Preface

Nucleases, enzymes that restructure or degrade nucleic acid polymers, are vital to the control of every area of metabolism. They range from "housekeeping" enzymes with broad substrate ranges to extremely specific tools ***(1)***. Many types of nucleases are used in lab protocols, and their commercial and clinical uses are expanding. The purpose of *Nuclease Methods and Protocols* is to introduce the reader to some well-characterized protein nucleases, and the methods used to determine their activity, structure, interaction with other molecules, and physiological role. Each chapter begins with a mini-review on a specific nuclease or a nuclease-related theme. Although many chapters cover several topics, they were arbitrarily divided into five parts:

Part I, "Characterizing Nuclease Activity," includes protocols and assays to determine general (processive, distributive) or specific mechanisms. Methods to assay nuclease products, identify cloned nucleases, and determine their physiological role are also included here.

Part II, "Inhibitors and Activators of Nucleases," summarizes assays for measuring the effects of other proteins and small molecules. Many of these inhibitors have clinical relevance.

Part III, "Relating Nuclease Structure and Function," provides an overview of methods to determine or model the 3-D structure of nucleases and their complexes with substrates and inhibitors. A 3-D structure can greatly aid the rational design of nucleases and inhibitors for specific purposes.

Part IV, "Nucleases in the Clinic," summarizes assays and protocols suitable for use with tissues and for nuclease based therapeutics.

Part V, "Nucleases in the Lab," includes protocols for the use of nucleases in cloning and in determining the activity of other proteins.

The experienced reader will immediately recognize several of the nucleases used as examples throughout this book, especially RNase A and restriction enzymes. However, new nucleases with novel specificity, often performing unexpected functions, are constantly being discovered. For example, a regulator of the unfolded protein response, identified initially as a kinase, is also a sequence-specific ribonuclease (Chapter 3). A human analog of a plant ribonuclease was discovered in the search for a tumor suppressor protein (Chapter 7), whereas angiogenin (Chapter 25) was cloned as a factor stimulating blood vessel formation. RNase L is one of the mediators of Interferon activity (Chapter 12).

Researchers who unmask a nuclease disguised as a cytokine, mating factor, toxin, and so forth should find the methods for characterizing their protein described in the first section of this book particularly useful. These chapters suggest questions to ask about the nuclease's activity or primary structure. Is the amino acid sequence novel or similar to one of the major families of nucleases (Chapters 7 and 18)? Is the cleavage processive or distributive, i.e., does the nuclease scan the nucleic acid polymer

and cleave repeatedly before separating, as has been shown for restriction endonucleases and glycosylases (Chapter 1) or, does it, in the fashion of RNase A (Chapter 2), cleave and simultaneously release the polymer, generating products that are at the same time novel substrates? Does the back (synthesis) reaction affect the kinetics of the cleavage process? Is the nuclease activity essential for metabolic activity, as McClure and coworkers (Chapter 5) have elegantly demonstrated for the stylar RNases? Finally, is the activity sensitive to known inhibitors or activators (Chapters 8, 9, and 12)?

These questions all pave the way for characterization of the 3-D structures of nucleases and their complexes with substrates and activity modulators. The chapters at the center of this book were selected to introduce the reader to methods that can be used to define the tertiary structure of nucleases. Of course, a complete tertiary structure determination by X-ray crystallography (Chapters 13, 14, 17, 19, and 20) or NMR (Chapter 16) requires a good deal of time and specialized techniques too complicated to be summarized here. However, if the sequence has significant identity to a protein for which a structure has been determined, tools now available on the Internet allow one to model the probable 3-D structure (Chapter 18). The methods described aid in the design of nucleases with new properties (Chapters 15 and 20) and improved inhibitors (Chapters 13 and 14).

Nuclease-based therapies and diagnostics are slowly coming into the clinic. DNase I therapy (Chapters 20 and 21) has improved the lives of thousands of cystic fibrosis victims. Nucleases with demonstrated antitumor activity (Chapter 24) stimulated clinical trials of other members of the RNase A family and modified forms (Chapters 23 and 26). Better understanding of nucleases that repair damaged DNA (Chapters 1 and 18), mediate retroviral integration and replication (Chapters 10 and 22), or play a role in cytokine and growth factor mechanisms (Chapters 9, 12, and 25) is important both in understanding disease progression and developing better therapeutic modalities. Antisense therapies, for example, depend on directing the activity of intracellular RNase H (Chapter 11).

As more medical professionals become aware of the importance of nucleases in metabolism and the improved assays for their activity, there will almost certainly be an increase in their use in diagnostics. A recent report, for example ***(2)***, correlated increases in the levels of eosinophil cationic protein in asthmatics allergic to grass pollen with the risk of onset of prolonged asthmatic symptoms.

Finally, nucleases are a major research tool in molecular biology. The exquisite specificity of restriction endonucleases (Chapters 19, 27, and 28) is routinely used in gene cloning. Exploiting the special qualities of a subclass, hapaxoterminers, can make subcloning and gene modification easier (Chapter 29). There are many uses for nonspecific nucleases as well. DNase I can be used to locate the binding sites of proteins on DNA (Chapter 30) and S1-nuclease (Chapter 31) or ribonuclease (Chapter 32) mapping to quantitate specific mRNAs. Degradation of nucleic acid polymers with nonspecific nucleases, including DNase I, RNase A, and the endonuclease from *Serratia* (Chapter 17), can be used to clarify lysates and ease protein purification.

All the chapters describe why and when to use the assays, and the thinking that went into the development of the protocols. These comments, and the Notes on the method, provide guidance and insight when things go wrong (i.e., not as planned),

and for how to go about correcting them. Protocols change in their details constantly. A Northern blot to check the expression in a tissue from various organs of mRNA for a specific protein, which 10 years ago required weeks to prepare, can be done today in a few hours starting with a commercially prepared membrane. The reader is welcome to simplify these protocols further as new developments allow.

We can anticipate that the model proteins used to develop new biophysical methods and clinical therapies, which have changed little in the past 30 years, will show more variety in the future. Most scientists will claim that they use RNase A since the protein is small, soluble, and easy to assay and to refold from a completely denatured state (Chapter 15). However, ready availability and force of habit contribute to the attention paid this "ideal" protein. RNase A was first crystallized ***(3,4)***, Bill Wyckoff has suggested only partially in jest, because a meat packing company made it available free of charge in a highly purified form. The easy purification of pancreatic RNase from the cadavers of zoo animals made this family a Rosetta stone for comparative biology as well. However, many new nucleases are commercially available, and the genome projects are revealing copious sequence information about nuclease families that may be more important metabolically. The examples in *Nuclease Methods and Protocols*, as varied as they are, are only starting points for exploration in the wide world of nucleases.

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