Candidate number ……..…….……
Course code ……..
Page no……...

NORGES TEKNISK-NATURVITENSKAPELIGE UNIVERSITET
INSTITUTT FOR FYSIKK

EXAM IN TFY4260 – CELL BIOLOGY AND CELLULAR BIOPHYSICS

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Date: 8 June 2017
Time: 09.00-13.00

All questions in Exercises 1 to 4 have the same weight (5 pts). Questions in Exercise 5 count with 1 pt each (15 in total). None of the questions require lengthy answers so answer as precisely and concisely as possible.
Good luck!

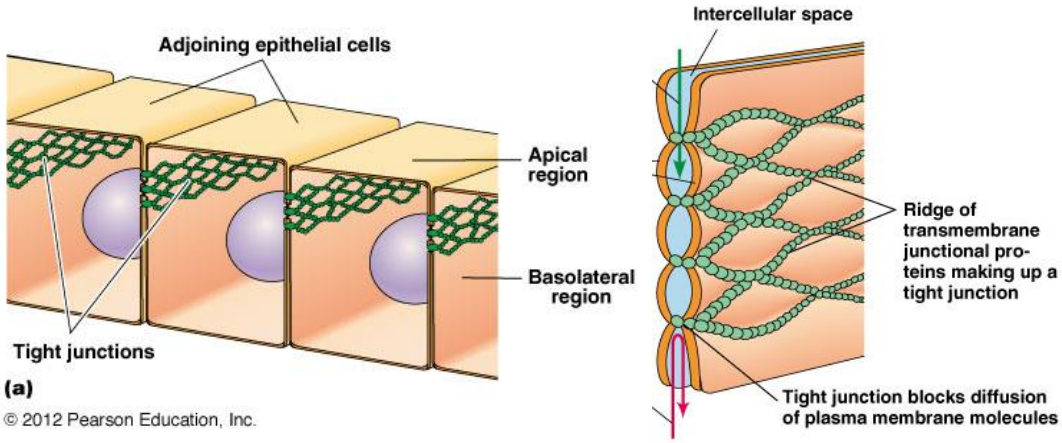
Exercise 1: Transport across membranes and cell junctions

a) Taking into account that the concentration of glucose is higher inside the epithelial cells that line the gut than in the gut or blood, explain how glucose is transported from the gut to the blood.
b) What type of cell-cell junction is responsible for preventing substances from diffusing between adjacent cells in the epithelium? Make a scheme of how such junctions look like.
c) Besides limiting the flow of substances between cells, the junctions from b) also define compartments in the plasma membrane. Explain how this is relevant in the context of glucose transport (a)).
d) You are given a sample with fluorescently labelled cells to visualize using confocal microscopy. What is the purpose of the pinhole in front of the detector in this technique?

Answers:

a) Since the concentration of glucose is higher inside the epithelial cell, the transport of glucose needs to be done by active transport.
A Na^+/glucose symporter is used to transport glucose inside the cell. Here, the fact that the concentration of Na^+ inside the cells is lower than in the gut is used. Two Na^+ ions are moved down their electrochemical gradient and the energy harvested in this process is used to move glucose against its gradient. For this to work continuously, Na^+/K^+ pumps are also present to ensure that the concentration of Na^+ inside the cell stays sufficiently high.
The glucose transporter is used to transport the glucose across the membrane from the cell to the blood and down its concentration gradient. No energy input is needed here.
b) Tight junctions. The scheme could be as follows:

Candidate number ……..…….……
Course code ……..
Page no……...



Here it would be important to point out that the junction forms a continuous belt around the cells. Otherwise the space between the cells would not be sealed.

c) The compartmentalizing role of the tight junctions is very important as it keeps the Na^+/glucose symporter on the apical region of the cells (towards the gut) and the glucose transporters on the basal domain (towards the extracellular matrix). This is fundamental to ensure that the glucose transport is done in the correct direction.

d) Reduces contribution from out-of-focus light. This increases contrast and resolution and allows imaging of thicker samples, optical sectioning and 3D reconstruction.

Exercise 2: Cell signaling and cell mobility

The G protein-linked receptor is responsible for many of the signaling cascades present in cells. It consists of a large transmembrane protein that crosses the membrane seven times. It possesses the N-terminus in the extracellular fluid and the C-terminus in the cytosol of the cell.

