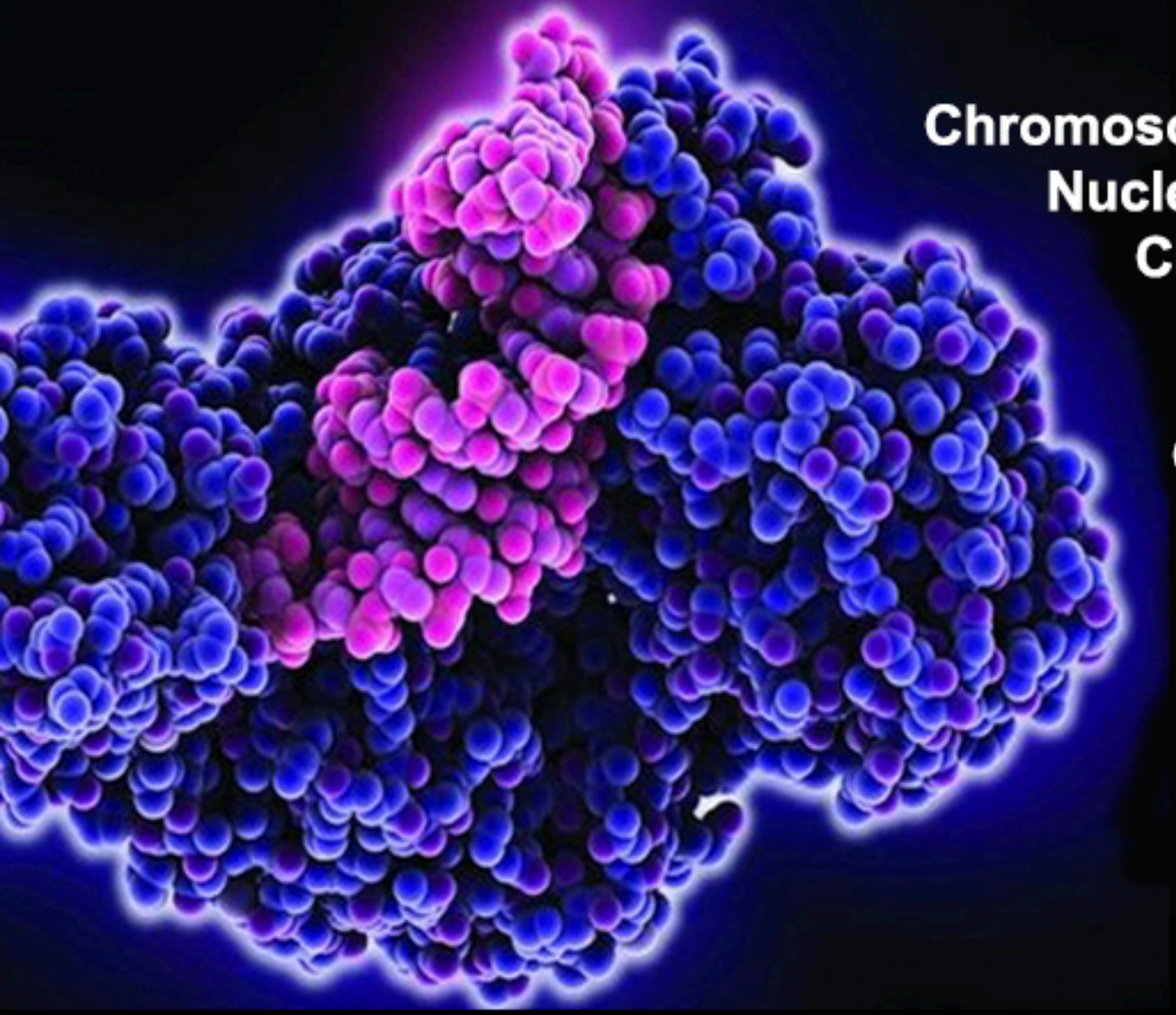
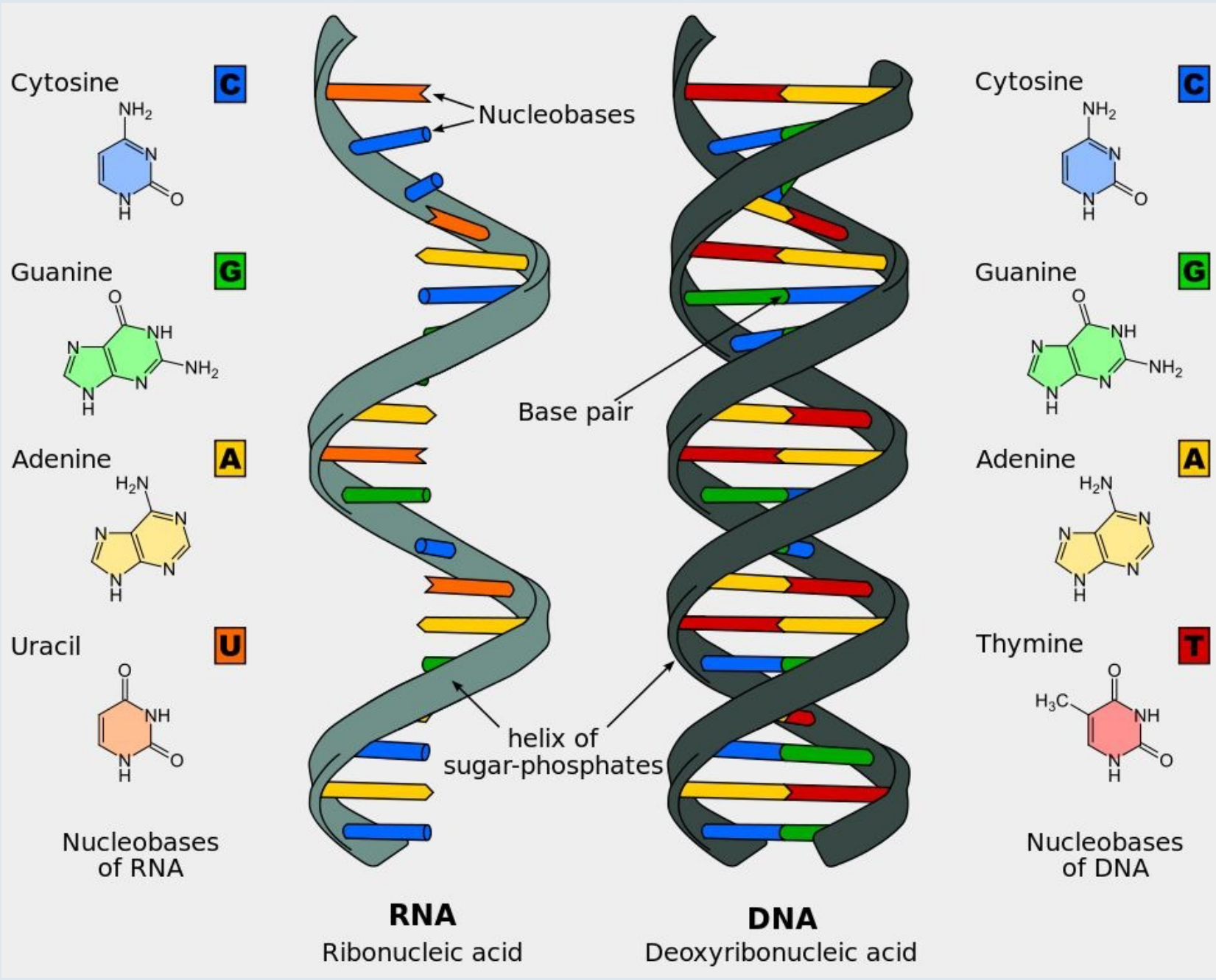
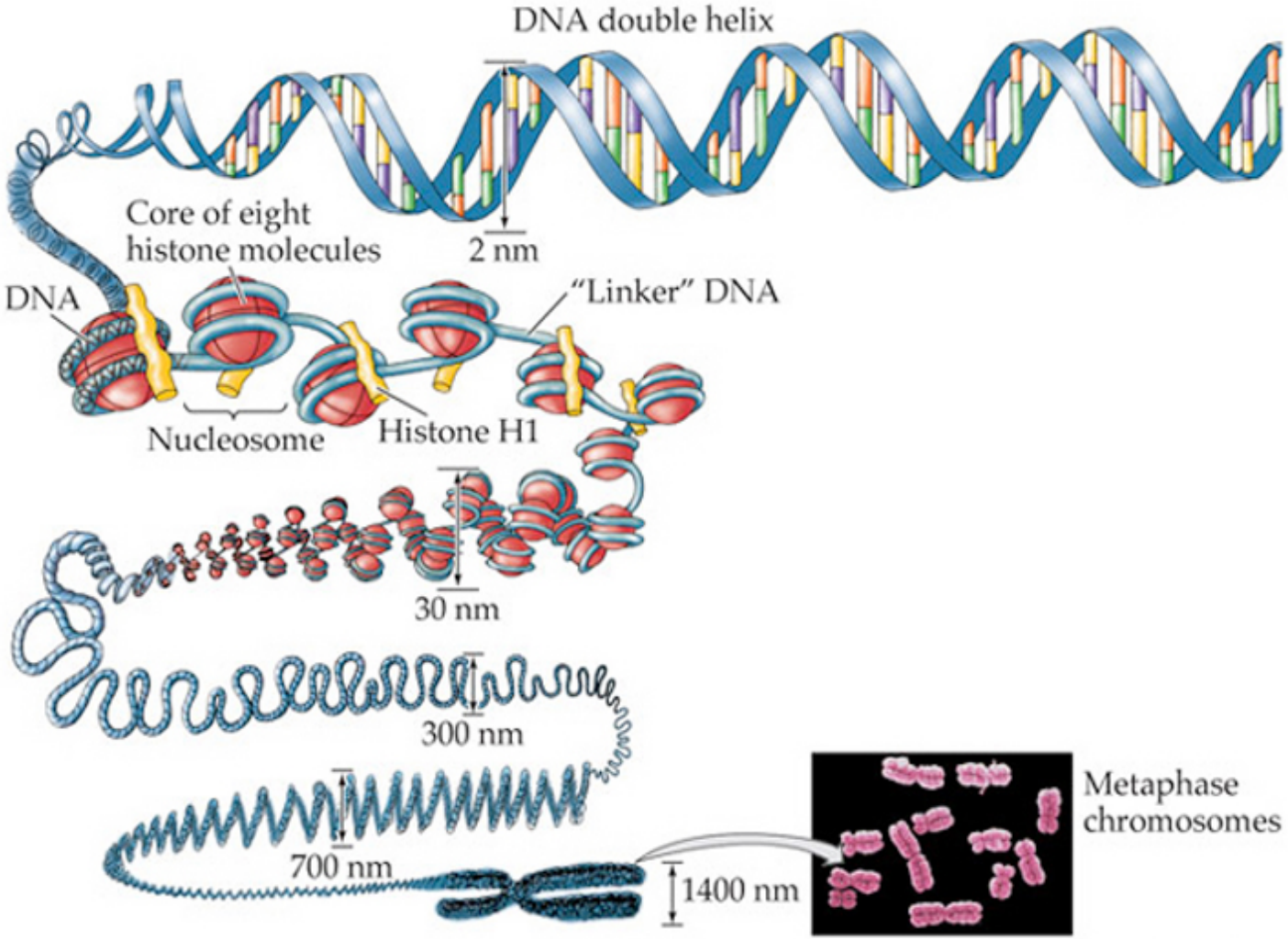
BIOL8620 Eukaryotic Genetics



Chromosomes, Nucleosomes and Chromatin I

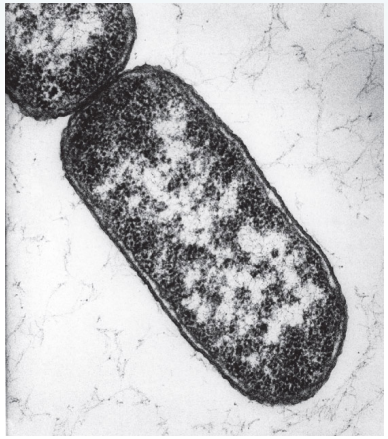
Chapters 7 & 8

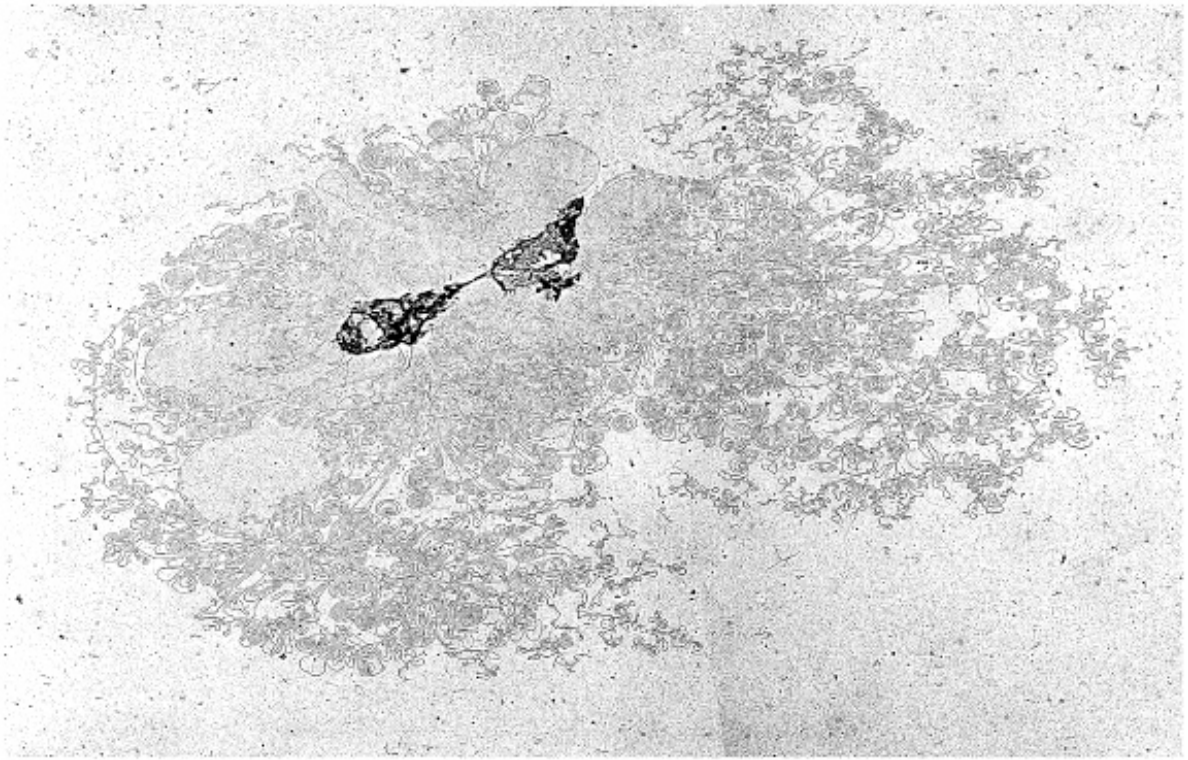

Cytosine
C
Guanine
G
Adenine
A
Uracil
U
Nucleobases of RNA
Nucleobases
Base pair
helix of sugar-phosphates
RNA
Ribonucleic acid
DNA
Deoxyribonucleic acid
Cytosine
C
Guanine
G
Adenine
A
Thymine
T
Nucleobases of DNA

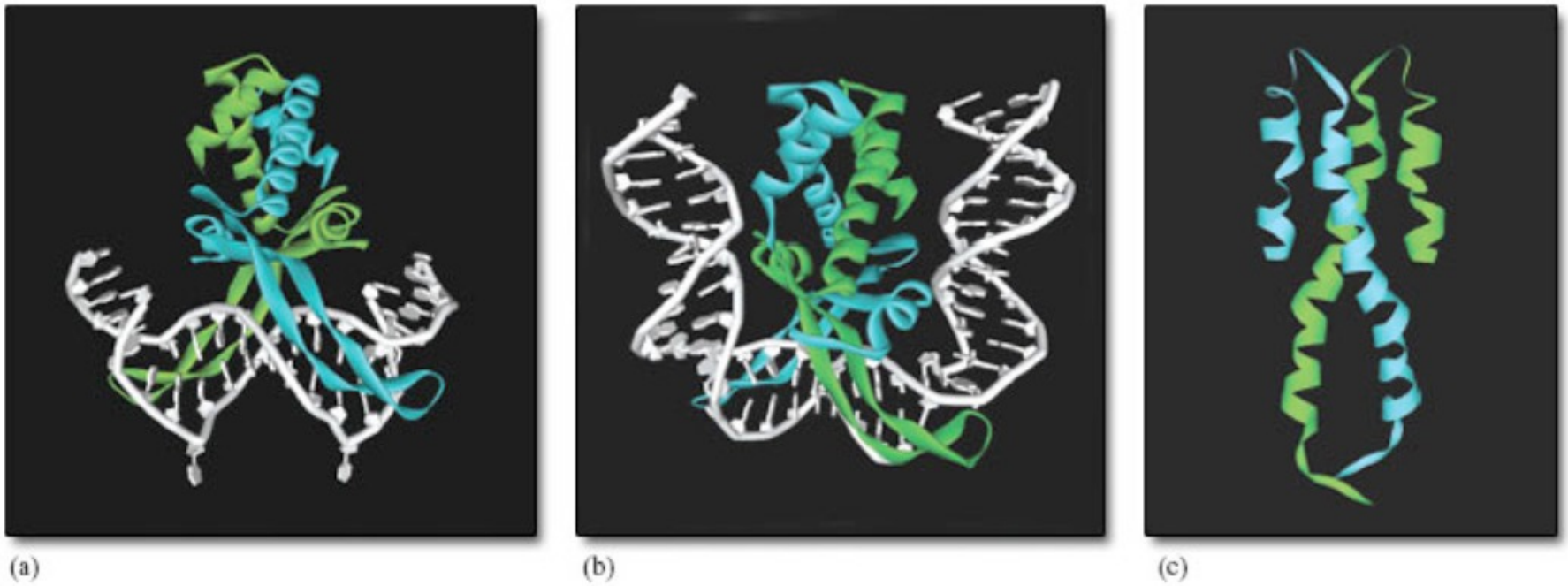


LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.6 DNA Packs into a Mitotic Chromosome

The Bacterial Genome Is Supercoiled







- **(a) The HU protein dimer complexed with DNA**
- **(b) Binding of an *E. coli IHF* dimer to DNA induces a 180° Turn**
- **(c) Structure of the N-terminal domain of *E. coli* H-NS dimer.**

All structures show protein secondary structures and tubular DNA

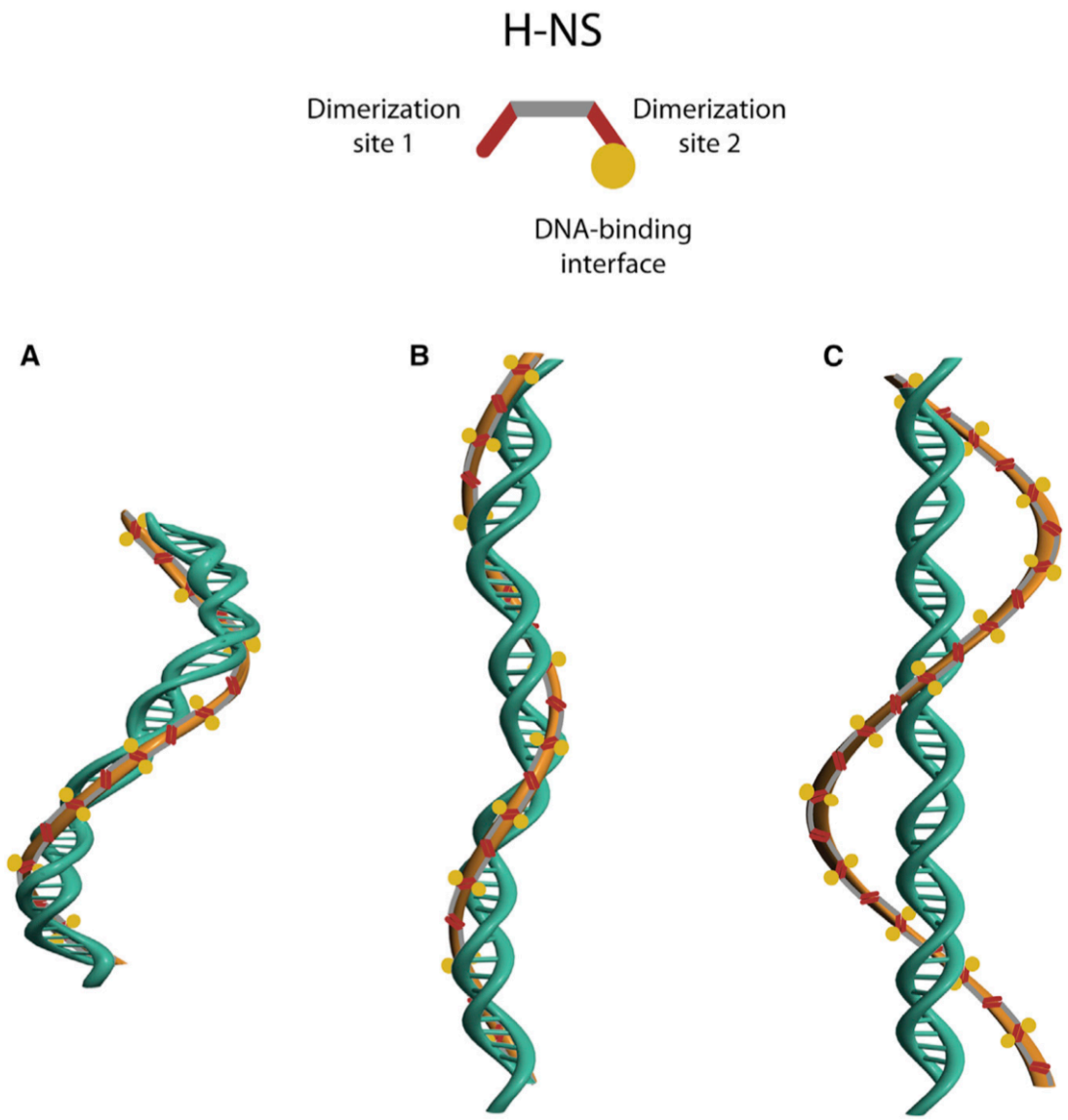
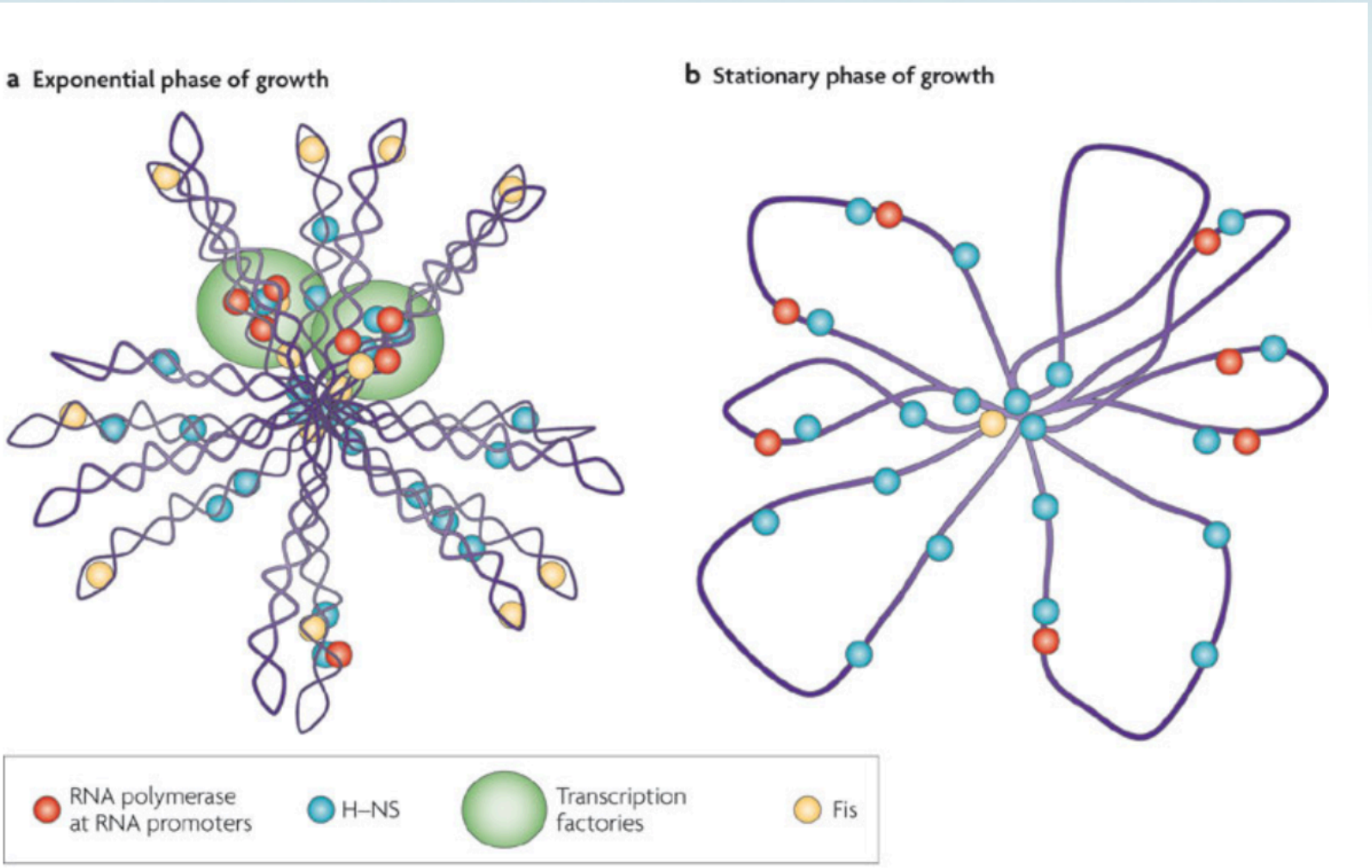


FIGURE 3 H-NS forms a right-handed helical nucleoprotein filament. An H-NS molecule has two dimerization domains (Fig. 1), which enable H-NS to form a chain of linked H-NS molecules on DNA. Three H-NS nucleoprotein filament structures are proposed. (*A*) DNA wraps around an H-NS helix, reducing the contour length to ~50%. (*B*) A flexible H-NS nucleoprotein filament allows deformation into a thinner and longer structure. (*C*) H-NS wraps around the DNA in a helix, forming thick nucleoprotein filaments. The DNA is drawn to scale to highlight the change in contour length. Yellow circles (free DNA-binding domains) that are directed toward the outside of these helical filaments may allow interaction with naked DNA, hence forming filament-mediated DNA bridges under certain conditions.

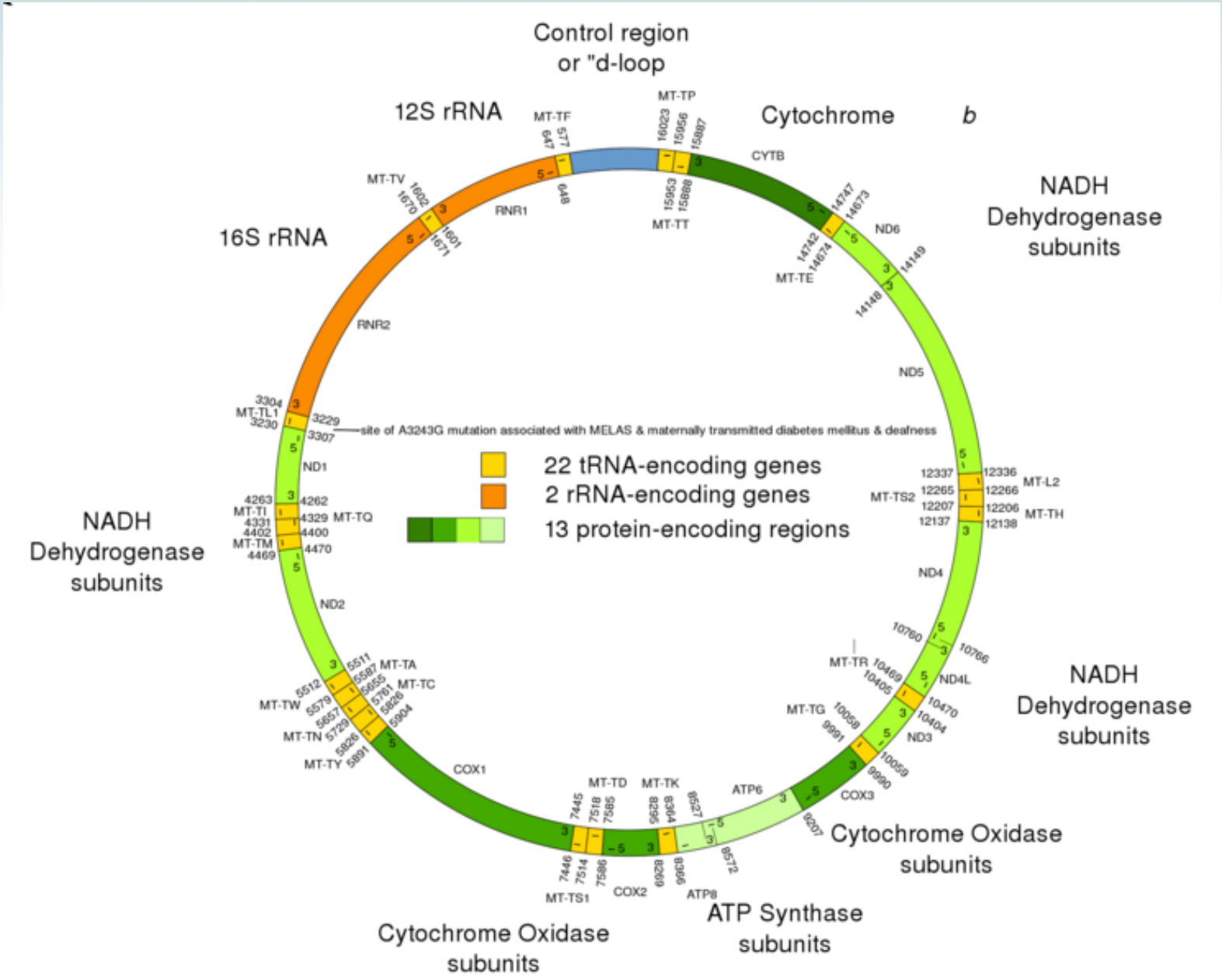
Volume 109, Issue 7, Biophysical Journal 6 October 2015, Pages 1321-1329



Bacterial nucleoid-associated proteins, nucleoid structure and gene expression

Shane C. Dillon & Charles J. Dorman

Nature Reviews Microbiology **volume 8**, pages 185–195 (2010)



Human Mitochondrial Genome16,569 base pairs (bp), 37 genes

Biochimie. 1994;76(10-11):909-16.

The mitochondrial histone HM: an evolutionary link between bacterial HU and nuclear HMG1 proteins.

Megraw TL, Kao LR, Chae CB.

Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill 27599-7260, USA.

Abstract

The mitochondrial histone HM is a very abundant protein in yeast mitochondria that wraps DNA and activates transcription in vitro and is required within the cell for proper maintenance of the mitochondrial chromosome. HM and the bacterial histone-like protein HU have similar activities in vitro and can substitute for each other in E coli cells and in yeast mitochondria. HM also appears to be functionally homologous to nuclear HMG1 proteins, with which it shares a high degree of sequence homology. We report here the isolation of extragenic suppressors of the yeast HM mutant temperature-sensitive phenotype. We also examined the effects of the lack of HM protein and of respiration deficiency on yeast cells mutant for the NHP6 proteins, the putative yeast nuclear HMG1 homologues.

PMID: 7748934 [PubMed - indexed for MEDLINE]

Protein Components of Mitochondrial DNA Nucleoids in Higher Eukaryotes*

Daniel F. Bogenhagen‡§, Yousong Wang‡, Ellen L. Shen‡¶, and Ryuji Kobayashi‖**

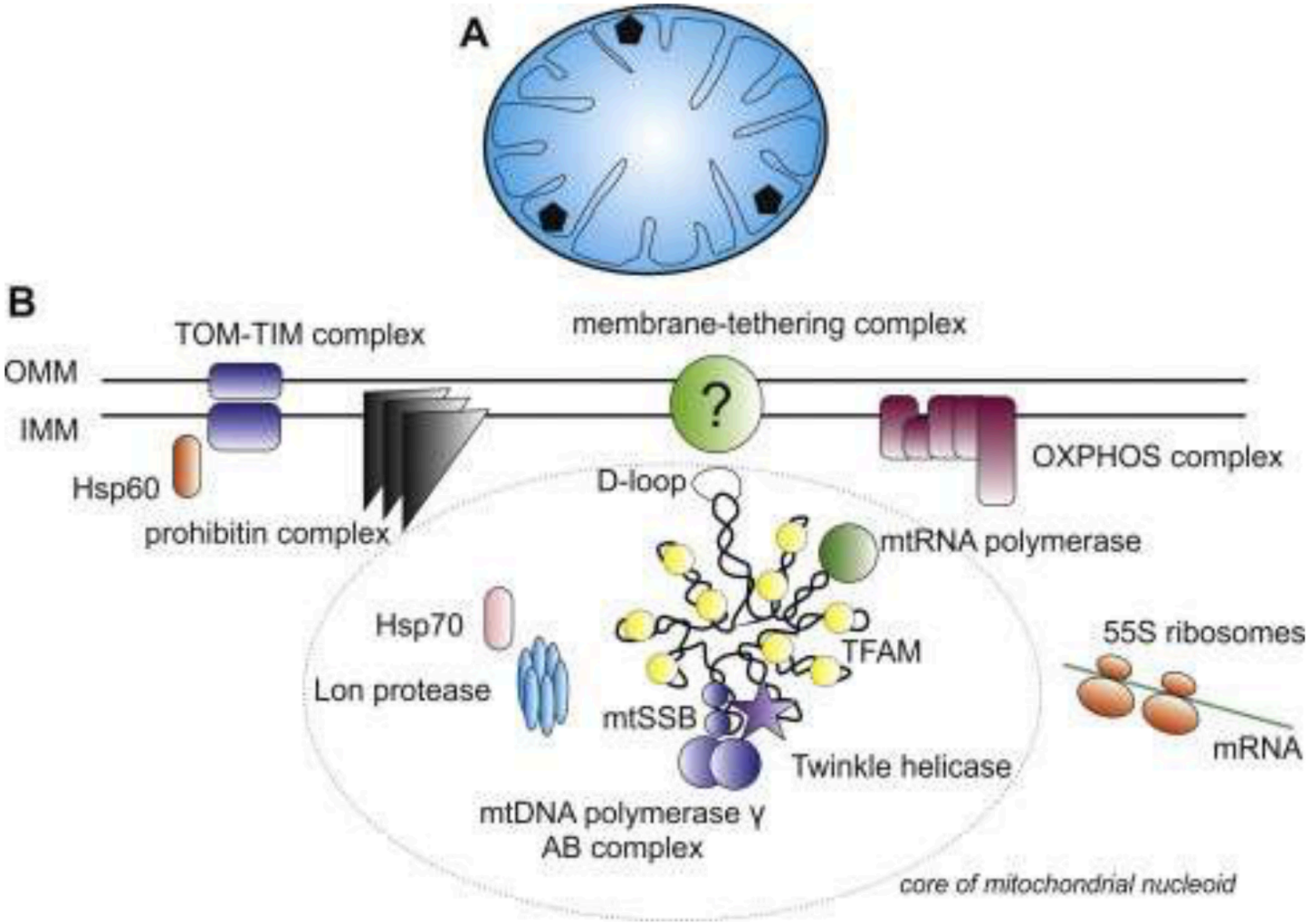
Mitochondrial DNA (mtDNA) is not packaged in nucleosomal particles, but has been reported to associate with the mitochondrial inner membrane. Gentle lysis of *Xenopus* oocyte mitochondria with nonionic detergent liberates a nucleoprotein complex containing mtDNA associated with a previously characterized DNA binding partner, mitochondrial transcription factor A (mtTFA), as well as a series of inner membrane proteins identified by sequencing. More extensive detergent treatment stripped most of these proteins from the DNA, leaving a limited number of proteins in a nucleoid core. Sequencing of the major proteins retained in association with mtDNA revealed the expected mtDNA binding proteins, mtTFA and mitochondrial single-stranded DNA binding protein (mtSSB), as well as four proteins not previously reported to associate with mtDNA. These include adenine nucleotide translocator 1, the lipoyl-containing E2 subunits of pyruvate dehydrogenase and branched chain α-ketoacid dehydrogenase and prohibitin 2. The association of several of these proteins with mtTFA-containing mtDNA nucleoids was confirmed by immunoprecipitation. ***Molecular & Cellular Proteomics 2:1205–1216, 2003.***

contrast to the wealth of information on the protein packaging of nuclear DNA in chromatin. The endosymbiotic hypothesis suggests that the DNA-protein complexes in mitochondria may resemble those in their bacterial ancestors. While eubacteria do not package their DNA in nucleosomes, they do contain abundant basic proteins like HU and INT that compact the DNA and exert a significant influence on gene expression (2).

mtDNA is associated with at least two basic proteins with clear bacterial homologs. The HMG-box protein, mitochondrial transcription factor A (mtTFA), which has been conserved from yeast to humans, is structurally related the bacterial HU protein. Indeed, the yeast mtTFA and *Escherichia coli* HU proteins can genetically substitute for one another (3). mtTFA plays a role as a transcription factor in vertebrates (4–6), but apparently not in yeast (7). Thus, it appears that the essential role that mtTFA plays in yeast mtDNA maintenance is as an architectural DNA binding protein, which accounts for its relative abundance (8). mtTFA is remarkably abundant in mitochondria of *Xenopus* oocytes and human HeLa cells, with hundreds of copies of the protein per mtDNA (5, 9–11). A second mtDNA binding protein that is relatively well-charac-

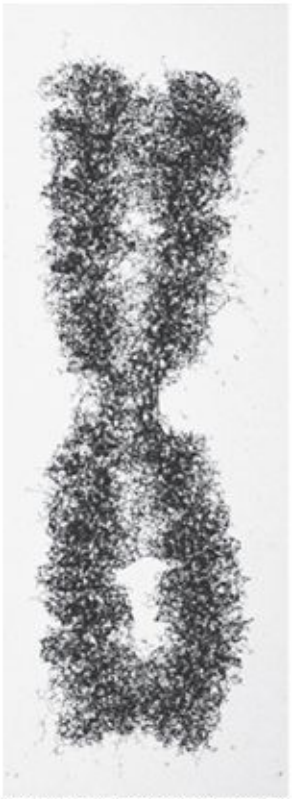
Nucleoids and the structure of life

July 7, 2014 by John Hewitt, Phys.org report



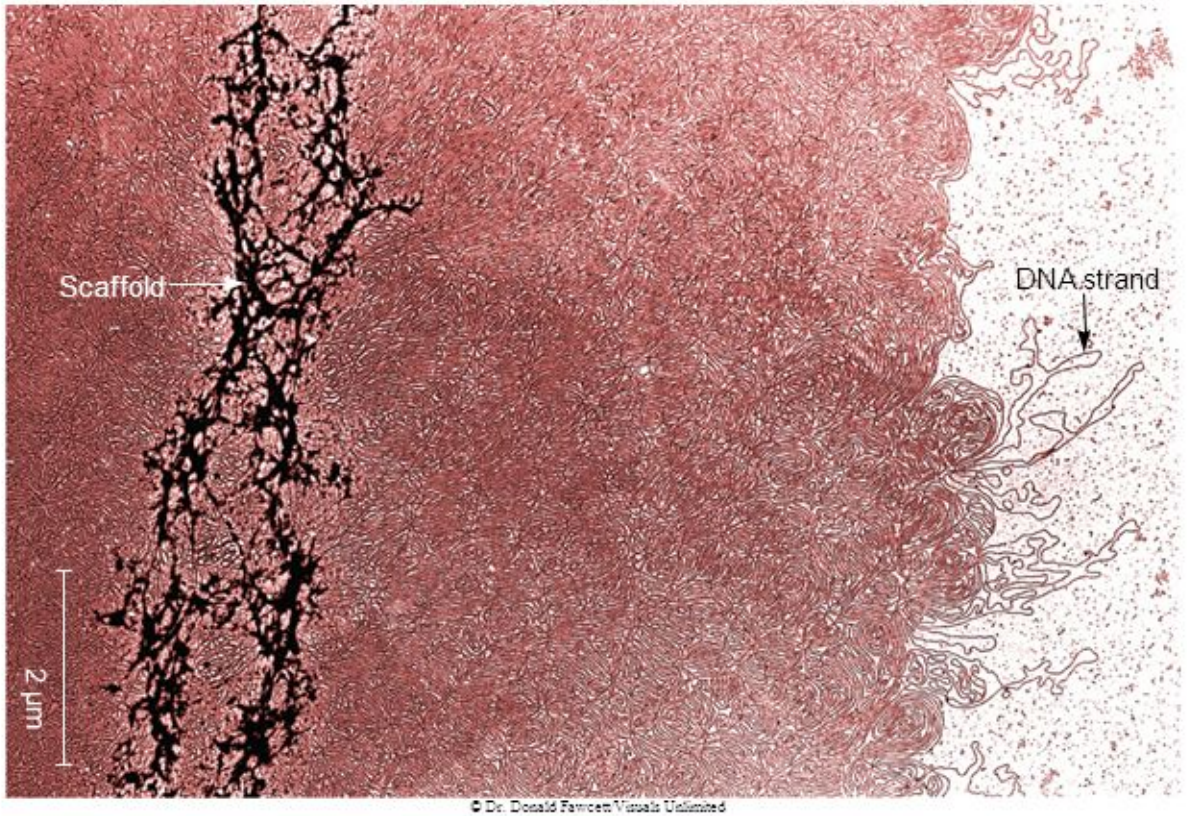
Mitochondrial Nucleoid Core. Credit: mol-biol4masters.masters.grkraj.org

Metaphase Chromosomes



© Peter Engelhardt/Department of Virology, Haartman Institute

Metaphase chromosome

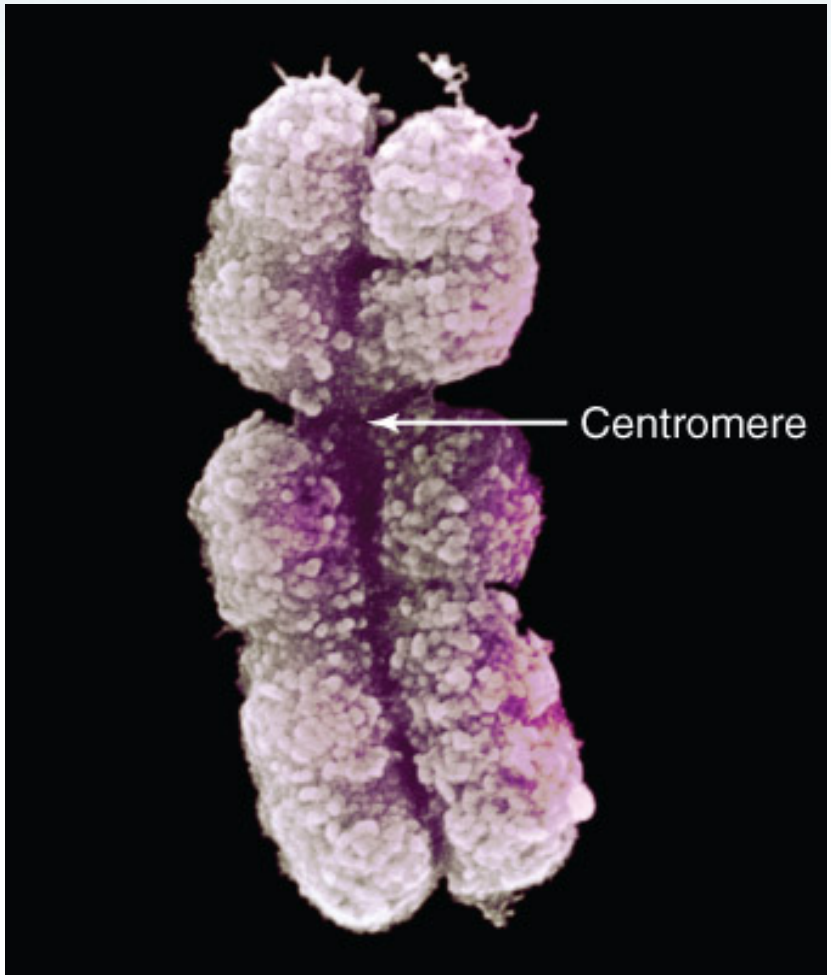


© Dr. Donald Fawcett/Visuals Unlimited

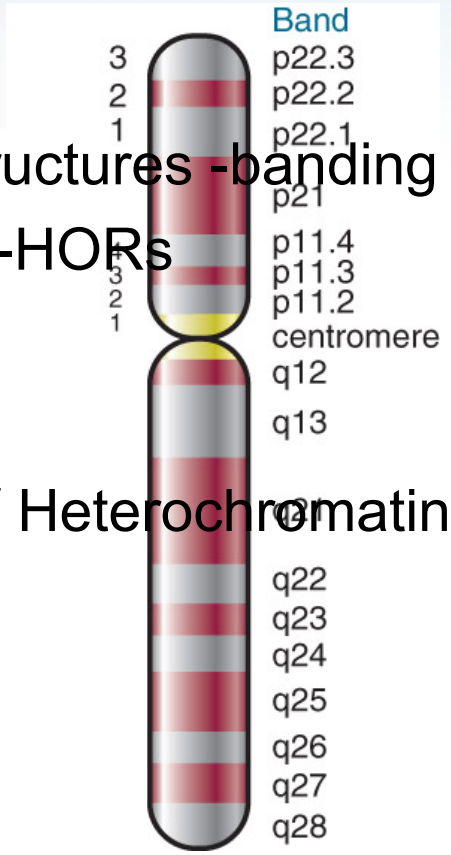
Metaphase chromosome treated with high salt to remove histone proteins

Brooker, Fig 12.18

The Eukaryotic chromosome



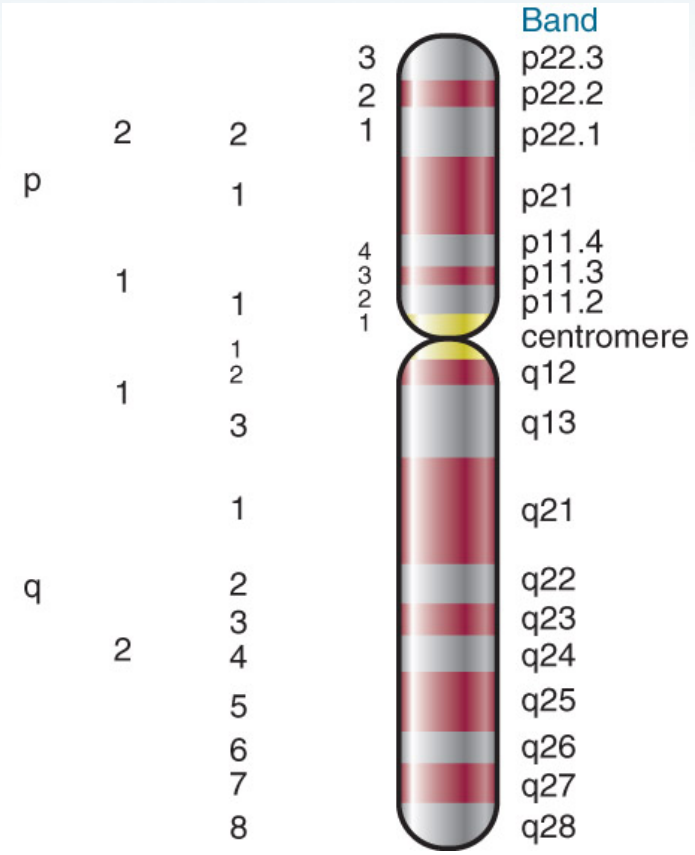
- Organized Structures -banding
- Centromeres -HORs
- Telomeres
- Nucleosomes
- Euchromatin / Heterochromatin



