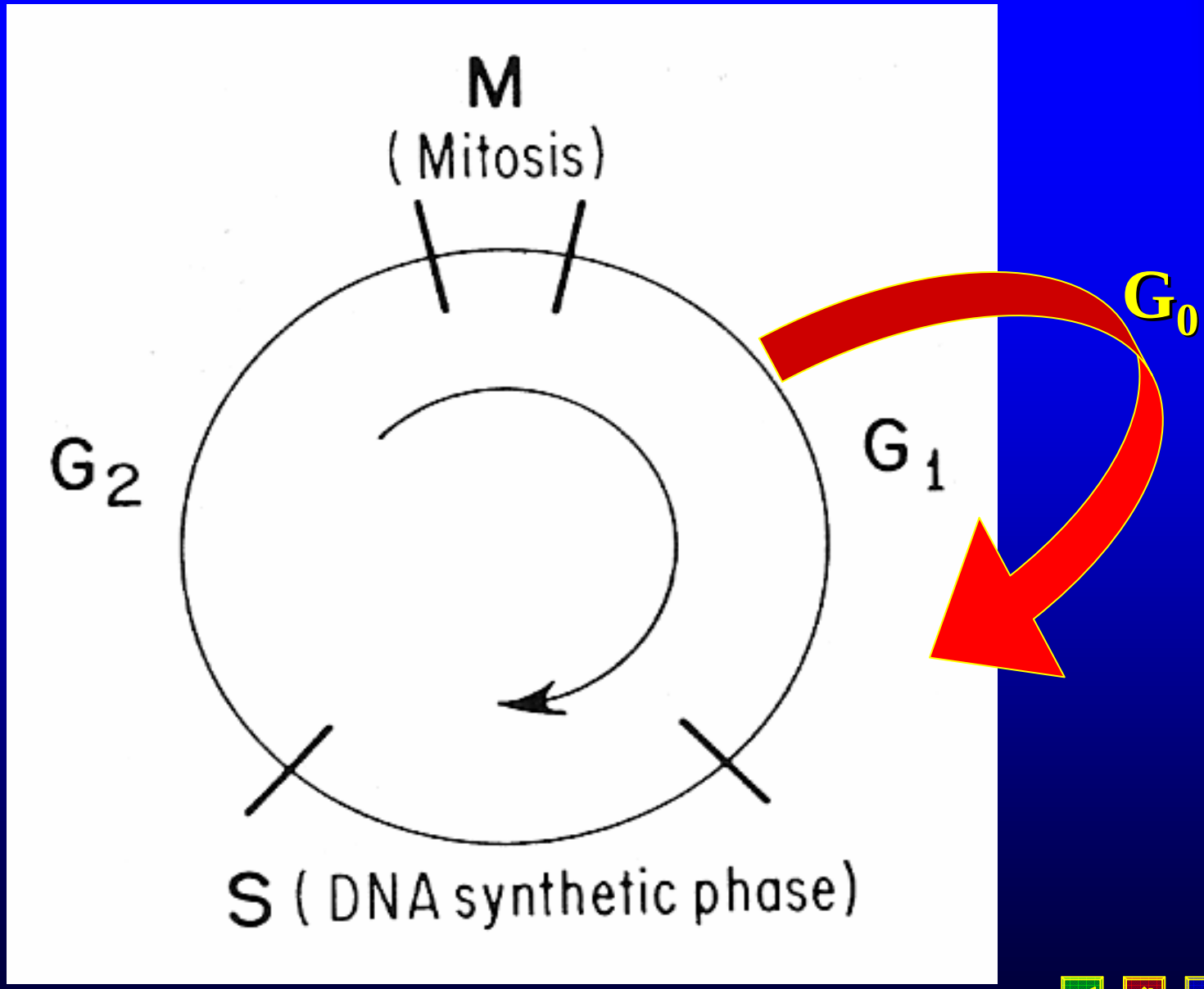
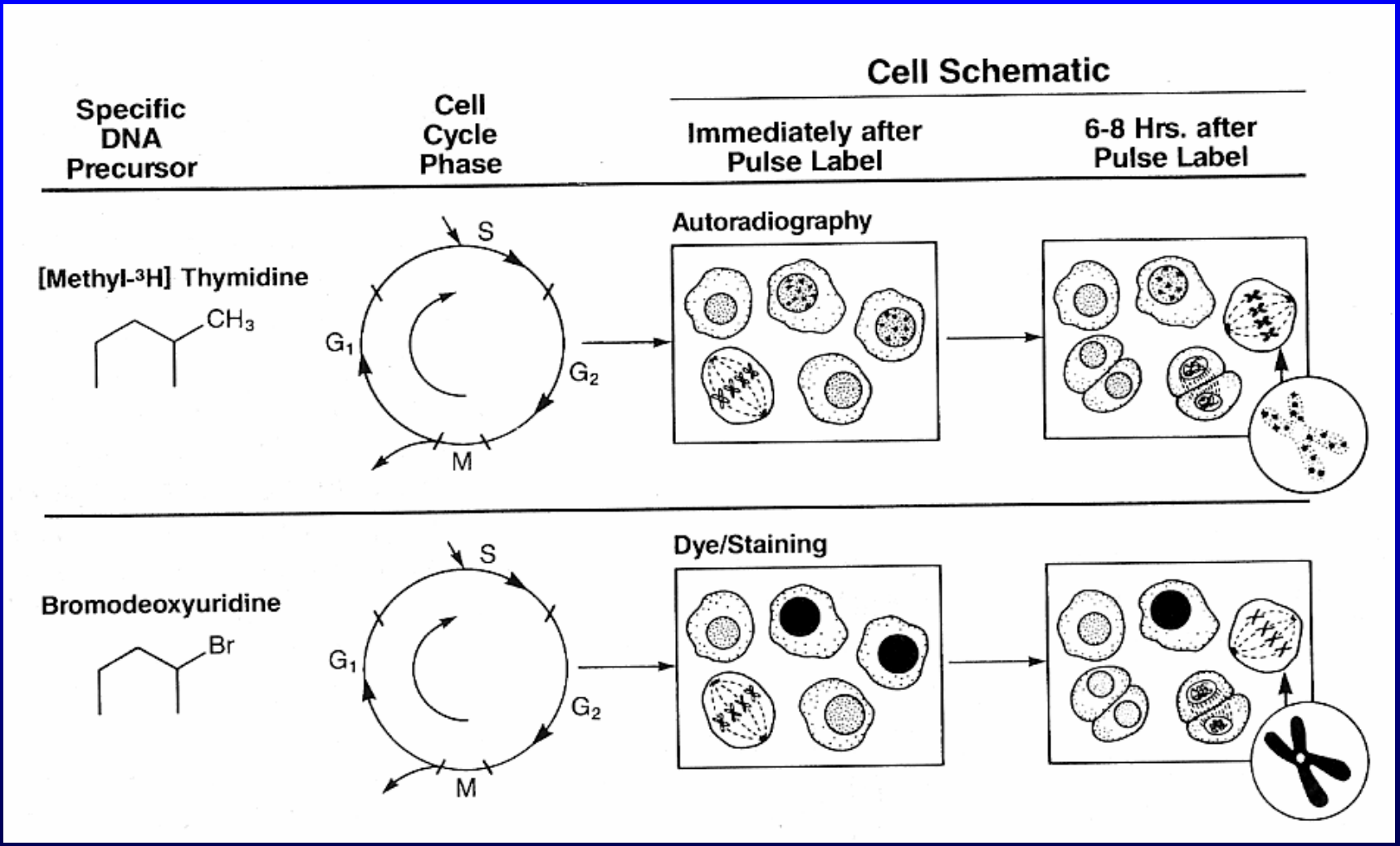
# Radiosensitivity and Cell Cycle (Chap. 4)

