

Molecular cell biology study guide exam assignment



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Protein Folding & Post-translational Modifications -What are post-translational modifications? – are modifications that a proteins undergoes to achieve its mature state. Such as cutting, folding, splicing and other processes. -How do proteins achieve their final conformation? – A protein achieves its final conformation by spontaneously folding. All the information that the protein needs as to how to fold is already located in the amino acid sequence of the protein. -Why is conformation important?

The two most important determinants of protein function are shape (conformation) and the cellular location. The protein MUST have the right shape and be localized in the right cellular location to be able to perform its job. Folding: -What's the role of chaperons? – Help maintain the protein unfolded. Preventing incorrect folding and protein aggregation. Also allows the protein to achieve its correct conformation -What do Chaperoning do? How is that different from what chaperons do? – Chaperons help maintain the protein in an open conformation, therefore preventing aggregation and misfiling.

Chaperoning provide and “ isolated” environment for proteins to fold in the absence of other interfering factors. What kind of physiological conditions trigger an perpetuation in the synthesis of chaperons? A/Heat shock and other stress conditions. Hence their name “ Heat Shock Proteins”. These proteins are thought to stabilize and facilitate the refolding of proteins that have been partially denatured as a result of exposure to elevate temperatures or other stress conditions. -What's the role of PDP (Protein Disulfide Isomerism)? How does it contribute to protein folding?

Does it play a role in the folding of cystolic proteins? Where is PDP located, I. E. In which cellular organelle? - Facilitates the formation of different disulfide bonds so that proteins can try different disulfide bonds until they find one that is more in agreement with the same that is dictated with the primary sequence of the protein. It is located in the Endoplasmic Reticulum (ER) and it is a critical chaperone and catalyst of protein folding. Protein Folding and Disease: -Are there any diseases associated to defects in protein folding? - Yes, Osteogenesis Imperfecta (OI) not being able to make bones the right way.

The most extreme lead to early abortions, spontaneous abortions due to the defect being so bad that the organism will not go through fetal development. Least extreme is people that tend to break their bones very easily. This is due to the shape of collagen is not the proper shape associated with mutations in the gene for collagen. Huntington Disease associated with hunting protein associated with defects in your central nervous system. Comes when the # of repeats of CAG (glutamine) more than the normal times in the protein, you end up with protein aggregation. The protein cannot fold and it aggregates leading to cell death.

This disease is associated with coordination and movement that's the reason why early symptoms they have shaky movements, movements they cannot control. Similar to Parkinson yet in this case its not only one limb it's the whole body. Protein aggregation can be toxic to cell = cell death -What is Huntington disease associated to? A/ Expansion of Poly-Glutamine (Q) repeats in the sequence of Huntingtin. 36 or less = normal protein; 36 or more = mutated Huntingtin - the protein is misfolded and misfiled - leads

to neuron death. (see answer above as well) -What is Alzheimer's disease associated to?

A/ Misfolded Amyloid Precursor Protein (APP). Cell death). Therefore these long ubiquitin's chains are added to the proteins to be guided to their final destination in the protease. -What's the main effect mediated by the conjugation of Ubiquitin to a target protein? A/ Ubiquitination usually targets the modified protein to be degraded by the PROTEASE. They contain many ubiquitin in a long poly tail. -By the way, what's the PROTEASE? – a protease (can be thought of as a shredder machine) it is found in the cell that shoots out the shredded proteins within the cell.

Elimination of proteins is due to age(they get old) which leads to taking shape that they shouldn't have (aggregation -> cell death). Therefore these long ubiquitin's chains are added to the proteins to be guided to their final destination on the protease. – We can deactivate the protease to test if a protein can be absentminded. By adding something. When we do that, we see 10-1 kDa with every ubiquitin is added. -What's the main effect mediated by the conjugation of SUMO to a target protein? -A place and a time for everything ... -Keep in mind where each type of post-translational modification occurs within the cell.

