Proteins that normally function in the endoplasmic reticulum (ER) are tagged on their carboxyl termini with Lys-Asp-Glu-Leu amino acid sequences. Name one protein that you would expect to contain a carboxyl-terminal Lys-Asp-Glu-Leu tag. (1 point)

Calnexin, signal peptidase, translocation channel, srp receptor, glyosylating enzymes, glucosyl transferase, scramblease, or any number of other ER proteins.

Proteins containing this Lys-Asp-Glu-Leu sequence at their carboxyl termini sometimes ‘escape’ the ER and travel to the Golgi. They are retrieved by receptor proteins that bind to the 4-amino acid sequence. These receptors must cycle back and forth between the ER and the Golgi to accomplish their task of ensuring that soluble ER proteins are retained in the ER lumen. In which compartment, the ER or the Golgi, does the receptor have a higher affinity for proteins tagged with the Lys-Asp-Glu-Leu sequence? Explain your rationale. (3 points)

We would expect a higher affinity in the Golgi because in that organelle, it is important for the Lys-Asp-Glu-Leu receptor to grab onto the escaped ER protein. In the ER, it is important that the receptor let go of its ‘cargo’ so that the ‘cargo’ (the resident ER protein) can do its job there.

Using DNA manipulation, you are able to add the 12 nucleotides that encode for the Lys-Asp-Glu-Leu protein sequence at the end of the RNA polymerase coding sequence, immediately “upstream” of the encoded stop UAG stop codon (TAG in the DNA). After transcription of this genetically-modified RNA polymerase gene and translation of its mRNA, you are able to use an antibody and immunofluorescent microscopy to localize the mature protein in the cell. Where do you find it? Explain. (3 points)

The polymerase would likely still be in the nucleus where it normally functions because the other tags that are necessary to target the polymerase to the ER (i.e., the hydrophobic signal sequence) is not present. Thus, even with the genetically-added ER-retention sequence, the polymerase protein will be completely synthesized in the cytoplasm, would fold (hopefully properly; see below), and would be transported to the nucleus using its nuclear localization signal (NLS)—which would necessarily be part of the protein given that its necessary function occurs in the nucleus. If addition of the Lys-Asp-Glu-Leu sequence at the carboxyl-terminal end of the polymerase protein precluded its ability to fold properly such that the NLS were not visible, then the polymerase would remain in the cytoplasm where it was synthesized—but only for a short while because the cell would have a very short lifespan without the ability to transcribe specific RNAs!
How does the cell ensure that proteins meant to be targeted to regulated secretory vesicles, rather than constitutively targeted to the plasma membrane, actually get to secretory vesicles? (2 points)

Although it has not yet been discovered or described, it is likely necessary that proteins meant to be targeted to regulated secretory vesicles have an additional signal (a signal patch, most likely) that can be recognized by the cell machinery. Recognition of these signals then results in trafficking these proteins properly to the appropriate vesicles.

Cop-I coated vesicles are employed for vesicular transport between the stacks of the Golgi. Assembly of these COP-I coated vesicles depends upon a monomeric G-protein, *Arf-1*, which functions in a manner similar to that of *Sar-1* in the formation of the Cop-II coated vesicles that are employed for vesicular transport from the ER→Golgi. When the rate of *Arf-1*’s GTP hydrolysis is tested, the results shown in the table below are obtained. Given this, describe the steps required for the formation of Cop-I coated vesicles and vesicular transport through the Golgi. (5 points)

<table>
<thead>
<tr>
<th>Components Added</th>
<th>Relative Rate of GTP Hydrolysis→ GDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arf-1</td>
<td>0</td>
</tr>
<tr>
<td>Arf-1 + GAP</td>
<td>1</td>
</tr>
<tr>
<td>Arf-1 + Cop I subunits</td>
<td>0</td>
</tr>
<tr>
<td>Arf-1 + Cop I subunits + GAP</td>
<td>1000</td>
</tr>
</tbody>
</table>

The data above simply tells us that hydrolysis occurs at a much more rapid rate when both GAP and Cop I subunits are present—too fast for assembly of the Cop I coats to form a vesicle. Therefore, exposure of vesicles to the GAP must occur after the vesicle has formed and bud off of the Golgi; the GAP may be somehow localized in between the stacks. If the mechanism of Cop I-coated vesicles follows the pattern of Cop II-coated vesicles that bud from the ER, the steps required for the formation of Cop-I coated vesicles are likely as follows:

1. A Golgi membrane-associated GEF facilitates the exchange of GDP for GTP in Arf-1.
2. GTP binding causes a shape change in Arf-1 such that a hydrophobic region is exposed; this region embeds itself in the Golgi-membrane such that Arf-1 is now membrane-associated.
3. Cargo-associated Sec (or sec-like) proteins gather cargo and cluster around the membrane-associated Arf-1.
4. Cop-I proteins also gather around the Arf-1 and Sec proteins to deform the membrane such that a vesicle is able to form and bud off of the Golgi membrane.
5. At some site distant from the membrane that just released a bud/vesicle, a GAP is encountered that causes Arf-1 to hydrolyze its bound GTP to GDP. The Cop-I coat disassembles as Arf-1 dis-engages from the vesicles membrane. This exposes Rab and SNARE proteins on the naked vesicle, which is necessary to allow the vesicle to eventually fuse with its target compartment.