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NORGES TEKNISK-NATURVITENSKAPELIGE UNIVERSITET
INSTITUTT FOR FYSIKK

EXAM IN TFY4260 – CELL BIOLOGY AND CELLULAR BIOPHYSICS

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Date: 26 May 2015
Time: 09.00-13.00
Examination aids: Dictionary Norwegian – English, English – Norwegian

Read the exercises carefully and answer all questions.
Good luck!

Exercise 1: Transport across membranes

a) What is the difference between simple diffusion, facilitated diffusion, and active transport? Describe, briefly, one process (three in total), that makes use of each of these types of transports.

Simple diffusion: Unassisted movement of molecules across a membrane, down a concentration or electrochemical gradient. Typically this type of movement is only possible for gases, nonpolar molecules, or small polar molecules such as water, glycerol, or ethanol.
Example: Transport of oxygen and carbon dioxide across the membrane of erythrocytes.

Facilitated diffusion: Most substances in the cell are too large or too polar to cross membranes by simple diffusion. These can only move in and out of cells with the assistance of transport proteins. If the solute diffuses as dictated by its concentration or electrochemical gradient the process is called facilitated diffusion.
Example: The erythrocyte is capable of glucose uptake by facilitated diffusion because the level of blood glucose is much higher than that inside the cell. Glucose is transported inwards by a glucose transporter (GLUT). GLUT1 is thought to transport glucose through the membrane by an alternating conformation mechanism. One conformational state, T1, has the binding site for glucose open on the outside of the cell. The other conformational state, T2, has the binding site open to the inside of the cell. The binding of glucose to the transporter leads to a change in conformation with the concomitant release of the glucose towards (generally) the interior of the cell where the glucose concentration is lower.

Active transport: Refers to the movement of solutes up a concentration or electrochemical gradient, away from equilibrium. This requires the input of energy that can be obtained by ATP hydrolysis (direct active transport) or by simultaneously transporting one solute down its gradient (indirect active transport).
Example: For example, glucose can be transported against its concentration gradient using a Na^+/glucose symporter (SGLT proteins). The transport of Na^+ down its electrochemical gradient provides the energy necessary to drive the transport of glucose up its concentration

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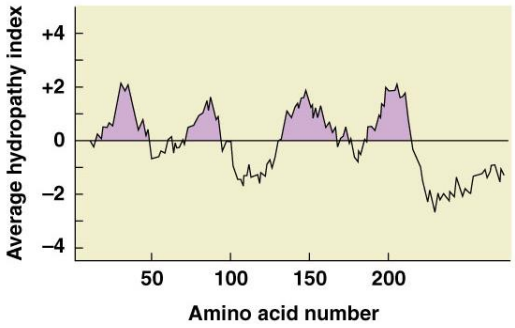
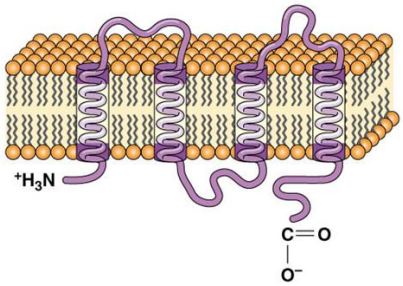
gradient. The steep Na^+ gradient is maintained across the plasma membrane via the Na^+/K^+ pump, another example of active transport, now using ATP hydrolysis.

b) The figure below shown the hydropathy plot of a protein involved in the transport across a membrane. What does the plot tell about the structure of the protein?



The plot tells us that the protein possesses four hydrophobic regions (regions with a positive hydropathy index), most likely corresponding to four transmembrane segments, as follows:



c) Why is such type of structure relevant from a transport point of view?

Lipids membranes are only impermeable to gases, nonpolar molecules and small polar molecules. Proteins that are directly involved in transport need to be able to transport molecules from one side to the other of the membrane and therefore need to cross the entirety of the membrane. The number and structure of each of the transmembrane segments, and the way they are organized will vary, depending on the type of molecule that should be transported.

