Cell Cycle and Cell & Tissue Kinetics

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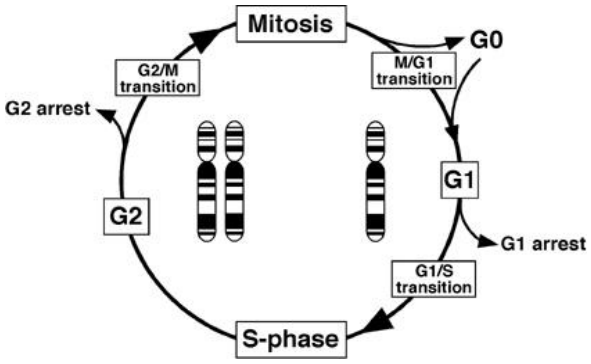
3:15 pm Thursday, May 2, 2013

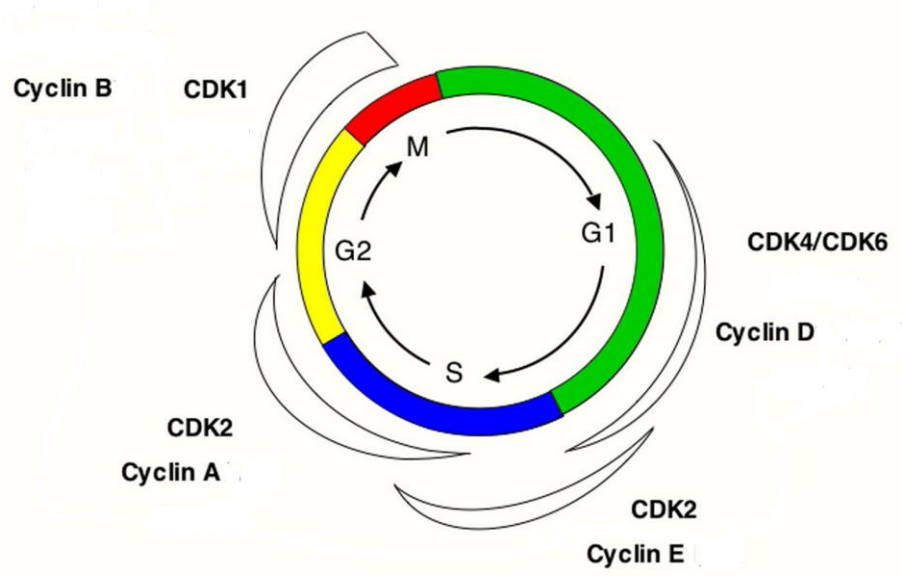
Cell Cycle Molecular Events

A. Cells progress through the cell cycle during which time their DNA content increases from 2n to 4n during S phase and at the end of the cycle cells divide and distribute their 4n quantity DNA equally amongst the two new cells.

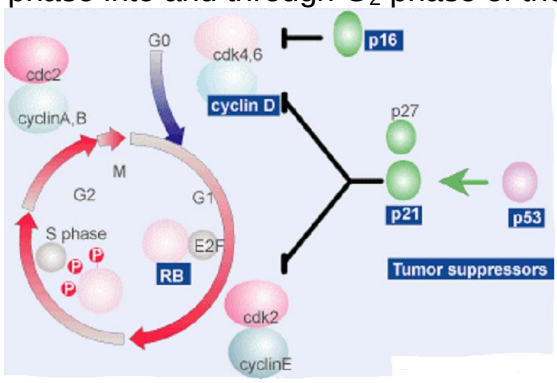


B. Useful definitions
   1. Cyclins: Proteins that complex with Cdks to regulate cell movement through the cell cycle. Cyclin levels rise and fall through the cell cycle (rate limiting).
   2. Cdks: Cyclin dependent kinases are proteins that complex with their cyclins to phosphorylate proteins to ultimately act on the promoter region of genes essential to the movement of cells through the cell cycle.
   3. Kinase: Protein that adds phosphate (PO_4) to other proteins.
   4. Phosphatase: Protein that removes phosphate group from proteins.

C. Cell cycle associated proteins



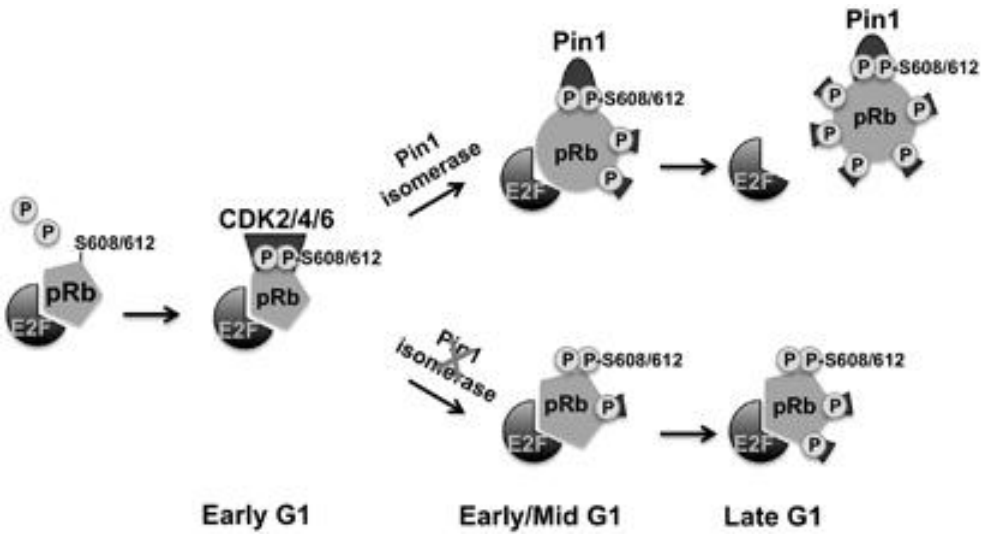
1. G_1 thru S-phase
   a. Cyclins D, E, and A and Cdks 6, 4, and 2
      Cyclin D combines with Cdk 6 and 4 to phosphorylate proteins essential for the movement of cells through G_1 phase of the cell cycle. Cyclin E combines with Cdk 2 in late G_1 to assist cell movement from G_1 phase into S phase. Cyclin A combines with Cdk 2 to move cells through S phase into and through G_2 phase of the mitotic cycle.

