

Molecular Mechanisms of DNA and Chromosome Damage and Repair

General Overview of DNA Strand Breaks

Measuring DNA Strand Breaks

DNA Repair Pathways

- Base Excision Repair (BER)
- Nucleotide Excision Repair (NER)
- DNA Double-Strand Break Repair
- Nonhomologous End-Joining (NHEJ)
- Homologous Recombination Repair (HRR)
- Crosslink Repair
- Mismatch Repair (MMR)

Relationship between DNA Damage and

Chromosome Aberrations

Chromosomes and Cell Division

The Role of Telomeres

Radiation-Induced Chromosome Aberrations

Examples of Radiation-Induced Aberrations

Chromosome Aberrations in Human Lymphocytes

Summary of Pertinent Conclusions

Bibliography

■ GENERAL OVERVIEW OF DNA STRAND BREAKS

There is strong evidence that DNA is the principal target for the biologic effects of radiation, including cell killing, carcinogenesis, and mutation. A consideration of the biologic effects of radiation, therefore, begins logically with a description of the breaks in DNA caused by charged-particle tracks and by the chemical species produced.

Deoxyribonucleic acid (DNA) is a large molecule with a well-known double helical structure. It consists of two strands held together by hydrogen bonds between the bases. The “backbone” of each strand consists of alternating sugar and phosphate groups. The sugar involved is deoxyribose. Attached to this backbone are four bases, the sequence of which specifies the genetic code. Two of the bases are single-ring groups (pyrimidines); these are thymine and cytosine. Two of the bases are double-ring groups (purines); these are adenine and guanine. The structure of a single strand of DNA is illustrated in Figure 2.1. The bases on opposite strands must be complementary; adenine pairs with thymine, and guanine pairs with cytosine. This is illustrated in the simplified model of DNA in Figure 2.2A.

Radiation induces a large number of lesions in DNA, most of which are repaired successfully by the cell and are discussed in the following sections of this chapter. A dose of radiation

that induces an average of one lethal event per cell leaves 37% still viable; this is called the D_0 dose and is discussed further in Chapter 3. For mammalian cells, the x-ray D_0 usually lies between 1 and 2 Gy. The number of DNA lesions per cell detected immediately after such a dose is approximately:

- Base damage, >1,000
- Single-strand breaks (SSBs), 1,000
- Double-strand breaks (DSBs), 40

If cells are irradiated with a modest dose of x-rays, many breaks of a single strand occur. These can be observed and scored as a function of dose if the DNA is denatured and the supporting structure is stripped away. In intact DNA, however, **SSBs** are of little biologic consequence as far as cell killing is concerned because they are repaired readily using the opposite strand as a template (Fig. 2.2B). If the repair is incorrect (misrepair), it may result in a mutation. If both strands of the DNA are broken and the breaks are well separated (Fig. 2.2C), repair again occurs readily because the two breaks are handled separately.

By contrast, if the breaks in the two strands are opposite one another or separated by only a few base pairs (Fig. 2.2D), this may lead to a **DSB (double-strand break)**, resulting in the cleavage of chromatin into two pieces. DSBs are believed to be the most important lesions produced in chromosomes by radiation; as described

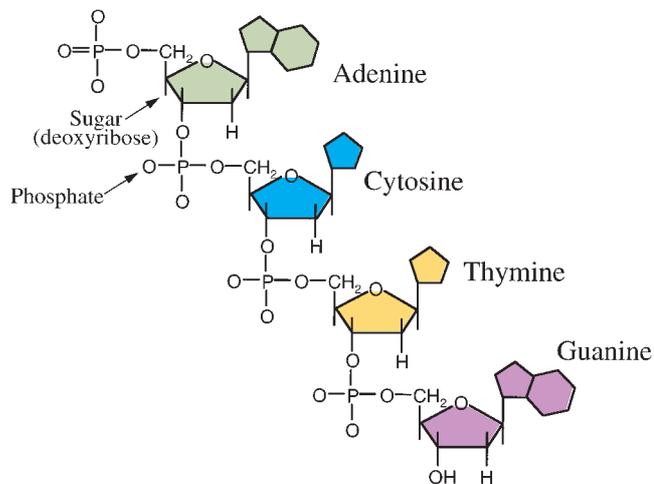


FIGURE 2.1 The structure of a single strand of DNA.

in the next section, the interaction of two DSBs may result in cell killing, carcinogenesis, or mutation. There are many kinds of DSBs, varying in the distance between the breaks on the two DNA strands and the kinds of end groups formed. Their yield in irradiated cells is about 0.04 times that of SSBs, and they are induced linearly with dose, indicating that they are formed by single tracks of ionizing radiation.

Both free radicals and direct ionizations may be involved in the formation of the type of strand break illustrated in Figure 2.2D. As described in

Chapter 1, the energy from ionizing radiations is not deposited uniformly in the absorbing medium but is located along the tracks of the charged particles set in motion—electrons in the case of x- or γ -rays, protons and α -particles in the case of neutrons. Radiation chemists speak in terms of “spurs,” “blobs,” and “short tracks.” There is, of course, a full spectrum of energy event sizes, and it is quite arbitrary to divide them into just three categories, but it turns out to be instructive. A spur contains up to 100 eV of energy and involves, on average, three ion pairs. In the case

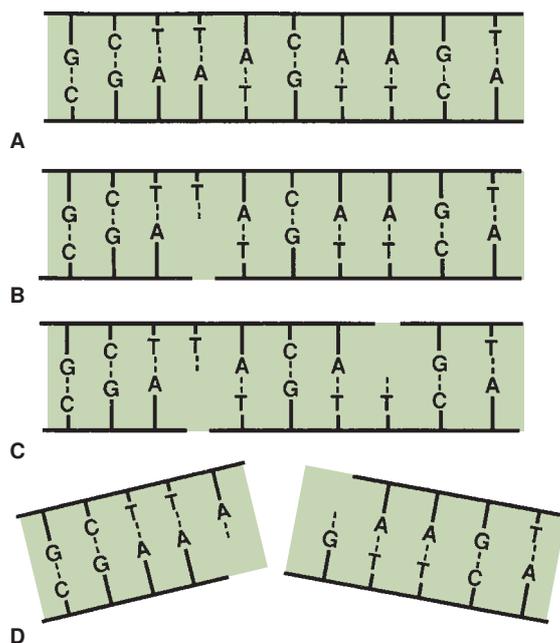
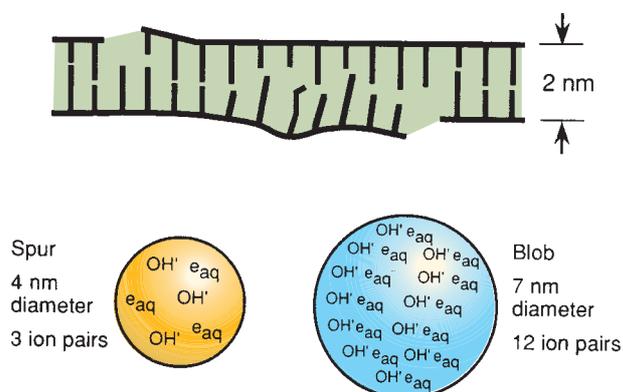


FIGURE 2.2 Diagrams of single- and double-strand DNA breaks caused by radiation. **A:** Two-dimensional representation of the normal DNA helix. The base pairs carrying the genetic code are complementary (i.e., adenine pairs with thymine, guanine pairs with cytosine). **B:** A break in one strand is of little significance because it is repaired readily using the opposite strand as a template. **C:** Breaks in both strands, if well separated, are repaired as independent breaks. **D:** If breaks occur in both strands and are directly opposite or separated by only a few base pairs, this may lead to a double-strand break in which the chromatin snaps into two pieces. (Courtesy of Dr. John Ward.)

FIGURE 2.3 Illustration of a locally multiply damaged site. Energy from x-rays is not absorbed uniformly but tends to be localized along the tracks of charged particles. Radiation chemists speak in terms of spurs and blobs, which contain several ion pairs and have dimensions comparable to the DNA double helix. A double-strand break is likely to be accompanied by extensive base damage. John Ward coined the term *locally multiply damaged site* to describe this phenomenon.



of x- or γ -rays, 95% of the energy deposition events are spurs, which have a diameter of about 4 nm, which is about twice the diameter of the DNA double helix (Fig. 2.3). Blobs are much less frequent for x- or γ -rays; they have a diameter of about 7 nm and contain on average about 12 ion pairs with an energy range of 100–500 eV (Fig. 2.3). Because spurs and blobs have dimensions similar to the DNA double helix, multiple radical attacks occur if they overlap the DNA helix. There is likely to be a wide variety of complex lesions, including base damage as well as DSBs. The term **locally multiply damaged site** was initially coined by John Ward to describe this phenomenon, but it has been replaced with the term *clustered lesion*. Given the size of a spur and the diffusion distance of hydroxyl free radicals, the clustered lesion could be spread out up to 20 base pairs. This is illustrated in Figure 2.3, in which a DSB is accompanied by base damage and the loss of genetic information.

In the case of densely ionizing radiations, such as neutrons or α -particles, a greater proportion of blobs are produced. The damage produced, therefore, is qualitatively different from that produced by x- or γ -rays and it is much more difficult for the cell to repair.

■ MEASURING DNA STRAND BREAKS

Over the years, various techniques have been used to measure DNA strand breaks, including sucrose gradient sedimentation, alkaline and neutral filter elution, nucleoid sedimentation, pulsed-field gel electrophoresis (PFGE), and single-cell gel electrophoresis (also known as the comet assay). Of these techniques, PFGE and single-cell gel

electrophoresis are still used to measure DNA strand breaks. In addition to these past techniques, radiation-induced nuclear foci has become a popular approach to visualize DNA damage through the recruitment of DNA repair proteins to sites of DNA damage.

PFGE is the method most widely used to detect the induction and repair of DNA DSBs. It is based on the electrophoretic elution of DNA from agarose plugs within which irradiated cells have been embedded and lysed. PFGE allows separation of DNA fragments according to size in the megabase-pair range, with the assumption that DNA DSBs are induced randomly. The fraction of DNA released from the agarose plug is directly proportional to dose (Fig. 2.4A). The kinetics of DNA DSB rejoining exhibit a fast initial rate, which then decreases with repair time. The most widely accepted description of this kinetic behavior uses two first-order components (fast and slow) plus some fraction of residual DSBs. Studies have supported the finding that rejoining of incorrect DNA ends originates solely from slowly rejoining DSBs, and this subset of radiation-induced DSBs is what is manifested as chromosomal damage (i.e., chromosome translocations and exchanges).

