Cellular and Molecular Biology I

BIO-205-3

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Extra questions

How is heterochromatin attached to the nuclear lamina?

- The nuclear lamina interacts with heterochromatin through proteins. This anchors silent chromatin at the nuclear periphery.
- The nuclear periphery is generally a transcriptionally repressive environment. By tethering heterochromatin there, the cell ensures genes in these regions remain silent.

The arrangement creates a functional separation:

- Euchromatin (active, loosely packed) is often more central in the nucleus.
- Heterochromatin (inactive, dense) is peripheral, adjacent to the lamina.

Extra questions

What is the difference between FISH and chromosome painting

- Both **FISH** and **chromosome painting** are cytogenetic techniques that rely on fluorescent probes to visualize DNA sequences, but they differ in **scope, purpose, and resolution**.

FISH (Fluorescence In Situ Hybridization)

- Definition: A technique where fluorescently labeled DNA probes bind (hybridize) to specific DNA sequences on chromosomes.
- Scope: Can target small, specific DNA sequences—for example, a single gene, a locus, or even repetitive sequences.
- Resolution: High—can detect specific genes or small chromosomal abnormalities like microdeletions, duplications, or translocations.
- Uses:
  - Location of a given DNA sequence in the nucleus
  - Diagnosing genetic disorders (e.g., Down syndrome, DiGeorge syndrome).
  - Detecting cancer-associated chromosomal rearrangements (e.g., BCR-ABL fusion in leukemia).
  - Identifying copy number variations.

Extra questions

What is the difference between FISH and chromosome painting

Chromosome Painting (a type of FISH)

- Definition: A special application of FISH where entire chromosomes (or large chromosomal regions) are "painted" with fluorescent probes that cover their full length.
- Scope: Whole chromosomes—probes are made from DNA libraries spanning an entire chromosome.
- Resolution: Lower than gene-specific FISH, since it labels the whole chromosome rather than pinpointing a small locus.
- Uses:
  - Identifying structural chromosomal abnormalities (e.g., translocations, insertions, complex rearrangements).
  - Karyotyping in cancer research.
  - Studying chromosome evolution across species (comparative genomics).

Extra questions

What is the difference between biotin or fluorescent probes?

Biotinylated Probes

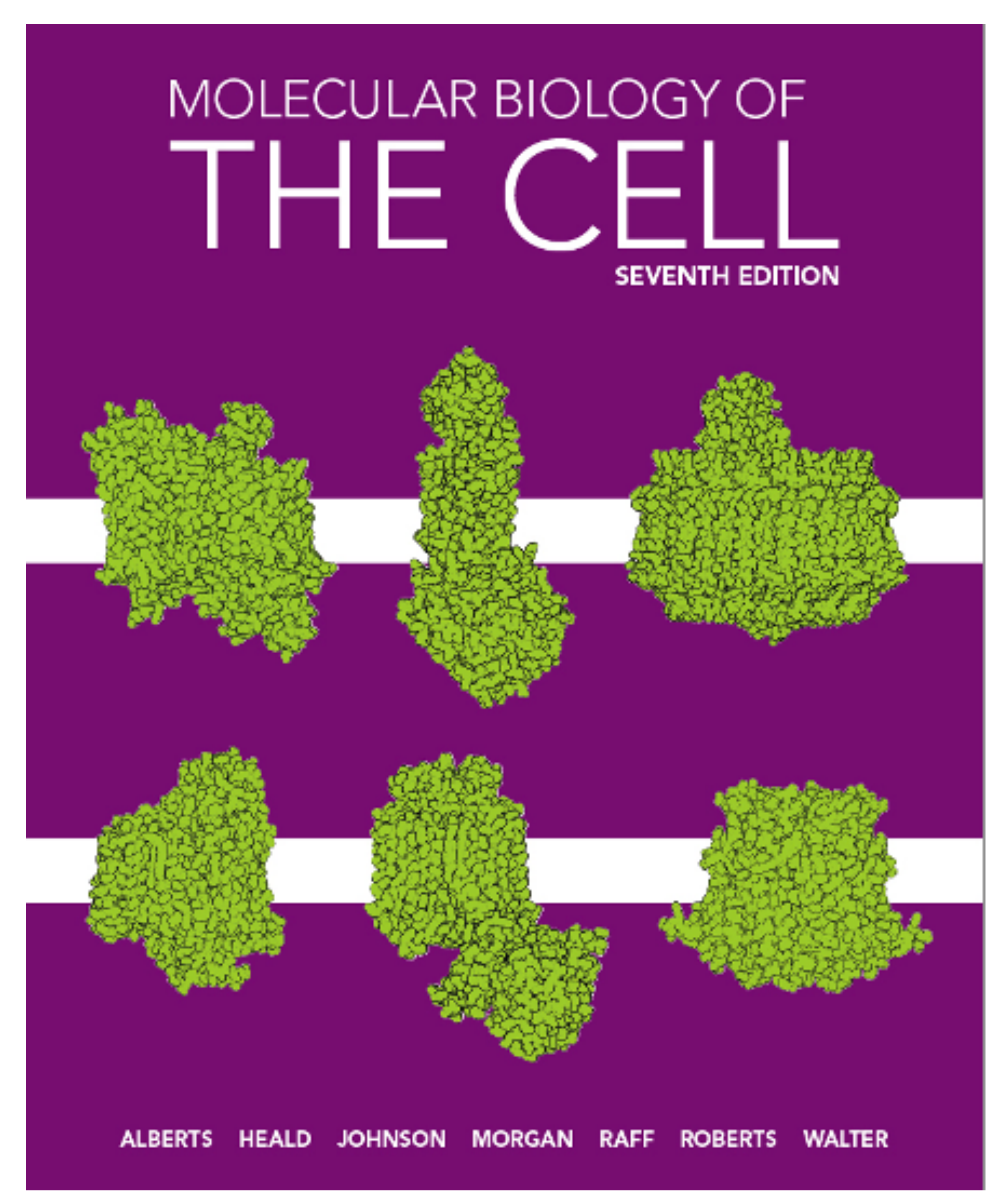
- How they work:
  - Probes are labeled with biotin (a vitamin).
  - After hybridization to the target DNA, a secondary detection step is needed: Biotin is recognized by avidin/streptavidin/antibody, which are conjugated to a fluorescent dye or an enzyme.
- Advantages:
  - Signal amplification: Multiple fluorescent molecules can bind to a single biotin, giving a stronger signal—useful for detecting low-copy sequences.
  - Flexibility: The same biotin probe can be detected with different labels (fluorescent or enzymatic).
  - Stable labeling: Biotinylation is chemically stable and easy to incorporate into probes.
- Disadvantages:
  - Requires extra steps (hybridization + detection), so more time-consuming.

Extra questions

What is the difference between biotin or fluorescent probes?

Fluorescent-Labeled Probes

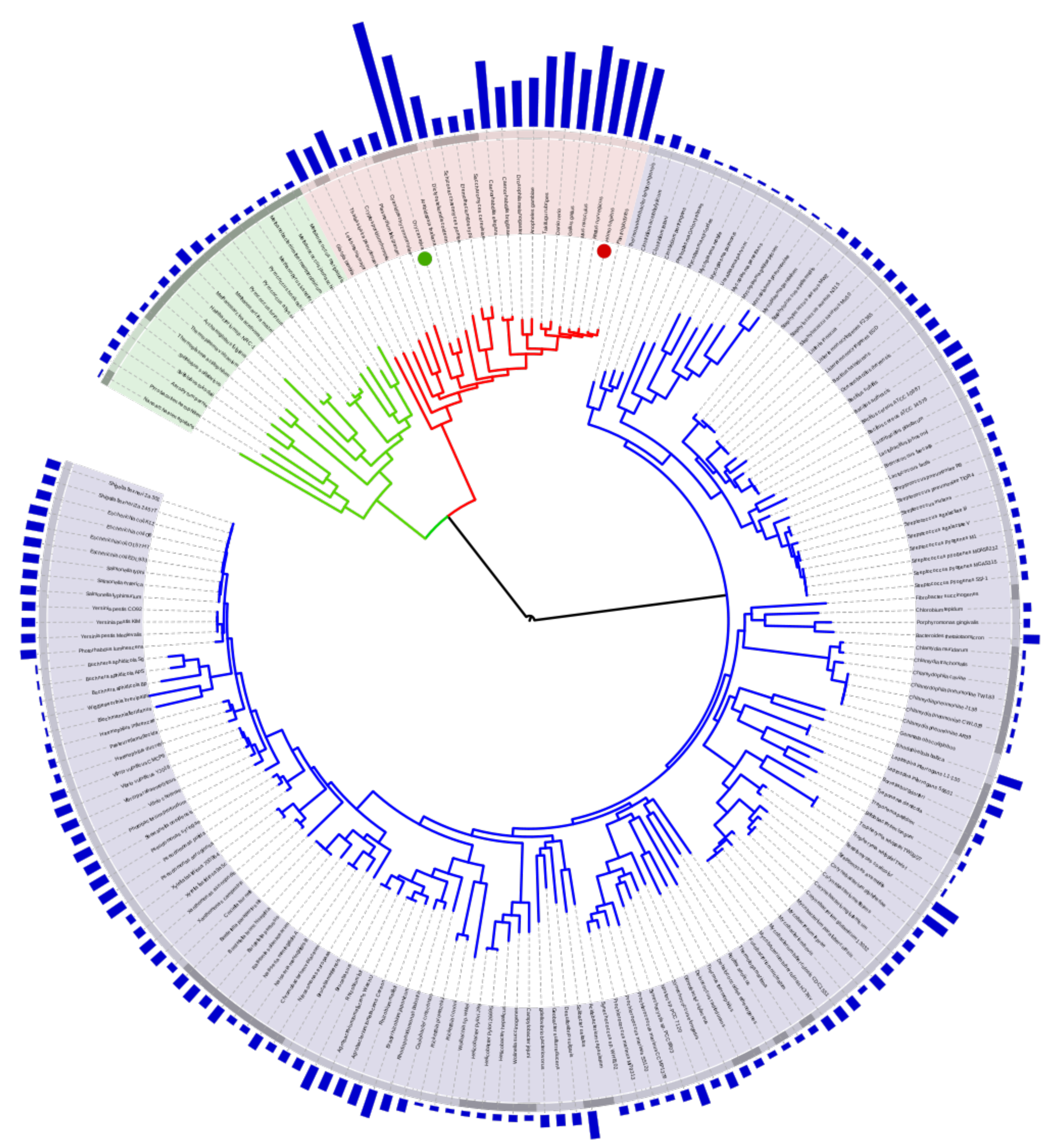
- How they work:
  - The probe itself is directly tagged with a fluorescent dye
  - After hybridization, you can visualize the probe immediately under a fluorescence microscope.
- Advantages:
  - Faster and simpler—no secondary detection step.
  - Lower background compared to biotin-based methods.
  - Great for high-copy targets (like repetitive sequences or chromosome painting).
- Disadvantages:
  - Lower sensitivity (since only one dye per labeled nucleotide).
  - Less flexible (if you want to change the color, you need to remake the probe).
  - Fluorescent dyes may photobleach faster.



Chapter 4

DNA, Chromosomes, and Genomes

Quick recap: genomes



Quick recap: genome evolution

- Evolution depends on **accident and mistakes** followed by **non-random survival**
- **Failures** in the mechanisms by which genomes are **copied or repaired**
- When errors (mutations) happen in **germ cells**, they are passed on to the next generation
- Errors are **“rare”** events: ~ 1 in 10^8 per generation (implying that each gamete has in average 30 mutations)

What are the different **types of mutations**?

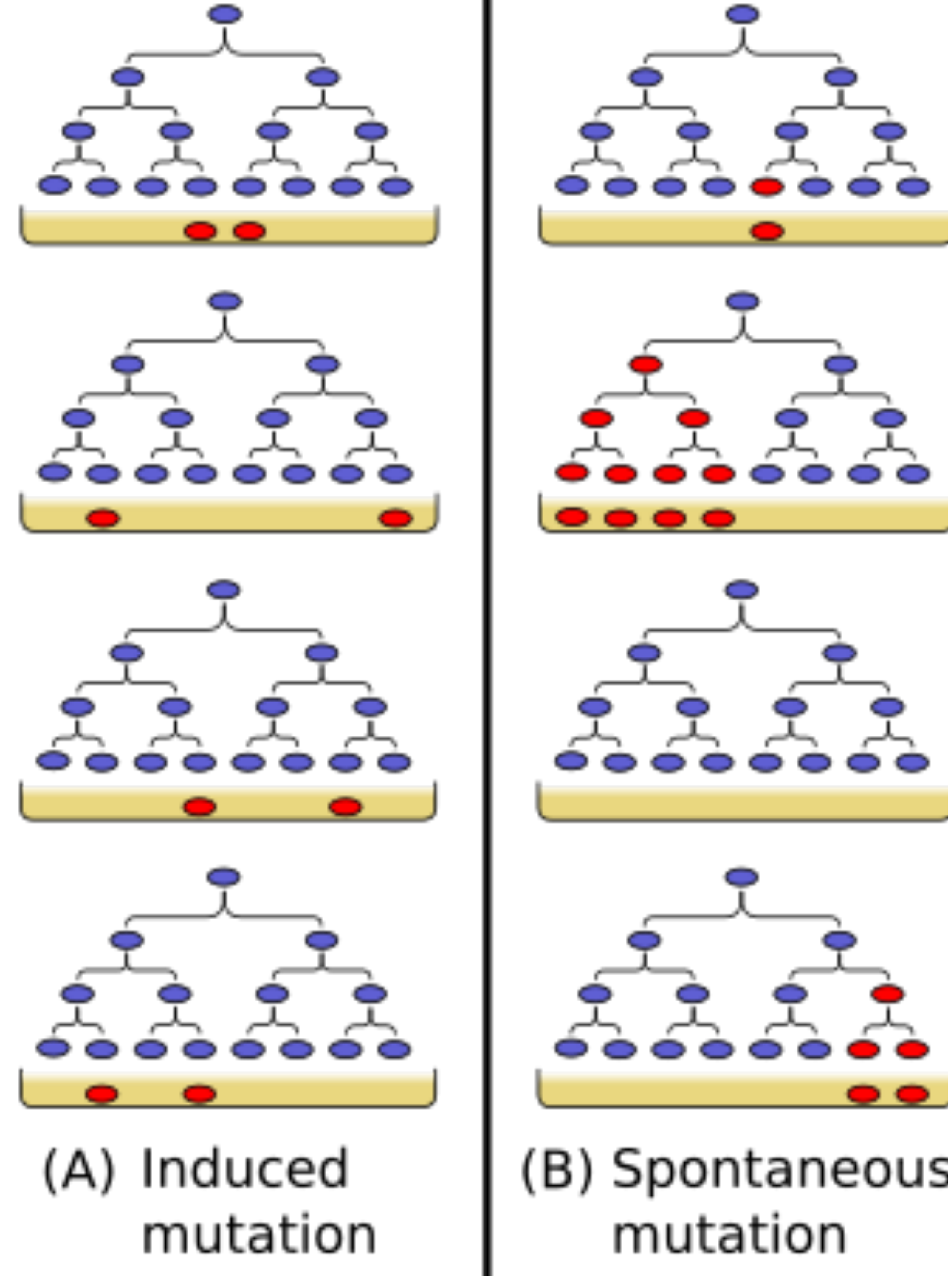
- Simple, local changes - **point mutations**
- Large-scale genome rearrangements - **deletions, duplications, inversion, translocations**
- <u>**In addition, important role of mobile genetic elements**</u>

Fluctuation test

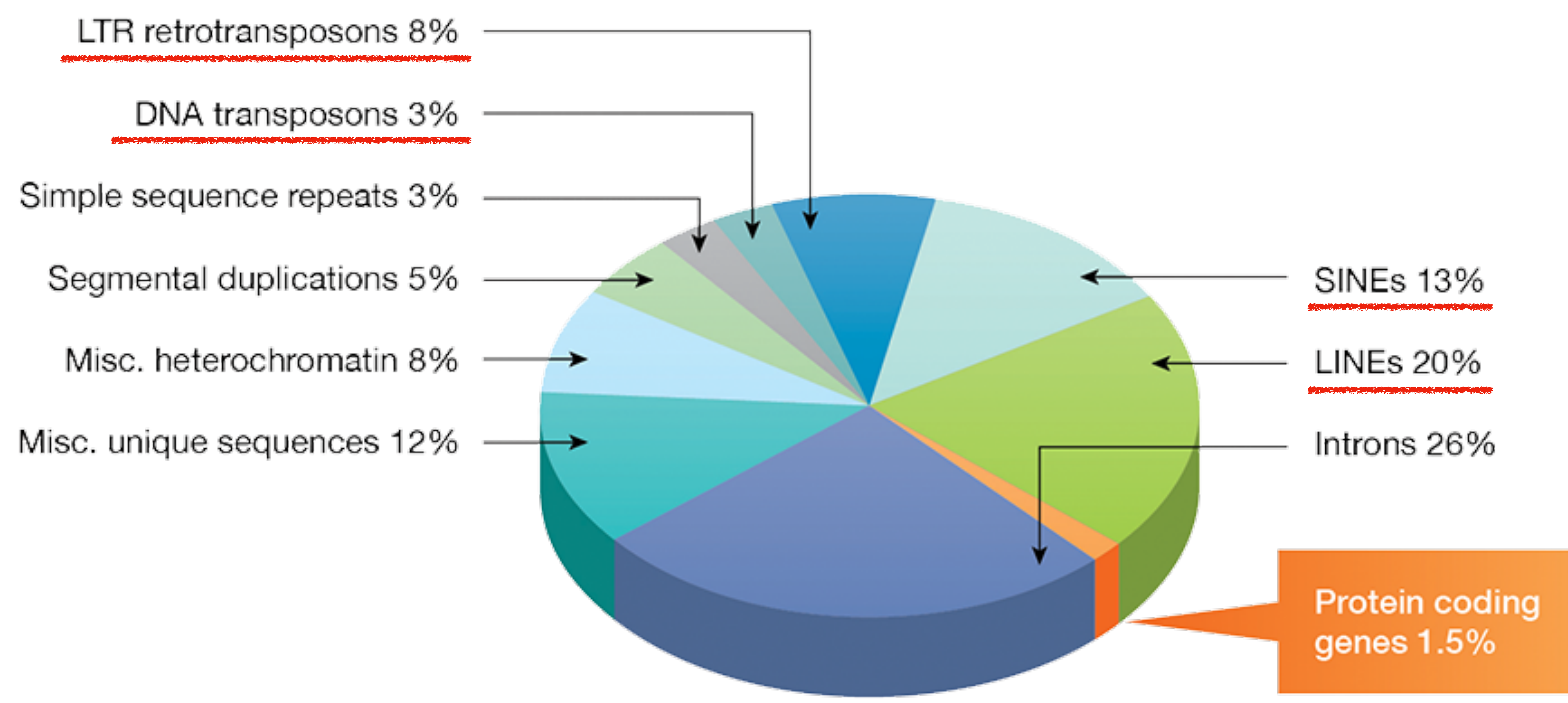
➡ How do we know if mutations are **induced by a given condition** or if they are **random** and **later selected** by the given condition?

