Chapter-17

INTRODUCTION TO RADIOBIOLOGY

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17.1. INTRODUCTION

Radiobiology, also known as radiation biology, is the branch of science that studies the interaction of ionizing radiation with living systems. It integrates principles from biology, physics, and medicine to understand how radiation affects cellular and molecular structures, and the biological consequences of such interactions. The field is essential in many disciplines, particularly in medical imaging, radiation therapy, nuclear medicine, radiation protection, and environmental health. Radiobiology serves as a cornerstone for developing safe and effective uses of radiation in both diagnostic and therapeutic contexts, as well as for implementing protective measures in occupational and public settings. Ionizing radiation refers to radiation with enough energy to remove tightly bound electrons from atoms, thereby producing ions [1]. The most common types of ionizing radiation include X-rays, gamma rays, alpha particles, beta particles, and neutrons. When such radiation interacts with biological tissue, it can cause a range of effects at the molecular, cellular, tissue, and systemic levels. The energy deposited by radiation in tissues can result in direct damage to critical biomolecules such as DNA, proteins, and membranes, or indirect effects via the radiolysis of water, leading to the production of reactive oxygen species (ROS) that, in turn, cause oxidative damage. Radiobiology, the scientific study of the interaction between ionizing radiation and living systems, has its origins deeply rooted in the discovery of radiation itself. This multidisciplinary field emerged as a response to the need to understand the biological effects of radiation exposure, both beneficial and harmful. It encompasses a comprehensive understanding of the mechanisms by which radiation interacts with biological molecules, cells, tissues, and organisms. Over the decades, radiobiology has evolved into a cornerstone of various medical and scientific disciplines, particularly radiation oncology, diagnostic radiology, nuclear medicine, radiation protection, and environmental health sciences.

Historical Foundations

The history of radiobiology is intimately connected to the discovery of X-rays and radioactivity at the turn of the 20th century. In 1895, Wilhelm Conrad Roentgen, a German physicist, discovered X-rays while experimenting with cathode rays. This marked the dawn of a new era in physics and medicine. The following year, Henri Becquerel discovered natural radioactivity, observing that uranium salts emitted invisible rays that could fog photographic plates. These discoveries were soon extended by Marie and Pierre Curie, who identified and isolated radioactive elements such as polonium and radium [2]. These groundbreaking findings not only revolutionized physics and chemistry but also laid the foundation for the biological exploration of radiation. Within a few years of these discoveries, reports began to emerge regarding the harmful effects of radiation exposure. Early X-ray operators and scientists experienced symptoms such as skin burns, hair loss, and even malignancies, long before the mechanisms of radiation damage were understood. These adverse effects prompted the need for systematic biological investigation into the nature of radiation injury. The field of radiobiology began to take shape as researchers started exploring how ionizing radiation affects cells and tissues. The first recorded biological experiment using radiation was conducted by Antoine Henri Becquerel, who carried radium in his pocket and developed a skin lesion resembling a burn, suggesting that radiation could penetrate tissue and induce damage. Further studies in the early 1900s by scientists such as Caspari, Zirkle, and Puck provided foundational insights into cellular radiosensitivity and radiation-induced mutations.

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One of the key early findings was that radiation caused chromosomal aberrations and could impair cellular reproduction, which later became central to understanding radiation-induced carcinogenesis. In 1906, Jean Bergonié and Louis Tribondeau, two French scientists, proposed what would become a fundamental principle of radiobiology—the Law of Bergonié and Tribondeau. This law stated that the radiosensitivity of cells is directly proportional to their reproductive activity and inversely proportional to their degree of differentiation. In essence, rapidly dividing, undifferentiated cells (such as those in bone marrow and the gastrointestinal tract) are more susceptible to radiation damage than mature, specialized cells. This principle has remained foundational in radiobiological research and clinical radiation therapy planning [3].

Radiobiology saw significant growth and institutional development during and after World War II, especially with the onset of nuclear weapons research and the atomic bombings of Hiroshima and Nagasaki in 1945. These events underscored the devastating biological potential of high-dose radiation exposure and spurred extensive studies into the short- and long-term effects of radiation on human health. The Atomic Bomb Casualty Commission (ABCC), and later the Radiation Effects Research Foundation (RERF), conducted extensive epidemiological studies on atomic bomb survivors, providing vital data on radiation-induced cancer risks, genetic effects, and dose-response relationships. Simultaneously, in the mid-20th century, radiobiology became more established as a scientific discipline. The development of in vitro and in vivo experimental techniques enabled researchers to examine the mechanisms of radiation damage at the cellular and molecular levels. Studies by Douglas Lea and Hermann Muller (who won the Nobel Prize in 1946 for showing that X-rays induce mutations in fruit flies) advanced understanding of radiation-induced genetic damage. Investigations into DNA repair mechanisms, cell cycle checkpoints, and programmed cell death (apoptosis) further enriched the field. The 1950s and 1960s also witnessed the development of dose fractionation principles in radiation therapy, spearheaded by studies on tissue repair kinetics and tumor control probability. These findings became integral to optimizing therapeutic radiation schedules, balancing effective tumor eradication with the preservation of normal tissue function.

With the advent of molecular biology and genetic engineering in the late 20th and early 21st centuries, radiobiology entered a new phase. Research began to focus on the molecular and cellular pathways involved in radiation response, including DNA damage signaling, repair pathways (such as non-homologous end joining and homologous recombination), and the roles of tumor suppressor genes like p53. The use of advanced imaging techniques, molecular assays, and omics technologies allowed for high-resolution studies of radiation effects at the gene and protein levels. Radiogenomics—an emerging subfield—aims to link genetic profiles with individual radiosensitivity, enabling personalized radiation therapy. Similarly, studies in bystander effects, adaptive responses, and radiation-induced genomic instability have reshaped the classical understanding that only directly irradiated cells are affected, suggesting complex intercellular signaling mechanisms play a role in the biological response to radiation. In tandem, advancements in radiation protection standards, driven by organizations such as the International Commission on Radiological Protection (ICRP), the National Council on Radiation Protection and Measurements (NCRP), and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), have relied heavily on radiobiological data to define safe exposure limits for workers, patients, and the public.

17.2. SIGNIFICANCE OF RADIOBIOLOGY IN MEDICINE AND RESEARCH

Radiobiology plays a vital role in both medicine and biomedical research, underpinning a wide range of applications that are central to diagnosis, treatment, radiation safety, and scientific discovery. Its principles are fundamental to understanding how ionizing radiation interacts with biological tissues, enabling clinicians and researchers to use radiation effectively while minimizing risks to patients and healthcare workers. In medicine, radiobiology forms the scientific backbone of disciplines such as radiation oncology, diagnostic radiology, nuclear medicine, and radiation protection, each of which relies on a deep understanding of the biological effects of radiation.

- In radiation oncology, radiobiology is crucial for designing and optimizing cancer treatment regimens that use ionizing radiation to destroy malignant cells while sparing normal tissues. Knowledge of tumor cell radiosensitivity, DNA repair mechanisms, the effects of dose fractionation, and the influence of oxygenation (hypoxia) helps oncologists tailor individualized treatment plans. Concepts such as the four Rs of radiobiology—Repair, Reassortment, Repopulation, and Reoxygenation—are fundamental to understanding tumor response and improving therapeutic outcomes. Radiobiology also informs the use of advanced modalities like stereotactic body radiotherapy (SBRT), intensitymodulated radiotherapy (IMRT), and proton therapy, each requiring precise biological modeling to maximize efficacy [4].

- In diagnostic imaging, including X-rays, CT scans, and nuclear medicine procedures, radiobiology guides the balance between image quality and radiation exposure. Understanding the stochastic risks of low-dose radiation exposure, especially in vulnerable populations such as children and pregnant women, is essential for justifying procedures and optimizing radiation doses. Radiobiological data support the implementation of dose-reduction strategies, such as the ALARA principle (As Low As Reasonably Achievable), ensuring patient safety without compromising diagnostic performance.
- In nuclear medicine, which involves the use of radiopharmaceuticals for both imaging and therapy, radiobiology provides insight into how radioactive isotopes interact with tissues at the cellular level. The design and application of targeted radiotherapies, such as radioimmunotherapy or theranostics, depend on a precise understanding of radiation dose distribution, cellular uptake, and biological effects on both tumor and healthy tissues.

Radiation protection and public health is another critical area where radiobiology is of immense significance. It informs the establishment of regulatory standards for occupational exposure, environmental monitoring, and emergency response in the event of nuclear accidents or radiological terrorism. Organizations such as the ICRP, IAEA, and NCRP base their recommendations on radiobiological evidence derived from both experimental studies and epidemiological data, including those from atomic bomb survivors and patients exposed to medical radiation. These standards are essential for protecting workers in radiological environments, ensuring public safety, and guiding policymakers in formulating evidence-based regulations.
From a research perspective, radiobiology continues to drive innovation and discovery in cell biology, genetics, and molecular medicine. Research into DNA damage response pathways, oxidative stress, and cellular signaling mechanisms has provided broader insights into cancer biology, aging, immune responses, and degenerative diseases. Radiobiological studies have led to the identification of biomarkers for radiation exposure and have contributed to the understanding of individual radiosensitivity, paving the way for personalized medicine. The emerging field of radiogenomics seeks to correlate genetic variations with individual responses to radiation, offering the potential to tailor radiation therapy based on genetic profiling. Radiobiology is also central to space medicine, where understanding the biological effects of cosmic radiation on astronauts is vital for planning long-duration space missions. Research in this area examines the potential risks of space radiation on cellular integrity, neurocognitive function, cancer development, and overall human health in microgravity and high-radiation environments [5].

