

**Part One**  
**Chemistry and Biology of DNA Lesions**



# 1

## Introduction and Perspectives on the Chemistry and Biology of DNA Damage

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### 1.1 Overview of the Field

The subject of this book, the chemical biology of DNA damage, is concerned with the chemistry that produces DNA damage, and the relationships between the structural features of the DNA lesions formed and their biological impact. The subjects and examples described illustrate the interdisciplinary approaches that can be used to bridge the gaps between the chemical aspects and biological endpoints of DNA damage, especially lesions generated by different endogenous and exogenous DNA-damaging agents. In Part One (Chapters 2–8), the focus is on the chemistry and biological impact of some representative and important DNA lesions. The topics of Part Two (Chapters 9–17) deal with recent and current research on the relationships between the chemical structure and physical properties of selected DNA lesions, and how the lesions are processed by the DNA repair, replication, and transcription machineries.

The chemistry of DNA damage is complex and the variety of DNA lesions is enormous. This book considers an important subset of DNA lesions that illustrate the relationships between the chemistry, structure, biochemistry, and biology of DNA damage. In this chapter, we provide a broad but brief overview of this vast field. Some of the established links between DNA damage and human diseases are highlighted. The objectives of this chapter are to situate the topics covered in this book within the overall field and to guide the interested reader to the original literature concerned with topics that either are or are not explicitly covered in the rest of the book.

We begin with an overview of the origins of DNA damage, followed by summaries of the relationships between DNA lesions and disease, and a brief overview of cellular DNA damage response (DDR) systems, and conclude with a brief description of the specific topics covered in this book and how they relate to the field overall.

## 1.2

### DNA Damage—A Constant Threat

The human genome is under constant attack from endogenous and exogenous reactive chemical species. A variety of genotoxic agents can induce chemical transformation of the nucleotides or damage the phosphodiester backbone of DNA with deleterious consequences for the cell. The relationships between cellular DNA damage caused by endogenous and environmental genotoxic agents, the cellular response, and the development and prevention of human diseases and aging are areas of great current interest in the medical, biological, and chemical research communities [1].

It has been estimated that there are tens of thousands of DNA-damaging events per day suffered by the approximately  $10^{13}$  cells within the human body [2] and that DNA damage associated with endogenous species is more extensive (greater than 75%) than damage caused by environmental factors [3]. Among the endogenous species that damage cellular DNA are reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive intermediates are produced under conditions of oxidative stress, a consequence of normal metabolic activity, and the inflammatory response [3, 4]. Other forms of endogenous DNA damage are depurination (and to a lesser extent depyrimidination) that arise from the hydrolysis of the glycosidic bonds between the nucleobase and deoxyribose residues, thus leading to the formation of apurinic (or apyrimidinic) sites [5]. The hydrolytic deamination of cytosine can also occur spontaneously and give rise to uracil [6]. Both forms of DNA damage, if not repaired by the normally efficient cellular base excision repair (BER) mechanism, can result in the mutagenic insertion of an incorrect base during error-prone translesion synthesis when the DNA is replicated past the lesion.

Among the external causes of DNA damage are ionizing radiation and solar UV radiation. Sunlight has been called the most prominent and ubiquitous physical carcinogen in our natural environment [7]. There are ample epidemiological data and a wealth of supporting animal model experiments that indicate that solar UV radiation is a major cause of skin cancer among the white Caucasian populations in the Western world [8]. The UV portion of the solar spectrum in the 290- to 300-nm region is absorbed by DNA and forms cyclobutane pyrimidine dimers (CPDs) that have been linked to the etiology of skin cancer [9]. Ionizing radiation is routinely used in medical diagnostic and chemotherapeutic applications. There are different forms of radiation that generate a variety of DNA lesions that include double- and single-strand breaks, as well as oxidatively modified nucleobases and deoxyribose moieties. The human population is also continuously exposed to environmental pollutants that are present in air, water, and food [10]. Many of these chemicals are metabolized in human cells to highly reactive intermediates that react chemically with the nucleobases to form deleterious DNA strand breaks and a variety of DNA lesions or adducts that are readily detectable in human cells [11–13]. Fortunately, nature has devised a host of cellular defense or DNA repair mechanisms that have been described [14] and reviewed in a comprehensive

monograph [15]. Some of the mechanisms that involve the removal of DNA lesions are discussed in Chapters 11 and 12. The effects of DNA lesions that escape repair can be bypassed during DNA replication by a damage tolerance mechanism that depends on the actions of a set of specialized polymerases [16, 17] or through homologous recombination mechanisms that leave the lesion intact on the damaged strand [18].

