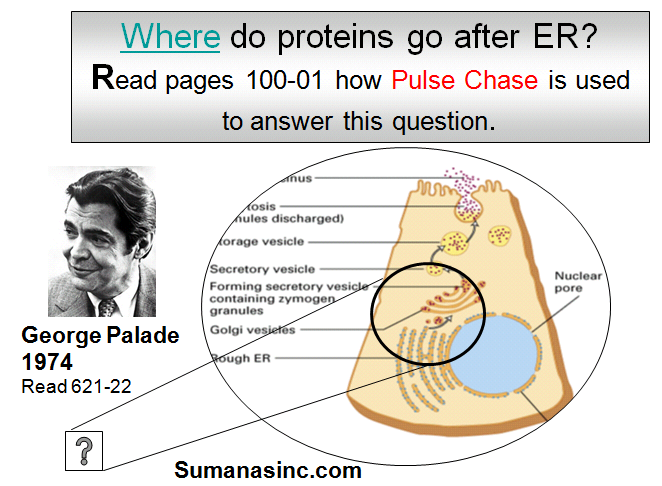
**Golgi and pr – Jan 25**

\*\*\*know pulse chase exp and how you would use

It to determine how pr moves in cell

**Pulse-chase (pc) experiments are useful for tracking**

**changes in pr intracellular location or modification**

- cell sample exposed to radiolabeled compound

that can be incorporated/attached to **pulse**,

(molecule of interest) then washed with buffer

- incubate with **chase** (unlabeled form of compound)

- samples taken periodically are assayed to determine

location/chem. form of radiolabel as function of time

- pc exps (pr detected by autoradiography) are used

to follow synth rate, modification and degradation by

adding radioactive aa precursors during pulse and detecting amts of radioactive pr during the chase

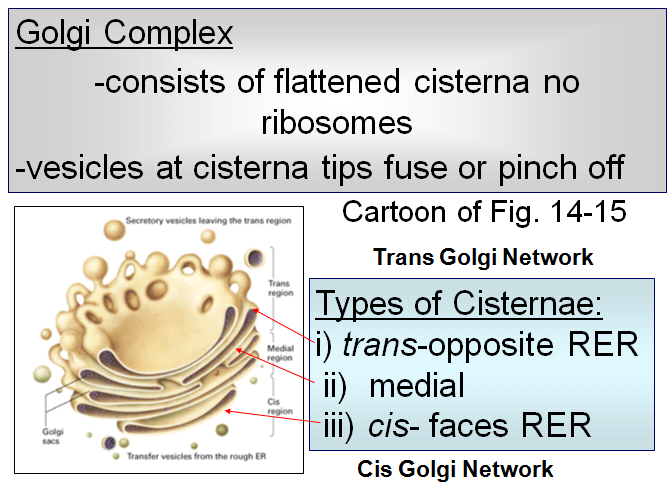
- pc was used to find pathway of secreted prs from ER to cell surface

E.G. cells incubated with 35-S Met (pulse) to label all newly synthesized prs and radioactive aa not incorporated was washed away

- cells were further incubated (chase) for varying times and samples from each time of chase were subjected to immunoprecipitation to isolate a specific pr

- SDSPAGE of immunoprecipitates followed by autoradiography permitted visualization of 1 specific pr, which is initially synthesized as a small precursor (p) and then rapidly modified to a larger mature form (m)

- could measure conversion and/or degradation rate



Golgi functions: modify/sort/package

- how do you go from cis -> medial -> trans?

2 explanations:

1. vesicles moving up: medial region just an older

cis region

2. entire cisternae moves: goes from cis -> medial ...

