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Protein palmitoylation

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Introduction

Protein palmitoylation was first described over 30 years ago, and at that time represented the first example of covalent lipid modification of proteins in eukaryotic cells. In the intervening years, palmitoylation has been joined by other forms of protein lipidation, including prenylation, myristoylation, GPI anchor addition, and cholesterol addition. An important difference between palmitoylation and these other forms of lipid modifications is that palmitoylation is reversible. Accordingly, palmitoylation has the potential to regulate function through cycles of acylation and deacylation catalyzed by protein acyltransferases and protein thioesterases. In this respect, palmitoylation can be thought of as the lipid version of protein phosphorylation.

In 1990, *Methods* dedicated one of the first issues to the “Covalent Modification of Proteins by Lipids” [Methods 1(3), 215–320 (1990)]. In that issue, edited by Pat Casey, protein prenylation, myristoylation, and GPI anchors dominated, with just one paper dealing with methods for labeling cells with tritiated palmitate to detect protein palmitoylation. At that time, protein palmitoylation was suspected to play an important regulatory role in cells, but tools for studying palmitoylation were limited. In vivo labeling of cells with tritiated palmitate was difficult and not quantitative. The existence of enzymes that catalyze palmitoyl transfer to proteins was a matter of lively debate, as was the functional significance of protein palmitoylation. However, the situation has changed dramatically in recent years with the identification of a family of protein acyltransferases (PATs) along with improved methods for studying the occurrence and functional significance of protein palmitoylation. The current issue of *Methods* returns to the issue of protein lipidation, this time focusing on protein palmitoylation.

The first article, by Drisdel et al., describes a new method for detecting protein palmitoylation using acyl-biotin exchange (ABE) chemistry. The ABE method is rapidly replacing the traditional method of metabolic radiolabeling of proteins in cells using tritiated palmitate and detection by fluorography. In vivo labeling was difficult to quantitate and resulted in low signal intensity that often required gels to be exposed for weeks to detect palmitoylation. ABE chemistry has made it possible to perform a proteomic analysis of protein palmitoylation. Roth et al. describe the method they used to obtain the first description of a palmitoylproteome, using

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yeast *Saccharomyces cerevisiae* as a model system. The method is now ready to apply to more complex systems.

The second set of papers in this issue deal with methods for purifying and characterizing PATs. The biochemical isolation of PATs eluded investigators for years, but this changed with the identification of two PATs in yeast through studies of Ras trafficking and mating pheromone signaling. These studies indicated that a family of DHHC domain-containing proteins might be the elusive palmitoyltransferases. Biochemical confirmation of this hypothesis followed. These studies opened the door to analysis of DHHC proteins in other systems. Methods for the purification and characterization of DHHC proteins in yeast and mammalian systems are described in papers by Budde et al. and Fukata et al. Meiringer and Ungermann describe methods for analyze palmitoylating activity at the yeast vacuole. The characterization of PATs has been greatly aided by design and synthesis of lipidated peptides and proteins. Two examples of how these peptides are synthesized and used to characterize PATs are presented in papers from Brunsveld et al. and Ducker et al.

While methods for detect palmitoylation and characterize PATs have proceeded briskly, significant advances have been occurring simultaneously in methodology to study the functional significance of protein palmitoylation. As described in the article by Resh, synthetic analogs and inhibitors of palmitoyltransferases have been developed to study the functional significance of palmitoylation. These methods provide an excellent foundation on which to synthesize lead compounds from drug development. Fluorescence-based methods have also emerged as effective tools for studying palmitoylation-dependent membrane association and subcellular trafficking of palmitoylated proteins. Two papers, one by Henis et al. and the other by Kenworthy, describe how FRAP can be used to advance our understanding of how palmitoylation and depalmitoylation cycles influence the trafficking of palmitoylated proteins

We thank the editors at Elsevier for providing us with the opportunity to assemble this issue. We especially appreciate the enthusiasm on the part of the authors and their investment of time and effort in the project. It is our hope that this issue of *Methods* will facilitate the entry of new investigators and contribute to exciting advances in the field of protein palmitoylation.