Eukaryotic chromosomes are linear and have **inherent features**

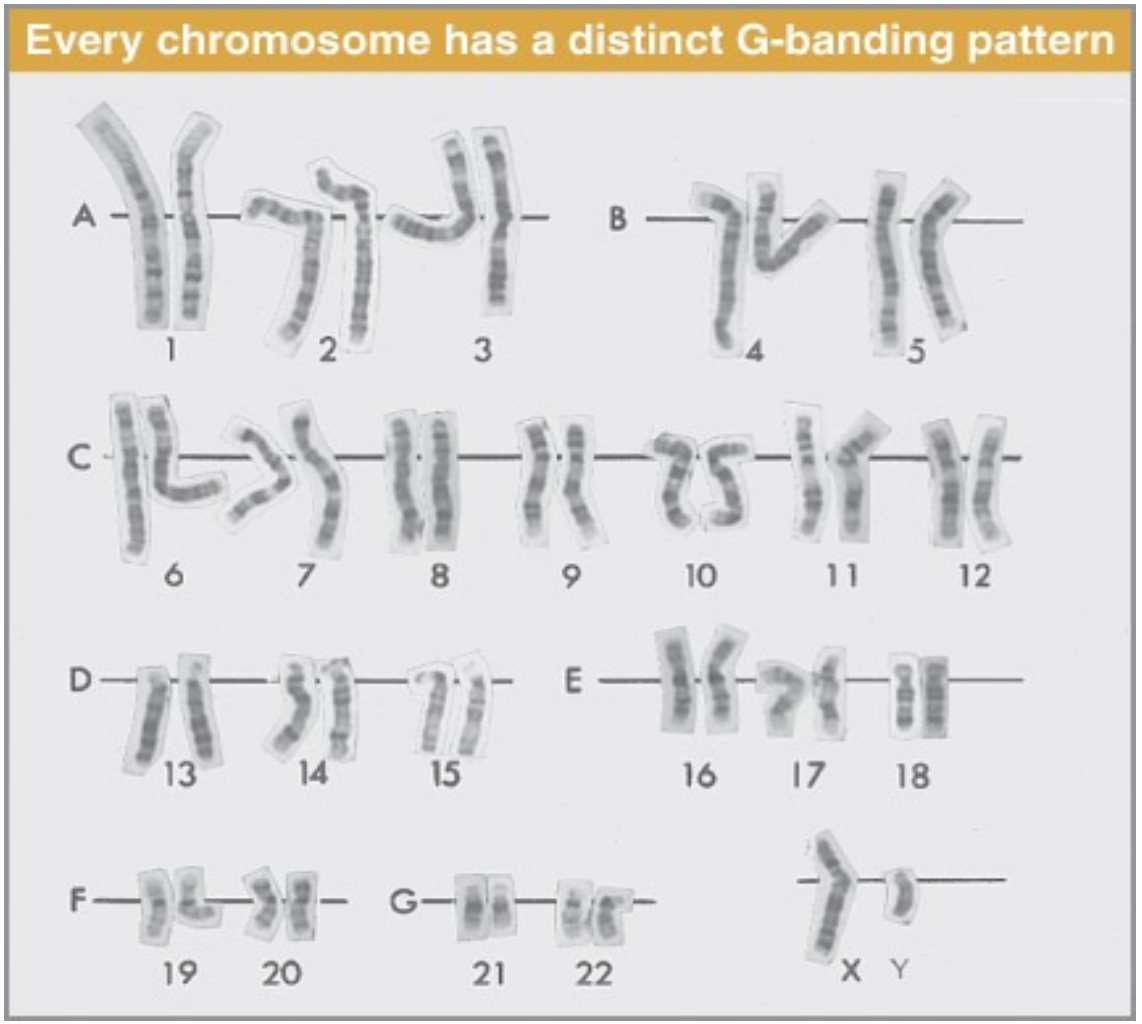
Chromosomes Have Banding Patterns

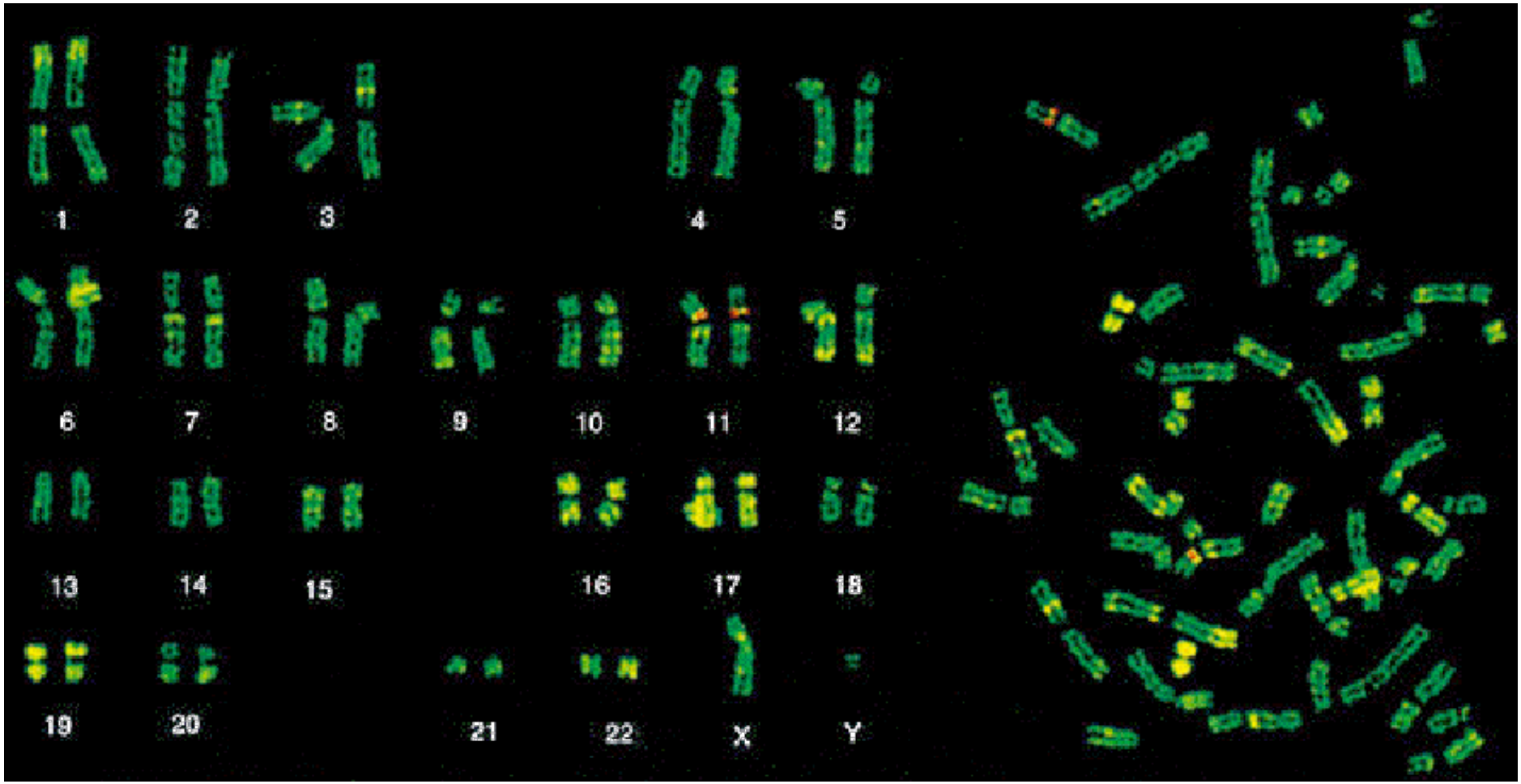
- Certain staining techniques cause the chromosomes to have the appearance of a series of striations, which are called **G-bands**.
- The **bands** are lower in G-C content than the **interbands**.
- Genes are concentrated in the G-C-rich interbands.



The human X chromosome, for example, can be divided into distinct regions by its banding pattern

- Certain staining techniques cause the chromosomes to have the appearance of a series of striations.
  - They are called G-bands.




1
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21
22
X
Y

Additional examples of chromosome banding….

R-banding procedures (modified Giesma or Hoechst stain) produce the reverse, or opposite banding pattern as **G-banding** and **Q-banding** and denotes the **GC-rich, euchromatic regions**. R-bands are also important for detecting minor inversions, for comparing length of homologues, for examining the ends of chromosomes, and for identifying subtle deletions or rearrangements that may go undetected using G- or Q-bands.

Previous figure showed Q-banding.

C-banding is a procedure which stains the **constitutive heterochromatin** that is localized in **pericentromeric** regions of all chromosomes and on the distal long arm of the Y chromosome. The process is similar to G-banding (except heat is used instead of proteolytic enzymes to open up the DNA). The use of C-banding facilitates the determination of **pericentric** inversions and also the identification of polymorphisms within the **centromeric** regions of chromosomes.

NOR staining is a silver staining procedure which stains the nucleolus organizer regions (NORs) of satellited chromosomes. It is particularly useful in studying variations in the size of the stalks and satellites of the acrocentric chromosomes.

•

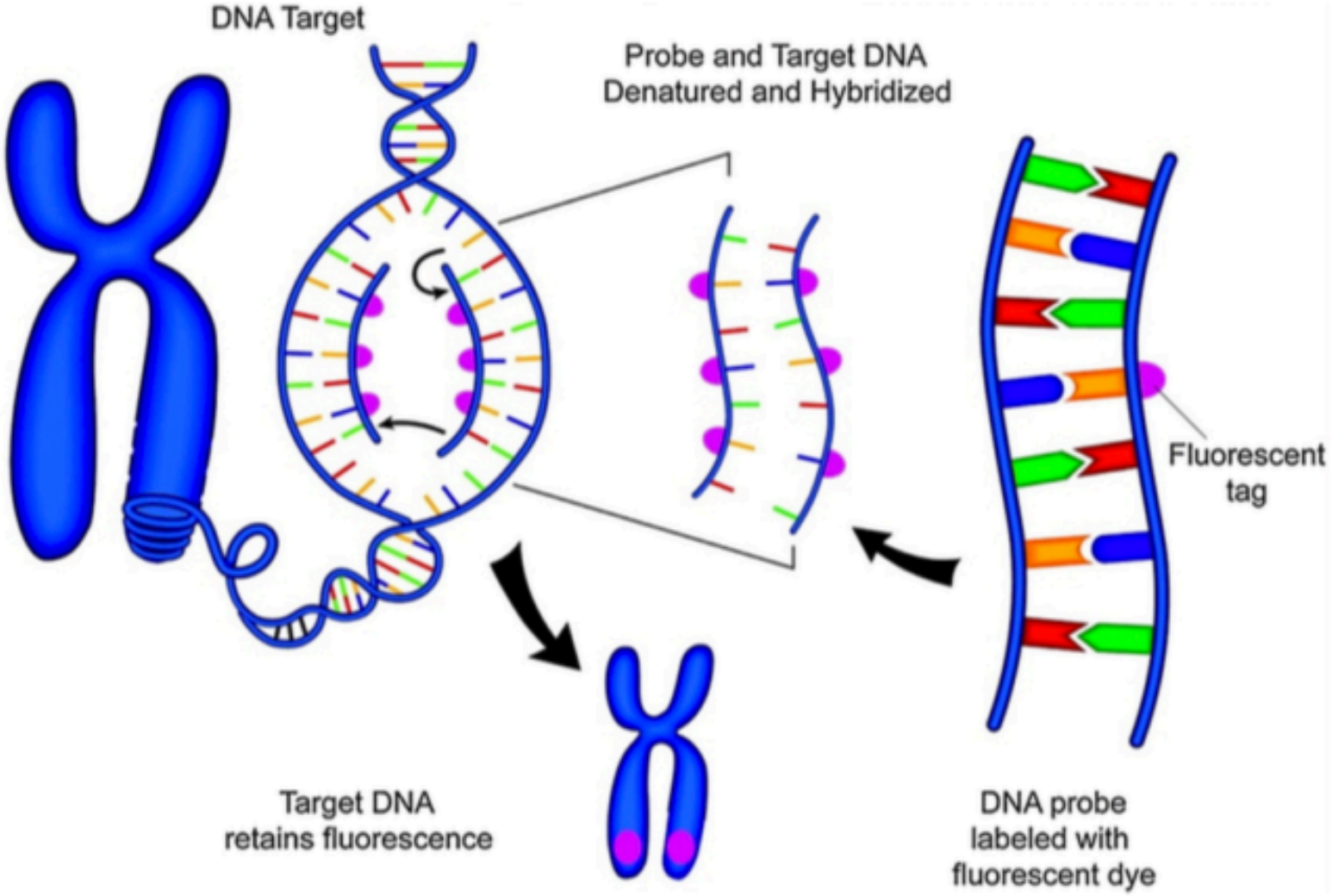
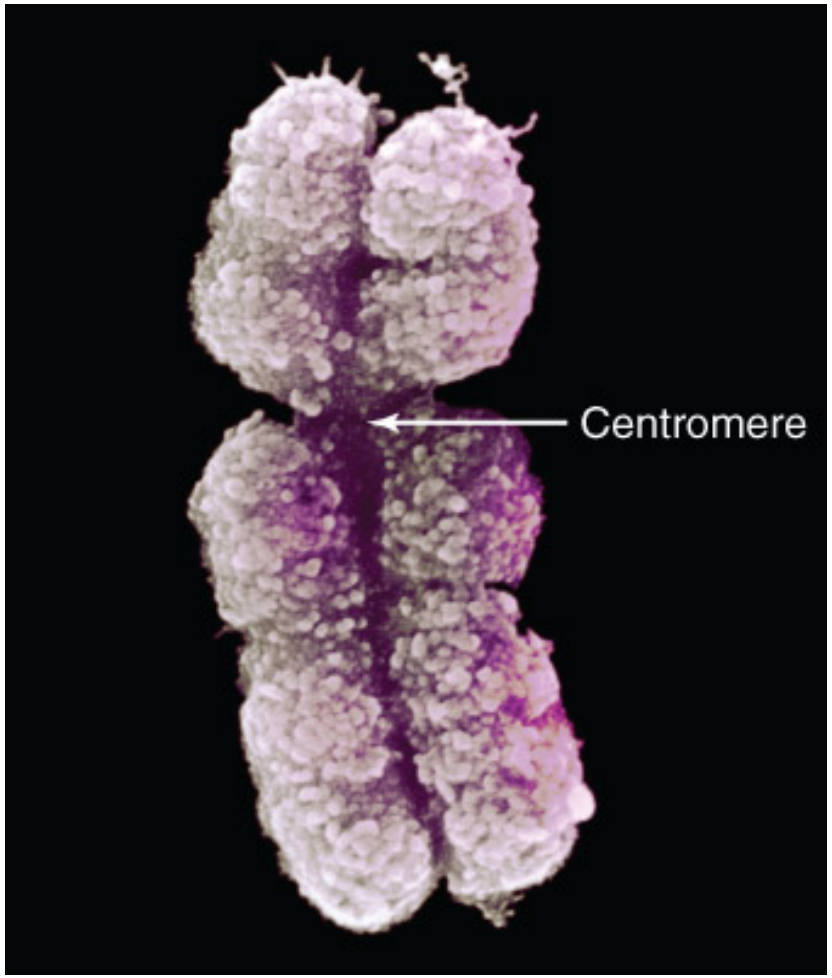


Fig. 8. Fluorescence In situ hybridization cytogenetic technique (FISH, http://www.abnova.com).

The Eukaryotic chromosome

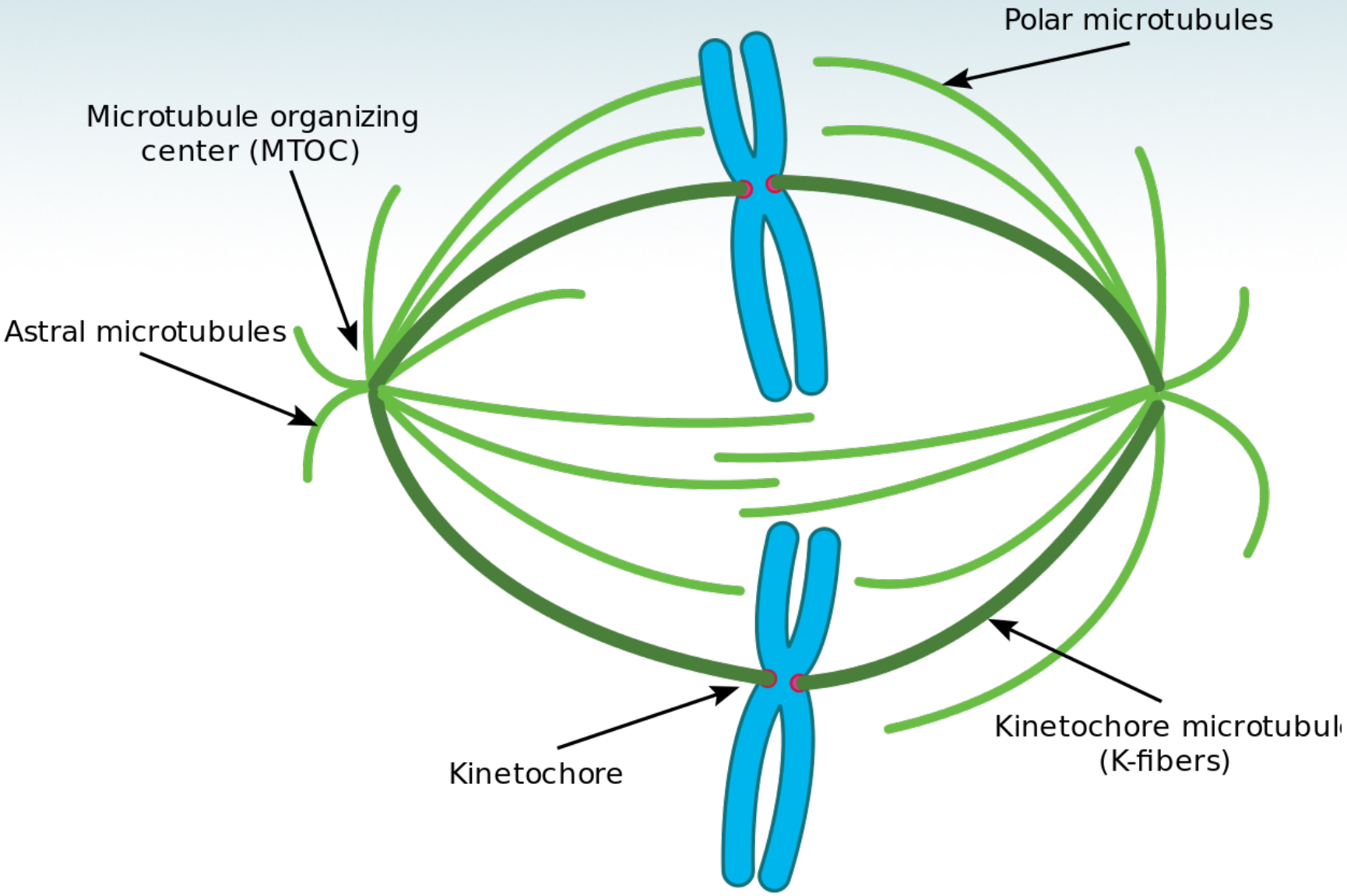


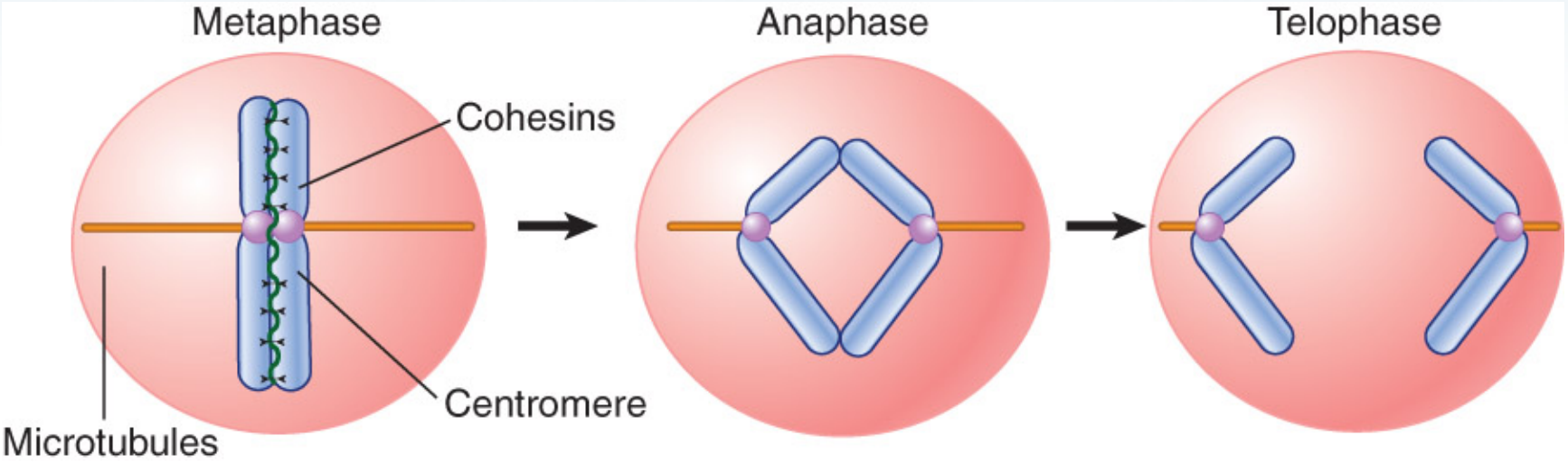
- Organized Structures -banding
- **Centromeres -HORs**
- **Telomeres**
- **Nucleosomes**
- **Euchromatin / Heterochromatin**

The Eukaryotic Chromosome Provides for Segregation

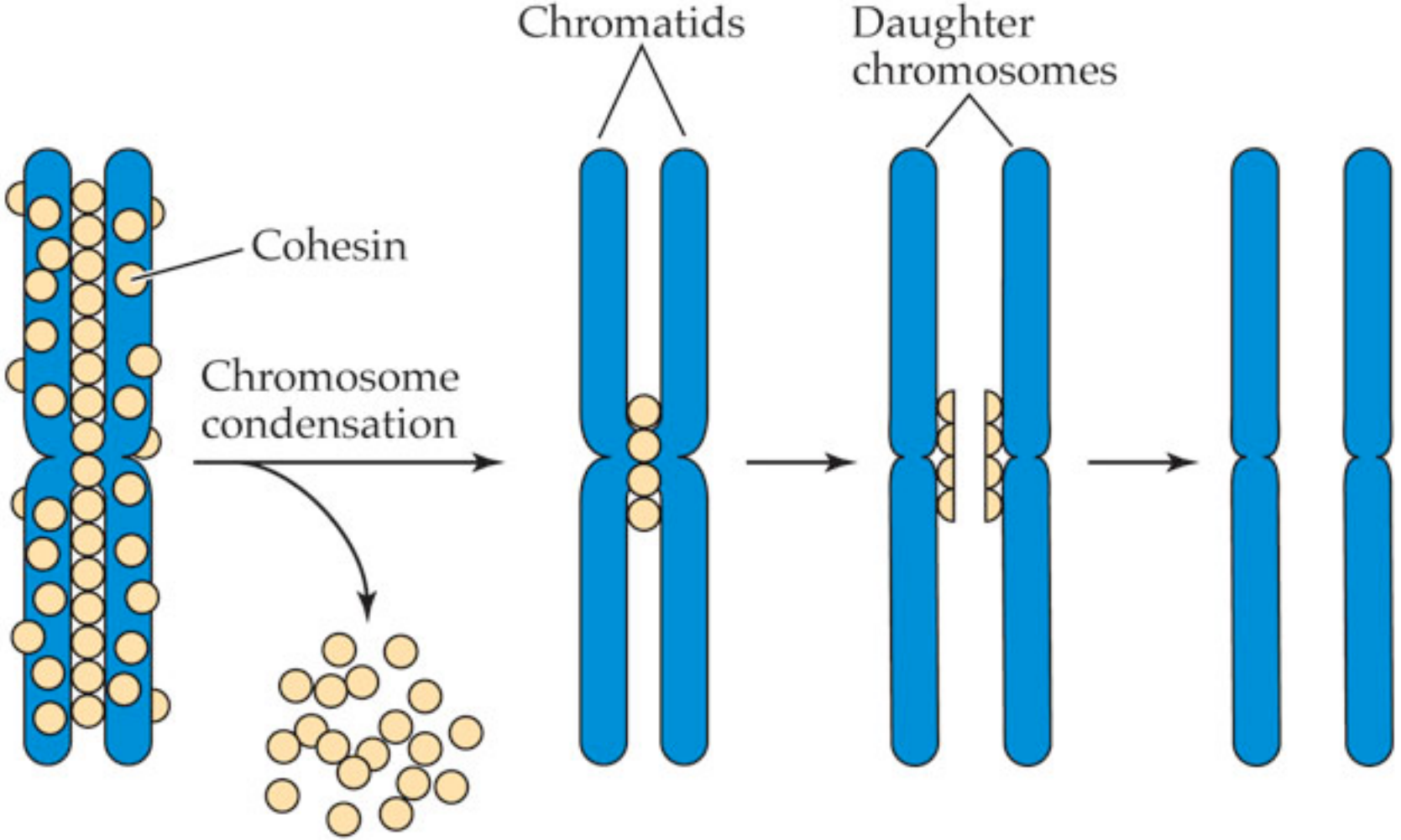
- A eukaryotic chromosome is held on the mitotic **spindle** by the attachment of microtubules to the **kinetochore** that forms in its centromeric region.

- **microtubule organizing center (MTOC) –** A region from which microtubules emanate.
  - In animal cells the centrosome is the major microtubule organizing center.

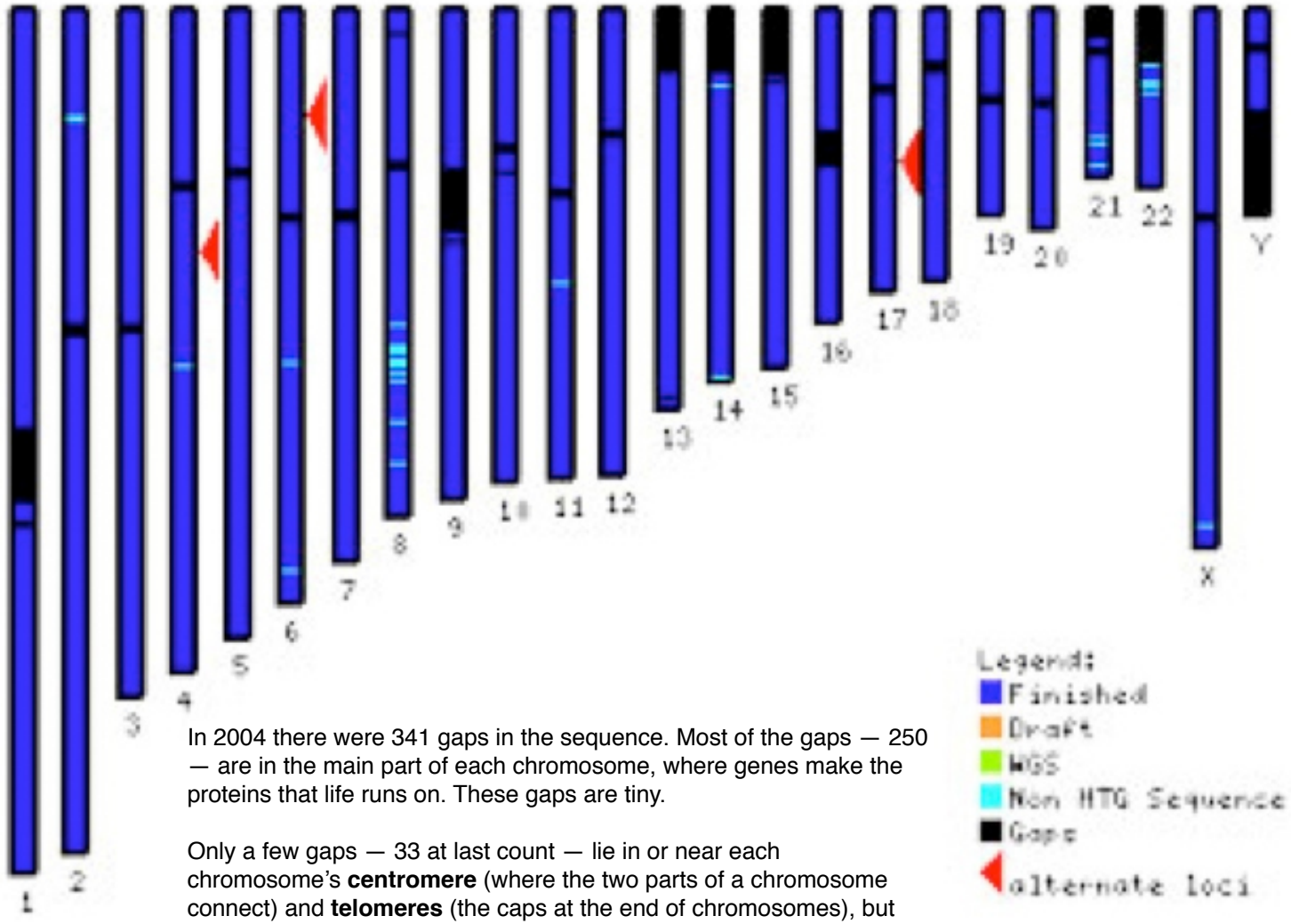

Polar microtubules
Microtubule organizing center (MTOC)
Astral microtubules
Kinetochore
Kinetochore microtubul
(K-fibers)



Chromosomes are pulled to the poles via microtubules that attach at the centromeres



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.9 Molecular Biology of Chromatid Attachment and Separation



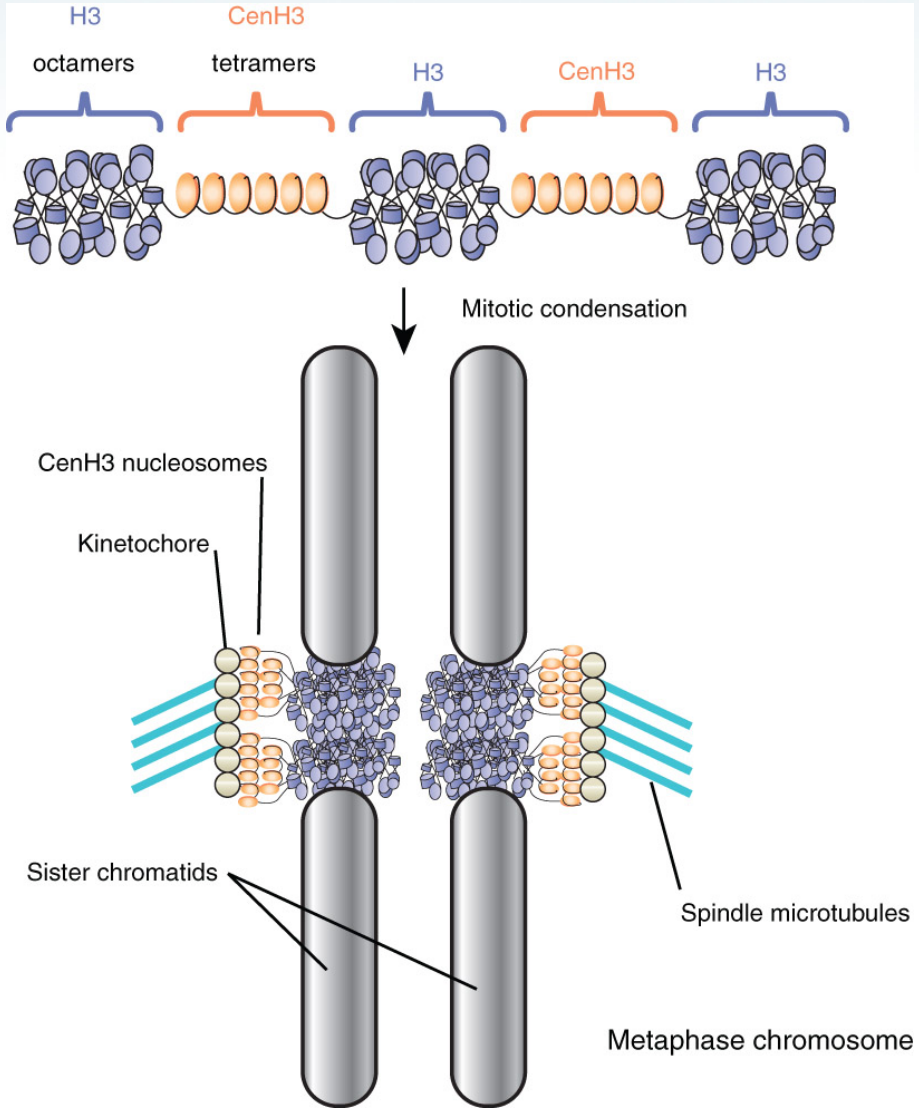
In 2004 there were 341 gaps in the sequence. Most of the gaps — 250 — are in the main part of each chromosome, where genes make the proteins that life runs on. These gaps are tiny.

Only a few gaps — 33 at last count — lie in or near each chromosome's **centromere** (where the two parts of a chromosome connect) and **telomeres** (the caps at the end of chromosomes), but these 33 are 10 times as long in total as the 250 gaps.

"It's very fair to say the human genome was never fully sequenced," Craig Ventner

"The human genome has not been completely sequenced and neither has any other mammalian genome as far as I'm aware," said Harvard Medical School bioengineer George Church, who made key early advances in sequencing technology.

Regional Centromeres Contain a Centromeric Histone H3 Variant and Repetitive DNA



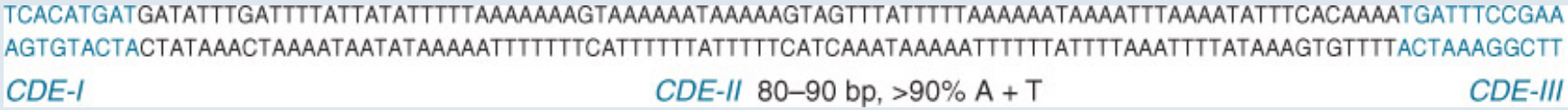
A model of the overall structure of a regional centromere

Adapted from Y. Datal, et al., Proc. Natl. Acad. Sci. USA 104 (2007): 15974-15981.

Centromeres in *S. cerevisiae* Contain Short, Essential DNA Sequences

- *CEN* elements are identified in *S. cerevisiae* by the ability to allow a plasmid to segregate accurately at mitosis.

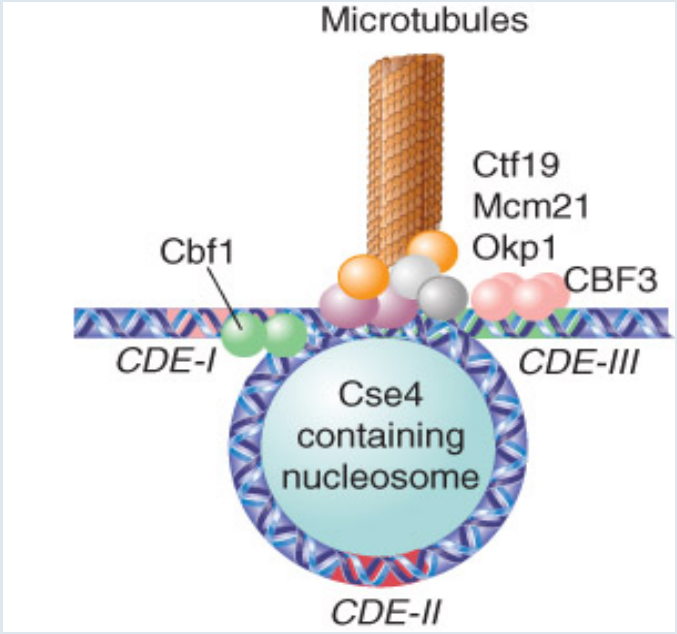
- *CEN* elements consist of the short, conserved sequences *CDE-I* and *CDE-III* that flank the A-T-rich region *CDE-II*.



Three conserved regions can be identified by the sequence homologies between yeast CEN elements.

In *S. cerevisiae* the Centromere Binds a Protein Complex

- A specialized protein complex that is an alternative to the usual chromatin structure is formed at *CDE-II*.
- The histone H3 variant Cse4 is incorporated in the centromeric nucleosome.
- The CBF3 protein complex that binds to *CDE-III* is essential for centromeric function.
- The proteins that bind *CEN* serve as an assembly platform for the kinetochore and provide the connection to microtubules.



The DNA at CDE-II is wound around an alternative nucleosome.

A unique chromatin complex occupies young α-satellite arrays of human centromeres

Jorja G. Henikoff[1], **Jitendra Thakur**[1,2], **Sivakanthan Kasinathan**[1,3], and **Steven Henikoff**[1,2,*]

[1]Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

[2]Howard Hughes Medical Institute, Seattle, WA 98109, USA

[3]Medical Scientist Training Program, University of Washington School of Medicine, Seattle, WA 98195, USA

Abstract

The intractability of homogeneous α-satellite arrays has impeded understanding of human centromeres. Artificial centromeres are produced from higher-order repeats (HORs) present at centromere edges, although the exact sequences and chromatin conformations of centromere cores remain unknown. We use high-resolution chromatin immunoprecipitation (ChIP) of centromere components followed by clustering of sequence data as an unbiased approach to identify functional centromere sequences. We find that specific dimeric α-satellite units shared by multiple individuals dominate functional human centromeres. We identify two recently homogenized α-satellite dimers that are occupied by precisely positioned CENP-A (cenH3) nucleosomes with two ~100–base pair (bp) DNA wraps in tandem separated by a CENP-B/CENP-C–containing linker, whereas pericentromeric HORs show diffuse positioning. Precise positioning is largely maintained, whereas abundance decreases exponentially with divergence, which suggests that young α-satellite dimers with paired ~100-bp particles mediate evolution of functional human centromeres. Our unbiased strategy for identifying functional centromeric sequences should be generally applicable to tandem repeat arrays that dominate the centromeres of most eukaryotes.

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http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4410388/pdf/nihms681867.pdf

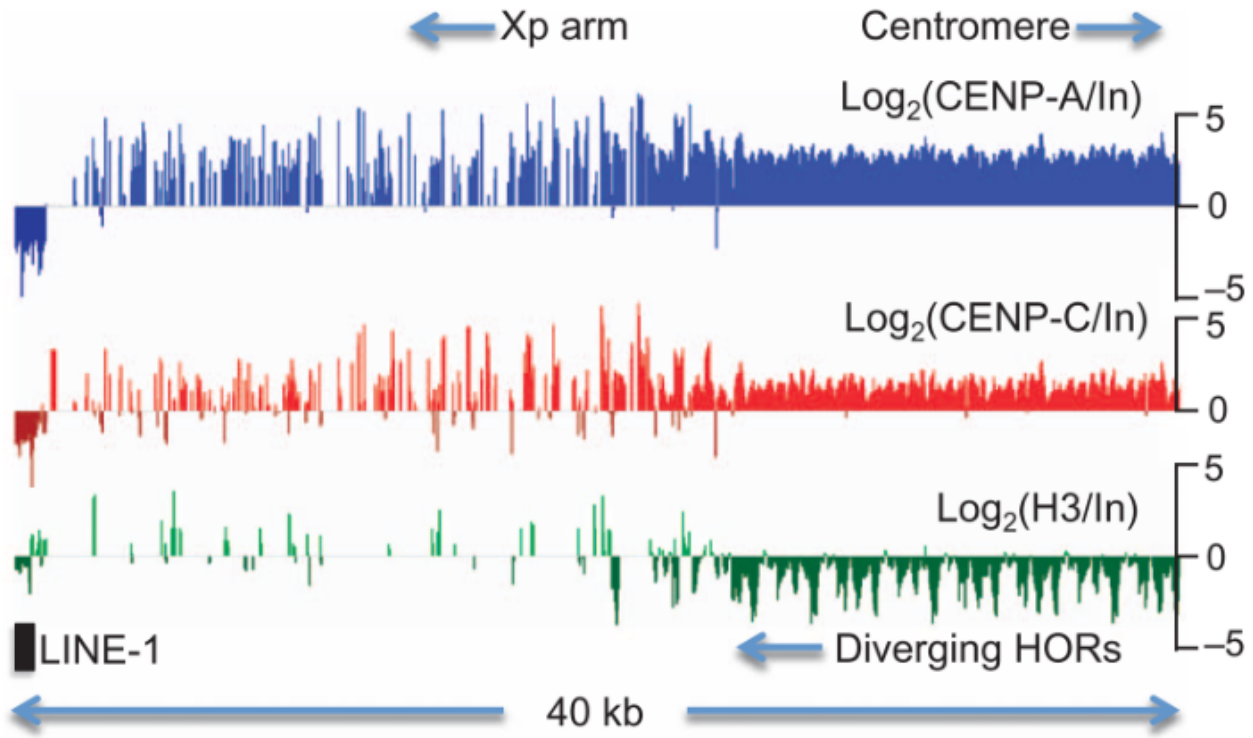


Fig. 1. CENP-A and CENP-C enrichment decreases withα-satellite divergence in pericentric heterochromatin

Log-ratio CENP-A, CENP-C, and H3 enrichment profiles spanning the 40-kb most proximal annotated segment of chromosome arm Xp, which spans the DXZ1 α-satellite HOR gradient (3). Dense CENP-A and CENP-C enrichment diminishes with distance from the centromere-proximal edge, and depletion of H3 diminishes ~20 kb from the edge. Diverged α-satellite occupies the Xp arm punctuated by LINE-1 and other elements where centromere protein enrichment is low.

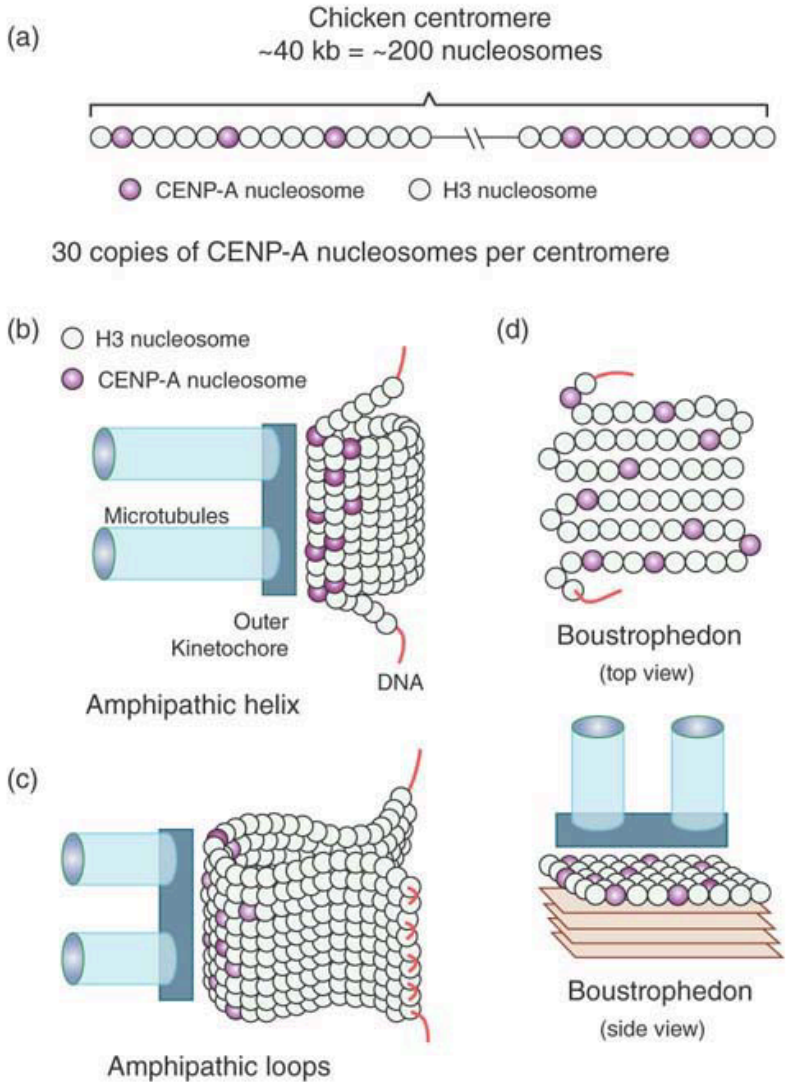
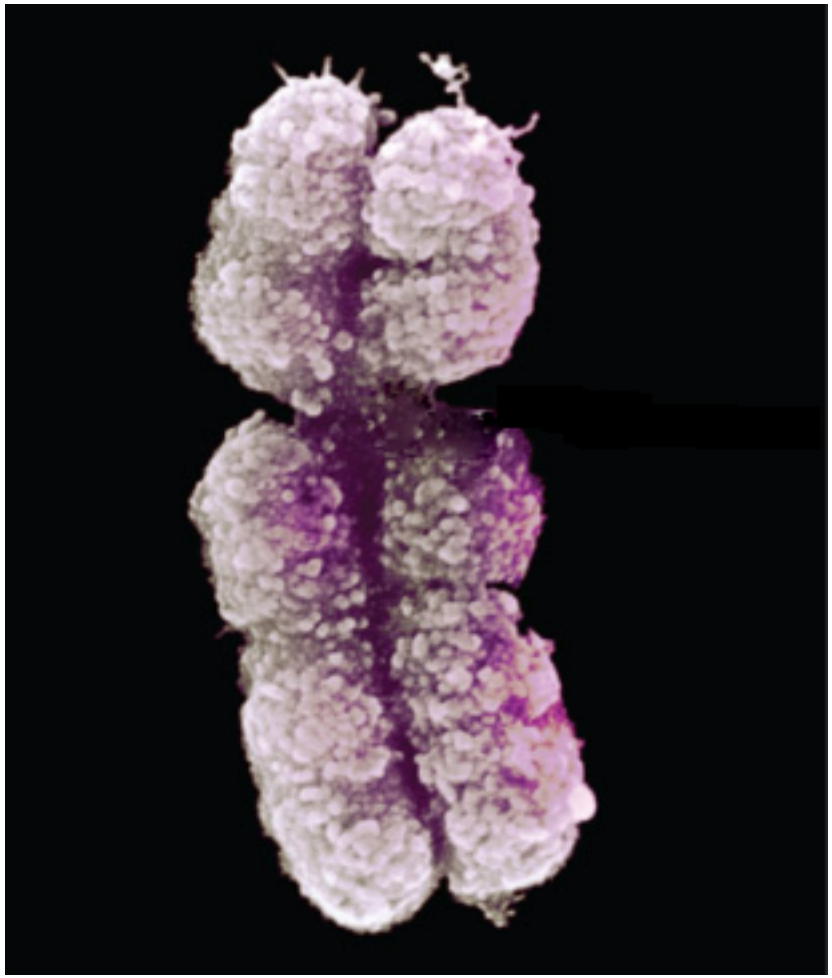
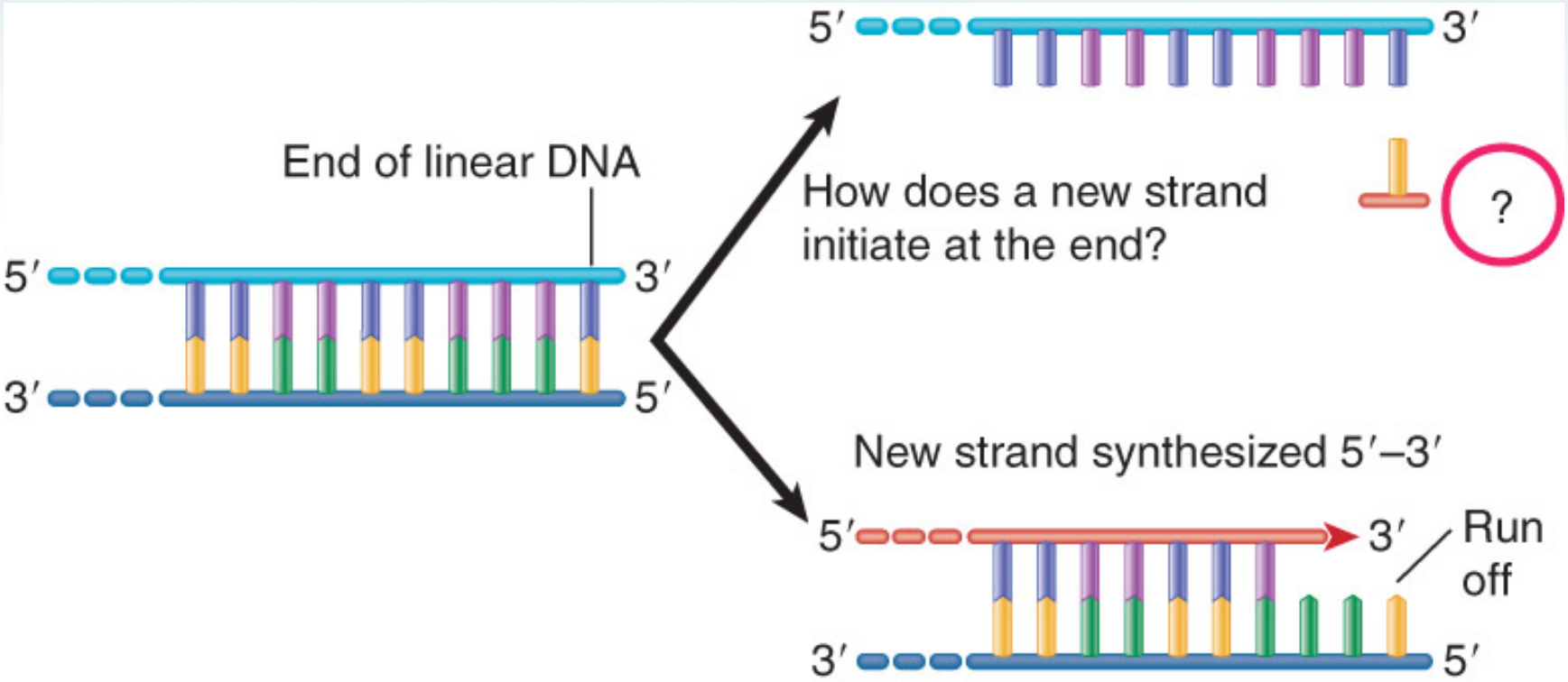


FIGURE 7.25 Organization of CENP-A and H3 Nucleosomes in Centromeres. (a) Centromeres are ~40 kb long in chicken, corresponding to 200 nucleosomes per centromere. Of these, 30 are predicted to contain CENP-A (roughly 1 in 6–8 centromeric nucleosomes). Thus, centromeric chromatin is largely composed of nucleosomes containing histone H3. (b and c) The CENP-A chromatin was originally suggested to form an amphipathic organization, with CENP-A on the exterior facing the kinetochore, and H3 largely on the interior. This chromatin was proposed to form either a helix or loop structure. (d) The boustrophedon model of centromeric CENP-A-containing chromatin was proposed based on super-resolution microscopy.