17.3. RADIATION INTERACTION WITH CELLs

Ionizing radiation exerts profound effects on the cell cycle by inducing DNA damage that activates a network of signaling pathways responsible for halting cell cycle progression, allowing for DNA repair, or initiating cell death if the damage is irreparable. The cell cycle is divided into four main phases: G_1 (first gap), S (DNA synthesis), G_2 (second gap), and M (mitosis). Each phase is governed by specific regulatory checkpoints that ensure proper DNA replication and segregation. When a cell is exposed to radiation, the most immediate consequence is the induction of DNA lesions, particularly double-strand breaks (DSBs), which are among the most lethal forms of DNA damage. These lesions are sensed by damage recognition proteins such as ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related), which activate downstream effectors including p53, Chk1, and Chk2. These molecules then mediate cell cycle arrest by inhibiting cyclin-dependent kinases (CDKs), halting progression at specific checkpoints [6]. The G_1/S checkpoint is one of the first barriers activated in response to radiation-induced damage. This checkpoint ensures that damaged DNA is not replicated during the S phase. The tumor suppressor protein p53 plays a central role here by inducing the expression of p21, a CDK inhibitor that halts the cell cycle by preventing the phosphorylation of the retinoblastoma protein (Rb), thereby blocking the transition from G_1 to S phase. In cells with functional p53, this mechanism serves as a critical line of defense against the propagation of mutations. In contrast, cells lacking p53 may fail to arrest properly, resulting in genomic instability. During the S phase, radiation-induced damage can lead to replication stress and fork stalling. The intra-S phase checkpoint becomes active to slow down or halt DNA synthesis, allowing the cell time to repair lesions before replication resumes. This is crucial for maintaining replication fidelity and preventing the formation of additional DSBs that can occur if replication forks collapse.

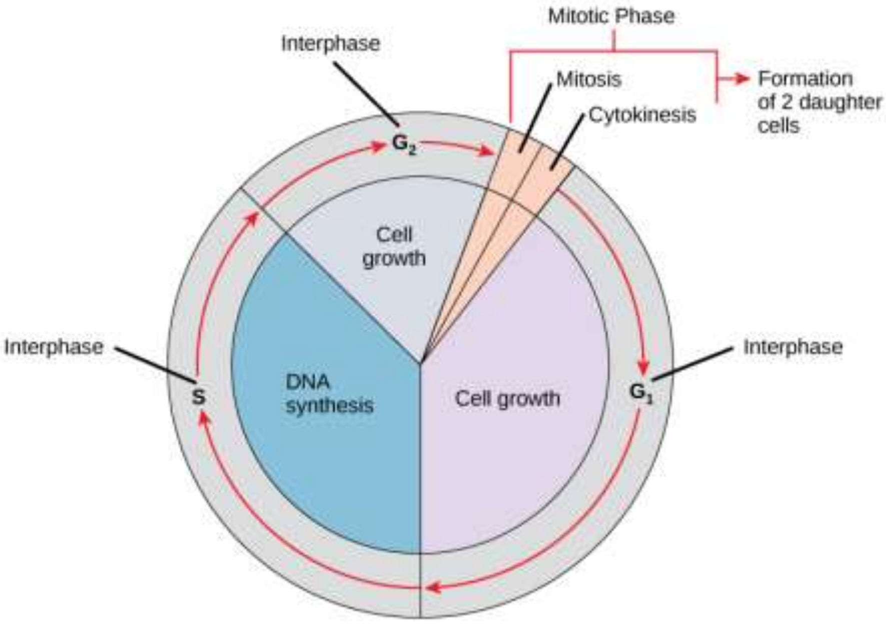

Fig: 17.1. Cell Cycle

The G_2/M checkpoint is another critical juncture affected by radiation. Prior to entering mitosis, cells check for unreplicated or damaged DNA. Radiation exposure activates the G_2/M checkpoint through Chk1/Chk2-mediated inhibition of CDC25 phosphatases, which are necessary for activating the CDK1-cyclin B complex that drives the cell into mitosis. This delay allows repair mechanisms, such as non-homologous end joining (NHEJ) and homologous recombination (HR), to correct the DNA damage. Cells that bypass this checkpoint and enter mitosis with damaged DNA often undergo mitotic catastrophe, a form of cell death associated with aberrant chromosome segregation and nuclear fragmentation. The effects of radiation on the cell cycle are also phase-dependent in terms of radiosensitivity. Cells are generally most sensitive to radiation during the G_2 and M phases due to the condensed chromatin structure and active mitotic machinery, which make DNA damage more impactful and less repairable. In contrast, cells in the late S phase are relatively more radioresistant, as homologous recombination repair is most active and DNA is less condensed, allowing greater access to repair proteins.

17.3.1. Radio-sensitivity

Radio-sensitivity refers to the susceptibility of cells, tissues, or organisms to the harmful effects of ionizing radiation. It is a central concept in radiobiology, with significant implications in radiation protection, medical diagnostics, and cancer therapy. The degree of radiosensitivity varies depending on biological and physical factors, including cell type, mitotic activity, DNA repair capacity, oxygenation level, and the quality of radiation received. One of the earliest and most enduring principles describing radiosensitivity is the Law of Bergonié and Tribondeau (1906), which states that cells are more radiosensitive if they are rapidly dividing, immature, and have a long mitotic future. Consequently, embryonic and stem cells exhibit high sensitivity to radiation, whereas mature, differentiated cells such as neurons and muscle fibers are more resistant. Radiosensitivity also fluctuates throughout the cell cycle. Cells are generally most radiosensitive in the **G_2 and** M phases, where chromatin condensation limits repair access, and least sensitive during the **late S phase**, due to active DNA repair via homologous recombination [7].

A critical determinant of radiosensitivity is the cell's ability to repair radiation-induced DNA damage. Cells equipped with efficient repair mechanisms, including non-homologous end joining (NHEJ) and homologous recombination (HR), are more likely to survive radiation exposure. However, mutations in key DNA repair genes, such as ATM, BRCA1/2, or TP53, can impair the cellular response to damage, thereby enhancing radiosensitivity and increasing the risk of carcinogenesis. The presence of oxygen also profoundly affects radiosensitivity. Known as the oxygen effect, this phenomenon occurs because oxygen "fixes" radiation-induced free radical damage, making it permanent and thus more lethal to cells. Oxygen enhancement is quantified by the Oxygen Enhancement Ratio (OER). Tumor cells in hypoxic regions are less radiosensitive and often resist conventional radiotherapy. This has led to the development of strategies like hyperbaric oxygen therapy and hypoxic radiosensitizers to increase the effectiveness of radiation treatment.

Additionally, the type of radiation influences cellular radiosensitivity. High-LET (Linear Energy Transfer) radiation, such as alpha particles and carbon ions, deposits energy densely along its path, creating complex DNA lesions that are harder to repair. These types of radiation exhibit greater biological effectiveness and are used in advanced forms of radiotherapy like carbon ion therapy. Conversely, low-LET radiation, such as X-rays and gamma rays, causes more sparse ionization and is less effective per unit dose. Understanding radiosensitivity is crucial in clinical oncology, where the goal is to maximize tumor damage while sparing healthy tissues. Tissues with high radiosensitivity require lower radiation doses for therapeutic effects, while radioresistant tumors may necessitate dose escalation, radiosensitizing agents, or alternative treatment modalities [8]. It is also vital in radiation protection, particularly for sensitive populations such as children and pregnant women, and for the development of personalized medicine approaches based on individual genetic susceptibility to radiation.

