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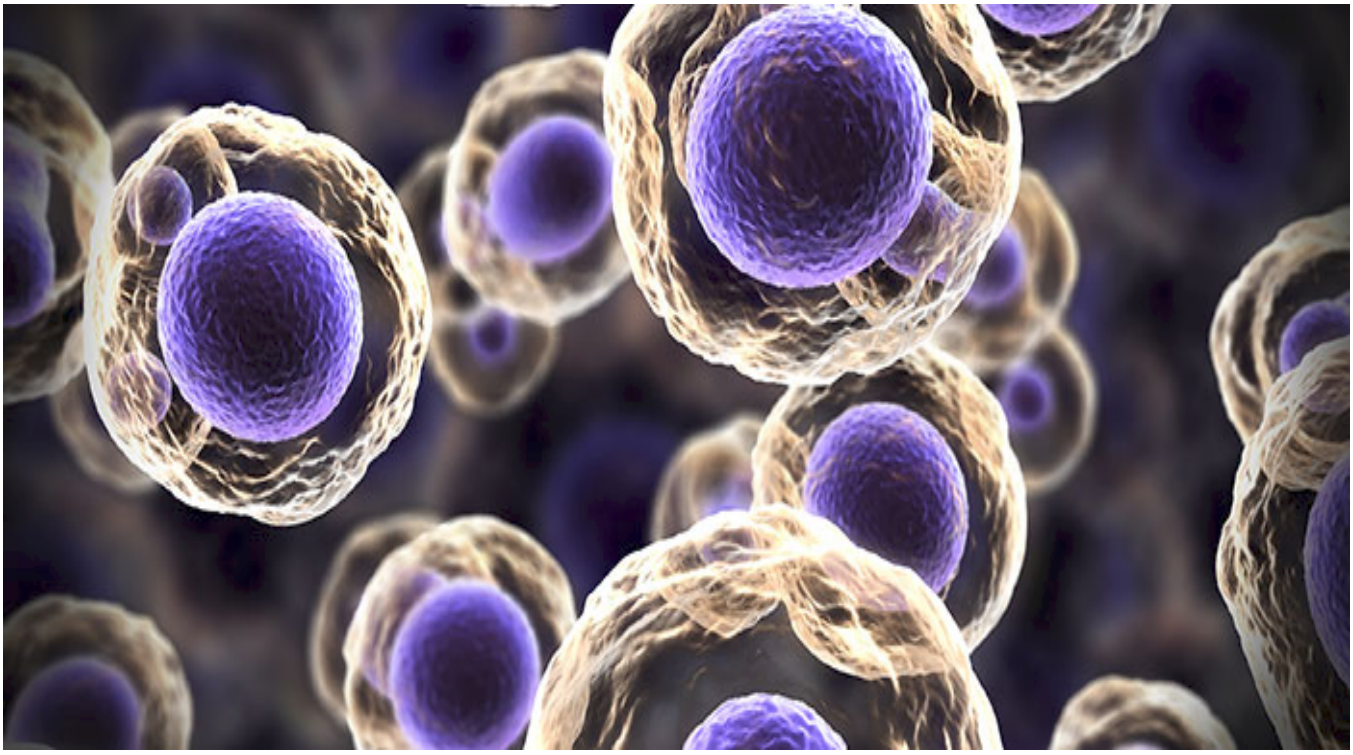
BIO 1140 FINAL CONDENSED NOTES



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Pre-Reading (1): Introduction to Cell Biology

Textbook: Chapter 2.1, 3.1, - 3.4

CHAPTER 2.1: Basic Features of Cell Structure and Function

Basic Structural and function units of living organisms contain organized systems of molecules like

A. DNA
B. RNA
C. Nucleic Acids

These molecules carry the hereditary information and control the manufacture of other molecules

- **Chemical molecule**s or **light** energy is used as energy sources

Cells are also able to change their internal environment in order to adapt to the external conditions.

Examples of **UNICELLULAR** organisms

- Almost all bacteria
- Archaea
- Protists like amoebas
- Fungi like yeast

Examples of **MULTICELLULAR** organisms

- Plants
- Animals

The Cell Theory: Cells are the basic units of all living organisms

CHAPTER 2.1 A: Cells are Small and Can Only Be Seen Under a Microscope

3 Divisions: Bacteria, Archaea and Eukarya (recently bacteria and Eukarya were grouped into the same domain Prokaryote) <- Disapproved recently since they are not evolutionarily related.

2 Common types of microscopes we use:

A. **Light microscopes** - using light to illuminate the specimen
B. **Electron microscopes** - use electrons to illuminate the specimen

Magnification: The ratio of the object as viewed to its real size
Resolution: minimum distance by which 2 points in the specimen can be separated and still be seen as 2 points. (Depends primarily on wavelengths)

QUESTION: Why are cells small?
ANSWER: Depends on the surface area - volume ratio. This relationship determines the amount of chemical activity that can take place within it.
Some cells increase ability to exchange materials with surroundings by flattening the surroundings. E.g. The intestinal cells in human have closely packed extensions to increase surface area which enhances the ability to absorb digested food.

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CHAPTER 2.1 B: Cells have a DNA- Containing Central Region That is Surrounded by Cytoplasm

All cells are bounded by plasma membrane by a **lipid bilayer.**
Lipid Bilayer:

- Hydrophobic barrier creating a passageway for water-soluble molecules
- Contain membrane bound proteins to transport non-water soluble molecules
- Selective movement of ions

Central region of the cells contain DNA and RNA (allows for the DNA to be copied into RNA)
Cytoskeleton: provides structure and support for cell shape and aids in cell division and chromosome segregation.

CHAPTER 2.1 C: Cells Occur in Prokaryotic and Eukaryotic Forms, Each with Distinctive Structures and Organization

2 Fundamentally different types of cells

A. Prokaryotes (lacks nucleus)
B. Eukaryotes (DNA is inside the nucleus)

CHAPTER 3.1 A: Seven Characteristics Shared by All Life-Forms

Definition of Life in 7 Characters:

1. Displays order
2. Harnesses and uses energy
3. Reproduces
4. Responds to stimuli
5. Exhibits homeostasis
6. Grows and Develops
7. Evolves

VIRUSES may seem biotic; however, they are abiotic because…

- Viruses lack cellular machinery and metabolism to synthesize their own proteins
- To create protein, they must infect living cells
- E.G. Bacteriophage

CHAPTER 3.1 B: The Characteristics of Life Are Emergent

7 characteristics of life stated before are called **emergent** because they come about from many simpler interactions that in themselves do not have the properties found in higher levels.
Example:
Ability to harness and use energy is not a property of molecules or proteins or biological membranes in isolation; rather the ability emerges from the interactions of all three of these part of a metabolic process.

In conclusion, the complexity of organisms and cells, comes from the sums of simpler units.

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CHAPTER 3.2 A: Earth is 4.6 Billions Years Old

CHAPTER 3.2 B: Earth lies within the Habitable Zone around the Sun

All components of the solar system were formed at the same time by the gravitational condensation of matter in an interstellar cloud which mainly consisted of hydrogen. The intense heat and pressure in this cloud formed the sun

The earth's size allowed for the gravitational force to be strong enough to hold its atmosphere around the planet.
Overtime earth radiated away some of its heat and the surface layer cooled down into a solid rock of crust. (500 million years to cool)
Existence of water was the reasoning for the origin of life to begin in planets.

CHAPTER 3.2 C: Biologically Important Molecules Can Be Synthesized Outside Living Cells

All forms of life are composed of major macro and micro molecules.
3 major hypotheses:

1. Reducing Atmosphere

The primordial atmosphere 4 billion years ago contained water vapour, hydrogen, carbon dioxide, ammonia and methane. COMPLETE LACK OF OXYGEN
Two scientists Oparin and Haldane independently proposed that organic molecules were essential to the formation of life (forming the primordial earth).
Their hypothesis stated that the large concentrations of the molecules stated above, contain lots of electrons and hydrogen and would have entered into reactions that would yield more complex organic molecules.
Lack of oxygen = no ozone layer
Because of this, the UV RAYs would be able to reach earth, and further provide abundance of energy for the organic molecules to react in millions of ways.

- Miller recreated this scenario (Miller-Urey experiment)
- Eventually important molecules were created such as purines and pyrimidines (nucleic acids)

2. Deep Sea Vents

Complex organic molecules could have originated from the ocean floor at the site of deep sea (hydrothermal) vents.
Located near volcanic or tectonic activities and release superheated nutrient-rich water as well as reduced molecules like methane ammonia and hydrogen sulfide.

3. Extraterrestrial Origins

Organic molecules came from outer space. The Murchison meteorite that landed in Australia in 1969 showed an assortment of biologically important molecules like amino acids including glycine, glutamic acid and alanine + purines and pyrimidines.

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CHAPTER 3.2 D: Life Requires the Synthesis of Polymers

Molecules like amino acids and nucleotides are **monomers**.
Nucleic acids and proteins are polymers - macromolecules formed from bonding together individual monomers
Many carbohydrates are polymers.
Polymers are synthesized through **dehydration synthesis**
The synthesis of proteins and nucleic acids require protein based catalyst called **enzymes**
Hypothesized that primordial earth was not a good environment for polymerization to occur, thus it is likely that polymerization occurred in solid surfaces like clay.

- Clay consist of thin layers of minerals separated by layers of water only a few nanometers thick
- The layered structure is charged allowing for molecular adhesion force to bring monomers together in orientation. Clay can also store potential energy that may have been used for energy requiring polymerization reactions.

This is the clay hypothesis on polymerization.

QUESTION: What is the difference between a reducing atmosphere and an oxidizing atmosphere?
ANSWER: Oxidizing atmosphere (what we have today) has high levels of oxygen and prevents electron rich molecules from being formed because oxygen is a strong oxidizing agent and would accept the electrons itself. Reducing atmosphere had a complete lack of oxygen, containing methane, CO2, hydrogen molecules and ammonia. These molecules contain an abundance of electrons and hydrogens and would enter a reaction with one another.

CHAPTER 3.3 A: Lipids Spheres May Have Led to the Development of Cells

Protobiont: group of abiotically produced organic molecules surrounded by a membrane of membrane like structure.

- Studies show protobionts could have formed spontaneously
- Early type of protobiont could have been the liposome = lipid vesicle where the lipid molecules form a bilayer similar to the cell membrane
- Liposomes can easily be made in labs and are selectively permeable
- Recent studies show that clay does not only catalyze clay for polymerization but also for accelerating the formation of lipid vesicles

CHAPTER 3.3 B: RNA Can Carry Information and Catalyze Reactions

The flow of DNA to RNA to protein is common to all forms of life, this is called the **Central Dogma.**
Scientists discovered a group of RNA molecules that could themselves act as catalysts. These are called **ribozymes**.

- Single stranded molecules can fold into specific shapes based on intramolecular hydrogen bonding
- Ribozymes revolutionized the thinking about origin of life

Because of this conclusion, RNA molecules could have been the first molecule to serve as a carrier of information

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CHAPTER 3.3 C: RNA Is Replaced by DNA for Information Storage and Proteins for Catalysis

Hypothesized small population of RNA molecules evolved that could catalyze the formation of very short proteins before developing ribosomes.
Cells that evolved the ability to use the information present in RNA to direct synthesis of small proteins would be at an advantage because
proteins are more versatile than RNA molecules for 3 reasons:

1. Catalytic strength of an enzyme is stronger than a ribozyme
2. RNA molecules can be composed of only 4 nucleotides, where as proteins have 20 different kinds of amino acids
3. Amino acids can interact chemically with each other in bonding arrangements not possible between nucleotides

Evolution of DNA followed after the evolution of proteins. DNA molecules are more complex than RNA molecules.
DNA - Double stranded, contains sugar deoxyribose

DNA is better than RNA for 3 reasons:

1. DNA is more chemically stable
2. The base uracil in RNA is not in DNA, replaced by thymine.
3. DNA is double stranded so in case of mutation of one strand, there is another strand to correctly repair damage

CHAPTER 3.3 D: Simple Oxidation-Reduction Reactions Probably Preceded Metabolism

REDOX reactions were probably one of the first energy releasing reactions.
Example would be how our cells oxidizes food molecules.
This wasted a lot of energy, thus over time multi step reactions were developed.
E.g. oxidation is slowly released

- Found in cellular respiration, greater efficiency stepwise energy release would have favoured development of intermediate carriers and opened the electron transport chains.
- ATP was established

QUESTION: In what ways is DNA better than RNA for storing genetic information?
ANSWER: DNA is double stranded therefore, if one strand mutated, the complementary strand can be used to repair the other strands. Additionally, DNA is more chemically stable than RNA because of its complex structure (sugar deoxyribose and helical structure). Finally, DNA has the base thymine where as RNA used uracil. By using thymine uracil is easily recognized as a damaged cytosine that needs to be repaired.

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Lecture (1):

What is a cell?
Robert Hooke was interested in playing around with lenses, trying to enlarge and portray objects with more details. He looked at cork and called the small "rooms" cellulae which is now known as cells.
From identifying cork --> Cells --> domains of organisms

QUESTION: Are there any living organisms that exist that do not have any cells?
ANSWER: No, there has to be at least one. (Viruses are not organisms; they need a host)

Cell Theory - mid 1800s
- To be an organism there needs to be at least one cell
- Must use energy
- Cell is the basic structural and functional unit of all living organisms
- Cells arise from division of pre-existing cells

Features of Cells:
Common characteristics
- Some sort of extension/appendages
- Have cell membrane
- Metabolism (ATP and cellular waste)
- DNA (they all have the same DNA)

Diversity:
- Shape and size
- Arrangements
- General or specialized roles

Cell Size and Scale
Be able to identify the range of sizes and compare the sizes between organelles/cells
Prokaryotes (1-5 nanometers) are much smaller than eukaryotes (10-100 nanometers)
Prokaryotes are unicellular so being small will allow it to survive impact
*switch from nano-micro- mili
*Which microscopes to use for which types of specimen?

Surface area to volume ratio:
Smaller cells will be much more efficient at interacting the surroundings
E.g. lung trying to achieve equilibrium of oxygen diffusion. If 0.1mm = 0.067s where as 1m = 78 days

Cells can remain single celled, separated from each other.
It can also form bundles to create tissues and organs. (working together)
E.G. Muscle tissue - Heart: how important is it to work together? All cells contract and relax because they have cell to cell communication (gap junction allow the transfer of ions through one cell to another).

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QUESTION: What elements of organisms can we use to classify them in phylogenetic trees?
ANSWER: The sequence of ribosomal RNA, so that you can see which is most similar. The cell membrane can help determine which branch it can go on.
Therefore, we can conclude that Archaea is much closer to Eukarya than we actually thought.

Types of cells
Prokaryotes - unicellular. Bacteria, archaea
Eukaryotes - Unicellular protists, multicellular (plants, fungi)

Prokaryotes
Very small cells
Highly adaptable because they are extremophiles and have much smaller genomes
Have plasmids
Eukaryotes
Much larger than prokaryotes
Elaborate and more complex structure for cellular functions
Larger genomes because it takes more cells to complete eukaryotes
Transport systems exist
Inside eukaryotes they have internal compartments called organelles and all specialize in different things
All organelles are attached, and there is an intriquete design called the cytoskeleton

Typical Bacteria (prokaryote): Escherichia coli
Flagella made of micro tubulin
No nucleus, DNA not encased in anything
lots of ribosome
Has pili

Typical animal (Eukaryote):
Lots of organelles etc.
Nucleus

Typical Plant Cells
Not round, and has a cell wall
Chloroplast
Large vacuole - contains mainly of water. Not always bean shaped, can always be in different forms.

COMPARE	Prokaryotes	Eukaryotes
Cell Size	Smaller 1-10um	Larger 10-100um
Cellular Organization		
Genome		
Organelles		

Eukaryotic cell organelles
Ribosomes are not organelles because there is no membrane (it acts as an organelle)
Therefore, organelles are surrounded by membranes
Functions are varied and depends on cells.