Single-cell electrophoresis (comet assay) has the advantage of detecting differences in DNA damage and repair at the single-cell level. This is particularly advantageous for biopsy specimens from tumors in which a relatively small number of cells can be assayed to determine DNA damage and repair. Similar to PFGE (described earlier), cells are exposed to ionizing radiation, embedded in agarose, and lysed under neutral buffer conditions to quantify induction

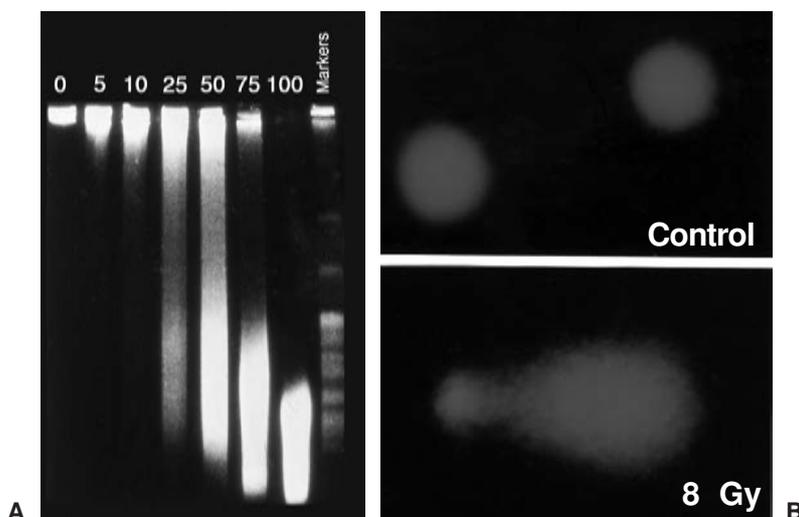


FIGURE 2.4 **A:** The effect of ionizing radiation on DNA strand break induction as measured by pulsed-field gel electrophoresis. As the dose of ionizing radiation increases from 5 to 100 Gy, the size of the DNA fragments as detected by ethidium bromide staining decreases. Thus, more DNA enters the gel with increasing dose of ionizing radiation. In these experiments, cells were embedded in agarose and irradiated on ice to eliminate the effects of repair. The number above each lane refers to the dose in Gy to which each group of cells was exposed. (Courtesy of Dr. Nicholas Denko.) **B:** Photomicrograph of control and 8-Gy irradiated cells as detected by the comet assay. Unirradiated cells possess a near-spherical appearance, whereas the fragmented DNA in irradiated cells gives the appearance of a comet when stained with ethidium bromide. (Courtesy of Drs. Ester Hammond and Mary Jo Dorie.)

and repair of DNA DSBs. To assess DNA SSBs and alkaline-sensitive sites, lysis is performed with an alkaline buffer. If the cells are undamaged, the DNA remains compact and does not migrate. If the cell has incurred DNA DSBs, the amount of damage is directly proportional to the migration of DNA in the agarose. As a result of the lysis and electrophoresis conditions, the fragmented DNA that migrates takes the appearance of a comet's tail (Fig. 2.4B). This assay has high sensitivity and specificity for SSBs and alkaline sensitive sites and to a lesser degree DNA DSBs. By changing the lysis conditions from an alkaline to a neutral pH, the comet technique can be used to measure DNA DSB repair.

Both of these assays are cell based, where DNA in cells is much more resistant to damage by radiation than would be expected from studies on free DNA. There are two reasons for this: (1) the presence in cells of low-molecular-weight scavengers that mop up some of the free radicals produced by ionizing radiation, and (2) the physical protection

afforded the DNA by packaging with proteins such as histones. Certain regions of DNA, particularly actively transcribing genes, appear to be more sensitive to radiation, and there is some evidence also of sequence-specific sensitivity.

DNA damage-induced nuclear foci (radiation-induced foci assay) in response to ionizing radiation represents complexes of signaling and repair proteins that localize to sites of DNA strand breaks in the nucleus of a cell. There are several advantages of assaying for foci formation over other techniques to measure DNA strand breaks, which include the ease of the protocol and that it can be carried out on both tissue sections and individual cell preparations. Technically, cells/tissues are incubated with a specific antibody raised to the signaling/repair protein of interest, and binding of the antibody is then detected with a secondary antibody, which also carries a fluorescent tag. Fluorescence microscopy detects the location and intensity of the tag, which can then be quantified.

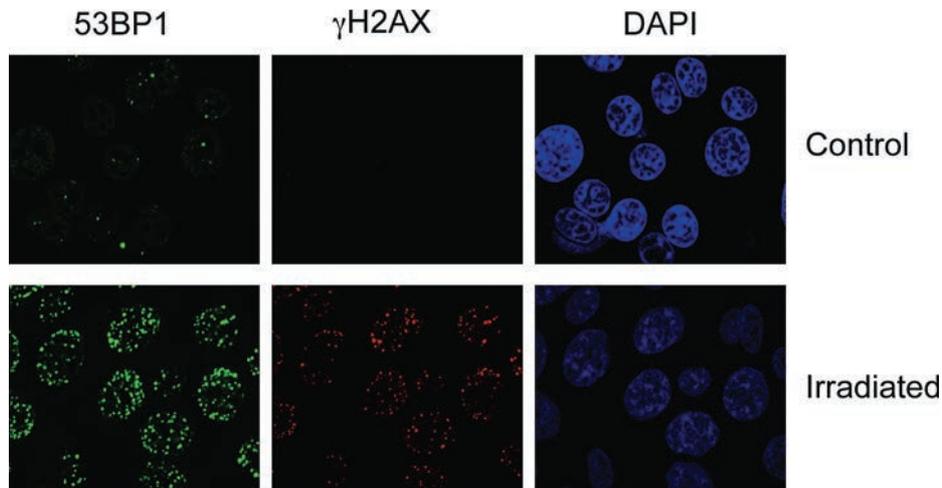


FIGURE 2.5 Photomicrograph of nuclear foci in control and 2-Gy irradiated cells as detected by staining with antibodies to 53BP1 (green) and γ H2AX (red). Cells were also stained with the nuclear stain 4',6-diamidino-2-phenylindole (DAPI) to show the location of nuclei. Without DNA strand breaks, there is little staining with γ H2AX and 53BP1 in foci. In contrast, staining for both proteins increases significantly after 2 Gy. (Courtesy of Dr. Ester Hammond.)

The most commonly assayed proteins for foci formation are γ H2AX and 53BP1 (Fig. 2.5). H2AX is a histone protein, which is rapidly phosphorylated in response to damage to form γ H2AX. Staining for the unmodified histone (H2AX) gives a pan nuclear stain or unchanging band on a western blot while γ H2AX is rapidly induced on a western blot in response to stress and can be seen to form discrete nuclear foci in damaged cells (Fig. 2.5). 53BP1 also becomes phosphorylated in response to stress and forms nuclear foci at the sites of DNA DSBs. In this case, antibodies to either the phosphorylated or unmodified form can be used to detect DSBs as the protein relocalizes to the damaged chromatin (i.e., it is not already part of the chromatin as is the case for H2AX). DNA damage-induced increases in γ H2AX or phosphorylated-53BP1 can also be quantified by flow cytometry. Other proteins also form foci in response to damage such as ataxia-telangiectasia mutated (ATM), replication protein A (RPA), RAD51, BRCA1 (discussed in subsequent sections).

Several γ H2AX or 53BP1 foci that form in a damaged cell directly correlate with several DSBs present. If this value is measured over time, then it also reflects the kinetics of repair (i.e., as the DSBs are repaired, the number of foci decreases). Recently, BRCA1 and RAD51,

two proteins involved in the repair of DNA damage by homologous recombination, have been used as biomarkers in a small pilot study by Willers et al. to detect repair defects in breast cancer biopsies.

■ DNA REPAIR PATHWAYS

Mammalian cells have developed specialized pathways to sense, respond to, and repair base damage, SSBs, DSBs, sugar damage, and DNA–DNA crosslinks. Research from yeast to mammalian cells has demonstrated that the mechanisms used to repair ionizing radiation-induced base damage are different from the mechanisms used to repair DNA DSBs. In addition, different repair pathways are used to repair DNA damage, depending on the stage of the cell cycle.

Much of our knowledge of DNA repair is the result of studying how mutations in individual genes result in radiation hypersensitivity. Radiation-sensitive mutants identified from yeast and mammalian cells appear either to be directly involved in the repair process or to function as molecular checkpoint-controlling elements. The pathways involved in the repair of base damage, SSBs, DSBs, sugar damage, and DNA–DNA crosslinks are discussed in the next sections and represent a simplified representation of our current state of understanding.

In Chapter 18, the syndromes associated with mutations in genes involved in sensing DNA damage or repairing DNA damage are discussed in more detail.

Base Excision Repair

Base damage is repaired through the base excision repair (BER) pathway illustrated in Figure 2.6. Bases on opposite strands of DNA must be complementary; adenine (A) pairs with thymine (T), and guanine (G) pairs with cytosine (C). U therefore represents a putative single-base mutation that is first removed by a glycosylase/DNA lyase (Fig. 2.6A). Removal of the base is followed by the removal of the sugar residue by an apurinic endonuclease 1 (APE1), then replacement with the correct nucleotide by DNA polymerase β , and completed by DNA ligase III–XRCC1-mediated ligation. If more than one nucleotide is to be replaced (illustrated by the putative mutation UU in Fig. 2.6B), then the complex of replication factor C (RFC)/proliferating cell nuclear antigen (PCNA)/DNA polymerase δ/ϵ performs the repair synthesis, the overhanging flap structure is removed by the

flap endonuclease 1 (FEN1), and DNA strands are sealed by ligase I (Fig. 2.6B). Although ionizing radiation–induced base damage is efficiently repaired, defects in BER may lead to an increased mutation rate, but usually do not result in cellular radiosensitivity. One exception to this is the mutation of the x-ray cross complementing factor 1 (*XRCC1*) gene, which confers about a 1.7-fold increase in radiation sensitivity. However, the radiation sensitivity of *XRCC1*-deficient cells may come from *XRCC1*'s potential involvement in other repair processes such as SSBs.

Nucleotide Excision Repair

Nucleotide excision repair (NER) removes bulky adducts in the DNA such as pyrimidine dimers. The process of NER can be subdivided into two pathways: global genome repair (GGR or GG-NER) and transcription-coupled repair (TCR or TC-NER). The process of GG-NER is genome-wide (i.e., lesions can be removed from DNA that encodes or does not encode for genes). In contrast, TC-NER only removes lesions in the DNA strands of actively

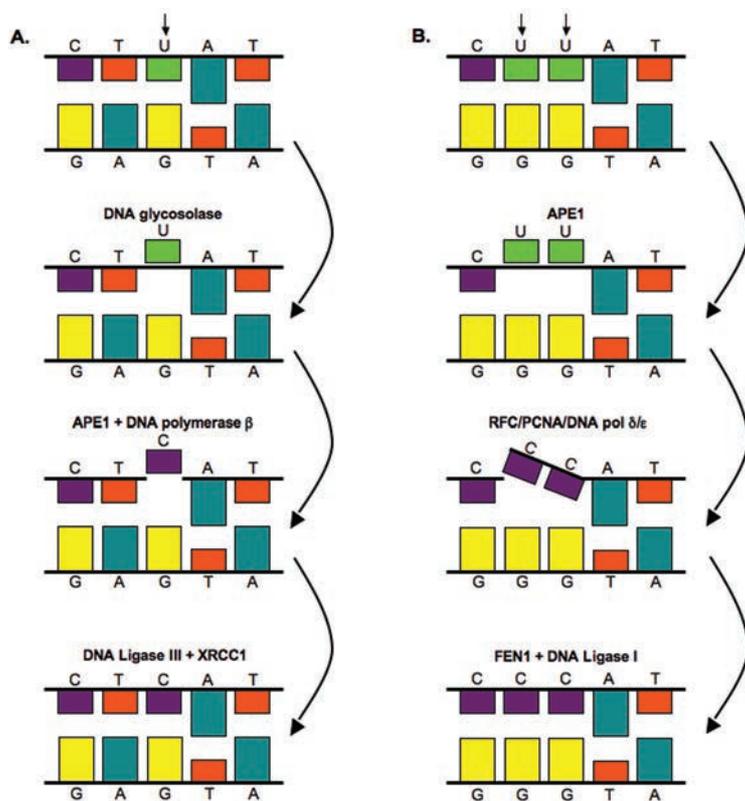


FIGURE 2.6 Base excision repair pathways. **A:** Base excision repair of a single nucleotide. Bases on opposite strands must be complementary; adenine (A) pairs with thymine (T), and guanine (G) pairs with cytosine (C). U represents a putative mutation that is first removed through a DNA glycosylase–mediated incision step. **B:** Base excision repair of multiple nucleotides. In this case, the double UU represents a putative mutation that is first removed through apurinic endonuclease 1 (APE1). See text for details.