### 1.3 DNA Damage and Disease

#### 1.3.1 The Inflammatory Response

Chronic inflammation in mammalian tissues can be caused by a variety of chemical, physical, and infectious factors that are not only cytotoxic, but can also increase the risk of malignant cell transformation and promote the development of various human cancers [19]. The inflammatory response includes the activation of macrophage and neutrophil cells that result in a complex spectrum of chemically reactive species that damage DNA and other biomolecules [4]. Activated macrophages overproduce nitric oxide (NO) and superoxide ( $O_2^-$ ) that combine rapidly to form peroxynitrite (ONOO<sup>-</sup>). The latter decomposes to reactive intermediates that can cause damage to DNA and other biomolecules (see Chapters 2–4). The activated neutrophils, on the other hand, contribute to the myeloperoxidase-mediated generation of hypochlorous acid (HOCl)—a potent oxidizing and halogenating agent [4]. While many of the DNA lesions formed are oxidized forms of DNA bases themselves [20, 21], more bulky DNA lesions can also arise from the endogenous peroxidation of lipids that generate highly reactive aldehyde derivatives that react with DNA [22] (see also Chapters 5 and 9). The generation of guanine radical intermediates also leads to the formation of cross-linking reactions with thymine [20, 23] as discussed in Chapters 3 and 4.

#### 1.3.2 Reactive Oxygen and Nitrogen Species

DNA lesions caused by reactions with ROS and RNS that are byproducts of the inflammatory response have also been implicated in the etiology of neurological diseases such as Alzheimer's [24] and Parkinson's [25]. Furthermore, oxidatively generated DNA damage has been implicated in aging, based on the hypothesis that DNA damage accumulation contributes to this natural phenomenon [26–28]. The elevated concentrations of ROS and RNS alter the intracellular signaling pathways via inflammatory cytokines; this can result in an imbalance between oxidative damage of cellular DNA and DNA repair processes, causing the accumulation of DNA lesions in the genome. If not removed by cellular DNA repair mechanisms, the cytotoxic lesions may result in abnormal cell physiology,

apoptosis, and cell death if DNA replication or transcription is inhibited, or may cause mutations and cancer if error-prone translesion bypass occurs.

### 1.3.3

#### **Early Recognition of Environmentally Related Cancers: Polycyclic Aromatic Hydrocarbons**

The connections between environmental chemicals and cancer have a long history [29], dating to the eighteenth century when the first correlation was made between exposure to soot and the high incidence of scrotal cancer among chimney sweeps in London. During the twentieth century, a combination of epidemiological and animal experiments has provided persuasive evidence that polycyclic aromatic hydrocarbons (PAHs), such as the well-known and representative compound benzo[*a*]pyrene (B[*a*]P), are key chemical carcinogens in soot and coal tar. While PAH compounds are chemically unreactive and are, at best, sparingly soluble in aqueous solutions, a seminal early observation documented that these and other bulky aromatic compounds are metabolically activated by microsomal P450 enzymes to oxygenated derivatives with higher water solubilities, thus facilitating their excretion [30]. Among these metabolites, however, are highly reactive electrophiles that can react chemically with nucleic acids to form covalent DNA adducts (Chapter 6). The link between DNA damage and cancer risk is difficult to establish in humans. However, decades of epidemiological evidence and studies of animal chemical carcinogenesis models point to DNA adducts as being of central importance in causing permanent genetic changes [10, 29, 31] that play an important role in the etiology of cancer. The overall hypothesis is that normal growth control is adversely affected when the mutations occur in critical codons of tumor suppressor genes or oncogenes [32–34]. Establishing causal relationships between human exposure to a suspected environmental carcinogen, the formation of DNA adducts, and cancer risk involves a complex series of steps. These steps include (i) the analysis of the tumorigenic activity of a suspected human carcinogen in animal model systems; (ii) the identification and development of specific chemical biomarkers (DNA and protein (e.g., albumin) adducts), urinary carcinogen metabolites, and nucleic acid adducts, (iii) establishing correlations between exposure, biomarkers, and the development of disease in animal models [10], and (iv) applying similar criteria, if feasible, to humans and connecting epidemiological evidence with biomarkers of disease.

