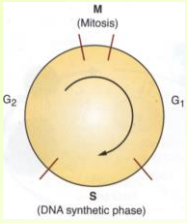
Radiosensitivity and Cell Age in the Mitotic Cycle

Chapter 4

Eric J. Hall., Amato Giaccia,
Radiobiology for the Radiologist

Introduction

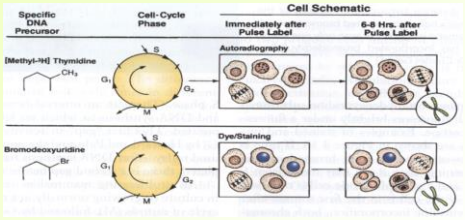


- Mammalian cells propagate by mitosis
- The cell cycle in all dividing mammalian cells is divided into four phases:
  - M phase can be identified through the light microscope
  - After the cells pass through M, there is a period of apparent inactivity, termed G1 (the first "gap" in activity in the cell cycle)
  - Next, the cells actively synthesize DNA during the S phase, identified through labeling
  - Between the S phase and the onset of the next mitosis, there is another gap in activity, G2
- The time between successive divisions defines the **cell cycle time T_C**

Cell labeling techniques

- Cell visualization during the S phase is based on feeding the cells with radioactive labels or fluorescent dyes, which are incorporated into duplicating chromosomes
- The medium is flushed after a short time period
- Cells are fixed and stained for ease of viewing after varying time interval
- Labeled cells are counted

Cell labeling techniques



- Tritiated thymidine (^{3}HTdR) incorporated into chromosomes emits β-particles forming a latent image on a layer of photo-emulsion coated over the sample (takes ~3-4 weeks)
- Labeling with dyes (e.g., BrdU) is more convinient; use fluorochrome-tagged antibody to BrdU, observable under a fluorescence microscope

Cell labeling techniques

- BrdU labeling movie:

http://academics.wellesley.edu/Biology/Concepts/Html/brdu.html

- Antibodies are extremely sensitive to location of a cellular component or a specific molecule (a tissue antigen)
  - Target can be inside or on the surface of a cell
  - Antibody is tagged with a fluorescing dye molecule

Cell labeling techniques

- To ensure free access of the antibody to its antigen, the cells must be fixed and permeabilized
- Sample preparation entails fixing the target cells to the substrate (microscope slide)
- Perfect fixation immobilizes the antigens, while retaining authentic cellular and subcellular architecture and permitting access of antibodies to all cells and subcellular compartments

Cell labeling techniques

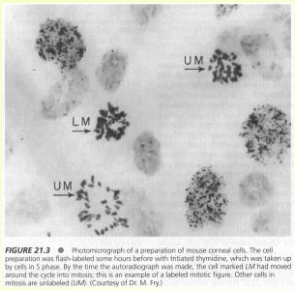


FIGURE 21.3 ● Photomicrograph of a preparation of mouse corneal cells. The cell preparation was flash-labeled some hours before with tritiated thymidine, which was taken up by cells in S phase. By the time the autoradiograph was made, the cell marked *LM* had moved around the cycle into mitosis; this is an example of a labeled mitotic figure. Other cells in mitosis are unlabeled (*UM*). (Courtesy of Dr. M. Fry.)

- Only a small portion of cells in a sample uptakes the label
- The technique is very labor-intensive, relying on the manual cell counting
- New automated approaches significantly speed up characterization

Flow cytometry

- A biophysical technique that allows counting and sorting cells in a sample based on their size, structure, and functionality
  - Functionality is determined through labeling with antigen-specific fluorescent dyes
- Can discriminate cells at speeds up to 70,000 cells/second (compare to 200 cells/min manually)
- Flow cytometry tutorials:

https://www.youtube.com/watch?v=EQXPJ7eeeuQ
https://www.youtube.com/watch?v=yt-cI-B4fB2&feature=player_embedded&v=vfWWxFBIipQ&x-yt-ts=1422327029

