

BI 2213 - CELL BIOLOGY

Membranes are very important

There are about 20,000 protein coding genes in cell humans.

64% - Intracellular

28% - Membrane bound protein

Other proteins are secreted and used elsewhere.

Human body - 10^{14} cells - 100 km^2 total membrane (250 HSERP campus)

Many proteins are related to cell membrane & we can see that the cell itself invests a lot in producing membranes - for the cell and the organelles.

Composition

Membranes are made of mainly lipids, proteins & carbohydrates. The proportion varies based on the type of cell and the type of protein.

Plasma membrane - Protein:lipid = 1

Inner mitochondrial membrane - P:L = 3.2

Myelin - P:L = 0.23

Membranes are formed spontaneously in cells i.e. the lipids produced organize spontaneously.

But there seems to be some organisation w.r.t. the composition based on which kind of membrane is being produced.

The problem with ratio is they're mass/mass - but protein and lipid mass is variable.

Lipid ~ 800 - 1000 Da

Protein - 50 kDa

When converted to molar ratio for 1:1 mass ratio, there are 50 lipid molecules (25 units for every 1 protein, in a bilayer).

This is barely enough to solvate the protein.

Nature of Biomembranes

Enzymes that build membranes are encoded, but the lipid composition of biomembranes is also dependent on the food we consume. i.e. composition can't be deduced using the genome.

Which lipids are digested?
Its decided based on the kind of 'head' - like choline and the body (fatty acid)

Cod liver oil - Polyunsaturated fatty acids
gets incorporated into membrane
through the diet

- Very thin, quasi-2D film of lipids and proteins. cell membrane thickness = ~5nm
- Held together by non-covalent interactions among membrane components. Covers a cell of size 10µm → very thin sheet
- Membranes are fluid & dynamic
- Supported by interactions with the cytoskeleton/anchoring proteins.

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Composition of lipids

- Glycerolipids** - Polar head group + phosphate, Glycerol, Fatty acid(s)
 - ↳ Many kinds (Sphingomyelin) Makes up 65% mol% Back bone
- Sphingolipids** - Polar head + phosphate / Glucose (Glycosphingo lipids), Sphingosine
 - Makes up 10% mol% Cans vary from 5 to 50 mol%
- Sterols** - Eg: cholesterol Makes up 25% cholesterol
 - 4 rings + fatty acid chain. Its head group - OH

1 and 2 are amphipathic - has a polar and non-polar part
When lipids are put in water, they self-assemble to form a membrane - sterols can't do that, they just form insoluble aggregates.

Mitochondrial glycerolipids of bacterial origin
[CL] Cardiolipin - synthesized by covalent bond b/w glycerol and two phospholipid
For 20% of inner mitochondrial membranes

Only found in bacteria & mitochondria

Phosphatidyl glycerol (PG), phosphatidylserine (PE) and P.ethylamine (PE) are other important lipids derived from prokaryotes

- Distribution:
- ER - P.choline (PC), PE
 - Mitochondria - PC, CL, PE
 - Plasma membrane - Cholesterol, PC, PE

These relative ratios of lipids are maintained.
Even though all organelles are connected, the cell manages to maintain these ratios. This is still a research topic.

CL's shape is like a traffic cone - so when stacked together, if doesn't form a plane - it's used to form a curve
PG, PS - -ve lipids PE - +ve lipid (+)

Lecture 3

Book: Life as a matter of fat

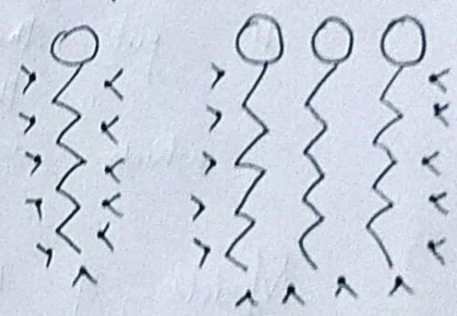
Recall: lipids are amphipathic - helps in self assembly

Formation of biomembrane

The dominant force that holds membrane together is due to the hydrophobic effect.

H₂O (in bulk matter) form hydrogen bonds that are constantly formed & broken → dynamically arranged and stabilized by entropy.

- When lipid molecules disperse in water, non-polar tail interrupts the hydrogen bond & gets surrounded by H₂O molecules
- When the aggregate, the surface area is reduced & water is freed to form hydrogen bonds again.



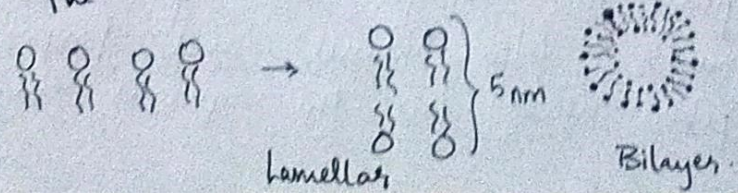
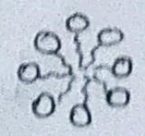
The nature of self-assembly is determined by the structure of the molecule.

This is how membranes are held together in place.
 ↳ Hydrophobic effect.

Micelles vs. Bilayers

- In a fatty acid, the size of polar head and the tail is the same i.e. cylinders
- In lipids, the polar head is much larger than the tails i.e. its like an ice cream cone - which can only self aggregate as a sphere
- Most lipids are cylindrical - the stack as planes and then two layers come together so that it minimises the area that's exposed to water

Micelles



Structure of Lipid Bilayer

Unlike other molecules, they can't be crystallised. So membranes are centrifuged to form multi-layer stacked* membrane and this is the closest we can get to a crystal.

When X-Ray is shined through it, we get some approximations & the size of the membrane can be estimated - about 5 nm, which has been experimentally

validated

↳ Didn't understand that part.

Experiment - Refer slide 13 - BOPC: dioleoylphosphatidyl choline.

These multilamellar stacks are put under X-ray diffractions

The spectrum obtained puts different moieties at a distance from the centre of the monolayer

It puts the phosphate group 15 Å from the centre, so the width of the monolayer is about 3 nm, which is close enough to 5 nm

Models of Biological membranes

01. Overton model (1895)

Suggested that thin membranes surrounding cells have properties of oil - determined it to be composed of lipids and cholesterol (amphipathic molecules)

Permeation of molecules is related to their partition coefficient between water & oil.

02. Langmuir model (1917)

- First model to propose the effect of hydrophobic molecules in the formation of membrane.

- From the composition of phospholipids, suggested that they form bilayers

- Developed Langmuir's apparatus / budget - trough filled with water with a sliding ruler

03. Gorter and Grendel model (1925)

Experimentally verified the bilayer nature of the membrane, using Langmuir's film balance and proposed that the polar heads face the aqueous environment.

* multilamellar stacks
* low-resolution spectra due to thermal motion

F & E (1970) - two different cells with different proteins are forced to fuse - proteins redistribute over the whole surface in 40 mins. (5) fluorescent

Experimentally extracted RBC membrane from human, dog, rabbit, sheep, guinea pig, goat. Spread on a water surface & area was measured in a Langmuir film balance.

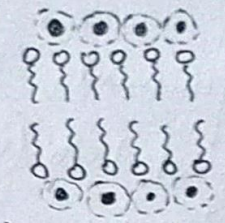


Monolayers with hydrophobic tail sticking out.

The measured surface area of RBCs using a microscope & found that area of monofilm was twice that of the RBCs (within error).

04. Danielli and Dawson (1935)

- The first model to incorporate proteins in the membrane.
- Took into account that membranes had a significant content of proteins adsorbed. Came about due to observation of alternating light & dark bands in myelin sheath.
- Suggested that a protein was tightly associated with the lipid layer.



05. Robertson model (1958)

Made observations in electron microscope. Described membranes of different organelles - mitochondria & nucleus.

Conclusion: Three-layered structure (lipid bilayer b/w ^{adsorbed} protein layers) which is about 7.5nm thick.

06. Singer and Nicholson - Fluid mosaic model (1972)

- Membranes constructed from lipids and proteins - peripheral and integral.
- They incorporated the fluidity of the membrane and the concept of a mosaic - non-uniform composition of lipids & proteins across the membrane.
- This idea came about because certain classes of lipids pack well together and others don't. Fluid ⇒ molecules can diffuse freely laterally.
- They didn't do any expt. just read the literature very well especially the Frye & Edidin (1970)* experiment.

Human - red phospholipase
None - green phospholipase

Singer & Nicholson characterised it as "proteins are like icebergs in a sea of lipids"

They were wrong about this - proteins are densely packed with about 50 lipids to 1 protein.

Frye & Edidin found that the diffusion of proteins was ATP independent but temperature dependent.

Fusion of cells - through virus and chemicals like polyethylene glycol → can induce fusion.
 → one protein - fusagen

Refinements to Singer & Nicholson -

⇒ Israelachvili (1978)

Domains emerge because of lipid-protein interaction
 Recognised the need for membrane proteins to adjust with lipids. Incorporated protein folding, pore formation and thickness variations along with heterogeneity

⇒ Lackmann (1995)

Emphasized the importance of extracellular matrix and cytoskeleton interactions on membrane organisation.