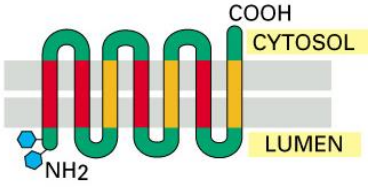
a) Where are these receptor proteins synthesized? Justify.
b) Make a simple scheme of the primary structure the protein indicating where you would expect to find the start-transfer and stop-transfer sequences. Justify. (*Hint:* do not forget to indicate the N- and C-termini in your representation.)
c) The activation of the G proteins by binding to the G protein-linked receptor is an amplification step, but the activation of adenylyl cyclase for cAMP synthesis is not. Why?
d) Neutrophils are phagocytic cells (part of the immune system), which have G protein-linked receptors all over the surface. When a chemoattractant binds to a receptor it starts two signaling cascades. One induces the activation of Rac (via PIP_3), leading to the polymerization of actin filaments. The other leads to the Rho pathway and induces actin-myosin contraction. How do these signal cascades contribute to the unidirectional motion of the cell by chemotaxis? (*Hint:* The PIP_3 second messenger is very short-lived).

Answers:

a) As all proteins, the synthesis of G protein-linked receptors starts in the cytosol of the cell. An aminoacid sequence in the nascent protein serves as a signal, stalling the synthesis and driving the ribosome to the ribosome receptors at the surface of the rough endoplasmic reticulum. The synthesis of the protein is resumed and further aminoacid signaling sequences will lead to the synthesis of a soluble protein inside the lumen of the ER or of transmembrane proteins in the membrane of the ER.
b) Since the N-terminus is outside the cell, in the extracellular matrix, and the C-terminus is inside the cell in the cytosol, this means that during protein synthesis in the ER the N-

Candidate number ……..…….……
Course code ……..
Page no……...

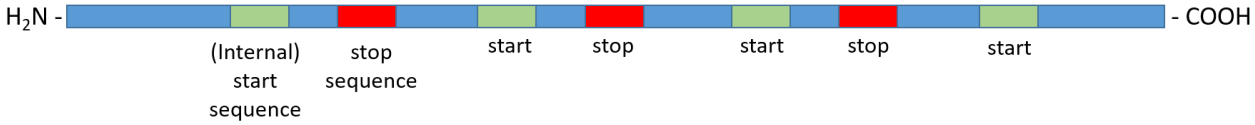
terminus will be facing the lumen (inside) of the ER and the C-terminus will be facing the outside of the ER, towards to cytosol of the cell:



A (very) simplified scheme of the primary structure of the protein would be as follows:

The region nearest to the N-terminus serves as a start-transfer sequence that causes the preceding N-terminal portion of the protein to pass across the ER membrane. Subsequent (hydrophobic) sequences function in alternation as start-transfer and stop-transfer. These correspond to pairs that occupies the translocator and gets released after the stop sequence.

Note: Answers where the "directionality" of the protein in the ER was not taken into account where also considered "correct". So, for a protein synthesized in the ER with the N-terminus facing the outside of the ER (cytosol of the cell) and the C-terminus toward to inside of the ER, the sequence of start and stops would look like:



Since the N-terminus is outside the ER, in the extracellular matrix, the start sequence cannot be placed to close to the N-terminus. It starts, thus, with an internal start sequence followed by a stop sequence. This corresponds to a pair that occupies the translocator and gets released after the stop sequence. Two more start-stop sequences follow, so the protein now spans the membrane 6 times. It is indicated that the proteins has 7 passes and that the C-terminus is inside the cytosol. This is consistent with a final start sequence signal.
Note that the scheme is very simplified; it suggests, for example, that the size of all the intra- and extra-cellular domains have the same length which is not true.

c) An amplification step means that one activate protein will give rise to many activated proteins in a signaling cascade. In this case it means that an activated G protein receptor will activate many G proteins. Adenylyl cyclase is an enzyme that catalyzes the synthesis of cAMP from ATP. Adenylyl cyclase gets activated by the formation of a 1:1 complex with the G_α protein, that is, there is no amplification in this step.

d) The movement of the neutrophil involves the formation of filopodia and lamellipodia inn the front of the cell (facing the activated G protein-linked receptors) and the contract of the cell, driven by actin and myosin contraction, in the opposite side of the cell. The two signaling events (Rac and Rho) directly inhibit each other. The Rac activation dominates in the part of the cell where the receptor was activated and leads to the formation of cell extensions by actin polymerization. Since the PIP_3 is very short-lived it is not able to diffuse far into the cell and so, Rho activation dominates in the opposite side of the cell, where the activation of actin-myosin contraction leads to the contraction of the part of the cell opposite to the activated receptors and helps the cells to move forward.