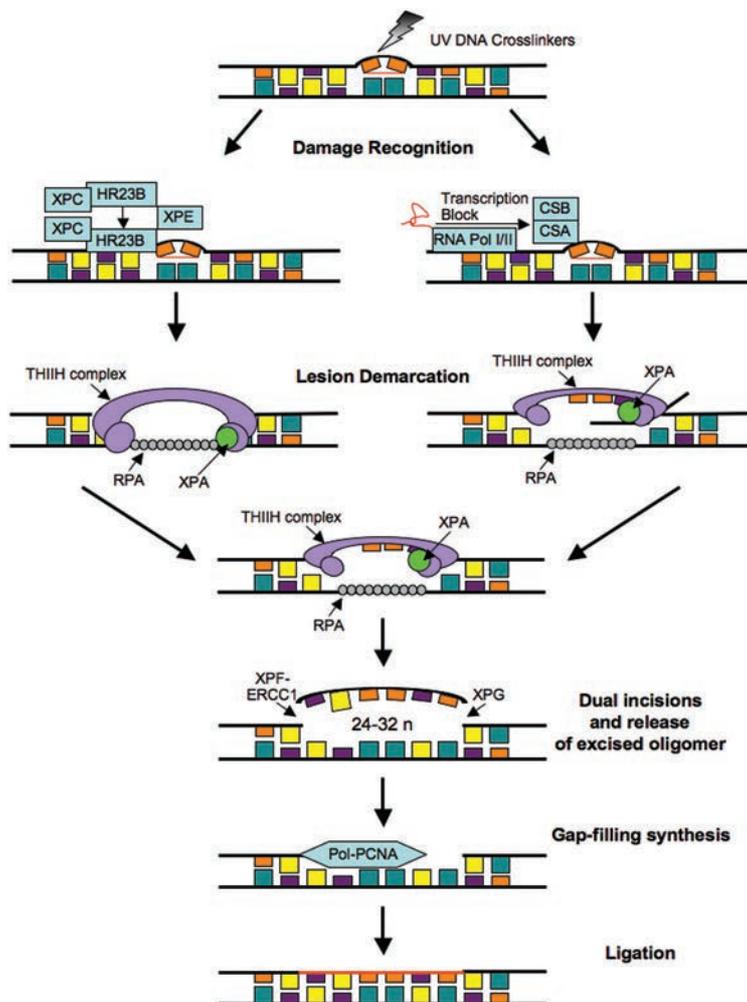
transcribed genes. When a DNA strand that is being actively transcribed becomes damaged, the RNA polymerase can block access to the site of damage and hence prevents DNA repair. TC-NER has evolved to prevent this blockade by RNA polymerase by effectively removing it from the site of damage to allow the repair proteins access. The mechanism of GG-NER and TC-NER differs only in the detection of the lesion; the remainder of the pathway used to repair the damage is the same for both. The essential steps in this pathway are (1) damage recognition; (2) DNA incisions that bracket the lesion, usually between 24 and 32 nucleotides in length; (3) removal of the region containing the adducts; (4) repair synthesis to fill in the gap region; and (5) DNA ligation (Fig. 2.7). Mutation in NER genes does not lead to ionizing radiation sensitivity. However, defective NER

increases sensitivity to UV-induced DNA damage and anticancer agents such as alkylating agents that induce bulky adducts. Germline mutations in NER genes lead to human DNA repair deficiency disorders such as xeroderma pigmentosum in which patients are hypersensitive to ultraviolet light.

DNA Double-Strand Break Repair

In eukaryotic cells, DNA DSBs can be repaired by two basic processes: homologous recombination repair (HRR), which requires an undamaged DNA strand as a participant in the repair as a template, and nonhomologous end-joining (NHEJ), which mediates end-to-end joining. In lower eukaryotes such as yeast, HRR is the predominant pathway used for repairing DNA DSBs. Homologous recombination is an error-free process because

FIGURE 2.7 Nucleotide excision repair pathways. The two sub-pathways of NER, GG-NER/GGR (global genome repair) and TC-NER/TCR (transcription-coupled repair), differ at the initial damage recognition step. GGR uses the XPC-HHR23B-XPE protein complexes, whereas in TCR, the NER proteins are recruited by the stalled RNA polymerase in cooperation with CSB and CSA. Following recognition, the lesion is demarcated by binding of the transcription factor IIH (TFIIH) complex, XPA and RPA. The TFIIH complex helicase function unwinds the DNA and generates an open stretch around the lesion, at which point the XPG and XPF-ERCC1 endonucleases make incisions at the 3' and 5' ends, respectively, releasing a 24–32 oligomer. The resulting gap is filled by the polymerases δ/ϵ aided by RFC and PCNA and the strand is finally ligated. (XPC, xeroderma pigmentosum, complementation group C; XPE, xeroderma pigmentosum, complementation group E; CSB, cockayne Syndrome B gene; CSA, cockayne Syndrome A gene; XPG, xeroderma pigmentosum, complementation group G; XPF, xeroderma pigmentosum, complementation group F; ERCC1, excision repair cross-complementation group 1 gene; RFC, replication factor C; PCNA, proliferating cell nuclear antigen.)



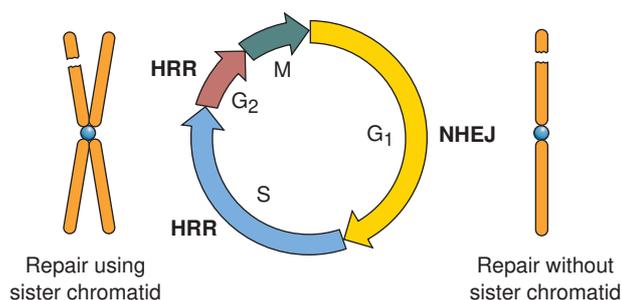


FIGURE 2.8 Illustration showing that nonhomologous recombination occurs in the G_1 phase of the cell cycle, at which stage, there is no sister chromatid to use as a template for repair. In contrast, homologous recombination occurs in the S and G_2 phases of the cell cycle, when there is a sister chromatid to use as a template in repair.

repair is performed by copying information from the undamaged homologous chromatid/chromosome. In mammalian cells, the choice of repair is biased by the phase of the cell cycle and by the abundance of repetitive DNA. HRR occurs primarily in the late S/ G_2 phase of the cell cycle, when an undamaged sister chromatid is available to act as a template, whereas NHEJ occurs in the G_1 phase of the cell cycle, when no such template exists (Fig. 2.8). NHEJ is error prone and probably accounts for many of the premutagenic lesions induced in the DNA of human cells by ionizing radiation. However, it is important to keep in mind that NHEJ and HRR are not mutually exclusive, and both have been found to be active in the late S/ G_2 phase of the cell cycle, indicating that other as-yet-unidentified factors, in addition to cell cycle phase, are important in determining what repair program is used.

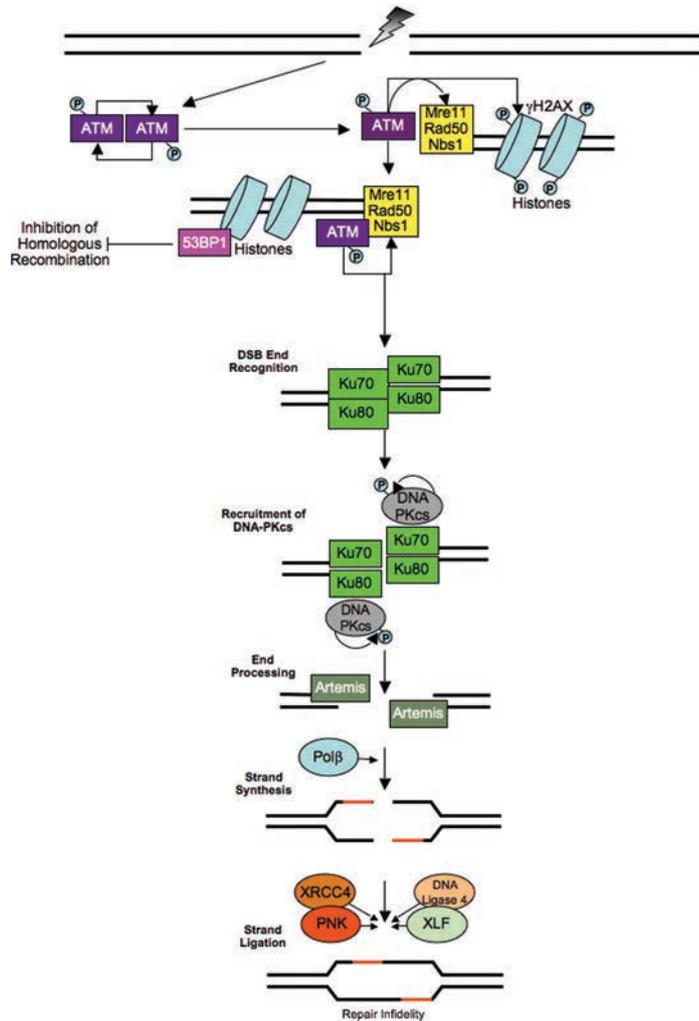
Nonhomologous End-Joining

The immediate response of a cell to a DNA DSB is the activation of a group of sensors that serve both to promote DNA repair and to prevent the cell from proceeding in the cell cycle until the break is faithfully repaired. These sensors, ATM and Rad3-related (ATR), are protein kinases that belong to the phosphatidylinositol-3-kinase-related kinase (PIKK) family and are recruited to the sites of DNA strand breaks induced by ionizing radiation. The competition for repair by HRR versus NHEJ is in part regulated by the protein 53BP-1. Functionally, ATM promotes the processing of broken DNA ends to generate recombinogenic single-strand DNA by regulating the activity of the NBS/MRE11/Rad50s protein complex (Fig. 2.9), and this resection activity of ATM is diminished by 53BP-1.

The ligation of DNA DSBs by NHEJ does not require sequence homology. However, the damaged ends of DNA DSBs cannot simply be ligated together; they must first be modified before they can be rejoined by a ligation reaction. NHEJ can be divided into five steps: (1) end recognition by Ku binding, (2) recruitment of DNA-dependent protein kinase catalytic subunit (DNA-PKcs), (3) end processing, (4) fill-in synthesis or end bridging, and (5) ligation (Fig. 2.9).

End recognition occurs when the Ku heterodimer, composed of 70-kDa and 83-kDa subunits, and the DNA-PKcs bind to the ends of the DNA DSB. Although the Ku/DNA-PKcs complex is thought to bind ends first, it is still unknown what holds the two DNA DSB ends together. Although microhomology between one to four nucleotides can aid in end alignment, there is no absolute requirement for microhomology for NHEJ. In fact, Ku does not only recruit DNA-PKcs to the DNA ends, but an additional protein, Artemis, which possesses endonuclease activity, forms a physical complex with DNA-PKcs. The Ku/DNA-PKcs complex that is bound to the DNA ends can phosphorylate Artemis and activate its endonuclease activity to deal with 5' and 3' overhangs as well as hairpins. End processing is followed by fill-in synthesis of gaps formed by the Artemis endonuclease activity. This aspect of NHEJ may not necessarily be essential; for example, in the ligation of blunt ends or ends with compatible termini. At present, it is unclear what the signal is for a fill-in reaction to proceed after endonuclease processing. However, DNA polymerase μ or λ has been found to be associated with the Ku/DNA/XRCC4/DNA ligase IV complex and serves as the polymerase for the fill-in reaction. In the final step of NHEJ, ligation of nicked

FIGURE 2.9 Nonhomologous end-joining. DNA strand breaks are recognized by the ATM and the MRN (Mre11-Rad50-Nbs1) complex, resulting in resection of the DNA ends. Homologous recombination is inhibited by the activity of 53BP1. The initial step of the core NHEJ pathway starts with the binding of the ends at the DSB by the Ku70/Ku80 heterodimer. This complex then recruits and activates the catalytic subunit of DNA-PK (DNA-PKcs), whose role is the juxtaposition of the two DNA ends. The DNA-PK complex then recruits the ligase complex (XRCC4/XLF-LIGIV/PNK) that promotes the final ligation step.



