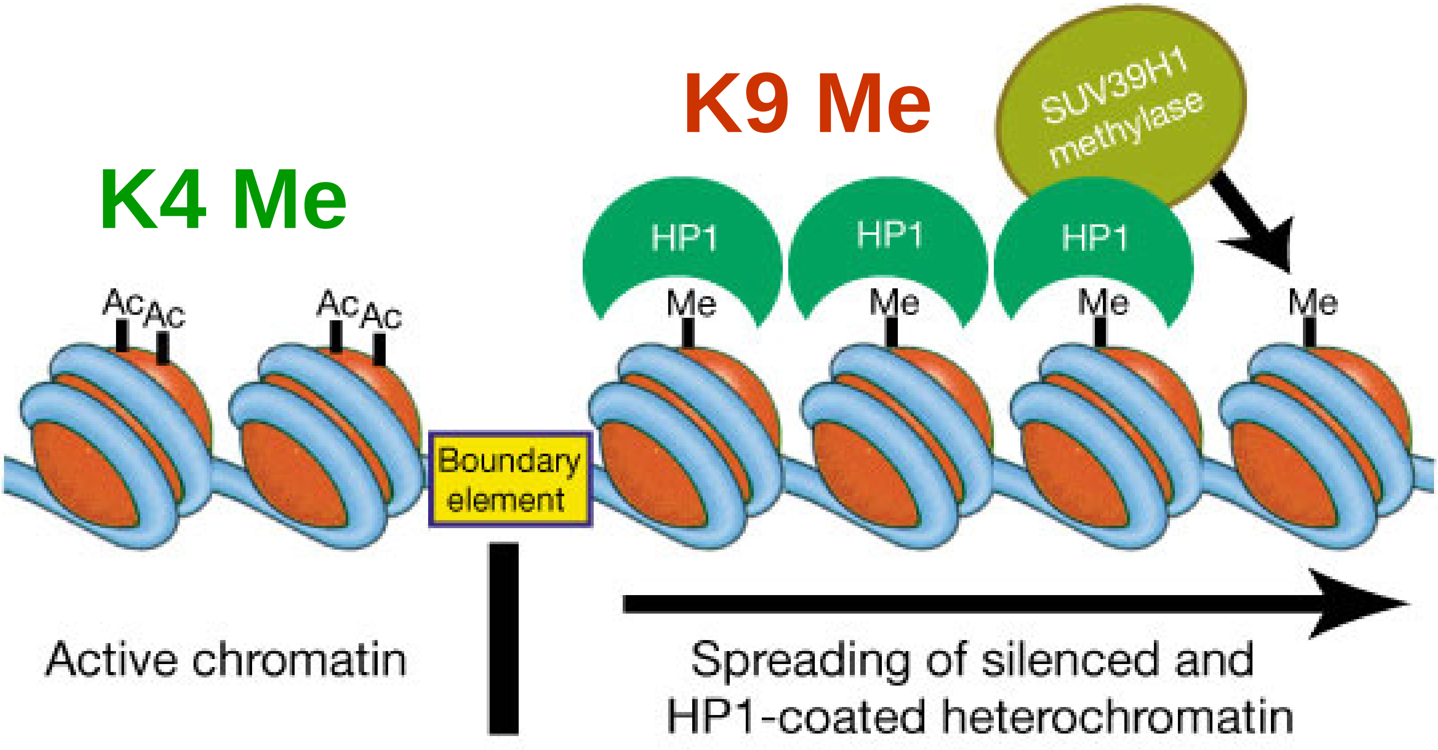


(Taken from Boggs BA et al. - Nat Genet., 2002)

Roles of H3 lysines 4 and 9 methylation

K9 Me

K4 Me

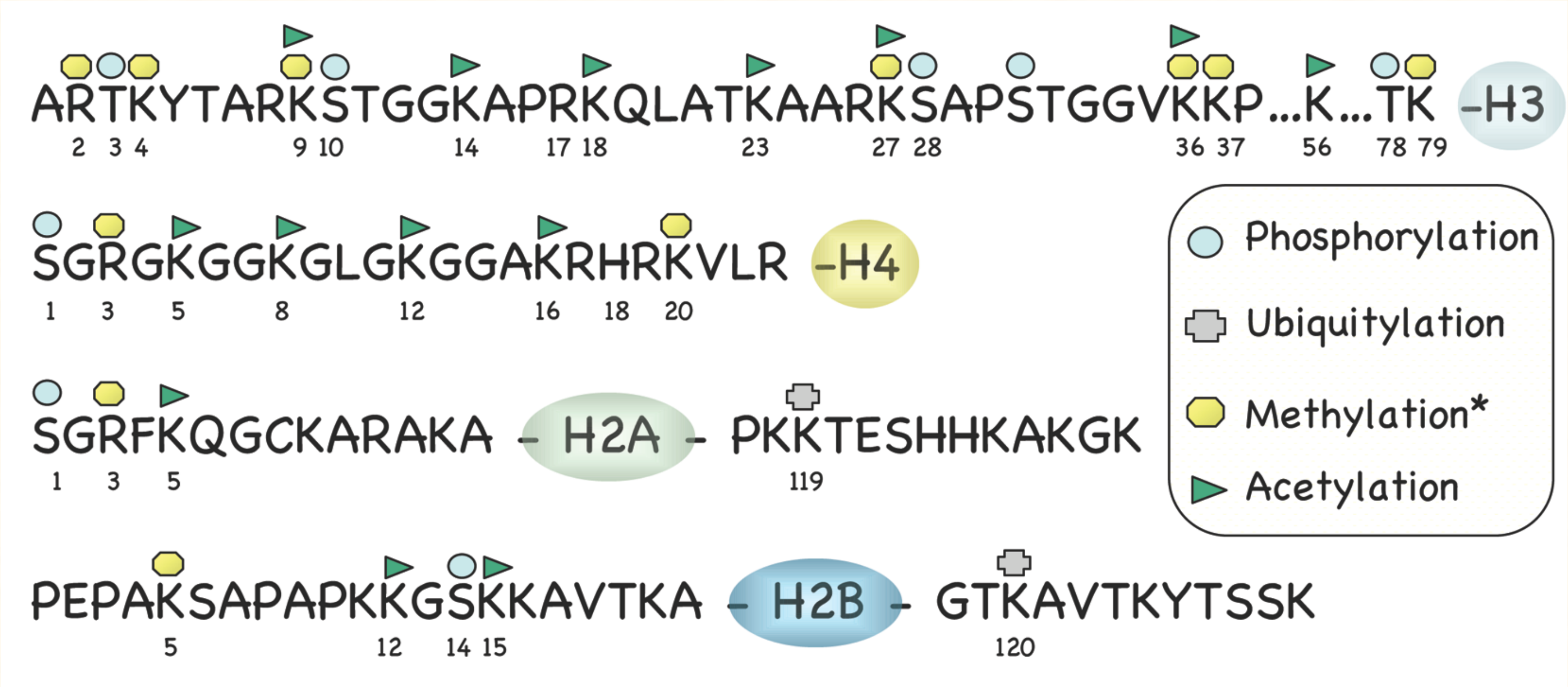


On

Off

(Taken from Bannister et al. - Nature, 2001)