Need for new model

Singer & Nicholson model assumes fluidity which is correlated with dynamics and hence randomness

But lipids & proteins can self-associate and form domains. lipid-lipid & protein interactions, cytoskeletal interaction can cause non-random, heterogeneous distribution

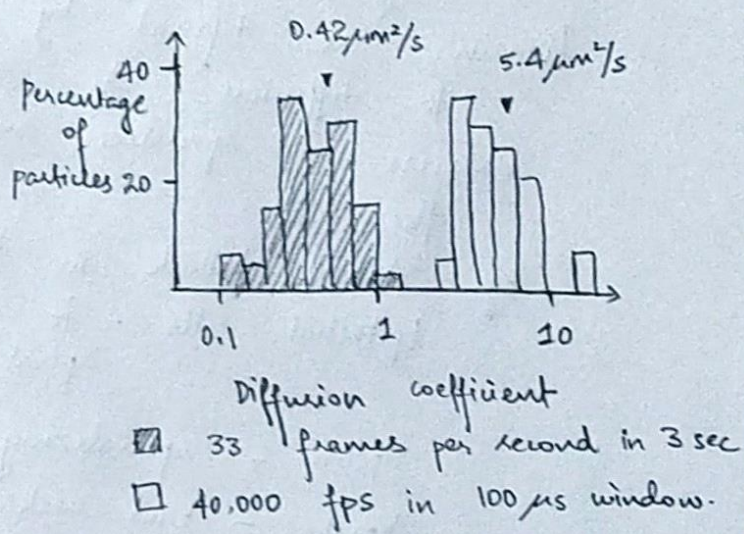
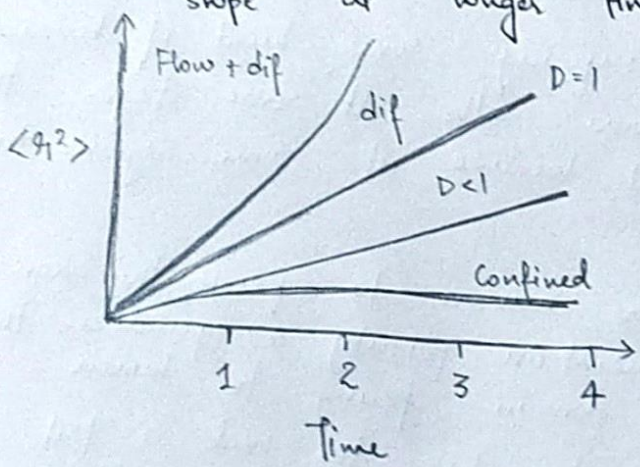
Contemporary model of cell-membrane

Kusumi et. al in their review described domains/cavals and showed how it correlated ^{with} the outlines of the cytoskeleton filaments underlying the membrane.

To quantify their hypothesis, they conducted experiments and analyzed membrane organisation by Single Particle Tracking (SPT) using a fluorescent or gold particle tagged lipid.

Through SPT, the measured the diffusion coefficient,
 $D = \frac{\langle r^2 \rangle}{\text{Time}}$ Means square displacement vs time
 Slope of \uparrow

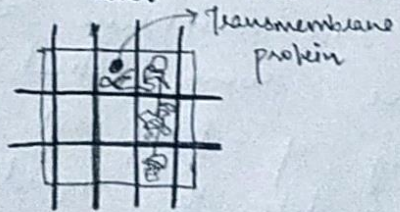
Certain range of values of D corresponds to diffusion and flow ($D > 1$ always), pure diffusion ($D = 1$), diffusing in presence of obstacles ($D < 1$) and confined movement (steady slope at longer time scale).



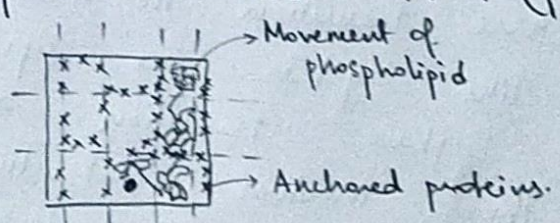
- They found that the avg diffusion coefficient for ≈ 30 fps is an order of magnitude lesser than when analysed at 40k fps.
- If free diffusion was happening all the time, the value of D should have stayed around 1.

Picket-fence model

They proposed that mosaics are formed in the membrane which are bounded/corralled by membrane cytoskeleton (fence) and anchored proteins associated with it (picket).



Bottom view (inside the cell)



Top view

As we can see its easy for the lipid to diffuse inside this corral/domain, but it is confined by the 'picket-fence', hence its diffusion coefficient decreases when analysed at longer time scale (3s)

- Phospholipids undergo hop-diffusion within 230 nm confined regions in cell membranes.
- So, long range diffusion is a reflection of diffusion within the confined regions & their tendency to hop across these regions.
- This explains why lipid diffusion is 10-fold slower in cell membranes than in model membranes.

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Lecture 5

This picket-fence model helps in facilitating or deterring signal transduction.

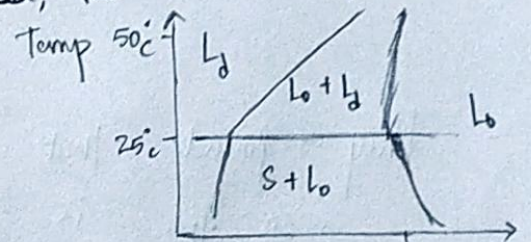
Domains are defined by imposing some kind of barriers to diffusion. In some cells, at some stages, these barriers specialize and localize at some regions in the cell.

Eg. Axonal hillock in the neuron - to separate the axon & dendrites

Epithelial cells - to maintain polarity & specialize the apical domain facing the lumen

Sperm - specializing the head, the neck & tail

Yeast - bud neck.



Membrane domains

Attributed to -

- apparent affinity b/w lipids & proteins
- segregation of lipids & proteins together because of matching physical properties
- segregation of lipids and proteins away because of mis-matching properties.

Phase transition in membranes

Like crystals, the lipids in membrane also undergo phase transition. above some critical temperature.

This involves restructuring of fatty acyl chains from solid ordered state to liquid disordered

Solid/liquid diffusion. Ordered/disordered refers to internal degree of freedom or rotational diffusion

Eg: DPPC - critical temperature: 41°C.

Cholesterol also adds integrity to membrane - decreases diffusion across the membrane (9)

Effect of cholesterol - Buffering function

- Rigid & planar steroid ring in cholesterol confers properties intermediate to ordered & fluid fatty acid chains in phospholipids
- Cholesterol exerts opposing effects depending on nature of host membrane -
 - In fluid membrane (above T_m), it orders disordered fatty acid chain (by asserting its planar structure)
 - In ordered membrane (below T_m) it induces disorder in ordered fatty acid chains
- High amounts of cholesterol homogenizes the physical properties of membranes to create a new phase called liquid-ordered phase.
- This is formed above 30 mol% of cholesterol. Its characterised by high translational mobility but low rotational mobility of host lipids
- This is ideal for supporting membrane functions.

Lipid rafts

- Nanoscale heterogeneities currently believed to be 20-80 nm wide (this is highly debated)
- Causative factors -
 - phase coexistence: liquid ordered domains in a fluid bulk membrane
 - Preferential interaction between cholesterol and lipids containing saturated fatty acid chains
 - Domains are thicker than other regions of membrane and distinct set of proteins partition into these domains.

Lipid rafts - discrete lipid domains present on the plasma membrane - enriched in cholesterol, glycosphingolipids, glycosyl-phosphatidyl-inositol & GPI anchored proteins. insoluble in low concentrations of non-ionic detergents

(?) Related to signal transduction. How?

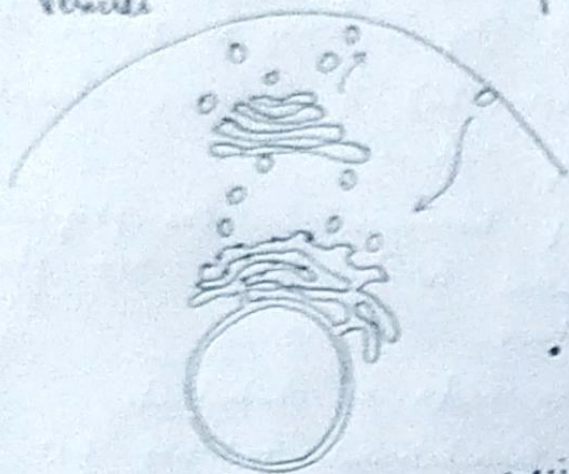
Endomembrane System.

It's comprised of membrane-bound compartments of cytoplasm - ER, Golgi body and vesicles.

It's one of the main differences b/w eukaryotes and prokaryotes.

Golgi - flattened stacks of membranes

Vesicles - smaller components that could be secretory.



The structure, abundance and ^{position} composition (?) is very dynamic and changes with the age, function and environment.

The proteins are produced in ER, transported to Golgi through vesicles where they are processed and

again vesicles bud out of golgi and reach the plasma membrane through microtubule assisted motor traffic.

- This vectorial nature of transport is because of organisation of microtubules - Anterograde pathway of vesicular transport
- If vesicles are just sent out, there will be imbalance. To balance this, a compensatory mechanism that returns the traffic is through endocytic pathway - many vesicles are formed from plasma membrane and they give rise to endolysosomal system. At steady state, half of plasma membrane gets turned over through this system in about 20 mins or so.

Endoplasmic Reticulum

- It is found in cytoplasm and has a network-like structure
- It is continuous with the outer nuclear membrane - its dense near the nucleus and on the periphery, its more homogenous and has tubular structure
- Two kinds - Rough ER - more like flattened stacks with bound ribosomes
Smooth ER - tubular, lack bound ribosomes.

Rough ER

Found near the nucleus
 Membrane is secreted protein synthesis
 Lipid synthesis
 N-linked glycosylation (lumen)
 - post-translational modification
 Exocrine cells have large fraction of rough ER

Smooth ER

Found at the periphery
 Steroid hormone secreting cells, hepatocytes that detoxify hydrophobic molecules.

The ratio b/w rough and smooth ER seems to be developmentally regulated

Specialisation of ER

SER extensively developed in kidney tubules, gonads, skeletal muscle & endocrine glands.

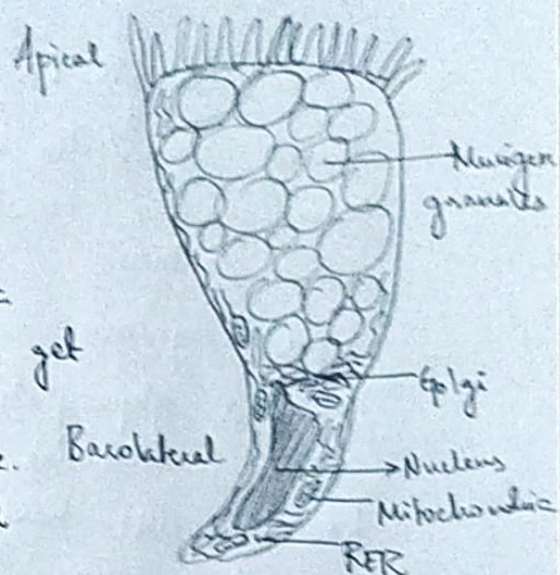
- Synthesis of steroid hormones → in each cell.
- Detoxification of organic compounds (barbiturates and ethanol)
 Converts hydrophobic to hydrophilic so they're more easily excreted. This function is because SER compartmentalises the cell and manages compounds that way.
- Sequesters Ca^{2+} in skeletal & cardiac muscle cells. Regulated release of Ca^{2+} from 'sarcoplasmic reticulum' triggers contraction of muscle.

