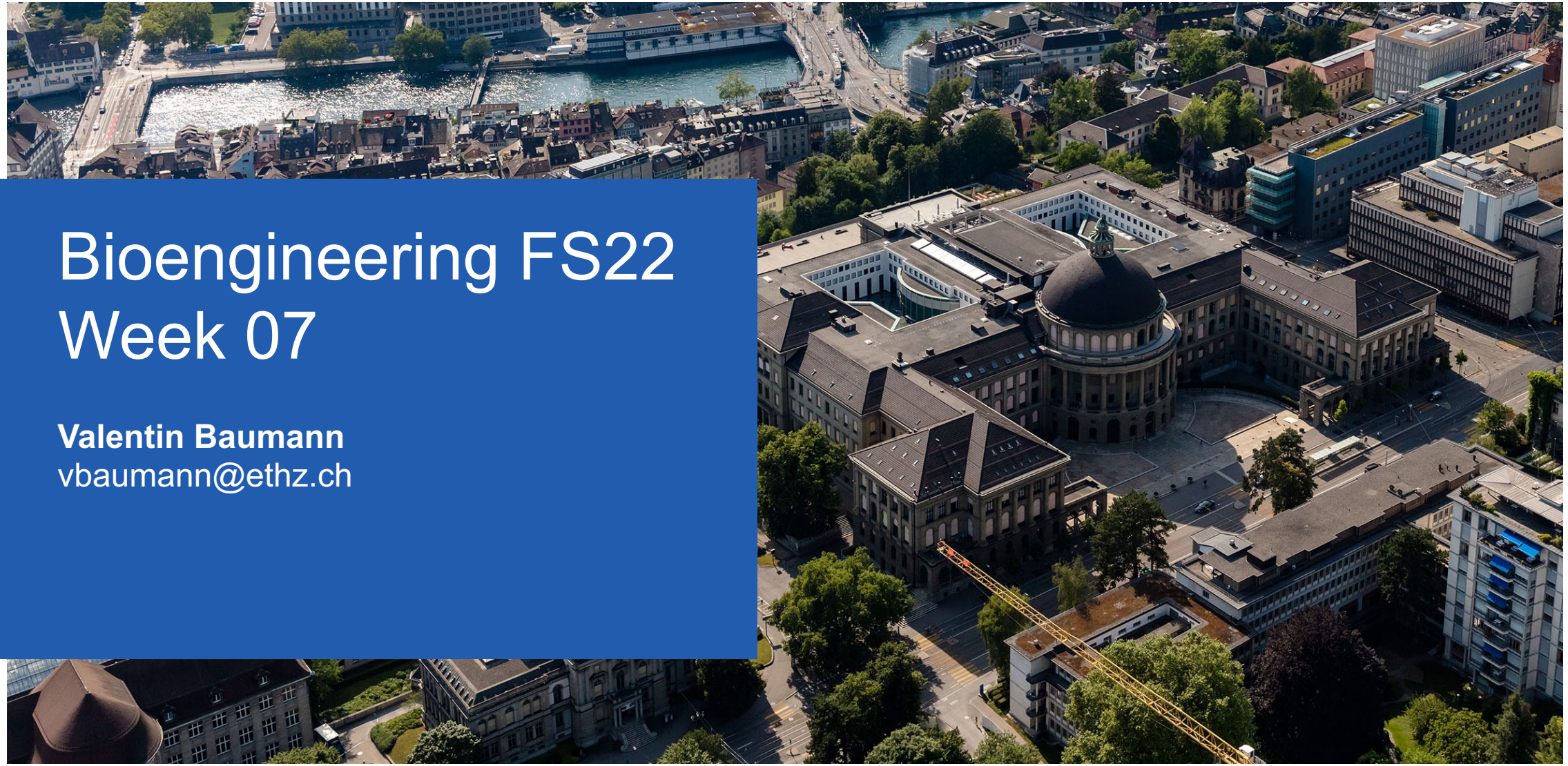
ETH zürich



# Bioengineering FS22
# Week 07

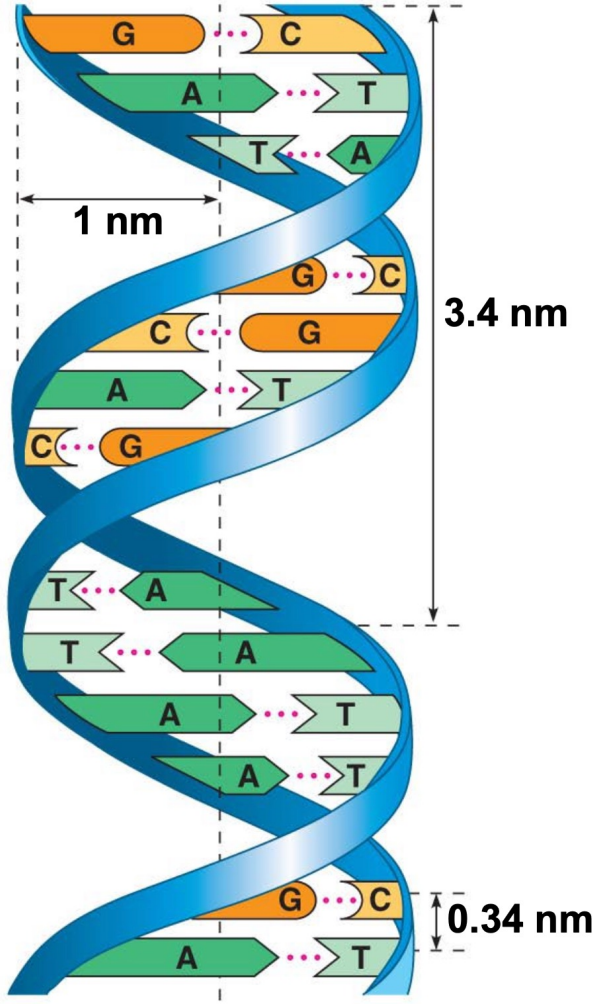
**Valentin Baumann**
vbaumann@ethz.ch

# Agenda heute

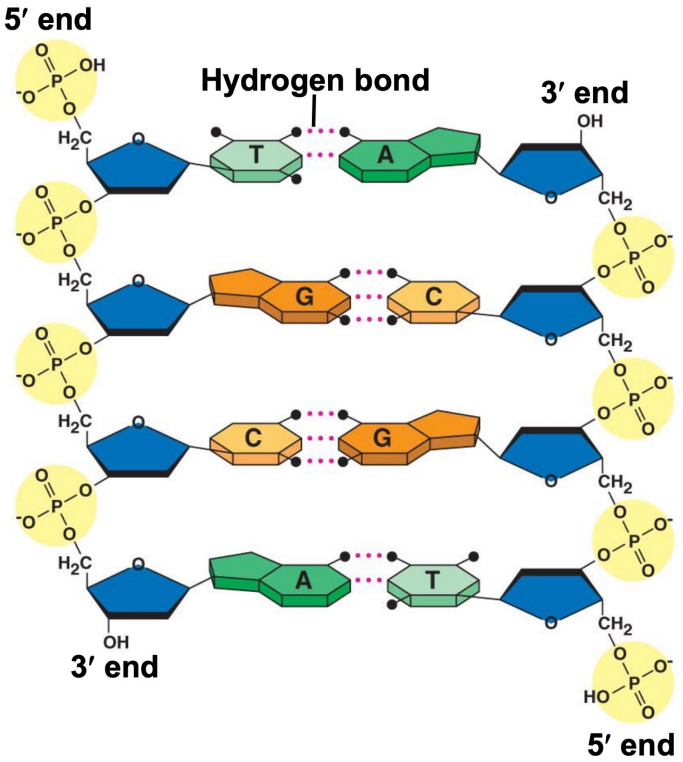
1. DNA-Replication
2. Cell Membrane
    1. Structure
    2. Function
    3. Transport