The Eukaryotic chromosome



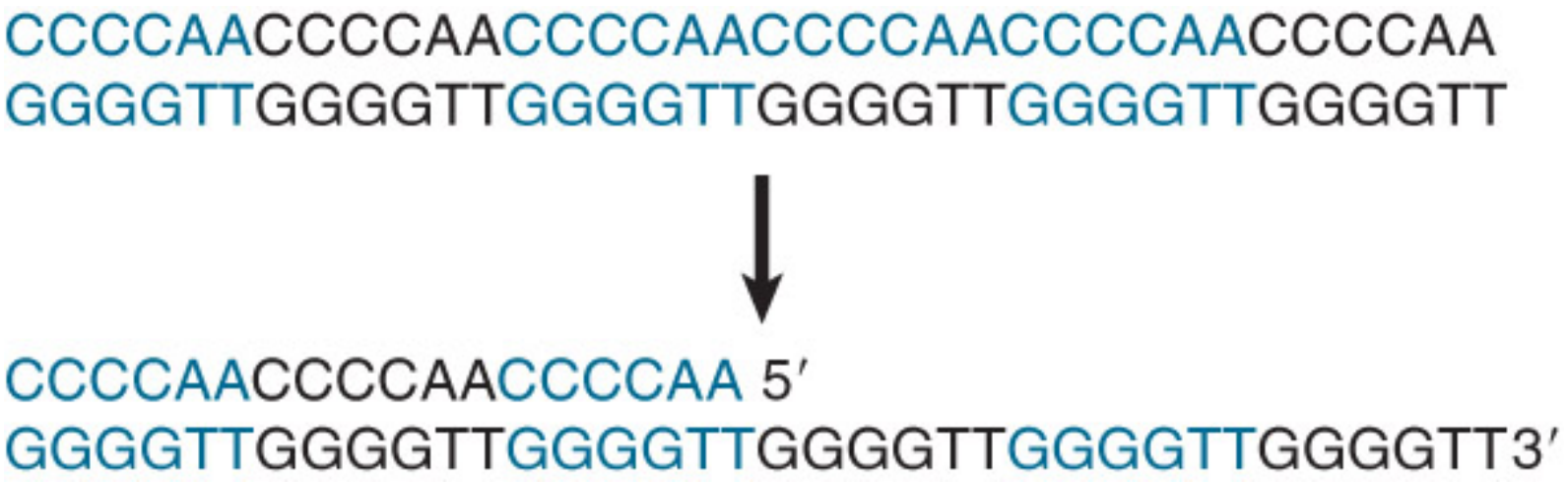
- Organized Structures -banding
- Centromeres
- **Telomeres**
- **Nucleosomes**
- **Euchromatin / Heterochromatin**



Replication could run off the 3’ end of a newly synthesized linear strand, but could it initiate at a 5’ end?

Telomeres Have Simple Repeating Sequences

- The **telomere** is required for the stability of the chromosome end.
- A telomere consists of a simple repeat where a C+A-rich strand has the sequence $C_{>1}(A/T)_{1–4}$, or conversely a G+T-rich has the sequence $(A/T)_{1–4}\,G_{>1}$



A typical telomere has a simple repeating structure with a G-T-rich strand that extends beyond the C-A-rich strand

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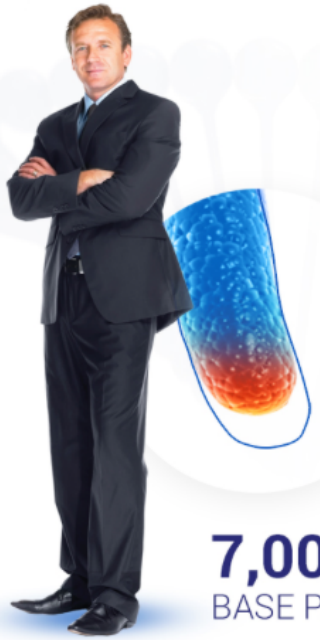
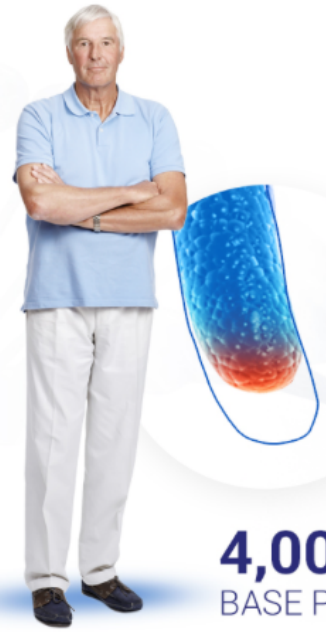
15,000
BASE PAIRS

10,000
BASE PAIRS



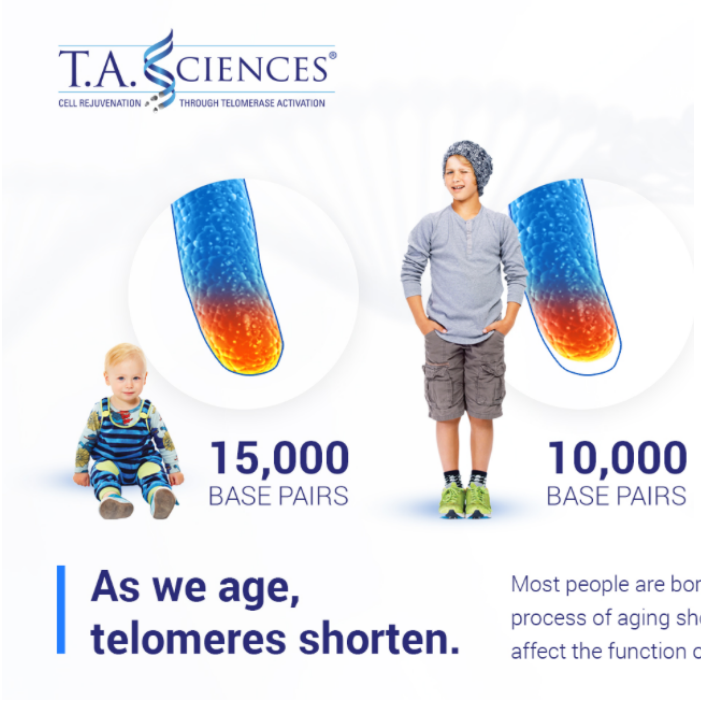
7,000
BASE PAIRS

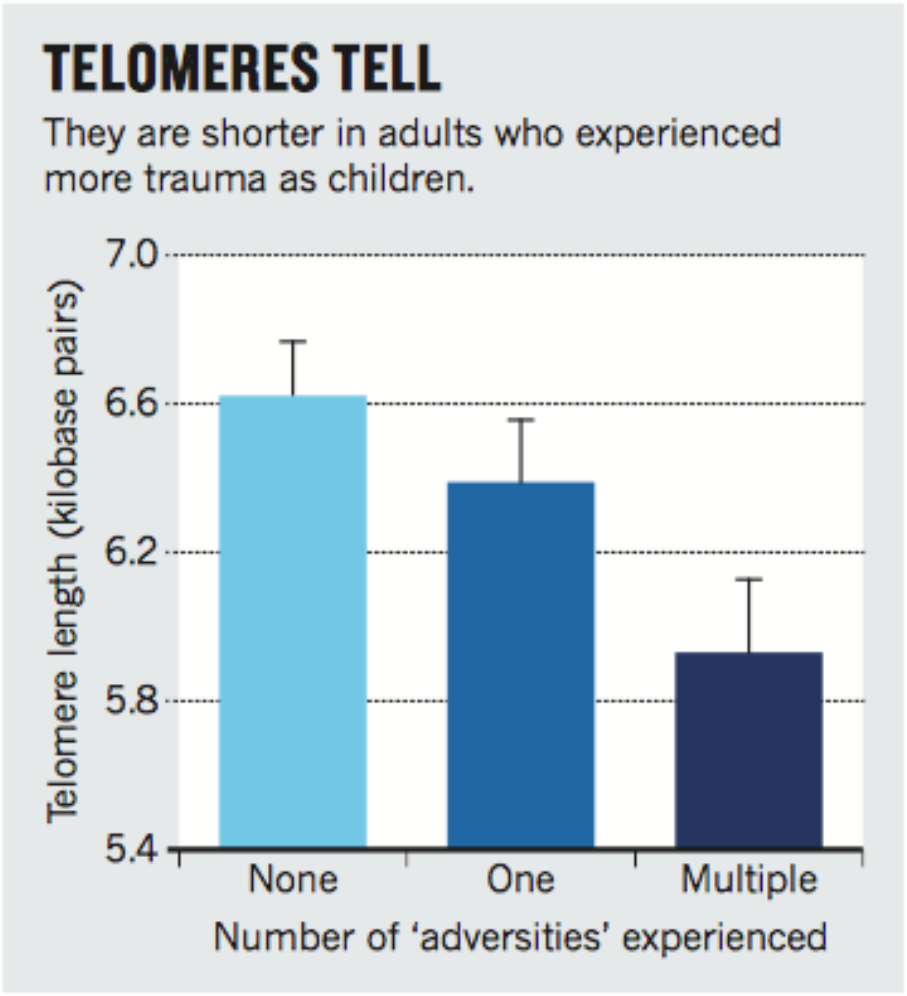


4,000
BASE PAIRS

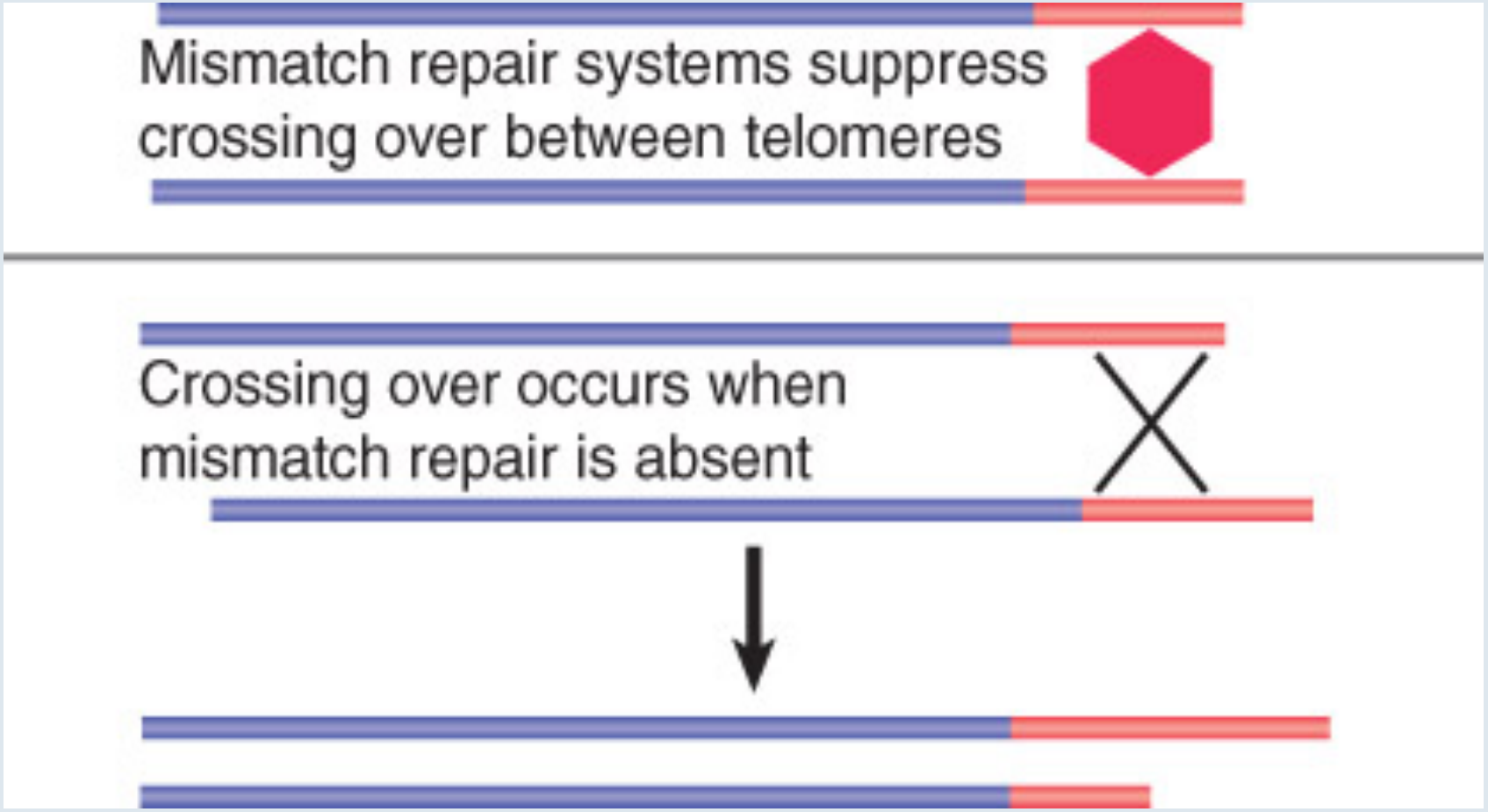
As we age, telomeres shorten.

Most people are born with telomeres over 15,000 base pairs long, but the natural process of aging shortens telomeres. When telomeres become critically short, it can affect the function of that cell.

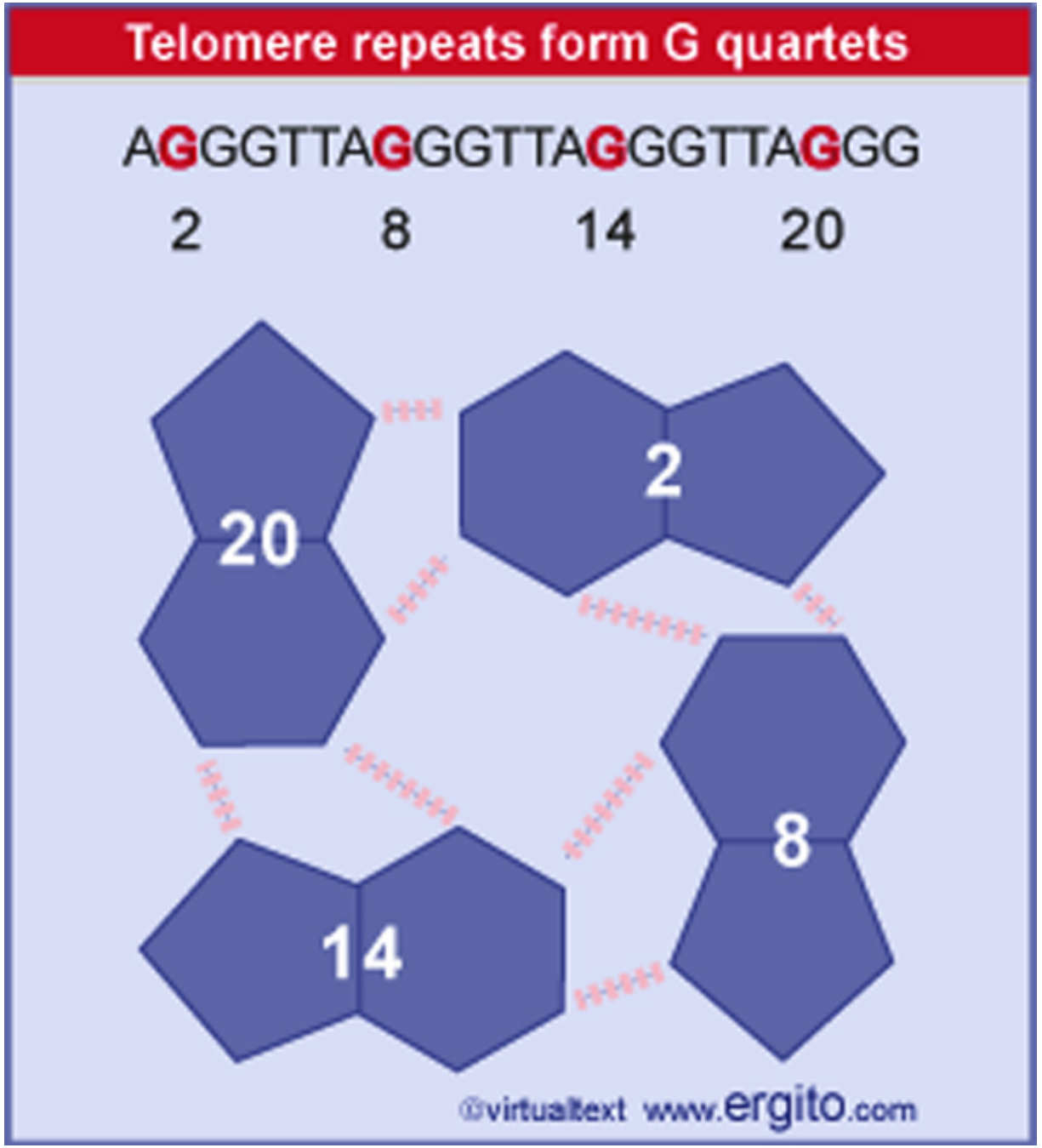




Blackburn EH, Epel ES. Too toxic to ignore. Nature. 2012;490:169-171



Crossing-over in telomeric regions is usually suppressed by mismatch-repair systems, but can occur when they are mutated.


Telomere repeats form G quartets
AGGGTTAGGGTTAGGGTTAGGG
2
8
14
20
20
2
14
8
©virtualtext www.ergito.com

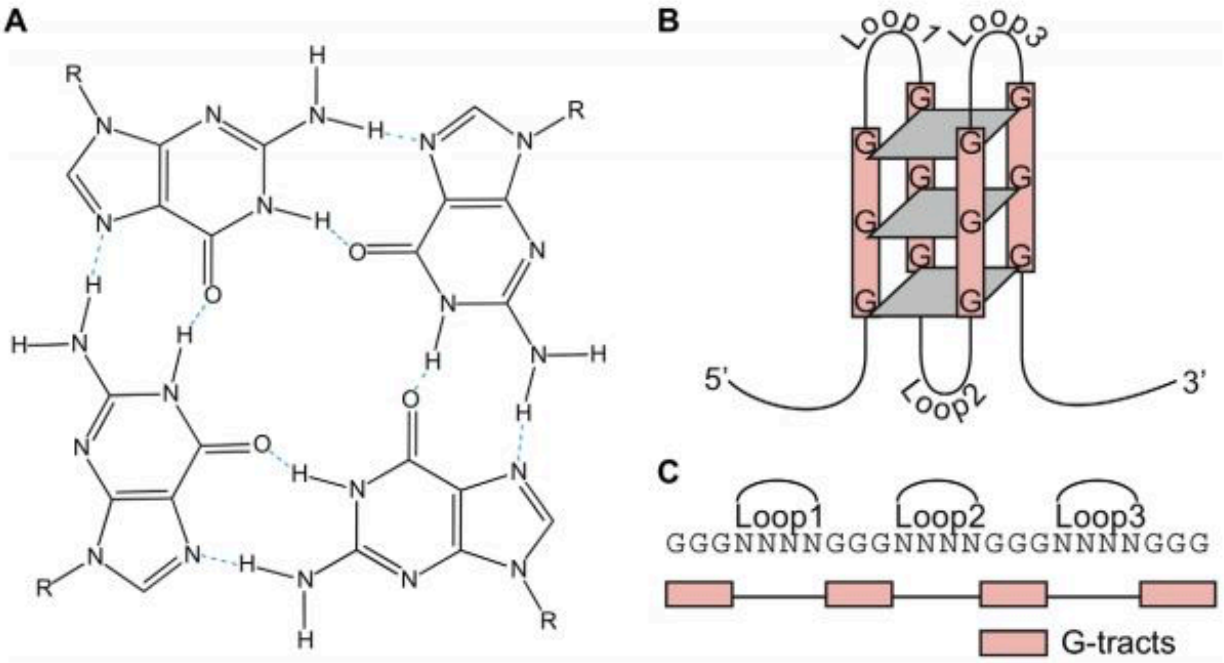
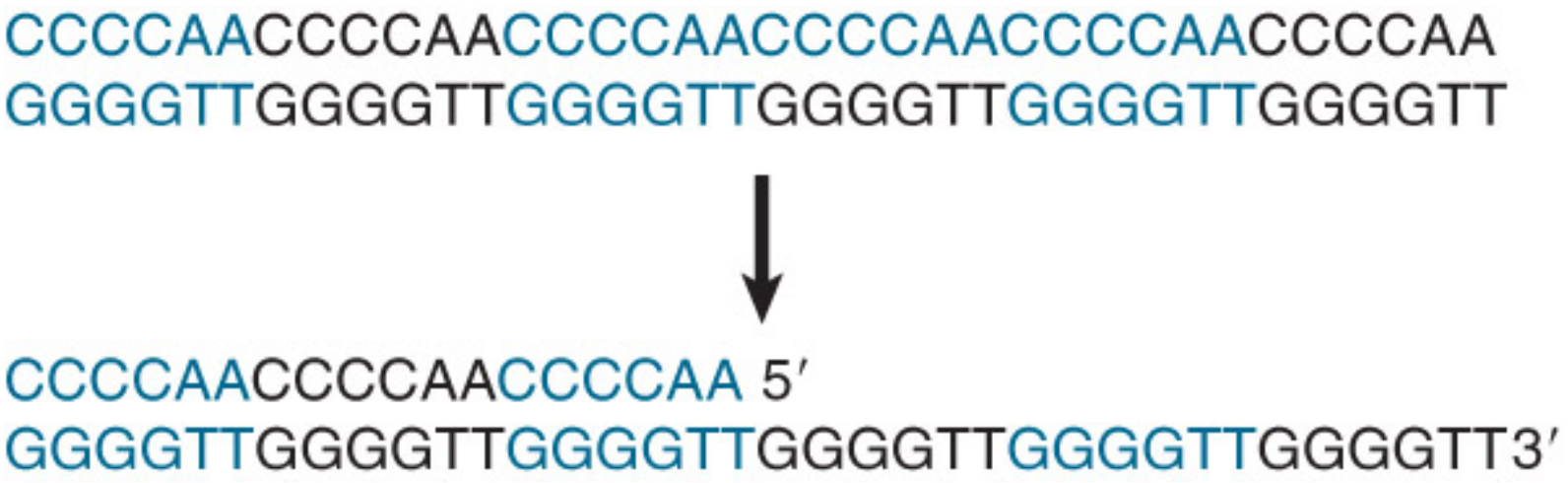


Figure 1. The G4 DNA structure and motif. (**A**) Structure of a G-quartet. The planar ring of four hydrogen-bonded guanines is formed by guanines from different G-tracts, which are separated by intervening loop regions in the intra-molecular G4 DNA structure. (**B**) Schematic of an intra-molecular G4 DNA structure consisting of three G-quartets. Inter-molecular G4 DNA structures can also form from two or four strands. (**C**) The G4 DNA motif sequence used in this study with four G-tracts of three guanines separated by loop regions.
doi:10.1371/journal.pcbi.1000861.g001

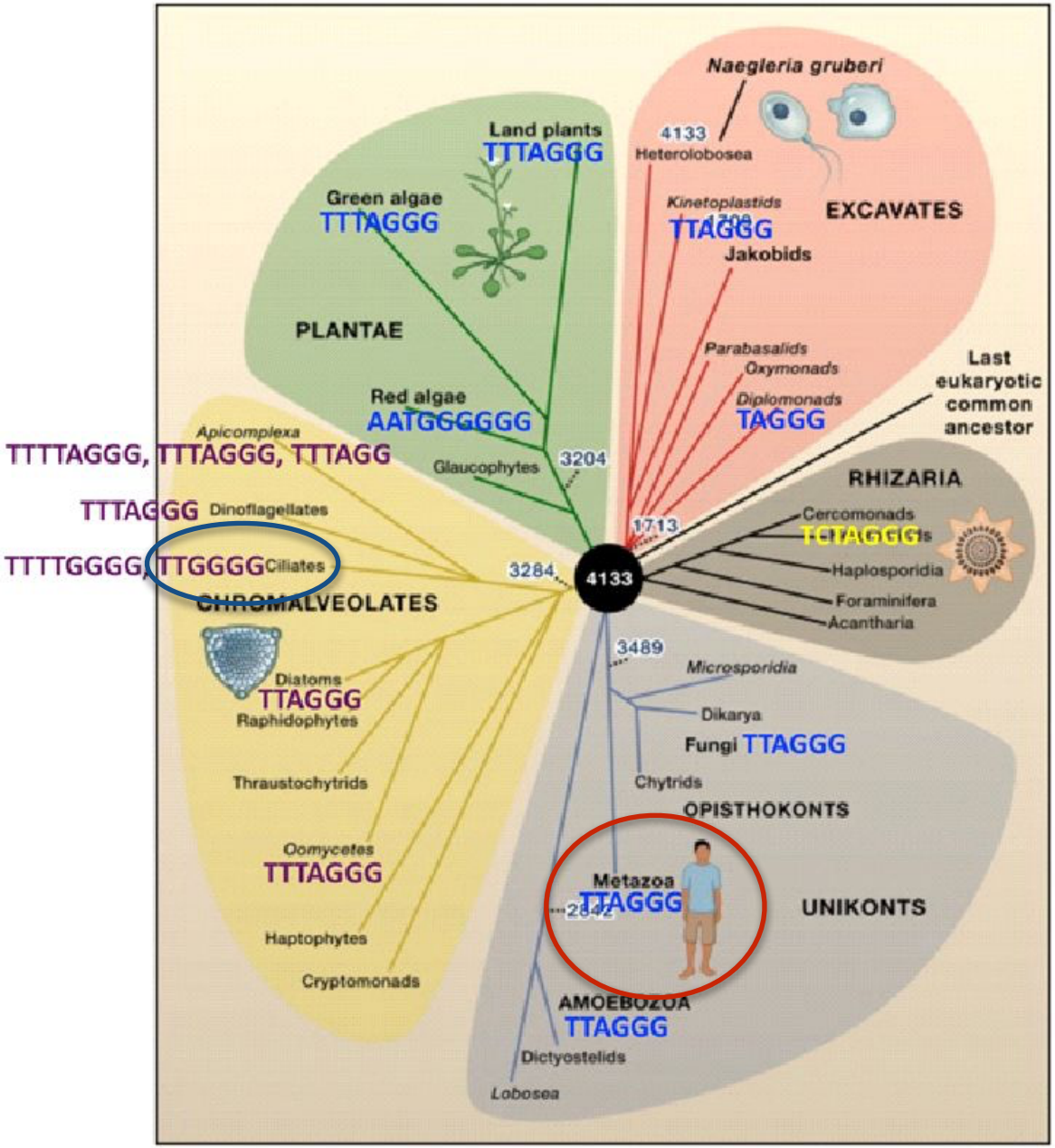
Fig. 1. Length-independent telomere damage. Telomere shortening that occurs naturally with each round of cell division ultimately leads to chromosome ends becoming exposed and activating a DNA damage response, which results in a permanent arrest known as replicative senescence. However, recent evidence suggests that telomeres may serve as highly sensitive sensors of stress. It is known that mild oxidative stress causes single-stranded breaks to accumulate at telomeres, leading to accelerate shortening and premature cell cycle arrest. However, it is possible that acute stresses induce telomeric double-stranded breaks which are not efficiently repaired. This results in a persistent DDR signaling, preventing cells from undergoing further rounds of replication irrespective of their telomere length, a state which can also be called "telomere length-independent senescence".

Telomeres Have Simple Repeating Sequences

- The **telomere** is required for the stability of the chromosome end.
- A telomere consists of a simple repeat where a C+A-rich strand has the sequence $C_{>1}(A/T)_{1–4}$, or conversely a G+T-rich has the sequence $(A/T)_{1–4}\,G_{>1}$



A typical telomere has a simple repeating structure with a G-T-rich strand that extends beyond the C-A-rich strand


Naegleria gruberi
4133
Heterolobosea
EXCAVATES
Kinetoplastids
TTAGGG
Jakobids
Land plants
TTTAGGG
Green algae
TTTAGGG
PLANTAE
Parabasalids
Oxymonads
Diplomonads
TAGGG
Last eukaryotic common ancestor
Red algae
AATGGGGGG
Apicomplexa
TTTTAGGG, TTTAGGG, TTTAGG
Glaucophytes
3204
RHIZARIA
TTTAGGG Dinoflagellates
Cercomonads
TTTAGGG
1713
TTTTGGGG, TTGGGG Ciliates
3284
4133
Haplosporidia
Foraminifera
Acantharia
CHROMALVEOLATES
3489
Microsporidia
Diatoms
TTAGGG
Raphidophytes
Dikarya
Fungi TTAGGG
Thraustochytrids
Chytrids
OPISTHOKONTS
Oomycetes
TTTAGGG
Metazoa
TTAGGG
UNIKONTS
Haptophytes
Cryptomonads
AMOEBOZOA
TTAGGG
Dictyostelids
Lobosea

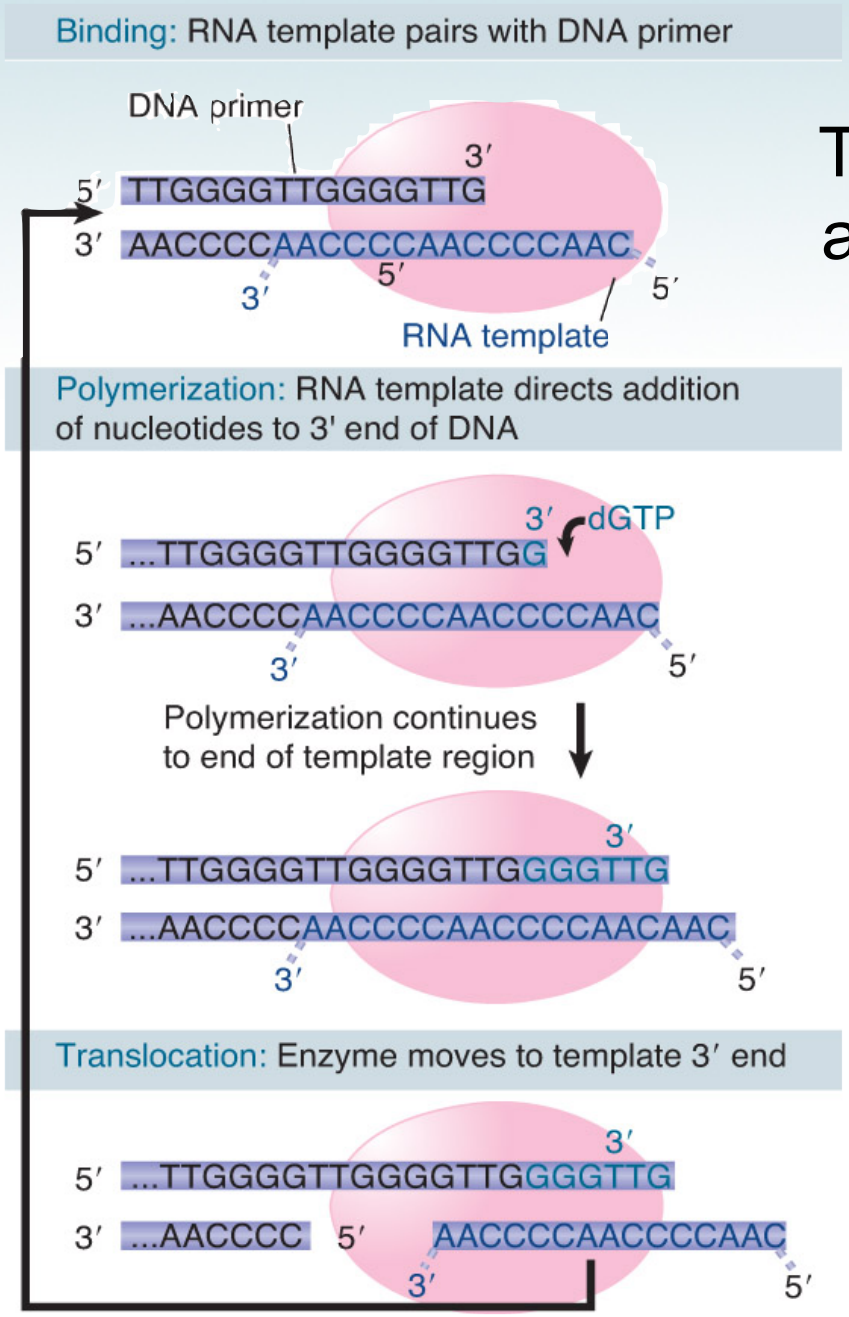
Telomeres Are Synthesized by a Ribonucleoprotein Enzyme

- **Telomerase** (TERT) uses the 3′–OH of the G+T telomeric strand to prime synthesis of tandem TTGGGG repeats (in Ciliates) and TTAGGG in humans.

- The RNA component of telomerase (TE**RC**) is a sequence of RNA of varying length (159 bases - 451 bases) and has a sequence that pairs with the C+A-rich repeats at the end of chromosomes.

- One of the protein subunits within the TERT is a reverse transcriptase that uses the RNA as template to synthesis the G+T-rich sequence (RNA Dependent DNA Polymerase; RDDP).

Telomeres Are Synthesized by a Ribonucleoprotein Enzyme, Telomerase (TERT)



Telomerase positions itself by base pairing between the RNA template and the protruding single-stranded DNA primer

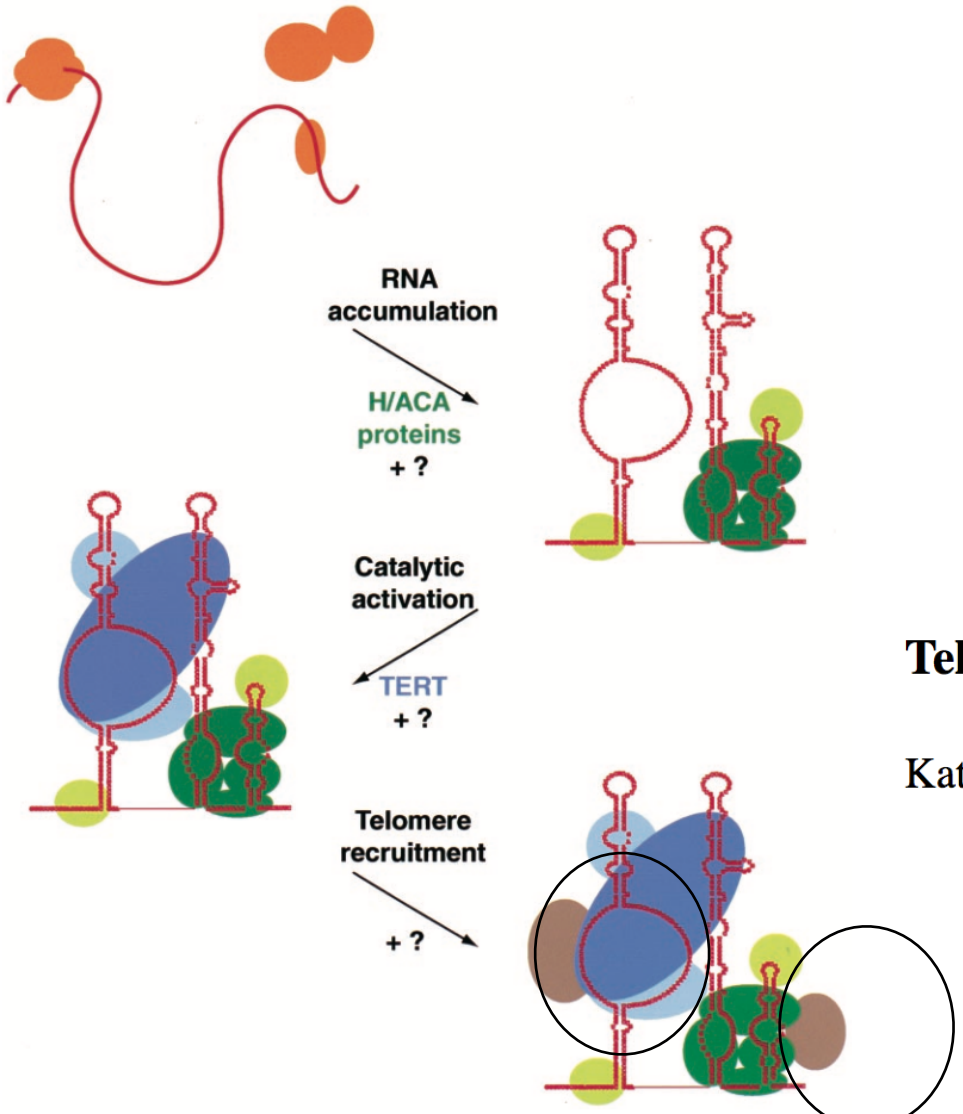
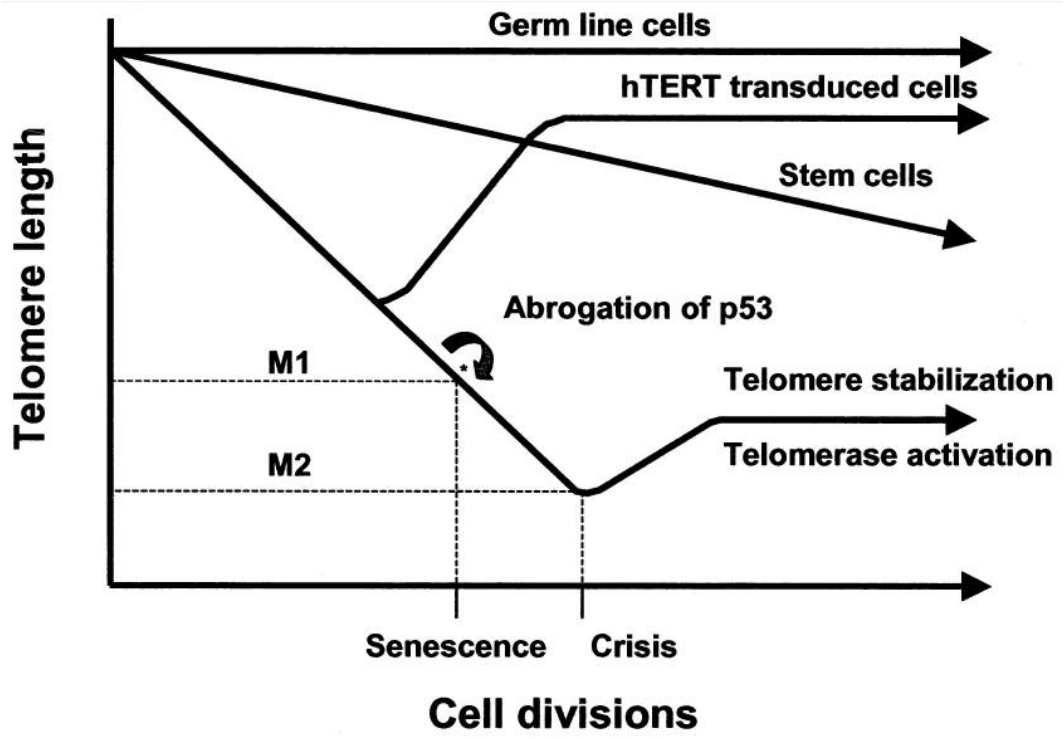


Figure 1 Biogenesis of biologically active human telomerase enzyme. Step 1: telomerase RNA synthesis and maturation require the participation of numerous cellular factors, which together will establish the level of mature RNA accumulation. Some factors (orange) will associate only transiently with telomerase RNA (red). H/ACA proteins (darker green) and other hTR binding proteins (lighter green) become bound to telomerase RNA, providing RNA stability. Step 2: catalytic activation requires the association of telomerase RNP with TERT (dark blue) and possibly other proteins as well (lighter blue). TERT binds directly to telomerase RNA in at least two separable regions. Step 3: active holoenzyme must be recruited to telomere substrates. Proteins that interact with active telomerase (brown) may regulate its recruitment to telomeres by interacting with telomeric chromatin directly or by affecting telomerase subnuclear localization

Telomerase in the human organism

Kathleen Collins and James R Mitchell[a]



Two-step hypothesis of cellular senescence and immortalization. Unlike **germ cells,** in which telomere length is maintained by telomerase, most human somatic cells have lower levels of telomerase or are telomerase negative and experience telomere shortening with each cell division.

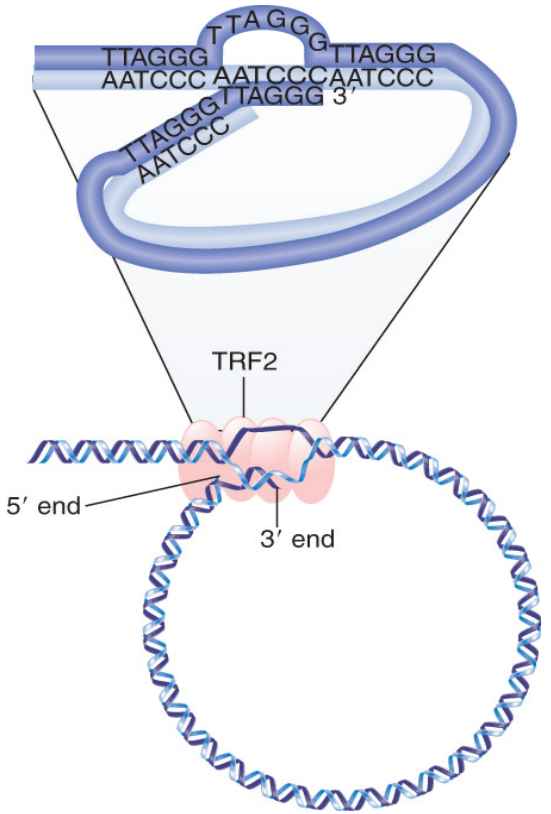
Pluripotent stem cells are **telomerase positive** -but DO NOT necessarily maintain full telomere length.
Telomere length shortens in stem cells at rates slower than that of telomerase-negative somatic cells.
Critically shortened telomeres may signal cells to enter senescence at, what is called the "**Hayflick limit",** or "**M1"**.

This proliferative checkpoint can be overcome by inactivation of **pRB/p16** or **p53**.
Such cells continue to suffer telomere erosion and ultimately enter crisis, or **M2**, characterized by widespread cell death. Rarely surviving cells acquire unlimited proliferative potential and stabilization of telomere length by reactivation of telomerase.

When cells are cultured in adequate conditions, ectopic expression of hTERT allows cells to bypass proliferation barriers and become immortal.

Telomeres Seal the Chromosome Ends and Function in Meiotic Chromosome Pairing

- The protein **TRF2** catalyzes a reaction in which the 3′ repeating unit of the G+T-rich strand forms a loop upstream of the end of the chromosome by displacing its homolog in an upstream region of the telomere -forming a D-loop.



A loop forms at the end of chromosomal DNA

Photo courtesy of Jack Griffith, University of North Carolina at Chapel Hill.