Exercise 2: Cytoskeleton and cell cycle

a) Describe the assembly process of microtubules.

The basic unit of a microfilament is a heterodimer of the proteins tubulin (one α-tubulin and one β-tubulin), the αβ-heterodimer. The heterodimers firstly associate to form αβ-αβ oligomers and protofilaments. About 13 of these protofilaments associate to form a microtubule, which grows further.

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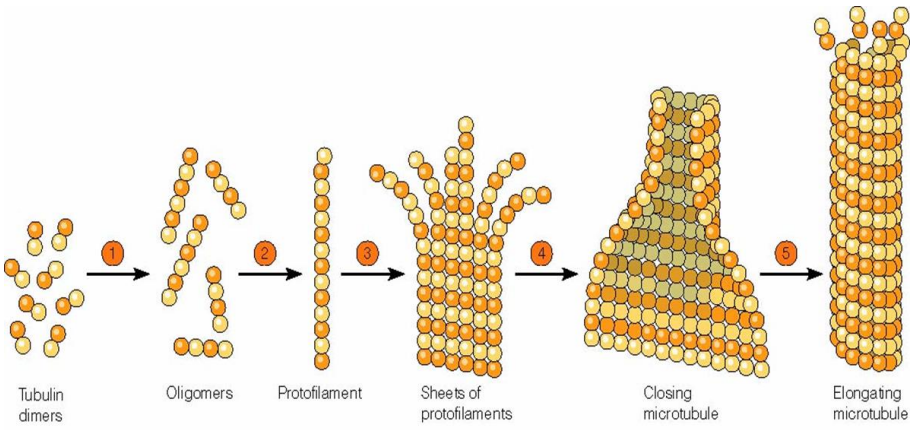
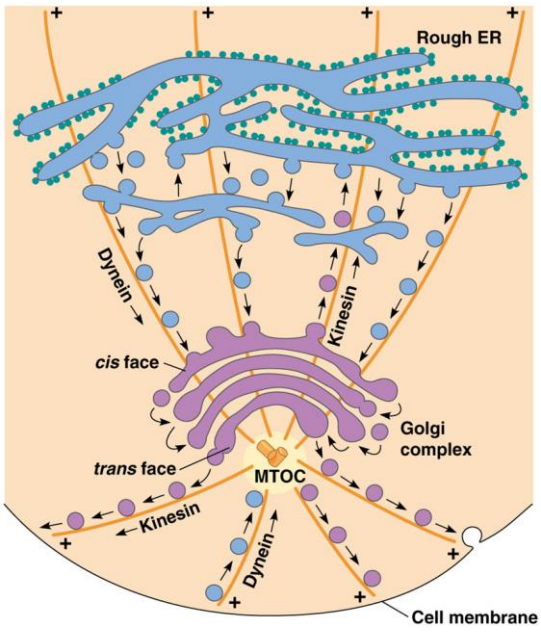


b) Microtubules are said to be polar. Explain what is meant with this and discuss the consequences of such polarity in terms of intracellular movement and cell division.

Because the two tubulin sub-units are chemically different and the association of tubulin dimers is always so that α-tubulin binds to β-tubulin, and not to other α-tubulin, the microtubules (MT) are aid to be polar. In other words, one extreme of the MT (say the α-tubulin, and 'minus' end) will be chemically different than the other extreme (say the β-tubulin, and 'plus' end).
The polarity of the MT is important for cellular movement because the different classes of motor proteins that "walk" along the MTs transporting vesicles, for example, will move in a pre-determined fashion; the kinesis walk towards the 'plus'-ends, and the dyneins move towards the 'minus'-end of the MT:



The polarity of MTs is also important in the mitotic spindle, which is responsible for chromosome movements during mitosis.
In cells, the MTs are anchored and grow from microtubule-organizing centers (MTOC). The MTs grow out from an MTOC with a fixed polarity; their 'minus'-ends are anchored to the MTOC while the 'plus'-ends extend out towards the cell membrane, as shown in the Figure above. In addition the MTs are dynamic structures with tubulin subunits being added and removed from both ends. In general, the 'plus'-end is the site favored for the addition of subunits and the 'minus'-end is the favored for tubulin subunit removal.