# DNA-Replication

# DNA & Mode of replication

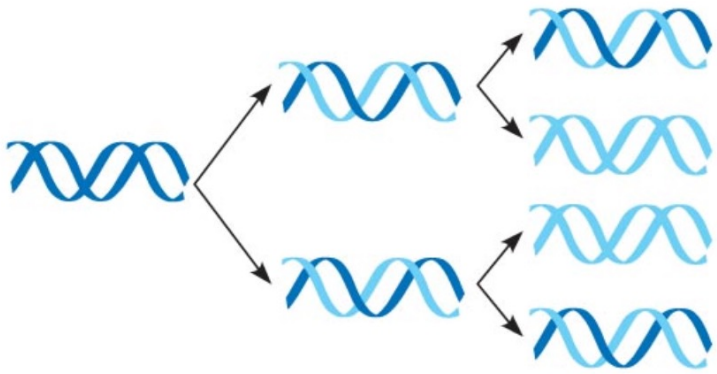



(a) Key features of DNA structure



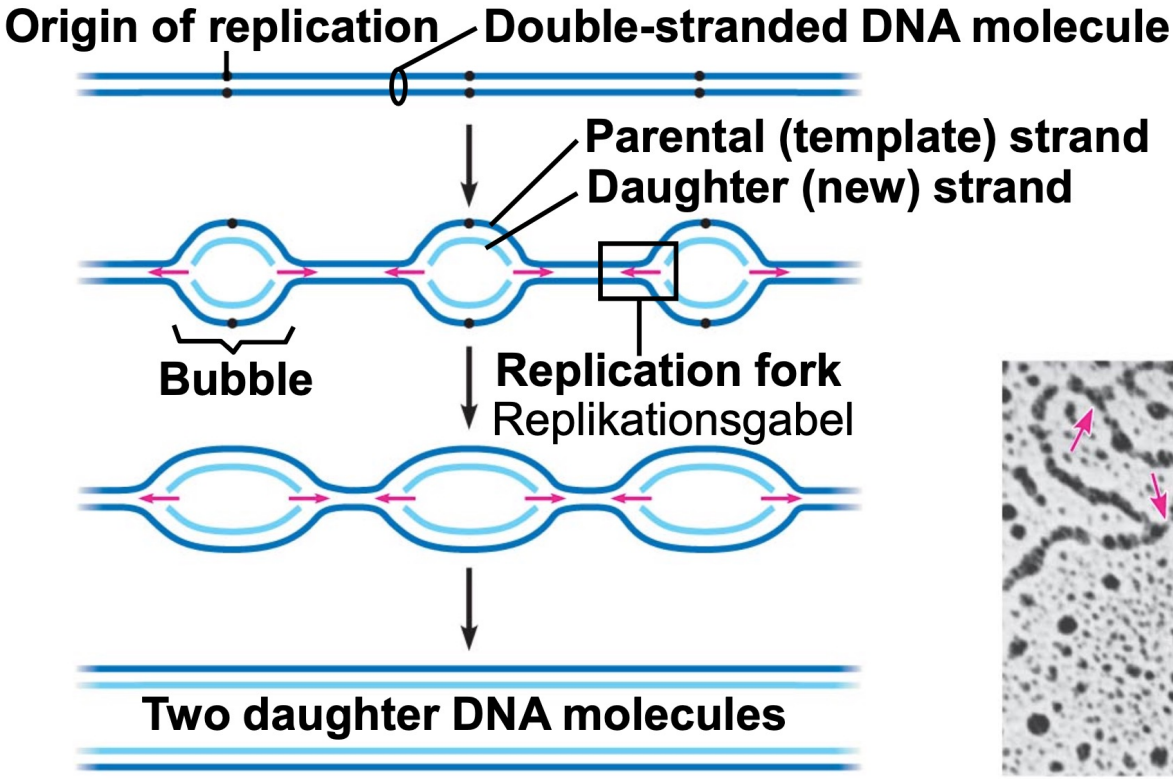

(b) Partial chemical structure

(b) Semiconserva-tive model



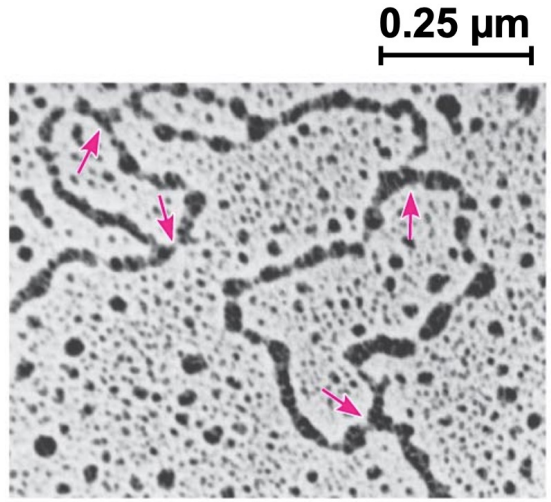
# Origins of replicaiton & DNA „bubbles“

- DNA is opened up at hundreds/thousands of sites along the chromosome → Origins of replication
- DNA strands are separated and **replication fork** forms at both ends of every resulting “bubble”
- Replication forks of same “bubble” move away from each other, elongating the new daughter strand, until replication forks from neighbouring “bubbles” collide
- Replication proceeds in both directions



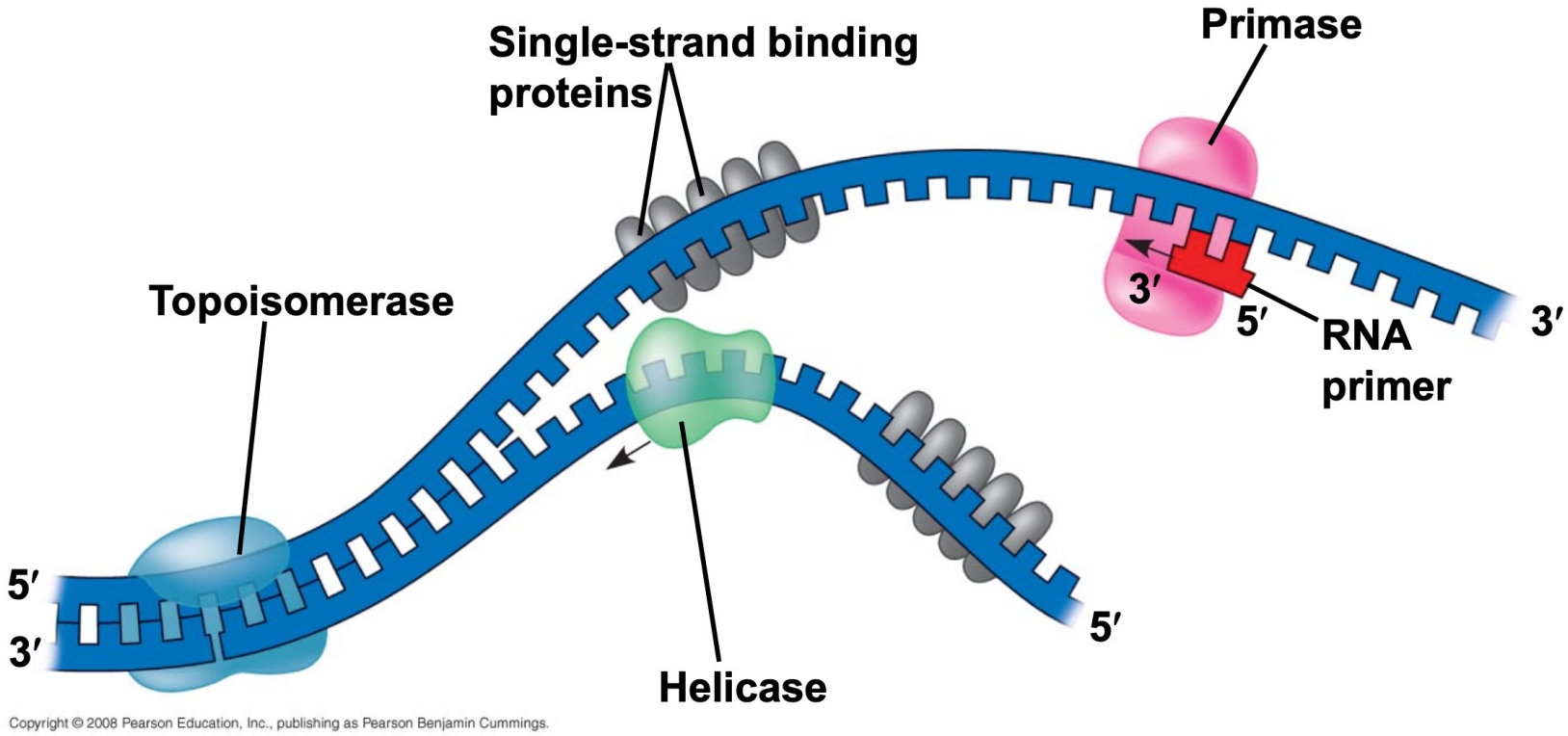

**(b) Origins of replication in eukaryotes**





# Replication fork

- **Helicase** is an enzyme that untwists the double stranded helix at replication fork (like a zipper)
- **Single-stand binding protein** binds and stabilizes the two now separated single strands of DNA
  - → allows Primase and later DNA polymerase to access the strands