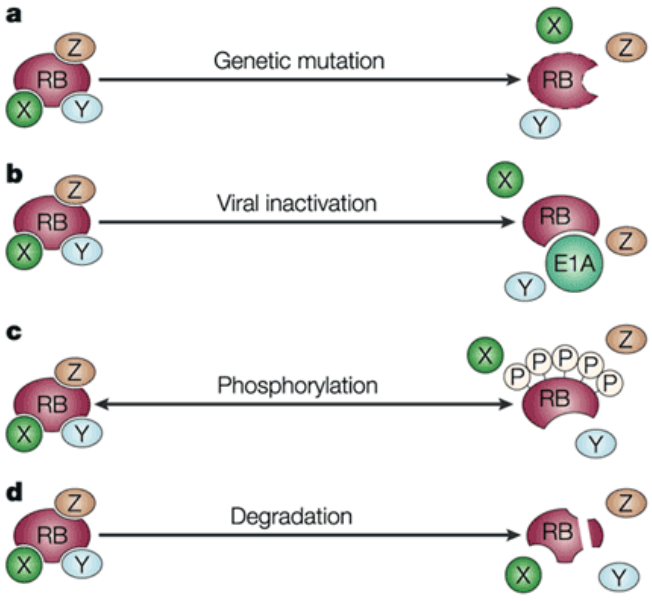


   b. Inhibitors of cyclin-Cdk complex formation
      Cyclin-Cdk complexes may be inactivated by cyclin-dependent kinase inhibitors (CKI) such as TGFβ (growth inhibitory factor), the INK4 family (p15, p16, p18, and p19) that binds to Cdk4 and Cdk6, and the Kip family (p21, p27, and p57) that inhibits all G_1- and S-phase Cdk enzymes. The protein product of p21 (also known as waf1/cip1) inhibits all complex formations of Cdk6, Cdk4, and Cdk2 with their associated cyclin (D, E, and A) and halts cells in G_1 and S phase.

      The gene p27 has a sequence that is related to p21 (and therefore redundant), and also binds to Cdk-cyclin complexes. Over-expression of p27 blocks progression of cells through S phase, and levels of p27 are increased when cells are sent into a quiescent state by treatment with TGFβ. p21 and p27 block the catalytic subunit of Cdk-cyclin from being a substrate for activation via phosphorylation.
   c. The Rb gene and its protein product
      Some tumors develop because of a **loss of function** such as the case when both alleles of the Rb gene are mutated or deleted. Some viral tumor antigens (SV40 T and E1A) bind to the non-phosphorylated form of Rb and permanently turn off Rb function by preventing the non-phosphorylated Rb protein from binding with E2F. Rb, a nuclear phosphoprotein, is not the only protein of its type. p107 and p130 have similar properties.

      Phosphorylation of the Rb gene protein causes decoupling from E2F (E2F is a transcription factor that binds to the promoter region of 20-30 genes whose protein products are essential during S phase) so that cells can proceed through the cell cycle. The Cdk-cyclin kinase complex phosphorylates the Rb protein.





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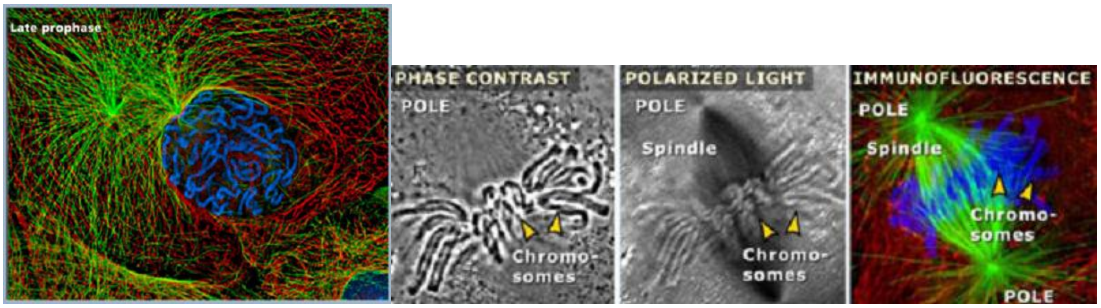
d. The master gene *p53*
Normal cells express normal, wild type *p53* (*Wtp53*) and mutants are designated Mtp53. p53 binds to promoter regions (p53 is a transcription factor). It activates p21, GADD45 (repair protein), and apoptosis in some circumstances.

