

Molecules, Cells and Processes

Part II



YEAR 1
2020, SPRING TERM

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Lecture 1: Introduction

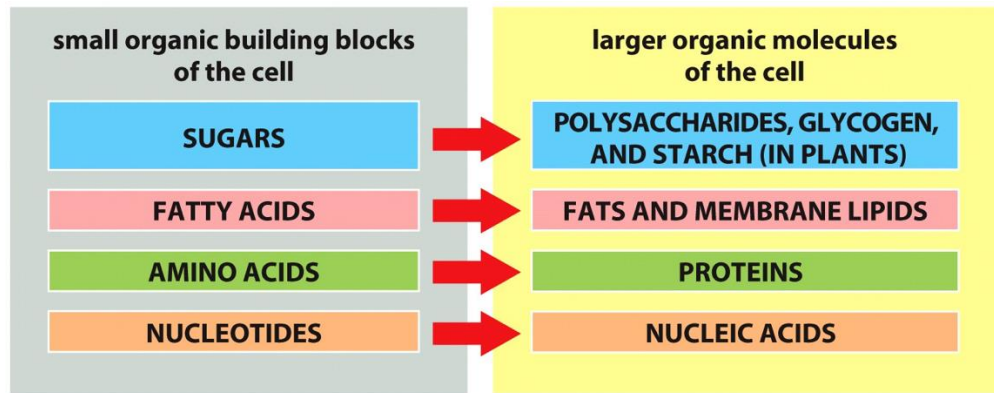


Figure 2-16 Essential Cell Biology, 4th ed. (© Garland Science 2014)

1.1 Sugar

- Function:
 - ⇒ Sugar is the primary source of energy for the cells - glycogen in animals, starch in plants.
 - ⇒ Sugar provides structural support - cellulose in cell walls of plant cells, chitin exoskeleton in insects.

- The simplest sugar is monosaccharides, have a general formula of $(CH_2O)_n$, $n=3\sim6$
- Sugars made from these monomers are called carbohydrates

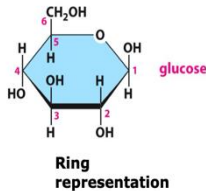
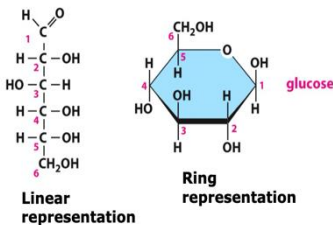
- For example: glucose - $C_6H_{12}O_6$

Three types of representations:

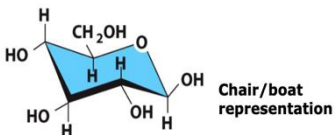
- ⇒ Linear: provides an approximation of how atoms are joined together
- ⇒ Ring: in water the molecule combines with a water molecule
- ⇒ Chair and boat: provide a real 3D shape

- Three types of most common sugar polymers made of glucose monomers:

- ⇒ Glycogen: stored in liver.
- ⇒ Cellulose: with a special arrangement in the form of beta sheets with abundant hydrogen bonds, which gives



glucose



cellulose an extraordinary strength. It is the structural component of the cell wall of plants.

⇒ Chitin: form the exoskeleton of insects and other arthropods (such as crustaceans -crabs, lobster, shrimps).

1.2 Lipid

- Function:

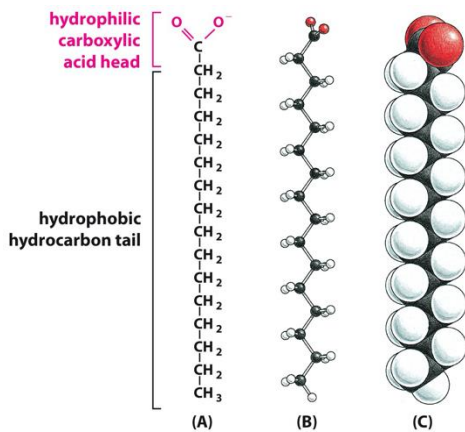
⇒ Lipid is the secondary source of energy in cells – triglycerides, triacylglycerol.

⇒ Lipid also provides structural support - the membrane that surrounds and protect cells and organelles are made of phospholipids and cholesterol.

1. Can be classified into two groups:

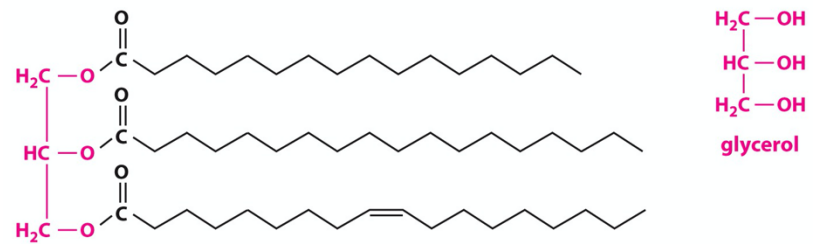
⇒ Triacylglycerols: storage of energy, for example: fatty acid

⇒ Phospholipid and cholesterol: fundamental components of membranes, for example: phospholipid



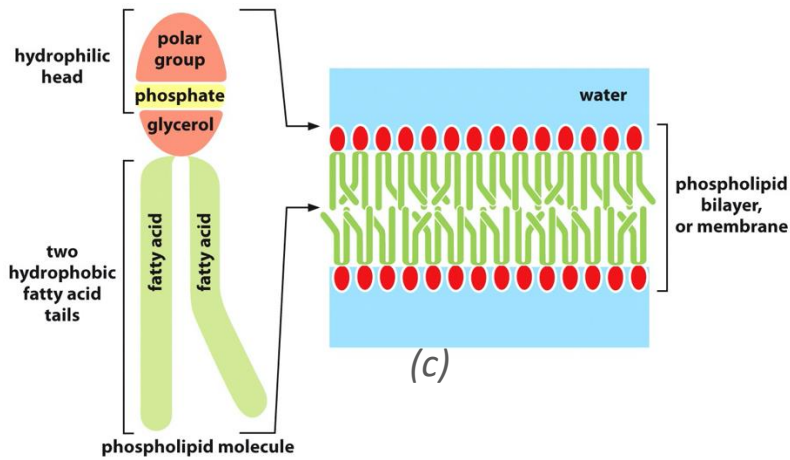
TRIACYLGLYCEROLS

Fatty acids are stored in cells as an energy reserve (fats and oils) through an ester linkage to glycerol to form triacylglycerols.



(a) Fatty acid - a hydrophilic head, a hydrophobic tail.

(b) Fatty acids are stored in cells as an energy reserve through an ester linkage to glycerol to form triacylglycerols.



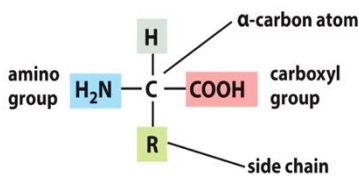
(c) Phospholipid – a hydrophilic head, a hydrophobic tail. The major constitution of cell membrane.

1.3 Protein

2. Function:

- ⇒ structure role(keratin)
- ⇒ enzymatic activity
- ⇒ transporters(hemoglobin)
- ⇒ hormones (insulin)

3. Proteins are polymers made of 20 different types of amino acids, linked by peptide bonds. an amino acid has the general formula: (see left)

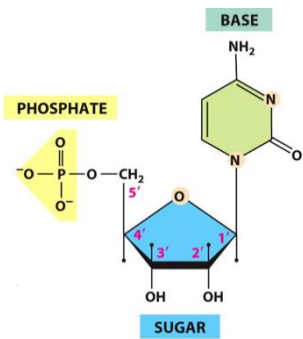


1.4 Nucleic acid

- DNA and RNA

• Made of nucleotides with the structure: sugar + nitrogenous base + phosphate:

- ⇒ The phosphates are normally joined to the C5 hydroxyl group of the sugar
- ⇒ The sugar in nucleotides is a ribose/deoxyribose (pentose)
- ⇒ Four types of base in DNA/RNA respectively:



Base	ABBR.	Type	Place
adenine	A	Purine	DNA, RNA
guanine	G	purine	DNA, RNA
cytosine	C	pyrimidine	DNA, RNA
thymine	T	pyrimidine	DNA

uracil	U	pyrimidine	RNA
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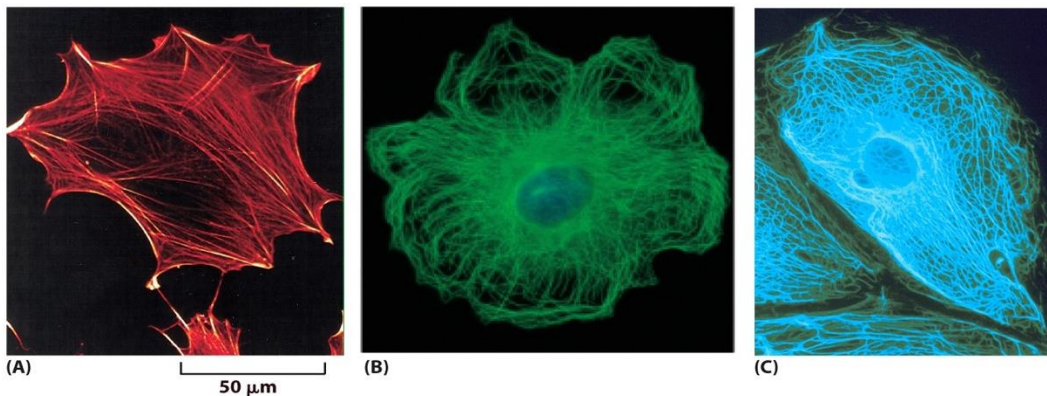
1.5 Scales

