METHODS IN MOLECULAR BIOLOGY

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cAMP Signaling

Methods and Protocols

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Cover Illustration: Epifluorescence image of a neonatal cardiac myocyte expressing a FRET-based reporter for detection of cAMP levels in real-time. The panels on the left and centre show the pseudocolor image of the cell in the cyan (480nm) and yellow (540nm) channels, respectively, acquired on excitation of the sample at 430nm. The panel on the right shows the image of the 480nm/535 nm FRET signal from the same cell, showing in red subcellular compartments with higher cAMP levels. The myocytes was treated with 10nM isoproterenol to trigger generation of cAMP. Image: M Zaccolo (University of Oxford, UK).

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Preface

Adenosine 3′,5′-monophosphate (cAMP), the prototypical intracellular second messenger, regulates a large variety of cellular functions and biological processes, including gene transcription, cell metabolism, proliferation, development, as well as more specialized functions depending on the cell type. In its simpler formulation, the cAMP signaling pathway involves a hormone (the "first" messenger) that binds and activates a specific G protein-coupled receptor that in turn activates adenylyl cyclases to synthesize cAMP. The intracellular (or "second") messenger cAMP then binds to a limited number of intracellular effectors, most notably to protein kinase A (PKA), which phosphorylates downstream targets leading to a specific functional outcome. Signal termination is mediated by phosphodiesterases (that hydrolyze cAMP) and phosphatases (that dephosphorylate PKA targets), enzymes that are modulated by complex regulatory mechanisms.

In the last 15 years, the field of cAMP signaling has witnessed an exciting development with accumulating evidence demonstrating that cAMP is compartmentalized and that spatial regulation of cAMP signals is critical for faithful signal propagation and for specificity of response. This realization has changed our understanding of cAMP signaling from a model where a linear pathway connects the receptor located at the plasma membrane with an effector and its function to a model where signal propagation occurs within a complex network of cAMP-dependent signaling pathways simultaneously operating within the same cell. The pathway or pathways the cAMP signal travels along are dictated by the overall state of the cell at the time the cAMP signal is generated, depending on the activity of on/off signals that operate on individual routes at that particular time. Based on this new model, the functional outcome of a signal mediated by cAMP depends strictly on local and temporal regulation. The hormonal specificity of cAMP action results from the generation of distinct pools of the second messenger which in turn mediate different functional outcomes via activation of different subsets of the cAMP effector PKA. PKA is largely localized to different subcellular compartments via binding to a family of scaffolding proteins known as A Kinase Anchoring Proteins (AKAPs). Apart from their common ability to anchor PKA, AKAPs show a high degree of structural variability which allows for different subcellular localization and binding to a variety of other signaling components. As a result, AKAPs serve as signaling centers, where elements of the cAMP signaling pathway and other regulatory molecules are organized for a particular task.

The realization of this extremely complex spatial organization and local regulation is providing novel mechanistic insight into cell physiology and is producing a novel framework for the identification of disease mechanisms. This new model also offers the potential to establish original avenues for the treatment of disease. New approaches have been developed that allow researchers to gain information that goes beyond a measure of cAMP activity at the whole cell or cell population level. In preparing this volume, I have tried to encompass new technological developments that specifically address questions related to cAMP compartmentalization, that probe relevant protein–protein interactions, that increase the spatial and temporal resolution of cAMP signals detection, and that can facilitate integration of the mounting complexity of the information that is becoming available on this signaling system.

I am extremely grateful to all authors for living with my deadlines and providing excellent and comprehensive methods and extensive notes with essential "tricks of the trade" that are so precious when troubleshooting a new technique. Finally, I thank the Senior Editor, John Walker, for giving me the opportunity to compile this volume in the excellent series, *Methods in Molecular Biology*. I hope the selection of methods will prove appealing and will be a real resource to researchers in the field.

Oxford, UK ***Manuela Zaccolo***

Contents

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