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Pre-Reading (2): The Cell

CHAPTER: 2.3: Eukaryotic Cells

Eukarya divided into 4 major groups

A. Protists
B. Fungi
C. Animals
D. Plants

Eukaryotes have a **true** nucleus enclosed by a nuclear envelope.
Membrane protein can be used as a channel for substances **OR** act as receptors for hormones and enzymes.
Plasma membrane also have **IDENTITY** by using a protein, allowing the cells to detect what type of cell it is.
A **Cell wall** is surrounded around the membrane in **fungi** and **plants**.

In a nuclear envelope there are two layers separated by the cytoplasm:

1. Layer just inside
2. Layer separated by a narrow space

Network of protein filaments called **lamins** lines an reinforces the inner surface of the nuclear envelope (IN ANIMALS CELLS) - Lamin is a type of **intermediate filament**
Proteins line the inner surface of nuclear envelopes in protists, fungi and plants.

Nuclear Pore Complex: large octagonal symmetrical, cylindrical structure formed by many proteins called the nucleoporins
Function: It exchanges components between the nucleus and cytoplasm and **prevents** the transport of material not mean to cross the nuclear membrane. A channel through the nuclear pore complex is called a **nuclear pore** and is a pathway to exchange large molecules like proteins and RNA molecules with cytoplasm.
EXAMPLE:
Some proteins, the enzyme for replicating and repairing DNA must be imported inside the nucleus for the function to be carried out. A specific protein recognizes and binds to the signal and moves the protein containing it to the nuclear pore complex.

Nucleoplasm: Liquid/semi-liquid substance in the nucleus.
Inside the nucleoplasm, it is filled with **chromatin** a combination of DNA and proteins.
Chromosome Vs. Chromatin
Chromatin refers to any collection of eukaryotic DNA molecules with their associated proteins.
Chromosome refers to one complete DNA molecule with the associated proteins.

Eukaryotic nuclei contain much more DNA than prokaryotic nucleoids.
Nucleoli: irregular masses of small fibres and granules. They form around genes coding for rRNA molecules of ribosomes.

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Eukaryotic Ribosomes:
Function is identical to prokaryotic ribosomes

- Use the information in mRNA to assemble amino acids into proteins

1. Freely suspended ribosomes in the cytosol

- Proteins made in free ribosomes remain free in the cytosol, it passes through the nuclear pores into the nucleus OR becomes a part of the mitochondria, chloroplast, the cytoskeleton or other cytoplasmic structures
- Proteins that enter the nucleus become part of the chromatin

2. Attached to membranes

- Some are attached to the nuclear envelope
- But most are attached to a network of membrane called the endoplasmic reticulum

The Endomembrane System:

Definition: collection of interrelated internal membranous sacs that divide the cell into functional and structural compartments.
Functions include:

- Synthesis and modification of proteins
- Transport of proteins into membranes and organelles or the outside of the cell
- Synthesis of lipids
- Detoxification of some toxins

Components include: nuclear envelope, endoplasmic reticulum, Golgi complex, lysosomes, vesicles and plasma membrane.

Endoplasmic Reticulum: an extensive interconnected network of membranous channels and vesicles called **Cisternae.**
A cisterna is formed by a single membrane that surrounds an enclosed space called the endoplasmic reticulum lumen.
2 types of endoplasmic reticulum:

1. Rough
2. Smooth

Rough endoplasmic reticulum consists of many ribosomes on the outer surface. The proteins created on these ribosomes enter the lumen where they **fold** into their final form
Chemical modifications of these proteins like **carbohydrate groups** to create glycoproteins occurs in the lumen.
These proteins are then delivered to the other regions of the cell using **small vesicles** that pinch off.
Usually the next destination of these proteins is the Golgi complex.
Smooth endoplasmic reticulum has no ribosomes on the outer surface of the membrane.
Various functions but in particular **synthesis** of lipids that become a part of the cell membrane
Contains enzymes that convert drugs, poisons and toxic by-products into substances that can be tolerated easily or removed from the body.
Both endoplasmic reticulum are often connected making the entire system a continuous network of interconnected channels in cytoplasm.

Golgi Complex consists of a stack of flattened, membranous sacs known as cisternae. (looks like stacked pancakes)
They are all **separate** sacs not interconnected! THIS IS THE **DISTRIBUTING CENTER**
There are approximately 3 to 8 cisternae but some organisms have several tens of cisternae.
The number and size vary depending on the cell type and metabolic activity.

- Usually located near concentrations of rough endoplasmic reticulum membranes and plasma membrane

It receives proteins and fuses into the Golgi membrane which releases the contents directly to the cisternae.
The vesicles contact the **cis** face of the complex, and the modified proteins are transported within the Golgi to the **trans** face of the complex.
Secretory Vesicles: Vesicles that secrete proteins from the cell to the plasma membrane using **exocytosis.**
Vesicles can also be reversed in a process called **endocytosis** creating an **endocytic vesicle.**
The substances carried to the Golgi complex are sorted and placed into vesicles for **routing** to other locations which may include lysosomes.

Lysosomes: Small membrane bound vesicles that contain more that 30 hydrolytic enzymes for digestion of many complex molecules like proteins, lipids, nucleic acids and polysaccharides.
Lysosomes are formed by the budding of the Golgi complex. Lysosome are acidic (pH of 5)
Hydrolytic enzymes therefore function best in acidic conditions (cytoplasm pH = 7.2)

- They digest food molecules entering the cell

Autophagy: a process in which a membrane surrounds the defective organelle, forming a large vesicle that fuses with one or more lysosomes. The organelle is degraded by hydrolytic enzymes.

Therefore, the endomembrane system is a **major traffic network** for proteins and other substances.

The Mitochondria: Membrane bound organelles where cellular respiration occurs.
Cellular Respiration: Process by which energy-rich molecules like sugars, fats and other fuels are broken down to water and carbon dioxide releasing ATP.
Mitochondria are enclosed by 2 membranes - Outer mitochondrial membrane and inner mitochondrial membrane
Inner mitochondrial membrane is expanded by cristae. Surrounds the mitochondrial matrix.
The matrix contains DNA and ribosomes that resemble the equivalent structures in bacteria.

Cytoskeleton: Internal organization and cells types are supported and maintained by the cytoskeleton. It reinforces the plasma membrane and functions in movements within and out of the cell.
In animal cells there are three major types of structural elements:

1. Microtubules
- Largest cytoskeletal element
- Use tubulin
2. Intermediate filaments
- Intermediate filament proteins
3. Microfilaments
- Actins

Microtubules: microscopic tubes with an outer diameter.

Constructs supportive structures of cells. Wall of the microtubule contains 13 protein filaments arranged side by side.
Filament: is a linear polymer of tubulin dimers each dimer consisting of one alpha-tubulin and one beta-tubulin
One end with the 1+ alpha sub units and the other end with one - beta sub units
They change length according to the addition or removal of the dimers
Intermediate Filaments: fibres intermediate in size, and usually occurs in parallel bundles and in interlinked networks.
Provides structural support in many cells and tissues E.g. nucleus in epithelial cells is held within the cell by a basketlike network of intermediate filaments made of keratin.
Microfilaments: Thin protein fibres that consist of two polymers of actin subunits wound around each other.
Used in locomotion and contractile elements like the muscle fibres of vertebrates. (roles of myosin and microfilaments)
Cytoplasmic Streaming: active flowing motion of cytoplasm. Used for transporting nutrients, proteins and organelles.
These filaments are used for dividing the cytoplasm

Flagella and Cilia

- Elongated slender motile structure on the cell surface
- Bundle of microtubule extends from the base to the tip of a flagellum or cilium
- Creates the 9+2 complex
- Dynein motor proteins slide the microtubules forming the 9+2 complex to produce the movement of a flagellum/ cilia
- Formation of flag/cilia: when the centriole moves to a position just under the plasma membrane. The 2 of the three microtubules of each triplet grow outward from one end of the centriole to form the ring of nine double microtubules

Basal Body: Protein structure found at the base of the flagellum or cilia. It is formed from a centriole and several other protein structure.

CHAPTER 2.4: Specialized Structures of Plant Cells

Chloroplast: sites of photosynthesis in plant cells.
Plastids: Members of a family of plant organelles.
Amyloplasts: Colourless plastids that store starch a product of photosynthesis
Chromoplast: contain red and yellow pigments and is responsible for the colours of ripening fruits or autumn leaves.
All plastids contain DNA genomes and molecular machinery for gene expression and the synthesis of proteins on ribosomes.
Chloroplasts are lens or disc shaped organelles surrounded by a smooth outer boundary membrane and inner boundary membrane.
These two boundary membranes completely enclose an inner compartment called the stroma.
Within the stroma is a third membrane system (closed sacs) called thylakoids.
Grana: Thylakoids stacked on top of another (found in higher plants)

Central Vacuole in Plants:
Are large vesicles that perform specialized functions unique to plants. The pressure in the central vacuole supports the cell. The membrane that surrounds the vacuole is called the **tonoplast** which contains transport proteins that move substances in and out of the vacuole. Plants increase in size depending on the pressure and volume of the vacuoles.

Cell Wall:
Extracellular structure because they are located outside the plasma membrane. Provides support for individual cells and contain the pressure produced by the central vacuole.
Consists of cellulose fibres giving it tensile strength to the walls.
Plasmodesmata: Minute channels in the cell wall. Most contain narrow tube like structure derived from the smooth ER. This channel allows ions and small molecules to move directly from one cell to another through connecting cytosol.
In some walls chitin exist instead of cellulose.

CHAPTER 2.5: Animal Cell Surface

Cell adhesion molecules bind cells together more complex cell junctions seal the spaces between cells and provide direct communication.
3 Types of cell junctions in animal tissues:

1. Anchoring junctions
- Forms button like spots that run entirely around cells "welding" cells together
2. Desmosomes
- Intermediate filaments anchor the junctions in the underlying cytoplasm also known as adherens junctions
3. Tight Junctions
- Regions of tight connections between membranes of adjacent cells
- It is tight so that it can prevent small particles from moving between the cells
- Found in internal organs like the stomach

Gap junctions open direct channels that allow ions and small molecules to pass through directly

Extracellular matrix provides protection and support. It forms mass of skin bones and tendons and forms many highly specialized structures like the cornea.
Glycoproteins are the main component and in animals the glycoproteins are the collagen fibres. It gives tensile strength and elasticity.
Proteoglycan: Glycoproteins that consist small proteins non covalently attached to long polysaccharide molecules.
Fibronectins: Aids in organizing the ECM and helps cells attach to it. They help the receptor proteins bind on to the plasma membrane.

CHAPTER 3.5: The Eukaryotic Cell and the Rise of Multicellularity

Present day eukaryotic cells have two characteristics

1. The separation of DNA and cytoplasm
2. Presence in the cytoplasm of membrane bound compartments

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Theory of Endosymbiosis and Mitochondria + Chloroplast
Chloroplast and mitochondria evolved from free-living prokaryotic cells.
Mitochondria descended from aerobic bacteria
Chloroplast descended from cyanobacteria
Prokaryotic ancestors engulfed larger prokaryotic cells forming mutually advantageous relationships called endosymbiosis.

Evidence of this Theory:

1. Morphology
- Form or shape of mitochondria and chloroplast is similar to bacteria and archaea
2. Reproduction
- Cells cannot synthesize mito/chloro.
- They are derived from pre-existing ones
3. Genetic information
- Mitochondria and chloroplast contain their own DNA
- Nucleus is linear, and DNA is circular
4. Transcription and Translation
- Both contain a complete transcription and translational machinery
5. Electron Transport
- Both have electron transport chains
- They are also found in free-living prokaryotic cells
6. Sequence Analysis
- Sequencing of RNA that makes up ribosomes of chloroplast and mitochondria establishes that they belong to the bacterial branch of life

Horizontal Gene Transfer
IF both mitochondria and chloroplast were free-living prokaryotes then they should have roughly the same number of genomes. BUT THEY DON'T
Mitochondria = 37
Chloroplast in algae = 99
Over evolutionary time some protein coding genes that were once part of the chloroplast/mitochondrial genome have been relocated to the nuclear genome.

Endomembrane derived from the Plasma Membrane

- Theory of the infolding of the plasma membrane creating the endomembrane system
- Hypothesis that cell lines leading prokaryotic cells to eukaryotes pockets of plasma membrane may extended inward and surrounded the nuclear region. Some fused with the DNA creating the nuclear envelope. Remaining infolds created the vesicles, ER and Golgi.

Evolution of Multicellular eukaryotes
This evolution led to increased specializations

- Coevolution

Lecture (2):

The Nucleus:
Compartment where DNA and protein is located
Site where transcription/ DNA replication occur

Nuclear pores are on the outer surface of the membrane allowing materials to pass through. Double membranes are made of cylindrical hollow proteins that open for RNA and ribosomes.
A double membrane making it different than other organelles.
Nucleolus - coding for ribosomal RNA (area within the nucleus), assembles ribosomes

QUESTION: Where does translation occur?
ANSWER: Translation occur where ribosomes are located. (in the cytoplasm where there are free ribosomes, or the endoplasmic reticulum.

Ribosomes
Made up of 2 sub units - large and small

Endoplasmic Reticulum
Rough - Ribosomes on the surface of the membrane
- Translation can occur simultaneously (more protein yield)

Smooth - Lacks the membrane bound ribosomes
- Synthesis of lipids
- Detoxification of metabolic waste and toxins in the cell (e.g. alcohol)

Golgi Complex
- Sorts, organizes and modifies proteins
- Post translational modification (after)
- Create glycoproteins by adding chemical functional groups, lipids, sulfyl groups
- Gives proteins different abilities, and how they interact within the cell
- They travel through the cis face travel through the stacks, and exit through the trans face
- It makes sure the protein is addressed properly that orients where it will go

Lysosome
- Inside the lysosome, there are enzymes have acidic environment
- They do not transport they degrade molecules
- Autophagy: self digestion
- They break chemical bonds and release it back so that the material can be reused
- It can take components outside the cell and ingest it and reuse what's useful

Mitochondria
- Correlates to the theory of endosymbiosis
- Acts as a cell within a cell
- They have their own DNA and very similar to bacteria
- Inherited from the pre-existing mitochondria
- Binary fission is how it reproduces according to its own schedule
- A cell can have more mitochondria than other cells
  - Cells are able to change the number of mitochondria depending on the amount required
- Uses sugar and oxygen and water to produce energy

Chloroplast
- Also produces energy through photosynthesis
- Uses photons of light to excite pigments in chloroplast (chlorophyll) that starts a chemical reaction producing sugar and oxygen

- Double membrane organelle
- Has plastids

Central Vacuole
- Filled with water and sometimes sugar and has tonoplast that makes the central vacuole have the appropriate amount of substances inside
- **Tonoplast** controls turgor pressure to maintain cell shape

Cell Wall
- Contributes to the shape of the plant cell because of its rigid structure
- Instead of gap junctions they have plasmodesmata, allowing passage of small ions across from one cell to another (because cell wall is rigid and harder to pass through)

Plants have similar vacuoles of a lysosome, that has acidic properties (recently proven)

Cytoskeleton
- 3 kinds of cytoskeletal fibres: microtubules, intermediate filaments and microfilaments
- Microtubules: largest tubulin fibre, have great tensile strength and provides elasticity
- Intermediate filament: Intermediate in size, and similar properties to microtubules, made up of
- Microfilaments: made up of actin, smallest and used during cellular division + locomotion

Make up a summary chart of organelles, roles and cell types ----

QUESTION: what distinguishes eukaryotes from bacteria and archaea
ANSWER:
- nuclear envelope (separating DNA from the rest of intracellular components)
- Membranous compartments with specific roles (ER, mitochondria)

Theory of Endosymbiosis
- Prokaryote engulfing another prokaryote that was aerobic, resulting it getting an aerobic property. This shows an symbiont relationship each prokaryote benefiting
- As time progresses they continue to become more complex

Evidence of the theory of endosymbiosis (6)
Morphology, reproduction, genome, transcription and translation, electron transport and sequence <-- found in pre-readings

Endosymbiosis Today
Sea slug (Elysia Chlorotica)
- Steals chloroplast so that it can undergoe photosynthesis (this process is called kleptoplasty)

Salamander and green algae
- Algae provides O2 while they use the N2 produced by salamanders

Coral reefs are clusters of animals related to anemones

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Pre-Reading (3): Macromolecules

Purple Pages F-8 to F-42

Isotopes
Atoms with the same atomic number but different atomic masses.

