Exam 2 **Name** _____

MCBII

1. GT is a type II transmembrane protein (N-terminus in the cytosol) localized to the Golgi. If you add a KDEL sequence to its N-terminus, where will the mutant protein localize in the cell? (4 pts)

   A. Still in the Golgi.

   B. ER.

   C. Secreted.

   D. Plasma membrane.

   E. Cytosol.

2. If you flip the topology of the Sec12 protein so that its cytoplasmic domain is now lumenal, which is likely true? (4pts)

   A. The COPII coat will assemble in the ER lumen.

   B. No COPII coat assembly.

   C. COPII coat is assembled but vesicles are not formed.

   D. Sar1 will be locked in its GTP bound state.

   E. COPII coat assembly and vesicle budding will be normal.

3. These nascent proteins are <u>not</u> bound by SRP? (4 pts)

   A. An ER protein with a transmembrane domain near its N-terminus.

   B. An ER protein with a transmembrane domain at its C-terminus.

   C. Ion channels on the plasma membrane.

   D. Golgi enzymes.

   E. Lysosomal enzymes.

4. Clearly explain how yeast cells and fluorescently tagged Golgi enzymes were used to prove the cisternal maturation model. (8 pts)

In yeast, cisternae are spatially separated. Therefore, could test whether an individual cisternae can mature. Labeled an *early Golgi enzyme* in one color (green) and a *late Golgi enzyme* in another (red) and asked using fluorescence microscopy if a cisternae went from green to yellow to red. This means that the cisternae matures as it loses cis markers and gains trans markers.

5. Randy Schekman did a yeast screen and identified a mutant in which the ER was pretty normal, the Golgi was small, and COPII vesicles were excessive. What might be the cause? (4 pts)

   A. SRP receptor can no longer bind to SRP.

   B. ER exit sites cannot concentrate cargo.

   C. NSF can no longer unwind v and t snare pairing.

   D. The COPII coat cannot disassemble.

6. The following affects the lipid composition at the plasma membrane? (4 pts)

A. Flippases that generate lipid asymmetry at the ER membrane bilayer.

B. The composition of lipids packaged into secretory vesicles.

C. Lipid transfer proteins found at ER-membrane contact sites.

D. All of the above.

7. Order the following events? (4 pts) **A, B, C, E, D (no partial credit)**

A. Lysosomal enzymes are translated.

B. Lysosomal enzymes are modified by N-linked glycosylation.

C. Lysosomal enzymes are modified with M6P.

D. Lysosomal enzymes are packaged into clathrin coated vesicles.

E. Lysosomal enzymes bind to M6PR.

8. Protein X is a type I integral membrane protein that contains 5 TM domains. Draw its topology on the ER and on the PM. Which termini can be glycosylated? (8pts)

N termini in the lumen, C termini in the cytoplasm at both ER and PM. N termini can be glycosylated because it is located in the lumen.

9. Where would a lumenal ER protein be translated and localized if you replace its N-terminal signal sequence with a mitochondrial signal sequence (4 pts)?

A. Translated in the cytosol, localized to the mitochondria.

B. Translated on the mitochondria and localized in the mitochondria.

C. Translated in the cytosol, and localized in the ER lumen.

D. Translated on the ER, and localized in the ER lumen.

10. A Golgi enzyme has a single transmembrane domain. It has a short N-terminal domain that is located in the cytosol. Which is likely to be a feature of its cytosolic domain? (4 pts)

A. It originally contained a signal sequence that was cleaved off.

B. It is modified by O-linked glycosylation

C. It is modified with M6P.

D. It contains positively charged residues flanking the transmembrane domain.

11. Which of the following types of vesicles are most likely to transport the voltage-gated K+ channel? (4 pts)

A. COPI vesicles.

B. COPII vesicles.

C. Clathrin vesicles.

D. Endosomes.

E. None of the above.

Points this page _____

12. Describe how a temperature sensitive viral protein and GFP was used to dissect the pathway taken by proteins in the secretory pathway (8 pts)

At high temperature, this GFP tagged PM protein does not fold and it is retained in the ER. After shift to permissive temperature, the protein will fold, and you use fluorescence microscopy to watch it traffic from the ER to the Golgi and then to the PM over time.

13. Which of the following is not considered a coated vesicle? (4pts)

A. COPI vesicles.

B. COPII vesicles.

C. The vesicles that traffic M6PR/M6P lysosomal enzymes from the Golgi.

D. Clathrin vesicles that form at the PM during endocytosis.

E. Secretory vesicles carrying neurotransmitters to the plasma membrane.

14. The signal recognition particle (SRP) hydrolyzes GTP upon binding: (4 pts)

A. SRP receptor.

B. The translocon.

C. The ribosome.

D. The ER signal sequence.

E. The signal peptidase.

15. ER associated degradation of misfolded protein does <u>not</u> involve the following? (4pts)

A. Transport of the misfolded protein from the ER into the cytosol.

B. The misfolded protein is ubiquitinated in the ER lumen.

C. Degradation of the misfolded protein by the proteasome.

D. The misfolded protein is recognized by ER chaperones.

16. Describe the five cytoplasmic components of the COPII coat, what do they do, and what is their order of assembly. (8pts)

Sar1 GTP binding, reveals a hydrophobic helix that inserts into the cytoplasmic leaflet and generates membrane curvature. (3 pts)

Sec23 binds Sar1 (1 pt)

Sec24 binds with Sec23 and binds/concentrates cargo, banana shape stabilizes membrane curvature. (2 pts)

Sec 13/31 bind as a dimer and form the lattice around Sec23/24. (2 pts)

17. Which of the following charged phospholipids is enriched in the cytoplasmic leaflet of the ER relative to the lumenal leaflet? (4pts).

A. Phosphatidylcholine (PC).

B. Phosphatidylethanolamine. (PE)

C. Cholesterol.

D. Phosphatidylserine (PS).

18. If you would like to target LDL receptors for lysosomal degradation, which would be the best approach? (4 pts)

A. Add a polyubiquitin chain to the cytoplasmic domain of the LDL receptor.

B. Add a single ubiquitin to the cytoplasmic domain of the LDL receptor.

C. Add a single ubiquitin to the lumenal domain of the LDL receptor.

D. Add a polyubiquitin chain to the lumenal domain of the LDL receptor.

19. If you artificially relocate the v-SNARE from secretory vesicles to the plasma membrane, which of the following is likely correct? (4pts)

A. Vesicle tethering to the PM by Rabs and their effectors would be prevented.

B. Neurotransmitters are released normally.

C. NSF would no longer dissociate the SNARE complex.

D. Cis-SNARE complex is formed.

20. How do KDEL receptors regulate directional retrieval of ER lumenal proteins from the Golgi to the ER? What allows KDEL receptor to localize to both the Golgi and to the ER? (8pts)

KDEL receptors bind KDEL sequence in the low pH Golgi lumen. Cytoplasmic domain also binds to COPI coat, which recycles it to the ER.

KDEL receptors release KDEL sequence at neutral pH ER lumen. Cytoplasmic domain also binds to COPII coat, which takes back to the Golgi for further rounds.