The cell cycle times

TABLE 4.1 Phases of the Cell Cycle for Two Commonly Used Cell Lines Cultured *In Vitro*

	Hamster Cells, h	HeLa Cells, h
T_C	11	24
T_M	1	1
T_S	6	8
T_{G1}	1	11
T_{G2}	3	4

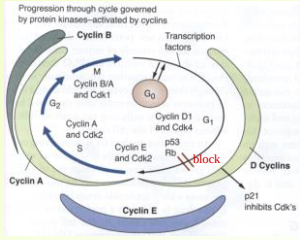
- Based on the number of counted labeled mitosis and varying the time between labeling and counting, can characterize the temporal transitions within a cycle
- The difference among mammalian cell cycle times, varying from ~10 to 100 hours among different cells in different circumstances, is the result of a dramatic variation in the length of the G1 period
- The remaining components of the cell cycle vary comparatively little

Origin of HeLa cells:
http://www.youtube.com/watch?v=0gF8bCE4wqA

Cyclins and protein kinases

- Cyclins and cyclin-dependent kinases were discovered by Hartwell, Hunt, and Nurse, who thus won the 2001 Nobel Prize in Physiology or Medicine "for their discoveries of key regulators of the cell cycle"
- Cyclins are a group of regulatory proteins that control the progression of cells through the cell cycle by activating cyclin-dependent kinase (CDK) enzymes
- Protein kinases are enzymes that modify the function of other proteins by attaching phosphate groups (PO4-) to them (phosphorylate proteins)
  - They are key controllers of most biochemical pathways and important in health and disease

Progression through the cell cycle



Current concept of the cell cycle and its regulation by protein kinases, activated by cyclins

- Each cyclin protein is synthesized at a discrete phase of the cycle: cyclin D and E in G_1 , cyclin A in S and G_2, and cyclin B in G_2 and M. Transitions in the cycle occur only if a given kinase activates the proteins required for progression
- Tumor suppressor genes (p53, Rb) can block cell division if DNA is damaged

Synchronizing cells within cycle

- Survival curves are collected with the assumption that the population of irradiated cells is asynchronous: cells distributed throughout all phases of the cycle
- Study of the variation of radiosensitivity with the position or age of the cell in the cell cycle was made possible only by the development of techniques to produce synchronously dividing cell cultures: populations of cells in which all of the cells occupy the same phase of the cell cycle at a given time

Synchronizing cells within cycle

- Two principal techniques of cell synchronization:
  - Mitotic harvest: physical separation of cells preparing for mitosis (works only on monolayer cell cultures)
  - Use of a drug, eliminating all cells in S phase, and imposing a block on cells at the end of G1 (works for cell and tissue samples)

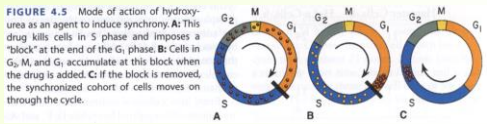


FIGURE 4.5 Mode of action of hydroxyurea as an agent to induce synchrony. **A:** This drug kills cells in S phase and imposes a "block" at the end of the G_1 phase. **B:** Cells in G_2, M, and G_1 accumulate at this block when the drug is added. **C:** If the block is removed, the synchronized cohort of cells moves on through the cycle.

Effect of x-rays on synchronously dividing cell cultures

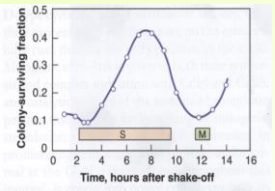
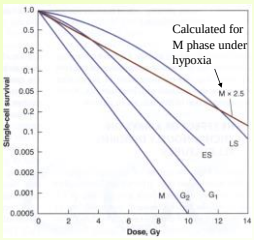


FIGURE 4.7 Fraction of Chinese hamster cells surviving a dose of 6.6 Gy of x-rays as a function of time. Time zero corresponds to the harvesting of mitotic cells. The surviving fraction increases to a maximum late in S phase.

