Name: Student number:

CHEM-E8120 Cell Biology, Examination December 11th, 2018

1. Duration: **4 hours**
2. Help: **"Cheat sheet" 1 A4 paper containing two pages of hand-written notes and hand-drawn illustrations (front & backside) marked with name and student number.**
3. **Return exam questions (marked with name) together with your answers and the cheat sheet.**

Questions 1 to 3: Problem solving, each question gives maximally 5 points.

Questions 4 and 5: Essay questions, Q4 gives maximally 10 points, Q5 maximally 5 points.

Questions 6: Match terms with their definitions, maximally 5 points.

Question 1.

A born skeptic, you plan to confirm for yourself the results of a classic experiment originally performed in the 1960s by Meselson and Stahl. They concluded that each daughter cell inherits only, one strand of its mother's DNA. To check their results, you "synchronize" a culture of growing cells, so that virtually all cells begin and then complete DNA synthesis at the same time. You first grow the cells in a medium that contains nutrients highly enriched in heavy isotopes of nitrogen and carbon (^{15}N and ^{13}C in place of the naturally abundant ^{14}N and ^{12}C). Cells growing in this "heavy" medium use the heavy isotopes to build all of their macromolecules, including nucleotides and nucleic acids. You then transfer the cells to a normal, "light" medium containing ^{14}N and ^{12}C nutrients. Finally, you isolate DNA from cells that have grown for different numbers of generations in the light medium and determine the density of their DNA by density-gradient centrifugation. Your data, plotting the amount of DNA isolated versus its density, are shown in Figure 1.

Are these results in agreement with your expectations? Explain the results.

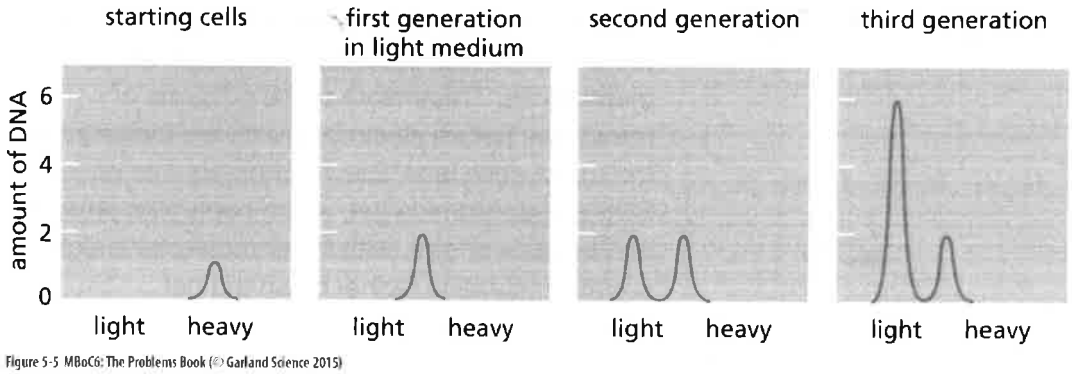

Figure 5-5 MBoC6: The Problems Book (© Garland Science 2015)

Figure 1. Density of DNAs isolated from cells that were grown for different times in "light" medium after initial growth in medium enriched for heavy isotopes of nitrogen and carbon. Equal culture volumes were analyzed for each time point. Amount of DNA is in arbitrary units, with the peak amount of DNA in the sample containing starting cells set equal to 1.

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Question 2

Microsomes are fragments of endoplasmic reticulum and attached ribosomes 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.

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 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 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 that are added in the ER. An electrophoretic analysis of these samples is shown in Figure 3.

A. Explain the experimental results that are seen in the absence of microsomes (Figure 2, lanes 1 to 4). (2 points)

B. Using the three criteria outlined above, decide whether the experimental results in the presence of microsomes (Figure 2, lanes 5 to 8) indicate that the protein is translocated across microsomal membranes. How would you account for the migration of the proteins in Figure 1, lanes 5, 6, and 8? (2 points)

C. Is the protein anchored in the membrane, or is it translocated all the way through the membrane? (1 point)

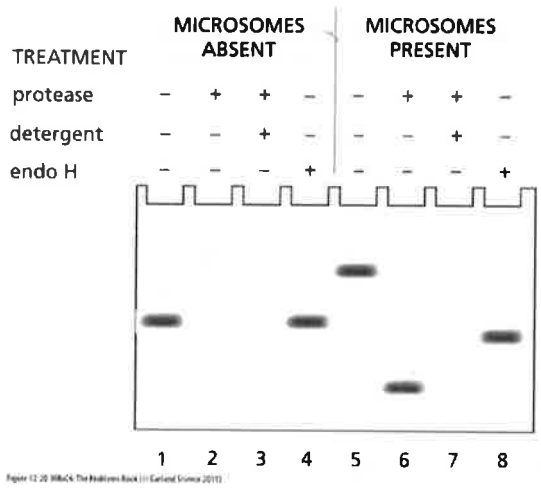

Figure 2. Results of translation of a pure mRNA in the presence and absence of microsomal membranes. Treatments of the products of translation before electrophoresis are indicated at the *top* of each lane. Electrophoresis was on an SDS polyacrylamide gel, which separates proteins on the basis of size, with lower molecular weight proteins migrating farther down the gel.

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Question 3

Akt is a key protein kinase in the signaling pathway that leads to cell growth. Akt is activated by a phosphatidylinositol-dependent protein kinase (PDK1), which phosphorylates threonine 308. At the same time, serine 473 is phosphorylated. Your advisor has been unsuccessful in purifying the protein kinase responsible for the phosphorylation of serine 473, but you think you know what is going on. You construct genes encoding two mutant forms of Akt: one carries a point mutation in the kinase domain, Akt—K179M, which renders it kinase—dead, and the other carries a point mutation in the domain required to bind to PDK1 (Akt-T308A), which cannot be activated by PDK1. You transfect each of these constructs, and a construct for wild—type Akt, into cells that do not express their own Akt. You treat a portion of the cells with an insulin—like growth factor (IGF1), which activates PDK1, and analyze the phosphorylation state of the various forms of Akt using antibodies specific for Akt or for particular phosphorylated amino acids (Figure 3).

