What is the structure of a microfilament?

· G-actin (g = globular) subunits assume a "U" shape when synthesized. · These are lined up through non-covalent interactions into a strand of actin monomers. o In this form, the strand is called an F-actin filament (f = filamentous) Two intertwined f-actin filaments form a microfilament

What gives microfilaments unique directionality?

· The "U" shape the G-actin assumes when synthesized creates a unique direction of polymerization. · The actin subunits bind nucleotides (ATP) in the apex of the U shape creating poles on the structure; a plus and minus end.

Describe the dynamic character of microfilaments

· Similar to microtubules. · G-actin binds with ATP and is added to the plus end of the structure. · This molecule now tends to hydrolyze ATP after time. Actin bound to ADP is most likely to dissociate at the minus end

Is there dynamic instability in microfilaments?

· No there isn't. There is no catastrophic shrinkage at the plus end of the structure. · They are more stable.

Are microfilaments organized? If so, how?

· Yes they are, by ABPs (actin binding proteins). · ABPs regulate the rate of polymerization of microfilaments in a cell and are produced based on the cell's needs (controlled by signal transduction pathways). There are many types: monomer sequestering (prevents polymerization), nucleating ABPs (increase speed of growth by orienting monomers), monomer depolymerizing ABPs, etc.

What are useful drugs to experiment with microfilaments?

· Cytochalasin – prevents actin monomers from polymerizing. o Same as colchicine in microtubules. o As a result, they increase the likelihood the filaments collapse. · Phalloidin – stabilizes microfilaments. Similar to taxol for microtubules.

What are the four primary functions of microfilaments?

· Shape/support; the cell cortex. · Absorption: microvilli; increases cell surface area. · Adhesion: Cell-to-cell and cell-to-substrate connections. Movement: muscle contraction & cell crawling

Describe the cell cortex.

· A network of microfilaments on the interior of the cell membrane. o This provides support and rigidity. · This also determines the shape of animal cells (no cell wall).

Explain what the function of microvilli is and how they work.

· Microvilli are small finger-like projections from the cell membrane. · They greatly increase the cell's surface area and thus absorption area. o Similar to cilia made from microtubules. · Minus end of microfilaments at base of structure, but no organizing centre (ex. basal body) at base. o They are anchored in a network of proteins. · The microfilaments are bound together via binding proteins (ex. villin & fimbrin). Especially found in the cells lining the gut.Motor proteins can haul nutrients from the absorption surface, down the microvillus, into the cell.

What are the two types of junctions that microfilaments can form?

· Adherens junctions; cell-to-cell connection. Focal adhesions; cell-to-substrate connection.

What 3 elements is the cytoskeleton made up of? Which is the largest/smallest?

Microtubules, intermediate filaments, microfilaments (largest to smallest).

What are the primary purposes of the cytoskeleton?

· 3 main purposes… o Structural Support: Particularly important in animal cells which lack a cell wall. § In plants, the cytoskeleton determines where the cell wall will form. o Organization: Positions organelles. Movement: vesicle transport within the cell, and cilia/flagella outside the cell.

What is the approximate diameter and shape of a microtubule?

About 25 nm long, a hollow tube structure

Describe the types of microtubules?

· Cytoplasmic: very dynamic, constantly growing and shrinking. Axonemal: Very stable and rigid; make up cilia/flagella.

What is the structure of a microtubule?

· 13 protofilaments align linearly to make a cylindrical microtubule. · Each protofilament is made up of tubulin heterodimers containing an alpha and beta tubulin subunit. · These subunits are the monomers of a microtubule. The repetitive arrangement of heterodimers gives the microtubule unique directionality.

What is unique directionality?

· A microtubule, more specifically a protofilament is formed in a unique direction. · The specific alternation between alpha and beta tubulin monomers gives a microtubule two poles; a plus and minus end. This influences at which end microtubules grow.

What is tubulin?

· A family of small, globular proteins. · Alpha & Beta are its most common members. · They are the monomers of a microtubule.

How does a microtubule grow and disassemble?

· GTP binds to a floating alpha-beta tubulin heterodimer. · This aligns the dimer and it is added to the plus end of the microtubule. · Once bound, the beta tubulin will eventually hydrolyze the GTP molecule into GDP. This heterodimer will dissociate from the microtubule and can be recycled*.

What is the dynamic instability model?

· Since microtubules are constantly polymerizing at the plus end and dissociating at the minus end, there is dynamic instability in their structure. · When the rate of polymerization and disassembly/hydrolysis are equal, this is known as tread milling. · However, when the rate of disassembly/hydrolysis exceeds the rate of polymerization, this is referred to as catastrophic shrinkage. · The dynamic instability model is when catastrophic shrinkage occurs. **Microtubules that experience catastrophic shrinkage are said to be inviable

What are MAPs and what is their significance?

· MAP stands for microtubule assembling proteins. · These proteins bind to the microtubules (varies which end based on which MAP) and can anchor them in place. · They can regulate the rate of polymerization and hydrolysis in the microtubule.

What are good experimental drugs to study microtubules?

· Colchicine: inhibits polymerization, halts growth. · Taxol: Stabilizes the structure so there is no polymerization or hydrolysis.

How are microtubules organized?

They are organized by structures called MTOCs

What is a MTOC?

· Stands for microtubule organizing centre. · These are structures in a cell that organize the formation of microtubules at a controlled speed and in a controlled direction. · The two most important types of MTOC in animal cells are the basal body which control cilia and the centrosome that controls cell division in mitosis. · These centers have y-tubulin (gamma) that binds to the minus end of the protofilaments and orients the polymerization of the microtubule.

What is the structure and significance of centrosomes?

· Centrosomes contain two centrioles at 90° to each other (perpendicular). · Each centriole, containing high concentrations of y-tubulin (gamma) is surrounded by pericentriolar material. · The pericentriolar material attracts tubulin subunits increasing the likelihood of polymerization. Spindle fibers are the microtubules grown out of centrioles in animal cells! These are what the cell uses to split into two

Give 3 examples of microtubule function

· Transport of molecules within the cell; vesicle transport. · Cell movement; cilia & flagella. · Axonal transport; transporting vesicles along microtubules that surround neural axons via dynein and kinesin motor proteins to increase speed. Chromatophores; transport of pigment granules within cells to change an organisms color.

How does vesicle movement along microtubules in animal cells happen?

· Microtubules provide a highway that families of motor proteins are capable of walking on. · Kinesin & Dynein are the two families that contribute to vesicle movement. · Both kinesin and dynein have a motor domain which resembles two feet capable of walking along the microtubule. · They also have a tail domain (kinesin's is longer) that can bind to "cargo" and haul/carry it along these microtubule highways. · Kinesin is plus-end oriented whereas dynein is minus-end oriented. o Kinesin is involved in carrying towards the peripheries of a cell or structure (+ end). o Dynein is involved in carrying towards the center or nucleus of a cell/structure (- end).

Compare and contrast the structure of cilia and flagella (Similarities).

· Cilia and flagella both have an identical core made up of 20 microtubules bundled together. · This core has a 9+2 arrangement in which 9 dublets of microtubules surround a central pair. o Within each pair there is an Alpha and a Beta tubule (alpha = 13 protofilaments complete, beta = 10-11) · Protein spokes hold the dublets in their 9+2 arrangement. · Nexin proteins hold the dublets together, in pairs. · At the base, a basal body binds to the minus end of the microtubules orienting them in the outward direction. · Dynein motor proteins attach their tail domain to an Alpha tubule, and their "feet" domain to the adjacent dublets Beta tubule. By hydrolyzing ATP and changing conformation, it forces the structure to bend and wiggle.

What are the differences between cilia and flagella?

· Cilia are short (Cilia exist in large numbers; usually only 1 or 2 flagella. · Cilia row like boat oars, flagella whip like fly fishers. · Cilia and flagella can move cells, but cilia can also be used to move things across cells (in the lungs).

What are the four functions of membrane proteins?

· Transport: sodium/potassium pump. · Enzymatic activity: electron transport chain (ATPase) · Signal transduction: these proteins bind to hormones and change conformation transducing signals into the cell. · Attachment/recognition: linking proteins (attachment to cytoskeleton) and cadherin proteins (cell-to-cell recognition).

What are the three types of membrane proteins we saw?

· Integral membrane proteins (aka transmembrane) · Peripheral · Lipid-anchored proteins

What is an integral membrane protein?

· A protein that either passes through the entire lipid bilayer or at least interacts with the hydrophobic core. · The region that interacts with the hydrophobic core of the bilayer is also hydrophobic/non polar, as a result of the non-polar 'R' groups of these amino acids. o These transmembrane domains are typically ~20 amino acids long. · They can pass through the membrane one or more times (single or multipass). · Difficult to remove from the membrane; require detergents that can also denature the proteins.

What is a peripheral membrane protein?

· A protein that does not have a hydrophobic domain/region and thus remains on the surface of the membrane (cytosolic or external side). · These can also bind to integral membrane proteins. · These bond to the membrane or integral proteins via non-covalent interactions (ionic and hydrogen bonds). · These are easier to remove than transmembrane/integral proteins; a change in pH or ionic strength.

What is a lipid-anchored membrane protein?

· COVALENTLY BONDED to the phospholipids in the bilayer. · Can either be fatty-acid anchored (inside OR outside the cell) or GPI anchored (ONLY outside the cell). · GPI = glycosylphosphatidylinositol which is a phospholipid with an inositol as the R group and a branching carbohydrate chain. · These proteins can be cleaved using a phospholipase C enzyme. It breaks the ester bond between the glycerol and phosphate in the phospholipid.

What are the two types of transport across the membrane?

· Passive and active o Passive: with a gradient o Active: against a gradient

What is passive transport?

· Does NOT use ATP (chemical energy) Can be either… · Simple diffusion: small and uncharged molecules can pass through the membrane (hydrophobic, non-polar core). o The most easily diffused molecules are O2 and CO2. · Facilitated diffusion: With a gradient but requires a 'facilitator.' o These facilitators are usually integral membrane proteins. o Because these molecules require proteins to be transported across the membrane, the diffusion can reach a saturation point (it displays saturation kinetics). *Lots of life's processes require polar, charged molecules (ex. Na+ and K+)

What are the types of proteins involved in facilitated diffusion?

· There are channel proteins and carrier proteins. o Both are integral membrane proteins. · Channels: provide hydrophilic passages. Allow polar and charged molecules to pass (water, K+, Na+, Ca2+). o They can have open, closed and intermediate stages. · Carriers: bind to a specific solute. o Uniporter – carries one solute across. o Symporter – carries two solutes in the same direction. o Antiporter – carries two solutes in opposite directions across the membrane. **Can be reversed

What is active transport?

· The movement of molecules against a gradient, and thus requiring ATP (chemical energy). o This movement always involves a carrier protein (like facilitated diffusion). o Therefore this also displays saturation kinetics. · There are two types: primary and secondary active transport.

What is primary active transport?

· This transport directly uses the energy from the hydrolysis of ATP. · When the carrier protein hydrolyzes the ATP into ADP and Pi it changes conformation and consequentially transports its molecule across the membrane. · Phosphorylation-type pumps (P-type): pump cations and are reversibly phosphorylated (SEE BELOW). · Vacuolar-type pumps (V-type): pump protons (H+) into organelles.

What is secondary active transport?

· Uses the gradient created by a primary active transport pump, so the energy from ATP hydrolysis is indirectly used. · These carrier proteins transport two solutes across the membrane at the same time in cotransport. **Can't be reversed

Describe cotransport.

There are two types… · Symport: the two solutes bind to the carrier protein on the same side of the membrane. · Antiport: the two solutes bind on opposite sides of the membrane. o This is also known as exchange diffusion. **Reversibly phosphorylated means an inorganic phosphate (Pi) can be put onto the pump making it change conformation and essentially run in reverse. On the other hand, if ions are allowed to diffuse through a P-type pump, ADP can be phosphorylated into ATP.

Explain the cell theory (and who made these principles)

· All organisms have 1 or more cells. (Schwann & Schleiden) · The cell is the basic unit of structure. (Schwann & Schleiden) · All cells come from pre-existing cells. (Rudolf Virchow)

Which of the following is a possible size for a eukaryotic cell? Prokaryotic cell? Eukaryotic ribosome? The endoplasmic reticulum (other organelles)

· Eukaryotic cell = 30-100 µm · Prokaryotic cell = 1-10 µm · Organelles = 1-10 µm · Ribosomes = 50 nm = 0.05 µm

How did eukaryotic cells overcome the surface area to volume ratio issue?

· Compartmentalization: endomembrane system; increases surface area. Solves the absorption issue. · Organelles: also compartmentalizes the cell, solves the 'adequate concentrations' issue. o A cell doesn't need to synthesize as much substrate. · Cytoskeleton: can transport nutrients around the cell without relying on diffusion.

Name and describe the use of a model organism.

· Mus musculus: 'Small' 'mouse' = house mouse. o Fast reproduction, easy to work with. o Similarities with humans. · Danio (rerio): zebra fish à Studied by Dr. Gilmour o Fast reproduction, super fast maturation (48 hours) · Drosophila melanogaster: house fly. o Practical to study; easy to care for and breed. · Arabidopsis Thaliana: a weed in the mustard family o Matures quickly, grown easily in lab. o Good for studying heredity; simple genome (like Mendel's pea plants). · C. Elegans: nematode round-worm. o Fast maturation and reproduction. o Fully sequenced genome and has exactly 959 cells (all of which are described). · Saccharomyces Cerevisiae: 'sugar' 'mushroom', 'of beer' o Grows quickly and easily in lab. o First fully mapped genome for eukaryotes. o Includes many types of yeast; also found in beer.

Describe endosymbiosis and its proofs.

· An aerobic bacteria was engulfed by a prokaryotic cell and held hostage. o They developed a symbiotic relationship. o Plants: cyanobacteria was engulfed (photosynthetic). o Animals: aerobic bacteria. · Proofs: aka similarities to prokaryotes o Circular DNA o Reproduce with binary fission o Have their own ribosomes o Same size as prokaryotes o Double membrane Parsimony: fossil records show that cells with endosymbionts were selected for and gave rose to eukaryotes.

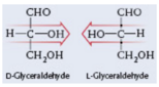
What is kleptoplasty?

· A modern day example of the endosymbiont theory. · Solar-powered sea slugs (Elysia Chlorotica) can extract the chloroplasts from the algae it eats and incorporate them into its own cells making it photosynthetic.

Distinguish between alpha and beta glucose

· Beta glucose has the hydroxyl group pointing upwards and polymerizes into cellulose. Alpha glucose has the hydroxyl group pointing downwards and polymerizes into glycogen

Distinguish between D (dexter) and L-glyceraldehyde



Hydroxyl group is either left (L) or right (D for dexter)

What is a glycosidic bond?

· A linkage between two monosaccharides usually formed between carbon's 1 and 4 involving a condensation reaction. o An oxygen molecule links the two sugars.

What are the components of a nucleotide?

· A sugar (ribose or deoxyribose), a nitrogenous base (adenine, guanine, cytosine, thymine, or uracil), and a phosphate group (up to three). · 1' carbon bonded to nitrogenous base. · 5' carbon bonded to phosphate. **A nucleoside contains only the sugar and nitrogenous base.

Distinguish between RNA and DNA (4 things)

· RNA is single stranded, DNA is double stranded. · RNA uses the pyrimidine uracil, DNA uses the pyrimidine thymine. · DNA uses deoxyribose sugar, RNA uses ribose sugar. · Function: DNA contains the genetic information, RNA transcribes and translates the genetic information.

What is a phosphodiester bond?

· Produced through a condensation reaction. The phosphate group of a nucleotide bonds to the hydroxyl of the 3' carbon of the sugar of another nucleotide

Describe the structure of DNA

· Backbone: phosphate groups and deoxyribose sugar. · Nitrogenous bases form the "rungs" of the alpha helix ladder. o They are connected with hydrogen bonds (non-covalent).

Who came up with the structure of DNA?

· Watson & Crick in 1953

What direction is DNA synthesized & read in?

· 5’ to 3’

What is an amino acid?

· N-terminus on the left, C-terminus (COOH) on the right. · An R group branching off the central carbon. There are 20 amino acids; their R groups are all different

What is a protein?

One or more peptide chains

Distinguish between a motif and a domain

· A motif describes a pattern of secondary structure (ex. alpha helix or beta-pleated sheet). · A domain is an area on a polypeptide that folds independently of other areas at the tertiary level. · Domains are like rooms in a house, motifs are like elements in a room like a light switch or a chair. · Ex. zinc finger, hairpin loop, helix-turn-helix are motifs.

Describe the different levels of structure in a protein.

· Primary – the linear sequence of amino acids. Listed from the N-terminus to the C-terminus (left to right). · Secondary – Hydrogen bonding in the backbone. o Alpha helices or beta pleated sheets. o This is where motifs appear in protein structure. · Tertiary – Disulphide bridges and non-covalent interactions appear (ionic, hydrogen bonds, and hydrophobic interactions). o Domains appear in this protein structure. · Quaternary – bonding between many polypeptide chains using the same interactions as the tertiary structure (disulphide bridges and non-covalent interactions)

Compare the three classes of lipids we studied.

