Brief answers are provided.

Question 1

1) The N-terminus is the signal sequence. Cleavage of the signal sequence yields the mature protein.

2) The hydrophobicity plot shows a hydrophobic signal sequence at the N-terminus.

lane 1: full-length protein that is not protected from proteases in the absence of import (lane 3)
lane 2: protein import into microsomes yield a signal sequence cleaved protein. Only the TM and lumenal portion of the protein are protected from proteases (lane 4)
lane 5: detergent disrupts microsomes and renders imported protein vulnerable to proteases. This is a control for protease activity.

3) The pathway would be the one for Type I single-pass transmembrane proteins. After the classical SRP-SRPR interactions, the nascent polypeptide chain would be threaded through the translocon co-translationally. The N-terminus containing the signal peptide opens the translocon; the N-terminus enters the ER lumen; the signal peptide is cleaved by signal peptidase. Co-translational import continues till the transmembrane segment ("stop-transfer anchor sequence) is encountered. The polypeptide exits the translocon laterally. As a result, the N-terminus is in the lumen of the ER, which topologically is equivalent to the outside of the cell.

4) The protein would be trapped in the ER lumen and COPII vesicles, due to the inability of COPII vesicles to fuse with the Golgi. This could be tested by immunofluorescence against the secreted factor using the polyclonal antibody, in normal cells and cells with depleted NSF. Alternatively, subcellular fractionation, followed by Western blotting, could be used.

5) The results would likely be uninterpretable because all new synthesized proteins would be labeled, and only a small subset is in the secretory pathway. The Palade experiment worked because pancreatic acinar cells are secretory machines.

Question 2

1) Sec12 is a guanine nucleotide exchange factor (GNEF) for Sar1. It acts as a GNEF *specifically* for Sar1, as opposed to other GTPases like Ypt1, ARF, or Ras2. Exchange activity leads to release of [^{32}P]GDP and binding of nonradioactive GTP. The [^{32}P]GDP passes through the filter.

2) Overexpression of the cytosolic fragment would inappropriately activate Sar1 in the cytosol, as opposed to the ER surface. Overexpression of Sar1 would titrate out the excess fragment, and allow activation at the ER surface.

3) Make a Sar1-GFP fusion. Track the dynamics of Sar1-GFP localization with time-lapse fluorescence microscopy in normal and Sec12 mutants cells. It would be necessary to test that the fusion protein is functional. In normal cells, some fraction of Sar1-GFP should associate with the ER membrane; in Sec12 mutants, this would not happen.

4) Timing is coordinated by the GTPase activating activity (GAP activity) of the coat protein. Therefore, binding of coat to activated Sar1 leads to increased Sar1 GTPase activity, leading to its inactivation and release of the coat. Mutations in the coat protein that disrupt the GAP activity of the coat would delay coat shedding.

5) GTPγS would constitutively activate Sar1. Coated vesicles would form and shedding of the coat would be delayed due to inability to hydrolyze GTP. Therefore, coated vesicles would be stabilized.

The cytosolic fragment of Sec12 would inhibit coated vesicle formation, as in (2).

Question 3

1) When a ribosome encounters a truncated mRNA (lacking a stop codon), it gets "stuck." The ribosome does not release the nascent polypeptide chain, so the translation and translocation process is frozen.

2) Sec61 is the translocon, which surrounds the translocating polypeptide chain. It is the major crosslinked protein.

3) The last ~30 residues are inside the ribosome and cannot crosslink to membrane proteins.

4) The residues immediately prior to the last 30 residues should crosslink to Sec61. It was found that residues 100-139 crosslink to Sec61.

5) Puromycin is structurally similar to tyrosyl-tRNA and goes into the A site of the ribosome. The polypeptide chain is transferred to puromycin via a peptidyl transferase reaction. The chain plus puromycin then is released from the ribosome. In normal translational termination, a release factor is recruited at a stop codon position, and it causes release of the polypeptide chain.

Question 4

1) Yeast cells defective in vacuolar proteases accumulate autophagic bodies upon nitrogen starvation. Yeast cells can be mutagenized and screened for cells that fail to accumulate autophagic bodies upon nitrogen starvation.

2) Autophagy mutants would be expected to show defects under nitrogen starvation.

3) LC3-I is produced by proteolytic processing of the LC3 precursor by ATG4. LC3-II is produced when LC3 is conjugated to phosphoethanolamine (PE). LC3-II is the form that associates with autophagosomes.

Upon starvation, LC3 gets lipidated, causing transient accumulation of LC3-II (0.5 and 2 hours). (But with prolonged starvation LC3-II is degraded because it is also a substrate in the autophagic process.)

Atg5-null cells cannot produce LC3-II. They have a defect in autophagy because they cannot produce the Atg5/Atg12/Atg16 complex. This system is apparently necessary for formation of LC3-II.

4) With prolonged starvation LC3-II is degraded because it is also a substrate in the autophagic process.

5) Increased LC3-II can indicate increased autophagic flux or defective autophagic progression. One way to distinguish between these 2 possibilities is to treat the cells with bafilomycin. In the former possibility, this treatment should further accumulate. In the latter, the levels of LC3-II would remain the same.