Table: 17.1. Radio-sensitivity of Various Human Tissues and Cells

Category	**Examples**	**Radiosensitivity Level**	**Remarks**
Highly Radiosensitive	Bone marrow stem cells, lymphocytes, spermatogonia, intestinal crypt cells	Very High	Rapidly dividing, undifferentiated cells; significant for acute radiation syndrome
Moderately Radiosensitive	Skin basal cells, salivary gland cells, lung epithelium, liver cells	Moderate	Can recover from sub-lethal damage; affected by dose and exposure duration
Radioresistant	Muscle cells, neurons, cartilage, connective tissue cells	Low	Highly differentiated, non-dividing; minimal DNA replication
Tumor Cells	Varies widely by type (e.g., lymphomas: high; gliomas: low)	Varies	Dependent on cell type, hypoxia, genetic mutations, and repair capacity
Embryonic and Fetal Cells	Embryo, fetal CNS, germ layers	Extremely High	High cell division rate; potential for developmental abnormalities

17.4. RADIATION EFFECTS ON DNA

DNA, the hereditary material in all living organisms, plays a vital role in cellular functions such as replication, transcription, and genetic expression. It is composed of two strands forming a double helix, made up of nucleotides containing a sugar-phosphate backbone and nitrogenous bases. This molecular structure is highly susceptible to damage by external and internal factors, among which ionizing radiation is one of the most potent. Exposure to ionizing radiation leads to a wide array of DNA damage, ranging from subtle base modifications to lethal double-strand breaks. The integrity of the genome is maintained through intricate DNA repair systems that identify and rectify damage, thereby ensuring genomic stability and cellular survival. Understanding the effects of radiation on DNA and the cellular responses to such damage is crucial for radiobiology, cancer therapy, radiation protection, and genetic toxicology [9].

17.4.1. Direct Action Mechanism

The direct action mechanism is one of the primary ways ionizing radiation causes damage within biological cells, particularly through its interaction with vital macromolecules like DNA. In this mechanism, the radiation deposits energy directly into the molecular structure of these targets, leading to ionization or excitation of atoms within the DNA itself. As a result of this energy deposition, critical chemical bonds in the DNA structure may break, producing various types of lesions. These include single-strand breaks (SSBs), double-strand breaks (DSBs), base modifications, and DNA-protein crosslinks. Such damage interferes with fundamental cellular processes like DNA replication and transcription, potentially compromising cell viability and genetic integrity. If not accurately repaired, these alterations can lead to mutations, carcinogenesis, or programmed cell death. Direct action is especially relevant in the context of high-linear energy transfer (LET) radiation, such as that emitted by alpha particles, neutrons, and heavy charged particles like carbon ions. High-LET radiation deposits a large amount of energy over a short path length, creating dense ionization tracks. This densely packed energy deposition results in clusters of ionization events in a localized region, thereby increasing the likelihood of the radiation interacting directly with DNA and other essential biomolecules. The track structure of high-LET radiation is complex and often leads to multiple types of damage within a small region of DNA, commonly referred to as clustered or complex DNA damage. These complex lesions are much more difficult for the cell to repair and pose a greater biological threat than damage caused by sparse, isolated ionizations typical of low-LET radiation [10].

The biological consequences of direct radiation action are profound. Among the various types of DNA damage, double-strand breaks (DSBs) are considered the most lethal. If DSBs are not repaired correctly, they can lead to chromosomal aberrations such as deletions, inversions, or translocations, which in turn can disrupt cellular function or initiate malignant transformation. Cells respond to such damage by activating a range of repair pathways, including non-homologous end joining (NHEJ) and homologous recombination (HR). However, the densely ionizing nature of high-LET radiation often results in damage that overwhelms or bypasses these repair mechanisms, leading to irreversible cell injury, apoptosis, or senescence. Moreover, even when repair is attempted, errors in repair can introduce mutations, further increasing the risk of oncogenesis. From a therapeutic perspective, the direct action mechanism forms the foundation of several advanced radiotherapy modalities that utilize high-LET radiation. Treatments such as proton therapy, carbon ion therapy, and neutron therapy exploit the heightened biological effectiveness of high-LET radiation to target tumors more precisely and destroy cancer cells that are resistant to conventional low-LET radiation, such as X-rays or gamma rays. These therapies benefit from a characteristic known as the Bragg peak, which allows the maximum dose of radiation to be delivered directly to the tumor with minimal exposure to surrounding healthy tissues. The clinical advantage of high-LET radiation lies not only in its superior dose distribution but also in its ability to induce complex DNA damage that cancer cells struggle to repair, thereby enhancing tumor control and improving patient outcomes.

17.4.2. Indirect Action Mechanism

The indirect action mechanism represents a major pathway by which ionizing radiation induces biological damage, particularly within cells that are predominantly composed of water. Unlike the direct action mechanism, which involves the immediate deposition of energy into critical macromolecules like DNA, indirect action relies on radiation interacting with the water molecules that make up approximately 70–80% of the cellular volume. This interaction leads to a process known as radiolysis of water, wherein ionizing radiation causes the water molecules to break apart, forming a cascade of highly reactive chemical species known as free radicals. The most important of these are the hydroxyl radical (•OH), the hydrogen radical (•H), and the hydrated electron (e^-_{aq}). These free radicals are chemically unstable and highly reactive, enabling them to diffuse short distances within the cell and subsequently react with critical biomolecules, including DNA, proteins, and lipids. Through these secondary interactions, indirect action can cause a variety of cellular damage, including single-strand breaks, base modifications, and lipid peroxidation, thereby impairing vital cellular functions. The generation of free radicals through the radiolysis of water follows a specific sequence of reactions. Initially, a water molecule absorbs energy from the radiation and becomes ionized, forming a positively charged water ion (H_2O^+) and an ejected electron (e^-). This process is represented by the equation:

$$H_2O + \text{radiation} \rightarrow H_2O^+ + e^-.$$

The water ion (H_2O^+) is highly reactive and immediately reacts with a neighbouring water molecule to produce a hydronium ion (H_3O^+) and a hydroxyl radical (•OH):

$$H_2O^+ + H_2O \rightarrow H_3O^+ + \bullet OH.$$

Simultaneously, the free electron (e^-) becomes hydrated upon interaction with water, producing a hydrated electron (e^-_{aq}), which can then lead to the formation of additional radicals such as the hydrogen radical (•H) and hydroxide ion (OH^-):

$$e^- + H_2O \rightarrow e^-_{aq} \rightarrow \bullet H + OH^-.$$

Among all the radicals generated, the hydroxyl radical is the most biologically significant due to its extremely high reactivity and ability to induce chemical alterations in the DNA, especially by abstracting hydrogen atoms from the sugar-phosphate backbone or attacking the nucleotide bases. Although it has a very short half-life, its high reactivity and proximity to essential macromolecules make it a potent agent of biological damage. The indirect action mechanism is predominantly associated with low-LET (Linear Energy Transfer) radiation such as X-rays and gamma rays, which are commonly used in both diagnostic radiology and radiation therapy. Unlike high-LET radiation, which deposits energy densely and is more likely to cause direct DNA hits, low-LET radiation distributes energy more sparsely as it passes through tissues. Consequently, the probability of direct ionization of DNA is relatively low in such cases. However, the abundance of water molecules ensures that ionizing events within the cellular milieu predominantly affect water, leading to prolific generation of free radicals. These radicals can then cause indirect DNA damage, making this mechanism the dominant mode of action for low-LET radiation in clinical and environmental exposure settings.

While the cellular damage resulting from indirect action is generally more diffuse and less clustered than that from direct action, it still holds significant biological importance. The resulting DNA lesions are often repairable through base excision repair and single-strand break repair pathways. However, the efficiency of repair varies depending on the extent of damage, the cell cycle phase, and cellular repair capacity. An important factor influencing the biological consequences of indirect action is the presence of molecular oxygen, which enhances the effectiveness of radiation. This phenomenon, known as the oxygen effect, involves oxygen reacting with radiation-induced free radical products or damaged DNA bases to produce permanent and irreparable lesions. As a result, oxygenated cells are more radiosensitive than hypoxic cells. This principle is central to various strategies in radiation oncology, such as hyperbaric oxygen therapy, which aims to improve tumor oxygenation, and the development of radiosensitizing agents designed to increase the effectiveness of radiation in oxygen-deficient tumor regions. The relative contributions of direct and indirect actions are influenced by factors such as radiation type, LET, oxygen availability, and cellular hydration status. For example, the presence of molecular oxygen enhances the fixation of radiation-induced DNA damage by forming peroxyl radicals, a phenomenon known as the oxygen enhancement effect, which is particularly significant for indirect action. This has important clinical implications in radiation therapy, where hypoxic tumor regions are more radioresistant due to reduced indirect DNA damage.

17.4.3. Types of Radiation-Induced DNA Damage

Ionizing radiation causes a variety of DNA lesions that differ in both frequency and biological significance. The extent and nature of this damage depend on the radiation quality, dose, and the physical and chemical environment of the cell at the time of exposure. DNA damage is the critical initiating event for many of the harmful cellular outcomes of radiation exposure, including mutation, genomic instability, cell death, and carcinogenesis. The most relevant types of radiation-induced DNA damage are base damage, single-strand breaks, double-strand breaks, crosslinking lesions, and complex or clustered DNA damage.