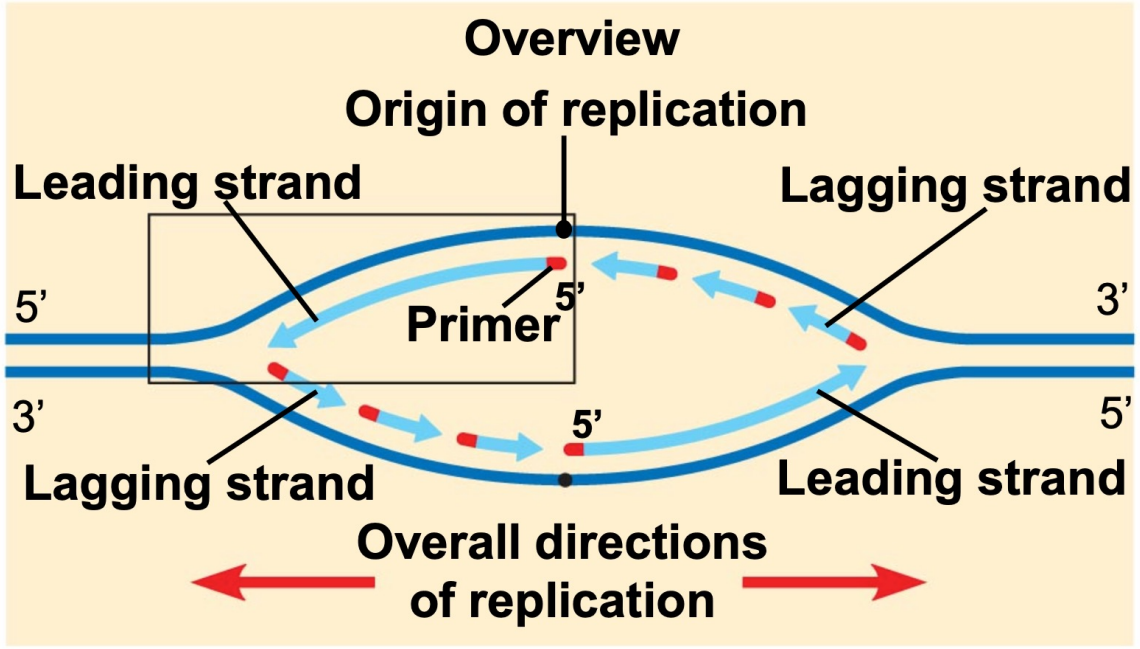


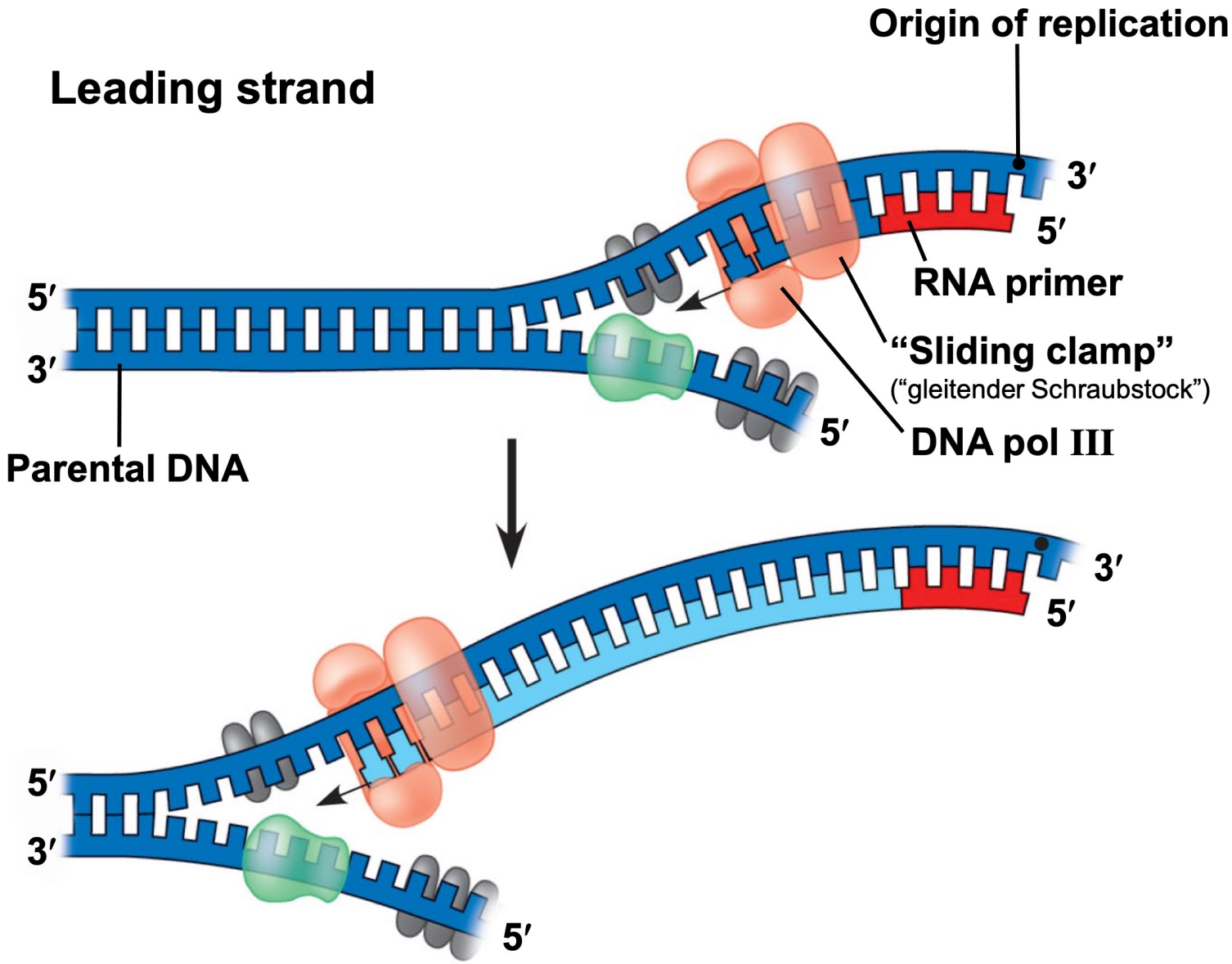
- **Topoisomerase** corrects overwinding of DNA, occurring due to untwisting by the Helicase (like separation of two intertwined strings)
  - Breaks, unwinds and re-joins the strands
- **Primase** creates RNA primers, which can be recognised by DNA polymerase later on and serve as starting point of daughter strand elongation

# DNA polymerase & Replication directionality

- While replication occurs in both directions on each strand, DNA polymerase can only work and elongate the daughter strand in one direction (5'-3') and thus only moves 3'-5' on the old template strand

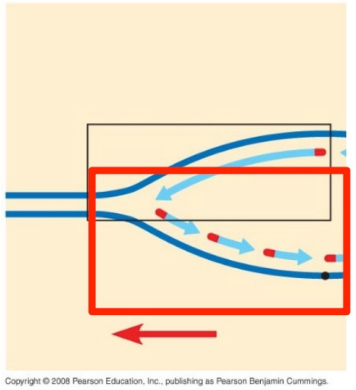
- Therefore the daughter strand is created in differently on both sides of the origin of replication
  - **Leading strand** -> continuous production of daughter strand behind replication fork
  - **Lagging strand** -> discrete production of daughter strand in smaller fragments **(Ozaki-fragments),** as polymerase moves away from replication fork, instead of following it
    - → Ozaki fragments later joined by DNA ligase