Alteration of function may be caused by changing its protein half-life (from 6-20 minutes to several hours), causing a protein conformational change (3D structural change that does not allow the protein to bind to DNA in its primary role as a transcription factor), switching its location from the nucleus to the cytoplasm, or being irreversibly bound to proteins (**Wtp53 protein can be inactivated by binding with proteins like those from viral and cellular oncoproteins (*E1B, SV40 large T antigen,* and *HPV E6*).**

p53 protein is degraded by the protein mdm2 (a proto-oncogene) by acting as an E3 ubiquitin ligase so that p53 undergoes ubiquitin degradation. An upstream protein of mdm2 can bind to it and directly prevent ubiquitination of p53.

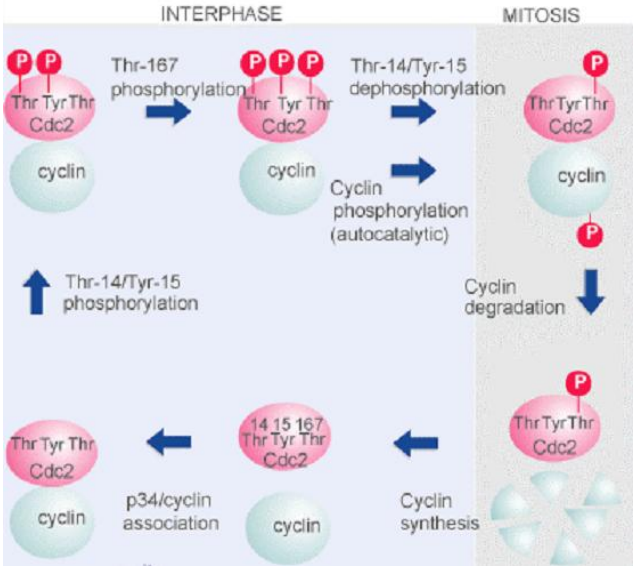
2. G_2-phase cells into and through M phase



a. Cyclins A and B and Cdks 2 and 1 (Cdk1 is also known as Cdc2)
Cyclin A combines with Cdk 2 in S phase and elevated levels persist into and through G_2 phase by phosphorylating proteins critical for cell progression. Cyclin B combines with Cdk 1 (Cdk1 protein is $p34^{cdc2}$) and levels rise in G_2. When levels reach a maximum, modification of Cdk1 is the critical event that triggers the G_2/M transition. As cells enter M phase, the following is visible:
   i. chromosomal condensation
   ii. cytoskeletal reorganization
   iii. nuclear envelope breakdown

b. The abrupt destruction of cyclin B/Cdk1 (because of ubiquitin degradation), cells move from M phase to G_1. The activation of $p34^{cdc2}$ is associated with the dephosphorylation of the phosphorylated tyrosine and threonine residues of the protein. Its kinase activity appears to be

associated with the tyrosine residue, **so dephosphorylation of this site appears essential for movement into M phase.**