· Triglycerides (Dr. Gilmour refers to these as fatty acids): a glycerol molecule and three fatty acid chains (16-18 C's). o Produced through a condensation reaction. o Used in energy storage. · Phospholipids: similar to triglycerides but have a phosphate group and a polar R group. o This amphipathic property explains why they make up the cell membrane. · Steroids: made up of four carbon rings derived from the isoprene molecule. o Contribute to the plasmamembrane structure as well. o Have a role in signaling and chemical messaging.

Describe the three types of sterols

· Cholesterol: animals
· Phytosterols: plants · Ergosterols: fungi
**Prokaryotes do not have sterols!

What happened in 1972?

· Singer and Nicolson invented the fluid mosaic model

What are some of the functions of the cell membrane?

· Defines the boundaries of a cell. · Organizes a cell. · Regulates solute transport into and out of the cell. · Responds to external signals. · Cell-to-cell communication.

How thick is an average cell membrane?

- ~10nm = 0.01 μm

How was membrane fluidity discovered?

· Components of the membrane of a mouse and human cell were marked fluorescently. These two cells were fused with a virus and the markers mixed together since they are free to move around in the membrane.

What is FRAP?

· FRAP is an experimental way of quantifying the fluidity of a membrane. · It stands for: o Fluorescence Recovery After Photobleaching · Process: The transmembrane proteins are fluorescently tagged and an area on the cell membrane is bleached using a laser. The time it takes for the proteins to recover and fill in the bleached area can be graphed with the intensity of the laser

Who are Gorter & Grendel?

· They discovered that the cell membrane is a lipid bilayer. · They measured the area of a cell and broke it open to measure the quantity of lipids… they found twice as much as they expected. · This happened in 1925.

How does hydrocarbon chain length affect fluidity?

· Shorter chains = higher fluidity because they can be more densely packed.

How does temperature affect fluidity?

· Colder = less fluid because the phospholipids are tightly bonded and they have lower kinetic energy.

How does saturation affect fluidity?

· Saturated fatty acids = lower fluidity because there are no double bonds/kinks in the chains; they can pack together more densely.

How does the presence of sterols affect fluidity?

· Sterols buffer membrane fluidity. · At high temperatures, their rigid structure lowers it. · At low temperatures, their rigid structure forces the phospholipids apart increasing fluidity.

How does the polarity of the head affect fluidity?

· More polar head groups = more fluid because they repel each other and create space. **Phosphatidylethanolamine has a more polar head group than phosphatidylcholine

How does an organism alter their membrane fluidity based on its surrounding conditions?

· They adjust by countering the effect of the environment. · Some organisms maintain a constant body temperature which maintains a constant membrane fluidity. · Other organisms counter the effect of the environment by altering the composition of the cell membranes. **A protist is expelled into a colder environment, what would it do to adjust? Answer: increase membrane fluidity

What is homeoviscous adaptation?

· The altering of the membrane (lipid composition) in order to maintain constant membrane fluidity. · **A protist is expelled into a colder environment, what would it do to adjust? Answer: increase membrane fluidity

What is special about the semi-palmated sandpiper?

· Main point: diet can affect membrane fluidity. · These birds fly 4500km from the Bay of Fundy to South America (Brazil) in 3 days of non-stop flying over the Atlantic ocean. · In the Bay of Fundy they rest for two weeks and can double their body weight by eating mudshrimps. · These are high in PUFA's (polyunsaturated fatty acids) which increase membrane fluidity. o Ex. PUFA's = omega 3 · The incorporation of PUFA's into muscle cell membranes improves flight muscle endurance. · This was tested by feeding a PUFA rich diet to couch-potato quails and it worked.

Compare and contrast adherens junctions and focal adhesions.

· Adherens junctions bind two cells together. Focal adhesions bind a cell to a substrate. · Adherens junctions involve transmembrane cadherin proteins that interact and bond like Velcro straps. · These are connected via linking proteins to actin microfilaments within each cell. Focal adhesions use the same mechanism but different proteins (which we don't need to know).

Describe the process of cell crawling.

· Actin microfilaments are polymerized in a desired direction of movement by the cell. · They push the cell forward and undergo focal adhesions (to the newly covered substrate). The remainder of the cell is hauled into the new space by myosin I motor proteins.

How do microfilaments participate in vesicle movement?

· Microfilaments can provide a "road" for myosin I motor proteins to haul vesicles along. · These are typically near the peripheries of the cell and act as "back roads" to vesicle transport whereas microtubules provide the "main highways."

What are myosin motor proteins?

· Globular head/motor domain : bind to actin microfilaments, hydrolyzes ATP into ADP and undergoes a conformation change to “walk” along the filament. · Tail region : anchors cargo (sometimes the myosin itself) and drags this along with it when undergoing conformation changes and “walking.” · Move towards the plus end of the microfilamentsMyosin I is used in various functions within the cell (some vesicle transport, cell crawling, etc.)Myosin II is "muscle myosin" and plays a role in muscle contraction within a sarcomere.

Describe the structure of a muscle cell and relate it to its function (Long answer).

· They are made up of many myofibrils bundled together forming a thick strand. · Each myofibril contains a long row of sarcomeres. · A sarcomere is bounded by two "Z lines," which contains a capping protein called Cap Z. o This is important because this binds to the + ends of microfilaments in the sarcomere and stabilizes them/fixes them in place. o These filaments are called thin filaments and are covered by troponin and tropomyosin requiring calcium ions to change their conformation, allowing myosin II to bind to them. · Thick filaments are composed of a bundle of myosin motor protein tail domains. o The head domains that stick out of the thick filaments can reach over and bind to the thin filaments (assuming Ca2+ is provided). o Through a process known as the cross-bridge cycle the myosin II motor proteins can pull the thin filaments inwards, shortening the sarcomere and creating mechanical potential energy.

Describe, in full detail, the cross-bridge cycle (long answer).

· The many head domains of the myosin II motor protein from the thick filaments are known as cross-bridges. · Process: walking in the plus end direction towards the Z lines where Cap Z binds and stabilizes the thin actin filaments*** · The head domains bind to the thin actin microfilaments; aka the cross-bridge state. · ATP has been hydrolyzed previously so the free, inorganic, phosphate (Pi) is released. · Releasing Pi allows the myosin head to change conformation and undergo the power stroke; the thin filaments are pulled inwards from the Z lines towards the center of the sarcomere, the M line (m = middle) · This shortens the sarcomere and thus the myofibril and thus the muscle cell. · With the conformation change, ADP is now released and new ATP can bind to the myosin head causing it to dissociate. · Once ATP is hydrolyzed into ADP + Pi, the myosin head returns to its resting conformation and can bond again to the thin actin microfilament starting a new cross bridge.

How does cytokinesis involve a similar process to the cross-bridge cycle?

· During cell division, actin microfilaments form around the equator of the cell and are bound to the membrane at various locations. Myosin I walks along the filaments creating a drawstring sort of action splitting the cell into two.

What are intermediate filaments?

· Intermediate filaments are a large family of fibrous proteins; they can be made of a variety of different proteins (ex. keratins, neurofilament proteins & nuclear lamins)

In what organisms and tissues are intermediate filaments (IFs) found?

· Found only in vertebrates and a small number of soft-bodied animals. · They are the most rigid cytoskeletal elements so are found in high concentrations in the epithelial cells of the skin. · Intermediate filaments made up of keratin can provide a cell-to-cell attachment called a desmosome. o These are made up of membrane-bound protein plaques that are held together by intermediate filaments. · IFs have high tensile strength so allow cells to be stretched and not rip.

What is the structure of an intermediate filament?

· Made up of 8 strands of protofilaments (a linear protein subunit chain) clumped together like a rope. · Each protofilament is made up of a chain of staggered tetramers. · Staggered tetramers are made up of pairs of coiled-coil dimers in anti-parallel orientation (C and N terminuses line up between dimers) · A coiled-coil dimer is made up of 2 monomer fibrous protein strands with a C and an N terminus which are paired when they coil each other in an alpha-helix strand. o The proteins that make these fibrous strands up can vary depending on the type of intermediate filament. *Can you imagine, in reverse now, from the monomer to a polymerized intermediate filament?

Do intermediate filaments have dynamic character like microtubules?

· No, because of the anti-parallel orientation of the coiled-coil dimers in each staggered tetramer. o They can be polymerized in any direction or at any point in the structure. The tetramers are simply bundled together like a rope. · Due to this lack in unique directionality, they also lack polarity and thus cannot exhibit dynamic characteristics. http://www.sumanasinc.com/webcontent/animations/content/intermediate_filaments.html

How do plant cells communicate with each other?

· Through plasmodesmata (sing. Plasmodesma)

What is the plant cell wall, what does it consist of and what purpose do these things serve?

· Structural fibers; provides strength & rigidity. · Matrix; Holds the structural fibers in place. · Adhesive molecules; connects cells and anchors the cell wall to the cell.

Describe the structure of cellulose microfibrils and how this relates to their function.

· Strings of cellulose molecules that act as a structural fiber in the plant cell wall. · The 1-4 glycosidic linkage between the Beta D-glucose forces the molecules to alternate/flip in orientation. o This makes these bonds stiffer and the molecule much denser than the alpha glycosidic bonds. This stiffness is required to provide structure to the cell wall.

What makes up the matrix in the plant cell wall?

· This is primarily made up of carbohydrates with some glycoprotein. · Main carbohydrates: Hemicellulose & Pectin (bulk of the matrix). · Main glycoprotein: Extensin (small component of the total).

What is the function of the matrix in the plant cell wall?

· Analogy = The matrix is like concrete, and the structural fibers are like the re-bars to reinforce the concrete.

What is hemicellulose?

· It is one of the polysaccharides found in the matrix of the plant cell wall. · It is an ensemble of carbohydrates with a simpler structure than cellulose. They have long side chains that link together to form a network.

What is the structure of a pectin and how does it relate to its function?

· Similar to hemicelluloses, pectins are an ensemble of heavily branched carbohydrates. · The backbones of pectins carry a lot of negative charges, which attracts H2O and keeps the matrix hydrated. · It is these molecules that makes the matrix gelatinous. **Pectin is used to make jam and contributes to the jelly character!

What is the primary adhesive molecule in the plant cell wall?

· Pectins: it holds the cell wall to the cell and can join to an adjacent cell.

How is cellulose synthesized, what does this synthesis allow for (long answer)?

· The monomers are synthesized within the cell and exported to the cell surface (otherwise it would be difficult to export such a large molecule from inside the cell). · There, the monomers are polymerized with the help of rosettes. · Rosettes are enzyme complexes that catalyze cellulose polymerization (see "What is a rosette" question). o These integral membrane proteins can be moved within the membrane, altering the pattern of formation of cellulose microfibrils in the plant cell wall (primary & secondary). o This increases the strength of the plant cell wall.

What is a rosette, why are they significant?

· An integral, transmembrane protein that has an extracellular and cytosolic domain. · The extracellular side has catalytic activity. · The cytosolic side links to microtubules by means of motor proteins. o These motor proteins can tow the rosettes through the membrane and thus control where cellulose is synthesized. · The primary and secondary plant cell wall can have different orientations of cellulose microfibrils giving much greater tensile strength.

How are the multiple layers of the plant cell wall created? What impact does this have on the structure itself (long answer)?

· The layers are synthesized on the outside of the cell from the outer most layer, the middle lamella, to the inner most layer. · The Middle Lamella is laid down; it is made of a layer of pectins. o This joins the primary (outer) cell walls of two cells together. o It is a gel-like layer that allows for a small amount of movement or adjustment but is similar to wet cement in that sense. · After the cell secretes the middle lamella, it secretes the primary cell wall. o The primary cell wall; has a loose network of cellulose microfibrils (no particular weaving-like orientation such as in the 2nd cell wall below). o Because of this, it can expand as it gets pushed further from the cell and the cell is not restrained to its original size after the primary cell wall is secreted à it can be expanded. · After the primary cell wall is laid down, layers of the secondary cell wall are laid down. o In each layer of this cell wall, the cellulose microfibrils have an opposite (or just different) orientation from the layer preceding it. o This gives it a structure like plywood making it much more rigid and not capable of expanding.

How does a plant cell have control over the structure and strength of its cell wall (long answer)?

· The rosette enzyme complexes, which catalyze the polymerization of cellulose and cellulose microfibrils, on the exterior of the cell are anchored to motor proteins. · They control the orientation of cellulose microfibrils in the layers of the cell wall (primary & secondary). · The pattern can be controlled by the cell's synthesis of microtubules and MTOC's within the cell. · Depending on the need of the cell, the layers of the cell wall can have higher or lower tensile strength; the microfibrils will be arranged in a stronger or weaker network. · This affects the direction and extent of growth for this cell.

With a thick, strong cell wall, how do plant cells communicate with each other? How does this method function?

· Plasmodesmata provide a cytosolic connection between plant cells. · Anything small enough to fit through the connection (desmotubules) can move between the cells: water, small ions, some hormones. o This is why the cells are said to be in electrochemical communication.

Describe the structure of a plasmodesmata.

· As the cell wall is forming, bits of smooth endoplasmic reticulum (smooth ER) stretch across an area of formation. o The cell wall forms around these extensions of the smooth ER. · Desmotubules; the extensions of the smooth ER while the cell wall is forming. · Plasmodesma; a gap in the cell wall that has a desmotubule through it.

What are the three examples of cell-to-cell connections animal cells are capable of?

· Adhesive/anchoring junctions; hold cells together with great tensile strength. · Tight junctions; seal the space between cells. · Gap junctions; a communicative junction.

Compare and contrast desmosomes and adherens junctions.

· Both contain cadherin, transmembrane glycoproteins, made of several subunits. · They connect to recognized cadherin proteins from adjacent cells like Velcro with non-covalent interactions. o Their cytosolic domain bonds via linking proteins to the cytoskeleton (microfilaments or intermediate filaments). · Desmosomes connect to intermediate filaments. o These are very strong connections and form tissues with integrity (hold their shape/form). o The linking proteins form a very dense plaque. **Found in tissues like the skin. · Adherens Junctions: connect to microfilaments. o These can integrate the cell cortex into the connection.

What is the structure of a tight junction and how does this relate to its function (long answer)?

· These junctions involve integral membrane proteins. · Claudins & Occludins: families of integral transmembrane proteins used in tight junctions. o Inserted in the membrane laterally so they run along the membrane around the cell similar to a belt around a sphere (the equator on the globe). o They bind with corresponding proteins on the membrane of an adjacent cell laterally. o The more proteins that are in the membrane binding to the adjacent cell, the tighter the junction is. The length and amount of rows of the claudins and occludins will determine how strong or leaky they are. · These junctions seal the PARACELLULAR SPACE between the cells forcing substances to move through the TRANSCELLULAR SPACE which the cell's have a large amount of control over. **Tight junction proteins also prevent the proteins in the plasma membrane of cells from lateral diffusion. This creates varying concentrations of proteins in the membrane around the cell and allows polarization

How does the structure of a gap junction relate to its function?

· A ring made of 6 connexin proteins is called a connexon. o These transmembrane protein structures provide a pore through the cell membrane. · Lining up the connexons of adjacent cells through non-covalent interactions creates a hydrophilic channel; water & other small molecules/ions can pass through. · These channels are important because they provide rapid communication between cells. · This allows a single cell to send a message to surrounding cells very quickly in the form of a wave starting from the central cell and spreading to all adjacent cells.

What are gap junctions?

· A communicating junction, a cytosolic connection found in animal cells found in animal cells. · **Equivalent of plasmodesmata in plant cells = Electrochemical communication between each cell (ions; ex. water & small molecules) o The gaps are even smaller than plasmodesmata so hormones are for the most part too large to fit through.

Give an example of where gap junctions are necessary in the human body.

· In the heart, cardiac muscle cells require rapid communication between each other. · The "pace-maker" cells send a signal via gap junctions that spreads across the atria and then the ventricles. · The spreading of this signal causes the atria to contract first, then the ventricles. · Also, it allows the ventricles to contract in unison.

What are the components of the animal extra-cellular matrix (ECM)?

*Same as plant cell wall, but more protein-based. · Structural fibers; provide strength to matrix. · Matrix; hold structural fibers in place. Adhesive molecules; connect ECM to cell and to adjacent cells.

What are collagens and elastins? Why are they significant?

· Collagens and elastins are proteins that act as structural fibers in the animal ECM. · Collagens are densely wound, alpha helical proteins that are very strong and rigid. · Elastins are a single coiled polypeptide chain and provide elasticity to the animal ECM. · They are significant because in different proportions the cells form tissues with varying strength. o The skin needs high elastins for more flexibility.

Relate the structure of collagen proteins to their function.

· Recall that collagen proteins are a structural fiber in the animal ECM. · They are homotrimers made up of 3 similar polypeptide chains wound together in a tight alpha helix. · The amino acid Glycine is rich in these proteins since its R group is hydrogen (the smallest amino acid) and can be densely packed together. · Depending on the stress that the tissue undergoes, they will be oriented differently; a mesh or an organized, alternating network.