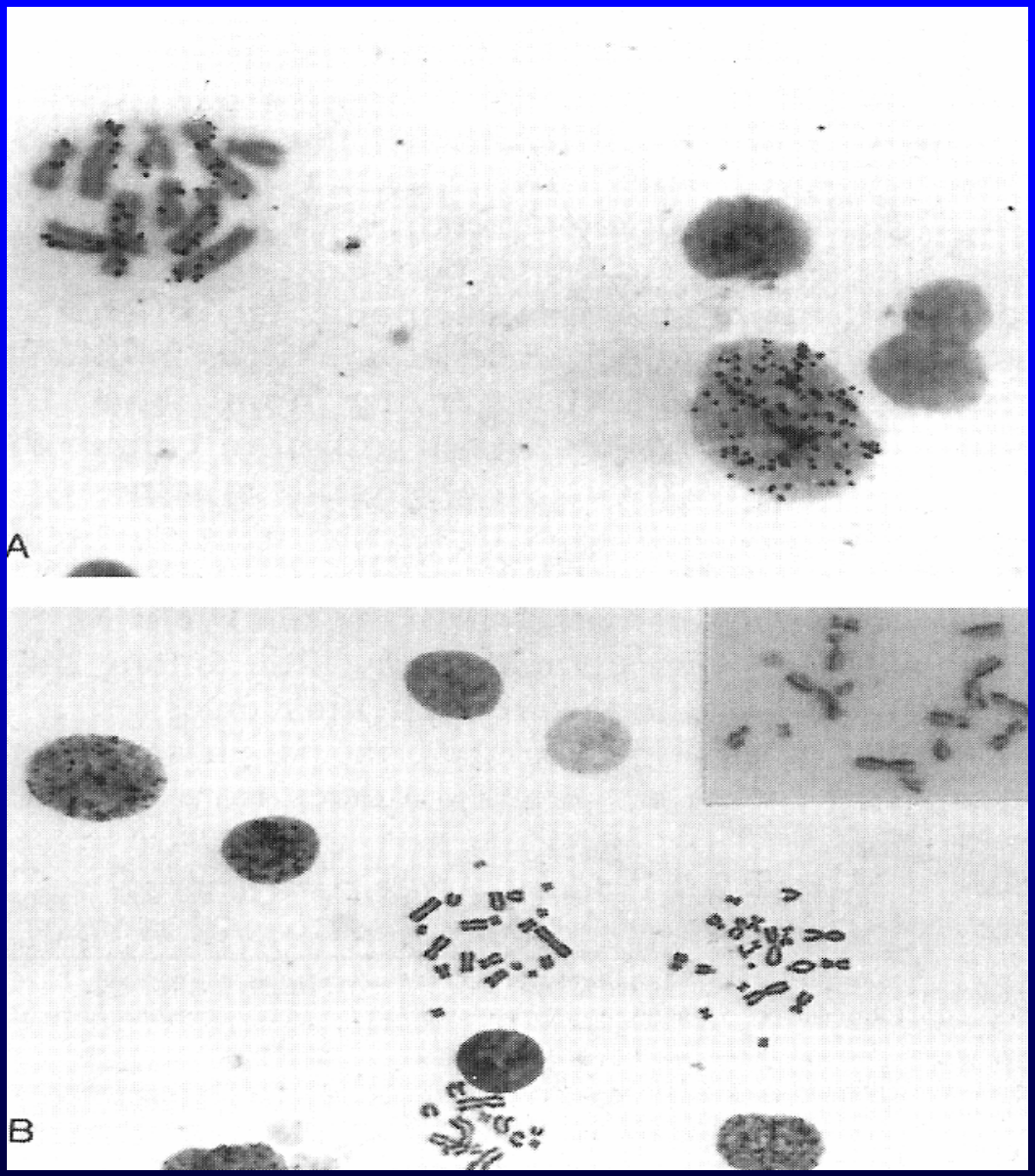
# Fig. 4-1: The Cell Cycle



# Fig. 4-2: The study of cell cycle time



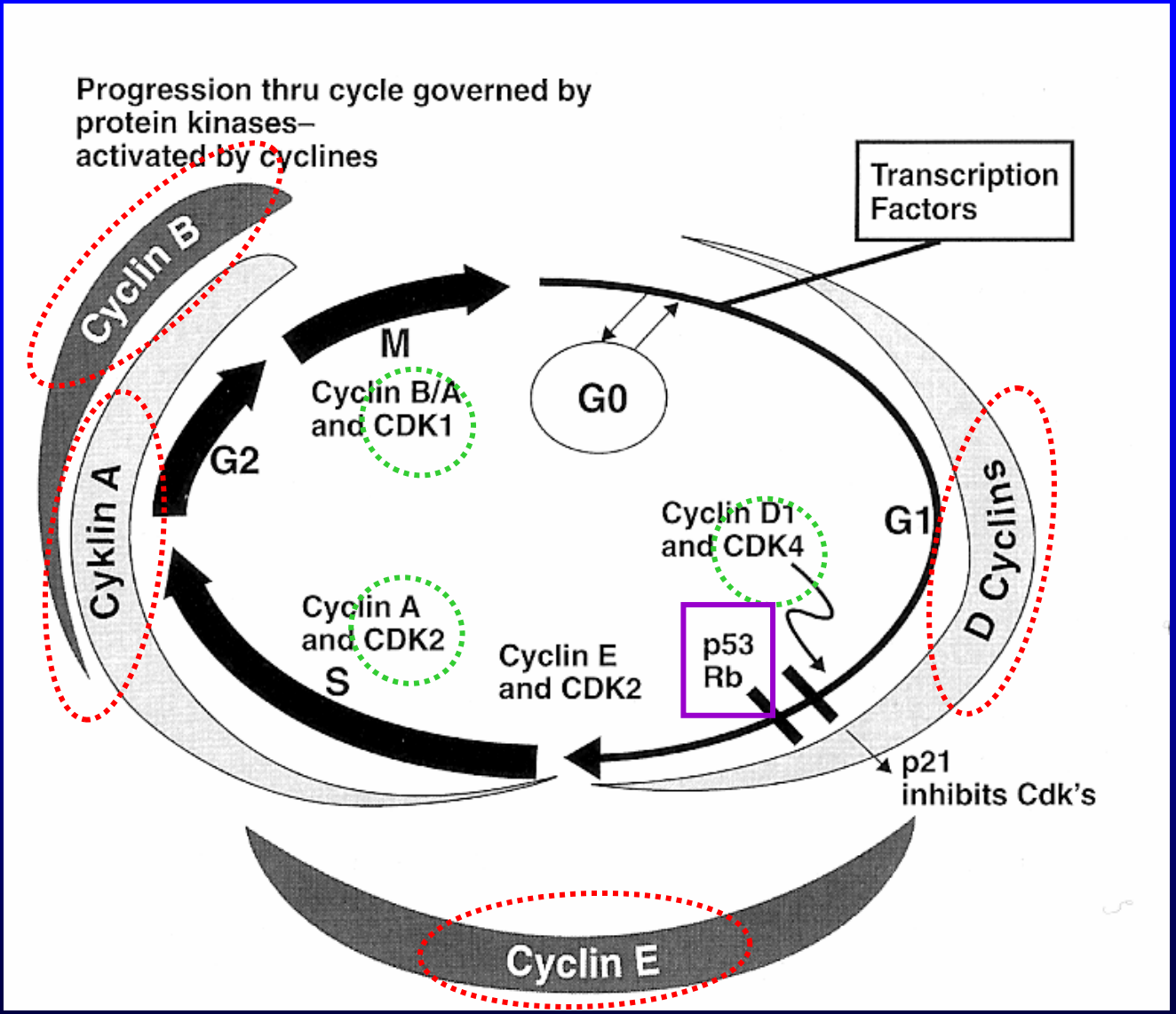
# Fig. 4-3: Study of Cell Cycle Time




$^{3}$H-Thy staining

BrdUrd staining

# Fig. 4-4: The regulation of cell cycle



# Cell Cycle and Growth Factors

- Cell cycle progression through restriction points requires growth factors
  - To activate resting cells from $G_0$ phase to enter G1
  - To pass mid-G1 phase
- S, G2, and M phases are growth factor independent
- Without growth factors, cells die.

# Cell Cycle Progression

**Cell Cycle Progression through each checkpoint requires:**

- **Retinoblastoma tumor suppressor gene family (especially G1-S)**
- **Is regulated by Cyclins that coordinate cell cycle progression and are synthesized at the appropriate time for each phase and then degraded. G1 cyclin expression is induced by growth factors.**
- **And Cyclin Dependent Kinases (CDK) that are activated by cyclins to phosphorylate targets required for the next cell cycle phase**
- **And by regulators of cyclins and Cdks**
  - **inhibitors block assembly of cyclin/cdk complexes or activation of the cdks to cause cell cycle arrest**
  - **Phosphorylation of certain sites on cdks inhibit progression**

# Cyclins

- Have no intrinsic enzymatic activity
- Bind and activate cdks
- Synthesized and degraded each cycle
- Cyclins A to J have been identified (no I)
- Cyclin families D and E are required for restriction point passage

# Cyclin Dependent Kinases

- Activated by binding to cyclins
- Serine/threonine kinases with multiple substrates e.g. pRb, p53, E2F, etc. that they activate/inactivate
- Have kinase and regulatory domains
- Present throughout cell cycle
- Cyclin D activates cdks 4 and 6
- Cyclin E activates cdk2

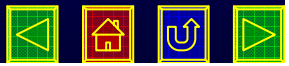
# Cell Cycle Inhibitors

| Phase | Complexes | Inhibitors |
|---|---|---|
| • G1 | cyclin D-Cdk4, 6 | p16 (INK 4a), $p19^{ARF}$, p15 (INK4b) |
| • G1/S | cyclin E-Cdk2, 3 | $p21^{CIP1}$, $p27^{KIP1}$ |
| • S | cyclin A-Cdk2 | p21, p57 |
| • G2/M | cyclin B-Cdk1 | p21 |

**Inhibitors (CKIs) belong to 2 families - INK4 and KIP/CIP**

**Generally compete with cyclins for cdks**

**P19 binds mdm2 to increase p53**



# pRb

- 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.
  - E2F is a transcription factor for 20-30 genes that are necessary for S phase gene expression.

