We have seen the enemy, and he is us!

—Churchy LeFemme, aka Walt Kelly

Radiation, Cell Cycle, and Cancer

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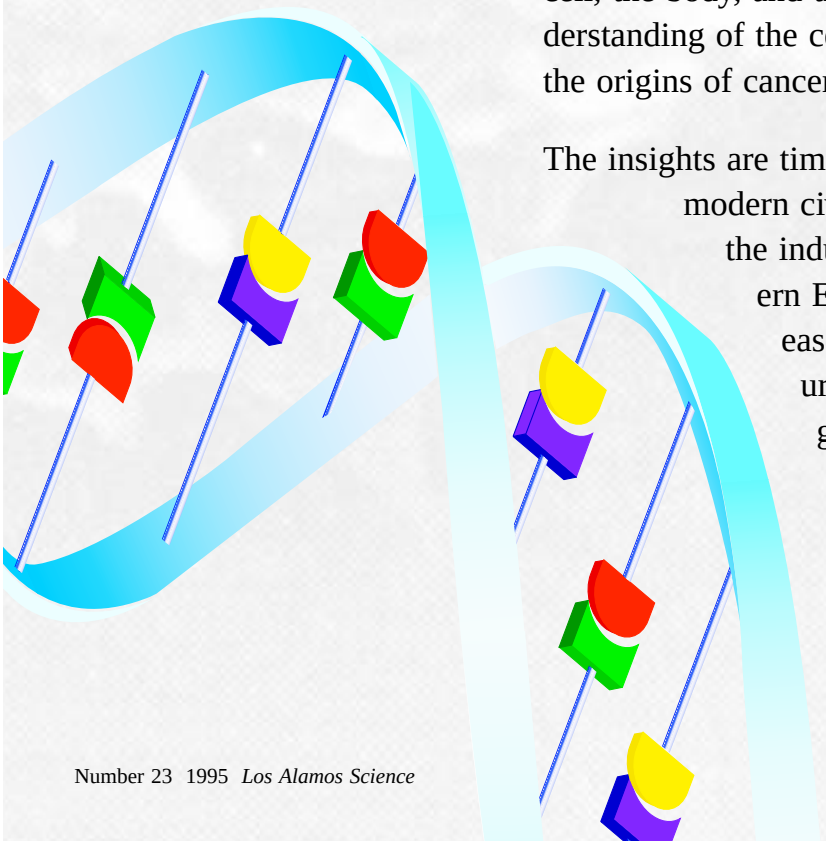
We live in remarkable times. The DNA within our cells—the entire human genome—is steadfastly being mapped and deciphered. That work combined with new results from molecular and cellular biology are enabling researchers to reconstruct the inner workings of cells in unprecedented detail. We are beginning to build a holistic framework for understanding the human organism, one that integrates the distinct yet interrelated roles of DNA, genes, the cell, the body, and the environment. With it comes a better understanding of the cellular origins of many diseases, including the origins of cancer.



The insights are timely. Cancer is one of the great scourges of modern civilization, for roughly one in five people in the industrialized nations of North America, western Europe, and Asia will die of it. It is a disease of the cell that develops because of failures in the mechanisms that regulate cell growth. An individual cell multiplies without restraint until it and its progeny eventually overwhelm tissues and organs. What initiates this process and how it progresses

has been the subject of theoretical and experimental investigation almost since the start of medical research. It has led to the identification of various cancer-causing substances, or *carcinogens*, in our diet and within our environment.

Ionizing radiation* is one of those carcinogens, and its ability to induce cancer is not in doubt. The tragic experiences of the radium-dial painters during the early part of this century and the sobering epidemiological studies of the atomic-bomb survivors of Hiroshima and Nagasaki bear witness to the fact that ionizing radiation can instigate a variety of cancer types. The bomb survivors, for example, display a small but statistically significant increase in the level of several cancers, including leukemia, breast, thyroid, and skin cancer. Radiation and cancer definitely correlate.

How does ionizing radiation cause cancer? How can a brief interaction with invisible particles smaller than an atom or the transient passage of massless electromagnetic waves cause a smoothly functioning, exceptionally well-organized cell to spiral chaotically out of control? Our cells for the most part are stable and predictable entities, yet exposing them to levels of radiation well below the lethal dose can induce behavior that will eventually lead to the death of an entire organism. How does this happen?

Answering these questions has proven to be extraordinarily difficult. Even today, the causes of cancer and the many ways the disease can progress are not completely understood. In the absence of a complete understanding, it has not been possible to determine the exact role that ionizing radiation plays in cancer induction. Nevertheless, a basic understanding does exist. Ionizing radiation can damage the DNA of chromosomes and potentially mutate the genes that reside on those chromosomes. Because genes ultimately dictate cell function and behavior, ionizing radiation, through its capacity to induce genetic mutations, can bring about a change in the basic nature of the cell. The cell becomes *transformed*, meaning that it is aberrant and is slowly evolving into a cancerous state.

Although this picture is correct, it is somewhat superficial. It does not take into account the rate of DNA damage or the particular type of damage that ionizing radiation induces, nor does it account for the powerful DNA repair mechanisms that help maintain the genome. It does not reveal that healthy cells have "defenses," or cellular responses, that can limit excess proliferation and prevent cancer from developing. Augmenting the basic picture and elucidating what is known specifically about radiation and *oncogenesis* (the causes of tumor formation) is the main objective of this primer. In attaining that goal, we will spend a considerable amount of time building concepts and vocabulary, beginning with *genes* and *gene expression*. We will relate gene expression to cell function and then expand upon the nature of cell regulatory processes. We will learn that once a cell has become transformed by some random, initial event, its progression towards cancer will be driven by the abnormal behavior or removal of specific, critical proteins. We will learn that within that set of "cancer-causing" genes, some are specifically correlated with DNA damage induced by ionizing radiation.

*We will restrict our attention to *ionizing raditation* in this primer, that is, only nuclear emissions and x rays. Effects due to lower-energy electromagnetic radiations, such as ultraviolet radiation and emissions from power lines, will not be considered.

Genes and Gene Expression

We are what we are because of our genes. This notion, along with the realization that DNA is the molecular carrier of heredity, are two of the seminal discoveries of modern science. It has been discovered that a gene is composed of a specific DNA sequence, and gene sequences are distributed throughout our chromosomes (see "DNA, Genes, and Protein Synthesis"). Each chromosome is a single, long DNA molecule that is woven around a complex protein structure. Every person inherits a set of 23 chromosomes from each parent, and for every chromosome passed to us by our mother, there is a corresponding chromosome contributed to us by our father. The 46 chromosomes that compose the human genome can be arranged into 22 pairs of matching, or *homologous*, chromosomes, plus one pair of sex chromosomes—the X and Y chromosomes. Females possess an XX pair, whereas males possess an XY pair (Figure 1). Because each chromosome in a homologous pair contains the same set of genes, our cells have two copies, or *alleles*, of every gene. The DNA sequences of two alleles are usually very similar but not identical—each contains information from one of the two parents. What happens when a cell makes use of dissimilar gene copies?

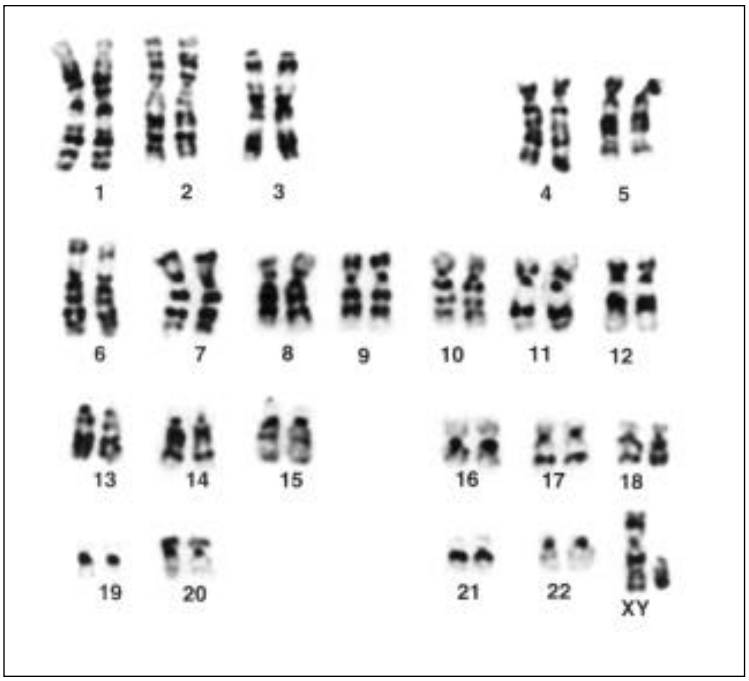


Figure 1. The Human Genome
The human chromosomes in this photograph were arranged to show the 22 pairs of homologous chromosomes, plus the one pair of sex chromosomes (lower right). The original photo was taken when the chromosomes had assumed their most condensed state. (For most of the life of a cell, a chromosome is in a very loose, threadlike form.) The chromosomes shown above were treated with a dye (Giemsa stain) that preferentially stains certain regions and thereby produces the unique banding patterns that are used to identify each chromosome. Because the two sex chromosomes are different (X and Y), or not homologous, the genome shown is that of a male, namely the well-known cytogeneticist T. C. Hsu of the University of Texas System Cancer Center. (Photo courtesy of T. C. Hsu.)

This question relates to gene expression, which was first systematically investigated by Gregor Johann Mendel (1822-1884), the "father" of modern genetics. Over the course of eight years, Mendel manipulated the breeding of several purebred strains of garden pea plants. He noted the manifestation of certain characteristics of the plants, say flower color or pea texture, and how often those traits appeared in each successive generation. From his observations, he was able to deduce the statistical laws of inheritance, using as a hypothesis the existence of two inherited "units" for each trait. (Mendel's units of heredity are what we now call genes. He used the word "Merkmale" to describe the units of heredity. The word gene was coined by the Dutch botanist Wilhelm Ludwig Johannsen (1857-1927).) Mendel was also able to deduce when certain traits would be observed, or expressed.

Take for example the trait flower color. Mendel found that a pea plant has a "gene" that dictates flower color, and that the gene has two "alleles," one for violet flowers and one for white flowers. He also found that the violet allele had a *dominant* mode of gene expression, that is, only one violet allele had to be present for the flowers to be violet. In contrast, the white allele had a *recessive* mode of expression, that is, both flower-color alleles had to be white for the flowers to be white.

Mendel's basic concepts about gene expression have been greatly expanded. The term "gene expression" is now used to describe the manifestation of traits at the molecular and cellular level. Expression begins with the processes of gene *transcription* and *translation* in which the DNA sequence that makes up a gene is used as a template to synthesize a protein (see "DNA, Genes, and Protein Synthesis"). That protein then produces certain observable characteristics in the cell. Thus a gene is said to be "expressed" when the protein that it specifies is actually synthesized and functioning in the cell.

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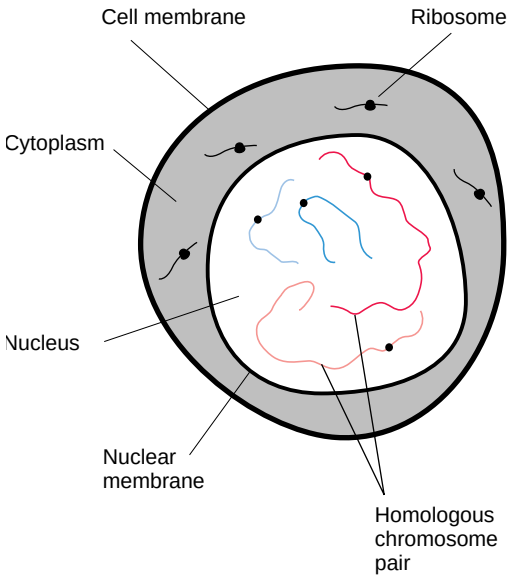
DNA, Genes, and Protein Synthesis

The cell is a marvelous ensemble of proteins, organic molecules, and organelles. Although it is on the order of ten microns or so in diameter, a cell is an incredible chemical factory with the capability to synthesize more than 10,000 different proteins and enzymes and the ability to oversee thousands of simultaneous chemical reactions. Figure A is a simple depiction of a mammalian cell in which we've selectively drawn only a few basic components (not to scale). The cell boundary is defined by an outer, bilayer lipid membrane. The cell interior is filled with an aqueous colloidal fluid called the cytoplasm. Floating within the cytoplasm are thousands of proteins and large, macromolecular structures. We've indicated a ribosome, which is a protein complex required for the synthesis of proteins. We've also conspicuously highlighted the cell nucleus, which houses all of the nuclear DNA (our genome).

Figure A. Basic Components of the Cell



If the DNA molecules found in a human cell were laid end to end and stretched out, the resulting line, though only 2 billionths of a meter wide, would be about two meters long, or about 200,000 times longer than the cell itself. Therefore, our DNA is packaged into dense constructs called chromosomes, each of which consists of a single, linear DNA molecule containing millions of base pairs. The DNA is twisted and packed around proteins called histones, and that structure is itself twisted into a secondary packing structure. There are at least four levels of twisting and packing, but the degree of packing and the chromosome appearance can vary, depending upon both transcriptional activity (described below) and the stage of the cell's reproductive cycle.

Human beings have a total of 46 chromosomes. Two of those chromosomes, called X and Y, determine the sex of the person. All males have an XY combination, whereas all females carry an XX combination. The other 44 chromosomes can be grouped into 22 pairs of “homologous" chromosomes. The individual members of each pair are very similar, but one is inherited from the mother and the other is inherited from the father (see Figure A and main article). For simplicity, we have depicted only four chromosomes, representing two homologous pairs.

As shown in Figure B, the double-stranded DNA that makes up a chromosome consists of two single-stranded molecules that are intertwined to form a double helix. The backbone of each single strand is a long chain consisting of repeating sugar-phosphate subunits. The sugars appear as pentagon-shaped rings in Figure B. (DNA is an acronym for deoxyribose nucleic acid. Deoxyribose is the particular type of sugar.) The sugar portion contains five carbon atoms, labeled 1' to 5', and the backbone is constructed by linking, through a phosphodiester bond, the 5' carbon of one sugar to the 3' carbon of the next. Because of the asymmetry in the phosphodiester linkage, the *phosphodiester backbone*, as it is often called, can be assigned an orientation, either 5' to 3' or 3' to 5'. The two strands of the DNA double helix actually have opposite orientations. One strand can be said to move “up,” whereas the other moves “down.” Many proteins that interact with DNA are sensitive to this orientation and can distinguish one strand from the other.

Attached to each sugar unit is one of four different nucleic acid bases: adenine (A), cytosine (C), guanine (G), and thymine (T). The bases can be further classified as purines (A and G) or pyrimidines (C and T). In forming the double helix, the bases will line up between the two DNA backbones, a base in one strand pairing with an opposing base in the complementary strand. The base pairs are chemically linked by hydrogen bonds. In the standard Watson-Crick base pairing, each pair must be

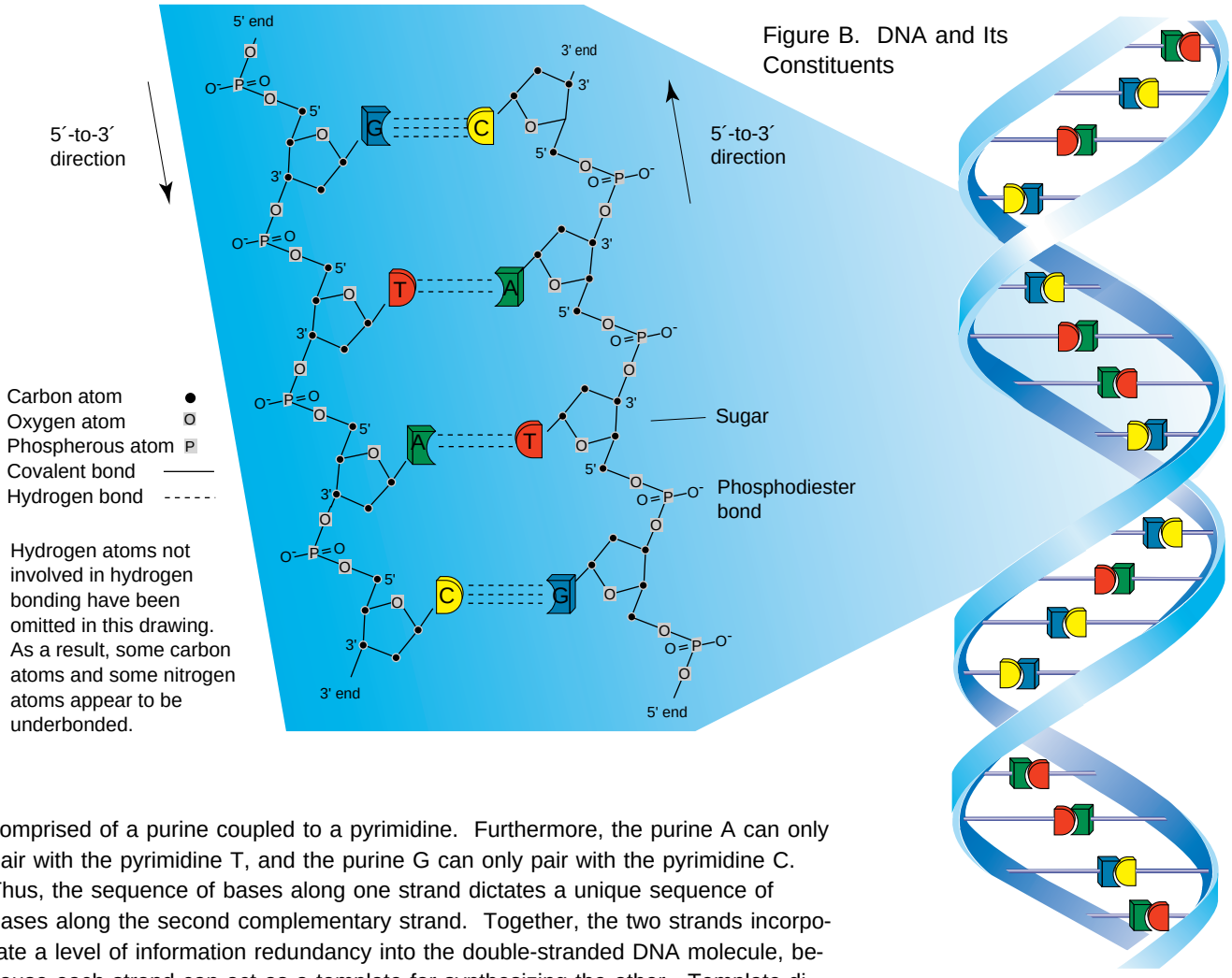


Figure B. DNA and Its Constituents

comprised of a purine coupled to a pyrimidine. Furthermore, the purine A can only pair with the pyrimidine T, and the purine G can only pair with the pyrimidine C. Thus, the sequence of bases along one strand dictates a unique sequence of bases along the second complementary strand. Together, the two strands incorporate a level of information redundancy into the double-stranded DNA molecule, because each strand can act as a template for synthesizing the other. Template-directed copying of each DNA strand is called replication.

The information encoded within the DNA molecule enables the cell to synthesize proteins. A gene, depicted schematically in Figure C, is that segment of DNA that codes for a single protein, and our genome contains roughly 50,000 to 100,000 genes dispersed among the 46 chromosomes. Because the overwhelming majority of cell processes are carried out by proteins, a cell goes to great lengths to ensure that the integrity of the base sequence is maintained. This is the primary role of DNA repair mechanisms (see “DNA Repair” on page 78).

To translate the information encoded by DNA into a protein product, the cell must go through a multistep process. First, the coding region of a gene is read, or transcribed, into a copy of the DNA sequence. The copy takes the form of a molecule of RNA, which is similar, with a few differences, to a single-strand of DNA. After some processing, the RNA will leave the nucleus and enter the cell's cytoplasm. The information contained in the RNA will be translated by a ribosome, a large macromolecule that guides the assembly of amino acids into the protein product.