Exercise 3: Cancer cells, cytoskeleton and immunology

Candidate number ……..…….……
Course code ……..
Page no……...

a) Which cells of the adaptive immune response do you expect to be activated during a viral infection? Justify.
b) Describe, schematically, two different mechanisms in which insertional mutagenesis, the integration of the viral genome into a cell, can lead to the development of cancer?
c) MSH2 is a gene commonly associated with familial nonpolyposis colorectal cancer. This gene encodes a protein that is involved in correcting mismatched nucleotides. Briefly explain why the individuals who possess a mutated MSH2 gene from birth are more likely to develop colon cancer than individuals without a mutated gene.
d) Intermediate filaments can be used in cancer diagnostics. Why?
e) Bending of cilia is driven by the action of axonemal dyneins. What would happen to the cilia if the nexin proteins, which join adjacent **microtubule** doublets, would be missing?

Answers:

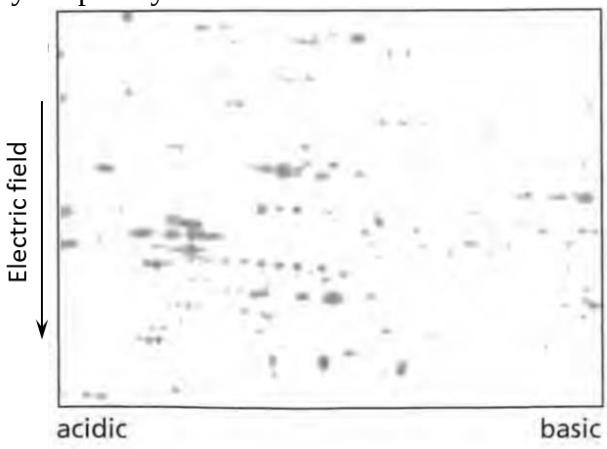
a) Cells that are infected by the viruses will present fragments of the viruses proteins at the surface of the cell (antigen-presenting cells) via the major histocompatibility complex I, which will in turn be recognized by cytotoxic T lymphocytes (T_C cells). The T_C cells will directly attack and destroy the antigen-presenting cells (full score).
It is also possible for B lymphocytes (B cells) to be directly activated by binding to antigens (aminoacid sequence, for example) at the surface of the virus. In addition, help T lymphocytes (T_H cells) can also be activated and activate T_C and B cells.
b) Insertional mutagenesis can lead to cancer due to the insertion of an oncogene directly into the genome of the host that could encode, for example, for a continuously activated tyrosine kinase receptor. Retroviruses can sometimes cause cancer even if they do not possess oncogenes in their genome. They achieve this by including their genome in the region of the host genome where a proto-oncogene is located. Such integration will lead to loss of regulation control of a normal protein, leading to the excess of such protein, which could be, for example, a transcription factor.
Note: This question was somewhat vague and many have answered with for example the two mechanism in which HPV can lead to cancer. These answers as well as other who presented and justified two different (and more specific) mechanism that may lead to cancer where considered correct.
c) The MSH2 gene encodes a protein that is involved in correcting mismatch, that is, this is a tumor suppressor gene. If one of the copies of the gene in the cell is functional, the protein needed for nucleotide mismatches correction is still synthesized, avoiding the appearance of cancer. So, in order for cancer to develop in these cells, both copies of the gene need to be mutated.
Now, all cell types in this individual, right from birth, have only one functional copy of the MSH2 gene, including the cells that make the colon (Genotype: MSH2+/MSH2-). The probability that one of the colon cells will acquire a mutation in the non-mutated MSH2 gene, which will lead to cancer, is larger than suffering a mutation in each of the two functional copies of the MSH2 gene in one colon cell.
d) Contrary to the microtubules and microfilaments that are built from the same monomers, intermediate filaments are composed of different proteins, with a similar tertiary structures, and their composition is different from cell to cell within the same organism. It is thus possible to analyze the composition of the IF in tumor cells and determine if those cells are originated from that particular tissue or is they are metastasis from some other tissue.
Note: Answers that involved the observation and comparison of the IFs in normal and cancer cells to look for differences in structure or concentration, due to the lacks of

cadherins were awarded 2.0 (in 5.0) points. Not sure it is actually correct but it was an interesting suggestion.