Luria and Delbrück (1943)

Fluctuation test



Quick recap: the human genome



Plan

- Quick recap
- Mobile genetic elements
- Comparing genomes
- The maintenance of DNA sequences
- DNA replication mechanisms

Mobile genetic elements

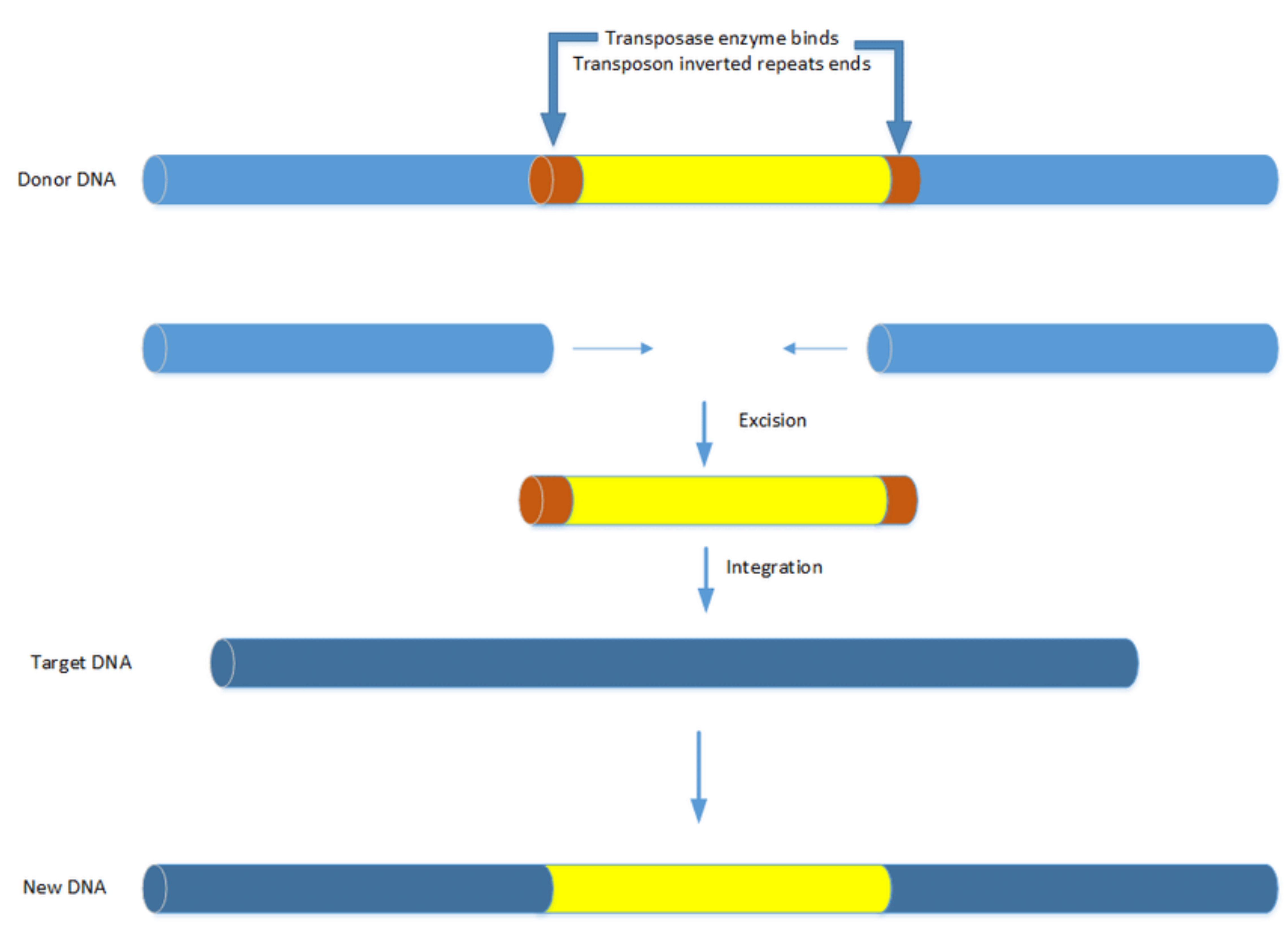
- **All cells** contain mobile genetic elements
- **Mobile genetic elements**
    - Typically between few hundreds to tens of thousands base pairs
    - Each carries a unique set of genes
    - Often, one of these genes catalyses the movement of that element
- They **move** within the genome
- In the process, they might **disrupt the function** or alter the **regulation** of existing genes

Mobile genetic elements

- They are typically part of the **repeated sequences** in our genome
- Overtime, random mutations affected their sequence and only a **small fraction is still active**
- They are considered to be **molecular parasites** (or selfish DNA) that persist in cells because they cannot get rid of them
- The genes they carry can provide an **advantage to the host** (e.g. antibiotic resistance in bacteria)
- Over long periods of evolution, mobile genetic elements are considers as **drivers of evolution and biodiversity**

Transposons

Transposable DNA elements (=**transposons**) are parasitic DNA sequences that can integrate and spread in the genomes they colonise (=one class of mobile genetic element)

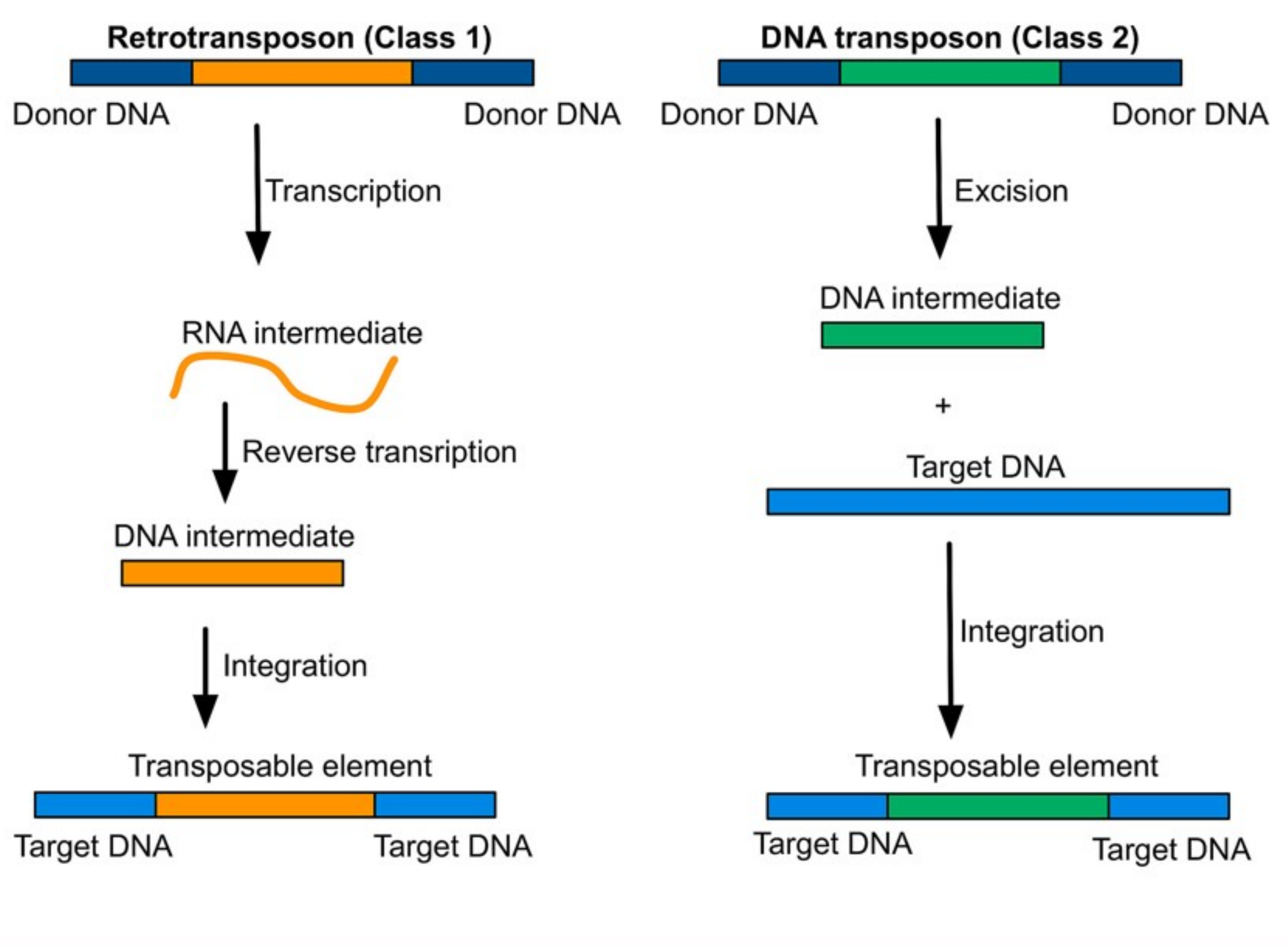


https://www.researchgate.net/publication/311653875_Local_Alignment_on_Highly_Unbalanced_DNA_Sequence_Lengths

Transposons

- Mobile elements that move by transposition are called **transposons**
- A **transposase** (usually encoded by the transposon) is an enzyme that acts on specific DNA sequences at each end of the transposon, causing it to insert at a new DNA location
- They are typically not very **selective** in choosing their target site
- Most transposons move **rarely**
- Transposons belong to **3 large classes**, based on their structure and transposition mechanisms

Transposons



https://www.onlinebiologynotes.com/transposable-elements-in-eukaryotes/

Transposons

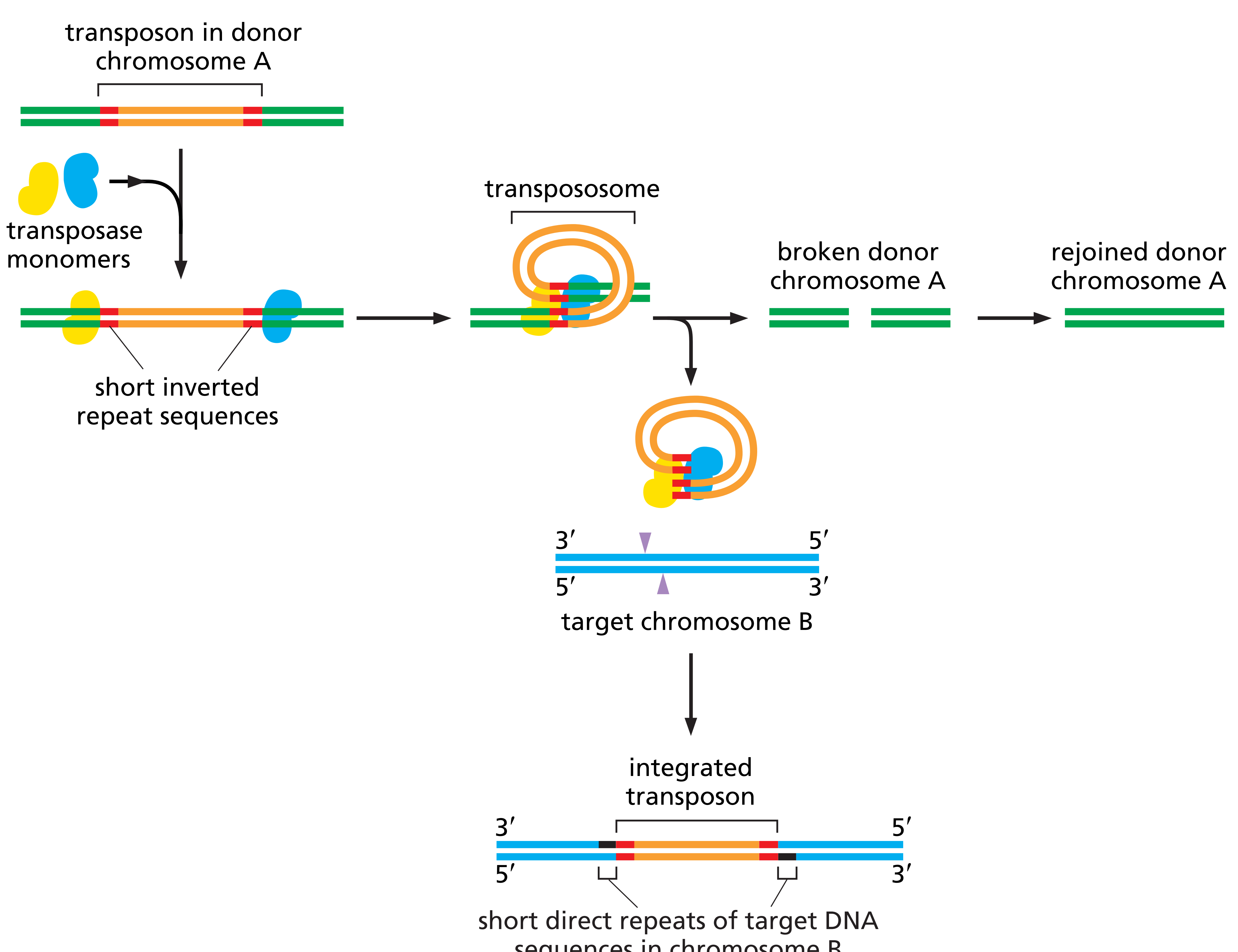
TABLE 5–4 Three Major Classes of Transposable Elements

Class description and structure	Specialized enzymes required for movement	Mode of movement	Examples
DNA-only transposons			
Short inverted repeats at each end	Transposase	Moves as DNA, either by cut-and-paste or replicative pathways	P element *(Drosophila)*, Ac-Ds (maize), Tn3 and Tn10 *(E. coli),* Tam3 (snapdragon)
Retroviral-like retrotransposons			
Directly repeated long terminal repeats (LTRs) at each end	Reverse transcriptase and integrase	Moves via an RNA intermediate whose production is driven by a promoter in the LTR	Copia *(Drosophila),* Ty1 (yeast), THE1 (human), Bs1 (maize)
Nonretroviral retrotransposons			
AAAA TTTT Poly A at 3′ end of RNA transcript; 5′ end is often truncated	Reverse transcriptase and endonuclease	Moves via an RNA intermediate that is often synthesized from a neighboring promoter	F element *(Drosophila)*, *L1* (human), Cin4 (maize)

These elements range in length from 1000 to about 12,000 nucleotide pairs. Each family contains many members, only a few of which are listed here. Some viruses can also move in and out of host-cell chromosomes by transpositional mechanisms. These viruses are related to the first two classes of transposons.

DNA-only transposons

- Predominate in **bacteria**
- Largely responsible for the spread of **antibiotic resistance** - these elements can be moved from one bacteria to the other by **horizontal gene transfer**
- Once inserted in a cell, it will be passed to the **progeny**
- **Cut-and-paste** transposition
- The "hole" left by excision is repaired by recombinational **double-strand break repair**
- **Short direct repeats** around the transposon allow to track transposition in genomes



Transposition used by viruses

- Some **viruses** are considered mobile genetic elements because they can insert their genome in the host DNA by transposition - but they can also infect other cells

- Transposition has a key role for specific viruses like retroviruses (e.g. HIV)

- Important role of the **retrotranscriptase** and **integrase**

- **Retroviral-like retrotransposons** move by a similar mechanism

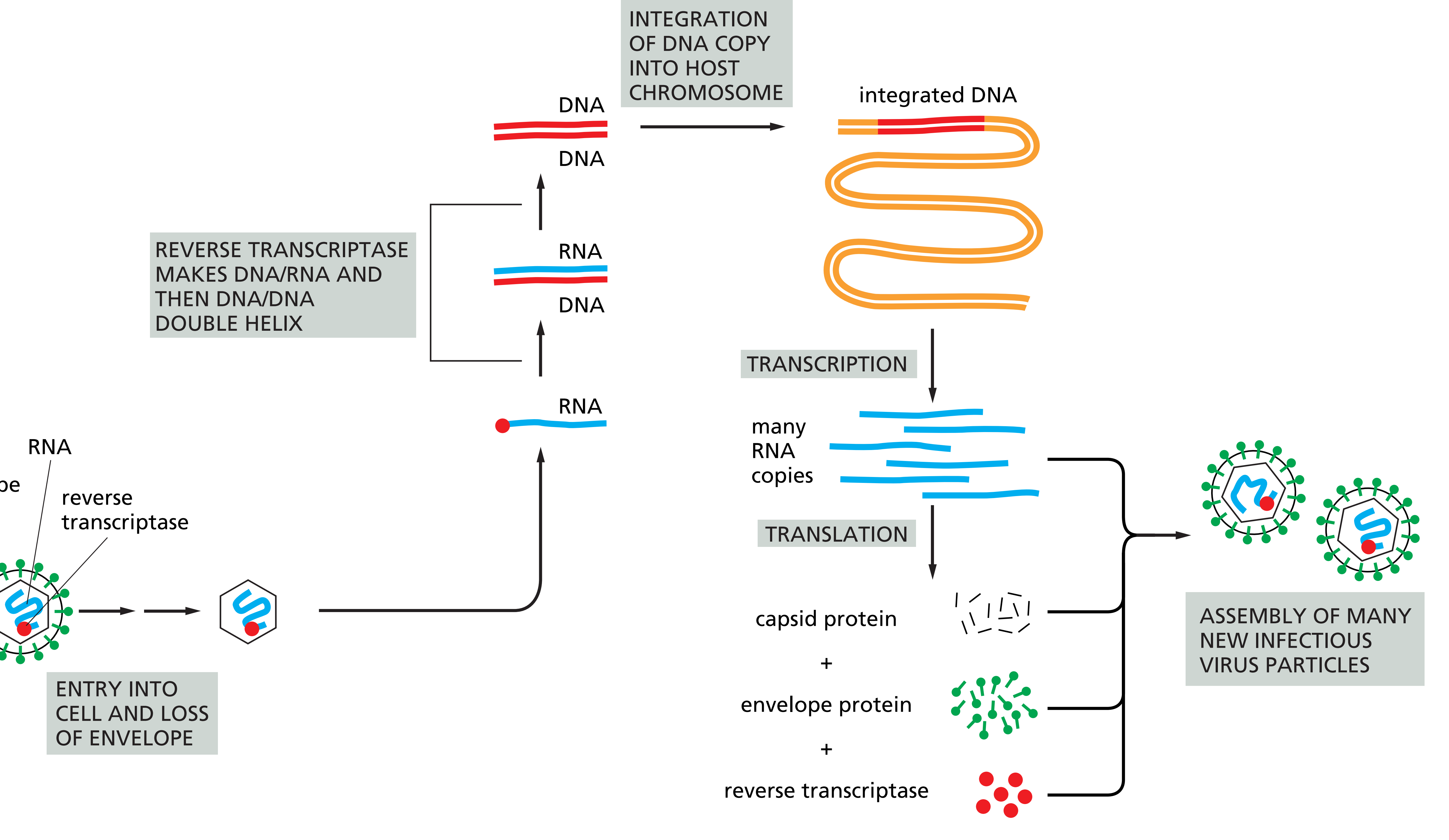
    1. Transcription of the whole transposon

