Six Classes of Chemical Messenger

Chemical Messenger	Chemical Properties	Communication Pathway	Action and Interaction
Steroids	Derived from cholesterol. Lipophilic and membrane permeable which allows it to pass directly to the nucleus.	Endocrine pathway. Will diffuse or be bound to transport protein (albumin).	Reaches intracellular receptors. <u>Gene transcription factors:</u> -Mineralocortocoids (ADH) -Glucocorticoids (Cortisol) -Sex Hormones
Eicosanoids	Derived from arachadonic acid, a lipid which modifies membrane phospholipids. <u>2 Pathways:</u> -Cyclooxygenase pathway leads to Prostaglandins, Prostacylins and Thromboxanes. -Lipooxygenase pathway leads to Leukotrienes. Lipophilic and membrane permeable.	Paracrine or autocrine pathway. Local response.	<u>2 Main Classes:</u> -Pain: Prostaglandins -Inflammation: Leukotrienes
Peptides/Proteins	Made of amino acids (peptide <50, protein >50). Hydrophilic, so packaged in vesicles and secreted by exocytosis.	Most indirect pathways. Too big for direct pathway.	Packaged together in vesicles as pro-proteins (inactivated). Inside is an enzyme for cutting off inactivator, waiting to be triggered.
Amines	Have an amine group (NH2), usually synthesized from an amino acid. Most are hydrophilic, except thyroid hormones which are hydrophobic.	Most/all indirect pathways.	Epinephrine, histamine, GABA, thyroid hormones.
Purines	Derived from nitrogenous bases adenine or guanine. Able to hydrogen bond.	Require a transporter or use exocytosis. Paracrine and neuronal pathways.	
Gases	Small molecules with short half-lives. Can passively diffuse.	Direct, Paracrine, Neuronal, and Endocrine pathways.	Ex: Vascular homeostasis: NO from RBCs triggers relaxation of smooth muscle in blood vessels causing vasodilation.

Cell Communication:

- Essential during: development, hormonal regulation, muscle contraction, immunity and self recognition, cancer, apoptosis etc...
- 3 Steps: reception, transduction, response.

Receiving the Message

- Receptors are proteins on cell surface or inside the cell.
- Ligand binding will induce a change in conformation of the receptor protein.

Four Classes of Receptors

Receptor Type	Class of Receptor		Ligand	Pathway	Action
Transmembrane	Ligand Gated Ion Channel		Ach...etc	LGICs are a channel made of multiple subunits. Ach binds to the 2 alpha subunits. This triggers a conformation change to open the pore and allow ions through. Mostly Na+ and Ca2+ in and some K+ out. This causes changes in membrane potential. Acetylcholine is destroyed by acetylcholinesterase and choline is returned to the neuron to be recycled.	Muscle contraction etc...
	Enzyme Receptors	**-Tyrosine Kinase** -Guanylate Cyclase -Serine/ Threonine Kinase	Insulin, Growth Factors	1. Hormone binds to receptor and causes the dimerization and autophosphorylation of tyrosine residues. 2. Binding of GRB2 and Sos tcouples receptor to inactivate Ras. 3. Sos promotes dissociation of GDP from Ras; GTP binds and Sos dissociates from active Ras. 4. Ras now can act as secondary messenger. It undergoes a series of phosphorylations to become MAPk. 5. MAPk is a gene transcription activator which allows BCL2 to remain active, thus promoting cell growth/differentiation and preventing apoptosis. <u>Neuronal Growth Factor</u> 6. The second dimer interacts with P3K which phosphorylates into inhibitors of apoptosis (IAPs) or into BADp which promotes apoptosis by activating BAK and BAX. (see apoptosis). Cell will undergo apoptosis if it is starved of growth factors. <u>Epithelial Growth Factor</u> 6. The second dimer interacts with PLCγ, activating it. It then reaches a target, PIP2, which cuts it into 2 pieces. 7. One portion becomes IP3 and the other becomes DAG (an arachidonic acid). 8. IP3 goes to IP3 receptor channels on ER and releases Ca2+ ions into the cytosol. (see mitochondria and calcium).	Survival, growth, proliferation and metabolism.
	G-protein Coupled Receptors		Neurotransmitters, hormones, odorants, tastants, and light photons. *Norepinephrine (NE)	- receptor portion has 7 transmembrane domains. - G proteins on intracellular side activate secondary messengers. - G protein has 3 subunits: alpha, beta and gamma. Alpha subunit is activated by releasing 1. Ligand binds to G-protein coupled receptor. 2. Alpha subunit releases GDP and binds GTP. 3. Activated alpha subunit or betagamma subunit move through membrane and interact with amplifier enzyme. 4. Amplifier converts secondary messenger (cAMP or IP3) to active form.	*Stimulate nervous system, activate glycogenolysis and glucogenesis, pupil dilation, and maximize blood flow to