Which ones occur in the cytoplasm? How about in the ER lumen? Also, keep in mind that each type of post-translational modification can be exerted only on specific amino acid residues. Finally, remember that each type of post-translational modification produces a change in the MOLECULAR WEIGHT of the protein affected (check the in-class activity!!!). [pick] The Nucleus

Nuclear Import requires the formation of a complex between the import factor and the target protein containing the Nuclear Localization Signal (NLS). Sometimes the import factor required is composed of two different subunits.

This is the case for many proteins carrying classical Nils. The two import factors recognize the NLS and demerit, and carry the cargo containing the NLS across the Nuclear Pore Complex (NP). Inside the nucleus, Arrant binds to the importing and induces a structural change in the importing which leads to the disassembly of the import complex. The cargo is therefore released inside the nucleus. Next, the Importing-Arrant complex is exported to the cytoplasm, where Arrant finds its Stage activating factor: Range. Range activates the Stage activity of Arrant, thus leading to the hydrolysis of GET to produce GAP.

This change from GET to GAP triggers a conformation change in Ran which destabilize the export complex therefore releasing the importing in the cytoplasm, ready for another cycle of nuclear import. Nuclear export requires the presence of GET to allow the formation of the trig-molecular nuclear export complex. This complex is formed by Arrant, the Exporting (CRM or some other exporting), and the cargo protein containing the Nuclear Export Signal (NEST). The trig-molecular complex goes through the NP and reaches the systolic side of the NP. There, the trig-molecular export complex finds Range which activates the Stage activity of Ran.

Ran hydrolysis GET to GAP and this produces the dissociation of the export complex in the cytoplasm. The continues traffic of Arrant to the cytoplasm

would over time lead to a decrease in the nuclear concentration of Ran. To prevent this, Ran is imported back into the nucleus by using another import specific for Ran: . NET. . NET forms an import complex with Ran and delivers Ran into the nucleus. In the nucleus, the presence of the Ran-Exchange Factor (REF) allows the exchange of GAP for GEF, therefore regenerating Ran in the nucleus. -What are the main structural components of the nucleus?

A/ Nuclear envelope (Outer Nuclear Membrane, Inner Nuclear Membrane), Nuclear Lamina, and Nuclear Pore Complexes. -How is the perinuclear space in comparison with the ER lumen? A/ Identical! -How is the Outer Nuclear Membrane in comparison with the ER membrane? A/ Identical!!! -How is the Inner Nuclear Membrane in comparison with the ER membrane and the Outer Nuclear Membrane? -A/ Different!!! The INN contains proteins that are specific to the INN and does not have ribosomes associated to it. -Two proteins specific to the INN are Emerin and Lamina B Receptor (LBR).

What's the role of LBR during the re-formation of the nucleus after mitosis? –

Due to the Lamina ability to associate to DNA, the DNA will begin to get coated by these vesicles, which will surround the DNA and then fuse (vesicle fusion). When these vesicles fuse they then begin to form a larger membrane that can now coat the chromosomal DNA and these separately coated chromosomes can then tuck into each other that then completely reform the nucleus. When the nucleus reforms, the Lamina are no longer phosphorylated which allows for the order of the scaffolding structure to return –

What's the main role of the nuclear lamina? The Nuclear Lamina is a mesh of filaments, proteins that provide physical support to the nuclear
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envelope. -What's the main structural component of the nuclear lamina?

How is it regulated? - Lamina, regulated by phosphorescently

(phosphorescently triggers the disassembly of the scaffolding structure formed by the lamina). The proteins or filaments that form that mesh are known as Lamina and there are four different Lamina (A, 81, 82, and C).

These proteins form homo dimmers in which the heads are attached to the heads and the tails are right next to the tails.

Each one of these dimmers that forms is then able to interact with another dimer and these interactions are head to tail interaction. These Lamina are post-translationally modified by the addition of lipids O(foresation). They also interact with proteins of the inner clear membrane such as Emerge and Lamina B-Receptor. Can also be disrupted by phosphorescently. -The nuclear pore complex is enormous; however it is made of only about 35 different - Each segment of the October is identical. Proteins. How come? -How many Nuclear Pores are there per nucleus? How is that of any relevance?

