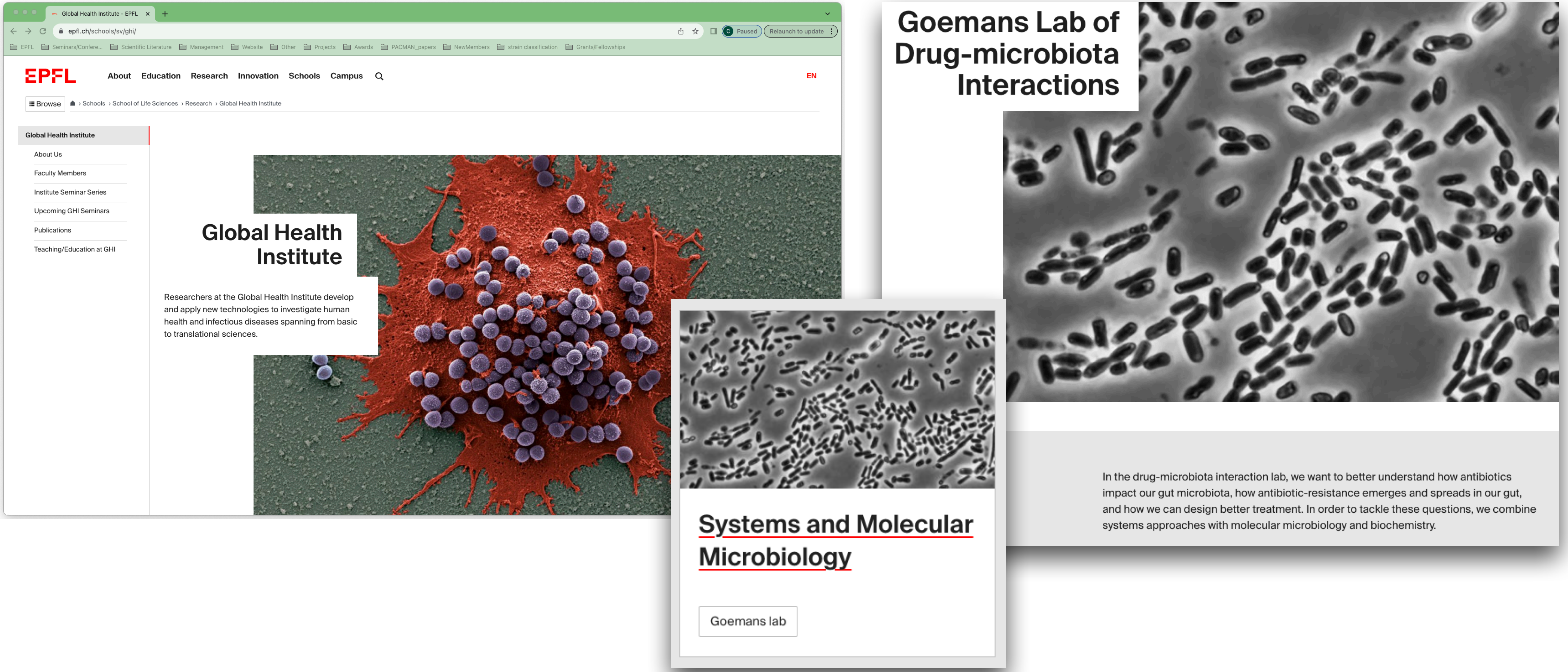
Welcome to Cellular and Molecular Biology I

BIO-205-1

Prof. Camille Goemans
EPFL-SV-GHI

Research interests of the Goemans Lab

Systems and Molecular Microbiology



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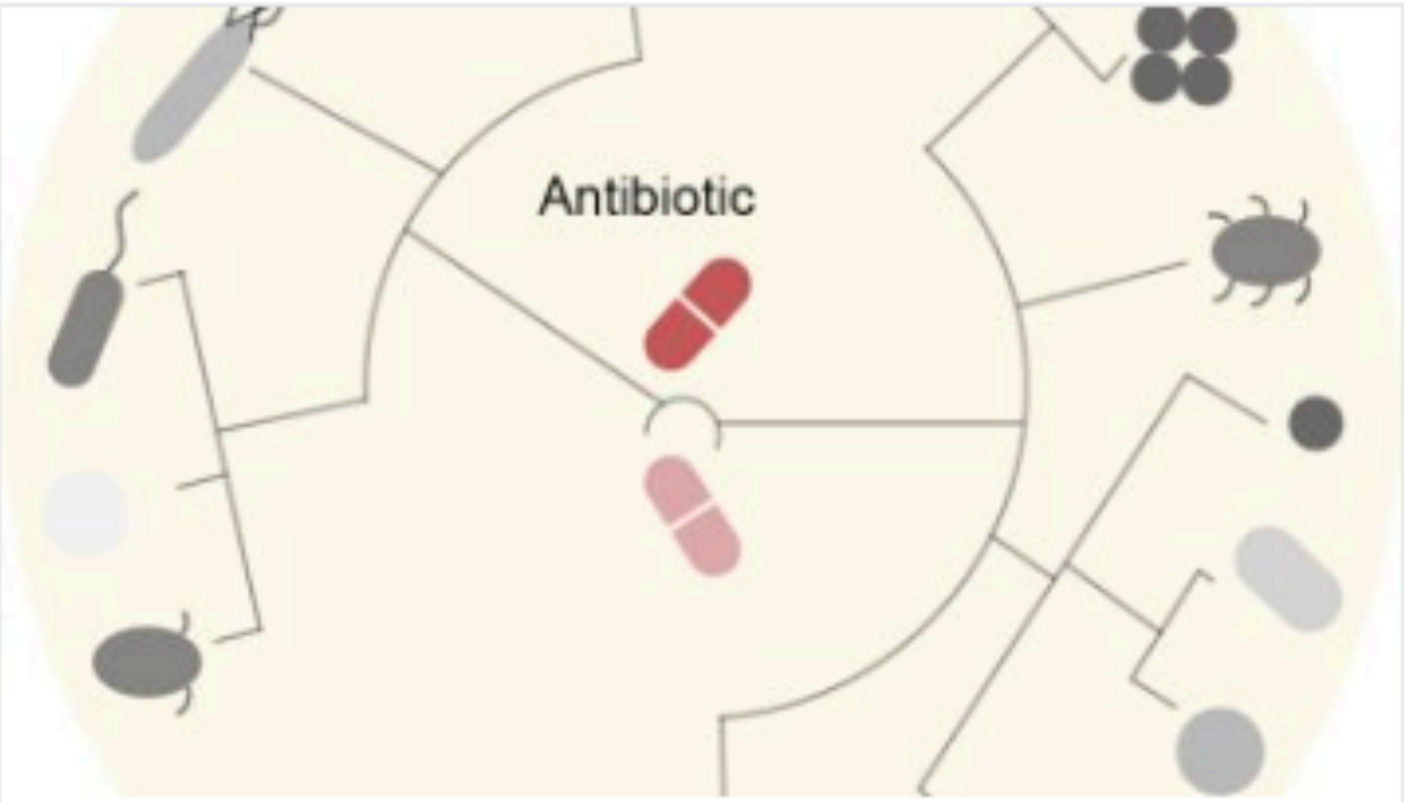
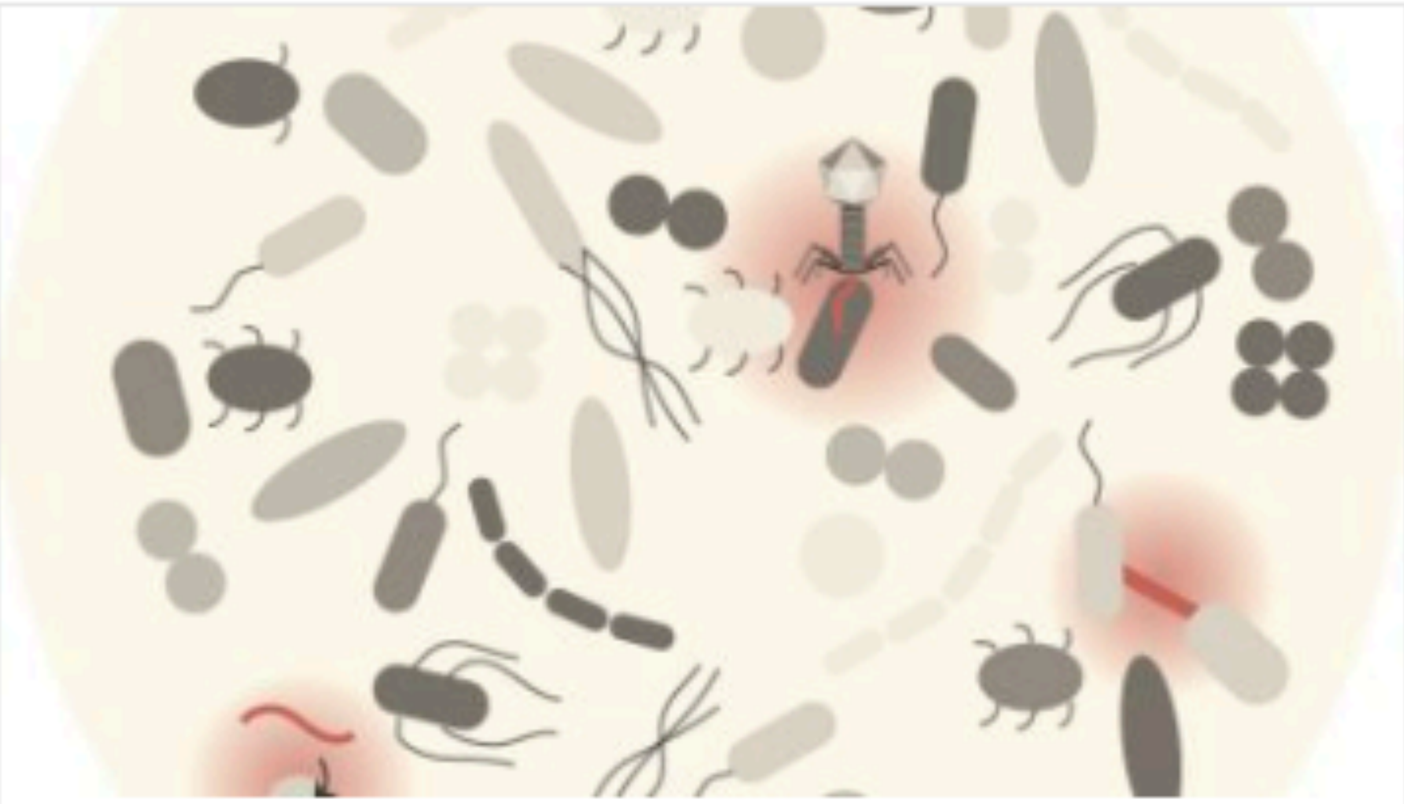


Antibiotics impact

How antibiotics impact diverse bacteria ?

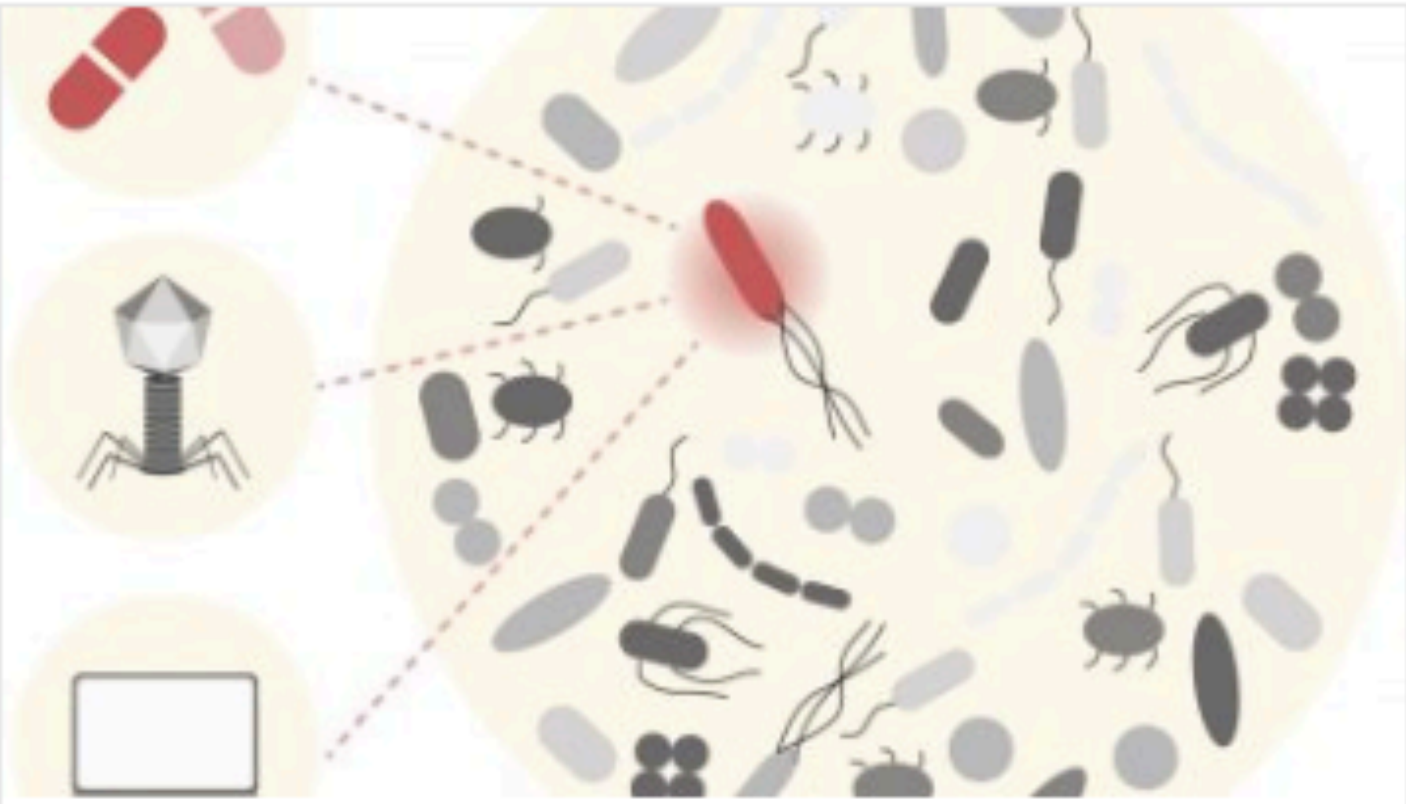
See more



Antibiotic resistance

How antibiotic resistance emerges and spreads in the gut?

See more



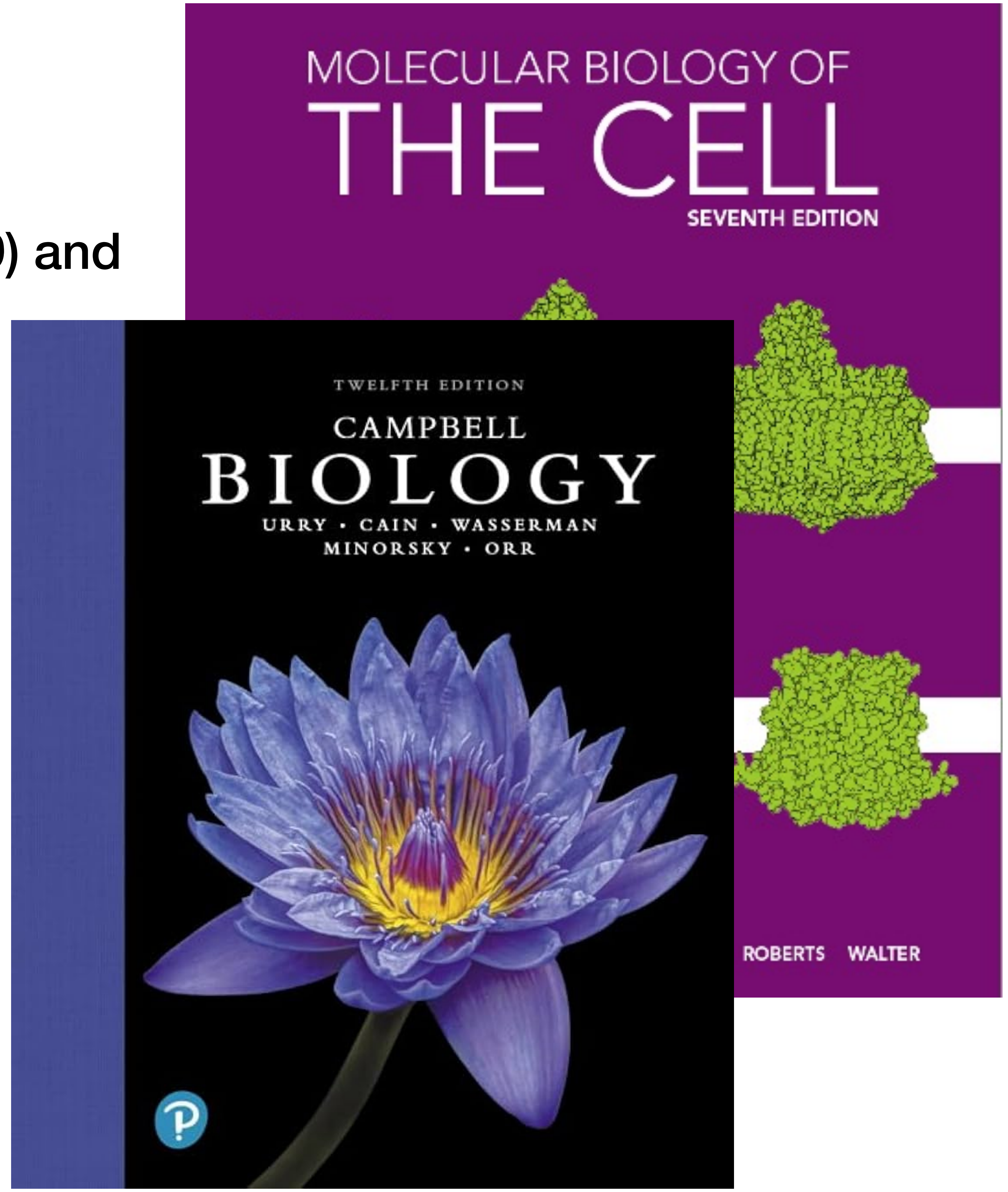
Treatment design

How to design better treatment

See more

General information about this course

- Slides and lectures are in **English** - with notes
- Reference Books are **Molecular Biology of the Cell (chapters 4-9) and Biology,** both available at the library - not needed
- **Images** are primarily from both books
- All **references** available upon request
- Background is **Biology I** with some overlap
- **Questions**: during the break
- **Interesting resources**: https://www.biointeractive.org/classroom-resources



General information about this course

- **Exam** (English) on lectures+exercise material; with open book (only slides and notes), similar to exercises
    - multiple choices
    - TRUE or FALSE
- **Questions/Feedback** during the break
- Interactive **quizz** during the course: please participate
- Student’s reps?

General information about this course

- Every **Wednesday** on **Moodle**:
  - **Slides** for the next class (material is **new** this year !)
  - **Exercises** for the next session
  - **Answers** to last week's exercises

Exercise sessions with TAs

- Exercises (English): Mondays 17.15-19.00
- Room CE1100 : students A-F (last name)
- Room CE1101 : students G-O (last name)
- Room CE1105: students P-Z (last name)
- **Do attend the exercise sessions as they prepare you for the exam !**

Let's make this a great experience

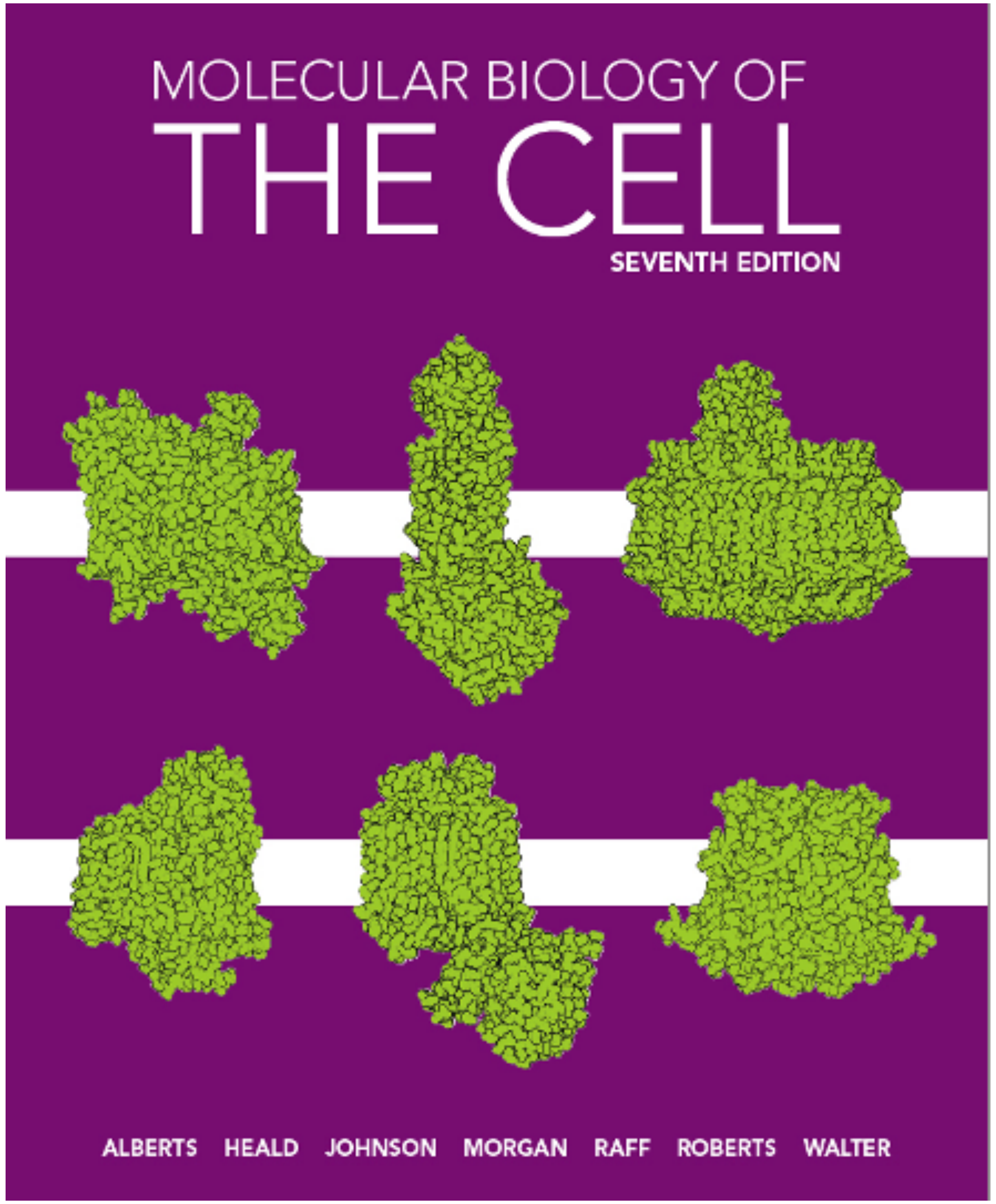
- I show up on time
- I prepare my classes so they are (hopefully) enjoyable
- I am open to feedback

- You show up on time/listen to the class
- You participate to the class when needed
- You provide me with feedback when necessary

General objectives of this course

- Understand **DNA organization**, **gene expression** and **protein function**
- **General principles** and **experimental approaches**

Now, let's start!



