Sidney P. Colowick and Nathan O. Kaplan

Methods in ENZYMOLOGY

Volume 159

Initiation and Termination of Cyclic Nucleotide Action

Edited by

Jackie D. Corbin

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Preface

It seemed logical to include in the same volume the methods used for studying enzymes and other substances which initiate and terminate the cascades of reactions that begin with the elevation of cyclic AMP or cyclic GMP. These substances include the cyclic nucleotides themselves, cyclic nucleotide-dependent protein kinases or other cyclic nucleotide receptors, phosphoprotein phosphatases, and phosphodiesterases. Most investigators in this field are actively engaged in studies of more than one of these steps.

The cyclic nucleotide pathway is similar to other cellular pathways in that it responds to hormones, neurotransmitters, and other agents in two opposing ways. A cascade of reactions is activated by the elevation of the intracellular cyclic nucleotide level, and this same cascade is concommitantly inhibited by the stimulation of enzymes that oppose cyclic nucleotide action. The inhibitory steps are necessary to prevent constant background stimulation and overstimulation or to terminate the agonist effect. One way that simultaneous activation and inhibition of the pathway could occur is through stimulation of a cyclic nucleotide-dependent protein kinase together with stimulation of an opposing phosphoprotein phosphatase by elevation of the phosphoprotein substrate. If inhibition of the latter enzyme should occur through substrate-independent mechanisms, it would amplify the signal generated by cyclic nucleotide elevation. In addition to phosphoprotein phosphatase, the protein kinase activation may also be opposed by simultaneous stimulation of cyclic nucleotide breakdown either by elevation of the cyclic nucleotide substrate itself, by covalent modification of the phosphodiesterases, or by binding of cyclic nucleotides to allosteric sites on the enzymes.

It should also be emphasized that there are several analogies and homologies among the enzymes involved in cyclic nucleotide metabolism, and many of the same methods are used by each investigator. The cyclic AMP- and cyclic GMP-dependent protein kinases are homologous proteins, as are several phosphodiesterases which have just recently been characterized at the protein chemical or molecular biological level. Although conjectural at present, the possibility should be considered that there are other homologies among the proteins of the cyclic nucleotide systems. The phosphodiesterases must have recognition sites for cyclic nucleotides, at both binding and catalytic sites, which could have an evolutionary relationship to the cyclic nucleotide binding sites of the protein kinases. The cyclic nucleotide binding sites of nonmammalian recep-

tors such as that of *Dictyostelium* may have a kinship to that of mammalian protein kinases or phosphodiesterases, and since protein kinases can act as phosphatases *in vitro,* it is conceivable that these enzymes could be related to the phosphoprotein phosphatases.

It was considered appropriate to begin this volume with theoretical considerations of cyclic nucleotide cascade systems. This should be useful to all investigators in this field. The remainder of the volume presents currently used methods for investigating cyclic nucleotides and the specific proteins which have been identified as being responsible for initiating and terminating cyclic nucleotide action. Even though most of these methods represent improvements over previous ones, the reader will also find informative the methods listed in the volumes of the "Hormone Action" series of *Methods in Enzymology.*

We are grateful to all the authors for their excellent contributions, and apologize to those, in a rapidly moving field, whose excellent work appeared too late to be included.

This volume is dedicated to Drs. Charles R. Park and Edwin G. Krebs for their guidance and inspiration.

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EDITED BY

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