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Bioorthogonal Chemical Reporters for Analyzing Protein Lipidation and Lipid Trafficking

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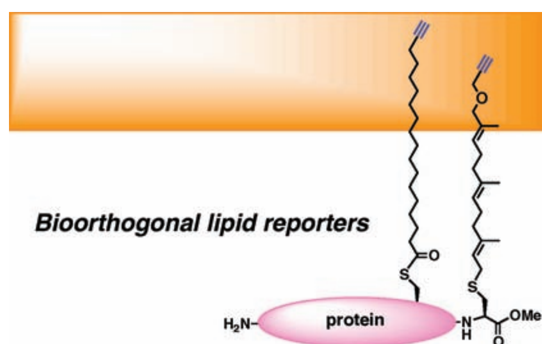

Protein lipidation and lipid trafficking control many key biological functions in all kingdoms of life. The discovery of diverse lipid species and their covalent attachment to many proteins has revealed a complex and regulated network of membranes and lipidated proteins that are central to fundamental aspects of physiology and human disease. Given the complexity of lipid trafficking and the protein targeting mechanisms involved with membrane lipids, precise and sensitive methods are needed to monitor and identify these hydrophobic molecules in bacteria, yeast, and higher eukaryotes.

Although many analytical methods have been developed for characterizing membrane lipids and covalently modified proteins, traditional reagents and approaches have limited sensitivity, do not faithfully report on the lipids of interest, or are not readily accessible. The invention of bioorthogonal ligation reactions, such as the Staudinger ligation and azide−alkyne cycloadditions, has provided new tools to address these limitations, and their use has begun to yield fresh insight into the biology of protein lipidation and lipid trafficking. In this Account, we discuss how these new bioorthogonal ligation reactions and lipid chemical reporters afford new opportunities for exploring the biology of lipid-modified proteins and lipid trafficking.

Lipid chemical reporters from our laboratory and several other research groups have enabled improved detection and large-scale proteomic analysis of fatty-acylated and prenylated proteins. For example, fatty acid and isoprenoid chemical reporters in conjunction with bioorthogonal ligation methods have circumvented the limited sensitivity and hazards of radioactive analogues, allowing rapid and robust fluorescent detection of lipidated proteins in all organisms tested. These chemical tools have revealed alterations in protein lipidation in different cellular states and are beginning to provide unique insights in mechanisms of regulation. Notably, the purification of proteins labeled with lipid chemical reporters has allowed both the large-scale analysis of lipidated proteins as well as the discovery of new lipidated proteins involved in metabolism, gene expression, and innate immunity. Specific lipid reporters have also been developed to monitor the trafficking of soluble lipids; these species are enabling bioorthogonal imaging of membranes in cells and tissues. Future advances in bioorthogonal chemistry, specific lipid reporters, and spectroscopy should provide important new insight into the functional roles of lipidated proteins and membranes in biology.

1. Introduction

The organization of lipids into discrete membranes provides an essential mechanism to compartmentalize living matter and signaling platforms for protein complexes.[1,2] Changes in lipid composition control membrane architecture and recruitment of proteins to membranes that have significant effects on organismal physiology.[1,2] Indeed, genetic mutations that regulate lipid homeostasis or membrane

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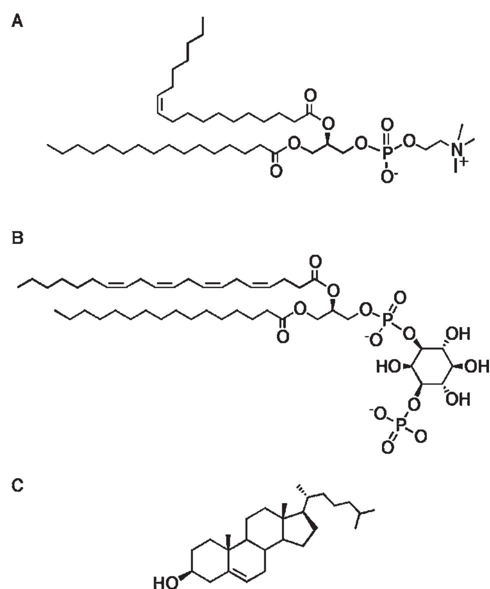

FIGURE 1. Examples of cellular lipids. (A) Phosphatidylcholine. (B) Phosphatidylinositol 3-phosphate (PI3P). (C) Cholesterol.

targeting of proteins are associated with a variety of human diseases ranging from cancer, neurological disorders, and atherosclerosis. The complexity of lipids[1] and diversity of membrane-targeting mechanisms for proteins[2] present significant challenges for understanding how alterations in lipid composition and protein recruitment to membranes influence complex signaling pathways for specific physiological functions. In this Account, we summarize how the advances in bioorthogonal chemistry are providing new tools to investigate lipid trafficking and protein lipidation in biology as well as innovative methods for biotechnology applications.

1.1. Lipids. Lipids control a vast array of cellular functions (Figure 1).[1] Beyond their fundamental roles in metabolism, lipids such as phospholipids are the primary building blocks of cellular membranes in animal cells (Figure 1A), which vary in fluidity and curvature depending on their lipid composition.[1] Additionally, phosphatidylinositol phosphates (PIPs) such as phosphatidylinositol 3-phosphate (PI3P) can recruit specific protein effectors to membranes for cell signaling (Figure 1B). Intracellular lipid droplets have also emerged as important structures for storage of cholesterol (Figure 1C) and fatty acids in cells. The regulation of these and other lipid structures provides important mechanisms to recruit proteins to specific membranes for cellular signaling.