There are -?? 3, 000 - 4, 000 pores per nucleus. -What goes through the Nuclear Pore Complex (NP)? - RNA, proteins, sugars, ions, amino acids, nucleotides, small proteins, etc. Protein Transport across the Nuclear Pore Complex: -What's the size limit for free diffusion across the NP? A/ Proteins larger than -?? 40 KDE are not able to diffuse freely across the NP and therefore require transport factors. -Why is it that the NP restricts the passage of proteins across its lumen? -RNA doesn't go through the NP unless accompanied by specific proteins that act as " carriers"- DNA does NOT go through it. Proteins larger then KDE don't go through the NP unless

accompanied by specific proteins that act as " carriers" (koshering). - All <https://assignbuster.com/molecular-cell-biology-study-guide-exam-assignment/>

other molecules (sugars, ions, amino acids, nucleotides, small proteins, etc.) go through without any specific control (aqueous channel). -What are Nucleotides? The proteins that are structural components on the NP are known as nucleotides (Naps). One important feature of the nucleotides, particularly the ones facing the aqueous channel, is that they contain repeats of two amino acids (Phew, Ugly or FOG repeats).

These FOG repeats are what provide the NP with selectivity by their very disorganized shapes that can form. -How is the Ran GTP gradient across the Nuclear Envelope generated? Where is the Ran GTPase Activating Protein (RanGAP) localized? Where is the Ran GEF Exchange Factor localized? What does each one of these factors do? - The regulator - Arrant jugulate the activity of both Importing and Exporting and it does so by regulating the formation and disruption of Importing as well as Exporting complexes.

Arrant is able to regulate import as well as export by forming what is known as the Arrant gradient. A. Ran is a GTPase (protein that can hydrolyze GTP). B. Ran exists in two different forms: 1. GEF-bound 1'. GAP-bound c. Like all GTPases, Ran has two co-factors: 1. GTPase Activating protein (GAP) - Ran GAP (GEE) - IIRC 1'. GEF Exchange Factor - Arrant is in very high concentrations inside the nucleus whereas RanGTP is in very high concentrations inside the cytoplasm. The reason for this gradient across the nuclear envelope is the location of the Ran GTPase Activating Protein and the Arrant Exchange Factor. B. The Arrant GTPase Activating Protein is located in the cytosolic side of the NP (associated to the cytosolic extensions of the NP), and on the other hand, the Arrant Exchange Factor is located in close association with the chromatin and is therefore located inside the nucleus. C. This is responsible for

generating a very high concentration of Ran in the nucleus and Ran in the nucleolus. -Why is the Ran gradient important for nuclear traffic?

Ran favors/prevents the formation of import/export complexes or the disruption of the already existing complexes (for instance, Ran is REQUIRED for the formation of Export complexes, whereas Ran triggers the DISRUPTION of Import complexes). -What's a Nuclear Localization Signal (NLS)? What's a Nuclear Export Signal (NES)? What's the main feature of a NLS? What's the main feature of a NES? Going into the nucleus must have a Nuclear Localization sequence (NLS)- usually a sequence of 4 or more adjacent OR closely located positively charged amino acids (e. G. - Proteins going out of the nucleus must have a

KICK, KIRK, KARRI). Nuclear Export Sequence (NES) - usually a series of Lysine residues with a characteristic spacing between them (e. G. LEXICALLY or LEXICAL). - In both cases, the signals... A. Can be located anywhere in the linear sequence of the protein. Mediate the interaction with the cargo and "carrier" proteins that regulate nuclear traffic -What are IMPORTING? What are EXPORTING? What are Ran's? Types: ; Two a. Importing: Recognize NLS's and mediate Nuclear Export (e. G. Importin a, Importin P). B. Exporting: Recognize NES's & mediate Nuclear Export (e. G. C.

Proteins of the importin alpha and importin beta families play a central role in neoclassical transport. -How are proteins imported into the nucleus. 7 which are the factors required? How are proteins exported out of the nucleus? Which are the factors required? How is nuclear import/export regulated? ; The gradient of Ran that is observed across the nuclear

envelope is what actually regulates the whole process of neoclassicisms traffic. When a cargo is located in the cytoplasm that contains a nuclear localization signal, that cargo will form a complex with importing α and in the cytoplasm.

