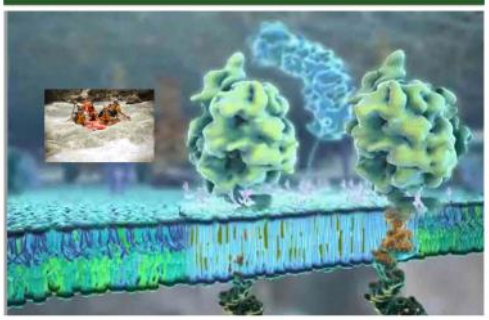
BIOLOGY 2B03

Cell Biology

Lecture 4
Lipid Rafts

Department of Biology — McMaster University

Row, row, row… my lipid rafts!

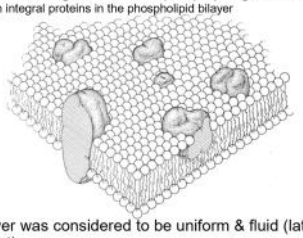


Plasma membrane-*Functions*

- Encloses the cell
- Barrier between internal/external environment
- Selective permeability
- Sensing the environment (receptors)
- Growth and changes in cell shape
- Anchoring cytoskeletal structures
- Mediate cell/cell interactions

Fluid mosaic model of the cell membrane

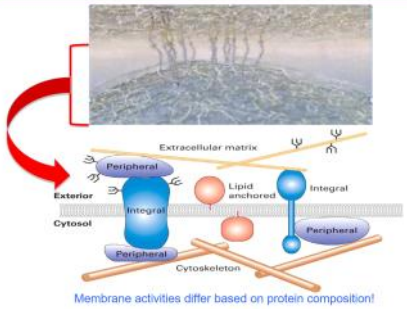
Original figure from Singer and Nicholson (1972) depicting membrane cross section with integral proteins in the phospholipid bilayer



The bilayer was considered to be uniform & fluid (lateral movement)

Long standing question: Why bother with the many classes of lipids found in biomembranes?

Components of the plasma membrane



Membrane activities differ based on protein composition!

Biomembrane composition

Phospholipid components can modify fluidity:

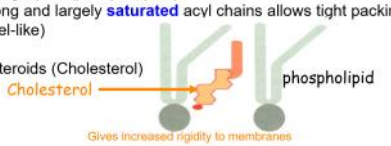
Phosphoglycerides (phospholipids)

- Short and largely **unsaturated** acyl chains that tend to kink and pack more loosely allowing rapid lateral movement (fluidity)

Sphingolipids (phospholipids)

- Long and largely **saturated** acyl chains allows tight packing (gel-like)

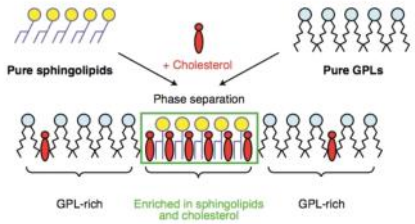
- Steroids (Cholesterol)



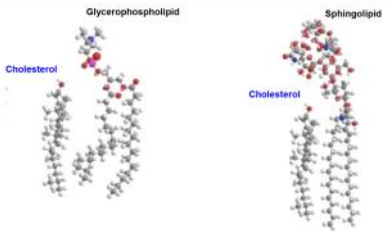
Gives increased rigidity to membranes

Cholesterol favours phase separation

- Cholesterol interacts preferentially (but not exclusively) with sphingolipids, and favours phase separation between sphingolipids and glycerophospholipids



Cholesterol favours phase separation



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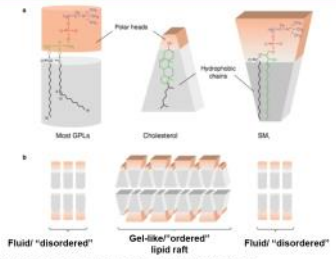
Organization based on lipid shape



- Glycerophospholipids are roughly cylindrical
- Sphingolipids and cholesterol are cone-shaped (fit together well)

The lipid raft concept

progress

Functional rafts in cell membranes

Kai Simons & Elina Ikonen

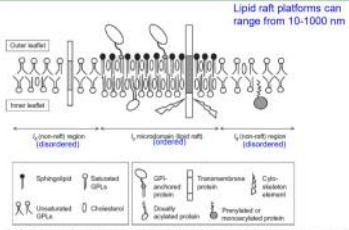
A new aspect of cell membrane structure is presented, based on the dynamic clustering of sphingolipids and cholesterol to form rafts that move within the fluid bilayer. It is proposed that these rafts function as platforms for the attachment of proteins when membranes are moved around inside the cell and during signal transduction.

Nature (1997)

Proposed:
Self-associated properties of sphingolipids and cholesterol *in vitro* could explain the basis of lipid sorting in biological membranes

What are lipid rafts?

Lipid raft platforms can range from 10-1000 nm



- Predominantly sphingolipid and cholesterol-rich microdomains in the **outer (exoplasmic) leaflet** of the plasma membrane *(…composition can change…)*
- Cholesterol and glycerophospholipids also present in **inner leaflet**
- GPI-anchored, acylated & transmembrane proteins also present in raft

Raft "lifetime" can vary from ns to mins (i.e. signaling)

Function of lipid rafts (health & disease)

- Signal transduction
- Lipid rafts for changes in shape during migration
- Adhesion rafts (i.e. immune cells –Inner Life of the Cell video)
- Lipid rafts that help form the blood-brain barrier

Disease:

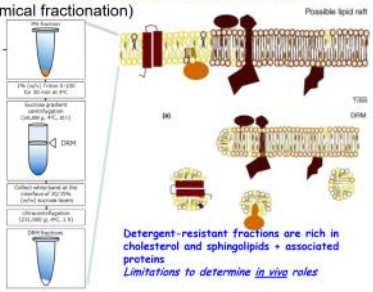
- Cancer cells have a higher level of lipid rafts
- Amyloid plaque accumulation –Alzheimer's
- Lipid rafts can be targets of different pathogens
- Raft localization of cellular prion protein which facilitates conversion to pathological form

Lipid rafts- *an animation*

http://www.youtube.com/watch?v=SjWnkFpbjgQ

Methods for studying lipid rafts

Detergent-resistant membrane fraction isolation
(biochemical fractionation)

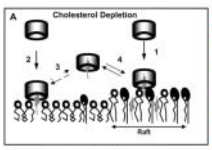


Detergent-resistant fractions are rich in cholesterol and sphingolipids + associated proteins
Limitations to determine in vivo roles

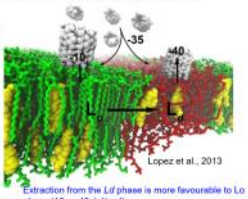
Methods for studying lipid rafts

Cholesterol depletion with cyclodextrins

- Have a hydrophobic cavity that extracts cholesterol
- May remove cholesterol from raft and non-raft areas along with other phospholipids with prolonged exposure
- Can use other chemicals: inhibit synthesis & sequestration



Zidovetzki and Levitan, 2007

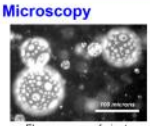


Lopez et al., 2013

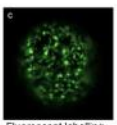
Extraction from the *Ld* phase is more favourable to Lo phase (10 vs 40 kJ/mol)

Methods for studying lipid rafts

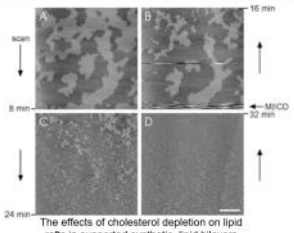
Microscopy



Fluorescence of giant unilaminar vesicles



Fluorescent labelling of stomach cells

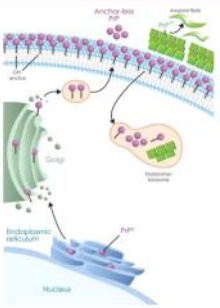


The effects of cholesterol depletion on lipid rafts in supported synthetic lipid bilayers with atomic force microscopy
+*cyclodextrins*
(nm resolution)

Other techniques: i.e. single molecule tracking, FRAP etc
Limitations to artificial membrane use

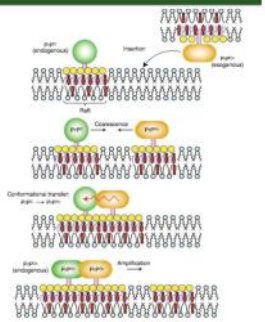
Recall: Prion proteins

- PrP^{C} proteins are synthesized, folded and glycosylated in the ER
- GPI anchor is a glycolipid that is attached to the C-terminus as a post-translational modification
- Two fatty acids in the GPI anchor the protein to the cell membrane
- PrP^{Sc} accumulates near PrP^{C} leading to potential for conversion
- Conversion can also occur in endosomes or lysosomes



Prions- *transmission from infected cells via lipid rafts*

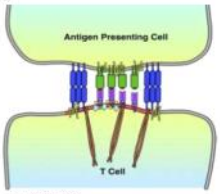
- Conversion of endogenous PrP^{C} requires insertion of PrP^{Sc} into the continuous membrane
- Coalescence of lipid rafts allows close contact between PrP^{C} and PrP^{Sc} proteins
- Allows PrP^{C} to PrP^{Sc} conversion to occur
- Infection is then propagated on the surface of the host cell



Lipid rafts and the immune response

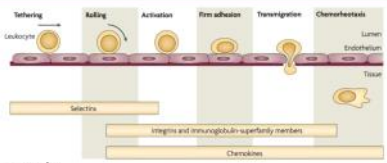
Dependent on the ability of leukocytes to:

- migrate to regions of infection (inflamed tissues)
- migrate to regions of antigen presentation
- respond to the inflammation



Head et al., 2014

Leukocyte response to vascular inflammation



Multistep cascade:

- Selectins on endothelial cells + leukocyte glycoprotein =initiate tethering
- Binding of chemokines to leukocyte GPCRs can occur as leukocyte slows down
- Triggers intracellular signaling pathways that activates leukocyte integrins, assembles lipid rafts and allows leukocyte to makes firm adhesion to endothelial intercellular adhesion molecules (ICAMs)
- Leukocytes follow chemokines (transendothelial migration)

Weber et al., (2007) Nat Rev Immunol

Lipid rafts are involved in multiple functions in the body

Inflammation of skin

A brake like mechanism on the human body

Allows homing of the cytokine, creates gradient through the body.

Tethering done by lipid rafts. When leukocytes start to slow down, they slow and start to roll.

Most of cells in blood are living in suspension, but they differ in many traits.

Activation to have it move on tissue is different. A cell that is mobile firmly adheres to and transmigrate the endothelial cells

As a result, we have inflammation. Cell uses the cellular adhesion molecules to

Random Quiz In class…

1) Which of these is a correct statement? D
2) HSP 70 family of chaperones does not include? B) ATCase
3) Phosphorylation involves the transfer of a phosphate group from ATP to the OH of which amino acids? D)

Cholera Diagram
-Shows what happens in the presence of cholera

-Bacteria secretes toxin that binds to GM1, which affects the lipid raft

Stimulates the CFTR gene, which induces water to be secreted into the intestine, which causes massive amounts of diarrhea.

Cholera is preventable and treatable. 100 000 deaths per year

Familial Hypercholesterolemia

- Low-density lipoprotein (LDL) and cholesterol elevated in blood plasma
- Premature arteriosclerosis
- Xanthoma in skin and tendons
- Decreased life expectancy
- Autosomal dominant
- Mutation in LDL receptor gene

1. General features

2. Xanthoma formation

3. Arcus lipoides

Genetic disease: Even if you are a carrier, you will show the negative phenotype. If you are Homozygous you will die early in life

Due to high amounts of LDL.

LDL is transported cholesterol. The function of LDL is to take cholesterol and provide it to cell.

It is actually good.

If you have a lot of LDL, the LDL get modified by other factors and becomes more sticky due to oxidation

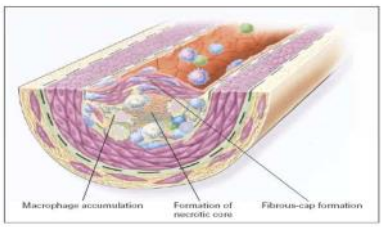
In this disease, Pt will naturally have a lot of LDL in blood.

Become Erthrogenic=deposits of lipids

Decreases life expectancy, a lot of patients will have

It is a result of a mutation in the receptor for LDL

Formation of an advanced, complicated lesion of atherosclerosis



(Ross R. N Engl J Med. 1999. 340(2):115-26)

Atherosclerosis: WE ALL HAVE THIS

This process occurs when you are still in womb. Its what we do afterword that determines whether this process becomes detrimental.

A lot of bad things change the properties of LDL

Heart is highly vascularized. The heart needs this. Blood cells migrate through the vessels.

Because of various irritants to the endothelial layer, it becomes inflamed. Cells start to come into the layers. These cells start to take up large amounts of LDL. It becomes a foam cell, highly irritated cell, and stays under the endothelial of the artery. Stimulates a lot more cells to come which leads to accumlation

Deposits start to narrow the lumen of the artery.

Area of accumulation of foam cells is known as a plaque formation

Fibrous cap=endothelial pushed up.

Lots of these=low oxygen to heart=Ischemia

When fibrous cap is ruptured, all of the plaque is exposed and a clot forms. Forms a occlusion of the artery and the heart stops.