1.2. Protein Lipidation. Covalent modification of proteins with lipids controls the subcellular localization and activity of diverse protein families in bacteria and eukaryotes.[2,3] Lipoproteins (LPPs) in bacteria are characterized by the diacylglyceryl modification of Cys residues encoded in lipobox motifs in conjunction with N-terminal fatty-acylation (Figure 2A).[3] LPPs play key roles in bacterial membrane biogenesis and are important recognition factors for activation of immune responses during infection.[3] Noncanonical fatty-acylation of bacterial proteins can also occur on Lys residues as in the case of hemolysin (Figure 2B).[4] In eukaryotes, *N*-myristoylation and *S*-palmitoylation comprise two major classes of protein lipidation for spatial and temporal control of protein activity.[2] *N*-Myristoylation is a cotranslational modification of myristic acid to N-terminal Gly residues through the action of *N*-myristoyltransferases (NMTs) (Figure 2C).[5] *S*-Palmitoylation (*S*-acylation) on the other hand describes the addition of palmitic acid or other long chain fatty acids onto Cys residue of proteins (Figure 2D).[6] *S*-Palmitoylation is enzymatically regulated through a conserved family of Asp-His-His-Cys-containing protein acyltransferases (DHHC-PATs) (Figure 3A).[6] No definitive consensus motif is available for *S*-palmitoylation, but predictive algorithms based on known sites of modification have been developed.[7] Dissecting specificity and regulatory mechanisms of *S*-palmitoylation presents a significant challenge, as the ~23 DHHC-PATs in humans or mouse exhibit differential and overlapping substrate specificities.[6] *S*-Palmitoylation is uniquely reversible among fatty-acylated proteins and is recognized as an important mechanism for dynamic targeting of proteins to membranes.[6] In addition to cytosolic protein fatty-acylation, secreted factors such as the Wnt- and hedgehog family of proteins can be lipidated by the membrane-bound *O*-acyltransferases (MBOATs).[8] MBOAT-mediated palmitoylation of sonic hedgehog (Shh) that bears an N-terminal Cys residue in its mature form results in *N*-palmitoylation (Figure 2E). MBOAT family members can also catalyze *O*-acylation with alternative fatty acids that result in palmitoleoylation of Ser residues on specific Wnt-isoforms or octanoylation of peptide hormones such as ghrelin (Figure 2F).[9,10]

Several classes of C-terminal lipidation have been reported. Protein *S*-prenylation (farnesylation and geranylgeranylation) are posttranslational modifications of Cys residues with isoprenoids through thioether linkages (Figure 2G).[11] Farnesyltransferase (FTase) and geranylgeranyltransferase-1 (GGTase-I) utilize isoprenoid pyrophosphate substrates to modify C-terminal CaaX motifs [C is site of modification, aa are aliphatic amino acids, and X determines selectivity for FTase (A, C, M, Q, or S) or GGTase-I (L or F)] (Figure 3B). After *S*-prenylation, the CaaX motif is proteolytically processed and methylated to yield a C-terminal methylester (Figure 3B). GGTase-II has no consensus sequence but primarily dually geranylgeranylates the Rab subfamily of GTP-binding proteins in complex with carrier proteins.[11] Prenylation occurs