- The proportion of cells that survive the dose increases rapidly with time as the cells move into S phase
- When the cells move out of S into G1 phase and subsequently to a second mitosis, the proportion of surviving cells decreases again

Effect of x-rays on synchronously dividing cell cultures



Cell survival curves for Chinese hamster cells at various stages of the cell cycle

- Cells are the most sensitive in M and G2: survival curves are steep and have no shoulder
- Cells in the latter part of S phase (LS) exhibit a survival curve that is less steep, but has a very broad shoulder
- The range of sensitivity between the most sensitive cells (mitotic) and the most resistant cells (late S) is of the same order of magnitude as the oxygen effect

Variation of radiosensitivity with cell age in the mitotic cycle

- Cells are most sensitive at or close to mitosis
- Resistance is usually greatest in the latter part of S phase. The increased resistance is thought to be caused by homologous recombination repair between sister chromatids that is more likely to occur after the DNA has replicated
- If G1 phase has an appreciable length, a resistant period is evident early in G1, followed by a sensitive period toward the end of G1
- G2 phase is almost as sensitive as M phase
- Exceptions to these generalizations have been noted for some cell lines

Molecular checkpoint genes

- Mammalian cells exposed to radiation tend to experience a block in the G2 phase
- In several strains of yeast, mutants have been isolated that are more sensitive than the wild type to both ionizing radiation and UV light by a factor between 10 and 100
- The mutant gene has been cloned and sequenced and found to be a "G2 molecular checkpoint gene"

Molecular checkpoint genes

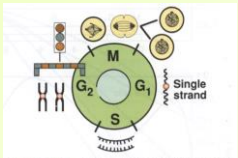


FIGURE 4.11 Diagram illustrating the site of action and function of the molecular checkpoint gene. Cells exposed to any DNA-damaging agent, including ionizing radiation, are arrested in G_2 phase. The function of the pause in cell cycle progression is to allow a check of chromosome integrity before the complex task of mitosis is attempted. Cells in which the checkpoint gene is inactivated are much more sensitive to killing by γ-rays or ultraviolet light. The mutant gene isolated from a sensitive strain of yeast functions as a checkpoint gene.

- Mutant cells that lose this G2 checkpoint gene function move directly into mitosis with damaged chromosomes
  - They are at a higher risk of dying - hence their greater sensitivity to radiation and other DNA-damaging agents
  - Cells that survive mitosis are likely to give rise to errors in chromosome segregation - hence more prone to carcinogenesis

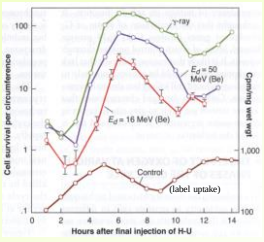
Effect of oxygen

- Effect of oxygen on cell survival curves is characterized by oxygen enhancement ratio

$$OER = \frac{D_{\%\,S}\,(hypoxic)}{D_{\%\,S}\,(aerated)}$$

- The OER was measured at 2.3 to 2.4 for G2 phase cells, compared with 2.8 to 2.9 for S phase, with G1 phase cells showing an intermediate value
- This small variation of oxygenation through the cycle is of little clinical significance in radiation therapy

The age-response function for a tissue *in vivo*



The survival of jejunal crypt cells exposed to γ-rays or neutrons as they pass through the cell cycle after synchronization with hydroxyurea

- The pattern of response in this organized normal tissue, with a sensitive period between G1 and S and maximum radioresistance late in S, is very similar to that characteristic of many cell lines cultured *in vitro*
- High LET radiation decreases the variation of radiosensitivity through the cell cycle

Mechanisms for the age-response function

- The patterns of radiosensitivity and radio-resistance correlate with the mechanism of repair of DNA DSBs:
  - Radiosensitivity correlates with nonhomologous end joining, which dominates early in the cell cycle and is error prone
  - Radioresistance correlates with homologous recombinational repair, which occurs after replication (in S phase) and is more faithful
- Not fully understood

