- Protein trafficking:
    - Destination of protein trafficking dependent on its sorting signals (sequence/patch)

Nuclear transport

- Nucleus structure
    - Double membrane structure with nuclear pore complex
    - Nuclear pore complex - largest structure of around 60 MDa structure, (<5kDa protein diffuse into nucleus, larger proteins move by active transport, >60kDa proteins move entirely by active transport)
    - Nuclear pore complex have FG repeats, interact with receptor proteins allow transport
- Nuclear transport
    - Nuclear localisation singals (NLS) directs nuclear proteins in/out the nucleus
        - Importing NLS: lysine-arginine rich sequence
        - Exporting NLS: leucine stretch
    - Folded polypeptide with NLS bind to importin nuclear import receptor in the karyopherin family, & pass through the nuclear pore complex.
    - Protein with exporting NLS bind with exportin receptor karyopherin, take protein out of the nucleus
    - Direction of transport: Ran-GTP/GDP
        - Nucleus Ran-GDP exchanged to Ran-GTP, via Ran-guanosine exchange factor (GEF)
        - Cytosol Ran-GTP exchanged to Ran-GDP in the cytosol via Ran-guanosine GTPase activating protein (Ran-GAP)
        - High [Ran-GTP] in nucleus, high [Ran-GDP] in the cytosol
        - Ran GTP binds with importin and exportin when exiting the nucleus, Ran-GTP hydrolysed and dissociate from localisation proteins.

- Mitochondrial import
    - Mitochondrial localisation signal: amphipathic(both hydrophobic + hydrophilic) α-helix structure
    - Mitochondrial import occur at places where inner membrane close to outer membrane.
    - Protein enters mitochondria via TOM complex and TIM 23 complex (Translocon of outer/inner membrane)
        - TOM complex contains TOM receptor and TOM channel
    - Localisation signalling amphipathic helix binds with TOM receptor, translocated into mitochondria in unfolded state
    - Hsp70 protein in cytosol keep protein unfolded using ATP hydrolysis
    - 3 energy potential involved in mitochondrial import:
        - ATP hydrolysis: Mitochondrial hsp70 pulls protein into cytosol and keep protein unfolded once it enters the mitochondria using ATP.
        - Membrane potential (-ve within mitochondria matrix) also contribute to moving protein across the membrane
        - Redox potential: Mia40 oxidise imported protein in the intermembrane space, protein form disulfide bond, prevent going back.
- Mitochondrial insertion
    - Outer membrane: Sorting & Assembly Machinery (SAM): Protein in intermembrane space bound to chaperones, inserted into outer membrane via SAM complex.
    - Inner membrane: 4 possible mechanisms:
        - Hydrophobic sequence behind amphipathic helix, when helix cleaved off, hydrophobic sequence in TIM complex laterally move into inner membrane
        - Metabolite transporter: uses electrical gradient across inner/outer membrane to insert intermembrane space

protein into inner membrane via another TIM complex (TIM22)

- OXA complex: Mitochondrial synthesised proteins with signal sequence inserted inside out via OXA complex
- Protein enters matrix, helix cleaved off, OXA localisation signal behind matrix cause it to be inserted via OXA complex.

Endoplasmic Reticulum transport:

- Sec61 dependent transport
  - Sec61 is the channel protein within the membrane
  - SRP (Signal recognition protein), soluble complex made up of 6 protein and rRNA
  - Signal of ER import made up of: positive amino acid followed by 6-12 hydrophobic amino acids on the N-terminus
  - During translation, SRP in the cytosol recognise signalling sequence, bind to N-terminus of mRNA, pausing translation.
  - SRP-mRNA bind to Sec61 complex and a GTP, emerging segment of mRNA transferred into the ER lumen, hydrolysis of GTP releases SRP, translation continues. (Both SRP and Sec61 contain GTPase subunit)
  - Signalling sequence cleaved off by signal pepsidase, protein folds (soluble) or move laterally to be inserted into ER membrane
  - ER membrane protein: C-terminus in cytosol is type 1, N-terminus in cytosol is type 2.
- Sec61 independent (Sec62/63) transport:
  - Sec62, Sec63 form complex with Sec61
  - ATP dependent, protein bind to polypeptide in cytosol to keep unfolded.
  - BiP in the ER lumen, is a Hsp70 ATPase, hydrolyse ATP to pull polypeptide into the ER
- TRC40 pathway
  - Transport proteins that have a C-terminal membrane domain/lipid anchor, does not enter ER lumen, stay on membrane
  - SRP independent, ATP dependent
  - Post translational polypeptide bound by chaperone proteins to remain unfolded.
  - Binding with TRC40 protein in cytosol bring it towards the WRB/CAML complex