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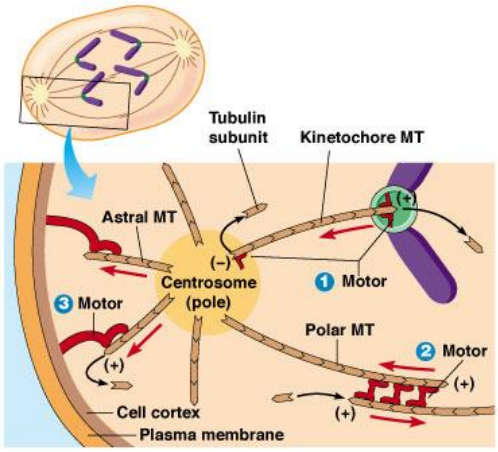
During the cell cycle, in the late prophase, MT growth speeds up and initiation of new MTs at the centrosomes increases. Once the nuclear envelope disintegrates at the beginning of the prometaphase, the binding between the 'plus'-side of MTs and chromosomal kinetochores is possible. These lead to the formation of kinetochore MTs. These type of MTs have motor proteins associated with the 'plus'-ends which will depolymerize the MTs, pulling the chromosome towards the spindle pole. In the meantime, another group of MTs (polar MTs) make direct contact with other (polar) MTs coming from the opposite centrosome. When the 'plus'-end regions of the two MTs of opposite polarity start to overlap, crosslinking proteins bind them to each other. These motor proteins cause the MTs to slide apart, forcing the spindle poles away from each other, at the same time as the MTs are extended by polymerization of tubulin subunits at the 'plus'-end. Finally, astral MTs motor proteins will link the 'plus'-ends of astral MTs to the cell cortex and exert a pull on the spindle poles by inducing astral MTs depolymerization at the 'plus'-end:



c) Describe briefly how one can obtain the fraction of T lymphocyte cells in the G1 and G2+M phases using flow cytometry.

1. Remove RNA and label DNA of cells with e.g. propidium iodide.
2. Generate fluorescence histogram. In the flow cytometer one cell at the time should be illuminated by a laser beam. This requirement can be tested by measuring time of flight. Fluorescent light from a cell is emitted in all directions and collected by an objective lens and detected by a photomultiplier tube. Optical filters placed before the detector ensures that only fluorescence light reaches the detector. The amount of fluorescence from a single cell is assumed to be proportional to the amount of DNA. G1 and G2+M can be identified in the histogram. The fraction of cells in each phase can be calculated by integration of the respective parts of the histogram.

Exercise 3: DNA packing and regulation of gene expression

The genome is composed of very long DNA molecules that, when extended, can measure close to one meter.

a) Describe the first two levels of DNA packing in eukaryotic cells.

In the first level of DNA packing, the DNA wraps nearly 2 times around the histone octamer (two of each histone H2A, H2B, H3 and H4), with about 146 base pairs. This structure is called a nucleosome, or nucleosome bead. Between these beads is about 50 base pairs of "naked" DNA called linker DNA. These structure, called the 10 nm chromatin fiber, looks like beads on a string:

