CELL CYCLE ,CHECK POINTS

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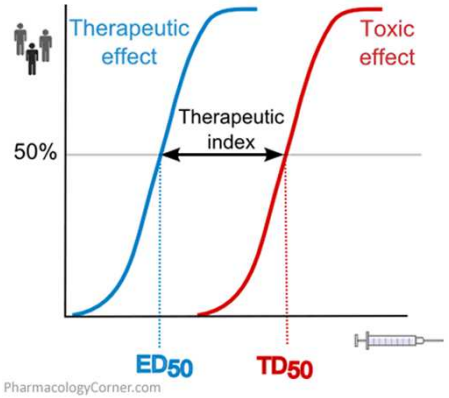
IN RADIATION ONCOLOGY

TUMOR

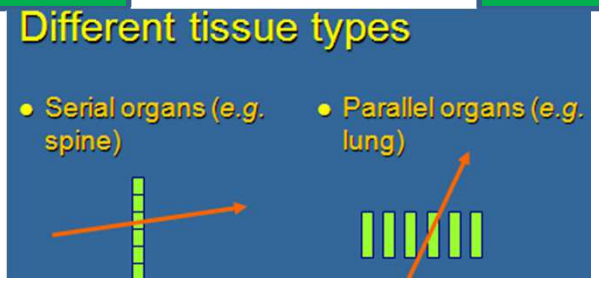
FRACTION OF CELLS SURVIVING DETERMINES THE SUCCESS OR FAILURE OF A TREATMENT REGIMEN



NORMAL TISSUE

ACUTE REACTING TISSUE

LATE REACTING TISSUE



TOLERANCE OF NORMAL TISSUE FOR RADIATION DEPENDS ON ABILITY OF CLONOGENIC CELLS TO MAINTAIN A SUFFICIENT NO OF MATURE CELLS SUITABLY STRUCTURED TO MAINTAIN ORGAN FUNCTION

CELL DEATH

- CELL DEATH AFTER IRRADIATION OCCURS MOSTLY AS CELLS ATTEMPT TO DIVIDE.
- RAPIDLY DIVIDING TISSUE:-DAMAGE EVIDENT QUICKLY
- SLOW/RARELY DIVIDING CELL:-RADIATION DAMAGE REMAIN LATENT FOR LONG PERIOD.

LAW OF BERGONIE' & TRIBONDEU:- Tissue appears to be more radiosensitive if their cells are **less differentiated**,**have a greater prolliferative capacity** and **divide more rapidly**.

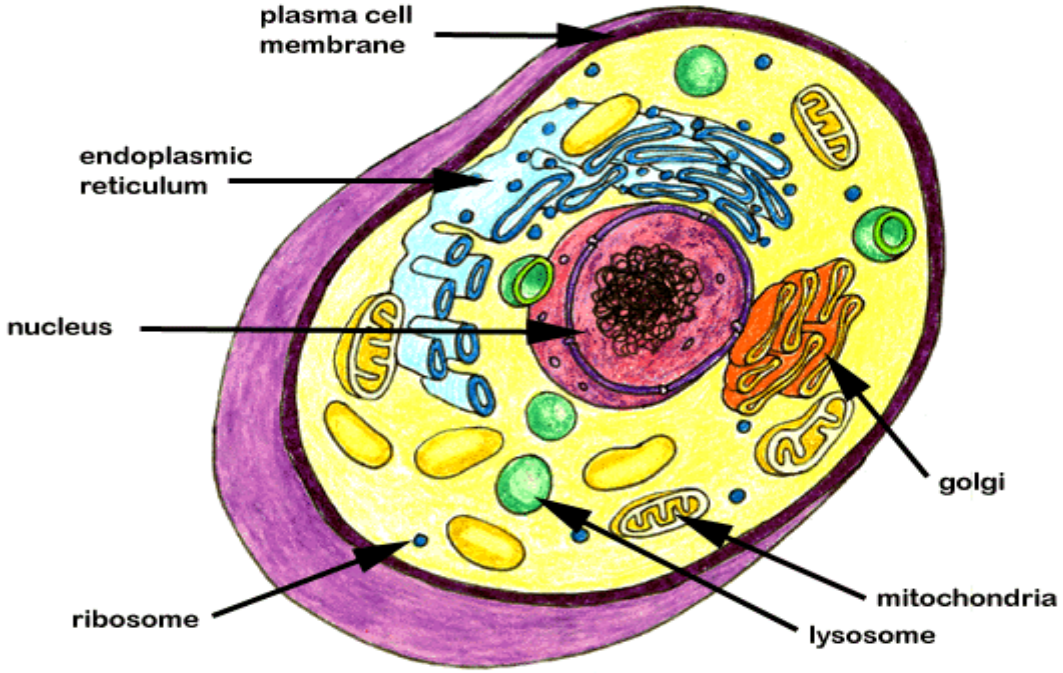
CASARETT'S CLASSIFICATION OF RADIOSENSITIVITY

	CELL TYPE	PROPERTIES	EXAMPLE	SENSITIVITY
I	VEGETATIVE INTERMITOTIC CELLS	DIVIDE REGULARLY.NO DIFFERENTIATION	ERYTHROBLAST INT CRYPTS GERMINAL CELL OF EPIDERMIS	HIGH
II	DIFFERENTIATING INTERMITOTIC CELLS	DIVIDE REGULARLY.SOME DIFFERENTIATION BETWEEN DIVN.	MYELOCYTE	HIGH
III	REVERTING POST MITOTIC CELLS	DO NOT DEVIDE REGULARLY,VARIABLY DIFFERENTIATED	LIVER,KIDNEY, PANCREAS	LOW
IV	FIXED POST MITOTIC CELL	DO NOT DEVIDE HIGHLY DIFFERENTIATED	NERVE CELLS MUSCLE CELLS	LOW

SENSITIVITY DECREASES

The CELL

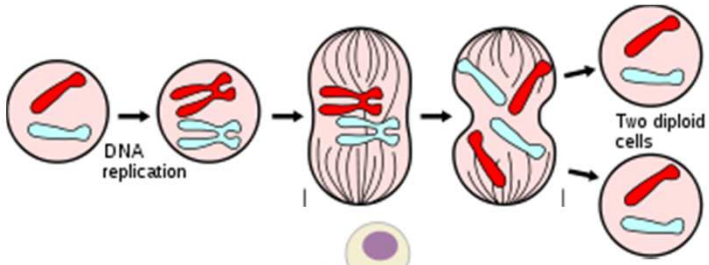
- Cell membrane
- Cytoplasm
- Nucleus
- Mitochondria
- Lysosomes
- Golgi complex
- Endoplasmic retic
- Ribosome

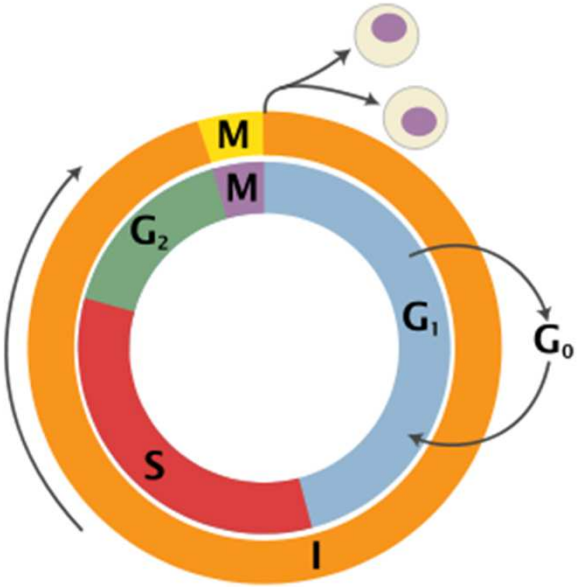


CELL DIVISION

PROKARYOCYTE

BINARY FISSION





EUKARYOCYTE

RESTING PHASE

INTERPHASE

MITOTIC PHASE

G1

S

G2

KARYOKINESIS

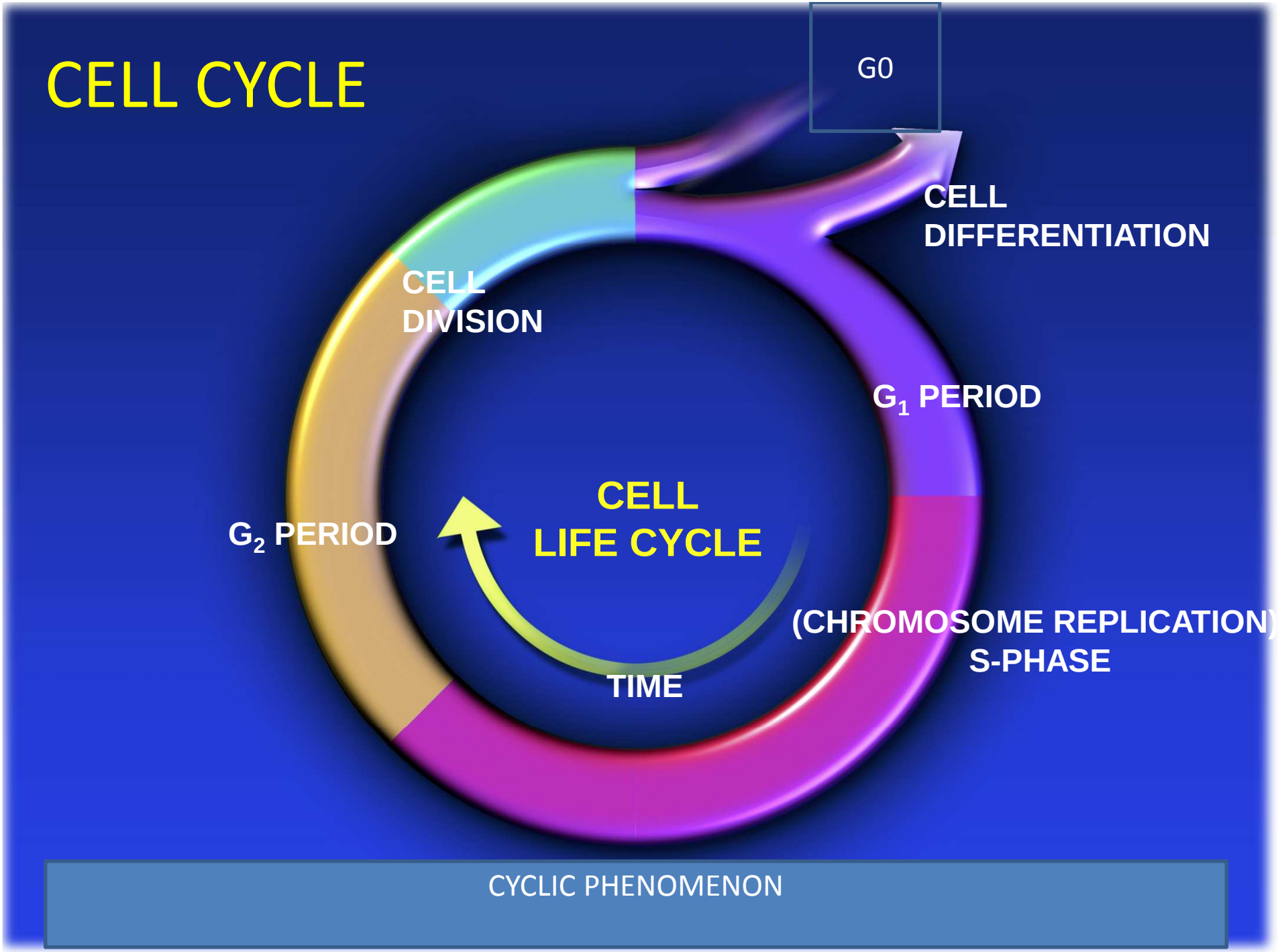
PROPHASE

METAPHASE

ANAPHASE

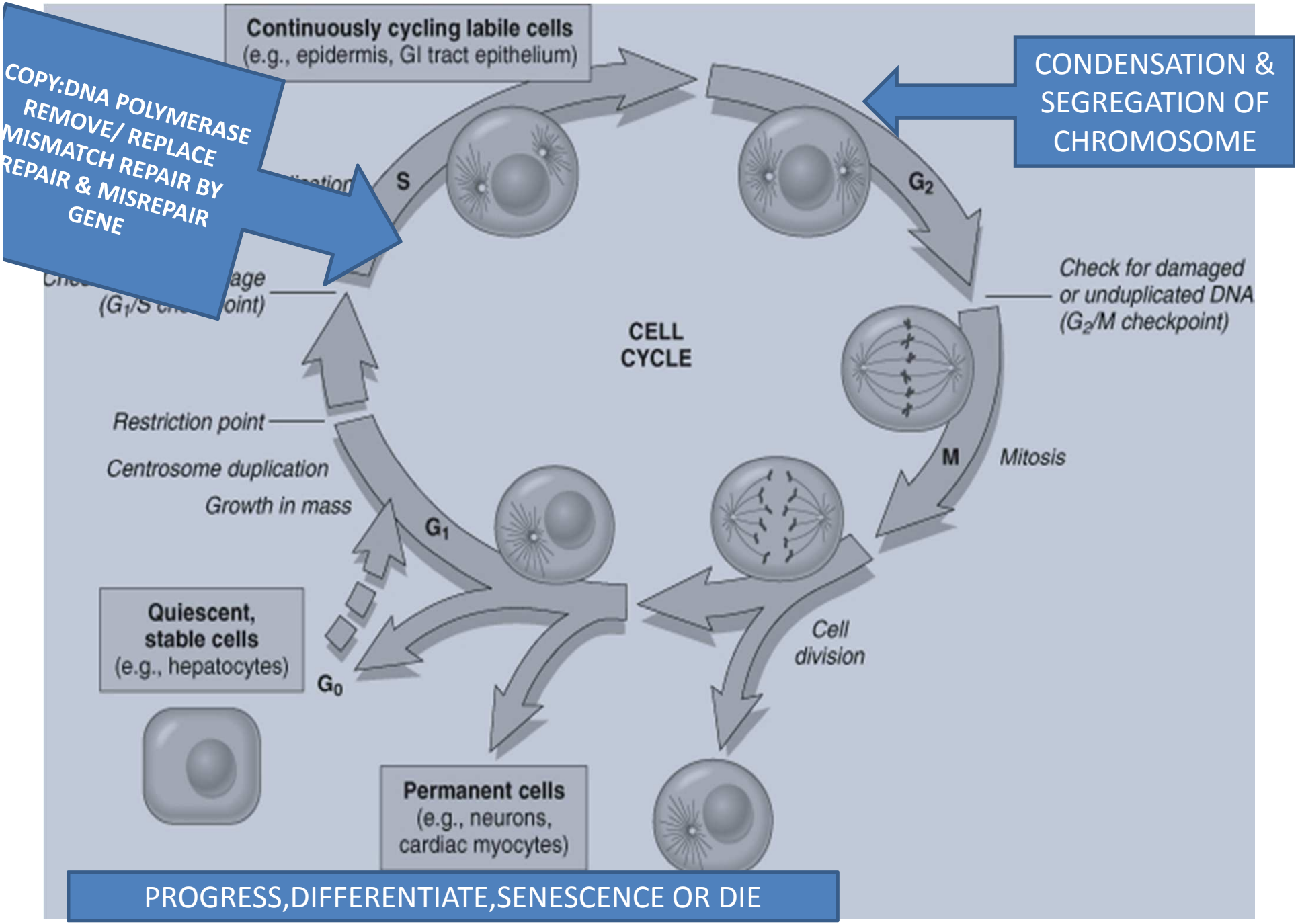
TELOPHASE

CYTOKINESIS


CELL CYCLE
G0
CELL DIFFERENTIATION
CELL DIVISION
G_1 PERIOD
CELL LIFE CYCLE
G_2 PERIOD
(CHROMOSOME REPLICATION)
S-PHASE
TIME
CYCLIC PHENOMENON