DNA ends that have been processed is mediated by a PNK/XRCC4/DNA ligase IV/XLF complex that is probably recruited by the Ku heterodimer. Polynucleotide kinase (PNK) is a protein that has both 3'-DNA phosphatase and 5'-DNA kinase activities and serves to remove end groups that are not ligatable to allow end-joining. XRCC4-like factor (XLF) is a protein that has a similar protein structure as x-ray repair complementing defective repair in Chinese hamster cells 4 (XRCC4) and stimulates the activity of DNA ligase IV. NHEJ is error prone and plays an important physiologic role in generating antibodies through V(D)J rejoining. The error-prone nature of NHEJ is essential for generating antibody diversity and often goes undetected in mammalian cells, as errors in the

noncoding DNA that composes most human genome has little consequence. NHEJ is primarily found in the G₁ phase of the cell cycle, where there is no sister chromatid.

Homologous Recombination Repair

HRR provides the mammalian genome a high-fidelity mechanism of repairing DNA DSBs (Fig. 2.10). In particular, the increased activity of this recombination pathway in late S/G₂ suggests that its primary function is to repair and restore the functionality of replication forks with DNA DSBs. Compared to NHEJ, which requires no sequence homology to rejoin broken ends, HRR requires physical contact with an undamaged chromatid or chromosome (to serve as a template) for repair to occur.

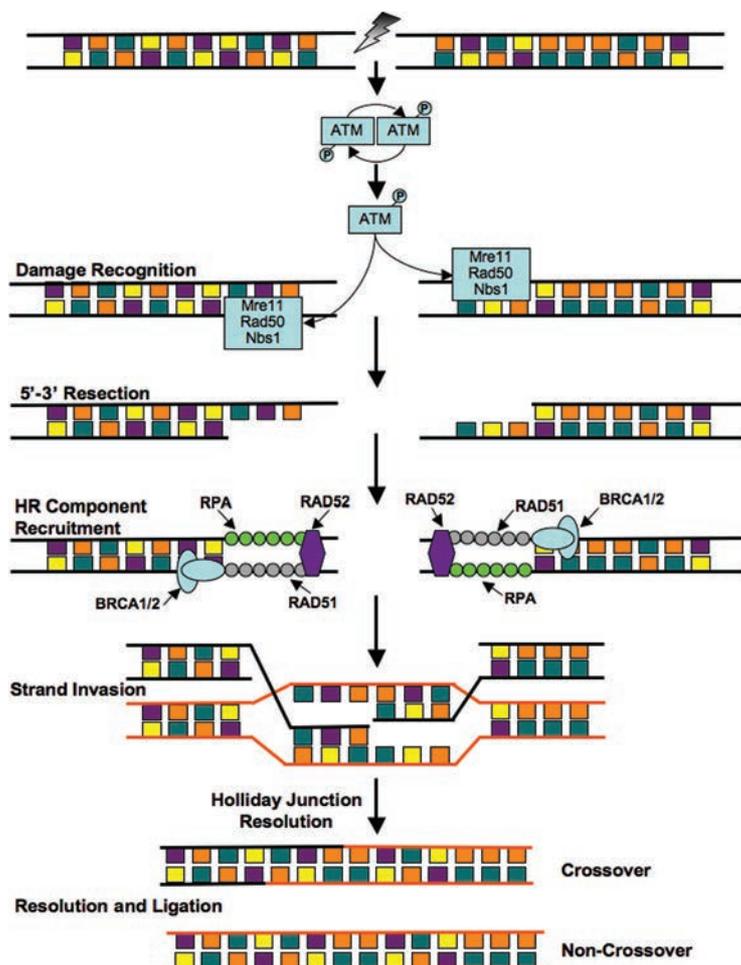


FIGURE 2.10 Homologous re-combinational repair. The initial step in HR is the recognition of the lesion and processing of the double-strand DNA ends into 3' DNA single strand tails by the MRN (Mre11-Rad50-Nbs1) complex, which are then coated by RPA forming a nucleoprotein filament. Then, specific HR proteins are recruited to the nucleoprotein filaments, such as RAD51, RAD52, and BRCA1/2. RAD51 is a key protein in homologous recombination as it mediates the invasion of the homologous strand of the sister chromatid, leading to formation of Holliday junctions. The Holliday junctions are finally resolved into two DNA duplexes. See text for details.

During recombination, evidence exists that ATM phosphorylates the breast cancer tumor suppressor protein BRCA1, which is then recruited to the site of the DSB that has been bound by the NBS/MRE11/Rad50s protein complex (Fig. 2.10). MRE11 and perhaps other yet unidentified endonucleases resect the DNA, resulting in a 3' single-strand DNA that serves as a binding site for Rad51. BRCA2, which is attracted to the DSB by BRCA1, facilitates the loading of Rad51 onto RPA-coated single-strand overhangs produced by endonuclease resection. Rad51 protein is a homologue of the *Escherichia coli* recombinase RecA and possesses the ability to form nucleofilaments and catalyze strand exchange with the complementary strand in the undamaged chromosome. Five additional paralogues of Rad51 also bind to the RPA-coated single-stranded region

and recruit Rad52, which protects against exonucleolytic degradation. To facilitate repair, Rad54 uses its ATPase activity to unwind the double-stranded molecule. The two invading ends serve as primers for DNA synthesis, and the so-called Holliday junctions are resolved by MMS4 and MUS81 by noncrossing over, in which case, the Holliday junctions disengage and DNA strand pairing is followed by gap filling, or by crossing over of the Holliday junctions, which is followed by gap filling. The identities of the polymerase and ligase involved in these latter steps are unknown. Because inactivation of HRR genes results in radiosensitivity and genomic instability, these genes provide a critical link between HRR and chromosome stability. Dysregulated homologous recombination can also lead to cancer by loss of heterozygosity (LOH).

Crosslink Repair

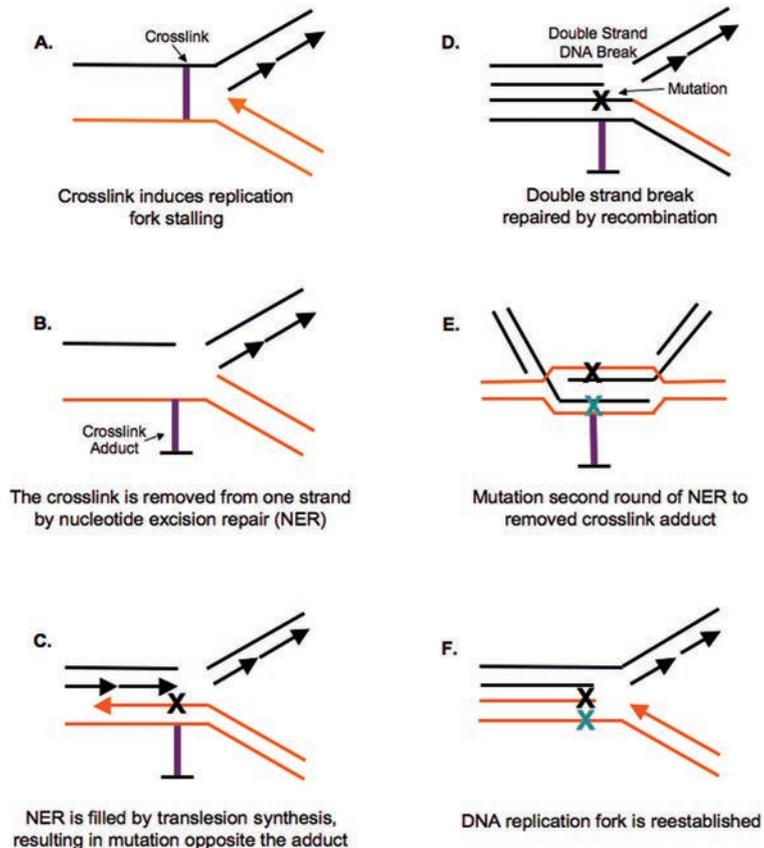
Several DNA–DNA and DNA–protein crosslinks induced by ionizing radiation have not been extensively studied to arrive at a quantitative estimate. Furthermore, the genes and pathways used for DNA–DNA or DNA–protein crosslink repair are still under investigation. The current thinking is that a combination of NER and recombinational repair pathways is needed to repair DNA crosslinks (Fig. 2.11). The predominant signal from a DNA–interstrand crosslink that signals for repair is stalling of the DNA replication fork. The crosslink is removed in a multistep process, first from one strand by a second round of NER, resulting in a strand break and a DNA adduct. DNA synthesis can proceed past the lesion, resulting in a point mutation opposite the lesion. However, the SSB will become a DSB, and seems to require HRR for restitution. Finally, the adduct that remains is removed by NER. Cells with mutations in NER and HRR pathways are modestly sensitive to crosslinking

agents. In contrast, individuals afflicted with the syndrome Fanconi anemia are hypersensitive to crosslinking agents. Chromatin that contains actively transcribed genes is more susceptible to DNA–protein crosslinks, and the crosslinked proteins are usually nuclear matrix proteins.

Mismatch Repair

The mismatch repair (MMR) pathway removes base–base and small insertion mismatches that occur during replication. In addition, the MMR pathway removes base–base mismatches in homologous recombination intermediates. See Figure 2.12 for schematic representation and an indication of the critical gene products. The process of MMR can be subdivided into four components: first, the mismatch must be identified by sensors that transduce the signal of a mismatched base pair; second, MMR factors are recruited; third, the newly synthesized strand harboring the mismatch is identified and the incorrect/altere nucleotides are excised; and in the fourth stage, resynthesis and ligation

FIGURE 2.11 DNA–DNA crosslink repair. The initial signal for DNA–DNA crosslinks is stalling of the replication fork (A). The crosslink is removed from one strand by nucleotide excision repair (B), followed by translesion synthesis, resulting in a mutation opposite the adduct (C). The resulting DNA double-strand break is repaired by homologous recombination (D) and the crosslink is removed from the DNA by another round of nucleotide excision repair (E–F). This schema for crosslink repair is still a work in progress.



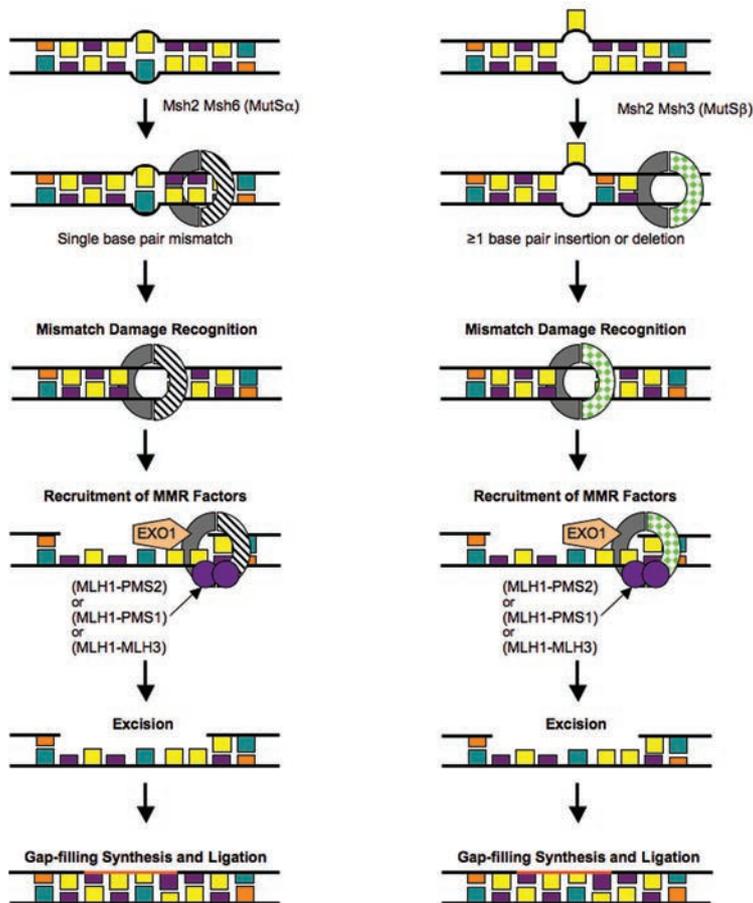


FIGURE 2.12 Mismatch repair. The initial step in the mismatch repair pathway is the recognition of mismatched bases through either Msh2-Msh6 or Msh2-Msh3 complexes. These recognition complexes recruit MLH1-PMS2, MLH1-PMS1, and MLH1-MLH3, alongside the exonuclease EXO1 that catalyzes the excision step that follows. A gap-filling step by polymerases δ/ϵ , RCF, and PCNA is followed by a final ligation step.

of the excised DNA tract is completed. MMR was first characterized in *E. coli* by the characterization of the *Mut* genes, of which homologues of these gene products have been identified and extensively characterized in both yeast and humans. Mutations in any of the mismatch *MSH*, *MLH*, and *PSM* families of repair genes leads to microsatellite instability (small base insertions or deletions) and cancer, especially hereditary nonpolyposis colon cancer (HNPCC).