e) The bending of cilia is driven by the "walking" of the axonemal dyneins, covalently-bond to a microtubule doublet, over the adjacent doublet. Since the MTs are bound together this "walking" will lead to the bending of the cilia. If the MTs are not bound to each other, the cilia will not show the beating movement. Instead, the microtubule doublets will be displaced from the other leading to the elongation and "thinning" of the cilia.

Exercise 4: Gene regulation and cell cycle

a) A small portion of a two-dimensional display of proteins from human brain is shown in the figure below. These proteins were separated using two-dimensional (SDS-PAGE) gel electrophoresis, that is, they were separated based on their size in one dimension and on their isoelectric point in the other. Not all protein spots in the gels are products of different genes; some represent modified forms of a protein that migrate to different positions. Indicate where in the gels you would expect to find proteins with larger molecular weight. Indicate sets of spots that could represent proteins that differ in the number of phosphates they carry. Explain your choices.



b) Some of the spots present in the electrophoresis gels are due to transcription factors. What are these and what is their function?

c) Describe the different phases the cells undergoes during cell division.

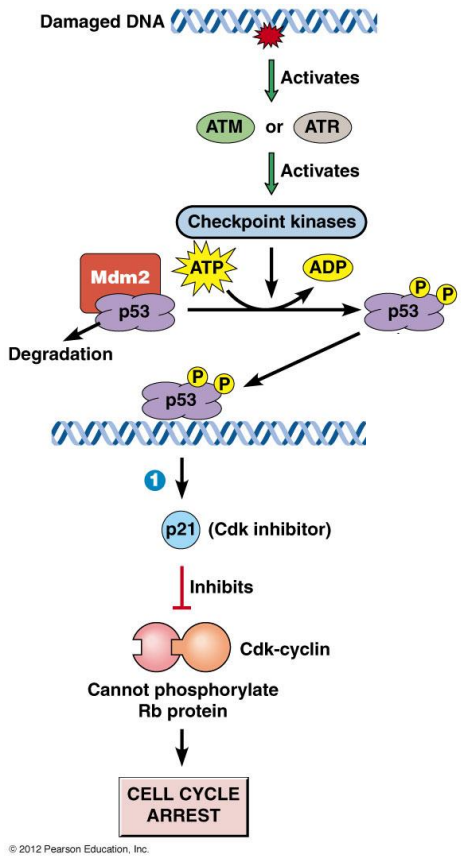
d) A person has been exposed to ionizing radiation and the DNA is damaged. To avoid replication of the damaged DNA, the cells are stopped/arrested in the cell cycle. Describe how this takes place. Some of the proteins involved are: ATM, p53, p21, cyclin, RB protein. What happens to the cells if the DNA is not repaired?

Answers:

a) The proteins with the largest molecular weight will be found on the top of the gels closer to the loading wells and lighter proteins will migrate longer in the gels. During the electrophoresis the proteins migrate through a gel network and so proteins with a larger molecular weight will reptate through the network more slowly than lighter proteins. Proteins that have a different number of phosphate groups, for example, phosphorylated enzymes in a signaling pathway will have a similar molecular weight but different charge, that is, different isoelectric points. One expects them to be the nearly-horizontal groups of spots in the gel.

b) Transcription factors are regulating proteins that act in combination to control gene expression in different cell types. Most cells in an unicellular organism have the same

Candidate number ……..…….……
Course code ……..
Page no……...



genome, that is, they can potentially produce the same gene products. On the other hand, cells that compose a specific tissue are high specialized, and produce only a specific set of proteins characteristic for the function and structure of those particular cells. Transcription factors bind to DNA regulatory sequences in the genome and regulate the expression of proteins by enhancing the transcription of some genes and by inhibiting the expression of others.