- **Base Damage:** Base damage is one of the most common outcomes of ionizing radiation, especially via the indirect effect where reactive oxygen species (ROS), such as hydroxyl radicals (•OH), oxidize DNA bases. These alterations include chemical modifications to the nitrogenous bases (adenine, guanine, cytosine, and thymine), which can affect base-pairing fidelity during DNA replication. A widely studied lesion is 8-oxoguanine (8-oxoG), an oxidized form of guanine that tends to mispair with adenine instead of cytosine, leading to G→T transversion mutations. Other important lesions include form amidopyrimidine, which results from purine ring cleavage, and thymine glycol, which distorts the DNA helix and blocks replication. While base lesions may not be lethal on their own, their mutagenic potential is high, and they can compromise genome stability if not efficiently repaired by the base excision repair (BER) pathway.
- **Single-Strand Breaks (SSBs):** Single-strand breaks occur when ionizing radiation cleaves the phosphodiester backbone of one DNA strand. SSBs are frequently induced by low-LET radiation such as X-rays and gamma rays and often arise from the attack of free radicals generated during radiolysis of water. These lesions are typically repaired efficiently by the BER pathway, which involves DNA glycosylases, AP endonucleases, DNA polymerases, and ligases. However, if not promptly repaired, SSBs can interfere with transcription and replication machinery, causing replication fork collapse or transcriptional arrest. Additionally, if SSBs occur in clusters or opposite each other on complementary strands, they can convert into double-strand breaks—a far more dangerous form of damage.

Double-Strand Breaks (DSBs): Double-strand breaks represent the most cytotoxic and mutagenic form of DNA damage induced by ionizing radiation. They involve the simultaneous disruption of both strands of the DNA helix within a few base pairs. DSBs can arise directly from high-LET radiation tracks or indirectly through the collision of replication forks with unrepaired SSBs or base damage. The repair of DSBs is carried out by two major pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). NHEJ is active throughout the cell cycle and operates quickly but can be error-prone, leading to insertions or deletions. HR, on the other hand, is a high-fidelity process restricted to the late S and G2 phases, utilizing the sister chromatid as a template. If DSBs are not accurately repaired, they can lead to severe consequences such as chromosomal translocations, deletions, dicentrics, aneuploidy, or cell death via apoptosis or mitotic catastrophe. High-LET radiation such as alpha particles, carbon ions, or neutrons tends to produce clustered and complex DSBs, making repair more difficult and outcomes more lethal.

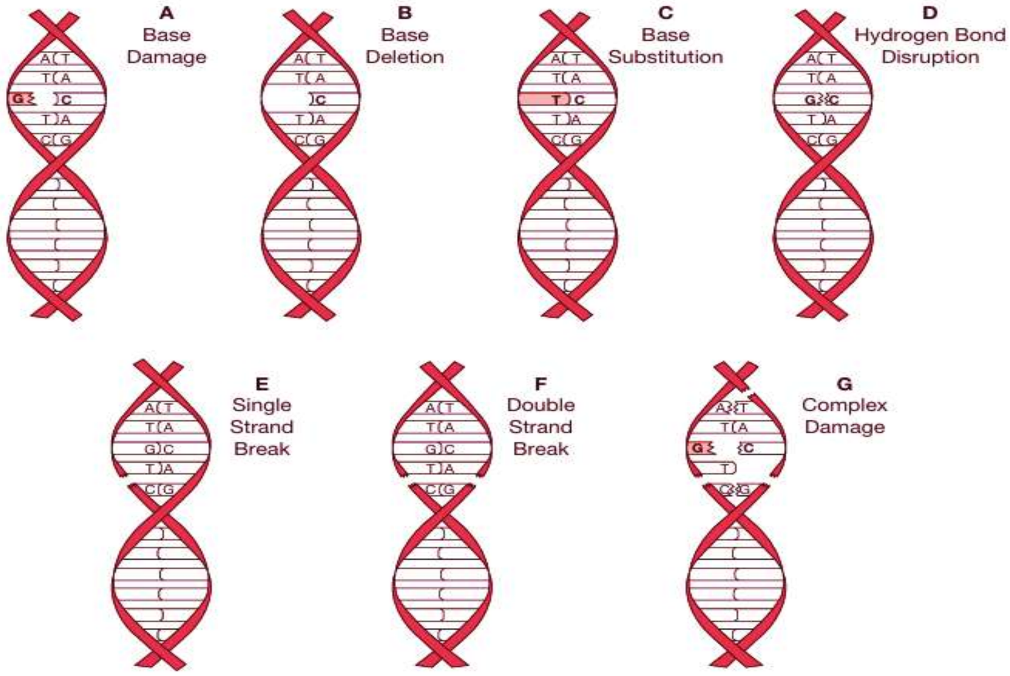

Fig: 17.2. Types of DNA Breaks

- **DNA Crosslinks:** Radiation-induced crosslinks are another serious form of DNA damage. Interstrand crosslinks (ICLs) covalently bond opposite DNA strands, preventing strand separation, while DNA-protein crosslinks (DPCs) tether DNA to nearby nuclear proteins such as histones or transcription factors. These lesions impede essential processes such as replication, transcription, and chromatin remodeling. Unlike SSBs or base damage, crosslinks are particularly challenging for the cell to repair and typically require a combination of multiple repair pathways, including nucleotide excision repair (NER), homologous recombination, and specialized enzymes such as Fanconi anemia proteins. Crosslinking lesions, if not effectively resolved, can lead to stalled replication forks, chromosomal breakage, and cell death. Although radiation is not as potent a crosslinking agent as certain chemotherapeutic drugs (e.g., cisplatin), significant crosslinking can occur, particularly under oxidative stress or in hypoxic conditions.
- **Clustered (Complex) DNA Damage:** Clustered or locally multiply damaged sites (LMDS) are hallmark lesions caused by high-LET radiation. These sites are defined as two or more lesions—such as base damage, SSBs, or DSBs—occurring within one or two helical turns of the DNA (approximately 10–20 base pairs). The high density of ionization events along the path of high-LET particles leads to these complex lesions. Clustered damage is far more difficult to repair than isolated damage because the presence of multiple lesions in close proximity can interfere with the binding and function of repair enzymes. For example, simultaneous repair attempts by BER and DSB repair mechanisms may lead to interference or misrepair, resulting in mutagenesis, chromosomal aberrations, or cell death. Clustered damage is a key reason why high-LET radiation has a greater relative biological

effectiveness (RBE) compared to low-LET radiation and forms the biological rationale behind its use in heavy ion therapy for cancer treatment.

17.5. DNA REPAIR MECHANISMS

Cells have evolved a complex and highly efficient network of DNA repair mechanisms to maintain genomic integrity in the face of constant endogenous and exogenous DNA damage. Ionizing radiation is a major source of exogenous DNA damage, producing lesions such as base modifications, single-strand breaks (SSBs), double-strand breaks (DSBs), and crosslinks. If left unrepaired or misrepaired, these lesions can lead to mutations, genomic instability, cell death, or cancer. To counter these effects, cells utilize several distinct DNA repair pathways, each specialized for specific types of damage. The primary DNA repair mechanisms relevant to radiation-induced damage include base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), non-homologous end joining (NHEJ), and homologous recombination (HR). Additionally, repair of crosslinks and complex damage often involves combined or accessory pathways.

17.5.1. Base Excision Repair (BER)

Base Excision Repair (BER) is a highly conserved and crucial DNA repair pathway that primarily handles small, non-helix-distorting base lesions in the genome. These lesions are typically the result of endogenous metabolic processes—such as reactive oxygen species (ROS) production—or external damaging agents, particularly ionizing radiation. Ionizing radiation causes damage directly by ionizing DNA or indirectly through radiolysis of water, generating free radicals like hydroxyl radicals (•OH), which in turn oxidize DNA bases. Common modifications include oxidized bases (e.g., 8-oxoguanine), deaminated cytosine (producing uracil), or alkylated bases, all of which can lead to mispairing if left unrepaired, ultimately resulting in mutations or chromosomal instability. The BER pathway is initiated by a class of enzymes known as DNA glycosylases, which are specialized for recognizing specific forms of base damage. Each glycosylase scans the DNA for lesions and, upon encountering a damaged base, cleaves the N-glycosidic bond between the base and the sugar-phosphate backbone. This action releases the abnormal base and creates an apurinic/apyrimidinic (AP) site, also referred to as an abasic site. Several DNA glycosylases exist in human cells, including OGG1 for oxidized guanine, UNG for uracil, and MPG for alkylated bases, allowing BER to respond to a wide range of chemically distinct lesions.

Following base removal, the DNA at the abasic site is processed by an AP endonuclease, most notably APE1 (apurinic/apyrimidinic endonuclease 1) in human cells. APE1 cleaves the DNA backbone 5' to the AP site, generating a single-strand break with a 3'-hydroxyl and a 5'-deoxyribose phosphate (5'-dRP) terminus. The repair now proceeds in one of two subpathways, depending on the complexity of the lesion and cellular context. In short-patch BER, a single nucleotide is replaced. This pathway involves the recruitment of DNA polymerase β, which possesses both DNA synthesis and dRP lyase activities. It removes the 5'-dRP moiety and fills in the correct nucleotide. The final step involves DNA ligase III, working in complex with XRCC1 (X-ray repair cross-complementing protein 1), which seals the remaining nick in the sugar-phosphate backbone. Alternatively, long-patch BER may be employed, particularly when the 5'-dRP terminus is resistant to removal due to oxidative modification or other structural hindrance. In this pathway, DNA polymerase δ or ε synthesizes a stretch of 2 to 10 nucleotides, displacing the existing strand and forming a flap structure. This flap is then cleaved by flap endonuclease 1 (FEN1), and the gap is finally sealed by DNA ligase I. BER is a vital mechanism in all living cells for maintaining genome stability, particularly in non-dividing cells like neurons where DNA is not frequently replicated. Defects in BER components are associated with increased cancer susceptibility, neurodegenerative disorders, and premature aging. Importantly, the efficiency and regulation of BER are also relevant in radiation biology, as cells exposed to ionizing radiation rely heavily on this pathway to counteract oxidative DNA damage and prevent the accumulation of mutations or apoptotic cell death. The fidelity and speed of BER are therefore essential for preserving genetic information, cellular function, and organismal viability in the face of continuous genotoxic stress.