■ RELATIONSHIP BETWEEN DNA DAMAGE AND CHROMOSOME ABERRATIONS

Cell killing does not correlate with SSBs, but relates better to DSBs. Agents (such as hydrogen peroxide) produce SSBs efficiently, but very few DSBs, and also kill very few cells. Cells defective in DNA DSB repair exhibit hypersensitivity to killing by ionizing radiation and increased

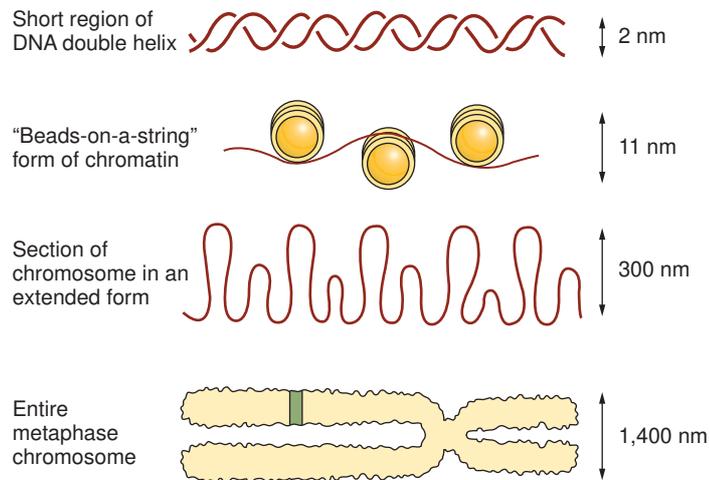
numbers of chromosome aberrations. On the basis of evidence such as this, it is concluded that DSBs are the most relevant lesions leading to most biologic insults from radiation including cell killing. The reason for this is that DSBs can lead to chromosomal aberrations that present problems at cell division.

■ CHROMOSOMES AND CELL DIVISION

The backbone of DNA is made of molecules of sugar and phosphates, which serve as a framework to hold the bases that carry the genetic code. Attached to each sugar molecule is a base: thymine, adenine, guanine, or cytosine. This whole configuration is coiled tightly in a double helix.

Figure 2.13 is a highly schematized illustration of the way an organized folding of the long DNA helix might be achieved as a closely packed series of looped domains wound in a tight helix. The degree of packing also is illustrated by the

FIGURE 2.13 Illustration of the relative sizes of the DNA helix, the various stages of folding and packing of the DNA, and an entire chromosome condensed at metaphase.



relative dimensions of the DNA helix and the condensed metaphase chromosome.

The largest part of the life of any somatic cell is spent in interphase, during which the nucleus, in a stained preparation, appears as a lacework of fine and lightly stained material in a translucent and colorless material surrounded by a membrane. In the interphase nucleus in most cells, one or more bodies of various sizes and shapes, called **nucleoli**, are seen. In most cells, little more than this can be identified with a conventional light microscope. In fact, a great deal is happening during this time: The quantity of DNA in the nucleus doubles as each chromosome lays down an exact replica of itself next to itself. When the chromosomes become visible at mitosis, they are each present in duplicate. Even during interphase, there is good evidence that the chromosomes are not free to move about within the nucleus but are restricted to "domains."

The various events that occur during **mitosis** are divided into several phases. The first phase of division is called **prophase**. The beginning of this phase is marked by a thickening of the chromatin and an increase in its stainability as the chromosomes condense into light coils. By the end of prophase, each chromosome has a lightly staining constriction known as a **centromere**; extending from the centromere are the arms of the chromosome. Prophase ends when the chromosomes reach maximal condensation and the nuclear membrane disappears, as do any nucleoli.

With the disappearance of the nuclear membrane, the nuclear plasma and the cytoplasm mix. **Metaphase** then follows, in which two events occur simultaneously. The chromosomes move to the center of the cell (i.e., to the cell's equator), and the spindle forms. The spindle is composed of fibers that cross the cell, linking its poles. Once the chromosomes are stabilized at the equator of the cell, their centromeres divide, and metaphase is complete.

The phase that follows, **anaphase**, is characterized by a movement of the chromosomes on the spindle to the poles. The chromosomes appear to be pulled toward the poles of the cell by fibers attached to the centromeres. The arms, particularly the long arms, tend to trail behind.

Anaphase is followed by the last phase of mitosis, **telophase**. In this phase, the chromosomes, congregated at the poles of the cell, begin to uncoil. The nuclear membrane reappears, as do the nucleoli; and as the phase progresses, the chromosome coils unwind until the nucleus regains the appearance characteristic of interphase.

■ THE ROLE OF TELOMERES

Telomeres cap and protect the terminal ends of chromosomes. The name *telomere* literally means "end part." Mammalian telomeres consist of long arrays of TTAGGG repeats that range in total length anywhere from 1.5 to 150 kilobases. Each time a normal somatic cell divides, telomeric DNA is lost from the lagging strand because DNA polymerase cannot synthesize

new DNA in the absence of an RNA primer. Successive divisions lead to progressive shortening, and after 40 to 60 divisions, the telomeres in human cells are shortened dramatically, so that vital DNA sequences begin to be lost. At this point, the cell cannot divide further and undergoes senescence. Telomere length has been described as the “molecular clock” or generational clock because it shortens with age in somatic tissue cells during adult life. Stem cells in self-renewing tissues, and cancer cells in particular, avoid this problem of aging by activating the enzyme telomerase. Telomerase is a reverse transcriptase that includes the complementary sequence to the TTAGGG repeats and so continually rebuilds the chromosome ends to offset the degradation that occurs with each division.

In tissue culture, immortalization of cells—that is, the process whereby cells pass through a “crisis” and continue to be able to divide beyond the normal limit—is associated with telomere stabilization and telomerase activity.

Virtually all human tumor cell lines and approximately 90% of human cancer biopsy specimens exhibit telomerase activity. By contrast, normal human somatic tissues, other than stem cells, do not possess detectable levels of this enzyme. It is an attractive hypothesis that both immortalization and carcinogenesis are associated with telomerase expression.

■ RADIATION-INDUCED CHROMOSOME ABERRATIONS

In the traditional study of chromosome aberrations, the effects of ionizing radiations are described in terms of their appearance when a preparation is made at the first metaphase after exposure to radiation. This is the time when the structure of the chromosomes can be discerned.

The study of radiation damage in mammalian cell chromosomes is hampered by the large number of mammalian chromosomes per cell and by their small size. Most mammalian cells currently available for experimental purposes have a diploid complement of 40 or more chromosomes. There are exceptions, such as the Chinese hamster, with 22 chromosomes, and various marsupials, such as the rat kangaroo and woolly opossum, which have chromosome complements of 12 and 14, respectively. Many plant cells, however, contain fewer

and generally much larger chromosomes; consequently, until recently, information on chromosomal radiation damage accrued principally from studies with plant cells.

If cells are irradiated with x-rays, DSBs are produced in the chromosomes. The broken ends appear to be “sticky” because of unpaired bases and can rejoin with any other sticky end. It would appear, however, that a broken end cannot join with a normal, unbroken chromosome, although this is controversial. Once breaks are produced, different fragments may behave in various ways:

1. The breaks may rejoin, that is, rejoin in their original configuration. In this case, of course, nothing amiss is visible at the next mitosis.
2. The breaks may fail to rejoin and give rise to an aberration, which is scored as a deletion at the next mitosis.
3. Broken ends may reassort and rejoin other broken ends to give rise to chromosomes that appear to be grossly distorted if viewed at the following mitosis.

The aberrations seen at metaphase are of two classes: *chromosome* aberrations and *chromatid* aberrations. **Chromosome aberrations** result if a cell is irradiated early in interphase, before the chromosome material has been duplicated. In this case, the radiation-induced break is in a single strand of chromatin; during the DNA synthetic phase that follows, this strand of chromatin lays down an identical strand next to itself and replicates the break that has been produced by the radiation. This leads to a chromosome aberration visible at the next mitosis because there is an identical break in the corresponding points of a pair of chromatin strands. If, on the other hand, the dose of radiation is given later in interphase, after the DNA material has doubled and the chromosomes consist of two strands of chromatin, then the aberrations produced are called **chromatid aberrations**. In regions removed from the centromere, chromatid arms may be fairly well separated, and it is reasonable to suppose that the radiation might break one chromatid without breaking its sister chromatid, or at least not in the same place. A break that occurs in a single chromatid arm after chromosome replication and leaves the opposite arm of the same chromosome undamaged leads to chromatid aberrations.

■ EXAMPLES OF RADIATION-INDUCED ABERRATIONS

Many types of chromosomal aberrations and rearrangements are possible, but an exhaustive analysis is beyond the scope of this book. Three types of aberrations that are *lethal* to the cell are described, followed by two common rearrangements that are consistent with cell viability but are frequently involved in carcinogenesis. The three lethal aberrations are the **dicentric**; the **ring**, which are chromosome aberrations; and the **anaphase bridge**, which is a chromatid aberration. All three represent gross distortions and are clearly visible. Many other aberrations are possible but are not described here.

The formation of a **dicentric** is illustrated in diagrammatic form in Figure 2.14A. This aberration involves an interchange between two separate chromosomes. If a break is produced in each one early in interphase and the sticky ends are close to one another, they may rejoin as shown. This bizarre interchange is replicated during the DNA synthetic phase, and the result is a grossly distorted chromosome with two centromeres (hence, dicentric). There also are two fragments that have no centromere (acentric fragment), which will therefore be lost at a subsequent mitosis. The appearance at metaphase is shown in the bottom panel of Figure 2.14A. An example of a dicentric and fragment in a metaphase human cell is shown in Figure 2.15B; Figure 2.15A shows a normal metaphase for comparison.

The formation of a **ring** is illustrated in diagrammatic form in Figure 2.14B. A break is induced by radiation in each arm of a single chromatid early in the cell cycle. The sticky ends may rejoin to form a ring and a fragment. Later in the cycle, during the DNA synthetic phase, the chromosome replicates. The ultimate appearance at metaphase is shown in the lower panel of Figure 2.14B. The fragments have no centromere and probably will be lost at mitosis because they will not be pulled to either pole of the cell. An example of a ring chromosome in a human cell at metaphase is illustrated in Figure 2.15C.

An **anaphase bridge** may be produced in various ways. As illustrated in Figure 2.14C and Figure 2.16, it results from breaks that occur late in the cell cycle (in G_2) after the chromosomes have replicated. Breaks may occur in both

chromatids of the same chromosome, and the sticky ends may rejoin incorrectly to form a sister union. At anaphase, when the two sets of chromosomes move to opposite poles, the section of chromatin between the two centromeres is stretched across the cell between the poles, hindering the separation into two new progeny cells, as illustrated in Figure 2.14C and Figure 2.16B. The two fragments may join as shown, but because there is no centromere, the joined fragments will probably be lost at the first mitosis. This type of aberration occurs in human cells and is essentially always lethal. It is hard to demonstrate because preparations of human chromosomes usually are made by accumulating cells at metaphase, and the bridge is only evident at anaphase. Figure 2.16 is an anaphase preparation of *Tradescantia paludosa*, a plant used extensively for cytogenetic studies because of the small number of large chromosomes. The anaphase bridge is seen clearly as the replicate sets of chromosomes move to opposite poles of the cell.