Radioisotopes
Radioactive decay - unstable atoms that decay giving off particles of matter and energy. This decay transforms unstable radioactive isotopes into an atom of another element.

Half-life - length of time it takes for one half of a sample of a radioisotope to decay.

Properties of Water
Hydrogen bonds with its neighbours forms an arrangement called a water lattice. These bonds hold the lattice together, and are able to break and reform allowing water molecules to break apart from the lattice and slip to another position.

Specific Heat and Heat of Vaporization
Hydrogen bond lattice slows the escape of individual water molecules when heated. Most of the heat contributes to the breaking of the hydrogen bonds. Therefore water has a high specific heat.
Heat of vaporization is the amount of heat required to break molecules motion to break loose from liquid to gas (or other phases)

Surface Tension
Cohesion - Hydrogen bond lattice of water results in water molecules staying together.
Related to surface tension which is a measure of how difficult it is to stretch or break the surface of a liquid.
Some spiders are able to stay on top of water because it is less dense than the surface tension of water.

Aqueous Solutions
The strong polarity of water readily surrounds other polar molecules creating a surface coat called **hydration shell**, reducing the attraction between molecules and promoting separation into a solution.

Dissociation of Water and pH
Hydrogen bond lattice is unrelated to the ability to dissociate. Proton is what actually leaves and the electron is left behind creating either an OH- or an H3O+. These changes make the new solution either acidic or basic depending on the salts used when dissolved in water.

Buffers
Organisms can maintain internal pH using buffers, which are substances that compensate for pH changes by absorbing or releasing hydrogen ions.
When hydrogen ions are released buffers combine with them and remove them from the solution.
When hydrogen ions are lacking, buffers release hydrogen to restore the balance.
Most buffers are either weak bases or acids or combinations of both.

Carbon Bonding
Carbons central role is its bonding properties.
It is able to assemble into astounding variety of chain and ring structures that form backbones of many biological molecules. (carbon has 4 unpaired electrons and readily shares to complete the outer most energy level)
Can create combinations of single, double or triple bonds.
Carbon atoms bond covalently to each other and other atoms.

Dehydration and Hydrolysis Reactions
When an OH- and a H atom are removed to produce water between two compounds, this reaction is called dehydration synthesis.
In hydrolysis the opposite reaction occurs, a water molecule is added to separate the compound into 2.

Functional Groups
Hydroxyl - OH
Carbonyl - C double bond O
Carboxyl - both carbonyl and hydroxyl
Amino - Nitrogen
Phosphate - phosphate molecule with oxygen
Sulfhydryl - Sulfur atom

Lecture (3):

QUESTION: Which of these functions is not accomplished by carbohydrates?
ANSWER: Plasma membrane fluidity is influenced by cholesterol, which is not involved with carbohydrate.
Carbs. Are not fat soluble and are highly polar, thus cannot be in the lipid bilayer.

Polarity
Electronegativity: The ability of an atom to attract an electron of another atom.
Be able to determine polarity by looking at the molecule and explain why and how.

Protein
Forms bonds by combining an oxygen from one amino acid and 2 hydrogens from another one (dehydration synthesis) and form a **peptide bond**. (produces water)
Proteins are oriented, meaning they have a beginning and an end. This is important when building protein for transcription.
Not much difference when comparing polypeptide and a protein (just the size and the number of amino acid)
QUESTION: why is the polarity profile of amino acids important for protein structure?
Polarity needs to exist in the amino acids so that they are able to form protein structures. How the protein folds by hydrogen bonds. Polarity affects structure which affects the location.

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Pre-Reading (4): The Cytoskeleton

CHAPTER 8.4: Formation and Action of the Mitotic Spindle

Spindle fibre is made up of microtubules. These form a major part of interphase cytoskeleton of eukaryotic cells.
When mitosis occurs, the microtubules disassemble from the cytoskeleton and form the spindles in the cell.
Asters: centrosomes at the spindle tips which form the poles of the spindles.
Centrosome of centrioles are not present in angiosperms (flowering plants) or in most gymnosperms like conifers.
Microtubules can be divided into two groups during the metaphase of mitosis:

1. Kinetochore microtubules
- Connect the chromosomes to the spindle poles
2. Non-kinetochore microtubules
- Extend between the spindle poles without connecting to chromosomes

Data now shows that chromosomes walk towards the poles of the cells instead of the spindle pulling them.

CHAPTER 42.6 A + B: Microtubules and microfilaments and change in cell shape

Embryonic cells undergo changes in shape that generate movement like infolding of surface layers to produce endoderm of mesoderm. Movement is produced by changes in rate of growth of microtubules. Changes in cell shape and movement play important roles in cleavage, gastrulation and organogenesis.

Changes in cell shape result from reorganization of the cytoskeleton.
E.G: in frogs
Neural plate in frogs the ectoderm flattens and thickens and cells in the ectoderm layer change from cube like to columnar in shape. Micro filaments slide over each other tightening the ring like a drawstring and narrowing the top of the cell. Entire cell layer invaginates.

Lecture (4):

Part of lecture 3 -->

Protein Structures

Secondary structure:
Alpha helix - charged polarity will be found in more globular structure
Beta pleated sheets - Has a more rigid structure/shape, non polar molecules will create a structured, pleated rigid sheet.
Tertiary Structure:

- They can either be a single strand
- Or they can be built by combining different structures together

You can assemble more tertiary structures into quaternary structures, with each individual structures called subunits.

Hemoglobin needs 4 subunits (2 alphas and 2 betas) and they need to be correctly assembled and folded so that they can maximize their ability to bind to oxygen and transport other molecules.
Sometimes assembly and folding will not be done properly, thus it must be degraded by **lysosomes** .
Sickle Cell Anemia:
A single change in amino acids results in the hemoglobin folded in the same secondary, tertiary and quaternary structure. (change from glutamate for valine) polar to non polar
They've become so inflexible that their shapes has become crescent shaped, not being able to travel when many of these sickle celled hemoglobin clump together at junctions/capillaries.

Functions of Proteins:
Hormonal proteins
Receptor proteins
Contractile and Motor proteins
Structural proteins
Enzymatic proteins
Defensive proteins
Storage proteins
Transport proteins
Explain each term ----

Cytoskeleton

Functions include:
Mitotic division (the spindle fibres made of microtubules attaching to the chromosomes) Microtubules made of dimers that can change the length of its fibres.
Intercellular transportation

Question: Given the cytoskeletons different roles what is an important property of these structures? (hint we said this was a 3D and a dynamic network)
Answer: No covalent bonds. Breaking a covalent bond requires less energy, thus making it easy to destroy and reattach the fibers.

Microtubules
Basic units of microtubules are called tubulin dimers (alpha and beta)
Different domains link up --> secondary structure
GTP - energy molecule within the microtubule. It requires energy so that it can grow.
Adding a beta and alpha tubulin together, we added polarity and orientation. Tubulin is always added as a dimer. One GTP isn't accessible so one end has GTP and one end does not. Thus, the subunits will orient themselves accordingly. Always formed interchangeably alpha beta pattern.
They have a hollow inside. 3D structure assembly of 13 and the angle explain the tubule shape.

Microfilaments
All the way to the end of the plasma membrane and spread throughout the entire cell (unlike the microtubule)
Functions to also protect the cell. Protects against sheering and tearing giving it elasticity.
Made up of a globular protein called actin, but once assembled it creates fibres (which is no longer globular/polar). They also have an orientation and energy molecule ATP like the microtubule. Barbed

end (positive) and the back end (negative) like an arrow. Actin microfilaments are not hollow and they are solid. Its assembled one after the other forming a right hand helix.
Polarity gives direction for motor proteins.

Intermediate Filaments
Made up of keratin or cytokeratin. They are fibrous proteins (like shoelace) many strands that are wrapped and braded together. Dimers associate head to tail to form tetramers. They protect against compression (particularly the nucleus).
Each monomer/strand of protein coils with another identical protein. Both ends are the same. No energy molecule is involved and does not have polarity. It does not harvest any energy either.

QUESTION: Why is polarity important for 2/3 of the cytoskeletal fibers?
ANSWER: It provides direction

2 species of frogs
They are different why?

- Pigments (melatonin concentration are either sparse or concentrated in one area of the plasma membrane)
- How the pigments were transported and distributed

How do we transport these pigments?
Something needs to hold on to the cargo. Motor proteins are mechanoenzymes and need two things, orientation (from polarity) and energy (ATP).
Mechanoenzymes have orientation:
Kinesins - goes towards the plus ends (anterograde/forward) towards the cell membrane
Dyneins - Goes towards the minus ends (retrograde/inwards) Towards the inside of the cell.
Microfilaments - use myosin (that can go in multiple direction depending on the structure)

Pre-Reading (5):

Chapter 5.1-5.3 + F40-43

CHAPTER 5.1: Structure of the Membrane

Fluid Mosaic Model: proposes that membranes are not rigid molecules locked in place but instead it consists of proteins that move around within this lipid molecule.
Maintaining the membrane in a fluid state is **crucial** for the membrane to function.
Mosaic aspect refers to that most membranes contain variety of proteins. Since proteins are larger they move more slowly in the membrane fluid and sometimes do not move.

Important characteristic -
Proteins and other components of one half of the lipid bilayer are different from the other half of the bilayer. This is called **membrane asymmetry**. Reflects the different functions performed by each half of the membrane.

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Membranes are fluid:
Human cell and mouse cell experiment conducted by David Frye and Michael A.
They tagged human or mouse membrane proteins with dyes. Human = red, Mouse = green. They fused the human and mouse cells and in minutes they discovered that the two different proteins mixed and in hours the colour has completely intermixed in the fused cells. This concluded that the different proteins moved around the fused membranes.

Membrane Asymmetry:
Key experiment was the freezing fracture technique.
Blocks of cells were rapidly frozen by dipping it in liquid nitrogen and then was fractured by hitting it with a microscopically sharp knife edge. Usually the fracture splits the bilayers into inner and outer halves exposing the interior. Using the electron microscope, it was discovered that membrane differed in size number and shape, proving that each sides were different.

Phospholipids
Phospholipids are **amphipathic** which means they contain both hydrophilic and phobic properties. Fatty acid chains are nonpolar and the phosphate head would be very polar.
Double bonded chains = unsaturated fat
Single bonded = saturated fat
When phospholipids are added to water, they self assemble into one of three structures:

1. Micelles
2. Liposomes
3. Lipid bilayer

Fatty Acid Composition and Temperature Affecting Membrane Fluidity
Fluidity of the membrane is influenced by 2 main factors:

1. Types of fatty acids making up the lipid molecules
2. Temperature

Fully saturated fatty acid chains are linear allowing lipids to pack tightly together. Whereas lipid molecules with unsaturated fatty acids are prevented from packing closely together because the double bonds introduce kinks in the fatty acid backbone. Therefore, unsaturated fatty acids **increase** membrane fluidity.

As temperature drops the random molecular motion of lipid molecules slows down. It reaches a point where fluidity is lost and phospholipids become semisolid gel. (E.g. Butter)
The more unsaturated the molecule is; the lower temperature they will have.
At high temperature, molecular motion increases that can result in the membrane becoming to fluid = loss of structure. Normally a balanced membrane is achieved with even numbers of saturated and unsaturated fatty acids.

Organisms Adjusting Fatty Acids:
Keeping membrane fluidity is very important for cells. Increase in membrane fluidity from high temperature can lead to mass leakage of ions like potassium, sodium, calcium. If temperature is too low than the electron transport chain will not receive enough required molecules for cellular respiration to occur. Most organisms can match their body temperature to the external environment. Example are plants, protists and insects.

Desaturase are groups of enzymes that catalyze fatty acid biosynthesis. Desaturase act on saturated fatty acids by removing 2 hydrogen atoms from neighbouring carbons and introducing the double bond. As temperature decreases the desaturase abundance increases, vice versa.
Besides lipids, a group of compounds called sterols influence membrane fluidity. **Cholesterol** is classified as a sterol found in animal cells. They act as buffers at high temperatures to help restrain the movement of lipid molecules, thus reducing fluidity of the membrane. However in lower temperature sterols disrupt fatty acids from occupying lots of space between lipids.

Key Functions of Membrane Proteins

1. Transportation
- Proteins provides a channel for large, or insoluble molecules through the membrane
2. Enzymatic activity
- Many membrane proteins are enzymes. E.g. respiratory and photosynthetic electron transport chains
3. Signal Transduction
- Membranes often have receptor proteins and allow for bindings of specific hormones and chemicals. This can cause chemical changes within the cell.
4. Attachment and Recognition
- Proteins exposed on the membrane act as an attachment site or allows for cell-cell recognition

Integral Membrane Proteins interacting with Membrane Hydrophobic Core
Proteins embedded inside the membrane are called integral proteins. These proteins have distinct regions called **domains** that differ in polarity. To find a transmembrane protein, it will have stretches of nonpolar amino acids from 17-20 amino acids. It interacts with the hydrophobic core.

Peripheral Membrane Proteins:
These proteins are positioned on the surface of the membrane and do not interact with the hydrophobic core. They are held onto the surface with non-covalent, hydrogen and ionic bonds. Key enzymes are involved with peripheral proteins because they don't interact with the hydrophobic core they are made of a mixture of polar and nonpolar amino acids.

F40-43: LIPIDS AND FATS

Lipids - Diverse group of water insoluble and primarily non polar molecules.
Isoprene and Fatty Acids:
Isoprene are five carbon molecules that when they are linked together they form long hydrocarbon chains. Their structural unit in steroids and phospholipids.
A fatty acid contains a single hydrocarbon chain and a carboxyl group at the end. This carboxyl group gives the fatty acid its acidic properties. As length of the fatty acid increases it becomes less water soluble and much more solid.

Fats:
Consists of 3 fatty acid chains linked to a single molecule of glycerol. Fats are often reffered to as tryglycerol or tryglyceride. As individual fatty acids and tryglycerides become less fluid the length of the fatty acid chains increases. They are mainly used as stored energy and insulation in animals.

Steroids:
Groups of lipids with structures based on a framework of four carbon rings that are derived from isoprene units. The most abundant steroids and sterols have a single polar OH group. Although they are almost hydrophobic, the single hydroxyl group at one end gives the molecule slight polarity. Therefore, sterols have dual solubility properties and like phospholipids. Cholesterol is an important sterol in animals, and the equivalent for plants is called phytosterols.

Lecture (5):

Cilia and Flagellum
These structures use microtubules to exert into different motions.
Cilia can be used to displace or attract nutrients to the cell.
Flagellum is mainly used to move the entire cell to move from one point to another. (uses rotary or whip like motions for locomotion). These movements will impose different type of movements.
Both emerges from basal body (microtubules), centrioles form them underneath the plasma membrane.
9 doublets with one doublet in the centre

QUESTION: Given the structure of cilia and flagella what would happen if there was no anchor (as provided by nexin)?
ANSWER: The microtubules doublets would slide apart. The reason is because the nexin holds the adjacent doublets together. They slide apart because one of the doublet is holding the cargo while the other motor protein is walking on it. If they weren't held by the cross holder then a protein like dynein would slide it apart instead of it bending properly.

Each doublet is held by a cross linking protein. The nexin makes sure the bend occurs instead of it sliding back down. After the bending motion the doublet can flick back up instantly.