### 1.3.4

#### **Exposure to Environmental Cancer-Causing Substances**

Many environmental chemical substances have been implicated in the etiology and promotion of human cancers and have been classified as such by the World Health Organization's International Agency for Research on Cancer (IARC). The IARC classifications are widely utilized to assess the degree of risk associated with human exposure to well characterized chemicals or mixtures (<http://>

monographs.iarc.fr/ENG/Classification/). For example, the well-known and widely studied PAH compound B[a]P has been classified by the IARC as a substance that is carcinogenic to humans. The PAH compounds are products of fossil fuel combustion and are therefore ubiquitous in our environment [35]. Other aromatic carcinogens associated with the human diet are the aromatic amines and heterocyclic amines (Chapters 7 and 10) that are produced by broiling meats at high temperatures. Such products are known to contribute to the etiology of gastrointestinal cancers as discussed in detail in Chapter 7 [10]. The well-established association between cigarette smoking and the high incidence of lung and other cancers is based on worldwide epidemiological data, and is supported by animal chemical carcinogenesis model studies [36]. There are over 50 carcinogens in cigarette smoke, including PAH compounds, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, 1,3-butadiene [36, 37], and aromatic amines and heterocyclic aromatic amines (Chapter 7). All of these compounds are metabolically activated to reactive intermediates that form premutagenic covalent adducts with DNA.

### 1.3.5

#### **Aflatoxins**

The aflatoxins are mycotoxins that are among the most toxic and cancer-causing substances in animals and humans that occur naturally [10, 38]. The members of the aflatoxin family are produced by fungal *Aspergillus* species that grow as contaminants in stored grains and other crops, particularly during storage in humid environments, and are thus dietary carcinogens. Extensive epidemiological studies have shown that chronic exposure to aflatoxins is associated with a high incidence of human hepatocellular carcinoma [38]. While there are more than a dozen types of naturally occurring aflatoxins, the B<sub>1</sub> type is the most toxic. Aflatoxin B<sub>1</sub> is metabolized in the liver to the highly reactive exo-8,9-epoxide that binds covalently to DNA [39]. There is a strong correlation between the DNA adducts formed, the G → T transversion mutation signature associated with this form of DNA damage, and the biological endpoint—carcinogenesis [38].

### 1.3.6

#### **Aristolochic Acid**

Another important example of a naturally occurring dietary carcinogen, aristolochic acid, has emerged recently [40]. A high incidence of human renal disease and urothelial carcinoma, termed Balkan endemic nephropathy (BEN), was noted in the Balkan areas of Europe. The occurrence of BEN was traced to the contamination of grains of wheat with seeds from the plant *Aristolochia clematidis*, native to these regions, that contain aristolochic acid. [40, 41]. It has also been found that users of certain herbal medicines that contain aristolochic acid can also develop nephropathy that is similar to BEN [42, 43]. It was shown recently that BEN is correlated with the binding of metabolically activated forms of aristolochic acid

with DNA, that such adducts are present in renal tissues from patients suffering from BEN, and that these DNA lesions cause mutations in the *p53* gene [40]. Thus, there is considerable evidence that aristolochic acid is implicated in the etiology and perhaps progression of human cancers associated with BEN [40].

### 1.3.7

#### **Estrogens**

Endogenous human estrogens have been classified as human carcinogens by the IARC. The mechanism of action involves hormonal activity that is related to the binding of estrogens to the estrogen-responsive element and the stimulation of cell proliferation by such receptor-mediated processes. However, a genotoxic mechanism that involves the metabolic activation of human estrogens to *o*-quinone intermediates that bind to cellular DNA and promote mutations, if not repaired, has also been proposed [44–46] (Chapter 8). Equine estrogen *o*-quinone metabolites such as equilin and equilenin, which are commonly used in hormone replacement therapy applications, also form covalent adducts with DNA [47]. Furthermore, a mechanism involving ROS derived from the redox cycling between the catechol and *o*-quinone derivatives of endogenous human and equine estrogens also leads to oxidatively damaged DNA [47–49], and can contribute to the etiology and progression of cancers. Interestingly, certain PAH compounds such as B[a]P can be metabolically activated to similar *o*-quinone derivatives that can redox cycle by a similar mechanism and oxidatively damage nuclear DNA by an analogous ROS mechanism [50] (Chapter 6). The nature of the two different mechanisms associated with the etiology of human cancers—hormonal versus genotoxic—is a topic of substantial current interest [48] (Chapter 8).