Gross chromosome changes of the types discussed previously inevitably lead to the reproductive death of the cell.

Two important types of chromosomal changes that are not lethal to the cell are symmetric translocations and small deletions. The formation of a **symmetric translocation** is illustrated in Figure 2.17A. It involves a break in two prereplication (G_1) chromosomes, with the broken ends being exchanged between the two chromosomes as illustrated. An aberration of this type is difficult to see in a conventional preparation but is easy to observe with the technique of fluorescent *in situ* hybridization (FISH), or *chromosome painting*, as it commonly is called. Probes are available for every human chromosome that makes them fluorescent in a different color. Exchange of material between two different chromosomes then is readily observable (Fig. 2.18). Translocations are associated with several human malignancies caused by the activation of an oncogene; Burkitt lymphoma and certain types of leukemia are examples.

The other type of nonlethal chromosomal change is a **small interstitial deletion**. This is illustrated in Figure 2.17B and may result from two breaks in the same arm of the same chromosome, leading to the loss of the genetic information between the two breaks. The actual sequence of events in the formation of a deletion is easier

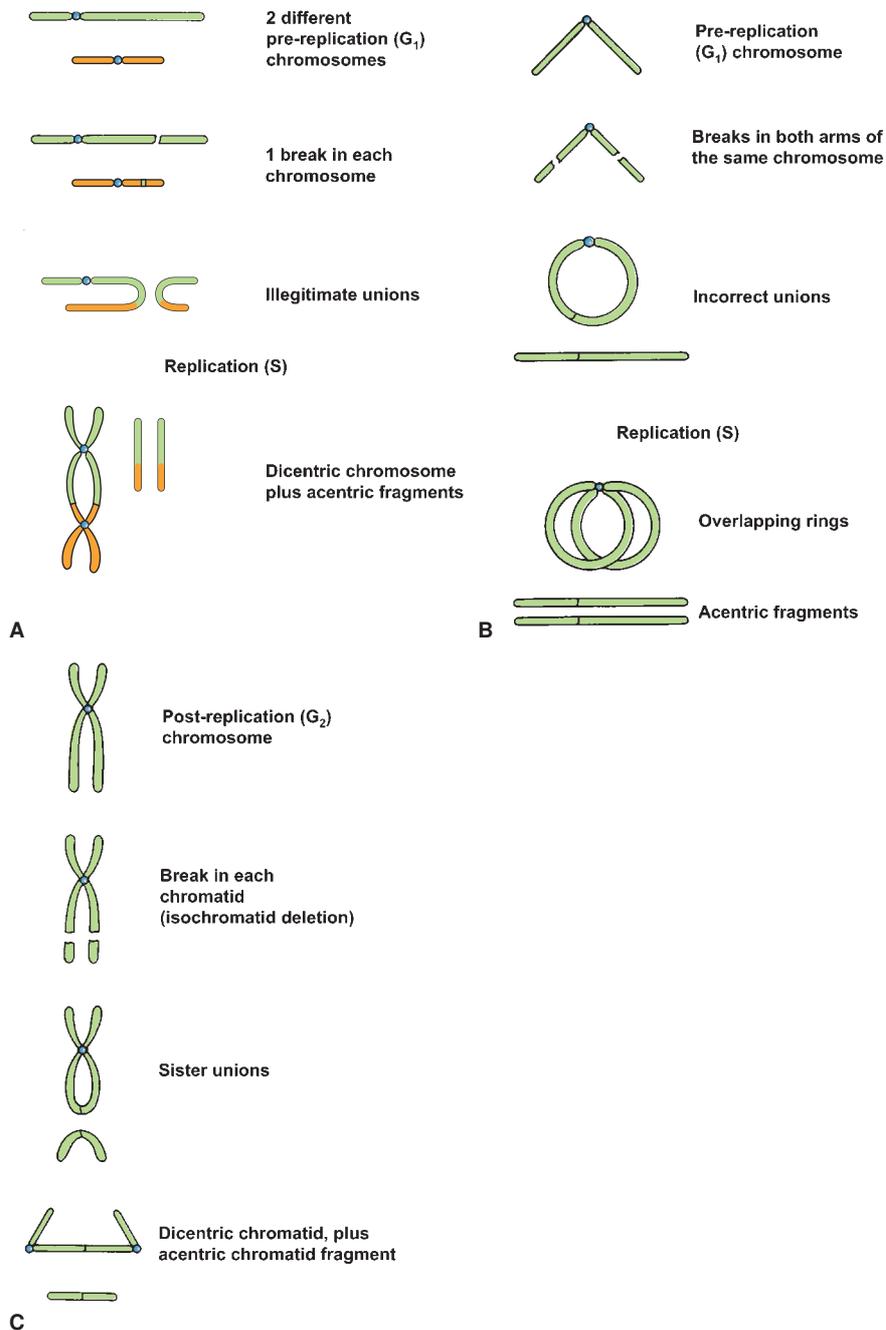
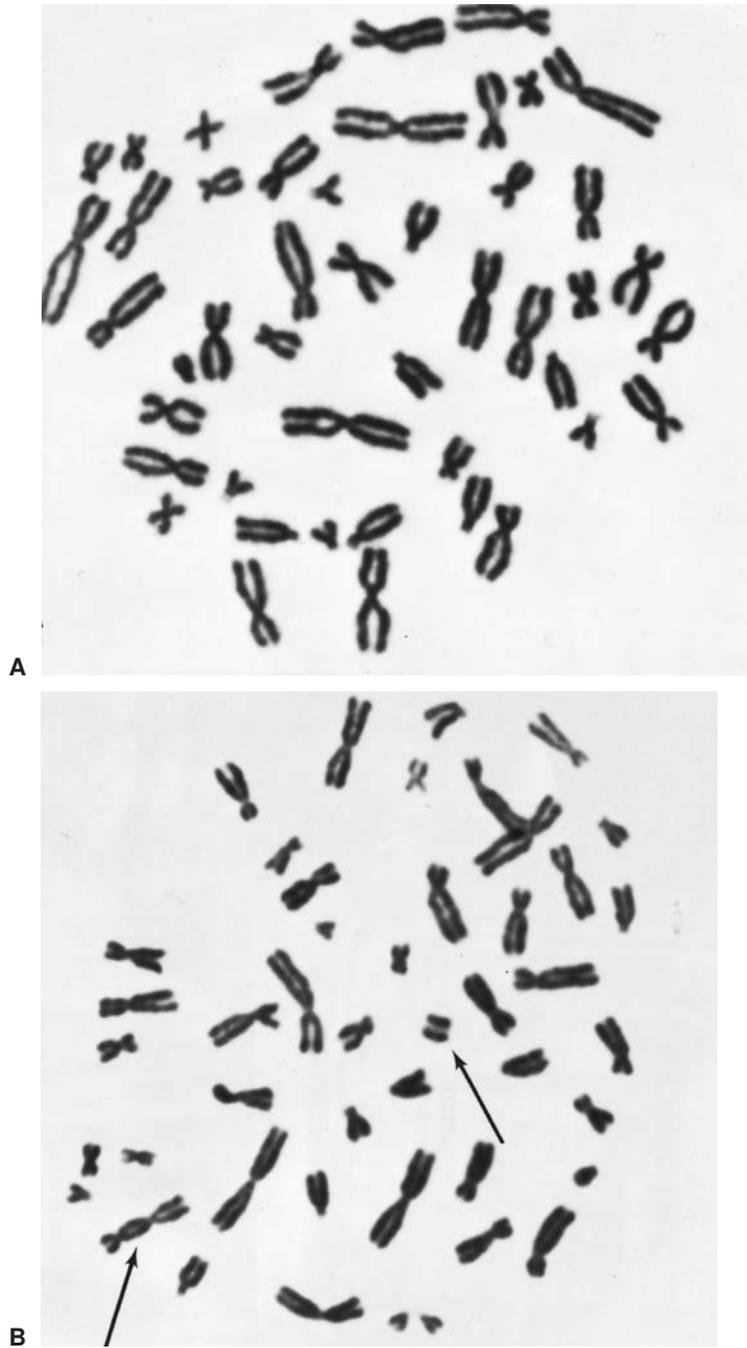


FIGURE 2.14 **A:** The steps in the formation of a dicentric by irradiation of prereplication (i.e., G₁) chromosomes. A break is produced in each of two separate chromosomes. The “sticky” ends may join incorrectly to form an interchange between the two chromosomes. Replication then occurs in the DNA synthetic period. One chromosome has two centromeres: a dicentric. The acentric fragment will also replicate and both will be lost at a subsequent mitosis because, lacking a centromere, they will not go to either pole at anaphase. **B:** The steps in the formation of a ring by irradiation of a prereplication (i.e., G₁) chromosome. A break occurs in each arm of the same chromosome. The sticky ends rejoin incorrectly to form a ring and an acentric fragment. Replication then occurs. **C:** The steps in the formation of an anaphase bridge by irradiation of a postreplication (i.e., G₂) chromosome. Breaks occur in each chromatid of the same chromosome. Incorrect rejoining of the sticky ends then occurs in a sister union. At the next anaphase, the acentric fragment will be lost, one centromere of the dicentric will go to each pole, and the chromatid will be stretched between the poles. Separation of the progeny cells is not possible; this aberration is likely to be lethal. (Courtesy of Dr. Charles Geard.)

FIGURE 2.15 Radiation-induced chromosome aberrations in human leukocytes viewed at metaphase. **A:** Normal metaphase. **B:** Dicentric and fragment (arrows). (Continued)





C

FIGURE 2.15 (Continued) **C:** Ring (arrow). (Courtesy of Drs. Brewen, Luipold, and Preston.)

to understand from Figure 2.19, which shows an interphase chromosome. It is a simple matter to imagine how two breaks may isolate a loop of DNA—an acentric ring—which is lost at a subsequent mitosis. Deletions may be associated with carcinogenesis if the lost genetic material includes a tumor suppressor gene. This is discussed further in Chapter 10 on radiation carcinogenesis.

The interaction between breaks in different chromosomes is by no means random. There is great heterogeneity in the sites at which deletions and exchanges between different chromosomes occur; for example, chromosome 8 is particularly sensitive to exchanges. As mentioned previously, each chromosome is restricted to a domain, and most interactions occur at the edges of domains, which probably involves the nuclear matrix. Active chromosomes are therefore those with the biggest surface area to their domains.

■ CHROMOSOME ABERRATIONS IN HUMAN LYMPHOCYTES

Chromosomal aberrations in peripheral lymphocytes have been used widely as biomarkers of radiation exposure. In blood samples obtained for cytogenetic evaluation within a few days to a few

weeks after total body irradiation, the frequency of asymmetric aberrations (dicentric and rings) in the lymphocytes reflects the dose received. Lymphocytes in the blood sample are stimulated to divide with a mitogen such as phytohemagglutinin and are arrested at metaphase, and the incidence of rings and dicentrics is scored. The dose can be estimated by comparison with *in vitro* cultures exposed to known doses. Figure 2.20 shows a dose-response curve for aberrations in human lymphocytes produced by γ -rays. The data are fitted by a linear-quadratic relationship, as would be expected, because rings and dicentrics result from the interaction of two chromosome breaks, as previously described. The linear component is a consequence of the two breaks resulting from a single charged particle. If the two breaks result from different charged particles, the probability of an interaction is a quadratic function of dose. This also is illustrated for the formation of a dicentric in Figure 2.20.