Centrosomes
Centrosomes are two centrioles in a cell. Centrioles are made up of 9 microtubule triplets arranged in a ring structure.
Cell to Cell interactions - Junctions
Tight junctions - Closes the spaces between the cells preventing the ions to pass
Anchoring junctions - Plaques of proteins interacting cells together
Gap junctions - allow passage for ions and molecules
Gap junctions are made of 6 proteins sub units that spin the membrane across the plasma membrane.
These proteins change shape (they change their orientation which in turn changes the structures).
ALP - an axon proposes a way this portion of a call can grow to reach a target.
ANSWER: microtubules want to grow towards the target cell. However, there is a double layered plasma membrane thus that wont be enough. The microtubule does not reach the membrane but microfilaments form networks that form rigid structures like bundles.
QUESTION: True or False: Membranes are only found on the outer surface of cells
ANSWER: true except some prokaryotes

Membranes
5 Reasons why we have them?
Fluid Mosaic Model the lipid portion makes it fluid

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Mosaic stems from the different proteins

Fluidity

The membrane is two layers of phospholipids. The membrane is very dynamic and active. All the phospholipids can change places. This is called lateral diffusion. Fatty acid chains start to flex and move around. All the fatty acid chains are no all the same length and are not all saturated. Each layer of the membrane is asymmetrical because of the different structures inside and outside the cell. They have to accommodate for different types of environments.

QUESTION: Why are both layers of the membrane different?
ANSWER: Both layers of membranes are asymmetrical because they need to accommodate for different environments. For examples the inner membrane within the cell need to accommodate for integral proteins within the cell, and different organelles travelling to and from the membrane. Where as the outer membrane can also have different types of proteins like receptors for hormones to attach and act upon. These 2 different environments influence how the plasma membrane can differ in each side. Difference between the inter and extracellular communication (what it needs to accomplish).

Membrane Symmetry

Translocation from one layer to the other is rare and requires energy (ATP)
Different ratios of main phospholipids, and cholesterol. Glycolipids are found on the outer membrane. Phosphatidylserine makes it negatively charged (found in the inner surface of the plasma membrane).

(lec.7)

Membrane Fluidity - how easily things can get across and how easy it is to move inside
Temperature
Chain length and degree of saturation
Cholesterol - how packed the phospholipids are <- also influenced by temperature, they work against the extremities of temperature. High temperature, the cholesterol will restrict the movement of the phospholipids. Adds a little bit of stability within the phospholipids. Sterols are the buffers of the membrane.
Polarity - polarity differs depending on the functional group on the phospholipid. There are 4 major types of phospholipids in the cellular membrane.

Membrane is Dynamic

Homeoviscous adaptation - the ability to change the membranes lipid composition according to the environment.
Class Example: Fish in different temperature water PC/PE ratios.

Trout is moved from 28 degrees 10 degrees. The membrane will become more ___ cholesterol will ____ and pc/pe ratio will ____.
ANSWER: Introduce double bonds, and more PE and less PE. (fluid, increase, decrease)

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Pre-Reading (6) Membrane Transport:

CHAPTER 5.3-5.6: Membrane Transport

CHAPTER 5.4: Passive Membrane Transport

Passive transport is based on diffusion. Without the need to expend chemical energy like ATP is what drives this system. Diffusion is the primary mechanism of solute movement within a cell. **Driving force** of diffusion is an increase in entropy (concentration gradient)
There are two types of passive transport:

1. Simple
2. Facilitated

Simple diffusion is the movement of molecules directly across a membrane with a transporter.
Facilitated Diffusion is the diffusion of molecules across the membrane with the aid of a transporter.
Diffusion of many polar and charged molecules like water amino acids sugar and ions.
In facilitated diffusion there are two groups of transport proteins:

1. Channel proteins:
   Forms hydrophilic pathways in the membrane through which molecules can pass. The channel aids the diffusion of molecules by providing an avenue that is shielded from the hydrophobic core of the bilayers. Diffusion of water is facilitated by water specific transport proteins called **aquaporins**.
2. Gated Channels:
   These transporters can open or close the "gate" that allows molecules to pass through. An example of this type of protein would be the sodium and potassium pump. Depending on the concentration and environment within the cell, the sodium potassium pump will open when the required ions are in place.

Osmosis is known as the passive diffusion of water. Water can passively move through the membrane in a process called osmosis. Depending on the solute concentration of the environment and the cell, osmosis can occur to maintain solute balance. Hypotonic refers to a low concentration of something. Hypertonic refers to a high concentration of something. And finally Isotonic refers to a balanced concentration of something.

CHAPTER 5.5.: Active Membrane Transport:

Active membrane transport required ATP and energy. Usually this type of transfer requires molecules and ions to go **against** the concentration gradient. Three main functions of the active transport of cells and organelles are:

1. Uptake of essential nutrients from the fluid surrounding cells
2. Removal of secretory or waste material
3. Maintenance of essentially constant intracellular concentrations of H+ Na+ K+ and Ca2+

There are also two classes of active transport:

1. **Primary active transport:** The same protein that transports the molecules also hydrolyzes ATP to power the transport directly.

2. **Secondary active transport:** The transport is indirectly driven by ATP. Uses a favourable concentration gradient for transport.

Primary Active Transport
All primary active transport moves only hydrogen Ca2+ Na+ K+ across the membrane. These ions require pumps and is essential for cell life. For example proton pumps are essential for the electron transport chain. Calcium pumps is widely distributed among eukaryotes. It pushes calcium from cytoplasm to cell exterior and from the cytosol to the vesicles. (Calcium is typicall high outside the cell)
Electrochemical gradient: Electrical charge difference on the two sides of the membrane. (found in sodium, K pumps)

Secondary Active transport:
Driving force for most secondary active transport in animal cells is the high outside/ low inside Na+ gradient. Therefore, transfer of Na+ into the cell is coupled with ion supply. It occurs in two mechanisms called **symport** and **antiport.**
The co-transported solute moves through the membrane channel in the same direction as the driving ion, a phenomenon called **co-transport.** In antiport, the driving ion moves through the membrane channel in one direction providing energy for the active transport. <--- Exchange diffusion

CHAPTER 5.6: Exocytosis and Endocytosis

The largest molecules transported through membranes are achieved by exo/endo cytosis
Exocytosis
Secretory vesicles move through the cytoplasm and fuse into the plasma membrane releasing the vesicles content into the cell exterior. All eukaryotic cells secrete materials to the outside through exocytosis like peptide hormones or milk proteins.
Endocytosis:
Proteins and other substances are trapped that bulge inward in the plasma membrane. The **bulk-phase endocytosis** (pinocytosis), is when the extracellular water is taken along with any molecules that happen to be in there. (cell drinking) In the second endocytic pathway, **receptor mediated endocytosis** is when the molecules are to be taken in are bound to the outer cell surface by receptor proteins. After binding their target molecules, the receptors collect into a depression in the plasma membrane called a **coated pit** because of the network of proteins called **clathrin** that coat and reinforce the cytoplasmic side. The enzymes within lysosome digest the contents of the vesicles breaking them down into smaller useful molecules.
Some cells in white blood cells can take in large aggregates molecules by a process related to receptor mediated endocytosis. This process is called **phagocytosis** meaning cell eating. It begins when the face receptors bind molecules on the substance to be taken in. Materials are digested and remained in residues.

Lecture 6:

QUESTION: Why are there proteins in the plasma membrane?
ANSWER: Proteins are required in the plasma membrane because they are necessary for the transport of small and large molecules, and ions. These proteins also maintain concentration gradient between the cell and the extracellular matrix which is vital for cellular respiration and other important functions for the cell to stay alive. ---- (transport, enzymes, signal transduction and attachment/ recognition.)

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3 Types of membrane proteins:

I**ntegral proteins** - Bound to the membrane. They need a portion of their domain to interact with the hydrophobic area of the membrane. And they form a hydrophilic core to isolate and allow substances to pass through this area.
Peripheral Proteins - Interacts with the membrane without being embedded in the membrane. They can be found on both inner and outer layer of the membrane.
Lipid Anchored Protein: Found only on one side of the lipid membrane, while anchoring a portion of its structure into the membrane.

Integral membrane:
- They span the membrane and go across
- Built using amino acids and bound in peptide bonds giving them an orientation (amino end and carboxyl end)

Peripheral Proteins:
- They interact with things that are bound to the membranes and the cytoskeleton
- All interactions use non-covalent bonds
- Therefore, the interactions they have can be easily modified
- For example, in red blood cells they need a lot of elasticity (ankyrin)

Lipid anchored proteins:
- They have to be partly embedded
- They do this with different types of anchors
- They are covalently bound with their anchors
- Intracellular will be anchored using fatty acid or phenyl groups
- Extracellular proteins are anchored with GPI anchors

Why are they anchored with different anchors?
Different environment requires different types of structures.
Extracellular proteins are useful for cell adhesion
Intracellular proteins are involved in mediating cell division and cell growth.

How to study membranes?
- Using microscopy
- Freeze Fracturing

Freeze the cells using liquid nitrogen (flash freeze). Then cut the membrane and separate it into two parts. Whatever is embedded in the lower layer will remain there and vice versa. View under electron microscope and see where it is actually located. (example of discovering a new protein, and determine where it is located). A con is that the cell dies.

Fluorescence recovery after Photo bleaching (FRAP)
- Dye the cell surface with fluorescence dye
- Then laser an area
- Then view under the microscope and wait
- Then observe how the fluorescence recovering, because some that weren't bleached are moving around. Therefore, you can measure how long it takes so you can measure how fluid your membrane is.

- Helps you identify that the membrane is fluid

QUESTION: What can we expect if we are bleaching membrane proteins that are anchored?
ANSWER: That anchored proteins will only remain in the same area as the phospholipids. Therefore, it will most likely remain in the same area unless the phospholipids that are being anchored to the protein move with the protein. You will not be able to see the recovery quickly. Since everything is covalently bond you need to break a lot of the bonds that require lots of energy and time.

Movement across membranes

Passive/Simple Diffusion:

- Spontaneous down the gradient
- Small ions and molecules
- Uncharged solutes
- Osmosis
- Gas (partial pressure gradient)
- Water is able to filter through the hydrophilic heads (sheer size and polarity)

Facilitated Diffusion:

- Requires some form a passage

Channel vs. Carrier

- Channel is a gated leaked channel (a pore or an open passage)
- Carrier is a change in structure that opens/closes (not always open)
- They still follow the concentration gradient

ALP - Glucose is required inside cells. It can passively transport across the membrane. Cells need glucose for ATP production. Another system for taking glucose inside the cell.

QUESTION: Explain the difference in the graph of glucose transport.
ANSWER: As concentration of the glucose increases, the rate of diffusion decreases because it must balance the concentration gradient (Passive transport). The other function is showing a facilitated diffusion because the channel/carrier because it will saturate at one point. There will a limit of which the molecules are able to open/close carriers or gates.

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MIDTERM 2

Lecture 1:

What is the role of the mitochondria in a cell with regards to energy production?
Mitochondria is essential for physiological functions like

- Metabolism
- Response to stress
- Cell death
- Calcium storage

The ultimate goal of the mitochondria is for ATP production using proteins
Enzymes are proteins that catalyze reactions. Some are ribozymes (RNA)

Glycolysis- occurs in the cytoplasm not the mitochondria! The end product are 2 pyruvate molecules which needs to pass the membranes into mitochondria. It will be oxidized into acetyl Co-A entering the Kreb's cycle. Electron carriers goes through ETC to go through oxidative phosphorylation to generate ATP.
There are two phases in glycolysis: investment (ATP) and payoff. Requires 2 ATP and creates 4 ATP.
Krebs cycle
This cycle describes the 8 chemical enzymatic reactions the furnace uses to burn the combustible pyruvate and generate ATP energy.
1 pyruvate = 1 acetyl co-A and 1 hydrogen and 1 NADH
acetyl co-a enters the citric acid cycle to get 3 NADH+ 3H ATP and FADH2

Chloroplast has electron transport system and it is different from the mitochondrial ETC. At the end of chloroplast transport system ATP can be produced ATP synthase or go into the calvin cycle to form glucose
This is called photophosphorylation powered through sunlight and photons.
Oxidative phosphorylation also known as chemiosmosis which builds a gradient
Bigger gradient = more force = more ATP produced
Understand the importance of calcium (availability, storage), what are the cellular functions
Calcium is stored in mitochondria and calcium can be free within cells or bound to protein. Calcium is required for cells however; it becomes toxic when not beings used.
Important because:

- Response to stimuli (vesicle secretion
- Muscle contraction
- Signalling (second messenger)
- Enzymatic cofactor (coagulation)
- Bones
- Metabolism

Mitochondria stores calcium because in order to produce ATP two important biochemical steps require calcium so it can be used in Krebs cycle.
Calcium is regulated by the concentration gradient, binding protein buffering, compartmentalisation channels transporters and the replenish reserves.
Understand key concepts of cellular energetics in relation to mitochondria and chloroplast
What happens in the outer membrane, intermembrane space, inner membrane and the matrix of the mitochondria? And the key elements?

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What is mitophagy? And why/how does it occur?
Mitophagy is the controlled regulation of the number of mitochondria according to the metabolic requirements. It is a process that involves recruiting various signalling protein and lysosomes. The process how mitochondria are chosen remains unclear. Important for aging, development and certain pathologies (AD,Parkinsons,etc.)

Pre-reading 1

CHAPTERS 4.1-4.6, 6.1, 6.2, 6.5, 7.1-7.2
CHAPTER 4.1: Energy and the laws of thermodynamics

Energy - The capacity to do work.
Energy is readily able to transform from one form to another. E.g. Chemical energy in a battery is converted into electrical energy that passes through the bulb.
All forms of energy are grouped into one of two types:

1. **Kinetic energy:** Energy that is in motion. (flow of electrons)
2. **Potential energy:** The stored energy from the position or chemical structure.

When an electron gains energy it moves up to the next energy level (energy absorption) = higher potential energy. When an electron loses energy it is lost and moves down a level.

Thermodynamics: Concerns how energy changes chemically and physically.
There are 3 types of systems:

1. **Isolated system:** does not exchange matter or energy with the surroundings. The only true isolated system is the universe itself. (thermos bottle)
2. **Closed system:** is able to exchange energy but not matter with the surroundings. Example would be a saucepan of water with a lid heating on a stove.
3. **Open system:** Both energy and matter can freely move between the system and surroundings. Example would be the ocean, it absorbs energy and releases it and has a hydrological cycle where water is constantly gained or lost through evaporation and condensation.

First law of thermodynamics:
Energy can be transformed from one form into another but it cannot be created or destroyed. Example is the Niagara Falls: has lots of potential energy at the top, and as water goes down the potential energy is transferred into kinetic energy.
Entropy: tendency of energy to become dispersed or spread out.

Second law of thermodynamics:
The entropy of a system and the surroundings will increase energy will always become more spread out. Entropy is the measure of how much energy has flowed from being localized to becoming more widely dispersed.
Therefore, sometimes always a portion of energy is lost elsewhere (cars are never 100% efficient).

CHAPTER 4.2: Free energy and spontaneous Process

Spontaneous process: A process that can occur without energy.

Why does oxygen readily diffuse without energy?
Enthalpy: Total potential energy of a system. (H)
Change in enthalpy helps determine whether a reaction occur spontaneously.

1. Reactions tend to e spontaneous if they are exothermic - The products have less potential energy than the reactants. E.g. in methane the products have lower potential energy because electrons are more tightly packed by the atoms of the products than the reactants.
2. Reactions tend to be spontaneous when the entropy of the product is greater than the entropy of the reactants. Transformations tend to occur if the energy of the product is more spread out than the energy in the reactants.

Exergonic process -> spontaneous process
Endergonic process -> non spontaneous

*GIBS FORMULA TO DETERMINE process

CHAPTER 4.3: Thermodynamics and Life

Life does not go against the second law of thermodynamics. Organisms are open systems thus are constantly using energy and matter that they bring from the environment to keep a low-entropy state. According to the 2nd law entropy should increase, however it actually states that entropy of a system plus its surroundings must increase. Thus organisms increase the entropy of the surrounds by releasing metabolic products.

The flow of energy through the biosphere

Earth does not exchange matter with the rest of the universe but does exchange a huge amount of energy. Life on earth exists because its positioned in the solar system allowed for heat by the sun. Its not the heat from the sun that allows for metabolism, but the light in packs called photons. Process of photosynthesis!

CHAPTER 4.4: Overview of Metabolism

Metabolism - Collection of chemical reactions present within a cell or organism.
Two fundamental pathways for metabolism:

1. Those that require energy t build molecules
2. Those that release energy by breaking molecules down

Catabolic Pathway: Chemical reactions that result in the breakdown of larger more complex molecules into smaller, less complex ones.

