Biology I

Basic Principles of Cell Biology

Structure and Organization of the Eukaryotic Chromosome

Genome, chromosomes and chromatin

Yiannis Drosos
Assistant Professor
Laboratory of Biology
Department of Medicine
University of Patras
idrosos@upatras.gr

2 meters of DNA in a 6 µm nucleus

~2 m

DNA in every diploid cell (6.2 billion bp × 0.34 nm)

~6 µm

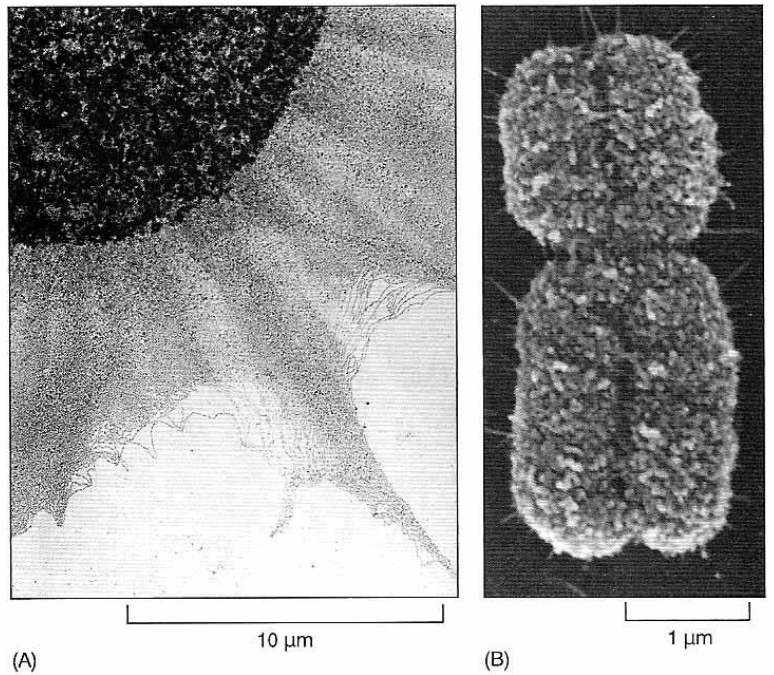
typical nuclear diameter

46

DNA molecules (chromosomes) in every somatic cell

×10,000

compaction in the mitotic chromosome



Metaphase chromosome in the scanning electron microscope (Alberts et al.)

Like fitting 40 km of fine thread into a tennis ball — and still being able to read it, copy it and share it out correctly.

Analogy: Alberts et al., Molecular Biology of the Cell, 7th ed., Ch. 4

Three questions for the next three hours

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

1

How is the genome divided into chromosomes, and how do we “read” them?

→ Part A (1 hour): genome, karyotype, centromeres, telomeres

2

How does DNA fit into the nucleus without losing its function?

→ Part B1: nucleosome, loops, TADs, nucleus

3

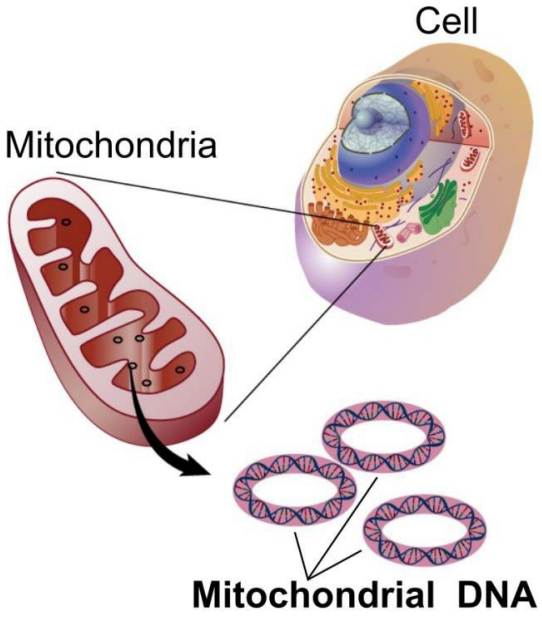
How does chromatin control genes — and what happens when it goes wrong?

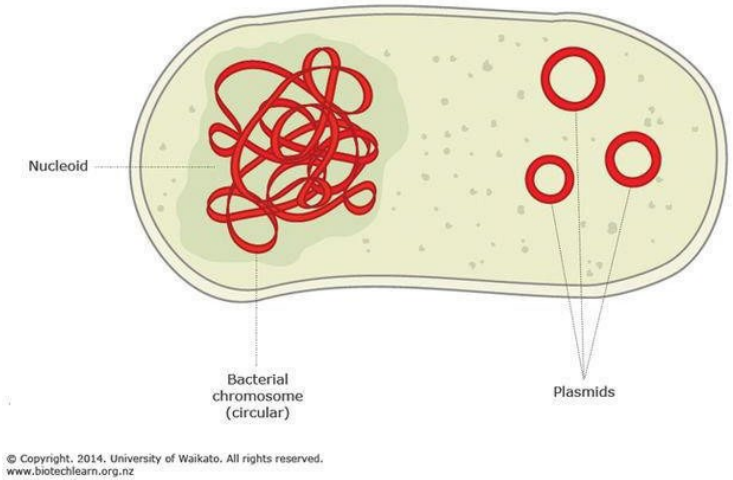
→ Part B2: heterochromatin, histone modifications, disease

From the previous lecture: DNA = double helix · gene · genome. Today: how it is organized inside the cell. Alberts, Essential Cell Biology, Ch. 5 · MBoC, Ch. 4.

Linear or circular: where DNA is found







Nuclear DNA

Linear chromosomes; 46 in humans, wrapped around histones (chromatin).

Mitochondrial DNA

Circular, 16,569 bp, 37 genes; hundreds of copies per cell; maternal inheritance.

Bacterial DNA

A single circular chromosome in the nucleoid + small circular plasmids (e.g., resistance genes).

The human genome in numbers

3.055 billion

base pairs (haploid) · complete T2T sequence, 2022

24

distinct chromosomes: 22 autosomes + X + Y

46

in every somatic cell (23 pairs)

~19,400

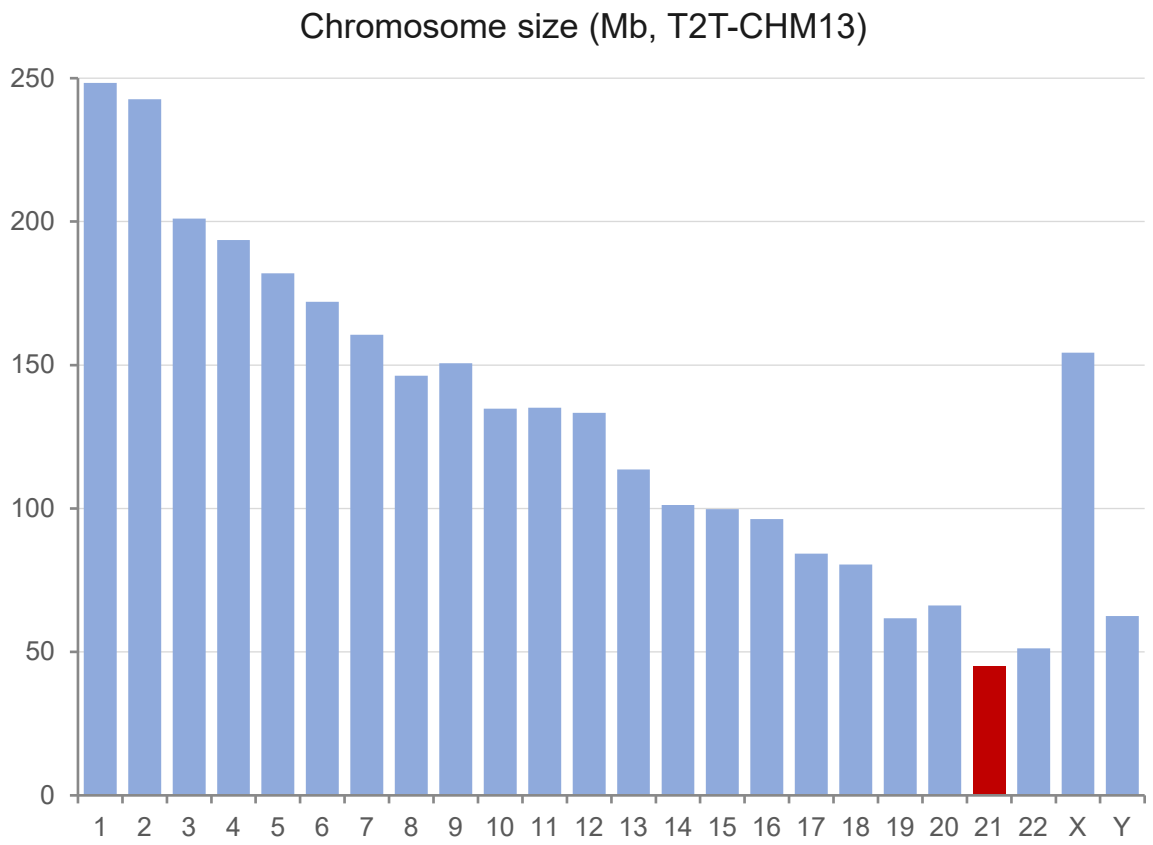
protein-coding genes (GENCODE v50)

~1.5%

of the genome codes for proteins

~50%

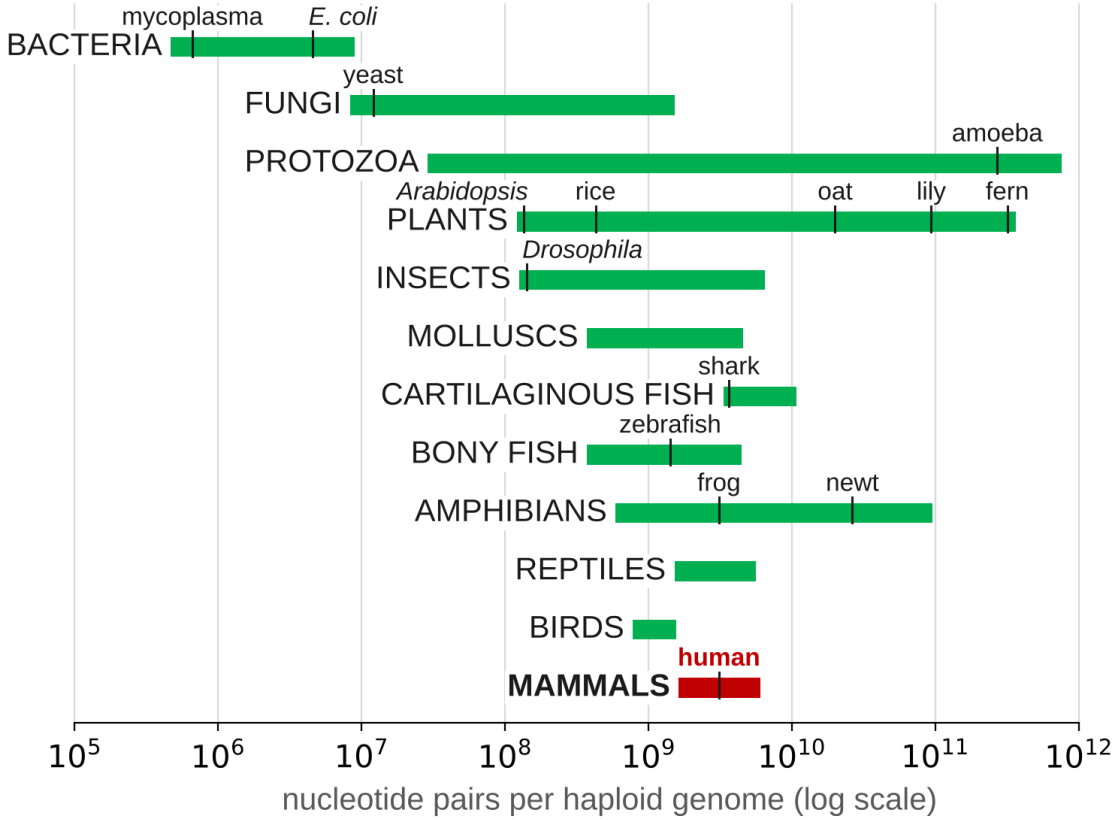
repetitive sequences



Chromosome 21 is the smallest (45 Mb) — not 22.

Nurk et al., Science 2022 · Rhie et al., Nature 2023 (Y) · GENCODE v50 (2026) · UCSC hs1.chrom.sizes

Genome size ≠ complexity



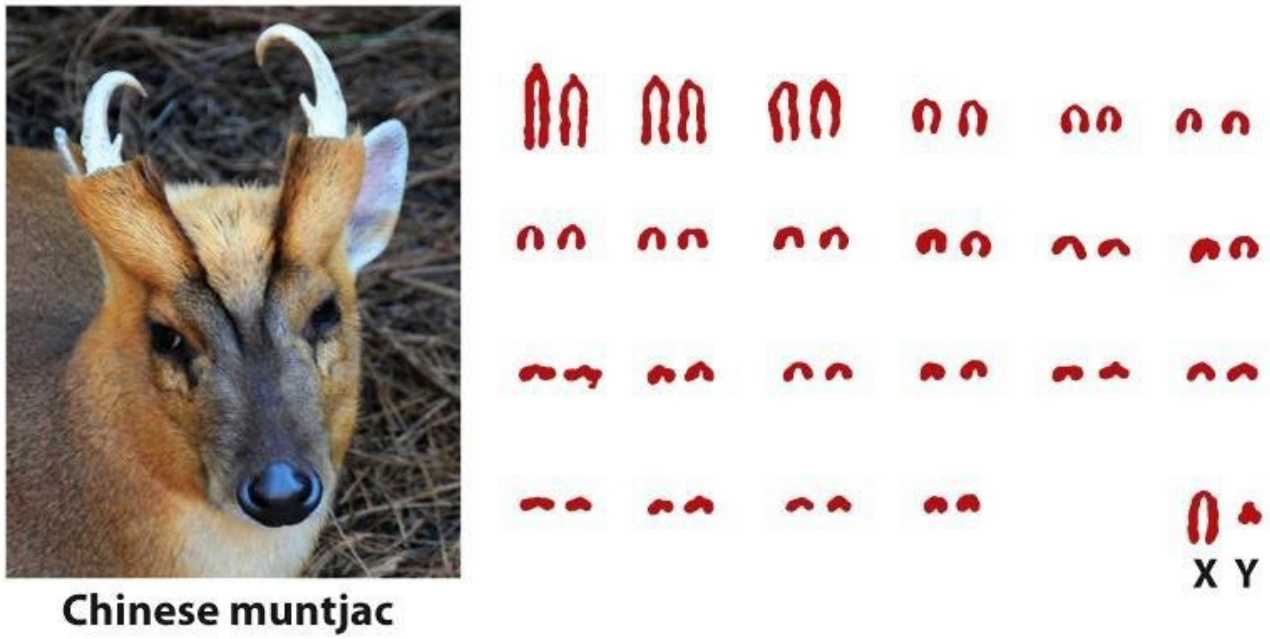
Range of haploid genome size (bp) by group of organisms

Organism	Mb	Protein-coding genes
E. coli	4.6	~4,300
S. cerevisiae (yeast)	12	~6,000
C. elegans (nematode)	100	~20,000
D. melanogaster	140	~14,000
A. thaliana (plant)	135	~27,000
Human	**3,100**	**~19,400**
Australian lungfish	~43,000	—

Why? The difference lies mainly in non-coding DNA (repeats, transposable elements, regulatory regions) and in regulation — not in the number of genes.

Chart redrawn from Alberts et al., Essential Cell Biology, 4th ed. · Meyer et al., Nature 2021 · GENCODE v50

…nor the number of chromosomes



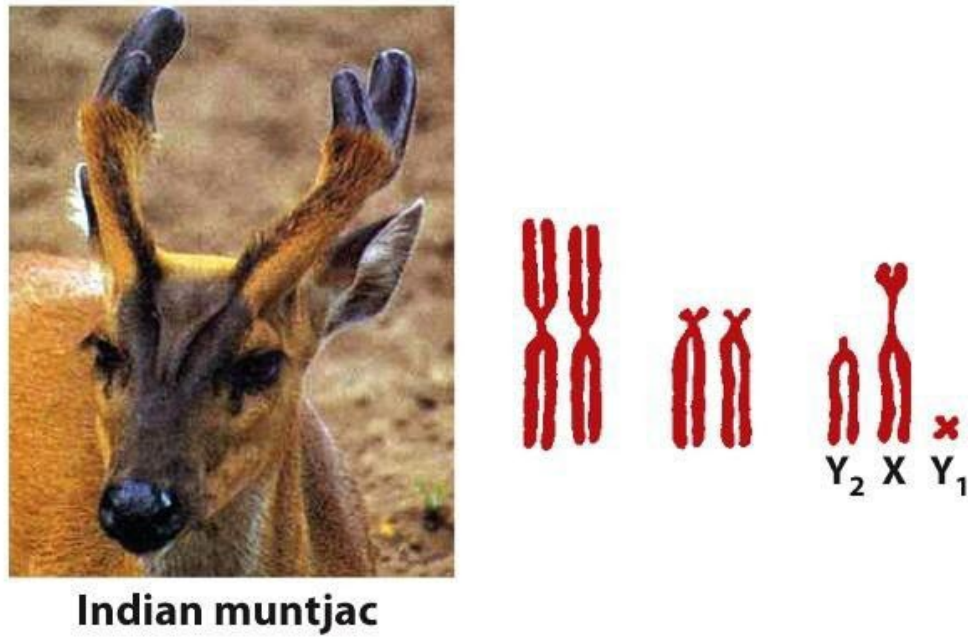


Figure 5-13 Essential Cell Biology, 4th ed. (© Garland Science 2014)

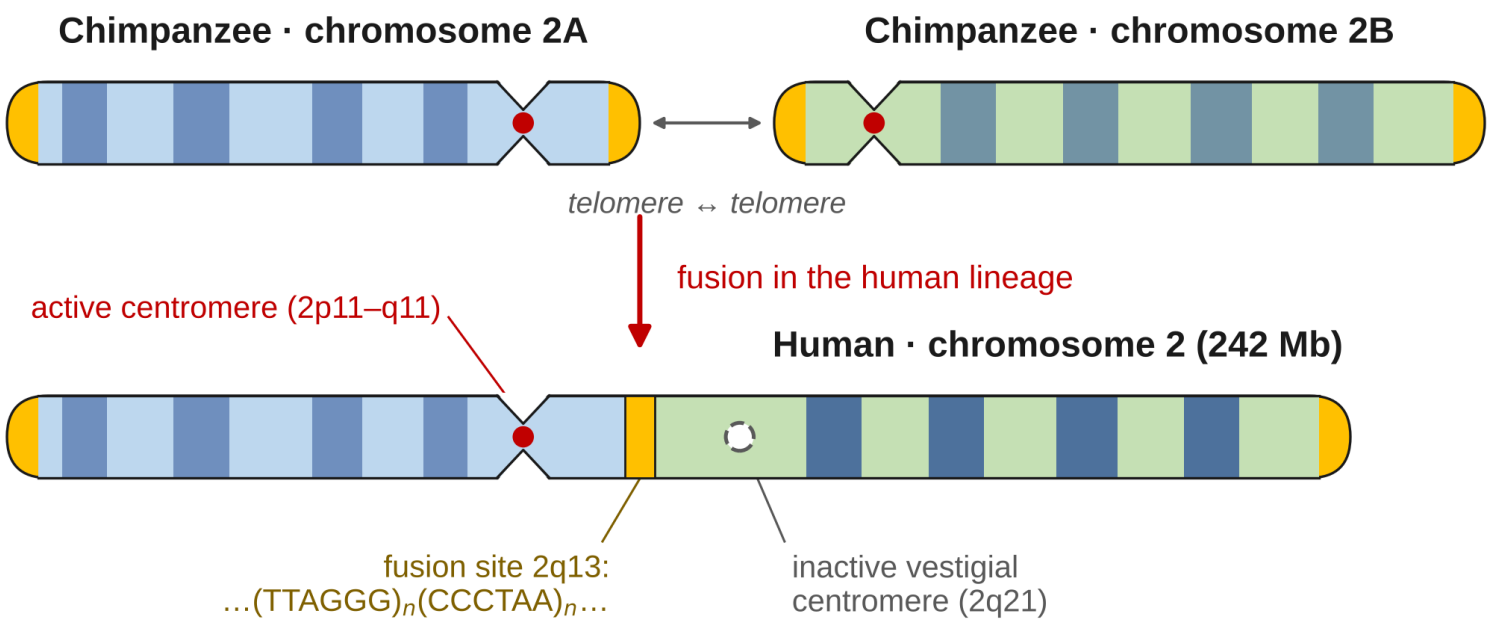
Chinese muntjac
2n = 46

Indian muntjac
2n = 6 (♀) / 7 (♂)

Roughly the same DNA, similar appearance; the difference is due to successive chromosome fusions.