    2. Production of the retrotranscriptase and integrase

    3. Double-strand DNA copy from the RNA

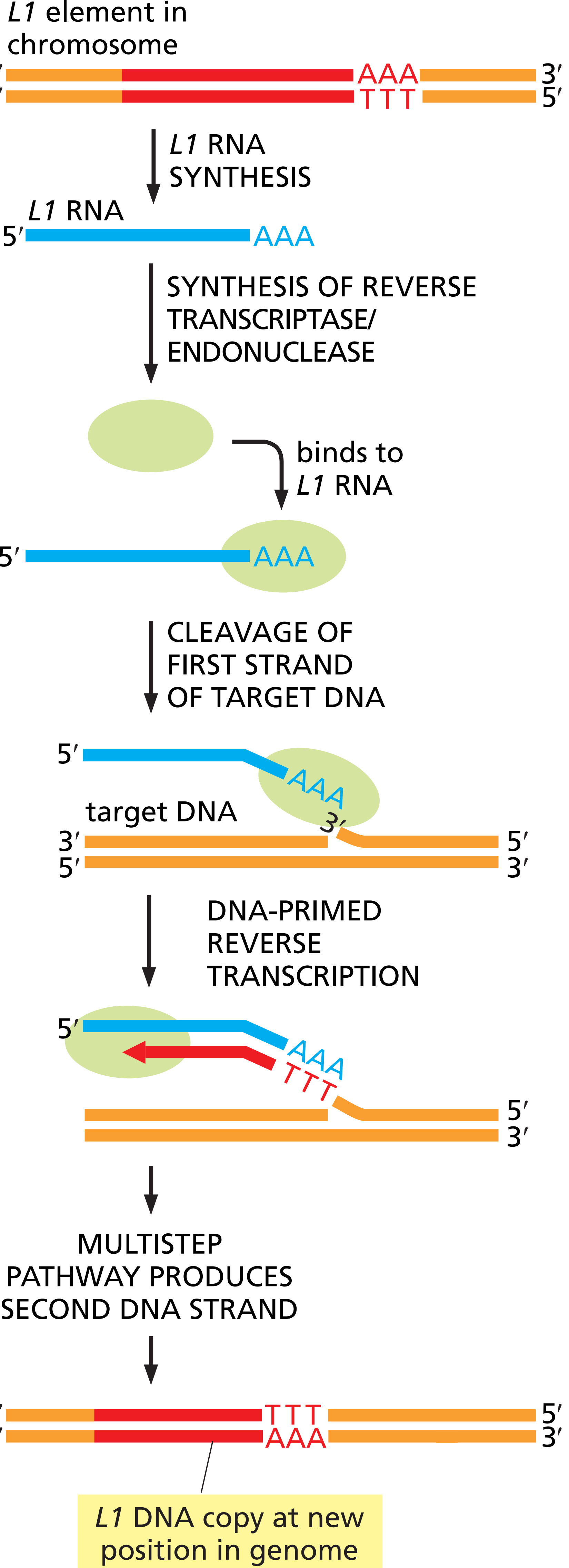
    4. Integration in the genome by integrase

- They cannot leave their host cell (unlike viruses)

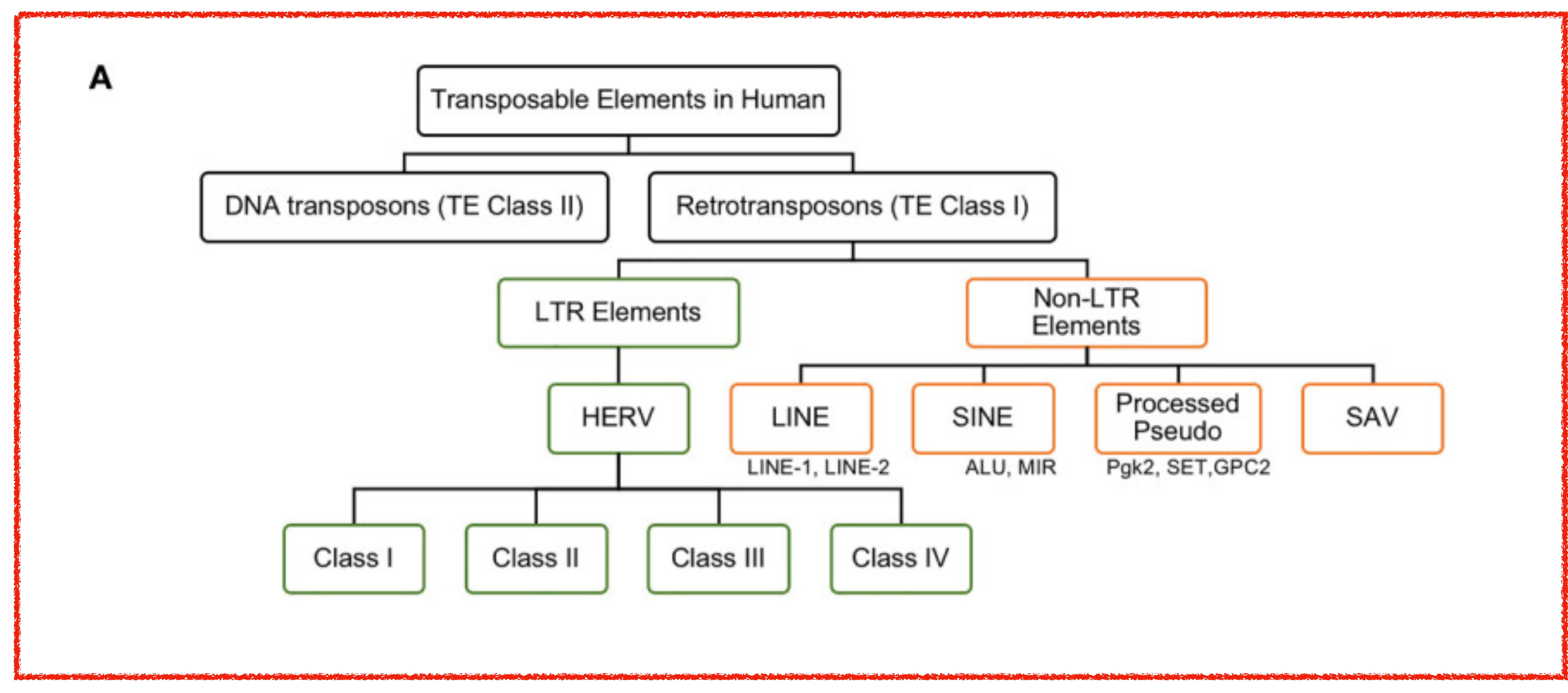


Nonretroviral retrotransposons

- A significant fraction of vertebrates chromosomes
- A few retained the ability to move
- Need an endonuclease and a reverse transcriptase
- LINEs and SINEs make up over 30% of our genome



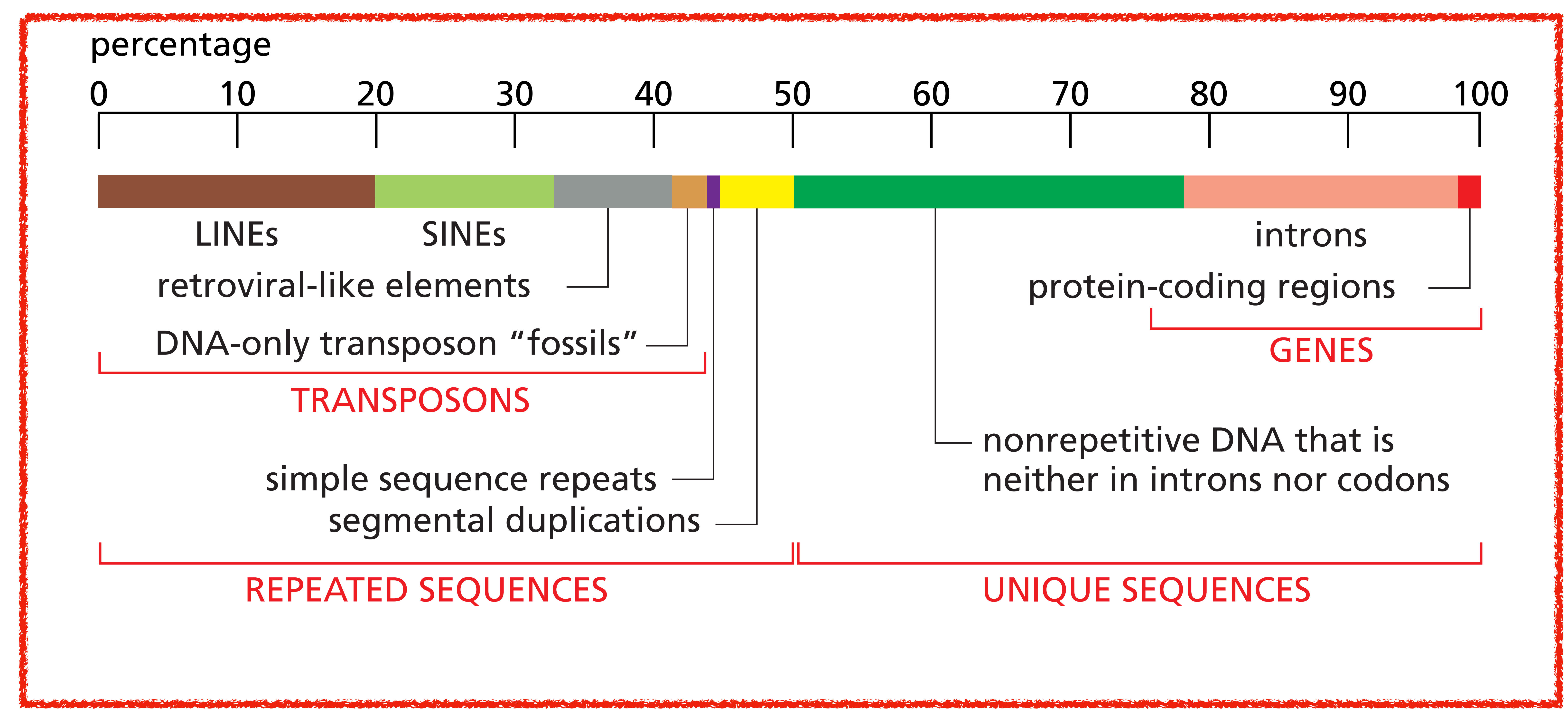
Mobile genetic elements in Humans



https://europepmc.org/article/med/34867957

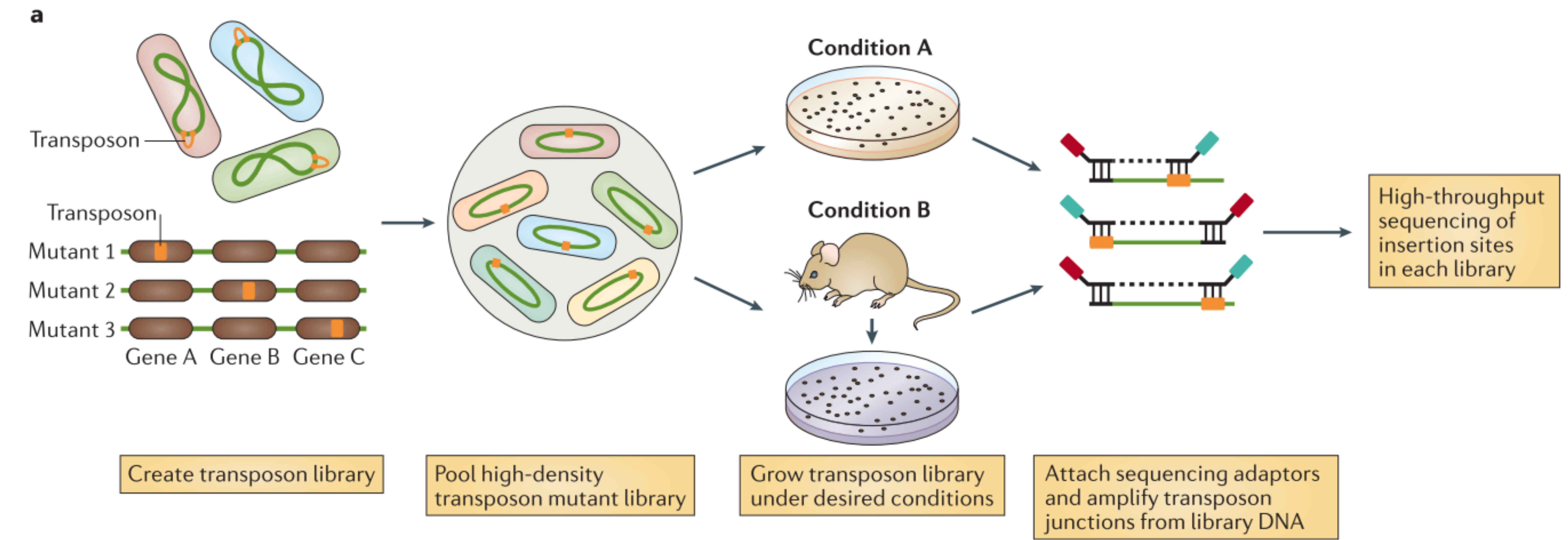
Mobile genetic elements in the human genome

Transposable DNA elements (=**transposons**) are parasitic DNA sequences that can integrate and spread in the genomes they colonise



Using transposons in research

Building Tn-libraries and Tn-Seq



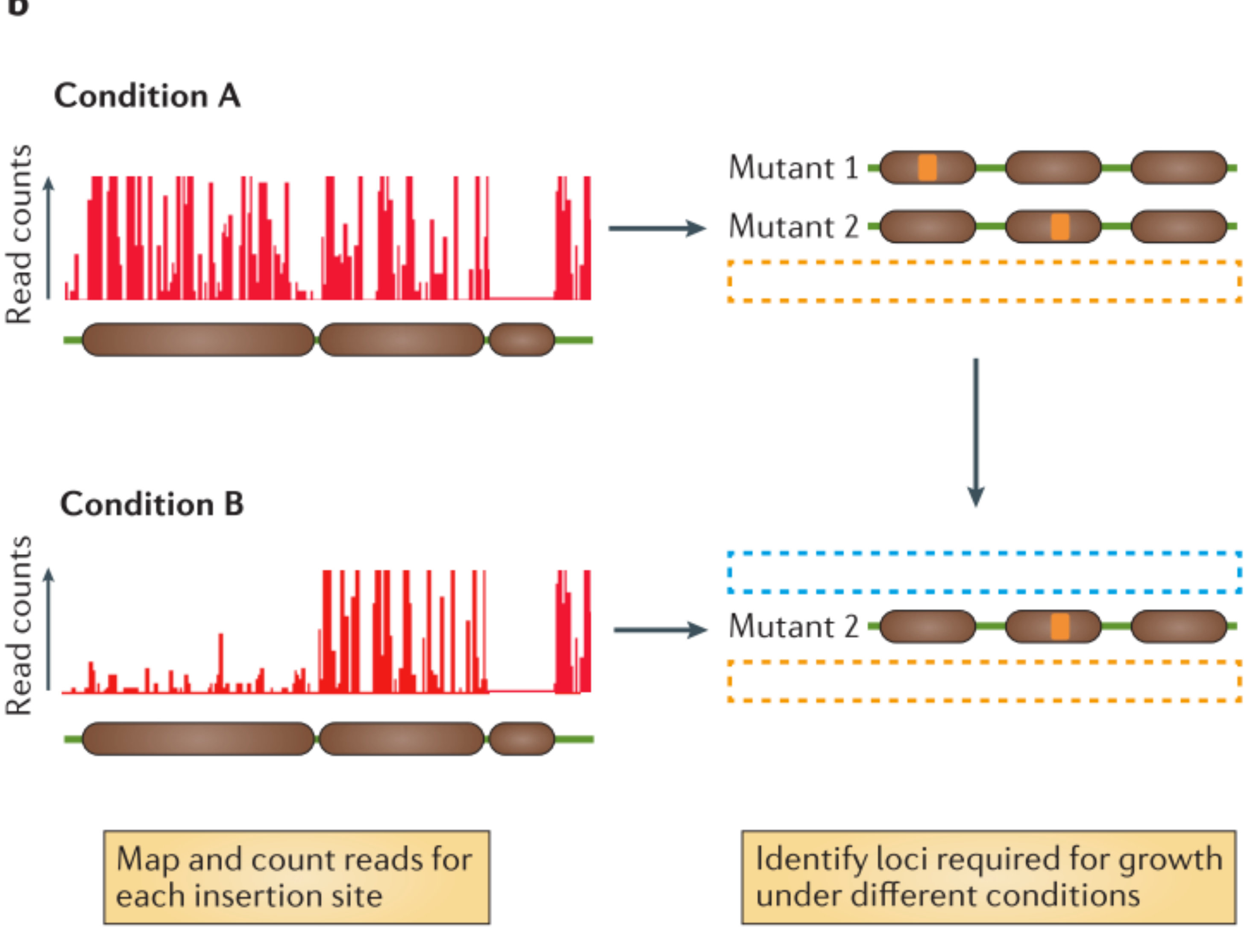


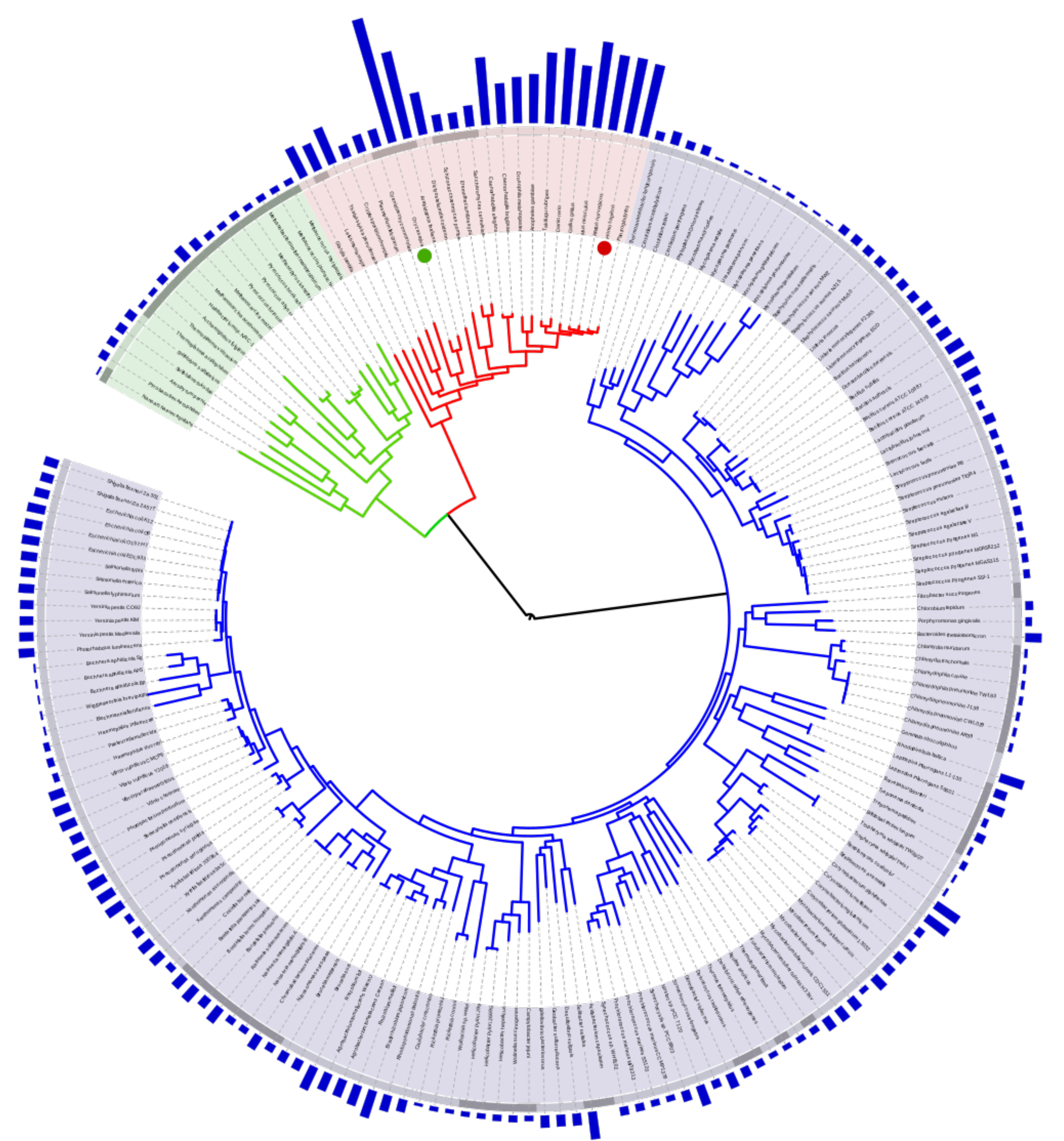
Figure 1: Transposon insertion sequencing method (from Chao *et al.* 2016)

