8/27/21

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 IISERP 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 ie the lipids produced organize spontaneously
But there seems to be some organisation wrt 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 in a bilayer) for every 1 protein.
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 (like cholesterol) i.e composition can't be decoded using the genome.

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

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

Cod liver oil - Polyunsaturated fatty acid. Gets incorporated into membrane through the diet

9/2/21

Composition of lipids

1. Glycerolipids - Polar head group + Phosphate, Glycerol, Fatty acid (2) → Back bone
   Many kinds (Sphingomyelin). Makes up 65% mol %
2. Sphingolipids - polar head + Phosphate / Glucose (Glycosphingo lipids), sphingosine
   Makes up 10% mol %. Can vary from 5 to 50 mol%
3. Sterols - Eg: cholesterol. Makes up 25% cholesterol. Its head group - OH
   4 rings + fatty acid chain.

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. Only found in bacteria & mitochondria
   For 20% of inner mitochondrial membes

Phosphatidyl glycerol (PG), phosphatidyl serine (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. How is still a research topic.

CL's shape is like a traffic cone - so when stacked together, it 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.

H_2O (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 H_2O molecules
- When the aggregate, the surface are 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.

Micelle vs. Bilayer

Micellar

- In a fatty acid, the size of polar head and the tail is the same i.e. cylinder ⇒
- 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

5nm

Lamellar

Bilayer.

Structure of Lipid Bilayers

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.

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

Experiment — Refer slide 13 — BOPC : bioleoylphosphatidyl choline.

These multilammelar 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 15Å from the centre, so the width of the monalyer is about 3nm, which is close enough to 5nm

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.

* F & E (1970) – two different cells with different proteins are forced to fuse – proteins redistribute over the whole surface in 40 mins

FLUID + DYNAMIC ✓

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.

Monolayer 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. Danieli and Davson (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 big 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 fluorophore

Mouse – green fluorophore

(6)

Lecture 4

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

⇒ Sackmann (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 can cause non-radom, heterogenous distribution

Contemporary model of cell-membrane
Kusumi et. al in their review described domains/corrals and showed how it correlated with the outlines of the cytoskeleton filaments underlying the membrane.

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

Through SPT, the measured the diffusion coefficient,

$D = \frac{\langle r^2 \rangle}{Time}$ Means square displacement vs time
Slope of ↑

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).

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 with 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.

16/2

Lecture 5

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

Domains are defined by imposing some kind of barrier 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 seperate the axon & dendrites
Epithelial cells - to maintain polarity & specialize the apical domain facing the lumen
Spern - specializing the head, the neck & tail
Yeast - bud neck.

Temp 50°c, 25°c, L_d, $L_o + L_d$, L_o, $S + L_o$, C_{14} lipid, 30 mol% cholestrol

Membrane domains
Attributed to -
a) apparent affinity b/w lipids & proteins
b) segregation of lipids & proteins together because of matching physical properties
c) 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 relates to positional freedom & translational diffusion. Ordered / disordered refers to internal degree of freedom or rotational diffusion
Eg: DPPC - critical temperature : 41°c.

Cholestrol also adds integrity to membrane - decreases diffusion across the membrane

Effect of cholesterol - Buffering function

- Rigid & planar steroid rig 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 acyl 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 beleived 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 acyl 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-phosphatidylinositol & GPI anchored proteins.
- insoluble in low concentrations of non-ionic detergents

(?) Related to signal transduction. How?

Lecture 06

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.

Steady state diagram

- The structure, abundance and composition (position?) is very dynamic and changes wrt 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 – Antrograde 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 endolysosomic system

At steady state, half of plasma membrane gets turned over through this system in about 30 mins or so

Endoplasmic Reticulum

- It is found in cytoplasm and has a network-like structure
- It is continuous with the outer nuclear membrane –
- It is dense near the nucleus and in the periphery, it's more homogeneous and has tubular structure
- Two kinds – Rough ER – more like flattened stacks (sacs) with bound ribosomes

  Smooth ER – tubular, lack bound ribosomes.

Rough ER	Smooth ER.
Found near the nucleus	Found at the periphery
Membrane & secreted protein synthesis	Steroid hormone secreting cells, hepatocytes that detoxify hydrophobic molecules
Lipid synthesis	
N-linked glycosylation (lumen) - post-translational modification	
Exocrine cells have large fraction of rough ER	

The ration 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 dies are used to distinguish SER. is used to distinguish RER.

? Translocon apparatus shoves the nascent polypeptide into lumen of ER
Protein channel

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

Apical

Zymogen granules

Golgi

Basolateral

Nucleus

Mitochondria

RER

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.

