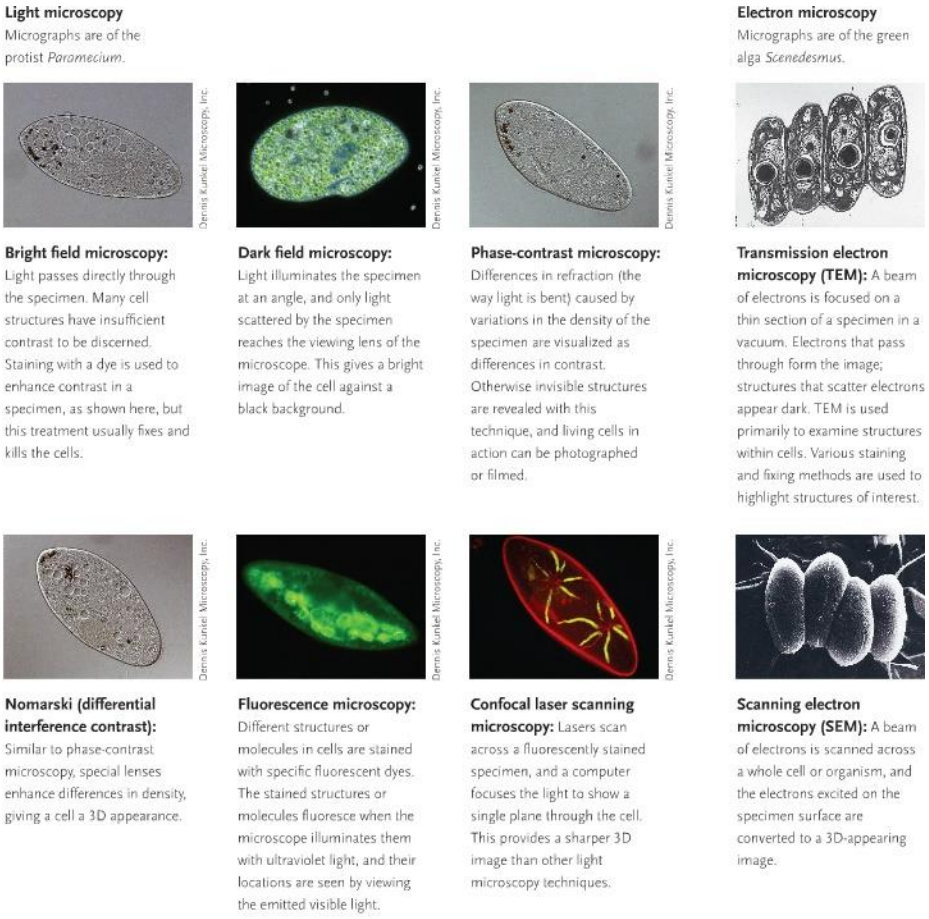
1. **What is a cell?**
    - Membrane-bound structures that occur as functional independent units of life (such as in unicellular organisms, e.g. bacteria, protozoa, *etc*.), or as structural or fundamental units in a biological tissue specialized to perform a particular function in multicellular organisms (e.g. plants and animals).
2. **What is a cell made of? Name the different organelles and the roles/functions for each**
    - **Ribosomes**
        - Made out of protein and rRNA
        - has a large and small subunit
        - produced in the nucleolus
        - can find ribosomes in the cytosol or in the rough endoplasmic reticulum
        - its job is to grab mRNA and translate the strand into proteins
    - **Nucleus**
        - Stores and protects DNA
        - Nuclear envelope serves to protect
        - Nuclear pore complexes differentiate things coming in by size & affinity; it only protects from entry
    - **Endoplasmic Reticulum**
        - Rough: synthesizes proteins, membrane is studded with ribosomes
            - Membrane is called cisternae
            - Proteins move to the golgi complex for further modifications after being translated in the rough ER
            - Folding of protein mostly takes place here
            - Network of flattened membrane
        - Smooth: synthesizes lipids
            - Tubular shape
    - **Golgi complex**
        - folds of membrane – cisternae
        - looks like a stack of pancakes
        - Proteins from the rough ER are sent here, these proteins are not functional yet, they need to be modified – addition of functional groups to the proteins (sugars, thiols, phosphates, etc.)
        - Golgi adds a chemical tag to the proteins, and all proteins with the same tags are packaged into a vesicle and shipped to their destination
        - Proteins come in through the cis face, and exit out of the trans face
        - Proteins can also go to the Golgi complex in order to be recycled
    - **Lysosome**
        - Organelle used to digest macromolecules
        - Acidic
        - Contain enzymes to break down – enzymes are proteins

- Contain 30-60 enzymes
- Lysosomes come from the Golgi complex

- **Mitochondria**
  - An animal cell's source of energy
  - Contains two membranes – an outer and an inner, in between there is a matrix
  - The inner membrane is made of neat folds – cristae
    - Folds increase surface area, the more surface area there is, the higher the proton gradient can build up – allowing for increased production of ATP
- **Chloroplast**
  - A plant cell's source of energy
  - Plastids: Chloroplasts, amyloplasts, chromoplasts
  - Amyloplasts store energy in the form of starch
  - Chromoplasts are colour pigments (these allow leaves to change colours every season)
  - Chloroplasts are made up of a series of disks called thylakoids which are built up by granum
    - In between thylakoids are stroma
    - Chloroplasts also contain two membranes
- **Central Vacuole**
  - Organelle in plant cells, holds water and is important for the cell's shape and pressure
  - Area of plant cell where digestion occurs
  - Maintains the cell's turgor pressure
- **Cell wall**
  - Plants have an outer and an inner leaflet, outer being the cell wall and the inner being the plasma membrane
  - There are small openings between cells across the cell wall called plasmodesmata
    - These are used for cell communication, ions and small molecules are able to cross these openings
- **Cytoskeleton**
  - Not an organelle
  - Organelles are anchored on the cytoskeleton
  - Cytoskeleton acts as a road for the transport that goes on within the cell

