AWARD NUMBER: W81XWH-17-1-0361

TITLE: Loss of ZDHHC-Mediated Scribble Palmitoylation Disrupts Cell Polarity and Promotes Prostate Cancer Progression

PRINCIPAL INVESTIGATOR:
Xu Wu, Ph.D

CONTRACTING ORGANIZATION:
Massachusetts General Hospital
Boston, MA 02114

REPORT DATE: August 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE
August 2018

2. REPORT TYPE
Annual

3. DATES COVERED
15 Jul 2017 - 14 Jul 2018

4. TITLE AND SUBTITLE

Loss of ZDHHC-Mediated Scribble Palmitoylation Disrupts Cell Polarity and Promotes Prostate Cancer Progression

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0361

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Xu Wu, Ph.D

E-Mail: xwu@cbrc2.mgh.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts General
Hospital, Boston, MA 02114
And
Northwestern University,
Chicago, IL 60611

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Progression and metastasis of prostate cancers (PCs) are major therapeutic challenges, with the underlying mechanisms remaining unclear. The apical-basal polarity of epithelial cells plays critical roles in regulating normal cell migration and proliferation in prostate. Loss of cell polarity leads to tissue disorganization, uncontrolled proliferation and migration, hallmarks of prostate cancer progression and metastasis. ZDHHC7-mediated palmitoylation of Scribble is critical for cell polarity and metastasis. In the first year of the study, we have analyzed the palmitoylation levels of Scribble in various prostate cancer cell lines, at different progression stage. We analyzed the localization of Scribble in these cells, and correlated its palmitoylation levels. We have generated ZDHHC7 gain-of-function cell lines, and evaluated the effects on Scribble.

15. SUBJECT TERMS

None

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	29	19b. TELEPHONE NUMBER *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

1. Introduction……………………………………………………………….1

2. Keywords……………………………………………………………1

3. Accomplishments………..………………………………………..1

4. Impact…………………………...……………………………………7

5.Changes/Problems...….………………………………………………8

6. Products…………………………………….……….….……………..9

7.Participants & Other Collaborating Organizations……………….9

8.Special Reporting Requirements……………………………………11

9. Appendices…………………………………………………………11

1. INTRODUCTION:

Prostate cancer (PCa) is a commonly diagnosed cancer in American men. However, a majority of these cancers recur and develop resistance to treatments. The apical-basal polarity of epithelial cells plays critical roles in regulating epithelial cell functions, including differentiation, migration, proliferation, and apoptosis, and is essential for normal development and tissue homeostasis. Loss of cell polarity leads to tissue disorganization, uncontrolled proliferation, epithelial-to-mesenchymal transition (EMT), and migration, which are hallmarks of progression of PCa. SCRIB has been characterized as an essential regulator of cell polarity, tumorigenesis and metastasis. SCRIB is frequently amplified and overexpressed in multiple human cancers, including PCa. Amplified, but mislocalized SCRIB could function as an oncogenic factor. Therefore, the mechanism that regulates SCRIB membrane localization might be an important molecular switch, critical for PCa progression. We identified that ZDHHC7 is the major palmitoyl acyltransferase regulating SCRIB. Loss of ZDHHC7 decreases SCRIB palmitoylation and lead to its mislocalization, activation of the oncogenic YAP pathway, and cell invasion. The overall objective of this project is to define the roles of cell polarity regulator SCRIB in PCa cell progression, and how misregulation of SCRIB palmitoylation contributes to the disease. We hypothesized that: loss of cell polarity plays major roles in prostate cancer progression, and the signal transduction network involving ZDHHC7, SCRIB and the downstream YAP, MAPK or PI3K/AKT pathways promotes prostate cancer progression. ZDHHC7 functions as a potential tumor suppressor in PCa cells, and restricts the downstream oncogenic factors. Loss of ZDHHC7 in PCa promotes SCRIB mislocalization. We will elucidate the mechanisms of ZDHHC7-mediated SCRIB palmitoylation in regulating SCRIB mislocalization and cell polarity in prostate cancers, determine the roles of the ZDHHC7-mediated SCRIB palmitoylation in prostate cancer progression using preclinical *in vitro* and *in vivo* models, and evaluate their expression in primary specimens, and identify the regulator(s) of SCRIB de-palmitoylation in prostate cancer cells, and to validate it as new therapeutic target for prostate cancer therapeutics.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Cell polarity, palmitoylation, Scribble, ZDHHC7, prostate cancer, metastasis

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The overall objective of this project is to define the roles of cell polarity regulator SCRIB in PCa cell progression, and how misregulation of SCRIB palmitoylation contributes to the disease. As shown below in the proposed SOW, we have focused our work in the first year in Aim 1, to elucidate the regulation of ZDHHC7 in Scribble palmitoylation, loacalization in prostate cancer cell lines.