Plan

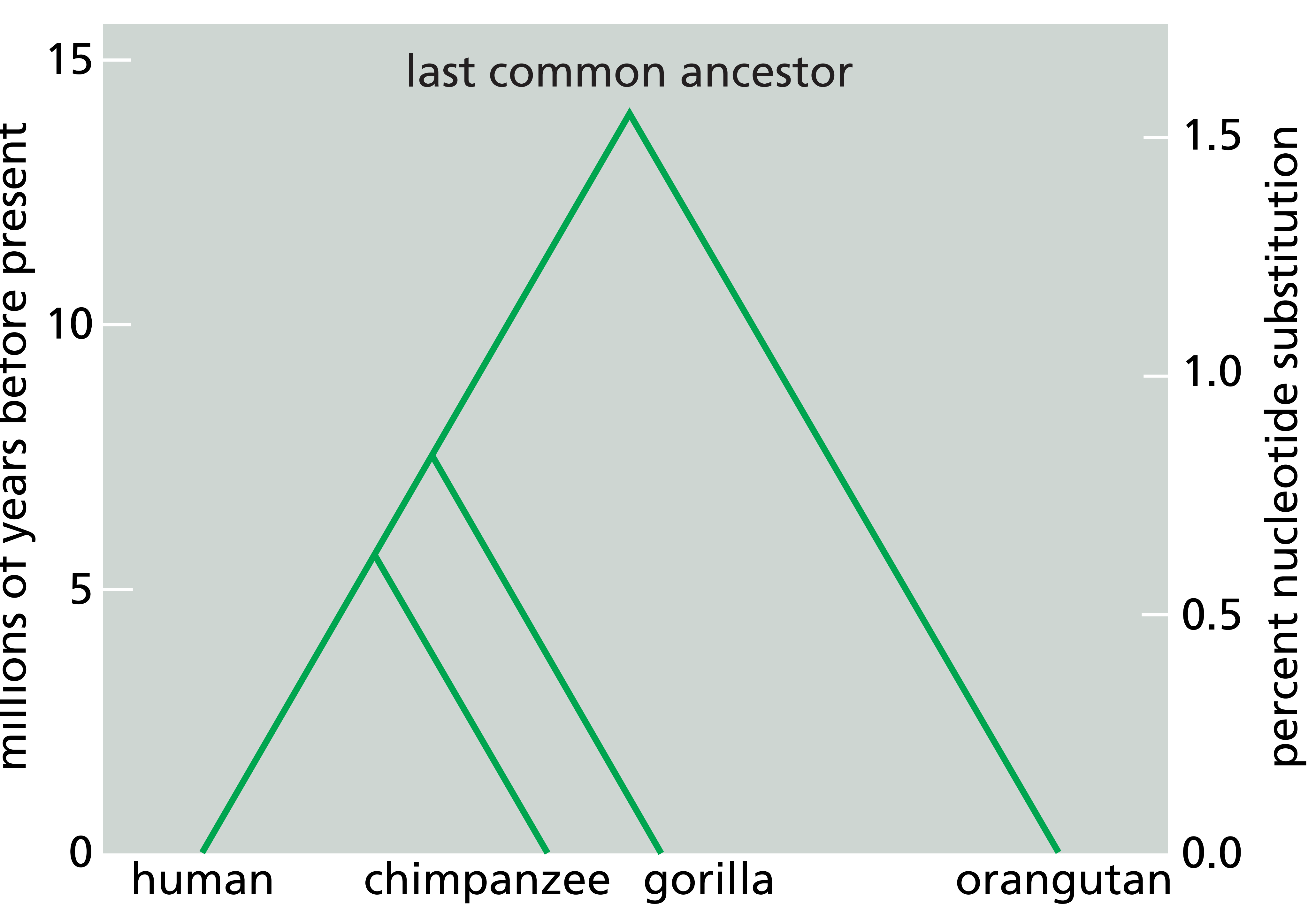
- Quick recap
- Mobile genetic elements
- Comparing genomes
- The maintenance of DNA sequences
- DNA replication mechanisms

How can we reconstruct evolution?

- Differences in genomes have accumulated over 3 billon years
- **Nucleotide substitution rate** reflects the time available for the accumulation of mutations
- Comparative genomics use **phylogenetic trees**, built using comparison of genes or protein sequences
- Timing has been calibrated with fossil record and mutations occur at a nearly constant rate (**molecular clock** for evolution)
- Some clocks run faster than others



How can we reconstruct evolution?



How can we reconstruct evolution?

- **Mutation rate** is different in different parts of the genome

Negative selection or purifying selection is the selective removal of alleles that are deleterious

exon ◄─► intron

mouse

```
GTGCCTATCCAGAAAGTCCAGGATGACACCAAAACCCTCATCAAGACCATTGTCACCAGGATCAATGACATTTCACACACGGTA-GGAGTCTCATGGGGGACAAAGATGTAGGACTAGA
GTGCCCATCCAAAAAGTCCAAGATGACACCAAAACCCTCATCAAGACAATTGTCACCAGGATCAATGACATTTCACACACGGTAAGGAGAGT-ATGCGGGGACAAA---GTAGAACTGCA
```

human

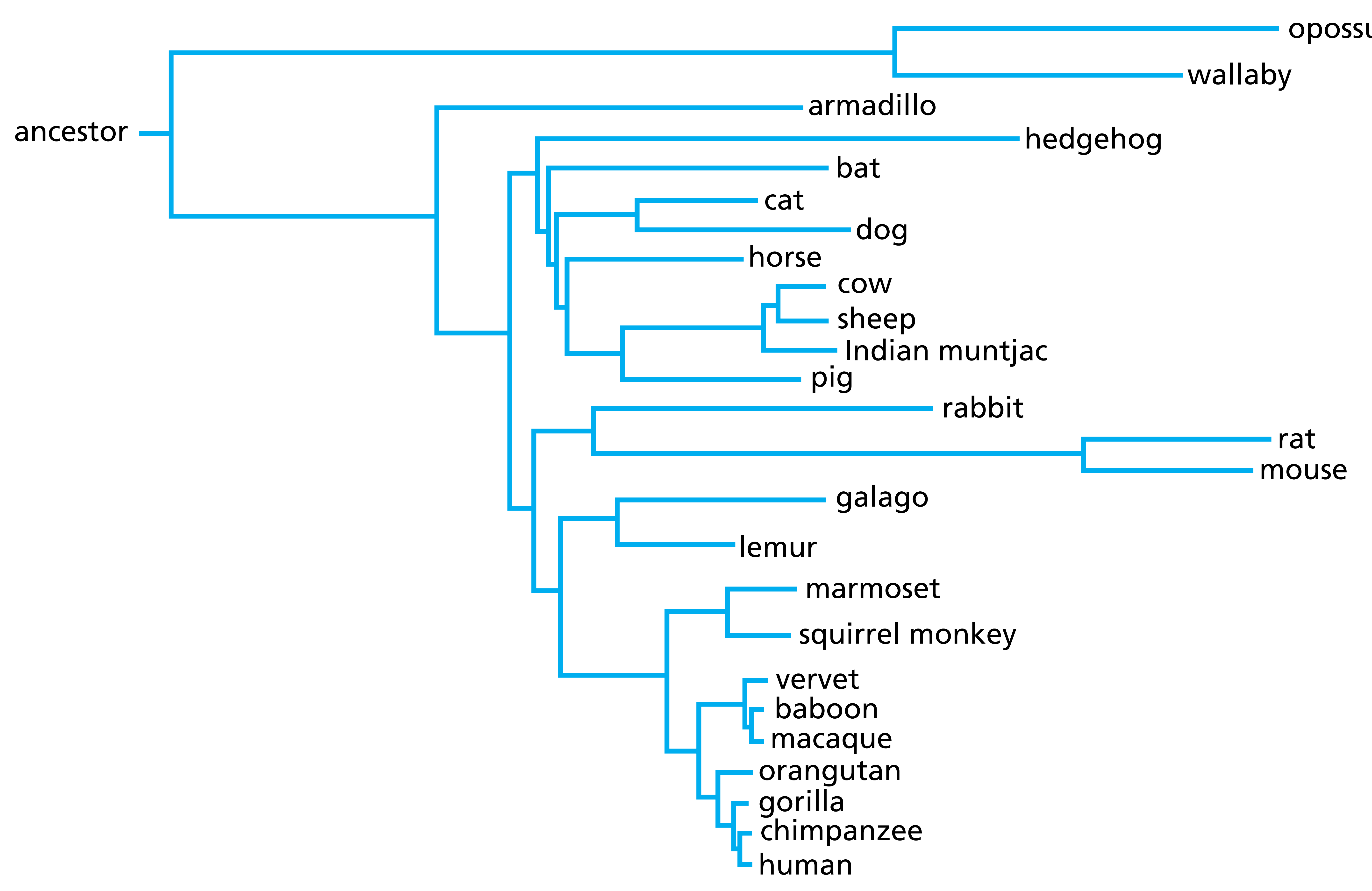
mouse

```
ACCAGAGTCTGAGAAACATGTCATGCACCTCCTAGAAGCTGAGAGTTTAT-AAGCCTCGAGTGTACAT-TATTTCTGGTCATGGCTCTTGTCACTGCTGCCTGCTGAAATACAGGGCTGA
GCCAG--CCC-AGCACTGGCTCCTAGTGGCACTGGACCCAGATAGTCCAAGAAACATTTATTGAACGCCTCCTGAATGCCAGGCACCTACTGGAAGCTGA--GAAGGATTTGAAAGCACA
```

human

Figure 4–65 **The very different rates of evolution of exons and introns, as illustrated by comparing a portion of the mouse and human leptin genes.** Positions where the sequences differ by a single nucleotide substitution are boxed in *green*, and positions that differ by the addition or deletion of nucleotides are boxed in *yellow*. Note that, thanks to purifying selection, the coding sequence of the exon is much more conserved than is the adjacent intron sequence.

Comparing human and mouse genomes



- Human and chimpanzee genomes are much more alike than mouse genome, although they have similar sizes and carry a nearly identical set of genes
- Mouse and human have diverged 80 million years ago (only 6 million for human and chimpanzees)
- Rodents have fast molecular clocks (generation time is shorter)

Comparing human and mouse genomes

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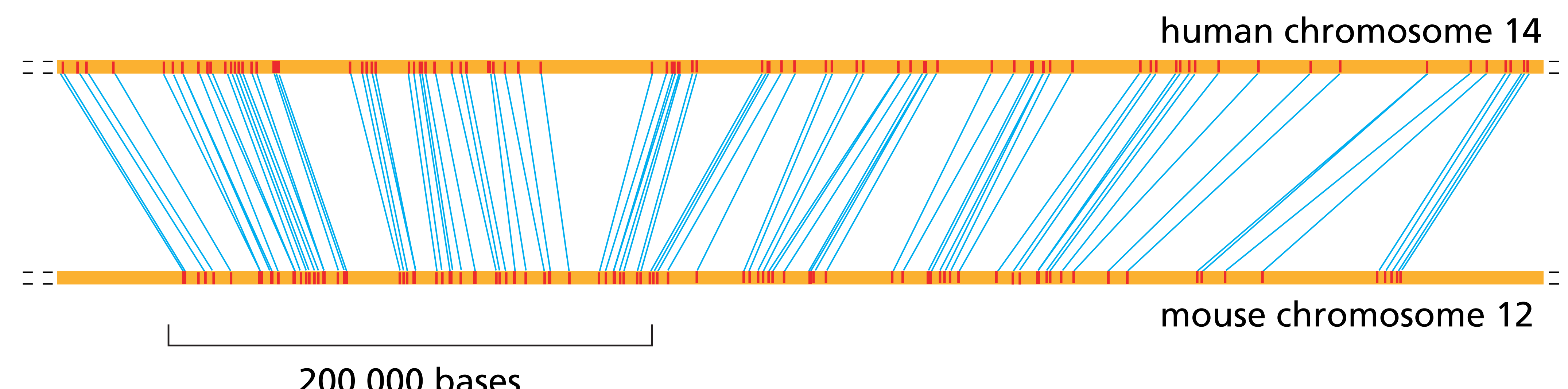
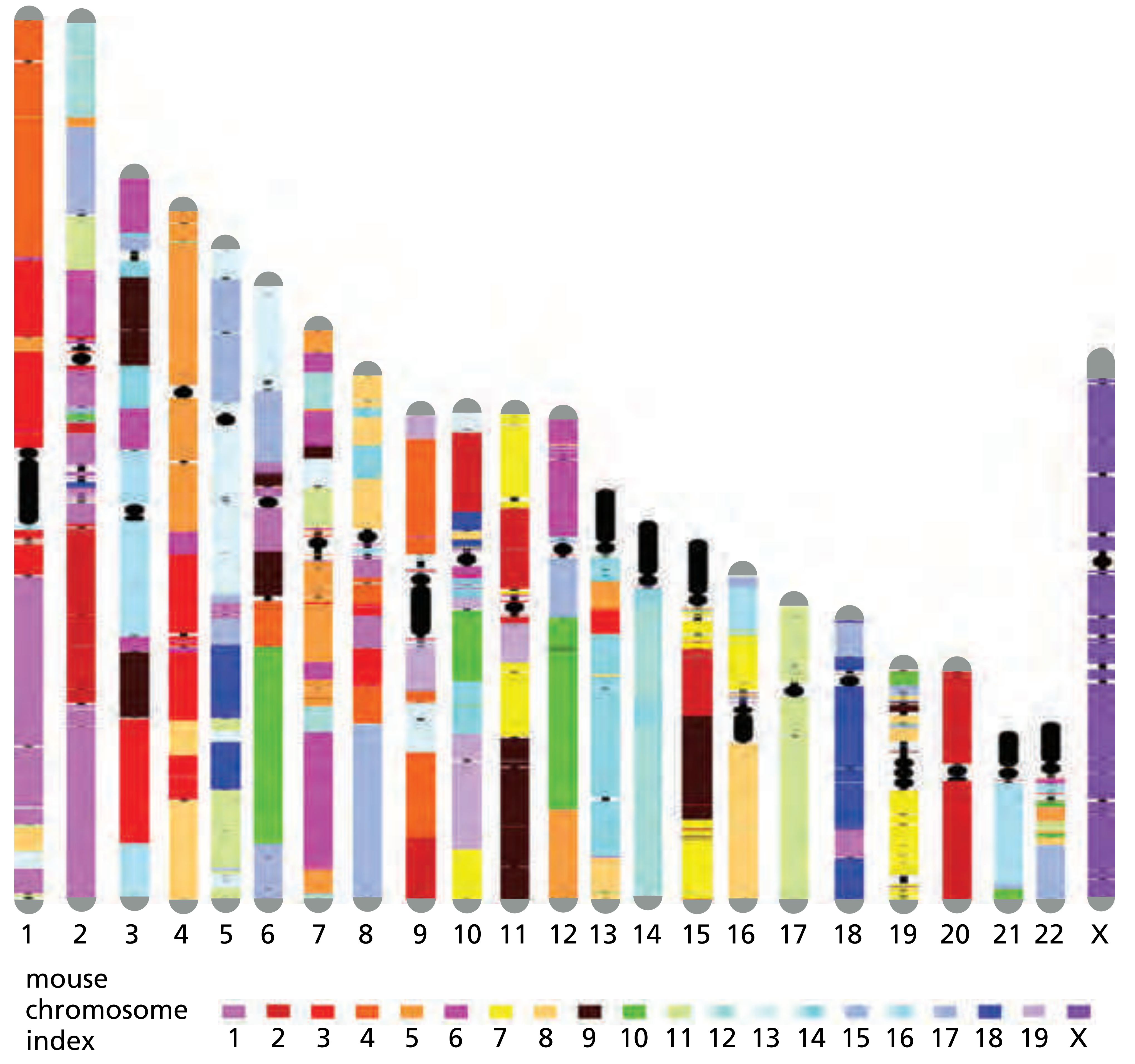
- Mouse (20 chromosome pairs); Human (23 chromosome pairs)
- Heavy **chromosomal rearrangement** during evolution (e.g. 180 breakage/fusion events)
- DNA was lost over evolution in mice

Stretches of DNA with conserved gene order are called **synteny**





Short introns do not affect gene function

- The size of vertebrate genomes reflects the rate of **deletion and addition of DNA**
- Large difference in genome size between similar organisms