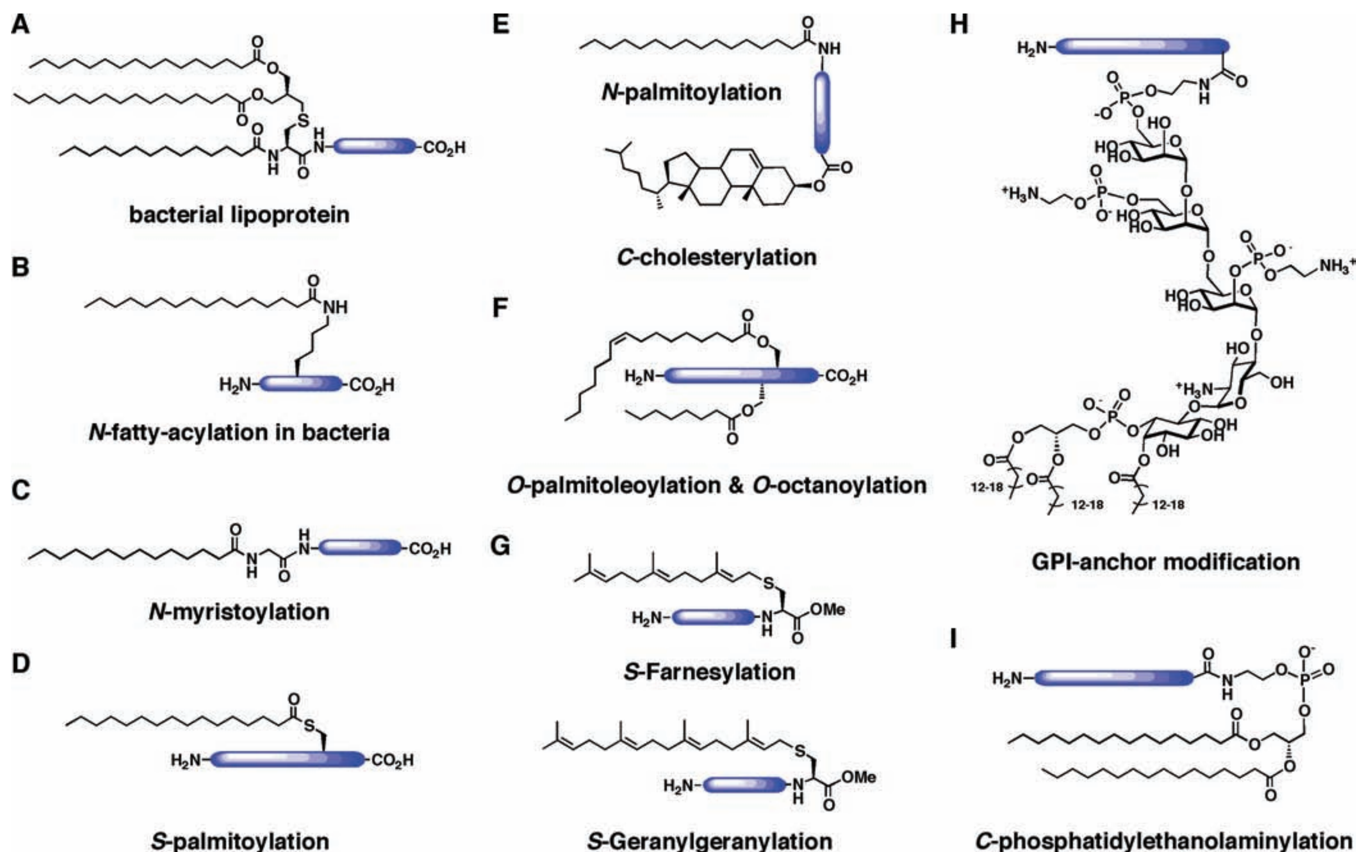

FIGURE 2. Survey of protein lipidation in bacteria and eukaryotes.

predominantly on membrane associated small GTPases that regulate diverse signaling pathways.[11] Farnesylation of K/H/N-Ras is particularly important for oncogenesis and has motivated the development of FTase inhibitors (FTIs) for chemotherapy.[11] Cholesterylation has only been found on the hedgehog family of secreted signaling molecules so far (Figure 2E).[8] Cholesterol is installed by autocatalytic processing of the Shh precursor resulting in a C-terminal thioester intermediate and nucleophilic attack by cholesterol.[8] Cholesterylation restricts the extracellular diffusion of Shh and generates morphogen gradients that controls cell signaling and vertebrate development.[8] Cholesterylation null mutants show gross mispatterning and embryonic lethality in *Drosophila* embryos and exhibit holoprosencephaly in humans.[8]

Glycosylphosphatidylinositoylation (GPI) has the most complex structure of lipid PTMs consisting of a phosphoethanolamine linker to the protein, a glycan core, and a phosphatidyl inositol tail (Figure 2H).[12,13] The GPI-anchor structure is heterogeneous and is installed by *en bloc* attachment of the preassembled glycolipid onto target proteins through a transamidase complex. GPI-anchor modification results in outer leaflet plasma membrane localization for eukaryotic proteins that is particularly important for the display of variant surface glycoproteins in trypanosomes, the parasite responsible for African sleeping sickness.[13] A variety of mammalian proteins such as CD14 receptor are also GPI-modified. The C-terminus of ubiquitin-like proteins such as Atg8 in yeast and its mammalian homologue LC3 can also be covalently modified with phosphatidylethanolamine (Figure 2I).[14] Lipidation of Atg8/LC3 is mediated by ubiquitin-like conjugation systems and is crucial for the induction of autophagy, a catabolic pathway that is important for cellular homeostasis and resistance to infection.[15] These examples highlight diverse and prominent forms of protein lipidation that play key roles in basic aspects of cell biology as well as disease.

2. Analytical Methods for Lipid Analysis

The significance of lipids and lipid-modified proteins in biology has motivated the development of many experimental methods for their functional analysis. Advances in chromatography and mass spectrometry (MS) have greatly facilitated the analysis of lipids extracted from tissues, cells, and proteins and are providing new opportunities for unbiased large-scale lipidomic studies.[16] While powerful, MS-based detection methods require extraction of lipids and