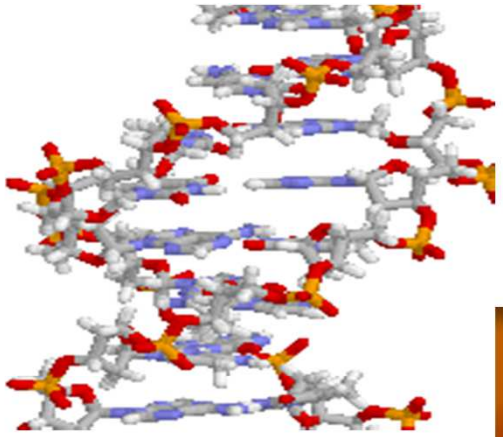
MITOTIC CYCLE TIME OR CELL CYCLE TIME(Tc)

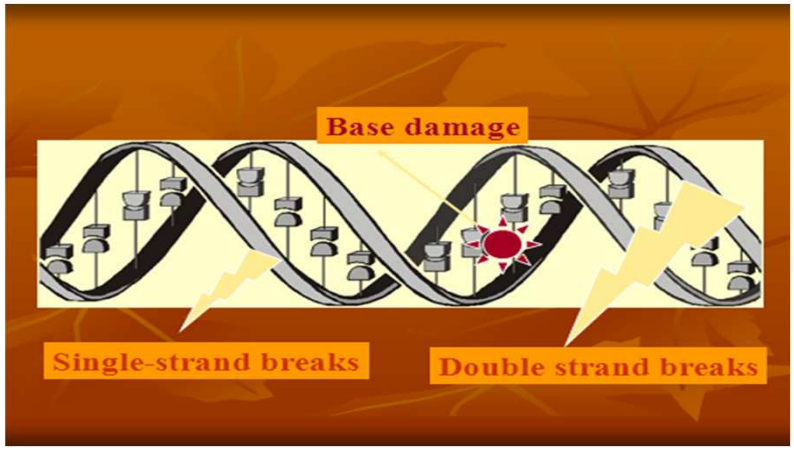
TIME BETWEEN SUCCESSIVE DIVISION OF CELL IS KNOWN AS MITOTIC CYCLE TIME

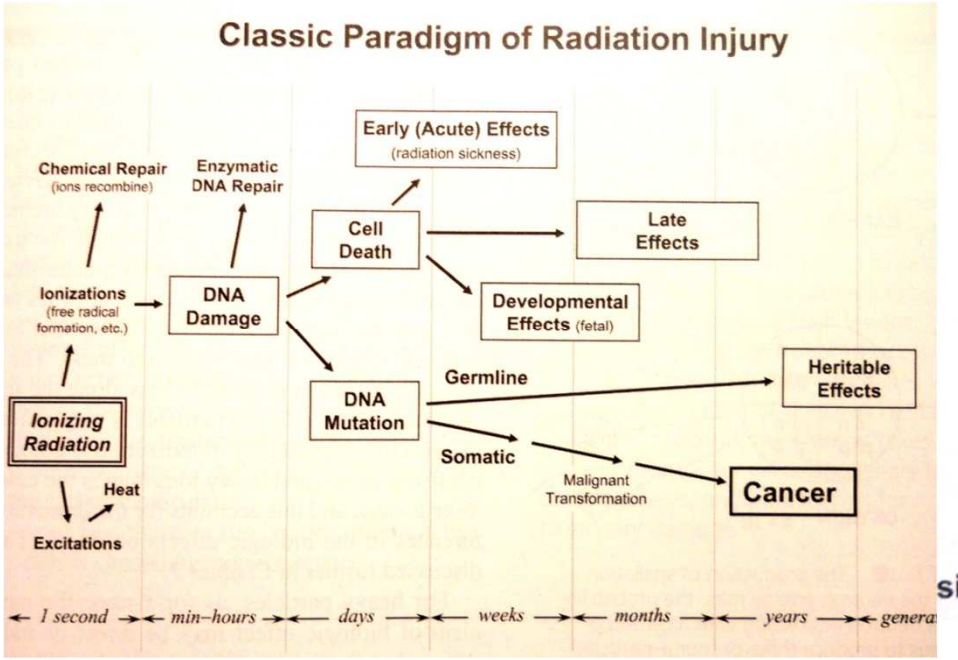

COPY:DNA POLYMERASE
REMOVE/ REPLACE
MISMATCH REPAIR BY
REPAIR & MISREPAIR
GENE
CONDENSATION &
SEGREGATION OF
CHROMOSOME
Continuously cycling labile cells
(e.g., epidermis, GI tract epithelium)
S
G2
Check for damaged
or unduplicated DNA
(G2/M checkpoint)
CELL
CYCLE
M
Mitosis
Restriction point
Centrosome duplication
Growth in mass
G1
Quiescent,
stable cells
(e.g., hepatocytes)
G0
Cell
division
Permanent cells
(e.g., neurons,
cardiac myocytes)
PROGRESS,DIFFERENTIATE,SENESCENCE OR DIE

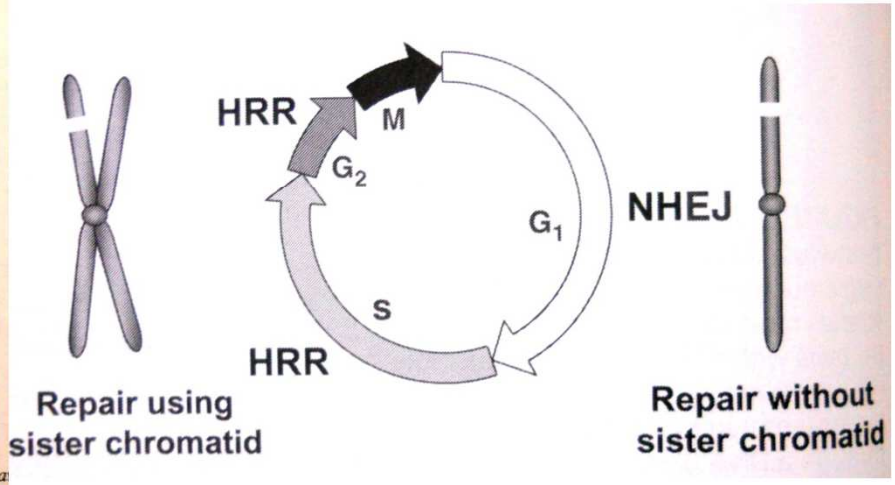
DIFFERENCE IN CELL CYCLE TIME IS MOSTLY DUE TO VARIATION IN LENGTH OF G1 PERIOD

	HAMSTER CELL 11HRS	HELA CELL 24 HRS
Tc	11	24
Tm	1	1
Ts	6	8
Tg1	**1**	**11**
Tg2	3	4



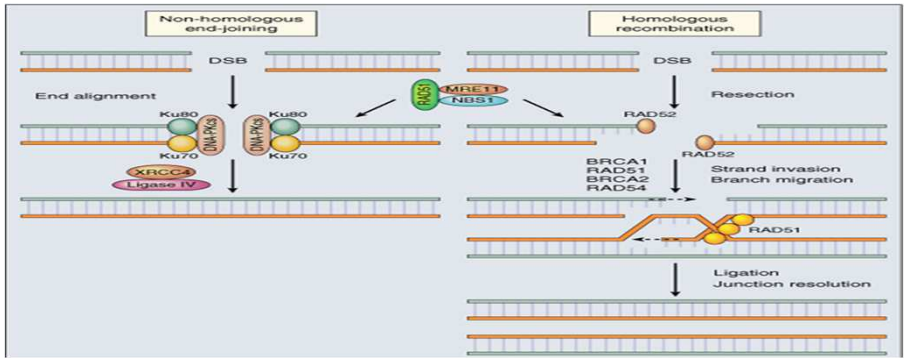

Base damage
Single-strand breaks
Double strand breaks


Classic Paradigm of Radiation Injury
Chemical Repair
(ions recombine)
Enzymatic
DNA Repair
Early (Acute) Effects
(radiation sickness)
Cell
Death
Late
Effects
Ionizations
(free radical
formation, etc.)
DNA
Damage
Developmental
Effects (fetal)
Germline
Heritable
Effects
Ionizing
Radiation
DNA
Mutation
Somatic
Malignant
Transformation
Cancer
Heat
Excitations
1 second
min–hours
days
weeks
months
years


HRR
M
G2
G1
S
HRR
NHEJ
Repair using
sister chromatid
Repair without
sister chromatid

REPAIR OF DOUBLE STRAND BREAK

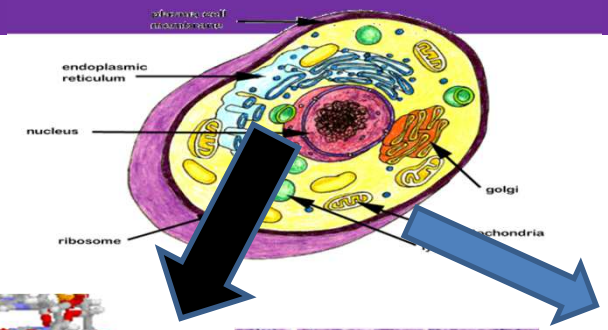
HOMOLOGOUS RECOMBINATION	NONHOMOLOGOUS END JOINING
LATE S,G2 PHASE	G1 & EARLY S
CRITICAL IN CELL CYCLE SIGNALLING	PREDOMINANT MECHANISM OF DOUBLE STRAND BREAK
REQUIRE AN UNDAMAGED DNA STRAND AS PARTICIPANT	MEDIATES END TO END JOINING
ERROR FREE	ERROR PRONE

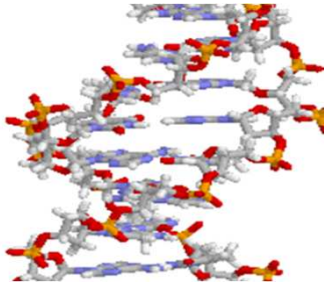


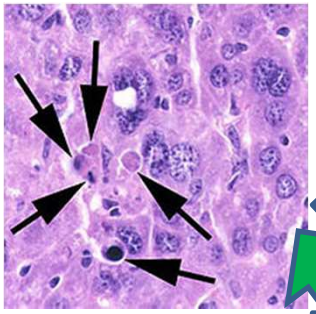
RADIATION INDUCED CELL DEATH

NECROTIC DEATH	APOPTOTIC CELL DEATH
PASSIVE PROCESS,CELL PASS THROUGH MITOSIS WITH UNREPAIRED DNA BREAK	ACTIVE PROCESS,CASCADE OF EVENT AFTER CELLULAR STRESS
LOSS OF MEMBRANE INTIGRITY,CYTOPLASMIC VESICLE,DEGRADATION OF DNA	FRAGMENTATION OF DNA,NUCLEAR FRAGMENTATION,CONDENSED CHROMATIN,EOSINOPHILIC CYTOPLASM