3. **Explain the theory of the cell and endosymbiosis.**
    - **Cell Theory**
        - All organisms are composed of one or more cells.
        - The cell is the basic structural and functional unit of all living organisms.
        - Cells arise only from division of pre-existing cells.
    - **Endosymbiosis**
        - Endo meaning inside, and symbiosis meaning a mutually beneficial relationship
        - Two prokaryotic cells joined together to form a eukaryotic cell
        - One of these two cells was an energy producing cell, and once they formed together, the energy producing cell eventually became a mitochondria
        - The energy producing cell used the host cell for protection, while the host cell used the energy producing cell for energy
        - It is similarly thought that chloroplasts were formed the same way
        - **Evidence:**
            - **Morphology:** shape of mitochondria and chloroplasts are similar to the size of bacteria and archea
            - **Reproduction:** they both reproduce by binary fission
            - **Genome:** they both have circular DNA
            - **Transcription and Translation:** they each have their own machinery
            - **Electron transport:** both have a double membrane with ETC
            - **Sequence:** mitochondria – proteobacteria, chloroplasts – cyanobacteria
4. **What are the main groups or types of cells and how are they different?**
    - **Prokaryotes**
        - 1-5 micrometres in size
        - Doesn't contain any membrane-bound organelles
        - whole organism is one cell
        - Able to inhabit harsh environments (extremely hot environments, cold, etc.)
        - contains plasmids – smaller circular genomes, easier to control & adapt
        - small genomes – 10^6 base pairs
    - **Eukaryotes**
        - 10-100 micrometres
        - Contains membrane-bound organelles (nucleus, mitochondria, endoplasmic reticulum, etc.)
        - Elaborate membranes allow compartments to organize cellular functions
        - Contain transport systems – cytoskeleton
        - Have larger genomes (10^6 to 10^9 base pairs) – linear DNA
        - not easy for eukaryotes to change and adapt
        - since there are more cells in eukaryotic cells, all cells have to agree to change

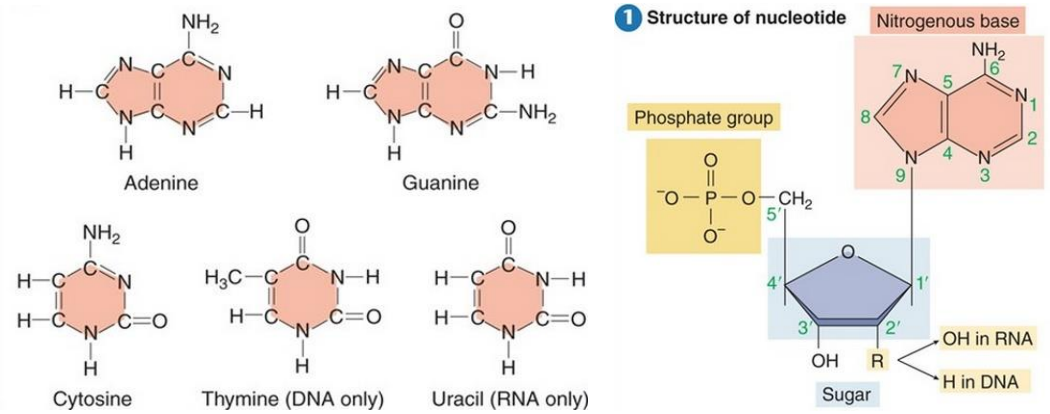
5. **Understand different types of microscopy and their requirements for use in cell biology.**



6. **What are the main classes of macromolecules, their structures, and their roles in cells?**
    - **Carbohydrates**
        - Mono-, di- or polysaccharides
        - Fuel source for chemical energy
        - Used for cellular recognition and communication
            - Antigens are a form of recognition of a cell
        - Glucose can come in two different types depending on where the hydroxyl groups are in the structure
            - Alpha and beta glucose, for alpha: the hydroxyl groups are on the same side, for beta: the hydroxyl groups are on the opposite sides
        - Depending on alpha and beta confirmations, the polysaccharides can have different shapes
        - Examples
            - Storage – glycogen and starch
            - Structure – cellulose and chitin

- **Lipids**
  - Four main groups: fatty acids and triglycerides, phosphoglycerolipids, glycolipids, steroids
  - **Amphipathic:** when a molecule has a polar and a non-polar region
  - **Fatty acids:** long chains, the length of chains itself give properties to fatty acid
    - The polar region of the molecule gives it high hydrophilicity while the carbon chain region gives the molecule high hydrophobicity.
    - Can be saturated (contains only single bonds in the carbon chain) or unsaturated (contains double bonds)
    - Plasma membrane is made up of saturated chains; if the fatty acids in the plasma membrane were unsaturated, the membrane wouldn't be as well organized
    - More difficult to line up fatty acids when there are unsaturated double bonds
    - The longer these chains are, the more chance there is for double bonds
    - Unsaturated fatty acids are more fluid at room temperature, i.e. oil
    - Saturated fatty acids are more solid at room temperature, i.e. butter
    - Can package more saturated fatty acids in the same amount of space compared to unsaturated fatty acids
    - Fat cells are important for metabolic energy, mechanical protection and thermal regulation
  - **Triglycerides:** stored in specialized cells in the liver – adipocytes
    - Can serve as energy reserves
    - Play an important roles in thermal regulation and isolation, as well as protection against mechanical pressure
  - **Glycolipids:** lipids to which a mono or oligosaccharide is fixed
    - Contain glycerol or sphingosine backbones
    - Part of the extracellular matrix – important in cellular recognition
  - **Steroids:** only in eukaryotes
    - Can be found in membranes
    - Important in cell signalling
    - All have the same basic structure: have a small polar end, a large sterol core (four aromatic rings) and a carbon tail
      - Differences in steroids come from changing the carbon tail or the functional groups on the sterol core