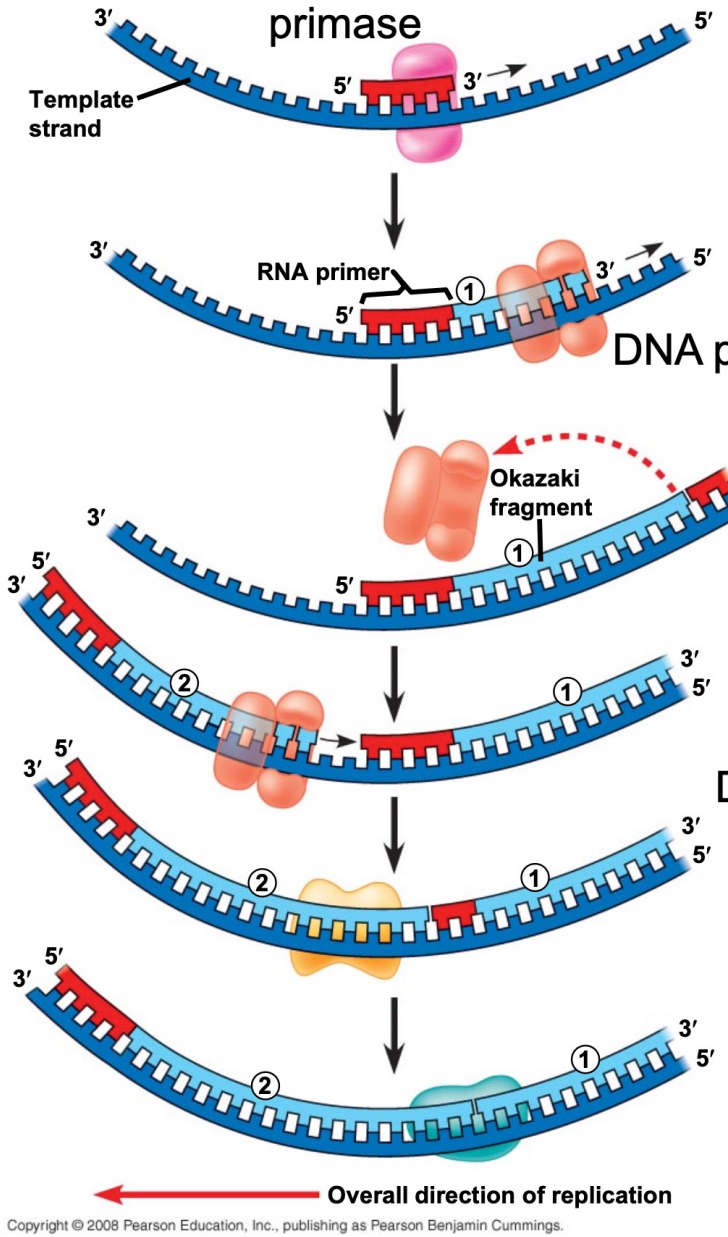



Origin of replication
Leading strand
3′
5′
RNA primer
5′
3′
“Sliding clamp”
(“gleitender Schraubstock”)
5′
DNA pol III
Parental DNA
3′
5′
5′
3′
5′

replication fork

Lagging strand





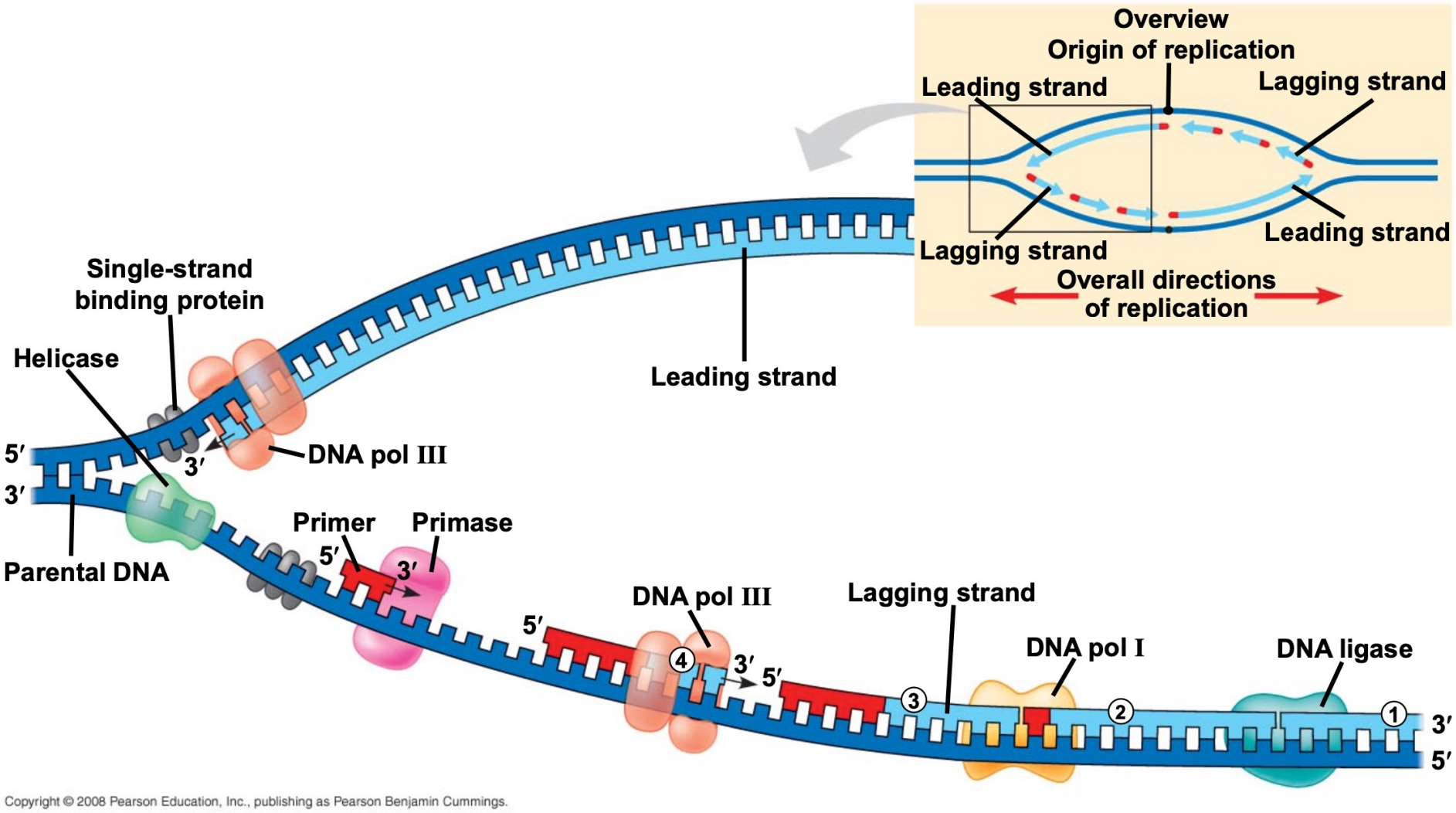
origin of replication

DNA pol I eliminates primer

DNA ligase closes the remaining gap in the DNA backbone ("ligation")