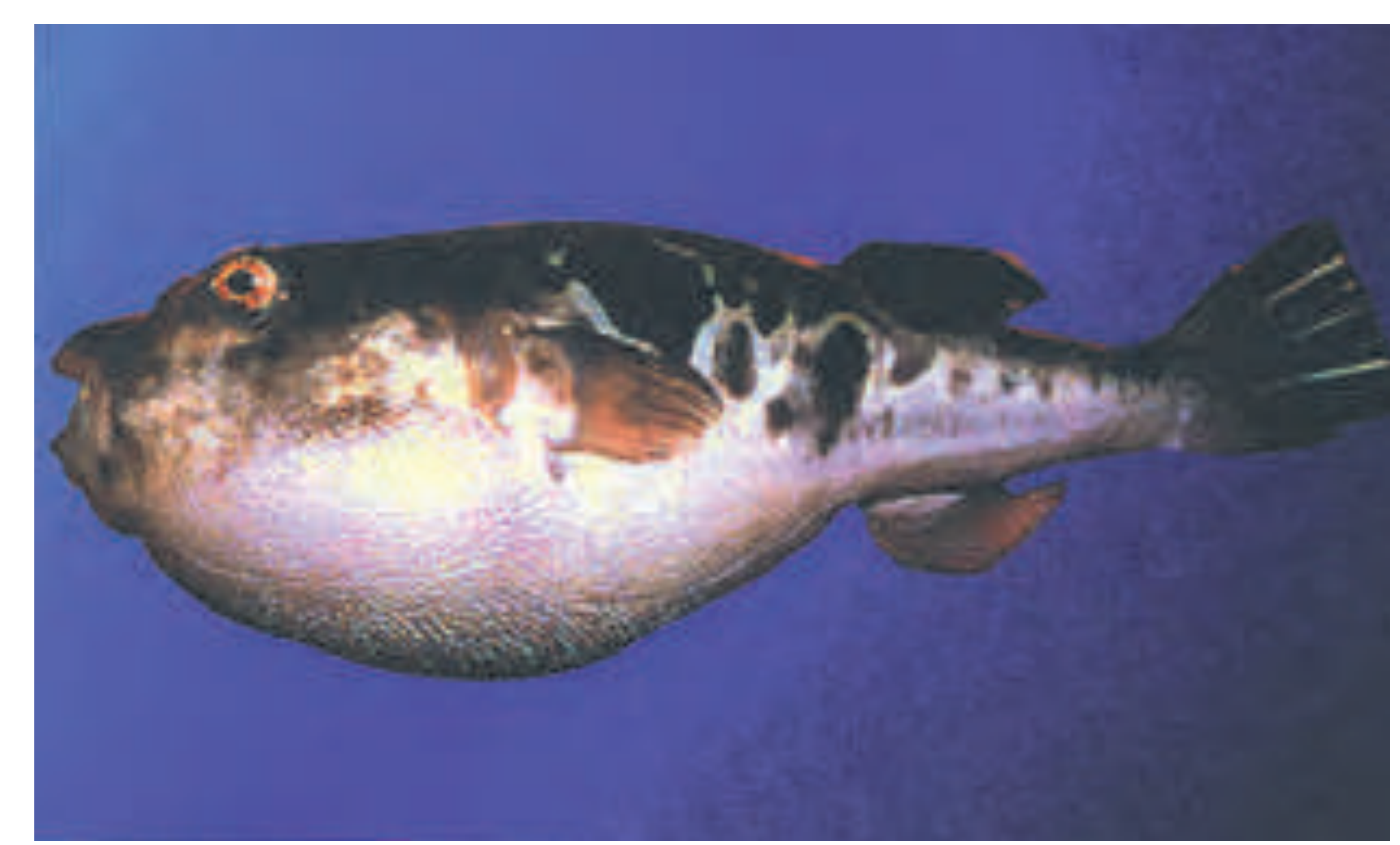
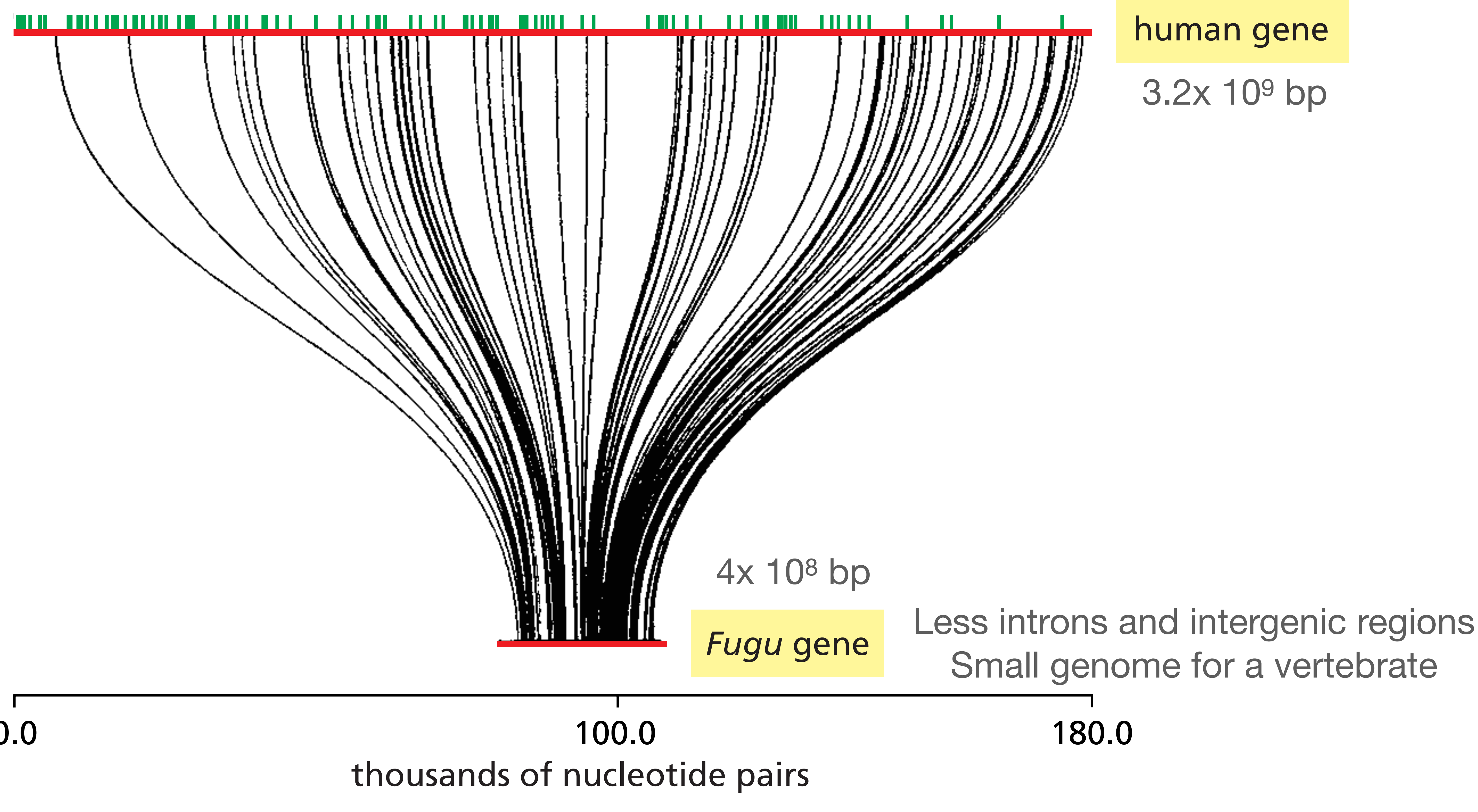


Figure 4–70 **The puffer fish, *Fugu rubripes*.** (Courtesy of Byrappa Venkatesh.)



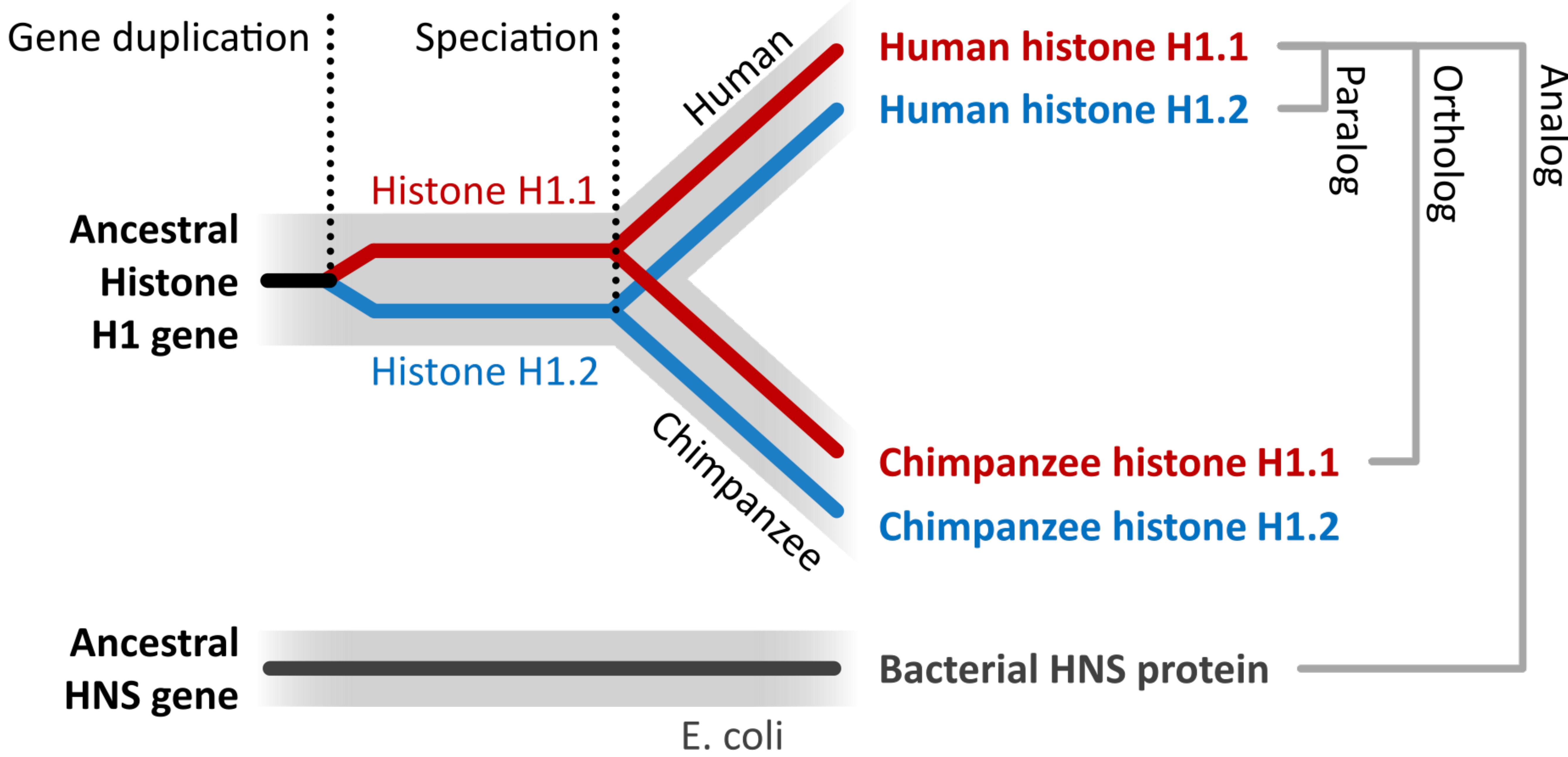
Conservation of genes across evolution

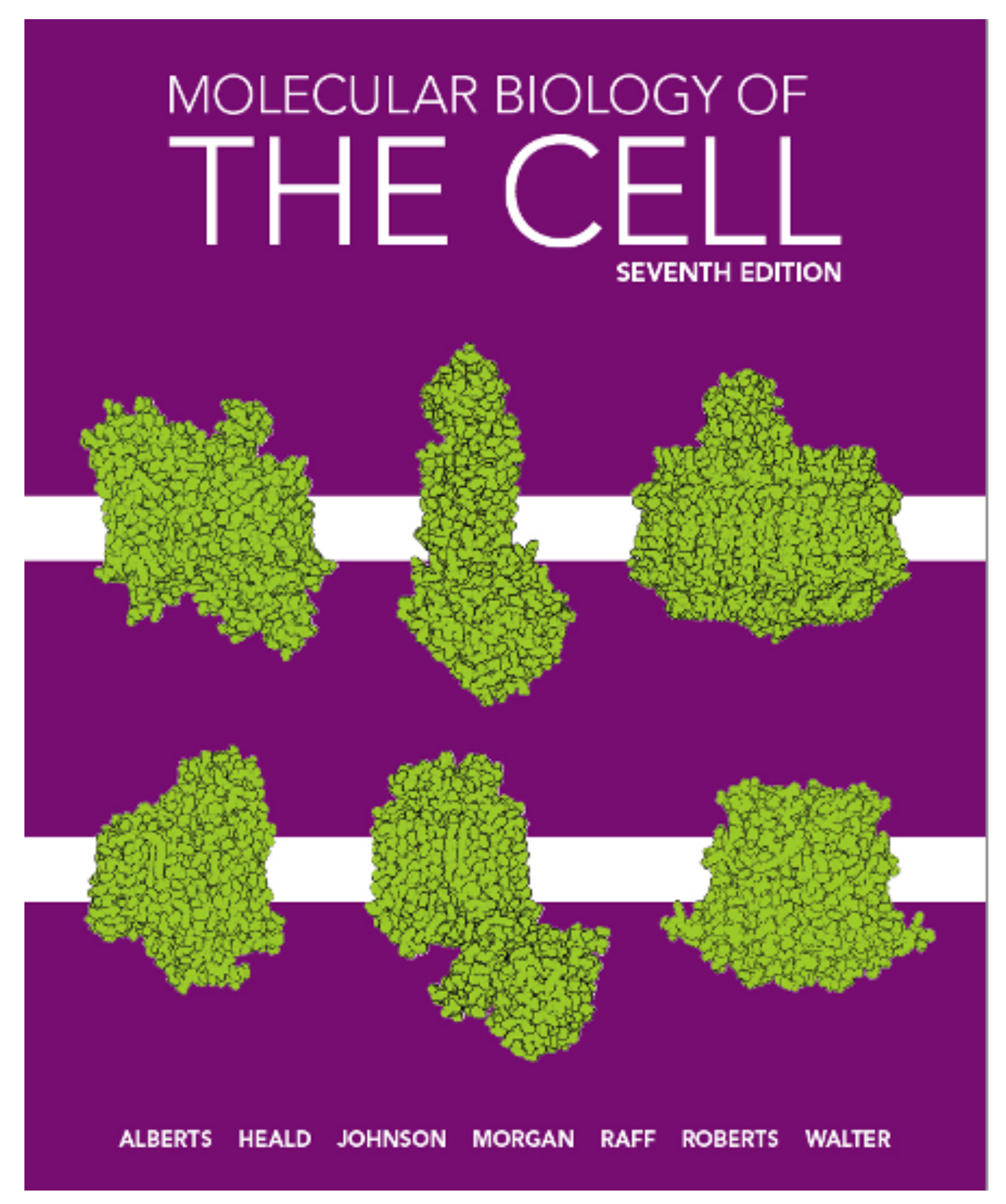
Homologous genes are genetic sequences inherited from a common ancestor. They are similar in sequence and can likely perform similar functions. Paralogues and orthologues are homologues.

Paralogues are homologues in the same genome that arose from a gene duplication event.

Orthologues are homologues in different genomes derived from a common ancestor.

Analogues are similar sequences in different genomes without a common ancestor.





Chapter 5

DNA Replication, Repair, and Recombination

Plan

- Quick recap
- Mobile genetic elements
- Comparing genomes
- The maintenance of DNA sequences
- DNA replication mechanisms

Maintaining DNA

DNA replication

- Accurate **duplication** of vast quantities of DNA
- Occurs before a cell can produce **two genetically-identical** daughter cells

DNA repair

- DNA is **continuously damaged** by chemicals, radiation, thermal accidents or reactive molecules inside the cells
- Protein machineries that **repair** DNA

Maintaining DNA

- Sexually-reproducing animals or plants have two types of cells: **germ cells and somatic cells**
- Both need to **protect their DNA**: germ-cells to maintain the species and somatic cells, to maintain the structure of the body
- Uncontrolled mutant proliferation in somatic cells = **cancer**

Germ-cells transmit genetic information from parent to offspring while **somatic cells** form the body of the organism

Plan

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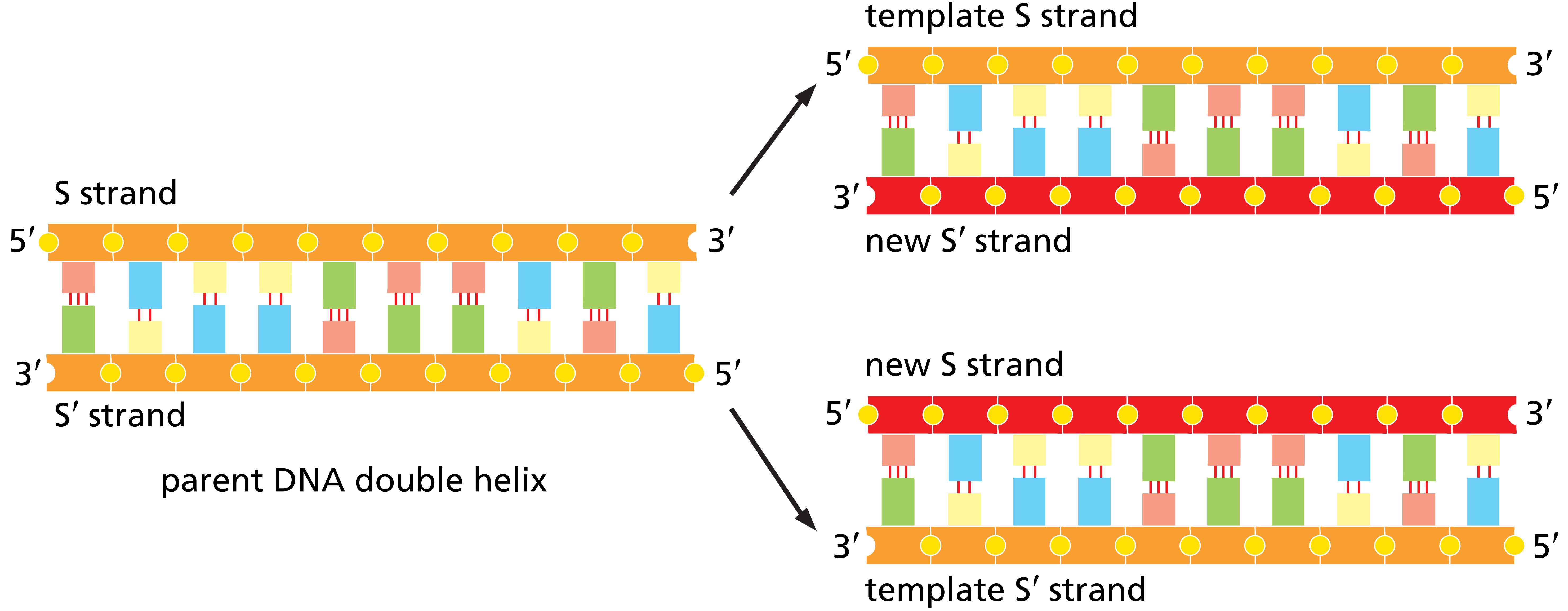
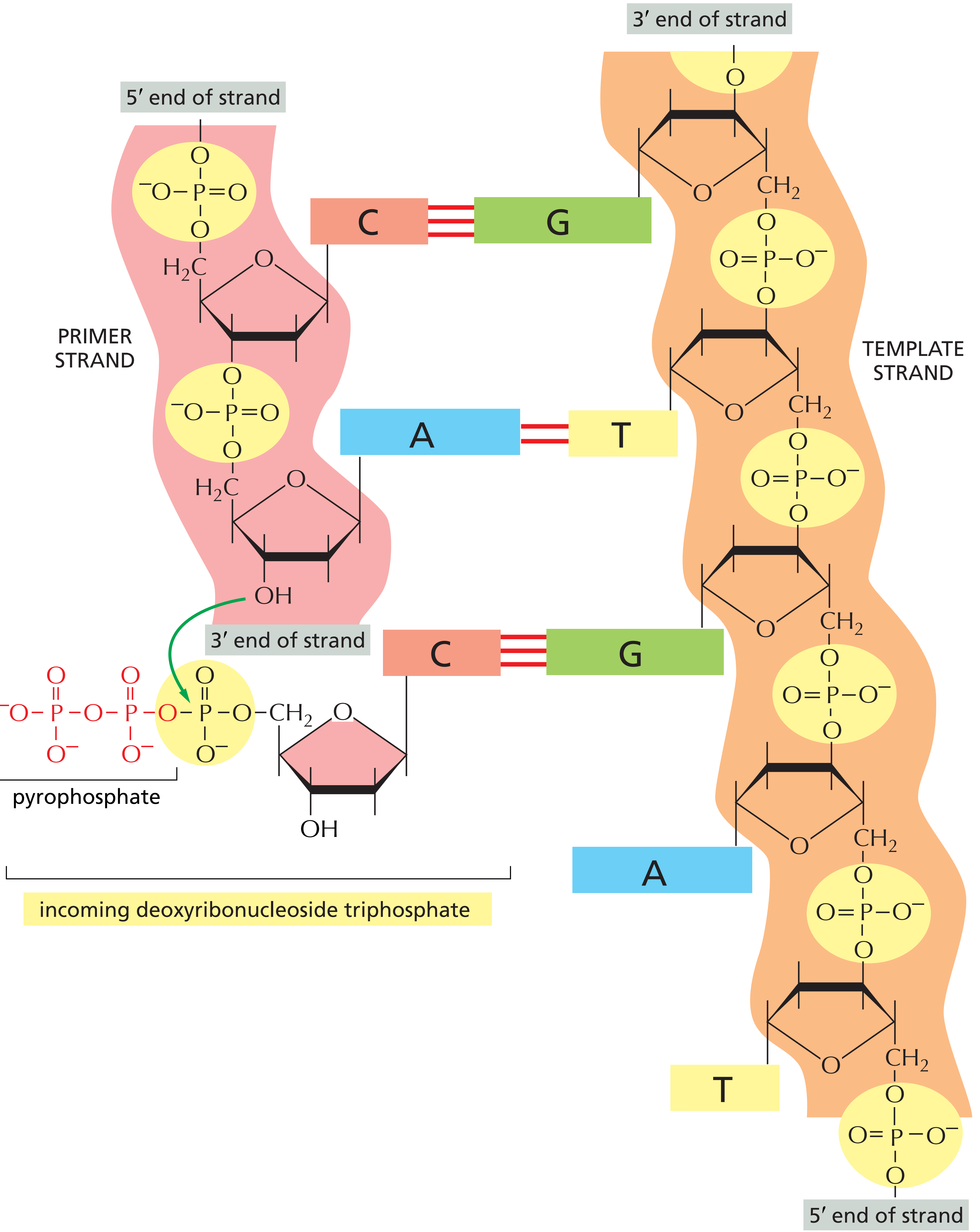
DNA replication principles