Relate the structure of elastin proteins to their function.

· Recall that elastin proteins are a structural fiber in the animal ECM. · They are made of a single polypeptide and interact with each other non-covalently. · They form cross-bridges, and in their mature form they have a curled/coiled shape. o This allows them to be stretched, and return to their original conformation after the stress is removed.

What makes up the matrix of the animal ECM and why is it this molecule?

· It is mainly composed of proteoglycans. · These transmembrane proteins have a very large extracellular carbohydrate component. o This carb component carries negative charges and attracts water molecules keeping the matrix hydrated.

Give an example of a highly hydrated ECM in the human body.

· Cartilage; acts as a cushion between bones and requires lots of water. o Water isn't easily compressed and helps absorb shock.

What are the two primary adhesive molecules in the animal ECM?

· Fibronectin: a glycoprotein dimer that connects the cell to the substrate or another cell. o The dimers are connected at each end by disulfide bridges; like an open zipper. · Integrin: A transmembrane, heterodimer (protein) that bind to adhesive molecules like fibronectin and connect them to the cell. o They bind via linking proteins to the cytoskeleton.

Compare and contrast focal adhesions and hemidesmosomes.

· These are two types of connections involving integrin proteins, linking proteins, and a cytoskeletal element. · Focal adhesions; integrin proteins, via linking proteins, connect to microfilaments. · Hemidesmosomes; the intracellular portion of a desmosome that specifically involves integrin proteins connecting via linking proteins to intermediate filaments.

What are the 3 types of cell-to-cell communication?

· Direct cytoplasmic link; gap junctions and plasmodesmata · Contact; adhesion · Chemical messengers: hormones

What are the 3 types of chemical messengers?

· Hormones: synthesized by endocrine cells, released & carried to distant target cell. · Neurotransmitters and neurohormones: released by neurons. o Neurotransmitters tend to act on adjacent cells (local action/mediator) o Neurohormones act on distant cells. · Paracrine Agents/Messengers: released by signaling cell (ex. clotting factors for blood clotting) and act on the neighboring cells. · "Para" meaning near = a local mediator

What are the primary, most important steps in any signaling system from beginning to end?

1. Synthesis and excretion of the signaling molecule. 2. Reception: target cells, those with the appropriate receptor, recognize the signal. o “Lock and key mechanism,” a physical fit. 3. Transduction: the signal is converted into a cellular response. 4. Cellular Response: the effect of the transduced signal is carried out. 5. Termination: the signal and its effects are stopped.

What are the two broad divisions of receptor molecules and what significant characteristic distinguishes them?

· Water-soluble receptors; signaling molecule binds extracellularly. · Lipid-soluble receptors; signaling molecule binds intracellularly.

What two ways can a transduced signal carry out an effect on its target cell? How long do each take?

· Alter gene expression in the DNA which changes protein synthesis. o Very long; hours or days. · Alter existing proteins inside the cell. o Very fast; seconds or minutes, “fight or flight.”

Give three examples of water-soluble signaling molecules.

· Insulin (hormone) · Acetylcholine · Adrenaline · Many other less important ones*

Describe the structure of a membrane receptor molecule and relate it to its function.

· These molecules are transmembrane/integral glycoproteins. o Can be single pass or multi-pass based on how many times they cross the membrane (how many transmembrane domains)** · External side of cell: ligand-binding domain; point where signaling chemical binds to the receptor. · Internal side of cell: A domain who's activity is altered by the binding of the signal to the receptor. The conformation of the glycoprotein is altered by the binding and turns on the intracellular domain initiating the signal's pathway.

What are the three types of membrane receptors?

· Ligand-gated ion channels. · Enzyme-coupled receptors. · G protein-coupled receptors.

How does a ligand-gated ion channel receptor function?

· These receptors act as a channel and a receptor. · They have a receptor site where the signaling molecule can bind; this binding changes the conformation of the protein's channel causing it to open or close. · This allows ions to pass through the channel.

Explain how muscle contraction is controlled (started and terminated) by ligand-gated ion channel receptors and a corresponding pathway.

· Skeletal muscles at the ends of nerves receive acetylcholine released by the nerve endings. · It binds to the cholinergic receptor (a ligand-gated ion channel) opening the channel. · This allows Na+ to enter the cell and continues the transduction of the electrochemical signal across the axons. This eventually triggers muscle contraction due to the influx of Na+ ions into the cell. · Termination: there are enzymes in the extracellular space that break down acetylcholine (the signaling molecule) so that the ligand-gated channels can close and muscle contraction stops.

What is curare, how does it work?

· Curare is a South American blow dart poison. · This molecule binds to ligand-gated channels in the skeletal muscles and inhibits the binding of acetylcholine. · This stops the muscle contraction process resulting in paralysis.

What type of receptor is the RTK (receptor tyrosine kinase) pathway a prime example of?

· The enzyme-coupled receptors.

What is a protein kinase, what does it do and how do is do this?

· An enzyme that catalyzes the phosphorylation of specific proteins. · It cleaves a phosphate from ATP and adds it to specific amino acid residues within a protein. · The amino acids with hydroxyl groups are most commonly phosphorylated; Tyrosine, threonine & serine. *Protein kinases need to be TURNED ON before they can perform any phosphorylation.

What are tyrosine kinases?

· There are a specific type of kinase ENZYME that catalyzes the phosphorylation of tyrosine amino acid residues.

Based on the name, what does a receptor tyrosine kinase pathway involve?

· It involves a kinase enzyme specifically tailored to phosphorylate tyrosine amino acid residues. *The tyrosine residues are “its” own (the receptors).

Explain the process of protein phosphorylation and its effect (short answer).

· Phosphorylation of a protein involves the addition of a phosphate group, PO42- to its structure. · These molecules carry a double negative charge and result in a conformation change in the protein. · This conformation change results in a change in function of the protein.

What are protein phosphatases, why are they significant?

· an ENZYME that removes the phosphate group from a protein. · These enzymes are typically always active or "ON." · Termination: These stop the transduction of the signal from the tyrosine-kinase receptors by cleaving a phosphate from the tyrosine amino acid residues and therefore deactivating them. * The resulting Pi (inorganic phosphate) can be recycled to form new ATP.

Describe the steps in the receptor tyrosine-kinase pathway.

· 2 signaling molecules bind to 2 individual receptor molecules. · This causes them to bond together or to dimerize. · The dimerization activates the tyrosine-kinase enzyme on the intracellular side allowing the receptor to phosphorylate its own tyrosine residues within the cell. o This is called auto-phosphorylation. · Now activated, it can attract and phosphorylate other proteins in the cell. This triggers the cellular response.

What molecules typically "turn on" RTK (receptor tyrosine-kinase) pathways?

· The RTK pathways are commonly turned on by growth factors (GF's) such as insulin-like growth factor (IGF), epidermal growth factor, nerve growth factor, etc.

What 4 things do growth factors typically regulate?

· GF's typically regulate cell division, cell growth, cell proliferation/multiplication, and cell differentiation/maturation (changing from "child" to "adult" cell form).

What is MAPK? Why is it significant?

· MAPK stands for Mitogen-activated protein kinase. o These enzymes are threonine/serine specific. The MAPK pathway is triggered by a growth factor and results in cell growth and division through the promotion of mitosis.

Explain the steps in the MAPK pathway (long answer).

· The signaling molecules, a growth factor, bind to two receptors. · The receptors dimerize and auto-phosphorylate their own tyrosine residues on the intracellular side. · The activated tyrosine-kinase receptor activates a protein called RAS via "adapter proteins" (RAS = subfamily of small GTPases that are involved in signal transduction). o RAS can hydrolyze GTP. It is inactive when GDP is bound to it & active when GTP is bound to it. o RAS drops a GDP molecule and picks up GTP and activates. · Activated RAS can phosphorylate the first MAP kinases (Mitogen-activated protein kinases; MAPK's). · This triggers a phosphorylation cascade where each activated MAPK phosphorylates/activates the next kinase in the pathway. · The final activated MAPK enters the nucleus and phosphorylates proteins which control specific gene expressions; transcription factors or regulators. · This produces a cellular response. · Termination: Phosphatase enzymes can cleave a phosphate from either the tyrosine amino acid residues or the RAS protein, deactivating them. · Visual: http://www.wiley.com/college/fob/quiz/quiz21/21-16.html

What is RAS?

· RAS is a type of G protein (G standing for GTPase) which is capable of binding to and hydrolyzing GTP molecules. · They are a family of proteins involved in signal transduction. · They are involved in receptor tyrosine-kinase pathways. o They act as the molecular switches for these pathways.

How is RAS involved in certain cancers?

· About 30% of cancers involve problems with RAS activation that keep them active so that the cell proliferation pathway is permanently turned on. · This could be related to the inability to hydrolyze GTP into GDP, a non-hydrolysable analogue, or a lack of phosphatase enzymes.

Explain the pathway used to lower blood sugar levels in our body (long answer).

*High blood sugar levels cause our pancreas to release insulin in order to increase cellular glucose uptake in the liver cells as well as increase its conversion to glycogen (lowers blood sugar). · Insulin activates this bodily response through a receptor tyrosine-kinase pathway. · 2 Signaling molecules, insulin, bind to 2 receptor tyrosine-kinase molecules which dimerize and auto-phosphorylate their tyrosine residues, activating the tyrosine-kinase domain. · The activated receptor protein complex phosphorylates IRS-1 (insulin receptor substrate I). · IRS-1 can activate: RAS (indirectly) which turns on the MAPK pathway and in liver cells, the activated genes code for the synthesis of enzymes involved in glycogen synthesis. · Can also indirectly activate a pathway with Akt (protein kinase B/PKB) as the endpoint; This promotes insertion of glucose transporters into cell membrane (increasing cell's ability to take up glucose) & increased synthesis of glycogen synthase. · Glycogen synthase converts glucose into glycogen. · **Coordinated response; all contribute to lowering blood sugar level**

What is significantly different between receptor tyrosine-kinase molecules and G protein-coupled receptors?

· G protein-coupled receptors have 7 transmembrane domains (7 TMD's) o By crossing the membrane more than once, they are called multipass transmembrane proteins. · The "molecular switch" for RTK's are RAS proteins, whereas a different type of G proteins are the molecular switches for G protein-coupled receptors.

What is a G protein (5 things)?

· The letter G stands for GTPase. · These proteins are fatty-acid anchored and are capable of binding to and hydrolyzing GTP molecules into GDP. o After time, hydrolysis will occur on its own. · They are heterotrimeric because they have 3 unique subunits; alpha, beta, and gamma. · They act as molecular switches in pathways since they are active when bound to GTP and inactive when bound to GDP.

What are the general steps in the transduction of a signal using a G protein-coupled receptor?

· A signaling molecule binds to the receptor molecule, activating it. · The activated receptor catalyzes the dissociation of GDP from the G protein, and binding of GTP. · The activated G protein activates an enzyme within the cell. · The enzyme produces a 2nd messenger that begins a phosphorylation protein kinase cascade. · This cascade results in a cellular response. *The kinases typically involved are threonine/serine specific.

How or why were 2nd messengers given their name (short answer). Give an example of one.

· Signaling molecules are typically regarded as primary messengers. · 2nd messengers are named given the fact that they carry on the transduction of the signal from a 1st or primary messenger. · 2nd messengers are usually produced by an enzyme in a cellular pathway. · A good example if cAMP: It is produced by adenylyl cyclase (an enzyme) and continues the transduction of the signaling molecule's message by starting a protein kinase cascade.

Why do G proteins involved in G protein-coupled receptors have 3 unique subunits. What are they?

· These G protein has an alpha, beta and gamma subunit. · The alpha subunit serves as a binding site for GTP. · When GTP binds, the subunit dissociates from the remaining beta-gamma complex. · Once separated, each part can carry out separate functions.

Why do G proteins involved in G protein-coupled receptors require a signaling molecule to be activated?

· When the signaling molecule binds to these receptors, their intracellular domain promotes the dissociation of GDP and the binding of GTP to the alpha subunit of the G protein. · With GDP bound, the G protein is inactive. Also, G proteins are capable of hydrolyzing GTP that is bound to it so that after time they were essentially "turn themselves off."

Describe the steps in a pathway that produce cAMP.

· G protein turns on adenylyl cyclase and this enzyme catalyzes the production of cAMP: o Adenylyl cyclase cleaves 2 phosphate groups from an ATP molecule. o This causes the one remaining phosphate to twist and bond back to the sugar (ribose) giving it a cyclic structure. o This is cAMP and acts as a second messenger in the cell. · Termination; a phosphodiesterase enzyme breaks the cyclic bond between the phosphate and the sugar giving regular AMP.

Differentiation the structure of cAMP and AMP.

· cAMP stands for cyclic adenosine monophosphate. · AMP stands for adenosine monophosphate. · In cAMP, the single phosphate group is bonded to the ribose sugar at two points, making it cyclic.

Explain the steps in the pathway our body follows to increase blood sugar levels. In what famous example does this happen (long answer)?

· This is a prime example of a pathway used during the "fight or flight" response. · The signaling molecule in this case is adrenaline. · Its binding to a G protein-coupled receptor activates it, promoting the dissociation of GDP and the binding of GTP to the fatty-acid anchored G protein. · The activated alpha subunit turns on, through phosphorylation, adenylyl cyclase, producing cAMP, which turns on protein kinase A and begins a kinase phosphorylation cascade: · Protein kinase A phosphorylates/activates phosphorylase kinase. · Active phosphorylase kinase phosphorylates/activates glycogen phosphorylase. · Active glycogen phosphorylase breaks down glycogen, producing glucose.

What two hormones control color in animals containing chromatophores? What are their effects?

· Alpha MSH: causes dispersion of pigment granules within chromatophore cells resulting in a darker looking organism. · Adrenaline/nor-adrenaline: aggregates the pigment granules in the center of the chromatophore cells resulting in a lighter/paler looking organism.

Based on two similar pathways in chromatophore cells, explain how the color of organisms can be changed and controlled by hormones (long answer).

· Darkening the organism: MSH (Melanocyte stimulating hormone) acts on its receptors; melanocortin 1 receptor (MC1R). · MC1R is a G protein-coupled receptor, its G protein activates (through binding GTP) and turns on adenylyl cyclase. · Adenylyl cyclase produces cAMP in the cell. · cAMP turns on protein kinase A (PKA), which then activates kinesin motor proteins (series of steps to activate kinesin are not well known). · Kinesin moves the pigment granules along the microtubules towards the peripheries of the cell (+ end oriented) giving a dark appearance. · Lighter/Paler Organism: Adrenaline acts on its receptors; alpha 2 adrenoreceptor. · These are also G protein-coupled receptors as well; their G protein inhibits/turns off adenylyl cyclase activity. · This stops the production and reduces levels of cAMP and as a result protein kinase A (PKA). · Dynein motor proteins are also activated by the binding of adrenaline to its receptor (series of steps not well known either) and aggregate the pigment granules in the centre of the cell giving the chromatophore a lighter appearance.

What is a chromatophore (1 point)? In what organisms are they found?

· Any cell or plastid that contains pigment.
· They are found in amphibians, fish, reptiles, crustaceans, and cephalopods.

Describe the steps involved in any phospholipase C-dependent pathway (very long answer).

· A signaling molecule binds to its G protein-coupled receptor in order to activate it. · That turns on the G protein (through binding of GTP) which splits into an alpha subunit and a beta-gamma complex. · The alpha subunit activates the enzyme phospholipase C. · This enzyme acts on phosphatidylinositol phospholipids in the membrane (those with an inositol head group). · It breaks the bond between the glycerol molecule & the phosphate group with the polar inositol head. This results in diacylglycerol, DAG and IP3 (inositol trisphosphate) molecules. o Both DAG and IP3 can act as a second messenger. · IP3 is now water soluble (without fatty acid chains) and can dissociate into the cytosol. · It binds to a receptor on the endoplasmic reticulum (ER). · This receptor is a ligand-gated ion (calcium) channel and opens with the binding of IP3 allowing Ca2+ to leave the ER and enter the cytoplasm (increasing its cytosolic concentration). · With an increase in cytosolic Ca2+ now, an ion channel in the cell membrane opens after DAG phosphorylates it and allows Ca2+ to simply diffuse into the cell. · Ca2+ binds to protein kinase C (PKC) proteins, causing it to be attracted to the membrane = "membrane-associated PKC." And preparing it for activation by DAG. · DAG (the other second messenger), having stayed anchored to the membrane activates the calcium-bonded protein kinase C (PKC). · Activated PKC can phosphorylate multiple targets resulting in a varied array of cellular responses.

