BIO LEARNING OBJECTIVES MIDTERM 1 (L1-L8) INCREASE DETAIL

Topic 1

• How did life evolve into organisms and cells? (Review from BIO1130)

- It is postulated that organic material was synthesized from inorganic material due to the fact that the primitive earth's atmosphere contained the necessary components to do so such as steam, UV rays, volcanoes and lightning which would catalyze the creation of small monomers and amino acids.

- Scientists have performed experiments in which this process is imitated in an apparatus that mimics earth's early atmosphere and the results support the possibility of this happening.

- Another thesis is that life started in hydrothermal vents or that organic material, sugars, proteins and amino acids were transplanted on earth by a meteorite.

- Next, larger molecules such as proteins would have been synthesized. Since this process is in need of an enzyme or catalyst and none were yet created, hot sand acted as the catalyst to create proteinoids which are believed to be the earliest proteins.

- Next, a cell with a semi permeable membrane with the capacity to carry out chemical reactions different from its external environment would be developed. This is called a protocel and an example is a lysosome which has the required membrane and the ability to absorb things such as RNA. This is evidence of the earliest metabolism in a cell.

- The next requirement would be the capacity to replicate, but DNA would prove as an illogical explanation since it's processes require enzymatic activity which would prove impossible in the given age since enzymes were not around.

- The solution is RNA, which has the capacity to replicate as well as catalyze. The basis of replication would have relied on RNA until natural selection eventually acted to produce a more stable means of replication in the form of DNA.

- These processes resulted in the basis of life (cells) that are the building blocks of all organisms. Tissues would be formed by the grouping and interaction of similar cells, organs would form from groups of tissues which would create organ systems which make up the organism.

• What are the different types or classes of organisms?

Bacteria, are the most unique of the three for various reasons,

- They have a peptidoglycan layer which can be gram-/+.
- They are the most diverse organisms which differ from eukarya in the sense that they are prokaryotic organisms that lack membrane bound organelles. (archaea)
- They have circular DNA (archaea)
- The lack a nuclear envelope (archaea)

Archaea, are alike both other classes but have more in common with eukarya**.**

- Archaea and eukarya are considered to be monophyletic and more alike due to their more complex RNA polymerases.
- They posses histones associated with DNA
- They both lack peptidoglycan

They are similar to bacteria in the sense that they:

- Have no nuclear envelope
- No membrane bound organelles
- Have circular DNA

Their own traits include:

- Extremophile nature
- They contain membrane lipids with branched hydrocarbons

Eukarya. Eukarya have the following exclusive traits

- Multicellularity, defining them as eukaryotes
- Sexual reproduction : meiotic cell division forming haploid gametes that form diploid gametes
- Highly organized cell interior:
- Membrane bound organelles
- Nuclear envelope

• What is a cell?

- A cell is the basic functional and structural unit of life
- It has the ability to replicate and has a metabolism.
- Different divisions of cells exist such as prokaryotic and eukaryotic.
- Cells may perform a variety of different chemical functions as well as are structurally different and mostly microscopic.
- Some contain organelles and others do not. Microscopic organisms typically consist of a single cell which can be eukaryotic or prokaryotic.

• What is a cell made of? Define the different features, organelles and explain the roles/functions for each.

Ribosomes:

- Create proteins using genetic instructions from the nucleus,
- Created by a combination of proteins and RNA in the nucleolus.
- They are suspended in the cytoplasm or embedded in the RER.
- Found within all cells.
- mRNA provides the sequence by which ribosomes link amino acids.

Lysosomes:

- Digests foods, wastes, organelles and viruses and bacteria using lyso-enzymes which cannot survive outside of the lysosome

Peroxisomes:

- Creates hydrogen peroxide as a by product of its metabolic activities and converts it into water
- Breaks down fatty acids which are used for membrane construction and as fuel for respiration.

RER:

- Studded with membrane bound ribosomes which produce proteins,
- Produces membrane and is continuous with the nuclear envelope.
- Proteins are sent to golgi apparatus

SER:

- Produces lipids and steroid hormones,
- Detoxifies toxins,
- Ribosome-less,
- Stores calcium.
- It can also adapt to drug use and increase a specific tolerance to a drug since it becomes larger as a response to frequent use.

Nucleus:

- Contains genetic info in the form of linear DNA in chromosomes (Eukaryotes only).

Nucleolus:

- Creates rRNA (ribosomal) with instructions from the nucleuses DNA and assembles proteins and rRNA into ribosomes.

Nuclear envelope:

- Separates the nucleus from the cytoplasm using a double membrane with pores that contain pore complexes within them which regulate influx and efflux of RNA, macromolecules and proteins.

Centrioles:

- Produce microtubules and occur in pairs as a centrosome.
- Oriented in 9 triplets of microtubules
- Form the cytoskeleton and are used for cell motility
- MORE DETAIL IN TOPIC 3

Golgi apparatus:

- Packages, stores, ships, receives and manufactures cell products.
- Receives proteins for ER and modifies them.

Mitochondria:

- Uses respiration in order to convert glucose and oxygen to CO2 and ATP, 32
- May vary in numbers depending on the cell's functions or needs.
- *Example:* Slow oxidative muscle cells require lots of mitochondria since they produce ATP aerobically.

Chloroplasts:

- Convert CO2 and water using light into oxygen and food for the cell in the form of sugar.
- Found only in plant cells they house thylakoid stacks and give most plants their green pigments.

Cell wall:

- Found in plant cells, made of sugars, glucose, polysaccharides that contain pores called;
- plasmodesmata which make the cytoplasm of it's adjacent plant cells continuous since they provide passageways, proteins and RNA may even be shared.
- Protect and support plant cells.

Plasma membranes:

- Double layered selective membrane with hydrophobic and philic properties that allow for lipid soluble molecules to diffuse through and non solubles to either be rejected or require facilitated transportation by a carrier or channel.
- Encloses the cell cytoplasm
- MORE DETAIL IN TOPIC 4

Flagellum:

- Motility structure of cells which allows them to navigate or simply displace.
- Attached to basal body and controlled by motor.
- Cluster of microtubules and extension of PM
- MORE DETAIL IN TOPIC 3.

Cytosol:

- Inner gel like space of cell that surrounds all organelles made up of mostly water and salt.
- Cytosol is the part of the cytoplasm that is not held by any of the organelles in the cell.

Cytoplasm:

- Is the part of the cell which is contained within the entire cell membrane
- Other than the nucleus

Pilli:

- Outer projection of some cells that increases the surface area with little volume increase as well as increase the adhesiveness of the cell.

Capsule:

- Surrounds bacterial cell walls and provides protection

Cytoskeleton:

- Reinforces cell shape, protects it and functions in cell movement, made of proteins below.
- MORE DETAIL IN TOPIC 3

Intermediate filaments:

- Provide support upon collisions yet do not contribute to cell motility
- MORE DETAIL IN TOPIC 3

Microtubule:

- Projections of centrioles that contribute to motility of organelles and participate in mitosis actions
- MORE DETAIL IN TOPIC 3

Microfilament :

- Contractile actin strands that resist stress and force as well as contribute to motility
- MORE DETAIL IN TOPIC 3

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

Cells are microscopically small for many reasons:

- A large SA/V ratio is ideal for cells like erythrocytes which participate in constant gas exchange.
- This is effective since the rate of diffusion (flow in and out of cell, nutrients and wastes) is more effective for higher ratios.
- Larger cells require more nutrients and create more waste, therefore smaller cells are more efficient due to their lesser cytoplasm volume. These cells still occupy the same space however and congregate in trillions to create organisms efficiently.

• Explain the theory of the cell and the theory of endosymbiosis – can you provide some examples?

- An endosymbiont cell is one that houses another cell in which these cells have formed a symbiosis (mutualism) of some kind.
- It is believed that ancient prokaryotes engulfed other prokaryotes as prey which facilitated this relationship.
- An example of this is early non photosynthetic oxygen consuming prokaryotic cells being absorbed and becoming what's known as mitochondria, which create ATP for the cell through respiration and receive shelter.
- It is believed that this is true since the organelles have their own double semi permeable membranes as well as their own DNA separate from the host cell indicating it was not synthesized by the host.
- Organelles have smaller genomes
- The size of prokaryotes is relative to the size of organelles
- Organelles divide by nuclear fission which is a prokaryotic means of dividing.
- Circular MDNA and CDNA

• What are the main groups or types of cells and how do they differ?

- All cells share some similar components including a plasma membrane, genetic material (DNA), ribosomes and cytoplasm.
- This is all that is in common between prokaryotes such as bacteria and eukaryotic plant and animal cells.
- Bacteria have nucleoids, capsules, plasmids of circular DNA and pilli while eukaryotic cells have membrane bound organelles, a larger genome, a nuclear envelope surrounding a nucleus containing linear DNA and are much larger in nature (10-100 um).
- Plant cells differ from animal cells among eukaryotes. Plant cells contain central vacuoles, cell walls, plasmodesmata and chloroplasts.

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

Light microscopes: Project visible light through a specimen and then through a glass lens. The lenses refract/ bend the light in order to magnify the specimen.

- We can observe things as small as individual cell's large organelles like mitochondria, nuclei and some of the smallest bacteria.
- Using super resolution microscopy, we are able to see viruses and even ribosomes.
- Magnification, resolution and contrast all play a part in microscopy. *Magnification* is the ratio of the images actual size and its observed size. *Contrast* is the dif in brightness between the images dark and light areas, and *resolution* is the images clarity, which is represented by the minimum distance two points can be separated and still distinguishable as separate. Resolution is the current limiting factor of microscopy.

Scanning Electron microscopes: A 3D image is portrayed on a screen by the activity of detected excited electrons.