Post-translational modifications decorate histones



Molecular mechanisms that influence chromatin structure and function

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2. Histone modifications
3. **Histone variants (e.g. H2A.Z, CENP-A, etc.)**
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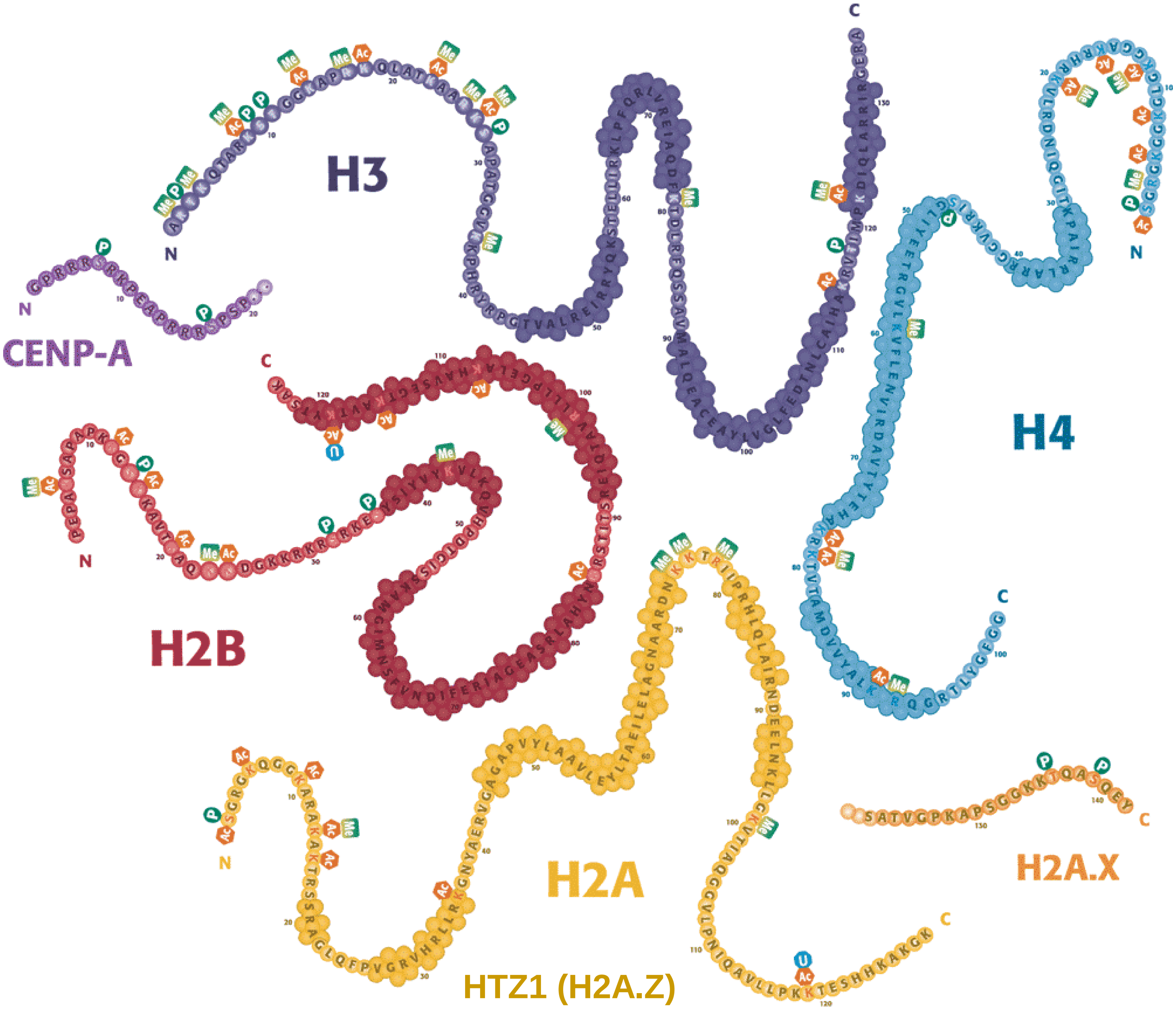