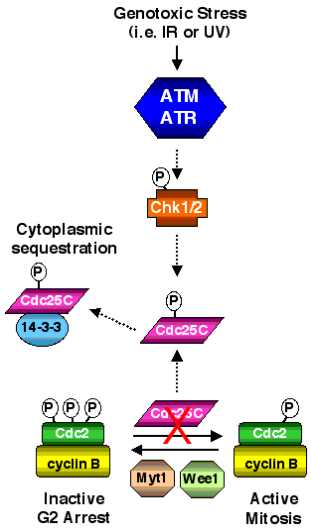


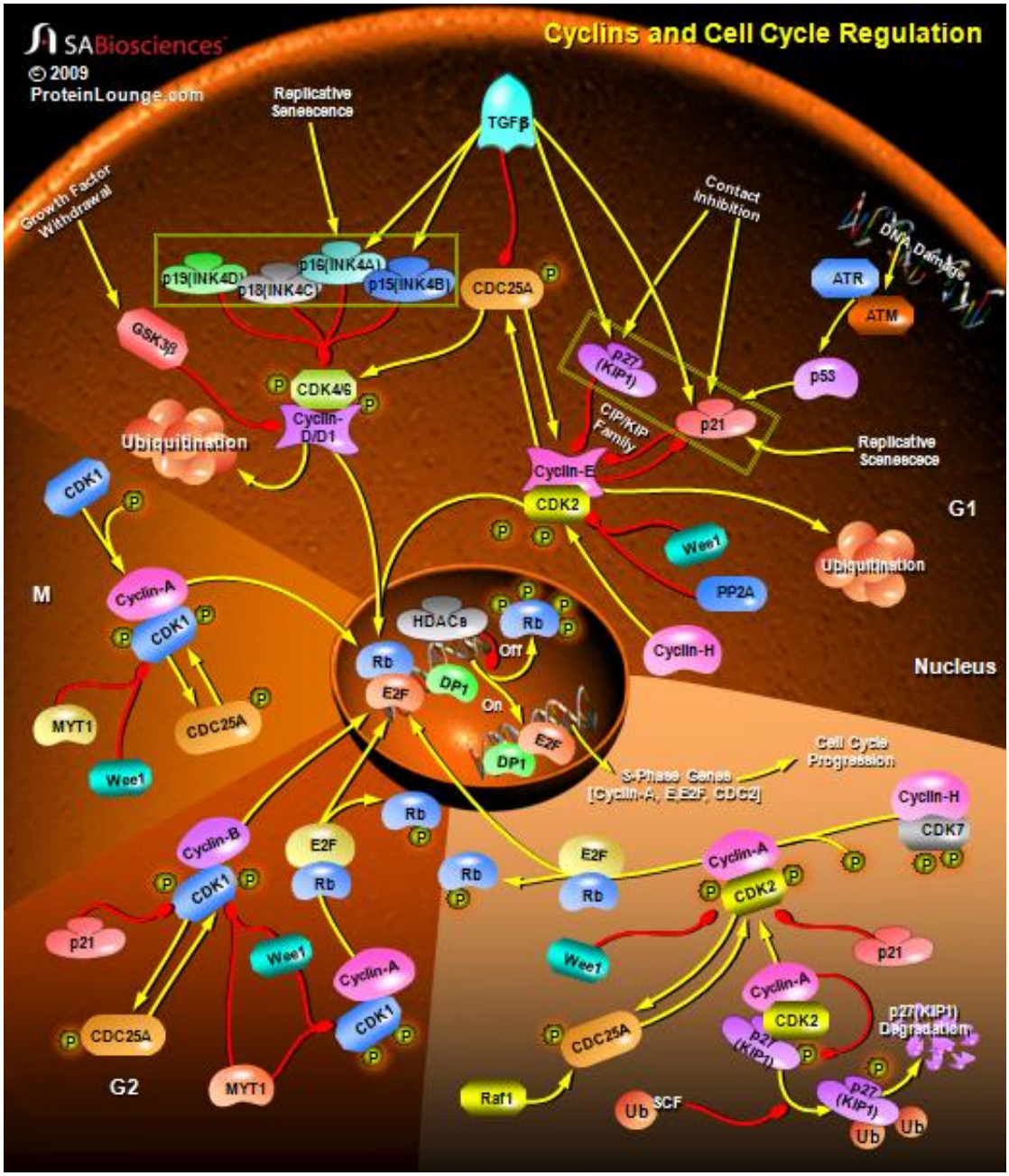
c. Inhibitors of cyclin-Cdk complexes

i. The protein product of p21 (also known as waf1/cip1) inhibits the formation of cyclin A and cyclin B with Cdk 2 and 1, respectively.

ii. The gene rad9 is an important regulator of cell cycle checkpoints and apoptosis induced by ionizing and non-ionizing radiation exposure. Rad9 is phosphorylated directly by atm.

iii. At the site of DNA damage, proteins accumulate and are joined by two transducer kinases, ATR (ATM and RAD 3) and ATM. These kinases phosphorylate and thereby activate effector kinases like Chk2 (RAD53) and CHK1, which then phosphorylates proteins (Cdc25, for example) that bind to the protein 14-3-3, which keeps it inactive and unable to dephosphorylate Cdk1 (Cdc2). Therefore, M phase cannot be activated (part of the radiation-induced G_2/M block).




SABiosciences
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ProteinLounge.com
Cyclins and Cell Cycle Regulation
Replicative Senescence
TGFβ
Growth Factor Withdrawal
Contact Inhibition
DNA Damage
p15(INK4D)
p18(INK4C)
p16(INK4A)
p15(INK4B)
CDC25A
ATR
ATM
GSK3β
p27 (KIP1)
p53
CDK4/6
Cyclin-D/D1
CIP/KIP Family
p21
Replicative Senescence
Ubiquitination
Cyclin-E
CDK2
G1
CDK1
Wee1
PP2A
Ubiquitination
M
Cyclin-A
HDACs
Rb
Off
Cyclin-H
Nucleus
E2F
DP1
On
MYT1
CDC25A
S-Phase Genes [Cyclin-A, E,E2F, CDC2]
Cell Cycle Progression
Wee1
Cyclin-H
CDK7
Cyclin-B
E2F
Rb
CDK1
p21
Wee1
Cyclin-A
CDK2
p21
CDC25A
Cyclin-A
CDK1
CDK2
p27(KIP1) Degradation
G2
MYT1
Raf1
Ub
SCF
Ub
Ub

Radiosensitivity Through the Cell Cycle

A. Cell cycle methods to determine phase times and cell synchrony.

1. Mitotic index $MI = \frac{\lambda T_m}{T_c}$ T_m = # of mitotic figures (related to time for mitosis) T_c = # of cells in view (related to time for cell cycle)

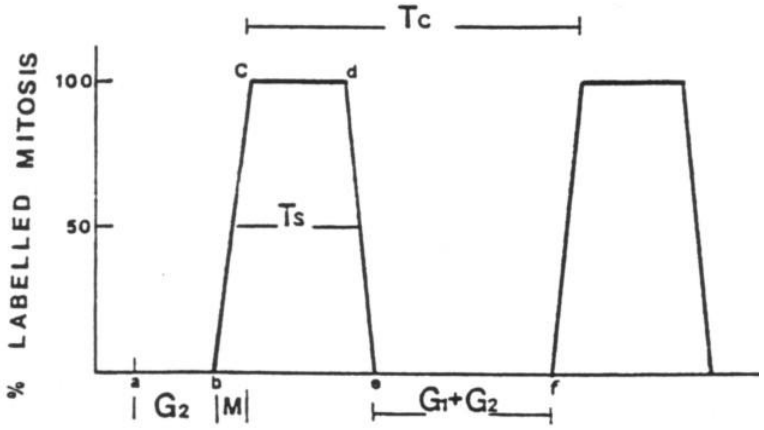
   λ = age distribution correction factor (varies from 0.7 to 1.0) and is relatively small and unimportant as a correction factor.