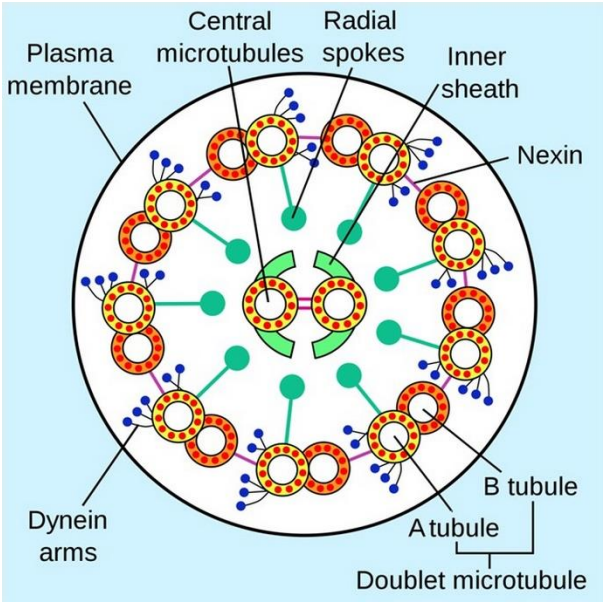
- **Nucleic acids**



  - Nucleotides assemble to form polymers; nucleic acids (DNA and RNA)
- **Protein**
  - Polymers of amino acids
  - There are 20 essential amino acids
    - These are all different, but have similar structural backbones
      - They all have a central asymmetrical carbon, along with an amino group, a carboxyl group and a hydrogen
      - Difference in structure comes from the radical group
  - 3 classes of amino acids: nonpolar, polar and electrically charged
  - Proteins have levels of structure: primary, secondary, tertiary and quaternary.
  - Secondary structure:
    - Alpha helices are flexible and more soluble areas in proteins
    - Beta pleated sheets are more rigid and the less soluble areas in proteins
    - When these are added, they start folding on each other, this forms tertiary and quaternary structures
  - Proteins are involved in many cellular functions
    - Hormonal proteins – coordination of an organism's activities
    - Receptor proteins – response of cell to chemical stimuli
    - Contractile and motor proteins – movement for cell
    - Structural proteins – support for cell
    - Enzymatic proteins – selective acceleration of chemical reactions
    - Defensive proteins: protection against disease (i.e. antibodies)
    - Storage proteins – storage of amino acids
    - Transport proteins: transport of substances around the cell

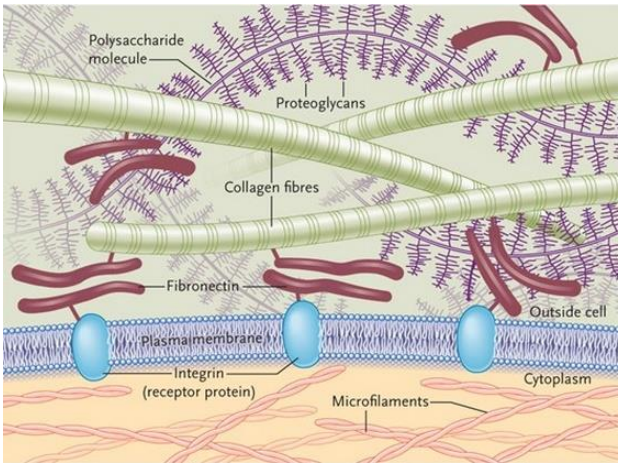
7. **How is each component of the cytoskeleton formed?**
    - **Microtubules**
        - Hollow long tubes (23 nm in diameter)
        - Assembled from tubulin protein
        - Two types of tubulin: alpha and beta – these form a dimer (no covalent bonds)
        - Both proteins can bind to GTP but easier to bind on to beta tubulin
        - Dimers always attach in the same orientation
        - B-tubulin is the positive end (growing end)
        - A-tubulin is the negative end (the base, MTOC (microtubule organization centre)
        - When GTP binds to tubulin, it helps to make it favourable to attach other dimers
        - Tubulin heterodimers assemble as a single row (protofilament)
        - These stack to form sheets
        - Their 3D structure assembly every 28 degrees explains the tubule shape
        - Has a hollow inside
        - Contained within the interior of the cell; main highway for transport
    - **Microfilaments**
        - 7 nm in diameter
        - Made of two strands of actin (globular protein)
        - Right hand helix formation
        - Anchor is negative, microfilaments elongate by the positive end – grows where the carboxylic acid group is
        - ATP makes it more favourable to join the monomers together
        - The + end – barbed end
        - The – end – pointed end
        - Microfilaments have the ability to branch
        - Microfilaments cover the entire surface area of the cell
    - **Intermediate filaments**
        - 10 nm in diameter
        - Forms cage-like structure around nucleus
        - Made up from collagen and keratin
        - Assembly is as a helix of proteins, which forms a coiled-coil
        - These dimers than associate head to tail to form tetramers
        - These tetramers will stack together in a staggered manner forming rope-like structures
        - Able to withstand a lot of stress (torsion, shearing, compression)
        - Contained under the plasma membrane along with microfilaments, as well as around the nucleus

8. **What are the main types of cell-to-cell interactions?**
    - **Junctions**
    - **Tight junctions –** fusion of proteins on external plasma membranes. Seals the area – even ions cannot pass
    - **Anchoring junctions –** plaques of proteins interacts with microfilaments (desmosomes) or intermediate filaments (adherens) and anchor cells together. (Mechanical force)
    - **Gap junctions –** channels formed by protein bridging cells – connexins. Allows passage of ions and small molecules – cell communication
9. **Cellular motility – how is it accomplished?**
    - Motility is the ability to move, using energy
    - **Cilia**
        - Many cilia, short
        - Move in whip-like fashion – back and forth
        - Moves liquid/substances on the cell surface (i.e. mucus)
    - **Flagella**
        - Usually a single, long flagellum
        - Moves in a smooth S-wave motion
        - Moves the cell in environment
    - Both of these have the same structure: a 9+2 complex
        - A circle of 9 double microtubules surrounding a double microtubule
    - Dynein allows the microtubules to slide over each other, while a linker protein – nexin, holds the doublets together
    - As dynein pushes the structure to one side, once the doublets move too far, the nexin pulls it back