Glycosylation: addition of sugar

- Core glycosylation/N-linked glycosylation:
  - Oligosaccharyl transferase is an enzyme attached to Sec61 complex and ribosome, transfer oligosaccharide groups from oligosaccharide-linked lipids onto asparagine residues.
  - Oligosaccharyl transferase detect N-X-S / N-X-T (X: anything but proline, N-asparagine, S-serine, T-threonine)
  - Glycosylation function: mark incorrectly folded proteins, resist degradation from proteases for transport, signal transmission

- ER associated degradation (ERAD)
  - Incorrectly folded sugars have reduced disufide bonds by disulfide isomerase
  - Misfolded protein stay longer in the ER, sugar will be overprocessed by lectin, trimmed by glycanse
  - Ubiquitin tag added by E3 ubiquitin ligase
  - Chaperone binding to the protein, target for degradation
  - Protein targeted for proteasome for degradation.

Methods to study membrane trafficking: GFP tagging, protein co-purification (fragment cell, centrifuge, purify, see if

attached), Genetic screen by inducing mutation in yeasts

Trafficking from the ER

- Biosynthetic secretory pathway: ER - Golgi - Surface membrane (can be directed to endosome, then lysosome)
- Endocytic pathway: Outside into the cell - endosome - lysosome
- Retrograde/recycling pathway: Golgi to ER, endosome to golgi. Endosome to membrane

General mechanisms involved in transport between compartments: vesicle transport (e.g. ER-Golgi) and compartment fusion (e.g. endosome + lysosome)

- Vesicle proteins (shown as electron dense coats under electron microscopy)
    - Coating proteins complex recognise, select and bind protein cargos tranported in the vesicle, form vesicle using GTPase hydrolysis
- COP2 vesicle: ER - Golgi
    - Made up of sec23/24, sec13/31
    - associated with GTPase sar1 (coat dis/assembly)
- COP1 vesicle Golgi - ER
    - 7 subunit coatomers
    - Associated with GTPase ARF
- Clathrin and adaptors
    - Clathrin forms the structural component of vesicle while adaptor proteins select the cargo
    - AP1 adaptor: Transgolgi <—> Endosome
    - GGA adaptor: Trans golgi to endosome
    - AP2 adaptor: endocytosis, form vesicle from plasma membrane to endosome
    - AP3 adaptor: golgi to lysosome
    - For clathrin vesicles, assembly requires ARF ATPase while disassembly requires Hsp70 ATPase

- Vesicle formation (e.g. clathrin vesicle formation)
    - Clathrin binds to receptor
    - AP2 adaptor binds to clathrin, bind between themselves
    - Bud formation, tip restricted by GTPase
    - When vesicle comes off, clathrin+adaptor disassembly via Hsp70 ATPase, produce naked vesicle

- Vesicle fusion mechanism
    - V-SNARES on vesicle, T-SNARES on target
    - Rab-GTP is found on the surface of the vesicle, Rab effector tethering protein found on the target membrane
    - Rab-GTP interact with Rab effector, being vesicle closer to the membrane.
    - At closer distance, V-SNARE(1 α-helix) T-SNARE(3-α helix) interact, form tran-SNARE complex, zippering brings vesicle to target membrane.
    - Fusion of membranes, fusion of contents
    - Rab-GTP hydrolysis, Rab dissociates
    - The SNARE complex is dissociated with NSF with ATP hydrolysis
- Mechanism involved in neurotransmitter release, tetanus toxin cleaves SNARE proteins, prevent release
- Many types of rab and SNAREs proteins provide specficity.