# Cyclins and Cdks


pRb dephosphorylation

Cyclin A/ cdk1 (cdc2)
Cyclin B/ cdk1 (cdc2)

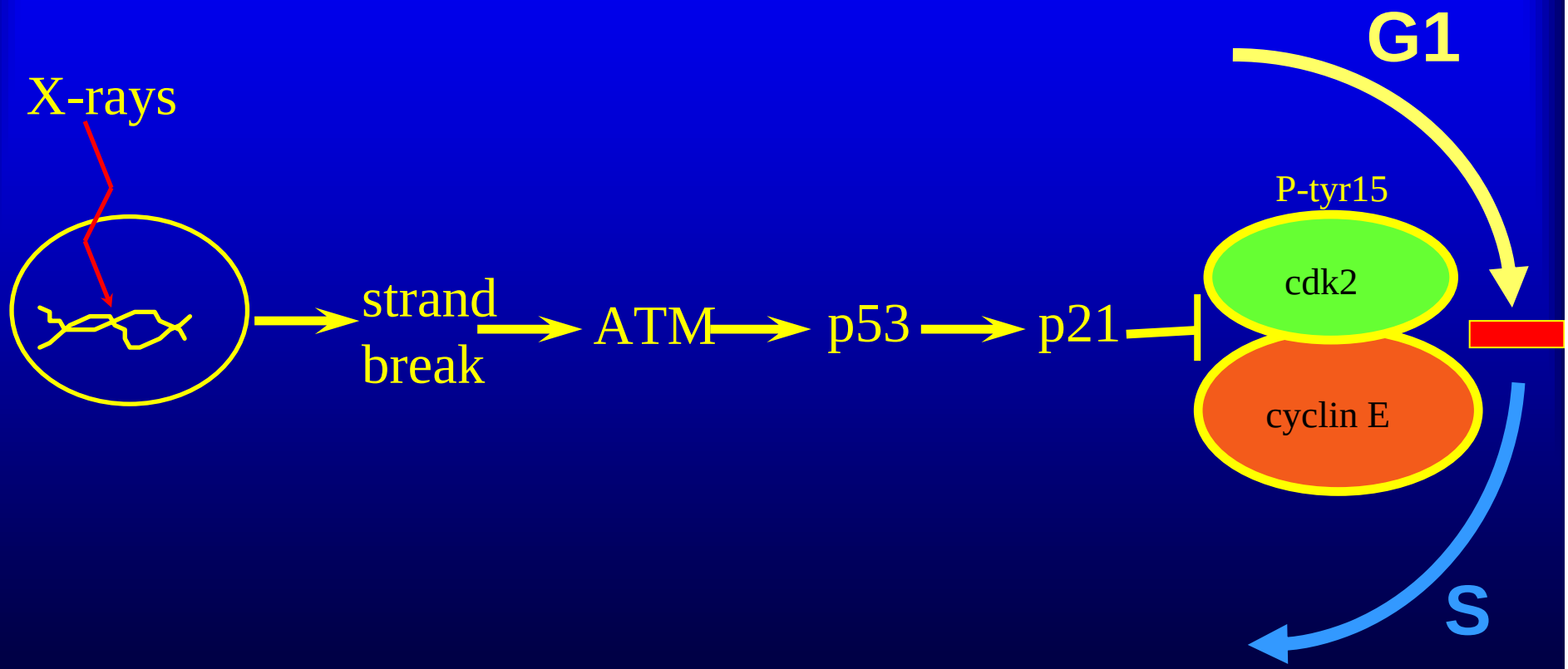
**Early - mid G1**
Cyclin D / cdk4
Cyclin D/ cdk6
Responsible for pRb phosphorylation

Cyclin A/ cdk2

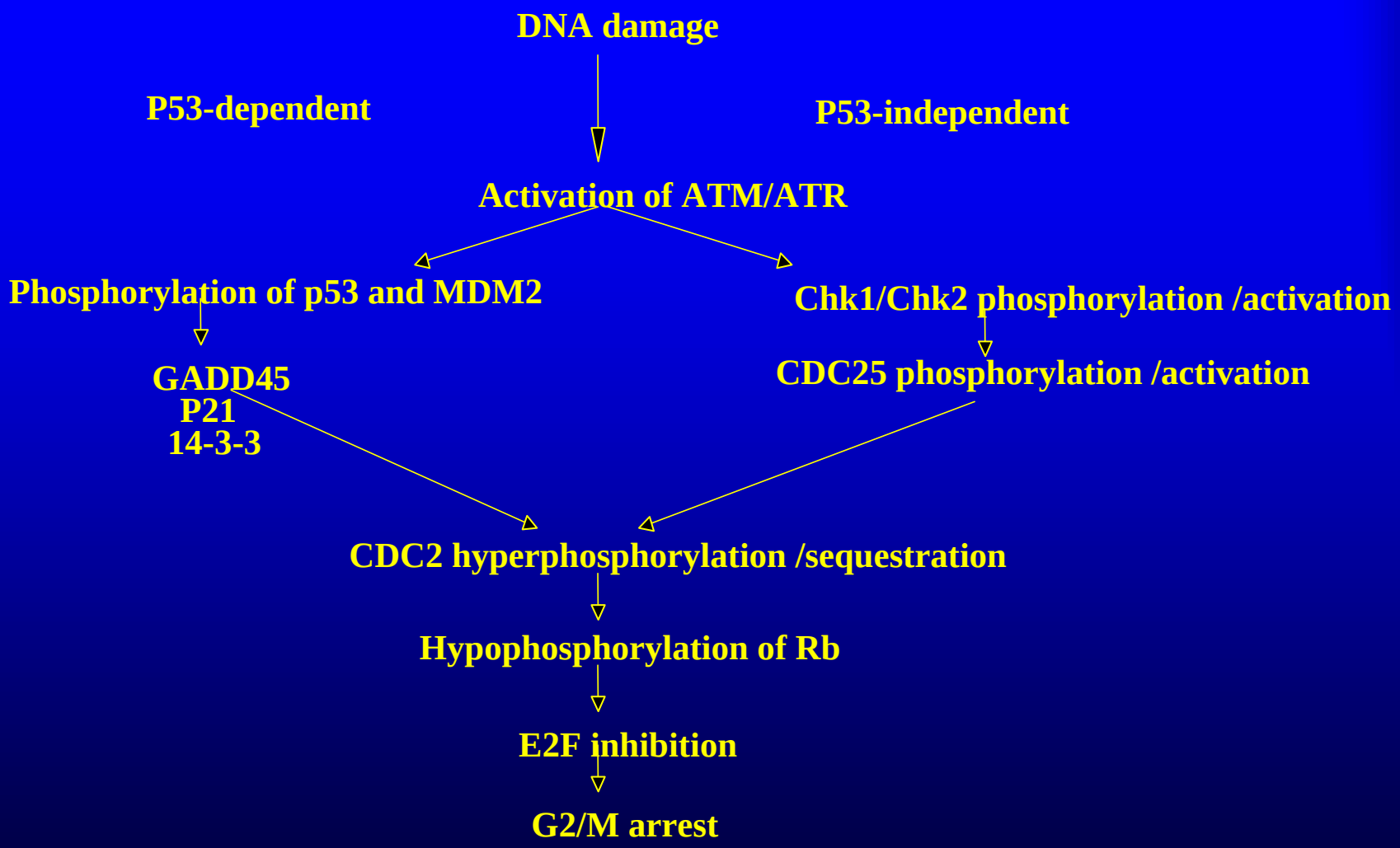
Cyclin E/ cdk2 Responsible for pRb
Cyclin A/ cdk2 phosphorylation