Again during the "fight or flight response," how can our bodies reduce blood flow to the viscera? What are viscera (singular; a viscus)? LONG ANSWER AGAIN

· First of all, viscera are internal organs in the main cavity of our body. For example, the intestines. o During "fight or flight" moments, blood flow is only wasted going to the viscera. · The reduction of blood flow to the viscera is driven by the phospholipase C pathway (PLC). · Adrenaline or nor adrenaline binds to its receptor (alpha 1 adrenergic receptor). · This G protein-coupled receptor activates its G protein via binding of GTP. The G protein splits into an alpha subunit and a beta-gamma complex. · The activated alpha subunit activates phospholipase C which splits phosphatidylinositol molecules in the membrane. · The head group portion, IP3 (inositol trisphosphate) binds to the ER releasing Ca2+ into the cell. · DAG phosphorylates a calcium channel in the membrane, opening it. · Through simple diffusion, Ca2+ ions diffuse into the cell from the surroundings due to the newly produced ion gradient. · The high amount of calcium ions in the cell turns on smooth muscle cells that are in the walls of the blood vessels, causing them to shrink. This shuts off blood flow. **Note that the alpha 2 adrenergic receptor receives adrenaline molecules in chromatophores, controlling cell color and pigment granule movement. In this pathway, an alpha 1 adrenergic receptor receives adrenaline to control blood flow in smooth muscle cells. Animation: http://bcs.whfreeman.com/lodish5e/content/cat_010/13010-01.htm?v=chapter&i=13010.01&s=13000&n=00010&o

What is significantly different between water-soluble and lipid-soluble receptors? (1 point)

· Lipid-soluble receptors can diffuse through the membrane and reach a receptor within the cell.

What is a big problem lipid-soluble signals would experience without carrier/binding proteins (2 points)?

· They would collect with the membrane since they are similar in structure and polarity. · They would not be able to maintain adequate concentrations within cells to carry out a cellular response.

What is the structure of lipid-soluble signal receptors and how do they function?

· These receptors have a ligand-binding domain for the lipid-soluble signaling molecule. · They also have a second domain; "DNA-binding domain." o This is a pair of embedded zinc finger motifs in the protein structure. · When the signaling molecule binds to the receptor it changes conformation so that the zinc finger motifs appear. · These recognize and bind to DNA.

What are response elements and why are they significant?

· The small regions on the DNA that allow for the signaling molecule to bind to are called the response elements. · Response elements are found in the promoter regions of genes. · Promoter regions act as binding sites for transcription factors that can transcribe the DNA; this regulates gene expression. · When the zinc finger motifs bind to a response element, it affects the genes that are "downstream" from that region (5' --> 3'). · The target gene that may be synthesized could code for another transcription factor causing more of the gene to be synthesized similar to the phosphorylation cascade in membrane protein receptors. Animation: http://glencoe.mcgraw-hill.com/sites/9834092339/student_view0/chapter9/how_intracellular_receptors_regulate_gene_transcription.html

Describe how testosterone enhances muscle growth. LONG ANSWER

· Testosterone is a steroid hormone, it is lipid soluble. o It is a derivative of cholesterol* · It is carried in the blood plasma by carrier/binding proteins. · It detaches from the carrier protein and diffuses through the cell membrane. · The molecule then binds to its receptor molecule; an androgen receptor. o Androgen = male sex hormone = "andro-" (male) + "-gen" (produced by). · This binding causes the androgen receptor to change conformation so that zinc finger motifs appear (in the protein structure). o An inhibitor protein was previously "covering up" the zinc finger motifs. · This also causes the receptor-hormone complex to move into the nucleus. · Within the nucleus, the zinc finger motifs can bond to the androgen (hormone) response element on the DNA which is found in the promoter region of a specific gene(s). · This triggers the production of mRNA with the transcribed DNA, which migrates out of the cell and combines with a ribosome to produce proteins. · These proteins that are produced cause muscle growth, one of the known results of high testosterone levels.

What is cross talk (short answer; 1 point)?

· Cross talk is when individual pathways communicate with each other. o They aren't running in isolation within the cell*

Between what types of pathways can cross talk occur? (very short answer)

· Any! Membrane receptor pathways, intracellular receptor pathways, etc.

Explain how the pathway involving cortisol and the pathway involving adrenaline can cross talk.

· This is an example of cross talk between an intracellular steroid hormone pathway (cortisol = stress hormone = lipid soluble) & a membrane receptor pathway (adrenaline = water-soluble signal). · Cortisol: a steroid hormone that acts on its receptor to regulate protein synthesis of various genes. o One of the proteins that is synthesized in this pathway is the adrenergic receptor*. · If more of these receptors are present in the cell membrane, this will affect its signaling pathway. o In this case, more adrenaline could be received and glycogen could be broken down.

Keeping cross talk in mind, what difficulties can be posed when trying to experimentally determine and prove a signaling pathway?

· Pathways interact on numerous levels; the proteins used, the receptors used, and the signaling molecules required. · In order to determine the steps in a signaling pathway, all its elements must be separated from what is happening in the rest of the cell. · This can be very difficult!

When trying to determine the steps of a signaling pathway, why would it be useful to identify which proteins are being phosphorylated? How can this be done experimentally?

· The proteins that are phosphorylated are most likely involved in the pathway. · Experimentally: Add the signaling molecule, allow the pathway to run, break up the cell and run the proteins out on a gel. · Using specific antibodies, phosphorylation sites can be identified. · These proteins could be involved in the pathway but also might be part of another pathway!

What does it mean if something is "hot"? How could "hot" ATP molecules help determine what proteins are involved in a signaling pathway?

· When something is "hot" it means it is radio-labeled. · When ATP is "hot" it has a radio labeled phosphate group. · As the phosphate group is transferred from ATP to the protein being phosphorylated, the protein becomes "hot" and it can be found on a gel as well. · This indicates what proteins might be involved.

What two techniques can be employed in order to experiment with terminating and prolonging a signaling pathway?

· Molecular biology techniques: these require extensive knowledge about the organism being studied. o Genetically modified proteins (analogues of GTP for example) can be added to the cells in order to observe results. · Pharmacological techniques: these require a wide selection of drugs. o Depending on the goal, or previous information on the pathway, second messengers can be targeted § Ex. activate adenylyl cyclase to produce more cAMP or inhibit phosphodiesterase to also increase cAMP.

What is co-immunoprecipitation?

· This is a technique used to help determine what proteins are interacting in a signaling pathway. · An antibody that recognizes one protein can be inserted into the cell performing the signaling pathway. · After pulling that protein out of solution, other proteins may be associated with it. · If there are, those proteins are bound to each other and are likely interacting as part of the signal transduction pathway.

Evidence to support the endosymbiont theory?

Evidence: Circular DNA, their own ribosomes (but smaller & simpler than those of eukaryotes), capable of translating/transcribing their genetic info, capable of reproducing through binary fission (like prokaryotes), have two outer membranes that are not similar to one another, similar in size to average prokaryotic cells

Distinguish between a polymer and a polysaccharide.

Polysaccharides are examples of polymers, but not all polymers are polysaccharides (they are made up of many monomers).

Provide an example of a carbohydrate used for energy storage

Glycogen or starch.

Glucose is a 6-carbon sugar or a

.

ose

Why are there å (alpha) and ß (beta) forms of glucose in the ring configuration but not in the linear configuration?

The linear configuration does not have isomers and thus Carbon #1 cannot bond in different ways. In the ring form, there are two isomers out of consequence.

What carbohydrate is formed from repeated units of ß-glucose? · Can humans digest this carbohydrate?

Cellulose; no we can't we lack the enzyme cellulase.

Distinguish between a nucleic acid and a nucleotide.

Nucleotides make up nucleic acids. Nucleic acid is a polymer, the monomer are nucleotides.

True or false – cytosine is a purine

False it is a pyrimidine (Cytosine and Guanine)

What is a nucleoside?

Nucleoside = Ribose & adenine. Adenine is attached at the 1' Carbon of the sugar.

List three differences between DNA and RNA.

DNA is double stranded, RNA is single-stranded. DNA contains the nitrogenous base thymine, RNA has uracil. DNA has the deoxyribose sugar, RNA has the ribose sugar (both 5 Carbons/pentoses)

Describe the structure of dGTP

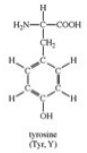
dGTP = deoxyguanine triphosphate. Has the deoxyribose sugar, the guanine (pyrimidine) base, and three phosphates attached.

Who elucidated the structure of DNA?

Watson & Crick

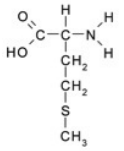
Nucleic acid sequences are always presented in the order _____ to _____.

5' to 3'.



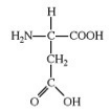
Deduce the chemical nature of the amino acid above by examining their side chains.

Non-polar, uncharged



Deduce the nature of the amino acid above by examining its side chains.

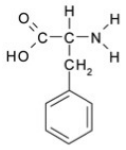
Polar, uncharged



aspartic acid
(Asp, D; Asx or B)

Deduce the nature of the amino acid above by examining its side chains.

Polar, negatively charged (acid gives up proton on -OH group)



Deduce the nature of the amino acid above by examining its side chains.

Non-polar, uncharged

Between what groups does a peptide bond form?

Amine & Carboxyl.

Distinguish between a polypeptide and a protein.

Polypeptide = 50+ amino acids, makes up the primary structure. · Protein = the conformation of a polypeptide. · Polypeptides are like a piece of paper, and proteins are like origami.

List three functions of proteins.

Structural support, transport, enzymes, cell signaling, synthesis

Distinguish between a motif and a domain

Motifs are found within domains of a protein. →Motifs = secondary structure →Domains = tertiary structure

For what level of protein structure is hydrogen bonding in the backbone responsible?

Secondary

True or false – Monomeric proteins do not exhibit quaternary structure.

True: Quaternary structure can't exist with a monomeric protein (one peptide chain)

Are lipids polymers?

no, lipids are not made of a chain of monomers (subunits).

Distinguish between a fatty acid and a phosphoglyceride.

Two fatty acids make up a part of a phosphoglyceride

What lipids play a role in signaling?

Steroids (ex. stress hormones) · Seen later in course (phosphoglycerides & lipids can also signal)

Define amphipathic. Use a sketch of a phosphoglyceride to illustrate your answer.

Amphipathic = a molecule that possesses polar and non-polar regions (or terminals). · In phosphoglyceride there is a polar phosphate and 'R' group but the two fatty acid chains are non-polar.

Would you expect to find sterols in the inner membrane of a mitochondrion or chloroplast? Explain your answer.

No, the inner membranes of organelles didn't originate from Eukaryotic cells and only eukaryotes have steroids (in specific sterols).

The lipid bilayer functions as a permeability barrier because.. a) the lipids are held together by covalent bonds that prevent unwanted movement across the membrane. B) it is difficult for hydrophilic molecules to traverse the hydrophobic core of the lipid bilayer. C) the lipid bilayer is thick, so that the diffusion distance becomes too great for rapid transit across the lipid bilayer. D) the glycolipids of the lipid bilayer recognize and bind molecules that the cell wishes to exclude. E) None of the above

b, lipid bilayer IS hydrophobic and thus hydrophilic molecules would be repelled.

Which of the following remedies is likely to be most effective against athlete's foot, a fungal infection of the human foot? a. Oseltamivir, an anti-viral agent better known as tamiflu. b. Micronazole, an inhibitor of ergosterol synthesis. c. Penicillin, an inhibitor of peptidoglycan (bacterial) cell wall formation. d. Sodium azide, an inhibitor of oxidative metabolism. e. None of the above

2. b, Ergosterols are the sterols used by fungi for signaling, membrane structure & fluidity. d, inhibiting oxidative metabolism would kill foot cells

A protist (Giardia) is suddenly expelled from a warm human intestine into cold water. Which of the following adjustments might the protist make to maintain membrane fluidity following this change in temperature? A) Decrease the length of the hydrocarbon tails in its membrane phospholipids. B) Decrease the proportion of unsaturated hydrocarbon tails in its membrane phospholipids. C) Decrease the amount of cholesterol in the membrane. D) Decrease the proportion of phosphatidylcholine relative to phosphatidylethanolamine. E) Both (a) and (d) would be appropriate responses for the protist to make to maintain the same level of membrane fluidity following the change in environmental temperature.

e... If we examine 'a' first… Decreasing the length of the hydrocarbon chains in its membrane phospholipids would increase fluidity as the phosphoglycerides wouldn't pack as easily/nicely. This would compensate for the automatic decrease in fluidity caused by the temperature drop. If we examine 'd' now… Phosphatidylcholine (PC) is less polar than phosphatidylethanolamine (PE). This would result in a net increase in the polarity of the head groups in the membrane phospholipid bilayer. The more polar groups cannot be as densely packed and thus would increase the membrane's fluidity.

Can you design an experiment to distinguish between facilitated diffusion and secondary active transport?

Using a cell model, the cell could be deprived of energy (ATP) and if it's active transport the system should stop working. The other way, manipulate the concentration gradients and see if the direction of transport can be reversed.

What is the dynamic instability model?

This model refers to the constant growth and shrinkage of microtubules. Since microtubules only grow at one end (the plus end) and are constantly hydrolyzing GTP into GDP and dissociating, they are dynamically unstable

Taxol is used in cancer chemotherapy. Suggest a possible mechanism of action.

We know that taxol acts as a stabilizer. The rate of dissociation and growth virtually stops when microtubules are in the presence of the taxol drug. If cancer cells could be targeted, their microtubule growth could be stopped and thus they wouldn't be capable of undergoing cell reproduction.
→Cell division uses spindle fibers/microtubules to physically split the cell into two. Cancer cells are dividing rapidly and thus tend to be affected "more" than regular somatic cells…but they will still be affected.

Distinguish between cytoplasmic and axonemal microtubules

Cytoplasmic microtubules are found in the cytoplasm of eukaryotic cells. · Axonemal microtubules are found surrounding the axons of nerve cells.

Distinguish between Dynein and Kinesin

Kinesin is plus-end oriented. Dynein is minus-end oriented. · Kinesin has a longer tail domain than dynein.

Distinguish between a cilium and a flagellum

Cilia are much shorter (Cilia exist in large numbers on the surface of a cell, flagella exist singly or in pairs. · Because of their lengths, cilia row like oars and flagella whip.

Distinguish between axonal transport and axonemal dynein

Axonal transport is the carrying of nutrients of molecules along the axons of nerve cells. · Axonemal dynein is the form of the dynein motor protein that exists in the axoneme of a cilium or flagellum. Its function is to make the structure bend.

Compare & Contrast Microvilli and Cilia · Points of similarity? · Points of difference?

Similarities: Minus end is at the membrane end and the plus end is at the tip of the structure -->Both are finger-like projections of the cell membrane -->Both usually in cell numbers -->Both contain cytoskeletal elements -->Both stable (no dynamic instability) Differences: -->No organizing centre bonded to the minus end in the cell membrane. Basal body is at the minus end of the microtubules in cilia. -->Microvilli = microfilaments ; cilia = microtubules -->Microvilli is much shorter than cilia -->Cilia = 9+2 arrangement ; microvilli = a bundle of microfilaments --> Function: Cilia are used for moving the cell or transporting things over it ; Microvilli are used to increase the cell's surface area (found particularly in the stomach for absorption) -->Cilia are motile ; microvilli are not (no motor protein)

Design an experiment to determine whether vesicle movement in a cell relies on myosin or kinesin motor proteins. Briefly describe the experimental approach that would be taken, the rationale for this experimental approach, and the expected results

Cytochalasin (myosin) vs. Colchicine (kinesin) 1. First, put a fluorescent marker on the vesicles. 2. Treat the cell with Cytochalasin. If the vesicles continue to move, it must be moving on microtubules and by means of kinesin motor proteins. If the vesicles stop moving, then it must be moving on microfilaments by means of myosin motor proteins.

AP.PNP is a non-hydrolysable analogue of ATP (modification of the phosphate groups prevents ATP from being hydrolyzed to ADP). How would use of AP.PNP in a cell affect... · Microfilaments? · Microtubules? · Motor proteins?

: Microfilaments: added stability and better growth à because the minus end can't hydrolyze ATP into ADP and dissociate. Growth continues regularly. Microtubules: Tubulin binds GTP so using AP.PNP wouldn't affect microtubule stability. Intermediate filaments: Don't use any nucleotides so using AP.PNP wouldn't have any effect. Motor proteins: Without being able to hydrolyze ATP into ADP, these proteins wouldn't create the energy necessary to change conformation and walk along microtubules and filaments meaning they can't "walk" or move anymore.

How are different patterns of cellulose microfibrils achieved in the different layers of the cell wall?

The different patterns of cellulose microfibrils in the cell wall are achieved by the different patterns of microtubules in the cell. -->The motor proteins that walk along these microtubules drag the rosettes through the membrane in special patterns. The cell can alter and control the pattern of microtubules throughout it, so that as the multiple layers of the secondary cell wall are being created the pattern of cellulose microfibrils can change.

Contrast and compare the structure and function of plasmodesmata and gap junctions.

Plasmodesmata: Found only in plant cells. -Both cytoplasmic connections between cells (electrical & chemical communication) -Allow larger molecules to pass through (than gap junctions) -The plasma membrane flows through the plasmodesma allowing two cells to connect via this. Gap Junctions: Found only in animal cells. -Both cytoplasmic connections between cells (electrical & chemical communication) -Only allow smaller molecules to pass through -Require connexin transmembrane proteins (a connexon) to create the junction between two cells.