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

- In the ECM, can find sugars and proteins, salts and ions, concentration gradient, lipids
- Holds cells together in tissues
- Helps filter material passing between different tissues
- Helps orient cell movement during embryonic development, growth and tissue repair
- Plays a role in chemical signalling from one cell to another
- **Integrins** – plasma membrane receptors, binds to the ECM and micro filaments
- **MMPs (Matrix metallo-proteins)** – can produce and degrade the ECM to allow cell movement and guidance in tissue, without these, the ECM would hinder the movement of the cell



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

- They act as a boundary; they are selectively permeable
- The organize and scaffold
- Regulate solute support
- Receive signals from other cells
- Cell-to-cell communication

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

- **Fluid lipid** molecules with embedded, free-floating or anchored proteins.
- Arranged in sheets, non-covalently bound
- Two facing sheets/fluid lipid layers – provide structure
- **Mosaic** of different proteins – allow various functions (specificity)

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

- Phospholipids are the most abundant lipids in the membrane bilayer
  - 1 polar head and 2 hydrocarbon tails
  - Presence of unsaturated double bonds produces a kink and influences the fluidity of the membrane

        - The four main phospholipids include: phosphotidylcholine, phosphotidylethanolamine, phosphatidylserine(-) and spingomyelin
    - Glycolipids also make up the membrane, they are 1 or 2 fatty acid chains with single or multiple sugar groups attached via glycerol or sphingosine
        - These are important for cellular recognition
    - Sterols also make up the membrane, they are made up of 4 aromatic rings and present only in eukaryotes
        - Play an important role in fluidity; both in terms of arrangement and in relation to temperature (buffers)
        - Examples of some sterols – animals, some protists: cholesterol, plants: phytosterol, fungi: ergosterol
        - Prevent holes in the membrane
    - Properties of the lipid bilayer: Membrane asymmetry, fluidity and dynamics
        - Membrane asymmetry: translocation from one layer to the other is rare and requires energy (ATP) this is necessary to maintain the asymmetry
        - Membrane fluidity: at high temperatures, most of the membrane becomes very fluid – this is due to the increase of desaturase (enzyme) at high temperatures
        - Membrane dynamics: **homeoviscous adaptation** - the ability to change the membrane's lipid composition in response to the environment in order to maintain membrane fluidity

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

- Transport across the membrane in most cases goes along the concentration gradient with the exception of active transport
- **Simple diffusion –** no help needed, molecules just go straight through the membrane
    - Small lipophilic molecules
- **Facilitated diffusion –** channel-mediated and carrier-mediated (integral proteins made up of connexins)
    - **Channel mediated**
        - highly selective
        - water and ions go through
        - gated
        - electrochemical gradients
        - i.e. aquaporins and voltage-gated ion channels
    - **Carrier mediated**
        - Highly selective
        - Ions, large polar molecules
        - Uniporters, co-transporters, exchangers
        - Driven by solute gradients
        - i.e. Na^{+}/Ca^{2+} exchanger, glucose transporter

        - Small or slightly larger hydrophilic molecules use facilitated diffusion
    - **Active transport –** goes against the concentration gradient and requires energy
        - Two possible sources of energy: using the concentration of another solute **(secondary transport)**, or using an input such as ATP **(primary transport)**
        - Two types of secondary transport
            - Symport: both the driving ion and the transported solute travel in the same direction
            - Antiport: the driving ion and the transported solute travel in opposite directions

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

- Membrane potential is due to the electrochemical gradient produced by ions on the inside and outside of the plasma membrane
- Normally there is a higher concentration of Na^+ and Ca^{2+} on the outside and a higher concentration of K^+ on the inside
- The cell however is slightly more negative on the inside compared to the outside of the cell – this is the cause of the membrane potential
- The membrane potential is the source of energy for secondary transport
- i.e. Glucose normally too large and polar to diffuse across the membrane by itself, so it uses a symporter along with sodium. When sodium diffuses across the membrane, it creates potential energy which can then be used to transport glucose across the membrane via facilitated diffusion using the Na^+ glucose symporter

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

- Fatty acid beta-oxidation (ATP via PhosOx)
- Krebs cycle
- Heme biosynthesis (haemoglobin, cytochromes)
- Steroidogenesis (mevalonate can convert cholesterol)
- Fe/S complex assembly (complex I and II; metalloproteins, ferredoxins, etc.)
- Amino acid metabolism (urea cycle)
- Gluconeogenesis
- Ketogenesis

17. Understand the importance of calcium

- **Availability & Storage**
    - Calcium is stored in the Endoplasmic reticulum as well as the mitochondria
    - Calcium is also located on the outside of the plasma membrane and can enter the cell through ion channels
    - Though calcium is important for numerous cellular functions, it is also toxic at high levels – triggering apoptosis (cell death)
- **Cellular functions**
    - **Physiological and biochemical functions that need calcium**
        - Response to stimuli (i.e. vesicle secretion)

- Muscle contraction
- Signalling (second messenger)
- Enzymatic cofactor (i.e. coagulation)
- Bones
- Metabolism