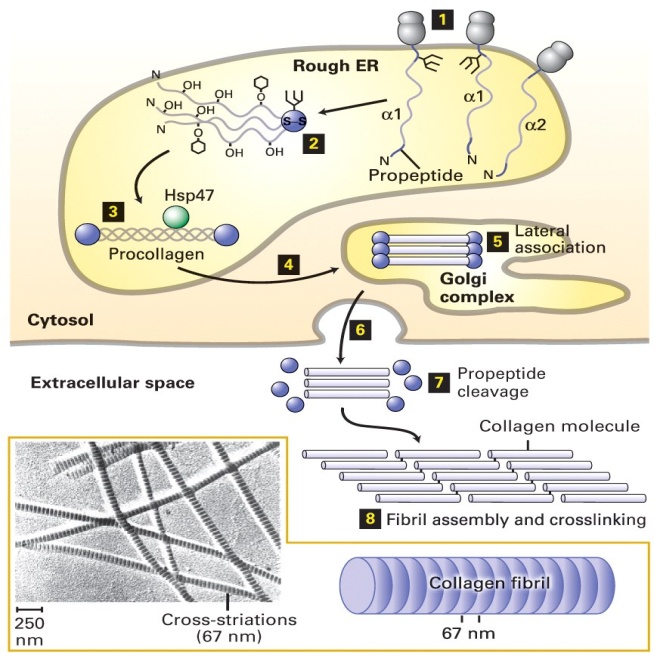
\*\*\*each region has unique functions!

- cis golgi network: vesicles pinching off near base of RER

- trans golgi network: prs not being processed but

getting ready for secretion?

Golgi Function: - “Scurvy” = vit c deficiency

-sugars are trimmed from polypeptide

-hydroxyl groups, added in RER, are used for folding (proline + lysine) Vitamin C

- fact that P and K can be hydroxylated is unique

-final synthesis/packaging for secretion

- collagen most abundant pr

- vitamin C is a cofactor for allowing proline and lysine

to be **hydroxylated** in RER

- RER is place where **glycosylation** takes place

- adding sugars to growing polypeptide

- moving from RER to Golgi, sugars trimmed and fine-tuned

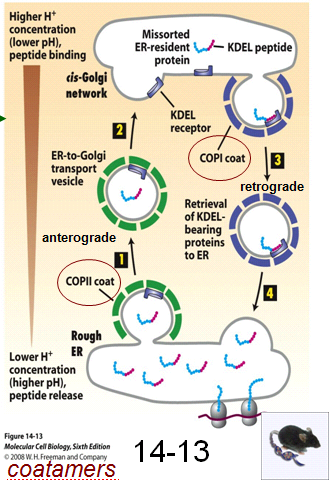
- collagen forms triple helix to help with chaperone i.e Hsp 47

- from RER to Golgi, there is some assembly of collagen but only

until it’s released

- at the pt. of release, fibril formed -> if this happened

inside cell, they it would be bombarded and rupture

Golgi also reroutes proteins back to RER or lysosomes

How?

-**KDEL** signal on proteins that should stay in RER lumen

*“Retention Signal”*

- chaperones are resident ER prs cuz need folding in RER

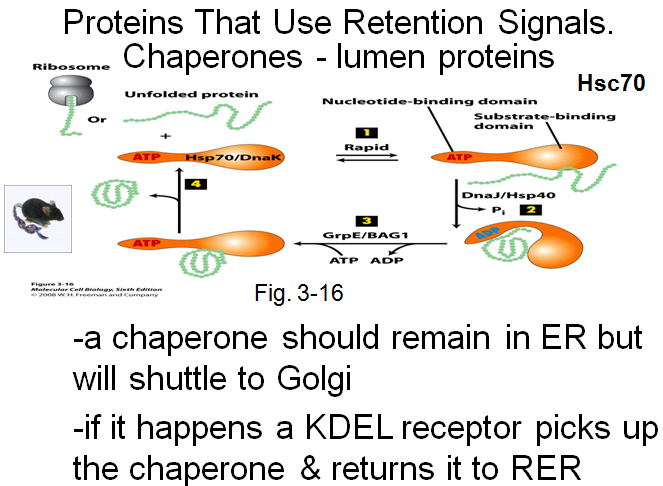
- coatamers: the type II family recognizes prs moving in anterograde

fashion (moving away from RER towards Golgi)

- receptor binds to pr in low pH -> any pr that has this specific-ordered

sequence KDEL will be recognized by KDEL receptor which causes:

- pr returned to RER, recycling what would be potentially damaging

**

- ATP required for chaperones to work

- chaperones in cytoplasm (not just lumen of RER)