* Lipid droplets - emerge from SER - like a droplet of oil with a small amphipathic boundary.
 So strong hydrophobic dyes are used to distinguish SER.

? Translocon apparatus is used to distinguish RER.
 Protein channel that shoves the nascent polypeptide into lumen of ER
 ER membrane is connected to nucleus & the lumen is open to the extracellular space - so the lumen & extracellular environment is oxidising and the intracellular environment is actually reducing.

Polarity of ER

Epithelial cell of intestine - polar cell. So the ER and other organelles are also polarised. They remain in position because of microtubules and other cytoskeletal elements that constrain them.



The structure of ER is very dynamic. During replication, the ER seems to get torn apart into two and later they grow based on this template. The nuclear membrane gets dissolved into smaller vesicles.

The tubes are connected to form 3-way junction.

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Lecture 7

ER Organisation during Cell Division

TP

The ER is marked in green and chromosomes in red. They tracked the structure through cell cycle.

During telophase and cytokinesis the ER is roughly severed into two ^{equal} networks, without forming vesicles.

During cell division, the inner nuclear membrane dissolves and merges back into the ER network.

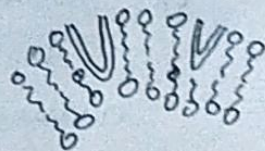
How is the ER made?

A bilayer generally forms a planar structure, so a lot of energy has to be invested to form membrane tubes.

- One theory proposes that ER tube is formed by pulling a vesicle along a microtubule through motor protein. But cytoskeletal structures are absent during cell division. So this doesn't hold up well.

- Another theory: certain integral proteins (Reticulons) are enriched in the ER. They are small with 2 α -helices, forming a hairpin-like structure.

They are embedded only in the outer membrane (outer leaflet).



This expands the area of the outer leaflet, thus stabilising the tube-like structure.

These proteins stay on the outer membrane because its energy-expensive for it to flip.

3-way junctions

They are formed and resolved continuously. This membrane fusion is facilitated by proteins. They do it by bringing together the membranes very close so that they fuse (Atlastins)

This fusion seems to be stochastic, only somewhat directional because of cytoskeletal assistance.

The sprouting of tube is directional but the fusion is random. But as ER is dense it'll end up hitting another tube and fusing. Process requires GTP hydrolysis.

→ - The model was tested in recent experiments. The team took artificial liposomes with Reticulons and Atlastins.

- Some fluorescent dye was added to see the liposomes. In absence of GTP, they remain as specks but if the presence of GTP, a network is formed very similar (organisation & architecture) to ER in cells.

- This was an important leap (by reconstituting the membrane) in corroborating the model.

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Lecture 08

The tube like network of ER -

- occupies 60% of membrane by area i.e surface available is increased
- helps transport substances i.e proteins.

Most integral proteins are produced in ER and embedded in the membrane.

Types of integral proteins

- * COTRANSLATIONAL
- Type I - A single transmembrane domain of protein with the N-terminal facing outside
 - Type II - Same as I, only C-terminal faces outside
 - Polytopic - multiple transmembrane domains. Most integral proteins are of this type. Major type in this are GPCRs - famous eg: Rhodopsin.

These proteins are produced, transported through ER and get inserted into the membrane - the conformation is very important here.

- * Post-translational proteins - produced in cytosol as soluble membrane, modified after translation and then inserted into membrane. They make up 10% stretches of

These proteins have a special property - 4-7 hydrophobic aminoacids so they can stay comfortably in the membrane.

Integral Membrane Protein (IMP) Biogenesis

- IMPs make up to 20-30% of the proteome
- Very diverse - channel proteins, signalling receptors, mediate intracellular trafficking, facilitate organelle biogenesis, and compose adhesion and gap junctions.
- Range from 1-20 transmembrane domains (TMD)
- IMPs are assembled in ER, integrated into membrane (stitched), final topology is determined and 3°-4° structures are formed. Finally, it is sorted to its ultimate location of function.
- If anything goes wrong, it is degraded in one of several quality-control pathways.

Secretory protein - insulin / hormones

IM protein - Receptors, channels

Soluble proteins - enzymes (kinases, polymerases)

- IMPs destined to be inserted into ER face biophysical challenges
- The hydrophobic TMDs need to be shielded from the cytosol ($\sim 300 \text{ mg/ml}$ protein), because otherwise it would promote potentially toxic aggregates in the cell.
- TMDs need to be -
 - recognised as they emerge from ribosome
 - put into the core of the lipid bilayer after getting past the polar surface
 - oriented properly with receptor on the outside
 - IMPs need to be targeted to the appropriate organelle

⇒ Secretory Protein Biogenesis

We can understand more about the process by looking at synthesis and processing mechanism of secretory proteins -

- They have a 8-amino acid long hydrophobic chain at the N-terminus called ER signal sequence
- It emerges from soluble pool of ribosomes
- It directs the ribosome to dock at the translocator
- It directs the protein in ER membrane
- Then the rest of translation and translocation (through the ER membrane) occurs simultaneously
- The signal sequence is cleaved off (kept in the membrane) and the nascent protein is put into the ER lumen for further processing.

⇒ Co-translational protein synthesis

- here too, the detection of ER signal protein sequence halts the translation. due to binding of SRP
- Signal Recognition Particle (SRP) - a small, soluble protein binds the nascent signal sequence and the ribosome

- SRP receptors on the ER binds the SRP-Ribosome complex and directs it to a translocon
- SRP receptors and SRP are released and recycled
- Translocon transfers the protein across the double layer and the translation continues.

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Lecture 09

Translocon apparatus helps transfer protein from cytosol to ER lumen in case of secretory protein.

But after co-translational protein is docked at the ER, if the translocon encounters an extremely hydrophobic segment (stop-transfer segment), it releases the segment into the lipid bilayer i.e. it partitions into the membrane.

Then the rest of the protein is translated

But the mechanism of this is not well known - how it changes the configuration of it so that the N-terminal stays out.

* The TMD of protein is hydrophobic, so when the nascent polypeptide is inserted, because of the hydrophobic environment, the structure folds by itself and forms secondary structures. No chaperone proteins required to fold.

Translocon structures (2009) it - organizes spontaneously. It might not be wide enough for two polypeptide chains. The plug is not another polypeptide - its just another part.

Translocon is actually a protein conducting channel - needs to be plugged, lest the ions leak out

* Structure solved through cryo-electron microscopy.

* because of their tendency to flip, the area of ER keeps growing even if lipids are inserted only into the upper leaflet. (17)

Lecture 10 Membrane biogenesis

PS on the extracellular side acts as a signal to initiate apoptosis because its not supposed to be there.

- Membranes are formed from pre-existing membranes. They grow as newly synthesized proteins and lipids are inserted into existing membranes. Only growth and expansion.
- Membranes move from ER to other compartments of cell, and as they move, its lipids and proteins are modified to give a unique composition.
- ER forms vesicles with necessary proteins which fuse with the Golgi. The resident enzymes there modify it and vesicles again pinch off and fuse with plasma membrane.
- Membranes are ① asymmetric - two phospholipid layers have different composition among themselves and between different organelles. This arises in ER.
- This asymmetry is maintained through retention signals i.e. when a vesicle fuses with Golgi, some proteins are retained while others are passed on.
- Phosphatidyl serine - enriched in the ER because its important for protein picket fence maintenance. Moreover, its present only on the inner leaflet - connected to cytoskeletal element.
- Lipids keep flipping* in the membrane, but the cell still maintains the asymmetry. so PS wont be a part of endosome.
- There's also some ② asymmetry wrt to proteins expressed on the membrane - their orientation (type I, II), composition and position.

- ③ Asymmetry wrt domains situated in ER at the -
 - Cytosolic surface: remains on cytosolic surface of vesicles, Golgi and internal surface of plasma membrane
 - Luminal surface: maintain orientation in Golgi & vesicles, but are found at external/exoplasmic surface of plasma membrane.

- Sphingolipids, glycolipids - exceptions - synthesis starts in ER but completed in Golgi
- Mitochondrial and chloroplast membranes are synthesized by enzymes that reside in those membranes.
- Enzymes involved in lipid biosynthesis are an integral proteins of ER, with their active side facing towards cytosol. \Rightarrow lipid molecules are inserted on the upper leaflet of the ER membrane.
- Those with a small polar head keep flipping on their own. But some domains are reversed to the opposite leaflet through the action of 'flippases'
- Lipids are carried from ER \rightarrow Golgi \rightarrow PM as part of bilayer that makes up walls of transport vesicles. \rightarrow 'Vesicular Transport Pathway'

Enriching certain lipids in particular organelles.

- ▶ Organelles have enzymes that convert lipids already present in a membrane to a type of phospholipid.
 - * PSerine is synthesized by modifying PEthanolamine
- ▶ Some types of lipids are preferentially included in the membranes of transport vehicles
- ▶ Some proteins bind and transport individual (!) lipids through the cytosol from one membrane to another.
 - soluble proteins: lipid transfer proteins.
 - They transfer lipid molecules from ER to other organelle membrane, individually. While transporting, the lipid molecule is shielded

The membrane/organelles are also asymmetrical in the kind of ~~enz~~ proteins enriched. The proteins formed in the ER are transported through vesicles.

But the proteins are retained in the ER/Golgi through specific signal sequences called Retention signals. - concentrate the proteins in certain kind of organelle

Lecture 17

Nucleus

High resolution image of nucleus is undistinguished.
 Differentiable structures - nuclear envelope & nucleolus.
 But it's actually quite organised

The nucleus is clearly separated. This results in decoupling of transcription and translation i.e. more regulation. Upside: lot of things have to be transported across nuclear membrane

Nuclear membrane

- Defines the nuclear compartment
- The outer membrane is continuous with the ER
- Two membranes separated by 10-50 nm
- Inner nuclear membrane has specific integral proteins
 - ↳ Outer one is more like ER
 - ↳ binding sites for chromatin and nuclear lamina (holds the nuclear envelope in place; analogous to cytoskeleton)
 - ↳ stabilize PM
- The membrane is penetrated by nuclear pores
- The whole envelope disassembles during cell division.

Nuclear pore complex

Formed by a set of integral membrane proteins and soluble proteins. It allows for selective transport of macromolecules.