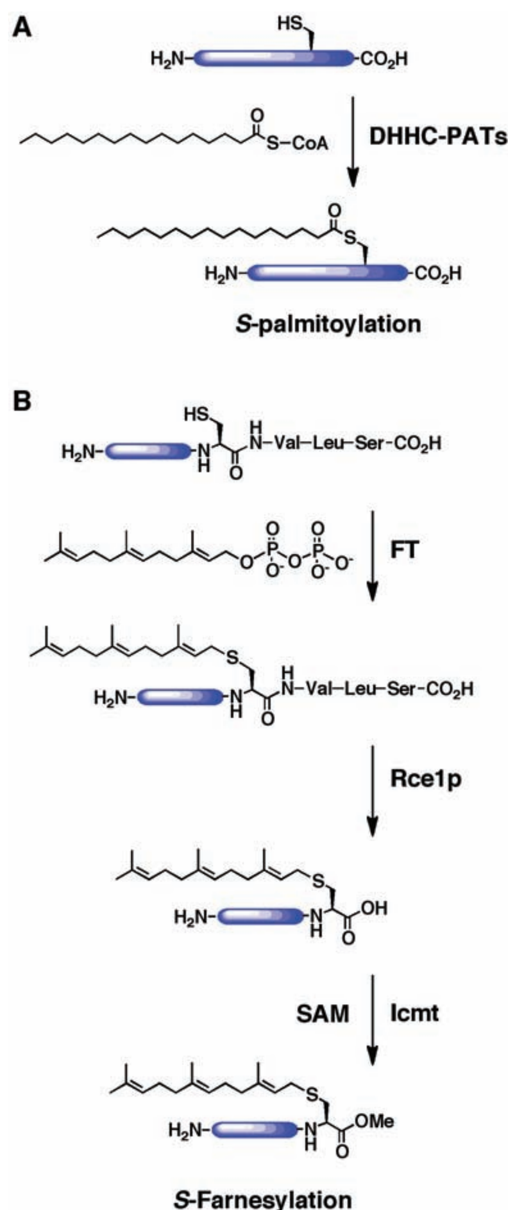

FIGURE 3. Enzyme-mediated protein *S*-palmitoylation and *S*-farnesylation. (A) Protein *S*-palmitoylation. (B) Protein *S*-farnesylation.

often require chemical derivatization for ionization. The precise analysis of some lipids also necessitates high precision mass spectrometers that may not be available to many researchers. Readily accessible reagents and methods are therefore still needed for analysis of specific lipids that complement MS-based approaches. Radioactive tracers have traditionally been employed to monitor lipid uptake, metabolism, and covalent attachment to proteins by scintillation counting or autoradiography.[17] However, the long exposure times (often weeks to months) for detection and hazards associated with radioactivity present major disadvantages for using ^{3}H- or ^{14}C-lipid analogues. ^{125}Iodinated lipids can

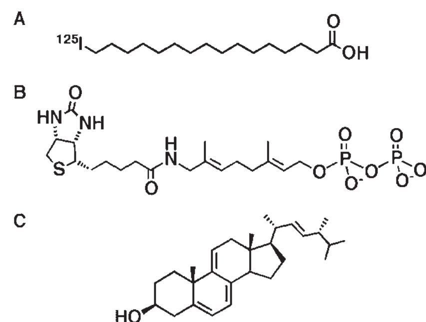

FIGURE 4. Survey of lipid analogues and imaging probes. (A) ^{125}I-ω-Palmitic acid. B) Biotinylgeranylpyrophosphate (BGPP). (C) Dehydroergosterol (DHE).

improve sensitivity, but these analogues have short shelf-lives and are still hazardous to use (Figure 4A).[17]

Nonradioactive lipid reporters afford convenient alternatives to radioactivity and enable detection of soluble lipids as well as protein lipidation.[18−20] While fluorescent lipid derivatives have been used in many contexts for tracking lipids in vitro, in cells and animals, chemical modifications that are often larger than the parent lipid can greatly alter their physical properties and biological behavior. For example, NBD- and biotinylated isoprenoids can function as substrates for prenyltransferases in vitro,[19] but biotinylated analogues such as BGPP require mutation of native enzymes for substrate utilization (Figure 4B).[21] Intrinsically fluorescent lipids such as dehydroergosterol (DHE) can provide more faithful lipid reporters (Figure 4C).[22] However, DHE does not rescue cholesterol auxotrophs and suffers from low quantum yield, rapid bleaching, and ultraviolet (UV) excitation and emission that requires specialized UV-transparent imaging equipment.[22] These nonideal biological and photochemical properties often complicate cellular loading with lipid reporter at nonphysiological concentrations that may significantly perturb biological pathways of interest. Reagents have also been developed to indirectly monitor lipids. Fluorescent membrane dyes such as filipin have been used to image cholesterol in cells, but the specificity of filipin for hydrophobic molecules in complex cellular environments is often unclear.[23] Many methods and reagents have been developed for analyzing lipids and protein lipidation, but new tools are still needed to analyze lipid trafficking and protein lipidation in many biological settings.

3. Bioorthogonal Chemical Ligation Methods

The development of bioorthogonal chemical ligation reactions has afforded readily accessible reagents and sensitive methods to monitor diverse biomolecules (Figure 5).[24]

