Q1.

a.

AP-1, where mannose-6-phosphate receptors accumulated, is a docking adaptor complex, composed of different, though related, adapter proteins. AP-1, interacted with clathrin, coats vesicles derived from the TGN to deliver cargo. GTPase ARF-1 is required for the binding of AP-1 to Golgi membranes.

Two pathways: cellular targeting secretion from TGN to lysosome; intracellular transport between TGN and early endosome.

b.

AP-1 takes charge of most lysosomal enzymes targeting. If AP-1 were defective, AP-3 may take over some work and target subset of these lysosomal enzymes to lysosome. AP-1 also takes part in the transport between TGN and early endosome. AP-4 may take over AP-1's basolateral sorting job. And there will be many proteins tagged with M6P still left, which was used to target to lysosome. These proteins will be secreted out into the extracellular fluid "by default".

c.

In general, the cells will show severe growth defect and a delayed developmental cycle.

As normal lysosome's function will be influenced. With less lysosomal enzymes, lysosome will become larger and denser. Many lysosomal proteins will be wasted into the extracellular fluid in the precursur's form, and it will result in: more slow development of the cells, more uptake of substrates used for protein synthesis and more energy needed (maybe more mitochondrial activities and more glucose-degradation activities).

And the endocytosis between the TGN and endosomes will be influenced too. You may see smaller endosomes than before. As only AP-4 will take over this endocytosis, there will be not enough clathrin-coated vesicles to form large endosomes.

d.

Cargo binding site: AP-1 transports proteins with M6P, so there is a cargo binding site on the AP-1, which has M6P specificity. Membrane binding site: AP-1 coats vesicles derived from TGN, and it stays in the TGN when it is not recruited. And in order to take charge of the transport between TGN and early endosome, Ap-1's membrane binding site should have different affinities on different membranes, with or without cargo attached. There also should be a GTPase ARF-1 binding site, which is needed for the interaction between ARF-1 and AP-1.

e.

As there are totally 4 binding sites important for AP-1's normal function, which should be conserved, you can alignment the beta1-adaptin's sequences with some already known sequences, focusing on these binding sites, to predict the critical amino acid residues. For example, to secret the protein with M6P sorting signal, there should be a conserved M6P receptor sequence on the beta1-adaptin.

Q2.

a.

Endosome, lysosome, plasma membrane, TGN.

b.

The protein, which serves as a transcription factor, only appears in the nuclear or mitochondrion. To transport into the nuclear, the protein should have nuclear localization signal (NLS), which is rich in the positively charged amino acids lysine and arginine, such as KKKRK. And the signal can be located almost anywhere in the amino acid sequence. To transport into the mitochondrial, the protein should have a signal sequence at the N terminus. And this signal sequence is folded into an amphipathic alpha-helix, in which positive charged residues are clustered on one side of the helix, while uncharged hydrophobic residues are clustered on the opposite side.

c.

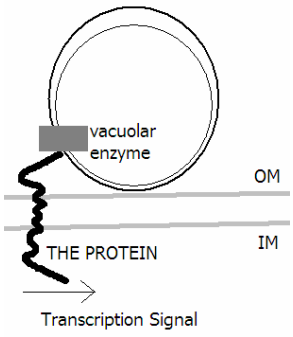
There are two methods can be used. 1, homogenize the cells, centrifuge and collect fractions to get different sub-cellular compartments; use the antibody of this protein to test. You should do the experiments with or without bafilomycin to figure out where are the proteolytic fragments. As bafilomycin will block the proteolytic cleavage of the protein, compared with no bafilomycin treated sample you will find some bands, which are the proteolytic fragments, disappear in the bafilomycin treated sample. 2, use immunofluorescent microscopy to test the co-localization of different proteins. You have the antibody of this protein labeled with green fluoresce, and another location specific protein (there are many proteins only locate in specific sub-cellular positions) labeled with red fluoresce. If you can get yellow spots on the overlay picture, you may conclude that these two proteins are co-localized, which means that these two proteins have the same sub-cellular location. Compared with experiments from with or without bifilomycin treatment, you will figure out where are the proteolytic fragments.

d.

Endoplasmic reticulum; Golgi; the matrix of mitochondrial.

e.