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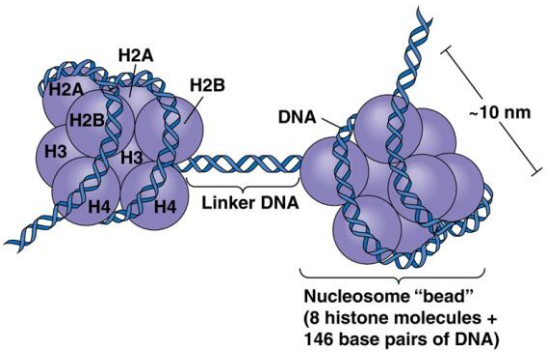
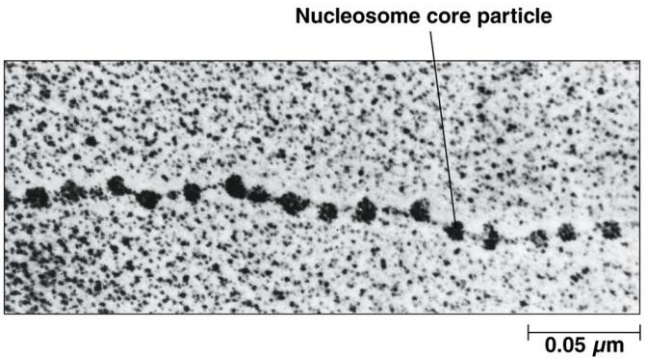
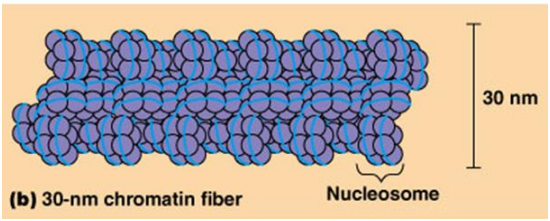




In the second level of packing the nucleosome beads in 10-nm chromatin fiber pack together to form an irregular, three-dimensional zig-zag structure called the 30-nm chromatin fiber. Both the H1 histone and the histone tails are believed to be important for the stability of this structure:



b) Give an example of genomic gene expression control. Justify.

Genomic gene expression control is the first level of genetic control. Here gene expression can be controlled by gene deletion or amplification, and by DNA methylation, for example. Chromatin condensation and decondensation, and histone modifications that lead to a higher or lower DNA condensation degree are other examples of genomic expression control.

c) Describe how prokaryotes and eukaryotes coordinate the expression of groups of related genes.

Prokaryotes coordinate the expression of groups of related genes by having these genes placed one after the other in an operon, such as the lac operon. In this way a group of genes with related functions can be turned on and off (regulated) simultaneously. In addition, and upstream from the promoter region, prokaryotes have a regulatory gene, which codes for a repressor protein. The repressor protein binds to the operon, which partially overlaps with the promotor region, preventing the binding of the polymerase to the promoter region and, consequently, transcription.

Eukaryotes, on the other hand, must turn on and off, simultaneously, genes that are scattered throughout the genome. To coordinate the expression of such physically separated genes, eukaryotes employ DNA control sequences called response elements to turn transcription on and off in response to a particular environmental or developmental signal. Response elements can function either as proximal control elements or as components of enhancers. In either case, placing the same type of response element next to gene residing at different chromosomal locations allows these genes to be controlled together even though they are not located next to each other.

d) The synthesis of Ferritin (iron-storage protein) is under negative gene regulation at the post-transcriptional level, with iron being the inducer. Draw a scheme of the translation control in response to iron.

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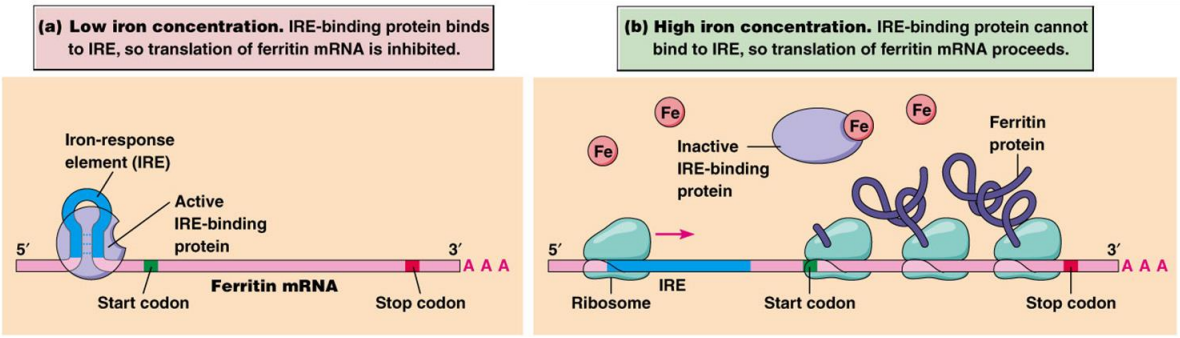
With negative gene regulation is meant that the synthesis of Ferritin is only activated in the presence of iron. Since the control is done at the post-transcriptional level, more specifically, at the translational level we know that it affects the translation machinery. From this we can conclude that a protein complex binds to the ferritin mRNA and stops the ribosome from binding to or progression along the mRNA. When iron is present, it binds to the protein complex, inactivating it. The iron-protein complex leaves the mRNA and translation can initiate and/or resume.
The scheme of the translational control is response to iron is as follows:



(There was no need for a big level of detail on the scheme.)

Exercise 4: Cell signaling and cancer cells

a) Describe the structure of a G-protein-linked receptor, as well as the structure, activation and deactivation of G-proteins.

The G-protein-linked receptor has seven transmembrane α helices and several extracellular and cytosolic loops. The ligand binding site is at the extracellular portion of the receptor. The binding of a ligand causes a configurational change to the receptor with the concomitant binding and activate a G-protein at the cytosolic portion of the receptor.
The G-protein that associated to a G-protein-linked receptor is of the heterotrimeric type, possessing three different subunits: G_α, G_β, and G_γ. The G_α unit is the largest and the one that binds to a guanine nucleotide (GDP or GTP). The G_β and G_γ units are permanently bound. As mentioned, when a messenger binds to a G-protein-linked receptor on the surface of the cell, the change in conformation of the receptor causes the G protein to associate with the receptor, which in turn causes the G_α subunit to release its bound GDP. The G_α then acquires a new, different molecule of GTP and detaches from the complex. Depending on the G protein and cell type, either the free GTP-G_α subunit or the $G_{\beta\gamma}$ complex can then initiate the cell signaling events by binding to a particular enzyme or protein in the cell. The activity of the G-protein persists for as long as the G_α unit is bound to the GTP, and the G_α and $G_{\beta\gamma}$ units are separated. When the GTP in the G_α subunit is hydrolyzed to GDP all three subunits recombine to form an inactivated G-protein.

b) *TRK* is an oncogene created by chromosomal inversion where an end of the tyrosine kinase (*NTRK1*) gene becomes fused with an end of the gene coding for nonmuscle tropomyosin (*TPM3*). Explain the mechanism of chromosomal inversion. Draw a scheme of the structure of the resulting fused protein and explain why such mutation leads to a cancer cell.

In chromosomal inversion, DNA undergoes breakage in two points along the sequence of two different genes, creating a DNA fragment, which gets oriented in the opposite direction and rejoined into the main DNA chain. This leads to the fusion of one end of one gene with the

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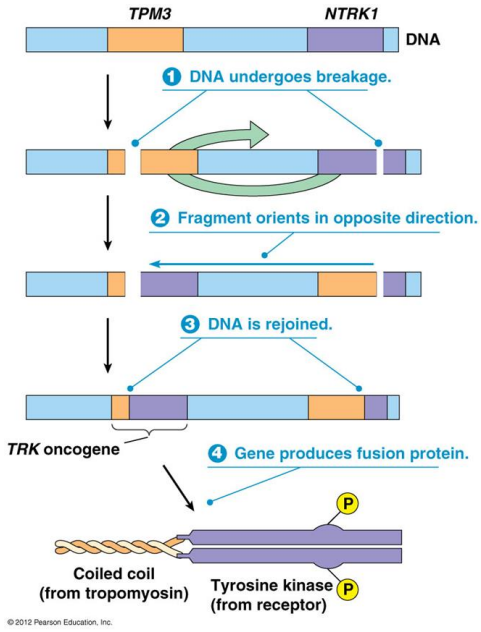
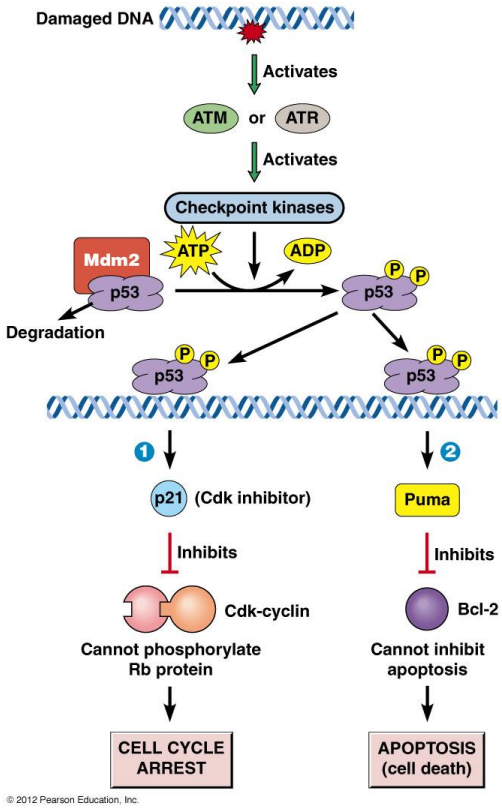


