The Early Endosome

- Receives material internalised from plasma membrane in the form of receptors
- Sort this to different destinations
  - ↳ back to PM
  - ↳ transfers to other organelles such as Golgi
  - ↳ delivers content to lysosomes

Membrane Features (basic features of biological membrane)

- sheet-like structures
- thickness 6–10nm ~ determined by chemical composition, hence it is NOT absolute
  - ↳ some membranes are designed to be thicker
  - ↳ some areas of the same membrane can be of different thickness
- tendency to form closed boundaries
- non-covalent assemblies
- asymmetric
- fluid; 2 dimensional liquid
- mostly electrically polarised, inside -ve

Membrane Composition

- composed of lipid, proteins & carbohydrates
- lipids (hydrophobic & hydrophilic areas – lead to formation of closed sheets)
- proteins (pumps, channels, receptors, enzymes)
- lipid : protein 1:4 to 4:1
- carbohydrates linked to lipids & proteins

Lipids : Any of the large group of fats and fat-like compounds which occur in living organisms and are characteristically soluble in certain organic solvents but only sparingly soluble in water

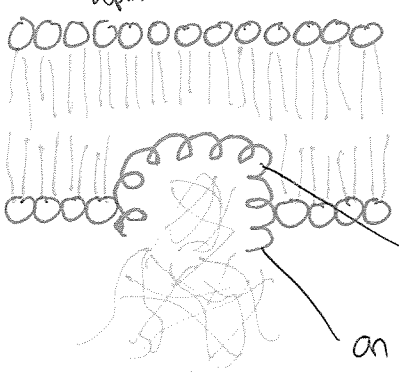
- phospholipids
  - charged
  - neutral
- sphingolipids
- glycolipids

} capable of spontaneous formation of membrane

steroids ~ incapable of spontaneous formation of membrane

Prostaglandin H2 synthase

Arachidonate (lipid-derived) → Prostaglandin H2 → inflammation
aspirin



hydrophobic amino acid side chains

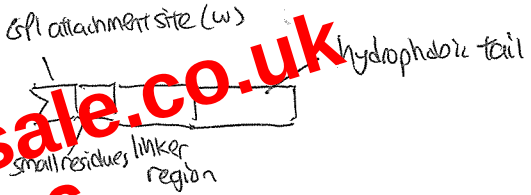
an amphipathic helix allows stable membrane insertion [hydrophobic side chains face the acyl chains of lipid bilayer
hydrophilic " " face the head groups]

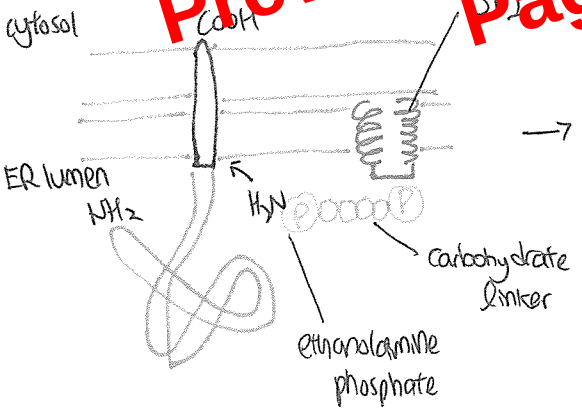
Lipid Modification of Proteins

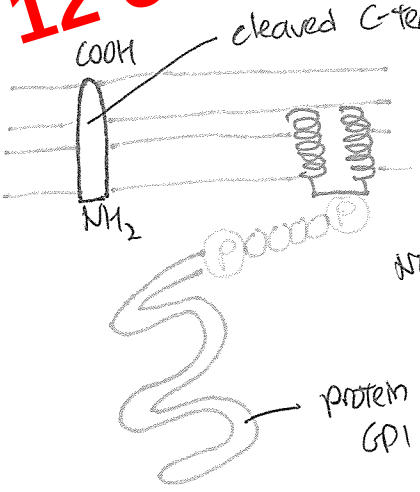
1) Anchoring to the outside ie. lumenal leaflet of the membrane

