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NORGES TEKNISK-NATURVITENSKAPELIGE UNIVERSITET
INSTITUTT FOR FYSIKK

EXAM IN TFY4260 – CELL BIOLOGY AND CELLULAR BIOPHYSICS

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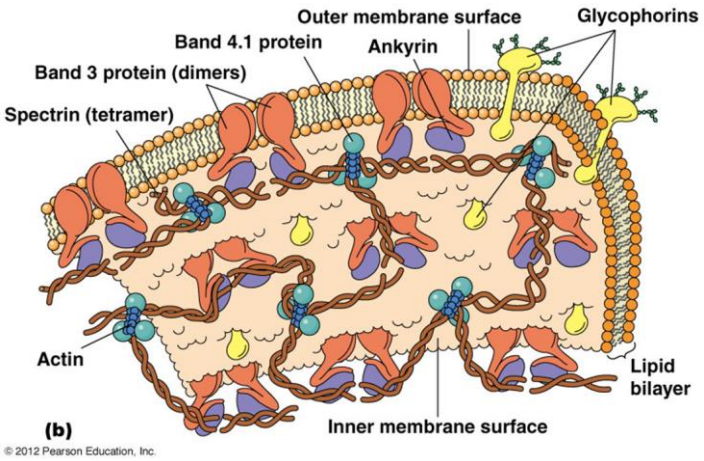
Date: 6 June 2018
Time: 09.00-13.00

All questions in Exercises 1 to 3 have the same weight (5 pts). Questions in Exercise 4 count with 1 pt each (20 in total). None of the questions require lengthy answers so answer as precisely and concisely as possible.
Good luck!

Exercise 1: Membranes and transport across membranes

In the capillaries of body tissue, O_2 is released by hemoglobin within the erythrocytes (red blood cells) and leaves the cell. CO_2 enters the cell and is converted to bicarbonate (H_2CO_3). Bicarbonate ions (HCO_3^-) are then transported out of the cell.

a) By which processes are CO_2 transported in and HCO_3^- transported out?
b) Why is HCO_3^- transported out of the cell?
c) How will an erythrocyte respond when placed in a hypotonic solution?
d) Below is a schematic representation of the erythrocyte lipid membrane and some associated proteins. Discuss two (general) functions of spectrin in this cell.



Answers:

a) CO_2 is a small non-polar molecule, which easily crosses the lipid membrane and diffuses into the cells where its concentration is much lower – Simple Diffusion.
HCO_3^- is an ion and therefore it will not cross the lipid membrane on its own. In this case one needs a transport protein to aid in the transport. In this particular case, the HCO_3^- is

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transported out of the cell towards a low concentration, so it is a facilitated diffusion process. However, since the ion is negatively charged, its transport is mediated by the simultaneous transport of Cl^- inside the cell, also down its concentration gradient. So, the transport of HCO_3^- is a facilitated diffusion processes mediated by an anion exchange protein.

b) The function of the bicarbonate in the erythrocyte is (at least) two-fold: (i) it keeps the concentration of CO_2 low inside the cell, so more CO_2 can be up taken by the cell in the body tissue; and (ii) the dissociation of the bicarbonate reduces the pH inside the cells which leads to the dissolution of O_2 from the hemoglobin and consequent release from the cells. Now, the reaction from CO_2 to H_2CO_3 will only proceed while the concentration of H_2CO_3 (and HCO_3^-) is sufficiently low; thus, the transport of HCO_3^- to outside the cell keeps its concentration low, which allows the absorption of more CO_2 by the cell.

c) In a hypotonic solution, the osmolarity of non-permeable molecules in the solution is lower. When a red blood cell is placed in a hypotonic solution, water molecules will diffuse into the cell until a steady state is achieved. The red blood cells will therefore swell and they could eventually break open (hemolysis).

d) As indicated in the figure, spectrin forms a two dimensional network underneath the lipid membrane of the red blood cells, providing the cell with structural support that allows it to squeeze through narrow blood vessels and regain its shape afterwards. As also indicated in the figure, the spectrin also anchors some of the membrane-associated proteins to the membrane, hindering these from diffusing along the membrane.