2. Labeling index: $L.I. = \frac{\lambda \text{ labeled cells}}{\text{total cells}} = \frac{T_s}{T_c}$

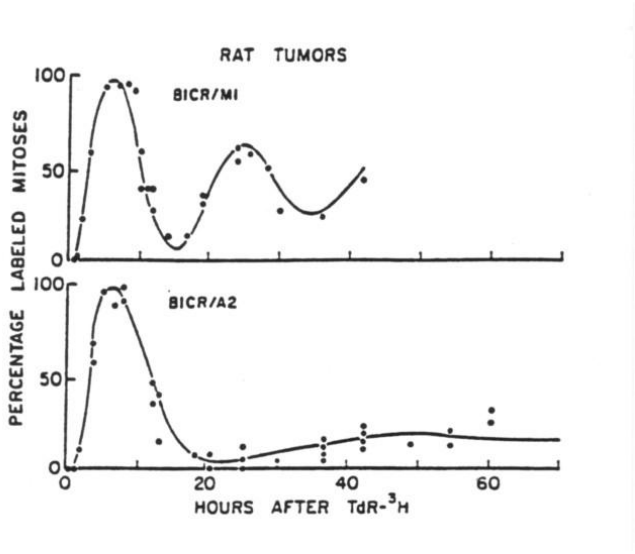
   Flash/pulse label cells with ^{3}HTdR to selectively label cells in S phase. MI and LI yield basic information about the proliferation status of the population but does not tell anything about how long each phase is.

3. Time for mitosis: If you treat cells with an agent to block cells in M phase and collect metaphases, the time required to accumulate twice the number of metaphases is the T_m value. For most mammalian cells, M phase is short (~1 h of a multi-hour total cycle time).

4. Percent labeled mitosis (PLM) techniques
   a. Identify a cohort of **asynchronously dividing cells** by labeling them in S phase and observing their progress through an identifiable window (M phase).



$$T_C = T_M + T_{G1} + T_S + T_{G2}$$



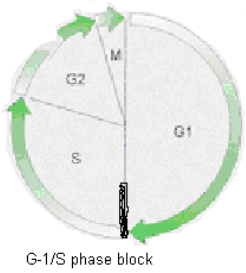
b. Plot PLM vs. time $LI = \lambda T_S/T_C$ $T_C = \lambda T_S / LI$

If LI = 5.2%, T_S = 12 h, what is T_c? Use $\lambda = 0.693$ if it is given on the test

$T_C = 0.693 \times 12\ h / 0.052$ T_c = 160 h (6 days and 16 h)

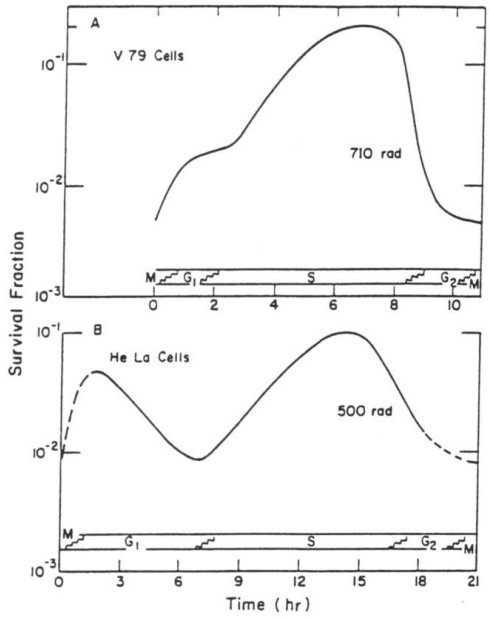
In general, the length of G_2, S, and M are reasonably constant. The major difference accounting for the wide **variation in T_c is in the length of G_1**.

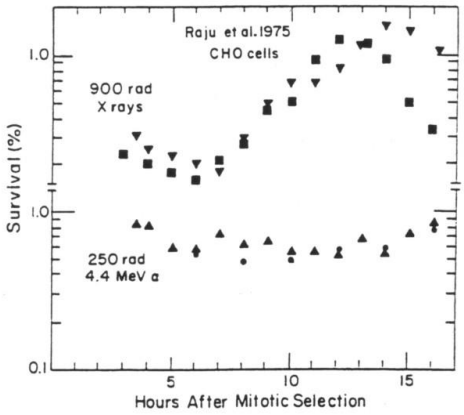
5. Cells may be synchronized in several ways. Two popular methods include mitotic shake-off and hydroxyurea (there are several other methods).