Alberts et al., Essential Cell Biology, 4th ed., Fig. 5-13 · Mudd et al., Commun Biol 2020

Chromosome 2: traces of a fusion



Great apes: 2n = 48 · Human: 2n = 46 — fewer chromosomes, same DNA content

Internal telomeric repeats

Head-to-head at 2q13 (IJdo et al., 1991).

A second, inactive centromere

Vestige at 2q21 — one centromere was “silenced.”

Confirmed in 2025

Complete (T2T) genomes of six ape species (Yoo et al., Nature).

Fusions and inversions change the number and shape of chromosomes during evolution — without changing the “content.”

IJdo et al., PNAS 1991 · Yoo et al., Nature 2025

Chromosomes are visible only during division

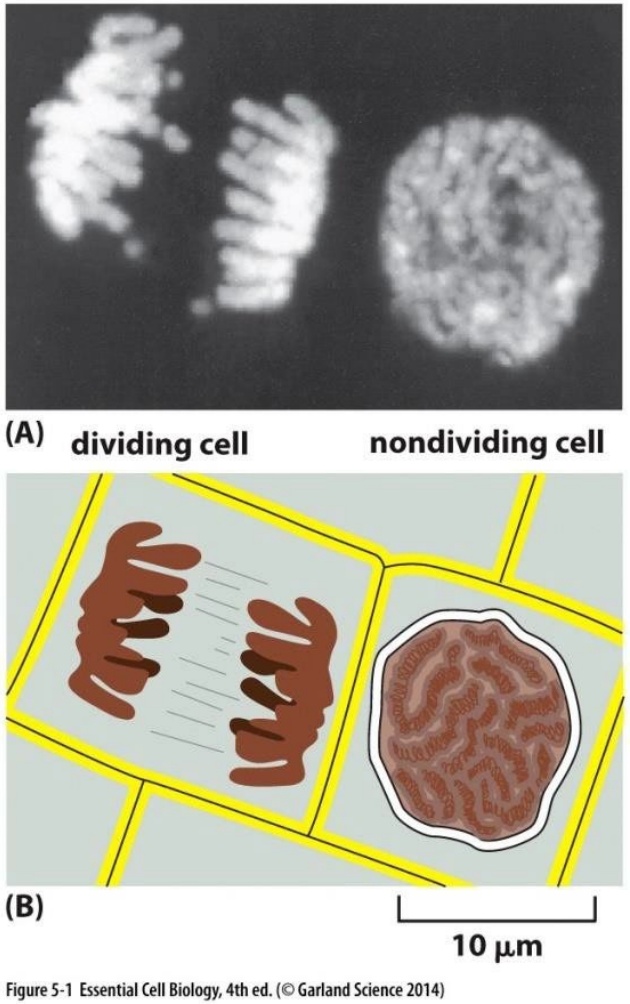
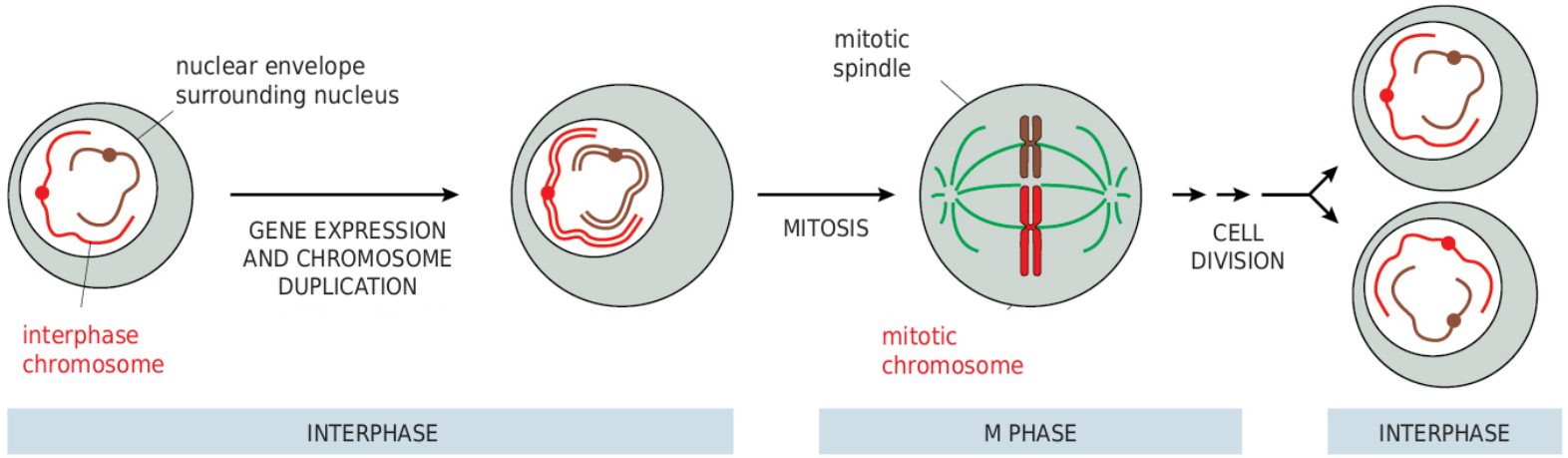


Figure 5-1 Essential Cell Biology, 4th ed. (© Garland Science 2014)



Interphase

Decondensed chromosomes; transcription and replication (S phase).

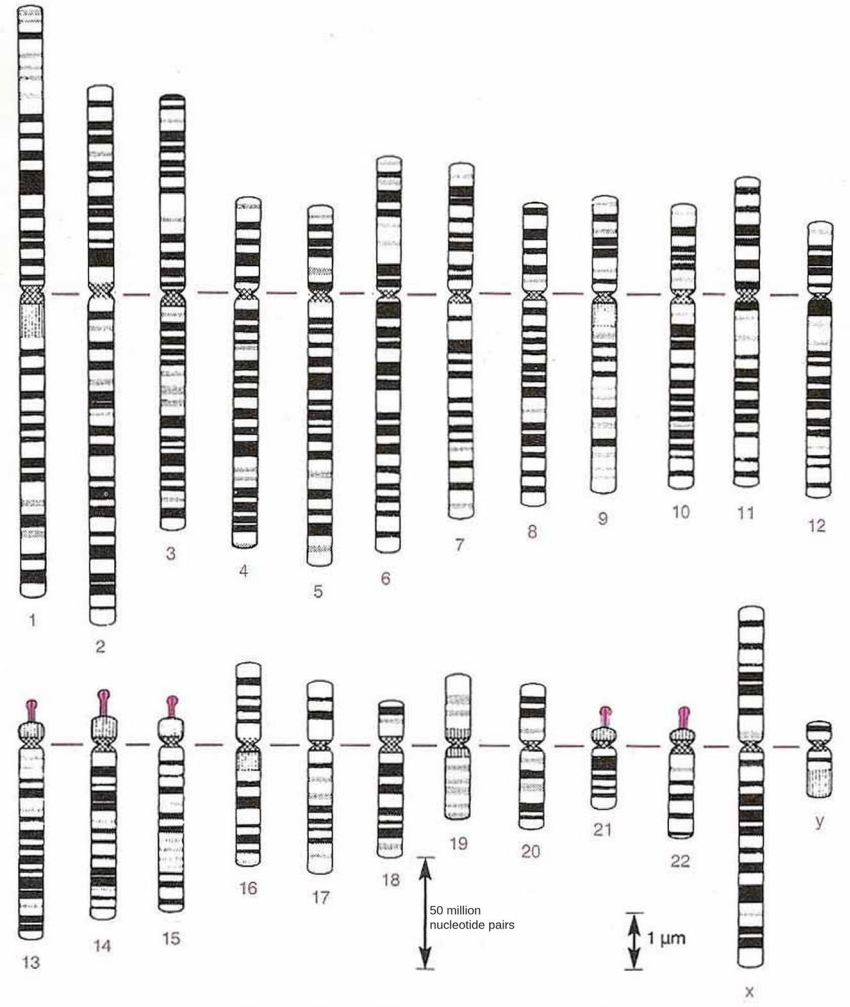
M phase (mitosis)

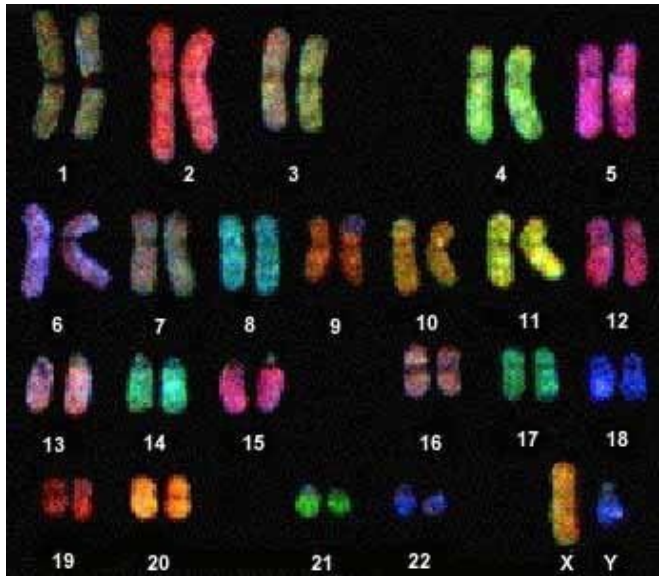
Condensed, with two sister chromatids; transcription almost stops.

▶ **Mitosis animation:** youtube.com/watch?v=_ug2nYVfWvo

Alberts et al., Essential Cell Biology, 4th ed., Fig. 5-1 and Ch. 5 (adapted)

Karyotype: a picture of the genome





Spectral karyotype (SKY): each pair in its own color

Classification

size · centromere position · banding pattern

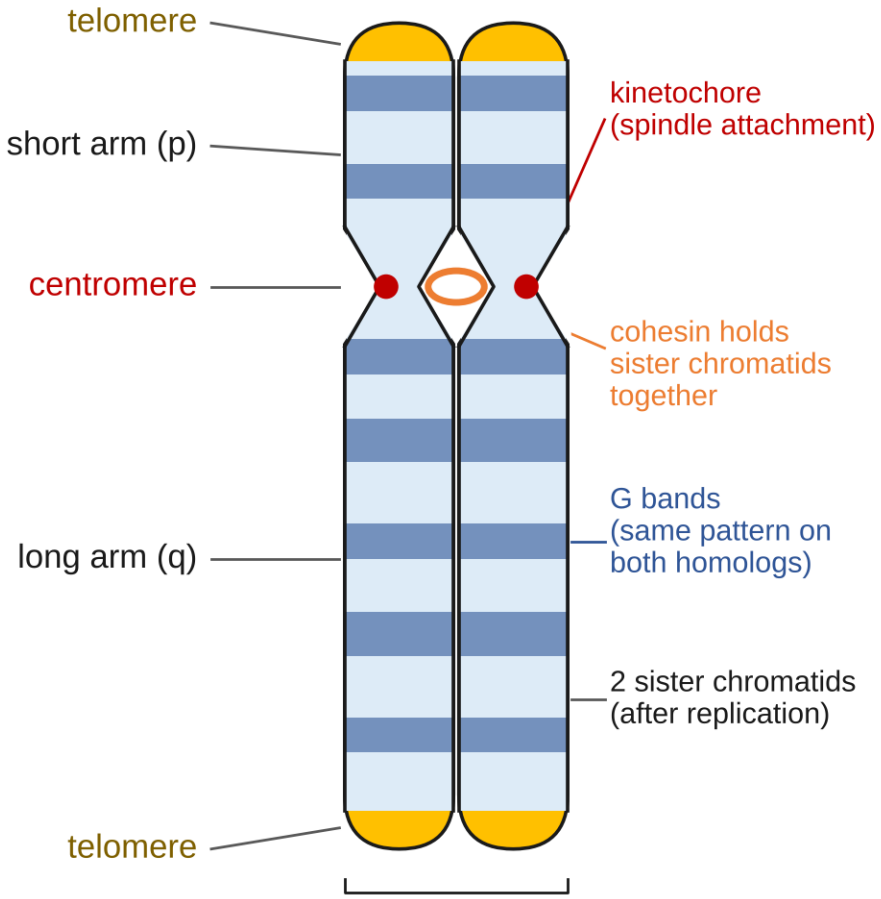
Notation (ISCN)

46,XX · 46,XY · 47,XY,+21 · 45,X

G-banding (Giemsa)

Dark bands: A–T-rich, gene-poor, late-replicating · light bands: G–C-rich and gene-rich.

Chromosome: anatomy and “addresses”



How to read a location:

7	q	3	1	.2
chromosome	long arm	region	band	sub-band

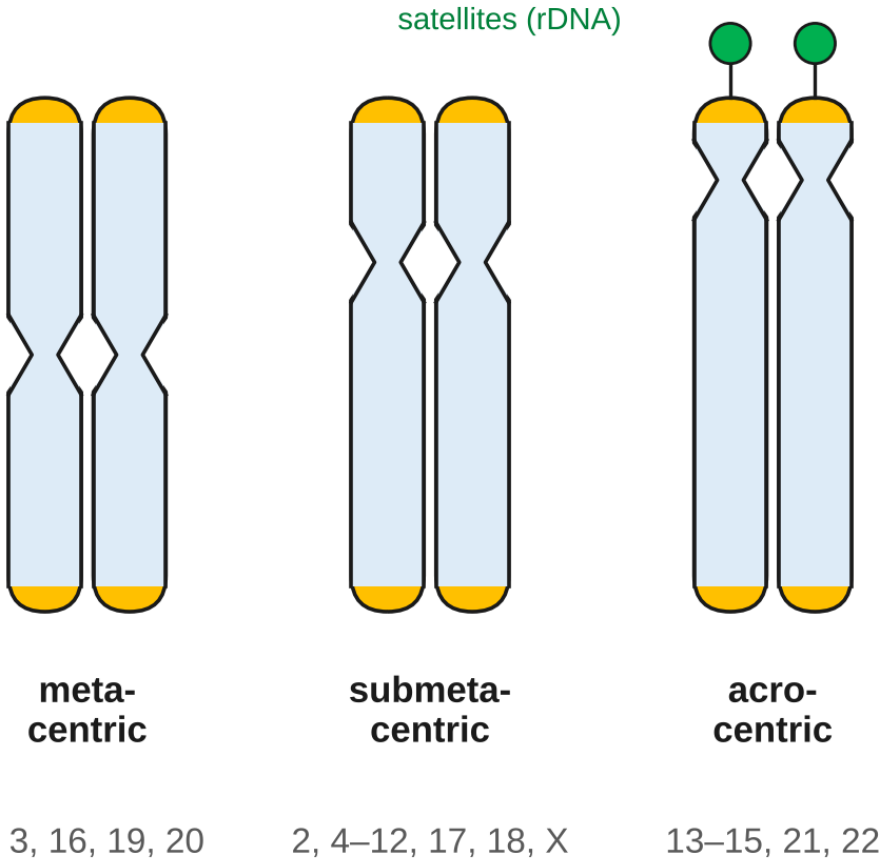
CFTR `7q31.2` · cystic fibrosis

HBB `11p15.4` · β-thalassemia, sickle cell disease

BCR::ABL1 `t(9;22)(q34;q11)` · Philadelphia chromosome · CML

ISCN nomenclature · gene locations: GRCh38/NCBI Gene

Chromosome types



Acrocentric: 13, 14, 15, 21, 22

The short arms carry rRNA gene arrays → they form the nucleolus.

Robertsonian translocation

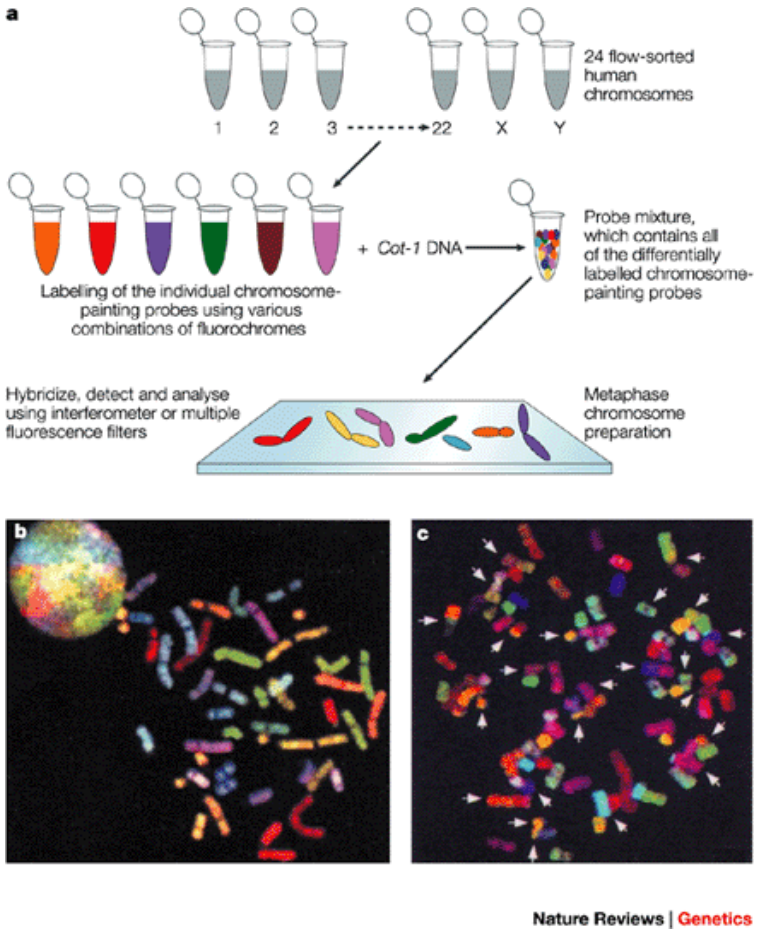
Fusion of two acrocentrics at the centromere, e.g., rob(14;21). ~1/1,000 people. Carriers are healthy (45 chromosomes), with a risk of offspring with trisomy (familial Down syndrome).

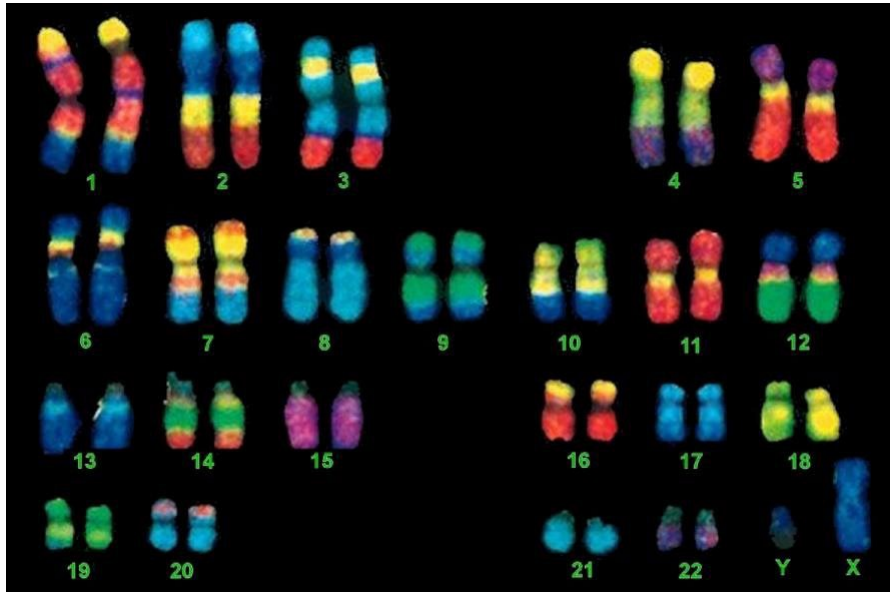
New data (T2T, 2023)

The p arms of the acrocentrics exchange sequences with one another.