APOPTOSIS







DAMAGE TO PLASMA MEMBRANE

CLEAVAGE MEMBRANE BOUND SPHINGOMELIN

SPINGOMYELINASE

CERAMID

CASPASE ACTVATION

ATM

p53

BAX

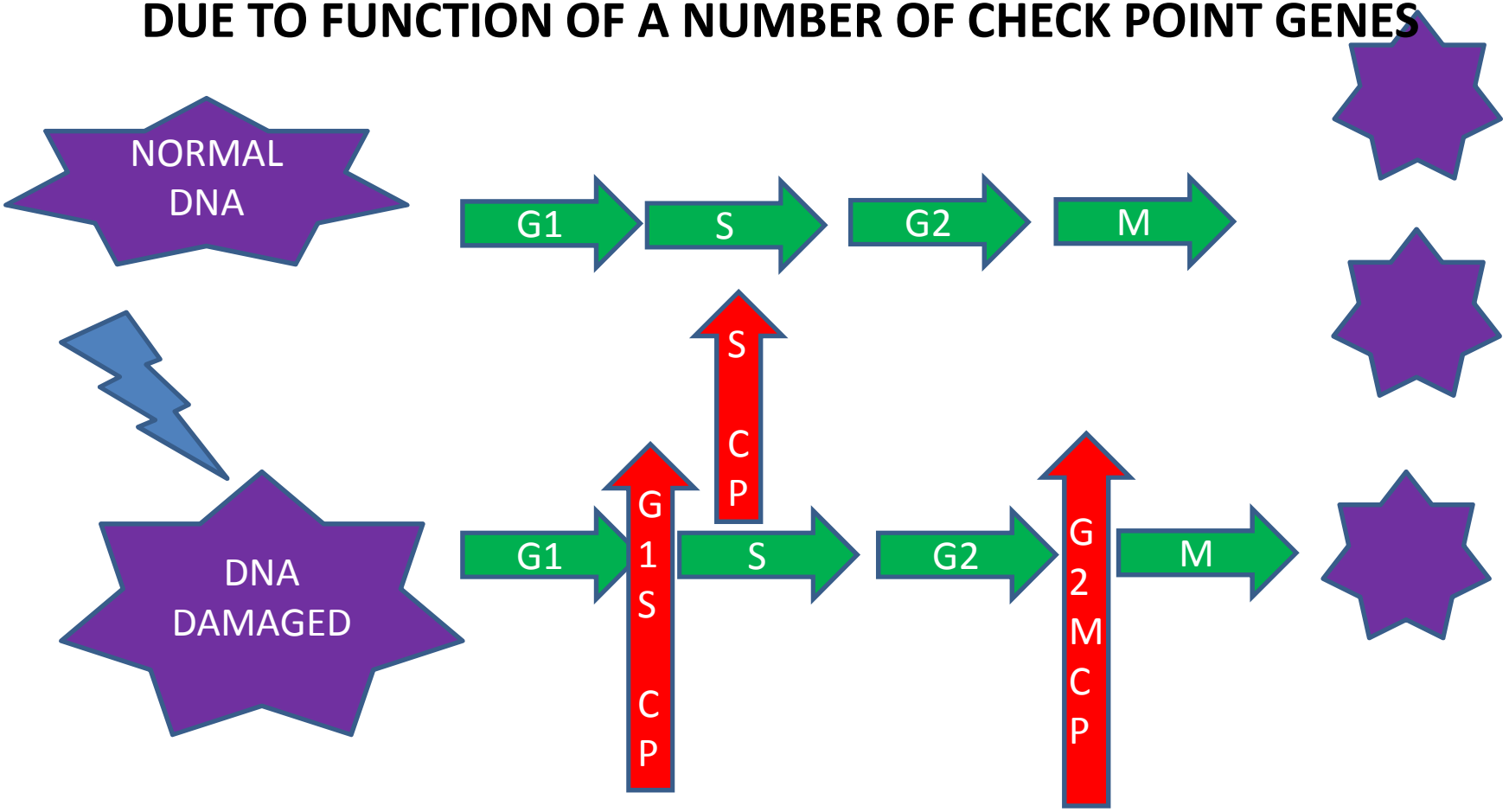
ACTIVATUION OF CASPASE 3,6,7

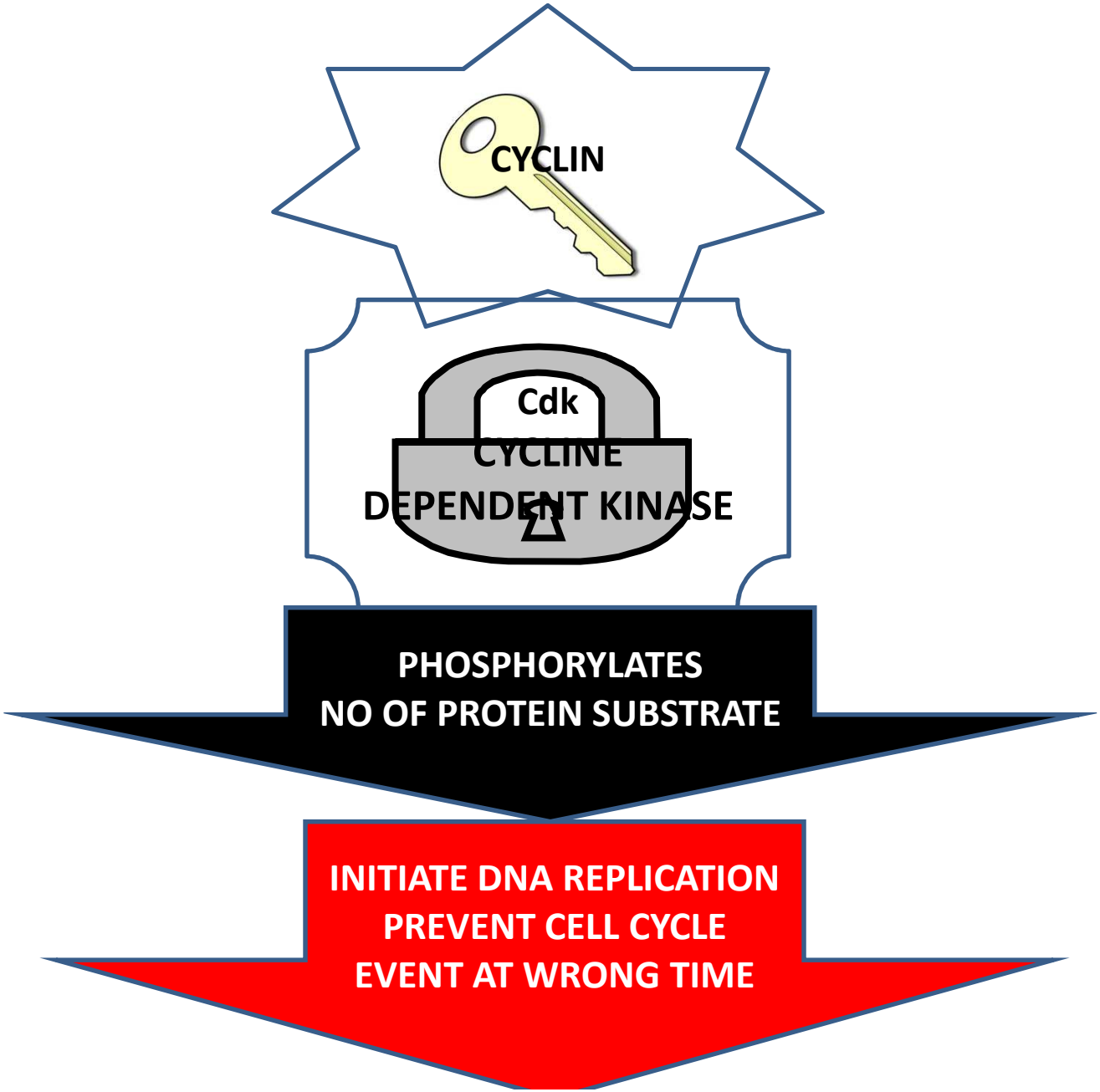
CYTOCHROME C

CELL DEFICIT IN SPHINGOMYELIN RESISTANT TO APOPTOSIS AFTER RT CERAMID

CHECK POINTS

EVENTS IN THE CELL CYCLE MUST TAKE PLACE IN SPECIFIC ORDER DUE TO FUNCTION OF A NUMBER OF CHECK POINT GENES




CYCLIN
Cdk
CYCLINE
DEPENDENT KINASE
PHOSPHORYLATES
NO OF PROTEIN SUBSTRATE
INITIATE DNA REPLICATION
PREVENT CELL CYCLE
EVENT AT WRONG TIME

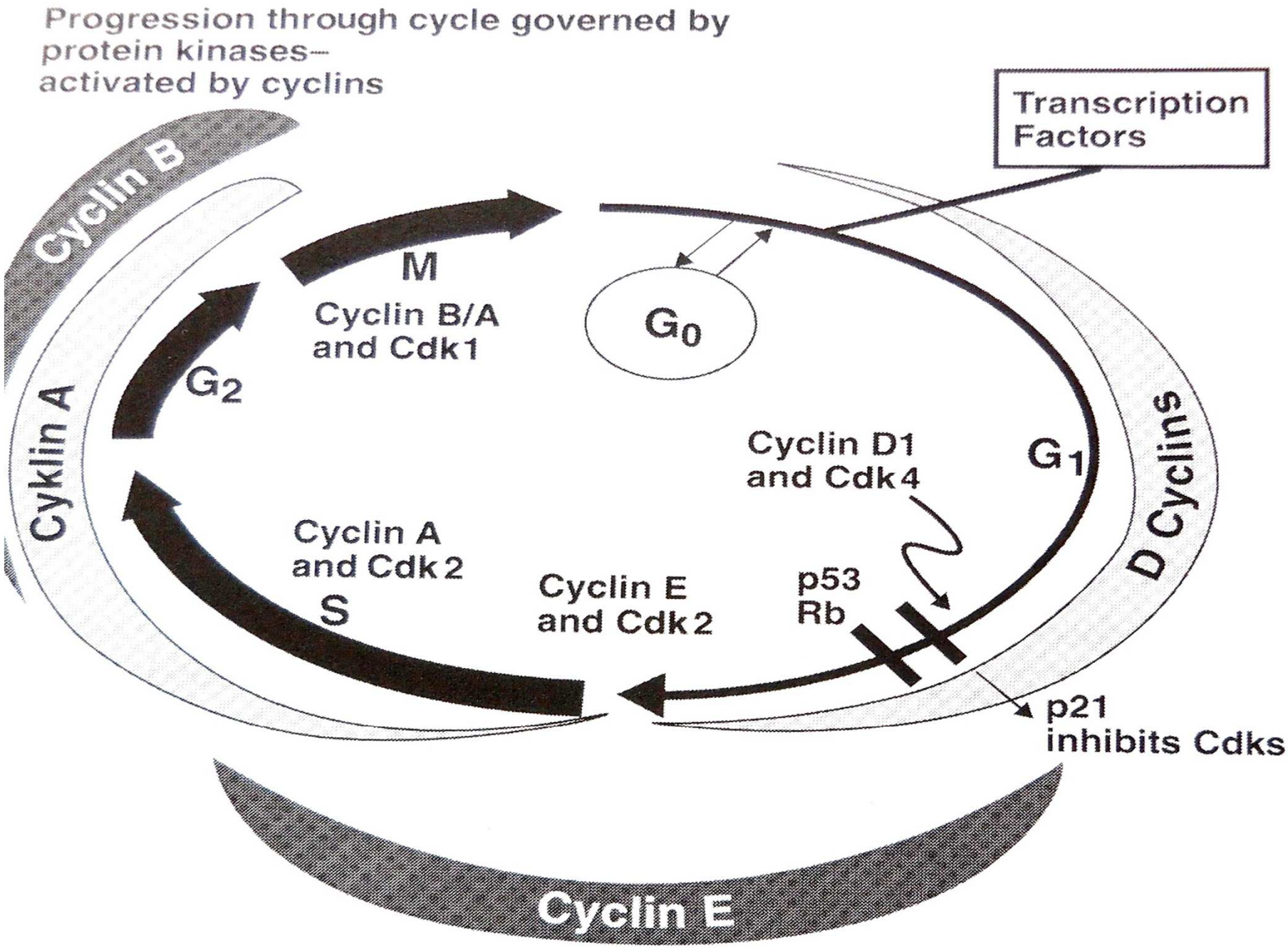
TYPE OF CYCLINS:-A,-H

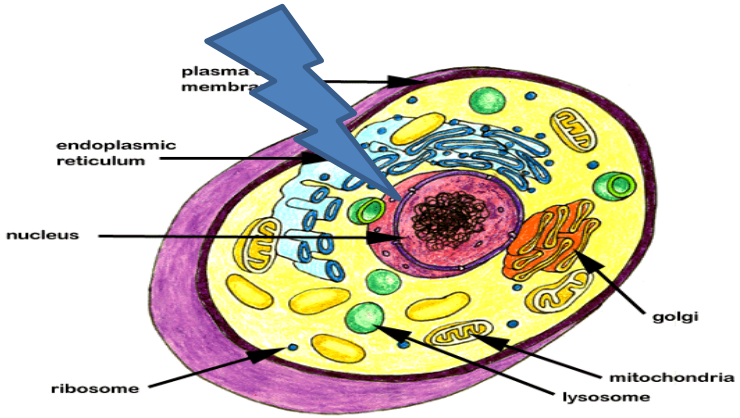
EACH CYCLIN SYNTHESIZES AT A PERTICULAR PHASE OF CELL CYCLE

G1:-cyclin D & E
S:-CYCLIN A
G2:-cyclin A
G2-M:- cyclin B

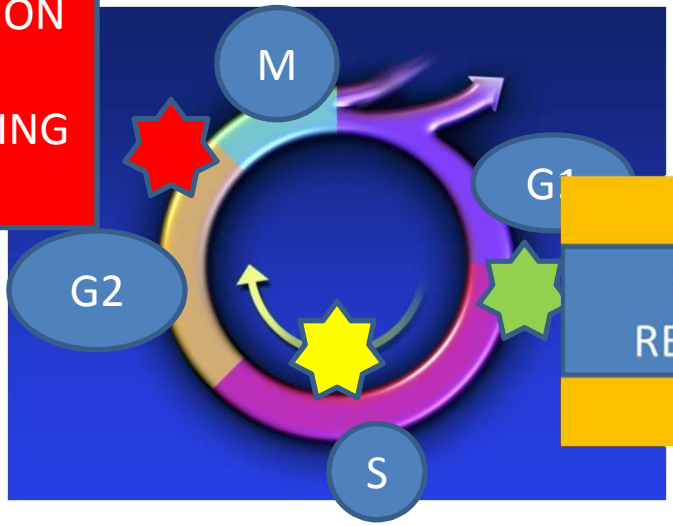
CYCLINE LEVEL OSCILLATE WITH THE PHASE OF CELL CYCLE

Cdk :- SEVEN TYPES AND LEVELS ARE CONSTANT THROUGH OUT THE CELL CYCLE.ACTIVITY OF CdK REGULATED BY ACTIVATING KINASES,CYCLIN REGULATORY SUBUNITS,Cdk INHIBITORS

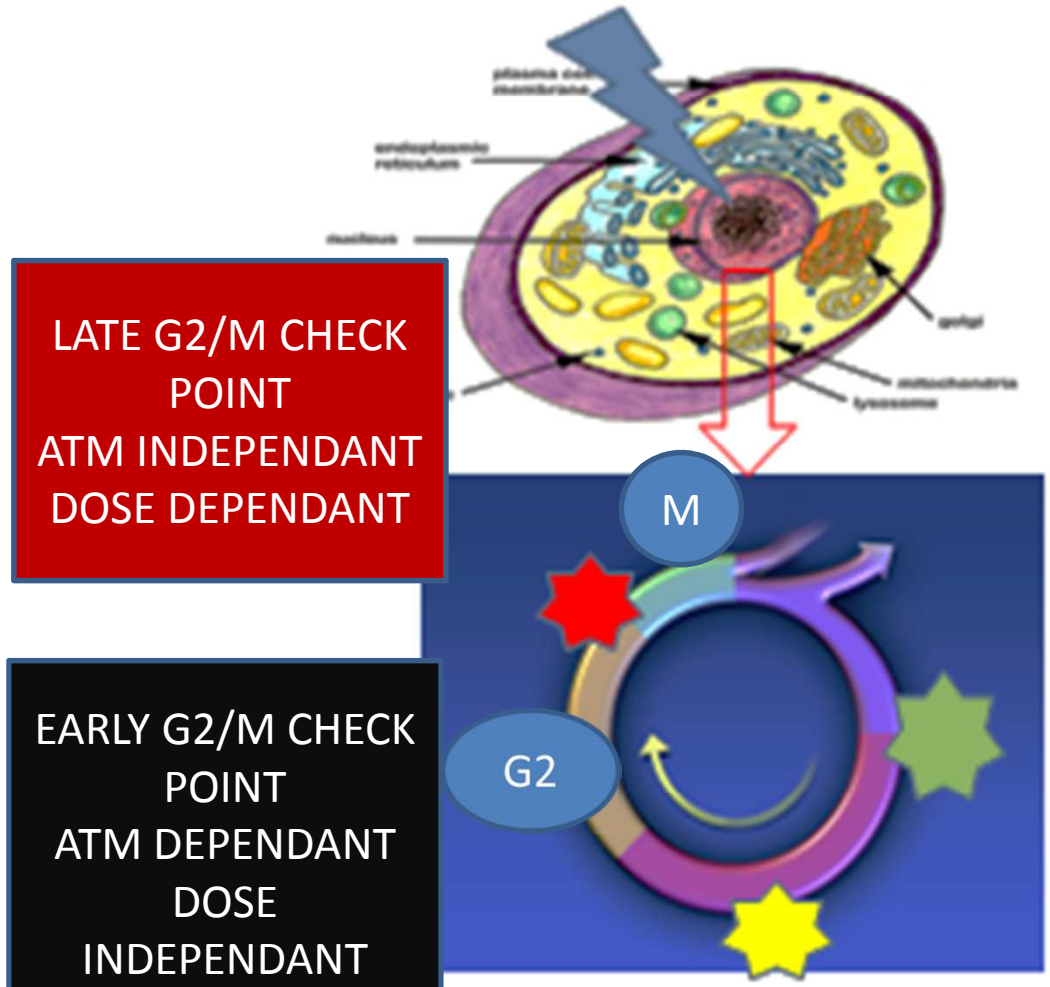

Progression through cycle governed by protein kinases– activated by cyclins
Transcription Factors
Cyclin B
M
Cyclin B/A and Cdk1
G0
G2
Cyklin A
Cyclin D1 and Cdk4
G1
D Cyclins
Cyclin A and Cdk2
S
Cyclin E and Cdk2
p53
Rb
p21 inhibits Cdks
Cyclin E

