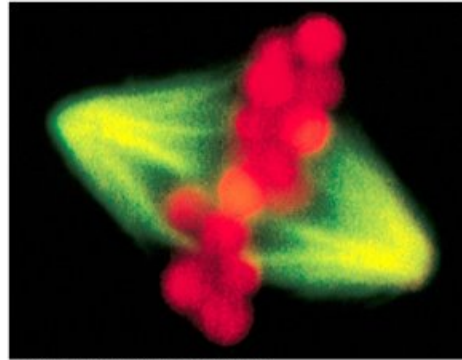


Week 7 & 8

Cellular reproduction: The cell cycle

Read Karp – 8th edition – Chapter 14
Cell cycle



Cellular reproduction overview

3rd tenet of cell theory!

- new cells originate only from other living cells

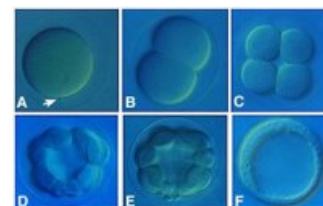
Single celled organisms

- cellular reproduction ensures propagation of species



Multicellular organisms

- 1 cell (zygote) divides to become highly organized complex of many of cells
- cell division continues through life
- adult humans - millions dividing at any moment to replace old, damaged cells

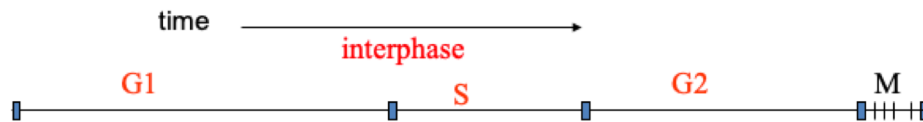


Sea urchin embryos

Cell cycle introduction

Cell cycle = the time from one division to the next division

Cell cycle is divided into 2 visible phases:



M phase (M for mitotic) – includes mitosis (nuclear division) and cytokinesis (cell division)

Interphase – cell growth and metabolism

- length of interphase can vary depending on cell type
- <30 minutes in early embryo
- 12-36 hours for rapidly dividing human cells (e.g. blood stem cells)
- Months for slow growing cells (e.g. liver)

Most cells in our body actually don't divide! They're 'stuck' at the end of G1... so we say these cells are in a G0 state. Also called 'quiescent' cells.

The cell cycle

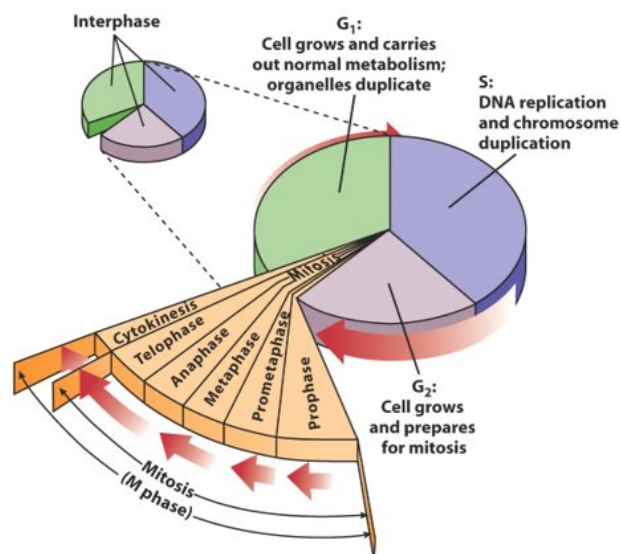
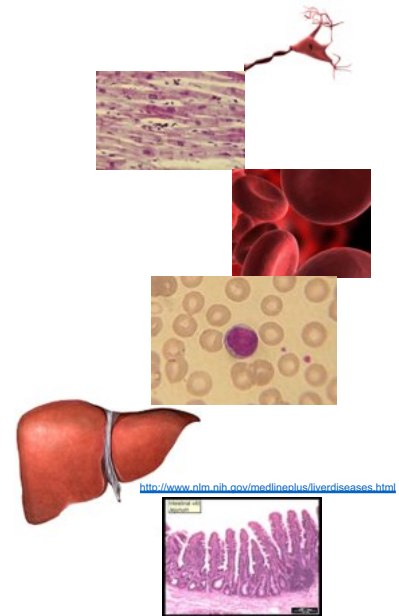


Figure 14-1 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Different cells go through the cell cycle at different rates

