

Questions 1 and 2: Problem analyzing and solving, each question gives maximally 5 points

Questions 3 and 4: Essay questions, Q3 gives maximally 5 points, Q4 gives maximally 10 points

Problem solving:

First, analyze the problem. If you are unsure about certain details of the problem clearly indicate it in your answers and mention your assumptions!

Question 1. (maximally 5 points)

Although the vast majority of transmembrane proteins insert into membranes with the help of dedicated protein-translocation machines, a few proteins can insert into membranes on their own.

You are studying a protein that inserts itself into the bacterial membrane independent of the normal translocation machinery. This protein has an N-terminal, 18-amino-acid hydrophilic segment that is located on the outside of the membrane, a 19-amino-acid hydrophobic transmembrane segment flanked by negatively and positively charged amino acids, and a C-terminal domain that resides inside the cell (Figure 1A). If the protein is properly inserted in the membrane, the N-terminal segment is exposed externally where it can be clipped off by a protease, allowing you to quantify insertion.

To examine the roles of the hydrophobic segment and its flanking charges, you construct a set of modified genes that express mutant proteins with altered charges in the N-terminal segment, altered lengths of the hydrophobic segment, and combinations of the two (Figure 1B). For each gene you measure the fraction of the total protein that is cleaved by the protease, which is the fraction that was inserted correctly (Figure 1B). To assess the contribution of the normal membrane potential (positive outside, negative inside), you repeat the measurements in the presence of CCCP, an ionophore that eliminates the charge on the membrane (Figure 1B).

- A. Which of the two N-terminal negative charges is the more important for insertion of the protein in the presence of the normal membrane potential (minus CCCP)? Explain your reasoning.
- B. In the presence of the membrane potential (minus CCCP), is the hydrophobic segment or the N-terminal charge more important for insertion of the protein into the membrane? Explain your reasoning.
- C. In the absence of the membrane potential (plus CCCP), is the hydrophobic segment or the N-terminal charge more important for insertion of the protein into the membrane? Explain your reasoning.

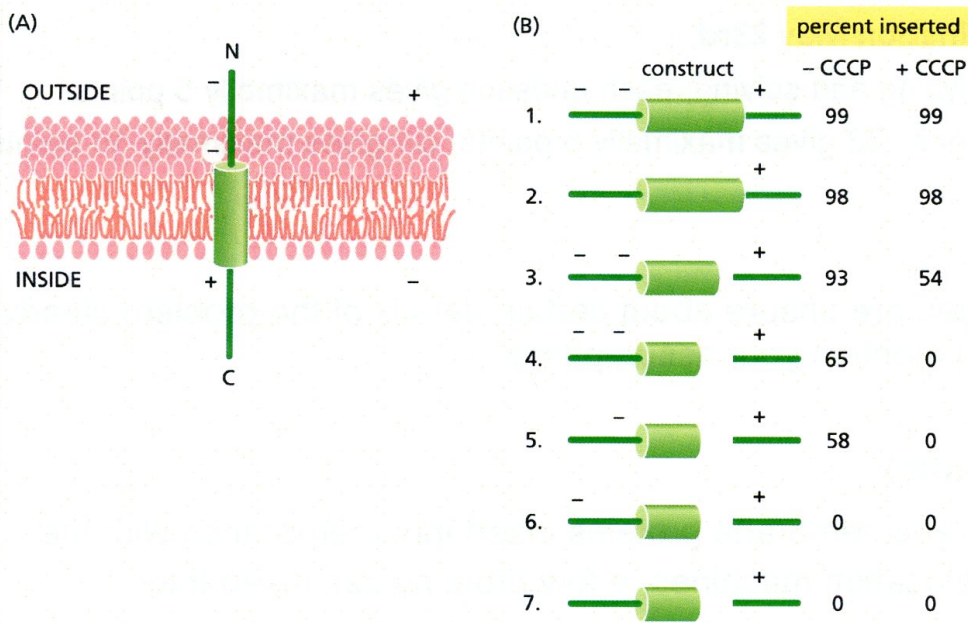


Figure 1. Insertion of a small protein into the bacterial membrane. (A) Normal orientation of the protein in the membrane. (B) Mutant proteins used to investigate the contributions of the N-terminal negative charges and length of the hydrophobic segment to membrane insertion. The presence of negative charges is indicated by -; *green cylinders* indicate the length of α -helical, transmembrane hydrophobic segments; deletions of the hydrophobic segments are shown as *gaps*. Percent inserted refers to the proportion of the total protein whose N-termini are sensitive to protease digestion.

Question 2. (maximally 5 points)

Activation (“maturation”) of frog oocytes is signaled through a MAP kinase signaling module. An increase in the hormone progesterone triggers the module by stimulating the translation of the mRNA for Mos, which is the frogs MAP kinase kinase kinase. Maturation is easy to score visually by the presence of a white spot in the middle of the brown surface of the oocyte Figure 2).