The implications of the age-response function in radiotherapy

- Since general population of cells in tissues is asynchronous, cells in more sensitive phases of the cycle are preferentially killed
- Variations in sensitivity through the cell cycle may be important in radiation therapy because they lead to "sensitization resulting from reassortment" in a fractionated regimen
- Reassortment is one of the “4 R’s of radiobiology”

Fractionated Radiation and the Dose-Rate Effect

Chapter 5

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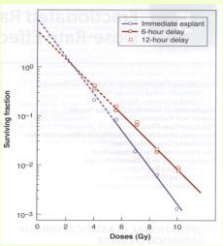
Radiobiology for the Radiologist

Introduction

- Radiation damage to mammalian cells can operationally be divided into three categories:
  - (1) **lethal damage,** which is irreversible and irreparable and, by definition, leads irrevocably to cell death
  - (2) **potentially lethal damage (PLD),** the component of radiation damage that can be modified by postirradiation environmental conditions
  - (3) **sublethal damage (SLD),** which under normal circumstances, can be repaired in hours unless additional SLD is added (e.g., from a second dose of radiation)

Potentially lethal damage repair



X-ray survival curves for density-inhibited stationary-phase cells, subcultured either immediately or 6 or 12 hours after irradiation

- PLD is potentially lethal since under ordinary circumstances it causes cell death
- The cell survival can be increased if postirradiation conditions are suboptimal for growth
  - cells do not attempt to divide
  - have time to repair the damage

Potentially lethal damage repair

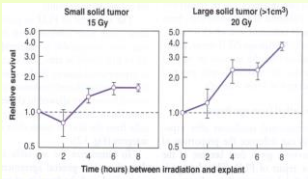


FIGURE 5.2 Repair of potentially lethal damage in mouse fibrosarcomas. The tumors were irradiated *in situ* and then removed and prepared into single cell suspensions. The number of survivors was determined by their ability to form colonies *in vitro*. The fraction of cells surviving a given dose increases if a time interval is allowed between irradiation and removal of the tumor, because during this interval, PLD is repaired. (Adapted from Little JB, Hahn GM, Frindel E, et al. Repair of potentially lethal radiation damage in vitro and in vivo. *Radiology*. 1973;106:689–694, with permission.)

- Observed both *in vivo* and *in vitro*
- Hypothesis: radio-resistant tumors have efficient mechanisms to repair PLD, while radiosensitive tumors do not

Sublethal damage repair

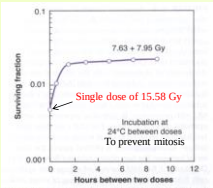


FIGURE 5.3 Survival of Chinese hamster cells exposed to two fractions of x-rays and incubated at room temperature for various time intervals between the two exposures. (Adapted from Elkind MM, Sutton-Gilbert H, Moses WB, Alescio T, Swain RB. Radiation response of mammalian cells in culture: V. Temperature dependence of the repair of x-ray damage in surviving cells (aerobic and hypoxic). *Radiat Res*. 1965;25:359–376, with permission.)

- SLD repair is the operational term for the increase in cell survival that is observed if a given dose is split into two fractions separated by a time interval
- Probability of damage repair increases if cells do not attempt to divide

Sublethal damage repair

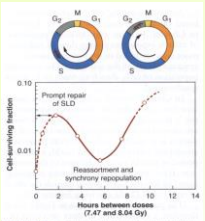


FIGURE 5.4 Survival of Chinese hamster cells exposed to two fractions of x-rays and incubated at 37° C for various time intervals between the two doses. The survivors of the first dose are predominantly in a resistant phase of the cycle (late S). If the interval between doses is about 6 hours, these resistant cells have moved to the G_2M phase, which is sensitive. (Adapted from

