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## Preface

Adenosine 3',5'-cyclic monophosphate (cAMP) and guanosine 3',5'-cyclic monophosphate are ubiquitous nucleotides that have been described as the first and second messengers. In concert with intracellular calcium and IP<sub>3</sub>, they play a major role in the control of intracellular signaling, which orchestrates normal and pathophysiological responses.

Downstream from the cyclic nucleotide synthesis by adenylyl and guanylyl cyclases, the multigenic family of cyclic nucleotide phosphodiesterases (PDEs), by specifically hydrolyzing cyclic nucleotides, controls cAMP and cGMP levels to maintain a basal state. Their critical role in intracellular signaling has recently designated them as new therapeutical targets. Several leading pharmaceutical companies are searching and developing new therapeutic agents that would potently and selectively inhibit PDE isozymes, notably PDE4 and PDE5. Nevertheless, the precise mechanism and the contribution of the various PDE isozymes in modulating intracellular signaling remain to be established.

The aim of *Phosphodiesterase Methods and Protocols* is to provide a palette of a variety of conceptual and technical approaches designed to solve questions concerning the role of PDEs, and ultimately of their different variants, in physiological functions as well as their implications in several pathologies.

During the four research decades spent characterizing cyclic nucleotide phosphodiesterases, PDE nomenclature (PDE1 to PDE11) was recently established according to their genes, biochemical properties, regulations, and sensitivities to pharmacological agents. Although PDE1 to PDE6 were first well characterized because of their predominance in various tissues, their specific contribution to tissue function and their regulatory rules in pathophysiology remain open research fields. Molecular biology as well as fluorescent cell imaging provide further insight into the knowledge of PDE implication in intracellular and subcellular signaling. This is particularly necessary for the PDE7 to PDE11 families, for which roles are not yet well established.

Many of the newest biotechnologies are reported by leader teams in PDE field in this book.

Chapters 1–4 deal with biosensors that allow the measurement of local variations of cyclic nucleotides in living cells as well as their visualization in a spatiotemporal manner. This approach is very helpful for analyzing the contribution of the various PDEs in cyclic nucleotide compartmentalization. Chapters 4–7, devoted to the localization and characterization of PDE activities in tissues and living cells, shed light on critical PDEs and their implications in

cellular functions, thus indicating them as targets for specific pathologies. Chapters 8–14, which deal with PDE overexpression, promoter identification, purification, and biochemical and structural studies, describe several approaches for assessment of the potential role of targeted PDEs in the rational development of specific tools and drugs. Chapter 15 describes how to generate PDE4 knockout mice. If no compensatory mechanisms take place, this transgenic approach, which may be extended to various PDEs, is necessary to demonstrate the potential role of targeted PDEs. Chapters 16–21 mainly focus on PDE regulation, by phosphorylation, dimerization, or protein interactions, giving some starting points for further studies on the central role of PDEs in intracellular signaling control.

*Phosphodiesterase Methods and Protocols* is intended for biochemists, molecular biologists, cell biologists, and pharmacologists who wish to initiate or deepen studies in the PDE field. It also provides a basis for new approaches in drug design for medicinal chemists and pharmaceutical companies. Furthermore, our work will point out a new way for clinicians to find and test novel therapies for numerous pathologies where the molecular origin remains unknown and the treatment is principally symptomatic. In many pathologies, such as inflammation, neurodegeneration, and cancer, alterations of intracellular signaling related to PDE deregulation may explain the difficulties observed in their prevention and treatment. By specifically inhibiting the deregulated PDE isozyme(s) with newly identified selective PDE inhibitors, one could imagine the potential restoration of normal intracellular signaling.

We are grateful to all authors for their excellent contributions, which make this book a useful aid not only for scientists wishing to work in the PDE field, but also clinicians working to develop new therapeutic proteins.

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2. Beavo, J. A. and Brunton, L. L. (2002) Cyclic nucleotide research-still expanding after half a century. *Nature Reviews*, 3, 710–718.

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