Isolation and characterization of the cell organelles

This process aims to break open a cell by disruption of plasma membrane and then separate the cell organelles in a sequence of centrifugation steps. The process is described below.

- First step is to rupture the plasma membrane and for this cells are suspended in an appropriate buffer solution. It is usually an isotonic solution of sucrose (0.25M) or a combination of salts similar in composition to the cell`s interior.
- Plasma membrane is now disrupted by
    - stirring the cells in a high speed blender or
    - by exposing it to the ultrahigh frequency sound (sonication) or
    - by using pressurized tissue homogenizers
- The cell solution during all this process is kept at $0^{o}C$ so that enzymes and other components are kept safe after their release from the cell.
- When disruption is done, we get a homogenate which is a mix of suspended cellular components. This homogenate is put to a series of centrifugation steps to separate the cell organelles.

Centrifugation for organelle separation

When homogenate is ready, it is filtered and we start with a series of differential centrifugation steps each with increasing speeds.

At each step, centrifugation is done for a fixed amount of time and supernatant is poured off and put through the next step of the centrifugation. This continues till the completion of the steps as is shown in the figure below.

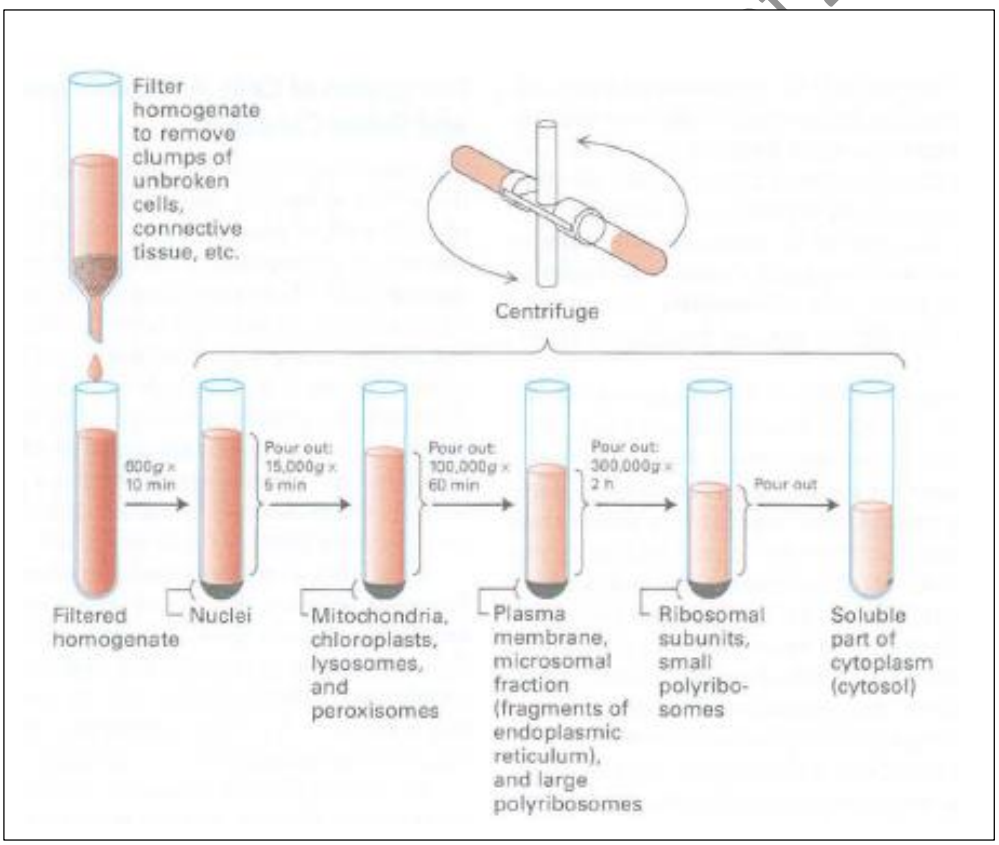

Figure: Fractionation of cellular organelles using differential centrifugation.