Glycosyl phosphatidyl inositol (GPI) modification
Anchors protein in outer leaflet of PM
Stable
"Flexible leash"
Essential for protein to remain attached to cell
Specific phospholipase can release protein from cell in response to signals

GPI made outside of the ER, in the cytosol







NOTE: GPI anchors have 2 saturated acyl chains

protein bound to membrane by GPI anchor

Eg of GPI proteins

1) PrPc: prion protein (function still unknown) associated w scrapie and BSE (Bovine spongiform encephalopathy) Mad cow disease

2) Trypanosoma brucei (sleeping sickness) variable surface glycoprotein (VSG)
↳ covered w GPI anchor
↳ prevent digestion by host cell

3) placental alkaline phosphatase (PLAP)

Clathrin-coated vesicles as a paradigm for vesicle formation

↳ clathrin-coated vesicles have a characteristic basket-like structure

↳ structure is formed by protein, clathrin, which is a major component of coat

Clathrin assembly into a coat

- Each clathrin subunit – triskelion comprised of 3 heavy and 3 light chains
- Assemble into a basket-like framework of hexagons and pentagons ⇝ alters membrane curvature
- Isolated triskelions can self-assemble to form cages in absence of membranes.
  They determine the geometry of the cage

* globular domain at the end of heavy chains bind to adaptor protein which bind to cargo protein

Clathrin-adaptor proteins : 2nd major component of clathrin-coated vesicles

Required for 1) attachment of clathrin to the membrane
2) recruitment of cargo proteins into vesicle

2 major classes : Monomeric adaptors
Dab 2 – PM
AP180 – PM
GGA – TGN

Tetrameric Adaptors
AP 1 TGN
AP 2 PM
AP 3 Early endosome
AP 4 TGN

AP complex structure : Heterotetramers with 2 large, 1 medium and 1 small (clathrin binding, cargo binding)

↳ AP complex links clathrin to the membrane through binding to cargo & lipids

Phosphoinositide lipids recruit proteins to membrane

↳ this includes AP 2 and certain other protein required for vesicle formation

Phosphoinositides (PIs)

- Found only on cytoplasmic leaflet
- Generated by differential phosphorylation of inositol ring at 3,4,5 positions
- Diff. PIs enriched in diff. compartments and even domains within the same membrane compartment
- can be hydrolysed to generate 2nd messengers or act as signals in their own right to recruit proteins to membranes
- Interconversion between diff PIs is mediated by specific kinases & phosphatases
- PI-4,5P_2 recruits AP 2 to the PM (PI3,4,5P_2 only found in PM)
  PI-3P recruits protein to early endosome eg: SNX (PI-3P only found on endosome)
- PIs recruit effectors to the membrane in a specific manner

Receptor-mediated endocytosis

- clathrin-dependent
- Efficient uptake of specific components via cell-surface receptors

  Eg: yolk lipoproteins in hen oocyte

Insights gained from disease

⇝ ~~Familial~~ Familial hypercholesterolemia (FH)

- genetically inherited disease
- high blood cholesterol resulting in early death by heart attack
- due to defective LDL uptake

Analysis of diff. patients: LDL receptor absent / defective
- some mutations in receptor abolish LDL binding
- other mutations in receptor that prevent incorporation into coated pits

Internalisation Signals

- Hundreds of receptor types internalised by RME
- Internalisation constitutive (LDL receptor) or induced by ligand binding (EGFR)

All receptors have internalisation signals

↳ NPXY motif in LDL receptor (Y mutated in some FH patients) ϕ → hydrophobic residue