17.5.2. Nucleotide Excision Repair (NER)

Nucleotide Excision Repair (NER) is a fundamental and evolutionarily conserved DNA repair mechanism designed to eliminate bulky, helix-distorting lesions from the genome. These lesions include a variety of structurally significant DNA modifications, such as cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts (6-4PPs), which are typically formed by ultraviolet (UV) radiation, but also include chemical adducts and certain oxidative lesions that can be generated through ionizing radiation (IR) and environmental mutagens. Unlike base excision repair (BER), which targets small, non-distorting base lesions, NER is capable of recognizing distortions in the DNA double helix regardless of the specific chemical identity of the damaged base. This property makes NER particularly versatile and essential for maintaining genomic integrity under various forms of genotoxic stress. NER is functionally divided into two sub-pathways based on the context in which the damage is recognized: global genome NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER operates throughout the entire genome and is responsible for scanning DNA for lesions in both transcribed and non-transcribed regions. The initial lesion recognition in GG-NER is mediated by the XPC-HR23B complex, which detects distortions in the DNA helix. In contrast, TC-NER is specifically activated when a lesion stalls the progression of RNA polymerase II during transcription. In this sub-pathway, the proteins CSA (Cockayne syndrome A) and CSB (Cockayne syndrome B) are responsible for initiating the repair response by recognizing the arrested transcription machinery and recruiting downstream repair factors.

Once damage recognition has occurred—either by XPC in GG-NER or by CSA/CSB in TC-NER—the repair process converges on a shared core mechanism. The TFIIH complex, which contains the XPB and XPD helicases, is recruited to the lesion site and initiates local DNA unwinding, creating a bubble-like structure of approximately 25–30 nucleotides. This step is critical for providing access to the damaged DNA strand. Subsequent verification of the lesion is conducted by XPA and RPA, which stabilize the opened DNA and facilitate precise positioning of the excision machinery. The next step involves dual incisions by specific endonucleases: XPF-ERCC1, which cuts the DNA strand on the 5′ side of the lesion, and XPG, which incises on the 3′ side. These cuts bracket the lesion and result in the excision of a short, 24–32 nucleotide-long oligonucleotide that contains the damaged base(s). This excision creates a single-stranded DNA gap, which is then filled in by DNA polymerase δ or ε, using the undamaged complementary strand as a template. Finally, the newly synthesized patch is sealed by DNA ligase I (in replicating cells) or DNA ligase III-XRCC1 complex (in non-replicating cells), restoring the integrity of the DNA.

17.5.3. Mismatch Repair (MMR)

Mismatch Repair (MMR) is a post-replicative DNA repair system that plays a critical role in maintaining genomic stability by correcting base-base mismatches and insertion-deletion loops (IDLs) that arise during DNA replication. These errors occur when the DNA polymerase incorporates incorrect nucleotides or slips on repetitive sequences, such as microsatellites. Although DNA polymerases have intrinsic 3’ to 5’ exonuclease proofreading activity, this mechanism is not flawless. MMR serves as a secondary, high-fidelity correction system that identifies and repairs mismatches that escape the proofreading activity of the replication machinery. MMR is essential for preventing point mutations and frameshifts, particularly in rapidly dividing cells, and is a major determinant of replication accuracy. The MMR pathway is initiated by the recognition of mismatched DNA by specific protein complexes. In eukaryotes, this role is played by MutS homologs: the MSH2-MSH6 heterodimer (also known as MutSα) primarily recognizes base-base mismatches and small insertion-deletion loops, while MSH2-MSH3 (MutSβ) is responsible for identifying larger insertion-deletion mismatches. Upon binding to the mismatched DNA, these complexes undergo a conformational change that facilitates the recruitment of the MutL homolog complex, most commonly MLH1-PMS2 (MutLα). This recruitment is essential for signal amplification and activation of downstream excision and repair steps.

Once the mismatch is recognized and marked, the erroneous DNA strand must be distinguished from the correct template strand. In prokaryotes, this is achieved via methylation patterns; in eukaryotes, strand discrimination is thought to be directed by nicks or gaps present in the newly synthesized lagging strand or generated during replication or repair. After strand discrimination, an exonuclease is recruited—typically EXO1 (exonuclease 1)—to remove a stretch of DNA containing the mismatch. This excision step is coordinated by the MMR complex and often involves helicases and single-strand DNA binding proteins like RPA to stabilize the unwound region. Following excision of the mismatch-containing DNA, the gap is filled by DNA polymerase δ or ε, using the complementary strand as a template, and the resulting nick is sealed by DNA ligase I, thereby restoring the correct DNA sequence. This multi-step process ensures that replication-associated errors do not persist and accumulate, preserving the fidelity of the genome.

Although MMR is not a frontline mechanism for repairing ionizing radiation-induced DNA damage, it plays a crucial supportive role in radiation biology. During base excision repair (BER) or nucleotide excision repair (NER)—both of which are directly involved in processing radiation-induced lesions—MMR can act to correct errors introduced during the DNA synthesis phase of repair. Additionally, some oxidative lesions caused by radiation may result in mispairing (e.g., 8-oxoguanine pairing with adenine), and MMR can help resolve these misincorporations if they arise during replication or repair synthesis. Importantly, deficiencies in MMR proteins—due to inherited mutations (as seen in Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer, or HNPCC) or somatic inactivation—lead to a phenomenon known as microsatellite instability (MSI). MSI is characterized by the accumulation of insertion or deletion mutations in repetitive DNA sequences, a hallmark of certain cancers, particularly colorectal, endometrial, and gastric cancers. MMR-deficient cells also display a mutator phenotype, characterized by an elevated rate of spontaneous mutations, which contributes to tumorigenesis.

17.5.4. Non-Homologous End Joining (NHEJ)

Non-Homologous End Joining (NHEJ) is the primary DNA double-strand break (DSB) repair mechanism in mammalian cells, particularly during the G_0 and G_1 phases of the cell cycle when a sister chromatid is not available to serve as a repair template. NHEJ is especially vital because DSBs are among the most cytotoxic forms of DNA damage; if left unrepaired, they can lead to chromosomal fragmentation, apoptosis, or oncogenic transformation. Unlike homologous recombination (HR), which requires sequence homology and is restricted to the S and G_2 phases, NHEJ is a template-independent, rapid, but error-prone repair pathway. It functions by directly ligating the two broken ends of DNA, which is especially important for cells that are not actively dividing, such as neurons and other terminally differentiated cells. The NHEJ process begins with the recognition of DSBs by the Ku70/Ku80 heterodimer, which rapidly binds to the exposed DNA ends. The Ku complex acts as a molecular scaffold that protects the DNA ends from excessive degradation and recruits additional repair proteins to the damage site. Once bound, Ku70/80 recruits and activates DNA-dependent protein kinase catalytic subunit (DNA-PKcs), forming the DNA-PK holoenzyme. This kinase complex serves not only to tether the DNA ends together but also to phosphorylate downstream substrates involved in end processing and ligation.

In many cases, the DNA ends at a DSB are not immediately compatible for direct ligation due to overhangs, blunt ends, chemical modifications, or mismatches. Therefore, a set of end processing enzymes is recruited to modify the termini and make them ligatable. Among the most important of these is Artemis, a nuclease activated by DNA-PKcs phosphorylation, which can remove damaged nucleotides and resolve hairpin structures. Additionally, template-independent DNA polymerases, such as Pol μ and Pol λ, may add nucleotides to fill gaps or create microhomology overhangs to facilitate alignment of the ends. This processing is highly variable and can introduce insertions or deletions (indels) at the junction site, contributing to the intrinsic error-proneness of NHEJ. Once the DNA ends are adequately processed, the final ligation step is carried out by the ligase complex consisting of XRCC4 (X-ray repair cross-complementing protein 4), XLF (XRCC4-like factor, also known as Cernunnos), and DNA ligase IV. XRCC4 stabilizes ligase IV and coordinates ligation, while XLF bridges and stabilizes DNA ends, facilitating end alignment and ligation. The activity of this complex ensures that the broken DNA is sealed, although the resulting junction may not be identical to the original sequence, leading to mutagenic outcomes if the repair is imprecise. In the context of ionizing radiation, NHEJ plays a central role in cellular survival, particularly following exposure to low-linear energy transfer (low-LET) radiation such as X-rays and gamma rays. However, with high-LET radiation (e.g., alpha particles or heavy ions), the damage is often more complex, with clustered DSBs and associated base damage or cross-links. Under such circumstances, NHEJ's tendency to perform minimal processing may result in chromosomal translocations, large deletions, or fusion of non-homologous DNA ends, increasing the risk of mutagenesis and genomic instability. This is particularly relevant in radiation oncology, where high-LET radiation is sometimes used to exploit this vulnerability in cancer cells. Despite its lack of fidelity, NHEJ is indispensable for genomic maintenance, especially in post-mitotic cells and during the development of the immune system, where it plays a physiological role in V(D)J recombination—the process of assembling antigen receptor genes in B and T lymphocytes. Deficiencies in key NHEJ components, such as DNA-PKcs, Artemis, or Ligase IV, result in radiosensitivity, immunodeficiency, and increased susceptibility to cancer, as seen in certain genetic syndromes like Severe Combined Immunodeficiency (SCID).