## 1.4

### **DNA Damage and Chemotherapeutic Applications**

Up till now we have focused on DNA damage caused by genotoxic exogenous and endogenous agents with the implication that such damage must be avoided for maintaining the integrity of the genome. In contrast, in cancer therapy applications, the opposite result is desired—to damage DNA in order to diminish the survival of tumor cells. Both ionizing radiation and chemotherapy are commonly utilized. In the case of ionizing radiation, double-strand breaks are the major but not unique forms of DNA damage, but other important intracellular and intercellular signaling pathways are induced that also play a critical role in destroying tumor tissue [51]. Platinum-based compounds are among the most extensively used agents in cancer chemotherapy applications [52], and cisplatin (*cis*-diamminedichloroplatinum[II]) is the original and the best-known representative of this group. In cells, cisplatin reacts with a variety of biomolecules, but its reaction with DNA plays a dominant role in killing tumor cells by generating double-strand breaks [53] and a variety of cross-linked adducts [54] that inhibit DNA

synthesis. The active forms of cisplatin are the aquated forms  $[\text{Pt}(\text{NH}_3)_2\text{Cl}(\text{OH}_2)]^+$  and  $[\text{Pt}(\text{NH}_3)_2(\text{OH}_2)_2]^{2+}$  that react with purines in DNA at their N7 positions to form several intrastrand cross-linked products in a sequence-dependent manner. The relative abundance of intrastrand cross-linked adducts is  $d(\text{G}^*\text{pG}^*) \gg d(\text{A}^*\text{pG}^*) \gg d(\text{G}^*\text{pNpG}^*)$  [55],  $d(\text{G}^*\text{pC}^*)$  [56], and smaller fractions of interstrand cross-linked lesions [56, 57] are deemed important because they are not easily removed by DNA repair mechanisms [53]. While normal cells are also sensitive to DNA-damaging agents such as cisplatin, tumor cells proliferate more rapidly than normal cells and are thus more sensitive to attack by chemotherapeutic compounds. One of the important issues in chemotherapy is that cancer cells eventually become resistant to further treatment, which may be related to a decreased susceptibility to DNA repair mechanisms [1].

## 1.5 The Cellular DNA Damage Response (DDR)

Cellular DNA damage elicits a variety of complex, tightly regulated transient pathways of DDR that arrests cell cycle progression. This delay allows the cells to cope with the deleterious effects of DNA strand breaks or DNA base or sugar damage and preserves the integrity of the genome before cell cycle progression is resumed. During the cell cycle arrest, a DNA damage signal transduction pathway not only slows cell cycle progression by inhibiting DNA replication, but also promotes relevant DNA repair mechanisms that correct or remove the damage. If DNA repair is successful, the DDR response is deactivated and cell progression is resumed. On the other hand, prolonged, chronic DNA damage, or defects in the DDR system, may overwhelm the cellular defense systems, thus resulting in apoptosis (programmed cell death), senescence, or to error-prone DNA replication that can lead to enhanced mutation rates and ultimately to genomic instability and cancer [1, 58, 59].

In eukaryotic cells, the DNA is complexed with histone proteins and packaged into chromatin where it is less accessible to DNA repair, transcription, and replication proteins than naked DNA. One important question in the DDR pathway is how the DNA damage that occurs within chromatin is recognized and how the DDR response is initiated. In response to signaling mechanisms and histone-modifying enzymes, the chromatin structure is altered (remodeled), and access to DNA repair and other proteins becomes possible. These remodeling processes involve the modification of critical histones by reversible acetylation, methylation, or phosphorylation events at well-defined sites in the proteins [60]. The signal transduction pathways can be viewed in terms of distinct steps that involve protein sensors that sense the presence of DNA damage and are recruited to these sites. These events activate the transducing DDR protein kinases ATR (ATM and Rad3-related) and ATM (ataxia telangiectasia mutated) that, in turn, activate the checkpoint effector kinases Chk1 and Chk2, respectively. The latter occurs via the phosphorylation of SQ/TQ clusters in Chk1 and Chk2 at sites that critically affect

protein–protein interactions [59, 60]. The Chk1 activation pathway via ATR is known to occur mainly in response to stalled replication forks arising from UV photodamage that involves the recognition of single-stranded DNA regions complexed with the single-stranded DNA-binding protein RPA (replication protein A) [61]. On the other hand, the activation of Chk2 by ATM involves the response to the processing of DNA double-strand breaks [62]. The question arises how the signal transduction mechanism can recognize the plethora of different DNA lesions. It has been suggested that the triggering of the effector kinase checkpoints involves common single-stranded DNA regions that are formed during the initial processing of a DNA lesion, which are independent of the physical or chemical nature of the lesion [58].