Exercise 2: Cell signaling, regulation of gene expression and cancer cells

a) Ras is a monomeric G protein that plays an important role as a second messenger in cell signaling initiated by receptor tyrosine kinases (RTK). Explain the role of such G proteins in cell signaling events.

b) Besides the activation of Ras, RTKs can also activate phospholipase C_γ. Make a scheme of the signaling pathway, which also involves IP_3 (inositol-1,4,5 triphosphate) and DAG (diacylglycerol) as second messengers. Each of these can lead to the activation of other second messengers. One of these processes is a key event in many signaling events. Which? Include it in your scheme.

c) Even when the transcription of a gene is said to be “off”, there is usually a low, basal level of transcription. Why is this important in relation to the synthesis of galactoside permease (carrier protein) in the lactose catabolism?

d) DNA methylation is said to be a mechanism that reinforces the cell memory regarding gene expression patterns. What is meant with this?

e) To which of the hallmarks of cancer is the lower expression of cadherins by the cancer cell related to?

f) A major goal in cancer therapy is to identify anticancer drugs that can be used to inhibit products of specific cancer-critical genes. Should such inhibitors target the products of oncogenes or the products of tumor suppressor genes? Justify.

g) Briefly explain primary and secondary cell culturing.

Answers:

a) (Monomeric) G proteins are small proteins that function as molecular switches, belonging to the larger group of enzymes called GTPases. The large majority of these proteins are

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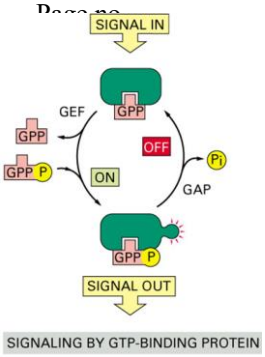
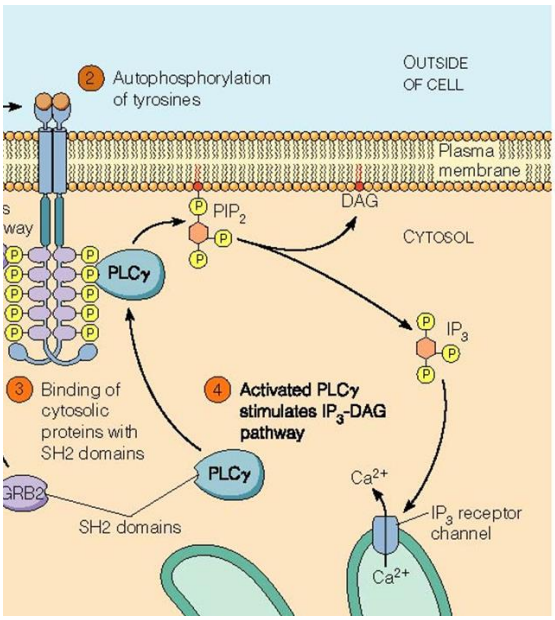
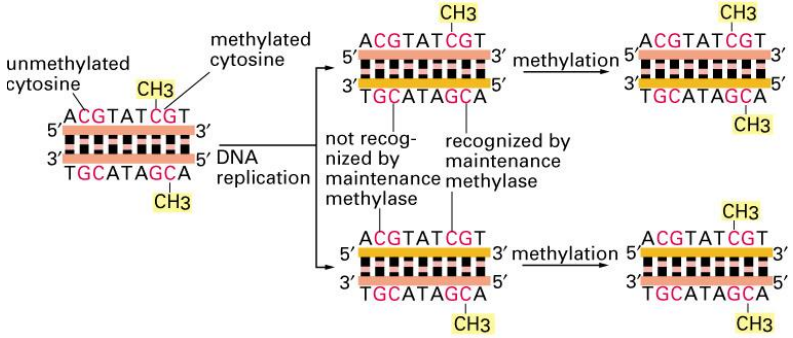
activated when bound to GTP and inactivated when bond to GDP but the opposite can be found too (guanylyl cyclase is an example of a G protein where the activated protein is bound to a GMP and the inactive form to GTP). Their activity is regulated by enzymes that replace bound GDP with GTP (guanosine exchange factors, GEF) and enzymes that hydrolyse GTP to GDP+P_i (GTPase activating protein, GAP).



