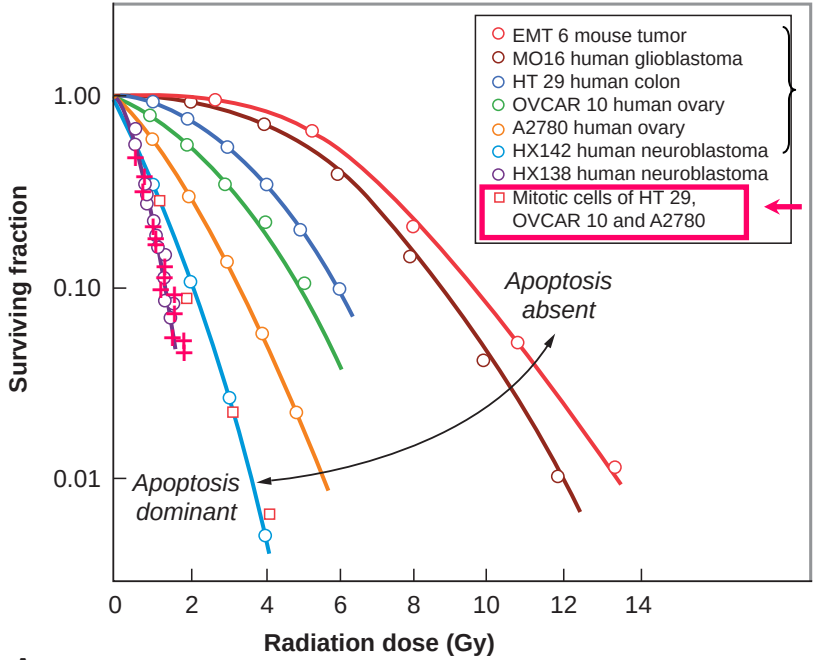
# Chapter 4 Radiosensitivity and Cell Age in the Mitotic Cycle Chapter 22 Cell, Tissue, and Tumor Kinetics (1st Part)

9/23/2024

# Outline

- **Studying the Cell Cycle**
- Age-Response in Synchronous Dividing Cell Cultures
- The Effect of X-rays of Synchronously Dividing Cell Cultures
- The Age-Response Function for a Tissue *in Vivo*
- Variation of Sensitivity with Cell Age for High LET Radiations
- Mechanisms for the Age-Response Function in Radiotherapy
- The Possible Implications of the Age-Response Function in Radiotherapy
- The Cell Cycle (Chapter 22)
- Checkpoint Pathways (Chapter 22)

# Survival Curve Shape and Cell Cycle




Asynchronous cell

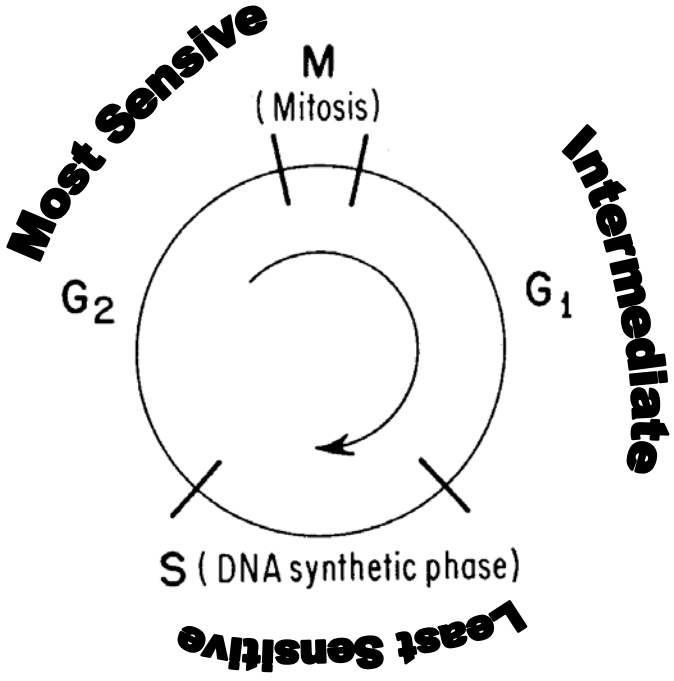
Mitotic cells

- Asynchronous cells show a wide range of radiosensitivity
- **Mitotic cells from all of the cell lines have essentially the same radiosensitivity**
- Implication – radiosensitivity is governed by DNA content and conformation

  (hence, position in cell cycle)

# Radiosensitivity and the Cell Cycle

- Cells are **most sensitive** to radiation during mitosis (M phase) and post-DNA synthesis/pre-mitosis ($G_2$ phase)
- **Less sensitive** during the preparatory period for DNA synthesis ($G_1$ phase)
- **Least sensitive** (or **most resistant**) during DNA synthesis (S phase)
- During mitosis (M), the metaphase is the most sensitive



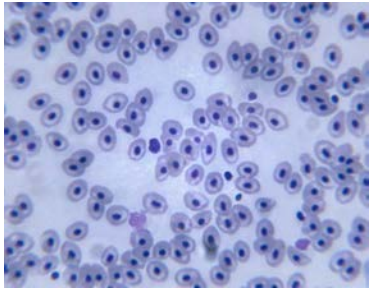
# The Big Picture

- The rest of the **chapter 4** deals with the scientific methods of studying the variation of radiosensitivity with the position of the cell in the cell cycle (aka the age-response); the effect of radiation qualities (i.e., high LET vs. low LET) on age-response

- **Chapter 22** (part I) concerns the molecular control of cell cycle progression and checkpoint pathways

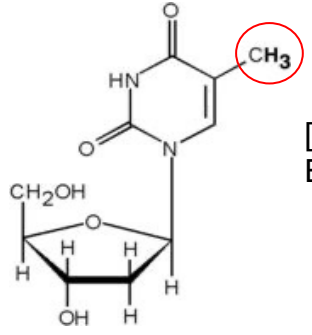
# Studying the Cell Cycle

- Using conventional light microscope, the only event that can be identified is mitosis



- The remainder of the cell cycle can be further divided by using some **marker of DNA synthesis**
- The original technique was autoradiography, introduced by Howard and Pelc in 1953

# Cell Labeling Techniques – Autoradiography



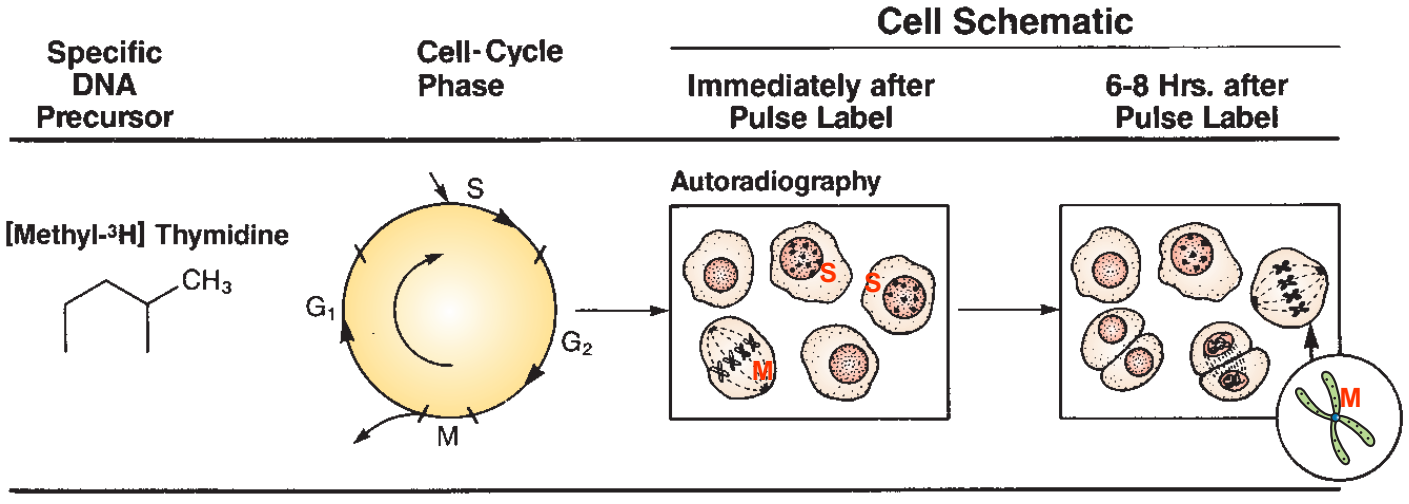

[Methyl-$^{3}$H] Thymidine
Emit β-particle

Cells growing in monolayer
↓
$^{3}$H-TdR (low intracellular pools)
↓
$^{3}$H-TdR incorporates into synthesized DNA
↓
Wash out excess $^{3}$H-TdR
↓
Fix, stain, photographic emulsion
↓
↓ ~1w - 1m.
Develop / Fix

The area through which a β-particle has passed appears as a black spot

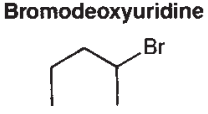
**Autoradiography** – the specimen itself is the source of the radiation, which originates from radioactive material incorporated into it (i.e., "self-labeling")

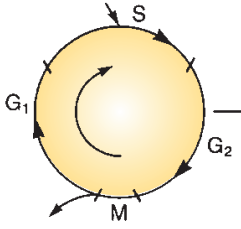
# Autoradiography

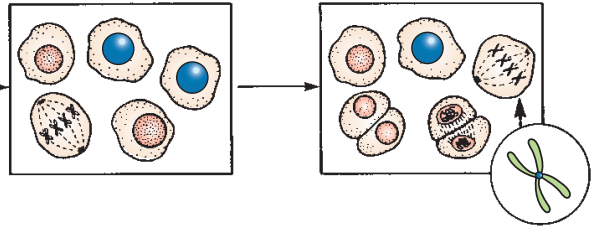


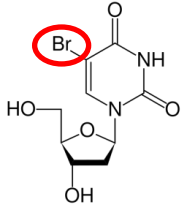

- Black spot is β-particle
- Observe/Count cells with black grains (DNA synthesis)
- ↑ magnification, observe chromosomes
- Then lengths of the various phases of the cycle can be determined by varying the lengths of time cells are incubated with label before fixing

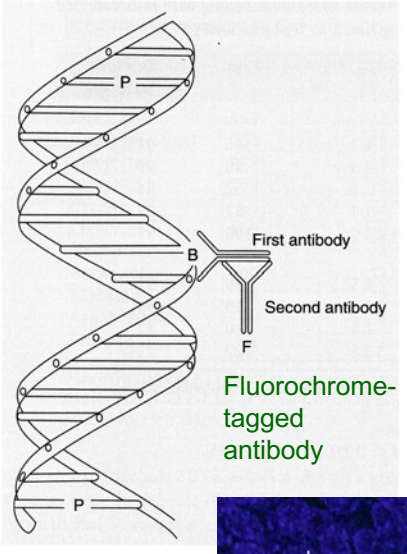
# BrdU Labeling

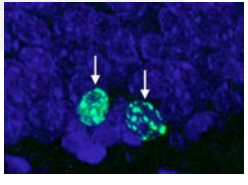


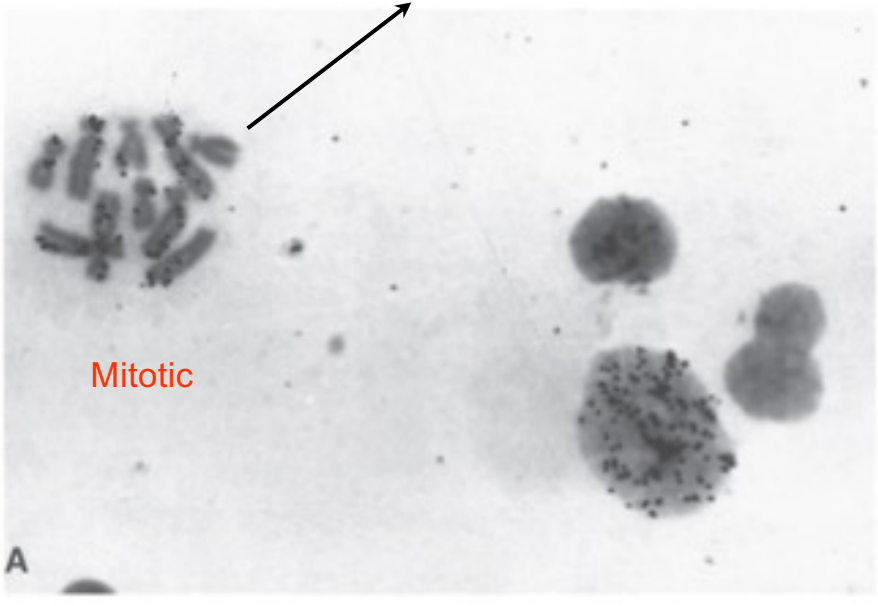



- 5-bromodeoxyuridine replaces tritiated thymidine
- Likewise, it is incorporated into cells in S
- Can be recognized by the use of a Giemsa stain or a monoclononal antibody to BrdU-substituted DNA
- **Advantages** – no radioactivity, faster because no emulsion period needed

# Labeling of Chinese Hamster Ovary (CHO) Cells

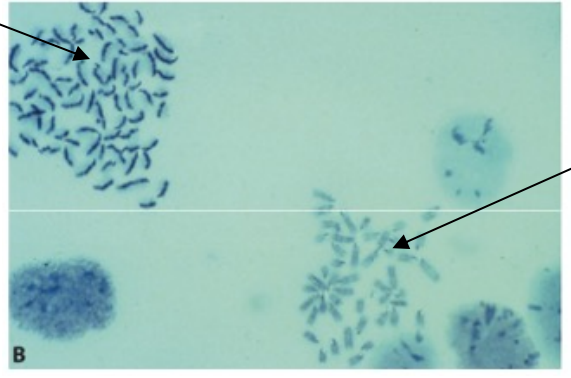
The cell was in S-phase when the culture was flash-labeled but moved to M phase before it was stained and autoradiographed



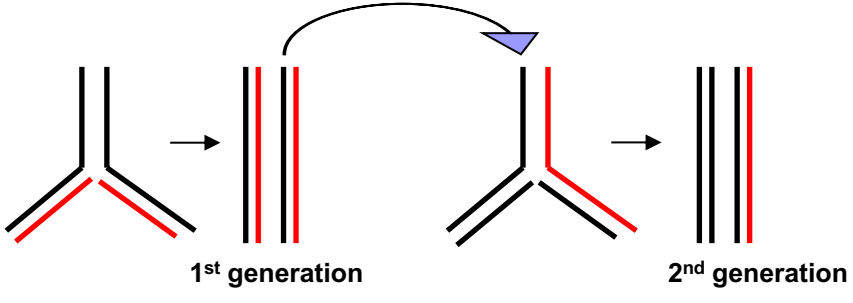
# Labeling of CHO Cells

**1st generation mitotic cells**
Both chromatids are stained

**BrdU Labeling**




**2nd generation mitotic cells**
Only 1 chromoatid of each chromosome is stained



# Conclusions from Cell Labeling Studies

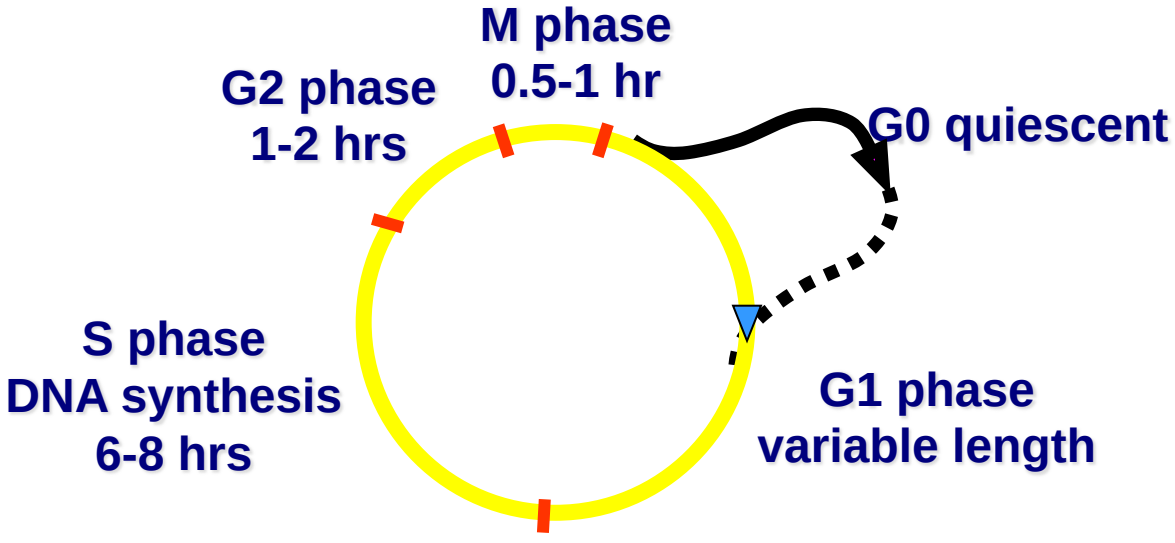
- Cells synthesize DNA only in discrete phase of the cell cycle (called **S**)
- There is an interval between mitosis (**M**) and S where no DNA synthesis occurs → Gap 1 or **$G_1$**
- There is another "gap" after DNA synthesis, before mitosis, called **$G_2$**
- This M→G1→S→G2 occurs in all mammalian cells
- The lengths of times for these phases vary from one cell line to another