Figure from Millipore/Upstate

Histone Variants

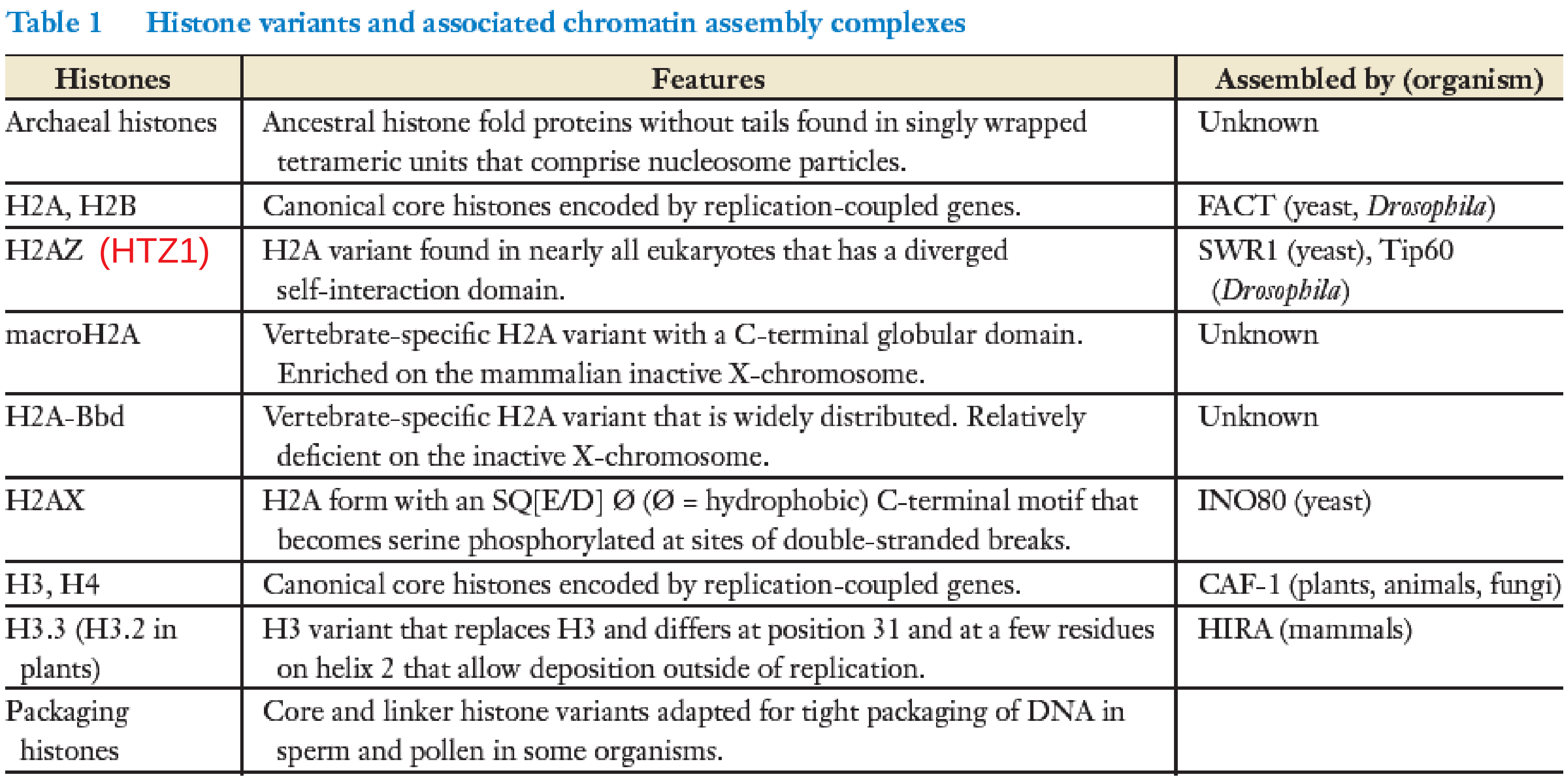


Table 1 Histone variants and associated chromatin assembly complexes

Histones	Features	Assembled by (organism)
Archaeal histones	Ancestral histone fold proteins without tails found in singly wrapped tetrameric units that comprise nucleosome particles.	Unknown
H2A, H2B	Canonical core histones encoded by replication-coupled genes.	FACT (yeast, *Drosophila*)
H2AZ (HTZ1)	H2A variant found in nearly all eukaryotes that has a diverged self-interaction domain.	SWR1 (yeast), Tip60 (*Drosophila*)
macroH2A	Vertebrate-specific H2A variant with a C-terminal globular domain. Enriched on the mammalian inactive X-chromosome.	Unknown
H2A-Bbd	Vertebrate-specific H2A variant that is widely distributed. Relatively deficient on the inactive X-chromosome.	Unknown
H2AX	H2A form with an SQ[E/D] Ø (Ø = hydrophobic) C-terminal motif that becomes serine phosphorylated at sites of double-stranded breaks.	INO80 (yeast)
H3, H4	Canonical core histones encoded by replication-coupled genes.	CAF-1 (plants, animals, fungi)
H3.3 (H3.2 in plants)	H3 variant that replaces H3 and differs at position 31 and at a few residues on helix 2 that allow deposition outside of replication.	HIRA (mammals)
Packaging histones	Core and linker histone variants adapted for tight packaging of DNA in sperm and pollen in some organisms.	

(Table from Henikoff and Ahmad, Annu. Rev. Cell Dev. Biol, 2005)

Molecular mechanisms that influence chromatin structure and function

1. Chromatin remodeling complexes (e.g. Swi/Snf)
2. Histone modifications
3. Histone variants (e.g. H2A.Z, CENP-A, etc.)
4. **DNA methylation**

DNA methylation

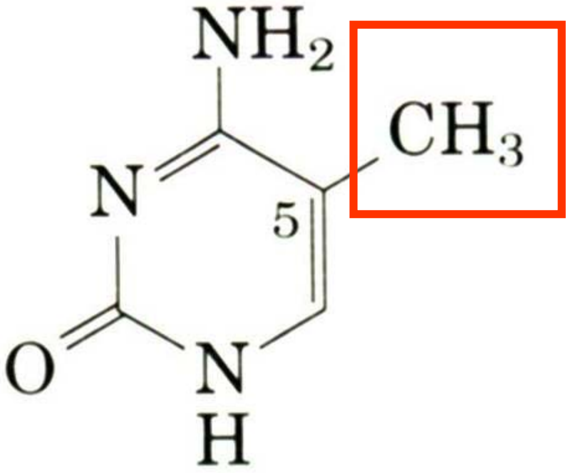
Occurs in:

(1) select organisms and (2) usually at CpG dinucleotide residues

1. Organisms found in:

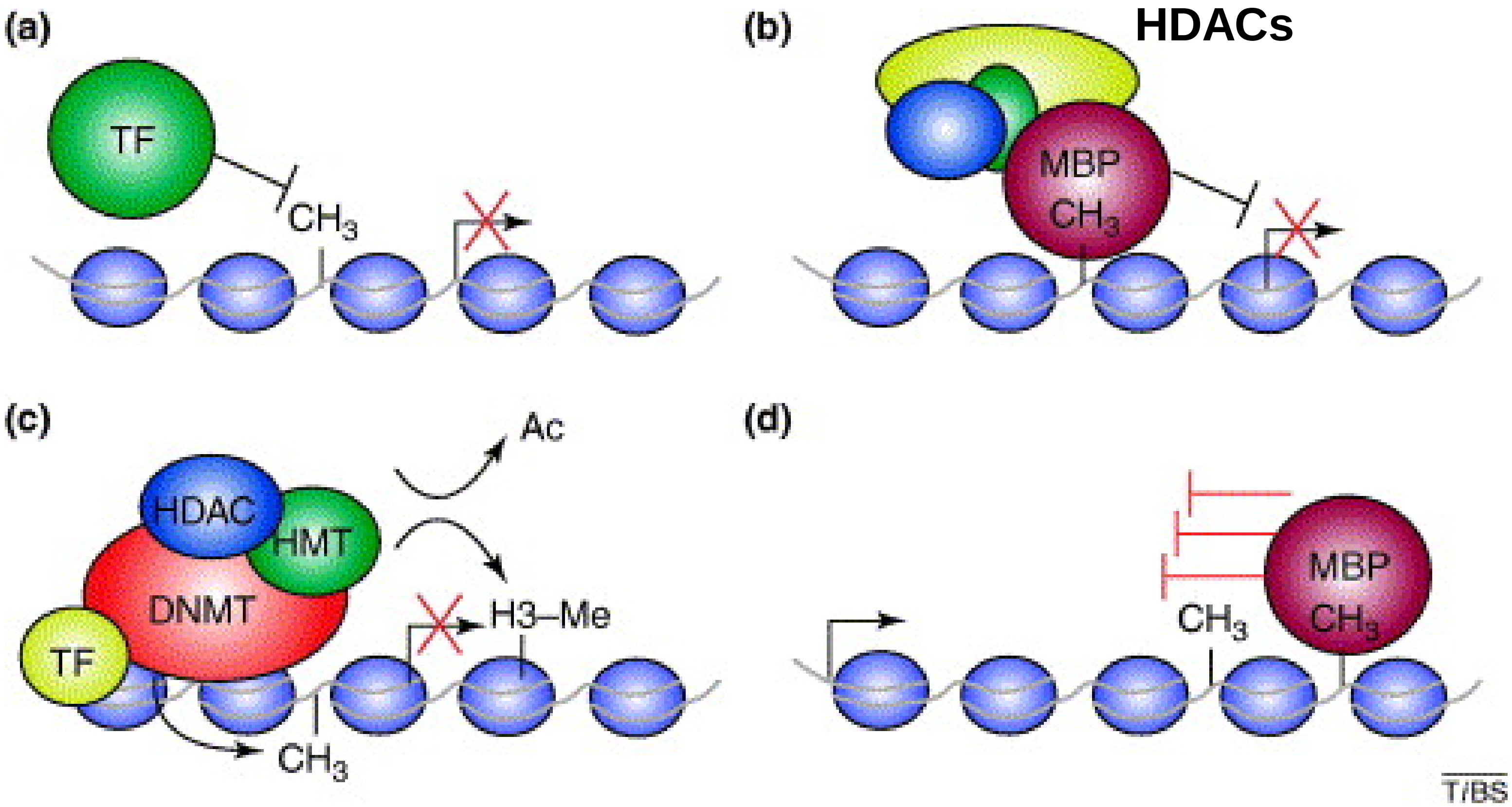
Humans
Mice
Frogs
Flies*(low levels and CpT)