17.5.5. Homologous Recombination (HR)

HR is a high-fidelity DSB repair pathway that operates during the S and G2 phases of the cell cycle when a sister chromatid is available as a template. HR is essential for maintaining genome stability and accurate chromosome segregation. The repair process begins with resection of the 5' DNA ends at the break site, producing 3' single-stranded overhangs. These are coated by RAD51, which facilitates strand invasion into the homologous sequence of the sister chromatid. DNA synthesis extends the invading strand, and the resulting joint molecule is resolved by recombination proteins such as BRCA1/2, RAD52, and the MRN complex (MRE11-RAD50-NBS1). Because HR requires extensive processing and strand homology, it is slower but much more accurate than NHEJ.

17.5.6. Repair of DNA Crosslinks

DNA crosslinks represent some of the most cytotoxic and replication-blocking forms of DNA damage, as they covalently link DNA strands or DNA to proteins, thereby preventing essential cellular processes like DNA replication, transcription, and chromatin remodeling. Crosslinks can be induced by endogenous aldehydes, reactive oxygen species, metabolic byproducts, or exogenous agents such as ionizing radiation, chemotherapy drugs (e.g., mitomycin C, cisplatin), and UV light. There are two primary types of crosslinks: interstrand crosslinks (ICLs), which covalently link the two complementary DNA strands, and DNA-protein crosslinks (DPCs), in which a protein is covalently attached to DNA.

Interstrand Crosslinks (ICLs): Mechanism of Repair

ICLs are particularly problematic because they prevent the two strands of DNA from separating, thereby stalling both transcription and DNA replication forks. Because of this, ICLs are lethal if not accurately repaired, making their resolution essential for cell viability and genome stability. The repair of ICLs is a multi-step, multi-pathway process that primarily involves the Fanconi anemia (FA) pathway, nucleotide excision repair (NER), translesion synthesis (TLS), and homologous recombination (HR). The pathway is generally activated during the S phase of the cell cycle, when a replication fork encounters an ICL. The ICL repair process is initiated by recognition of the stalled replication fork. This triggers activation of the Fanconi anemia (FA) pathway, a tumor suppressor pathway composed of over 20 genes. The core complex, including FANCA, FANCB, FANCC, and others, functions as an E3 ubiquitin ligase complex that monoubiquitinates FANCD2 and FANCI, which then localize to chromatin at the site of damage. This activation recruits structure-specific endonucleases such as SLX4, FAN1, and XPF-ERCC1, which make endonucleolytic incisions on one DNA strand to "unhook" the crosslink and separate the strands—creating a single-stranded gap and a double-strand break (DSB) on the opposite strand.

Once the crosslink is unhooked, the lesion remains on one strand and must be bypassed. This is achieved via translesion DNA synthesis (TLS), which involves error-prone specialized DNA polymerases such as Pol η (eta) and Pol κ (kappa) that can synthesize DNA across damaged bases, albeit with reduced fidelity. The unhooked ICL remnant is subsequently removed, often by NER enzymes, while the DSB generated on the complementary strand is resolved through homologous recombination (HR). HR uses the sister chromatid as a template to restore the original sequence, ensuring high-fidelity repair. This integration of TLS, NER, and HR with the FA pathway illustrates the multifaceted and highly regulated nature of ICL repair. Disruption in any component of this coordinated process, particularly in FA proteins, leads to Fanconi anemia, a rare genetic disorder characterized by bone marrow failure, congenital abnormalities, and a strong predisposition to cancer. FA-deficient cells exhibit hypersensitivity to DNA crosslinking agents, reinforcing the critical role of this pathway in maintaining genome integrity.

DNA-Protein Crosslinks (DPCs): Mechanism of Repair

DPCs arise when proteins become covalently trapped on DNA, often as a result of abortive enzymatic reactions (e.g., topoisomerase I/II failures), aldehyde damage, or ionizing radiation. These lesions are bulky and obstructive, impeding DNA metabolism similarly to ICLs. Due to the dual nature of DPCs—containing both a protein and a DNA component—the repair requires the coordinated degradation of the protein moiety and excision of the DNA lesion. The repair of DPCs is typically initiated by proteolytic degradation of the covalently bound protein. One of the key enzymes in this process is SPRTN (Spartan), a metalloprotease that becomes activated during DNA replication and directly degrades proteins crosslinked to DNA. This proteolysis step exposes the underlying DNA lesion and generates a smaller peptide-DNA adduct that is more amenable to traditional DNA repair pathways. After proteolytic degradation, the residual peptide-DNA crosslinks can be removed via nucleotide excision repair (NER) or base excision repair (BER), depending on the size and chemical structure of the remaining damage. NER is typically involved in the excision of bulky lesions, while BER may play a role if the peptide adduct mimics smaller base damage. Tyrosyl-DNA phosphodiesterases (TDP1 and TDP2) also contribute to DPC repair, particularly in cases involving topoisomerase-DNA complexes. The repair of DPCs is particularly important in mitotically active and post-mitotic cells, such as neurons, where DPCs can accumulate and contribute to genomic instability and neurodegeneration. Mutations in SPRTN are associated with Ruijs-Aalfs syndrome, a progeroid disorder characterized by early-onset hepatocellular carcinoma and genomic instability, underscoring the biological importance of DPC repair pathways.

17.5.7. Repair of Clustered (Complex) DNA Damage

Clustered DNA damage, also referred to as complex DNA damage, describes a constellation of two or more distinct DNA lesions located within one or two helical turns (typically 10–20 base pairs) of the DNA double helix. This form of damage is especially challenging for the cell to repair due to the close spatial proximity of lesions, which often includes combinations of base damage, abasic (AP) sites, single-strand breaks (SSBs), and double-strand breaks (DSBs). These clusters interfere with the canonical, sequential repair processes normally employed for isolated lesions. Clustered DNA damage is most commonly induced by high-LET radiation sources such as alpha particles, neutrons, or heavy ions, as used in certain types of radiation therapy (e.g., carbon ion therapy). In contrast to low-LET radiation (like X-rays and gamma rays), which tends to produce dispersed DNA lesions, high-LET radiation deposits dense energy tracks along its path, generating multiple lesions within nanometers. These clustered lesions are a major contributor to the increased biological effectiveness of high-LET radiation and are associated with higher rates of cell death, chromosomal aberrations, and mutagenesis.

Structural Complexity and Repair Difficulty

The repair of clustered DNA damage is significantly more complex than that of isolated lesions due to several overlapping issues:

1. Steric Hindrance and Enzyme Interference: When lesions are too close together, the structural distortion in the DNA helix can physically block repair enzymes from accessing their target sites. Additionally, when multiple repair enzymes are recruited to adjacent lesions simultaneously, they may interfere with each other's activity, leading to stalled or incomplete repair.
2. Pathway Competition and Crosstalk: The lesions in a cluster may fall under the jurisdiction of multiple repair pathways, such as base excision repair (BER) for oxidized bases or abasic sites, nucleotide excision repair (NER) for bulky lesions, and double-strand break repair pathways like non-homologous end joining (NHEJ) or homologous recombination (HR) for strand breaks. The activation of one repair pathway can block or alter the substrate for another, causing misrepair or delay.
3. Increased Risk of Double-Strand Breaks: If two SSBs or abasic sites are located on opposite strands and are not coordinated properly during repair, the intermediate steps of repair (such as strand incision) can convert these into a DSB, which is a more lethal and harder-to-repair lesion.
4. Error-Prone Repair Outcomes: The complexity of clustered lesions often overwhelms high-fidelity repair systems, resulting in mutagenic repair, large deletions, or chromosomal rearrangements. These erroneous repair events are particularly dangerous in genomic regions encoding essential genes or regulatory elements.

Repair Pathway Coordination

Despite these challenges, cells attempt to manage clustered DNA damage through coordinated and sequential engagement of repair pathways:

- Initial Damage Recognition: Lesions are typically first recognized by BER or NER enzymes, depending on their chemical nature. For example, 8-oxoguanine may be processed by OGG1, while bulky adducts are substrates for XPC in global genome NER.