That is due to the fact that when importing α and are together they have the ability to recognize those nuclear localization signals (NLS). This forms a very tight complex that allows them to bring the cargo into very close proximity with the NP. This proximity will then enhance the passage of the cargo together with the importing through the channel of the NP and into the nucleus. Once the complex reaches the nucleolus the high concentration of Arrant that is present in the nucleus will then allow the binding of Arrant to the importing β .

Whenever this binding takes places, Arrant triggers a conformational hanged in importing which then makes importing release importing α . Once importing α is released, the entire complex falls apart, therefore releasing the cargo inside the nucleus. These complexes cannot reform within the nucleus because as long as you have a high concentration of Arrant, importing will not be able to bind again with importing α . If the two are not bound together they will not be able to recognize the NLS that is present in the cargo. For the nuclear export of proteins the cargo (the protein that will be exported out of the nucleus) must contain a Nuclear Export Signal (NES). That NES is recognized by the exporting (e. . CRM). However CRM by itself is not able to recognize the NES and in order to do that CRM must first be able to recruit Arrant. When this happens, Arrant allows CRM to then

recognize the NES in the cargo and form a very tight complex between the <https://assignbuster.com/molecular-cell-biology-study-guide-exam-assignment/>

exporting, cargo, and Arrant that is then able to locate in close proximity to the NP and then allow the passage of the whole protein complex.

Once the exporting, cargo, Arrant complex is in the systolic side of the NP will then move in very close proximity with Ran GAP which is in close proximity to the systolic extensions of the NP. This will then trigger the hydrolysis of GET by Ran, and once it is hydrolysis, the whole complex will fall apart because CRM is only able to hold the cargo when it is associated with Arrant. The cargo has now been successfully exported to the systolic side. Nuclear Organization & Nuclear Compartments: -How are chromosomes distributed within the nucleus? What are Replication Factories, Nuclear Speckles, Pro-Myeloid Leukemia Nuclear Bodies (ML-NBs), and Nucleoli (Nucleolus)? 1 . Replication Factories ; Clustered sites for DNA replication and where it takes place. Approximately 30, 000 origins of replication in the entire genome. But only see -?? 200 discrete sites of replication and this area is referred to as a replication factory. ; Replication factories are places where you accumulate all the determinant factors required for DNA replication. ; Approximately 40 replication forks are clustered in a replication factory. 2.

Nuclear Speckles ; Actively transcribed genes appear evenly distributed throughout the nucleus. ; Splicing machinery are concentrated to discrete sub nuclear domains. Concentrated to 20-50 locations or nuclear speckles in active cells. ; Though to be storage for sites for splicing components that are recruited from the speckles to reinitiate genes and are located within very specific defined areas of the nucleus to be readily accessible and easily used

when needed. 3. Protectively Leukemia Nuclear Bodies (ML-NBs) ; Cells from PAL individuals have fragmented ML bodies.

Treatment with all- trans-retention acid (ATRIA) allows these bodies to reform and, remarkably, this correlated with remission from disease. ; Storage depot for numerous transcription factors and chromatin-modifying factors within the cell. ; Assembly of and protein targeting to ML-NBs are both tightly connected with Simulation. 4. Nucleolus The nucleolus is the only domain that can be seen without using any particular type of dye under a microscope. ; Most prominent substructure. ; Site of RNA processing and ribosome assembly. ; All copies of RNA genes (-200 [ass= ass, ass, 5. s]/ - 2000 [as]) are clustered together. ; Following each cell division, nucleoli form around the chromosome regions that contain the RNA genes. ; The genes are therefore called “ nucleolus organizing regions”. A single nucleus. ; Initially, separate nucleoli form and they then fuse to form The Nucleus During Mitosis: -What happens to the nucleus during mitosis? ; It disappears. The structural components are disassembled but are rapidly re- assembled at the end of mitosis. -What’s the critical event that triggers nuclear disassembly?