- Used for detailed study of the topography of a specimen,
- They may interpret things as small as lipids and proteins on the plasma membrane.

Transmission electron microscopes: An electron beam is aimed through a very thin section of a specimen that has been stained with a heavy metal. The electrons scatter more in the denser regions therefore fewer are transmitted. The image displays the transmitted electrons. Both EMs use electromagnets rather than lenses in order to bend electrons to focus the image. The downside of EMs is that they kill the cells during the preparation, which may introduce artifacts unseen in the living specimen.

- Used to study the internal structure of cells.
- A cross section is given

Fluorescence microscopes: Fluorescence is used to differentiate different cell components. Fluorescent substances absorb ultraviolet radiation and emit visible light which can help us observe cell structures more accurately.

Topic 2

Understand the role, identify areas and determine the importance of a molecule's polarity (electronegativity) of different biologically relevant macromolecules (particularly amino acids and proteins)

Role

- It can determine if a protein is soluble or not which indicates if it will dissolve in water or not. This can be expressed by being hydrophilic or hydrophobic.
- It can determine which other molecules it interacts with based on the other molecules polarity as well its own.
- Polarity can determine the structure and function of aminos and macromolecules such as proteins, whose structure depends on its amino acid chain (primary structure).

Identify areas

- An amino acid will be polar if it contains a charge, such as lysine which contains a positively charged ammonium group at the end of its side chain, resulting in an uneven distribution of the electron cloud.
- Another criteria is if it is asymmetrical in terms of its side chain. In which case certain regions of the molecule will hold more or less electrons based on which molecule is making it unsymmetrical.
- Also, polarity depends on an atom's electronegativity, which is the tendency of an atom to pull an electron to itself. The result of this is an uneven distribution of charge. Most biologically important electronegative elements are O, S, N.
- For this reason the location of certain large atoms play a large role in a molecules polarity, for example exposed Oxygen atoms at the end of a side chain will make a molecule polar due to it's high electronegativity.

Importance

- The polarity of amino acids is important since they form the primary structure of proteins by being linked together in the hundreds. This in turn influences the secondary structure which can either be an a-heleses or a b-pleated sheet.
    - B-pleated sheets are rigid, insoluble and mostly comprised of non polar aminos which will in bulk determine a protein's structure and function, a greater amount of sheets in a tertiary structure will make the protein fibrous thus making the protein strong such as spiders silk.
    - A-helexis are formed by polar aminos and result in soluble and flexible spirals that make tertiary structures more hydrophilic. Collagen is an example of a quaternary structure made up of 3 helical polypeptides.

Explain the relationship between polarity, molecular structure, cellular location and function – particularly for proteins and lipids.

Proteins

Structure: Polarity of amino acids in total, hydrophobic interaction
Location: Microfilaments in the cytoskeleton, channel proteins
FXN: Enzymes, ability to bond - antibodies marking viruses

- Polarity of amino acids will determine the molecular structure of a protein by determining its secondary structures.
- More polar amino acids in the primary chain will determine folding into a a-helices which in bulk will make a protein more flexible and soluble. Three of these helices may wrap around each other to make a long durable fibrous protein known as collagen, whose function is to resist force.
- Additionally, More hydrophobic areas of a protein's tertiary structure will be found on the inside of it's large structure, shielded by it's hydrophilic parts which are in contact with the aqueous cytoplasm or ECF. This is called a **hydrophobic interaction**. Whereby water determines a protein's structure and the cellular location of certain aminos.
- The location of a protein in a cell depends on it's structure and function for example globular proteins such as actin are found in tn the cytoskeleton of the cell as microfilaments, functionally, they serve to contract and withstand force.
- Non polar proteins will form integral proteins in the membrane.
- Function is also determined by its polarity, since structure is determined by polarity.
- The function of a protein depends on it's ability to bind and recognize other molecules.
- Antibodies function by mimicking a virus's structure and marking it for destruction.

Lipids

- **Phospholipids** for example are composed of a hydrophilic, polar glycerol head and 2 hydrophobic, nonpolar long hydrocarbon chains. They form a bilayer with their heads facing the aqueous ECF and inner cytosol of the cell. Their nonpolar tails face inwards in a non aqueous environment. They function to regulate the permeability of the cell by allowing lipid soluble gases and small molecules to diffuse through, while others are rejected. For this reason, they make up the plasma membrane on the exterior of cells and organelles

- **Triglycerides** are similar in structure but instead have a third hydrocarbon tail attached to it's glycerol, for this reason they are strictly non-polar and are not soluble in water. For this reason they have immense energy storage capabilities and are found in bulk in fat cells as storage of fuel, insulating material for animals and cushioning for organs.

- Lastly there are **steroids** which have a structure of 4 fused carbon rings and a substituent group that determines its function and location. They are also non polar due to their large amount of hydrocarbon. Their location and function can vary, one example is cholesterol which is found in the plasma membrane and regulates its fluidity and rigidity.

What are the types of chemical bonds present in cellular macromolecules and how are they relevant to cell biology?

Van der waals attractions

- is a general term used to define the attraction of intermolecular forces between molecules. There are two kinds of Van der Waals forces: weak London Dispersion Forces and stronger dipole-dipole forces.
- The occur in polypeptides to help hold them together

Ionic bonds

- These occur between two oppositely charged atoms in which electrons are donated by one atom.
- These occur in polypeptides between negative and positive side chains and help determine and stabilize it's structure.

Hydrogen bonds

- These occur when two electronegative atoms share an electropositive hydrogen.
- These bonds between polar side chains of polypeptides help stabilize the structure.
- They also hold together b- sheets and a- helecis
- In water they can be broken down or weakened due to the polarity of water and its strong ability to hydrogen bond

Peptide bonds

- When two amino acids are side by side their back bones will bond covalently by a dehydration reaction (removal of an H2O molecule) in which the carboxyl group bonds to the amino group.
- This process is repeated over and over forming polypeptides. Which is a polymer of amino acids, that form proteins.
- These bonds occur in order with an N-terminus (amino) at the beginning of the chain and a C-terminus (carboxyl) at the end.
- There may be hundreds of these bonds in a polypeptide.

Covalent bonds

- Bonds between two nonmetallic atoms in which electrons are shared evenly or unevenly.
- An example is carbon carbon bonds, peptide bonds, disulphide bridges and glycosidic linkages.

Double bonds

- Found in the hydrocarbon chains of unsaturated fats.
- This makes them liquids (oils) at room temperature.
- Hydrogenation is a process in which hydrogen is added in order to saturate these bonds.

Glycosidic linkages

- Occur between two monosaccharides as a covalent bond formed by a dehydration reaction.
- The accumulation of these bonds for di and polysaccharides

Carbon-carbon bonds

- Found in hydrocarbons and are essential to the structure of lipids and some amino acid side chains.
- This makes lipids non polar.

Ester linkages

- These are bonds between carboxylic acid and an alcohol group.
- They occur between the hydrocarbon and glycerol of phospholipids and triglycerides

Disulphide bridges

- When 2 cysteine monomers with sulfhydryl groups on either side are brought close together by folding of a polypeptide and they bond covalently. Structural integrity of polypeptides.

What is the importance of water in biology

- Water is the most abundant molecule of the earth
- It consists of an O bonded covalently to two H's by hydrogen bonds which also attract other water molecules and are strong.
- It has various properties that make it biologically important

1. **Freezing properties**

Ice is less dense than water therefore it sits on water, and insulates aquatic environments below.

2. **High latent heat of fusion**

Cell contents are slow to freeze in cold environments since water requires large amounts of heat to be removed to form ice.

3. **High latent heat of evaporation**

Large amounts of heat are required to evaporate water. Provides an effective cooling mechanism for mammals by sweating.

4. **High transparency**

Light penetrates water and allows visibility and photosynthesis in water.

5. **High surface tension**

Water molecules are held together by hydrogen bonds. This allows formation of a continuous water column in plants.

6. **High polarity**

Water is the universal solvent due to its high polarity. Water can dissolve many substances and hence facilitates chemical reactions and serves as transport medium.

7. **Incompressibility**

Water is incompressible and provides turgidity for plants and support for animals with hydroskeleton.

Know and identify the main classes of macromolecules, recognize their **general structures**, and interpret these in relation to their **cellular location** and their **roles in cells.**

Proteins

- Amino acids consist of a back bone with an amino group and carboxyl group attached to an alpha carbon with a hydrogen and side chain attachment. The side group is what determines the polarity of the acid and in the grand scheme of things, the structure, function and location.
- If a secondary structure is composed of mostly non-polar aminos, it will form B-sheets. If these exist in bulk in a tertiary structure, the result is a fibrous polypeptide that will be insoluble soluble and create fibrous proteins. For example spider silk, which is stronger than a steel fiber of the same size due to plenty hydrogen bonds.
- Or if a-helexes are formed from polar amino acids, tension bearing proteins can result such as collagen, which is a helix of 3 helical polypeptides that is found in the ECM for structural support and anchorage of glycoproteins. Another example is microfilaments composed of actin which are globular proteins that form a 3D network in the cortex of the cell and turn the cytoplasm into a gel. This network helps support the cell structure.

Lipids

- Lipids structure makes them non polar, for example triglyceride and phospholipids contain a glycerol head and three and two hydrocarbon tails respectively.
- Not only is this symmetrical but in the case of the phospholipid, the third bonding site of glycerol binds to a phosphate group which is what gives it its water loving properties; it's large amount of oxygen.
- Functionally, triglycerides are used for fuel and are stored in adipose cells for animals since their structures allow for extremely condensed storage of energy rich hydrocarbons. Phospholipids on the other hand form the selective bilayers of plasma membranes with their hydrophobic tails facing inwards and their heads facing the aquatic interior and exterior.