# Length of Time in Phases of the Cell Cycle

| Phase of cell cycle | CHO cells (hours) | HeLa cells (hours) |
|---|---|---|
| Tc | 11 | 24 |
| $T_M$ | 1 | 1 |
| $T_S$ | 6 | 8 |
| $T_{G2}$ | 3 | 4 |
| $T_{G1}$ | 1 | 11 |

The difference in the total cell-cycle time between these two cell lines is accounted for almost entirely by the difference in the **length of the G1 period**

# Mammalian Cell-Cycle Times

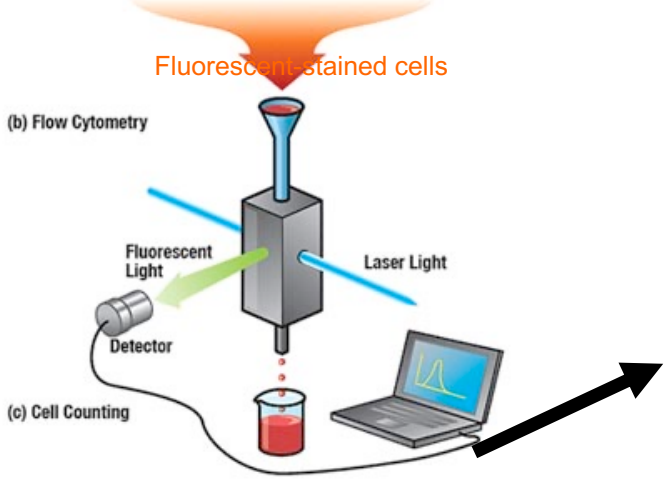


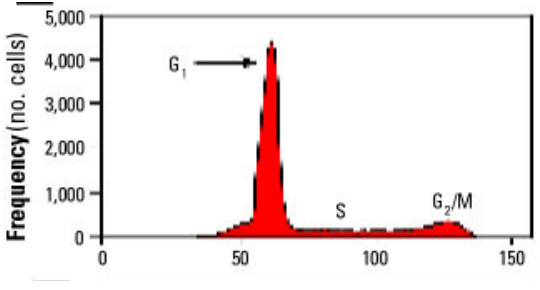

**Tc** – mitotic-cycle time (aka cell-cycle time)

| | |
|---|---|
| Crypt cells in mouse | 9-10 hours |
| Stem cells in resting mouse skin | 200 hours |
| Most human cells actively dividing | 12-24 hours |
| Human tumors | 48 hours (15-125 hours) |

# Pulsed Flow Cytometry

- **Conventional autoradiography** give precise, meaningful answers, but are laborious and slow
- Now largely replaced by **pulsed flow cytometry** which can count thousands of cells per second



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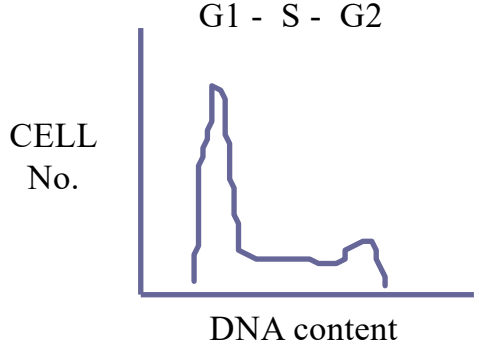
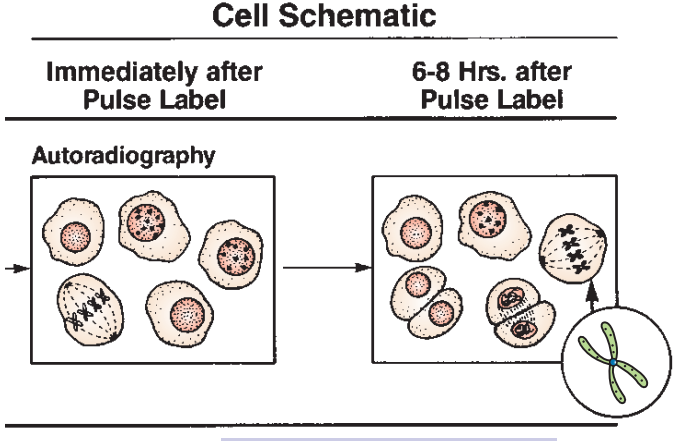
# Synchronously vs. Asynchronous Dividing Cell Cultures

- Asynchronous cell culture consists of cells distributed throughout all phases of the cell cycle




Flow Cytometry



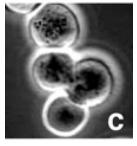

Autoradiography

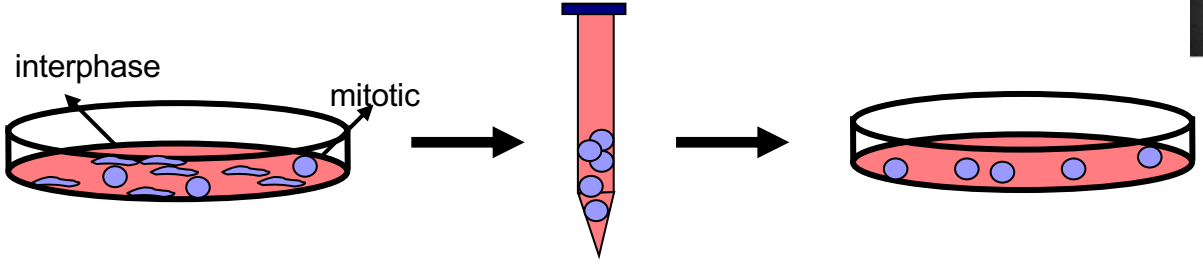
# Synchronized Cells

- Study of the variation of radiosensitivity with the position or age of the cell in the cell cycle requires **synchronously dividing cell culture**, i.e. populations of cells in which all of the cells occupy the same phase of the cell cycle at a given time
- Approaches to synchronize
  - Mitotic Harvest or Mitotic Shake-off
  - Drugs to affect cell cycle progression

# Mitotic Harvest (Mitotic Shake-off)

- When cells are close to mitosis they round up and become loosely attached to the surface



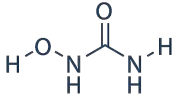

Asynchronous culture

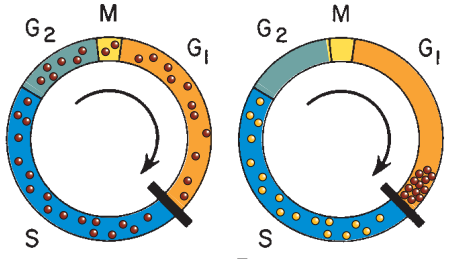
Synchronous mitotic cells

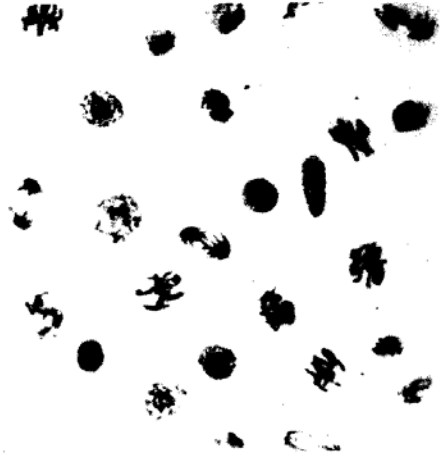
- Works only for cells that grow in monolayer

# Hydroxyurea (HU)

**Hyroxyurea**
1) Kills only cells in S phase
2) Blocks cells in G1

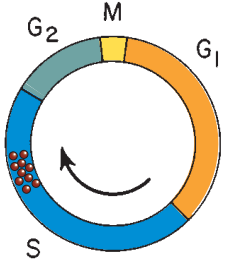


**Root tip of a Vicia seedling 11 hours after synchrony was induced with HU**

**HU added** – cells in G2, M and G1 accumulate at this block




**HU removed** – synchronized cells moves on through the cycle

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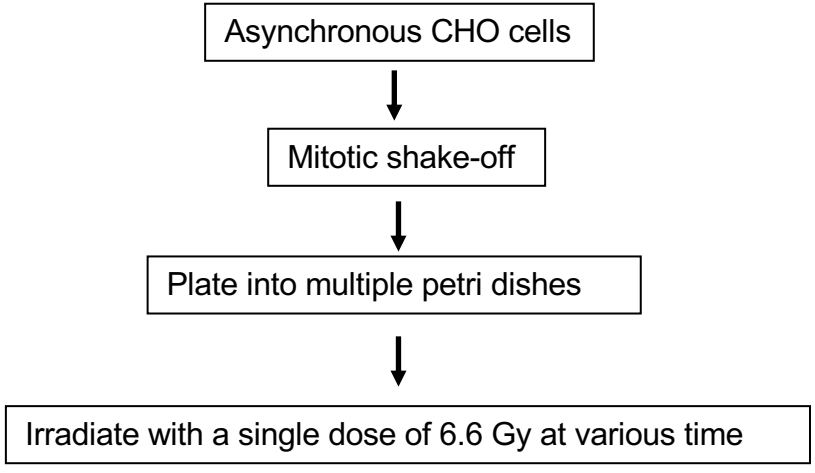
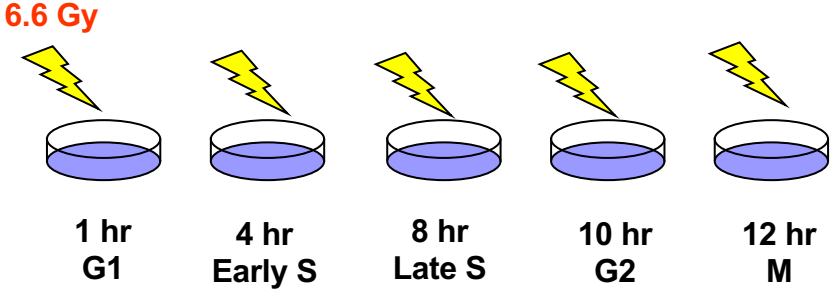
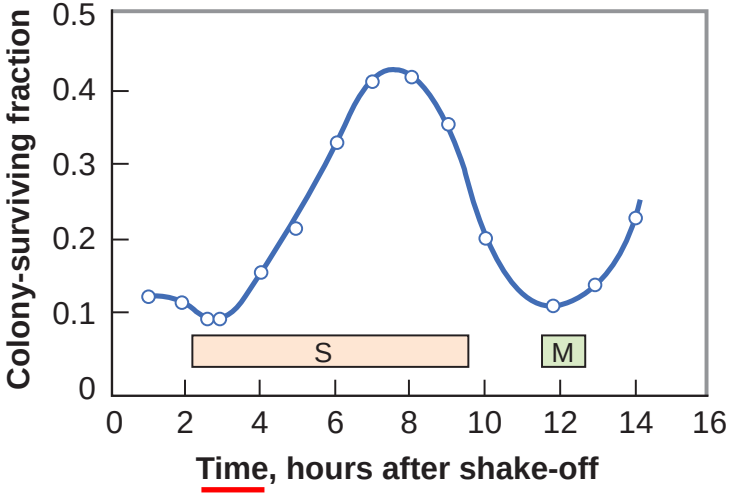
# Radiosensitivity of Synchronized CHO Cells

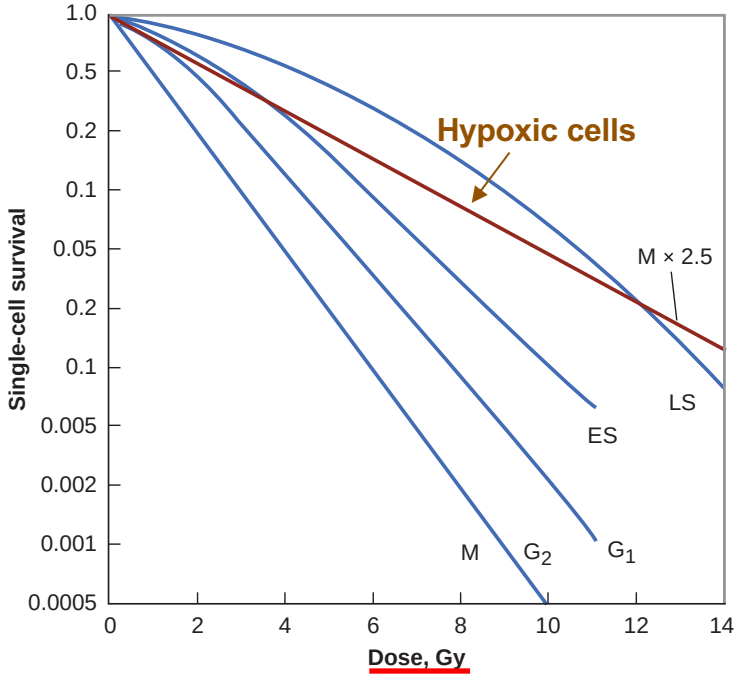



Single dose of X-ray

# Complete Cell Survival Curves at Various Cell-Cycle Phase




**CHO Cells**

Cells are most sensitive to radiation during M and $G_2$ phase – note that the curve is steep and has no shoulder (M more sensitive than G2 at lower dose)

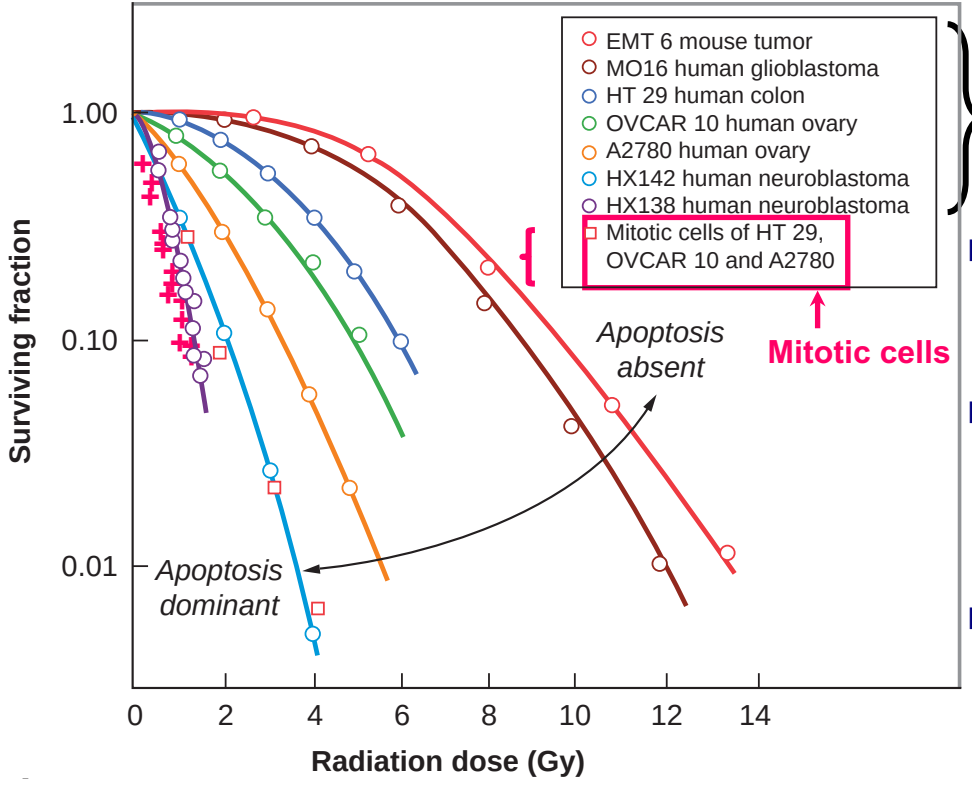
Cells are least sensitive in late S phase – note that the curve is less steep and has a very broad shoulder

Cells in $G_1$ and early S have intermediate radiosensitivity

Hypoxic cells are ~ 2.5 x more radioresistant compared to aerated cells (more in Chapter 6)