			<u>cAMP</u> 1. Ligand binds to a Gs-protein coupled receptor, causing a conformational change. 2. The alpha subunit releases GDP, binds GTP, moves through the membrane and activates adenylate cyclase. 3. Activated adenylate cyclase catalyzes the conversion of ATP to cAMP. 4. cAMP binds to the regulatory subunit of protein kinase A (PKA), which dissociates from the catalytic subunit, activating it. 5. The activated catalytic subunit phosphorylates proteins, causing a response. 6. The phosphorylated proteins are rapidly dephosphorylated by serine/throenine phosphates, terminating the response. 7. When ligand binds to Gi-protein coupled receptor, the alpha subunit inhibits adenylate cyclase, inhibiting the signal transduction pathway. <u>(PKA can also translocate to the nucleus and activate genses via the CRE)</u>	skeletal muscle and survival organs.
			<u>PIP/ IP3</u> 1. Ligand binds to a G-protein coupled receptor, causing a conformational change. 2. The alpha subunit of the G protein releases GDP and binds GTP and moves through the membrane. 3. The activated alpha subunit activates phospholipase C, which cleaves PIP2 into IP3 and DAG. 4. DAG is cleaved in the membrane to form arachidonic acid, the substrate for the synthesis of chemical messengers called eicosanoids. 5. IP3 is released into the cytoplasm. 6. IP3 can be phosphorylated to IP4, which has diverse effects. 7. IP3 also binds Ca2+ channels on the endoplasmic reticulum, releasing Ca2+ into the cytoplasm. 8. The Ca2+ binds to calmodulin, causing diverse effects within the cell. 9. The Ca2+ also stimulates protein kinase C (PKC) to move to the membrane where it interacts with DAG. 10. DAG activates the PKC which then phosphorylates proteins, stimulating a phosphorylation cascade.	
Intracellular (in cytoplasm or in nucleus)		Lipophilic or very small ligands	Ligand-receptor complex acts as a transcription factor. In the nucleus, it binds to specific areas of DNA to activate or inhibit gene transcription.	Glucocorticoids: cortisol reduces insulin synthesis in pancreas.

6 Ways of Ending the Response:

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

Lipid Rafts

- Sphingolipids and cholesterol form highly ordered microdomains/rafts. SL hydrocarbon tails are longer and saturated.
- Rafts are produced in the ER and sent to the plasma membrane. They can accomodate proteins with long transmembrane domains.
- Rafts organize and cluster proteins to function together.

Famous People!

- Neuronal Growth Factor: Discovered by Rita Levi-Montalcini, who observed that sarcoma tissue released “neurotrophins”. Won her Nobel Prize in 1986.
- GPCRs: Brian Kobilka and Robert Leftowitz won chemistry Nobel Prize for their work on structure and function of GPCRs with crystallography.
- X-ray Crystallography: use x-rays to determine structure by looking at diffraction/interference pattern when crystallized protein is bombarded with x-rays. Problems: time consuming (first took 22 years), large amount of material required, and insoluble protein crystallization is hard.

Specificity of Responses (Epinephrine)

- Same receptors but different intracellular proteins: epinephrine binds to beta receptor on liver cell and blood vessel causing different responses.
- Different receptors: on blood vessels, epinephrine binds to beta receptors in skeletal bv causing dilation and alpha receptors in intestinal bv causing constriction.

Coordination of Responses:

- Antagonism between insulin and glucagon on the pancreas avoids futile cycle of increasing and dropping blood glucose concentration and maintains homeostasis.

Mitochondria and Apoptosis

Cell Death:

- Size of organism/organ depends on total cell mass, thus the number of cells must be controlled.
- Cells can grow, divide and/or die.
- Control of apoptosis by direct, autocrine, paracrine, endocrine and neuronal signalling.

Cell Death	Necrosis	Apoptosis
Trigger	Damage to the cell.	Intrinsic or extrinsic stress signal. Chemical messengers (growth factors, mitogens (cell division) and survival facors), toxicity, and cell cycle checkpoints.
Process	- Ischemia (loss of blood flow to the tissues) and excitotoxicity (nerve cells killed by excessive stimulation of neurotransmitters). - Calcium surplus in the cell.	Intrinsic Pathway -Internal stressor (loss of survival factor, DNA damage). -Activate proapoptotic protein by dephosphorylating it. It activates Bad. Bad inhibits Bcl2 (anti-apoptotic orevents IP3 from binding) and activates BAX and BAK.