2. Occurs on Cytosine:



5-Methylcytosine

How DNA methylation regulates gene repression?



A) By sterically blocking the binding of transcription factors (e.g. E2F, NF-kB, CTCF
B) & C) By recruiting chromatin modifying activities
D) By affecting RNA Polymerase II transcription

(figure from Klose & Bird, Trends Biochem Sci., 2006)

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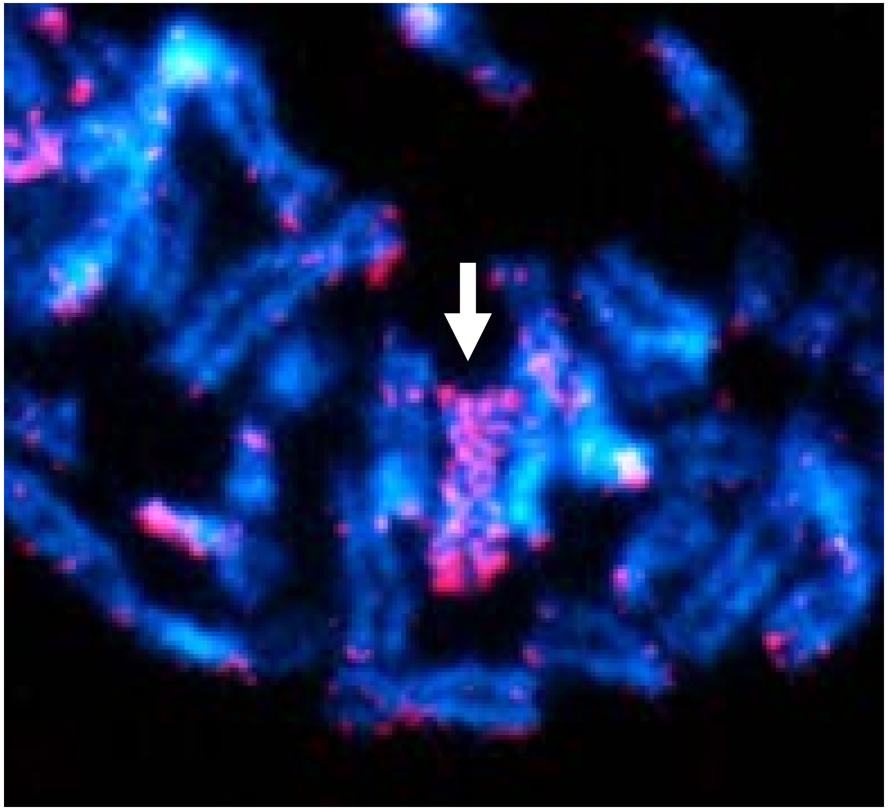
Histone modification-specific antibodies have enabled the study of chromatin!