At each step we also find a pellet in the tube. This pellet contains a mixture of organelles as can be seen in the figure above. This organeller pellet fraction is impure and is purified i.e. separated into its components so that we can get each organelle in a purified form.

- This purification is done by equilibrium density gradient centrifugation which separates cellular components according to their density.
- To achieve this, we resuspend the pellet fraction in a suitable buffer and then layer it on the top of a solution which contains a gradient of a dense, non-ionic substance such as sucrose or glycerol.
- The tube is then centrifuged at high speed for several hours.
- This allows each particle to migrate to an equilibrium position where density of the liquid is same as the density of the particle.
- The different layers are then removed carefully.

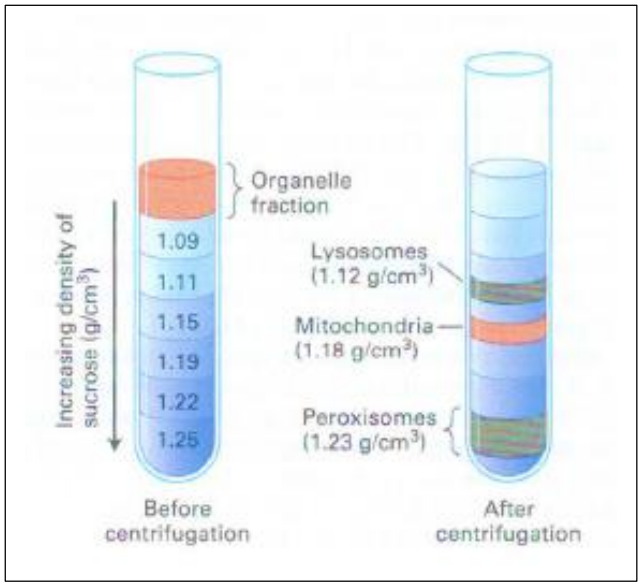

Figure: Density gradient centrifugation

- Contents of each layer are then assessed for purity. This can be done by using electron microscopy or quantification of organelle specific molecular markers.
- E.g. Cytochrome c is present only in the mitochondria. If it is also present in lysosome fraction, then it indicates contamination of mitochondria in lysosomal fraction.
- Other specific molecular markers are tabulated below:

Organelle	Molecular Marker
Peroxisome	Catalase
Lysosomes	Acid phosphatase
RER and cytosol	Ribosomes
SER	Cytidylyl transferase
Plasma membrane	Amino acid permease

<u>Preparation of highly purified organelle samples:</u>

- In this method, we use monoclonal antibodies (mab) against organelle specific proteins.
- The organelle preparation is treated with a specific mab and incubated for sufficient time.
- After this, the preparation is incubated with protein A/G linked to agarose/sepharose beads which bind to the mabs.
- After incubation, we gently centrifuge the mixture to precipitate the mabs bound to protein A/G.
- Because the mabs are also bound to the organelle via specific protein, the organelle is also pulled with the mabs.

- This preparation is highly pure.
- E.g. Purification of endocytic vesicles using anti-clathrin antibodies, separation of GLUT4 containing vesicles by using antibodies that can bind to the cytosolic projections of the GLUT4 etc.