Cells can be grouped into 3 broad categories with respect to cell cycle

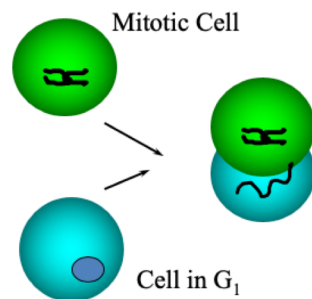
- *Cells that do not cycle, but enter a permanent arrest stage (G_0)*
 - e.g. neurons, muscle cells, RBCs – once differentiated, they remain in that state until they die
- *Cells that normally do not divide, but can be induced*
 - e.g. liver cells, lymphocytes
- *Cells that divide regularly*
 - e.g. stem cells that produce the epithelial cells that line body cavities and surfaces



Just from a number of cells point of view, *most* cells are in G_0

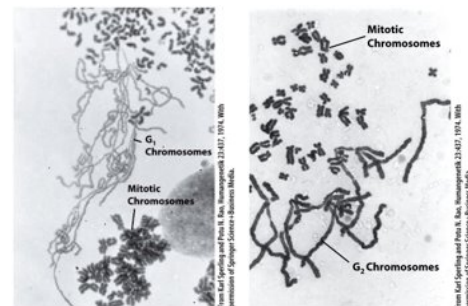
Early experiments into cell cycle regulation

Experiment by Rao and Johnson (1970) fused mitotic and interphase cells:



Found: if interphase cells were in G_1 , they observed “premature chromosomes” (slightly condensed) i.e. the non-mitotic cell tried to enter M phase

Conclude: a “factor” present in the mitotic cell directs cells to undergo mitosis (i.e. it’s under positive control)



What they actually observed

Same experiment, but fused a mitotic cell with a cell in G_2 . G_2 chromosomes are thicker because they’ve gone through the S phase!

Control of the G2 to M transition

Entry into mitosis requires a protein complex called Maturation Promoting Factor or MPF

MPF is a complex of two proteins:

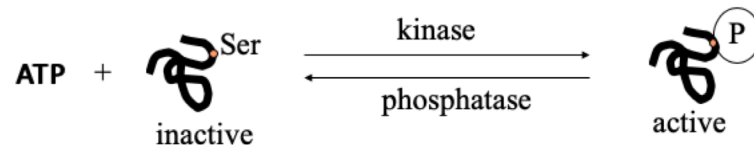
1: Cyclin b

- regulates Cdk1 activity
- levels rise and fall throughout cell cycle (as in, it cycles, get it?)

2: Cdk1 (cyclin dependent kinase)

- a **Kinase** is a protein that adds a phosphate from ATP to other proteins; the activity of the target protein is modified.
- As a note: Cdk1 is a kinase, but it also a target of kinases and phosphatases

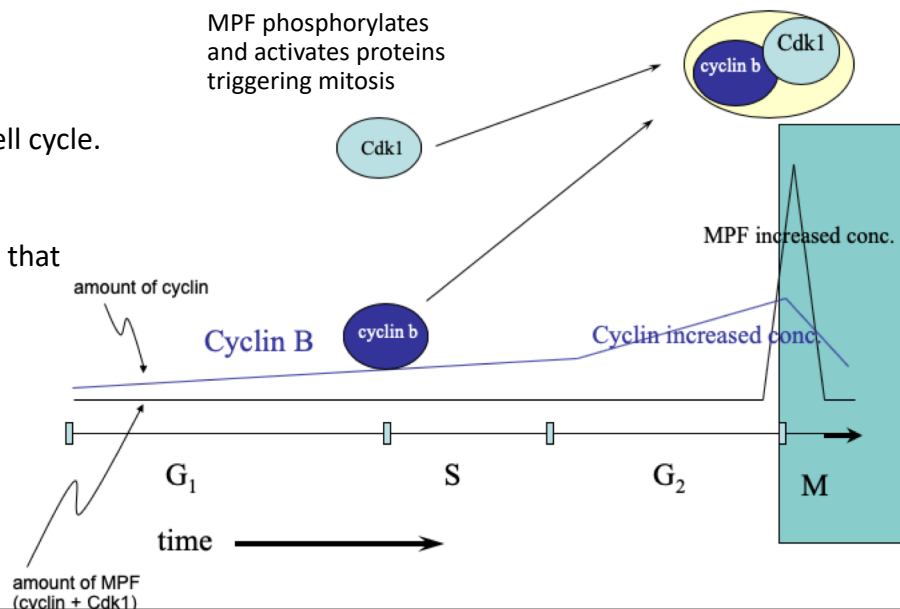
Generalized schematic of kinase/phosphatase switches:



Control of the G2 to M transition

Cyclin levels increase throughout cell cycle. Cdk1 stays constant.