- same prs made in cytoplasm have to be folded

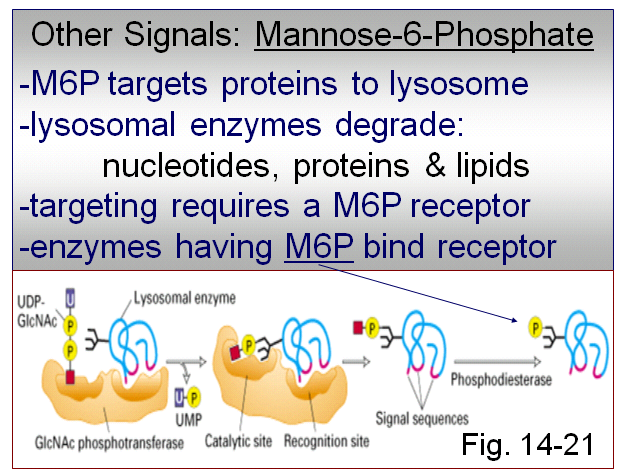
correctly

- region for binding ATP and region for binding pr

- pr binds to chaperone

- give chaperone ATP and ready to release pr and pick

up another one to start procedure again -> recycling aspect



- Tay Sachs: 1 in 25-30 Ash. Jews

- neurons are packed full of lipid that can’t be broken down

- causes blind, deaf, inability to swallow, seizures, muscle

atrophy

- lysosomal hydrolases (hydrolyze prs, lipids, nucleic acids)

-> **all have a M6P signal on it**

- this occurs in **cis-Golgi**: addition of P group onto mannose sugar

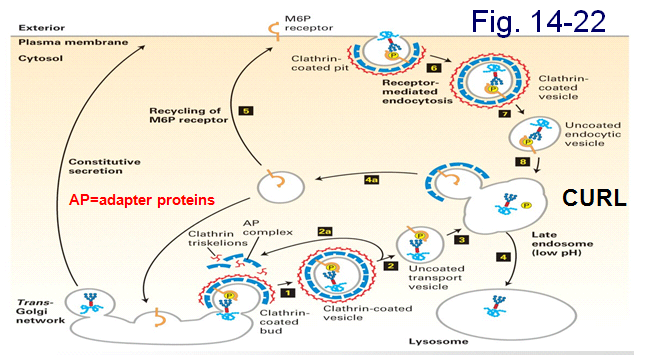
- sugar phosphotransferase (don’t need to know specific names)