Because this protein interacts with a vacuolar enzyme (suggesting it is a membrane protein), and is also a protein functioning like a transcription factor (suggesting it is in nuclear or mitochondrial), I think it is a membrane protein crossing both inner and outer membranes of mitochondrial, maybe like TIM23 complex. The right figure here shows how the protein achieves the functions. It is a membrane protein, so it may have problems to bind to the mito-DNA directly. I think it may trigger other proteins to bind the DNA and this protein may regulate the transcription by changing its matrix-part conformation.



Q3.

a.

Hsp60 chaperones are large, barrel-shaped oligomeric ring complexes. They function post-translationly, and help fully synthesized but partially folded polypeptides efficient fold (binding at the large hydrophobic surfaces inside the central cavity). They can prevent the aggregation and provide an environment favorable for folding. They are ATPase, and use ATP to control binding and dissociation of substrate polypeptides.

Cytoplasm and mitochondrial will be impacted.

b.

Endoplasmic reticulum would not be affected.

c.

Hsp60 helps fully synthesized proteins to fold correctly. Loss of functional hsp60 will lead to the formation of protein aggregates (cells become denser) and eventual cell death. As hsp70 still functional, loss of hsp60's function may enhance hsp70's activities, and slower the development speed of the cell (mutation of hsp60 wastes ATP). As more other chaperones are needed were hsp60 fully mutated, transcription in the nuclear and chaperones synthesis in the cytosol/ER will be increased, and uptake of substrates will be increased. Or the cells may "shrink" because of using up substrates while uptake too slow.

d.

ATP is needed not only by hsp60, but also hsp70, etc. So the depleting ATP may result in that all the ATPase protein can't work correctly and the cells will die.

If the depleting is not consistent, the cells maybe recover after the quick ATP depleting: mitochondrial will be activated by the depleting ATP shock to produce more energy. And some protein aggregated in the cytosol may be sent to the peroxisome for degradation later.

Q4.

a.

Caveolae and raft pathways mediate the internalization of glycolipid binding toxins such as cholera toxin. There should be endosomes formed to transfer cholera toxin.

b.

After contaminated by *Vibrio cholerae*, cholera toxin attaches to the membrane. B subunits may attach the membrane directly and transfer A subunit into the membrane. Then caveolae-coated vesicles will form to transfer A subunit into Golgi, through PM, early endosome, late endosome, and TGN, during which A subunit is proteolytic modified. As there are many KDEL receptors in the Golgi (mostly in the CGN) cycled by the retrograde transport, A subunit with KDEL sequence will be treated just as a soluble ER resident protein, recognized by the receptor, and "returned" back to the ER by COPI coated vesicle from Golgi through retrograde transport pathway (docking on the ER is operated by the SNARE interaction). The accumulation of cholera toxin in the ER will accelerate the "default" transport from ER to PM, and the toxin reach the cytoplasm, where A subunit activates the adenylate cyclase and induces the intestinal Cl^- secretion.

c.

I will use site directed mutagenesis to test the requirement of the KDEL. I will construct plasmid expressing the toxin with mutant KDEL, such as AAAA, or even truncated it. Infect cells with mutant A subunit and wild type B subunits, and see the phenotype changes of the cells. Use centrifugation to collect the cytoplasm fraction, and immunoprecipitate to test whether the A subunit's sub-cellular location changes. Also, we can construct plasmids that can express EGFP-[mutant A subunit]. Infect cells with these tagged cholera toxin complex, and use fluorescent microscopy to trace and locate the A subunit in the cells (secretion protein will diffuse, and can't be seen by microscopy).

d.

GTPase is used in glycosylating and also involved in the vesicle formation. GTPase inhibitor will block the normal cell vesicle formation and transportation, which may dramatically influence the formation of caveolae vesicles and retrograde transports. Cholera toxin will not be taken in. And the existing cholera toxin will not be returned to the ER, and it will not be ably to reach the cytoplasm too. The cell will not secrete Cl^- after depleting the already existing cholera toxin on the PM. And glycolipid formation will also be blocked, which may change the morphology of the cells, and decrease the binding sites available for cholera toxin protein complex.

e.

In this example, how exactly the A subunit transfers from ER to PM is not clear; how the proteolytic modification make to the A subunit during transport is not clear. For general, KDEL retrograde transport is based on the sub-cellular compartmentation of KDEL receptors' binding affinity, how it changes is not clear now, maybe pH?