What is the identity of the enzyme (PDK1 or autophosphorylation by Akt) that phosphorylates serine 473 on Akt? Explain your reasoning.

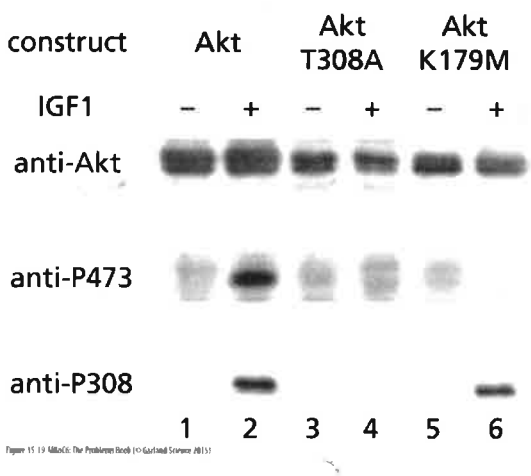

Figure 3. Expression levels of various forms of Akt and their degree of phosphorylation in the presence and absence of IGF1 (Problem 15—102). Anti-Akt recognizes all three forms of Akt regardless of their phosphorylation state; anti—P473 specifically recognizes the phosphorylated serine at position 473; anti—P308 specifically recognizes the phosphorylated threonine at position 308.

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Question 4. Essay question (write approximately 1,5 pages), 10 points.

Discuss in total 10 important aspects related to this question, two in part A, eight in part B. Depending on the depth of the discussion, you will get a full point or reduced amounts of points 0.75, 0.5 or 0.25 per item discussed.

Cellular uptake of galactose is mediated by the highly specific galactose transporter, which is a multi-pass membrane protein localized in the plasma membrane. The transporter is only expressed if galactose is present in the extracellular environment. Galactose stimulates a plasma membrane localized receptor tyrosine kinase (RTK). After activation of the RTK, 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.

A) Explain how the RTK is converting the extracellular stimulus into an intracellular signal and how this signal might be relayed into the nucleus. (2 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.) (8 points)

Question 5, Write approximately 0,5 page. Maximally 5 points.

Define the following terms and describe their relationships to one another:

a. TATA box
b. promoter
c. general transcription factors
d. cis-regulatory site

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Question 6, maximally 5 points, for each missing/incorrect pair reduction of 0,28 points.

Find the correct term for each definition. For each definition, add the number of the correct terms into the table.

Chromosomal DNA and packaging			**Membrane transport**			**Mechanisms to control gene expression**		
Definition	Term		Definition	Term		Definition	Term	
A			A			A		
B			B			B		
C			C			C		
D			D			D		
E			E			E		
F			F			F		

Chromosomal DNA and packaging

1. Cell cycle
2. centromere
3. chromatin
4. chromosome
5. exon
6. histone
7. histone H1
8. homologous chromosome
9. intron
10. karyotype
11. nucleosome
12. replication origin
13. telomere

A. Constricted region of a mitotic chromosome that holds sister chromatids together.
B. Any one of a group of small abundant proteins, rich in arginine and lysine, that form the primary level of chromatin organization.
C. The orderly sequence of events by which a cell duplicates its contents and divides into two.
D. Complex of DNA, histones, and non-histone proteins found in the nucleus of a eukaryotic cell.
E. One of the two copies of a particular chromosome in a diploid cell, each copy being derived from a different parent.
F. Beadlike structure in eukaryotic chromatin, composed of a short length of DNA wrapped around a core of histone proteins.

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Membrane transport

1. cargo
2. clathrin-coated vesicle
3. coated vesicle
4. coat-recruitment GTPase
5. COPI-coated vesicle
6. COPII-coated vesicle
7. lumen
8. Rab protein
9. Rab effector
10. SNARE protein (SNARE)
11. transport vesicle
12. t-SNARE
13. v-SNARE

A. General term for a membrane-enclosed container that moves material between membrane-enclosed compartments within the cell.
B. Any of a large family of monomeric GTPases present in the plasma membrane and organelle membranes that confer specificity on vesicle docking.
C. Protein that facilitates vesicle transport, docking, and membrane fusion once it is bound by an activated Rab protein.
D. General term for a member of the large family of proteins that catalyze the membrane fusion reactions in membrane transport.
E. The interior space of a membrane-enclosed compartment.
F. General term for a transport vesicle that carries a distinctive cage of proteins covering its cytosolic surface.

Mechanisms to control gene expression

1. Cap-Independent Initiation
2. Internal ribosome entry site (IRES)
3. alternative splicing
4. Post-transcriptional control
5. mRNA decay
6. Noncoding RNA
7. Small interfering RNA
8. microRNA
9. RNA interference
10. CRISPR

A. A way to generate different proteins from the same gene by combining different segments of the initial RNA transcript to make distinct mRNAs.
B. General term for a regulatory event that occurs after RNA polymerase has bound to the gene's promoter and begun RNA synthesis.
C. A sequence in the interior of an mRNA that folds into structures that bind translation initiation proteins.
D. Natural defense mechanism in many organisms that is directed against foreign RNA molecules, especially those that occur in double-stranded form.
E. A class of short noncoding RNAs that regulated gene expression.
F. A defense mechanism in bacteria that allows them to destroy viral invaders they have seen before.