Light microscopes:

- allow us to magnify cells 1000 times. With the common normal light microscopes, the limit is 200 nm or 0.2 micro which means that we cannot see molecules and many organelles.
- The samples are needed to be conveniently prepared (stained with specific fluorophores)

Fluorescence microscopy:

- needs more sample elaboration as you label the cells with specific fluorescent dyes but the capabilities for differentiating different cellular structures are greater.
- Example: cytoskeletons - action filaments, microtubules, intermediate filaments



Lecture 2: Cell Organelles

2.1 Organisms can be classified into 3 groups: bacteria, archaea, eukaryotic, based on evolutionary level:

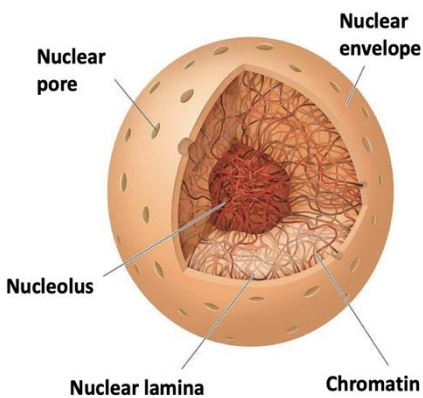
- Bacteria are prokaryotic unicellular cells:
 - ⇒ Does not have nucleus: DNA is dispersed in cytoplasm
 - ⇒ Ribosomes are used to synthesis protein
 - ⇒ Have both cell membrane and cell walls

- ⇒ Some of them have flagellum or pilus for locomotion
- Animals and plants are eukaryotic multicellular organisms.
However, there are some differences:
- ⇒ Plants have cell wall for protection
- ⇒ Plants have chloroplasts to capture energy from sunlight
- ⇒ Plants have vacuole to store liquids

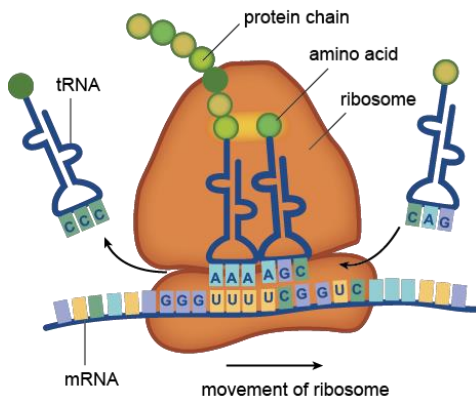
Some organelles in eukaryotic cells:

2.2 Nucleus

- Function: storage the genetic information, place for transcription - known as the regulator of all functions in cells.
- Structure:
 - ⇒ The nucleus is surrounded by the nuclear envelope that contains nuclear pores to facilitate the transport of molecules in and out of the nucleus, e.g. transcription factors (in), mRNA (out), rRNA(out).
 - ⇒ The nuclear lamina is a network of intermediate filaments which provides stability. Intermediate filament is a type of cytoskeleton.
 - ⇒ DNA are stored in chromosomes - only visible during cell division. There're specialized proteins inside the nucleus that organize the DNA before cell division, the complex of DNA and protein is chromatin.
 - ⇒ Chromosomes does not only fit the DNA inside the nucleus, allowing it to be properly distributed into two daughter cells, also allowing enzymes to come into it to replicate, repair and read it information

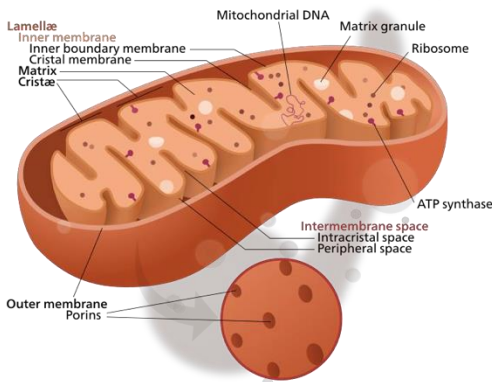


2.3 Ribosome



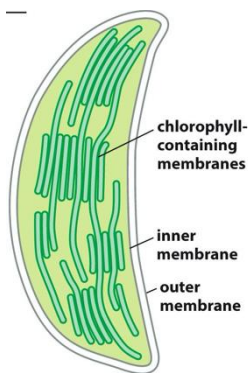
- Function: protein factory
- Structures:
 - ⇒ small unit(lower) and large unit(upper)
 - ⇒ Small subunit binds to mRNA, responsible for the base pairing between codons and anticodons
 - ⇒ Large subunit is responsible for the formation of peptide bonds.

2.4 Mitochondrion



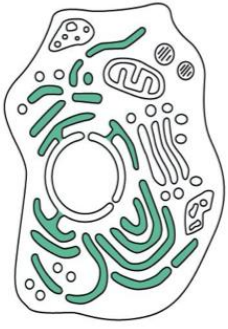
- Function: generate ATP, used as a source of chemical energy
- Structure:
 - ⇒ Double membrane: outer/inner
 - ⇒ Have intermembrane space
 - ⇒ Have matrix contains its own DNA - Krebs cycle
 - ⇒ Vary considerably in size, shape, number in different cells
 - ⇒ Not visible after staining

2.5 Chloroplasts



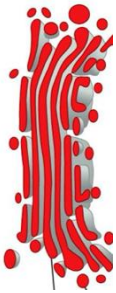
- Only in plant cells
- Function: place for photosynthesis. Produce macromolecules from small molecules from sunlight
- Plants are able to sense the loss of light by chemical receptors. They can register day-length changes and undergo physical and chemical changes, e.g. change of color, leave dehydration.

2.6 Endoplasmic reticulum



- Function: make cell-membrane components and materials for secretion. Also correct folding of all proteins
- Two types: rough (RER)/ smooth (SER)
 - ⇒ RER is caused by the attachment of ribosomes. Made of flattened sheets.
 - ⇒ SER is more made of tubular.
 - ⇒ Function of SER: modify, sort, package and transport proteins; form vesicles.

2.7 Golgi apparatus



- Function: modify and package the particles made in ER
 - ⇒ Protein synthesized in ER is packaged into vesicles and then fuse with Golgi apparatus.
 - ⇒ These proteins are modified and destined for secretion via exostosis or for use in the cell.
 - ⇒ Also involved in lipid transport and lysosome formation

2.8 The origin of mitochondria and chloroplasts in eukaryotic cells:

- Mitochondria most likely evolved from engulfed bacteria
- Chloroplasts almost certainly evolved from engulfed photosynthetic bacteria
- Thus, eukaryotic cells may have originated as predators

Lecture 3: DNA and Chromosomes

- Nucleic acids (DNA, RNA) are two-chain polymers made of subunits of nucleotides. Nucleotides are made of *sugar + nitrogenous base + phosphate*.

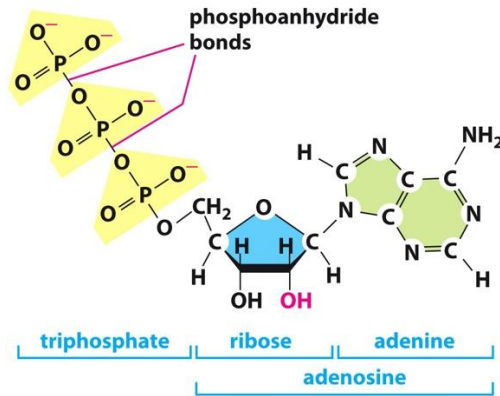
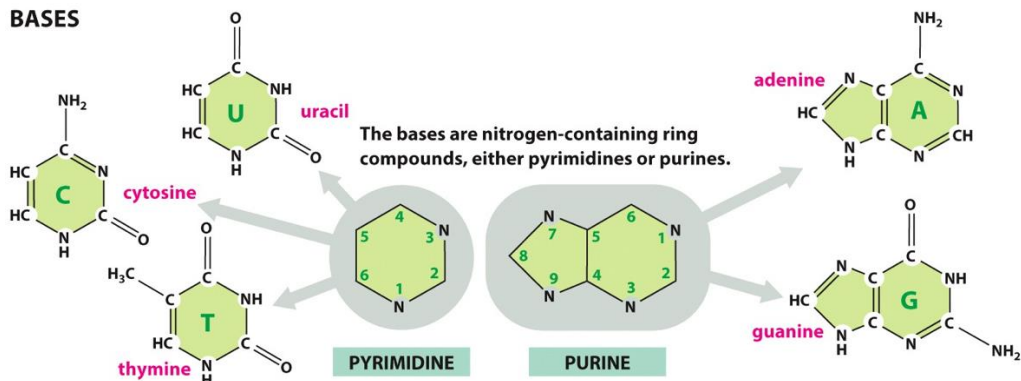


Figure 2-24a Essential Cell Biology, 4th ed. (© Garland Science 2014)

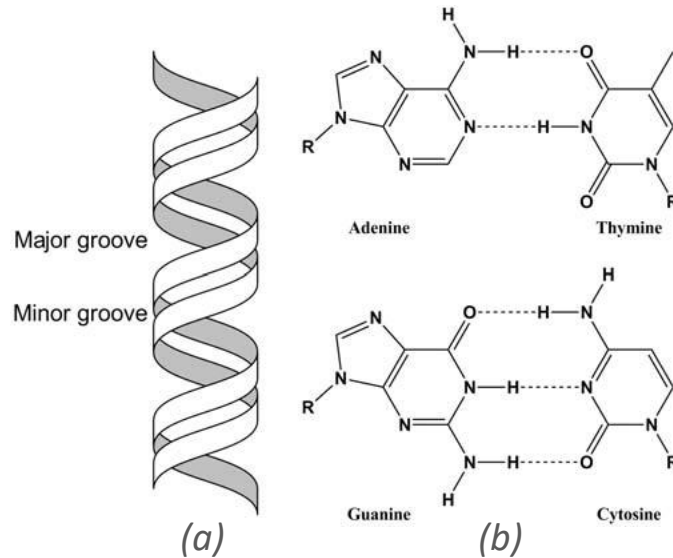
- Bases can be classified into two groups: *purine* and *pyrimidine*
- ⇒ Purine has double rings, pyrimidine have single ring