Chapter 4

DNA, Chromosomes, and Genomes

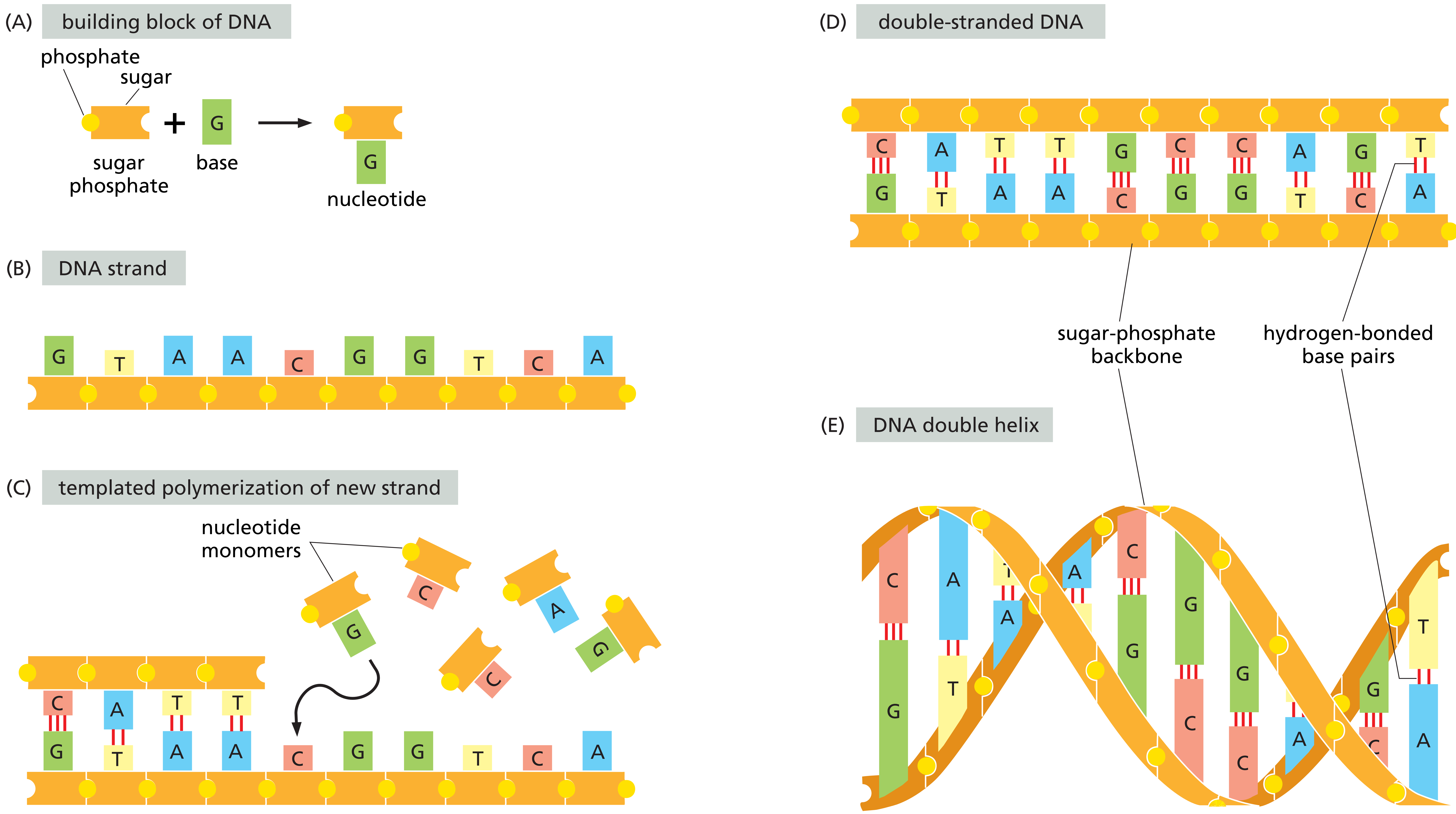
Now, let's start!

- Brief introduction on Biology basics
- The structure and function of DNA
- Chromosomal DNA and its packaging into chromatin
- The effect of chromatin structure on DNA function

Brief introduction on Biology basics

- Living organisms are **diverse**
- They are all made of **cells**
    - Membrane-enclosed units
    - Filled with a concentrated aqueous solution of chemicals
    - Ability to grow and divide in two
- All cells store their **hereditary information** in the same chemical linear code: **DNA**

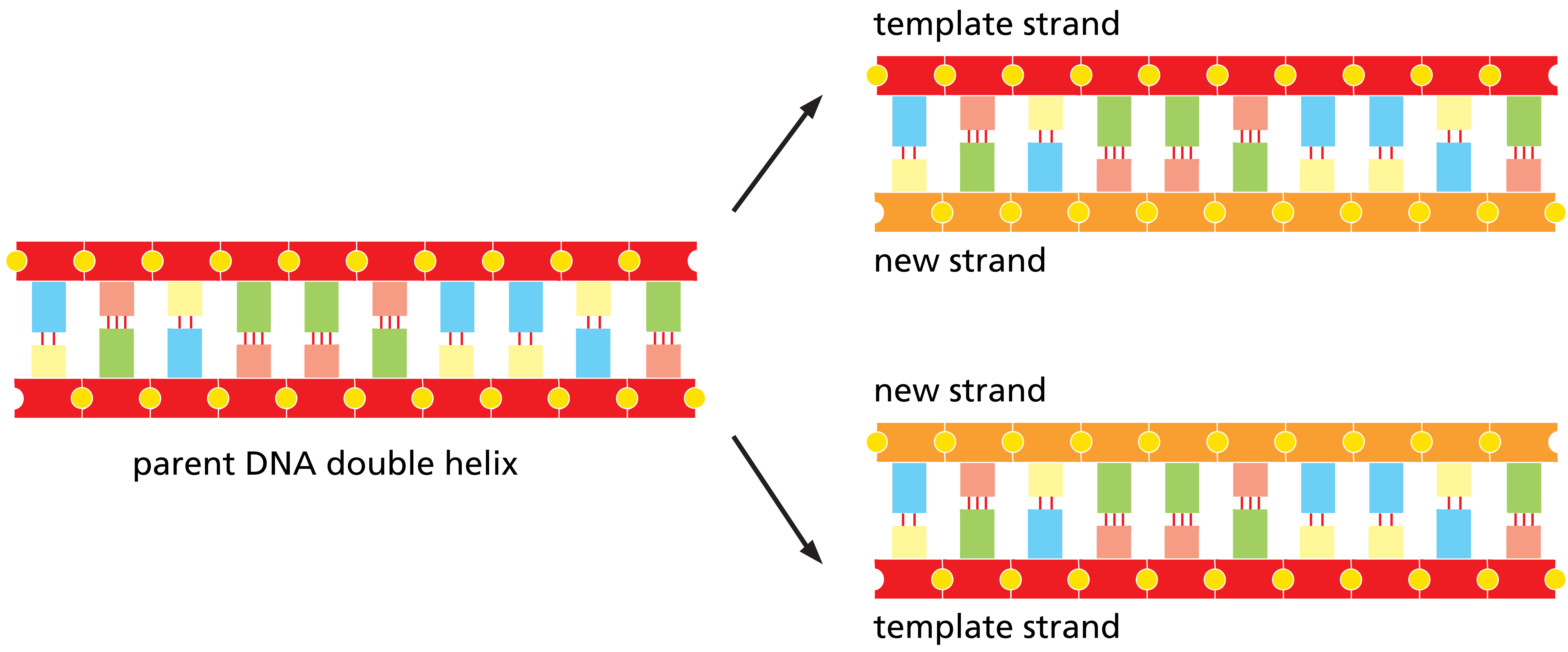
What is DNA ?



(A) building block of DNA
phosphate
sugar
G
sugar phosphate
base
G
nucleotide
(B) DNA strand
G T A A C G G T C A
(C) templated polymerization of new strand
nucleotide monomers
C A T T
G T A A C G G T C A
(D) double-stranded DNA
C A T T G C C A G T
G T A A C G G T C A
sugar-phosphate backbone
hydrogen-bonded base pairs
(E) DNA double helix

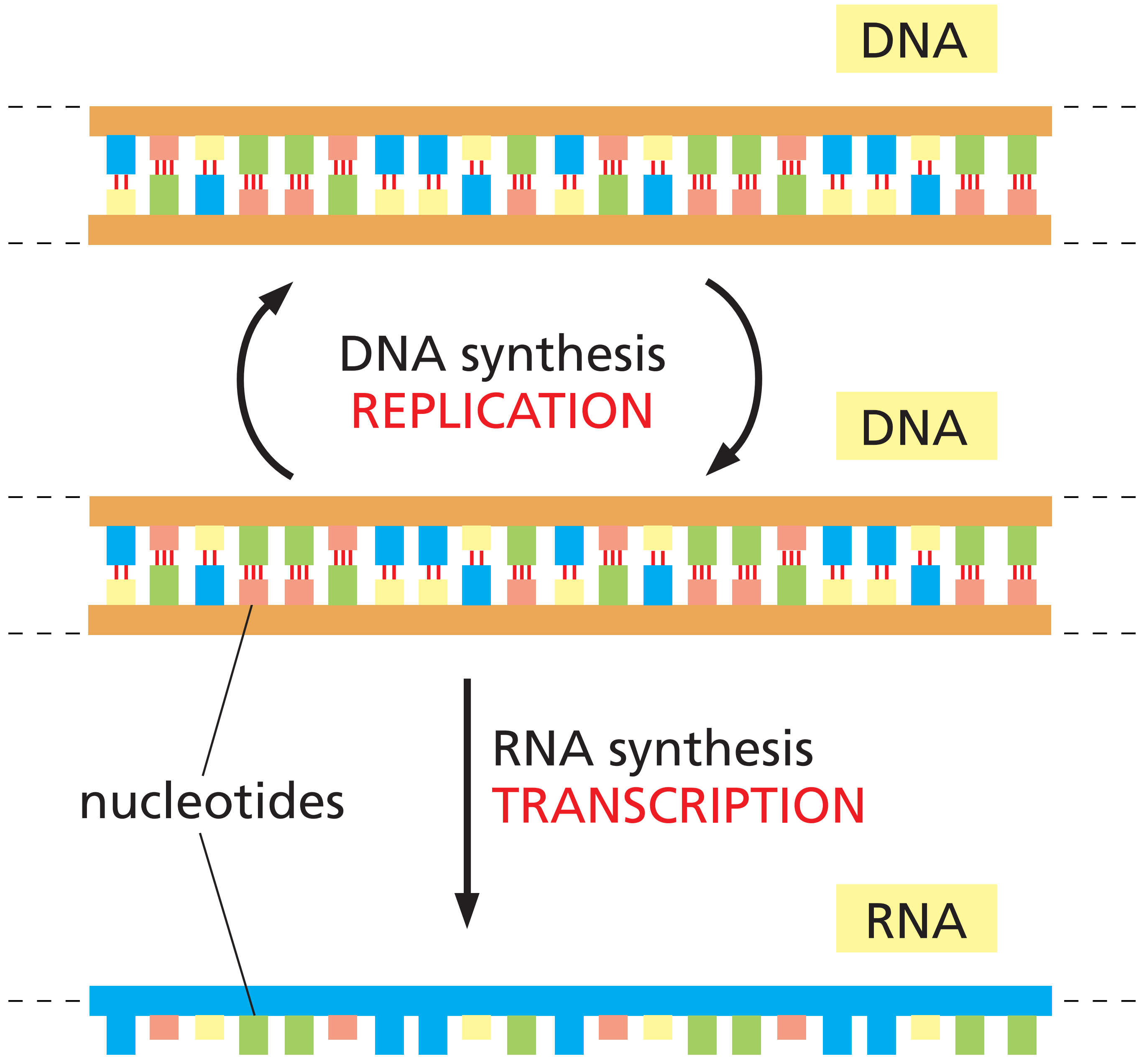
DNA replication

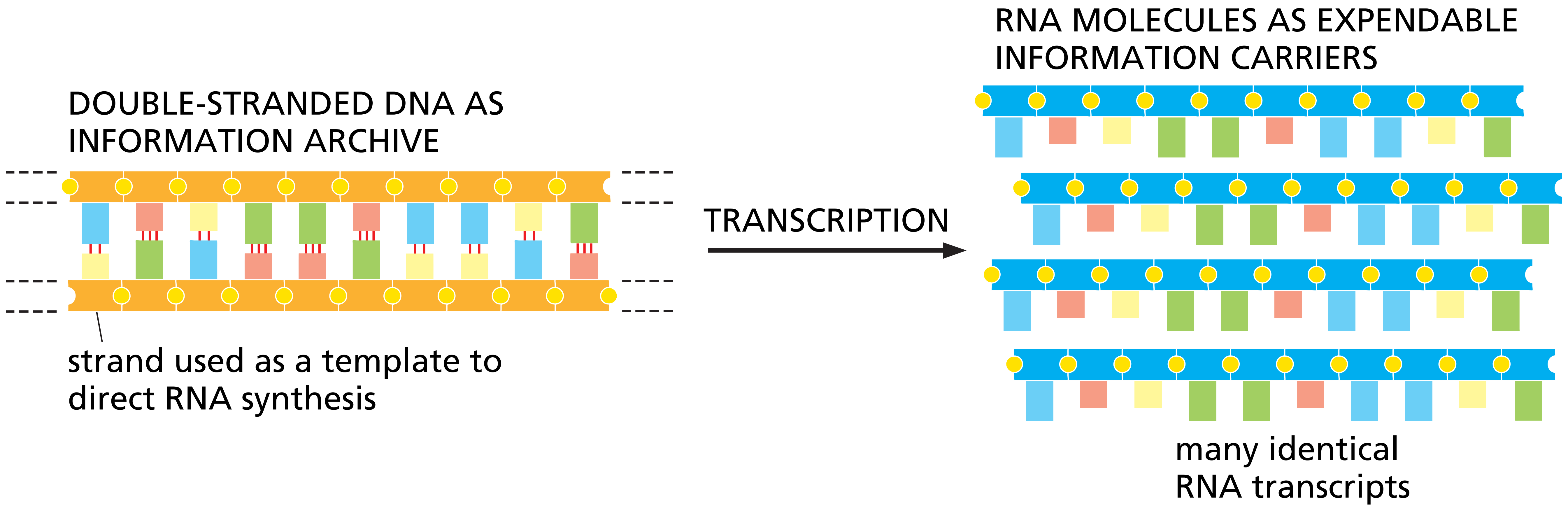
- All cells replicate their DNA by **templated polymerization**



Transcription

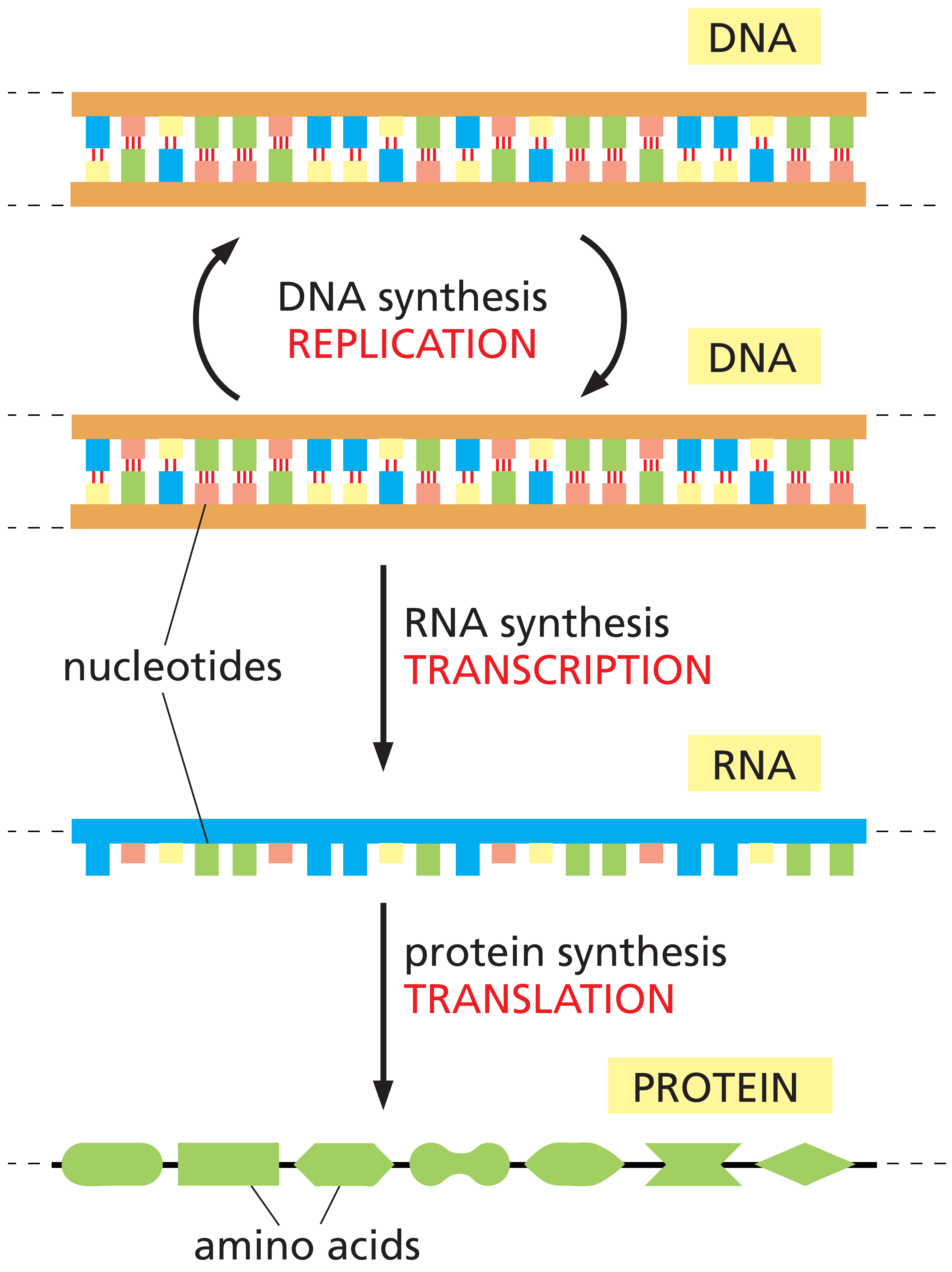
- All cells transcribe portions of their **DNA** into **RNA**





Translation

- All cells **translate RNA into proteins** the same way



What are proteins?

Proteins are

- Long polymers of **amino acids** (20 types) = polypeptide
- Important **3D structure** linked to a function
- Can act as **enzymes, structure, generation movement, sensing signals,**...

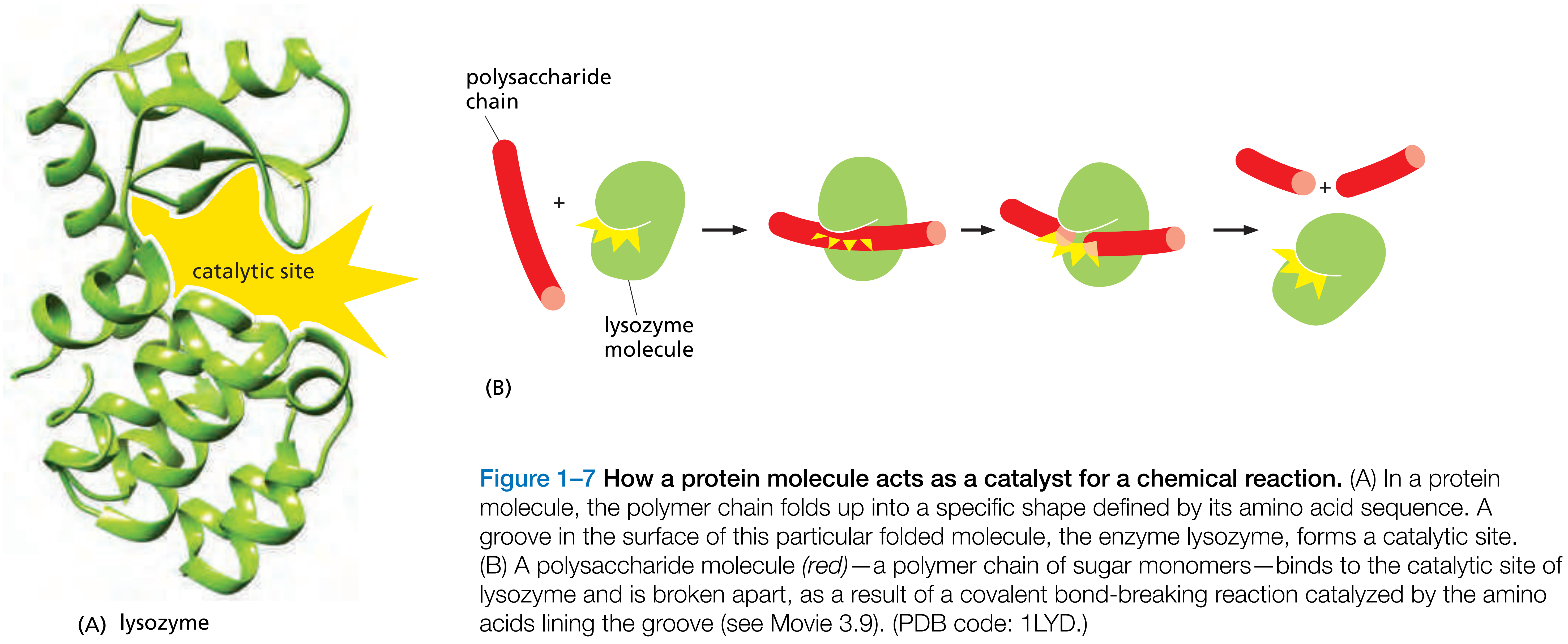


Figure 1–7 **How a protein molecule acts as a catalyst for a chemical reaction.** (A) In a protein molecule, the polymer chain folds up into a specific shape defined by its amino acid sequence. A groove in the surface of this particular folded molecule, the enzyme lysozyme, forms a catalytic site. (B) A polysaccharide molecule *(red)*—a polymer chain of sugar monomers—binds to the catalytic site of lysozyme and is broken apart, as a result of a covalent bond-breaking reaction catalyzed by the amino acids lining the groove (see Movie 3.9). (PDB code: 1LYD.)