- Energy is released into catabolic pathways because the overall free energy of the final product of the pathway is less than the free energy of the starting molecules.

Anabolic Pathway: Series of reactions that result in the synthesis of larger more complex molecules from simpler starting molecules. Also called biosynthetic pathways require energy because the overall free energy of the product of the pathway is greater than the free energy of the starting molecules.

Hydrolysis reactions releases free energy. The nitrogenous base adenine joined to a chain of 3 phosphate groups is what ATP is. ATP + H2O -> ADP + Pi

Although ATP releases free energy when it is hydrolyzed this doesn't mean that it is a reactive molecule. The rate of ATP hydrolysis in an aqueous environment like cytosol of a cell is slow. If ATP was reactive it would be impossible for metabolism involving ATP to be tightly controlled. Its hydrolysis would release heat possibly causing damage to the cell.
Therefore, hydrolysis is an exergonic reaction that can harness energy through coupling reactions.

ATP breakdown is an exergonic process then ATP synthesis from ADP and Pi is endergonic. The continuous breakdown and resynthesis of ATP is called the ATP cycle.

CHAPTER 4.5: Role of enzymes in biological reactions

For a chemical reaction to occur, established bonds to need be broken and new bonds need to be formed. For bonds to be broken they must be strained or made less stable (requiring energy). The initial energy investment required to start a reaction is called the activation energy. Molecules that gain the necessary activation energy occupy what is called transition states where bonds are unstable and are ready to be broken.

Enzymes accelerate reaction by reducing the activation energy.
Catalysts are chemical agents that speeds up the rate of a reaction without itself taking part in the reaction. It reduces the activation energy.
Many enzymes require a cofactor, a non protein group that binds very precisely to the enzyme.
Cofactors are usually metals, like iron, copper, zinc or manganese. They are necessary for enzymes.
Some cofactors are called **coenzymes** which are organic molecules that are often derived from vitamins.

How do enzymes reduce the activation energy?

1. Bringing the reacting molecules together. Reacting molecules can assume transition state only when they collide Binding to an enzyme active site brings the reactants together in the right orientation for catalysis to occur.
2. Exposing the reactant molecule to altered charge environments that promote catalysis.
3. Changing the shape of a substrate molecule. The active site may strain or distort substrate molecules into a conformation that mimics that transition state.

CHAPTER 4.6: Factors that affect enzyme activity

1. **Enzyme and substrate concentration**

- The rate of the reaction depends on the concentration of substrate or enzymes in the environment
- As enzyme concentration increases the rate of reaction increases (forever linear function)
- As substrate concentration increases the rate of reaction increases but slows down at the saturation level (curve)

2. **Enzyme activity altered by competitive and non-competitive interactions**

- Molecules that can bind to an enzyme

- Competitive inhibition - blocks the substrate from being able to bind to the enzyme's active site (can be solved by increasing the amount of substrate relative to the inhibitor
- Competitive regulators differ in how strongly they bind to the active site

3. Non-competitive Regulation

4. Temperature and pH

- Each enzyme has an optimal pH
- As temperature increases rate increases (if too much enzyme degenerates)
- As temperature decreases rate decreases

CHAPTER 6.1: Chemical Basis of Cellular Respiration

C-H bonds are non covalent bonds and can easily be replaced. This allows for electrons to easily be given and taken.
The potential energy in molecules are released when electrons are lost (oxidized). When a molecule gain electrons it is a reduced.
Process: Glucose -> releases electrons -> transferred to oxygen -> Reduced to water -> the carbon on glucose become CO2
For glucose to combust, it must reach the transition state by having enough required energy.
The most common energy carrier is NAD+ (oxidized version of NADH)

CHAPTER 6.2: Cellular Respiration

Cellular respiration is divided into 3 phases:

1. Glycolysis
- Enzymes break down glucose into 2 pyruvate molecules. Some ATP and NADH is synthesized
2. Pyruvate oxidation + Citric Acid Cycle
- Acetyl CoA is formed during the oxidation of pyruvate enters a metabolic cycle that is completely oxidized to CO2. Some ATP and NADH is synthesized.
3. Oxidative Phosphorylation
- The NADH is now oxidized by taking the electrons and passing them down the electron transport chain, until they are transferred to oxygen which produces water. This generates a proton gradient from the ECM which is used to generate mass amounts of ATP.

Glycolysis and citric acid cycle occurs in the cytosol in prokaryotic organisms, and oxidative phosphorylation occur
in the internal membranes. In eukaryotic organisms, oxidative phosphorylation and citric acid cycle occur in the mitochondria, and glycolysis occur in the cytoplasm.

CHAPTER 6.5: Oxidative Phosphorylation and Chemiosmosis

The electron transport chain coverts the potential energy in NADH and FADH2
The proteins do not transfer the electrons, however the prosthetic groups (non-protein molecules). These are redox active cofactors, that are able to alternate between oxidized and reduced states. A common example is the heme, a component found in the cytochrome C protein. It is biologically important which is also found in hemoglobin (carries oxygen). Central to its function it contains a redox active iron atom that alternates between Fe2+ and Fe3+.

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ATP synthase is a molecular motor. Embedded in the inner mitochondrial membrane.
Electron transport and chemiosmosis can be uncoupled *

CHAPTER 7.1: Photosynthesis

A redox process, producing glucose molecules from the use of photons.

Pre-Reading 2

Chapter 8.5h, 42.7f + online document

CHAPTER 8.5h: Some cells are programmed to die

Apoptosis: programmed cell death.
-> Found to be an ancient mechanism for many multicellular eukaryotes. Initiation of this mechanism results from the internal or external signals of the cell.
Example: Nematode is an organism that uses these signalling as it always as the exactly same number of cells in its body.
The main executioner enzyme is normally an inactive protease called **capsases** coded by the cell death abnormal gene.

If a cell is coded to die it begins when the internal developmental cues stimulate an expression of a gene called egg laying deficient (EGL-1) . This protein bind to a CED-1 protein resulting in the release of the CED-4 bound protein and forms the active apoptosomes. The causes of the death are nuclear DNA degradation and disrupted mitochondrial function. The dead cells are engulfed by neighbouring cells. Removing cell that are surplus for development is one function of apoptosis. Its beneficial for organisms to preform apoptosis to severe DNA damaged cells, leading to uncontrollable replication of mutations.

CHAPTER 42.7f: Cell death genes - Apoptosis

Apoptosis plays a role in breakdown of a tadpoles tail and in many other patterns of developments in vertebrate and invertebrates.
Example: In humans fingers and toes are initially connected by tissue forming paddle shaped structures which are later deconstructed through apoptosis.
Apoptosis results from a gene activation in response to molecular signals from receptors on the surfaces of marked cells. Therefore, the signals are death notices delivered at a specific time during embryonic development. The C. elegans (a death signal molecule) binds to the receptor of the plasma membrane of the target cell and initiates this mechanism. Activation leads to proteins that kill the cell. In the absence of a death signal the membrane receptors are inactive.

Studies in mutants helped understand the role of cell-death genes. Lacking normal Ced-3 or ced-4 genes the marked 131 cells failed to die, producing disorganized embryos.

Lecture 2:

What are the triggers of cell death?

- There are growth factors (absence of growth will tell the cell to stop growing)
- Mitogens signals the cell to die
- Different stresses to the cell like toxicity damage dyshomeostasis
- There is cell cycle check points to see if cells are healthy, if they can be repaired or should they instead just die

Reasons for cell death are numerous.
Essentially there are two key mechanisms why cells die.

QUESTION: Why was the nematode C. Elegan a good model for apoptosis?
ANSWER: Nematodes had exactly the same number of cells at all times, thus shows a good model of how well the signalling mechanism worked. The very limited number of cells would be much easier than an organism that has millions of cells. Identifying homologs and observing cell death is much harder in a complex model.

In the nematode the same 131 cells die every time. They discovered that ced-3 gene encodes proteins similar to proteases.

Cell Death: Necrosis Vs. Apoptosis
Main difference between these two mechanism is that apoptosis shrivels up where as necrosis showed that the cell swelled.
Under apoptosis, the cell dying does not impact the neighbouring cells. It is a very clean and organized mechanism. It never impacts the environment.
Under necrosis, the membrane ruptures. All the content of the cytoplasm is released and digestive enzymes is in the cells environment which will become harmful to neighbouring cells.
List of differences found on the power-point

Necrosis
- Something happens to the cell (mutation, damage to the membrane, damage to DNA) will result in an increased calcium concentration. The damages convinces the endoplasmic reticulum to releases all the calcium from storage. Therefore, all the calcium found in cytoplasm is toxic to the cell. The cell responds by making proteases active. It goes straight to the lysosome and disintegrates the membrane and the lysosome ruptures. All the powerful digestive enzymes are released, thus able to chomp on everything inside the cell. An example is cathepsin will attack the cytoskeleton, membrane etc. = no chance of survival.
- This is a very disorganized process with a lot of collateral damage
- Key features is that calcium increases = cell dies

Apoptosis
- A very well orchestrated process. The cell is packaged into smaller portions (like vesicles) called apoptotic bodies. Once the cell receives a message that it needs to undergo apoptosis, it condenses the chromatin. The nuclear envelope shrinks. Then the cytoplasm begins to shrink and the cytoskeleton disintegrates, microtubules unravel. Everything inside the cell becomes less anchored and loose, and becomes easy to package. Breakdown of the DNA sections to smaller sections. Loss of ability to attach to neighbouring cells (no cell junctions), and loses adhesion. They begin to form bubbles called blebs. These blebs become apoptotic bodies are taken up by neighbouring cells by phagocytosis into their system.

- Does not damage the neighbouring cells and a very organized process.

Phagocytosis
Asymmetric distribution of plasma membrane is lost. The negatively charges phosphatidylserine becomes exposed on the outside cell. The cell is then marked for phagocytosis carried out by a macrophage.
These phosphatidylserines trigger this mechanism
Something must flip these PDS!
Flipping does not occur very often, however using enzymes they are able to. When undergoing apoptosis it uses the enzyme called scramblases (prefers flipping phosphatidylserine) where as the other three enzymes prefers flipping for membrane asymmetry.
Capsases is an enzyme in the cell that activates apoptosis.
Two main apoptotic signalling pathways:

- Extrinsic
- Intrinsic (focus on this course)

Intrinsic pathway of apoptosis:

- Has an internal stressor
- Leads to dephosphorylating and activation of bad (pro-apoptotic)
- As soon as they become active they are able to promote apoptosis, and inhibit proteins that prevent apoptosis
- Pro apoptic

Capsases

- Family of proteases (enzymes that cleaves proteins)
- The executioner capsases act on the cell itself (shrinking the nucleus, losing adhesion, breaking the cytoskeleton etc.)
- There are also initiator capsases
- Protein kinases disrupt cell adhesion triggered by capsases
- Lamins = disassembly of nuclear envelope
- Cytoskeleton = change cells shape and size (unraveling the microtubules)
- They also activate an enzyme called DNAse - they cut the DNA fragmentation

Apoptosis in C. Elegans

Role of mitochondria in apoptosis

- The cell no longer receives to keep growing or surviving
- It will initiate its own suicide
- To trigger death you must activate dephosphorylation
- The pro apoptotic initiates the process
- Receives cytochrome C from mitochondria

QUESTION: What rearrangements within the mitochondria need to occur for cytochrome C to be released?
ANSWER: Cytochrome is found in the inner mitochondrial membrane (cristae). (answer..)

What is going to change the mitochondria for it to be released? (BAD BACs and …) <- are proteins inside the cell, there needs to be a concentration gradient. Calcium is toxic to cells, if BCl2 is

contributing to making the cell healthy. The ER has a channel that depend on IP3. It binds to that channel and opens up, releasing all of the calcium. <- BCl2 prevents doing this (if its inhibited BACS and BAD come in). In addition BACs and BAD are going to increase the infinity for that channel making it easier for the binding to occur. The mitochondria is going to respond b sucking up the calcium (it can be thousand times more concentrated than the cytoplasm). When the mitochondria becomes too concentrated the pores form cristae is released and cytochrome C is released.

In summary…

1. Bad is activated when a death signal is triggured
2. Inhibition of BCL-2 and activation of BAX and BAK (IP3)
3. Increase in calcium opening of PTP (permeability pore =release of CytoC)
4. Apoptosomes are formed (Cyto. C) <-- acts as an armour for capsases KNOW TERM NOT WAGON WHEEL
5. Nuclear condensation DNA fragmentation cytoplasmic shrinkage apoptotic bodies and phagocytosis occurs

Important for many reasons like embryonic development.
Interdigital tissue - cell undergoes apoptosis and spaces are formed between the digits on the hand.

Pre-Reading 3

CHAPTER 5.7, 43.1-43.2

CHAPTER 5.7: Role of Membranes in Cell Signalling

Living things have the ability to sense and respond to changes in the environment because of signal transduction.
Signal pathways follow three steps:

1. **Reception** - Binding of a specific molecule with a specific receptor of target cells.
- Target cells have receptors that are specific for the signal molecule which distinguishes them from cells that do not respond to the signal molecule.
- Most receptors are found on plasma membrane but some are found on the internal membranes like the ER
- Receptors are soluble proteins that are found in the cytoplasm
2. **Transduction** - Process whereby signal reception triggers other changes within the cell necessary to cause the cellular response
- Involves reactions with several different molecules "signalling cascade"
3. **Response -** Transduced signal causes a specific cellular response. Different signalling pathway lead to different downstream responses.
- Signal transduction lead to direct activation of specific enzymes, while others often trigger changes in gene expression.

Membrane Surface Receptors
Membrane receptors that bind molecules are integral proteins. The fit is similar to an enzyme and a substrate.

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The binding of a signal molecule to a plasma membrane receptor is sufficient to trigger the activation of the signalling cascade. The signal molecule does not make a direct response when injected into the cell. Un related molecules that mimic the structure of the normal extracellular signal molecule can trigger or block a full cellular response as long as they can bind to the recognition site.
Common characteristic of signalling mechanisms is that the signal is relayed inside the cell by **protein kinases** which are enzymes that transfer a phosphate group from ATP to one or more sites on particular proteins.
Protein kinases act in a chain catalyzing series of phosphorylation reactions called a **phosphorylation cascade** to pass the signal forward.
The 1st kinase catalyzes phosphorylation of the 2nd which then becomes active and phosphorylates the third.
The last protein in the cascade is the target protein.
Protein phosphatases - group of enzymes that remove phosphate groups from target proteins, unlike protein kinases which are active only when a surface receptor binds a signal molecule.

Another characteristic of signal transduction pathway is **amplification.** Which is an increase in the magnitude of each step as a signal transduction pathway proceeds. Amplification occurs because many of the proteins that carry out individual steps in the pathways including the protein kinases are enzymes. Once activated, each enzyme can activate hundreds of proteins including other enzymes. Generally, the more enzyme catalyzed steps in a response pathway, the greater the amplification.

CHAPTER 43.1: Hormones and their Secretions

There are four types of cell signalling in the endocrine system:

1. **Autocrine Regulation** - Local regulators acts on the same cells that release it. Common mechanism used by cells to either reduce or increase their sensitivity to other stimuli
2. **Paracrine Regulation** - Cell releases a signalling molecule that diffuses through the extracellular fluid and acts on nearby cells. On both of these instances regulation is *local* rather than at a distance. Many growth factors that regulate cell division and differentiation act in both an autocrine and a paracrine fashion
3. **Classical endocrine regulation** - Hormones are secreted into the blood or extracellular fluid by the cells of ductless secretory organs called endocrine glands. Hormones are circulated throughout the body in the blood or or the body fluids and as a result most body cells are constantly exposed to a variety of hormones. Only target cells of a hormone with receptor proteins recognize and bind to hormones.
4. **Neuroendocrine regulation** - Neurosecretory neurons respond to and conduct electrical signals but rather than synapsing with target cells they release a neuro-hormone into the circulation when appropriately stimulated. Hormone is produced in the cell body and packaged in membrane bound vesicles that are transported along the axon to the release sites.

