Endocytosis

For a brief overview of endocytosis, we will need to keep in mind the following points:

- Endocytosis is a process by which cells take up components from the plasma membrane surface
- Endocytosed cargo → ① receptor-ligand compleres ② nutrients and their carriers ③ ECM components ④ cell debris ⑤ bacteria, viruses and other cells

- way of regulating plasma membrane composition in response to changing extracellular conditions

- Main process: ⓐ material to be ingested is progressively enclosed by a small portion of the plasma membrane
  ⓑ plasma memb pinches off to form endocytic vesicle
  ⓒ endocytic vesicle fuses with early endosome → sorting
  ⓓ some cargo returned to membrane via recycling endosome
  ⓔ others remain in endosome as it matures from early → late → endolysosomes & are degraded

① Receptor mediated endocytosis ② Phagocytosis ③ Pinocytosis
④ Endosomal sorting and maturation

① Receptor mediated endocytosis

- also called clathrin mediated endocytosis (clathrin coated pits & vesicles)
- macromolecules bind to complementary transmembrane receptor proteins
  ↓
  accumulate in clathrin coated pits
  ↓
  enter cell as receptor-macromolecule compleres in clathrin coated vesicles

- ligands are selectively captured by receptors – selective & efficient concentrating mechanism

Case study – import of cholesterol

- cholesterol import needed to make new membranes & prevent atherosclerosis

- cholesterol imported as cholesterol esters in the form of LDLs
- LDLs → low density lipoproteins – lipid droplets bearing a core of triacylglycerol, free cholesterol, and cholesteral esters, stabilised by protein B
- when cell needs cholesterol for membrane synthesis, it makes transmembrane receptors for LDL

↓

LDL receptor diffuses until an endocytosis signal binds the adaptor protein AP2

↓

AP2 recruits clathrin to initiate endocytosis

↓

coated pits rapidly pinch off to form coated vesicles → any LDL bound to receptors is rapidly internalised

↓

after shedding coat, vesicles deliver coats to early endosomes

Recycling endosomes

- early endosomes are the main sorting stations in the endocytic pathway
- in the mildly acidic environment of the early endosome, many receptors change their conformation → ligands thus released are mostly degraded
- some others remain bound, and share the fate of the receptors

In context of cholesterol internalisation,

the receptor for LDL dissociates from LDL and is carried back to the plasma membrane for reuse, while the LDL is carried off to lysosomes

How does the process of recycling work?

The recycling transport vesicles bud from long, narrow tubules that extend from early endosomes (geometry of tubules helps membrane proteins localise)

Recycling endosomes regulate plasma membrane composition

- receptors might be returned to the same plasma membrane domain or to a different domain (transcytosis), while some remain and are degraded

- transcytotic pathway is not direct – receptors move from early

endosome to recycling endosome.

- different receptors follow a variety of different pathways from early endosomes to plasma membrane, implying that they also have sorting signals to guide them to the appropriate pathway

Endocytosis can be broadly classified into dynamin dependent and dynamin independent pathways.

Key examples of dynamin dependent pathways include clathrin-mediated and caveolin-mediated pathway
Key examples of dynamin-independent pathways include the clathrin-independent carriers (CLICs) and Arf6-dependent pathways

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Exocytosis

First, we will be talking about the constitutive and secretory pathways. The fusion of the vesicles with the plasma membrane is exocytosis.

Formation of secretory vesicles

- secretory vesicles form from the TGN and release their contents to the cell exterior via exocytosis in response to specific signals

Mechanism of formation:

1. Selective aggregation of secretory proteins in the TGN using some common sorting signal
2. clumps are then segregated and packed into secretory vesicles, either with the help of receptors or some sort of enclosing.
3. clathrin coated buds pinch off from these vesicles, removing extra volume.