Lipid rafts and the inflammation response

http://multimedia.mcb.harvard.edu/anim_innatelife.html
Narrated: http://www.youtube.com/watch?v=FzoTgroMdZk

Video

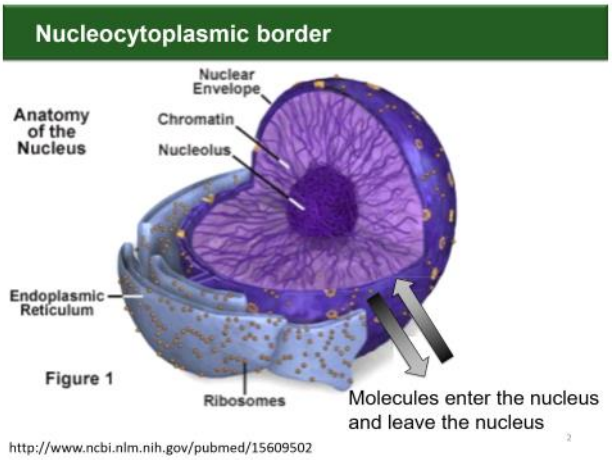
Lipid rafts- *questions remain*

- Structure and function hard to determine
- No consensus on lipid raft size (10-1000 nm)
- No known time scale for lipid raft existence
- Without complete understanding of structure and function, it is hard to understand the precise physiological role of these lipid microdomains
- However, scientific approaches do confirm this compartmentalization and role in many cellular processes & role in health and disease

Wrap up

Nuclear ports: it is an intriguing part of the cell.

We spoke of the nucleus at the city hall. It has to be pretty well protected, but there has to be an efficient way to bring things in and out



The nuclear envelope has holes in it. Allows the entry of regulated materials

They are in continually. ER and Nuclear envelope shares the same membrane.

It is a same sheet of membrane that follows around the nucleus.

Nuclear pores

They do an image of the nucleus

There is so much in and out of the cells. The nucleus has so many gates.

First evidence of protein transport into nucleus

John Gurdon:
Injection of histone proteins into cytoplasm

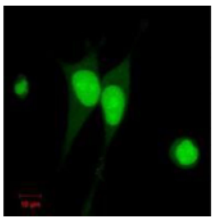
Isolated histones and injected it into the cytosol

William Bonner:
Labelled nuclear proteins injected into cytoplasm

Took proteins labelled with a radioactive dye, and injected them

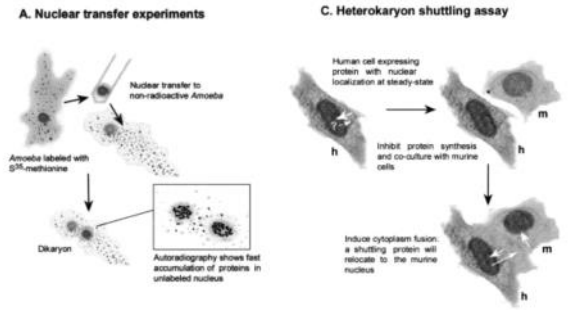
Is there a nuclear localization signal?
Can we test what is necessary?

Can we relocalize GFP to the nucleus?



See that all of the GFP is inside the nucleus of the protein

Nuclear shuttling: Import and export



Is there a separate NLS and NES?

http://www.sciencedirect.com/science/article/pii/S0014579301024875

The experiment is as good as the data it creates

You take nucleus from and put radioactive amino acids.

Dikaryone. You can see that the radioactive proteins are showing up in both nuclei

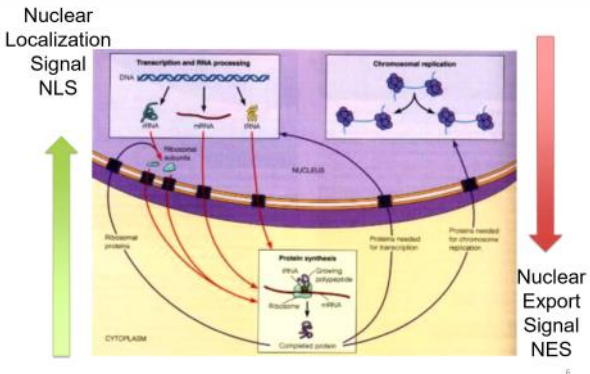
Proteins are transferred between both nuclei. Shows that it is an in and out process.

Transport takes stuff in and out

Heterokaryon cells have multiple nuclei.

Human cell without cell transfer, add molecules that disallow from fusing. Inhibit protein synthesis

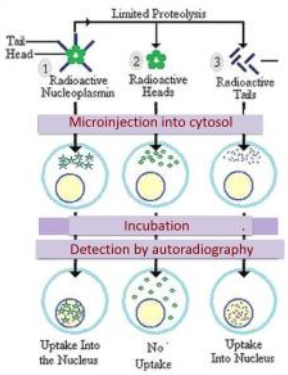
What kinds of proteins are transported?



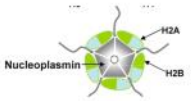
What kinds of proteins are transported?

Are all proteins going in or are they selected.

First evidence of a nuclear transport signal



A domain in the tail specifies transport of nucleoplasmin (pentamer) into the nucleus.



Dingwall C, Sharnick SV, Laskey RA. 1982. Cell. (2):449-58.

What is the evidence for a nuclear transport signal?

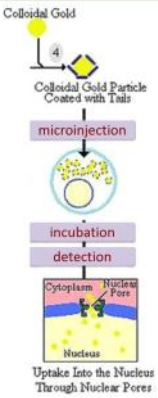
We can GFP, or we can break up the protein

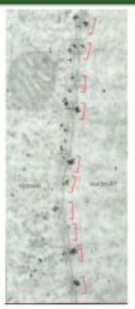
You have to stop an area that is responsible for the protein.

We cut up proteins slowly, we know that it is in a specific region.

Take radioactive parts and put into solution with

Sufficiency of signal:





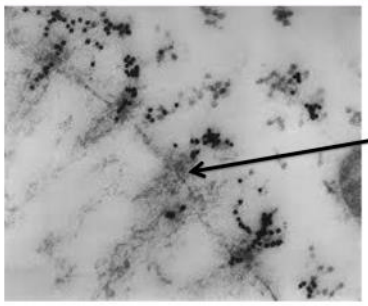
Visualization of gold particles in TEM (electron-dense, black spots)

This an older technique, label with gold molecules which are electro dense

We see as black spots

We can see pores and the

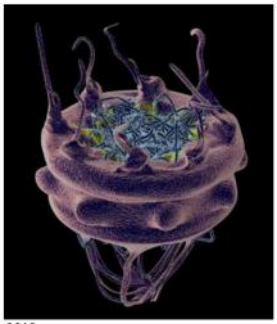
TEM view of gold particles entering the nucleus



The nuclear pore complex (NPC) acts as a selective barrier between the nucleus and the cytoplasm and is responsible for mediating communication by regulating the transport of RNA and proteins.

Label with gold particles for it to enter the nucleus and the cytoplasm

Nuclear import is a two-step process

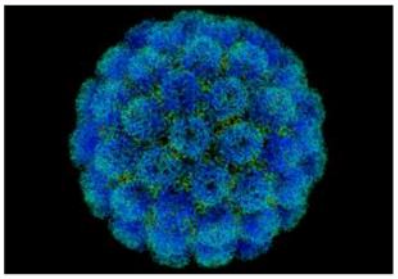


S.Patel and M.Rexash, 2012

https://www.youtube.com/watch?v=UyhqLpjicZg
https://www.youtube.com/watch?v=k4YcpGmn4rk

This is a 3d structure of the nuclear pore

Identifying a nuclear localization signal (NLS)



SV40 consists of an unenveloped icosahedral virion with a closed circular dsDNA genome of 5.2 kb. The virion adheres to cell surface receptors of MHC class I by the virion glycoprotein VP1. Penetration into the cell is through a caveolin vesicle. Inside the cell nucleus, the cellular RNA polymerase II acts to promote early gene expression.

The SV40 large T-antigen has been used as model to protein to study nuclear localization signals (NLSs).

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Viruses, are by small little particles that can infect the cell.

A virus is like a parasite, infects and modifies DNA.

The most important thing for a virus to do is to enter the nucleus.

All they have to do is get into the nucleus, this involves the gates

SV40 virus Double stranded DNA virus. It gets into the cell into the lipid raft.

Has a structure that has things sticking out. Virus are classified based on their genome. This is a small thing.

It has to hijack and reprogram the DNA of the cell. Most viruses work like this

Getting into the cell is part of the problem

Some Viruses need another virus to function well.

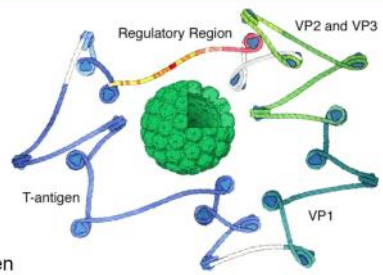
All need to get to the nucleus, which they have to go across.

SV40 binds to cell surface, MHC class receptors. Binding to the surface allowing it to penetrate through.

Cavleolin is on a lipid raft, which the virus passes through as it passes into the membrane.

Creates an antigent which is related to the breast cancer

SV40 genome: 4 genes



T-antigen
Required for viral genome replication

VP1, VP2, VP3
Genes for capsid proteins

http://www.rcsb.org/pdb/101/motm.do?momID=47

12

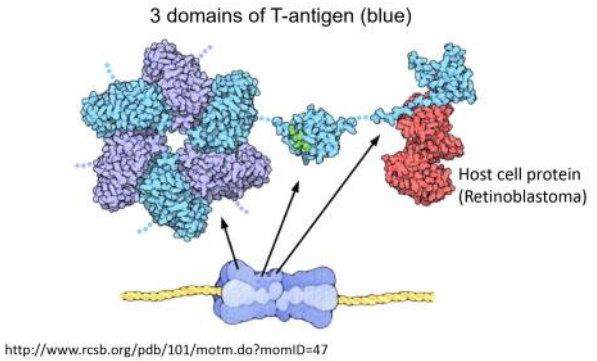
T-antigen= required for genome replication

Hijacks the DNA

T-Antigen is required for viral genome replication. It has a very small genome.

Viruses hijack cells and use it to create more clls. Regulatory components attract transcription factors. Directs the application of viral genomes.

T antigen directs replication of viral genome



http://www.rcsb.org/pdb/101/motm.do?momID=47

T antigen gains entry to nucleus

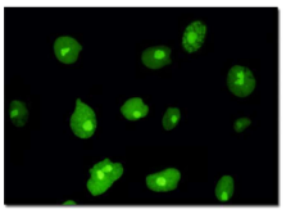
The SV40 virus can hijack the cells nuclear import machinery to get the T-antigen into the nucleus.

The T antigen protein is the only viral component required to get access to the cell.

How do we know?

The T antigen protein was tagged with GFP and injected into the cytoplasm and it traveled to the nucleus.

http://www.microscopyu.com/



How do they hijack the cell nuclear machinery?

Sends T-antigen, inside the cells. Hijacks the cells.

We took and injected the T-antigen, we can see it enter the cell.

Which part of the t-antigen is responsible for the cell?

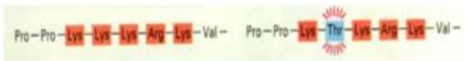
We start deleting parts until we nail the part that is responsible.

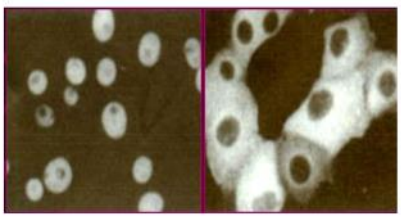
We want to find out how the cell finds the cells nuclear genome port?

T-Antigen=tagged with fluorescent tag.

Which sequences are necessary?

Design an experiment to determine which sequences of SV40 T-antigen are necessary for nuclear transport.





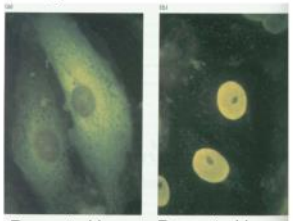
How to determine which component of this protein is passed through the pore.

Tag GFP to the protein

Mutate a part of the proteins, we see that change in one protein prevents it from going to the nucleus.

Which sequences are sufficient?

Design an experiment to determine which sequences of SV40 T-antigen are sufficient for nuclear transport.



Pyruvate kinase Pyruvate kinase + NLS

The T antigen sequence required for entry is: proline

NOT PART OF THE MIDTERM!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

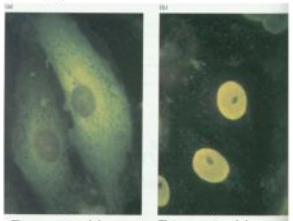
Only up to LIPID raft is lecture

Tag it to a marker. We want to find which proteins is sufficient. Attach to another protein and see if it enters the nucleus.

We can also put it into a computer to find sequences.

Which sequences are sufficient?

Design an experiment to determine which sequences of SV40 T-antigen are sufficient for nuclear transport.



Pyruvate kinase Pyruvate kinase + NLS

The T antigen sequence required for entry is: proline-lysine-lysine-lysine-arginine-lysine-valine (PKKKRKV)

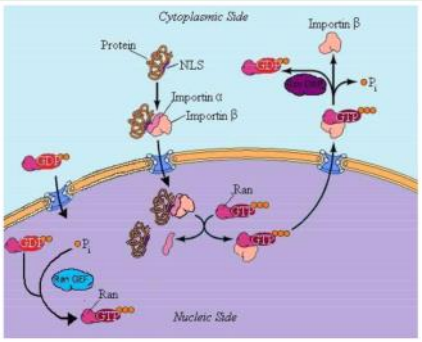
NOT PART OF THE MIDTERM!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!

