

PREFACE

Studies on the effects of radiation on biological organisms in the 1930s and subsequent work on mutagenesis and the discovery of DNA as the genetic material in the 1940s spawned an entire field of science focused on DNA repair. Since that time, the field has discovered a myriad of sources, types, and biological consequences of chemical transformation to DNA (i.e., DNA damage). This led to elucidation of a number of distinct DNA repair pathways that preserve the integrity of the genetic information, some of which function to restore aberrant DNA to an undamaged state, while others help the cell tolerate, or function in spite of, DNA damage. Genetic defects in these repair pathways are often linked to chromosome instability and predisposition of individuals to cancer and other diseases. Importantly, our understanding of the fundamental science behind DNA repair and the mechanisms of the enzymes involved has led to a number of therapeutic strategies to treat these diseases.

In these two volumes of *Methods in Enzymology*, leading investigators in the field of DNA repair present some of the most important, cutting-edge techniques used to probe DNA repair mechanisms across multiple scales—from cells to atoms. Each chapter focuses on a specific type of DNA damage or repair pathway, including base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), double-strand break repair (DSBR), replication-coupled DNA damage response (DDR), homologous recombination (HR), and DNA synthesis by specialized polymerases. Many of the methods described here are not specific to a particular enzyme or pathway, and thus the chapters are organized by a technique rather than by a specific type of DNA repair. Some authors provide detailed protocols and considerations when carrying out a particular experiment, while others present their philosophies for integrating multiple fields of investigation. The first volume (591) focuses largely on cellular, molecular, and chemical biology methods to investigate DNA damage and repair functions in a genomic or cellular context. The second volume (592) is focused on structural, single-molecule, and kinetic methods aimed at elucidating detailed enzymatic and mechanistic information.

Volume 591 ("DNA Repair Enzymes: Cell, Molecular, and Chemical Biology") begins at the DNA replication fork. The first two chapters (Wiest and Tomkinson; Cortez) describe benefits, limitations, and improvements to

the use of iPOND (isolation of proteins on nascent DNA) to identify DDR activities at sites of DNA synthesis. This is followed by the uses of DNA fiber analysis to monitor replication fork progression and stalling in the presence of DNA damage, as presented by Quinet et al. In the fourth chapter, Mondal and Guo describe a powerful method to detect strand-specific repair of low levels of DNA damage in cells, followed by a chapter by Saha and colleagues detailing how to monitor DSBs at various stages of the cell cycle. In the sixth chapter, Marsden and coworkers outline an integrated computational and cell biology pipeline to identify and characterize functionally significant mutations in BER genes, followed by a chapter from Standley et al. describing methods to detect bacterial mutations. In the eighth chapter, Fleming and colleagues describe innovative sequencing methods to detect 8-oxoguanine, a principal product of DNA oxidation in the genome. Chapters 9 and 10 provide protocols for the use of cell-free extracts of *Xenopus* oocytes; the first by Sannino et al. describes general aspects of replication-coupled DDR, and the second by Graham et al. outlines ensemble and single-molecule approaches to study DSBs mechanisms. In Chapter 11, Matos and West provide protocols to measure enzymatic activity of structure-selective endonucleases from yeast and human extracts. In vitro and cellular methods to monitor transcription-coupled NER are outlined in Chapter 12 by Epshtein et al., followed in Chapter 13 by an in vitro system by Kwon and colleagues to reconstitute repair DNA synthesis activities at D-loops during HR. In Chapter 14, Guillian and Doherty provide a historical and practical description of measuring DNA primase activities in vitro. In Chapter 15, Barton and colleagues provide a comprehensive review of their innovative DNA electrochemistry platform that takes advantage of DNA charge transport properties to probe binding and catalysis of redox-active proteins, including iron-sulfur cluster-containing DNA repair systems. Volume 591 concludes with useful instructions by Castaño and colleagues for preparation of stable DNA interstrand cross-links that can be used in vitro and in cells to study all aspects of DNA metabolism.

Volume 592 ("DNA Repair Enzymes: Structure, Biophysics, and Mechanism") includes a series of papers on structural techniques, including X-ray crystallography, small-angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR), and electron microscopy, as well as single-molecule and enzyme kinetic approaches to probe mechanistic details of enzymes involved in all types of excision and break repair. The first two chapters (Gradia et al. and Rees et al.) provide state-of-the-art methods for efficient cloning to produce multisubunit complexes in large quantities for structural and biochemical studies. Thompson and colleagues in the third chapter describe SAXS and

NMR methodologies to study conformational states and dynamic properties of multidomain proteins, using DNA primase and replication protein A as examples. In the fourth chapter, Friedhoff and coworkers share their cross-linking approach to trap transient conformational states of proteins involved in MMR. Chapters 5–7 focus on several contemporary challenges to X-ray crystallographic studies. Malaby et al. recount how they overcame challenges in expression and purification of full-length and active deletion constructs of human DNA pol θ , a specialized DNA polymerase involved in DSBR alternative end-joining. Next, Figiel and Nowotny use RNase H2 to summarize crystallographic approaches to protein–nucleic acid complexes, followed by strategies from Chirgadze and colleagues used to push the resolution of the crystal structure of the 4128-residue DNA-dependent protein kinase catalytic subunit important for regulation of nonhomologous end-joining DSBR. The next four chapters (Sawicka et al., LeBlanc et al., Kong et al., Soniat et al.) focus on the examination of physical behaviors of DNA repair machines using single-molecule methods, including electron, atomic force, and fluorescence microscopies coupled to innovative DNA visualization techniques such as DNA tightropes (Kong et al.) and DNA curtains (Soniat et al.). In the 12th chapter, Samara and coworkers illustrate their powerful time-resolved X-ray crystallographic methods that have pushed the boundaries of our understanding of catalysis of nucleic acid synthesis and degradation. Chapters 13–15 beautifully describe different methods and uses of enzyme kinetics—Powers and Washington include a variety of approaches to monitor catalysis and binding activities of translesion DNA polymerases; Coey and Drohat detail the design, execution, and interpretation of single- and multiple-turnover kinetics experiments using DNA glycosylases as an example; Hendershot and O'Brien follow with kinetic strategies to characterize DNA-binding and nucleotide-flipping mechanisms used by DNA glycosylases and many other DNA processing enzymes. The two-volume series concludes with a discussion from Brosey and colleagues, who tie together elements of both volumes by discussing examples of studies that integrate information from structural biochemistry and cell biology to develop a comprehensive understanding of these multifaceted DNA damage responses.

This collection is the culmination of 80 years of ingenuity and discovery in nucleic acid biology and is timely addition to the field, following the 2015 Nobel Prize in Chemistry to Modrich, Lindahl, and Sancar for their mechanistic studies of DNA repair. My hope is that these chapters will help inspire new innovations at the frontier of DNA repair research, while also serving as a guide to scientists engaged in all aspects of molecular biology. It is an

exciting time as we address the next challenges focused on how mechanisms, pathways, and regulation of DNA repair intersect with those of human disease, mutagenesis, and evolution. As in other volumes of *Methods in Enzymology*, the approaches described here are applicable across disciplines and therefore have the potential to cross-pollinate and inspire new ideas in other areas of investigation.

BRANDT F. EICHMAN

Vanderbilt University