MCB130 Midterm Key

Name:
SID:
GSI's Name:

1. Peroxisomes are small, membrane-enclosed organelles that function in the degradation of fatty acids and in the degradation of H_2O_2. Peroxisomes are not part of the secretory pathway and peroxisomal enzymes are targeted directly from the cytoplasm to the lumen of peroxisomes via a peroxisomal targeting signal (PTS).
   a. Design a *general* experimental strategy to identify the PTS in your favorite peroxisomal protein 1 (FPP1). [15 pts]

The question is asking to describe the experiments to find a peroxisomal targeting signal sequence without assuming the signal is one that has already been described. You cannot assume that the signal sequence is cleaved, this is not a valid assumption and experiments based solely on this assumption received only minimal credit for experimental design.

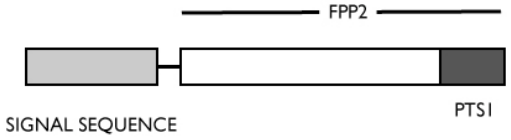
Need to specifically explain how to first find the PTS in the FPP1 protein, need to explain an experiment that would narrow down to a specific region or find it based on mutagenesis. It is important to not only explain how to test a potential PTS but also explain how the PTS was found in the first place. After the PTS sequence is found, need to show specifically that the PTS is both necessary and sufficient for localization to the peroxisome. [**2 pts** for clearly finding the PTS sequence from the FPP1 protein and recognizing that it needs to be necessary and sufficient]

To show that the PTS sequence is necessary for localization of the FPP1 protein to the peroxisome, need to either make a point mutation or small deletion in the PTS of FPP1. Then it is important to have an experiment to show that the FPP1 protein with a defective PTS is not able to localize to the peroxisome. [**5 pts**. for clearly defining the PTS as necessary.]

Examples of possible experiments include 1) tagging FPP1 with GFP and using microscopy to visualize the cellular localization (need to mention control that FPP1-GFP is able to localize to peroxisome and FPP1-PTS* mutant-GFP was not able to localize to the peroxisome) or 2) doing an *in vitro* experiment where the localization of FPP1 was assayed by a protease protection assay; in the experiment need to either label the FPP1 with radioactivity or have another means of detection such as an antibody and then run a gel to determine if FPP1 could get into the peroxisome and the FPP1-PTS* mutant was excluded. [**3 pts** for clearly describing the experimental assays]

To show that the PTS sequence from FPP1 is sufficient for localization of non-peroxisomal molecules to the peroxisome, need to attach the PTS sequence to a cytosolic protein or a gold particle and assay for the incorporation into the peroxisome. Again need to specifically explain an experiment along with how the outcome would be assayed / visualized. [**5 pts**. for clearly defining the PTS as sufficient.]

1. b. Previous research has shown that many peroxisomal enzymes contain a PTS at their very carboxy-terminus. This signal, termed PTS1, consists of the three amino acids Ser-Lys-Leu. A researcher fuses the ER signal peptide to his favorite peroxisomal protein 2 (FPP2) and expresses this fusion protein in cells:

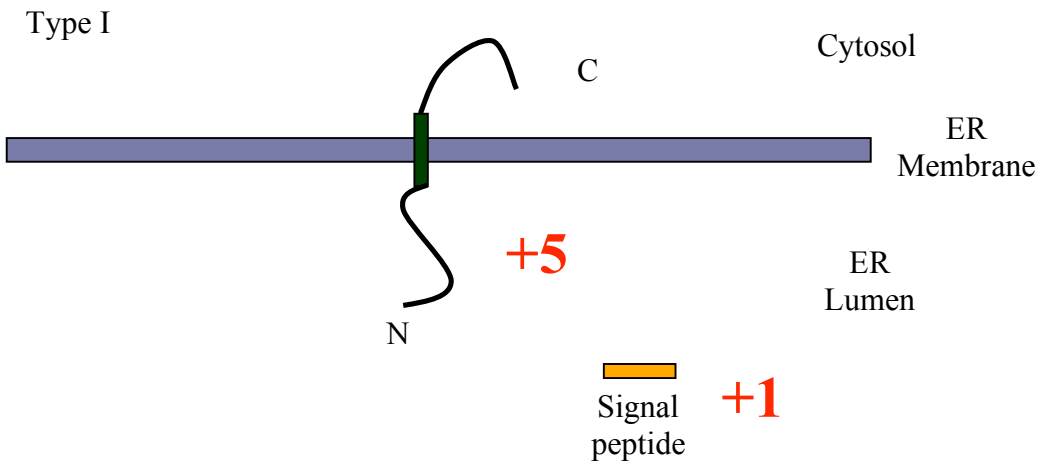


Upon expression, in which cellular compartment will he find the fusion protein? Explain your answer. [15 pts]