Distinguish between connexin and claudin.

Connexin vs. Claudin →Connexin = gap junction. Cytoplasmic link between cells. Require 2 connexons (each w/ 6 connexins) to make a gap junction →Claudin = tight junction. Seal the space between cells.

Distinguish between cellulose and cadherin.

Cellulose vs. Cadherin
→Cadherin = transmembrane protein. Link 2 cells together in adhesive junctions.
→Cellulose = polysaccharide. Present in cell wall (structural fiber)

Distinguish between a tight junction and a gap junction.

Tight junction vs. Gap junction →tight junction = seals the spaces between cells (creates a permeability barrier). Uses claudins →Gap junction = continuous cytoplasmic link (communication route). Uses connexons

How can we show experimentally that binding of the signal molecule to a surface receptor is what triggers the cellular response, i.e. that the signal molecule itself does not enter the cell?

Experimental methods: i. If the molecule is a protein it could be fluorescently tagged and observed. ii. Eliminate the receptor and see if the response still happens… Iii. Inject the signaling molecule into the cell. It wouldn't need a receptor if it were an intracellular molecule…

GTP(gamma) S is a non-hydrolysable analogue of GTP. How would use of GTP(gamma) S in a cell affect G protein signaling?

· G proteins are active when GTP is bound to them. Without the hydrolysis of GTP into GDP, the G protein would remain active. · The signal wouldn't be able to be turned off. · This would become a problem for the cell. **About 30% of cancers are due to gene problems with RAS. The pathways get turned on permanently, and the cell divides uncontrollably.

What is the advantage of a phosphorylation cascade?

The cascade amplifies and increases the speed of the response. · By turning on cAMP, each molecule can turn on a molecule of protein kinase A (PKA), each PKA can turn on several of the next kinase, etc. · Multiple regulation points: much more control over the pathway.

Insulin and adrenaline have opposite effects on liver glycogen levels. Why?

Recall that insulin causes blood sugar to go down and adrenaline causes it to increase. · These two molecules are part of different pathways with different receptors. · They turn on different pathways, with the same endpoint, but effect it in different ways.

What do the different responses to adrenaline of liver cells, blood vessel smooth muscle cells, and chromatophores tell us about cellular responses to a chemical messenger?

Recall, we've seen adrenaline causing: · Smooth muscle contraction in blood vessels, · Glucose release from liver cells, · Pigment granules to move in chromatophores. The receptors all bind adrenaline, but the pathways that they turn on differ in every case. Depending on the cell, its tissue, and its receptor, a different pathway will be activated resulting in varying cellular responses. *A single cellular response can be affected by different signaling molecules & A single signaling molecule can affect multiple cellular/signaling pathways.

Distinguish between a ligand-gated channel and a ligand-activated transcription factor.

A ligand-gated channel is a membrane receptor that receives water-soluble signaling molecules. · A ligand-activated transcription factor is a receptor that receives lipid-soluble signaling molecules capable of diffusing into the cell without the help of another receptor. o These transcription factors are activated by the binding of these signaling molecules, or ligands.

Distinguish between a kinase and a phosphatase

These are both involved in phosphorylation. · Activated kinase proteins add phosphate groups to proteins. · The phosphatase enzyme de-phosphorylates proteins.

Distinguish between a DNA-binding domain and a ligand-binding domain

A ligand-binding domain is the region where a signaling molecule can bind to a receptor. · These exist on both membrane receptors and intracellular receptors (for lipid-soluble signals) · A DNA-binding domain is found on an intracellular receptor and is usually occupied by an inhibitor until the signaling molecule/ligand binds to it. o The removal of the inhibitor reveals two zinc finger motifs on this domain, capable of binding to DNA.

Distinguish between a receptor and a response element

· A response element is a small region of DNA that allows the binding of a receptor-ligand complex. o These regions are usually found in the promoter sequences of DNA that are responsible for the expression and transcription of a specific gene(s). · A receptor is a molecule that receives a ligand, and begins the transduction of a signal.

Design an experiment to determine whether activation of the urea excretion mechanism of toadfish relies on a G protein-coupled receptor signal transduction pathway involving cAMP. Briefly describe the experimental approach that would be taken, the rationale for this experimental approach, and the expected results.

NOTE: This question is not fully answered because this is not necessary to know for this course. In fact, this is a potential 4th year research project that could be done as an honour student's thesis.First (Possible) Approach: Measure water/urea levels over time. · Feed the toadfish an infusion of caffeine and see if it produces an increase in urea excretion. o Inhibits phosphodiesterase activity, yielding more cAMP, and therefore more urea. Second (Possible) Approach: Prepare a culture of cells, and treat them with an analogue of cAMP that is always on. · In order to observe urea excretion, the culture of cells would need to be capable of it themselves, or incorporated into a live toadfish…

What are four functions of nucleotides?

· Make up nucleic acids; information storage. · Energy transfer · Responsible for protein synthesis · Signaling functions

What is axonemal dynein and why is it significant?

· Axonemal dynein is the motor protein in cilia & flagella. · By hydrolyzing ATP and walking along microtubules it causes the movement/bending of the structure which results in cell movement.

What is a MTOC and why is it significant?

· MTOC stands for microtubule organizing center. In animals it has two centrioles and pericentriolar material. · The centrioles contain gamma (y) tubulin which bonds to the minus end of microtubules orienting them in a specific direction. · The pericentriolar material draws in tubulin subunits making it more likely that a microtubule polymerizes.

Distinguish between "tread milling" and "dynamic instability"

· Tread milling is when the rate of polymerization at the plus end (beta end) equals the rate of dissociation AT THE MINUS END · Dynamic instability is when the PLUS END grows or shrinks rapidly making it unstable.

Differentiate between Passive Transport and simple diffusion:

Simple diffusion is a type of passive transport. Simple diffusion requires no chemical energy, or ATP. Whereas passive transport, the general category, can be either simple or facilitated which does require ATP.

Differentiate between an integral membrane protein and a channel protein

A channel protein facilitates diffusion through the membrane. It is a type of transport protein. An integral membrane protein is the general name for a protein that is embedded in the membrane. They are amphipathic.

Differentiate between a channel and carrier

A carrier changes conformation to allow molecules across the membrane. A channel is like a pore in the membrane which is only involved in facilitated diffusion. A carrier can also be involved in active transport (requires ATP to change conformation).

Differentiate between a symporter and an antiporter

A symporter carries two or more solutes through the membrane in the same direction. An antiporter carries two solutes across the membrane in opposite directions.

Differentiate between secondary active transport and facilitated diffusion

Secondary active transport requires energy, ATP. Facilitated diffusion does not. a. The solute that is moved through facilitated diffusion can travel in either direction. b. In secondary active transport, the solutes must move in the same direction. These proteins are said to have directionality. i. This also requires two proteins. A pump which establishes a gradient, and a carrier.

What is the cross-bridge cycle and why does it matter?

· Found specifically in muscles; in the sarcomeres. · Cycle: action of myosin motor protein with the microfilaments. Standard motor protein cycle, binds, hydrolyzes ATP, changes conformation and steps along the microfilament. · This is important because; muscle contraction happens because of this. Without the cross-bridge cycle, our muscles can't contract.

What is the function of intermediate filaments? How does their structure suit them for this function? (structure-function type question)

· Provide structure within the cell; strength and support. · Only found in animal cells and some soft bodied invertebrates. · Intermediate filaments are found in the nuclear envelope of all eukaryotic cells. · Fibrous proteins that are wound around each other, stronger than globular proteins. o Two proteins wind around each other, pair up, and build into a rope-like structure (8 of these wound proteins) o This high tensile strength is useful in preventing cells or nuclear membranes from being ripped apart.

AP.PNP is a non-hydrolysable analogue of ATP (looks like it but modification of how one of the phosphate groups is joined so it can't be hydrolyzed to ADP). How would use of AP.PNP in a cell affect…1. Microfilaments2. Microtubules3. Intermediate filaments4. Motor proteins

· Microfilaments: added stability and better growth à because the minus end can't hydrolyze ATP into ADP and dissociate. o Growth continues regularly. · Microtubules: Tubulin binds GTP so using AP.PNP wouldn't affect microtubule stability. · Intermediate filaments: Don't use any nucleotides so using AP.PNP wouldn't have any effect. · Motor proteins: Without being able to hydrolyze ATP into ADP, these proteins wouldn't create the energy necessary to change conformation and walk along microtubules and filaments meaning they can't "walk" or move anymore.

Relate the structure of the plant cell primary wall to its function

Structure made of cellulose and because of its BETA 1-4, glycosidic bonds, the glucose molecules can be densely packed together making a rigid fibrous substance. This protects the cell and makes it rigid.

What is a "tight junction" and why is it significant?

· Permeability barrier between epithelial cells/tissues that forms a seal between compartments in an organism. · Substances can't cross these junctions, separating the compartments. Ex. the gut lining and the blood vessels around it. · Structure: ridges of claudins and occludins

What is “signal transduction” and why is it significant?

· Taking a signal and converting it to a response by the cell fall into the category of signal transduction pathways. o It matters because without these pathways, a signal is meaningless to a cell.

What structural element is absolutely required by a cell for it to respond to a signaling molecule?

· Receptors: if a cell doesn't have a receptor it will not respond to the signal.

Why are their Alpha and Beta forms of glucose in the ring conformation and not in the linear conformation?

· In the linear conformation there are no isomers and therefore there cannot exist an alpha and beta form.

Between what two amino acids can a disulfide bridge form? What level of protein structure does this contribute to?

· Between two cystein amino acids (the only ones with reactive sulfhydryl groups). This contributes to tertiary structure.

Which carbon in glucose would no longer be assymetrical(*) in the linear conformation.

· Carbon 1. NOTE: Gilmour uses the word asymmetrical here to represent chirality. · Carbon 1 is bonded to 4 different substituent groups in the ring form, but not in the linear form.

Why isn't there cholesterol in the membrane of mitochondria?

Prokaryotes lack sterols.

A biologist working on the Ottawa river isolated DNA from two unknown organisms P and Q. She discovers that the adenine content of P is 15% and the cytosine content of Q is 42%. This means that · 1. the amount of guanine in P is 15% · 2. the amount of guanine and cytosine combined in P is 70% · 3. it takes more energy to unwind the DNA of P than the DNA of Q. · 4. the amount of thymine in Q is 21% · 5. the amount of adenine in Q is 42%

2

Is heterochromatin found only in heterochromatic cells?

Heterochromatic cells do not exist, no.

True or false; Topoisomerases are needed for all DNA synthesis.

Answer: False, in circular DNA the genetic material doesn't need to be unwound. In linear or helical structures it does.

Every day, the number of times you repair DNA damage is greater that the earth's population. T/F?

Answer: True, the number of repair events in a human body is about 1016-1018 whereas the Earth's population is 7 x 109.

Histone H5 replaces histone H1 in chicken erythrocytes. Therefore, histone H5 must bind to linker DNA. True or false?

Answer: True. If H5 replaces H1 it must also assume its function, which is to bind to linker DNA.

Some DNA polymerases function in “repair synthesis” as they have a 5’ to 3’ exonuclease activity. TRUE OR FALSE

Answer: False, 3' to 5' exonuclease activity allows proofreading to occur. It's how DNA polymerase can backtrack and replace mispaired nucleotides.

During replication DNA is synthesized. This implies that: · 1. successive nucleotides are added to the 3'OH end of the newly forming chain · 2. ligase unwinds DNA in a 5' to 3' direction · 3. primase on the 3' end of the replicating strand. · 4. because both strands are replicated nearly simultaneously, replication must be continuous on both · 5. the template is read in a 5' to 3' direction

1

Heterochromatin is a highly-condensed state of the chromosome. · 1. Heterochromatin is stable and thus genes within it are never expressed. · 2. Heterochromatin may not exist since DNA has many forms. · 3. Heterochromatin can be converted to euchromatin and the genes can be expressed. · 4. Heterochromatin can be converted to euchromatin and but the genes cannot be expressed.

3

What group of proteins associated with DNA could be described as a small, well-defined basic protein group that exhibits very high lysine and/or arginine content? · 1. histones · 2. acidic proteins · 3. nonhistone proteins · 4. succinate dehydrogenases · 5. DNA helicase

1

Amino acid residues in histone tails are most susceptible to _____.
· 1. methylation · 2. acetylation · 3. phosphorylation · 4. sulfation · 5. a, b and c

5

Proteins in your cells called histones bind to DNA to change the structure. If a cell did not have histones what types of proteins would you expect would you expect to find bound to the DNA? · 1. none, as the DNA could still fold up and form a compact structure · 2. small, acidic proteins so that they could bind the DNA and fit inside the cell. · 3. small, basic proteins so that they could bind the DNA and fit inside the cell. · 4. small proteins so that they could bind to the DNA and fit inside the cell.

3

The basic biochemistry of DNA synthesis is the same in your cells and in E. coli. True or false

True

Which chromatin stays condensed in all cells at all times and thus represents DNA that is permanently silenced? · a. constitutive euchromatin · b. constitutive heterochromatin · c. euchromatin · d. facultative heterochromatin

B

In you cells different types of DNA synthesis require different DNA polymerases. These different types of DNA synthesis can be summarized as · 1. chromosomal, mitochondrial, repair · 2. chromosomal, mitochondrial · 3. chromosomal, mitochondrial, lysosomal, repair · 4. chromosomal, mitochondrial, Lysosomal

1

What is the most common mechanism for repairing damage to DNA? · a. simplistic repair of the damage · b. altruistic repair of the damage · c. selective excision of the damaged section and use of the complementary strand to replace excised portion · d. simple removal of damaged portion without replacement · e. direct repair of the damage

C

What determines the characteristic shape of mitotic chromosomes? · 1. the length of DNA · 2. the thickness of the DNA · 3. the position of the centromere · 4. the position of the telomeres · 5. a and c

2

What is the advantage of the highly condensed state of the DNA of mitotic chromosomes? · a. The highly condensed state favors delivery if an intact package of DNA to each daughter cell. · b. The condensed state favors DNA repair prior to mitosis. · c. The condensed state allows replication to occur more rapidly as the cell enters mitosis. · d. The condensed state allows chromosomes to fuse more easily. · e. This state allows gene expression needed during mitosis.

A

Following histone H1 removal, the octomer no longer binds 146bp of DNA. True or False

False

Prokaryotic DNA · 1. is surrounded by densely packed histones · 2. is packaged as euchromatin and heterochromatin · 3. is packaged as a large circular chromosome · 4. has many sites for the initiation of DNA replication · 5. has both strands synthesized in the same direction

3

In a right-handed double helix, if one looks down the central axis of the molecule _____. · a. each strand follows a counterclockwise path moving toward the observer · b. one strand follows a clockwise path moving away from the observer, the other follows a clockwise path moving toward the observe · c. each strand follows a clockwise path moving away from the observer · d. each strand follows a counterclockwise path moving away from the observer · e. each strand follows a clockwise path moving toward the observer

C

The X-ray picture of DNA taken by Rosalind Franklin was used to define the DNA width and repeat distance. True or false?

Answer: True, the X-ray diffraction pattern obtained from the sample of DNA indicated that the DNA molecule is cylindrical and 2nm in diameter (wide). W Watson and Crick also determined that 0.34nm separated the base pairs and one turn of the helix was 3.4nm.

Which of the following statements about DNA is false? · 1. DNA is bidirectional in synthesis · 2. phosphate is linked to the 5' and 3' carbons of adjacent desoxyribose molecules · 3. each side of the helix is antiparallel to the other · 4. Avery identified DNA as the transforming factor in crosses between smooth and rough bacteria. · 5. the binding of adenine to thymine is through three hydrogen bonds.

Answer: Five is false, cytosine and guanine form 3 hydrogen bonds, adenine and thymine form 2.

During replication in a eucaryote, DNA synthesis from one ARS meets DNA synthesis from a neighboring ARS. T/F?

Answer: True, neighbouring ARS (origins of synthesis) will meet each other unless they are at the end of a chromosome.

Genes in heterochromatin are permanently repressed. True or false

Answer: False, by remodeling the chromatin the genes can be expressed again.

Bacterial chromosomes are organized into looped structures called nucleoids. True or false?

True

Bacterial chromosomes are organized into looped structures called nucleoids. True or false?

True

During DNA replication all the important events occur at the replication fork. True or false?

True

What caused the uniformity of DNA fragments when chromatin was treated with nonspecific nucleases? · 1. It is a normal property of DNA to be broken down into equal-sized fragments. · 2. At regular intervals, DNA contains sequences that are immune to attack by nonspecific nucleases. · 3. Treated chromatin was protected by proteins associated with the DNA, except at certain periodic intervals along its length. · 4. Treated chromatin was denatured and so was susceptible to the enzymes only at periodic intervals. · 5. a and b

3

Chromatin remodeling · a. results in specific patterns of gene expression. · b. is subject to well-characterized stages of regulation by the cell. · c. blocks access to genes that should not be expressed in a particular cell. · d. is unaffected by alterations to the DNA sequences of a cell.