Only up to LIPID raft is lecture

Tag it to a marker. We want to find which proteins is sufficient. Attach to another protein and see if it enters the nucleus.

We can also put it into a computer to find sequences.

Import machinery



http://en.wikiversity.org/wiki/Membrane_Assembly:_Signal_Hypothesis

Two proteins that are important
Importin A and B. They will bind to you and take you into the nuclear core.

Alpha and Beta will pass through, dissociate.

Ran dissociates the Importin to dissociates from the protein.

NLS

This limits the speed of nuclear protein.

Viral exploitation of host machinery

- Entry into host cell of of viral capsids
- Transcriptional and translational machinery:
Transcription of viral genome to produce viral proteins:
e.g. capsid proteins, replication factor (SV40 T antigen)
- Replication machinery:
Replicate multiple copies of viral genome independently of host S-phase.
- Exploiting the import machinery:
Translated factors are imported into nucleus
e.g. SV40 T antigen

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Evolution has come up with many ways to prevent virus from entering cells. However, so will virus.

Exploitation of transcriptional and translation machines. CASPID and SV40 T Antigen

Virus has to be acting independently of what is happening in the cell.

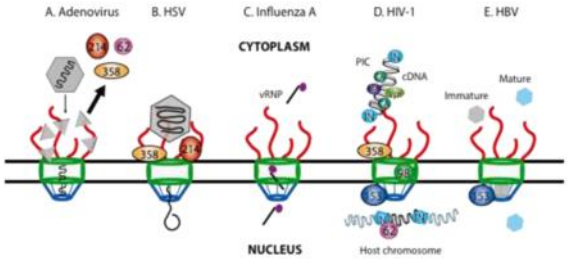
Uses that to make more virus. The virus is filled with viral material, until it bursts. Work with the cell to keep it alive,

NLS signal is figured out if by virus for it to enter the cell. Utilized by its own proteins.

It's how to virus hijacks the cell.

Exploiting import machinery for genome import

Viruses have evolved various mechanisms for accessing the host cell nucleus:



http://www.mdpi.com/1999-4915/5/8/2019/htm

19

The first thing we get infected with is the cold virus.

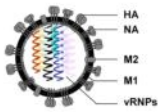
Immunization allows for protection of the body from the virus.

All viruses must interact with a nuclear pore. Every virus has its own way to deal with the nuclear envelope.

Influenza has an NLS signal. Huge diversity of mechanisms for the infiltration of the cell.

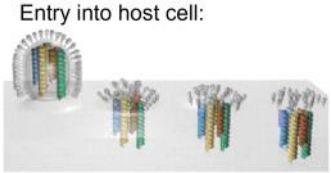
Influenza A virus capsid and genome

Viral capsid:



The genome:
eight single-stranded, negative-sense RNA molecules (complementary to mRNA)

Entry into host cell:



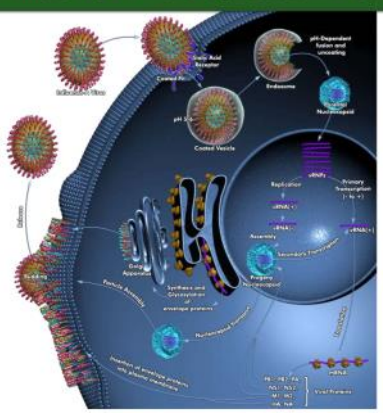
20

Influenza A is a famous virus. It was deadly at some point in human history. Pls take flu shot.

It is an RNA based virus, 8 single stranded, negative sense RNA molecules.

It has complementary RNA, which means. It is needed for reverse transcription. Which is used for creating viral DNA

Influenza A virus replication virus



1918-20 Spanish Flu Pandemic killed more than 100 million people

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Spanish Flu Epidemic killed 100 million people. Puts a lot of stress on the body.

In the nasal, binds to a cell, connects to the flu virus.

Two proteins are located on cell HA and NEU proteins. HA binds to sugar on cell. We can rinse nose with the sugar to clear virus.

HA AND SYALIC ACIDS BIND TO EACH OTHER THIS IS ON EXAM!!!!!!!!!!!!!!!!!!!!

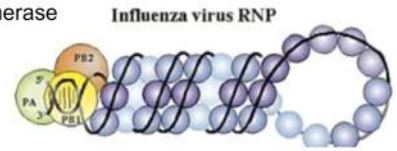
Lysosomes have low pH…

***Lysosome is a structure involved in recycling. Recycling is dependent on low ph. Ph is about 4.5, these enzymes are only active at around 4.5. If lysome is broken, they will not be functional!**

Virus breaches lysosomal membrane to which is it free into the lysosome. The Viral DNA is packed as little rod.

Influenza A viral ribonucleoprotein particle vRNP

RNA polymerase



Each genomic RNA is individually packed with several copies of the viral nucleoprotein (NP) into ribonucleoprotein particles (vRNPs).

37 to 97 copies of NP per RNA

22

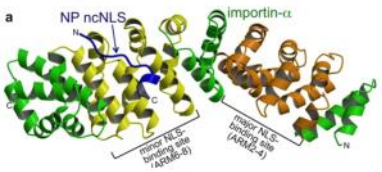
This is viral DNA, called RNP. This this packed.

You send this to nucleus, as well as accessory proteins

For every 1 entry, you have 1000 exits

Nucleoproteins (NP) have two NLS sequences

NP contains at least two putative NLSs, one at the N-terminus (NLS1) and one in the middle (NLS2) of the protein. NP NLSs have been shown to mediate the nuclear import of recombinant NP molecules.



Which NLS mediates the nuclear import of influenza vRNP complexes?

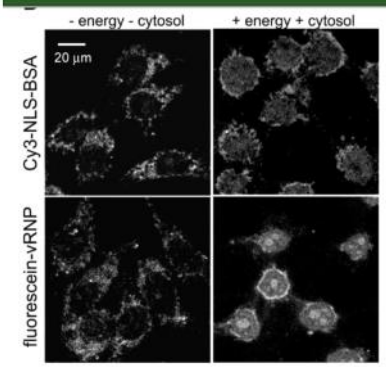
May 2015
http://www.nature.com/articles/srep15055

How does the rod and the protein transported to the nucleus. If we interfere, we can prevent infection of viruses.

Virus has made two NLS.

We crystallize Importin A, recognize NLS. Internal NLS, leads to tight binding

vRNPs are competent for nuclear import.



Fluorescein-labeled influenza vRNPs are competent for nuclear import.

Wu et al, 2009.

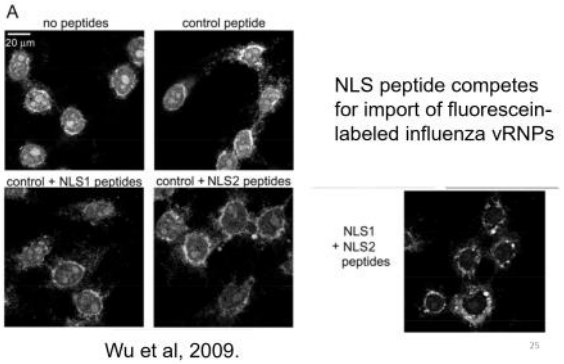
24

vRNPs and we wanted to see which NLS is functional. Try to manipulate it to get functional proteins

We infected cells with the virus, we see that there is less transport.

If you provide cytosolic components, the cell does well and is imported.

NLS peptide inhibits import of vRNPs



NLS peptide competes for import of fluorescein-labeled influenza vRNPs

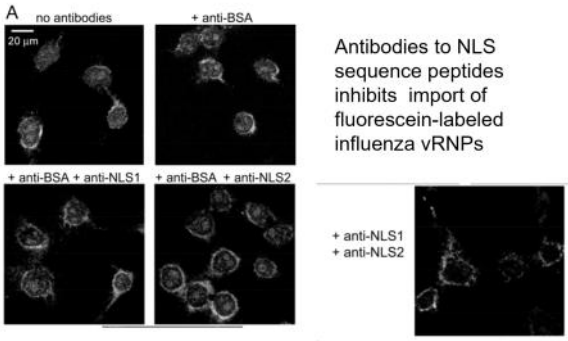
Wu et al, 2009.

25

NLS peptide. Competing between the ligand and the binding protein. If we add a peptide that resembles NLS to compete. NLS will not have access with importin A.

We want to figure out which NLS works. We use a peptide to compete out NLS 1, we do the same for the NLS 2, for both, we see that they both work to get the virus into the cells. If you add both peptides to compete with NLS 1 and 2, than there is no transport at all!

Antibodies to NLS inhibit import of vRNPs



Antibodies to NLS sequence peptides inhibits import of fluorescein-labeled influenza vRNPs

Wu et al, 2009.

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Summary

- Inhibition of either NLS1 or NLS2 on NP proteins reduces import of nRNP.
- Inhibition of NLS1 and NLS2 has an additive effect, so both are important for nuclear import of vRNP and viral infection.
- Development of antiviral therapeutics will require targeting both NLS1 and NLS2 (maybe a third?)

Wu et al. 2007. Nuclear import of Influenza A viral ribonucleoprotein complexes is mediated by two nuclear localization signals on viral nucleoprotein. Viral J. 4: 49.

Inhibit of NLS1 or 2 reduces import,

Anti-viral need to target NLS 1 and NLS 2.

PROTEIN HAS HA AND NEU, syaladase. It is involved with the release of the virus. Can we find inhibs that block this?

Syaladase in humans is similar to virus. This has a bad thing when inhibited.

But we came up with Tamiflu. Inhibits the viral version and not the human. Said that it would reduce the time sick.

Prof gave it to mice, 14 days and same dose to humans. This drug will fuck you up.

BIOLOGY 2B03

Cell Biology

Lecture 6

GPCRs and Drug Design

Department of Biology — McMaster University

Signals induce intracellular responses

- A signal will target a cell
- The signal is received
- Receiving of the signal is dependent on specific receptors for that signal
- Signals can include: physical stimulus, chemical molecules (small molecules, gases, peptides, soluble proteins, proteins bound to surface of cell or ECM)
- The end result of a signal cascade can lead to short term or long term effects

Which are fast? slow?

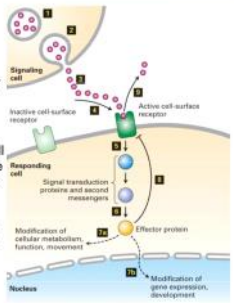


Fig 15.1

Oxygen Can act as the signal, as well as nitrous oxide.

Nitroglycerine is a example of this, this is a vasodilator.

Hydrogen sulphide is also an example. Rotten eggs farts. Release of gas

More common or peptides

Insulin is also a signal receptor

LH and FSH in the body as well. LH goes from the brain to the ovaries.

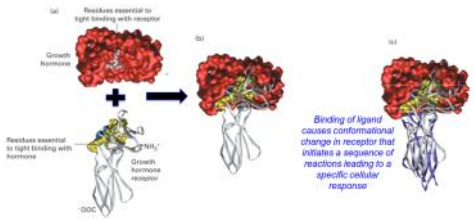
End results can be long term or short term. Signalling has multiple components.

A lot of research has gone to signalling

EGFR is an example,deletion of a kinase. Drugs targert the kinase to allow patients to live cancer. Adenocarcinoma EGFR

Binding of ligands activates receptors on target cells

- Receptor proteins are located within target cells
- Each receptor binds a specific ligand, or closely related molecules
- Ligand binding depends on weak, multiple noncovalent forces (ionic, van der Waals, hydrophobic) & molecule complementarity



Basic idea. When two molecules bind tightly, they affect how the structure is of both proteins.

When they bind, they change the cytosolic portions of the receptors.

GTP-binding proteins are common On/Off switches

- Switch proteins turn downstream proteins on or off in a signal cascade
- This includes the GTPase superfamily
- A signal will activate the GTPase though a GEF
- Hydrolysis of the bound GTP converts the GTPase back to the inactive form

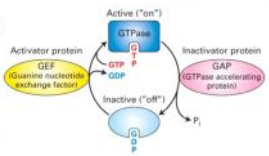


Figure 15.6
Molecular Cell Biology, Seventh Edition
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Examples of GTPase switches:
-Trimeric G proteins (Bound to receptors)

-Monomeric G proteins (not bound to receptors, include Ras proteins)

G proteins associated with GTP binding proteins.

Can turn on or off certain pathways.

GEF is an activator, GAP is a inactivator. G

GEF=GDP to GTP
GAP= removes the P groups.

Protein kinases and phosphatases are everywhere!

- The human genome encodes about 600 protein kinases and 100 different phosphatases
- Kinases add phosphate groups to specific amino acids (in animals: serine, threonine, tyrosine)
- Phosphatases remove phosphate group from specific residues on target proteins
- Kinases can be cytosolic, or a receptor itself can posses intrinsic kinase activity or be tightly bound to a cytosolic kinase

Figure 15.4
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Why phosphorylate?
In many cases, adding a phosphate group allows for a second protein to bind and interact with the first protein

Cells depend on other cells.

Phosphorylation happens on certain amino acids.

Theronine, serine and tyrosine. These guys are the site of phosphorylation

Kinases phosphorylate these, phosphatases remove this.

600 kinases and 100 phosphatases

Receptor can self phosphorylates itself. All it involves is adding a phosphate group to the protein.

Kinase interacts it, now it can interact with other proteins.