Carbohydrate

- Monomers called monosaccharides which are basically any form of sugar or isomer of glucose, form the simplest structure of carbohydrates, these bond by dehydration reactions forming glycosidic linkages to synthesize poly or disaccharides.
- The diversity of these structures can vary immensely dependent on the location of functional groups and the arrangement of parts around asymmetric carbons. This will result in a different function and location, for example
- Cellulose and starch share the same composition (glucose) yet differ in their arrangement. Cellulose monomers are arranged in Beta configuration which means the hydroxyl group is on the opposite side of each one of it's neighboring monomers. While in starch, they are in alpha configuration - all hydroxyl groups on the same side.
- The function of starch will be energy storage since it will be able to pack together more closely, while the function of cellulose is to form strong bonding networks with their exposed hydroxyl groups and provide structural support for plants.
- Plants store starch as granules within cellular structures known as plastids.
- Glycogen is stored in muscle cells, which is also a glucose polymer that is extensively branched in order to have more free ends available for hydrolysis providing fuel.

Topic 3

• How is the cell's interior organized?

- Things are not simply floating around in the cell. Intermediate filaments anchor many organelles in place, including the nucleus by forming the nuclear lamina, which lines the interior of the nuclear envelope.
- Microtubules on the other hand help organize the cell interior by acting as transport routes for organelles and vesicles. This occurs with the help of motor proteins such as dynein and kinesin, which appear to walk across microtubules either in the plus or negative direction. Dynein moves towards the minus end of microtubules and kinesin moves towards the plus. Organelles equipped with both motor proteins are capable of movement in both directions.
- Intermediate filaments however are not capable of transport since they do not possess a positive or negative end (no Polarity).
- Microtubules may also move organelles using their quick retraction and extension abilities due to the weak hydrogen bonds and van der waals forces at their plus end that can toggle tubulin.
- An example is during mitosis where centrioles will migrate to either end of the cell and microtubules will split the chromosomes and drag them to either end for cell division.
- For this reason the centrioles are placed close to the nucleus for easy access to genetic material. Also, the Rough endoplasmic reticulum is continuous with the nuclear envelope since the ribosomes it's studded with that create proteins originate in the nucleolus, therefore they do not travel far.

• Compare how each component of the cytoskeleton is formed

Microtubules

- Are formed starting with and Alpha tubulin protein with a GTP molecule facing outwards, this is dubbed the minus end since it's GTP is buried by a Beta tubulin that precedes it.
- This structure is called a dimer and it is linked continuously by weak hydrogen bonds and van der waals forces that allow the plus end (beta end) to form and break bonds with tubulin molecules very rapidly in a constant process called ***dynamic instability,*** which conserves energy of the cell.
- Tubulin heterodimers form as single rows called protofilaments that stack to form sheets. This process forms hollow tubes lengthwise. 9 triplets of microtubules form together in a ring called a centriole.

Microfilaments

- Are formed by actin subunits that appear as a helix. This structure is formed unit by unit however, not by the intertwining of two strands. The globular protein actin, bonds together in a staggered manner with a barbed plus end bearing an ATP molecule and a pointed minus end with it's ATP concealed. These may also branch off due to ARP complexes and cap off due to capping proteins.

Intermediate filaments

- Are formed by various monomers of proteins from the keratin family.
- These do not disassemble and are the permanent fixtures of the cell that may remain even after the cell dies.
- This resilience is due to their tightly woven coiled structures that resemble cables.
- This occurs starting with a helical alpha monomer, then dimers are coiled, then tetramers forming, and then 8 tetramers coiling together to create and ultimately strong structure.
- These do not have energy molecules associated with them, for this reason there is no orientation to these filaments and they do not participate in transportation by motor proteins.

• Relate the structure of the cytoskeletal fibers to their roles in the cell

Microtubules

- Tubular structure acts as a pressure resisting girder that resists compression.
- Their structure also allows for multiple exposed GTPs for quick addition and subtraction of tubulin at the plus end.
- Their structure can give them different roles in the cell, for example microtubules of cilia and flagella originate from basal bodies where they are arranged differently.
- The length of the microtubules define them as flagella or cilia, long microtubule structures will result in a flagella that propels the organism is the direction of its axis using a swimming or undulating motion.
- If its structure is short and often numerous, they will produce a power stroke whiplike motion that sweeps the immediate area perpendicular to its axis. These can be found in our trachea where mucus is sweat up.
- Cilia or flagella are arranged in 9 doublets forming a circle with a doublet in the middle.

- The basal body, which is the anchor for the cilia or flagella microtubule, is arranged in 9 triplets in a circle much like centrioles. In fact some basal bodies of sperm may become centrioles in an egg.

Microfilaments

- The intertwined structure of microfilaments compliments it's ability to bear tension, which is why they are found in bulk in the sarcomeres of myofibrils which form muscle tissue where tension is the greatest.
- Microfilaments branch extensively by arp complexes and due to this, they form the structure of surface area increasing microvilli.
- Cells that crawl along a surface (amoeboid movement) use actin to move in this way. They do so by continuously adding and removing actin protein to the **microfilaments using arp complex branching**, which pushes and pulls the membrane.
- Their branching also forms a network of cortical filaments just within the plasma membrane called the cortex. The bulk of filaments found here make the cytoplasm gel like, and they modulate the plasma membrane.

Intermediate Filaments

- Are tightly coiled structure that begin with a helical region of a protein monomer and twist around another to form a dimer.
- This structure is staggered against another one to form a tetramer which is then staggered against 7 other tetramers which all coil together to form an ultra tension bearing cable that forms the structural framework of the cell.
- It's sturdy structure allows it to anchor organelles easily and form the nuclear lamina which encloses the nucleus and protects it.
- Perhaps the most important **function** of **intermediate filaments** is to provide mechanical support for the plasma membrane where it comes into contact with other cells or with the extracellular matrix.
- No motility

• What are the main types of cell-to-cell interactions and their characteristics?

Desmosomes (A form of anchoring junction) fxn like rivets to anchor adjacent cells into strong sheets. Intermediate filaments anchor desmosomes in the cytoplasm. Proteins such as keratin also form part of the Muscle cells contain desmosomes because of the large amount of tension they bear. Muscle tears can be associated with desmosome rupture.

Gap Junctions Provide communication channels for small ions, sugars, amino acids, and small molecules to adjacent cells. They are like plasmodesmata in plants. They provide communication between tissues like the heart, and animal embryos. Their structure is 6 connexin proteins that form a conexon which binds with another and opens and closes due to concentration gradients.

Tight Junctions contain proteins that bind plasma membranes of two cells very tightly forming a continuous seal. This establishes a barrier that prevents leakage of fluid across epithelia. This is why our skin is waterproof. The ECM is also squished.

Plasmodesmata are found within the cell walls of plants and act as cytoplasmic channels between adjacent plant cells. These small pores are lined with a pore complex that regulates the passage of certain molecules, small solutes and water may pass freely between cells making the internal environment of plants very alike. RNA and some proteins may also be able to pass through cells making them even more alike.

• Discuss how cellular motility and mobility are different and how they are accomplished

Motility is the ability of living systems to exhibit motion and to perform mechanical work at the expense of metabolic energy. The scope of motility, as it is presently understood, includes a variety of diverse phenomena:

(a) bacterial (prokaryotic) flagellar movement;
(c) saltatory motion of particles in cytoplasm;
(d) organelle movements (deformation or translocations of chloroplasts, mitochondria);
(e) cytoplasmic streaming (in protists, plant, animal, and fungal cells) ;

(f) amoeboid movement (cell movement by means of cytoplasmic streaming in lobopodia, filopodia, axopodia, retraelopodia, etc);
(i) contractility (of muscles)
(k) mitotic movements;
(m) eukaryotic flagellar and ciliary movement.

Cell mobility generally refers to motility, but may also refer to other ways of activation, such as cell differentiation and cell proliferation. Cell proliferation is the process that results in an increase of the number of cells. Cellular differentiation is the process by which a less specialized cell becomes a more specialized cell type. Differentiation occurs many times during the development of a multicellular organism.

• Describe what the extracellular matrix is made of, and interpret why it is important to cells

The extracellular matrix consists of collagen fibers attached to integrins of the plasma membrane by fibronectin. Collagen fibers are embedded in a web of proteoglycan complexes which are made of proteoglycan molecules that branch extensively off of polysaccharide molecules by covalent bonds, which increase strength of attachment. All of these components are secreted by the cell and are thus part of the cell.

The roles of the cytoskeleton include:

- communicating extracellular information to the interior of the cell via response transmitted through integrins. Integrins integrate the ECM and the cytoskeleton allowing for dual ended communication.
- Providing support due to it’s collagen make up which resists tension and may shield the cell. Proteoglycans may also provide cushioning for the cell due to their large quantity forming a buffer.
- ECM is involved with getting cells close together so that junctions can bind them.

• Associate the different cytoskeletal fibers with their preferred molecular motors and contrast their characteristics with respect to their roles

Microtubules: Contribute in organelle transport around the cell. In order for this to occur they need one or two specific motor proteins that bind to the organelle and appear to walk along the microtubule. Dynein is a motor protein that transports organelles towards the minus end of microtubules only due to the polarity difference. Kinesin is a motor protein that moves along with its cargo towards the plus end. An organelle bound to both of these motor proteins may change directions at any time.

These proteins work together to drive the movement of flagella and cilia. Dyneins are found linking each microtubule doublet of cilia or flagella all along their lengths in a 9 doublet formation. Adjacent microtubules are also crosslinked by a protein called nexin that keep doublets intact while dynein imposes mouvement by moving to its favored minus end in a wave like or whip like motion depending on length. Without this connection, one doublet would simply slide off the other. There needs to be mouvement in the other direction or for cilia a recovery stroke. This is facilitated by kinesin moving the dynein back towards the plus end.

