(1) Antitrypsin, which inhibits certain proteases, is normally secreted into the bloodstream by liver cells. Antitrypsin is absent from the bloodstream of patients who carry a mutation that results in a single amino acid change in the protein. Antitrypsin deficiency causes a variety of severe problems, particularly in lung tissue (emphysema), because of uncontrolled protease activity. Surprisingly, when the mutant antitrypsin is synthesized in the laboratory, it is as active as the normal antitrypsin at inhibiting proteases. Why then might the mutation cause the disease? How would you prove that this is the case? What would you target to try and treat the disease?

The actual explanation is that the single amino acid change causes the protein to misfold slightly so that, although it is still active as a protease inhibitor, it is prevented by chaperone proteins in the ER from exiting the cell. It therefore accumulates in the ER lumen and is eventually degraded. Alternative interpretations might have been: (1) the mutation affects the stability of the protein in the bloodstream so that it is degraded faster than the normal protein; (2) the mutation inactivates the ER signal sequence and prevents the protein from entering the ER; or (3) the mutation created an ER (or Golgi) retrieval signal so that the protein was continually returned to the ER (or Golgi). One could distinguish among these possibilities by using fluorescently tagged antibodies against the protein to follow its transport in the cells.

(2) The KDEL receptor is a transmembrane protein that shuttles 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 different binding affinities in the two compartments? If you were designing the system, in which compartment would you have the highest concentration of KDEL receptor? What specific trafficking signal or signals (coat interacting motifs) would you expect to find on the KDEL receptor?

The KDEL receptor binds its ligands more tightly in the Golgi apparatus, where it captures proteins that have escaped the ER, so that it can return them. The 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. The basis for the different binding affinities is thought to be the slight difference 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. This 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 efficient 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. That signal allows it to be incorporated into COPI-coated vesicles, which are destined to return to the ER.

(3) In mammalian cells entirely lacking the enzyme GlcNAc phosphotransferase (normally found in the cis Golgi), how is trafficking of lysosomal enzymes changed? Why? What happens to the cells?

In cells lacking GlcNAc phosphotransferase lysosomal enzymes will not be targeted to the lysosome because they will not be recognized by the mannose 6-phosphate receptor. Lysosomal function in these cells is therefore compromised.

(4) True or False: Critical to normal function of the lysosome is its pH, which is optimal at pH = 9.

False. Its pH is closer to 5.

The protein that maintains lysosomal pH is called the vacuolar ATPase.

True or False: The ER maintains a pH of <5. False. Its pH is closer to 7.

True or False: All of the glycoproteins in intracellular membranes have their oligosaccharide chains facing the lumen, and all those in the plasma membrane have their oligosaccharide chains facing the outside of the cell.

True. The oligosaccharide chains are added in the lumens of the ER and Golgi apparatus, which are topologically equivalent to the outside of the cell. This basic topology is conserved in all membrane budding and fusion events. Thus, oligosaccharide chains are always topologically outside the cell, whether they are in a lumen or on the cell surface.

(5) At least three different coats form around transport vesicles. Name three coats. What two principal functions do these different coats have in common?

Clathrin, COPI, COPII
The ability to concentrate cargo and coordinate membrane deformation and scission.

(6) Insulin is synthesized as a pre-pro-protein in the β cells of the pancreas. Its pre-peptide is cleaved off after it enters the ER lumen. To define the cellular location at which its pro-peptide is removed, you have prepared two antibodies: one that is specific for pro-insulin, and one that is specific for insulin. You have tagged the anti-pro-insulin antibody with a red fluorophore and the anti-insulin antibody with green fluorophore, so that you can follow them independently in the same cell. When you incubate a pancreatic β cell with a mixture of your two
antibodies, you obtain the results shown below. In what cellular compartment is the pro-peptide removed from pro-insulin?

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis Golgi network</td>
<td>red</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>red</td>
</tr>
<tr>
<td>Golgi cisternae</td>
<td>red</td>
</tr>
<tr>
<td>Immature secretory vesicles</td>
<td>yellow</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>none</td>
</tr>
<tr>
<td>Mature secretory vesicles</td>
<td>green</td>
</tr>
<tr>
<td>Nucleus</td>
<td>none</td>
</tr>
<tr>
<td>trans Golgi network</td>
<td>red</td>
</tr>
</tbody>
</table>

The pro-peptide is removed from pro-insulin in immature secretory vesicles. The red fluorescence in compartments from the ER through the trans Golgi network indicates that they contain only pro-insulin. The green fluorescence in mature secretory vesicles indicates that they contain insulin. The yellow fluorescence, which arises when both the red and green fluorophores are excited in the same place—the combination of red and green light is yellow—indicates that pro-insulin and insulin are both present in immature secretory vesicles. Thus, immature secretory vesicles must be where the pro-peptide is removed. The absence of label in lysosomes, mitochondria, and nuclei (and other compartments) provides assurance that you are indeed following the secretory pathway.