b) The scheme below shows the RTK as a dimer that gets activated (autophosphorylated) upon the binding of a ligand. The phospholipase C_γ, possessing the necessary SH_2 domain, binds to the phosphorylated tyrosine and gets activated. As mentioned, the activated enzyme can stimulate the IP_3-DAG pathway, by cleaving the PIP_2 lipid, present in the cell membrane. The PIP_2 is cleaved into two molecules, the DAG, which contains the hydrocarbon chains and remains in the membrane where it can activate the protein kinase C (outside the scope of the question), and the IP_3, which is a soluble molecule that diffuses through the cytosol. The increase in concentration of the cytosolic IP_3 will lead to the opening of IP_3 receptor Ca^{2+} channels which will lead to the transport of Ca^{2+} from the lumen of the ER, where it is present at large concentration, to the cytosol. The release of Ca^{2+} from the ER is a key event in many signaling processes.



c) DNA methylation is a heritable epigenetic mark involving the covalent binding of a methyl group (CH_3) to the cytosine bases of DNA by DNA methyltransferases. Generally these modifications affect gene expression, by blocking or reducing the access of polymerases and transcription factors to genes. Methylation occurs most often in DNA locations where the cytosine is bound to a guanine. Furthermore, methylation of cytosines is catalyzed by the presence of methylated CG groups in the complementary bases (see middle representation in the figure below). So, when DNA is replicated, the methylations of the cell are inherited to the daughter cells. It is therefore said that the cells have memory regarding gene expression patterns.



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d) The lactose catabolism refers to the degradation of lactose for obtaining energy. Degradation of lactose is regulated by the lactose operon. That is, all genes associated with the degradation process are concentrated in an area of the genome and regulated at the same time. Like in other catabolic processes in bacterial cells, the synthesis of the enzymes required for the degradation of lactose is regulated by the presence of the lactose (substrate induction), or actually, in this case, by the presence of allolactose, an isomer of lactose. Allolactose binds to the repressor at the operator of the operon and increases the level of expression of these genes by a few fold. Now, one of the enzymes that is expressed by the lactose operon is the galactoside permease, a membrane protein responsible for facilitating the transport of lactose across the bacteria membrane and into the cell. If these genes would be completely off, then the cell would not produce the carrier protein and would not have been able to detect the presence of lactose.
e) Cadherins are proteins present at the surface of the cells, responsible for cell-cell adhesion. A lower expression of these molecules in cancer cells leads to a weaker cell-cell adhesion and a higher probability that the cancer cells detach for other cells in the tumor. This, together with the secretion of proteases that digest the extracellular matrix and the increased motility (stimulated by signal molecules from the cancer cells or their neighbors), is the basis for “tissue invasion and metastases”, one of the hallmarks of cancer cells.
f) Oncogenes is the denomination given to genes whose products can trigger the development of cancer. Tumor suppressor genes, on the other hand, are genes that, in normal cells inhibit cell proliferation and so their loss or inactivation can lead to cancer. Anticancer drugs design to inhibit gene products must therefore target the products of oncogenes, as these are the ones that may trigger cancer. Inhibiting the products of tumor suppressor genes would, on the other hand, enhance the problem.
g) Primary cell cultures derive from cells, which are directly isolated from the parental tissue or blood of an organism by enzymatic, chemical or mechanical dissociation. These cells resemble the parental tissue in morphology and can only undergo a limited number of cell divisions before they die out. There are adherent primary cells derived from organs such as liver or kidney and suspension primary cells from the blood system such as lymphocytes. Secondary cell cultures are an established sub-culture (passage) of the primary cell culture. The subculture can give rise to finite cell lines, which derive directly from the primary cell culture and have a limited life span or continuous cell lines with an unlimited life span. Continuous cell lines are generate by in vitro transformation with immortal cancer cell lines. Finite and continuous cell lines are present as adherent and suspension cell lines.