Panel 2-6a Essential Cell Biology, 4th ed. (© Garland Science 2014)

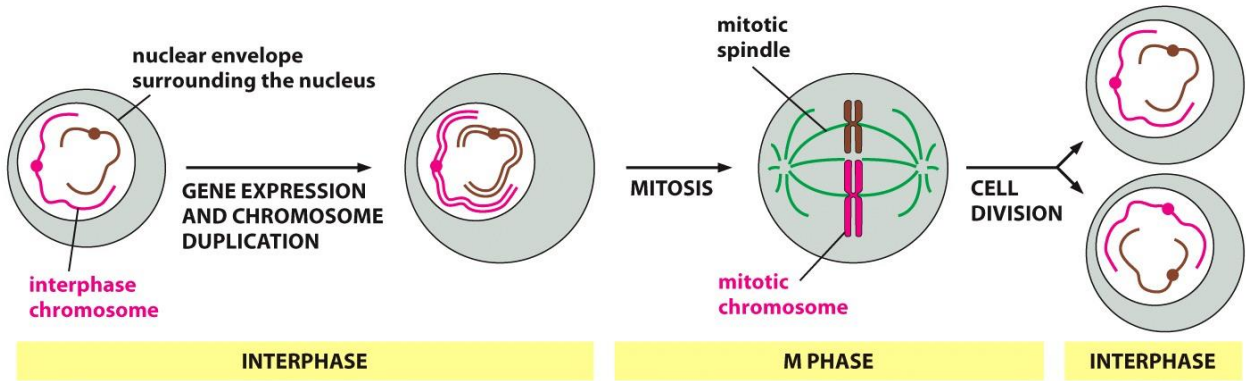
- ⇒ In DNA, four bases are guanine, adenine, cytosine, thymine
- ⇒ In RNA, four bases are guanine, adenine, cytosine, uracil
- ⇒ Bases are linked with sugars by covalent bonds.
- The backbone of DNA: all bases are on the inside of the molecule, the backbone of sugar and phosphates on the outside of the molecule.
 - ⇒ Reason: Nucleotides are joined together by phosphodiester bonds between 5' and 3' carbons via phosphate groups.
 - DNA has polarity, both ends are *asymmetrical* (5'-phosphate, 3'-hydroxyl)
 - ⇒ Reason: two chains run in antiparallel directions, oriented in different polarities.

- Two strands are linked together by base pairing with hydrogen bonds (see (b) below)
 - ⇒ Adenine (A) pairs with Thymine (H) with two H bonds
 - ⇒ Cytosine (C) pairs with Guanine (G) with three H bonds
- Two complementary strands are wound into a *double helix*, creating a major groove and a minor groove. (see (a) below)

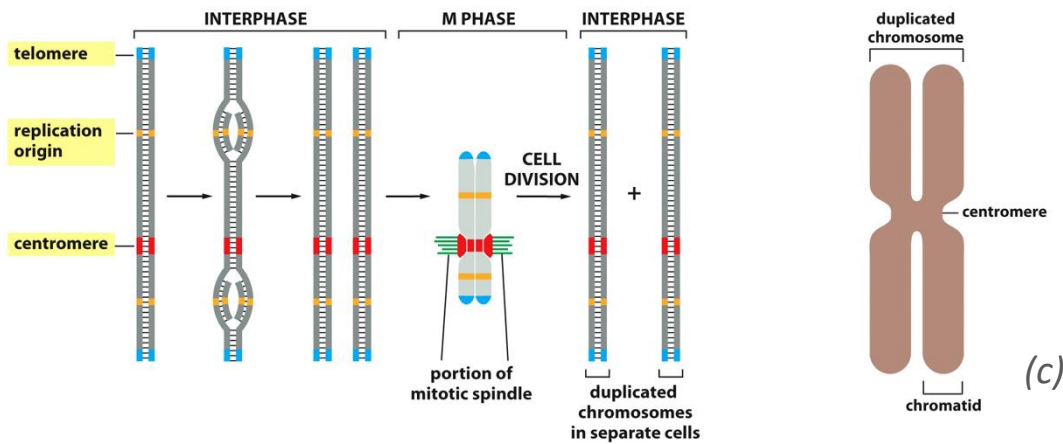


- The DNA molecules contains the information in gene (sequence of nucleotides) for the synthesis of proteins.
- *Chromosomes*: In the nucleus, DNA are packed into chromosomes, only visible during mitosis. Before mitosis, chromatin is the form in the nucleus.
- *Human karyotype* has a full set of sequence of human chromosomes. (*Exception*: germ cells, contains 2 copies of chromosomes, known as homologous chromosome)
- *Genome* is the total genetic information carried by all chromosomes in eukaryotic organisms.

- To form a functional chromosome, a DNA molecule must be able to duplicate and segregate equally and reliably into the 2 daughter cells.



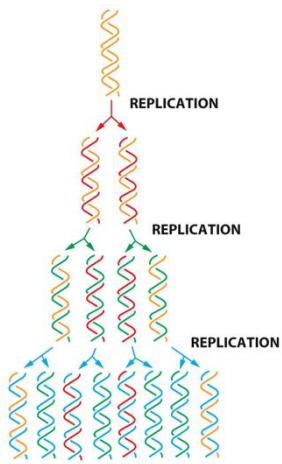
- DNA during mitosis:



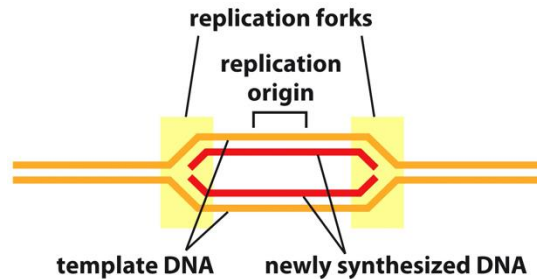
- ⇒ During the interphase, the DNA starts duplicating at the *replication origins* (a specific nucleotide sequence).
- ⇒ The *telomeres* (protect DNA from degradation) forms special caps at the ends of the chromosomes help in the replication of chromosomes ends.
- ⇒ During the M phase, the chromosomes attach to the mitotic spindle, and *centromeres* (separate the chromosomes) allows duplicated chromosomes to be separated.

*In a mitotic chromosome, each duplicated DNA molecule is called chromatid. (see (c) above)

Lecture 4: DNA replication

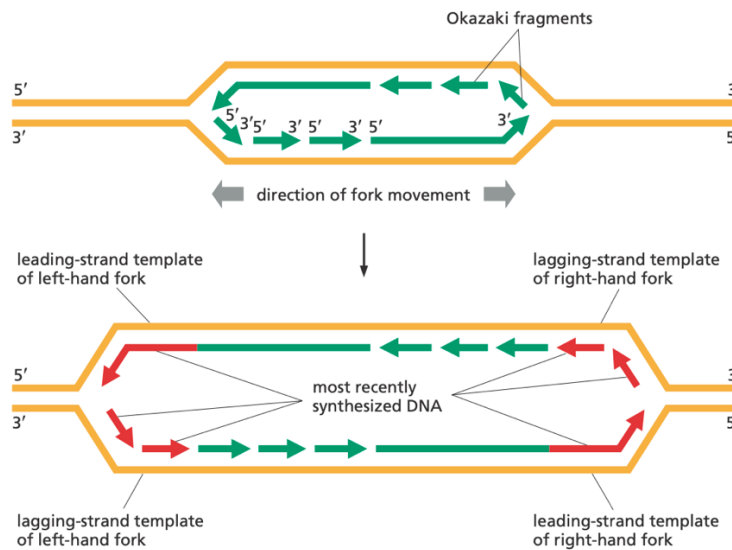


- DNA is replicated during S (synthesis) phase before mitosis.
- The replication process is *semiconservative*: each parental strand serves as a template for one new strand, end up with one DNA is consists of one original strand and one new strand.
- DNA replication starts at replication origins:
 - ⇒ The initiation protein (helicase) splits the two strands by breaking H bonds (A-T pairs typically) between bases to form two *replication forks*.
 - ⇒ The replication forks move rapidly in opposite directions (bidirectional) from the replication origins.

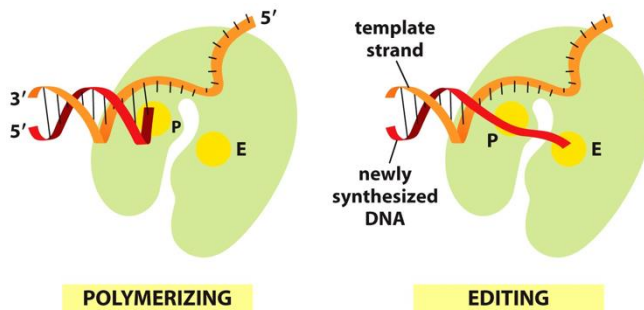


- *DNA polymerase* catalyzes the formation of phosphodiester bond. The energy for polymerization comes from ATP hydrolysis.
- The replication fork is *asymmetrical*
 - ⇒ Reason:
 - DNA has polarity
 - Two strands run in antiparallel
 - ⇒ Result: DNA polymerase runs in one direction in leading strand (S' strand). In the lagging strand (S strand), the DNA polymerase moves backwards with regards to the replication fork to form discontinuous

DNA pieces, known as Okazaki fragments. Okazaki fragments later join together by enzyme ligase to form a continuous strand.