replication fork

origin of replication

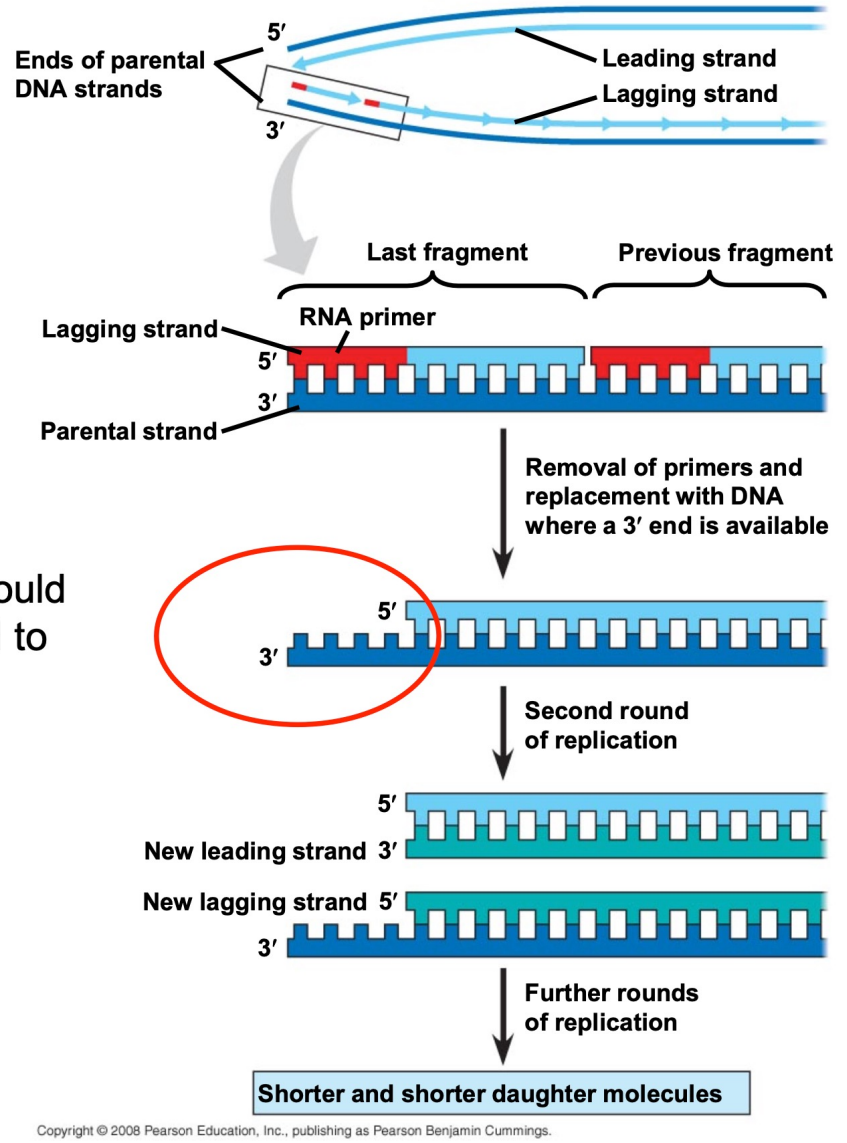


# Telomeres

- 5’ end of daughter strand cannot be completely produced (lagging strand)
- To ”prepare” for this situation, eukaryotic cells have **telomeres,** which are non-coding nucleotide sequences at the end of the chromosome
- **Telomeres** are eroded during lifetime of cells throughout all their divisions and thus postpones erosion of coding sequences of the chromosome (genes)
- Shortening of telomers also act as “aging” of the cells, whereby ”old” cells with short telomeres are killed by apoptosis to prevent uncontrolled/cancerous growth
- In germ and stem cells which divide for much longer time than somatic cells, we have **telomerase** enzymes that catalyze lengthening of the telomeres

**The «end problem»**

DNA Pol I would need a 3’OH to get started!

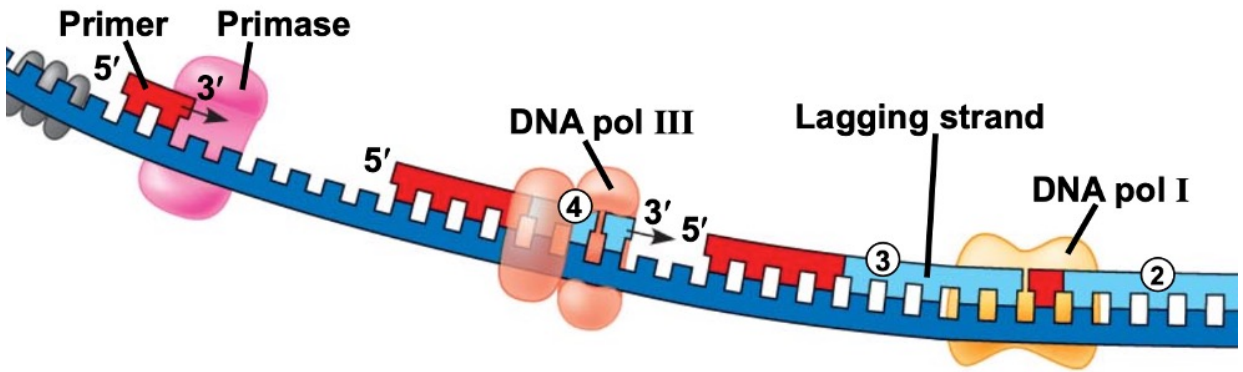


# Polymerases - Overview

- **Primase** – can produce RNA fragment on DNA matrix without the neeed of a pre-existing 3’ OH end of a previous nucleotide as starting point (same for RNA Polymerases)

- **DNA Polymerase III** – Needs pre-existing 3’ OH end of previous nucleotide to start (→ Therefore need for primer); Cannot decompose RNA or DNA in 5’-3’ direction (→ Cannot decompose primer and produce DNA in its place); Can decompose RNA or DNA in 3’-5’ direction (→ allows correction of falsely produced nucleotide, by moving back a little)

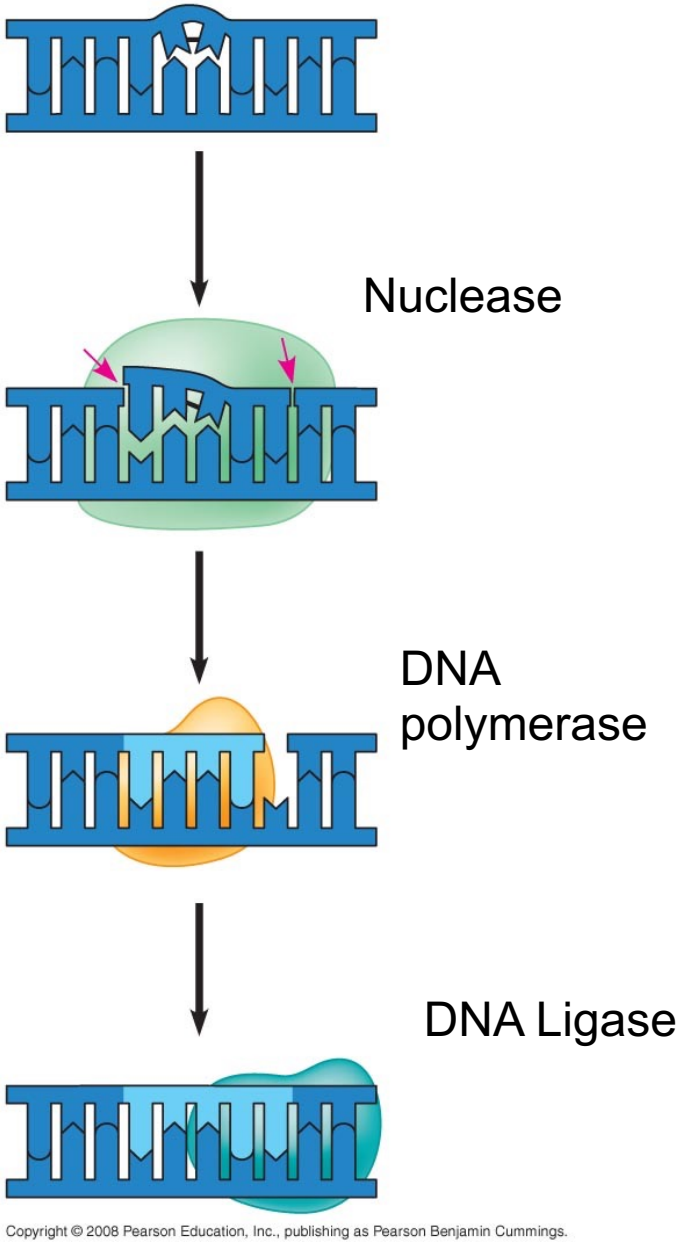
- **DNA Polymerase I** – Needs pre-existing 3’ OH end of previous nucleotide to start (→ normally previously created DNA strand from DNA pol III; Can in contrast to DNA pol III decompose DNA in 5’-3’ direction, so can ”substitute RNA primers needed before with DNA



# Proofreading and Repairing DNA

- **Proofreading**
  - Incorrect nucleotides in newly produced DNA
  - Mismatch repair (see diagram on the right)

- **Repairing**
  - Damaged DNA by chemicals, radioactive emissions, etc. Corrected by nucleotide excision repair, which is similar to mismatch repair but differs in participating proteins (longer stretches of DNA than for mismatch repair)