Aim 1. To elucidate the mechanisms of ZDHHC7-mediated SCRIB palmitoylation in regulating SCRIB mislocalization and cell polarity in prostate cancers	Timeline	Site 1	Site 2	% of completion	Actual complete dates

Major Task 1: Evaluate the palmitoylation levels of SCRIB in prostate cancer cells	Month s				
Subtask 1: evaluate the palmitoylation levels of SCRIB in different prostate cancer cell lines	1-3	Dr. Wu		100	Nov.2017
Subtask 2: evaluate the localization of SCRIB in different prostate cancer cell lines	1-6	Dr. Wu	Dr. Yu	100	Jan. 2018
Major Task 2: Establish that loss of ZDHHC7 leads to loss of SCRIB palmitoylation and its mislocalization in prostate cancer cells.					
Subtask 1: evaluate the ZDHHC7 expression levels in prostate cancer cell lines by western blot	3-6		Dr. Yu		
Subtask 2: generate ZDHHC7-stably knockdown or knockout cell lines using shRNA or CRISPR/Cas9-mediated knockout methods	6-10	Dr. Wu	Dr. Yu	80	KO cells need more validation
Subtask 3: evaluate the SCRIB palmitoylation levels in ZDHHC-deleted PC cells	9-12	Dr. Wu		50	Delayed dues to CRISPR KO selection
Subtask 4: evaluate the SCRIB localization status in prostate cancer cell lines at different stage and in ZDHHC-deleted cell lines	9-12	Dr. Wu	Dr. Yu	50	
Major Task 3: Determine that SCRIB mislocalization leads to loss of cell polarity in prostate cancer cells					
Subtask 1: examine the expression of cell junction markers in prostate cancers	12-15		Dr. Yu	0	
Subtask 2: examine cell junction markers in ZDHHC7-stably knockdown or knockout PC cells	12-15	Dr. Wu		0	
Subtask 3: express SCRIB C4/10S mutant in prostate epithelial cell lines or benign cells and evaluate the cell polarity markers	15-18	Dr. Wu		0	
Aim2: To determine the roles of the ZDHHC7-mediated SCRIB palmitoylation in prostate cancer progression using preclinical in vitro and in vivo models, and evaluate their expression in primary specimens.					
Major Task 4: Evaluate the expression levels of ZDHHC7 and SCRIB in localized and metastasized prostate cancer specimens					
Subtask 1: perform IHC of SCRIB in PC samples	12-18		Dr. Yu	0	
Subtask 2: Perform IHC of ZDHHC7 in PC samples	12-18		Dr. Yu	0	
Major Task 5: Determine the tumor suppressor roles of ZDHHC7 in prostate cancer cell lines in vitro and in vivo					

Subtask1: will examine the effects of re-expression of ZDHHC7 in prostate cancer cell lines	18-24	Dr. Wu		0	
Subtask2: test the tumorigenesis potential of these cell lines in vivo in tumor initiation (sub-cu xenograft model) and metastasis models	20-26		Dr. Yu	0	
Subtask 3: generate stable knockdown or knockout cell lines of ZDHHC7 in benign cell line; as well as androgen dependent cell lines	18-24	Dr. Wu	Dr. Yu		
Subtask 4: evaluate cell growth, colony formation, cell invasion in ZDHHC7-deleted cells	24-28	Dr. Wu		0	
Subtask 5: test ZDHHC7-deleted cells in vivo	24-30		Dr. Yu	0	
Major Task 6: Determine the activation of downstream oncogenic pathways upon ZDHHC7 knockdown or expression of SCRIB palmitoylation deficient mutant, and the effects result in EMT, cell migration and metastasis in vitro and in vivo.					
Subtask 1: generate prostate cancer cells (LNCAP, C4-2B, 22RV1 and PC3) with expression of SCRIB WT or C4/10S mutant	24-30	Dr. Wu		0	
Subtask 2: examine the downstream signaling activities of MAPK, AKT and YAP. Western blots of p-MEK, p-ERK, p-AKT, and p-YAP will be studies	30-33	Dr. Wu		0	
Subtask 3: YAP nuclear localization and transcriptional activities will be evaluated by co-focal imaging or qRT-PCR of downstream target genes (CTGF, Cyr61 etc.)	33-36	Dr. Wu		0	
Subtask 4: evaluate the effects of EMT, cell migration and metastasis in vitro and in vivo	30-36	Dr. Wu	Dr. Yu	0	
Aim3: To identify the regulator(s) of SCRIB de-palmitoylation in prostate cancer cells, and to validate it as new therapeutic target for prostate cancer therapeutics.					
Major Task 7: Identify the de-palmitoylating enzymes that regulate SCRIB in prostate cancer	Months				
Subtask 1: clone these genes into a retroviral vector, and carry out a gain of function screen for SCRIB depalmitoylation in HEK293 and C4-2B cells	24-28	Dr. Wu	Dr. Yu	0	
Subtask 2: reconfirm their activities by co-IP and mutagenesis studies	28-30	Dr. Wu		0	
Subtask 3: examine whether overexpressing of the candidates will decrease the half-life of SCRIB palmitoylation	30-33	Dr. Wu		0	
Subtask 4: generate the shRNA constructs of the candidate depalmitoylating enzymes	30-33	Dr. Wu		0	
Major Task 8: Validate the effects of the de-palmitoylating enzymes in restoring SCRIB palmitoylation and blocking prostate cancer progression using shRNA knockdown or CRISPR/Cas9-mediated knockout.	Months				