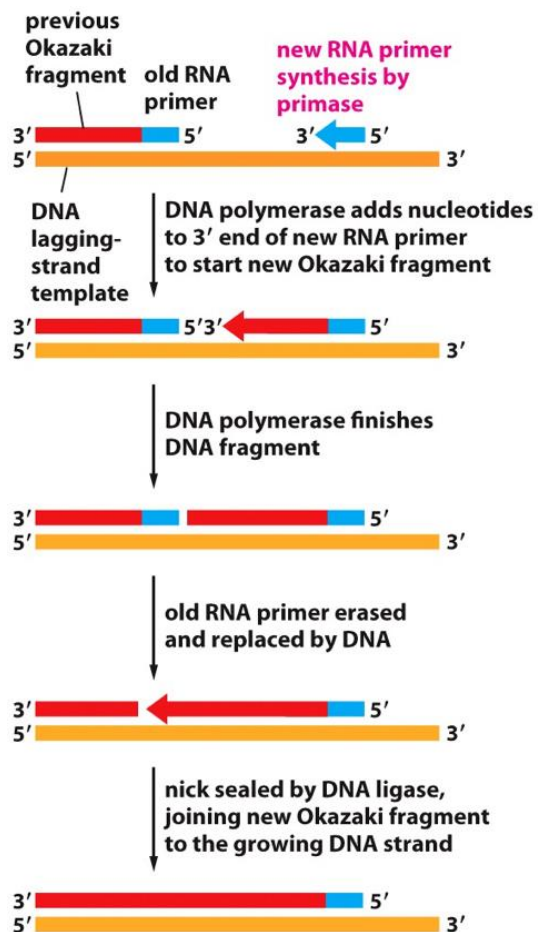


- DNA polymerase is self-correcting (proofreading).
 ⇒ If an incorrect nucleotide is added to a growing strand, the DNA polymerase cleaves it from the strand and replaces it with the correct nucleotide before continuing.



E is the catalytic site to remove the incorrect nucleotide

- Replication starts with an RNA primer:
 ⇒ Primase is an RNA polymerase that synthesizes RNA primers.
 ⇒ In leading strand, one primer is needed; in lagging strand, several primers are needed.
- The Okazaki fragments join together by removing several RNA primers AND replacing with DNA

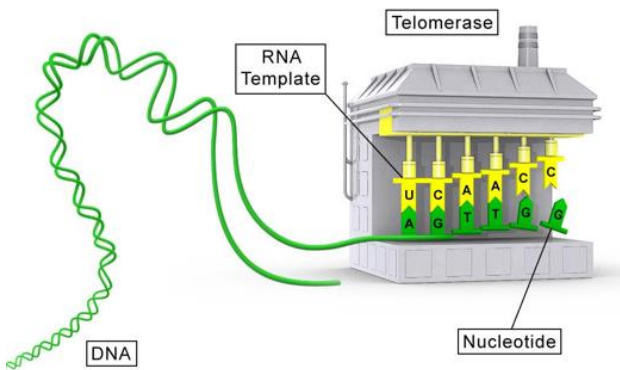
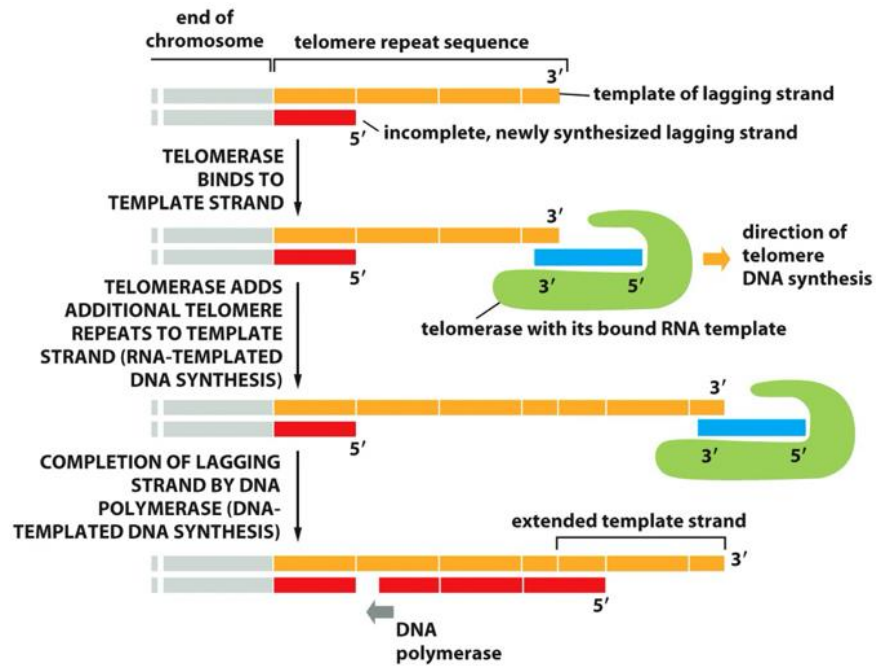
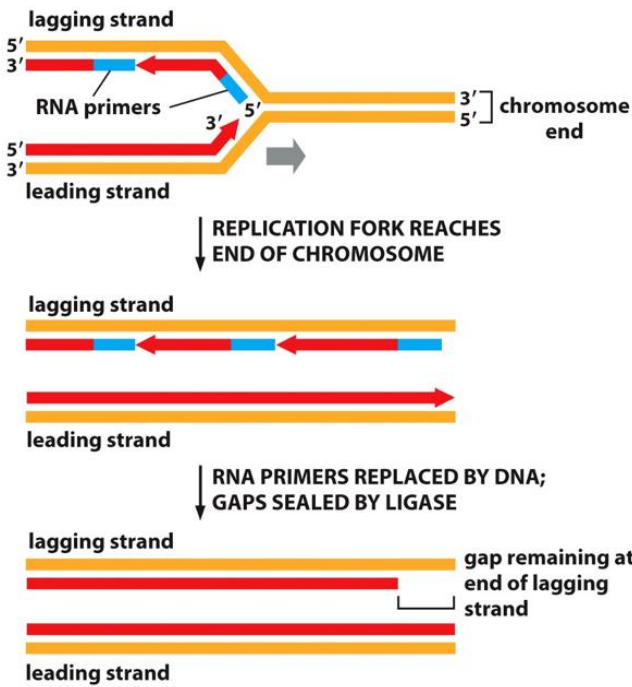


Nuclease: Degrades the RNA primers.

Ligase: Joins the 5'-phosphate end with the adjacent 3'- hydroxyl end using ATP

- DNA replication **creates tension** in strands
 - ⇒ Reason: the helicase unzipped the helices from the replication forks. The DNA twists excessively then generates torsional stress. As the chromosomes are too large to rotate fast, tension is built up.
 - ⇒ Strategy: DNA topoisomerase will relieve tension by producing transient nicks in DNA backbone temporarily and reseal the nicks afterwards.
- DNA replication in the end stage:
 - ⇒ By removing the final RNA primer in lagging strand, no DNA would replace the RNA, so the new DNA sequence would be shorter than its parental strand.

⇒ Strategy: for eukaryotic cell, *telomerase* replicates the ends of chromosome repeatedly to extend the sequence in template.

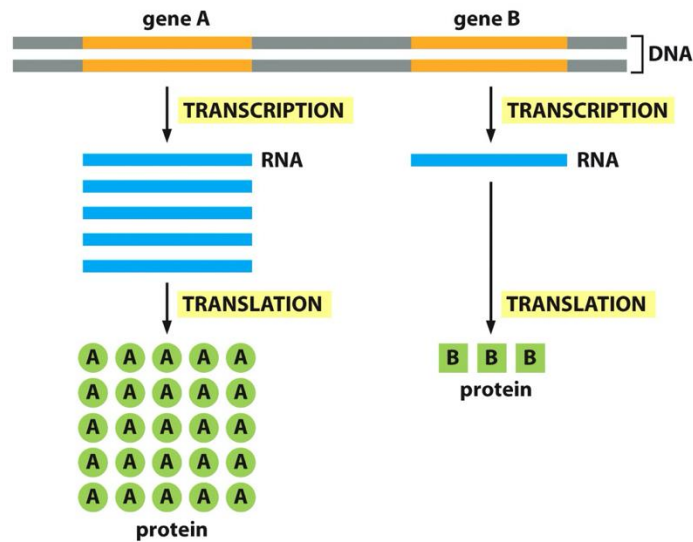


Telomerase adds DNA using its own RNA as template.

⇒ The strategy for bacterial cell is they using a circular DNA.

Lecture 5: DNA transcription

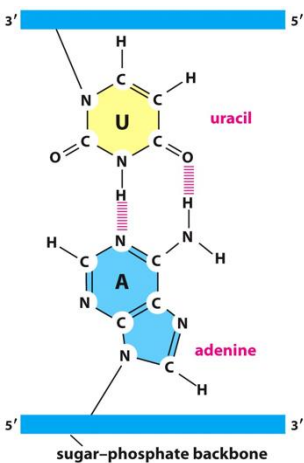
- Protein synthesis is gene expression by two steps - transcription and translation. The first step is transcription - it copies the information in DNA to mRNA.