22/2

Lecture 7

ER Organisation during Cell Division

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 in important leap (by reconstituting the membrane) in corroborating the model.

23/2/21

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.

* COTRANSLATIONAL

Types of integral proteins

- 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%

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

Integral Membrane Protein (IMP) Biogenesis

- IMPs make upto 20-30% of the proteome
- Very diverse - channel proteins, signalling receptors, mediate intracellular traficking, 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.

Secretary 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 (~300mg/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 translocon 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 receptor on the ER binds the SRP - Ribosome complex and directs it to a translocon
- SRP receptor and SRP are released and recycled
- Translocon transfers the protein across the double layer and the translation continues.

25/2

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 it - organizes spontaneously

Translocon structures (2009)
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 cryoelectron microscopy.

* Because of their tendency to flip, the area of ER keeps growing even if lipids are inserted only onto the upper leaflet.

2/3

Lecture 10

Membrane biogenesis

- 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 maintanence. Moreover, its present only on the inner leaflet - connected to cytoskeletal element so PS won't be a part of endosome
- Lipids keep flipping* in the membrane, but the cell still maintains the asymmetry.
- 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.

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

PP

Slide 66.

- 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. ⇒ 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 → Golgi → PM as part of bilayer that makes up walls of transport vesicles.
↳ '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 compartment to another.
↳ soluble proteins : lipid transfer proteins.
They transfer lipid molecules from ER to other organelle membrane, individually. While transporting, the lipid molecule is sheilded

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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 its actually quite organised

The nucleus is clearly seperated. This results in decoupling of transcription and translation i.e. more regulation. Flipside: 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 seperated 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.

The models of its formation and insertion in the membrane are contentions. NPC is quite complex and particular and needs to reassembled / preserved through cell division when nuclear membrane is dissolved. (← Prepore vs. Insertion model)

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

- Newly synthesized INM-destined integral membrane are co-translationally inserted into ER and distributed to INM and ONM by diffusion
- NPCs prevent free diffusion & passing of membrane proteins with domains > 60 kDa. Smaller proteins / particles are free to diffuse about. This dictates the size of INM-bound proteins
- However only proteins that bind efficiently to 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 74

Movement of materials through NPC

- Expt: Gold was injected into cytosol and obsered 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 octogonal 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.

Phe - Gly : Two AAs that can't be replaced by any other amino acids. FG domains get organised disorderedly (34)

Green fluorescent protein — 3nm 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.

8/3/24

Lecture 12

Flexible FG nucleoporins 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 order of nm)
* # AFM — has a cantillever 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 + cantalliver can be used to get an idea of the nature of material by indenting on it.
* In the paper, the 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.
* ⇒ 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.

Good analogy : FG domain ≡ Kelp forests

like signal sequence ← Nuclear localisation Signals (NLS)

They're short sequences of positively charged (lys/arg) amino ā which ~~when~~ ~~attach~~ are on to proteins & gives it the pass through nuclear membrane

Can be located anywhere in a protein (loops/surface)

* There are some bona fide sequences that can be attached to any protein to localize it in nucleus.

like SRP ← 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 :

Cytoplasmic

Pi ← GAP ← GTPase (GTP)

GTPase (GDP) → GEF → GDP out, GTP in → GTPase (GTP)

Nuclear

GAP : GTPase Activating Protein

GEF : Guanosine Exchange Factor (nucleotide)

GAP — Ran BP1 , RanGAP1

GEF → RCC1

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

RanBP1 , RanGAP1 — Cytosolic ~~Ran~~CC1 – chromatin associated nuclear protein

NPC ER outer

Inner membrane

This asymmetric ~~exchang~~ 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 mainly 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 Ran GDP.

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 Ran GTP hydrolyse to RanGDP.

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

Ran GEF : Nuclear   RanGAP : Cytosolic.

RanGTP is v. 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

P

Import

Nuclear transport receptor (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, RanGAP1 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 riding 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-proteobacterium and Asgard archaea
- Slowly, M lost is 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

DNA
Ribosome
ATP synthase
Cristae
Matrix

- ATP production occurs through TCA cycle – which breaks down nutrients to produce ATP by creating a protonmotive force across the inner membrane

- 2 things happened over evolution –
  - The nucleus controls when the M divide
  - Also makes the M fuse and fiss, through proteins (family: dynamins – Opa (I) & mitofusin (O)) 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 the cytoskeletal structure, especially the mito

Interphase – Network like structure
Cell division – occurs as single entities so they're seperated 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 Arfaptin 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 the 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 degraged once it's inside the mitochondria. Also translocation into mitochondria is post.
- The SS is α-helical, amphipathic — half of signal is charged (+ve) residues and other half is hydrophobic
- SS is recognised by translocons 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 its 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

OCR
Basal respiration
Oligomycin
oxidative phosphorylation
control
FAM92A1
Time

→ Comparing immunofloresence 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 ~~cisternae~~ 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 the membrane formed tubule like structures.
This shows that FAM is also sufficient to cause / form cristae structures in membranes.