- Base Excision Repair (BER): BER enzymes such as glycosylases, APE1, and DNA polymerase β are often involved early in the process. However, BER may be aborted or stalled if the opposite strand contains a closely located lesion, preventing safe strand cleavage or gap filling.
- Strand Break Management: If strand breaks are involved or generated during repair, NHEJ is recruited in the G_0/G_1 phase, while HR takes precedence in the S/G_2 phase, where sister chromatids are available for accurate repair.
- Chromatin Remodeling: The complexity of the damage often necessitates chromatin remodeling to allow access for large repair complexes. ATP-dependent chromatin remodelers like SWI/SNF and INO80 are sometimes recruited to expose the damage region.
- Damage Signaling: ATM (ataxia telangiectasia mutated) and ATR (ATM and Rad3-related) kinases play pivotal roles in signaling complex DNA damage, initiating cell cycle checkpoints, and orchestrating the recruitment of repair machinery through phosphorylation of histone H2AX and activation of 53BP1, BRCA1, and other mediator proteins.

Biological and Clinical Relevance

From a biological perspective, clustered damage represents a unique repair challenge and is more likely to persist in the genome than isolated lesions. The incomplete or misrepair of such clusters can result in point mutations, indels, or chromosomal translocations, contributing to carcinogenesis or cell death, depending on the cell type and repair capacity. In clinical settings, the difficulty of repairing clustered DNA damage is therapeutically exploited in cancer treatments using high-LET radiation (e.g., proton or carbon ion therapy), which preferentially induces these lesions in tumor tissues while sparing normal cells through precise dose delivery. Moreover, understanding how tumor cells respond to clustered DNA damage is crucial for developing radiosensitizers and synthetic lethality approaches, particularly in tumors with deficiencies in HR or BER.

17.5.8. Factors Influencing DNA Repair Efficiency

The efficiency and fidelity of DNA repair mechanisms are influenced by various biological and physical factors. These factors not only determine a cell's ability to respond to genotoxic insults like ionizing radiation but also play a pivotal role in the outcomes of radiation therapy, radiosensitivity, and genomic stability. A detailed understanding of these factors is essential in radiobiology and medical research.

- **Cell Cycle Phase:** One of the most critical determinants of DNA repair efficiency is the phase of the cell cycle during which radiation exposure occurs. The type of repair pathway activated often depends on the cell's position in the cell cycle. Homologous recombination (HR), a highly accurate repair process, is restricted to the late S and G2 phases when a sister chromatid is available as a template for repair. In contrast, non-homologous end joining (NHEJ), a faster but error-prone repair mechanism, is active throughout the entire cell cycle, including G1. Cells in S and G2 phases thus tend to favor high-fidelity repair, whereas those in G1 primarily rely on NHEJ. This differential activation affects both the accuracy and outcome of DNA repair following radiation-induced damage.
- **Radiation Quality (Linear Energy Transfer, LET):** The quality of radiation, especially its linear energy transfer (LET), significantly impacts the nature and complexity of DNA damage and thus the repair process. Low-LET radiation, such as X-rays and gamma rays, typically produces sparse and isolated DNA lesions that are more easily accessible and repairable by the cell's machinery. In contrast, high-LET radiation, including alpha particles and heavy ions, causes dense ionization tracks that result in complex, clustered DNA damage. These clusters can include double-strand breaks (DSBs), base modifications, and DNA-protein crosslinks within a localized region, making repair difficult and often incomplete. Consequently, high-LET radiation poses a greater challenge to cellular repair systems, leading to higher levels of cell death or misrepair, which may contribute to mutations and carcinogenesis.
- **Cellular Oxygenation:** Cellular oxygen levels also play a vital role in modulating DNA damage and repair efficiency. Oxygen acts as a radiosensitizer by reacting with and stabilizing free radicals generated during the radiolysis of water, thereby increasing the likelihood of permanent DNA damage. This phenomenon, known as the "oxygen enhancement effect," means that well-oxygenated cells are more susceptible to radiation-induced damage. Conversely, hypoxic cells—commonly found in the cores of solid tumors—are more resistant to radiation because the transient radicals produced in the absence of oxygen often revert to non-damaging species. Hypoxia not only reduces the extent of initial DNA damage but also influences the cellular microenvironment, leading to the selection of more

aggressive and repair-deficient cancer cell subpopulations that are resistant to therapy.

- **Genetic Makeup of the Cell:** The genetic constitution of a cell, particularly with regard to DNA repair genes, significantly influences its ability to recognize, signal, and repair DNA damage. Mutations in key genes involved in the homologous recombination and NHEJ pathways can severely compromise DNA repair capacity. For instance, mutations in BRCA1 or BRCA2, which are central to homologous recombination, reduce the ability of cells to accurately repair DSBs, thereby increasing the risk of genomic instability and tumorigenesis. Similarly, mutations in the TP53 gene—often referred to as the "guardian of the genome"—can impair the cell's ability to arrest the cell cycle or initiate apoptosis in response to DNA damage. Defects in ATM, RAD51, or other DNA damage response genes can also result in increased radiosensitivity or susceptibility to radiation-induced malignancies. Therefore, genetic makeup not only determines inherent repair proficiency but also influences the cellular response to radiation therapy.

17.6. CHROMOSOMAL ABERRATIONS INDUCED BY IONIZING RADIATION

Ionizing radiation is a well-established genotoxic agent capable of inducing a wide spectrum of DNA damage, with chromosomal aberrations representing some of the most significant structural manifestations of genomic injury. Chromosomal aberrations refer to alterations in the number, structure, or organization of chromosomes resulting from direct or indirect radiation-mediated damage to the DNA within the nucleus. These aberrations serve as critical biomarkers in cytogenetics, radiation biodosimetry, and radiation risk assessment due to their correlation with radiation dose and biological effect.

17.6.2. Mechanisms of Radiation-Induced Chromosomal Damage

Ionizing radiation interacts with cellular DNA through both direct and indirect mechanisms. In direct interactions, radiation deposits energy directly onto the DNA molecule, resulting in ionization and cleavage of chemical bonds. Indirectly, radiation ionizes water molecules (which comprise ~70% of the cell), generating reactive oxygen species (ROS) such as hydroxyl radicals (•OH), superoxide (O_2^-), and hydrogen peroxide (H_2O_2). These ROS can diffuse and react with DNA, leading to base modifications, single-strand breaks (SSBs), and most significantly, double-strand breaks (DSBs)—the primary lesion responsible for chromosomal aberrations. When DSBs occur simultaneously or in close proximity within the genome, incorrect or incomplete repair by non-homologous end joining (NHEJ) or homologous recombination (HR) pathways can result in misrejoining, leading to a variety of chromosomal abnormalities.

17.6.2. Classification of Radiation-Induced Chromosomal Aberrations

Radiation-induced chromosomal aberrations can be categorized based on their structure, stability, and temporal persistence in the cell cycle.

1. Structural Chromosomal Aberrations

Structural aberrations result from the physical rearrangement of chromosome segments due to erroneous repair of DSBs. These may be clastogenic (involving breakage) or rearrangement-type aberrations.

- Dicentric Chromosomes: Formed when two chromosome segments, each containing a centromere, fuse abnormally. These are inherently unstable and typically result in cell death during mitosis due to improper segregation.
- Ring Chromosomes: Created when terminal ends of a single chromosome join together post-breakage, forming a circular structure. These are unstable and often lost during cell division.
- Acentric Fragments: Chromosomal fragments lacking a centromere. These cannot attach to the mitotic spindle and are lost during anaphase, contributing to genomic instability.
- Translocations: Result from exchange of segments between non-homologous chromosomes. These are often stable and transmissible through multiple cell divisions, and are associated with oncogenesis (e.g., BCR-ABL in chronic myeloid leukemia).
- Inversions: Occur when a chromosome segment breaks off, flips in orientation, and reinserts into the same chromosome. Paracentric (not involving centromere) and pericentric (including centromere) types exist.
- Deletions: Loss of chromosomal material due to unrepaired or misrepaired DSBs, potentially leading to loss of essential genes and cellular dysfunction.

- Duplications/Insertions: Extra copies of chromosomal segments may be introduced due to replication errors following DNA damage, potentially activating oncogenes or disrupting gene regulation.

2. Numerical Chromosomal Aberrations

While less directly caused by DNA strand breaks, ionizing radiation can disrupt the mitotic apparatus, leading to errors in chromosomal segregation:

- Aneuploidy: Gain or loss of individual chromosomes (e.g., monosomy, trisomy), which may result in cell cycle arrest or transformation.
- Polyploidy: Doubling of the entire chromosome set due to mitotic failure, which is usually incompatible with normal cellular function in somatic tissues.

3. Stable vs. Unstable Aberrations

- Stable Aberrations: Persist through successive cell divisions and include reciprocal translocations, small deletions, and some inversions. These aberrations may lead to long-term genomic instability and carcinogenesis.
- Unstable Aberrations: Often lethal to the cell or lead to mitotic catastrophe. These include dicentrics, rings, and acentric fragments, which disrupt mitotic spindle attachment or chromosome segregation.