Propose vs. Invention model

The models of its formation and insertion in the membrane are contentious. NPC is quite complex and particular and needs to be reassembled / preserved through cell division when nuclear membrane is dissolved.

What molecules enter the nucleus are determined by the diameter of NPC - ~60 kDa (*Ribosomes squeezed in)

- 50
- Newly synthesized IMM-destined integral membrane are co-translationally inserted into ER and distributed to INM and ONM by diffusion
 - NPCs prevent free diffusion & pairing of membranes with proteins with domains $> 60 kDa$.
Smaller proteins / particles are free to diffuse about
 - This dictates the size of INM-bound proteins that bind efficiently to
 - However only nuclear components will become enriched in nuclear interior.
 - Consistent with a diffusion-retention-based process, the INM-bound proteins don't depend on active transport guided by a consensus sorting signal.

Images - Slide 44

Movement of materials through NPC

- Expt: Gold was injected into cytosol and observed at regular intervals. Observed that gold particle pass through NPC in a line to cross into nucleus.
- The NPC is not an open tunnel - there is some electron dense structure in the middle

Nuclear Pore Complex

- huge, supramolecular structure (15-30x ribosome).
- Exhibits octagonal symmetry
- can rearrange to change diameter of opening from 20 nm to 40 nm.
- Contains 30 different proteins - nucleoporins - that are conserved from yeast to vertebrate.
- Inner lining contains stretches of phenylalanine and Glycine (FG) repeats - forming FG domain.
- They're both sufficiently hydrophobic. Has a disordered structure that gives them an extended and flexible organisation.

Pro - Gly : Two side that can't be replaced by any other amino acids. FG domains get organized hierarchically (34)

- * Green fluorescent protein - 3000 in size
- * The FG domains form a hydrophobic meshwork or sieve that blocks the diffusion of large macromolecules (> 40 kDa)
- * Density of NPC correlates with nuclear activity - typically, 3000 - 4000 complexes.

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Lecture 12

Flexible FG nucleoporus as entropic barriers to nucleocytoplasmic transport.

- * Biophysical nature & mechanical response of individual and cluster of FG molecules was studied using an atomic force microscope (resolution of orders of nm)
- * # AFM - has a cantilever and tip made of material with high spring constant so it can respond to minute forces.

The tip moves over the sample and the back of the tip reflects a laser so that the position of tip can be measured to an accuracy of atomic length.

This tip + cantilever can be used to get an idea of the nature of material by indenting on it.

- * In the paper, they dragged the tip over FG repeats and measured the drag.

They found that the FG repeats don't form a tight domain, rather the chains arrange loosely so they form a brush-like structure.

- * \Rightarrow Molecules face resistance going through the NPC, but they're not totally hindered.

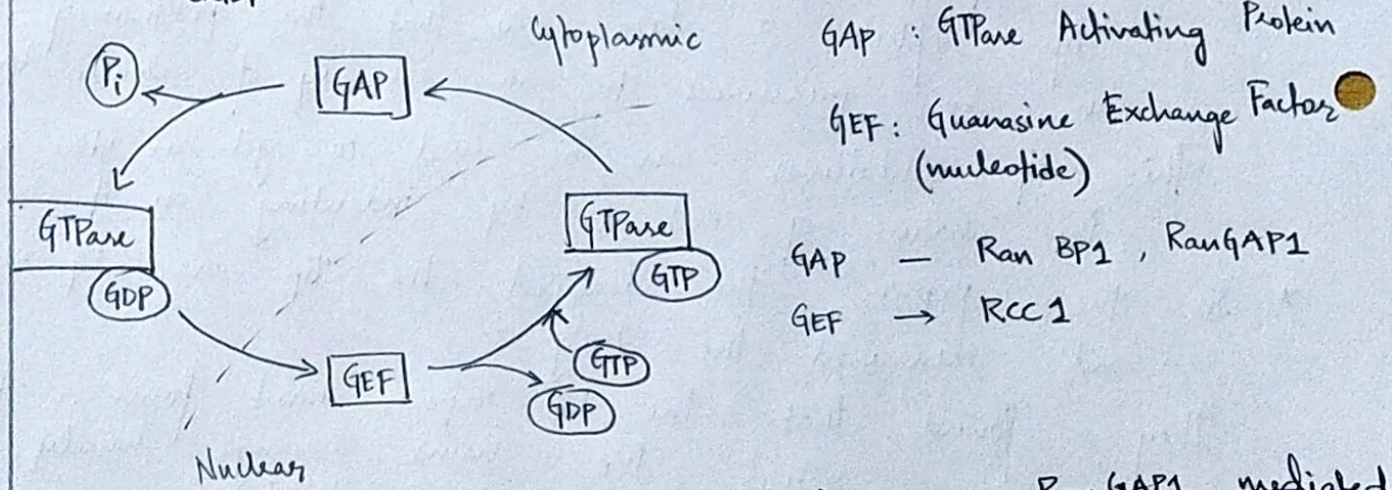
- * When put in less polar condition, they undergo a reversible collapse transition. So FG domains act as entropic barriers and a selective trap.

52) Good analogy: FG domain \equiv Kelp forests

like signal sequence \leftarrow Nuclear localisation Signals (NLS)
 They're short sequences of positively charged (lys/arg) amino acids which when attached on to proteins gives it the pass through nuclear membrane
 can be located anywhere in a protein (loops/surface)
 like SRP \equiv There are some bona fide sequences that can be attached to any protein to localize it in nucleus.

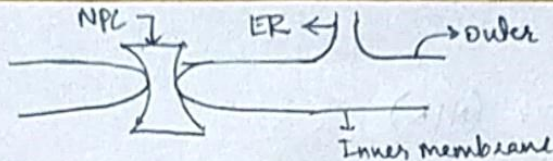
- \leftarrow Nuclear import and export receptor
- Nuclear import receptors bind NLS and nucleoporins
 - Soluble cytosolic proteins
 - FG repeats on nucleoporins bind receptors on cytosolic side
 - Nuclear export receptors rely on those signals that bind to it.
 - Nuclear import and export receptors are structurally related

The gradient of transport depends on the conc. of proteins called Ran
 Its biochemistry is similar to that of GTPase - it can exist in GTP or GDP bound state:



GAP: GTPase Activating Protein
 GEF: Guanine Exchange Factor (nucleotide)
 GAP - RanBP1, RanGAP1
 GEF \rightarrow RCC1

RanBP1 binds to Ran-GTP and increases RanGAP1 mediated nucleotide hydrolysis. Conversely, RCC1 (GEF) increases the conc. of Ran-GTP
 RanBP1, RanGAP1 - Cytosolic
 RCC1 - chromatin associated nuclear protein



This asymmetric exchange distribution of GAP and GEF across the nuclear envelope predicts that RanGTP - nuclear and RanGDP - cytosolic. This distribution plays a key role in determining the directionality of nuclear transport.

Evolutionarily, the role of GTP as a signalling molecule and that of ATP as an energy molecule has diverged

9/3

Lecture 13

Using Nuclear import and export receptors is limiting - all of them may get engaged. So another method of transport has to be used which depends on gradient of G protein - called Ran - across the nuclear membrane. RanGTP and RanGDP.


This binding of GTP/GDP, causes distinct conformation changes - so you can get, essentially, two proteins from one gene.



Generally, the rate of hydrolysis is very low. So, only when bound to GAP, does RanGTP hydrolyse to RanGDP.


The relative abundance of GAP and GEF is different inside the nucleus and cytoplasm.

Ran GEF : Nuclear
 RanGTP is r. small and can travel through the nuclear envelope freely. But RanGTP is concentrated in nucleus and a gradient of RanGTP is established in the cell.
 RanGAP : Cytosolic.

Import

Nuclear transport receptors (NTR) 

Ran GTP / GDP  / 

Cargo 

1. Complex of NTR and NLS bearing cargo is formed in cytoplasm and NTR also binds to FG repeats
2. After translocation across NPC, RanGTP displaces the cargo from NTR and binds to it. This occurs because chromatin associated RanGEF keeps increasing the conc. of RanGTP inside the nucleus.
3. The NTR - RanGTP complex goes to cytoplasm through NPC and there, RanGAP2 stimulates GTP hydrolysis releasing NTR to bind with another cargo.

This makes it possible for cell to enrich a certain protein inside the nucleus by siding this gradient.

Note: NTR - general notation. Import and export receptors are different.

Export

1. Formation of trimeric complex of cargo - NTR - RanGTP in nucleus.
2. After NPC passage, this complex dissociates due to RanGTP hydrolysis, releasing the cargo, RanGDP and NTR (export) into the cytoplasm.
3. The export NTR diffuses back to the nucleus. n/s

Lecture 14

Mitochondria and Chloroplast

Endosymbiotic theory - some bacteria fused with others.

M - 2 membranes, 2 compartments - matrix, intermembrane space

C - 3 membranes, 3 compartments - thylakoid space, stroma (matrix), intermembrane

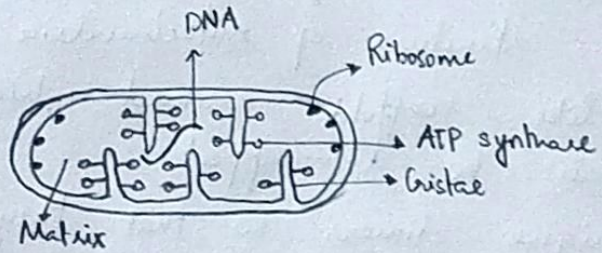
How do such organelles emerge in cells?

- Mitochondria arose ~ 2 bya from engulfment of α -prokaryote and Asgard archaea
- Slowly, M lost its autonomous functions but maintained core functions of ATP production.
- The host evolved genes to produce the proteins in mitochondrial proteome (Nucleus - 5000+, M - 10-100 proteins)
- No protein produced by M helps in cellular function
- In the 5000 genes -
 - ~ 1000 - mitochondria
 - ~ 1000 - ER
 - ~ 3000 - cytoplasm

⇒ M is not autonomous, its dependent on nucleus.

- But M has its own ribosome and other enzymes in the matrix. Other proteins are imported from cytoplasm & sorted into compartments by outer and inner translocase machines.