	- Loss of ATPase activity, electrochemical gradient and decreased ATP. - Membrane swells due to changes in osmotic pressure (permeable to water and ions) causing excessive diffusion and inflammation. - Loss of membrane integrity so cell will rupture (lysis) and die. - Neighbouring cells are affected by enzymes loose in the intracellular matrix.	- BAX/BAK facilitate IP3 binding to calcium channel on ER to release calcium. Calcium: - ER releases calcium via IP3 channels which increase calcium levels in the cell and mitochondria (absorb calcium to protect the cell). - Excess calcium in mitochondria makes cristae rearrange to form an opening called the permeability transition pore (PTP) which spans both membranes. (Mitochondria can thus remain healthy until the cell dies completely). - BCl2 can inhibit IP3 (stop release of Ca2+) while BAX/BAK activate IP3 (promote Ca2+ release). - Release of cytochrome c through PTP into cytosol which forms apoptosomes (activate initiator caspases). Caspase cascade: -Caspases are enzymes (proteases – cleave protein). Sorted into initiator and executioner caspases. Initiators responsible for activating executioners. - When all are activated, they stop various protein kinases which makes cell adhesion mechanisms and lamins (maintain nuclear envelope) inefficient. - Blebbing occurs and nuclear envelope disintegrates. - Caspases activate DNAases to chop up long pieces of DNA for DNA laddering. - Caspases deactivate cytoskeleton so that the cell can shrink into apoptotic bodies. - Caspases activate scramblase to move PS to the outer leaflet of the membrane, signalling the macrophages to engulf it via phagocytosis.
Appearance	- mitochondria has no cristae and no longer useful to the cell. - cell is enlarged.	- mitochondria still functional and effective. - cell is blebbed and shrinks, then separates into apoptotic bodies.

Membrane Flipping: Phosphatidylserine for signalling of phagocytosis

- Spontaneous: bidirectional movement, no energy required, non-specific.
- P-type Flippase: inward movement, ATP required, specific.
- ABC Flippase: outward movement, ATP required, specific.
- Scramblase: bidirectional movement, no energy required, non-specific, Ca2+ and caspase dependent.

Mitophagy:

- Reduce number of mitochondria (ageing, changes in activity level or pathologies) or get rid of a damaged one.
- During damage, the mitochondria will break off the healthy portion which will fuse with another healthy mitochondria.
- PINK1 will attached to membrane of damaged portion and recruits parkin. Parkin is an E3 ubiquitin-ligase which promotes the ubiquitination of membrane protein, signalling autophagocytosis. Lysosome will degrade the damaged portion.
- Under severe stress where most or all mitochondria are damaged, cytochrome c will be released and trigger apoptosis.

DNA

DNA The Hereditary Molecule:

- 1911: Thomas Hunt Morgan able to associate traits (genes) to a chromosome by studying sex-linked inheritance in Drosophilia melanogaster.
- 1928: Frederick Griffith identified properties of strains of Streptococcus peneumoniae related to their virulence in mammals.
- 1940: Oswold Avery determined that these pro-proteins were related to DNA (not RNA or protein).
- 1952: Alfred Hershey able to finally demonstrate that DNA was indeed the hereditary molecule by using phasge and Escherichia coli.
- 1953: James Watson and Francis Crick determined the molecular structure of DNA. Awarded Nobel Prize in 1962 (with Maurice Wilkins, without Rosalind Franklin).
    - Knew DNA was made of A, T, G and C (purines and pyrimidines). Trying to figure out the 3D structure and had different arrangement possibilities.
    - Had to be a helix of 2nm, the only possible arrangement was A-T, G-C.
- 50 years later we were able to sequence entire genomes.

DNA Structure

- Nucleotides:
    - Nitrogenous bases: Adenine, Guanine (purines) Thymine, Cytosine (pyrimidines), a five carbon sugar and a phosphate group.
    - Each nucleotide attached by phosphodiester linkage between 3rd carbon of sugar and P group of next nucleotide.
    - Hydrogen bonding attaches complimentary bases.
- Orientation of strand:
    - First nucleotide is 5' end presenting phosphate group and last nucleotide is 3' end presenting OH on sugar.
    - Elongation always occurs from 5' to 3'.
- Double Helix:
    - Both strands run in opposite directions with nitrogenous bases facing inside (h bonds) and the sugar and phosphate form the backbone.
- Packing
    - DNA is wrapped around histones to form nucleosome.
    - Nucleosomes wrap into chromatin.
    - Chromatin coils into solenoids.
    - Chromosomes are formed from chromatin.