Intracellular second messengers transmit signals

- Ligands=first messengers which lead to a change in the concentration of low-molecular-weight second messengers
- Second messengers bind to other proteins and modify their activity
- Include: calcium, cAMP, cGMP, DAG, IP3

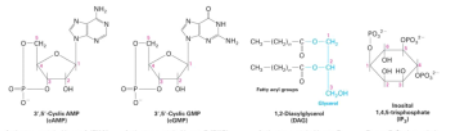

Figure 15.8
Molecular Cell Biology, Seventh Edition
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Second messengers need a first messengers.

But first messengers are the ligands that bond to the receptor.

Second is the other proteins that go to the first messenger.

Our nervous system is all done through signal transduction, happen very fast.

These all happen to fill memory.

cAMP does a lot of stuff, binds to protein and allows protein translation

IP3 and DAG, each of all of these have a job. KNOW IP3 RELEASE CALCIUM

Release of calcium into the cytosol creates exocytosis. Uses IP3

CAMP protein kinase a activation
cGMP protein kinase G activation

DAG activates kinase C

Signal transduction allows for signal amplification

Binding of one ligand can lead to activation of hundreds of thousands of proteins

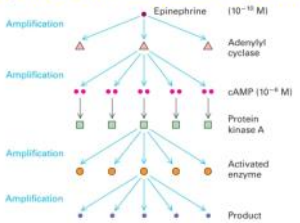

Figure 15.9
Molecular Cell Biology, Seventh Edition
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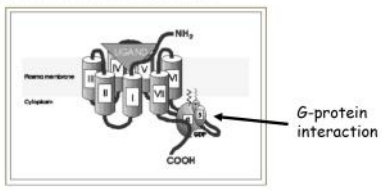
You have a cascade

Amplification steps happen.

This is what signalling is all about. Imagine how a system control so much. These are all controlled by multiple systems.

G Protein-Coupled Receptors (GPCRs)

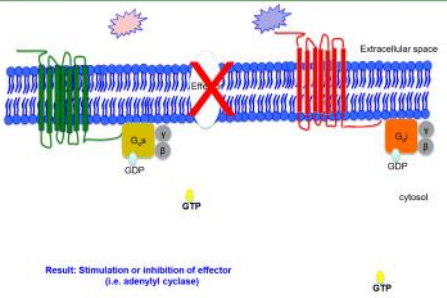
- All share a common seven transmembrane spanning domains
  - -7 Transmembrane α-helices
  - - 4 extracellular segments (E1-4)
  - - 4 cytoplasmic segments (C1-4)
- Linked to small, trimeric G-proteins



Proteins with multiple transmembrane domains.

Very hard to create. Cytosolic parts convey the signals

GPCRs interact with G-proteins once ligand is bound



Components of cytosolic are the transduction pathway.

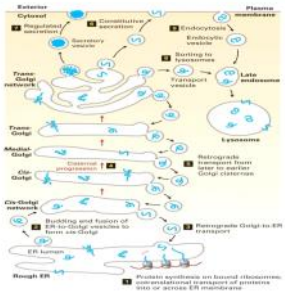
GTP binds to effector molecule

Can have a negative signal.

This is a dual effect system.

Turn it off or on.

Overview of protein traficking



ER is a shoe factory. Passes through the golgi, inspected and modified and sent.

A lot of different area where you want to send your product too.

Receiving products is also very important.

Shoe factory (ER) needs to have factory workers (proteins) that needed to keep in the ER.

Signal on ER molecules that bring them back.

KDEL is the signal that brings the proteins back. You are trying to keep the proteins you need in the ER, if you cannot do this, you lose factory workers, you need a retrieval mechanism

Use the same way to look at other factories.

There was a guy who disputed the golgi.

JP LeBlanc
Graduated in 1942

He was the first one to postulate that there is a golgi

The Canadians did it first. George Palady got the nobel prize for this

What's the relationship between Golgi and ER. ER and Golgi are separate entities.

ER are not as dynamic as people think.

Kris discovered a substance that could disintegrate the golgi.

Found mechanism for moving stuff from one compartment to another

Making vesicles that form golgi. A critical size in which the Cis matures enough to move up. It is a continuous progression of cisternae. From CIS golgi to trans golgi, dependent on vesicles moving in and out, called specialization

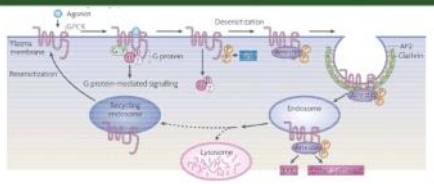
Manno 6 phosphate. How do you get specialized golgi sacs? VESICLE RETRIEVAL

Once retrieval is too inefficient, the cisternae move up. As it is dynamic, it is highly specialized.

Sugar are added on, but why? They determine how long something remains in the blood. Determines circulation.

Regulated: Stored in cell until you get a signal. Calcium is regulated, IP3 On TEST

GPCR activity can be regulated



Nature Reviews | Molecular Cell Biology

- Binding of an agonist to a GPCR leads to the activation of heterotrimeric G proteins
- With persistent stimulation, GPCRs are phosphorylated by GPCR kinases (GRK) and association with arrestins
- Arrestins interact with clathrin and clathrin adaptor (AP2)
- GPCRs are internalized into endosomes, targeted to lysosomes for degradation or dephosphorylated and recycled back to the cell surface

GPCR could be various forms, but they are called coupled because they are coupled to G protein, used as on and off switch

Receptor receives a ligand(agonist).

Signal is transduced. G-protein dissociates, and allows for phosphorylation.

Attracts Arrestin, which induces, endocytosis. Endocystosis=down regulation of receptor.

Involves clathrin, brings it into a coated pit. Becomes an endosome, woth arrestin.

Can be sent to lysosome to be degraded, or upregulated

LDL goes through the same pathway. Mutation in LDL receptors can lead to hyperchol

Mutations can be on receptor, or can be something associated with receptor.

GPCRs and Homeostasis

Table 14–3 Average Daily Water Gain and Loss in Adults

Intake	
In liquids	1400 mL
In food	1100 mL
Metabolically produced	350 mL
Total	2850 mL
Output	
Insensible loss (skin and lungs)	900 mL
Sweat	50 mL
In feces	100 mL
Urine	1800 mL
Total	2850 mL

Vanders Human Physiology

Normal water intake = Normal water Loss

One of the most important process is water intake and output.

Electrolytes, effect a lot of stuff

Daily intake and output is mostly the same. The body tries to balance this amount

Look at bloodwork to see how stable a patient is. Tells you this person is in an acceptable homeostatic position

Electrolyte is the most telling vital.

Normal water intake should equal normal water loss

Kidney regulates this

Proteins that are continually made=Constitutive secretion

Mannose 6 phosphate=brings it to the lysosome. This is lyosomal transport signal.

Chloride channel and proton ATPase start being sent to the lysome. These produces HCL in the lyosome, lowers the PH of the lysosome

Ph is a protection, if lyosome bursts, they will not eat the cell

Cis golgi is also known as intermediate cisternae.

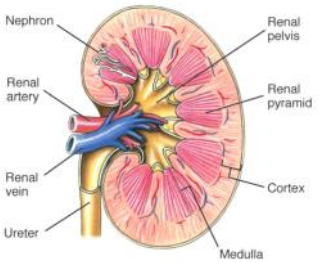
Flattened sacs known as cisternae.

Secretory granules are stored in the cell. A signal will trigger a release. Calcium influx causes secretion

Late endosome with more and more enzymes turn into lysosomes

Cytosol is 7-7.4
Transgolgi-6.5

Water balance is regulated at the nephron



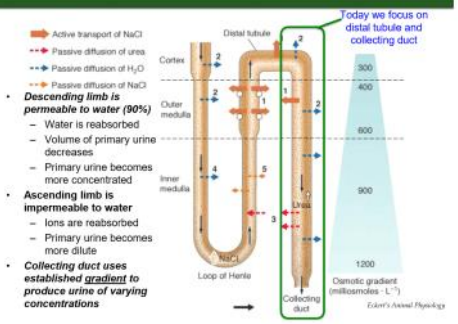
Kidney tubes rely on osmatic difference

Difference changes from outside to the middle

Nephron is distillation area, blood purification

Nephron created to have many functions.

Osmoregulation at the nephron of the kidney



- ***Descending limb is permeable to water (90%)***
  - Water is reabsorbed
  - Volume of primary urine decreases
  - Primary urine becomes more concentrated
- **Ascending limb is impermeable to water**
  - Ions are reabsorbed
  - Primary urine becomes more dilute
- ***Collecting duct uses established gradient to produce urine of varying concentrations***

Nephron is loops of tubes

Traits start to change as you move around the nephron.

ANATOMY IS IMPORTANT,STUDY THIS

Osmatic gradient is different from outside to inside. Coupled with receptors to allow active transport

Kidney main function is to balance blood, get rid of urea.

Loop of Henle=active transport of NaCL.

Diffusion of urea as you go back up

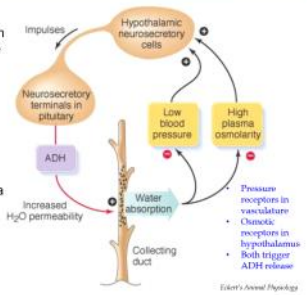
Cortex=massive active transport of sodium chloride.

Trap urea out of the blood

Collecting duct is the last run, water is diffused out of the body.

Constant monitoring to maintain homeostasis

- Osmolarity of blood is under feedback regulation by **antidiuretic hormone (ADH)** on the collecting duct
- **Increases** water permeability (*stippled area)*
- Counteracts low blood pressure and high plasma osmolarity



How does it happen?

Distal tubule and collecting duct

This is under the control of the brain.

ADH is a hormone that involved in many diseases.

For any disorder, ADH is can be the cause
Obesity is a multifactorial problem, many things can be used to cause it.

Blood pressure is multifactorial.

ADH is related to blood pressure. It starts with hypothalamus.
Secretes ADH from the pituitary.
ADH to kidney.
Increase water permeability to intake more water.

Water absorption will cause low BP and high plasma osmolarity.

This causes the brain to release more impulses for ADH. Always have a negative or a positive feedback in endocrinology.

Counteract=the red circles

Hypothalamus is running the show. Tiny motor in the brain that controls a lot of things. Controls pituitary.

Controls menstrual cycle.

ADH receptors and aquaporin channels

Basolateral ADH receptor
&
Luminal Aquaporin2

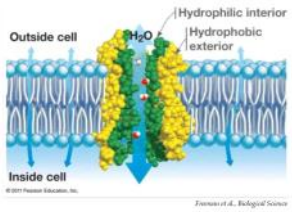
are needed for water permeability of principal cells of the collecting duct

How does ADH work?

ADH binds to aquaporin. Hole for water. Those two component are crucial

Aquaporins allow rapid transport of water

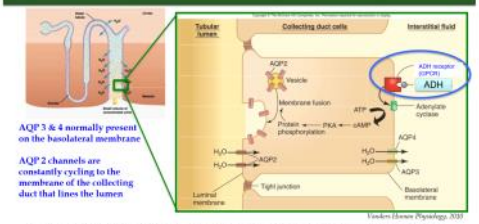
- Aquaporins are exclusively permeable to water
- These protein channels allow water to move across the cell membrane through osmosis
- Movement across is concentration gradient dependent



Aquaporins allow water to move across the cell membrane. Only dependent on the gradient. How does Adh increase water permeability?

ADH bi

ADH increases water permeability



- The activity of the ADH receptor leads to activation of protein kinase A which leads to AQP-2 receptor phosphorylation & an increase in AQP-2 receptor vesicle fusion; reduces the rate of endocytosis (receptor recycling)
- End result: more AQP-2 receptors are inserted & remain at the luminal (apical) surface, increasing water permeability

ADH binds to receptor. Forms CAMP,activates the pathway. Activates PKA,

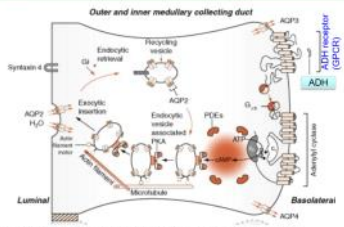
One molecule for thousands signals

PKA phosphorylates Aquaporin. When phosphorylated. Conformation change and internalization of receptor. If its internalized, it is done.

We are able to control hormone that comes into the brain, receptor ignites whole messenger, PKA activates by Camp, acts on target.

A signal that comes through the body binds to a receptor, makes Camp, stimulate or induce PKA, enable to aqp-2 to bind to cell membrane

ADH receptors are GPCRs



- Located on the basolateral (non-lumen) side of the distal tube/collecting duct
- Binding of ADH allows G_{α} subunit to activate adenylyl cyclase, which synthesizes cAMP from ATP
- cAMP activates protein kinase A (PKA) which helps with exocytic insertion of AQP-2 into luminal membrane, and allows water into collecting duct cells for reabsorption into the blood

Bichet, (2009) *Progress in Molecular Biology and Translational Science 89:15-29*

Slightly more details. More info to fill in the holes. ADH binds to the receptor, GPCR, stimulation component, makes CAMP and activates pkA, CAMP binds to PKA and binds to vesicle and proteins associated.

If it is internalized, allows vesicles to be recycled back.

This involves phosphorylation.