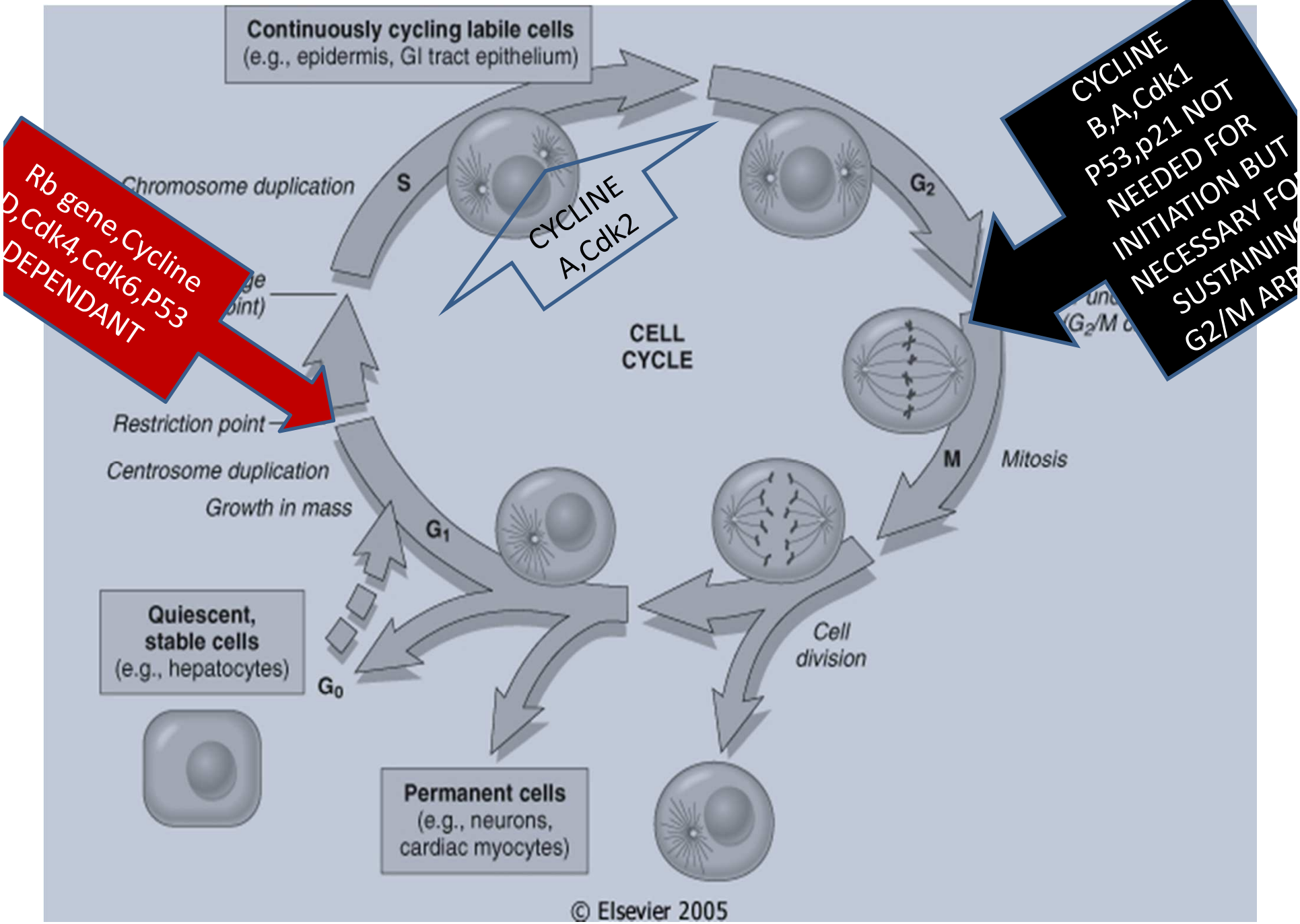


PREVENT SEGREGATION OF ABERRANT CHROMOSOME DURING M PHASE



PREVENT REPLICATION


LATE G2/M CHECK POINT
ATM INDEPENDANT
DOSE DEPENDANT
M
G2
EARLY G2/M CHECK POINT
ATM DEPENDANT
DOSE INDEPENDANT


Continuously cycling labile cells
(e.g., epidermis, GI tract epithelium)
Rb gene,Cycline D,Cdk4,Cdk6,P53 DEPENDANT
CYCLINE A,Cdk2
CYCLINE B,A,Cdk1 P53,p21 NOT NEEDED FOR INITIATION BUT NECESSARY FO SUSTAININ G2/M ARR
Chromosome duplication
S
G2
CELL CYCLE
Restriction point
Centrosome duplication
Growth in mass
G1
M
Mitosis
Cell division
Quiescent, stable cells
(e.g., hepatocytes)
G0
Permanent cells
(e.g., neurons, cardiac myocytes)
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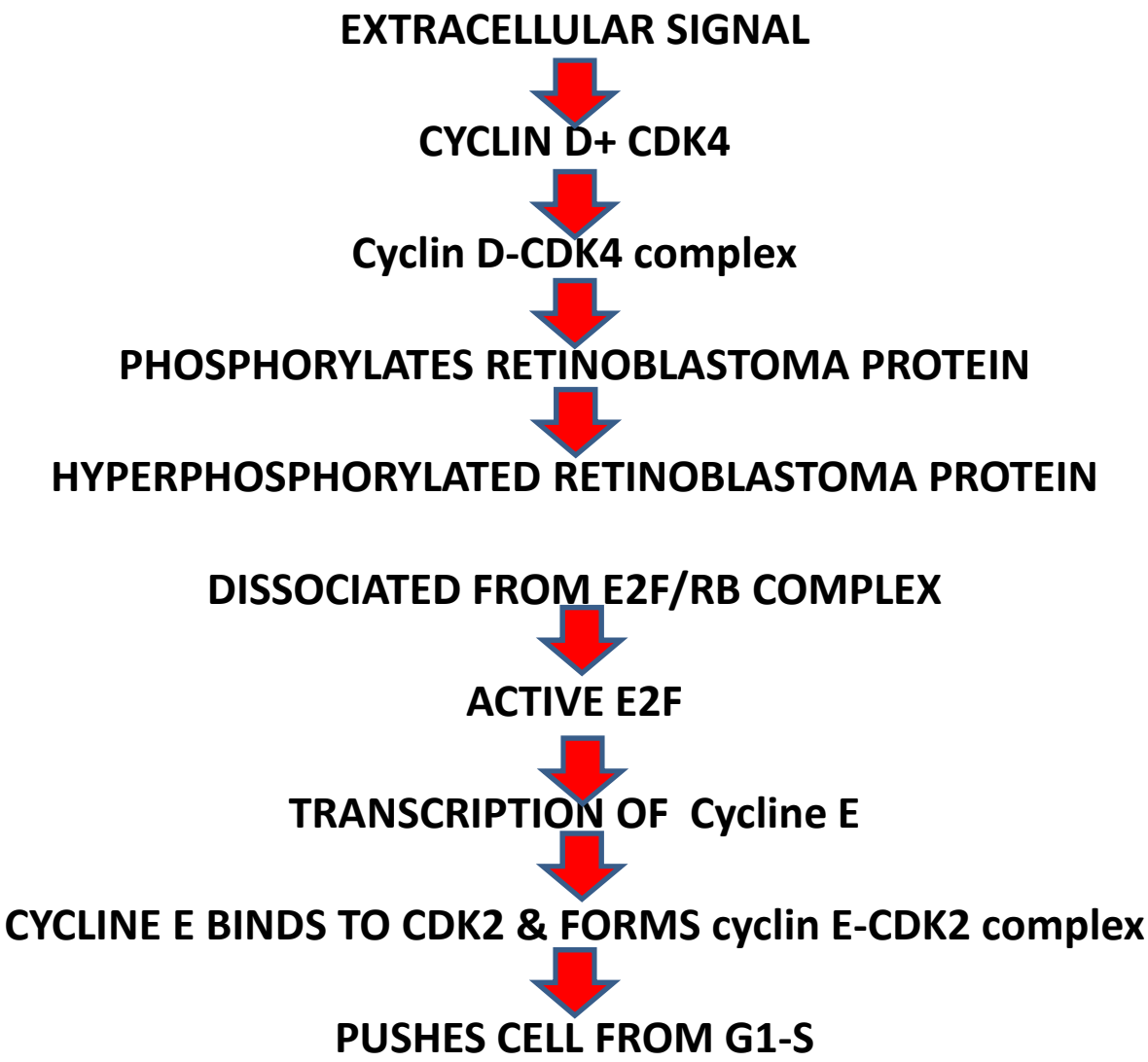
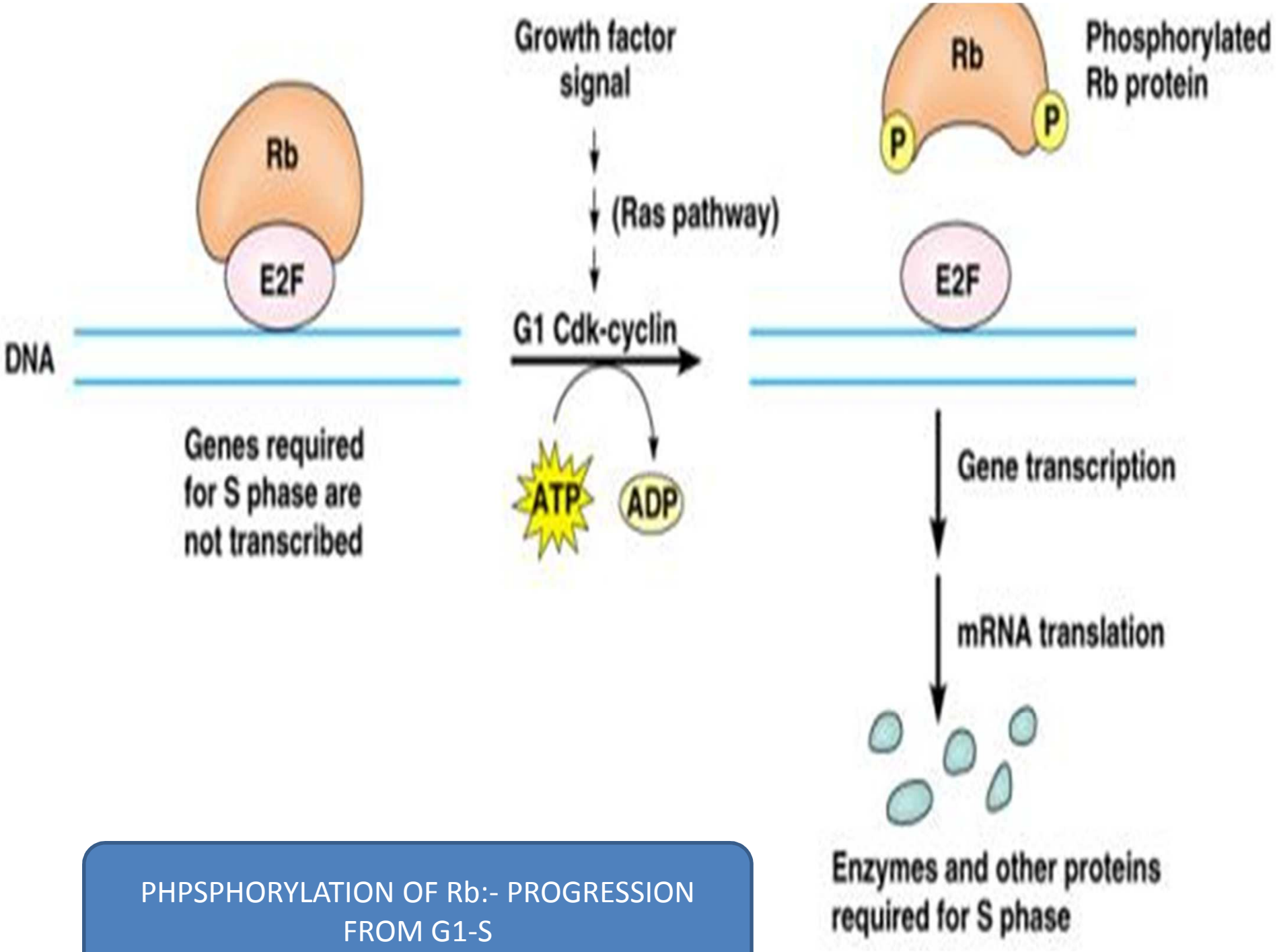
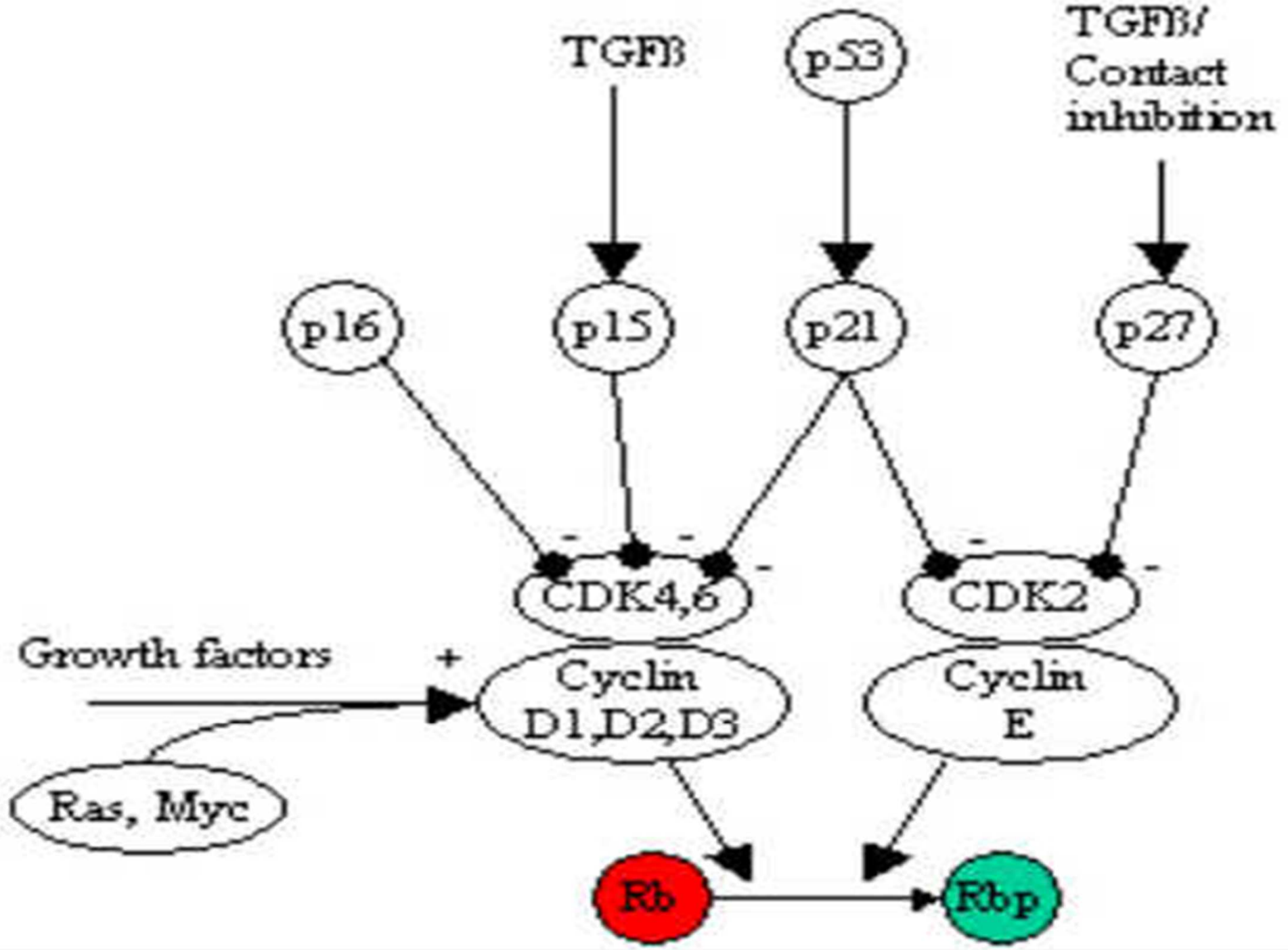

EXTRACELLULAR SIGNAL
CYCLIN D+ CDK4
Cyclin D-CDK4 complex
PHOSPHORYLATES RETINOBLASTOMA PROTEIN
HYPERPHOSPHORYLATED RETINOBLASTOMA PROTEIN
DISSOCIATED FROM E2F/RB COMPLEX
ACTIVE E2F
TRANSCRIPTION OF Cycline E
CYCLINE E BINDS TO CDK2 & FORMS cyclin E-CDK2 complex
PUSHES CELL FROM G1-S



PHPSPHORYLATION OF Rb:- PROGRESSION FROM G1-S


TGFβ
p53
TGFβ/
Contact
inhibition
p16
p15
p21
p27
CDK4,6
CDK2
Growth factors
+
Cyclin
D1,D2,D3
Cyclin
E
Ras, Myc
Rb
Rbp