At a critical threshold, enough MPF is formed that triggers mitosis.



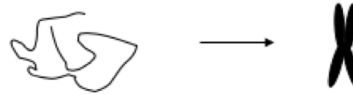
What does MPF do???

It phosphorylates and thus activates a whole bunch of proteins needed for mitosis:

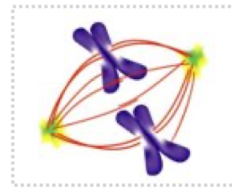
- Phosphorylates lamin proteins – causes nuclear envelope to break up



- Alters histones – causes DNA to condense



- Activates proteins of mitotic spindle



Yeast genetics was used to understand cell cycle control

Conditional mutant: protein works fine at low temperature, not functional at a higher temperature

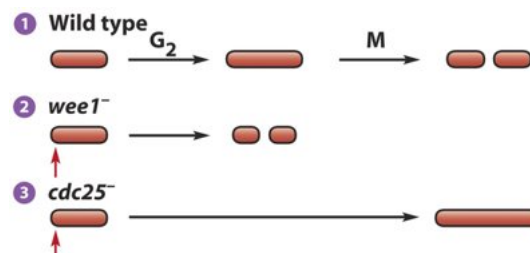


Figure 14-4b Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Wee1 protein is an inhibitor of MPF (prevents mitosis)

Wee1- mutant lacks inhibitor – results in early division

Cdc25 protein activates MPF

Cdc25- mutant lacks activator, division fails to occur

MPF is activated in a multi-step process

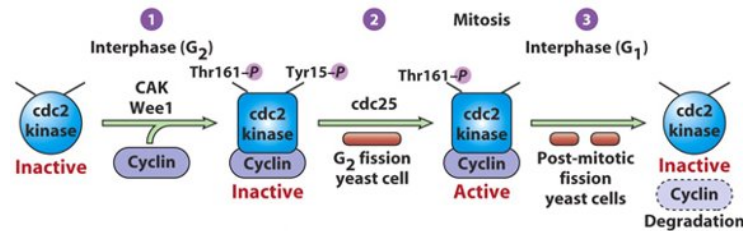
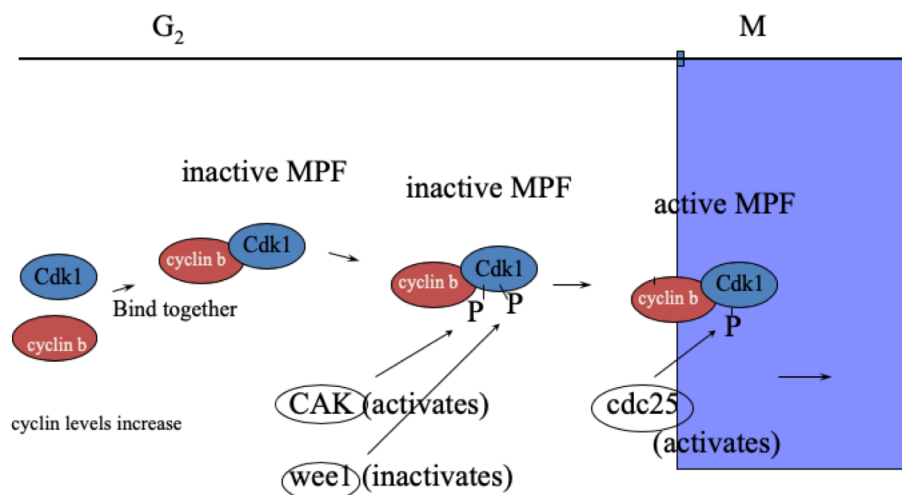