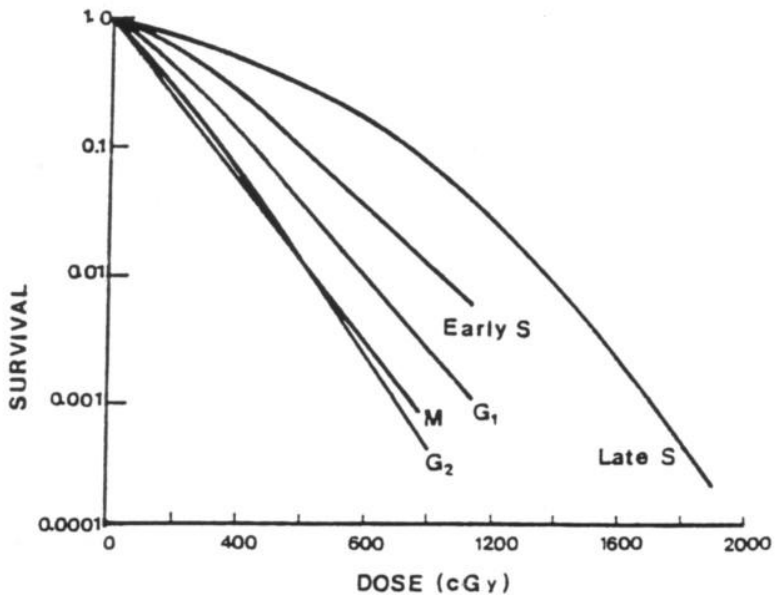


G-1/S phase block

B. Cell cycle-dependent radiation effects
   1. Cycling cells are more sensitive than non-cycling cells (non-cycling cells have more time to repair damage than cycling cells before entering the cell cycle and faithfully synthesize DNA and successfully survive M phase).
   2. Cells in **M-phase are the most radiosensitive** (G_2 cells are also sensitive) and **late S-phase cells are the most radioresistant** (if G_1 phase is extended, early G_1 cells are resistant, and late G_1 cells are sensitive). Resistance of cells during S phase is related to DNA content and an increase in cellular glutathione in resistant phases. **Cell cycle-dependent effects are not as pronounced for cells exposed to intermediate to high LET radiation.**







3. Radiation sensitivity of dividing cells
   a. Minor difference in the inherent radiosensitivity amongst normal cells.
   b. The time interval between exposure and the expression of damage depends upon the division cycle, sensitivity of cells in the maturing compartment, and the time to progress to mature functioning cells or tissues.
   c. Division delay rule of thumb is 1-2 min/cGy up to about 8Gy.
   d. Delay is primarily with G_2 cells that normally progress into M phase (to a lesser extent from G_1 into S phase).
   e. Delay allows for repair.

4. Oxygen effect through the cell cycle. YES (but not important in radiotherapy)
   a. 2.3 for G_2-phase cells, 2.8 for S-phase cells and intermediate for G_1-phase cells.
   b. Oxygen is a dose modifier (in general, OER is the same at all doses).
   c. OER is smaller at high survival (low doses) and larger at low survival. Since S phase is the predominant phase (typically ~33% of the total cell cycle time), S-phase OER drives the oxygen effect through the cell cycle.

Cell and Tumor Kinetics

A. Doubling times
   1. Tumor volume doubling time (T_{vol}) characterizes tumor growth (often determined by measuring the diameter doubling time and converting to volume doubling time).
   2. Potential volume doubling time (T_{pot}) is rarely measured. When tumor is very small, $T_c = T_{pot}$ (growth fraction is 1 and cell loss is nil).

	T_{pot} (days)	T_{vol} (days)	Growth Fraction (%)	Cell Loss (%)
Embryonic Tumors	2-4	27	90	93
Lymphomas	1.1	2.8	100	70
Mesenchymal Sarc	23	44	15	40
Colorectal Adenoma	3-4	95	35	96
Breast Cancer	10	90	25	90

   2. Conversion of diameter doubling time to volume doubling time.
      Volume doubling time increases three times faster than the diameter doubling time (or six times for quadrupling time of diameter)

      If T_d = doubling time for diameter
      Then $T_d = 3T_{vol}$
      If T_q = quadrupling time for diameter
      Then $T_q = 6T_{vol}$

Sample Calculations

Sample 1: If the diameter of a tumor increases from 1 cm to 2 cm in 15 days, what is its volume doubling time (T_{vol})?
Divide 15 days by 3 to get 5 days for volume doubling.

Sample 2: If the diameter of a tumor increases from 0.5 cm to 2 cm in 60 days (quadrupling), what is its tumor volume doubling time (T_{vol})?
Divide 60 days by 6 to get 10 days for volume doubling.

B. Potential volume doubling time (T_{pot})

1. The volume doubling time that would be measured in the absence of cell loss

$$T_{pot} = \frac{\lambda T_s}{LI}$$

T_s = Duration of S phase LI = Labeling index

λ = age distribution correction (0.693 to 1.0)

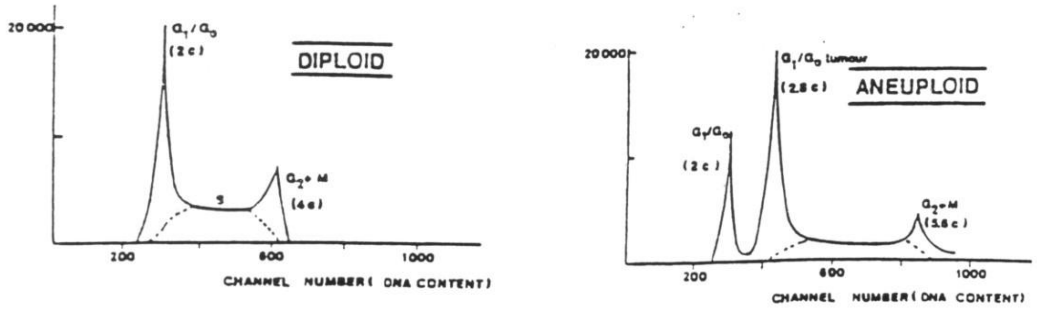
2. Typical values for T_s, and L.I.

