1 Protein Sorting and Modification

Path of a transmembrane protein from translation to the plasma membrane (PM) proceeds as follows:

- cytosol →ER
  - translocation into the ER membrane (cotranslation translocation)
  - requires a signal sequence
- ER → Golgi Glycolysation lipids and proteins go in the Golgi.
- Golgi *to* PM

1.1 Integral Membrane Protein

Synthesis of an integral membrane protein still involves SRP and SRP receptors.

Hydrophobic transmembrane segment makes up the internal signal sequence.

Orientation of the protein depends on the distribution of charges on the molecule. The more negatively charged side of the transmembrane segment is directed towards the ER lumen.

Translocator protein assumes a variety of configurations, and therefore allows for different ways of insertion.

1.2 Golgi Apparatus

The size of a Golgi apparatus

Vesicles transpot proteins/lipits from the ER. The side which is close to the endoplasmic reticulum is called a *cis side*.

Each protein is inserted into the membrane in the ER in a specific manner. This protein assymetry is maintained through the vesical point even beyond the insertion by a translocon protein.

1.3 Lysosomes and Endosomes

Early endocytosed material is found in the early endosome. Lysosomal proteins delivered in vesicles from the Golgi are usually degested and eventully become called as late endosome

1.4 Lysosomes are the main site of intracellular digestion

Lysosomes, acidified by a proton pump, contain approx 40 types of hydrolytic enzimes. Note that ATP and ADP contentrations matter in determination whether the protein is of f- or v-type ointo th

Lysosomes are the main site of intracellular difeston. Low pH requirement of enzymes is necessary to protect the contents of cytosol itself.

1.5 Formation of Lysosomes

Lysosomal proteins from the ER and Golgi are incorporated into endosomes at different stages.

Plant vacuoles, occupying 30-90% of the cell volume, are typically acidic.

A large increase in the volume of a plant cell can happen without increasing the total volume.

The turgor pressure is an internal pressure of the cell that pushes it against the cell walls.

1.6 Peroxidsomes

This agent uses molecular oxygen to oxidise chain reactions.

2 Protein Sorting

There are two types of sorting: post-translation and co-translational. When in mitochondria or plastids, post-translational protein

How does a nuclear protein get into the nucleus? Transport is gated, and the process proceeds via the nuclear pore complexes.

2.1 Gated Transport

Nuclear pore complexes provide selective transport of macromolecules, as well as free diffusion of small molecules (< 50000 daltons).

2.2 Estrogen Receptors

The Estrogen Receptor is a ligand-modulated regulator of transcription. When estradol is not present, then estrogen receptor is in the cytosol. When estradiol enters the cell, it binds to the estrogen receptor. Ligand-bound ER moves into the nucleus thorugh NPCs. Estradiol then binds to nehancer sites in the genome and activates transcription of target genes.

3 Vesicles

3.1 Cytoskeleton

- Provides structural support
- Postions organelles
- Directs vesicular transport
- Involved in locomotion
- Required for cell divison

There are three types of filaments:

- microfilaments
  Actin, diam = 5-9 nm
- intermediate filaments
  intermediate filament proteints, diam = 10 nm
- microtubules (13 of them form a hollow cylinder)
  tubulin, diam = 25 nm

3.1.1 Immunofluorescence

A techinque used to determine the location of proteins with in the cell. Cells are fixed, and a primary antibody is used binding specifically to the protein of interest. A secondary antibody binds to the first antibody and is covalently tagged with a fluorescent molecule. A fluorescence microscope is used to excite the fluorescent molecule and visualise the light emitted.

3.1.2 Limits of Light Microscopy

The light microscope has a resolution limit due to diffraction. With an electron microscope, where the wavelength of the beam is much shorter, the resolution is better by a factor of about 250.

3.2 Dynamics

For cell motility/crawling, the actin filaments must rapidly disassemble and reassemble at the leading edge.

Most interphase microtubules radiate from one microtubule organising centre, and they are recognised to form the bipolar mitotic spindles in dividing cells.

3.3 Microtubules

Microtubules are involved in intracellular transport, sturctural support, cell organisation, mitosis processes and cell motility (flagella and cillia). Microtubules are made of tubulin, long hollow tubes, which are stiff and inextensible.

The structure of microtubules is made of individual subunits of α-tubuling and β-tubulin. Two closely related globular proteins form tubulin heterodimers. This regular arrangement of α and β subunits give the microtubule polarity. It has a plus end β and a minus end α.

3.4 Microtubule Protofilaments

13 parallel protofilamets make up the hollow tubule. All the bonds between the individual subunits are non-covalent. The bonds between protofilaments are weaker than the bonds within each protofilament. Growth and disassembly of microtubules can occur at the ends.

After the heterodimers have been in this structure for a while, GTP is cut to GDP.

If there are GDP-bound heterodimers at the end, they will disassemble.

In vitro, microtubule growth is faster at the plus end.

3.5 Tubulin Dimers

Free dimers are bound to GT. Tubulin subunits are enzymes that hydrolyse GTP. When this occurs in the filament, GDP is trapped in the tubulin subunits.

The microtubule GTP cap stabilises the plus end, which is the faster growing end. The GTP cap stabilises the plus end and favours tubule growth. Dimers in the T form bind more strongly to other dimers in the tubule. Hydrolysis of bound GTP reduces the binding affinity of the subunit.

3.6 Microtubules

In cells microtubules are nucleated at the MTOC (microtubule organising gentre). The centrosome is a MTOC which nucluates the formation of microtubules, with the minus end stabilised and the plus end dynamic.

3.7 γ-Tubulin ath the MTOC

γ-TuRCs is a complex of proteins forming a ring structure. γ-Tubulin binds the ring structure and acts as an attachment site for λ/β

Nerve cells in the spinal cord can exted to the finger tips. These neurons can be more than a meter long.

Kinesin and dyening are motor proteins that walk along microtubules.

Dyenin movement is towards the cell body, while kinesin movement is towards the axon terminus.

Organelles are assocated with microtubules, which are walked to specific location.

3.8 Movement of vesicles through the endomembrane system

The Golgi apparatus is also a MTOC but it's different from the centrosome.