Most hormones and local regulators can be grouped into 4 classes based on their chemical structure:

1. **Amine hormones** - involved in classical endocrine signalling and neuroendocrine signalling. Most are based on tyrosine. With one exception, they are hydrophilic molecules which diffuse into blood and ECF. When reaching the target cell they bind to receptors.

- Includes: Dopamine, epinephrine, norepinephrine, protostomes, octopamine which are all neurotransmitters released by some neurons

2. **Peptide Hormones** - consists of amino acid chains ranging in length from as few as 3 amino acids to more than 200. Mostly hydrophilic hormones, and they are released into the blood or ECF by exocytosis when cytoplasmic vesicles containing the hormones fuse with the plasma membrane. Large group of peptide hormones are the **Growth factors** which regulate the division and differentiation of many cell types in the body. Many growth factors act in both a paracrine and an autocrine manner as well as classical endocrine because they can switch cell division on or off.
3. **Steroid Hormones** - involved in classical endocrine signalling. All are hydrophobic molecules derived from cholesterol and are sparingly soluble in water. They combine with hydrophilic carrier proteins to form water-soluble complexes that diffuse into blood or other fluids. When contacting a cell the hormone is released from its carrier proteins passes through the plasma membrane of the target cell and binds to internal receptors in the nucleus or cytoplasm.

- Includes aldosterone, cortisol, vertebrate sex hormones, ecdysone, the hormone that governs formation of new cuticles
- Steroids can act via membrane receptors controlling cellular events like apoptosis and cell proliferation.

4. **Fatty Acids -** Specialized category of hormones. In arthropods and annelids hormones derived from farnesoic acid include juvenile hormones that govern metamorphosis and reproduction. Prostaglandins and relatives are important local regulators derived from arachidonic acid. They are involved in paracrine and autocrine regulation in all animals.

- First discovered in semen they enhance the transport of sperm through the female reproductive system

Secretions of many hormones is regulated by **feedback pathways** some of which operate partially or completely independently of neuronal controls. Most pathways are controlled by negative feedback in which a product of the pathway inhibits an earlier step in the pathway.

- In vertebrates, secretion by thyroid gland is regulated by a negative feedback loop.
- Neurosecretory neurons in the hypothalamus secrete TRH into a vein
- In response the pituitary gland releases TSH into the blood
- Which stimulates the thyroid gland to release thyroid hormones.

Concentration in the blood increases it begins to inhibit TRH.

Body processes are regulated by coordinated hormone secretion
Most body processes are affected by more than one hormone. The blood concentration of glucose, fatty acids, and ions like Ca2+, K+, and Na+ are regulated by coordinated activities of several hormones.
Many of theses systems negative feedback loops adjust the levels of secretion of hormones that act in **antagonistic (opposing)** ways creating a balance in the effects that maintains body homeostasis.

CHAPTER 43.2: Mechanisms of Hormone Action

Secreted hormones may not be in an active form

Many hormones are secreted in an inactive or less active form = prohormone, which is then converted by a target cell or enzymes in the blood to the active form.

Ecdysone, a steroid, governing the formation of new cuticles in insects. It is converted to the much more active functional hormone 20-OH ecdysone by the addition in the target cells of a single hydroxyl group.
Peptide hormones are commonly synthesized as prohormones that undergo post-transitional conversion to the active forms.

Angiotensin - a hormone that governs blood pressure is secreted by the liver as angiotensinogen by an enzyme. This inactive form is converted to the active hormone by angiotensin converting enzyme (ACE). ACE inhibitors are often prescribed for control of high blood pressure.

Hydrophilic Hormones Bind to Surface Receptors, Activating Protein Kinases Inside Cells

Hormones that bind to receptor molecules in the PM, produce their responses through signal transduction pathways. Typically signal transduction involved protein kinases which are enzymes that add phosphate groups to proteins. By adding phosphate groups to proteins may activate or inhibit it depending on the protein and the reaction.
Types of target proteins

1. Tyrosine kinase molecule:
- A receptor with a built in protein kinase on the cytoplasmic side of the receptor itself
2. **G Protein-coupled Receptor:**
- secondarily activates protein kinases within the cell.
- These hormones act on functional proteins that are already present in the cell like enzymes, ion channels and transport proteins

Hydrophobic Hormones Bind to Receptors inside Cells, Activating or Inhibiting Genetic Regulatory Proteins

After passing the PM they hydrophobic steroid and thyroid hormones bind to internal receptors in the nucleus or cytoplasm. They bind to receptors then bind to a control sequence of specific genes. Depending on the gene binding the control sequence can either activate or inhibit its transcription leading to changes in protein synthesis.

Aldosterone shows this mechanisms triggered by internal receptors.

- If blood pressure falls below optimal levels aldosterone is secreted by adrenal glands. The hormone circulates throughout the body in the blood but affects only cells that contain the aldosterone receptor in the cytoplasm. When activated the receptor binds to the control sequence of a gene leading to the synthesis of proteins that increase reabsorption of Na+ by the kidney cells.

Target Cells may respond to more than one hormone and different target cells may respond differently to the same hormone

A single target cell may have receptors for several hormones and respond differently to each hormone.
Mechanisms by which hormones work have 4 major features:

1. Only the cells that contain surface or internal receptors for a particular hormone respond to that hormone

2. Once bound by their receptors, hormones may produce a response that involves stimulation or inhibition of cellular processes through the specific types of internal molecules activated by the hormone action.
3. Because of the amplification that occurs through both the surface and internal receptor mechanisms, hormones are effective in very small concentrations
4. The response to a hormone differs among target organs

Lecture 3:

Communication is important for many different metabolic activities in organisms:

- Development
- Immunity
- Physiology
- Hormonal regulation and homeostasis
- Cell Growth, survival and cancer

Cell communication -

1. Nature of the message
2. How is it received
3. Interpreting the message
4. Acting upon the message

QUESTION: The target tissues for steroid hormones do not have receptors on the membrane surface for these hormones because these hormones…
ANSWER: Steroid hormones are soluble in the lipid bilayer.

1. The chemical messengers

There are six different classes of chemical messengers

A. Steroids:

- Lipophilic/hydrophobic, thus cannot be stored in the vesicles
- Derived from cholesterol
- They enter the cell and act as transcription factors

There are 3 classes of steroids:

1. Mineralocorticoids (aldosterone)
2. Glucocorticoids (Cortisol) <- response to stress
3. Sex Hormones (Testosterone/ Estrogen)

Generally recognize a steroid
What would you look for?
The rings (they have 4 rings)

B. Fatty Acids (Eicosanoids):

- Mostly derived from arachidonic acid
- Lipophilic (they act locally and are lipid hormones)

C. Peptide / Proteins:

- Depending on their folding's, they can be active or inactive
- When translated by ribosomes, they can be packaged into secretory vesicles, which can wait. Resulting in pool of vesicles containing the proteins. They are released using exocytosis when needed.
- When the cells receive the message that the protein must be released the vesicles will release the contents by fusing into the membrane and exocytosis to the ECF

D. Amines

- Has an amino group (NH2)
- Derived form amino acids
- Very useful as neurotransmitters like dopamine, epinephrine etc.
- All are hydrophilic except thyroid hormones (they are hydro phobic), thus cannot be packaged into vesicles. Thus they will need intracellular receptors like steroids

E. Purines

- Derived from nitrogenous bases adenine and guanine
- They need a transporter or use exocytosis

F. Gases

- Small molecules and can passively diffuse
- They are able to use direct and indirect pathways
- For example NO, O2 and CO
- They can use gap junctions or go to neighbouring cells using paracrine and autocrine signalling

QUESTION: Neural activity transmission of information via ____ ; in contrast, endocrine activity involves transmission of information via _____?
ANSWER: Electrochemical events; hormones transport to target tissues.

Cell Communication Paths

Are all the messengers going to reach the target in an indirect path?

- Further they travel it will have a decreased chance of being able to bind to the designated receptor

Difference between autocrine and paracrine?
Autocrine - Cell releases the messenger and will act upon releasing cell. *local
Paracrine - Cells will release the messenger and go to neighbouring cells *local
Endocrine - Using the bloodstream to reach very far cells

Chemical property of the messenger will determine the very nature of its job.
Lipophilic molecules can use proteins to travel through the bloodstream.

1. Purine
2. Amine
3. Gas
4. Peptide
5. Eicosanoid

6. Steroid

Receiving the Message
(4 classes)

QUESTION: Which of the following hormones enter cells and have the primary action to increase or decrease mRNA production
ANSWER: Steroids and Thyroid Hormones (because they are lipophilic)

How does the cell decide which gene gets turned on or off?
The promoter. (more on lecture 13 and 14)

QUESTION: Which of these types of proteins is not a potential candidate to act as a transmembrane receptor?
ANSWER: GPI anchored protein (they do not span the whole membrane --- Only on the outer membrane)

Transmembrane Receptors

- Integral proteins that span the membrane
- Ligands binding domain outside the cell
- Ligands are mostly hydrophilic
- Ligands do not enter the cell
- The intracellular domain will interact with other key molecules in the cell, which will help amplify the message.

3 Classes:

1. Ligand gated ion channels (many types) - when bound, changes shape, allows for transfer of an ion
2. Enzyme Receptors (3 main classes) - Binding the ligand turns on the enzyme (found in the intracellular membrane)
3. G-Protein coupled receptors (GPCRs; many types) - receptors that are coupled to G-proteins that will interact with the receptor portion only when ligands are bound to it

Lipid Rafts

G- protein spans the membrane 7 times
Sphingolipids and cholesterol form highly-ordered micro domains or rafts
Rafts are produced in the ER and sent to the plasma membrane

- Receptors are proteins but where are they built? The rough ER
- As you build that, its going to embed the protein right away in the ER = longer saturated fatty acid chains, thus compact
- That's why we have cholesterol to maintain fluidity since these proteins are very tight

Lecture 4:

QUESTION: How can one cell, alter gene expression of another cell?

ANSWER: By sending a chemical messenger that will trigger a signal transduction

Must be bound to a ligand in order to start the signal transduction. Receptor activation leads to relaying and amplifying signal inside the cell. Maximum cellular response with minimal ligand.

Intracellular Receptors

- Lipophilic messengers will be released by an organism.
- It will reach the target cell and find the receptor inside the cell (cytoplasm or nucleus).
- It forms a complex that act as transcription factor.
- Response elements (patterns in the promoter).
- Within the regulatory portion there are patterns that help it to bind to. As long as it is bound it will undergo transcription

Example: Glucocorticoids

- Cortisol is secreted by adrenal glands when under stress
- Distributed by the blood stream (endocrine pathway)
- They are kept inactive because there are no ligands or binding proteins
- It can bind like a lock and key and is now active: now a transcription factor
- It will find among all the genes, that have specific gene sequences and "sit" on that gene and either turn on or off transcription

Transmembrane receptor 1: Ligand gated ion channels

- Ion channels that need to be opened or closed
- They need a messenger to be opened
- They need hydrophilic messenger
- The ligand will be in the extracellular portion
- When the messenger binds the confirmation changes (the shape), which allows the passage of ions - hence the name ligand gated
- You change the membrane potential: a difference of charges inside and outside the cell
- The difference in charge gives potential energy (membrane potential) can be measured in volts
- Goes from potential energy to kinetic energy

Membrane Potential

- Distribution of ions is unequal with more + charge outside and so the inside is less positive
- This uneven distribution creates potential energy (MP) it can be measured in volts

Example: Acetylcholine

- Released by presynaptic cells and post synaptic cell receives it (in muscle contraction)
- Very important neurotransmitter
- They travel down the neuron through action potentials across the axon
- Released by presynaptic cleft and binds to a receptor in a post synaptic cleft (paracrine)
- Confirmation change
- Ach are made of 5 subunits, this opens the gate and sodium and calcium to rush in

- Used in neuromuscular junctions

KNOW WHERE Ach binds in the 5 subunits and what happens

QUESTION: Which of these statements best explains how the message Ach brings is interpreted by the muscle cell
ANSWER: The change in membrane potential in the muscle cell membrane allows entry of more ions, triggering and amplifying the cellular response

How is the muscle receiving the message from the neuron?
How are you going to put an end to that?

- Action potential comes down, and the voltage gated calcium channel opens rushing the calcium in, that triggers the vesicles to release their content (in this case Ach) - since they are hydrophilic they are able to be packaged into vesicles.

- When Ach binds little bit of K+ rushes out and lots of Na and Ca2+ rushes in. Enough change in ions, the membrane potential will become an action potential

- It opens voltage gated sodium channels that allows more sodium inside

- The sarcoplasmic reticulum is sensitive to changes in voltage and concentration, which allows it to release calcium

- Now we have a rise in intracellular calcium concentration

- Troponin is the calcium regulator, and calcium binds to troponin (confirmation change) and reveals myosin binding sites

- The message ends because of acetylcholine esterase (an enzyme that degrades Ach), that tells how we stop telling the muscle to contract
- But for it to relax we stop the calcium reuptake

QUESTION: An amino acid derived ligand binds to a receptor. A cytosolic protein with an SH2 domain was recruited and the cascade initiated resulted in release of calcium from the ER. Which type of receptor was involved?
ANSWER: Tyrosine kinase receptor enzyme

Transmembrane Receptor 2: Receptor Enzymes

- What do they do? They are involved in interacting ligands that have similar objectives. They signal the cell to grow, thrive, survive which are positive messages.
- 3 main classes - focus on tyrosine kinase receptors

All the receptors are monomers (single trans member protein)
The change in confirmation gives greater affinity for like monomers

They form dimers. When they form a dimer the two structures together will have enzymatic activities and phosphorylate on the intracellular domain.
Focus on what happens when the growth factor binds

Growth factor binds to a monomer -does not activate
Its going to form a dimer and auto phosphorylate - now active
It needs to send a message along the cell, so it needs to interact with other proteins that have SH2 domains
When this happens, they are able to recruit other proteins
It can pass the message along to the second messenger as long as it is bound

Ras is a lipid anchored protein, when inactive it is bound to GDP
We need to change the affinity, we need Ras to have less affinity and more affinity to GDP
Sos recruits Ras and creates a confirmation change

CONTINUED REWATCH

Lecture 5:

QUESTION: Why does DAG (diacylglycerol) remain with the membrane after the cleavage PIP2?
ANSWER: because it is a fatty acid anchored protein.

G-Protein coupled receptors (GPCRs)

- Similar to the Ras

cAMP pathway

- The alpha subunit is inactive, so we need to exchange GDP to GTP
- It goes on to the amplifier enzyme
- This allows the ATP to be converted to cAMP

Should PKA be active forever?
In order to shut the PKA down, the regulatory subunit must be taken off the catalytic subunit. Therefore, there should be a stop in the production of these subunits. Also to detach the subunits and free floating in the cytoplasm, the enzyme must be degraded.
The ligand binds to the Gi protein and inhibits the pathway. Therefore, the enzyme is no longer able to produce cyclic AMP.

PIP/IP3 Pathway

- Cleaves and 2 messenger groups are produced during this pathway
- Calcium is required which is obtained from one of the messengers (IP3) -> opens the IP3 gated calcium channel
- Calcium with DAG activate protein kinase C
  These pathways influence other pathways.
  - Very similar to tyrosine kinase pathway

What happens when one ligand bind to different receptors? Will it lead to the same response (no). Norepinephrine (NE) is a catecholamine and bind to andrenergic receptors. It stimulates the nervous system. They interact differently within the cell

Alpha 1: you bind the same ligand and the protein kinase C will activate the calcium channel. E.g. muscle contraction
Inhibitory G-protein inhibits the entry of calcium. E.g. muscle relaxation
Beta 2: activates PKA and phosphorylates the receptor allowing calcium
*look at Coordination of response of glucagon - endocrine system - pancreatic pathway

EXAMPLE: Growth factor NGF - looking at signal transduction

IAP - are inhibitors of apoptosis
Growth factors insures that there are pro-survival situations inside the cell.

