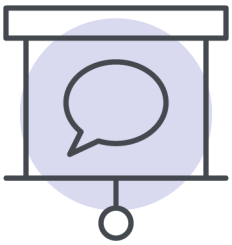
UOttawa

BIO1140
FINAL EXAM
STUDY GUIDE



Lecture Notes

Topic 1 Notes: Introduction to Cell Biology

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Objectives:

What is a cell?

What is it made of? Name the different organelles and the roles/functions for each

What are the different scales/sizes for cells and organelles?

Explain the theory of the cell and endosymbiosis

What are the main groups or types of cells and how are they different?

Understand different types of microscopy and their requirements for use in cell biology

The Discovery of Cells:

Mid 1600s: Hooke built a rudimentary device to allow him to see smaller things that could not be seen with the naked eye

- He first looked at cork and saw similarly shaped, small, organized compartments – he called them cellulae
- What Hooke observed were really just dead cells and their remaining cell walls

At the same time, van Leeuwenhoek was working on a similar device – the first microscope

These devices allowed scientists to look at the world around them an to collect data

- After 200 years of gathering information, scientists were able to come up with the tree of life and establish cell biology

Cell Theory:

Mid 1800s

All organisms are composed of one or more cells

The cell is the basic structural and functional unit of all living organisms (Schwann)

Cells arise only from division of pre-existing cells (Virchow)

Cell Features:

Diversity:

- Wide range of sizes
- Simple or complex shapes – related to function
- Generalized or very specific role

Common characteristics:

- Components
- Basic chemistry
- Metabolism (uses ATP)
- DNA

Cells can remain individual (ex. In blood) or assemble together to form functional tissues and organs

Cell Size and Scale:

Relevant units:

- 1 μm = 10^{-6} m
- 1 nm = 10^{-9} m

Typical cell sizes:

- Prokaryote: 1 – 5 μm
- Eukaryote: 10 – 100 μm

Relative size (largest to smallest):

- Length of some nerve and muscle cells (~1 m)
- Chicken egg (~ 0.1 m)
- Frog egg (~ 1 mm)
- Plant and animal cells (10 – 100 μm)
- Nucleus, most prokaryotes, mitochondrion (1 – 10 μm)
- Smallest prokaryote (~ 100 nm)
- Viruses (<100 nm)
- Ribosomes (~20 nm)
- Proteins (~10 nm)
- Lipids (~5 nm)
- Small molecules (<1 nm)
- Atoms (~0.1 nm)

Microscopy:

Microscopy: a technique for producing visible images of objects that are too small to be seen by the human eye

Light microscopes: use light to illuminate the specimen

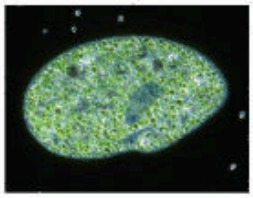
- Have lower resolution

Electron microscopes: use electrons to illuminate the specimen

- Have higher resolution

Light microscopy
Micrographs are of the protist *Paramecium*.

Bright field microscopy: Light passes directly through the specimen. Many cell structures have insufficient contrast to be discerned. Staining with a dye is used to enhance contrast in a specimen, as shown here, but this treatment usually fixes and kills the cells.



Dark field microscopy: Light illuminates the specimen at an angle, and only light scattered by the specimen reaches the viewing lens of the microscope. This gives a bright image of the cell against a black background.

Phase-contrast microscopy: Differences in refraction (the way light is bent) caused by variations in the density of the specimen are visualized as differences in contrast. Otherwise invisible structures are revealed with this technique, and living cells in action can be photographed or filmed.

Electron microscopy
Micrographs are of the green alga *Scenedesmus*.

Transmission electron microscopy (TEM): A beam of electrons is focused on a thin section of a specimen in a vacuum. Electrons that pass through form the image; structures that scatter electrons appear dark. TEM is used primarily to examine structures within cells. Various staining and fixing methods are used to highlight structures of interest.

Nomarski (differential interference contrast): Similar to phase-contrast microscopy, special lenses enhance differences in density, giving a cell a 3D appearance.

Fluorescence microscopy: Different structures or molecules in cells are stained with specific fluorescent dyes. The stained structures or molecules fluoresce when the microscope illuminates them with ultraviolet light, and their locations are seen by viewing the emitted visible light.

Confocal laser scanning microscopy: Lasers scan across a fluorescently stained specimen, and a computer focuses the light to show a single plane through the cell. This provides a sharper 3D image than other light microscopy techniques.

Scanning electron microscopy (SEM): A beam of electrons is scanned across a whole cell or organism, and the electrons excited on the specimen surface are converted to a 3D-appearing image.

<u>Why are cells small?</u>

SA:V ratios

- As cells expand, the surface area and the volume don't increase at the same rate (by the same ratio)
- The larger the cell grows, the greater the discrepancy between the SA and the V becomes
- Smaller cells have greater SA:V ratios
- It's more efficient to have a high number of smaller cells occupying the same space than to have fewer large cells – maintains a larger SA:V ratio

Rates of diffusion

- Larger SA:V ratio makes it easier to maintain the rates at which molecules diffuse into or out of the cell
- It allows there to be more surface area for substances to diffuse through
- Cells rely on diffusion to get in nutrients and let waste out, so a high SA:V ratio requires less effort for these substances to diffuse

Adequate concentrations or synthetic capacity

- Diffusion occurring easily allows the concentrations of substances inside the cell to be more consistent
- Increasing the cell's size can cause concentrations to differ throughout the cell – this can affect how the cells can perform their functions
- [H+] can change, which changes the pH
    - Proteins operate differently in different pH's

The size of a cell is crucial to that cell's ability to carry out its functions and to survive

- However, not all cells are the same size and not all cells of the same type are the same size

The Tree of Life

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The Tree of Life:

3 domain: bacteria, archaea, eukarya

Prokaryotes:

- Unicellular (bacteria, archaea)
- Stay small – roughly the size of a mitochondrion (1 – 5 μm)
- Keep it simple
- Highly adaptable (extremophiles)
- Small genomes (10^6 base pairs) – easier for prokaryotes to change their genome to adapt because their genomes are smaller
- Bacteria and archaea:
    - Fairly similar, especially in appearance
    - Archaea used to be part of the bacterial domain
    - Archaea has quite a few differences with bacteria and quite a few similarities with eukarya
    - Bacteria, archaea, and eukarya have ribosomes, but they all have different ribosomes
        - Ribosomal DNA shows that archaea and eukarya are very similar
    - The phospholipid bilayer of archaea are very similar to those of eukarya

Eukaryotes:

- Unicellular (protists)
- Multicellular (plants, fungi, animals)
- Larger (10 – 100 μm or larger)
    - Can be 5 – 20x the size of a prokaryote
- Elaborate membranes allow compartments to organize cellular functions – has internal compartments*
- Transport systems
    - Requires energy and a transport system to move substances around in the cell due to specialization of certain elements
    - Specific proteins can use ATP to transport materials across the cell
- Larger genomes (10^6 to 10^9 base pairs) with associated proteins
    - Linear, often in multiple pieces (chromosomes)
    - DNA is encompassed within a double membrane in the nucleus
- Don't have the ability to adapt
    - Becoming multicellular makes it harder to adapt

    - The more complex, the more specificity, the more things involved – everything must agree to change in the same manner and at the same time in a collaborative effort
        - This is harder to do with multiple cells vs. one cell
- Lower SA:V ratio

Viruses are not considered to be a form of life – viruses aren't cells (Cell theory: the basic unit of life is the cell – no cell, no life)

- They are an organism on the edge of a life form

Main Components of a Bacterial Cell:

Cell membrane:

- Composed of three layers
    - Plasma membrane
    - Cell wall
    - Capsule (glycocalyx): can be very rigid or rather slimy – has a protective role
        - Difference between rigid or solid is due to the amount and the kind of sugars that compose the capsule

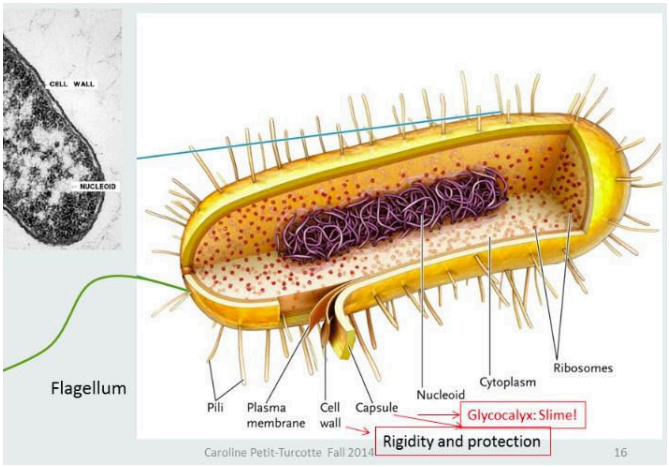
Ribosomes compose most of the cellular cytoplasm

DNA is contained in a central area – the nucleoid, but it is not bound by a membrane (therefore, it is not a nucleus)

Pili and flagellum: made out of protein

- Pili: composed of piline protein
    - Shorter
    - Whipping motions
    - Anchoring, projection, small movements
- Flagellum:
    - Longer
    - Wave-like motion
    - More efficient in terms of mobility

Prokaryotes have no mitochondria



Typical Animal Cell:

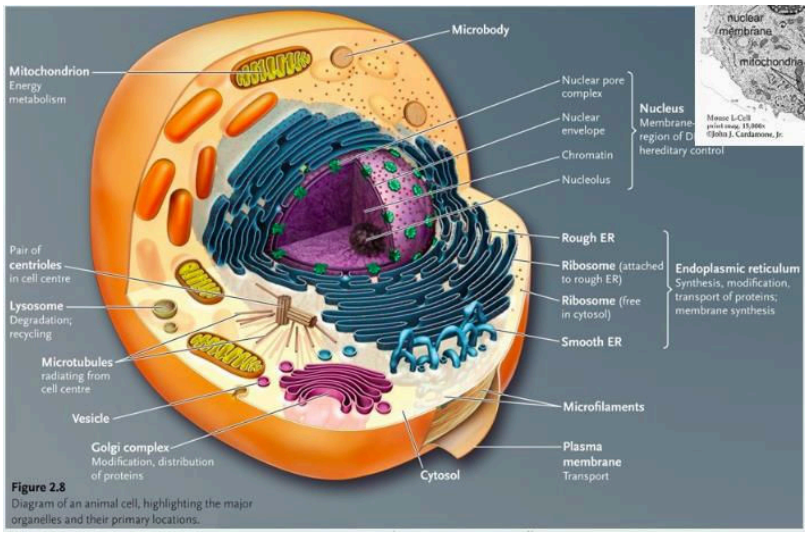
Has the centriole: manages microtubules and the cytoskeleton

- Important in cell division

No capsule – single shelled plasma membrane instead

Transport mechanism (needed due to the compartmentalization):

- Motor proteins move along the microtubules and microfilaments
- Vesicles – carried by motor proteins



Typical Plant Cells:

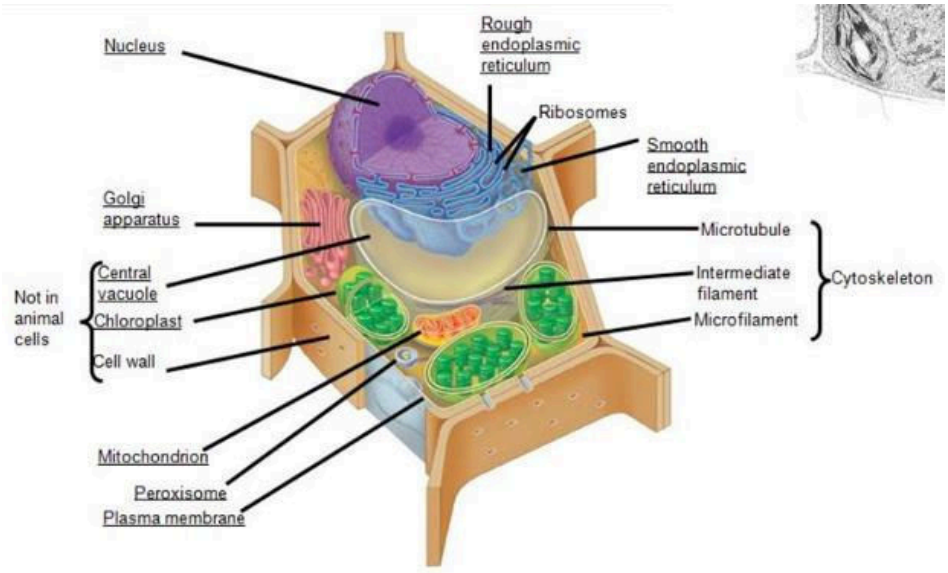
Has a cell wall composed of cellulose

Has chloroplasts

Still has mitochondria

Large central vacuole

Peroxisome: similar to lysosome but carries out oxidoreductive activities, not specifically digestive activities



What distinguishes eukaryotes from bacteria and archaea?

1. Nuclear envelope: separates DNA from the rest of the intracellular components
2. Membranous compartments with specific roles (ER, mitochondria, etc.)