Plan

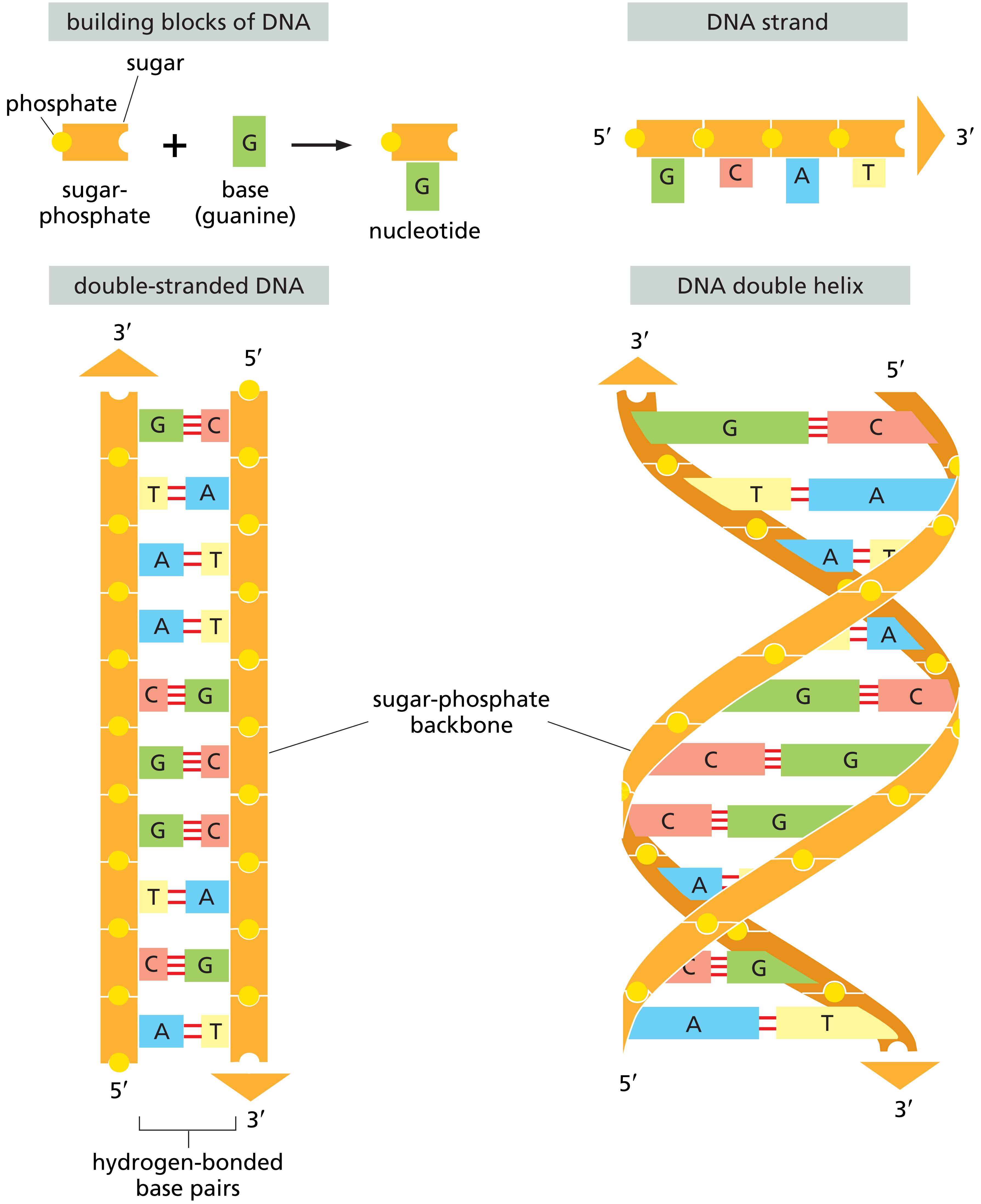
- Brief introduction on Biology basics
- <u>The structure and function of DNA</u>
- Chromosomal DNA and its packaging into chromatin
- The effect of chromatin structure on DNA function

The structure of DNA

- DNA is passed from one cell to its daughter cells at **cell division**
- Instructions are stored as **genes**, which define the characteristics of a **species**
- In the 1940s, it was difficult to imagine that a molecule as **simple** as DNA could be the genetic material
- In the 1950s, x-ray diffraction analysis showed it had **two strands and formed a helix**
- 1953: **model of DNA** by Watson and Crick

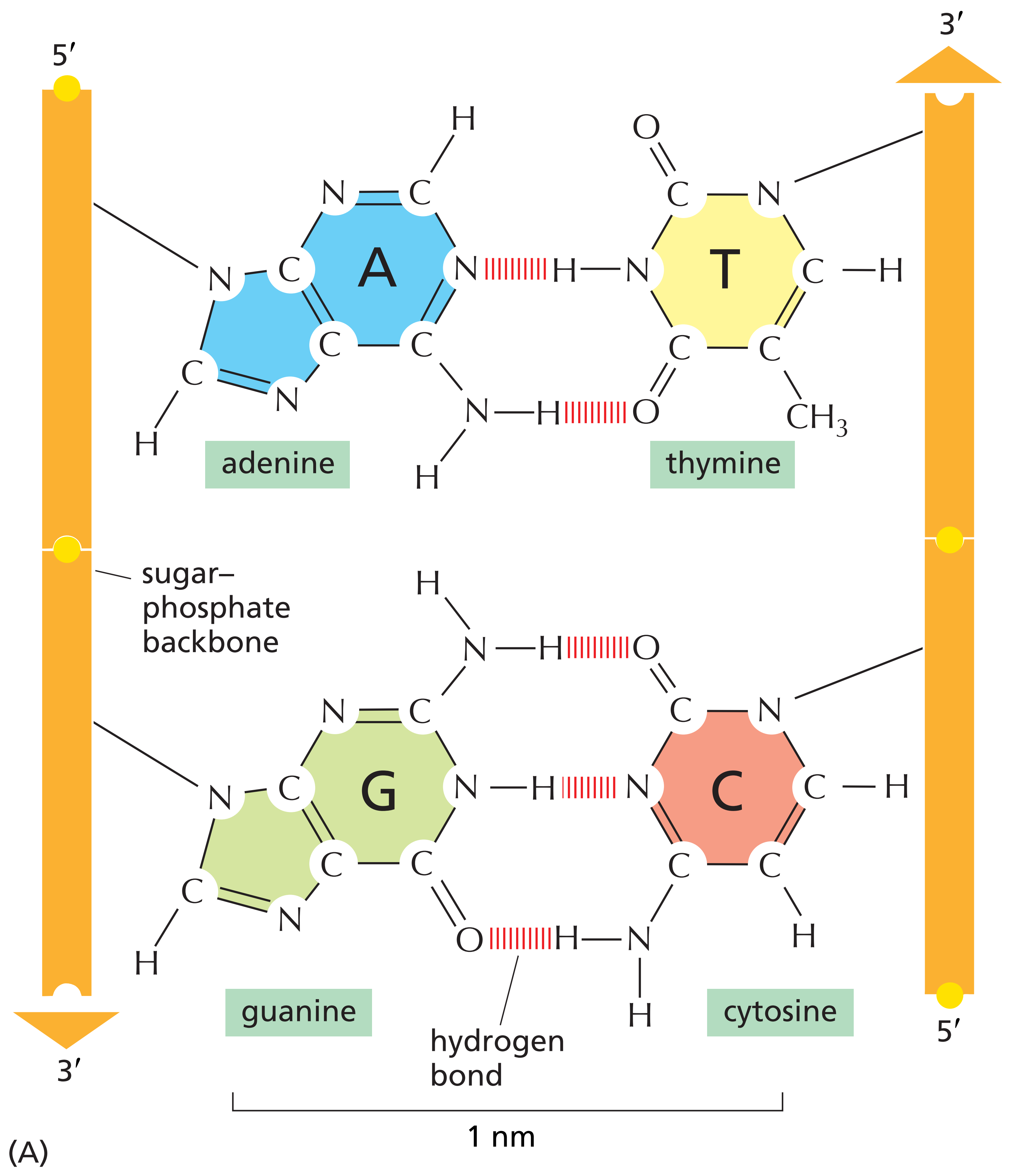
The structure of DNA

- **DNA** = Deoxyribonucleic Acid
- **Nucleotides** are the building blocks of DNA
- **Nucleotides** are formed by a **sugar** (deoxyribose) linked to a **phosphate** group and a nitrogen-containing **base** (adenine, cytosine, guanine, thymine)
- **Covalent link** to form the **sugar-phosphate backbone** = DNA strand
- Two long **polynucleotide chains**
- **Antiparallel** to each other (5' end and 3' end can be distinguished)
- Linked by **hydrogen bonds**



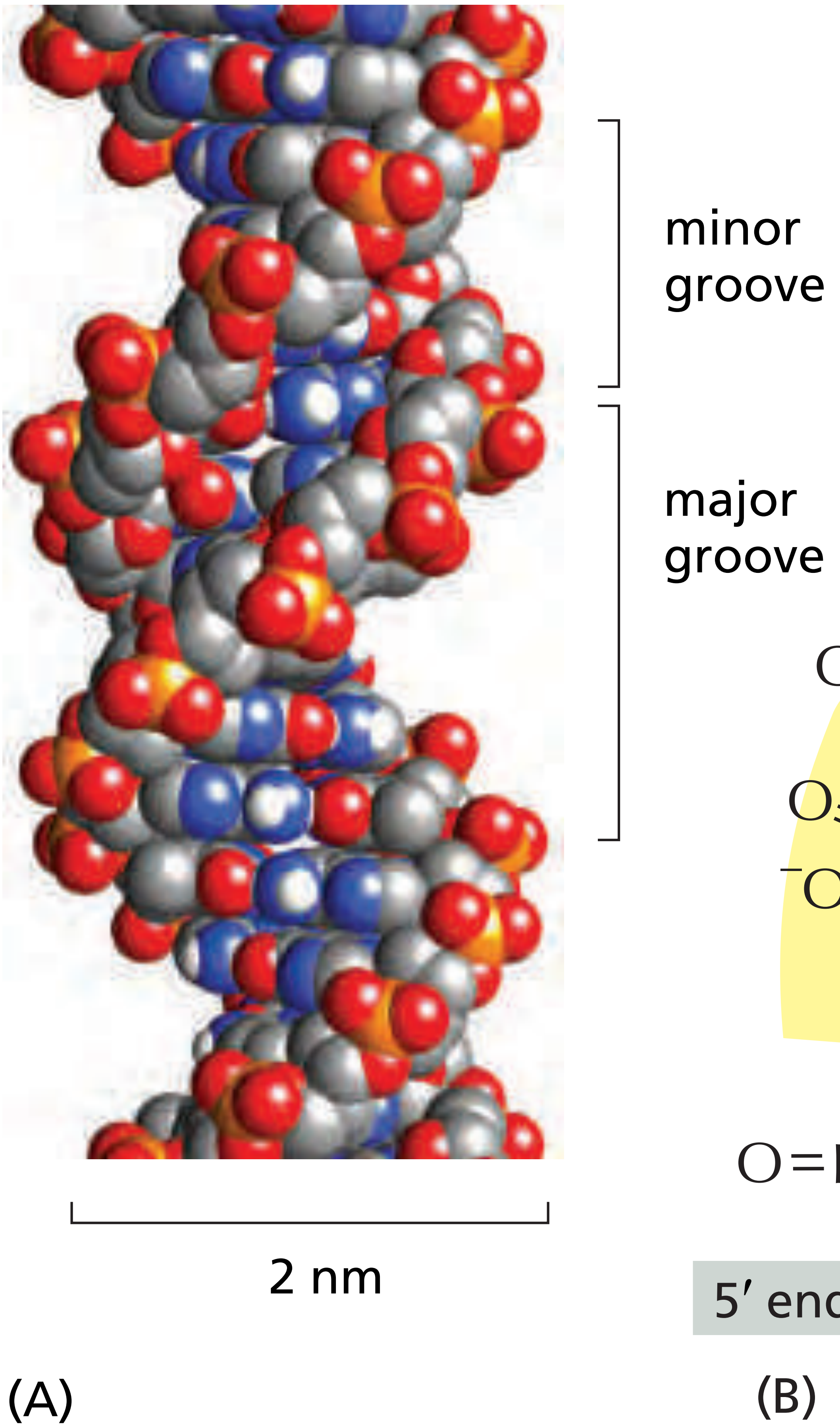
The structure of DNA

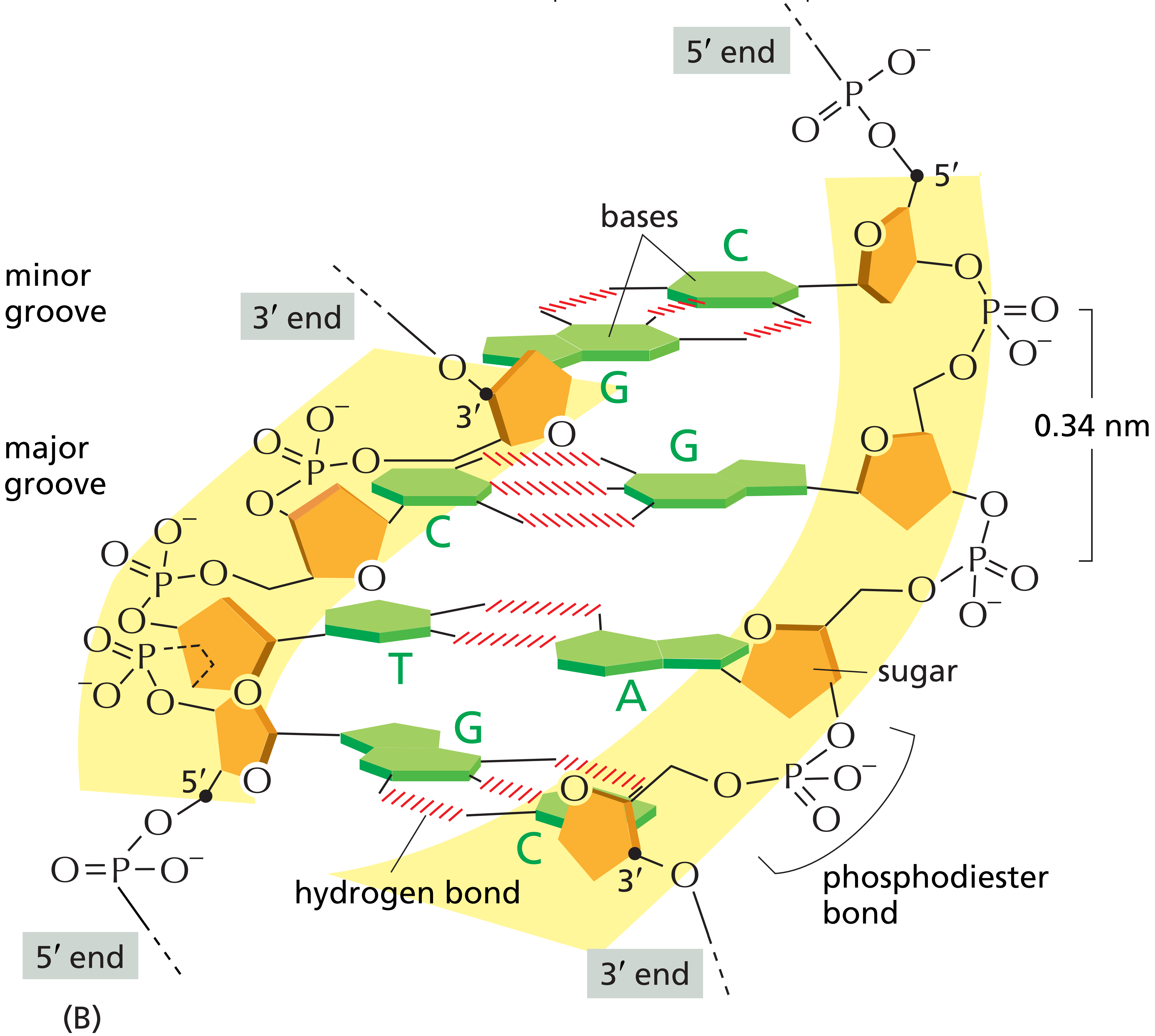
- A pairs with T; and G with C
- 2-ring base = purine
- 1-ring base = pyrimidine



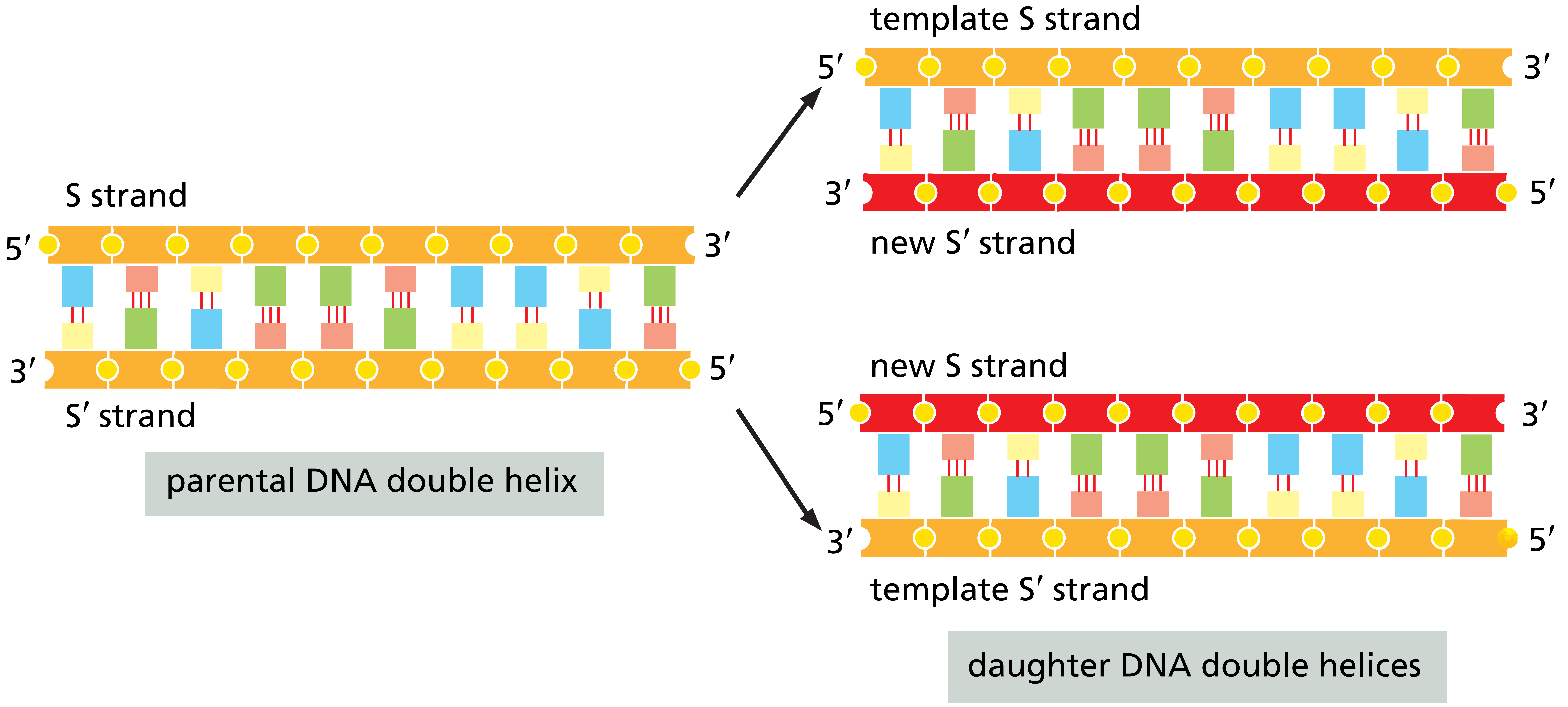
The structure of DNA

- The two backbones wind around each other to form a **helix** with a turn per 10 base pairs



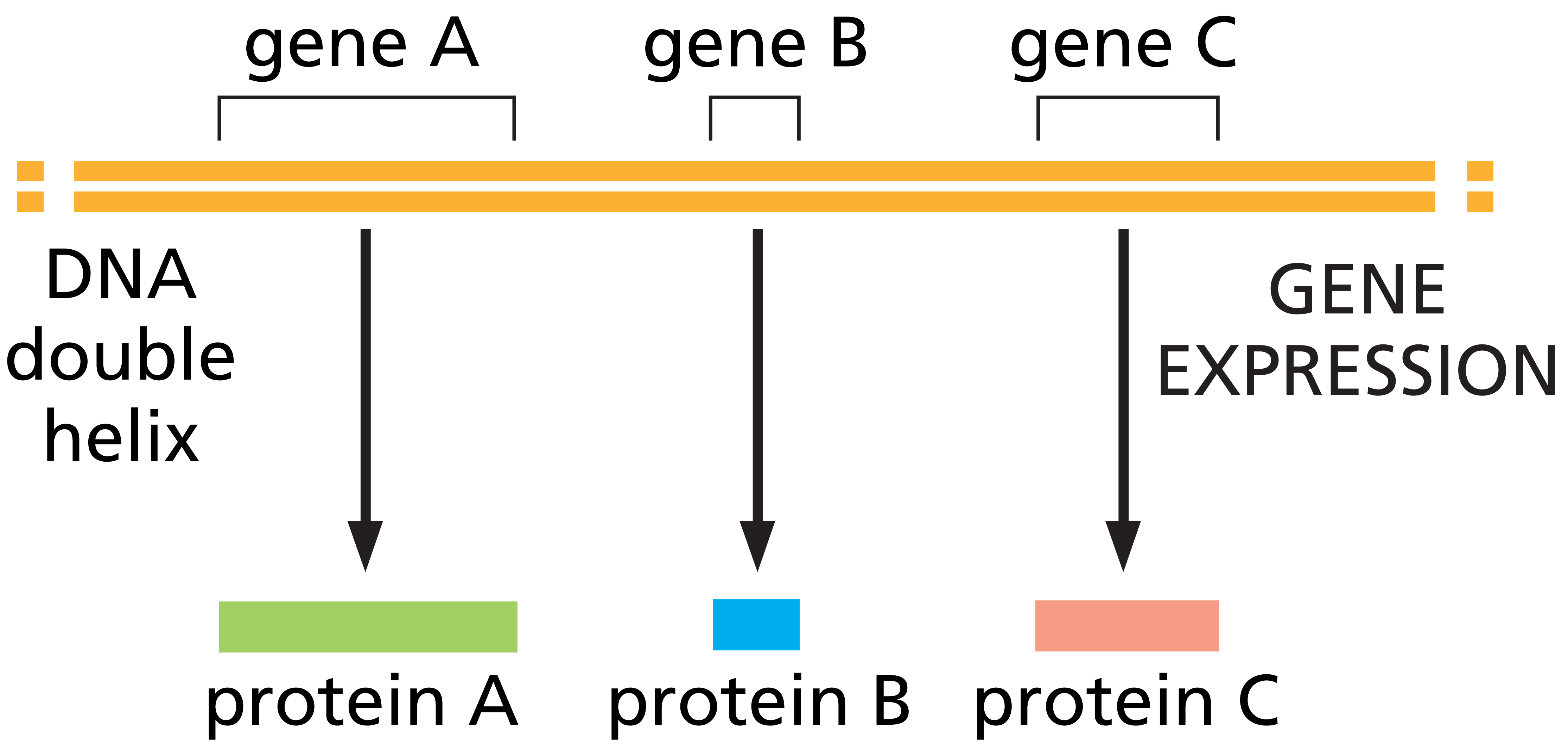


How is information copied from generation to generation?



How is information stored?