Microfilaments use myosin as their motor protein

Topic 4

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

The plasma membrane is the selectively permeable barrier that regulates what enters and exits the cell due to it's phospholipid bilayer and membrane proteins. All of its functions with their descriptions follow.

Protecting the cell

- The plasma membrane serves as a barrier for the internal and external environment of the cell due to it's tightly packed structure of phospholipids who make the membrane interior nonpolar, allowing only what is non-polar to diffuse through it.

Cell structure

- Since the membrane is attached to the cytoskeleton via microfilaments that form the cortex of the cell, a gel like barrier of the cell that supports cell structure, it does so also.
- Sterols in the cell membrane act as a buffer for it's rigidity, making the cell more less rigid by spacing out the surrounding phospholipids and keeping them from compacting during cold temperatures. It also prevents the membrane from becoming less rigid by anchoring phospholipids and limiting their movement during warm temperature.

Selective permeability

- Due to the orientation of the phospholipids with their hydrophilic heads facing the interior and exterior cell, and hydrophobic tails facing inwards, The plasma membrane is only permeable to certain small solutes and gases which are lipid soluble. These can diffuse directly down their concentration gradients. If this was not the case, large molecules like glucose or amino acids could leave the cell at will or simply end up in the wrong cell and being destroyed or hurt the cell.
- Other means of transport exist which the cell membrane accepts other molecules

Cell communication / interaction

- Cells communicate via chemical messengers or electrical impulses. The cell membrane reacts to ligands by activating G proteins which activate enzymes and order them to carry out a specific task such as mitosis, protein production, hormone production etc.
- They may also link to each other via tight, gap or anchoring junctions. These have different functions per structure.

Organize the cytoplasm

- They do this by defining compartments of the cell or organelles. Certain membranes of bound organelles are permeable to different things.

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

The membrane is fluid in the sense that it is mobile and has a certain degree of rigidity. It is a mosaic in the sense that it contains diverse and dynamic components arranged sporadically.

- Phospholipids can move laterally around the cell at a high speed, 2 um per second on average which is the size of a bacteria. They can also switch leaflets by flip flopping on rare occasions since this requires more energy. Another thing that maintains the fluidity of the membrane is cholesterols wedged in between phospholipids. These are buffers of fluidity that respond to temperature changes.
- Integral proteins penetrate the plasma membrane and cross both layers. These serve multiple functions.
- Peripheral proteins also contribute the the mosaic, they span only 1 leaflet of the membrane and often attach to integral proteins.
- Glycoproteins and glycolipids anchor to the cell possessing their own functions.

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

Integral proteins

- Span both leaflets of the plasma membrane, may be one of three types worth mentioning.
- An alpha helix which crosses the membrane once and is used for receptors and recognition, since they are short routes for a signal to travel.
- Beta barrels, which are intertwined pleated sheets with a polar core that facilitates transport, for this reason it is a channel protein that may transport polar solutes.
- Helical bundles which span the membrane multiple times and are used for enzyme activity, transporters and receptors.
- They also function as sites of anchoring and attachment such as for fibronectin of the ECM, these also allow for signaling of the external environment to the cytoskeleton of the internal cell. These types of signals can tell the cell to undergo mitosis, grow or signal.

Peripheral Proteins

- Peripheral proteins only interact with one leaflet on the plasma membrane and they often interact with integral proteins.
- These are not covalently bound and function as enzymes, anchorage and carriers.
- They can provide structural support and elasticity, for example red blood cells possess a membrane with an intricate array of peripheral proteins that allow them to condense their structure through small capillaries and reform once it has space.

Lipid anchored proteins

- Covalently bonded to the outside or inside of the cell,
- Internal proteins such as fatty acid or prenyl groups are involved in mediating cell division and cell growth.
- Extracellular proteins like GPI is a phospholipid, these have strong relationships with hard to break bonds, for this reason they are used for cell to cell adhesion

Sterols

- These are lipid molecules that form 4 rings and an R group that determines their function. In animal cells, cholesterol acts as a buffer for the fluidity of the plasma membrane.

Phospholipids

- These provide the cell it's bilayer and fluidity, they are not covalently bonded instead weak forces like hydrogen bonds and van der waals holds them together which allow for lateral movement which is the definition of fluidity.
- If they are saturated they may pack together tightly and reduce fluidity, the opposite can occur.
- Their amphipathic nature is what gives them their selective properties.

Glycoproteins/Glycolipids

- Usually short, less than 15 monomers of sugar, they covalently bond to a protein or lipid.
- They form hydrogen bonds with the water molecules surrounding the cell and thus help to stabilise membrane structure.
- They are used for cell recognition, for example the human blood types are defined by the presence of certain glycoproteins.

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

Diffusion Molecules of small size that are lipid soluble and membrane permeable molecules or liquids may diffuse directly across the PM with no form of facilitation. Factors that this depends on are permeability of the solute, fluidity of the plasma and it's concentration gradient.

Facilitated diffusion (channel mediated/carrier mediated) Integral proteins provide a route for larger non membrane permeable (polar) molecules to enter and exit the cell. They may take the form of a channel, such as b-barrels which intertwine as a series of b-pleated sheets surrounding a polar area which provide a passage for hydrophilic molecules to diffuse down their gradients, examples are aquaporins and ion channels. Carriers transport specific solutes down their concentration gradients by changing shape when one binds to them. These processes require no energy and happen spontaneously.

Active transport This form requires a carrier integral protein as well as energy in order to facilitate transport against a molecules concentration gradient. The most common example of this is the Na/K pump. In this example Na is a higher concentration outside the cell than inside. Na bonds to the pump and a phosphate group from ATP phosphorylates the pump causing it to change shape and release 3 sodiums into the extracellular space. Potassium, which has a higher concentration inside the cell that outside, then bonds to the pump on the outside. The phosphate group is removed and the cell exposes potassium to the intracellular space.

Endocytosis Broadly, this term describes the process of a cell intaking a molecule through the plasma membrane folding and forming a vesicle.

Phagocytosis Cellular eating consists of the cell swallowing prey or food by extending pseudopodia extended by microfilaments and engulfing a prey. The food vesicle is then transported along microtubules using the motor protein dynein to and fuse with lysosomes to be digested by lysozyme enzymes that can't survive outside of the lysosome.

Pinocytosis Cellular drinking, is when the cell gulps droplets of ECF by forming infoldings of the PM and pinching off the vesicle. Anything may be absorbed by this method, making the pit coated vesicles non selective. Pit coating is a proteins that forms a fuzzy layer around the vesicle.

Receptor-Mediated Endocytosis Special type of pinocytosis that allows the cell to uptake whatever they their receptor is coded for in bulk quantities. Thes vesicles are also coated with pit proteins.

Exocytosis This is the means by which molecules exit the cell in a vesicle created by the golgi apparatus. These molecules may be products secreted by the cell like insulin in the pancreas. They move along microtubules toward the membrane and fuse with the membrane in order to release their cargo, this is where they stay.

Cotransport This means of transport is when the transport of one molecule depends on anothers gradient. For example the proton pump continuously pumps H out of the cell with the help of ATP. This maintains the electrochemical gradient of H+ and it will happily diffuse back into the cell through the sucrose/H cotransporter, dragging sucrose along with it. This occurs in plants.

• How are membrane dynamics important for transport?

It's double layer provides a dual barrier which is **amphiphilic** meaning it has both polar and nonpolar characteristics. This helps exclude certain molecules from freely diffusing into or out of the cell. The **membranes rigidity** plays a role in the transportation of phospholipids on the PM. If the cell is rigid and phospholipids are packed closely together, they will not be able to move as freely, Temperature can have this effect and sterols regulate this. Some molecules may be rejected due to **tight junctions** that create a continuous surface along epithelial cells in which no molecules can diffuse through. Increasing amounts of cis unsaturated and polyunsaturated fatty acids can cause for a less rigid membrane with more fluidity due to the spacing out of phospholipids. Length of fatty acid chains can influence the PM fluidity, shorter chains are more fluid. Increasingly polar head groups cause for more fluid membranes as well.

Phosphotydylserine contributes to membrane asymmetry by existing solely on the internal leaflet and carrying a negative charge. Glycolipids do not exist on the inner layer since there is no cell recognition to be done on the inner layer. Phospholipids are in constant motion laterally attributing to asymmetry and flip flop as well.

