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## Preface

Our current knowledge of the mechanisms that regulate DNA repair has grown significantly over the past years with the great help of technological advances such as RNA interference, advanced proteomics, and microscopy as well as high-throughput screens. These technological advances have revealed a tight interplay between DNA repair, recombination, and DNA replication through complex protein network that allows the timely, efficient, and coordinated activation of cellular responses to genomic insult. However, we are far from understanding exactly how cellular pathways protect the genome, and current research frontiers include enzymatic details, lesion accessibility in the context of chromatin, regulatory modes such as posttranslational protein modifications of DNA repair and recombination pathways and not at least, how cells assign the respective pathways depending on the nature of damage or genotoxic replication stress.

This edition of *DNA repair protocols* should supply researchers in the field of DNA repair with a protocol handbook suitable for daily use. It provides detailed step-by-step instructions for studying the various aspects of the eukaryotic response to genomic insult including recent advanced protocols as well as standard techniques used in the field of DNA repair. Both mammalian and nonmammalian model organisms are covered in the book, and many of the techniques can be applied only with minor modifications to other systems than the one described.

*DNA repair protocols* is organized into five parts and begins with an introduction kindly written by Vilhelm Bohr, which discusses the history and more recent developments in the field of DNA repair. Then follows Part I, Identification of repair genes and generation of repair mutants, which covers three chapters; the first two describe how to screen for repair and/or checkpoint mutants using RNAi-based screen in *Drosophila* and *Caenorhabditis elegans*, respectively. Chapter 4 describes targeted gene disruption of repair genes in DT40 cells, which still remains a powerful tool to investigate the regulation and function of gene and protein in vertebrate.

Part II, Assessing DNA damage and repair (Chapters 5–9), describes assays for measuring the level of DNA damage and repair. Chapter 5 presents an electrophoretic mobility shift assay, which is used to study proteins that bind to DNA structures generated by DNA damaging agents. Chapter 6 describes the widely used comet assay, a microgel electrophoresis technique for detecting overall damage in individual cells. Combination of the Comet assay with FISH (Chapter 7) allows the detection of DNA damage or repair at higher level of resolution and provides a mean for visualizing region-specific damage and repair. The use of lower eukaryotes and prokaryotes in the comet assay has been hampered by the low cellular DNA content; however, a recent improved comet assay protocol described in Chapter 8 now permits sensitive and reproducible measurement of DNA damage and repair using *Saccharomyces cerevisiae* as model system. Chapter 9 presents a general QPCR assay for measuring any kind of polymerase-blocking DNA lesion such as single-strand breaks, abasic sites, and bulky adducts.

Part III, Repair of incorrect or damaged DNA bases brings together a panel of approaches to characterize repair proteins and repair pathways. Chapters 10 and 11 detail the preparation of mismatched substrates used with nuclear extracts from human or yeast

cells. Chapters 12–14 present three different methods for detection and/or repair of DNA photolesions such as cyclobutane pyrimidine dimers and (6-4) photoproducts, which are processed by the nucleotide excision repair pathway. One of the most harmful types of DNA damage is the roadblock forming interstrand crosslinks (ICL), where the repair mechanism is still poorly understood. Chapter 15 details the preparation of plasmid DNA substrates containing a single ICL at a specific site, and Chapter 16 describes several assays that examine specific steps in ICL repair making use of the plasmid DNA substrate together with *Xenopus laevis* egg extract. The remaining chapters in this part are allocated to base excision repair assays either using mammalian cell extract (Chapter 17), *Arabidopsis* cell extracts (Chapter 18), or yeast extracts (Chapter 19), whereas Chapter 20 outlines protocols for isolating mitochondria from mammalian cells and rodent tissues and describes in vitro assays for measuring the enzymatic steps of BER in the lysates of isolated mitochondria.

Part IV, DNA strand breakage and repair, describes methods for detecting strand breakage (single- or double-strand break), for inducing specific strand breaks in the DNA and measuring repair. Chapter 21 presents a modified alkaline gel electrophoresis assay coupled with a neutral *O*-hydroxylamine to measure true single-strand break formation. This is followed by a chapter on pulse-field gel electrophoresis, which is used to analyze DNA double-strand break formation in human chromosomal DNA (Chapter 22). Chapters 23 and 24 both describe methods to detect and quantify the formation of single-stranded DNA, which is a DNA repair, recombination, and replication intermediate. Chapter 23 presents the QAOS method based on qPCR, whereas Chapter 24 describes a rapid yeast genomic DNA extraction procedure that can be used in a simple fluorescent in-gel assay to measure single-stranded DNA. Chapters 25–28 all describe in vivo systems to study repair of strand breakage. One of the most powerful in vivo systems to study double-strand break repair is the HO-endonuclease system from budding yeast described in Chapter 25. The rare-cutting I-SceI endonuclease is also exploited to analyze DSB repair and is described for DT40 cells (Chapter 26) and mammalian cells (Chapter 27). Chapter 28 presents a method to induce single-strand breakage by the Flp recombinase and at the same time to study repair of a protein–DNA adduct. Chapter 29 describes how the immunoglobulin loci of DT40 cells can be used to study homologous recombination in a chromosomal context. Chapter 30 details the use of gene targeting designed to investigate mechanisms of homologous recombination in somatic mammalian cells. Live cell microscopy has added significantly to our understanding of how a DNA damage response is orchestrated in the cells. Chapter 31 reviews useful fluorescent markers and genotoxic agents for studying the DNA damage response in living yeast cells and provides protocols for live cell imaging, time-lapse microscopy, and for induction of site-specific DNA lesions. This chapter has specific focus on proteins involved in recombinational repair. The last two chapters in this part both describe in vitro assays for analyzing double-strand break repair. Chapter 32 describes an in vitro assay that allows the study of DSB rejoining in genomic DNA using either “naked” DNA or DNA organized in chromatin, whereas Chapter 33 presents an in vitro end-joining assay employing linear oligonucleotides.

In addition to pathways involved directly in repairing DNA lesions cells have also developed strategies of damage tolerance (postreplication repair), which allows for lesion bypass and thereby completion of DNA replication in the presence of damage. Part V gathers together a few methods for studying damage tolerance and checkpoint responses. Chapters 34 and 35 both describe assays to measure translesion DNA synthesis with plasmid-based

systems. Modifications of the eukaryotic sliding clamp PCNA play a significant role both for translesion synthesis and template switching, as well as for hindring unscheduled recombination. Chapters 36 and 37 deal with methods for detecting PCNA modifications in vivo as well as in vitro PCNA modification assays. It has for long time been known that DNA damage has a detrimental effect on the replication process possibly controlled by checkpoints. Chapter 38 describes a method to measure in vitro DNA replication using cytoplasmatic extracts from cells that have sustained DNA damage. Chapter 39 provides methods for detecting checkpoint activation due to DNA damage with focus on the central checkpoint kinase Chk1, whereas Chapter 40 describes a few standard protocols for detecting H2AX phosphorylation, which in recent years have attracted much attention as a specific marker for DNA damage.

Finally, I wish to take this opportunity to thank all authors for their commitment, cooperation, and contributions that made my first editing job a very enjoyable experience. A special thanks to Dr. John Walker for his excellent advice during the process.

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