↳ YXXϕ motif found in many receptors

↳ LL motif*

⇒ bind to clathrin adaptors (AP2) ARH / Dab2 for LDL receptor

Early Endosome Fusion

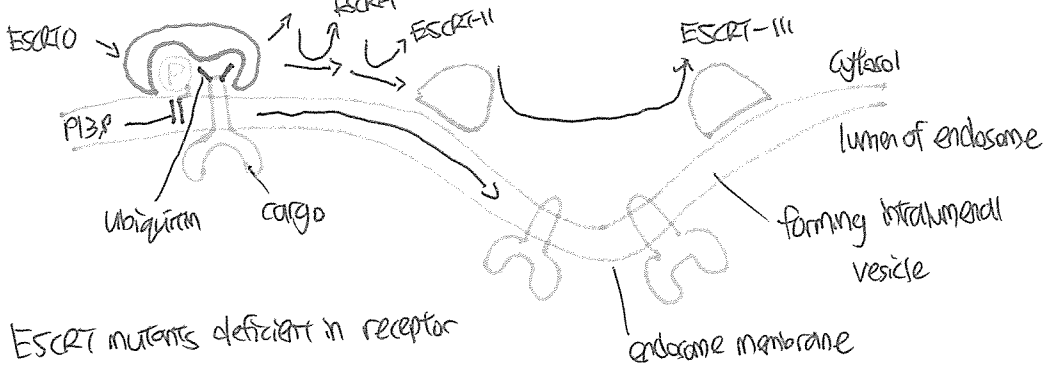
- endocytic vesicles uncoat and fuse with early endosomes
- early endosomes also have the capacity to fuse with each other → homotypic fusion
- Both fusion events regulated by Rab5

Early Endosomes

- Main sorting station in endocytic pathway
- acidic environment → most receptors release cargo

Receptors can be
- recycled to PM
- transported to diff domain of PM (transcytosis)
- transported to lysosomes

ESCRTs act sequentially for receptor sorting



ESCRT mutants deficient in receptor sorting to intraluminal vesicles

↓

persistent mitogenic signalling and cancer

Storage of PM proteins in recycling endosome

↳ recycling endosome can act as a repository for some PM proteins

eg: GLUT 4 glucose transport in insulin-responsive cells

- GLUT 4 normally stored in specialized recycling endosomes - Upon insulin signal, the GLUT4 receptors are transported to PM for glucose uptake

Cargo is sorted at the TGN

- cargo is sorted into carriers for delivery to different destinations
- cargo without signals is delivered to the PM by default constitutive secretion

Constitutive Exocytosis – constant stream of transport containers move from the TGN to the PM

- supplies PM with new protein and lipids made in the ER allowing
  1) PM expansion before cell division
  2) protein secretion

Transport containers

↳ vesicular / tubular in nature

↳ formed by motor-driven extrusion of TGN along microtubules

↳ Move along microtubules and fuse with PM

↳ Extrusion and movement requires kinesin (MT + and motor)

↳ NO coat protein identified : consistent with no selection of cargo

Regulated Exocytosis

- Specialised secretory cells make hormones, neurotransmitters, enzymes, sorted into secretory vesicles that bud from TGN and accumulate near PM
- only fuse with PM and release content after stimulation by extracellular signal eg: [blood glucose] ↑ → ↑ insulin

Sorting of cargo into secretory vesicles

- selective aggregation of secretory proteins (hydrophic patches of protein come together)
- probably mediated by signal patch on the proteins
- aggregates sorted into vesicles
- sorting mechanism not known

Maturation of secretory vesicles

dump maturation, cargo concentration ↑

↳ maturation: cargo concentration

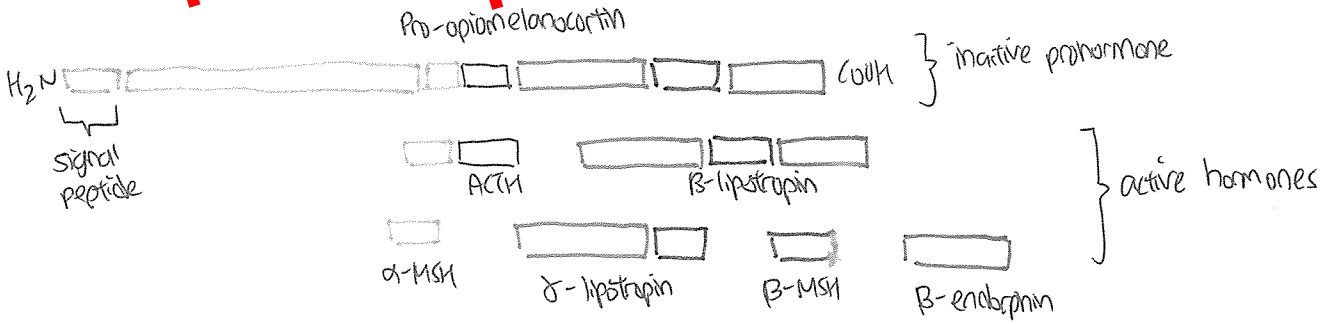
due to: 1) retrieval of membrane in clathrin coated vesicles [clathrin takes away excess membrane from secretory vesicles]

