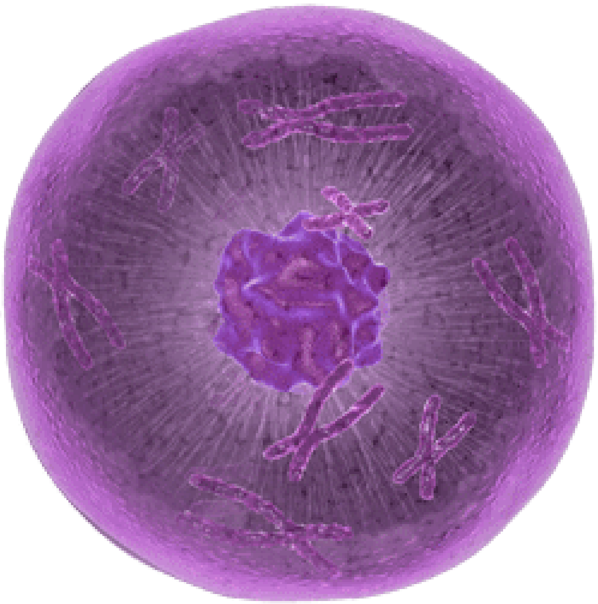
Summer term 2016
Nanobiophysics Module

Introduction to Molecular and Cellular Biology

LECTURES 12-13:

Cellular nucleus

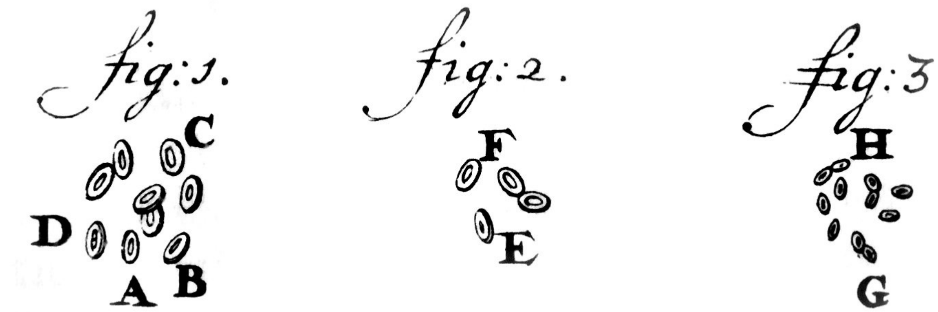


LECTURES 12-13: CELLULAR NUCLEUS

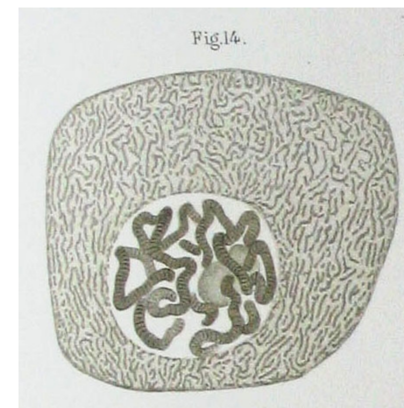
- Nucleus structure
- Nuclear transport
- DNA organization in nucleus:
  - chromosomes
  - nucleosomes
  - global structure of chromosomes

HISTORY

- 1719, Leeuwenhoek: salmon red cells
- 1804, Bauer: description
- 1838, Schleiden: “cytoblast”
- 1850s, Remak, Virchow: cell are generated from cells
- 1873, Weismann: cells => heredity
- 1878, Hertwig: fertilization of oocyte, nucleus fusion
- Beginning of XX century: nucleus function through mitosis



Leeuwenhoek, 1719



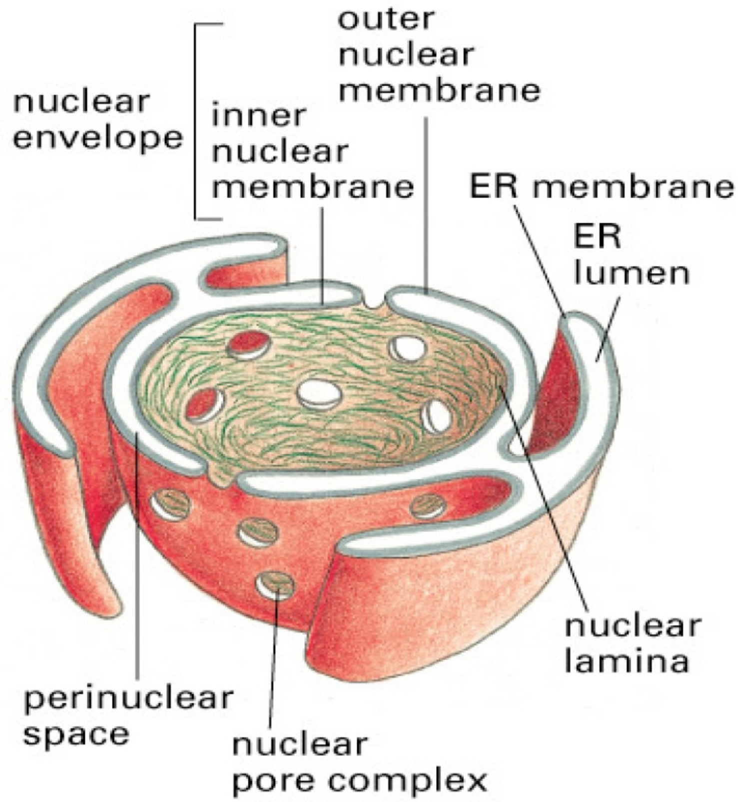
Flemming, 1882

- Evolution of nucleus:
  - symbiotic: archea + bacteria
  - protoeukaryotic cells evolution
  - viral eukaryogenesis
  - exomembrane hypothesis
- Evidence:
  - histones structures
  - plastomycetes bacteria
  - linear DNA, RNA-capping

NUCLEUS STRUCTURE

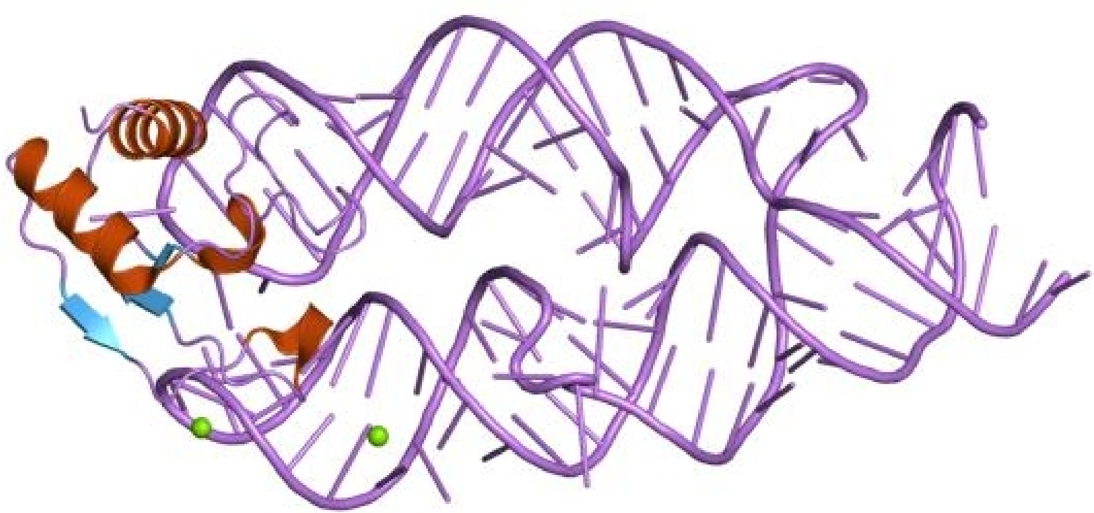
Double-membraned organelle, large round body in the middle of the cell

- **Nuclear envelope: encloses DNA and defines nuclear compartment**
- **Nuclear pore: large protein complexes in nuclear envelope**
- **Nuclear lamina: fibrillar network inside the nucleus**
- **Inner nuclear membrane ~ 50 nm**
- **Perinuclear space: space between two membranes**
- **Outer nuclear membrane => membranes of ER**
- **Nucleolus**

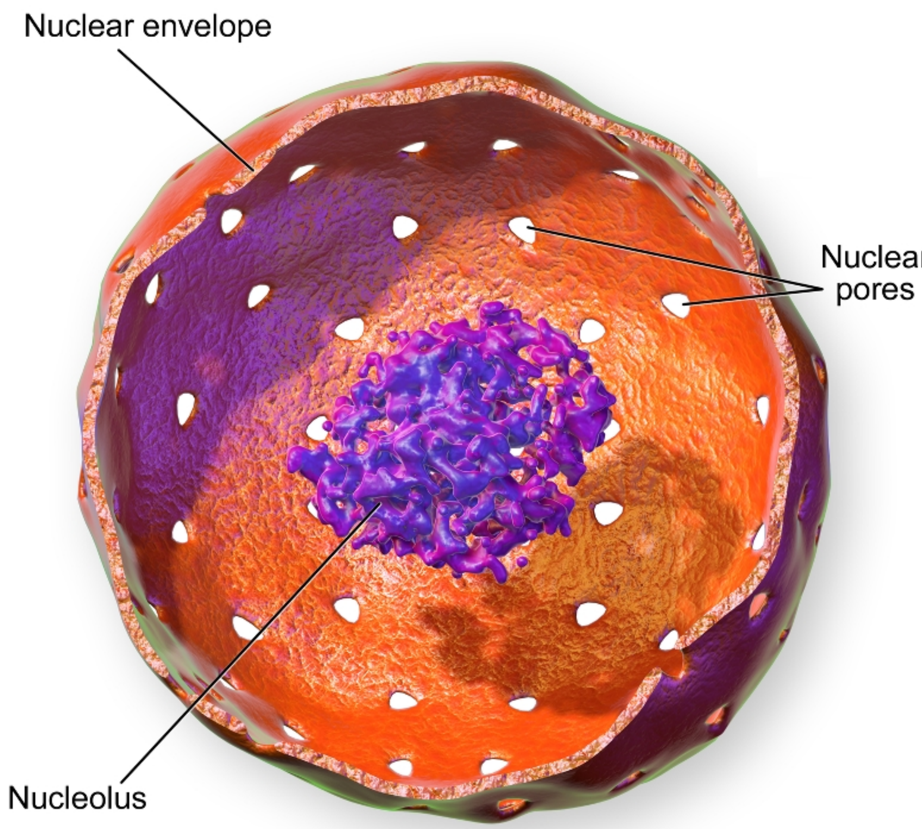


NUCLEUS STRUCTURE: NUCLEOLUS

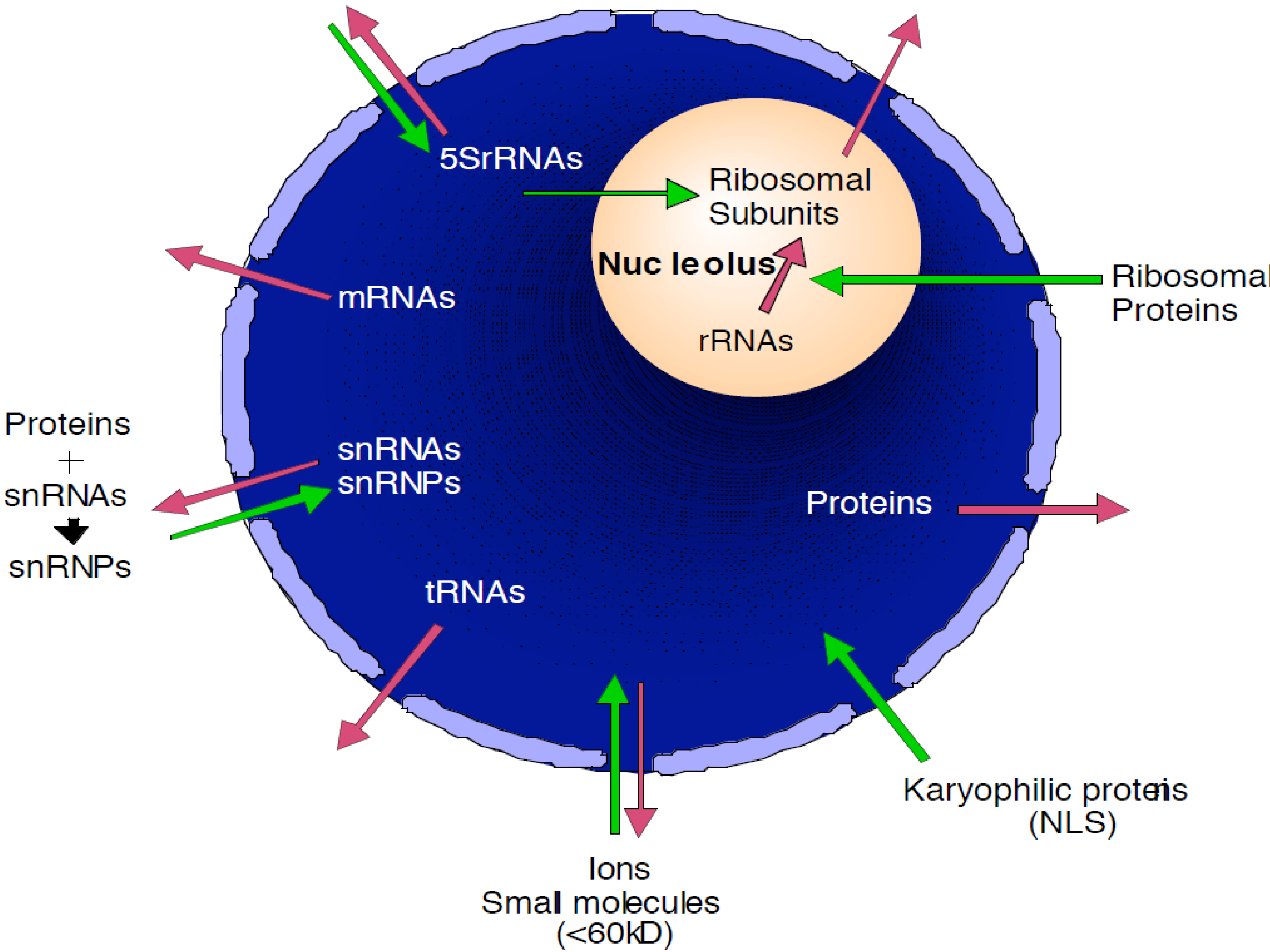
- Site of ribosome sythesis and assembly
- Assembly of signal recognition particles
- Components:
  - fibrillar center
  - dense fibrillar component
  - granular component



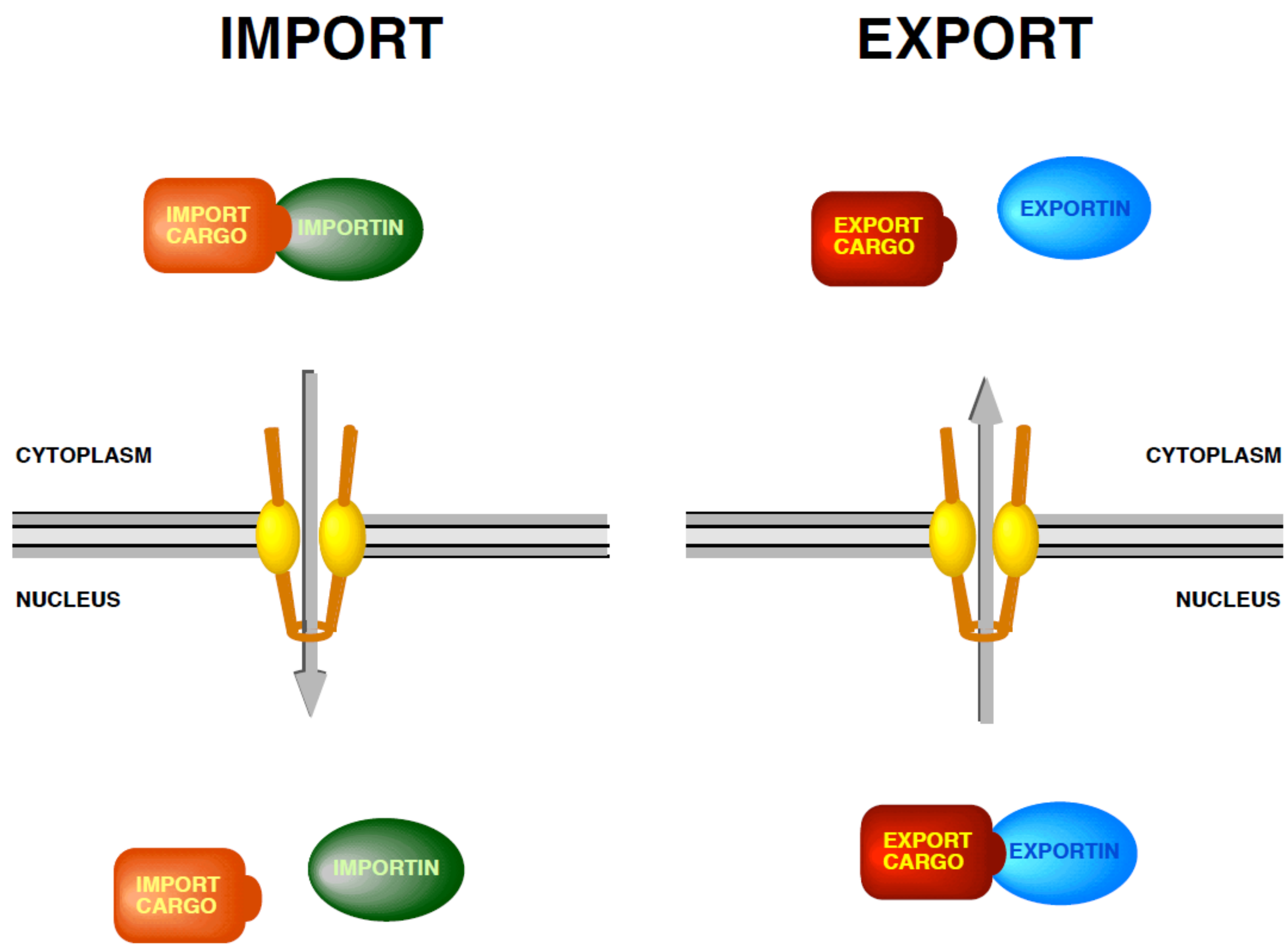
Signal recognition particle



NUCLEAR TRANSPORT OVERVIEW



NUCLEAR TRANSPORT: TWO DIRECTIONS



NUCLEAR PORE COMPLEX

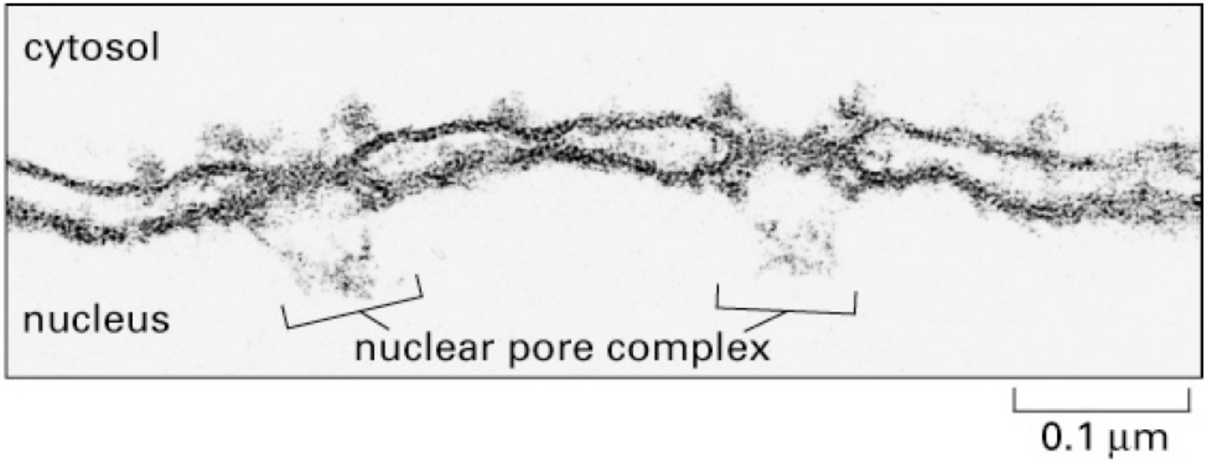
125 000 000 Da

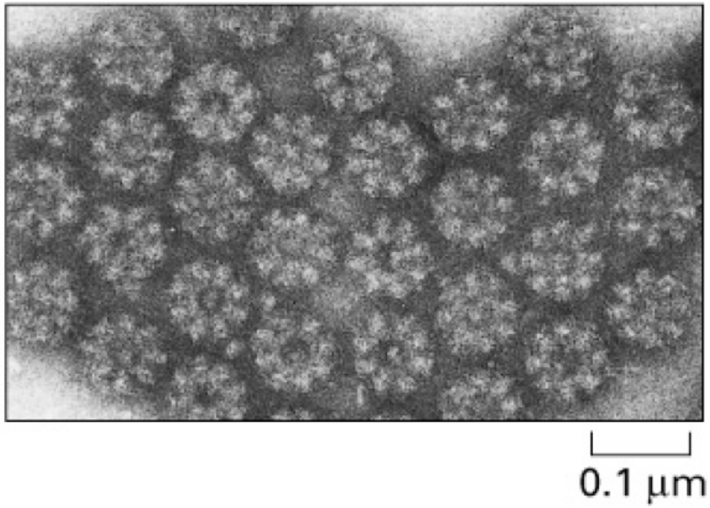
50 proteins (nucleoporins)

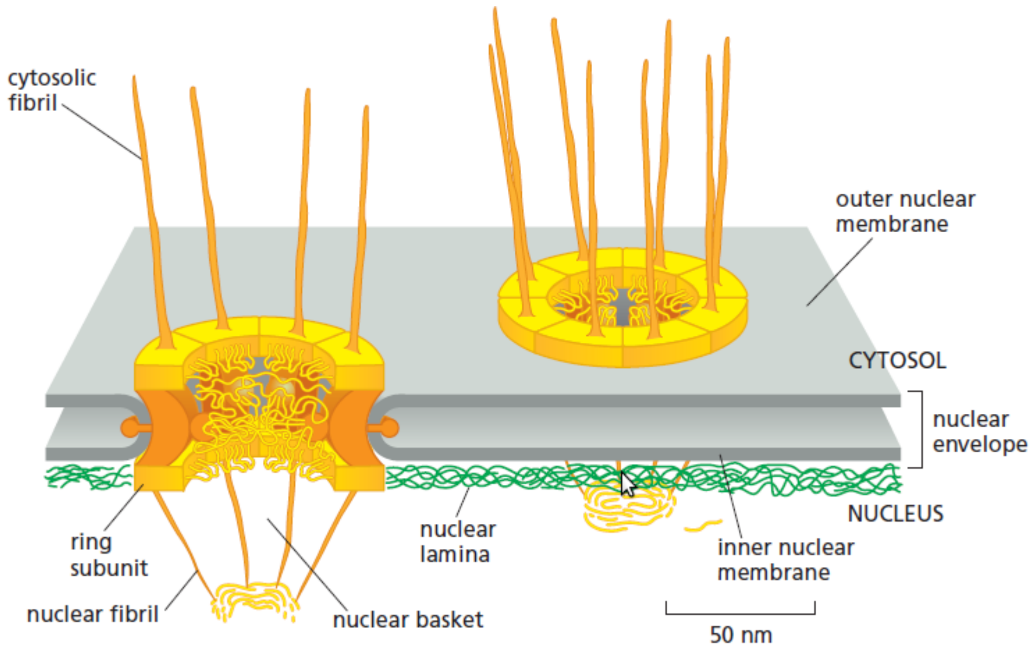
3000-4000 pores

100 histones/minute

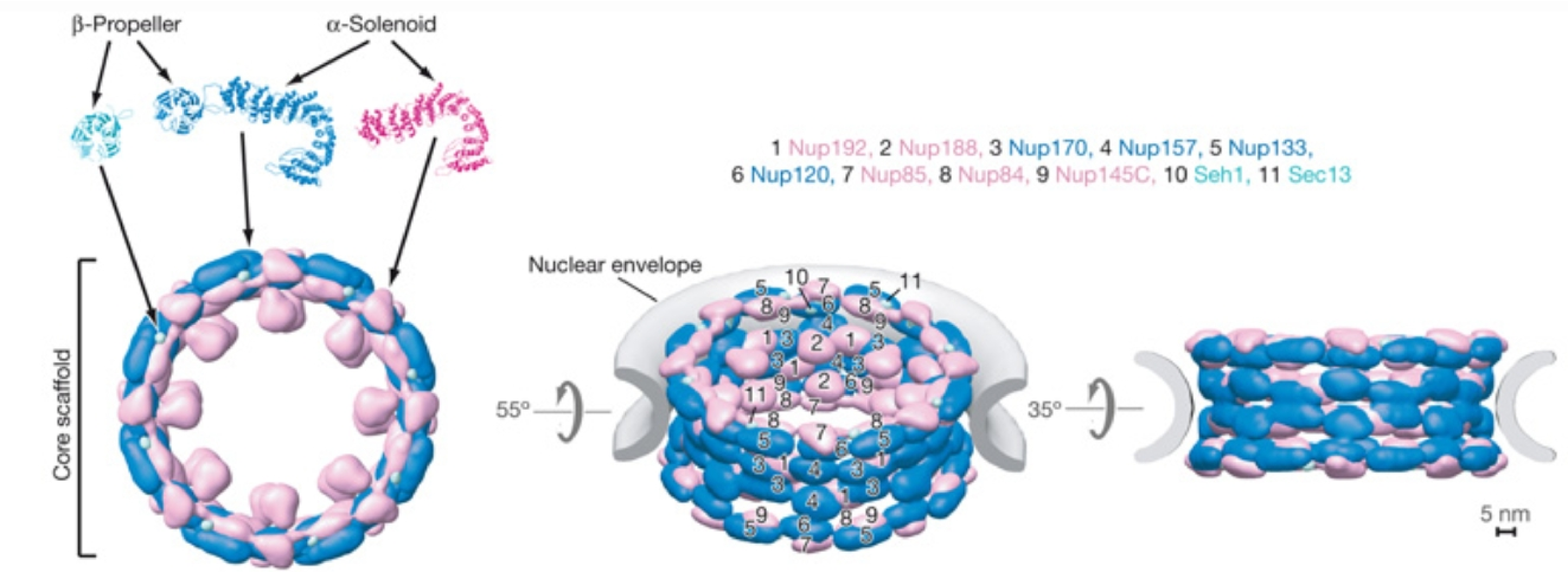
6 ribosomal SU/minute



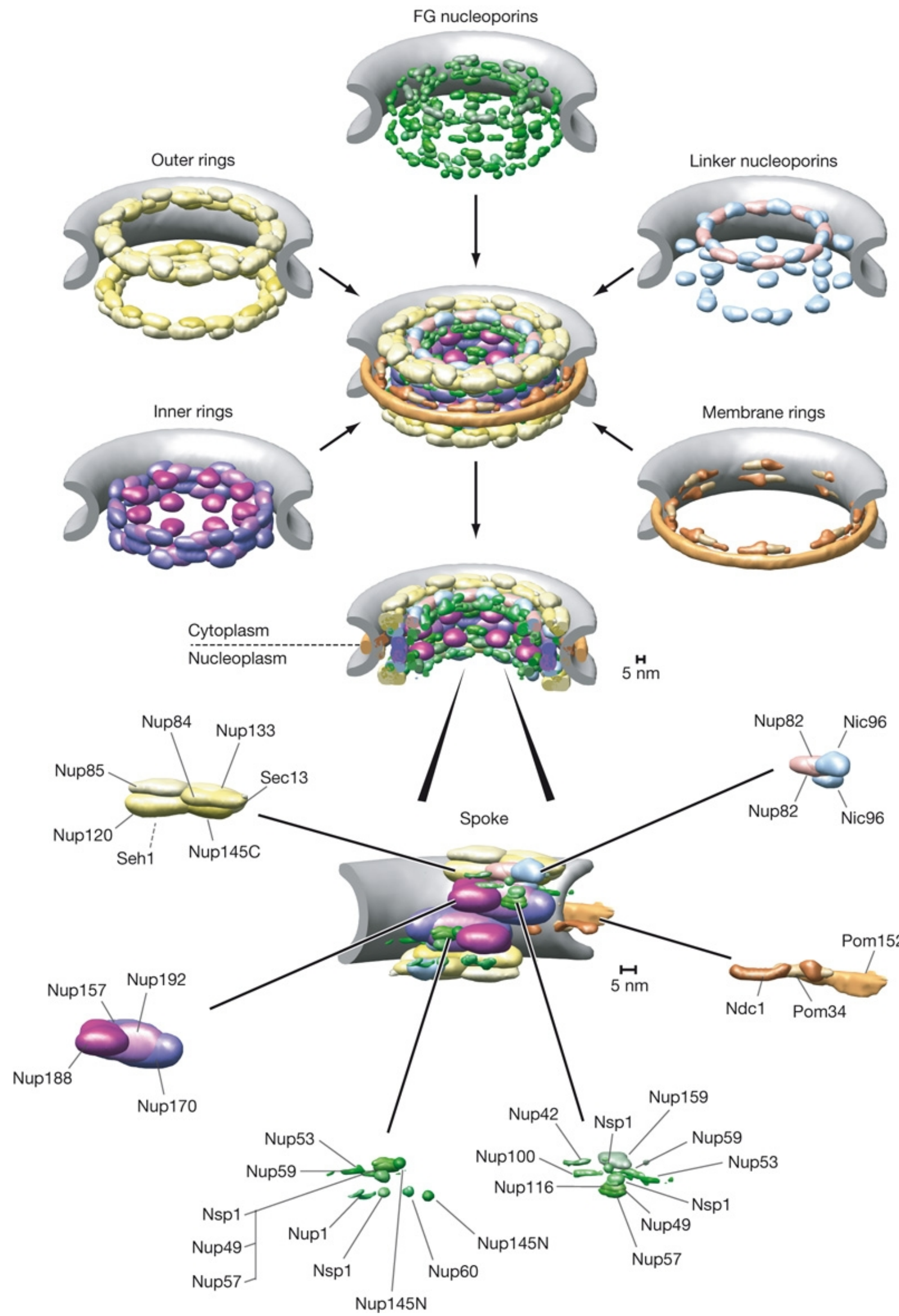




NUCLEAR PORE COMPLEX

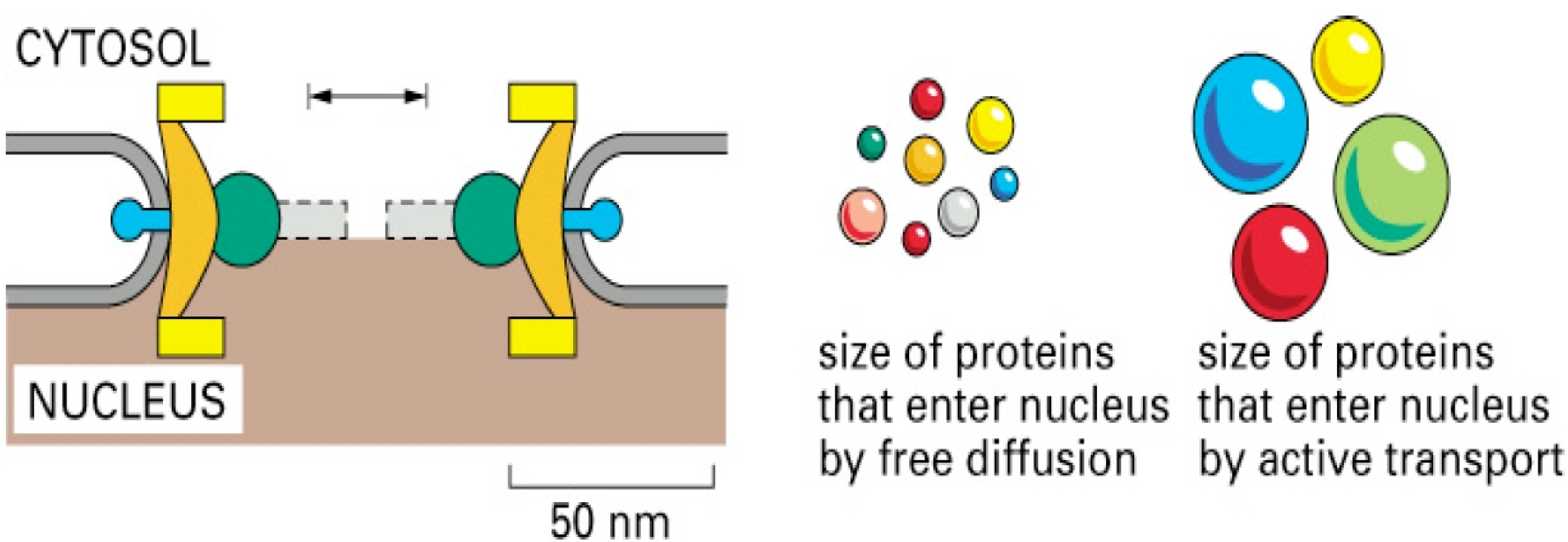


NUCLEAR PORE COMPLEX

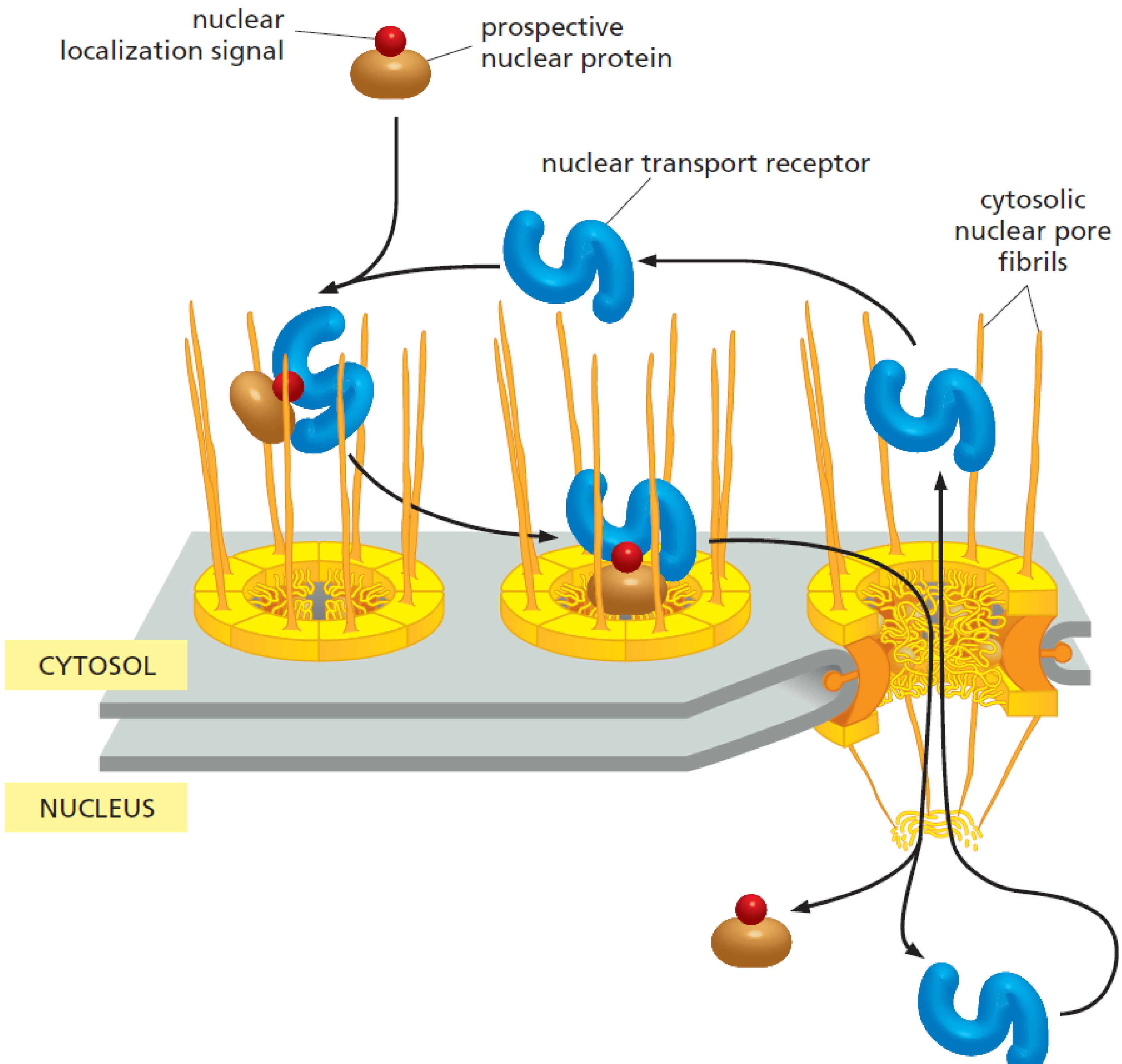


NUCLEAR TRANSPORT: CARGO SIZE

- **Pore diameter: 9 nm**
- **Pore length: 15 nm**
- **Rapid free diffusion: < 5000 Da**
- **Free diffusion: < 60 000 Da**
- **Active transport: polymerases, RNA etc.**



NUCLEAR LOCALIZATION SIGNAL

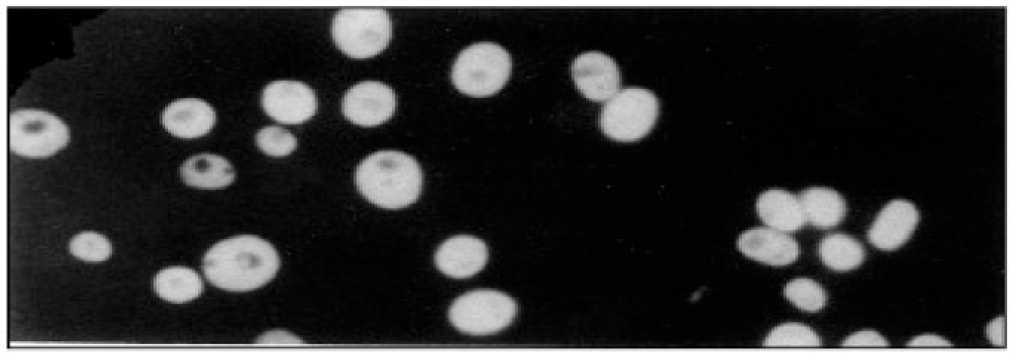


NUCLEAR LOCALIZATION SIGNAL

Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val – not cleaved after the delivery

