Answers

1) recycled to the original membrane

2. destroyed in the lysosome
3. transcytosed across the cell to a different membrane

2) The lysosomal enzymes are all acid hydrolases, which have optimal activity at the low pH (about 5.0) found in the interior of lysosomes. If a lysosome were to break, the acid hydrolases would find themselves at pH 7.2, the pH of the cytosol, and would therefore do little damage to cellular constituents.

3) W—3 (defect in mannose-6-phosphate receptor)
X—2 (defect in phosphotransferase)
Y—1; Z—1 (defect in lysosomal hydrolases); these will be defects in two different lysosomal acid hydrolases.

A cell that has no mannose-6-phosphate receptor will be able to make all the lysosomal hydrolases properly but will not be able to send them to the lysosome and will also not be able to scavenge hydrolases from the external media. Hence, this cell line cannot be rescued by a culture medium that has had lysosomal hydrolases secreted into it and thus will not be rescued by any of the media tested here. A cell line that has no phosphotransferase will be able to scavenge hydrolases from the external medium, but because all of the cell's own hydrolases will lack the mannose-6- phosphate tag, it will be rescued only by medium from a cell line that is able to make all of the hydrolases. Cell lines lacking one hydrolase will be rescued by medium from any cell line that is able to secrete that hydrolase in a mannose-6-phosphate-tagged form; in addition, media from cultures of cells lacking a hydrolase will rescue any cell line with another type of defect.

4)
A: As there is no "retention/return to the ER signal" the protein would slowly be secreted from the cell.
B: If we add KDEL a "retention/return to the ER signal" to a protein it would be localized to the ER
C: 1) A N-terminal ER localization sequence to ensure translocation to the ER during/after protein synthesis (Chapter 12)
2) Transmembrane spanning sequences, alpha helices are the conventional structure
3) An ER-retention signal on the c-terminus like KKXX

5) The KDEL receptor binds its ligands more tightly in the Golgi apparatus, where it captures proteins that have escaped the ER, so it can return them. he receptor binds its ligands more weakly in the ER, so that those proteins that have been captured in the Golgi apparatus can be released upon their return to the ER. he basis for the diferent binding ainities is thought to be the slight diference in pH; the lumen of the Golgi apparatus is slightly more acidic than that of the ER, which is neutral. Since the

primary job of the KDEL receptor is to capture proteins that have escaped from the ER, it would be reasonable to design the system so that the receptors are found in the highest concentration in the Golgi apparatus. his is, in fact, the way it is in the cell. You would be correct if you predicted that the KDEL receptor does not have a classic ER retrieval signal; after all, the receptor is designed to spend most of its time in the Golgi apparatus, and a classic signal would ensure its eicient return to the ER. It does, however, have a “conditional” retrieval signal; upon binding to an ER protein in the Golgi apparatus, its conformation is altered so that a binding site for COPI subunits is exposed. hat signal allows it to be incorporated into COPI-coated vesicles, which are destined to return to the ER.

6) If the KDEL signal and the KDEL receptor were all that was required to retain a protein in the ER, then addition of KDEL to a secreted protein should result in its retention in the ER. Clearly, addition of KDEL to rat growth hormone or human chorionic gonadotropin did not result in their eicient retention in the ER. Presumably, their slower rate of secretion was due to the KDEL system, since changing KDEL to KDEV abolished the efect. A comparable efect is also seen for ER residents that have had their KDEL signals removed; they are secreted, but at signiicantly slower rates than true secreted proteins. One explanation that might account for both these efects is kin recognition, which embodies the idea that residents of the ER might have a general ainity for one another, making it more diicult for any of them to leave the compartment. According to this idea, ER proteins that are missing their KDEL signal are secreted slowly because they still retain their ainity for other ER residents. Similarly, secreted proteins with an added KDEL signal would not be expected to have a general ainity for ER residents, and thus would escape the ER at a higher rate than true ER residents.

7) In the vesicle transport model, vesicles carry proteins across the stack by budding from one cisterna and fusing with the next. It is this role in the forward movement of proteins that is the critical diference between the two models. Vesicles are also required to maintain the identity of each cisterna by capturing resident proteins that have escaped and returning them to the appropriate cisterna. his retrograde low is also used to capture ER resident proteins that have escaped into the Golgi apparatus and return them to the ER. In the cisternal maturation model, vesicles are not required to move proteins across the Golgi apparatus. Movement of the stacks themselves accomplishes the forward movement of proteins. Vesicles are still required to maintain the identity of individual cisternae, but in this model they are not returning escaped proteins, but rather are transferring proteins in a retrograde direction to a new residence because their old residence has changed identities, from a *cis* cisterna to a medial cisterna, for example. In this model, as in the vesicle transport model, vesicles are responsible for returning escaped ER proteins to the ER. The critical diference between the two models is that the forward movement of proteins is accomplished by vesicles in the vesicle transport model and by movement of the cisternae themselves in the cisternal maturation model.

8)

A) Endocytosis B) Multivesicular body C) Macrophage D) Pinocytosis
E) Caveola F) Clathrin-coated pit G) Receptor-mediated endocytosis H) Caveolin
I) Early endosome J) Phagocytosis

9) In a cell capable of regulated secretion, the three main classes of protein that must be sorted before they leave the *trans* Golgi network are (1) those destined for lysosomes, (2) those destined for secretory vesicles, and (3) those destined for immediate delivery to the cell surface.

10)

A) Vesicles on the endocytic pathway will contain transferrin, and thus be labeled with colloidal gold; vesicles on the exocytic pathway will contain albumin, and thus be labeled with ferritin.
B) Clathrin-coated vesicles are rapidly uncoated after they pinch of from the plasma membrane, so some will be caught with their coats of, while others will still have their coats on.

11)

D. Ricin is a large protein, so it must be taken into the cell via endocytosis. Choices A and C are not correct because clathrin and SNARE proteins work in the cytoplasm, so they would not be accessible to ricin outside the cell. Choice B is incorrect because there are no known pore complexes in the plasma membrane.

B. Once ricin enters the cell via endocytosis, it makes its way from endosomes to the ER via the Golgi apparatus. Retrograde transport from the Golgi to the ER uses COPI-coated vesicles. Choice A is not directly involved in the fusion of vesicles with their target membranes, a process that involves SNAREs. After fusion, however, the SNAREs need to be pried apart by NSF before they can be used again. hus, NSF would be required for a continuous low of ricin to the ER, but would not be required for the initial delivery. Choice C is incorrect because M6P receptors are used to concentrate various lysosomal hydrolases into clathrin-coated vesicles for antegrade transport from the *trans* Golgi network to lysosomes. Choice D is not correct because the Sar1 protein is required for assembly of COPII coats at the ER membrane and those vesicles are used for antegrade transport to the Golgi apparatus.

D. To get from the ER to the cytosol, ricin must cross the ER membrane, and the only known route is the pathway that translocates unfolded proteins into the cytosol for degradation. Ricin triggers this pathway by partially unfolding in the lumen of the ER. It avoids degradation by refolding correctly in the cytosol. Choices A, B, and C are not correct because they do not describe known pathways.