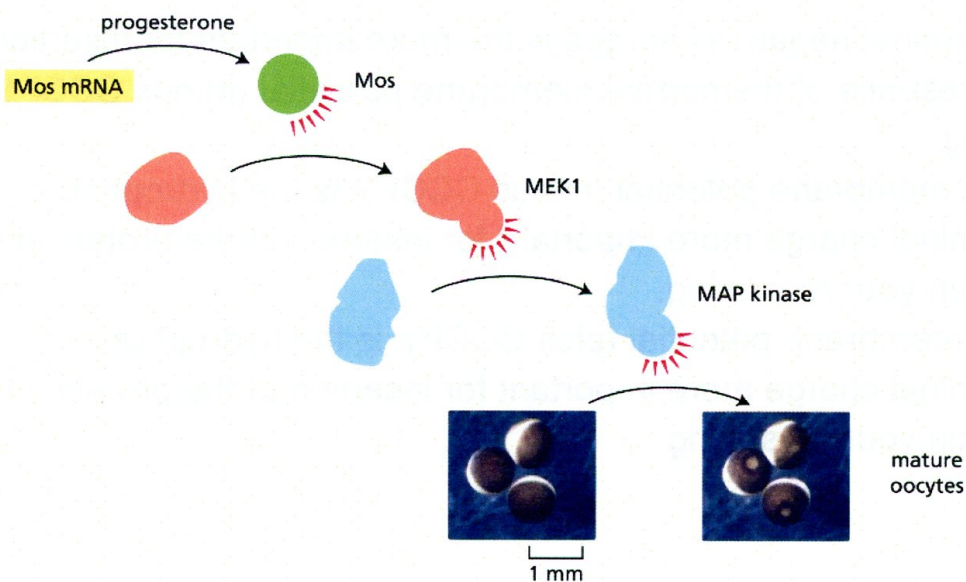


Figure 2. Progesterone-induced MAP kinase activation, leading to oocyte maturation. MEK1 is the frog’s MAP kinase kinase.

To determine the dose-response curve for progesterone-induced activation of MAP kinase, you place 16 oocytes in each of six plastic dishes and add various concentrations of progesterone. After an overnight incubation, you crush the oocytes, prepare an extract, and determine the state of MAP kinase phosphorylation (hence, activation) by SDS polyacrylamide-gel electrophoresis (Figure 3A). This analysis shows a graded response of MAP kinase to increasing concentrations of progesterone.

Before you crushed the oocytes, you noticed that not all oocytes in individual dishes had white spots. Had some oocytes undergone partial activation and not yet reached the white-spot stage? To answer this question, you repeat the experiment, but this time you analyze MAP kinase activation in individual oocytes. You are surprised to find that each oocyte has either a fully activated or a completely inactive MAP kinase (Figure 3B).

- Which mechanism is used to convey the signal through the MAP kinase signaling module (1 point)
- What is an all-or-none response? (1 point)
- How can an all-or-none response in individual oocytes give rise to a graded response in the population? (3 points)

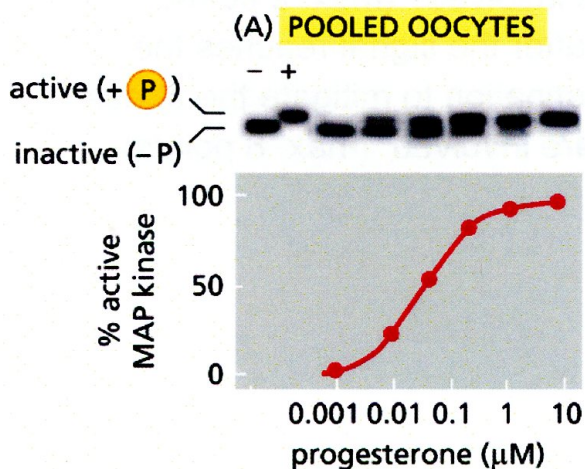
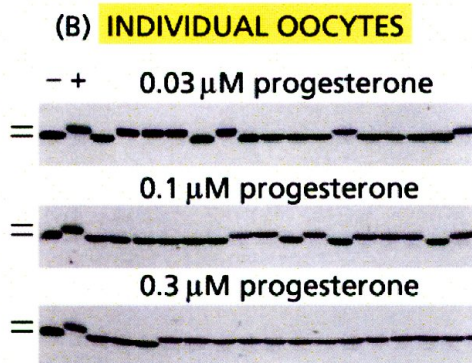


Figure 3. Activation of frog oocytes (A) Phosphorylation of MAP kinase in pooled oocytes. (B) Phosphorylation of MAP kinase in individual oocytes. MAP kinase was detected by immunoblotting using a MAP-kinase-specific antibody. The first two lanes in each gel contain nonphosphorylated, inactive MAP kinase (-) and phosphorylated, active MAP kinase (+).



Essay questions

Question 3. Essay question, maximally 5 points

Discuss the role of Histones in the chromatin organization and gene expression. Start with a description of Histone(s) and how their activity is controlled. Then discuss how Histones contribute to chromatin organization and gene expression.

Question 4. Essay question, maximally 10 points

The unfolded proteins response is a cellular reaction to cope with the accumulation of unfolded proteins in the Endoplasmic reticulum. Ultimately, its activation leads to the induction of the gene expression, for example of a chaperone.

- A. Give a rough account how the signal initiating the UPR is generated and relayed into the nucleus. What are the key steps and molecules involved? (max. 2 points)
- B. Give a detailed account of the events taking place after the signal reaches the nucleus until said chaperone reaches the target destination to mitigate the stress. Clearly indicate also which cellular compartments are involved. (max. 8 points)