2) Acidification of lumen by ATP-driven H^+ pumps

dense packing allows rapid release of large amounts of material

Polypeptide Processing in Secretory Vesicle

- many polypeptide hormones, peptide and digestive enzymes made as inactive protein precursors
- proteolysed to release active molecules



- peptides are too small for ER targeting (some hormone ~ 50aa, ER & targeting signal ~ 200aa, hence cannot enter ER)
- Don't want active products until release eg: digestive enzymes

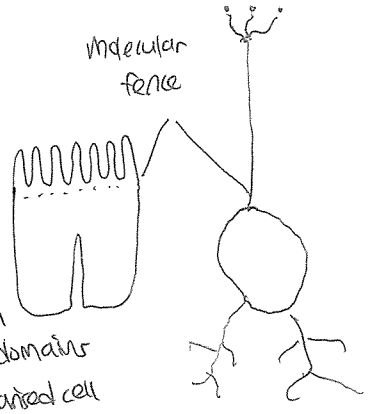
Secretory vesicles wait near PM for signal to fuse

eg: Neurons → synaptic vesicles accumulated near site of release

→ cluster of synaptic vesicles docked at nerve terminal PM of lamprey reticulospinal neuron

Polarised cells

- 2 domains of PM: apical & basolateral
- separated by molecular "fence" (tight junction / axonal hillock)
- diff. domains have distinct lipid and protein content



↓ prevent lateral diffusion between diff. domains of a polarised cell

Sorting of PM proteins in a polarised epithelial cell

a) direct sorting of membrane proteins in TGN
b) indirect sorting via endosomes

Sorting signals guide proteins to apical / basolateral membrane

Signalling from Membranes

Information transfer across the PM – information from outside needs to produce an appropriate response inside the cell

stimulus → detection → relay (signalling intermediates) → response

molecular mechanism of information transfer

Extracellular stimuli

Act via receptors: specialised proteins that detect a specific stimulus

physical stimuli eg: light
↳ specialised proteins convert into chemical form
[vast majority of extracellular stimuli]

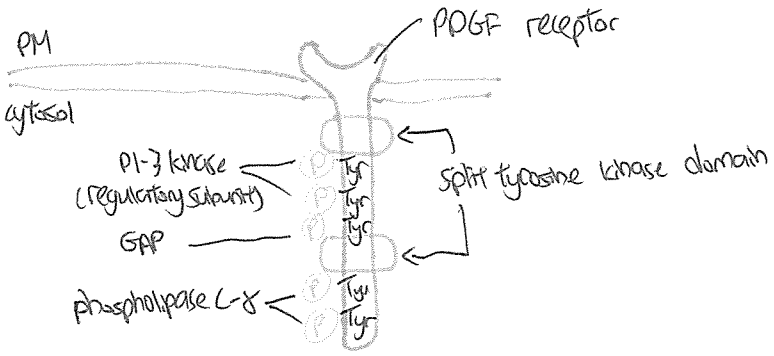
Chemical stimuli: hormones, neurotransmitters etc

- small molecules eg: Ach, larger peptides eg: insulin
- a few can cross PM, most do NOT
- receptors for impermeant stimuli are membrane proteins, stimulatory molecule (ligand) binding site exposed to extracellular environment
- multicellular organisms coordinate the function of diff. tissues via intercellular communication

long range coordination — synaptic signalling
— endocrine signalling

Dynamic Recruitment of Effector Proteins

- enhances signalling efficiency (as all proteins brought to 1 place)
- recruited protein recognises specific phosphotyrosine residues
- multiple effectors: signal bifurcation (one receptor can recruit various signalling molecules)