methyl (**Lys 9**) H3



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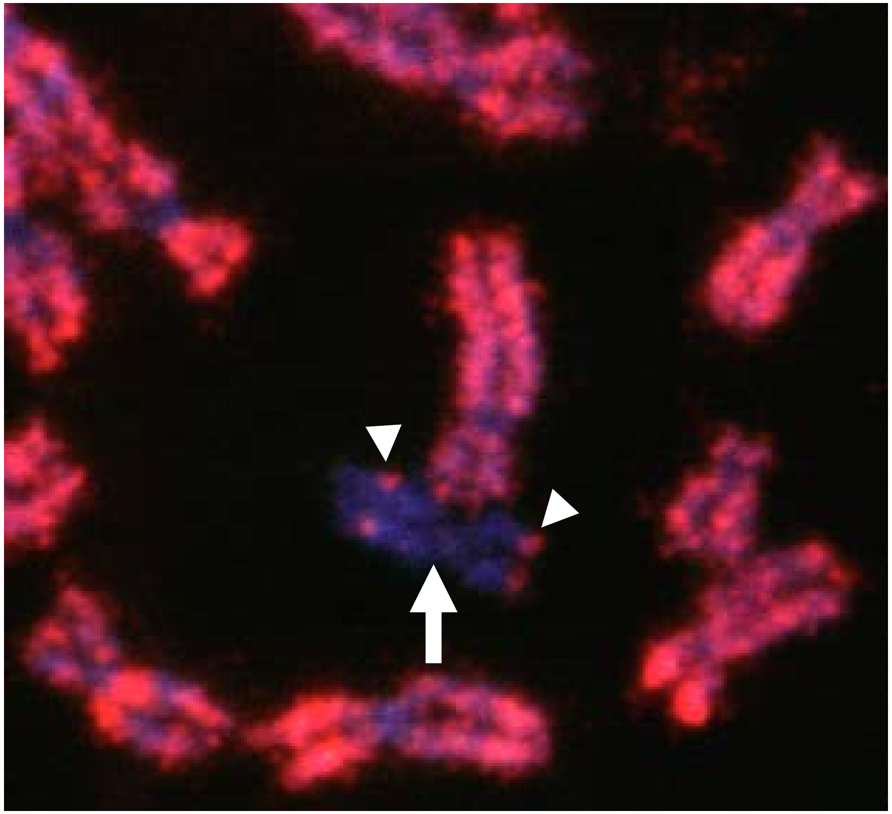
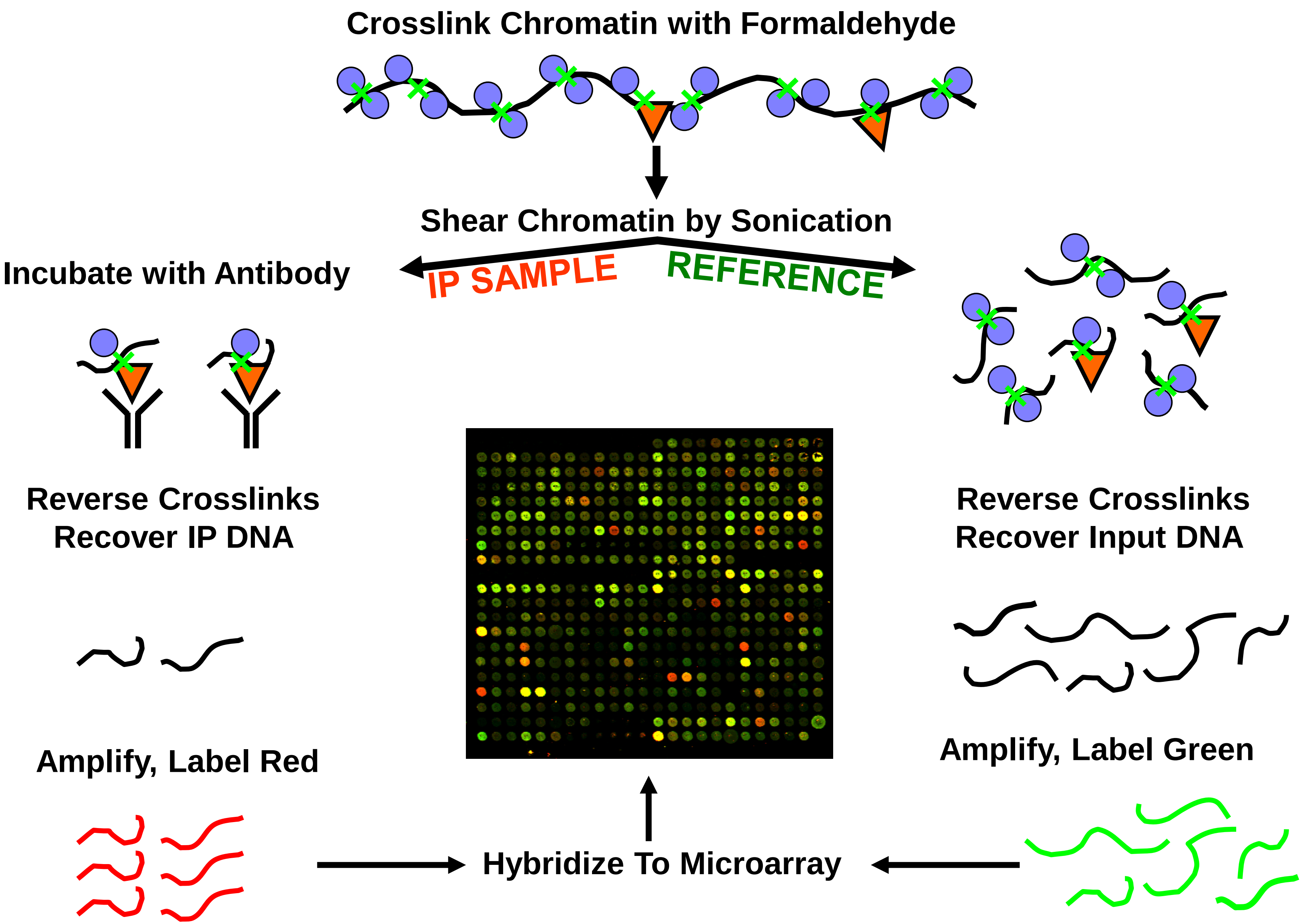


(Taken from Boggs BA et al. - Nat Genet., 2002)

The ChIP-chip procedure



Crosslink Chromatin with Formaldehyde

Shear Chromatin by Sonication

Incubate with Antibody

Reverse Crosslinks
Recover IP DNA

Reverse Crosslinks
Recover Input DNA

Amplify, Label Red

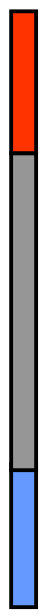
Amplify, Label Green

Hybridize To Microarray

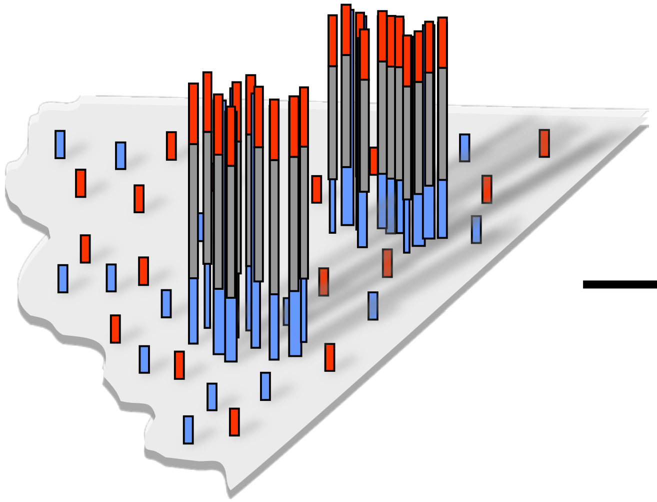
(Provided by Jason Lieb, UNC)

Solexa Sequencing (Illumina)

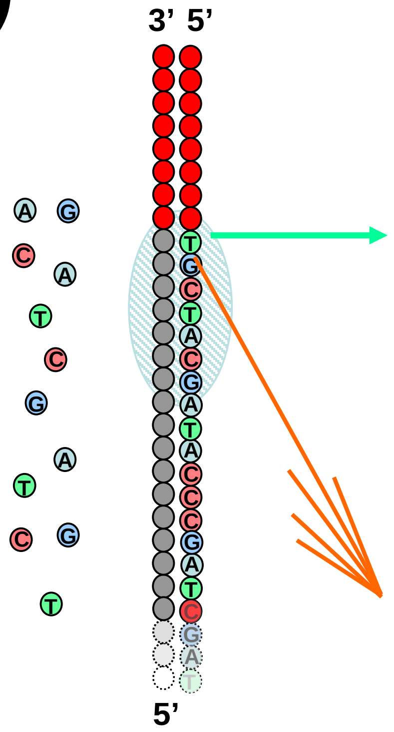
DNA
(0.1-1.0 ug)



Sample preparation



Cluster growth



Sequencing

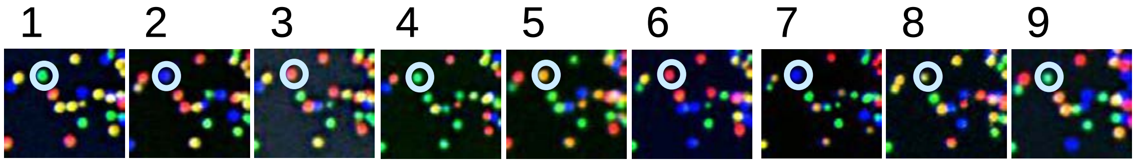
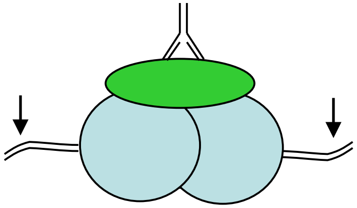