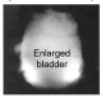
THIS IS IMPORTANT

Congenital nephrogenic diabetes insipidus

- **Inherited in an X-linked recessive fashion and is caused by mutations in the ADH receptor**
  - -Almost all XNDI patients are male; women can be carriers
- Characterized by excessive thirst, and large amounts (>3L/24 h) of hypotonic urine (<250mmol/kg)
- Can lead to life-threatening dehydration, fatigue and even seizures due to electrolyte imbalance along with enlarged bladder

Leads to nephron malfunction

- (i.e. Insensitivity or no response to ADH) How???



Most diseases caused by GPCR dysfunction are based on genomic alterations

Disease is an x-linked disorder. Caused by mutations of LADH receptor.

Low BP, hypotensive
Almost exclusively males. Due to x-linked linked. If it was dominant, we see no gender.

Electrolytic messing up. Electrolyte balance in the body is very sensitive and crucial.

Lack of receptor leads to nephron malfunction. No response to ADH. Very large water.

Most disorders that are mediated by G proteins are typically genetic based.

Whole human genetic course, covers a lot of genetic diseases

Excessive thrust and excessive urinating. Leads to imbalance of electrolytes, which can cause fainting and seizures.

Classification of GPCR defects

Receptors folded in the ER, processed in the Golgi & inserted into the membrane

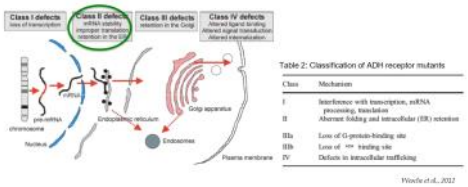


Table 2: Classification of ADH receptor mutants

Class	Mechanism
I	Interference with transcription, mRNA processing, translation
II	Aberrant folding and intracellular (ER) retention
IIIa	Loss of G-protein-binding site
IIIb	Loss of [illegible] binding site
IV	Defects in intracellular trafficking

Wiesle et al., 2011

- Mutations can induce gain or loss of physiological function

Trying to tie how genetic diseases could manifest itself.

ADH receptor mutants, we get patients and look at mutations. From patients we can classify class. Class has implications to life span and quality of life.

Each class has mutation, ADH receptor gene.

Class one=Interference with Transcription RNA, no RNA, class one. No trace of receptor. Most common cause is mutations in the start condon. Or adding premature stop. MOST SEVERE

Class two=mutations that cause protein misfolding. Most common mutation. Body deals with it and over comes it. This is detrimental if strong enough,

Class three A=binding site of G protein is affected. G protein ligand is affected.

Class four =Defects in cellular trafficking.

These classes are important.

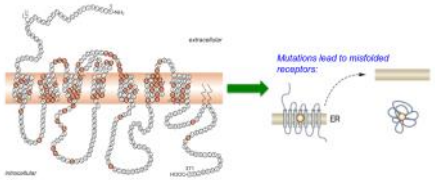
Different consequences.

Frequency of these mutations is widespread, but they all have different variety of severity.

Genetic diseases

Cause of nephrogenic diabetes insipidus

Inactivating mutations: impairment/abolishing receptor function
(Identified as class II based on sequencing and functional analysis)



- Currently **221 known** ADH receptor gene mutations that cause XNDI
- Misfolded ADH receptors trapped in the endoplasmic reticulum
- Result: loss of ADH signal and so no AQP 2 expression or transport to luminal membrane

These lead to misfolding in the ER.

Shoes are defective, UPR response to the ER. Turns on the warning alarms. Alarms are not going to do anything, it is a problem for the whole system.

221 known mutations that cause XNDI. Loss of signal of ADH, dire consequences of diseases

If you have a mutation, and the protein is misfolded. How do we fix misfolding.

We want to get some product out, which is better than none. So we make these drugs to help them get through, even with a mutation

Knowing structure and how it passes through the ER, we can determine severity of mutation

Sometimes mutations can cause bends or kinks, ER tags it to be degraded

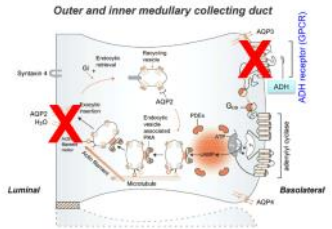
Quality control is detrimental to use if we have small mutations. Small mutation that has no affect could be degraded. Happens more often then we think.

Single point mutations leads to overeaction of the ER, causing the protein to be degraded even though the mutation isn't that bad.

Mechanism of disease: nephrogenic diabetes insipidus

- Mutations in the ADH receptor lead to ADH receptor absence in basolateral membrane & lack of aquaporin channels in the luminal membrane of the collecting duct

Outer and inner medullary collecting duct

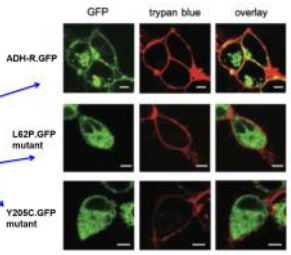


WE ARE ONLY TALKING ABOUT POINT MUTATIONS.

Visualizing class II defects of ADH receptors

Disease causing mutants are retained in secretory pathway (ER and/or Golgi)

- Wildtype & Mutant ADH receptors tagged @ C-terminal with GFP and transiently transfected in Human Embryonic Kidney (HEK) cells
- Wild-type ADH.GFP receptor was located at the plasma membrane (Trypan blue is a membrane indicator)
- GFP-mutant ADH receptors were located to cell interior within the ER and Golgi



Hermosilla et al., (2004)

Some of these mutations, we can test them. Part of investigating is knowing what happens to proteins when it is synthesized.

To be sure, you have to synthesized. Tag it with GFP. Create an effusion protein. PUT THE GFP AT TAIL (C-Term)

Normal (top) all them are targeted to the membrane

Mutant (middle) L62P=Leucine to proline at 62. Proline change is very bad, due to it being special protein. Compare=most of the protein is trapped in the ER, not on membrane

Y205C (bottom) very bad mutation. Protein does not go where it is supposed to go.

Emerging therapies to target class II ADH receptor defects

~Development of pharmacological chaperones~

- Pharmacological chaperones are synthetic small molecules that assist in correctional folding proteins
- Interact with target proteins through non-covalent interactions (i.e. van der Waals, hydrogen bonding etc)

End result:
- Stabilization of the native folding of a protein, preventing aggregation
- Proteins can then be routed correctly throughout the cell

Pharmacochaperone. Small molecules that assist in the folding of proteins.

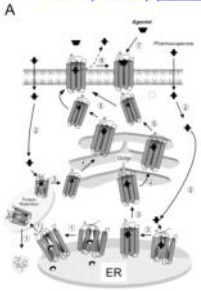
Drug that comes to stabilize the protein. Design a chemical that interferes with mutation. This is very difficult to do.

Want non-covalent way.

Emerging therapies to target class II ADH receptor defects

~Development of pharmacological chaperones~

A



Cellular protein folding is faciliated by molecular chaperones:

1. If folding fails, misfolded proteins are retained in ER and targeted for degradation

2-3. Pharmacochaperones diffuse into the cell, bind misfolded proteins and influence folding

4. Allows then for correct routing to the Golgi complex
4. Mature proteins can then be delivered to the cell surface
4. Pharmacochaperone dissociates
4. Receptor can now interact with its ligand

Approach this with a pharma chaperone.

Substance that binds to receptor as it is synthesized. Allowing it to move out of the cells

Problems with ER. If you do not fold properly, you do not leave

Chaperones that bind and lets it get out of ER, it can give enough function for patient to survive and do well.

Tay-sachs=allows misfolded protein to survive shoe factory. Even if some small function. Pharma chaperones.

Chaperone mediated therapy=overcome the improper folding in the ER, fake the ER to allow it to export.

With some mutants, It is sent out of ER and degraded.

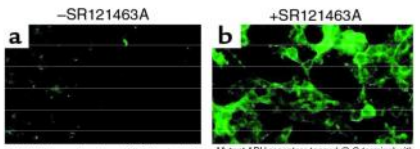
Chaperone can cross membrane and goes to the ER. Binds to pocket in the protein as it is made, allows it to be stable so the ER does not recog is it mutant.

Er allows it to pass, binds to the cell membrane,

Skeptism=this is hard, and at that time is was an inhibitor. If you modify, you can try to find a way to correct it.

Emerging therapies to target class II ADH receptor defects

- Treatment of human embryonic kidney (HEK) cells expressing ADH receptor mutant with *pharmacological chaperone* (+SR121463A) could rescue receptors by promoting proper folding, increased localization at the membrane and responsiveness to ADH



Mutant ADH receptors tagged @ C-terminal with GFP fail to localize to cell membrane in Human Embryonic Kidney (HEK) cells

Mutant ADH receptors tagged @ C-terminal with GFP **localize** to cell membrane in Human Embryonic Kidney (HEK) cells when treated with pharmacological chaperone

Morello et al., 2000

HEK cells=most used cells in research created by McMaster

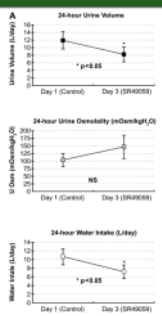
They found that the receptor is rescued, localized more to the membrane. This chaperone has made it through clinical trials. Some of the

Evidence of effectiveness of pharmacological chaperones

Administration of pharmacological chaperone compound in 5 adult males with X-linked NDI

Observed results:
- decrease in urine volume
- increased urine osmolarity
- constant blood plasma osmolarity
- decrease in water intake

Most promising to date



Bernier et al., 2006

Most promising drug to date

Most encouraging sign

Big impact on medical community

You can try to design molecules that will work. You have to design chaperones that are tailored to mutation

Misfolding is dependent on mutation, which is why chaperones must be tailored to each mutation.

Chaperones only stabilize, can't change to amino acids.

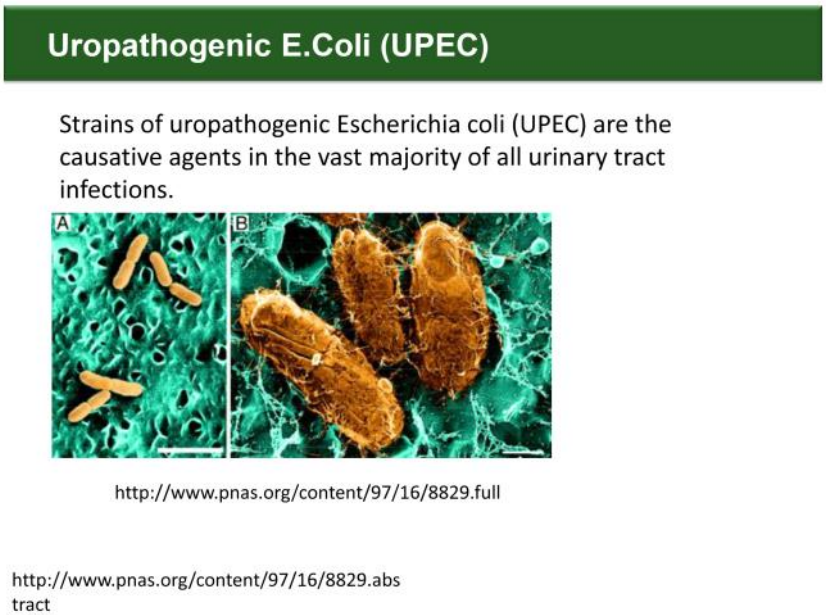
Main goal of chaperone is to exported out of the ER. Stay on until they get to their destination.

Enzyme of receptor does not have to be 100% to work. We can have a mutation that reduces effectiveness, but do not manifest.

We all have mutations.

Lecture 7 Bacterial Adhesion

November 17, 2016 4:31 PM



Strain e coli that affect urinary tract infections

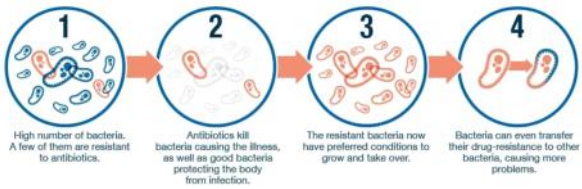
Treatments? Antibiotics

More than 15 million women suffer from urinary tract infections (UTIs) annually in the United States, with an estimated cost exceeding $2.5 billion.

Common treatment? antibiotics

Problems?

- Antibiotic resistance
- Perturbance of beneficial gut microbiome



Perturbance= Screws up gut environment

If you have an infection, anti-biotic kills most bacteria. Even the good ones. The length of dosage=so it can kill every single cell.

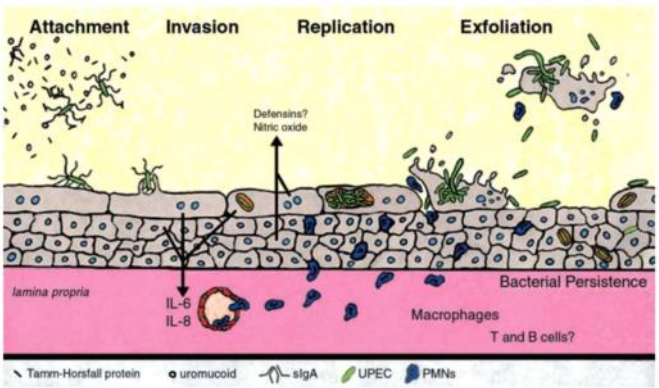
This leads to antibiotic resistance.

This is very scary. Setting certain lines of antibiotics from different institution.

The body deals with infection at a local level and a systematic level. Liver can induce various function.

Sepsis=liver does this, thinks it's a massive infection, shuts down the body.