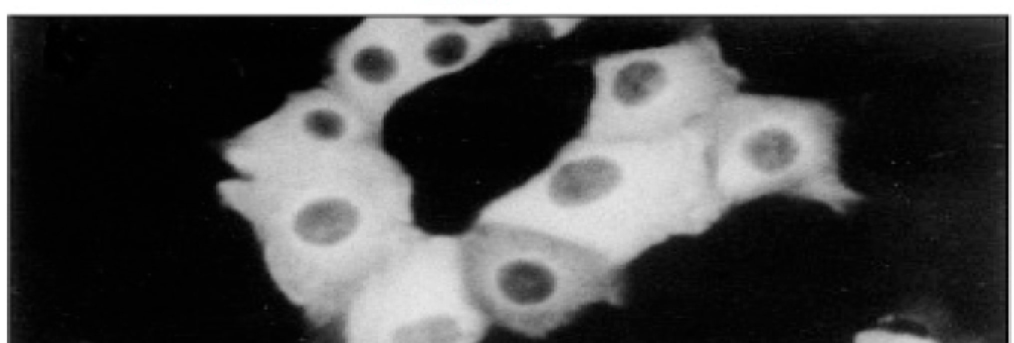
LOCALIZATION OF T-ANTIGEN CONTAINING
ITS NORMAL NUCLEAR IMPORT SIGNAL

Pro—Pro—Lys—Lys—Lys—Arg—Lys—Val—

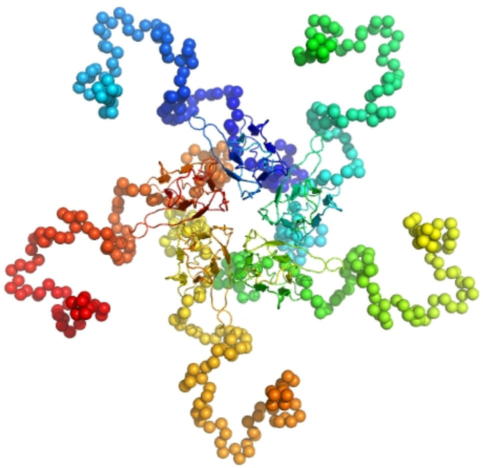


LOCALIZATION OF T-ANTIGEN CONTAINING
A MUTATED NUCLEAR IMPORT SIGNAL

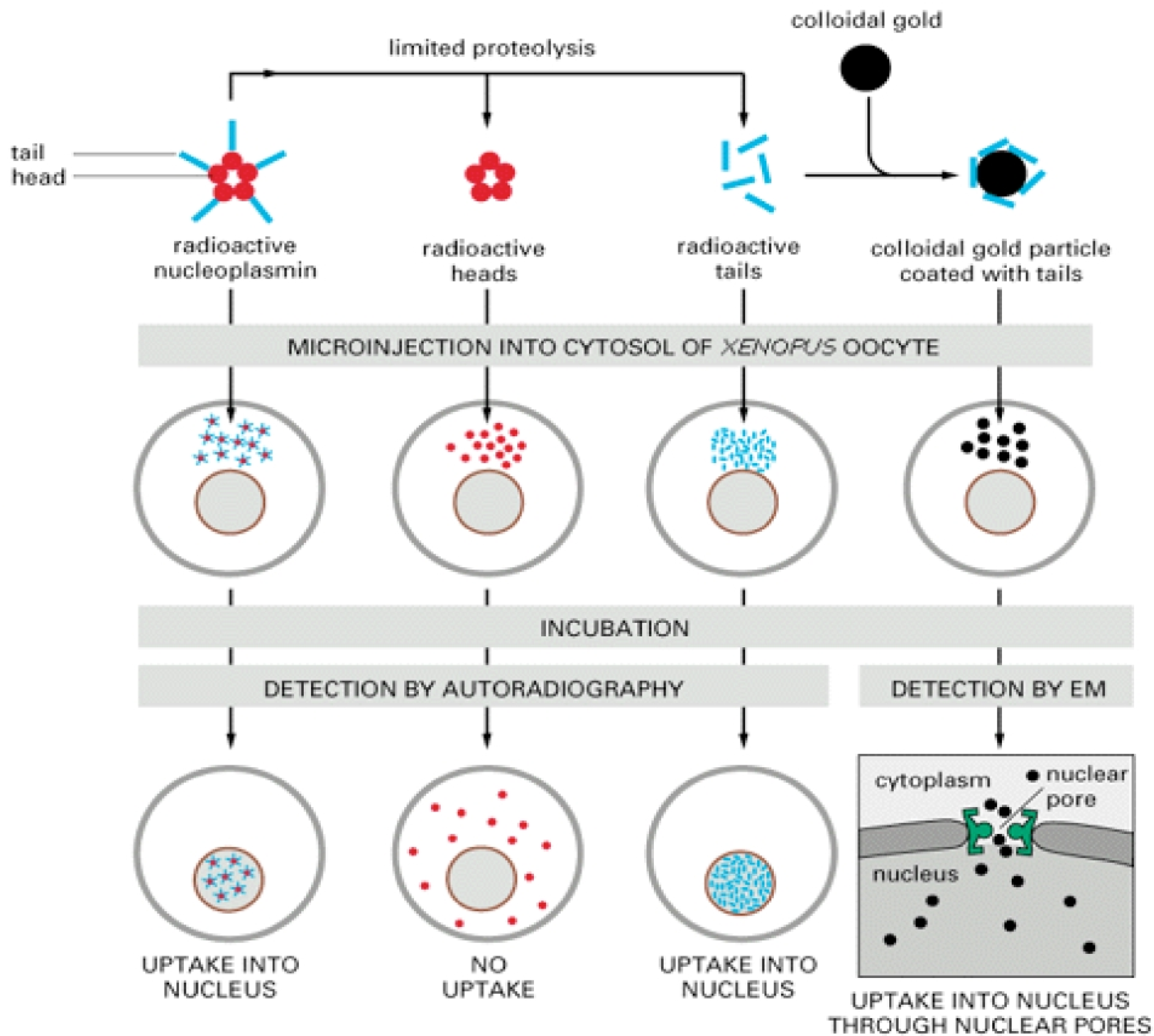
Pro—Pro—Lys—Thr—Lys—Arg—Lys—Val—



DETECTING NUCLEAR LOCALIZATION SIGNAL



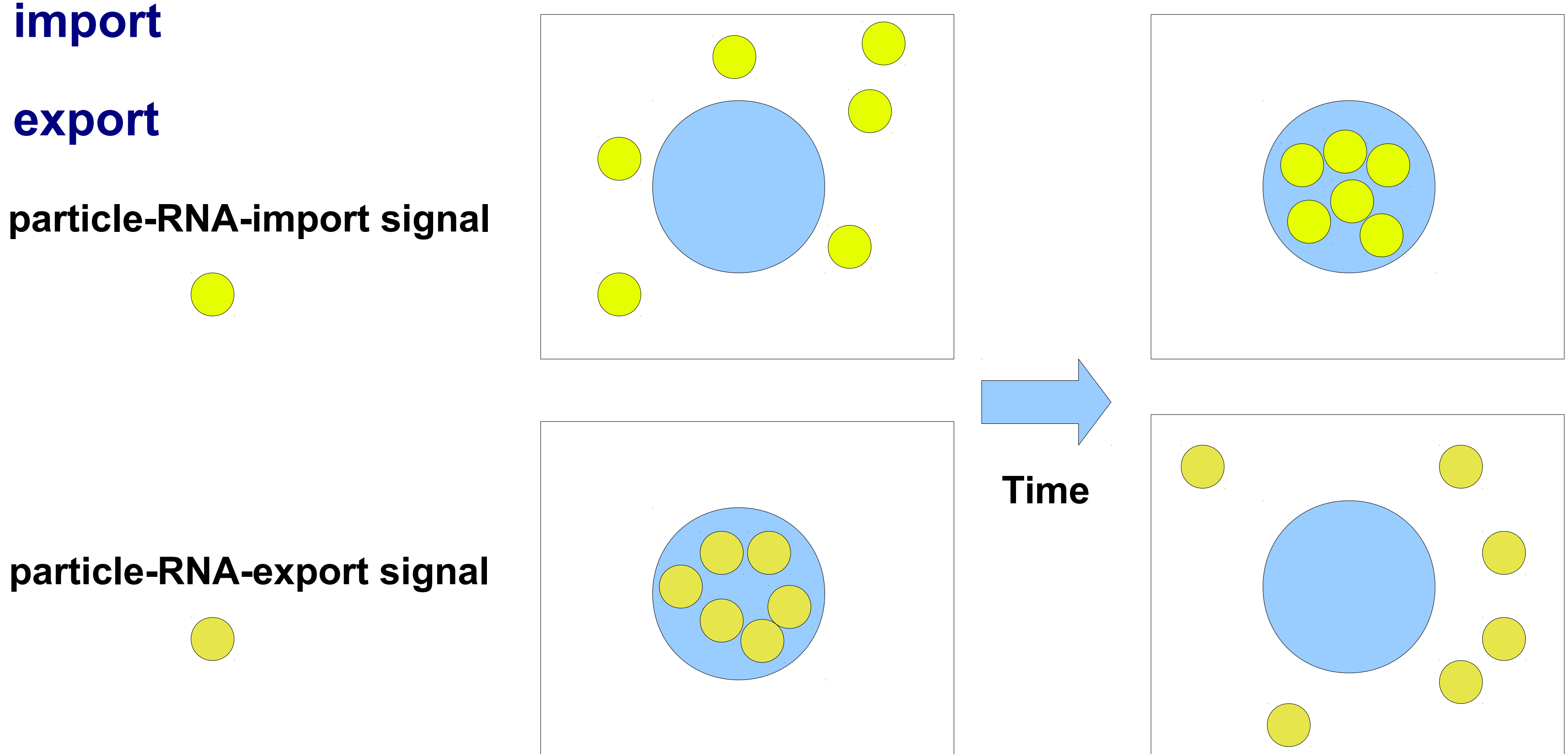
Nucleoplasmin



NUCLEAR RECEPTOR

- Bind to FG-repeats in nuclear pore
- Return to cytosol after the transport event
- Use adaptor proteins (structural similarity)
- 14 genes in yeast (karyopherins)
- Pores work in both directions:

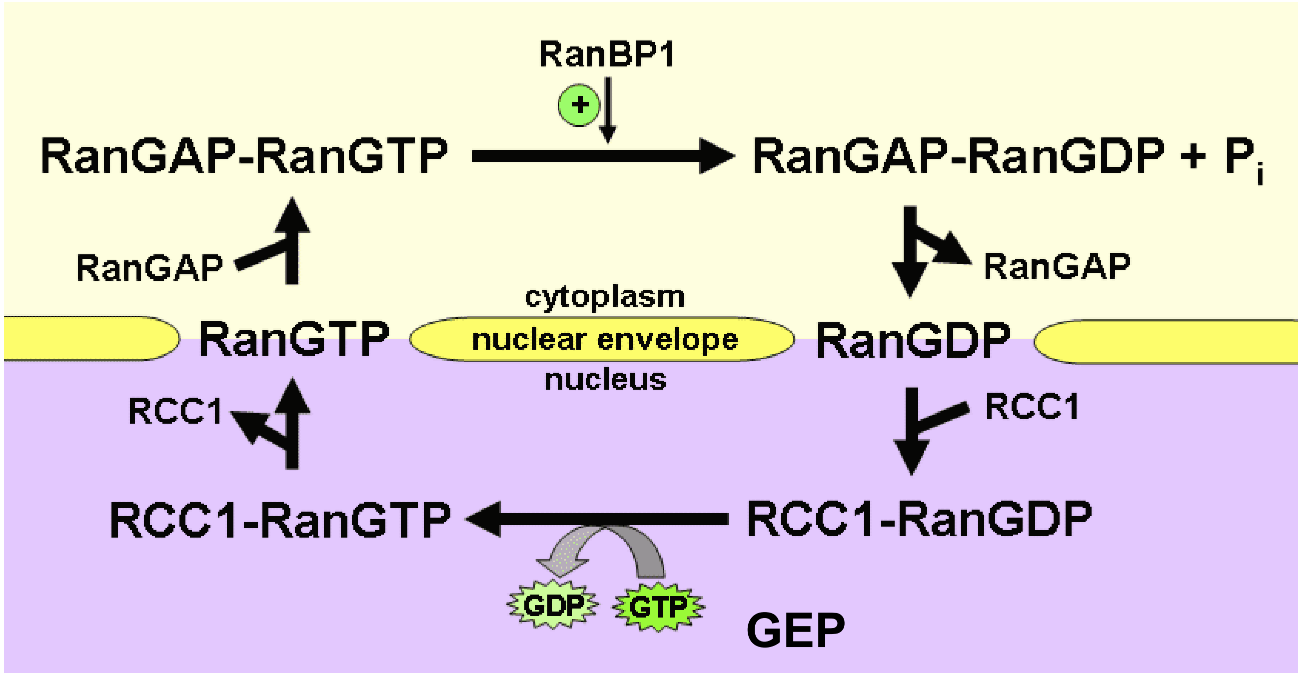
  - import
  - export

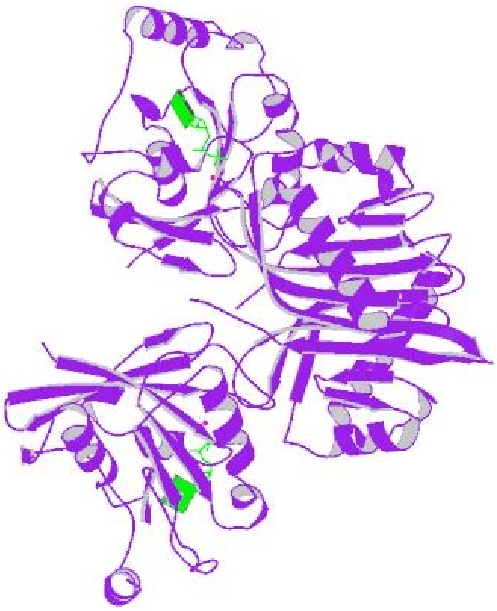


RAN GTPASE ROLE IN NUCLEAR TRANSPORT

Ran (RAs-related Nuclear protein), GTP-binding nuclear protein Ran

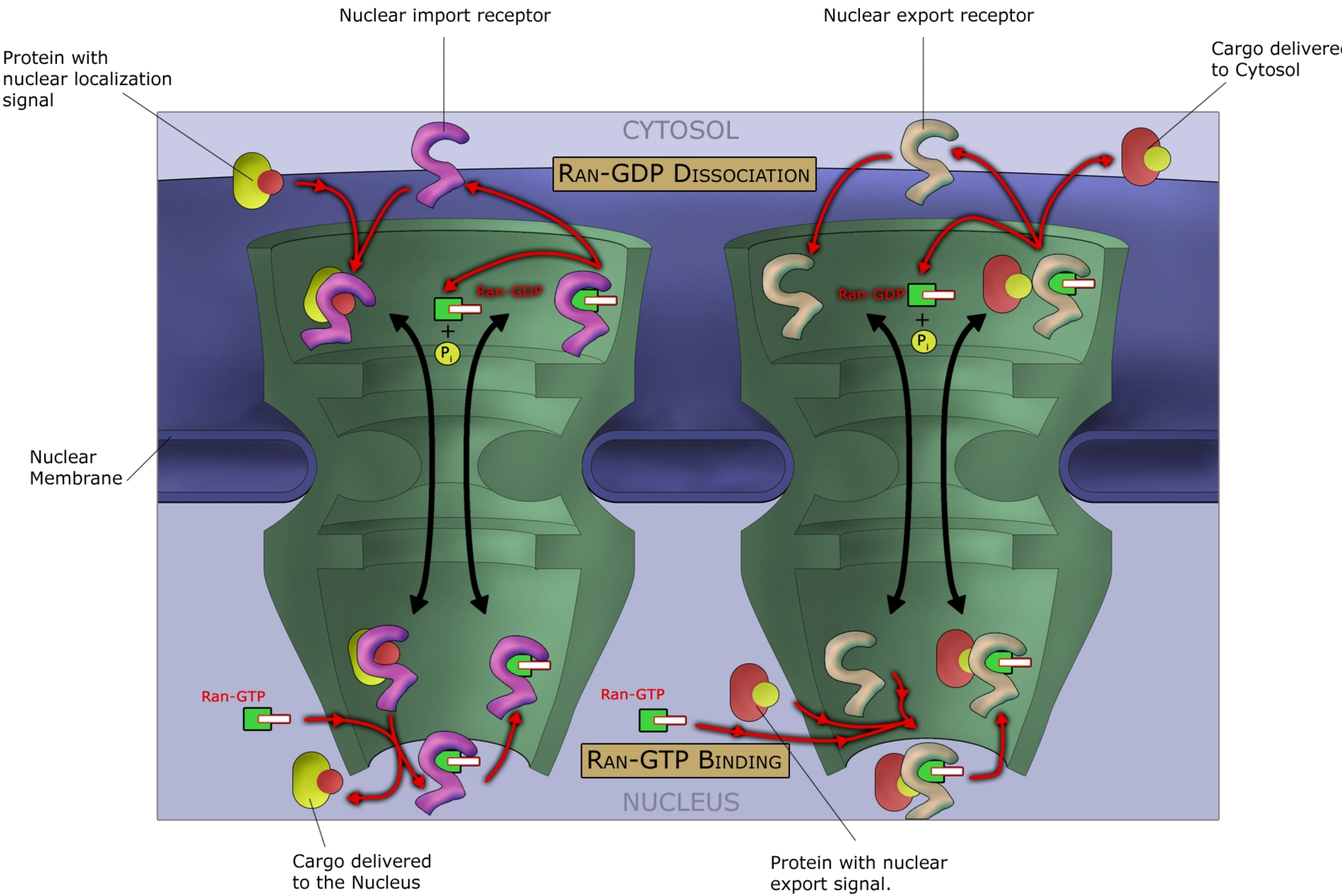
- **Both in cytosol and nucleus**
- **GAP (GTPase activating protein): cytosole**
- **GEF (Guanine exchange factor): nucleus**





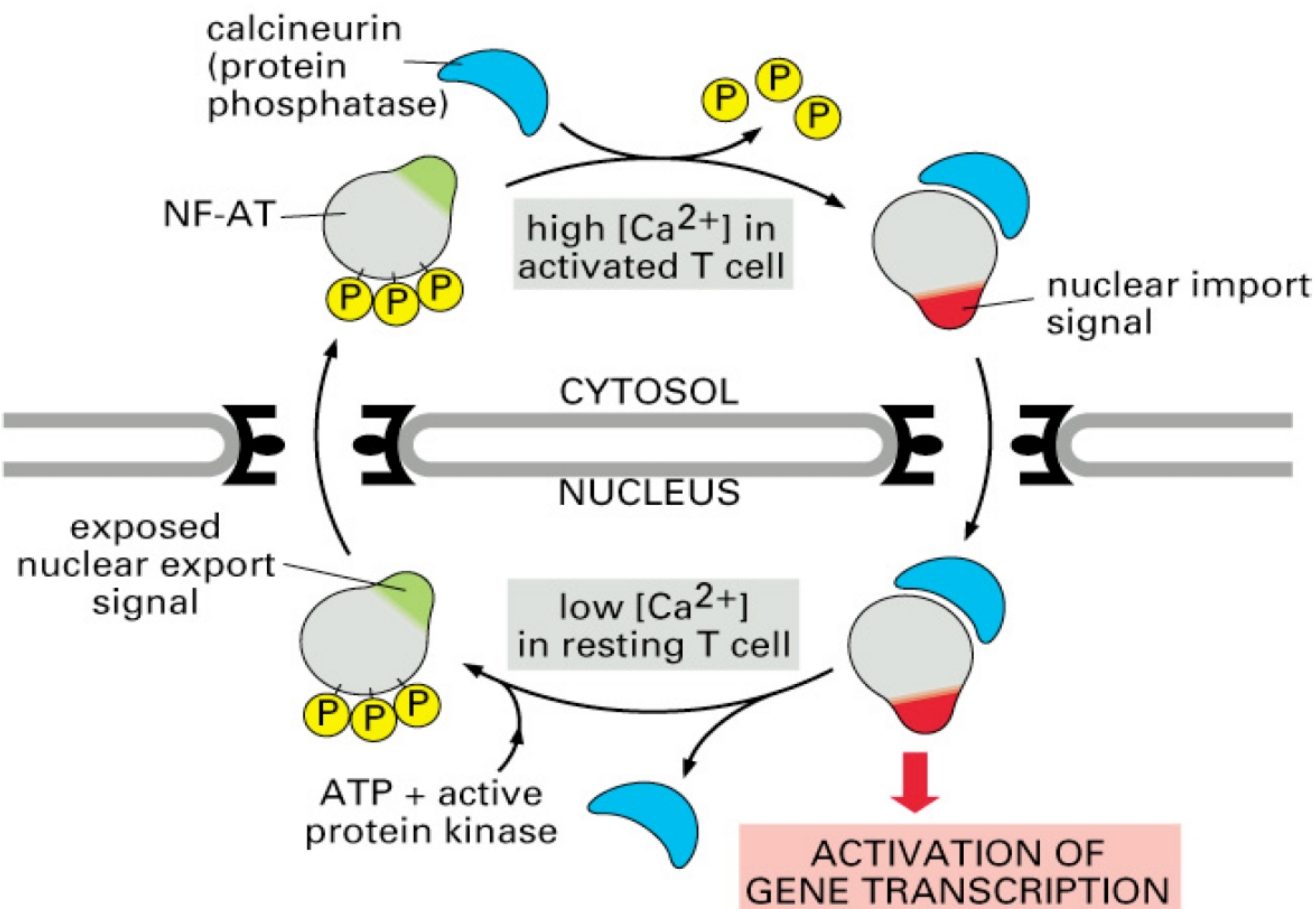
Ran-GTPase

RAN GTPASE ROLE IN NUCLEAR TRANSPORT



NUCLEAR TRANSPORT REGULATION

- Shuttling proteins: migrate between cytosol and nucleus
- Phosphorylation
- Export/import-signals association proteins (f.i. masking signals)

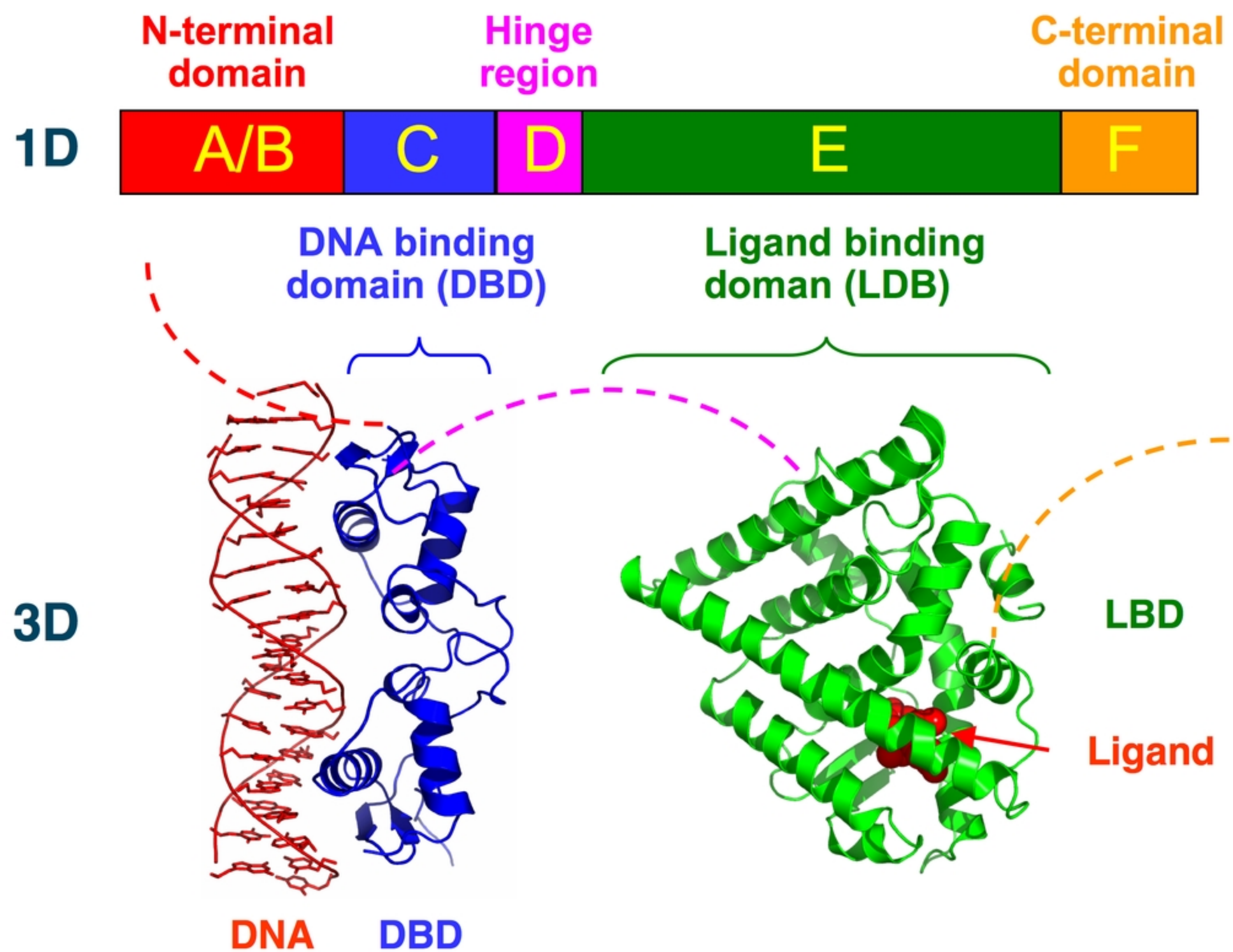


Nuclear import in T-cell activation

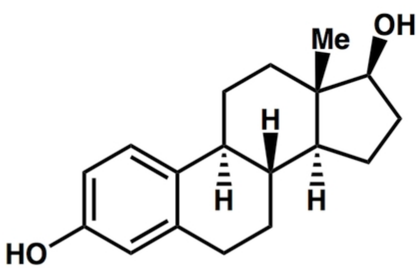
NUCLEAR TRANSPORT REGULATION: NUCLEAR RECEPTOR

Shuttling proteins sensitive to hormones (and other signals)

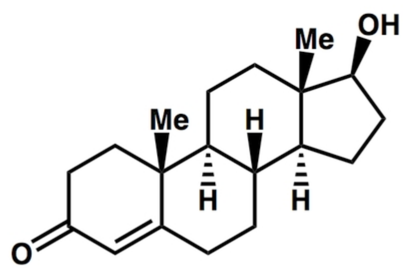
4 different types



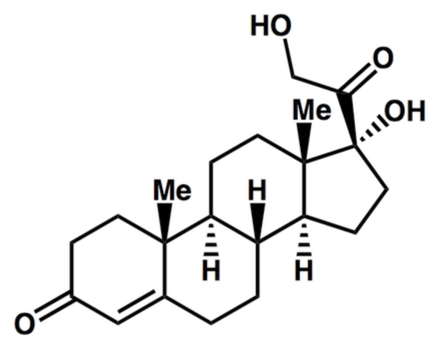
NUCLEAR TRANSPORT REGULATION: NUCLEAR RECEPTOR LIGANDS



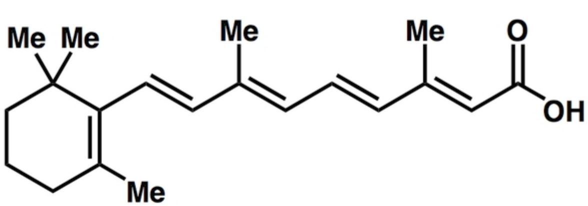
17-β-estradiol
estrogen receptor
(ER)



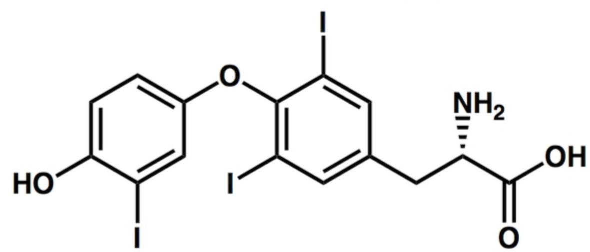
testosterone
androgen receptor
(AR)



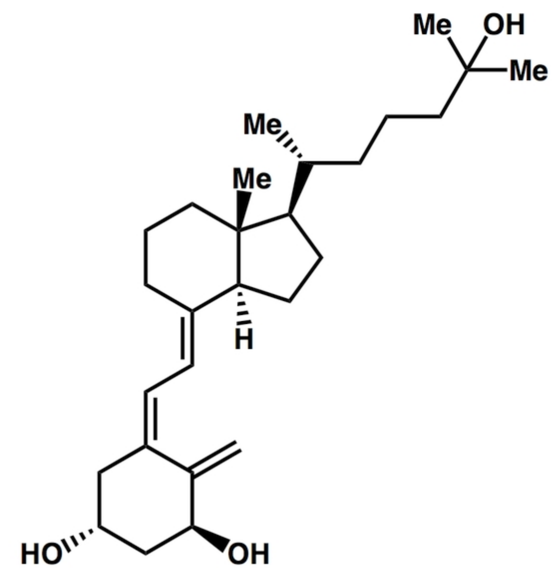
cortisol
glucocorticoid receptor
(GR)



vitamin A
retinoic acid receptor
(RAR)

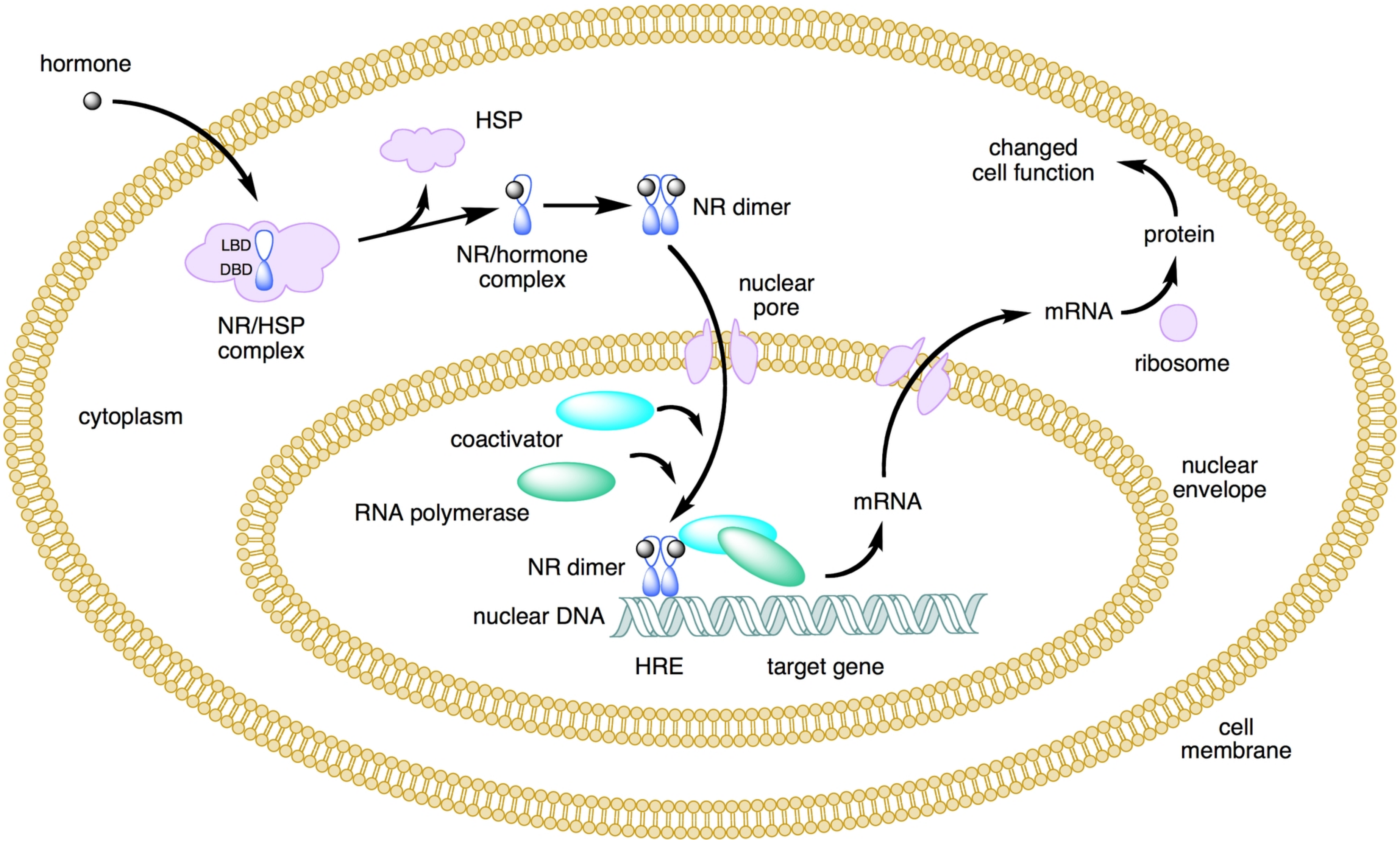


triiodothyronine (T_3)
thyroid hormone receptor
(TR)

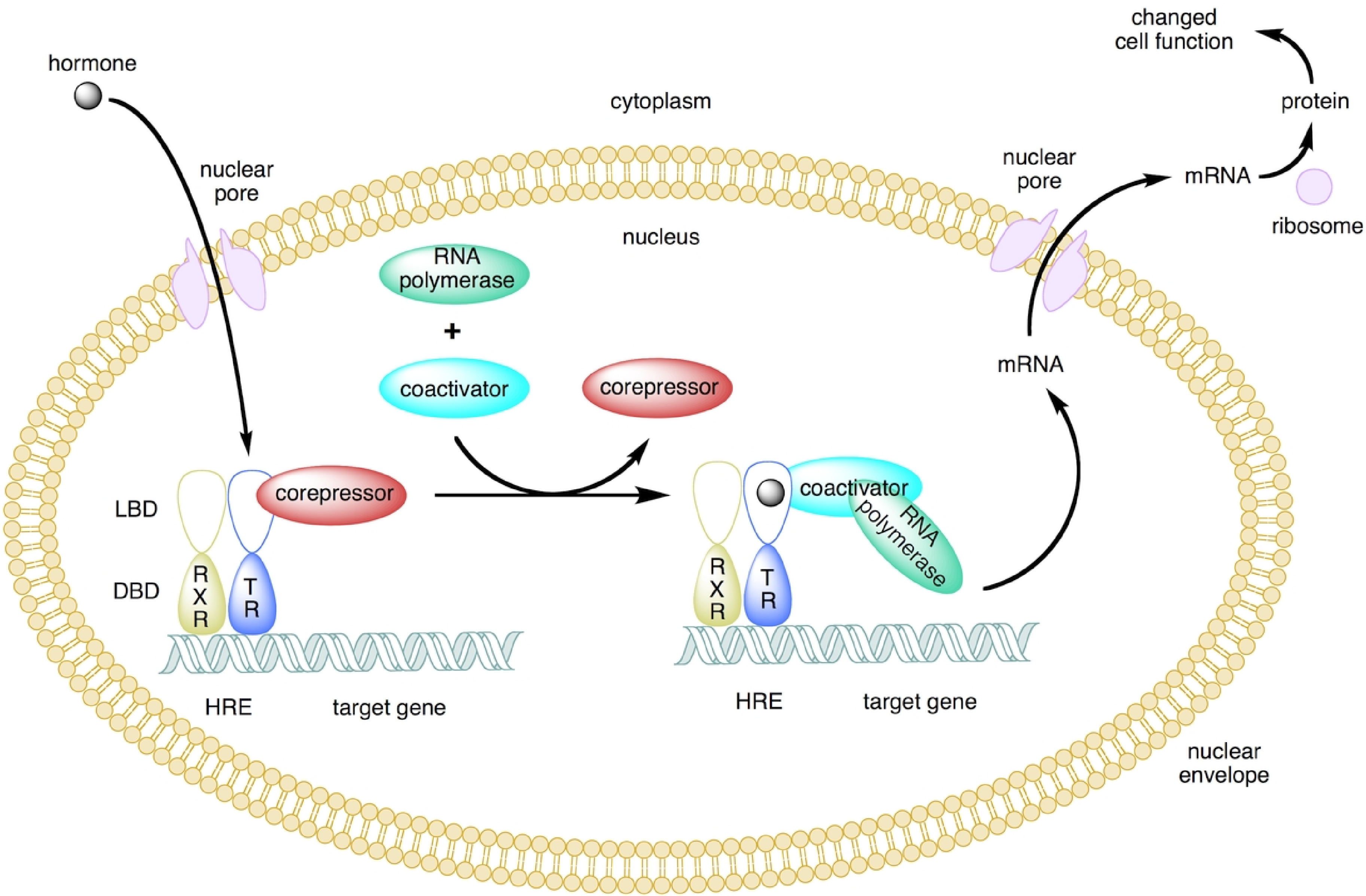


calcitriol
vitamin D receptor
(VDR)