Exercise 3: Immunology, the endomembrane system, and cellular motility

a) Macrophage motility is essential for normal development and immune function. Briefly describe the steps of cell crawling, pointing out the main players in the process.
b) While most cells have the ability to present antigens at their cell membrane, macrophages and dendritic cells are called **professional** antigene presenting cells. Why?
c) In the process of presenting an antigen from an intracellular pathogen to the surface, the antigen is transported from the cytosol to the lumen of the endoplasmic reticulum. Why?
d) How are the major histocompatibility complex protein and antigen transported to the surface of the cell?
e) Extracellular pathogens are ingested and degraded in the lysosomes of antigen presenting cells. How are lysosomes formed?

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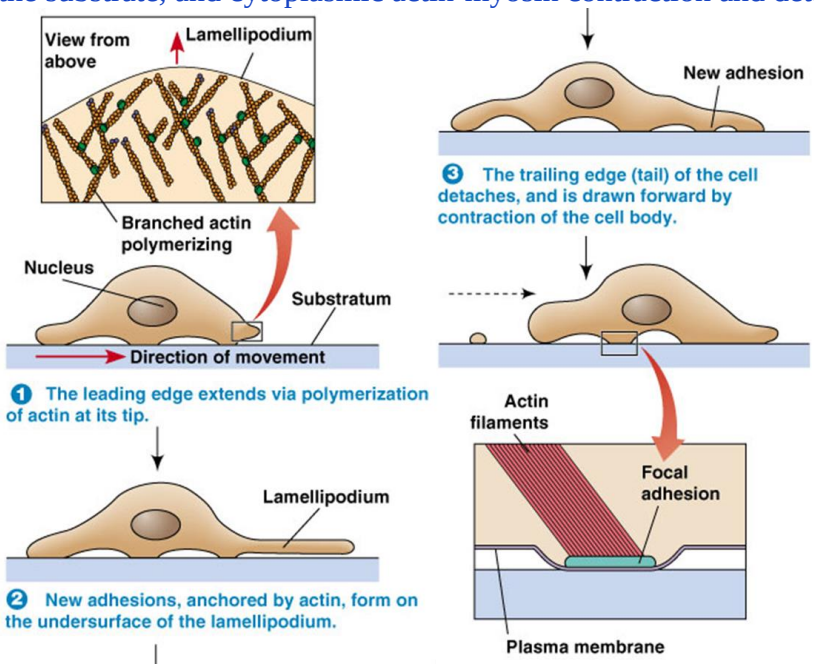
Answers:

a) Guided migration of macrophages involves sequential protrusion of filopodia and lamellipodia at the leading edge, focal complex-dependent adhesion of the leading edge to the substrate, and cytoplasmic actin-myosin contraction and detachment of the trailing edge:



b) Macrophages and dendritic cells are called professional because they travel to the closest lymph organs and present the antigens to other cells of the immune system, activating these and initiating the adaptive immune response. These cells are the helper T cells, who activate B and cytotoxic T cells, cytotoxic T cells, which travel to the site of infection, and B cells who initiate the production of soluble antibodies.

c) The MHC protein, the receptor that binds to the antigen and presents it towards the extracellular matrix, is a membrane protein. As such it is synthesized in the rough endoplasmic reticulum and transported to the cell membrane by coated vesicles. Now, in order to be able to present the antigen to the outside of the cell, the antigen-binding site on the MHC protein needs to face the inside (lumen) of the ER. As such, the antigen needs to be transported into the ER.

d) The MHC protein – antigene complex is after assembly at the rough ER transported, via de Golgi complex, to the surface of the cell by coated vesicles, more specifically by COPI-coated vesicles, and the movement of motor proteins, called kinesins, along microtubules.