Also, all eukaryotic cells have some sort of energy transforming unit (mitochondria, chloroplast)

Theory of Endosymbiosis:

The common ancestor to all eukaryotes must have been a simple, single celled organism
It became complex when one prokaryote engulfed an aerobic bacteria – became the mitochondria
The mitochondria became part of the host organism
Endo = inner, symbiosis = mutually beneficial

- Host gets energy, mitochondria gets protection

Theory of endosymbiosis: the mitochondria got engulfed and the cell gained complexity
It is thought that chloroplasts were incorporated in the same way – helped us get more complex organisms that are an entirely different domain → eukarya
NB: mitochondria and chloroplasts were their own organism that got incorporated into the cell
Plants and some protists have chloroplasts and mitochondria
Animals, fungi, and some protists only have mitochondria
*Evidence:

- Morphology: shape and size of mitochondria and chloroplasts are similar to prokaryotes
- Reproduction: only reproduce by binary fission
- Genome: circular DNA
    - The also have all the necessary components to synthesize proteins and are capable of synthesizing a small number of proteins
- Transcription and translation: have the machinery
- Electron transport: double membrane with ETC
- Sequence: bacterial branch on the tree of life
    - Mitochondria = proteobacteria
    - Chloroplasts = cyanobacteria

Current examples of endosymbiosis in nature:

- Fungi and plant roots
- Bacteria and digestive system
- Spotted salamanders and green algae: algal cells infiltrate the embryos to use the N2 produced by the salamanders and give them O2 in return

Eukaryotic Organelles

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Eukaryotic Cell Organelles:

Subcellular compartments within the cytosol surrounded by a membrane

- Ribosomes are the only organelle without a membrane

Their functions are varied, and depend on the cell's role

Ribosomes:

Found on the endoplasmic reticulum, scattered within the cytoplasm, and on the nuclear membrane

Ribosomes are produced in the nucleolus (area within the nucleus) and then leave the nucleus

- They leave the nucleus in stages, and that's why they're found on the nuclear membrane

Free ribosomes: within the cytoplasm

- Proteins from free ribosomes always remain within the cytosol

On the ER:

- On the RER
- Proteins from the RER always stay in a membrane (through the membrane in the endomembrane system and the to the PM)

They are found in the cytoplasm or on the RER depending on which proteins they build

- Free ribosomes produce soluble proteins within the cytoplasm
- RER ribosomes latch on to the ER wile translation continues
    - The protein will end up in the Golgi apparatus to be processed to be secreted or sent somewhere else in the cell
    - These proteins go through the ER because they need a maturation process

The Nucleus:

Protects/stores DNA

- DNA codes for proteins – genes need to be turned on

Nuclear pores allow transcription factors (ex. Hormones, proteins, steroids, small molecules) to enter the nucleus to turn on genes and trigger gene activation/inactivation

- Steroid hormones can diffuse straight through the membrane, but everything else must go through the pores

Nuclear pore complexes exclude substances from the nucleus by:

1. Size
2. By affinity (physical, chemical, properties)
   They protect against entry into the nucleus

RNA leave through the pores – they are 2-way

Nuclear pore complexes are formed by proteins embedded in the nuclear membrane

Nuclear envelope: serves to protect the DNA

Endoplasmic Reticulum:

Starts from the nucleus and goes towards the center of the cell

Has cisternae (folds)

Two kinds: rough and smooth

- RER's membrane is studded with ribosomes, and synthesizes proteins within the lumen (ER's inner space)
- RER is a network of flattened membranes, while the SER is more tubular in shape
- SER synthesizes lipids and helps detoxify the cell

Golgi Complex:

Comes after the ER

Like the ER, it is also composed of folds of the membrane

Proteins from the ER come to the Golgi

Directional organelle:

- Cis face: "entry face"
  - The side where vesicles from the ER fuse
- Trans face: "exit face"
  - The side where vesicles leave from the Golgi
- Sending things through the trans face instead of the cis face can cause substances to be repurposed/recycled

Functions:

- Modifies proteins at the beginning, folds them, can ionize them, and adds functional groups after the cis face
  - Functional groups give the proteins their chemical properties, can act as "labels"
- Sorts and packages proteins towards the end, right before the trans face
- Helps group the proteins together – the same kinds have the same destination and will end up in the same vesicle

*Most important: adds functional groups

Golgi "finishes the produce" – the protein

Lysosome:

Only in animal cells

The digestive unit of the cell – sorts and breaks down substances

Acidic environment containing digestive enzymes

- Contains 30 – 60 different enzymes that will break down macromolecules
- The pH in lysosome is more acidic than in the cytosol

They bud out from the Golgi apparatus

Mitochondria:

The energy source of the cell

Parts:

- 2 membranes: inner and outer
    - Inner: has folds (cristae) to increase the surface area
        - More surface area allows a larger gradient to be created, allowing more ATP to be produced – without the cristae, the mitochondrion would need to be huge to produce the same amount of ATP
        - Also has the machinery required to build a proton gradient to fuel the phosphorylation of ADP to ATP

Plastids:

Only in plant cells

Amyloplasts: store energy in the form of starch

Chromoplasts: store pigments

- Ex. Allows leaves to change colour – they accumulate the hue

Chloroplasts: produce glucose and undergo photosynthesis

- Composed of a series of disks (thylakoids) that form a functional unit called a granum
- Stroma: fluid interior
- Also has an inner and outer membrane

Central Vacuole:

Only in plant cells

Acts like the plant's lysosome and more

Serves as a digestive area – can contain enzymes

Mostly water

Surrounded by the tonoplast (a membrane) that helps the vacuole contribute to turgor pressure

Cell Wall:

Contains plasmodesmata: small perforations across the cell wall

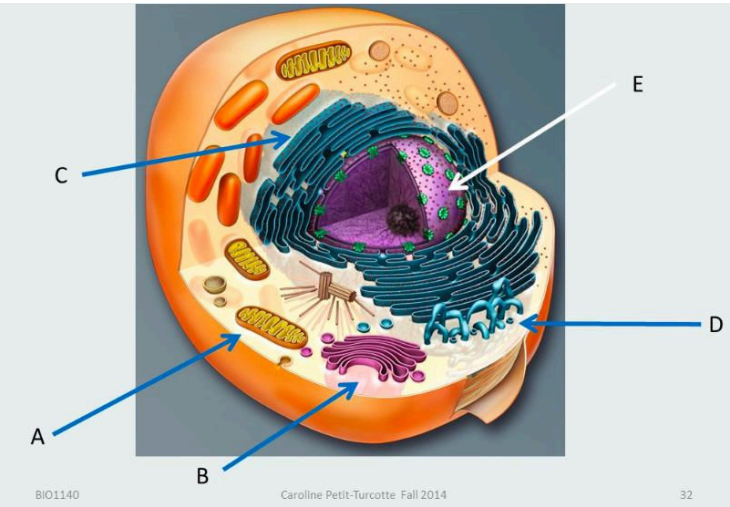
- Helps connect one cell to another
- Allows cells to communicate with each other
- Mostly allows ions or small molecules to cross
    - Na+, K+, Ca2+ - important communication ions

Cytoskeleton:

Organelles: have a specific function to accomplish and are membrane bound (except for ribosomes) – so the cytoskeleton is not an organelle, but it is an essential sub cellular compartment

Anchors cellular compartments – nothing is free floating

Creates "roads" for motor proteins



A: Mitochondrion
B: Golgi apparatus
C: Rough endoplasmic reticulum
D: Smooth endoplasmic reticulum
E: Nucleus/nuclear membrane

Organelle	Role	Cell types
Nucleus	Store DNA (w. membrane)	E
Nucleoid	Area (no membrane) where DNA stored	P
Nucleolus	Assembly of ribosomes	E
Mitochondria	Energy metabolism	E
Lysosomes	Degradation and recycling	E
Rough endoplasmic reticulum (RER)	Protein synthesis, maturation, sorting	E
Smooth endoplasmic reticulum (SER)	Lipid synthesis, detoxification	E
Ribosome	Protein synthesis (translation)	E + P
Golgi complex	Protein maturation and sorting	E
Cytoskeleton	Structure, support, signalling	E
Central vacuole	Digestion, cell growth, support, storage	E (Plants, fungi)
Chloroplast	Photosynthesis, starch storage	E (Plants)
Membrane	Specific differences	E + P
Cytosol	Aqueous media within cell	E + P

Topic 2 Notes: Macromolecules Carbohydrates

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Macromolecules Objectives:

Understand and evaluate polarity of molecules

What are the types of chemical bonds relevant to biology

Discuss the importance of water in biology

What are the main classes of macromolecules, their structures, and their roles in cells

Carbohydrates:

Mono, di, or poly saccharides

- All sugars can come as monomers or as polymers (polysaccharides)

Fuel sources for chemical energy

Structural molecules

Cellular recognition and communication

- Sugars attached to proteins on the cell surface help determine cell identity (Ex. Blood types)

*Sugars can come in two conformations:

- The shapes are determined by the location of hydroxyl groups
- Alpha conformation: both hydroxyls are on the same plane (ex. Both on top/bottom)
- Beta conformation: the hydroxyls are on different planes (one is on top, one is on bottom)
- Conformation determines what form/shape of polymer is obtained

Polysaccharides:

From 3 to millions of single sugar units attached together

- Oligosaccharides (3 – 10 monomers)

3 functions:

- Storage
  - Glycogen (animals)
  - Starch (plants)
- Structure
  - Chitin (animals)
  - Cellulose (plants)
- Recognition

Proteins

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Proteins:

Polymers of amino acids

- 20 essential amino acids = proteinogenic amino acids
- There are other aa's that are derivatives of these 20

Formed with peptide bonds linking the aa's

Units are Daltons (Da)

- 1 Da = 1 H
- 1 aa is ~110 Da

Amino Acids:

Central asymmetric carbon (asymmetric – each bond is formed with a different substance)

The C has an R side chain, an amino group, a hydrogen, and a carboxyl group that are always oriented in the same way

The R side chain is the difference between the amino acids

Aa's attach to each other in an oriented manner – the covalent bond between two aa's always form in the same orientation

- Always with two hydrogens of the amino group (of the upcoming aa) with the oxygen of the carboxyl group (of the previous aa)
- Forms a peptide bond
- The same reaction is repeated to form proteins
- This occurs in ribosomes

pH in a cell is typically 7.2 – most aa's will be in their ionized form

Aa's are sorted by polarity due to the differences in radical groups (R chains)

- Nonpolar
- Polar
  - R chains with highly electronegative atoms tend to be polar
- Electrically charged
  - Contain R chains with ions

Why is polarity important?

- The cell has a high water content – mostly a polar environment
- Polar aa's are hydrophilic and are water soluble – they can diffuse easily and travel, retain their shape, exercise their function

Levels of Protein Structure:

Primary: same backbone, different R groups; sequence of aa's

Secondary structure: formation of alpha helixes and beta pleated sheets

- Alpha helix: very flexible, very solid
- Beta pleated: rigid, less soluble; folds on each other

Tertiary structure: bonds are being formed between the folds as the protein is being folded

- H bonds or disulphide bonds

Quaternary structure: 2 or more proteins (subunits) join together to form a larger protein

- Ex. Hemoglobin = 2 alpha subunits + 2 beta subunits

Structure is important for function

- Changes in structure can affect function
- Ex. Sickle cell is caused by the change of just one amino acid – causes long non-flexible rigid protein chains that are attached to each other and have low oxygen carrying capacity
    - Starts changing the shape of blood cells

<u>Functions of Proteins:</u>

Hormonal proteins: coordinates an organism's activities

- Ex. Insulin

Receptor proteins: response of cell to chemical stimuli

- Ex. Receptors built into the membrane of a nerve cell detect signaling molecules released by other nerve cells

Contractile and motor proteins: movement

- Ex. Actin and myosin

Structural proteins: support

- Ex. Keratin

Enzymatic proteins: selective acceleration of chemical reactions

- Ex. Digestive enzymes catalyze the hydrolysis of bonds in food molecules

Defensive proteins: protection against disease

- Ex. Antibodies

Storage proteins: storage of amino acids

- Ex. Casein, the protein of milk
- Ex. Ovalbumin ins egg whites

Transport proteins: transport of substances

- Ex. Hemoglobin

Lipids/Nucleotides

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Lipids:

Small to large hydrophobic molecules

NB: they are not polymers

4 main groups sorted based on their structures (not functional groups):

- Fatty acids and triglycerides
- Phosphoglycerolipids (phospholipids)
- Glycolipids
- Steroids

Fatty Acids:

Very good for the cell

A very long chain

- Length of the chain gives that fatty acid its properties

Has a polar region (the carboxyl group) and a non-polar region (a hydrocarbon chain)

- The hydrocarbon chain can be saturated (palmitic acid) or unsaturated (palmitoleic acid)
- Saturated: only single bonds
- Unsaturated: has double bonds
    - Causes the fatty acid to bend
    - Bends cause it to be difficult to easily line up the fatty acids

Amphipathic: has a polar region and a non-polar region

Triglycerides:

Triglyceride = glycerol + 3 fatty acids

Stored in adipocytes (especially in the liver)

- Adipocytes are specialized – they have reorganized their structure to be able to better store fats
- They flattened the nucleus and moved it to the periphery to create more space for storage

Function:

- Energy reserves
- Thermal regulation and isolation
- Protection against mechanical pressure

Can be saturated or unsaturated

- Saturated: solid at room temperature (ex. Butter)
    - More can be stored in the same amount of space

- Unsaturated: liquid at room temperature (ex. Olive oil)

Phosphoglycerolipids:

Phospholipids = glycerol + 2 fatty acids + hydrophilic head (phosphate and other compounds)

- Allows part of it to be hydrophilic, unlike in triglycerides

Amphipathic

Make up the cell membrane (and organelle membranes)

- Amphipathic molecules placed in water will spontaneously arrange in a bi-layer
- They tend to assemble with each other with the hydrophobic tails facing one side and the hydrophilic heads facing the other
- This conformation will be observed in membranes, micelles, liposomes (and lipoproteins)
    - Micelles: one layer of phospholipids
    - Liposome: has the bilayer, like a vesicle
        - Can be used to transport substances that otherwise would not have been favorable in the cell's environment
        - Has the outer polar heads – can easily travel in polar environments
        - Anything that prefers to be in a lipid environment can be "hidden" in a liposome to be transported
- This conformation is characteristic of eukaryotes

Glycolipids:

Lipids to which a mono or oligo saccharide is fixed

- Added sugars instead of polar head groups

Glycerol or sphingosine backbones

Part of ECM

Important in cellular recognition (ex. Blood types)

Helpful for microvilli:

- Cytoskeleton supports the growth of the projection
- Microvilli increase surface area and slow down the food to maximize absorption
- The glycocalyx helps slow down the food, makes passage less fluid

Steroids:

Steroids = polar end + sterol core (4 aromatic nonpolar rings) + carbon tail (nonpolar)

- Carbon tail can be changed, steroid will always have the sterol core – can have functional groups added to it

Only in eukaryotes

Main steroid in vertebrates: cholesterol

- In plasma membrane an lipid rafts
- Is able to withstand being within the bilayer because it's just as hydrophobic as the fatty acid tails and the small polar end can stay with the phosphate heads

Part of the plasma membrane

- Cholesterol can be inserted as a buffer to modify the fluidity of the membrane

Precursor for many hormones

<u>Nucleotides:</u>

Bases of DNA and RNA

Nucleotide = nitrogenous base + sugar + phosphate group

5 possible nitrogenous bases: 4 in DNA and 4 in RNA

Difference between DNA and RNA is the sugar that is used

Phosphate + sugar forms the backbone for the helices in DNA or RNA

Nucleotides assemble to form polymers (nucleic acids)

Topic 3 Notes: Cytoskeleton and the Extracellular Matrix

Cytoskeleton

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Objectives:

How is the cell's interior organized?

How is each component of the cytoskeleton formed?

What are the main types of cell-to-cell interactions?

Cellular motility – how is it accomplished?

What is the extracellular matrix made of, and why is it important to cells?