- blue is hydrolase -> how do you tell pr to go to lysosome

- only place where it will work

- take mannose and attach a P residue

- once enzyme has **6P**, it will be recognized and sent to lysosome



CIS-GOLGI NOT TRANS

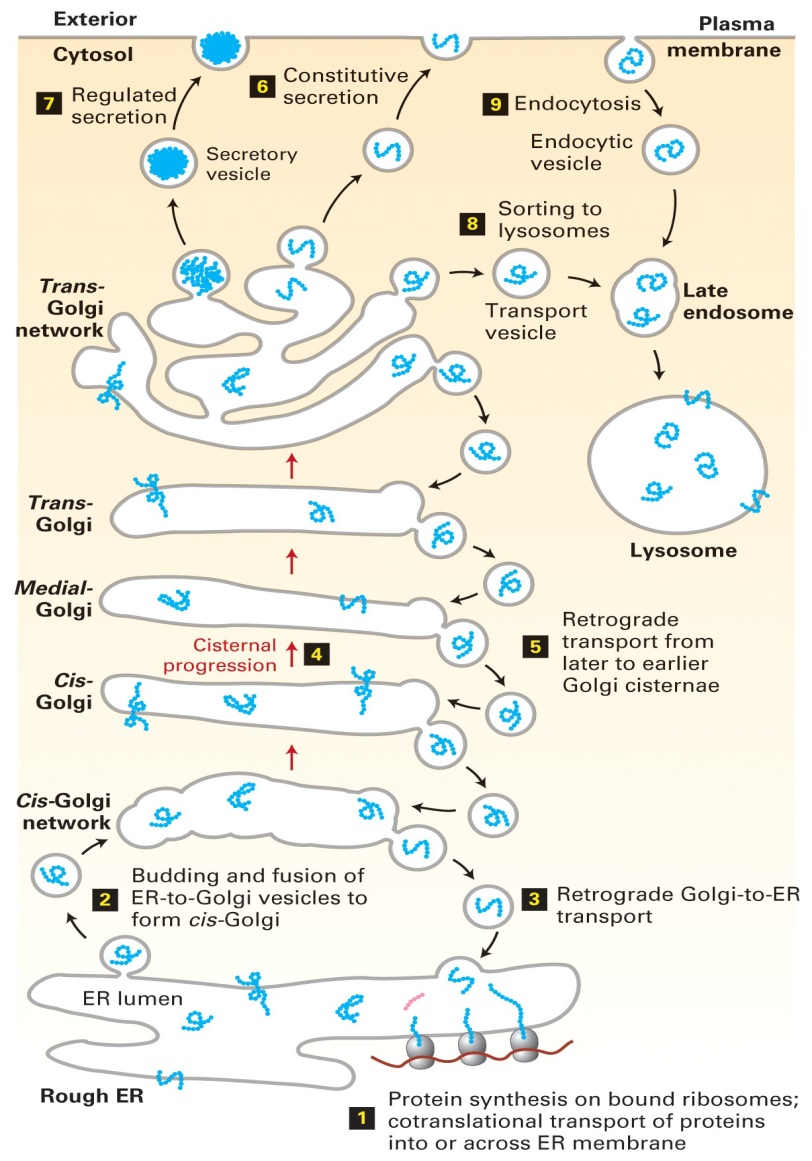
- receptor specifically recognizes M6P and hundreds packaged

- adaptor prs ensure receptors stay where they’re supposed to

- vesicle pinches off and clathrin pr (triskelions bind to vesicles, the more vesicle buds off)

- once vesicles are in place, cytoskeleton carries “cargo” to lysosome

- late endosome/CURL: compartment where you have uncoupling of ligand from receptor

- cells uses receptor and concentrates lysosomal enzymes (works at low pH)

-receptor binding occurs ~ pH 7

-at low pH M6P detaches from receptor

-M6P receptors return to Golgi or to PM

Why receptor on surface? If lysosomal enzyme

secreted out of cell, this is a fail-safe mechanism

to bring it back to cell and send it off to CURL

-> Look at step 6 and 7

EM of just cis, med, trans golgi?

- how would you know pointing to cis?

- what technique? Tag with P using heavy metal

- label pr with antibody:

- purify sugar phosphotransferase

- make antibody

- label antibody with gold particle

- do EM and take antibody and incubate onto

tissue sections?