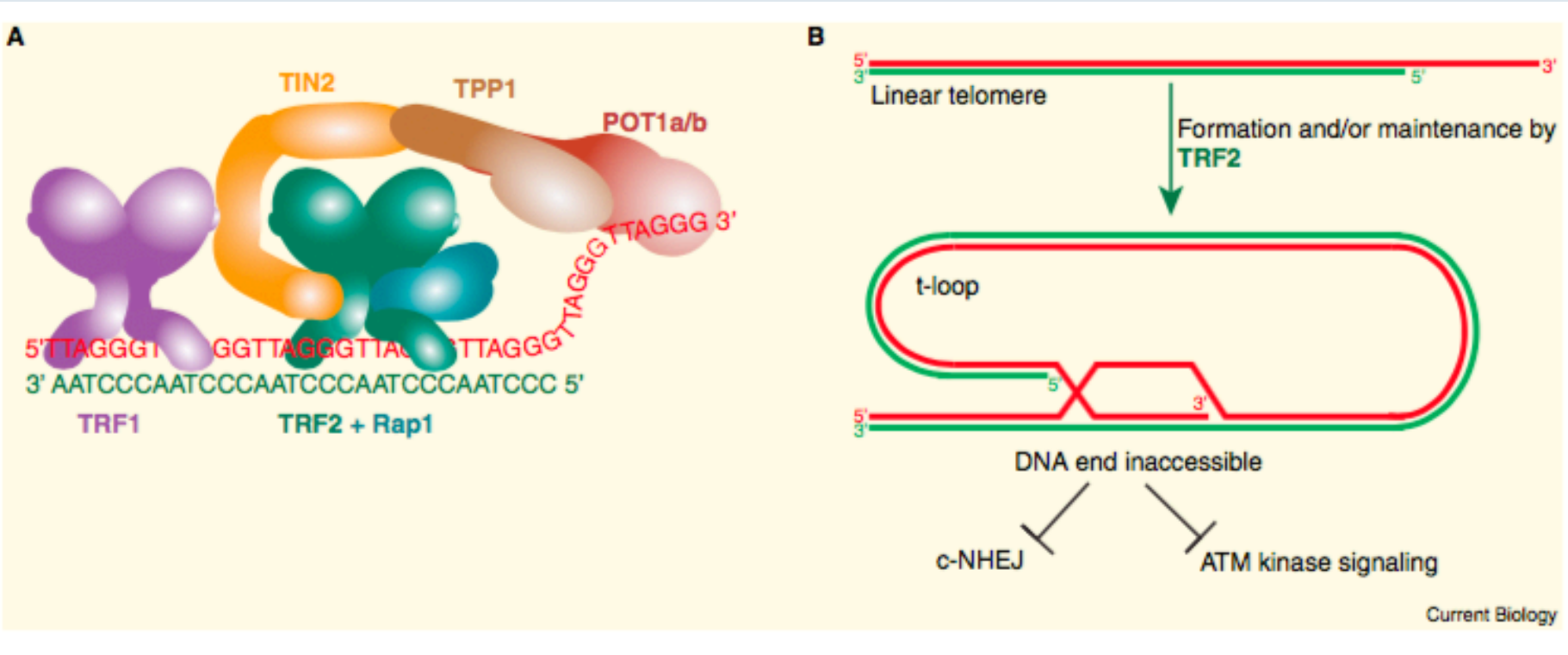
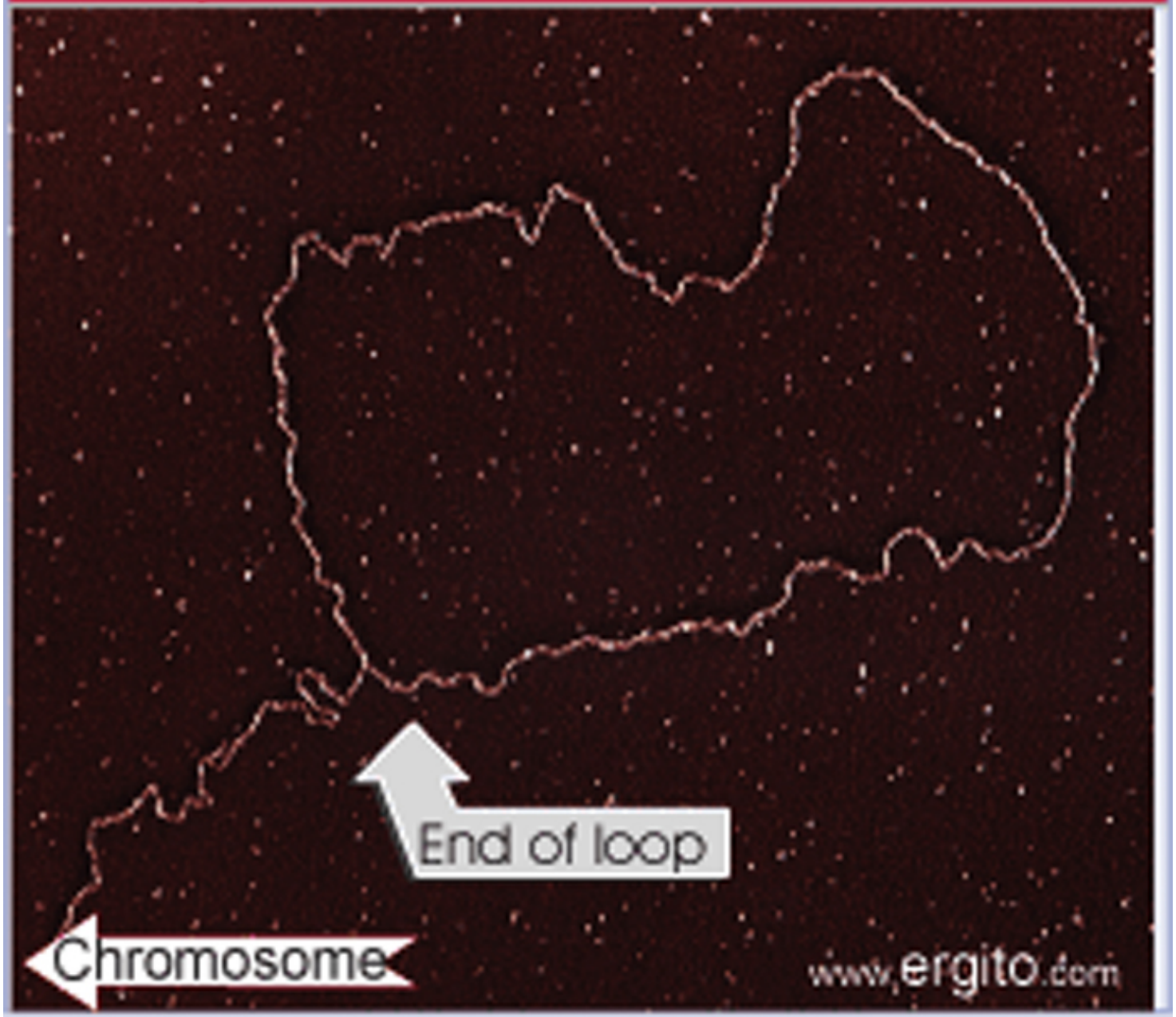


Figure 1. The shelterin complex protects telomeres from DNA damage signaling and repair.

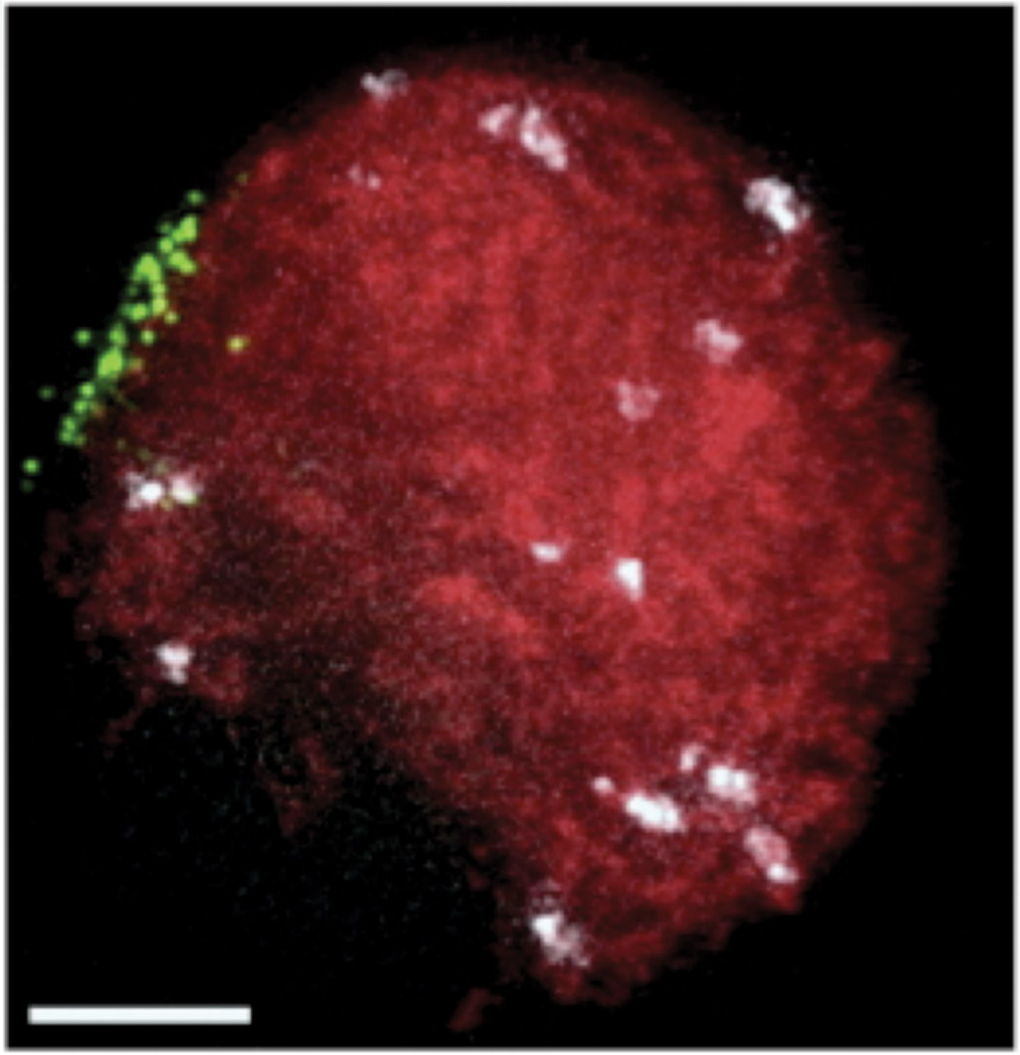
At mammalian telomeres, the presence of shelterin (A) ensures genome integrity by repressing DNA damage signaling and repair, promoting semi- conservative replication of the telomeric DNA, and regulating telomerase-mediated telomere maintenance. The t-loop structure (B) is crucial for repression of c-NHEJ and ATM kinase signaling.

Current Biology 26, R387–R407, May 23, 2016

A loop seals the end of the chromosome



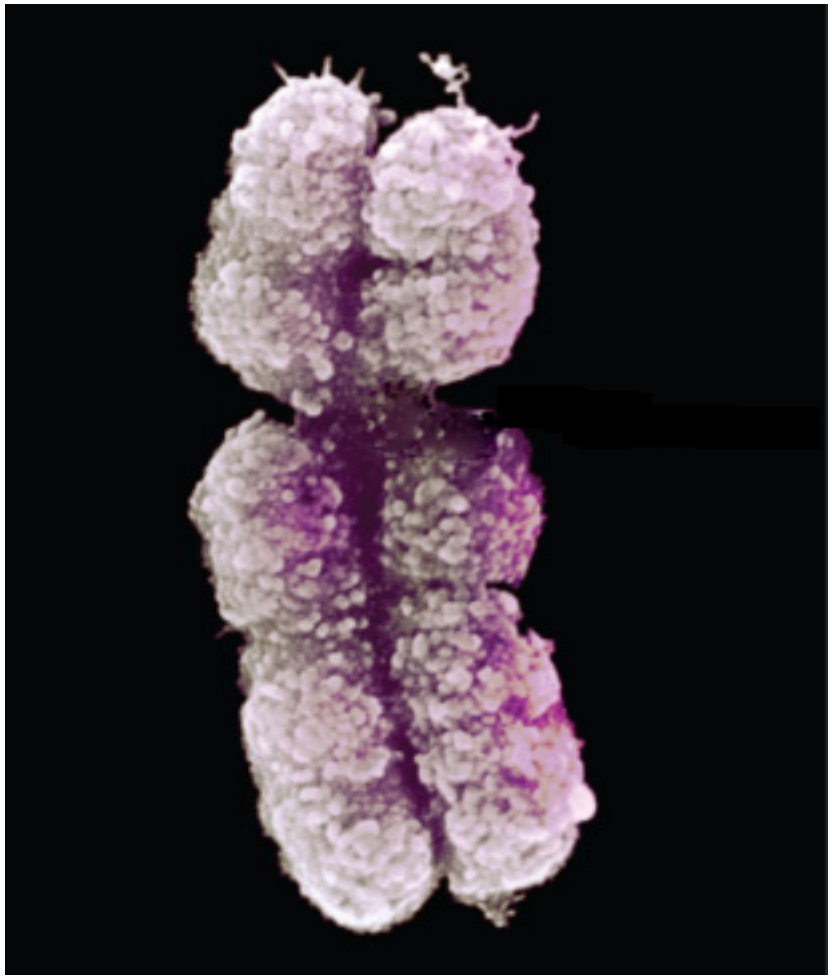
Telomeres Seal the Chromosome Ends and Function in Meiotic Chromosome Pairing



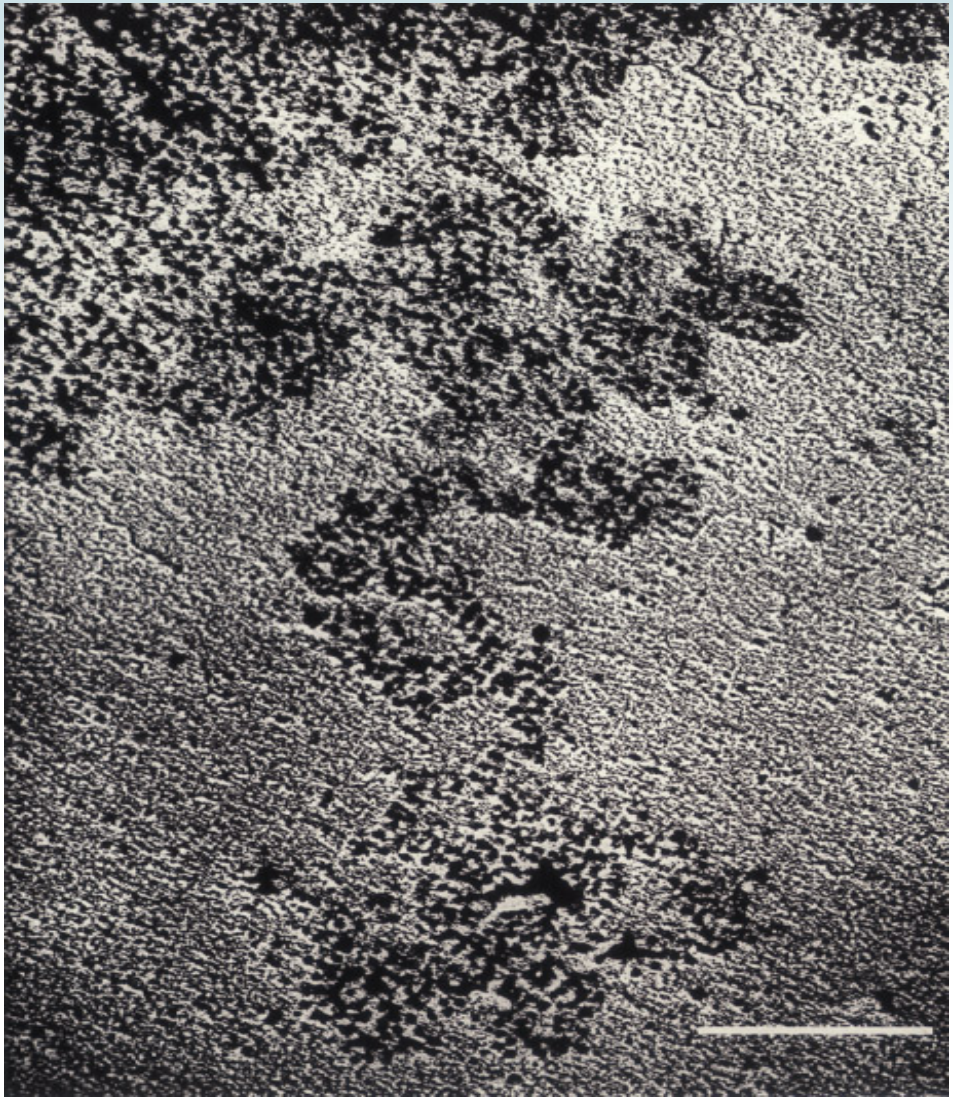
The meiotic telomere cluster is visualized by telomere **FISH**

Photo courtesy of S. P. Murphy and H. W. Bass, Florida State University.

The Eukaryotic chromosome



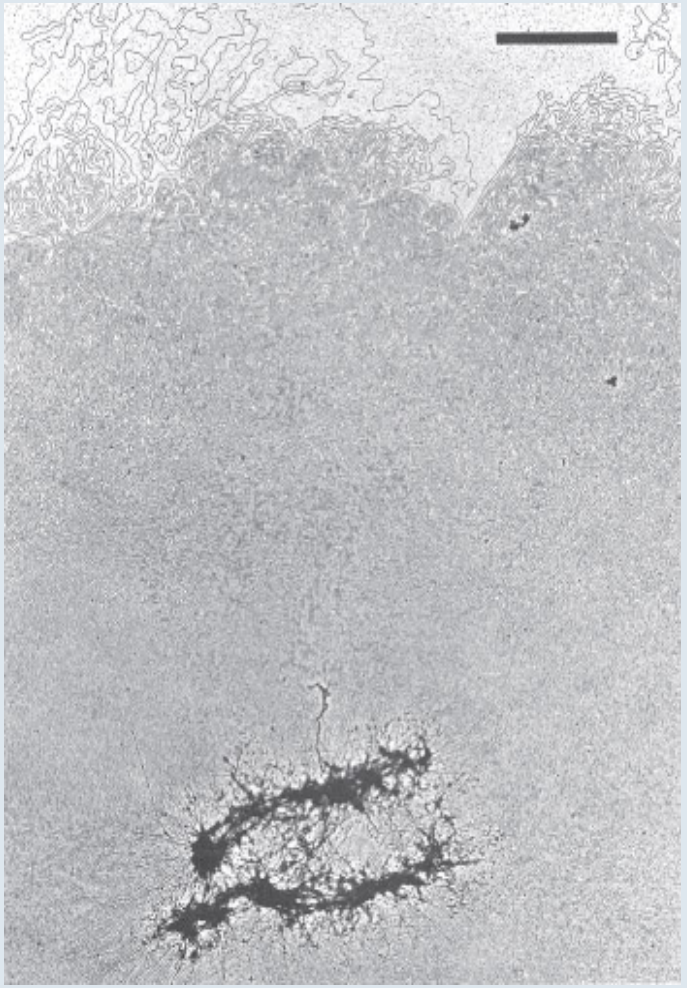
- Organized Structures -banding
- Centromeres
- Telomeres
- **Nucleosomes**
- **Euchromatin / Heterochromatin**



Chromatin spilling out of lysed nuclei consists of a compactly organized series of particles. The bar is 100 nm

- DNA of interphase chromatin is negatively supercoiled into independent domains of ~85 kb.

- Metaphase chromosomes have a protein **scaffold** to which the loops of supercoiled DNA are attached.



Histone-depleted chromosomes consist of a protein scaffold to which loops of DNA are anchored.

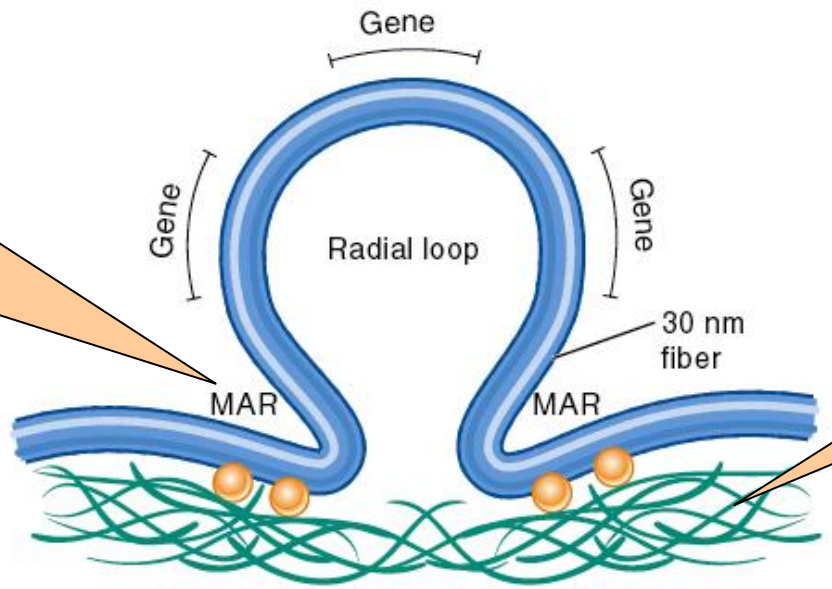
Further Compaction of the Chromosome

- An additional level of compaction involves interaction between the 30 nm fibers and the nuclear matrix

Matrix-attachment regions

or

Scaffold-attachment regions (SARs)

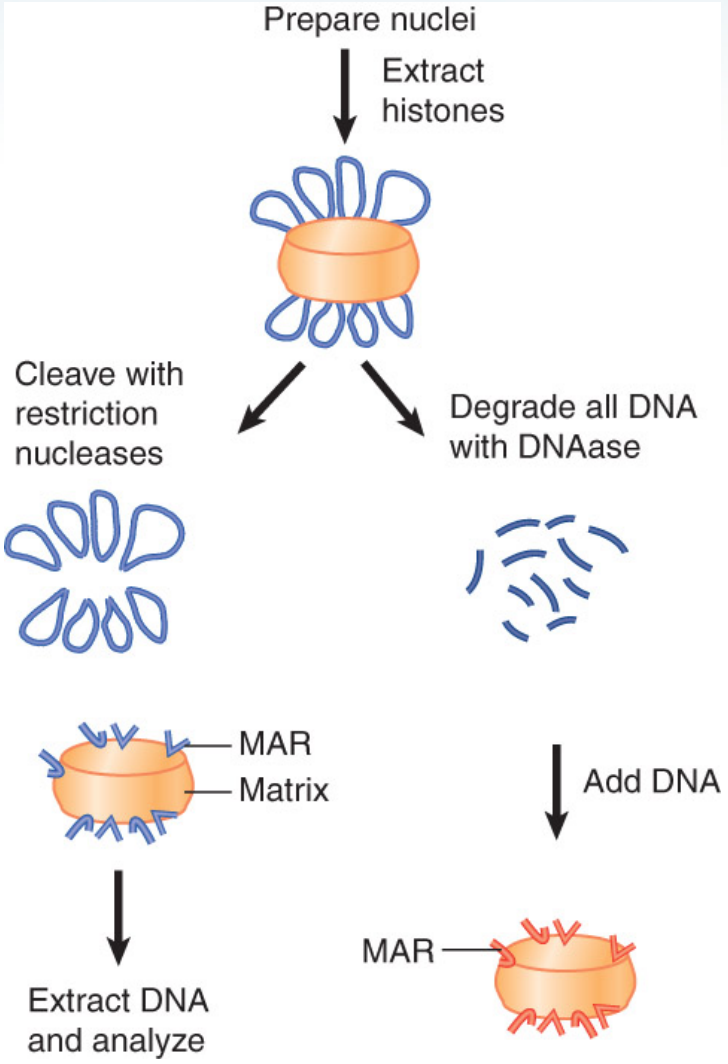


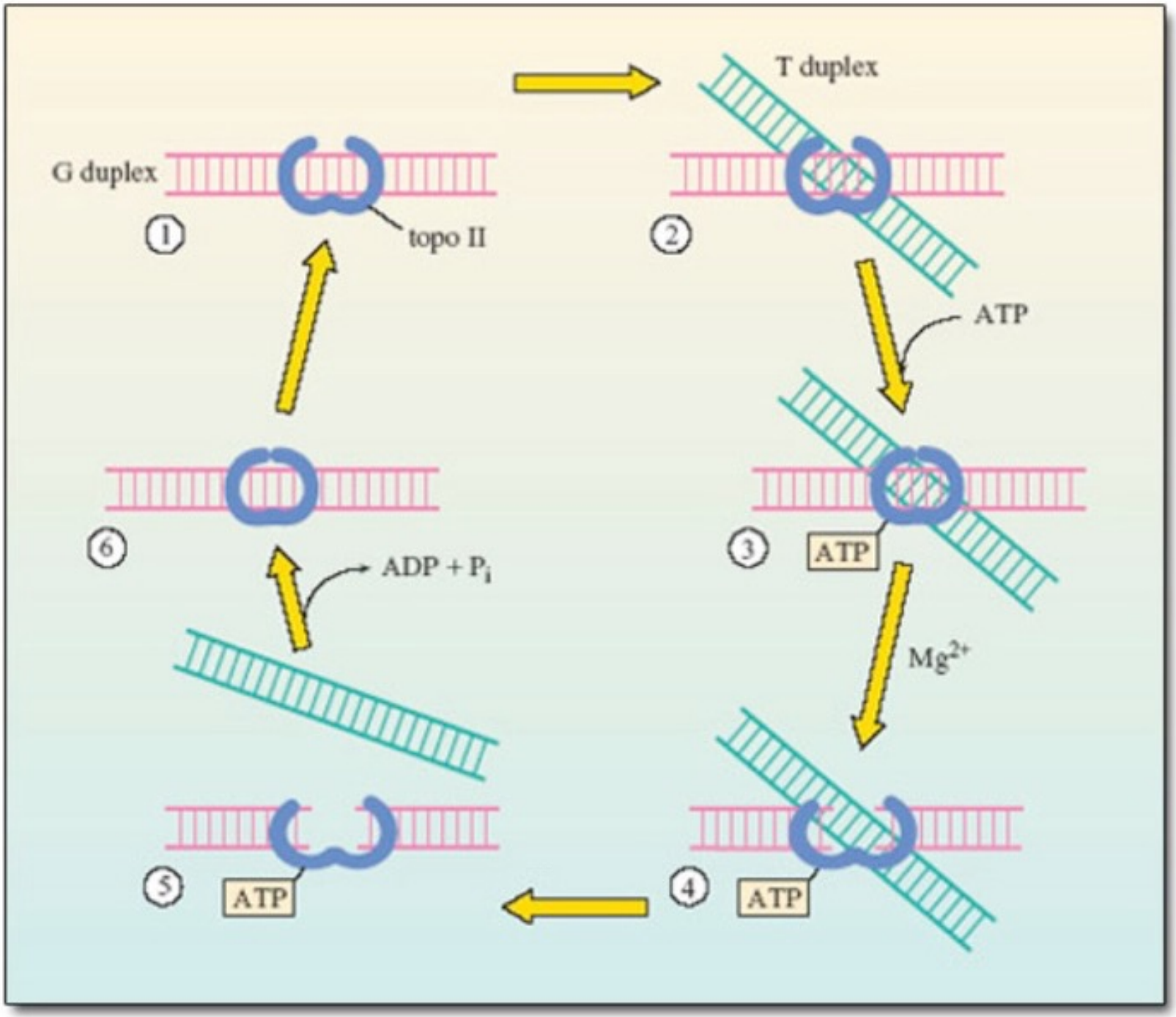
MARs are anchored to the nuclear matrix, thus creating radial loops

Specific Sequences Attach DNA to an Interphase Matrix

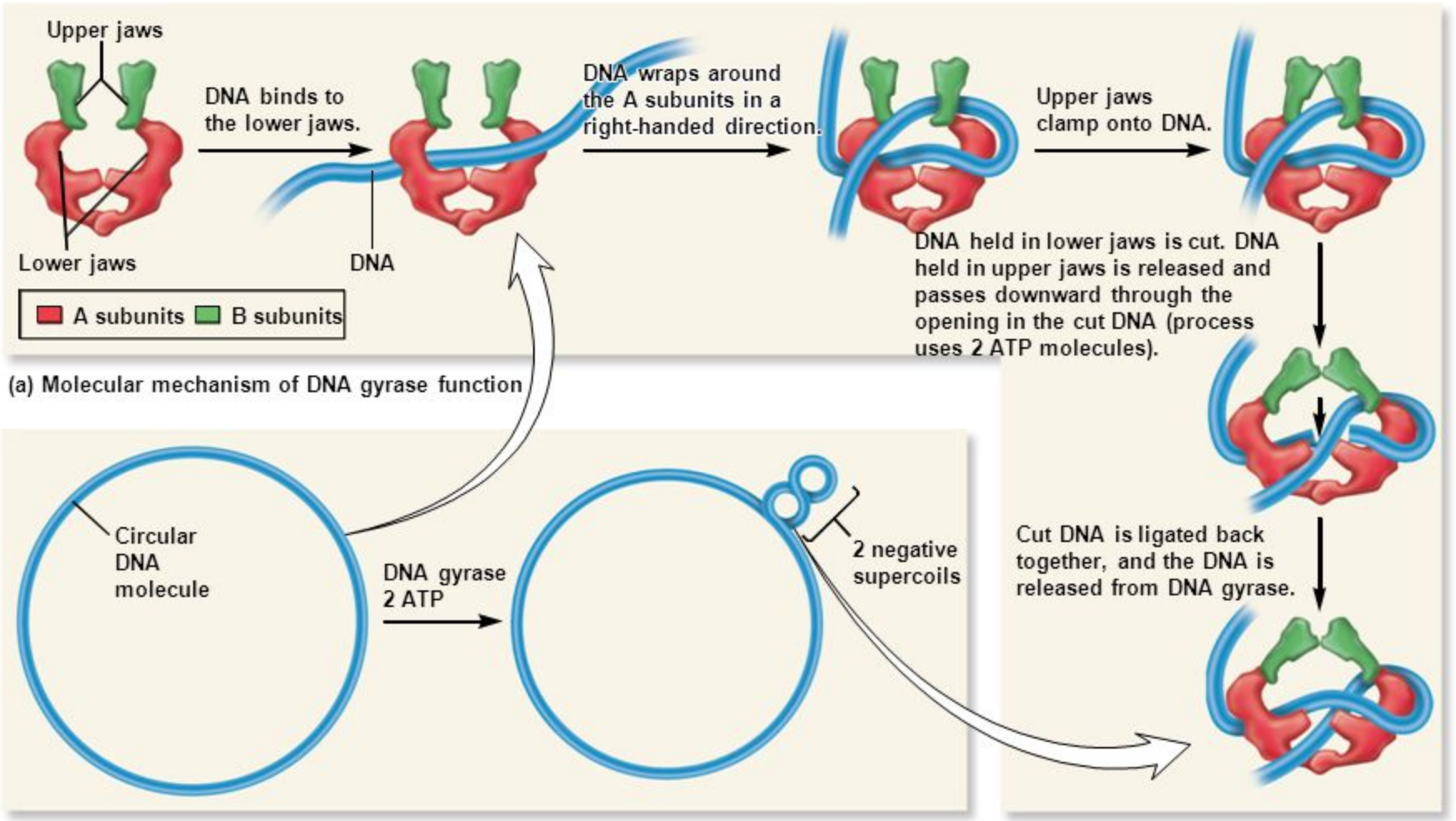
- DNA is attached to the nuclear matrix at specific sequences called **MARs** or **SARs**.
- The MARs are A-T-rich but do not have any specific consensus sequence.
- **Chromosome scaffold –** A proteinaceous structure in the shape of a sister chromatid pair, generated when chromosomes are depleted of histones.

Identifying matrix-associated regions of DNA

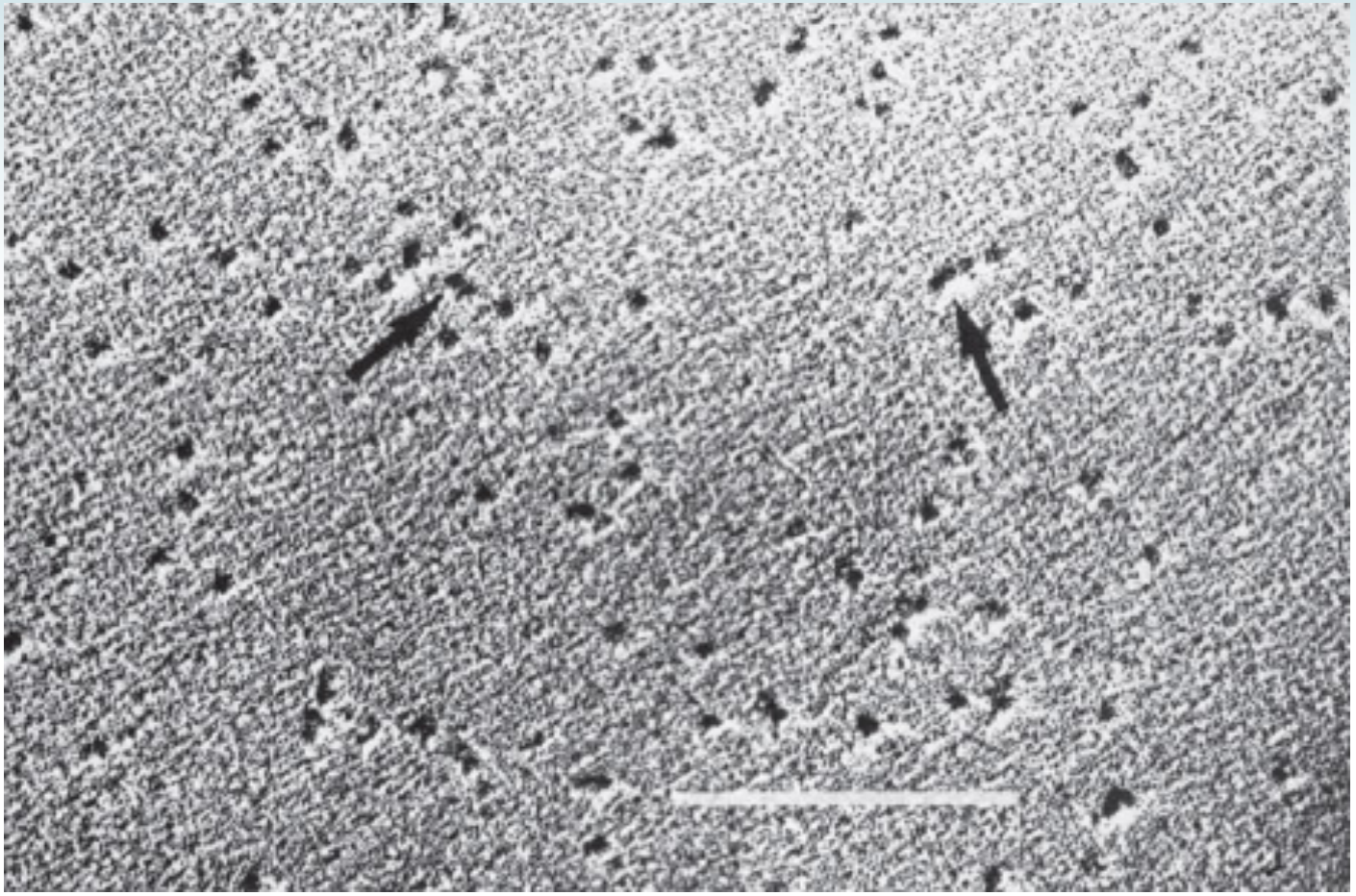



G duplex
1
topo II
T duplex
2
ATP
3
ATP
Mg2+
4
ATP
5
ATP
6
ADP + Pi

Model for coiling activity of Topoisomerase II (Gyrase)



Brooker, Fig 12.6



Individual nucleosomes are released by digestion of chromatin with micrococcal nuclease. The bar is 100 nm

There are effectively **five classes of histones**.

The core of a nucleosome contains eight histone molecules, two each from four of the histone classes.

There are **147 - 160 base pairs** of DNA wrapped around the core, or >1.65 turns of DNA.

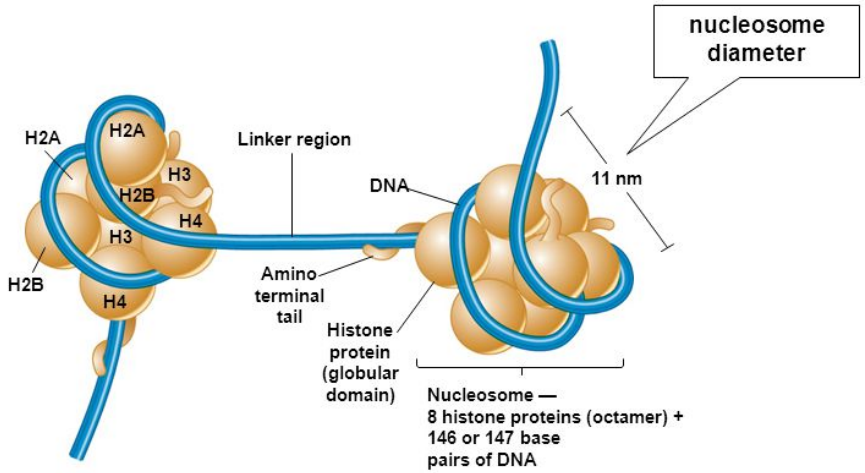
One molecule from the remaining histone class, histone **H1**, clamps the DNA to the core, and helps form the next level of packaging.

During **mitosis** and **meiosis**, the **chromatin** becomes even more heavily coiled and condensed.

Regions of chromatin that remain densely packed throughout interphase are termed **heterochromatin.**

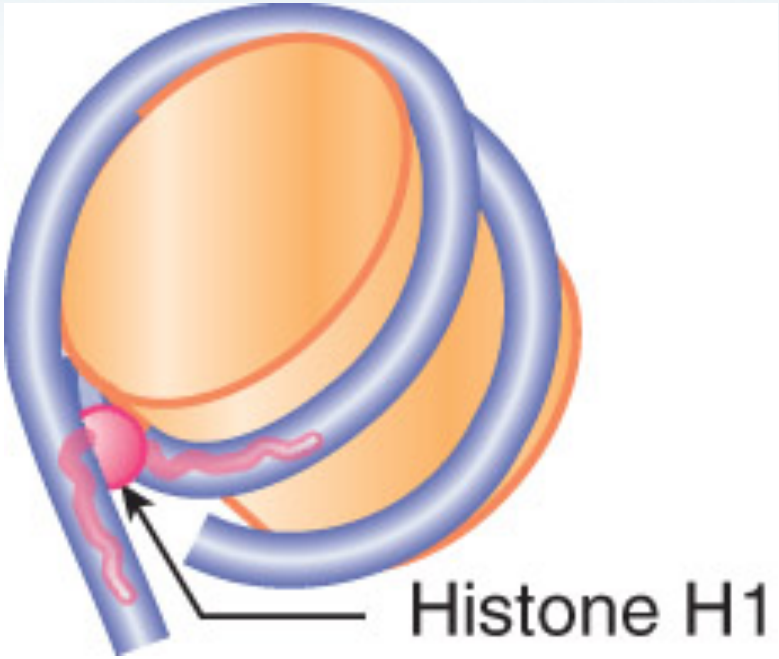
Chromocenter – An aggregate of heterochromatin from different chromosomes.

Nucleosomes shorten DNA ~seven-fold



(a) Nucleosomes showing core histone proteins

Brooker, Fig 12.10a



- Linker DNA is the region of 8 to 114 bp, and this is the region that is susceptible to early cleavage by the enzyme.
- Changes in the length of linker DNA account for the variation in total length of nucleosomal DNA.
- H1 proteins are associated with linker DNA and may lie at the point where DNA enters and leaves the nucleosome.

Possible model for the interaction of **histone H1** with the nucleosome

table 24–3

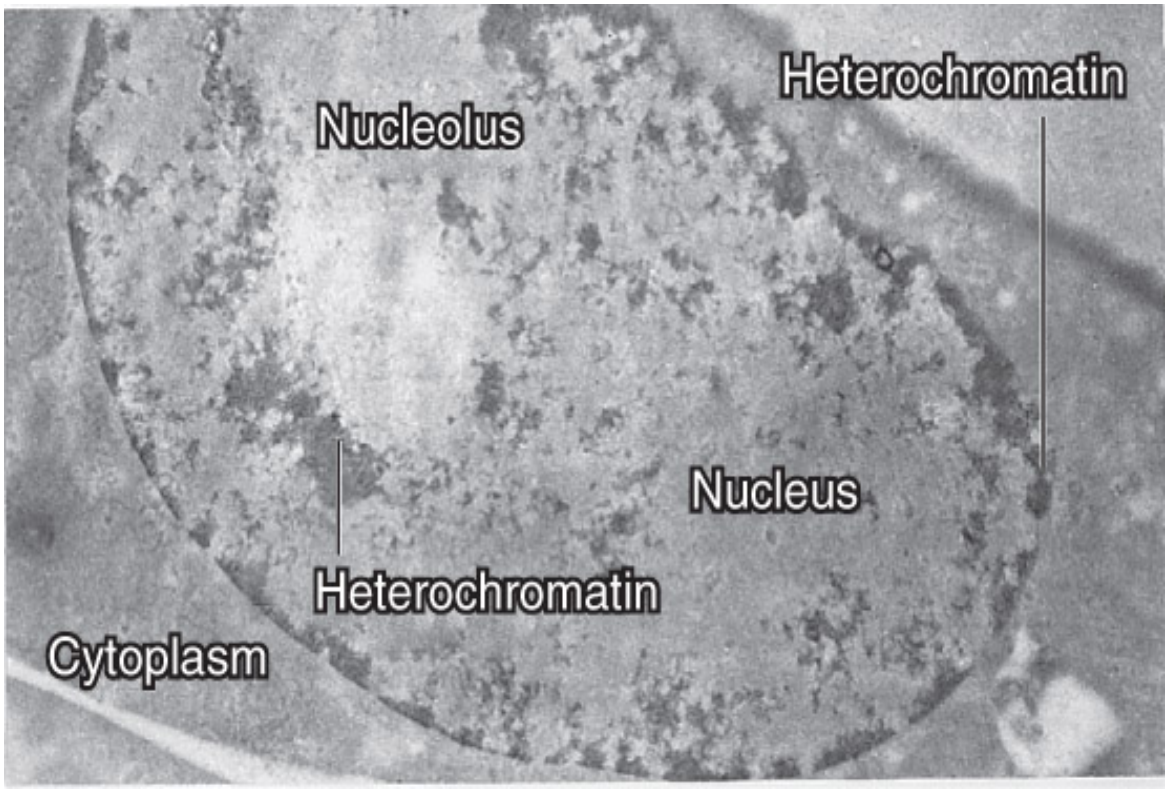
Types and Properties of Histones

Histone	Molecular weight	Number of amino acid residues	Content of basic amino acids (% of total)	
			Lys	Arg
H1*	21,130	223	29.5	1.3
H2A*	13,960	129	10.9	9.3
H2B*	13,774	125	16.0	6.4
H3	15,273	135	9.6	13.3
H4	11,236	102	10.8	13.7

*The sizes of these histones vary somewhat from species to species. The numbers given here are for bovine histones.

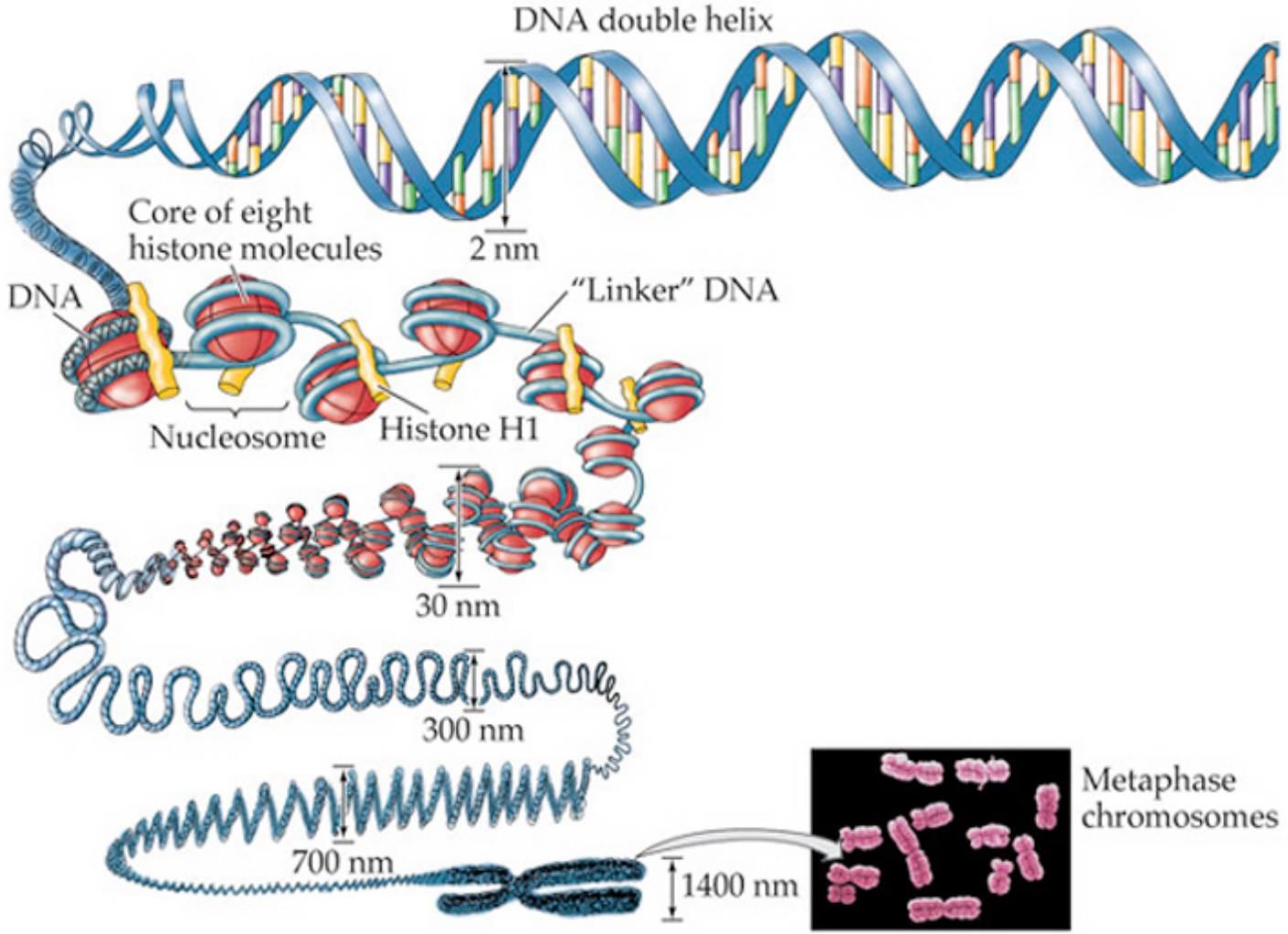
Chromatin Is Fundamentally Divided into Euchromatin and Heterochromatin

- Individual chromosomes can be seen only during mitosis.
- During interphase, the general mass of chromatin is in the form of **euchromatin**, which is slightly less tightly packed than mitotic chromosomes.



Regions of compact heterochromatin are clustered near the **nucleolus** and **nuclear membrane**

Photo courtesy of Edmund Puvion, Centre National de la Recherche Scientifique

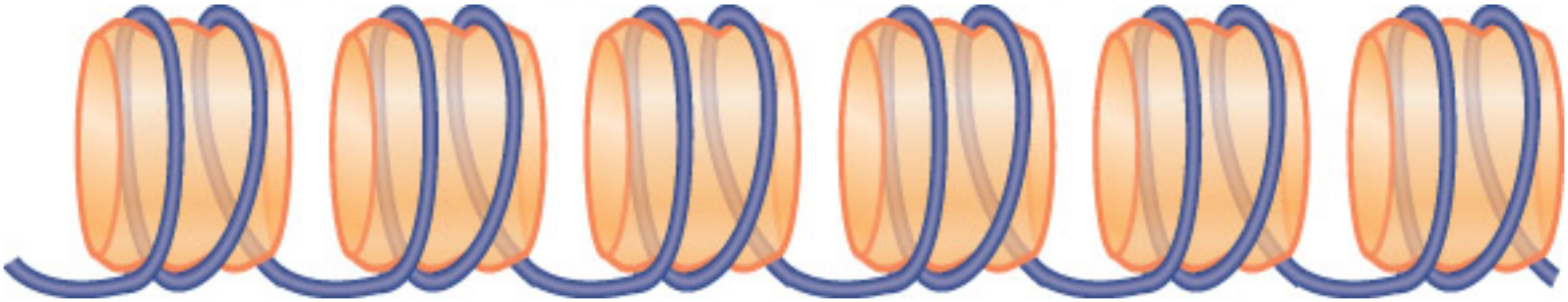


LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.6 DNA Packs into a Mitotic Chromosome

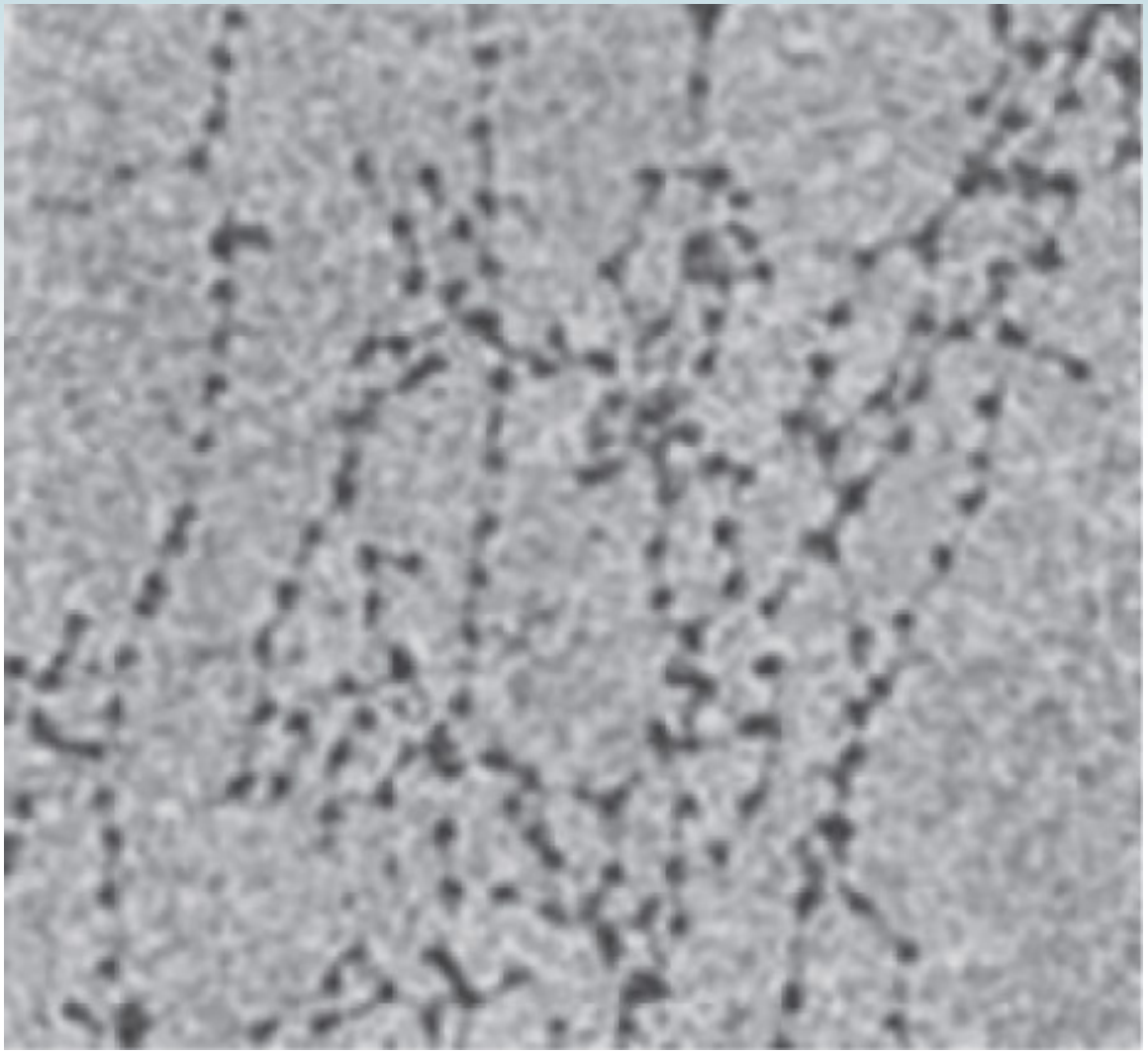
Chromatin: Basic Structures

- **nucleosome –** The basic structural subunit of chromatin, consisting of ~200 bp of DNA wrapped around an octamer of **histone** proteins.