The Cytoskeleton:

Cytoskeleton = microtubules + microfilaments + intermediate filaments

Extensive

Branches out from the nucleus

Not found in the nucleus

There are more microtubules in the center and they radiate towards the periphery of the cell, but they don't quite make it all the way

- They are involved in cell division – makes sense that microtubules

The periphery of the cell is mostly microfilaments

Intermediate fibers tend to stay towards the middle of the cell

Functions include:

- Provide structure and support
- Intracellular transport
- Position organelles within the cell
- Generate force for cell movement
- Contribute to cell division

Is a dynamic, 3D network

Found in eukaryotic cells (although bacteria have similar structures)

Cytoskeletal Fibers:

3 types:

- Microfilaments
  - Smallest
  - Most flexible
- Intermediate fibers
  - Intermediate size
  - Intermediate flexibility

- Microtubule
  - Largest of the three
  - Most rigid

Monomers of proteins connected end to end to forms polymers
Connected by weak bonds to be able to reassemble easily, but also to be able to disassemble quickly – no covalent bonds between subunits
Different motor proteins are mechanoenzymes that use ATP hydrolysis as a source of energy – walk along the cytoskeleton
Diversity comes from combination of fibers, proteins, and proportions
Protects the cell by allowing it to change its shape in order to be able to withstand the forces being applied on it
Role in movement:

- Cytoskeleton as a road
  - The road along which motor proteins travel
- Cytoskeleton reorganizes network
  - Pushes, pull, changes direction of movement
  - Can convince the membrane to change its shape, grow in a particular direction
  - Directs how to move the entire cell (flagellum, cilia) or parts of the cell
  - Phagocytosis: due to movement of the cytoskeleton fibers
- Proteins pull on network

<u>Microtubules:</u>
Basic units are tubulin dimers: composed of an alpha and a beta tubulin

- Tubulin is a globular, soluble protein

GTP binding site location confers polarity

- Causes there to be a positive (+) end on the beta tubulin where the binding site is easily accessible
- There is a negative (-) end on the alpha tubulin because it's binding site is hidden

Originate from a central point, the microtubule organization center (MTOC) at the centrosome (near nucleus, at the – end)

- However, some can originate in the cytoplasm

Formation:

- Tubulin dimers assemble as a single row (protofilament)
  - Not all protofilaments have to be the same length – the growing edge can be jagged

- These will stack to form sheets
- Their 3D structure assembles 28° from each other, creating the tubule shape
- Forms a hollow tube
- The dimers assemble so that there is always a + end and a – end so that the GTP binding site is readily available, which is phosphorylated to allows the dimers to attach/detach
    - The position of the GTP binding site makes it more favorable to orient the dimers in this way
    - Allows the directional growth
    - The + end is the growing end

Microfilaments:

Not hollow

Thin fibers, 5 – 7 nm in diameter

Made of two strands of actin (a globular protein)

- Has a + end: the barbed end with the COOH
- Has a – end: the pointed end
- Has ATP instead of GTP binding sites

Forms a right hand helix

- NB: it is not two strands that wrap around each other – actin subunits are sequentially added to each strand

Growing end: grows where the carboxyl end is

ATP encourages the linkage

Have the ability to branch, unlike microtubules

Can form bundles and branches – leaves room for mechanoenzymes to interact with more than one filament

Involved in cytoplasmic streaming

Intermediate Filaments:

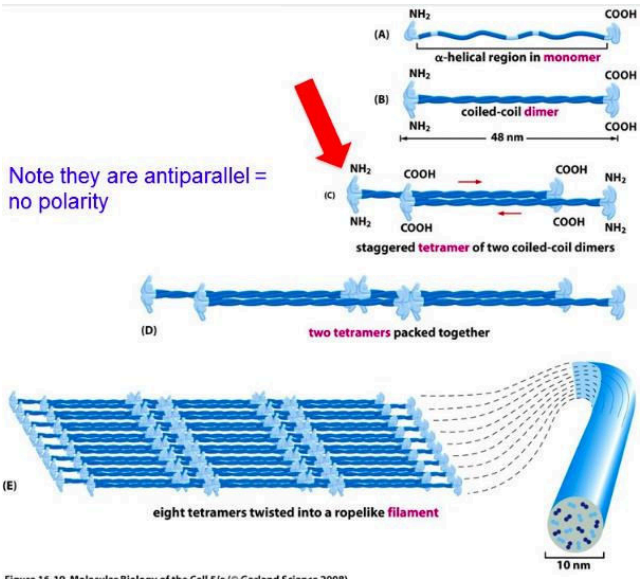
Formed by assembly of various proteins such as keratins

Long, rigid, fibrous proteins that assemble together and braid – like a rope

Formation:

- Form coiled dimers
- Attach two coils in a staggered manner to form a tetramer

- Attach two tetramers together end to end
- Twist eight tetramers together to form a package of 16 dimers
- This structure (coiled and staggered) is what gives it both strength and flexibility
- NB: assembled antiparallel (no + or – end)



Assembly is as a helix of proteins, which formed a coiled-coil

Not hollow

Strong, can withstand a lot of stress (torsion, shearing, compression)

- Helps protect against forces

Very flexible – gives opportunity for the cell to change shape and withstand the mechanical stress placed upon it

Skin, muscle, cartilage, and digestive cells benefit from having a lot of intermediate filaments

- Anything that lines blood vessels, surrounds an organ

Found just below the PM alongside microfilament and around the nucleus

Extensive H bonds

Monomers are fibers instead of globular proteins

No jagged edge, only blunt ends

*No energy requirements to assemble these structures (no ATP or GTP)

<u>Cytoskeletal Tracks:</u>

Microtubules and microfilaments have a polarity that allows directed transport of vesicles, pigments, organelles, etc. in the cell

- Molecular motors (ex. Dynein and kinesin walk in specific directions)

Vesicles carry different content to many locations

Diversity is in the many isoforms of motor proteins and the multiple combinations

- Myosin walks on microfilaments
- Dynein and kinesin walk on microtubules

<u>Motor Proteins:</u>

Mechanoenzymes – use ATP as fuel

Drive motion in cells

- Along the polar cytoskeletal fivers
- Vesicles, organelles, pigments, membrane, motility and mobility, etc.

Cargo can be passed from one type of motor to the other and molecular motors can even carry each other (ex. Kinesin can carry dynein back to its starting point)

Pigments can be carried along microtubules – causes different pigmentation patterns

- Proteins/hormones trigger the displacement of pigments and the pattern of how the pigments are deposited causes the pattern of pigmentation

Kinesins:

- Anterograde (forward) transport
- Walk towards the + end of microtubules (towards the PM)

Dyneins:

- Retrograde (inward) transport
- Towards the – end of microtubules (towards the center of the cell

Actin and myosin functions:

- Vesicle transport
- Microvilli: actin supports the extensions
- Amoeboid motion
- Muscular contraction

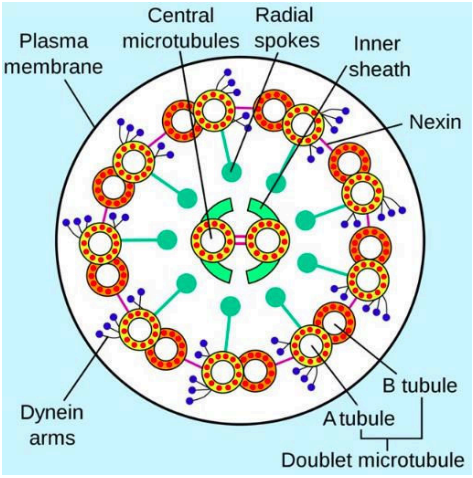
<u>Cell Motility and Mobility:</u>

Motility is the ability to move using energy

Mobility refers to motility but can be used for proliferation or differentiation

We will concentrate on motility – locomotion

Cell motility – cilia and flagella:



- Made of microtubules
- Anchored to the basal body but then project outwards form the cell
- Both have the same structure: 9 + 2 complex
  - Circle of 9 double microtubules surrounding a double microtubules
  - Dynein allows the microtubules to slide over each other
  - Nexin: a linker protein that holds the doublets together; exercises a limit to pull the microtubules back
- Small arrangements: cilia – usually a large quantity present in the cell
- Large arrangements: flagella – usually only one

- Cilia:
  - Many shorter cilia
  - Whip-like (oar-like) movement – goes back and forth
  - Move liquid/substances on cell surface
- Flagella:
  - Usually a single longer flagellum
  - Smooth s-wave motion
  - Move the cell in the environment

<u>Centrosome/Centrioles:</u>

Each cell has a centrosome, made up of 2 centrioles that are perpendicular to each other

Centrioles are made of 9 microtubules triplets that are arranged in a ring structure (with no central triplet)

Will serve as anchor to cilia and flagellum

Acts as the MTOC

Mitotic spindle formation

Placed near the nucleus – microtubules originate in the middle of the cell

- Good for them to be near the nucleus, where they perform an important function in cell division

Cilia can have multiple basal bodies, but flagella will only have one

The Extracellular Matrix

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Extracellular Matrix:

Proteins that are secreted by cells in or near the matrix

- Collagen, fibronectin: fibrous proteins – strong, not very soluble/easily diffused, good at anchoring, protection, rigidity

Polysaccharides, proteoglycans: sugars – cell signaling

The ECM holds cells together in tissues

Hinders the cell from extending its membrane

Helps filter material passing between different tissues

Helps orient cell movement during embryonic development, growth, and tissue repair

Plays a role in chemical signaling from one cell to another

Communication between ECM and cytoskeleton is important

Integrins:

- Plasma membrane receptors
- Binding of ECM and microfilaments; bound to compounds that span the membrane

MMPs (Matrix metalloproteins):

- Produce and degrade ECM allowing cell movement and guiding in tissue
- Degrade the rigid structures in the ECM on top of the membrane for a short period of time to allow the microfilaments to keep growing and push the membrane out

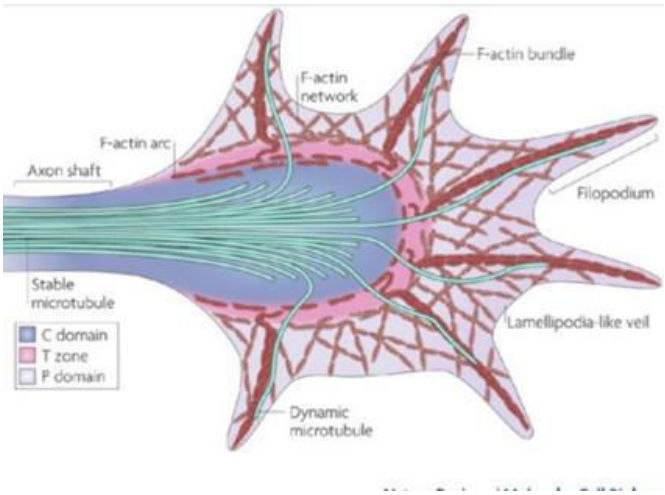
Cytoskeleton and ECM Working Together – Neuronal Growth Cones:

Neurons don't divide, but they can increase/reduce the number of projections at the end of the axon

Microfilaments can form tight bundles to support the membrane to extend it

Filapodium: the "finger" like projections, contains more of the thicker bundles

Lamelipodia: the "will" in between the filapodia; contains more of smaller branches of microfilaments



Microtubules extend from the exon into portions of these projections, but not all the way

- They are still needed because the kinesins and the dyneins are needed to carry substances such as neurotransmitters

Cell to Cell Interactions – Junctions:

In addition to the cytoskeleton and ECM guiding a cell's interaction with the outside, there are also structure to organize physical interaction between cells

Tight junctions:

- Fusion of proteins on external plasma membranes
- Seals that area – even ions cannot pass
- Absolutely nothing can go through

Anchoring junctions:

- Plaque of proteins interact with microfilaments (MFs) or intermediate filaments (IFs) and anchor cells together
    - Anchoring junctions are desmosomes when the plaques interact with MFs
    - They are adherens when they interact with IFs
- MFs form the anchoring junctions
- Plaques: dense areas of proteins within the membrane
    - Interact with the cytoskeleton on both sides to form the junctions

Gap junctions:

- Channels formed by proteins bridging cells (connexins)
- Made up of proteins called connexins to form a connexon
- Forms a small cylindrical passageway between cells
- Allows passage of ions and small molecules – cell communication

Conditions (ex. Temp, pH) will impact the shape of proteins

- The shape of the proteins and the conditions will determine whether of not the gap junctions are opened
- Can act as a switch for opening/closing gap channels
- Ions (especially Ca^{2+}) can drive the opening and closing of gap junctions

Topic 4: Membranes

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Objectives:

Discuss the role of membrane, and in particular the plasma membrane in a cell

Understand and explain the fluid mosaic model. Include the membrane's properties in the discussion.

What is the membrane made of? Describe and organize the different molecules involved and link them to the membrane's properties.

Compare the different types of transport across the membrane and give examples for each.

How is membrane potential formed and why is it useful in a cell?

Membrane Functions:

Boundary; selective permeability

- Maintains internal environment

Organize and scaffold

- Compartmentalizes things, helps form organelles

Regulate solute transport

Receive signals

Communication

Types of Membranes:

There are various types, but they all have similar features

Show diversity in lipid/protein content

- Also types of lipids and proteins

Glycolipids and glycoproteins

Plasma Membrane – Fluid Mosaic Model:

Fluid lipid molecules with embedded, free-floating or anchored proteins

Arranged in non-covalently bonded sheets

Two facing sheets/fluid lipid layers – provide structure

Phospholipid bilayer and their arrangements give the membrane its fluidity

Mosaic of different proteins – allow various functions (specificity)

- Ex. Communication, transportation, signaling, structure

Fluidity:

Within each layer, the lipids continuously change places

- NB: not all of the fatty acids are the same

Movements:

- Lateral diffusion: occurs very quickly
- Rotation: kinks in fatty acids du to double bonds can interact differently depending on its orientation
- Flexion: fatty acid tails can move due to the introduction or removal of double bonds – the saturation can be modified
- Rarely, a phospholipid can flip from one layer to another

Composition of the Lipid Layers:

Phospholipids: are the most abundant lipids in the membrane bilayer

- Presence of unsaturated double bond produces "kink" and influences fluidity of the membrane
- 4 main phospholipids:
    - Most polar:
        - Phosphatidylethanolamine
        - Phosphatidylserine: predominantly towards the inside of the cell
    - Less polar: predominantly towards the outside of the cell
        - Phosphatidylcholine
        - Sphingomyelin

Glycolipids: important for cellular recognition

- Change how phospholipids interact and contribute to the fluidity of the membrane

Sterols:

- Present only in eukaryotes
- Play an important role in fluidity, both in terms of arrangement and in relation to temperature (buffers)
    - State of the membrane changes depending on temperature
- All have the same steroid core but different functional groups
- Animals, some protists: cholesterol
- Fungi: ergosterol
- Plants: phytosterol

Properties of the Lipid Bilayer:

Membrane asymmetry:

- Both layers are different
- The inner layer is going to be more negative – enhances communication
- Translocation from one layer to the other is rare and requires energy in order to maintain the asymmetry of the bilayer

- This is performed by an enzyme called flipase

Membrane fluidity:

- Chain length and degree of saturation
  - The longer the chain, the stronger the forces, which decreases fluidity
- Polar head groups (variable polarity)
  - Nature of the polar head group influences lateral diffusion and rotation
- Sterols (buffering effect)
- Temperature
  - Low temperature: less energy, moves less, more solid

Membrane is dynamic:

- Homeoviscous adaptation: the ability to change the membrane's lipid composition in response to the environment in order to maintain membrane fluidity
  - Ratio and amount of phospholipids in the membrane can be changed
  - Higher temperatures: more fluid
    - To fix can saturate the fatty acids or change the phosphotidalcholine vs. phosphatidalethylene ratio in the membrane (PC:PE ratio)
    - PE confers more fluidity than PC
  - This ability to adapt:
    - Allows the cell membrane to change shape
    - Helps permeability
    - Helps create boundaries and not allowing things to leave or preventing things from coming in
    - Helps keep the internal environment different from the external environment
    - Allows cell to cell communication
    - Allows cell signaling
    - Helps transport
- Desaturase: an enzyme that introduces a double bond
- Amount of desaturase changes in reaction to temperature
- Ratio and amount of phospholipids in the membrane can be changed
- Higher temperatures: more fluid
  - To fix can saturate the fatty acids or change the phosphotidalcholine vs. phosphatidalethylene ratio in the membrane (PC:PE ratio)
    - PE confers more fluidity than PC

Membrane Proteins – The Mosaic:

Main functions of proteins in the membrane:

- Transport
- Enzymes (catalytic activity)
- Signal transduction
- Attachment/recognition

3 types: sorted based on the way they interact with the membrane, not the tasks that they carry out or their functions

- Integral proteins: anything that spans the membrane
  - Aka transmembrane proteins
  - Ex. Connexons, integrons
  - Properties:
    - Strings of non-soluble amino acids within the fatty acid chains of the membrane
    - Soluble polar areas that are on the outside and the inside of the cell (outside the membrane)
  - Can span the membrane more than once (single, double, or multipass)
  - N or C terminus can face inside or outside the cell
  - Assembled by ribosomes from the RER and then assembled within the membrane that originates from the ER
  - Link between the membrane and the cytoskeleton
  - Helps maintain the cell's structure, provides support and elasticity
  - Always held together with weak bonds
- Peripheral proteins: will interact either outside or inside the cell
  - Interact with the membrane or compounds within the cell, can also interact with integral proteins
  - Never span the membrane – don't enter it at all
  - Can be on the inside or the outside of the cell membrane
  - Non-covalently bound to another element (integral protein or cytoskeleton)
  - Can serve to provide structural support (elasticity) or mediate binding
- Lipid-anchored proteins: anchored via a lipid component
  - Can either be within or outside the cell
  - The protein part never enters the membrane, but the lipid portion does
  - Proteins can be anchored (covalently) on the outside or inside of the cell
  - There are 2 types of anchors:

        - Extracellular proteins: GPI anchors
        - Intracellular proteins: fatty acid or prenyl groups
    - Has different functions depending on the location and type of anchor
        - Extracellular proteins: cell adhesion
            - Also cell signaling and recognition
        - Intracellular proteins: involved in mediating cell division and cell growth

Studying Membranes:

Microscopy

Freeze-fracture: useful to study inside the membrane

- Freeze cells using liquid nitrogen
- Split membrane using a knife
- View under electron microscope

Fluorescence Recovery after Photobleaching (FRAP): useful to study the ability of molecules to move within the membrane

- Use a dye to see the phospholipids – they get stained
- Take a laser and bleach a specific area (gets rid of the dye in that area)
- Can measure how long it takes for the fluorescence to return to the bleached area
- Allows you to study how fast that phospholipids are moving around in the membrane

Membrane Potential:

Membrane potential: potential energy sotred across the membrane

- Energy comes from the concentration gradient

Distribution of ions is unequal with more positive charge outside, making the inside less positive

This uneven distribution creates potential energy – can be measured in voltage (V)

As excitable cells are stimulated, channels allow the passage of ions, changing the ionic distribution

- This is called action potential
    - When sodium channels are opened, sodium rushes in the cell and the inside of the cell becomes more positively charged – the voltage increases

    - This triggers the neighboring potassium voltage gated channels to open, which causes potassium to rush out of the cell, causing the cell to become more negative
    - The Na/K ATPase the works to helps restore the resting membrane potential
- Changes the ionic distribution, hence potential energy and hence V

Depends on charges instead of vesicular transport – allows faster messages

Membrane Transport

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Movement Across Membranes:

A few general principles:

- Relative permeability of molecules
  - Some compounds can pass through easily while others can't
  - Selective permeability is essential to allow concentration gradients to be built
- Concentration gradients vs. electrochemical gradients
  - Concentration gradients: depends on chemical concentrations
  - Electrochemical gradients: take concentration into account, but also the charge
    - Can only have an electrochemical gradient with charged compounds
- Gradients store potential energy
  - Both chemical and electrochemical gradients store potential energy

Passive (Simple Diffusion):

Down/along the gradient (high concentration to low concentration)

Solutes must be small, non-polar (lipophilic)

- Uncharged solutes (concentration gradient)
- Water (osmotic gradient)
- Ions (electrochemical gradient)
- Gases (partial pressure gradient)

Spontaneous, random

Facilitated Diffusion:

Small or slightly larger hydrophilic molecules

Goes down the concentration gradient

Channel mediated:

- Water, ions
- Highly selective
- Gated channels: controlled opening and closing (ex. Voltage gated, ligand gated, mechanically gated)
- Leakage channels: always open
- Follow electrochemical gradients

Carrier mediated:

- Ions, large polar molecules

- Highly selective
- Uniporters, co-transporters, exchangers
- Driven by solute gradients
- Can get saturated – there's a limited number of carrier proteins

Active Transport:

Uses ATP

Only type of transport across membrane that goes against the concentration gradient – requires energy

Two possible sources of energy:

- Use the concentration gradient of another solute – this is secondary transport
- Using an energy input, such as ATP – this is primary transport

Can get saturated – there's a limited number of carrier proteins

2 types of secondary transport:

- Symport: both the driving ion and the transported solute travel in the same direction
    - Ex. Na-glucose symporter in intestinal microvilli
    - Glucose goes against gradient, uses sodium powered symport
- Antiport: the driving ion and transported solute travel in opposite directions
    - Ex. Na-Ca exchanger

Primary active transport – ATP pumps:

- Small molecule pumps:
    - P-type: ion ATPases (ex. Na/K ATPase, Ca/ATPase)
    - Na/K ATPase is found in the plasma membrane
    - Ca ATPase can be found in the plasma membrane where it pumps Ca2+ out of the cell or in the sarcoplasmic reticulum where it pumps it into the SR
        - Na/K ATPase:
        - Pumps out 3 Na+ for every 2 K+ pumped in
        - Sodium is highly concentrated outside of the cell, potassium is highly concentrated inside the cell
        - Has three binding sites inside the channel with within the membrane for Na+ and two binding sites for potassium
            - NB: binding sites are specific to certain ions
        - Cycle:

        - 3 Na+ ions bind to their binding sites when the protein opens facing the inside of the cell
        - The protein gets phosphorylated by ATP, which causes it to change shape and it opens facing the outside of the cell – causes the sodium to be released
        - 2 K+ ions bind to their binding sites
        - The pump is dephosphorylated and the pump changes its conformation is opens towards the inside of the cell, causing the potassium to enter the cell
        - The cycle is now ready to start again
      - This is an electrogenic exchange: the exchange is not the same amount of charge (contributes to a difference in charge)
    - F or V types: H+ pumps
      - NB: they only pump hydrogen
      - F type: found in the mitochondrial inner membrane
- ABC (ATP Binding Cassettes): pump large molecules (ex. Drugs, toxins, etc.)

<u>Exocytosis:</u>

The vesicle and the membrane fuse, forcing the contents out of the cell

Vesicles can contain neurotransmitters, hormones, etc.

- No lipid or protein based macromolecules – only soluble molecules

Vesicle membranes contain integral proteins – come from the RER, soluble content within the vesicle can come from the free ribosomes

- These proteins will then become embedded with the PM after the fusion

<u>Endocytosis:</u>

Pinocytosis:

- Replenishes water and solute content of the cell
- Happens fairly often
- Pocket forms and grabs what is immediately outside the cell – extracellular fluid (water, small ions solutes)
- The pocket forms a vesicle and enters the cell
- The cytoskeleton (especially microfilaments) helps the membrane pull in to make the "pocket"
    - Peripheral and anchor proteins can also help

Receptor mediated endocytosis:

- A messenger binds to the receptor to trigger the formation of a pocket, which will form a vesicle
- The vesicle contains whatever was outside of the cell in that area, the receptors, and the messenger bound to the receptors
- The vesicle then goes to the lysosome that will digest everything
    - The vesicle can also get sent to the golgi apparatus to recycle certain aspects (ex. The receptors)

Phagocytosis:

Solid particles are engulfed and sent to the lysosome

Small vacuole from the lysosome is sent off containing anything not digested by the lysosome to be expelled from the cell

Different from exocytosis because there is no fusion with the membrane

Topic 5: Mitochondria and Energetics 2/13/2015 10:22:00 PM

Objectives:

Explain what is the role of mitochondria in a cell with regards to energy production

Understand the importance of calcium

- Availability, storage
- Cellular functions

Understand key concepts of cellular energetics in relation to mitochondria

Mitochondria:

Essential for many physiological functions:

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

Metabolic Functions:

Fatty acid beta-oxidation (ATP via phosphorylative oxidation)

Krebs (TCA) cycle

Heme biosynthesis (ex. Hemoglobin, cytochromes)

Steroidogenesis (mevalonate can convert cholesterol)

Fe/S complex assembly (complex I and II; metalloproteins, ferredoxins, etc.)

Amino acid metabolism (urea cycle)

Gluconeogenesis

Ketogenesis

Studying Structure and Function:

Methods to isolate mitochondria:

- Centrifugation
- Subcellular fractionation
- Differential centrifugation
- Density gradient centrifugation
- Equilibrium density centrifugation

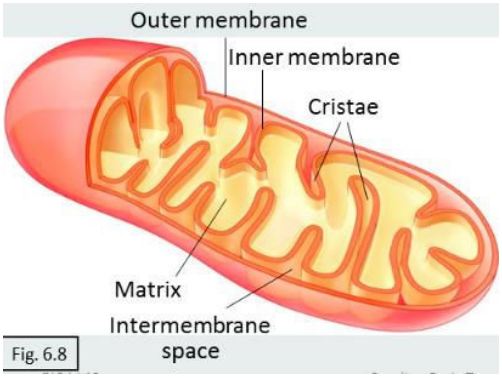

Fig. 6.8

Cellular Respiration:

The objective: power up the F type ATPase – the F1F0 ATPase

- Cristae increase surface area and gives more room for F type ATPases to create a bigger ATP gradient – larger ATP synthesis capacity

Glycolysis: occurs in the cytosol – outside the mitochondria

- Transforms glucose into NADH and 2 pyruvate molecules
- Makes 2 ATP
- Depends on the cell's metabolic requirements and on the availability of oxygen

Pyruvate oxidation:

- Pyruvate is shuttled across the membrane by the pyruvate hydrogenase where it is transformed to acetyl-CoA and can be used to power the Krebs cycle
    - Pyruvate dehydrogenase requires calcium to transport pyruvate into the mitochondria

Krebs cycle (aka TCA or citric acid cycle): in the mitochondria

- Produces FADH2 and NADH2
- Makes 2 ATP

Oxidative phosphorylation (electron transport chain): inner mitochondrial membrane

- Uses electrons to build up the H+ gradient to power the ATP synthase
- Produces heat, water, a proton gradient (H+) and Reactive Oxygen Species (ROS)
- Makes 32 – 34 ATP
- Protein complex, consisting of 4 multi-subunit proteins and 2 electron carriers
    - Complex I, II, III, and IV
    - Oxygen is the final electron acceptor
    - Each complex uses electrons to help contribute to the proton gradient
- Responsible for transferring the electrons from reducing equivalent
- The proton gradient is pushed thank to the proton motive force
    - Proton motive force: potential energy
    - The ETS forms an electrical gradient and a chemical gradient (by exchange of protons and electrons, pH gradient, and membrane potential)
    - This forms the proton motive force, which can be used to power other activities
    - Loses heat in the form of heat and ROS
    - Chemiosmosis: harnessing the proton-motive force to do work
- Phosphorylation: the F1F0 ATPase
    - Complex V, the F1F0 ATPase uses the energy from the proton gradient to phosphorylate ADP to generate ATP using Pi
    - Is reversible, so it can also break down ATP to contribute to the H+ gradient if needed
    - Affected by:

    - Pyruvate dehydrogenase
    - Also H+ gradient, ADP and Pi levels, amount of NADH and FADH2, and amount of pyruvate/glucose available

Limitations on ATP generation in eukaryotes:

- Entry of e^- from glycolysis into mitochondrion
- Use of mitochondrial proton gradient for transport
- Oxidative phosphorylation is regulated by supply of ADP

Importance of Calcium

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Importance of Calcium:

Physiological and biochemical functions that need Ca2+:

- Response to stimuli (Ex. Vesicle secretion)
- Muscle contraction
- Signaling (second messenger)
- Enzymatic cofactor (ex. Coagulation)
    - Some enzymes interact with calcium, which can either be integral to its function or can maximize the enzyme's function
- Bones
- Metabolism

Krebs cycle is reliant on calcium

Calcium is toxic to cells – calcium balance must be maintained to avoid calcium toxicity

Calcium Regulation:

Gated channels

The membrane is impermeable to calcium

Active transport to pump out calcium

Calcium binding proteins: globular, soluble

- Essentially hides the calcium
- It is no longer free ionic calcium so it doesn't contribute to the intracellular concentration
- It is now quickly and readily accessible

Stored in the endoplasmic reticulum (sarcoplasmic reticulum in muscles) and in mitochondria

- Neither of their membranes are permeable to Ca2+
- Concentration is higher inside the compartment
- Uses Ca2+ ATPase to pump calcium in
- Some gates allow the calcium to be accessed

All of these methods are driven by the intracellular concentration

Topic 6: Mitochondria - Apoptosis

3/15/2015 9:22:00 PM

Objectives:

Describe and differentiate the two cell-death mechanisms and explain the role of mitochondria and calcium in each

What is mitophagy and how/why does it occur?

Why do cells die?

The size of an organ or organism depends on the total cell mass

The cell number must be controlled

Death helps maintain organ/organism size and helps maintain total cell number

Other reasons: cells are dysfunctional, they have outlived their usefulness, they have become infected

Some brain cells die to allow for improved connections

Cell Death Triggers:

Chemical messengers:

- Growth factors
- Mitogens: stimulate cell division
- Survival factors

Toxicity

Damage

Cell cycle checkpoints

All triggers lead to changes in the cell, which lead to its demise. The mechanisms of the death are the differences.