- **Genes** encode the information to make **proteins**
- If genes are made of DNA, DNA can be somehow turned into proteins
- **Genetic code**: correspondance between DNA and amino-acid
- The complete store of information of an organism is called **genome**

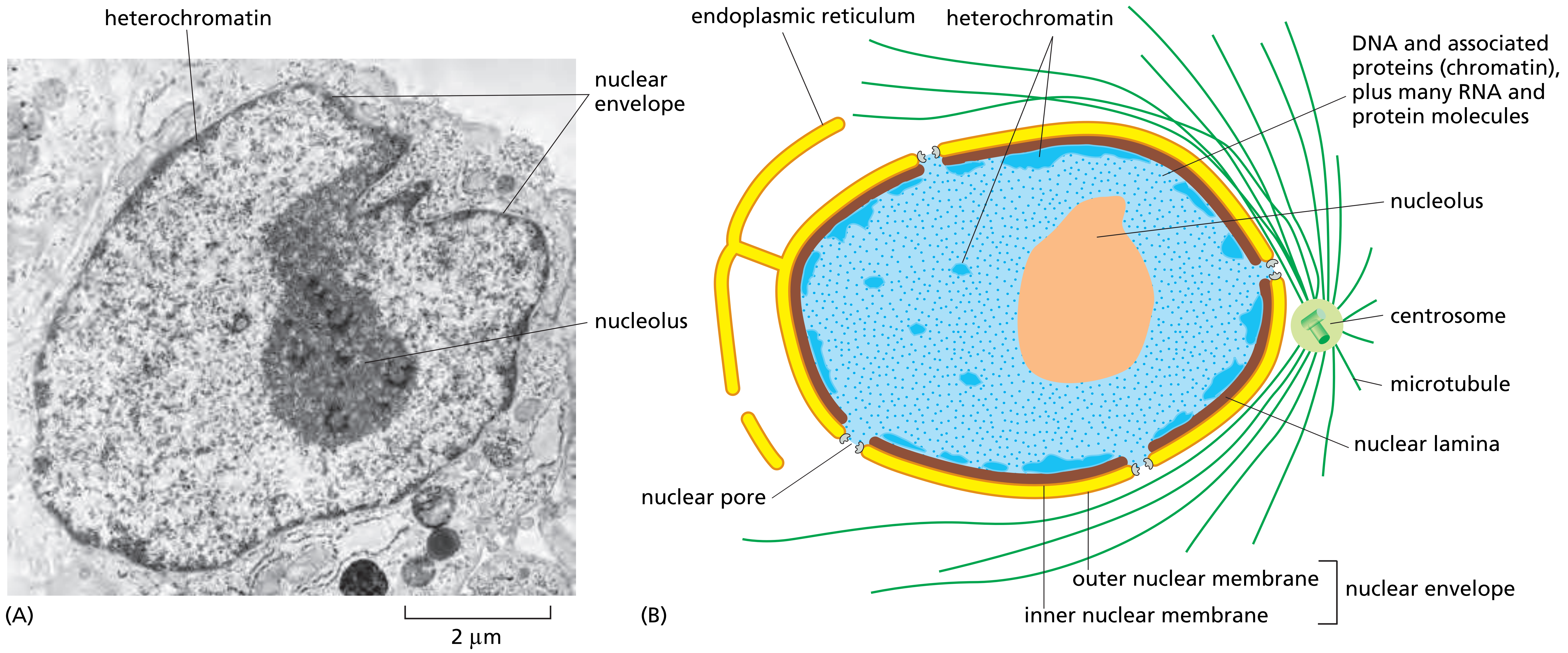


Plan

- Brief introduction on Biology basics
- The structure and function of DNA
- <u>Chromosomal DNA and its packaging into chromatin</u>
- The effect of chromatin structure on DNA function

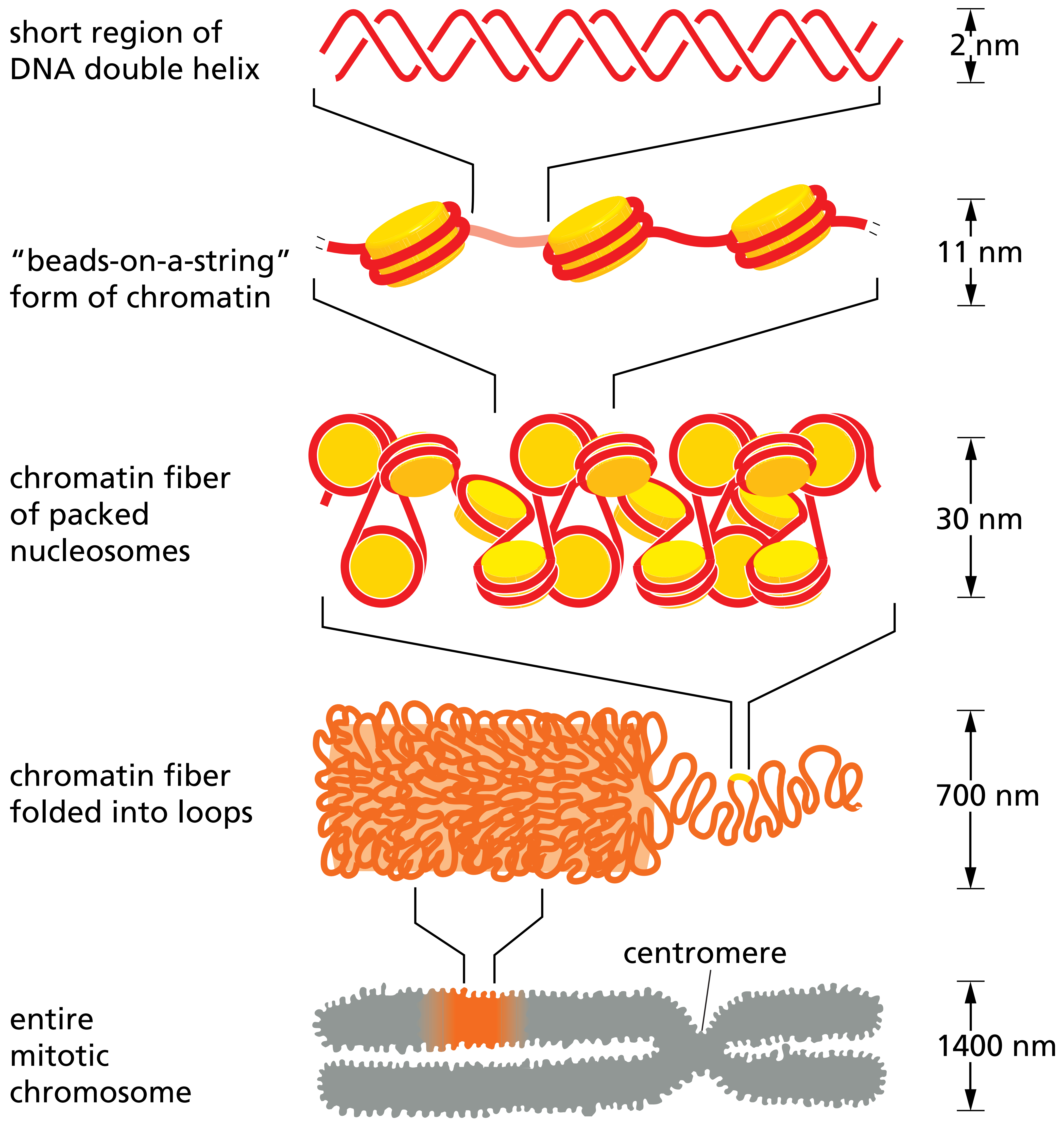
Chromosomal DNA and chromatin

- In Eukaryotes, DNA is packed in a **nucleus** (10% of the volume of the cells), separated by **nuclear envelope**
- Nuclear envelope is a **two concentric lipid bilayer membranes** punctured by large **nuclear pores**
- Mammalian **DNA is 2-3 m** long but the nucleus has a **diameter of ~ 10 um**



How is DNA packed?

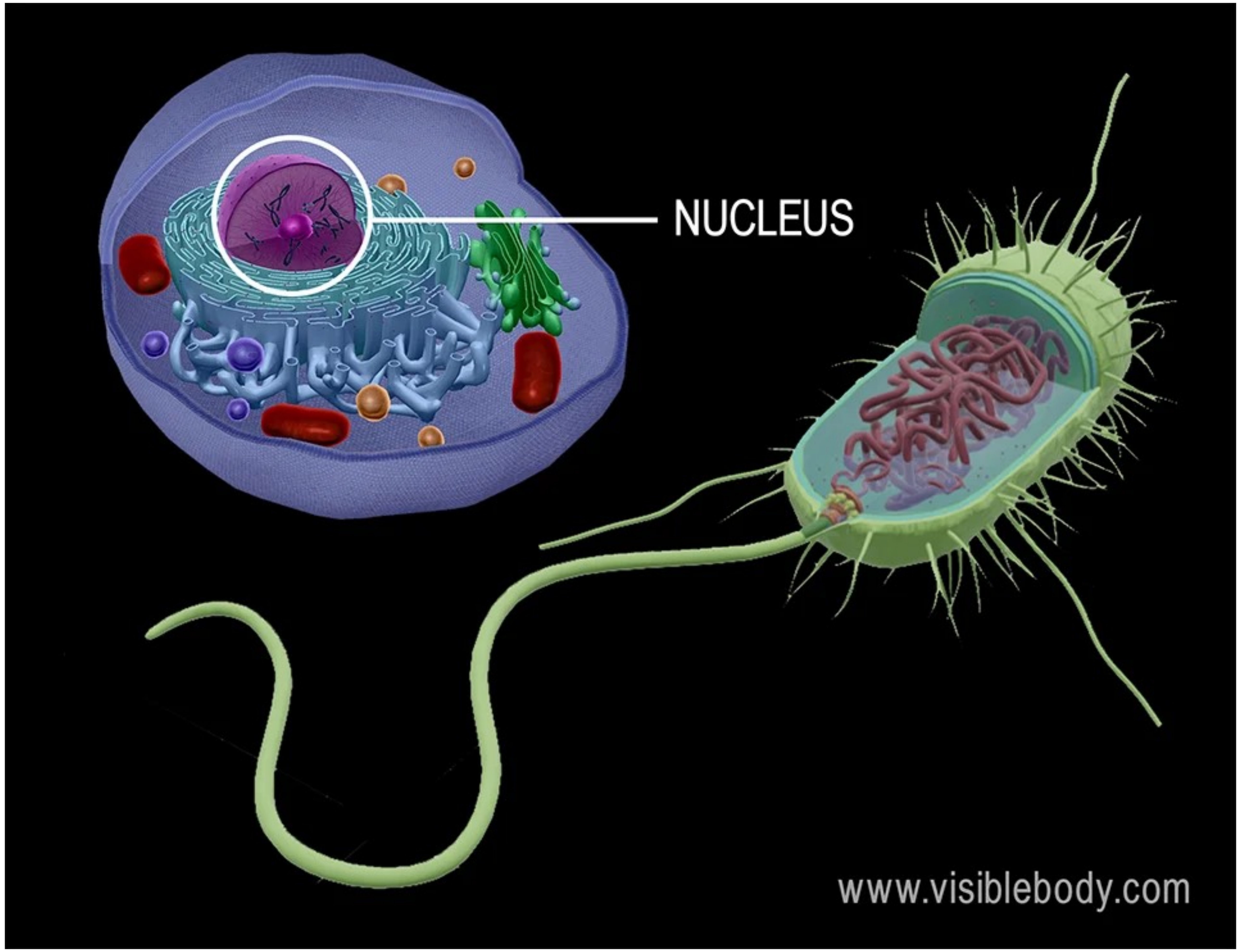
- Level 1: DNA - **double helix**
- Level 2: DNA and proteins form **chromatin**
- Level 3: Chromatin is condensed into **chromosomes**
- Each **chromosome** is a single long DNA molecule with proteins that pack it into the right structure
- Chromosomes reach their highest level of compaction during **mitosis**
- **Bacteria** do not have a nucleus and typically have one chromosome (different from the ones in eukaryotes)



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS FULLY EXTENDED LENGTH

Prokaryotes and eukaryotes

- **Prokaryotes:**
    - **Circular** DNA molecule
    - Associated with a **few** proteins
    - Inside the **cell** (no nucleus)
- **Eukaryotes:**
    - **Linear** DNA molecules
    - **Large amount** of proteins
    - Inside the **nucleus**



Human chromosomes

- Human cells (except gametes and highly-specialised cells like red-blood cells) have **two copies of each chromosome** (one from the father, one from the mother)
- The maternal and paternal chromosomes are called **homologous chromosomes**
- The **sex** chromosomes in males are **non homologous**
- Each human cell comprises **46 chromosomes: 22 pairs of autosomes and 2 sex chromosomes**

Human chromosomes

- Two DNA molecules produced during **DNA replication,** each called a **sister chromatid**
- Linked at the **centromere** (highly-condensed regions)

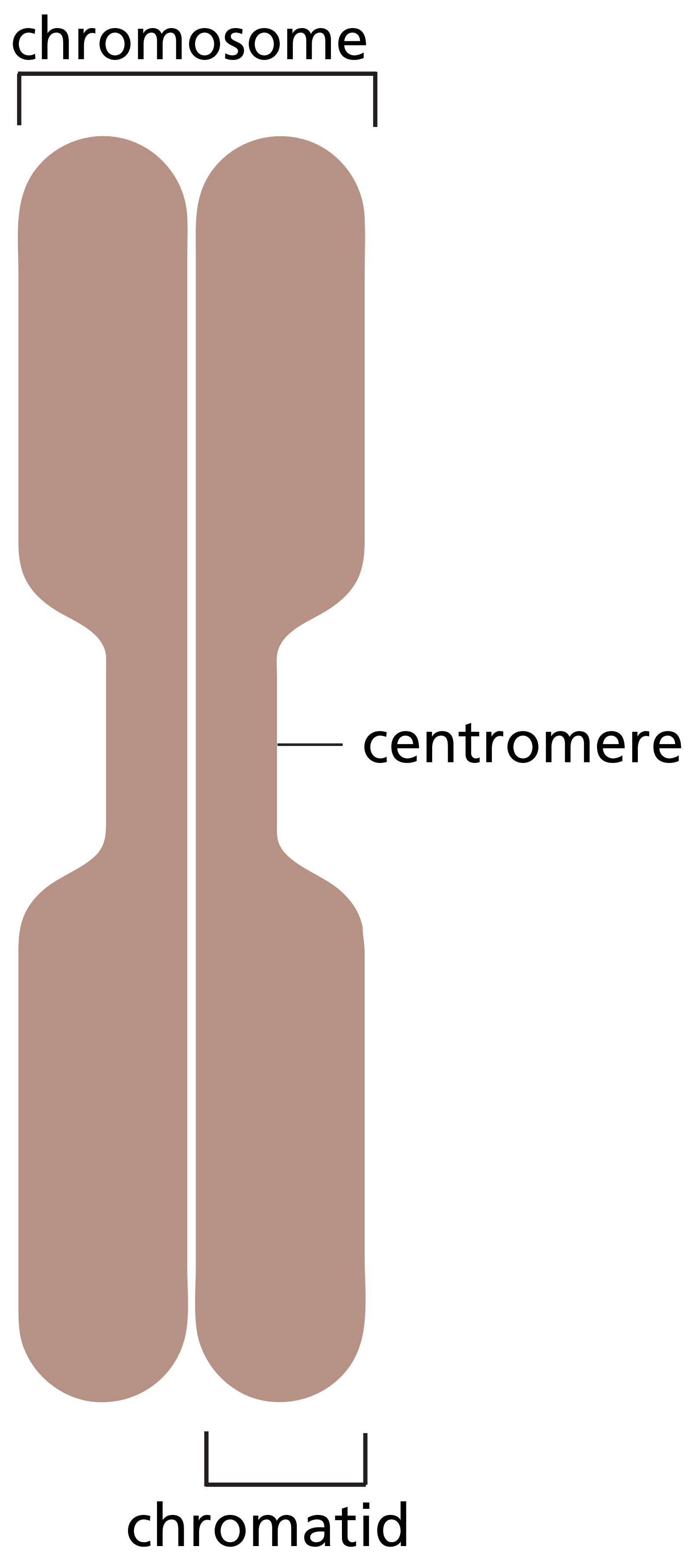
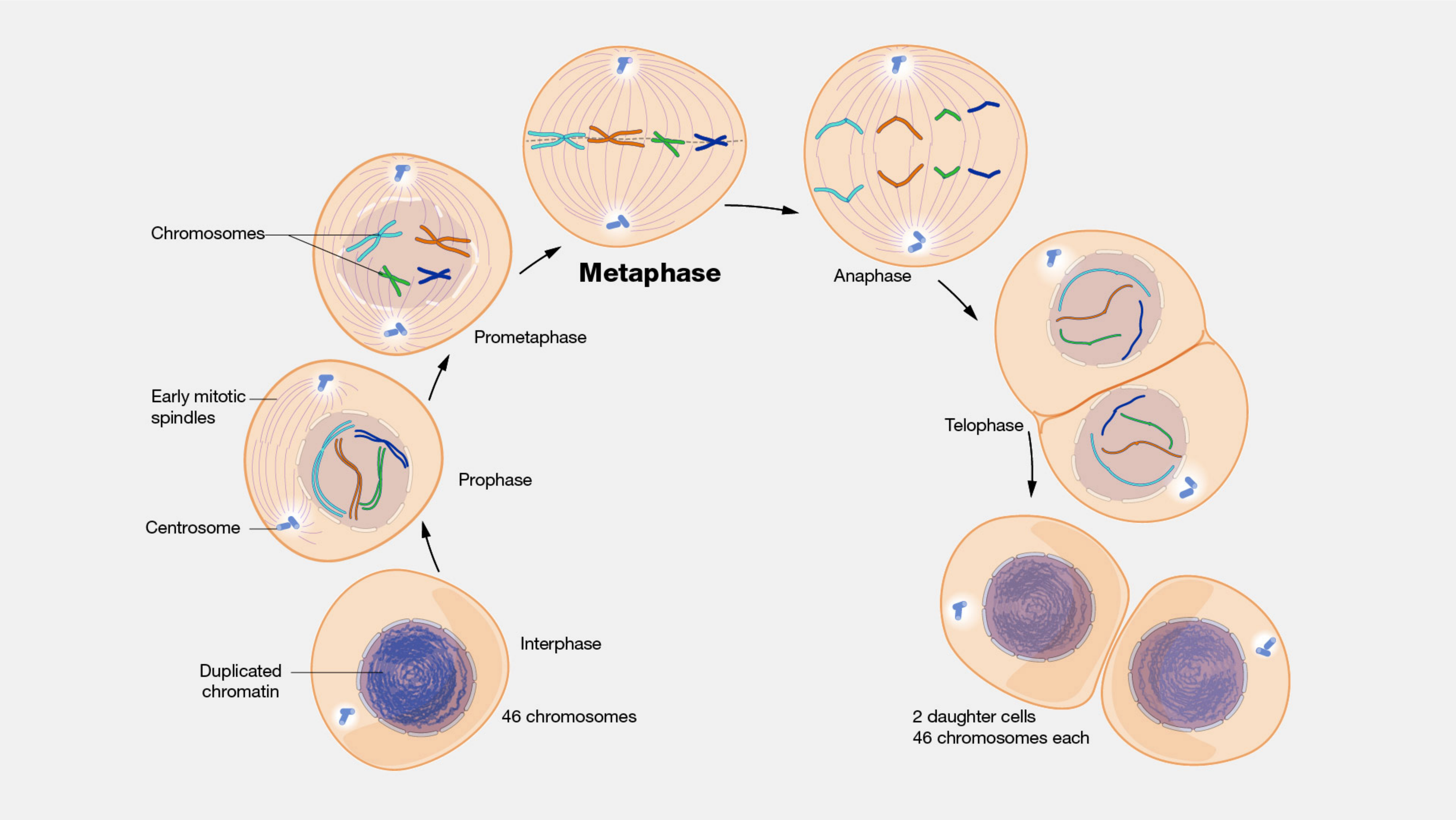


Figure 4–59 A typical mitotic chromosome at metaphase. Each sister chromatid contains one of two identical sister DNA molecules generated earlier in the cell cycle by DNA replication (see also Figure 17–21).

Human chromosomes

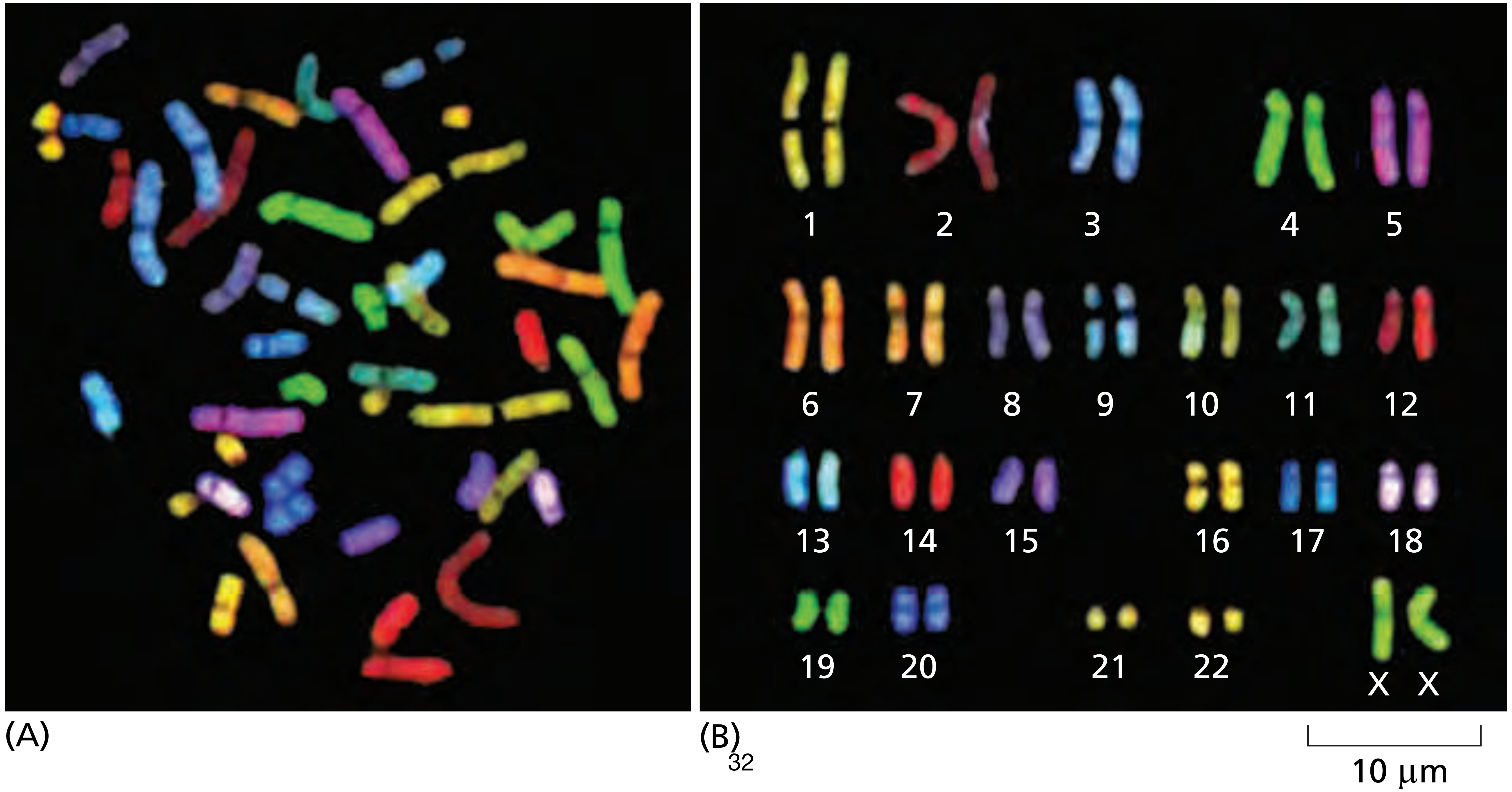


- During **mitosis**, **genes are silenced**
- Chromosomes are **highly compacted** with help of proteins (condensins and cohesins)
- The **centromere** is the region where the **mitotic spindle** will attach to the chromosomes to separate them in two cells