Guarracino et al., Nature 2023 · Alberts et al., Essential Cell Biology, 4th ed., Ch. 5

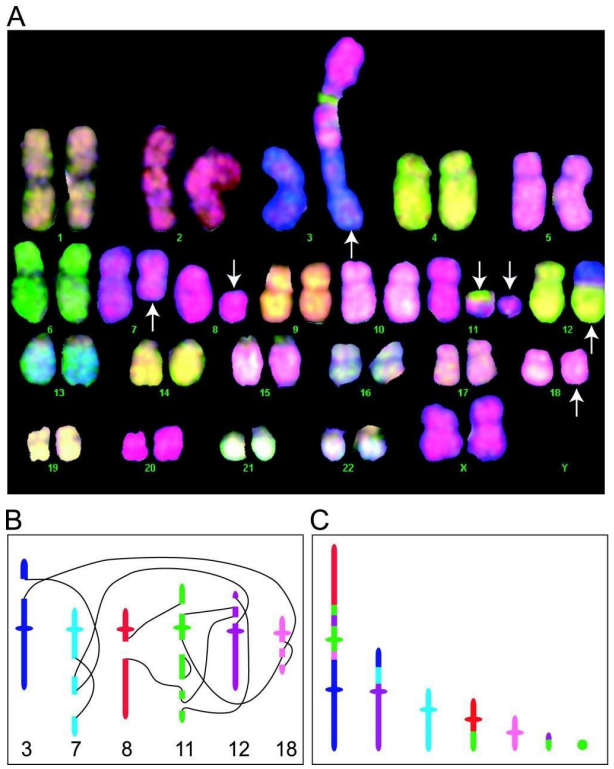
Chromosome imaging: M-FISH and SKY





The principle

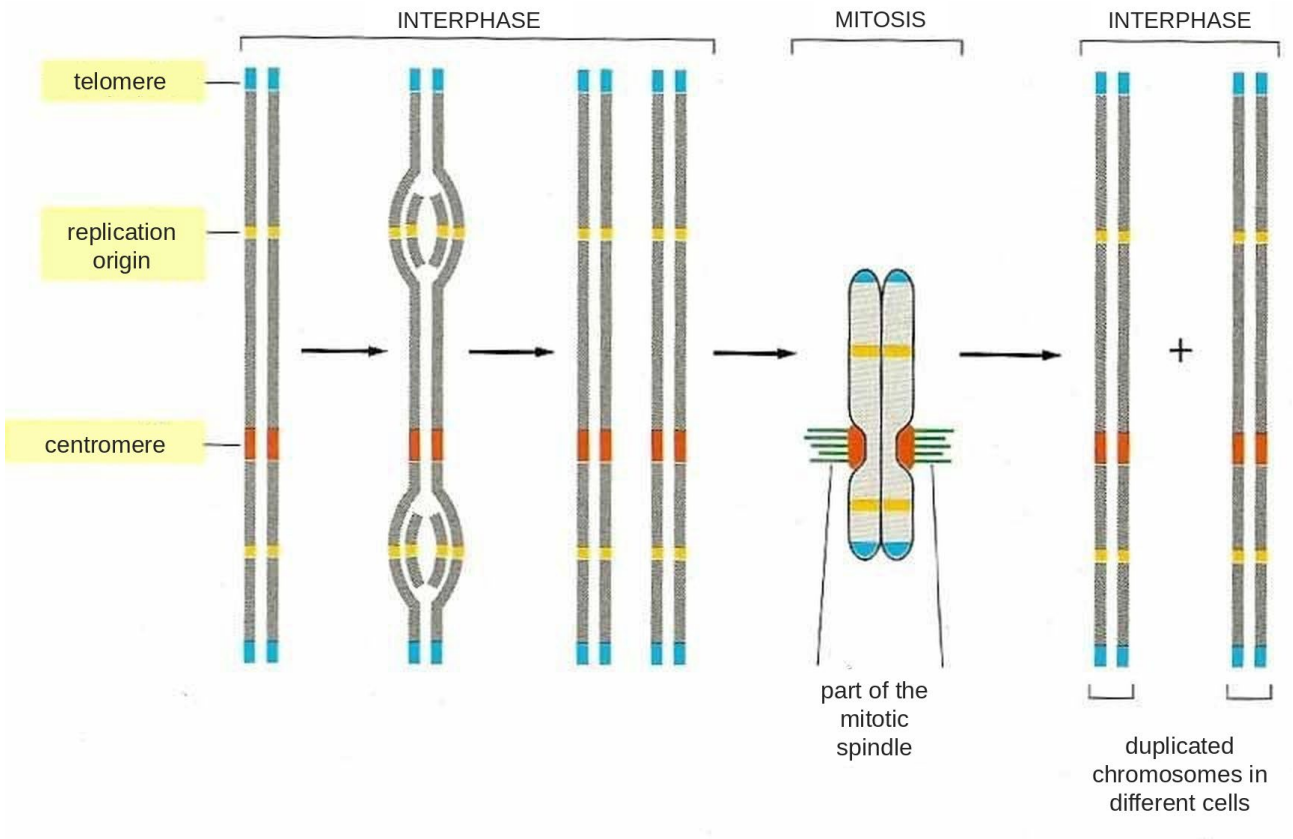
24 probes, each with its own combination of 5 fluorochromes → every chromosome in “its own color.”



Reveals translocations and complex rearrangements (tumors, leukemias).

Speicher et al., Nat Genet 1996 · Schröck et al., Science 1996 · Speicher & Carter, Nat Rev Genet 2005

What does a chromosome need?



1 **Replication origins**
Many on each chromosome (tens of thousands in the genome) → replication in a few hours.

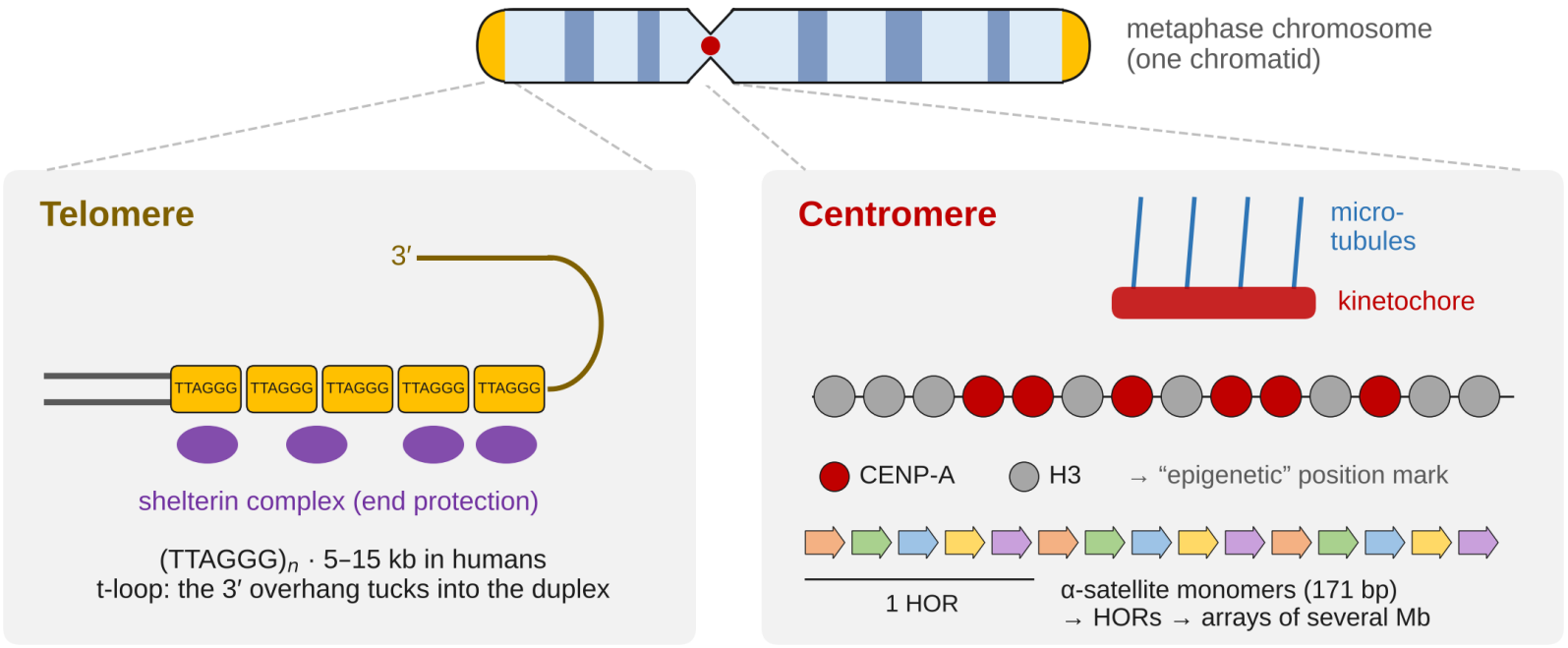
2 **Centromere**
Forms the kinetochore → attachment to the spindle and correct segregation.

3 **Telomeres**
Protect the ends and solve the “end-replication problem.”

The proof: yeast artificial chromosomes (YACs) are stable only when they carry all three elements.

Alberts et al., Essential Cell Biology, 4th ed., Ch. 5 (adapted)

Telomeres and centromeres



Clinical link: telomere biology disorders (e.g., dyskeratosis congenita) → bone marrow failure, pulmonary fibrosis; telomerase reactivation “immortalizes” cancer cells.

Telomeres and aging

Shorten with every division; telomerase extends them in stem cells and in ~85–90% of cancers.

Centromere = epigenetic locus

Defined by CENP-A nucleosomes, not by sequence alone (neocentromeres).

T2T genome (2022)

Satellite repeats = 6.2% of the genome; first complete centromere maps.

Nurk et al., Science 2022 · Altemose et al., Science 2022 · MBoC 7th ed., Ch. 4–5

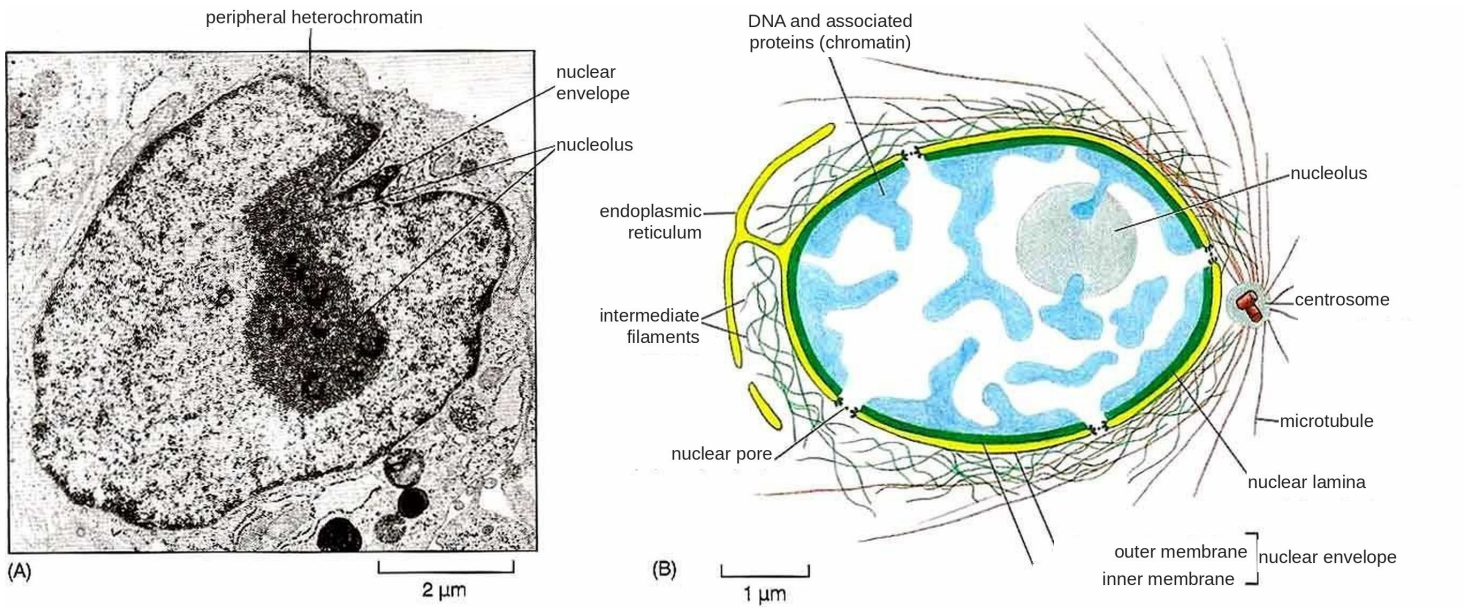
IN-CLASS QUESTION #1 · 2 MINUTES

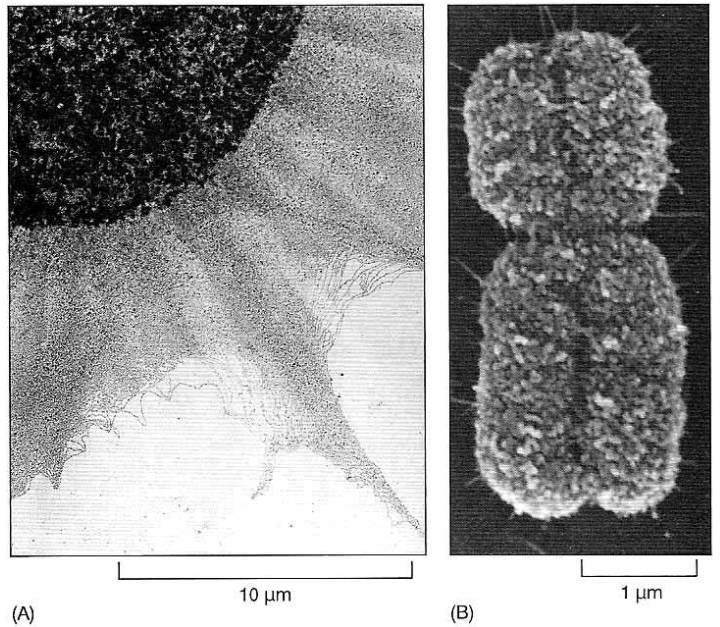
Design an artificial chromosome

Three constructs in yeast cells. Each one lacks a single element. Match each construct to its outcome.

1	no centromere	A	Not replicated, so it is “diluted out” and lost within a few divisions.
2	no replication origins	B	Replicated, but distributed randomly to the daughter cells.
3	no telomeres	C	Replicated, but its ends are degraded or fused.

How much space do chromosomes take up?





5–8 µm

nuclear diameter

~2 m

DNA length per nucleus

10^3–10^4 ×

compaction in mitosis

2

sister chromatids per metaphase chromosome

Alberts et al., Essential Cell Biology, 4th ed., Ch. 5

Chromosome territories

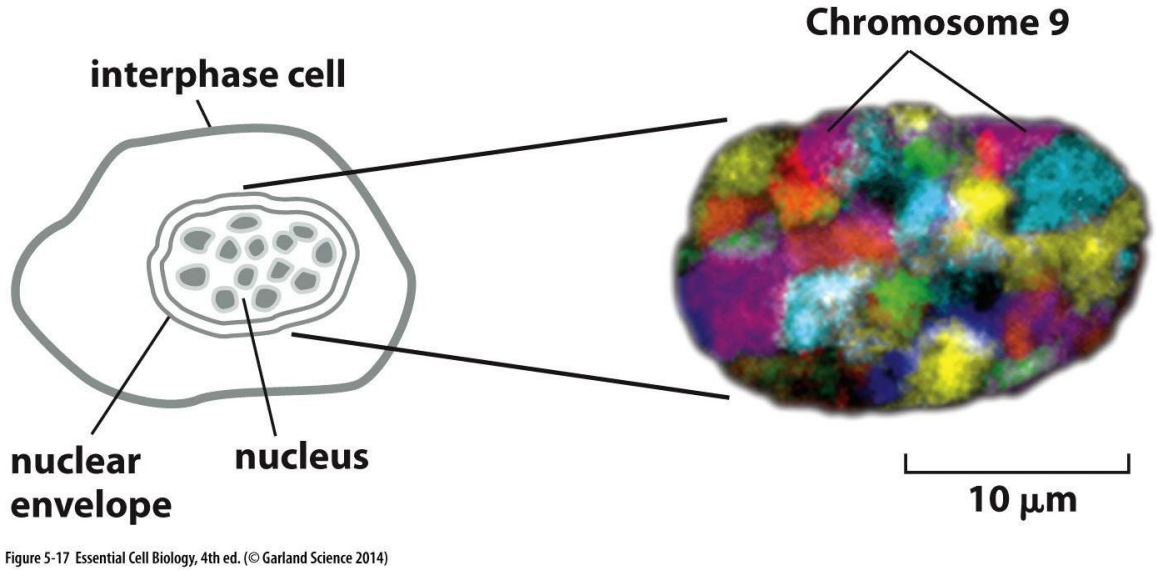


Figure 5-17 Essential Cell Biology, 4th ed. (© Garland Science 2014)

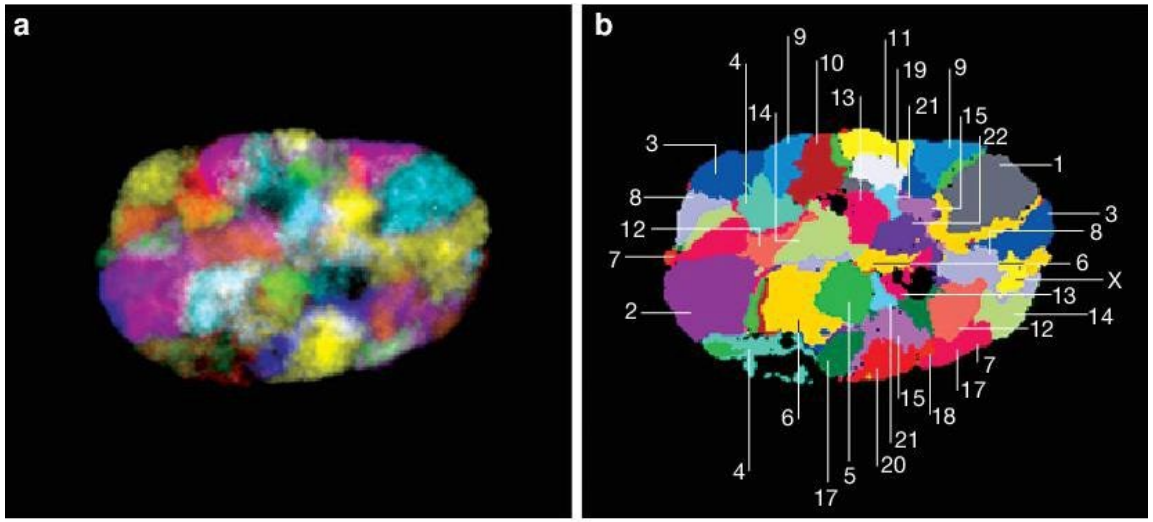


Figure 5 | **Studying genome organization using three-dimensional fluorescence *in situ***

Each chromosome in its own "territory"

Homologs usually do not lie next to each other.

Position is not random

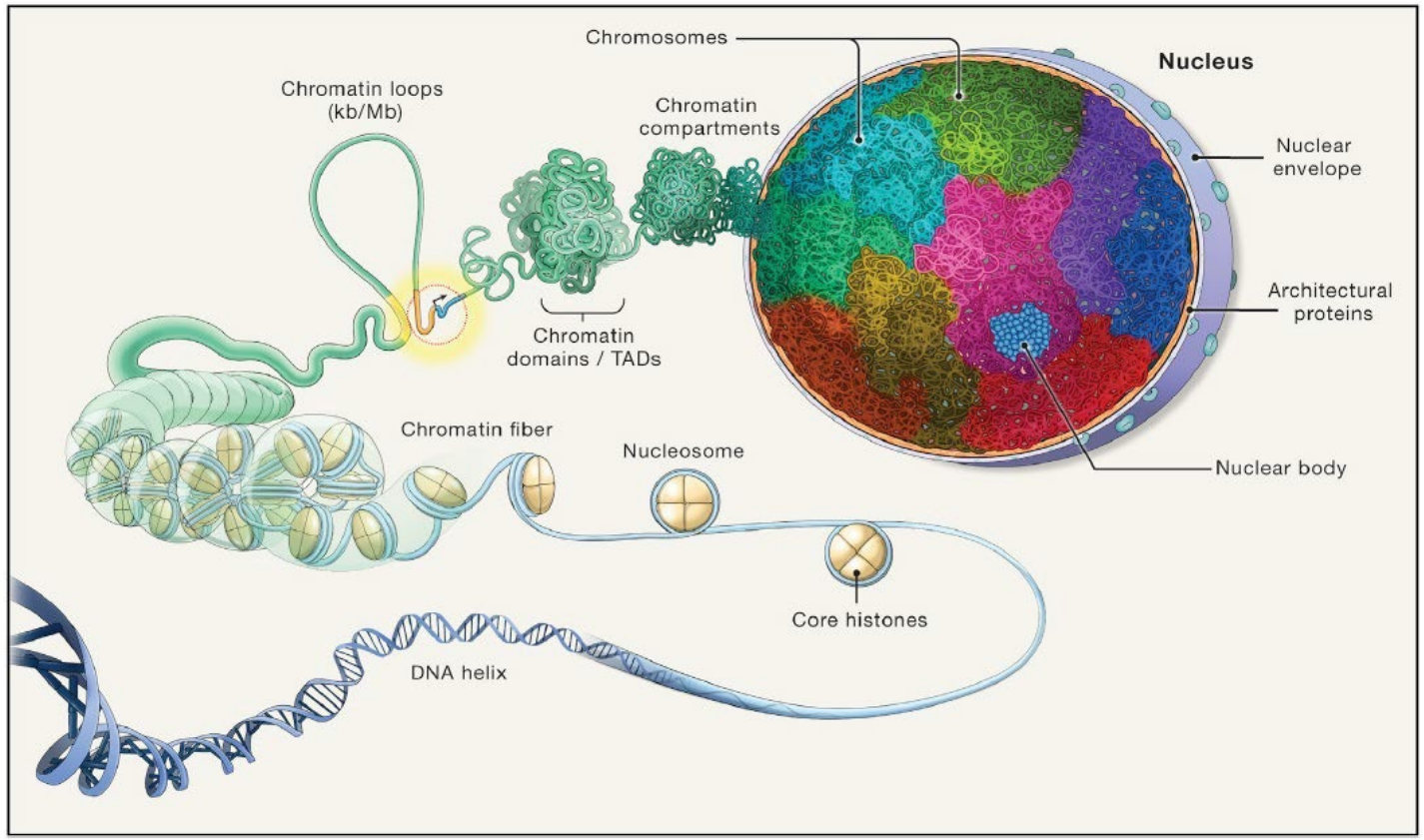
Gene-dense regions are organized in the interior of the nucleus, gene-poor regions at the periphery.