T_S = 12 h to 24 h (we use 18 h below)

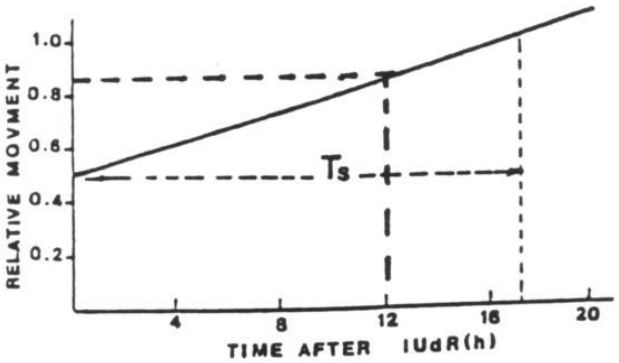
λ = 0.7

L.I. = 5 - 30% (we use 10% below)

T_{pot} = 0.7 x 18 h/0.1 = 126 h (5.25 d)



By using flow cytometry (labeling with BrdUrd, to indicate the proportion of S-phase cells, and staining total DNA with propidium iodide), both T_S and LI can be determined from a single sample.



Sample problem: If a biopsy specimen is obtained 12 h after administration of BUdR and the relative DNA content is 0.85, how long is T_s?

Solution: *Use the concept of similar triangles (proportionality)*

FOR CHANGE IN DNA CONTENT, START AT 0.5 LEVEL

Difference between start (0.5) and end level of DNA (0.85) = 0.35

0.35/0.5 = 12 h/ T_s

T_s = 6 h/0.35

T_s = 17.1 h

C. Growth Fraction (GF): Proportion of cells proliferating.
   1. Growth fraction = # of proliferating cells/ (# proliferating + # of quiescent cells)
   2. Measured by PLM technique. Inject ^{3}HTdR (or BrdUrd) into patients with tumors and several generations later prepare autoradiographs (or immunohistochemical stains) of tumor slices.

      GF = fraction of cells labeled
           fraction of mitoses labeled
   3. Proliferating cell nuclear antigen (PCNA) is present in proliferating cells (higher in G_1 & S). Ki67 is the antibody that binds to PCNA.
   4. Growth fraction is about three times the labeling index.

D. Cell loss factor (CLF)
   1. Overall growth of tumors is the result of a balance between cell proliferation and loss of tumor cells.
   2. Tumors usually grow much slower than would be expected on the basis of T_c and GF. The difference is due to loss by:
      Exfoliation, metastasis, apoptosis, lysis

Cell Loss Factor (ϕ) is estimated by comparing the rate of production of new cells with the observed growth of the tumor.

$$CLF = 1 - \frac{T_{pot}}{T_{vol}}$$ where T_{pot} is the potential tumor volume doubling time and T_{vol} is the volume doubling time of the tumor.

If CLF = 1, no growth, if CLF= 0.5, 1/2 of the tumor cells produced, are lost. Therefore, as CLF increases from 0 to 1, the rate of tumor growth decreases from rapid growth to no growth.

Sample calculation 1:
If the diameter of a tumor increases from 1 cm to 2 cm in 27 days and its T_{pot} is 5.4 days, what is its volume doubling time and CLF?
27 days for diam. to double ÷ 3 = 9 days for T_{vol}
CLF = 1-5.4 days/9 days CLF = 1-0.6 CLF = 0.4 or 40%

Sample calculation 2:
If CLF = 60%, and T_{vol} = 20 days, what is the T_{pot}?