Gene segments range from thousands to millions of base pairs in length. Therefore, Figure C depicts the DNA as a solid bar containing different subregions. We have indicated the coding region, which contains the actual sequence used for protein synthesis, and two regulatory DNA sequences that are used to control the

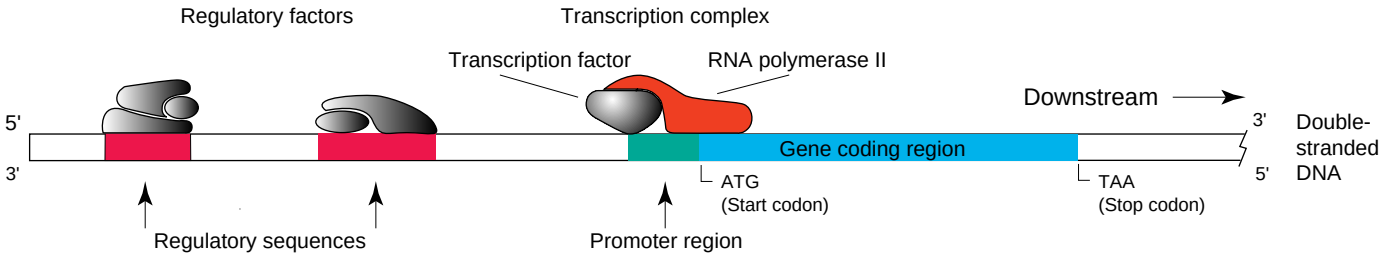


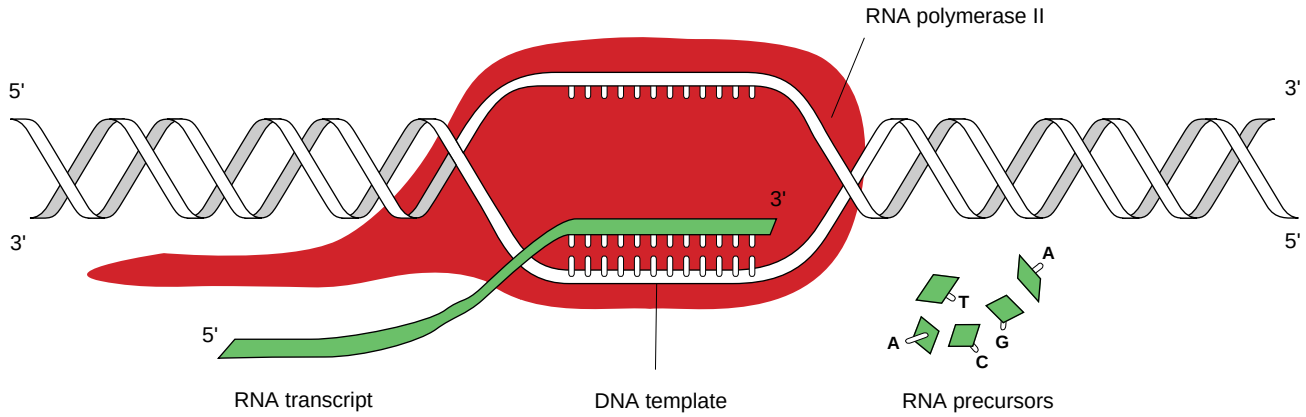
Figure C. Gene Structure and Transcription

rate and frequency of transcription. The double helix is actually a fairly open structure that permits access to the chemical groups of the DNA bases. Proteins called *regulatory factors* will recognize these groups and selectively bind to specific DNA sequences. By physically distorting the helix (bending and folding the DNA strands) or by promoting protein-protein interactions, the regulatory factors can either facilitate or inhibit transcription. The regulatory regions may be far removed from the coding region and may even be located "downstream."

Transcription of the DNA sequence into an RNA copy is initiated at the promoter region, which also contains a specific DNA sequence (TATA) that is recognized by a *transcription factor*. This factor is a protein that binds to the DNA and initiates the self-assembly of a transcription complex consisting of perhaps 10 or more proteins, including RNA polymerase II (RNA Pol II). The RNA Pol II complex will transcribe the DNA coding sequence. Thus, the initial step in creating a protein is tied to the presence (or sometimes the absence) of transcription factors and regulatory proteins. That is one way the cell has of regulating the expression of a gene.

As depicted in Figure D, RNA Pol II instigates the unwinding of the DNA double helix, which enables it to "read" and transcribe one of the two DNA strands. Because of the Watson-Crick base-pairing rules, the RNA molecule that is produced contains all of the information that was originally encoded in the DNA strand. As RNA Pol II moves along, the relaxed strands of previously transcribed DNA sections rewind. After the gene has been completely transcribed, RNA Pol II will leave the DNA and some processing of the RNA molecule occurs. The resulting RNA strand (now called messenger RNA, or mRNA) leaves the cell nucleus and will be used as the template for protein synthesis.

Figure D. Transcription of DNA to RNA



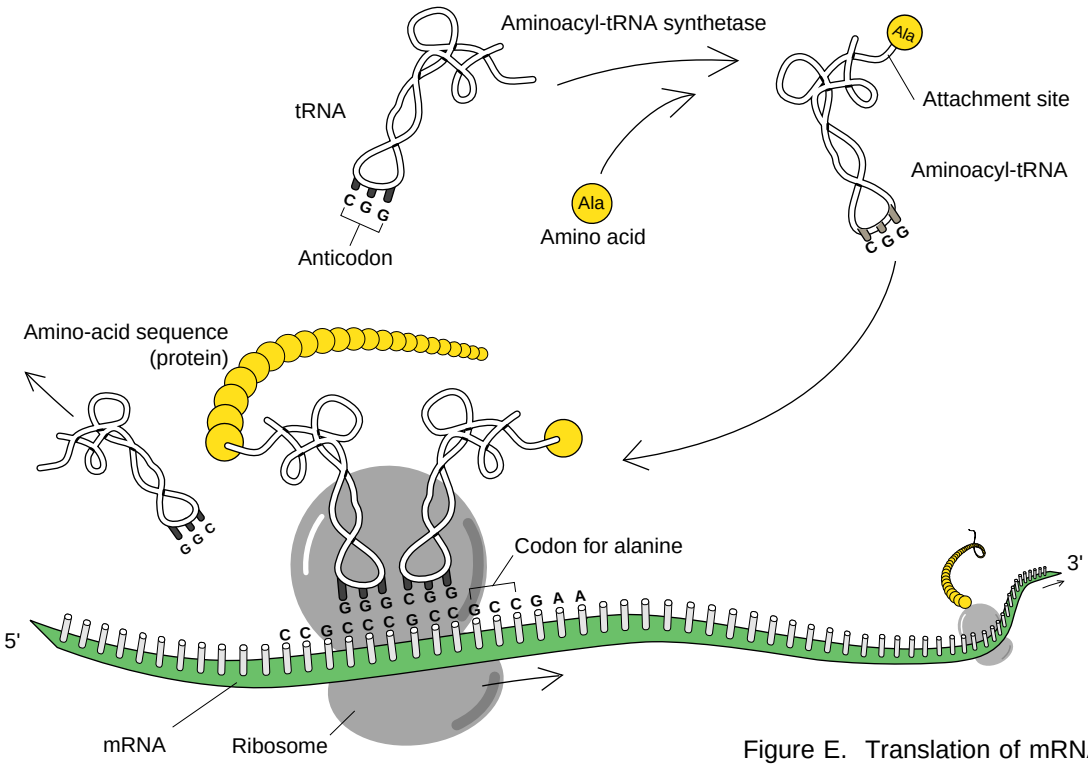


Figure E. Translation of mRNA to Protein

The process of converting the mRNA template into an actual protein is called translation, as shown in Figure E. Translation takes place at the ribosome. The sequence of RNA bases contained in the mRNA transcript is interpreted as a series of "words," or codons, consisting of three consecutive RNA bases. With some exceptions, each codon corresponds to a specific amino acid. These are the small molecules from which proteins are constructed. For example, the DNA base sequence GCC codes for the amino acid alanine. The exceptions are three *stop* codons (TAA, TAG, TGA) that are used as punctuation and indicate the termination of an amino-acid sequence.

A molecule called transfer RNA (tRNA) is the actual link between a codon and an amino acid. One end of the tRNA has an "anticodon" that pairs according to Watson-Crick rules with a codon in the mRNA template. The other end of the tRNA is bound to an amino acid that corresponds to that codon in the mRNA template. The top of Figure E shows the reaction that places the correct amino acid onto the corresponding tRNA. That reaction is catalyzed by a family of specific enzymes called the aminoacyl-tRNA synthetases. The ribosome facilitates the pairing of the anticodon region of a tRNA molecule to the mRNA codon and catalyzes the transfer of the amino acid to the growing protein chain. The ribosome steps along the mRNA molecule, adding an amino acid to the chain at each step, until it reaches a stop codon. At that point, the protein product is finished, and the ribosome detaches. Numerous ribosomes will often attach to the same mRNA, so that many copies of the same protein are produced for each DNA transcription event. It is clear, then, that DNA plays a critical role in protein synthesis. A single gene can get transcribed many times, and each time it is transcribed, many identical proteins are produced. If a gene coding for a major regulatory protein becomes mutated, then that single mutation can mean the difference between a normal and a dysfunctional cell. ■

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But a question remains. We have approximately 50,000 to 100,000 genes. Does every cell make use of all the genes that are encoded in its genome? The answer is no, and the reason has to do with a much more fundamental concept of gene expression—the notion of regulation. A gene embodies not only DNA sequences that code directly for protein construction but also regulatory sequences that control various aspects of the transcription process. Regulatory sequences include the *promoter* region, where transcription is initiated, and regions that control the rate and frequency of transcription. Those regulatory regions are recognized by *regulatory factors*, which are a class of proteins that bind to certain DNA sequences and either directly or indirectly (by attracting other proteins) inhibit or enhance transcription.

Therefore, the mere presence of a gene within our genome does not guarantee that it will be expressed. Instead, the production of a protein from the gene is dependent upon a very complicated relationship between DNA, regulatory proteins, and protein synthesis. In fact, the cell has at least six levels of control on gene expression, beginning with regulation of the promoter region and ending with the breakdown and removal of the protein product. Once produced, however, many proteins must first be activated by other proteins, form a complex, or both, before being able to play a part in cell processes. Protein activation and participation in protein complexes are but two examples of how a gene product can be regulated. The expression of a particular gene and the behavior of its protein product can therefore change due to a number of factors. Abnormal behavior can certainly be the direct result of a DNA mutation, but it may be expressed through a type of domino effect that links the action of one protein to the function of another.

Cell Differentiation

Cancer is a disease of cells, and human beings have lots of them. We are composed of approximately 10^{13} to 10^{14} individual cells, most of which are not identical. Instead, they have differentiated into roughly 350 types. Differentiation means that a cell has become specialized in function and structure and has compromised its independence and some of its capabilities in favor of being a cooperative member of a tissue and organism. Our cells, for the most part, are immobile, and therefore, the specialized cells in any given tissue depend heavily on other tissues to provide nutrients and basic resources, to remove waste, to create environmental stability, and to provide protection.

This interdependence is distinct from a single-cell organism. A free living cell is self-sufficient and behaves in a manner that best aids its own survival. Certainly, one survival mechanism is proliferative advantage. For example, the rod-shaped bacteria *Escherichia coli* can divide and produce two new bacteria every 30 minutes. In principle, then, *E. coli* has the reproductive capacity to produce well over 200 trillion progeny in just 24 hours!*

Clearly, the differentiated cells of a multicellular organism cannot exhibit this type of proliferative behavior, nor can they be insensitive to the needs of other cells. Instead, everything about a differentiated cell, including when it reproduces, its shape and size, and the chemicals and proteins it synthesizes, is essentially determined by the needs of the tissue and the organism of which that cell is a part. Ex-

*This number is a theoretical extrapolation. The actual number of bacteria that would be generated is limited by the availability of resources and by the necessity to remove heat and waste by-products.

erting its influence through a multitude of intricate, intercellular controls, the body ensures that the behavior of individual cells is directed towards sustaining the overall health of the organism. This paradigm of specialization is obviously a successful one that imparts survivability to all the cells of the body and to the entire organism.

How do cells differentiate? The process is only beginning to be understood. It is not simply that each specialized cell has a different set of genes, because all the cells of the body are genetic clones and possess the same genome. Nor does differentiation result from a change in the information content of the DNA or the amount of DNA present. Rather, specialization comes about because only a particular set of genes are expressed. Those expressed genes determine if the cell will be a nerve cell or a skin cell, if it is mobile within tissue (such as a macrophage), or if it grows slowly or rapidly. In one sense, the genome is analogous to a library that contains books on all subjects. When we wish to specialize our area of interest, we select from the library only those books that are appropriate for our needs and leave the other books undisturbed.

An *epigenetic* change within the genome—one that modifies gene expression without changing the information content of the DNA—appears to be the mode by which cells differentiate. Many differentiated cells pass their traits on to their progeny; that is, a liver cell begets a liver cell, which implies that the epigenetic changes to the genome are conferred to daughter cells. The transmission is believed to happen through a chemical modification of DNA sequences known as methylation. The methylation patterns are maintained during DNA replication, but the way in which they are originally established and the way they become modified is not fully understood.

However, not all cells are descendants of fully differentiated cells. Instead, the specialized cells of many tissues and organs originate from a class of relatively undifferentiated cells called *stem cells*. The successive progeny of stem cells display increasing degrees of specialization, and that process may continue for several cell generations. The stem cells are highly unusual. Their specific role in the tissue is to renew lost or damaged cells, but at the same time, they must maintain their own population. As illustrated in Figure 2, stem cells have the peculiar property of generating dissimilar progeny. One of the cells that is produced remains a stem cell, whereas the other cell begins to specialize in response to external signals. Those signals evidently help trigger the epigenetic changes. But each type of stem cell is slightly different, and the pathway of specialization that their progeny follow is also distinct. Thus, basal cells are ultimately the source of epithelial cells (those cells that make up the skin layers and the lining of the intestinal and respiratory tracts), whereas the hemopoietic stem cells are the precursors of about ten or more different cell types that make up the blood and the immune system.

As the progenitor of many tissue cells, the stem cells perform a function that highly differentiated cells have relinquished, namely repeated cell division. The terminally differentiated cells of the skin or the hemopoietic system are so specialized that they rarely divide. Stem cells renew those nondividing cells, and therefore, the role of the stem cells within the scheme of the organism is that of proliferation. Unlike *E. coli*, however, the reproductive potential of a stem cell is strictly regulated (as it is for all other types of cells). Cells will only multiply as a consequence of having received numerous extracellular signals. Growth factors, or *mitogens*, are positive regulators that stimulate proliferation. Other signals will inhibit growth and are considered to be negative regulators. Normally, cellular

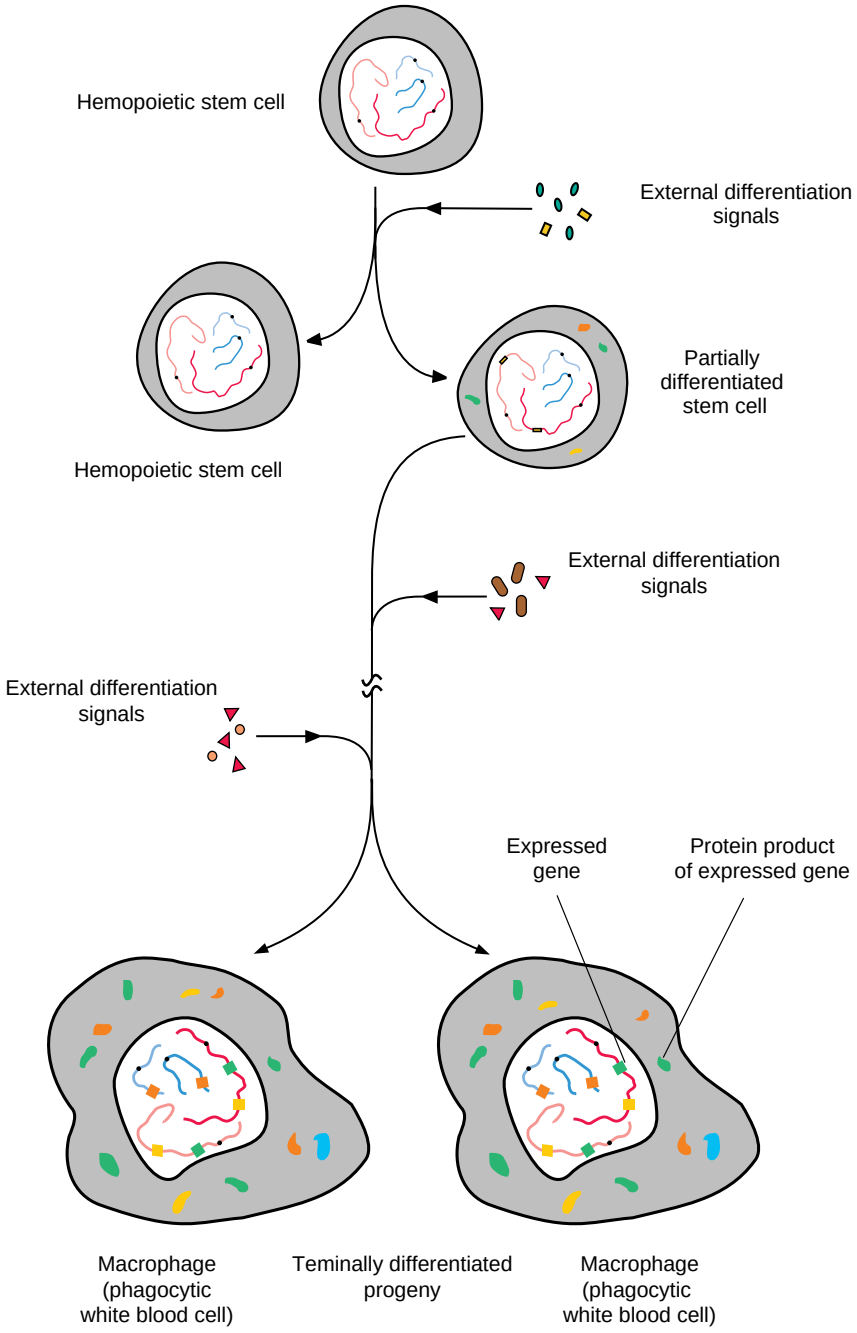


Figure 2. Cell Differentiation and Stem Cells

The hemopoietic stem cells are responsible for generating about a dozen different types of cells, including the various kinds of blood cells and the cell types that make up the immune system. The figure illustrates the generation of a highly specialized cell, the macrophage (a cell that lives within tissue and is descended from a white blood cell), from a relatively undifferentiated stem cell. The process of differentiation takes place over several cell generations, and it occurs because of the expression of different genes. When a stem cell divides into two cells, one of them will remain a stem cell. The other, in response to external signals, begins to specialize. This daughter expresses new genes (colored segments on the chromosomes) that are transcribed and translated into proteins (colored shapes in the cytoplasm). The new proteins modify the cell's function and appearance. The process of specialization continues through several more generations until finally a cell type reaches a terminal stage of differentiation. If a macrophage divides, both of its progeny will remain as macrophages. Because stem cells and their partially differentiated progeny divide frequently, cancers often emerge from those cell types.

proliferation is controlled by the cell's interpretation and response to these reciprocal types of regulation.

