1) Name three possible fates for an endocytosed molecule that has reached the early endosome.

2) If a lysosome breaks, what protects the rest of the cell from lysosomal enzymes?

3) Fibroblast cells from patients W, X, Y, and Z, each of whom has a different inherited defect, all contain "inclusion bodies," which are lysosomes filled with undigested material. You wish to identify the cellular basis of these defects. The possibilities are:

1. a defect in one of the lysosomal hydrolases
2. a defect in the phosphotransferase that is required for mannose-6-phosphate tagging of the lysosomal hydrolases
3. a defect in the mannose-6-phosphate receptor, which binds mannose-6-phosphate-tagged lysosomal proteins in the *trans* Golgi network and delivers them to lysosomes

When you incubate some of these mutant fibroblasts in a medium in which normal cells have been grown, you find that the inclusion bodies disappear. Because of these results, you suspect that the constitutive exocytic pathway in normal cells is secreting lysosomal hydrolases that are being taken up by the mutant cells. (It is known that some mannose-6-phosphate receptor molecules are found in the plasma membrane and can take up and deliver lysosomal proteins via the endocytic pathway.) You incubate cells from each patient with medium from normal cells and medium from each of the other mutant cell cultures, and get the results summarized in the Table

Indicate which defect (1, 2, 3) each patient (W, X, Y, Z) is most likely to have.

	Medium				
	From normal cells	From cultures of W cells	From cultures of X cells	From cultures of Y cells	From cultures of Z cells
Cell line					
Normal	+	+	+	+	+
W	–	–	–	–	–
X	+	+	–	–	–
Y	+	+	–	–	+
Z	+	+	–	+	–

+ indicates that the cells appear normal;
– indicates that the cells still have inclusion bodies

4) KDEL function

A: Describe what would happen to the localization of a soluble protein that is normally resident in the ER lumen if the KDEL sequence is deleted.

B: What would happen if we added a KDEL sequence to a protein that normally does not have it?

C: You are designing a new ER resident multipass transmembrane protein, what protein structures and amino acid sequences would you include and why?

5) The KDEL receptor must shuttle back and forth between the ER and the Golgi apparatus in order to accomplish its task of ensuring that soluble ER proteins are retained in the ER lumen. In which compartment does the KDEL receptor bind its ligands more tightly? In which compartment does it bind its ligands more weakly? What is thought to be the basis for its diferent binding ainities in the two compartments? If you were designing the system, in which compartment would you have the highest concentration of KDEL receptor? Would you predict that the KDEL receptor, which is a transmembrane protein, would itself possess an ER retrieval signal?

6) When the KDEL retrieval signal is added to rat growth hormone or human chorionic gonadotropin, two proteins that are normally secreted, the proteins are still secreted, but about six times more slowly. If the C-terminal L in the signal is changed to V, the proteins are once again secreted at their normal rate. By contrast, bona ide ER resident proteins rarely, if ever, are secreted from the cell; they are usually captured and returned very eiciently. How is it, do you suppose, that normal resident proteins with a KDEL signal are eiciently retained in the ER, whereas secreted proteins to which a KDEL signal has been added are not eiciently retained? Is this what you would expect if the KDEL signal and the KDEL receptor accounted entirely for retention of soluble proteins in the ER?

7) Two extreme models—vesicle transport and cisternal maturation—have been proposed to account for the movement of molecules across the polarized structure of the Golgi apparatus. In the vesicle transport model, the individual Golgi cisternae remain in place as proteins move through them (Figure 13–9A). By contrast, in the cisternal maturation model, the individual Golgi cisternae move across the stack, carrying the proteins with them (Figure 13–9B). Transport vesicles serve critical functions in both models, but their roles are distinctly diferent. Describe the roles of the transport vesicles in each of the two models. Comment speciically on the roles of vesicles in the forward movement of proteins across the Golgi stack, in the retention of Golgi resident proteins in individual cisternae, and on the return of escaped ER proteins to the ER.

