Methods in Enzymology

Volume 195

ADENYLYL CYCLASE, G PROTEINS, AND GUANYLYL CYCLASE

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Adenylyl Cyclase, G Proteins, and Guanylyl Cyclase

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Preface

Since the first volume of *Methods in Enzymology* on cyclic nucleotides (Vol. XXXVIII) was published in 1974, substantial progress has been made in cyclic nucleotide research, particularly on the enzymes involved in their synthesis, degradation, and mode of action. Cyclic nucleotide-dependent protein kinases, cyclic nucleotide phosphodiesterases, and quantitative assays of cAMP and cGMP levels were updated in Volumes 99 and 159. This volume emphasizes methods for the assay, purification, and characterization of adenylyl cyclases, guanine nucleotide-dependent regulatory proteins (G proteins), and guanylyl cyclases. Research in each of these areas has grown rapidly in the past sixteen years, especially recently with the application of molecular biological approaches that augment biochemical techniques.

One consequence of the rapid growth is that it has become impossible to have an absolutely current book describing these advances. Although adenylyl and guanylyl cyclases have been purified and characterized from numerous sources, it is becoming clear that each is, in fact, a family of enzymes. For adenylyl cyclase this is most easily recognized by differences in function and distribution of mammalian forms that are either sensitive or insensitive to calmodulin. These are dealt with in depth in this volume. Additional members of the adenylyl cyclase family are currently being purified and/or cloned from numerous prokaryotic and eukaryotic sources. These are only briefly discussed. The soluble and particulate forms of guanylyl cyclase, while appearing diverse due to differences in their distribution and in their sensitivities to specific peptide hormones and to nitrous oxide, also form a growing family of enzymes. The "snapshot" of the field presented suggests substantial future developments. For both adenylyl and guanylyl cyclases and for the G proteins it is becoming clear that in addition to the established modes of regulation, e.g., of the cyclases through G proteins or hormones, there are likely other mechanisms through which cells may regulate the activities of these important enzymes. Prominent among these are covalent modifications, e.g., phosphorylation–dephosphorylation, as well as allosteric regulation, e.g., inhibition of adenylyl cyclases by specific cell-derived adenine nucleotides. Thus, future direction of research will certainly include additional details of the number and structure of the various members of the families of adenylyl and guanylyl cyclases and of the modes of their regulation.

The impact of rapid growth in a research area is most obvious with the G proteins. Given that important aspects of our current interest and understanding of G proteins derive from investigations on their role in the

regulation of adenylyl cyclases, it is obviously imperative that a section on G proteins be included in any volume dealing with adenylyl cyclases. In part, G proteins were discovered due to the effects of GTP to mediate hormonal activation of adenylyl cyclases initially described by Rodbell and co-workers. G proteins were later found to be involved also in mediating hormonal inhibition of this enzyme and to be targets for ADP-ribosylation by cholera and pertussis toxins. However, the explosion in G-protein research, in the number and variety of G proteins, and in the myriad of actions they mediate force a limitation on coverage. The emphasis in this volume is limited to the purification and quantification of those G proteins mediating stimulatory (G_S) and inhibitory (G_i) effects on adenylyl cyclases and to the low molecular weight proteins that enhance the actions of cholera toxin on G_S. While G proteins mediate the effects of stimulatory and inhibitory hormones on the activity of adenylyl cyclases, we thought it beyond the scope of this volume to deal with their interactions with each of the numerous hormone receptors with which they are known to interact. We have limited the treatment of these interactions simply to general aspects of hormone receptor–G protein–adenylyl cyclase reconstitution. Similarly, the actual mechanisms by which G proteins mediate activation or inhibition of adenylyl cyclases are skirted since they are presently very poorly understood. Another volume on this topic will become necessary as our understanding of these enzymes and their regulation develops.

We are grateful to the authors for their excellent contributions, and we apologize to those who have made many contributions to these fields but whose work may not be adequately recognized here. The inevitable omissions have been due to editorial oversight, to potential authors being already overcommitted, and to the rapid rate at which research in these areas has occurred and, hence, to timing.

This volume is dedicated to Dr. Martin Rodbell for his very many contributions to our understanding of the hormonal regulation of adenylyl cyclases.

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