REVIEW SECTION

Topic 1 (Reiterate these learning objectives [2,4,6,7,8] - Relate them to any other topic)

- Endosymbiosis - refer to DGD rubric
- Straight forward topic, use learning objectives
- Be able to implicate these concepts to other topics

Topic 2 (Reiterate these learning objectives [1,2,5] - do exercises with polarity)

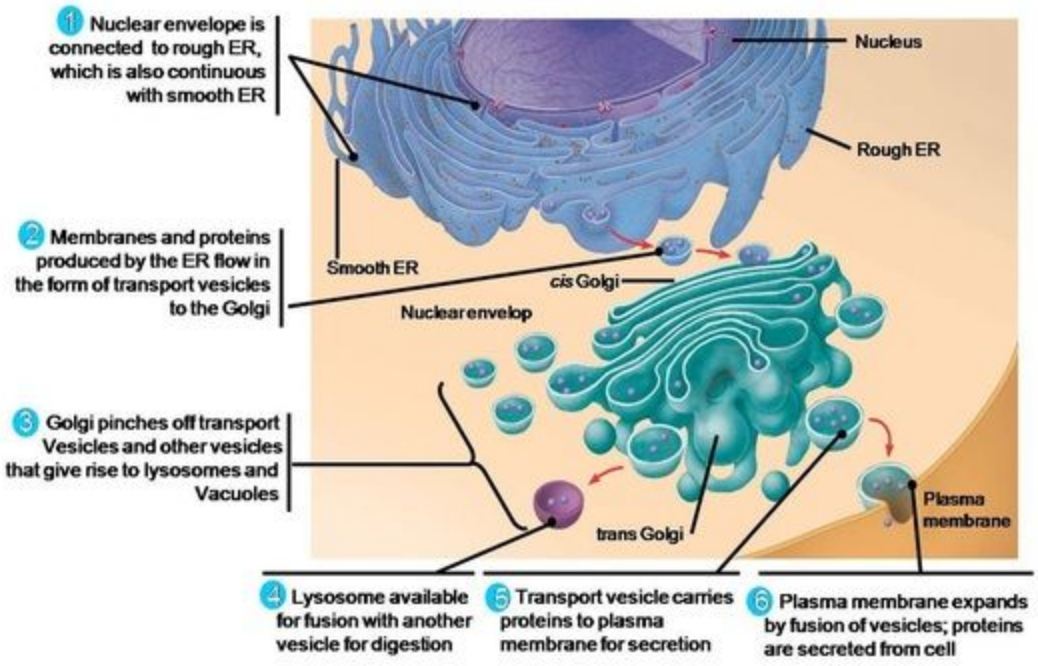
- Understand macromolecules structure, location and functional relationships.
- Macromolecules interacting physically refer to DGD 2. Where, fxn
- Amino acid analysis of polarity in depth, contribution to secondary structures.
- Know structure of proteins, lipids, carbohydrates. Be able to interpret them, what tasks can it achieve, where is it in the cell.
- Function of secondary and tertiary structures.

Topic 3 (Reiterate these learning objectives [2,3,4,5,] - diagrams, tables)

- Cytoskeleton fibers, function, structure, location
- Junctions
- Motility, mobility
- Cilia and flagella
- Motor proteins 101
- Be able to understand broken structures of organelles

Topic 4 (Reiterate these learning objectives [1,2,3,4] - Use diagrams, tables)

- Fluid mosaic model
- How its built and how it achieves what it does
- Key roles of the membrane and how it's structure contributes to it
- Proteins of the membrane
- Membrane transport - in detail - relationship between membrane, cytoskeleton, vesicle content, direction of vesicles to and from within the cell.
- Observe diagrams of cellular processes and explain what's happening - membrane transport


1 Nuclear envelope is connected to rough ER, which is also continuous with smooth ER
Nucleus
Rough ER
2 Membranes and proteins produced by the ER flow in the form of transport vesicles to the Golgi
Smooth ER
cis Golgi
Nuclear envelop
3 Golgi pinches off transport Vesicles and other vesicles that give rise to lysosomes and Vacuoles
Plasma membrane
trans Golgi
4 Lysosome available for fusion with another vesicle for digestion
5 Transport vesicle carries proteins to plasma membrane for secretion
6 Plasma membrane expands by fusion of vesicles; proteins are secreted from cell

Mock Middy

Define the term kleptoplasty. Provide an example (2)

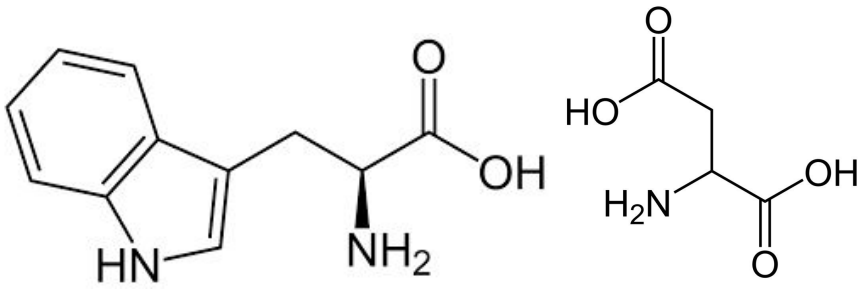
Integral proteins are amphipathic, what does this mean? Provide an example. (2)

In order to transport a vesicle from the RER to the golgi apparatus, which motor protein and cytoskeletal fiber will be used. (2)

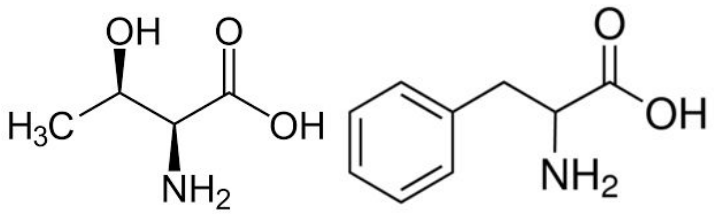
Explain how the plasma membrane remains asymmetric (2)

The plasma membrane has multiple roles in the cell, choose and name two of those roles and explain what properties or features of the membrane allow it to carry out that role. (4)

Which molecule is the most polar, explain your choice by comparing them.



Do It again



A mouse has been modified so it cannot express the gene for nexin. With the help of a structural description, explain what the consequences will be of no longer producing this cross linking protein. You can use a drawing appropriately labeled. (5)

Using two lines of evidence explain why the theory of endosymbiosis is the most plausible theory for the appearance of eukaryotes. (4)

Discuss the role of the centrioles with respect to the cytoskeleton (5)

You wish to localize and quantify a specific protein, titin. Which is found in the contractile unit of skeletal muscles. You have the choice between various chemical markers or antibodies to label the protein. You have other experiments to conduct after

you observations with this sample. Which microscope would be best for you to achieve your obj? Justify (3)

Using amino acid building blocks, propose a sequence for an integral protein, refer to picture. (10)

Midterm 2 Learning Objective

Energetics

Explain, contrast and compare, the role of mitochondria and chloroplasts in cells with regards to energetics

Mitochondria:

- This is the site of respiration where pyruvate potential energy is used to synthesize ATP through oxidation and reduction reactions that involve movement of electrons.
- ATP is a compound that stores potential energy in its phosphate bonds in order to do work in the cell.
- cells may contain 10-1000s depending on metabolic needs.
- Glycolysis features the splitting of two glucose molecules into pyruvate in the cytoplasm of the cell. This generates 4 ATP molecules and consumes 2 ATP. this can occur with or without oxygen and is a ten step process.
- Potential energy of glucose is harvested step by step using specific enzymes and carriers. NAD+ may transfer electrons from glucose by binding a H-, it can switch easily between oxidized and reduced states of NADH. Electrons lose very little potential energy when attached to NAD. The next step occurs in the electron transport chain of the intermembrane space where a series of carrier molecules accept the H from NADH. Since they are increasingly electronegative, small amounts of energy is released powering the creation of ATP. The terminal electron acceptor is oxygen which has the highest affinity for electrons and forms water. This process yields 26-28 ATP molecules.
- If Oxygen is present, pyruvate in the mitochondria will undergo a transformation2 into acetyl CoA during pyruvate oxidation that takes place within the matrix. 2 NADH are produced.
- The citric acid cycle / calvin cycle is initiated by Acetyl CoA by feeding its acetyl groups, which will generate 1 ATP molecule per turn via substrate level phosphorylation. This is per pyruvate, since glucose splits into two, 1 is essentially two per turn in terms of overall output. This process reduces NAD x3 and FAD allowing them to shuttle electrons to the electron transport chain. CO2 x2 is also created as waste.
- Oxidative phosphorylation features the coupling of chemiosmosis and the ETC.

- The chain is used as mentioned above, starting with the donation of H to complex 1.
- Note that FADH2 adds its H's directly to complex 2, meaning at a lower energy level causing ⅓ less energy to be harnessed.
- Chemiosmosis features ATP synthase, an enzyme that makes ATP from ADP. This pump utilizes the H gradient established by the ETC in the intermembrane space in order to power its catalytic sites by spinning its rotor.

Chloroplast ;

- Present in plant cells and photosynthetic eukaryotic organisms. Essential to the production of sugars the plant needs to survive and releases oxygen as a waste product into the atm.
- Chloroplasts also produce ATP, although at a much lesser rate and yield than mitochondrion.
- Light reactions use chemiosmosis to power addition of phosphate groups to ADP. This process is photophosphorylation which powers the calvin cycle.
- Light generates ATP and NADPH using the two photosystems embedded in the thylakoid membrane of chloroplast. This occurs during linear electron flow utilizing ETCs and enzymes. This occurs when an electron is transferred from and excited P680 to a primary acceptor that is capable of supplying the ETC with a hilltop energy source from water (not food as in mito) in order to create ATP.
- Chemiosmosis uses the H reservoir in the thylakoid space created by linear electron flow through photosystem 1 - 2 to power ATP synthase in the stroma.
- Briefly the calvin cycle converts co2 with the help of ATP and NADPH to generate 1 G3P per turn, While returning NADP and ADP to the Light reaction area (thylakoid) to be re-phosphorylated.
- In this cycle, after carbon fixation and reduction, Rubisco (RuBP) is regenerated by 3 molecules of ATP in order to restart carbon fixation with the help of rubisco.

Understand the importance of calcium homeostasis

- Availability, Storage, regulation (later with signal transduction)

- Many types of channels and transporters gently regulate the gradient for calcium.
- It can be reduced by binding proteins such as calbindin, parvalbumin, calsequestrin.
- Mitochondria and ERs regulate their concentrations and compartmentalize by storing and releasing them.
- During stress ER releases ca through IP3 gated channels (RyR)
- Mitochondria absorb Ca

- **Provide examples of cellular functions that rely on calcium**
- Response to stimuli (vesicle secretion)
- Muscle contraction
- Signaling (second messenger)
- Enzymatic cofactor - coagulation
- Storage in bones
- metabolism .
- Closing of stomata

What is mitophagy and how/why does it occur?