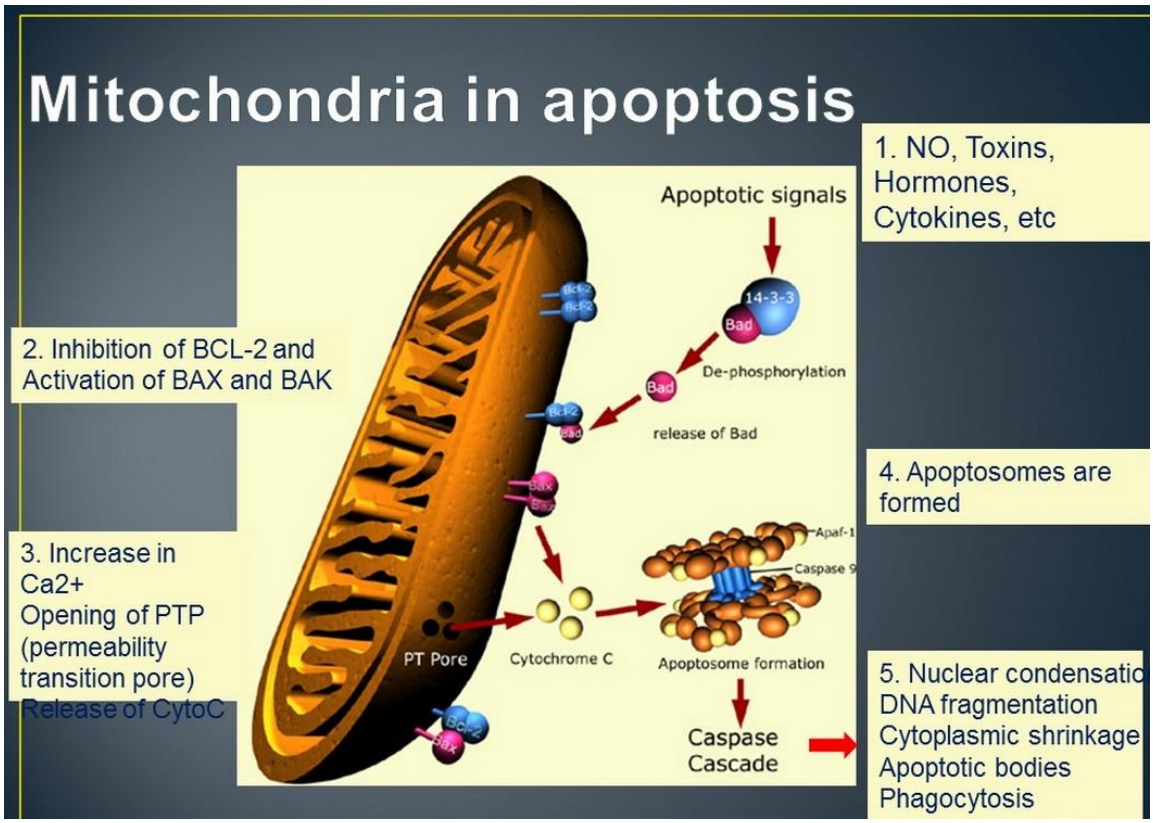
18. Understand key concepts of cellular energetics in relation to mitochondria.

- **Cellular respiration**
  - **Goal is to produce enough energy via the concentration of protons to power up a rotor – F-type ATPase**
  - F-type ATPase can spin in two different directions
  - **Will use protons or ATP as its energy source**
  - **Concentration gradient will flow through the rod and will provide enough energy to spin the portion within the matrix**
  - **As the ATPase spins, it phosphorylates ADP into ATP**
  - Increased surface area in the mitochondria from cristae allows it to have a huge proton gradient and many F-Type ATPases to produce as much ATP as possible
  - When it spins in the reverse direction, it uses protons to make ADP
  - **Four main stages in cellular respiration: glycolysis, pyruvate oxidation, krebs cycle and oxidative phosphorylation**
  - **Glycolysis**: takes place in the cytosol, it produces 2 pyruvates, 2 ATP and 2 NADH from one molecule of glucose
  - **Pyruvate oxidation:** takes place in the mitochondrial matrix, pyruvate is converted into acetyl-coA by an enzyme – pyruvate dehydrogenase – the result of this process is 2 acetyl-coA and 2 NADH
  - **Krebs cycle:** takes place in the mitochondrial matrix, it yields 2 ATP, 6 NADH, 2 $FADH_2$ and 4 CO_2
  - **Oxidative phosphorylation:** takes place on the inner membrane and intermembrane space, it yields about 32 ATP.
  - In total, this whole process yields about 36 ATP.

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

- **Necrosis**
  - There is a change in osmotic pressure which causes the selective permeability of the plasma membrane to change; this allows water to rush into the cell
  - The transport systems and membrane system shuts off
  - The cell will eventually swell and explode – cell lysis
  - This is dangerous to neighbouring cells since there are enzymes in the cell that has exploded that can damage other cells

- **Apoptosis**
  - Apoptosis occurs when there is a trigger – stress, absence/inactivation of a growth factor, toxins, hormones, cytokine, etc.
  - The dephosphorylation of BAD activates the intrinsic apoptotic cascade
  - BAD activates BAX and BAK
  - The activation of BAX and BAK leads to changes in affinity of IP3-gated calcium channel on the ER – opening it and allowing calcium to rush out into the cytosol
  - BCL2 was what was keeping the IP3-gated channel from opening
  - The calcium released from the ER will be absorbed by the mitochondria
  - Once the concentration of calcium in the mitochondria gets too high, the cristae in the mitochondria will rearrange and the PTP will be formed
  - Once the PTP is formed, cytochrome C is released by the mitochondria
  - Cytochrome C will bind to Apaf-1 and caspase 9 to form apoptosomes
  - Apoptosomes allow the activation of the caspase cascade
  - This leads to apoptosis – cytoskeletal and cytoplasmic shrinkage, DNA laddering, blebbing and phagocytosis of apoptotic bodies
  - Caspases and calcium are necessary in order for scramblase to flip the plasma membrane in order to allow phosphotidylserine to be on the outside in order to mark the apoptotic bodies for phagocytosis.



20. What is mitophagy and how/why does it occur?

- Mitochondria-specific apoptosis
- When the mitochondria is under stress, it has the ability to split into two parts – healthy portion and unhealthy portion
- The healthy portion can go to another mitochondria and fuse with it
- The unhealthy portion binds to PINK1 and Parkin – Parkin is an E3 ubiquitin-ligase which promotes ubiquitination of membrane protein, signalling autophagocytosis.

21. Explain and give examples of cellular communications and its role.

- Cell communication is basically a conversation between cells
- Occurs so that basically all cellular processes can occur
- Essential during: development, hormonal regulation, muscle contraction, immunity and recognition of self, cancer, apoptosis, etc.