NUCLEAR TRANSPORT REGULATION: NUCLEAR RECEPTOR MECHANISM 1

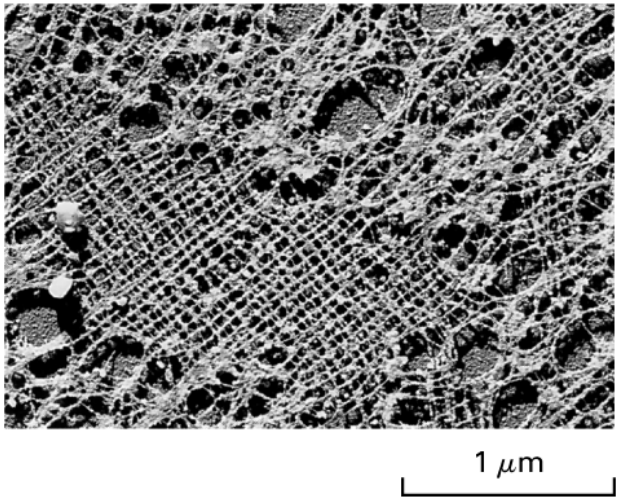


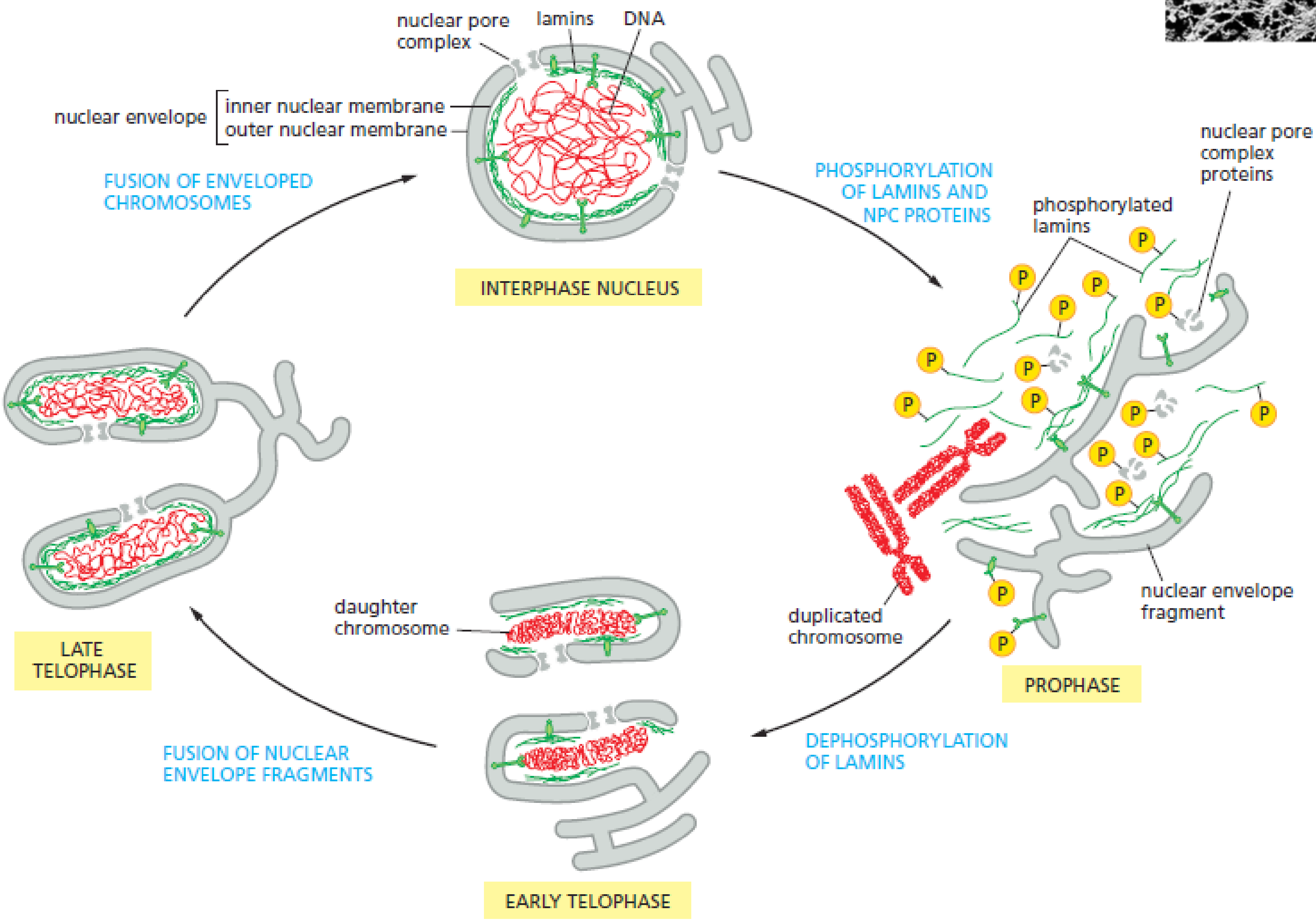
NUCLEAR TRANSPORT REGULATION: NUCLEAR RECEPTOR MECHANISM 2



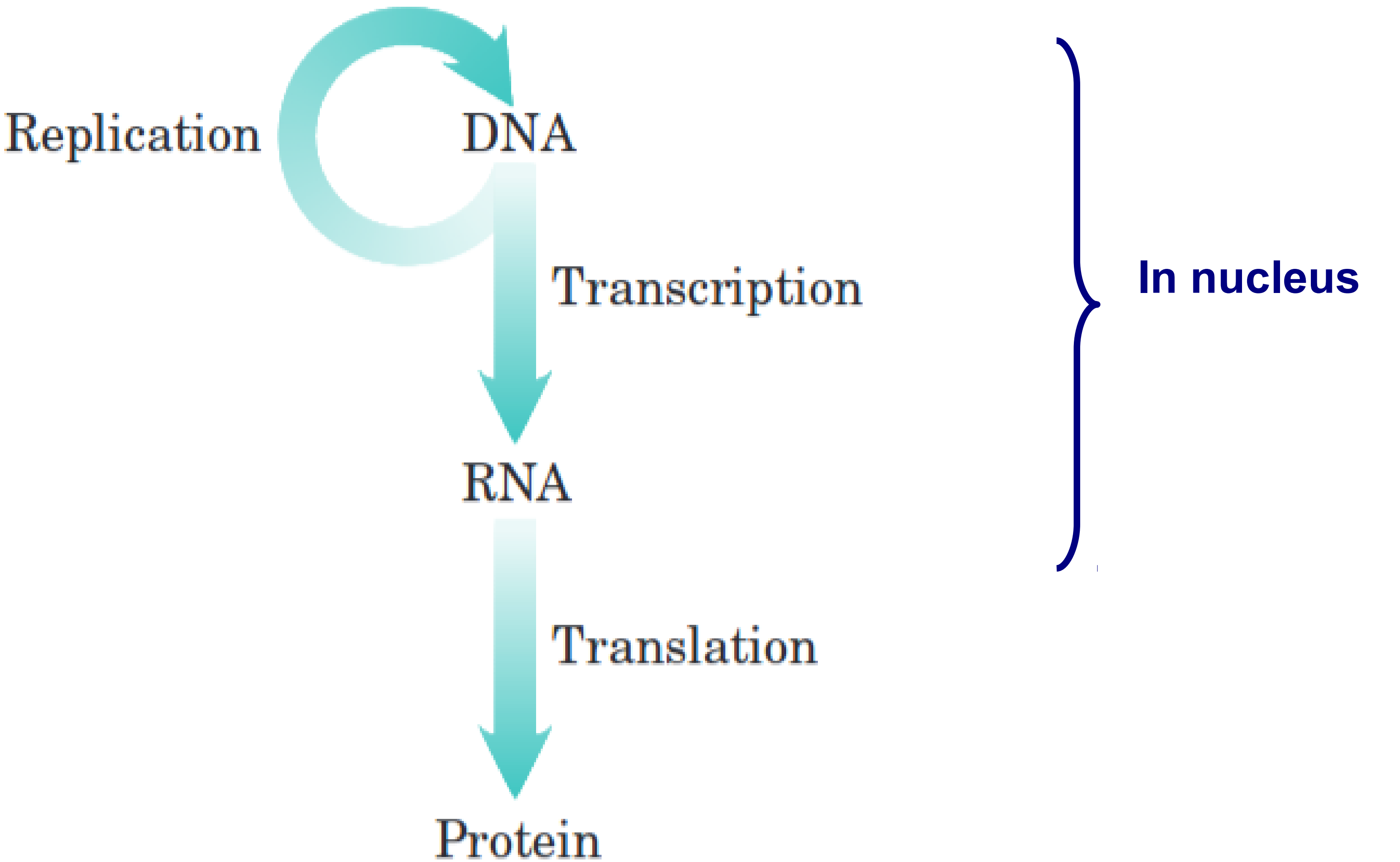
NUCLEAR ENVELOPE DISSASEMMBLY IN MITOSIS

- Lamins interact with chromatin
- Depolymerization in mitosis
- Cyclin-dependent kinase: phosphorylation



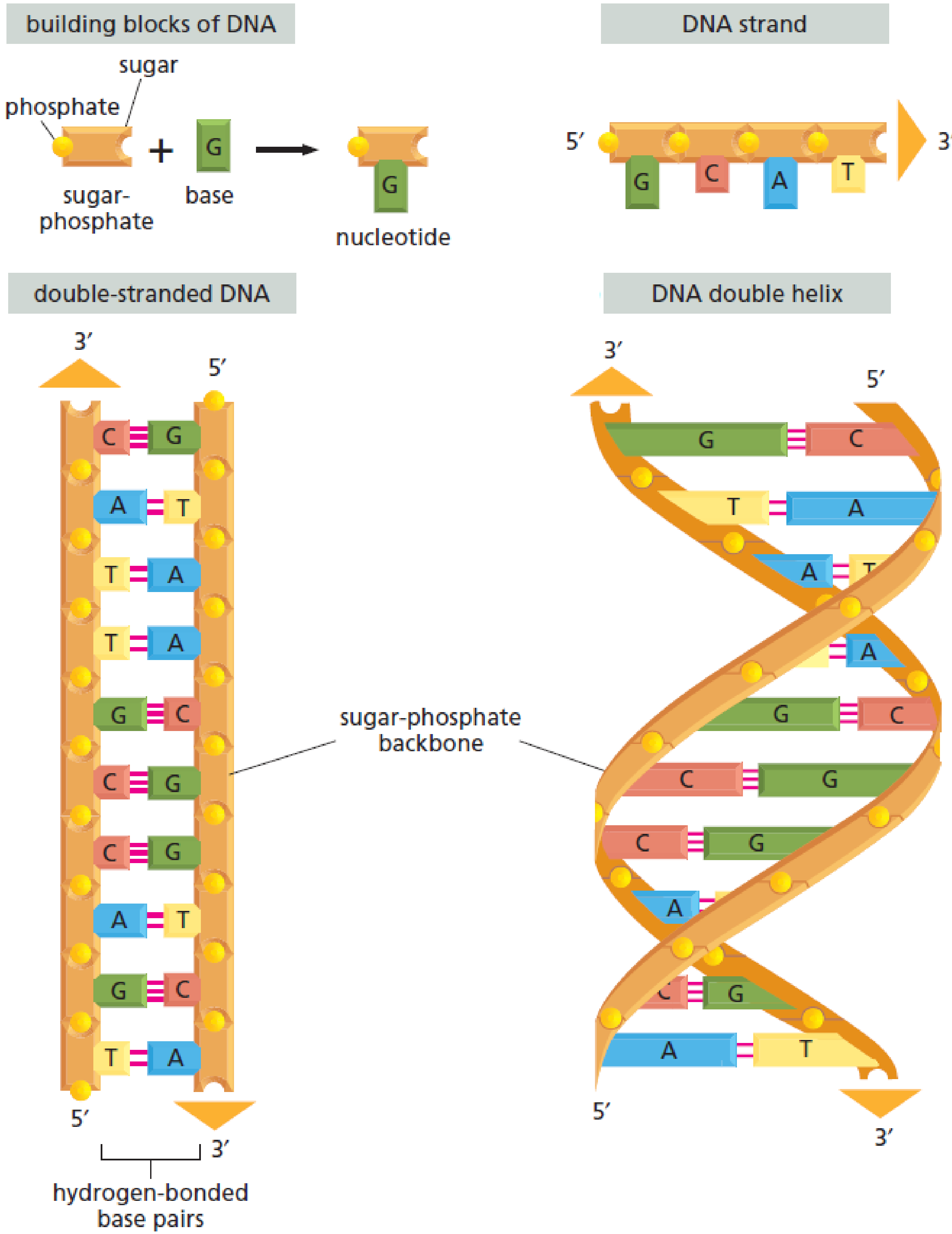


CENTRAL DOGMA OF MOLECULAR BIOLOGY

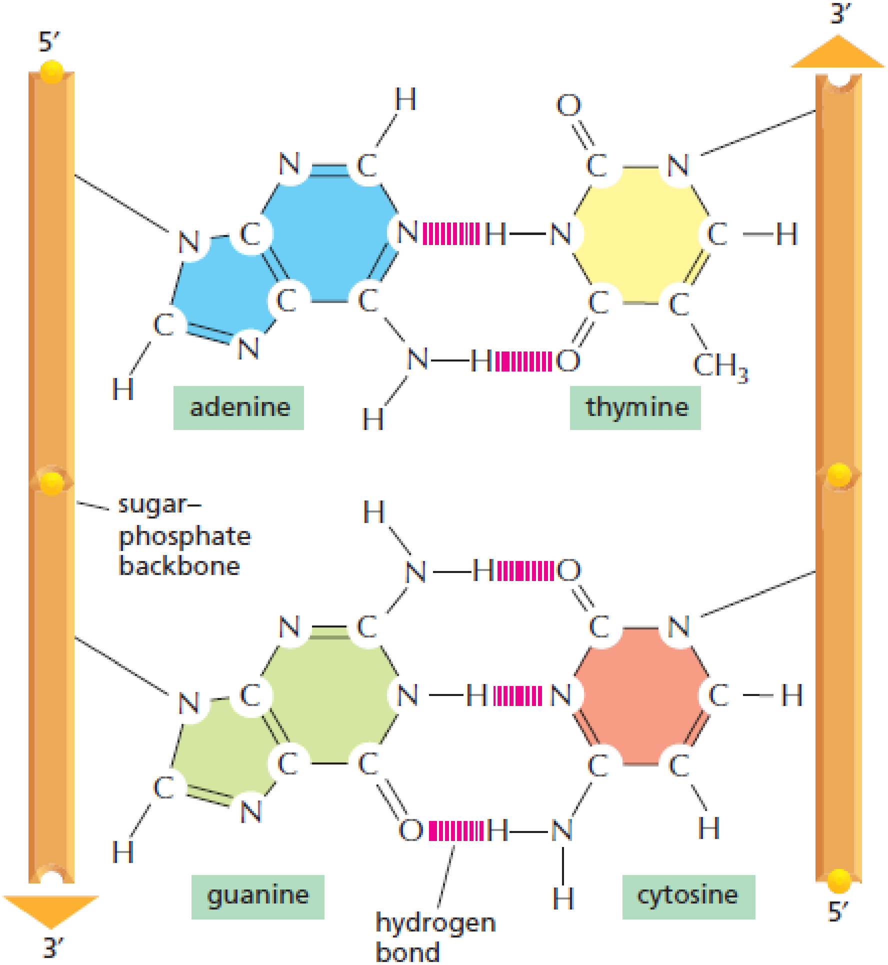


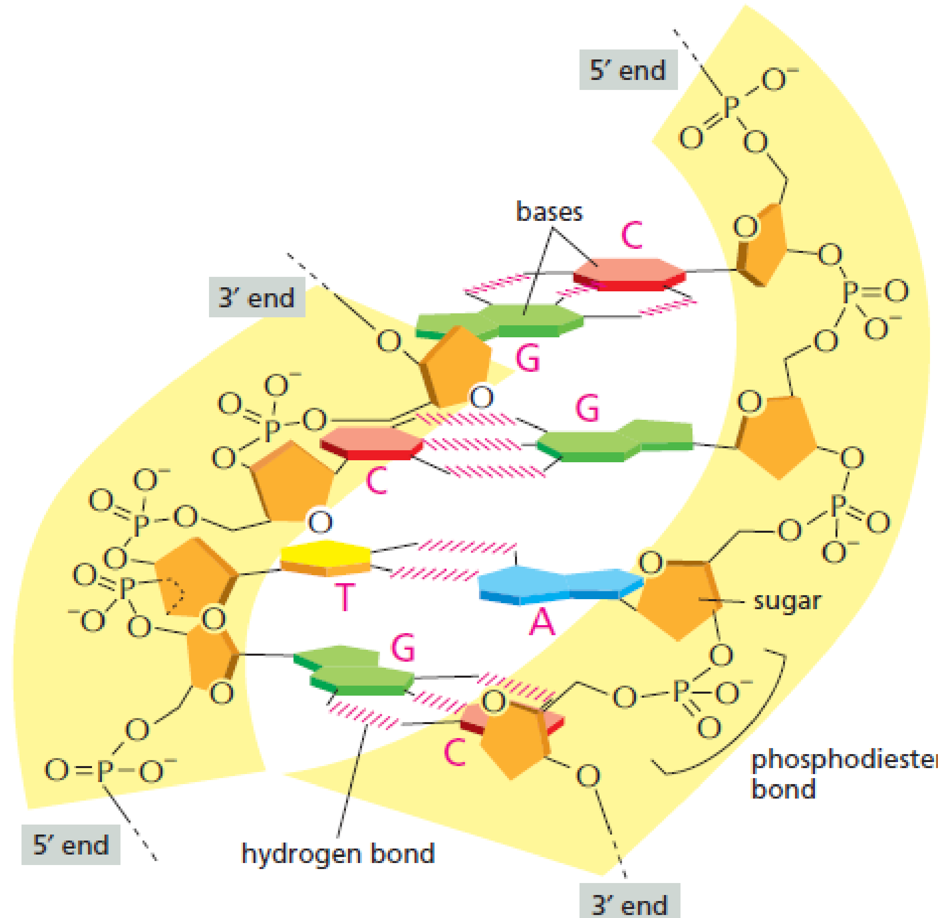
Gene: the basic physical and functional unit of heredity

DNA


building blocks of DNA
sugar
phosphate
G
sugar-
phosphate
base
G
nucleotide
DNA strand
5′
G
C
A
T
3′
double-stranded DNA
3′
5′
C G
A T
T A
T A
G C
C G
C G
A T
G C
T A
5′
3′
hydrogen-bonded
base pairs
sugar-phosphate
backbone
DNA double helix
3′
5′
G C
T A
A T
A
G C
C G
C G
A
C G
A T
5′
3′

DNA

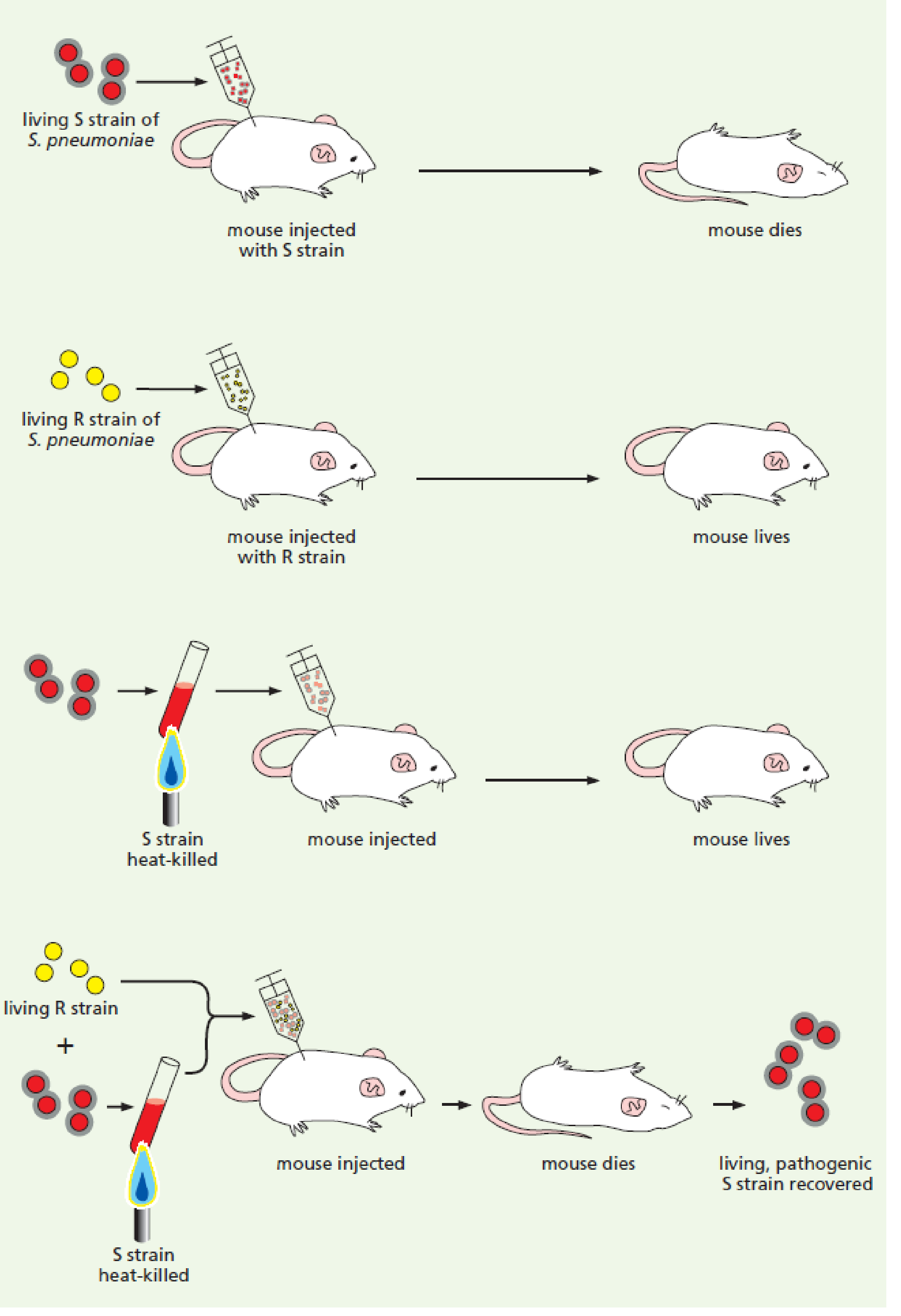




DNA structure implies the mechanism of heredity

GENES ARE MADE OF DNA

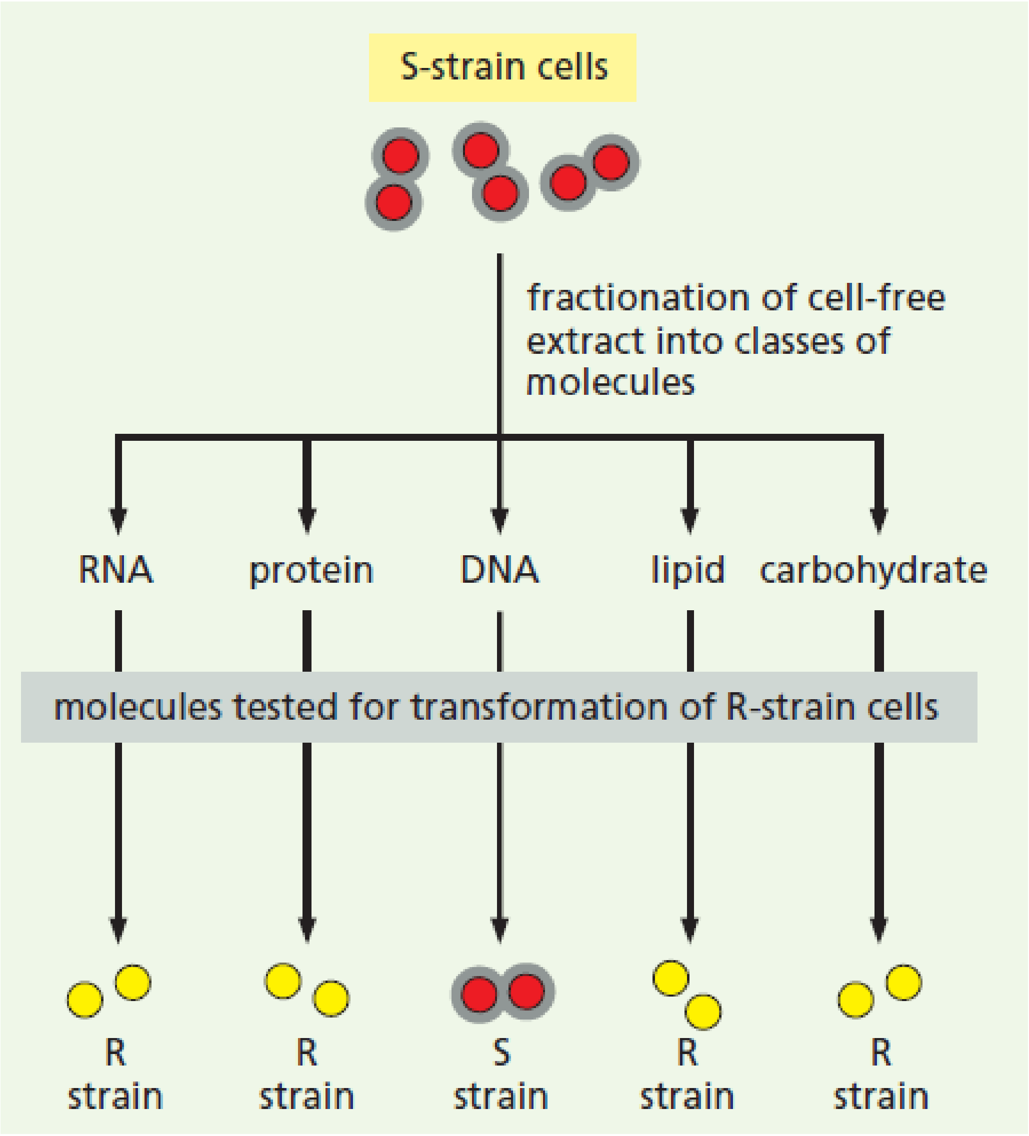
- **Griffith's experiment (1920s):**
  - **- S form (pathogenic)**
  - **- R (non-pathogenic)**



GENES ARE MADE OF DNA

McLeod/McCarty explained Grifith's results:

DNA is genetic material

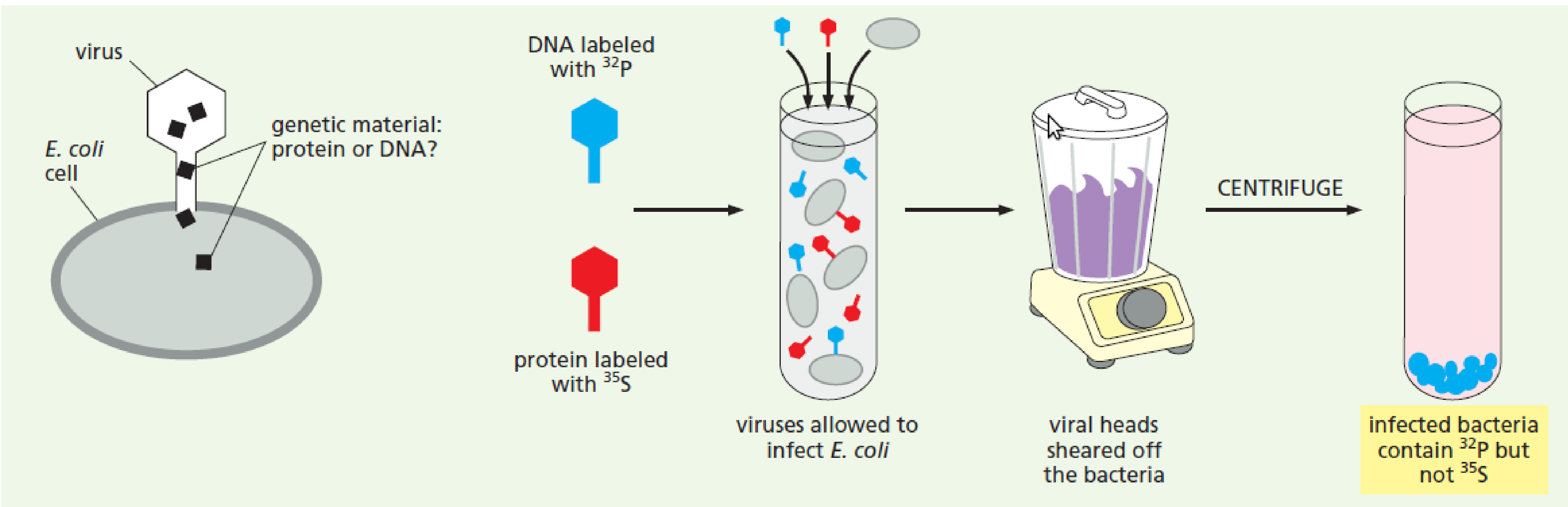


GENES ARE MADE OF DNA

- **Hersley/Chase experiment (1952):**

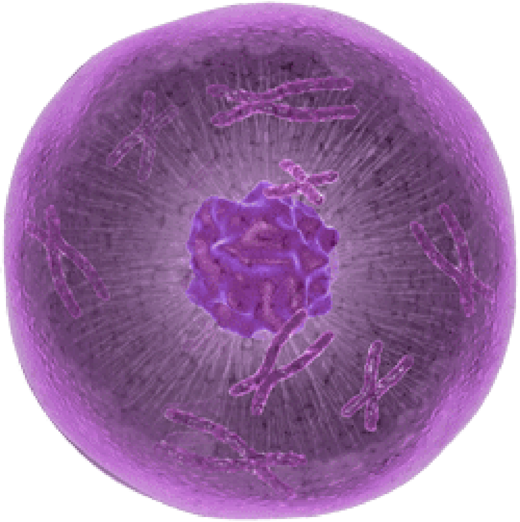
  - T2-virus: protein + DNA

  - Specific radioactive labels



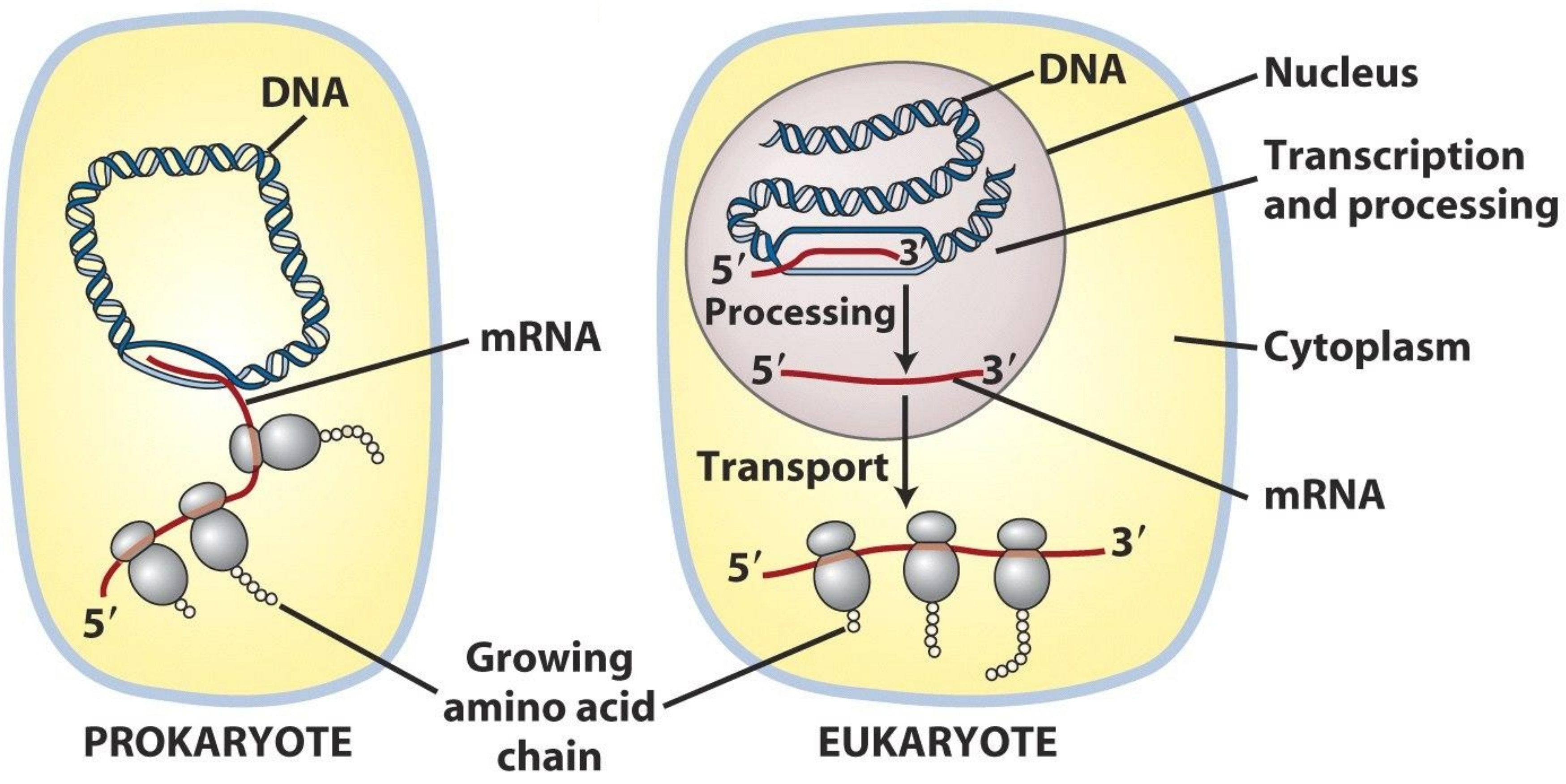
DNA IN CELL

- Human DNA: 2 meters long
- Cell nucleus ~ 5-8 µm



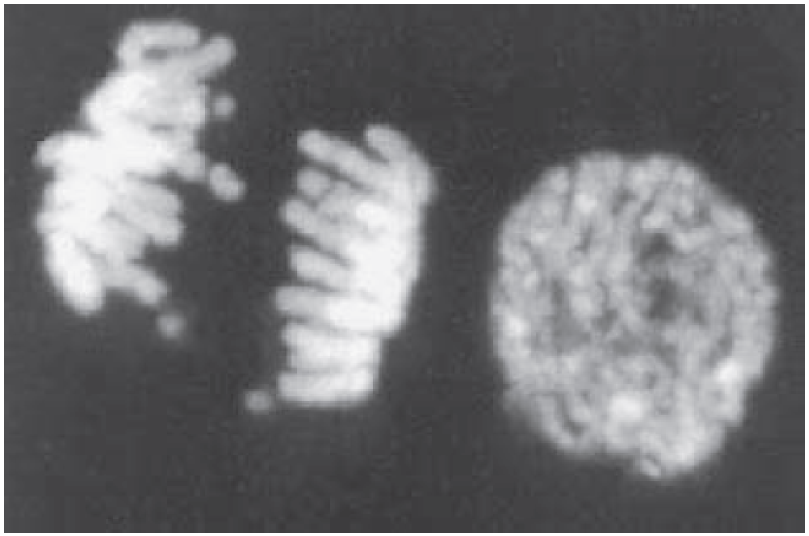
CHROMOSOME

- DNA + proteins around => chromosome
- Histones: protein core of DNA wrapping
- DNA is accessible
- DNA: linear (eukaryotes) and circular (prokaryotes)

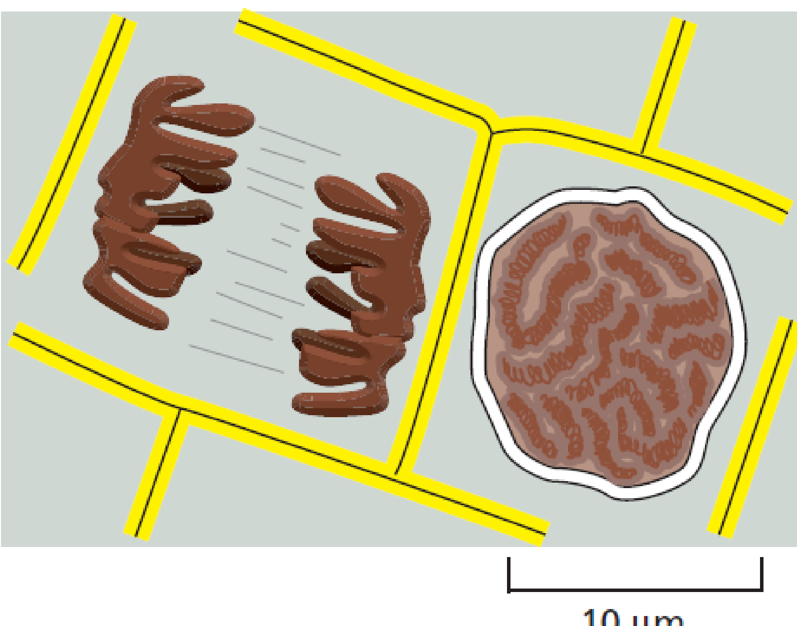


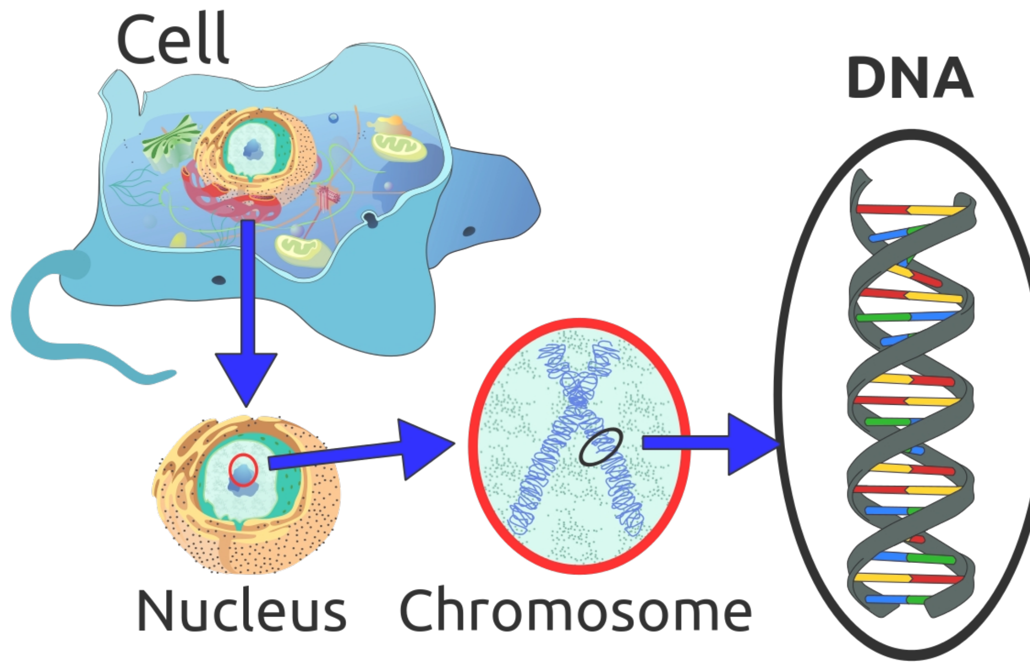
CHROMOSOMES:

Chromosomes:
thread-like structures located inside the nucleus containing DNA.