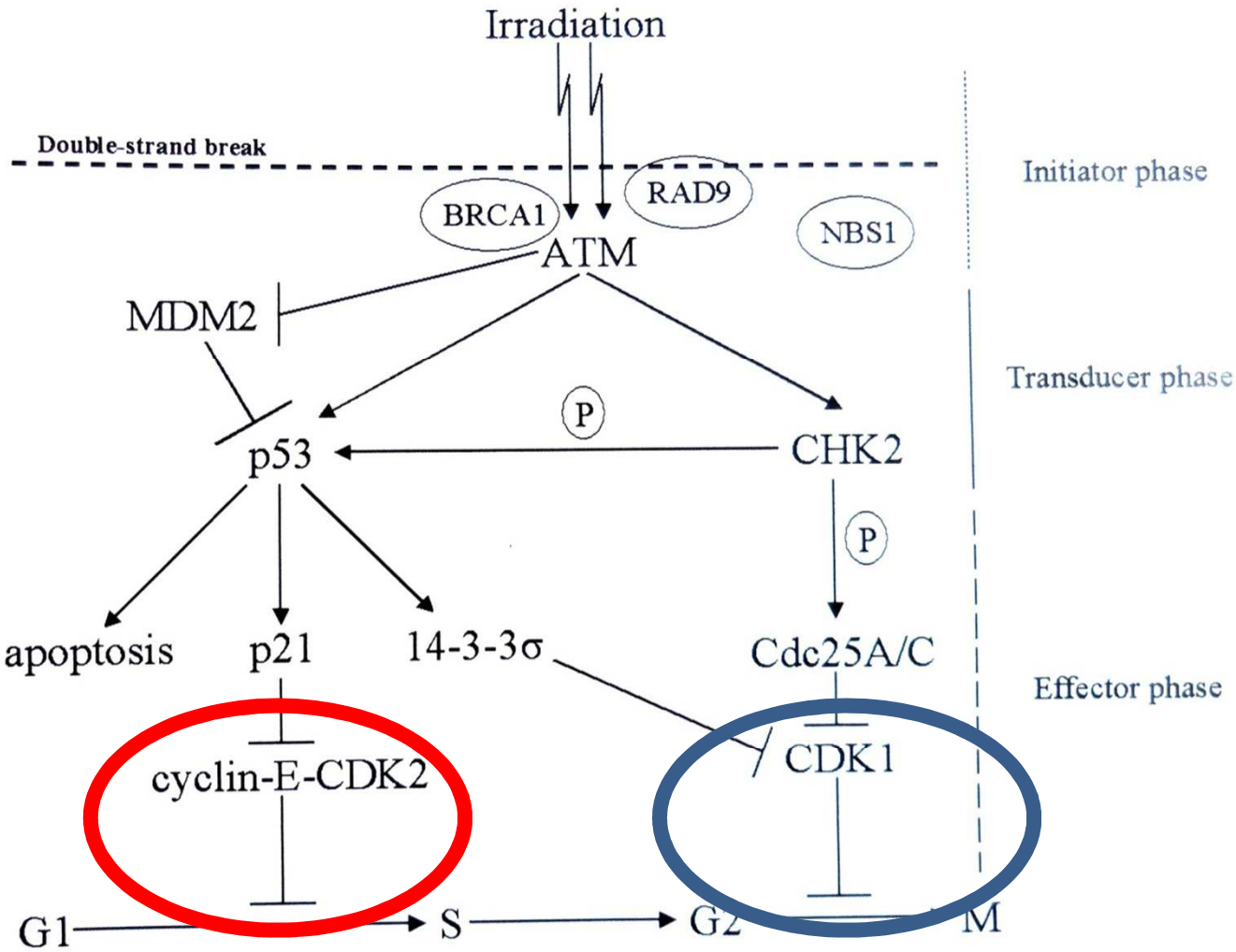
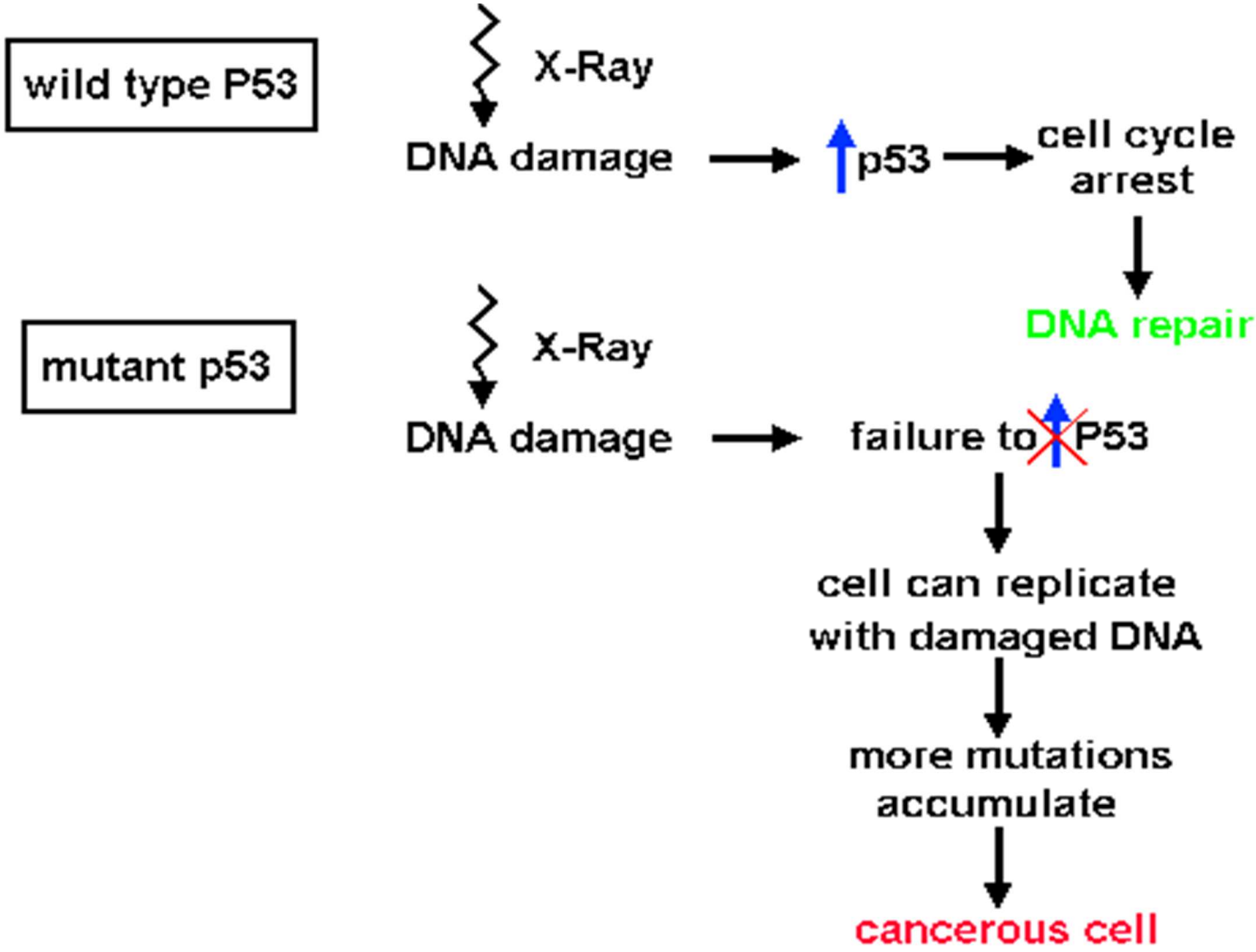


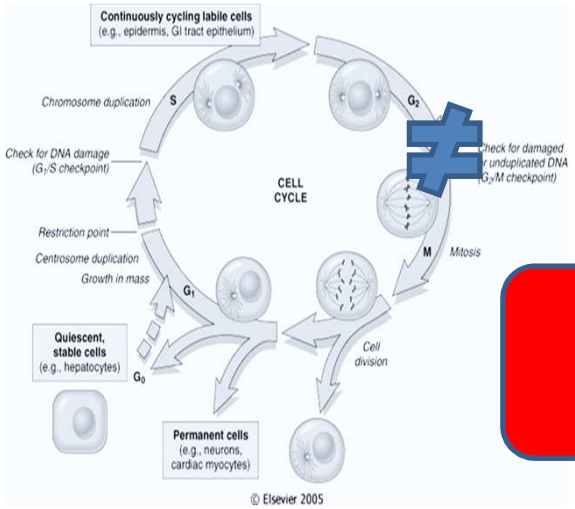
Fig. 2. Ionizing radiation rapidly induces the protein kinase activity of the *ATM* gene, which in turn interacts wit broad network of proteins to block progression through the cell cycle, allowing time for DNA repair. ATM activ both p53 and CHK2, leading to either a G_1/S or G_2/M cell cycle block, depending on interactions with downstre target genes. Adapted from Samuel T, Weber HO, Funk JO. Linking DNA damage to cell cycle checkpoints. *Cell C*


wild type P53
X-Ray
DNA damage
p53
cell cycle arrest
DNA repair
mutant p53
X-Ray
DNA damage
failure to P53
cell can replicate with damaged DNA
more mutations accumulate
cancerous cell