22. Differentiate between the 3 steps of cell communication.

- **Reception –** binding of ligand onto receptor
- **Transduction –** taking the message from the ligand and amplifying the message all over the cell
- **Response** – activation of cellular response

23. Know and differentiate the 6 paths of communication.

- **Direct cell pathway –** messengers go through gap junctions, cells are connected
- **Autocrine pathway –** a cell can release a ligand, the ligand can eventually find its way back on a receptor on the same cell
- **Paracrine pathway –** same as autocrine except the ligand can eventually find its way back on a receptor of a different cell
- **Endocrine pathway –** ligand is released into the blood stream and can travel throughout whole organism, can potentially reach all organs and cells connected to blood
- **Exocrine pathway –** ligand interacts with glands, glands secrete stuff……
- **Neuronal pathway –** chemical message is received by a cell and is turned into an electrical signal, travels down axon – at the synapse, the electrical signal is turned into a chemical messenger

24. Identify and differentiate the 6 messenger classes and their characteristics.

- **Steroids**
  - Derived from cholesterol
  - Lipophilic
  - Membrane permeable – can reach intracellular receptors
    - Act as gene transcription factors
  - 3 classes of steroids
    - Mineralocorticoids – aldosterone
    - Glucocorticoids – cortisol
    - Sex hormones – testosterone/estrogen

- **Eicosanoids**
  - Most are derived from arachidonic acid
  - Lipophilic
  - Follow the paracrine pathway
  - Two main pathways that lead to two main classes
    - Cyclooxygenase pathway leads to the production of prostaglandins which are for pain
    - Lipoxygenase pathway leads to the production of leukotrienes which are for inflammation
- **Peptides**
  - Made up of a few to many amino acids – less than 50 (more than 50 = protein)
  - Hydrophilic
  - Packaged in vesicles
  - Secreted by exocytosis
- **Amines**
  - Derived from amino acids
  - Have an amine NH_2 group
  - Hydrophilic – except for thyroid hormones which are hydrophobic
  - Many neurotransmitters are amines
  - i.e. epinephrine, histamine, GABA, thyroid hormones
  - Can move freely in the cytosol, but can't move through the membrane
- **Purines**
  - Derived from adenine or guanine
  - Need a transporter or use exocytosis
  - Mainly use paracrine or neuronal paths
  - Two ring structure
- **Gases**
  - very small
  - can diffuse through everything
  - can go through most pathways
  - have short half lives
  - gases can come from metabolism – by products of all chemical processes going on in a cell
  - cells are efficient – cells can accomplish a task and end up with a by product and use that by product as a signal
  - i.e. NO, O_2, CO, etc.

25. Know the 4 different classes of receptors.

- **Ligand-gated ion channel**
  - Ligand binds to receptor located on the membrane, the channel will change conformation – this will either open or close the channel

        - This allows or restricts ions from going in or out
        - Ligand gated ion channels can be multiple subunits associated together, when the ligand binds, the conformation of the entire structure changes
        - When the gate opens, ions are able to rush in and the electrochemical gradient would be changed – depends on which way ions are going through, and which way they are going through
        - i.e. IP3 gated channel
    - **Receptor-enzyme**
        - Doesn't change conformation to open and close, doesn't let through any ions
        - When the ligand binds to the ligand binding domain, the conformation changes and on the intracellular side, the catalytic domain induces enzymatic activity
        - 3 families: guanylate cyclase, tyrosine kinase and serine/threonine kinase
    - **G-protein coupled receptor**
        - Ligand binds to receptor, receptor changes conformation
        - Change in conformation recruits G-protein
        - Once recruited this leads to the activation of the alpha subunit – in order for it to be activated, need to exchange GDP to GTP and release the beta and gamma subunits
        - Once alpha subunit is activated, it goes to the amplifier enzyme which are able to convert inactive secondary messengers into activated secondary messengers
        - These signals keep going while the ligand remains bound to the receptor
    - **Intracellular receptor**
        - Can be found in the cytoplasm or in the nucleus
        - Lipophilic or very small ligands will diffuse across the membrane to reach these receptors
        - The binding of a ligand forms a ligand-receptor complex and this acts as a gene transcription factor – in the nucleus, bind to specific areas of DNA (promoter) and can activate or inhibit gene transcription

26. Define cellular signalling and transduction.

- **Cell signalling**
    - When cells need to communicate with each other, they send chemical messengers to relay their messages – ligands – that land on the receptors of other cells
- **Transduction**
    - Any process by which a biological cell converts one kind of signal or stimulus into another

27. Know the basic signalling pathways:

- **Intracellular**
  - Ligand (usually lipophilic or can diffuse across membrane) binds on to an intracellular receptor, creating a ligand-receptor complex
  - This ligand-receptor complex acts as a gene transcription factor
  - The complex can then go into the nucleus and land on a hormone response element (HRE) and can trigger or inhibit transcription
- **Ion channels**
  - Ligands bind on to the ion channels on the plasma membrane, inducing a change in conformation to either open or close the ion channel
  - This allows or restricts ions from going in or out
  - The opening of these channels on the membrane creates a membrane potential
- **Enzymatic receptors (Particularly Tyr-K)**
  - Ligand binds on the monomeric subunits of the Tyr-K receptors and promotes dimerization
  - Once a dimer, the receptor gains the ability to autophosphorylate and its catalytic domain becomes active
  - Once phosphorylated, the receptor becomes activated
  - To bind to Ras, an effector protein, the receptor must bind with GRB2 which has an SH2 domain which will then bind with Sos with an SH3 domain and Sos can bind with Ras
  - Once bound to Ras, it can exchange GDP into GTP – changing its conformation
  - Once the conformation is changed, Ras can no longer bind to Sos and the membrane and becomes a second messenger
  - Ras can then go on and activate MAPK through a series of steps; MAPK can act as a gene transcription factor
  - Meanwhile, phospholipase C (PLC) can also bind directly to the receptor since it contains an SH2 domain
  - PLC is able to cleave phospholipids, specifically PIP2, this separates IP_3 and DAG – allowing IP_3 to become cytosolic and DAG stays in the membrane
  - IP_3 is able to open IP_3 receptor channel in order to release calcium into the cytosol – BCL2 makes IP_3 and IP_3 receptor binding difficult, but BAX and BAK makes this easy
  - DAG can also be converted into arachidonic acid & activates PKC which can phosphorylate proteins
- **GPCR (cAMP and PIP/IP3 Pathways)**
  - ligand binds to receptor, receptor changes conformation and recruits G-protein
  - alpha subunit exchanges its GDP into GTP which activates it, unbinding from the beta and gamma subunits