other end of the other. In the given example, the tyrosine kinase gene becomes fused with an end of the gene coding for nonmuscle tropomyosin and so the resulting oncogene produces a fusion protein of the tyrosine kinase receptor with the tropomyosin segment. When two receptor kinases associate as a response to the binding of a ligand, the tropomyosin segments in each receptor will form a coiled coil, leading to the stabilization of the receptor dimer and its permanent activation. Since the tyrosine kinase receptors are growth factor receptors, their permanent activation may lead to an uncontrolled proliferation of cells, i.e. cancer.

c) Two of the hallmarks of cancer are sustained angiogenesis and evading apoptosis. Describe an experiment that has shown the requirement of angiogenesis for tumor growth. Describe the role of *p53* proteins in cell apoptosis and how they are targeted by the human papiollomavirus (HPV).

One of the experiments that have shown the requirement of angiogenesis for tumor growth was the growth of cancer cells on the iris or anterior chamber of the eye. Cancer cells were either injected in the liquid-filled anterior chamber of a rabbit's eye, where there are no blood vessels, or they were placed directly on the iris. It was found that the tumor cells in the anterior chamber stop growing before the tumor reached a millimeter diameter, while the cancer cells implanted in the iris, quickly got infiltrated by blood vessels, allowing the tumors to grow to thousands of times their original mass.



p53 protein is involved in a few different processes pertaining the cell cycle control, playing a central role in the DNA damage checkpoints that exists to monitor for DNA damage and halt the cell cycle at various points. The altered DNA triggers the activation of an enzyme called ATM protein kinase that catalyzes the phosphorylation of kinases known as checkpoint kinases, which is turn phosphorylate the p53 protein. The phosphorylated p53 does not interact with *Mdm*2, a protein that marks the p53 for destruction by marking it with ubiquitin. This way the p53 protein will accumulate in the cell. This activates two processes, cell cycle arrest and cell death (see figure on the left-hand side). In the latter mechanism, the phosphorylated p53 protein activates genes coding for a number of proteins that promotes apoptosis by binding to, and blocking the

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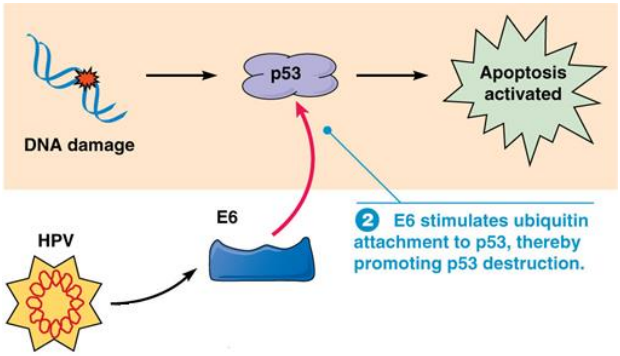
action of the apoptosis inhibitor, Bcl-2. One of these proteins is called Puma (p53 upregulated modulator of apoptosis).
The p53 proteins are targeted by the HPV virus as follows. The virus possesses an oncogene that produces a protein called E6. The E6 protein stimulates the attachment of ubiquitin to the p53 and targets it for destruction. As a result, p53 can no longer trigger apoptosis in cells with damaged DNA.