- If cells are allowed to progress through the cell cycle, than only those in S phase at the time of irradiation repair the damage and survive
- They become radiation sensitive again as they move to M phase (6 hours dose split time)
- The cell population doubles if time > T_C

Sublethal damage repair

- Repair of sublethal radiation damage has been demonstrated both *in vivo* and *in vitro*
- Split dose experiments illustrate three of the "four Rs" of radiobiology:
  - Repair
  - Reassortment
  - Repopulation
- The effect of the fourth "R" – reoxygenation has also been demonstrated

Sublethal damage repair

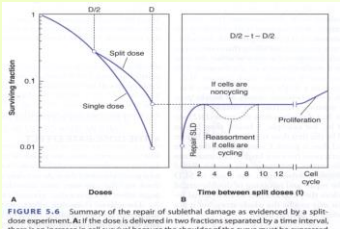
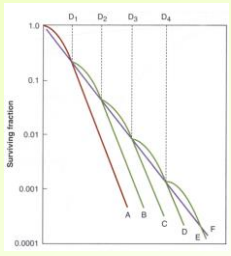


FIGURE 5.6 Summary of the repair of sublethal damage as evidenced by a split-dose experiment. **A:** If the dose is delivered in two fractions separated by a time interval, there is an increase in cell survival because the shoulder of the curve must be expressed each time. **B:** The fraction of cells surviving a split dose increases as the time interval between the two dose fractions increases. As the time interval increases from 0 to 2 hours, the increase in survival results from the repair of sublethal damage. In cells with a long cell cycle or that are out of cycle, there is no further increase in cell survival by separating the dose by more than 2 or 3 hours. In a rapidly dividing cell population, there is a dip in cell survival caused by reassortment. However, as shown in Figure 5.7, if the time interval between the split doses exceeds the cell cycle, there is an increase in cell survival owing to proliferation or repopulation between the doses.

- Broad shoulder of a cell survival curve is an indication of SLD repair
- For high LET radiation there is very little of SLD repair
- DSB from two separate events: damage is repaired before the 2nd hit

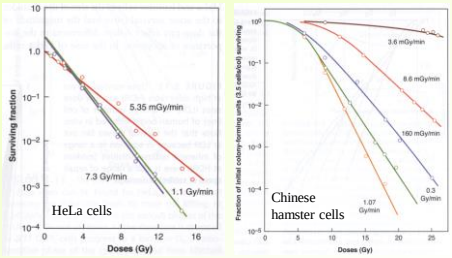
The dose-rate effect



Multiple small fractions approximate to a continuous exposure to a low dose rate

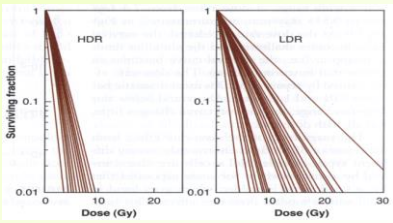
- For x- or γ-rays, dose rate is one one the principal factors that determine the biologic consequences of a given dose
- As the dose rate is lowered the biologic effect of a given dose generally is reduced
- LDR irradiation may be considered as an infinite number of infinitely small fractions
  - the survival curve has no shoulder
  - shallower than for single acute exposures

The dose-rate effect



- Different cell lines demonstrate different degree of the dose rate effect depending on the efficiency of SLD repair
- The effect is most pronounced between 0.01 and 1 Gy/min dose rates

The dose-rate effect



- HDR and LDR survival curves for a large number of cell lines of human origin in vitro
- LDR curves fan out due to the difference in SLD repair

