Cell Cycle: Sequence of changes in a cell starting with the moment the cell is created by cell division, continuing through the doubling of the DNA and other cellular contents, and ending with cell division into 2 daughter cells

Phases of the cell cycle:

I. **Interphase** (G1, S, and G2): the part of the cell cycle that excludes mitosis
   - G1: Gap between previous cell division (M) and DNA replication (S)
   - S: DNA replication
   - G2: Gap between DNA replication (S) and cell division (M)

II. **Mitosis** (M): where copied DNA and cytoplasm are divided between two daughter cells

Cells can exist in a quiescent state (G0) where they are not in an active cell cycle. The decision to enter into an active cell cycle depends on extra-cellular signals and nutrients levels that signal to the nucleus. A complex molecular circuitry (“The Cell Cycle Clock”) integrates all of these signals to decide whether to enter into an active cell cycle.

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Figure 8-1 The Biology of Cancer (© Garland Science 2007)
Cell Cycle Effects of Radiation

Mitosis can be subdivided into prophase, metaphase, anaphase, and telophase:

**Prophase:** Chromosomes condense and become visible under microscope. Centrosomes assemble at opposite poles and bind to microtubules that will form the mitotic spindle.

**Metaphase:** Chromosomes align in a symmetrical fashion as they become attached at the mitotic spindle. Nuclear membrane disappears.

**Anaphase:** The two copies of the DNA are pulled into opposite directions.

**Telophase:** Chromosomes de-condense and a nuclear membrane forms around each set of chromosomes. The cell completes division into 2 daughter cells.

Prophase | Metaphase | Anaphase | Telophase
--- | --- | --- | ---
![Prophase](image1.png) | ![Metaphase](image2.png) | ![Anaphase](image3.png) | ![Telophase](image4.png)

Blue = chromosomes
Green = microtubule fibers of the mitotic spindle

Centrosome: is made up of 2 centrioles that are each composed of 9 triplets of microtubules
The Cell Cycle Through the Ages

1) **The Pre-1953 Cell Cycle:**

In the (really) old days, the only cell cycle “feature” that could be distinguished was mitosis, identifiable microscopically by the appearance of chromosomes.

When not in mitosis, cells were said to be in the rather generic “interphase”.

2) **The Cell Cycle: 1953 – 1970:**

a) the identification of discrete phases within “interphase” was only made possible because a new marker was discovered that was specific for cells synthesizing DNA (which had only been discovered recently itself), that is, S phase

1. the original marker used was $^{32}$P- and $^{14}$C-adenine, quickly replaced by **tritiated thymidine** ($^{3}$H-TdR), a radioactively-tagged version of the nucleotide thymidine, one of the DNA bases; this marker was only incorporated into DNA when supplied to cells that were actively undergoing DNA synthesis

2. in order to detect the incorporation of the marker into individual cells, Howard and Pelc developed the technique of autoradiography (ref: Heredity 6 (suppl): 261-273, 1953)

(a) autoradiography is a technique in which a liquid photographic emulsion is layered on top of a microscope slide containing fixed and stained cells, and allowed to incubate in total darkness (for upwards of several weeks!) until the low-level radioactive decays from the $^{3}H$-TdR expose the emulsion
3. thus, with two cell cycle markers available, it was then easy to identify the other phases — G₁ and G₂ — as "gaps" falling in between the markable phases (S and M)

G₁ ("Gap 1"): the age interval following mitosis but before DNA synthesis

S ("Synthesis"): the age interval during which DNA is reproduced

G₂ ("Gap 2"): the age interval following mitosis but before cell division

M ("Mitosis"): the age interval during which chromosomes condense, the mitotic apparatus assembles (and disassembles), chromosomes are equally segregated into two "poles" of the cell, and the cell divides

b) armed with this new knowledge that the cell cycle consisted of four phases instead of two, researchers began to question what the actual purpose of the cell cycle was in a general sense, and in particular, what was going on during the "mysterious" phases of G₁ and G₂

1. in the broadest sense, the cell cycle is the highly-regulated, uni-directional process of preparing a cell for propagation of its genetic material and for division, both being absolutely crucial during embryonic development, and later, for the growth and maintenance (and reproduction!) of the organism throughout its lifetime

(a) minimally, four "things" have to happen to make the cell cycle possible...which probably explains why it is composed of four distinct phases:
**Cell Cycle Effects of Radiation**

- (Hopefully accurate) Replication of the DNA, followed by packaging into chromosomes
- Reproduction of the centrioles that anchor the cell division apparatus
- Assembly, followed by disassembly, of the mitotic “machinery”
- Generation of sufficient mass and energy reserves to power the above processes


During growth, eukaryotic cells continually progress through the four stages of the cell cycle, generating new daughter cells. In most proliferating cells, the four phases of the cell cycle proceed successively, taking from 10–20 hours depending on cell type and developmental state. During interphase, which consists of the G1, S, and G2 phases, the cell roughly doubles its mass. Replication of DNA during S leaves the cell with four copies of each type of chromosome. In the mitotic (M) phase, the chromosomes are evenly partitioned to two daughter cells, and the cytoplasm divides roughly in half in most cases.

c) So is *that* the whole cell cycle story? **Answer:** Nope!