Exercise 5: Mark the correct alternative with a cross. Deliver these pages together with the answers of the other exercises. Do not forget to indicate the candidate number.

a) A particular plasma membrane possesses three main proteins, A, B, and C. When the membranes are treated with a high-salt solution, protein B is no longer detected in the membrane. The salt treatment has no effect on proteins A and C. What can we conclude?
- Protein B might be a lipid-anchored membrane protein.
- Protein B might be a peripheral membrane protein.
- Protein B might be a singlepass integral membrane protein.

b) Glycolipids are found
- in the cytosol of the cell.
- imbedded in the plasma membrane and facing the exterior of the cell.
- imbedded in the nuclear membrane and facing the nucleoplasm.

c) Cristae in mitochondria are related to
- ATP synthesis.
- packing of mitochondrial DNA.
- ATP storage.

d) The synthesis of proteins destined to the plasma membrane starts in the
- cytosol.
- ER.
- Golgi complex.

e) ER to Glogi traffic is done
- along microtubules.
- using clathrin-coated vesicles.
- via retrograde transport.

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f) Mannose-6-phosphate tags enzymes for
- destruction by proteasomes.
- transport to lysosomes.
- transport to the ER.

g) What role do voltage-gated calcium cation channels play in the transmission of signal across synapses?
- They substitute for potassium cation channels.
- They bind to neurotransmitters such as acetylcholine.
- They enable an influx of calcium to trigger neurotransmitter secretion.

h) The transmission of an action potential along a myelinated axon is often called saltatory conduction. Why?
- Because the action potential jumps from node to node.
- Because the electric signal propagates faster in the myeline portions and more slowly in the nodes.
- Because neurotransmitters are released to the synaptic cleft.

i) Ras is
- activated by a GTPase activating protein.
- activated by Raf.
- a membrane bound protein.

j) Actin filaments
- can be organized in 1, 2 and 3 dimensional structures.
- are associated with gap junctions.
- have no polarity.

k) Myosin proteins are **not** involved in
- muscle contraction.
- flagella motility.
- vesicle trafficking.

l) In relaxed muscle, calcium is found in high concentrations in the
- T tubules.
- sarcolemma.
- Sarcoplasmic reticulum.

m) Cell fusion experiments showed that fusion of a cell in S phase with one in G1 phase leads the nucleus that was in G1 to begin S phase. The best interpretation of this finding is that _____.
- the DNA polymerase in the nucleus of the S cell moved to the G1 nucleus and started DNA replication
- the G1 nucleus senses the plasma membrane fusion event and then enters S phase
- the cytoplasm of the S cell contains a diffusible signal that causes the G1 nucleus to enter S phase

n) During the cell cycle, and in order to be active, a Cdc-cyclin needs to be
- singly phosphorylated.

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- acetylated.
- doubly phosphorylated.

o) In recombinant technology, what is the purpose of the amp^R gene in the plasmid pUC19?
- To enable a scientist to use detection of the protein β-galactosidase to find plasmids carrying the gene of interest
- To provide a series of restriction enzyme sites convenient for making recombinant plasmids
- To enable a scientist to kill bacterial cells that are not carrying pUC19 or a recombinant derivative of pUC19

p) The rolling of leukocytes along the endothelium cells that line blood vessels is mediated by
- selectins.
- integrins.
- cadherins.

q) Tight junctions
- allow the passage of solutes with molecular weight above 1200.
- block the lateral movement of proteins.
- bind cells to the basal lamina.

r) Genes that, when present, trigger the development of cancer are known as
- tumor suppressor genes.
- oncogenes.
- proto-oncogenes.