c) Mitosis is divided into prophase, prometaphase, metaphase, anaphase and telophase. During prophase, replicated chromosomes condense into paired sister chromatids while centrosomes initiate assembly of the mitotic spindle. In prometaphase, the nuclear envelope breaks down and chromosomes then attach to spindle microtubules and move to the spingle equator, where they line up at metaphase. At anaphase, sister chromatids separate and the resulting daughter chromosomes move toward opposite spindle poles. During telophase the chromosomes decondense, and a nuclear envelope reassembles around each daughter nucleus.

d) The figure above schematizes the response of the cell to damaged DNA. To avoid replication of damaged DNA, DNA is checked before starting phase S (restriction point), in the G2 and M phases. Damaged DNA activates the ATM (or ATR) protein kinase, leading to the activation of checkpoint kinases, which leads to the phosphorylation of the p53 protein. Phosphorylation stabilizes p53 by blocking its interaction with a protein (Mdm2) that would mark p53 for degradation. The phosphorylated p53 acts as a transcription factor, binds to DNA and regulates the transcription of a gene coding for protein p21. p21 is a Cdk inhibitor and the resulting inhibition of Cdk-cyclin prevents phosphorylation of the Rb protein, leading to cell cycle arrest at the restriction point.

If the DNA damage cannot be repaired the cell will undergo apoptosis.

Exercise 5: 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.

a) Protein/Lipid ratio is largest in the
- myelin sheath of nerve axons
- **rough endoplasmic reticulum**
- outer membrane of mitochondria

b) Transport of HCO_3^- in and out of the erythrocytes (red-blood cells) is driven by
- simple diffusion.

Candidate number ……..…….……
Course code ……..
Page no……...

- a HCO_3^--pump.
- **a HCO_3^-/Cl- antiport carrier protein**

c) ATP synthases can be found in the
- outer membrane of mitochondria
- intermembrane space of mitrochondria.
- **inner membrane of mitochondria.**

d) Newly synthesized lipids are predominantly transported to the mitochondria via
- coated vesicles.
- **lipid carriers.**
- translocases.

e) The core oligosaccharide side chain added to glycoproteins in the endoplasmic reticulum is composed of:
- **mannose (Man), galactose (Gal) and N-acetylglucosamine (GlcNAc).**
- mannose (Man), sialic acid (Sia) and N-acetylglucosamine (GlcNAc).
- mannose (Man), galactose (Gal) and N-acetylneuraminic acid (NANA).

f) Fusion of vesicles with target membranes is mediated by
- **SNARE proteins.**
- dynamin.
- actin.

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

h) An action potential occurs in the following steps:
- Opening of K^+ voltage-gated channels, membrane depolarization, inactivation of K^+ channels, opening of Na^+ voltage-gated channels, membrane hyperpolarization.
- Opening of K^+ voltage-gated channels, membrane hyperpolarization, inactivation of K^+ channels, opening of Na^+ voltage-gated channels, membrane depolarization.
- **Opening of Na^+ voltage-gated channels, membrane depolarization, inactivation of Na^+ channels, opening of K^+ voltage-gated channels, membrane hyperpolarization.**

i) Nerve transmission in the heart muscle is done via a
- adhesive junctions.
- **gap junctions.**
- tight junctions.

j) Striated muscle contraction is driven by
- Microtubules and kinases.
- **Microfilaments and myosin.**
- Intermediate filaments and vimetin.

Candidate number ……..…….……
Course code ……..
Page no……...

k) The protein class, whose fibers have a high tensile strength and account for much of the strength of the extracellular matrix is named
   - **collagen.**
   - elastin.
   - fibronectin.

l) Which class of enzymes has allowed the development of recombinant DNA technology?
   - DNases.
   - **Restriction endonucleases.**
   - Topoisomerases.

m) RNA splicing in an example of
   - genomic control.
   - **post-transcriptional control.**
   - translational control.

n) During screening process of T cells in the thymus, these are presented to cells presenting self-peptides. T cells undergo apoptosis if the affinity of their receptor to that of the MHC-bounded self-peptide is
   - strong.
   - **weak.**
   - none.

o) Lack of cadherins in cancer cells contributes to
   - evading apoptosis and cell immortality.
   - Sustained angiogenesis and uncontrolled growth.
   - **enhanced cell mobility and metastases.**