RNA Structure

- Uracil instead of Thymine. Different sugar (ribose). Forms single stranded helix.
- Types: mRNA (messenger), tRNA (transfer), rRNA (ribosomal), snRNA (small nuclear), ncRNA (non coding), and iRNA (interfering).

DNA Replication

- Conservative model: parental DNA remains complete and new DNA is a copy.
- Dispersive model: Random parts of parental DNA are copied and pieced together.
- Semiconservative model: One strand of parental DNA is copied and transfers to each new DNA molecule.
    - Meselson-Stahl experiment:
- Leading Strand: orientation of elongation of daughter strand is 5' to 3' so it is replicated in a continuous fashion.

- Lagging Strand: orientation of elongation of daughter strand would be 3' to 5' which is impossible, so it is done in sections from 5' to 3'. Lagging strand will have primer on 5' end which will not be able to be replaced with DNA. Thus, when primer is removed, there will be a 5' gap and a 3' overhang. Each replication cycle will shorten the 5' end. Will have as many as 5000 non coding nucleotides to protect DNA.
- Okasaki Fragments: **********
- Enzymes:
  - Helicase: unwinds DNA helix.
  - Single stranded binding proteins (SSBs): Stabilize single stranded DNA and prevent the two strands at the replication fork from reforming double stranded DNA.
  - Tropoisomerase (DNA Gyrase): Avoids twisting of the DNA ahead of the replication fork by cutting the DNA, turning the DNA on one side of the break in the direction opposite to that of the twisting force, and rejoining the two strands.
  - Primase: Assembles RNA primers in the 5' to 3' direction to initiate a new DNA strand.
  - DNA Polymerase 3: Extends RNA primer by adding DNA nucleotides. Only binds to double strands, which is why primase exists. Corrects its own mistakes by backtracking and replacing incorrect nucleotides.
  - DNA Polymerase 1: enzyme that uses 5' to 3' exonuclease activity to remove the RNA of the previously synthesized Okasaki fragment, and uses its 5' to 3; polymerization activity to replace the RNA nucleotides with DNA nucleotides.
  - Sliding Clamp: Tethers DNA polymerase 3 to the DNA template, making replication more efficient.
  - DNA ligase: seals nicks left between adjacent bases after RNA primers replaced with DNA.
  - Endonuclease: Can attach to already made double stranded DNA to correct problems. Will break the backbone of the new strand on each side of the mismatch, and remove the bases leaving a gap in the DNA. (DNA polymerase 3 will come in and fill it).
  - Telomerase: Binds to single stranded 3' end of chromosome. It synthesizes new telomere DNA (TTAGGG) using RNA as a template . 3' overhang will result from primer on the end.

Working With DNA

- DNA Cloning: using bacteria to uptake pieces of enzymes, grow them in cultures, find the gene you are studying, and grow more.
- Restriction Enzymes: cut pieces into fragments as a way of isolating a gene, can then be transferred to another genome cut with the same enzyme.
- Polymerase Chain Reaction: heat DNA to separate it, cool to allow primers and nucleotides to be attached, heat again to separate new DNA...

DNA Transcription

- Gene: made up of coding and regulatory sequences.
- DNA: 3'-5' strand is the template or non-coding strand. 5'-3' strand is coding strand that will not be copied in transcription.
- RNA polymerase II reads 3'-5' DNA strand. The RNA strand produced is elongated in the 5'-3' direction, therefore the mRNA obtained it 5'-3'.
- RNA polymerases:
  - RNA polymerase I: rRNA (ribosomal). Provides the ribosomes.
  - RNA polymerase II: mRNA (messenger). Only one that leads to protein (translation).
  - RNA polymerase III: tRNA (transfer – translation). Brings the amino acids.
- Initiation: Within the promoter, some proteins and transcription factors bind to specific sequences and form the transcription initiation complex.
  - TATA box Binding Protein (TBP) binds to the TATA sequence on DNA.
  - Additional proteins come and complete the transcription initiation complex (TIC), and provide stability to allow RNA polymerase II to come.
- Elongation:

    - RNA polymerase II recognizes the initiation complex, separates the DNA, reads the template strand and prepares the complementary RNA strand in the 5'-3' direction. RNA polymerase II adds 60 nucleotides per second, however it does not proofread or correct.
- Termination:
    - The complete RNA molecule is released from the template DNA. RNA polymerase II leaves the DNA and the doublt helix reforms.
    - In eukaryotes, the pre-mRNA molecule is processed to produce translatable mRNA.
- Pre-mRNA
    - 5' cap: 7 guanines added after transcription of the first 20-40 nucleotides. Gausnine's 3' OH has been reversed (5') and is attached with 3 phosphate groups to the pre-mRNA. Protects the mRNA from degradation and is the site of attachment at the start of translation.
    - UTR (untranslated regions): Exons are coding sequence, introns are non-coding sequence and must be removed by splicing.
        - Splicing: Small nuclear ribonuclear proteins (snRNPs) and other proteins together form spliceosomes. They recognize the ends of introns and catalyze their cleavage. The ends of exons after removal of introns are joined together for a continuous coding sequence.
        - Alternative Splicing: spliceosome can make use of different recognition sites on introns to remove some exons on the same gene to give a different coding sequence. Ex: tropomyosin is different on smooth and skeletal muscle and the different shapes are caused by the removal of exons. Different cell types will favour one configuration over the other so the muscles exhibit the proper tropomyosin.
    - 3' poly A tail: 50-200 adenines added at the 3' end by the poly-A-polymerase. Used for recognition to exit the nuclear pore and as protection from enzymes that cut DNA/RNA.
- Transposons: all organisms have small portions of DNA that are able to change location within the genome and be inserted anywhere (even within coding sequences). Transcription of that gene will have a mutation: frame shift, silencing... can be bad or good consequences.
    - Bad consequence: bacterial anitbiotic resistance.
    - Good consequence: genetic diversity and acquiring new traits.
    - Alu transposon: notes on lecture tools
    - Central Dogma: telomeres have RNA template to create DNA. Can reverse.
    - Retrotransposons: Some mRNA will not go on to translation but rather be reverse transcribed back to DNA and inserted back into the genome. There is no proofreading or correction mechanism which means there are many errors. Happens in retroviruses:
    - Retroviruses: using a reverse transcriptase, they convert viral RNA into a complementary strand of DNA in the host cell. The host's DNA polymerase makes it into a double strand of DNA by displacing the RNA strand and adding the complementary strand of DNA. Integrase allows 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. Mutations make it harder to eradicate.

DNA Translation

- Start codon is AUG Methionine. Not all proteins start with Methionine because they are not fully matured when they leave the ribsosome. Go to Golgi to be matured.
- Stop codons are UAA, UAG and UGA.
- More than one codon for an amino acid. Only one amino acid for each codon. 64 codons, 3 that don't code for amino acids.
- Wobble effect: first 2 nucleotides in a codon have to be an exact match. Third can be wobbly. Felxibility is in the third nucleotide of the codon – will bring the same nucleotide. Amino acids match tRNA according to complementary sequence.
- Exons are functional domains in protein. Each domain corresponds to an area in protein and a specific job of the protein. Ex: extracellular part, intramembranous part, and intracellular part.

- TRNA: 75-90 nucleotides folded into structure with 3 hairpins. One carries the anticodon (3'-5') which is the complementary RNA to the mRNA codon. Amino acids carried at 3' end. Oscillation means that we only need 45 tRNAs to accomodate the 61 codons.
- Aminoacyl tRNA synthetase: Each amino acid has its own enzyme. Enzyme will bind its specific amino acid and use an ATP. To secure the bond will hydrolyse ATP, release 2 phosphates, now have a complex. Now we recruit tRNA with anitcodon complementary to codon that corresponds to the amino acid. Bond between amino acid and tRNA at the 3' end. Enzyme will release the complex. Now have aminoacyl tRNA.
- Ribosomes: 2 subunits – small (30s) and large (50s).
  - A (aminoacyl) site: aminoacyl tRNA arrives with proper amino acid.
  - P (peptidyl) site: peptide bond forms. Sever bond between amino acid and tRAN and forms peptide bond between amino acid and the aa chain.
  - E (exit) site : Exit of growing polypeptide chain out of ribosome. Empty tRNA leaves the ribosome and picks up another of the same amino acid.
  - Shifting from A->P->E until reach the stop codon. Trigger for tRNA to move into a new site is energy – called translocation. Subunits come apart, shift over and come back together.
- Amino acid chain: peptide bond formed between amino acids. Carboxyl group of one group binds to amine group.
- Intiation: Methianine is the first AA and the only one that goes directly to the P site. Methionine enters the small subunit with GTP. The mRNA strand 5' cap base pairs with the anticodon of Met-tRNA. The larger ribsosmal subunit binds and GTP is hydrolyzed, completing initiation. Elongation initiation factor causes the formation of a peptide and causes methionine to move over.
- Elongation: An aminoacyl-tRNA binds to the codon in the A site (GTP hydrolyzed; facilitated by elongation factor EF). Peptidyl trasnferase, an enzyme in the large ribosomal subunit, cleavses the amino acid from the tRNA in the P site and forms a peptide bond between it and the amino acid on the tRNA in the Asite (catalyzed by peptidyl transferase). When the reaction is complete, the polypeptide chain is attached to the A site tRNA and an empty tRNA (no AA) is in the P site. The ribosome translocates along the mRNA to the next codon, using energy from GTP hydrolysis and an EF. During translocation the 3 tRNAs remain bond to their respective codons so thies step positions the peptidyl tRNA (tRNA with growing polypeptide) in the P site and generates a new vacant A site.The empty tRNA is now in the E site. When translocation is complete, the empty tRNA in the E site is released. With the A site vacant and the peptidyl tRNA in the P site, the ribosome repeats the elongation cycle.
- Termination: Stop codons reached. No tRNA with that anticodon. Release factor (RF) protein occupies the A site, promoting the last peptide bond formation and translocation. Peptide released after translocation. RF promotes separation of the ribosomal subunits which can reassemble again with another mRNA.
- Polysomes (polyribosomes): an mRNA can be translated by more than one ribosome at a time, giving rise to multiple polypeptides. Ribosomes in cytosol, in eukaryotes and prokaryotes.
- Soluble proteins are made by free cytosolic ribosomes.
- Transmembrane proteins- cotranslational translocation:
  - Signal peptides in cytosol bind to specific sequences of amino acids as they come out of the ribosome. This binding pauses translation and brings it to the ER where it attaches to a specific receptor next to a translocating protein complex (contains an enzyme). The signal peptide will be handed off to the receptor and now translation is resumed. Since it is anchored within a protein that acts like a pore, the peptide grows into the ER lumen. Just before termination, signal peptidase cleaves signal peptide and it is no longer anchored so that the finished polypeptide is in the lumen.
  - In addition to the signal sequence to direct translation to ER, there is a stop-sequence that halts co-translational import. Translation is completed, but not import resulting in a transmembrane protein. Membrane forms into vessicle and goes to Golgi.