17.6.3. Dose–Response Relationship

Radiation-induced chromosomal aberrations demonstrate a clear dose-dependent relationship, wherein the frequency of aberrations—particularly dicentric chromosomes—increases with the magnitude of radiation exposure. This response typically follows a linear-quadratic (LQ) model, which is mathematically expressed as $Y = \alpha D + \beta D^2$. In this equation, Y denotes the observed frequency of chromosomal aberrations, D represents the absorbed radiation dose, α corresponds to the linear component indicative of damage caused by single ionizing tracks, and β reflects the quadratic component associated with the interaction of two separate radiation tracks. This dual-component model accounts for both low-dose linear responses and high-dose synergistic effects, offering a robust framework for predicting biological outcomes across a wide range of exposure levels. The LQ model is particularly critical in the field of cytogenetic biodosimetry, where it is employed to estimate unknown radiation doses in individuals involved in accidental, environmental, or occupational exposures. By analyzing the frequency of chromosomal aberrations—especially unstable types like dicentrics—scientists and health professionals can retrospectively reconstruct the dose received and assess the potential health risks associated with the exposure.

Detection Techniques for Chromosomal Aberrations

Table: 17.2. Various cytogenetic techniques are used to detect and quantify chromosomal damage

Method	Description	Application
Conventional Karyotyping (Giemsa Staining)	Identifies gross structural changes in metaphase chromosomes	Routine cytogenetic analysis
Fluorescence In Situ Hybridization (FISH)	Uses fluorescent probes to identify specific sequences or translocations	Detection of stable aberrations (e.g., translocations)
Micronucleus Assay	Detects acentric fragments or lagging chromosomes in binucleated cells	Rapid screening for radiation exposure
Spectral Karyotyping (SKY)	Uses multiple fluorescent dyes for full chromosome visualization	Complex or multiple rearrangement analysis
Chromosome Painting	Highlights entire chromosomes or segments with FISH probes	Evaluates translocations and complex exchanges

Clinical Implications of Chromosomal Aberrations

Chromosomal aberrations have significant clinical consequences depending on the extent, location, and type of aberration:

- Acute Radiation Exposure: High doses can induce lethal unstable aberrations, contributing to bone marrow failure, gastrointestinal syndrome, or central nervous system syndrome depending on dose level.

- Chronic and Low-Dose Exposure: Accumulation of stable aberrations increases the risk of delayed effects, such as cancer, fibrosis, and teratogenesis.
- Carcinogenesis: Chromosomal translocations and deletions may activate oncogenes or inactivate tumor suppressor genes, forming the molecular basis of many radiation-induced malignancies (e.g., thyroid cancer, leukemia, sarcomas).
- Heritable Genetic Effects: Radiation-induced mutations in germ cells may lead to transmission of chromosomal defects to progeny, although such effects are rare in humans based on current epidemiological evidence.

Table: 17.3 Common Radiation-Induced Chromosomal Aberrations

Aberration Type	Mechanism	Stability	Clinical Impact
Dicentric Chromosome	Fusion of two centromeric fragments	Unstable	Cell death, radiation biodosimetry
Ring Chromosome	End-to-end fusion of a single chromosome	Unstable	Mitotic failure
Acentric Fragment	Chromosome fragment without centromere	Unstable	Lost during cell division
Translocation	Exchange between non-homologous chromosomes	Stable	Oncogenesis, long-term monitoring
Inversion	Reversed reinsertion of chromosome segment	Usually stable	Gene disruption
Deletion	Loss of genetic material	Stable or unstable	Developmental and functional deficits

17.7. BIOLOGICAL CONSEQUENCES OF DNA DAMAGE

Exposure to ionizing radiation poses a significant threat to cellular integrity due to its capacity to induce a wide array of DNA lesions. These include single-strand breaks (SSBs), double-strand breaks (DSBs), base modifications, crosslinks, and complex clustered damage that can occur within a few nanometers of DNA helix. The cellular response to such insults is governed by the DNA Damage Response (DDR), a multifaceted system encompassing damage detection, signal transduction, cell cycle regulation, and DNA repair. The biological consequences that follow depend on various factors including the type of lesion, its location in the genome, the phase of the cell cycle during which the damage occurs, and the efficiency of the cell’s repair mechanisms. When DNA repair is successful, genomic stability is maintained. However, when damage is extensive, misrepaired, or left unresolved, cells may undergo apoptosis, senescence, or neoplastic transformation, all of which have profound implications for tissue function, organismal health, and disease development.

- **Cell Cycle Arrest:** A primary early consequence of DNA damage is the activation of cell cycle checkpoints, which temporarily arrest the cell cycle to prevent replication or segregation of damaged DNA. Key regulators in this process include the ATM (Ataxia Telangiectasia Mutated) and ATR (ATM and Rad3-related) protein kinases. ATM responds primarily to double-strand breaks, while ATR is activated by replication stress and single-stranded DNA regions. Once activated, these kinases phosphorylate downstream effectors such as Chk1, Chk2, and the tumor suppressor p53. In the G1/S checkpoint, p53 activation leads to upregulation of the cyclin-dependent kinase inhibitor p21, which blocks CDK2 activity, halting DNA replication initiation. In the G2/M phase, Chk1/Chk2 phosphorylate and inhibit CDC25 phosphatases, preventing activation of CDK1/cyclin B complexes necessary for mitosis. This controlled delay in the cell cycle allows the cell time to initiate and complete DNA repair. If the damage is repaired successfully, normal cell cycle progression resumes. Otherwise, the cell must decide between other fates such as apoptosis or senescence.
- **Apoptosis, or programmed cell death**: It serves as a critical mechanism to eliminate severely damaged or genetically unstable cells. The intrinsic (mitochondrial) pathway of apoptosis is typically triggered by internal signals, such as DNA damage, and involves the release of cytochrome c from mitochondria following the permeabilization of the outer mitochondrial membrane. This event is tightly regulated by the Bcl-2 family of proteins, which includes both pro-apoptotic members like Bax and Bak and anti-apoptotic proteins such as Bcl-2 and Bcl-xL. Once released, cytochrome c binds to Apaf-1 and pro-caspase-9 to form the apoptosome, activating the caspase cascade that culminates in

cell dismantling. Alternatively, the extrinsic pathway is initiated by ligand binding to death receptors (e.g., Fas, TNF receptor), leading to caspase-8 activation. Both pathways ultimately activate executioner caspases such as caspase-3, which cleave cellular proteins and result in morphological and biochemical hallmarks of apoptosis, including cell shrinkage, chromatin condensation, and DNA fragmentation. This form of cell death is crucial for preventing the propagation of mutations and for maintaining tissue homeostasis after radiation exposure.

- **Senescence**: In cases where the damage is not immediately lethal but repair mechanisms fail to fully restore genomic integrity, cells may enter a state of permanent cell cycle arrest known as senescence. Senescence is distinct from apoptosis in that the cell remains viable and metabolically active, but it no longer divides. This state is maintained by the sustained activation of tumor suppressor pathways, particularly the p53-p21 and p16^INK4a-pRB axes. The retinoblastoma protein (pRB), when in its active hypophosphorylated form, binds and inhibits E2F transcription factors, thereby preventing the transcription of genes required for DNA synthesis and cell cycle progression. Senescent cells frequently exhibit increased activity of senescence-associated beta-galactosidase (SA-β-Gal), enlarged morphology, altered chromatin organization, and the persistent presence of DNA damage foci. Moreover, senescent cells secrete a range of pro-inflammatory cytokines, growth factors, and proteases collectively termed the senescence-associated secretory phenotype (SASP). While senescence serves as a potent barrier to tumorigenesis by halting the proliferation of potentially oncogenic cells, chronic accumulation of senescent cells can contribute to tissue dysfunction, inflammation, and age-related pathologies.
- **Carcinogenesis:** When misrepaired or unrepaired DNA lesions persist, there is a significant risk of oncogenic transformation and cancer development—a process known as carcinogenesis. Ionizing radiation can induce point mutations, deletions, insertions, and complex chromosomal rearrangements such as translocations and aneuploidy. If these genetic alterations affect key regulatory genes—such as the activation of proto-oncogenes (e.g., MYC, RAS) or inactivation of tumor suppressors (e.g., TP53, RB1)—the cell may gain a selective growth advantage and begin unregulated proliferation. For instance, the Philadelphia chromosome, a translocation between chromosomes 9 and 22 that generates the BCR-ABL fusion gene, is a classic example of radiation-induced chromosomal aberration linked to leukemia. The latency period between radiation exposure and cancer development can span years to decades, highlighting the need for long-term surveillance in individuals exposed to ionizing radiation. Epidemiological evidence from atomic bomb survivors, radiation therapy patients, and nuclear industry workers strongly supports the causal relationship between radiation exposure and increased cancer risk.

End of Chapter

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