CHEM-E8120 Cell Biology, Examination December 10th, 2020

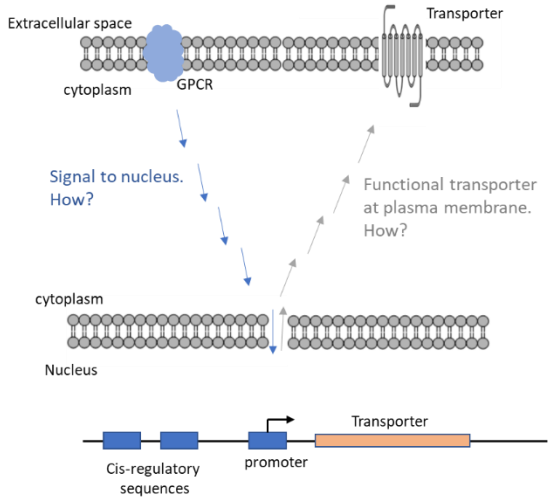
1. Open book exam
2. Duration: 4 hours
3. The exam contributes 50% to the course grade
4. **Submit your answers in a single PDF file that is marked with your name.**

Question 1: Essay question (maximally 20 points).

Questions 2 to 4: Problem solving, each question gives maximally 5 points.

Question 1. Essay question. (maximally 20 points)

Cellular uptake of galactose is mediated by the highly specific galactose transporter that is localized to the plasma membrane. The transporter is an integral membrane protein with 7 membrane-spanning α-helices and it contains two N-linked oligosaccharides. The transporter is only expressed if galactose is present. Galactose stimulates a plasma membrane localized G-protein coupled receptor (GPCR). After activation of the GPCR, the signal is conveyed through a signaling cascade to the nucleus. When the signal reaches the nucleus, expression of the gene encoding the galactose transporter is induced (See sketch below; please note that this is a fictional set-up that is based on real biological processes and molecules!).



a) Explain how the GPCR is converting the extracellular stimulus into an intracellular signal. Then, design a putative signaling pathway that could relay the signal to the nucleus in order to turn on gene expression (8 points).

b) Give a detailed account of the events taking place after the mRNA encoding the transporter has left the nucleus until the transporter reaches its destination in the plasma membrane. (Consider cellular processes, important proteins and other molecules, cellular compartments, etc.) (12 points)

Question 2. (maximally 5 points)

When activated, the platelet-derived growth factor (PDGF) receptor phosphorylates itself on multiple tyrosines. These phosphorylated tyrosines serve as assembly sites for several SH2-domain containing proteins that include phospholipase C-y (PLCy), a Ras-specific GTPase-activating protein (GAP), a subunit of phosphoinositide 3-kinase (PI3K), and a phosphotyrosine phosphatase (PTP) (Figure 1A).

PDGF binding stimulates several changes in the target cell, one of which is an increase in DNA synthesis that can be measured by incorporation of radioactive thymidine or bromodeoxyuridine into DNA. To determine which of the bound proteins is responsible for activation of DNA synthesis, you construct several mutant genes for the PDGF receptor that retain individual or combinations of tyrosine phosphorylation sites. When expressed in cells that do not make a PDGF receptor of their own, each of the receptors is phosphorylated at its tyrosines upon binding of PDGF. As shown in Figure 1B, DNA synthesis is stimulated to different extents in cells expressing the mutant receptors.

What roles do PI3K, GAP, PTP, and PLCy play in the stimulation of DNA synthesis by PDGF? Clearly explain how you reach your conclusion.

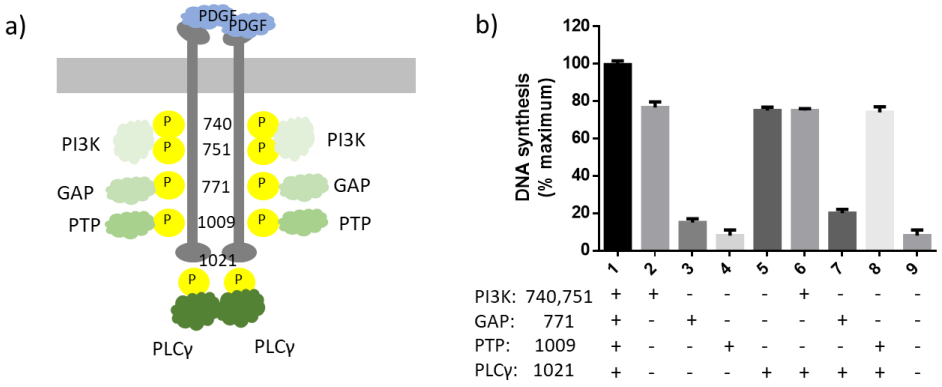


Figure 1. A) The signaling complex assembled on the PDGF receptor. Numbers refer to the positions of the phosphorylated amino acids in the sequence of the PDGF receptor. B) Stimulation of DNA synthesis by the PDGF receptor and by receptors missing selected phosphorylation sites. Stimulation of DNA synthesis by the normal receptor is set at 100%. The presence of a phosphorylation site is indicated by +; the absence of a site by –