- ATP production happens in Matrix which occurs means most proteins are imported there



- ATP production occurs through TCA Cycle - which breaks down nutrients to produce ATP by creating a proton motive force across the inner membrane

- 2 things happened over evolution -
 - The nucleus controls when the M divide and fission, through proteins (family: dynamin - Opa (I) & mitofusin (0)) present on the outer and inner membrane.
 - So the mitochondria can exist as single units or as a connected network of mitochondria.
 - Fission - controlled by Drp1 protein which binds around the mitochondria and cleaves it.

The mitochondria are also loosely organised along the cytoskeletal structure, especially the microtubule network.

Interphase - Network like structure

Cell division - occurs as single entities so they're separated proportionately. The activity of cell cycle Drp1 is cell cycle dependent i.e. it gets de-phosphorylated in interphase.

The long tubular structure is less efficient at producing ATP. So in cells which have greater energy need, the mitochondria exist as single entities.

15/3

Lecture 15

How is the shape of the mitochondria determined?
The matrix contains mtDNA and transcription enzymes required for mitochondria.


The enzymes required for oxidative phosphorylation are associated with the cristae - folds of the inner membrane. The shape translates to function.

⇒ Paper - FAM92A1 is a BAR domain protein required for mitochondrial ultrastructure & function

⇒ Fam determines the structure of mitochondria
BAR domain proteins - like scaffold around which a membrane can be wrapped.

- They're present as unique dimers so that they're concave shaped - it induces curvature in the membrane attached to it.

- The nature and extent of curvature varies - from deep concave to mild convex.

- BAR proteins are soluble proteins. For eg. BAR Asfaptin 
- binds on the golgi and causes it to bud

- FAM is localised in the mitochondria. It was identified using a bioinformatics approach.
Is it in the matrix or intermembrane space.

- To figure this out, proteinase K is added to —
 outer membrane (+)
 after swelling i.e. intermembrane space (+) [osmotic shock]
 after lysing both membranes (-) → matrix.

This way it was ascertained that the protein was localised in the matrix.

- Antibody against FAM — anti-FAM binds to FAM. When section of mitochondria is exposed to it and observed under microscope. We can see that FAM is bound to the cristae on two matrix side.

16/3

Lecture 16

Mitochondria is generally very visible because it has Fe-S complexes that make it very electron dense.

FAM is coded in the nucleus, synthesized in cytosol and then inserted into mitochondria. The pathway is conceptually similar to how proteins are inserted in ER lumen.

- Mitochondrial precursor proteins have a signal sequence (SS) at their N-terminal. SS is necessary & sufficient for import of proteins.

- Its transplantable sequence. Its quickly degraded once its inside the mitochondria. Also translocation into mitochondria is post.

- The SS is α -helical, amphipatric — half of signal is charged (+ve) residues and other half is hydrophobic.

- SS is recognised by translocins on mitochondrial membrane — TOM complex (outer membrane) and TIM complex (inner membrane) that is associated with TOM.

If the protein needs to go into the matrix, it gets translocated through TOM + TIM. If it needs to remain in intermembrane, it comes through a 'free' TOM and remains in the space.

FAM comes with its own mitochondrial target signal sequence. If it removed, its not localised inside the mitochondria.

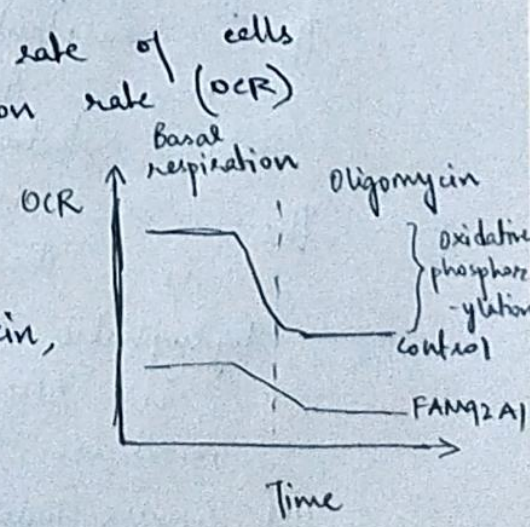
Also attaching the sequence to Apex protein localises it in the matrix. This establishes that FAM is definitely in the matrix.

→ Then they removed FAM 92 using siRNA technology. Over 72 hours, it was almost removed. Then they checked the performance of mitochondria when given glucose (lesser performance) and galactose (significantly lesser). This measured as growth rate over 5 days.

Glucose - just glycolysis
Galactose - oxidative phosphorylation is required. If its hampered, then in this medium, growth rate actually falls.

≠ siRNA control - another siRNA that doesn't affect mitochondrial function to make sure that the performance isn't going down because of siRNA binding.

→ They also measured the respiration rate of cells by measuring oxygen consumption rate (OCR). To make sure that the difference is because of mitochondria only, normal rate was compared to oligomycin, (binds to ATPase and block oxidative phosphorylation)



→ Comparing immunofluorescence images of control and siRNA samples, we can see that the mitochondria in control exists as long tubular structures. But in siRNA, the mitochondria are small, rounded and vesicle-like. EM microscopy images also show that siRNA mitochondria are also very simple empty - less cristae

→ If Is FAM92A1 sufficient to form cristae? They added the protein to liposomes with PC: PE and PC: PE: Cardiolipin. The full length protein ~~bound~~ bound well to the membranes and induced positive curvature and tubule like structures. This shows that FAM is also sufficient to cause / form cristae structures in membranes.

PART 2

30/3
4/04

Lecture of Cellular microenvironment - local environment around cell that contains signals (phy/chem) that influence cellular behaviour (individual or a group of cells).

There are some disadvantages to studying cells in isolation

Cellular microenvironment could be the matrix, something matrix has captured or other cells.

All components of cell work in tandem to keep the cell functional. They respond to biochemical & biophysical cues in the microenv. to possibly create a structure very different from the single cells themselves.

Microenv - ECM Soluble factors
Neighboring cells Physical fields

- There is a distinct 3D architecture to the microenv that influences how the cells think & function

- This 3D space can also recruit growth factors & integrin binding sites, thus creating a biochemical aspect to the microenv. along with physical aspect.

- Together, they can create local concentrations and gradients by influencing how things move.

- There is also a definite physical aspect - how cells are shaped and arranged in and shear and facilitate to minimise an example of that

endothelium blood flow is an example of that
When exposed to laminar, shear flow, the endothelial cells align themselves along the direction of the flow and the flow can drastically influence cells to have different shape, secreted factors etc based on flow connected/neighboring cells.

Many factors affect (or get affected) the cell -

- ⇒ 3-dimensionality
- Cell-matrix adhesions
- Chemical/physical matrix characteristics
- Autoregulation (niche formation)
- Growth factors, cytokines
- Cell-cell junction & communication
- Nutritional status
- Mechanical stresses.

Extracellular Matrix (ECM)

- It's made by cells - they secrete large molecules into this space and organize them in various ways. These macromolecules is ECM.
- The meshwork formed by these
- Tissues vary in their ECM: quality (composition), quantity (size)

TP: Arrangement of collagen in different kinds of tissues

- ECM is not inert. It influences all aspects of cell migration, proliferation, shape, function, survival, development, behavior.
- ECM can also as -

Functions

1. Anchorage
2. Migration barriers/tracks
3. Signal reservoir (signals can be trapped in ECM with varying efficiency - think growth factors)
4. Low affinity coreceptor (aligning signal molecule [ligand])
5. Functional fragments (ECM fragments also affects cells)
6. Biomechanical force - in resisting it?

Composition

Mainly two kinds of macromolecules -

Exception: hyaluronic acid

1. Proteoglycan (PGs) - Perlecan
 - PG = Glycosaminoglycan (GAG) + Specific protein core
 - PGs have been classified based on core protein, localisation & GAG composition -
 - Small leucine-rich PGs (SLRP)
Involved in signaling pathway & activation of factors
 - Modular PGs
Modulate cell adhesion, migration, proliferation
 - Cell-surface PGs
Act as co-receptors facilitating ligand encounters
 - These molecules are extremely hydrophilic
⇒ adopt extended conformations - hydrogel formation
enables the matrix to withstand high compressive forces.

2. Fibrous proteins - Collagen, Elastin
 - Collagen - most abundant: 30% of protein mass of organism
 - ↳ 28 different types
 - Strongly associated with elastin
 - Synthesis of collagen involves a lot of enzymatic, post-translational modifications which involves strengthening the fibrils through crosslinking
 - covalent cross-linking b/w lysine residues of constituent collagen by lysyl oxidases (LOX)

Lecture 02

Structure of collagen - GN Ramachandran
 Triple helical model - 1954
 Ramachandran plot - 1963

Fibronectin (FN)
 Component of ECM involved in mediating cell-attachment
 and function.

FN can be stretched several times over its resting length
 FN is secreted as a dimer.

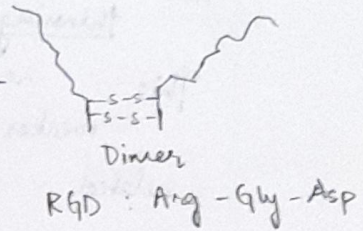
MMPs - membrane metallo proteins matrix metalloproteases
Important for homeostasis \rightarrow for degrading the matrix (33)
tightly & complexly controlled

FN has receptor binding sites to other FN dimers,
collagen, heparin and cell-surface integrin receptors.

Cell surface binding of FN is essential for its
assembly into fibrils. FN has multiple binding sites for
integrins for

* Cell architecture influences FN-fibril assembly thus
influences architecture of ECM

Fibronectin have an RGD peptide
sequence that's considered an important
factor in identifying FN allowing integrins



TP Stain of fibronectin matrix ~ 22:10

Collagen - Triple helix - left handed turns
These could be many post-translational mechanisms

The features / aspects of cells change significantly when
grown in a 3D ECM, as compared
to 2D petri dishes

There's a way to evaluate the matrix of any
tissue after taking out the cells.

There are processes to study the matrixome (composition)
and other features and see how they
alter in diseased conditions so much diversity? slide 15

What's the point of

Interactions between ECM components and cell is imp.
Paper describes how ECM changes at various
stages of cell (wounded, aged etc)

1. Normal - Type I, III Collagen + Elastin, FN, PGs
Fibroblast metallo-proteinases (secreted by some cells) go &
chew up the ECM; & it's hindered by its
inhibitors. This regulates the extent / shape of ECM &
helps maintain tissue homeostasis by mmp

Controlled activity of other enzymes (LOX, transglutaminases) create crosslinks and stiffen the ECM

2. Aged The composition and assembly of ECM changes - PGs decrease, FN increases, elastin network degrades, thinning of basement membrane.