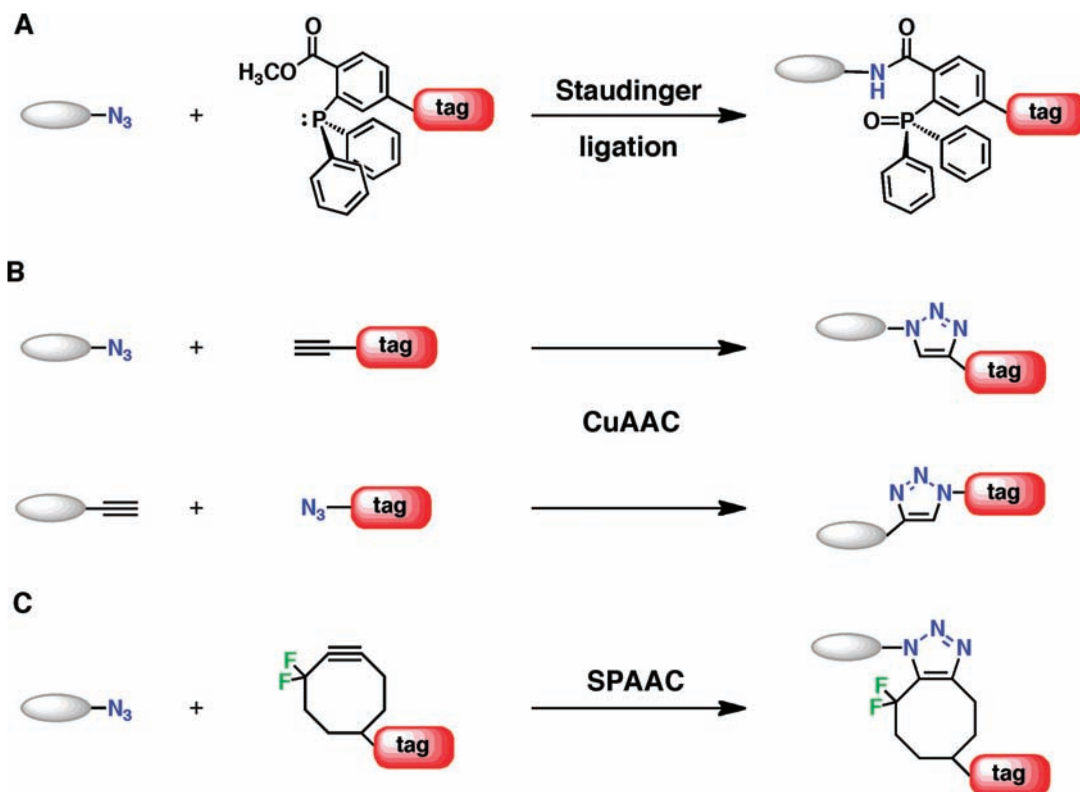

FIGURE 5. Bioorthogonal ligation methods. (A) Staudinger ligation. (B) Cu^I-catalyzed azide–alkyne cycloaddition (CuAAC). (C) Strain-promoted azide–alkyne cycloaddition (SPAAC).

Building upon chemoselective ligations that enabled the assembly of complex biopolymers such as proteins and glycans in aqueous conditions,[25] the invention of the Staudinger ligation by Saxon and Bertozzi provided the first example of a "bioorthogonal" chemical ligation reaction where an alkyl azide and triarylphosphine ester could selectively react to form a covalent adduct in aqueous and aerobic conditions with minimal cross-reactivity to other functional groups present in biopolymers and metabolites (Figure 5A).[26] The subsequent development of the Cu^I-catalyzed [3 + 2] azide–alkyne cycloaddition (CuAAC) by Meldal and co-workers,[27] as well as Sharpless and co-workers[28] based upon earlier studies by Huisgen,[29] provided a second example of bioorthogonal ligation reaction (Figure 5B), which is often termed "click chemistry".

Significant advances in bioorthogonal chemistry have also been achieved to enable more rapid labeling on living cells and animals. Notably, Bertozzi and co-workers have developed a strain-promoted alkyne–azide cycloaddition (SPAAC) that has faster reaction kinetics and circumvents the need for copper that is toxic to cells and animals (Figure 5C).[30] More efficient syntheses of reactive cyclooctyne derivatives have also been reported to improve the availability of these reagents.[30] Alternatively, copper ligands with increased stability and reactivity for CuAAC have been developed to reduce toxicity in animals.[31] In addition to the Staudinger ligation and azide–alkyne cycladditions, several new bioorthogonal ligation methods have been developed, but they are beyond the scope of this review and have been summarized elsewhere.[32] These bioorthogonal chemical reactions have firmly launched the two-step labeling approach using small azide/alkyne-functionalized probes/reporters and detection tags to analyze or modify various classes of biological molecules and small molecule-protein interactions (Figure 6).[24] This approach is particularly attractive, since azides and alkynes are relatively small, nonpolar, and stable functional groups that can be readily installed onto metabolites or drugs with minimal structural perturbation and retain biological activity.

4. Biological Applications of Lipid Reporters

The challenges in understanding how lipids control membrane trafficking and protein function in many physiology pathways and diseases have motivated the development of diverse bioorthogonal lipid chemical reporters.[19,20] Given the modularity of the two-step bioorthogonal labeling,