Figure 14-6a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

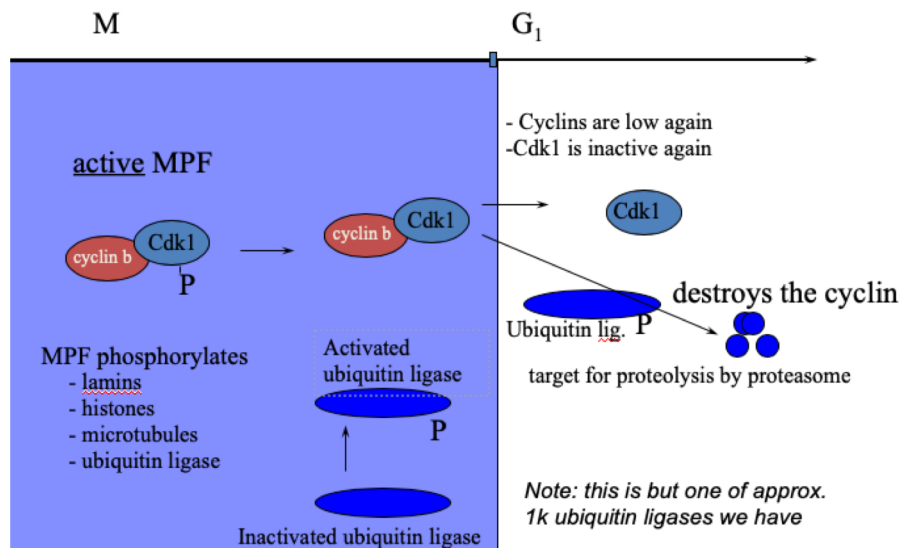
1. Cdk (cdc2 kinase) is inactive, even after bound to cyclin
 - because wee1 adds phosphate to Tyr15 – inhibits activity
 - CAK adds a phosphate to Thr161 which is required for activity
2. Cdc25 is a phosphatase – removes P from Tyr15 – Cdk is now active
3. At end of mitosis, another phosphatase removes P from Thr161, cyclin detaches, and is degraded

Note: because this is in yeast, the names are a bit different. Cdc2 kinase here = cdk1 in MPF

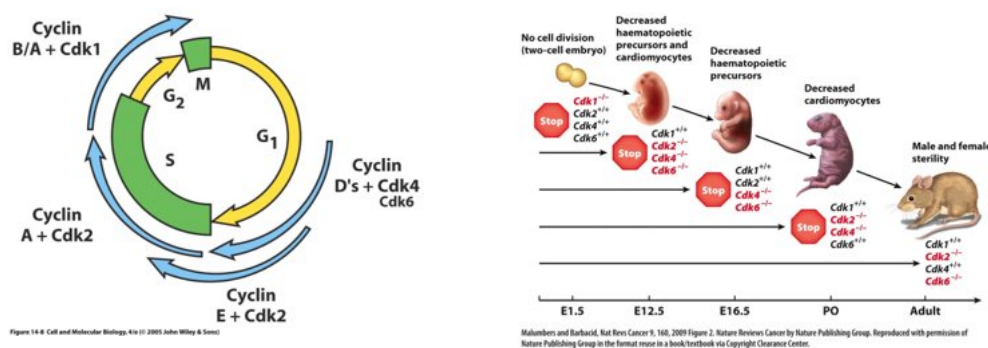
MPF activation and the cell cycle



Cyclin action is turned back off by activating ubiquitin ligase, which terminates MPF activity by causing proteolysis of cyclins



Humans have different cyclins and cdks that operate at different checkpoints and cell cycle stages

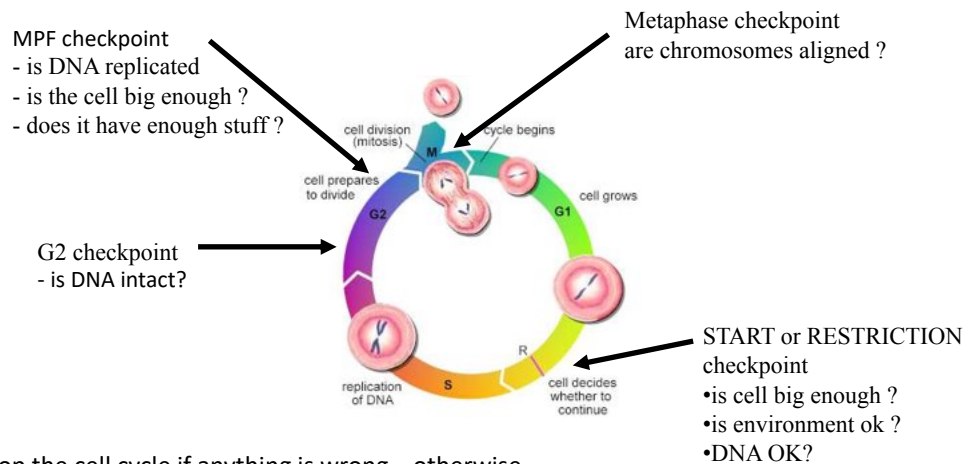


There's some overlap in their function though.

Missing cdk1 results in no cell division.

Missing all others, but with a functional cdk1 results in some cell division... but the mouse dies *in utero*. So, cdk1 is partially *redundant* for the other cdks.