This results in a more stiff, less elastic i.e. weaker tissue. This can promote age related diseases

3. Wounded cells Wounding is a rapid event, as compared to aging Wound response - fibrin clot, stimulates monocyte infiltration, recruited fibroblasts synthesize and dump large quantities of ECM proteins.

This can induce transdifferentiation of fibroblasts Myofibroblasts - promote formation of large, rigid collagen bundles which are crosslinked and help mechanically strengthen/stiffen the tissue

Eg: formation of scar tissue

4. Tumors Increased PG, collagen, elastin and FN Tumors = wound that fails to heal surrounding tissue. Tumors are stiffer than ECM is remodeled by resident fibroblasts and there's increased contractility of epithelium

How to build a molecule that recognizes and works with the ECM?

POW 1

- Why is it important for ECM to be dynamic?
 Why couldn't it have been inert, without biochemical & biomechanical cues?
Changes in microenvironment act as better signalling than cell-to-cell transfer. ECM talks to cells and provides cues, and not just act as a scaffold and reservoir.

Correlation b/w complexity of organism and function of ECM (giving another level of control)?

ECM provides more efficient directionality?

- Why so much diversity? - Redundancy, finer control.
 Having more diversity - lesser risk of something going wrong due to a mutation
 more knobs (parameters)

Essentially giving you more control.
 More binding sites => more regulation of concentrating hormones / GFs.

20/4

Lecture 07

A receptor that interacts with the ECM should respond to biochemical & biomechanical cues - if should bind to components of ECM and transduce the information, downstream to the cell.

Should the receptors and biophysical cues bind and respond to biochemical and biophysical cues?

The biochemical receptors - Integrins - are designed to detect both together. (24) types in humans
 The tail and of receptor would trigger the same process either way.

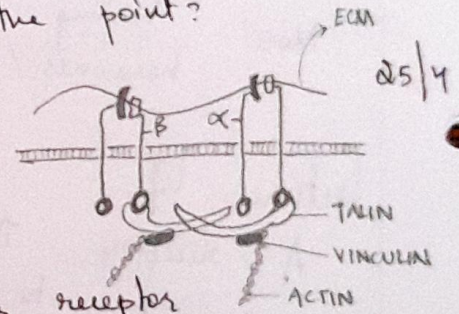
Actin-linked cell-to-matrix junctions can be small & transient OR large, permanent & durable like in focal adhesions and hemidesmosomes structures

Integrins - Structure and Function

- They control cell adhesion to ECM and in turn affect cell growth, migration, differentiation etc.
- Heterodimeric adhesive receptors - α & β subunit
- In mammals, 24 canonical integrins - 18 α & 8 β
- Cytoplasmic interactions are mediated by cytoplasmic tail of β subunit.

Integrin domain structure * Transmembrane, * Heterodimer
 Transmembrane and intracellular domains are important
 Tailin & α -actinin bind to tail ends of α and β subunits

The crystal structures are well-studied & illustrated
 The receptor opens and closes - Similar to tongs
 The binding sites show a diversity but all occur through tailin and α -actinin only. What's the point?



Lecture 05

Receptor diversity

- Expression of receptors
- Affinity of a particular receptor

The kinetics and nature of these 2 factors of reaction is based on

Integrin cytoplasmic tail - β tail mainly recruits downstream processes.
 molecules and regulates downstream processes.
Talin is the first molecule that binds to integrin.

When tension on integrin increases, it strengthens its hold on ECM & intracellular components \Rightarrow it can translate (37) mechanical signals to molecular, & vice versa.

The activation status of a molecule is a through an integration of all the signals coming from different receptors on cell surface

Integrin adhesion - a network of signalling pathways very dense, proliferate network triggered by one receptor.

This has a very specific spatial context i.e. cells will not grow/live if not attached to ECM response to growth factors. Anchorage dependence of the cell to the substrate. This is anchorage dependence which helps tissues overcome heat better.

This is because there's crosstalk b/w integrin-mediated adhesion and growth factors. Crosstalk is also important for cell migration.

Sensitive to force \neq The function of ~~recept~~ integrins is sensitive to external, mechanical force. Integrin can regulate the intensity of the signal by clustering on the cell surface. Also has a role in regulating signal pathways.

These through intercellular also linked through the cell, short and long loops. Force mediated actin by retrograde flow is also regulated by integrin signalling.

SUBSECONDS

MINUTE/DAYS

Intracellular

Structural reorganization of cytoskeleton → Signal propagation from adhesion site to nucleus

Alteration of protein expression

Adhesion site

Extracellular

Tension applied causes opening of cryptic site

Integrin recruitment & translocation, matrix assembly

Matrix remodelling & stretching; switching matrix functionality

Bidirectional integrin signaling

Integrins can transmit signals from inside the cell to outside and vice versa. Binding of a molecule on the tail causes the receptor to change, modulating its affinity. It can have a -ve feedback loop. - can change responsiveness of cell

Inside-out signaling

allows integrins to transmit forces required for cell migration & ECM assembly. Binding of talin - high affinity integrin through conformational change. This drives the extracellular portion of integrin go into its active conformation

Two models of Activation

Direct & Indirect. The molecules that bind to alpha and beta subunits are different - almost no overlap. They bind to various regions of receptor.

Lecture of

There would be change in lateral mobility of receptors to regulate signalling

Clustering of integrin

is critical for downstream signalling. Integrin function can be blocked by preventing ligand binding, clustering or targeting downstream effectors.

- 1. Outside-In Activation: When integrin binds to ligand in ECM, cell reacts by tying cytoskeleton to point of attachment so force can be applied
- 2. INSIDE-OUT: Talin competes with tail of alpha to bind to tail of beta subunit. When it binds, it goes into its active conformation

Caveolin - integral membrane protein that helps in forming imagination. (39)

The affinity of receptors and the strength and duration of its signalling is controlled by the clustering, which in turn is modulated by the plasma membrane.

Anything that influences lateral mobility of receptors affects the signalling of receptors. (eg: cholesterol)

Cholesterol such domains (lipid rafts) that influence the way receptors move & in turn their signalling.

Lipids get endocytosed? - lipids marked with (cholesterol) sphingo) cholera toxin + fluorescein are internalised. So signals moving on the normal adherent cell and non-adherent cell is different.

Clustering → Strong adhesions
"Vincos principle"

Caveolin mediated domain internalisation.

Integrin mediated adhesion regulates membrane order. Laurdan - dye whose emission varies based on phase in suspension time, for cells with internalised

* with caveolin - cholesterol & all are internalised ⇒ Fluidity increases with time. For cells with lacking cells, the GP value remains *

For caveolin constant. In response to loss of adhesion?

These lipids are inserted back in membrane through exocytosis. The cell basically uses endocytosis and exocytosis to change the composition of the membrane signalling, which in turn, influences

Study: Integrin signalling generates nanodomains of the plasma-membrane that promote cell spreading

Actin formation - recruits talin & vinculin - allows regulation of receptor clustering

Integrins - Syndecans clustering

There's recycling of integrins through endocytosis and exocytosis.

Adhesion dependent membrane targeting of lipid rafts regulates Pak, Erk, Akt (signaling molecules in adhesion - GF network) activation.

This confers adhesion dependence to anchorage signaling. Pak Erk & Akt

Downstream signaling molecules - Rab, Ras, Arf, Rho

Cytoskeletal Network Functions - Actin - shape of cell surface necessary for whole-cell locomotion; Microtubules - direct intracellular transport determine position of membrane-bound organelles; Intermediate filaments - mechanical strength

- Mechanical support
- Fibers act as geodesic dome to stabilize and balance opposing forces (even external)
- Provides anchorage for organelles
- Acts as transport highways
- Dynamic structure - dismantles and reassembles to optimize the shape of cell/organelle to their respective function.

FUNCTIONS
1. Architectural framework => polarity of cell
2. Spindle apparatus that segregates chromosomes
3. Internal transport network to

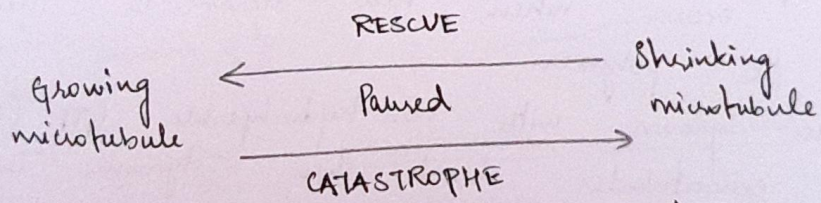
Lecture 8, 10 - Microtubules traffic vesicles mediated by microtubule associated proteins to form a rigid internal core which can be used by cilia + flagella to generate movement

These 2 features drive the function of microtubules

Microtubule Associated Proteins (MAP) - Kinesin & Dynein

α, β - binding site for 2 GTP
 GTP bound to α tubulin is trapped, but β tubulin can have GTP or GDP bound to it & they are exchangeable

- Microfilaments are hollow, unlike actin
- α and β tubulin heterodimer subunits assemble linearly to form a protofilament.
- 1 microtubule = 10-15 protofilaments that associate laterally to form a 24 nm tube with a 15 nm lumen. It has a β (+ end) and α (- end) structural polarity.
- The 2 ends have different polymerisation ends rates. The β (+) is faster growing (& faster depolymerisation).
- The γ -subunit, along with capping protein is attached to centrosome. Acts as template for correct assembly of microtubules.



This ability allows microtubules to grow and shrink as needed. Eg. To separate chromosomes. Cytoskeleton made of multiple protofilaments: stronger & more stable.

Mechanism
 + end - the β -subunits have a GTP cap that stabilises the microtubule.

[The formation of microtubule depends on the conc. of free subunits. If conc. is above critical conc., then this process will happen spontaneously. The cell can couple this unfavorable reaction to make it happen.]

The subunits have a nucleotide binding site. Usually free tubulins are bound to GTP, whereas those in the polymer get hydrolysed i.e. G-form

CHECK
 General case to illustrate mutation RES

Microtubule lattice is mainly composed of GDP-tubulin & depolymerisation is characterised by rapid loss of D form subunits.