Cytoskeleton Dynamics

Actin

- filaments are helical polymers of actin protein
- flexible with a diameter of 8 nm that organise into 2D & 3D structures
- concentrated in cortex.
- three mammalian isoforms of actin — α, β, γ, that differ slightly in their amino acid sequences and functions
- accessory proteins crosslink and bundle the filaments together - rigid structures

Actin polymerisation:

1. actin subunits can bind to one another, but association is unstable unless oligomers or nucleus is formed (filament nucleation)
2. time course of actin polymerisation involves nucleation, elongation and steady state
3. nucleation is the rate limiting step - instability of smaller actin oligomers make nucleation inefficient — hence no filaments visible in lag phase

Critical concentration (C_c) — as the conc. of actin monomers declines, system approaches a steady state where rate of addition of new filaments = rate of subunit dissociation

At this equilibrium, $k_{on} C = k_{off} \Rightarrow \boxed{C_c = \frac{k_{off}}{k_{on}}}$

Below C_c → dissociation more
At C_c → both equal
Above C_c → elongation more

What differentiates the plus end from the minus end?

The plus end is fast-growing, the minus end is slow growing.

Points to consider:

- k_{on} and k_{off} have different values for each end, but their ratio must be same at both ends

Accessory proteins:
① Nucleators ② Monomer-binding proteins ③ Severing proteins

Nucleators

- soluble monomer concentration well above critical concentration - yet only a small fraction of actin monomers polymerise
- this is because a large no of nucleators tightly control polymerisation, close to a membrane surface - mostly Arp 2/3 or formins
- Arp 2/3 —
  - contain Actin related proteins (45% identical to actin)
  - nucleates actin filament growth
  - remains bound to minus end, allows rapid elongation at the plus end
  - requires the activity of a nucleation-promoting factor (NPF)
  - further stimulated when it attaches to the side of a preexisting actin filament
  - Arp 2/3 complex activation generates a branched array of actin filaments adjacent to a membrane, building individual filaments into a tree like network

- Formins -
  - dimeric proteins that nucleate the growth of unbranched filaments
  - along with other proteins, form parallel bundles
  - nucleates polymerisation by capturing two monomers
  - dramatically accelerates actin filament growth

Monomer binding proteins

- maintenance of free subunit resource

- Profilin —
  - binds to the face of the actin monomer opposite to the ATP binding cleft
  - blocks the side of the monomer that would attach to the filament ⊖ end
  - leaves it free to bind to the plus end - when it binds to the plus end, profilin falls off due to conformational change in actin
  - maintains a large pool of actin monomers for polymerisation at plus ends
  - bind to nucleators and are thus directly recruited to the sites of filament elongation

- Thymosin – • competes with profilin for actin binding

Severing Proteins

- regulating depolymerisation and generating new filament ends
- depolymerisation of old filaments while newly formed ends nucleate filament elongation

- Gelsolin – • activated by high levels of cytosolic Ca^{2+}
  - interacts with the site of the filament, with two sites
  - one site binds to exposed site on filament and the other hidden b/w adjacent subunits
  - when thermal fluctuation creates a gap b/w adjacent filaments, gelsolin inserts itself into the gap and severs the filament
  - after severing, remains attached to the filament and caps the new (+) end

- Cofilin – • binds along the length of the filament, forcing the filament to twist a little more tightly
  - mechanical stress induced weakens the contact b/w actin filaments
  - thus, filament is easily severed by thermal motions – most actin filaments in cells are therefore short-lived.
  - binds preferentially to ADP-actin, hence severs old filaments
  - crucial for polarised, directed growth of actin network

Regulation of actin filament behaviour

- side binding and capping proteins regulate filament dynamics and organisation

- Tropomyosin – • elongated protein binding simultaneously to six or seven adjacent subunits along each of the two grooves of the actin filament
  - stabilises and stiffens the filament
  - also prevents interaction of actin with other proteins

- Cap Z – • prevents actin filament from depolymerising rapidly, by binding to plus end
  - stabilises plus end by rendering it inactive
  - greatly reduces rates of filament growth and depolymerisation

- Tropomodulin – • caps actin filaments in muscle
  - binds tightly to the minus end of actin filament coated and stabilised by tropomyosin

- regulates filament length and stability

- Fimbrin – tight packing of parallel bundles of actin filaments in long cellular protrusions

Actomyosin

- skeletal muscle myosin – generates force for muscle contraction
- myosin II is an elongated protein (two heavy chains + two copies each of two light chains)
- each heavy chain – globular head domain at N-terminus + very long α-helical coiled coil to mediate heavy chain dimer
- each light chain – bind close to N-terminal head domain
- tails bundle with other myosin tails and form thick filaments
- each myosin head binds and hydrolyses ATP – using energy to walk to plus end of the filament
- actin filaments form thin filaments around these thick filaments in myosin that contract – in skeletal muscles