Subtask 1: examine its expression levels in different stages of prostate cancer samples	28-32		Dr. Yu	0	
Subtask 2: analyze its expression at protein level in various prostate cancer cells	30-32		Dr. Yu	0	
Subtask 3: examine its expression in primary prostate cancer specimen using IHC.	30-33		Dr. Yu	0	
Subtask 4: generate lentiviral shRNA constructs for stable knockdown or tet-inducible shRNA constructs for inducible knock-down	28-32	Dr. Wu		0	
Subtask 5: evaluate whether it can inhibit EMT, cancer cell proliferation, cell migration and induces apoptosis in these cells. Synthesize tool inhibitors and test compound in vitro	32-36	Dr. Wu	Dr. Yu	0	
Subtask 6: carry out in vivo experiments to validate that knock down or knockout of the depalmitoylating enzyme could inhibit prostate cancer cell growth in vivo	32-36		Dr. Yu	0	

What was accomplished under these goals? As shown in the above SOW table, our work has been focused on Aim 1 in the last funding period. We have developed methods to detect Scribble palmitoylation levels in prostate cancer cell lines, and evaluated the localization of Scribble in various prostate cancer cell lines using confocal microscopy. We have tested the ZDHHC7 expression levels in cell lines. We will discuss the detailed objective, results and conclusions below.

Specific Aim 1. To elucidate the mechanisms of ZDHHC7-mediated SCRIB palmitoylation in regulating SCRIB mislocalization and cell polarity in prostate cancers

Major Task 1: Evaluate the palmitoylation levels of SCRIB in prostate cancer cells

Subtask 1: evaluate the palmitoylation levels of SCRIB in different prostate cancer cell lines

Methods: We have used palmitoylation reporter (16-CYA and 18-CYA), which metabolically label palmitoylated proteins in cells, followed by Click reaction with biotin azide, pull-down by streptavidin, and western blotting of Scribble antibody. We can detect the palmitoylation levels of endogenous Scribble in cells. We have compared the palmitoylation levels of Scribble in various prostate cancer cells.

Results: As shown in **Figure 1**, in benign prostate epithelial cell line BPH1, we have detected strong palmitoylation signal of Scribble. However, in advanced prostate cancer cell line PC3, we do not detect Scribble palmitoylation.

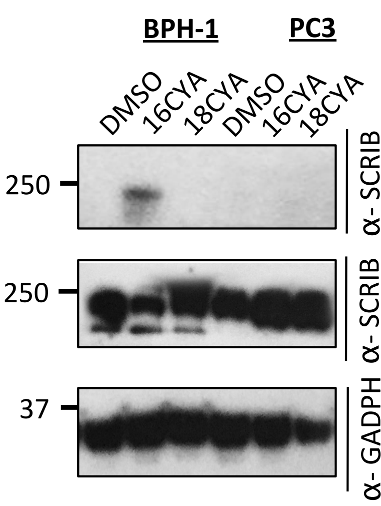

Figure 1. Metabolic labeling of palmitoylated protein by palmitoylation reporters (16CYA and 18CYA), followed by immunoprecipitation and western blotting, showed that Scribble is strongly palmitoylated in benign BPH1 cells, but not in advanced prostate cancer cell line (PC3).