If a sufficient number of metaphases are scored, cytogenetic evaluations in cultured lymphocytes readily can detect a recent total body exposure of as low as 0.25 Gy in the exposed person. Such studies are useful in distinguishing between “real” and “suspected” exposures, particularly in those instances involving “black film badges” or

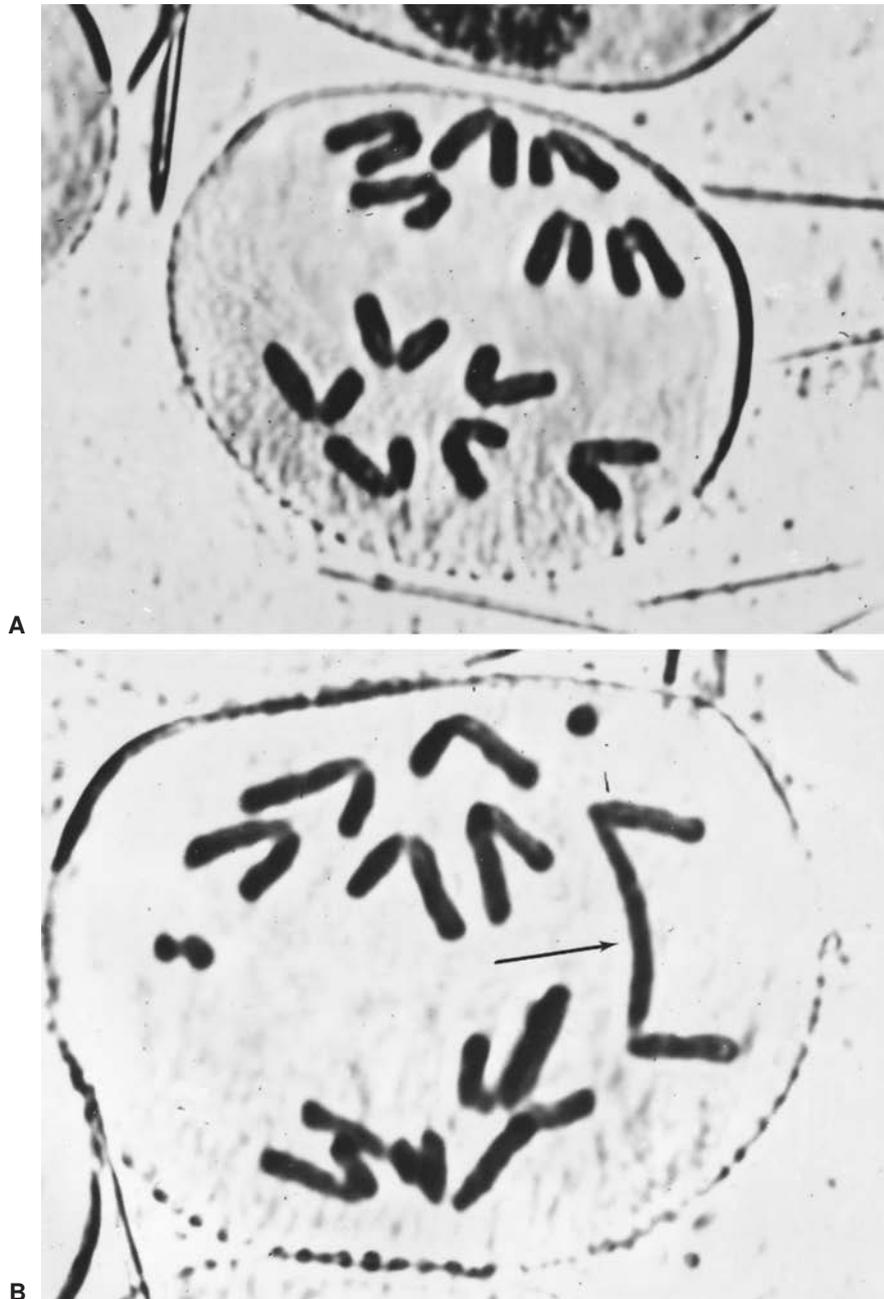


FIGURE 2.16 Anaphase chromosome preparation of *Tradescantia paludosa*. **A:** Normal anaphase. **B:** Bridge and fragment resulting from radiation (*arrow*). (Courtesy of Drs. Brewen, Luippold, and Preston.)

in potential accidents in which it is not certain whether individuals who were at risk for exposure actually received radiation doses.

Mature T lymphocytes have a finite life span of about 1,500 days and are eliminated slowly from the peripheral lymphocyte pool. Consequently, the yield of dicentrics observed in

peripheral lymphocytes declines in the months and years after a radiation exposure.

During *in vivo* exposures to ionizing radiation, chromosome aberrations are induced not only in mature lymphocytes but also in lymphocyte progenitors in marrow, nodes, or other organs. The stem cells that sustain asymmetric aberrations

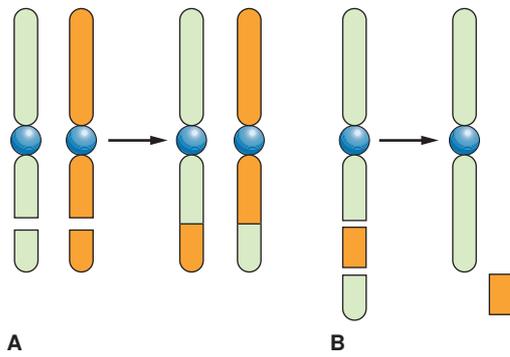


FIGURE 2.17 **A:** Formation of a symmetric translocation. Radiation produces breaks in two different prereplication chromosomes. The broken pieces are exchanged between the two chromosomes, and the “sticky” ends rejoin. This aberration is not necessarily lethal to the cell. There are examples in which an exchange aberration of this type leads to the activation of an oncogene. See Chapter 10 on radiation carcinogenesis. **B:** Diagram of a deletion. Radiation produces two breaks in the same arm of the same chromosome. What actually happens is illustrated more clearly in Figure 2.18.

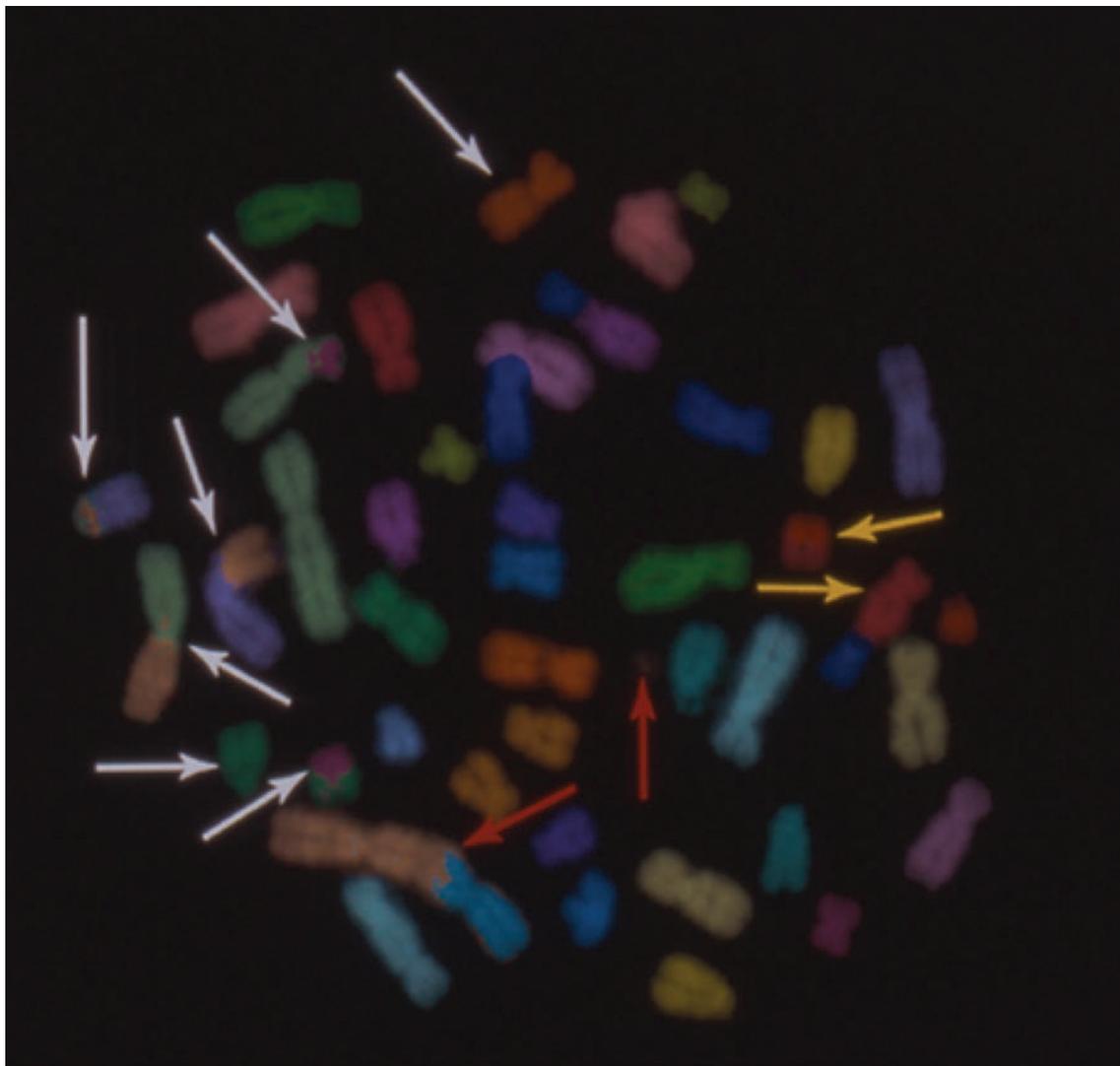
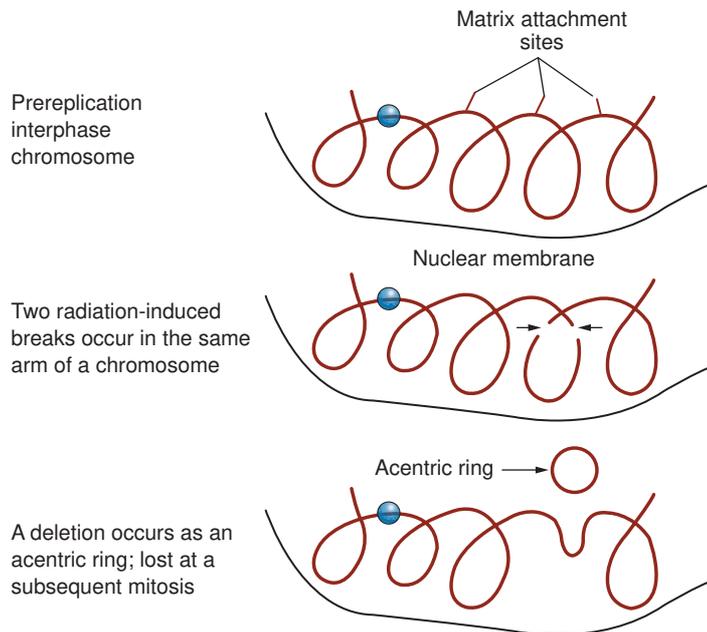


FIGURE 2.18 Fluorescence *in situ* hybridization of a metaphase spread from a cell that received 4 Gy. The hybridization was performed with a cocktail of DNA probes that specifically recognize each chromosome pair. Chromosome aberrations are demarcated by the arrows. (Courtesy of Dr. Michael Cornforth.)