PART 2

30/3
4/04

Lecture 01

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, Neighboring cells, Soluble factors, Physical feilds

- 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 endothelium to minimise shear and facilitate blood flow is an example of that

When exposed to laminar, shear flow, the endothelial cells align themselves along the direction of the flow – influenced by the flow and the connected / neighboring cells. The flow can drastically influence cells to have different shape, secreted factors etc based on flo

Many factors affect (& 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 stress.

Extracellular Matrix (ECM)

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

IP : Arrangement of collagen in different kinds of tissues.

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

ECM can acts as –

Functions
1. Anchorage
2. Migration barrier/track
3. Signal reservoir (signals can be trapped in ECM with varying efficiency - think growth factor)
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 —

1. Proteoglycan (PGs) - Perlecan

- PG = Glycosaminoglycan (GAG) + Specific protein core (Exception: Hyaluronic acid)
- 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 extendended 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 5/4 collagen by lysyl oxidases (LOX)

Lecture 02

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

Fibronectin (FN) → Contribute to organizing the matrix & attaching cells to ECM
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 ↳ for degrading the matrix
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

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

Fibronectin have an RGD peptide sequence that's considered an important factor in ~~identi~~ allowing integrins to identify FN

Dimer
RGD : Arg - Gly - Asp

IF Stain of fibronectin matrix ~ 22:10

Collagen – Triple helix – left handed turns
There 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 matrisome (compositⁿ) and other features and see how they alter in diseased conditions

Slide 15

What's the point of so much diversity?

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 (secreted by some cells) go & Fibroblast metallo-proteinases 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. Tumor
Increased PG, collagen, elastin and FN
Tumor ≃ wound that fails to heal
Tumors are stiffer than surrounding tissue.
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?

12/4

POW 1

- Why is it important for ECM to be dynamic? Why couldn't it have been inert, without biochemical & biomechanical cues?

<u>Changes</u> in <u>microenvironment</u> 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

  Essentially giving you more knobs (parameters) to control.

More binding sites ⇒ more regulation of concentrating hormones / GFs.

20/4

Lecture 07

A receptor that interacts with the ECM should respond to <u>biochemical</u> & <u>biomechanical</u> cues — it should bind to components of ECM and <u>transduce</u> the <u>information. downstream</u> to the cell.

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

The receptors — <u>Integrins</u> — are designed to detect both together. → (24) types in humans

The tail end of receptor would trigger the same process either way.

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

Integrins - Structure and Function

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

Integrin domain structure ★ Transmembrane, ★ Heterodimer
Transmembrane and intracellular domains are important
Talin & 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 downstream signalling occurs through talin and α-actinin only. What's the point?

25/4

ECM
β
α
TALIN
VINCULIN
ACTIN

Lecture 05

Receptor diversity
- Expression of receptors
- Affinity of a particular receptor

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

Integrin cytoplasmic tail - β tail mainly recruits 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 ⟹ it can translate mechanical signals to molecular, & vice versa

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

Integrin adherome – a network of signalling pathways
A very dense, proliferate network triggered by one receptor.
This has a very specific spatial context

→ Anchorage dependence
How the cell responds (response to) to growth factors depends on adherence of the cell to the substratum. This is anchorage dependence i.e cells will not grow/live if not attached to ECM. This feature also helps tissues heal better.
cancer cells overcome which
This is bcuz there's crosstalk b/w integrin-mediated adhesion and growth factors. Crosstalk is also important for cell migration

→ Sensitive to force#
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~~ receptors intercellular also traficked through the cell, in short and long loops.
Force mediated actin retrograde flow is also regulated by integrin signalling

SUBSECONDS ——————————→ MINS/DAYS

Intracellular: Adhesion site → Structural reorganisation of cytoskeleton → Signal propagation from adhesion site to nucleus → Alteration of protein expression

Extracellular: Adhesion site → 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

Binding of talin - high affinity integrin through conformational change.

Inside-out signalling controls adhesion strength and allows integrins to transmit forces required for cell migration & ECM assembly

Two models of Activation - Direct & Indirect

The molecules that bind to α and β subunits are different - almost no overlap. They bind to various regions of receptor.