## 1.6 Repair Mechanisms that Remove DNA Lesions

In contrast to the cell signal transduction mechanism, there are various types of DNA repair mechanisms [14, 15, 63] that are specialized in removing different kinds of DNA lesions.

### 1.6.1 Repair of Single- and Double-Strand Breaks

Single-strand breaks are converted to double-strand breaks upon DNA replication. The double-strand breaks are repaired by one of two complex mechanisms: homologous recombination [64] and nonhomologous end-joining mechanisms [65]. In the nonhomologous end-joining mechanism, Ku proteins bind to the two ends of the DNA and recruit a number of end-processing proteins that effect the repair; errors can occur during this type of repair. On the other hand, in the homologous recombination system, the participation of a sister chromatid sequence favors more accurate repair.

### 1.6.2 Alkylating Agents

Small alkylating agents that readily form DNA lesions include the directly acting nitrogen mustard and mustard gas, and those that require enzymatic activation, such as alkylnitrosamines, methylnitrosourea, dimethylsulfate, vinyl chloride, butadiene, chloroacetaldehyde, formaldehyde, and so on. Among the well-known repair enzymes are *O*<sup>6</sup>-alkylguanine methyltransferases (AGTs) that irreversibly transfer alkyl groups from *O*-alkylated nucleobases by a direct and error-free mechanism to an internal cysteine residue, thus deactivating the enzyme in the process (“suicide” enzyme) [66]. Another family of proteins, comprising AlkB from *Escherichia coli* and the human homologs ABH2 and ABH3, utilize a unique oxidative mechanism to remove alkyl groups from 1-methyladenines, 3-methylcytosines,

1-methylguanine, and 3-methylthymine. This mechanism is unprecedented because it involves an iron-oxo intermediate to oxidize the methyl substituents that leads to the regeneration of the normal nucleobases [67, 68].

### 1.6.3

#### **Base Excision Repair**

This mechanism repairs lesions such as uracil and relatively small oxidatively generated DNA lesions such as 8-oxoguanine [14]. It is an example of an excision repair mechanism that removes the damaged nucleobase and replaces it by the normal base (when the replacement proceeds in an error-free manner). BER (Chapter 11) is initiated by DNA glycosylases that catalyze the hydrolysis of the *N*-glycosidic bond, thus excising the damaged base from double-stranded DNA and leaving behind an apurinic (AP) site. A 5'-AP endonuclease cleaves the strand with the AP site leaving a 3'-OH terminal strand and a 5'-terminal deoxyribose phosphate group. The latter is excised by DNA deoxyribophosphodiesterase, the single nucleotide gap is filled in by the insertion of a nucleotide catalyzed by a polymerase, and the repair is completed by sealing the remaining nick with a DNA ligase.

### 1.6.4

#### **Mismatch Excision Repair**

Mismatched base pairs can arise from errors that occur during recombination repair. A variety of prokaryotic and eukaryotic mismatch repair proteins repair mismatched base pairs by a variety of mechanisms that involve the excision of one of the bases [69, 70].

### 1.6.5

#### **Nucleotide Excision Repair**

This is a complex multiprotein system in prokaryotes and eukaryotes that specializes in the repair of bulky lesions (Chapters 11 and 12). Both nucleotide excision repair (NER) systems recognize distortions and deviations from the normal DNA structure instead of the DNA lesions [63, 71]. Instead of excising the damaged lesion itself, entire lesion-containing DNA sequences, around 14 and 24–32 nucleotides in length in prokaryotes and eukaryotes, respectively, are excised. The resulting gap is filled in with nucleotides by a polymerase using the residual undamaged strand as a template and the nick is sealed by a ligase [63, 71]. There are two mechanisms of NER: (i) transcription-coupled (TC)-NER and (ii) global genomic (GG)-NER. Both mechanisms are similar except that TC-NER is triggered by RNA polymerases stalled at the sites of the DNA lesions, while GG-NER involves recognition of the DNA lesions by the eukaryotic NER factor XPC/HR23B (or currently named XPC-RAD23B, Chapter 11). The impact of DNA lesions on the elongation of transcripts catalyzed by RNA polymerases, as well as the differences between TC-NER and GG-NER, are discussed in detail in Chapter 17.