- The receptor will not be activated with the NGO
- BAD will not be phosphorylated
- Mitochondria is full and permeability is transformed, cytochrome C is released, and forms the apoptosomes
- Nuclear envelope
- Fragmentation of DNA
- Destroys the interior of the cell
- Scramblase and phosphotidelase on the exterior
- Loss of adhesion and the apoptotic bodies form

QUESTION: Propose at least 2 ways for a cell to put a end to a signal transduction cascade?
ANSWER: Stop the cell from receiving growth factors (how?) Inhibitor enzymes.

- Ligand removed by distant tissues
- Ligand taken up by adjacent cells
- Ligand degraded by extracellular enzymes
- Ligand receptor complex removed by endocytosis
- Receptor inactivation
- Inactivation of signal transduction pathway

TRANSCRIPTION PT.1

Regardless of the complexity of the genome, all of them have similar structure.
QUESTION: True or false - Before transcribing a gene to mRNA DNA must first be replicated
ANSWER: False. An entire DNA does not need to be replicated in order to transcribe mRNA.

VIDEO slide 5 -

The enzyme unzips the DNA, and creates a complementary strand of RNA.
This occurs in prokaryotes and eukaryotes but occurs differently in both.
How does this happen? Which strand is actually being transcribed

QUESTION During transcription from a DNA template in which direction are RNA molecules synthesized?
ANSWER :5' to 3'

RNA strand produced is elongated in the 5' to 3' direction. And the RNA polymerase II reads the 3' - 5' DNA strand (template strand)

The mRNA obtained 5' to 3'

Lecture 6:

In prokaryotes DNA is directly transcribed to mRNA
In eukaryotes DNA is transcribed to a pre-messenger RNA that will be matured into mRNA and then be transported out of the nucleus.

QUESTION: During transcription from a DNA template, in which direction are RNA molecules synthesized?
ANSWER: 5' --> 3'

Which strand does what?

The RNA strand produced is elongated in the 5' to 3' direction

The RNA polymerase II reads the 3'-5' DNA strand (the template strand)
The mRNA obtained is 5' to 3'

The RNA polymerase holo-enzymes first recognizes the promoter region and binds to the full promoter.

How does it start in eukaryotes?
There are different RNA polymerase for different tasks.
The transcription initiation complex (TIC) -

Organization of the Eukaryotic Gene

There is a regulatory region

- TATA BOX:
- Proximal elements ahead of the promoter region
- Regulatory sequences, places where you can change the amplitude or how often you can trigger transcription

Coding regions are exons
Non-coding are introns

QUESTION: What role does the TATA box play in transcription in eukaryotes?
ANSWER: It is important as a promoter element for transcription initiation.

Part 1: Initiation

- Your polymerase is lined up in the promoter region
- Transcription will initiate

- The RNA polymerase has the ability to separate the 2 strands, thus acts slightly like a helicase (unwinds strands), and synthesizes a complementary strand

Part 2: Elongation

- RNA polymerase adds 60 nucleotides per second
- DNA Polymerase does not proofread or make corrections

Part 3: Termination

- How does the polymerase know when to stop?
- There is a sequence at the end, and will be lead to the untranslated region

Prokaryotes

- The sequence itself will form a specific structure or recruit a protein that detaches or stops transcription
- Rho dependant process: ATP dependant unwinding enzyme at 3' end
- Rho independent (intrinsic): GC rich sequences at end
- Hairpin loop which pulls RNA away from DNA

Eukaryotes

- Specific sequences that differ depending on which RNA polymerase
- Example for mRNA sequence AAUAAA to which proteins bind this trigger end of transcription (this is related to the poly acid tail)

5' G-CAP

- At the beginning of transcription, guanine is added backwards
- It will have a methyl group that provides a specific ending to the RNA transcript
- It accomplishes something very important

Cutting out introns is called splicing

QUESTION: When comparing transcription in prokaryotes vs. eukaryotes; which of these does not correspond to a difference between them?
ANSWER: The organization of DNA before transcription.

Poly A Tail -

- 50-200 A added at the 3' end by the poly-A polymerase

QUESTION: Why are snRNP (snurp) molecules important to eukaryotic transcription?
ANSWER: snRNP's are critical to the identification and removal of introns

RNA Maturation Splicing

snRNPs: Small nuclear ribonucleic proteins

They recognize the ends of introns and catalyse their cleavage
The ends of exons after removal of introns will be joined together for a continuous coding sequence
A pre-mRNA can be 27000 NTs mRNA needs 1200 NTs to be translated to a protein of 400 A's

QUESTION: A nucleotide mismatch has been left uncorrected and lies right at the position of the signal sequence for intron 1. What will happen during processing of the pre-mRNA
ANSWER: Splicing will occur normally at the other introns but intron 1 will remain part of the mRNA.

Alternative Splicing

Pre-reading 7:

Prokaryotes will regulate transcription as translation occurs. They regulate it by using something called operons. These genes are under the control of a single regulatory unit.

Operator **-** an on or off switch for gene expression.

LACTOSE OPERON:

There is an upstream of promoter and operator. The regulatory gene is called Lac L, The repressor is bound to the operator region, the RNA polymerase is unable to move along through the operon (but can still bind to the promoter region) it just cannot move forward.

QUESTION: The product of the transcription of an operon is one _____..
ANSWER: Set of related mRNAs that code for functionally related proteins.

2 CONDITIONS IN THE OPERON

A. Lack of lactose

- Nothing is bound to the repressor protein
- Therefore, the repressor protein is bound to the operator region
- Transcription is blocked
- Small amounts of the enzymes can still be produced because of the repressor protein having very little chances of being unbound slightly (comes off randomly)
- But it will want to come back because it has high affinity for the operator region

B. Lactose present

- Lactose will be modified chemically into allolactose, (B-galactosidase does this)
- The allolactose (inducer) will bind to the repressor making it inactive and changing its shape
- Now the repressor protein will not have an affinity for the operator region

Prokaryotes have the ability to make mRNA to translate more than one peptide, whereas eukaryotes can only code for one protein. Mono/poly____ <-- pre-readings

QUESTION: When E.coli are grown in the absence of lactose, proteins involved with lactose metabolism are not produced because _____.
ANSWER: Lac repressor binds to the operator

REGULATION OF GENE EXPRESSION IN EUKARYOTES

QUESTION: In eukaryotes differences in gene expression between various cell types in one individual are best explained by _____.

ANSWER: Enhancers recognized by activators found only in specific cell types

You can have the exact same gene sequence in all cells, but not all cells would use all of those gene sequences.

QUESTION: RNA interference is best described as the phenomenon of silencing a gene post-transcriptionally by _____
ANSWER: miRNA or siRNA binding by complementary base pairing to part of the mRNA

siRNA can bind mRNA and target to degradation = no translation

Micro RNAs are important for regulation of gene expression. Their patterns of nucleotides make them fold over themselves. These are much smaller, these hairpin loops meet up with an enzyme called a dicer, which cuts off the loop portion. This leaves a double stranded short sequence of RNA. Afterwards this complex removes one of the 2 strands, leaving the dicer, the one strand and the protein complex.
This is called the miRISC (induced silencing complex) which halts translation and/or reduces available mRNA.

What if the match is not perfect? What will happen then?

- It can translate again only when it gets rid of the complex
- If the lining up is not perfect, as the mRNA is bound to the complex it will not be able to be translated
- It's a way to control how much mRNA is left and how long it can be present in the cell
- It can reduce the amount translated or save them to be translated later on

TRANSPOSONS
"jumping genes"

They are short sequences that have the ability to separate from the rest of the DNA and insert itself back in further.

- In humans they account for 50% of the genome

The Alu transposons are one of the most important ones
It is associated with diseases like alzheimers, hemophilia and cancer
15% of the genome

RETROVIRUSES + RETROTRANSPOSONS

mRNA will not go on to translation but rather be reverse transcribed back to DNA and inserted back into the genome
There is no proofreading or correction mechanism therefore many mutations can occur

Viruses- Using a reverse transcriptase they convert viral RNA into complementary strand of DNA
The host DNA polymerase makes it into a double strand of DNA by displacing the RNA strand and adding the complementary strand of DNA
Integrase allows to introduce this double stranded DNA into the hosts genome

When transcription occurs, viral RNA will be translated to viral protein which can be used to rebuild virus within the host.

Lecture 7:

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Lecture 8:

Translation - Protein Synthesis

mRNA - directed protein synthesis
For each codon there's a corresponding matching amino acid.

AUG (methionine) - Start Codon
- Not all proteins have methionine for their start codons

UAA, UAG or UGA - Stop Codon

Some amino acids have more than one codon, but there is no codon for two different corresponding amino acids. Therefore, there are synonyms but no ambiguity. Not more than one amino acids for a codon BUT there are more than one codons for an amino acid.

QUESTION: Using the codon table, determine the sequence of amino acids obtained with the following mRNA; 5' AUG-GUA-UAU….
ANSWER: Met - Gly - Tyr - Ser - Thr - Thr
- To make this a more difficult question she can give us the DNA or 3' to 5' or putting a stop codon in the middle

Oscillation/The Wobble Effect

Certain amino acids are associated with more than one codon and the difference lies in the 3rd nucleotide. This is how the synonyms arise. It gives flexibility in the reading the sequence.
- It is the ability to have more than one nucleotide with the sets of codon given

Exons Correspond to Protein Domains

Each domain can have a different role or bind to different location. Example: ligand binding, transmembrane and or catalytic domain.

tRNA - Transfer RNA

The tRNA is the chemical messenger that holds the amino acid. They carry the amino acids at the 3' end and recognize the mRNA sequence in their anticodon region.
tRNA has the amino acid on the 3' to 5' end and on the other side the tRNA has the set of anticodons that is used for reading the mRNA.
BECAUSE of the wobble effect we only need 32 tRNAs to accommodate the 61 codons existing.

QUESTION: How many different aminoacyl-transferases are there?
ANSWER: 20 aminoacyl-transferase because there are only 20 different amino acids.

What is an aminoacyl-transferase?
- An enzyme that attaches the appropriate amino acid onto the tRNA.

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Amino acylation - Specific Pairing

THE CYCLE:

1. ATP and the amino acid bind to the aminoacyl-tRNA synthase and the enzyme catalyzes the joining of the amino acid
2. The energy released by the breakdown of ATP is retained in the aminoacyl AMP molecule (we need this AMP molecule in order to bind the tRNA to the enzyme)
3. The correct tRNA binds to the Enzyme
4. The enzyme transfers the amino acid from AA-AMP to the tRNA forming AA-tRNA and the AMP is released
5. AA-tRNA is released from the enzyme and the enzyme is ready to enter another reaction series!

QUESTION: Which portion (region) of the mRNA is important for binding and stability with the ribosomes?
ANSWER: The 5' G Cap

- The start codon is not in the beginning to protect it from degradation and to allow the subunit to assemble and align it on the right spot.

Ribosomes

They are formed by 2 protein subunits (30S and 50S) and some rRNA
There are 3 different sections within the ribosomal subunits.

The number corresponds to the order in which the process undergoes
E - Exit Site (**3**)

- Exit of growing polypeptide chain out of ribosome

P - Peptidyl Site (**2**)

- Peptide bond is formed

A - Aminoacyl Site (**1**)

- Aminoacyl tRNA arrives with proper amino acid

QUESTION: Why is it important at the start of translation for an initiator tRNA to pair with the AUG start codon on the mRNA?
ANSWER: To establish the correct reading frame.

STEP 1: Initiation

The methionine is the first amino acid and is the only one that goes straight to P site. mRNA is recruited and a large subunit then completes the ribosome.

- Starts at the 5' G cap - and it will move until it lines up to the corresponding start codon.
- When that happens it will be ready to recruit the large subunit
- which leaves you with the empty E site since there are no amino acids yet, and will also leave you with an empty A site.

QUESTION: Once peptidyl transferase forms the peptide bond with the latest amino acid, where is the polypeptide?
ANSWER: On the most recent t-RNA in the A-Site

STEP 2: Elongation

Recognition of codon by aminoacyl-tRNA in the A-site
Peptide bond formation peptidyl transferase by 50S in the P-Site
Translocation P to E and new arrival at A
eIF = elongation initiation factors which aids in binding in the A-Site

QUESTION: Translation is terminated when a stop codon arrives at the _____ Site?
ANSWER: Aminoacyl

STEP 3: Termination

Stop codon is reached and no tRNA with the anticodon
Release factor (RF) protein occupies the A-Site, promoting last peptide bond formation and translocation.
The peptide released after translocation
RF promotes separation of the ribosomal subunits which can reassemble again with another mRNA.

QUESTION: A lipophilic drug is added to cells grown in culture. This drug binds to the E-SITE of ribosomes. What will be the consequence?
ANSWER: Binding of the drug will halt elongation

Polysomes (polyribosomes)

An mRNA can be translated by more than one ribosome at a time giving rise to multiple polypeptides.
This is for free cytosolic ribosomes ONLY!
Doesn't happen every time.

What about in prokaryotes?

There is no nucleus therefore there are no process, splicing or maturing. Therefore, its already a mature mRNA and you can transcribe and translate at the same time.

Lecture 9:

QUESTION: What is the function of the protein-RNA complex called the signal recognition particle?
ANSWER: It temporarily blocks translation it docks the ribosome to the ER membrane.

This process is called Co-translation. At the same time your translating it is transporting into the ER. It guides the whole ribosomal complex to the ER. There are two things. A signal recognition particle and a signal peptidase (an enzyme the cuts peptide bonds. The particle will bind to the receptor and the growing polypeptide will be cut by the peptidase.

The entire translation does not need to complete, just as all the amino acids are bound it is cleaved and released in the lumen of the ER. This peptide will be folded and tagged wherever it needs to go next. Translation will continue all the way to the end, but the import will be in the midst of the process.

In addition to the signal sequence that direct translation to ER there is a stop sequence that halts co-translational import
Translation is completed but not import; resulting in a transmembrane protein.
READ CO-TRANSLATION LATER

QUESTION: What is the function of a chaperone protein
ANSWER: To assist a polypeptide to fold into its 3-dimensional shape

Chaperone Proteins

Protects the protein and facilitates the proper folding of nascent (newly formed) proteins.
There are larger chaperone proteins that are barrel shaped, and they engulf the entire polypeptide AFTER translation. In doing so they isolate the polypeptide creating a new micro-environment allowing them to fold them. This can happen in the cytosol or the Golgi complex.

When you add ubiquitin it tags the molecule and signals a vesicle to package it and secrete the waste out.
At this point it can be packaged to the Golgi complex or to the lysosome depending on if the protein is a dud or good. The cytoskeleton has motor proteins that helps direct the vesicle depending on the content of the vesicle.
Kinesin will bring it to the Golgi and will be fused into the cis face. (anterograde transport)
Dynein (retrograde transport)

From the Golgi it can go anywhere else. As the protein travels through cisternae, the protein is modified and is placed into different compartments (sorted). By the time it reaches the trans face, the vesicles are already packaged that go towards the same destination.

Post transitional modifications
- Beings in ER continues in Golgi

Final locations and functions
- Add sugar to amino acid side chain
- Acetylation: adds acetyl group to N-term for stability
- Disulfide bond: Links S between residues (cys)
- Lipidation: adds lipids ubiquitination: adds ubiquitin (targets for degradation)

Regulated Secretory Pathway: Proteins are packaged but only secreted in response to a specific signal such as neural or hormonal stimulation.
Constitutive Secretory Pathway: proteins are continuously secreted from the cell, regardless of environmental factors. No external signals needed to initiate this process. Much faster

When things go wrong: Mutations

- Errors during DNA replication or transcription

- Mutagenic agents (radiation, chemicals, UV)
- Viruses and microorganisms
- These can lead to different types of changes to genes or how they are expressed

QUESTION: A certain base-pair mutation does not alter the amino acid sequence specified by a gene. This is possible because _____.
ANSWER: of the degeneracy of the genetic code, some base-pair substitutions do not change the amino acid specified by a codon.

QUESTION: What is the result of a nonsense mutation in a base-pair change in DNA?
ANSWER: A codon will be altered from an amino acid coding codon to a termination codon in the mRNA resulting in a shorter polypeptide.