24/4

Lecture 07

There could be change in lateral ~~signalling~~ mobility of receptors, to regulate signalling

Clustering of integrin is critical for downstream signalling. Integrin function can be blocked by preventing ligand binding, blocking 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 α to bind to tail of β subunit. When it binds, it forces the two apart. This drives the extracellular portion of integrin to go into its active conformation

Caveolin — integral membrane protein that helps in forming invaginations.

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

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

∃ cholesterol rich domains (lipid rafts) that influence the way receptors move & in turn their signalling.

Lipids get endocytosed? — lipids marked with (cholesterol / sphingo) cholera toxin + fluorosceine are internalised.

So signals moving on the normal adherent cell and non-adherent cell is different.

Clustering ⇒ Strong adhesions
"Velcro principle"

Caveolin mediated domain internalisation.

Integrin mediated adhesion regulates membrane order

Laurdan - dye whose emission varies based on phase ie indicates fluidity of membrane

With increase in suspension time, for cells with caveolin — cholesterol & all are internalised
* ⇒ Fluidity increases with time *

For caveolin lacking cells, the GP(?) value remains constant.

In response to loss of adhesion?
These lipids are inserted back in membrane through exocysts, based on signals from other molecules. The cell basically uses endocytosis and exocytosis to change the composition of the membrane which in turn, influences signalling.

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~~ anchorage dependence to Pak Erk & Akt signaling.

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.

Lecture 8,10 – Microtubules
- Dynamic nature
- Can bind distinct motor proteins

These 2 features drive the function of microtubules

FUNCTIONS
1. Architectural framework ⇒ polarity of cell
2. Spindle apparatus that segregates chromosomes
3. Internal transport network to traffick vesicles mediated by microtubule associated proteins
4. To form a rigid internal core which can be used by cilia + flagella to generate movement

Microtubule Associated Proteins (MAP) – Kinesin & Dynein

α, β – binding site for 1 GTP
GTP bound to α tubulin is trapped, but β tubulin can have GTP or GDP bound to it & they are exchangeable

- Microtubules 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 (15 nm lumen)
- α and β are arranged specifically – it has a structural polarity – α (- end) and β (+ end)
- The 2 ends have different polymerisation rates
  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

RESCUE
Growing microtubule ← Paused → Shrinking microtubule
CATASTROPHE

This ability allows microtubules to grow and shrink as needed
Eg: To seperate chromosomes

Cytoskeleton made of multiple protofilaments ∴ stronger & more stable

Mechanism

- + end – the + end have a GTP cap that usually 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 with an ...]

CHECK
General case to illustrate that nucleation is the RDS

- 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

- Microtubule lattice is mainly composed of GDP - tubulin & depolarisation 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 will have a GTP cap

1. Rate of growth / shrinkage 2
3. Catastrophe / Rescue 4 frequency - freq of shifting from growing ⇄ shrinking state

What powers the dynamic behavior?

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

but what? X

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 ∵ conc necessary for polymerisations of D-form >> T-form several orders of magnitude greater

Methods of study have improved

Lot of studies are done with drugs which can stabilise / destabilise microtuble.

Taxol - stabilizes (loses dynamism?)

Nocadizol Colchicine - depolymerisation

There are reversible changes that affects rate

Nucleation : RDS in polymerisation reactions

If monomer (free) conc is greater than critical conc. of T form but less than D form, * filament adds units in + end while simultaneously losing subunits in − end. This property of filaments is called **treadmilling**

Centrosome — Microtubule organising centre (MTOC) (could be golgi?)

They are particularly organised, duplicated and taken apart during cell division.

Study : Golgi could be the nucleating centre for microtubules

These microtubules are implicated in stabilizing, organising and maintaining integrity of Golgi

TIP of the microtubule is distinct and can be labelled because of association of certain complex proteins like EB1. [one among MANY].

The Tip is involved in polymerisation, depolymerisatn and interaction of MT with other cellular components, like during chromosome seperation

Stabilisation of MT ⇒ continuous maintanence MT helps set up polarity, especially important for cell migration.

8/5

Lecture 11 - Actin filaments / Microfilaments

Microfilament — two intertwined strands of actin 7nm in diameter

Each actin filament consists of G-actin (subunit) which has an ATP binding site

Subunits assemble to form filament with distinct polarity

2 parallel protofilaments twist around each other in right-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 :–

$$\text{G-actin} + \text{ATP} \xrightarrow{\text{hydrolysis}} \text{G-actin} - \text{ADP} + \text{iP}$$

The actin filament is synthesized by spontaneous 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°

7 subunits ← ARP 2/3

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 it turn 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 ARP 2/3 + WASp

ARP 2/3. → It manages to attach ATP bound G-actin to the strand which begins nucleation.