A

Yeast, like bacteria, organize their DNA into nucleoids. T or F?

Answer: False, yeast is a form of fungus which is a eukaryotic cell. Eukaryotes do not have nucleoids.

What bonds connect the nitrogenous bases in DNA? How many exist between the base pairs?

Hydrogen bonds. 2 between adenine and thymine, 3 between cytosine and guanine.

What charge is on a molecule of DNA? Where does it come from?

A molecule of DNA has a negative charge. This comes from the phosphate groups in the backbone linking the deoxyribose sugars together through phosphodiester bonds.

Is double stranded DNA always the genetic material in all organisms? If no, what else can be and where?

No it is not always the genetic material. There are certain organisms such as RNA viruses that use RNA as the genetic material (this is what they inject into the host cells). Also prions are “infectious agents” that are made up of misformed/misfolded protein; protein is their genetical material!

Compare and contrast pyrimidines and purines. Which base is which?

Both are aromatic compounds. Pyrimidines consist of one carbon and nitrogen rings whereas purines consist of two. Adenine and Guanine are purines, cytosine and thymine are pyrimidines.

What is the structural difference between uracil and thymine? What is the functional difference?

Uracil is the same structure as thymine without a methyl group on a carbon in the ring. Functionally, uracil is used in RNA strands whereas thymine is used only in DNA.

Compare and contrast a nucleoside and a nucleotide.

A nucleoside is a sugar (deoxyribose or ribose) linked to a nitrogenous base (pyrimidine or purine). A nucleotide has a phosphate group bonded via an ester linkage to the sugar.

What is the handedness of A, B, and Z DNA?

B and A DNA is a right-handed helix. This means that looking down on the helical structure, it will coil clockwise away from you. Z DNA is a left-handed helix.

Describe the structure and details of B DNA (think of what separates it from A and Z models). Who proposed it, what is its width, handedness, grooves.

B DNA is the model proposed by Watson & Crick which we all are familiar with. It is a right-handed helix 2nm in width. It's grooves have similar depth but the major is 50% wider than minor. 10 base pairs per 3.4nm turn in the helix. *It is the most common in living organisms because of the high hydration levels in their cells (assumes this shape naturally).

What are the key characteristics of A DNA (compare to B form; handedness, size/width, grooves, conditions it is found in)?

A DNA is also a right-handed helix but is much wider than the B form. Deeper major groove, shallower minor groove (compared to B form). Occurs in partially de-hydrated conditions. Never found in functional organisms.

What are the key characteristics of Z DNA (compare to B form; handedness, shape/appearance, grooves, tightness of coil/helix, conditions it is found in and why)?

Z DNA is a left-handed helix; it looks more like a Z than a helix. Major and minor grooves are symmetrical. Each turn only has 2 base pairs. Found in GC rich DNA. Guanine and cytosine form three hydrogen bonds, in GC rich DNA, the structure will be coiled more tightly such as Z DNA.

What is supercoiling? What enzyme is involved in this?

It is the coiling of the double helix structure into more or less (negative supercoiling) strained configurations. This changes the accessibility of regions on the DNA to be transcribed and accessed by enzymes or proteins. Topoisomerase is the enzyme during DNA replicating that "unwinds" the double helix also called negative supercoiling.

How does a bacterial cell compact its circular chromosome to fit? What is the nucleoid? "The matrix?"

The circular chromosome is first attached to several binding proteins. These binding proteins pull the circle inwards and form loop domains. Dr. Johnson refers to these binding proteins with loop domains as "the matrix." Gyrase and topoisomerase further coil the loop domains to fold in on themselves. This collection of folded genetic material forms the nucleoid.

Explain with detail how chromatin is made and how it makes up a chromosome (long answer). Include as many key words as possible *Difficult*

1. The double stranded helix of DNA wraps twice around each histone core (in a left-handed helix) made up of 2 of each proteins H2A, H2B, H3 and H4. · One of these structures is called a core nucleosomes. · A core nucleosomes contains ~146 base pairs. 2. The entire nucleosomes includes H1 proteins that control the proximity of neighboring nucleosomes. · This fiber is called the 10nm fiber (made up of nucleosomes). 3. 6-8 nucleosomes form a ring structure called a solenoid. Solenoids can make a fiber as well called the 30nm fiber (wider but shorter/more compact). · The 30nm fiber makes up chromatin and is continuous throughout an entire chromosome! · The 30nm fiber of solenoids (32,000,000 nucleosomes) is further folded into loops and coils to make up a chromosome. **2m of DNA is compacted to fit into a 10μm nucleus (diameter)!

Compare and contrast the structure of heterochromatin (3 points) and euchromatin (2 points).

Euchromatin: · Lightly packed chromatin (loose solenoids in the 30nm fiber) that is rich in gene sequences. o It makes up the most active portion of the genome. o It stains lightly; less condensed. · This is the only form of DNA in prokaryotes; heterochromatin must have evolved in eukaryotes. Heterochromatin: · Densely packed chromatin fibers that have inactive genes. · Can be considered constitutive or facultative (and anything in between). o Constitutive is never converted to euchromatin. § Occurs at centromeres and telomeres (these have structural functions only) o Facultative can be converted to euchromatin depending on an activation factor (ex. environmental conditions).

What is the histone code? What are the three modifications we know of?

An assembly of histone protein modifications that a cell can carry out to change the structure of the chromatin. · Methylation: adding a methyl group to the histone core causes the DNA to be more tightly wrapped around the nucleosomes (genes de-activated). · Acetylation: addition of acetyl groups to the nucleosomes loosens the association between the DNA and the histone proteins. · Phosphorylation: adds a negative phosphate group to the positive histone proteins, this neutralizes their charge and loosens their association with the DNA. o Genes become more transcriptionally active.

What is the difference between the histone code and chromatin remodeling?

The histone code involves modifications done directly to the histone proteins that will affect the transcription of genes in the DNA. Chromatin remodeling is the theory that certain binding sites on the DNA or histones allows for a complex of enzymes called the "remodeling complex" to indirectly affect the histones. This remodeling complex binds to these sites and sequesters/triggers the addition of other enzymes (varies from cell to cell) that can alter the chromatin structure.

Describe Griffith's experiment and its significance (long answer).

In 1928 Frederick Griffith was studying a pathogenic strain of bacteria called "Streptococcus pneumoniae" (causes severe pneumonia in mammals). Two strains of the bacteria were grown in an "agar medium." · S-Strain: reacts by producing smooth even coating on dish due to a polysaccharide coat secretion. · R-Strain: reacts by producing uneven rough coating on dish since no polysaccharide coat is secreted. When injected into mice… · S-Strain: triggered fatal pneumonia. o The polysaccharide coat allowed these bacteria to go undetected by the immune system. · R-Strain: Harmless. o Easily detectable (no polysaccharide coat). · Heat-shocked S (dead): Harmless. · Dead S-Strain and Live R-Strain (normally harmless): Fatal. S cells (transformed from living R cells) were found in mice's blood. Conclusion: Substance in heat-killed S-Strain transformed the harmless R-Strain into harmful – "Genetic transformation" · There must be an active substance in dead S-Strain which they called a transforming principle. *This would trigger further experiments to determine what this transforming principle is.

Describe Avery and his coworker's experiment and its significance (long answer).

In 1944 Avery, McLead, and McCarthy were investigating bacterial transformation in the hopes of determining the transforming agent. · Avery, McLeod and McCarthy first broke up cells from a culture of S-strain virulent bacteria. · These samples were treated with enzymes that broke down either protein, RNA or DNA one at a time. · They found that when only protein or RNA was left in the cells (DNA was broken down), no transformation happened with other live R-strain bacterial cells. · Eventually, they boiled down the cell extracts to only a pure form of DNA and found that it transformed the R bacteria! Their conclusion: Transforming agent must be DNA! *HOWEVER, many scientists at the time believed that protein was the genetic material and accused Avery and his co-workers of cross-contamination (protein traces left in DNA samples). It took another experiment to convince them…

What did Hershey and Chase do that was significant? Describe their experiment (long answer).

In 1952, Alfred Hershey and Martha Chase demonstrated that DNA is the genetic material of viruses. · Significance: A confirmation of the Avery/McLeod/McCarthy experiment. Bacteriophage: A virus that attacks bacteria. They focused on bacteriophage type "T2." There were two possibilities; either proteins or DNA are the transforming agent (only components of a virus). · Prepared two batches of "T2" bacteriophage particles in same medium as the bacterial cells. · Viruses were cultivated in an 35S medium (S = sulphur; incorporates into protein cystein in the virus) with bacterial cells. o The phages replicated via the bacterial cells and incorporated the same 35S yellow radioactive color. · These labelled phages were added to unlabelled bacterial cells and infected them. · Viruses in the 32P medium (P = phosphate; incorporates into DNA of the virus) also grew with bacterial cells. o The phages replicated and incorporated the blue 32P radioactive label. · These phages were added to unlabelled bacterial cells and infected them. · The mixtures of bacteriophages and bacteria cells were put in a blender to remove the protein coat of the viruses from the bacterial cell surface. Results: · 65% of 32P

Explain Chargaff's discovery.

In 1952 Chargaff wanted to know the base composition of DNA. · He used chromatographic methods used to quantify the relative amounts of the four bases: Adenine, Guanine, Cytosine, and Thymine. Results: · DNA from different cells has same definite ratio between bases (pyrimidines = purines). o Relative amounts of A and T versus G and C in DNA of different organisms DIFFER. *Amount of A = T and amount of G = C. · (A+G = C+T) "CHARGAFF'S RULE"

What did Watson and Crick do? How did they do this? What were the details of their discovery (there are many; think structure of DNA)?

In 1952 (the same year of Chargaff's discovery) Rosalind Franklin took an X-ray diffraction picture of DNA and derived that it must be a helical structure. · Watson and Crick took that information (from the diffraction picture) and produced a DNA model consisting of 2 intertwined strands – a double helix. o Sugar-phosphate backbones of strands on outsides of helix o Bases face inward to center and form "steps" or "circular staircase" o Helix = right-handed (coils clockwise when looking from above) o 10 nucleotide pairs per turn. § Width allows for 1 purine & 1 pyrimidine only! o Consistent with Chargaff's rules of A = T and G = C · Hydrogen bonding holding strands together fits only when they form between the base adenine (A) in one chain and thymine (T) in the other or between the base Guanine (G) and C in the other. · Complementary strands: the base sequence of one determines the other. · Model suggested mechanism by which genetic information could be replicated.

Why does tRNA assume a "T" shape?

This is an example of tertiary structures depending on secondary depending on primary. tRNA assumes a "t" shape because there is hydrogen bonding between certain bases; stem structures, and no hydrogen bonding in others; loop structures. This hydrogen bonding pattern can be determined from the primary structure of the molecule and thus dictates the higher structures of tRNA. *One of the four loop structures in a tRNA molecule is the anticodon which provides a binding site for the codon of an mRNA strand.

Who came up with the one gene-one enzyme hypothesis? What was the experiment that founded it? What was it renamed to and why?

Beadle and Tatum discovered this and it was later renamed to one gene-one polypeptide since not all proteins are enzymes. The experiment used fungi that could normally grow on a minimal medium and produce their own arginine protein. When exposed to X-rays, they discovered there were four different mutagens. We now know that arginine synthesis is a multi-step process involving 4 intermediate enzymes. Each type of mutagen was incapable of synthesizing one of these 4 intermediate enzymes and needed it to be present in their culture medium instead. Their conclusion was that each gene encodes one enzyme responsible for a specific function. *Some people believe this was the birth of molecular biology.

Give the general outline of the process a gene undergoes to be converted into a polypeptide (include where the material travels).

First RNA polymerase enzymes transcribe the gene from DNA into a strand of mRNA (messenger). The mRNA strand leaves the nucleus after being processed and binds with a ribosome. The ribosome translates the mRNA strand into a chain of amino acids to make up a polypeptide.

What is a difference in the relationship between translation and transcription in prokaryotes? How is this possible?

These two processes can happen simultaneously in the cytoplasm since the prokaryotes don't have a nucleus.

What are the four letters of the genetic code of DNA (full names)? Which are purines and pyrimidines?

Adenine, Guanine – purine. Thymine, Cytosine – pyrimidine.

What are the four letters of the genetic code of RNA (full names)? Which are purines and pyrimidines?

Adenine, Guanine – purine. Uracil, Cytosine – pyrimidine.

Explain, using a very brief mathematical proof, how scientists deduced that a codon must contain three nitrogenous bases (Hint: they knew there were only 20 amino acids).

If there are 4 bases, depending on the # of bases/codon, the possibilities of amino acids is limited. Ex. if a codon = 1 base, only 4 codons/amino acids are possible. If a codon = 2 bases, there are 42 (16) possible amino acids. Therefore, since they knew there were 20 amino acids each codon must have 3 bases = 43 possibilities (64).

What is the significance of having 64 possible codons and only 20 amino acids? How does this relate to the key properties of the genetic code?

If there are 64 codons and 20 amino acids, there must be degeneracy or redundancy. The genetic code is degenerate meaning that more than one codon can encode the same amino acid.

What is the un-ambiguity of the genetic code?

The genetic is unambiguous. This means that the each codon encodes one amino acid, and one amino acid only. For example, ACG codes for threonine and only threonine. This makes the codons unambiguous (even though, in contrast, multiple codons can code for the same amino acid).

What direction is a codon read? By what?

Codons are read in the 5' to 3' direction, as any genetic material is. They are read by a ribosome.

What amino acid does every polypeptide start with? Why is this the case?

Every polypeptide begins with the amino acid methionine. Methionine is the amino acid coded for by the codon AUG, also called the start codon. This triplet of nitrogenous bases is recognized by the ribosome and begins transcription.

What are the stop/termination codons? What is another common name for them? What do these codons cause?

There are 3 stop codons, aka non-sense codons. These are: UAA, UAG, UGA. These codons mark the end of a transcription unit, and cause the ribosome to dissociate and release the polypeptide chain.

What two amino acids defy the rule of degeneracy in the genetic code? As a result, how many amino acids are coded for by multiple codons?

Tryptophan (UGG) and methionine (AUG) are the only two amino acids that have a single codon. This means there are 20-2 = 18 amino acids coded for by multiple triplets (these are degenerate).

By applying the understanding of how codons are read, explain how a frame shift can be so damaging to a polypeptide.

Codons are read as triplets of nitrogenous bases. The start codon normally aligns the ribosome to begin reading in the correct "reading frame." If a frame shift occurs, the triplets become out of sync and the polypeptide produced becomes complete gibberish (genetically speaking).

Compare and contrast transcription with DNA replication (4 points).

· Only one strand serves as the template (because RNA polymerase can only synthesize one strand at a time). · Only a small portion of a DNA molecule is transcribed because not all DNA encodes genes for polypeptides. · RNA polymerases synthesize the new strand of mRNA (instead of DNA polymerases). · The product mRNA is single stranded instead of double stranded DNA.

What are the three major steps in transcription? Give one key event that happens in each one.

Initiation: RNA polymerase binds to the promoter sequence immediately upstream of the gene. Elongation: the synthesized mRNA strand elongates as RNApol transcribes the gene. Termination: the RNA polymerase enzyme reaches the termination sequence in the transcription unit and dissociates from the DNA molecule.

What does RNA polymerase need to bind to a DNA strand and initiate transcription? Where does this binding occur? (Key: In Eukaryotes*)

RNA polymerase needs to bind to the promoter sequence in order to start transcription. In eukaryotes, transcription factors (proteins) bind to TATA boxes that then recruit RNApol to the promoter sequence.

Explain the difference between eukaryotic and prokaryotic RNA polymerase enzymes (number & function). What are their functions (brief)?

In prokaryotes, there is only one RNA polymerase that carries out all transcription processes. In eukaryotes, there are 3 RNA polymerases (I, II, III). RNA polymerase I transcribes units encoding polypeptides. RNApol II and III transcribe non-polypeptide genes.

Although termination of transcription in eukaryotes is not well understood, prokaryotes are known for having two possible mechanisms to stop RNA polymerase. What are they?

Rho-dependent termination: A rho protein binds to the transcribed mRNA and causes it to form a stem-loop structure, which halts the RNApol enzyme. The rho protein then travels to the RNApol and causes it to dissociate. Rho-independent termination (intrinsic): A stem-loop structure forms without a rho protein (due to GC rich sequences) and has the same effect as above.

What must happen to mRNA before it can exit the nucleus and bind to a ribosome? Briefly describe the two processes.

Only in eukaryotes mRNA directly transcribed from DNA is called pre-mRNA (pre = precursor). These strands must be processed first so that they are not digested in the cytoplasm. Capping: a capping enzyme attaches a molecule to the 5' end of the synthesized RNA strand during transcription. · This is what allows the strand to bind to a ribosome to begin translation. Polyadenylation: The polyadenylation signal sequence (where a 3' poly(A) tail is to be added) in the pre-mRNA strand binds cleavage factors and stabilizing proteins to the sequence. · They cleave the strand at that point and a Poly(A) polymerase adds a long sequence of adenine nucleotides to the 3' end of the cleaved strand. This protects it from RNA digestive enzymes in the cytoplasm.