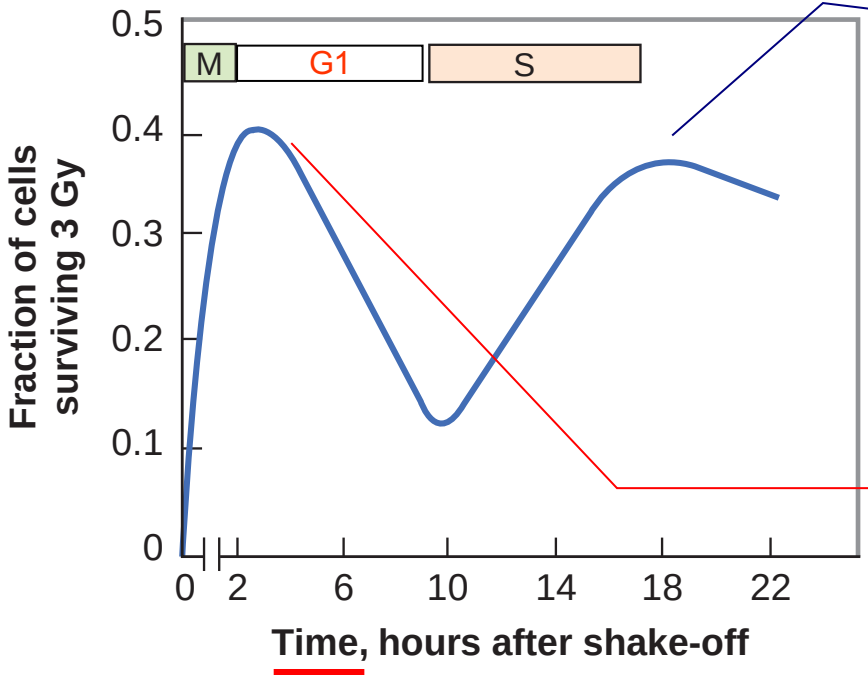
Cells in G0 are the most resistant (compared to cells that are cycling)

# Survival Curve Shape and Cell Cycle (Chapter 3)




- Asynchronous cells show a wide range of radiosensitivity
- **Mitotic cells from all of the cell lines have essentially the same radiosensitivity**
- Implication – radiosensitivity is governed by DNA content and conformation

# HeLa Cells

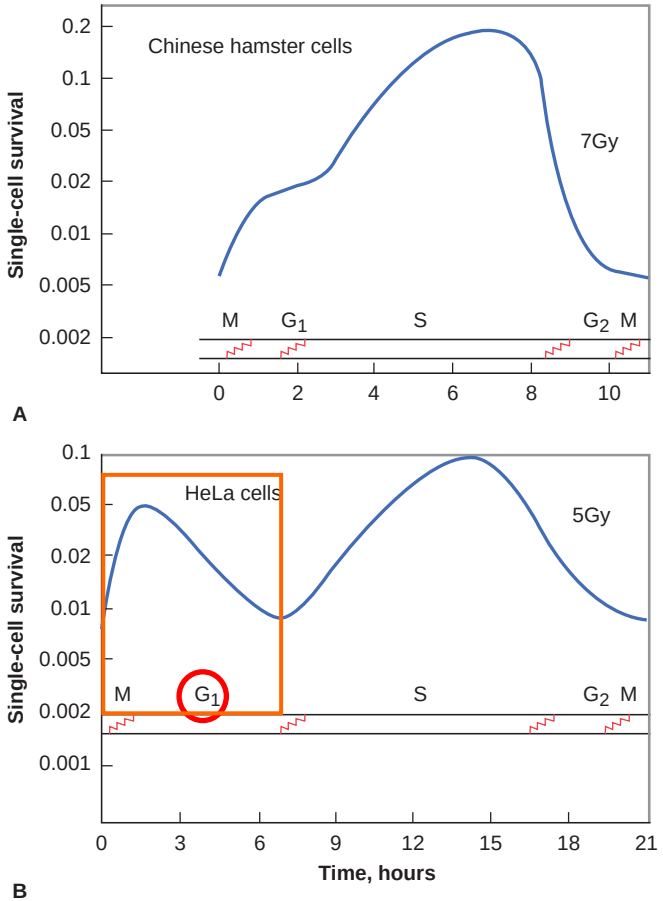



Increasing radioresistance in late S

At the beginning of $G_1$, there is a peak of resistance, followed by a sensitivity trough toward the end of G1

Single fraction of 3 Gy

# HeLa Cells vs. Hamster Cells




The general pattern of cyclic variation is very similar

Difference is in the G1 phase – HeLa cells have **a fine structure** during this period, i.e., a peak of resistance in early G1 followed by a sensitivity trough toward the end of G1

Remember the difference in $T_{G1}$

| Phase of cell cycle | CHO cells (hours) | HeLa cells (hours) |
|---|---|---|
| $T_{G1}$ | 1 | 11 |

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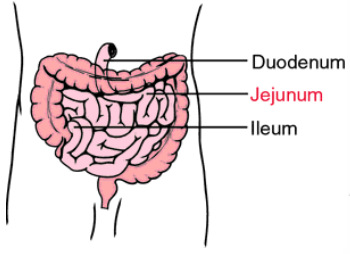
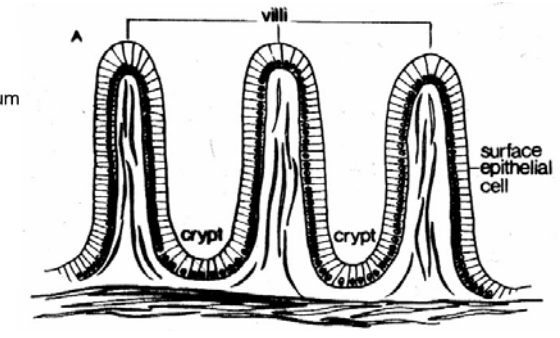
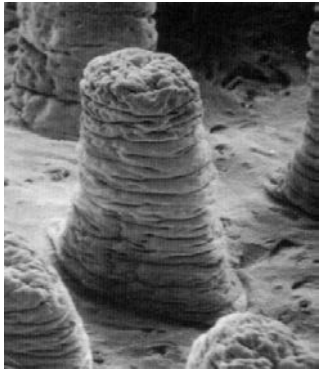
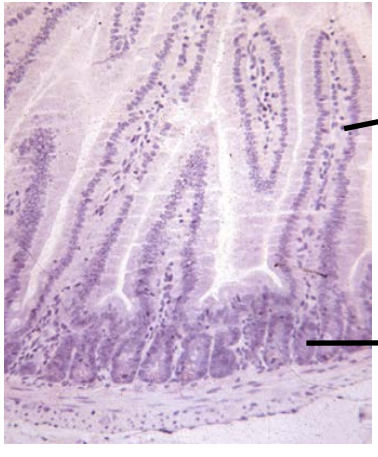
# Crypt Cells of the Mouse Jejunum



Scanning EM of jejunal villi



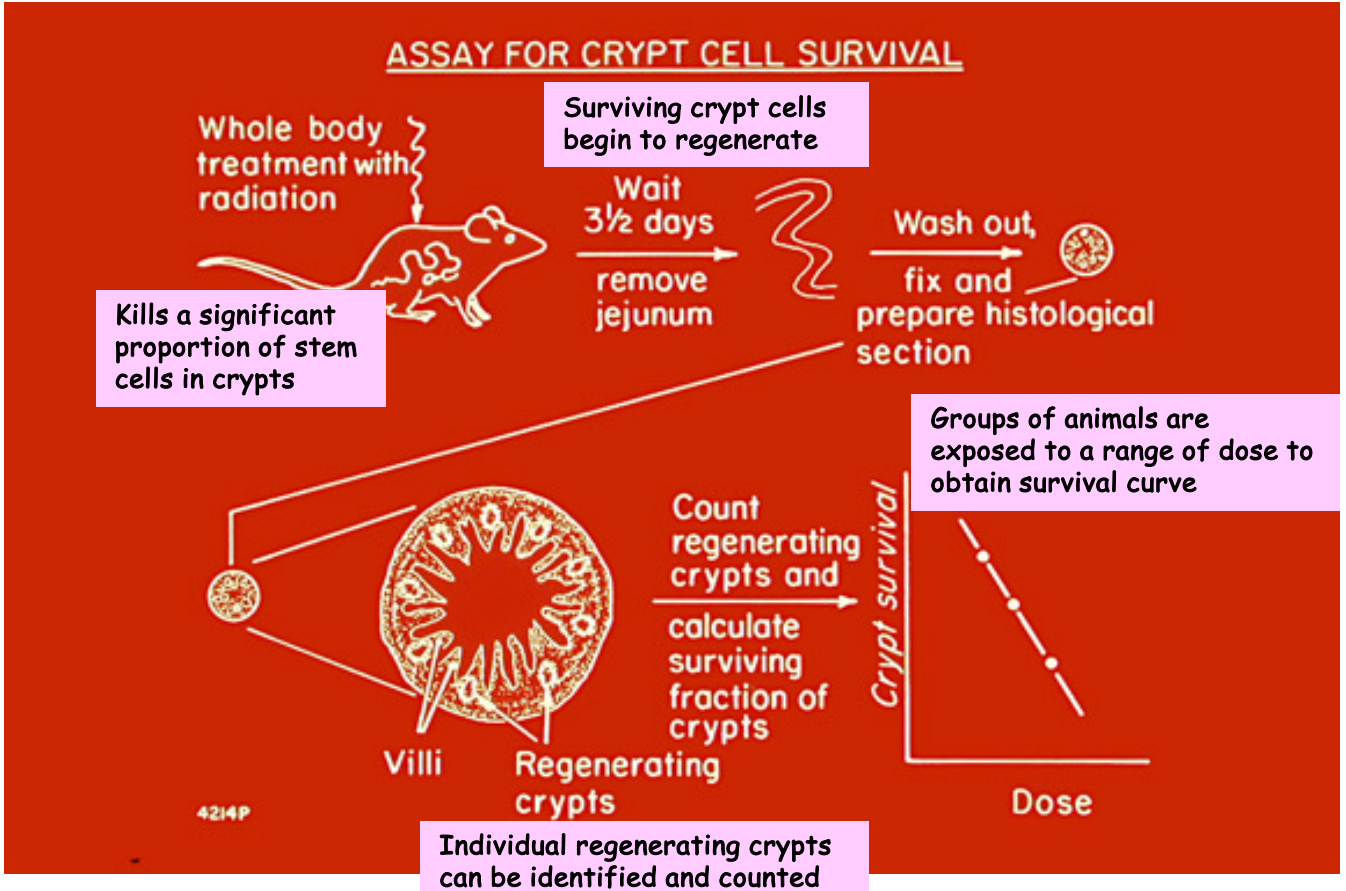
Villi

crypt

Actively dividing cells

The lining of the jejunum is a classic example of self-renewal system

# Crypt Cell Survival Assay



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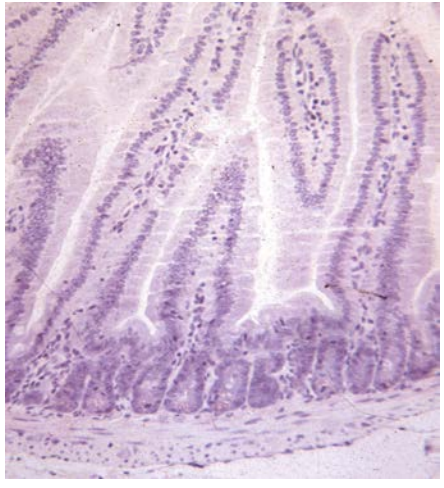
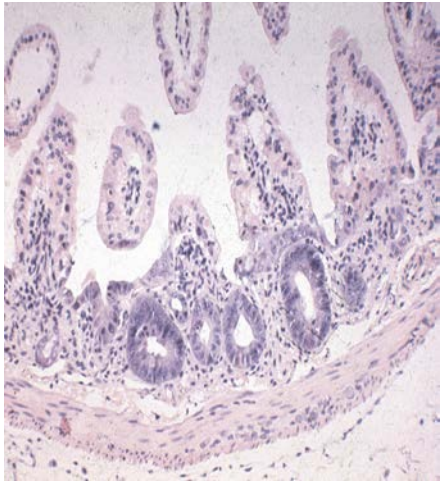


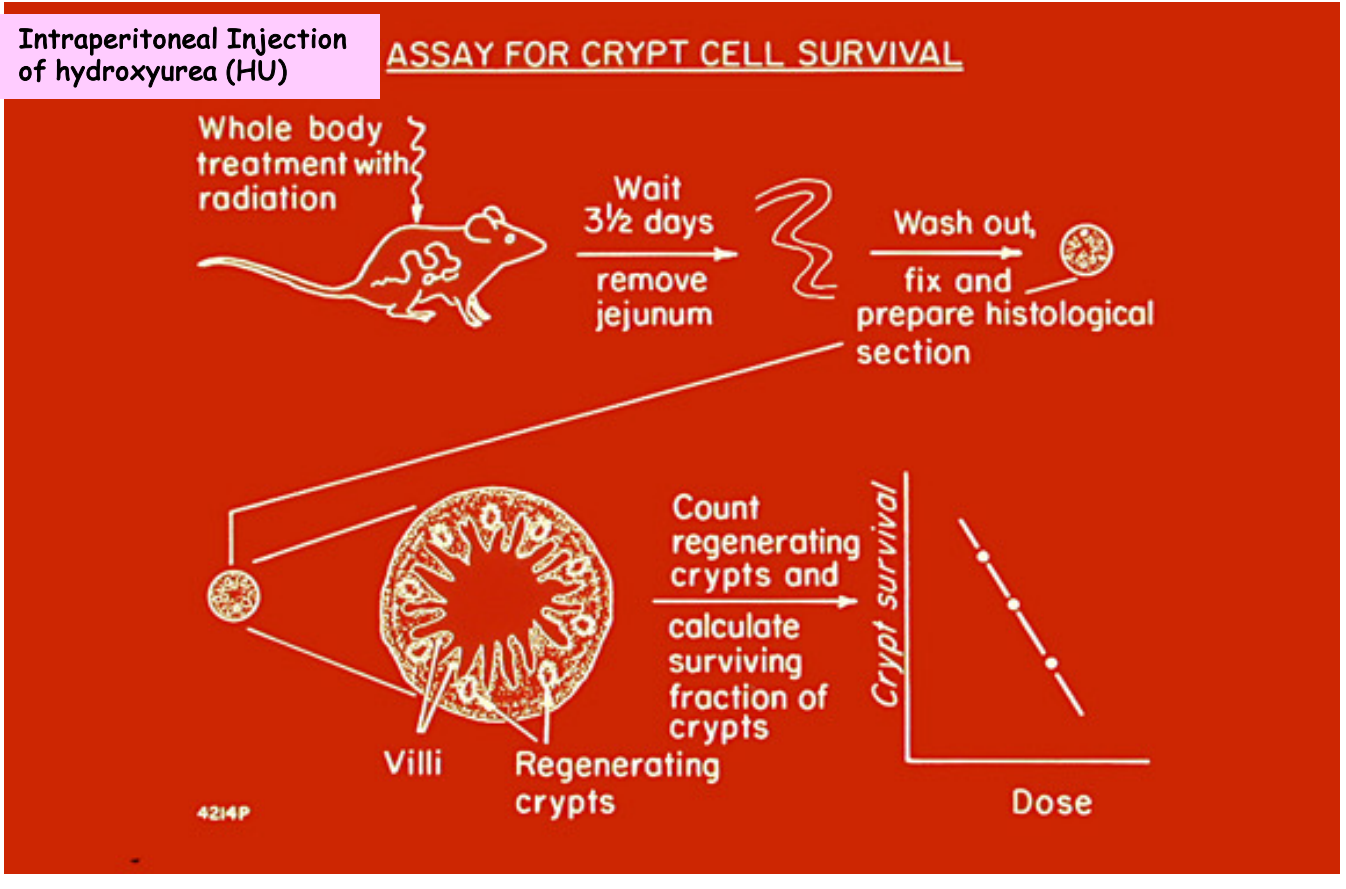
Unirradiated jejunum



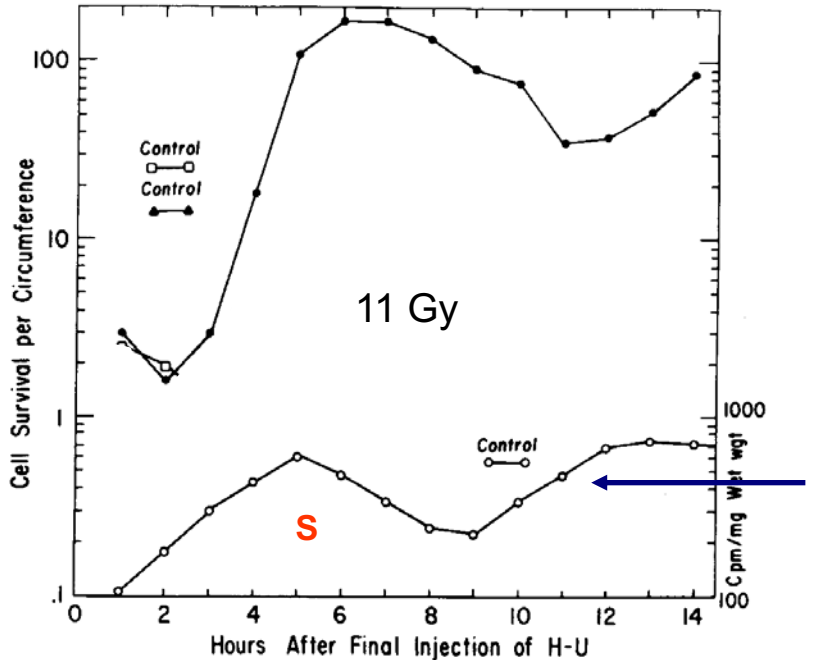
Regenerating crypts seen at 3.5 days following irradiation

The number of regenerating crypts per circumference of the sectioned jejunum as a measure of radiation damage