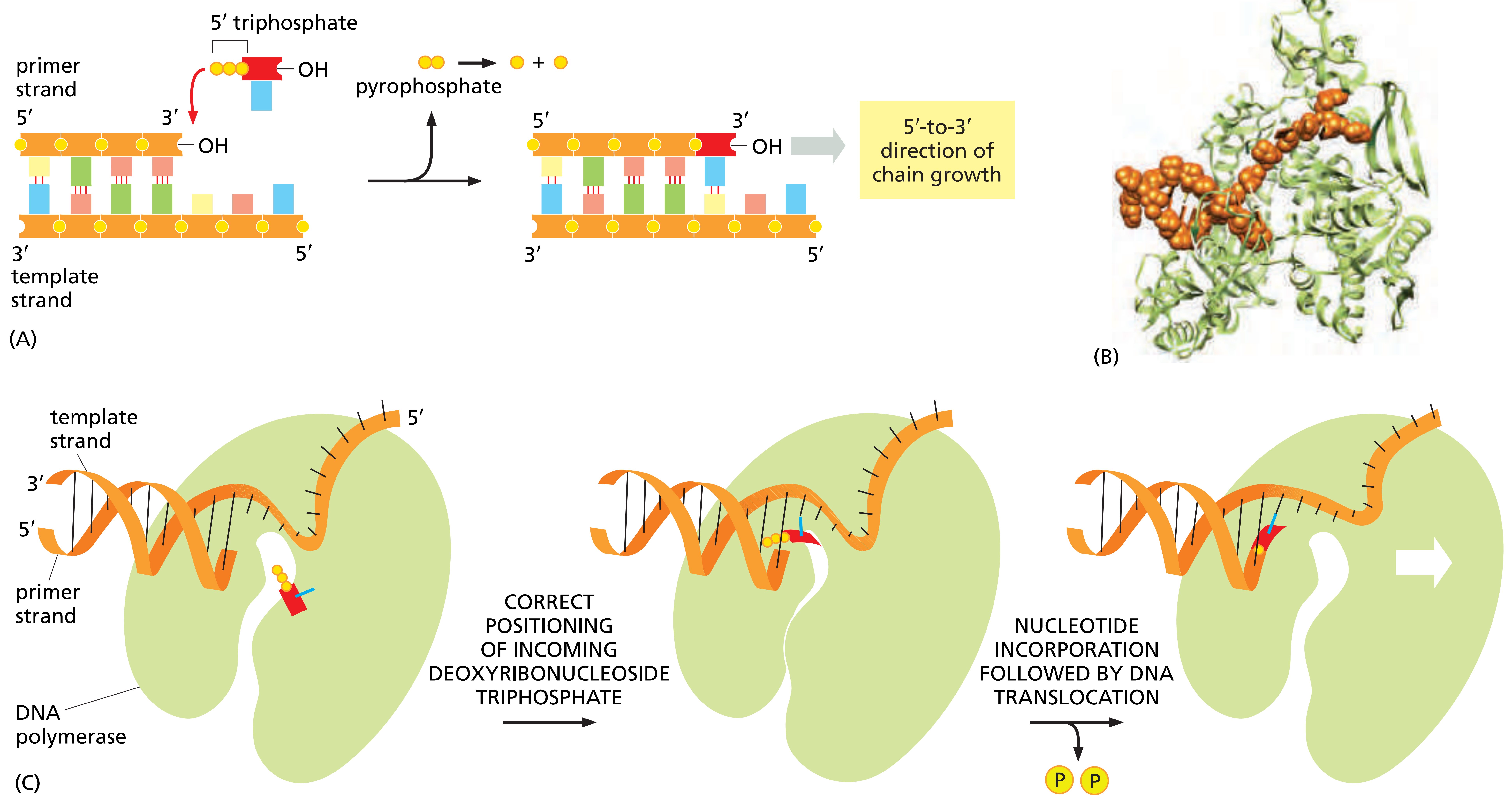
- **Separation** of the DNA helix into two strands
- **Recognition** of each template nucleotide by a free complementary nucleotide (deoxyribonucleotide triphosphate)
- Polymerisation of the nucleotide by the **DNA polymerase**
- Most mechanisms uncovered in **bacteria** and viruses



DNA replication mechanism

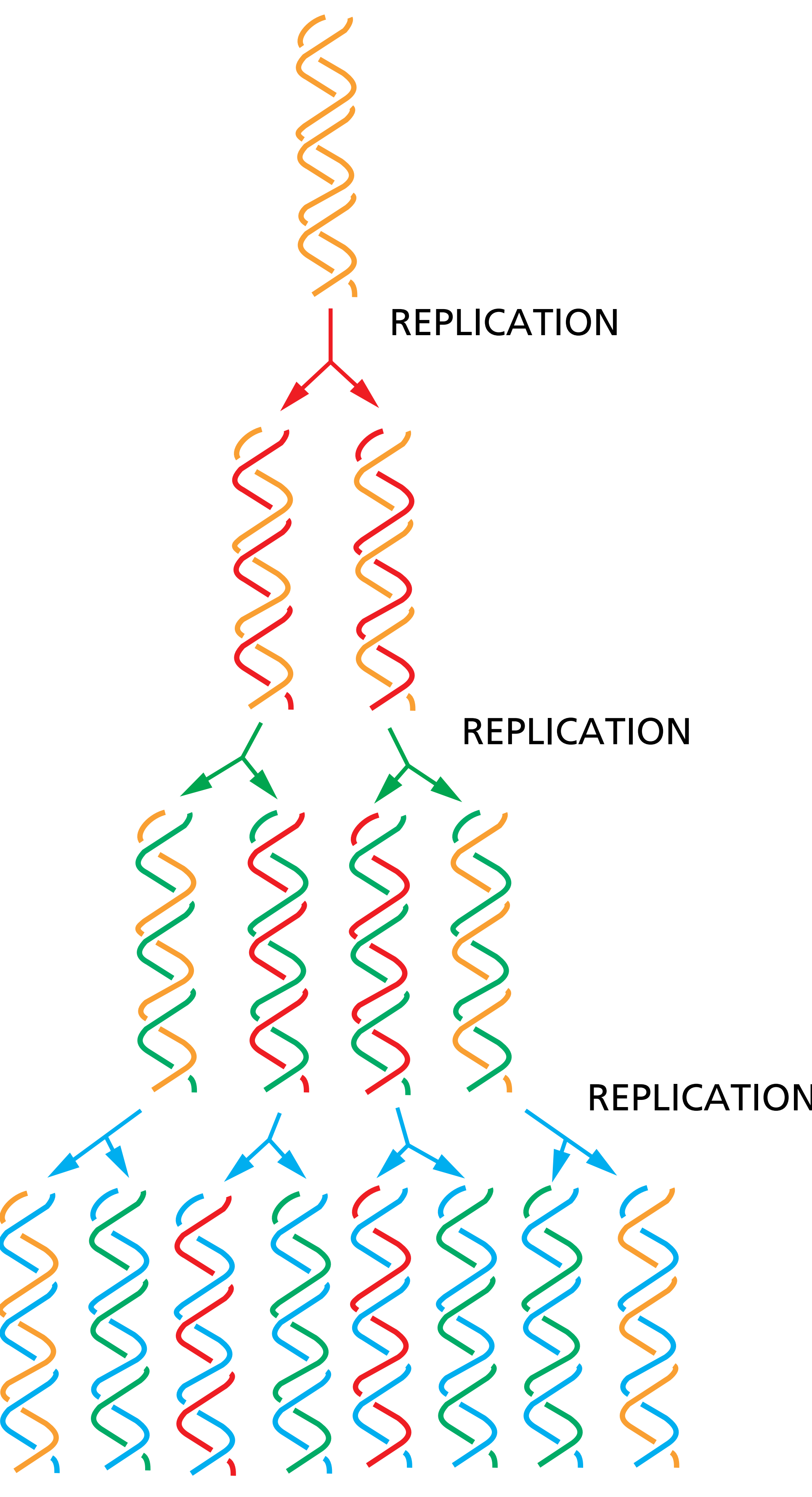


DNA replication mechanism



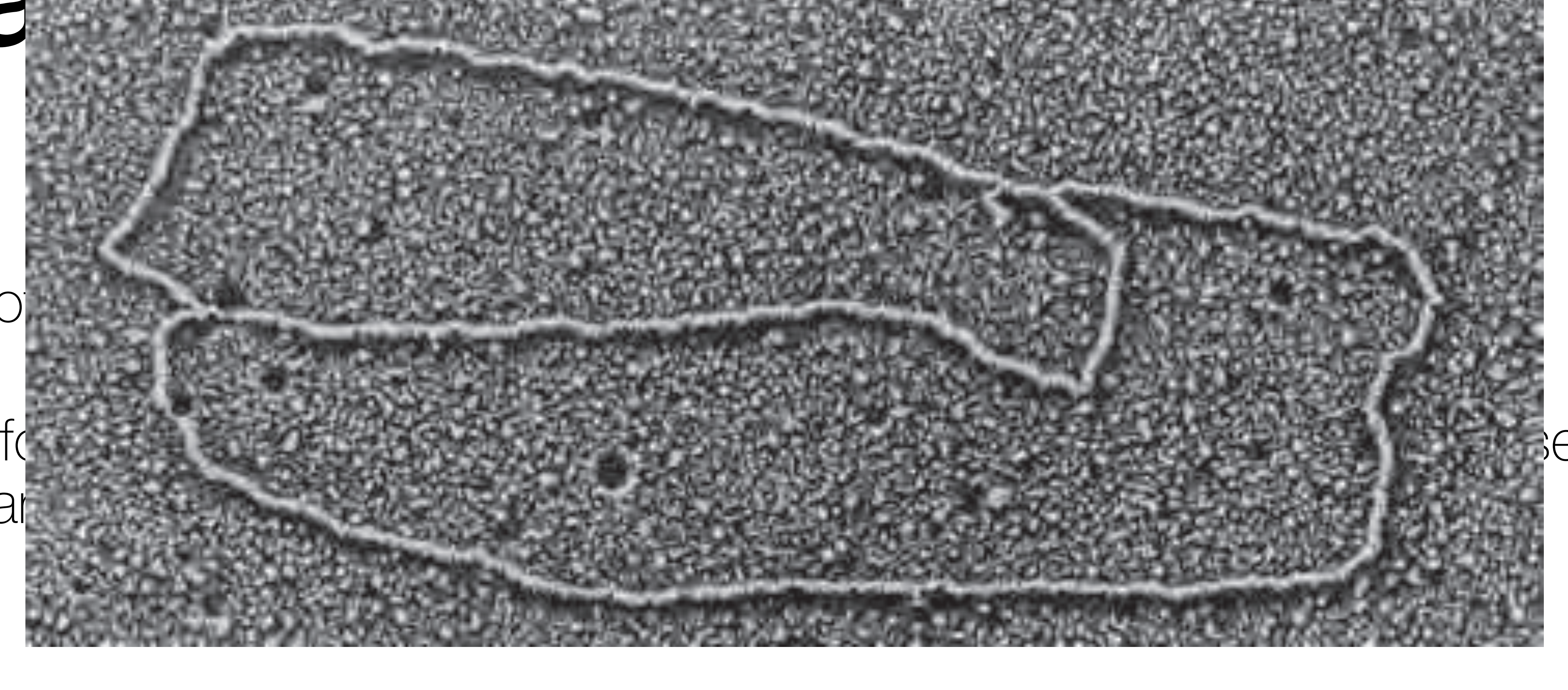
DNA replication principles

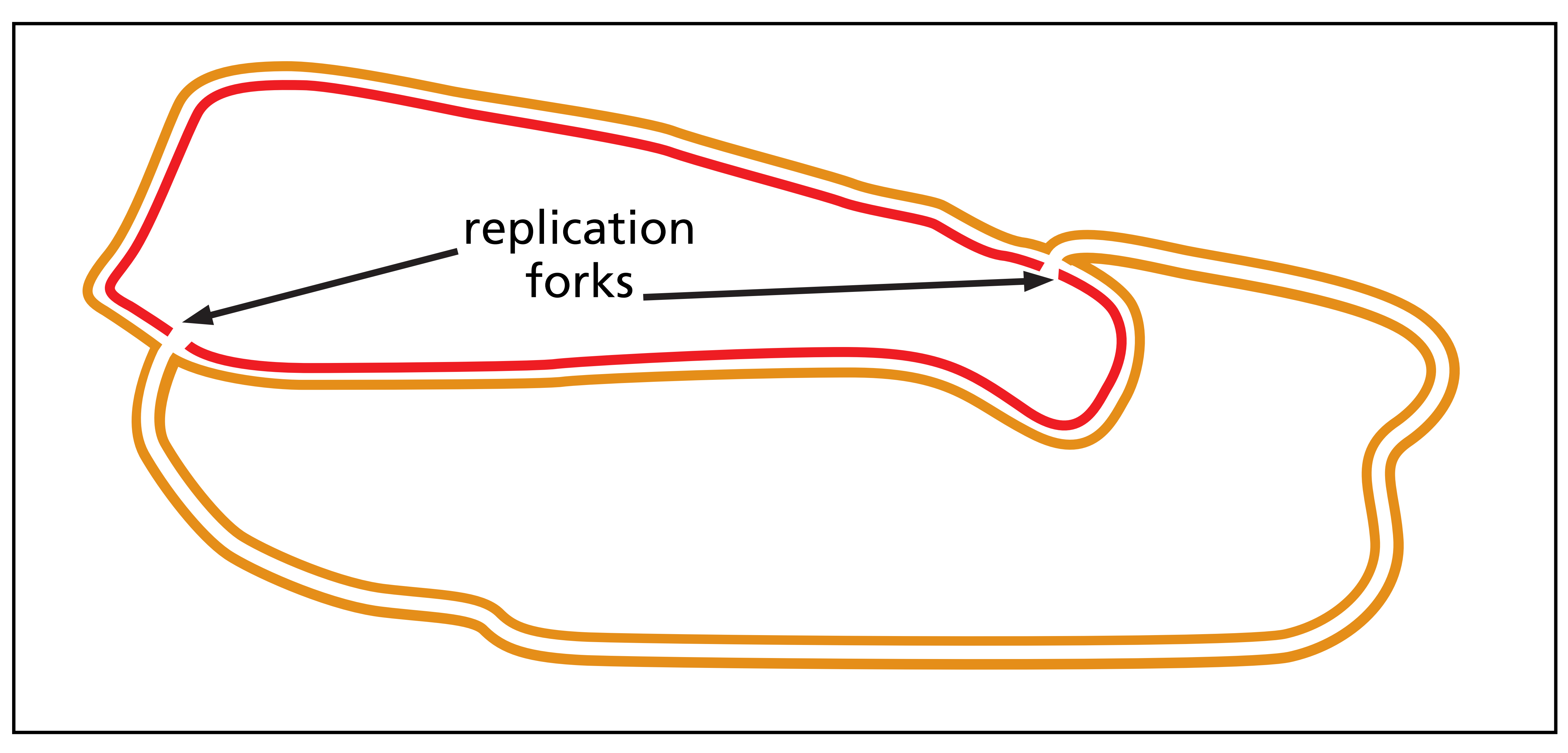
- DNA replication is **semiconservative** as the two daughter cells will inherit a double helix that contains one “original” and one “new” strand



DNA replication principles

- Localised region o
- At the replication fo ... ses the DNA of both new daughter stra



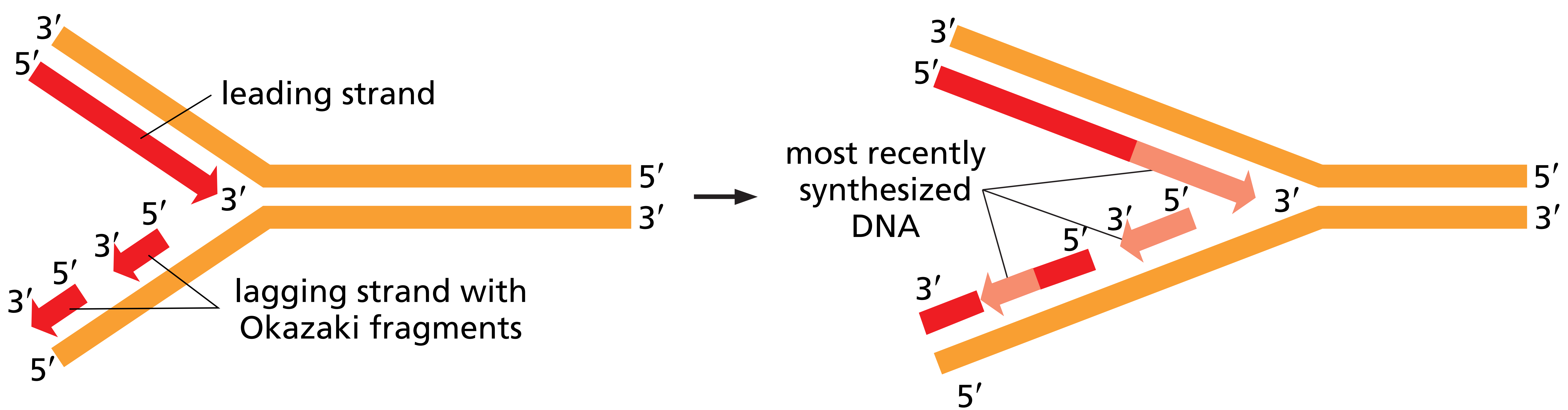


DNA replication principles

DNA polymerase can only polymerise **from 5’ to 3’ —>** How does it work for the **other strand**?