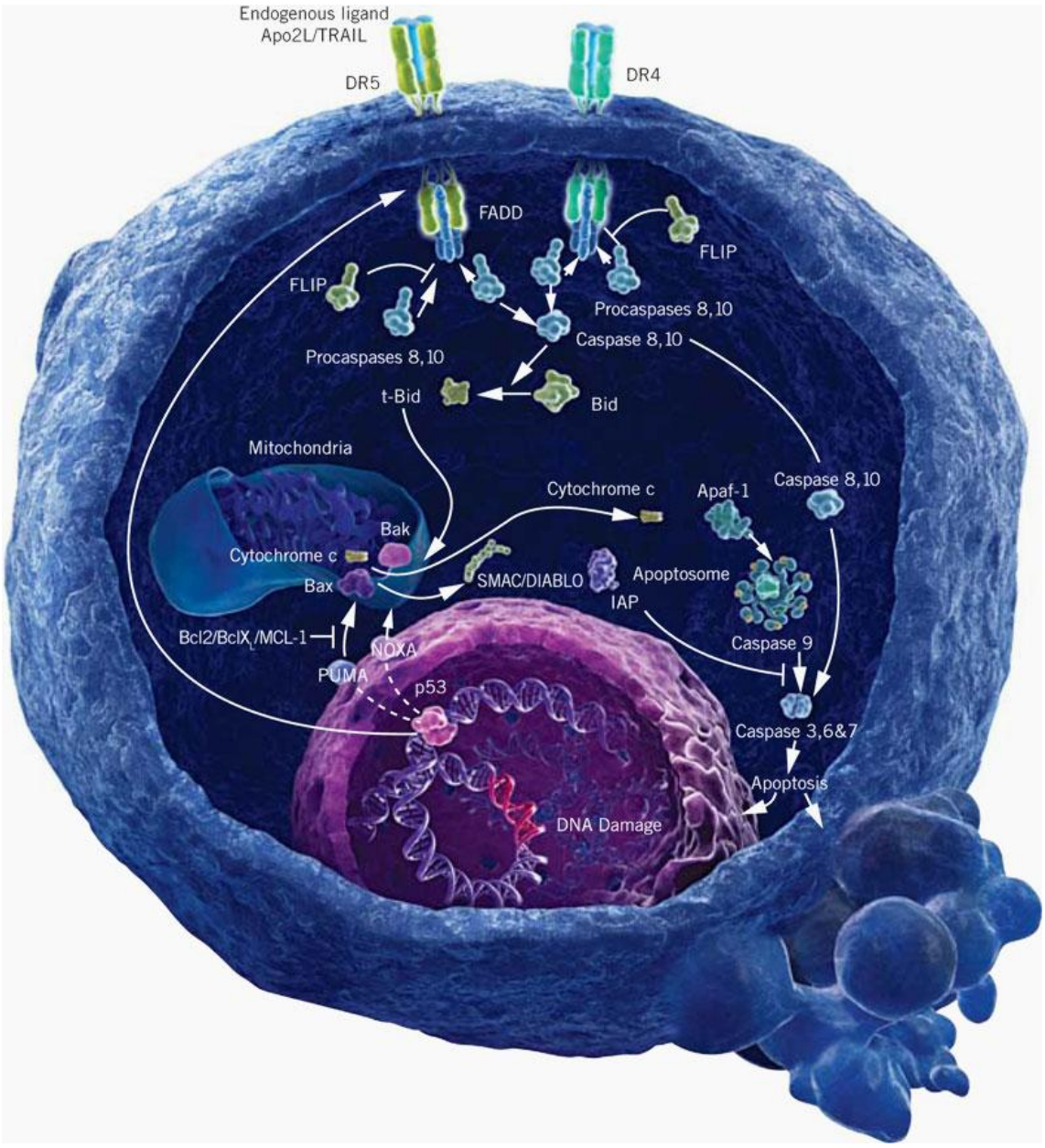
Summary
In animal tumors, growth fractions range from 30% to 50% (**remember 30%**).
In animal tumors, cell loss ranges from 0% to more than 90%.
Cell loss tends to be large for carcinomas and small for sarcomas.
Cell cycle time of malignant cells is less than for normal tissues.
Irradiation lengthens T_c in tumor cells and shortens T_c in normal cells.
In 90% of human tumors, T_c has a modal value of 48 h (range from 15 h to 125 h).
In human tumors, T_s has a modal value of 16 h (9.5 h to 24 h).
The mean duration of T_c in human tumors is **3 times T_s** (as a rule of thumb)

Growth fraction is more variable in human tumors than in rodents and correlates with gross T_{vol}.

Cell loss factor for human tumors is **~77%**. Therefore, cell loss factor is the most important factor that determines the pattern of tumor growth and decline.

E. Human tumor kinetics
   1. Tumors with a **high growth fraction** and a **high percentage of cell loss** will **regress rapidly** during and after treatment, regardless of whether they were growing rapidly or slowly before treatment. Rapid regression will also occur in tumors with a high growth rate and low cell loss.
   2. Regression rates are **not necessarily good predictors** of therapeutic success. Rapid regression could mean:
      i. Good prognosis – low growth rate, high cell loss
      ii. Poor prognosis – early reduction of growth fraction and cell loss but rapid regrowth of remaining clonogens
   3. Tumors with a high rate of cell loss are more common and therefore rapid regression is **usually** a good sign.
      Slow regression may be because of **low proliferation**, **low cell loss**, **residual stroma**, or **treatment failure**. Examples: prostate Ca, some nodular sclerosing Hodgkin's disease, teratocarcinomas of the testis, some soft tissue sarcomas, choroidal melanomas, meningiomas, pituitary adenomas, chordomas, and glomus tumors generally display slow regression because of persistent slow growth and/or excessive extracellular stroma. **Slow regression of a tumor type that usually regresses rapidly is not good.**
   4. Reducing the total therapy dose when a tumor regresses rapidly is not a good idea.
   5. Protracting treatment time for slow growing tumors is not recommended.

F. Apoptosis
   1. Pro-apoptotic gene products:
      Bax, Bak, Bok, Boo, Bclg, Bclb
      Bik, Bad, Bim, Perp, Noxa, Puma
      Most human cancers (>50%) contain point mutations that fail to activate the Bax promoter.
      p53 (a transcription factor) induces bax, puma, noxa, and perp.
      Bid & Bax make mitochondrial membranes more permeable (channels become larger) to cytochrome c.
   2. Anti-apoptotic gene products:
      Bcl-2, $Bclx_L$, Bclw, Mcl-1, Bcl-w, Bclb, Bag-1, Bfl-1, Brag-1.


Endogenous ligand
Apo2L/TRAIL
DR5
DR4
FADD
FLIP
FLIP
Procaspases 8,10
Caspase 8,10
Procaspases 8,10
t-Bid
Bid
Mitochondria
Cytochrome c
Apaf-1
Caspase 8,10
Bak
Cytochrome c
Bax
SMAC/DIABLO
Apoptosome
IAP
Bcl2/BclX_L/MCL-1
NOXA
PUMA
p53
Caspase 9
Caspase 3,6&7
Apoptosis
DNA Damage