- A means of removing damaged mitochondria or to reduce their numbers selectively by autophagy. Mitophagy may occur if the cristaea do not create much SA increase, if they are limp the ATP synthesis will decrease due to lack of intramembrane space and H reservoir. Increased ROS decreased H gradient.
- Kinase PINK phosphorylates MFN2 which acts a beacon to recruit Parkin which joins Ub, which recruits the lysosome which recognizes the proteins and engulfs the mitochondria, saving the cell if mild cell stress is induced and the mitochondria is digested. Severe stress is hard to manage since mitophagy cannot occur fast enough and mitochondria may burst releasing calcium.

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

Cells die by the following ways: Chemical messengers - Growth factors, Survival factors, Mitogens (cell division). Stress and cell cycle checkpoints.

Necrosis:

- The messy and destructive process by which digestive enzymes and cell contents are released into the ECS.
- Necrosis is caused by factors external to the cell or tissue, such as **infection**, toxins, or **trauma**
- Lysozyme is spilled out of the cell along with all the cellular content during necrosis. Lysozyme eat away at neighbouring cells ECM's proteins, glycocylax
- The calpain-cathepsin pathway is triggered by an necrosis initiating insult in which the ER is prompted to release calcium which activates the protein calpain that digests the lysosomes membrane leading to the protease cathepsin spillage, and thus the digestion of cellular content and cell death.
- Cathepsins can survive outside of the lysosomes acidic environment and will digest all protein content of the cell interior. -cytoskeleton etc. Everything in cell has some protein content. cell will swell and lysis.
- A cell cannot recover from necrosis. It may reverse the process however depending on the threat to the cell.

Apoptosis:

- No lysis of the cell occurs during apoptosis, only during necrosis. Apoptosis compartmentalizes the cell contents.
- Caspases are a family of proteases that cleave proteins, divided into two groups - initiators and executioners. Caspases look for specific proteins and activate them. They can shut down cellular processes and adherence, they can break down lamins in order to break up DNA, they allow the cytoskeleton to degrade. all this facilitates blebbing. This is how we recognize apoptosis.
- Dna is broken down so that it is easily packaged and easier to phagocytize.
- membrane phospholipids on apoptotic bodies signal phagocytic cells to eat them. Phosphotydylserine will become flipped onto outer layer and act as the signal. Scramblase can flip lipids in both directions only when intracellular Ca rises and caspase activation.
- Proapoptotic proteins can be activated by the lack of a protein. 14-3-3 is a safeguard that restricts apoptotic proteins. Bad restricts the antiapoptotic protein bcl2 which restricts IP3 thus restricting calcium release. Bad activates bax and bak which promote release of Ca through ER by promoting IP3.
- Mitochondria will take up Ca, when it's threshold is reached, cytochrome C is released which meets up with apaf 1 and caspase 9 to form an apoptosome. Initiator caspases turn on executioner caspases which trigger death. This process is irreversible.
- Nematode is a good model for this since it only has 1000 cells which researchers have decoded the entire history for each. Apoptosis occurs 131 times during its normal development in sequence activated by proteins coded by the ced 3,4 and 9 genes.

Relate cell death to the cell cycle (We will discuss this later in the cell cycle regulation learning session

Signal transduction

Explain and give examples of cellular communication and the role it plays in cellular biology

- Cellular communication is essential for multiple cellular processes including development, immunity, physiology, cell growth / survival / cancer, hormone regulation and homeostasis. Signals can be interpreted different depending on ligands, receptors and mechanisms that lead to different stimulus.
- Different classifications of cell com include long distance and local signalling.
- Cell junctions and cell-cell recognition are forms of signaling where direct contact occurs between cells.

Define the 3 steps of cell communication

Reception:

- The target cells detection of a signaling molecule coming from outside the cell, A chemical message is detected when the signal molecule binds to a receptor protein on the PM or within the cell.

Transduction:

- The ligand binding changes the receptor in some way, triggering the transduction pathway. Transductions goal is to convert the signal into a form that can bring about the desired specific cellular response. Ex the binding of epinephrine to a receptor protein in the liver cell leads to activation of glycogen phosphorylase. Transduction may occur in a single step or multiple steps involving multiple relay proteins in a process called a signal transduction pathway.

Response

- The transduced signal finally triggers a specific cellular response. This response may be any imaginable cellular activity and has most likely been amplified multiple times by one ligand. Different cells may respond to different signals based on thier transduction pathways and proteins.

Identify and differentiate the 6 messenger classes and their characteristics

The structure of messengers determines:

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

Steroids:

- Derived from lipids (4 ringed structure) lipophilic therefore cannot be stored in vesicles.
- Can diffuse or bind to transport proteins, reaches intracellular receptors.(endocrine path)
- Act as gene *transcription factors*
- 3 classes exist: Mineralocorticoids (aldosterone) , Glucocorticoids (cortisol) and sex hormones (test / estrogen).

Eicosanoids:

- Most are derived from arachidonic acid
- Lipophilic, paracrine pathway
- 2 main pathways lead to main classes: Pain (prostaglandins) / inflammation - (leukotrienes)
- Response to allergy.

Proteins / peptides

- Hydrophilic, packed in secretory vesicles
- Secreted by exocytosis (regulated secretion)
- Most indirect paths
- Enzymes are packaged with inactive proteins in vesicles.
- Insulin

Amines

- Characterized by an amine group
- Most hydrophilic (reserve pool in vesicles)
- Many are neurotransmitters (epinephrine, histamine, GABA)
- Thyroid hormones (hydrophobic)

Purines:

- Derived from nitrogenous bases adenine and guanine.
- Need a transporter or can use exocytosis.
- Paracrine and endocrine pathways.

Gases:

- Small molecules, short half life
- Passively diffuse
- Direct and indirect paths
- NO, O_2, CO

Know and differentiate the 6 paths of communication between cells

- Paracrine signaling (local): A secreting cell acts on a nearby target cell by secreting molecules of a local regulator (a growth factor).
- Synaptic signalling / neural regulation (local): a nerve cell releases neurotransmitter molecules within a synapse, stimulating the target cell such as a muscle or a neuron. Messenger is converted from electrochemical, to chemical)
- Endocrine (hormonal) signalling (long): Specialized endocrine cells secrete hormones into body fluids such as blood. Hormones reach virtually all body cells but are bound only by specific cells.
- Autocrine: local regulators acts on same cell as it is secreted by
- juxtacrine signalling: (or contact-dependent signalling) is a type of cell / cell or cell / extracellular matrix signalling in multicellular organisms that requires close contact. *Cell junctions:* Animal and plant cells have junctions that allow, molecules to pass through readily between adjacent cells without crossing the PM. *Cell-cell recognition:* Two cells in an animal may communicate by protruding molecules on their surfaces.
- Neuroendocrine: NTs/regulators secreted by neuron and carried by blood to tissue it acts on

• Note: target tissues for steroids DO NOT have steroid-receptors on their membrane surface because steroids are soluble in the lipid bilayer (receptors not necessary)

Know and recognize the 4 different classes of receptors

Reception requires specific ligands binding to specific receptors. This binding generally causes a shape change which triggers transduction. Some receptors reaction to ligands are to immediately bind other receptors. Most receptors are found on the plasma membrane and transmembrane receptors which are integral proteins. Their ligand binding sites are found outside of the cell and the ligands which they bind are mostly hydrophilic. The ligand never enters the cell. Three types include the following.

<u>Ligand gated ion channel:</u>

- Feature a gate which opens in response to ligand binding, which will close once the ligand detaches.
- Ions may pass through these channels depending on their gradients due to facilitated transport
- The presence of these ions within the cell may cause various cellular responses such as the neurotransmitter effect in muscle contraction. When NTs such as acetylcholine binds to postsynaptic terminal channels such as ACh ligand gated channels, these channels open causing influx of ions such as Na+ and Ca+ or efflux K+ triggering a change in membrane potential and a possible action potential.
- This potential may be propagated by the opening of voltage gated ion channels which respond to membrane potentials.
- *Contraction:* Vesicles are transported from cytoplasm to the membrane by microfilaments and myosin due to calcium concentration. membrane potential increases which open sodium channels which propagates an action potential. Sarcoplasmic reticulum receives the action potential through T-tubules. The calcium is released and binds to troponin which removes tropomyosin revealing the actin binding sites. Calcium is then actively transported back into the SR against its gradient. this pump is called Serca. This occurs when ACH is broken down by acetylcholinesterase and the muscle is not being signalled to contract.

<u>Enzymatic receptor:</u>

- Intracellular catalytic domains act as enzymes when signalled by a ligand, phosphorylation cascades occur to amplify the ligands impact on the target cell.
- Receptor Tyrosine Kinases (RTKS / Tyr-K) have such domains that catalyze the transfer of phosphate groups from ATP to the amino acid tyrosine on a substrate protein.
- They are the largest family of enzyme receptors, involved in survival, growth, metabolism and proliferation. Ligands may be insulin or growth factors.
- Signalling is initiated via an area called an SH2 domain (src homology). Main second messengers are phospholipids and Ras.