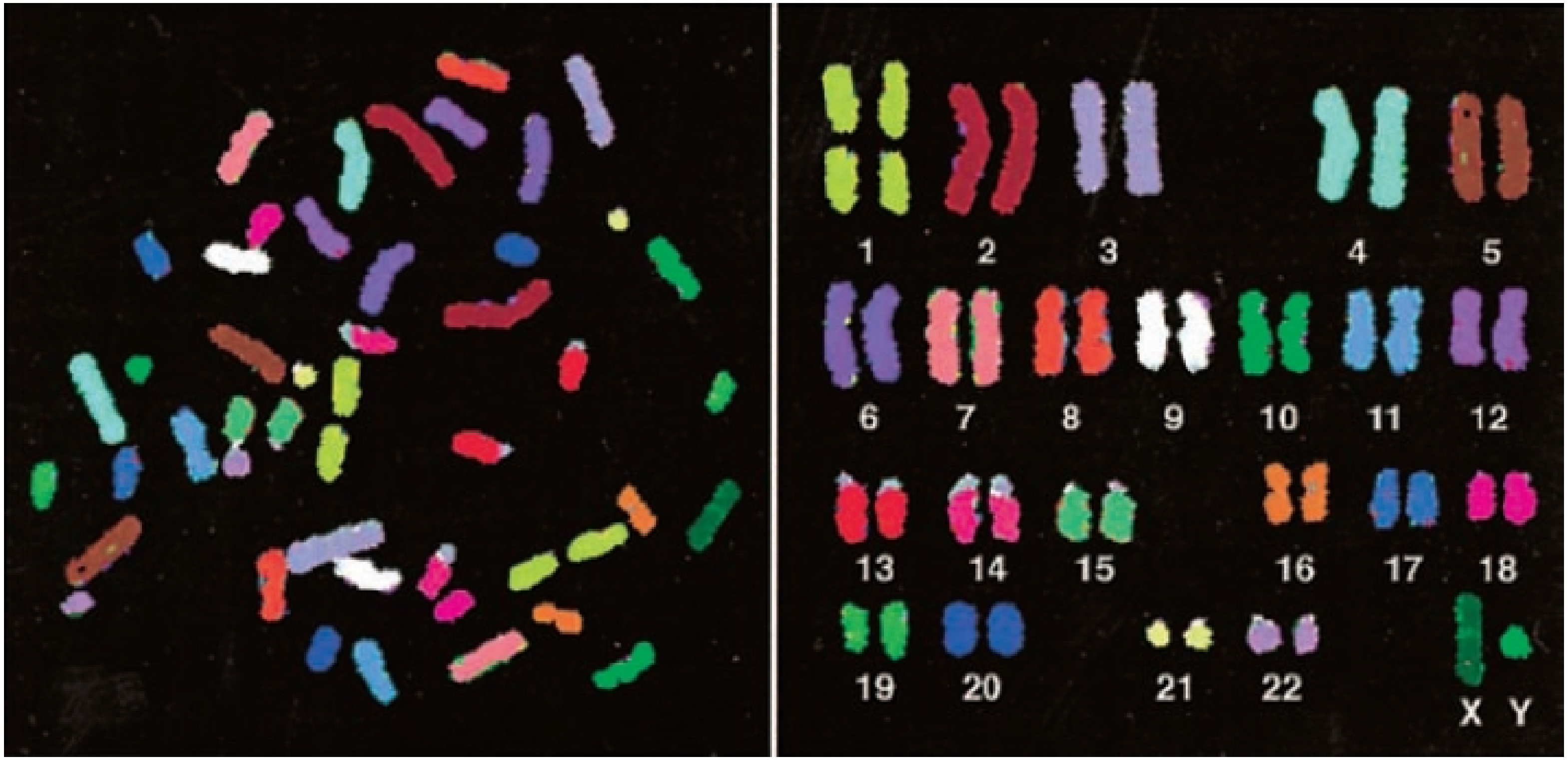
dividing cell nondividing cell



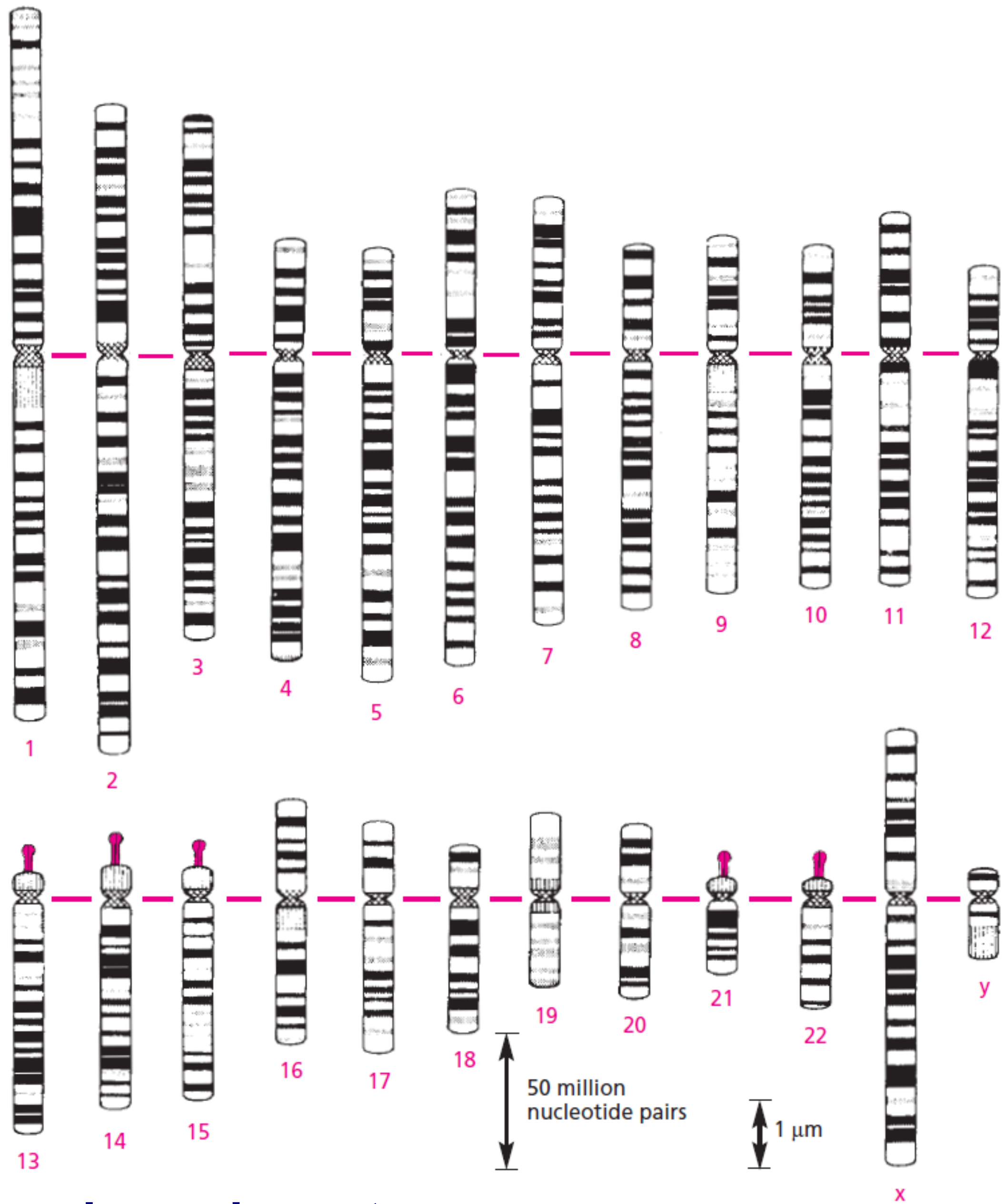


CHROMOSOMES IN HUMAN

- Human: $3.2 \cdot 10^9$ nucleotides
- Chromatin: DNA and protein (folding and packing) complex
- Chromatin + associated enzymes = chromosome
- Somatic chromosomes are paired, sex chromosomes are not (X,Y)

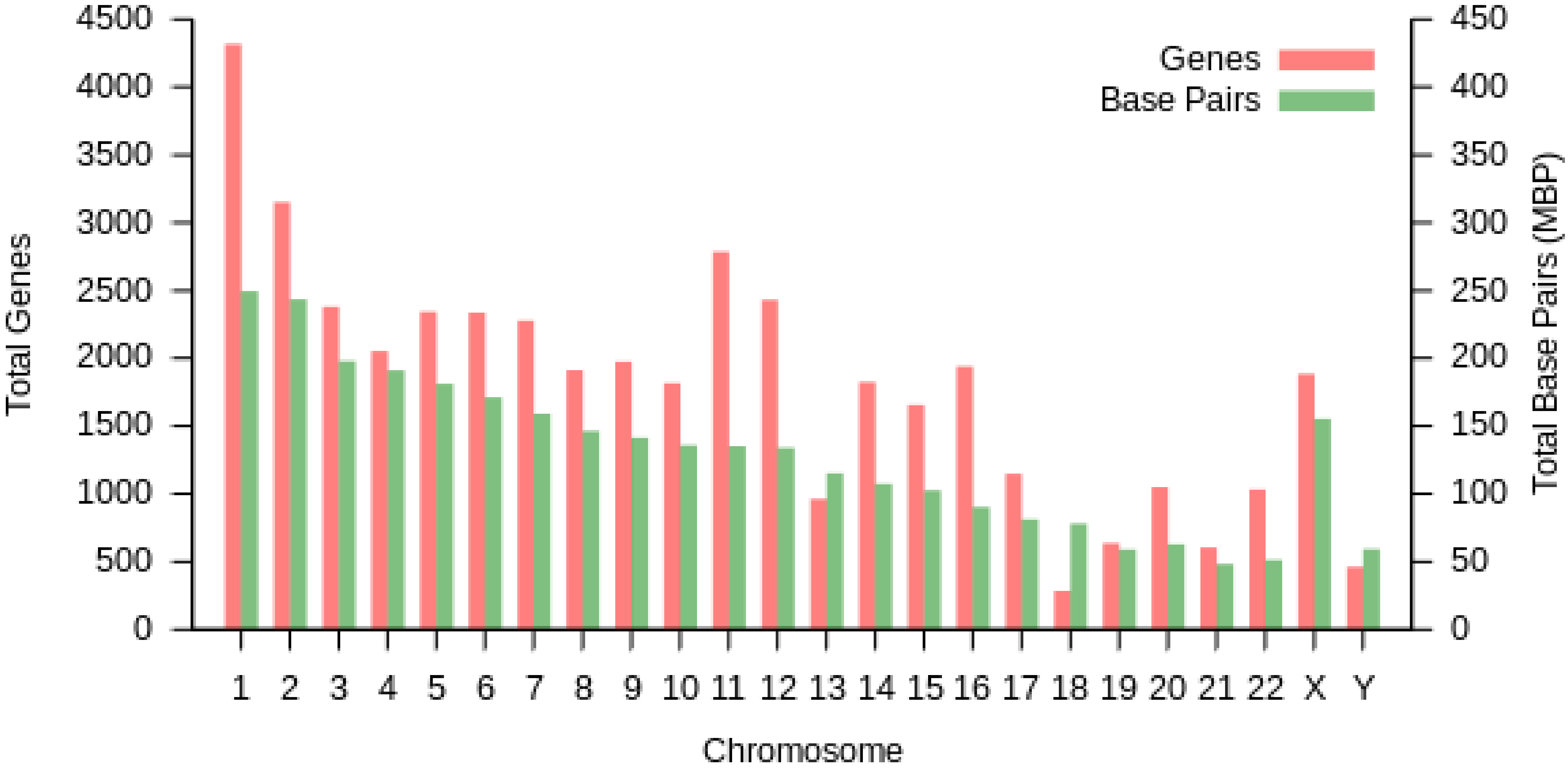


CHROMOSOMES IN HUMAN



- 46 chromosomes make up karyotype
- Many chromosome-related disorders

CHROMOSOMES IN HUMAN



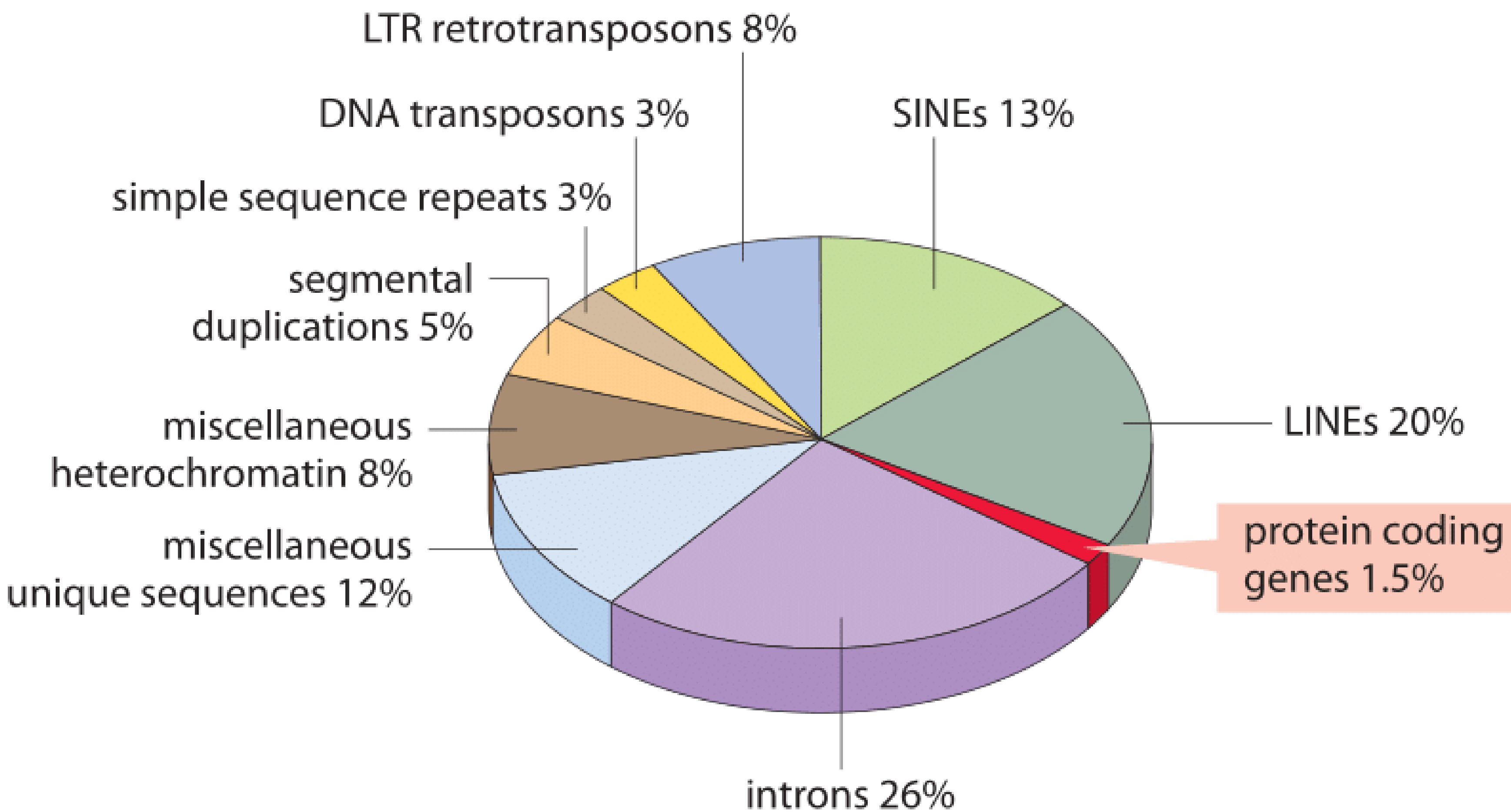
HUMAN GENOME STATISTICS

	CHROMOSOME 22	HUMAN GENOME
DNA length	48×10^6 nucleotide pairs*	3.2×10^9
Number of genes	approximately 700	approximately 30,000
Smallest protein-coding gene	1000 nucleotide pairs	not analyzed
Largest gene	583,000 nucleotide pairs	2.4×10^6 nucleotide pairs
Mean gene size	19,000 nucleotide pairs	27,000 nucleotide pairs
Smallest number of exons per gene	1	1
Largest number of exons per gene	54	178
Mean number of exons per gene	5.4	8.8
Smallest exon size	8 nucleotide pairs	not analyzed
Largest exon size	7600 nucleotide pairs	17,106 nucleotide pairs
Mean exon size	266 nucleotide pairs	145 nucleotide pairs
Number of pseudogenes**	more than 134	not analyzed
Percentage of DNA sequence in exons (protein coding sequences)	3%	1.5%
Percentage of DNA in high-copy repetitive elements	42%	approximately 50%
Percentage of total human genome	1.5%	100%

* The nucleotide sequence of 33.8×10^6 nucleotides is known; the rest of the chromosome consists primarily of very short repeated sequences that do not code for proteins or RNA.

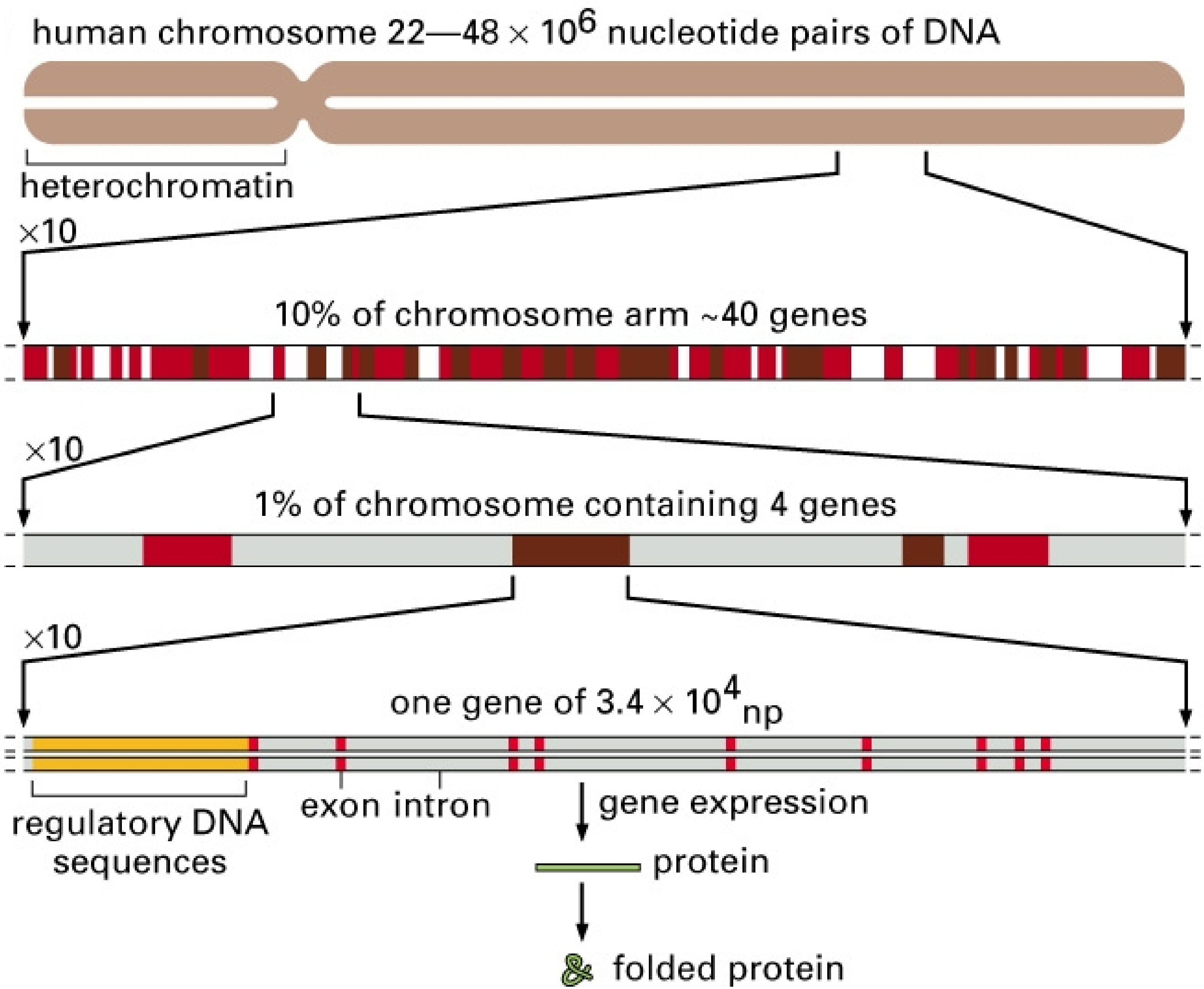
** A pseudogene is a nucleotide sequence of DNA closely resembling that of a functional gene, but containing numerous deletion mutations that prevent its proper expression. Most pseudogenes arise from the duplication of a functional gene followed by the accumulation of damaging mutations in one copy.

HUMAN GENOME COMPONENTS



- **Transposons, SINEs, LINEs, LTRs: repeated elements**
- **Exon: coding part of the gene => mRNA**
- **Intron: non-coding part of the gene**

HUMAN GENE ORGANIZATION



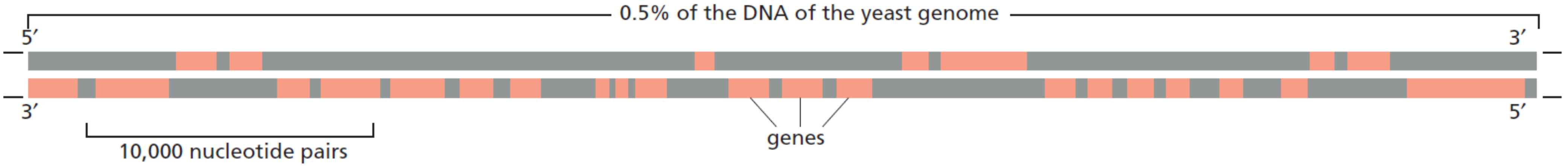
DNA/CHROMOSOMES IN DIFFERENT SPECIES

- **Complexity ~ number of genes (human 25 000, bacteria 500)**
- **“Junk DNA”:**

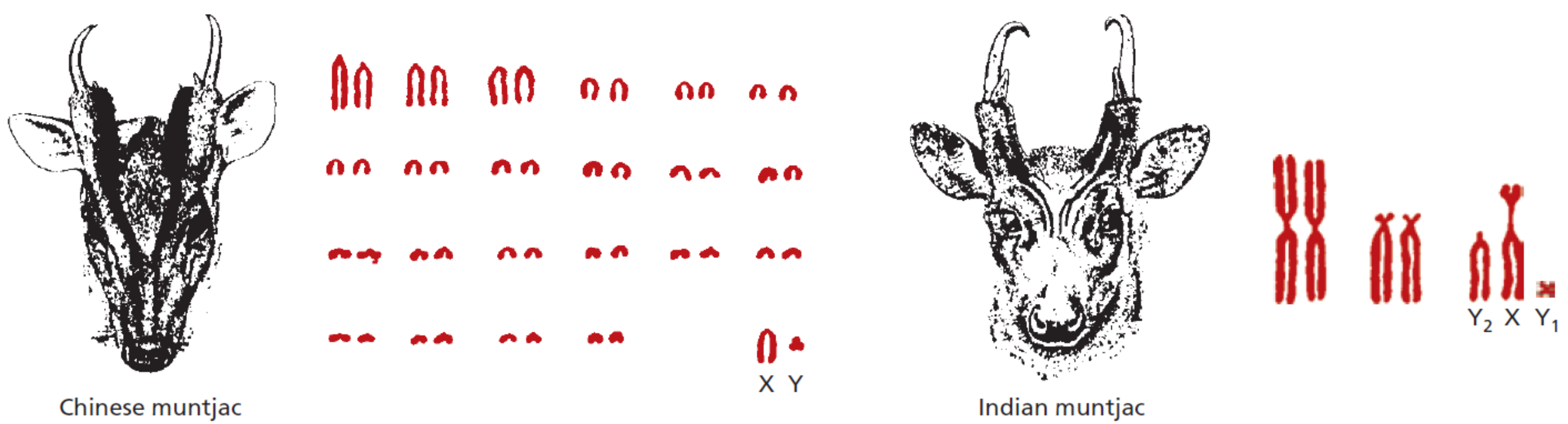
  human DNA ~ 30 yeast DNA

  human DNA < plant DNA

  human DNA ~ 1/60 amoeba DNA



- **Chromosome number does not correlate with species proximity**



22 **7**

DNA/CHROMOSOMES IN DIFFERENT SPECIES

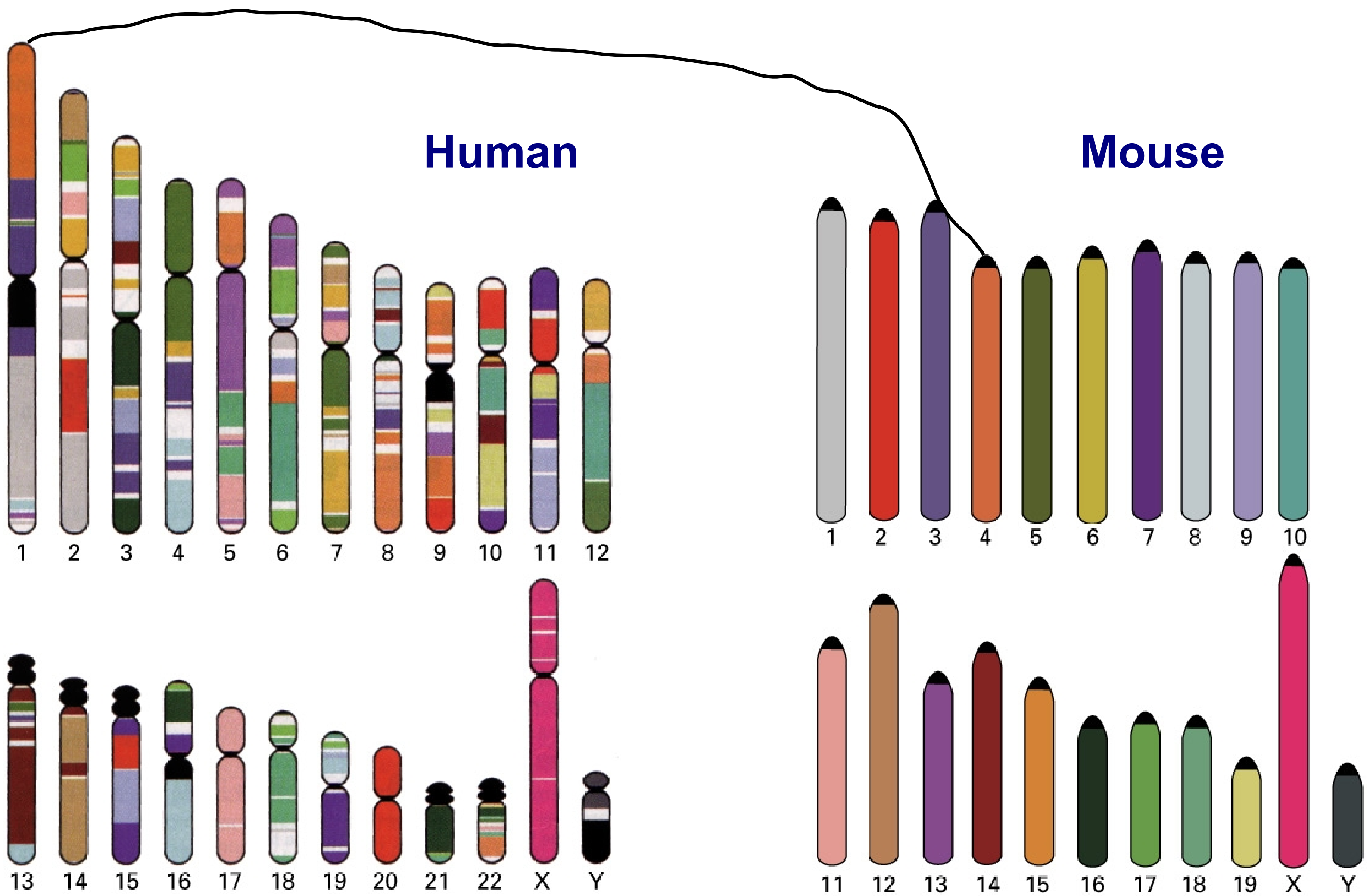
Kingfisher: 132 chromosomes

Adder's tongue fern:

~1200 chromosomes

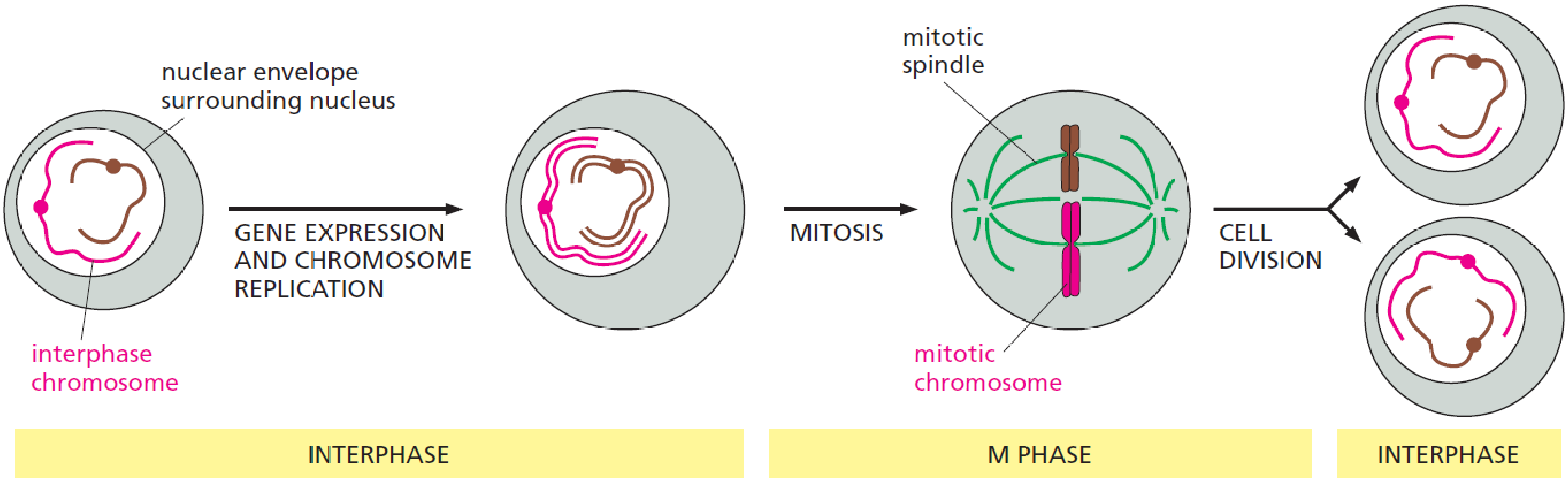
GENE IN DIFFERENT SPECIES

- Conserved/non-conserved regions
- Conserved syntony: conservation of homologous blocks
- Human/mouse = 97.5 % similarity of genes

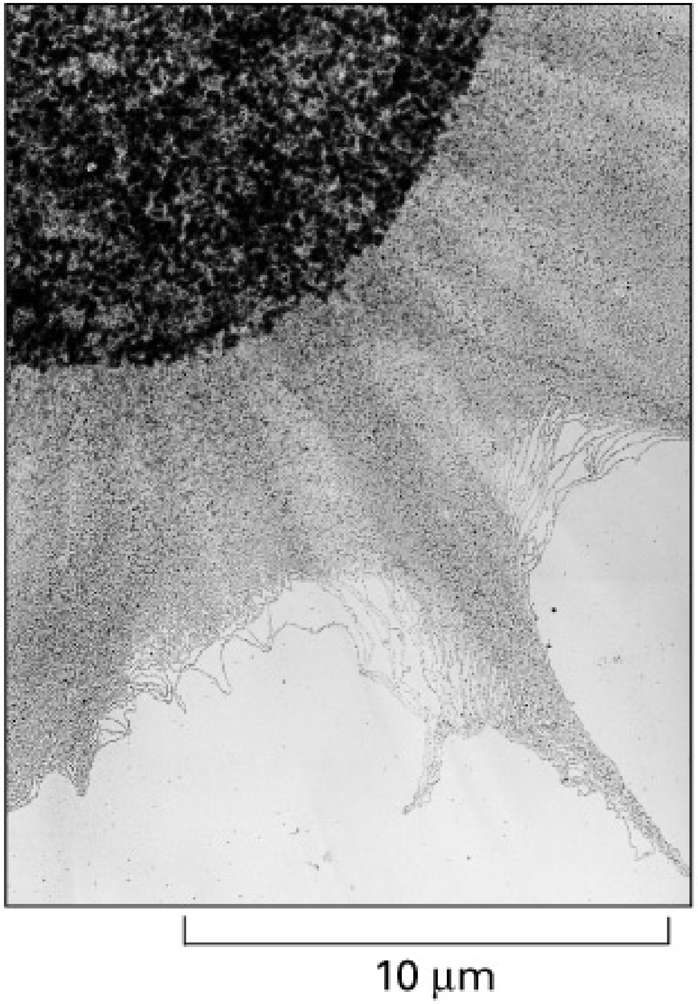


DIFFERENT STATES OF CHROMOSOMES

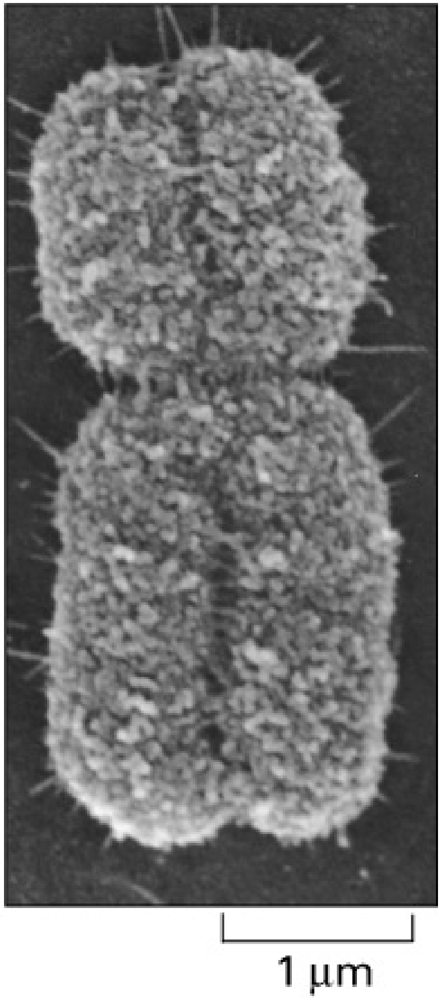
- Cell cycle (L21-24): cell division cycle
- Interphase: chromosomes are replicated
- Mitosis: chromosomes are condensed (10^5 times more)



DIFFERENT STATES OF CHROMOSOMES

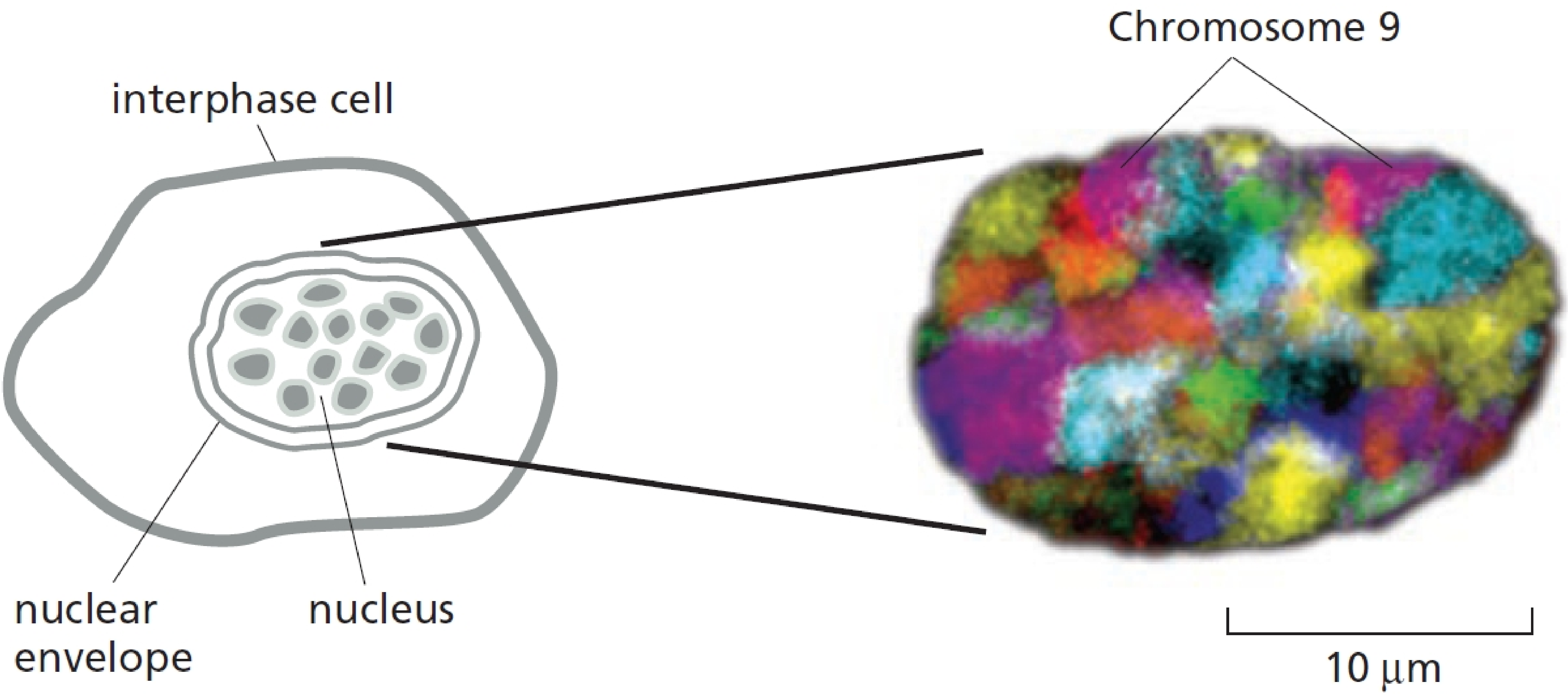


Interphase chromosome



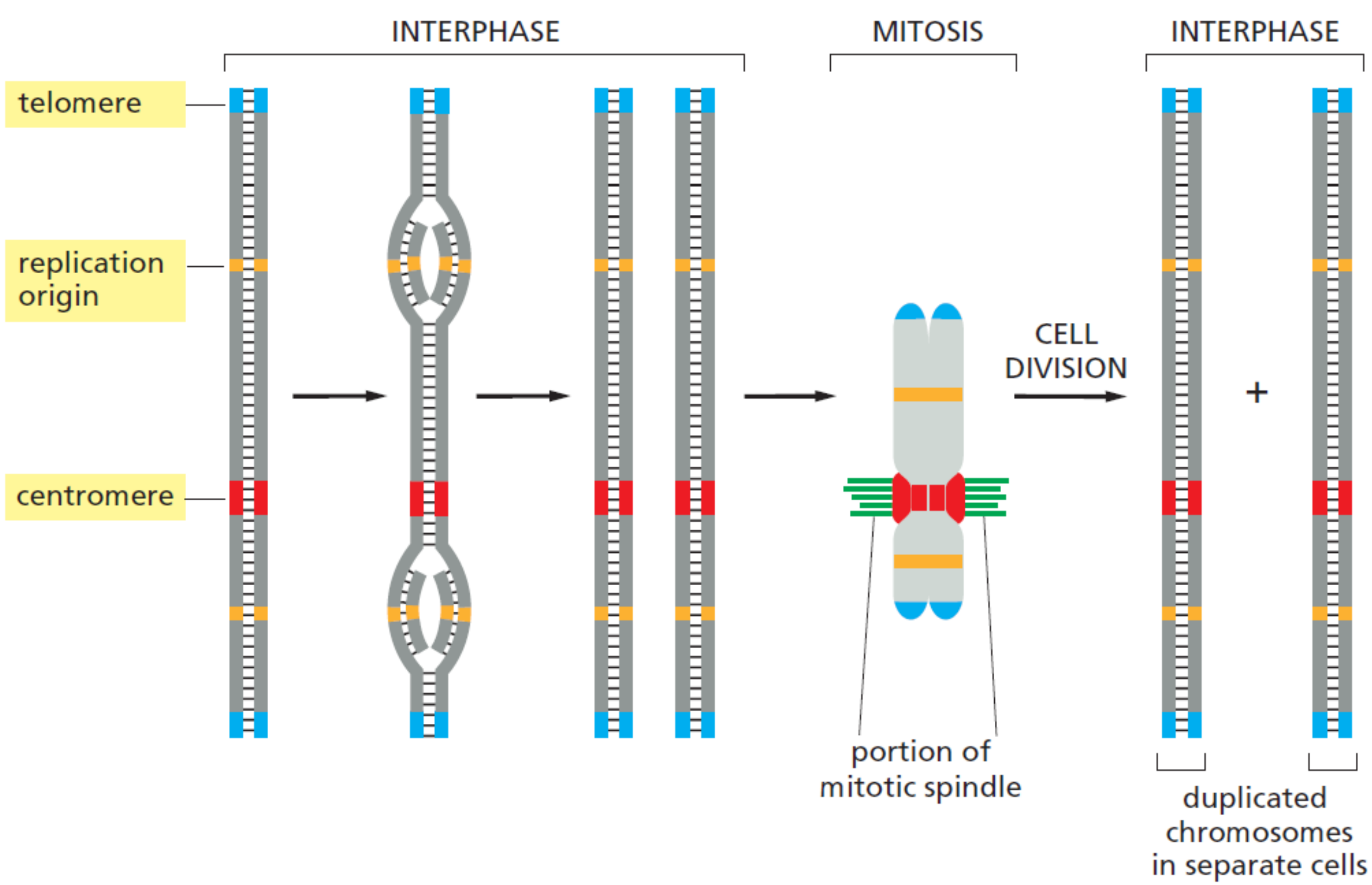
Mitotic chromosome

CHROMOSOMES LOCALIZATION IN NUCLEUS

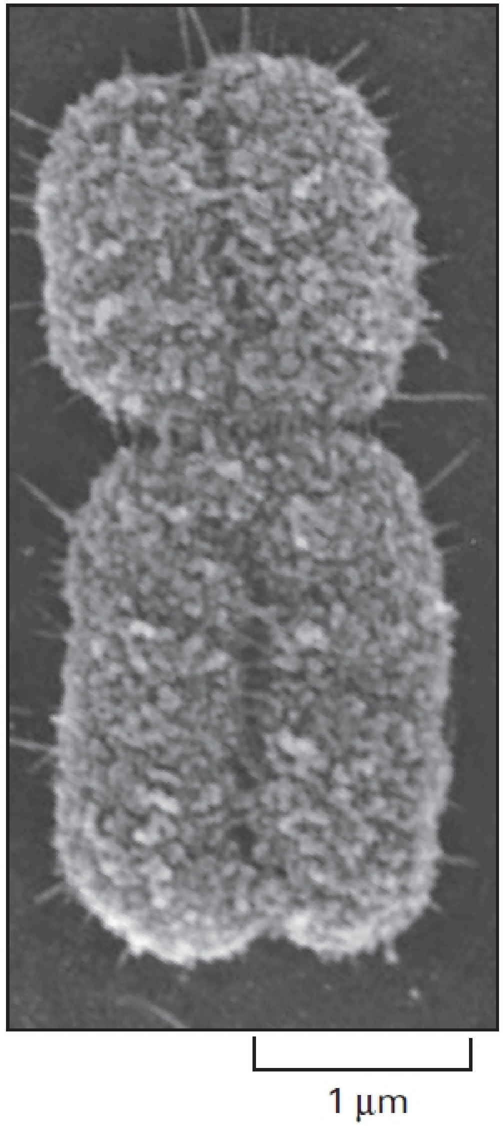


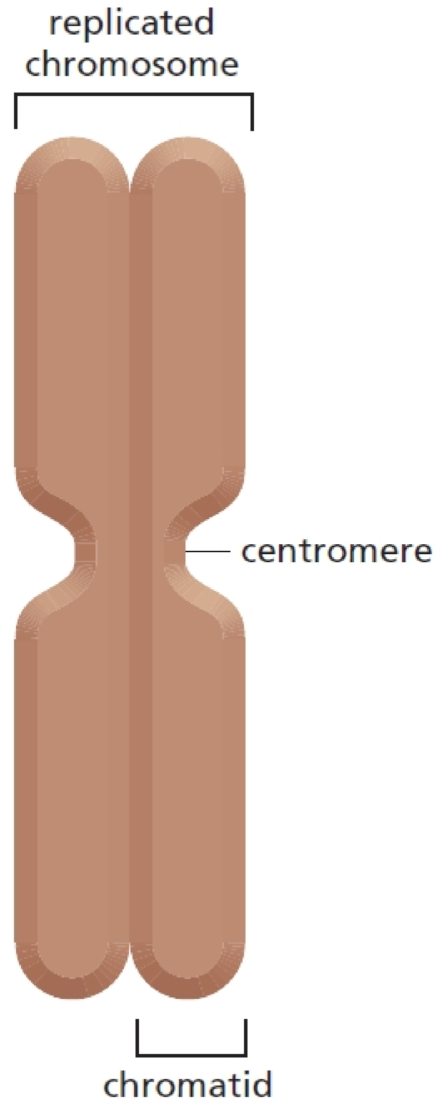
CHROMOSOME MORHOLOGY

- Replication origin: point of replication initiation
- Chromatid: one of the two identical parts of the chromosomes in S-phase
- Centromere: point of touch between two chromatids
- Telomers: ends of chromatids