- phospho-tyrosines are not all equivalent, allowing signal bifurcation

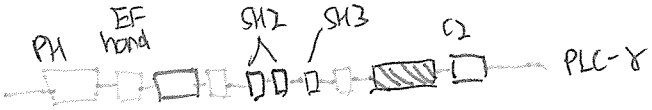
- Recruitment may not amplify signal directly, BUT many recruited proteins are enzymes - activation allows amplification

Phosphotyrosine binding

- signalling proteins interact with specific tyrosine residues only when they have been phosphorylated
- specificity due to amino acids around phosphotyrosine & amino acids sequence of binding domain
- 2 kinds: SH2 domain (Src homology 2) } 2 kinds of phosphotyrosine binding modules
  PTB (phospho-tyrosine-binding)
- protein complex only forms when tyrosine is phosphorylated: inappropriate activation less likely

Interaction domains in signalling

PH = Pleckstrin homology



Lipid binding domains (when Ca^{2+} ↑)

1) C2 - binds phospholipid, calcium-dependent
2) C1 - binds DAG
3) PH - binds inositol phospholipids

Protein-binding domains

1) SH2 binds phosphotyrosine residues
2) SH3 binds proline-rich domains (PxxP)
3) PTB binds phosphotyrosine residues

Other domains:
EF hand binds calcium

* Important for recruitment and activation/regulation of signalling proteins.
complex formation often dynamic: only when appropriate

Inactivation of G-proteins : GTPase activity

- G-proteins reset themselves → allow signalling to occur before deactivation
  - ↳ low but significant GTP hydrolysis (usually several minutes) ; built-in clock
- can be accelerated : by binding to effector protein (downstream of signalling pathway)
  - : by binding to Regulator of G-protein Signalling (RGS) protein, function as a GAP

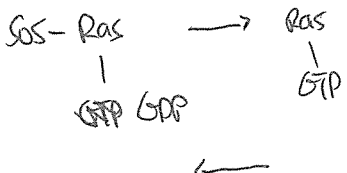
* once GTP hydrolysed to GDP, G_α re-associated with $G_{\beta\gamma}$

Heterotrimeric G proteins and disease : cholera

- caused by Vibrio cholerae
- profuse water diarrhoea : >5oml fluid lost per hour (dehydration)
- major killer in areas w poor sanitation
- cholera toxin : covalently modifies $G_{s\alpha}$, preventing GTP hydrolysis - loss of off switch
- result : cAMP levels 100x basal, CFTR activated, large Cl^- efflux, H_2O into lumen of gut

Pertussis (whooping cough) caused by pertussis toxin covalently modifies $G_{s\alpha}$ so that it's always in GDP bound state, hence unable to regulate its protein

RAS GTPase activity



- monomeric G proteins also inactivated by GTP hydrolysis
- GAP proteins ↑ GTPase activity of Ras
  - ↳ important for inactivation
  - ↓ [GTP-Ras]
  - ↓ t½ : 1-5 hour
  - ↳ Ras system - [illegible]
  - ↳ injected GTP-Ras into oocyte - all GTP hydrolysed in 5min.

- Ras-GAPs recruited by activated RTKs : help to down-regulate signalling
- several forms of Ras ; K-ras is mutated in 30% of human tumours (90% of pancreatic tumours)
- mutations ↓ GTPase activity of Ras - loss of off switch
  - ↳ K-ras is constitutively activated

Ras-GAPs and disease : Neurofibromatosis type I

- multiple neurofibromas (benign growth arising from Schwann cells) develop under skin and in nervous system
- predisposition to cancer
- affects 1 in 3000 people
- caused by mutations in neurofibromin (NF1) : a Ras-GTP GAP - reduced Ras GAP activity
- over-active Ras (and consequently MAPK and PI-3K) signalling