- **histone tails** – Flexible amino- or carboxy-terminal regions of the core histones that extend beyond the surface of the nucleosome.
  - Histone tails are sites of extensive post translational modification.



The 10 nm fiber is a continuous string of nucleosomes



The 10 nm fiber in partially unwound state can be seen to consist of a string of nucleosomes

Photo courtesy of Barbara Hamkalo, University of California, Irvine.

Chromatin: Basic Structures

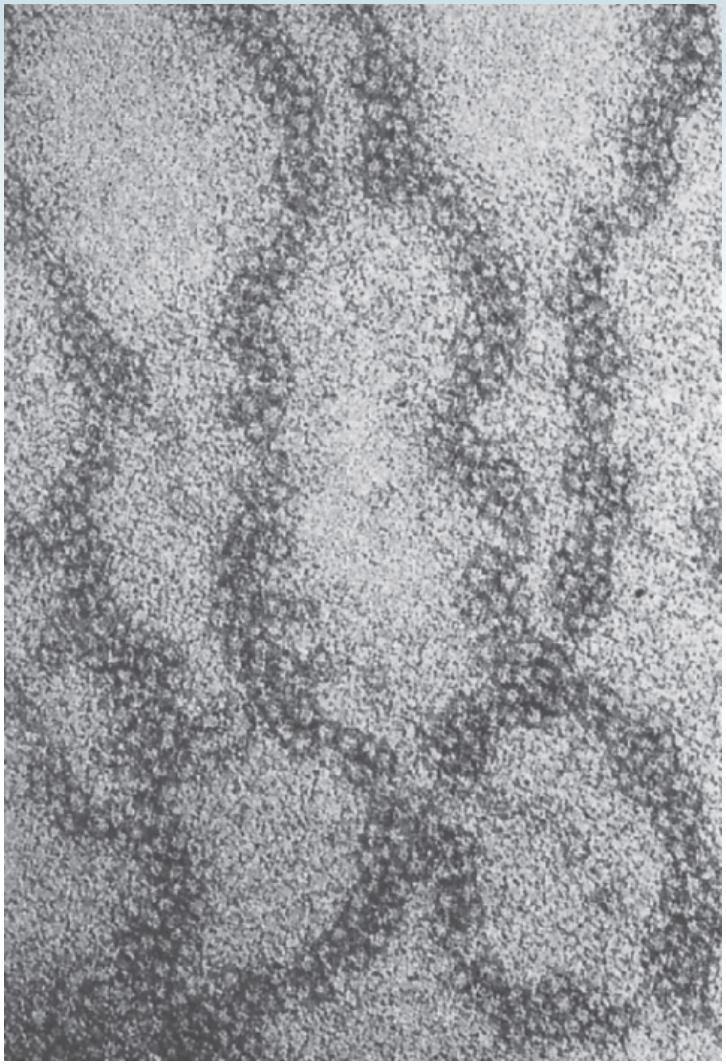
- **10 nm fiber** – a linear array of nucleosomes generated by unfolding from the natural condition of chromatin.

- **linker histones** – a family of histones (substantially the histone **H1 family**) that are NOT components of the nucleosome core.
  - Linker histones bind nucleosomes and/or linker DNA and promote formation of the **30 nm fiber**s.

Chromatin: Basic Structures

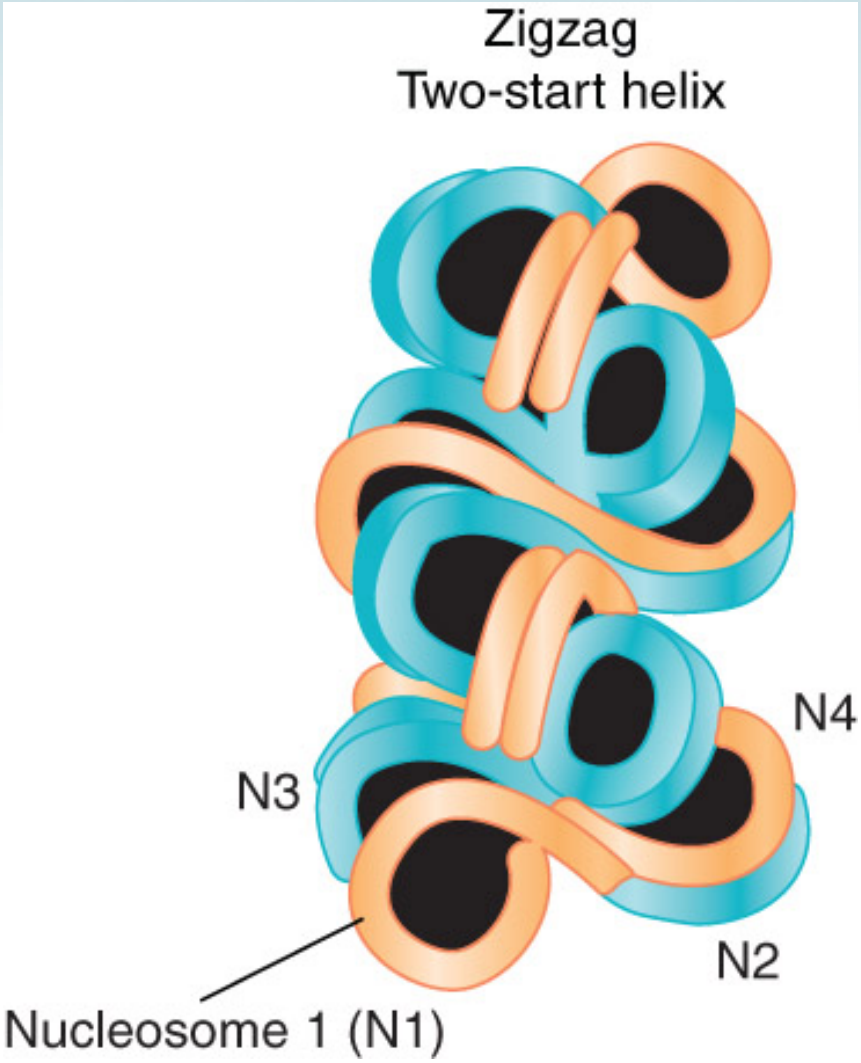
- **30 nm fiber** – A coil of nucleosomes.
  - It is the basic level of organization of nucleosomes in chromatin.

- **nonhistone** – Any structural protein found within a chromosome with the exception of histones.



The 30 nm fiber has a coiled structure

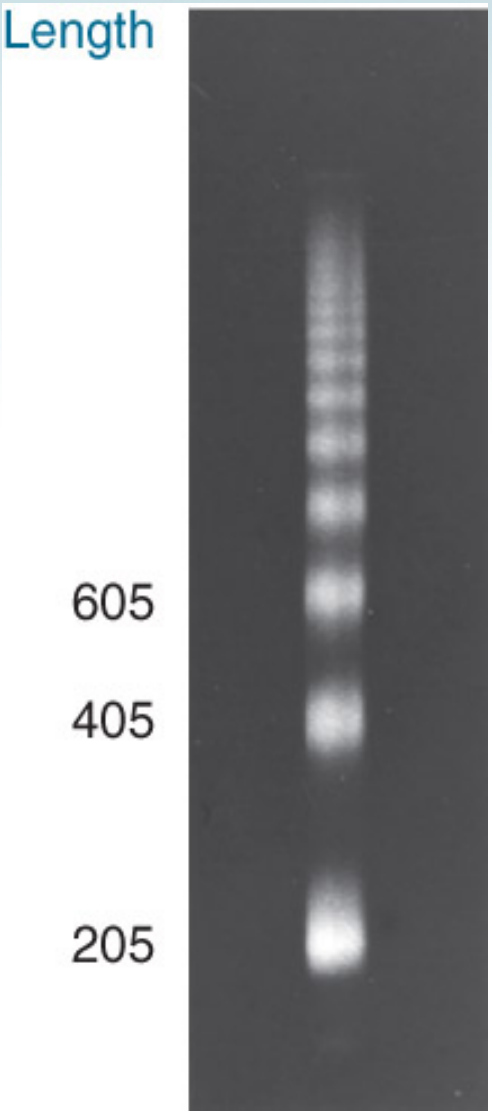
Photo courtesy of Barbara Hamkalo, University of California, Irvine.

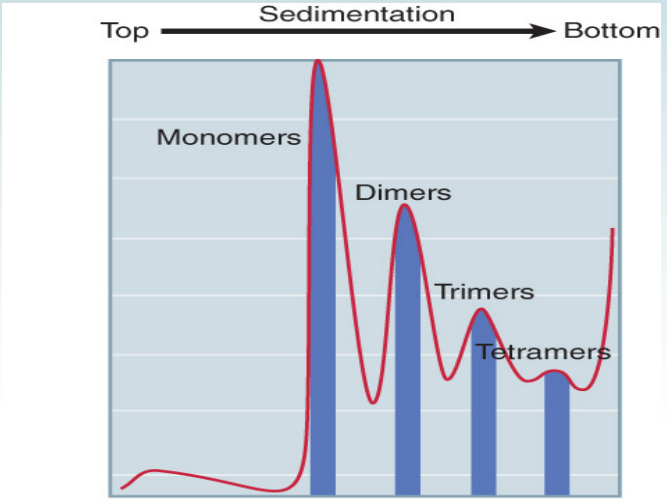


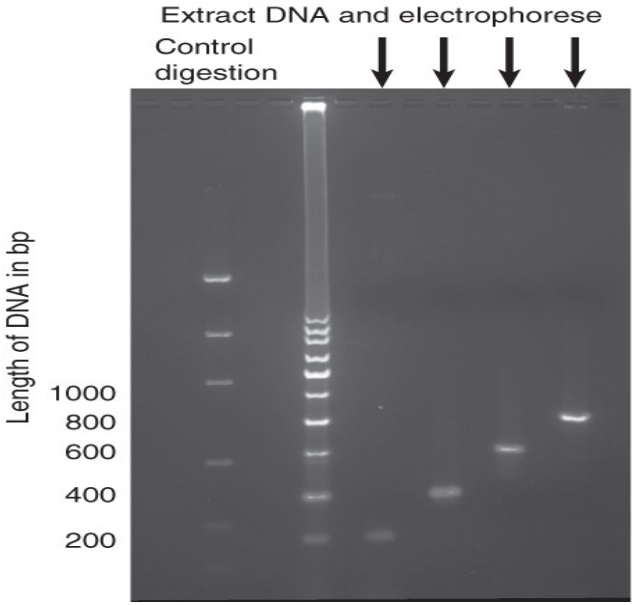
The 30 nm fiber is a two start helix consisting of two rows of nucleosomes coiled into a solenoid

DNA Is Organized in Arrays of Nucleosomes

- **MNase (micrococcal nuclease)** cleaves linker DNA and releases individual nucleosomes from chromatin.
- >95% of the DNA is recovered in nucleosomes or multimers when **MNase** cleaves DNA in chromatin.
- The length of DNA per nucleosome varies for individual tissues or species in a range from **154** to **260 bp**.
- Nucleosomal DNA is divided into the core DNA and linker DNA depending on its susceptibility to MNase.

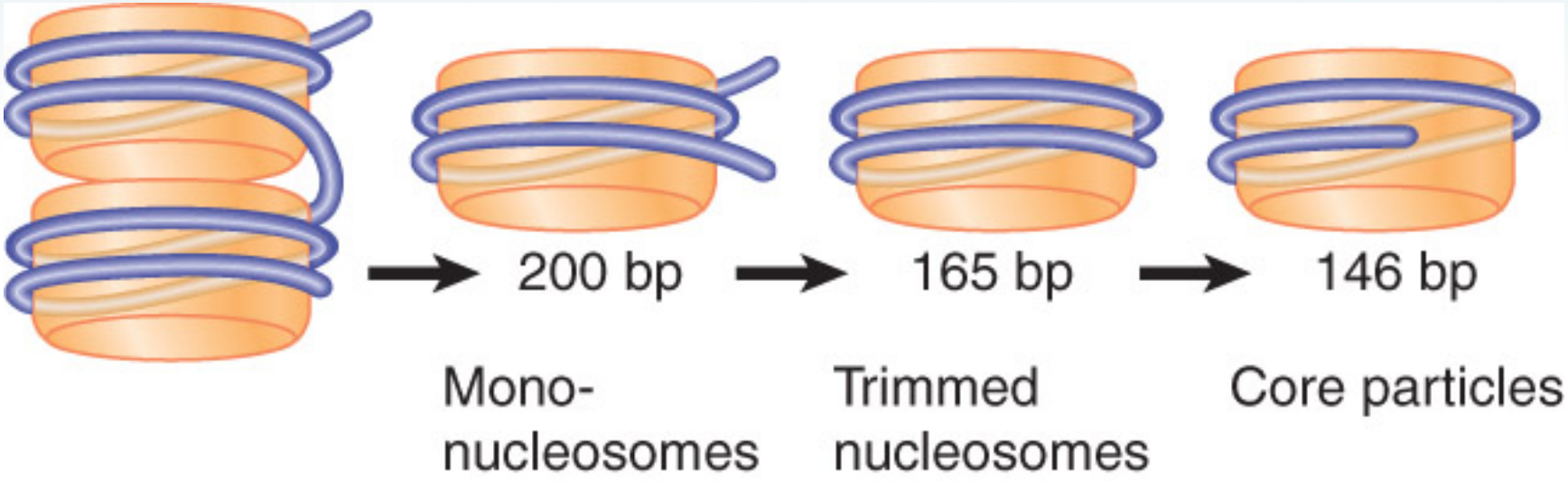




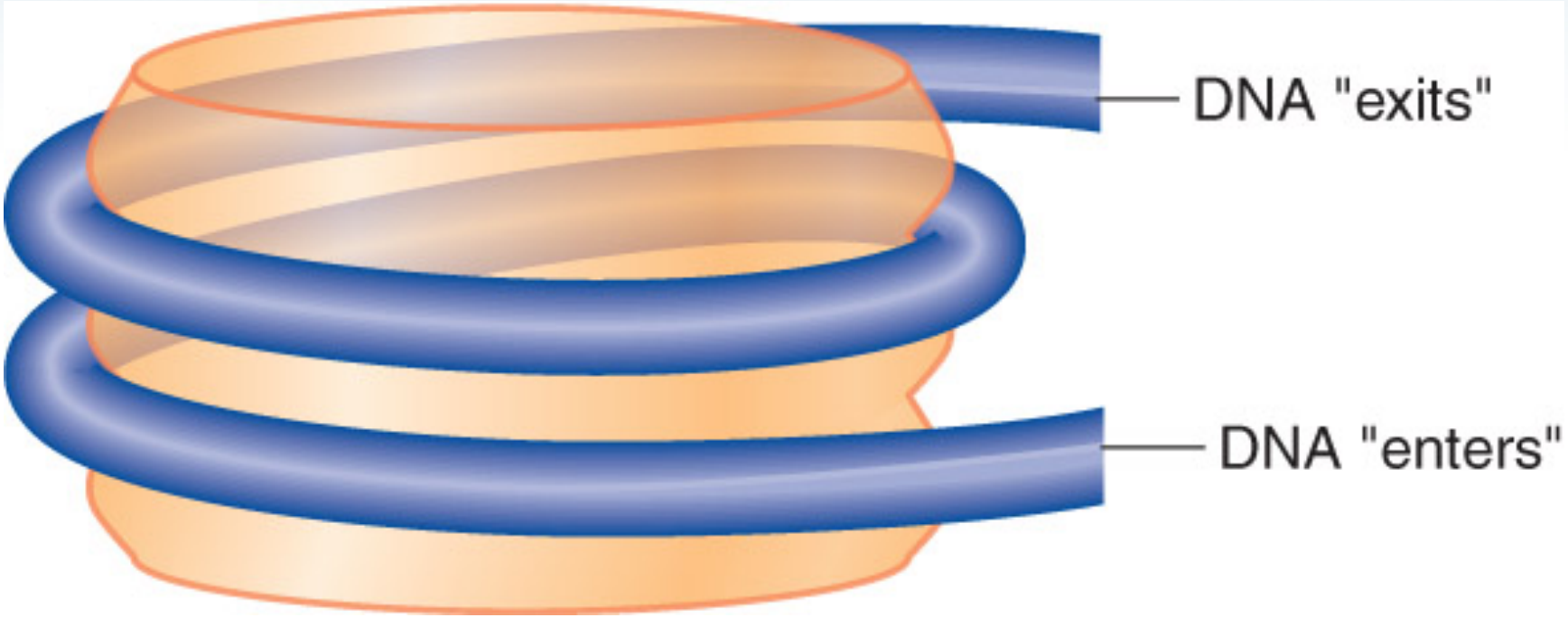


Micrococcal nuclease digests chromatin in nuclei into a multimeric series of DNA bands that can be separated by gel electrophoresis. Each multimer of nucleosomes contains the appropriate number of unit lengths of DNA

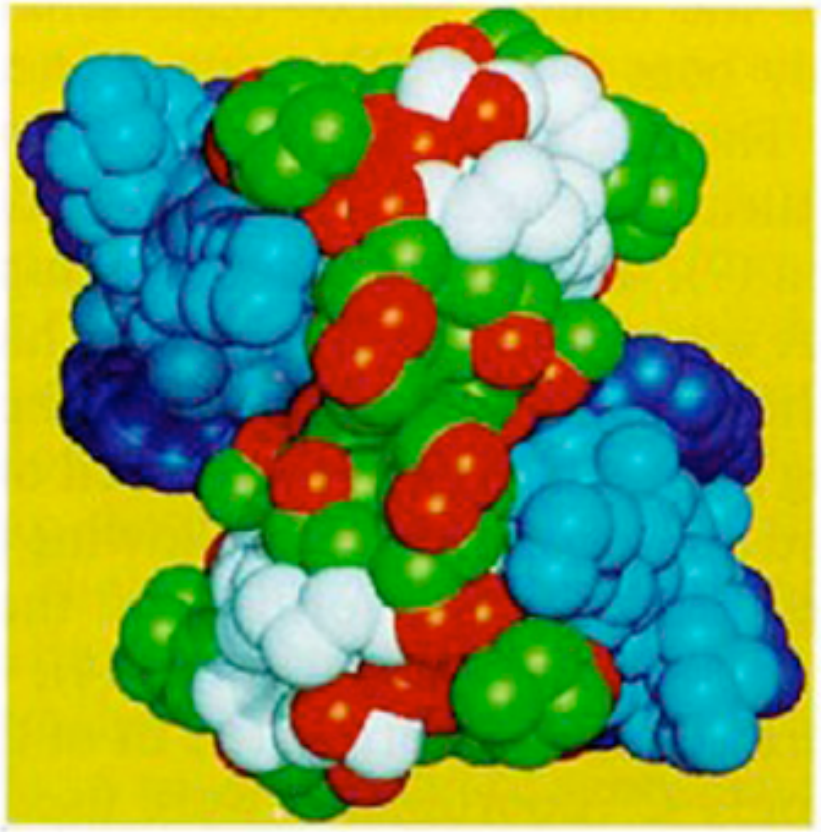
Photo courtesy of Markus Noll, Universität Zürich.



Micrococcal nuclease initially cleaves between nucleosomes



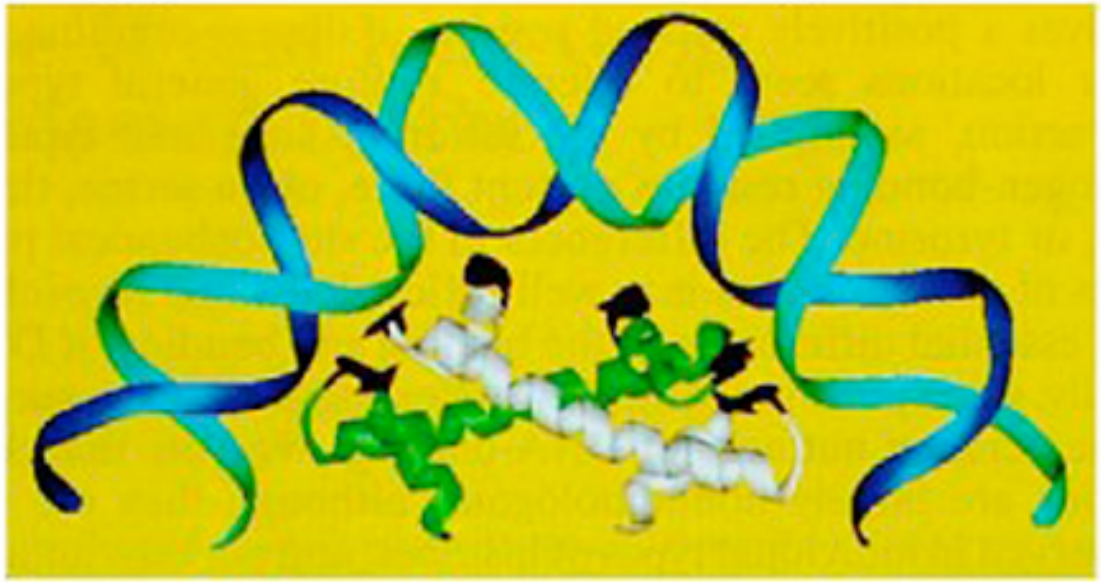
The nucleosome can be considered to be a cylinder with DNA organized into ~1 2/3 turns around it's surface



H4	white
H3	green
H2A	light blue
H2B	dark blue

11 nm

6 nm

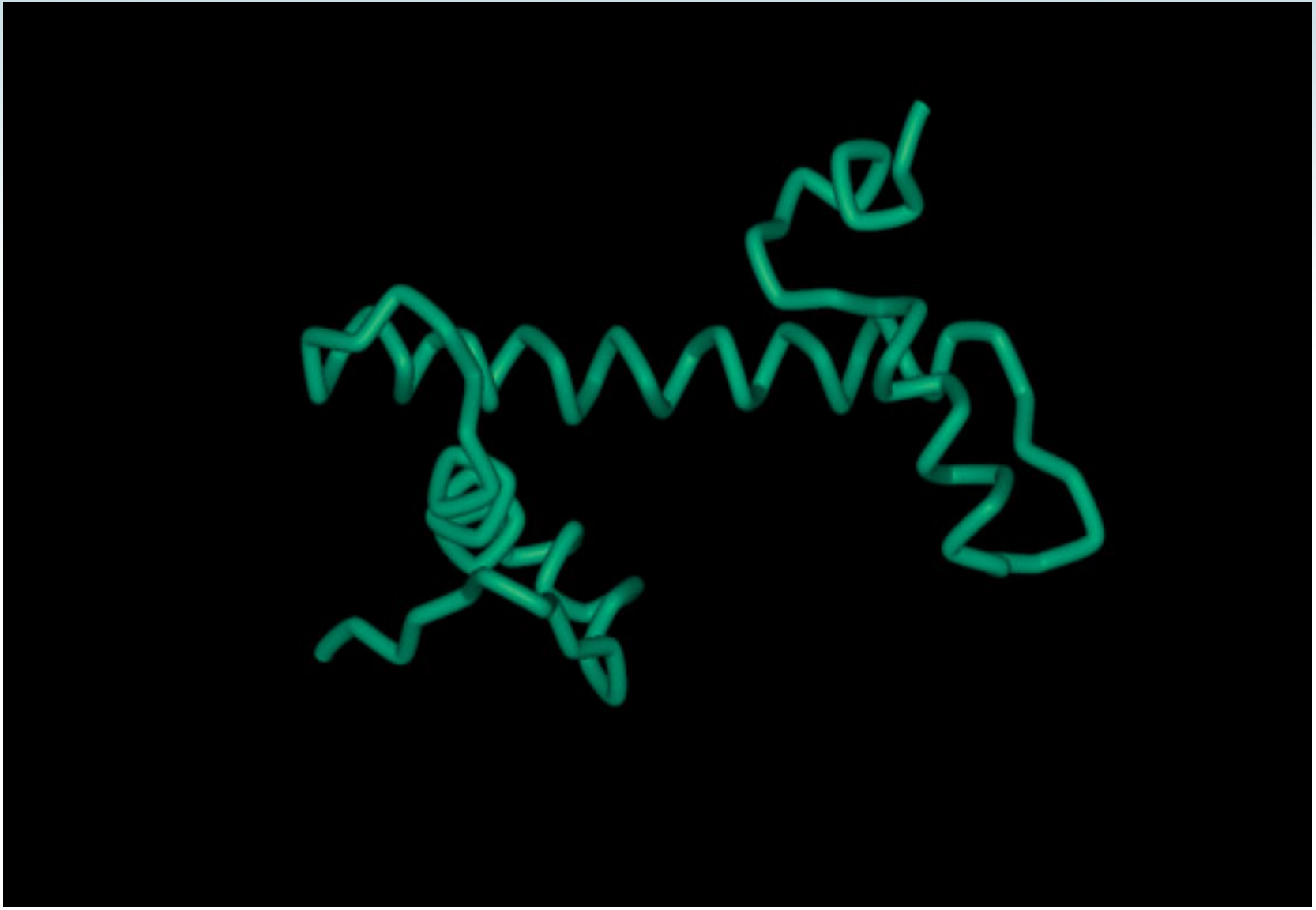


- Each core histone dimer
  has 6 DNA binding surfaces
  that organize 3 DNA turns;
- The histone octamer
  organizes 145 bp of DNA
  in 1 3/4 helical turn of DNA:

48 nm of DNA packaged in a disc of 6 x 11nm

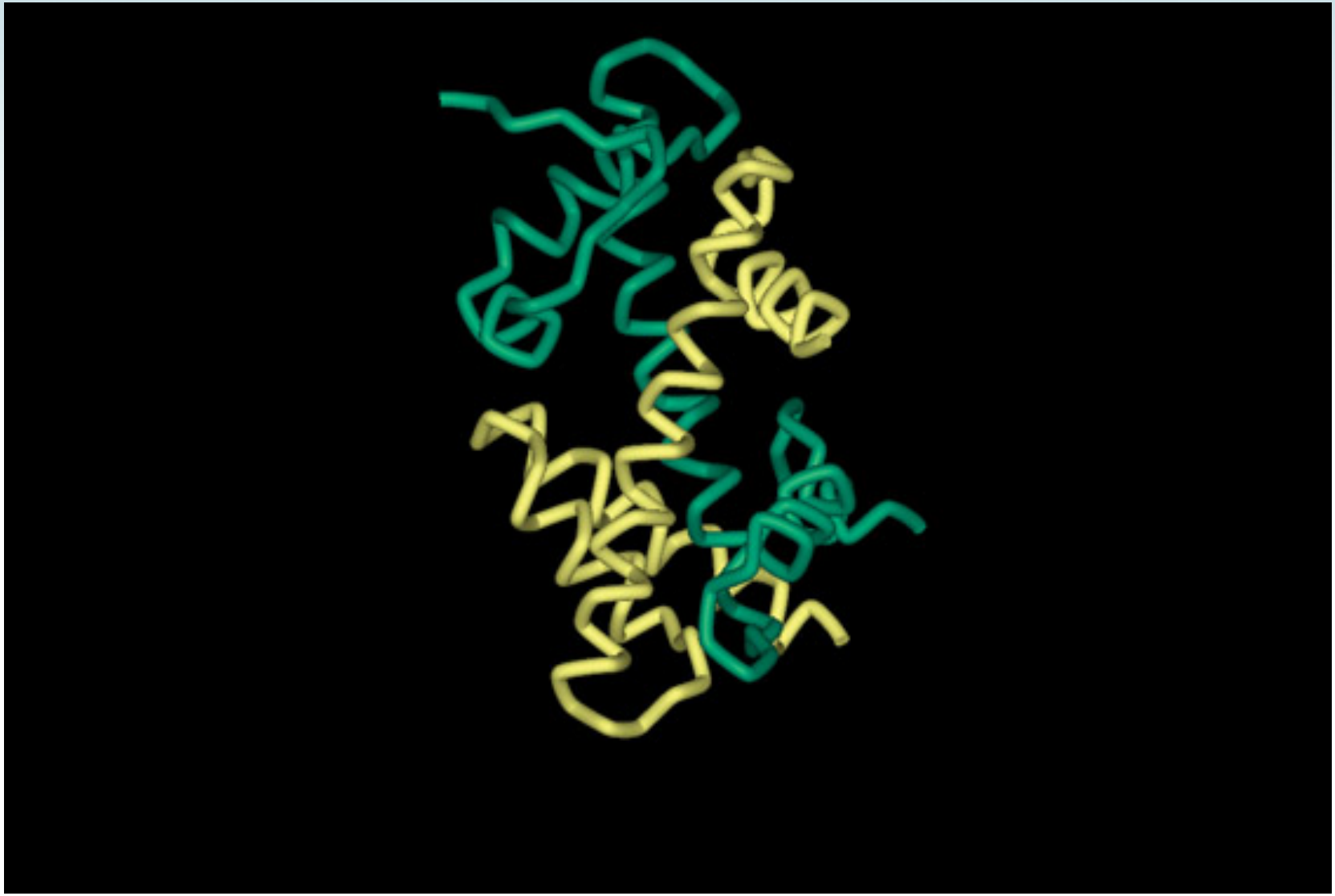
www.dnalc.org

http://**www.youtube.com**/watch?v=gbSIBhFwQ4s



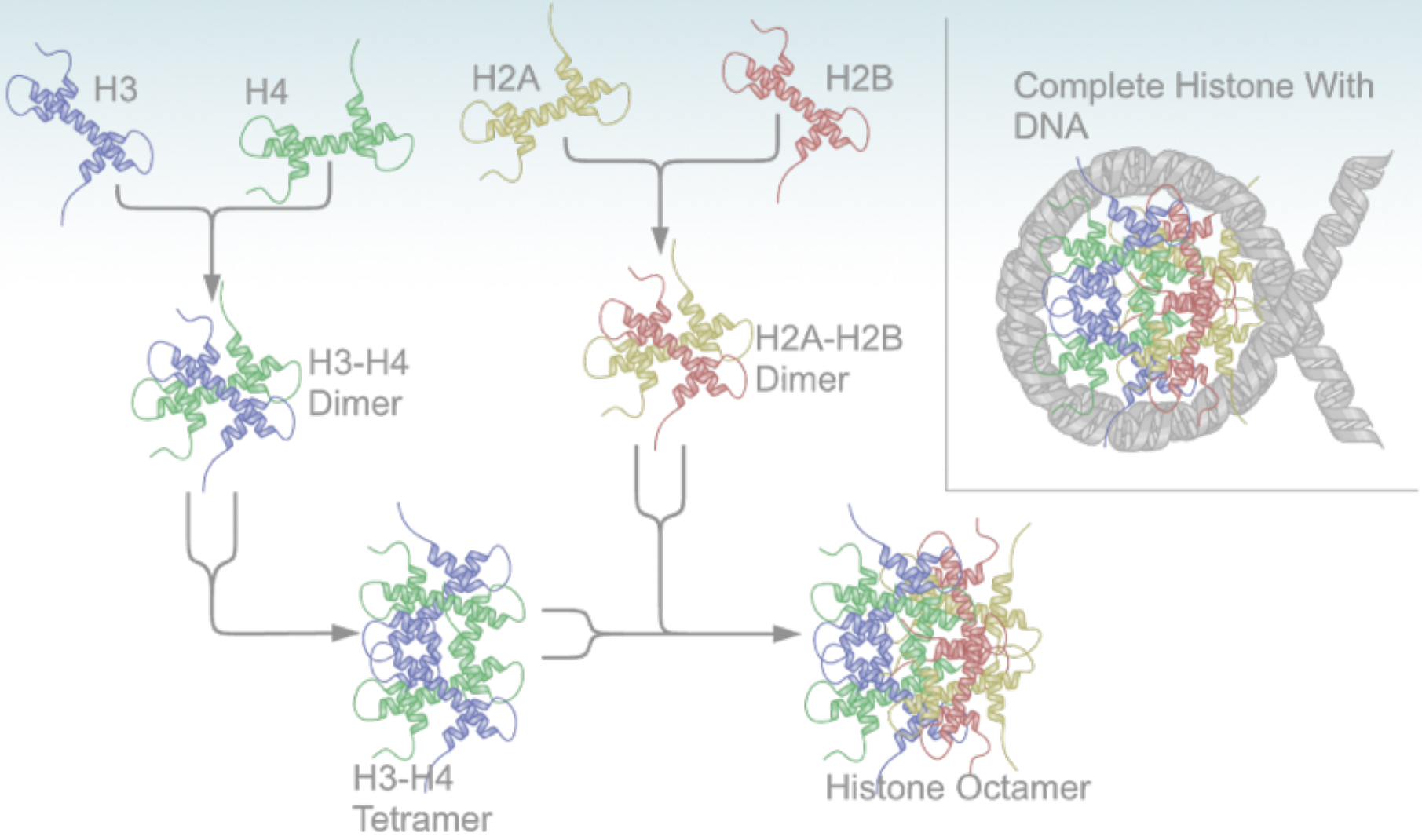
The histone fold consists of two short a-helices flanking a longer a-helix

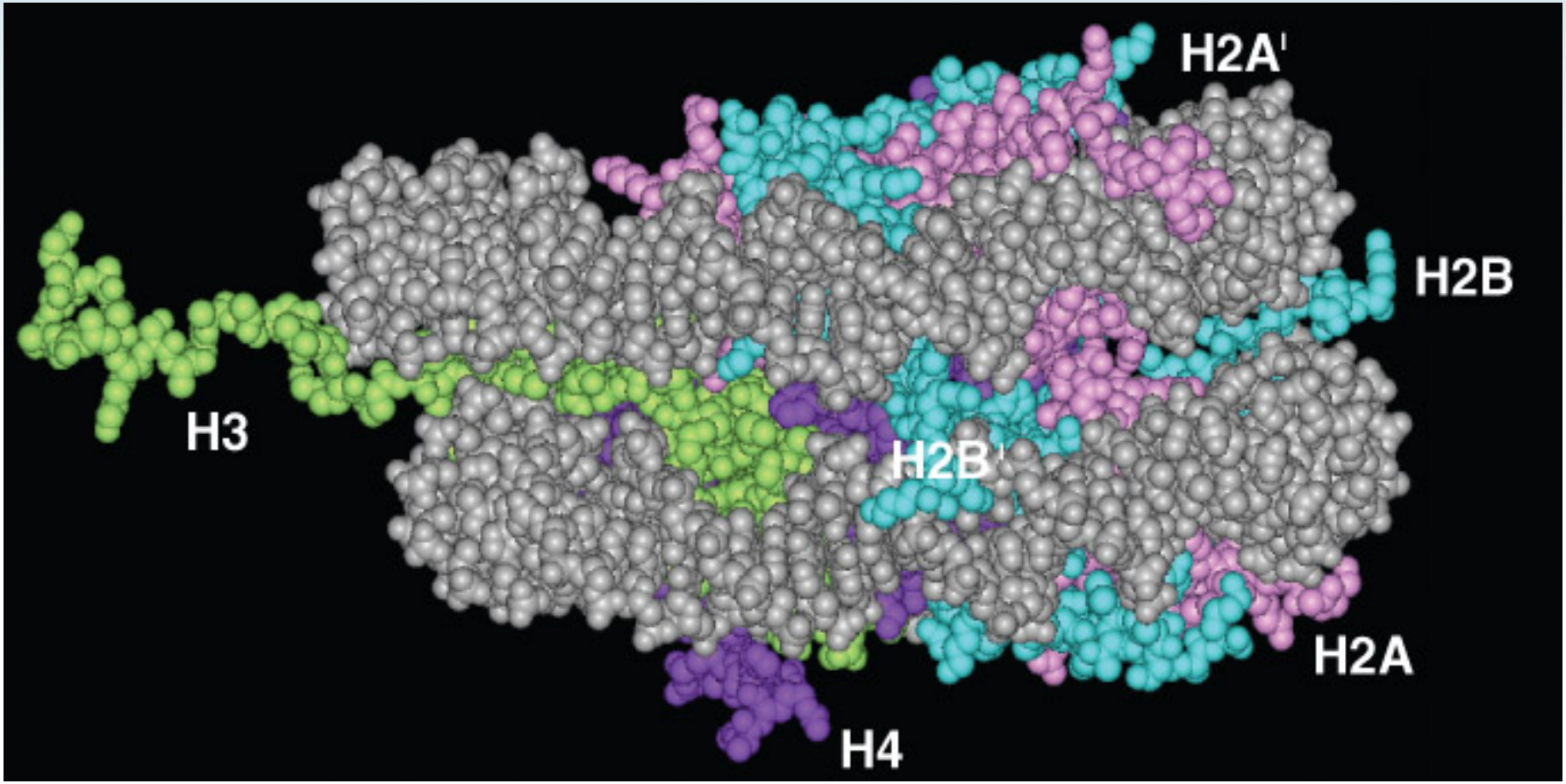
Structures from Protein Data Bank 1HIO. G. Arents, et al., Proc. Natl. Acad. Sci. USA 88 (1991): 10145-10152.



Histone pairs (H3 + H4 and H2A + H2B) interact to form histone dimers

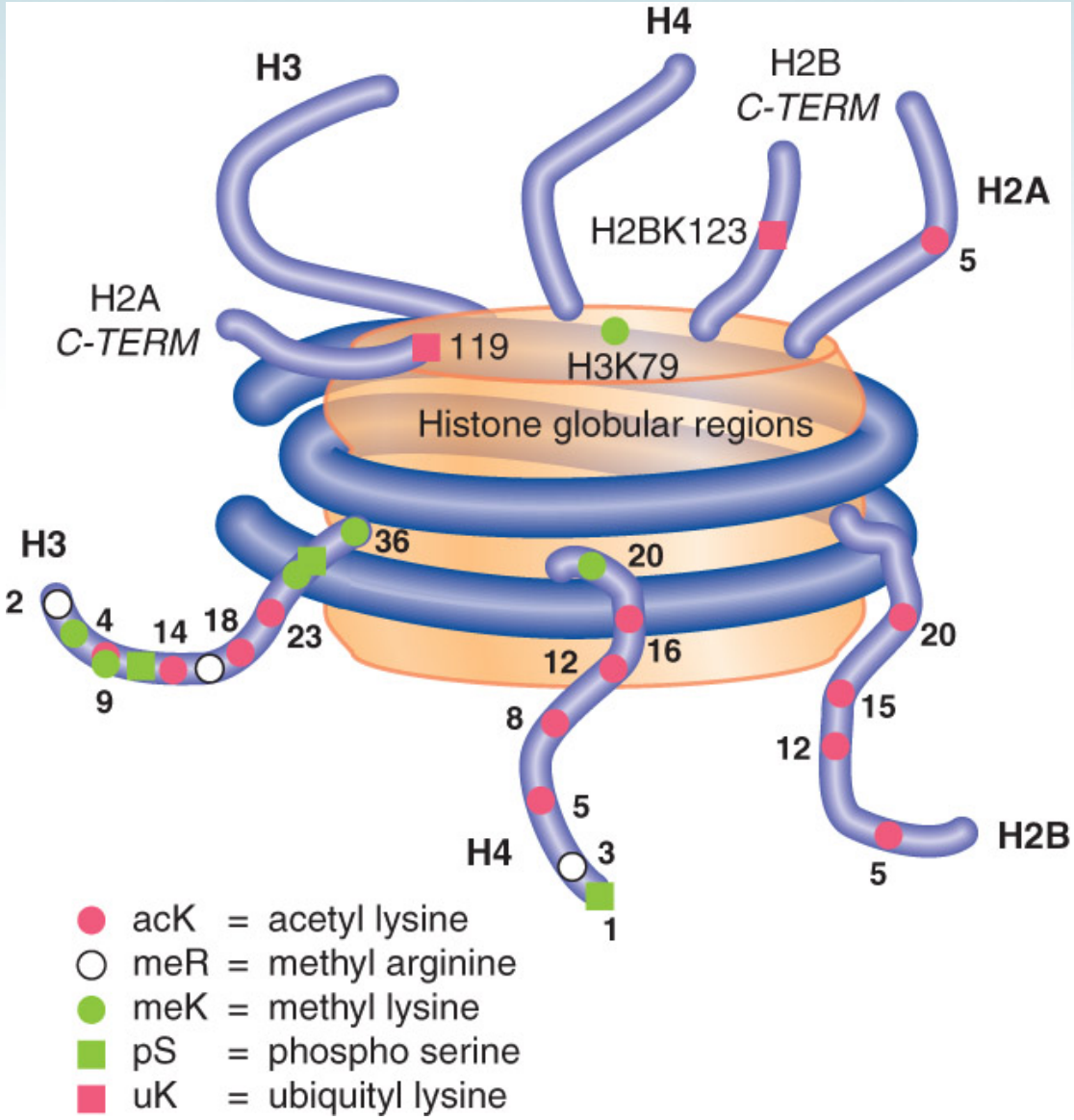
Structures from Protein Data Bank 1HIO. G. Arents, et al., Proc. Natl. Acad. Sci. USA 88 (1991): 10145-10152.


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



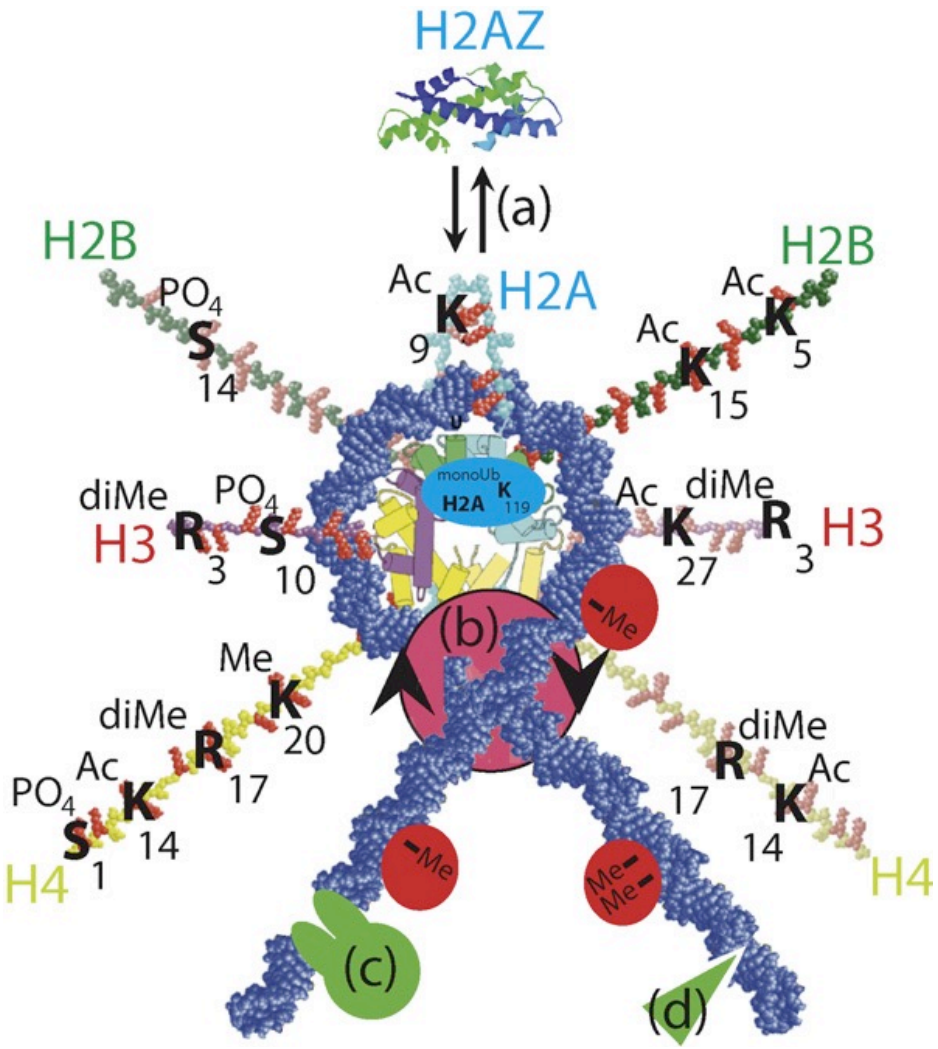
The histone tails are disordered and exit from both faces of the nucleosome and between turns of the DNA

Structure from Protein Data Bank 1AOI. K. Luger, et al., Nature 389 (1997): 251-260.