- EM: everywhere with dots tells Cis

Conceptual Recap

Go over Fig. 14-1 on Protein Secretion

- one cell sends signal to another cell to

Trigger cell div

- signals direct prs to diff destinations

e.g. KDEL on resident ER prs

e.g. M6P signal attached in cis-Golgi instructs

(via M6P receptor) hydrolases to move from trans-

Golgi to lysosome

- M6P attached to enzyme dictats where

It will go

-> pr is NOT secreted, only trafficked to

Where it’s required

Trans-Golgi Network is sorting station

- constitutive v. regulated secretion (i.e waiting

for hormonal or NT signal), also place

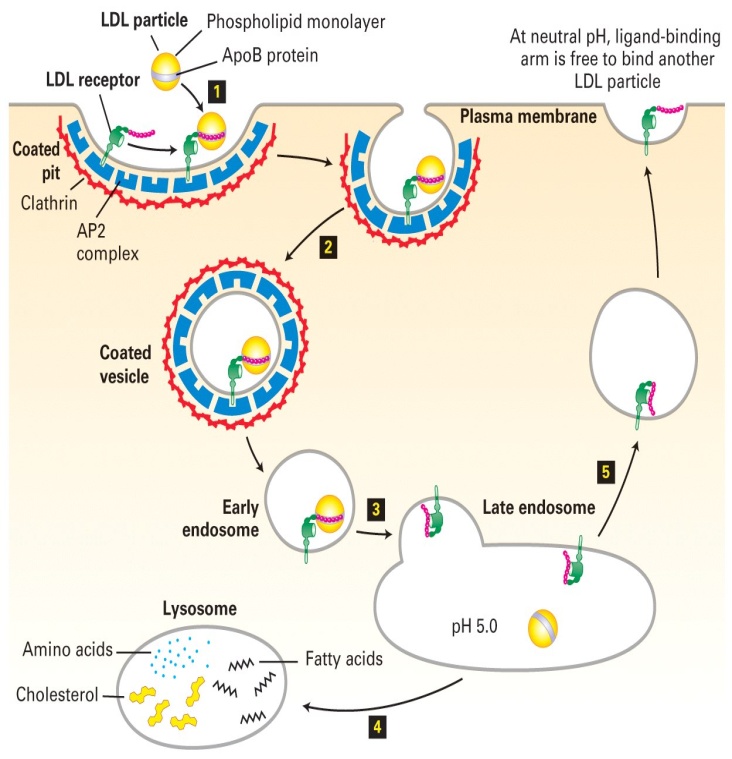
Of final proteolytic processing

Vesicles use adapter prs and clathrin to facilitate

Transport – involves cytoskeleton and motor prs

-

-

**Receptor-Mediated Endocytosis**

The Concept: What is it?

A method of selective internalization

- pino: “cell sipping”

- phagocytes: for large particles

- RME: receptor is specific to ligand

i.e. LDL particle => cholesterol

- there are many LDL receptors for efficiency

- adapter prs present and form interaction with clathrin

- clathrin facilitates pinching in and coated vesicle forming

- once clathrin has internalized particle, it’s recycled

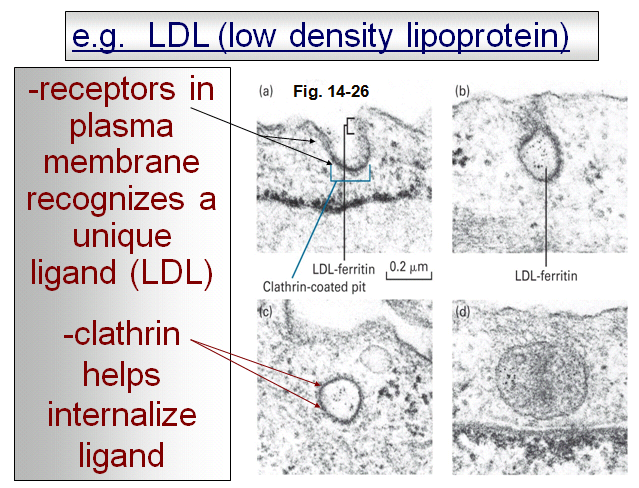
- late endosome = CURL (uncouple receptor from ligand)

- proton pumps all along compartment (pumping protons

Into CURL) -> M6P hydrolases only work at low pH

- when active, they break it down into components

- cholesterol = building blocks for hormones



- TEM

- fuzzy coat = clathrin

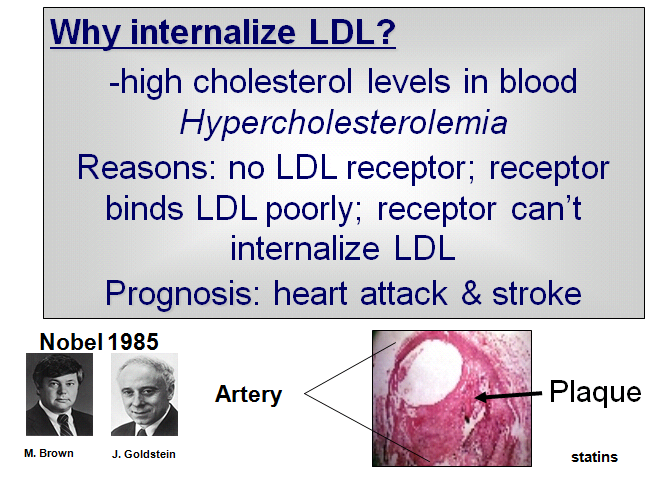
------------------------------------------------------------------------------------------------------------🡪