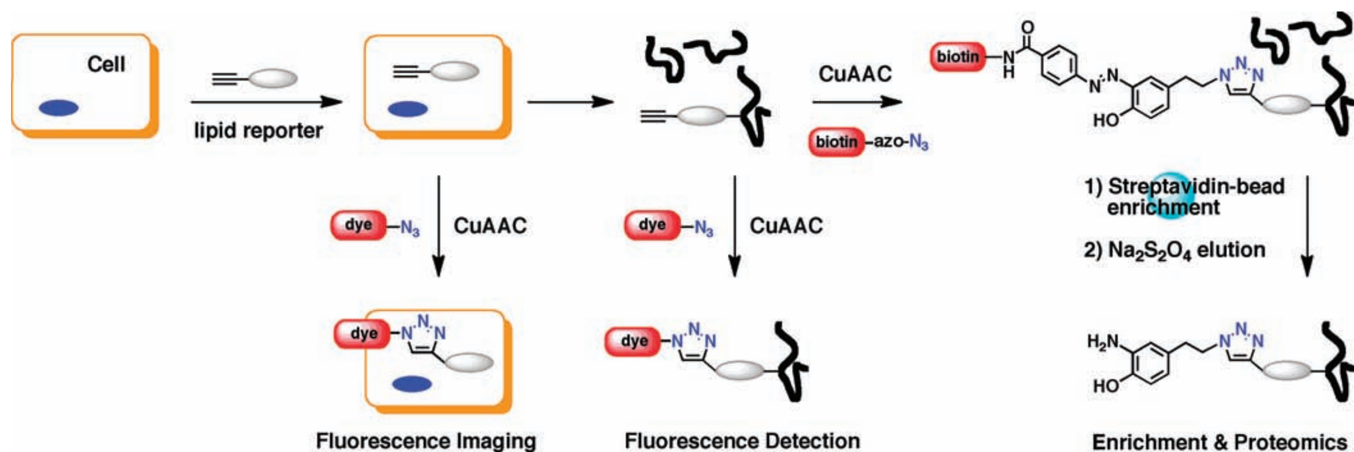

FIGURE 6. Two-step chemical labeling approach for bioorthogonal imaging, detection, and identification of lipid reporters.

azide- and alkyne-functionalized lipid chemical reporters provide powerful tools for monitoring lipid trafficking and metabolism, protein modification, as well as biotechnology applications (Figure 7).[20,33]

4.1. Fatty Acid Reporters. Fatty acid analogues functionalized at the ω-position with an azide or alkyne from 10−18 carbons in length provide useful lipid reporters for visualizing and identifying fatty-acylated proteins in bacteria, yeast, and mammalian cells.[20,33] Early studies of fatty acid analogues demonstrated that transporters and biosynthetic enzymes involved in protein fatty-acylation could tolerate unnatural substrates.[5] Following these studies, azido-fatty acid labeling of mammalian cells revealed *N*-myristoylated and *S*-palmitoylated proteins could be readily visualized after Staudinger ligation or CuAAC ligation of cell lysates or known substrates with various detection tags.[34−38] Alkynyl-fatty acids also proved to be efficient lipid reporters for monitoring fatty-acylation in mammalian cells (Figure 7A−C).[35,39−41] Comparative analysis of lipid reporters, bioorthogonal ligation methods, and detection modes revealed alkynyl-fatty acid reporters in conjunction with CuAAC and in-gel fluorescence detection afford the most sensitive protocol for visualizing lipidated proteins.[35]

The improved detection of fatty-acylated proteins with bioorthogonal lipid reporters has provided unique opportunities to discover lipidated proteins, evaluate their changes upon cellular activation as well as underlying regulatory mechanisms. For example, in-gel fluorescence profiling of alkynyl-fatty acid labeled mammalian cell lines highlighted the abundance and diversity of fatty-acylated proteins between various cell types.[35] The application of these fatty acid chemical reporters and their corresponding acyl-CoA derivatives along with cellular fractionation has revealed discrete profiles of fatty-acylated proteins in the mitochondria[37,42] as well as posttranslationally *N*-myristoylated proteins during apoptosis.[36] Notably, fatty acid reporter proteomics of Jurkat T cells identified *S*-palmitoylation of serine hydrolases from 125 high-confidence protein hits[41] as well as *S*-acylation of histone H3 variants from 178 high-confidence hits[43] (Figure 7A−C). Alternatively, proteomic analysis of alk-16 labeled dendritic cell line (DC2.4) identified 157 high-confidence hits and uncovered a family of *S*-palmitoylated interferon-induced transmembrane proteins (IFITMs).[44] *S*-Palmitoylation of IFITM3 in particular was shown to be crucial for host defense against influenza virus infection.[44] A fatty acid chemical reporter with an oxy-ether linkage, 15-hexadecynyloxyacetic acid, can also be metabolically installed onto known *S*-palmitoylated proteins, which may circumvent degradation of alkynyl-fatty acids via the β-oxidation pathway that may result in labeling of metabolic enzymes or potential lysine-acetylated proteins.[45] Indeed, short chain alkynyl-fatty acids (ω-butynyl and pentynyl acids) and their corresponding acyl-CoA derivatives are efficient bioorthogonal chemical reporters for monitoring lysine protein acetylation (Figure 7D).[46] In bacteria, metabolic labeling with alkynyl-fatty acids with variable chain length revealed alk-14 afforded the optimal profiling of canonical lipoproteins and also identified *S*-acylation of unpredicted substrates (Figure 7A).[47] For proteomic analysis of azide/alkyne-modified proteins described above,[43−47] the use of clickable biotinylated tags such as azido-azo-biotin (Figure 6) that can be cleaved with sodium dithionite ($Na_2S_2O_4$) has been particularly helpful for elution of captured polypeptides from streptavidin beads for subsequent protein identification or Western blot validation.[46,48] From bioorthogonal fatty acid reporter proteomics[41,43−45] and complementary studies using *S*-acyl-biotin exchange,[49−53] many new candidate *S*-