GLUT 1 Deficiency Syndrome / De Vivo disease

- Rare autosomal dominant disorder due to mutations in GLUT 1
- impaired glucose transport across the blood-brain barrier
- microcephaly (small head), mental and motor developmental delays, infantile seizures refractory to anticonvulsants, ataxia, dystonia, spasticity
- seizures start between 1 and 4 months in 90% of cases
- Treatment: a ~~keto~~ ketogenic diet (low carbohydrate)

ataxia: loss of control of bodily movements
dystonia: abnormal muscle tone
~~resulting in~~
↳ paralysis, ↑ tendon reflex, hypertonicity

- Glut 2 facilitates glucose absorption through basolateral membrane in GI tract.
- GLUT 5 (fructose transporter) on the apical membrane
- GLUT 1 ~~does~~ transport glucose

~~GLUT~~ GLUT 4 is regulated by insulin
- In adipocytes and muscle cells, insulin stimulates translocation of GLUT 4 to the PM

Primary Active Transporters
↳ P-type ATPase
↳ Na^+-K^+-ATPase
↳ V type (vacuolar) H^+ ATPase
↳ F-type ATPase (generate energy in mitochondria)

Ion pumps are enzymes
↳ Hydrolysis of ATP provides energy to move ions against energetically unfavourable gradients

Categories
1) P-type ATPase (E1E2-ATPase): Na^+K^+-ATPase, Ca^{2+}-ATPase, H^+K^+-ATPase, metal ion pumps, flippases
2) V-type ATPase: 'vesicular' H^+-ATPase
3) F-type ATPase (F_0F_1-ATPase): Mitochondrial ATP synthase
4) ABC transporters – ATP binding ~~case~~ cassette (ABC) domains
↳ P-glycoprotein, MsbA multi-drug resistant protein

Na^+-K^+-ATPase
- the 'Sodium' pump
- Hydrolysis of ATP provides energy for active transport
- it is a carrier and an enzyme
- maintains gradients for excitability and 2° active transport
- most important pump in animal cells
- only 1° active Na^+ pumps in animal cells

- responsible for inward Na^+ gradient that drives many 2° active processes

Ion channel structures : Basic Profiles

- Ion channels are multi-meric proteins and have a wide number of arrangements and combinations of structures
- K-channels form from a minimum of 4 subunits with each subunit ~~containing~~ → encoded by 4 diff. genes
  contributing to the pore. Auxiliary potens will also bind to the core tetrameric structure
- Voltage-gated Na and Ca channels have a single functioning subunit containing four domains which have functional importance
  ↳ encoded by a single ~~domain~~ gene
  ↳ fusion of the 4 subunits of K+ channels through evolution

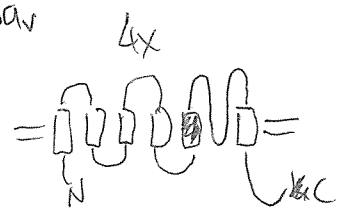
Ion channel structures : K+ channels

- There are 80 genes that encode K-channels subunits
- Several subfamily groups - Voltage-gated K-channels, Kv
  - Voltage- and Ca-gated K-channels, Kca
  - Inward rectifier K-channels, Kir
  - Twin-Pore K-channels

Some calcium-voltage activated K+ channels have an extra TM domain, causing N-termini to be extracellular

The 6TM family : includes Voltage-gated K-channels (Kv)

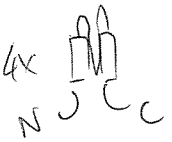
- The 6TM is a basic structural blueprint of 6TM ion channels and Cav & Nav
- TM4 - voltage sensor [detect changes in membrane ~~symmetry~~] causing chan
- the loops between TM5 and [illegible] form part of the "filter" and the "pore" (K+)
- often referred to as the α-subunit



required for tetrameric voltage-gated K+ channel

The 2TM family of K-channels : includes inward rectifier K-channels (Kir)