# Crypt Cell Survival Assay



# *In Vivo* Age-Response Curves




Mouse jejunal crypt cells synchronized with HU

Note that the pattern is very similar to that of *in vitro* cell lines

DNA synthetic activity is monitored by injecting with tritiated thymidine at hourly intervals after last injection of HU

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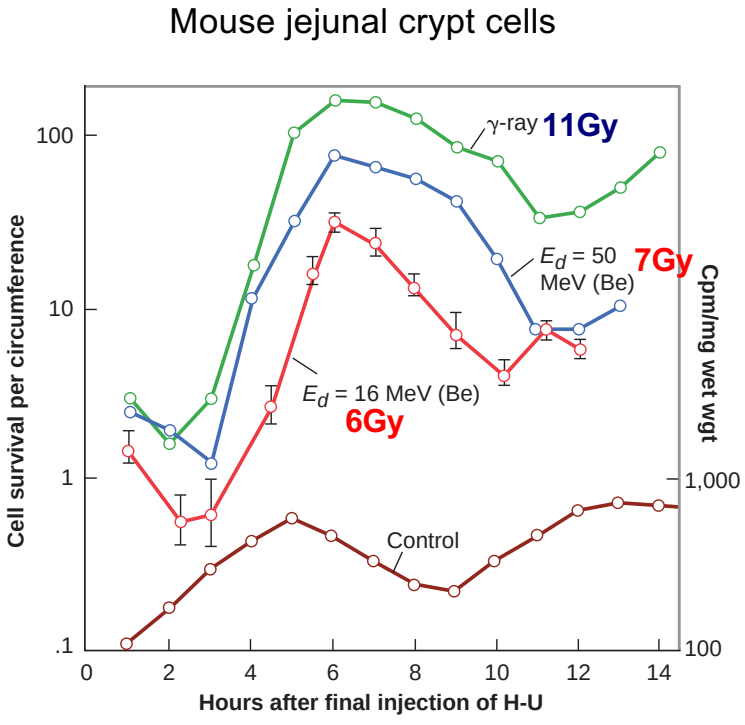
# Variation of Sensitivity with Cell Age for High-LET Radiations




The pattern is similar for neutrons and x-rays

As LET increases, the variation in radiosensitivity through the cell cycle decreases, i.e., **High LET responses are less affected than low LET radiation**

# Outline

- Studying the Cell Cycle
- Age-Response in Synchronous Dividing Cell Cultures
- The Effect of X-rays of Synchronously Dividing Cell Cultures
- The Age-Response Function for a Tissue *in Vivo*
- Variation of Sensitivity with Cell Age for High LET Radiations
- **Mechanisms for the Age-Response Function in Radiotherapy**
- The Possible Implications of the Age-Response Function in Radiotherapy
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# Mechanisms for the Age-Response Function

- The reasons for the sensitivity changes through the cell cycle are not understood
- Possible explanations
  - The level of sulfhydryl compounds (natural radioprotectors) is highest in S phase and lowest near mitosis ↔ the pattern of resistance and sensitivity
  - Radiosensitivity correlates with repair of DSBs (undamaged sister template available in late S for homologous recombination)
  - DNA is the primary target for radiation lethality → the amount or form of the DNA might result in variations in sensitivity

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# Implications for Radiotherapy

Single Fraction Regimen

Cells in more radiosensitive phases of cell cycle are killed / more resistant survive

⇩

Majority of cells left are in resistant phase of the cell cycle

⇩

**Tends to synchronize cell population**

# Implications for Radiotherapy

Fractionated Regimen

Between dose fractions, cells progress into more sensitive phases, thus increasing the number of sensitive cells to the next dose fraction **(Reassortment)**

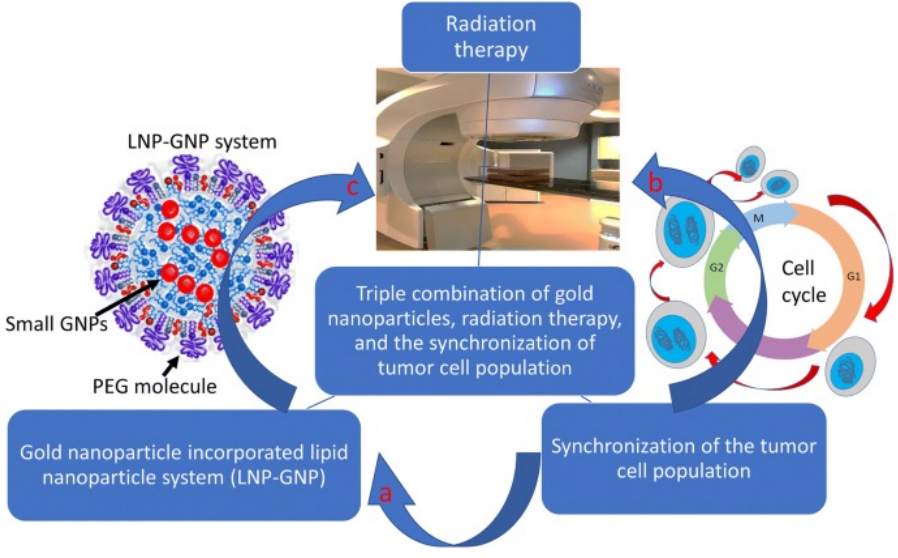
Reassortment occurs only in **rapidly dividing cells** and not in late-responding normal tissues

**Thus, "sensitization due to reassortment"** results in therapeutic gain

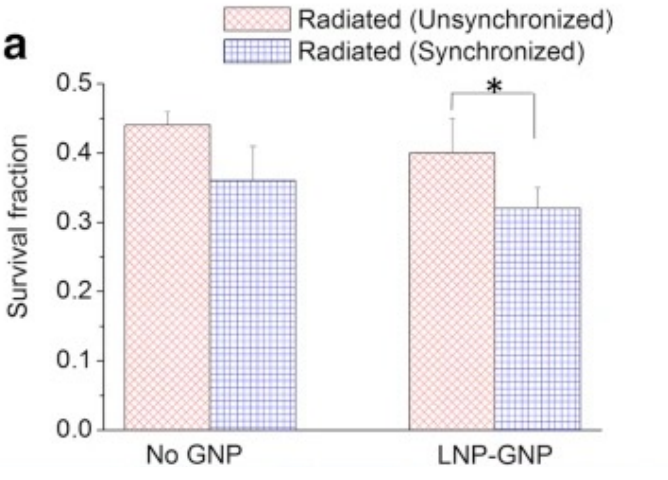
# Exploitation of Age-Response

- In 1970s, there was much interest in ***synchronization therapy***, i.e., treating with a 2nd agent at the optimum time interval after a priming treatment such as radiation
- However, results were disappointing, likely due to the fact that tumors tend to be very heterogeneous from a kinetic point of view: induced cell synchrony is quickly lost and/or impossible to achieve for the entire tumor

# Synchronization Therapy




breast cancer cell line




Cell cycle synchronization, gold nanoparticles, and radiation therapy may be combined to improve outcome of cancer therapy.

Bromma 2019; Cancer Nano 10(1)

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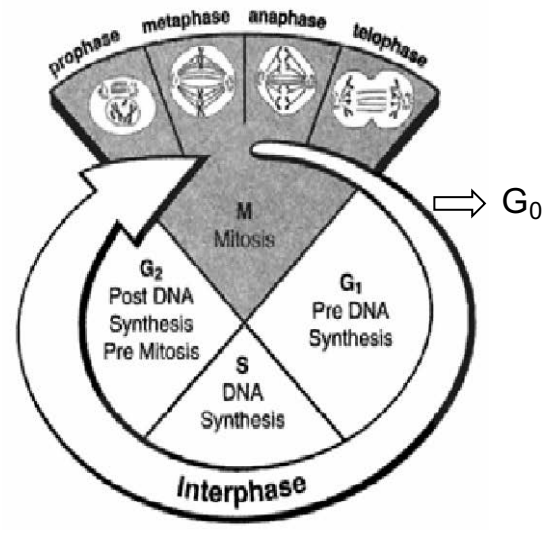
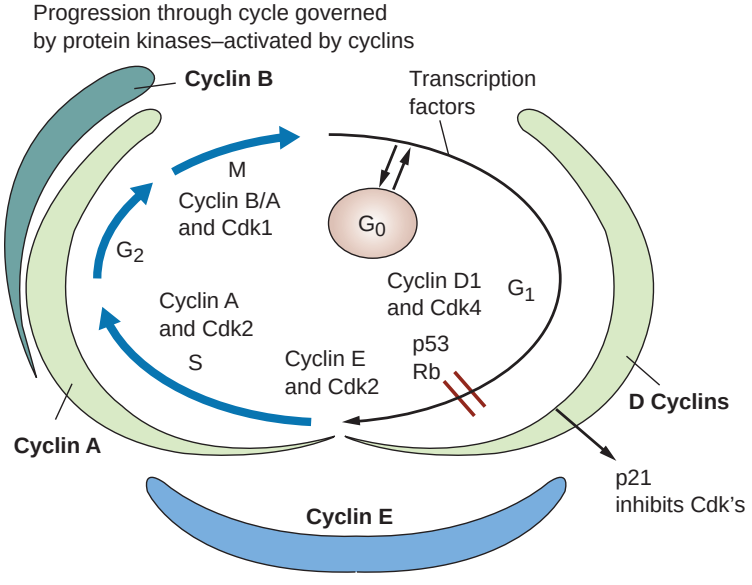
# Phases of the Cell Cycle



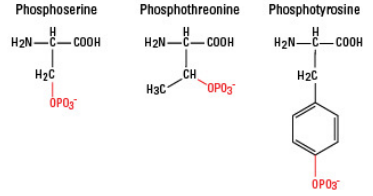
# Growth Factors

- $G_0$ through $G_1$ to S requires **growth factors** (GF)
  - To activate resting cells to enter G1
  - To allow cells to pass through G1 phase
  - To gain competence to progress into S phase
- The GF that are required vary with the cell type; e.g., for fibroblasts
  - **PDGF** (platelet-derived GF) activates cells
  - **EGF** (epidermal GF) and **insulin** act as competence factors
  - **IGF** (insulin-like GF) promotes progression into S
- Through S, G2, and M, cycling is GF-independent

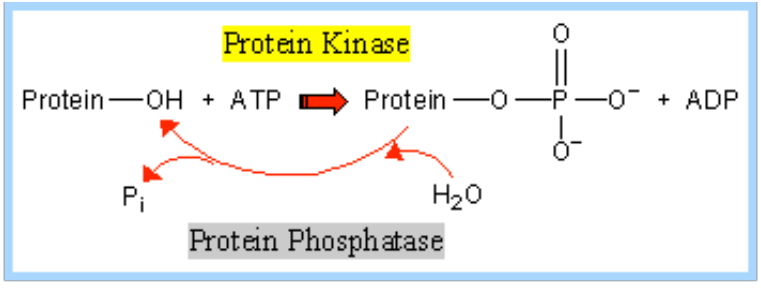
# Control of the Cell Cycle – 3 Components

- **Cyclins**
  - Synthesized at the appropriate time for each phase and then degraded to coordinate cell cycle progression
  - Cyclin expression in $G_1$ is induced by **growth factors**
- **Cyclin-Dependent Kinases (Cdk)**
  - Activated by cyclins to **phosphorylate** targets required for the next cell cycle phase
- **Regulators of Cdk/cyclin**
  - Activating phosphatases
  - Inhibitory kinases
  - Non-kinase inhibitors

# Kinase & Phosphatase




Many enzymes are regulated by covalent attachment of **phosphate**, in ester linkage, to the side-chain hydroxyl group of a particular amino acid residue (**serine, threonine or tyrosine**).

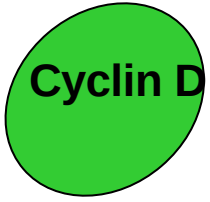


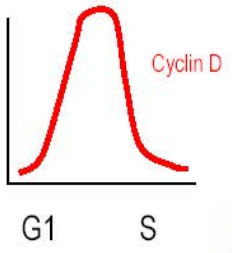

A **protein kinase** transfers the terminal phosphate of ATP to a hydroxyl group on a protein

A **protein phosphatase** catalyzes removal of the phosphate by hydrolysis

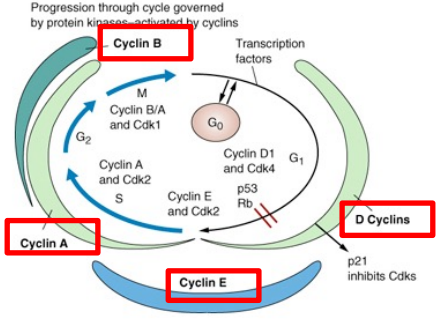
- Phosphorylation may directly alter activity of an enzyme, e.g., by promoting a **conformational change**
- Alternatively, altered activity may result from **binding another protein** that specifically recognizes a phosphorylated domain

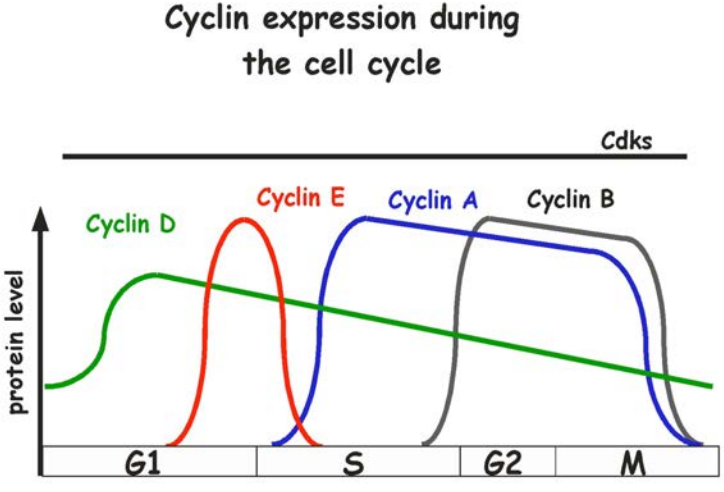
# Cyclins



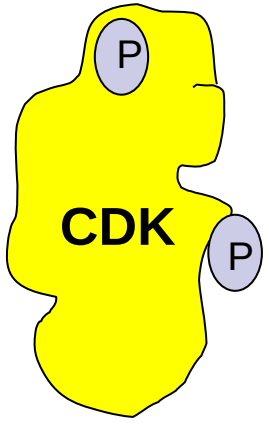




- Have no intrinsic enzymatic activity
- Cyclins A to J have been identified (no I)
- Synthesized and degraded during each cell cycle phase
  - Cyclin D ($G_1$ phase)
  - Cyclin E (S Phase)
  - Cyclins A (S & $G_2$ phase)
  - Cyclins B ($G_2$ & M-phase)
- Bind and activate Cdks