https://www.genome.gov/sites/default/files/media/images/tg/Metaphase.jpg

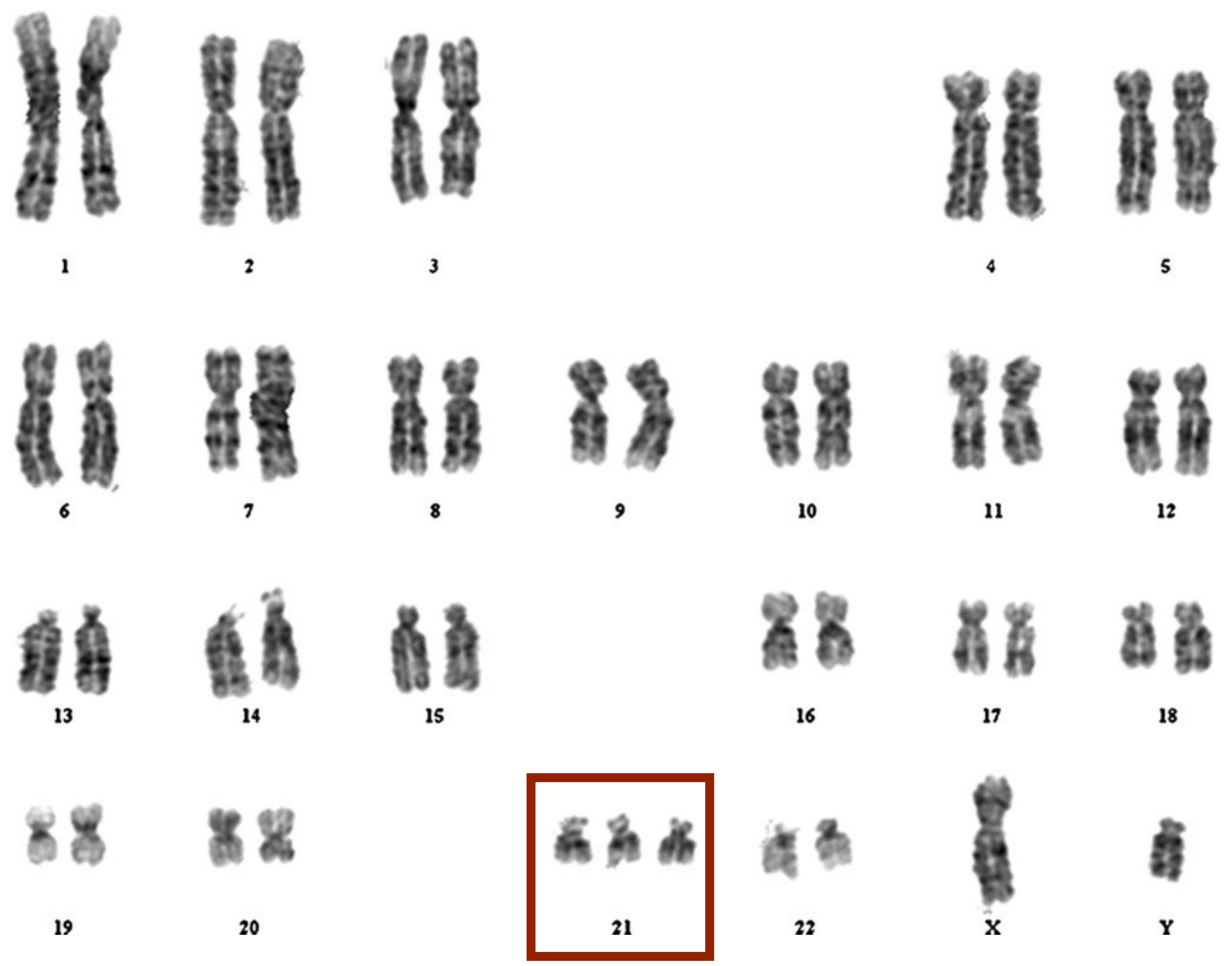
Human chromosomes

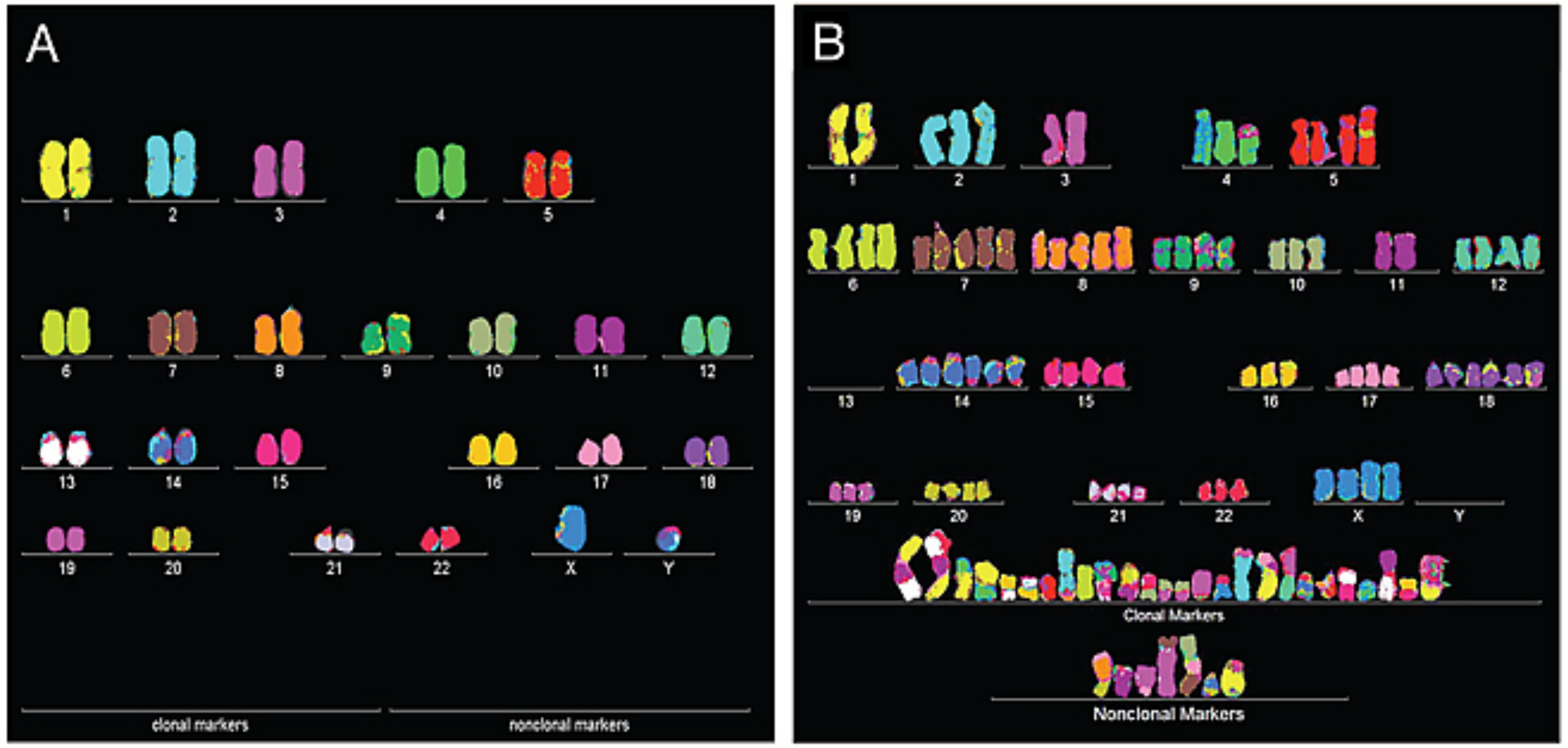
- Chromosomes can be distinguished by **“painting” or DNA hybridisation** (see later)
- A short strand of nucleic acid with a fluorescent dye serves as a probe that binds a complementary DNA sequence
- The display of the 46 chromosomes at mitosis is called **karyotype** and is used to detect inherited chromosome abnormalities



Chromosome aberrations

- **Genetic** (trisomy) vs. **Somatic** diseases (cancer)

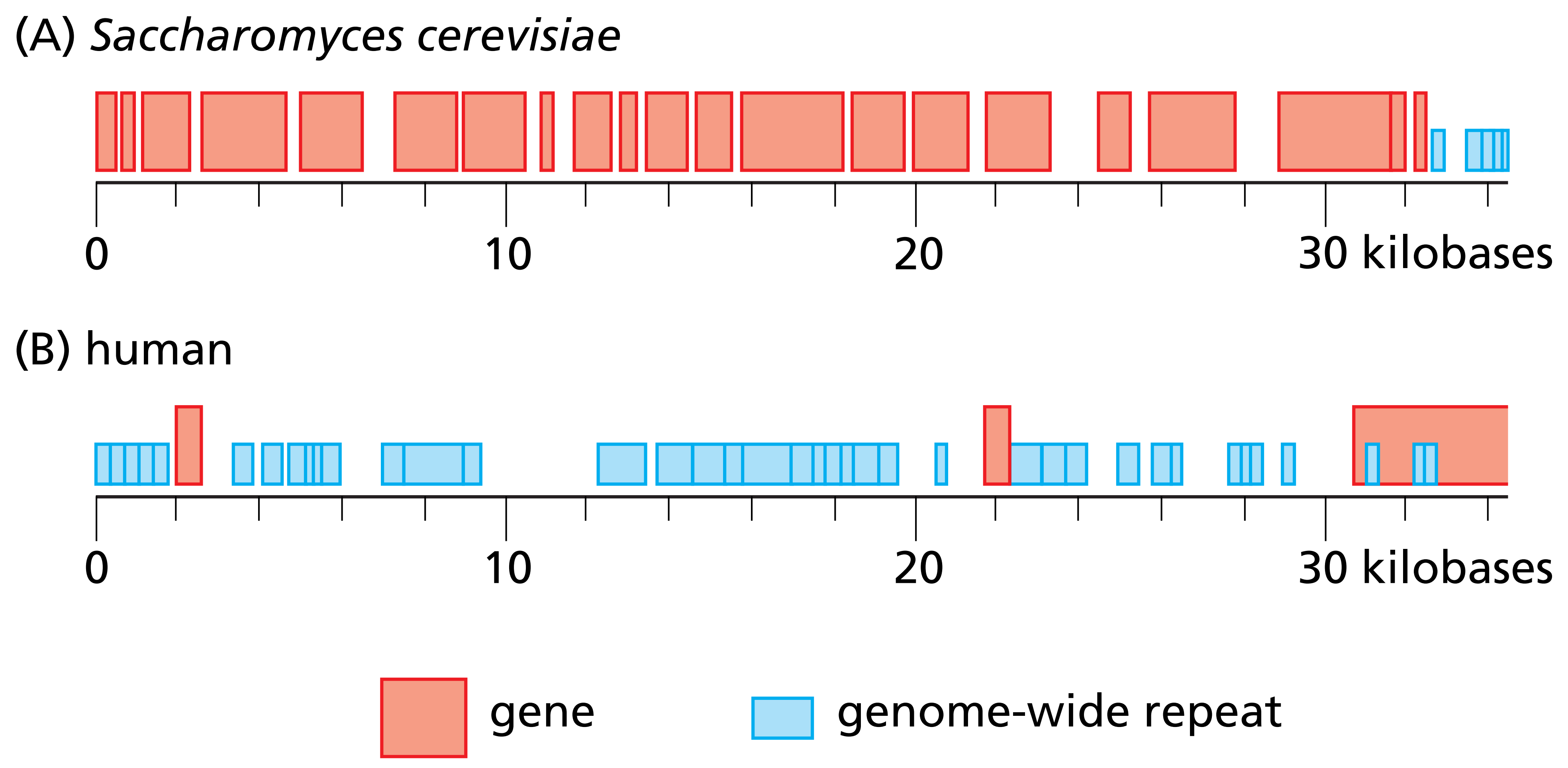




 https://vcresearch.berkeley.edu/sites/default/files/news_images/karyograms670.jpg

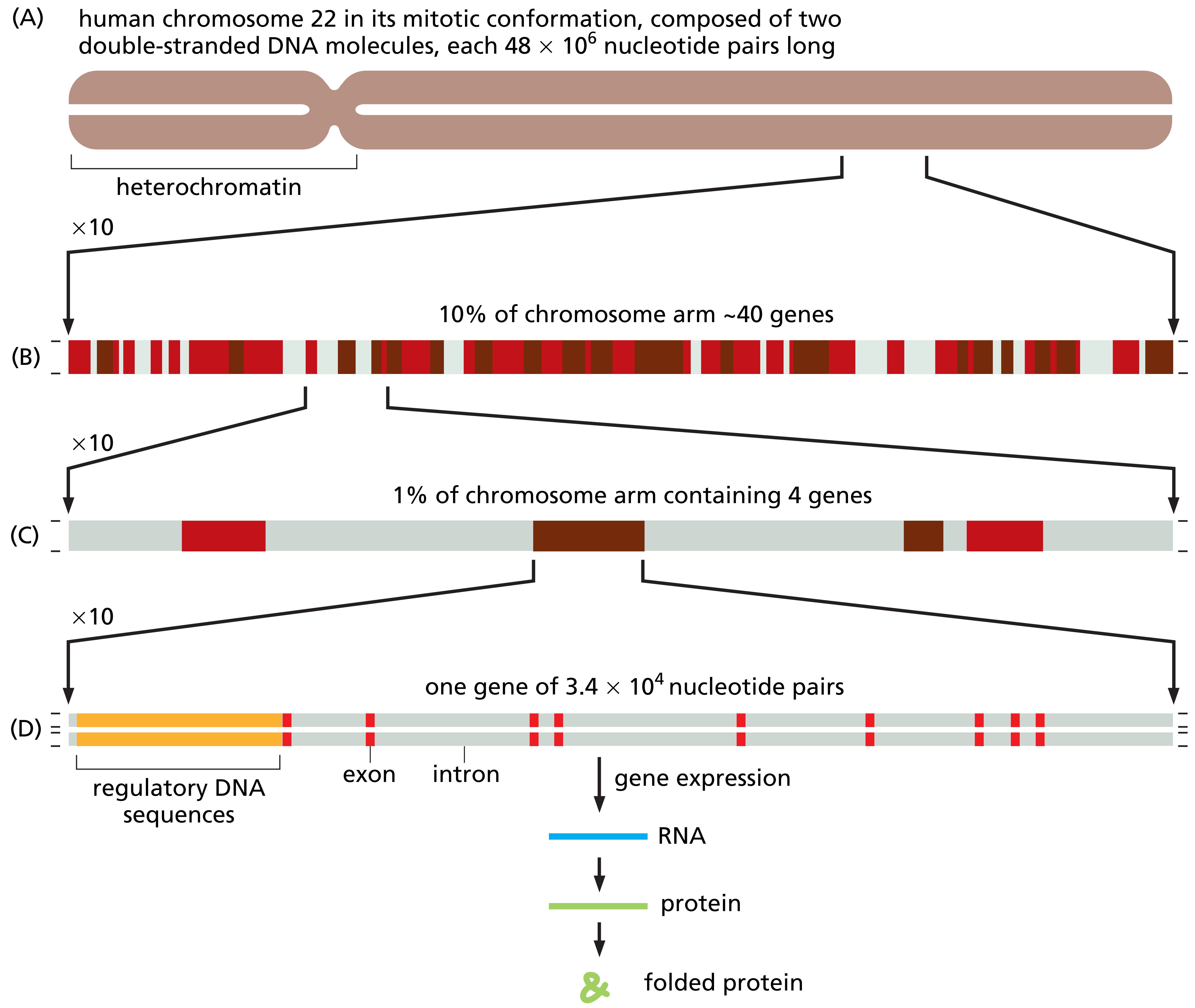
Zooming into the chromosomes

- Chromosomes carry **genes** = segments of DNA that contain the instructions to make **particular proteins**
- Some genes are **RNA genes, with RNA as a final product**
- Correlation between the **complexity of an organism and its number of genes** (~3K in bacteria vs. 30K in humans, incl. 21K coding for proteins)
- In multicellular organisms, large quantity of **interspersed DNA between genes** whose function is poorly understood - crucial for the control of **gene expression** = **non-coding DNA**



https://www.ensembl.org/index.html

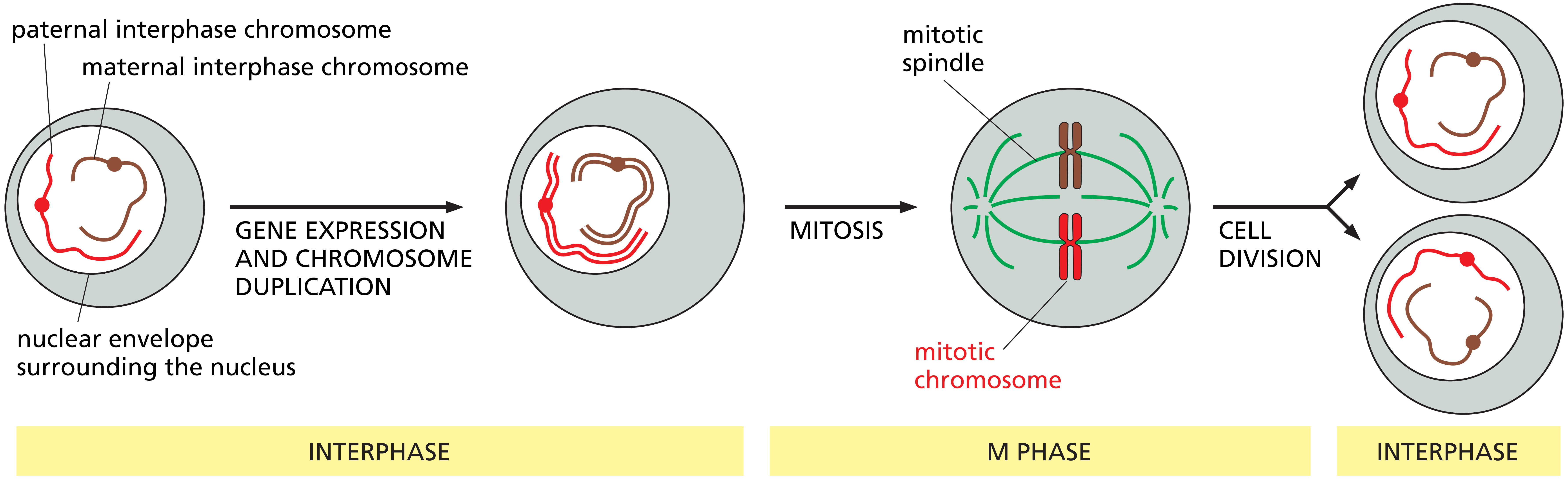
Zooming into the chromosomes



- In 2004, full sequence of the **human genome**
- We see **how genes are arranged** within chromosomes
- Only **1.5% of DNA** is coding for proteins
- ~50% is made of **mobile DNA**, inserted gradually over evolutionary time
- Average gene size is 27Kbp, which is long: **introns (non-coding DNA) between exons (coding sequences)**

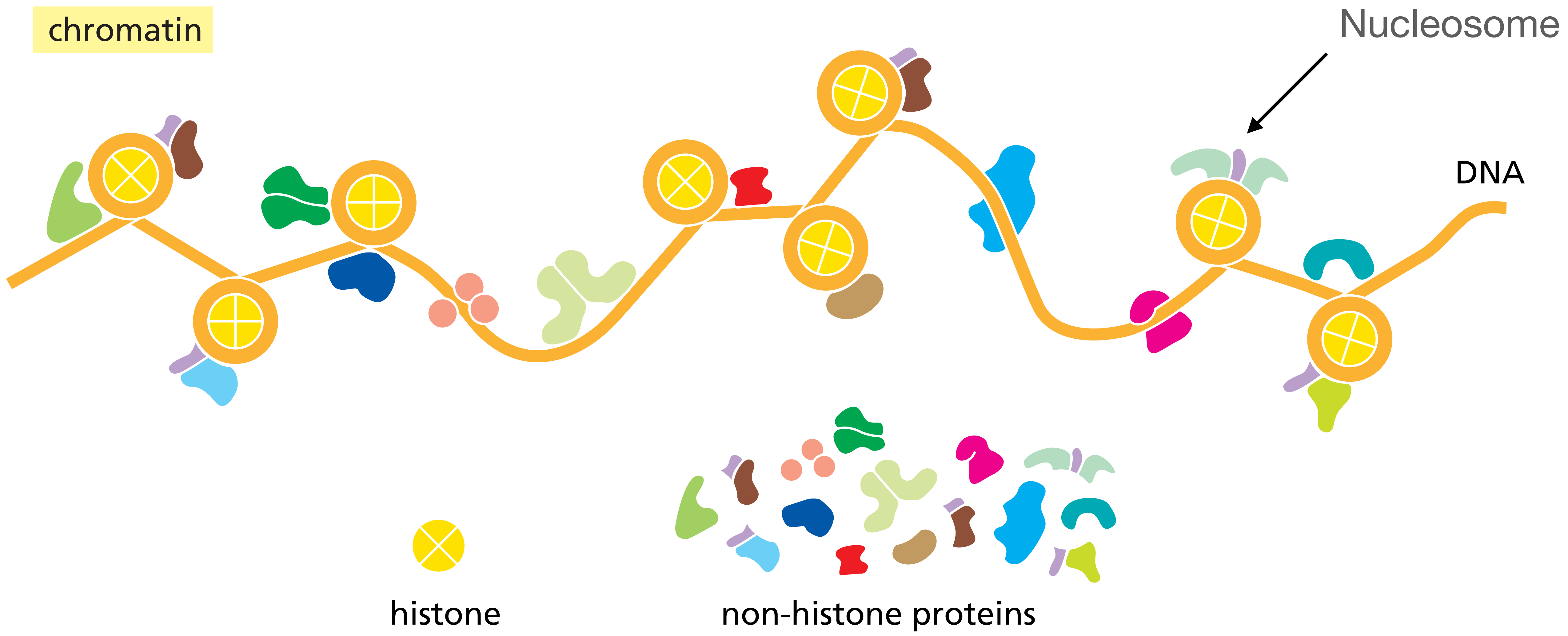
Chromosomes and cell cycle

- A **functional chromosome** must be able to **replicate**, be **separated** and reliably **partitioned** into daughter cells at each division = cell cycle
- During the **Interphase**, **genes are expressed and chromosomes replicated**; the two sister chromatids stay together
- At **mitosis**, chromosomes are **condensed** (= mitotic chromosomes)



Chromatin organization

- DNA molecules are **highly condensed** in chromosomes
- **Proteins** that coil and fold the DNA into higher levels of organization
- Chromosome structure is dynamic - they **decondense for replication, gene expression or DNA repair**
- Proteins that bind to DNA to form eukaryotic chromosomes are the **histones and the non-histone chromosomal proteins**
- Histones+ non-histone chromosomal proteins+DNA = **chromatin**



Nucleosomes

- Histones are responsible for the first level of chromosome packing, the **nucleosome**
- **“Beads on a string”** structure