One of the major differences between a normal cell and a cancer cell is that the latter responds in an unbalanced manner to regulatory signals and proliferates at inappropriate times. A precancerous, or *neoplastic* cell, might undergo changes in the way it responds to regulatory signals, and it might divide independently of the needs of the tissue. If the modified behavior results in a proliferative advantage for a cell line, the uncontrolled growth can ultimately lead to the disruption of tissue function. Because stem cells are the most rapidly and frequently dividing cells in our body, they are in one sense "primed" to express proliferative advantages. Most cancers originate from the various types of stem cells or from the partially

differentiated progeny of stem cells. Because different sets of genes are expressed in those stem cell variants, the cancers that develop also differ from each other. They might present different characteristics and have altogether different consequences.

Proteins and Signal Transduction

We have stressed that cancer is a distortion of cell behavior and that a cancer cell differs from a normal cell largely because of malfunctions in processes that control cellular proliferation. From a mechanistic point of view, this translates into the failure of proteins to properly regulate what is called the *cell cycle*, which is the sequence of stages that a cell passes though when it undergoes reproduction. But the initiation and regulation of the cell cycle cannot be appreciated without a better understanding of how proteins work and how they can act as catalysts.

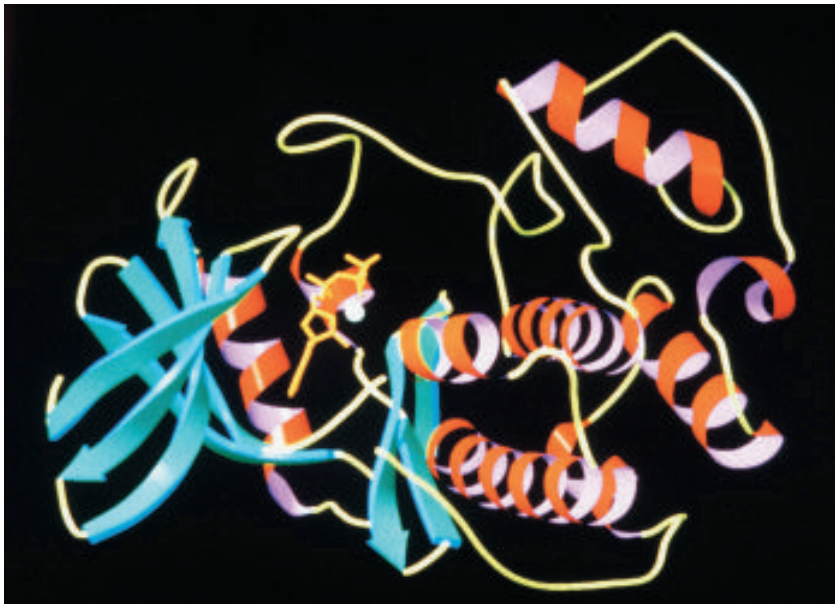


Figure 3. Cdk2 Protein

The cyclin-dependent kinases, or Cdk, are a major class of regulatory proteins of the cell cycle. This representation of Cdk2 shows, not atomic detail, but the amino-acid chain as a continuous ribbon. That depiction gives a feel for the protein's three-dimensional structure, which can provide insight into the protein's function. The catalytic binding site of the Cdk lies at the center of the protein. The cofactor, a molecule of ATP (yellow pentagon with branches), already lies within that binding pocket. A ligand will also bind to that site, and one of the phosphate groups making up ATP will be transferred to the ligand, thus bringing about its modification. Photo courtesy of Prof. Sung-Hou Kim, UC Berkeley. Reprinted with permission from *Nature* 363: 595-602 (© 1993, Macmillan Magazines Limited).

A protein consists of a chain of amino acids strung together like beads on a string. There are 20 common amino acids, which are distinguished from each other by a unique chemical side chain that is attached to a "core" carbon atom. Interactions between the various side chains will fold a protein molecule into a convoluted, three-dimensional shape. That shape is a critical feature that is central to a protein's function, for it affects the accessibility and position of individual amino acids. Typically, most of the protein merely serves as a means to configure a small subset of amino acids into a uniquely contoured region. That region, which is often in the form of a cavity or small protrusion, is called a *binding site.* Because of its unique shape, and because the amino acids that compose it have specific sizes, affinities, and chemical properties, the binding site allows only a select group of *target molecules*, called *ligands,* to interact and bind to the protein at that area. A ligand can be any type of ion or organic molecule, including proteins.

The binding site of a protein can simply allow the protein to adhere to its target molecule. For example, the binding site of a regulatory factor has an affinity for the exposed chemical groups of specific DNA sequences and thereby enables the factor to bind selectively to those particular segments of DNA. But a binding site often plays another, more ubiquitous role. It can hold a ligand within close proximity to another small molecule (a cofactor) for the sole purpose of facilitating, or catalyzing, a chemical reaction between those two molecules. Any protein catalyst is called an *enzyme.* Figure 3 is a computer-generated rendering of an enzyme, in this case, the protein Cdk2. This protein is one of several that are at the heart of cell-cycle regulation. Cdk2 is shown holding a molecule of ATP (the cofactor) within its binding site. ATP contains three phosphate groups, and Cdk2 will catalyze the transfer of one of those phosphate groups to a ligand that binds to Cdk2. The transfer and covalent attachment of a phosphate group to a target protein is called *phosphorylation,* and the enzymes that catalyze phosphorylation are called *kinases.* Thus Cdk2 is an example of a kinase.

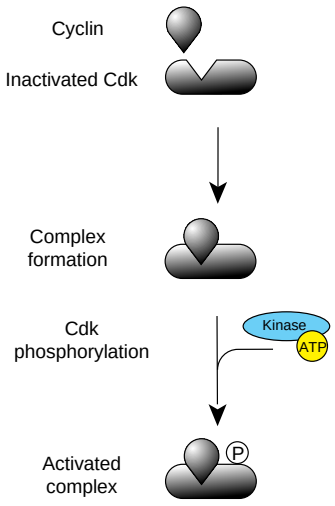


Figure 4. Activation of a Cyclin-Cdk Complex

The Cdk protein has a potential kinase activity that enables it to phosphorylate other proteins. To become an active kinase, the Cdk protein must first complex with a cyclin molecule. The binding initiates a structural change that enhances accessibility to the Cdk binding site and to the ATP cofactor that lies within that site. Once the complex has formed, activation still requires that the Cdk protein itself become phosphorylated. Cdk activity may also be regulated through phosphorylation at yet another site (not shown in the figure). Activation would then require removal of the inhibitory phosphate (dephosphorylation). The three separate steps of complex formation, phosphorylation, and dephosphorylation are the means by which Cdk is regulated. In reference to Figure 3, the cyclin binds to the Cdk along the blue ribbon area on the left side of the image.

Phosphorylation is a very common means of activating (or inhibiting) the function of a protein. Kinases, therefore, can play a regulatory role within the cell by helping to turn target proteins functionally on or off. The kinase itself may be regulated in that some type of protein interaction is required to activate its enzymatic capability. For example, Cdk2 is a *c*yclin-*d*ependent *k*inase, which means it must couple to a cyclin protein before it can catalyze phophorylation. (The cyclin family of proteins, like the Cdk, are major components of cell-cycle regulation. The cyclins will be discussed in the following section.) The joining with a cyclin modifies the Cdk protein and allows access to the Cdk's binding site (also called its binding pocket). But complex formation with a cyclin is only a required first step in activating the kinase activity of a Cdk. The states of phosphorylation at two distinct sites are also involved. The phophate group at one site keeps the binding pocket "open," whereas the phosphate group at the other site is an inhibitor of the Cdk's kinase activity. Thus, Cdk activation requires cyclin binding, phosphorylation at one site, and the absence of a phosphate group at a second site. A cyclin-Cdk complex and its activation are illustrated in Figure 4.

Proteins play equally important roles in the link between extracellular conditions and the cell-cycle control elements. When a cell initiates a new reproductive cycle, it is usually in response to external growth signals. These signals are relayed into the nucleus through a chain of interacting proteins that form a *signal transduction cascade.* The chain will convert the growth stimulus, which may be in the form of a hormone or a mitogen, into an action that is carried out by the cell. Often, this action is manifested through the transcription and expression of specific genes. Figure 5 shows a simplified version of one such cascade, in which a cell in a tissue has been stimulated to grow by a mitogenic signal. The mitogen may have been excreted into the extracellular medium by other cells of the tissue or else may have been released from distant organs and transported to the tissue by way of the circulatory system. In either case, the signaling molecule will bind to protein structures embedded in the cell membrane called receptors.

A fairly common class of receptors, the tyrosine kinase-linked receptors, exert their influence through the phosphorylation of specific tyrosine amino acids on target proteins. These receptors have two functional regions. One region protrudes through the cell membrane and is exposed to the extracellular medium, whereas the other remains inside the cell's cytoplasm and carries a latent kinase activity. Once a mitogen has bound to the receptor, the cytosolic (or intracellular) part of the receptor becomes enzymatically active. It will phosphorylate its target, which will then go on to activate additional proteins.

What follows is a deliciously complicated series of chemical reactions—a multiplexed chain of events mediated principally by phosphorylation events—that will sequentially activate (or inhibit) subsequent proteins in the cascade. In our figure, we've indicated one such protein, pRas, which is a participant in many different cascades and is important for the induction of several types of cancer. What is important for our discussion now, however, is that our representative cascade terminates in the cell nucleus. There, the activation of one or more transcription factors will stimulate the transcription of their respective target genes. The proteins produced from those genes will then effect some sort of trait, which in our example would be the formation of protein complexes that herald the start of a new reproductive cycle.

A similar chain of events can occur when the cell intercepts an inhibitory signal, one that prevents growth and cell division. That signal might initiate a signal-

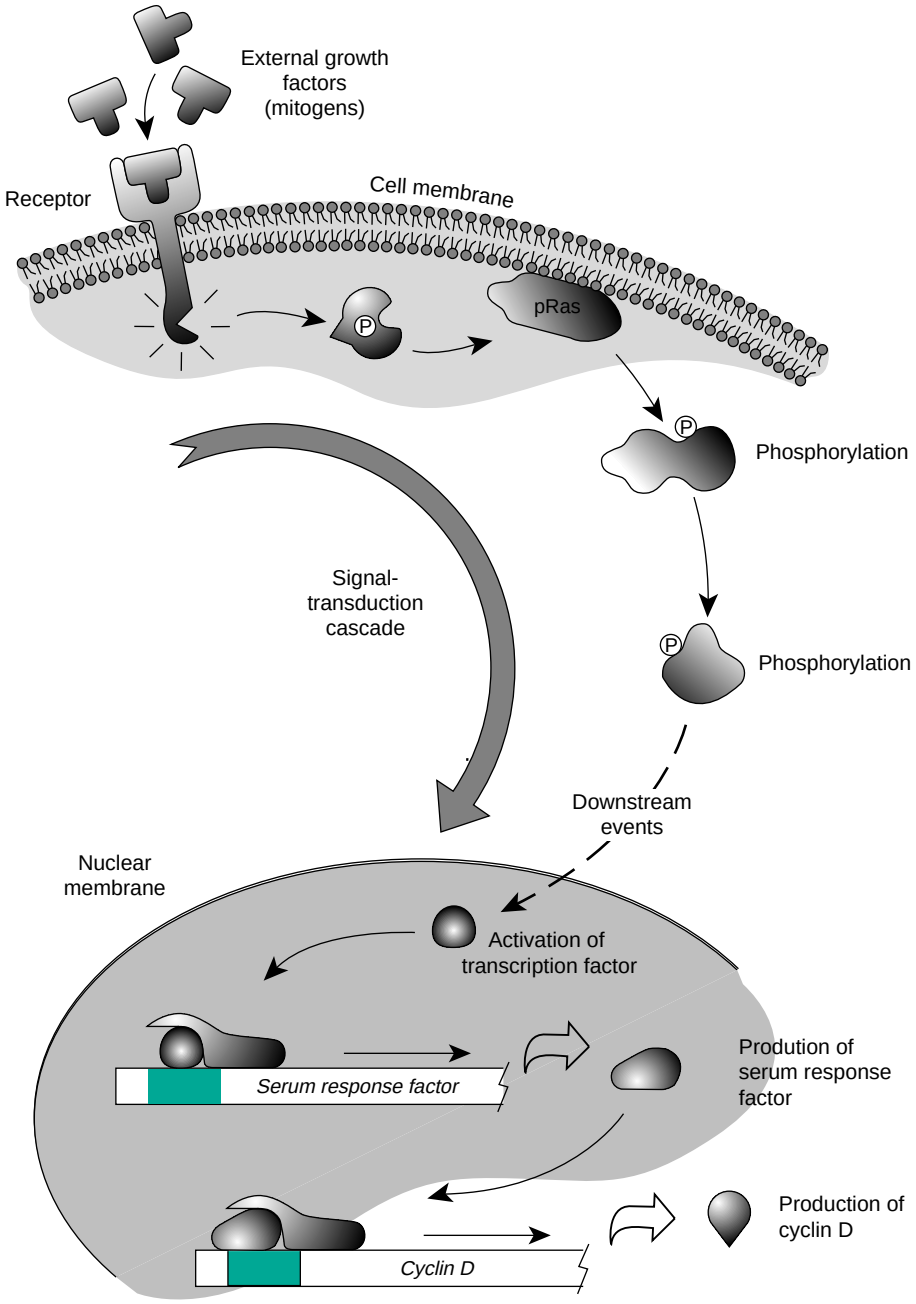


Figure 5. Signal-Transduction Cascade

Signal-transduction cascades relay external signals from the tissue or from other parts of the body into the cell. The one we've shown here is representative of a cascade that might stimulate an epithelial cell to grow and divide. The cascade starts with the binding of a signaling molecule, in this case a mitogen, to the extracellular part of a receptor. The part of the receptor that lies inside the cell then becomes enzymatically active and can initiate a cascade of protein phosphorylation events. In this diagram, the signal passes through pRas, a critical enzyme that is involved in many cascades. The message continues to be propagated by other proteins until it is eventually relayed into the cell nucleus. There a transcription factor becomes activated that will initiate the production of other transcription factors. We've indicated the production of serum response factor, which can then go on to help transcribe the cyclin-D gene. As a major regulatory protein of the cell cycle, the presence of cyclin-D protein within the nucleus is necessary to initiate cell division. Although the signaling pathways may proceed through any of several routes, often those different routes are channeled through one or two critical proteins. Improper expression of those proteins can therefore lead to an abnormal response to external signals and erratic cell behavior.

transduction cascade that could conclude in any number of ways, such as the blocking of a second cascade that is transmitting a positive growth signal. The cascade might terminate by blocking the transcription of a positively regulated gene. It might result in the production of a protein that will ultimately carry out some regulatory function through a direct interaction with another protein. Although the surface receptors, specific proteins, and endpoints of various cascades may all be different, most pathways use similar mechanisms to transmit signals from the external environment to some intracellular target.

Figure 5 and our description greatly understates the complexity of signal transduction. It is a far more intricate process than we have indicated, with many enzymes often participating in multiple pathways. A particular enzyme may be positively regulated in one pathway and thus help stimulate growth, whereas it may be negatively regulated in another pathway. Proteins can be activated not only by the addition of phosphate groups but also by their removal. A protein may further be inhibited by either of those methods. The cell employs all mechanisms, and it is not unusual to find several different modes of activation or inhibition acting within the same protein. The complexity of the cascades and the myriad interconnections are, in one sense, both an asset and a liability. Like a massive government bureaucracy, the redundancy tends to make a cascade fairly robust and insensitive to minor breakdowns. There is almost always a way to get around a dysfunctional part of the system. Likewise, however, it also means that there are many ways in which the system can break down.

The Cell Cycle and Basic Cell-Cycle Control

Both the body and the tissue tell the cell when to begin a new cell cycle. They do so through various growth-stimulating factors that instigate signal-transduction cascades. The cascades relay the information into the cell body or into the cell nucleus where processes that help coordinate and carry out cell division will be initiated. Coordination is essential because cellular reproduction is an enormous undertaking. The entire volume of the cell must double so that cells can divide repeatedly without decreasing in size, and all cellular substructures and organelles must be reproduced. The cell's genome must be exactly duplicated, which, for a human cell, entails the faithful replication of some 6 billion nucleic-acid bases and the synthesis of 46 new chromosomes. Eventually, new nuclear and cellular membranes must form as the parent cell cleaves itself in half.

Cell growth takes place more or less continuously as the cell cycle progresses. The overall protein and organelle content of the cell also tends to increase at a fairly uniform rate. In contrast to that continuous and nondistinct growth are the discrete events of DNA replication, chromosome separation, and the actual division of the cell. Those events occur at particular times and permit the cell cycle to be partitioned into four phases. As illustrated in Figure 6, those phases are termed G_1, S, G_2, and M. Newly generated cells are born into G_1 phase, and it is there that slowly dividing cells will typically spend the majority of their lives. In particular, a nondividing cell may enter a resting state often referred to as G_0. During G_0, the cell-cycle machinery is partially dismantled, and the cell may acquire specialized characteristics or may differentiate. Under the proper conditions, a G_0 cell can re-enter G_1 and, thus, continue cycling.

During S phase (for DNA synthesis), DNA replication duplicates the entire

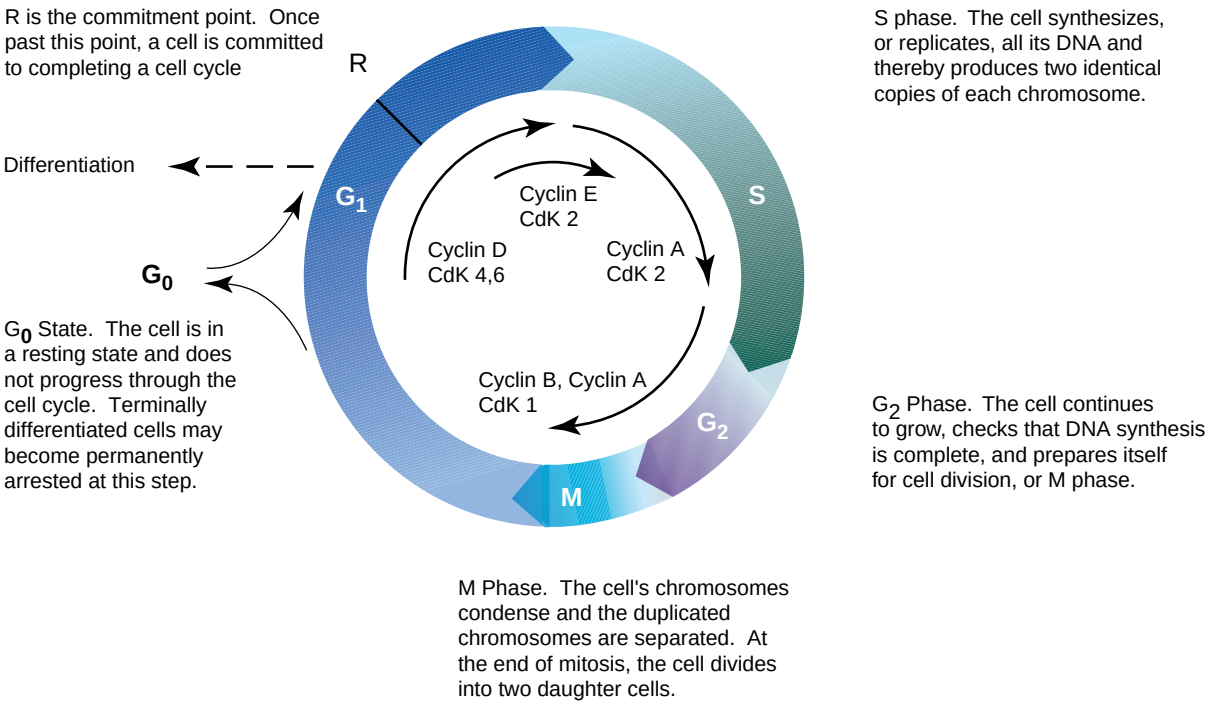


Figure 6. The Cell Cycle

The stages of cell division are collectively called the cell cycle. A newly created cell is "born" into G_1 phase. Cell differentiation will emerge from G_1 phase. Also from G_1, a cell can enter a nonreproductive state, called G_0, from which it will perform its usual functions. When new cell growth is called for, the cell will re-enter G_1 to begin a new cell cycle. The cell will begin to produce cyclin D and cyclin E and form active cyclin-Cdk complexes. Those will help advance the cell to S phase. The various activated cyclin-Cdk complexes that will regulate the progression of the cell cycle from one phase to the next are indicated in the center of the diagram. The cycle is completed after M phase with the cell dividing into two new cells.

genome and produces two copies of each chromosome. During G_2 phase, the cell continues to grow as it prepares for mitosis, or division of the cell nucleus. During the cell-division, or M (for mitotic), phase, the duplicated chromosomes will condense to their most compact form, align themselves along a central axis, and split into single chromosomes, which are then segregated to opposite sides of the cell. Next, new nuclear membranes form, creating two nuclei, and at the end of M phase, the cell divides into two cells. Each new cell is complete, and each has received an entire copy of the genome. The steps involved in mitosis are illustrated in Figure 7. If these steps are not carried out in a proper, sequential fashion, or if DNA replication or other cellular activities are not duly coordinated, one, or possibly both, daughter cells may be born incomplete. By necessity then, the entire cell-division process is extremely well regulated. That regulation is carried out by a series of protein-protein interactions and protein modifications.