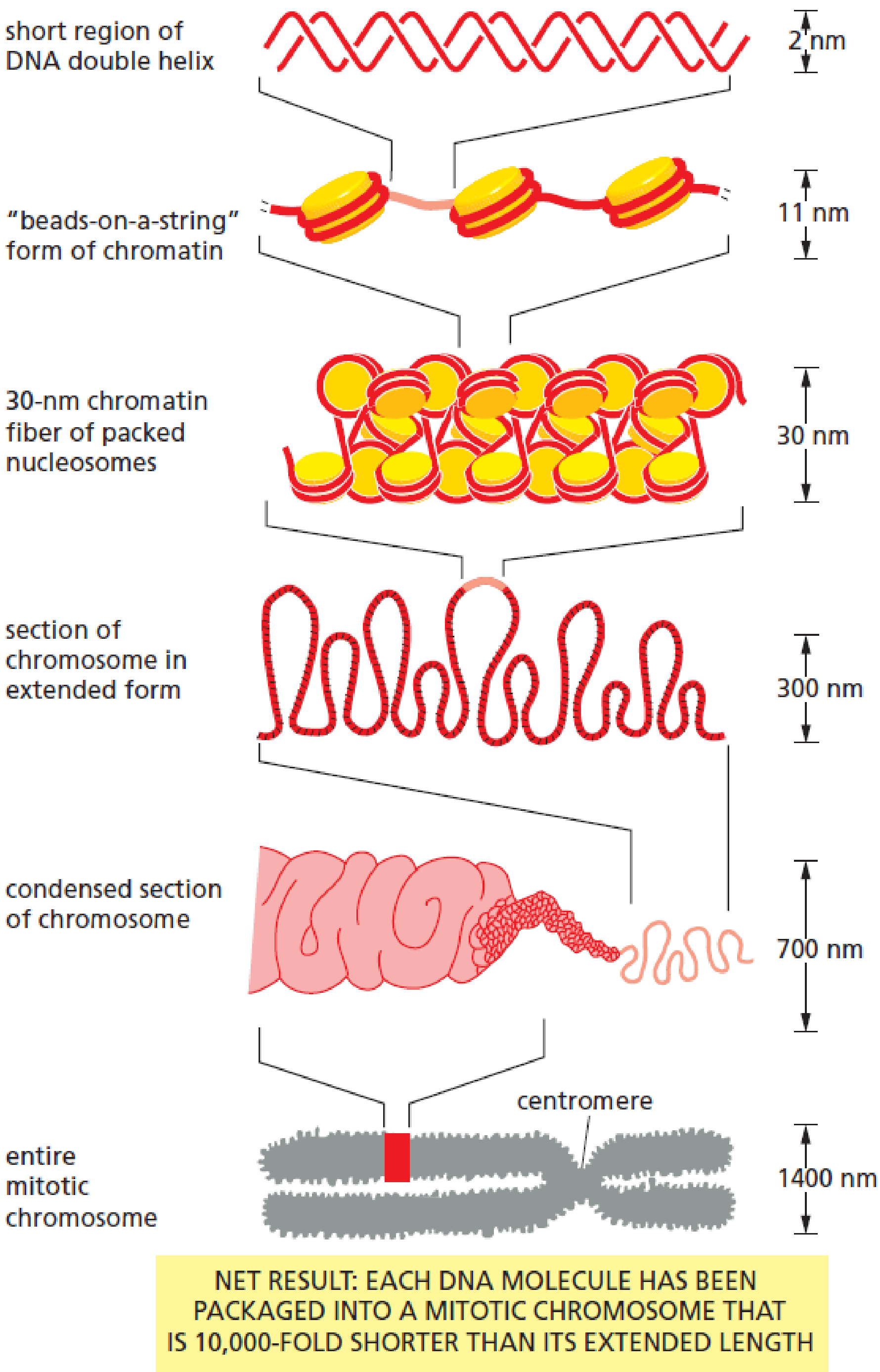
CHROMOSOME MORHOLOGY





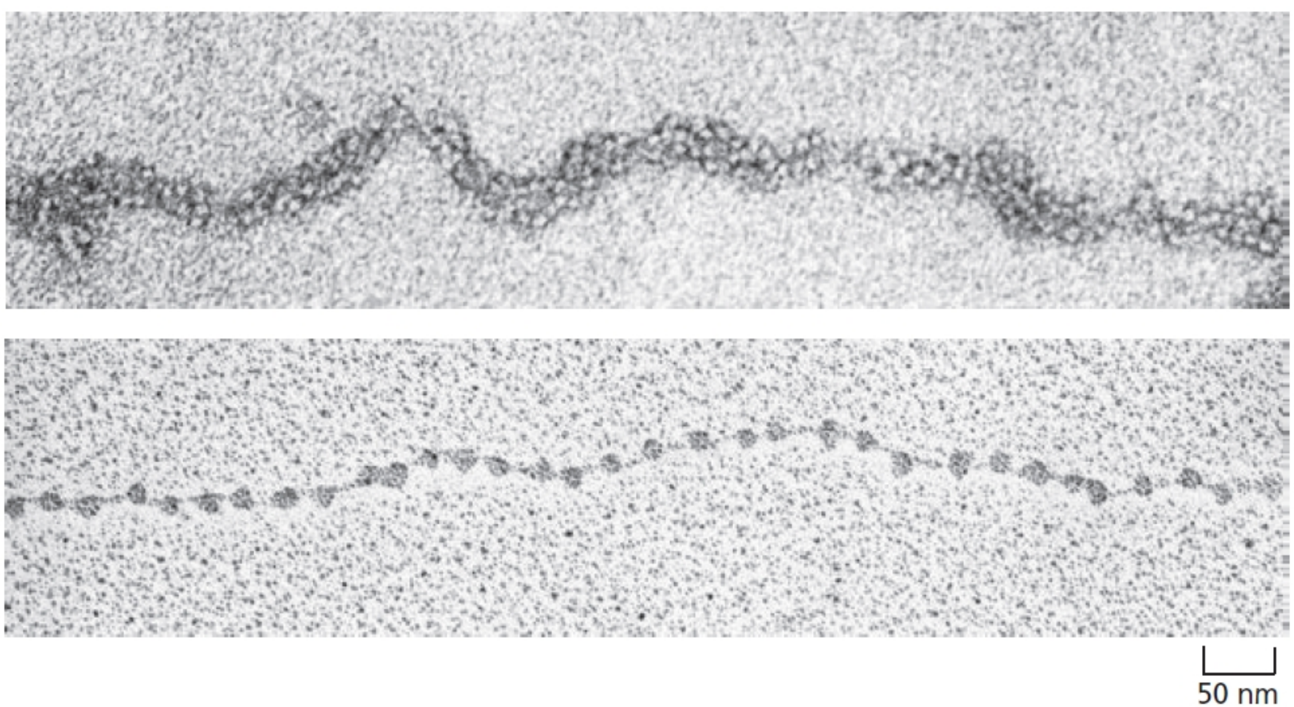
LEVELS OF DNA STRUCTURAL ORGANIZATION

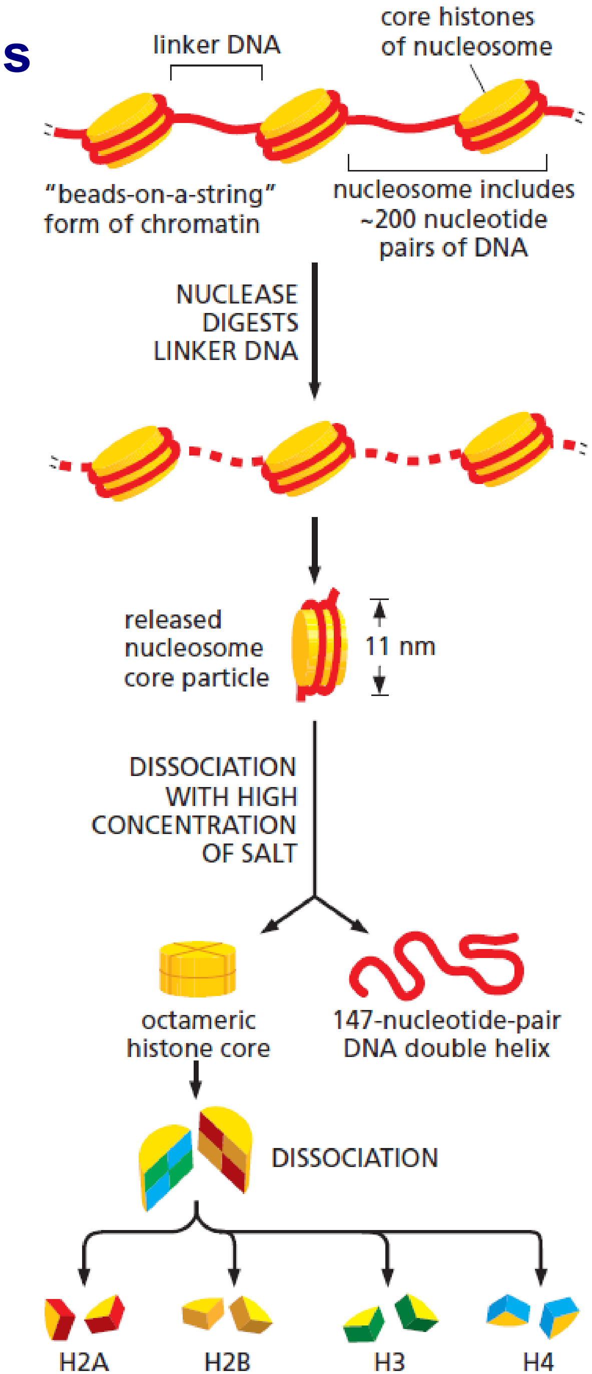
- **DNA**
- **Nucleosomes**
- **"Bead-on-a-string"**
- **Fibers**
- **Active chromosome**
- **Mitotic chromosome**



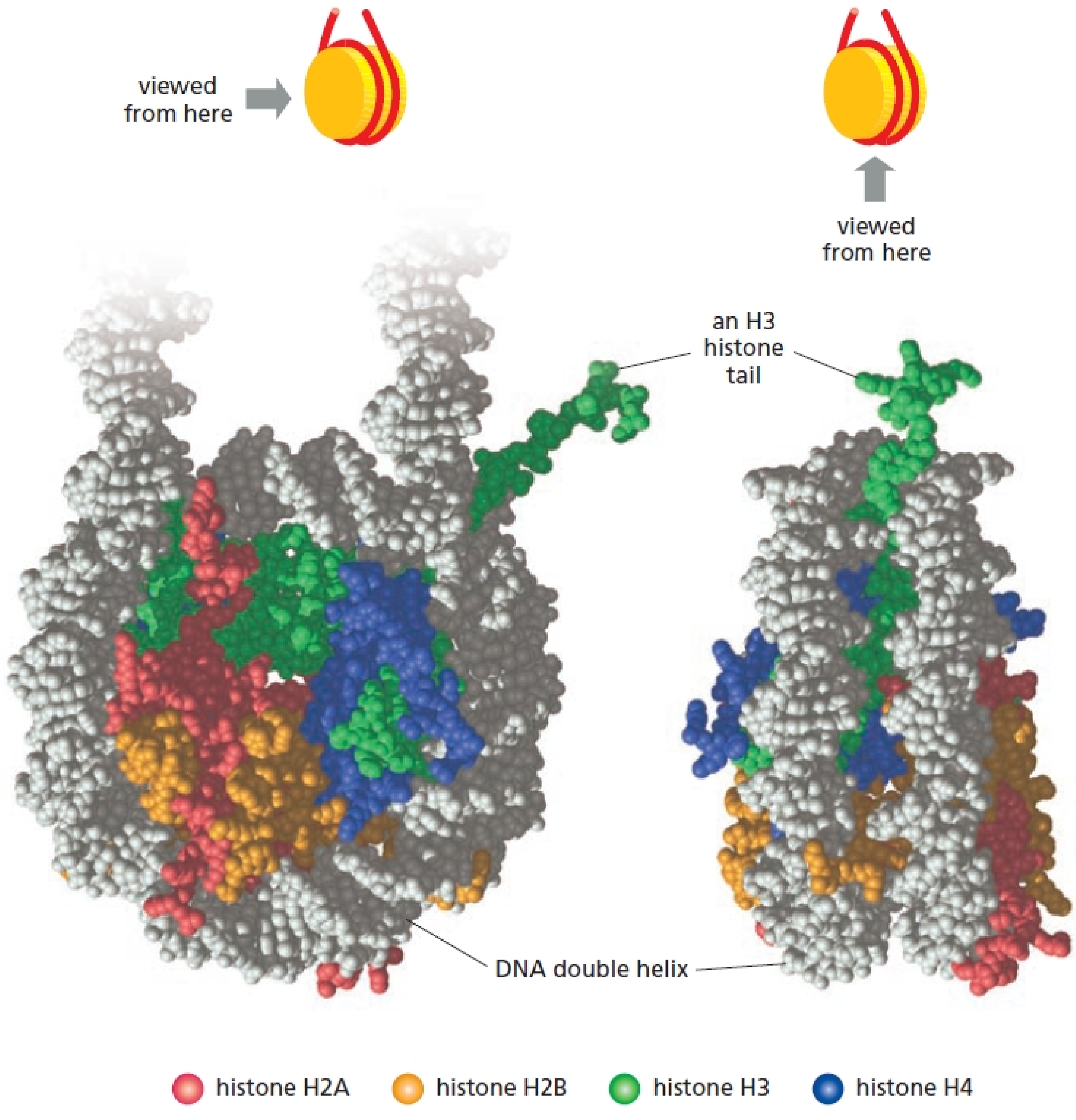
NUCLEOSOMES

- Chromosome = DNA + histones + nonhistone proteins
- ~ $6 \cdot 10^7$ histones in the cell
- Histone octamer forms a core: H2A, H2B, H3, H4
- Nucleosome repeat ~ 200 bp
- ~ $3 \cdot 10^7$ nucleosomes

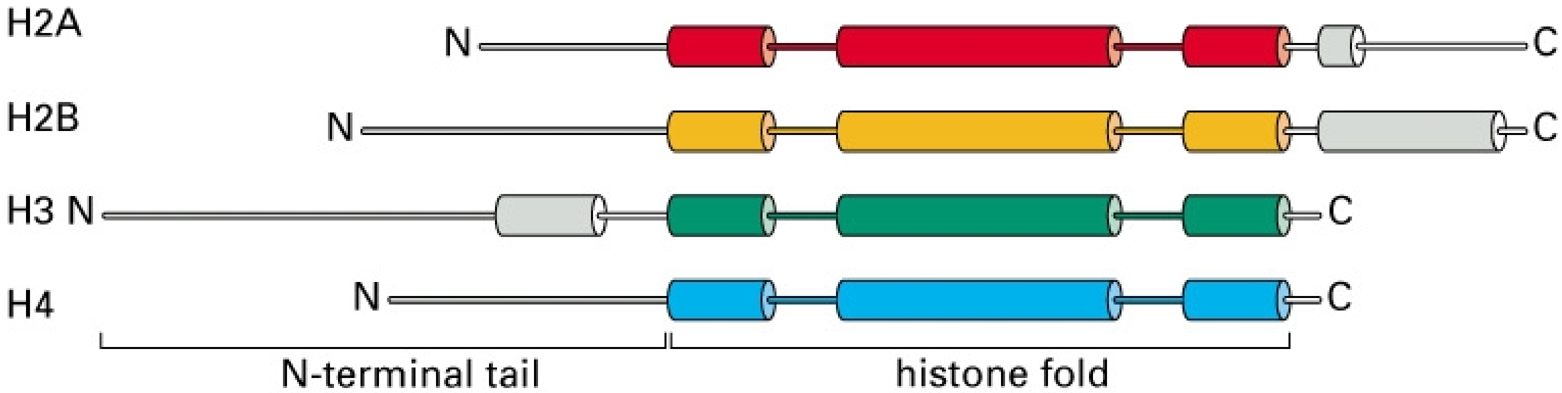


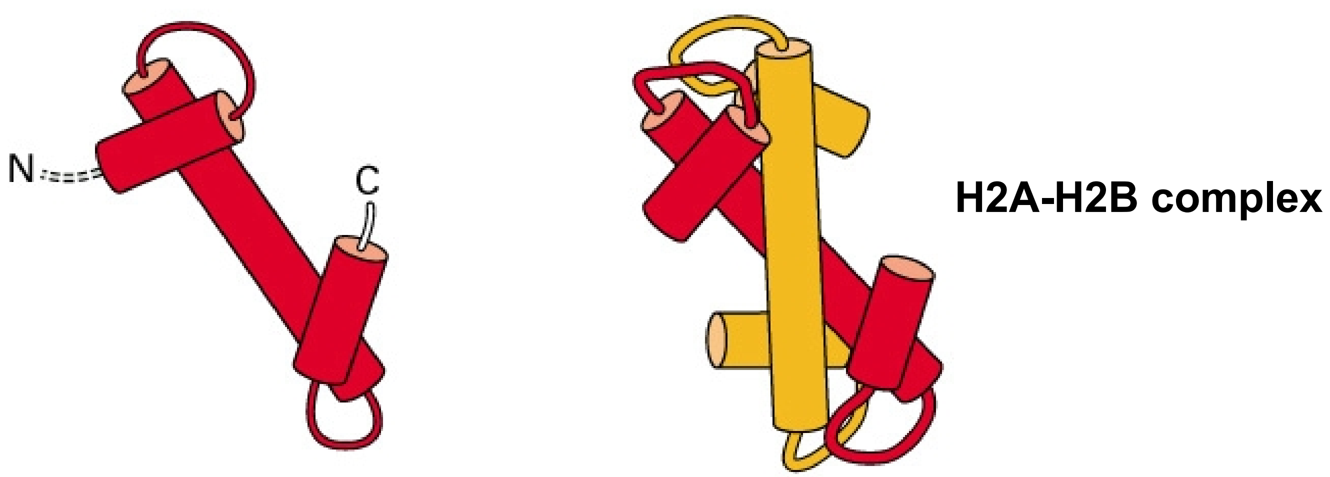


NUCLEOSOME: CRYSTAL STRUCTURE



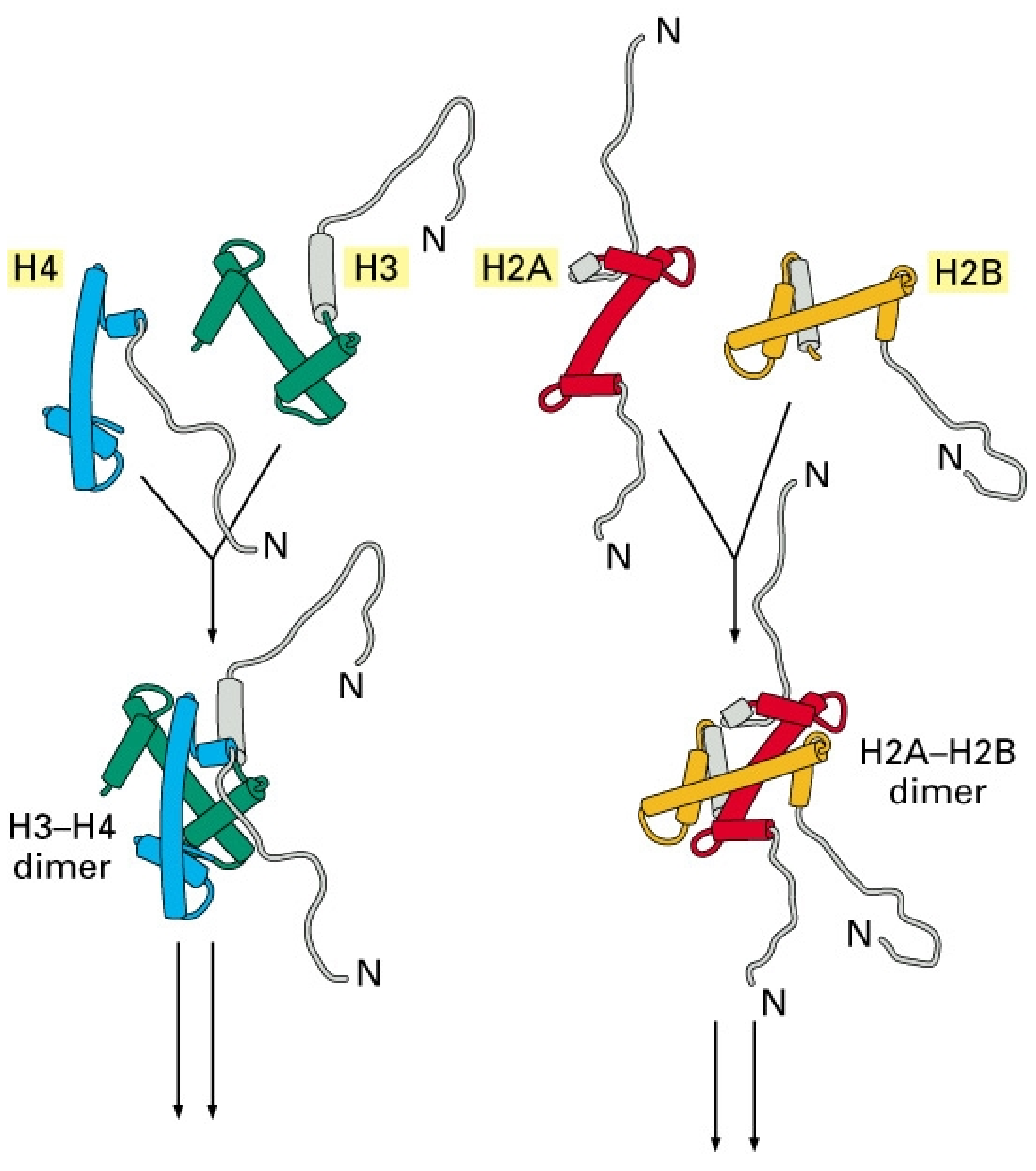
HISTONE DOMAINS



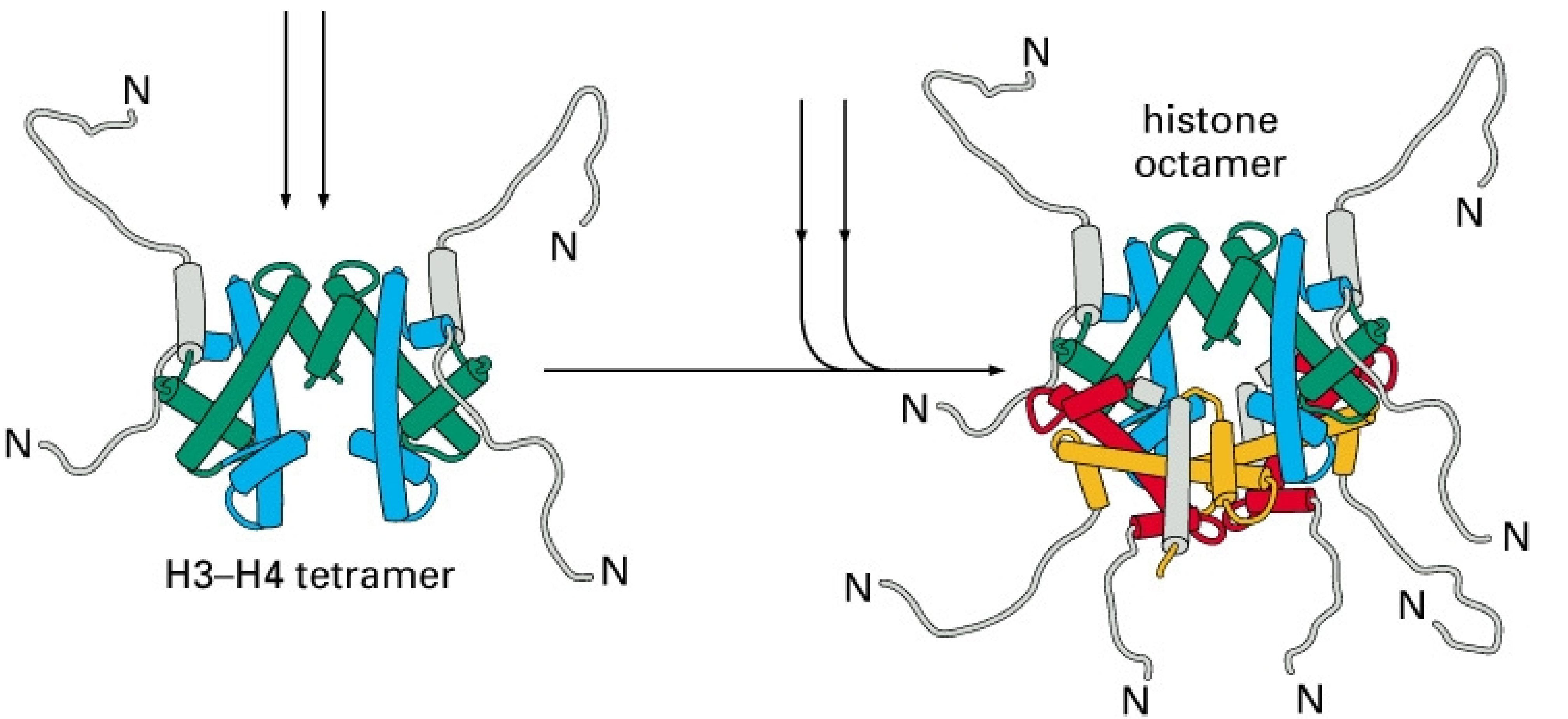


- Histones: 102-135 amino acids, histone fold
- Rich in Lys and Arg
- Highly conservative (f.i. H4 in cow and pea: 2 mutations per 102 aa)
- Many isoforms

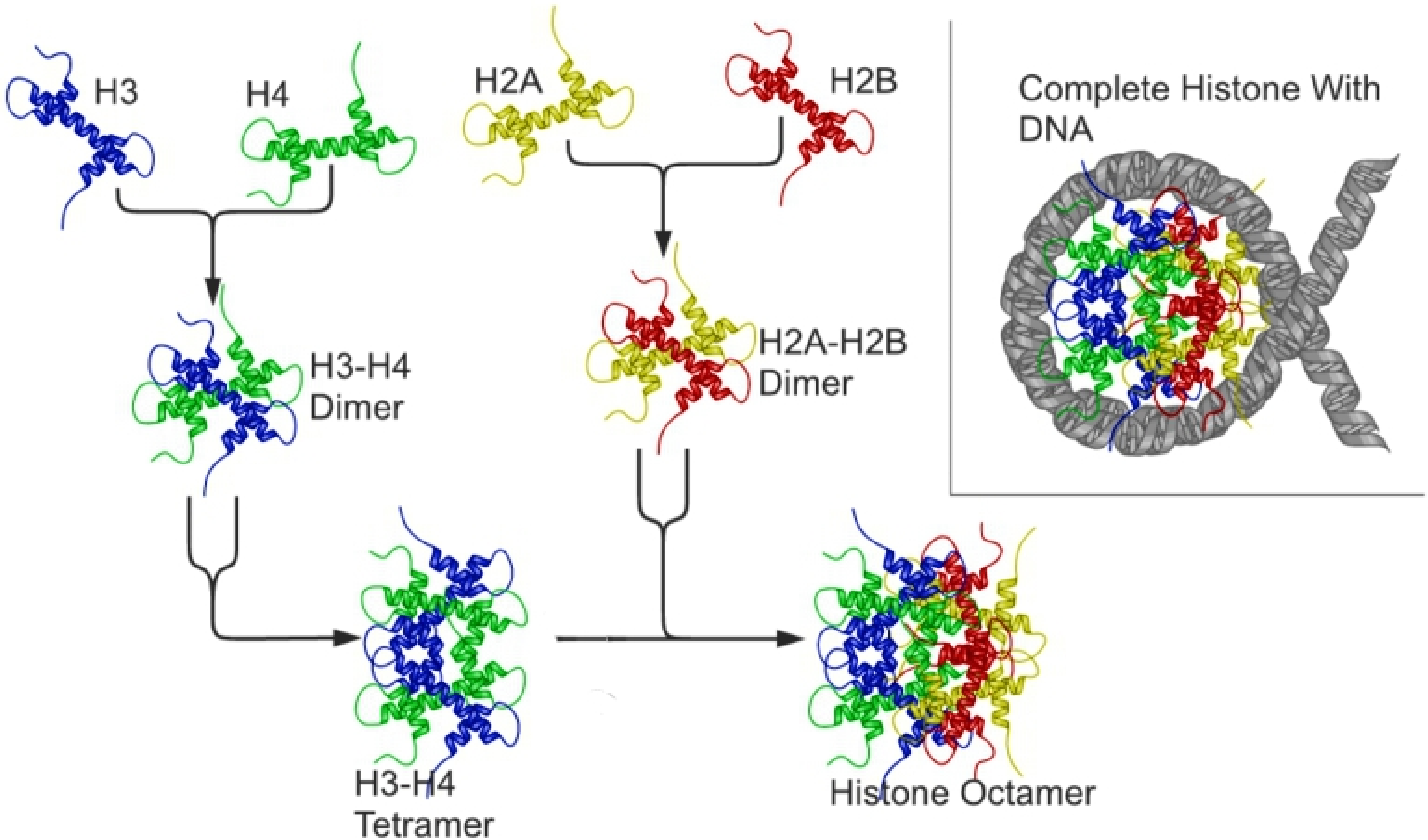
HISTONES ASSEMBLY



HISTONES ASSEMBLY



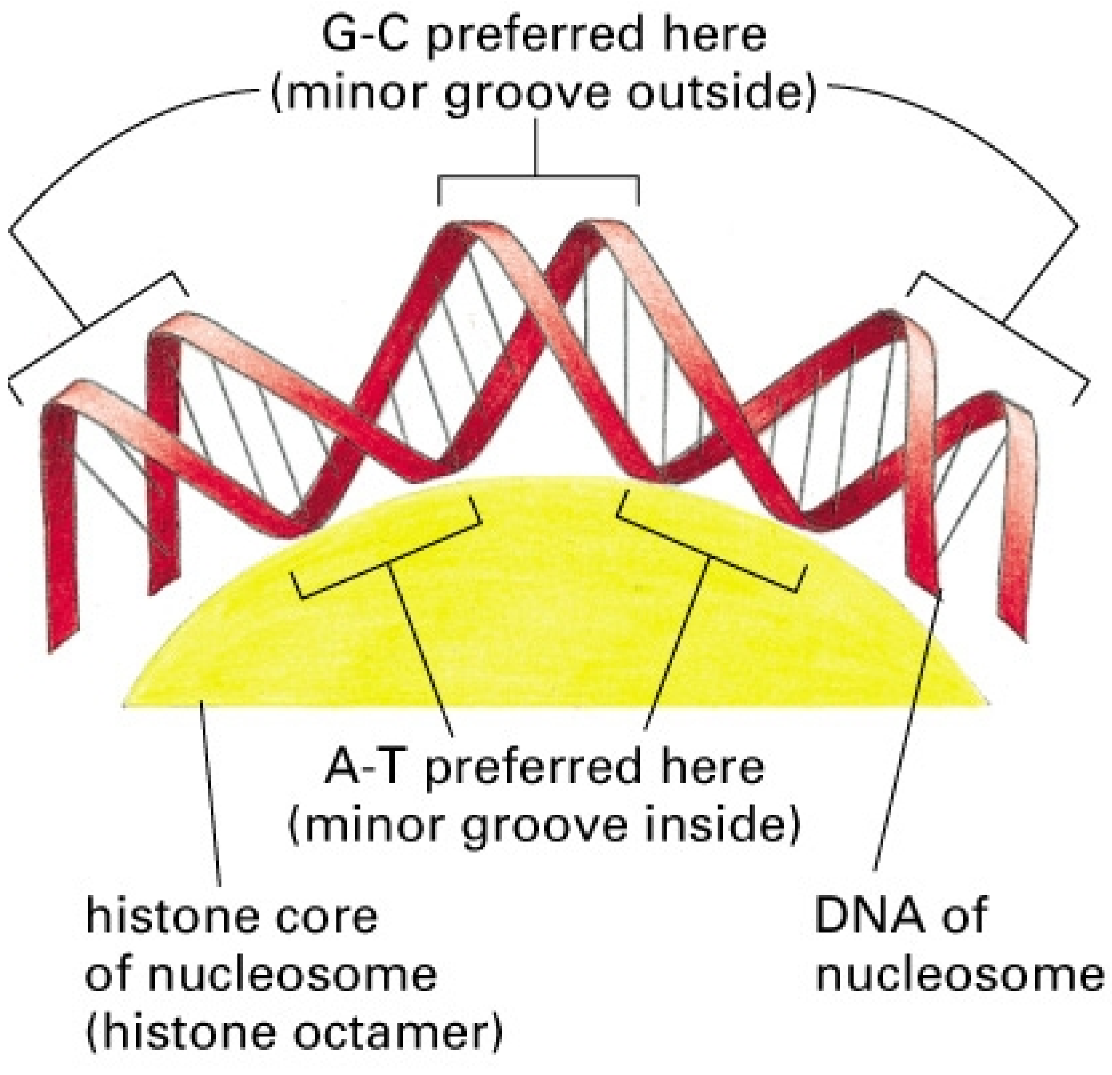
HISTONES ASSEMBLY



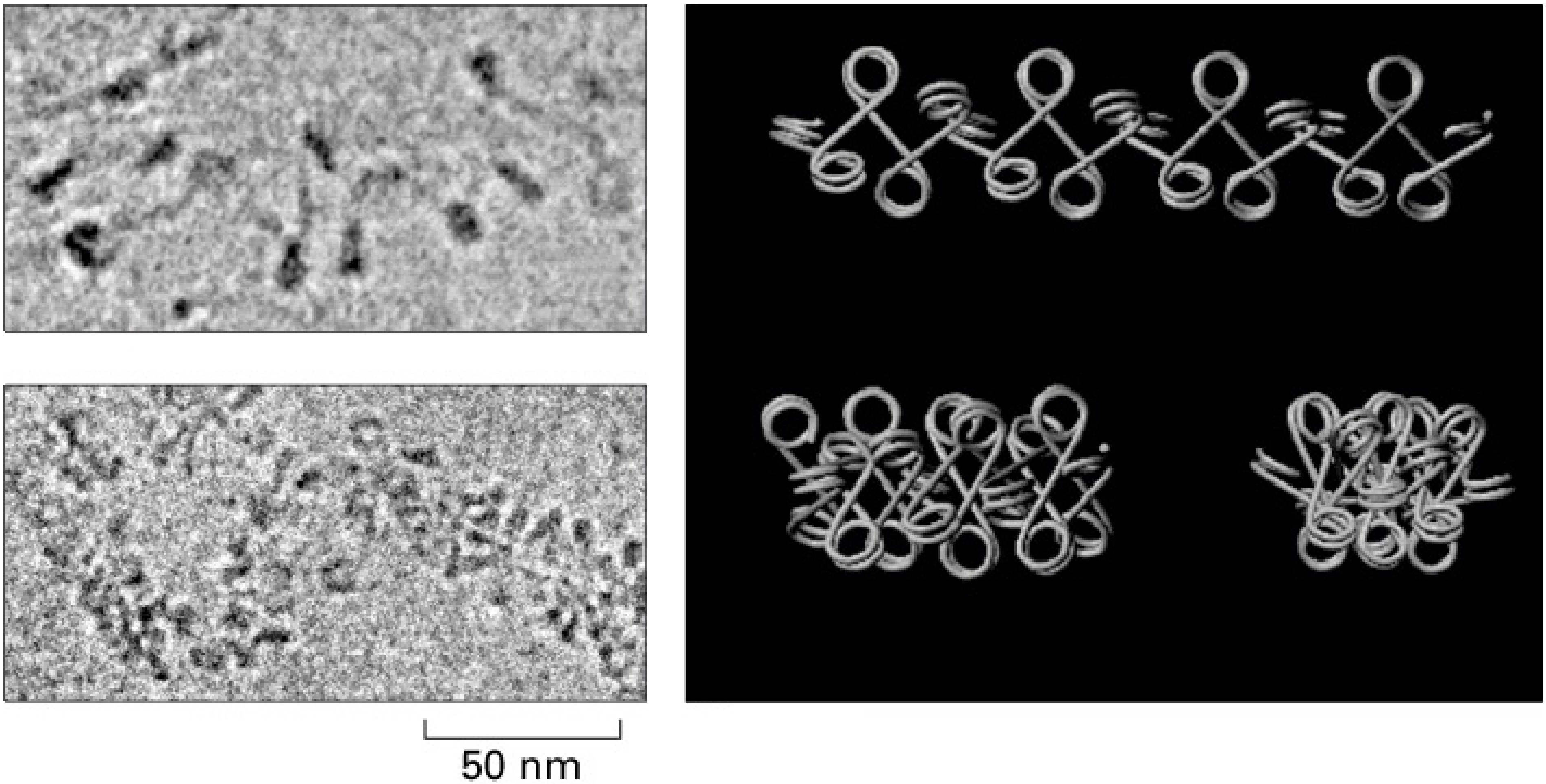
- **Histones-DNA: 142 H-bonds**
- **50% H-bonds: main chains of histone – main chain of DNA**