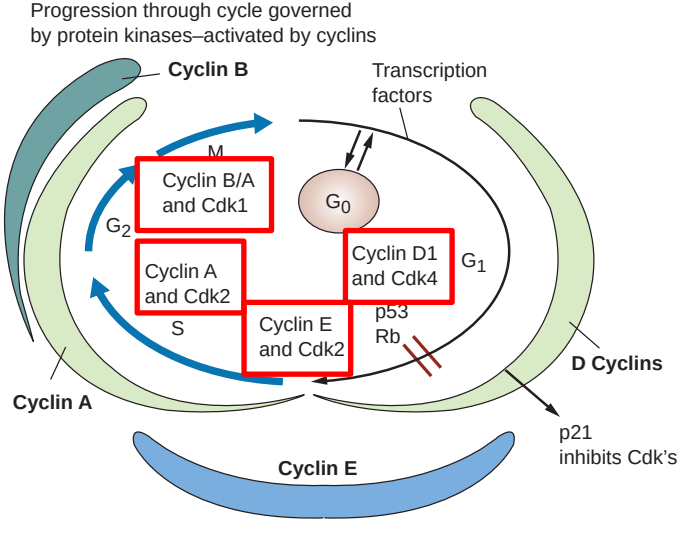


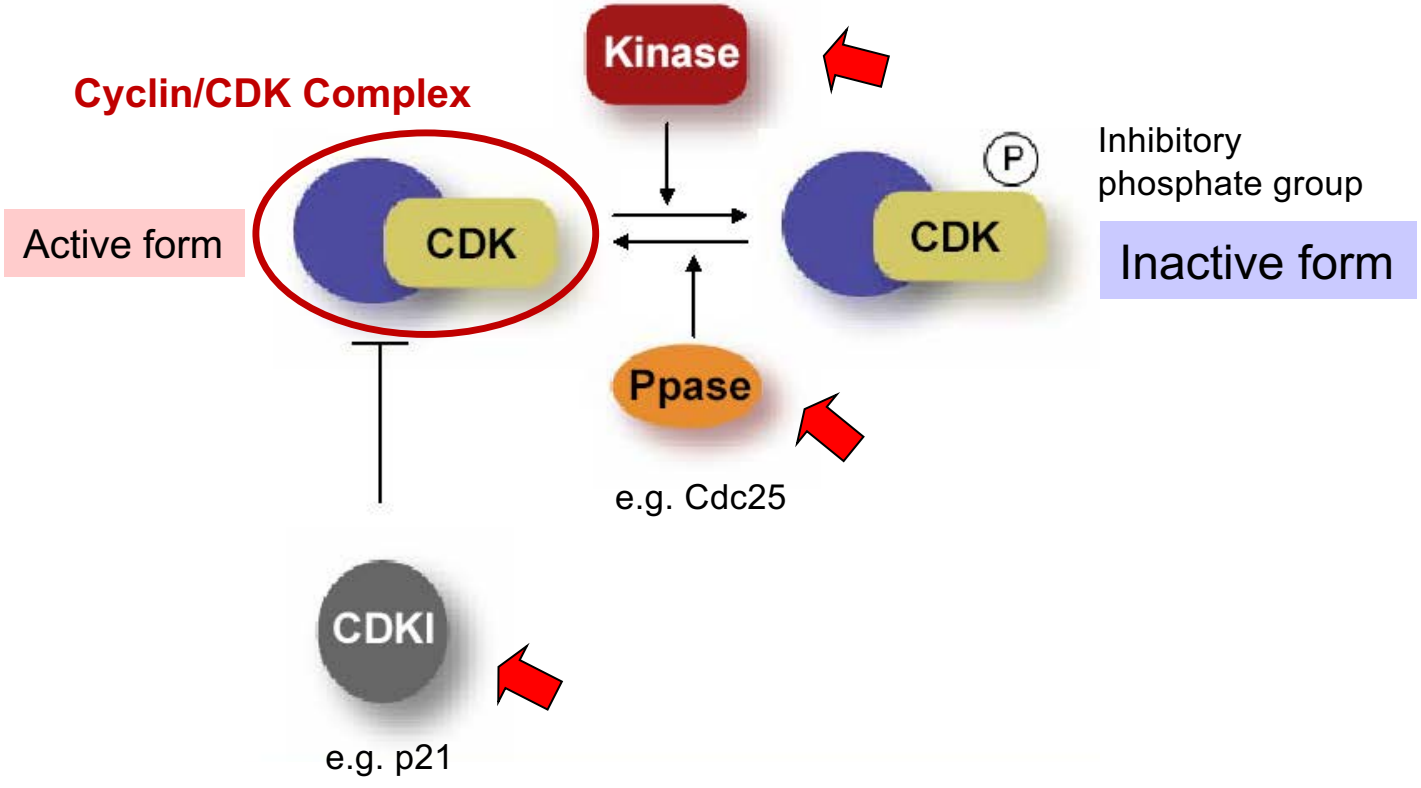
# Cyclin-Dependent Kinases



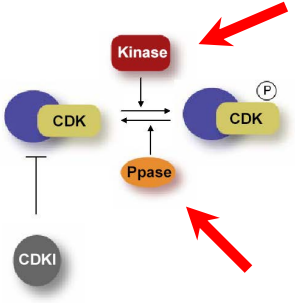

- Present throughout cell cycle
- Serine/threonine kinases with multiple substrates
  - e.g. pRb, p53, E2F, etc. that they activate/inactivate
- Activated by binding to cyclins
  - **Cdk4/6-cyclin D**
  - **Cdk2-cyclins E, A**
  - **Cdk1-cyclins A, B**
- Regulated by
  - Cyclin levels
  - Cdk inhibitors
  - Cdk phosphorylation/dephosphorylation



# Regulation of Cdk/Cyclin



# Activating Phosphatases/Inhibiting Kinases

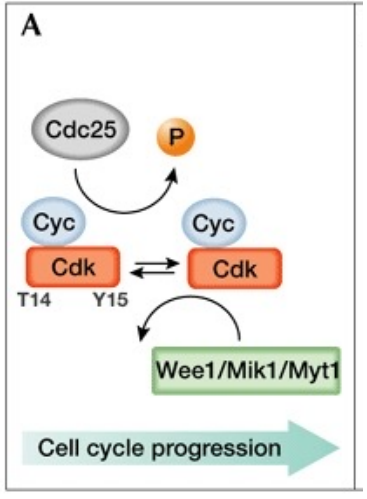



- There are kinases which adds a phosphate group to cdk, thereby inactivating it (e.g., **Wee1**)
- **Cdc25** removes phosphate from Tyr-15 on cdk, thereby activating the cyclin/cdk complex

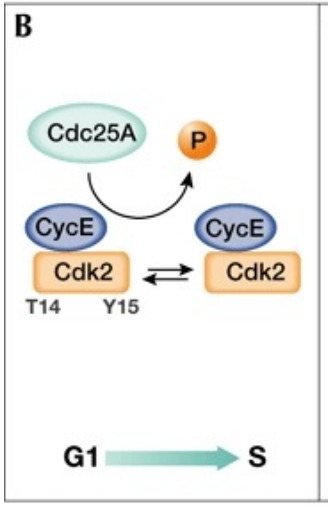
An example of activating phosphatase

| Phase | Complex | Activators |
|---|---|---|
| $G_1$/S specific? | Cyclin E – Cdk2 | Cdc25A |
| S-phase exit | Cyclin A – Cdk2 | Cdc25B |
| $G_2$/M | Cyclin B – Cdk1 | Cdc25A/B/C |

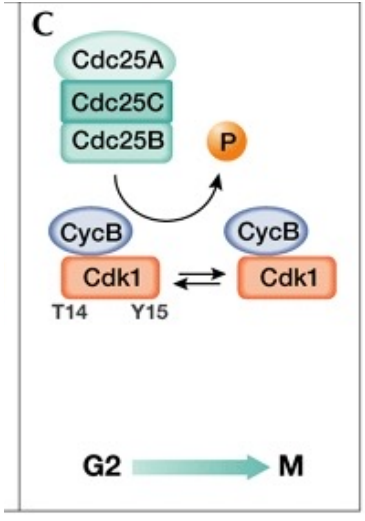
# Cdk Regulation by Cdc25 Phosphatases




Cdks are maintained in an inactive state through the phosphorylation of T14 and Y15
The rate-limiting step in the activation of Cdks is dephosphorylation of these residues by Cdc25 dual-specificity phosphatases

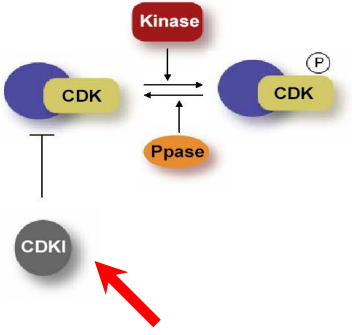



Cdc25A regulation of Cdk2/cyclin E during G1 and S phase




Cdc25A, Cdc25B and Cdc25C regulation of Cdk1/cyclin B during G2 and M phase

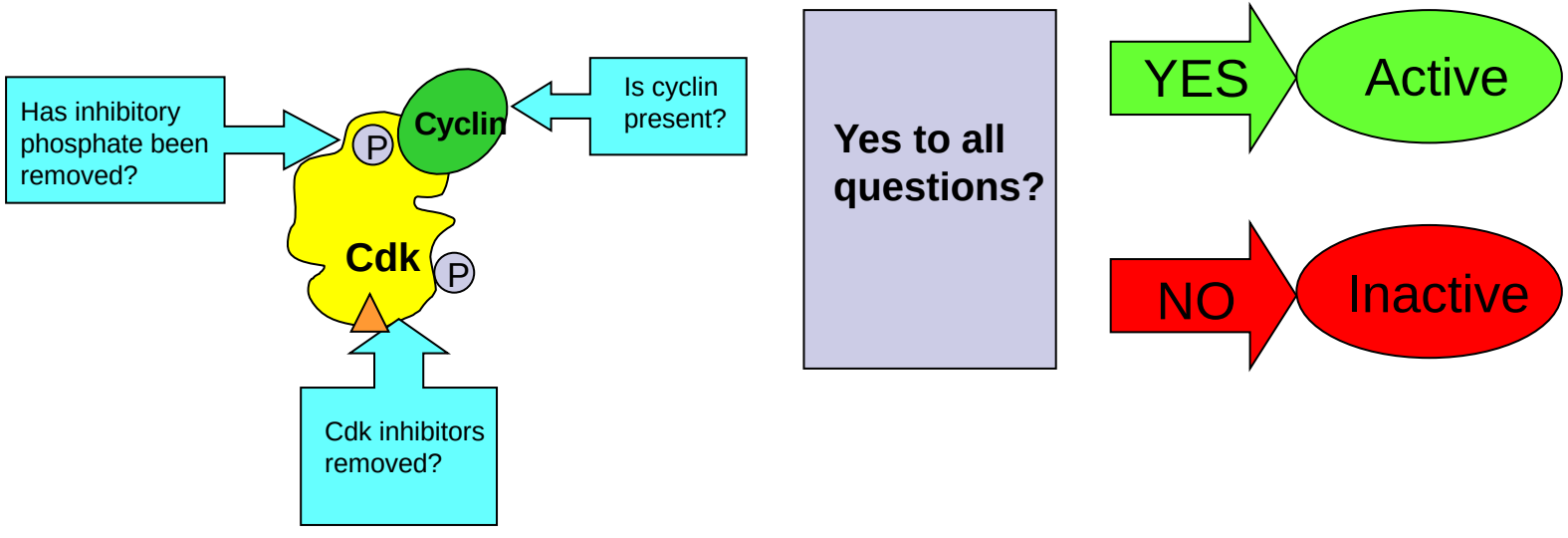
# Cdk Inhibitors




- Cdk Inhibitors (**CKIs**) belong to 2 families
  - **INK4** and **KIP/CIP**
- Generally compete with cyclins for Cdks

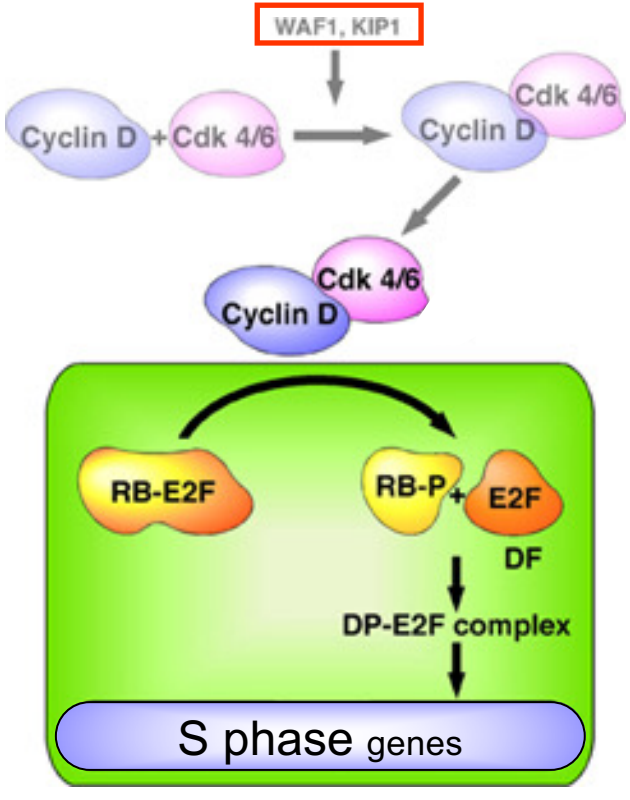
| **Phase** | **Complex** | **Inhibitors** |
|---|---|---|
| $G_1$ | Cyclin D – Cdk4,6 | p16 (INK4a), $p19^{ARF}$ (INK4a), p15 (INK4b) |
| $G_1$/S | Cyclin E – Cdk2, 3 | $p21^{CIP1}$, $p27^{KIP1}$ |
| S | Cyclin A – Cdk2 | p21, p57 |
| $G_2$/M | Cyclin B – Cdk1 | p21 |

# Regulation of Cdk/Cyclin

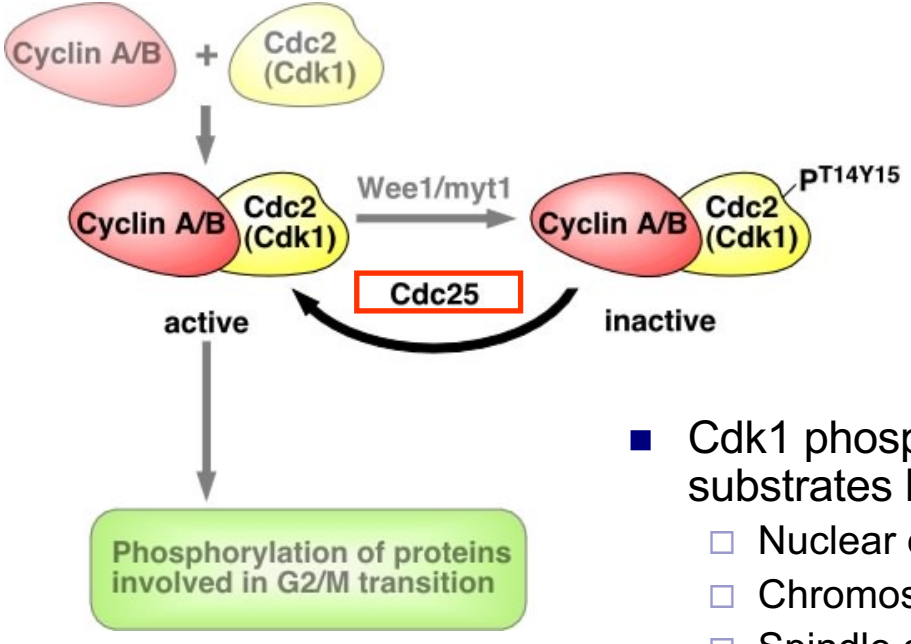


# G1/S Transition

- Cyclin D and E are needed to phosphorylate **Rb** which is essential for cell cycle progression into S
- This releases **E2F**, which is normally bound by Rb and inactive
- E2F is a transcription factor for 20-30 genes that are necessary for S phase gene expression



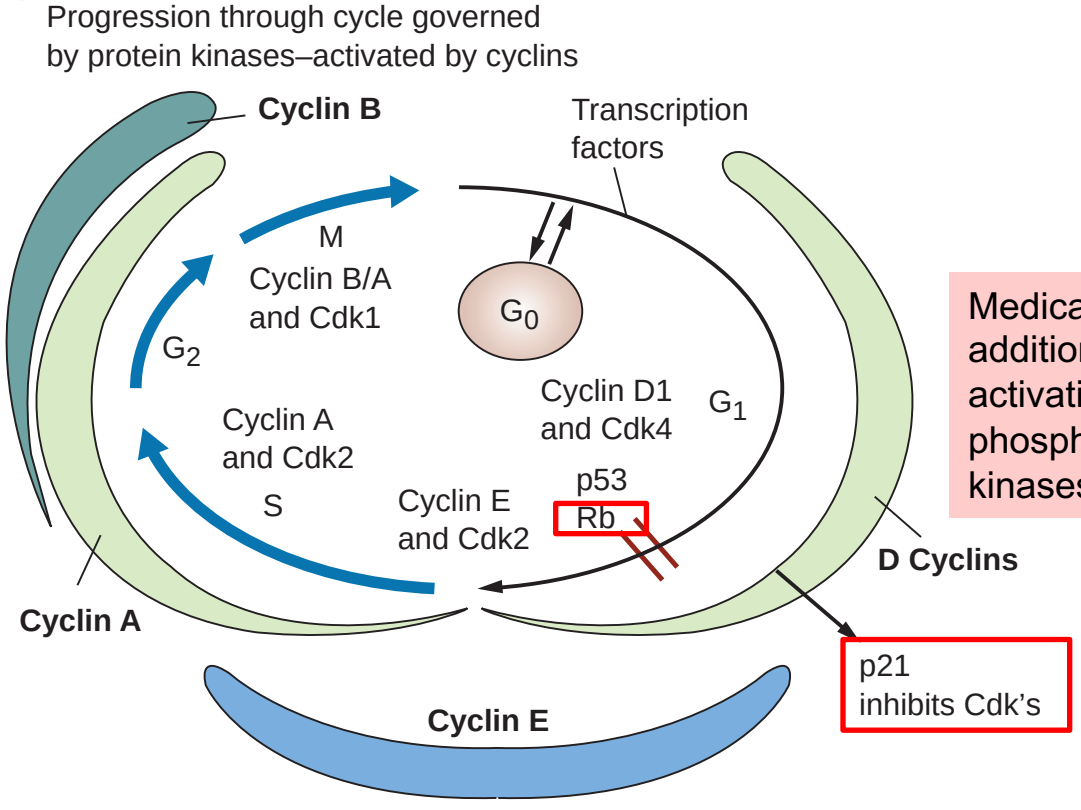
# G2/M Transition




- Cdk1 phosphorylates substrates leading to
  - Nuclear envelope breakdown
  - Chromosome separation
  - Spindle assembly
  - Chromosome condensation

# The Current Concept of Cell Cycle and Its Regulations

Physics students need to know components as illustrated on this diagram




Medical students need to additionally know activating phosphatases/inhibiting kinases, ckd inhibitors