1. the idea that certain subpopulations of G1 cells were actually quiescent (temporarily out of the cell cycle, but still clonogenic and able to be recruited back into cycle when needed) was first introduced by Patt *et al.* in 1968—**these were called G0 cells (when talking about normal tissues) or Q cells (when talking about tumors)**

2. it was also clear that, **while some quiescent cells were recruitable back into cycle, most were not because they were terminally differentiated and accordingly had permanently lost reproductive capacity**
Cell Cycle Effects of Radiation

Cell Cycle and Differentiation of Normal Tissue Stem Cells

Normal tissue stem cells have 2 defining properties:

1. self-renewal
2. differentiation

Cell Cycle and Differentiation of “Cancer Stem Cells”

Cancers can follow the clonal evolution model or the cancer stem cell model (or may be a mix of the two models). Emerging evidence suggests that cancer stem cells may be resistant to radiation.

Reya et al Nature 2001
d) What was going on radiobiologically during these early years of study of the mammalian cell cycle? Answer: Lots of stuff, both conceptually and technologically (much of which proved quite useful in our subsequent understanding of the molecular biology of the cell cycle some 20 or more years later!)

### Age Response Through the Cell Cycle

1. Cells at different positions in the cell cycle have different sensitivities to radiation—this was first noted by Terasima and Tolmach, and subsequently studied exhaustively by Sinclair (1960s).

![Graph showing cell survival and DNA synthesis](image)

The upper curve shows the fraction of cells surviving reproductively after receiving 300 rads of x-rays administered at different times in the division cycle, in which mitosis is taken as zero hours. Each symbol represents a separate experiment, the timing of which have all been normalized to a minimum interdivisional time of 18 hours. The lower curve shows the pattern of DNA synthesis found in 3 separate experiments with synchronized cells. The fraction of cells showing incorporation of H³-thymidine after 20 minute sojourns in medium containing this tracer is plotted against the time after mitosis, normalized as in the upper curve.

(a) In order to do these types of experiments, synchronized populations of cells are needed; how the heck did they accomplish this back in the early 1960's?

1. **Answer:** the Mitotic Selection Procedure (also developed by Terasima and Tolmach)
   
   - this easy (in principle) method of synchronization takes advantage of the fact that cells in mitosis "round-up" and partially detach from the surface of a culture vessel; by shaking such a flask or petri dish, mitotic cells are dislodged into the media and can be collected
   - by repeatedly shaking flasks, collecting medium, adding new medium and shaking again at short time intervals, lots of mitotic cells can be collected with a purity of 95% or better
Cell Cycle Effects of Radiation

Interphase Cell  Mitotic Cell

- by leaving the cell suspension in an ice bath, cells are prevented from completing division, but as soon as cells are returned to incubator temperatures, they progress into G1, and around the rest of the cell cycle in a synchronized manner.

Radiation Age Response: General Findings

Cells in mitosis are the most sensitive to ionizing radiation, with G1 cells not far behind.

Cells are most resistant in S phase, particularly late S phase.

Cells in G1 phase are usually of intermediate radiosensitivity, although sensitivity can vary somewhat at different points in G1.

Also, please note the “dip” in radiosensitivity at the boundary of G1 and S phase, that is more pronounced for human HeLa cells than rodent CHO cells. This feature is most prominent for cells with long cell cycle times (and in particular, a long G1 phase duration).

Age-response curves for cells with short G1 phase, represented by hamster cells (A), and cells with long G1 phase, represented by HeLa cells (B). The time scales have been adjusted so that S phase has a comparable length on the figure for both cell lines. (From Sinclair WC, Dependence of radiosensitivity upon cell age. In Proceedings of the Carmel Conference on Time and Dose Relationships in Radiation Biology as Applied to Radiotherapy, pp 97-107. BNL Report 50203 (C-57). Upton, NY, 1969.)
2. How does the age response function manifest itself in terms of complete cell survival curves (as opposed to cell survival following a single dose)?

![Age Response Effects in Synchronized Chinese Hamster Ovary Cells Exposed to Graded Doses of X-Rays](image)

- **G2/M cells** have steep survival curves (low D₀'s) and little or no survival curve shoulders.
- **late S cells** have somewhat shallower survival curves (higher D₀'s), but mainly, have very broad shoulders in the low dose region.