Pr is part LDL and part ferretin

- ferretin transports Fe (heavy mental) into cells

- Fe needs to be carried through

- if RME, specific for one ligand, clathrin needed



- significant component of hypercholest. is genetic

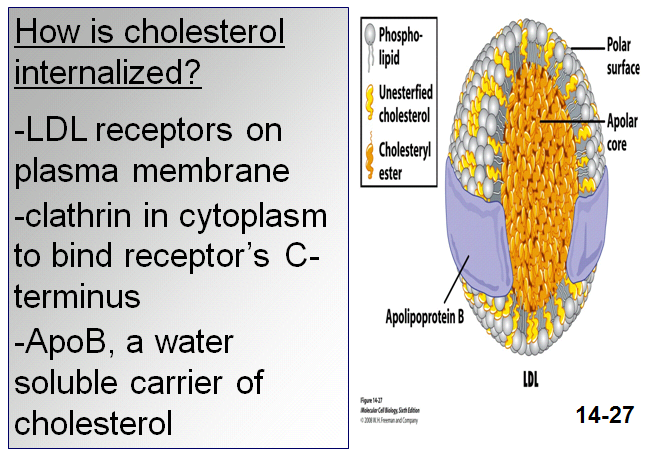
- foam cells?

- build up occluding coronary artery which leads to heart attack

- binding poorly: 90% of LDL still circulating

- girl given liver transplant: liver stores a huge amt of LDL

And puts it back into bloodstream



- cholesterol lipid

- ApoB wraps around cholesterol molecules

- if mutated ApoB that couldn’t transport

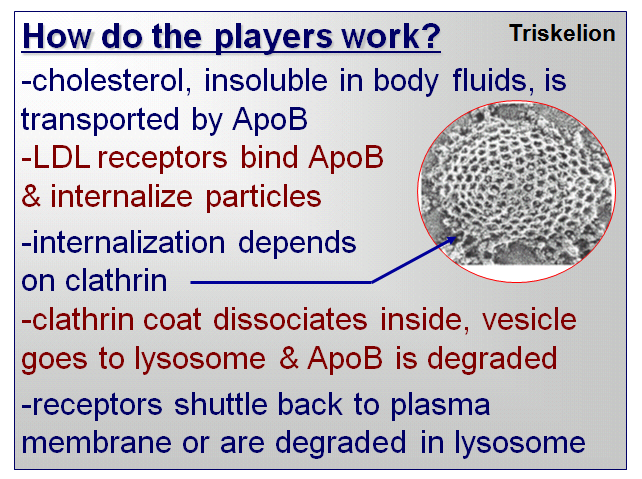
Cholesterol, you’d prolly still have heart attack

- ApoB is pr recognized by receptor

- extra digits: sonic hedgehog signalling pathway

- need cholesterol for embryonic development

- you’ll have it anyways, but needs to be internalized



- if you purify clathrin, it’ll self assemble to

Form clathrin basket

- SEM

- lose clathrin, lose trafficking of prs

- once clathrin has internalized vesicles, than

Clath falls off

-

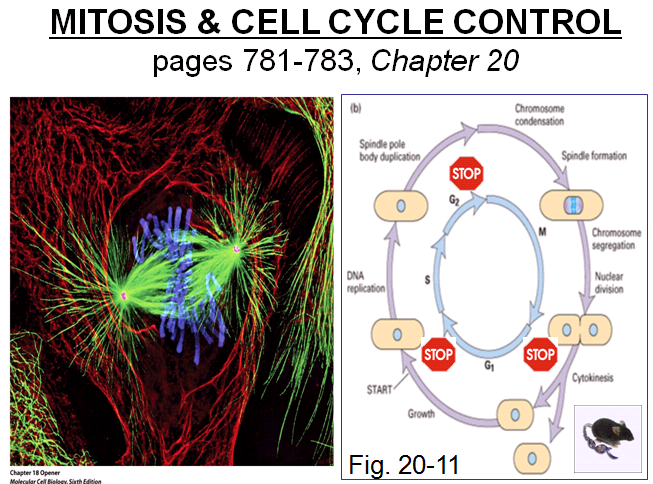
Signal Transduction

- cells respond to signals without having to internalize them

- signals may be secreted growth factors i.e. EGF instructing cells to divide

How does the cell respond? Positively and negatively?