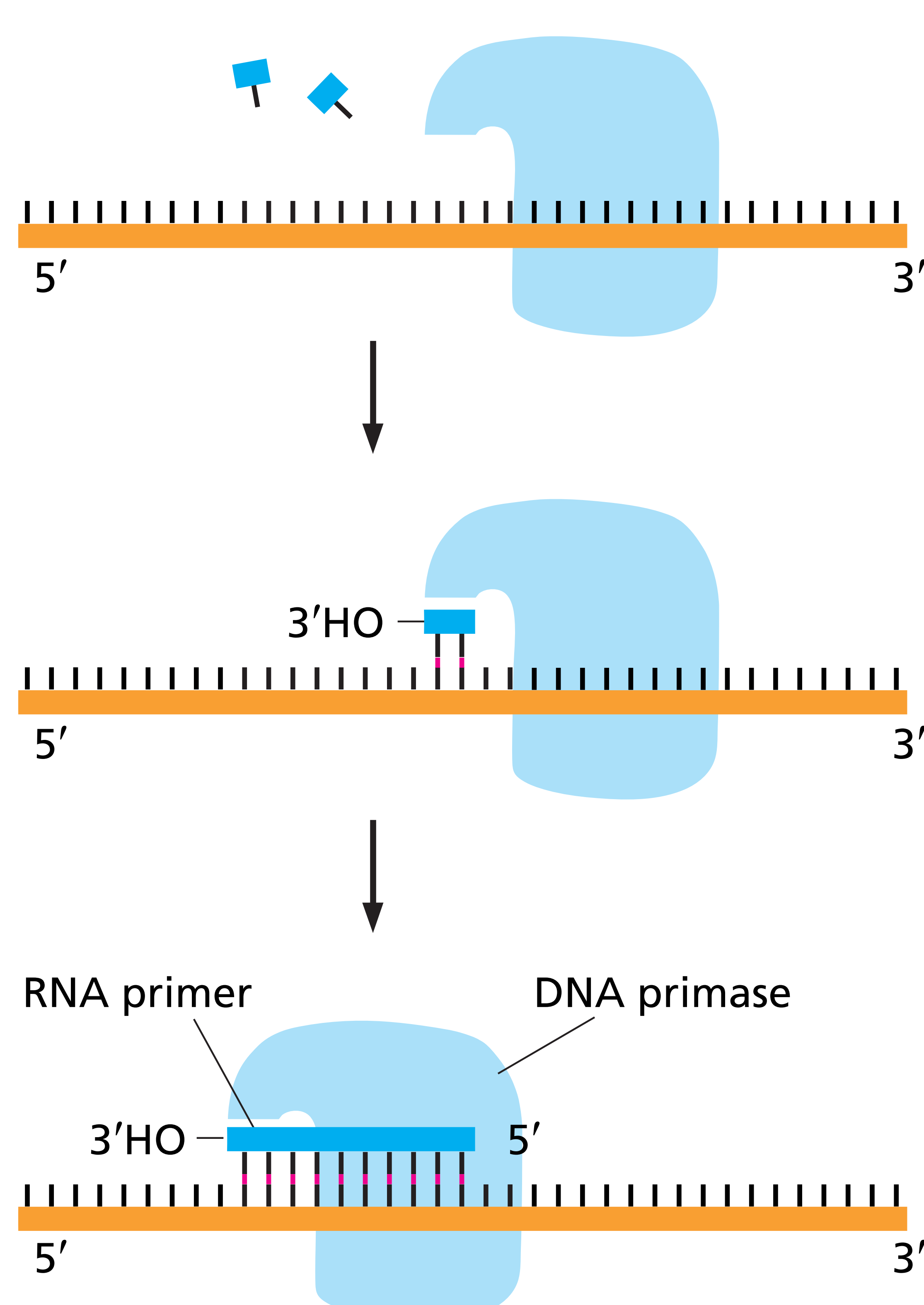
DNA replication principles

- Transient existence of pieces of DNA that are ~ 100-1000 nucleotide long at the replication fork = **okazaki fragments**
- They are synthesised **from 5’ to 3’** and **joined together after synthesis**
- The replication fork has therefore an **asymmetric structure** with a **leading strand** (synthesised continuously) and a **lagging strand** (synthesised non-continuously)



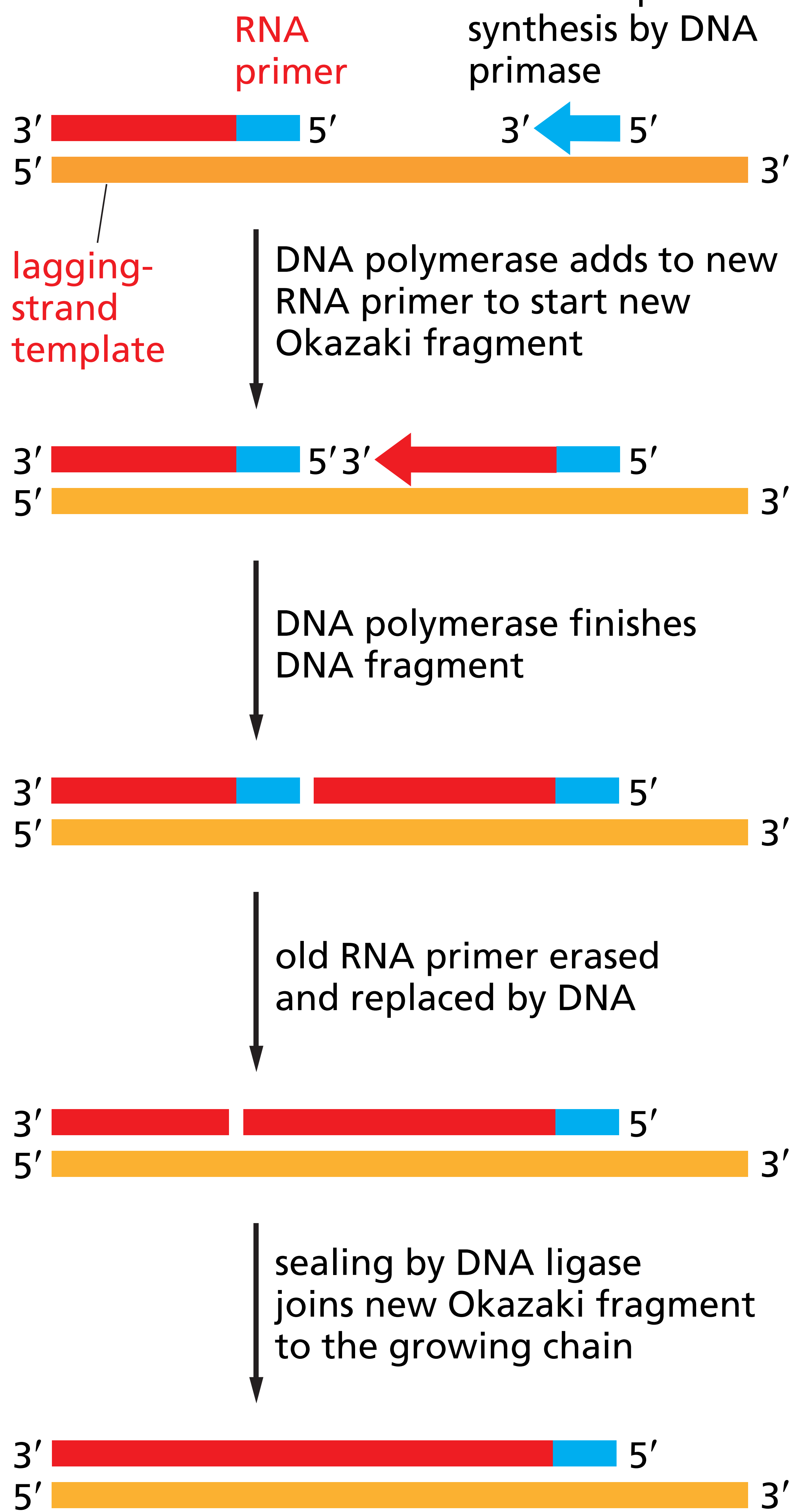
Building an RNA primer

- DNA polymerase **cannot start synthesis *de novo* without a primer**
- For the leading strand, a primer is needed at the start of replication
- For the lagging strand, a primer is needed at each new Okazaki fragment
- This mechanism depends on the **DNA primase** which uses ribonucleotides triphosphate to synthesise short **RNA primers** on the lagging strand
- In eukaryotes, those are about **10 nucleotide long** and are made at intervals of 100-200 nucleotides on the lagging strand



DNA synthesis on the lagging strand

- On the lagging strand, DNA synthesis **stops when the DNA polymerase runs into the next RNA primer**
- A special **DNA repair system** acts quickly to **erase the RNA primer** and **replace it with DNA**
- A **DNA ligase** then **joins** the 3’ end of the new fragment to the 5’ end of the old fragment



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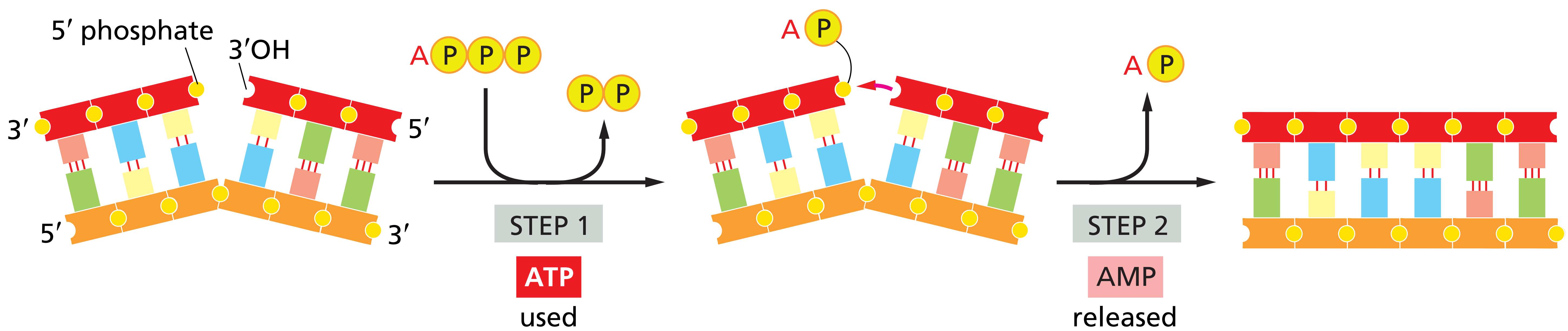
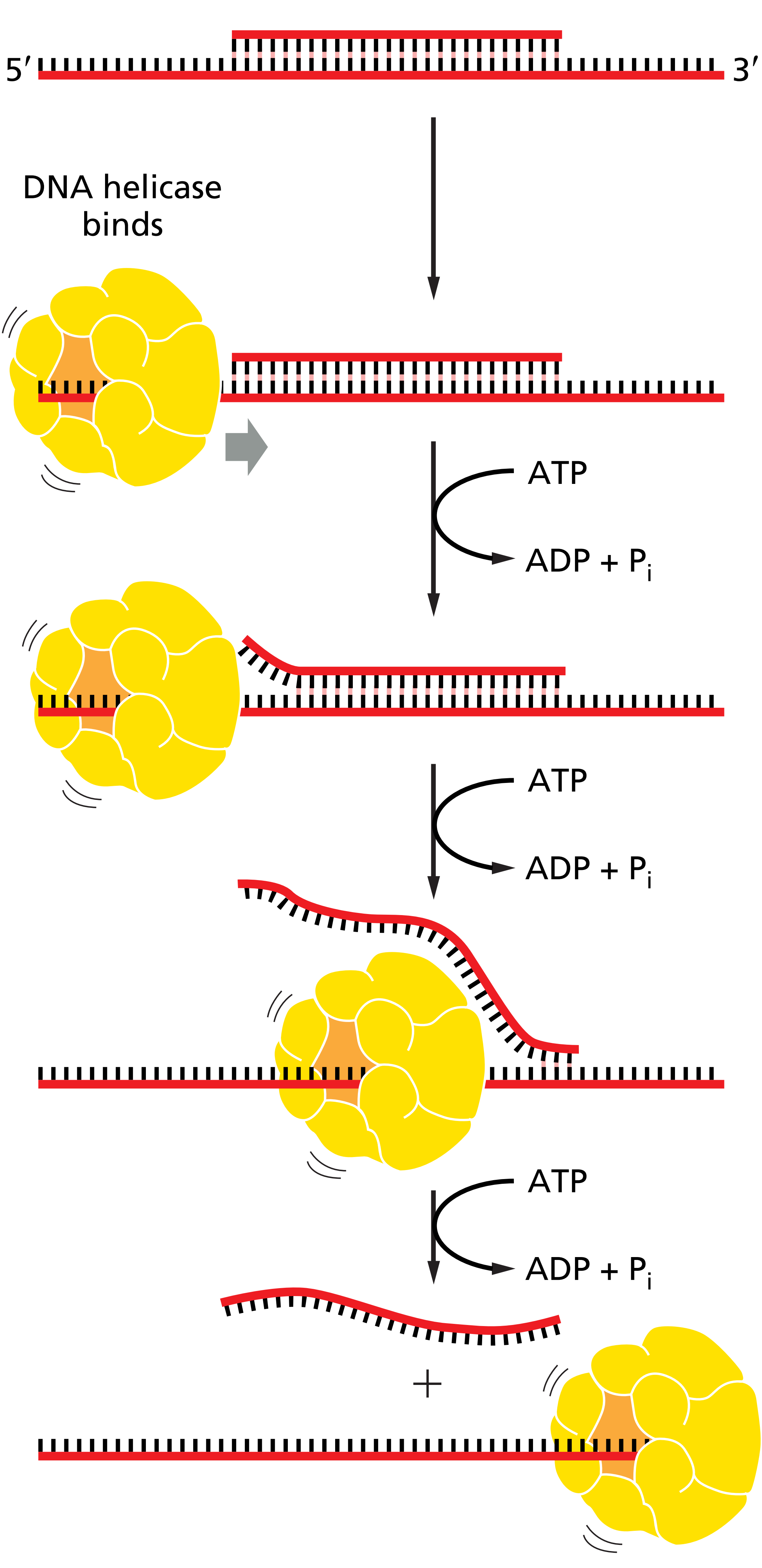
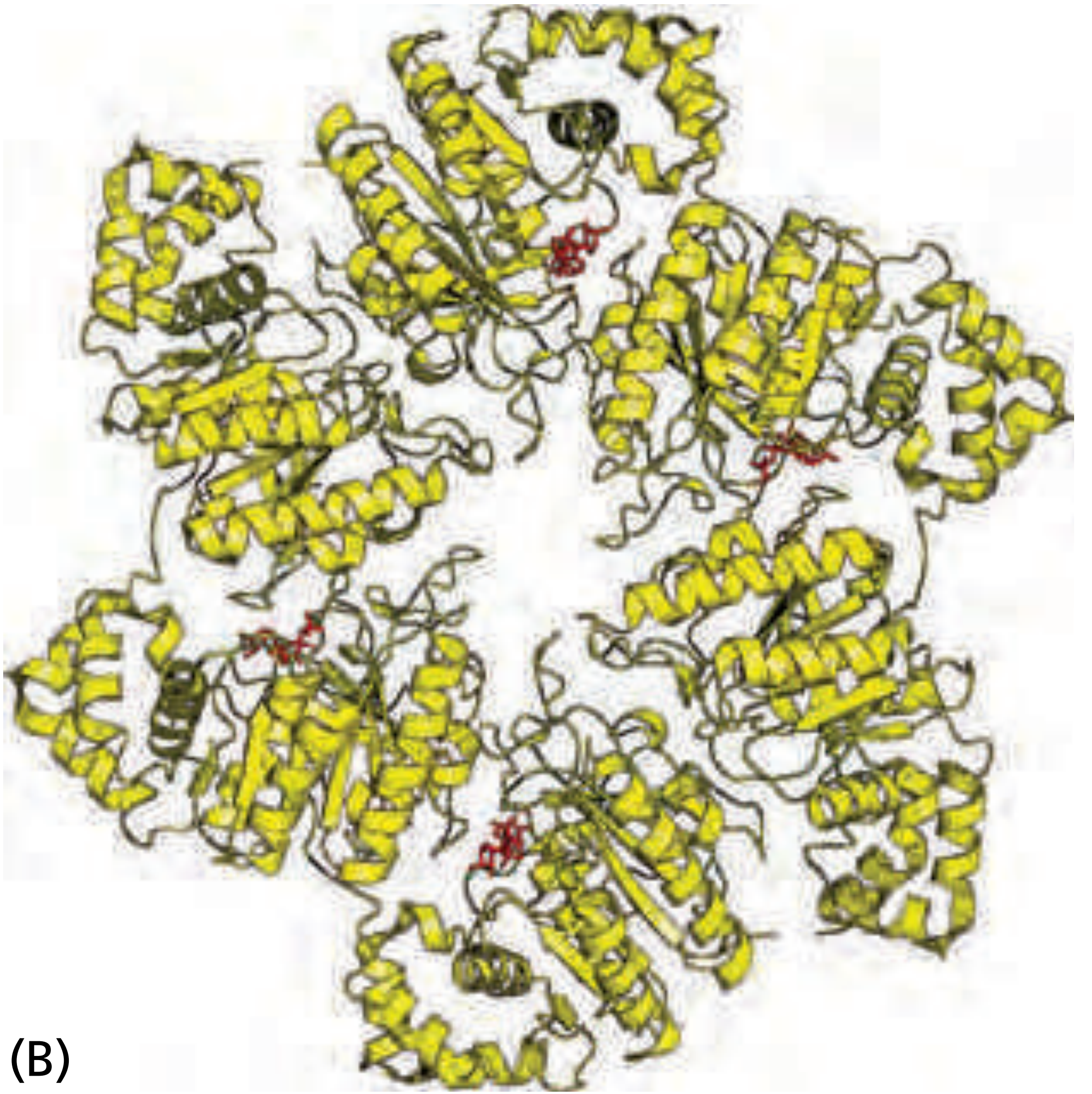