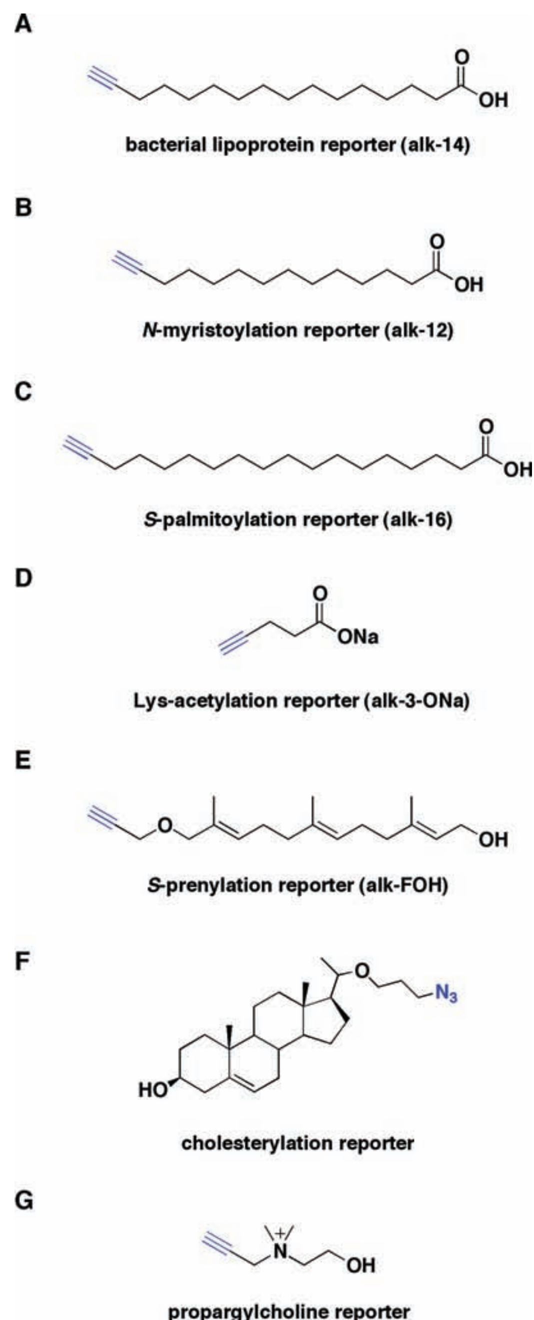

FIGURE 7. Survey of bioorthogonal lipid chemical reporters for metabolic labeling. Readers are referred to other reviews for a more complete list of lipid chemical reporters.[20,33]

palmitoylated proteins have now been identified and suggest that ~1−2% of the protein encoding open-reading frames in eukaryotes are covalently modified with fatty acids.

Fatty acid reporters are also beginning to reveal changes in protein fatty-acylation during different cellular states. To monitor the bulk distribution of fatty-acylated proteins in cells, alk-16 palmitate reporter labeled cells can be fixed, permeabilized, extracted with methanol or detergent (Triton-X 100) to remove soluble lipids, subjected to CuAAC labeling, and imaged by fluorescence microscopy.[35] After this protocol, alk-16 labeling is primarily associated with membrane compartments as judged by costaining with known cellular markers.[35] Interestingly, the analysis of PC3 tumor cells undergoing cytokinesis revealed an enrichment of alk-16 labeling at the cleavage furrow, suggesting that *S*-palmitoylated proteins may be recruited to specific membranes during cell division.[40] The reversibility of protein *S*-palmitoylation has long suggested that this dynamic posttranslational modification plays key roles in protein targeting to membranes for cell signaling. However, quantitative biochemical analysis of palmitoylation/depalmitoylation cycles has been very challenging with radioactivity. With improved fluorescent detection of *S*-palmitoylation, alkynyl-palmitic acid reporter (alk-16) pulse-chase studies have revealed differential regulation of individual palmitoylation sites on membrane proteins such as β1-adrenergic receptor.[54] In addition, dual pulse-chase labeling of cells with alk-16 and azidohomoalanine (AHA) or azido-myristic acid (az-14) followed by sequential CuAAC reaction with orthogonal fluorophores provides a robust method for simultaneously monitoring depalmitoylation and protein turnover of specific substrates.[55] The sensitivity and accuracy of this protocol enabled the analysis of pharmacological agents that can affect depalmitoylation rates in mammalian cells and also revealed accelerated depalmitoylation of Lck upon T-cell activation, which suggests dynamic membrane targeting of this Src-family kinase may be crucial for cell signaling.[55] Future studies with fatty acid chemical reporters should provide additional insight into the mechanisms that regulate protein *S*-palmitoylation.

4.2. Isoprenoid Reporters. A variety of in vitro and cellular studies demonstrated that isoprenoid biosynthetic enzymes and protein prenyltransferases could utilize unnatural substrates.[20,33] Metabolic labeling of statin-treated mammalian cells with azido-farnesol (az-FOH) and its pyrophosphate derivative (az-FPP) showed that prenylated proteins could be visualized by bioorthogonal detection.[56] Affinity enrichment of az-FPP labeled proteins in COS-1 cells resulted in the identification of 18 putatively farnesylated proteins as well as known substrates.[56] Following these studies, several other azide and alkyne-derivatives of farnesol, geranylgeraniol, and their pyrophosphate analogues have been shown to function as isoprenoid reporters in vitro and in cells.[19,20] Geranylgeranylated proteins in mammalian cells have also been profiled after CuAAC labeling using two-dimensional electrophoresis and in-gel fluorescence.[57] Purification and

proteomic analysis of azido-geranylgeraniol (az-GGOH) labeled polypeptides revealed 10 previously described geranylgeranylated proteins of the Rab and Ras families from MCF-7 cells.[57] In comparison with in vitro biotinylation methods with engineered prenyltransferases,[21] bioorthogonal proteomics with isoprenoid reporters has been less effective.[56,57] Prenylome profiling in general is currently limited by the need to deplete endogenous isoprenoids with statins for efficient labeling of prenylated proteins, which precludes comparative studies of different cellular states and analysis of regulatory mechanisms without significant metabolic perturbation of cells. Alkynyl-isoprenoids that afford more sensitive detection of prenylated proteins compared to their azide counterparts (Figure 7E)[58,59] and improved affinity enrichment methods[48] may circumvent this technical limitation for large-scale analysis of prenylated proteins.