- One RTK may activate 10 or more different transduction pathways for cellular response. This is contrary to GCRPs activation of only 1 transduction pathway.
- Receptors exist as single units called monomers before the signal binds, an alpha helix is present in the membrane and a protruding intracellular tail.
- Once ligands have binded two monomers, they become dimerized, which allows their tyrosine kinase regions of each monomer to become active. Each tyrosine kinase phosphorylates the adjacent monomer kinase using ATP. Once active the phosphorylated dimer may be recognized by specific relay proteins which change shape once they become active and activate transduction pathways.
- SH2 and SH3 are Src homology domains that bind to Tyr-P : Grb2, PLCy, PI3K
- SH2 domain on GRB2 provides the ability to be an adaptor protein. it can attach to a phosphorylated tyrosine. Sos is binded to the SH3 domain on Gbr2 and it activates Ras since it can attach Ras. It is a guanine exchange factor, forces Ras to swap GDP for GTP. Ras then detaches from Sos but stays membrane bound. Ras's will be activated as long as the complex is attached. This is the amplification effect. This path may lead to transcription via Raf - MEK - MAPK.
- Ras is a GTPase which is activated by GTPase activating proteins (GAP) and Guanine exchange factors such as Sos.
- Phospholipase Cy triggers the splaicing of PIP2 into IP3 and DAG which stays membrane bound, IP3 may influence IP3 receptor channels on the ER to promote calcium release into the cytoplasm.
- PI3K - Phosphatidylinositol 3 kinase can influence cell proliferation and growth, they may activate IAP - inhibitors of apoptosis.

<u>G- protein coupled receptor</u>

- Largest family of receptors (metabotropic), multiple ligands, some of which bind multiple GPCRs. Ex ACh binds 5 different receptors, adrenalin - 4.
- G-proteins are trimeric.
- Diverse range of ligands - Nt, hormones, odorents, tastents, photons of light.
- They interact with G-proteins, main pathways PIP2, cAMP. Some functions of GPCRs are embryonic development and sensory reception.
- G proteins are molecules that bind to GTP, GPCRs are made of several transmembrane a-helices with loops for binding sites of ligands and G-proteins on opposite sides.
- Bacterial infections may arise from malfunctions in GPCRs along with other diseases. 60% of all medicines exert their effects through GPCRs.
- G-proteins are loosely bound to the intracellular side of the PM and are activated by the presence of GTP instead of GDP, which is triggered by a ligand binding to a GPCR.
- The active G=protein will dissociate and diffuse across the membrane and bind to an enzyme which will change its conformation and catalyze cellular response.
- Ligand concentration outside the cell determines how often a ligand binds and the frequency of response.

- This binding of G-protein to enzyme is short lived since they act as GTPases which hydrolyze the bound GTP into GDP and inorganic phosphate and are ready for reuse.
- This allows rapid shutdown when the ligand is no longer bound.

Intracellular receptors:

- These receptors may be found in the nucleus or in the cytoplasm
- Lipophilic / hydrophobic ligands like steroids and thyroid hormones may diffuse across the membrane and bind receptors. Nitric oxide also has an intracellular receptor.
- Cytoplasmic or nuclear receptors once bound to a ligand form a hormone-receptor complex transcription factor which may trigger a hormone response element (HRE) that activates transcription by interacting with DNA.
- Glucocorticoids such as cortisol are released by adrenal glands and may bind intracellularly to influence stress response and metabolism.
- Intra cellular pathways usually lead to transcription factors which regulate transcription of DNA. Transcription factors determine which genes are turned on, aka which genes are transcribed into mRNA.
- Molecules such as aldosterone and steroids act as transcription factors. The receptor protein itself acts as the transcription factor and transduction part of the path.
- Some are found in or outside of the nucleus, these hormone receptor complexes while in the nucleus bind specific genes. The bound protein (transcription factor) stimulates the trans of the gene into mRNA which is translated into a protein.

Define what is cellular signalling and transduction

- Proteins often relay the info that the ligand is trying to transmit by transducing the signal. The ligand's signal is transduced into a different form at each step often involving phosphorylation. It is also amplified at each step.
- Amplification can occur due to length of an effector bound (ligand) and the quantity of speed of binding the next molecule in the transduction pathway. For example, cAMP is usually catalyze by the hundreds, while each molecule binds ten protein kinases. The response of one ligand in the glycogen breakdown path, is 10^8 molecules of glucose from glycogen.
- An enzyme that transfers a phosphate group from ATP to a protein is called a protein kinase. Enzymes called protein phosphatases remove phosphate groups from proteins (dephosphorylation) making them inactive and ready for reuse. These molecules are involved in phosphorylation cascades.

Identify and compare the basic signalling pathways:

Intracellular receptors:

- Peep above i guess

Ligand gated ion channels

- Peep above i guess

Enzymatic receptors (We will focus particularly on tyrosine kinase receptors)

- Peep above i guess

GPCR (the cAMP pathway):

- A trimer subunit (G- protein) is a molecular switch, interaction with the receptor will change its conformation (*amplification since multiple G- proteins can be triggered while ligand still bound*). Alpha subunit will separate from beta and gamma once G-protein has GTP bound. Alpha will activate the enzyme that produces cAMP. As long as alpha is bound to adenylate cyclase, it will produce cAMP from ATP *(Amplification)*. cAMP (the second messenger) activates protein kinase A. PKA has 4 subunits, cAMP must bind normal subunits in order to activate catalytic PKA subunits which phosphorylates multiple proteins *(amplification*). PKA may also translocate to nucleus and activate genes via CRE (cAMP response element). This cAMP response does not persist long in the absence of the hormone since an enzyme called phosphodiesterase turns it back into AMP. Other G-protein systems may inhibit Adenylyl cyclase by activating an inhibitory G-protein via another receptor, shutting down the pathway.

GPCR (the IP3 pathway):

- Phospholipase C is amplifier enzyme activated by an alpha subunit of a G-protein. While activated PIP2 is cleaved into DAG (membrane bound) and IP3. (Amp occurs in receptor that may activate many trimers), (enzyme amplifies IP3 and DAG formation). IP3 activates calcium channel on ER membrane causing release due to concentration gradient (amplification due to amount of Ca). Ca can bind calmodulin - other fxns. DAG activates PKC for protein phosphorylation (amplification) or arachidonic acid which can produce eicosanoids.
- Ca, IP3 and DAG are all second messengers in this pathway.
- Ca may cause cellular responses such as muscle contraction, metabolism and memory functions

Describe in detail an example of a signal transduction cascade starting from ligand binding to cellular response

- Descriptions of Ras - MAPK, phospholipase C_Y pathway, cAMP, PIP3 - G.

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

For a multicellular organism to remain capable of responding to signals, molecular changes brought upon by ligands may only last a short time. The ability of a cell to receive signals depends on its ability to reverse the changes produced by prior signals. As the concentration of ligands fall, there are less ligands bond at any given moment. Once the ligand is detached, cellular processes are reversed, GTPases hydrolyze GTP into GDP on G-proteins, cAMP is turned into AMP by phosphodiesterase, protein phosphatases inactivate phosphorylated kinases and other proteins. The result is the capability to respond to a fresh signal.

Ligand remove by distant tissues

- Ligand may diffused into a capillary and degraded in the kidney or liver

Ligand taken up by adjacent cells

- An adjacent cell may endocytose the ligand into a membranous vesicle.

Ligand degraded by extracellular enzymes

- Enzymes may digest ligands such as acetylcholinesterase digesting ACh in the synaptic cleft of a NMJ, terminating contraction.

Ligand-receptor complex removed by endocytosis

- The target cell itself may endocytose the receptor along with the ligand and degrade it in the lysosome.

Receptor inactivation

- A receptor may be inactivated by phosphorylation of that receptor.

Inactivation of signal transduction pathway

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Transcription

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

The first step of transcription involves transcription factors binding to DNA in order to signal and attach RNA polymerase II (Initiation). Once it is bound RNA polymerase II unzips DNA 3’ - 5’ while transcribing complementary base pairs from the template strand to an RNA strand 5’ - 3’. Coding region begins at the end of the promoter, Polymerase starts reading and transcribing downstream here (Elongation). In its wake the DNA helix reforms and is capable of acting as another template for an new RNA polymerase. Eventually the pre-mRNA transcript detaches along with the polymerase once enzymes catch up to it. (Termination) this occurs due

to a recognizable signal called the polyadenylation signal sequence (AAUAAA). Next the pre-mRNA must be modified (In eukaryotes) in order to be translated. This process occurs as splicing along with the alteration of both ends of the mRNA. These additions are 5' caps and poly A tails. More detail on the start sequence, and mRNA refining follow.

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

- In prokaryotes, transcription is initiated by the RNA polymerase binding to the promoter with help of the sigma factor which helps make sure it attaches securely and in the right place - lines up the two strands and ensures you start at the first coding nucleotide. sigma factor detaches once polymerase is stable. holoenzyme needs a protein as a cofactor. No helicase is involved here, RNA polymerase does everything.
- In eukaryotes the initiation process is different. TATA box binding proteins binds the TATA box sequence on DNA which positions the helix for transcription and forms the promoter. Additional proteins form the initiation complex such as transcription factors and regulatory elements. RNA polymerase II binds to the promoter along with more trans factors. The RNApol2 unzips the helix and begins transcribing at the start point on the template strand that is a few nucleotides down from the TATA box.
- Steroid and other intracellular ligands or signal pathways may produce or act as transcription factors.

Explain and demonstrate RNA maturation and splicing

- Before mRNA is formed and can leave the nucleus it must undergo processes of maturation which turn pre-mRNA into mRNA. Firstly both ends of the transcript are altered.
- The 5' end is synthesized first, it receives a modified form of guanine nucleotide added onto the 5' end after transcription of the first 20-40 nuecleotides.
- The 3' end receives a poly A tail. After the polyadenylation signal AAUAAA, enzymes add 50-250 more adenine molecules which form the tail.
- The cap and tail serve important functions such as facilitating export of the mRNA via nuclear pores. They help prevent degradation of the mRNA by enzymes, and they help ribosomes attach the 5' end at the beginning of translation.
- Next is RNA splicing which occurs in order to remove the introns from our pre-mre. Introns are regions of the transcript that must be removed since they are non coding. Exons remain as the coding regions of mRNA. The removal of these introns may shorten the strand from 27000 to 1200 and is facilitated by molecules called snRNPs (small nuclear ribonucleoproteins). snRNPs along with other proteins form spliceosomes that are capable of recognizing the start codons of introns and excising them by breaking phosphodiester bonds. The spliceosome then stitches together the ends of each exon.

