
Preface

Detection and analysis of DNA damage is of critical importance in a variety of biological disciplines studying apoptosis, cell cycle and cell division, carcinogenesis, tumor growth, embryogenesis and aging, neurodegenerative and heart diseases, anticancer drug development, environmental and radiobiological research, and others.

Individual cells within the same tissue or in cell culture may vary in the extent of their DNA damage and, consequently, can display different reactions to it. These differences between individual cells in the same cell population are detected using *in situ* approaches.

In situ is a Latin term meaning “on site” or “in place.” It is used to denote the processes occurring or detected in their place of origin. In molecular and cell biology this usually refers to undisrupted mounted cells or tissue sections. In that meaning “*in situ*” is used as part of the terms “*in situ* PCR,” “*in situ* transcription,” “*in situ* hybridization,” “*in situ* end labeling,” and “*in situ* ligation.” Sometimes the “*in situ*” term is applied at the subcellular level to cells disrupted in the process of analysis, for example, in the detection of specific sequences in chromosomes using fluorescent *in situ* hybridization (FISH). Historically, the term was used primarily in methods dealing with nucleic acids.

In situ methods for the detection of DNA damage can label DNA alterations directly in tissues and individual cells. They differ from the biochemical methods, which label DNA damage in bulk DNA samples obtained after tissue homogenization and cell disruption. *In situ* methods are advantageous in the analysis of heterogeneous cellular populations and in assessing the dynamics and morphological distribution of cellular reactions to various factors. Their attractive features include single-cell detection level, potential to co-localize DNA damage and cellular proteins, ability to use cellular morphology to verify cellular phenomena, small sample size, and, in many cases, the simplicity of performance. The importance of *in situ* approaches is further increased by the conclusion of the human genome mapping, which has put a new emphasis on co-localization of cellular phenomena with various cellular proteins.

Although other volumes dealing with the detection of DNA damage detection have been published, they primarily described biochemical methods

to study DNA alterations. *In Situ Detection of DNA Damage* is the first publication solely dedicated to the *in situ* format. The term “*in situ*” incorporates analysis of both tissue sections and individual cells. The volume brings together all major *in situ* techniques developed to study DNA damage and apoptosis. It also expands the utility of the presented methods by showing how approaches originally designed to label apoptotic cells can be used for DNA damage analysis (and vice versa). It includes many new cutting-edge protocols that have become possible as a result of the significant progress occurring in the field during the last five years.

In Situ Detection of DNA Damage is divided into six sections. Each of the major methods to study DNA damage *in situ* is discussed in detail in a separate section consisting of two to six chapters. The first two chapters in Sections 1–4 are theoretical and technical reviews summarizing accumulated information about the techniques described. The remaining chapters in each section concentrate on useful applications.

Sections 1–4 each discuss a single technique or a group of closely related techniques. The complete set of enzymatic approaches to study DNA breaks *in situ* is presented. These techniques were originally introduced to solve a utilitarian task of labeling apoptotic cells and therefore require detailed analysis from the DNA damage detection point of view. The techniques include those designed to detect specific single- and double-stranded DNA breaks in tissue sections using terminal transferase (Chapters 1–6), DNA polymerase I or its Klenow fragment (Chapters 7–9), T4 DNA ligase and T4 polynucleotide kinase (Chapters 10–12). Chapters 13–16 present methods for detection of DNA breaks in agarose-trapped cells and describe the comet assay and related techniques. Detection of modified bases and apurinic/apyrimidinic sites in tissue sections is discussed in Chapters 17 and 18. Chapters 19, 21, and 22 deal with such markers of DNA damage and apoptosis as poly (ADP-ribose) polymerase, p53, and active caspases. In recent years, instrumental techniques for studying DNA damage in tissue sections, single cells, and *in vivo* have grown significantly in sophistication and power. The novel instrumental techniques presented here describe *in situ* applications of both flow and laser-scanning cytometry for analysis of DNA strand breaks and apoptosis (Chapter 6) and the use of ultrasound for *in vivo* and *in situ* detection of apoptotic DNA damage (Chapter 20).

In Situ Detection of DNA Damage is a comprehensive source of information on every method described. It contains technical reviews discussing specificity, sensitivity, advantages, and limitations of the described techniques in comparison with alternative approaches. Different *in situ* approaches are reviewed with emphasis on their relative merits and shortcomings.

In Situ *Detection of DNA Damage* can be equally useful for both novice scientists and experienced researchers. For a scientist new to the area of *in situ* DNA damage detection, the book will help to select and use the technique most suitable for his/her specific field of study. Detailed explanation of the concepts underlying the methods and the types of DNA damage they label will render it easy for the reader to understand the possible pitfalls in each technique described and to properly interpret experimental results. Experienced researchers actively working in the field will find the book useful because it describes the new approaches, with each method being presented and discussed in greater detail than is generally found in the research literature. Since it provides deeper insight into the types of DNA damage labeled by current apoptosis detection techniques, it is also a helpful resource for molecular scientists studying apoptotic cell death.

Researchers in the fields of cell biology, molecular biology, embryology, toxicology, radiobiology, experimental and clinical pathology, oncology, experimental pharmacology, drug design, and environmental science can benefit from the book.

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