Starting within the G_1 phase, and then again at distinct times during the cell cycle, the concentrations of specific cyclins increase within the nucleus. These cyclins associate with an appropriate Cdk to form a cyclin-Cdk complex. Recall that complex formation is a necessary step in the ability of a Cdk to act as a kinase (Figure 4) and that by controlling phosphorylation, kinases can regulate other proteins. Cyclin-Cdk complexes are the main control elements that regulate the progression and activity of each phase of the cell cycle.

Cyclins are actually a family of closely related proteins, and to date, eight general types, cyclin A through H, have been identified. (Some types—for example, cyclin D—are themselves families consisting of several related proteins.) With the important exception of cyclin D, each type of cyclin is synthesized during a unique and relatively discrete period of the cell cycle. Each is also degraded at a second, relatively discrete point. The cyclin concentration, therefore, varies over time, and as it changes, new cyclin-Cdk complexes are formed, thereby activating the Cdk. Each time a different kinase becomes active, the cell moves through a given phase or initiates some process, as indicated in the center of Figure 6. For example, cyclin-D-Cdk4, cyclin-D-Cdk6, and cyclin-E-Cdk2 all control the pro-

M phase

Prophase. The chromosomes condense into microscopically visible threads. Microtubules radiating from the two centrosomes collectively compose the mitotic spindle.

Prometaphase. The centrosomes migrate to opposite sides of the cell. The nuclear membrane disintegrates so that the microtubules can bind to each chromosome at the centromere.

Metaphase. The chromosomes have assumed their most condensed state. The X shape is a result of the two identical chromosomes being joined at the centromere. Each chromosome is aligned along the midplane of the cell.

Anaphase. The bond joining the chromosome breaks, and each moves towards opposite sides of the cell. The cell begins to elongate and narrow at the midplane.

Telophase. A new nuclear membrane forms around each segregated set of chromosomes, the chromosomes begin to decondense, and the cell begins to divide.

G_1 phase. The cell has cleaved into two cells. The chromosomes decondense to their normal extended state for the resumption of normal cell activities.

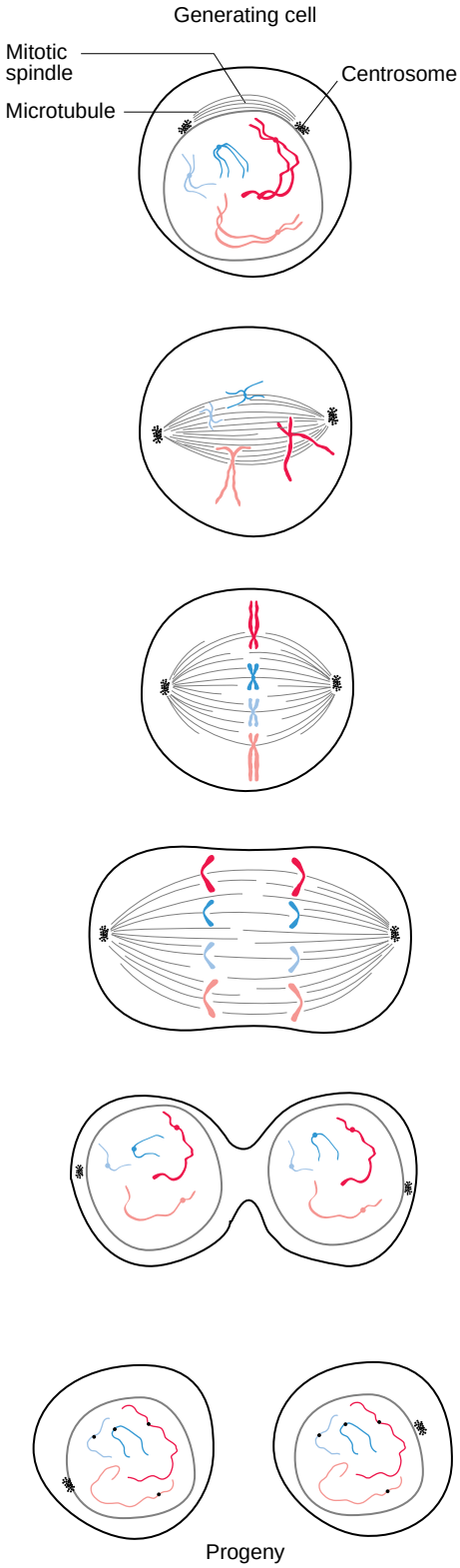


Figure 7. Mitosis and the Birth of Cells

Mitosis is the process whereby a nucleated cell segregates, partitions its already duplicated genome, and divides in two. The result is a set of chromosomes, identical to those initially possessed by the generating cell, being transmitted to each of the progeny cells.

gression of the cell cycle through G_1 and the transition into S phase, whereas cyclin-A-Cdk2 is necessary for progress through S phase. Typically, a phase or process will end when the cyclin is degraded. (In mitogenically stimulated cells the regulation of cyclin D comes about through the control over its subcellular localization and degradation rate.)

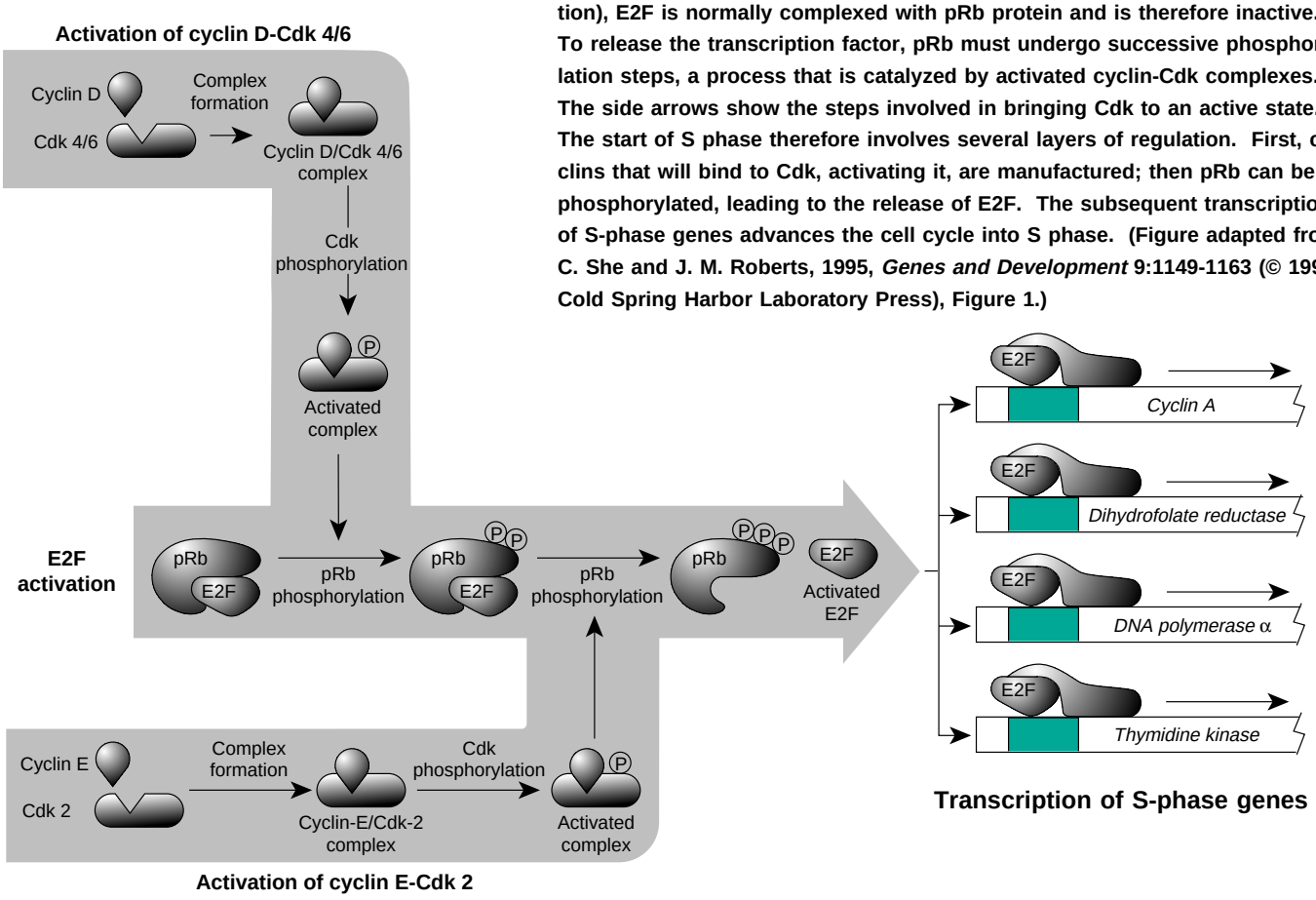
It has been learned that many of the proteins that operate within G_1 phase facilitate cancer development when they fail to function properly. We will therefore describe the G_1-to-S-phase transition in some detail. Also, many cellular responses to DNA damage are triggered from a point in G_1 called the G_1 checkpoint, and thus a section describing checkpoint control will follow. Although necessarily simplified, our description highlights those elements that are important for our later discussion of radiation-induced DNA damage and oncogenesis.

A new cell cycle and the progression through G_1 into S begins when a cell is stimulated to divide by mitogenic signals. Among other things, this initiates the positive regulation, or enhanced transcription, of cyclin D and cyclin E. Loosely associated with this transcriptional activity is a point in G_1 called R (for restriction point). Prior to R, cell-cycle progression remains sensitive to a variety of negative regulatory signals that can counter the effects of mitogenic signals. The cell can either return to G_0 or else begin to differentiate. Once past R, however, the cell is committed to continuing with the rest of the cycle.

Much of the metabolic activity that occurs during G_1 is directed towards preparing the cell for S phase. It is during S phase that the entire genome is duplicated, and so a substantial number of proteins and nucleotide precursors must be synthesized. The start of S phase, therefore, involves a significant amount of transcriptional activity, much of which is promoted by a transcription factor called E2F. (E2F is actually a family of transcription factors, E2F-1, E2F-2, and so on. Our discussion will be limited to E2F-1) If E2F is available, transcription and, hence, S phase can begin. But the availability of

Figure 8. G1-to-S Phase Transition

DNA replication requires the production of numerous proteins, including thymidylate synthetase, which is necessary for producing nucleotides, DNA polymerase α, which is needed to replicate DNA, and cyclin A, which will regulate processes occurring during S phase. Collectively, we've called the genes that produce those proteins S-phase genes. We've shown the regulation of a key step involved in the transcripton of S-phase genes: the release of the transcription factor E2F. As the central arrow indicates (E2F activation), E2F is normally complexed with pRb protein and is therefore inactive. To release the transcription factor, pRb must undergo successive phosphorylation steps, a process that is catalyzed by activated cyclin-Cdk complexes. The side arrows show the steps involved in bringing Cdk to an active state. The start of S phase therefore involves several layers of regulation. First, cyclins that will bind to Cdk, activating it, are manufactured; then pRb can be phosphorylated, leading to the release of E2F. The subsequent transcription of S-phase genes advances the cell cycle into S phase. (Figure adapted from C. She and J. M. Roberts, 1995, *Genes and Development* 9:1149-1163 (© 1995, Cold Spring Harbor Laboratory Press), Figure 1.)



E2F is carefully regulated by several mechanisms. Early in G_1 phase, E2F is bound to and inactivated by a protein called pRb. This inactivation prevents the premature transcription of S-phase genes. As illustrated in Figure 8, E2F is released only after pRb undergoes a structural modification caused by a series of phosphorylation events that are mediated by none other than the activated cyclin-Cdk complexes discussed earlier.

In fact, the cyclins may be considered the ultimate regulators of the transition from G_1 to S phase. Without them, Cdk would not become active, and the release of E2F from pRb would not occur. Conversely, if the cyclins were overexpressed or overly active, they could phosphorylate pRb prematurely and cause the early release of E2F and an abnormal progression through G_1 into S phase. The entire chain of events involving the cyclins D and E, the Cdk's, pRb, and E2F must function properly to regulate the transcription and expression of S-phase genes. If mutations to the genes that produce those proteins cause some of them to malfunction, the cell cycle may be compromised or it may simply break down. Thus, we have returned to our basic picture. Gene mutations that affect the function of certain proteins can lead to erratic or even deranged cell growth and cell behavior.

All other phases of the cell cycle behave in a manner similar to the G_1-to-S-phase transition. They are controlled by cyclin-Cdk complexes that, once formed, are able to activate proteins that can initiate a chain of subsequent events. The chores of a specific phase get carried out, and the cell advances to the next stage of its reproductive cycle. But the cell is not a simple, rigidly behaving automaton. The progression of the cell cycle and the advancement to each new phase can be regulated in response to environmental signals or internal triggers. The entire reproductive process can even be arrested. The decision to suspend cell-cycle progression is made at what are called the *cell-cycle checkpoints.*

Checkpoints and the G_1-Checkpoint Response

The only goal of cell division is to generate two viable cells, each inheriting an exact replica of the parent cell's genome. Given the potential consequences of transmitting incorrect genetic information, it should not be surprising to find that the status of the cell's DNA is internally monitored at the various checkpoints located within each phase of the cell cycle. If abnormalities in DNA structure or conditions that might affect DNA integrity are detected, then a checkpoint may slow the cell-cycle progression so as to allow time for the damage to be repaired. Through checkpoint control, the cell can prevent complications that result from attempts to replicate or segregate damaged DNA, and a cell can thereby minimize the consequences of the initial damage. Alternatively, a checkpoint may also respond to external signals, thus permitting the cell to halt reproduction if, for example, it detects that the tissue is not providing a favorable environment.

Checkpoints in G_1 and throughout S phase safeguard against damage in the DNA template. The G_1 checkpoint also controls the entry into S phase. A checkpoint in G_2 monitors the completion of DNA replication and the absence of chromosome damage, and it regulates the entry into M phase. A final checkpoint in M phase arrests mitosis if chromosomes are not properly aligned along the mitotic spindle (Figure 7: Mitosis). Thus, each checkpoint monitors different aspects of DNA replication and chromosome segregation, and each regulates a different phase of the cell cycle. Each is also sensitive to various environmental influences. As a DNA damaging agent, ionizing radiation triggers checkpoints in G_1, S, and G_2. But all of the checkpoints save G_1 are located beyond R, the cell-cycle commitment point. They can, therefore, only suspend the cycle's progression, without stopping it. Only the G_1 checkpoint can permanently bring the cell cycle to a halt.

Thus, the G_1 checkpoint seems to play the most important role in cell-cycle decision making. It is at the G_1 checkpoint that a cell can respond in any of several ways, depending on the nature of the damage or the environmental trigger. One response is to enter an arrested state that, like the G_2 arrest, suspends the reproductive cycle. Another cell response is *apoptosis.* This is in reality cellular suicide, or a metabolically activated form of cell death. Apoptosis can apparently be triggered from almost any phase of the cell cycle and by a variety of signals. It is a process that leads to the rapid elimination of the affected cell. Apoptosis appears to respond to the abnormal accumulation of cells that is characteristic of malignantly transformed cells and, thus, might help limit excess proliferation. It is further thought to respond to the loss of genetic integrity and to serve as a means of eliminating cells that have sustained unusually high levels of DNA damage.

A third cellular response is called *cell senescence,* which refers to a permanent quiescent state that is triggered after a cell has undergone a finite number of divi-

sions. The entire cell line simply stops dividing. First observed in cell cultures of human-skin fibroblasts, it is generally believed that this phenomenon applies to cells under normal physiological conditions as well. Senescence can be thought of as an internal constraint on the life-span of a cell line. By limiting the total number of cell doublings and hence the number of progeny, cell senescence will also constrain the effects that any individual cell can exert on the rest of the tissue.

These three cellular endpoints—the G_1 arrest, apoptosis, and cell senescence (see "Apoptosis" and "Senescence and Immortality")—can be viewed as defense mechanisms that are summoned to prevent the propagation of an altered cell. The first two are invoked in response to a broad range of insults, from DNA damage to nutritional deprivation, whereas senescence is more of a fail-safe method for limiting an excessive number of cell divisions. However, some cell types, especially those that are not prone to apoptosis, appear to become prematurely senescent following exposure to ionizing radiation. Those cells seem to use senescence more as a defensive response. Figure 9 summarizes the points just made concerning checkpoints and the G_1-checkpoint responses.

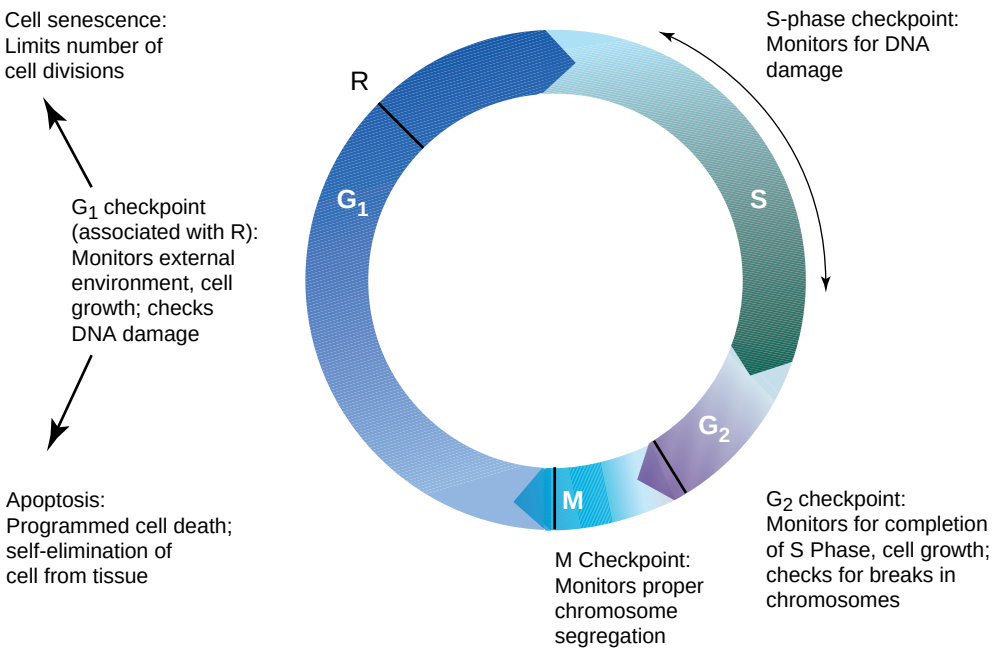


Figure 9. Cell-Cycle Defenses against DNA Damage

Checkpoints regulate the progression of the cell cycle. Both the G_1 and G_2 checkpoints are sensitive to environmental signals, such as nutritional status or tissue requirements, and will suspend the advancement to the next phase if DNA damage or a chromosome abnormality is detected. Faced with DNA damage, the G_1 checkpoint can trigger a cell-cycle arrest. It can also trigger an apoptotic response, which is a form of cell death. An excessive number of transits through the cell cycle can trigger cell senescence, which is thought to arise from the G_1 checkpoint. The checkpoint in M phase is triggered by faulty chromosome segregation, which is a severe breakdown of normal mitosis. The cell typically dies before passing this abnormality onto its progeny.