Image acquisition

Base calling

ChIP-Seq

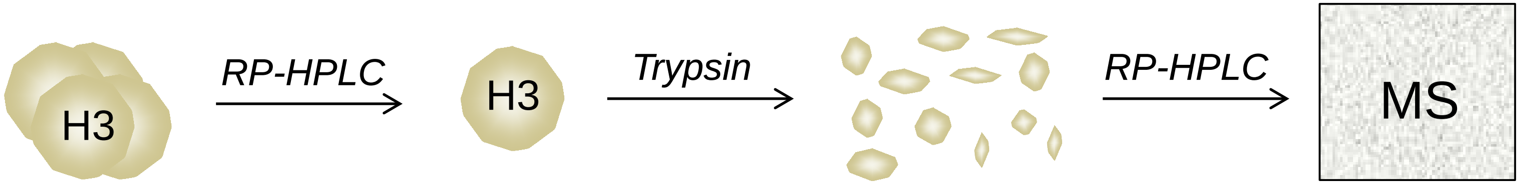
- **Follow standard ChIP procedure**
- **Identify uniquely aligned sequences in human genome**



Enriched by ChIP

Mass spectrometry is a vital tool in combinatorial PTM discovery

A. Bottom-up MS



H3

RP-HPLC

H3

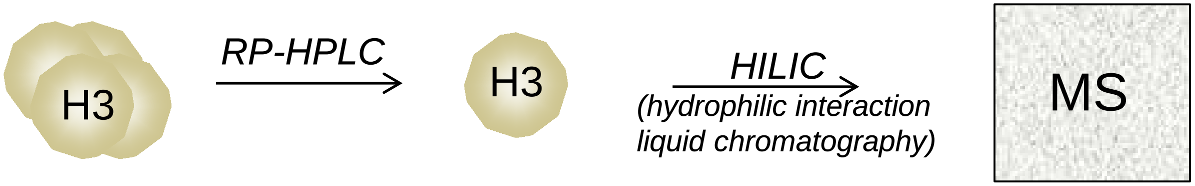
Trypsin

RP-HPLC

MS

ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFQRLVREIA
QDFKTDLRFQSSAVMALQEASEAYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA-134

B. Top-down MS



H3

RP-HPLC

H3

HILIC
(hydrophilic interaction
liquid chromatography)

MS

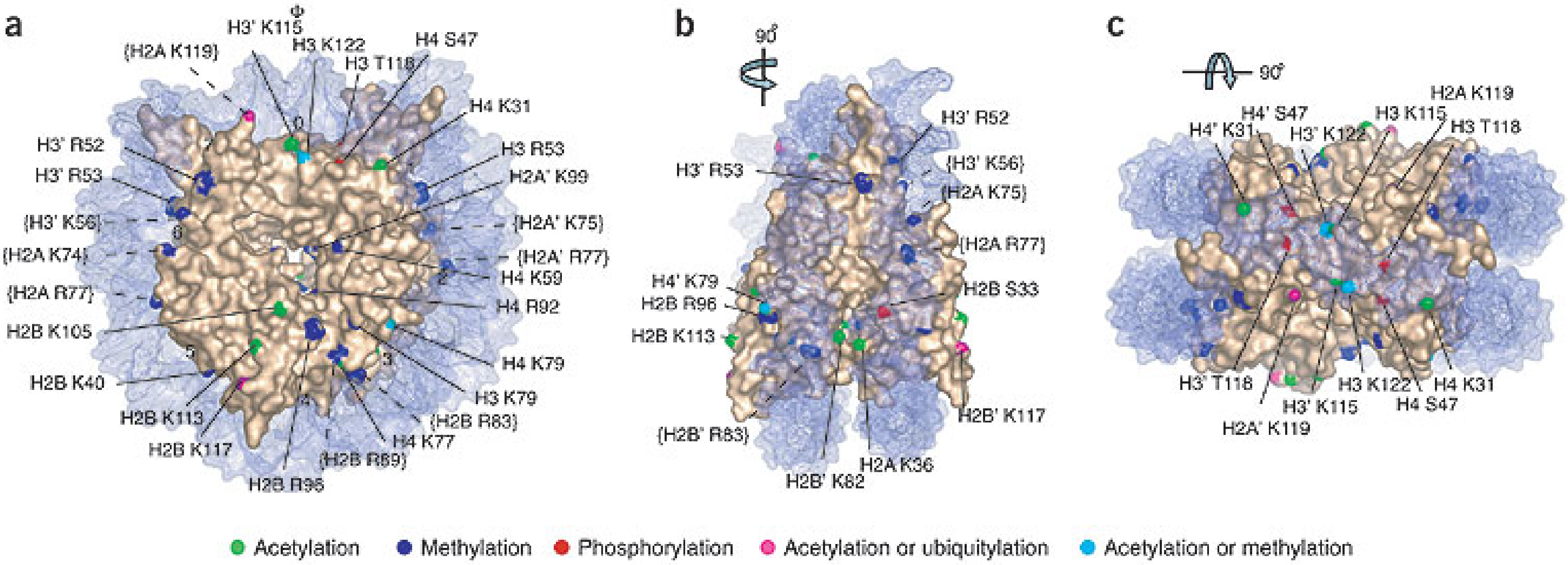
ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFQRLVREIA
QDFKTDLRFQSSAVMALQEASEAYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA-134

Mass Spectrometry technologies have revealed novel histone ‘marks’ and specific histone codes

nature
structural &
molecular biology

Regulated nucleosome mobility and the histone code

Michael S Cosgrove[1], Jef D Boeke[2,3] & Cynthia Wolberger[1,4]



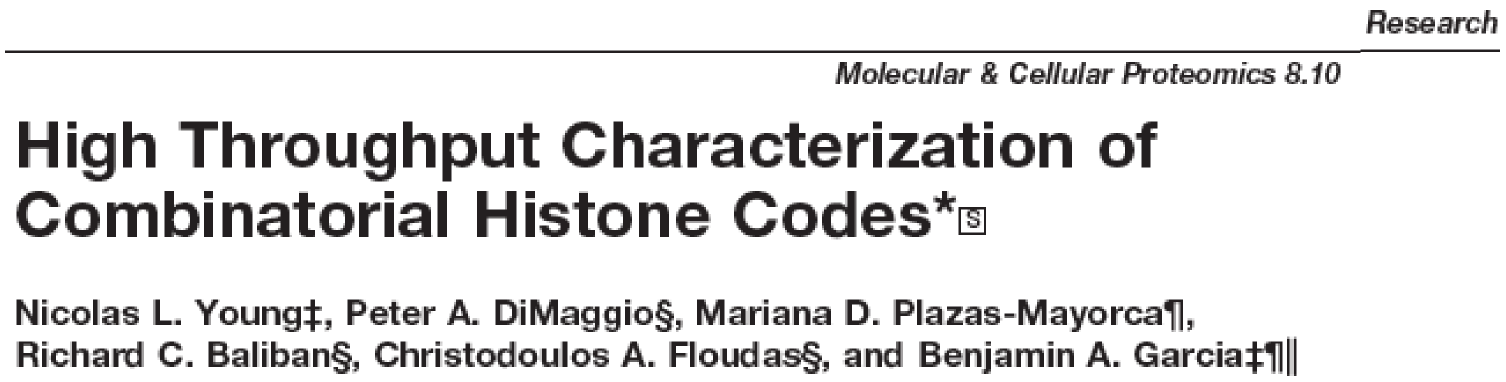
Mass spectrometry is a vital tool in combinatorial PTM discovery