- there is no voltage sensor
- TM 1 and TM 2 are equivalent to the last 2TM of TM6 family
- the loops between TM 1 and TM 2 forms part of the "filter" & "pore"



required for Kir (inward rectifier)

A novel 2TM family member : ATR-sensitive K-channels

[KCNJ11 + ~~ABCC~~ ABCC8]

an ATP binding cassette protein

[Hetero-octameric complex]

KCNJ11 / ~~ABCC~~ ABCC8 Gene defects

"loss of function" mutation - congenital hyperinsulinism

"gain of function" - neonatal diabetes, predispose to T2DM

NaV : core unit (voltage-gated Na channels) - made up of α-subunit

↳ a single pattern with 4 functional domains = channel

Non-voltage gated Na-channels eg: Epithelial Na channel (ENaC)

NaV: full complex

Heteromer: α, β1, β2

needed for function of NaV

CaV: full complex

Heteropentamer: α1, β, α2-δ, γ

Voltage-gated Ca-channel (CaV)

L-type Ca channels activated by strong depolarisation

T-type Ca channels ..... weak ..

non-... ...

eg: IP_3 receptors, RyR receptors

* if its NOT voltage-gated → totally different structure

Linking structure to Function : Ion selectivity

Example : K+ channels

- K+ channels are highly selective for K+
- 100 - 1000x more selective for K+ over Na+
- How can K+ channel recognises K+ over Na+ when Na+ is smaller?

[0.95Å vs 1.35Å in atomic diameter]

(Na+) (K+)

- Model studies on bacterial K+ channels
- K+ selectivity filter GYG motif

↳ Confirmed via directed mutagenesis

The weaver mouse an inherited channelopathy

- gene defect in KCNJ #7 - a 2TM channel
- GYG → SYG (non-polar → polar amino acid)
- No longer K+ selective, but conducts Na+

Mouse phenotype

- uneven gait, ataxia, seizures
- cerebellar granule cell death
- depleted dopaminergic neurones

} human homologue of Parkinson's disease

Andersen-Tawil syndrome : a human channelopathy

KCNJ 2 defect, a 2TM channel (Kir 2.1) - no longer conducts K+, now conduct Na+

"loss of function" defect in GYG

- ventricular arrhythmias
- prolonged QT interval
- periodic paralysis
- low-set ears
- micrognathia - undersized jaw } craniofacial region
- clinodactyly - bending of digits

Dominant disease

- A dominant disease that result in loss-of-function may arise by:

1) a 50% reduction in the protein content may NOT be sufficient for normal function

2) The mutant protein may inactivate the wild-type protein and reduces its functional activity

↳ the dominant negative effect: a single mutant subunit in within a multimeric channel complex results in defective channel function

only 1/16 chance to produce functional ion-channel with a tetrameric structure

Why study channelopathies?

- obvious medical benefit to individuals
- channelopathies are generally rare disease "natures experiment" and by defining the pathobiology, we can understand the basis of health and more common disorders

Examples: Hyperinsulinism → β-cell membrane potential, diabetes

LQTs → cardiac APs, cause of arrhythmias

LQT-syndrome: disease of ventricular cells

LQT: elongation of QT interval – heart is trying to relax while electrical signal stimulates it to contract

- 13 genes associated w LQTs
- LQTs result from a abnormally long delay between electrical excitation and relaxation of ventricles
- typical QT intervals: healthy adult male < 0.43s