Each of those responses involves a critical protein called p53. This protein is a *transactivating* protein, which means that it initiates the transcription of other genes. In the case of the G_1 arrest, p53 will induce the production of negative regulators that inactivate G_1-cyclin-Cdk complexes. As stressed in the previous section, those complexes are the major control proteins of the cell cycle, and their deactivation prevents a cell from advancing to the ensuing phase. Specifically, the G_1 arrest is actuated by preventing the cyclin-D and cyclin-E complexes from interacting with the E2F-activation pathway. As in most cell processes, this happens in a somewhat convoluted manner.

DNA damage triggers a G_1 arrest through its effects on the stability of p53 protein. Under normal conditions, p53 is synthesized continuously throughout the cell cycle. Its concentration is controlled by its relatively rapid rate of degradation. In the presence of DNA damage, however, the protein is modified in a manner that makes it more resistant to degradation. This has the net effect of increasing its overall concentration and, thus, increasing its transactivating potential. The exact steps involved in going from DNA damage to this structural modification are presently unknown, although one possible mechanism is presented in "DNA Repair" on page 78.

The stabilized p53 protein is a positive regulator for the transcription of several

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Apoptosis

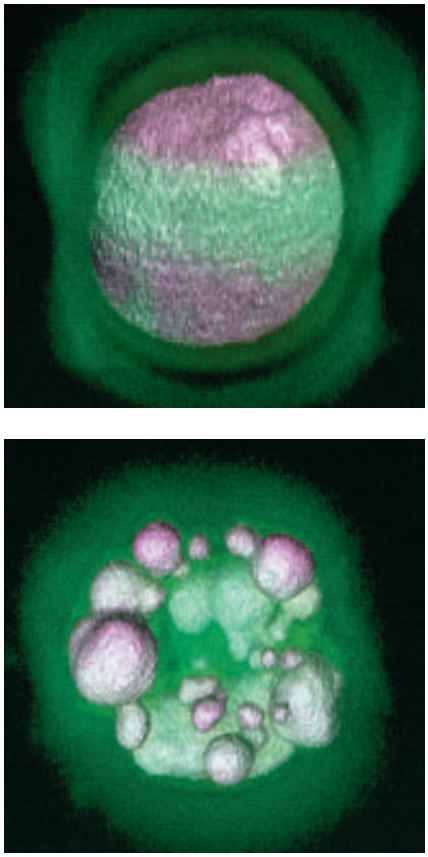


Figure 1. Apoptotic Nucleus
A DNA-binding fluorochrome was used to stain the cell's chromosomes, and a series of optical images, using a fluorescence, laser-scanning confocal microscope, were taken at different depths within the cell. The images were then used to reconstruct a three-dimensional image of the chromosomes within the nucleus. The top figure of a normal human lymphocyte shows that the chromosomes are uniformly distributed throughout the roughly spherical nuclear volume. The bottom picture shows an apoptotic cell. The chromosomes and the nucleus have fragmented and collapsed into small vessicles (apoptotic bodies). The cell will induce its own death by attracting a macrophage that will engulf and destroy the cell. (Photos courtesy of B. L. Marrone, Los Alamos National Laboratory.)

Apoptosis is a metabolically triggered form of cell death that is defined by progressive, cytologically observable changes in cell structure. It can occur in any one of several different situations, including the normal developmental process of eliminating unwanted tissue, such as webbing between fingers and toes, the physiological response to excess cellular division (hyperplasia), and the suicidal response to excessive damage to cellular DNA. Apoptosis also appears to respond to the abnormal accumulation of cells characteristic of malignantly transformed cells and may be inactivated in tumorigenic cells. Each of these individual processes meets the cytological criteria for apoptosis, and as a group, they appear to share biochemical features. To what extent they are actually mediated by the same or similar molecular processes, however, remains unclear.

The induction of apoptosis by DNA-damaging agents shares many features with the induction of G_1 arrest (see main article). Not only are both triggered by DNA damage, but both also appear to depend upon the transactivation of the *p21* and *gadd45* genes by the p53 protein. Apoptosis and G_1 arrest are mutually exclusive responses. The exact mechanisms regulating which response actually takes place have not been elucidated, but it is believed to depend, at least in part, upon the ratio of p21 to Gadd45 proteins. Irradiation of mitogenically stimulated cells favors the induction of G1 arrest over apoptosis. Irradiation of unstimulated cells favors apoptosis. Mitogenic signals are positive regulators of p21 synthesis but have no effect on the synthesis of Gadd45 and, therefore, act to increase the p21 to Gadd45 ratio. The p53 protein, the levels of which rise in response to p53 stabilization in irradiated cells, probably induces both p21 and Gadd45 to similar extents. It is therefore believed that mitogenic signals act through their influence on the levels of p21 and that a high p21 to Gadd45 ratio favors G_1 arrest over apoptosis, whereas a low ratio favors apoptosis.

Apoptosis is also responsive to a second set of regulatory proteins, Bcl-2 and Bax. As with p21 and Gadd45, the ratio of Bcl-2 to Bax seems to be important. In this case, Bcl-2 acts to inhibit apoptosis. However, Bcl-2 can become complexed with Bax, and in this form, it is no longer able to inhibit apoptosis. What controls the relative levels of Bax and Bcl-2? Both Bax and Bcl-2 are regulated at the transcriptional level by p53. The p53 protein stimulates the transcription of the *bax* gene while it represses synthesis of the *bcl-2* gene. Under normal conditions, Bcl-2 is continuously present and apoptosis is repressed. When p53 concentrations increase, as they do after cellular exposure to ionizing radiation, then the concentration of Bax increases relative to Bcl-2, and apoptosis is favored.

At this time, the relationship between regulation by p21-Gadd45 and Bcl-2-Bax systems is unclear. It is not known if these are sequential switches in a common pathway or if they represent parallel responses. Furthermore, it is not known if these switches are in some way linked to each other or if they are regulated by systems that are totally independent of each other. But it is clear that apoptosis is an important response for cells that have sustained DNA damage and, in this role, may act to eliminate severely damaged cells. It is also clear that apoptosis is important for maintaining the appropriate density of cells, such as B-lymphocytes, and that a breakdown in this process initiates a hyperplastic state that can progress into leukemia. ■

Senescence and Immortalization

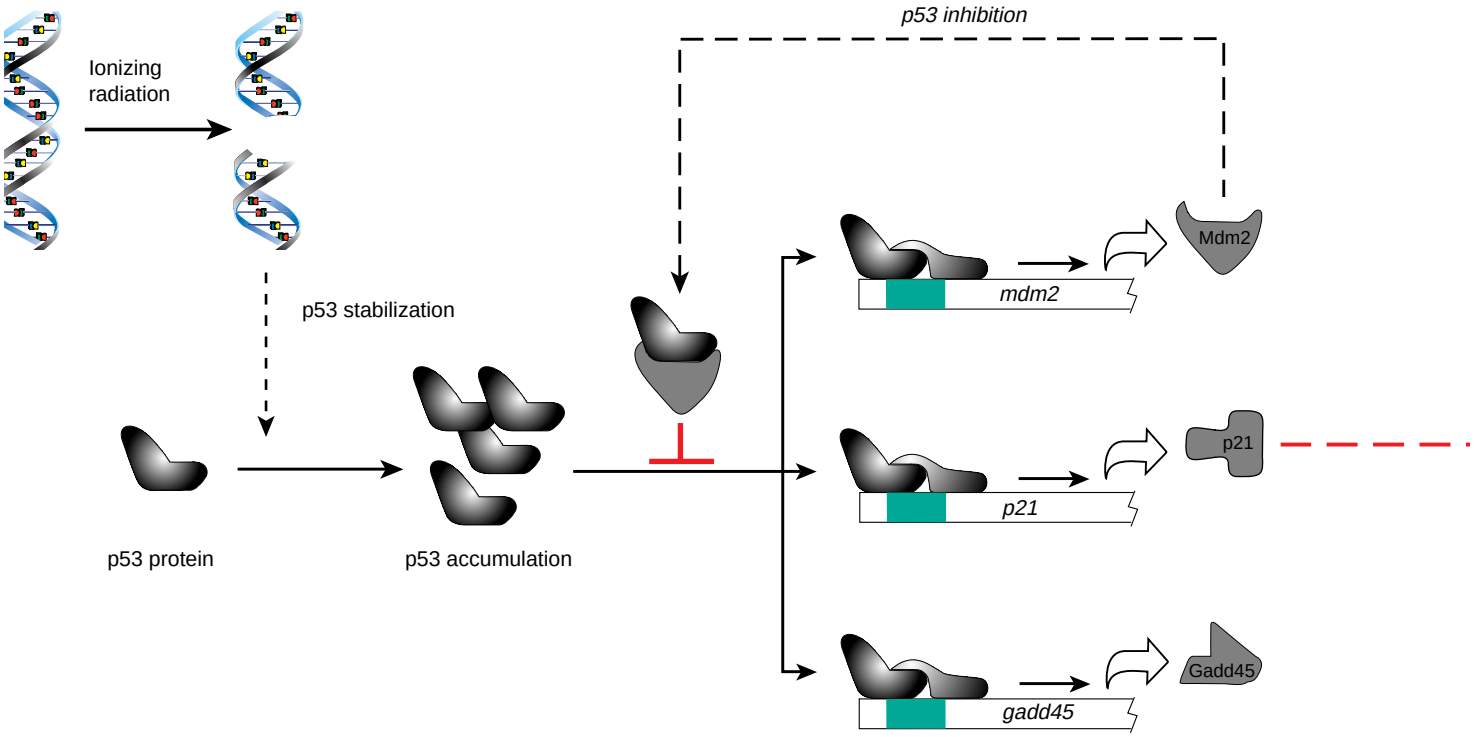
Senescence is a nonproliferative state that normal cells grown in culture will enter after a finite number of cell doublings. Senescent cells will no longer enter S phase, even under mitogenic stimulation, and they will no longer undergo cell division. Cells can be transformed such that senescence is abrogated and the number of cell doublings is extended. This extended life-span is usually accompanied by an escalating accumulation of chromosomal aberrations with each new cell division and an increased likelihood of cell death. Eventually, at a stage termed crisis, nearly all cells die. Under the appropriate conditions, however, a few transformed cells survive crisis and divide indefinitely. These cells acquire immortality. It is believed that to generate life-threatening tumors, a tumor cell must first escape the proliferative controls imposed by senescence and then must acquire immortality.

Table 1. Similarities Between G1 Arrest and Cell Senecence

	G1 arrest	Cell Senescence
Can enter S-phase?	yes	no
DNA content	diploid	diploid
Gene Expression		
cyclin D1	high	high
cyclin E	high	high
Cdk activity	low	low
p21	elevated	elevated
pRb	hypo-p	hypo-p
cyclin A	absent	absent
cyclin B	absent	absent
cdc2	absent	absent
metabolically active	yes	yes

In many ways, senescent cells resemble cells arrested at the G_1 checkpoint (Table 1). This suggests that the mechanisms mediating these two cellular responses may also be similar. A model has been proposed in which senescence is viewed as a specialized form of the G_1 checkpoint response, only triggered by a naturally occurring chromosome instability. Each end of chromosomal DNA terminates in a specialized repeating structure, the telomere. In normal cells, the number of repeats forming a telomere gradually decreases with each population doubling. Maintenance of telomere length would normally be the responsibility of an enzyme called telomerase, but the synthesis of telomerase is apparently terminated early in embryonic development. Thus, the absence of telomerase activity in adult cells results in the gradual erosion of telomere length.

It has been proposed that, at some point, one or more telomeres become too short to perform their normal function of masking DNA termini. Unmasked termini may be recognized as DNA lesions and trigger a G_1 arrest. This is the senescent state. It has been demonstrated, however, that transformed cells with inactive forms of p53 lose the ability to initiate a G_1-checkpoint response (which is also required for the maintenance of the senescent state) and would, therefore, be expected to continue cell-cycle progression even in the presence of DNA damage. Those cells, by continuing their proliferation, would further reduce the telomere length of their chromosomes and accumulate additional DNA damage in the form of nonfunctional telomere units. This predicted accumulation of DNA damage is consistent with the increasing amounts of genomic instability associated with extended life-span proliferation. Protection from the continued loss of telomere function and the resulting accumulation of DNA damage is provided by the restoration of telomerase activity. This is apparently a rare event that may arise as a result of the genomic instability manifested during extended life-span proliferation. The restoration of telomerase activity would lead to restoration of missing telomeres and to the stabilization of telomere length during DNA replication. With the loss of checkpoint controls and the restoration of telomere stability, crisis would be avoided, and the surviving cells would presumably be able to grow indefinitely and, therefore, be immortal. ■



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genes, including the *p21* gene.* The *p21* gene is an important player in checkpoint responses, and the central role of this protein can be appreciated from the variety of ways in which the gene encoding this protein has been cloned. For example, *p21* has been cloned as *cip1* (for *c*yclin-dependent-kinase *i*nhibiting *p*rotein 1) by a group that was seeking negative regulators of cyclin-dependent kinases. It was also cloned as *waf1* (an acronym for *w*ild-type p53-*a*ctivated *f*actor 1) by a second group of investigators that was looking for genes that might be responsive to changes in p53 levels. Finally, *p21* was cloned as *sdi1* (which stands for *s*cenescent-cell *d*erived *i*nhibitor 1) by a third group that was attempting to isolate genes that mediated the cell-senescence response. It is now known that each of these genes codes for the same 21-kilodalton protein, referred to simply as p21.** That protein binds to and is a negative regulator of several G_1 proteins, including cyclin-D-Cdk and cyclin-E-Cdk2 complexes.

As illustrated in Figure 10, the p21 protein blocks the kinase activity of the cyclin-Cdk complex. It does so through a protein-protein interaction, although the exact mechanism is not known. (Other than p21, a class of proteins called the cyclin-dependent kinase inhibitors (CKI's) can regulate the activity of a cyclin-Cdk complex. This interaction is not shown in the figure. The p21 protein is also considered to be a CKI.) The end result is that the cyclin-Cdk complexes are prevented from phosphorylating pRb, and thus, the E2F transcription factor is not released. The S-phase proteins necessary for the G_1-to-S transition are not synthesized, and the cell cycle cannot advance into S phase. Instead, the cell remains in an arrested state in G_1.

*We will designate the name of a gene in italics, and the protein product of that gene in normal type; for example, *p21* gene and p21 protein.

**The full name is $p21^{cip1/waf1/sdi1}$; throughout this article, it will be shortened to *p21*.

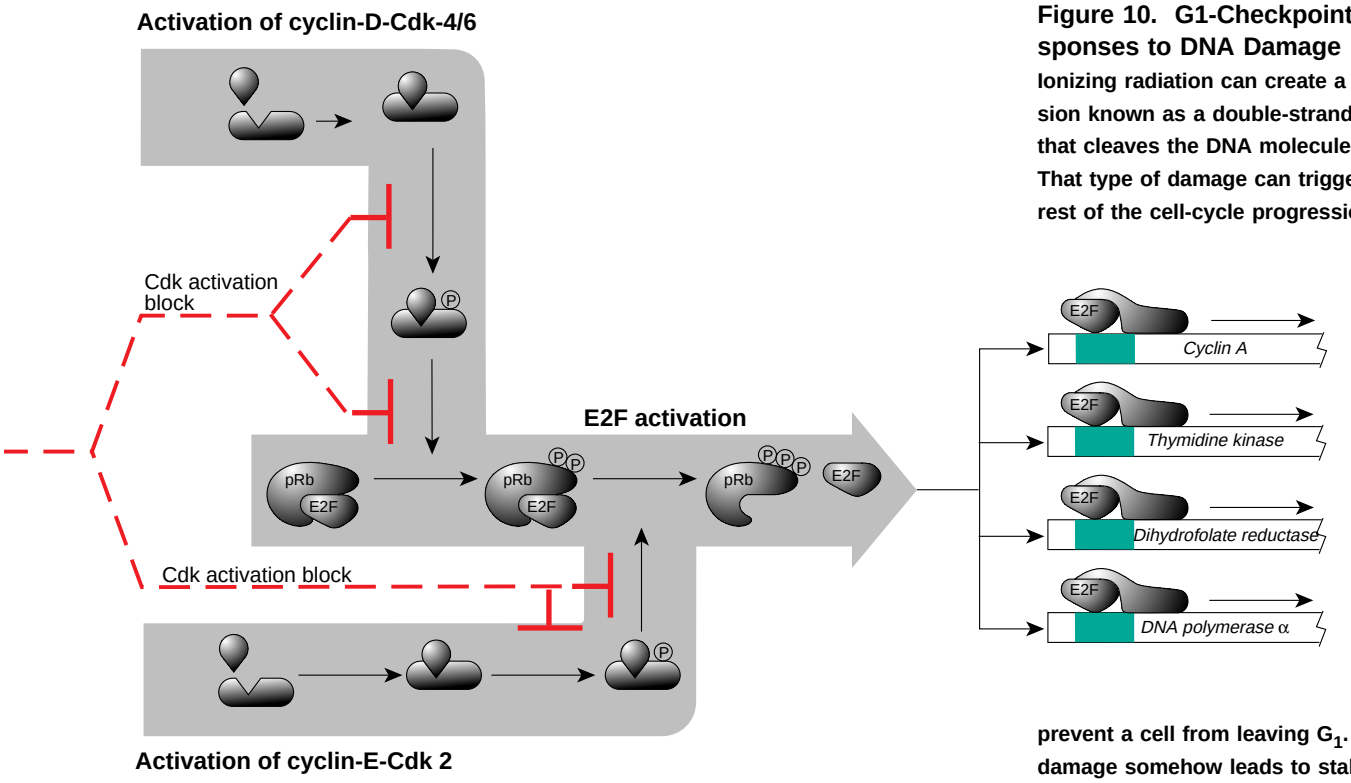


Figure 10. G1-Checkpoint Responses to DNA Damage

Ionizing radiation can create a DNA lesion known as a double-strand break that cleaves the DNA molecule in two. That type of damage can trigger an arrest of the cell-cycle progression and prevent a cell from leaving G_1. The damage somehow leads to stabilization and accumulation of p53 protein (the steps leading to p53 stabilization are not known). The p53 protein promotes transcription of several genes, including the three shown here. The p21 protein is the key protein involved in the arrest. It can block the kinase activity of cyclin-Cdk complexes. Those enzymes are no longer able to phosphorylate pRb, and the crucial transcription factor (E2F) that will transcribe S-phase genes is not released. The cell cannot begin S phase and remains in an arrested state within G_1. The p53 protein also initiates transcription of the *mdm2* gene which produces a protein that can inhibit the transcriptional activity of p53. Thus, the *mdm2* gene becomes part of a negative feedback loop that limits the duration of the G_1 arrest. The third gene shown here to be transcribed by p53 is *gadd45.* In conjunction with p21, the protein product of this gene helps to trigger the apoptotic response. Whether DNA damage results in a G*1* arrest or apoptosis depends in large part upon other factors. In either case, by triggering one of these two responses, the cell minimizes the potential consequences of attempting to replicate a damaged genome.