Cell Death Mechanisms:

Necrosis: normal cell → swelling → disintegration

- Possible reasons:
  - Change in osmotic pressure
  - Cell membrane fails to act as a boundary – its permeability to water has changed
  - Cell is no longer able to cope
- Everything from inside the cell is released
- Some of the substances that are released can break down other neighboring cells – causes collateral damage
- Caused as a response to damage
- Ischemia, excitotoxicity

- Ca^{2+} surplus
- Loss of ATPase activity, loss of electrochemical gradient, decreased ATP

Apoptosis: normal cell → condensation → fragmentation → apoptotic bodies

- No collateral damage – everything remains surrounded by a membrane
- The apoptotic bodies are phagocytosed and degraded
- In response to a stress signal (intrinsic or extrinsic)
- Programmed cell death (autophagy)
- Not always in response to a negative stimuli – it can also be essential to development
    - Ex. Tadpoles get rid of the tail to grow legs, embryos get rid of interdigit membrane to get digits
- Increase in Ca^{2+} (cell and mitochondria)
- Pro-apoptotic protein: mitochondria
- Opening of PTP (permeability transition pore)
- Cytochrome c release (changes to cristae)
- Activation of apoptosomes
- Signaling cascade (caspases)

Apoptosis

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Structural Changes:

Cell decides it needs to die and starts the "cascade of death"

Can have an internal or external signal, but the process is the same

1. Chromatin condenses, shrinkage of cytoplasm
2. Nucleus fragmented, DNA laddering, blebbing, cell fragmentation
    - DNA laddering: DNA becomes cut into smaller portions
    - Anchoring junctions are no longer functioning
    - Blebbing: cell forms small pockets surrounded by membrane. These blebs detach from the cell.
3. Apoptotic bodies are phagocytosed by other cells (especially macrophages)

Phagocytosis:

Apoptotic bodies are marked for phagocytosis due to the presence of phosphatidylserine on the outside of the cell, making them appear as foreign bodies or pathogens

The asymmetric distribution of the PM is lost

Negatively charged phosphatidylserine then becomes exposed on the outside of the cell

The cell is then marked for phagocytosis by a macrophage

The phosphatidylserine appears on the outside of the cell due to flippases:

- Some of it is spontaneous, but that rarely occurs
- P-type flipase: move from outside to inside, used to maintain membrane asymmetry
    - ATP required, specific
- ABC flipase: moves from inside to outside
    - ATP required, specific
- Scramblase: bidirectional
    - No energy needed, non-specific
    - CA^{2+} dependent
    - Inactive until the become activated by calcium and caspase
        - Caspase is activated by the "cascade of death"
        - Scramblase can transport in both directions, but favors moving to the outside

Signaling Pathways:

Two main apoptotic signaling pathways:

- Intrinsic pathway
- Extrinsic pathway

The Intrinsic Pathway:

Possible triggers are internal stressors: loss of survival factor, DNA damage

Leads to dephophorylation and activation of bad (pro-apoptotic protein)

Bad inhibits BCl_2 (anti-apoptotic protein)

Bad also activates Bax and Bak (changes to calcium regulation in ER and mitochondria)

Causes the caspase cascade

- Caspases:
  - A family of proteases (enzymes that cleave protein)
  - Are split into 2 groups: initiator and executioner
    - Initiator: first to become activated, wake up the executioner caspases
    - Executioner: inhibits various protein kinases
  - Caspase targets:
    - Inhibits protein kinases: disrupt cell adhesion
    - Lamins: disassembly of nuclear envelope
    - Inhibits cytoskeleton: change cell shape and size
    - Activates DNAse: DNA fragmentation
  - They also activate the scramblase instinct to bring phosphatidylserine to the outside of the PM

Calcium and the Process:

Bad is activated, which inhibits BCl_2 and activates bax and bak

BCl_2, prevents the binding of IP_3

BCl_2 is inactivated, allowing bax and bak to promote IP_3 binding

This allows the endoplasmic reticulum to release Ca^{2+} via IP_3-dependent channels

The mitochondria absorbs the Ca^{2+} to protect the cell

The Ca^{2+} concentration inside the mitochondria becomes too high, and the cristae rearrange themselves, and punch a hole in the outer mitochondrial membrane to create a permeability transition pore (PTP)

The PTP allows a protein called cytochrome C to exit the mitochondria and trigger the caspase cascade

Cytochrome C forms an apoptosome by binding with caspase 9 and APAF-1, which activated initiator caspases and then the executioner caspases

Despite this, mitochondria remain healthy until the last apoptotic body is engulfed

- In necrosis, there are no functional mitochondria

NB: calcium is a consequence or a trigger or initiating necrosis, but it is a part of the regulation/process of apoptosis

Mitophagy

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Mitophagy:

Controlled regulation of the number of mitochondria according to the metabolic requirements

- Can be used to reduce the number of mitochondria or to get rid of damaged portions of mitochondria

Process that involves recruiting various signaling proteins and lysosomes

The process how mitochondria are "chosen" remains unclear

Important for aging, development, and certain pathologies (AD, Parkinson's, etc.)

Is a normal process during aging or a decrease in activity levels

Process:

PINK is a kinase on the surface that recruits Parkin

Parkin adds ubiquitin groups to the cell surface, which signals autophagocytosis (the mitochondria is sent towards the lysosome)

Topic 7: Cell Signaling

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Objectives:

Explain and give examples of cellular communication and its role

Differentiate between the 3 steps of cell communication

Know and differentiate the 6 paths of communication

Identify and differentiate the 6 messenger classes and their characteristics

Know the 4 different classes of receptors

Cell Communication:

Conserved mechanisms (unicellular and multicellular organisms)

- Conversation between one or multiple cells

Essential during:

- Development
- Hormonal regulation
- Muscle contraction
- Immunity and self-recognition
- Cancer
- Apoptosis

3 steps:

- Reception: the binding of a signal molecule with a specific receptor
- Transduction: the process whereby signal reception triggers other changes within the cell necessary to cause the cellular response
- Response: the transduced signal causes a specific cellular response

Communication Pathways:

Direct cell signaling: uses gap junction to allow the passage of ions between neighboring cells

Indirect signaling:

- Autocrine and paracrine signaling:
    - Autocrine: one cell releases a chemical messenger that diffuses into the extracellular space and binds to receptors on its own cell surface
    - Paracrine: one cell releases a chemical messenger into the extracellular space that binds to receptors on the surface of neighboring cells
        - The messenger doesn't travel very far – depends on diffusion
- Endocrine signaling: one cell releases a chemical messenger that enters the blood stream and then travels to a target cell
    - The messenger travels throughout the entire organism

- Neural signaling: a message is received through a cell that is interpreted and converted into an electrical signal, which travels down the axon where the electrical signal is changed into a chemical messenger (neurotransmitter)
- Exocrine signaling: a message signals a gland to secrete a product through a duct

Reception

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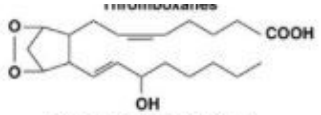
Messengers/Ligands:

Their structure determines:

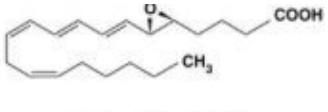
- Their chemical properties (hydrophobic vs. hydrophilic)
- The communication path taken to reach target cell
- Their mode of action/interaction with the cell and receptors

There are 6 classes of messengers:

- Steroids:
  - Derived from cholesterol
  - Lipophilic – cannot be stored in vesicles (endocrine path)
    - Will diffuse or be bound to a transport protein
  - Membrane permeable – can reach intracellular receptors
  - Act as gene transcription factors
  - 3 classes:
    - Mineralcorticoids (aldosterone)
    - Glucocorticoids (cortisol)
    - Sex hormones (testosterone/estrogen)
  - Has four hydrocarbon rings
- Lipids (Eicosanoids):
  - Most are derived from arachidonic acid
    - Phospholipase removes the polar head group from phospholipids in the PM and slightly changes the structure of the glycerol backbone to make arachadonic acid
    - Arachidonic acid is modified by the cyclooxygenase pathway (changes the structure of the acid, adds a hemicycle) or the lipooxygenase pathway (changes the structure, but not as much – no hemicycle)
      - Cyclooxygenase pathway produces prostaglandins, which manage pain
      - Lipooxygenase pathway produces leukotrienes, which manage inflammation



Example: prostaglandin H_2



Example: leukotriene A_4

  - Lipophilic
  - Paracrine path
  - Local hormones

    - Structure: has two long fatty acids
- Peptides/proteins:
    - Few to many aa's (<50 = protein, <50 = peptide)
    - Hydrophilic: can be packaged
        - Can be packaged in an inactive form, can be quickly activated
    - In vesicles
    - Secreted by exocytosis
    - Most indirect paths
- Purines:
    - Derived from nitrogenous bases adenine or guanine
    - Need a transporter or use exocytosis
    - Mainly use paracrine and neuronal paths
    - Have two cyclic rings
    - Ex. Caffeine, theobromine (chocolate)
- Amines:
    - Have an amine (NH_2 group)
    - Usually biosynthesized from an amino acid
    - Most are hydrophilic (reserve pool in vesicles)
        - Thyroid hormones are hydrophobic
    - Many neurotransmitters are amines (ex. Epinephrine, histamine, GABA)
- Gases:
    - Small molecules, short half-life
    - Passively diffuse
    - Direct, paracrine, neuronal, and endocrine paths

Having different types of ligands (messengers) allows different messages, different speeds, more efficient messaging, and triggering only the desired response

<u>Receiving the Message:</u>

The interaction with the receptor is the first level of discrimination (sets the path to the cell's response)

Receptors are proteins – can be on the cell surface or inside the cell

Ligand binding will induce a change in the conformation of the receptor protein, which acts as the trigger for the 2nd phase (transduction)

<u>Receptors:</u>

Ligands can bind to more than one type of receptor – increases the change of a ligand reaching a receptor
Cells express a different array/pattern of receptors in multiple copies to accept a large amount of the receptors
Affinity of the receptor for the ligand changes how long the "conversation" lasts
4 classes:

- Ligand-gated ion channel:
  - Facilitated diffusion
  - Ligand binds and changes the shape of the receptor to either allow ions into or out of the cell
- Receptor-enzyme:
  - Ligand binds and changes the conformation of the protein
  - It now gains enzymatic ability on the intracellular side
- G-protein coupled receptor:
  - Spans the membrane 7 times
  - Ligand binds, conformation changes and starts interacting with G-proteins (large proteins inside the cell) that are activated and go interact within the cell
- Intracellular receptor

All except the intracellular receptors are transmembrane receptors

- Intracellular: binds to lipophilic or small ligands
  - Can be found in the cytoplasm or in the nucleus
  - The ligand-receptor complex acts as a transcription factor
    - Enters the nucleus and binds to specific areas of DNA (the promoter sequence – area right before the gene)
      - The promoter sequence contains the response element, which is where the transcription factor binds
    - Activates or inhibits gene transcription
  - The ligand always enters the cell
- Transmembrane: binds to hydrophilic ligands
  - Integral protein that spans the membrane
  - Ligand binding domain outside the cell
  - Ligand binding induces a change in conformation that relays the massage inside the cell – second messengers
  - Ligand bonding sites depends on the sequence of aa's to affect the shape of the receptor – only accepts certain types

- Affinity of the receptor for the ligand changes how long the "conversation" lasts
- Size and placement of the receptor within the membrane will affect function
    - There are different areas within the membrane that are better equipped for certain receptors
    - Lipid rafts:
        - Sphingolipids and cholesterol form highly-ordered microdomains or rafts
        - SL hydrocarbon tails are longer and saturated (not kinked)
        - Rafts are produced in the ER and sent to the PM
        - Can accommodate proteins with long transmembrane domains
        - Organize and cluster proteins to function together
        - Less fluid (due to saturation) – withstands the stress of adding the large protein
        - Membrane is thicker in this area (due to the longer tails)
- The ligand will never enter the cell

Transduction

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Objectives:

Define what is cellular signaling and transduction

Know the basic signaling pathways:

- Intracellular
- Ion channels
- Enzymatic receptors (particularly RTK/Try-k)
- GPCR (cAMP and PIP/IP_3 pathways)

Identify and define the type of cellular responses

Describe in detail an example of a cellular response with its signal transduction cascade

Regulation and modulation:

- Specificity and coordination

Recognize and describe the mechanisms (6) that can terminate a cellular response

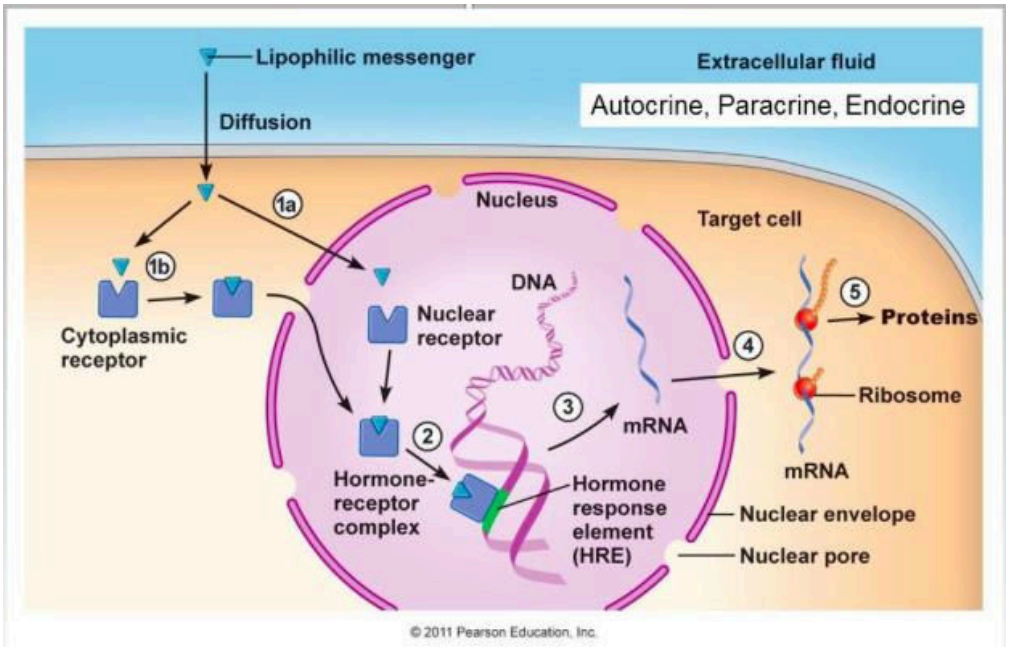
Transduction:

The response to the signal

Receptor activation leads to relaying and amplifying the signal inside the cell

- Amplification: one molecule triggers a large number of molecules within the cell to start working and to amplify the response
- Allows for maximum cellular response with minimal ligand

Intracellular Receptors:



Glucocorticoids:

Cortisol: long term stress response

Reduces insulin synthesis by pancreas – reduces ability to control blood glucose

Sequence of amino acids gives it its functional domains

Transmembrane Receptors 1: Ligand Gated Ion Channels

Very important for the nervous system – used by many neurotransmitters

Some channels are specific to the passage of certain ions, while others are simply open passage

Process: ligand binds to the ligand binding site, causing the channel's shape to change, either opening or closing it

Allowing the movement of ions changes the cell's electrochemical gradient – can cause AP's, etc.