- Chaperones: protect and sometimes drag a peptide along, and facilitate folding of nascent (newly folded proteins). Larger structures will fully encompass a polypeptide and trim off some amino acids and structures to ensure that it is properly folded and fully functional.
  - Small chaperones: interact with portion of polypeptide (translocation).
  - Large chaperones: serve to engulf and isolate the entire polypeptide. Can be matured and folded within the chaperone and then released.
- Vesicles and Cytoskeleton: vesicles travel using motor proteins and cytoskeleton. Vessicles moving from ER to Golgi and Golgi to plasma membrane use microtubules and kinesin to move. Near the plasma membrane, must use microfilaments and myosin.
- Golgi: modifications that occur are glycosylation (add sugar to amino acid side chain), acetylation (add acetyl group to N-term to increase stability), disulfide bonding (link S between residues), lipidation (adds lipids), ubiquitination (adds ubiquitin for degradation). Sorting occurs in the trans face.
- Constitutive secretory pathway: no trigger or signalling required and happens nonstop in the cell. As soon as protein is mature it will go to plasma membrane and fuse.
- Regulated secretory pathway: compounds that lie in reserve need a signal to be released (ex: insulin, acetylcholine). Vessicles can remian in space between Golgi and membrane or very close to the membrane (docking) waiting for a signal from neighboring cell. This will trigger the vessible to release its contents by exocytosis. Need calcium to go to membrane, therefore cascade leads to increase in intracellular calcium.
- Point Mutations – Single Nucleotides:
  - Missense mutation: change of one nucleotide will lead to a change in the amino acid that is translated. Will impact the protein structure if polarity or relative size of amino acid is different than original. Could change structure from alpha helix to beta pleated sheet or influence folding.
  - Nonsense mutations: codon has become one of the three stop codons. Will have impact on function of the protein.
  - Frame shift mutation: insertion or deletion of nucleotides that shifts the reading frame and will change the entire sequence of amino acids. Protein may not be able to carry out its task.
  - Silent mutation: on the third nucleotide of a codon and thus will not affect the amino acid due to the wobble effect.

Cell Cycle

- G1: cell grows by increasing cytosol, cytoskeleton, membrane, mitochondria, and energy storage.
- G0: if the cell reaches S phase and is not large enough, it will enter G0 for extra time to grow. Some cells get stuck in G0. All cells can enter G0.
- 3 Checkpoints:
  - G1/S checkpoint: At the end of G1
  - G2/M checkpoint: At the very end of G2
  - M-phase checkpoint: In the transition from metaphase to anaphase during M phase.
- Checkpoints: signalled by GFs such as NGF, EGF or others. The signalling cascade they activate lead to activation of protein that allow regulation of cell cycle.
- Restriction point: influenced by GFs, nutrients, cell size and DNA damage.
- NGF: cascade leads to formation of cyclins (protein) and Cdk (enzymes). They interact and form a complex called the maturation promoting factor (MPF) which must be in sufficient concentration in order to pass each checkpoint.
  - Cdk are kinases that depend upon cyclins to be activated. The level of Cdks (and cyclins) rise and fall during the cycle which controls each step. They are sorted according to the checkpoint they govern:
    - G1/S cycline: bind to Cdk2 near end of G1 and lead cell into DNA replication.