NUCLEOSOME POSITIONING

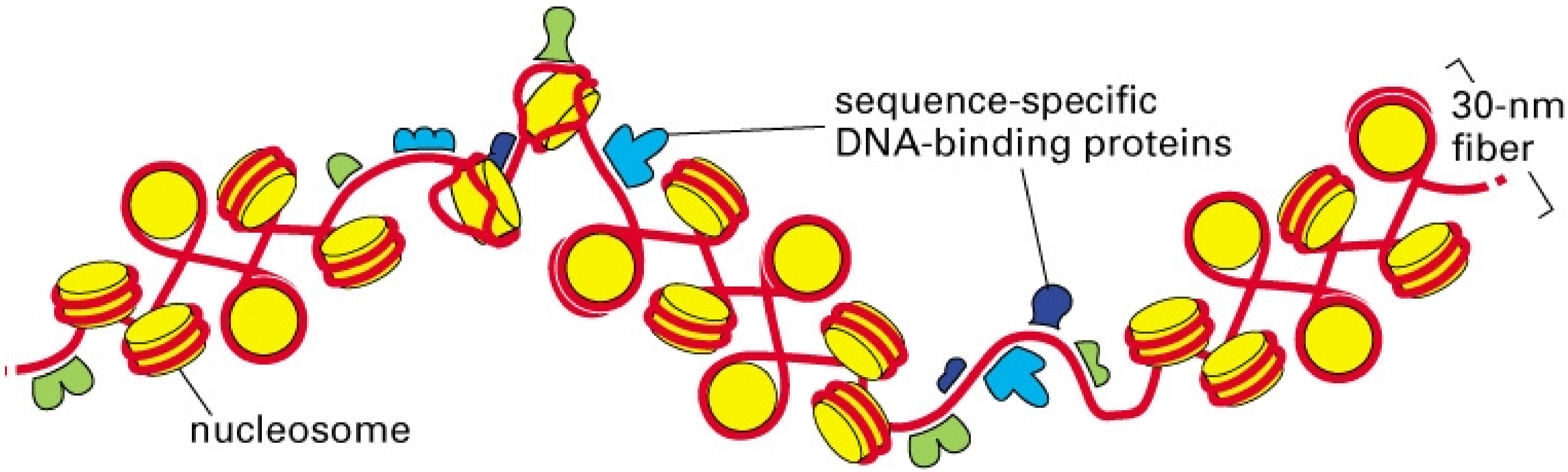
- DNA flexibility A-T vs. G-C
- Other protein participants



NUCLEOSOMES PACKING: CHROMATIN FIBER



NUCLEOSOMES PACKING: CHROMATIN FIBER

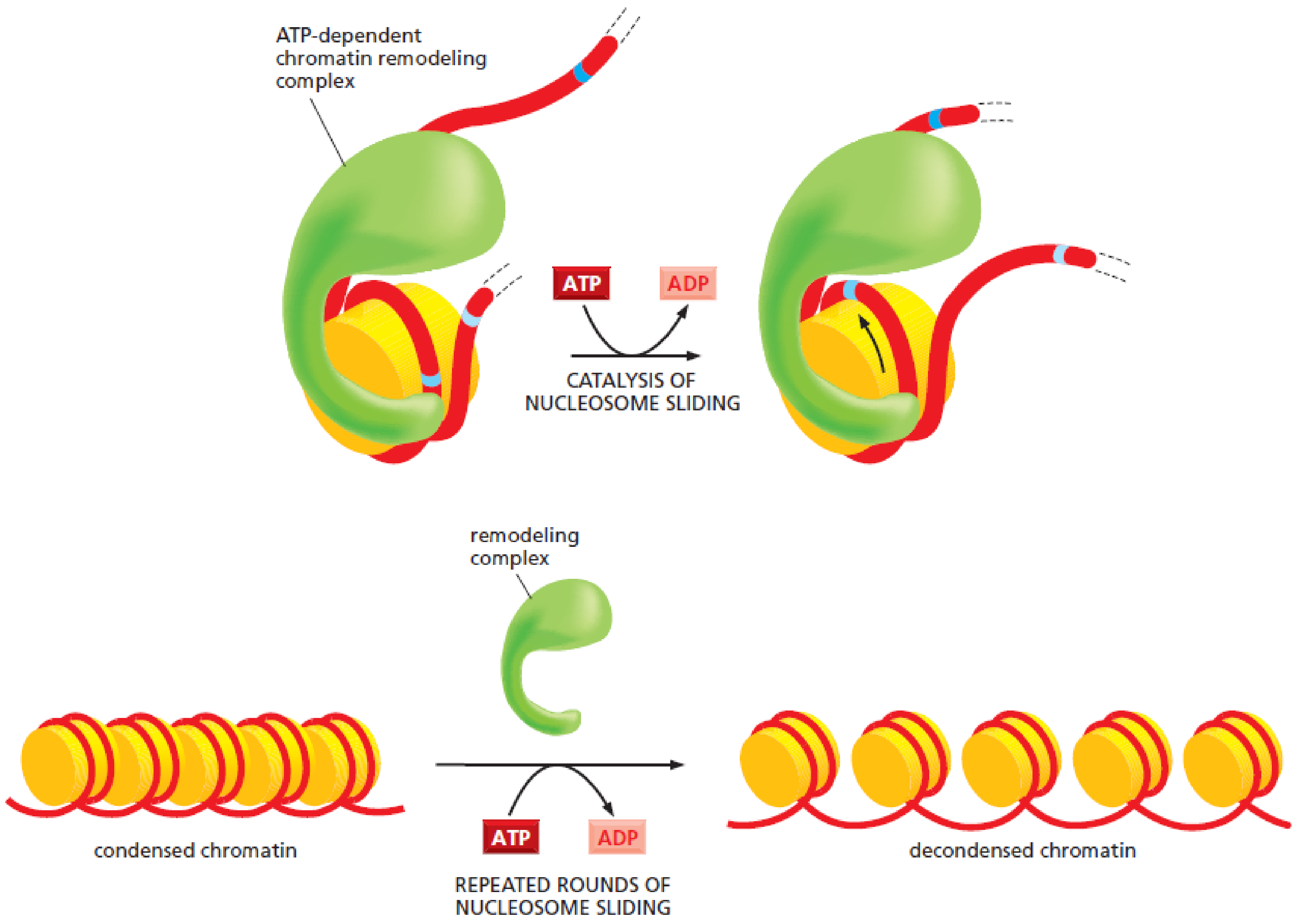


- **Nucleosomes remodelling: ATP-dependent remodelling complexes**

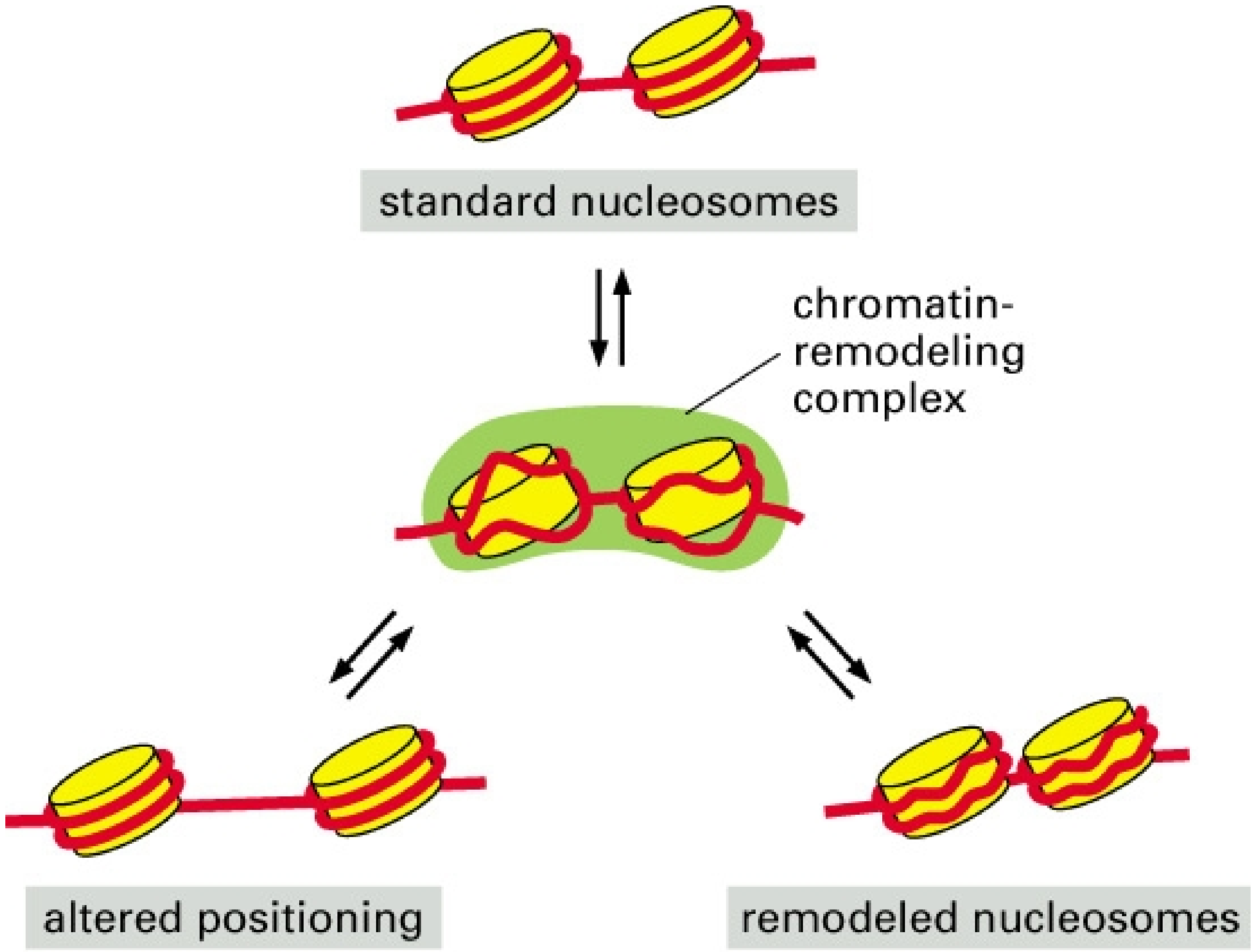
  - DNA accessibility for DNA-associated processes

  - catalysis of movement of histone octamers

NUCLEOSOMES REMODELLING COMPLEX

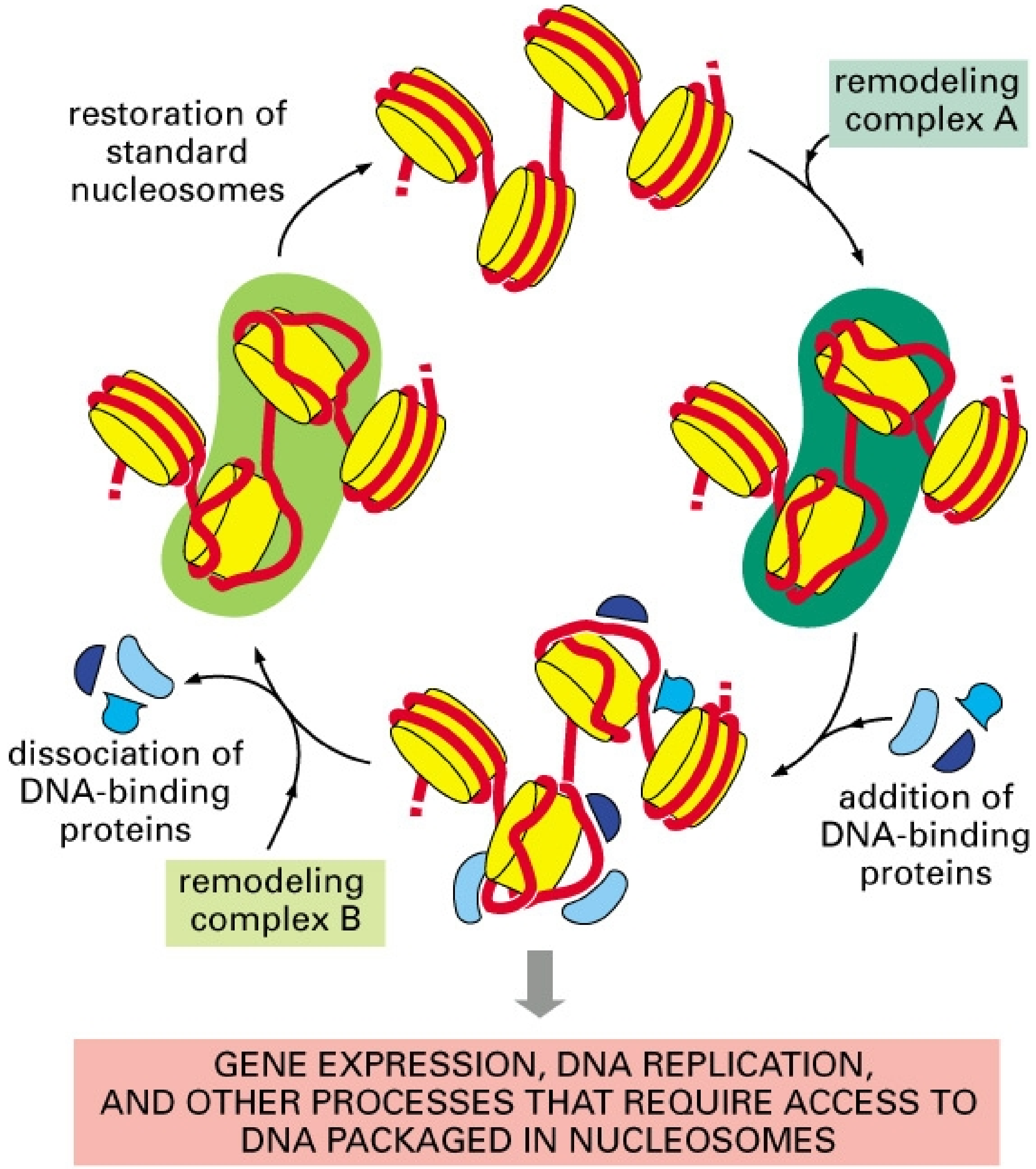


NUCLEOSOMES REMODELLING COMPLEX

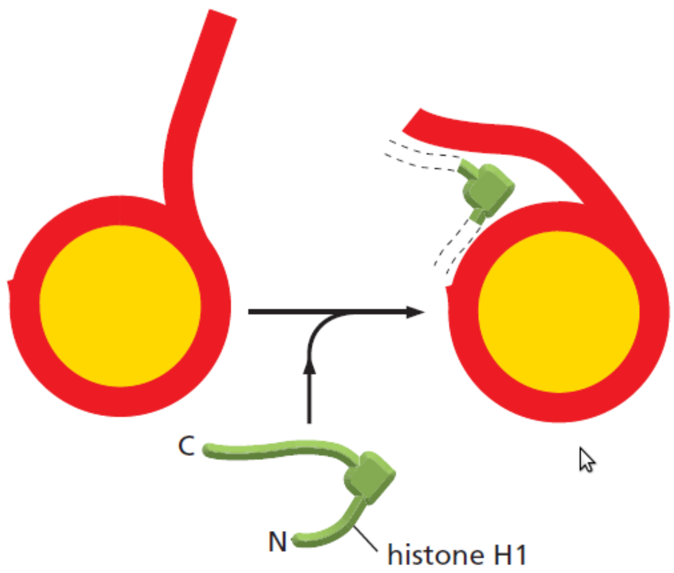


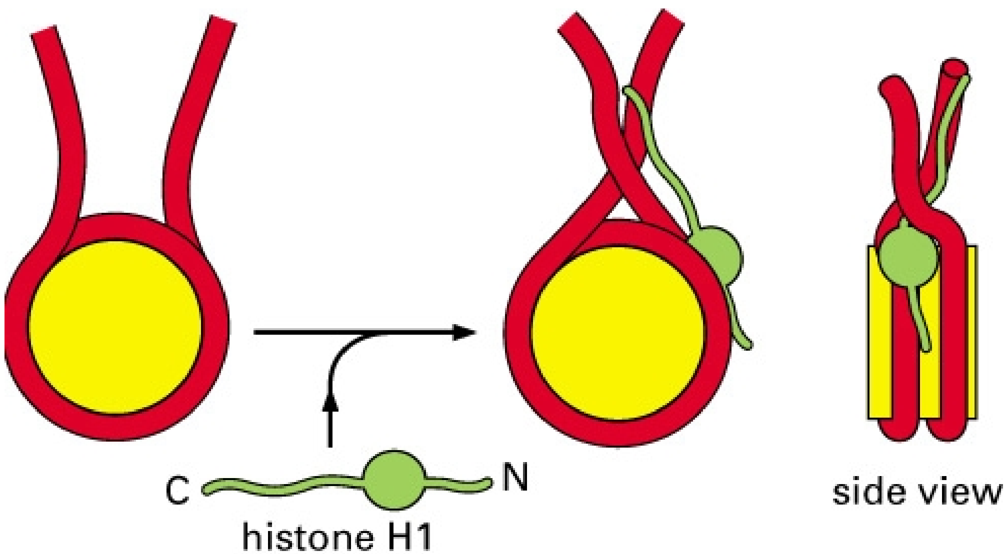
- ➢ **Rate of interconversions is increased dramatically**

NUCLEOSOMES REMODELLING COMPLEX



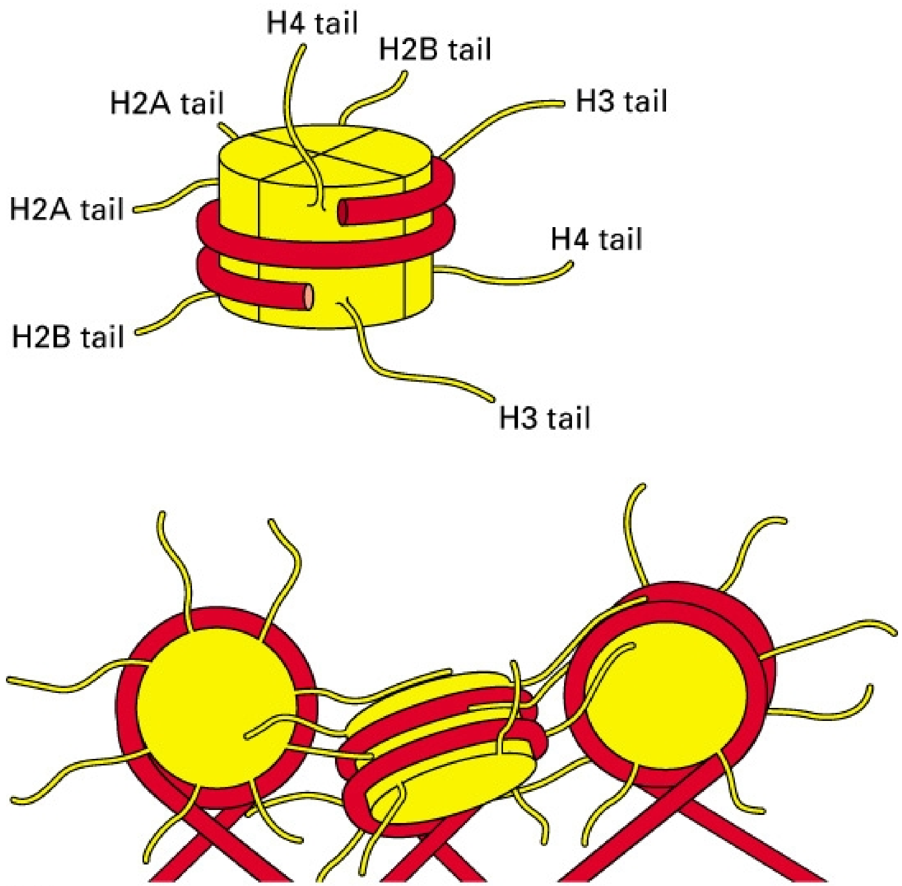
H1 PUTATIVE FUNCTION





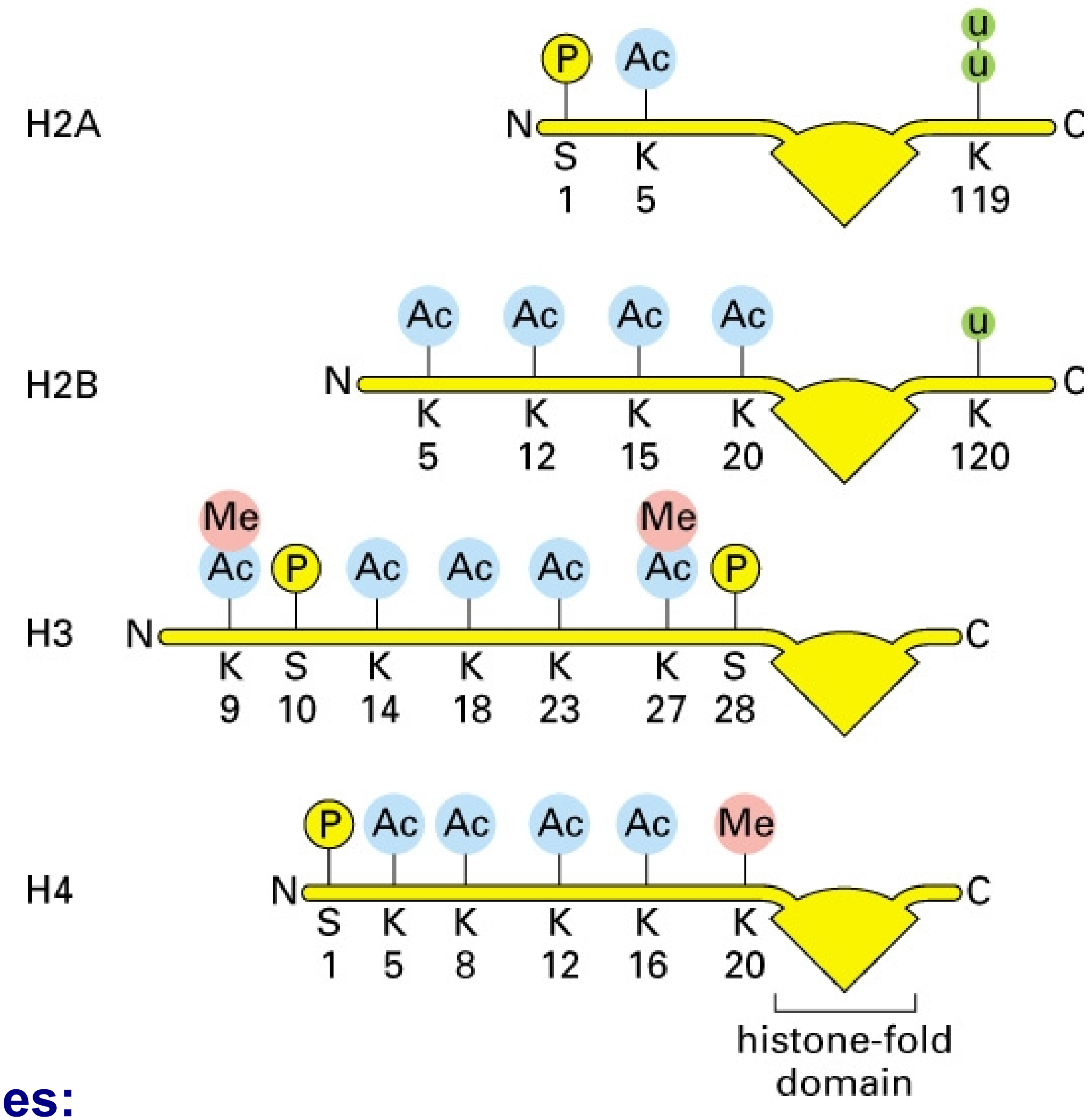
- Influence on DNA path
- H1 is not vital though important for compactization

THE ROLE OF HISTONE TAILS



- Highly conservative sequence
- Regulation of chromatin compactness
- Covalent modifications play crucial role (epigenetics)
- Example: histone acyltransferases (HATs), deacetylases (HDACs)

HISTONE TAILS MODIFICATIONS

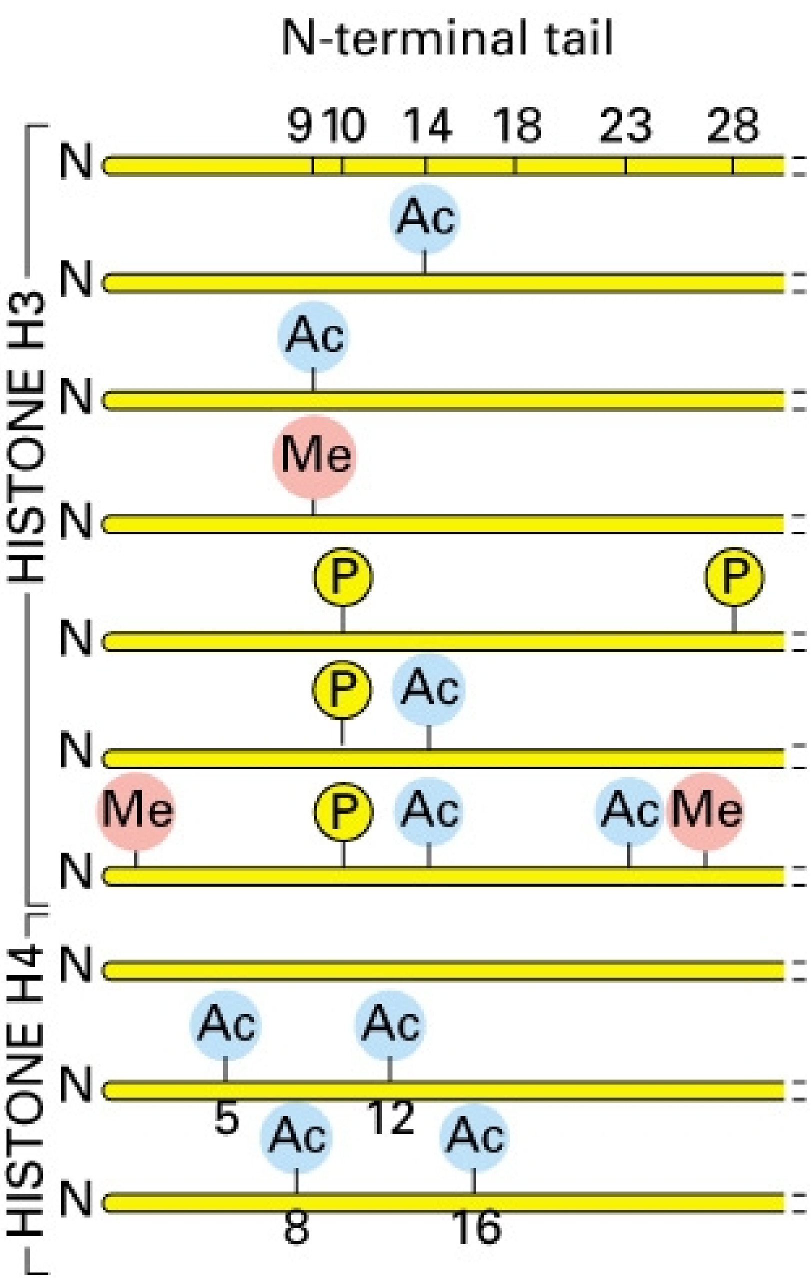


- **Effects bases:**

  - simple physical nature

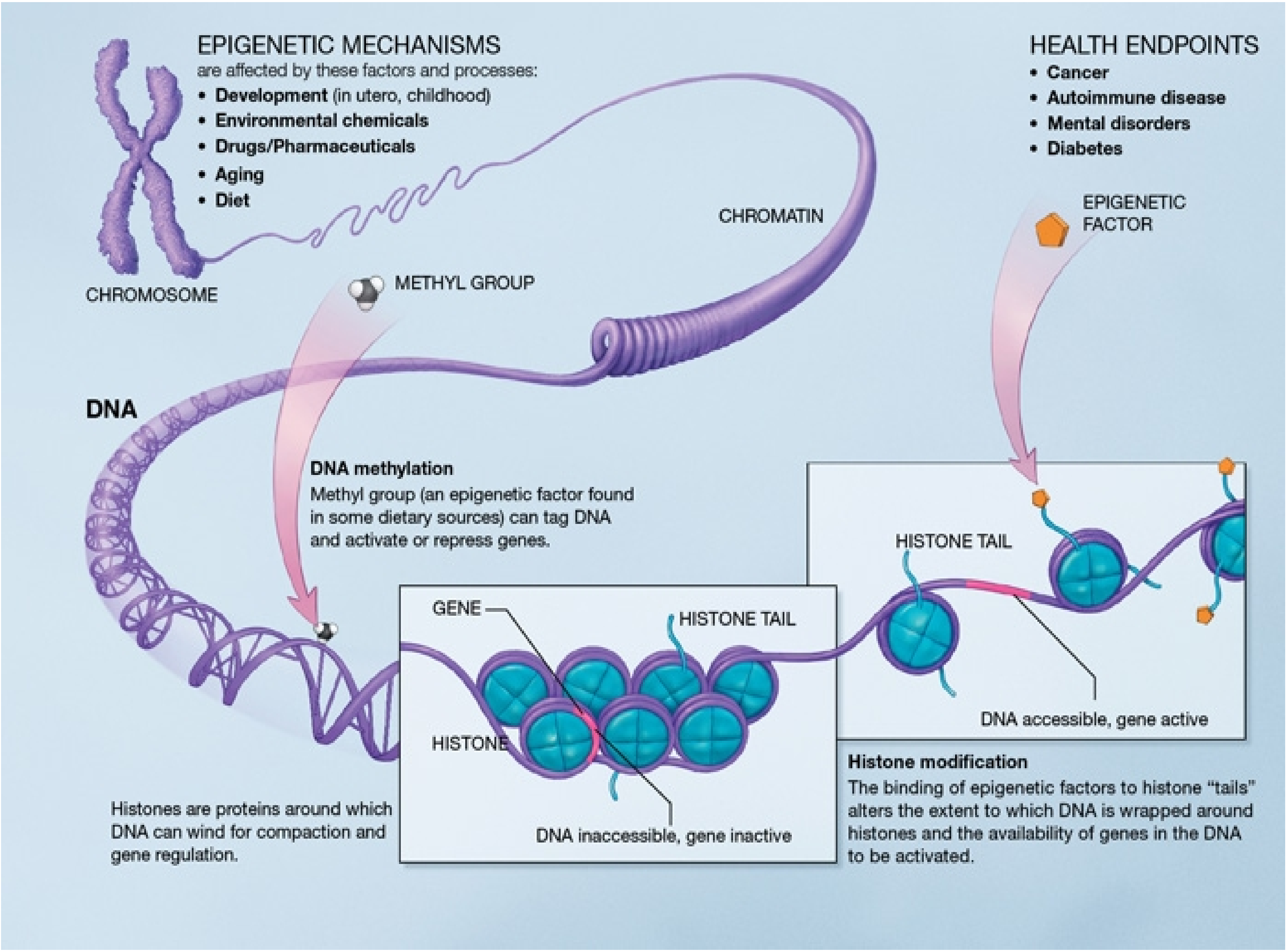
  - “specificity”

HISTONE TAILS MODIFICATIONS

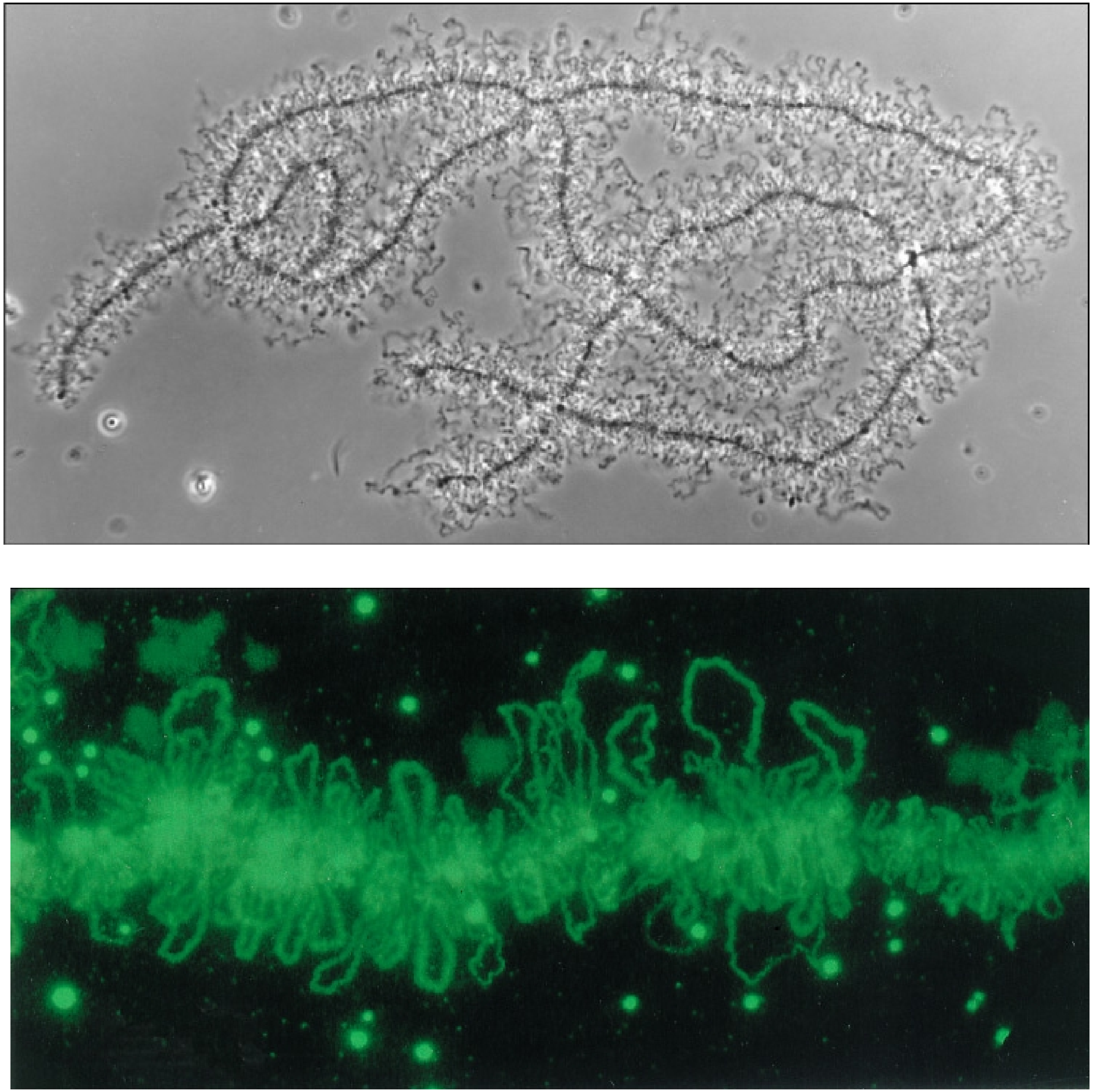


modification state	"meaning"
unmodified	gene silencing?
acetylated	gene expression
acetylated	histone deposition
methylated	gene silencing/ heterochromatin
phosphorylated	mitosis/meiosis
phosphorylated/ acetylated	gene expression
higher-order combinations	?
unmodified	gene silencing?
acetylated	histone deposition
acetylated	gene expression