# Radiation-induced G1/S Arrest

**Effector Pathway:**
**p21 (WAF1/cip1/sdi1) transactivated**
**P21 inhibits CYCLIN E or A / cdk2; CYCLIN D / cdk4**



# Radiation-induced G2/M arrest

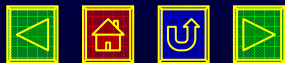


# Importance of cell cycle regulation

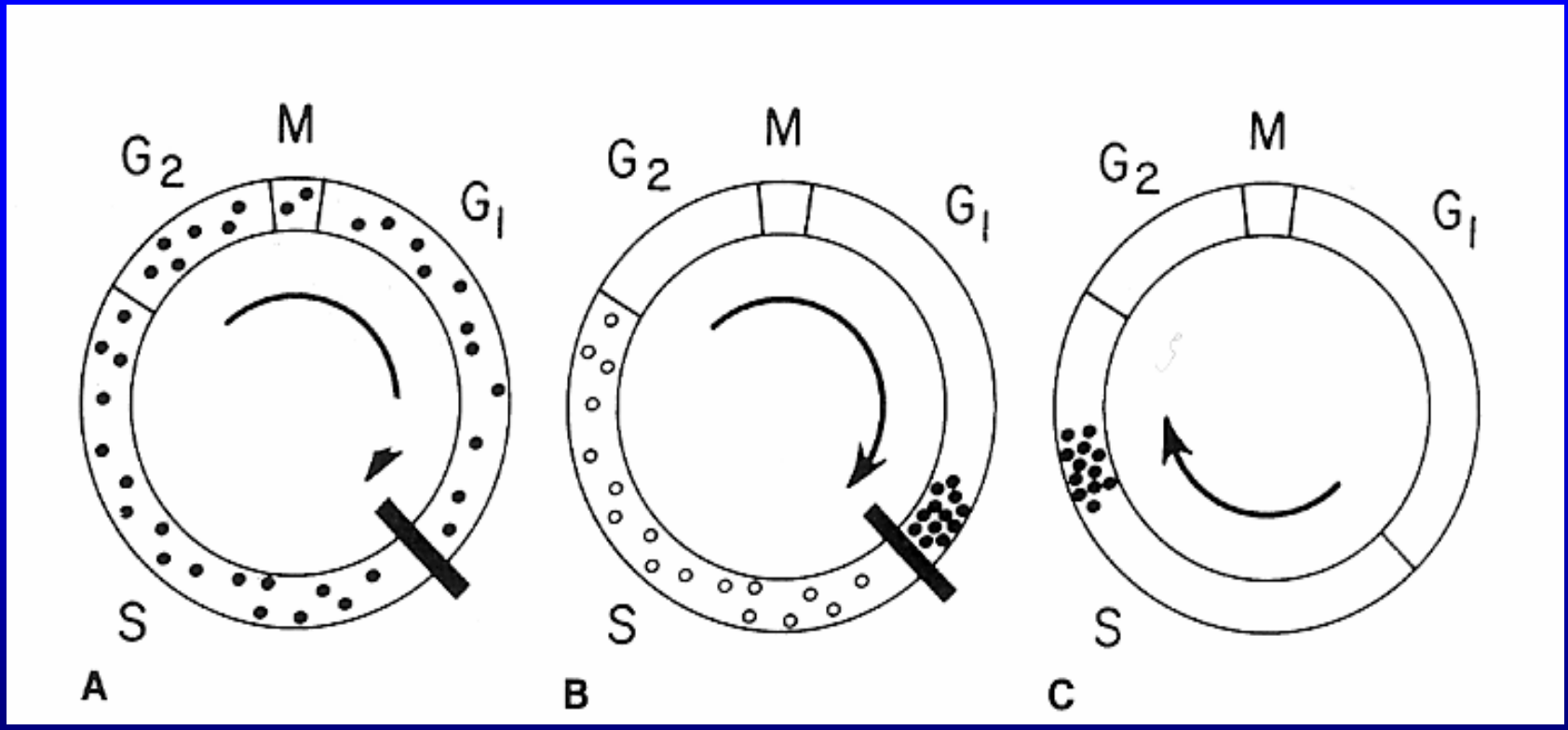
- If p53 or any other molecule governing cell cycle arrest is mutated, genetic instability results as well as more rapid cell cycle progression.
- Cyclin, cdks, CKIs and other molecules involved in cell cycle progression are frequently mutated or have altered expression in cancer e.g. cyclin D amplification and/or p16 deletion or silencing and/or p53 mutation in Head and Neck Ca.

# Cell Cycle Synchronization

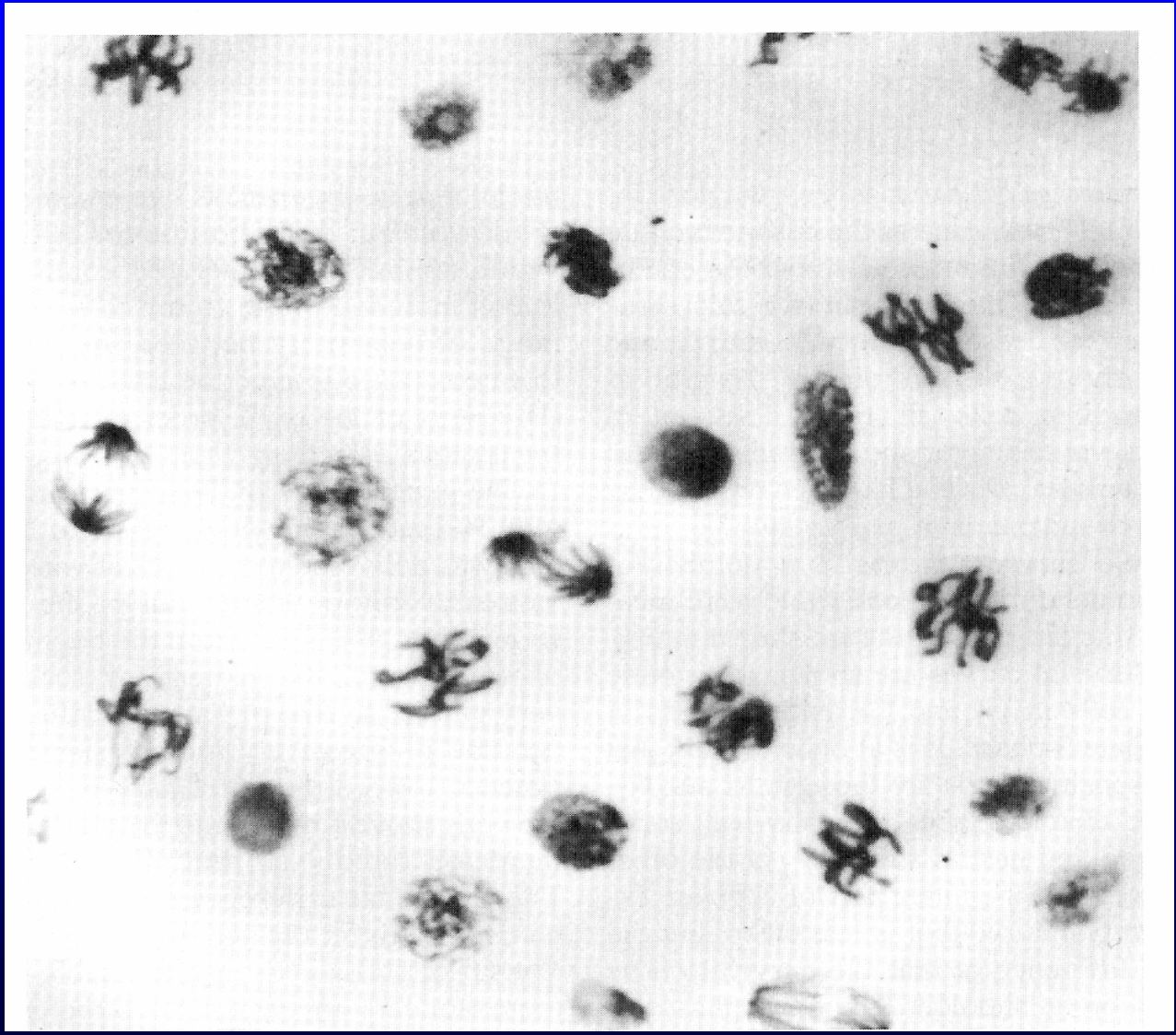
- **Mitotic Harvest: by Terasima & Tolmach, 1961**
  - **Many adherent cells become less adherent during mitosis and can be collected by shaking the plate**
- **Drug: hydroxyurea:**
  - **kills cells in S phase and (reversibly) blocks cells from going to S from G1**
- **Cell size difference:**
  - **Centrifugation**
  - **FACS**
- **Radiation**



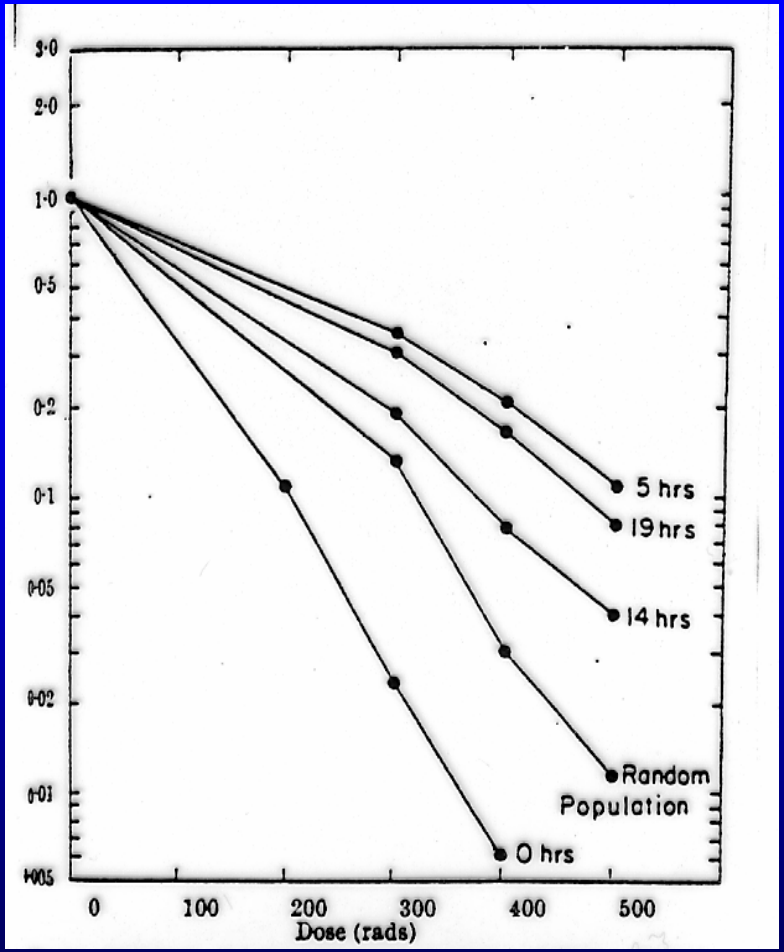
# Fig. 4-5 : Mode of action of hydroxyurea