MUTATIONS -

Missense mutation: change in nucleotide sequence leads to change in amino acids.
Mutation can also arise from a deletion or insertion of a base pair - resulting in a frame-shift.

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FINAL

Lecture 19: DNA Replication

Chapter 12.1

Watson and Crick determined that it was a double helix, and the orientation of the DNA. But they were not the key players in determining the genomes.

QUESTION: The type of bond holding the strands of DNA together at the centre (see red arrow) are _____
ANSWER: Hydrogen bonds
During replication and transcription these bonds must be able to break apart.

The alpha helix must be approximately 2nm in diameter and the only possible arrangement was A-T and G-C. They got Rosalind Franklin's data, which gave them measurements to help solve the genome. She shot X-Ray beams at the DNA sample creating a splatter (called X-ray refraction).

QUESTION: In a DNA nucleotide, the phosphate group attaches to the sugar deoxyribose at the _____ position and the nitrogenous base (ATCG) attaches at the _____ position.
ANSWER: 5' : 1'

At the 3' end there is a hydroxyl group that helps bring other nucleotides, bound to the other respective sugar. On the 5' end there is a phosphate group and each nucleotide are attached by a phosphodiester linkage. This is why elongation is always 5' to 3'.

How do we organize the DNA so that its not a big mess?
1. Accessibility
2. The space

Histones wrap the DNA, that structure is called a nucleosome which has a space between other nucleosomes called a linker. Solenoid is this whole structure (like electrical wire). When you need to replicate it can be unwound separated replicated, and then repackaged.

How to make an exact copy
- DNA replication occurs **before** mitosis
- Is mistakes were found in the replicated DNA, it will not be allowed to undergo mitosis

QUESTION: What is the mechanism by which DNA is replicated?
ANSWER: Semi-Conservative

QUESTION: Explain how this was demonstrated by Meselson and Stahl
ANSWER:
1. They grew bacteria in 15N in a heavy medium
2. They transferred some bacteria to 14N (light) medium and the bacterial growth continues
3. Takes samples after 0-20 minutes after one round of replication and 40 minutes (two rounds of replication)

4. Before the bacteria reproduces for the first time in the light medium (at 0 minutes) all DNA (parental) is heavy

Conclusion: This pattern could only have been observed if each DNA molecule contains a template strand from the parental DNA; thus DNA replication is semiconservative.

DNA Replication

The circular genome of prokaryotes is replicated from a single point of origin (ori)
In eukaryotes there can be multiple simultaneous replication forks (autonomous replication sequences)

Replication Requirements

Origin of replication - is the site where replication begins, towards the fork on each strand in each direction. Replication occurs on both parental strands at both ends.

Unwinding of the DNA must occur with the use of helicase enzyme
The longer it stays in a bubble the better replication will be
Single stranded binding proteins that place along the parental strand that prevents the strands from recoiling.
Lots of tension builds up and we use DNA gyrase (topoisomerase) which cuts between nucleotides (small nicks) and relieves tension in the DNA. It is sufficient enough so that DNA can unwind but not enough to break the DNA.

DNA polymerase III will only make a complementary DNA strand to the origin of replication only if it has a double strand.
RNA primase -
Difference between the leading and lagging strand?
Leading strand needs one primer because elongation occurs from 5' to 3' so its replicated in a continuous fashion
Lagging Strand needs more than one primer since it is replicated 5' to 3' but it faces in 3' to 5' (replicating in 3' to 5' is impossible) therefore multiple replication mechanism is required.

Lecture 20: DNA Replication Part 2

Setting up DNA replication:

1. Unwind the DNA
- Helicase separates the two strands

To prevent them from recoiling, the single stranded binding protein separates them.
Topoisomerase will be further out than helicase that will prevent twisting as DNA unwinds (releasing tension).

QUESTION: Which enzyme catalyzes the synthesis of short RNA primers in the 5' direction?
ANSWER: Primase

We need to add primer because DNA polymerase III can only bind to double stranded DNA. To trick the polymerase, it will recognize and fill in the gap. The primase adds 6-10 nucleotides of RNA and detaches and adds primers elsewhere.

QUESTION: If you have a DNA strand with the following sequence, what would be the sequence of the RNA primer that the primase adds? DNA strand: 5' CGCGATCTCGTT 3'
ANSWER: AACG

DNA Polymerase III

Adds it on the 3rd carbon via the phosphate group via 5th carbon of the incoming nucleotide. To make sure DNA polymerase III remains stable when interacting with DNA (it goes all the way to the end) there's another protein that accompanies it. It make sure it stays on, it is called **sliding DNA clamp**. It ONLY contributes to make sure DNA polymerase III is interacting with the template strand.

QUESTION: Which portion represents the lagging strand in DNA replication?
ANSWER: Lagging strand is 3' to 5'
Instead of making it continuous, it must be done in small portions and sequences. Starts the same (origin to the fork) but the primase places the primer and DNA polymerase makes the first Okazaki fragment.

Primers are removed via DNA polymerase I, because we don't want a part DNA and part RNA. It removes RNA nucleotides and replaces them with DNA nucleotides. The difference between RNA and DNA nucleotides are the sugars. That leaves a gap between the first and second fragment (there's no bond between them). Therefore, we need a **ligase** that bonds the two fragments together.

QUESTION: The enzyme DNA _____ removes the RNA primer, replacing it with the DNA nucleotide, leaving a nick between newly synthesized segments that will close with the help of DNA _____.
ANSWER: Polymerase I, Ligase

Now there are fully replicated daughter strand with a parental strand. This is why it is a semi-conservative process. Dispersive = replicates little bits of pieces. Conservative = two huge long strand and cant allow them to recoil and keep them separate and find a matching pair.

Practice drawing a replication bubble and compare with your neighbour

Proofreading: DNA polymerase III can correct mistakes.
Exonucleic
Nucleotide excision repair: other proteins can correct mismatches, but it cannot do it for the one mismatch, a small section must be removed and filled in by DNA polymerase. A small nick will be made therefore; DNA ligase will bond the division.
If it is still mismatch a mutation occurs.

QUESTION: Do you need a primase to set an RNA primer before DNA polymerase I comes to repair the excised portion?
ANSWER: no because on either side of the excised portion the DNA is double stranded.

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Lecture 21: DNA Replication and Cell Cycle

Telomeres: Non-coding nucleotides and extends the length of DNA, protects the coding portion of DNA. DNA gets shorter every time it replicates because ----------
The telomere is a buffer, a long sequence of nucleotides, but because it's a non-coding region it doesn't have a consequence when it shortens.

Additional DNA, the sequence TTAGGG (humans) repeated thousands of times.
With replication that sequence shortens but protects the coding regions of our chromosomes.

The last primer is at the end where the Okazaki fragment is. When the primer is removed there is no polymerase to fill in that gap.

QUESTION: One special problem in replicating eukaryotic chromosomes is the loss of segments at the ends of the linear DNA molecules with each cell division. Which enzyme helps preserve these segments?
ANSWER: Telomerase

Protection in Eukaryotes: Telomeres

1. Chromosome end after primer removal
2. Telomerase binds to the single stranded 3' end of the chromosome by complementary base pairing between the RNA of telomerase and the telomere repeat
3. Telomerase synthesizes new telomere DNA using telomerase RNA as the template
4. Telomerase moves to the 3' end of the newly synthesized telomere DNA
5. Telomerase synthesizes more new telomere DNA using telomerase RNA as the template
6. Telomerase leaves the extended template strand and a primer is added by a primase
7. New end of the chromosome after replication
8. Short, single-stranded region remains after primer removal.

Still left with a 3' overhang - We got further away from our gene so its not worse. Gives you that length of telomere that can be shortened with replication, you reached the hayflick limit and stop dividing.

Working with DNA
Knowing the entire sequence of an organism's genome has opened the door to numerous genomics advances. Here are 3 techniques that are essential to any work on DNA or specific genes:

Cloning Genomic DNA in a Bacterial Plasmid

1. Isolate genome DNA containing gene of interest from cells and cut the DNA into fragments
2. Cut a circular bacterial plasmid to make it linear
3. Insert the genomic DNA fragments into plasmids to make recombinant DNA molecules. Here, the recombinant DNA molecules are the recombinant plasmids.
4. Introduce recombinant molecules into bacterial cells; each bacterium receives a different plasmid. As the bacteria grow and divide the recombinant plasmids replicate, amplifying the piece of DNA inserted into the plasmid.

5. Identify the bacterium containing the plasmid with the gene of interest inserted into it. Grow that bacterium in culture to produce large amounts of the plasmid for experiments with the gene of interest.

How do we cut the plasmid?
- Restriction enzymes

Restriction Enzymes

There are catalogues full of restriction enzymes. These are the molecular tweezers and scissors. They selectively cut the DNA sequence by looking for specific patterns.

E.g. ECOR1 - looks for a specific sequence pattern and cuts two pieces that have overhangs called sticky ends. Now it is easy to take this piece and slide it in and reattach.

How do we reattach the cut piece and the DNA we want together?
- DNA ligase

We need to rebuild the phosphodiester bonds.

Polymerase Chain Reaction (PCR)
- Need primers
- Nucleotides
- Ligase

Forcing a replication of specific sequences of DNA in a test tube

1. Denaturation: Heat DNA containing target sequence to 95 degrees to denature to single strands
2. Annealing: Cool the mixture to 55-65 degrees C to allow the two primers to anneal their complementary sequences at the two ends of the target sequence.
3. Extension: Heat to 72 degrees the optimal temperature for DNA polymerase to extend the primers using the four nucleoside triphosphate precursor to make complementary copies of two template strands. This completes cycles 1 of PCR the end results is two molecules.
4. Repeat the same steps of denaturation annealing of primers and extension in cycle 2, producing a total of 4 molecules
5. Repeat the same steps in cycle 3, producing a total of eight molecules. Two of the eight math the exact length of the target DNA sequence

Cell Cycle Regulation

Mitosis

QUESTION: While in the process of dividing a cell is exposed to colchicine (a drug that interferes with spindle formation). At what stage will mitosis be arrested?
ANSWER: Metaphase

New Cell begins a life cycle

Interphase takes most of the time, it is divided into 3 parts

G1: Signal to grow all the signalling mechanisms that needs to prepare itself for division. Time where ATP production increases by ATP synthase, increase number of mitochondria and ATP production in the mitochondria.

S: Period when DNA replicates and chromosomal proteins are duplicated. This is where DNA replication occurs.

G2: Period after DNA replicates and prepares for cell division

QUESTION: What can the cell use as signals to determine if the cell cycle should proceed normally or be interrupted? Be specific.
ANSWER:

What are these signals the cell is looking for to make the decision?
The cell uses information obtained externally and internally.

Lecture 22: The Cell Cycle

Internal Signals Serving as Molecular Switches
(Cyclins and Cyclin dependant Kinases Cdk)

Cyclins - regulatory proteins
Cyclin dependant kinases phosphorylate target protein
They need to be bound to Cyclin in order to be active.

Concentrations of the kinases remains constant, but the activity fluctuates ----
The concentration rises and falls depending on the cell cycle

Draw diagram of the cell cycle diagram and the relative concentration of the MPF and cyclin.
MPF - Maturation promoting factor = cyclin + Cdk complex

QUESTION: How are the cyclin concentrations reduced?
ANSWER: They are ubiquitinated and degraded.

Check points

There are different checkpoints depending on the cell cycle.

G1/S cyclins (E): leads the cell into DNA replication, and builds throughout the growth phase in order to bind into the CDK and maximize the concentration and activity of the cell.

S-cyclins (A): Binds to Cdk during the S phase and is required for DNA replication

M-Cyclins (A,B): Binds to CDK1 to promote events of mitosis

G1-Cyclins (D): Bind to Cdk 4,6 to promote passage through restriction points in late G1

QUESTION: Cyclin E forms a complex with Cdk2 and allows progression from G1 to S phase. Which statement is correct?
ANSWER: Free Cyclin E concentration is greatest in G1
It only starts being built up through G1 and maximized during G1 because after S-phase it will be degraded.

G1/S restriction point
Quiescence - Cells are differentiated but cell cycle is arrested in G0. This can be permanent or temporary E.g. liver, neurons

P53 -has the ability to place the cell in G0, cell repair is activated and after the DNA is repaired it resumes G1/S

P53 stimulates the various proteins (don't need to know these specific proteins)
Which inactivates CDK2 - inhibiting S phase
And inactivating CDK1 - inhibiting M phase

QUESTION: After which checkpoint is the cell committed to go on through the M phase?
ANSWER: G1

Right after m-phase the DNA condenses ---

QUESTION: Which of the following is an important external signal to animal cells to not begin mitosis
ANSWER: contact inhibition

The M Checkpoint

During metaphase all the chromosomes are aligned on the equatorial plate using the kinetochores.
When microtubules grow there are 2 types

1. Kinetochore - bound to the chromatids and push and pull the chromosome at the metaphase plate.
2. Astrol - don't bind directly to kinetochore

All the chromosomes are aligned at the metaphase plate and they are pushed and pulled. The length of the microtubules will detubulize.
At which end do microtubules grow?

- At the + end (beta tubulin and has GTP)
- You lose dimers in the - end

The dimers are lost in the + end where the kinetochore is during the beginning of anaphase when the chromosomes are split.
Which motor walks to the + end normally?

- Kinesin (anterograde)

However, the molecular motor is pushing the kinetochore forward while the tubulin is being removed therefore it is anterograde. (NOT DYNEIN)

Non disjunction/ non segregation

During anaphase one pair of chromosomes is not able to make it during metaphase. Therefore, the two new cells die because one copy has an extra pair and the other cell has one less.

QUESTION: Cytochalasin B is a drug that blocks the function of actin. Which aspect of the cell cycle will mostly be disrupted?
ANSWER: cleavage furrow formation and cytokinesis

Lecture 23: End of Cell Cycle

What if the cell fails to meet those conditions? (P53 fails)
DNA damage (mutations, misreplication, telomere length)
P53 is responsible for triggering apoptosis!
Triggers miRNA because it wants to limit translation of the mutated strand of the DNA.
Therefore, if it fails DNA damaged G0 and trigger apoptosis

Otherwise the damaged DNA: G0 and cell repair is activated if it was unsuccessful it goes back to G0 and triggers apoptosis.

P53 the Cellular Watchdog

When we want to initiate cell death. P53 is a protein that is a gene regulatory protein that influences the expression of certain genes. More like an activator or like an enhancer, an plays an important role for key proteins are transcribed translated and formed so that it can keep the apoptotic cascade going.

QUESTION: What is an oncogene?
ANSWER: An altered gene that promotes uncontrolled cell division.

P53 is a gene regulatory protein and it is a tumour suppressor protein. It prevents uncontrollable division.
P53 suffers missense mutation in DNA binding domain or leading to change in folding.
P53 can have a mutation (missense) which wont do its job properly. When a cell is no longer able to pass through G1and you need to signal it to go to apoptosis. P53 will be unable to promote that cell to go to apoptosis. This is when we call it an oncogene because this should not be dividing and replicated and alive.

What is the key difference between somatic cells and germ cells?

- In somatic cells the cells don't need to be reproduced often. Telomeres shorten
- In germ cells, are the gamete cells. You want the telomerase to be still active

QUESTION: Besides the ability of some cancer cells to overproliferate, what else could result in a tumor?
ANSWER: Lack of appropriate cell death

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Final Questions REVIEW

1. Where are lipids synthesized

Smooth ER

2. What are plasmodesmata?

Plasmodesmata are small channels in the cell wall and the plasma membrane for the transport of molecules.

3. Where is keratin synthesized?

Free cytosolic ribosomes because keratin is what makes up intermediate filaments

4. Name the type of membrane transport: Thanks to the existing Na+ gradient towards the cytosol H+ can be released and raise intracellular pH.

Anti-port - secondary active transport

LONG QUESTION

You have skipped breakfast so your blood levels of glucose are quite low. Focusing on the cell communication between the pancreas and the liver:

A. Name and identify the class of the messenger that will be sent
B. Describe the steps involved in this mechanism
C. How the primary messenger is released
D. Which Cell communication path
E. Which signalling cascade
F. End your answer with the desired cellular response (be sure to include any elements of transport)