EPIGENETICS MECHANISMS



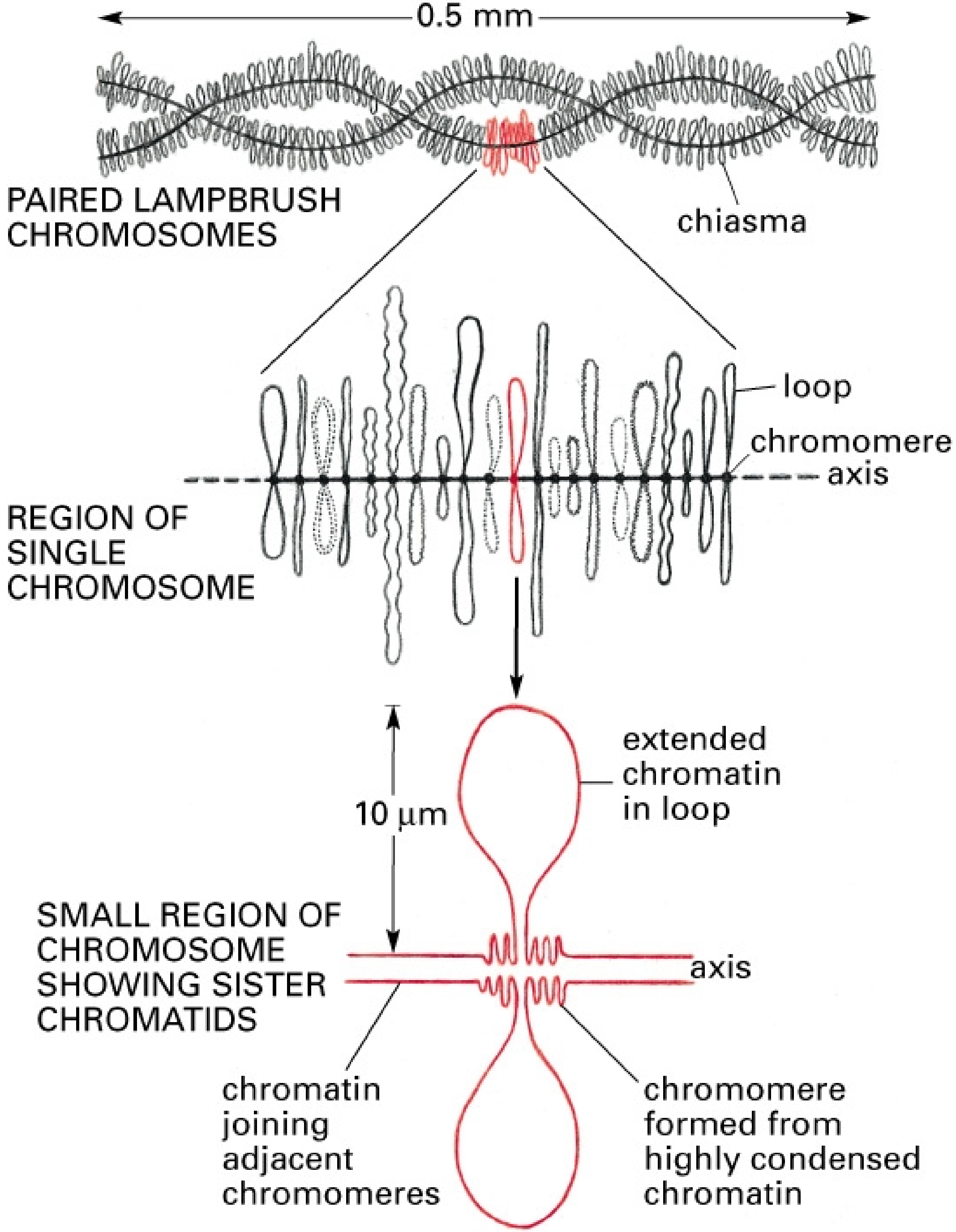
GLOBAL STRUCTURES OF CHROMOSOMES



Lambrush chromosome: (amphibian oocyte, immature eggs), interphase

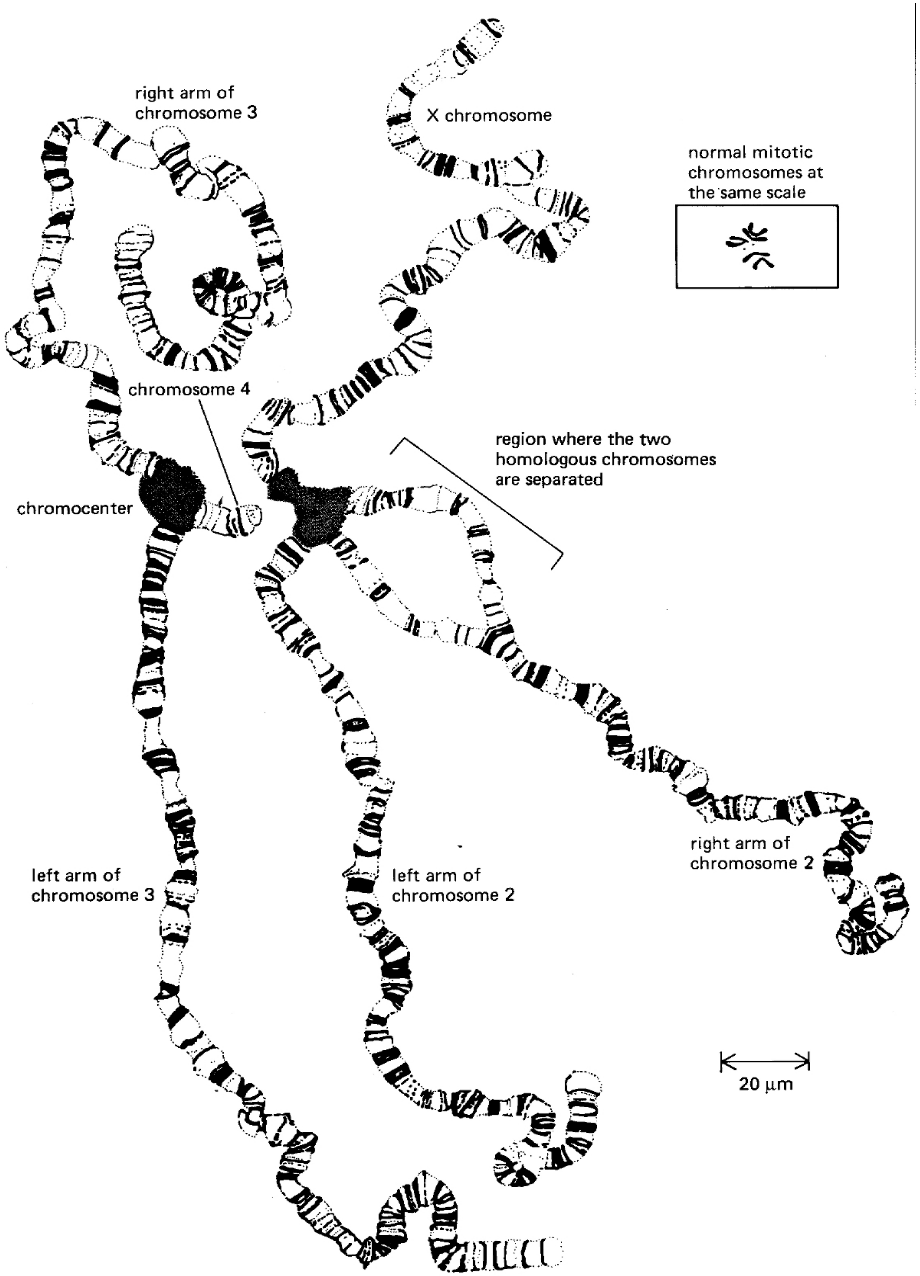
MODEL OF LAMBRUSH CHROMOSOME

- **DNA:**
  - **- highly condensed**
  - **- loops**



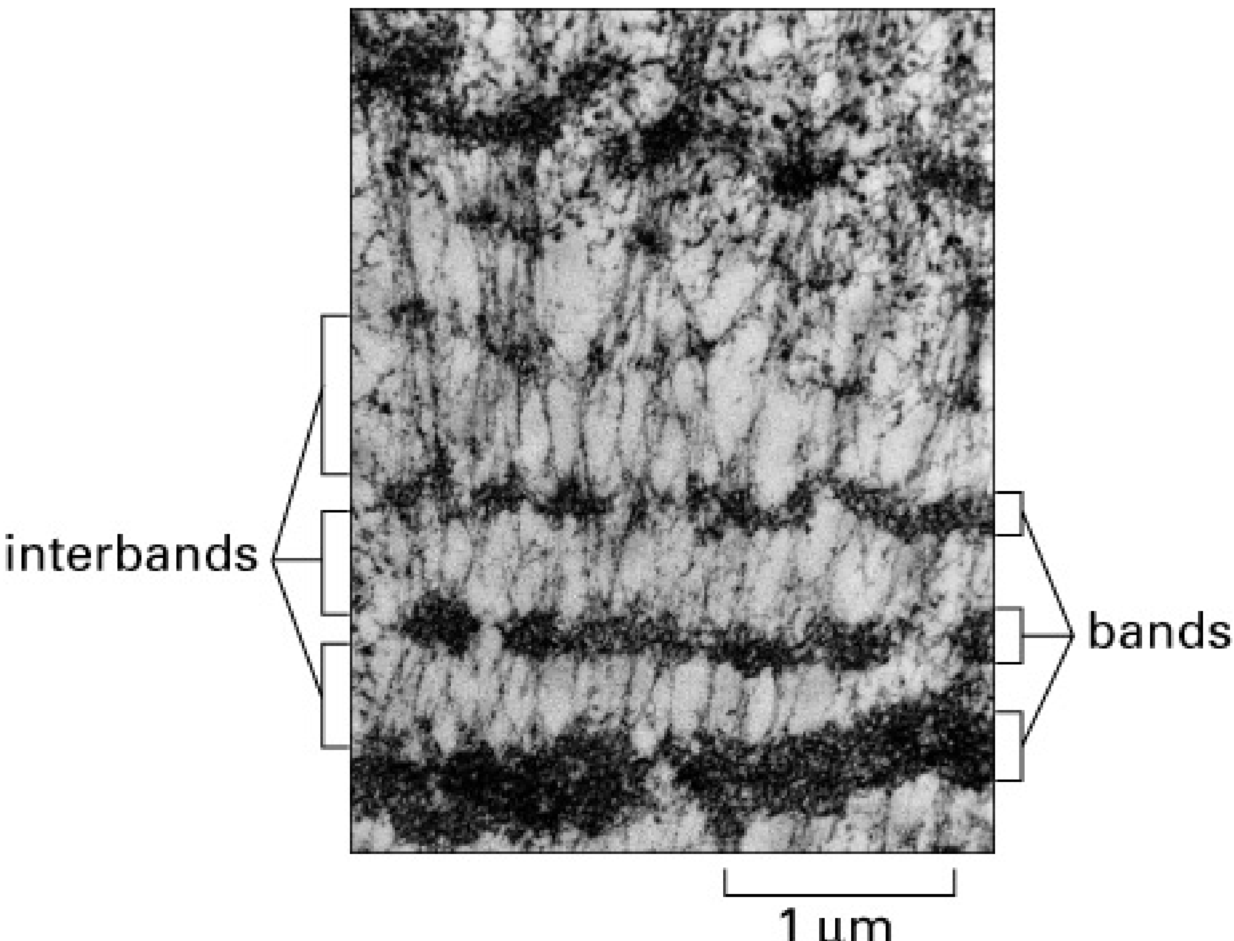
DROSOPHILA POLYTENE CHROMOSOME

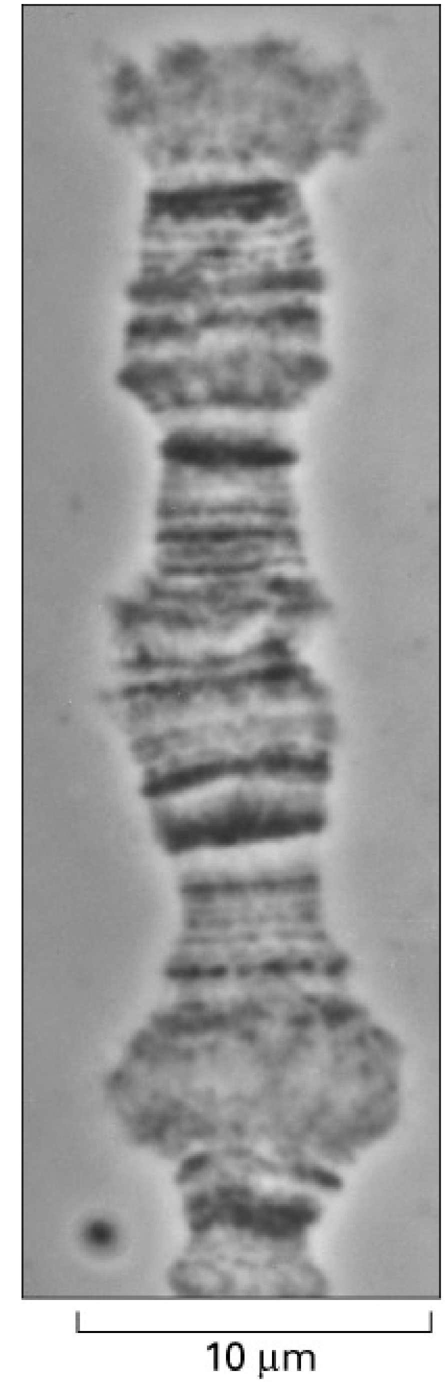
- **Polyploid cells: increased number of standard chromosomes**
- **Polytene chromosome: homologous chromosome copies**
- **Many replication events without cells separation**



DROSOPHILA POLYTENE CHROMOSOME

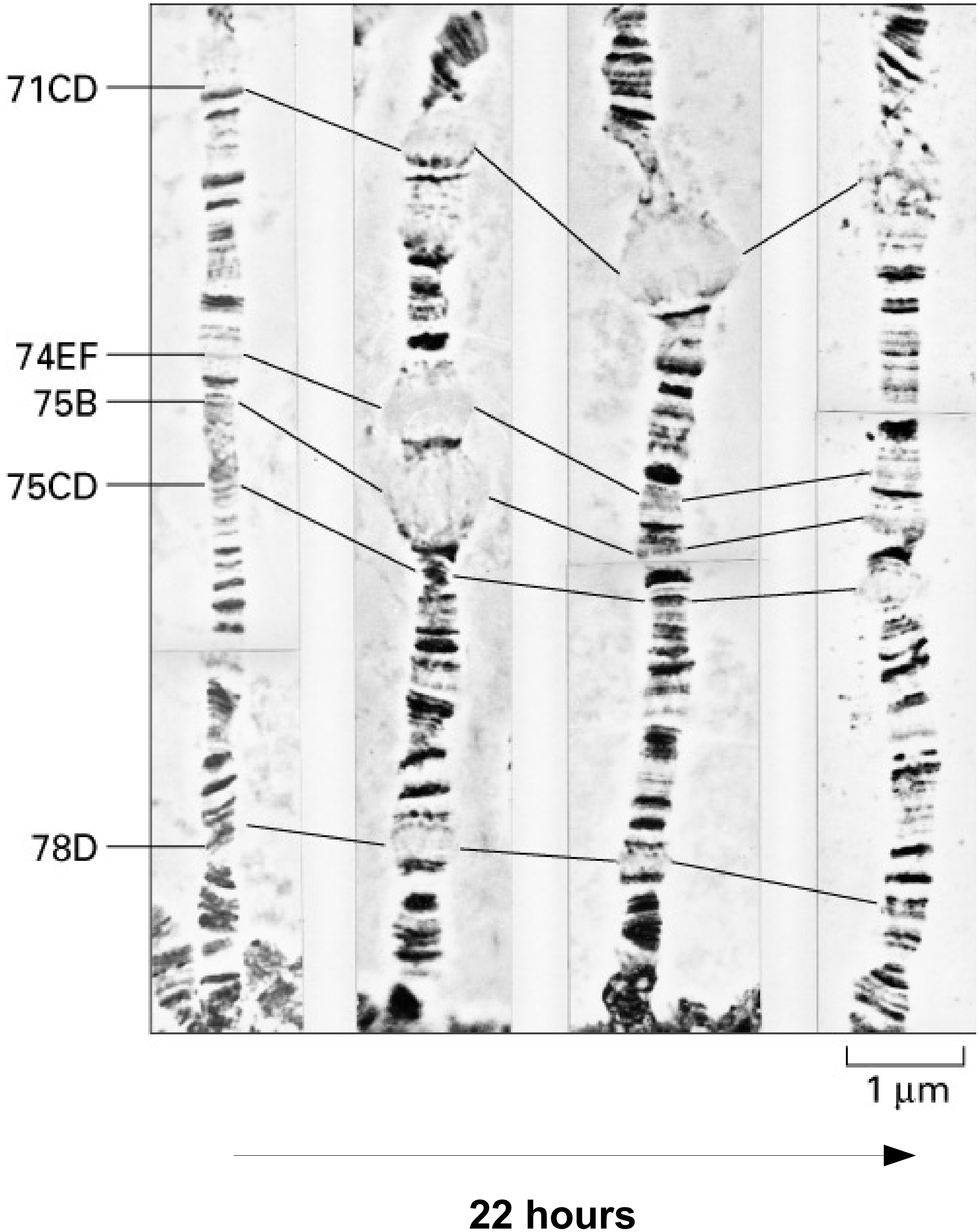
- **95% DNA: bands (condensed)**
- **5% DNA: interbands**
- **Band 10^3-10^5 pb**
- **~5000 bands/interbands**



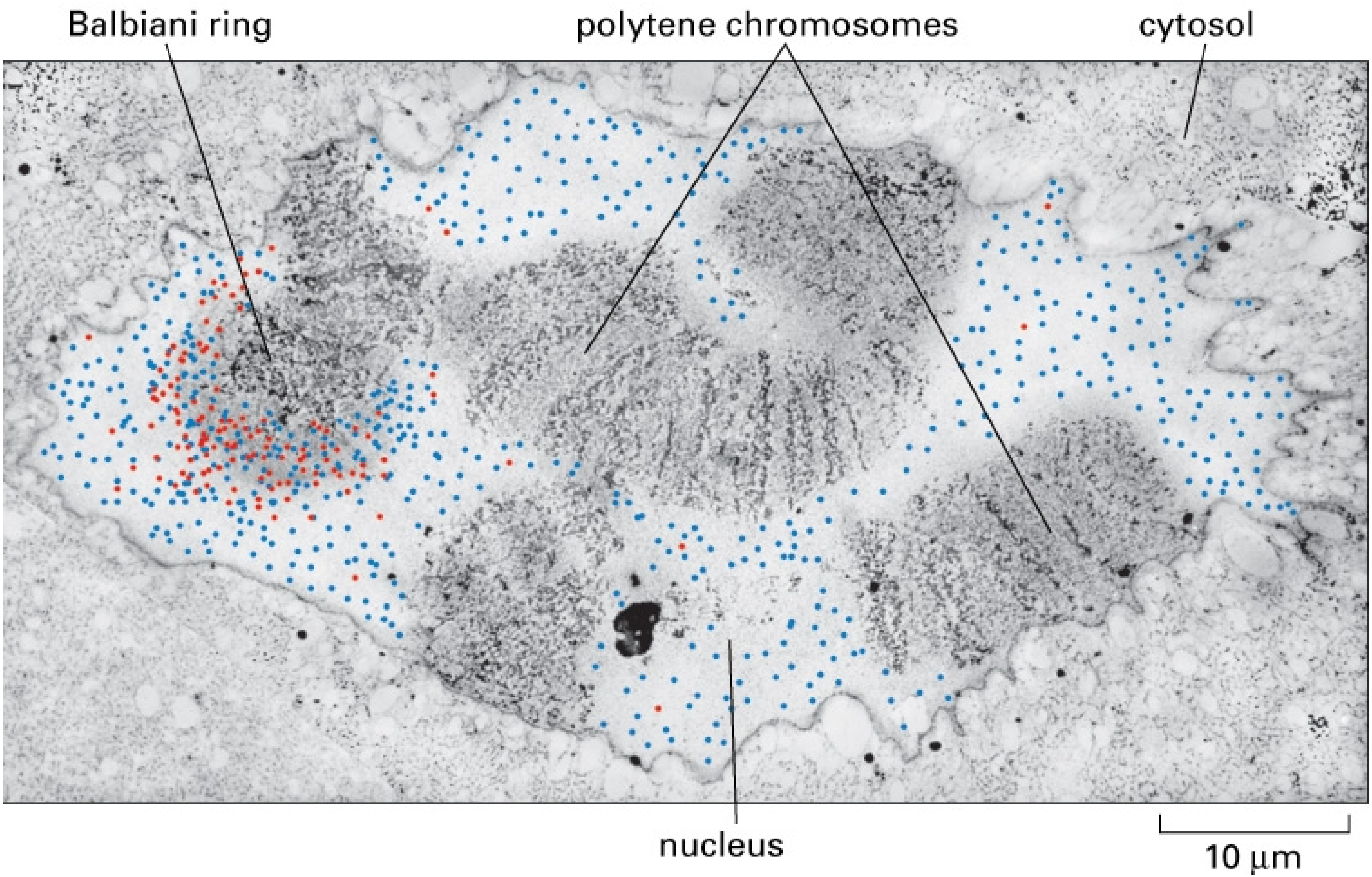


CHROMOSOME PUFFS

- Ecdysone => change of expression => change of puffs (condensated regions)

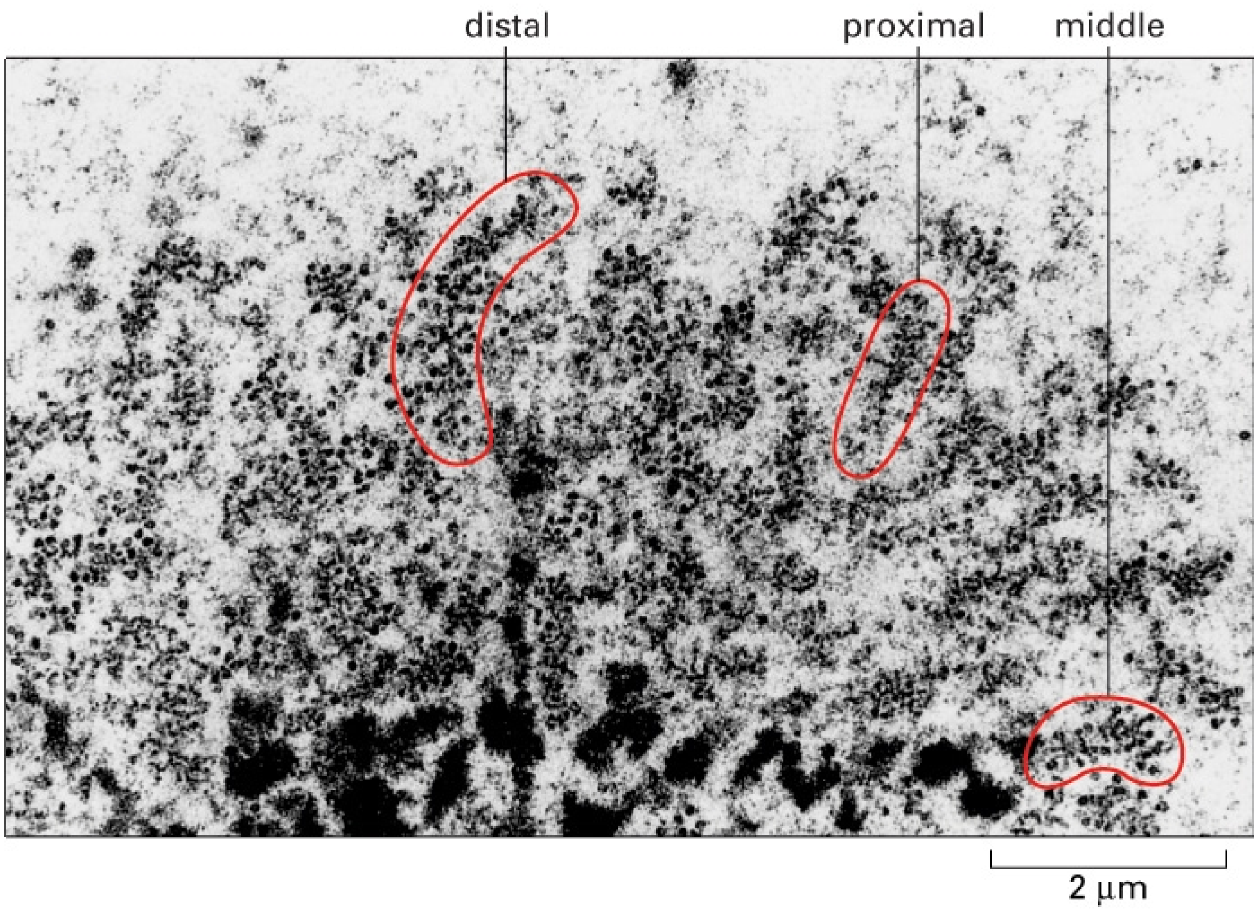


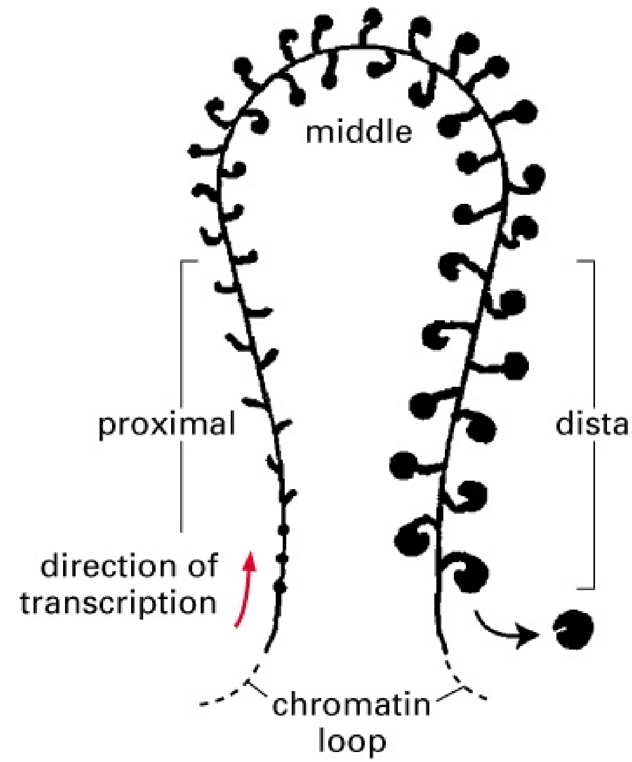
CHROMOSOME PUFFS: RNA SYNTHESIS

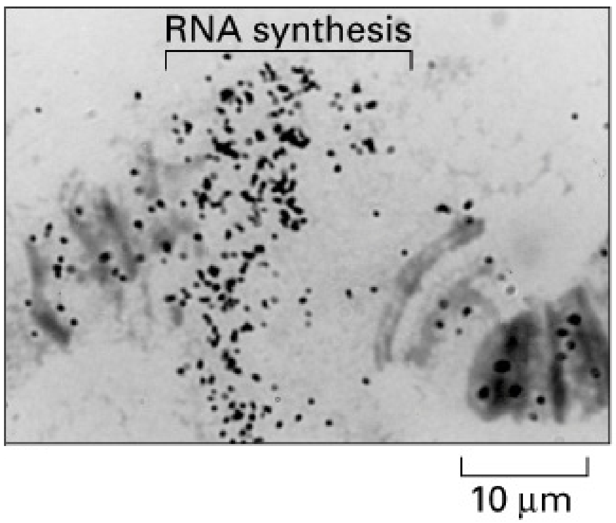


Localization of RNA after BrU addition and RNA before BrU addition

CHROMOSOME PUFFS: RNA SYNTHESIS



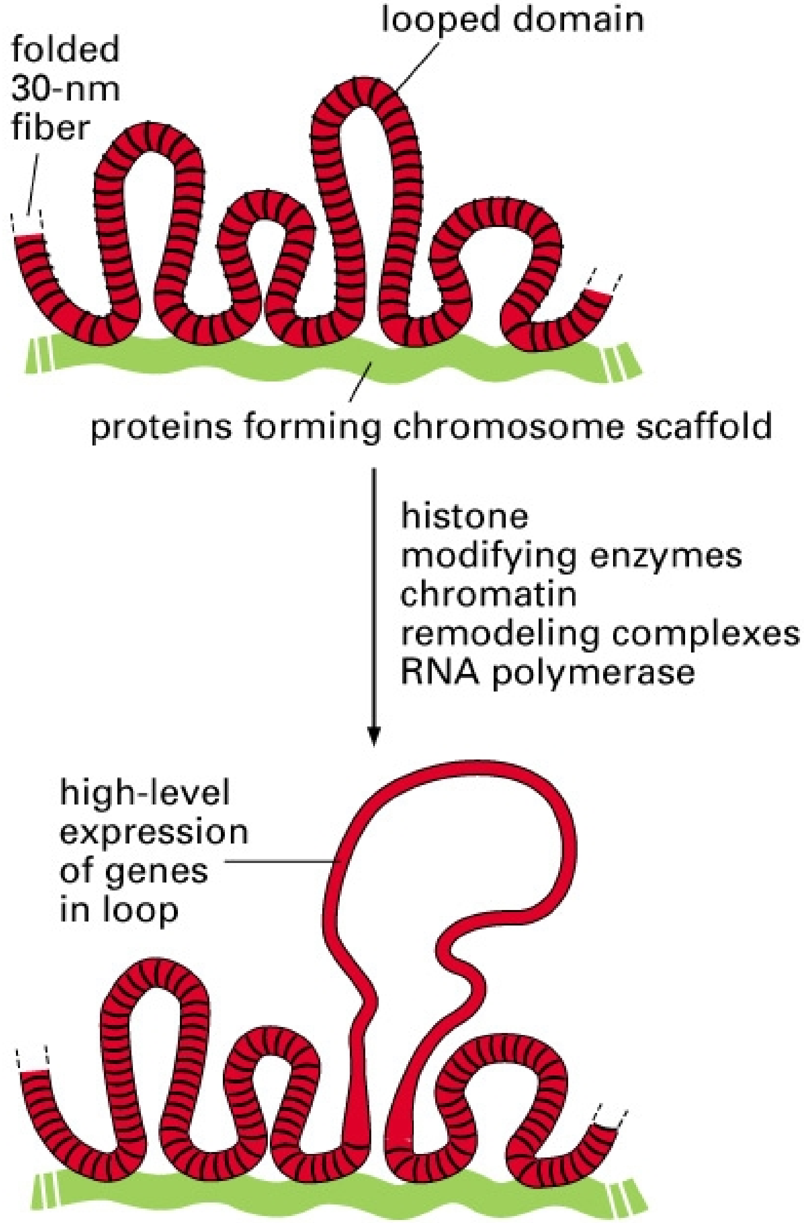




Expression is observed in loops

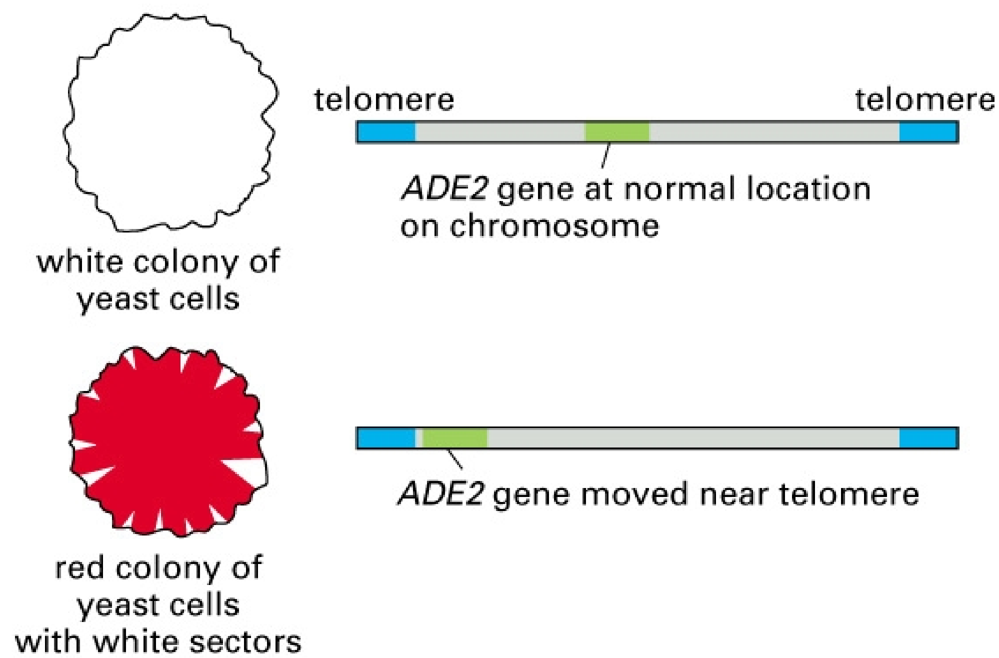
HETERO- AND EUCHROMATIN IN INTERPHASE CHROMOSOME

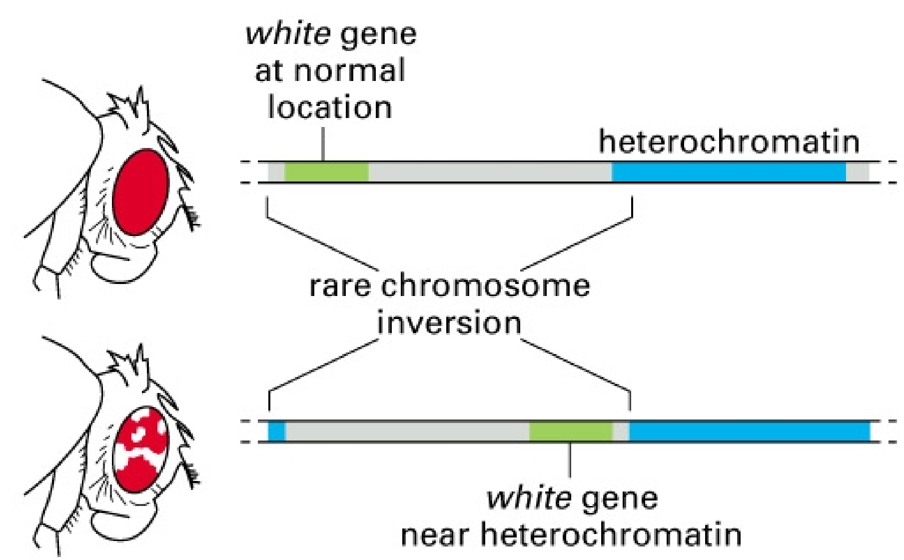
- **Heterochromatin:**
  - highly condensed
  - ~10% of genome
  - most DNA does not contain genes
- **Euchromatin: less condensed**
- **Loop (expressed) ~ 10^4-10^5 bp**



HETERO- AND EUCHROMATIN IN INTERPHASE CHROMOSOME

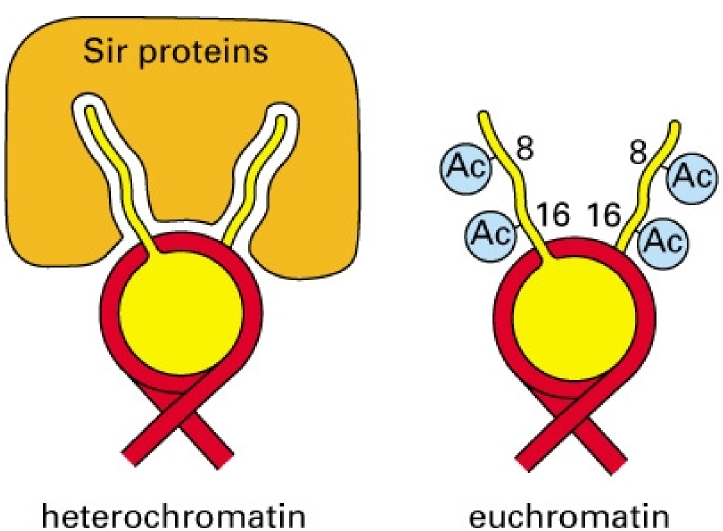
- **Position effects:**
  - **- silencing**
  - **- variegation**
- **Eu/hetero is inherited**

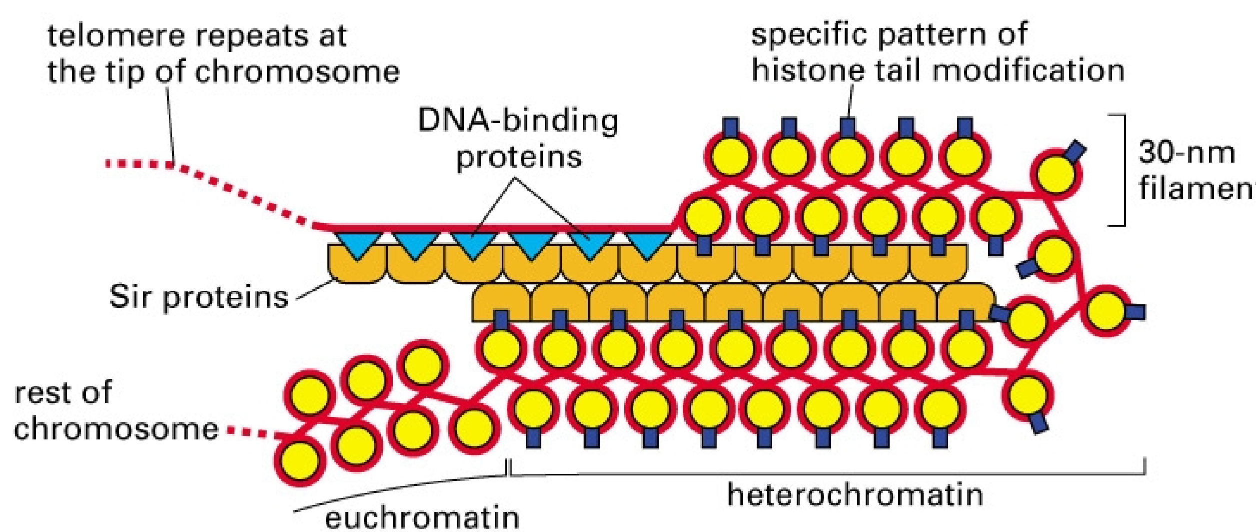




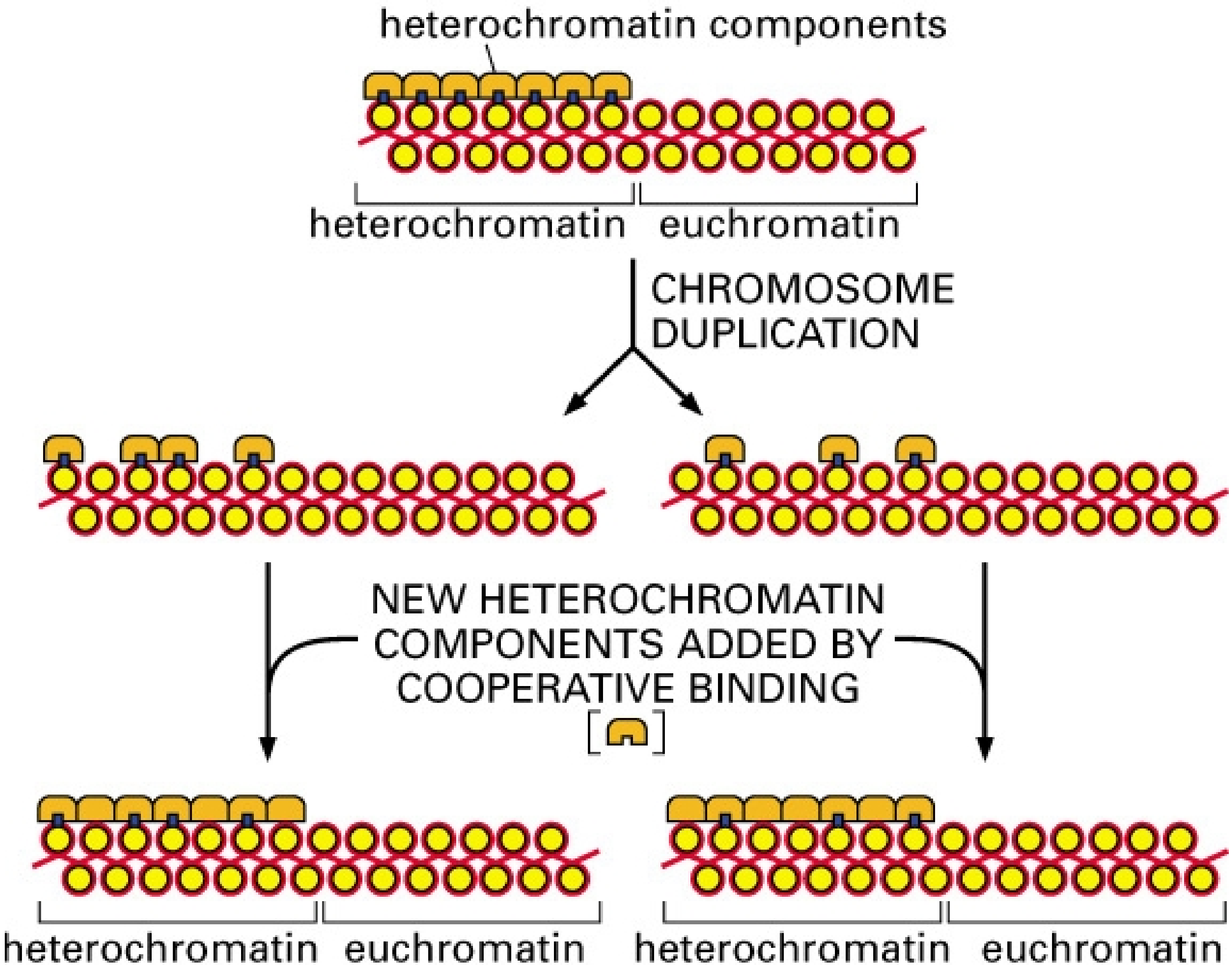
HETEROCHROMATIN AT CHROMOSOME ENDS

- ~ 5000 bp at the ends are resistant to gene expression
- Sir (Silent information regulator):
  - Sir2: NAD+-dependent histone deacetylase <= regulation
- Spreading effect
- Functions of heterochromatin at ends:
  - protection
  - regulation of telomer length
  - pairing in mitosis