Figure 10 also shows that the stabilized p53 protein induces the expression of at least two more genes. Gadd45 protein is believed to play a role in the G_1 checkpoint and apoptotic responses to DNA damage. In particular, the relative concentrations of Gadd45 to p21 protein might be important in determining which response is triggered (see "Apoptosis"). Another interesting pathway in the figure involves the protein product of the third gene transcribed, Mdm2. It acts to block the ability of p53 to transactivate its normal target genes. By negatively regulating its own enhancer, Mdm2 effectively establishes a negative feedback loop on the entire transactivation pathway. That is one established way that the G_1 arrest itself is regulated and controlled, and it is potentially the means by which the cell will shut off the blocking mechanism so that it can continue with a new cell cycle.

Once again, a mutation in any of the genes involved in the G_1 checkpoint, including *p53, p21,* and *mdm2,* could result in abnormal cell-cycle regulation. In fact, the importance of a mutated p53 in carcinogenesis is underscored by the fact that more than fifty per cent of human tumors have cells containing mutations in this protein. But it must be noted that the checkpoints, by providing time to repair corrupted DNA, also help maintain the fidelity of the genome. Thus, a mutation that inactivates p53 may allow some cells to advance into S phase and replicate DNA even in the presence of DNA damage. That damage can potentially lead to more mutations that can then be passed to the cell's progeny. A *genomic instability*, or progressive accumulation of chromosome abnormalities, is very characteristic of cancer cells. So is a variability in chromosome number, which would indicate a breakdown in either chromosome segregation controls (G_2 or M-phase checkpoint responsibility) or possibly a dysfunction in DNA-replication controls. A cell that incorrectly expresses some of its cell-cycle regulatory proteins therefore establishes within itself a positively reinforced mechanism that is destined to bring about the improper expression of even more genes. The slightly transformed, aberrant cell can become the seed of cancer within our bodies.

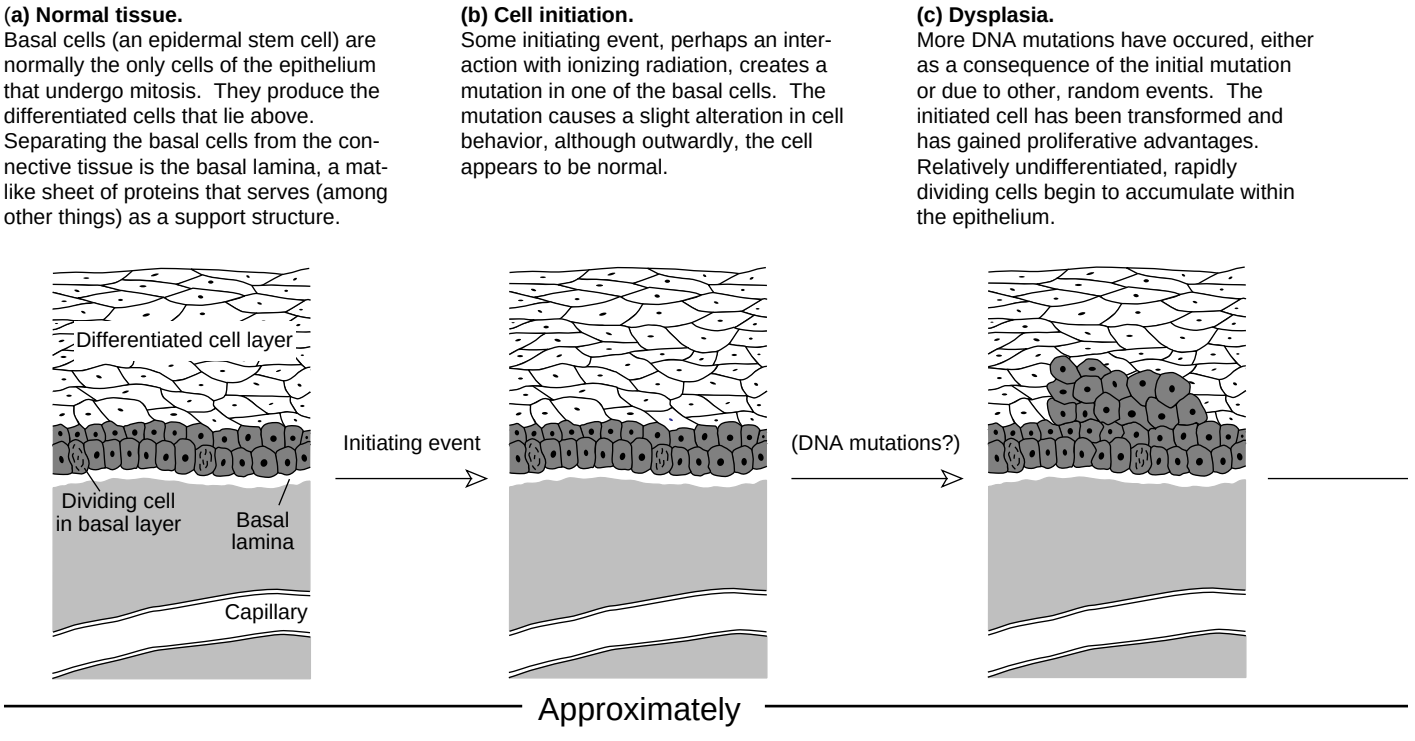
Cancer

Cancer is a gross distortion of cell behavior caused by numerous gene mutations and numerous abnormalities in the production and functioning of proteins. The specific abnormalities vary greatly, depending on the type of cancer as well as the type of tissue from which the cancer originated. Thus, there is not a single description of cancer or oncogenesis, because cancer is not a single disease. It is really a class of diseases all pertaining to unlimited cell growth that is potentially fatal to the organism. Broadly speaking though, carcinomas are cancers of epithelial cells, sarcomas are cancers of connective tissue or muscle cells, and leukemias are cancers of the blood and lymph systems. In the normal human population, over 90 per cent of all human cancers are carcinomas.

A substantial body of evidence now suggests that cancer initiates from a single cell that has been transformed due to a particular change in its DNA. Some event, such as exposure to radiation or exposure to a chemical carcinogen, creates a change in the genome. This may be a DNA mutation, or an epigenetic modification. Then, either through direct action or indirectly through a complex web of interacting proteins, the mutation changes the overall expression of some of the cell's genes. The cell continues to function, albeit slightly differently. Typically, the initial behavioral modification may be difficult to detect, but the functional change is passed on to future cell generations.

In general, a precancerous, transformed cell progresses through the characteristic stages and changes that are discussed in Figure 11. In comparison with a normal cell, a neoplastic cell is hyperresponsive to growth factors, underresponsive to growth inhibitors, and has an increase in metabolic transport capabilities. A cancer cell tends to have an irregular shape, an abnormally appearing nucleus, is more mobile, is invasive, and generally shows a genomic instability. Thus, cancer cells

Figure 11. Cancer Progression
This series of drawings depicts several stages typical of a cancer that initiates in an epithelial layer (such as cervical or colon cancer). (Figure adapted with permission from Alberts, et al., 1994, *Molecular Biology of the Cell*, third edition, New York and London: Garland Publishing, Inc., Figures 24-10 and 24-16.)



look different, grow excessively, and behave abnormally. The time scale needed to accumulate sufficient genetic damage to produce these derangements in cellular traits is typically decades, but for certain leukemias, it may be as short as a few years.

An autonomously growing, solid mass of cells like that shown in Figure 11d is called a tumor, or a neoplasm. (Not all neoplasias form tumors. Leukemias are a result of an unregulated increase in white blood cells, but these cells continue to circulate as individual cells within our bodies.) By necessity, a tumor requires an enhanced blood supply to provide nutrients and to remove waste. Called angiogenesis, tumor cells will help stimulate the production of blood vessels that help the neoplasm grow. By the time it is visible to the naked eye, a tumor may consist of over a billion cells, both normal and transformed.

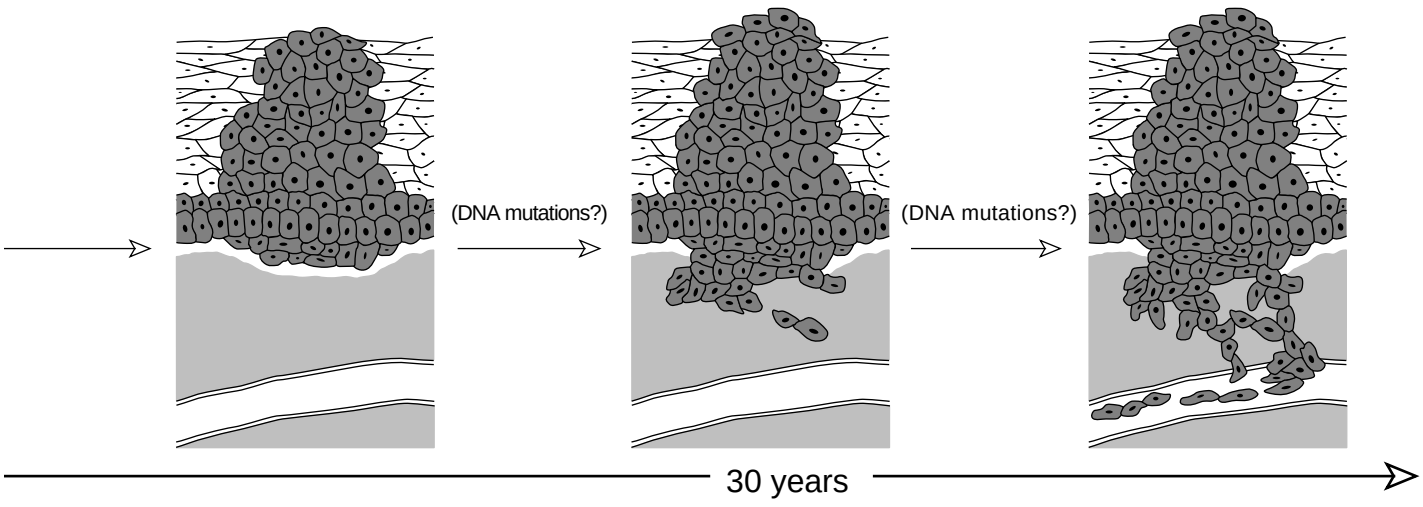
But a tumor is not necessarily cancerous. Cell senescence may still limit the proliferative potential of each cell and, thus limit, the size of the growing cell mass. In that case, the mass may have little physiological effect. A tumor may also be *benign*, which simply means that the neoplasm remains as a well defined cluster that does not spread into neighboring cells. Benign tumors in humans can often be identified and removed surgically with generally favorable results.

To be diagnosed as cancer, a tumor must become *malignant* (Figure 11e). It must gain the capacity to invade the surrounding tissue. This necessitates that individual cells acquire the ability to destroy or disrupt the proteins responsible for holding adjacent cells together. The disruption of intercellular adhesion enables invasive tumor cells to insert themselves between cells in the surrounding tissue and to migrate. Those cells can then disperse themselves throughout healthy tissue and form new growths.

(d) Benign tumor
More changes within the genome of the proliferating cell line leads to full tumor development. All layers of the epithelium contain undifferentiated cells.

(e) Malignant tumor
The tumor breaks through the basal lamina. This increased mobility and invasive property is characteristic of the malignant tumor. The cells are typically irregularly shaped, with an enlarged and irregularly shaped nucleus, have a noticeable genomic instability, and the cell line is immortal.

(f) Tumor metatasizes
Cancer cells break through the wall of a lymphatic vessel or blood capillary. The cells can now migrate throughout the body and potentially seed new tumors in different organs.



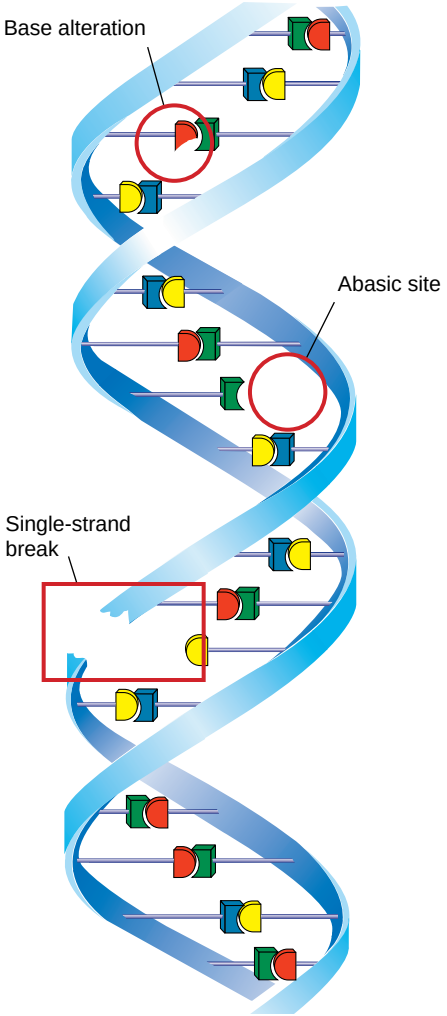


Figure 12. DNA Damage I: Base Alterations and Single-Strand Breaks

DNA bases consist of either one or two ring-like structures that contain both nitrogen and carbon atoms. A base alteration occurs when additional bonds between atoms are formed or broken or new chemical groups attach to the base. All of those situations result in a modified base structure that must be repaired. An abasic site occurs when a base separates from the sugar, leaving behind an unpaired base. Single-strand breaks in the phosphodiester backbone arise largely from hydroxyl radical attack at sugar units comprising the backbone. A gap opens in the normally intact DNA. All three of these general types of lesions are repaired with only a slight risk of genetic change.

The disease develops. Plagued with many growing tumors that are difficult to eradicate, the tissue gradually loses its ability to carry out its normal functions. Its resources are diverted to feed the growing cancer. The cancer may metastasize (Figure 11f), which means that the tumor cells will leave the original tissue, travel by way of the circulatory or lymph system, and invade other organs. As it spreads throughout the body, the tumor can invade and destroy tissue until one or more organs becomes so compromised that death ensues.

Radiation and DNA damage

This primer is about radiation and how radiation can act as a carcinogen to induce cancer. So far, we have only hinted at that relationship by stating that ionizing radiation can damage DNA and that DNA damage can lead to cancer. In reality, those are two separate statements and two separate research areas that must be linked together. Establishing that link, however, has plagued researchers for decades. The remainder of this primer will examine each piece and discuss what is currently known about the link.

Radiation is everywhere. It's invisible and penetrating. Radiation emanates from the soil, seeps as radon into the basements of our homes, and can be a product of the atomic bomb. Much is often assumed about exposing our bodies to radiation, but what *does* happen when an ionizing particle or photon passes through our cells? The radiation deposits energy in that aqueous environment and so creates reactive chemical species. In particular, radiation will produce a highly reactive species known as the hydroxyl free radical ($OH^{\bullet}$). This radical can easily break chemical bonds. An attack on the sugar to which a nucleic-acid base is attached can result in a single-strand break because all or nearly all of the sugar is typically lost. In that case, the break is actually a one-base-wide gap in the DNA backbone. Ionizing radiation can also cause simple modifications to individual DNA bases, creating numerous types of *base alterations.* An entire base can also become separated from the sugar, creating what is called an *abasic site*. Figure 12 illustrates some of the above mentioned DNA lesions.

Although ionizing radiation can lead to the creation of single-strand breaks, the rate at which it does so is negligible compared to a cell's normal metabolic processes. The latter produces copious amounts of hydroxyl radicals. It is estimated that for every single-strand break induced by background radiation, there are about ten million breaks induced by radicals generated during normal cellular metabolism. However, even though the total rate of single-strand breaks from such processes is high, the consequences of single-strand breaks are usually minimal. A cell possesses efficient and accurate mechanisms for rapidly repairing single-strand breaks (see "DNA Repair"). The repair makes use of the information redundancy built into the double-stranded DNA molecule and uses the undamaged complementary strand to restore the DNA to its original state. The vast majority of single-strand breaks are repaired without loss of information and with only a slight risk of genetic mutation. Although single-strand breaks might be lethal lesions to a cell if they are present during DNA replication, the result of DNA repair is that those particular circumstances are typically avoided.

Base alterations and abasic sites, on the other hand, can result in single base changes to the DNA strand known as point mutations. Damaged bases must be repaired, because they might possess altered or ambiguous Watson-Crick pairing properties. As for an abasic site, the DNA structure is compromised due to the in-

ability to form hydrogen bonds between the complementary DNA strands. In both cases, however, the DNA backbone is intact, and during S phase, DNA replication past those lesions will be attempted. The lesions can cause the replication to be error prone, potentially resulting in changes in the nucleotide sequence of the newly synthesized strand. Because the change in base sequence can affect the amino-acid structure and, hence, the protein structure, point mutations might alter the activity or regulation of the gene's protein product. Like single-strand break damage, however, generation of base alterations and abasic sites within the genome are dominated by processes other than ionizing radiation, and the repair of those lesions is similarly rapid and efficient. Probably as a consequence of that repair, ionizing radiation is a relatively poor inducer of point mutations compared with most chemical carcinogens.

Although single-strand breaks, abasic sites, and base alterations are induced by both ionizing radiation and normal metabolic processes, one particularly dangerous type of DNA lesion, the *double-strand break*, is induced preferentially by ionizing radiation. This is due to the manner in which radiation creates radical species within the cell, versus that of metabolic processes. Normal metabolism generates radicals one at a time and at essentially random locations throughout the cell volume. DNA lesions resulting from metabolically derived radicals, therefore, tend to occur at relatively isolated positions along the DNA molecule. Ionizing radiation, in contrast, deposits energy unevenly along the narrow track that is traversed by the ionizing photon or particle. As a result, many radical species are formed in a relatively limited area and tend to form clusters of radicals. If a radical cluster of this type envelops a DNA molecule, then multiple independent lesions might be induced within a localized region of the DNA and both DNA strands might become damaged, broken, or both. Not surprisingly, ionizing radiation can induce very complex lesions comprised of abasic sites and base alterations in addition to strand breaks, as illustrated in Figure 13.

The probability of a double-strand break occurring in any given cell is actually quite low. Thermal diffusion and chemical annihilation will quickly reduce the free-radical density within a radiation track. It has been estimated from Monte Carlo simulations that if the track passes at a distance greater than 2 nanometers from the DNA strand, the probability for DNA damage is slight. It has been estimated from cell-culture studies that approximately twenty to forty double-strand breaks occur per genome at 100 rad of exposure. At that rate, exposures equivalent to ordinary background radiation (typically about 0.3 rad per year) should produce only one double-strand break per ten cells per year!

A double-strand break is usually a mess, and repairing it can be problematic. Even a fairly clean double-strand break, wherein the two backbones are broken directly opposite from each other, results in at least a one-base-pair deletion and a disruption of the linkage between the two DNA segments. The passage of densely ionizing particles, such as alpha particles or neutrons, may break several proximal DNA molecules and cause base damage within each strand that can span several nanometers, or fifteen to twenty base pairs. Not surprisingly, the damaged bases are often excised as the free DNA ends are made ready for repair. The excision permanently removes bases. Simple rejoining of the exposed DNA ends is probably the major mechanism for the repair of double-strand breaks, but this mechanism would result in a loss of genetic information. Remarkably, another mechanism, called *homologous recombination*, exists within the cell that can restore missing information while repairing double-strand breaks discussed in detail in "DNA Repair"). At present, it is not clear what fraction of double-strand breaks

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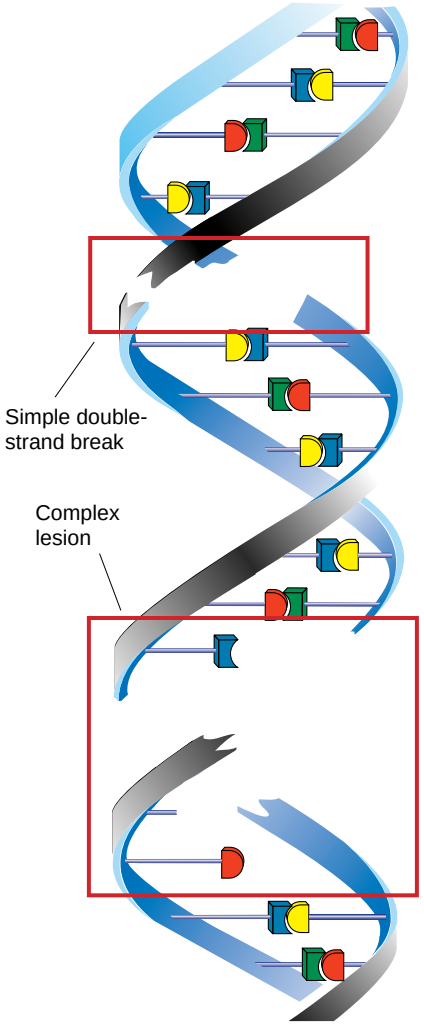


Figure 13. DNA Damage II: Double-Strand Breaks

Double-strand breaks result from two single-strand breaks that are induced at closely opposed positions in the complementary strands. Simple double-strand breaks (upper red box) can often be repaired by a simple end-joining procedure. Ionizing radiation often induces a complex lesion (lower red box) with base alterations and base deletions accompanying the breaks.