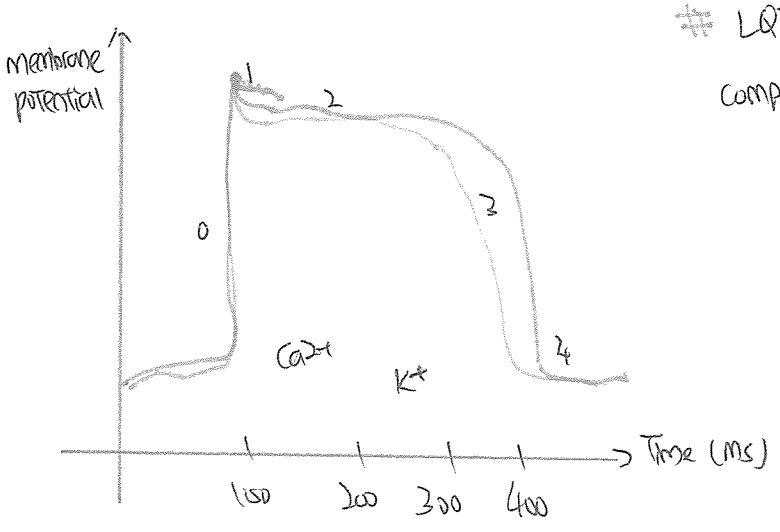
LQT adult male > 0.48s

<5% change between normal

Syncope: fainting

- arrhythmias, syncope on with exercise/excitement, ventricular fibrillation, death
- disease of the young → < 25 years old

prevalence 1 in 5000–10,000



LQT 3 & 10 caused by defects in different components of a Na_v resulting in impaired channel -inactivation (affect phase 0)

LQT3: SCN5A

LQT10: SCN4B

0 - depolarisation

1 - & fast repolarisation

2 - plateau phase

3 - terminal repolarisation

4 - resting

General Properties of Secondary Active Transporters

- can move solute uphill – against an electrochemical gradient
- does NOT consume ATP, but instead harness existing gradients eg: Na^+, K^+, H^+ (driver molecule)
- saturable and defined affinities

Symport
A B

Driver (A) and driven (B) in same direction

antiport / exchangers
A B

Driver (A) and driven (B) in opposite direction

Lactose Permease

- H^+ coupled symporter found in E. coli
- transports lactose by harnessing directed H^+ gradient
- proton-motive force
- proton was used by bacteria as the world before was rather acidic
- 12 TM α helices

Molecular Mechanism of bacterial lactose permease

- lactose & H^+ binding sites are accessible to extracellular space – arginine (R) 144 bonds with glutamic acid (E) 126 leaving E269 free to bind with H^+ lactose binds and conformational change that exposes binding sites to cytosol
- H^+ leaves and lactose binding site is disrupted due to rearrangement of helices. Lactose is unbond and R144 binds to E269

mechanism of transport: protonation-induced transition

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* Insulin was first determined structurally by X-ray crystallography

K_{ATP} (hetero-octamer Kir6.2 and SUR1 subunits)

CCK, GLP-1 → Gq
Ach → Gq
Glucagon → Gαs
β-adrenoreceptor → Gαs
(↑ insulin)

Somatostatin → Gαi
galanin → Gαi
α-adrenoreceptor → Gαi
(↓ insulin)

galanin = neuropeptide released by GI tract

Mutation in insulin receptor
↓
leprechaunism / Donohue syndrome

Some ppl make autoantibodies to IR which ↓ insulin binding or acts as insulin mimetic

Secondary active transport and facilitative transport working on concert

↳ 98% glucose is reabsorbed in proximal convoluted tubule

epithelial solute transport

via SGLT2 in early parts due to ↑ amount of glucose present in the tubule

SGLT1 in later parts due to ↓ amount of glucose present

In diabetic patients, ↑ this mechanism fails to reabsorb all the glucose leading to glycosuria

Glucose Galactose Malabsorption (GGM)

- Rare genetic disorder caused by a defect in glucose & galactose transport across intestinal brush border
- GGM is characterized by neonatal onset of watery and acidic severe diarrhoea, which is fatal within a few weeks unless lactose is removed from diet
- fructose absorption is unaffected
- mutations in SGLT1 gene causes GGM
- 23 GGM missense mutations in the secondary structure of SGLT1.
- MOST mutations result in either truncated SGLT1 protein / mistrafficking of the transporter in the cell

Divalent metal ~~transp~~ transporter aka NRAMP2 / DCT1 DCT: divalent cation transporter NRAMP: natural resistance - associated macrophage protein

- 561 aa ~ 60kDa polypeptide
- predicted to have 12 TM domain
- glycosylated extracellular loop
- N- and C-terminal in cytosol
- IRE in 3'UTR - sensitive to iron