Dynamic organization

Position correlates with activity and changes with differentiation.

Alberts et al., Essential Cell Biology, 4th ed., Fig. 5-17 · Bolzer et al., PLoS Biol 2005 · Croft et al., J Cell Biol 1999

Levels of genome organization



Misteli, Cell 2020

- **Double helix** 2 nm
- **Nucleosome** 11 nm
- **Chromatin fiber** ~10–30 nm
- **Loops & TADs** 0.1–1 Mb
- **A/B compartments** several Mb
- **Territory** whole chromosome
- **Nucleus** ~6 µm

→ **Part B**

Summary of Part A

1. The genome (3.1 billion bp) is divided into 23 pairs of linear chromosomes: size and number do not predict complexity.
2. The karyotype classifies chromosomes (size, centromere, bands) and gives “addresses” such as 7q31.2.
3. Each method—karyotype, FISH, CMA, OGM, sequencing—has its own resolution and blind spots.
4. Origins, centromere and telomeres are essential for replication and segregation.
5. In interphase, chromosomes occupy territories; in mitosis they condense ~10,000-fold.

Part B: Chromatin

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

B1

Hour 1 · From the nucleosome to the 3D genome

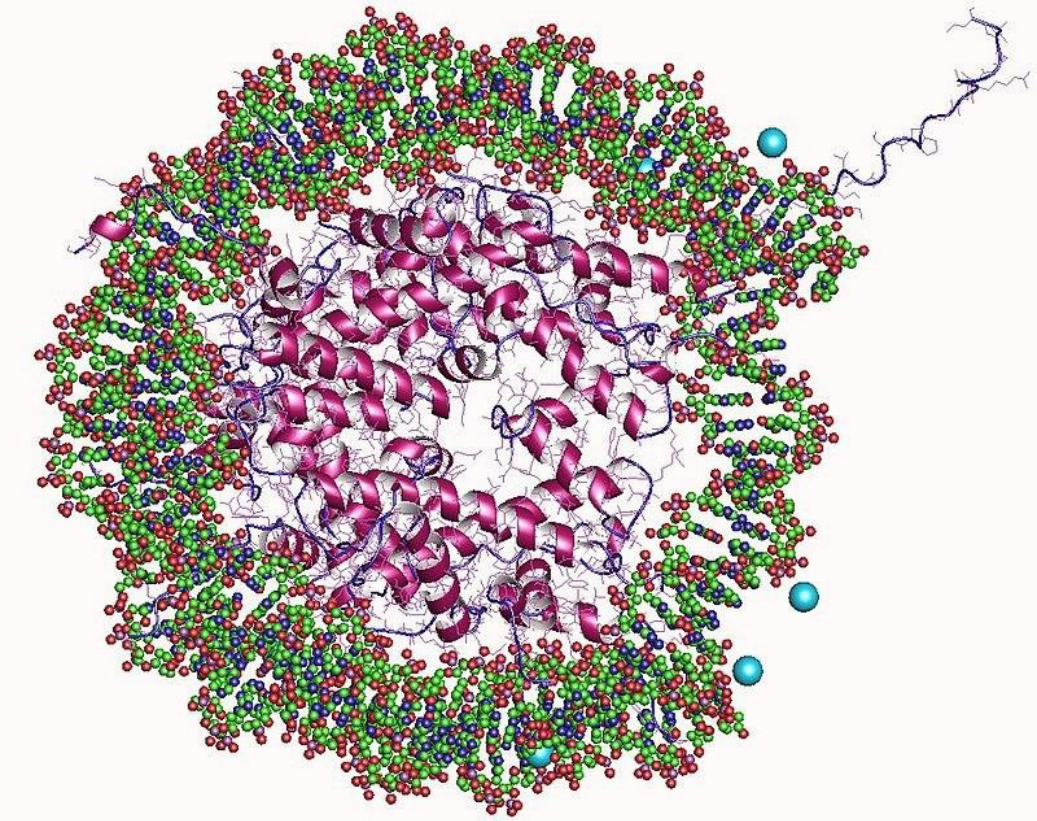
- Histones and the nucleosome
- Chromatin remodeling
- The “30 nm fiber” today
- Loops, TADs, compartments
- The nucleus as an organized space

B2

Hour 2 · Chromatin, gene expression and disease

- Euchromatin – heterochromatin
- X inactivation, position effect
- Histone modifications and their enzymes
- DNA methylation
- Epigenetic “memory”
- Chromatinopathies, oncohistones, drugs

Chromatin: DNA + proteins



Nucleosome core particle (PDB)

Chromatin: the DNA–protein complex of the eukaryotic chromosome.

histone mass

≈ DNA mass

~60 million

molecules of each histone type per cell

Non-histone proteins

transcription factors · remodeling complexes · modifying enzymes · cohesin, condensins, CTCF · HMG proteins · polymerases

Alberts et al., MBoC 7th ed., Ch. 4 · Essential Cell Biology, 4th ed., Ch. 5

Histones: small, basic, conserved

Human histone H4 (102 amino acids) — K and R in red

SG**R**G**K**GG**K**GL G**K**GGA**KR**H**RK** VL**R**DNIQGIT **K**PAI**RR**LA**RR** GGV**KR**ISGLI
YEET**R**GVL**KV** FLENVI**R**DAV TYTEHA**K**R**K**T VTAMDVVYAL **KR**QG**R**TLYGF GG

yellow: the only 2 positions where pea H4 differs from cow H4 (60, 77)

5 types

H2A, H2B, H3, H4 (nucleosome core) + H1 (linker)

~25% K + R

positive charge → strong attraction to the DNA phosphates

2 / 102

differences in H4 between a plant and a mammal

Histone fold

common structural motif + flexible N-terminal tails

Sequence: UniProt P62805 · Alberts et al., MBoC 7th ed., Ch. 4

Histone genes in humans

The following is a list of human histone proteins, genes and pseudogenes:[10]

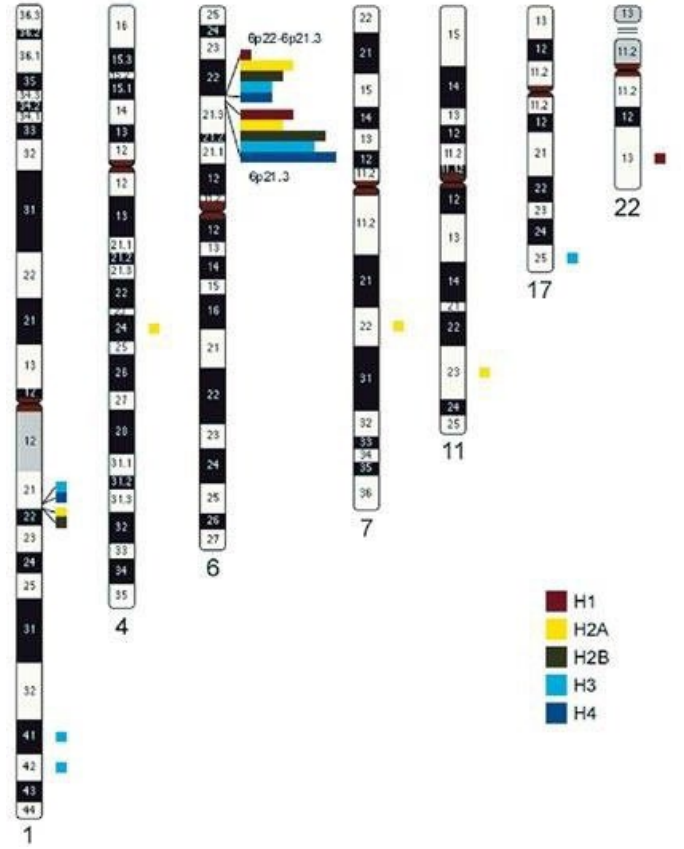
Super family	Family	Replication-dependent genes	Replication-independent genes	Pseudogenes
Linker	H1	H1-1, H1-2, H1-3, H1-4, H1-5, H1-6	H1-0, H1-7, H1-8, H1-10	H1-9P, H1-12P
Core	H2A	H2AC1, H2AC4, H2AC6, H2AC7, H2AC8, H2AC11, H2AC12, H2AC13, H2AC14, H2AC15, H2AC16, H2AC17, H2AC18, H2AC19, H2AC20, H2AC21, H2AC25	H2AZ1, H2AZ2, MACROH2A1, MACROH2A2, H2AX, H2AJ, H2AB1, H2AB2, H2AB3, H2AP, H2AL1Q, H2AL3	H2AC2P, H2AC3P, H2AC5P, H2AC9P, H2AC10P, H2AQ1P, H2AL1MP
	H2B	H2BC1, H2BC3, H2BC4, H2BC5, H2BC6, H2BC7, H2BC8, H2BC9, H2BC10, H2BC11, H2BC12, H2BC13, H2BC14, H2BC15, H2BC17, H2BC18, H2BC21, H2BC26, H2BC12L	H2BK1, H2BW1, H2BW2, H2BW3P, H2BN1	H2BC2P, H2BC16P, H2BC19P, H2BC20P, H2BC27P, H2BL1P, H2BW3P, H2BW4P
	H3	H3C1, H3C2, H3C3, H3C4, H3C6, H3C7, H3C8, H3C10, H3C11, H3C12, H3C13, H3C14, H3C15, H3-4	H3-3A, H3-3B, H3-5, H3-7, H3Y1, H3Y2, CENPA	H3C5P, H3C9P, H3P16, H3P44
	H4	H4C1, H4C2, H4C3, H4C4, H4C5, H4C6, H4C7, H4C8, H4C9, H4C11, H4C12, H4C13, H4C14, H4C15	H4C16	H4C10P

Replication-dependent

Many copies; no introns; mRNA without poly(A); expressed in S phase.

Histone variants

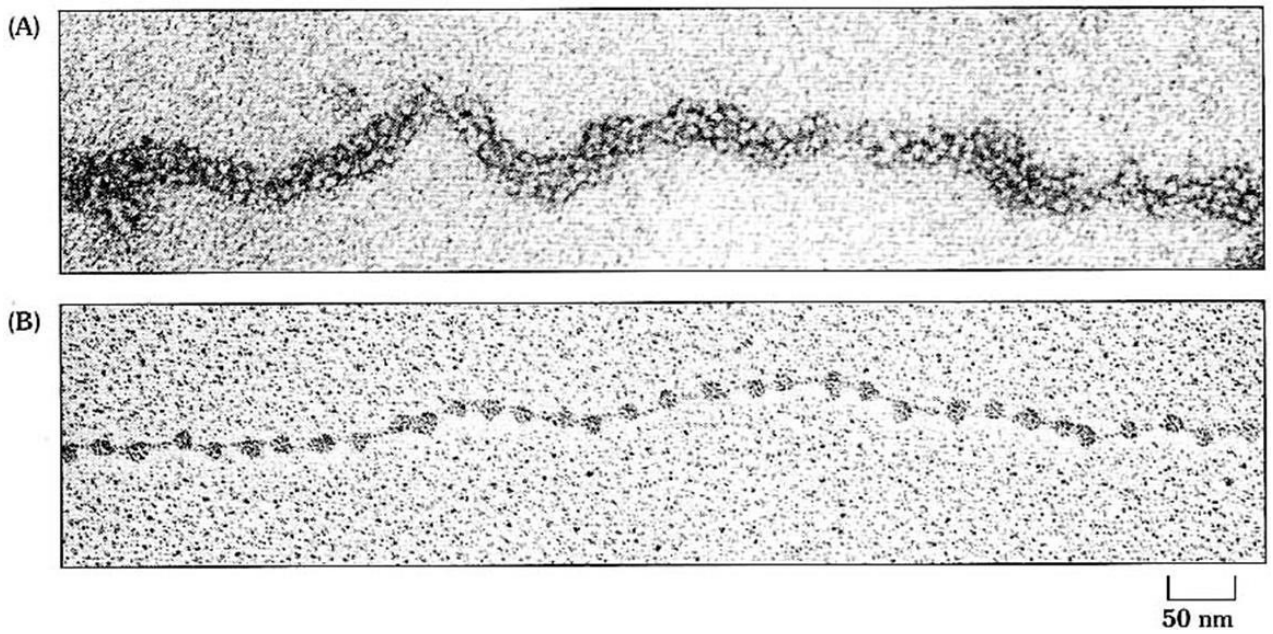
H3.3, H2A.Z, H2A.X, macroH2A, CENP-A · with introns, throughout the cell cycle.

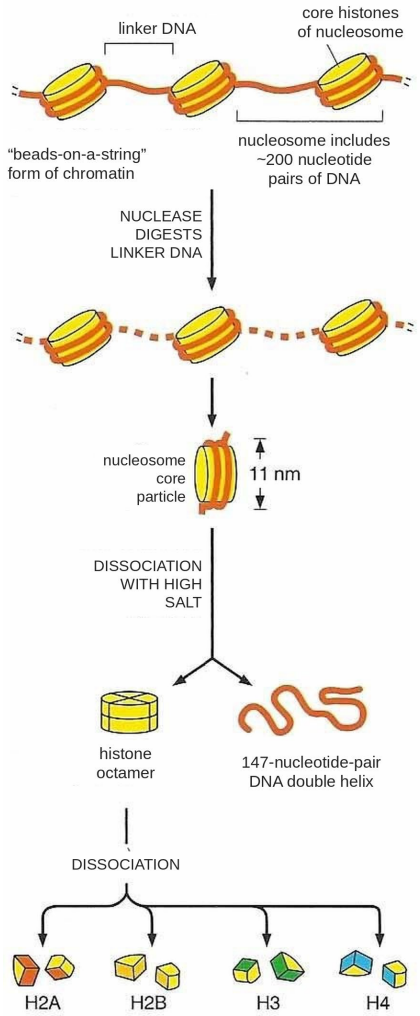


Histone gene clusters (e.g., 6p22, 1q21) — from successive duplications

Seal et al., Epigenetics Chromatin 2022 (standardized nomenclature)

Nucleosome: “beads on a string”





11 nm

diameter

147 bp

around the octamer

~200 bp

per nucleosome (+ linker)

The experiment

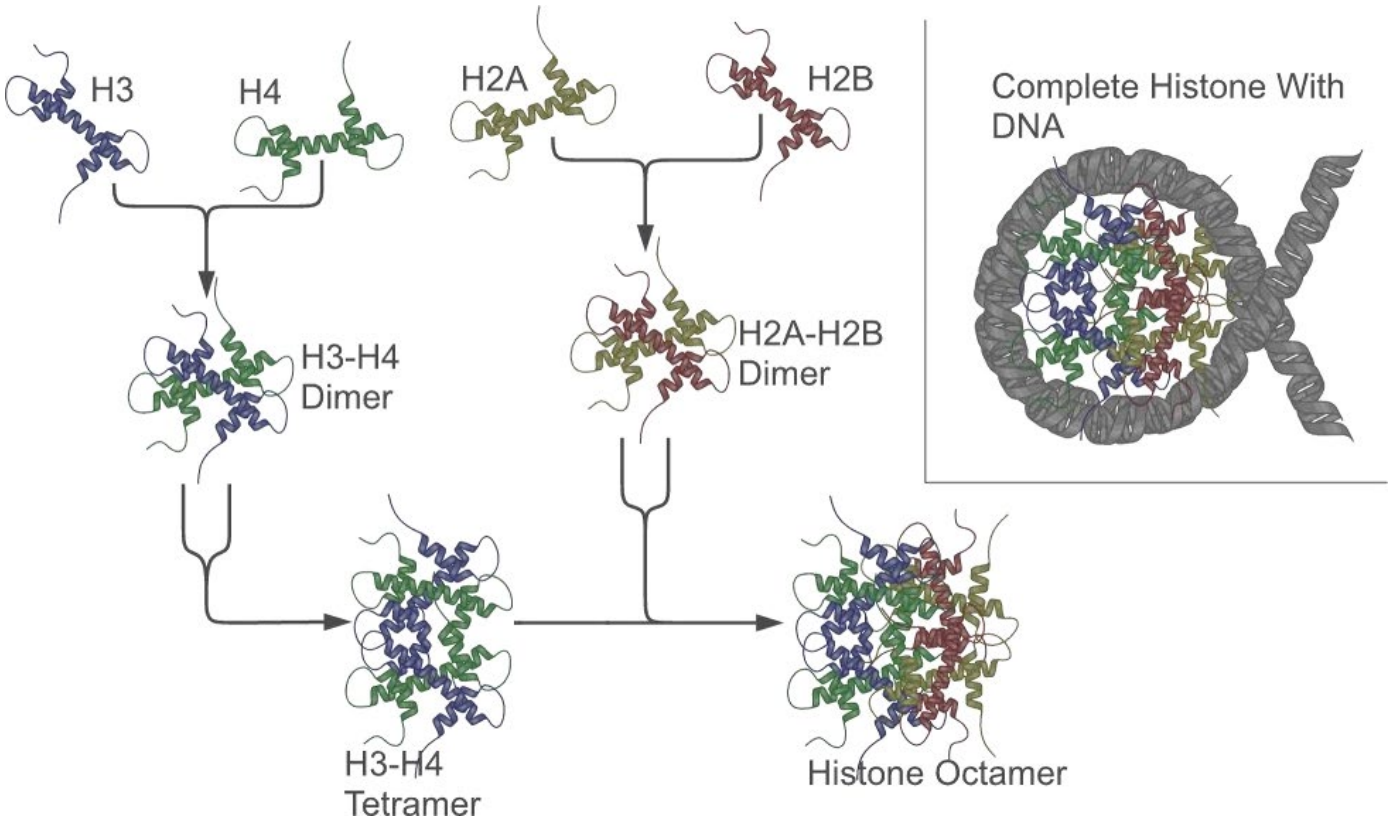
Nuclease cuts only the linker DNA → fragments of ~200 bp and their multiples.

Milestones

Olins & Olins, Kornberg (1974): the repeating unit · Luger et al. (1997): 2.8 Å crystal structure.

Alberts et al., Essential Cell Biology, 4th ed., Figs. 5-21/5-22 · Kornberg, Science 1974 · Luger et al., Nature 1997

Nucleosome assembly



1. The H3–H4 tetramer binds DNA first.
2. Two H2A–H2B dimers complete the octamer.
3. Histone chaperones: CAF-1 at the replication fork · HIRA for H3.3.