The dose-rate effect

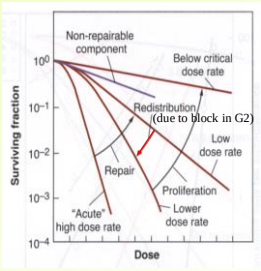


FIGURE 5.15 The dose-rate effect resulting from repair of sublethal damage, redistribution in the cycle, and cell proliferation. The dose-response curve for acute exposures is characterized by a broad initial shoulder. As the dose rate is reduced, the survival curve becomes progressively more shallow as more and more sublethal damage is repaired, but cells are "frozen" in their positions in the cycle and do not progress. As the dose rate is lowered further and for a limited range of dose rates, the survival curve steepens again because cells can progress through the cycle to pile up at a block in G_2, a radiosensitive phase, but still cannot divide. A further lowering of dose rate below this critical dose rate allows cells to escape the G_2 block and divide; cell proliferation then may occur during the protracted exposure, and survival curves become shallower as cell birth from mitosis offsets cell killing from the irradiation. (Based on the ideas of Dr. Joel Bedford.)

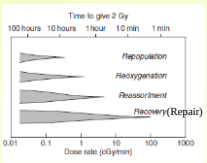
Red arrow shows "inverse dose rate effect"

Brachytherapy

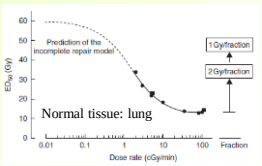
- Name comes from the Greek word for "short range"
- Utilizes a wide range of dose rates, from HDR (>12 Gy/hr* or 20cGy/min**) to LDR (0.2 – 2 Gy/h)
- Two distinct types: intracavitary and interstitial
- Were developed to an advanced stage in the beginning of the 20th century due to availability of the sources

*ICRU report 38; there is also a medium dose rate, MDR
** EBRT dose rate ~1-5 Gy/min (could be much higher for FFF beams)

Brachytherapy



The range of dose rates over which different R's modify radiosensitivity



The dose-rate effect for pneumonitis in mice: correlates with incomplete repairs

- Lowering dose rate allows for more tissue sparing
- Lower dose rate better discriminates between tissues with high and low radiosensitivity

Kogel et al., Basic Clinical Radiobiology, 4th Ed, 2009

Brachytherapy

- If brachytherapy is used as a sole treatment, a commonly used dose is 35 to 70 Gy in 5 to 9 days
- Total dose should he adjusted for dose rate (low DR – higher total dose)
- Clinical studies show that both tumor control and late effects vary with dose rate for a given total dose
- Brachytherapy is often used as a boost to external beam therapy, and only half the treatment is given with brachytherapy

Brachytherapy

TABLE 5.1 Characteristics of Radionuclides for Intracavitary or Interstitial Brachytherapy

Radionuclide	Photon Energy, keV: Average	Photon Energy, keV: Range	Half-Life	HVL,[a] mm Lead
Conventional				
Cesium-137	662	—	30 y	5.5
Iridium-192	380	136–1060	74.2 d	2.5
New				
Iodine-125	28	3–35	60.2 d	0.025
Gold-198	412	—	2.7 d	2.5
Americium-241	60	—	432 y	0.125
Palladium-103	21	20–23	17 d	0.008
Samarium-145	41	38–61	340 d	0.06
Ytterbium 169	100	10–308	32 d	0.1

[a]HVL, half-valve layer, the thickness required to reduce the incident radiation by 50.
Data computed by Dr. Ravinder Nath, Yale University.

In HDR afterloader

Brachytherapy

- HDR afterloaders use Ir-192; typically, several fractions are delivered
  - Examples: partial breast irradiation, cervical cancer treatments
- Permanent implants (LDR): I-125, Pd-103
  - Example: prostate cancer treatment
  - Radiobiological advantage of LDR: considerable sparing of normal tissues

Summary

- Labeling techniques are used to measure durations of cell cycle phases; they vary dramatically between cell lines, mostly due to differences in G1 length
- Cell radiosensitivity is a strong function of the position in the cell cycle
- Capability of a cell to repair sublethal damage strongly correlates with radiosensitivity
- Dose rate effect is governed by efficiency of sublethal damage repair; other R's on dif time scales