## 1.6.6

**Translesion Bypass of Unrepaired Lesions by Specialized DNA Polymerases and RNA Polymerases**

A specialized set of DNA polymerases cooperate to successfully replicate the strand containing the damaged nucleotide (Chapters 13–17). However, this mechanism of DNA damage tolerance is error-prone, and the fidelity of translesion bypass in human cells depends on the DNA lesion and the polymerase [17]. The progress of RNA polymerases depends generally on the size and shape of the DNA adduct, the local DNA sequence (Chapter 17), and the structure of the active site of the RNA polymerase [72, 73].

## 1.7

**Relationships between the Chemical, Structural, and Biological Features of DNA Lesions**

Advances in this area became feasible when technology became available for constructing oligodeoxyribonucleotides of defined base composition and sequence containing single, chemically defined DNA lesions [74]. The chapters in Part Two (Chapters 9–17) are devoted to different aspects of the structural and/or biological characteristics of such well-defined DNA lesions. The relationships between the structures of bulky aromatic DNA lesions and prokaryotic and eukaryotic NER susceptibilities are addressed in Chapters 10 and 12, respectively, while the general mechanisms of NER are reviewed in Chapter 11. The mutagenic characteristics of such site-specific DNA lesions have been studied extensively *in vitro* or in cellular environments [75–79]; specific examples are discussed in detail in Chapters 13–17.

Structural information on site-specific DNA lesions can be gained by high-resolution nuclear magnetic resonance (NMR) methods, X-ray crystallography, and molecular dynamic simulation methods. The latter, computational approach has the unique capability of providing insights into the dynamics of structural characteristics of DNA lesions by studying the evolution in time of dynamic ensembles (Chapter 14). The applications of NMR methods have, over the past two decades, yielded rich insights into the structural properties of bulky PAH-derived DNA adducts [80] (Chapter 12), adducts derived from aromatic amines (Chapter 10) and many other DNA lesions [81]. However, there are challenges in studying the structures of DNA lesions in solution by NMR methods, mainly because the lesions in solution can be highly mobile and heterogeneous in structure; this can make data interpretation difficult and defining structures even impossible in some cases. X-ray crystallography has provided some representative crystal structures of lesions in duplex DNA. Examples are intrastrand [82] and interstrand [83] cross-linked cisplatin adducts, a B[a]P 7,8-diol 9,10-epoxide-*N*<sup>2</sup>-deoxyguanosine adduct [84], and CPD [85] in double-stranded DNA, and 6-4 pyrimidine-pyrimidone [86] and CPD [87] photodimers in DNA in complexes

with photolyases—enzymes that restore the photodimers to their undamaged condition. Considerable progress has been made recently in determining the crystallographic structures of lesions in DNA in complexes with polymerases and repair proteins [88]. These include a variety of oxidatively generated and other types of lesions in double-stranded DNA bound to BER proteins [89, 90]. Examples, include *O*<sup>6</sup>-alkylguanine lesions in complexes with AGT [66] and in alkyltransferase-like proteins [66]. There are fewer examples of crystallographic structures of bulky lesions in complexes with NER-related proteins. The existing structures include a fluorescein-derived DNA lesion in a complex with the prokaryotic NER protein UvrB [91], CPD lesions in double-stranded DNA in a complex with Rad4, a yeast ortholog of the human DNA lesion-recognizing XPC/HR23B protein complex [92], and a 6-4 photodimer in double-stranded DNA in a complex with DDB1–DDB2 [93]—proteins that facilitate the identification and repair of UV photolesions in chromatin.

Considerable progress has been made in characterizing the structures of various DNA lesions in complexes with polymerases, including small [94, 95] and bulky adducts at or near the single/double-strand junctions positioned close to the active sites of polymerases [96–98]. Various aspects of these topics are addressed in Chapters 13–16. A number of reviews of this highly active field have been published [88, 95, 99–102]. While crystallography can provide outstanding resolutions of the structural properties of DNA lesions in proteins, challenges remain in growing crystals of sufficient high quality to yield high-resolution structures (resolutions below around 2 Å). This can be a serious obstacle in the case of DNA-containing lesions, where conformational flexibility and heterogeneity diminish the resolution. Of course, the same problem is encountered in solution NMR studies. Standard biochemical methods that provide detailed information on the error-prone or error-free rates of nucleotide incorporation opposite the lesion and neighboring DNA template sites provide valuable insights into the kinetic and potentially mutagenic bypass of DNA lesions catalyzed by polymerases [94, 95, 103]. These techniques, when complemented with NMR or crystallographic structural information, can provide valuable insights into the relationships between the structural features of DNA lesions and their impact on DNA replication.