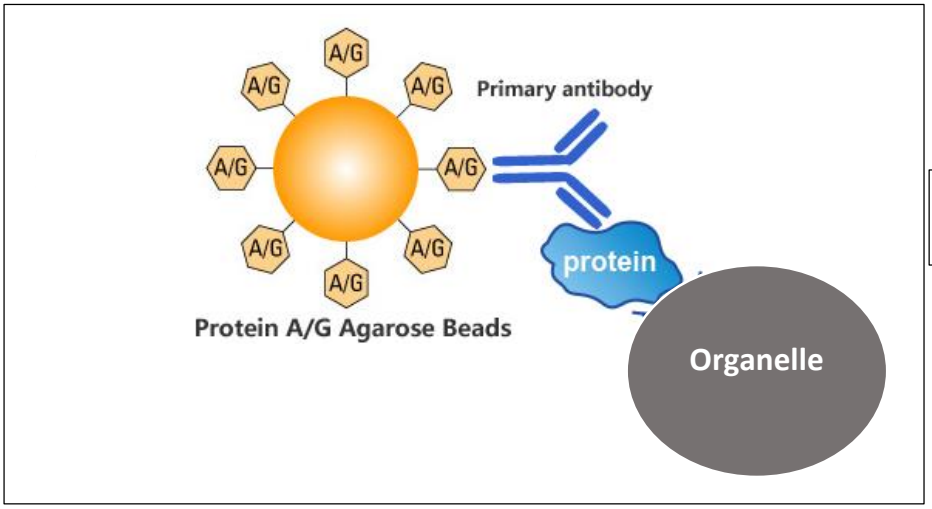

Figure: Immunoprecipitation of organelles.

Drugs in Cell Biology research:

Drugs are routinely used in cell biology research to perturb specific processes and study the effects of the perturbation on a given function. The list of drugs and their functions is given in the table below:

Drug	Effect
Aphidicolin	Eukaryotic DNA polymerase inhibitor
Campothecin, Etoposide	Eukaryotic DNA topoisomerase inhibitors
α-Amanitin	Eukaryotic RNA polymerase II inhibitor
Actinomycin D	Eukaryotic transcription elongation inhibitor
Rifampicin	Bacterial RNA polymerase inhibitor
Thiolutin	Bacterial and yeast RNA polymerase inhibitor
Cycloheximide	Translation inhibitor in eukaryotes
Genticin/G418, Hygromycin, Puromycin	Translation inhibitors in bacteria and eukaryotes
Chloramphenicol	Translation inhibitor in bacteria and mitochondria
Tetracycline	Translation inhibitor in bacteria
MG-132, Lactacystin	Proteasome inhibitors
E-64, Leupeptin	Serine and/or cysteine protease inhibitors
Phenylmethanesulfonylfluoride (PMSF)	Serine protease inhibitor
Tosyl-L-lysine chloromethyl ketone (TLCK)	Trypsin-like serine protease inhibitor
Phalloidin, jasplakinolide	F-actin stabilizer
Latrunculin, Cytochalasin	F-actin polymerization inhibitors
Taxol	Microtubule stabilizer
Colchicine, Nocodazole, Vinblastine, Podophyllo toxin	Microtubule polymerization inhibitors
Monastral	Kinesin-5 inhibitor
Brefeldin A	Secretion inhibitor
Leptomycin B	Nuclear protein export inhibitor
Dynasore	Dynamin inhibitor
Tunicamycin	N-linked glycosylation inhibitor
Genistein, Rapamycin, Gleevec	Tyrosine kinase inhibitors
Wortmannin, LY294002	PI3 kinase inhibitors
Staurosporine	Protein kinase inhibitor
Roscovitine	Cell cycle CDK1 and CDK2 inhibitors
Cyclosporin A, FK506, Calyculin	Protein phosphatase inhibitor

Okadaic acid	Inhibitor of serine/threonine phosphatases
Sodium orthovanadate	Tyrosine phosphatase inhibitor
Forskolin	Adenylate cyclase activator
A23187	Ca^{2+} ionophore
Valinomycin	K^{+} ionophore
BAPTA	Divalent cation sequestering agent
Thapsigargin	Endoplasmic reticulum Ca^{2+} ATPase inhibitor
Oubain	Sodium-Potassium ATPase inhibitor
Propranolol	β-adrenergic receptor antagonist
Statins	HMG-CoA reductase inhibitors as a result block cholesterol synthesis