In the context of transcription, what is the significance of the terms “cis” and “trans”?

In transcription, initiation usually involves an initiation complex. This complex is made up of a promoter sequence and DNA binding proteins. The promoter sequences are called “cis-acting sequences,” and the proteins are called “trans acting proteins/factors.” *“cis” means ‘on the same side as’ and indicates that these sequences are on the same DNA molecule as the gene they initiate. *“trans” in molecular biology means “acting from a different molecule.”

About what percentage of our genome encodes proteins?

~1.5%

What is an mRNA's intron/exon structure? What does a cell do to process/fix this (what complex is involved and how)?

An intron/exon structure is found in most mRNA strands. Introns are non-coding regions of DNA and their origin is still being researched. Since exons are the coding regions, the introns must be spliced out of the gene. A spliceosome: snRNA (small nuclear RNA) combines with various proteins to form a large snRNP complex. These complexes combine with pre-mRNA strands and splices introns and joins exons to form a mature mRNA strand. This is done when the snRNP's bind to either end of the intron and fold it inwards, forcing it into a loop structure and bringing the exon ends closer together. The intron is released as a loop and the exons are joined. (see next question*).
http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120077/bio30.swf::How Spliceosomes Process RNA

What is a lariat structure? What happens to these in the cell?

Lariat structures are the loop form of an intron released from the pre-mRNA strand. These are degraded by enzymes.

What is a ribozyme?

Ribozymes are any RNA molecule that is capable of acting like an enzyme. Some introns can splice themselves acting as a ribozyme!

What is the structure (# of nucleotides, shape/form, degree of structure) and function of a tRNA molecule?

Structure: only about 75-90 nucleotides long. · Secondary structure: Four regions in a strand of tRNA are capable of hydrogen bonding with themselves to form the "t" shape. o One of the arms of the "t" is the acceptor arm (binds to amino acids) and the one opposite this is the anti-codon arm (contains an anti-codon corresponding to its specific amino acid). · Tertiary structure: "t" shape further folds into a coiled L (search google image for tertiary structure tRNA for a picture).

What is the wobble hypothesis?

There are 64 different combinations of the 4 nucleotides into triplet codons. Three of these code for stop codons, and 61 code for amino acids (AUG; methionine is included). There are not 61 different tRNA molecules, so the hypothesis states that a tRNA binds precisely to the first 2 nucleotides in a codon but the third can be changed without needing a different tRNA molecule. Ex. a tRNA could bind to UUU or UUC.

What is Aminoacylation? What is another common name for this process?

Aminoacylation is the addition of an amino acid to a tRNA molecule. This is aka charging because free-energy is added to the molecule (by the amino acid).

What is an Aminoacyl-tRNA? What "converts" tRNA molecules into these? How many of these "converters" are there?

An Aminoacyl-tRNA is a tRNA bound to its "correct" amino acid. Aminoacyl-tRNA synthetases are responsible for catalyzing the addition of the amino acid onto the tRNA molecule. There are 20 of these for the 20 amino acids.

Where are ribosomes found in the eukaryotic and prokaryotic cells?

In prokaryotes they exist throughout the cell. In eukaryotes, they exist in the cytoplasm or anchored to the membrane of the rough endoplasmic reticulum (also in mitochondria & chloroplasts).

What is the general* structure of a ribosome? What are it's "pieces" made of? Is there a difference between prokaryotic and eukaryotic ribosomes? What is it?

A ribosome consists of a large and a small subunit. These subunits are made out of rRNA (ribosomal RNA) and other ribosomal proteins. Prokaryotic and eukaryotic ribosomal proteins are quite different, so a drug that affects one is capable of not having any effect on the other (since they're different).

Describe the large subunit of a ribosome and what it consists of.

The large subunit is made up of three binding site; E, P, and A (from left to right). A: Aminoacyl site binds a new tRNA molecule with its corresponding amino acid. P: the peptidyl site is where the tRNA molecule bonded to a growing peptide is. E: the exit site ejects a tRNA molecule after its amino acid has been transferred to the growing peptide.

What are the three general steps in translation (give one key event of each step)? How do these relate to those for transcription?

Initiation: The ribosome associates with the mRNA strand. Elongation: The ribosome begins reading codons and assembling an amino acid sequence. Termination: The ribosome reaches a stop codon and dissociates from the mRNA as well as the polypeptide. These are the exact same as the three steps in transcription.

What is a Shine-Dalgarno sequence?

Initiation of transcription in prokaryotes involves the small subunit of the ribosome binding to a specific nucleotide sequence (Shine-Dalgarno sequence) that is immediately upstream (5' direction) of the start codon. The initiator methionine-tRNA molecule can then bind to the small subunit and the large subunit can bind to this complex and elongation can start.

How does translation in eukaryotes initiate? What marks the end of the initiation stage?

The initiator tRNA (complementary to AUG start codon; methionine) and the small subunit of a ribosome associate with a strand of mRNA. This tRNA binds with the small subunit to the mRNA strand at the 5' cap of the mRNA molecule and scans it for the AUG/start codon. The large subunit then binds to this tRNA-small subunit complex. The end of initiation is when the methionine-tRNA molecule is in the P site of the formed ribosome.

What nucleotide triphosphate is hydrolyzed by the ribosome to allow it to shift along the mRNA strand?

Guanosine triphosphate.

Explain how the elongation step of translation occurs (how subsequent tRNA's move into and out of the ribosome).

After the methionine tRNA binds to the P site of the ribosome, the next codon recruits the corresponding tRNA molecule to the adjacent A site. The A site can only bind Aminoacyl-tRNA (tRNA with an amino acid), whereas the P site can only bind tRNA with a growing peptidyl chain (as well as methionine tRNA). The binding of Aminoacyl-tRNA triggers the shifting of the ribosome (hydrolysis of GTP) to the next codon, moving the growing peptide chain on the tRNA that was originally in the A site. The tRNA that was in the P site enters the E (exit) site and the A site tRNA with the peptide chain occupies the P site in its place. This leaves an available A site for the next corresponding Aminoacyl-tRNA.

What is a release factor and how is it related to the process of translation?

Release factors (RF's) are the equivalent of tRNA's but bind only to the 3 stop codons (UAA, UAG, UGA). The binding of an RF to a stop codon in the A site of the ribosome causes the ribosome to dissociate from the mRNA strand and release the polypeptide.

What is a polysome?

Polysome is a shortened word for poly-ribosomes. This is a strand of mRNA with several ribosomes attached to it and undergoing translation. If you drew a polysome, the polypeptide chains exiting the ribosomes would grow longer and longer as the ribosomes are farther along the mRNA strand.

How and where is simultaneous transcription and translation possible?

This can only occur in prokaryotes or organisms with no nucleus! A strand of DNA in these organisms can be transcribed into a strand of mRNA and simultaneously this strand of mRNA can be attached to and translated by one or several ribosomes (on the same strand) to produce a peptide chain. This means a strand of DNA could have several strands of mRNA branching off it from RNA polymerase enzymes, and each mRNA strand could be part of a polysome being translated into several polypeptides.

What is the main reason gene regulation is crucial for an organism to function properly? Give an example using food sources in the surrounding environment and dynamic gene expression.

Organisms have to be dynamic. They need to be able to adapt to the environment they find themselves in. Depending on their environment, an organism may want or need to change the expression of certain genes. If two food sources are present, but one is more favorable to digest, the genes encoding enzymes for digestion must be expressed selectively.

List the key components of a generic operon. In what organisms are operons found?

Operons belong solely to prokaryotes. An operon is a DNA sequence that is involved in the transcription and regulation of one or more genes in the organism's genome. Operator sequence: a short sequence directly upstream of the transcription unit (gene sequence). Regulator proteins (repressors & activators) can bind here. Promoter sequence: Found within or adjacent to the operator sequence. RNA polymerases bind here. *Other elements can exist depending on the operon, these are in the next few questions.

What is the role of a regulatory protein in transcription? Where do they bind in prokaryotes? What are the two types of regulatory proteins (what do they do)?

In prokaryotes, these proteins bind to the operator sequence upstream of the transcription unit (contains the genes) and control whether RNA polymerase can bind or not. Regulatory proteins are either repressors or activators. Self-evidently, a repressor inhibits the binding of RNApol and an activator promotes it.

What are the three genes of the lac operon? What is their combined function?

LacZ (B-galactosidase), LacY (permease), LacA (transacetylase; function unknown). Their overall function is to digest lactose.

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Describe the major elements of the lac operon and how they contribute to its transcription and/or regulation.

Operator: directly adjacent to the transcription unit. This is a site of binding for the repressor molecule. Promoter: upstream and adjacent to the operator sequence, this is the site of binding for RNApol. CAP binding site: Directly upstream to the promoter sequence. This is a binding site for the cAMP-CAP complex that recruits RNA polymerase. Transcription Unit: Contains 3 genes; LacZ, LacY, LacA. *LacI Gene: Upstream of the entire operon but still considered to be part of it. It encodes the repressor molecule.

How does the Lac repressor molecule act on the Lac operon (affect on RNA polymerase)? How is it synthesized and in what "state" or form?

The Lac repressor molecule is coded for by the LacI gene upstream of the Lac operon (but still considered a part of it). The repressor is synthesized in active form, and binds to the operator sequence directly upstream of the lac operon's transcription unit. It's binding prevents RNA polymerase from transcribing the lac operon's 3 genes.

Using your knowledge of the lac operon, explain how the presence and absence of lactose affects its transcription. Apply this to explain why lactose is called an inducer.

The lac repressor molecule is synthesized in the active form by the LacI gene. When lactose is absent, the lac repressor can bind to the operator sequence and prevent RNA polymerase from transcribing the genes. When lactose is present, it binds (actually a semi-digested form does; allolactose) to the repressor molecule and changes its conformation. The repressor molecule can no longer bind to the operator and the genes for lactose metabolism are transcribed. Since the presence of lactose increases transcription, it is called an inducer. *Extra info: B-galactosidase (encoded by LacZ gene) converts lactose into allolactose that binds to the repressor molecule. This enzyme is encoded by other genes, so its presence is not solely controlled by the expression of the lac operon.

What drugs can be used to study lactose metabolism in the lab (there are 2)?

IPTG is an analogue of allolactose that can not be broken down. Also X-gal is an analogue of lactose that turns blue when it is broken down by B-galactosidase. Possible applications: X-gal could be used to test for the presence of B-galactosidase. IPTG could keep the lac operon turned on continuously as it can't be broken down.

What is negative control? Explain with aid of an example from the lac operon.

Negative control is when a gene is regulated by a repressor and whether or not it is active. For example, the lac repressor molecule is synthesized in the active form and negatively controls these genes as it prevents the binding of RNA polymerase.

What is positive gene control? Explain with aid of an example from the lac operon.

Positive control is when a gene is regulated by an activator. An activator is a molecule that promotes the binding of RNA polymerase and induces transcription. In the control of the lac operon, a catabolite activator protein (CAP) is synthesized in its inactive form. When glucose is present in the cell's medium, little cAMP is produced in the cell. cAMP activates CAP by binding to it, which can then bind to the CAP site directly upstream to the promoter sequence. This complex recruits RNA polymerase to the promoter region, and activates transcription (assuming a repressor isn't binded to the operator sequence).

What is tryptophan, and why do prokaryotes need an operon for it?

Trytophan is an amino acid that is involved in the synthesis of proteins. Prokaryotes need an operon for its synthesis because the cell needs it whether it is present in the medium or not. If Trp is not present in the medium, the cell must produce it by its own means.

What are the main sequences/regions of the Trp operon (there are 4)?

Transcription Unit: Contains genes TrpE to TrpA (left to right). Trp Operator Sequence: directly upstream of the transcription unit. This is the binding site for the repressor molecule (no activator in this operon). Trp Promoter Sequence: directly upstream of the operator sequence. This is the binding site for RNApol. TrpR gene: similar to the LacI in the lac operon; upstream of all other elements. This gene encodes the repressor molecule that is synthesized in it's inactive form.

How does the Trp repressor molecule work? Why is tryptophan considered to be a co-repressor?

When tryptophan is present in the cell's medium, its biosynthesis pathway does not need to be turned on. As a result, tryptophan binds to the repressor and activates the repressor molecule allowing it to inhibit transcription of the 5 genes by binding to the operator sequence and preventing RNApol from binding. For this reason, tryptophan is considered to be a co-repressor as it "helps" repress the transcription unit.

What is a repressible operon? Explain this term by applying it to the Trp operon in prokaryotes.

A repressible operon is an operon that is always active unless it is repressed. In prokaryotes, unless tryptophan is present in the medium, its biosynthesis pathway needs to be active continuously since it is needed in the synthesis of all proteins.

What is a regulon?

A regulon is a group of operons that are all controlled by one common regulator. An example is cAMP; it controls many operons encoding digestive enzymes.

Why are there considered to be more ways a eukaryotic cell can regulate gene expression compared to a prokaryote? What are these 4 ways?

The processes of transcription and translation occur separately in eukaryotic cells. The nuclear membrane physically prevents them from happening simultaneously, so numerous levels of control exist. The 4 ways are: transcriptional, post-transcriptional, translational, post-translation.

What are the 4 ways of regulating gene expression at the transcriptional level in eukaryotes?

Chromatin remodeling: altering the accessibility of promoter regions for RNApol to bind. Transcription initiation: changing the ability of RNApol to bind to the promoter region. Combinatorial Gene Regulation: the sum of repression and activation of the expression of a gene. DNA Methylation: Methylation of the DNA or more specifically the promoter regions of genes silences them.

What is chromatin remodeling an example of? What is Dr. Johnson's common saying about the mechanism of ALL these models (hint: cis & trans)?

Chromatin remodeling is the altering of the chromatin structure to change the accessibility of promoter regions where RNApol enzymes bind. The 3 models are: *"They all involve the mechanism of a cis-acting sequence and a trans-acting protein."

How does chromatin remodeling work (the 3 models)? *Long answer*

Activator-Complex Model: an activator protein (trans-acting protein) binds to a regulatory sequence (cis-acting protein) upstream of the target gene. The activator recruits a remodeling complex. This remodeling complex essentially takes over the H1 linker proteins and separates two adjacent nucleosomes, revealing the promoter sequence of the target gene to RNApol enzymes. Acetylation Model: An activator protein (trans-acting protein) binds to a regulatory sequence (cis-acting sequence) upstream of the target gene. This recruits an acetylase enzyme that acetylates the histone proteins in the nucleosomes where the promoter of the target gene is. When histones are acetylated (adding CH3CO) they lose their association with the DNA making the promoter accessible to RNApol enzymes. De-acetylation Model: This is the same as the previous model except de-acetylase enzymes are recruited to make promoters of target

How is transcription initiation regulated by a cell (hint: the three regions/sequences on the DNA/operon that can regulate RNApol binding and activity)?

TATA boxes: in eukaryotes, these bind transcription factors that recruit RNApol enzymes. Promoter proximal regions: these are binding sites for activator proteins such as CAP in the lac operon that recruit RNApol enzymes. Enhancers: can maximize RNApol rate of transcription.

What is a TATA box? How does it work?

In eukaryotes, these are sequences (TATAT) within a promoter region neighboring its gene involved in transcriptional gene regulation. These are binding sites for general transcription factors which can in turn recruit RNA polymerase II to transcribe the gene. The transcription-initiation complex can then begin transcription. RNApol II cannot bind to the promoter region otherwise. *On its own, this complex produces few mRNA strands (maximal rate not enhanced by enhancer; 2 questions following this).

What is a promoter proximal region? How does it work (with respect to transcriptional gene regulation)?

Promoter proximal regions are nucleotide sequences directly adjacent to and upstream of the promoter region. They contain promoter proximal elements: part of a system to increase the rate of transcription for the given gene. These elements are binding sites for Activator proteins. Activator proteins induce the binding of transcription factors and promote the binding of RNApol. *An example is the CAP binding site in the lac operon (a promoter proximal element & region).

What is an enhancer region? What is its function in relation to transcriptional gene regulation?

Enhancers are distant from the beginning of the gene and contain regulatory sequences that control the gene's maximum transcription rate. These regulatory sequences provide binding sites for specific activator proteins (other activators bind to promoter proximal regions). These proteins then interact with a large multi-protein complex (floating around) and bond. Now the enhancer region, bound to an activator protein, bound to a large multi-protein complex (a co-activator) is pulled towards the promoter proximal region where other activator proteins then bind to the large multi-protein complex for stabilization. This makes the DNA strand form a loop and the enhancer is now directly above the promoter region of its gene. This activity stimulates RNApol II to transcribe at its maximal rate. http://bcs.whfreeman.com/thelifewire/content/chp14/1402002.html

What is combinatorial gene regulation?