Urinary tract infections (UTI)



Adhesion is required so that the organisms are not swept away by the natural cleansing mechanisms of the host.

http://www.pnas.org/content/97/16/8829.full.pdf

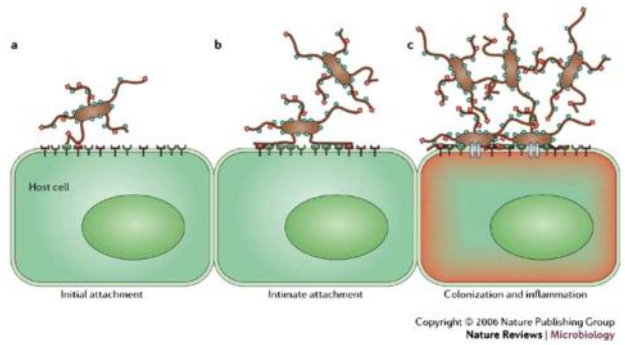
UTI=bacteria attaches to the endothelial layer of the urinary tract

Causing them to internalize. Can lead to immune response. Leukocytes, secretes cytokines to indicate

Bacteria can multiply, causing cells to break down, lose attachment and make more bacteria. Causes massive infection. Even though the body is responding, issue gets worse and we need anti-biotics

1st step in pathogenesis: adhesion

Adhesion allows the pathogen to stay at the site of infection long enough to multiply and to cause damage.



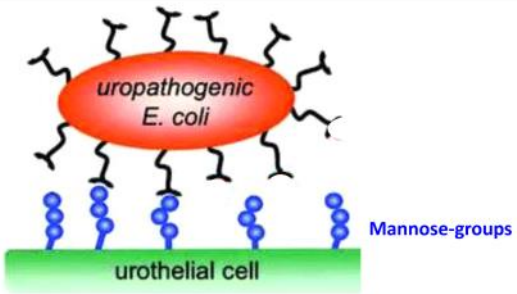
http://www.efsa.europa.eu/sites/default/files/efsa_rep/blobserver_assets/amrinfographic150226_800_a.jpg

Pathogen adheres to the cells. Adhesion molecules are also utilized by pathogens.

Sometimes a receptor can be hijacked by a pathogen.

Adhesion allows to stay, bind to cells

UPEC fibriae or pilli mediate attachment



Bacterial adhesion: Physicochemical property of fimbriated bacteria of attaching to cells, tissue, and nonbiological surfaces. It is a factor in bacterial colonization and pathogenicity.

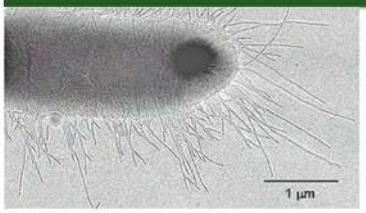
http://pubs.acs.org/doi/abs/10.1021/jm300192x

Pathogen has little hairs that allow them to bind to the mannose groups on the endothelial layer

By interfering with this process, we can disrupt endothelial later

Hairs are

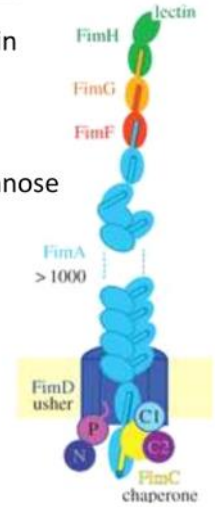
Type 1 pillus



Fim H is the adhesin

Fim H is a lectin

FimH target is mannose



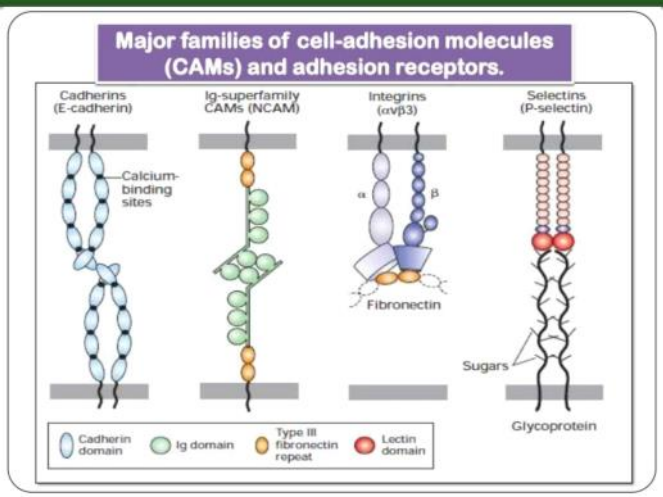
Lectins on the E. coli surface recognize oligosaccharide units on the surfaces of target cells. These lectins are located on slender hairlike appendages called fimbriae (pilli).

http://rsta.royalsocietypublishing.org/content/373/2036/20130153

Hairs=fims

Lectin=protein that binds to a sugar. Fimh is involved in the adhesion that targets the mannose. Targets any protein that has sugars that have mannose

Cell adhesion molecules: lectins



We know all of these. All of these are normal cell adhesion molecules in the body that can be targeted by bacteria.

Lectins

Lectins are carbohydrate-binding proteins, macromolecules that are highly specific for sugar moieties.

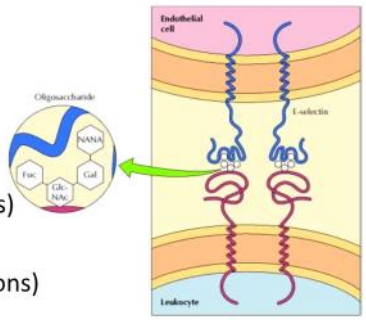
Examples include:

Calnexin (protein folding chaperone)

Selectins (mediate cell-cell interactions)

Influenza haemagglutinin (viral infections)

Plant lectins (often toxic)



LECTIN BINDS TO SUGARS

Highly specific to certain sugars. We use lectins as tools in lab, labelled with fluro dye.

Most from plants.

Calnexin=binds sugars in the ER, enables the protein to fold.

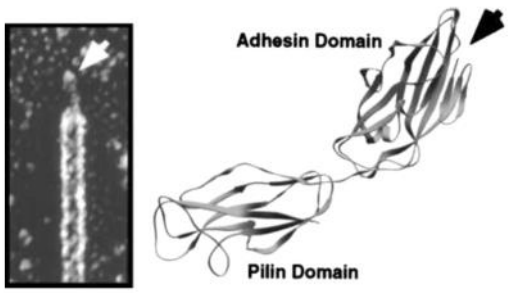
Selectin=endothelial layers

Influenza=HA for viral infections

Plant lectins=toxic. Used for assays

Binds to 4 sugars that allows the selectin to bind too. Leukocytes use this. Lectins have important function

Adhesin, FimH, lies at the end of the pili



The FimH adhesin consists of two domains: a COOH-terminal pilin domain involved in the incorporation of FimH into Type 1 pili and an NH3-terminal adhesin domain that contains a carbohydrate binding pocket capable of binding to a D-mannose residue.

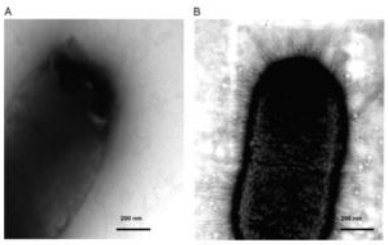
Binding part of pillus (hair) lies at the tip

Called a FimH protein. Adhesion domain and Pilin domain. Adhesion domain binds to mannose.

No hair=no infection

FimH is necessary for assembly of Type 1 fibriae

FimH-deficient mutants lack the ability to initiate infection.

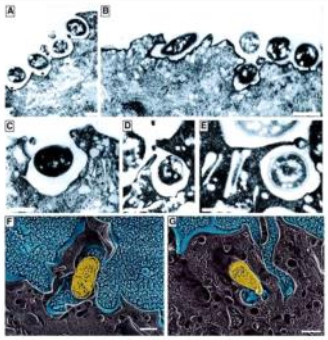


No fibriae
No infection

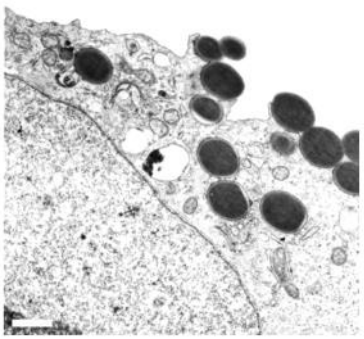
Fibriae
Infection

http://iai.asm.org/content/80/9/3289/F3.expansion.html

Internalization of *E.coli* requires FimH



Internalization of E.coli



Internalization of FimH-coated latex beads

Persistent and recurrent infections are associated with internalization.

http://www.pnas.org/content/97/16/8829.full

12

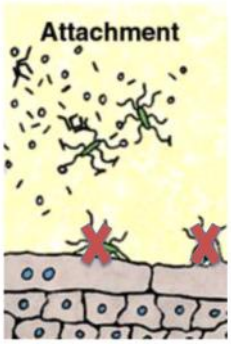
When cells are bombarded by pathogens, they start to take them in.

Took latex beads with FimH will cause internalization.

Now we know molecule to cause infection, we should come up with drug

Blocking adhesion?

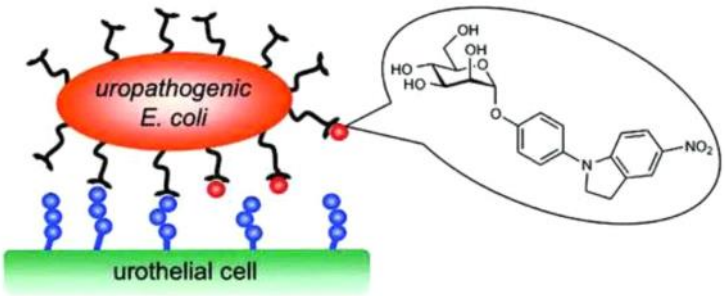
If the bacteria are unable to bind to the urinary tract lining, they will be eliminated simply by the flow of urine, before they can start an infection.



http://www.laboratory-journal.com/science/life-sciences-biotech/solid-state-nmr-insights-bacterial-autotransport-process

IF we block adhesion, can we block the mannose and free manoses to block

Carbohydrates as anti-adhesion drugs?

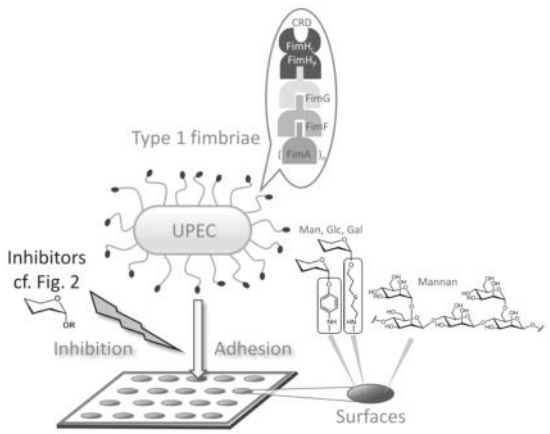


http://www.laboratory-journal.com/science/life-sciences-biotech/solid-state-nmr-insights-bacterial-autotransport-process

Use sugars to bind to the FimH, so it can block. Problem is that the bacteria has so many pili that we cannot possibly block all of it.

How do we overcome the number of hairs

Large scale screens for FimH inhibitors

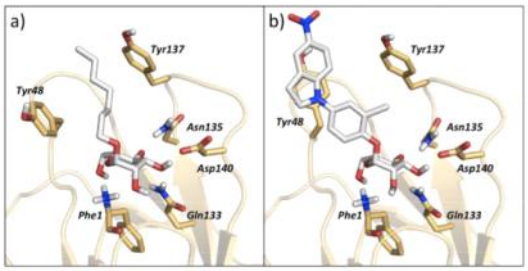


http://www.mdpi.com/2079-7737/2/3/1135/htm

Taking various amounts of mannose and testing which of them can prevent bacteria from binding.

We can narrow down something that can reduce binding.

Rational design



The x-ray crystal structures of FimH binding domain for α-d-mannose, and mannose derivatives (mannosides) can be used to rationally design FimH inhibitors with excellent cellular potency and low molecular weight .

http://www.mdpi.com/1422-0067/14/1/684/htm

Study of structure of FimH= allows us to know more about the binding of the structure.

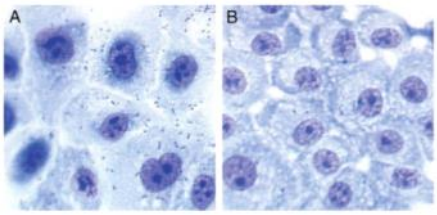
What do we have?

Competitive was weak, this was a poo poo way.

We create Glycodedrimers. Little spheres covered in many many many mannoses. Can inhibit the bacteria. Because of the shear number= overwhelms bacteria.

Other anti-adhesion molecules

Cranberries contain a specific group of polyphenol molecules called proanthocyanidins.



Control Cranberry extract

- Reduced adherance (AFM)
- Reduced infectivity
- Acts through FimH

Infection of Primary BECs (bladder epithelial cells)
Decreased bacterial adherence from 6.9 to 2.2 bacteria per cell (T-test p <0.001).