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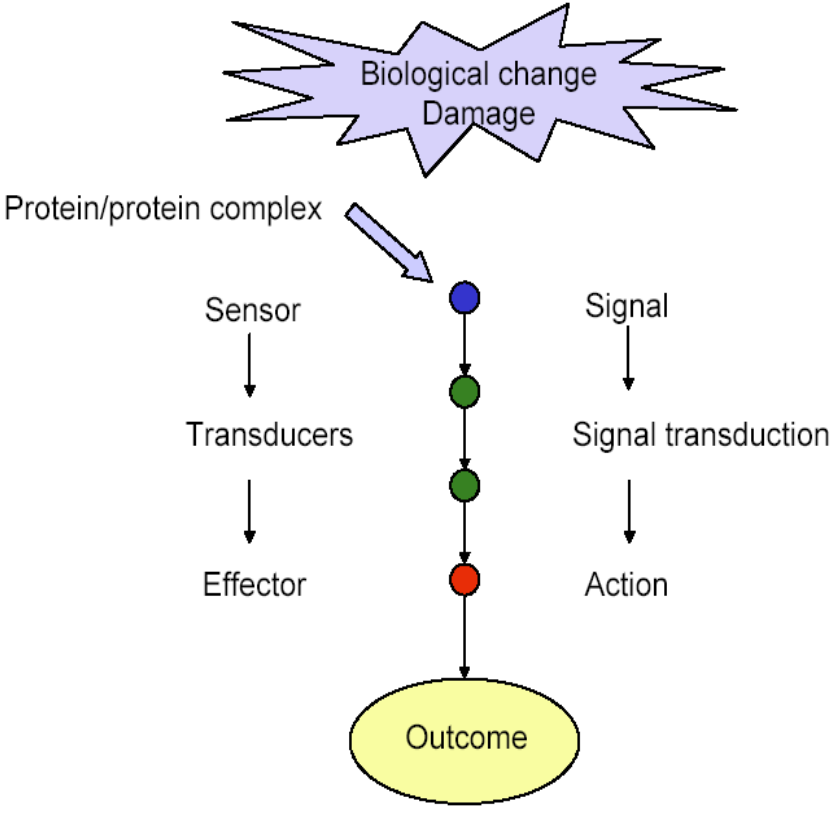
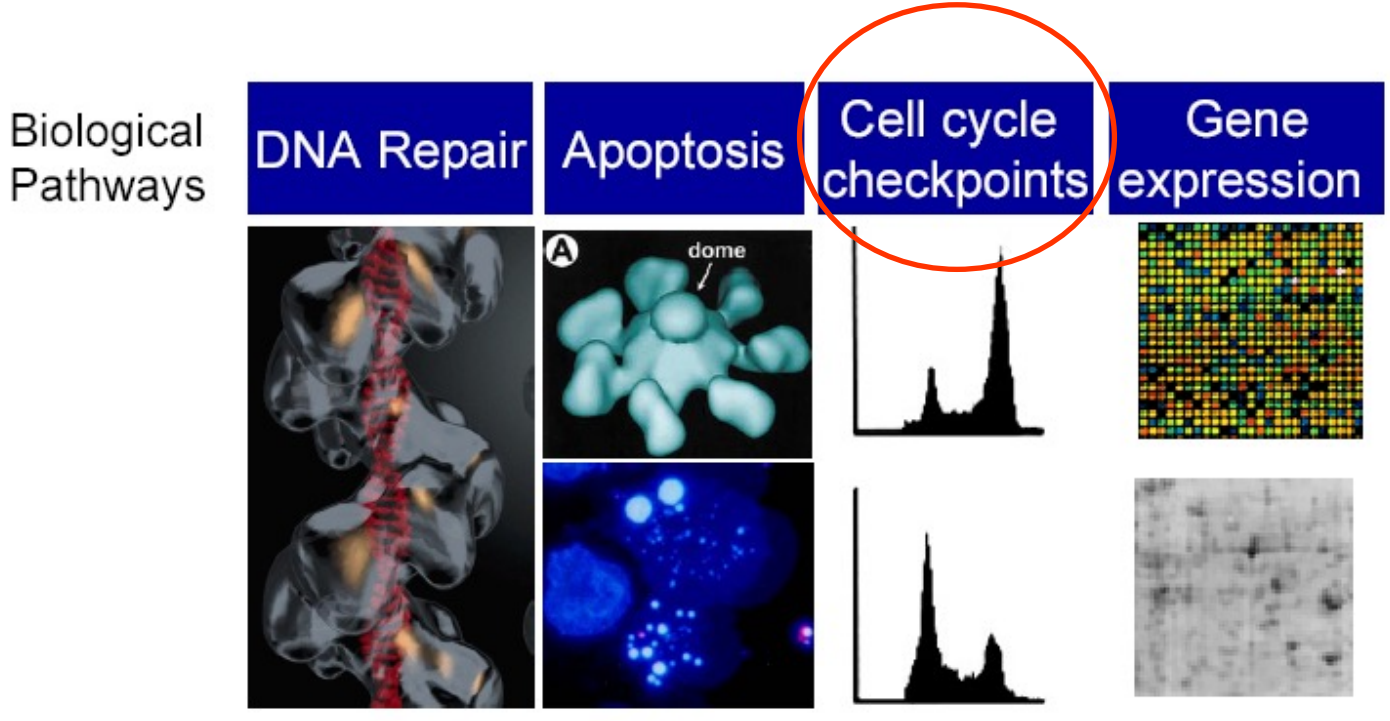
# Biological Response Pathways

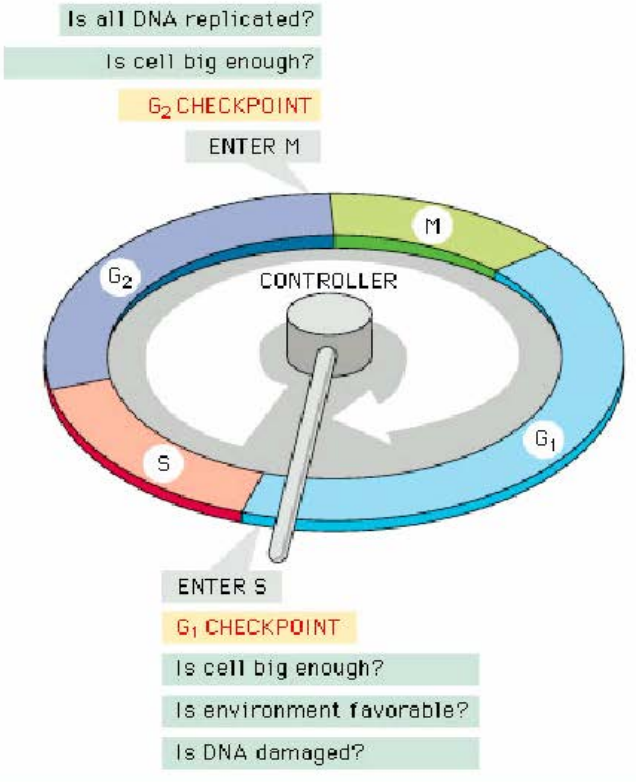


# Key Radiation Induced Pathways



# Cell Cycle Checkpoints

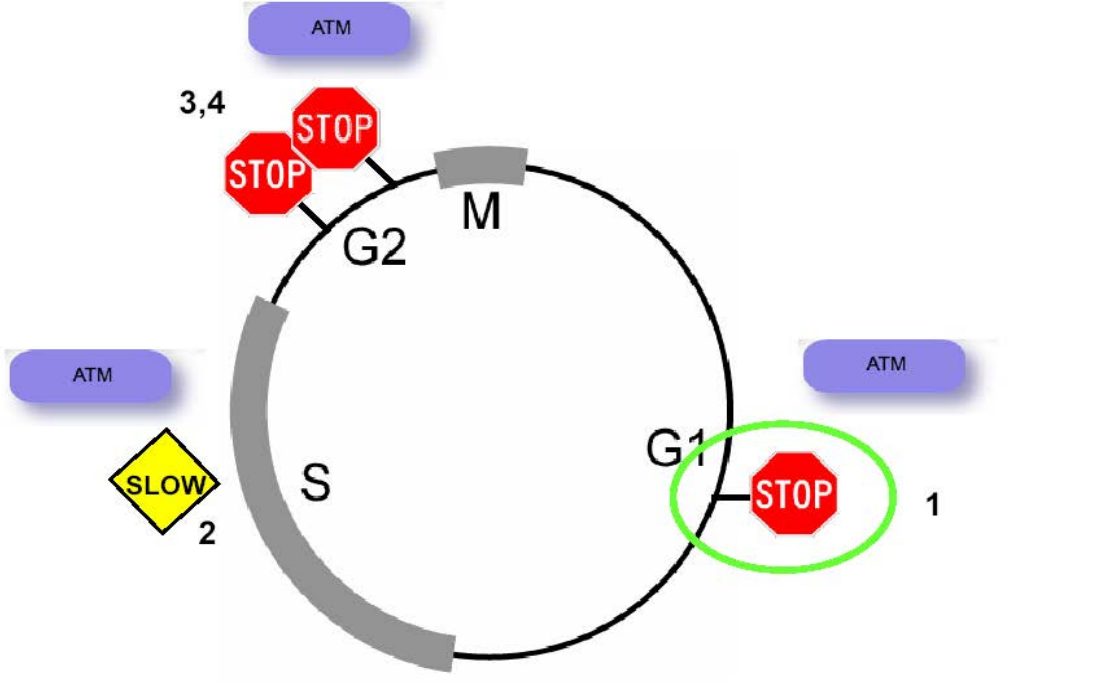
There are checkpoints during which the cell "checks" whether to continue progressing through the cell cycle




**Purpose**

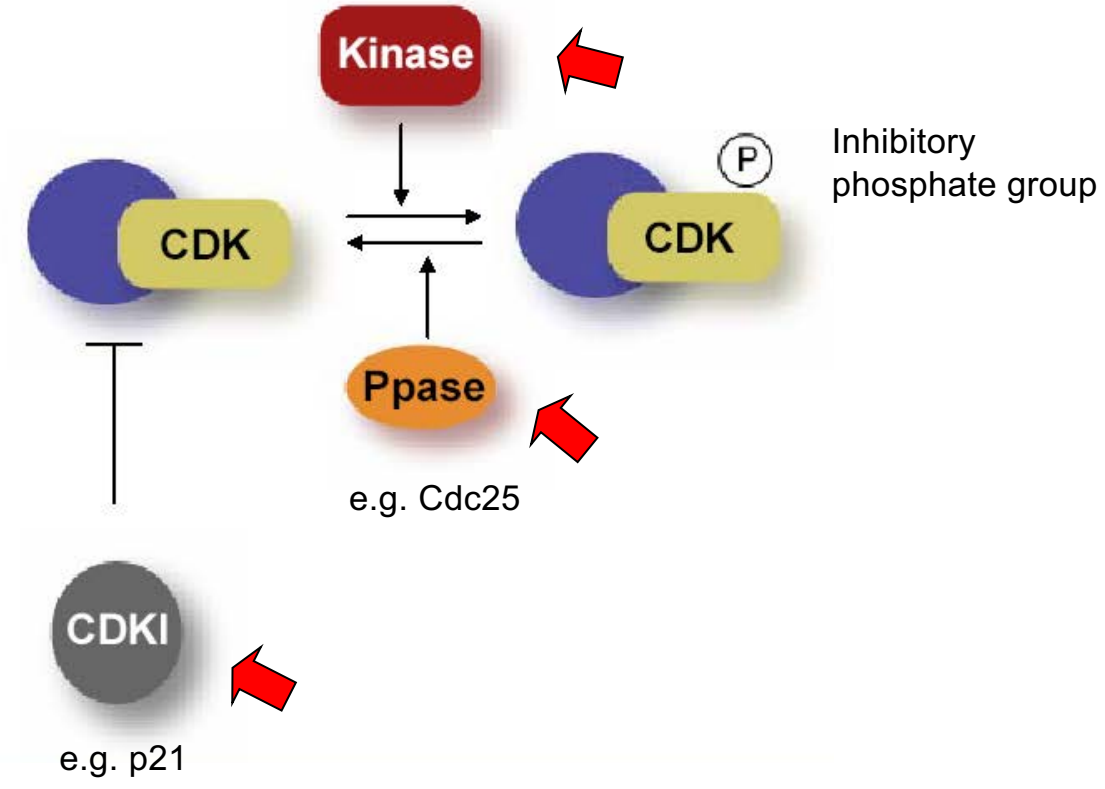
- Prevent or delay progression through the cell cycle when
  - Crucial events have not been completed
  - DNA is damaged
- Enforce dependency in the cell cycle
- Provide time for DNA repair

# Ionizing Radiation Induces 4 Distinct Checkpoints



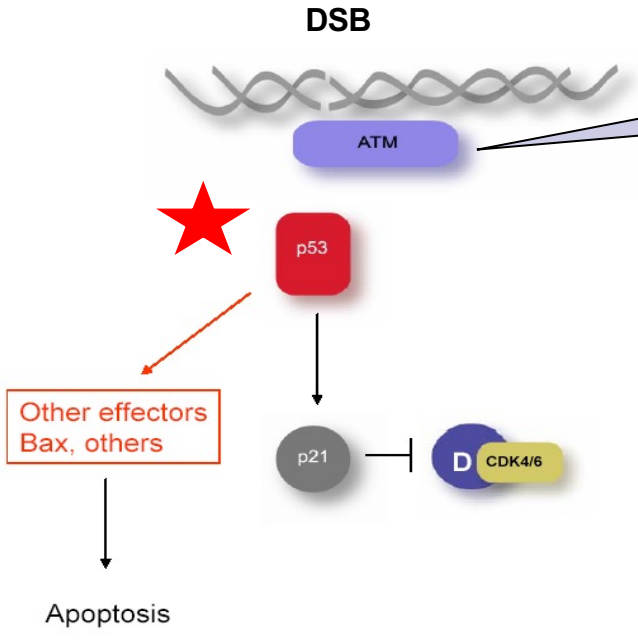

Rest of the slides are for medical residents only

# Regulation of Cdk/Cyclin



# Radiation Induced **G1/S** Checkpoint

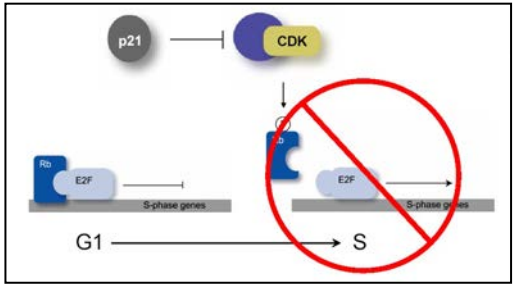
- **ATM** = Ataxia Telangiectasia Mutated
- It is an important cell checkpoint kinase and functions as a regulator of a wide variety of downstream proteins including tumor suppressor proteins p53, BRCA1, checkpoint kinase



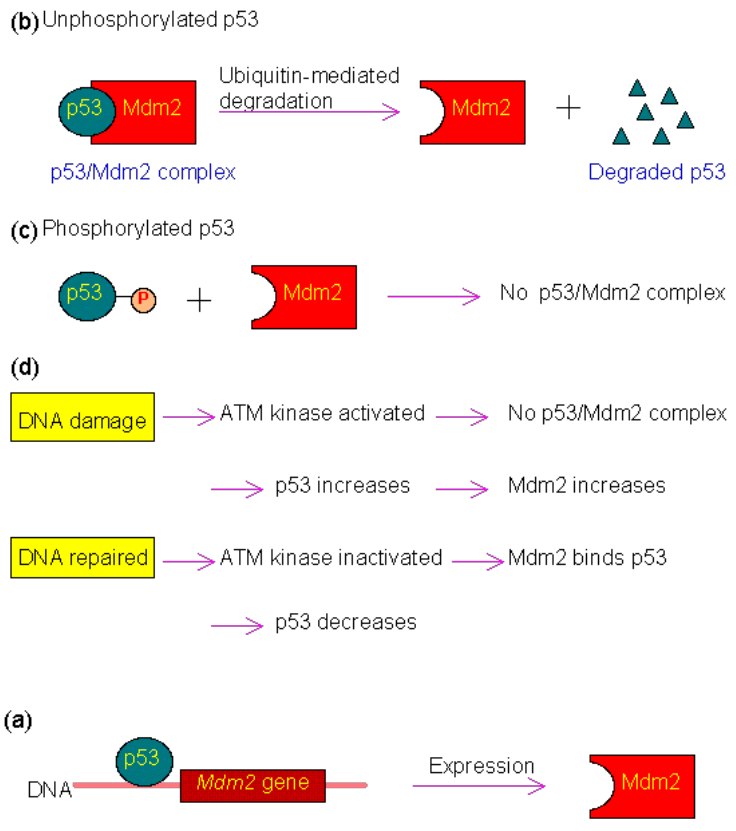

Sensors:
ATM
Other

Transducers:
P53/MDM2
Chk2

Effector:
p21



# Regulation of p53




The cellular concentration of *p53* must be tightly regulated

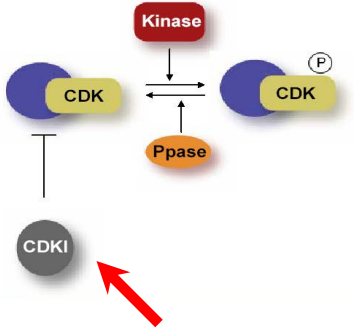
The major regulator of *p53* is Mdm2, which can trigger the degradation of *p53* by the ubiquitin system