- to transduce signal, you don’t have to internalize it

**Mitosis**

p. 781-783 (nice pic)

- once individual chrs are duplicated in S phase,

they are held together along their length by

prs called cohesins

- mitosis: process where duplicated chrs are

segregated to daughter cells

Prophase

- interphase MTs replaced by asters as duplicated

centrosomes become more active in MT nucleation

- provides 2 sites of assembly of MTs => asters

- 2 asters move and become opposite poles of

mitotic spindle (MT-based structure that separates

chrs)

- pr synth stopped, nucleolus breaks down and chrs

condense

- cohesins are degraded except at centromeric region where 2 sister chromatids remain attached by cohesins

- events coordinated by rapid increase of mitotic cyclin-CDK complex activity: kinase that phosphorylates prs

Prometaphase

- initiated by breakdown of nuclear envelope and pores and disassembly of lamin-based nuclear lamina

- MTs capture chr pairs at specialized structures called kinetochores

- sister chromatids align equidistant from 2 spindle poles => congression

Metaphase is the stage when all chrs are aligned at the metaphase plate

Anaphase

- induced by activation of the anaphase-promoting complex/cyclosome (APC/C)

- activated APC/C leads to destruction of cohesins

- anaphase A: chrs pulled to poles by MTs attached to kinetochore

- anaphase B: mvt of spindle poles farther apart

Telophase is stage where nuclear envelope reforms, chrs decondense, and contractile ring assembly

Cytokinesis happens when cell is pinched into 2 daughter cells by the contractile ring and interphase MT reforms

- green MT

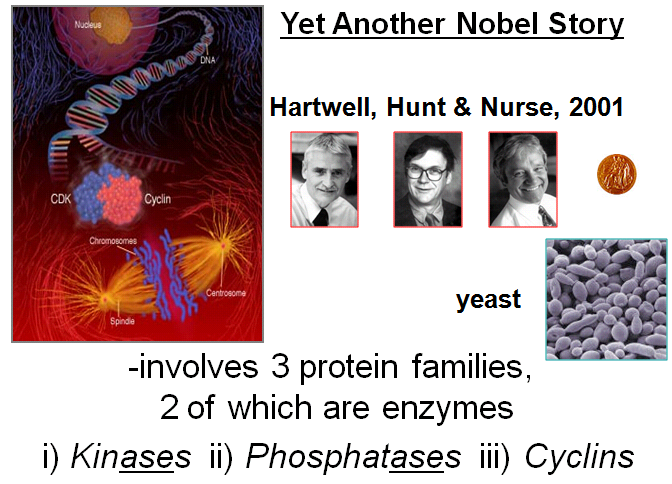
- blue DNA

- red cytoskeleton

- can use fluorescence microscopy in

Live cells

-



Concept:

-cell proliferation depends on signals

-progression through cell cycle is regulated: G2 -> M checkpoint

-check points ensure things should proceed:

Is all DNA replicated? Important for all

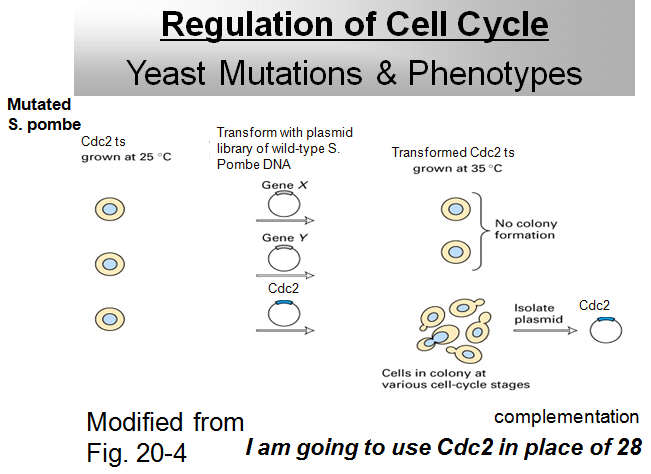
Is cell big enough? Yeast will be able to detect

Is environment favorable? Doesn’t apply for humans

Is DNA damaged? If DNA damaged, repaired or shutdown (apoptosis)

- there is apoptosis in yeast

- micrograph?