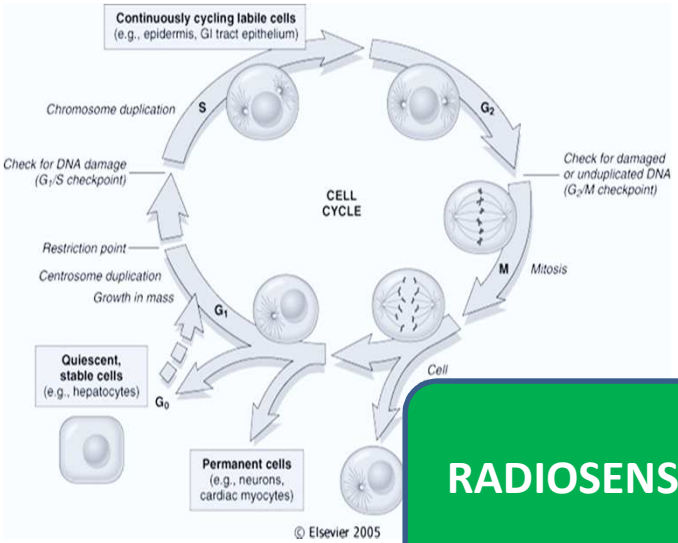
G2/M CHECK POINT

MOST REGULATED OF ALL CHECKPOINTS AND MOST IMPORTANT IN PREVENTING IN APPROPRIATE ENTRY OF DAMAGED CELL IN TO MITOSIS

CELLS LACKING G2 CHECK POINT ARE RADIOSENSITIVE AS THEY CAN NOT REPAIR DAMAGED CHROMOSOME BEFORE ENTERING M



NORMAL



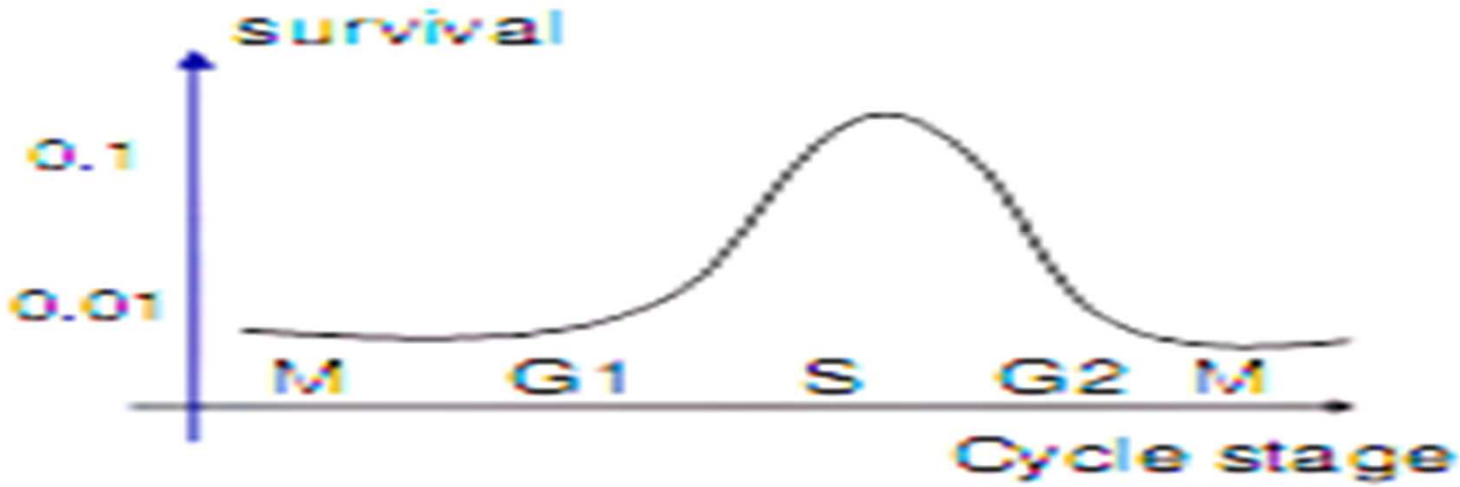
RADIOSENSITIVE

CLINICAL IMPORTANCE

- CELL AGE
- REASSORTMENT
- LET VS CELL AGE
- CHEMORADIATION
- DAMAGE THE CHECK POINTS

AGE RESPONSE

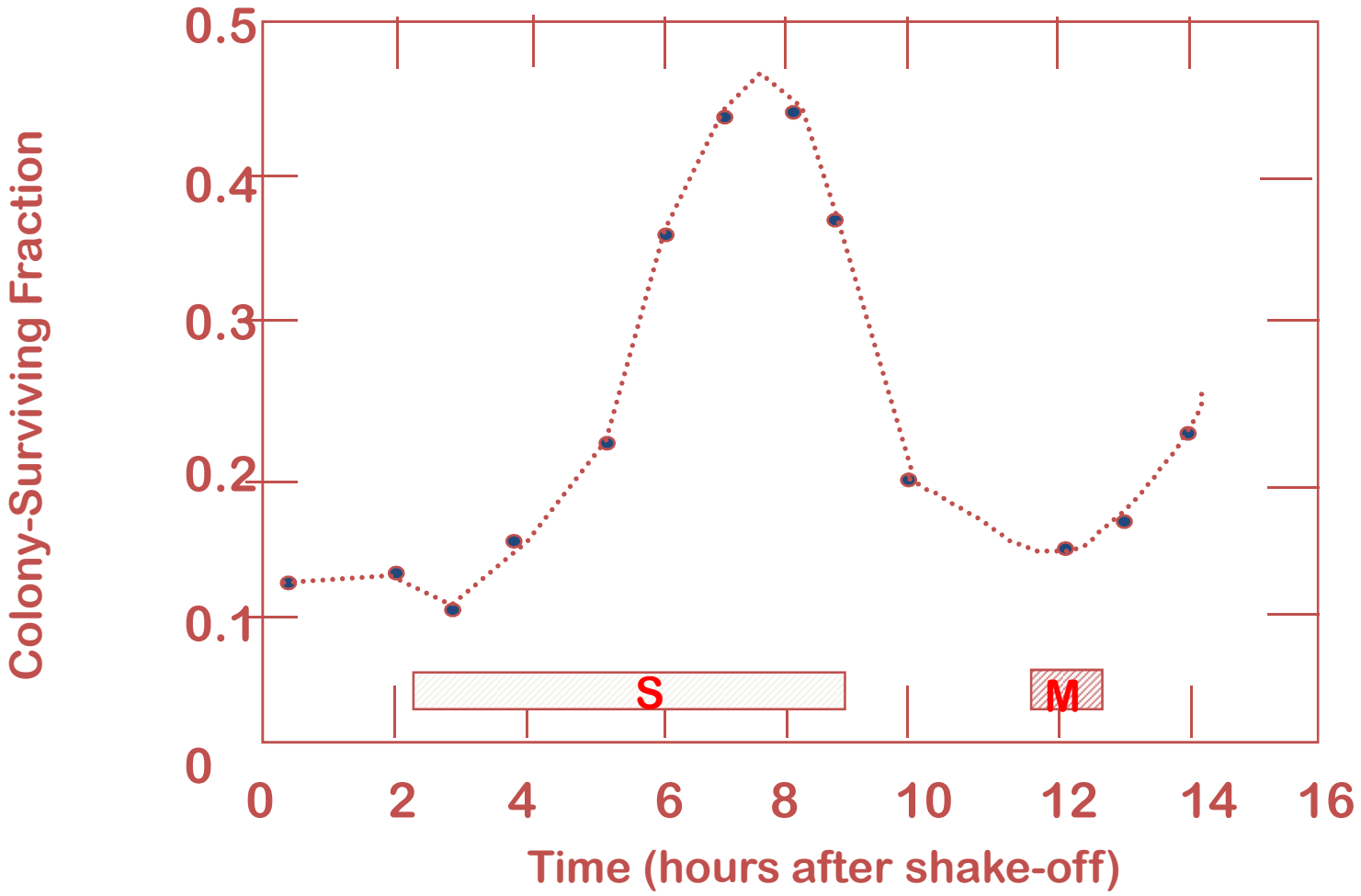
CHANGING RADIOSENSITIVITY DURING THE CELL CYCLE IS OFTEN CALLED AGE RESPONSE



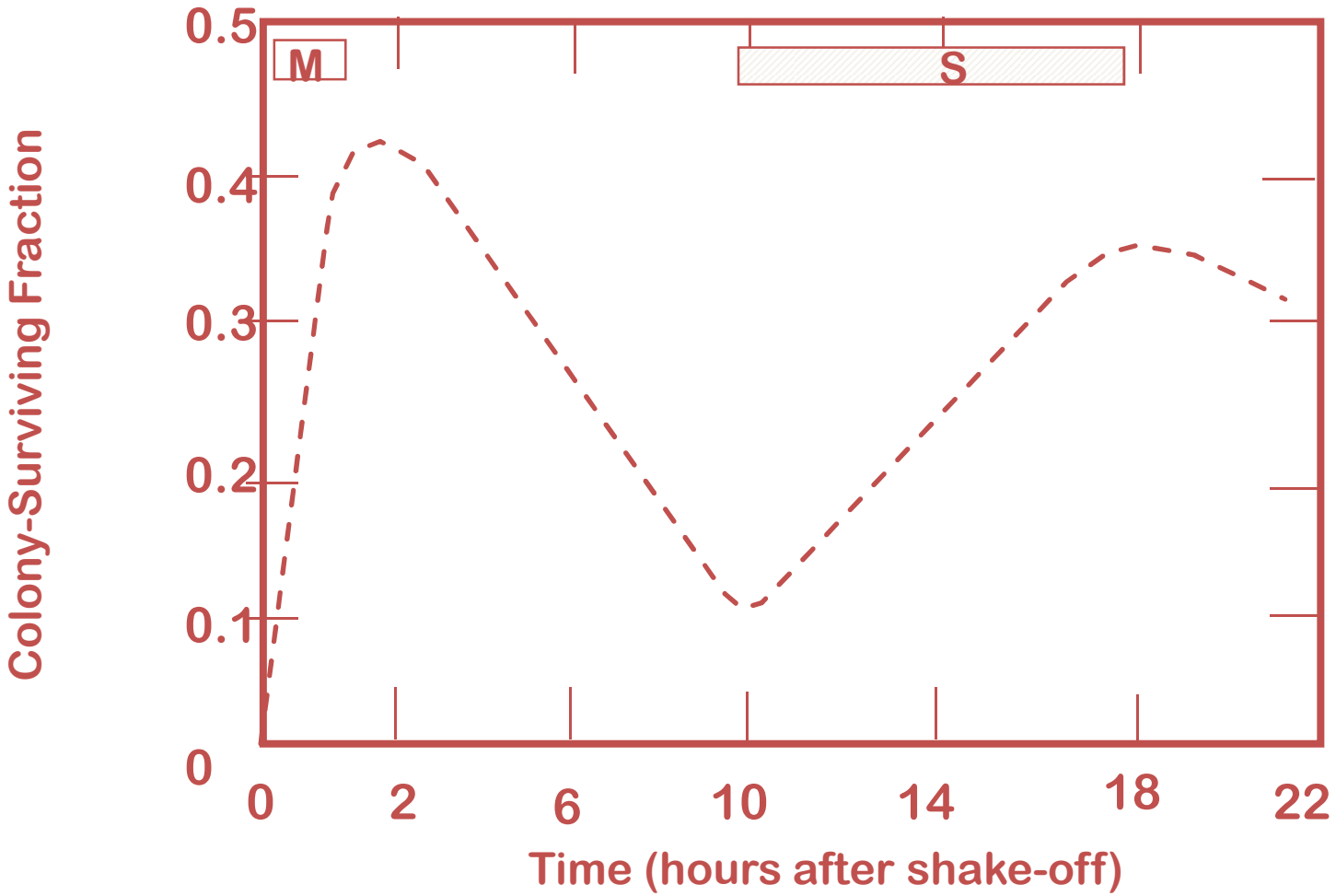
CELL CYCLE AND SENSITIVITY TO RADIATION

- **CELLS ARE MOST SENSITIVE AT OR CLOSE TO MITOSIS**
- **RESISTANCE IS GREATEST IN LATER PART OF S PHASE**
- **IF G1 PERIOD IS PROLONGED,A RESISTANCE PERIOD IS EVIDENT AT EARLY IN G1,FOLLOWED BY A SENSITIVE PERIOD TOWARDS THE END OF G1.**
- **G2 PHASE IS USUALLY SENSITIVE,PERHAPS AS SENSITIVE AS M PHASE**

Synchronously Dividing Chinese Hamster Cell



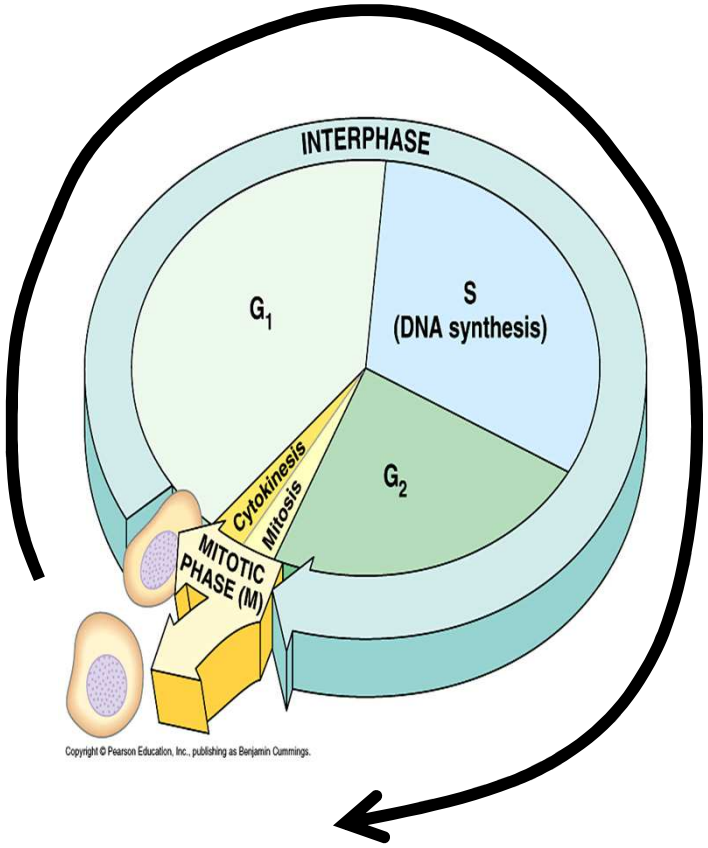
Surviving Fraction of HeLa Cells



WHY THE CHANGE IN SENSITIVTY

- CHANGE IN AMOUNT OR FORM OF DNA RESULT IN VARIATION OF SENSITIVITY.IN S PHASE DNA CONTENT IS DOUBLED AND CHROMOSOME MATERIAL CONDENSES IN TO DISCRETE ENTITIES.
- CHANGING LEVEL OF NATURALLY OCCURING SH GROUP
- NHEJ(Non homologous end joining is primarily found in G1 PHASE OF CELL CYCLEWHERE THERE IS NO SISTER CHROMATID
- IN LATE S/G2 ,UNDAMAGED CHROMATID ACTS AS A TEMPLATE FOR REPAIR i.e HOMOLOGOUS RECOMBINATION REPAIR TO REPAIR DOUBLE STRAND BREAK.
- SO G1 CELLS ARE MORE SENSITIVE DUE TO NHEJ AN ERROR PRONE REPAIR AND S PHASE CELLS ARE RADIORESISTANCE DUE TO ERROR FREE HRR PROCESS.
- ALSO DEPENDS ON EFFICENCY OF REPAIR PROCESS

Redistribution or Reassortment

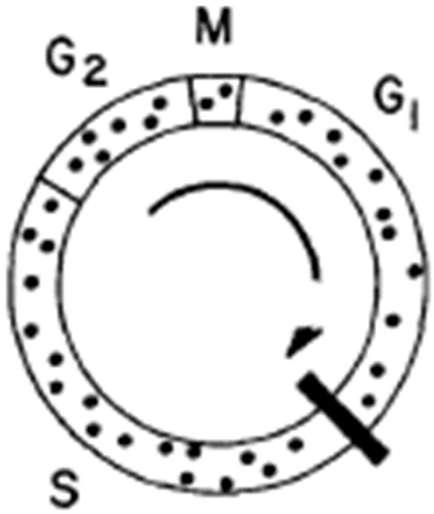


During fractionation of RT, cells in sensitive phase are killed and before next fraction, cells progress through cell cycle and again come to sensitive phase.This process is known as

REDISTRIBUTION/ REASSORTMENT.

Redistribution or Reassortment

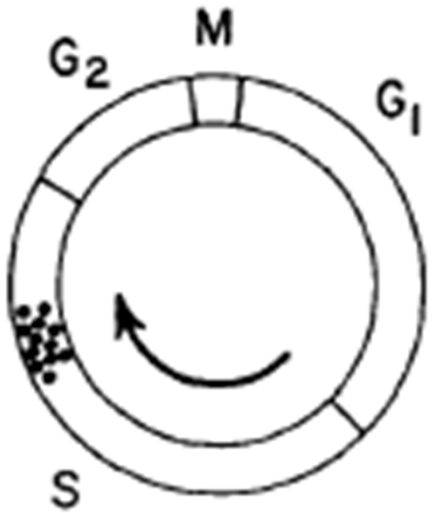
- Asynchronization:-
  - The dividing cells are distributed throughout all phases of cell cycle known as asynchronization.



Redistribution or Reassortment

- Synchronization:-

  When If all the dividing cells occupy the same phase of the cell cycle and then all cells progress through various phases of cell cycle simultaneously.



Hydroxyurea kills all the cells in S-phase and blocks the cells at the end of G1-phase.

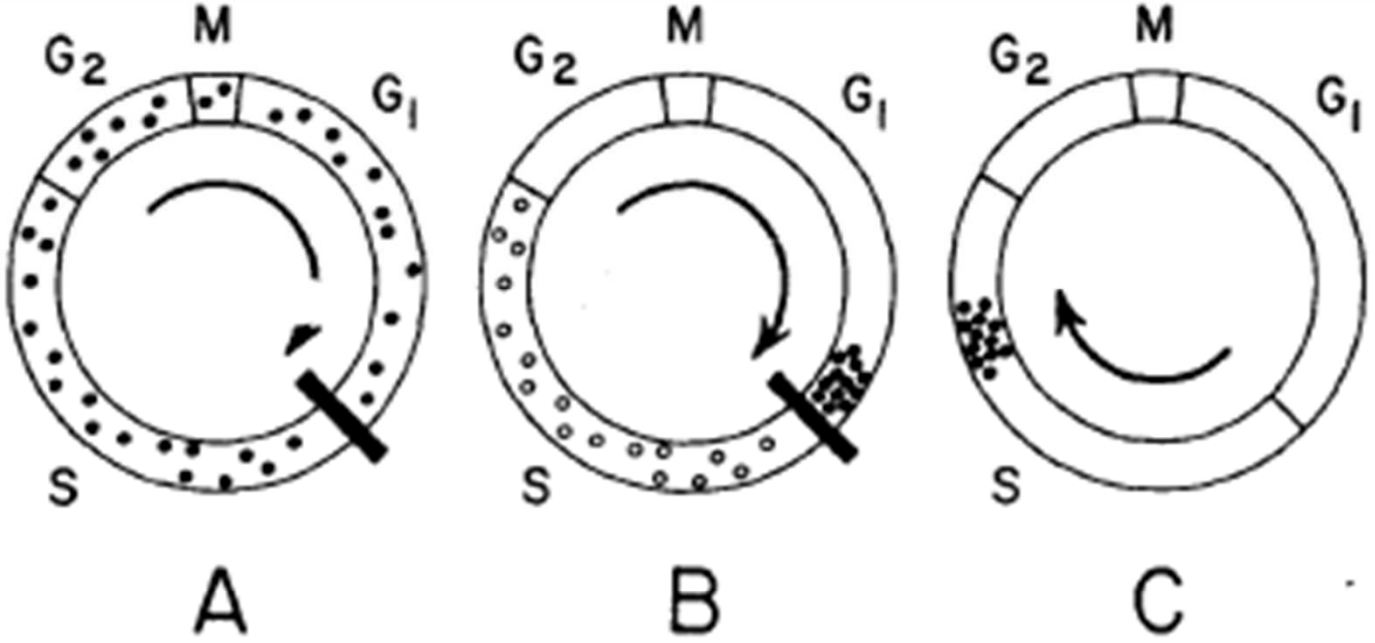


Figure 5–4. Mode of action of hydroxyurea as an agent to induce synchrony. This drug kills cells in *S* and imposes a "block" at the end of G_1. Cells in G_2, *M*, and G_1 when the drug is added accumulate at this block. When the block is removed, the synchronized cohort of cells moves on through the cycle.

Redistribution or Reassortment

- So by making the cells in synchronization and knowing the time when they pass through G2, M phase, which is the most sensitive phase to radiation, we may achieve maximum kill by scheduling fractionation accordingly.

EFFECT OF CELL SYNCHRONY ON RADIOSENSITIVITY

- **Cells are more sensitive to radiation during mitosis and in G2 phase, least sensitivity in S phase.**
- **Thus the concept of synchronizing tumor cells in a phase of the cell cycle that is sensitive to radiation was recognized as a potentially important way to enhance the clinical efficacy of RT**
- **Redistribution – split / # doses of RT may be more efficacious in part, by inducing a transient cell cycle arrest, after which second RT # is administered.**

SYNCHRONISED TUMOR CELLS IN A PHASE OF THE CELL CYCLE THAT IS SENSITIVE TO RADIATION WAS RECOGNISED AS A POTENTIALLY IMPORTANT WAY TO ENHANCE THE CLINICAL EFFICACY OF RADIATION

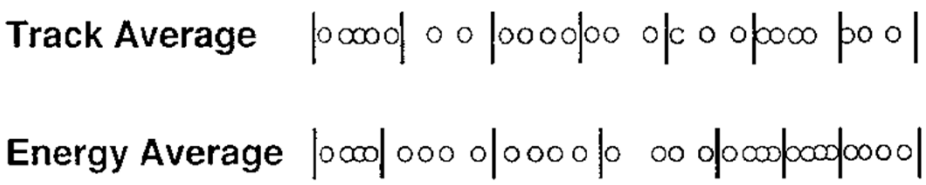
- **Therapeutic application of synchronization has certain limitations**
  - **Timing of synchronization varies in different cell lines and that optimal synchronization is, therefore, seldom achieved.**
  - **Synchrony of cell populations is difficult to maintain and many human tumors display a kinetic heterogeneity even after synchronization.**
  - **Synchronization of cell populations before each RT fraction would be cumbersome and difficult to achieve.**

Energy Deposition

- Low-LET (sparsely ionizing radiation)
  - x-rays
  - gamma
  - betas (higher energy)
- High-LET (densely ionizing radiation)
  - alphas
  - betas (lower energy)
  - protons
  - neutrons

Linear Energy Transfer (LET)

- LET is the average energy locally imparted (deposited) per unit track length (keV/μm)
- Different than "stopping power" (energy loss)
- Track averaged vs energy averaged



Some typical values

RADIATION	LET (keV/μm)	
Cobalt-60 γ-rays	0.2	
250-kV x-rays	2.0	
10-MeV protons	4.7	
150-MeV protons	0.5	
	Track Avg.	Energy Avg.
14-MeV neutrons	12	100
2.5-MeV α-particles	166	
2-GeV Fe ions	1000	

LET Vs CELL CYCLE

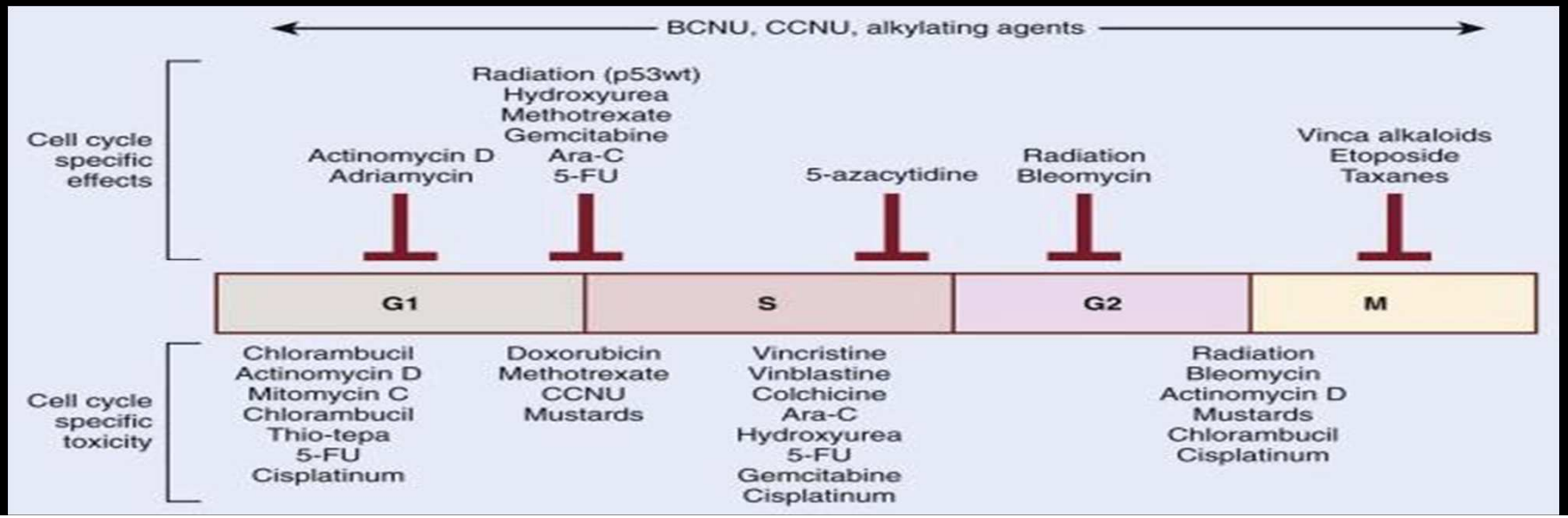
- **AS LET INCREASES THE VARIATION IN RADIOSENSITIVITY THROUGH CELL CYCLE DECREASES.**
- **AT VERY HIGH LET THE AGE RESPONSE FUNCTION IS ALMOST FLAT I.e RADIOSENSITIVITY VARIES LITTLE WITH THE PHASE OF CELL CYCLE.**