- Electrochemical gradient can also be used to fuel activities, such as active transport
- The electrochemical changes can also lead to something that will interact with DNA

ACh:

- A very important neurotransmitter
- Excitatory or inhibitory depending on the tissue
- Hydrophilic: can be passaged in vesicles and can make a pool/reserve
- Uses 2 types of receptors: ligand-gated ion channels (nicotinic receptors) or GPCRs (muscarinic receptors)
- Process:
    - ACh is made from choline and acetyl CoA (from mitochondria)
    - Changes in membrane potential triggers voltage gated calcium channels to open
    - Calcium bind to the vesicles containing ACh and triggers for them to bind to the membrane and release their contents
    - ACh diffuses across the synaptic cleft and binds to the receptors on the other cell (ligand-gated ion channels or G-protein channels)
    - In the synaptic cleft ACh is rapidly broken down by the enzyme acetylcholinesterase
    - Choline is transported back into the axon terminal and is used to make more ACh
- Ligand gated ion channels:

- ACh needs to bind to the alpha subunits
  - 2 alpha subunits per receptor – 2 ACh needed per receptor in order to open the ion pore
- When binding sites are filled, conformation changes, pore opens and lets ions through
  - Mostly Na+, Ca2+ into the cell; some K+ out
- Ions going in causes the membrane potential to become more positive and changes the membrane potential
- This change gets propagated throughout the entire membrane, including the T-tubules
- The potential is passed through the T-tubules to the sarcoplasmic reticulum, causing voltage gated channels to open and release calcium
- The calcium binds to troponin C, causing the tropomyosin to change its shape and expose the myosin binding sites, allowing muscular contractions to occur
- Note that the change in membrane potential in the muscle cell membrane allows entry of more ions, triggering and amplifying the cellular response

<u>Transmembrane Receptors 2: Receptor Enzymes</u>

Intracellular catalytic domain acts as an enzyme

- Ligand binds to the binding site, the receptor changes shape, and the catalytic area inside the cell starts working (guided by change in shape)

Initiates a cascade of phosphorylation-dephosphorylation that amplifies the ligand's impact on a target cell

- Unlike ligand gated ion channels, they don't change their conformation to open/close pores and they don't let ions through

3 main classes:

- Guanylate cyclase: converts CTP to cyclic GMP (cGMP)
- Tyrosine kinase: phosphorylates tyrosine aa's
- Serine/threonine kinase: phosphorylates serine or threonine aa's

Tyrosine kinase receptors (Tyr-K, RTK):

- Largest family of enzyme receptors (over 20 types)
- Involved in pathways linked to survival, growth, proliferation, and metabolism
  - All lead to positive cell processes (promote cell processes, cell survival promoters)
- Ligand examples: insulin, growth factors

- Activation requires dimerization and autophosphorylation:
  - The receptors lie in the membrane as monomers in an inactive form
  - When they bond to their ligands they change their conformation, allowing two nearby monomers to join and dimerize (form a dimer)
  - Once the dimer has formed the receptors autophosphorylate (the tyrosines) and triggers their catalytic ability
    - 6 – 10 tyrosines can in the catalytic domain
  - The receptor becomes active once phosphorylated and it can now interact with proteins inside the cell
    - The second messenger will depend on which tyrosine was phosophorylated
- Signaling is initiated via an area called an SH2 domain
  - SH2 domain: areas that a protein needs to have to interact with tyrosine receptors
  - The protein also has an SH3 domain so that it can interact with the next molecule in the cascade
  - SH = Src homology → structure is similar to Src protein
- Main second messengers: Ras and phospholipases
- Generic cascade: the Ras cascade
  - Growth factor binds to its own monomeric inactive subunit of the receptor. They change their conformation, which promotes dimerization
  - The dimer can autophosphorylate becomes activated once all of its tyrosines have been phosphorylated. The catalytic domain is now able to interact with the effector proteins on the intercellular side that will carry out the response
  - The effector proteins that interact with the receptor will go deliver the message to multiple places throughout the cell
  - Ras is an effector protein that doesn't have an SH2 domain, so it requires an adaptor protein
  - GRB2, a protein with an SH2 domain binds to the receptor
  - GRB2 has an SH3 domain that allows it to interact and bind with Sos
  - Sos recruits Ras and binds to in
    - In Ras' inactive state, it is a lipid anchor protein that is also bound to GDP. When GRB2 an Sos bind to the receptor they recruit Ras to come bind to Sos

    - When it binds to Sos, its affinity for GDP decreases and it releases it. It now has a high affinity for GTP and it exchanges it GDP for GTP
        - NB: it exchanges GDP for GTP, it doesn't phosphorylate it
    - Binding to GTP activates Ras and changes its shape and causes it to detach from Sos
    - Ras can now go pass on the message to various parts of the cell
- The case of nerve growth factor (NGF)
    - NGF: a family of growth factors that helps nerve grows
    - NGF acts as the ligand by binding to tyrosine kinase receptors and triggers the Ras cascade
    - Ras gets activated and it goes and activated various other proteins
    - The end result is activating MAP Kinase (MAPK), which is one of the ways to ensure that BCl_2 (apoptosis inhibitor) remains active
    - This cascade also ensures that Bad remains inactive (pro-apoptotic protein)
- The case of epithelial growth factor:
    - Also activates the Ras pathway
    - Ras activated Map Kinase (MAPK), which triggers gene translation through the activation of transcription factors
    - Epithelial growth factor can also activate PLC (phospholipase C), which has an SH2 domain and can directly interact with the receptor
        - PLC cuts the phosphate groups off of phospholipids
        - PLC targets PIP2 and cuts off the phosphate groups, which frees IP3, which can interact with various molecules within the cell
        - After IP3 is freed DAG (diacylglycerol) gets left behind, which can act as a second messenger
        - IP3 is needed to activate the calcium channel of the ER
    - Note the amplification: EGF activated not only MAPK, but also PLC and then they interact various other substances
    - 30% of cancers can be associated with dysregulation of one of the steps between Ras and MAPK

<u>Transmembrane Receptors 3: G-Protein Coupled Receptors (GPCRs)</u>

Largest family of receptors (metabotropic)

Brian Kolbika (structure) and Robert Lefkowitz (mechanism) helped define the structure and mechanism of signaling

- Structure: span the membrane 7 times, forming "pockets"
- The extracellular portion

Multiple ligands – some bind to many different GPCRs

- ACh can bind to 5 different types of GPCRs
- Adrenaline (4 main receptors)

Mediate responses to a diverse range of ligands (ex. Neurotransmitters, hormones, odorants, tastants, and photons of light)

Diversity of receptors for a given ligand translates to multiple responses

They interact with GTP-activating proteins (G-Proteins)

- G-proteins are composed of three subunits: alpha, beta, and gamma
- When all three subunits are together, the G-protein is inactive

2 main pathways:

- cAMP
- PIP (IP3)

Generic cascade:

- Ligand binds to a GPCR, causing a conformational change
- The activated receptor signals to an associated G-protein, causing the alpha subunit to exchange GDP for GTP, causing a change in conformation and it becomes activated
- The activated alpha subunit dissociates from the betagamma subunits, and it moves through the membrane and interacts with an amplifier enzyme
    - The alpha subunit will either activate the cAMP pathway or the PIP pathways, but either way a second messenger will be produces
    - Note that the betagamma subunits can also move on to interact with enzymes
    - It is possible that the alpha subunit can inhibit the amplifier enyzme
- The amplifier enzyme will activate the second messenger
    - Possible second messengers: cAMP, cGMP, DAG, Ca2+, IP3
- The activated second messenger activates or inhibits cellular pathways
    - The G-protein can be stimulatory and activated adenylate cyclase (G_s) or inhibitory (G_i)

The cAMP pathway:

- The activated alpha subunit activates adenylate cyclase
- Activated adenylate cyclase catalyzes the conversion of ATP to cAMP
- cAMP binds to the regulatory subunits of protein kinase A (PKA), which dissociates from the catalytic subunit, activating it

- The activated catalytic subunit phosphorylates proteins, causing a response
- The phosphorylated proteins are rapidly dephosphorylated by serine/threonine phosphatases, terminating the response
- PKA can also translocate to the nucleus and activate/inactivate genes via the CRE (cAMP response element)

The PIP/IP3 pathway:

- The activated alpha subunit activates phospholipase C (PLC), which cleaves PIP2 into IP3 and DAG
    - DAG is cleaved in the membrane to form arachidonic acid, the basis of eicosanoids
- IP3 is released into the cytoplasm
- IP3 can be phosphorylated to IP4, which has diverse effects
- IP3 also binds to Ca^{2+} channels on the endoplasmic reticulum, releasing Ca^{2+} into the cytoplasm
    - The Ca^{2+} binds to calmodulin, causing diverse effects within the cell
    - The Ca^{2+} also stimulates protein kinase C (PKC) to move to the membrane where it interacts with DAG
    - DAG activates the PKC, which then phosphorylates proteins, stimulating a phosphorylation cascade
    - PKC also leads to the production of arachadonic acid and eicosanoids

One ligand – many responses:

- Norepinephrine is a catecholamine (NT and hormone)
- Responsible for concentration
- Binds to adrenergic receptors
- Stimulates nervous system
- Activates glycogenolysis and gluconeogenesis
- Pupil dilation
- Maximize blood flow to skeletal muscle and survival organs
- Can bind to an alpha-1 receptor, it triggers the PLC pathways
    - Leads to activation of PKC, which phosphorylates the calcium channel on the membrane
    - Promotes entry of calcium into the cell
    - IP3, the other product of this, also allows calcium to enter the cell by leaving the mitochondria
- Can shut down adenylate cyclase – no cAMP, PKA is not activated
    - Receptor remains in its inactive conformation

    - cAMP pathway is shut down
    - Calcium is not allowed to enter the cell
- Can bind to a beta-2 receptor, activates adenylate cyclase using the cAMP pathway
    - Calcium channel is phosphorylated, calcium is allowed to enter the cell

X-Ray Crystallography:

Helps study receptors

Takes a protein that's membrane soluble and makes it a solid structure that can be hit with x-rays

- The x-ray will be refracted and the pattern of refraction allows patter, shape, size, etc. to be determined

Problems:

- Time consuming
- Large amounts of material required
- What about insoluble proteins?

Response

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Specificity of Responses:

Same receptors but different intracellular proteins cause different responses

Different receptors cause different responses

Same ligand and same receptor but different cell types can cause different responses

Coordination of Response:

Antagonism between insulin and glucagon on the pancreas avoids a futile cycle of increasing and dropping blood glucose concentration and maintain homeostasis

Insulin uses tyrosine kinase receptors, glucagon uses GCPRs and cAMP pathways

Co-ordination of signals: one pathway can take precedence over another

Ending the Response:

Ligand can be removed by distant tissues

- Guided by concentration gradient

Ligand can be taken up by adjacent cells

Ligand can be degraded by extracellular enzymes

Ligand-receptor complex may be removed by endocytosis

The receptor may be inactivated

The signal transduction pathway may be inactivated

Topic 8: DNA Replication
DNA/RNA

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Objectives:

Understand the structure and organization of DNA (including nucleotides, etc.)

Understand and demonstrate the steps involved in replication

Understand the structure and organization of RNA

Explain the DNA protection and correction mechanisms

DNA History:

1911: Thomas Hunt Morgan was able to associate traits (genes) to a chromosome by studying sex-liked inheritance in *Drosophila melanogaster*

1928: Frederick Griffith identified properties of strains of *Streptococcus pneumonia* related to their virulence in mammals

1940: Oswold Avery determined that these properties were related to DNA (not RNA or protein)

1952: Alfred Hershey demonstrated that DNA was the hereditary molecules by used phages and *E. coli*

1953: James Watson and Francis Crick determined the molecular structure of DNA

50 years later we were able to sequence entire genomes

READ 12.1!!!!!

DNA:

The genetic language used by all organisms

A helix of ~2 nm in diameter – the only possible arrange is A-T and G-C in order to achieve that width

- A purine (A or G) is always paired with a pyrimidine (C or T)

Structure: a sequence of linked nucleotides

- Each nucleotide is attached by a phosphodiester linkage between the 3rd C of the sugar and the P group of the next nucleotide
- This gives orientation to the strand: the first nucleotide is the 5’ end (it presents with a phosphate group). The last nucleotide is the 3’ end
- Elongation is always 5’ to 3’

The double helix:

- Both strands run in opposite directions
- Nitrogenous bases are facing inside the helix and provide stability by hydrogen bonding
- Sugars and phosphate form a ribbon backbone

Organizing DNA:

- DNA is wrapped around histone proteins, forming a nucleosome
- Nucleosomes are assembled together to form a solenoid
- These need to be unwound to replicate the DNA or to access a gene
- Allows you to compress the large structure in a small space in an organized and efficient manner

RNA:

Built in a similar fashion, but uses Uracil instead of Thymine

Forms a single stranded helix

Many different types:

- mRNA (messenger)
- tRNA (transfer)
- rRNA (ribosomal)
- snRNA (small nuclear)
- ncRNA (non-coding)
- iRNA (interfering)

The Flow of Genetic Information – the Dogma of Molecular Biology:

DNA → RNA → Protein

Unidirectional

DNA Replication

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DNA Replication:

Follows a semiconservative model

- Both parental strands separate and a copy will be made complementary to those parental strands, forming two new strands of DNA
- Each new strand has one parent and one daughter strand

Meselson-Stahl experiment: helped prove that it followed a semi-conservative model

- Used two isotopes of nitrogen
- Grew bacteria in a N heavy medium
- Transferred some bacteria to a N light medium, the growth continues
- Take samples after 0 minutes, one round of replication and two rounds of replications
- Results:
  - After 2 generations, half the DNA was intermediate and half of it was light only
  - There was no heavy only DNA
- Conclusion: this could have only occurred if each DNA molecule contains a template strand from the parental DNA, thus DNA replication is semiconservative

Circular genome: replicated from a single point of origin

Linear genome: multiple replication origins that eventually meet up together

- However, machinery is still the same for circular and linear DNA

Replication Requirements:

Unwind DNA and make sure it doesn't supercoil on the other side

2 separate anti-parallel mother strands – make 2 complimentary daughter strands (respect orientation of elongation)

Obtain 2 identical sets of DNA, each with a mother-daughter strand in a double helix

Leading and Lagging Strands:

Elongation only occurs 5' to 3', but replication occurs from the origin towards the replication fork in both directions on both strands at the same time

Can only add nucleotides on the 3' extremity of the parent strand (the 5' end of the daughter strand)

Leading strand: orientation of elongation of daughter strand is 5' to 3' so it is replicated in a continuous fashion

Lagging strand: orientation of elongation of daughter strand would be 3' to 5', which is impossible

- It is therefore accomplished by sections in 5' to 3' (forming Okazaki fragments)

Replication Process:

Replisome: a replicating area

Two strands are separated, forming a replication bubble

Helicase splits the two strands of DNA apart

Single stranded binding proteins come and bind to the single strands to prevent them from reannealing (winding back together)

DNA gyrase (topoisomerase) prevents supercoiling and torsion on the ends

Primase introduces a short complementary strand (4 – 10) strand of RNA nucleotides to the DNA – makes an RNA primer

- DNA polymerase can only attach to double stranded sequences, but primase can attach to single strands

DNA polymerase III introduces nucleotides that are complementary to the parental strand

Sliding clamp protein comes in at the same time of DNA polymerase III and ensures that the DNA polymerase doesn't detach until the entire complimentary strand has been synthesized

DNA polymerase I removes the RNA primer and replaces it with DNA nucleotides

- Replaces each RNA nucleotide with the equivalent DNA nucleotide, not the complimentary one
    - Replaces A for A, G for G, C for C, U for T