Conclusion: This result is consistent with our hypothesis that loss of Scribble palmitoylation is correlated with the progression of prostate cancer.

Subtask 2: evaluate the localization of SCRIB in different prostate cancer cell lines.

Methods: We have used anti-Scribble antibody to detect endogenous Scribble, and carried our confocal immunoflurescent staining. We detected the localization of Scribble in different prostate cancer cell ines.

Results: As shown in **Figure 2**, we have successfully detected the localization of Scribble in various prostate cancer cell lines. In benign BPH1 cells, Scribble is mainly localized at the cell-cell junction, consistent with its functions in maintaining cell polarity and epithelial structures. However, in protate cancer cell line DU145 and LnCap, we have observed mislocalization of Scribble, increase cytoplasmic location of Scribble. In PC3 cells, we have observed strong cytoplasmic localization of Scribble, and loss of cell junction of epithelial layer as observed in BPH1 cells.

Conclusion: Scribble is mislocalized in prostate cancer cells. Loss of cell-junctional structure and Scribble membrane localization is characteristic to the progression of prostate cancer cells.

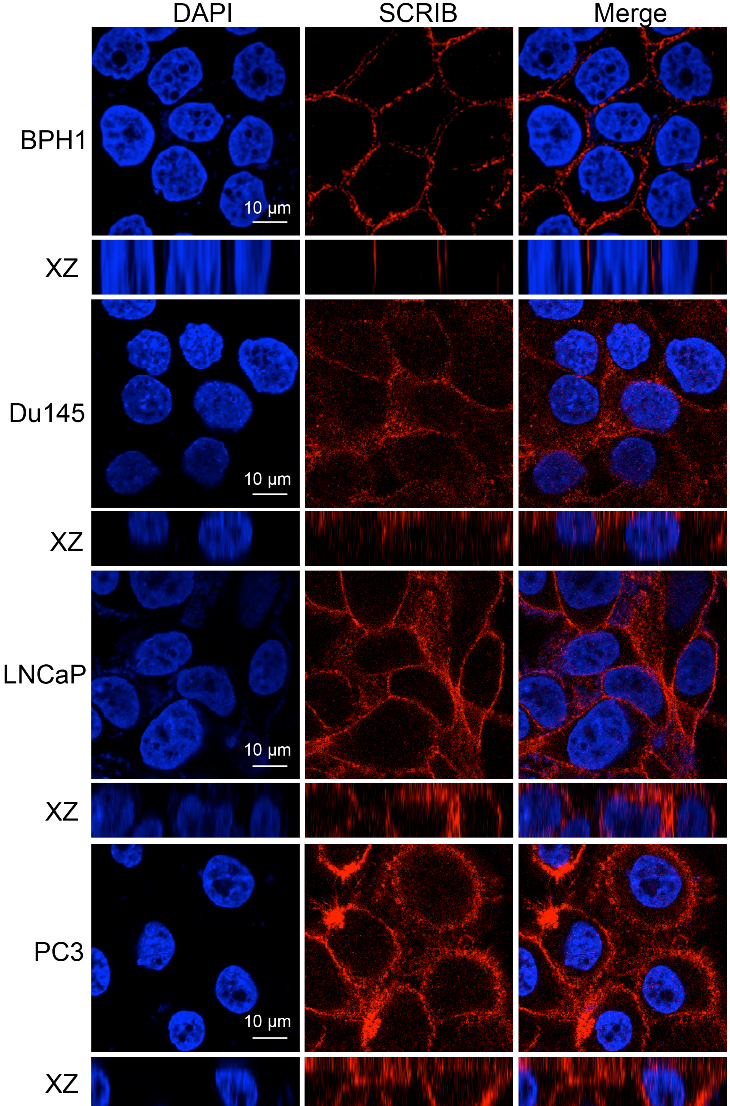

Figure 2. Confocal immunofluorescent staining of Scribble in different prostate cancer cell lines. Loss of Scribble membrane localization and loss of cell-cell junctional structures are observed in advanced prostate cancer cell lines.

Major Task 2: Establish that loss of ZDHHC7 leads to loss of SCRIB palmitoylation and its mislocalization in prostate cancer cells.

Subtask 1: evaluate the ZDHHC7 expression levels in prostate cancer cell lines by western blot

Methods: We have used western blotting to evaluate the expression levels of ZDHHC7 in different cell lines. However, there is no good antibody to detect ZDHHC7, and the results need to be further repeated with different anti-ZDHHC7 antibodies.