Chemoradiation
Mechanism of Interaction

- **Increase in initial radiation damage**
- **Inhibition of cellular repair**
- **Cell cycle effects**
  - **Elimination of radioresistant S phase cells**
  - **Arrest in radiosensitive G2 or M**
- **Counteracting radioresistance caused by hypoxic cells**
  - **Elimination of hypoxic cells**
  - **Increase in tumor oxygenation through cell loss**
- **Overcoming accelerated repopulation**

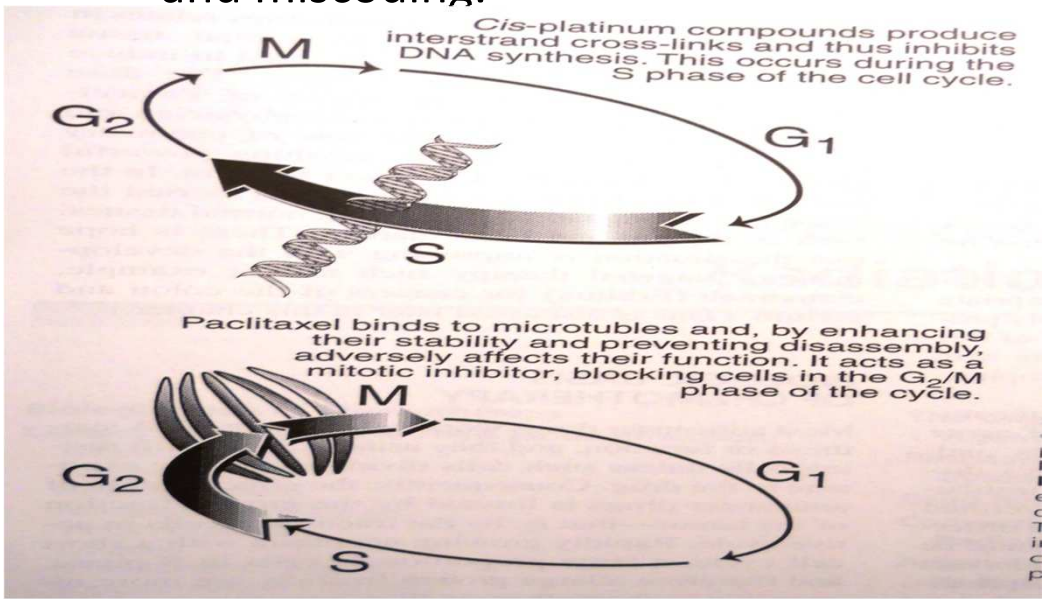
KINETICALLY BASED ADMINISTRATION OF CT & RT

- **Different types of cytotoxic drugs arrests cells at specific checkpoint**
  - **Vinca alkaloids arrest cells in S phase**
  - **Taxol blocks cells in radiosensitive G2M phase**
  - **Flavopiridol (CDKI) accumalates cells in G1 & G2 phase**
  - **5-fu increases RT sensitivity by killing cells in S phase**
  - **Gemcitabine acts on cells in S phase**



CISPLATINUM

- COMMON DRUG USED AS RADIOSENSITIZERS
- Reacts with cellular DNA to form intrastrand and interstrand crosslinks producing its characterstic biologic effect.
- Inhibition of DNA replication and RNA transcription leading to DNA breaks and miscoding.



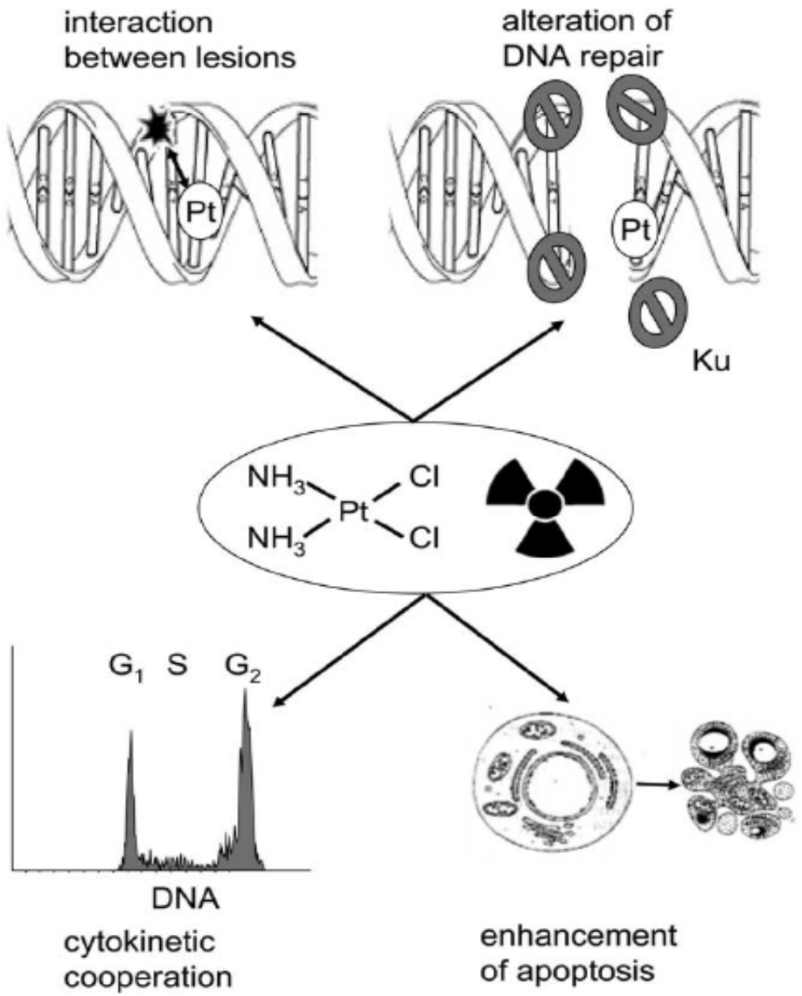
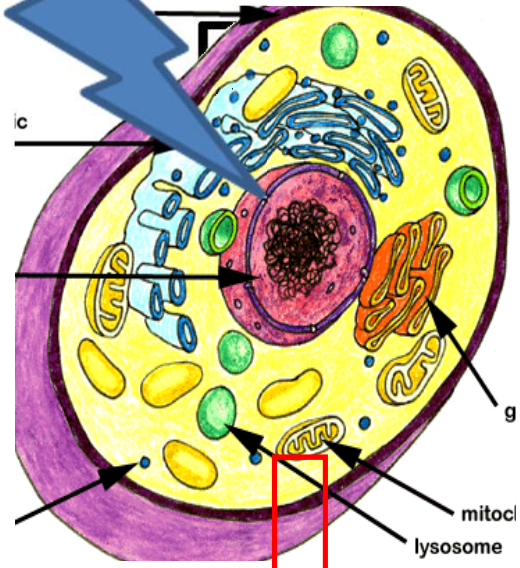


Figure 2 Interactions of cisplatin with radiation.

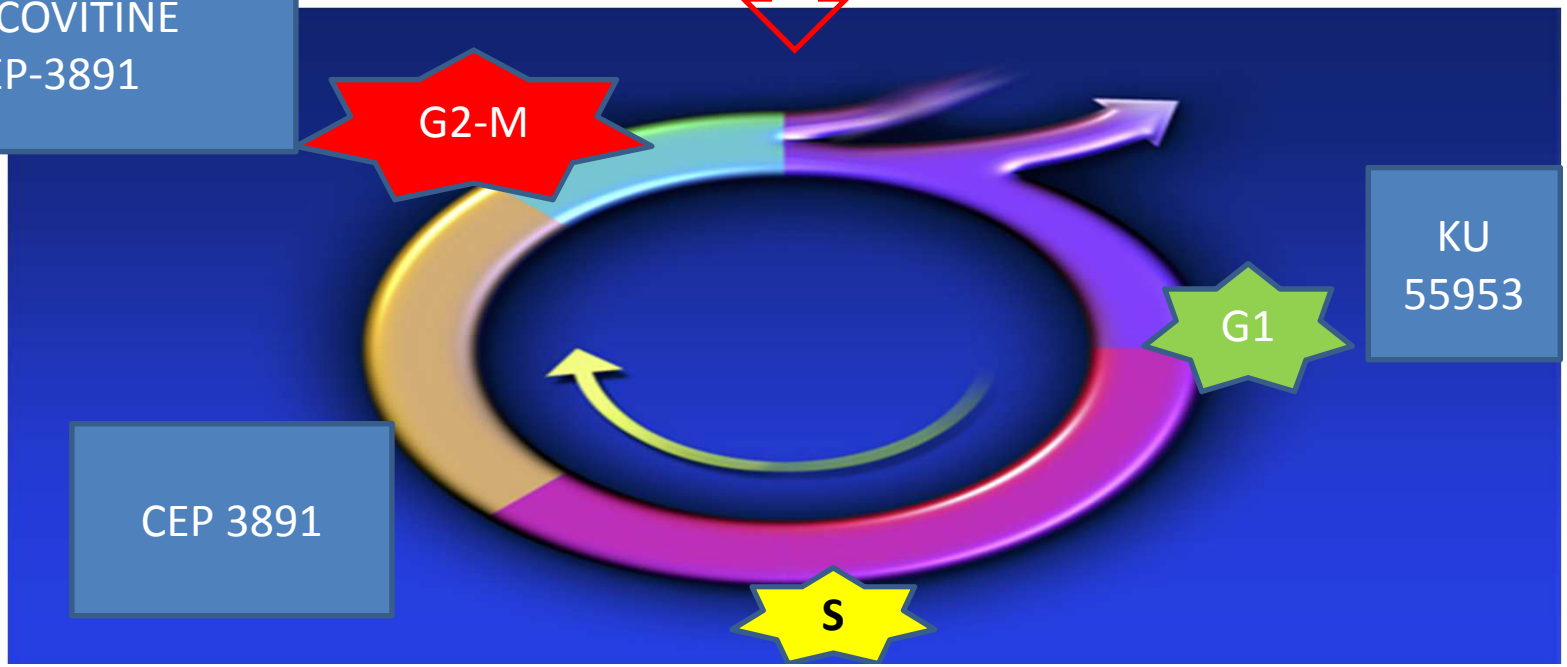
Molecular Checkpoint Genes

- Cellular progression through cycle is controlled by checkpoint genes
  - to ensure completion of events prior to progression
  - at G_2, cells are halted to inventory & repair damage before mitosis
  - cells where checkpoint gene is inactivated ...
    - move directly to mitosis, even with damaged chromosomes
    - are more sensitive to UV or ionizing radiation (or any DNA damaging agent)

CHEMICAL INHIBITORS OF CHK POINT



UCN-O1
FLAVOPIRIDOL
ROSCOVITINE
CEP-3891



KU 55953

CEP 3891

Table 1 Evidence for Radiosensitization Using Chemical Inhibitors of Cell-Cycle Checkpoint Control, DNA Repair, and the Proteasome

Agent	Target/Action	Evidence for Radiosensitization
Checkpoint inhibitors		
UCN-01	CHK1; inhibits G2 checkpoint	In vitro: sensitizes colon, breast, lymphoma, cervix, lung cancer cell lines In vivo: synergistic growth delay in murine fibrosarcoma following fractionated radiotherapy in vivo; clinical trials ongoing
Flavopiridol	Non-specific for CDK; inhibits G2 checkpoint and DNA-dsb repair	In vitro: sensitizes esophageal and colon cancer cell lines In vivo: increased growth delay in murine tumors and colon cancer xenografts; clinical trials ongoing
Roscovitine(CYC-202)	CDK1/CDK2/CDK5; inhibits DNA-dsb repair	In vitro: sensitizes breast cancer cell lines In vivo: increased growth delay in breast cancer xenografts; clinical trials ongoing
CEP-3891	CHK1; inhibits S and G2 checkpoint	In vitro: sensitized osteosarcoma cells
KU55953	ATM; inhibits G1 and G2 checkpoints and DNA-dsb repair	In vitro: sensitized HeLa cells
AdenoWTp53	p53; inhibits cell cycle arrests and DNA-dsb repair and induces apoptosis	In vitro: sensitizes brain, lung, prostate, and head and neck cancer cell lines In vivo: complete and partial clinical responses in lung cancer; clinical trials ongoing
ONYX-015	p53; targets MTp53-expressing tumors through cell cycle arrest and apoptosis	In vitro: sensitizes colon and thyroid cancer cell lines In vivo: sensitized glioma xenografts in vivo; clinical trials ongoing
DNA repair inhibitors		
Vanillin, SU1172, IC886, NU7163, NU7026	DNA-PKcs and NHEJ; inhibits DNA-dsb repair	In vitro: sensitizes ovarian, HeLa, glioma, CHO, and fibroblast cells
Imatinib	c-ABL; inhibits RAD51/HR and DNA-dsb repair	In vitro: sensitizes leukemic and glioma cells
3-AB, ISQ, NU1025, KU0058684 or AG14361	PARP; inhibits DNA-ssb repair and homologous recombination	In vitro: sensitizes prostate, lung, and lymphoma cancer cell lines In vivo: AG14361 sensitized colon cancer xenografts
Proteasome inhibitors		
PS-341 (bortezomib)	26S proteasome; altered apoptosis, cell cycle arrest and signal tranduction	In vitro: sensitizes melanoma, lymphoma, colon cancer cell lines In vivo: sensitized colon cancer xenografts; clinical trials ongoing
Ritonavir, saquinavir	26 proteasome; HIV protease	In vitro: sensitizes glioma and prostate cancer cell lines

Abbreviations: NHEJ, nonhomologous end-joining; HR, homologous recombination; PARP, poly(ADP-ribose) polymerase-1; HIV, human immunodeficiency virus; DNA-dsb, DNA double-strand break; MTp53, mutant p53. Data from the following references.[1,2,7,8,10,11,13,14,16,18,28,31,32,37,41,44,48]

Take home message:-1

- Cell death occurs only after cell attempt to divide.
- Less differentiated cell having greater proliferative capacity →More radiosensitive.
- According to Casaretts classification, 4 types of cells depends on position of cells in cell cycle and sensitivity accordingly.
- In eukaryocyte, there is resting phase, Interphase and Mitotic phase.
- Interphase consists of G_1, S, G_2 .

  Mitotic phase – Karyokinesis and cytokinesis.

Take home message :- 2

- Time between successive division of cells is known as Mitotic cycle time.
- Difference in cell cycle time is mostly due to variation in length of G_1 period.
- DNA break may be single stranded/double stranded.
- NHEJ mostly occurs at G_1 and HRR occurs in S and early G_2 phase. NHEJ is error prone.
- Radiation induced cell death can be either necrotic death/ Apoptotic cell death.
- 3 check points-G_1-S, S , G_2M.

Take home message:-3

- Cyclin+ CDK is the key of checkpoint.
- G_1S checkpoint prevent replication of DNA and G_2M check point prevent segregation of aberrant chromosome during M phase.
- G_1S checkpoint depends on Rb gene and G_2M on CHK2. p53-p21 needed for sustaining G_2M arrest.
- Phosphorylation of Rb – progression from G_1-S.
- Changing radiosensitivity during the cell cycle is called age response.
- Cells are more sensitive at S1 close to mitosis and resistance in later part of S phase.
- Making cells synchronise and knowing G_2M phase, we may achieve maximum cell kill.
- As LET increases, the age effect is lost.
- Kinetically based administration CT and RT and prevention of check points are various option.