~3 × 10⁷

nucleosomes in a diploid human cell

Alberts et al., MBoC 7th ed., Ch. 4 · figure: course material

The nucleosome at atomic resolution

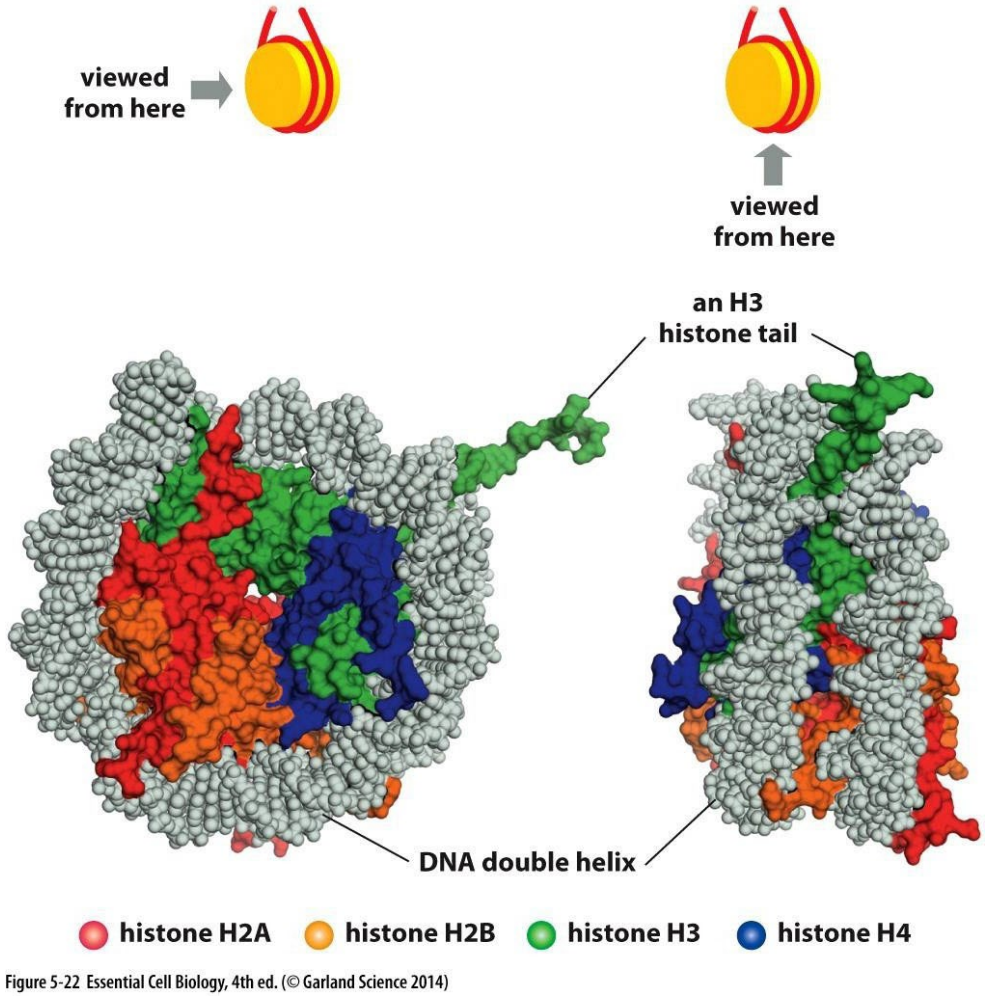


Figure 5-22 Essential Cell Biology, 4th ed. (© Garland Science 2014)

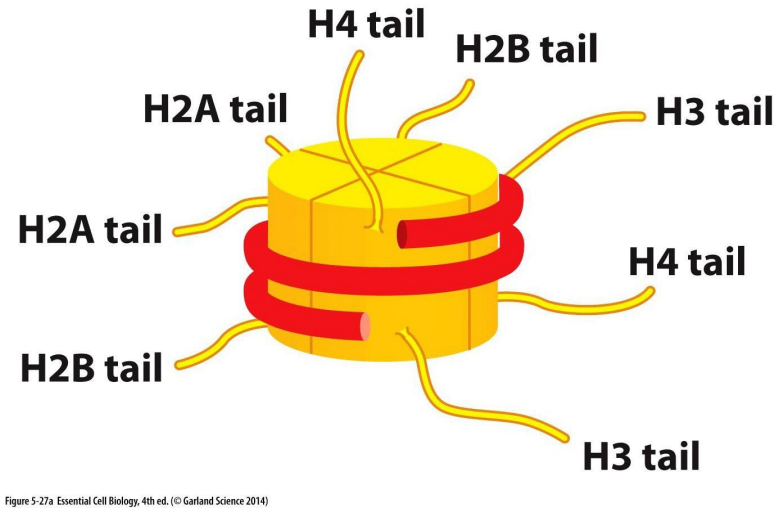


Figure 5-27a Essential Cell Biology, 4th ed. (© Garland Science 2014)

Tails

The N-terminal tails protrude → sites of modifications (B2).

1.7 turns

of a left-handed DNA superhelix around the octamer

142

hydrogen bonds, mostly with the backbone — not with the bases

Bending

A–T-rich sequences bend more easily → positioning “preference”

Alberts et al., Essential Cell Biology, 4th ed., Figs. 5-22 and 5-27 · Luger et al., Nature 1997

IN-CLASS QUESTION #2 · WORK ON PAPER

Count the nucleosomes

Data: diploid genome ≈ 6.2 × 10^9 bp · one nucleosome per ~200 bp · S phase ≈ 8 hours

a

How many nucleosomes does a diploid human cell have?

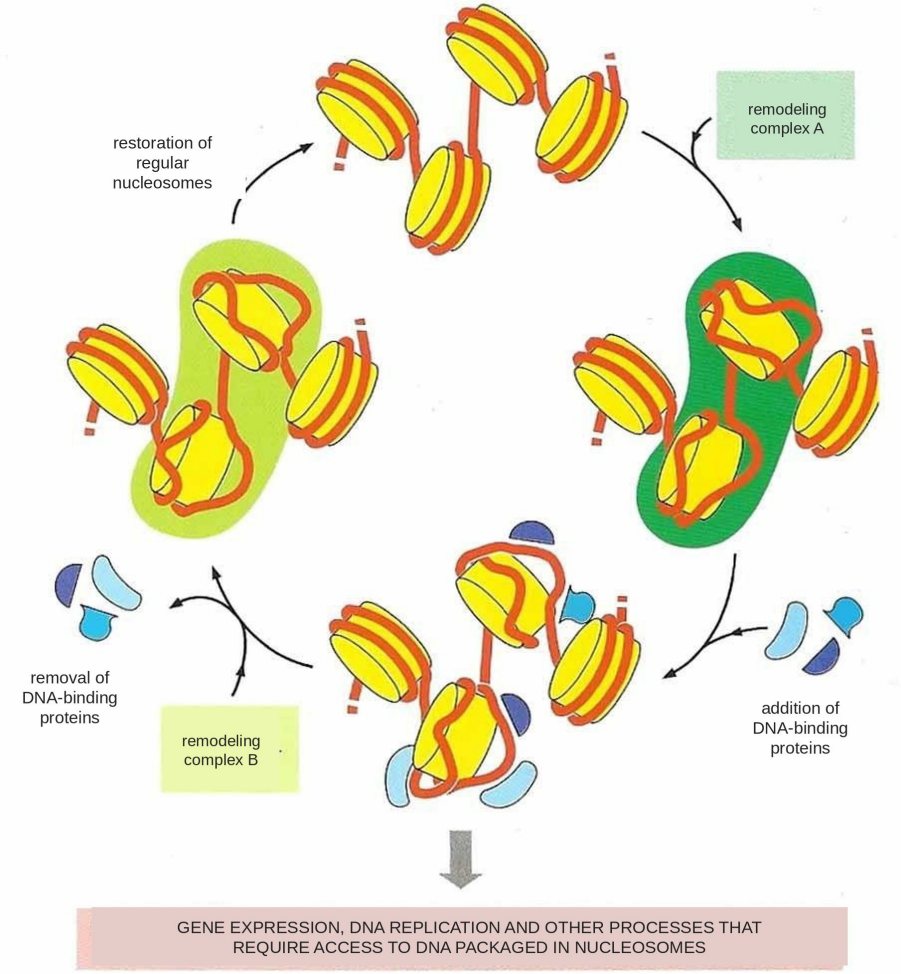
b

How many histone H4 molecules are needed?

c

How many new nucleosomes must be made every second in S phase? What does this tell us about the histone genes?

Nucleosomes are dynamic



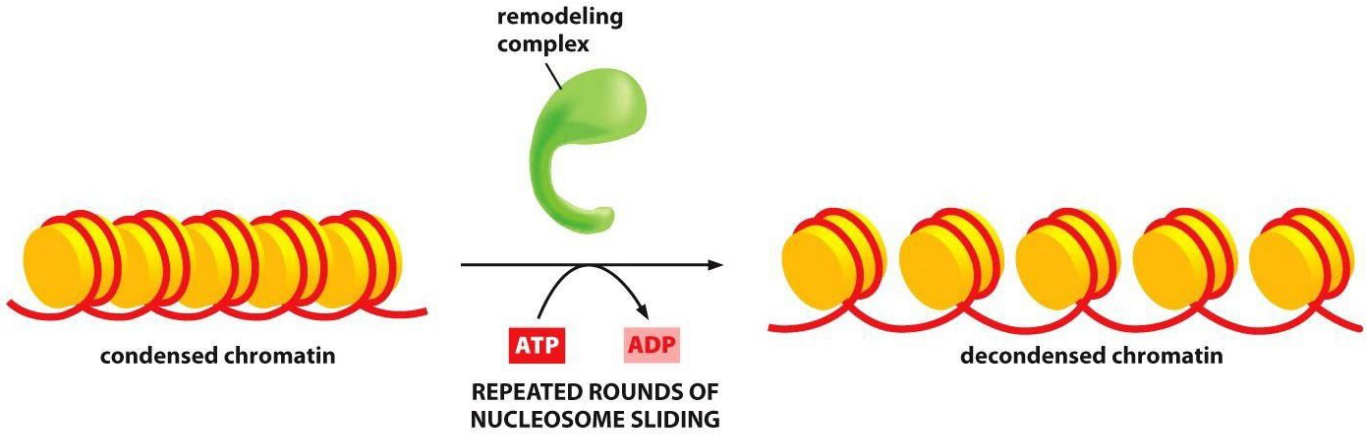


Figure 5-26b Essential Cell Biology, 4th ed. (© Garland Science 2014)

Spontaneously

Each end unwraps ~4 times/s for 10–50 ms.

With ATP

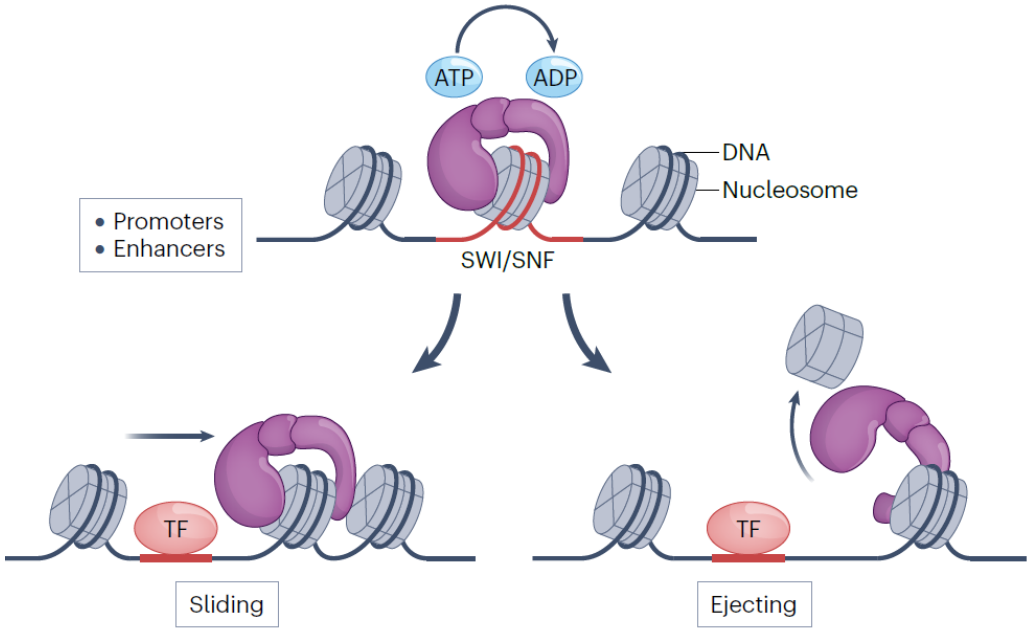
Remodeling complexes: sliding, removal, histone exchange.

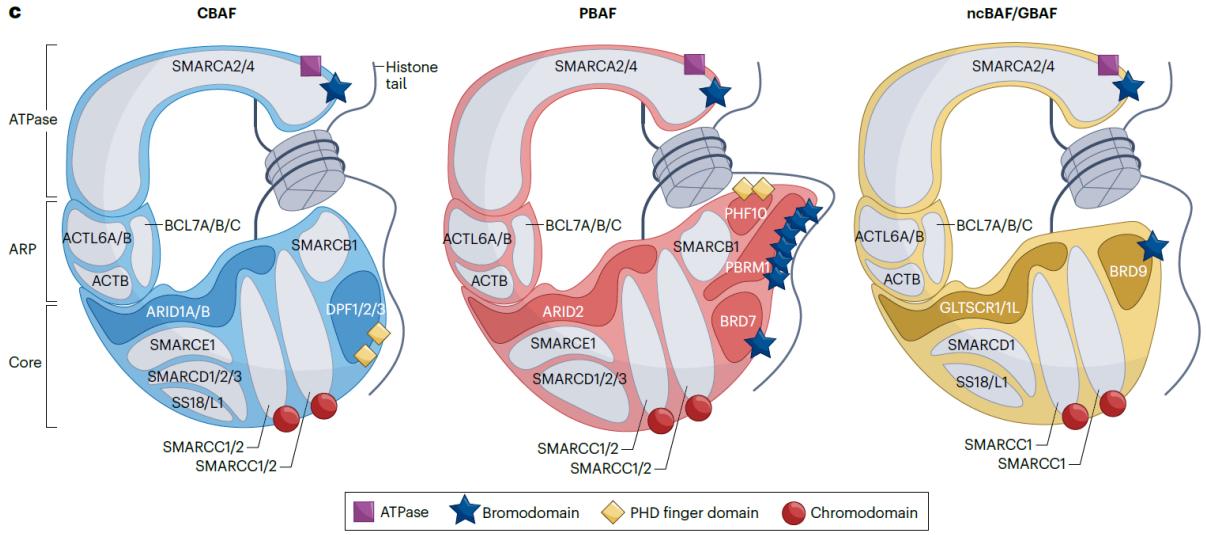
Why?

Transcription, replication and repair need access to DNA.

Alberts et al., Essential Cell Biology, 4th ed., Fig. 5-26 · MBoC 7th ed., Ch. 4

Remodeling complexes: SWI/SNF





4 families

SWI/SNF · ISWI · CHD · INO80

Cancer

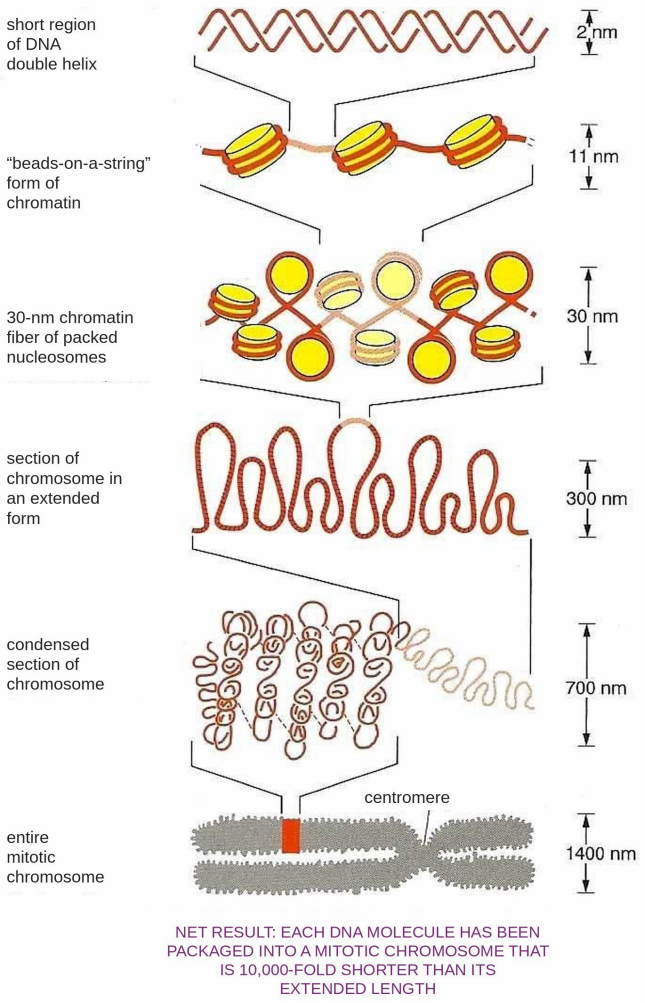
SWI/SNF subunits are mutated in ~20% of cancers (e.g., SMARCB1, ARID1A).

Developmental syndromes

Coffin–Siris (ARID1B, etc.) — germline mutations.

Kadoch et al., Nat Genet 2013 · Honer et al., Genes Dev 2024 · cBAF/PBAF/ncBAF structures: course material

Packaging levels: the classic scheme

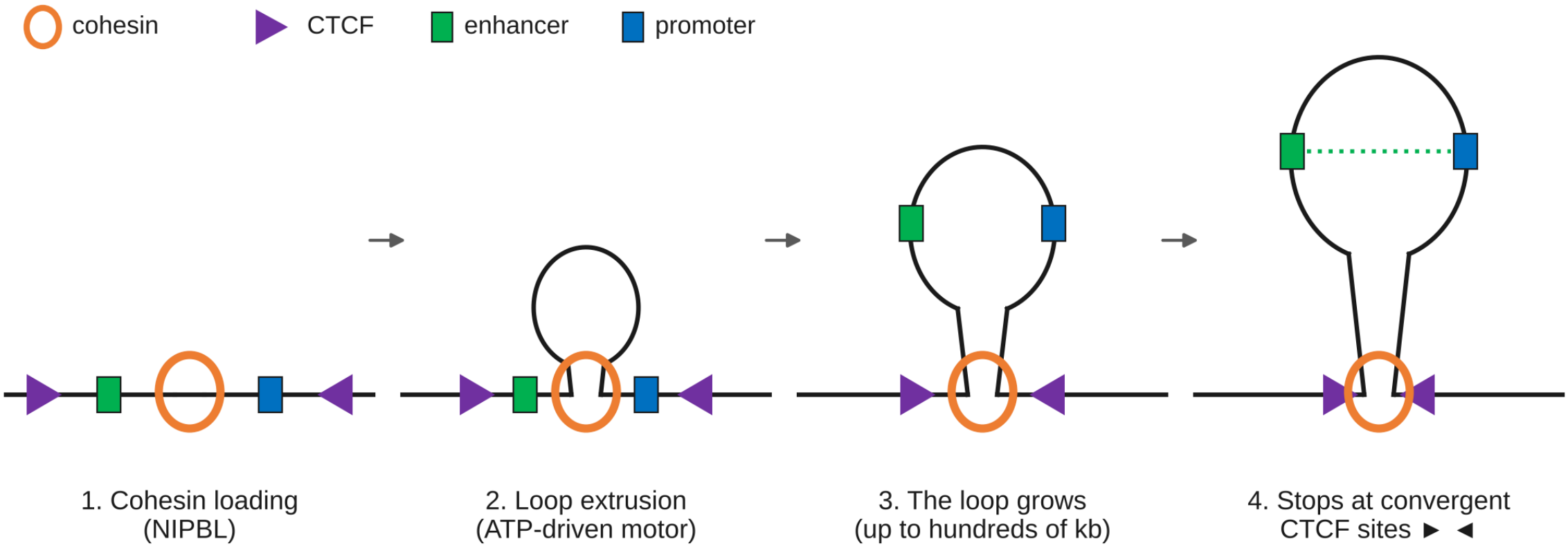


Level	Thickness	What stabilizes it
DNA double helix	2 nm	base pairing & base stacking
Nucleosomes	11 nm	histone octamer
Chromatin fiber	~10–30 nm	H1, histone tails (variable)
Loops / TADs	0.1–1 Mb	cohesin, CTCF
Mitotic chromosome	~1.4 μm	condensins, axis

Result: each DNA molecule becomes a mitotic chromosome ~10,000 times shorter than its extended length. The scheme is a useful model — not a literal picture (see previous slide).