Stochastic loss of G-cap at the + end triggers this.

At the - end, no GTP cap.

Rapid interconversion between growing & shrinking state, at uniform subunit conc is called dynamic instability.

Microtubule dynamic instability can be empirically characterised by - if the rate of polymerisation is fast, microtubule

- 1. Rate of growth / shrinkage will have a GTP cap
- 2. Catastrophe / Rescue frequency - freq of shifting from growing \leftrightarrow shrinking state

What powers the dynamic behavior?

* Energy required comes from GTP hydrolysis which occurs when new dimer is incorporated into the polymer. \rightarrow at the α & β subunit

When grown with non-hydrolysable GTP (GMPCPP), the microtubules don't show dynamic instability

* GTP-tubulin subunits at the + end (GTP cap) are thought to contribute to stability

* Upon hydrolysis of GTP cap, the D-form polymer quickly disintegrates \therefore conc necessary for polymerisations of D-form \gg T-form several orders of magnitude greater

Methods of study have improved

lot of studies are done with drugs which can stabilise / destabilise microtubule.

Taxol - stabilizes (loses dynamism?)

Colchicine - depolymerisation

These are reversible changes that affects rate

but what?

nocadizol

Nucleation: RDS in polymerisation reactions filament adds units in + end while simultaneously losing subunits in - end. This property of filaments is called treadmilling (13)

* If monomers (F-actin) are thicker than critical conc. of T form but less than D form, *
could be Golgi? They are particularly organised, duplicated and apart during cell division.

Centrosome - Microtubule organising centre (MTOC) are particularly organised, duplicated and apart during cell division.

Study: Golgi could be the nucleating centre for microtubules are implicated in stabilizing, and maintaining integrity of Golgi. These organising complex of the microtubule is distinct and can be labelled because of association of certain proteins like EB1. [one among MANY]. The Tip and cellular separation is involved in polymerisation, depolymerisation with other during chromosome interaction of MT like components.

Stabilisation of MT helps for cell migration. \Rightarrow continuous maintenance of polarity, especially important

Lecture 11 - Actin filaments / Microfilaments

Microfilament - two intertwined strands of actin in diameters
Form of actin filament consists of G-actin (subunit) which has an ATP binding site with distinct polarity. Subunits assemble to form filament with distinct polarity. 2 parallel protofilaments twist around each other in sight-handed helix.

Actin filaments are individually flexible but in a cell several filaments are cross-linked & bundled to create rigid, strong large-scale actin structures

Actin has a system in place so it can create branchings. Microfilaments are limited in this feature. The actin filament also has +/- ends which look different structurally.

Actin is like a mesh - they are enriched in the edges. They allow the cell to be spread and stretched out. Microtubule - backbone, radiating skeleton. Actin is the component that's moving the lamellum and lamellipodium

Visualizing actin - fluorescent labelling of actin. The conc. of label was wrongly added and this allowed us to see the retrograde flow of actin (∴ only some actin molecules were visible). This speckle microscopy allowed us to visualize the edge of the cell, and the flows / movement of actin.

≠ Actin (not microtubule) is the one supporting the membrane + end - barbed - end - pointed

Movie : - G-actin + ATP $\xrightarrow{\text{hydrolysis}}$ G-actin - ADP + iP.
The actin filament is synthesized by nucleation. As the filament grows at the + end, the subunits at (-) end get hydrolysed and disengage. This is called actin treadmilling.
The conc. of G-actin, ATP and ADP controls the rate of polymerisation & depolymerisation.

Actin accessory proteins - profilin, cofilin
They regulate different aspects of nucleation and polymerisation. Profilin inhibits polymerisation.
Others accelerate depolymerisation after severing to fragments.

Branching of actin network is done by ARP 2/3 protein — nucleates new filaments at 70° pushing the membrane forward

Filaments elongate, pushing the membrane forward

At steady state, actin filaments can be capped so no further polymerisation & actins winds up in ADP form. Its susceptible to depolymerisation by cofilin.

ARP 2/3 is activated by WASP protein which exists in autoinhibited state, which is activated by cdc42 so it can then activate ARP 2/3. [# cdc42 is a GTPase]

ARP 2/3 binds to WASP. This complex can weakly bind to an actin strand. This is strengthened by a G-actin - Wasp complex, which binds with ATP bound G-actin. If manages to attach, ATP bound G-actin to the strand which begins nucleation.

Actin dynamics in filopodia

Multiple actin strands are brought together and if pushes the membrane as it grows. Fibropodia allows the cells to probe the microenvironment — cell itself doesn't have to cue.

Listeria bacteria — has a tail of actin to propel itself forward (refer to AG's physiology notes)

Microtubules are central structure supporting cilia and flagella, along with motor proteins. which creates net movement of cilia/flagella.

(46)

The bending of cilia is driven by arms of motor protein called dynein. The arms grab, move, release the outer microtubules (requires ATP)

(+) end - barbed
(-) end - pointed.

Microfilaments [Intermediate filament]

Thinnest class of cytoskeletal fibers

Solid rods of G-actin (~~not~~ F-actin - twisted double chain)

They're designed to resist tension.

With other proteins, they form 3D network just below the membrane.

9/5

Lecture 12

Actin undergoes treadmilling. Cofilin depolymerises from the (-) end. Profilin slows polymerisation.

Crosslinking proteins (fibrin) and bundling proteins (fascin) undergo help form a network of actin to form filopodia/lamellipodia.

There are also capping (gelsolin) and severing proteins.

A number of drugs can bind to different components of actin, regulating polymerisation.

(thaloidin, Jasplakinolide) and depolymerisation (cytochalasin).
They're also used to study & visualise actin.

Intermediate filaments are primary component of cytoskeleton, although they're not seen in ^{all} eukaryotes,

they're absent in plant & fungi

They extend through cytoplasm and inner NM, consisting of 5 protein classes.

IF assembly begins with folding of IF proteins into a

Conserved α -helical rod, followed by polymerisation and annealing events that lead to IF of diameter 8-12 nm

They lack polarity, they're more stable & their subunits don't bind to ATP/GTP.

Protein	dimer	constituting IF —	hair, nails, horns
Type I and II	: <u>Keratins</u>		Epithelial cells & derivatives
Type III	: <u>Desmin</u>		Muscle
Type IV	: <u>Neurofilaments</u>		Neurons
Type V	: <u>Lamins</u>		Nuclear lamina

Assembly — each strand is brought together into dimer → tetramer → unit length filament → filament. They're bulky and built for strength.

IF are heterogenous — greater variation in their proteins which allows them to form more diverse structures

IF genes are conserved only in metazoans — in fact insects have only one class of proteins.

Metazoan lineage evolved & IF was adapted to support special kinds of cells & tissues.

- IF lack polarity; subunits don't bind nucleotides; subunit exchange is not confined to ends.
- They lack motor proteins (\therefore no polarity) have slower dynamics \Rightarrow inherently more stable
- They have ↑ tensile strength & are resistant to compression, twisting & bending forces

- Elastic nature : due to staggered assembly of subunits & high deg of latitudinal vs longitudinal interactions within filaments
=> strength, toughness & elasticity over long run

Phylogenetics of cytoskeletal elements.
Refer IP sent by Krishna (Shivani's notes)

Lecture 13

Motor proteins (MP)

Actin based MP are members of myosin superfamily
Skeletal muscle myosin - first identified

Myosin II - elongated protein
2 heavy chains + 2 x (2 light chain)
Has a head, neck & tail

Each heavy chain has a globular head domain at N-terminus that contains force generating machinery, followed by long aa sequence that forms an extended coil that mediates dimerization.

C terminus : cargo binds there

There's diversity in forms of MP across different life forms.

They're different kind of compartment of cell, kind of cargo they carry, rate at which they walk, the way they're regulated

These myosins bind specifically to actin & not MT.

The function of MP requires energy in form of ATP. MP's are essentially enzymes that hydrolyse ATP and use energy to "walk"

On the other end, myosin could bind to adaptors, receptors, lipid moieties, b/w a actin filaments, protein and ribonucleo-protein complexes.

Different kinds of myosins are regulated to specific compartments in cell, which defines their functⁿ. They are retained through specific interaction.

Actin-MP interaction generates force as its important in formation of fibropodia.

Also responsible for muscle contraction and cleavage furrow in cell division.

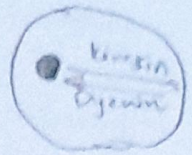
Actin gets recruited to focal adhesion structures. here MPs work with actin to create actin architecture based on the kind of force generated / sensed. This kind of force generation also occurs at 'leading edge' of cell migration.

Durotaxis - cells tend to move from soft/compliant to stiff matrix (to optimal stiffness). This movement is called durotaxis, its driven by MPs.

Cell-cell junction and polarity of cell i.e positioning of cellular organelles - actin-motor protein play an important role. Also imp for integrin clustering, elongation of cell, cell-cell interaction.

Membrane shaping - endocytosis, fibropodium, golgi morphology - use the actin cytoskeleton extensively. Position of golgi also drives vesicular transport, mediated by cytoskeleton.

Actin - myosin interaction are also important
 The myosin head has tails that also can shift as if they're on a hinge.
 This allows two myosins to maneuver large cargo when they come across each other.



Kinesin superfamily

Kinesin - moves along microtubules
 Kinesin structurally similar to myosin - 2 heavy chains, 2 light chains per motor.
 2 globular head motor domain & an elongated coil responsible for dimerisation.

Kinesin (like myosin) is a member of large protein superfamily where motor domain is the only common element

- * { Kinesin - walks away from nucleus to periphery
- Dynein - walks towards the center

Humans : 40 types of kinesins.