DNA Repair

Ionizing radiation induces four major types of DNA lesions. These are nucleic-acid base alterations, abasic sites, single-strand breaks, and double-strand breaks. Severe DNA damage might involve combinations of all three different lesions.

Most DNA base alterations are repaired by an enzymatic mechanism referred to as base-excision repair. This is a generalized repair mechanism that fixes many of the base alterations that are induced by ionizing radiation. The steps are outlined in Figure 1. Briefly, the damaged base and its associated sugar are removed from the DNA helix in a two-step process that leaves a one-base deletion. The missing base is replaced, using the undamaged complementary strand to ensure that the gap is filled with the correct base. Abasic sites are repaired in a similar manner. Repair of both base alterations and abasic sites by base excision restores the original nucleotide sequence.

Figure 1. Base-Excision Repair

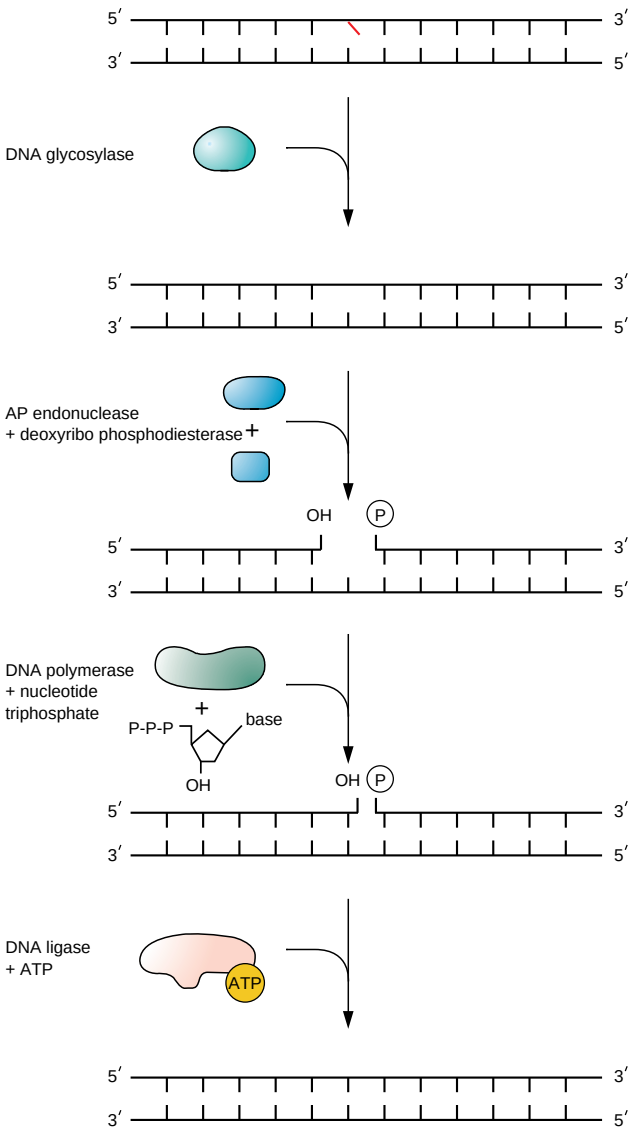
An altered base (slanted red line) results in a minor disruption of the DNA structure.

The lesion is recognized by a class of enzymes known as DNA glycosylases, which release the damaged base, leaving behind an abasic site.

An incision is made to the 5'-side of the abasic site by an AP-endonuclease and the remaining sugar is released by a deoxyribo phosphodiesterase.

The resulting one-base gap is filled by a DNA polymerase. Watson-Crick base pairing will dictate which base is used for the repair. The polymerase leaves a nick in the DNA backbone.

The repair is completed when a DNA ligase seals the nick. The DNA has been repaired with no loss of genetic information.



Single-strand breaks, which are lesions in the DNA backbone, frequently result from hydroxyl radical ($OH^{\bullet}$) attack on the deoxyribose sugar unit. The radical attack initiates the rupture of the sugar unit, leading to the release of the attached nucleic-acid base and most of the sugar unit from the DNA molecule. The result is a small, single-strand deletion, which is also repaired in a manner similar to base-excision repair. An exonuclease removes any sugar remnants, along with the phosphate group on the 5' side of the sugar. The resulting gap is filled by a DNA polymerase, again using the complementary strand as a template, and closure of the remaining single-strand nick is catalyzed by a DNA ligase.

Because there is an intact complementary DNA strand that is used in both base-excision repair and single-strand-break repair, these lesions pose little or no risk of permanent genetic change. This is not the case with double-strand breaks, which can be thought of as individual single-strand breaks that occur in opposite strands and within several bases of each other. The DNA molecule is completely broken in two. This type of damage usually results from severe insult to the DNA and is sometimes accompanied by significant base alterations in both strands. Thus, there may be no complementary strand immediately available with which to initiate repair.

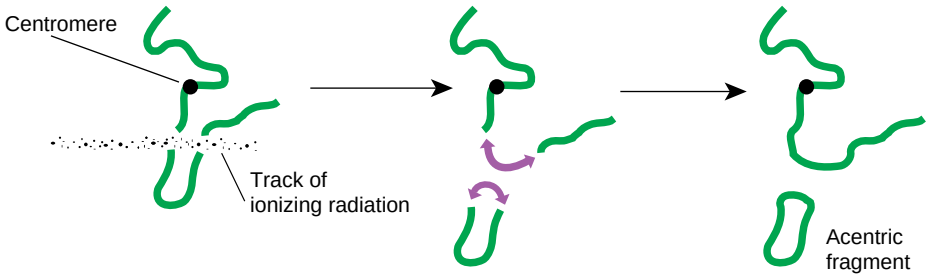


Figure 2.
Chromosome Deletions
Chromosomal deletions can arise if two double-strand breaks occur within the same chromosome, creating a DNA molecule that is broken into three pieces. If two of the end pieces are rejoined, such that the middle section of DNA is unattached, it is no longer associated with a centromere. This free floating piece, called an acentric fragment, may be lost during mitosis.

Double-strand breaks are often rapidly repaired by the simple mechanism of joining free ends, and this is likely to be a significant source of DNA mutations. In nonhomologous recombination, the free ends of broken DNA molecules are brought together and joined without reference to an intact partner. There are several mechanisms by which this can occur, the simplest employing a DNA ligase that ligates the two ends together. DNA topoisomerases I and II have also been identified as mediating the untemplated fusion of two DNA molecules. Although these mechanisms serve to rescue parts of a broken chromosome, they do so at the risk of introducing mutational changes and random genetic rearrangements.

Because end-joining reactions do not employ templates to guide the rejoining process, there is no means to replace missing information. Recall that each single-strand break is actually a one-base deletion. Joining the free ends of two single-strand breaks that are immediately opposite each other (creating a very simple type of double-strand break) will still result in the deletion of one entire base pair, and the formation of a deletion mutation. Furthermore, without a template, simple end-joining cannot even ensure that the ends being joined were from the same initial break. A chromosome deletion can occur if two or more double-strand breaks occur within the same chromosome, as illustrated in Figure 2. Because this deleted section is not associated with a centromere, the section (called an acentric fragment) is often lost when chromosomes are segregated during mitosis. This deprives a cell of a fraction of its genetic heritage.

A direct interaction between ionizing radiation and a cell's genome, however, can produce many proximal double-strand breaks. Joining of unrelated ends from

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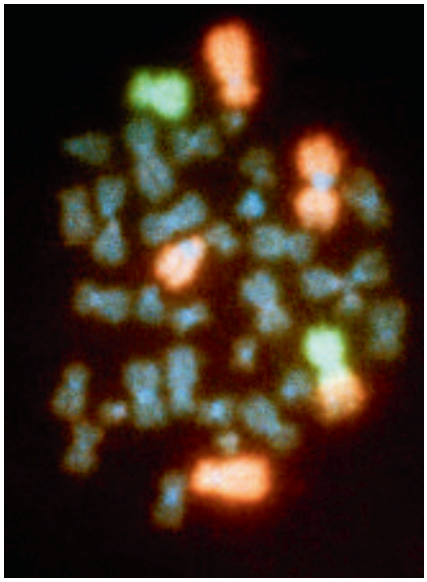


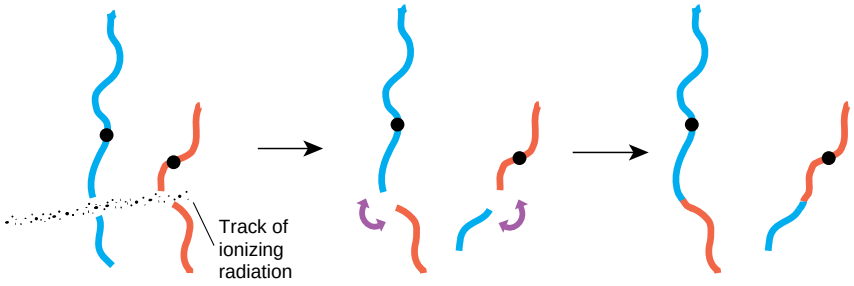


Figure 3. Chromosomal Translocation

A translocation occurs when large sections of genetic material are swapped between two or more chromosomes. In the illustration on the upper right, ionizing radiation creates double-strand breaks in two different chromosomes. The ends interchange when they are spliced back together.

The photo directly above of metaphase chromosomes from a human lymphocye shows a translocation between chromosome 1 and chromosome 8. Three chromosome pairs (1, 4, and 8) were labeled with colored markers that are specific for certain chromosomes. The two orange-colored chromosomes at the top of the photo are both chromosome 4. Chromosome 1 also appears orange. Chromosome 8 is marked with green. The orange-green chromosome is a translocation. A portion of chromosome 1 (center of photo) apparently became reattached to chromosome 8. It is not clear whether chromosome 8 remained intact or whether a portion was lost because of the translocation. (Photo courtesy of L. Gayle Littlefield, Ph.D, Oak Ridge Institute for Science and Education)

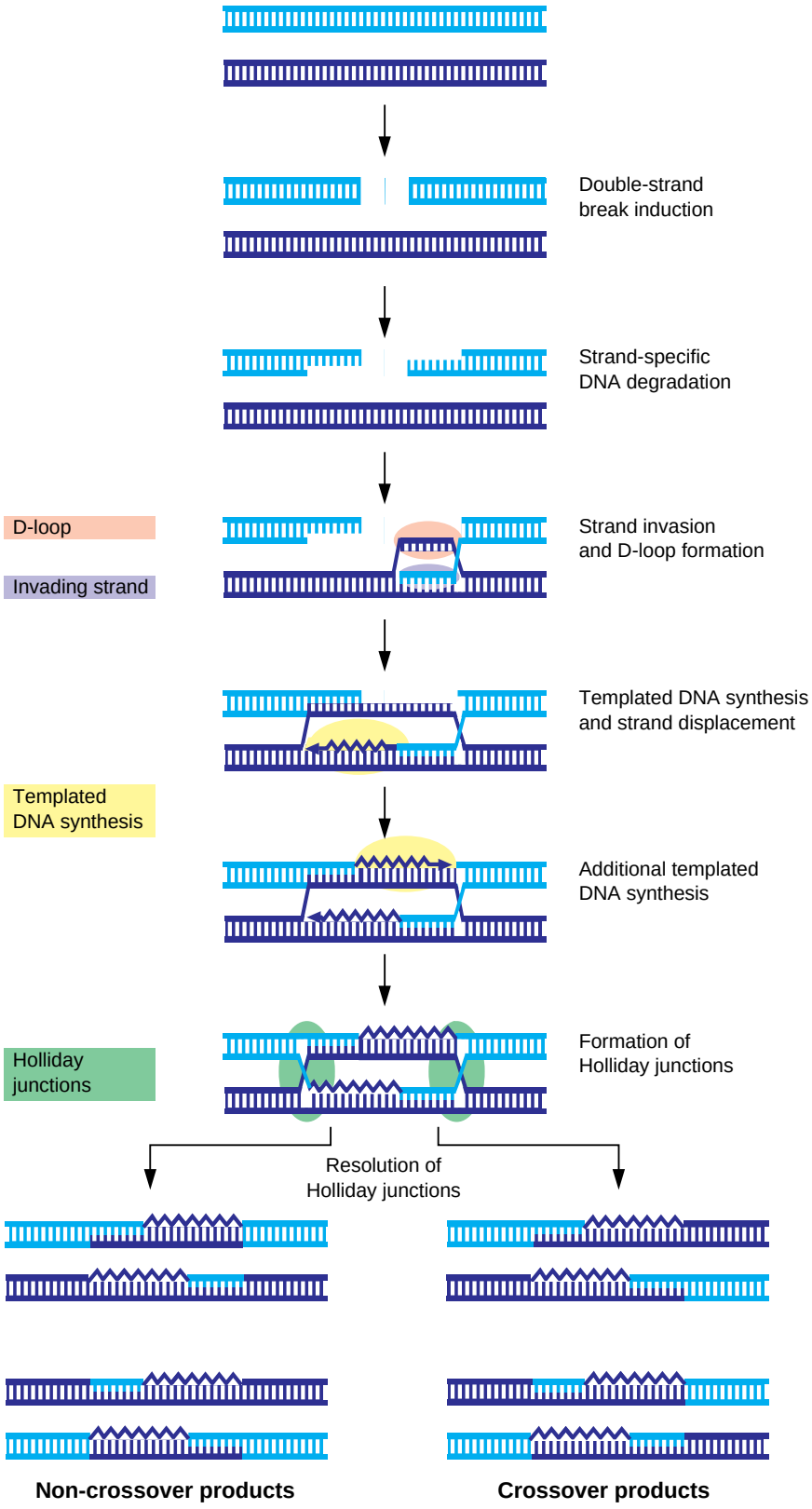
breaks in different molecules can result in genetic recombination, which is expressed at the chromosome level as chromosomal rearrangements and translocations. Figure 3 shows one such translocation.

Remarkably, there is a mechanism that is thought to be active in human cells that allows for the restoration of missing information. Called homologous recombination, it requires that a second, intact version of the DNA sequence be present in the cell. This can potentially be found on a homologous chromosome. The intact version is used as a template on which the fragments of the broken DNA molecule can be aligned in proper register. Figure 4 describes homologous recombination.

Recently, V(D)J recombination, which is the process that mediates the development of antibodies as part of the normal immune response, has been linked with the repair of double-strand breaks in DNA molecules. As such, it may provide an intriguing possible link between ionizing-radiation-induced DNA damage, p53 stabilization, and cell-cycle control.

An antibody "gene" is assembled from three different genetic elements, referred to as the variable (V), diversity (D) and joining (J) regions. Constructing the gene requires that V, D, and J sequences be selected from the collection of those available and that the selected sequences be joined to form the final antibody gene. The V(D)J-recombination system does this by inducing its own double-strand breaks and providing for their subsequent repair. Several different components of the V(D)J recombinase (the collection of proteins that mediate V(D)J recombination) have been identified and partially characterized. Two enzymes, RAG1 (*r*ecombination *a*ctivating *g*ene 1) and RAG2, are necessary and probably sufficient for introducing the site-specific cleavages. The resulting double-strand breaks are then bound by a complex of a least three proteins, including pKu70, pKu80 and p350, which is a DNA-dependent protein kinase (DNA-PK). The p350 protein expresses the kinase activity only when it is part of the pKu complex that is bound to a DNA end.

What is intriguing is that p53 is known to also bind to the DNA termini created by double-strand breaks and that p350 is capable of phosphorylating p53 in cell systems. It is speculated that the phosphorylation by DNA-PK is the mechanism by which p53 becomes stabilized and that this is the direct link between double-strand breaks and p53 stabilization that triggers the G_1 checkpoint (see main article) Recent findings have demonstrated that the V(D)J recombinase does participate in the repair of double-strand breaks induced by ionizing radiation and that this process contributes to the normal cellular resistance to the lethal effects of ionizing radiation. But it has not been proven that V(D)J recombinase directly helps induce the G_1 checkpoint nor that the particular site phosphorylated by p350 leads to an increase in p53 stability. ■



Adapted from Terry L. Orr-Weaver and Jack W. Szostak, 1985, Fungal recombination, *Microbiological Reviews* 49: 33-58.

Figure 4. Homologous Recombination

Homologous recombination requires two regions of identical or nearly identical DNA sequence. (In this diagram, two homologous DNA segments are indicated as separate molecules, but they might also be separate regions of the same molecule.)

The introduction of a DNA double-strand break into one of the two regions of homology provides the starting point for homologous recombination.

Unprotected ends generated by the double-strand break provide sites for strand-specific exonucleolytic degradation and the generation of protruding single-strand ends.

Invasion of the second DNA molecule by a protruding end can result in base pairing between the invading strand and a complementary sequence. The invading strand then serves as a primer for the initiation of DNA synthesis along the complementary strand of the intact molecule. Displacement of the second strand of the intact molecule (the D-loop) provides a single-strand region that can pair with the remaining end of the damaged molecule.

DNA synthesis proceeds along the displaced D-loop strand. Again, missing information is restored. This stage of the repair leaves two single-strand nicks.

Two cross-strand structures (Holliday junctions) form when residual single-strand nicks are sealed by a polynucleotide ligase.

Each Holliday junction can be resolved in two possible ways, resulting in four general types of recombination products. Two of these products retain flanking regions from the same parental molecule and are therefore noncrossover products. The other possible products are comprised of flanking regions from different parental molecules and are therefore crossover products. No matter which of these products is formed, missing DNA sequences have been replaced and the double-strand break sealed.

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are repaired using this mechanism. It is known, however, that ionizing radiation induces many deletion mutations and that these mutations probably arise during the repair of the double-strand breaks.

Because the repair of a double-strand break is generally nonspecific, free ends that arise from multiple breaks in chromosomes can get mixed and spliced back together arbitrarily. The result is a chromosomal rearrangement. These rearrangements include *chromosome deletions*, in which an entire section of a chromosome is spliced out, or a *translocation*, in which a piece of one chromosome is reattached to another chromosome. Chromosomal rearrangements that result in large DNA deletions, multiple translocations, or incomplete or distorted chromosomes are frequently fatal to a cell line. A surprising number of such aberrations, however, are not fatal. Stable translocations that don't result in cell death are readily found within the cells of healthy people, as well as in the cells of an irradiated population. Almost fifty years after the exposure, stable translocations can still be observed in the atomic-bomb survivors of Hiroshima and Nagasaki.