+5 pts localizes to the ER

+10 pts (2 pts per):
- signal sequence is translated first
- SRP binds to the signal sequence
- SRP causes translation to pause
- the protein is cotranslationally translocated
- therefore PTS1 never sees the cytoplasmic peroxisomal transport machinery

2. Topology of the human growth hormone receptor:



Design an experiment to test whether your prediction is correct:

1. Purify smooth ER from cells by differential centrifugation and gradient centrifugation.
2. In vitro system, IVT experiment with receptor mRNA and radiolabel aa + RM

} +3

3. Incubation to allow the translocation to occur
4. Purify the RM again and do protease digestion

} +3

5. Lyse the RM with detergent
6. Run the lysis on the SDS gel

} +3

7. Analyze the radio labeled peptides on gel

If the prediction is correct, C terminus should be completely diegested and N terminus with TM domain should be protected. So you will only get a band around 27kD.

OR:

1. Purify cell membrane (or ER) from cells with the expression of the hormone receptor by differential centrifugation } +3
2. Digest the microsome by protease } +3
3. Lyse the microsome and run on SDS gel
4. Do a western blotting or sequencing to identify the peptides from the receptor.
5. Analyze the peptides } +3

If the prediction is correct, C terminus should be completely diegested and N terminus with TM domain should be protected. So you will only get a band around 27kD.

3. Explain how secretory vesicles form at the membrane of the endoplasmic reticulum (ER). [15 pts]

2 pts Sar1 GEF in the ER membrane (must specify location)

1 pts Sar1 GEF converts Sar1GDP to Sar1GTP

2 pts Sar1GTP binds to ER membrane

5 pts Sar1 recruits COPII coat proteins
(2.5 pts for coat proteins without recruitment by Sar1)

2.5 pts ER membrane deforms

2.5 pts Vesicle buds

-1 pt for membrane curvature before coat recruitment

-2.5 pts for dynamin involvement

-3pts for describing CCV instead of COPII

-1 or -2 pts for other errors

4. VSV-G is a viral protein that travels through the secretory pathway and receives an N-linked complex oligosaccharide. Researchers have identified a temperature-sensitive variant of VSV-G, termed VSV-G^{ts}. At 40°C, VSV-G^{ts} is unable to leave the ER because it is not correctly folded. However, upon temperature shift to 35°C, VSV-G^{ts} folds rapidly and immediately leaves the ER to continue its journey towards the plasma membrane. Design an experiment in which you take advantage of the enzyme EndoH to determine the kinetics of the ER to Golgi transport of VSV-G^{ts}. [15 pts]

2 pts – Start growing cells that express VSV-G^{ts} at 40°C so that all the VSV-G^{ts} is in the ER.

3 pts – Shift cells to 35°C to allow for release of VSV-G^{ts} from the ER. This is the zero timepoint.

3 pts – Purify VSV-G^{ts} at various timepoints after the shift to 35°C and test for EndoH sensitivity.

2 pts – If VSV-G^{ts} is resistant to EndoH then it has reached the Golgi. Specifically it has encountered mannosidase II in the medial cisternae.

2 pts – EndoH is an enzyme that cleaves off N-linked complex oligosaccharides.

3 pts – The time between the shift to 35°C (release of VSV-G^{ts} from the ER) and the appearance of EndoH resistant VSV-G^{ts} is the time of ER to Golgi trafficking.

5. Statins are a class of widely used hypolipidemic drugs (that include the brands Lipitor and Zocor). Statins function by inhibiting the enzyme HMG-CoA reductase, the rate-limiting enzyme of the mevalonate pathway of cholesterol biosynthesis. Explain why statins lower both the cholesterol and LDL levels in the bloodstream. [15 pts]

2 pts By inhibiting HMG-CoA reductase, statins inhibit cholesterol synthesis and therefore lower intracellular cholesterol levels.

4 pts Reduced intracellular cholesterol levels are sensed by SCAP, a transmembrane protein located in the ER membrane and associated with SREBP. SCAP then escorts SREBP to the Golgi, where SREBP is cleaved to produce a soluble fragment of SREBP.

1 pt An NLS directs cleaved SREBP to the nucleus, where it serves as a transcription factor for SRE genes. Transcription of HMG-CoA reductase (1 pt) and the LDL receptor, or LDLR (2 pts) are upregulated.

2 pts HMG-CoA reductase is expressed, but is further inhibited by statins, thereby lowering overall cholesterol synthesis.

3 pts Increased levels of the LDLR are expressed and delivered to the plasma membrane. The LDLR recovers LDL (and cholesterol) from the bloodstream, thereby lowering levels of LDL in the blood.