Actin dynamics in filopodia

Multiple actin strands are brought together and it pushes the membrane as it grows. Filopodia allows the cells to probe the microenvironment — cell itself doesn't have to move unless there's a 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.

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

Microfilaments [Intermediate filament]

(+) end - barbed
(−) end - pointed.

Thinnest class of cytoskeletal fibers.
Solid rods of G-actin (~~...~~ 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 (filamin) 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 (Phalloidin, Jasplakinolide) and depolymerisation (cytochalsin D.)
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 classes constituting IF —

Type I and II : Keratins — Epithelial cells & derivatives (hair, nails, horns)
Type III : Desmin — Muscle
Type IV : Neurofilaments — Neurons
Type V : Lamins — 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 donot bind nucleotides; subunit exchange is not confined to ends.
- They lack motor proteins (∵ no polarity)
- They have slower dynamics ⇒ 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 v/s longitudinal interactions within filaments
  ⇒ strength, toughness & elasticity over long ra...

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

Lecture 13 — 12/5

Motor proteins (MP)

Actin based MP are members of myosin superfamily

Skeletal muscle myosin – first identified
↓
Myosin II – elongated protein
2 heavy chains + 2 × (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 aā 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 based on compartments 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. MPs are essentially enzymes that hydrolyse ATP and use energy to "walk"

basically anything

On the other end, myosin could bind to basically anything: adaptors, receptors, lipid moieties, b/w 2 actin filaments, protein and ribonucleo-protein complexes.

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

Actin-MP interaction generates force & its important in formation of filopodia.

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

Actin gets recruited to focal adhesion structures. there 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, filopodium, 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 ~~hige~~. hinge.
This allows two myosins to manuever large cargo when they come across each other.

Kinesin
Dyenin

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
  Dyenin - walks towards the center

Humans : 40 types of kinesins.
Kinesin - + end MP i.e they walk towards + end binding site in the tail
Most carry a vesicle or another microtubule for either a
The tails can be of variable length
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 Kinesis

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

Kinesin

Microtubule

attached

detached

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.

Dyenins - minus-end motor proteins

Composition & conformation of dyenin (similar to kinesin) allow it to 'walk' using ATP.

They're important for cell migration, seperation of chromosomes, ciliary / flagellar movement etc. They're localised to acheive there

Motor domain

Their structures are mostly preserved

← stalk →

Kinesin Myosin Dynein

David Goodsell – structural biologist + artist

14/3

Lecture 15

Cell Migration

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 – sperms, macrophages, protozoa, wound healing etc.

Velocity of cell spans a wide range –
Nerve (10^{-2} μm s^{-1}) to Tetrahymena (10^{2} μm s^{-1})

Macrophage chasing bacteria
Wound healing by sheet movement of epithelial layer

Cell polarization – responds to a certain kind of stimulus

Regulators	Side/Rear	Front
	PTEN	Activated Cdc42 & Rac
		Cdc42 / PARs / aPKC / PIP_3
	Myosin II	Activated integrin
		MTOC / Golgi
		Microtubules.

There's an integration of adhesion and growth factor signalling pathways

PI3 Kinase – important crosstalk-er b/w 2 pathways.

Rho-GTPase switch (Rho, Rac, Cdc42)

These GTPases are activated near the front. They're directly downstream of integrins & GFs.

GF & Integrin engagement
GEF
ATP → Activation → ADP
RHO-GTP | Effector → cytoskeleton and cell regulation
RHO-GDP ⟷ RHO-GDP
RHO-GDI
Sequestration
Inactivation
GAP
P_i

Rho-GTP interacts with effector 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 ~~to~~ rapidly.

The dynamic nature & spatial + temporal localization of the cycle are also v. 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 ⇒ action 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 signalling

Almost same

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

Rho : assembly of actin : myosin filaments
Rac : actin polymerisation to form lamellipodium
Cdc 42 : ——— 〃 ——— to form filopodial 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 & determine protein conformation.

When far away, probes emit diff. freq. emissions 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 GTPases.

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 cell's
Activated Rac

Photo activable Rac used to regulate cell polarity
Similar activation profile of Rho and Cdc 42.

Cdc 42 - 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 center (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.

- Signalling involved in cell migration is extremely tagged complex
Polarization in dictyostelium — PIP 3 is lighted up

Cell 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.

Stable MT
Capping proteins
Unstable

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.
radom – 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, occur 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 polymerisatⁿ & integrin engagement activates Rac

* Rap1 & PK3 increase integrin affinity