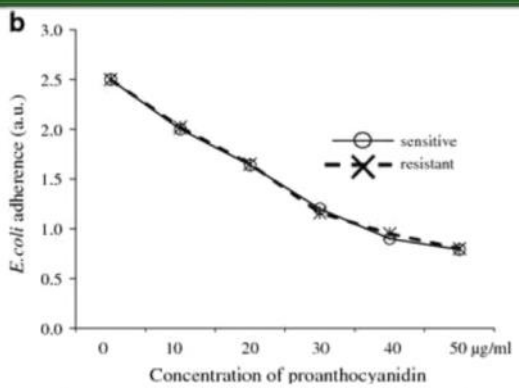
http://www.ncbi.nlm.nih.gov.libaccess.lib.mcmaster.ca/pmc/articles/PMC3684265/

Cranberries have a natural substance that have a natural substance.

Have proanthocyanidins; fights off the adherennce of the bacteria. Fights off FimH

Adding cranberry shows that we can prevent infection.

Effect of proanthocyanidin on adherence



Adherence of UPEC in absence and presence of various concentrations of proanthocyanidin of both sensitive (empty circle with light thin line) and multi-drug resistant (cross with dark thick dotted line) *E. coli.*

http://link.springer.com.libaccess.lib.mcmaster.ca/article/10.1007/s00240-011-0398-2/fulltext.html

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Effect of this molecule of the cranberry juice.

Cranberry juice helps infections.

Lingering challenges

Many bacterial pathogens carry a variety of adhesive structures.

- Develop cocktails of anti-adhesive agents that target a variety of different adhesive structures, rather than to rely on a single compound with a single target.
- Alternatively, anti-adhesion agents could be combined with conventional antimicrobial agents.

https://www.microbemagazine.org/index.php?option=com_content&view=article&id=6389:made-to-stick-anti-adhesion-therapy-for-bacterial-infections&catid=1238&Itemid=1519

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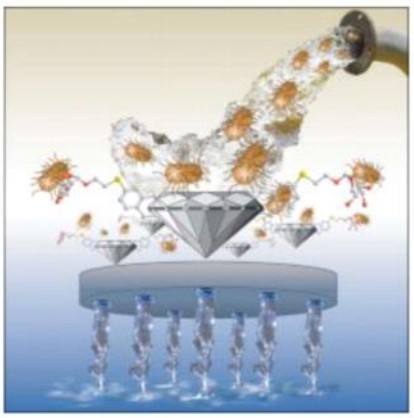
Many pathogens have variety of adhesive structure.

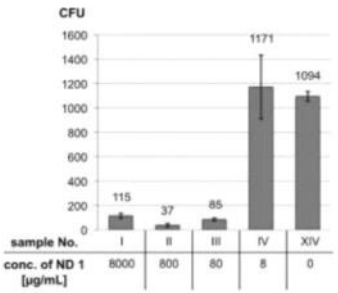
We want to create a cocktail of anti-adhesive targets.

Combine with anti-biotics

Water purification



Reduction of bacterial (colony forming units, CFU) by mannose linked nanodiamonds



http://www.otto-diels-institut.de/lind/Chemistry_2012.pdf

This can purify water.

Summary

- Adhesion of bacterial cells to host cells in the first step in pathogenecity.
- FimH lectin is required for adhesion of uropathogenic E.coli and infection.
- Blocking adhesion can prevent infection.
- Molecular mechanisms for blocking adhesion? FimH mannose-binding domain

Of further interest:

Mulvey, M.A. et al. 2000. Bad bugs and beleaguered bladders: Interplay between uropathogenic Escherichia coli and innate host defenses. PNAS. 97(16): 8829-8835.

http://www.pnas.org/content/97/16/8829/F1.expansion.html

Hartman, M. et al. 2012. Saccharide-Modified Nanodiamond Conjugates for the Efficient Detection and Removal of Pathogenic Bacteria. Chemistry. 18(21):6485-92.

http://www.otto-diels-institut.de/lind/Chemistry_2012.pdf

Lecture 8
Mitotic spindle dynamics

Department of Biology — McMaster University

Using motor proteins to power cell dynamics


A) Establishing a bipolar mitotic spindle in prophase and attaching chromosomes in prometaphase/metaphase

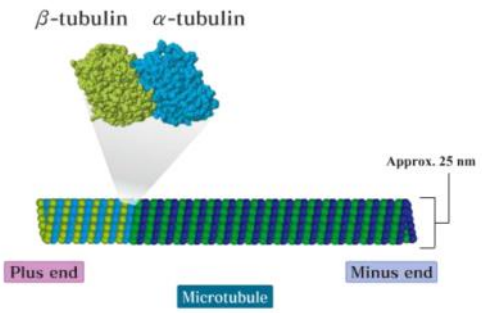

B) Orchestrating chromatid separation in anaphase


How are motor proteins employed in each of these?

Multipolar tumour cells??

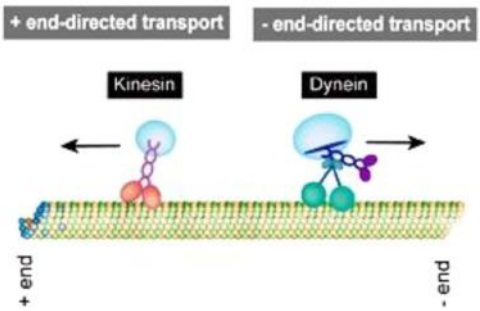
2

Point 1: microtubules are polar and dynamic



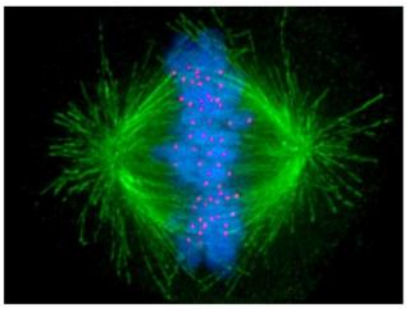
http://csls-text.c.u-tokyo.ac.jp/flash/0612_1.html

Point 2: motor proteins move in one direction



The mitotic spindle: how does a cell get to here?

How can we use the biology of microtubules and motor proteins to make this?



Green = Microtubules

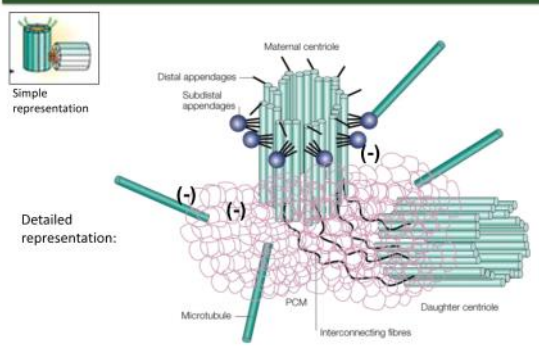
Blue = DNA

Red = kinetochore (DNA centromeres to microtubules

Chromosomes have to line up, attached by microtubles that originate from the centrosome.

Allows seperation of two sister chromatids.

Spindle assembly: A centrosome nucleates microtubules



- Minus-end of microtubules is in pericentriolar material (PCM)

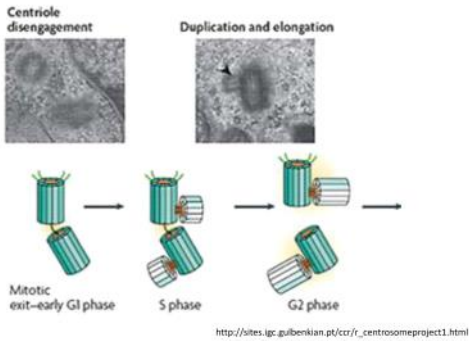
Centrosome is made up of two components. Centrosomes form two little microtubules.

Form a centre from which a microtubule forms. Negative end forms attaches to the centrosome.

- Ends of the microtubles are in the materials around the centrosome.

Centrosome duplication in G1/S

Duplication of each centriole to create two pairs.

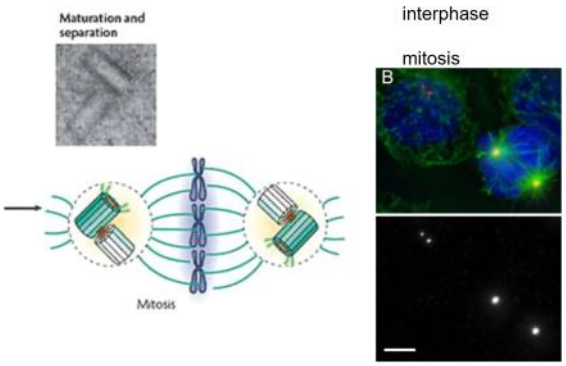


http://sites.igc.gulbenkian.pt/ccr/r_centrosomeproject1.html

Centrosome dupliaction begins very early. They seem to be intermingled with the microtubules.

Centrosome seperates during mitosis

Centrosome separation in mitosis

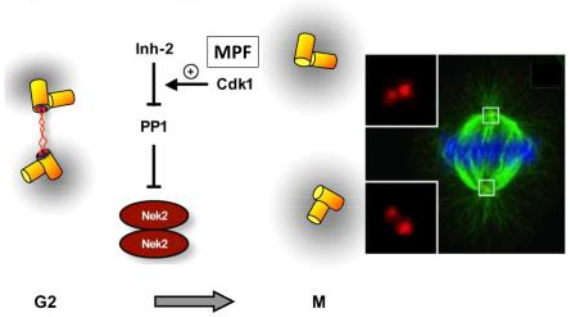


Seperation of centrosome is linked directly to the beginning of mitosis.

Uncoupling

How is centrosome separation linked to mitosis?

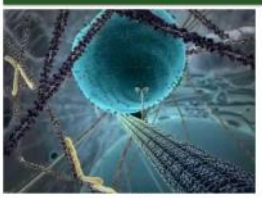
MPF indirectly leads to activation of Nek2 kinase that enables separation of centrosomes.



NEK 2 kinase is unlocked, promoting seperation of mitosis. Mitosis has to come in to create the seperation for complete centrosome.

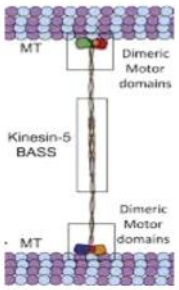
How do they move to different poles.

Separation is mediated by kinesin-5



Conventional kinesin

Kinesin-5 binds to two microtubules

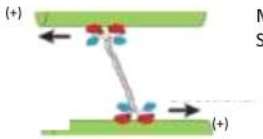


How might kinesin 5 direct centrosome separation?

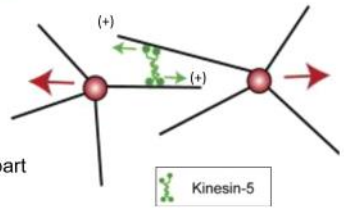
There are mutations that cause central nervous disease, linked to kinesin 5

Model for centrosome separation?

Filament sliding



Motor "walks to plus end"
Slides microtubules (MTs) apart



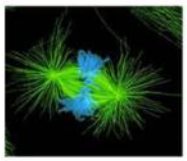
centrosomes

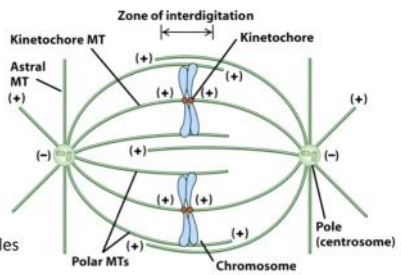
Pushes centrosomes apart

Kinesin can move very fast on microtubules. Binds to both tubules and walks negative to positive, forces the microt to expand.

Kinesin 5 pauses at the plus end of the microtubule, enchances the polymerization.

Result: Bipolar spindle



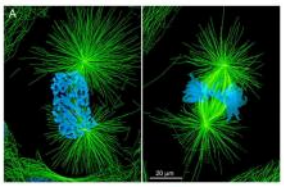


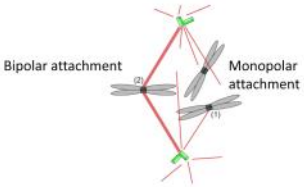
- Kinetochore microtubules
- Polar microtubules
- Astral microtubules

What happens is that you need to create bipolar spindles. The microtubules. Have to create kinetochores.

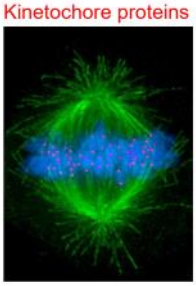
Kinetochores are hooked from both ends.

Kinetochore MTs: capturing chromosomes in prometaphase





Kinetochore proteins

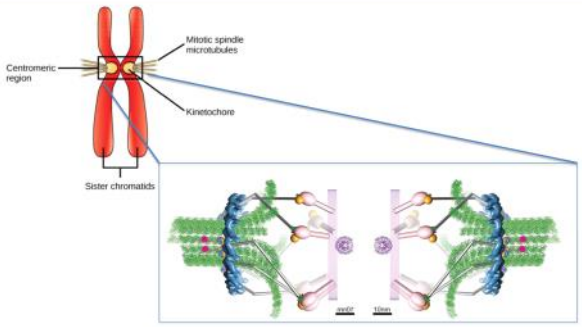


Monopolar attachment at first, but once it is stabilized, there is bipolar attachment.

Alignment of these chromosomes in the middle of the cell.

It has to be so precise, because the cell will screw up if you do not split these as needed.

Kinetochore proteins



Attachment of kinetochore proteins to a dynamic (elongating and shortening) microtubule.

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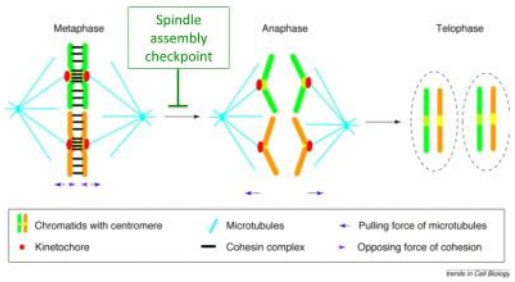
We want to focus what happens at the kinetochore.