However, there is a gap between the last nucleotide from the former RNA primer and the first DNA nucleotide. Gyrase had also introduced small nicks to help relieve the tension

DNA ligase comes and forms a bonds (the phosphodiester bond) between the two to close the gap and to close the nicks caused by gyrase

Proofreading:

DNA polymerase III:

- Rarely, DNA polymerase adds a mispaired nucleotide
- But proofreading occurs as replication does
- DNA polymerase III recognizes the mismatched base pair
- The enzyme reverse and removes the mispaired nucleotide from the strand
- DNA polymerase then resumes its polymerization activity in the forward direction

Nucleotide excision repair:

- Repair enzymes move along the completed DNA scanning for distortion in the double helix due to a mispaired base

- The enzymes break the backbone of the new strand on each side of the mismatch and remove several to many bases, including the mismatched base
- This leaves a gap in the DNA for DNA polymerase to fill in
- DNA ligase then seals the nick

DNA Protection in Eukaryotes: Telomeres

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Telomeres:

Regions at the ends of chromosomes

Long sequences of repeating non-coding nucleotides

Allows you to have an extra length of DNA to preserve the genes

- Every time DNA replicates it gets shorter and shorter
- Telomeres ensures that the non-coding nucleotides go missing, not any of the coding DNA

There is a limit though – once the telomere runs out the cell starts so age (enters senescence)

Telomere Synthesis:

After primer is removed, there is a single stranded region left at the end of the chromosome

Telomerase come in with its own RNA primer

It comes in, finds a position in the overhang, matches, and uses its RNA template to add DNA nucleotides at the 3' end, extending the overhang

- It repeats this quite a few times until it is a little longer

The telomerase leaves, taking its primer with it

A primase will be recruited and then DNA polymerase III can come to start replicating the DNA

The RNA primer created by the primase will be removed leaving an overhang

But, the overhang is repetitive so it can fold over itself and close off the overhang

- Consequences of sticky end if this isn't done properly: it is possible that two chromosomes with sticky ends will stick together

Working with DNA

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DNA Cloning:

Isolate genomic DNA containing a gene of interest from cells and cut the DNA into fragments

Insert each fragments into a different plasmid to make recombinant DNA molecules

Introduce the recombinant molecules into bacterial cells, with each bacterium receiving a different plasmid

As the bacteria grow and divide, the recombinant plasmids replicate, amplifying the piece of DNA inserted into the plasmid

Identify the bacterium containing the plasmid with the gene of interest

Grow that bacterium in culture to produce large amounts of the plasmid with the gene of interest

Restriction Enzymes:

Enzymes that look at DNA sequences and recognize patterns and cut the DNA is a certain fashion

Some will but blunt, some will cut unevenly to produce sticky ends

- Sticky ends are very useful for insertion

Polymerase Chain Reaction (PCR):

PCR is essentially amplified controlled replication

Identify a particular sequence of interest that is needed in large amounts

- The sequence must be known

The target sequence is isolated and the DNA is heated to use the H-bonds to break and the strands are separated

A RNA primer is created just ahead of the target sequence

The DNA is then cooled to allow the primers to anneal to their complementary sequences at the ends of the target sequence

The DNA is heated again (not as much as the first time), allowing a polymerase that mimics DNA polymerase III then starts to add the complimentary strand

This process is then repeated with continuous heating and cooling cycles

Topic 9: RNA Transcription and Regulation

RNA Transcription

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Objectives:

Understand, describe, and demonstrate the step involved in transcribing DNA to mRNA

Be familiar with the elements comprised in a transcription initiation complex and its role

Explain and demonstrate RNA maturation and splicing

Compare and contrast RNA transcription in prokaryotes vs. eukaryotes

Know, describe and/or compare the different elements of control for gene expression in prokaryotes vs. eukaryotes

- Activators, enhancers
- Repressors
- Alternative Splicing
- Operons
- Interference

Transposons and retrotransposons – be familiar with what they are, be able to briefly explain how they occur

Genes:

Each cell contains the entire genome in an organized manner (histones → nucleosomes → solenoid)

The genes are organized in such a manner that there is a series of genes and between the genes are intergenic (noncoding) regions

Each chromosomes have genes for many different proteins

Some genes can activate individually and some can activate along with others (in clusters)

Gene Organization:

Regulatory sequence: controls the timing, location, and amount of gene expression

- In front of the gene
- Non coding, doesn't get transcribed
- Determines whether or not transcription occurs, the rate of transcription, and for how long the transcription occurs
- Contains the promoter: a sequence that is recognized by transcription factors that they bind to
    - A repetitive sequence of A's and T's – the TATA box
- Just outside the promoter are the proximal near-regulatory elements: transcription factors, activators, and repressors will bind to this region

Coding sequence: determines the sequence of amino acids in the protein

- What leads to mRNA, translation, and the protein

Distal regulatory sequence: in the intergenic space; binding of proteins (TF, activators, repressors) can influence transcription further down the DNA

Gene Expression:

The gene is activated and the DNA is transcribed to mRNA in the nucleus

- Only RNA can leave the nucleus – helps keep DNA protected
- Allows many transcripts (copies) to be obtained from the same DNA
- Transcripts leaving the DNA allows many transcripts to be transcribed at the same time

mRNA gets transported to the ribosomes for translation

Transcription

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RNA Polymerases:

RNA polymerase I = rRNA

RNA polymerase II = mRNA

- Has the ability to split the two strands of DNA, read the template strand, and make a complementary mRNA strand

RNA polymerase III = tRNA

Only RNA polymerase II leads to proteins (translates)

RNA polymerase I and III contribute to this by providing the ribosomes and by bringing the amino acids respectively

Transcription:

A transcription factor interacts with the promoter in the regulatory sequence

RNA polymerase II reads the DNA strand 3' to 5' and builds a complementary strand in the 5' to 3' direction

- The template strand is the 3' to 5' strand of DNA
- The complementary strand of RNA that is obtained is the same as the 5' to 3' strand of DNA
  - The coding strand of the DNA is the 5' to 3' strand
  - Note: U instead of T

The mRNA that is obtained is 5' to 3'

- Nucleotides can only be added to the 3' end of the ribose sugar

Occurs in three stages:

- Initiation
- Elongation
- Termination

Initiation:

TATA binding proteins (TFs, activators, repressors) bind to the TATA box to form the transcription initiation complex

RNA polymerase II comes in and gets included with the transcription initiation complex

Transcription can now begin – will begin at the coding sequence (at the end of the regulatory sequence)

- First nucleotide in the coding sequence: +1

Elongation:

Nucleotides are added to obtain a complementary 5' to 3' sequence

- The complementary strand has the equivalent RNA nucleotides
- Ex. A for A, C for C, G for G, U for T

Behind RNA polymerase II, DNA strands reform into the double helix

Unlike DNA polymerase III, DNA polymerase II doesn't proofread and doesn't correct its mistakes

Termination:

The complete RNA molecule is released from the template DNA

RNA polymerase II leaves the DNA and the double helix reforms

Prokaryotes vs. Eukaryotes

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Prokaryote vs. Eukaryote Transcription:

Prokaryotes:

- Start of transcription:
  - Binding to the promoter of polymerase subunits (particularly the sigma subunit) – serves as a guide to place RNA polymerase
  - RNA polymerase initiates transcription
- Termination:
  - Rho dependent
    - ATP-dependent unwinding enzyme at 3' end
    - Rho comes and interacts with the sequence to promote the disassociation of the RNA from the DNA
  - Rho independent (intrinsic)
    - GC rich sequences at end
    - Creates a hairpin loop which creates lots of tension/pressure and pulls RNA away from DNA
- Product:
  - Produces a mature mRNA – it's ready to go

Eukaryotes:

- Start of transcription:
  - Binding to the promoter of transcription factors, activators, repressors
    - Activators/repressors: can enhance/decrease transcription
  - Formation of transcription initiation complex (TFs and protein)
  - Then RNA polymerase II is recruited to initiate transcription
- Termination:
  - Specific sequences of nucleotides signals to RNA polymerase II that it is nearing the end of the coding sequence
  - RNA polymerase II leaves the sequence in anyways and finishes transcription
  - The signal sequence at the end recruits polyA polymerase, which recognizes the sequence at the 3' end of the pre-mRNA and adds a sequence of A (50 – 200 A's → makes a polyA tail)
  - The polyA tail (3' end) is necessary for it to be matured and to be able to leave the nucleus through the nuclear pores; it also protects against degradation
    - Nuclear pores will recognize the polyA tail and allow it to pass through

        - Without the polyA tail, the pre-mRNA will remain in the nucleus and be degraded by nucleases
- Product:
    - Produces an immature mRNa → pre-mRNA
        - It needs to be processed (RNA maturation) before leaving the nucleus
        - Certain things need to be changed and certain sections need to be changed
        - Maturation:
            - Exons and introns:
                - Exons are coding regions and introns are non-coding regions
                - Splicing: removing the introns
                    - Alternative splicing: genes can be spliced differently to keep different exons in order to get different versions of a protein coded for by that gene
                        - Ex. Smooth vs. skeletal muscle tropomyosin
                        - Two different products can be achieved from the same gene
                - Spliceosome: structure made of small nuclear RNAs and proteins
                    - Specifically recognizes sequences at the beginning and end of the introns that bind to the RNA and form a complementary sequence and the area where binding occurs is cut out
                    - The intron will be degraded in the nucleus
                    - The ends of exons after removal of introns will be joined together
            - 5′ cap: 7 G's were added to the 5′ end while elongation was occurring (after transcription of the first 20 – 40 nucleotides were added)
- Modify chromatin (histones) to reach DNA sequence

<u>Regulation of Gene Expression:</u>

Prokaryotes:

- Regulation is achieve through operons (genetic switches that control transcription – on/off)
- Operons: comprised of the genes under the operon's control, the promoter, and the operator (the actual on/off switch)
- Ex. Lac operon
    - Lactose is broken down by three proteins
    - The genes for all three are located beside each other, and when transcription is initiated all three are transcribed and all three proteins will be translated
    - Low lactose: prokaryote will not expend energy transcribing those genes
        - Repressor comes from a regulatory gene, upstream from the lac operon
            - The regulatory gene transcribes it RNA into the repressor
        - Repressor protein attaches to the operator, there is now not enough room for RNA polymerase to interact with the promoter – no transcription occurs
        - The repressor may be displaced, and low levels of transcription can occur
    - High lactose:
        - Permease molecules transport lactose into the cell
        - Beta-galactosidase conert some of the lactose into allolactose
        - Allolactose binds to the Lac repressor, inactivating it by altering its shape so that it cannot bind to the operator
        - RNA polymerase binds to the promoter
        - Transcription of the lac operon structural genes occur
        - Once all the lactose has been catabolized, the repressor will bind to the operator and terminate transcription
- *Only responsible for lactose

Eukaryotes:

- In addition to the transcription initiation complex, there are activators and repressors that control which genes are expressed and the rate at which transcription occurs
    - Instead of having one repressor per gene, there is a pool of available activators and repressors

    - The combination of the activators and repressors that are used change which genes undergo transcription
- siRNA and miRNA occus after maturation
- siRNA (short interfering RNA) can bind to mRNA and target it for degradation by nucleases – prevents the genes from leaving the nucleus and being translated
- miRNAs (micro RNA) are important for the regulation of gene expression (time-sensitive expression)
    - They are the product of gene transcription and fold over itself, creating a hairpin loop
    - Leaves the nucleus and encounters a nucleus called dicer
    - Dice removes the loop from the miRNA, leaving a double stranded RNA
    - A protein complex degrades one of the miRNA strands, leaving behind a short miRNA strand in the protein, forming a miRISC (miRNA induced silencing comlex)
        - The miRISC can either stop translation or reduce the available mRNA (it gets degraded)
    - The miRNA in the miRISC binds to target mRNAs that have a complementary or nearly complementary base sequence
    - Matching between the miRNA to the target mRNA can be perfect or imperfect
        - Perfect pairing causes the mRNA to be sent to be degraded
        - Imperfect pairing of the miRNA to the target mRNA causes translation to be halted – delays when it will occur

Transposons and Retrotransposons

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Transposons:

Discovered in the 40s by Barbara McClintock, while she was studying maize

They are portions of DNA, transposable elements, that move from one location in the genome to another

They are observed in all organisms

- They account for 50% of the human genome

There are DNA transpsosons and retrotransposons

How they migrate:

- The transposase enzyme recognizes the beginning and the end of the transposon and cuts the DNA at these points, releasing the transposable element into the nucleus
- The released transposons can incorporate themselves into other points of the genetic material

Results of migration: vary depending on where they land

- Within a gene: can silence it or cause a mutation
- They can introduce a new sequence – a new gene
- Some transposons can be silenced by interfering RNA (miRNA or siRNA)
- Can also stimulate evolution – bring adjoining sequences alons when they jump, increasing genetic diversity

The Alu Transposon:

This sequence of around 300 base pairs can account for approximately 15% of the human genome

Thought to be the key to understanding the evolution of primates and humans

Associated with diseases such as: some forms of cancer, Alzheimer's disease, hemophilia, and others

Retrotransposons:

Need a reverse transcriptase to transcribe RNA to DNA before they transpose

- Counters the central dogma of DNA

Some mRNA does not go on to translation, but is reverse transcribed back to DNA and inserted back into the genome

- The mRNA meets reverse transcriptase, which reverse transcribes it to DNA (there is now one strand of DNA attached to the mRNA
- The cell doesn't like the RNA-DNA duplex and synthesizes a second strand of DNA to replace the RNA

- The double stranded DNA can now act like a transposon and inserts itself into the genome

Similar process to the RT telomerase

There is no proofreading or correction mechanism – many errors/mutations

- Increases genetic variability

Retroviruses:

Using a reverse transcriptase, they convert viral RNA into a complementary strand of DNA

- You now have a RNA-DNA matrix in the host cell

The host's DNA polymerase makes it into a double strand of DNA by displacing the RNA strand and adding the complementary strand of DNA

Integrase allows this double stranded DNA to be introduced into the host's genome

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

Mutations make the virus difficult to eradicate

Topic 10: Translation and Protein Sorting

Translation

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Objectives:

Understand and demonstrate the different steps involved in translation

Know the components and roles of a ribosome

Using the codon table, be able to obtain the mRNA and amino acid sequence corresponding to a given DNA strand

Define and explain, using an example, the different genetic point-mutations and possible mutagens

Translation:

Codons: a set of three nucleotides that pertains to a certain amino acid

The code is degenerate (redundant), there are synonyms but no ambiguity

- Only one AA per codon but many codons per AA

Oscillation or the wobble effect:

- Certain amino acids are associated with more than one codon – the difference lies in the 3rd nucleotide
- This is how the synonyms arise
- It gives flexibility in the reading sequence
- Allows only 45 tRNAs to be needed to achieve the 20 amino acids and accommodate the 61 codons

The code is read from 5' to 3'

The reading frame is determined by the start codon (AUG – Met)