NATURE METHODS | VOL.4 NO.6 | JUNE 2007 |

BRIEF COMMUNICATIONS

Pervasive combinatorial modification of histone H3 in human cells

Benjamin A Garcia[1], James J Pesavento[2],
Craig A Mizzen[1,3] & Neil L Kelleher[1,4]



Research

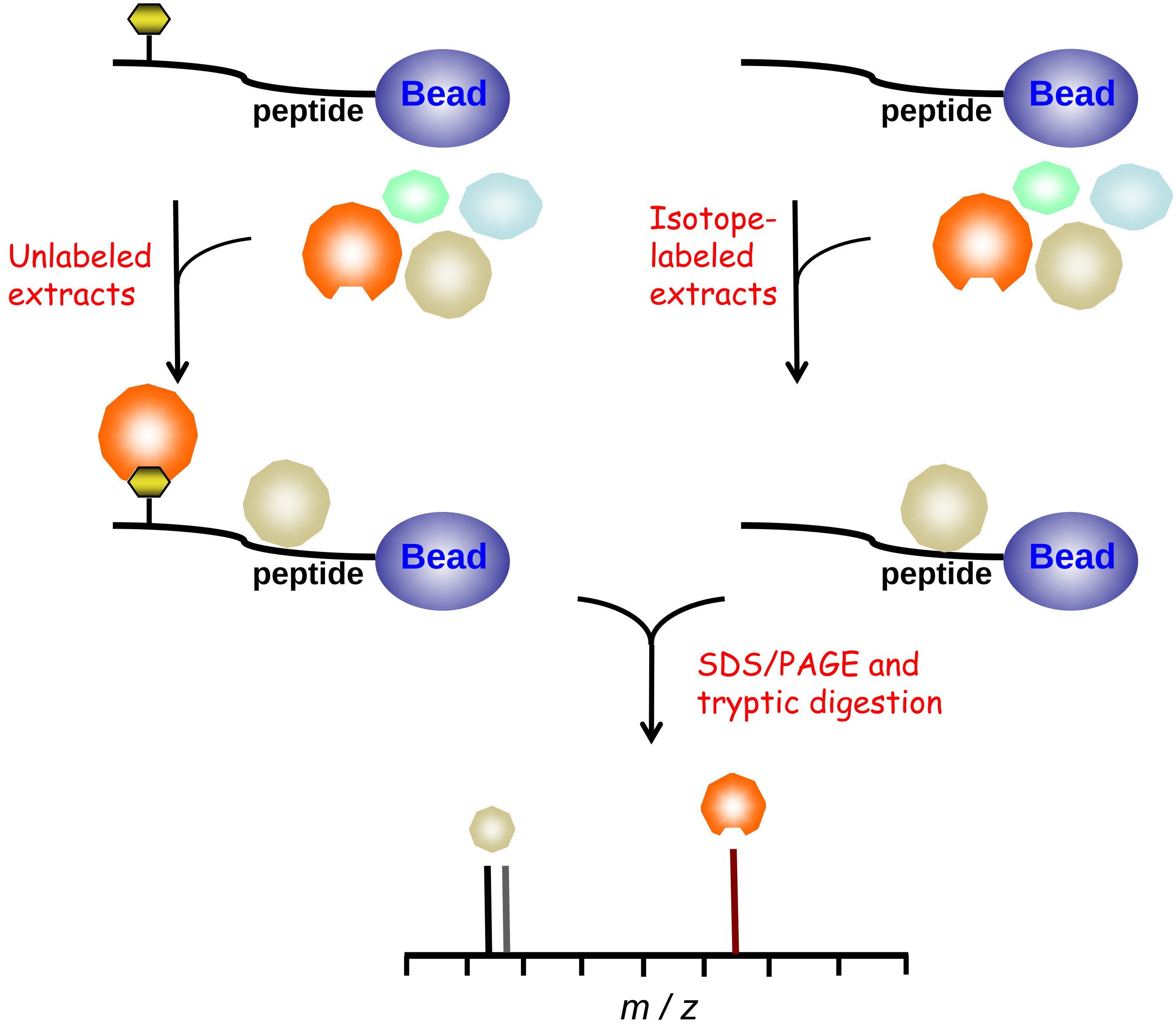
Molecular & Cellular Proteomics 8.10

High Throughput Characterization of Combinatorial Histone Codes*Ⓢ

Nicolas L. Young‡, Peter A. DiMaggio§, Mariana D. Plazas-Mayorca¶,
Richard C. Baliban§, Christodoulos A. Floudas§, and Benjamin A. Garcia‡¶‖

SILAC-based approaches are unlocking identification of novel effector proteins

(Stable isotope labeling by amino acids in cell culture)