We haven't been able to isolate the kinetochore structure. It is dynamic.

Microtubules attached dynamically

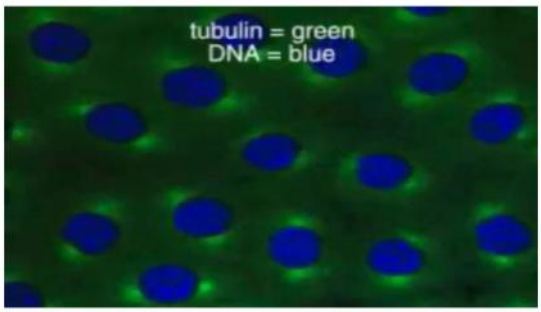
Metaphase: bipolar attachment

All chromosomes must achieve a bipolar attachment for the cell to enter anaphase.



There is a spindle assembly checkpoint, must be aligned before it goes into anaphase.

Movie showing anaphase in Drosophila



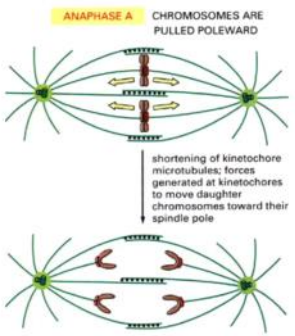
http://www.dnatube.com/video/4173/Mitotic-Spindles-in-a-Fly-Embryo

Anaphase in drosophila.

Movie showing anaphase in Drosophila

Video

Anaphase A: kinetochore microtubule shortening

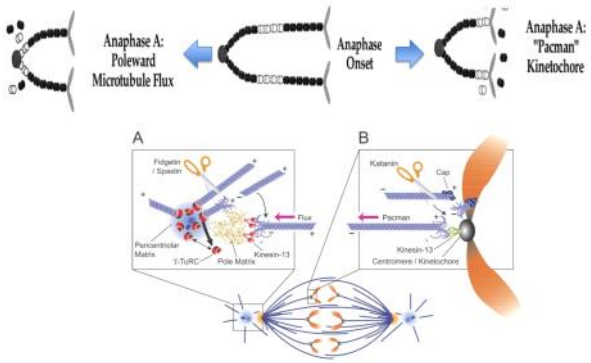


You have aligned the chromosome, now you must separate them.

MT shortening, the forces generated at the kinet will move the chromosome away from each other

Anaphase A: kinetochore microtubules shorten

At which end does shortening occur?



You have aligned the chromosome.

Which end does shortening occur?

Positive or negative end?
It occurs at both ends.

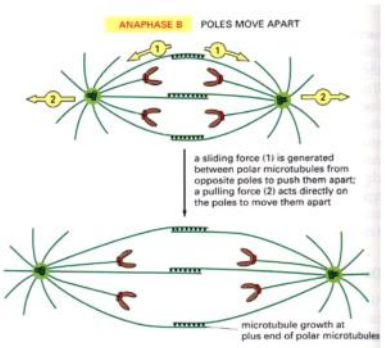
Two events

One at centrosome, one at kinetochore

Kinetochore: kinesin 15 allows the MT to depolymerize. Kinesin 15 eats the positive end of the microtubules, making them shorter

Centrosome: Gamma Turc must be removed to shorten the MT. Fidgetin remove that end. Kinesin 15 disassembles= shortening of both ends.

Anaphase B: separation of spindle poles



How might motor proteins separate centrosomes?

Forces the cell to have complete component of both ends

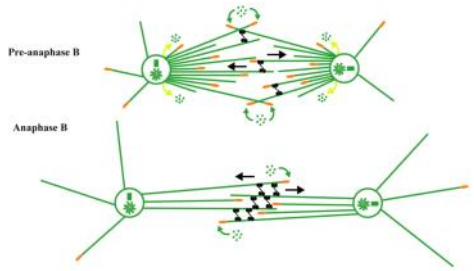
Kinesin works on both ends.

Kinesin 5 pushes the centrosome apart.

Some microtubules are holding onto the membrane, called astro microtubules. These adhere to cell membrane so they have ability to pull

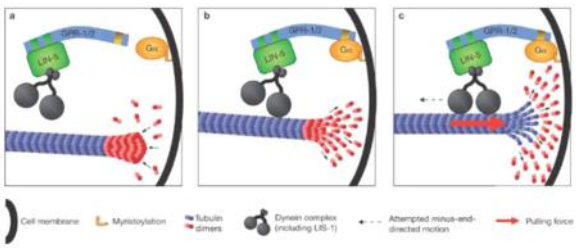
Anaphase B: sliding of polar MTs

Sliding of polar microtubules = pushing poles apart



KLP61F = kinesin 5

Anaphase B: shortening of astral MTs



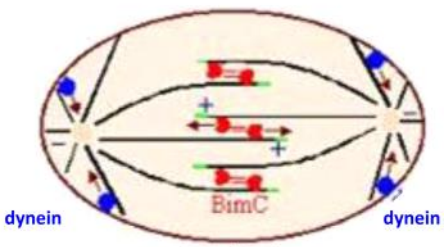
dynein

Why Dynein?

Similar process, need movement to go positive to negative. Can't use kinesin, you use Dynein.

Move the microtuble against the membrane, allows it to depol and shorten

Anaphase B: pushing and pulling centrosomes



BimC = kinesin 5

You are basically pushing and pulling th cnetrosomes

Pushes the sections apart

Summary

A) Establishing a bipolar mitotic spindle in prophase and attaching chromosomes in prometaphase/metaphase:

- Separation of centrosomes requires kinesin
- Attachment of kinetochores to KT microtubules – dynamic attachment

B) Orchestrating chromatid separation in anaphase

- Anaphase A (microtubule shortening)
- Anaphase B (kinesin and dynein)

One of the early things, MT interference=interference of cell division

If you inhibit cancer cell growth, you effect cancer cells more than normal

Taxol is chemo

Chemo=affecting cell growth, affects cells more. Fast dividing cells are also very hurt.

Applying new knowledge: targets for new cancer therapies

RESEARCH ARTICLE

CANCER

Proteins Required for Centrosome Clustering in Cancer Cells

Blanka Leber,[1]* Bettina Maier,[1]* Florian Fuchs,[2] Jing Chi,[1] Phillip Riffel,[1] Simon Anderhub,[1] Ludmila Wagner,[1] Anthony D. Ho,[3] Jeffrey L. Salisbury,[4] Michael Boutros,[2] Alwin Krämer[1]†

(Published 26 May 2010; Volume 2 Issue 33 33ra38)

"Inhibition of this centrosomal clustering, with consequent induction of multipolar spindles and subsequent cell death, would specifically target cancer cells and overcome one limitation of current cancer treatments."

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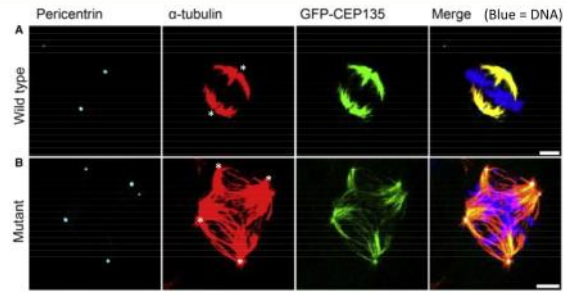
Multipolar spindles usually lead to aneuploidy and cell death



Yet, many of the cells seen in cancers are multipolar - how do they survive?

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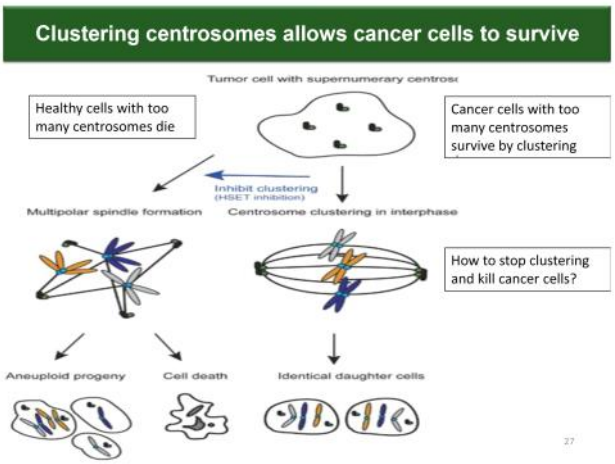
Idea: In cancer cells, observation is made is that there is multiple centrosomes in cancer cells.

You think it would make it more susceptible to dying, due to improper splitting of chromosomes

However, you see tall these spindles cause a weird arrangment in cancer cells (Bottom)



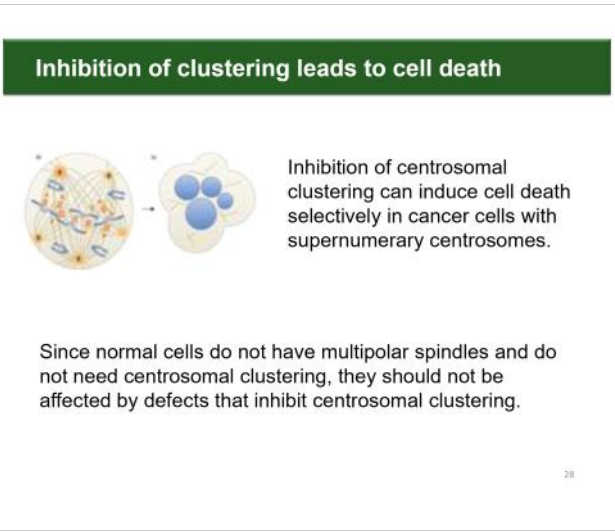
Clustering, start cooperating to make their own spindles.

Tumours with more centrosomes cluster and allows them to survive.

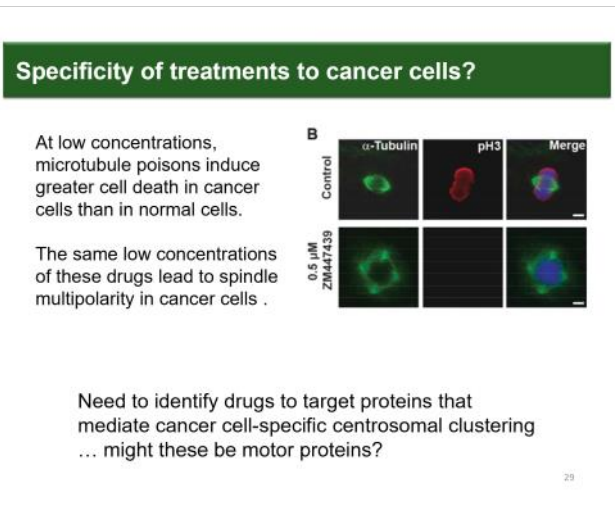
Clustering allows them to undergo a reasonable division. In cancer cells, biggest problem is lost of chromosome.

Every cancer is different. Cancers transforms. Cancers are mutliple diseases, many different phenotypes.

How to deaggreate cells.



Inhibition of centrosomal clustering can induce cell death in cancer cells. Selectively.



Culture C= plants extract that had value against cancer. Inhibits MT at low levels.

Taxol at certain concentrations can kill cancer cells.

Review Lecture

We looked at cell and various organelles. Finer details of the cell. We put forward an analogy for the cell

ER factory
Membrane Border
Golgi=label and transport
Lysome to breakdown stuff
Mito=powerhouse

Looked at proteins and structures, membranes

Looked at Main components of cell.
Looked at how ER manufacture the ER. The minute an RNA is available, used to synthesize proteins. The minute it is translated with signal sequence, brings toward ER.
EVERY GLYCOPROTEIN has to PASS THROUGH THE ER.

Events in the ER
- Glycosolation: covalent addition of polysaccharides
- Protein folidng
- Disulphide bridges
- Proteolytic cleavage

Typically immdiately. ASPARAGINE gets sugar. Lectin=protein that binds to sugar. Calnexin is a lectin. Cal nexin binds to sugar to keep it straight out. Calreticulin does this too. Chaperones are protein that covers hydrophobic so they do not misfold. Bip Does this to protect the protein. Key step of cells.

UPR response is also important to know. Hac 1 transcription factor is good for creating more chaperones for the UPR.

Cis network. Or intermediate compartments. Used to return ER proteins called KDEL signal. Trans network of golgi is where proteins are separate. Constitutive secretory pathway. Goes to late endosome, sorting of proteins, recycling or going to surface. We talked about Cop 1 and Cop 2. Retro and all that stuff.

Pulse-chase labeling. First one to discover the pathway of protein. Radioactive material to label proteins. Add them into the cell of the animals. Mannose is added into ER, track down radiography. Realize that it is ER, Golgi, secretory vesicles. Hint of sequence of glycosylation.

Cell responses: Fast and slow, Fast=activates enzyme. Slow response involving molecules and receptors. The Cytokine was an example of fast response. Jak stat inhbits apoptosis.

Cytoskeleton. Held together by bridges, roads, use One thing we notice in cancer cells is that the stress fibres are usually gone. Microtubules.

Cell cycle is most important on the test. Cell cycle is 25% on exam.

KNOW THE PROTEINS FROM THE CELL CYCLE VERY WELL. ESSENTIAL. Checkpoints are usually inactive/attacked by cancer cells.

We talk about apoptosis. Morphological change

ALL LECTURES
100 questions
Each question has 4 choices
6 Diagrams, many questions associated on it.