Preface

The study of lipid-modified proteins is in a period of tremendous growth. The field has emerged from its initial phase of primarily structural descriptions to one in which the enzymology and biology of these modifications are main areas of investigation. This volume is organized around the three major classes of lipids that have been identified in covalent attachment to proteins in eukaryotic cells: isoprenoids, saturated fatty acyl groups (palmitoyl and myristoyl), and glycosylphosphatidylinositol (GPI). Each of these lipids and the enzymes which catalyze their attachment to proteins have quite distinct chemical and biological characteristics, necessitating the development of unique experimental approaches specific for each lipid. An additional section highlights further modifications specific to isoprenoid-modified proteins which, while not truly lipid modifications per se, appear to play important roles for these proteins. While each of these sections contains articles which utilize physical–chemical approaches for structure determination, a particular emphasis of this volume is on methods that evaluate the functional significance or biological impact of these lipid modifications.

The role of lipid modifications in protein localization and function is most visibly recognized in cellular signaling pathways, as the majority of molecules which participate in the membrane-associated events in these pathways are lipidated. Examples include myristoylation of nonreceptor tyrosine protein kinases, palmitoylation and prenylation of the Ras-type GTP-binding proteins (G proteins), and multiple modifications of subunits of the heterotrimeric G proteins. The actual receptors which initiate the signaling responses can also be subject to lipidation; examples include palmitoylation of G protein-coupled receptors and the elaborate GPI modifications of numerous cell-surface adhesion molecules and receptors. A diverse set of methods useful for investigating the roles of attached lipids in the function of these molecules are covered in this volume. These include biochemical and molecular analyses of the cellular machinery involved, application of modern techniques in yeast genetics, and use of synthetic organic chemistry and spectroscopy to assess the structural basis of lipid-mediated specificity in the function of modified proteins.

New aspects of lipid modification are continually being discovered. Recent examples covered include dynamic turnover of palmitate on receptors and G proteins in response to activating signals and the finding that GPI-anchored proteins may be markers of specific membrane subdomains important in certain types of transmembrane signaling. Other pro-

cesses for which methods are still under development include removal of myristoyl groups previously presumed to be permanent additions to proteins, identification of novel lipids such as palmitoleic acid and arachidonate attached to proteins, and regulation of enzyme activity by direct modification of the active site with long-chain fatty acids. These discoveries will undoubtedly lead both to novel methods for studying lipidated proteins and to a greater appreciation of the role of lipid-modified proteins in cellular processes.

A 1990 issue of the companion journal to this series, *Methods: A Companion to Methods in Enzymology* (Vol. 1, Number 3), was devoted to "Covalent Modification of Proteins by Lipids." It contained several articles concerned with the identification of lipid-modified proteins and analysis of the lipid constituents. Most of the techniques described in that issue remain the preferred methods of analysis, and the reader is encouraged to refer to that journal for additional coverage of techniques pertaining to the identification and structural characterization of isoprenoids and GPI species attached to proteins, reconstitution of protein myristoylation in *Escherichia coli,* and detection of carboxyl-terminal methyl esters on prenylated proteins.

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