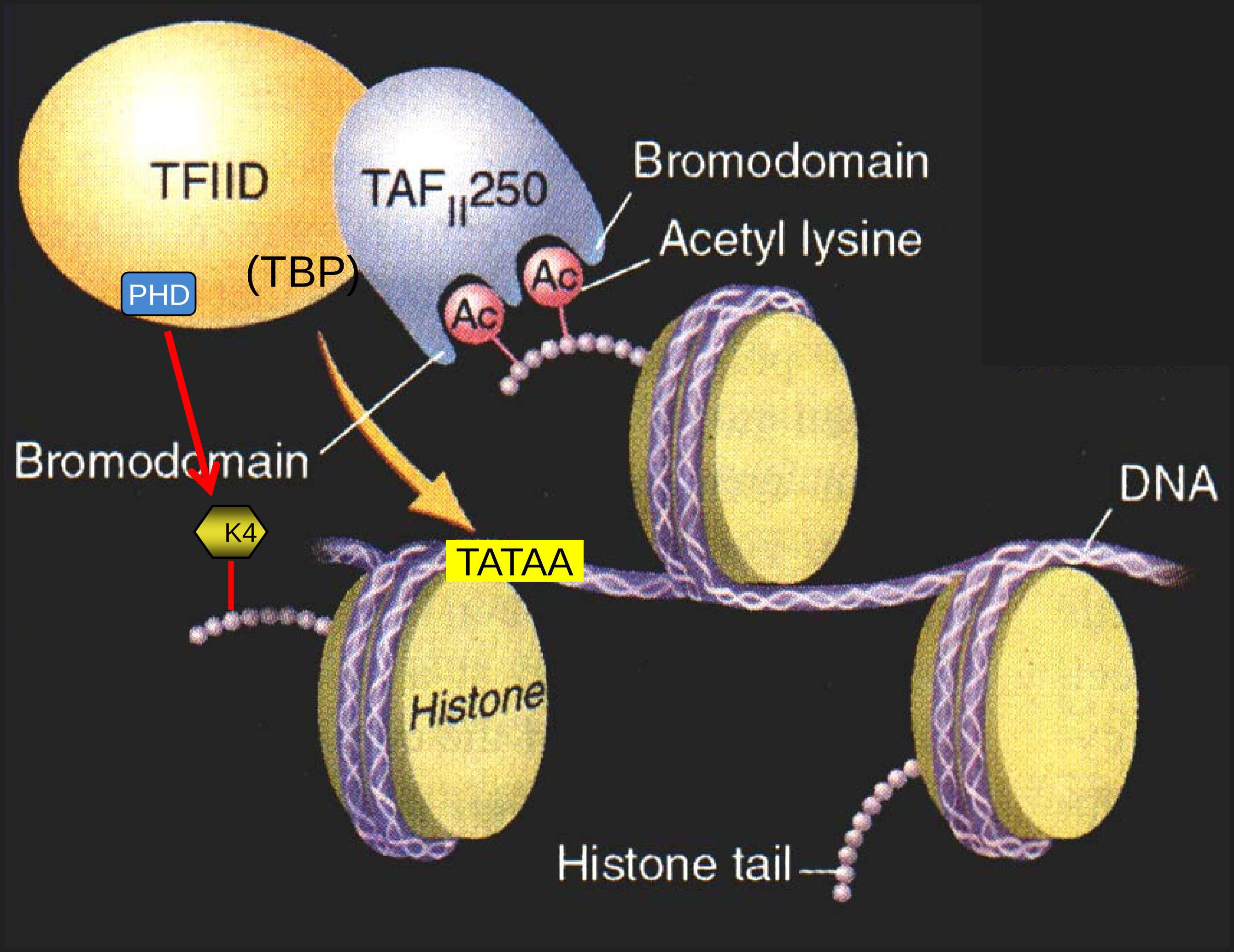


Unlabeled extracts

Isotope-labeled extracts

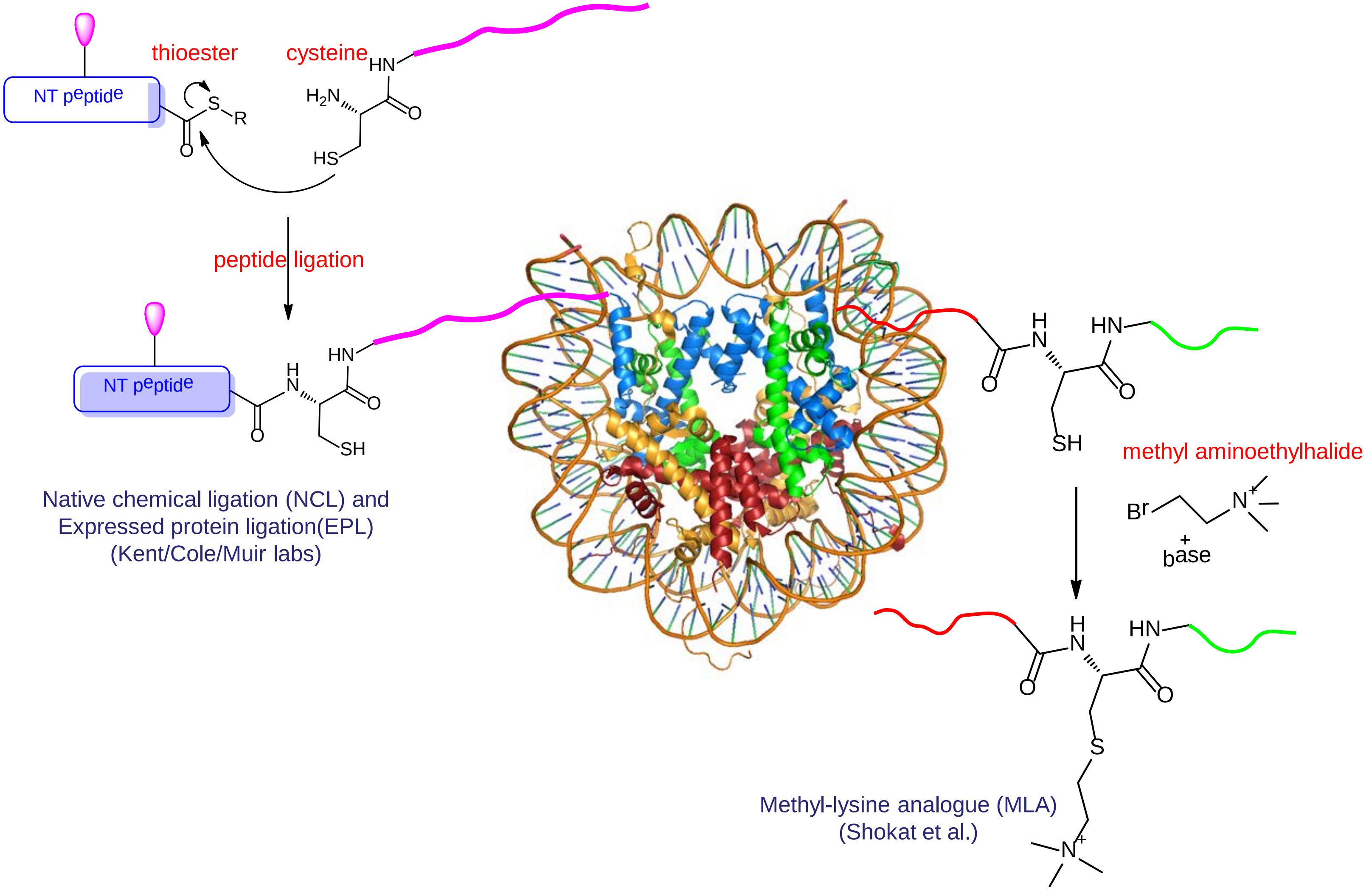
SDS/PAGE and tryptic digestion

Bromodomain-containing proteins can bind to acetylated histones



(Taken from E. Pennisi - Science, 2000)

Semi-synthetic modified nucleosomes explore multivalent engagements in chromatin



Native chemical ligation (NCL) and
Expressed protein ligation(EPL)
(Kent/Cole/Muir labs)

Methyl-lysine analogue (MLA)
(Shokat et al.)

Thank you!