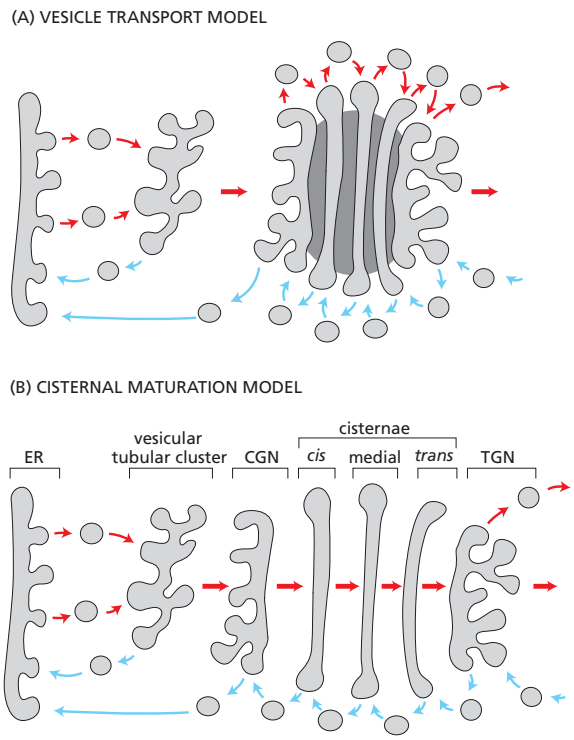

Figure 13–9 Two models for the movement of molecules through the Golgi apparatus (Problem 13–48). (A) The vesicle transport model. (B) The cisternal maturation model. In (B), the individual cisternae have been separated for illustration purposes. ER, endoplasmic reticulum; CGN, *cis* Golgi network; TGN, *trans* Golgi network.

8) Match each definition below with its term from the list.

Caveola, caveolin, clathrin-coated pit, early endosome, endocytic vesicle endocytosis, endosome maturation, ESCRT protein complexes, Late endosome, low-density lipoprotein (LDL) , macrophage, macropinocytosis, multivesicular body, neutrophil, phagocytosis, phagosome, pinocytosis, receptor-mediated endocytosis, recycling endosome, transcytosis, transferrin receptor

A) General term for the process by which cells take up macromolecules, particulate substances, and even other cells into membrane-enclosed vesicles.

B) Complex vesicle with invaginating buds and internal vesicles involved in the maturation of early endosomes into late endosomes.

C) Phagocytic cell—derived from a hematopoietic stem cell—that ingests invading microorganisms and plays an important role in scavenging senescent cells and apoptotic cells.

D) Type of endocytosis in which soluble materials are taken up from the environment and incorporated into vesicles for digestion.

E) Invagination that forms from lipid rafts at the cell surface and buds of internally to form a pinocytic vesicle.

F) Region of plasma membrane of animal cells that is covered with the protein clathrin on its cytosolic face; it will bud of from the membrane to form an intracellular vesicle.

G) Process by which macromolecules bind to complementary transmem- brane receptor proteins, accumulate in coated pits, and then enter the cells as receptor–macromolecule complexes in clathrin-coated vesicles.

H) One of a family of structural proteins in caveolae that are unusual because they extend multiple hydrophobic loops into the membrane from the cytosolic side, but do not cross the membrane.

I) Membrane-enclosed compartment just beneath the plasma membrane, to which external molecules are irst delivered by endocytosis.

J) Specialized form of endocytosis in which a cell uses large endocytic vesicles to ingest large particles such as microorganisms and dead cells.

9) In a cell capable of regulated secretion, what are the three main classes of protein that must be separated before they leave the *trans* Golgi network?

10) You are interested in exocytosis and endocytosis in a line of cultured liver cells that secrete albumin and take up transferrin. To distinguish between these events, you tag transferrin with colloidal gold and prepare ferritin-labeled antibodies that are speciic for albumin. You add the tagged transferrin to the medium, and then after a few minutes you ix the cells, prepare thin sections, and react them with ferritin-labeled antibodies against albumin. Colloidal gold and ferritin are both electron-dense and therefore readily visible when viewed by electron microscopy; moreover, they can be easily distinguished from one another on the basis of size.

A. Will this experiment allow you to identify vesicles in the exocytic and endocytic pathways? How?

B. Not all the gold-labeled vesicles are clathrin-coated. Why?

11) Ricin is one of the most toxic substances known: less than 2 mg injected into the bloodstream will kill an adult human. Ricin is produced by the castor bean plant as a 65 kd protein heterodimer composed of an A chain and a B chain. he B chain is a lectin that binds to carbohydrates on the cell surface. he A chain is an enzyme that modiies a highly conserved site in rRNA, leading to inhibition of translation. After entering the cell, ricin eventually ends up in the lumen of the endoplasmic reticulum (ER), and from there it moves into the cytosol, where it inactivates ribosomes.

A) What is the most likely mechanism by which ricin enters the cell?

a) Binding to clathrin proteins
b) Entry through pore complexes
c) Interaction with SNARE proteins
d) Internalization via endocytosis

B) Which one of the following is required in order for ricin to be delivered to the ER?

a) *N*-ethylmaleimide-sensitive factor (NSF)
b) Golgi-derived COPI-coated vesicles
c) Mannose 6-phosphate (M6P) receptors
d) the Sar1 monomeric GTPase

C) Which one of the following describes the most likely scenario for how ricin gets into the cytosol?

a) Ricin has a signal sequence that allows it to be transported across the ER membrane into the cytosol.
b) Ricin is packaged into vesicles that form vesicular tubular clusters, which release proteins into the cytosol.
c) Ricin is transported from the ER to the Golgi apparatus to the lysosome, where it is released into the cytosol.
d) Ricin, by mimicking an unfolded protein, is tagged for transport across the ER membrane into the cytosol.