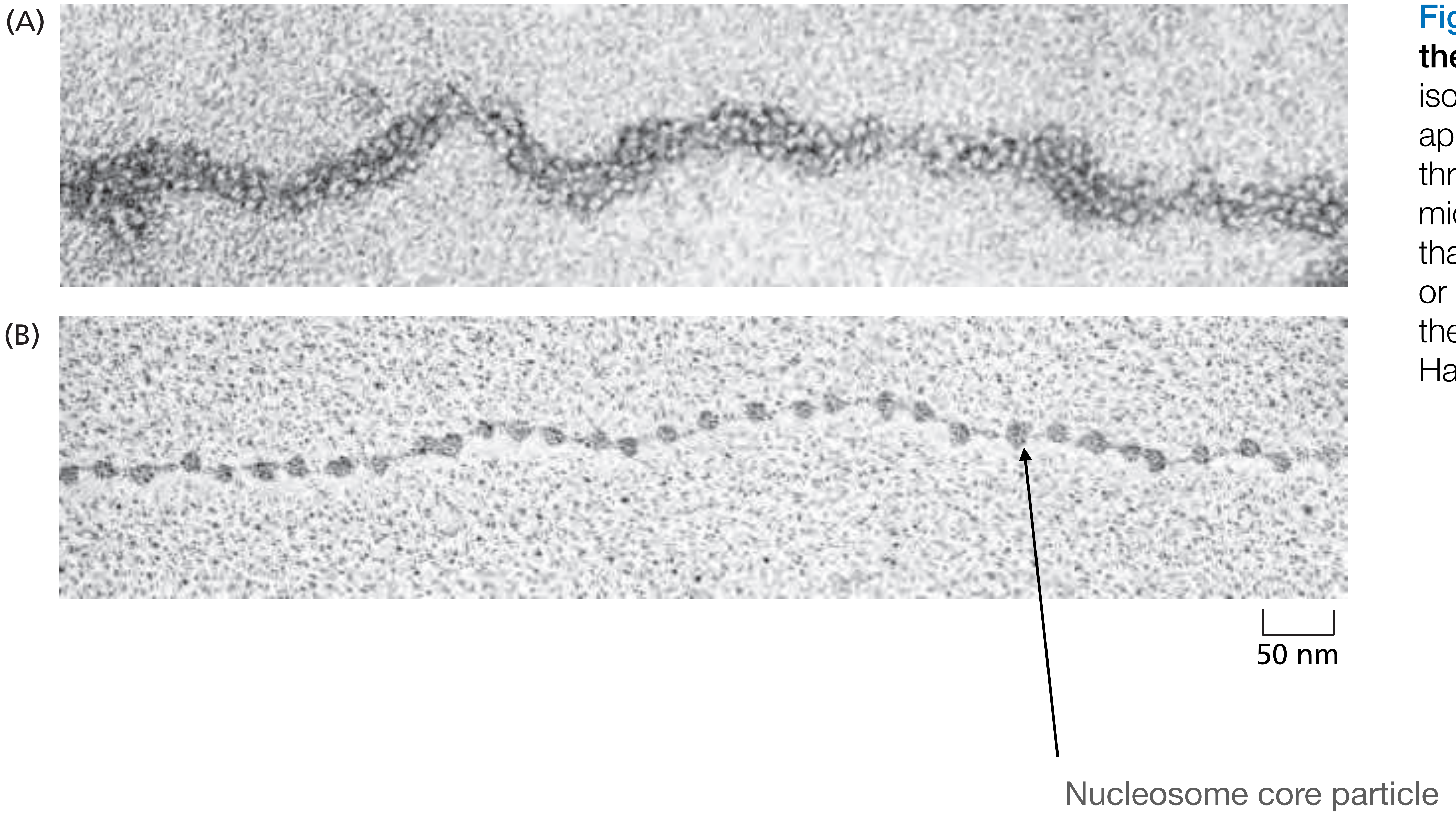
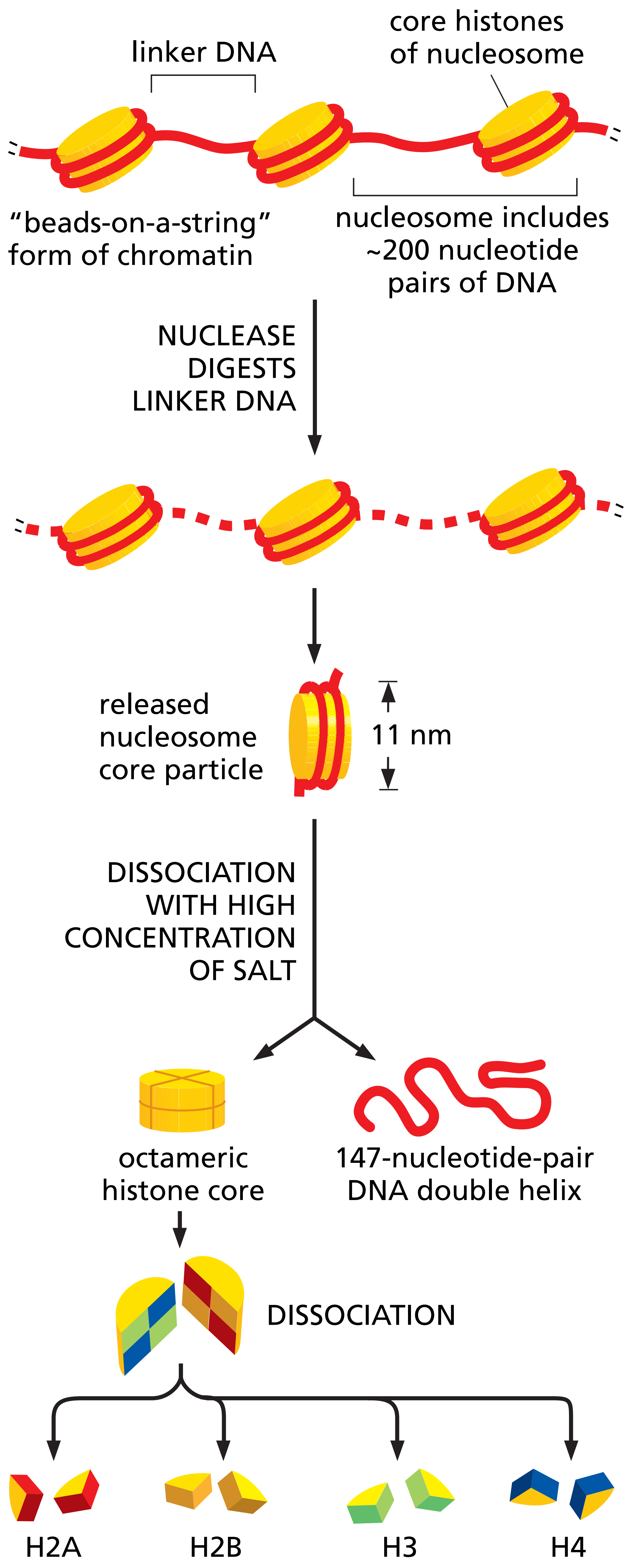


Figure 4–21 Nucleosomes as seen in the electron microscope. (A) Chromatin isolated directly from an interphase nucleus appears in the electron microscope as a thread about 30 nm thick. (B) This electron micrograph shows a length of chromatin that has been experimentally unpacked, or decondensed, after isolation to show the nucleosomes. (A, courtesy of Barbara Hamkalo; B, courtesy of Victoria Foe.)

Nucleosome structure

- Each individual **nucleosome core particle** consists of a complex of **8 histone proteins** and **double-stranded DNA**
- 4 different histones in duplicate = **octamer**
- Each histone octamer forms a **protein core** around which the DNA is wound
- Nucleosomes repeat at intervals of **~200 nucleotide** pairs
- **Linker DNA** is the DNA located between two nucleosomes
- **Compacts** DNA ~**3x**



Linker DNA and histone-1

- Nucleosomes are packed together into a **compact chromatin fiber** (diameter 30 nm)
- How this happens is unclear but involves **histone tails** and an additional **histone H1**

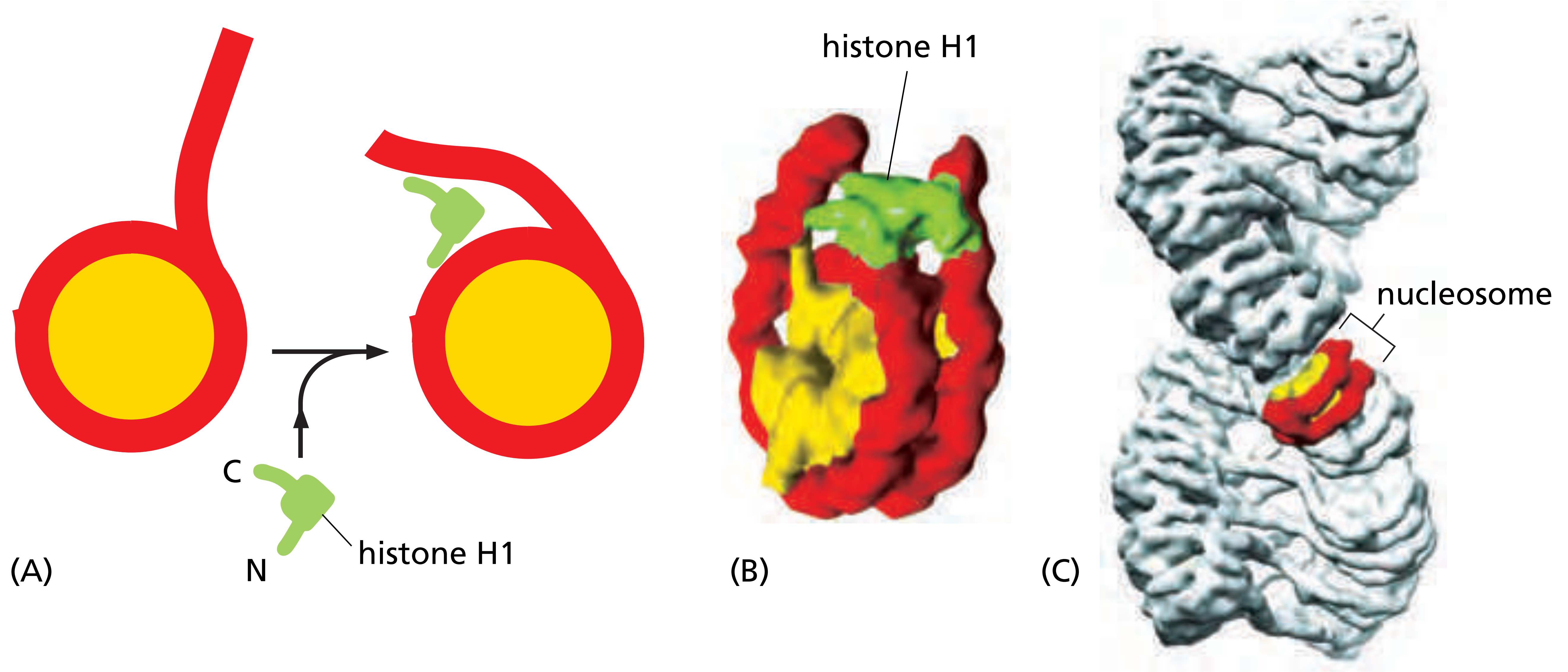


Figure 4–30 How the linker histone binds to the nucleosome. The position and structure of histone H1 is shown. The H1 core region constrains an additional 20 nucleotide pairs of DNA where it exits from the nucleosome core and is important for compacting chromatin. (A) Schematic, and (B) structure inferred for a single nucleosome from a structure determined by high-resolution electron microscopy of a reconstituted chromatin fiber (C). (B and C, adapted from F. Song et al., *Science* 344:376–380, 2014.)

Plan

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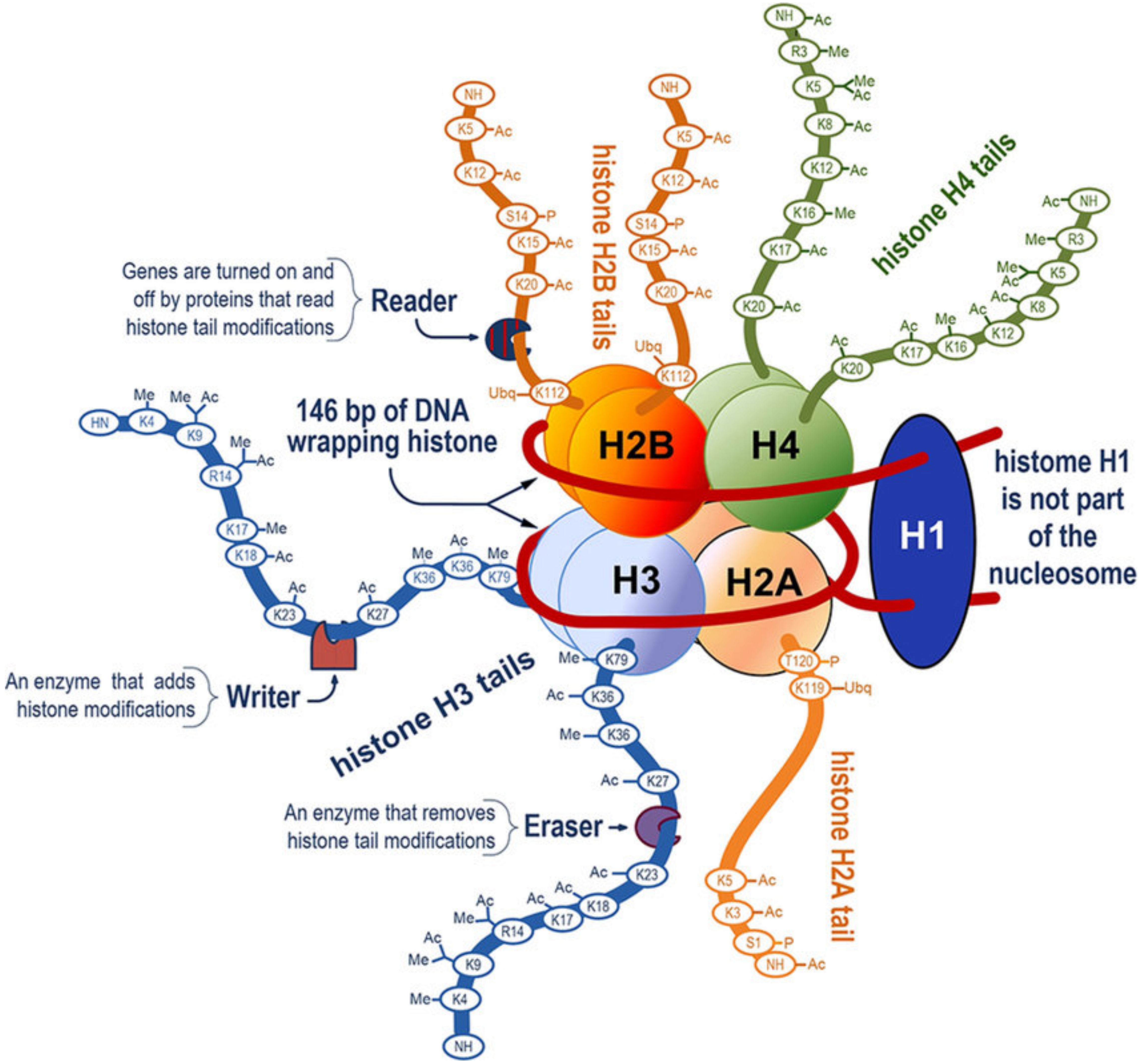
Histones characteristics

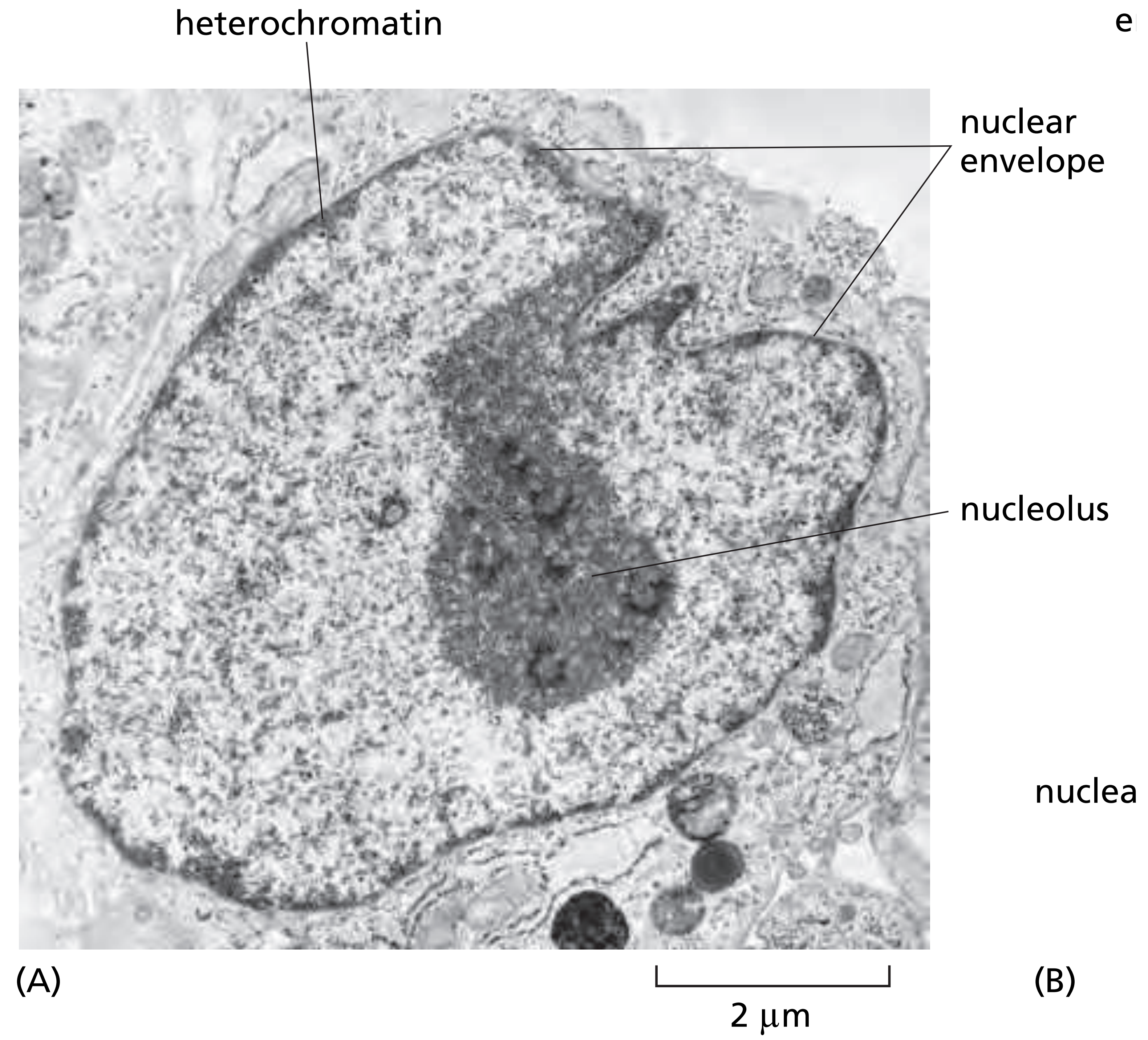
- **Positively charged** - neutralise negative charges from DNA
- Core histones are highly **conserved** across evolution
- Nucleosomes are **dynamic** to allow replication and transcription
- **N-terminal tails** of histones stick out from the nucleosome and are subject to **modifications**

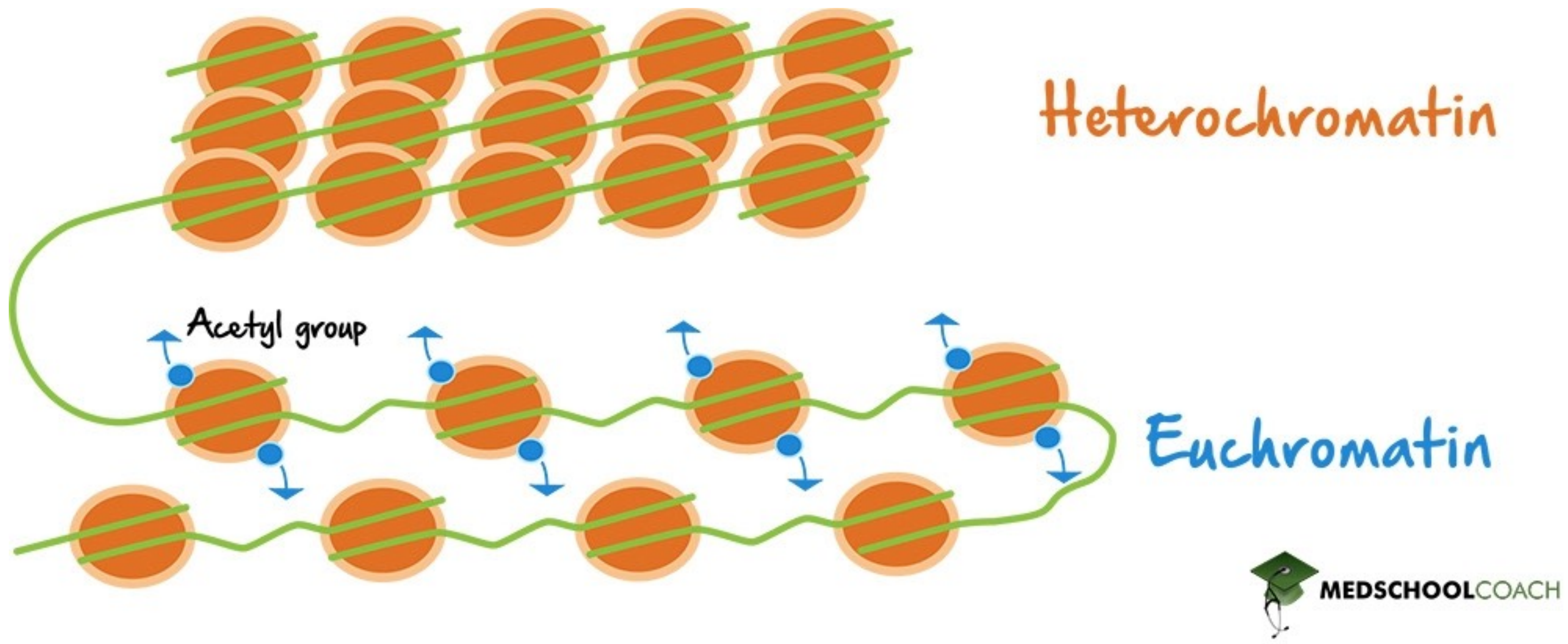


DOI:10.1021/acs.analchem.7b05007

Chromatin dynamically changes

- In the 1930s, light microscopy distinguished two types of chromatin: **heterochromatin (highly condensed- inactive) and euchromatin (less condensed- active)**
- Heterochromatin is highly concentrated in **certain regions** (centromeres and telomeres), but also present in other regions **depending on the physiological state of the cell**
- Heterochromatin can be **constitutive** or **facultative**
- Heterochromatin contains **few genes**
- Through chromosome breakage, if a part of **euchromatin ends up in heterochromatin**, this causes the **silencing of normally active genes** = **position effect**