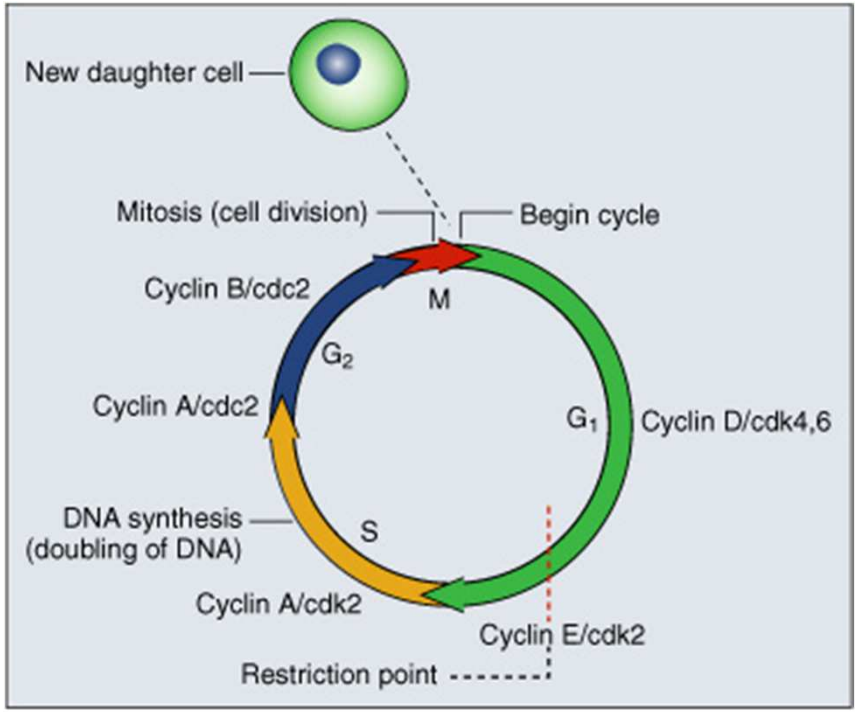
PHASE OF CELL CYCLE	CYCLIN	Cdk	GENE	
G1	D,E,A	Cdk4,Cdk6		D-GROWTH FACTOR SENSOR
G1/S TRANSITION	E	Cdk2,	Rb:-DECREASE GO TO S P53,p21:-DECREASE GO TO S	ARREST AT G1/S JUNCTION AVOID REPLICATION OF DAMAGED DNA
S	A	Cdc 25A		AVOID REPLICATION OF DAMAGED DNA REMOVE REPLACE MISMATCH REPAIR
G2/M	B,A	Cdk1	INCREASE CYCLIN A - ARREST	ARRESTED IN G2 TO ALLOW DNA REPAIR CELLS LACKING G2 CHECK POINT ARE RADIOSENSITIVE AS THEY CAN NOT REPAIR DAMAGED

Effects of Oxygenation

- Oxygen enhancement ratio (OER)
  - aerated cells are more radiosensitive (due to “fixing”)
  - oxygen reacts with free radicals to produce peroxide, which constitutes non-repairable damage
  - typical values: 2.5 - 3 for γ and x-rays
- OER OF G_1: ~ 2.3 TO 2.9
- S: ~ 2.8
- G_2: 2.3-2.4
- Provides implications for radiation therapy modes

5-FU and Radiation

- Synergistic effect
- Perturbating cell cycle regulation and abrogating radiation induced G_2 arrest
- Reducing time for cell repair.
- Imbalance in deoxynucleoside triphosphate – altered DNA repair.



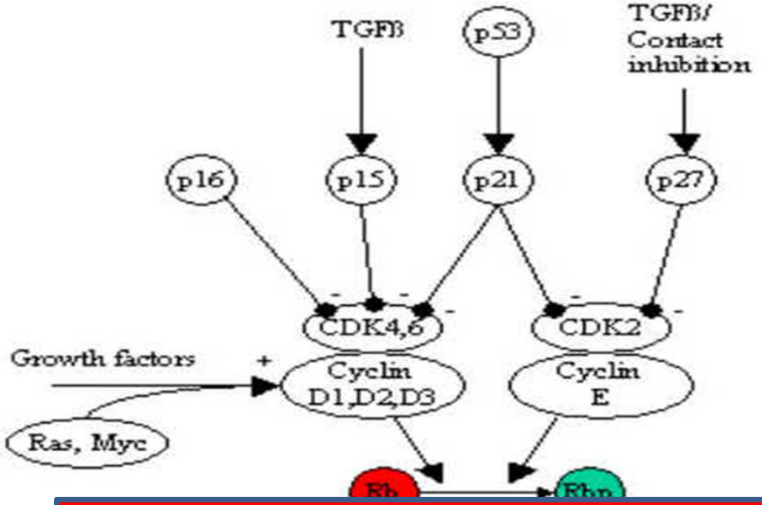
- **CLINICAL IMPLICATIONS**
  - **Cells defective in G2 checkpoint are more sensitive to RT and abrogation of G2 checkpoint by caffeine make cells more sensitive to RT.**
  - **Telomerase provides another cell cycle related, tumor specific target.Inhibition of its activity would theoretically lead to eventual cessation of cellular replication**

Cont..

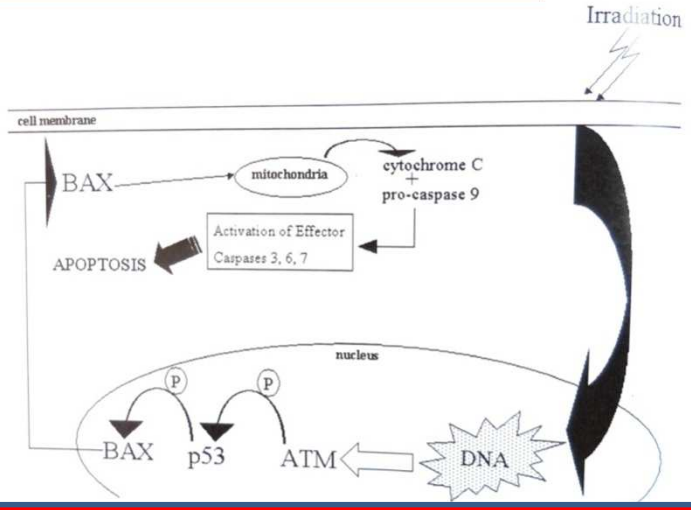
- **Semin Radiat Oncol 2002**
  - **The molecular basis of radiosensitivity&chemosensitivity in treatment of cancer breast**
  - **Powell SN**
- **BRCA 1 & 2 deficient tumour shows marked sensitivity to radiation & drug that produces ds breaks.**
- **Overexpression of cyclin D1 is associated with mild radioresistance**
- **P53 & ATM gene also place a significant role in determining response to therapy**

p53

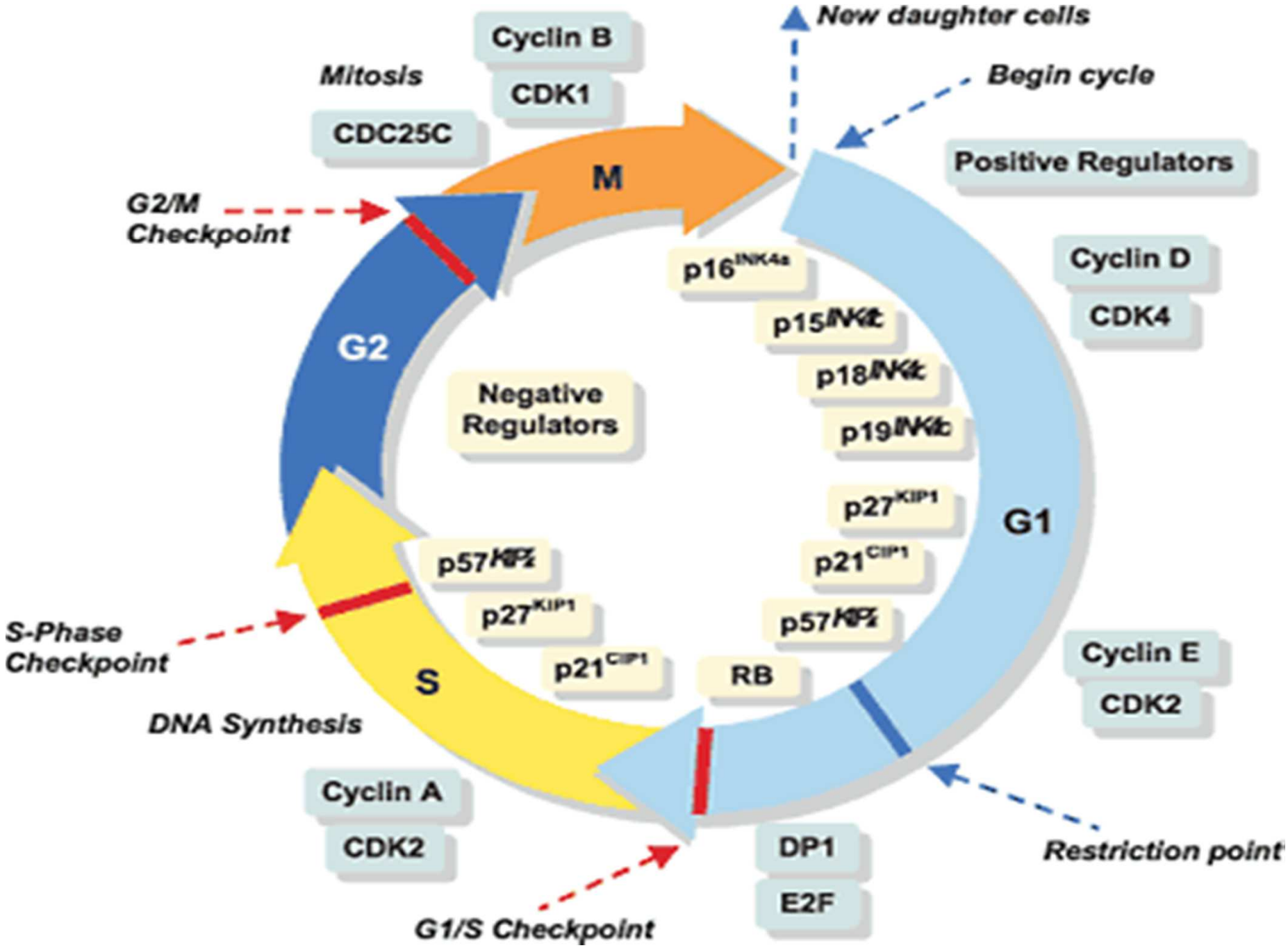
MEDIATES PROGRESSION THROUGH CELL CYCLE

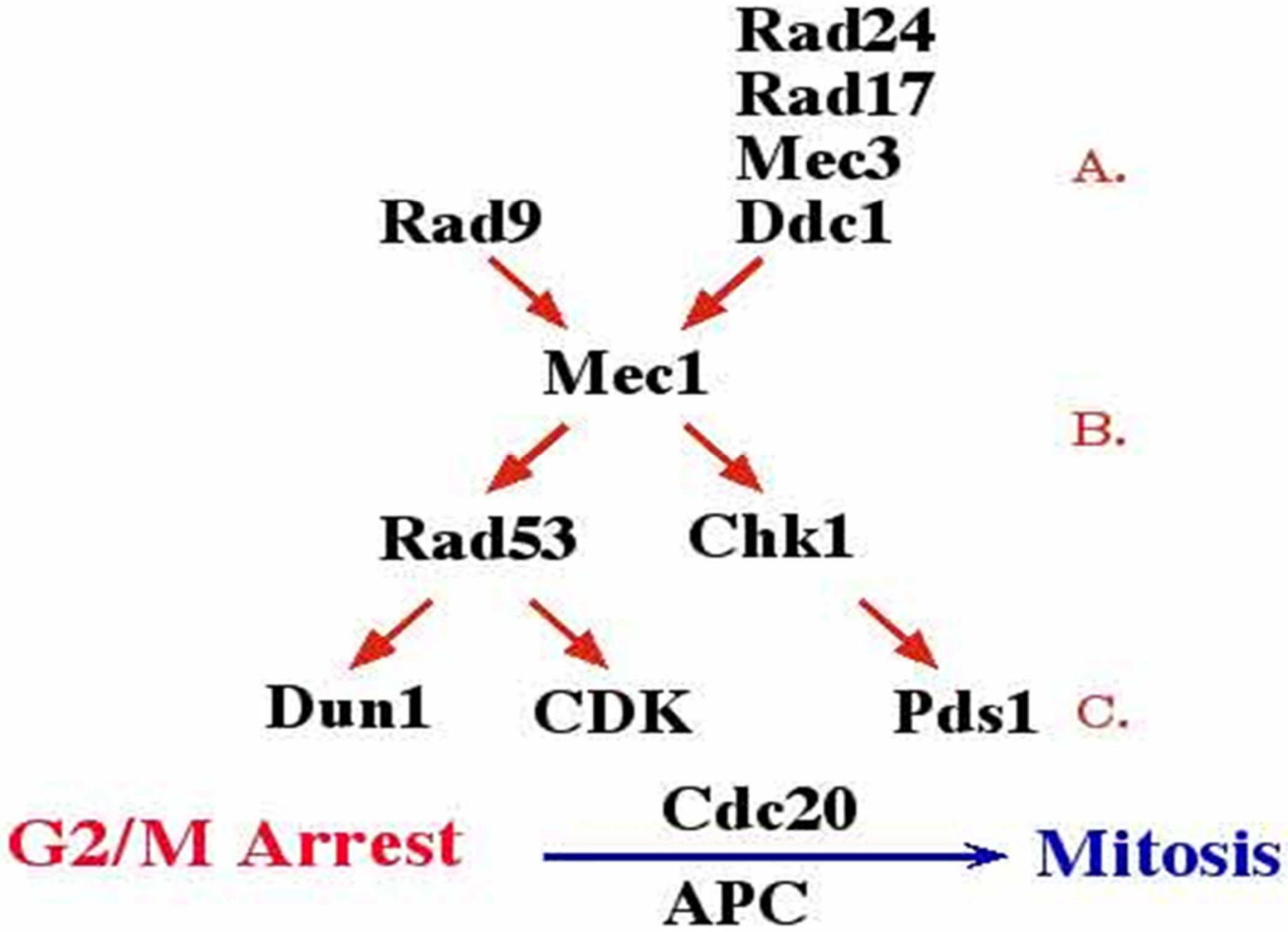


INDUCE APOPTOSIS (ACTIVATOR OF APOPTOSIS)



IN GENERAL LOSS OF p53 IS ASSOCIATED WITH RADIORESISTANCE PHENOTYPE.
P53 MEDIATED RESISTANCE IS MORE IMPORTANT IN CELL THAT DEPEND ON APOPTOSIS
P53,p21 REQUIRED FOR SUSTAINING G2/M ARREST AFTER DNA DAMAGE
DISRUPTION OR ABROGATION OF G2/M CHECK POINT LEADS TO RADIOSENSITIZATION OF p53 MUTATED CELLS
Pp21 MUTATION SENSITIZE THE TUMOR TO RADIATION
DISRUPTION OF G2 CHECK POINT BY CAFINE /PENTOXYPHYLLINE SENSITIZES THE CELL TO RT.


New daughter cells
Begin cycle
Cyclin B
CDK1
Mitosis
CDC25C
M
Positive Regulators
G2/M
Checkpoint
G2
Cyclin D
CDK4
p16INK4a
p15INK4b
p18INK4c
p19INK4d
Negative
Regulators
p27KIP1
p21CIP1
p57KIP2
G1
p57KIP2
p27KIP1
p21CIP1
RB
Cyclin E
CDK2
S-Phase
Checkpoint
S
DNA Synthesis
Cyclin A
CDK2
DP1
E2F
Restriction point
G1/S Checkpoint


Rad24
Rad17
Mec3
Ddc1
A.
Rad9
Mec1
B.
Rad53
Chk1
Dun1
CDK
Pds1
C.
G2/M Arrest
Cdc20
APC
Mitosis