(7) Clathrin-coated vesicles bud from eukaryotic plasma membrane fragments when adaptor proteins, clathrin, and dynamin-GTP are added. What would you expect to observe if the following modifications were made to the experiment? Explain your answers.

A. Adaptor proteins were omitted.
B. Clathrin was omitted.
C. Dynamin was omitted.
D. Prokaryotic membrane fragments were used.

A. Clathrin-coated vesicles cannot assemble in the absence of adaptor proteins, which link the clathrin to the membrane. At high clathrin concentrations, and under the appropriate ionic conditions, clathrin cages assemble in solution, but they are empty shells, lacking membranes and other proteins. Self-assembly of clathrin into baskets shows that the information for clathrin baskets is contained in the clathrin molecules themselves.

B. Without clathrin, adaptor proteins still bind to receptors in the membrane, but no clathrin coat can form. Thus, no clathrin-coated pits or vesicles will form.

C. In the absence of dynamin, clathrin-coated pits can form and proceed toward vesicle formation, but the last step—membrane fusion—is blocked without dynamin. As a result, deeply invaginated coated pits will be observed in the absence of dynamin.

D. Procaryotic cells do not perform endocytosis. A procaryotic cell does not contain any receptors with appropriate cytosolic tails that could mediate the binding of adaptor proteins. Therefore, no clathrin-coated vesicles will form.
(8) Correct the following description, as necessary. “Sar1 protein is a COPII-recruitment GTPase that facilitates the unidirectional transfer of COPII vesicles from the ER membrane to the Golgi membrane. A unique directionality is imposed on the transfer by the locations of a guanine-nucleotide exchange factor (GEF) and a GTPase-activating protein (GAP). The GEF, which is located in the ER membrane, stimulates vesicle formation by mediating attachment of Sar1-GTP to the ER membrane, where it recruits COPII subunits. The GAP, which is located in the Golgi membrane, stimulates vesicle docking by stimulating hydrolysis of Sar1-bound GTP. Sar1-GDP causes disassembly of the COPII coat, which prepares the vesicle for fusion with the Golgi membrane.”

Most of the statements in this description are correct. The GAP, however, is not located in the Golgi membrane. The COPII subunits—probably in association with another protein—act as the GAP, stimulating hydrolysis of GTP by Sar1. Thus, the GAP is associated with the vesicle membrane, not the Golgi membrane. Sar1–GDP then causes disassembly of the coat almost as soon as the vesicle has formed. The uncoated vesicle carries other proteins—a Rab protein and a vSNARE—that orchestrate docking and fusion with the Golgi membrane.

(9) How can it possibly be true that complementary pairs of specific SNAREs uniquely mark vesicles and their target membranes? After vesicle fusion, the target membrane will contain a mixture of t-SNAREs and v-SNAREs. Initially, these SNAREs will be tightly bound to one another, but NSF can pry them apart, reactivating them. What do you suppose prevents target membranes from accumulating a population of v-SNAREs equal to or greater than their population of t-SNAREs?

There will always be some v-SNAREs in the target membrane. Immediately after fusion, the v-SNAREs will be in inactive complexes with t-SNAREs. Once NSF pries the complexes apart, v-SNAREs may be kept inactive by binding to inhibitory proteins. Accumulation of v-SNAREs in the target membrane beyond some minimal population is thought to be prevented by active retrieval pathways that incorporate v-SNAREs into vesicles for redelivery to the original donor membrane.

(10) Rab GTPases recruit organelle-specific effectors to intracellular membranes. You are interested in the early secretory pathway (trafficking between ER and Golgi), and set out to define novel effector proteins for rab1. In one approach you carry out a yeast two-hybrid screen using a rab1 GTP-locked mutant as bait. You recover and confirm that the following proteins behave as prey. Motifs present in the proteins are indicated

A:  

| SS | KDEL |
Which of these would you consider promising candidates to study further, and why?

Of the three candidate proteins, protein C is the most attractive candidate. It has structural features similar to other known rab effectors, and is correctly localized within the cell to be involved with ER to Golgi transport. Protein A is likely to be a two-hybrid artifact because it will be present within the lumen of the ER while rab1 is cytosolic. Protein B is a possibility, but would require some regulation of its NLS to be present near ER and Golgi membranes (i.e. outside of the nucleus). For biochemical purification of rab1 effectors, any well explained approach will be considered favorably. A key feature of effectors is that they bind to rab1-GTP but not rab1-GDP. For full credit one of the controls should involve comparing binding to GTP and GDP bound proteins.