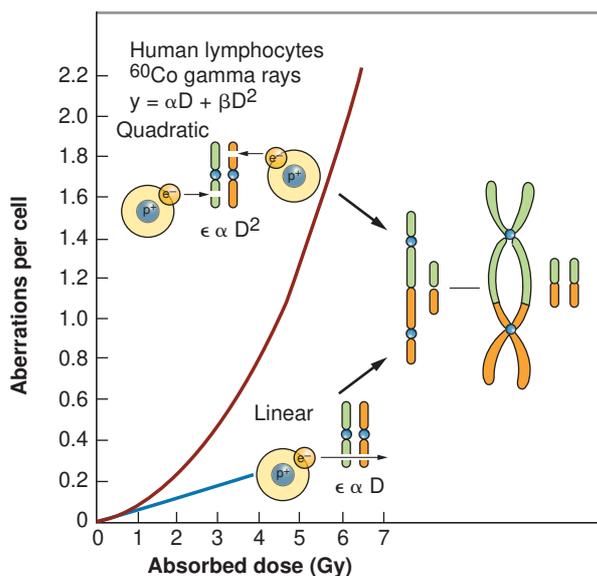
FIGURE 2.19 Formation of a deletion by ionizing radiation in an interphase chromosome. It is easy to imagine how two breaks may occur (by a single or two different charged particles) in such a way as to isolate a loop of DNA. The “sticky” ends rejoin, and the deletion is lost at a subsequent mitosis because it has no centromere. This loss of DNA may include the loss of a suppressor gene and lead to a malignant change. See Chapter 10 on radiation carcinogenesis.



(such as dicentrics) die in attempting a subsequent mitosis, but those that sustain a symmetric non-lethal aberration (such as a translocation) survive and pass on the aberration to their progeny. Consequently, dicentrics are referred to as “unstable” aberrations because their number declines with time after irradiation. Symmetric translocations, by contrast, are referred to as “stable” aberrations because they persist for many years. Either type of aberration can be used to estimate dose soon after irradiation, but if many years have elapsed, scoring

dicentrics underestimates the dose and only stable aberrations such as translocations give an accurate picture. Until recently, translocations were much more difficult to observe than dicentrics, but now the technique of FISH makes the scoring of such symmetric aberrations a relatively simple matter. The frequency of translocations assessed in this way correlates with total body dose in exposed individuals even after more than 50 years, as was shown in a study of the survivors of the atomic bomb attacks on Hiroshima and Nagasaki.

FIGURE 2.20 The frequency of chromosomal aberrations (dicentrics and rings) is a linear-quadratic function of dose because the aberrations are the consequence of the interaction of two separate breaks. At low doses, both breaks may be caused by the same electron; the probability of an exchange aberration is proportional to dose (D). At higher doses, the two breaks are more likely to be caused by separate electrons. The probability of an exchange aberration is proportional to the square of the dose (D^2).



SUMMARY OF PERTINENT CONCLUSIONS

- Ionizing radiation induces base damage, SSBs, DSBs, and DNA protein crosslinks.
- The cell has evolved an intricate series of sensors and pathways to respond to each type of radiation-induced damage.
- DNA DSBs, the most lethal form of ionizing radiation-induced damage, is repaired by nonhomologous recombination in the G₁ phase of the cell cycle and homologous recombination (mainly) in the S/G₂ phase of the cell cycle.
- Defective nonhomologous recombination leads to chromosome aberrations, immune deficiency, and ionizing radiation sensitivity.
- Defective homologous recombination leads to chromatid and chromosome aberrations, decreased proliferation, and ionizing radiation sensitivity.
- There is good reason to believe that DSBs rather than SSBs lead to important biologic end points including cell death, carcinogenesis, and mutation.
- Radiation-induced breakage and incorrect rejoining in prereplication (G₁) chromosomes may lead to chromosome aberrations.
- Radiation-induced breakage and incorrect rejoining in postreplication (late S or G₂) chromosomes may lead to chromatid aberrations.
- Lethal aberrations include dicentrics, rings, and anaphase bridges. Symmetric translocations and small deletions are nonlethal.
- There is a good correlation between cells killed and cells with asymmetric exchange aberrations (i.e., dicentrics or rings).
- The incidence of most radiation-induced aberrations is a linear-quadratic function of dose.
- Scoring aberrations in lymphocytes from peripheral blood may be used to estimate total body doses in humans accidentally irradiated. The lowest single dose that can be detected readily is 0.25 Gy.
- Dicentrics are “unstable” aberrations; they are lethal to the cell and are not passed on to progeny. Consequently, the incidence of dicentrics declines slowly with time after exposure.

- Translocations are “stable” aberrations; they persist for many years because they are not lethal to the cell and are passed on to the progeny.

BIBLIOGRAPHY

- Alper T, Fowler JF, Morgan RL, et al. The characterization of the “type C” survival curve. *Br J Radiol.* 1962;35:722–723.
- Andrews JR, Berry RJ. Fast neutron irradiation and the relationship of radiation dose and mammalian cell reproductive capacity. *Radiat Res.* 1962;16:76–81.
- Bakkenist CJ, Kastan MB. Initiating cellular stress responses. *Cell.* 2004;118:9–17.
- Barendsen GW, Beusker TLJ, Vergroesen AJ, et al. Effects of different ionizing radiations on human cells in tissue culture: II. Biological experiments. *Radiat Res.* 1960;13:841–849.
- Bender M. Induced aberrations in human chromosomes. *Am J Pathol.* 1963;43:26a.
- Blackburn EH. Telomeres. *Annu Rev Biochem.* 1992;61:113–129.
- Bonner WM, Redon CE, Dickey JS, et al. Gamma H2AX and cancer. *Nat Rev Cancer.* 2008;8:957–67.
- Bunting SF, Callen E, Wong N, et al. 53BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. *Cell.* 2010;141:243–254.
- Carrano AV. Chromosome aberrations and radiation-induced cell death: II. Predicted and observed cell survival. *Mutat Res.* 1973;17:355–366.
- Cornforth MN, Bedford JS. A quantitative comparison of potentially lethal damage repair and the rejoining of interphase chromosome breaks in low passage normal human fibroblasts. *Radiat Res.* 1987;111:385–405.
- Cornforth MN, Bedford JS. X-ray-induced breakage and rejoining of human interphase chromosomes. *Science.* 1983;222:1141–1143.
- Cremer C, Munkel C, Granzow M, et al. Nuclear architecture and the induction of chromosomal aberrations. *Mutat Res.* 1996;366(2):97–116.
- Cromie GA, Connelly JC, Leach DRE. Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. *Mol Cell.* 2001;8:1163–1174.
- Elkind MM, Sutton H. Radiation response of mammalian cells grown in culture: I. Repair of x-ray damage in surviving Chinese hamster cells. *Radiat Res.* 1960;13:556–593.
- Evans HJ. Chromosome aberrations induced by ionizing radiation. *Int Rev Cytol.* 1962;13:221–321.
- Frankenberg D, Frankenberg-Schwager M, Harbich R. Split-dose recovery is due to the repair of DNA double-strand breaks. *Int J Radiat Biol.* 1984;46:541–553.
- Gasser SM, Laemmli UK. A glimpse at chromosomal order. *Trends Genet.* 1987;3:16–22.
- Geard CR. Effects of radiation on chromosomes. In: Pizzarello D, ed. *Radiation Biology.* Boca Raton, FL: CRC Press; 1982:83–110.
- Georgiev GP, Nedospasov SA, Bakayev VV. Supranucleosomal levels of chromatin organization. In: Busch H, ed. *The Cell Nucleus.*, Vol 6. New York, NY: Academic Press; 1978:3–34.
- Gilson E, Laroche T, Gasser SM. Telomeres and the functional architecture of the nucleus. *Trends Cell Biol.* 1993;3:128–134.
- Grell RF. The chromosome. *J Tenn Acad Sci.* 1962;37:43–53.
- Hammond EM, Pires I, Giaccia AJ. DNA damage and repair. In: Libel S, Phillips TL, Hoppe RT, Roach M, eds. *Textbook of Radiation Oncology.* Philadelphia, PA: Elsevier Publisher; 2010: Chapter 2.

- Ishihara T, Sasaki MS, eds. *Radiation-Induced Chromosome Damage in Man*. New York, NY: Alan R Liss; 1983.
- Jackson SP. Sensing and repairing DNA double-strand breaks. *Carcinogenesis*. 2002;23:687–696.
- Jeggo PA, Hafezparast M, Thompson AF, et al. Localization of a DNA repair gene (XRCC5) involved in double-strand-break rejoining to human chromosome 2. *Proc Natl Acad Sci USA*. 1992;89:6423–6427.
- Lea DEA. *Actions of Radiations on Living Cells*. 2nd ed. Cambridge, UK: Cambridge University Press; 1956.
- Littlefield LG, Kleinerman RA, Sayer AM, et al. Chromosome aberrations in lymphocytes-biomarkers of radiation exposure. In: Barton L, Gledhill I, Francesco M, eds. *New Horizons in Biological Dosimetry*. New York, NY: Wiley Liss; 1991:387–397.
- Littlefield LG, Lushbaugh CC. Cytogenetic dosimetry for radiation accidents: “The good, the bad, and the ugly.” In: Ricks RC, Fry SA, eds. *The Medical Basis for Radiation Accident Preparedness*. New York, NY: Elsevier; 1990:461–478. *Clinical Experience and Follow-up Since 1979*; vol 2.
- Mahaney BL, Meek K, Lees-Miller SP. Repair of ionizing radiation-induced DNA double strand breaks by non-homologous end-joining. *Biochem J*. 2009;417:639–650.
- Marsden M, Laemmli UK. Metaphase chromosome structure: evidence for a radial loop model. *Cell*. 1979;17:849–858.
- Moorhead PS, Nowell PC, Mellman WJ, et al. Chromosome preparation of leukocytes cultured from human peripheral blood. *Exp Cell Res*. 1960;20:613–616.
- Muller HJ. The remaking of chromosomes. In: *Studies in Genetics: The Selected Papers of HJ Muller*. Bloomington, IN: Indiana University Press; 1962:384–408.
- Munlandy PA, Liu J, Majumdar A, et al. DNA interstrand crosslink repair in mammalian cells: step by step. *Crit Rev Biochem Mol Biol*. 2010;45:23–49.
- Munro TR. The relative radiosensitivity of the nucleus and cytoplasm of the Chinese hamster fibroblasts. *Radiat Res*. 1970;42:451–470.
- Petrini JHJ, Bressan DA, Yao MS. The rad52 epistasis group in mammalian double strand break repair. *Semin Immunol*. 1997;9:181–188.
- Puck TT, Markus PI. Action of x-rays on mammalian cells. *J Exp Med*. 1956;103:653–666.
- Revell SH. Relationship between chromosome damage and cell death. In: Ishihara T, Sasaki MS, eds. *Radiation-Induced Chromosome Damage in Man*. New York, NY: Alan R Liss; 1983:215–233.
- Ris H. Chromosome structure. In: McElroy WD, Glass B, eds. *Chemical Basis of Heredity*. Baltimore, MD: Johns Hopkins University Press; 1957:215–233.
- Sancar A, Lindsey-Boltz LA, Ünsal-Kaçmaz K, et al. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem*. 2004;73:39–85.
- Schultz LB, Chehab NH, Malikzay A, et al. p53 binding protein 1 (53BP1) is an early participant in the cellular response to DNA double-strand breaks. *J Cell Biol*. 2000;151:1381–1390.
- Spear FG. On some biological effects of radiation. *Br J Radiol*. 1958;31:114–124.
- Thacker J, Zdzienicka MZ. The mammalian XRCC genes: their roles in DNA repair and genetic stability. *DNA Repair*. 2003;2:655–672.
- Thompson LH, Brookman KW, Jones NJ, et al. Molecular cloning of the human XRCCI gene, which corrects defective DNA strand break repair and sister chromatid exchange. *Mol Cell Biol*. 1990;20:6160–6271.
- Ward JF. DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation and repairability. *Prog Nucleic Acid Res Mol Biol*. 1988;35:95–125.
- Ward JF. Some biochemical consequences of the spatial distribution of ionizing radiation produced free radicals. *Radiat Res*. 1981;86:185–195.
- Willers H, Taghian AG, Luo CM, et al. Utility of DNA repair protein foci for the detection of putative BRCA1 pathway defects in breast cancer biopsies. *Mol Cancer Res*. 2009;7:1304–1309.