In normal cells, *p53* is maintained at low level by Mdm2

DNA damage may activate protein kinases to phosphorylate p53, thereby disrupting its binding with Mdm2, leading to an increase of *p53*

Expression of Mdm2 is activated by p53, forming an autoregulatory loop

# Cdk Inhibitors

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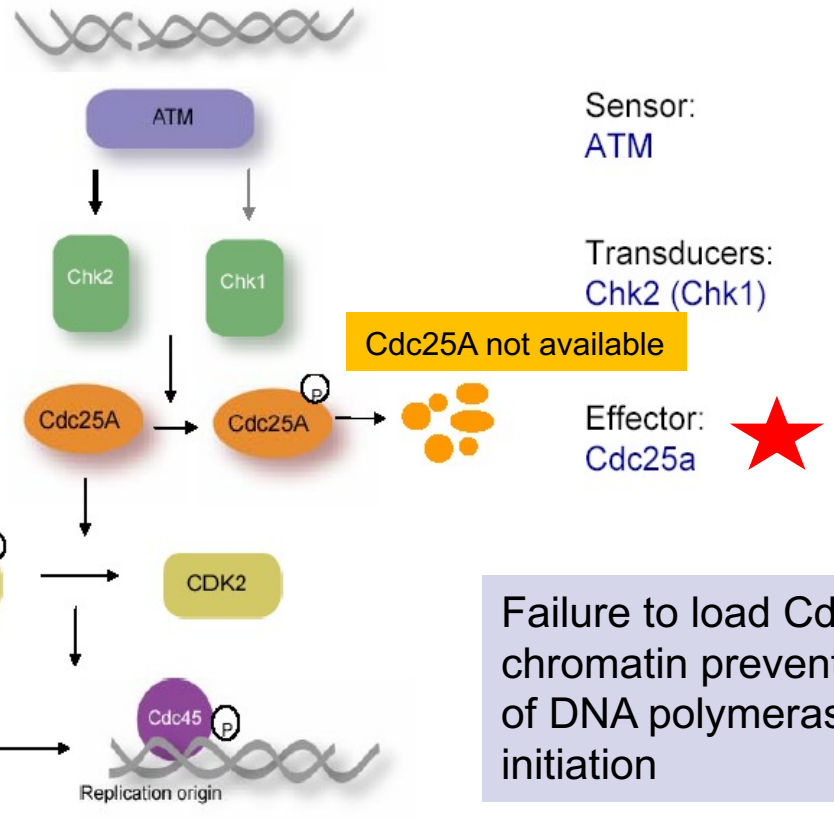
  - **INK4** and **KIP/CIP**
- Generally, compete with cyclins for Cdks

| Phase | Complex | Inhibitors |
|---|---|---|
| $G_1$ | Cyclin D – Cdk4,6 | p16 (INK4a), $p19^{ARF}$ (INK4a), p15 (INK4b) |
| $G_1$/S | Cyclin E – Cdk2, 3 | $p21^{CIP1}$, $p27^{KIP1}$ |
| S | Cyclin A – Cdk2 | p21, p57 |
| $G_2$/M | Cyclin B – Cdk1 | p21 |

# S-Phase Checkpoint




ATM activates Chk2 and Chk1, which phosphorylate Cdc25A, targeting it for degradation

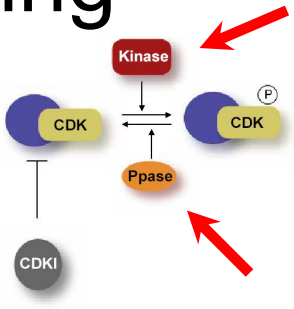
Cdc25A is required to remove the inhibiting phosphate group in the CDK2/Cyclin A complex

Cdc45 can't be phosphorylated without active CDK2/Cyclin A

Failure to load Cdc45 onto chromatin prevents the recruitment of DNA polymerase $\alpha$ and replicon initiation

# Activating Phosphatases/Inhibiting Kinases

An example of activating phosphatase




- **Cdc25** removes phosphate from Tyr-15 on cdk, thereby activating the cyclin/cdk complex

| Phase | Complex | Activators |
|---|---|---|
| $G_1$/S specific? | Cyclin E – Cdk2 | Cdc25A |
| S-phase exit | Cyclin A – Cdk2 | Cdc25B |
| $G_2$/M | Cyclin B – Cdk1 | Cdc25C |

- There are also kinases which adds a phosphate group to cdk, thereby inactivating it (e.g., **Wee1**)

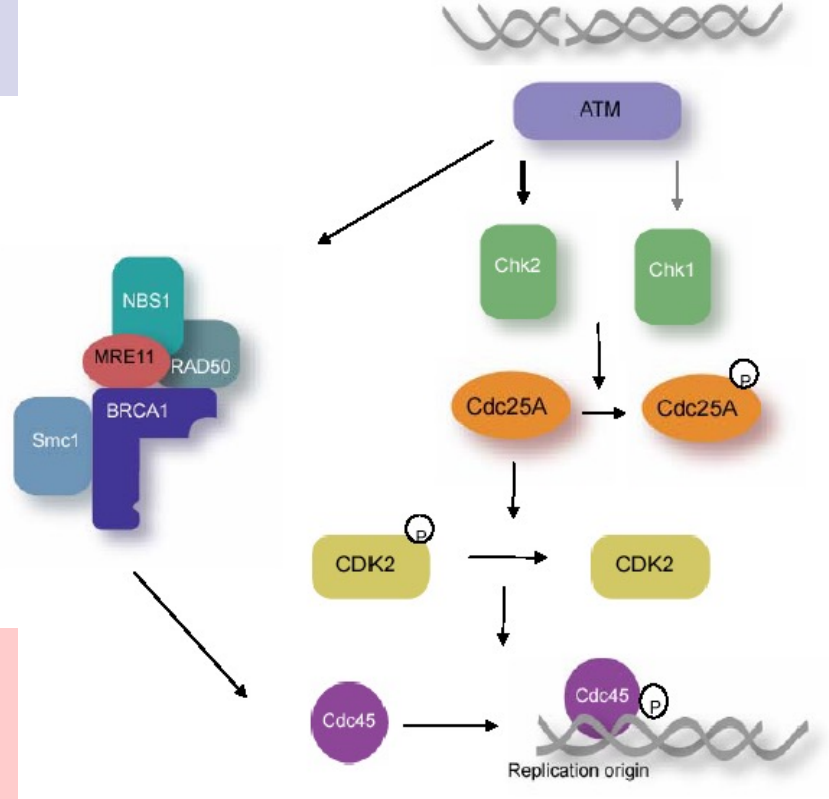
# S-Phase Checkpoint

A 2nd mechanism for S phase arrest is signaled by phosphorylation of NBS by ATM

Sensor:
ATM

Transducers:
BASC

Effector:
?




The importance of the S phase checkpoint is in protecting replication forks from trying to replicate through DNA strand breaks

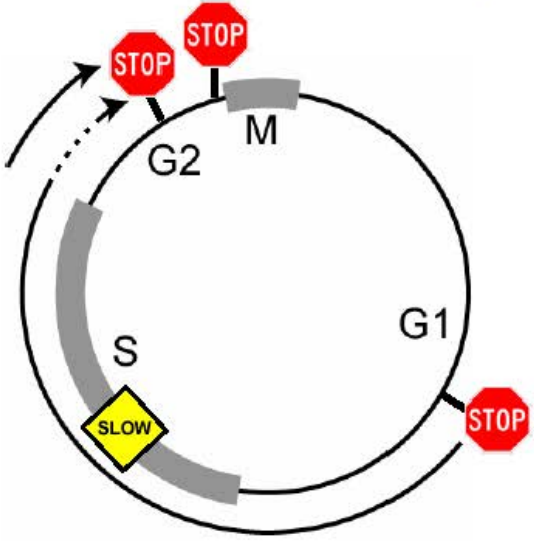
# **G2** Checkpoints Induced by IR

**Early**

- Dose independent (1-10Gy)
- Applies to cells irradiated in G2
- ATM dependent
- Does not affect radiosensitivty

**Late**

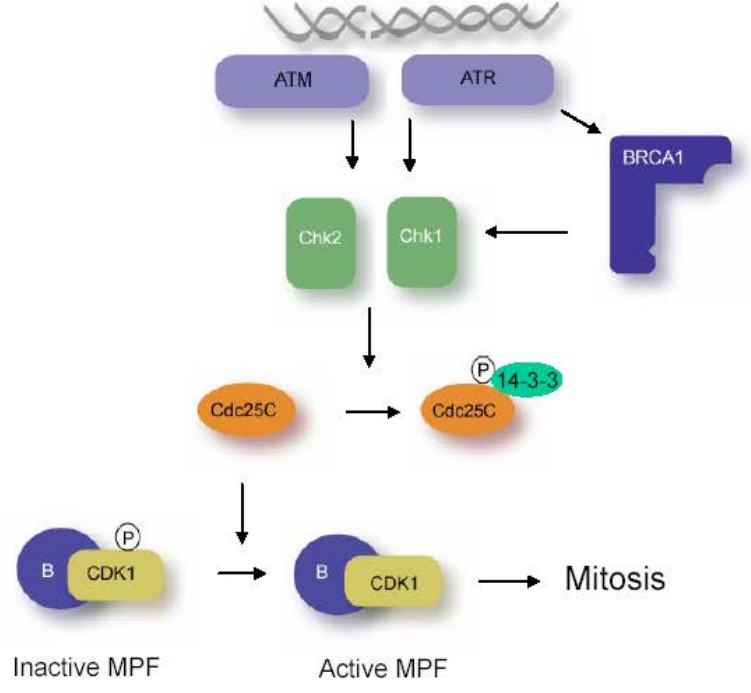
- Dose dependent
- Applies to cells irradiated in G1 or S-phase
- "classical" G2 delay
- ATR dependent
- May affect radiosensitivity




The arrest of cells in $G_2$ following DNA damage is observed readily in mammalian cells and was studied by radiation biologist for decades before checkpoints were understood at the molecular level

G2 checkpoint is the most regulated of all checkpoints

# Early G2 Checkpoint




Sensors:
ATM
ATR

Transducers:
Chk2
BRCA1
Chk1

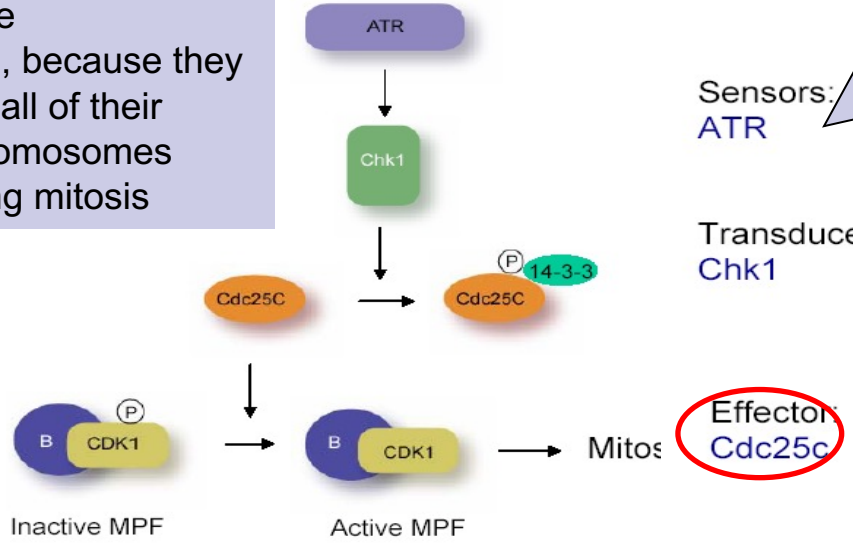
Effector:
Cdc25c

MPF = mitosis promoting factor

Applies to cells irradiated in G2 – blocks mitotic entry

# Late G2 Checkpoint

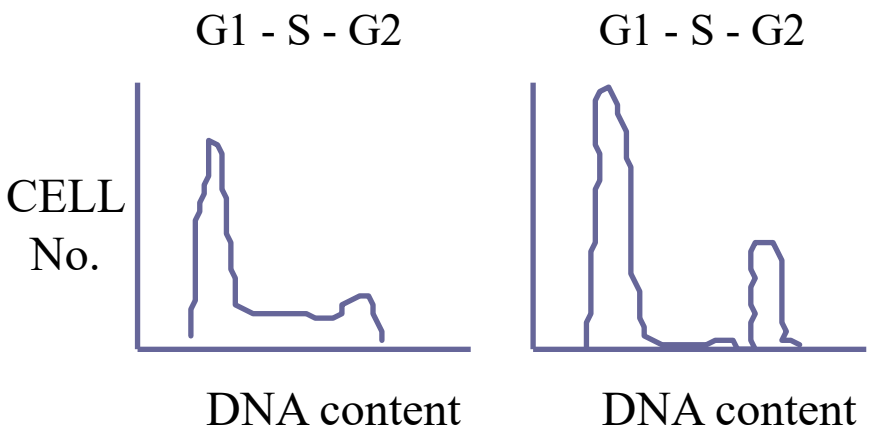
Cells lacking the late $G_2$ checkpoint are radiosensitive, because they cannot repair all of their damaged chromosomes before entering mitosis




- **ATR** = AT and Rad3-related
- Mutations are associated with Seckel Syndrome
- The protein belongs to the PI3-kinase family and is most closely related to ATM
- It functions both in parallel and cooperatively with ATM, but whereas ATM is primarily activated by DNA double-strand breaks induced by ionizing radiation, ATR has been shown to respond to a much broader range of DNA damage
- Upon activation, ATR phosphorylates several important tumor suppressors, including p53, BRCA1 and CHK1.

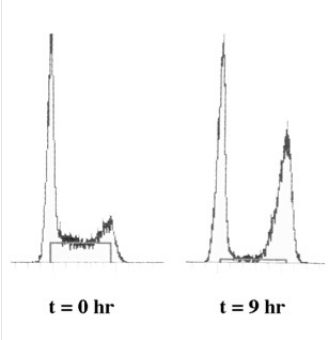
Applies to cells irradiated in G1/S – accumulation in G2

# Cell Cycle Arrest

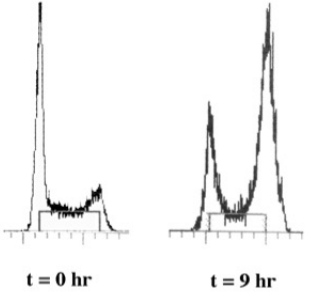



Asynchronous

X-ray treated
$G_1$/S block
$G_2$/M block
(6-9 hours)




wild-type




loss of G1/S in
p53 deficient
cells

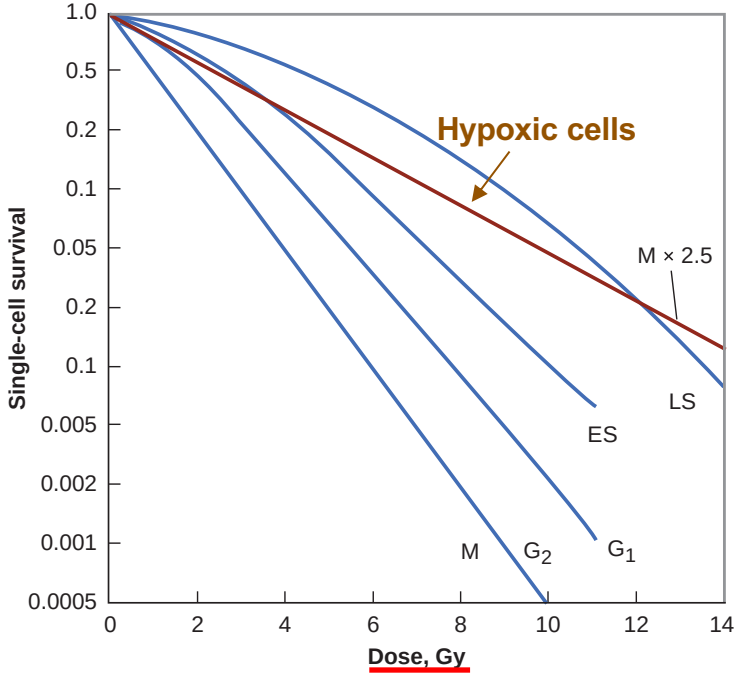
# Review Questions

# Question 1

The phase of the cell cycle that generally exhibits the greatest resistance to ionizing radiation is:

A. M
B. G1
C. Early S
D. Late S
E. G2

# Complete Cell Survival Curves at Various Cell-Cycle Phase




**CHO Cells**

Cells are most sensitive to radiation during M and $G_2$ phase – note that the curve is steep and has no shoulder

Cells are least sensitive in late S phase – note that the curve is less steep and has a very broad shoulder

Cells in $G_1$ and early S have intermediate radiosensitivity

Hypoxic cells are ~ 2.5 x more radioresistant compared to aerated cells (more in Chapter 6)

# Question 2

Which pair of cell cycle phase and active CDK or enhanced cyclin level is INCORRECT?