        - S-cyclins: bind to Cdk2 during S phase and are required for DNA replication.
        - M-cyclins: bind to Cdk1 and promote the events of mitosis.
        - G1-cyclins: (in most cells) promotes passage through restriction point in late G1.
    - Cyclins are degraded after they pass checkpoint, but Cdk1 and Cdk2 are reused in the cell. This causes the rise and fall of concentrations.
    - Termination of cell cycle by the activation of separase which pulls the chromosomes apart.
- DNA damage triggers p53, the cell's watchdog. It interacts with other proteins inside the cell and allows the cell to be put into G0 and reenter G1. If DNA is too damaged, p53 will initiate apoptosis in the cell.
- Mitotic Spindle: Chromosomes separated by separase enzyme. Bring the chromosomes to separate poles by deploymerizing the distal ends of the microtubules, as dynein brings the chromosomes to the centrioles.

	Eukaryotes	**Prokaryotes**
Cell Size	(most animal and plant cells) 10-100µm	1-5µm. Shape can be spherical, rodlike or spiral.
Cellular Organization and Organelles	Compartmentalization: - Nuclear double membrane: containing nuclear pore complexes. - Nucleolus: inside nucleus where ribosomes are produced. - Endoplasmic Reticulum: made up of membrane folds (cisternae) which extend from the nucleus and span out towards the interior of the cell. - Rough ER: contains ribosomes and involved in protein sythesis, maturation and sorting. - Smooth ER: no ribosomes and involved in lipid synthesis and detoxification. - Golgi Apparatus: made up of folds (saccules) and involved in protein maturation and sorting. - Lysosomes: contains digestive enzymes which break down macromolecules. They bud off the Golgi. More acidic than the cytosol. - Mitochondria: Cristae increase surface area for optimal energy production. Have double membrane, DNA and ribosomes. - Cytoskeleton: structure, support and signalling <u>Plants</u> - Central Vacuole: tonoplast (membrane) contains water and stores salts, organic acids, sugars, storage proteins, pigments and waste products. Vacuole maintains turgor pressure and contains digestive enzymes (acts as lysosome). - Cell Wall: made of cellulose and used for rigidity. Contains plasmodesmata for direct communication between cells by allowing the movement of small molecules and ions. - Plastids: have their own genomes and double membranes.	No compartmentalization. - Nucleoid: area where DNA is stored - Cell wall: Provides rigidity and protection. - Capsule: Can be coated with glycocalyx as slime layer (cell wall) to protect from damage and may enable cell to attach to a surface. - Plli Theory of Endosymbiosis - Morphology: shape of mitochondria and chloroplasts and size are similar to bacteria and archea. - Reproduction: only by binary fission. - Genome: circular mDNA and cpDNA - Transcription and Translation: machinery in place (ribosomes). - Electron Transport: double membrane with ETC. - Sequence: bacterial branch on tree of life (mitochondria = proteobacteria; chloroplasts = cyanobacteria)

	- Chloroplasts: perform photosynthesis to create energy. Stacked thylakoids form granum, contained within the stroma (fluid). - Amyloplasts: store starch (in roots) - Chromoplasts: contain red and yellow pigment and are responsible for colours of leaves in automn and ripening fruit. - Kleptoplasty: stealing of plastids shown by spotted salamander and green algae. Sea slugs steal chloroplasts.	
Organelle Similarities	- Ribosomes - Plasma Membrane - Cytosol - Cytoskeleton? - Flagellum?	
Genome	Multiple strands of linear DNA. Larger genomes 10^6 to 10^9 bp. Have associated proteins (histones...).	Single strand of circular DNA and plasmids. Smaller genomes 10^6 bp.
DNA Replication	- Linear genome can have multiple simultaneous replication forks. Pieces will meet up and form a complete strand. - Unwind DNA, and make sure no supercoiling occurs. - 2 separate antiparallel daughter strands - Obtain 2 identical sets of DNA each with mother-daughter strand in a double helix.	- Cicular genome is replicated from a single point of origin. Forks move along the circle until they meet.
DNA Transcription	-Transcription occurs inside the nucleus for protection. -Containt a pre-mRNA stage which must be matured before leaving the nucleus. -Nuclear pores let mRNA through the nucleus into the cytosol and assures that mRNA has properly matured. -START: binding of promoter of transcription factors, activators and repressors. Formation of transcription initiation complex (TFs and protein). Then RNA polymerase II recruited to initiate transcription. -Transcription: produces a pre-mRNA molecule with a 5' cap and 3' poly A tail. -TERMINATION: Specific sequences that differ depending on which RNA polymerase. Ex: for mRNA, sequence of AAUAAA to which proteins bind, this triggers end of transcription (related to the poly A tail).	-Transcription occurs in the cytosol. -DNA transcribed directly into mRNA (no pre-mRNA). -START: Binding to promoter of polymerase subunits, particularly the sigma subunit, which serves as a guide to place RNA polymerase. -RNA polymerase initiates transcription. -TERMINATION: Rho dependent:ATP-dependent unwinding enzyme at 3'end. Rho independent (intrinsic): GC rich sequences at end form hairpin loop which pulls RNA away from DNA.
Genes	-Enhancer: contains regulatory sequences that control the rate of transcription of the gene. Involved in the folding of the complex.	