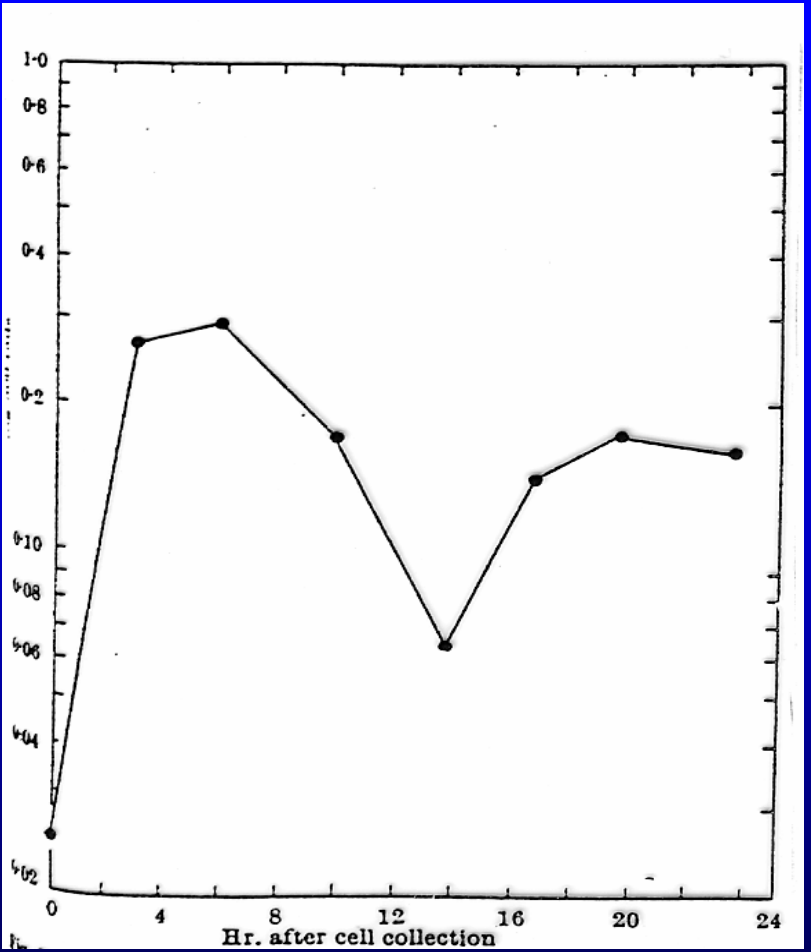


# Fig. 4-6: The root tip cells following hydroxyurea treatment



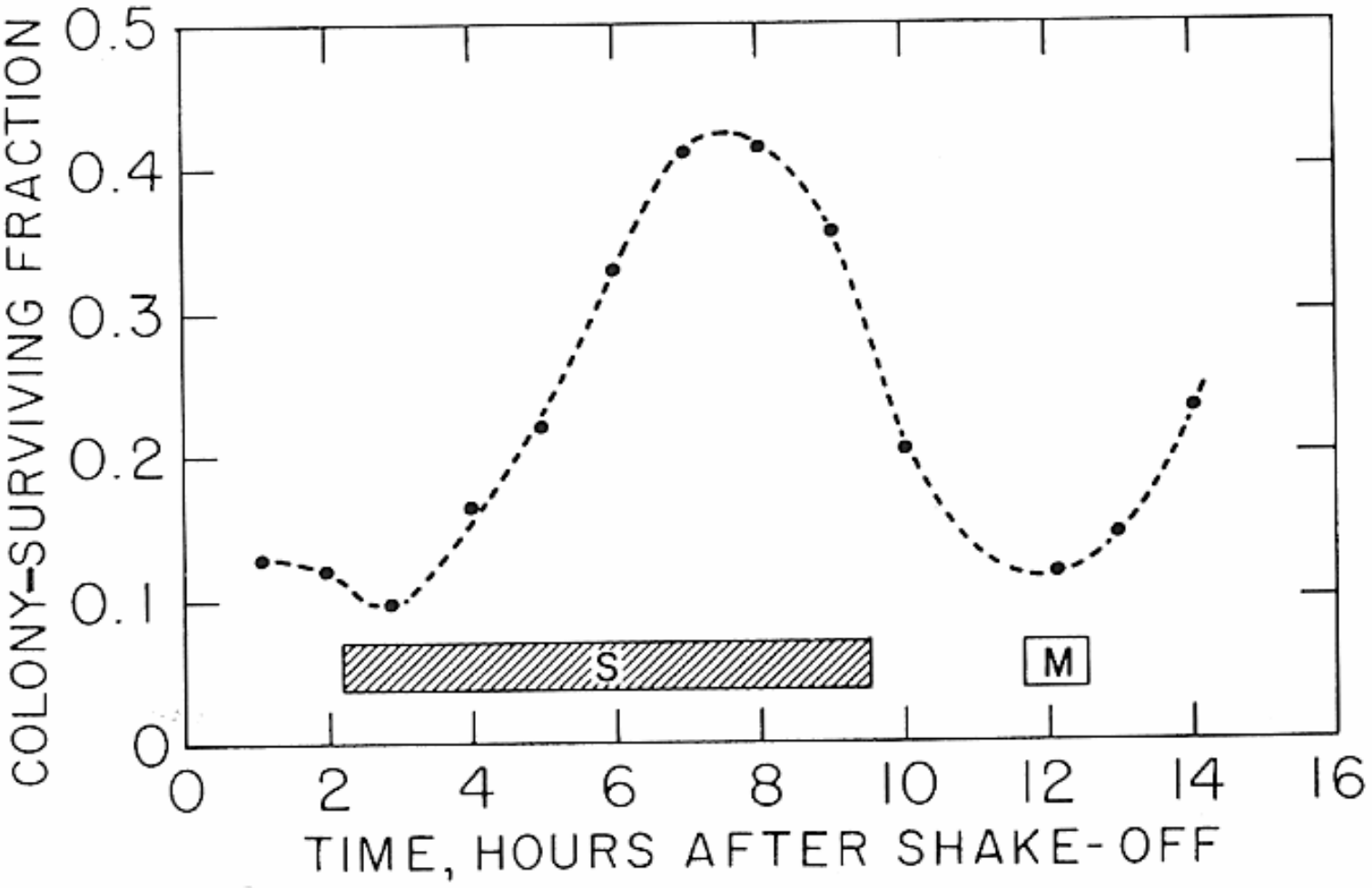
# Fig. 4-9: The effects of X-rays on synchronously dividing cell cultures (HeLa cells)