A. G1 - CDK1

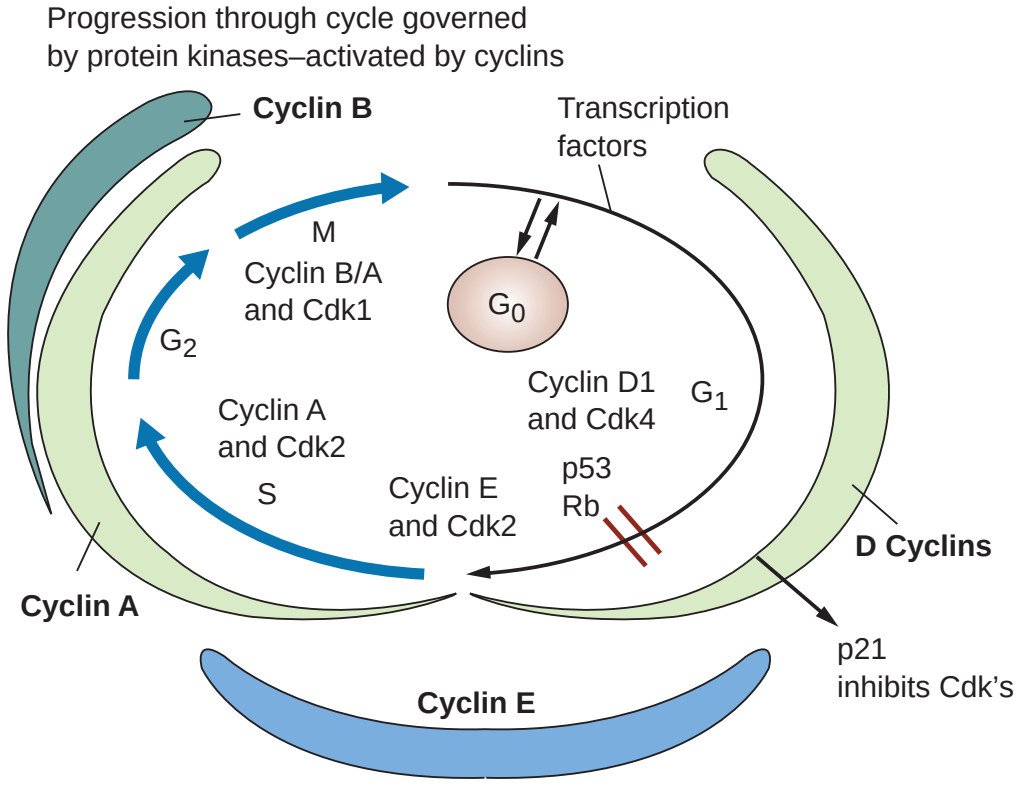
B. S - CDK2

C. G1 - CDK4

D. G2 - cyclin B

E. G1 - cyclin D

# Cell Cycle Control

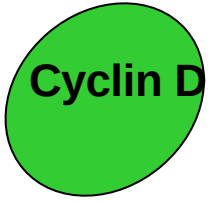


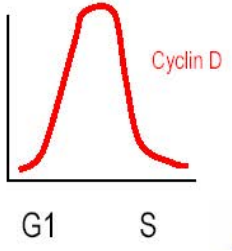
# Question 3

Cyclins are

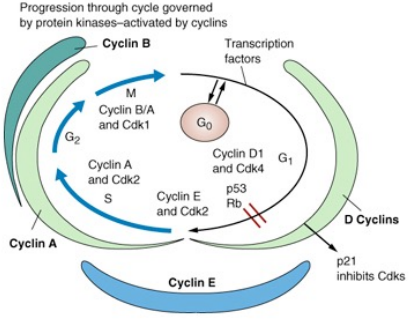
A. lipid kinases
B. protein phosphatases
C. regulatory proteins for CDKs
D. present only in the G0 phase of the cell cycle
E. regulators of DNA repair

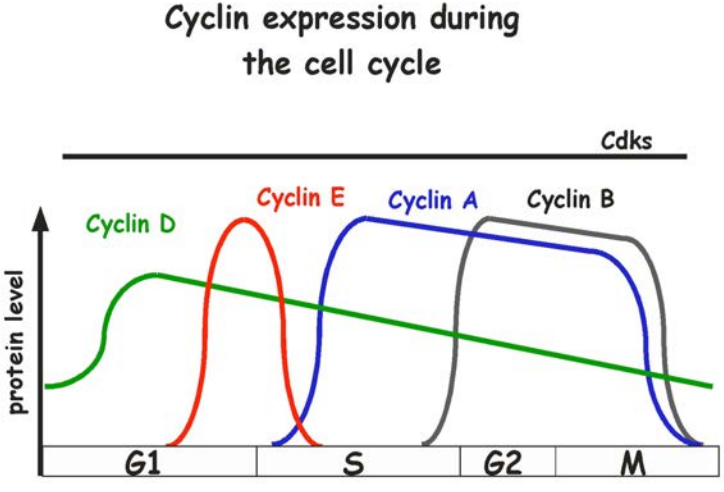
# Cyclins




- Have no intrinsic enzymatic activity
- Cyclins A to J have been identified (no I)
- Synthesized and degraded during each cell cycle phase
  - Cyclin D ($G_1$-phase)
  - Cyclins E & A (S-phase)
  - Cyclins B & A (M-phase)
- Bind and activate Cdks



# Question 4

Generally speaking, the most variable stage of the cell cycle is

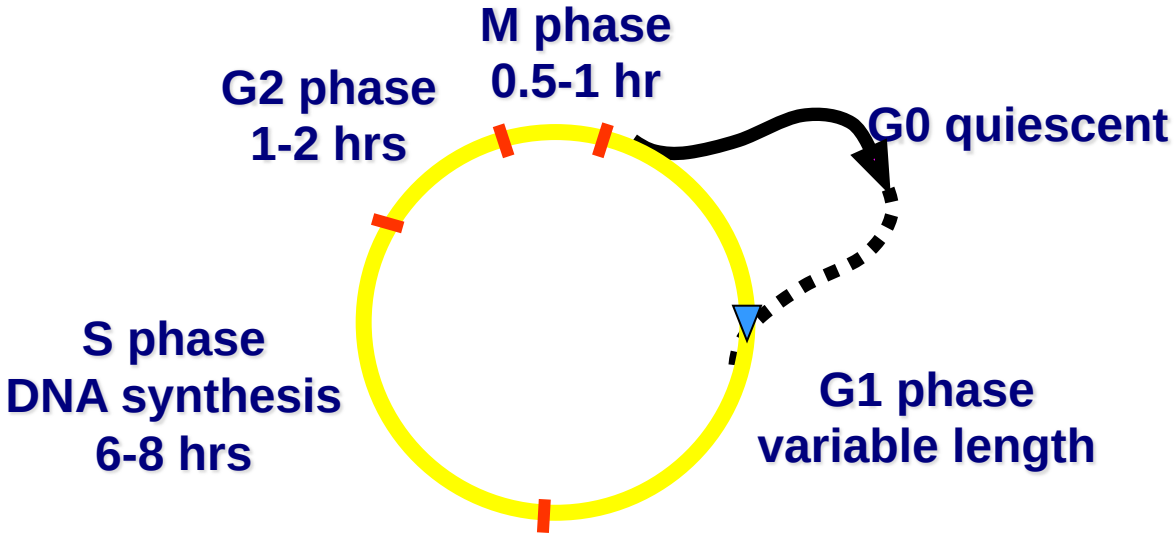
A. M phase

B. G1 phase

C. G2 phase

D. S phase

# Mammalian Cell-Cycle Times




**Tc** – mitotic-cycle time (aka cell-cycle time)

| | |
|---|---|
| Crypt cells in mouse | 9-10 hours |
| Stem cells in resting mouse skin | 200 hours |
| Most human cells actively dividing | 12-24 hours |
| Human tumors | 48 hours (15-125 hours) |

# Question 5

Medical Residents Only

Chk1/chk2 proteins are

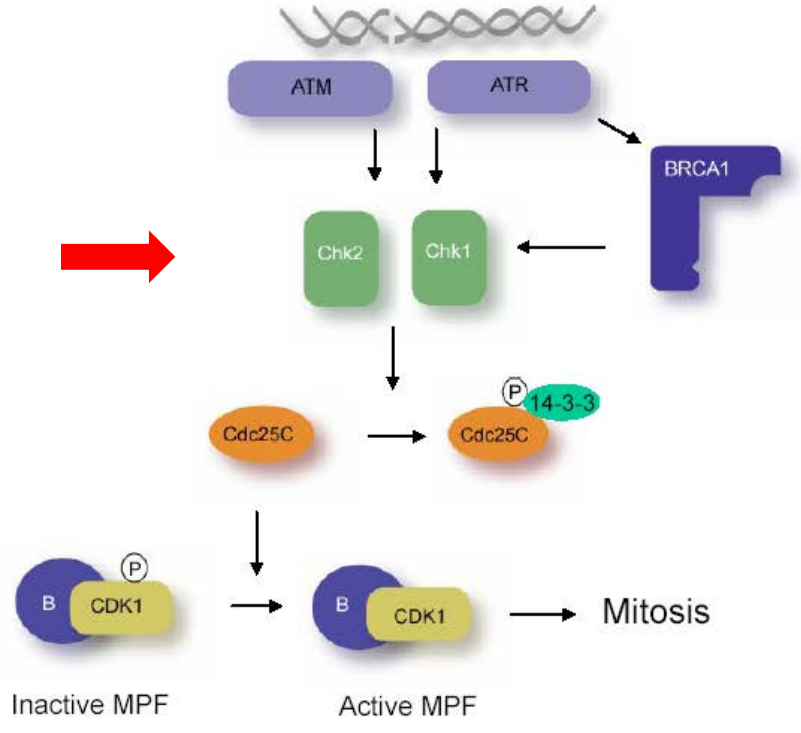
A. upstream of the ATM protein

B. are degraded by MDM2 protein

C. are kinases that phosphorylate the cdc25 protein

D. are ligases that degrade the cdc25 protein

# Chk1 & Chk2

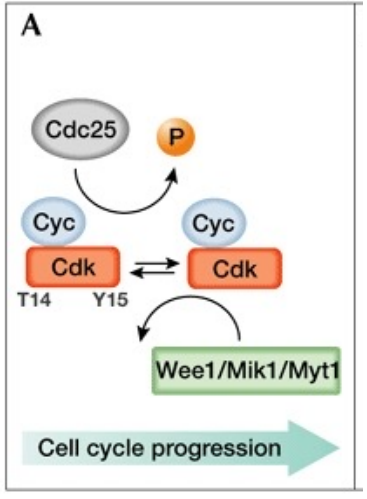



Sensors:
ATM
ATR

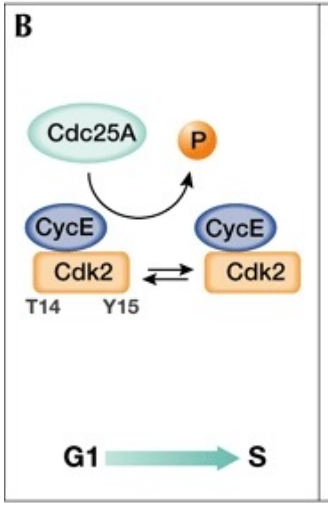
Transducers:
Chk2
BRCA1
Chk1

Effector:
Cdc25c

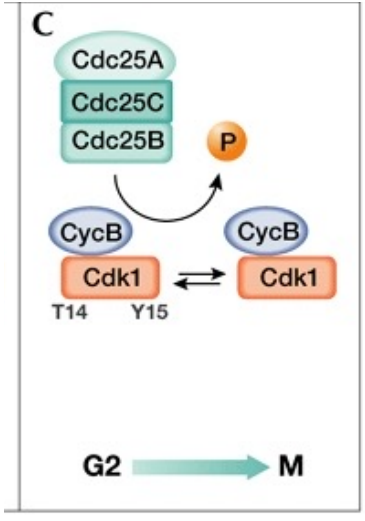
# Cdk Regulation by Cdc25 Phosphatases




Cdks are maintained in an inactive state through the phosphorylation of T14 and Y15
The rate-limiting step in the activation of Cdks is dephosphorylation of these residues by Cdc25 dual-specificity phosphatases




Cdc25A regulation of Cdk2/cyclin E during G1 and S phase




Cdc25A, Cdc25B and Cdc25C regulation of Cdk1/cyclin B during G2 and M phase

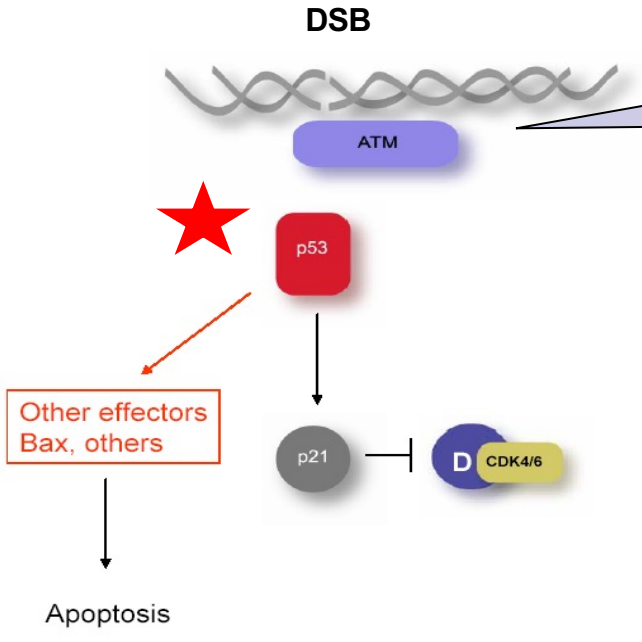
# Question 6

Medical Residents Only

Following exposure to ionizing radiation, cells that lack functional p53 are most likely fail to arrest in which phase of the cell cycle?

A. G1
B. S
C. G2
D. M
E. The cells will not arrest.

# Radiation Induced **G1/S** Checkpoint

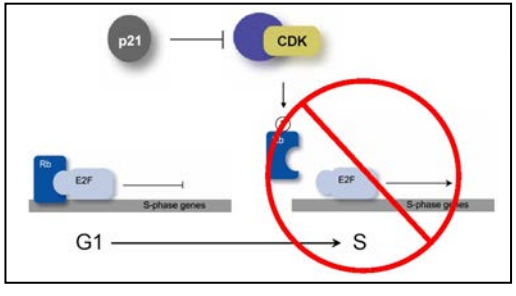



Sensors:
ATM
Other

Transducers:
P53/MDM2
Chk2

Effector:
p21

- **ATM** = Ataxia Telangiectasia Mutated
- Mutations are associated with AT, which is an autosomal recessive disorder
- The protein belongs to the PI3-kinase family
- It is an important cell checkpoint kinase and functions as a regulator of a wide variety of downstream proteins including tumor suppressor proteins p53, BRCA1, checkpoint kinase



# Question 7

Medical Residents Only

Which statement is TRUE concerning the role of p53 and p21 in the response of the cells to radiation?

A. p21 phosphorylates NBS1, thereby stimulating homologous recombination repair of DNA double-strand breaks

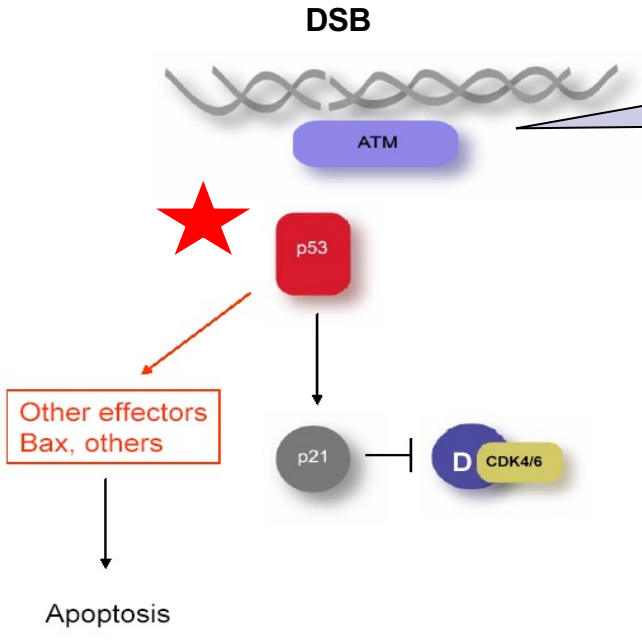
B. p53-mediated G1 phase arrest results form the inactivation of p21

C. A decrease in the amount of p53 can trigger apoptosis or G1 arrest

D. p21 inhibits CKD-cyclin activity thereby decreasing phosphorylation of RB1

E. DNA damage initiates a signal transduction pathway that results in a marked increase in transcription of *p53* gene

# Radiation Induced **G1/S** Checkpoint

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