- UTR is untranslated regions that positions RNA at the first codon, its a small buffer that is not translated, these are left un-spliced.

Compare and contrast RNA transcription in prokaryotes vs eukaryotes, other details added.

- Transcription occurs in the cytoplasm of prokaryotes followed directly by translation. In eukaryotes transcription occurs in the nucleus followed by a mandatory maturation phase before it is able to leave the nucleus for translation.
- Prokaryotes have only 1 RNA polymerase that may bind at will to promoters with only the help of the sigma factor which is also absent in eukaryotes. Eukaryotes however require 3 polymerases to carry out all forms of transcription: poly 1 = rRNA, poly 2 = mRNA, poly 3 = tRNA. They also require the formation of a transcription initiation complex which is comprised of many factors.
- Structure and location of DNA is different. Eukaryotes reserve transcription for growth cycles, prokaryotes can perform this any time.
- Unlike prokaryotes, eukaryotes have histones, which is what DNA wraps around tightly in nucleosomes and have effect on transcription. When histone is acetylated, it unwinds chromatin and permits transcription. Methylation on the other hand leads to condensed chromatin structure.
- A major difference is the presence of operons in prokaryotes which allows them to regulate transcription for multiple genes of similar fxn under the control of one promoter. Prokaryotes put all of the genes that work together under the same regulatory area. 1 promoter and 1 operator, once transcription occurs, all of the genes under the operon get transcribed. a Repressor (also a protein) located upstream of the operator may block the passage.
- In eukaryotes, coordinate control of dispersed genes often occur in response to a transcription signal. A steroid for example may bind an intracellular receptor and form a hormone - receptor complex that serves as a transcription activator. Every gene whose transcription is controlled by a given steroid will react to this stimulus simultaneously, increasing transcription yield of the specific RNA. Genes with the same control elements are activated by the same signals.
- Bacterial cell mRNA has short life span and may degrade within minutes of their synthesis. This allows them to change their patterns of protein synthesis quickly in result of a changing environment such as lactose or tyrosine presence.
- In contrast, mRNA of eukaryotes may last weeks so that translation can repeat.

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

Activators, Enhancers

- Distal control elements which may be found 1000s of nucleotides upstream of the promoter are called enhancers which may be plentiful. The rate of gene expression may be strongly influenced by the binding of certain transcription factors - activators or repressors - to enhancer control elements. Activators bound to enhancer regions may be brought into contact (by bending proteins) with mediator proteins and trans factors which interact with the promoter. This interaction helps assemble and position the initiation complex on the promoter. Activators may also bind to proteins that acetylate histones in order to promote transcription.
- Precise control of transcription depends largely on binding of activators to DNA control elements in eukaryotes. For the many genes expressed in eukaryotes, the number of completely different nucleotide sequences found in control elements is very small. On average each enhancer is composed of 10 control elements which each bind 1 or 2 specific trans factors. It is the specific combo of control elements in an enhancer that that is associated with a gene rather than one that matters. Each cell type contains a different group of activator proteins.

Repressors

- Repressors may also bind to enhancers or elsewhere, others may bind directly to activators and inhibit them. Some repressors may recruit proteins that remove acetyl groups from histones leading to reduced transcription - referred to as silencing. Repressors in prokaryotes function in codon pathways in order to influence transcription.

Alternative splicing

- Is a method by which one gene may code for multiple proteins, thus reducing the necessary amount of genes and getting more bang for buck. In alternative splicing, all the exons may be left in the transcript, or alternatively, some exons may splice out an exon between two introns along with both those introns changing the mRNA structure and thus the protein. Different combo of snRNPs that recognize different introns, some exons are redundant 3 , 4 carry the same role yet they have different structures, therefore they won't be sensitive to ca to the same degree, may contribute better or worse to contraction. Exons may be spliced depending on the snrp nucleotide sequence pattern. What is the advantage? multiple protein isoforms being encoded by a single gene, tweaked protein function without having more DNA.

Operons

- Two types of operons are mentioned in the literature, repressive(trans usually on but can be inhibited) and inducible ones(vica versa). Both of the following occur in E. coli.
- The trp operon depends on the presence of tryptophan in the stomach. When level are low, the repressor will be inactive and will not bind to the operator. In this condition, the RNA polymerase transcribes the consecutive genes of tryptophan under the codon/ promoters control. Once tryptophan is present, it activates as a co-repressor which will cooperate with the repressor in order to switch the operon off by binding to the operator.
- The lac operon usually has it's repressor bound to the operator, ceasing the transcription of B-galactosidase which digests lactose by turning it into monosaccharides (galactose, glucose). The gene for B is part of the lac operon and is needed when lactose is around. Once lactose is around, an inducer called allolactose binds to the repressor and inactivates it, causing trans of B to occur.
- Both these pathways are considered negative gene control. Gene control may be positive when a regulatory protein interacts directly with the genome to switch trans on.
- Cyclic AMP accumulates when glucose is scarce. The regulatory protein CAP is an activator - a protein that binds to DNA and stimulates transcription. When cAMP binds to CAP when glucose is scarce but lactose is present, CAP binds the the promoter upstream and increases the affinity for RNA polymerase to the promoter. This is positive because the CAP protein directly stimulates gene expression. CAP controls the rate of trans, while the repressor determines if trans occurs.

Know the components and roles of a ribosome

- Ribosomes have the role of binding mRNA and tRNA in order to catalyze translation in the cytosol or ER membrane to create proteins by catalyzing peptide bonds.
- Ribosomes may toggle between being free or bound depending on a signal recognition particle SRP that recognizes a signal peptide sequence for an ER type protein and binds the ribosome to the pore of the ER.
- Ribosomes have small subunits that bind and fasten mRNA as well as tRNA, which then bind large subunits with E(exit), P (peptidyl), A (aminoacyl) sites that help with translocation of tRNA. There is also an exit tunnel where polypeptides exit.
- Ribosomes consist mostly of rRNA and some proteins

Understand and demonstrate the different steps involved in translation

- AA pail is what the mRNA needs to leave the nuclear pores. UTR means ribosomes do not translate this region. Ribosomes must determine the beginning and end on translating region. 3 nucleotides = 1 codon are read at a time. Nucleotide combos (codons) are the exact same for every organism. AUG is the only codon that codes for methionine, every single coding region starts with Met. There are also 3 distinct stop codons that cause the ribo to stop UAA, UAG, UGA. Even though there are synonyms for amino acid codes, meaning many

codons for 1 amino. Yet no ambiguity, no codons correspond to more than one amino.

- Wobble effect = third nucleotide can toggle and produce the same amino. This produces redundancy
- Protein is built N to C terminus.
- RNA is transcribed 5’ to 3’ since the ribosome moves 5’-3’. The correct tRNA with the anticodon for the sequence in the A site must bind in order for the correct amino to bind to the chain.
- Reason for shape of tRNA is due to amino attachment. Anti codon is the complementary sequence to the codon we want to bond / the amino we want. There are 32 codons due to wobble effect.
- Something must attach the amino to the tRNA, aminoacyl-transferase does this by providing a template enzyme and strongly covalently bonding them. 20 of these exist, 1 for each amino acid.
- Small subunit with mRNA binds tRNA at P site starting at start codon and then binds large subunit using GTP. GTP is used by an Elongation factor to bind a tRNA to the A site and cycle begins. UTR allows for stability of subunits and hangs out.
- the A site using GTP. EF is released as it is hydrolyzed. Peptide bond between A site amino and C terminus of P site amino gets hydrolyzed by peptidyl transferase. Another set of EFs promote translocation of tRNAs exposing a free A site.

Moch Middy

Define proton motive force and provide an example of how the cell benefits from it (2 makrs)

Explain how the alpha subunit a_s of a G-protein is activated(2 marks)

What is an amplifier enzyme? Give a description and provide a specific ezample (2 marks)

What makes the nematode C elegans a good model to study apoptosis? Provide 2 reasons(2 marks)

Name two reasons why the cell uses mitophagy (2 marks)

Briefly describe two different ways cells can terminate a signal transduction pathway (2 marks)

Describe Alternative RNA splicing and provide a diagram explaining two possible scenarios. (5 marks)

Explain what a spliceosome is and its role (2 marks)

What advantage do lipid rafts contribute in terms of cell signalling (1 mark)

Describe chronologically how the transcription initiation process is formed (2 marks)

Define membrane potential and explain how it is maintained(2 marks)

Name 2 ways to terminate a signalling pathway(2 marks)

When considering RNA what are three things that are different from DNA (3 marks)

Name and explain the process involving interference with mRNA during post transcriptional gene regulation in eukaryotes. Provide an example for 1 consequence this may have on the cell (5 marks)

Some epithelial cells in culture are dying. Describe two manipulations that you could do experimentally and what observations each result should provide for you to be able to conclude the mechanism by which those cells are dying. (3 marks)

What should the consequence be if the regulatory subunit of PKA became insensitive to cAMP? Use the key steps of the transduction pathway to support your answer (4 marks)

Vividly describe how the cells of a mouse undergoing mitochondria damage will respond in order to ensure the survival of the mouse. Name the process and provide vivid explanation (6 marks)

Growth factor contributes to a cells growth, survival and its differentiation. Using a drawing of a cell, explain how NGF's signal is transduced by the cell and how it relates to ensuring survival and preventing the initiation of apoptosis. (

Acetylcholine is often used to relax muscles of patients. Using your knowledge of the ACh signalling cascade, propose a detailed explanation of the signal transduction cascade responsible for the observed molecular response with the alternate compound(10 marks)