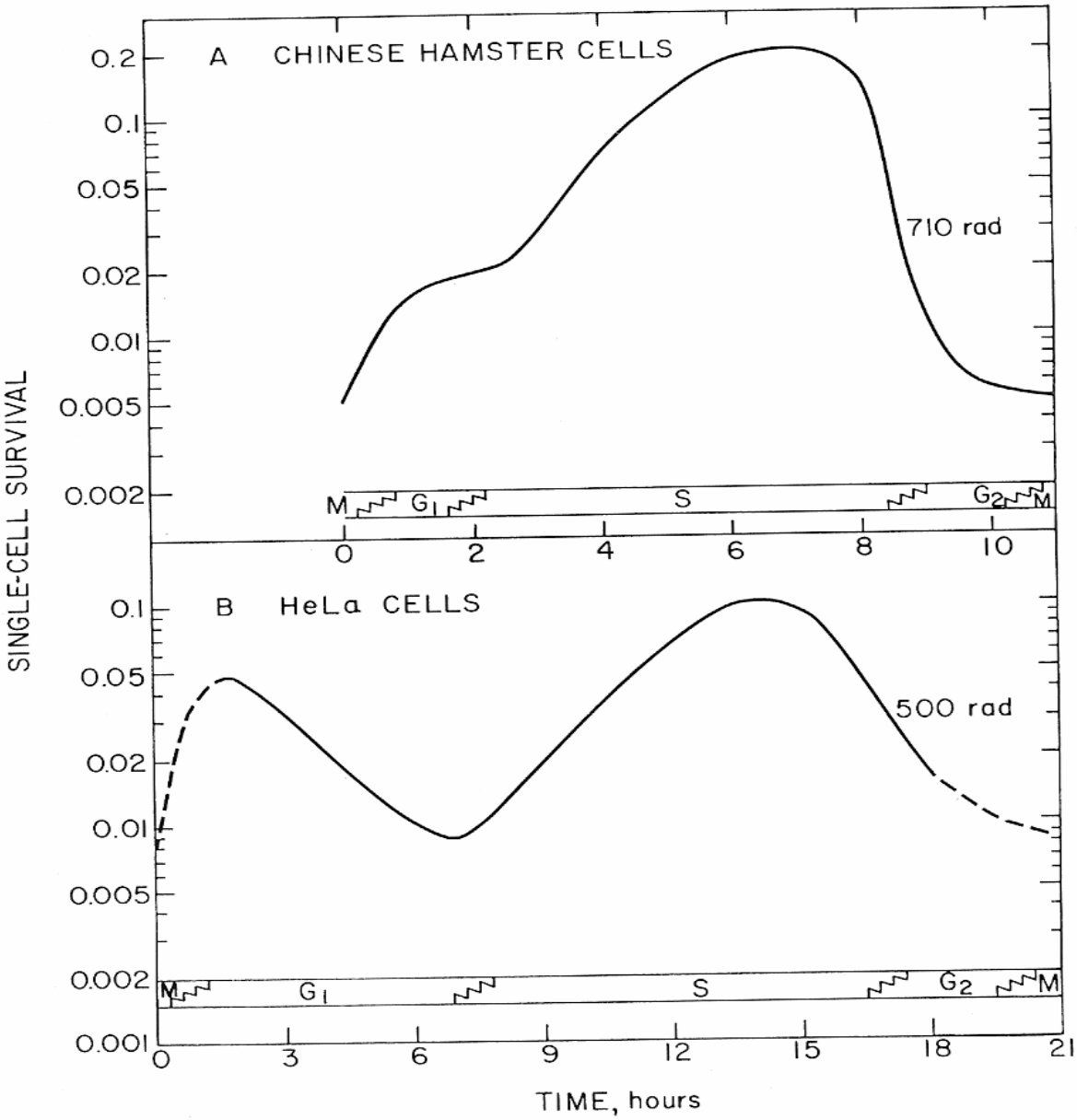




Terasima, T. & Tolmach, L.J.
Nature, 1961

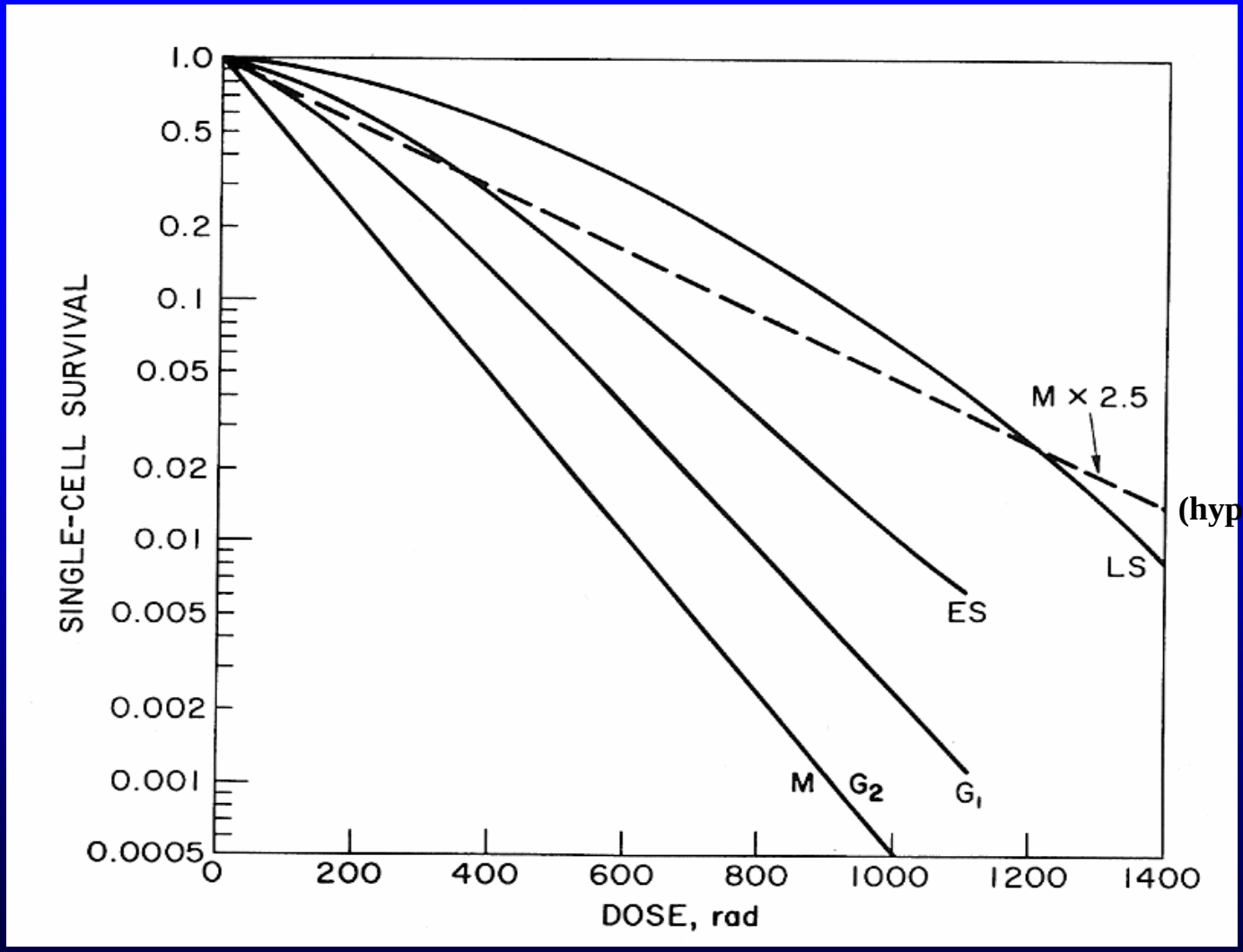
# Fig. 4-7: Cell cycle effects on CHL cells, 1966