3. Why is there an age response through the cell cycle? the answer: nobody knows for sure!

   - there are some tempting possibilities however --

   1. **"the DNA hypothesis"**: the idea being that the times of maximum and minimum sensitivity to a given dose of radiation also correspond to times of major DNA conformational changes (i.e., in M phase, DNA is in the process of being condensed into chromosomes, and in S phase, it is being unwound, partially stripped of chromatin and split into single strands); the suggestion is that DNA may have conformations more or less susceptible to radiation damage, or more or less accessible to repair enzymes.

   2. **"the radioprotector hypothesis"**: there is also a correlation between the changing radiosensitivity through the cell cycle, and the varying levels of naturally occurring sulphydryl compounds in the cell; glutathione, a major cellular sulphydryl, is known to be a potent radioprotector and is at its highest intracellular concentration during S phase.

   3. "the DNA repair hypothesis": the newest proposed explanation for the cell cycle age response, albeit still not the whole story...the idea being that cells in G₁ phase repair DNA double strand breaks using non-homologous end joining, an inherently error-prone process, whereas cells in S phase repair double strand breaks using homologous recombination, an error-free process, theoretically; this might explain the greater radioresistance of S phase cells compared to G₁.
Cell Cycle Effects of Radiation

A conceptual model of cell cycle variation in radiosensitivity and DNA repair. The solid line represents variation in radiosensitivity. The dashed line represents the relative contribution of NHEJ and the dotted line indicates the relative activity of HR. The positions of the checkpoints are indicated by the check boxes.


4. Implications of the Age Response Function for "Radiosensitivity" of Cell Populations

a. the survival curve for a heterogeneous population of cells with respect to cell age is actually a composite of many different survival curves

b. the overall shape of the curve depends on the relative proportions of "sensitive" and "resistant" subpopulations

**RADIATION-INDUCED “DIVISION DELAY”**

1. Division delay (sometimes called “mitotic delay”) is an operationally-defined phenomenon characterized by a delay time in the expected appearance of mitotic cells in an exponentially growing population of cells after irradiation, with the duration of the delay increasing with increasing dose

The effects of radiation on the progression of cells into mitosis after the treatment. At time zero, the cells are placed in medium containing colcemid, a drug that arrests cells in mitosis, and the percentage of cells that accumulate in mitosis is plotted as a function of time. The decline in the curves at late times is a result of cells escaping the drug-induced block or dying. The mitotic delay due to a radiation dose of 5.5 Gy displaces the curves for the radiation-treated cells to the right.

Cell Cycle Effects of Radiation

Changes in the number of mitoses (as a percentage of the mitotic index before irradiation) in chick fibroblasts after irradiation by radium-\(\gamma\)-rays at a dose rate of 20 Gy h\(^{-1}\).

a. Division delay is a consequence of radiation-induced slow-downs in transit through cell cycle phases (occurs mostly when doses are very high) and/or transient blocks at one or more discrete points upstream of mitosis (occurs even at low doses)

b. \(G_2\) Block/Delay: historically, the major, and most studied, upstream block responsible for division delay occurs in mid-to-late \(G_2\) phase, at a point called – back in the pre-molecular biology days – the "X-ray transition point" (XTP)

1) All cells, regardless of what phase they were in at the time of irradiation, and whether they will ultimately live or die, experience this block

2) The duration of the \(G_2\) block is cell age dependent, i.e., cells irradiated at an "age" closer to the block point (late S and early \(G_2\)) experience a longer delay than "younger" cells (\(G_2\) or early S)...however, on average, the duration of the \(G_2\) block is 1-2 hours per Gy

Cells are irradiated when in different phases of the cell cycle and the mitotic delay observed is plotted as a function of radiation dose.

Delay in cell division measured in populations x-irradiated at the times after mitosis indicated. The time scales have been normalized to a minimum interdivisional time of 18 hours for each experiment, each denoted by a different symbol.

From: Biophys J 3, 11-33, 1966


a) the early 1970’s was a banner time for further study of the cell cycle with the development of the flow cytometer, a fluorescence-based, analytical device capable of distinguishing (and ultimately, sorting) cells in different cell cycle phases on the basis of DNA content.

DNA distribution analysis using flow cytometry involves staining cells with a fluorescent dye that binds stoichiometrically to DNA, followed by passing a stream of stained cells through a laser beam that excites the dye and emits a fluorescent signal. The signal is quantified for each cell, and a frequency histogram generated, that corresponds to the number (or fraction) of cells in each phase of the cell cycle. Phase durations can also be determined.

The (relative) ease, versatility and high throughput possible with flow cytometry has led to it replacing many of the older cell cycle kinetic techniques.