Molecular dynamic simulation methods, in addition to being essential for interpreting NMR data at the atomic level, also augment experimental studies in a number of other ways [101]: modeling techniques can (i) yield structural information where experimental data has not yet been acquired, (ii) expand on experimental data through simulations that yield dynamic trajectories whose analysis provides unique information on lesion mobility, and (iii) provide thermodynamic insights by ensemble analysis using statistical mechanical methods. Furthermore, reaction mechanisms can now be determined with some confidence by combined quantum mechanical and molecular mechanical methods [104, 105].

Part One of the book, entitled *Chemistry and Biology of DNA Lesions*, will introduce the reader to the fundamentals of the chemical characterization, biochemical

properties, and biological effects of some representative and important sets of DNA lesions, as well as their impact on human health when such information is available. The material covered provides insights into the approaches used in this field that can serve as a blueprint for analyzing other forms of DNA damage. In Chapter 2, M.S. DeMott and P.C. Dedon discuss the chemistry of RNS associated with the inflammatory response and the chemistry of deoxyribose oxidation. Chapter 3 by J. Cadet, T. Douki and J.-L. Ravanat describes the chemistry of ROS, and their reactions with the nucleobases of DNA *in vitro* and in cellular environments. The role of free radical reactions with DNA is addressed in Chapter 4 by V. Shafirovich and N.E. Geacintov. In Chapter 5, C.G. Knutsen and L.J. Marnett discuss the chemistry of lipid peroxidation and the types of endogenous DNA adducts formed under oxidative stress. The different metabolic pathways of PAHs and the DNA lesions formed are reviewed by T.M. Penning in Chapter 6. The chemistry of aromatic amines and heterocyclic aromatic amines present in food and tobacco smoke, as well as the variety of DNA adducts formed, is described by R.J. Turesky in Chapter 7. The last chapter in Part One, Chapter 8 by J.L. Bolton and G.R.J. Thatcher, deals with genotoxic estrogen pathways of reactions with DNA of endogenous estrogens and estrogen derivatives used in hormone replacement therapy.

Part Two, entitled *New Frontiers and Challenges: Understanding Structure–Function Relationships and Biological Activity*, is focused on topics that deal with recent and current research on the relationships between the chemical structure and physical properties of selected DNA lesions, and how they are processed by the DNA repair, replication, and transcription machineries. In Chapter 9, Stone *et al.* discuss the relationships between the structural features and functional properties of interstrand cross-linked lesions derived from the reactions of  $\alpha,\beta$ -unsaturated aldehydes with DNA. Chapters 10–12 deal with different aspects of DNA repair, and Chapters 13–17 are focused on the impact of lesions on DNA replication and transcription. In Chapter 10, B. Cho describes the relationships between the structures and biochemical functions of aromatic amine-DNA adducts. O.D. Schärer and A.J. Campbell summarize the mechanisms of BER and NER (Chapter 11), while Y. Cai *et al.* address the molecular basis of the experimentally observed variable efficiencies of recognition and removal of PAH-derived bulky DNA adducts by the eukaryotic NER system (Chapter 12). The impact of structural features of different DNA lesions on translesion synthesis catalyzed by replicative and bypass DNA polymerases are discussed by R.L. Eoff, M. Egli, and F.P. Guengerich in Chapter 13, while insights into the relationships between the structures of DNA lesions in solution and their function in polymerases are discussed in Chapter 14 by S. Broyde *et al.* In Chapter 15, S. Chandani and E.L. Loechler provide an overview of the field and describe their work on mechanisms of translesion bypass utilizing model system *E. coli* polymerases. In Chapter 16, Z. Livneh summarizes the work of his laboratory on the molecular mechanisms of translesion DNA synthesis in human cells. Last, but not least, Chapter 17 by K. Dreij *et al.* represents a comprehensive description of the effects of structurally diverse DNA lesions on transcription elongation.

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