# Fig. 4-10: Influence of cell cycle time on cell age effects

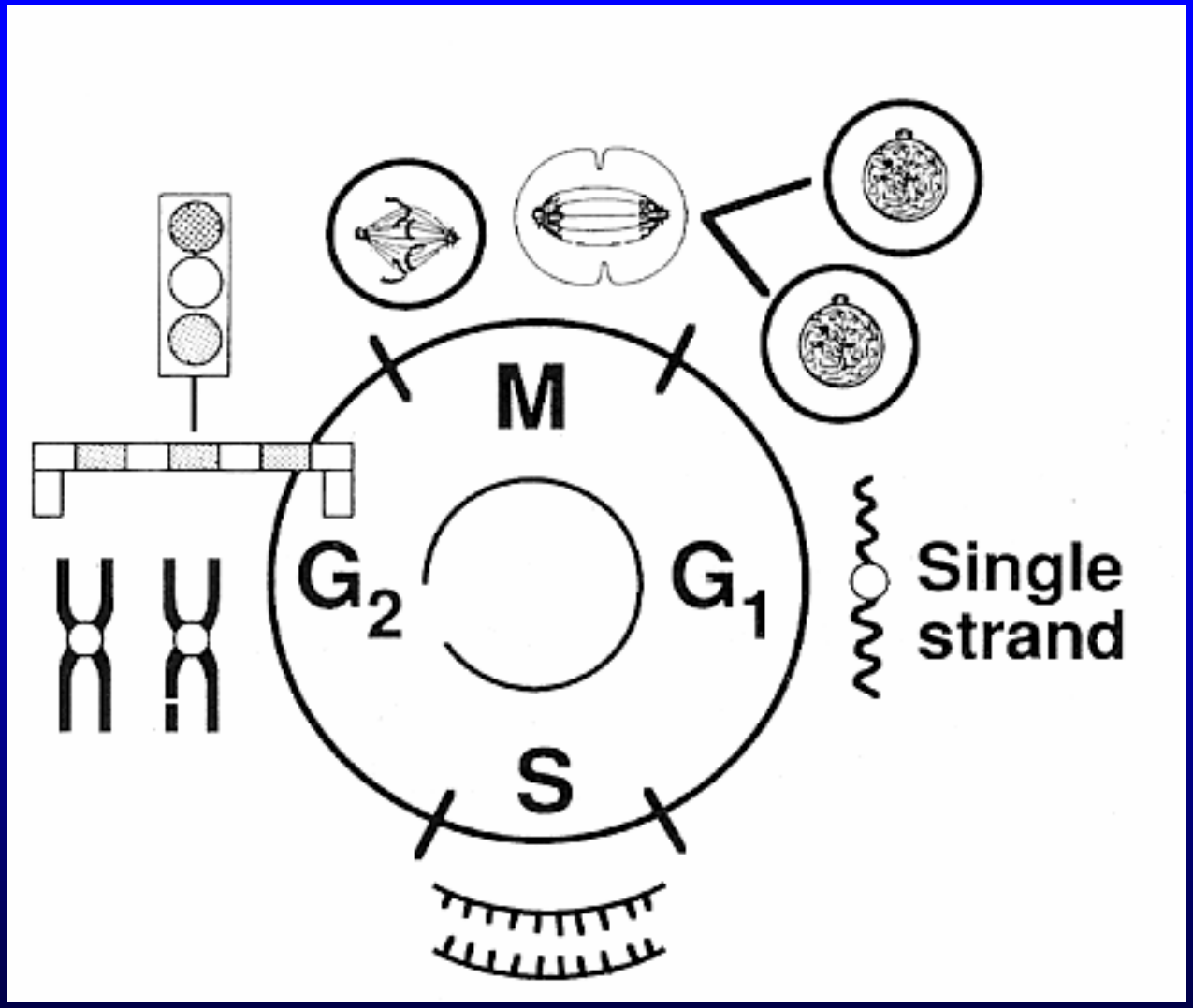


# Fig. 4-8: Summary of the cell cycle effects on CHL cells

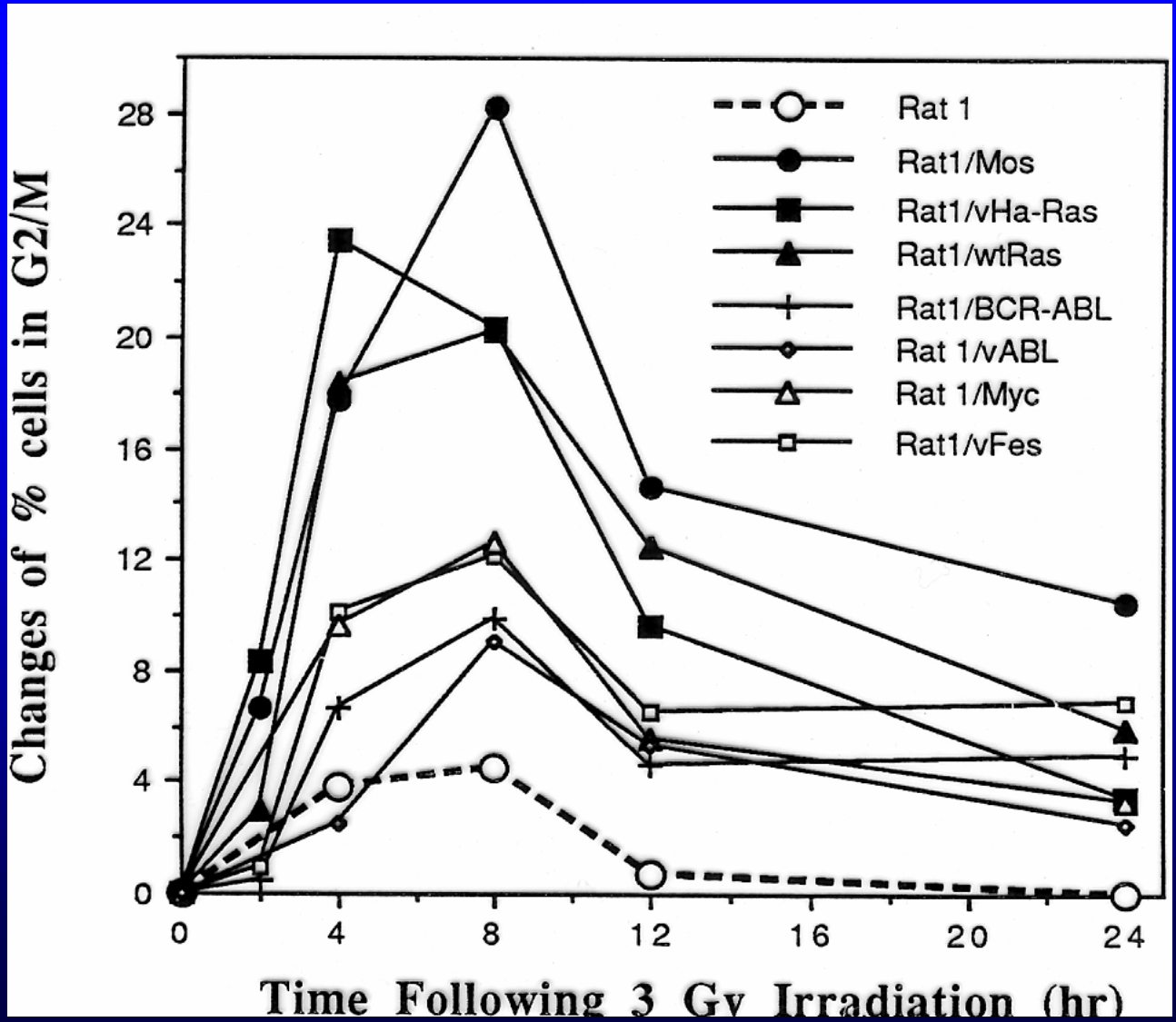



(hypoxic M cells)