Alberts et al., Essential Cell Biology, 4th ed., Fig. 5-30

Chromatin loops: “loop extrusion”



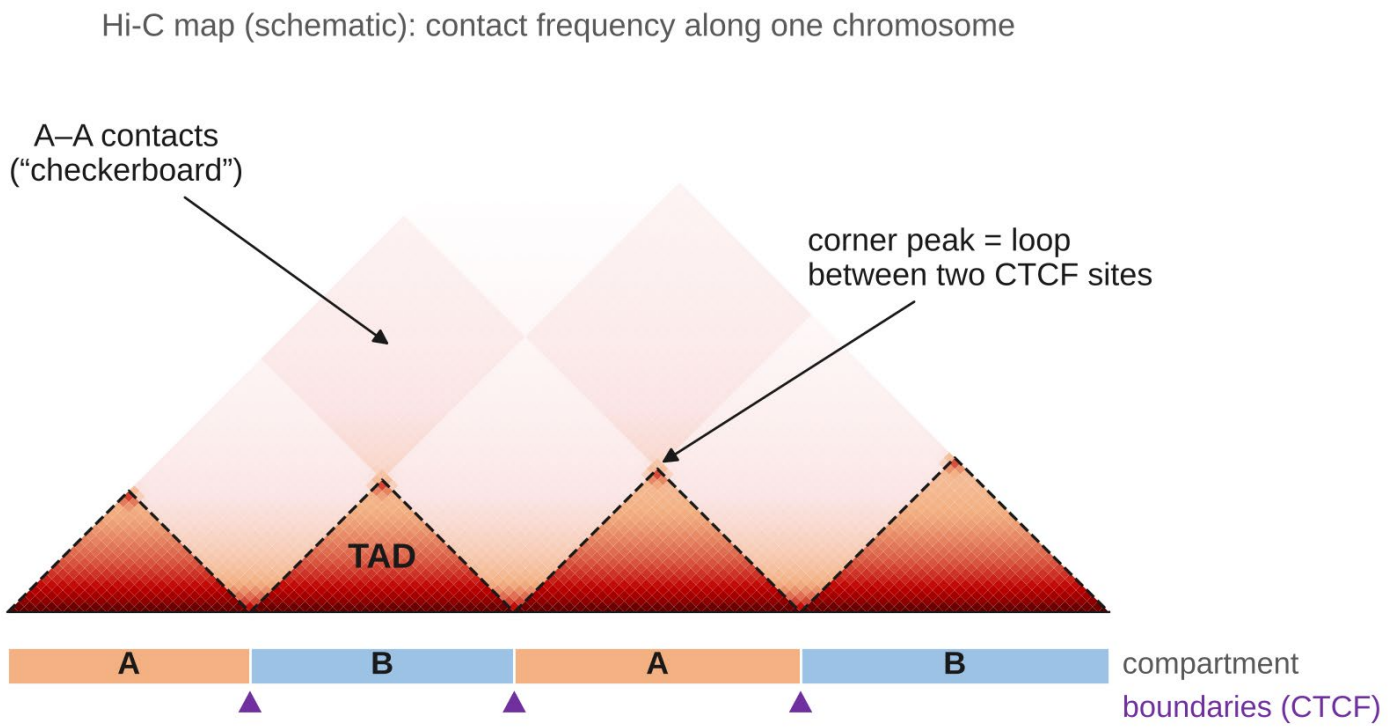
Cohesin (interphase) · **condensins** (mitosis)

Direct observation: in vitro 2018–2019 · in living cells 2025

Clinical: Cornelia de Lange syndrome (NIPBL)

Ganji et al., Science 2018 · Davidson et al. & Kim et al., Science 2019 · Sabaté et al., Nat Genet 2025

TADs and compartments: the Hi-C map



Hi-C

Measures which regions of the genome lie close together in space.

TADs (0.1–1 Mb)

“Neighborhoods” where contacts stay inside; boundaries marked by CTCF.

~10,000 loops

Mostly between convergent CTCF sites.

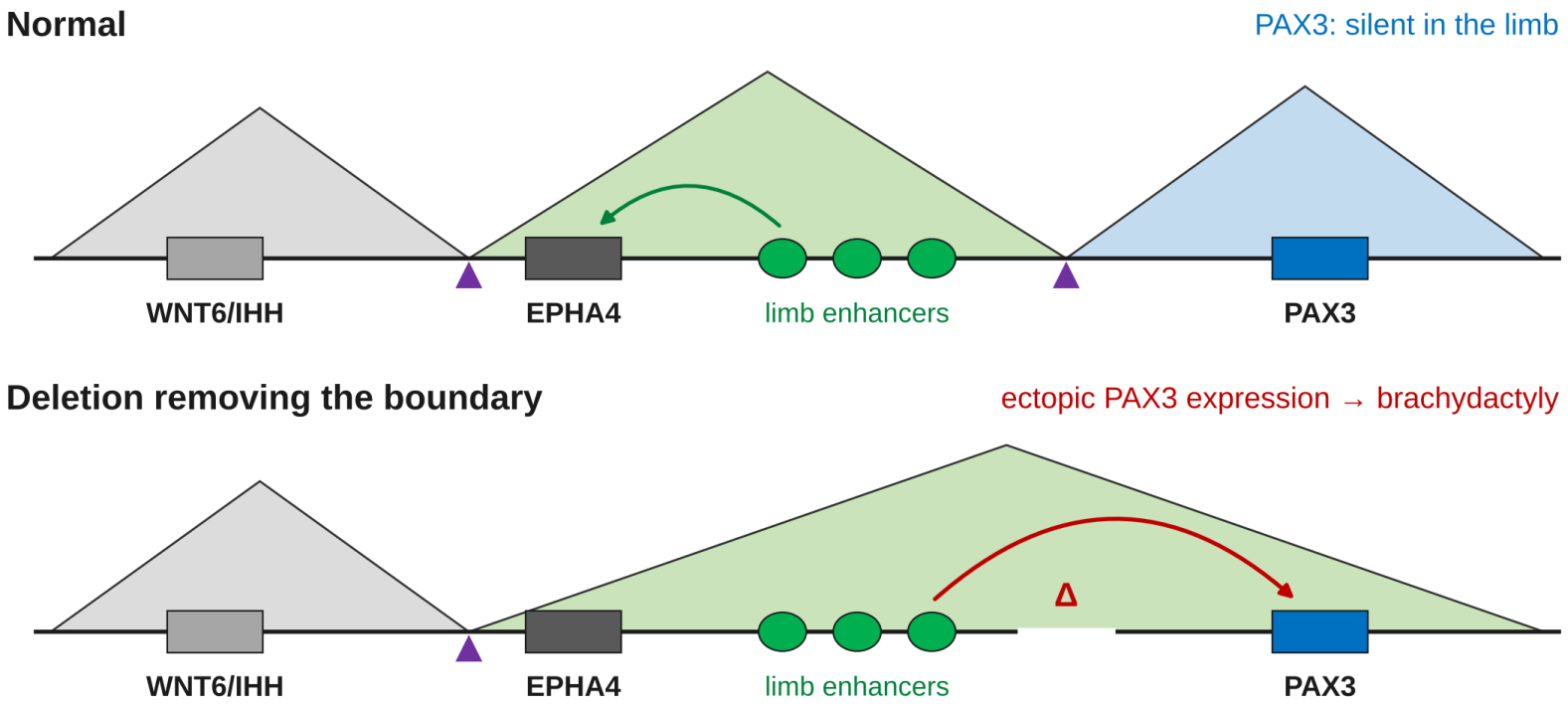
A / B

A: active, in the interior · B: inactive, at the periphery.

Within a TAD, an enhancer “finds” its promoter; the boundaries prevent wrong connections.

Lieberman-Aiden et al., Science 2009 · Dixon et al., Nature 2012 · Rao et al., Cell 2014

When a TAD boundary breaks



Boundary deletion
→ PAX3 → brachydactyly

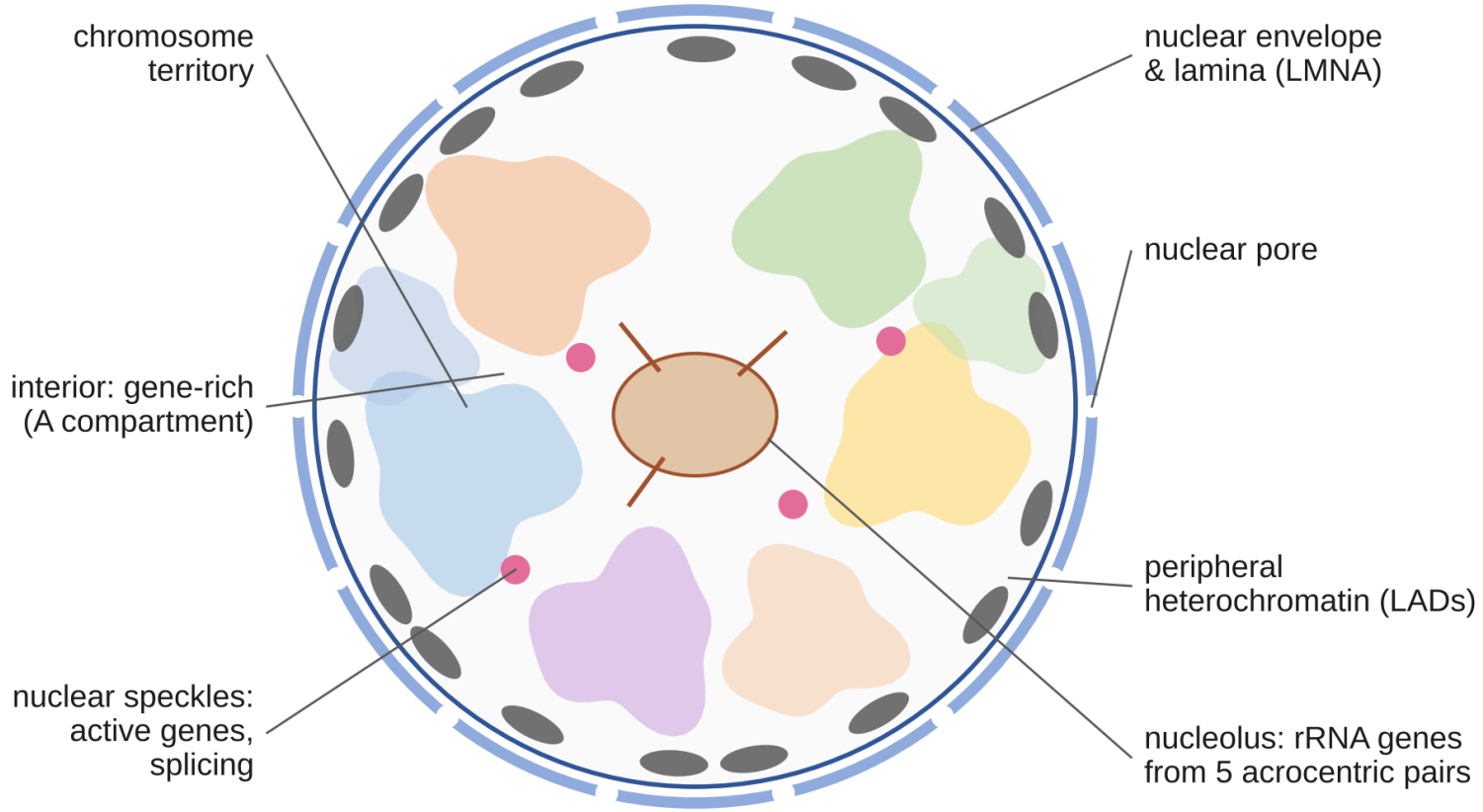
Inversion
→ WNT6 → F-syndrome

Duplication
→ IHH → polydactyly

Message: a mutation that alters no gene can still cause disease by changing the 3D “wiring” — critical for interpreting genomic tests.

Lupiáñez et al., Cell 2015

The nucleus: an organized space



Organization without membranes: compartments formed by interactions of DNA, RNA and proteins.

Nuclear lamina

Lamin network beneath the envelope; “anchors” heterochromatin (LADs).

Nucleolus

Forms around the rRNA genes of the acrocentric chromosomes.

Clinical

Hutchinson–Gilford progeria (LMNA); lonafarnib, FDA 2020.

Misteli, Cell 2020 · Alberts et al., MBoC 7th ed., Chs. 4 and 6

Summary B1

1. Nucleosome: 147 bp around a histone octamer — the basic, dynamic unit.
2. Remodeling complexes and histone chaperones open and close access to DNA.
3. The regular 30 nm fiber is an in vitro model; in vivo, chromatin is irregular.
4. Loops (cohesin/CTCF) → TADs → compartments → territories: the genome in 3D.

Videos for review

HHMI: How DNA Is Packaged

How is DNA packaged?

Chromatin, histones and modifications

B2 · Chromatin, expression and disease

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

1

Why does a gene “go silent” when it is moved to a different location?

2

How does a cell “remember” which genes are active after each division?

3

What happens when the chromatin “machinery” itself is mutated?

The answer to all three: chromatin is not just packaging — it is a regulator.

Euchromatin and heterochromatin

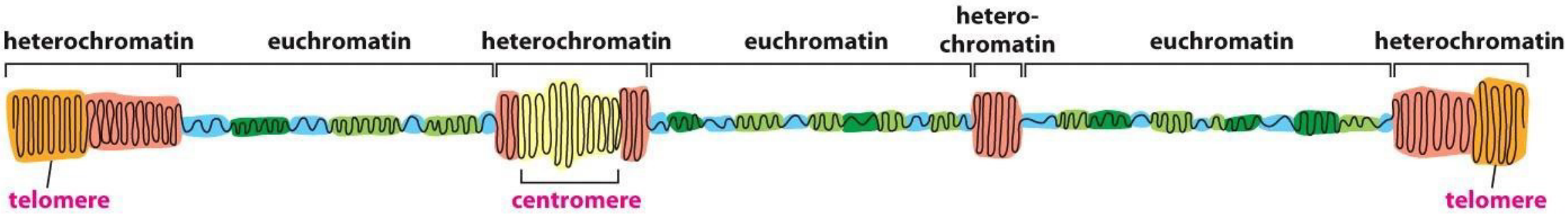


Figure 5-28 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Euchromatin

- less condensed
- rich in active genes
- acetylation, H3K4me3
- compartment A, interior

Constitutive heterochromatin

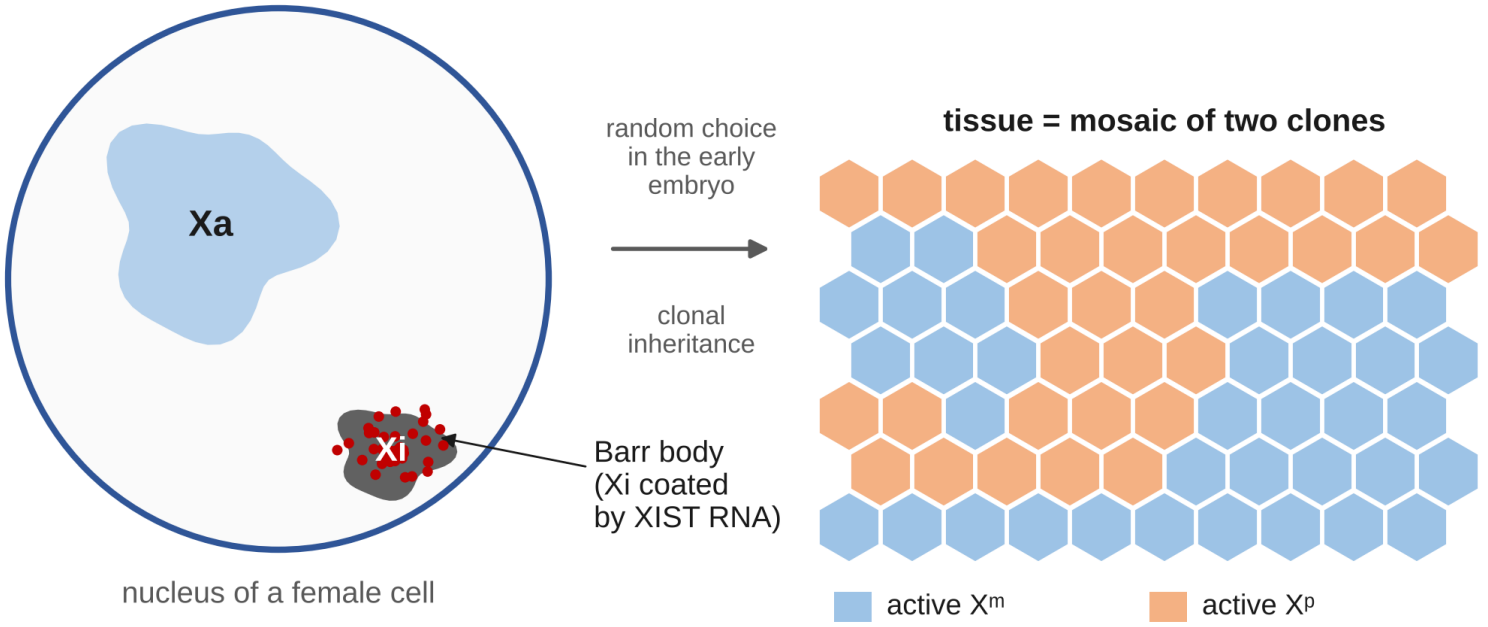
- centromeres, telomeres, satellites
- H3K9me3 + HP1
- same in all cells
- genome stability

Facultative heterochromatin

- developmental genes, Xi
- H3K27me3 (Polycomb)
- differs by cell type
- cell identity

Alberts et al., Essential Cell Biology, 4th ed., Fig. 5-28 · Allis & Jenuwein, Nat Rev Genet 2016

X-chromosome inactivation



XIST

Noncoding RNA that coats the X → H3K27me3, macroH2A, DNA methylation.

Dosage compensation

One active X in every cell, in both XX and XY.

Clinical relevance

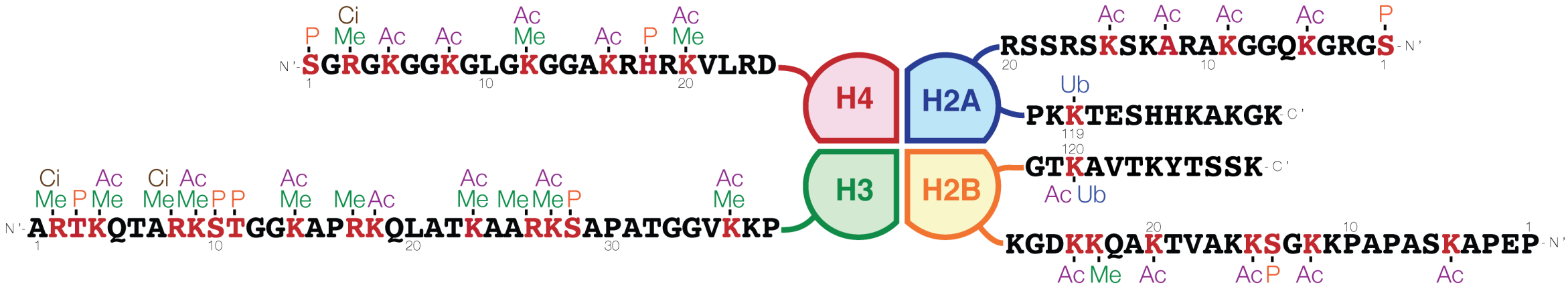
Heterozygous carriers of X-linked diseases are mosaics; “skewed” inactivation explains mild symptoms.

New (2024)

XIST–protein complexes are autoantigens — a possible explanation for the higher frequency of autoimmunity in women.

Dou et al., Cell 2024 · Alberts et al., MBoC 7th ed., Ch. 7

Histone tail modifications



Ac

Acetylation
lysines (K)

Me

Methylation
K (me1/2/3) and R

P

Phosphorylation
S, T, Y

Ub

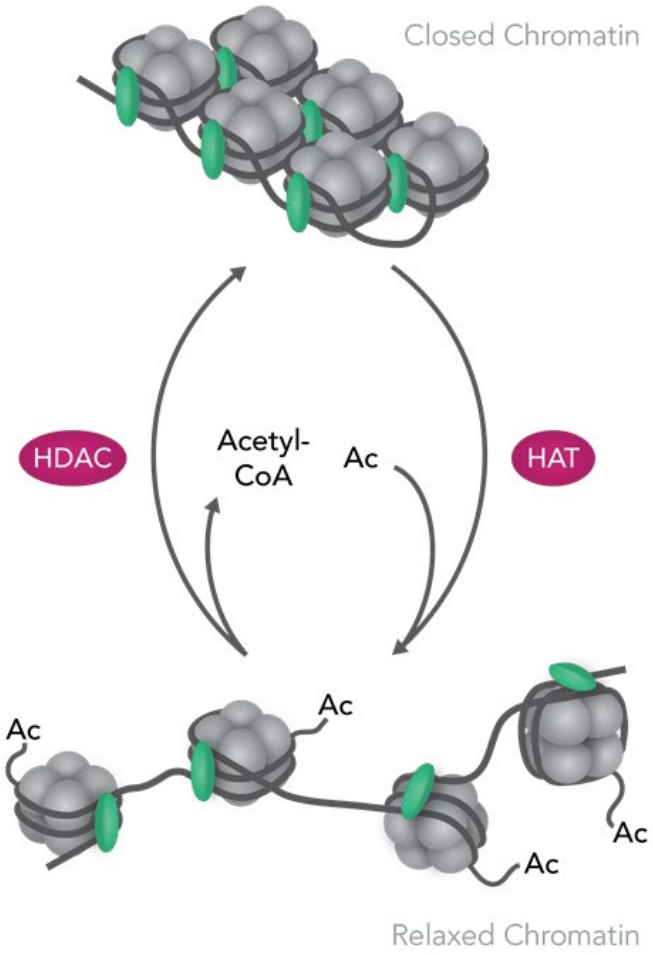
Ubiquitination
lysines (K)



C. David Allis (1951–2023) · 2018 Lasker Award with M. Grunstein

Rodríguez-Paredes & Esteller, Nat Med 2011 · Allis & Jenuwein, Nat Rev Genet 2016

Acetylation “loosens” chromatin



Lysine
$-CH_2-NH_3^+$
positive charge

HAT →

← HDAC

Acetyl-lysine
$-NH-CO-CH_3$
neutral

Weaker histone–DNA attraction → more “open” chromatin, access for factors.