e) Lysosomes are formed from endosomes. Endosome are membrane compartments containing enzymes needed to digest biomolecules, such as nucleic acids, proteins and polysaccharides (acid hydrolases). These compartments start off as vesicles that bud out of the Golgi complex with the acid hydrolases, and merge into early endosomes. These are merged by, in this example, a phagocytic vacuole containing the extracellular pathogen, and get matured into late endosomes. Lysosomes are formed when the hydrolases are activated, that is, when the pH in the late endosomes decreases to values of around 5. This is achieved by the pumping of H^+ (against the electrochemical gradient) into the late endosomes by ATP-ase pumps.

Exercise 4: Mark the correct alternative with a cross. Deliver these pages together with the answers of the other exercises. Do not forget to write down the candidate number.

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a) Bacteria cultures that are grown at low temperature are likely to produce membrane lipids with
   - a larger number of carbon atoms in the hydrocarbon chain.
   - **a larger number of double bonds.**
   - longer heagroups.

b) Membrane receptors involved in signal transduction belong to the class of
   - **integral membrane proteins.**
   - peripheral membrane proteins.
   - lipid-anchored membrane proteins.

c) Active transport of substances against their electrochemical gradient can be driven by
   - ATPases.
   - carrier proteins.
   - **both ATPases and carrier proteins.**

d) Most proteins found in mitochondria
   - **are synthesized in the cytosol and post-translationally imported.**
   - are synthesized in the ER and transported in coated vesicles.
   - possess a KREL tag.

e) The intermembrane space of mitochondria is characterized by the
   - **large concentration of protons.**
   - large concentration of ATP synthases.
   - large concentration of proteases.

f) The function of the smooth ER includes the synthesis of
   - neurotransmitters.
   - **cholesterol.**
   - messenger RNA.

g) Peroxisomes are characterized by possessing
   - a low pH value.
   - **a large concentration of urate oxidase.**
   - a large concentration of proteases.

h) Inactivation of the Na^+ channels is important in the context of nerve transmission because
   - it prevents subthreshold depolarization to initiate an action potential.
   - it allows the opening of K^+ channels and membrane repolarization.
   - **it keeps the Na^+ channels closed long enough to assure the directionality of the action potential.**

i) The plasma membrane of neurons is
   - equally permeable to all ions.
   - **more permeable to potassium ions.**
   - more permeable to sodium ions.

j) The nodes of Ranvier are rich in

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- myelin sheath.
- **voltage-gated ion channels.**
- neurotransmitters.

k) Flagella motility is driven by
- **Microtubules and dyneins.**
- Microfilaments and myosin.
- Intermediate filaments and vimetin.

l) Proteins enter the nucleus
- **via the nuclear pores.**
- if they possess a nuclear export signal.
- via the nuclear lamina.

m) The nucleolus
- is surrounded by a thin nucleolar membrane.
- is the site for synthesizes of ribosomal proteins.
- **concentrates the rRNA genes.**

n) Contact between the microtubules and chromosomal kinetochores occurs
- **when the nuclear envelope disintegrates.**
- in the anaphase.
- when the DNA is replicated.

o) Cytokinesis begins
- in the metaphase.
- **in the anaphase.**
- after the telophase.

p) Apoptosis is activated by
- the binding of a growth factor to the respective receptor.
- **damages to the DNA.**
- the binding of antibodies to antigen

q) Tight junctions
- connect the cytoplasm of two neighbouring cells.
- **seals the connection between two neighboring cells in the epithelium cell layer.**
- anchors the cells to basal lamina.

r) Resistance against compression forces in tissues is due to:
- collagen.
- elastin.
- **proteoglycans.**

s) In some cancer cells it is observed an abnormal amplification of genes encoding proteins involved in cell signalling. Which level of gene regulation does it refer to?
- **Genetic control.**
- Transcriptional control.

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- Translational control.

t) When a gene is under negative regulation, the effectors (ligands)
   - act as inducers.
   - act as repressors.
   - **can act both as inducers and repressors.**