- the activated alpha subunit will then go and activate adenylate cyclase, an amplifier enzyme, which will then activate cAMP by converting ATP into cAMP – cAMP are second messengers
- cAMP can bind on to PKA's regulatory subunit and free it of its cap to activate it
- PKA can phosphorylate proteins, causing a response
- The phosphorylated proteins can also be dephosphorylated by serine/threonine phosphatase, terminating the response
- PKA can also translocate to the nucleus and act as a transcription factor
- To stop the signal, a ligand must bind to the G_i-protein coupled receptor, activating the $alpha_i$ subunit which inhibits adenylate cyclase

28. Regulation and modulation

- **Specificity**
  - Specificity of responses depending on the type of cell and receptor
- **Coordination**
  - Coordination regulate additive, synergic or antagonistic chemicals to generate the desired effect

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

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

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

- DNA is made up of deoxyribose sugars, phosphates and nucleotides
- Nucleotides include adenine, thymine, guanine and cytosine
- Adenine binds to thymine and guanine binds to cytosine – this is due to the size
  - DNA is 2nm, C-G = 3 H-bonds and A-T = 2 H-bonds
- Phosphodiester linkage between the third carbon on pentose sugar and the phosphate group of the next base
- Antiparallel double helix

31. Understand and demonstrate the steps involved in DNA replication.

- DNA helicase unwinds the DNA strand, while single stranded binding proteins attach to the DNA strands to prevent them from coiling back
- DNA gyrase or topoisomerase binds ahead of the unwinding to prevent torsion
- Primase assembles RNA primers in the 5' to 3' direction (4-10 nucleotides)
- DNA pol III and sliding clamp can now bind on to the primer and start polymerizing DNA nucleotides in the 5' to 3' direction

- The leading strand is built continuously, but the lagging strand is built in parts called Okazaki fragments
- DNA pol I can replace the RNA primers, leaving behind a nick – which is sealed by DNA ligase
- Telomerase then adds a telomere to the 3' overhang using its built in RNA primers

32. Understand the structure and organisation of RNA.

- Ribose sugars
- Uracil bases instead of thymine
- Single stranded

33. Explain the DNA protection and correction mechanisms.

- DNA pol III can proofread the strand, and fix it in the 3' to 5' direction
- Endonucleases can cut out sections of DNA with mistakes, and DNA pol III can fill in the gap in the 5' to 3' direction – while DNA ligase fills in the nicks
- Telomeres – additional DNA, the sequence is repeated thousands of times
    - With replication, this sequence shortens, but protects the coding regions of chromosomes

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

- Within the promoter, there is a TATA box in which TATA binding protein binds to
- After this is bound, additional proteins come and complete the transcription initiation complex – transcription factors, activators, repressors, basal transcription factors, coactivators
- RNA polymerase II is then placed at the beginning of the coding sequence and transcription starts
- RNA polymerase II adds 60 nucleotides per second, but it does not proofread or makes corrections, meaning that any mistakes made during transcription will get carried on to translation
- In eukaryotes, there are specific sequences that differ depending on which RNA polymerase in order to signal termination
- This signal lets the polymerase know it's reaching the end of the sequence, this is part of RNA maturation – one of the things that needs to happen is that the signal sequence will recruit poly A polymerase
- Poly A polymerase recognizes the little sequence at the 3' end of the pre-mRNA and adds a series of adenosine nucleotides (50-300) which is the Poly A tail
- Without a Poly A tail, the mRNA strand would not be able to get through the nucleoporins, and the tail protects the strand from degradation by nucleases
- About 20-40 nucleotides into transcription, the 5' G Cap is added

- Within the transcribed mRNA strand, there are UTR (untranslated regions) – exons are coding sequence and introns are non-coding sequences -> must be removed by splicing
- Small nuclear ribonuclear proteins (snRNPs) are able to recognize the ends of introns and catalyze their cleavage – which is done by spliceosomes (snRNPs and other proteins)
- Once the introns are cleaved, the spliceosome is also able to fuse the ends of exons in order to make a continuous coding sequence
- The result is a mature mRNA

35. Compare and contrast RNA transcription in prokaryotes vs. eukaryotes.

- **Prokaryotes**
  - DNA is transcribed directly into mature mRNA
  - Sigma subunit (polymerase subunits) binds to the promoter on a gene, it serves as a guide to place RNA polymerase – RNA polymerase initiates transcription
  - RNA polymerase builds in the 5' to 3' direction, using the 3' to 5' strand of DNA as the template
  - To terminate transcription, there are two ways: Rho dependent and Rho independent
  - Rho dependent – ATP-dependant unwinding enzyme (Rho factor) at the 3' end
  - Rho independent (Intrinsic) – GC rich sequences at the end, creating a hairpin loop which pulls RNA away from DNA
- **Eukaryotes**
  - DNA is transcribed to a pre mRNA, that will be matured into mRNA and transported out of the nucleus – RNA maturation
  - Transcription factors, activators and repressors bind to the promoter of a gene – this causes the formation of a transcription initiation complex
  - Within the initiation complex, there are activators and repressors – these proteins can increase and decrease the rate at which transcription occurs (by binding on to enhancers and silencers)
  - There is also the basal transcription factor that positions RNA polymerase at the start and initiates transcription
  - Once initiation is complete, the product is a pre –mRNA, it needs to go through RNA maturation
  - About 20-40 nucleotides into transcription, the 5' G cap (guanine) is added
  - After transcription ends, the signal at the 3' end recruits poly A polymerase which forms the poly A tail (50-300 adenosine) – this is necessary in order for the mRNA strand to be protected from degradation and allow it to move past the nucleoporins in order to go into the cytosol

- The 5’ Cap is necessary in order to promote translation once in the cytosol from ribosomes