New binding site → bromodomains “read” acetyl-lysine and bring in activators.

Result → accessible promoter, increased transcription.

Alberts et al., Essential Cell Biology, 4th ed., Chs. 5 and 8 · image: course material

Writers, Erasers and Readers

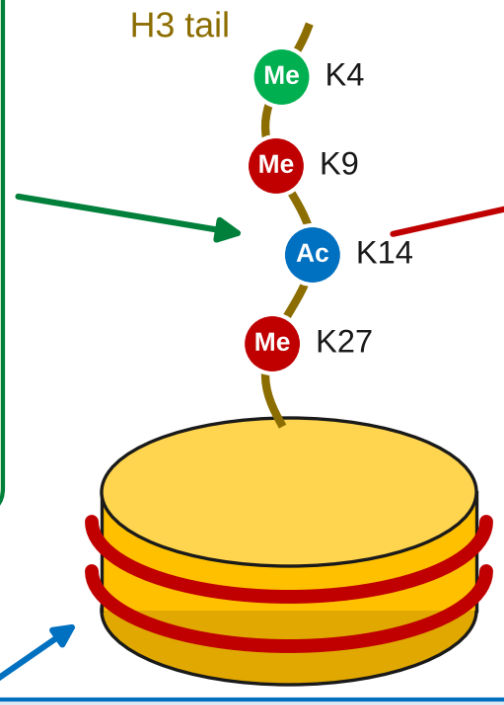
WRITERS · add marks

HATs (CBP/p300) → acetylation

HMTs: EZH2 (H3K27me3),

SUV39H1 (H3K9me3), MLL (H3K4me3)

drug: tazemetostat (EZH2 inhibitor)



ERASERS · remove marks

HDACs → deacetylation

KDMs: LSD1, JmjC

→ demethylation

drugs: vorinostat, givinostat (HDAC inhibitors)

READERS · bind marks and recruit partners

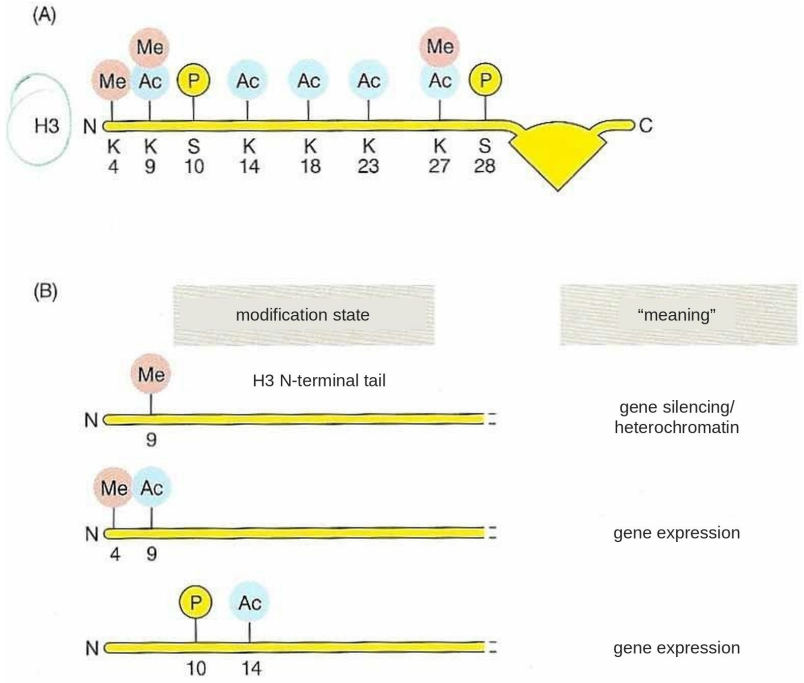
bromodomain → Ac (BRD4) · chromodomain → Me (HP1: H3K9me3 · Polycomb: H3K27me3) · PHD → H3K4me3

The modifications are reversible — which is why the enzymes that add or remove them are drug targets.

Allis & Jenuwein, Nat Rev Genet 2016 · Honer et al., Genes Dev 2024

What does each mark “mean”?

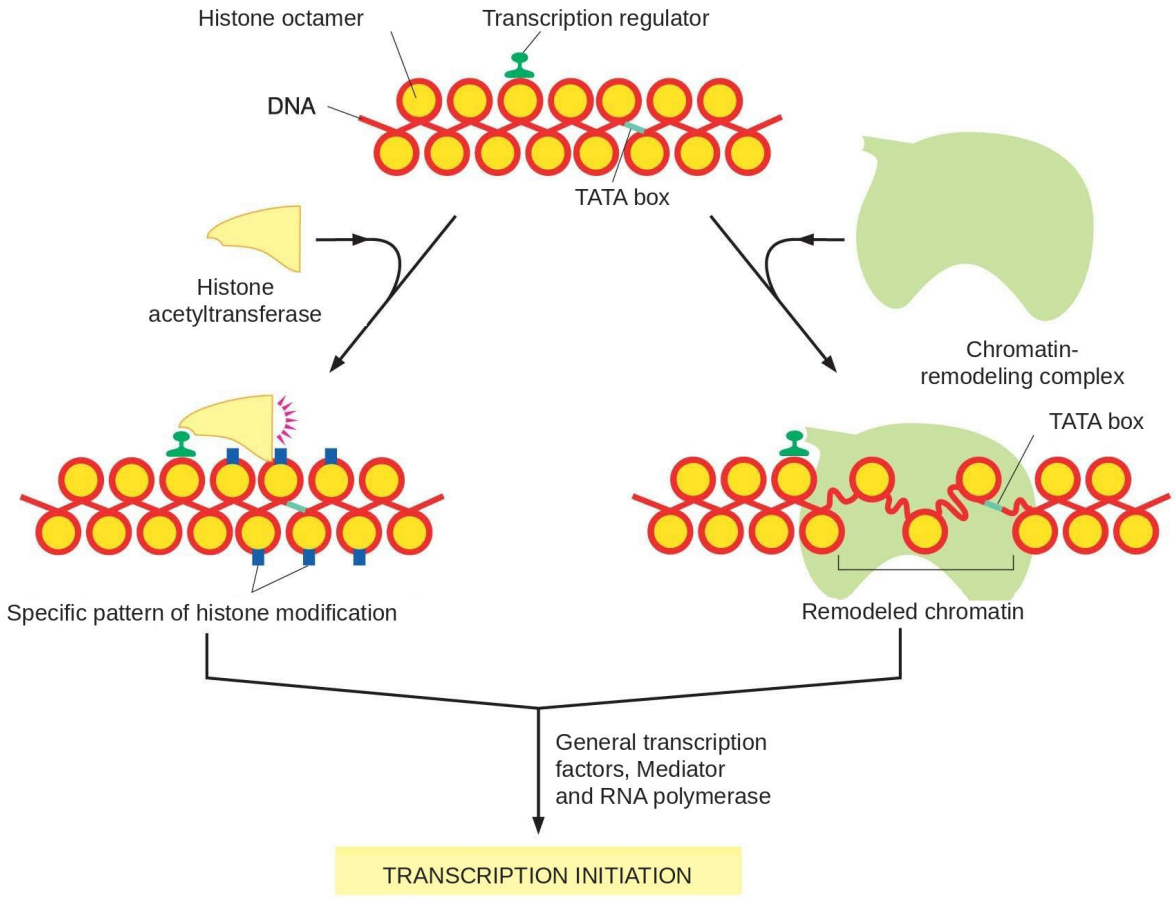
Mark	Where we find it	Meaning
H3K4me3	promoters	active genes
H3K27ac	enhancers and promoters	active regulation
H3K36me3	body of transcribed genes	elongation, transcriptional fidelity
H3K9me3	centromeres, repeats	constitutive heterochromatin (HP1)
H3K27me3	developmental genes, Xi	facultative silencing (Polycomb)

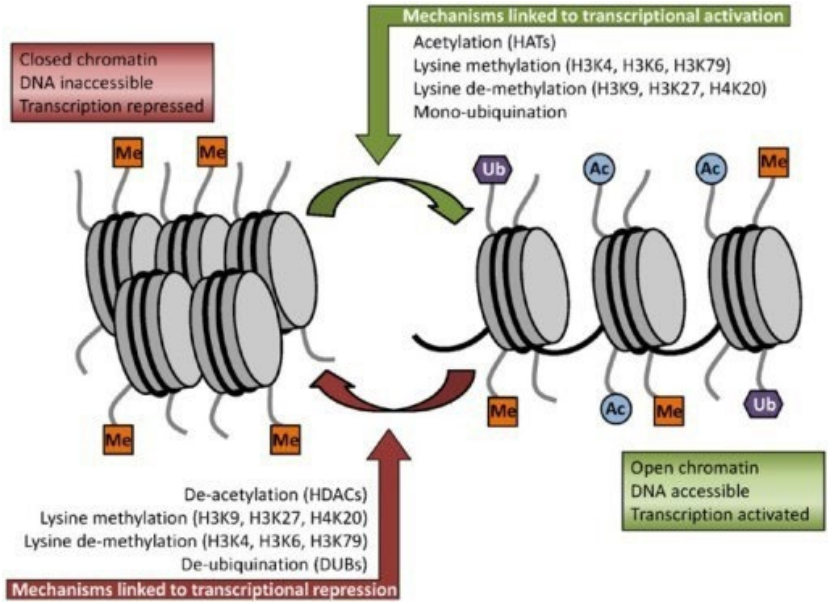


The combination matters: e.g., H3K4me3 + H3K27me3 together = “bivalent” promoters in stem cells — poised to switch on or off during differentiation.

Allis & Jenuwein 2016 · Alberts et al., Essential Cell Biology, 4th ed., Fig. 5-27

Who brings enzymes to the right site?





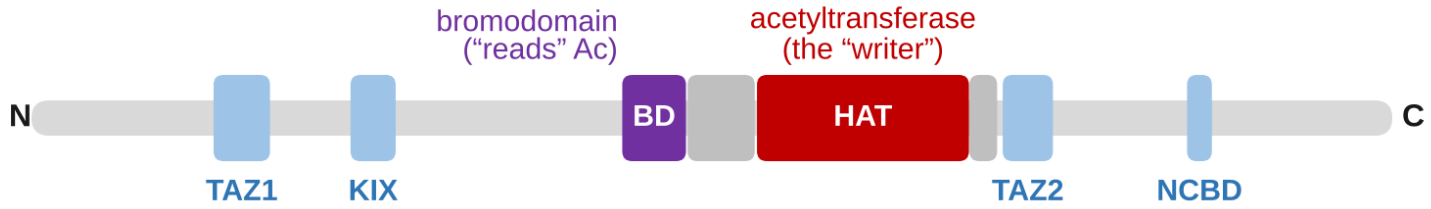
Coming up

Details in the lecture “Regulation of gene expression” (Ch. 8): enhancers, Mediator, repressors.

Alberts et al., Essential Cell Biology, 4th ed., Fig. 8-11

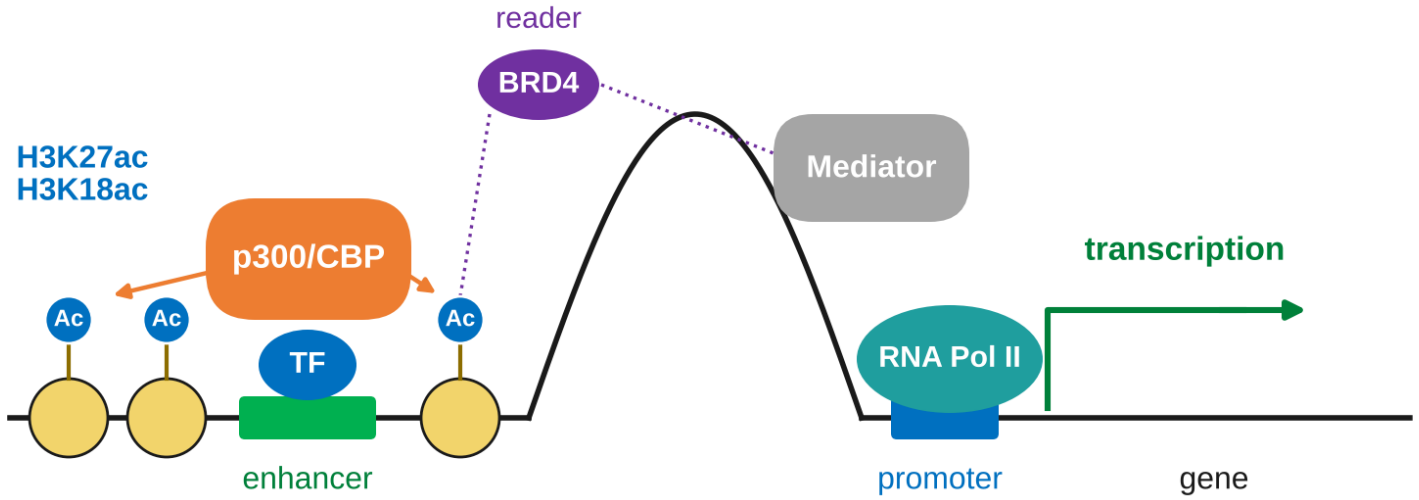
EP300/CBP: acetylation writers

A. Organization of the EP300 protein (2,414 amino acids; schematic)



blue: transcription-factor binding regions

B. At the enhancer: from transcription factor to transcription



Acetyl-CoA → acetyl groups on lysines: less positive charge, a binding site for bromodomains

Coactivators

Bind hundreds of transcription factors and write H3K27ac/H3K18ac at active enhancers.

Not only histones

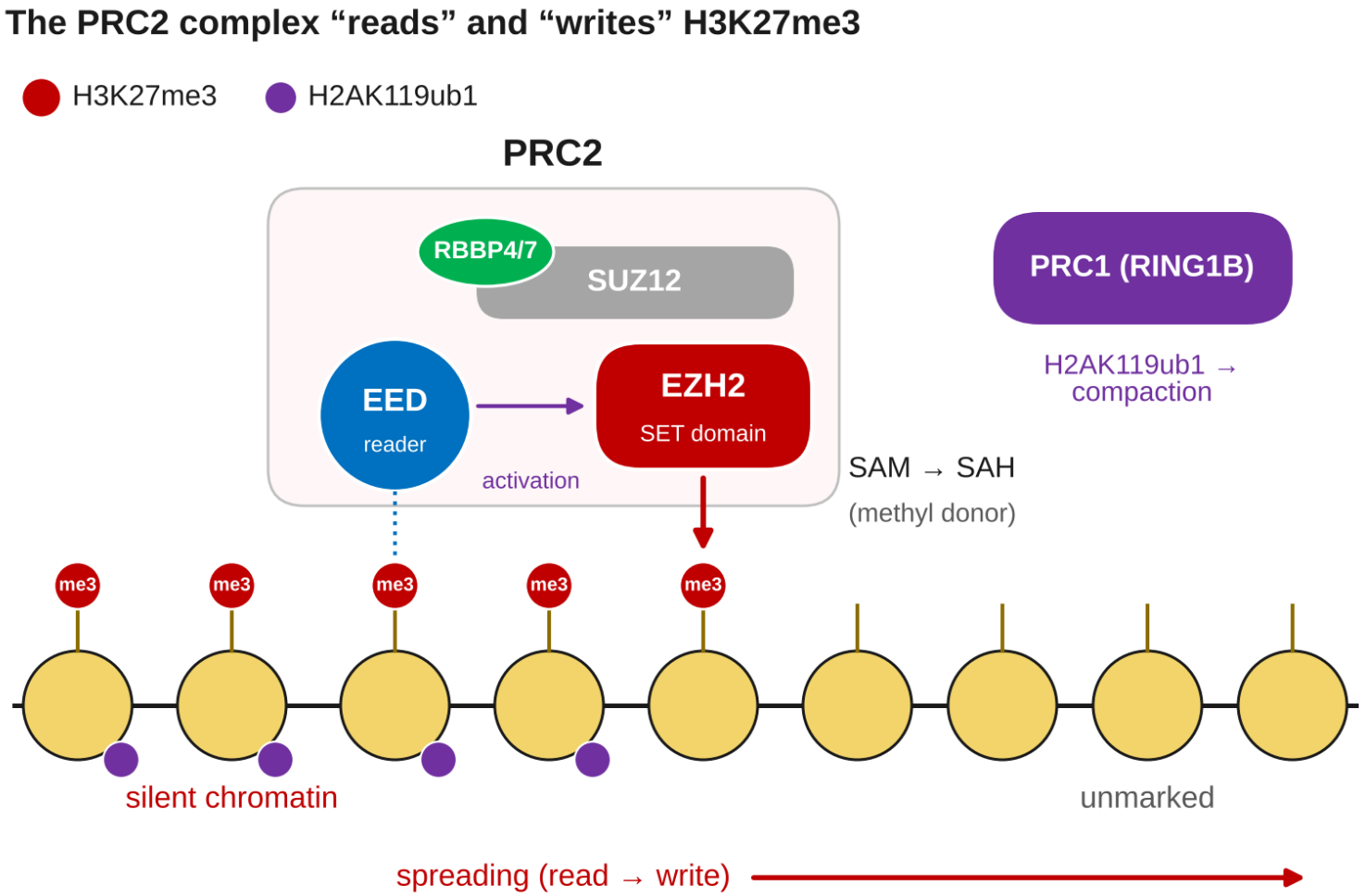
They also acetylate p53 and other transcription factors.

Clinical

Rubinstein–Taybi syndrome: CREBBP or EP300 haploinsufficiency. Lymphomas: CREBBP/EP300 inactivated in ~40% of DLBCL and FL.

Ogryzko et al., Cell 1996 · Gu & Roeder, Cell 1997 · Visel et al., Nature 2009 · Petrij et al., Nature 1995 · Pasqualucci et al., Nature 2011

EZH2/PRC2: Polycomb silencing



Mechanism

EZH2 (SET domain) transfers methyl groups from SAM to H3K27. EED “reads” H3K27me3 and activates the complex → spreading and inheritance.

Physiology

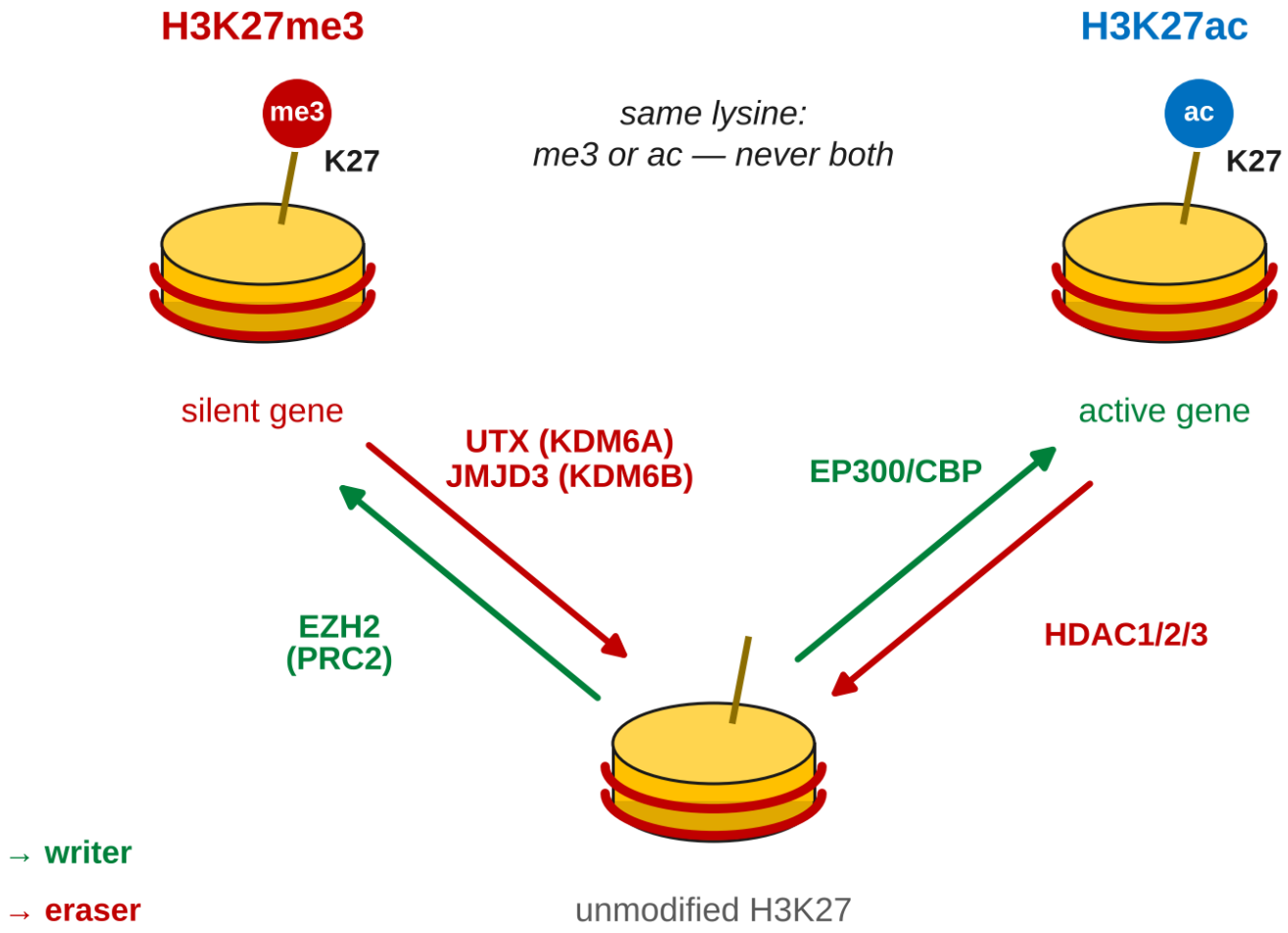
Silencing of developmental genes (e.g., HOX); Xi; cell identity.