# Fig. 4-11: Molecular Checkpoint Genes



# Relationship between radiosensitivity and radiation-induced $G_2$/M block



# Radiation-induced $G_2$/M block in AT

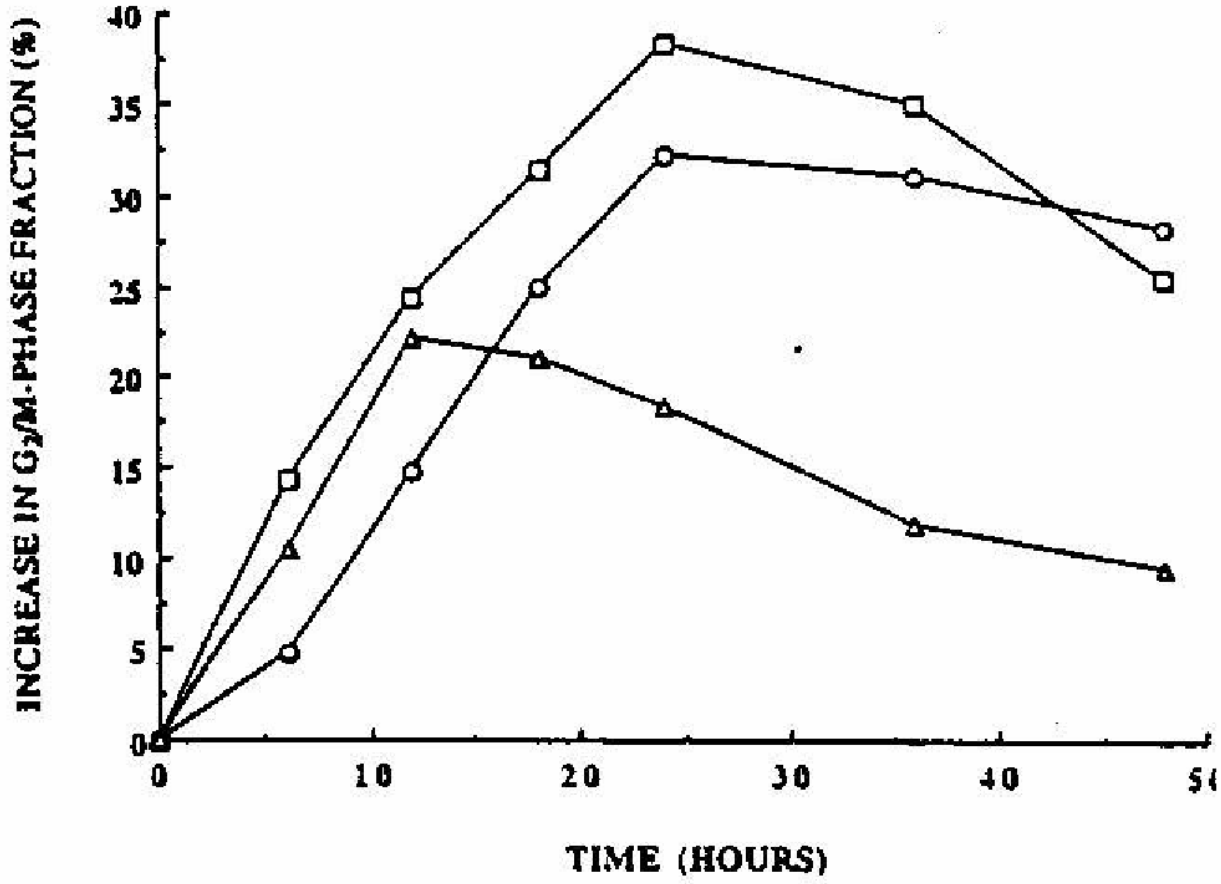
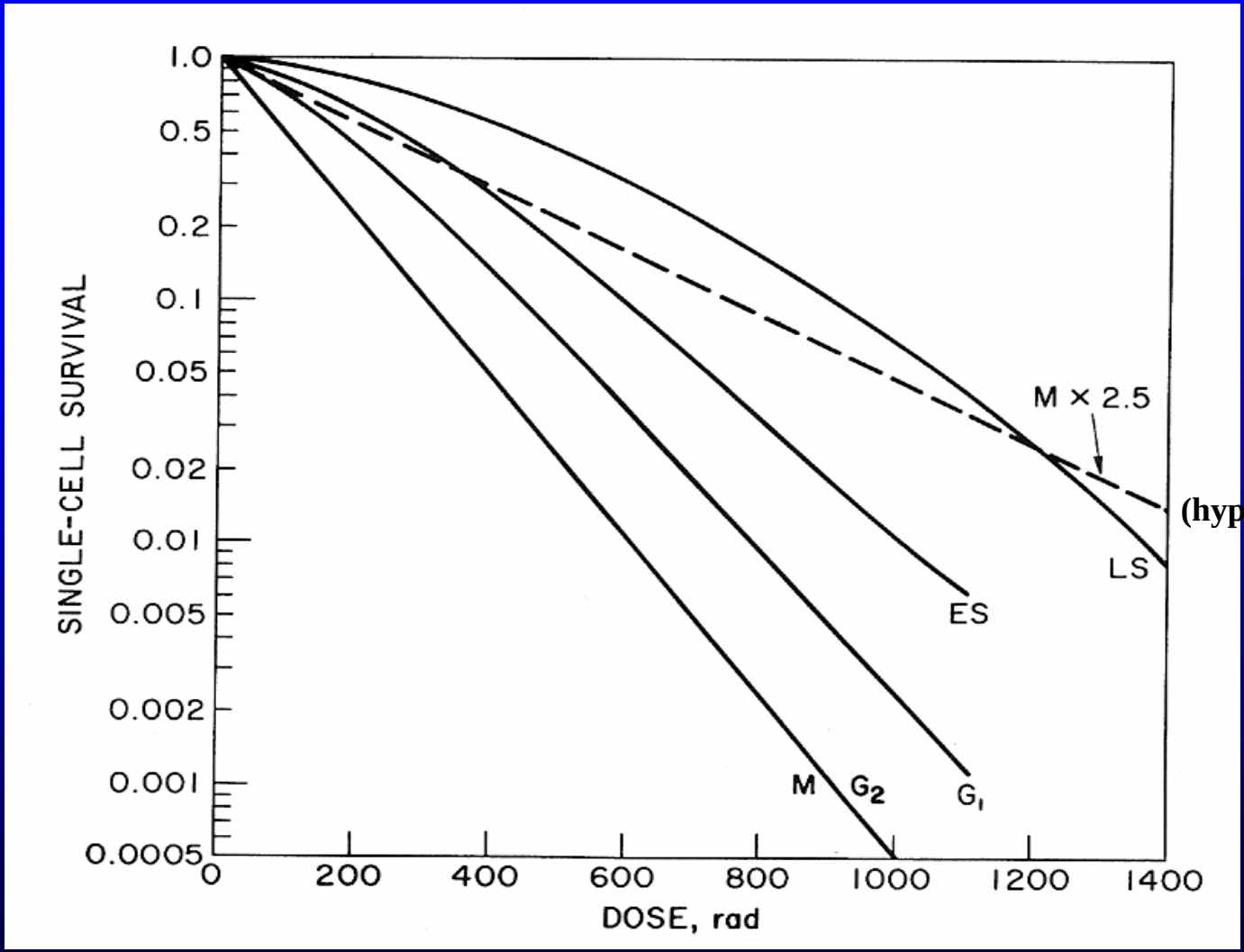



FIG. 1. A representative example of $G_2$/M-phase accumulation in
and control LCLs after 2 Gy irradiation. (□) CSA-LCL: AT. comp
mentation group C; (○) RJO-LCL: AT, group A; (△) NAT-8: norı
control. The percentage of cells in $G_2$/M phase at time x minus the p
centage of cells at time zero is shown.

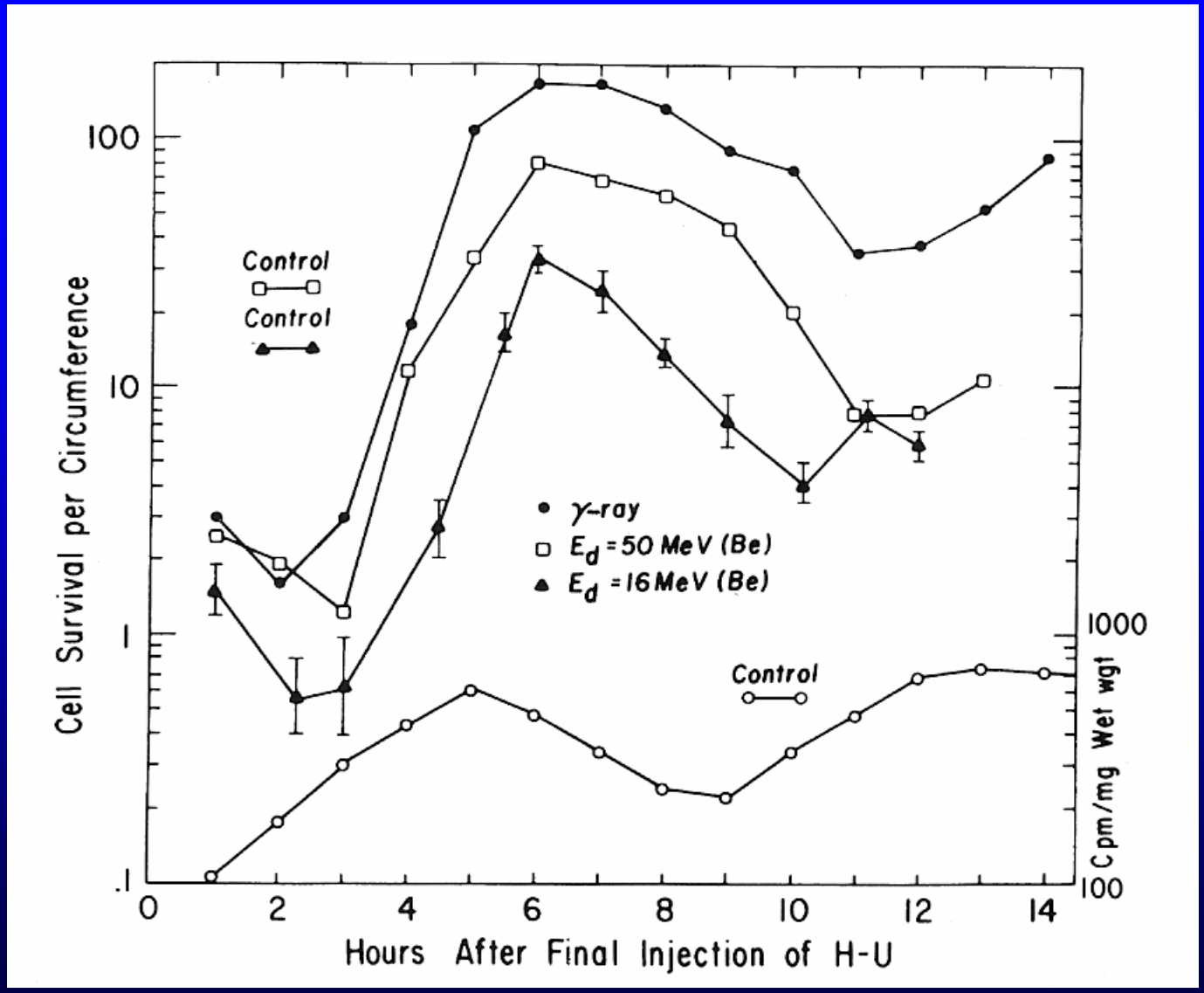
Hong, et.al.
1994
Radiat. Res.
140: 17~23

# Fig. 4-8: Summary of the cell cycle effects on CHL cells




(hypoxic M cells)

# Fig. 4-12: The age-response function for a tissue *in vivo*



# The Effect of Oxygen at various phase of the cell cycle

- **$G_2$ phase: OER = 2.3 ~ 2.4**
- ***$G_1$ phase: OER = 2.4 ~ 2.8***
- **S phase: OER = 2.8 ~ 2.9**

# Variation of sensitivity with cell age for neutrons

- Qualitative similar to X or γ-ray
- But quantitative difference to X or γ-ray

# Mechanisms for the Age-Responsive Function

- Repair capacity
- Contents of –SH compounds

# The possible implications of the age-response function in radiotherapy

# End