Contraction Mechanism Flowchart

① Myosin head lacking bound ATP is locked tightly onto actin filament

→

② ATP molecule binds to large cleft on back of head – causes conformational change

↓

③ Leads to rotation in converter domain, causing lever arm to swing out, and the head to be displaced along the filament by 5 nm

←

④ ATP hydrolysis occurs, but ADP and P_i remain bound to protein

↓

⑤ Myosin head binds weakly to new site on actin filament, releasing P_i → tight binding & regaining original information (power stroke)

• Muscle cell --> Myofibril ···> Sarcomere

Sarcomere

- miniature, precisely ordered array of parallel and partly overlapping thin & thick filaments
- thin filaments are formed from actin and associated proteins
  - they are attached to a Z-disc at each end of the sarcomere (with their plus ends)
- myosin filaments are arranged in a regular hexagonal lattice, with the actin filaments evenly spaced b/w them
- Sarcomere shortening — • caused by myosin filaments sliding past the actin thin filaments
  - bipolar thick filaments walk along the actin filaments
  - each myosin head remains bound to the actin only for a short period of time, so that they do not hold each other back (no coordination)
  - enables sarcomere to shorten by 10% of its length in less than one-fiftieth of a second.
- fast contraction is possible because individual myosin heads remain bound only for a short period of time & because a specialised membrane system relays the incoming signal rapidly throughout the entire cell.

Mechanism

① Incoming action potential activates Ca^{2+} channel in T-tubule membs → ② Triggers the opening of Ca^{2+} release channel in the closely associated sarcoplasmic reticulum.

↓

④ Ca^{2+} is pumped back immediately into sarcoplasmic reticulum via ATP dependent Ca^{2+} pump ← ③ Ca^{2+} floods into cytosol → contraction of each myofibril at once

- Troponin — a complex of 3 polypeptides - T, I, C
  - ↳ T-I complex binds to tropomyosin and pulls it into a position that interferes with myosin head binding
  - ↳ when Ca^{2+} ↑, troponin C binds to Ca^{2+} — causing Troponin I to release actin — allowing myosin heads to walk along actin filament

In smooth muscle cells, calmodulin instead of troponin

- elevated Ca^{2+} → Ca^{2+} bound calmodulin activates myosin light-chain kinase (MLCK) → phosphorylation of smooth muscle myosin on one of its two light chains → myosin head can interact with actin filaments and cause contraction

- Myosin V transports cargo by walking along actin filaments

Microtubules

- more complex than actin filaments
- polymers of the protein tubulin – heterodimer of α-tubulin and β-tubulin
- α-tubulin & b-tubulin – each binds to 1 molecule of GTP : catch is that the one on α can never be hydrolysed or exchanged.

- hollow cylindrical structure built from 13 parallel protofilaments
  - each composed of αβ tubulin heterodimers stacked head to tail & folded into a tube

- two kinds of protein-protein contacts :

both are tight – hence addition or loss of subunits occur only at ends

- multiple subunit-subunit contact make microtubules stiff and difficult to bend (persistence length → 10X of actin)

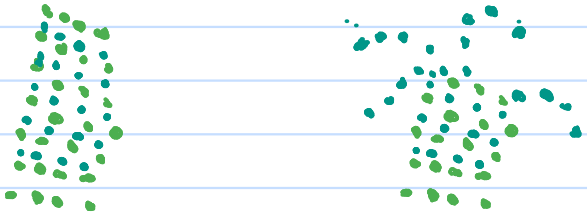
- α-tubulins exposed at ⊖ end and β-tubulins at ⊕ end
  - ⊕ end grows and shrinks much rapidly than ⊖ end

Dynamic Instability

- GTP for microtubules as bound to β-tubulin – hydrolysis responsible for microtubule dynamics
- GTP hydrolysis is accelerated when they are incorporated into microtubules — tubulin can be in T form or D form
- some of the energy of phosphate bond hydrolysis is stored as elastic strain in lattice — free energy change for dissociation of a subunit from D-polymer is more negative
  - $\frac{k_{off}}{k_{on}}$ for GDP-tubulin >> GTP-tubulin : T form tends to polymerise & D form tends to depolymerise
- whether the tubulin subunits at the end of the polymer are in (T/D) depends on rate of subunit addition
  - low → D form
  - high → T-form (GTP cap)