Histone tails have many sites of modification

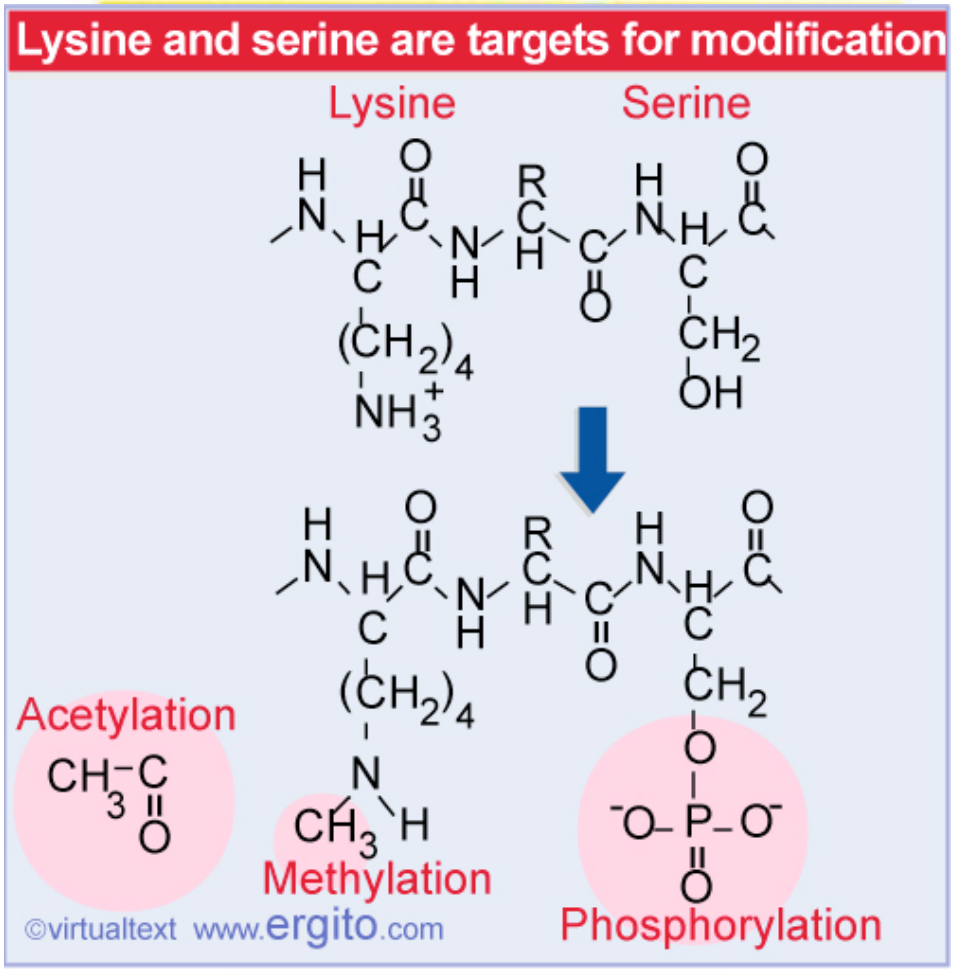
Adapted from The Scientist 17 (2003): p. 27.

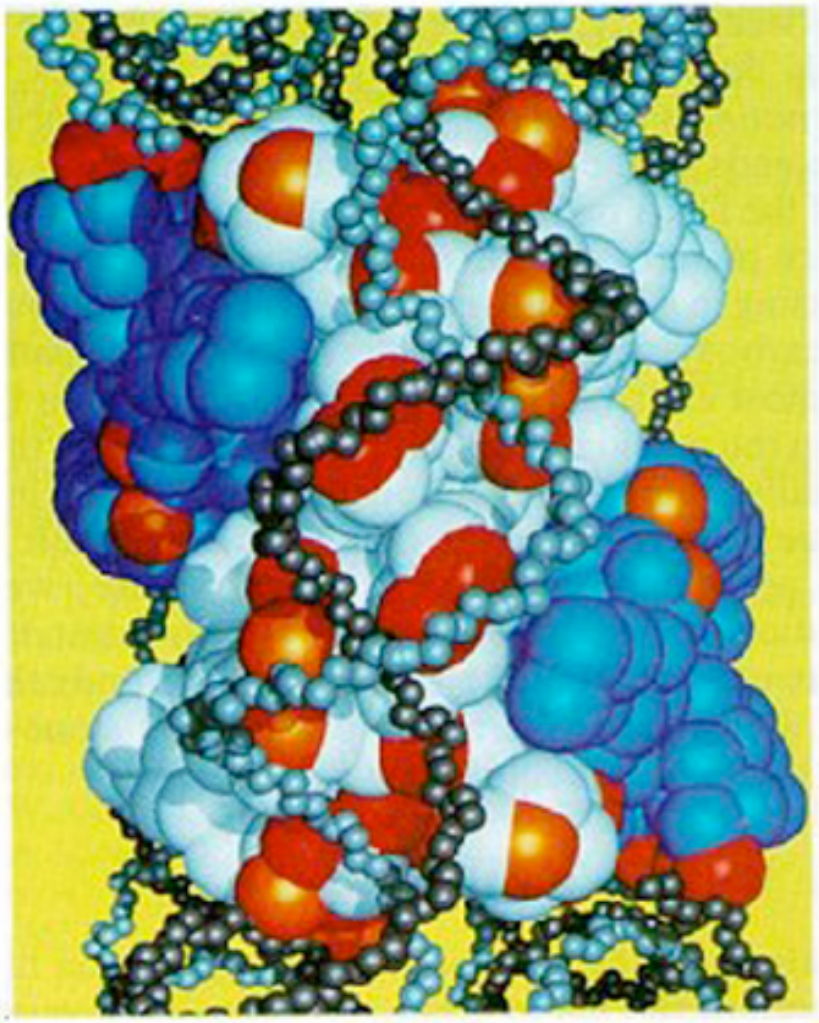


The covalent marks on chromatin. Chromatin can undergo post-translational modifications: (a) Histone exchange, (b) position change of nucleosome mediated by ATP-dependent remodeling complexes, (c) double-stranded DNA breaks by topoisomerase, and (d) single-stranded DNA breaks by topoisomerase.

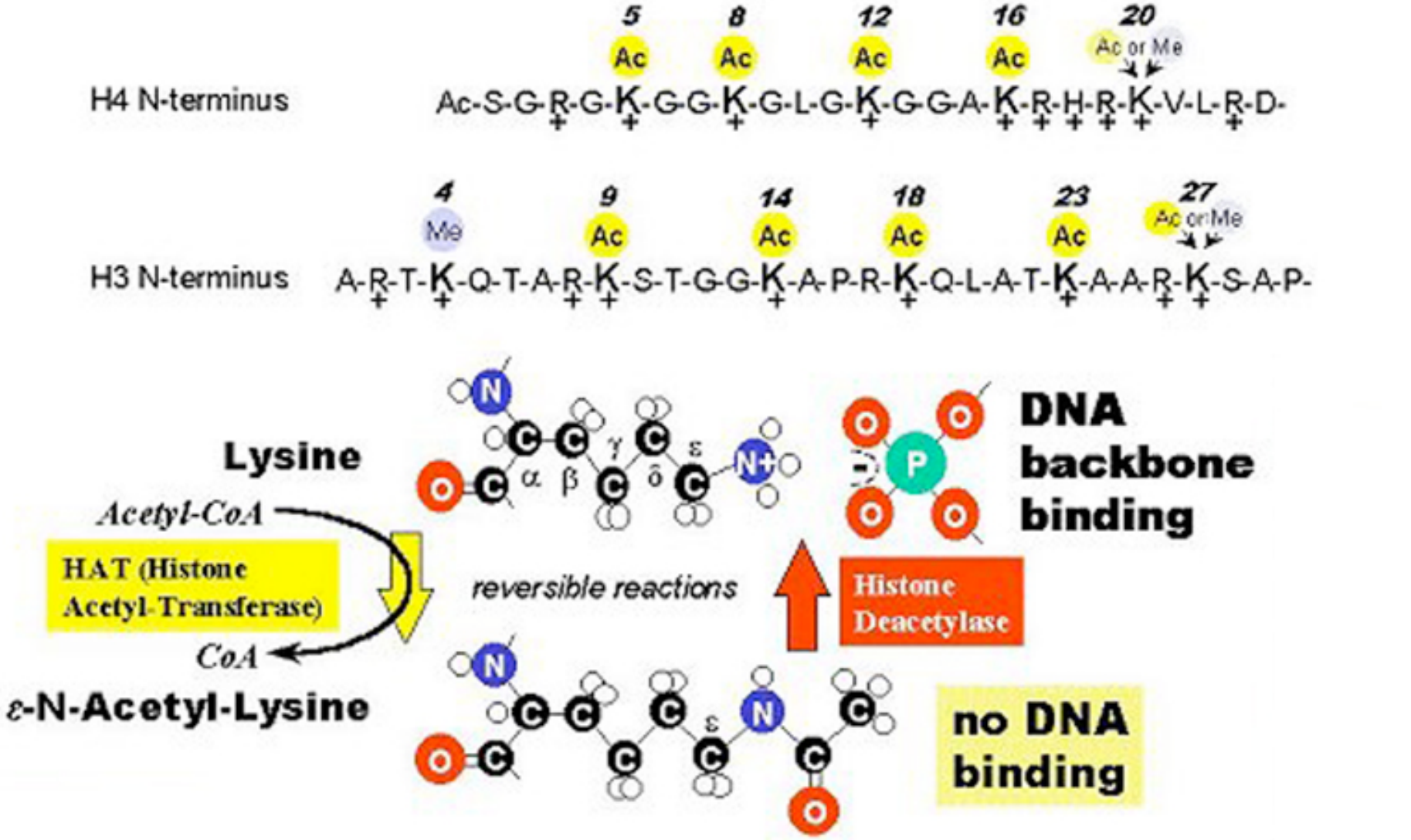
Reid G., Gllais R., Metivier R. (2009). Marking time: The dynamic role of chromatin and covalent modification in transcription. The International Journal of Biochemistry & Cell Biology 41 (2009) 155–163

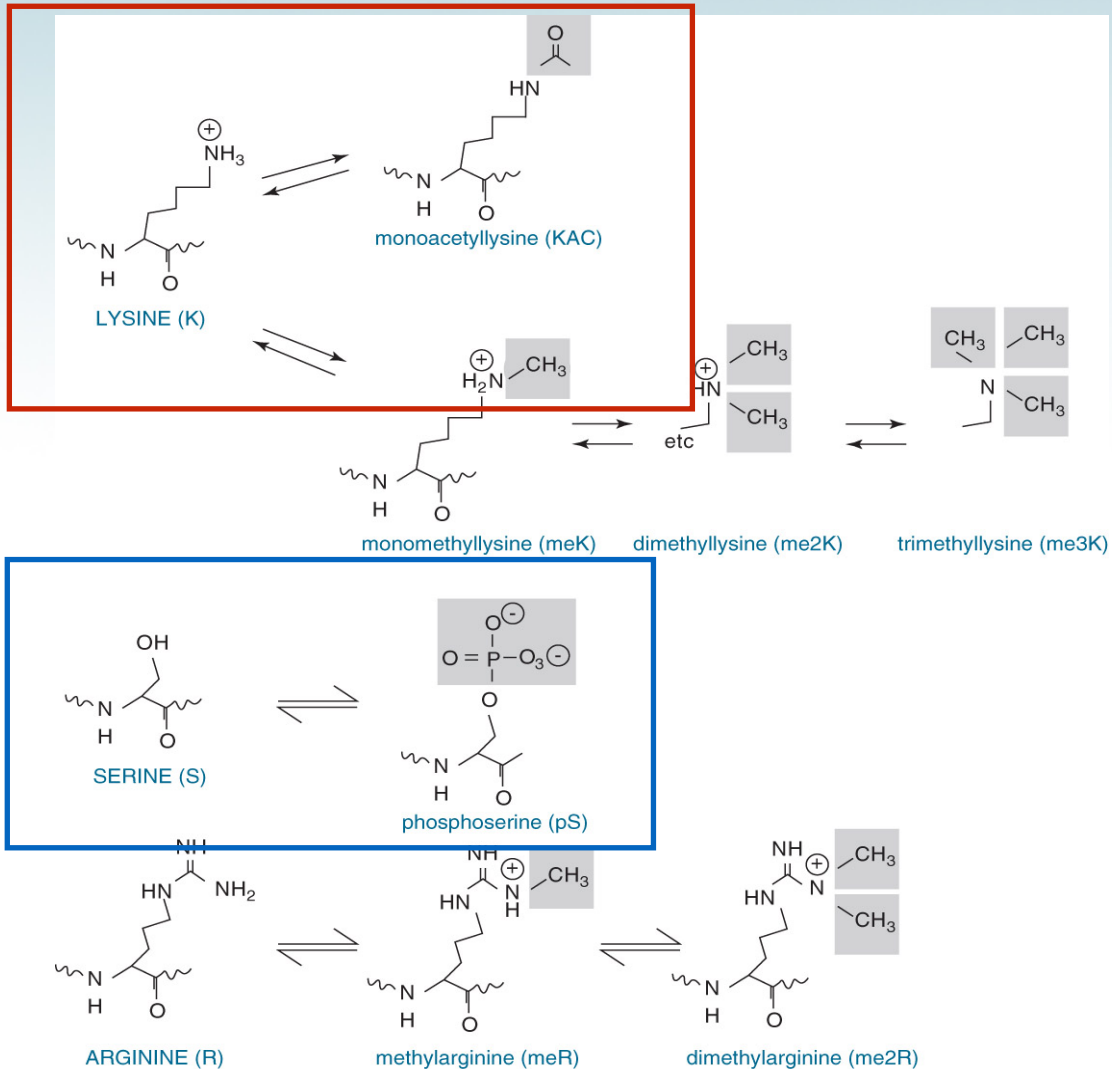
Lysine and serine are targets for modification





red: + (arg, lys) orange: -OH (ser, thr)


5
8
12
16
20
Ac
Ac
Ac
Ac
Ac or Me
H4 N-terminus
Ac-S-G-R-G-K-G-G-K-G-L-G-K-G-G-A-K-R-H-R-K-V-L-R-D-
4
9
14
18
23
27
Me
Ac
Ac
Ac
Ac
Ac or Me
H3 N-terminus
A-R-T-K-Q-T-A-R-K-S-T-G-G-K-A-P-R-K-Q-L-A-T-K-A-A-R-K-S-A-P-
Lysine
Acetyl-CoA
HAT (Histone Acetyl-Transferase)
CoA
ε-N-Acetyl-Lysine
reversible reactions
Histone Deacetylase
DNA backbone binding
no DNA binding



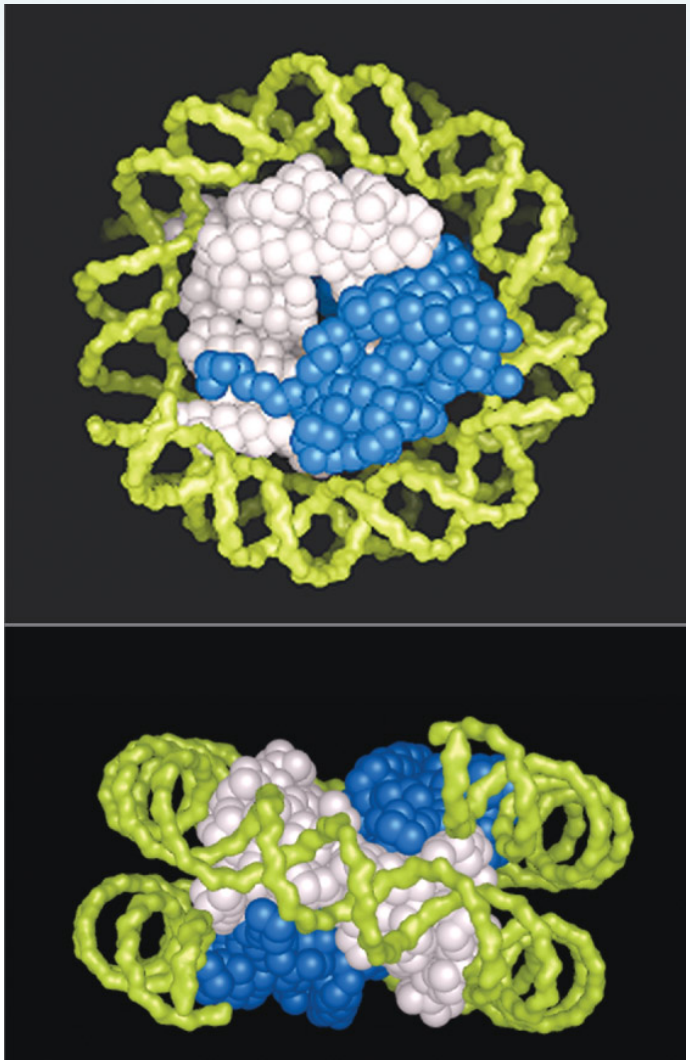
Among other changes… “polar” **Serine** residues can be made negatively charged by phosporylation, and the positive charge on **lysine** can be neutralized upon acetylation, while **methylated** lysine and arginine retain their positive charges, but now more “bulky”

Histone	Site	Modification	Function
H3	K-4	Methylation	Transcription activation
H3	K-9	Methylation	Chromatin condensation
	K-9	Methylation	Required for DNA methylation
	K-9	Acetylation	Transcription activation
H3	S-10	Phosphorylation	Transcription activation
H3	K-14	Acetylation	Prevents methylation at Lys-9
H3	K-79	Methylation	Telomeric silencing
H4	R-3	Methylation	
H4	K-5	Acetylation	Nucleosome assembly
H4	K-12	Acetylation	Nucleosome assembly
H4	K-16	Acetylation	Nucleosome assembly
	K-16	Acetylation	Fly X activation

Most modified sites in histones have a single, specific type of modification, but some sites can have more than one type of modification

The Nucleosome Is the Basic Subunit of All Chromatin

- Each histone is extensively interdigitated with its partner.
- All core histones have the structural motif of the histone fold. N- and C-terminal histone tails extend out of the nucleosome.
- H1 is associated with linker DNA and may lie at the point where DNA enters or exits the nucleosome.



The crystal structure of the histone core octamer is represented in a space-filling model

Photos courtesy of E. N. Moudrianakis, Johns Hopkins University.

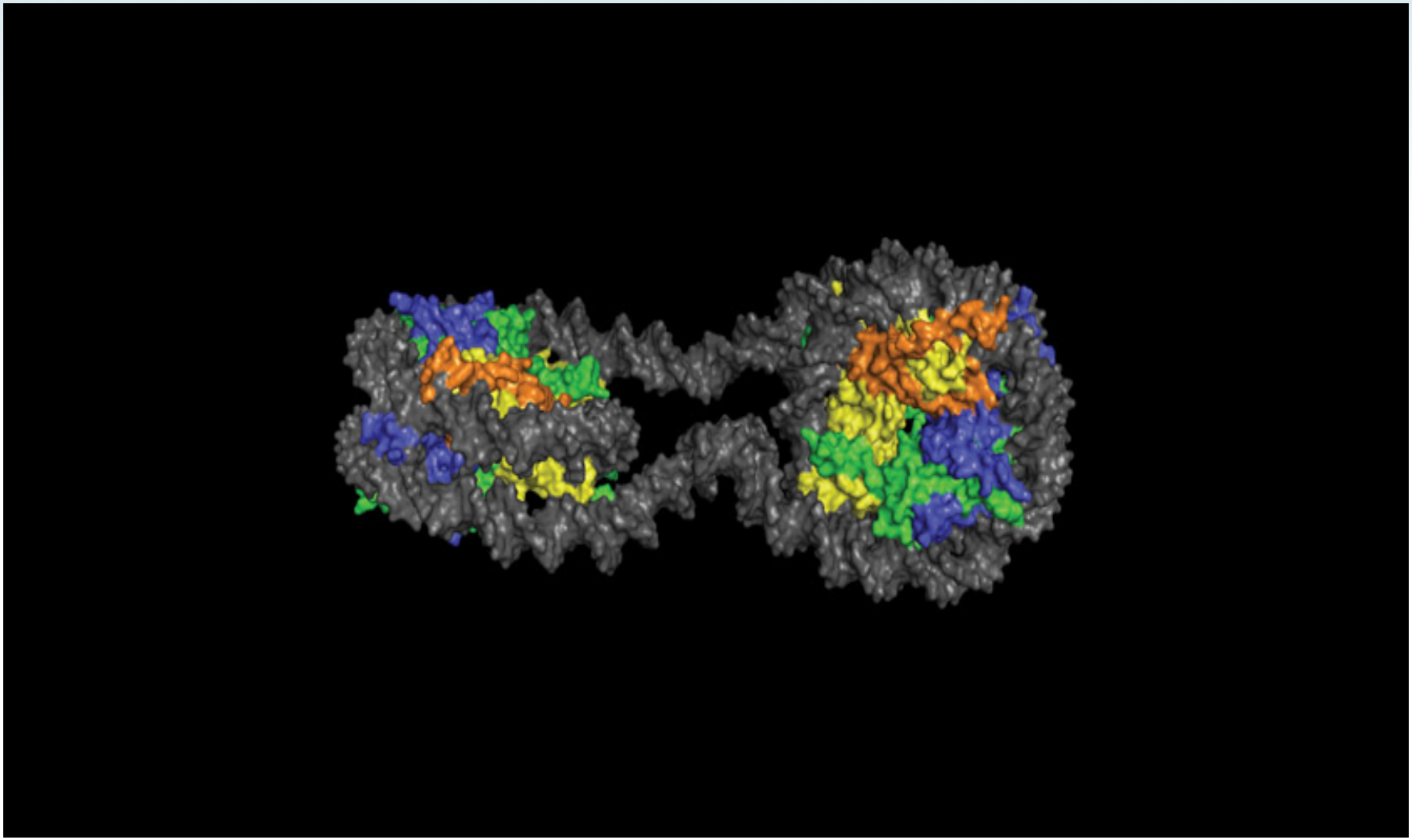
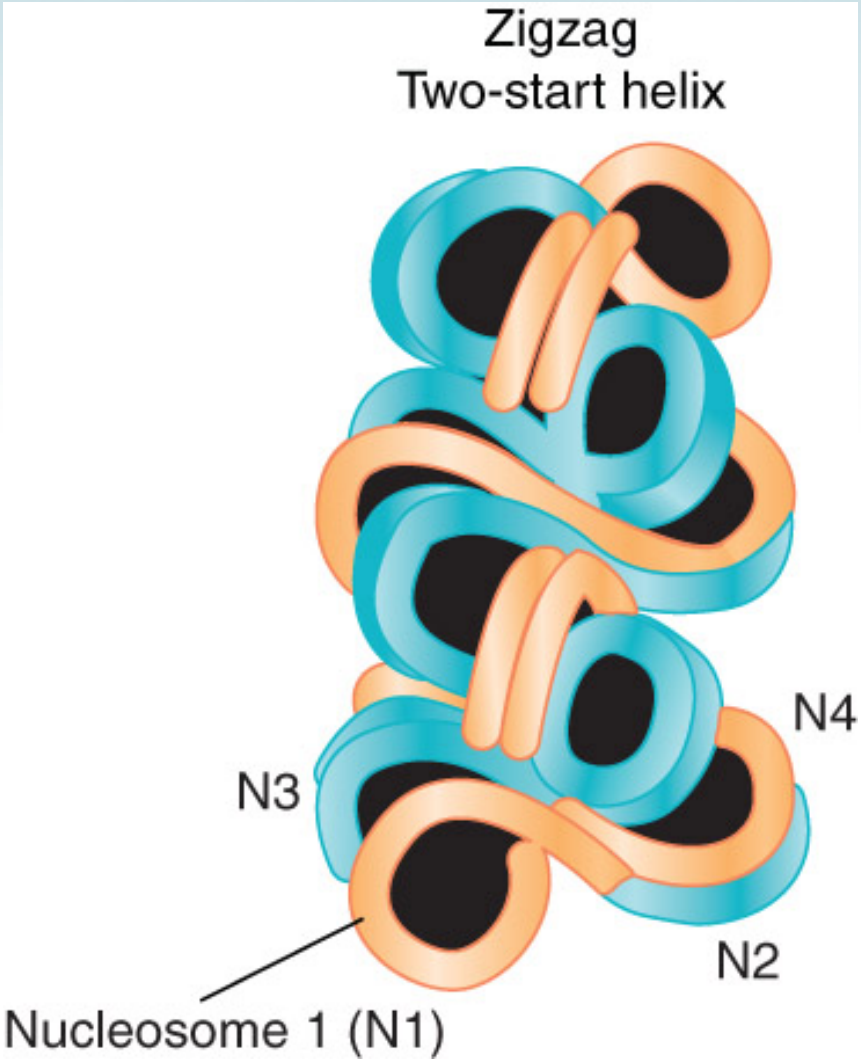
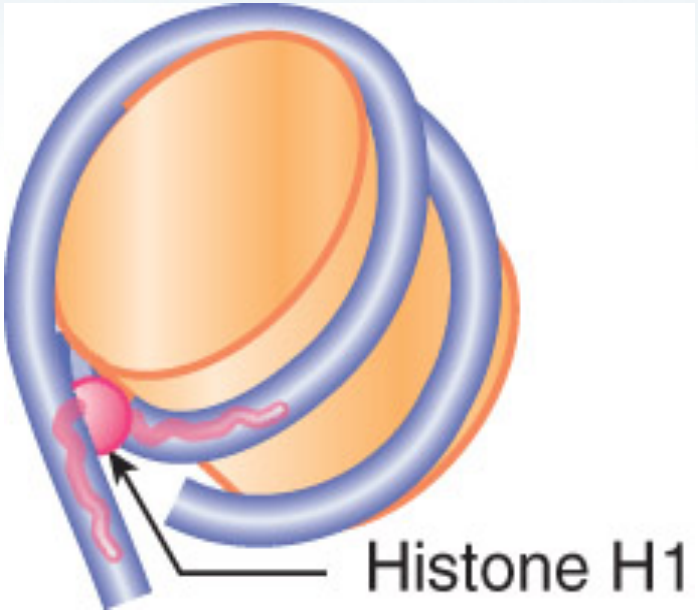


FIGURE CO: Chromatin

Structure from Protein Data Base 1ZBB. T. Schalch, et al., Nature 436 (2005): 138-141. Photo courtesy of Chris Nelson, University of Victoria.

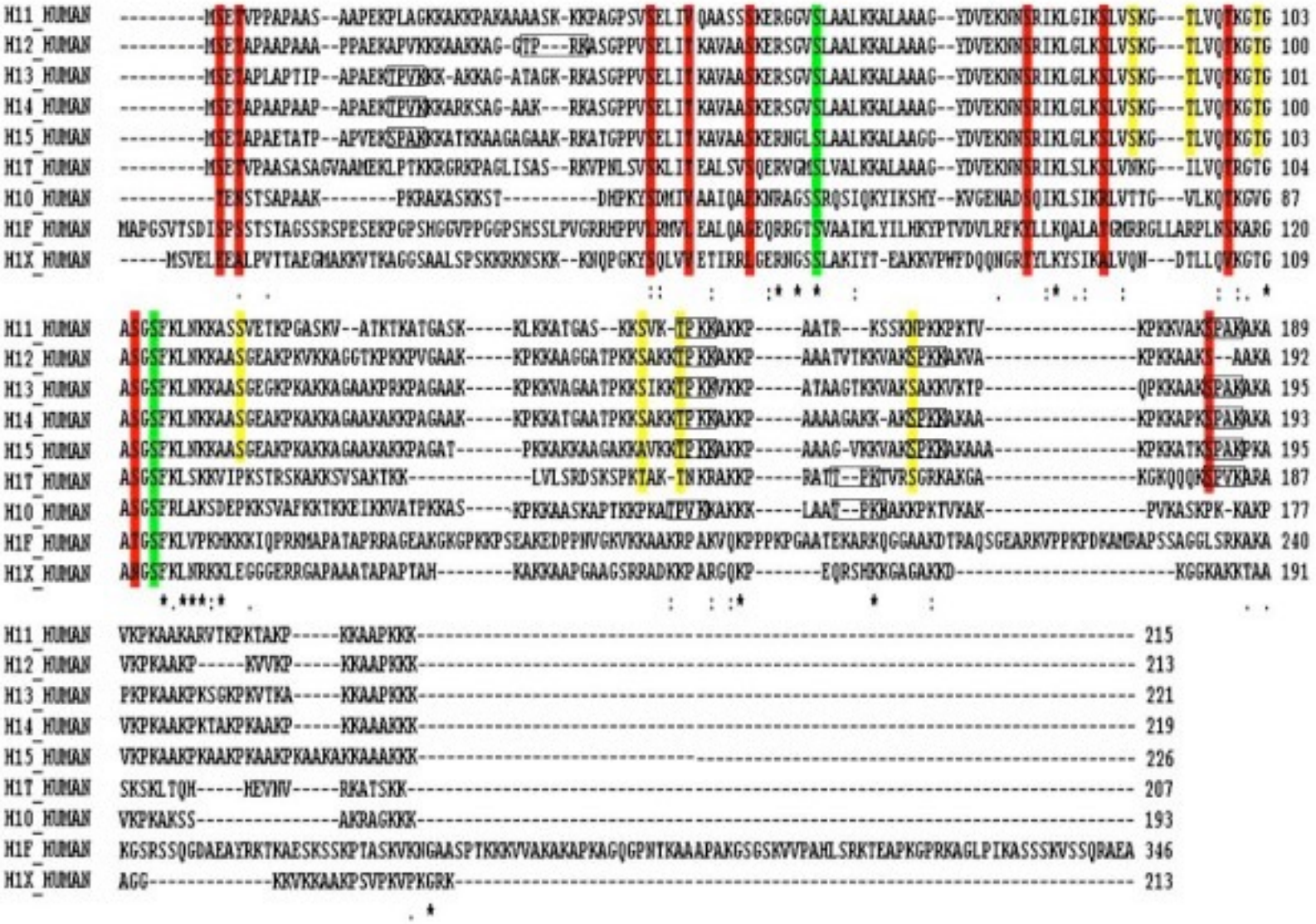


The **30 nm fiber** is a two start helix consisting of two rows of nucleosomes coiled into a solenoid



- Linker DNA is the region of 8 to 114 bp, and this is the region that is susceptible to early cleavage by the enzyme.
- Changes in the length of linker DNA account for the variation in total length of nucleosomal DNA.
- **H1 proteins** are associated with linker DNA and may lie at the point where DNA enters and leaves the nucleosome.
- which are also the points on the DNA that, having been twisted around the nucleosomal core is at its “weakest” and is most likely to break

Possible model for the interaction of histone H1 with the nucleosome



Alignment of the H1 variants in humans

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Cell Div. 2011; 6: 15.
Published online 2011 July 12. doi: 10.1186/1747-1028-6-15

PMCID: PMC3149562

Human linker histones: interplay between phosphorylation and O-β-GlcNAc to mediate chromatin structural modifications

Waqar Ahmad,[✉1] Khadija Shabbiri,[2] Noreen Nazar,[2] Shazia Nazar,[2] Saba Qaiser,[2] and Mirza Abid Shabbir Mughal[2]

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Abstract

Go to:

Eukaryotic chromatin is a combination of DNA and histone proteins. It is established fact that epigenetic mechanisms are associated with DNA and histones. Initial studies emphasize on core histones association with DNA, however later studies prove the importance of linker histone H1 epigenetic. There are many types of linker histone H1 found in mammals. These subtypes are cell specific and their amount in different types of cells varies as the cell functions. Many types of post-translational modifications which occur on different residues in each subtype of linker histone H1 induce conformational changes and allow the different subtypes of linker histone H1 to interact with chromatin at different stages during cell cycle which results in the regulation of transcription and gene expression. Proposed *O*-glycosylation of linker histone H1 promotes condensation of chromatin while phosphorylation of linker histone H1 is known to activate transcription and gene regulation by decondensation of chromatin. Interplay between phosphorylation and *O*-β-GlcNAc modification on Ser and Thr residues in each subtype of linker histone H1 in *Homo sapiens* during cell cycle may result in diverse functional regulation of proteins. This *in silico* study describes the potential phosphorylation, o-glycosylation and their possible interplay sites on conserved Ser/Thr residues in various subtypes of linker histone H1 in *Homo sapiens*.

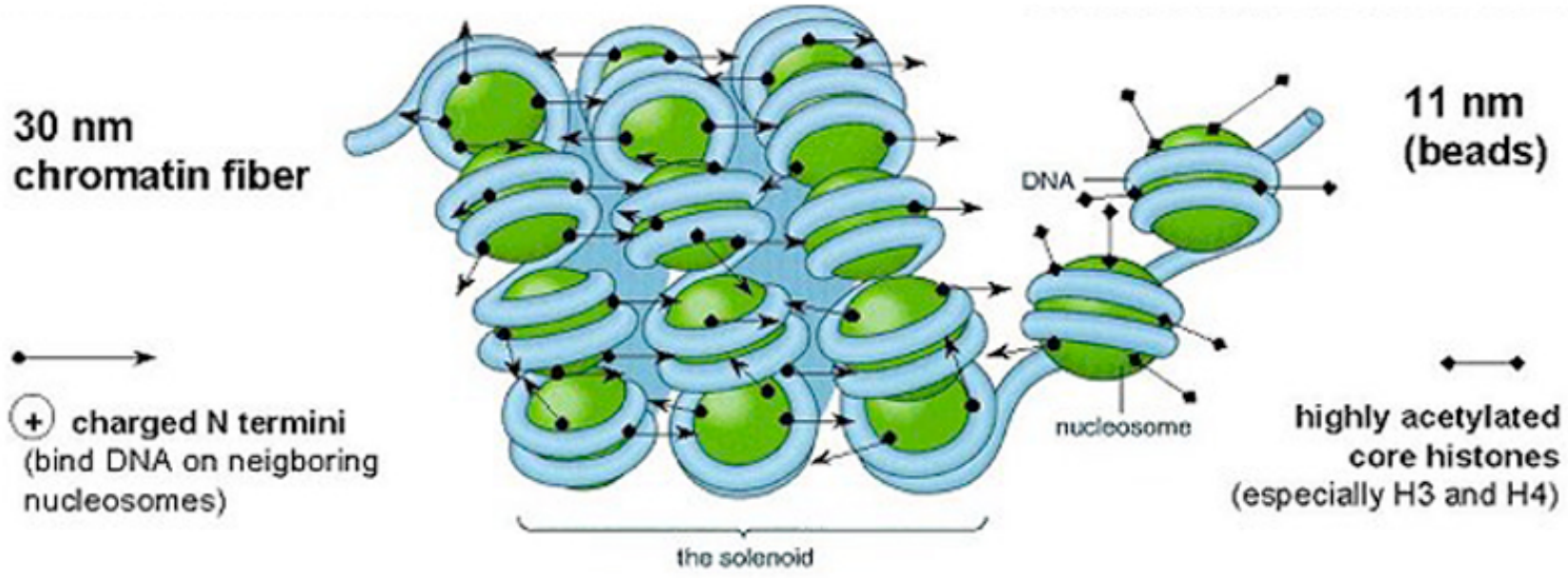
Introduction

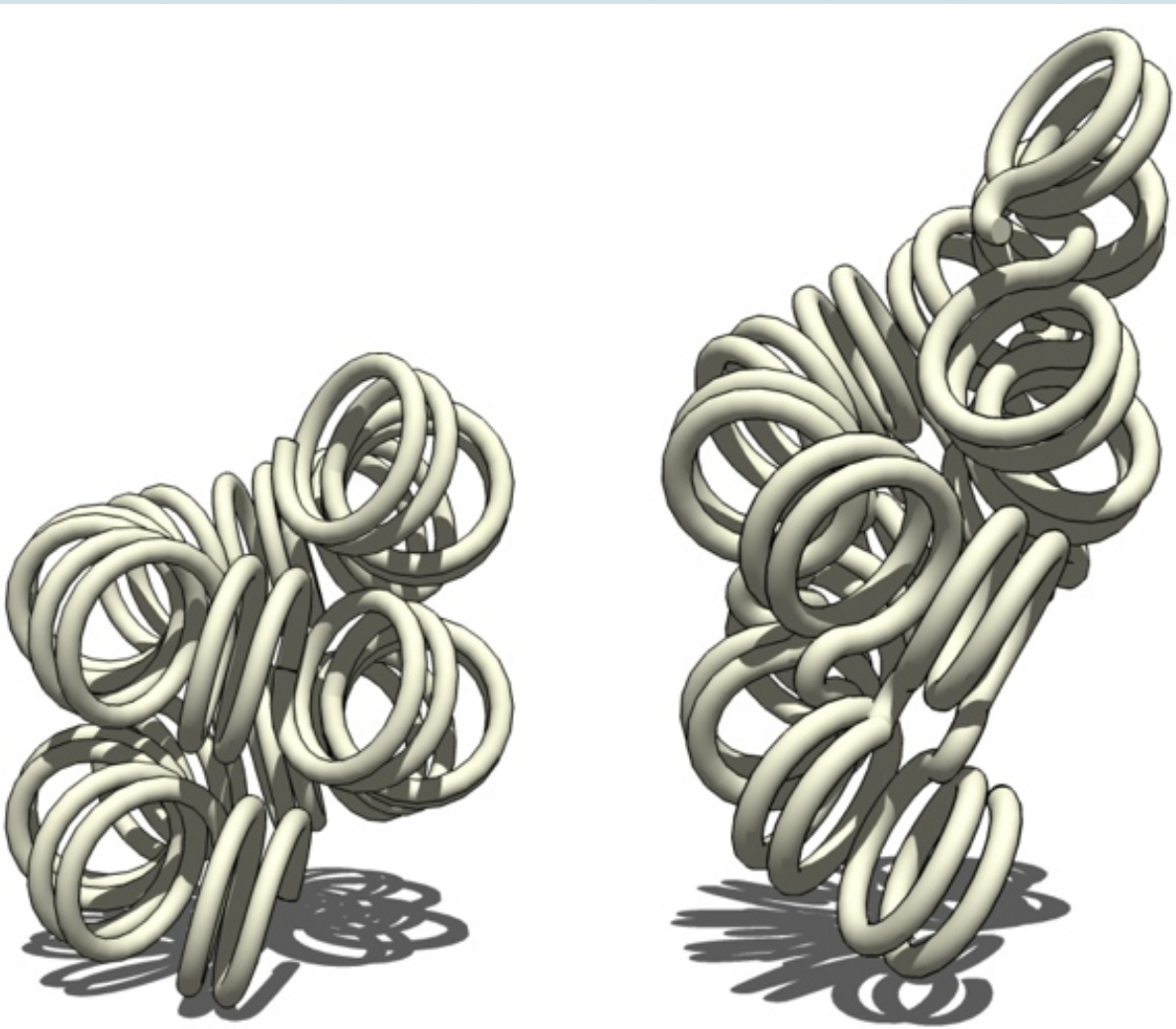
Go to:

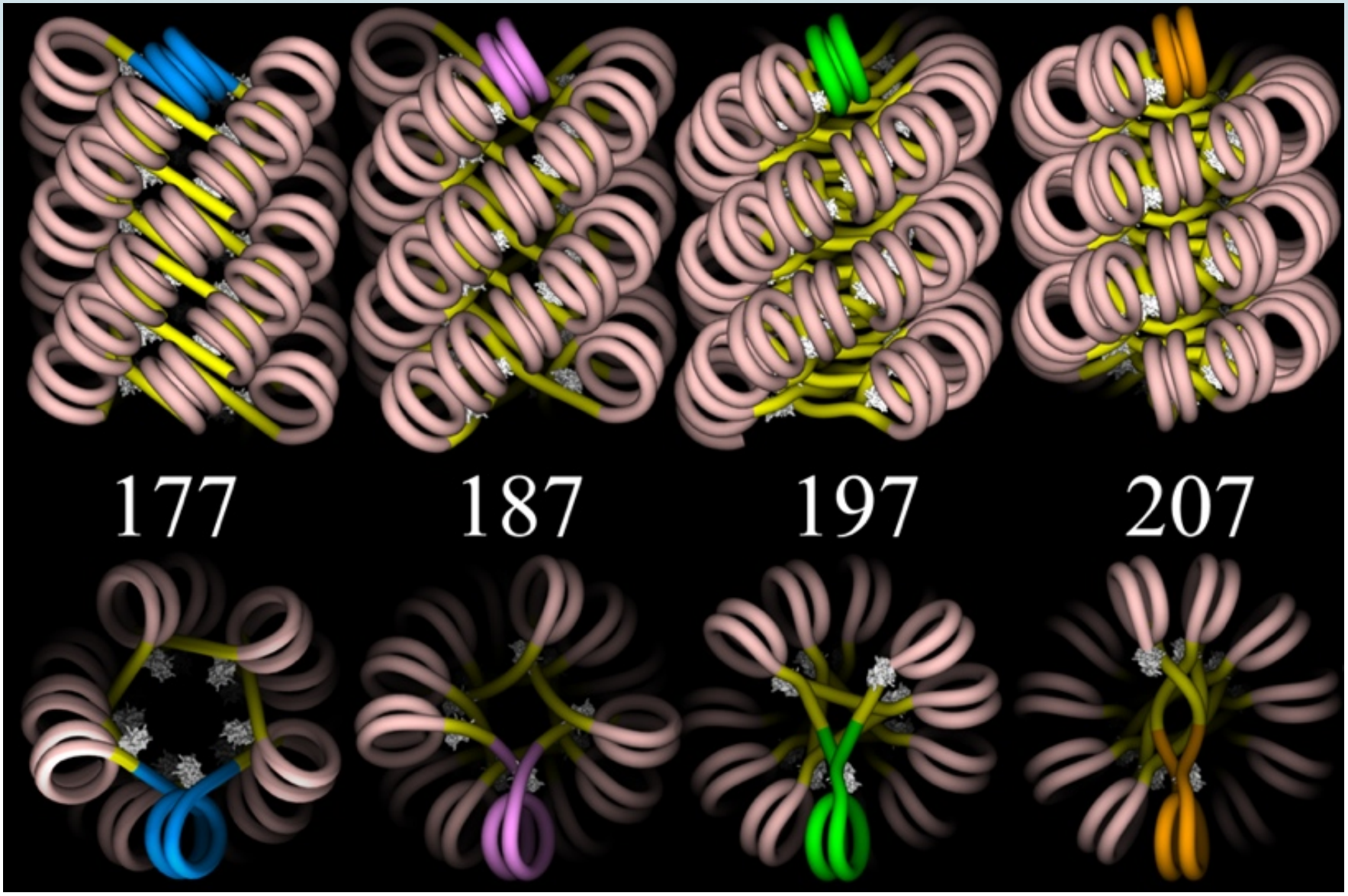
Eukaryotic genome is packaged into a structure known as chromatin. The basic structural unit of chromatin called as nucleosome is composed of DNA and proteins [1]. The major proteins involved in chromatin structure are histone proteins. Histone proteins are of five types: H1, H2A, H2B, H3 and H4 [2-4]. Histone H1 is known as linker histone while the other four histone proteins are collectively known as core histones. This DNA-protein complex is the tempelate for a number of essential cell processes including transcription recombination, repair and replication. Histone H1 is located on the linker DNA that goes between the nucleosomes in chromatin structure [5]. Linker DNA which is associated with linker histone H1 interconnects core particles, varies in length, depending on species and tissue [6]. Organization of DNA into nucleosomes by histone proteins and folding of nucleosomes into higher-order chromatin structure is generally believed to compact DNA and make it inaccessible to transcription factors [7]. Linker histones H1 are necessary for modulating chromatin structure and function at multiple levels [8].

