Preface

Neuronal loss that occurs in the central nervous system as a result of injury or neurodegenerative diseases is a devastating problem because of the lack of regenerative ability of the human body to functionally replace these neurons. Although there is promise of eventual replacement of these lost neurons through stem cell technology, inhibition of neuronal cell death in many instances would also provide a chance to reestablish their function. In many neurodegenerative diseases, however, there is no clear understanding of the cell death mechanism. Considerable debate focuses on whether these neurons undergo apoptosis, necrosis, or another yet undefined mode of cell death. The enigma is complicated by cell-type and by insult- and species-specific mechanisms of neuronal cell death. It is obvious that the identification and characterization of the mechanism of neuronal cell death can be resolved only through extensive research of the several types of neurons. Only when a fundamental understanding of the mechanisms of neuronal demise is achieved will we be in a position to identify the key pathways involved in human neurodegenerative diseases.

Since publication of the first edition of the *Apoptosis Techniques and Protocols* in 1997, the study of apoptotic mechanisms has boomed, and a number of key proteins involved in neuronal apoptosis have been identified. It has become clear that Bax and the family of caspases that is regulated through mitochondrial cytochrome-*c* release or through an extrinsic receptor-mediated pathway are key pro-apoptotic regulators of neuronal cell death. The first three chapters of this book present comprehensive technical approaches to study Bax (Hsu and Smaili), cytochrome-*c* (Ethell and Green), and caspases (Bounhar, Tounekti, and LeBlanc) in neurons. The following two chapters describe two methods, viral infections (Maguire-Zeiss, Bowers, and Federoff) and microinjections (Zhang and LeBlanc), to assess the importance of apoptotic proteins in cultures of primary neurons and in brain. Though key regulators of apoptosis have been uncovered, undoubtedly there are additional factors involved in neuronal apoptosis. Therefore, we must continue to search for proteins that may be responsible for neuronal loss in neurodegenerative

diseases. DNA microarray assay is one of the current techniques used to identify differentially expressed genes in human disease (Eastman and Loring) and in transgenic mice models of neurodegeneration (Tucker and Estus) . These chapters provide helpful insight into the design of an appropriate experimental protocol and in the interpretation of data from these microarrays. It is evident that as pro-apoptotic proteins are discovered, inhibitors of their functions in neuronal apoptosis must be sought. Berry and Ashe describe the role of differentially expressed G3PDH as an early marker of apoptosis and how one can isolate drugs against such pro-apoptotic molecules.

Though extensive research on apoptosis is performed and key mechanisms provided for many cell types, these do not necessarily apply to neurons. Indeed, we must consider the very specialized architecture of the neuron. Neurons are compartmentalized and polarized cell types. It is possible that specific pathways of apoptosis may be restricted to a specialized compartment of these cells and that activation of certain apoptotic mechanisms could result in cellular dysfunction prior to complete cell death. It has recently been proposed that the apoptotic process may occur in synapses. The technical and theoretical aspects of neuronal compartmentalization and the study of synaptosis are explained in the next two chapters, by Campenot and colleagues and by Cole and Gylys.

As we define the mechanisms of apoptosis that regulate cell death of neurons in cultures, it is essential to extend the studies to the brain tissue of individuals who have suffered from neurodegenerative diseases. It is hoped that this will lead to the identification of key processes that may eventually be controlled to prevent neuronal demise. The chapters by Roth on *in situ* detection of apoptosis and Smith and colleagues on the role of oxidative stress and apoptosis in Alzheimer's disease describe technical approaches associated with *in situ* detection of apoptosis.

The field of apoptosis research has grown exponentially in the past few years, and it would be impossible to describe each aspect of apoptosis as it applies to neurons. The importance of signal transduction pathways, transgenic animal models to study apoptosis, the other Bcl-2 family members, and the role of cell cycle gene expression in neuronal apoptosis has not been addressed in this book. These are equally important aspects of neuronal apoptosis.

Apoptosis Techniques and Protocols, Second Edition is intended to provide a handbook for the laboratory as well as a description of the limitations and advantages of the techniques proposed. I hope that it will be useful to both new and seasoned investigators who have an interest in unraveling the molecular mechanisms of neuronal cell death.

Andréa C. LeBlanc