- Different copies of a gene may transcribe different mRNA molecules, rapidly synthesis large amount of proteins.

5.1 RNA structure

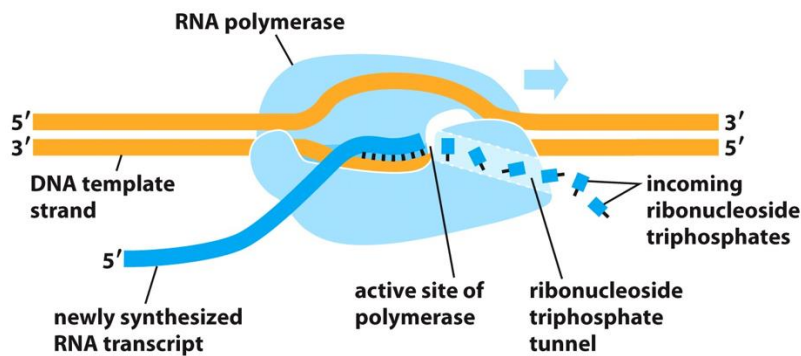
- ribose + nitrogenous base + phosphate
- Made of 4 types of nucleotide subunits, linked together by phosphodiester bonds
- For bases: Uracil replaces thymine, pair with adenine with 2 H bonds; guanine pairs with cytosine.
- Single-stranded
- Can be folded into many shapes - allows it be have more versatile roles than DNA - information conveyor, structural, catalytic, regulatory



Several types of RNA

Type of RNA	Function
messenger RNAs (mRNAs)	code for proteins
ribosomal RNAs (rRNAs)	form the core of the ribosome's structure and catalyze protein synthesis
microRNAs (miRNAs)	regulate gene expression
transfer RNAs (tRNAs)	serve as adaptors between mRNA and amino acids during protein synthesis
other noncoding RNAs	used in RNA splicing, gene regulation, telomere maintenance, and many other processes

- Transcription produces a mRNA complementary to its DNA template.
- *RNA polymerase* is needed. It moves from 3'- to -5' along the template.



There are similarities/differences between DNA polymerase and RNA polymerase:

Similarities

- ⇒ Catalyzes the formation of the phosphodiester bonds
- ⇒ Add nucleotides to the 3'- hydroxyl end of the chain
- ⇒ Can only move in the 3'- to -5' direction of the template

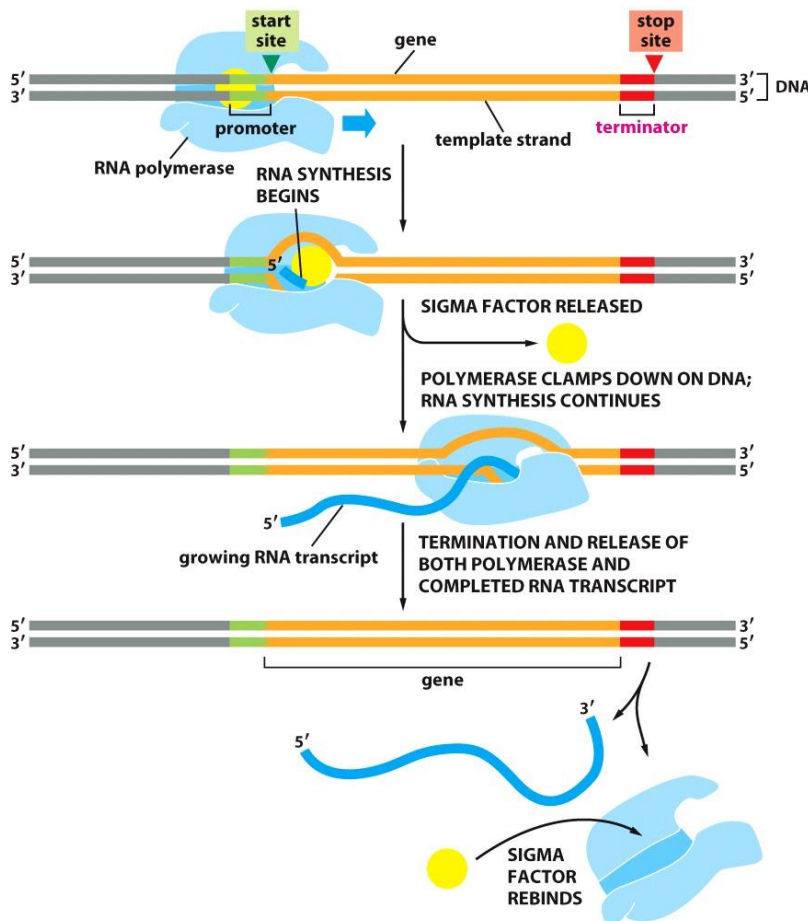
Differences

- ⇒ Can unwind the DNA, no need of helicases
- ⇒ Can start transcription without a primer
- ⇒ Does not promote the formation of H bonds between bases, the newly forming RNA molecule does not remain attached to the DNA template

- ⇒ Transcription does not need to be as precise as DNA replication, RNA is not passed from generation to generation and errors have less impact.
- ⇒ RNA does not have proof reading function
- ⇒ RNA polymerase: 1 error in 10^4 - DNA polymerase: 1 error in 10^7

5.2 The transcription in bacteria

- the DNA in bacteria has two specific sequences to initiate (promoter) and terminate (terminator) transcription.



⇒ RNA polymerase sticks weakly to DNA when approaching

⇒ When encounter the promotor, the *sigma factor* detaches from the RNA polymerase. RNA polymerase binds firmly to DNA, unzip the double helix, one strand serves as template

⇒ RNA polymerase adds nucleotides to the chain until finds the stop signal

⇒ RNA polymerase detaches from the DNA, rebinds the sigma factor.

Which DNA strand the polymerase use for transcription?

The promotor has polarity itself. It has two nucleotide sequence that positions the RNA polymerase in one direction. RNA polymerase can only add from 5' to 3', so it must be placed in 3' to 5' in template.

5.3 The transcription in eukaryotic nucleus

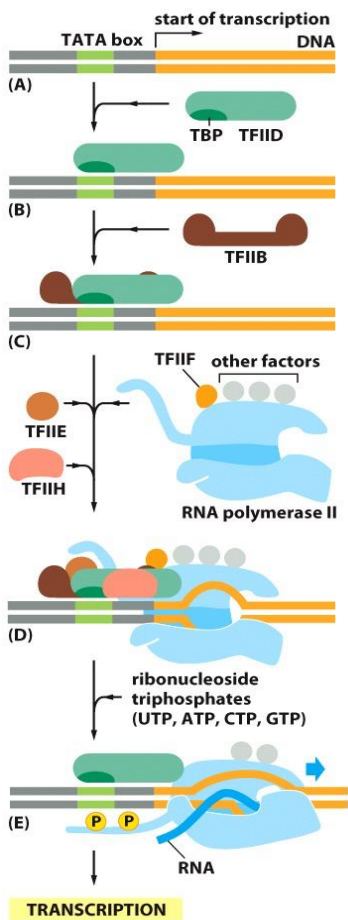
- Many principles are the same with bacteria transcription. But have some differences:

⇒ Three types of RNA polymerase: (only one type involves in bacteria transcription)

Type of Polymerase	Genes Transcribed
RNA polymerase I	most rRNA genes
RNA polymerase II	all protein-coding genes, miRNA genes, plus genes for other noncoding RNAs (e.g., those in spliceosomes)
RNA polymerase III	tRNA genes 5S rRNA gene genes for many other small RNAs

⇒ Requires general transcription factors: a group of proteins assembles on promotor, positioning the RNA polymerase. Similar to the sigma factor in bacteria transcription.

⇒ Eukaryotic transcription happens in nucleus, bacteria's in cytoplasm.



The process:

- *TBP* (TATA box binding protein, a subunit of *TFIID*) from *TFIID* (a type of transcription factor) binds to *TATA box* (a short sequence in DNA made of A and T);
- other factors (*TFIIB*, *TFIIE*, *TFIIH*) and RNA polymerase bind to the promotor;
- RNA polymerase is released from the complex to begin the synthesis of mRNA;
- Another transcription factor (*TFIIH*) introduces a phosphate group to the tail of the RNA polymerase;
- Once finished, the transcription the phosphate group is stripped off the molecule by phosphatases and ready to bind a new promoter.

5.4 Three steps involved in RNA processing in eukaryotic cells

- RNA capping at 5'-end

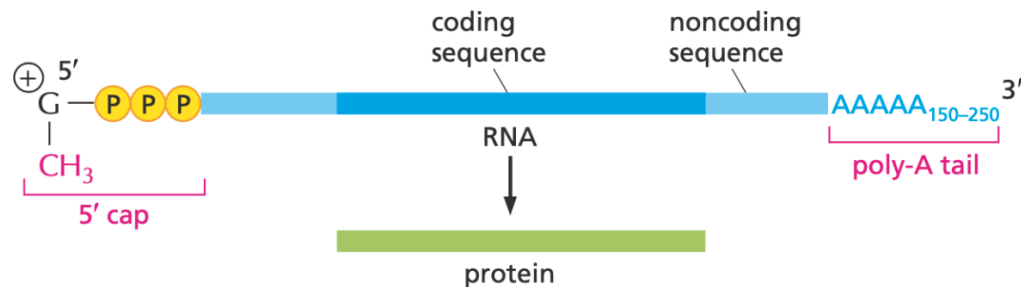
⇒ Addition of a methylated guanine at 5' region

⇒ Occurs after the RNA polymerase has produced around 25 nucleotides of RNA

- RNA polyadenylation at 3'-end

⇒ Trim the 3'- end that cuts the chain with a particular sequence

⇒ Adds a series of repeated adenine (*poly-AAA tail*) nucleotides to the 3'- end.