Reading ends with stop codons: UAA, UAG, or UGA

tRNAs will bring an amino acid to the growing chain

- Comprised of three hairpin folds
    - One of the loops contains the anticodon: it is complementary to the codon on the mRNA currently being read by the ribosome
- Carry the amino acids at their 3' end, and recognize the mRNA sequence (codon) in their anticodon region

Exons correspond to protein domains

- Domains can carry out different abilities (ex. Ligand binding, transmembrane region, catalytic domain)

tRNAs will bring an amino acid to the growing chain

- Carry the amino acids at their 3' end, and recognize the mRNA sequence (codon) in their anticodon region

Translation is comprised of three stages:

- Initiation
- Elongation
- Termination

Aminoacylation:

The process of attaching the amino acids to the tRNA

An aminoacyl-tRNA synthetase (an enzyme) will recognize the tRNA and an amino acid

They synthesize a covalent bond between an amino acid and a tRNA

- ATP and the amino acid bind to they aminoacyl-tRNA synthetase
- The enzyme catalyzes hydrolyzes ATP and releases two phosphates, allowing it to join the amino acid to AMP
- The correct tRNA binds to the enzyme
- The enzyme transfers the amino acid from AA-AMP to the tRNA, forming AA-tRNA
    - The AMP is released
- AA-tRNA is released from the enzyme and the enzyme is ready to enter another reaction series

Each amino acid can bind only to one specific tRNA and uses a specific enzyme to do so

- Each enzyme can only bind to one amino acid at its binding site

Ribosomes:

Assembled in the cytosol when binding to mRNA (30S to mRNA then 50S completes after)

Formed by 2 protein subunits (30S – small subunit; and 50S – large subunit) and some rRNA

- Small subunit: grabs the mRNA and helps snake it through the ribosome
- Large subunit has three sites:
    - A site: aminoacyl site
        - Aminoacyl-tRNA arrives with the proper amino acid
        - Reading the code happens here, the codon is matched to the anticodon on the tRNA
    - P site: peptidyl site
        - Peptide bond is formed between amino acids
        - The polypeptide grows in an N-term to C-term orientation
    - E site: exit site
        - Exit of growing polypeptide chain out of ribosome

Recruit tRNA once the start codon has been reached, catalyze peptide-bond formation

Free cytosolic ribosomes synthesize soluble proteins

Membrane bound ribosomes synthesize proteins that will be transmembrane proteins or that will be secreted

Initiation:

The small subunit of the ribosome reads the mRNA until it reaches the start codon

- tRNA carrying Met comes along with GTP and is already associated with the small subunit
- It is known that the start codon is reached because it matches the anticodon on the tRNA

Once the start codon has been reached, the large subunit will be recruited, along with an elongation initiation factor (eIF – a protein)

The large subunit will bind to and GTP will be hydrolyzed

The ribosome is now ready for the next stage of translation - elongation

NB: Met is the first AA and is the only one that goes straight to the P site

- The next tRNA will arrive at the A site

Elongation:

The eIF signals that the ribosome is ready to translate

tRNA can now be recruited to the available A site

- The tRNA with a matching anti codon to the codon in the A site will be recruited

A peptide bond will be formed between the carboxyl group on Met and the amino group on the next amino acid

Peptidyl transferase transports Met onto the second tRNA

- There is now an empty tRNA in the P site and a tRNA containing two amino acids in the A site

Other elongation factors come along with the tRNAs and help hydrolyze the GTP to help the translocation of the tRNAs

tRNAs will be translocated from the P to the E site and from the A to the P site

The A site is now empty and a new tRNA can arrive

The cycle will continue, and during translocation, the tRNA from the E site will leave the ribosome

Termination:

The stop codon is reached – there is no tRNA with that anticodon

Release factor (RF) protein occupies the A site instead, promoting the last peptide bond formation and translocation

After translocation, the peptide is released

- The RF stimulated peptidyl transferase to cleave the polypeptide from the P site tRNA

RF promotes separation of the ribosomal subunits, which can reassemble again with another mRNA

NB: not all of the proteins that are produced begin with Met

- After translation, protein is immature
- Must be folded, combined with other peptide chains, functional groups need to be added, chain needs to be cleaved, be transported to their effector area, etc.

Polysomes (Polyribosomes):

An mRNA can be translated by more than one ribosome at a time

This creates multiple polypeptides

Each of these ribosomes translate the entire length of the mRNA – this causes multiple copies of the protein to be produced

- NB: it is NOT that each of the ribosomes translate a portion of the mRNA and the portions will be joined together

Chaperones:

Protect protein and facilitate the proper folding of nascent (newly formed proteins)

They are small proteins that interact directly with the polypeptide in order to protect it (maintains it structure, prevents it from being degraded by enzymes, etc.)

There are also large chaperone protein complexes: a large structure that completely surrounds and "engulfs" the polypeptide

- In addition to protecting the polypeptide, they place it in a favorable environment isolated from the rest of the cell
- They contribute to folding and ensure that the protein accurately obtains its tertiary structure

Chaperones should always be used – they always accompany translation

Different types of chaperone proteins depending on the polypeptide being synthesized

Post-Translational Modifications:

Being in ER, continue in Golgi

- Soluble proteins from free ribosomes undergo co-translation to get to the ER
    - Co-translation:
        - While undergoing translation, the ribosome will move to the ER

- The chain of amino acids extending from the ribosome are accessible and are in the cytosol
  - These amino acids have a particular sequence and can be recognized
  - These are signal peptides and signal to transport them to the ER
  - NB: the signal sequence is at the N terminus
- Signal recognition proteins/particles (proteins in the cytosol) recognize the signal peptides and attach to it, pausing translation
- The ribosome remains assembled and the signal recognition particle will guide the ribosome to the ER and will bind to a signal recognition particle receptor
  - This ensures that the ribosome is correctly lined up with a translocating protein (protein complex spanning the membrane that forms a channel)
- The signal recognition particle hands off the ribosome to the translocating protein and translation continues
- The protein is now anchored inside the ER and will continue to grow
- Just before termination is reached, signal peptidase (an enzyme in the translocating protein) cleaves the signal peptide from the growing polypeptide
- Translation is now complete and the peptide is free and soluble inside the lumen of the ER
- The ribosomal subunits can now dissociated

- Proteins are transported from the ER to the Golgi by vesicles

Occur after translation is complete

Proteins that need to go through the ER:

- Membrane proteins, hydrophobic proteins
- Proteins that will be excreted from the cell
- Anything that will be directed to another organelle (ex. Lysosomal proteins)

Important for folding, sorting, protection, and final location/function

Modifications:

- Glycosylation: add sugar to amino acid side chain
  - Sorting in Golgi, functional
  - Easier to add to polar amino acids

- Acetylation: add acetyl group to N-term
  - Increase stability
- Disulfide bond: links S between residues (Cys)
- Lipidation: add lipids
- Ubiquination: adds ubiquitin
  - Targets for degradation, amino acids will be reused

Transmembrane Proteins:

In addition to the signal sequence to direct translation to the ER, there is a stop-sequence within the sequence of amino acids that halts co-translational import

- As the protein grows, the translocating protein recognizes the stop sequence and translocation stops
- Translocation continues to completion
- Peptidase cleaves the signal sequence, as normal
- Once translation is complete, the translocating protein changes conformation and releases the protein, which stays within the membrane as a transmembrane protein

Translation is completed, but import is not

Vesicles and the Cytoskeleton:

At the end of translation proteins will either be transmembrane proteins in the ER, proteins within the ER's lumen, or cytosolic proteins

Cytosolic proteins are do not need to be further transported

Vesicles are used to transport the other proteins to the Golgi for maturation

- Vesicles are made of the ER's membrane
- Transmembrane proteins will be within the vesicle's membrane
- Proteins within the lumen will be within the vesicle

The vesicles will use motor proteins and microtubules to move to the Golgi

In the last few cisternae of the Golgi, sorting will occur based on the modifications made to the proteins

Proteins with similar destinations will be packaged in the same vesicle

Prokaryotes:

In the absence of a nuclear membrane and since the mRNA is mature, transcription and translation will occur simultaneously

Recall: transcription occurs in the nucleus in eukaryotes, translation occurs outside the nucleus
This cannot occur in prokaryotes

Secretion:

Constitutive secretory pathway:

- Involved in maintaining regular amounts/flow of protein, receptors, porins, compounds secreted outside the cell
- Doesn't need to be triggered
- As soon as maturation is done at the end of the Golgi, the proteins are packaged in a vesicle and sent to the membrane

Regulated secretory pathway:

- Proteins are packaged in vesicles but are not secreted right away
    - Docking: there are some vesicles that are very close to the membrane so that they do not need to travel to the membrane once they have been triggered
    - The other vesicles are a little bit farther from the membrane
- Requires a trigger (ex. Peptide based hormones)
- Calcium triggers for the release of the vesicles
    - Can enter through ligand-gated ion channels
    - PIP pathway

Mutations

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Point Mutations:

Changes to single nucleotide pairs on the complementary DNA strands

Missense Mutation:

Change in nucleotide sequence leads to change in amino acids

- Ex. Get an A instead of a C

This slight change in sequence has a profound impact on structure and hence, protein function

Ex. Sickle cell anemia

Nonsense Mutation:

A change in a nucleotide causes the stop sequence to be produced

This causes premature termination of the polypeptide

Impact depends on where the mutation occurs and on which protein it occurs

Silent Mutation:

A change in nucleotide has no affect

There is no change in amino acid

Possible due to the Wobble effect (oscillation)

Frameshift Mutation:

Insertion or deletion of a base pair causes the reading from to be off by one from there on

- Ex. The fat cat sat → hef atc ats at

The amino acids are altered due to the frame shift

Topic 11: Cell Cycle Regulation
The Cell Cycle

4/12/2015 8:38:00 PM

Objectives:

Describe and compare the role of the different phases of the cell cycle, including the various molecular checkpoints

Explain how the cell cycle is regulated

Understand and discuss the relationship between the cell cycle's regulation and the cell's mechanisms for survival

The Cell Cycle:

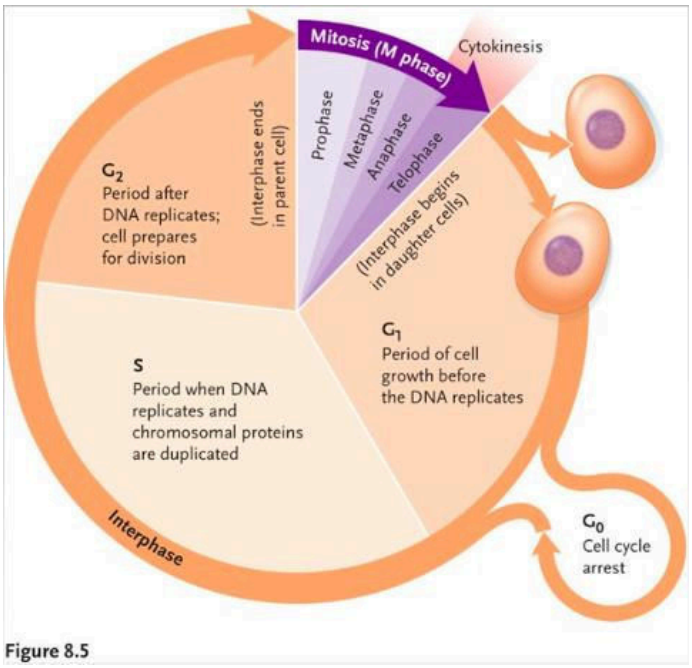

Figure 8.5

How does the cell progress from one phase to another?

What if one phase goes wrong; should the cell be able to interrupt division?

- Should be interrupted after metaphase if the chromatids have not properly attached to the spindle fibers
    - If not, the DNA will not be divided properly

Proteins and enzymes act as molecular switches

- Their concentrations rising and falling at different stages of the cell cycle to determine if the cell is in the proper state and can move forwards
- If it isn't, the cell will be given more time (G0) or will be marked for degradation (apoptosis)

Cell Cycle Regulation:

There are 3 main checkpoints that look for molecular signals and determine the cell's fate (G0 or apoptosis)

- G1/S checkpoint or G1 restriction point: at the end of G1
  - At the end of G1, there should be a larger healthy cell
  - If this is not the case, we will not proceed to the S phase
  - Is environment favorable?
  - Influenced by: growth factors, nutrients, cell size, DNA damage
    - GFs: the signaling cascade they activate lead to activation of protein that allow regulation of cell cycle
- G2/M checkpoint: at the end of G2
  - If the DNA is replicated, if there is enough, if it is ready for mitosis then it will move into the M phase
  - Is all DNA replicated? Is environment favorable?
  - Influenced by: cell size, DNA damage, DNA replication
- M-phase checkpoint: in the transition from metaphase to anaphase during the M-phase
  - The chromatids should have properly attached to spindle fibers to form the metaphase plate in order to continue
  - Are all chromatids attached to spindle fibers?
  - If they are, trigger anaphase and proceed to cytokinesis
  - Influenced by: chromosome attachments

<u>Growth Factors:</u>

The signaling cascade leads to the activation of transcription factors

Transcription is initiated and proteins are produces (ex. Cyclins and Cdk)

- Cyclins: essential and are expressed at the same time as enzymes that depend on cyclins (Cdk)
- They work together and their concentration rises and falls throughout the cell cycle
- Their concentrations allow the cell to pass through a checkpoint

<u>Cdk (Cyclin Dependant Kinases) Control the Cell Cycle):</u>

Cdk are kinases that depend upon cyclins to be activated

- They depend upon each other

The cell ensures that there is always a little bit of kinase and the buildup of cyclin allows a checkpoint to be passed

- The cyclin and the Cdk interact and form a cyclin-Cdk complex (maturation promoting factor), and the concentration of the complex is what allows the checkpoint to be passed
- After the checkpoint is passed, the cyclin will be degraded
- The complex is no longer active and the concentration falls

Different checkpoints have different cyclins and Cdk's

- They are sorted according to the checkpoint they govern:
  - G1/S cyclins: bind Cdk near the end of G1 and lead the cell into DNA replication
  - S-cyclins: bind Cdk during S phase and are required for DNA replication
  - M-cyclins: promote the events of mitosis
  - G1-cyclins: (in most cells) promotes passage through restriction point in late G1

<u>P53:</u>

Responsible to get the cell into the G0 phase

It also allows the cell to return to G1 if necessary growth or repair have been accomplished

It can also mark the cell for apoptosis

- It is capable of dephosphorylating Bad and recruiting APAF1

It also triggers miRNAs

<u>Mitotic Spindles:</u>

Motor proteins (dynein) attach to the kinetochores of the chromatids and walk along the microtubules of the spindle fibers to move the chromatids to opposite sides of the cell

Once the motor protein has walked past a certain section of the microtubule, it will depolymerize

Will break the bonds between the tubulin subunits (ionic, and hydrogen bonds)

<u>Nondisjunction:</u>

Metaphase plate was not properly formed and not all of the chromosomes attached to kinetochores

Leads to an inequity in genetic material in the daughter cells

Can have various effects depending on the type of cell and on the organism