What’s the post-translational modification that regulates it? ; During mitosis, the nuclear lamina is disassembled through phosphorescently of Lamina, which removes the ability to form the protein scaffold. Phosphorescently disrupts the head to tail structures that provide order and keep the nuclear lamina intact. Once the nuclear lamina is depilatories (eliminated) then the nuclear envelope begins to bubble, which forms vesicles that get distributed throughout the cytoplasm. The Secretors Pathway -Which are the cellular organelles involved in the secretors pathway? What are the potential cellular <https://assignbuster.com/molecular-cell-biology-study-guide-exam-assignment/>

destinations followed by a protein synthesized in free cytosolic ribosome? How about a protein synthesized in ER membrane-bound ribosome? ; Generally speaking, proteins that are destined to remain in the cytosol or in the nucleolus are synthesized within free ribosome in the cytosol. Since proteins are synthesized from proteins that are synthesized in the cytoplasm, those proteins are destined to either stay in the cytoplasm or to be later on trafficked to the nucleus. There are a few additional compartments that these proteins can be targeted to and one of them is the mitochondria.

The other two compartments, the peroxisomes and chloroplasts are alternative pathways that can be followed by some of these proteins that are synthesized by free-floating ribosome in the cytosol. However, if the protein is to be targeted to the main membrane bound organelles within the cell that is to the endoplasmic reticulum, Golgi apparatus, lysosomes, secretory vesicles, or plasma membrane to be secreted outside the cell, proteins need to be synthesized in ribosome that are bound to membranes in the Endoplasmic Reticulum. What's the difference between membrane-bound and free ribosome? ; One important thing to keep in mind about this important decision making process is that the ribosome that are going to be involved in the synthesis of proteins that will remain in the cytosol as well as proteins that will be targeted to different organelles within the cell are exactly the same. There are no differences between those ribosome. -What determines whether a ribosome remains free in the cytoplasm until it finishes synthesizing a protein or becomes membrane bound? This comes down to the same cues that regulate other proteins within the cells and

these are in the form of protein signals known as ERE signal sequence (ERRS) or signal sequence (AS). - What's an ERE Signal Sequence? What constitutes an ERRS? What's the minimum length of an ERRS to be functional? ; Those signal sequences are usually located at the amino terminus of the protein and are recognized by a complex of RNA and protein known as the Signal Recognition Particle (SIR). A AS is a sequence anywhere between 12 and 15 amino acids that are very highly hydrophobic in nature.

Any sequence of 12-15 amino acids can constitute a signal sequence (AS) and this sequence is efficient for recognition by the SIR. -What's the Signal Recognition Particle? What is it made of? What's its function? ; The SIR structure constitutes of 6 different polypeptides and an RNA component, which allows for the recognition of the 12-15 hydrophobic amino acid sequence in the protein that needs to be targeted to the secretory pathway. - What does the SIR bind to in the surface of the ERE? ; Transmembrane domain - What's a transmembrane domain? Can transmembrane domains be predicted using bioinformatics methods (i.e. Computer algorithms)? ; In order to have a sequence that will allow membrane insertion of the protein, the number of hydrophobic amino acids has to be longer (in this case longer than 15 amino acids). These sequences will then constitute transmembrane domains within the proteins that they are incorporated into. Most transmembrane domains adopt the structure of an α helix which are particularly good at crossing membranes because the natural tendency of amino acids is to form hydrogen bonds within its environment and is not possible within the environment of transmembrane domains.

These transmembrane domains allow proteins to remain permanently anchored to membranes and since they are permanently anchored they will have specific orientations in those membranes. They may either have the N-terminus toward the cytosol or they may have the N-terminus oriented toward the lumen of the ER. There are proteins that have several transmembrane regions and these are proteins that are very important for cell physiology. ; Based on the hydrophobic and hydrophilic nature of those amino acids in the linear sequence of a protein, it then becomes possible to predict which regions within a protein will likely to be transmembrane domains.

Does the topology of a membrane protein change during the different steps involved in the secretory pathway? ; No, the topology is maintained throughout the entire secretory pathway. -Which ones are the most frequent post-translational modifications enacted upon proteins during their traffic in the secretory pathway? The Endoplasmic Reticulum -What's the translation? -What's the main function provided by protein oscillation for proteins in the ER? - What's the role of Calnexin? ; Determines if protein has been folded correctly or not. If not then protein will be refolded.