Table 1 Phosphorylation and *O*-β-GlcNAc site map of *Homo sapiens*

Substrate		Phosphorylation Sites by NetPhos	Experimentally known	Predicted by Motif	Yin Yang sites	Conserved	Conserved sub
H1.1	SER	33, 41, 51, 52, 53, 91, 106, 114, 115, 123, 135, 145, 148, 164, 165	1, 35, 103, 183	183	33, 52, 53, 114, 164, 165	41, 43, 51, 53, 60, 106, 183	1, 48, 52, 91, 103
	THR	94, 151, 161, 173, 199, 203	151	151	161, 173, 199, 203	94	101, 151, 11, 164, 203
H1.2	SER	35, 50, 54, 104, 112, 149, 172, 187	1, 172	172	30, 50, 187	1, 40, 58, 77, 102, 104, 172, 187	35, 85, 88, 112
	THR	30, 91, 145, 153, 166	30	30, 145, 153	145, 166	44, 91, 95, 98	3, 153
H1.3	SER	36,51, 55, 104, 113, 150, 173, 188, 204	188	173, 188	35, 51, 188, 204	36, 41, 51, 58, 79, 89, 102, 104, 188	1, 86
	THR	18, 92, 146, 154, 167, 179	18	18, 146, 154	146	3, 45, 92, 96, 99	154
H1.4	SER	26, 35, 50, 54, 103, 112, 150, 171, 186	35, 171, 186	171, 186	35, 50, 186	1, 35, 40, 50, 54, 57, 78, 85, 88, 101, 103, 112, 150, 171, 186	172, 188
	THR	17, 91, 145, 153, 202	17	17, 145, 153	17, 145, 202	3, 17, 91, 95, 98, 145	141, 153, 202
H1.5	SER	17, 43, 53, 106, 115, 172, 188	17, 172, 188	17, 172	17, 43, 53	1, 43, 60, 80, 104, 106, 115	17, 53, 88, 91, 172
	THR	10, 24, 38, 94, 137, 154	137,154	10, 137, 154	10, 38	38	3, 8, 47, 98, 101, 154
H1.0	SER	6, 18, 21, 44, 48, 65, 70, 97, 103, 123, 130, 185	123		6, 21, 44, 97, 103, 123, 130	4, 6, 21, 28, 44, 45, 55, 65, 70, 89, 91, 103, 130, 170, 184, 185	18, 97, 115
	THR	109, 118, 134, 140, 152, 161		118, 140, 152	134, 161	1, 5, 22, 76, 77, 83, 109, 118, 123, 134, 140, 152	161
H1.T	SER	8, 42, 52, 54, 86, 107, 111, 118, 126, 128, 137, 140, 142, 165, 180, 187, 204	177	142, 180	8, 54, 118, 180, 204	1,42, 44, 52, 54,61,81, 105,107,140, 142,165, 180	8, 35, 126, 128, 137, 187, 189, 204
	THR	131, 148, 158, 159, 162, 203	158, 159		148, 158, 159, 162, 203	3, 21, 99, 102, 148, 158	10, 48, 131, 145, 203
H1oo	SER	8, 11, 13, 14, 16, 20, 21, 23, 26, 32, 42, 73, 161, 211, 229, 230, 235, 243, 245, 246, 260, 262, 263, 276, 336, 337, 340, 341		276	8, 13, 14, 16, 26, 73, 229, 262, 336, 337, 340, 341	5, 8, 12, 13, 20, 67, 110, 118, 221, 236	7, 122, 219, 231, 241, 249
	THR	72, 194, 256, 278, 319			256, 319	66, 81, 97, 116, 231	19, 209
H1.X	SER	31, 33, 39, 92, 113, 154, 171	31, 33		33	49, 65, 66, 92, 113,	27, 31, 133
	THR	55				101	12, 13, 55, 87


30 nm
chromatin fiber
11 nm
(beads)
DNA
nucleosome
+ charged N termini
(bind DNA on neigboring
nucleosomes)
highly acetylated
core histones
(especially H3 and H4)
the solenoid

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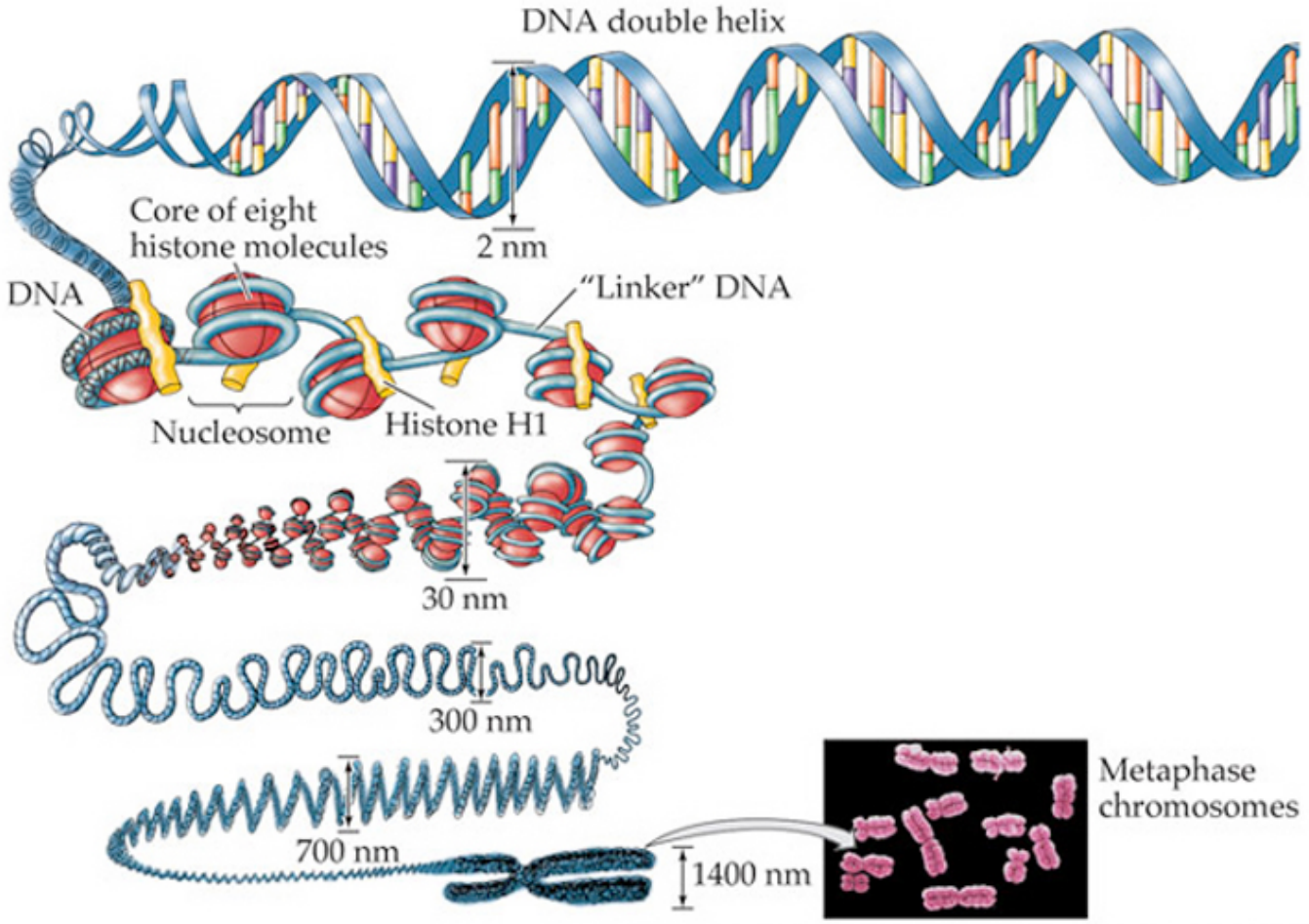
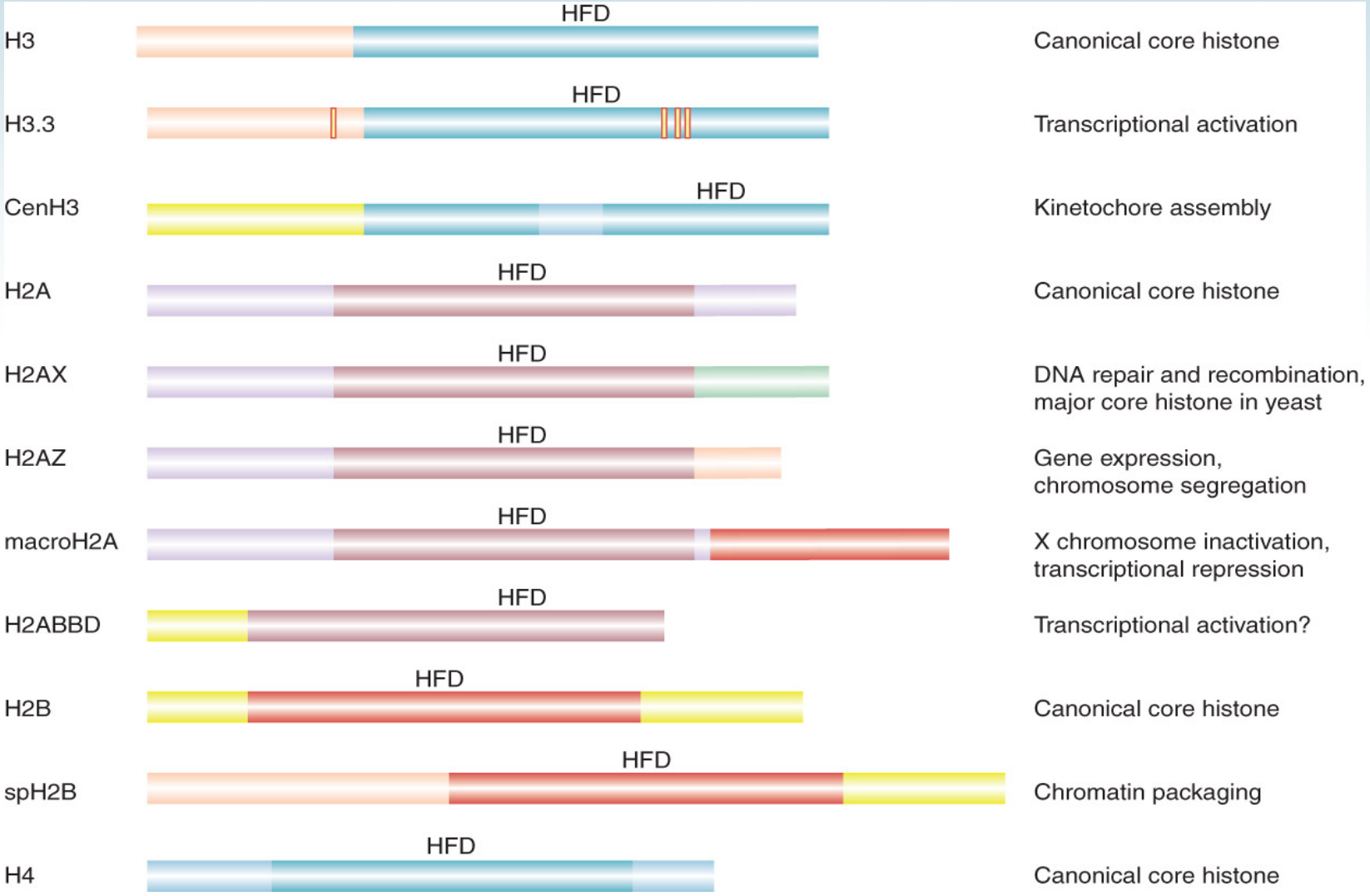


LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.6 DNA Packs into a Mitotic Chromosome

Histone Variants Produce Alternative Nucleosomes, and potentially Alternative Nucleosomal Organization

- All core histones except H4 are members of families of related variants.
- **Histone variants** can be closely related or highly divergent from canonical histones.
- Different variants serve different functions in the cell.



The major core histones contain a conserved histone-fold domain

Adapted from K. Sarma and D. Reinberg, Nat. Rev. Mol. Cell Biol. 6 (2005): 139-149.

PNAS
Proceedings of the National Academy of Sciences of the United States of America

Heterochromatic deposition of centromeric histone H3-like proteins

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+ Author Affiliations

Communicated by Gerald M. Rubin, University of California, Berkeley, CA (received for review September 9, 1999)

Abstract

Centromeres of most organisms are embedded within constitutive heterochromatin, the condensed regions of chromosomes that account for a large fraction of complex genomes. The functional significance of this centromere–heterochromatin relationship, if any, is unknown. One possibility is that heterochromatin provides a suitable environment for assembly of centromere components, such as special centromeric nucleosomes that contain distinctive histone H3–like proteins. We describe a *Drosophila* H3–like protein, Cid (for centromere identifier) that localizes exclusively to fly centromeres. When the *cid* upstream region drives expression of H3 and H2B histone–green fluorescent protein fusion genes in *Drosophila* cells, euchromatin–specific deposition results. Remarkably, when the *cid* upstream region drives expression of yeast, worm, and human centromeric histone–green fluorescent protein fusion proteins, localization is preferentially within *Drosophila* pericentric heterochromatin. Heterochromatin–specific localization also was seen for yeast and worm centromeric proteins constitutively expressed in human cells. Preferential localization to heterochromatin in heterologous systems is unexpected if centromere–specific or site–specific factors determine H3–like protein localization to centromeres. Rather, the heterochromatic state itself may help localize centromeric components.

centromeres heterochromatin Drosophila

Footnotes

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PNAS January 18, 2000 vol. 97 no. 2 716–721

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Human HeLa Centromeres
with
D. melanogaster H3-GFP

Human HeLa Centromeres
with
C. elegans H3-GFP

Human HeLa Centromeres
with
S. cerevisiae H3-GFP

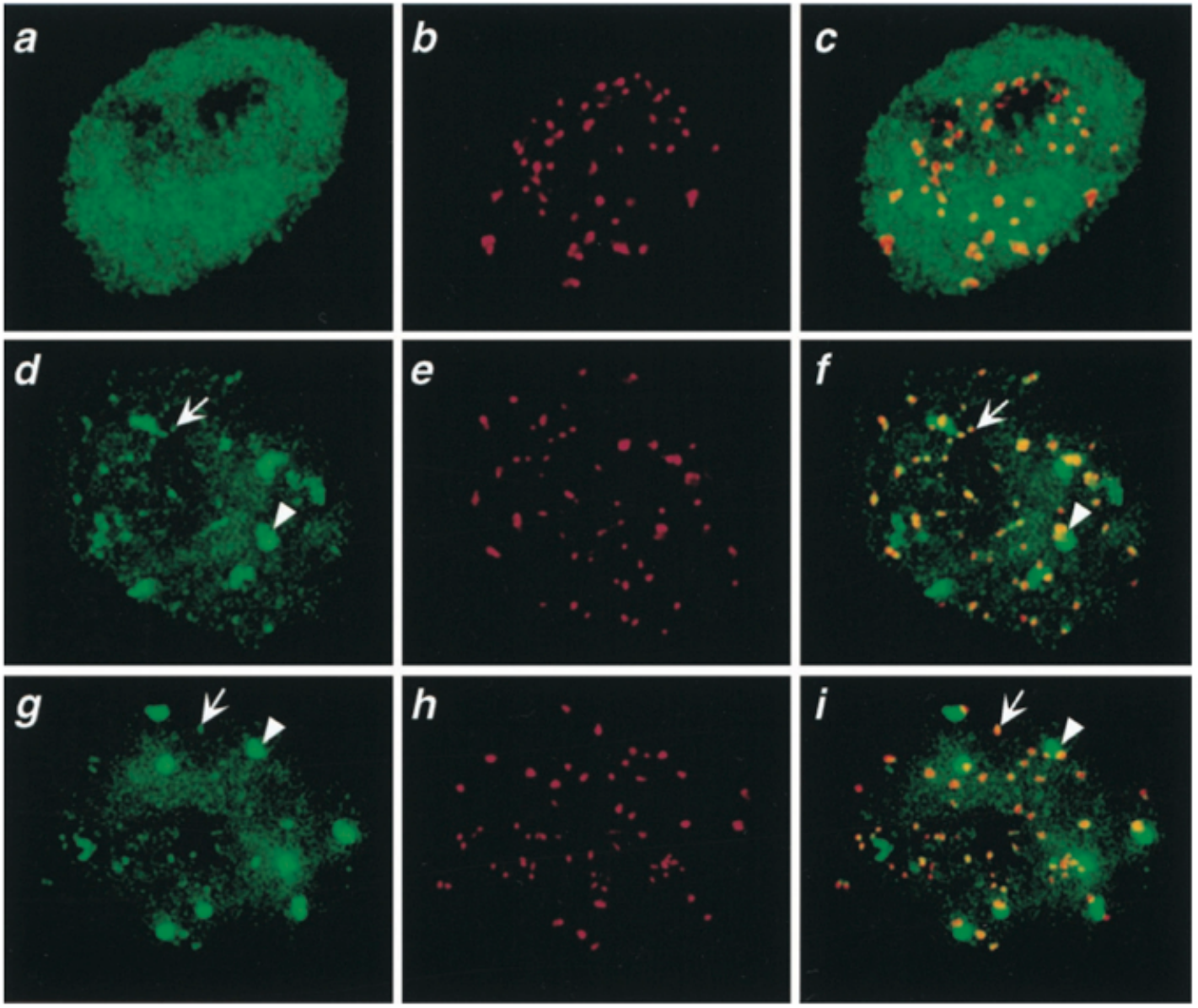
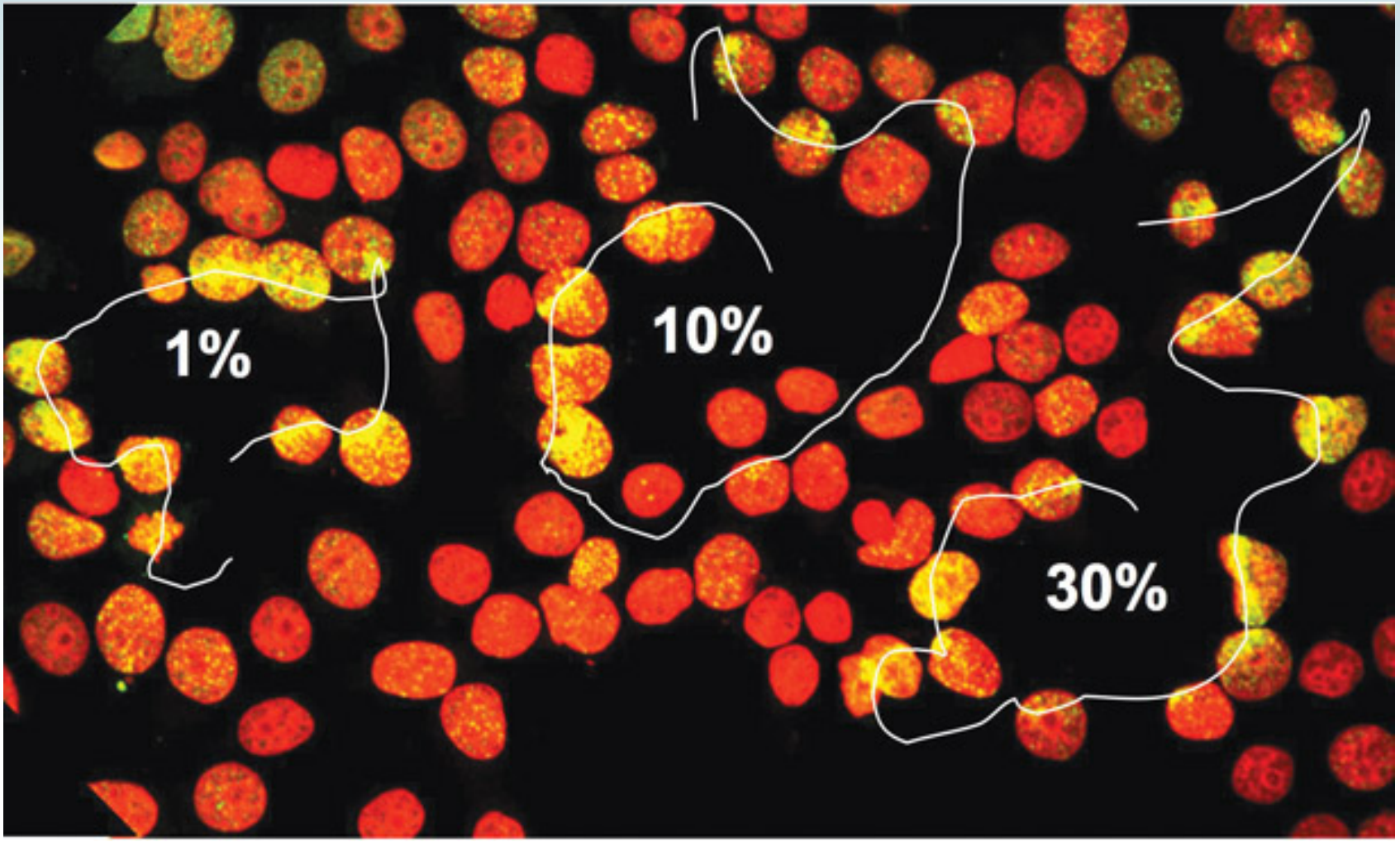
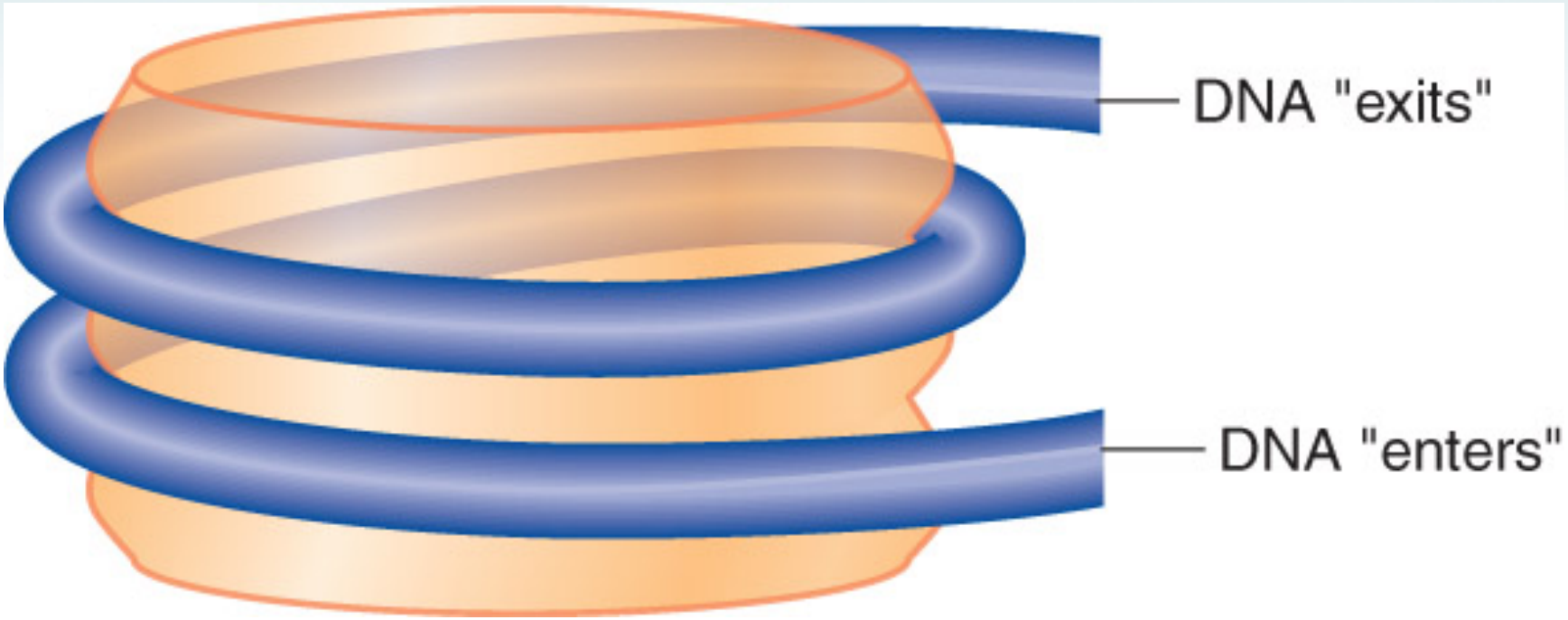


Fig. 5. Colocalization of H3-like proteins with centromeres in human interphase nuclei. HeLa cells were transfected with GFP fusions of *D. melanogaster* H3 (*a*–*c*), *C. elegans* HCP-3 (*d*–*f*), or *S. cerevisiae* Cse4p (*g*–*i*) driven by the constitutive cytomegalovirus promoter. Cells were fixed and stained with ACA serum against centromeres (red); the GFP signal is shown in green. Representative interphase nuclei are shown. We chose the 60 brightest GFP spots in *d* and *g*; 70% of these spots colocalized with the ~60 ACA spots (*f* and *i*). Arrows point to representative small spots and arrowheads point to large spots.



γ-H2AX is detected by an antibody (yellow) and appears along the path traced by a laser that produces double-strand breaks.H2AX has SEQL/Y in C-term which can be phosphorylated.



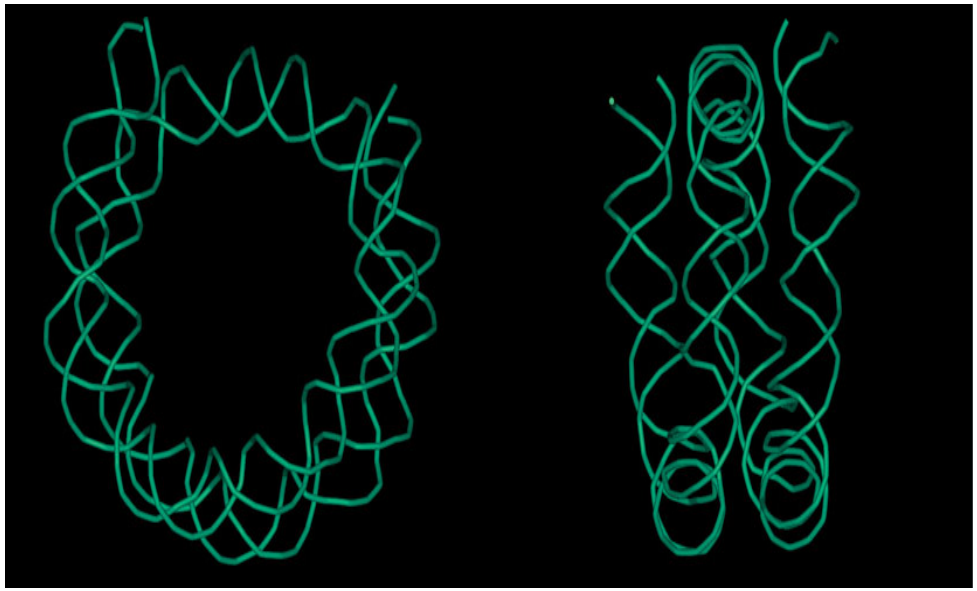
The nucleosome is a cylinder with DNA organized into ~1 2/3 turns around the surface

DNA Structure Varies on the Nucleosomal Surface

- DNA is wrapped **1.67 times** around the histone octamer.

- The structure of the DNA is altered so that it has:
  - an increased number of base pairs/turn in the middle
  - a decreased number at the ends

- DNA is wrapped 1.67 times around the histone octamer.
- DNA on the nucleosome shows regions of smooth curvature and regions of abrupt kinks.
- The structure of the DNA is altered so that it has an increased number of base pairs/turn in the middle, but a decreased number at the ends.

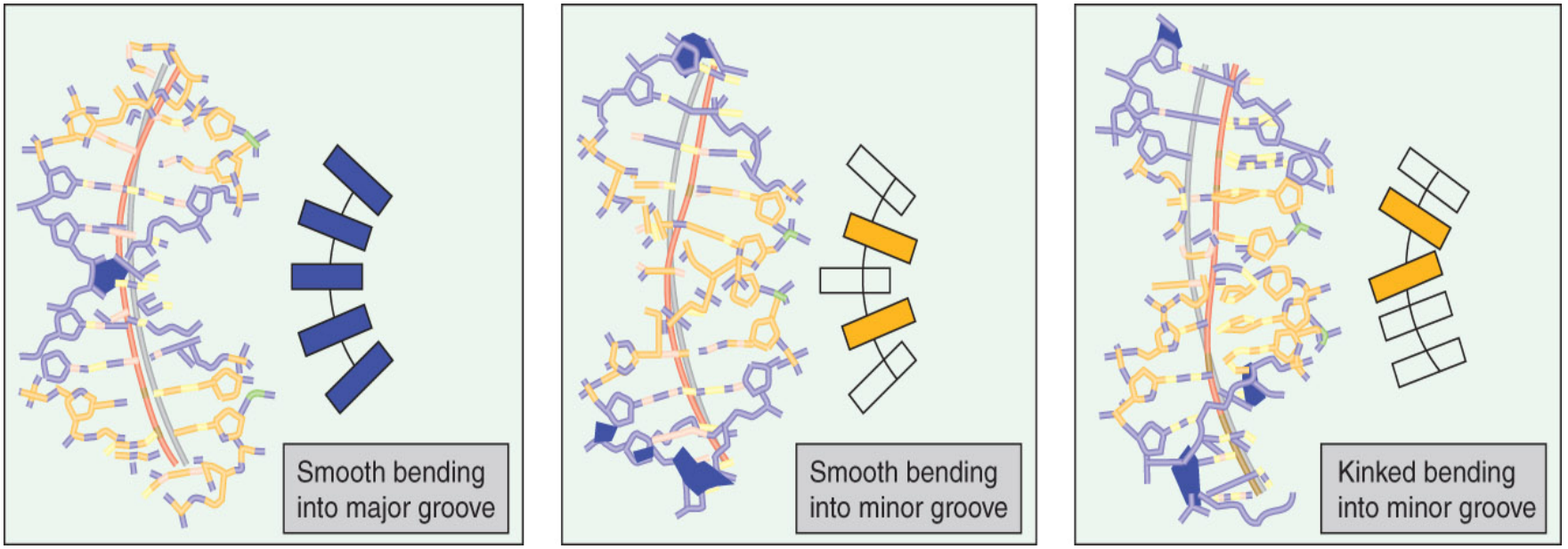


DNA structure in nucleosomal DNA

Structures from Protein Data Bank: 1P34. U. M. Muthurajan, et al., EMBO J. 23 (2004): 260-271.

- ~0.6 negative turns of DNA are absorbed by the change in bp/turn from 10.5 in solution to an average of 10.2 on the nucleosomal surface.
    - from 10.5 in solution
    - to an average of 10.2 on the nucleosomal surface

  – which explains the linking-number paradox.



DNA structure in nucleosomal DNA

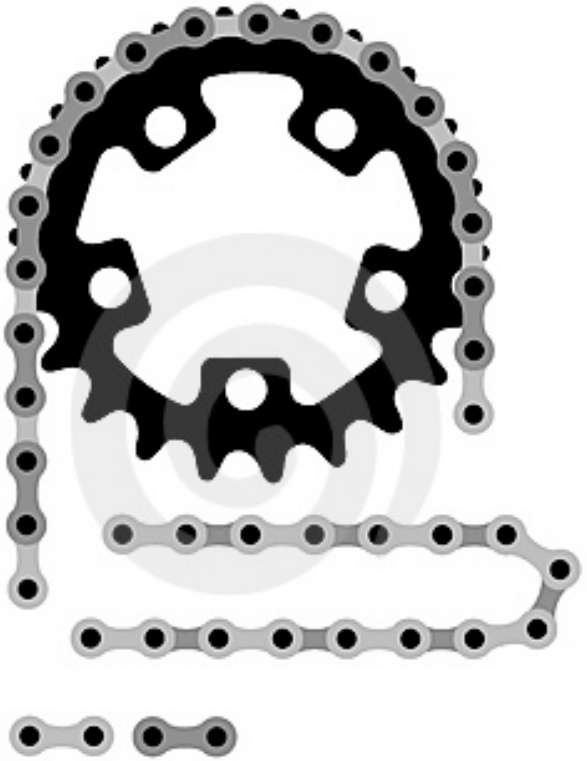
Adapted from T. J. Richmond and C. A. Davey, Nature 423 (2003): 145-150.

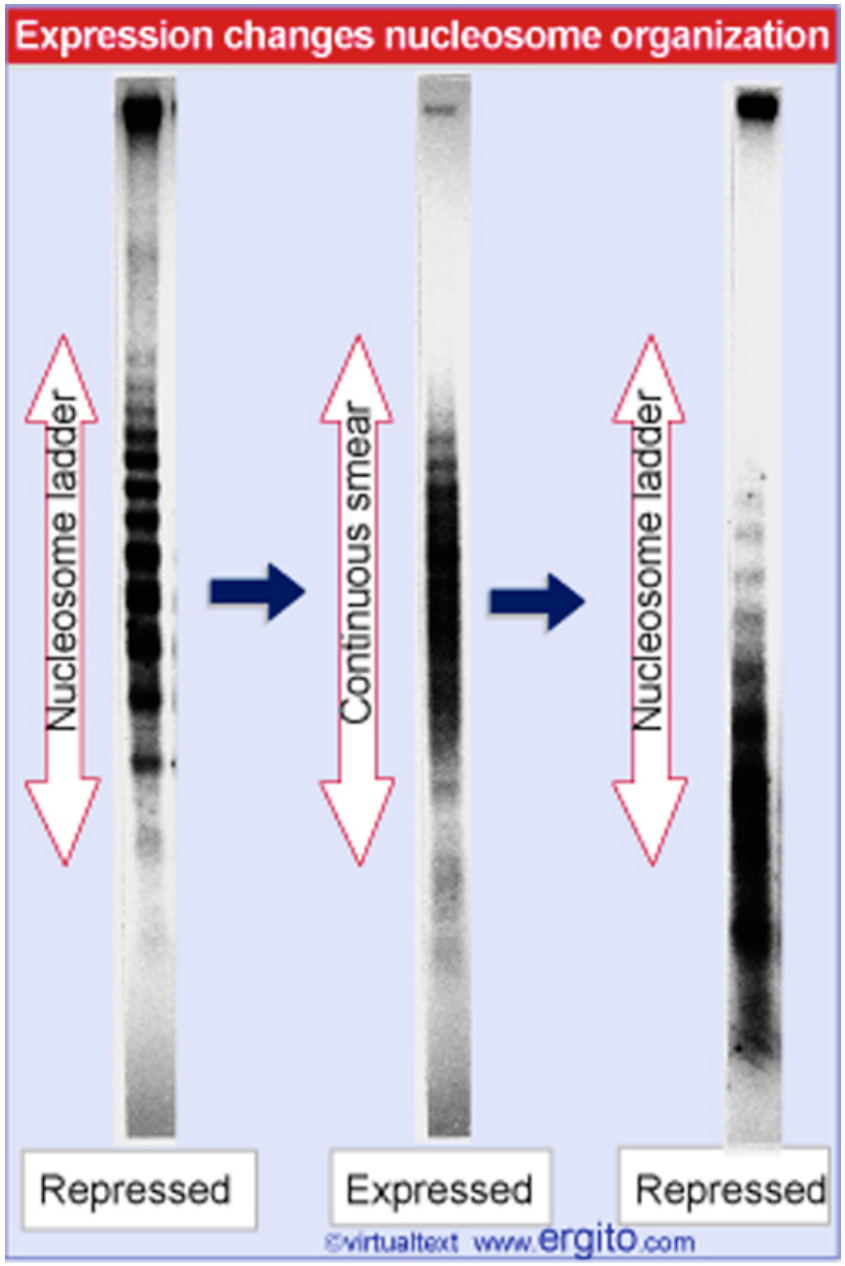
- Organized bending of chains around a spherical or circular object.....

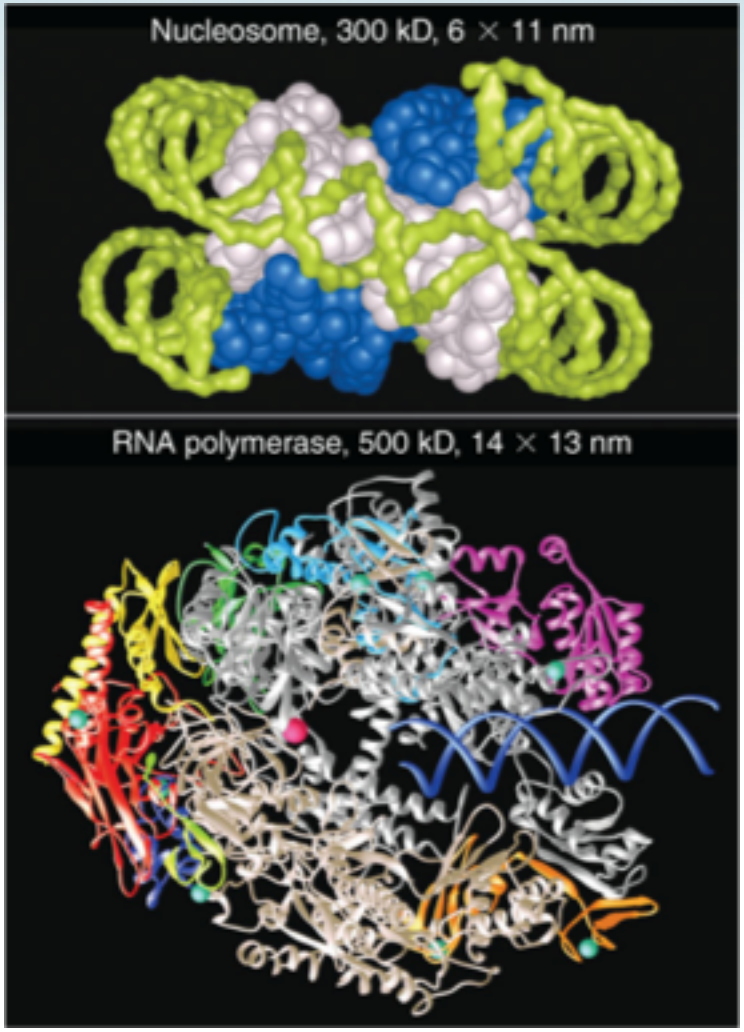
nucleosomes

and

bicycle chains.

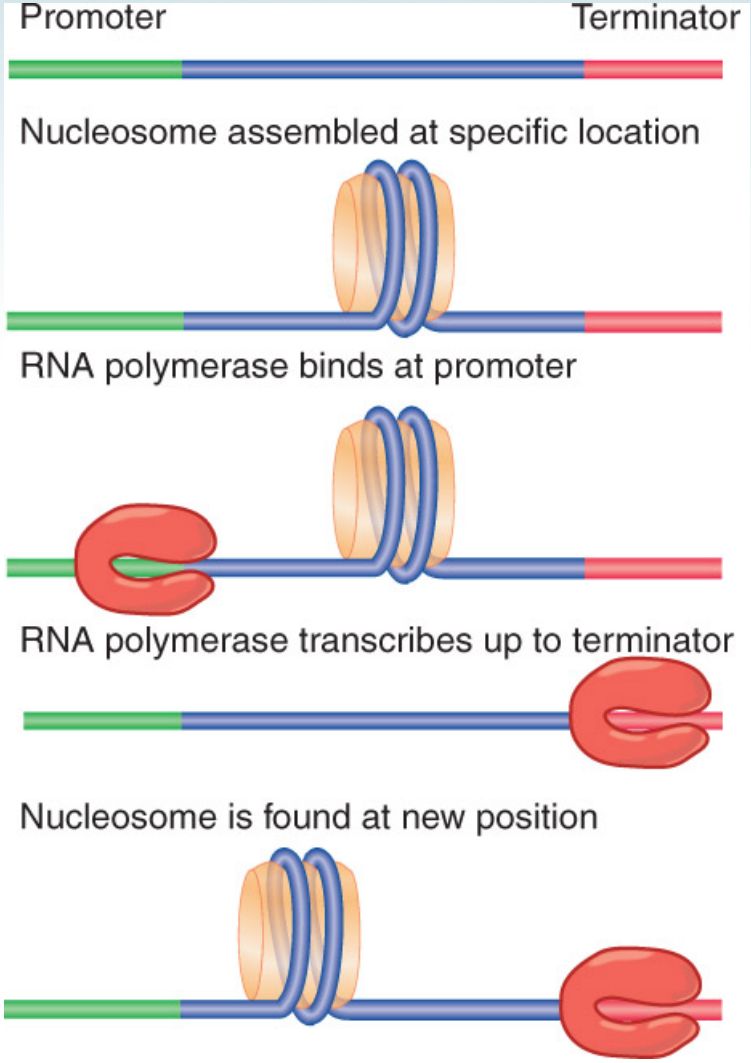



Expression changes nucleosome organization
Nucleosome ladder
Continuous smear
Nucleosome ladder
Repressed
Expressed
Repressed
©virtualtext www.ergito.com

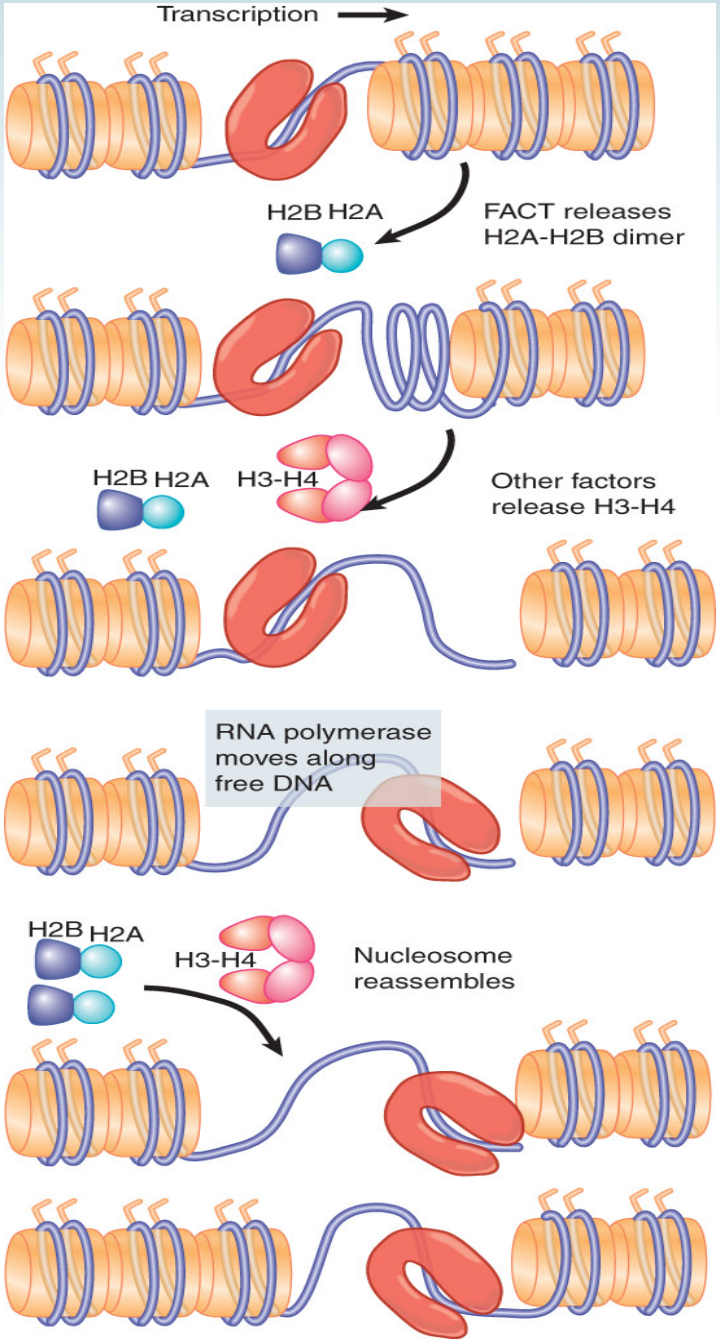


RNA polymerase is comparable in size to the nucleosome and might encounter difficulties in following the DNA around the histone octamer

Top photo courtesy of E. N. Moudrianakis, Johns Hopkins University.
Bottom photo courtesy of Roger Kornberg, Stanford University School of Medicine.



An experiment to test the effect of transcription on nucleosomes shows that the histone octamer is displaced from DNA and rebinds at a new position



- Ancillary factors are required both:
  - for RNA polymerase to displace octamers during transcription
  - for the histones to reassemble into nucleosomes after transcription

Histone octamers are disassembled ahead of transcription to remove nucleosomes

Published in final edited form as:
Annu Rev Genet. 2013 ; 47: 483–508. doi:10.1146/annurev-genet-110711-155440.

Control of Transcriptional Elongation

Hojoong Kwak and **John T. Lis**
Department of Molecular Biology and Genetics, Cornell University

Hojoong Kwak: hk572@cornell.edu; John T. Lis: jtl10@cornell.edu

Abstract

Elongation is becoming increasingly recognized as a critically controlled step in transcriptional regulation. While traditional genetic and biochemical studies have identified major players of transcriptional elongation, our understanding of the importance and roles of these factors is evolving rapidly through the recent advances in genome-wide and single-molecule technologies. Here we focus on how elongation can modulate the transcriptional outcome through the rate-liming step of RNA polymerase II pausing near promoters, and how the participating factors were identified. Among the factors we describe are NELF and DSIF, the pausing factors, and P-TEFb, the key player in pause release. We also describe non-exclusive models for how pausing is achieved by making use of high resolution genome-wide mapping of paused Pol II relative to promoter elements and the first nucleosome. We also discuss Pol II elongation through the bodies of genes and the roles of FACT and Spt6, the factors that allow Pol II to move through nucleosomes.

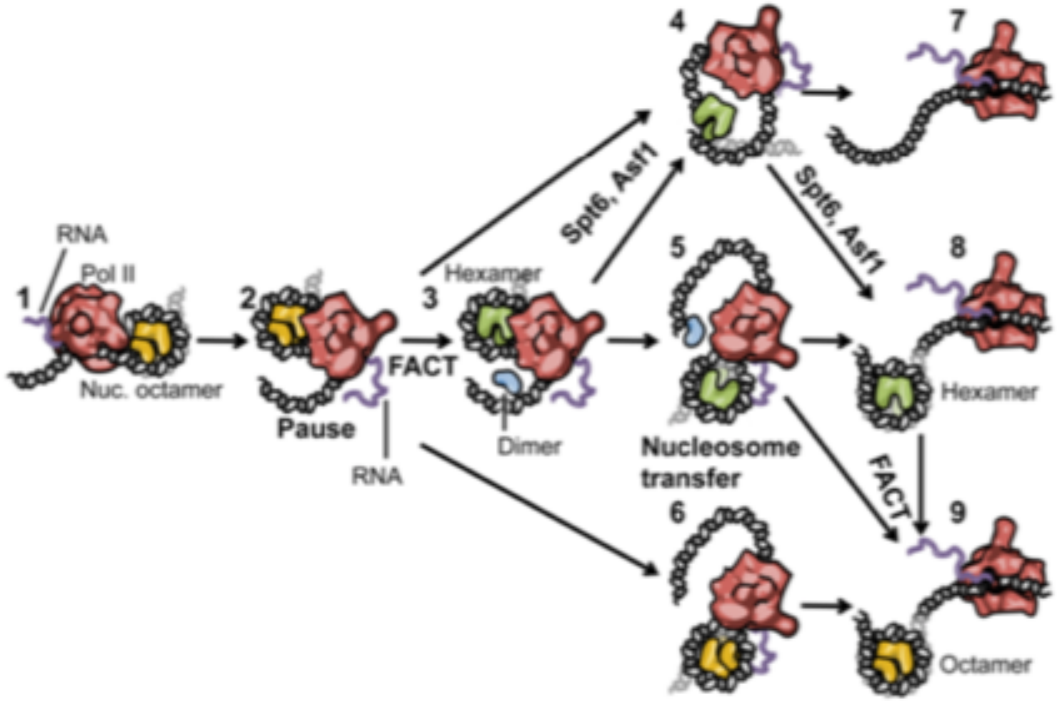


Figure 4. Pol II transcribing through gene body nucleosomes

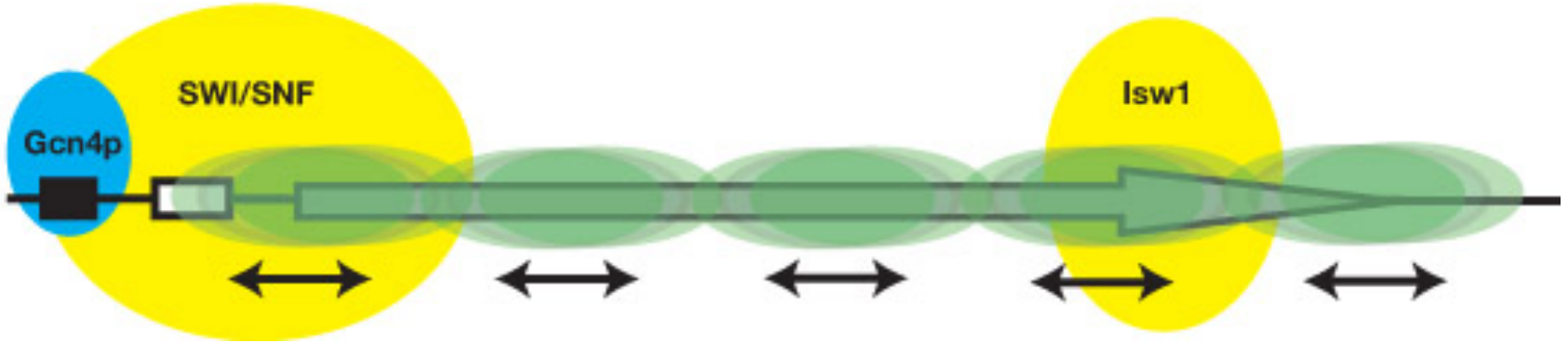
Pol II may use multiple mechanisms to get through a nucleosome, and not all the steps are used. Step 1, Pol II approaches and makes contact with a nucleosome. Step 2, the outer wrap of nucleosomal DNA can be easily unwrapped (17), and Pol II moves into the nucleosome near the dyad axis. Pol II active site is at around −40 from the dyad axis. The nucleosome binding is strong at this point and Pol II often pauses transiently (25, 52, 65). Step 3, H2A/H2B dimer is dissociated from the DNA and the nucleosome is now a hexamer. A dissociated dimer can still remain through its association with FACT and be re-deposited later (9). Step 4, H3/H4 core nucleosomal particle is evicted from DNA. H3 can remain associated and be re-deposited by Spt6 or Asf1 (13, 118) Step 5, nucleosome hexamer transfers upstream of Pol II while Pol II transcribes into downstream region. A looping intermediate may form during the transfer. Step 6, nucleosome octamer transfers upstream of Pol II, which can be facilitated by histone chaperones. Step 7, Pol II evicts the nucleosome by transcribing through it. Step 8, Pol II transcribes through the nucleosome leaving a hexamer. Step 9, Pol II transcribes through the nucleosome leaving an octamer.

Basal *HIS3* chromatin is static:

Dominant array with little remodelling activity.

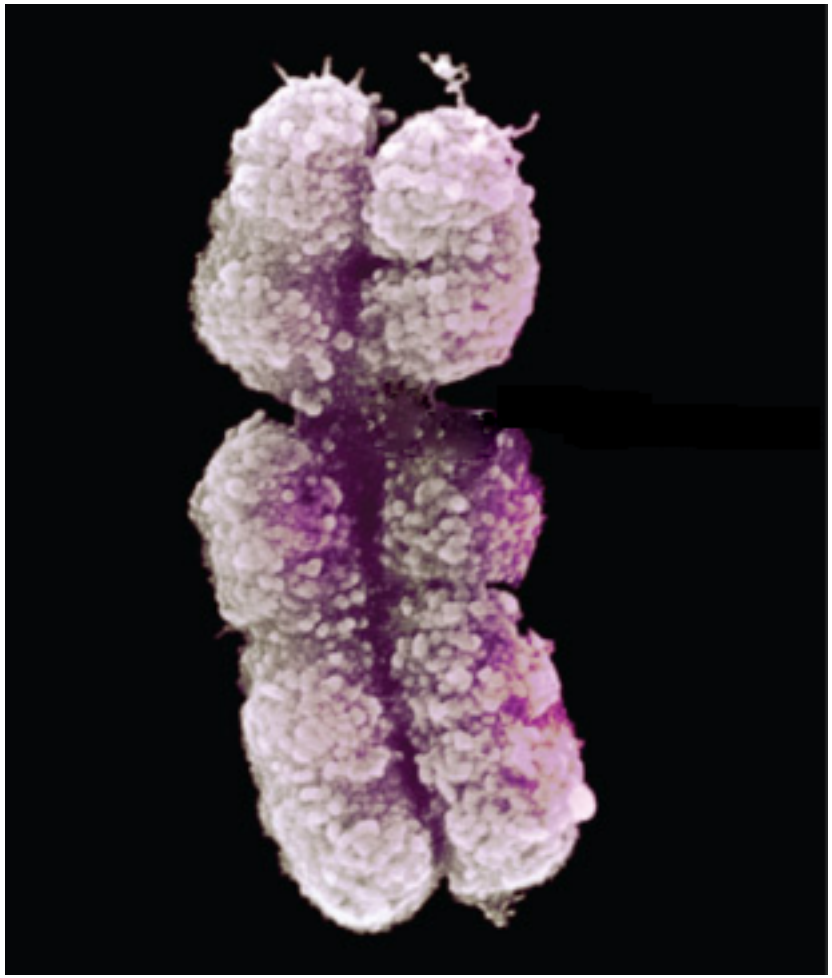
Activated *HIS3* chromatin is dynamic:

Nucleosomes are in flux as they are continually mobilised into different arrays by the competing activities of SWI/SNF, Isw1 and other remodelling complexes.