MPF regulates one of several cell cycle checkpoints



Checkpoints stop the cell cycle if anything is wrong... otherwise disastrous things could happen like passing mutations onto daughter cells. . . .

What happens when checkpoints get messed up???

The DNA damage checkpoint was discovered by patients with a very rare disease

Occurs twice... once before entering S phase and once before the G2 to M transition

Discovered accidentally in patients with **Ataxia-telangiectasia (AT)**

- Neurodegenerative disease (ataxia = loss of motor control), but affects many other tissues
- telangiectasia = tiny red spider veins (often starting in eyes)

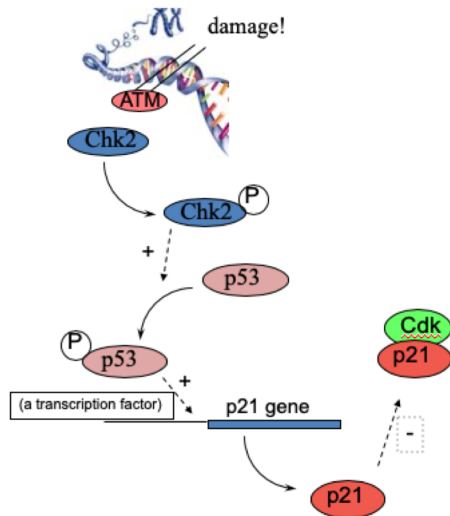


See image at : http://see.visualdx.com/diagnosis/ataxia-telangiectasia_syndrome

Patients susceptible to cancer

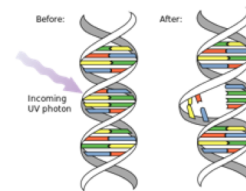
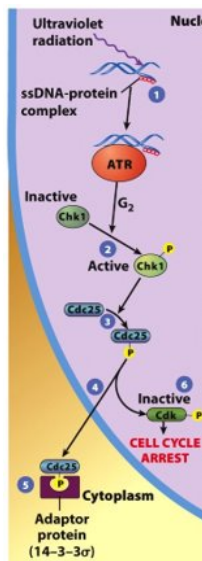
Also very susceptible to DNA damage from ionizing radiation

The DNA damage checkpoint (pathway A)



1. ATM protein detects a break in DNA strand in G1
2. ATM is a kinase. It phosphorylates another kinase Chk2, thus activating it.
3. Chk2 is also a kinase. It phosphorylates the protein p53 (stabilizes it)
4. When phosphorylated, p53 turns on p21 gene – p21 is synthesized
5. p21 protein binds Cdk and inhibits it
6. Cell is prevented from entering S phase until DNA repaired

The DNA damage checkpoint (pathway B)



"DNA UV mutation" by derivative work: Mouagip Licensed under Public Domain via Wikimedia

1. UVB light causes adjacent pyrimidines on DNA strands to fuse into dimers (not good!)
2. ATR recognizes these and phosphorylates Chk1
3. Chk1 phosphorylates cdc25 (the phosphatase needed to activate cdk)
4. This phosphorylated cdc25 gets bound to a 14-3-3 protein and exported out of the nucleus, thus can't activate cdk and the cell cycle is arrested

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Defects in cell cycle genes are often found in cancers

- Uncontrolled cell divisions result in cancer
- These cell cycle checkpoint proteins prevent the cell cycle from proceeding when it shouldn't. Thus, when they don't work cells can potentially grow uncontrollably
- 50% of human tumor cells have mutations in the p53 gene
- 1% of human genes implicated in cancer (approx. 3000)
- Cancer genome project aims to identify all gene defects associated with cancers



<http://www.sanger.ac.uk/genetics/CGP/>

Components of a checkpoint.

1. **Sensor.** *is DNA damaged ?*

- ATM/ATR proteins. One break stops the whole cell cycle.

2. **Transducer(s):** create a signal

- Cdc25, p53
- the MPF proteins
- this is one reason that the cell works in a multi-step fashion; different control systems interact. Points for feedback regulation (which we didn't talk about) and *amplification* of the signal.

3. **Effector:** change key enzyme action/protein

- In the first example, it's the synthesis of p21 that binds cdk... thus cell can't proceed to the next stage of the cell cycle

WYSKFTS

- Stages of cell cycle
- Speed and frequency of cycle for different cells
- Identification and functions of MPF
- Steps involved in MPF activation/de-activation
- Checkpoints – functions and components
- Steps involved in DNA damage checkpoint