Oncogenes and Tumor-Suppressor Genes

The correlation of specifically mutated genes with specific cancers and the identification of two major classes of "cancer-causing" genes represent major breakthroughs in cancer research. One gene type, the *oncogenes*, are activated by the mutation or amplification of normal genes (called *proto-oncogenes*). Oncogene activation can be thought of as a gain of gene function, in that the overexpression or dysregulation of those mutated genes helps promote cell transformation. Due to this gain of function, oncogenes act in a dominant fashion, and expression of the transforming trait requires only one abnormal allele to effect change. Mutations in *tumor-suppressor genes*, on the other hand, are recessive in nature. Both alleles must be mutated or eliminated to disrupt cell functioning. Tumor suppressors normally act in a manner that regulates or impedes progression through the cell cycle, and it is the absence of that regulation that allows tumor development. The presence of either normal allele would result in the production of functional proteins, and therefore, both alleles must be inactivated. The inactivation of both alleles represents a loss of gene function. These concepts are illustrated in Figure 14. Due to the redundancy of cell-cycle regulatory processes, however, cancer development typically requires more than just the activation of one oncogene or the inactivation of one pair of tumor-suppressor genes. If one were to analyze the genome of a typical cancer cell, one would probably discover multiple mutational changes and find a complex mixture of oncogene activation and tumor-suppressor inactivation.

Many proto-oncogenes are part of regulatory pathways and exert their influence through the phosphorylation of target proteins, the formation of protein-protein complexes, or the regulation of transcriptional activity of target genes. Tellingly, most proto-oncogenes participate in the regulation of cellular proliferation or progression through the cell cycle. As previously mentioned, a disruption of those regulatory processes can result in abnormal proliferation and cell transformation.

Proto-oncogenes become oncogenes through the induction of one of several different genetic events, including DNA point mutations. For example, the *ras* gene is a proto-oncogene that is frequently made oncogenic by point mutations. The protein product of *ras* is pRas, the protein mentioned earlier as part of a signal transduction cascade (see Figure 5). A single point mutation at a critical site in *ras*

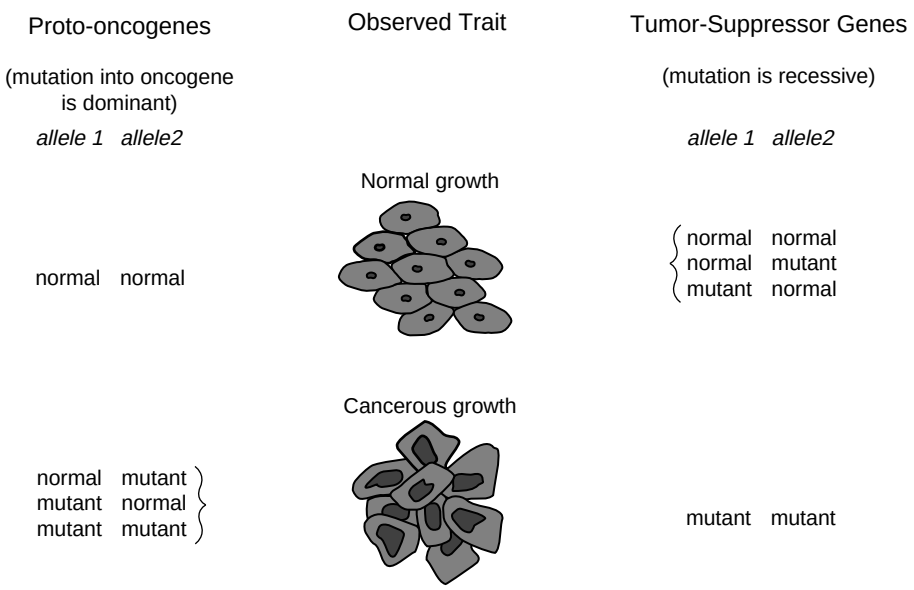

Figure 14. Proto-Oncogenes and Tumor-Suppressor Gene Action
The mutation of a proto-oncogene into an active oncogene represents a gain of gene function. The abnormal gene behaves in a dominant fashion and, therefore, only one of the two alleles normally present need be mutated (or overexpressed) to promote abnormal cellular proliferation. The mutant forms of tumor-suppressor genes represent a loss of function and are, therefore, recessive to the normal allele. Because the mutant forms are recessive, both alleles of tumor-suppressor genes must be inactivated by mutational changes.

may be sufficient to produce an abnormally fuctioning pRas protein that is always in an enzymatically active state. It no longer requires an activating signal to phosphorylate other proteins in the pathway. The entire cascade behaves erratically and continually sends a growth stimulatory signal to the nucleus. The cell either responds abnormally or else counteracts the signal via negative regulatory proteins. The *ras* oncogene is found to be mutated in about thirty per cent of all human cancers, including bladder and colon cancer.

Oncogenes may also be activated through a variety of cytogenetic events, such as large chromosome deletions, inversions, and translocations. Deletions result from the removal and loss of DNA segments, whereas inversions are the result of a DNA segment that has been removed from the chromosome and then reinserted at the same postion but in the opposite orientation. A translocation occurs when a section of one chromosome becomes reattached to a breakpoint in a second chromosome. Each of those processes results in the movement of large DNA segments, which may include a gene, from one position in the genome to a second position. If the breakpoints occur at the appropriate positions, the rearrangement can link a proto-oncogene to the distal end of another actively transcribed gene. In this way, transcription of the proto-oncogene can come under the control of a different and potentially more active gene. As an alternative scenario, the fusion protein may have altered properties, such as increased stability or resistance to negative effectors.

An example of oncogene activation through a translocation is the *bcl-2* proto-oncogene on the long arm of chromosome 18, which can become fused with the immunoglobulin heavy-chain (Ig H-chain) locus on the long arm of chromosome 14. This translocation is frequently associated with human B-cell lymphomas and has been identified in up to 85 per cent of the cases examined. A B cell, or B lymphocyte, is a type of white blood cell that is important for immune responses. The translocation positions the *bcl-2* gene downstream of the Ig H-chain promoter, which results in enhanced transcription of *bcl-2* and an overexpression of the Bcl-2 protein in B cells. This appears to prevent the B cells from undergoing apoptosis. At nominal Bcl-2 protein levels, external signals such as high cell densities

will trigger an apoptotic response in some cells. The B-cell population will be stabilized due to an equilibrium between cellular reproduction and apoptotic death. Cells that overexpress Bcl-2, however, become abnormally resistant to apoptosis and exhibit an extended life-span. The continued proliferation of B lymphocytes in the absence of appropriate cell death results in clonal expansion and an abnormal accumulation of cells that is ultimately recognized as leukemia.

Oncogene activation can be achieved by another process known as *gene amplification.* That phenomenon occurs when a region of DNA is replicated many more times than other regions of the cell's genome. Prior to gene amplification, a cell might contain anywhere between one and four copies of a given gene. After gene amplification, a gene might have hundreds or even thousands of copies per cell. If each amplified gene were transcribed at the same rate as its unamplified precursor, the encoded protein would be overexpressed in proportion to the increased representation of the gene. The cyclin-D1 oncogene was recognized as an amplified gene associated with parathyroid cancer. Cyclin-D1 protein was previously discussed in the context of regulating the E2F-activation pathway, and it is one of the major cell-cycle control proteins. Excess cyclin D1 is known to hasten the progression from G_1 to S phase and to reduce the influence of negative effectors on cell-cycle control. Under those circumstances, cellular proliferation is favored and a tumor can result.

But the regulation of cell reproduction is a result of a balance between positive and negative effectors. As outlined above, oncogenes are positive regulators that tend to stimulate cell growth (or to protect cells from apoptotic death). The tumor-suppressor genes act in a complementary fashion. Their normal role in cell regulation is to inhibit cell growth, and their protein products act as brakes on the cell cycle. The presence of any functional product, therefore, tends to limit cell growth and to suppress tumor formation. As a corollary, the complete absence of the gene (that is, loss of both alleles) enhances cell transformation and fosters neoplastic development.

Mutational inactivation of tumor-suppressor genes occurs by many of the same mechanisms that activate oncogenes, as well as additional mechanisms that result in a loss of function. Tumor-suppressor genes may be inactivated through the induction of point mutations, chromosome rearrangements, or the loss of part or all of a chromosome. Large deletions can eliminate the gene from the genome entirely or else remove so much genetic material that the protein product is not functional. But it is not required that the protein disappear altogether from the cellular pathways. The growth inhibitory function merely has to be compromised. Thus, single point mutations within a critical binding site can prevent the protein from functioning properly. The normal inhibitory function of the tumor-suppressor gene can then become inactivated.

The p53 gene, which was discussed earlier in connection with G_1 checkpoint regulation and apoptosis and which may also be involved directly in DNA repair, is the most notorious tumor-suppressor gene found to date. It is apparently mutated in one way or another in over fifty per cent of all human cancers. In the majority of cases examined, the inactivating mutations were found to be point mutations. The p53 protein contains a site-specific DNA binding region, the so-called *core domain.* The core domain specifies the sequence for a stretch of 190 amino acids, which are critical for the sequence-specific binding and transactivation properties of p53. Mutations at positions throughout this core domain have been found to correlate with human cancer.

Table 1. Some Oncogenes and Tumor-Suppressor Genes Active in the Cell Cycle

Oncogene	Activation	Human Cancer
cyclin A	disruption by viral infection	hepatocarcinoma
cyclin D	inversion	B-cell lymphomas
cyclin D	translocation	B-cell lymphomas
cyclin D	gene amplification	breast (~20%), gastric, esophageal carcinomas, parathyroid adenomas
bcl-2	translocation	B-cell lymphoma (85%)
E2F	overexpression	Cell culture and animal model systems
cdk6	overexpression	osteosarcoma
cdk4	gene amplification	sarcomas
mdm-2	gene amplification	soft-tissue sarcomas, metastatic osteosarcomas, malignant gliomas
ras	point mutation	~10% of all human cancers, including colorectal cancer (~30%), lung (~20%) pancreatic cancer (70-80%)
p53	point mutations (deletion mutations) (chromosome loss) (chromosomal rearrangement)	~50% of all human cancers, including breast, colon, liver, and lung
rb	deletion mutations chromosome loss (point mutations)	retinoblastoma (~100%) osteosarcoma

More than 70 oncogenes and about a dozen tumor-suppressor genes have now been identified within the cell. A partial listing of genes identified as oncogenes or tumor suppressors and discussed in this article is given in Table 1. These genes are "built" into our genomes and cannot be eliminated because they are so intimately tied to the proper functioning of the cell. For example, the *rb* gene, whose protein product, pRb, is essential in regulating the E2F transcription factor, is a well-known tumor-suppressor gene. A mutation of *rb* can lead to retinoblastoma, a rare cancer of the retina that typically shows up in childhood. Just as *rb* is central to the cell cycle, so is E2F, which is known to be an oncogene from cellular and animal studies. Those and all other oncogenes or tumor suppressors have been identified as such *precisely* because they are the genes that regulate cell behavior, and their improper expression, therefore, leads to neoplastic transformation. This lends itself to an interesting observation. Many "cancer-causing" genes specifically regulate cellular reproduction and thereby enable an organism to

maintain itself through growth and repair of tissue. Thus, cancer, which can bring about an organism's death, cannot be separated from those processes that help sustain an organism's life.

Ionizing Radiation and Cancer

Ionizing radiation can damage DNA, and that damage can lead to various changes in the genome, including gene mutations. From the point of view of the radiation biologist, the damage tends to be nonspecific. Genetic mutations appear in irradiated cells but with no apparent bias towards any particular gene. From the alternative perspective of the cancer researcher, specific oncogenes and tumor-suppressor genes have been correlated with specific cancers. Therefore, one concludes that genetic mutations do lead to cancer. Despite the apparent connection between the two viewpoints, there is still a crucial gap. Although many of the activating mutations in the genes *can* arise from interactions with ionizing radiation, we cannot in general state that they *did* arise as the result of a particular exposure. That is, it is usually not possible to say that radiation has induced a specific gene mutation and, further, that that specific mutation then results in a specific type of cancer.

There are several reasons for this lack of connectivity. The types of cancer normally found to be elevated in irradiated populations are also observed in nonirradiated populations. Frequently, the increased risk due to radiation exposure is small in comparison with the nominal risk. Therefore, one cannot deduce with any degree of certainty that a given cancer was due to a given exposure, as opposed to being the result of other factors. Furthermore, the development of cancer is a complex process, normally requiring multiple mutational changes that are accumulated over a period of many years. It is difficult to determine the order in which these changes arise. Establishing which of those genetic changes was the specific consequence of a radiation exposure that occurred many years prior to tumor formation is likewise very difficult. None-the-less, some correlations between radiation-induced DNA damage and cancer have been found.

One of the least ambiguous cases of a radiation-induced gene mutation concerns the *ret* oncogene. This oncogene has been associated with papillary adenocarcinomas of the thyroid, the predominant type of thyroid cancer found among the atomic-bomb survivors. The *ret* proto-oncogene is believed to encode a cell-surface receptor similar to those known to function in signal-transduction pathways. Since the *ret* proto-oncogene is known to be expressed more in fetal tissue than in adult tissue, it is hypothesized that the pathway is important for developmental growth and the maturation of tissue.

The oncogene was actually first recognized as a rearranged gene associated with thyroid cancer, and radiation-induced activation is thought to occur by this mechanism. The activating translocation occurs between a point within the *ret* gene, located on the long arm of chromosome 10, and a specific second locus, also on the long arm of chromosome 10. The ability of ionizing radiation to induce this specific rearrangement has been confirmed with cultured human cells. Moreover, in a recent study examining thyroid cancers in children from areas contaminated by the Chernobyl accident, this rearrangement of the *ret* oncogene was found in four out of a total of seven cancers examined, a remarkably high correlation.

Ionizing radiation is also believed to activate another oncogene, *bcr/abl*, through the induction of a specific translocation. The *c-abl* gene, located on chromosome 9, be-

comes fused with the *bcr* gene on chromosome 22. Each gene was originally located on one side of the breakpoint, and the fusion results in an abnormal, but still functioning, protein product. The *bcr/abl* oncogene has been strongly linked with a major form of radiation-induced B-cell leukemia called chronic myelogenous leukemia. Under normal conditions, the *abl* gene product is believed to act as a negative regulator of apoptosis in B lymphocytes. The *bcr/abl* fusion product becomes resistant to those signals that would normally override its protective influence. The increased resistance to apoptotic signals conferred by the *bcr/abl* oncogene leads to a clonal expansion and abnormal accumulation of the cells and, thus, to leukemia.

The only tumor-suppressor gene that is specifically correlated with radiation exposure is *rb*, which is a gene located on the short arm of chromosome 13. Mutations in both gene copies of *rb* are necessary to bring about retinoblastoma, which is a rare type of malignant cancer arising from the neural precursor cells in immature retinas. Retinoblastoma occurs in childhood and affects about one in every 20,000 children. Children that inherit only one defective *rb* gene have a predisposition for the disease. In the past, retinoblastomas were successfully treated by radiation therapy. Treatment with radiation, however, carries an increased risk of radiation-induced tumors when used to treat children with a familial defect in the retinoblastoma gene. Both primary and secondary retinoblastomas are characterized by the loss or inactivation of both rb alleles, and it is reasonable to assume that the increased risk observed with irradiated patients results from the inactivation of the normal *rb* allele.

Although many cancers have been correlated with ionizing-radiation exposure, the direct association with oncogenes or tumor-suppressor genes is frequently tenuous. Take as an example, lung cancer and the *p53* gene. Increased levels of lung cancers have been found in uranium miners exposed to radon gas. (Radon is a decay product of uranium, and so it will always be found in uranium mines.) Mutations of *p53* have been found in a number of those cancer cases. Most of those *p53* mutations are point mutations. We previously described how point mutations could arise from radiation-induced DNA base alterations, but we also indicated that radiation was not particularly good at creating that type of genetic change. Thus, correlating the *p53* mutations with radon is a little suspect. That suspicion should be coupled with research that indicates that *p53* inactivation seems to be a late-occurring mutation, that is, it appears in cells that have already been initiated and transformed. Late-appearing point mutations might be the result of some genomic instability that facilitates the accumulation of additonal mutations. Most of those mutations would be silent or have no effect on the cell. When a mutation appears in a critical gene, such as *p53*, the cell's transformation would get significantly advanced. Lastly, the uranium miners work in a hazardous enviroment and were exposed to elevated levels of silicates and diesel smoke. Many of them were also heavy cigarette smokers. Thus, in terms of epidemiology, this population is exposed to a complex mixture of carcinogens, and it cannot be concluded that ionizing radiation was the cause of the *p53* mutation in the lung cells of the uranium miners. It can only be stated that because of the exposure to elevated levels of radon gas and other environmental factors, uranium mine workers have an increased risk for developing lung cancer.

Conclusion

Ionizing radiation is a threat to human cells and ultimately to the entire organism. This threat begins with the induction of DNA damage, which may then be converted

into permanent genetic change. Ionizing radiation is an activator of onocogenes and an inactivator of tumor-suppressor genes. Oncogene activation promotes cellular proliferation, and the checks on this proliferation are removed through the inactivation of tumor suppressors. Together, these genetic events, when induced in cells that still grow and divide, can lead to unregulated cellular proliferation and cancer.

Yet, ionizing radiation doesn't seem to have *any* favored role in the induction of cancer; it is simply one of the many different types of carcinogens. The cancers that are strongly correlated with radiation exposure are also observed in "unirradiated" control populations. And although the data are still limited, radiation probably initiates cancer by mechanisms similar to those mediating the induction of "spontaneous" cancers and that are stimulated by other carcinogens. For example, activation of the *ret* proto-oncogene appears to be commonly associated with thyroid cancer in both irradiated and unirradiated populations. Activation of *abl* is commonly associated with B-cell lymphomas irrespective of radiation exposure. Most of the spontaneous and radiation-induced cancers in individuals with one normal and one mutant allele of rb probably involve inactivation of the remaining normal *rb* allele. Each of these genes is involved in the regulation of cellular proliferation, and similarities between spontaneous and radiation-induced cancers are striking.

Although radiation can induce DNA damage, human cells are not without their defenses. Many of the DNA lesions induced by ionizing radiation are similar or identical to those induced as a consequence of normal metabolic activity. DNA repair mechanisms can act to reduce the consequences of this damage. Most single-strand breaks and base alerations can be repaired perfectly. Even double-strand breaks are repaired so as to minimize genetic change. Simple end-joining reactions are optimized for retaining chromosomal linkage relationships and preserving the ability to segregate genetic information in a normal manner during mitosis. Homologous recombination can actually restore missing information (although this process is believed to mediate the repair of only a small fraction of double-strand breaks). Together these processes act to reduce the risk posed by the induction of DNA damage. But the damage induced by ionizing radiation poses more than one risk, and it should be remembered that the very process of DNA repair may actually create genetic alterations as part of an attempt to prevent cellular death.

Clearly, the induction of cancer is a complicated process, and our understanding of radiation's role in its induction is far from complete. But these are remarkable times. Research is progressing at an increasingly rapid pace, and amazing insights are reported daily. We have only recently begun to appreciate the remarkably active role that the cell plays in preventing the full development of cancer. Through cellular senescence and apoptosis, a cell effectively limits its own proliferative potential. How the cell triggers those responses and their contribution to the complexity of the cell cycle has only been gleaned within the past three or four years.

Current and future research into the cell cycle, the regulation of cellular proliferation, and the impact of radiation on these processes will undoubtly lead to additional insights. New proto-oncogenes and tumor suppressors will be discovered. Precise roles for known and soon-to-be-discovered genes will be defined. Our knowledge of DNA repair mechanisms and their contribution to genomic stability and genetic change in irradiated cells will be expanded. Each of these discoveries will move us closer to understanding the aberrations in cellular metabolism that enable the development of cancer. And each will advance us towards our goal of cancer prevention. In a few years, this primer will need to be rewritten. Perhaps then it will optimistically be called "Radiation, the Cell Cycle, and the Prevention of Cancer." ■

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