	-Promoter proximal region: contains regulatory sequences called promoter proximal elements. -Promoter: TATA box is where RNA polymerase comes in to transcribe. -Transcription unit: coding (exon) and noncoding (intron) regions. Get rid of introns.	
Regulation of Gene Expression	-Receptor initiator complexes. -Cells have access to a cerntain number of activators and repressors. Different combinations will initiate or interrupt gene transcription at different time and decide which exons will be ignored or included in the sequence. Activators and repressors control WHICH genes are expressed and the RATE at which transcription occurs. -Posttranscriptional regulation: some junk DNA has the purpose to control how much mRNA is translated. -Short interfering RNA (siRNA): will direct mRNA to degredation if there are too many. -Micro RNAs (miRNA): important for regulation of gene expression (ex: time-sensitive expression). They form miRNA indiced silencing complex (miRISC) which halts translation and/or reduces available mRNA. -Complementary sequence folds over with a hairpin loop so now the short double stranded RNA can get out of the nucleus. It then meets a dicer which cuts off the loop. It now looks like a short sequence of DNA which is recognized by a silencing protein complex. This silences translation of RNA and gets rid of one strand (goes to degradation). Now have a single stranded RNA attached to a protein complex. The RNA can easily be complimentary to other sequences of RNA. It will fish for mRNA and make a complementary match (imperfect or perfect). -Imperfect match: the strands will hang onto each other for an amount of time and then dissociate so that translation can occur. -Perfect match: the mRNA will be degraded without being translated.	-Achieved through operons, genetic switches that control transcription. Operon is the ability to control the promoter, transcription of genes and control the expression of more than one gene at the same time. Group genes together that accomplish very different tasks but are all essential for one process. -Lac Operon in metabolism of lactose. 3 proteins: beta-galactosidase, permease and transacetylase. -For 3 genes, one promoter and operator (on off switch). Regulatory gene determines the status of the operon (controls whether the switch will be on or off). -Repressor protein is what interacts with the operator. In absence of lactose it is bound to operator – switched off. RNA polymerase has no room to get to promoter and initiate transcription. -Lactose is a sugar (membrane not permeable) must be brought in by facilitated diffusion. -Get rid of repressor when lactose is present. Lactose will interact with beta-galactosidase to become allolactose which increases affinity between repressor and allolactose. Repressor will let go of operator and bind to allolactose. RNA polymerase will bind to promoter and will transcribe all 3 genes.
Translation	-Compartmentalization means that translation and transcription are two separate processes. DNA is more protected.	-In the absence of a nuclear membrane, and since the mRNA is mature, transcription and translation can occur simultaneously. DNA is more vulnerable.

Macromolecules

Macromolecule	Function	Component(s)	Other

Sugars/Carbohydrates	cellular recognition and communication, fuel source (glucose), fuel storage (glycogen, starch) and structural molecules (cellulose, chitin).	alpha and beta glucose: - When alphas bond they form starch. - When betas join they form cellulose.	Cellulose cannot be digested because enzymes cannot break down beta linkage.
Lipids	Storing energy (triglycerides), signalling (glycolipids) and acting as structural components (phosphoglycerolipids, steroids).	fatty acids: long carbon chains with a carboxyl group on the end. Amphipathic: has polar and non-polar region. Can be saturated or unsaturated.	
Nucleic Acids	Encoding, transmitting and expressing genetic information.	nucleotides: nitrogenous base, sugar and phosphate group.	
Proteins	Transport, enzymatic activity, signal transduction, and attachment/recognition	amino acids: 20 total, some polar, non-polar or charged.	