4.3. Other Lipid Chemical Reporters. The initial discovery of Shh cholesterylation also suggested other proteins may be covalently modified with sterols.[60] The synthesis of an azide-modified cholesterol reporter has enabled metabolic labeling and fluorescence detection of Shh after CuAAC ligation (Figure 7F).[61] Bioorthogonal cholesterol reporters should provide new reagents for identifying novel cholesterylated proteins that have biological functions beyond secreted morphogens.

Several azide/alkyne lipid reporters have been developed for imaging membranes.[62,63] Metabolic labeling with propargylcholine resulted in biosynthetic incorporation into phosphatidylcholine lipids in CHO cells and in mice (Figure 7G).[62] In both cases, membrane structures such as the plasma membrane were clearly labeled.[62] The use of imaging regents with differential cell permeability also allowed the distinction between surface exposed and internalized choline reporter.[62] As with fatty acid reporters,[35,40] propargylcholine labeling did not colocalize with any specific subcellular markers in CHO cells.[62] Three alkynyl-phosphatidic acid reporters, including a cyclooctyne analogue, have been synthesized for visualization of cellular membranes.[63] The phosphates of all analogues were modified with an *S*-acetylthioethyl group (SATE) to facilitate uptake and cleavage by esterases in living cells.[63] Fluorescence imaging of the three different phosphatidic acid reporters yielded general labeling of membranes in RAW264.7 macrophages by CuAAC after fixation or live cell SPAAC of the cyclooctyne using a fluorogenic azide-functionalized coumarin dye. Azide analogues of diacylglycerol (DAG) have also been synthesized and can be incorporated into vesicles for biochemical studies with membrane-binding proteins.[64,65] Moreover, azide-phosphatidylcholine analogues bearing photochemical cross-linking groups can be employed to identify lipid interacting proteins or other lipids.[66] The modularity of bioorthogonal lipid reporters may provide a useful means to decouple metabolic labeling and partition into membranes from subsequent imaging. These preliminary studies suggest that lipid reporters in conjunction with new bioorthogonal ligation reactions and imaging methods may provide unique insight into lipid trafficking in the future.

4.4. Biotechnology Applications of Lipid Chemical Reporters. Bioorthogonal lipid chemical reporters have also be adapted for biotechnology applications. For example, introduction of prenylation CaaX-motifs on the C-terminus on recombinant proteins enables metabolic tagging with azides or alkynes for specific installation of fluorophores for imaging applications or affinity tags for immobilization on surfaces for protein microarray applications.[67−72] Alternatively, *E. coli* expressing NMT can be used to label recombinant proteins bearing an N-terminal NMT recognition sequence with azido/alkynyl-fatty acids.[73,74] Surface proteins engineered with lipoic acid ligase modification sequences can also be enzymatically labeled with azido caprylic acid and visualized after SPAAC with fluorophores for protein trafficking studies in living cells.[75]

5. Conclusions and Future Outlook

Bioorthogonal chemistry is beginning to make a significant impact on functional studies of protein lipidation and lipid trafficking in biology, but many challenges still lie ahead for application of these chemical tools to human physiology and disease. For protein lipidation studies, the current set of chemical reporters target many protein substrates in cells. The site-specific incorporation of bioorthogonal lipid reporters onto individual proteins could greatly facilitate functional studies in living cells to monitor protein trafficking and lipidation levels in concert. Enzyme-specific lipid chemical reporters would also help elucidate the substrate specificity of lipid transferases such as the DHHC-PATs. Bioorthogonal ligation methods allow the installation of fluorophores and affinity tags, but the addition of these detection reagents could significantly interfere with membrane partition of labeled lipids and may also required permeabilization of cells for labeling. The application of lipid chemical reporters in living animals akin to the studies with glycan chemical reporters[76] could enable the analysis of lipid trafficking and protein modification in animal models of human diseases.

Finally, the recent advances in infrared and Raman spectroscopy have already enabled the visualization of azide/alkyne-labeled proteins in membranes[77,78] or nucleic acids in cells[79] that should allow direct spectroscopic imaging of lipid chemical reporters in vivo.

BIOGRAPHICAL INFORMATION

Howard C. Hang was born in Ho Chi Minh City, Vietnam in 1976. He obtained his B.S. degree in chemistry from the University of California, Santa Cruz in 1998 with Professor Joseph P. Konopelski. In 2003, he completed his Ph.D. in chemistry at University of California, Berkeley with Professor Carolyn Bertozzi with an American Chemical Society, Organic Division Graduate Fellowship. He then worked with Professor Hidde Ploegh at Harvard Medical School and the Whitehead Institute of Biomedical Research at Massachusetts Institute of Technology from 2004 through 2006 as a Damon Runyon Cancer Research Foundation Postdoctoral Fellow. In 2007, he joined the faculty at The Rockefeller University and is currently an Assistant Professor and Head of the Laboratory of Chemical Biology and Microbial Pathogenesis.

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FOOTNOTES

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