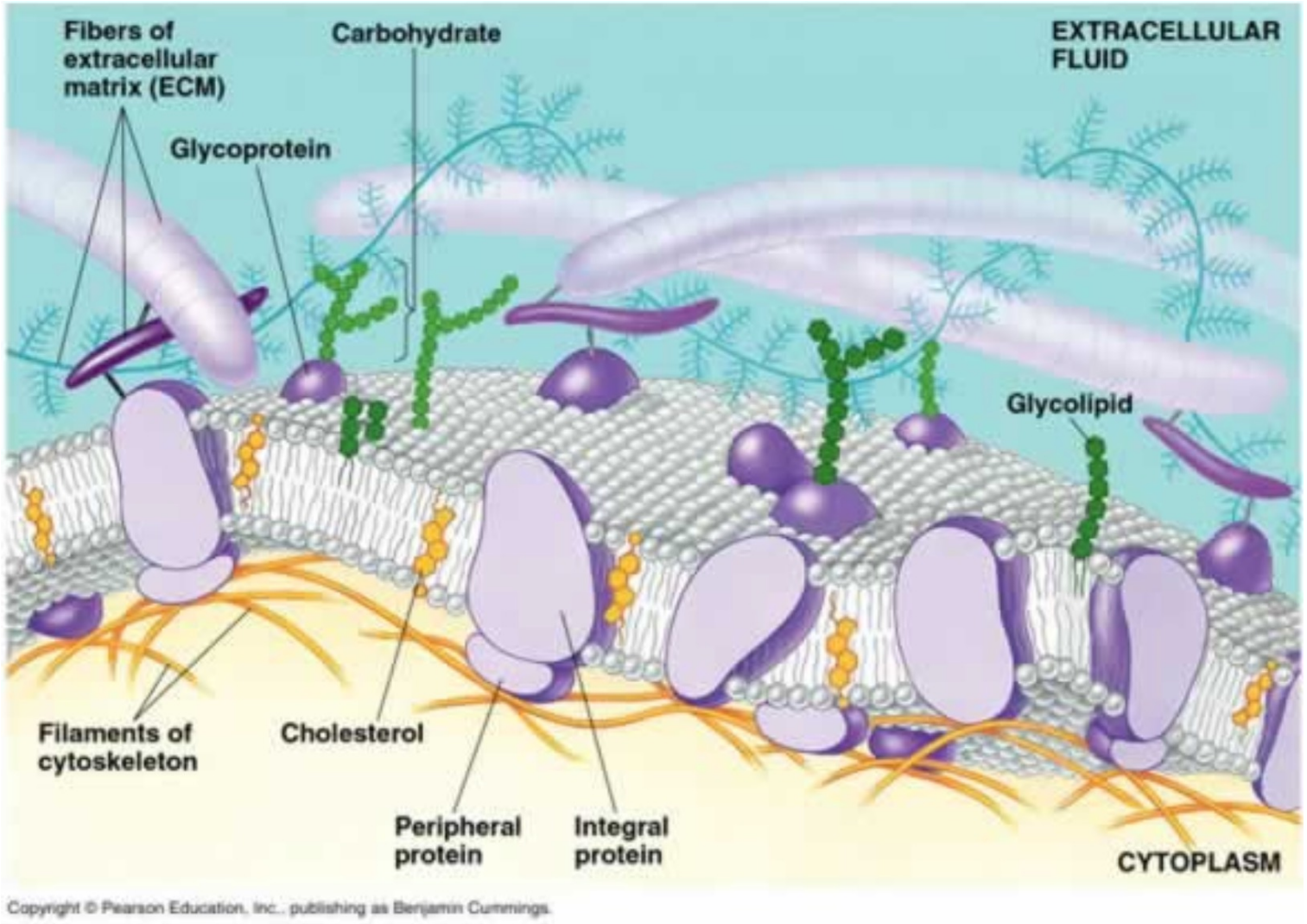
# Cell Membrane

# Cell Membrane - Grundstruktur

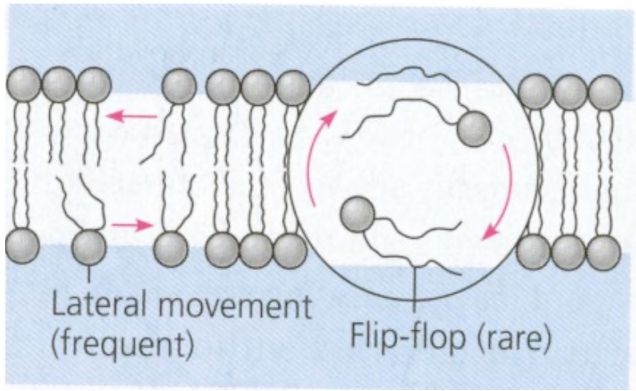
- Phospholipid double layer
- Proteins anchored wihtin phospholipids

→ Fluidic-mosaic model (membrane and content moves around)

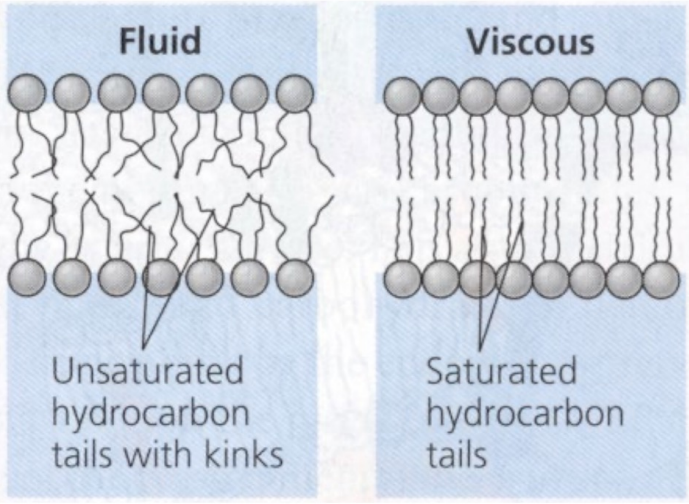
- Carbohydrate chains connected to lipids and proteins on the outside of the membrane
  - Glycolipids
  - Glycoproteins
- Membrane in constant and interactive contact with cytoskeleton within the cell and the extracellular matrix without it



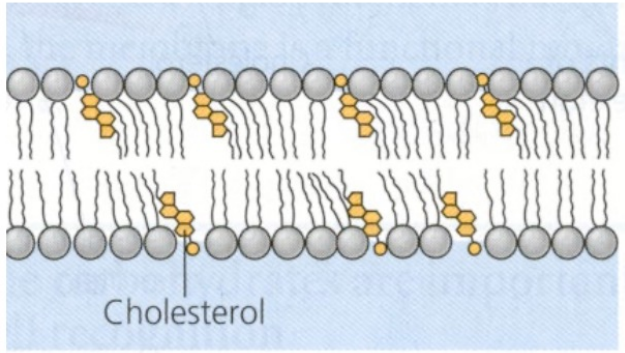
# Cell Membrane – Phospholipids (PL) & Cholesterol

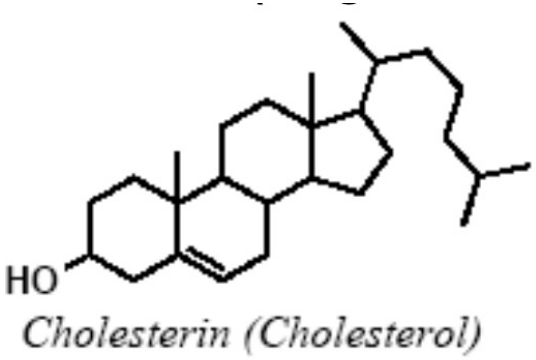



Phospholipids can move around → fluidity




Fluidity of membrane regulated by amount of double bonds (unsaturated) in the fatty acids of phospholipids




Cholesterol (mixed between phoshpolipids) regulates membrane fluidity:

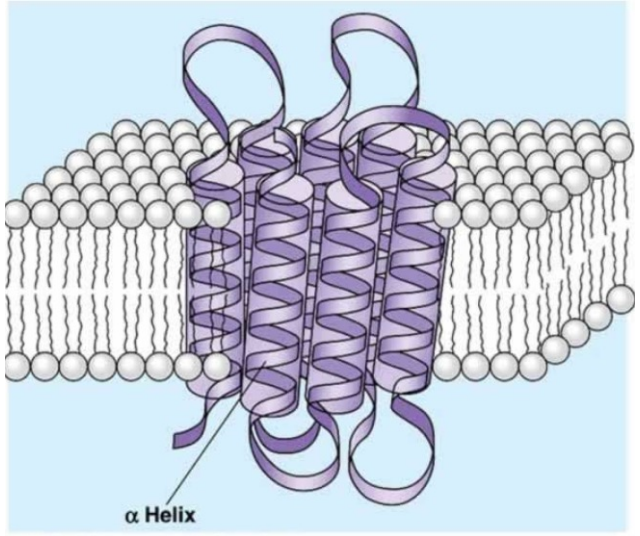
- Reduction of PL movement at high temperatures -> less fluid
- Inihibition of regular organisation of PL at low temperatures -> more fluid