Question 3. (maximally 5 points)

Microsomes are fragments of endoplasmic reticulum and attached ribosomes that are obtained after eukaryotic cells are broken-up and membranes are isolated. These microsomes can be used for *in vitro* studies of processes connected to the endoplasmic reticulum such as protein synthesis, translocation, signal peptide cleavage and protein N-glycosylation.

Translocation of proteins across rough microsomal membranes can be judged by several experimental criteria: (1) the newly synthesized proteins are protected from added proteases, unless detergents are present to solubilize the lipid bilayer; (2) the newly synthesized proteins are glycosylated by oligosaccharide transferases, which are localized exclusively to the lumen of the ER; (3) the signal peptides are cleaved by signal peptidase, which is active only on the luminal side of the ER membrane. Use these criteria to decide whether a protein is translocated across the rough microsomal membranes.

The mRNA is translated into protein in a cell-free system in the absence or presence of microsomes. Samples of newly synthesized proteins were collected and treated as indicated in the figure legend. After treatment, the samples were analyzed on an SDS polyacrylamide gel (Figure 2).

A. Explain how you can tell whether the protein is sensitive to protease, is glycosylated or the signal peptide is cleaved of. Which lanes do you need to compare? (2.5 point).

B. Using the three criteria outlined above, decide and explain whether the experimental results in the presence of microsomes (Figure 1, lanes 5 to 8) indicate that the protein is translocated across microsomal membranes. If it is translocated, is the protein anchored in the membrane, or is it translocated all the way through the membrane? How can you tell? (2.5 point)

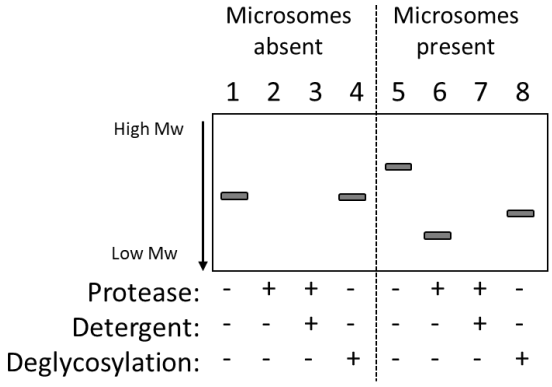
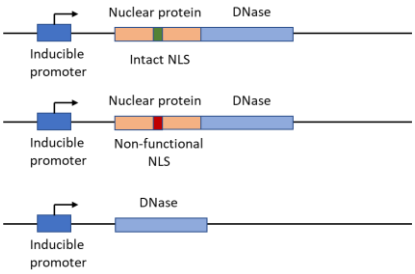


Figure 2. Results of translation of a pure mRNA in the presence and absence of microsomal membranes. Samples are treated in four different ways: (1) no treatment, (2) addition of a protease, (3) addition of a protease and detergent, and (4) disruption of microsomes and addition of endoglycosidase H (endo H), which removes N-linked sugars (= deglycosylation). Treatments are indicated at the *bottom* of each lane. Electrophoresis was done on an SDS polyacrylamide gel, which separates proteins according to size.

Question 4 (maximally 5 points)

You study the nuclear transport machinery and you received three plasmids. Each plasmid contains a gene encoding a DNase that is under the control of an inducible promoter. Two of the plasmids contain a hybrid gene that is a fusion between a gene whose product is normally imported into the nucleus and the gene for the DNase. The first plasmid contains an intact nuclear localization signal (NLS); the second plasmid does not have a functional NLS; the third plasmid contains only the DNase (See sketch below).



You introduce the three constructs into cells and then assay the transformed cells in presence or absence of the inducer. The promoter enables transcription of the genes only when the inducer is present in the growth medium. The results are shown in the Table.

	Repressing medium	Inducible medium
Intact NLS	proliferation	cell death
Non-functional NLS	proliferation	proliferation
Only DNase	proliferation	proliferation

a) Why do only cells with the intact NLS proliferate in the repressing media (absence of inducer) but die in the presence of the inducer, whereas cells with the non-functional NLS or the DNase only proliferate in both media? (2 points)

b) Describe a detailed set-up how this system might be used for a selection assay to isolate cells that are defective in nuclear transport? Explain what the expected results would be and why you expect to obtain these. (3 points)