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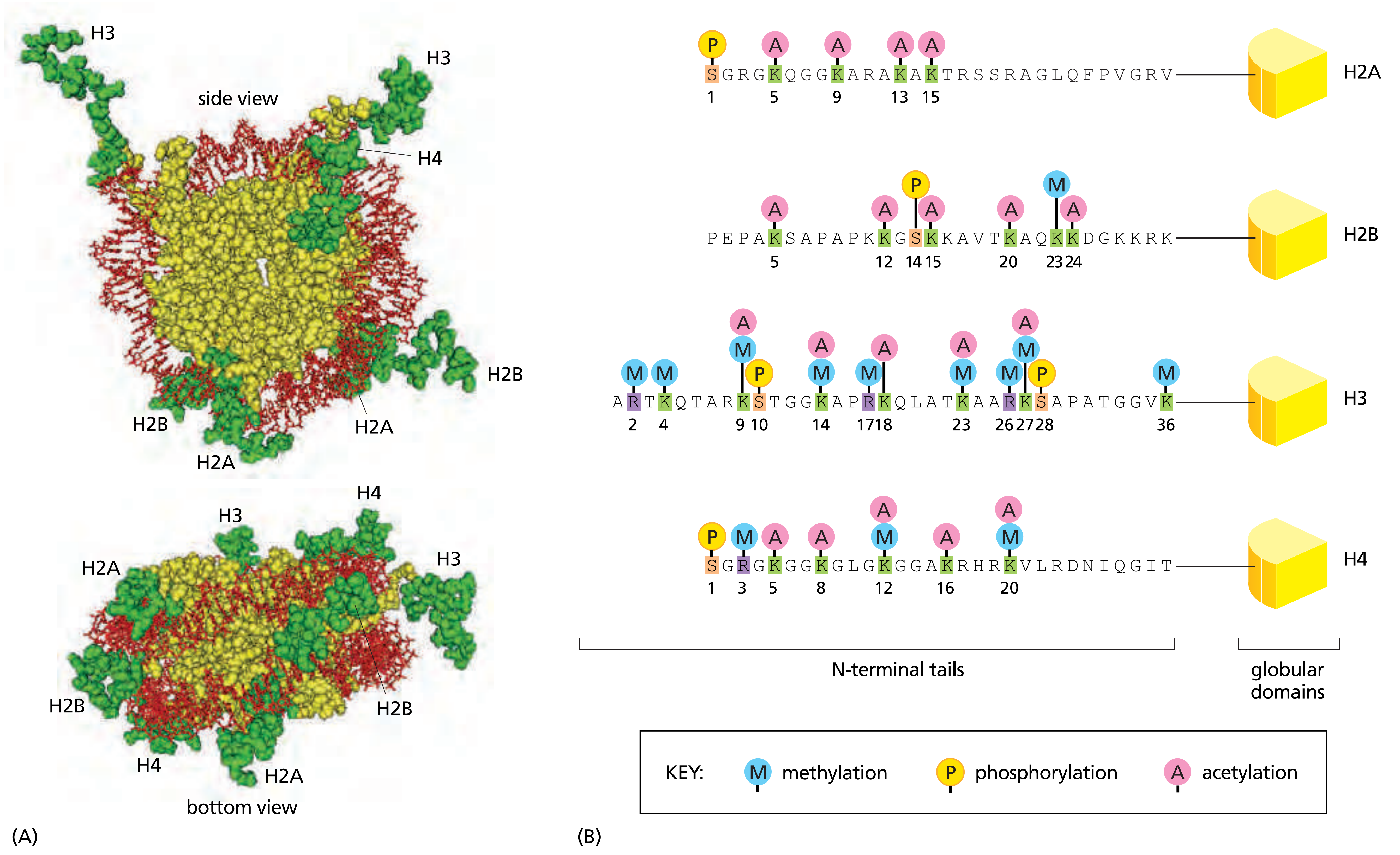
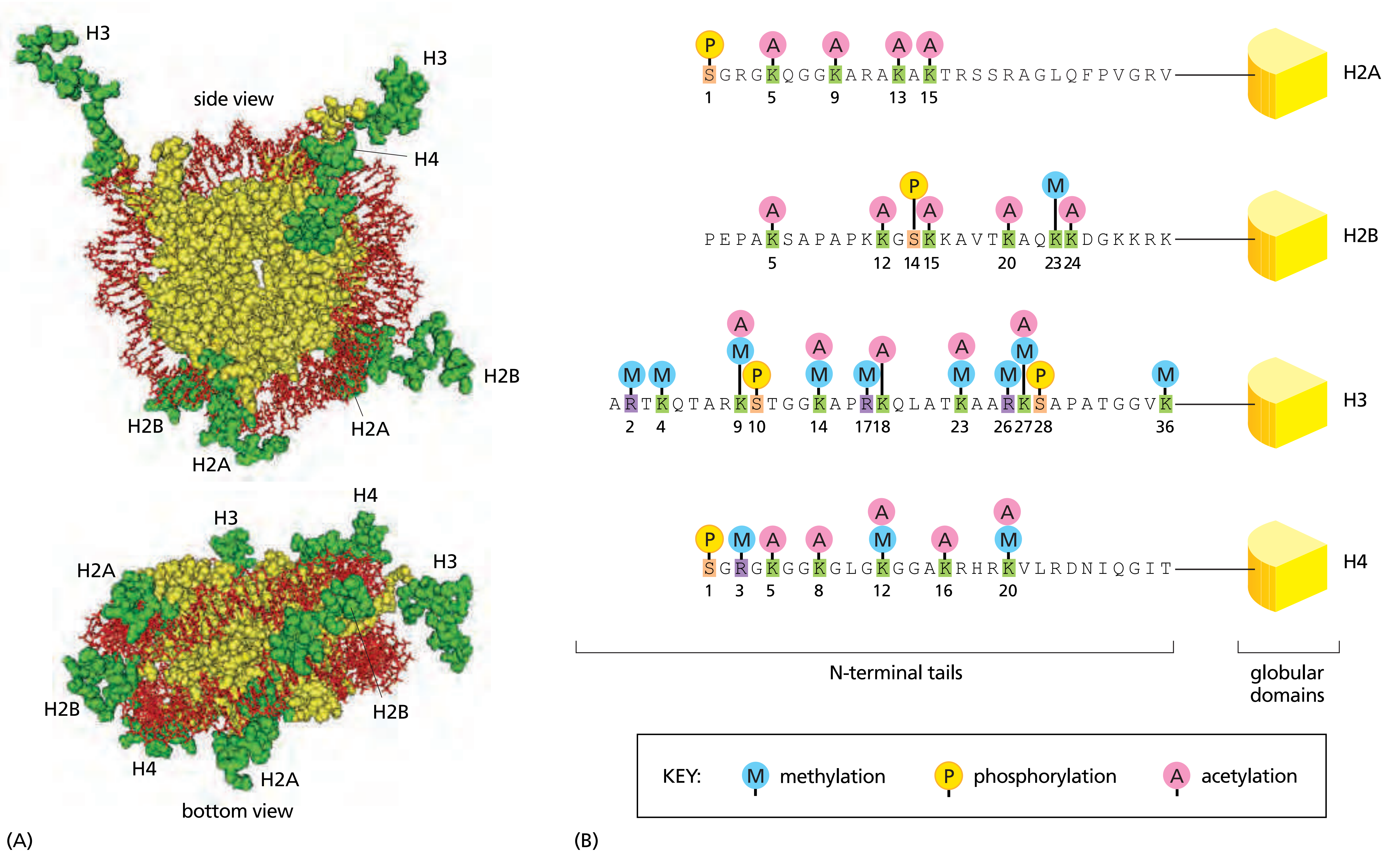
Histone modifications

- The **amino-acid side-chain of the <u>core</u> histones** are subjected to many covalent mutations (acetylation of lysines, methylation of lysines or phosphorylation of serines)
- Primarily on **histone tails** but also on **nucleosome** core
- Modifications are reversible with **dedicated enzymes** to add or remove them (e.g. HATs and HDACs)
- Result in change in **chromatin organization**
- Are referred to as the **“histone code”**



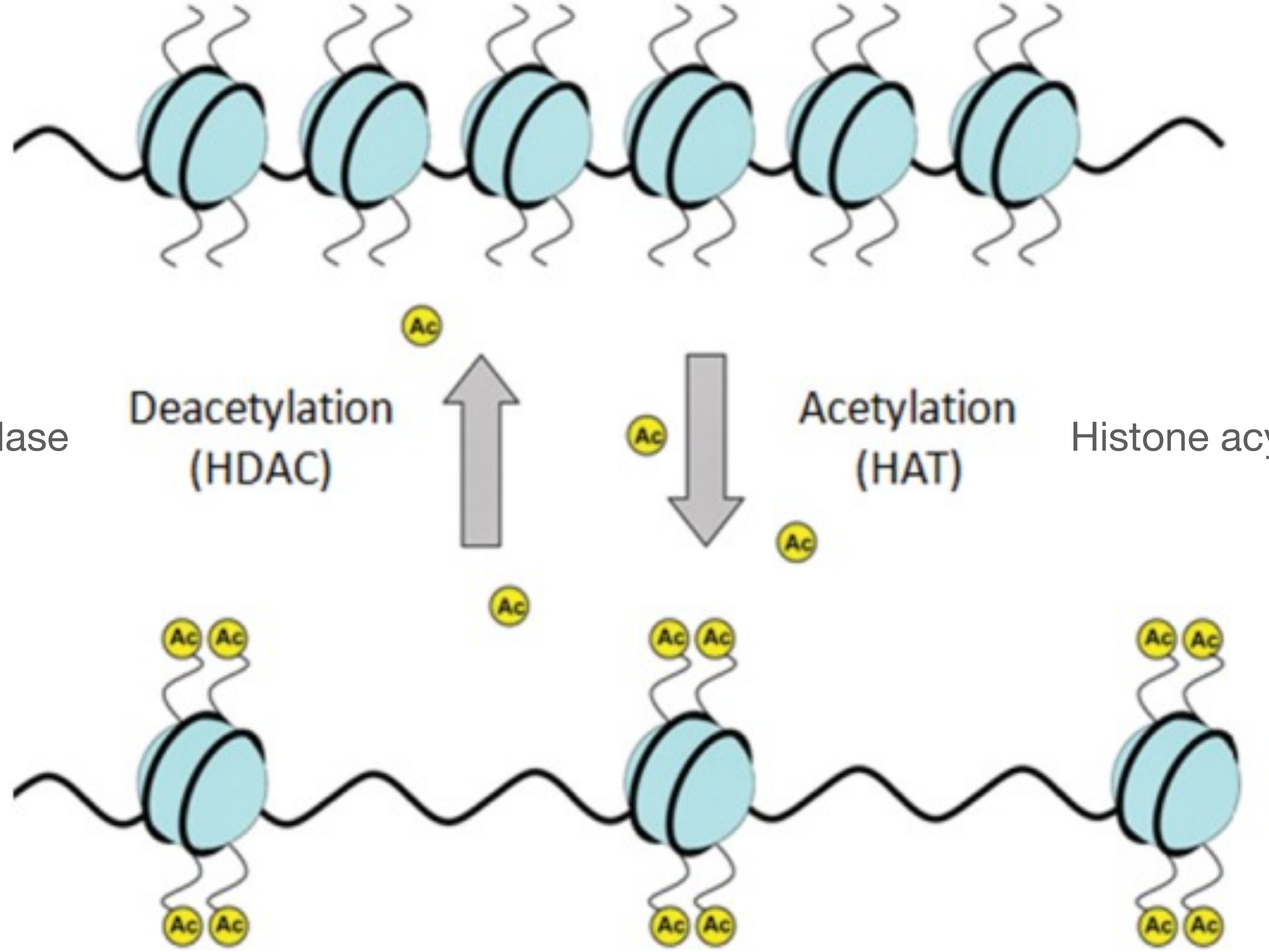
Histone modifications

- Modification enzymes bind to **DNA sequences**, so DNA determines **how histones are modified**
- Some modifications **persist in time**

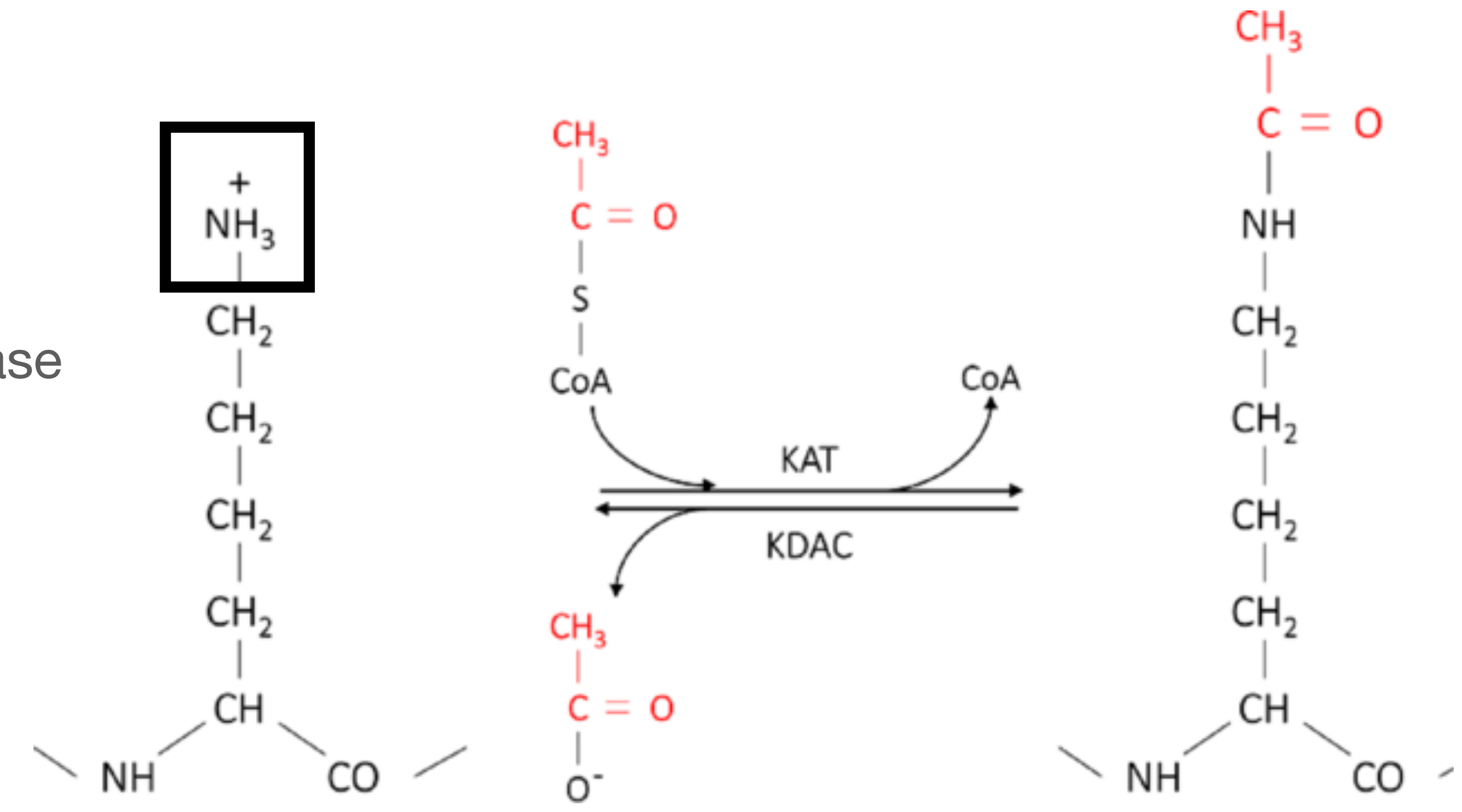


Example: Histone acetylation

Histone deacetylase



Histone acyltransferase



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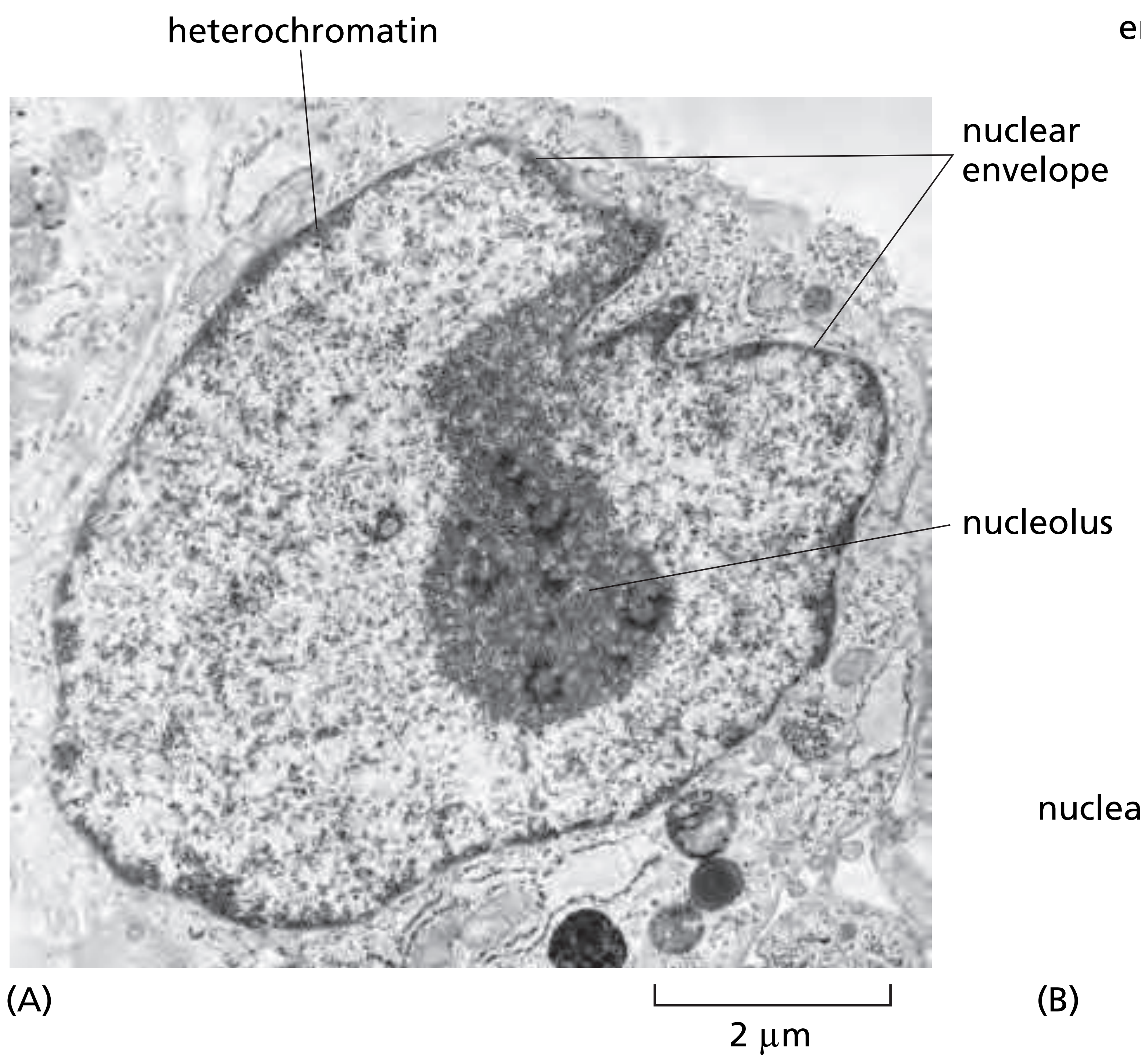
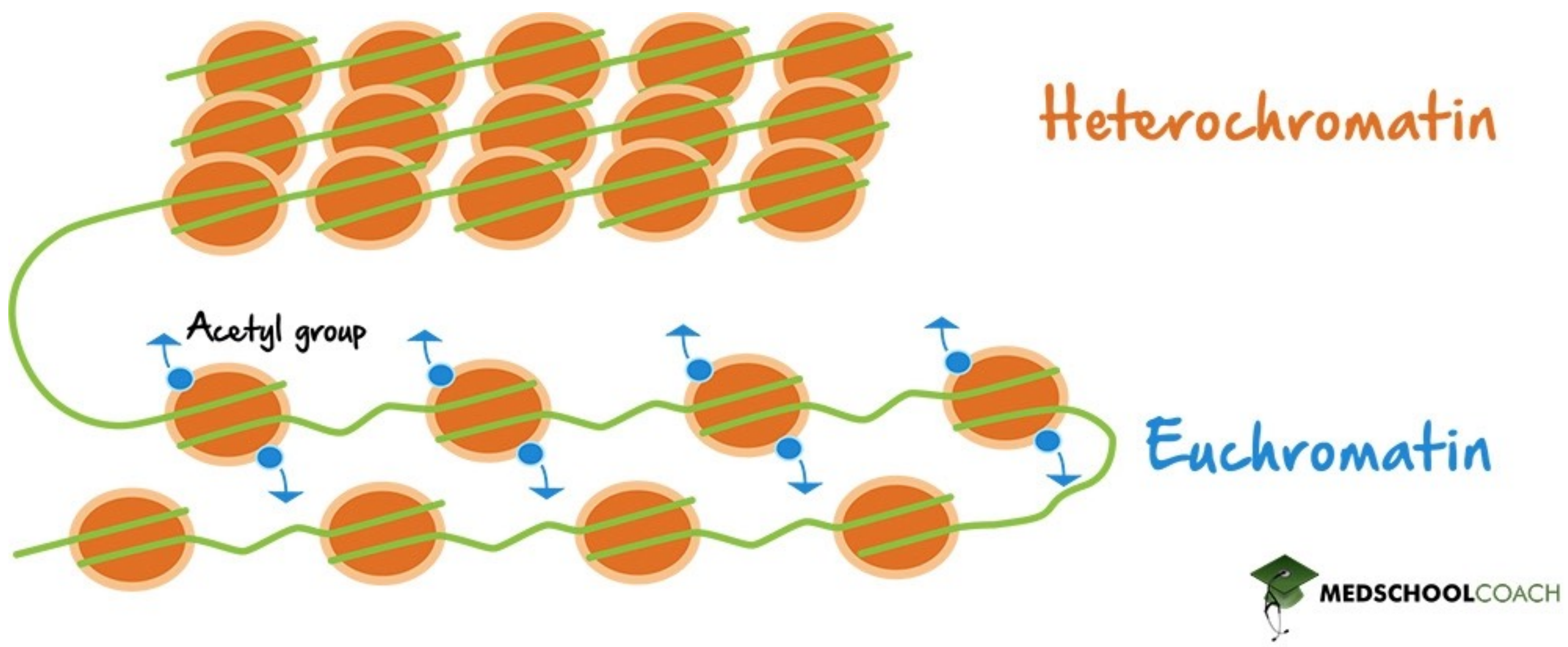
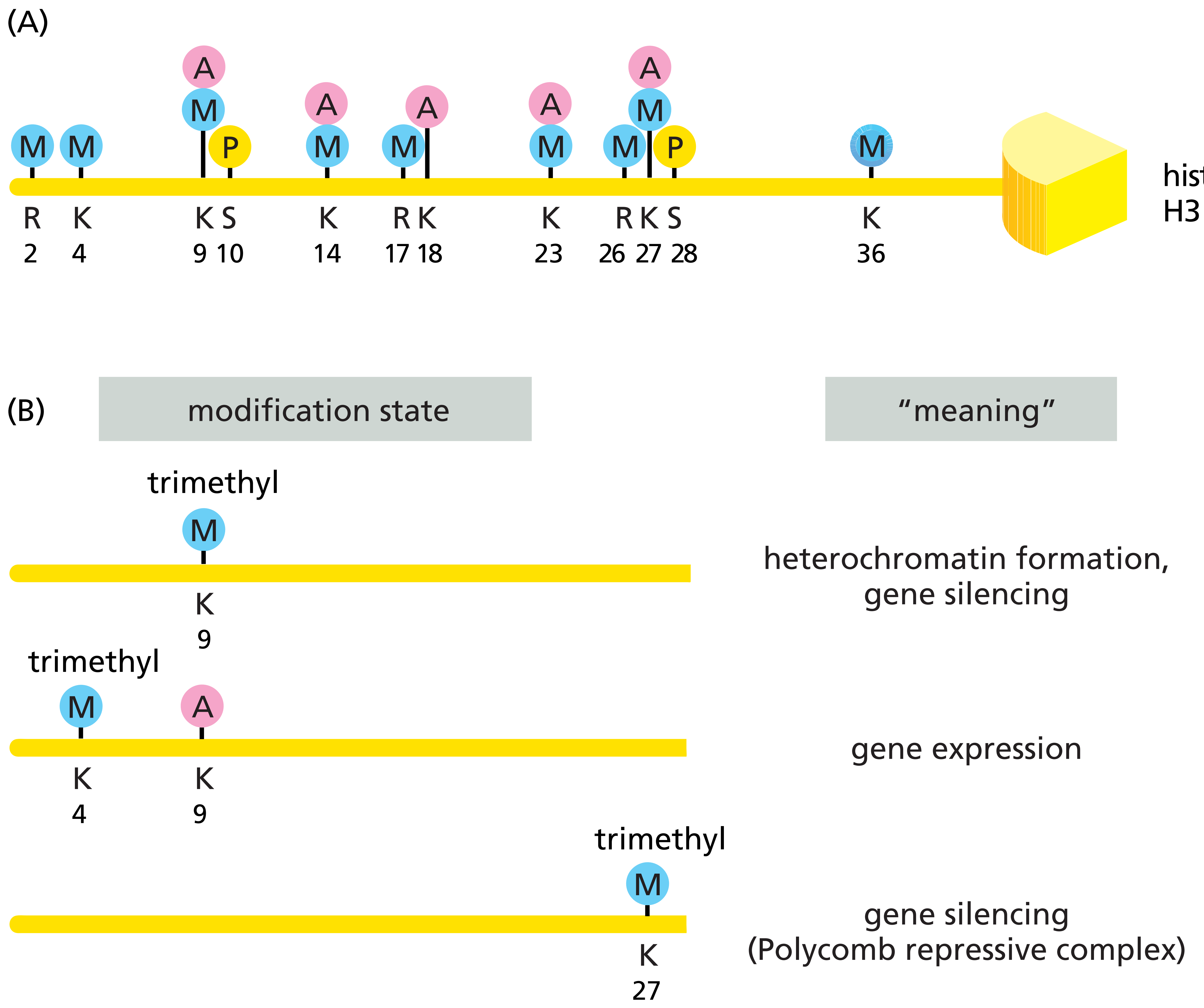
Histone modifications influence gene expression