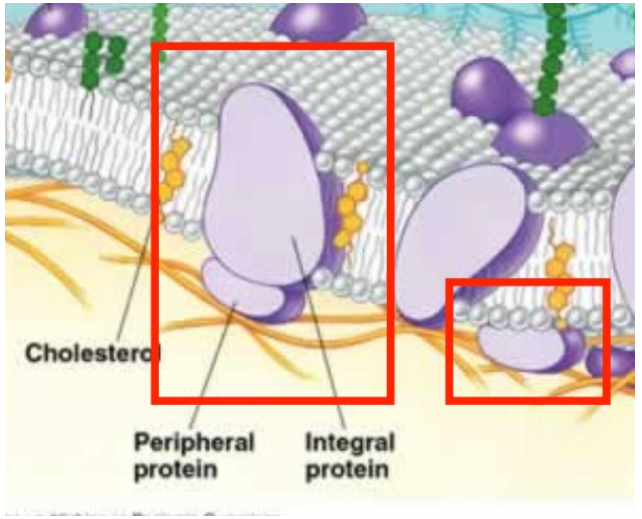
# Cell Membrane – membrane proteins

- Proteins differently anchored in membranes

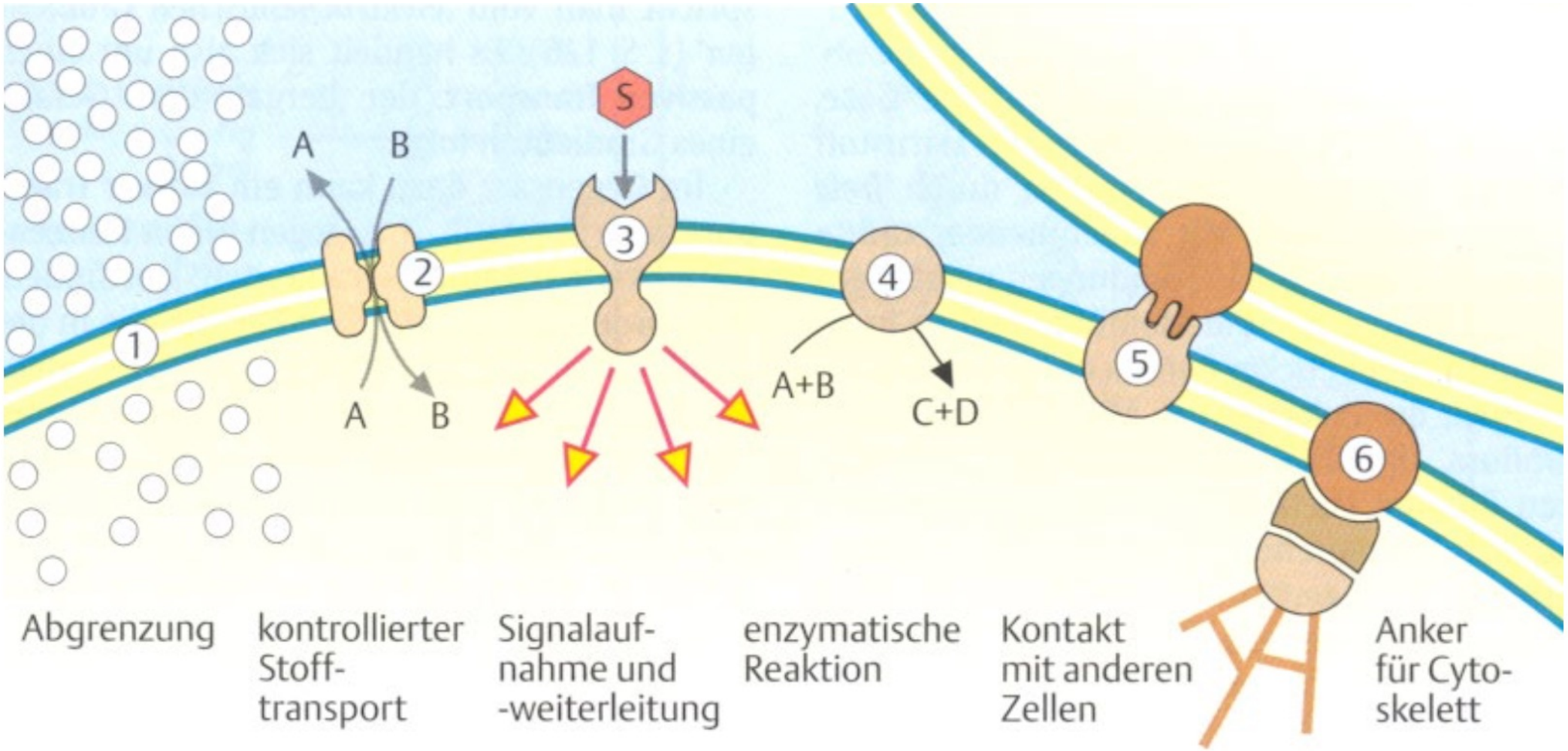
- Integral membrane proteins
  - Going through the membrane -> also in contact with hydrophobic core of PL double layer
  - 7 hydrophobic, trans-membrane alpha-helices & non helical parts in contact with hydrophilic phase outside of membrane (typical structure)

- Peripheral membrane proteins
  - Associated with hydrophilic parts of PL‘s and integral membrane proteins