- RNA splicing

⇒ Most eukaryotic cells

⇒ Introns (non-coding regions) are removed from pre-mRNA.

There are specific sequences at the beginning and ending of the introns which splicing machinery can identify them. Leave exons.

5.5 Transport of mature mRNA

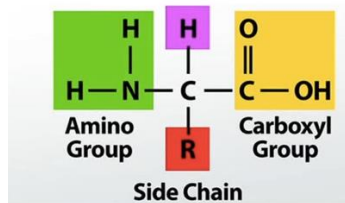
⇒ From nucleus to the cytoplasm

⇒ Driven by nuclear pore complexes (NCPs). NCPs act as gates control macromolecules enter/leave nucleus.

⇒ mRNA must bind to a specific set of protein. These proteins include poly-adenine binding proteins, a cap-binding protein, and proteins that bind the mRNAs that have been appropriately spliced.

Lecture 6: Gene translation

- Proteins are macromolecules made of small subunits - *amino acid*, by *translation*.

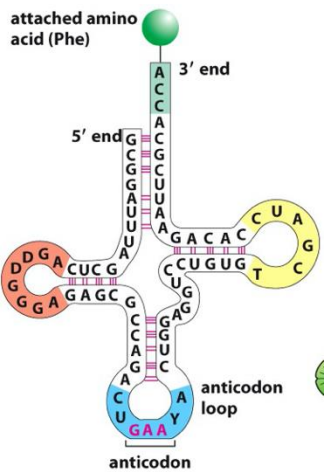


- Totally 20 amino acids that can form proteins
- The *genetic code* provides information that how the genetic information from RNA that can be transferred into proteins. They use *codon*, which is a sequence of three consecutive nucleotides that codify a specific amino acid.
- The genetic code is *redundant*: $4 \times 4 \times 4 = 64$ combinations of the genetic code, for 20 amino acids.

		Second letter				
		U	C	A	G	
First letter	U	UUU } Phe UUC } UUA } Leu UUG }	UCU } UCC } Ser UCA } UCG }	UAU } Tyr UAC } UAA Stop UAG Stop	UGU } Cys UGC } UGA Trp UGG Trp	U C A G
	C	CUU } CUC } Leu CUA } CUG }	CCU } CCC } Pro CCA } CCG }	CAU } His CAC } CAA } Gln CAG }	CGU } CGC } Arg CGA } CGG }	U C A G
	A	AUU } Ile AUC } AUA Met AUG }	ACU } ACC } Thr ACA } ACG }	AAU } Asn AAC } AAA } Lys AAG }	AGU } Ser AGC } AGA Stop AGG Stop	U C A G
	G	GUU } GUC } Val GUA } GUG }	GCU } GCC } Ala GCA } GCG }	GAU } Asp GAC } GAA } Glu GAG }	GGU } GGC } Gly GGA } GGG }	U C A G
						Third letter

Why addition and deletion of nucleotides are more dangerous mutations than the replacement of one nucleotide by another?

An mRNA sequence can be translated in any one of the three different possible reading frames but only one of the three reading frames specifies the correct protein.



- mRNA cannot directly recognize the amino acids. tRNA carries anticodons (the sequence of three consecutive nucleotides that is complementary to the codon) and corresponding amino acids.
- ⇒ Anticodons pair with codons
- ⇒ After pairing, the amino acid is added to the peptide chain

How does tRNA identify the one amino acid in 20 that is the right partner?

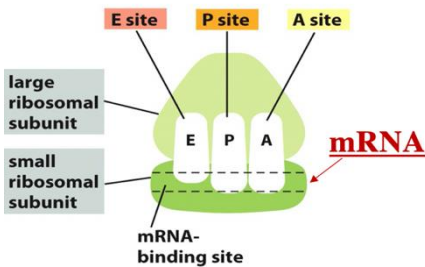
An enzyme called aminoacyl-tRNA-synthetase covalently links each amino acid to its appropriate set of tRNA molecules.

- The translation takes place at *ribosomes* in cytoplasm

⇒ composed of small proteins and rRNA molecules. A cell has millions of ribosomes in cytoplasm, can be free or bound to the ER.

⇒ two subunits - small subunit and large subunit. Small subunit sits at bottom, large subunit sits at top.

⇒ A ribosome has three binding sites: A, P, E

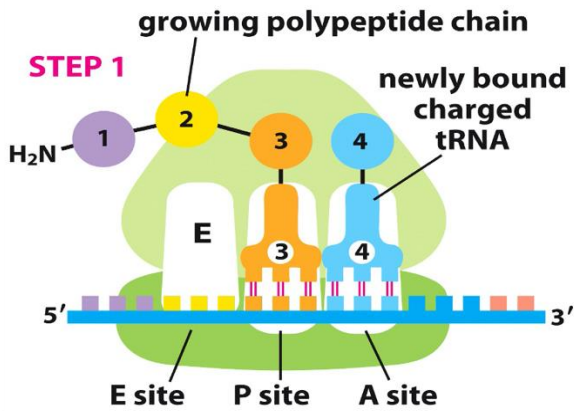


- *A site*: the first binding site and the point of entry for the aminoacyl tRNA molecules to the ribosome.
- *P site*: the place that the peptidyl tRNA is bound to the ribosome.
- *E site*: the exit site of the tRNA after it gives its amino acid (uncharged tRNA) to the peptide chain.

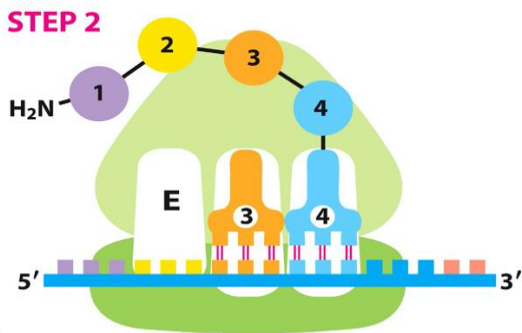
- The translation process:

⇒ At translation, ribosomes move along the mRNA from 5'-to-3'

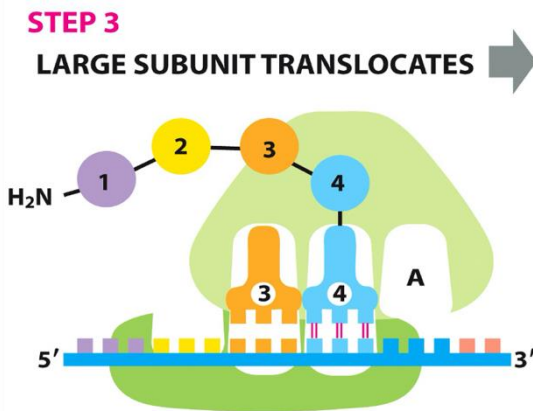
- ⇒ The small subunit matches the tRNA to the codons in the mRNA, the large subunit catalyzes the formation of the peptide bond that covalently link the amino acids.
- ⇒ Four steps for protein translation:



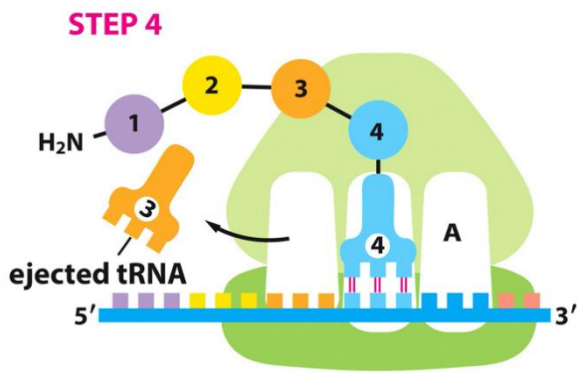
tRNA enters the A site by base pairing with the complementary codon on the mRNA molecule



The amino acid in the A site linked to the amino acid in the P site.
The carboxyl end of the polypeptide chain is uncoupled from the tRNA in the P site and joined by a peptide bond to the free amino acid linked to the tRNA in the A site. This reaction is catalyzed by an enzymatic site in the large subunit.



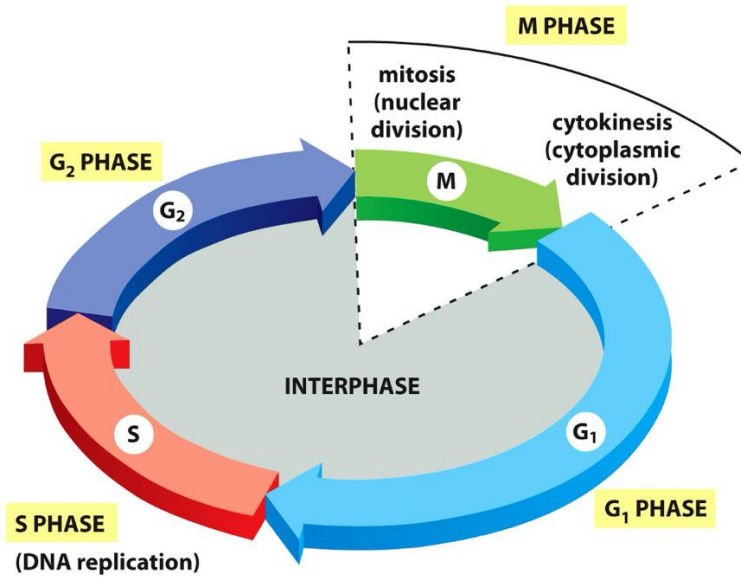
The large subunit shifts forward, moving the spent tRNA to the E site before ejecting it.