Kinesin - + end MP i.e they walk towards + end
 Most carry a vesicle or another microtubule for either a variable length

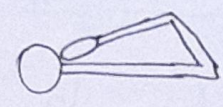
The tails of these motor proteins are enhanced in certain regions of the cell, so their functions are specific and unique

Kinesin cargo could also be variable - there is selectivity but not specificity in binding of MP and its cargo

Regulation of kinesin - cargo binding by 3 different mechanisms -

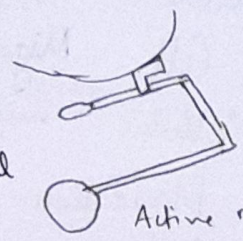
- 1. Phosphorylation
- 2. Rab & GTPase cycle
- 3. Calcium

Architecture / Folding of Kinesin
 When not bound to cargo, the tail and cargo-binding site are close to each other

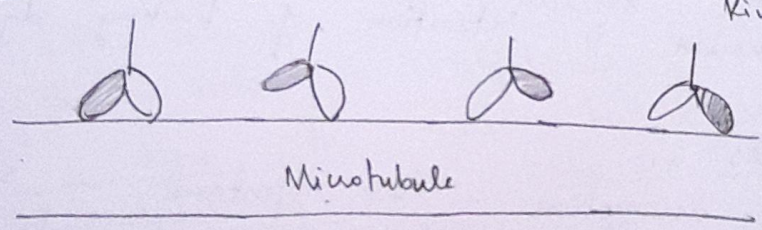


Inactive

Docking onto cargo activates the protein, initiating conformational change that relieves the inhibition of motor domain.

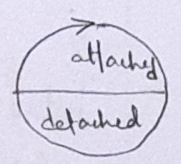


Active motor domain



Microtubule

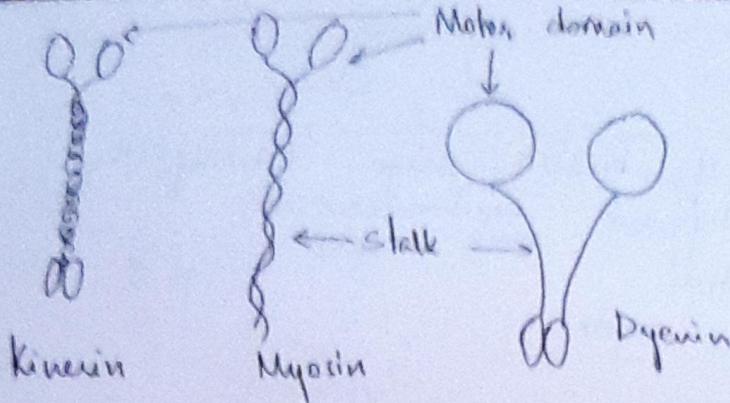
Kinesin



Hydrolysis cycle

In myosin cycle, the head remains bound to actin only 5% of entire cycle, unlike kinesin which is bound half the time.
 When we talk about speed of MP, we're talking about the rate of hydrolysis.

Dyneins - minus-end motor proteins
 Composition & conformation of dynein (similar to kinesin) allow it to 'walk' using ATP.
 They're important for cell migration, separation of chromosomes, ciliary / flagellar movement etc.
 They're localised to achieve these



Their structures are mostly preserved

David Goodsell - structural biologist + artist

Lecture 15

Cell Migration

14/5

Cellular migration is mediated by 4 major steps

4 steps

- Polarization of cell
- Protrusion of leading edge
- Formation of adhesive contacts with ECM
- De-adhesion & retraction of trailing edge

Intro slides

Cellular locomotion - vv important - sperm, macrophages, protozoa, wound healing etc.

Velocity of cell spans a wide range -

Nerve ($10^{-2} \text{ cm s}^{-1}$) to Tetrahymena (10^2 cm s^{-1})

Macrophage chasing bacteria
Wound healing by sheet movement of epithelial layer

Cell polarization - responds to a certain kind of stimulus
front

Regulators

Side/Rear

PTEN

Myosin II

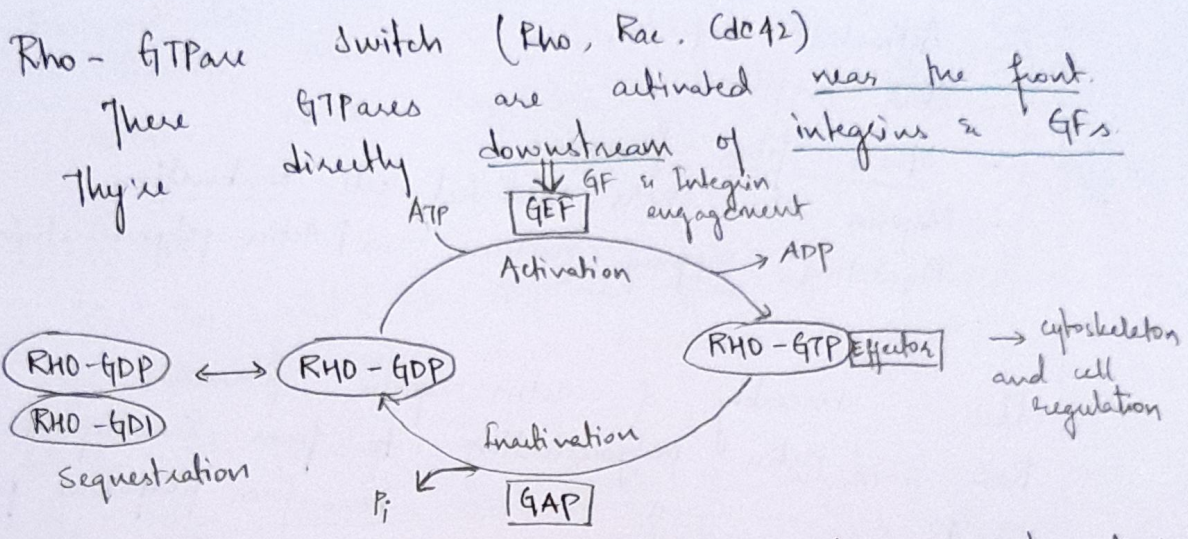
Activated Cdc42 & Rac
Cdc42 / PARs / aPKC / PIP₃

Activated integrin

MTOC / Golgi

Microtubules.

There's an integration of adhesion and growth factor signalling pathways.
PI3 Kinase - important cross-talks b/w 2 pathways.



Rho-GTP interacts with effectors and causes the effect.
The cycle is regulated by GEF and GAP.
GDI - block the cycle by sequestering and solubilizing the Rho-GDP form.

This cycle happens very rapidly.
The dynamic nature is spatial + temporal and are also important.

This allows for downstream signalling - to affect target proteins and recruit microtubules to the lamellipodium for extension.

Rac / cdc42 signaling in cell migration
The activation of these GTPases causes -

- Uncapping of actin capping protein
- Arp 2/3 → actin polymerisation
- Blocks cofilin (which causes actin depolymerisation)

- Vesicle recruitment through myosin
 - focal complex turnover - point adhesions pg. 57
- For these to occur, the signal integration of both Rac and cdc42 are required.

Rho signaling

- Activates Arp2/3
- Block cofilin
- Stress fiber formation
- Myosin like chain mediated cell contraction
- Regulating capping proteins => ↑ Actin polymerisation

Almost same

Rho : assembly of actin:myosin filaments
 Rac : actin polymerisation to form lamellipodium
 Cdc 42 : " " to form fibropodial protrusion

Together (based on relative activation), they regulate migration
 The intensity, localisation of activation of these components ultimately regulates cell migration

Efficiency and energy transfer - read yourself.
 FRET Probes - allows you to look at emissions & determine protein conformation.
 When far away, probes emit diff. freq, but when closer, they transfer energy & emit an entirely different wavelength.
 The FRET signal tells us how closely these probes are sitting. This allows us to understand the conformation of ATPases.
 We can also detect their localisation and activation levels.

Rac is present everywhere in cell but activated
Rac is only present at edge of lamellipodium.
ie leading edge of migrating cells Activated Rac

Photo activatable Rac used to regulate cell polarity
Similar activation profile of Rho and Cdc42.
Cdc42 - master regulator of cell polarity

12/6

Lecture 16

Rho, Rac and Cdc together affect the lamellipodium edge
Refer to schematic, which represents the activity.

Cdc42 - Cell polarity

- Its active towards the front of migrating cells, and inhibition and global activation could disrupt the direction of migration
- It influences polarity by restricting where lamellipodium forms.
- Also influences polarity by localising the microtubule organizing centres (MTOC) and golgi in front of nucleus, oriented toward the leading edge.

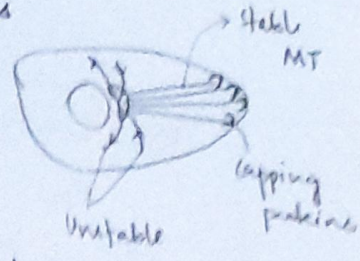
So movement of MTOC and golgi to the front & nucleus following was the idea of yore. Its no more.

But a study published in 2005 showed that nucleus moves back (?) so it looks like MTOC + golgi stays ahead. It was an observational find. ie MTOC stays where it is. tagged

- Signalling involved in cell migration is extremely complex
Polarization in dictyostelium - PIP3 is lighted up

② All polarisation requires stable microtubules in specific locations. This is done through capping proteins.

The stabilization allows for steady transport of vesicles.



Rho GTPases regulate MTs through their appropriate effectors.

Membrane polarization

There's accumulation of lipids at the leading edge - membrane cholesterol and sphingolipid enriched microdomains (rafts) localize there. This localization is regulated by integrin mediated adhesion.

2nd step in cell migration: Protrusion of leading edge

It is mediated through actin polymerisations. 2 types of structures: lamellipodia & filopodia.

Actin polymerization produces "pushing" forces. Recall actin dynamics - the mesh.

We now know - 2 regions of different rates of actin polymerisation in leading edge and lamellipodium. Different kinds of adhesions are also present.

Random vs Directed cell migration
directed - result of a chemoattractant.
random - spread out in all directions.

3rd step: Formation of adhesive contact with ECM
Adhesions are stable, right behind the leading edge. They stabilize the protrusion. Integrins are a major family of migration-promoting receptors.
We also need detachment at the tail.

Focal adhesions vary -

1. focal complex : small, point-contact, dynamic, occurs at the edge
2. focal adhesion: more stable, bigger, occurs just behind the lamellipodium.
3. Fibrillar adhesion: very big & strong. lots of stress fibres, occurs near the centre of cell, stable => responsible for cell anchorage

Step 4 : De-adhesion and retraction of trailing edge

Polarising the cell

- PI3K & PTEN
 - PI3K - leading edge - generates PIPs which amplify chemoattractant gradient.
 - PTEN - rear & sides - removes PIPs

- Rac - Stimulates PI3K recruitment; PI3K - activates Rac GEF
- Rac stabilises MT; induces integrin clustering
- MT polymerisation & integrin engagement activates Rac

* Rap1 & PK3 increase integrin affinity