From: Gray and Darzynkiewicz, Techniques in Cell Cycle Analysis, 1987
Cell Cycle Effects of Radiation

b) thanks in part to the development of the flow cytometer, it became possible to do “higher resolution” studies of the cell cycle, including measuring the appearance and disappearance of key proteins specific to certain cell cycle phases

1. some key findings in this regard were:

   • that the cell cycle is “distributed” probabilistically, i.e., that, even for a synchronized, homogeneous population of cells, phase durations and overall cell cycle times vary somewhat around an average value (practical implication: that cells don’t stay “synchronized” for long)

   ![Uniform cycle](image1)
   ![Distributed cycle](image2)

   • that the variability in cycle times for different cell types was largely due to differences in the duration of G1 phase (most of the other phases are fairly uniform in duration)

   • that the resting, G0 phase of normal tissue cells was not necessarily the same as that for tumor cells; the former occurs under normal physiological conditions, but the latter may be due more to nutrient deprivation than some inherent genetic programming

2. in addition, researchers began to wonder what some of these proteins were that appeared and then disappeared as cells moved through different phases, i.e., were they actually controlling cell cycle progression or a consequence of it?

c) finally, a new radiation-induced cell cycle block was identified: one located near the border of G1 and S phase, called the Gs Block (’d uh!); this likewise contributes to the overall amount of division delay experienced by the population as a whole

1. however, this Gs block was NOT noted for all cells, but rather, was observed preferentially in slowly- or non-cycling, untransformed cells (i.e., in most cases, tumor cells did not show this block)

Delay in the entry of serum-stimulated, normal human amnion cells into S phase after prolonged quiescence.

There was a dose-dependent decrease in the rate of flow of cells from G0 into S phase for doses as low as 10 cGy (not shown), and certainly for doses 1 Gy or higher. After 10 Gy, cells remained blocked in G1 phase for at least 10 days!

The Modern-Day Cell Cycle

**Cell cycle checkpoints**: Control mechanisms to ensure that the next step in the cell cycle does not proceed until the specific molecular events have occurred to complete the current phase of the cell cycle. Many of the cell cycle checkpoints are related to the status of DNA (DNA damage) and can be triggered by radiation.
To move from one phase of the cell cycle to the next, proteins are phosphorylated by a complex of a **cyclin** and a **cyclin-dependent kinase** (CDK):

- **G1**: Cyclin D-CDK4 or CDK6
  - Cyclin E-CDK2
- **S**: Cyclin A-CDK2
  - Cyclin A-CDC2
- **M**: Cyclin B-CDC2

Levels of cyclins change across the cell cycle.

How does radiation cause cell cycle arrest?

**CDK Inhibitors** are up-regulated by radiation and activate cell cycle checkpoints.
**Cell Cycle Effects of Radiation**

**G1 Checkpoint:**
Prevents S phase entry until after DSB is repaired.

Radiation activates ATM and CHK2 protein kinases that phosphorylate p53 protein and increase p53 protein levels. p53 increases p21 levels that block cyclinE-cdk.

\[
\text{IR} \rightarrow \text{ATM} \rightarrow \text{CHK2} \rightarrow \uparrow \text{p53} \rightarrow \uparrow \text{p21 (cdk inhibitor)}
\]

**CDKs are regulated** not only by the levels of cyclins and CDKIs, but also by the phosphorylation status of the CDK.

**G1 Checkpoint:** For CDK2 to be active, CDK2 must also have an inhibitory phosphate removed by the Cdc25A phosphatase. CHK2 phosphorylates Cdc25A and targets it for degradation. Without CDC25A, CDK2 retains an inactivating phosphorylation.

\[
\text{Inactivating} \quad \rightarrow \text{Degradation} \quad \rightarrow \text{E-CDK2} \rightarrow \uparrow \text{p53} \rightarrow \uparrow \text{p21 (cdk inhibitor)}
\]
CDKs are regulated by the phosphorylation status of the CDK to control entry into mitosis.

Passage through mitosis requires high levels of cyclin B and active Cdc2. For Cdc2 to be active, specific amino acids must not have phosphate groups. This adds another layer of regulation with phosphatases (like Cdc25c) that remove phosphate groups to activate Cdc2 and kinases (like Wee1) that add phosphate groups to inactivate Cdc2.

The phosphorylation of CDKs can change after radiation, which is another way that radiation can alter cell cycle progression.

Question: Why do most tumor cells not have a G1 checkpoint after radiation?

Summary:
1) Cell cycle progression depends on the activity of cyclin-CDK complexes
   a) Cyclin levels change in different phases of the cell cycle
   b) CDKs are also regulated by phosphorylation
   c) CDKI’s block cyclin-CDK activity
2) DNA damage response to radiation can alter CDK activity through these layers of regulation