The small subunit moves to its original position relative to the large subunit

The tRNA in the E site is ejected, A site is available to start the process again

Lecture 7: Mitosis



- M phase: mitosis (nuclear division) + cytokinesis (cytoplasmic division), usually takes up 1h in mammalian cells
- Interphase: G₁ - S - G₂
 - ⇒ G₁ (gap) and G₂ is the phases for cell growth, protein synthesis and gene transcription
 - ⇒ G₁ and G₂ phases also contains particular checkpoints for cells to decide whether to proceed to the next phase or pause to wait
 - ⇒ S (synthesis) is the phase when the cell replicates DNA

7.1 The cell-cycle control system

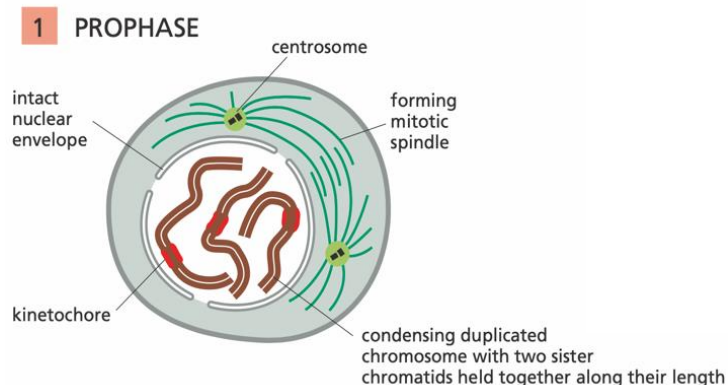
- The *cell-cycle control system* ensures all DNA and organelles are divided in an orderly manner.
- The control system is regulated itself at certain critical points of the cycle by feedback from the process currently being performed
- The system achieve the task by employing molecular brakes (*checkpoints*) to pause the cycle at certain transition points.

Phase	Checkpoints
G ₁	sufficient nutrients and extracellular signal molecules
G ₂	the DNA is undamaged and fully replicated
Mitosis	duplicated chromosomes are properly attached to mitotic spindle

- G1 to S is particular important. Extracellular signals would decide whether the cell proliferation is needed

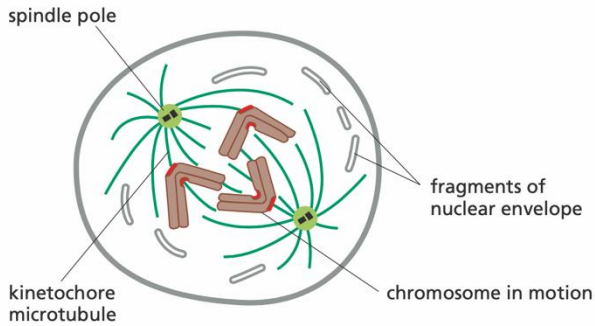
7.2 The process of mitosis

- Mitosis is consisted of five phases:



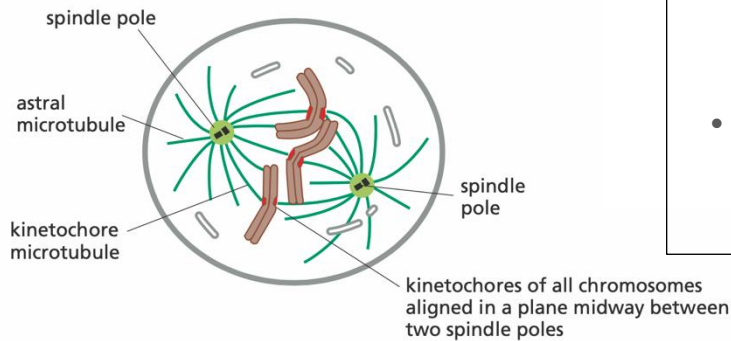
- All chromosomes condense
- The mitotic spindle assembles after the reassembling of microtubules

2 PROMETAPHASE



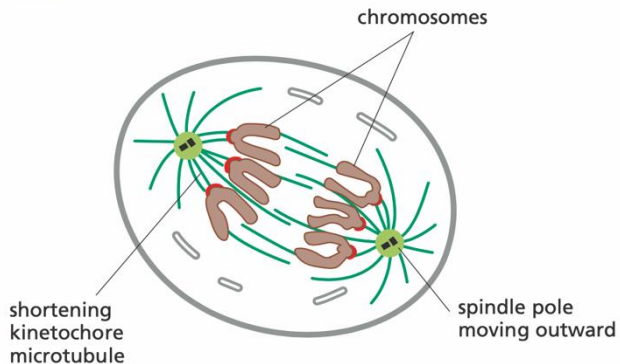
- Nuclear envelopes disappear (lamins get phosphorylated)
- Chromosomes attach to the mitotic spindle

3 METAPHASE



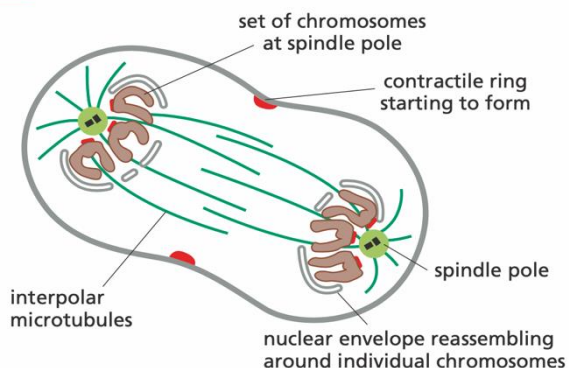
- All chromosomes are aligned at the equator of the mitotic spindle
- Kinetochores attach chromosomes to the mitotic spindle

4 ANAPHASE



- The duplicated chromosomes separate and migrate toward two opposite poles of the cell

5 TELOPHASE

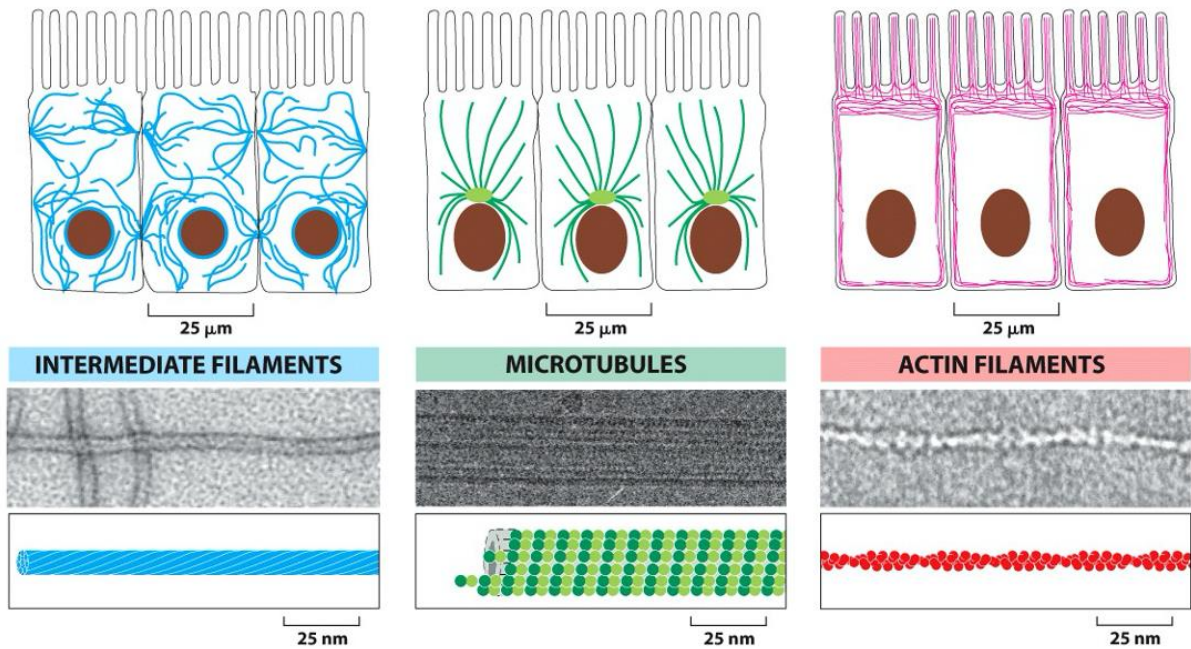


- The chromosomes arrive at the poles
- The nuclear envelope reassembles (lamins get dephosphorylated)

In cytokinesis,

- ⇒ The contractile ring of actin and myosin is formed which divides the cytoplasm
- ⇒ The overlapping interpolar microtubules that form the central spindle recruit and activate proteins that signal the cell cortex to assemble the contractile ring in a position midway between the spindle poles

Lecture 8: Cytoskeleton



Intermediate filaments are ropelike fibers with a diameter of about 10 nm; they are made of fibrous intermediate filament proteins. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength and distributing the mechanical stresses in an epithelial tissue by spanning the cytoplasm from one cell-cell junction to another. Intermediate filaments are very flexible and have great tensile strength. They deform under stress but do not rupture. (Micrograph courtesy of Roy Quinlan.)

Microtubules are hollow cylinders made of the protein tubulin. They are long and straight and typically have one end attached to a single microtubule-organizing center called a *centrosome*. With an outer diameter of 25 nm, microtubules are more rigid than actin filaments or intermediate filaments, and they rupture when stretched. (Micrograph courtesy of Richard Wade.)