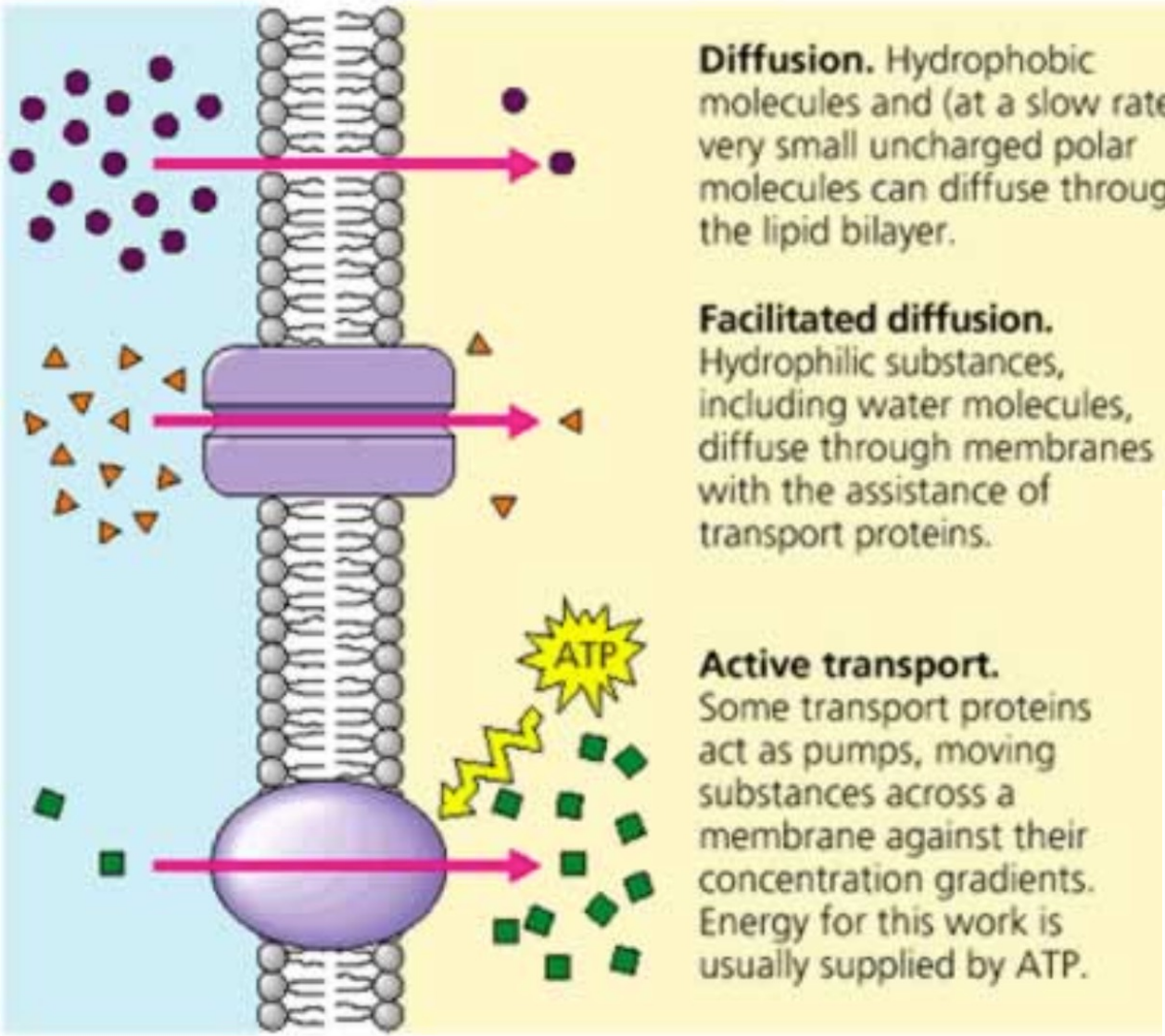


# Cell Membrane - Function



# Cell Membrane - Transport

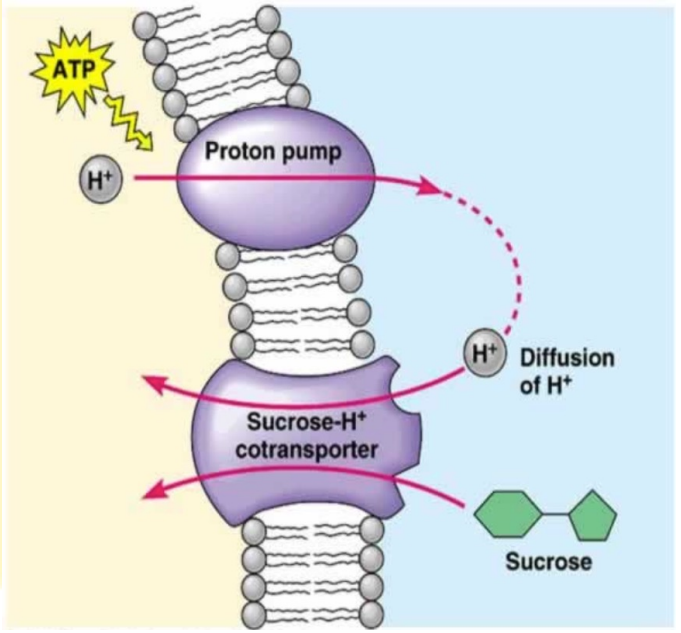



**Diffusion.** Hydrophobic molecules and (at a slow rate) very small uncharged polar molecules can diffuse through the lipid bilayer.

**Facilitated diffusion.** Hydrophilic substances, including water molecules, diffuse through membranes with the assistance of transport proteins.

**Passive transport.** Substances diffuse spontaneously down their concentration gradients, crossing a membrane with no expenditure of energy by the cell.

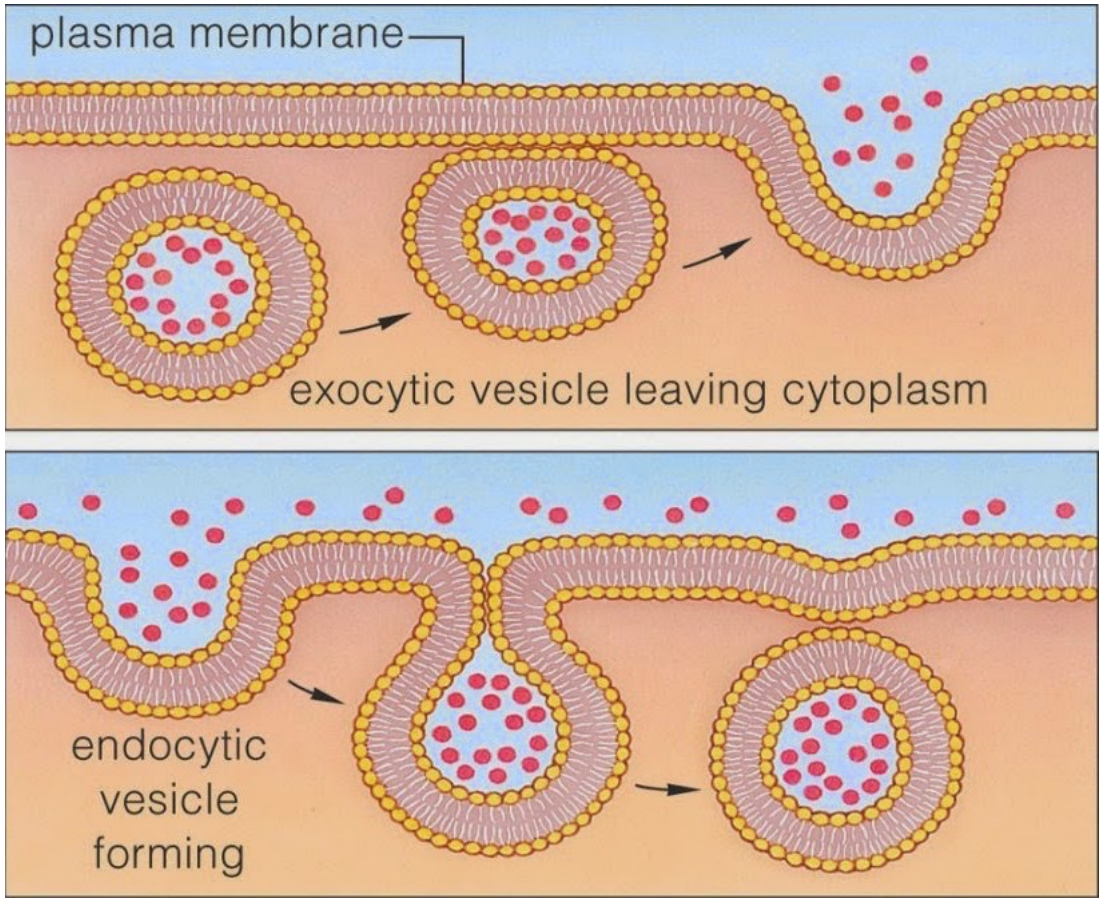
**Active transport.** Some transport proteins act as pumps, moving substances across a membrane against their concentration gradients. Energy for this work is usually supplied by ATP.




Transport of one substance (sucrose) passively enabled by active transport of another substance (H+) and subsequent gradient establishment

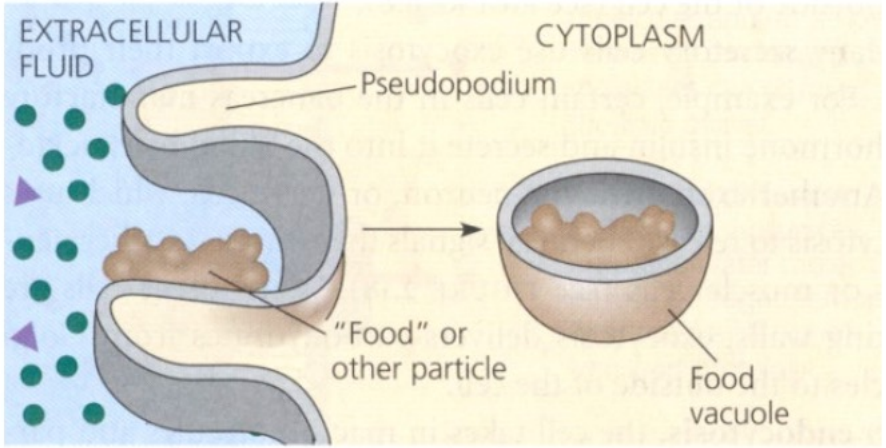
# Cell Membrane – Endo- & Exocytosis

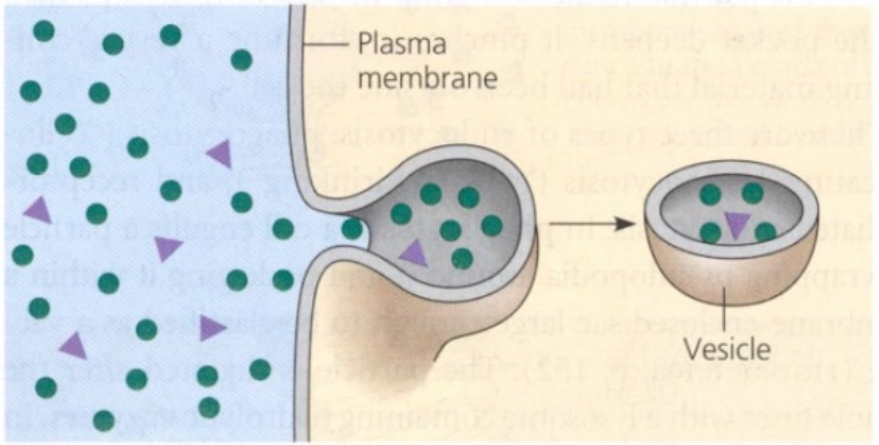
Exocytosis




Endocytosis

Phagocytosis (large particles in vacuoles)




Pinocytosis (liquid in vesicles)