Catastrophe

- intermediate free tubulin concs b/w D-tubulin C_c and T-tubulin C_c (necessary for T form assembly, but below for D form)
- on a single microtubule, an end might grow for a certain length in T form but might suddenly change to D form (catastrophe)
- rescue
- rapid interconversion between growing and sinking state (dynamic instability)
- microtubules spring up when they lose GTP cap and depolymerise

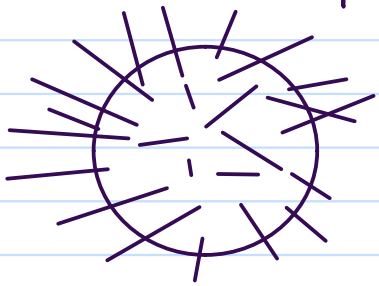


Nucleation of Microtubules

- concentration of free tubulin needed for spontaneous nucleation of microtubules is very high — help needed from other factors
- nucleation happens at specific intracellular location called (MTOC), where γ-tubulin is enriched
- γ-tubulin ring complex is necessary for nucleation:
  1. two accessory proteins bind directly to γ-tubulin
  2. creates a spiral ring of γ-tubulin molecules
  3. template to create microtubule with 13 protofilaments

Centrosome

- well-defined MTOC located adjacent to nucleus, nucleated microtubules at their minus ends
- recruits more than 50 copies of γ-TuRC
- not necessary for nucleation, can happen without it



- centrioles are cylindrical arrays of short modified microtubules arranged into cylindrical shape — they recruit the pericentriolar material where nucleation takes place

Microtubule-binding proteins (MAPs)

- short C-terminal ends that protrude from microtubule are enriched in glutamic & aspartic acids — hence net negative charge of microtubule surface
- MAPs are ⊕ vely charged and bind to microtubule surface through electrostatic interactions
- MAPs have one domain that binds to microtubule surface, and another one that projects outwards — projecting domain length determines how closely

MAP-coated microtubules pack together

Plus end binding proteins

- plus ends efficiently explore and probe the entire volume of the cell
- proteins like catastrophe factors influence dynamic instability — they bind to plus end and pry protofilaments apart to facilitate catastrophe
- others help in rapid microtubule growth by concentrating free tubulin at plus end. (XMAP215)
- plus end tracking proteins (+TIPs) – bind to plus end that is growing and dissociates when it shrinks

Tubulin-sequestering and Severing proteins

- Stathmin — binds two tubulin heterodimers and prevents their addition to tubule ends
  — decreases effective conc of tubulin and promotes catastrophe
  — phosphorylation inhibits stathmin and suppresses dynamic instability
- Katanin — severs 13 longitudinal bonds using ATP hydrolysis energy
  — extracts tubulin subunits, weakens structure and promotes breakage
  — releases microtubules from MTOC

Interestingly, severing leads to a decrease in tubulin conc.

- after severing lost GDP subunits are replaced by GTP subunits
- if sufficient no. of subunits accumulate before severing, polymerise due to GTP cap
- severing promotes growth of more polymer

Motor Proteins

- microtubule based motors – dyenins and kinesins
- functions – ① move cargo like organelles and macromolecules over long distances
  ② slide microtubules along each other to rearrange them
  ③ regulate microtubule dynamics

Kinesin

- large protein superfamily with common motor domain of heavy chain
- walk towards (+) end of microtubule (when motor domain at N-terminus)
- some like kinesin-13 do not walk at all – depolymerises microtubule ends

Dyenins

- minus end directed microtubule motors
- one/two/three heavy chains & a large & variable no. of intermediate & light chains
- cytoplasmic dyenins – organelle and mRNA trafficking, centrosome & nucleus positioning and mitotic spinde formation during cell division
- axonemal dyenins – rapid & efficient microtubule sliding movements for cilia & flagella beating

Intermediate Filaments

- only in some metazoans — mechanical strength for squishier animals
- related to nuclear lamins
- elongated proteins with conserved α-helical domain forming coiled coil dimer

- do not contain ATP or GTP binding sites
- tetramer formed of two dimers pointing in opp. direction — X polarity
- filament — eight parallel protofilaments made of tetramers (32 indiv α-helical coils)
- easily bent and stretchable — difficult to break
- mechanical stability & resistance to shear stress