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

- **Alternative splicing**
  - When some exons are not needed, they get spliced along with introns when they are spliced
  - Different exons coding for the same protein can be spliced based on the type of cell it is
  - Depending on which exons are kept and removed, you will end up with similar genes but with different functions
  - I.e. tropomyosin in smooth vs. skeletal muscles – smooth muscles don’t contain troponin, so the exons that contain troponin binding sites on the tropomyosin gene for smooth muscles
- **Operons**
  - Regulation of gene expressions – genetic switches that control transcription
  - Lac operon is what’s used to digest lactose
  - In order to digest lactose, need three enzymes – beta-galactosidase, permease and transacetylase
  - These three enzymes are side by side in the gene sequence, meaning they will be transcribed one after another
  - The operator on the gene is the actual on/off switch
  - The operator for the lac operon is usually bound to the lac repressor (produced upstream from the regulatory gene) – when bound, it puts up a roadblock, turning off transcription
  - Once in a while, the repressor falls off and low levels of these enzymes are produced
  - Lactose is a sugar, and the membrane is impermeable to sugar – permease is already present to allow lactose into the cell
  - Once into the cell, there are low levels of beta-galactosidase present which converts lactose into allolactose
  - Allolactose can bind onto the repressor, changing its conformation – unbinding it from the operator
  - RNA polymerase binds on to the promoter and transcription of the lac operon occurs
  - Transcription will go on until there is no more lactose – once there is no more lactose, the repressor will have nothing to bind to and will bind back onto the operator

- **Interference**
  - microRNA (miRNA) and small interfering RNA (siRNA)
  - miRNA: short sequences transcribed that fold over itself to form a double strand with a hairpin loop
    - able to exit the nucleus – able to interact with an enzyme called dicer which cuts off the hairpin loop
    - Silencers are then able to recognize the double stranded RNA and attaches to it while getting rid of one of the two strands
    - The single stranded RNA fishes for other mRNA with matching complimentary base pairs – once found, it leads the mRNA to degradation
    - If an imperfect match is found, translation is halted
  - siRNA: small pieces of RNA that can bind to mRNA with complimentary base pairs and target it to degradation

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

- **Transposons**
  - Jumping genes – segments of DNA that can be excised and placed somewhere else along the sequence
  - Transposase can recognize the beginning and the end of the transposons and cuts the DNA ribbon at these points, releasing the transposable elements into the nucleus of the cell
  - Once released, the transposons can incorporate themselves into other points of the genetic material, sometimes inside a gene or next to it
  - If it lands within a gene sequence, it can silence it or cause a mutation
  - They can also introduce a new sequence; a new gene
  - Some transposons can be silenced themselves by interfering RNA
  - Transposons can also stimulate evolution; when they jump, they can bring adjoining sequences along with them – exon shuffling, this increases genetic diversity
  - i.e. bacteria use transposons to acquire antibiotic genes
- **Retrotransposons**
  - Some mRNA will not go on to translation, but rather be reverse transcribed from RNA into DNA and inserted back into the genome
  - To do this, they need reverse transcriptase enzyme
  - There is no proofreading or correction mechanism – many errors (mutations)
- **Retroviruses**
  - Using a reverse transcriptase, they convert viral RNA into a complementary strand of DNA in the host cell (RNA-DNA)

- 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 (DNA-DNA)
- Integrase allows to introduce this double stranded DNA into the host's genome
- When transcription occurs, viral RNA will be translated to viral protein which can be used to rebuild virus within the host

38. Understand and demonstrate the different steps involved in translation.

- Translation occurs in the 5' to 3' direction
- tRNA (transfer RNA) are RNA structures that have a particular folding and are responsible for carrying amino acids to the ribosome during translation
- tRNA knows it's time to bring an amino acid since within one of the loops of tRNA, there is an anticodon which is complementary to the codon on mRNA that is currently being read on the ribosome
- tRNA and amino acids are covalently bound together by an enzyme called aminoacyl tRNA synthetase
- the enzyme needs specific amino acids and ATP
- once it has these two, it hydrolyzes ATP and gets rid of two phosphates
- within the enzyme's catalytic domain, there's the amino acid and AMP
- now the tRNA which has the anticodon which matches the amino acid is needed – the remaining energy from AMP is used to form a covalent bond between the 3' end of tRNA and amino acid
- the small ribosomal subunit will assemble with the tRNA that carries methionine, the tRNA is placed in the P site (this is the only tRNA that starts at the P site)
- the large ribosomal subunit comes in after tRNA and mRNA is matched
- along with the large ribosomal subunit, there is also a protein called elongation initiation factor (eIF)
- eIF triggers or singals in a way that we are ready to translate, it recruits tRNA that are carrying the amino acids
- since methionine is in the P site, the A site is available
- a tRNA that has the complementary anticodon to the codon at the A site is recruited
- in the large ribosomal subunit, there is peptidyl transferase activity – ability to transfer amino acid from tRNA forming a peptide bond with amino acid on the second tRNA – this leaves an empty tRNA on the P site and tRNA containing both amino acids in the A site
- other elongation factors that are coming along with tRNAs help hydrolyze one GTP in order to translocate the two ribosomal subunits moving each of the tRNAs one position over
- process repeats until it reaches a stop codon

- once the stop codon is reached, there is no tRNA with the anticodon, so a release factor (RF) protein occupies the A site
- the release factor stimulates peptidyl transferase to cleave the polypeptide
- the release factor also stimulates the separation of the ribosomal subunits
- mRNA and ribosome are recycled

39. Define and explain, using an example, the different genetic point-mutation and possible mutagens.

- **Point mutations** are changes to a single nucleotide
- **Missense mutations** – change in nucleotide sequence which leads to a change in amino acid – can impact tertiary structure, and affect protein function
    - **i.e. sickled RBC**
- **Nonsense mutations** – changes in a single base pair resulting in changing the codon that would have translated into an amino acid into a stop codon
- **Silent mutation –** changes in a single base pair with no effect thanks to the wobble effect
- **Frameshift mutation –** the insertion or deletion of a single nucleotide – this shifts the reading frame, creating a whole different series of amino acids

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

41. Explain how the cell cycle is regulated.