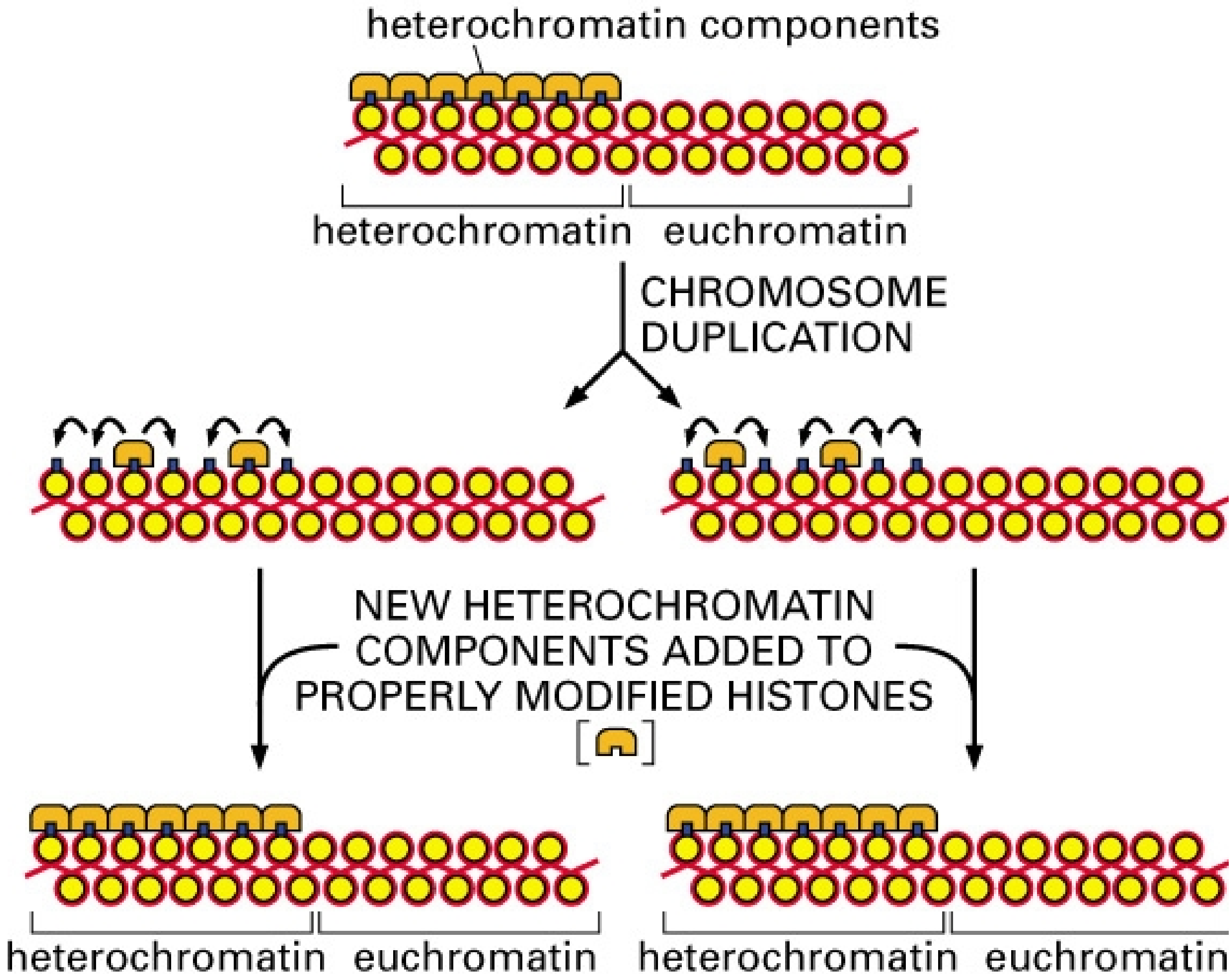




HETEROCHROMATIN INHERITANCE: MODEL 1



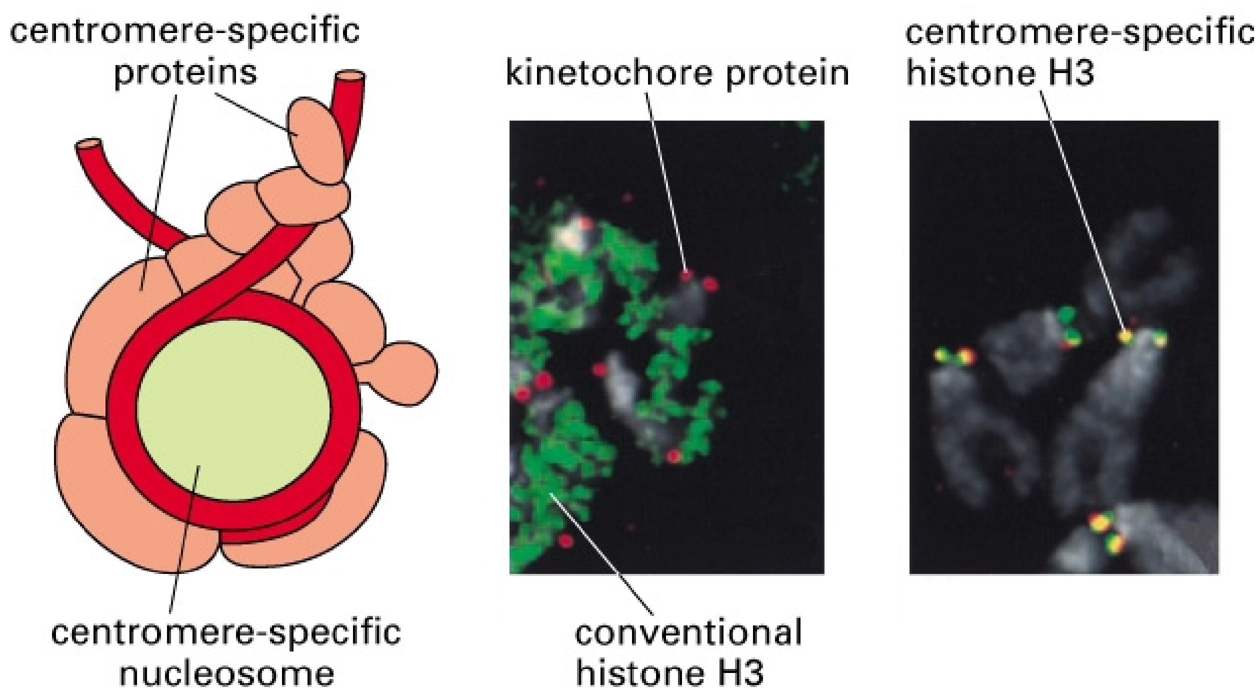
HETEROCHROMATIN INHERITANCE: MODEL 2



HETEROCHROMATIN AT CENTROMERS

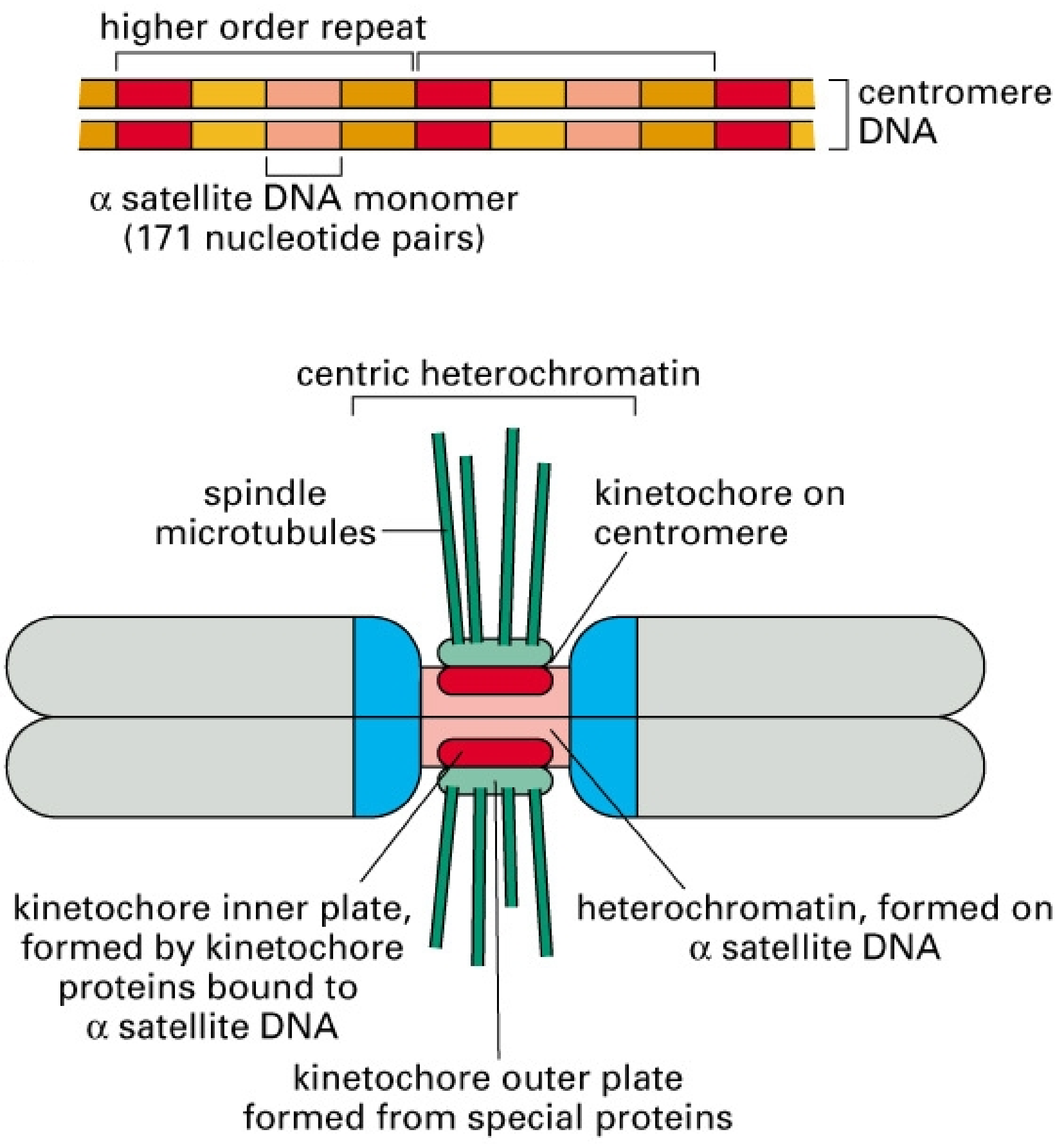
- Special case of heterochromotin: additional proteins
- Centromere:

  - 125 bp is an essential length in yeast
- Specific H3-variant
- Cooperative addition of proteins

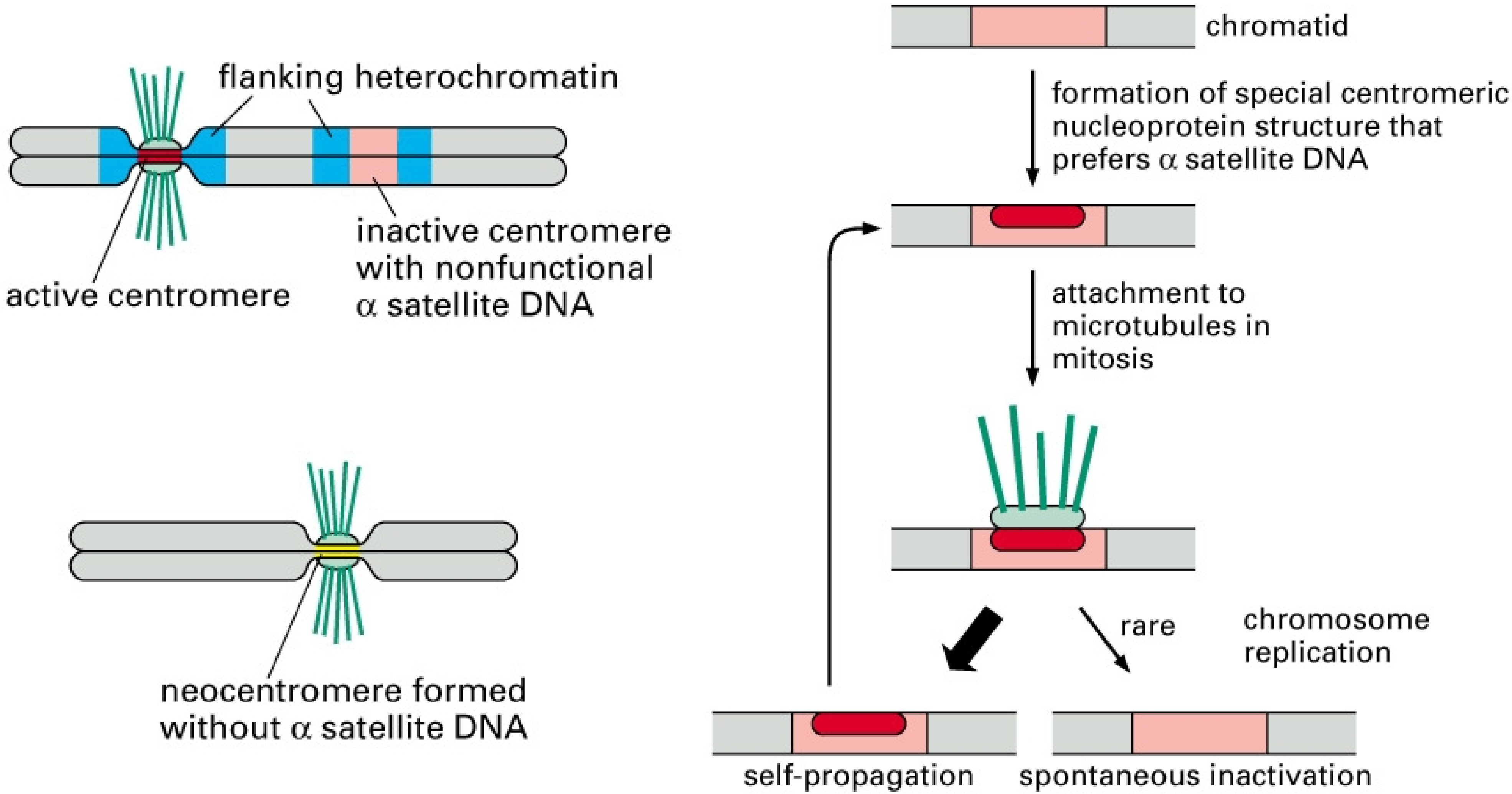


HETEROCHROMATIN AT CENTROMERES: HUMAN

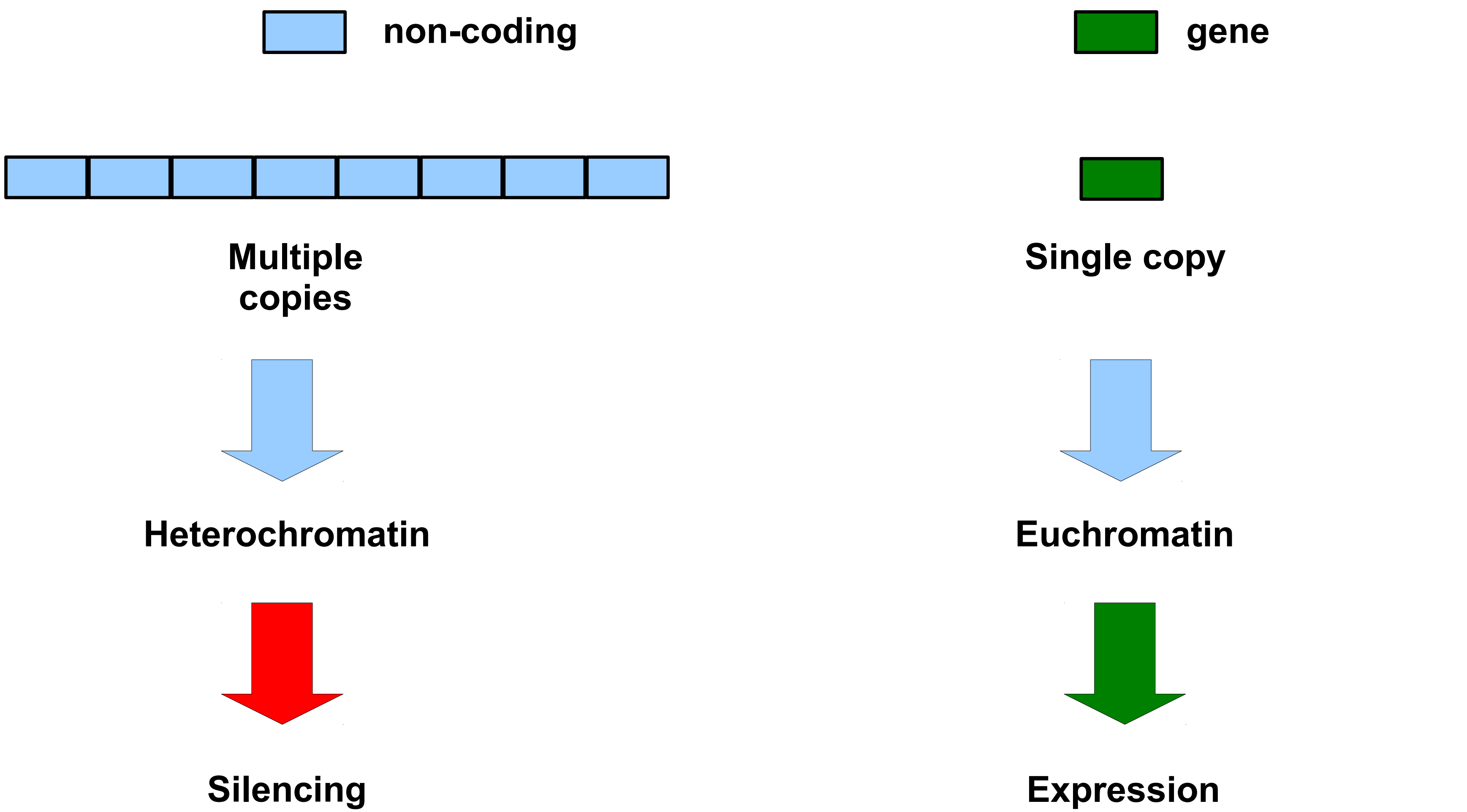
- **Centromere:**
  - **10^5 bp in fly and human**
  - **alpha sattelite DNA**
  - ***de novo* formation**



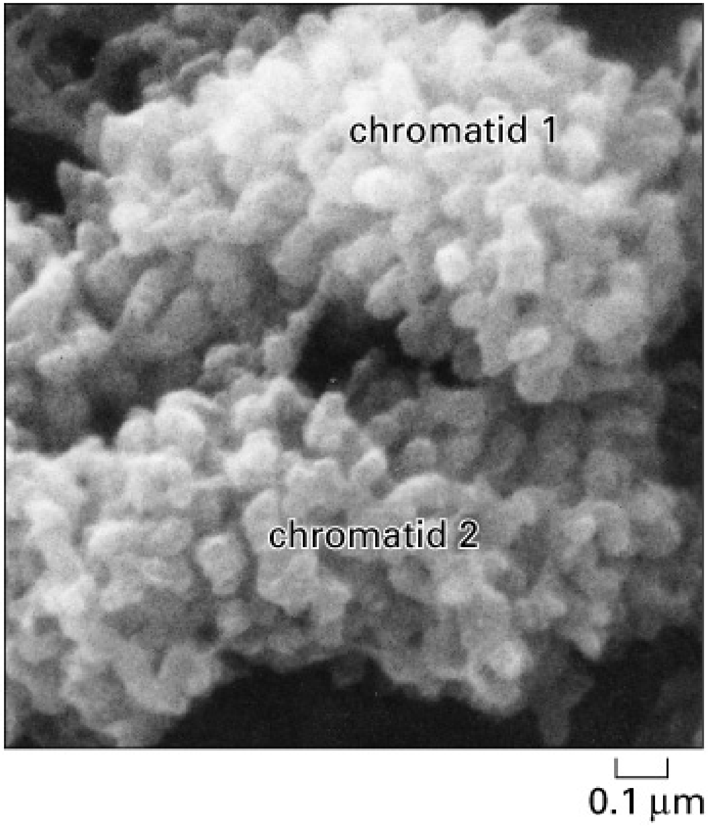
PLASTICITY OF HUMAN CENTROMERES FORMATION

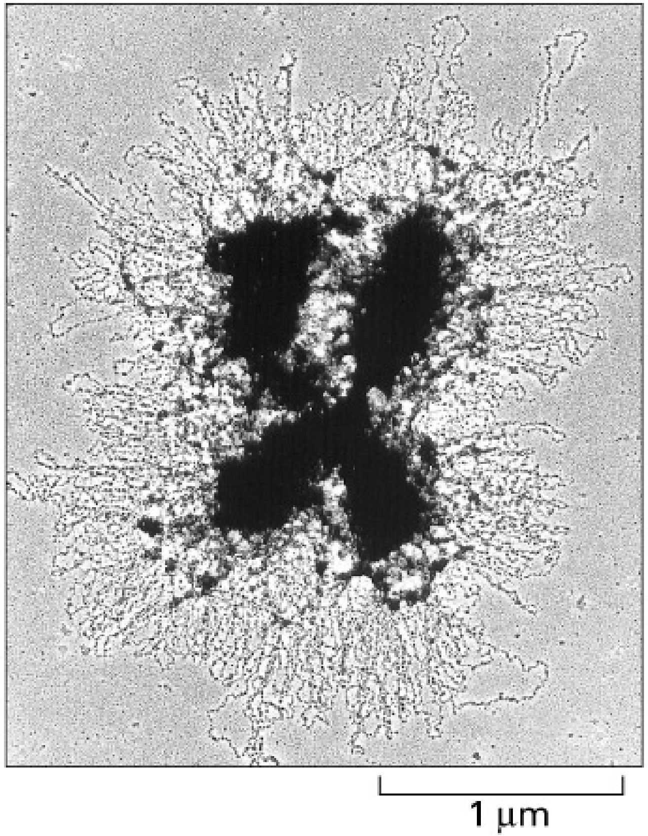


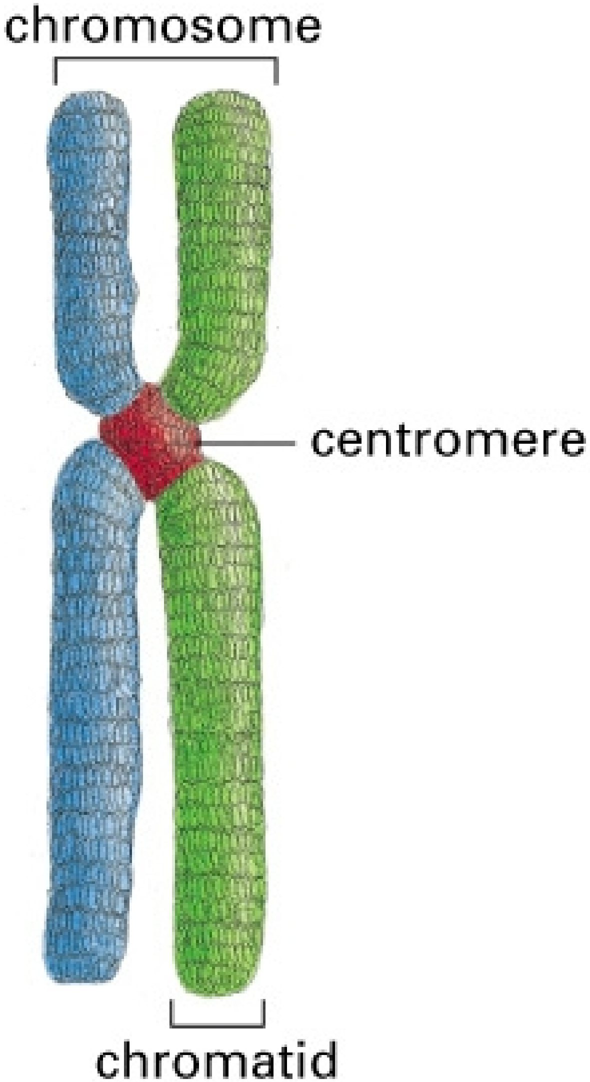
REPEATS AND HETEROCHROMATIN



MITOTIC CHROMOSOMES: MOST CONDENSE





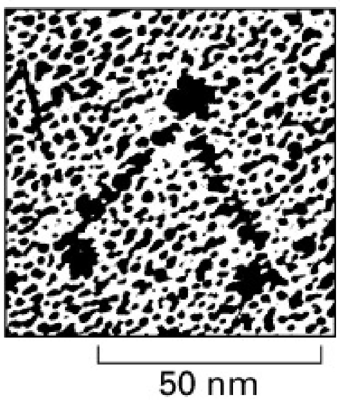


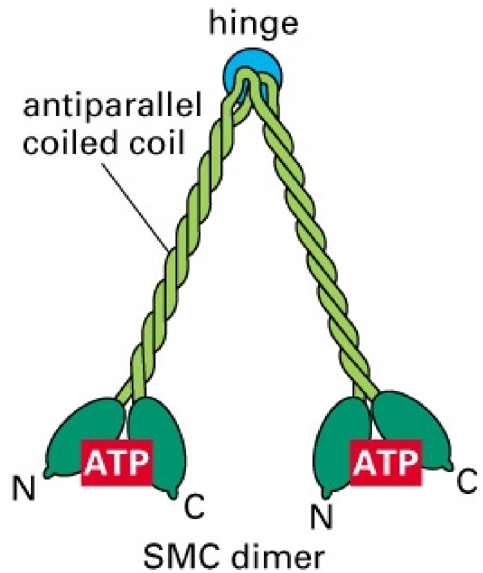
- **Deprived of RNA-protein complexes**
- **Function:**

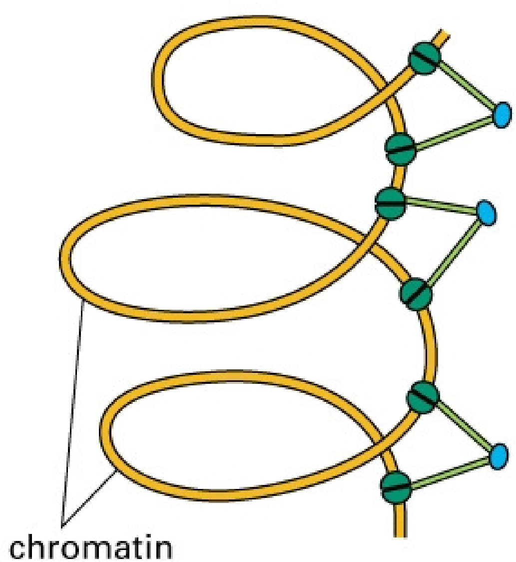
  - protection

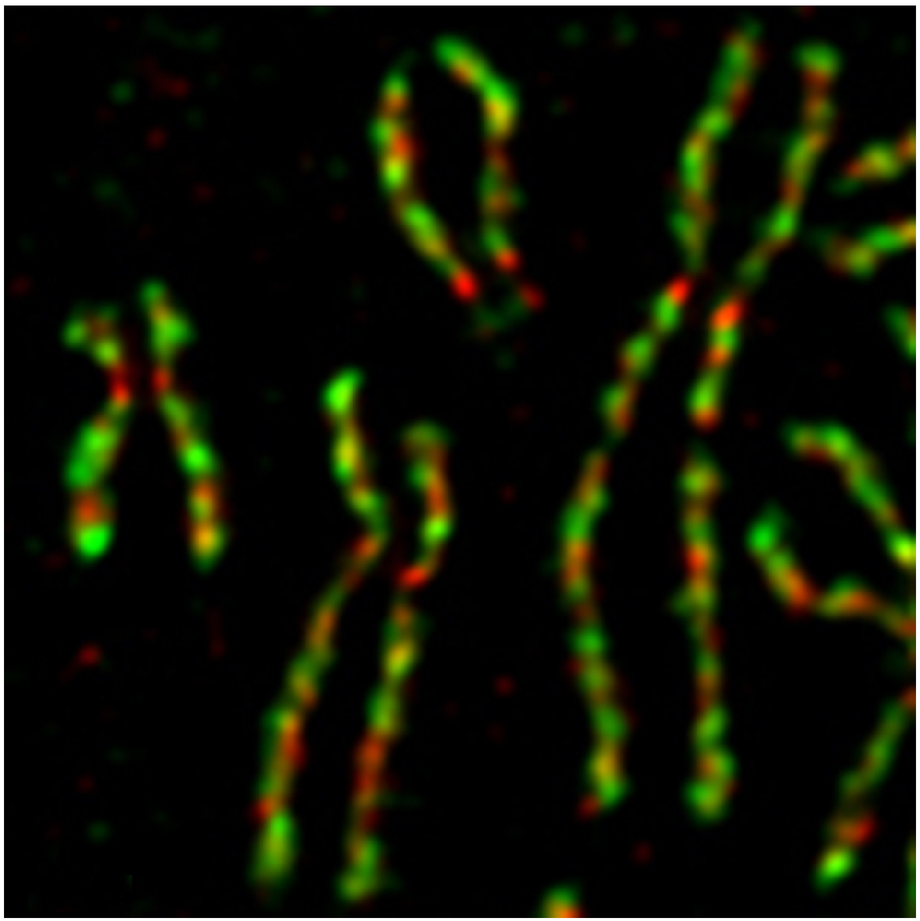
  - clear separation in mitosis
- **Condensins (ATPases)**

STRUCTURAL MAINTENANCE OF CHROMOSOMES: CONDENSINS (ATPASES)







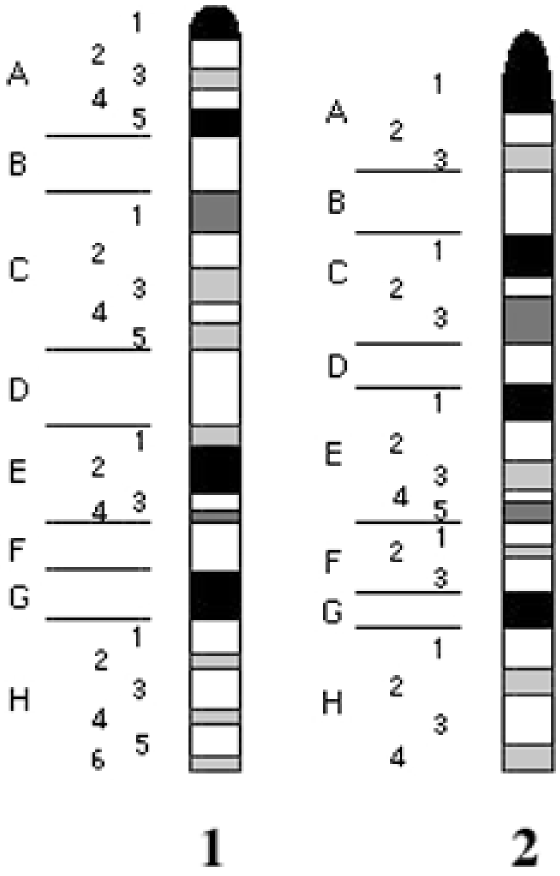


Condensin I

Condensin II

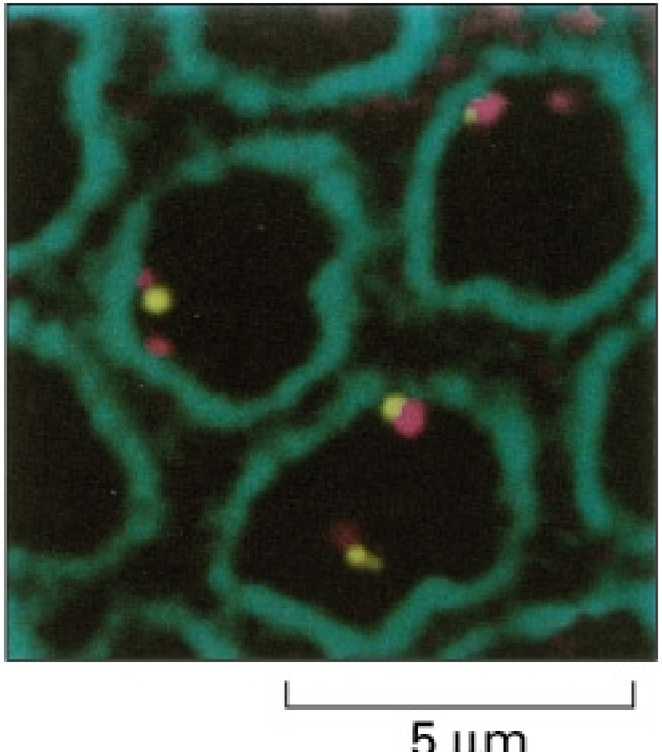
GIEMSA PATTERN SHOWS SIMILARITY BETWEEN CHROMOSOMES OF DIFFERENT SPECIES

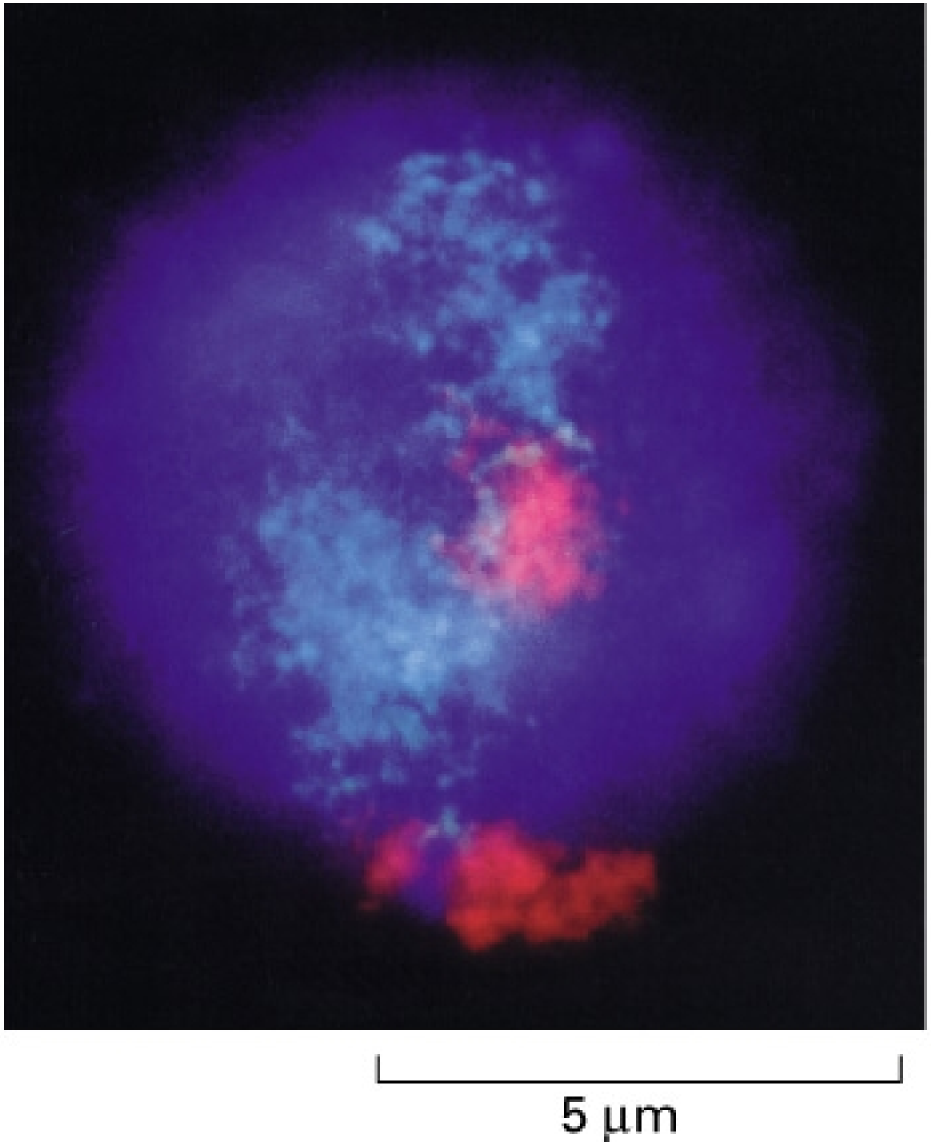
- ~ 2000 distinguishable bands
- Bands are thicker and fewer in mitosis
- Each band has > 10^6 bp
- Large non-random blocks with highr CG-content
- CG-rich blocks: “house-keeping” genes
- Karyotyping



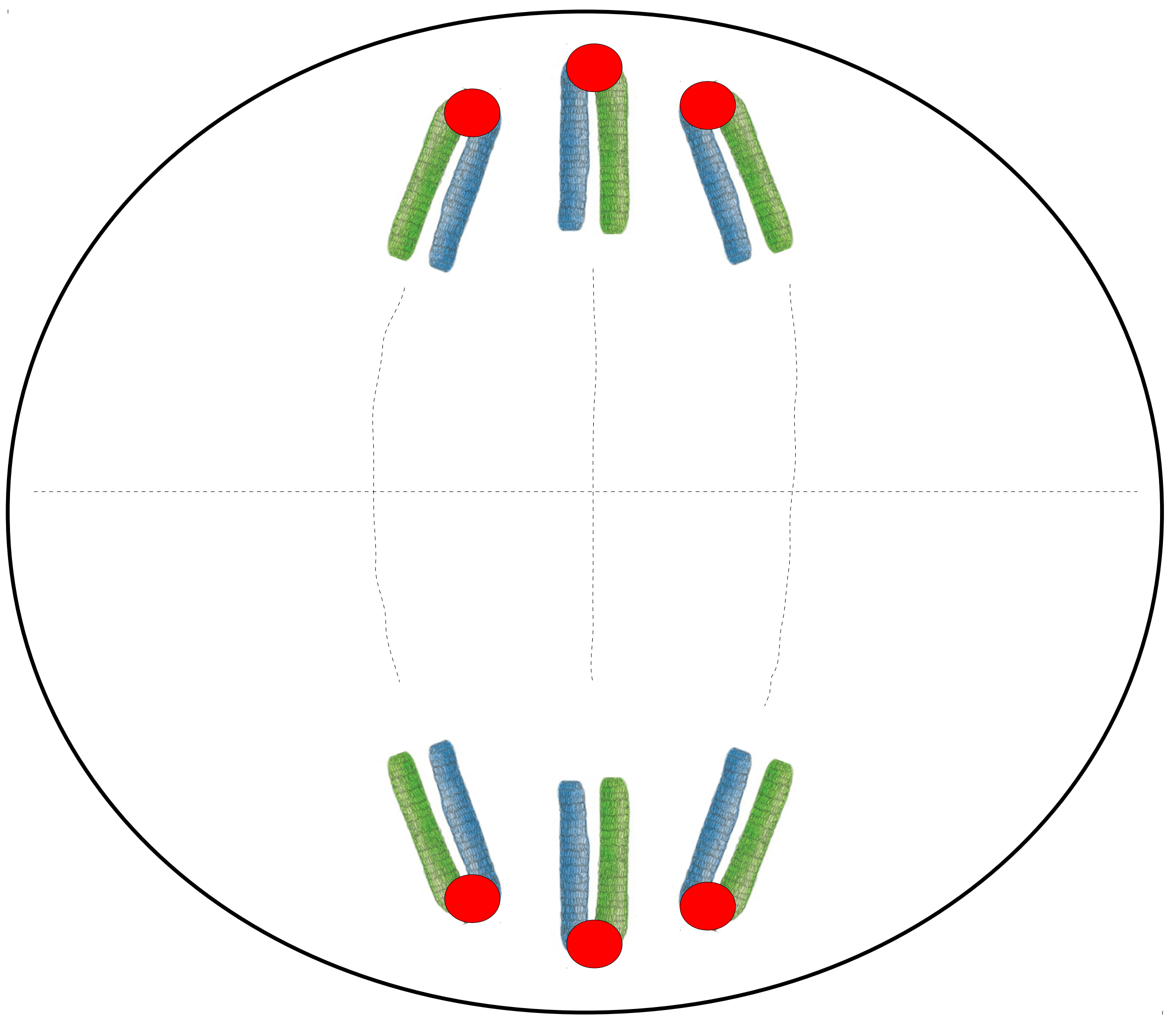
DIFFERENT CHROMOSOMES ARE DIFFERENTLY LOCALIZED IN INTERPHASE

- Chromosomes occupy discrete and small regions in nucleus
- SARs, MARs: DNA regions associated with scaffolds and matrix





RABL ORIENTATION BEFORE THE CELL DIVISION



LECTURES 12-13: CELLULAR NUCLEUS

- **Nucleus structure**
- **Nuclear transport**
- **DNA organization in nucleus:**

  - chromosomes

  - nucleosomes

  - global structure of chromosomes