This is the combination of activator and repressor molecules and their net effect on the transcription of a gene. This regulation involves all forms of activation by promoter proximal regions, activator proteins, and enhancer regions. Repression involves repressor molecules.

What is the effect of DNA Methylation? Why is it significant to a cell's gene regulation (hint: silencing)?

DNA Methylation is the addition of methyl groups to cytosine bases (producing 5-methylcytosine). The addition of methyl groups to the cytosine bases in a promoter region inhibits the binding of RNApol and therefore silences the gene.

What are Barr bodies? What is their function in humans?

Barr bodies are extremely condensed female X chromosomes. Their purpose is to equalize the amount of gene expression on X chromosomes between men and women (since women have XX and men have XY).

What is genomic imprinting? What does it signify? What "code" is it a part of?

Genomic imprinting is the "imprinting" of a gene that keeps this imprinting when it is passed on to their offspring. The expression of certain genes in parents will change throughout their lifetime and experiences. Depending on each parent, some silenced genes may be passed on to their offspring who would share the same gene expression! It is part of the epigenetic code.

What is the basis of post-transcriptional regulation? (Hint: availability of _____)

The basis of this type of regulation is to change the availability of mRNA to ribosomes in the cytoplasm.

What are the three possible variations in pre-mRNA processing we learned? How do they regulate the translation of these genes? (Hint: all these happen/start before exiting the nucleus)

Alternative splicing – Various regulatory proteins can bind to the pre-mRNA strand to influence its splicing of introns. This will affect or regulate the translation of the protein into a peptide chain. Masking proteins – various proteins can bind to mRNA making them unavailable to ribosomes. RNA breakdown – proteins and complexes especially including the exosome can breakdown RNA before it leaves the nucleus (and also in the cytoplasm). *Exosomes exist in the cytoplasm and in the nucleus.

What is miRNA? How does miRNA regulate the expression of a gene? (Hint: there are 2 ways it affects mRNA in the cytoplasm)

miRNA stands for micro RNA. There are genes in our genome that encode miRNA. When an RNApol enzyme transcribes these genes, the pre-mRNA product hydrogen bonds with itself and forms a hairpin (stem-loop) structure. After exiting the nucleus, an enzyme dicer removes the loop leaving double stranded miRNA with a few nucleotides overhanging each end of the strands. These overhangs make it subject to binding with RISC (RNA induced silencing complex) that unwinds the duplex and one strand is degraded. The remaining single strand and RISC can either bind to mRNA being transcribed and physically inhibit the ribosome or bind to a free mRNA strand and recruit slicer that splices the mRNA at two points making it aberrant and targeted for degradation.
http://www.biosolutions.info/2007/10/microrna.html

What is siRNA? Where does it come from? How does siRNA regulate the expression of a gene? (Hint: very similar to miRNA, previous question)

siRNA stands for small interfering RNA. This can only come from either an RNA virus or an artificial lab injection. Once in the cytoplasm of a cell, the enzyme dicer cleaves the duplex and leaves a short overhang on each end. This can then bind to RISC (RNA-induced silencing complex). RISC unwinds the duplex and one of the strands is degraded. The RISC-single strand siRNA complex can then do two things: bind to an mRNA undergoing translation and physically inhibit the ribosome, or bind to a free mRNA and recruit slicer which cuts the mRNA strand at two points making it aberrant and targeted for degradation. http://www.youtube.com/watch?v=Fa4skYBJHoI

How can a cell regulate gene expression at the translational level? (hint: degradation)

This regulation involves controlling the "survival" time of mRNA in the cytoplasm. This has to primarily with the length of the poly-adenosine tail on the mRNA strand. Certain enzymes in the cytoplasm can shorten and lengthen this. The tail is in direct proportion to the length of time the mRNA will remain intact in the cytoplasm.

What is the key concept in post-translational regulation? (hint: functional proteins) What are the three examples we learned in class?

Post-translational regulation involves the modification of proteins after they have been translated from a ribosome. These modifications will change whether or not the protein is functional. If the protein is modified to be non-functional, this is equivalent to silencing its respective gene. We learned three examples: Chemical Modification – addition or removal of chemical groups from proteins can change their functionality. Ex. phosphorylation. Processing – Some proteins leave the ribosome "unfinished," as a precursor molecule. These must be processed afterwards to become functional. This step can be controlled by the proteins that are involved in it. Ubiquitin Degradation – after a protein is translated it can be tagged by a chain of ubiquitin proteins. This chain is recognized by a proteasome which is a large protein complex in the cytoplasm. This complex digests the protein into its amino acids. http://www.sinauer.com/cooper5e/animation0802.html

What is the overall concept of protein targeting?

This idea is that every cellular compartment has proteins. Most proteins start translation in the cytoplasm. Therefore, in order to end up in the right cellular compartment they must be “shipped” or targeted.

What is a pre-protein and a pre-pro-protein?

A pre-protein is a protein with 2 signals. A pro-protein is a protein with 1 signal.

What is the basic model for any protein targeting pathway? (Hint: 4 steps starting with translation)

Translation: Initiation of protein synthesis. Interaction with receptor (unfolding) · Protein interacts with a receptor that recognizes it, usually they undergo some folding/unfolding process by chaperons. Translocation: the protein passes from one compartment to the other. Refolding/processing: chaperons again. *Sometimes the signal is cleaved by proteases (not always)!

What signal is involved in the default pathway? How does a protein with this signal get translocated into the lumen of the ER? What happens once it gets there? What are the other possible final destinations of these proteins?

This is the pathway followed by many proteins with a signal. This pathway only includes proteins with an amino terminal signal (the end that is first to exit the ribosome during translation). The protein with an amino terminal signal binds with a protein-RNA complex called the signal receptor particle (SRP). This SRP-ribosome complex stalls translation and translocates the translation complex to the outside of the rough ER (it is called rough because of the ribosomes on its membrane). The SRP-ribosome complex binds to an SRP receptor on the membrane of the rough ER which resumes translation. The protein being translated grows through the receptor into the lumen of the rough ER. Once inside, a signal peptidase cleaves the amino terminal signal and a chaperone protein covers the translocated protein to make sure it re-folds correctly. Depending on the protein, it can either remain in the ER, be transported to the Golgi complex, or exported from

What must a protein have if it is to remain in the lumen of the rough ER? What differs between plants and animals in this retention signal?

A signal, either HDEL or KDEL (below) is a C terminus signal that instructs proteins in the rough ER to be retained. KDEL is recognized in animals (proteins with this signal are instructed to be retained in the rough ER—"anchored"). KDEL and HDEL are recognized in plants (same effect as in animals).

If a cell is to be transported from the lumen of the rough ER to the Golgi complex, what must it have? How does the translocation happen?

In the ER there are enzymes that add sugars to proteins. These sugars are very large and are added in a process called N-linked glycosylation (these sugars usually look like a Christmas tree). It is called N-linked glycosylation because it involves the addition of a sugar to the N terminus of a protein. There has to be a signal for these enzymes to add these complex sugars. These glycoproteins are then budded off of the ER in vesicles towards the cis face of the Golgi complex. *The cis face is the side of the Golgi nearest the ER.

What signal must a protein have to make sure that it is sent to the lysosome (in animals) from the Golgi complex? If it doesn't have this signal, what is its destination?

A protein must have a mannose-6-phosphate signal in order to "instruct" the Golgi complex to send it to a lysosome (in animals). Without this signal, the protein is transported through the Golgi, budded into a vesicle, and sent to the plasmamembrane for export.

When a protein has the M6P signal, what happens to it starting from the Golgi complex?

With the mannose-6-phosphate signal, a phosphatase enzyme phosphorylates the protein. This protein passes through the layers of the Golgi and reaches a mannose-6-phosphate (M6P) receptor. This receptor anchors the protein so that it can be budded into a vesicle that ships it to the lysosome (from the trans face of the Golgi). In the lysosome, the acidic environment dissociates the protein from the anchored receptor and it forms a mature lysosomal hydrolase. The receptor is then re-budded back to the Golgi for recycling. *Hydrolases are used to break down waste in the cell.

Why must proteins be imported into the mitochondria?

The vast majority of proteins used by the mitochondria are encoded in the genome within the nucleus of the cell. Therefore they must be sent from their point of translation to the mitochondria.

If a protein has the signal that "ships" it to the mitochondria, what are the two possible destinations of this protein? (hint: think about the membranes of the mitochondria)

The mitochondria has a double membrane system. A protein can either be shipped directly into the lumen of the mitochondria (through both membranes) or to the intermembrane space.

A protein has the signal that sends it to the lumen of the mitochondria. How is it translocated to its destination?

A "Tom" complex binds to the signal on the protein. The protein is pulled into the lumen of the mitochondria across both membranes by the aid of chaperone proteins. Chaperone proteins also help the protein to refold. A signal peptidase then cleaves the signal from the protein and it assumes its function in the lumen. Ex. DHFR undergoes this exact pathway.

A protein has the signal that sends it to the intermembrane space of the mitochondria. How is it translocated to its destination?

A protein-receptor complex called "Tom" recognizes the signal and pulls it into the mitochondrial lumen with the aid of chaperone proteins. A signal peptidase cleaves the signal, however the protein has a second signal. The second signal from the matrix of the mitochondrion to the intermembrane space through a similar mechanism. The second signal binds to its receptor. The receptor pulls the protein into the intermembrane space. Ex. Cytochrome C1 undergoes this pathway as it functions in the intermembrane space of the mitochondria.

How many signals does a protein need to get from the cytoplasm to the thylakoids of a chloroplast? Why is this the case? How does this relate to proteins entering the intermembrane space of the mitochondrian?

A protein needs two signals to enter the thylakoids of a chloroplast. This is because it needs one to be shipped across the double membrane of the organelle, but needs another to be receive on the outer membrane of the thyalkoids and shipped into them. A protein entering the intermembrane space of a mitochondria uses the same number of signals and a similar mechanism.

Can a pre-pro-protein enter the thylakoids of a chloroplast? (Hint: how many signals do these proteins have?) If no, why not? If yes, how is it done and what is the final product of these translocations?

Yes it can, a pre-pro-protein has two signals. The first is used to be received by the receptor on the outer membrane of the chloroplast. The protein is translocated into the stroma of the chloroplast where a signal peptidase cleaves this first signal. Once cleaved, the second signal is revealed allowing the receptor on the thylakoids to bind to it and translocate the protein into the lumen of the thylakoids. Once inside the thylakoids, the second signal is cleaved and the pre-pro-protein is now just a protein (both signals have been removed).

Since most proteins and RNA's are too large to passively diffuse into the nucleus through the nuclear pores, what are the two ways these proteins can be transported into the nucleus? What happens once they are in the nucleus?

Proteins with a specific sequence of amino acids called a nuclear localization signal (NLS) can enter the nucleus. These signals bind to receptors on the nuclear membrane (they are part of the nuclear pores). The protein is then pulled through the membrane by the same model as any other translocation (chaperone un/folding). However, this signal is not usually cleaved since NLS's are not terminal and by cleaving them the protein would be destroyed. The other mechanism involves proteins that are capable of binding to other molecules in the cytoplasm that have NLS's. The same mechanism imports these proteins into the nucleus and they are not cleaved either.

We saw four different protein pathways in this course (default, mitochondria, chloroplast, nucleus). How do these pathways differ in terms of signal cleavage? (Hint: think of where the signal is on the protein)

In all but the nuclear entry pathway, the signals are terminal. Therefore all these signals are cleaved once they enter their destination. A nuclear localization signal is internal and therefore is not cleaved since it would render these proteins non-functional. *An exception to this rule is found in Peroxisomes where the signal is terminal (carboxy terminus) but not cleaved.

What is the secretory pathway?

The secretory pathway is essentially the default pathway a protein undergoes. When a protein has the SRP signal (signal receptor particle) it is translocated to the lumen of the rough ER. From there, unless it has an ER retention signal (H/KDEL) it will undergo glycosylation and be sent to the Golgi complex. Unless these proteins have a mannose-6-phosphate signal, they will be budded in vesicles that are carried by kinesin motor proteins to the peripheries of the cell. Once they fuse with the plasmamembrane their contents are released to the outside.

Give a brief explanation of the different stages in interphase.

G0: cellular arrest. The cell usually enters cellular arrest from G1 but certain cells can enter G0 from G2 (liver cells). G1: growth; synthesis of macromolecules, duplication of organelles. The cell is basically growing/developing mass S: synthesis of new DNA (DNA replication) and DNA-related proteins (histones). G2: Growth, "things" for division (centrioles, early spindle fibers).

Why are checkpoints necessary in the cell cycle? (Hint: two main reasons; viability, external coordination)

Checkpoints are needed to ensure that all the processes of the cell cycle happen at the same speed so that mitotic division only occurs when the cell is prepared for it. Otherwise, inviable or non-identical daughter cells would be produced through mitotic division. Also, multicellular organisms must grow at the same rate so coordinated growth is necessary.

What are cyclin-dependent kinases?

These are protein complexes that phosphorylate other proteins. They are only active when bound to cyclin.

What is cyclin?

A family of proteins that are involved in the regulation of the cell cycle and its transition between phases.

How can cyclin proteins be regulated in the cell? (Hint: think gene regulation; ex. transcriptional)…3 ways

Transcriptional control: Gene expression for cyclin proteins. Post-translation control: degrading cyclin proteins that are present in the cell. · The ubiquitin target pathway: · A proteasome degrades ubiquitin-tagged proteins through proteolysis. Location control: the expression of the genes encoding cyclin proteins are not changed, but their location is. They can be moved between the nucleus and the cytoplasm so that their concentration in certain compartments of the cell changes and thus controls its activity.

What chemical is used in the lab to observe levels or concentrations of cyclin in the cell?

GFP; green fluorescent protein.

What is an MPF? Why is it significant to the cell cycle?

MPF stands for maturation promoting factor. It is a complex of cyclin and cyclin-dependent kinases. When cyclins bind to a cyclin-dependent kinase, it activates and can phosphorylate key proteins involved in cell division. Phosphorylation is like a "switch" for proteins, turning them on or off. In our course, we learned that MPF triggers the transition from G2 to M (mitosis) phase. **Dr. Johnson believes that MPF represents only the CDK1-cyclin B complex. He told us that we can refer to any CDK-cyclin complex as an MPF however. Just remember that MPF triggers the G2 to M transition.

With the knowledge that an MPF is made of cyclin and CDK, and triggers the transition from G2 to M phase, list the phases of the cell cycle with a statement regarding the levels of cyclin and CDK. (Hint: do CDK levels change?)

G1: Very low cyclin and CDK levels constant but inactive (see why below; questions on P27). Practically no MPF. S: Increasing levels of cyclin, CDK levels constant but inactive. G2: Higher levels of cyclin, CDK levels constant. Significant amounts of MPF. M: Peak level of cyclin quickly begins to lower as MPF concentrations are maximum (MPF complexes involve cyclin, so their concentrations are inversely proportional).

What mechanisms can regulate the activity of CDKs?

Although the concentration of CDK's doesn't change, their activity can be regulated. Phosphorylation state: CDK's need to be phosphorylated to be active and capable of binding to cyclins. Inhibitors: These bind to CDK's and prevent cyclins from binding.

Where do cyclin proteins exist in a cell at the different phases of mitosis? (Hint: interphase vs. M phase)

In interphase, cyclins are in the cytoplasm or other organelles in the cytoplasm. In M phase cyclins are shuttled to the nucleus so that they can carry out their function by combining with CDK here and phosphorylating key proteins involved in cell division.

What is P27? How does it affect the cell cycle?

P27 is a class of enzymes that inhibit cyclin-dependent kinases (CDK's). CDK's bind with cyclin proteins to form an MPF complex that transitions the cell from G2 to M phase. P27 prevents this transition by inhibiting CDK from binding to cyclin.

With the knowledge that P27 enzymes are targeted by ubiquitin for degradation, how does its concentration in a cell relate to the level of CDK activity distributed over the cell cycle? (Hint: CDK activity starts to increase during G1). Why is this important for the control of the transition from G2 to M phase?

P27 proteins are tagged with ubiquitin to be degraded at a specific time in the cell cycle. P27 is an inhibitor of CDK, lowering its activity when P27 exists. G0: CDK activity very low, P27 high levels. G1: CDK activity slightly higher, P27 lower levels (beginning of ubiquitin-tagged degradation). S: CDK activity continuing to raise, P27 levels continue to decrease. G2: CDK activity reaches a peak and P27 levels reach a minimum. Through this regulation, CDK levels are only high during G2 phase when cyclins are present and can bind to form an MPF complex responsible for the transition from G2 into M phase.

How does cytokinesis differ between eukaryotes and prokaryotes?

In eukaryotes, a structure called the contractile ring made up of actin filaments and myosin II filaments pinches the two halves of the cell apart (similar to muscle contraction mechanism). In prokaryotes, a structure called the Z ring carries out this function.

How does cell division occur in eukaryotes vs. prokaryotes? Which method came first?

Eukaryotes undergo mitotic cell divison. Prokaryotes undergo binary fission. Since binary fission is a more primitive method, mitosis must have been derived from it.