Clinical

Gain of function: EZH2 Y641 in ~22% of GCB-DLBCL → tazemetostat (FDA 2020). Loss: Weaver syndrome. H3 K27M inhibits PRC2.

Cao et al., Science 2002 · Margueron et al., Nature 2009 · Morin et al., Nat Genet 2010 · Gibson et al., Am J Hum Genet 2012

The H3K27 “switch”: me3 or ac



Mutually exclusive

The same lysine cannot be methylated and acetylated at once; changing state first requires an eraser.

In differentiation

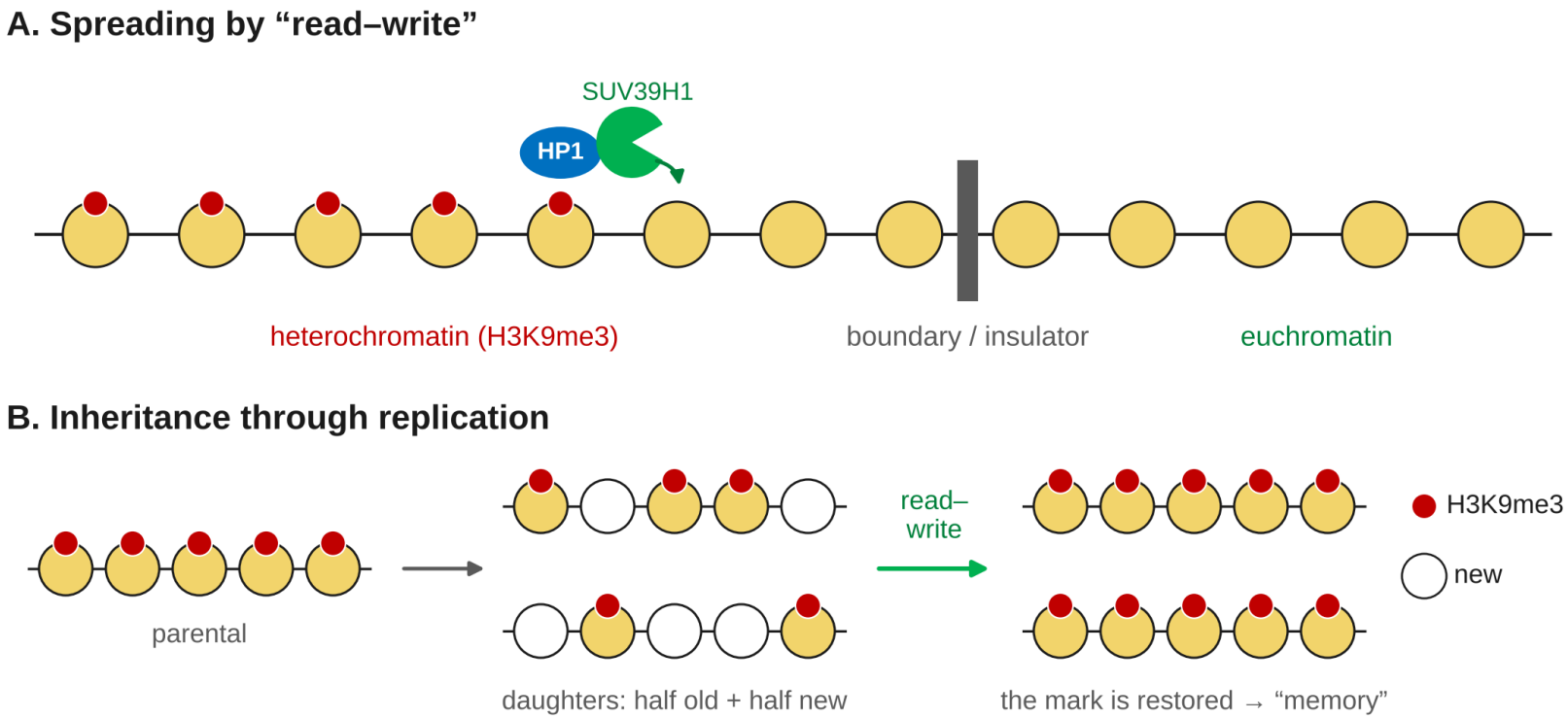
A developmental gene “switches on” when UTX removes me3 and p300 acetylates the same lysine.

In cancer

In lymphomas, CREBBP loss and EZH2 gain of function push toward silencing; this is the rationale for EZH2 and HDAC inhibitors.

Agger et al., Nature 2007 · Pasqualucci et al., Nature 2011 · Morin et al., Nat Genet 2010 · Allis & Jenuwein, Nat Rev Genet 2016

Read–write: spreading and memory



HP1 reads H3K9me3 and brings in SUV39H1 → the mark spreads.

Boundaries (insulators) stop the spreading.

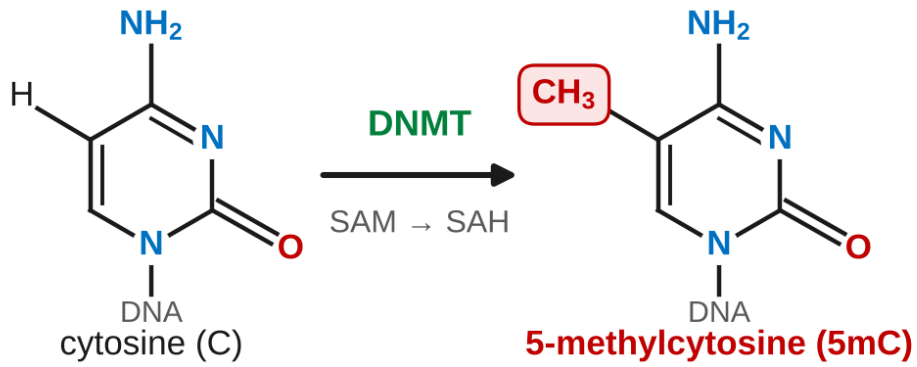
Phase separation: HP1 forms droplets (2017).

Epigenetics: a heritable change in gene function without a change in DNA sequence — histones · DNA methylation · noncoding RNAs.

Allis & Jenuwein 2016 · Larson et al. & Strom et al., Nature 2017

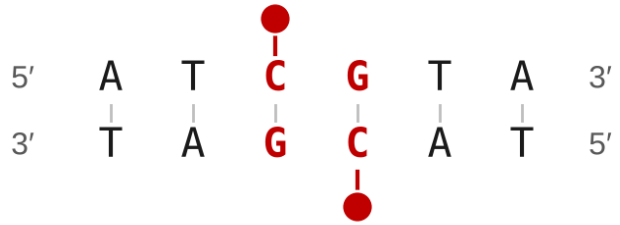
DNA methylation: 5-methylcytosine

A. The reaction



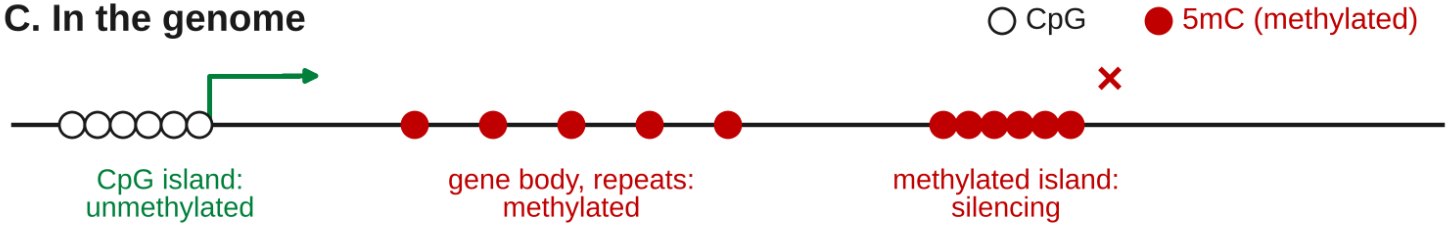
The methyl group at position 5 projects into the major groove without altering pairing with G

B. Target: the CpG dinucleotide (symmetric)



After replication, the "parental" strand shows where the new one must be methylated

C. In the genome



~32 million

CpGs in the genome (T2T)

70–80%

of CpGs methylated

~72%

of promoters with CpG islands

5mC → T

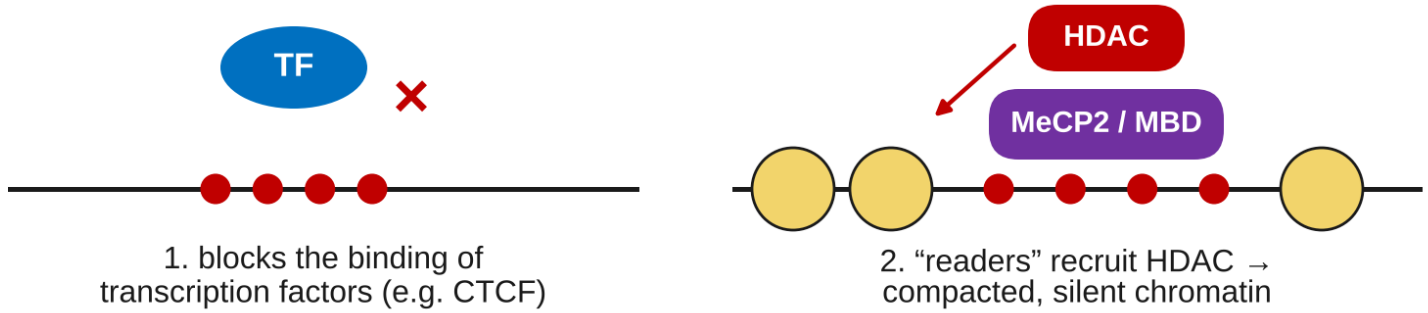
deamination: a mutation "hotspot"

Why so few CpGs? 5mC deaminates to T; during evolution most CpGs were lost (~¼ of expected remain), except in the islands, which stay unmethylated.

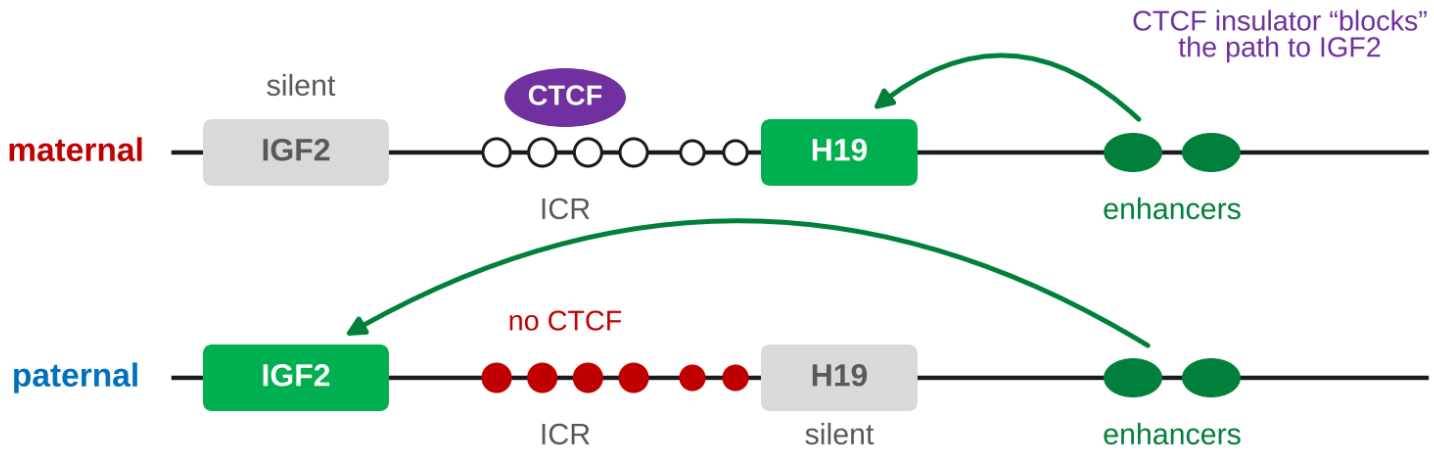
Gershman et al., Science 2022 · Lister et al., Nature 2009 · Saxonov et al., PNAS 2006 · Bird, Genes Dev 2002

DNA methylation: silencing, imprinting

A. How a methylated gene is “silenced”



B. Genomic imprinting: H19/IGF2 (11p15.5)



Functions

Silencing of transposable elements and repeats · imprinting · X inactivation · stable cell identity.

Imprinting

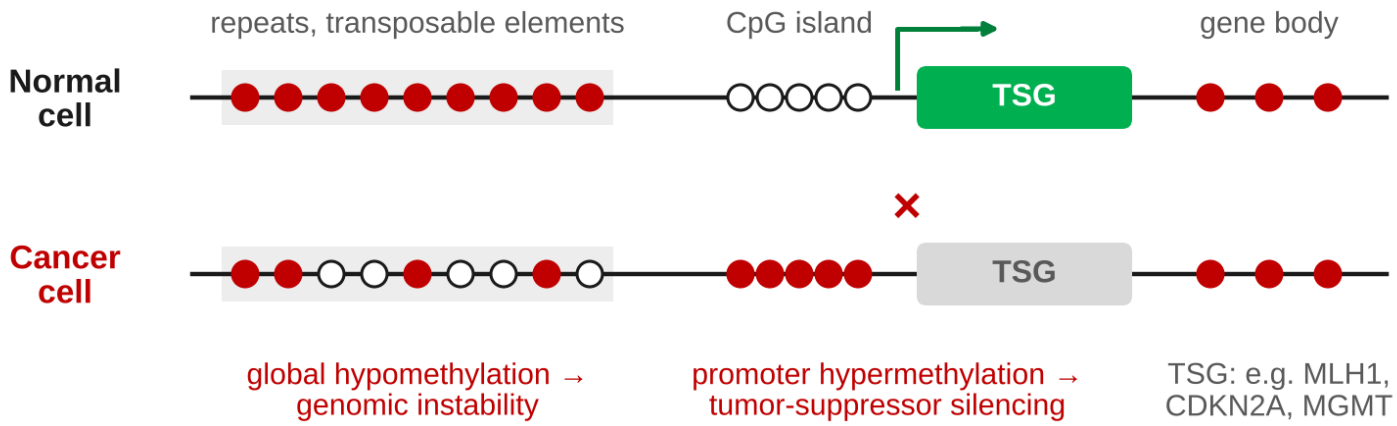
~100–200 genes are expressed only from the maternal or only from the paternal allele; the “mark” is set in the gametes.

Clinical

Prader–Willi/Angelman (15q11–q13) · Beckwith–Wiedemann/Silver–Russell (11p15): diagnosis by methylation testing.

Bell & Felsenfeld, Nature 2000 · Hark et al., Nature 2000 · Nan et al., Nature 1998 · Greenberg & Bourc’his, Nat Rev Mol Cell Biol 2019

DNA methylation and disease



Genetic syndromes

Fragile X: (CGG)n >200 → hypermethylation and silencing of FMR1 · Rett: MECP2 · ICF: DNMT3B.

Hematology

DNMT3A mutated in ~22% of AML; DNMT3A/TET2 in age-related clonal hematopoiesis. IDH1/2 → 2-HG → TET inhibition.

Diagnosis and prognosis

MGMT methylation → better response to temozolomide (glioblastoma). Classification of CNS tumors by methylation profile.

"Epigenetic clocks"

Methylation at ~350 CpGs estimates the biological age of a tissue (Horvath 2013).

Drugs

Azacitidine, decitabine (MDS, AML) · vorasidenib (IDH inhibitor, glioma, FDA 2024).

Herman & Baylin 2003 · Hegi et al. 2005 · Capper et al. 2018 · Horvath 2013 · Ley et al. 2010 · Jaiswal et al. 2014 · Figueroa et al. 2010 · Mellinghoff et al. 2023

Chromatinopathies

Syndrome	Gene	Role	Syndrome	Gene	Role
Rubinstein–Taybi	*CREBBP, EP300*	**writer (HAT)**	CHARGE	*CHD7*	**remodeler**
Kabuki	*KMT2D, KDM6A*	**writer / eraser**	Coffin–Siris	*ARID1B, SMARCB1…*	**remodeler (BAF)**
Sotos	*NSD1*	**writer (H3K36)**	ATR-X	*ATRX*	**remodeler**
Weaver	*EZH2*	**writer (H3K27)**	Cornelia de Lange	*NIPBL, SMC1A*	**cohesin (loops)**
Rett	*MECP2*	**reader (5mC)**	ICF	*DNMT3B*	**DNA methylation**

Common features

Intellectual disability, growth disorders, multisystem anomalies.

Why?

Dosage sensitivity: a single mutated allele is enough (haploinsufficiency).

Bjornsson, Genome Res 2015 · Honer et al., Genes Dev 2024

Drugs that target chromatin

ΠΑΝΕΠΙΣΤΗΜΙΟ ΠΑΤΡΩΝ
UNIVERSITY OF PATRAS

2004

Azacitidine
DNMT inhibitor · MDS

2006

Vorinostat
HDAC inhibitor · CTCL

2020

Tazemetostat
EZH2 inhibitor · epithelioid sarcoma

2024

Revumenib · Givinostat
menin–KMT2A (leukemias) · HDAC (Duchenne)

2025

Ziftomenib · Dordaviprone
menin (NPM1 AML) · H3 K27M glioma

Rationale
Epigenetic changes are reversible — unlike mutations.

Challenge
Selectivity: the enzymes act across the whole genome → side effects.

FDA approval dates · Honer et al., Genes Dev 2024

Summary

1. The genome (3.1 billion bp) is divided among 23 pairs of chromosomes; the karyotype and molecular methods “read” their structure.
2. Every chromosome needs replication origins, a centromere and telomeres.
3. Nucleosome: 147 bp around a histone octamer — the basic, dynamic unit of chromatin.
4. Loops (cohesin/CTCF), TADs and compartments organize the genome in space; their disruption causes disease.
5. Eu-/heterochromatin, histone modifications (writers–erasers–readers) and DNA methylation control expression and “remember.”
6. Mutations in the chromatin machinery → chromatinopathies and cancer; new drugs target it.

I. Comment briefly (1–3 sentences): true or false?

A Homologous chromosomes always lie next to each other in the interphase nucleus.

B A G-banded karyotype can detect a 50 kb deletion.

C Acetylation of lysines in histone tails neutralizes their positive charge.

D Chromosome 21 is the smallest human autosome.

E Heterochromatin consists only of noncoding DNA and is the same in all cells.

For study and review

Animations & videos

HHMI BioInteractive: How DNA Is Packaged

How is DNA packaged?

Chromatin, histones and modifications

Mitotic division (animation)

NCBI Genome Data Viewer (chromosomes)

References

Alberts et al. Essential Cell Biology, 6th ed. (2023), Ch. 5 · Molecular Biology of the Cell, 7th ed. (2022), Ch. 4

Nurk et al. Science 2022 (T2T) · Altemose et al. Science 2022

Misteli, Cell 2020 · Ou et al., Science 2017 · Davidson et al., Science 2019

Rao et al., Cell 2014 · Lupiáñez et al., Cell 2015

Allis & Jenuwein, Nat Rev Genet 2016 · Bjornsson, Genome Res 2015

Kouzarides, Cell 2007 · Margueron et al., Nature 2009

Greenberg & Bourc'his, Nat Rev Mol Cell Biol 2019 · Bird, Genes Dev 2002

Honer et al., Genes Dev 2024 · Arrillaga-Romany et al., J Clin Oncol 2024

Next lectures: DNA replication and repair (Ch. 6) · From DNA to protein (Ch. 7) · Regulation of gene expression (Ch. 8). Questions: idrosos@upatras.gr