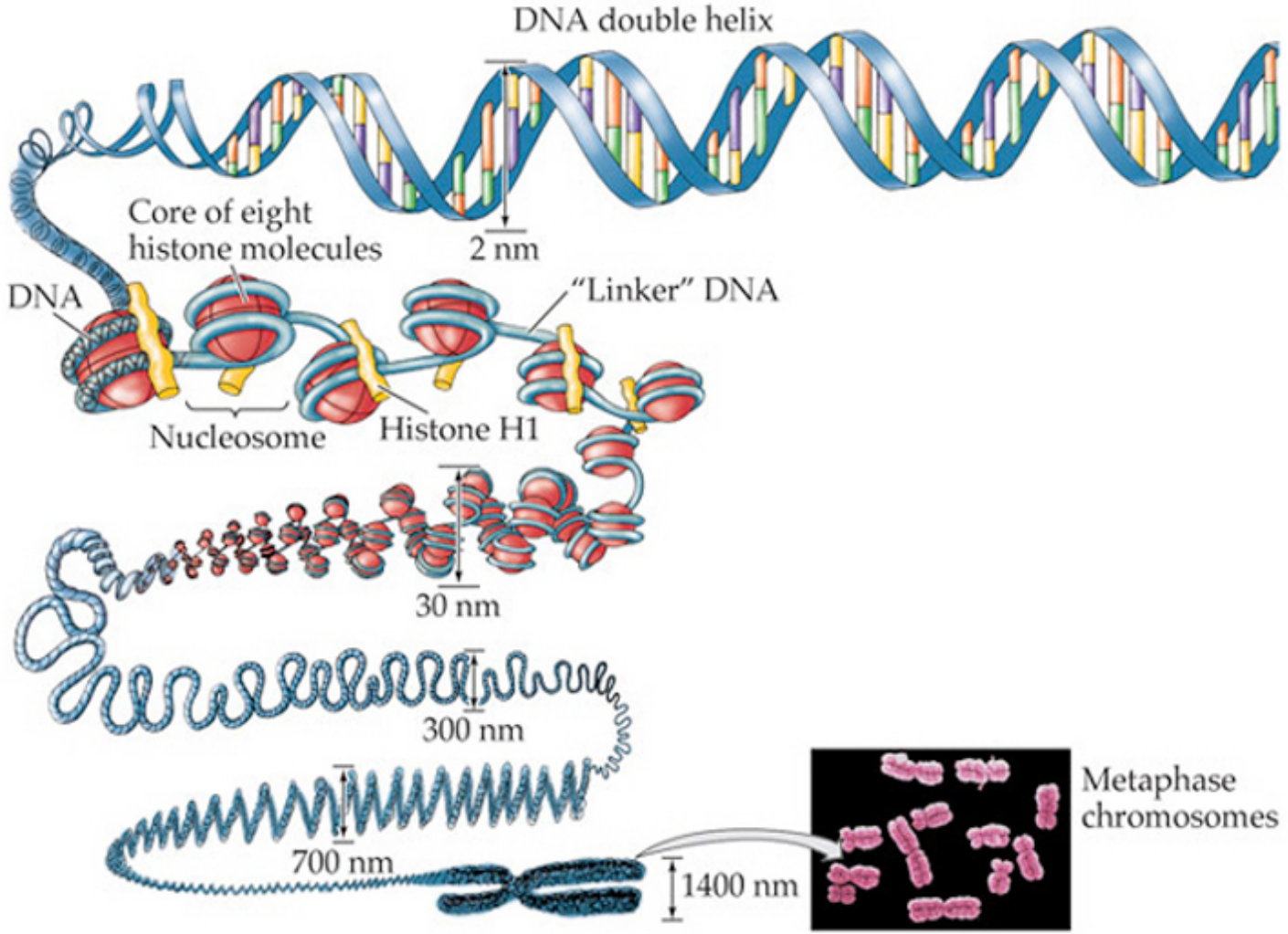


Basal/unactivated HIS3 chromatin is static and shows a single dominant nucleosomal array with little remodeling activity (top panel) Basal/unactivated HIS3 chromatin is static and shows a single dominant nucleosomal array with little remodeling activity (top panel)

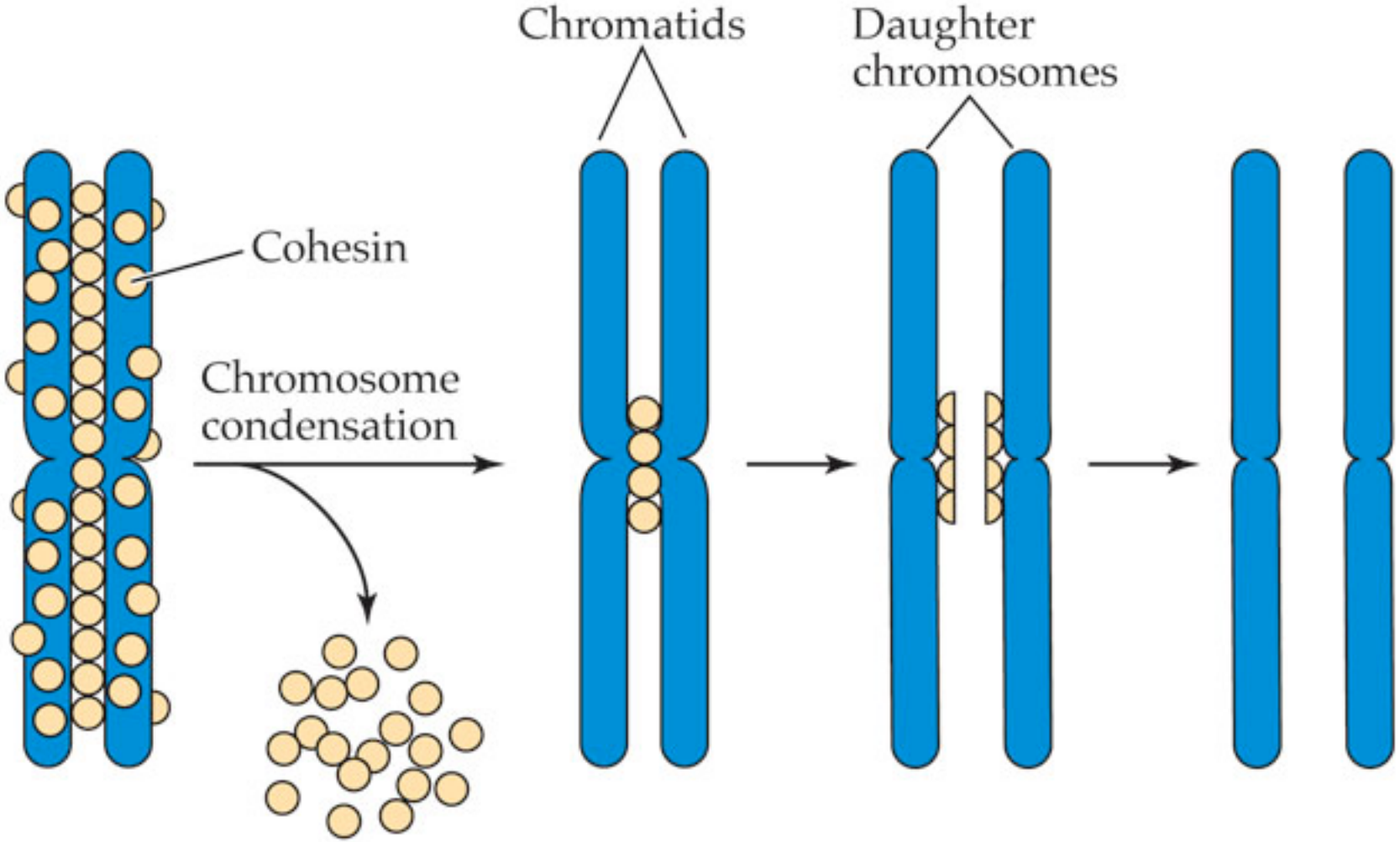
The Eukaryotic chromosome



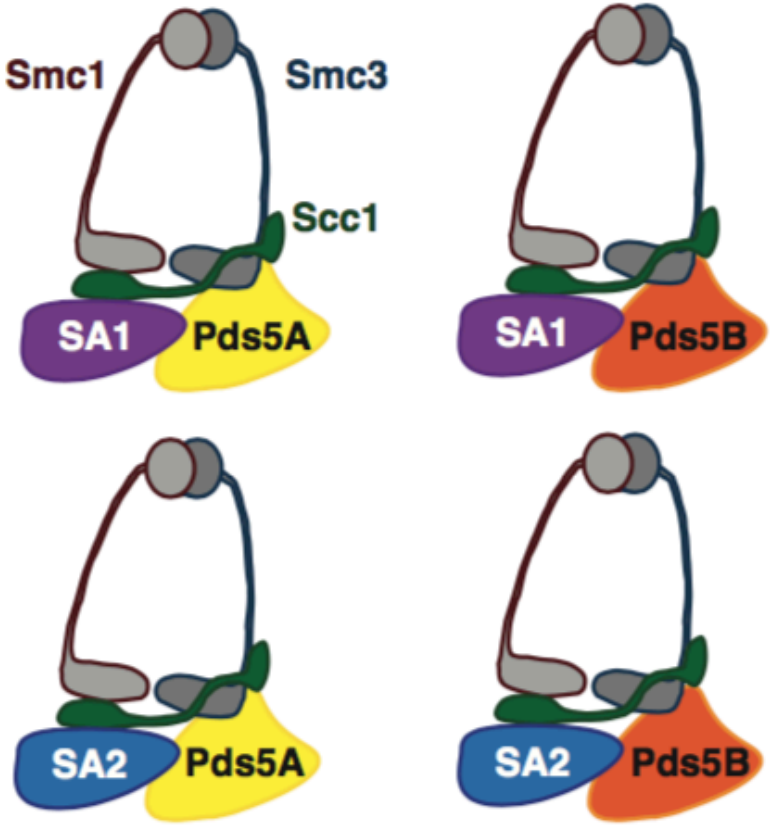
- Organized Structures -banding
- Centromeres
- Telomeres
- Nucleosomes
- **Euchromatin / Heterochromatin**
- **Higher Orders of Chromosomal Structure**



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.6 DNA Packs into a Mitotic Chromosome



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.9 Molecular Biology of Chromatid Attachment and Separation



Cohesins more than just sticky proteins, they are required to snare freshly replicated DNA and have two globular domains separated by a hinge like structure … different variants involved in different cell types that are undergoing different meiosis or mitosis…. Also help to build higher levels of chromatin organization…

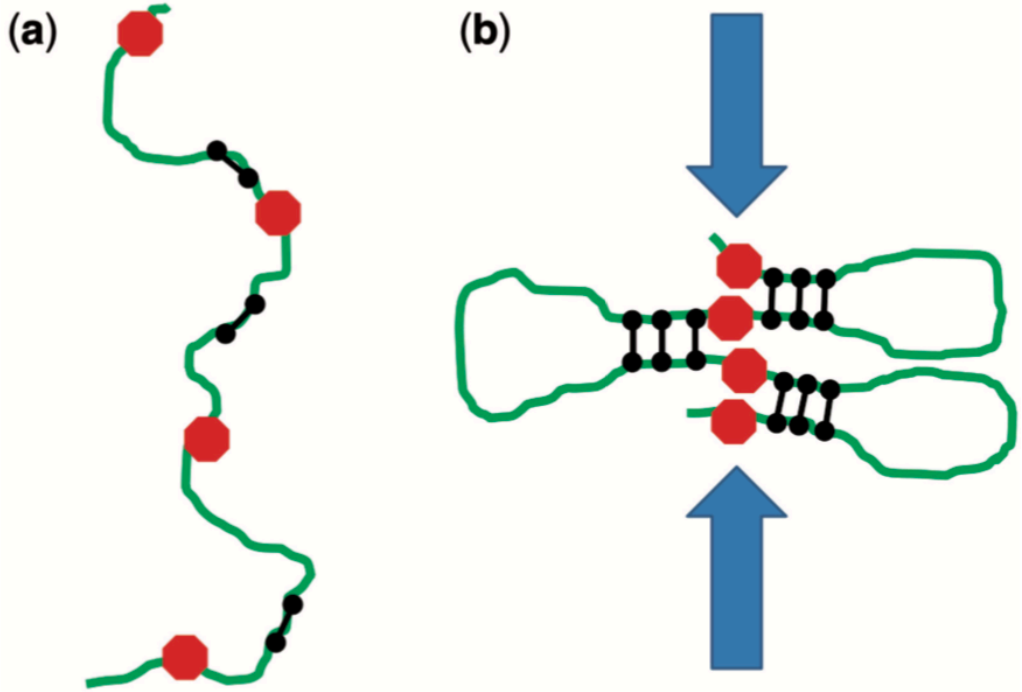


Figure 6. Condensation of multiple loop domains. (**a**) Initially condensins (black dumbbell shapes) bind along a long stretch of chromatin (green), between condensation boundary elements (red octagons). (**b**) As condensation proceeds, condensins organize into a 'stacked' configuration at the bases of chromatin loops defined by the boundary elements. The resultant crowding of chromatin at the bases of the loops generates inter-chromatid tension that will drive topo II to remove inter-chromatid entanglements.


small scale
10 bp - few kb
compaction of DNA into chromatin
double helix
nucleosomes

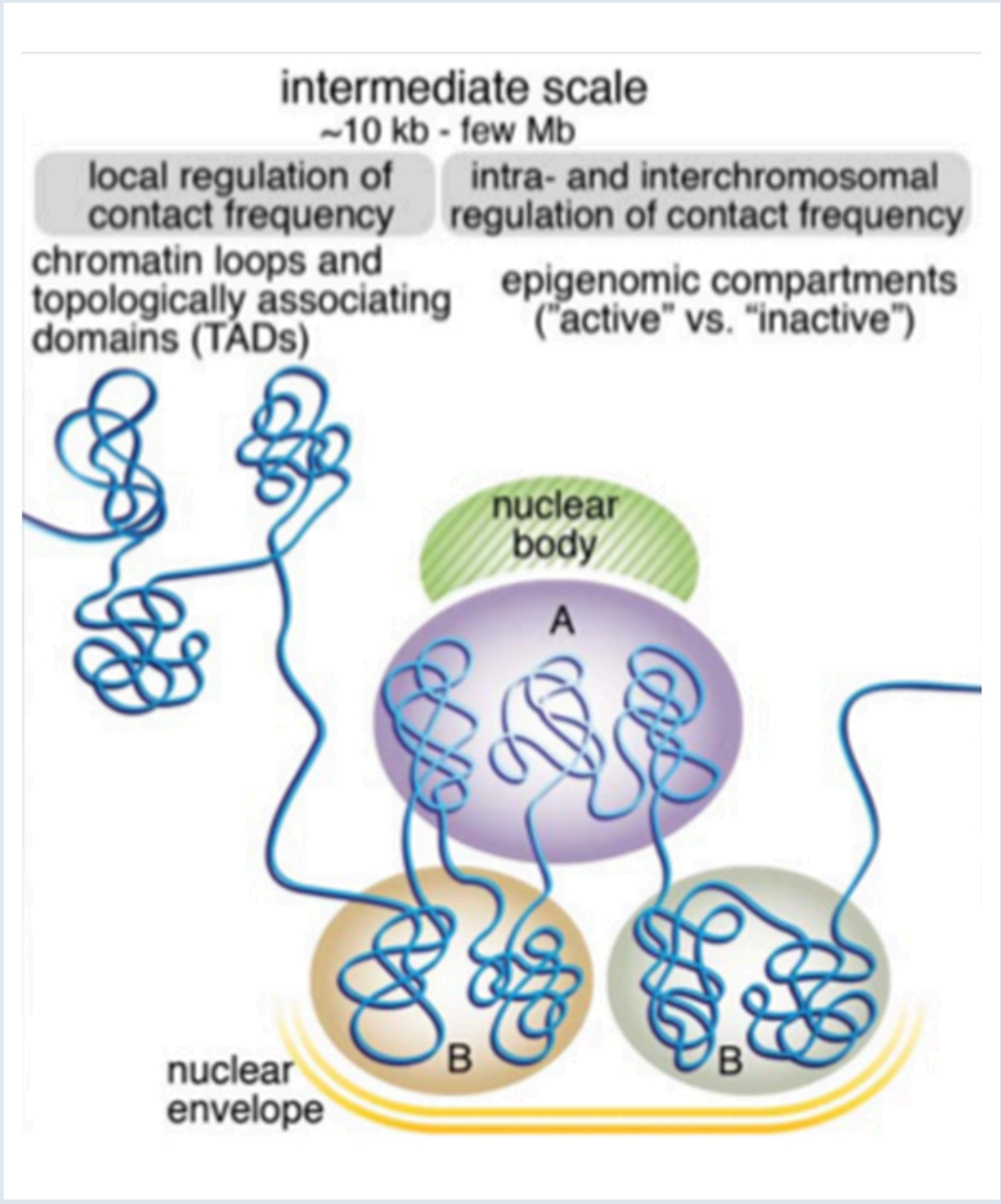

intermediate scale
~10 kb - few Mb
local regulation of contact frequency
intra- and interchromosomal regulation of contact frequency
chromatin loops and topologically associating domains (TADs)
epigenomic compartments ("active" vs. "inactive")
nuclear body
A
B
B
nuclear envelope

Figure 1. Chromosome structure and function is organized at multiple scales. At the smallest scale, DNA is folded into a double helix, which gets compacted into »11 nm nucleosomes, whereby **147 bp of DNA wrap around a histone octamer.**

Functionally, nucleosomes regulate access of DNA-binding proteins and serve as modules for epigenetic modifications, which regulate gene expression. At the intermediate scale of tens of kilobases to a few megabases, chromatin is organized into **Topologically Associating Domains** (**TADs**) with a median size of a few hundred kilobases.

Functionally, TADs are characterized by preferential contact of loci within them, and critically control enhancer-promoter interactions, and relative insulation from adjacent TADs. At a similar scale of TADs, chromatin is also organized into **epigenomic** A/B "compartments", whereby **active chromatin (A) tends to contact with other segments of active chromatin and localize in proximity of certain nuclear bodies such as nuclear speckles**, while **inactive chromatin (B) tends to contact with inactive chromatin and be associated with the nuclear lamina**.

At the largest scale, particular chromosomes tend to associate with other chromosomes and form stereotyped chromosome territories inside the cell nucleus. The image used to illustrate chromosome territories was generously provided by Stevens et al.[76]

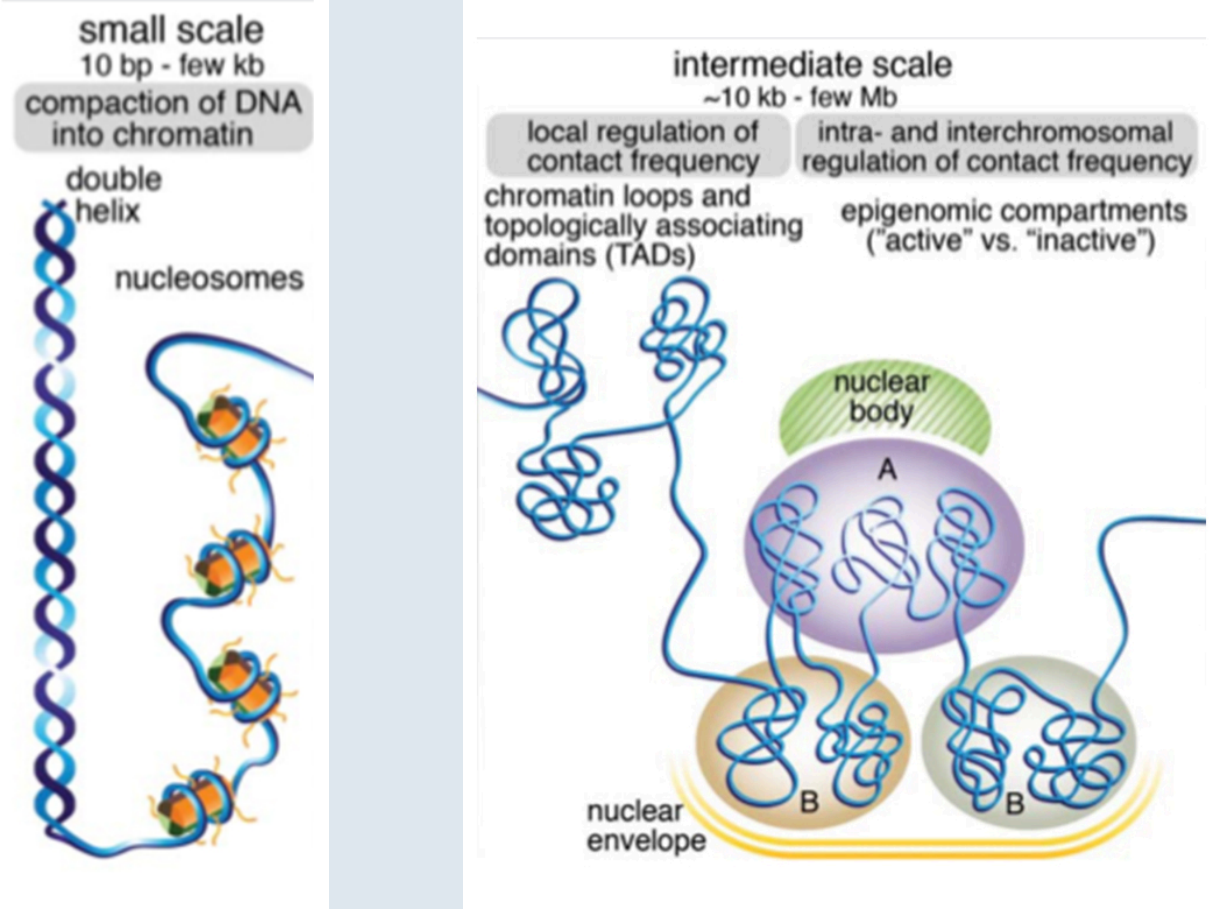


Figure 1. Chromosome structure and function is organized at multiple scales. At the smallest scale, DNA is folded into a double helix, which gets compacted into »11 nm nucleosomes, whereby 147 bp of DNA wrap around a histone octamer. Functionally, nucleosomes regulate access of DNA-binding proteins and serve as modules for epigenetic modifications, which regulate gene expression. At the intermediate scale of tens of kilobases to a few megabases, chromatin is organized into Topologically Associating Domains (TADs) with a median size of a few hundred kilobases. Functionally, TADs are characterized by preferential contact of loci within them, and critically control enhancer-promoter interactions, and relative insulation from adjacent TADs. At a similar scale of TADs, chromatin is also orga- nized into epigenomic A/B "compartments", whereby **active chromatin (A) tends to contact with other segments of active chromatin and localize in proximity of certain nuclear bodies such as nuclear speckles, while inactive chromatin** (B) tends to contact with inactive chromatin and to be associated with the nuclear lamina. At the largest scale, particular chromosomes tend to associate with other chro- mosomes and form stereotyped chromosome territories inside the cell nucleus. The image used to illustrate chromosome territories was generously provided by Stevens et al.

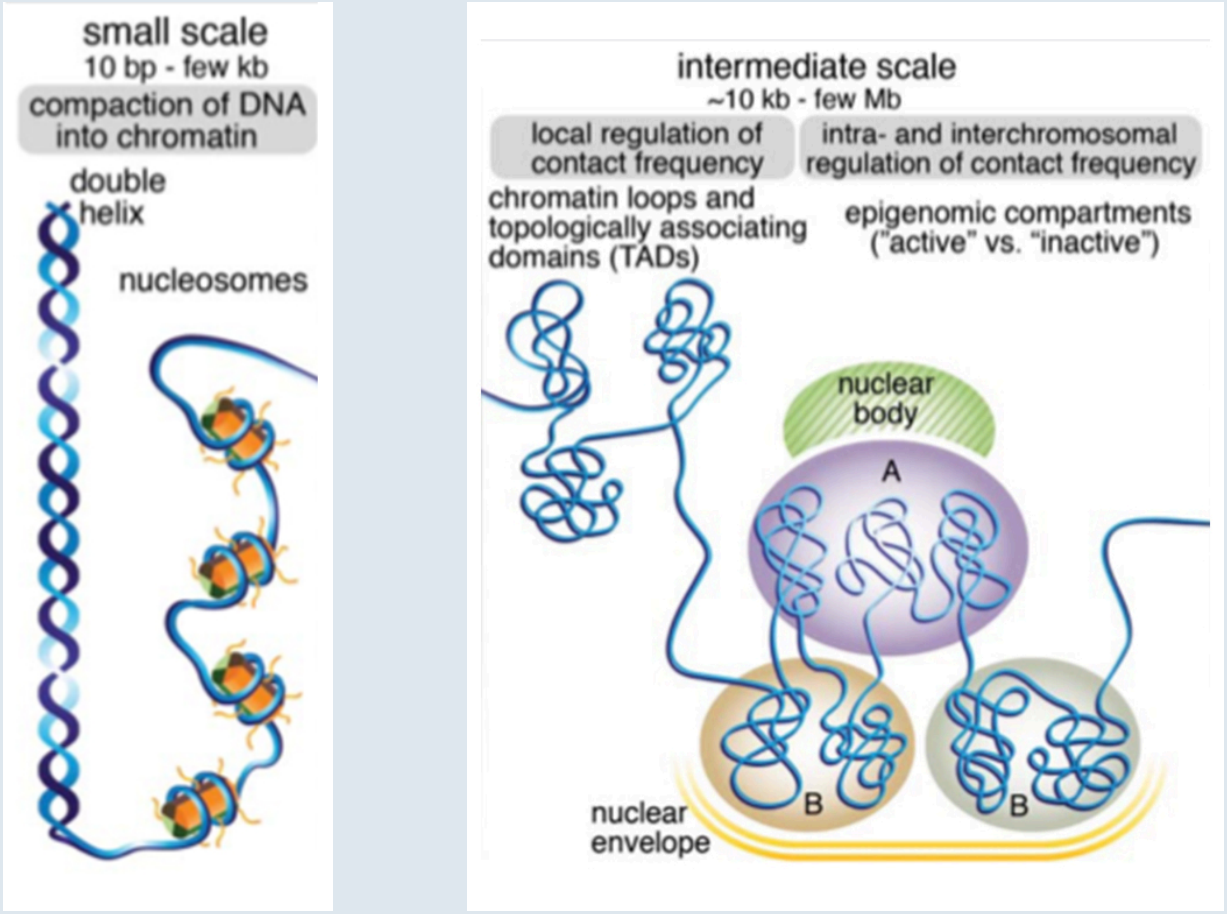


Figure 1. Chromosome structure and function is organized at multiple scales. At the smallest scale, DNA is folded into a double helix, which gets compacted into »11 nm nucleosomes, whereby 147 bp of DNA wrap around a histone octamer. Functionally, nucleosomes regulate access of DNA-binding proteins and serve as modules for epigenetic modifications, which regulate gene expression. At the intermediate scale of tens of kilobases to a few megabases, chromatin is organized into Topologically Associating Domains (TADs) with a median size of a few hundred kilobases. Functionally, TADs are characterized by preferential contact of loci within them, and critically control enhancer-promoter interactions, and relative insulation from adjacent TADs. At a similar scale of TADs, chromatin is also orga- nized into epigenomic A/B "compartments", whereby **active chromatin (A) tends to contact with other segments of active chromatin and localize in proximity of certain nuclear bodies such as nuclear speckles, while inactive chromatin (B) tends to contact with inactive chromatin and to be associated with the nuclear lamina. At the largest scale, particular chromosomes tend to associate with other chromosomes and form stereotyped chromosome territories inside the cell nucleus**. The image used to illustrate chromosome territories was generously provided by Stevens et al.[76]

NUCLEUS, 2018
VOL. 9, NO. 1, 20–32
https://doi.org/10.1080/19491034.2017.1389365

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EXTRA VIEW

Recent evidence that TADs and chromatin loops are dynamic structures

Anders S. Hansen [a,b], Claudia Cattoglio [a,b], Xavier Darzacq [a] and Robert Tjian [a,b]

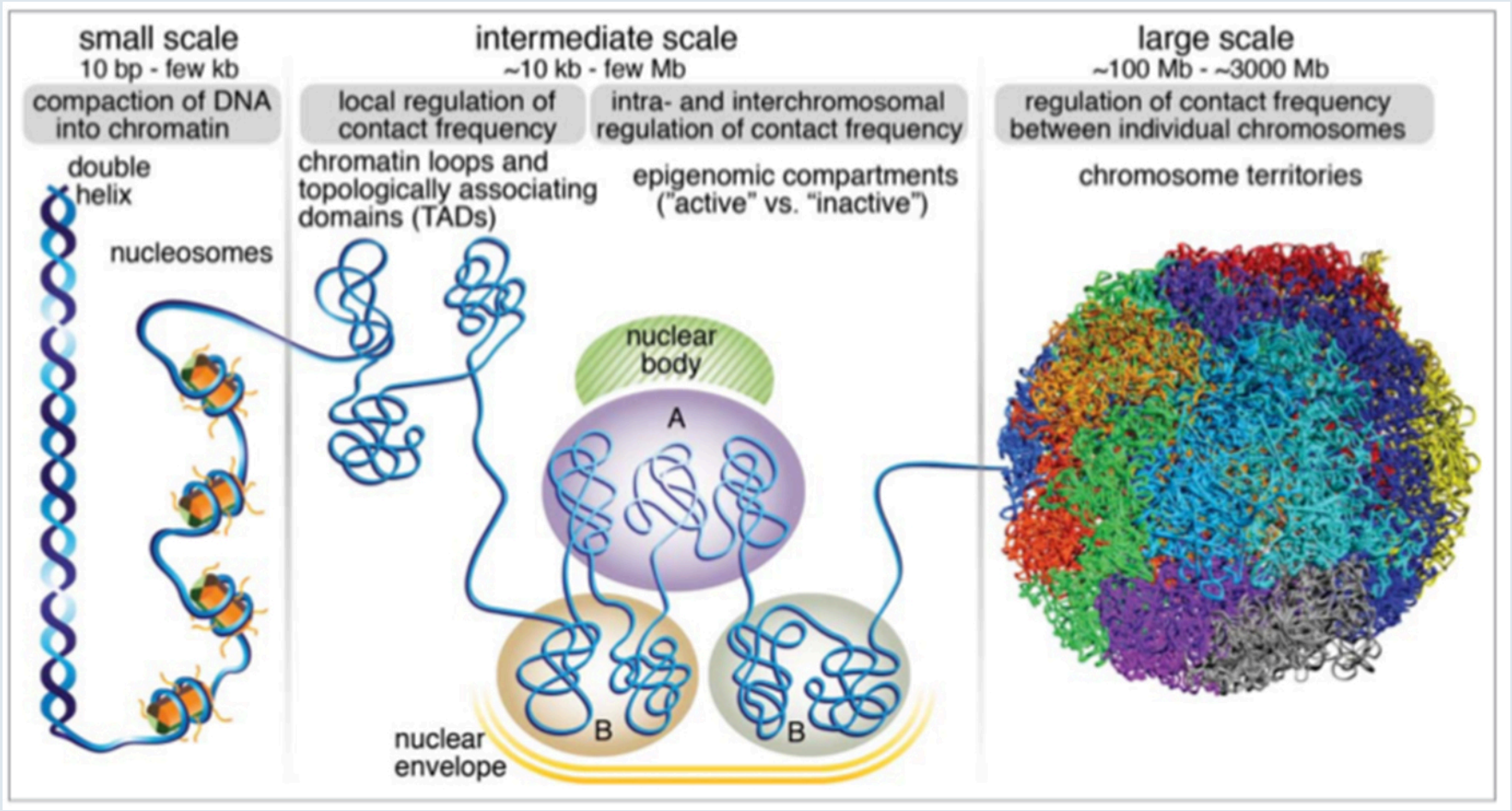
[a]Department of Molecular and Cell Biology, Li Ka Shing Center for Biomedical and Health Sciences, CIRM Center of Excellence, University of California, Berkeley, CA, USA; [b]Howard Hughes Medical Institute, Berkeley, CA, USA

ABSTRACT

Mammalian genomes are folded into spatial domains, which regulate gene expression by modulating enhancer-promoter contacts. Here, we review recent studies on the structure and function of Topologically Associating Domains (TADs) and chromatin loops. We discuss how loop extrusion models can explain TAD formation and evidence that TADs are formed by the ring-shaped protein complex, cohesin, and that TAD boundaries are established by the DNA-binding protein, CTCF. We discuss our recent genomic, biochemical and single-molecule imaging studies on CTCF and cohesin, which suggest that TADs and chromatin loops are dynamic structures. We highlight complementary polymer simulation studies and Hi-C studies employing acute depletion of CTCF and cohesin, which also support such a dynamic model. We discuss the limitations of each approach and conclude that in aggregate the available evidence argues against stable loops and supports a model where TADs are dynamic structures that continually form and break throughout the cell cycle.

ARTICLE HISTORY
Received 7 September 2017
Revised 29 September 2017
Accepted 3 October 2017

KEYWORDS
CTCF; cohesin; 3D genome; single-molecule imaging; dynamics; FRAP; topological domains; chromatin loops; loop extrusion; modeling


small scale
10 bp - few kb
compaction of DNA into chromatin
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local regulation of contact frequency
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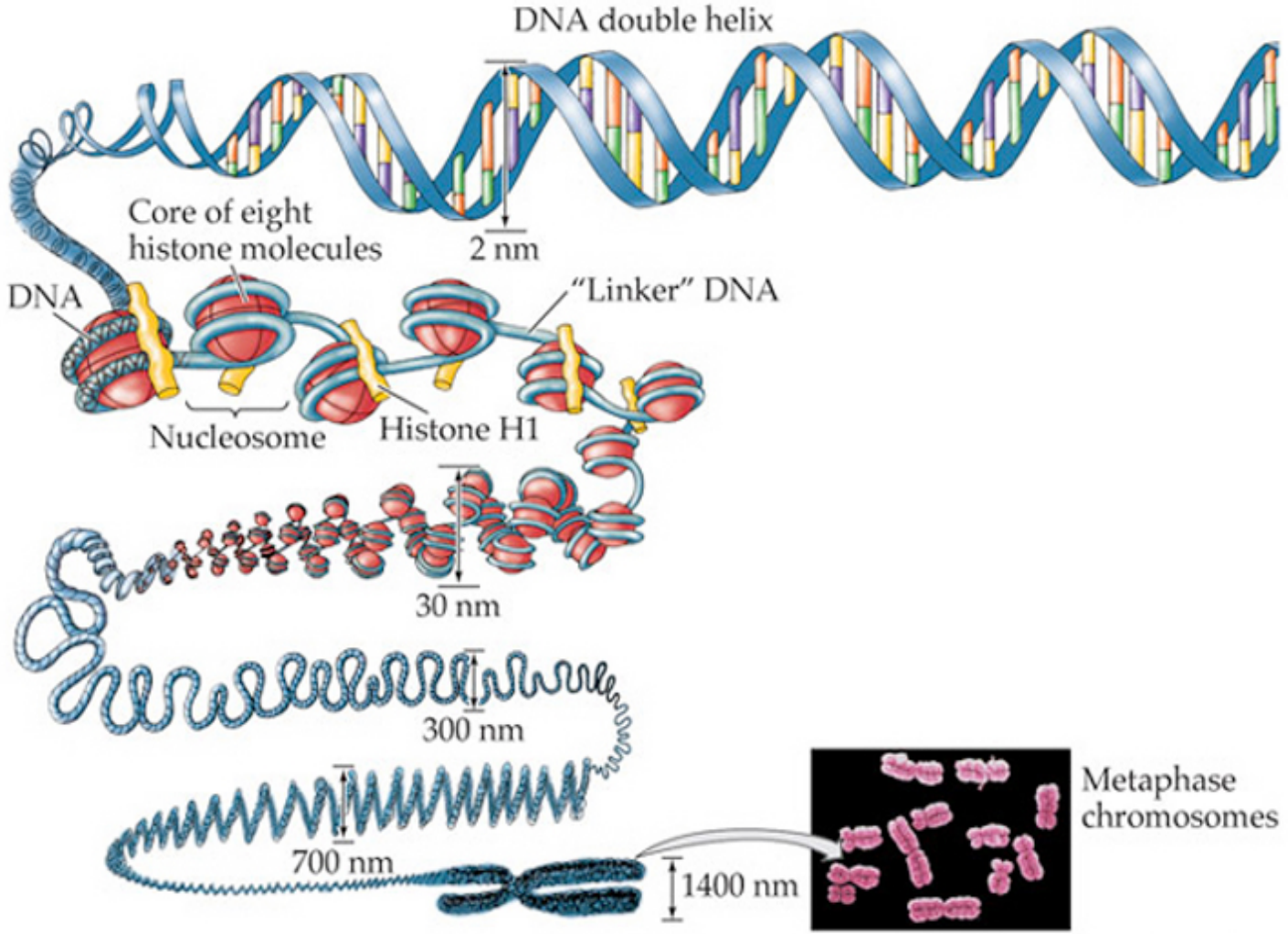
epigenomic compartments ("active" vs. "inactive")
nuclear body
A
B
B
nuclear envelope
large scale
~100 Mb - ~3000 Mb
regulation of contact frequency between individual chromosomes
chromosome territories



LIFE: THE SCIENCE OF BIOLOGY, Seventh Edition, Figure 9.6 DNA Packs into a Mitotic Chromosome

REVIEW

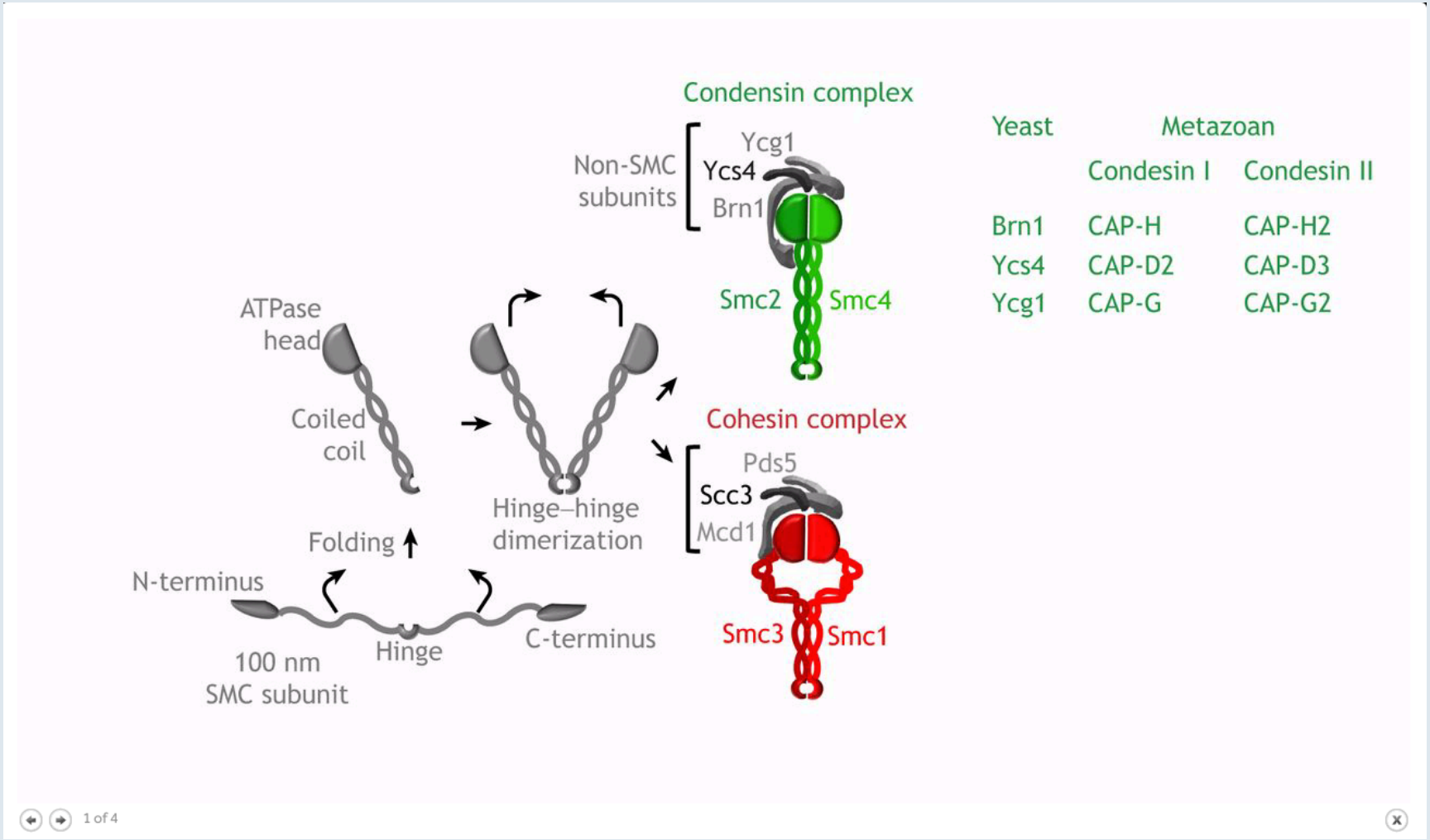
Condensins and cohesins – one of these things is not like the other!

Robert V. Skibbens

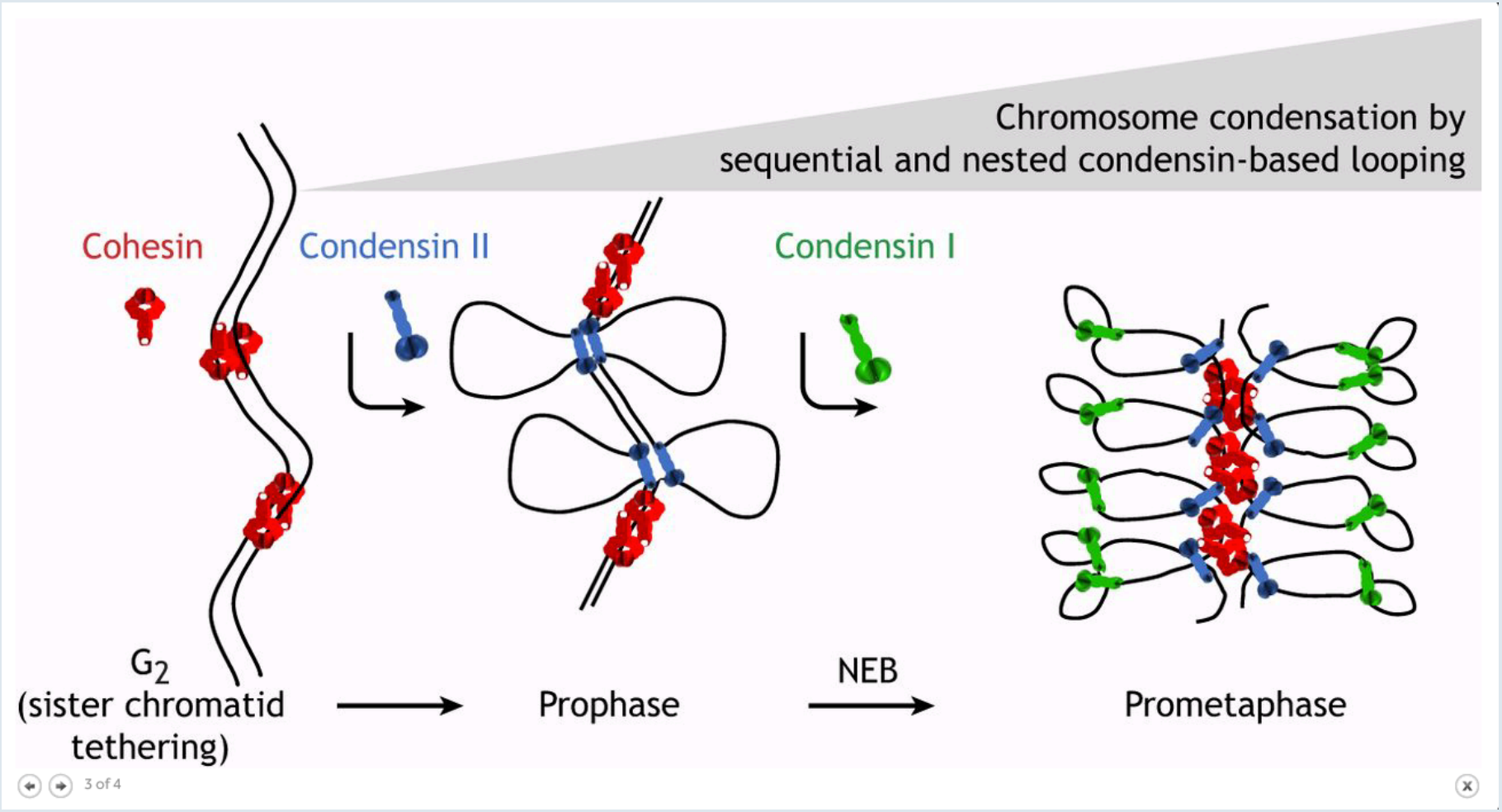
Journal of Cell Science 2019 132: jcs220491 doi: 10.1242/jcs.220491 Published 7 February 2019

ABSTRACT

Condensins and cohesins are highly conserved complexes that tether together DNA loci within a single DNA molecule to produce DNA loops. Condensin and cohesin structures, however, are different, and the DNA loops produced by each underlie distinct cell processes. Condensin rods compact chromosomes during mitosis, with condensin I and II complexes producing spatially defined and nested looping in metazoan cells. Structurally adaptive cohesin rings produce loops, which organize the genome during interphase. Cohesin-mediated loops, termed topologically associating domains or TADs, antagonize the formation of epigenetically defined but untethered DNA volumes, termed compartments. While condensin complexes formed through *cis*-interactions must maintain chromatin compaction throughout mitosis, cohesins remain highly dynamic during interphase to allow for transcription-mediated responses to external cues and the execution of developmental programs. Here, I review differences in condensin and cohesin structures, and highlight recent advances regarding the intramolecular or *cis*-based tetherings through which condensins compact DNA during mitosis and cohesins organize the genome during interphase.



SMC assemblies. The stereotypical folding and dimerization of SMC subunits is shown in gray; 100-nm-long SMC proteins fold at a central hinge and allow for N- and C-terminal binding to form ATPase heads. SMCs dimerize predominantly through hinge-hinge binding but also head–head binding. Condensins (shown in green) are formed from Smc2 and Smc4 heterodimers that recruit the non-SMC subunits Brn1, Ycs4 and Ycg1 to form flexible rods (see Fig. 2). Cohesins (shown in red) are formed from Smc1 (SMC1a or SMC1b in metazoans) and Smc3 heterodimers that recruit the non-SMC subunits Mcd1, Scc3 and Pds5 to form flattened rings (shown) but can adopt other conformations, such as rods, open V-shapes or C-clamps (not shown). Yeast contain single copies of Brn1, Ycs4 and Ycg1, but metazoan cells contain paralogs, which give rise to unique subtypes termed condensin I (CAP-H, CAP-D2 and CAP-G, encoded by NCAPH, NCAPD2 and NCAPG, respectively) and condensin II (CAP-H2, CAP-D3, and CAP-G2, encoded by NCAPH2, NCAPD3 and NCAPG2, respectively). Metazoan cells also contain subunit paralogs (RAD21 or RAD21L for Mcd1, SA1/STAG1, SA2/STAG2 or SA3/STAG3 for Scc3, and PDS5a or PDS5b/APRIN for Pds5), but the assembly of non-SMC subunits into cohesin complexes is less well defined. Note that metazoan cells also contain Sororin, which is absent in yeast



Cell-cycle-regulated chromosome compaction by sequential recruitment of condensins II and I promotes the formation of nested loops. Replicated sister chromatids are tethered together by cohesins (red). During prophase, condensin II (blue) binds DNA and extrudes loops. After nuclear envelope breakdown (NEB), condensin I (green) binds the looped DNA and forms new loops that are nested within the condensin II-generated loops. As cells progress into late prometaphase, DNA loop extrusion and compression of the helical scaffold continues. Not shown is the role for cohesin in generating cis-based compaction, which also promotes chromosome condensation.

A pathway for mitotic chromosome formation

Gibcus, Samejima, Goloborodko et al., Science (2018)