Phosphoinositide lipids: membrane identity specification

- Inositides can be phosphorylated at 3', 4', 5' positions, will represent different identities.
    - E.g. PI3P is found in endosomes, PI4P found in exosomes, PI4,5P belong to surface membrane.

Golgi modification: protein modified as it pass from cis-golgi network - cis golgi cisternae - medial cisternae - trans cisternae - trans network

    - As protein pass through the golgi appatus, Asparagine-linked oligosaccharides undergoes sugar trimming and sugar modification
    - Pass a certain point, the glycolysed protein becomes resistant to EndoH enzyme.
    - Level of modification reflect its previous position in the golgi apparatus
    - In the end, asparagine is attached to a complex oligosaccharide
- O-linked glycosylation
    - Sugar added to serine/threonine in N-X-S/T sequence, catalysed by glycosyl-transferase enzyme in the golgi
- Models of intra-golgi transport
    - Vesicle transport model: Forward transport of proteins, modification in each compartment
    - Cisternal maturation model: backward transport of enzymes

Lysosome

- Low pH vesicle resulted by proton pump, containing catabolic enzymes
- Fuse with endosome for digestion, and is recovered
- E.g. nuclease, protease, lipase, phoaphatase.

Protein sorting

1) Retrieve that need to stay in the ER

- Retrieval signal KDEL (Lys-Asp-Glu-Leu) at C terminus of soluble protein in ER, last four amino acids
- Retrieval signal KKXX (Lys-Lys-X-X) at C-terminus of transmembrane protein in ER
- COP2 vesicle with KDELR and KKXXR on membrane surface move to Golgi body
- Golgi-body have lower pH, facilitate signal-receptor binding, retreval via COP1 vesicle

2) Protein sorting and excytosis

- Similar mechanism as COPII vesicle trafficking
- Constitutive secretion (default secretion when not regulated) vs regulated secretion (form vesicle, secrete upon signal)

3) Delivery of soluble protein into the lysosome

- Mannose-6-phosphate tags the protein as a lysosomal protein.
- M6P Receptor bind to M6P protein, AP3 vesicle form from the golgi, fuse into the lysosome
- Lysosomal protein detatch from M6PR due to low pH, loses a phosphate becomes active
- Retrieval of M6P receptors back into golgi via retromer coating proteins.
- Mucolipidosis when enzymes are not transported into the lysosome but surface membrane instead, no lysosome function, death by 10.

4) Endocytosis of Low density lipoprotein - cholesterol uptake

- LDL bind to LDL receptor on the membrane surface

- Endocytosis, AP2-clathrin assembly with ARF GTPase, form vesicle
- Retrieval of LDLR from vesicle back to plasma membrane (retrieval signal F_NPXY (phenylalanine upstream of asparagine-proline-random-tyrosine).
- Endosome mature, fuses with lysosome (contain hydrolytic enzymes), absorb cholesterol

5) EGF signal turn-off

- EGF receptor on the cell surface endocytosed by clathrin-AP2 complex, tagged by ubiquitin tag.
- Endocytosis signal: YXX-hydrophobic
- Endosome Intracellular domain still exposed into the cytosol from the endosome
- Ubiquitin tag on the EGFR induce invagination, form multivesicular body, sequestrating EGF signalling.
- Fusion of late endosome with lysosome, degradation of both EGF and EGFR.

ESCRT proteins cause reverse budding of the membrane induced by ubiquitin tag

- ESCRT0 bind to PI3P, ubiquitin tag
- ESCRT1, 2 also binds with membrane lipid.
- ESCRT3 allow the constriction of the budding, away from cytosol (exocytosis/invagination into multivesicular body/ cytokinesis/HIV/Ebola)

Nuclear transport

- Nucleus structure
- Signal sequence
- Components involved
- Direction control

Mitochondria

- Structure
- Transport
- Energy potentials
- Insert into outer membrane
- Insert into inner membrane

ER

- 3 pathways
    - proteins involved
    - Signal
    - Energy source

ERAD

2 Glycosylation

Methods to study

Types of vesicles 3

Coating, adaptor, GTPase

Vesicle formation

Vesicle docking

Toxins

PIP

Golgi structure function

Lysosome

5 examples of vesicular transport and protein sorting.