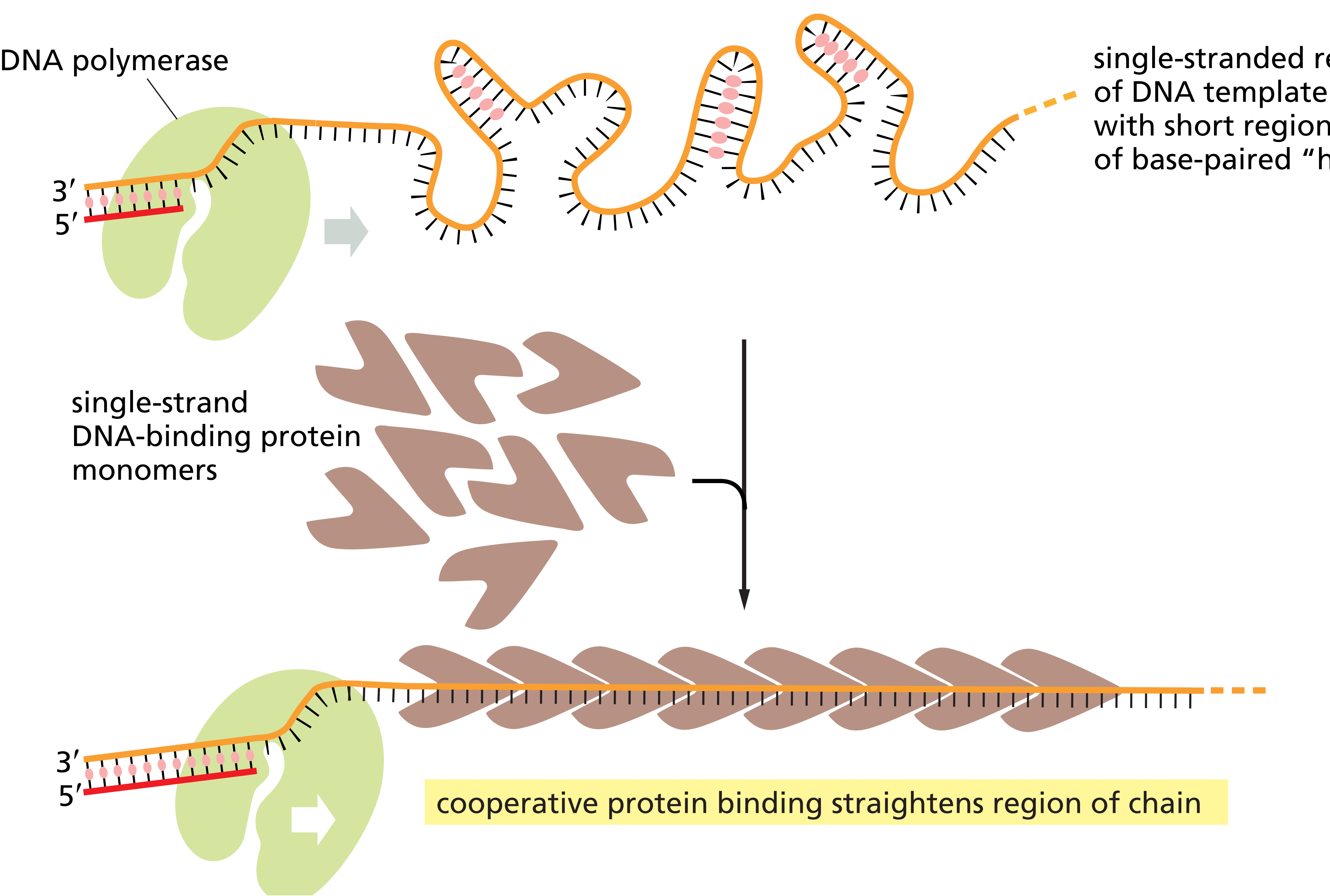
Opening up the double helix



- The double helix should be **opened** ahead of the replication fork
- DNA is very stable, in the lab, we use **very high temperatures** to separate two DNA strands
- **DNA helicases** hydrolyze ATP when they are bound to single strands of DNA, they move along the strand and open the helix once they encounter double-stranded DNA (1000 nt/sec)



Opening up the double helix



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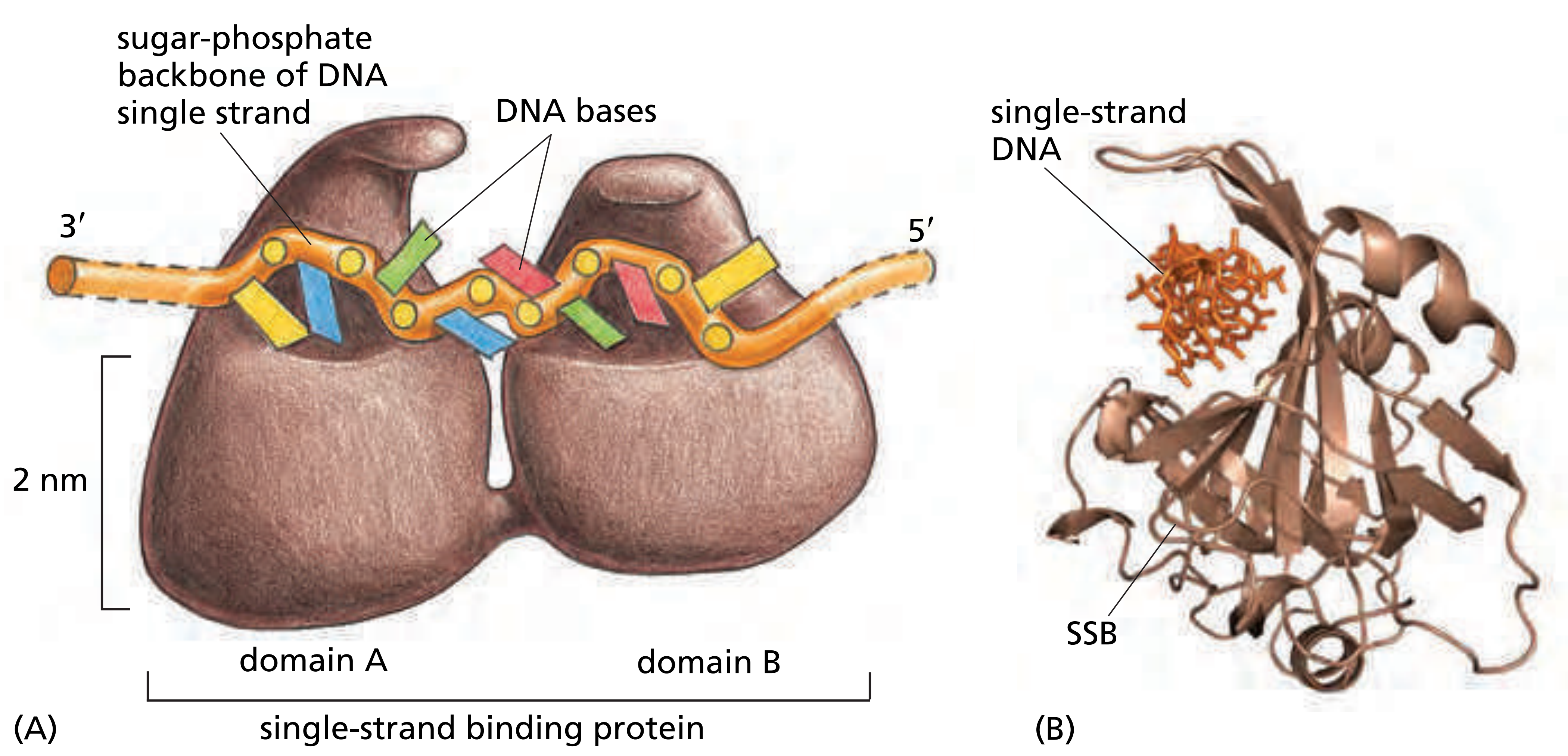
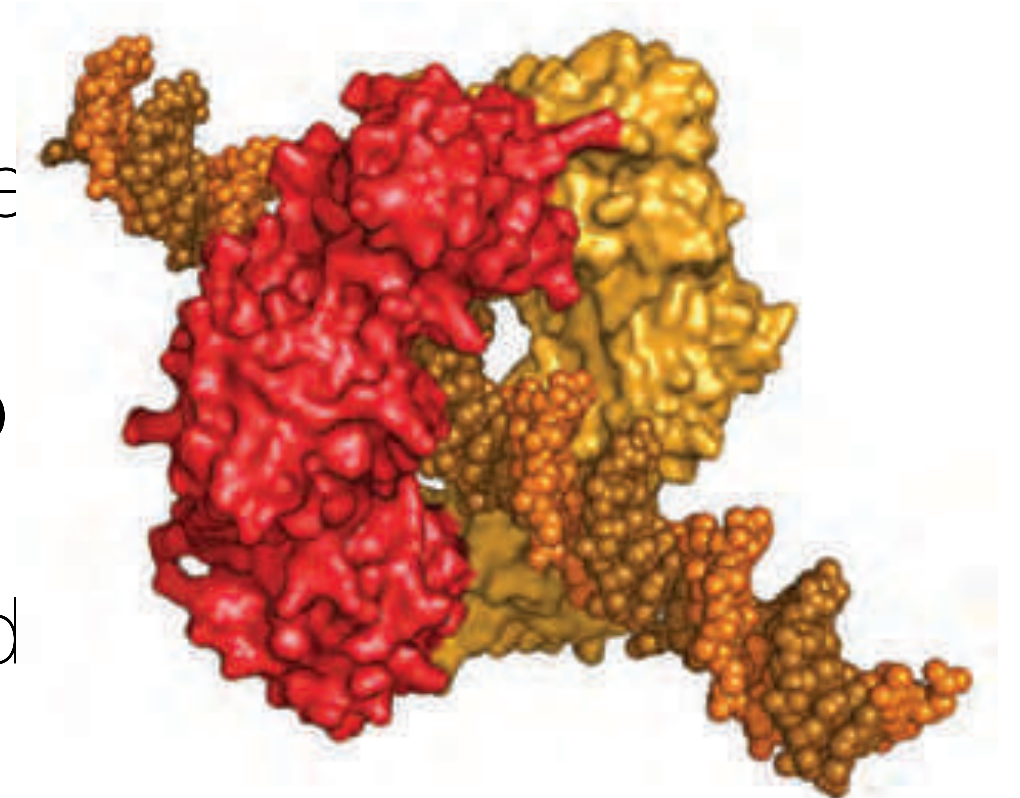
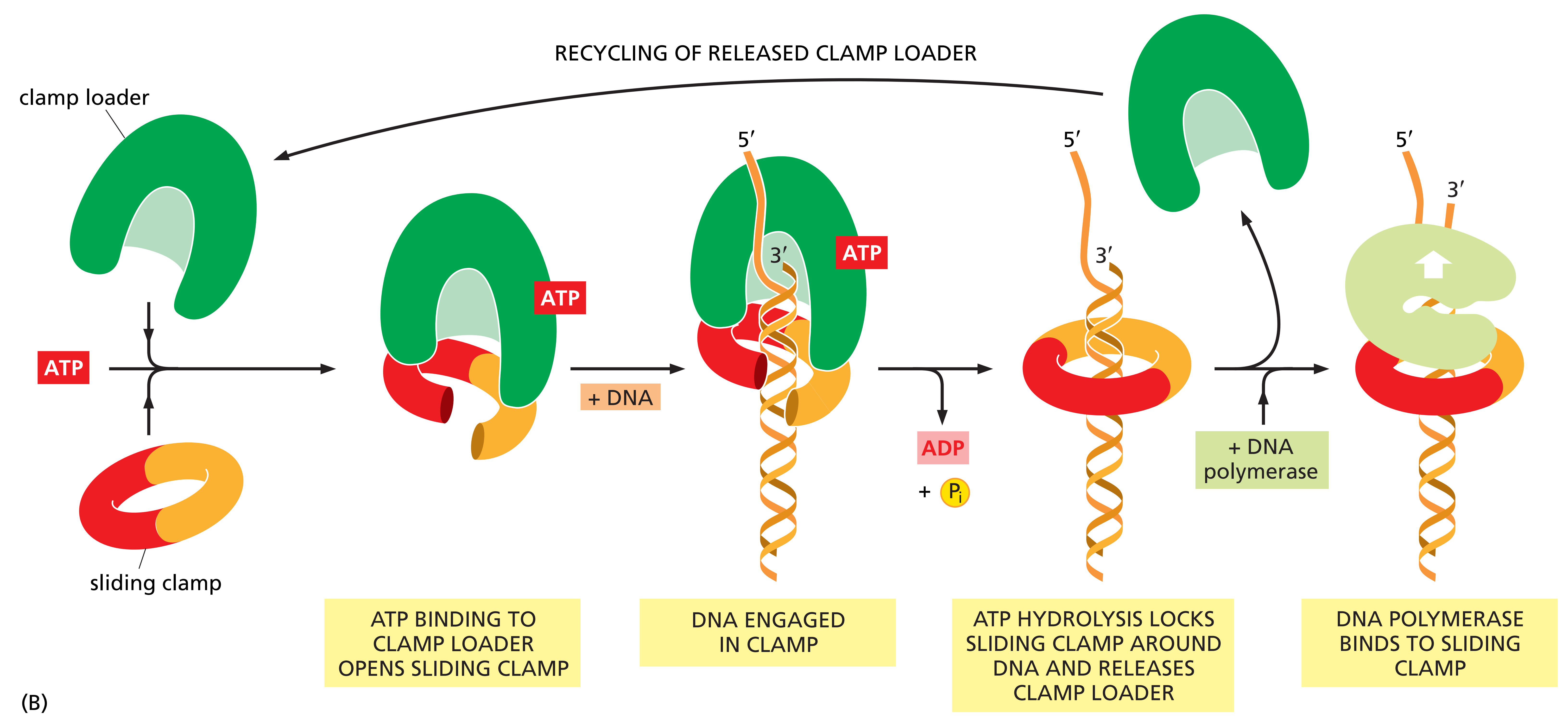
- **Single-strand DNA binding proteins** (SSB) bind to single-stranded DNA without covering the bases (available as templates)
- They **help** helices by **stabilising and straightening** the DNA
- They are **unable to open** a long DNA helix



Holding the DNA polymerase onto the DNA

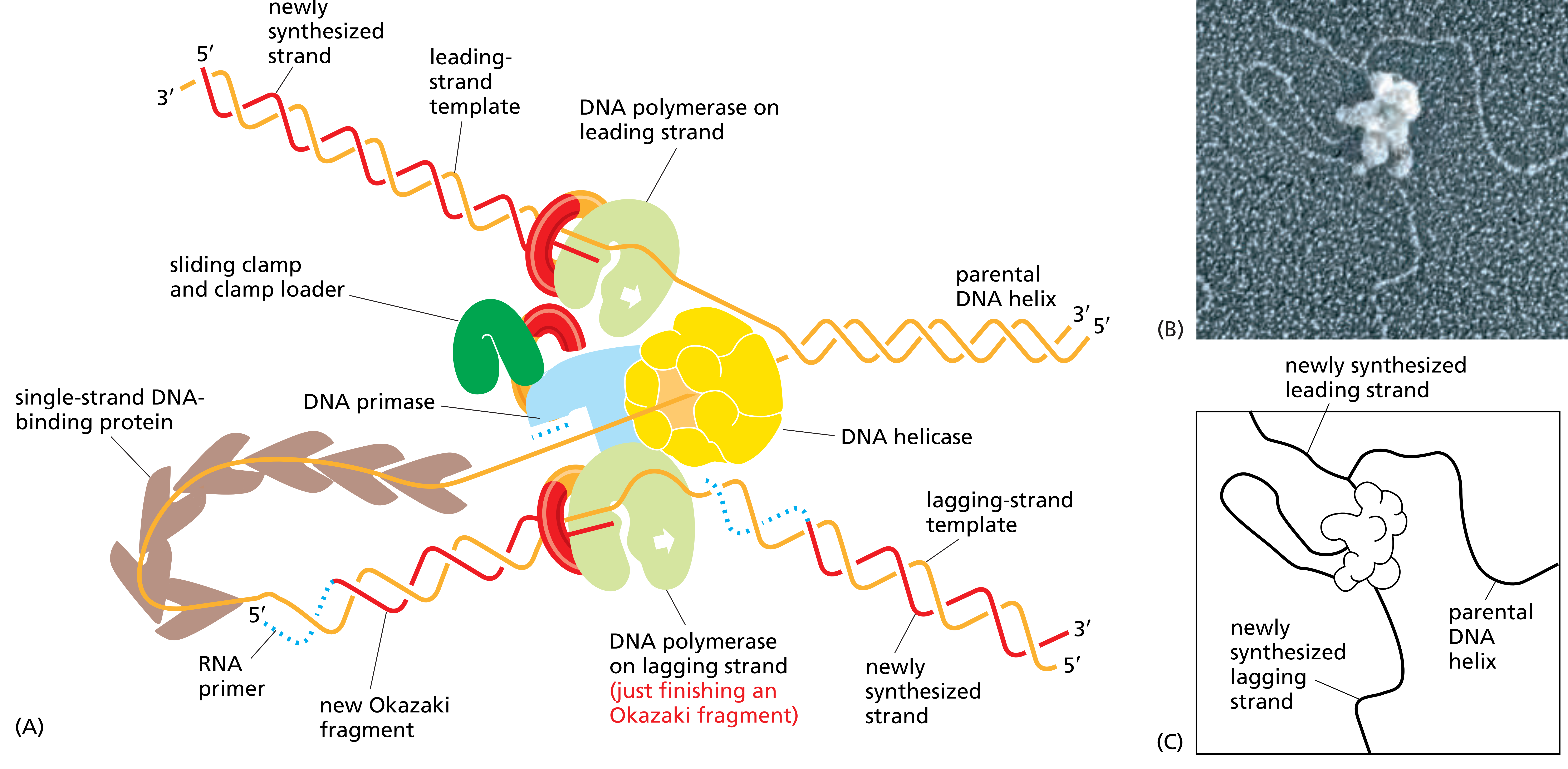
- DNA polymerases need to be able to **“fall off” the DNA** to synthesise
- On the leading strand, it needs a **sliding clamp** (proteins) and **clamp**
- The clamp **keeps the polymerase onto the DNA** until running into d





The replication machine

- Most of the proteins discussed before act together as a large **multi-enzyme complex** that rapidly synthesizes DNA



Bacterial replication fork

Have a nice day!