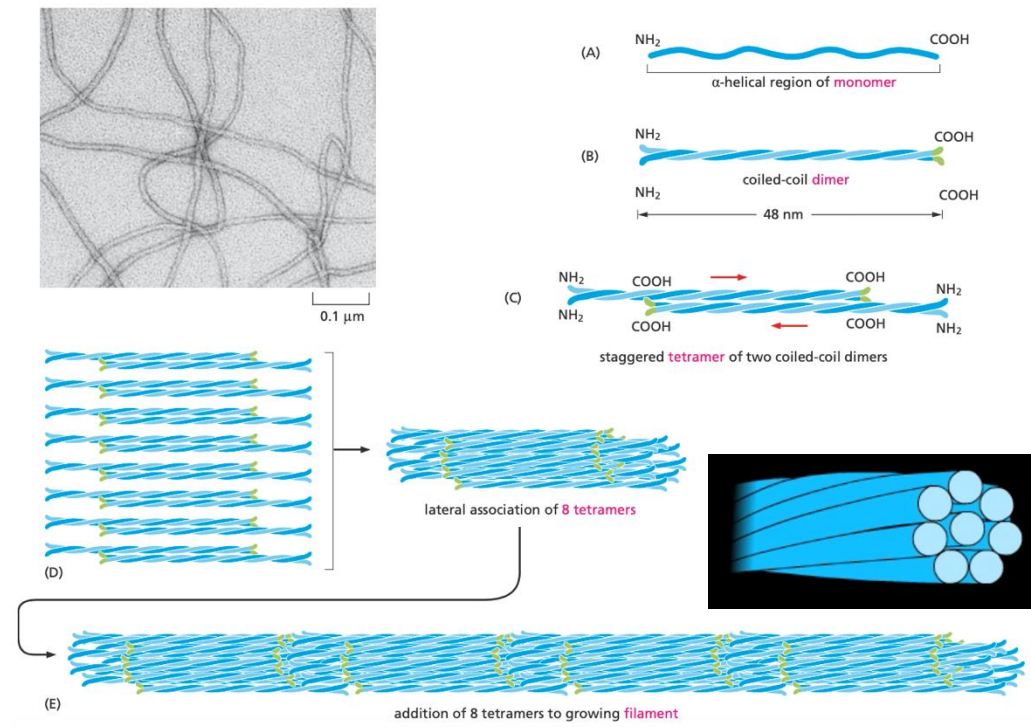
Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures, with a diameter of about 7 nm, that are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, the layer of cytoplasm just beneath the plasma membrane. (Micrograph courtesy of Roger Craig.)

- The ability for the cells to form different shapes, organize the many varieties of components in their interior, interact mechanically with the environment and to perform coordinated movements depends on the cytoskeleton
- The cytoskeleton is made of three types of protein filaments:
 - ⇒ Intermediate filaments: provide mechanical strength
 - ⇒ Microtubules: organize the cytoplasm of eukaryotic cells
 - ⇒ Actin filaments: support the cell surface and allow fibroblasts and other cells to move or migrate
- They support the large volume of the cytoplasm a function which is very important in animal cells given that these cells do not have cell wall

8.1 Intermediate filaments

- Have great tensile stress which enable the cells to withstand the mechanical stress when the cells are stretched
- Can be found in cytoplasm of most of the animal cells
- Often anchored to the plasma membrane and cell-cell junctions called desmosomes
- The structures:
 - ⇒ Made of fibrous subunits of alpha helical proteins
 - ⇒ Two monomers wrap around each other to form a dimer
 - ⇒ Two dimers run in opposite directions to form a tetramer, which has no polarity and soluble

⇒ Tetramers associate together to give the final intermediate filaments

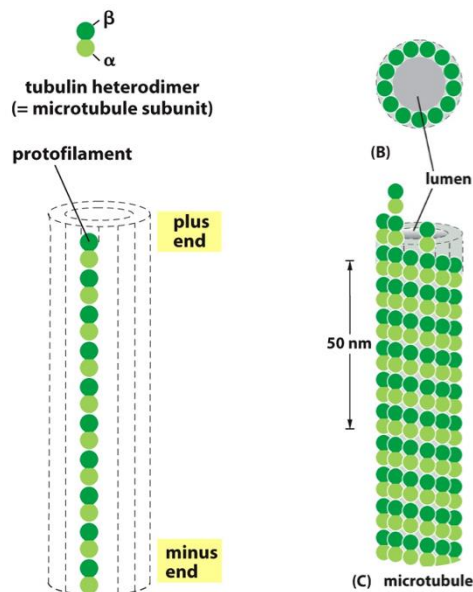


⇒ The overlapping structure allows the tetramers to form an extensive covalent bond network which gives the intermediate filaments great tensile strength

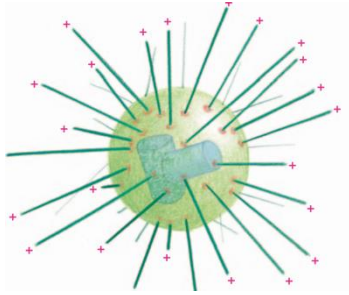
- Abundant in cells which need great tensile strength e.g. epithelial cells in skin, also present in nerve cells providing essential reinforcement to these long protrusive cells.
- Can be classified in four groups:
 - ⇒ Keratin filaments in epithelial cells - the most diverse class
 - ⇒ Vimentin filaments in connective - tissue cells, muscle cells, and supporting cells of the nervous system (glial cells)
 - ⇒ Neurofilaments in neurons
 - ⇒ Nuclear lamina that strengthen the nucleus of most animal cells

8.2 Microtubules

- Have fundamental organizing role in all eukaryotic cells, made of proteins, can rapidly disassemble from one location and assemble in another (unstable)
- Function: transport and position membrane-enclosed organelles within the cell for guiding the intracellular transport of macromolecules
- Typically, grow from centrosome extending out to cell periphery, creating tracks for transportations.
 - ⇒ When mitosis, the cytoplasmic microtubules disassemble and then reassemble into an intricate structure called the mitotic spindle.
 - ⇒ The mitotic spindle provides the machinery that will segregate the chromosomes equally into the two daughter cells just before cell division.
- Microtubules are built from subunits – molecules of tubulin - each of which is itself a dimer composed of two very similar globular proteins known as alpha tubulin and beta tubulin, bound together by non-covalent interactions.
- The tubulin dimers stack together again by non-covalent bonding to form the wall of the hollow cylindrical microtubule.

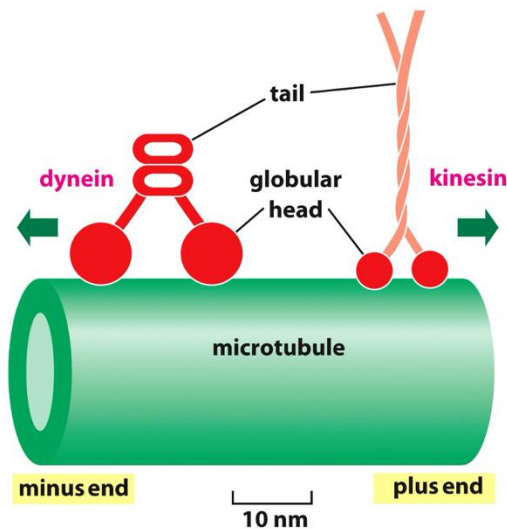


- The structure
 - ⇒ made of 13 parallel protofilaments, each a linear chain of tubulin dimers with alpha and beta tubulin alternating along its length.
 - ⇒ has polarity as an alpha tubulin unit exposed at one end and a beta tubulin unit at the other. If no polarity, cannot guide intracellular transport



- Microtubules radiates from centrosomes with the minus end embedded in centrosomes.

- Two motor proteins:



⇒ *Kinesin* and *dynein* are motor proteins that use the energy derived from the ATP hydrolysis to transport organelles, macromolecules or vesicles along the microtubules.

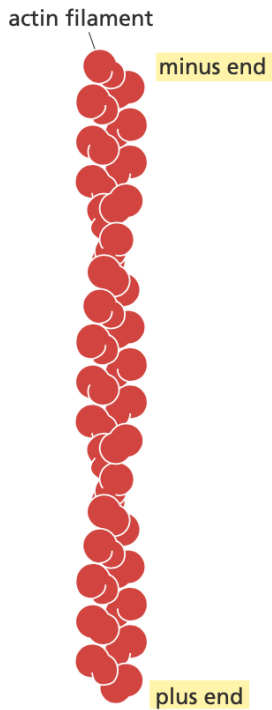
⇒ They attach to the microtubules using their globular head and the cargo protein is connected at the other end, which is known as the tail.

⇒ Kinesin moves toward the plus end of the cell (outward from the cell body).

⇒ Dynein moves toward the minus end (toward the cell interior).

8.3 Actin filaments

- Present in all eukaryotic cells which is important in cell movements, especially those involving the cell surface.
- Unstable, but can be stable binding with other proteins
- Made of *actin monomers*, twisted in a same direction - have polarity with a plus end and a minus end



- The hydrolysis of ATP to ADP reduces the strength of binding between the monomers, thereby decreasing the stability of the filament.

⇒ Thus, in the plus end, ATP binding to monomers promotes polymerization while at the minus end ADP binding to actin monomers promotes depolymerization.

- The actin monomer concentration is high in cytoplasm.

⇒ specific proteins (e.g. thymosin, profilin) bind to them preventing the polymerization to reserve them;

⇒ When actin monomers needed, other binding proteins (e.g. formins) will then promote the polymerisation