- Acetylated histones - open chromatin (euchromatin)- transcriptional activity

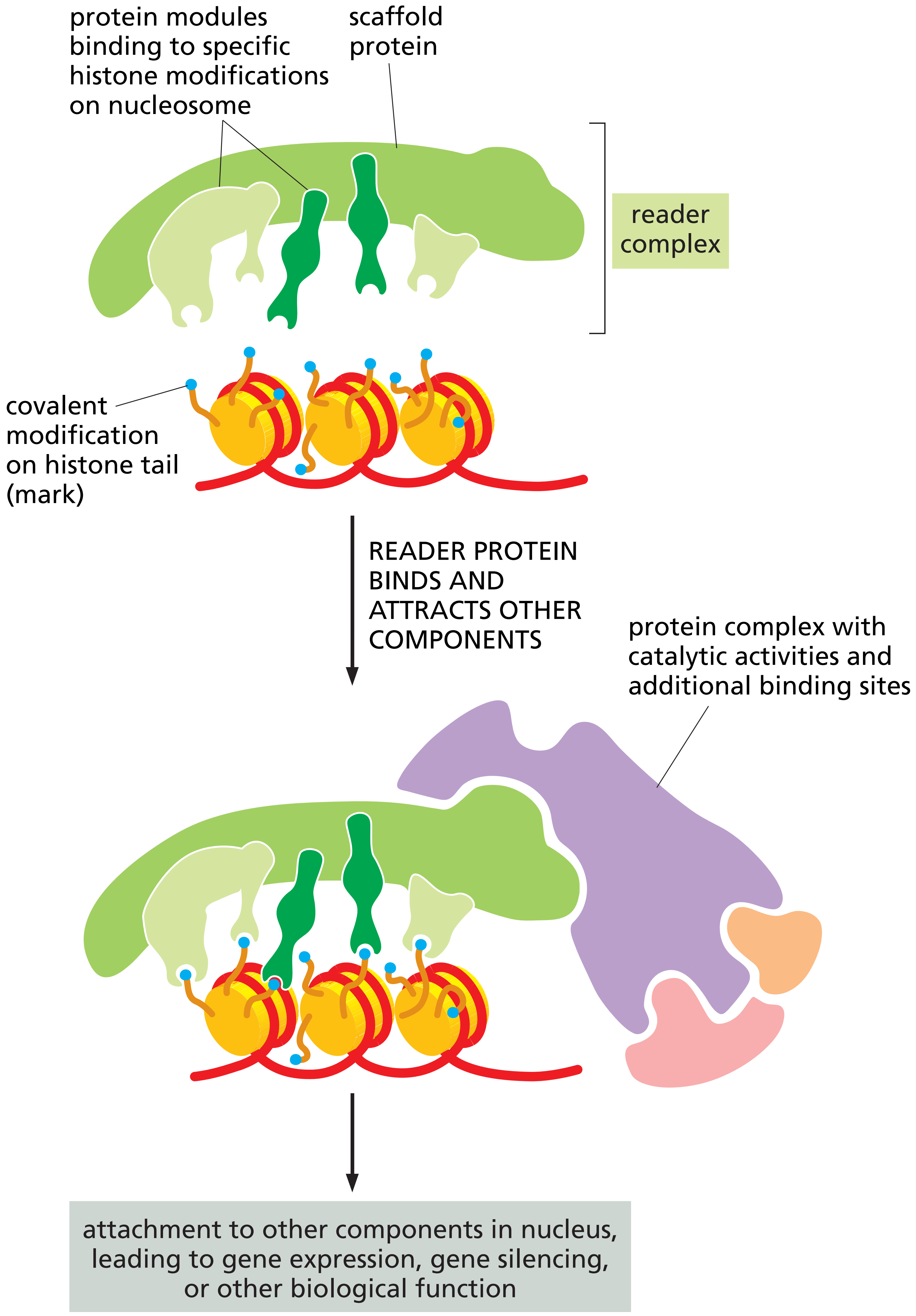




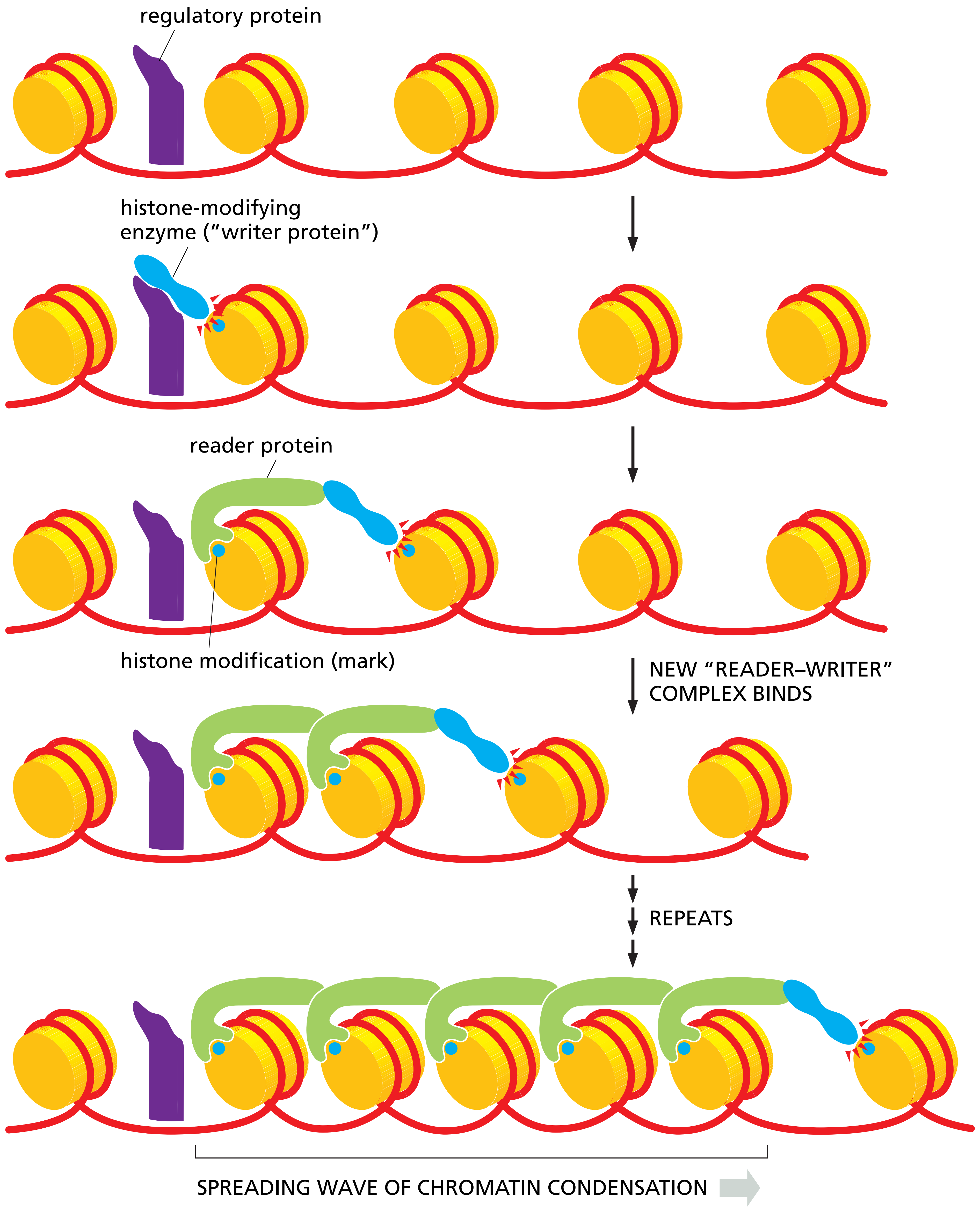
Example: histone code (H3)



Reader complexes read the histone code



The spread of heterochromatin (position effect)



What blocks the spread of heterochromatin?

- This can be problematic as each chromosome is one long stretch of DNA
- **DNA sequences** have been identified and mark the boundaries of chromatin domains = barrier sequences. If those are deleted, euchromatin can be invaded by heterochromatin
- Cluster of binding sites for **acetylation enzymes**
- Acetylation of a lysine side-chain is **not compatible** with methylation of the same side-chain
- Specific **lysine methylations are required to spread heterochromatin** so histone **acetylases** are logical candidates for barrier regions

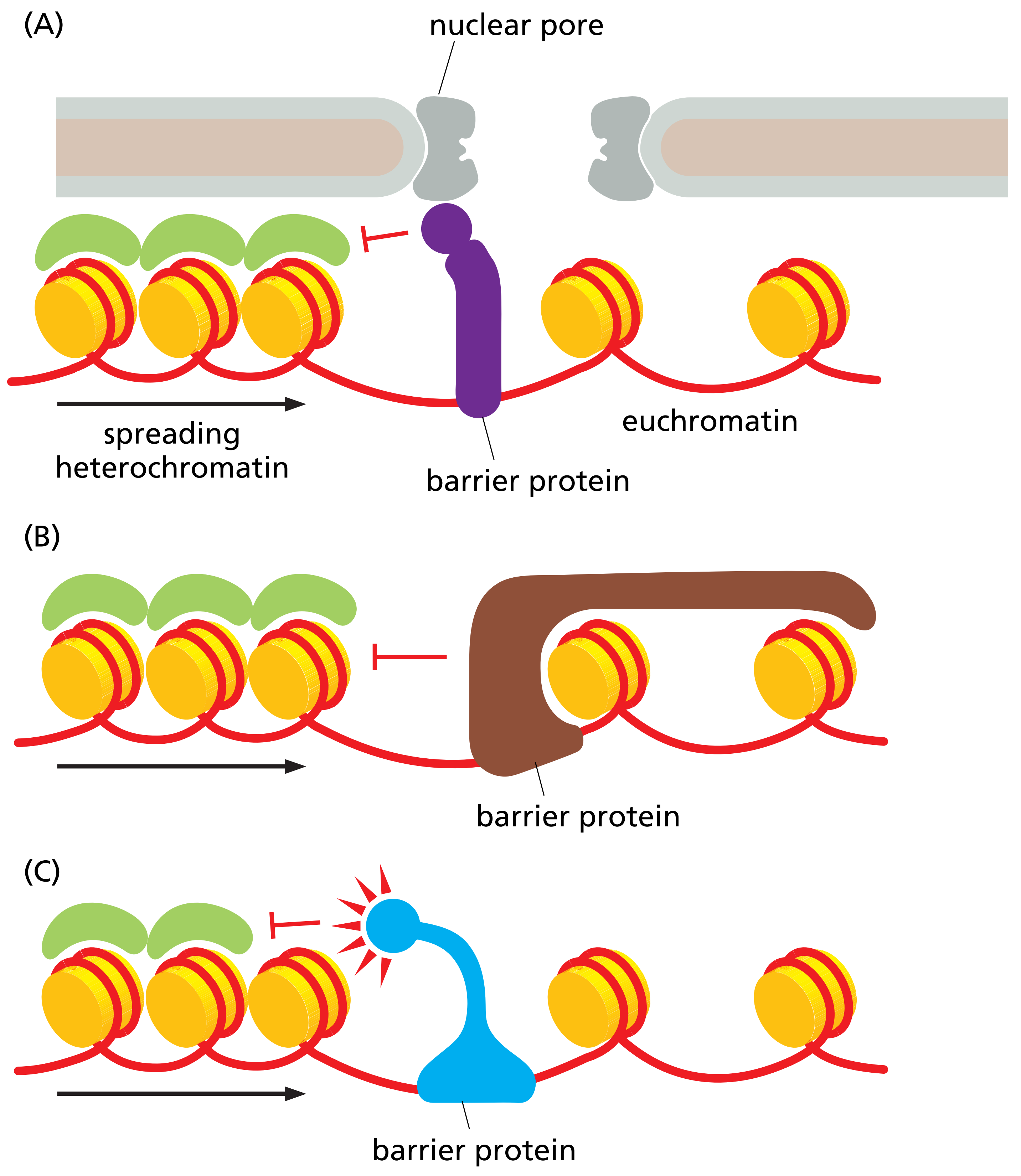
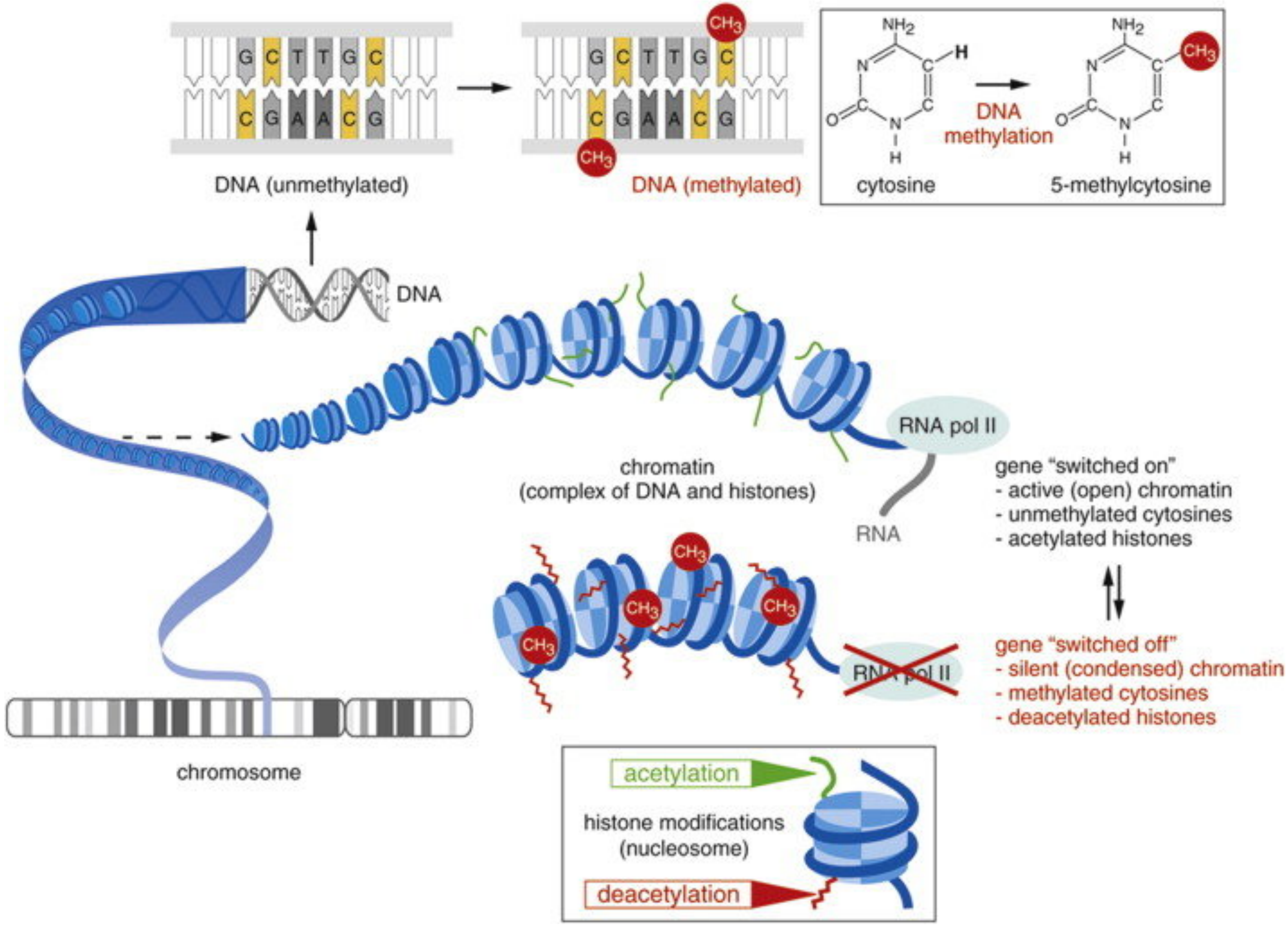


Figure 4–41 Some mechanisms of barrier action. These models are derived from experimental analyses of barrier action, and a combination of several of them may function at any one site. (A) The tethering of a region of chromatin to a large fixed site, such as the nuclear pore complex illustrated here, can form a barrier that stops the spread of heterochromatin. (B) The tight binding of barrier proteins to a group of nucleosomes can make this chromatin resistant to heterochromatin spreading. (C) By recruiting a group of highly active histone-modifying enzymes, barriers can erase the histone marks that are required for heterochromatin to spread. For example, a potent acetylation of lysine 9 on histone H3 will compete with lysine 9 methylation, thereby preventing the binding of the HP1 protein needed to form a major form of heterochromatin. (Based on A.G. West and P. Fraser, *Hum. Mol. Genet.* 14:R101–R111, 2005. With permission from Oxford University Press.)

In addition to histones, is DNA also modified?

- **DNA methylation** on **cytosine**
- DNA methylation influences **gene expression** but not the chromatin organisation (hetero- and euchromatin)



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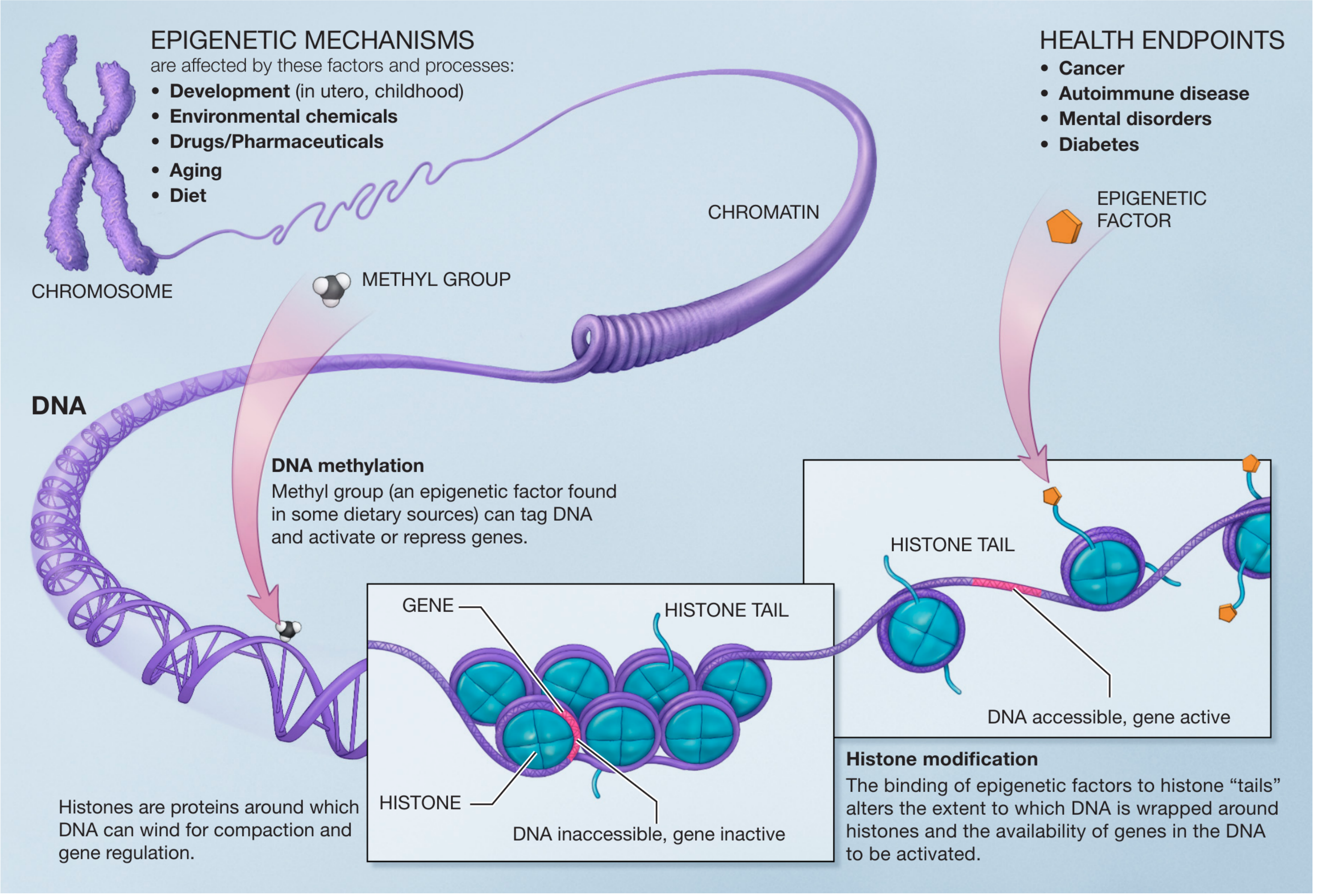
Genetics vs. Epigenetics

Genetic code	Epigenetic code
Conserved during division	Semi-conserved during division
Strong evolutionary conservation	Rapid evolutionary divergence
Identical in all cell types	Differs between cell types, responsive to environment



Have a nice day!