- cyclins used at checkpts

- from yeast 2 hybrid: fixing mutation so

You can make histidine

- fixed it by recreating TF -> complementing

Mutation by introducing a WT gene

- think “gene therapy” for yeast

- 2 genes but in diff strains of yeast

- functional complementation

- start with pool of yeast and mutate genome

- grow yeast at optimal and only temp (25)

- if you raise temp, they won’t grow

- introduce WT genes back into mutated strains

- then raise temp -> temp selection

- no growth

- then introduce another gene and if no growth,

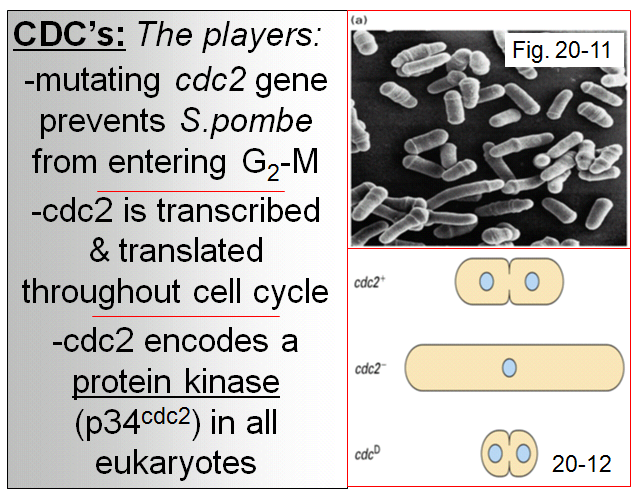
Tells you that WT gene didn’t complement mutation

- they could figure out that original mutation

Would be in cdc2 gene

- if cdc corrects mutation and allows cells to

Undergo mitosis =>



- trying to find which one was mutated in first way

- get around by introducing WT version

- SEM

- cells spend most of time in interphase

- cdc mutated blocks G2-M checkpoint

- how can it behave as a regulator of cell cycle

- kinase phosphorylates

- 34,000 D encoded by cdc2 genes

- if yeast is mutated, incorporate are own cdc2

- cdc2+ = normal

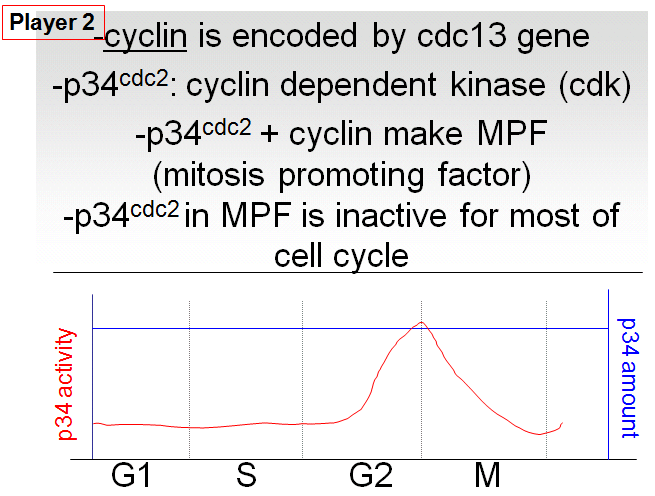
- cdc2- grows until it dies

- cdcD -> constantly being turned on (excessive)

- DNA won’t have time to replicate and this cell

Will die as well

-



- cyclin regulates cdc2

- no pr works in isolation

- looking at cell cycle: amt of p34 -> increases

In G2-M

- kinase phosphorylates

- pr sitting in cell doing nothing but waiting for signal

- once signal is there, change it’s biological activity

And it becomes active