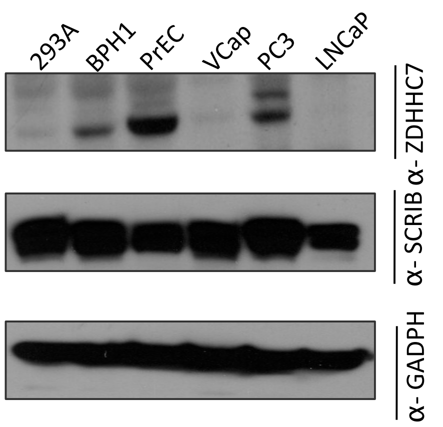

Figure 3. Expression of ZDHHC7 in different prostate cancer cell lines. Decreased ZDHHC7 expression was observed in prostate cancer cell lines. (PrEC: prostate epithelial cells).

Results: As shown in Figure 3, we have analyzed ZDHHC7 expression levels in different prostate cancer cell lines. The results are not completely consistent as the commercial antibody gives many nonspecific bands, and complicated with the detection.

Conclusion: Decreased ZDHHC7 protein levels are detected in prostate cancer cell lines. However, the results need to be repeated with a different antibody, or with qRT-PCR to make a solid conclusion.

Subtask 2: Generate ZDHHC7-stably knockdown or knockout cell lines using shRNA or CRISPR/Cas9-mediated knockout methods

Methods: We have generated two guide-RNAs for CRISPR/Cas-sytem to stably knockout ZDHHC7. In preliminary studies, we have validated these guide-RNAs. Due to antibody issue, it has been difficult to evaluate whether we have achieved complete knockout in cells.

Results: As shown in Figure 4, we should have knocked out ZDHHC7 in several cell lines. Guide-RNA1 has shown good knockout efficiency in PrEC cells. Further validation of the knockout is underway to show loss of ZDHHC7 expression (using different antibodies or qRT-PCR).

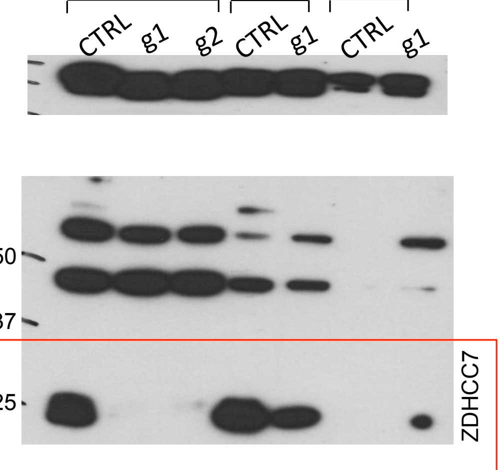

Figure 4. CRISPR-Cas mediated knockout of ZDHHC7 in cells.

Conclusion: Need to repeat and confirm that guide RNA1 or 2 can stably KO ZDHHC7 in cells.

What opportunities for training and professional development has the project provided?

Postdoctoral fellows and graduate students at the Cutaneous Biology Research Center (CBRC) receive in depth training within their PI's laboratory. They gain first hand experience in state of the art experimental approaches. As an important part of training, fellows are continuously challenged and prompted to prepare early written drafts of ongoing work, in which initial results are already framed within clearly formulated working hypotheses. Research goals are achieved through direct personal discussions with their PI supervisors, as well as other colleagues in the laboratory and more formal weekly lab meetings. There are a number of seminars and tutorials, held at various locations throughout the institution and Boston area that faculty, staff, pre and postdoctoral trainees, and graduate students are encouraged to attend. Each Tuesday at CBRC, a

tutorial is presented by a staff member (or pre or postdoctoral fellow or graduate student) on work in progress or review of fields that are being considered for expansion. The CBRC Seminar Series is presented monthly. World-renowned scientists from a variety of specialties are invited to spend the day meeting with faculty, having a private lunch with fellows and students and giving a seminar that is attended by staff throughout the institution.

Within our research groups, the PI provides continuous supervision, advice, and guidance related to ongoing research projects in the laboratory as well as career counseling and personal development.

This project has provided training and professional development of postdoctoral fellows in Wu lab: Baoen Chen and Carla Guarino. Both of them have gained significantly progresses in their molecular and cell biology, and chemical biology. Through interactions with our collaborator Jindan Yu's lab, they have also learned techniques to culture and test prostate cancer cell lines. We focused on improving their understanding to cell polarity and prostate cancer progression, and improving their technical skills of various experiments. I believe they are becoming more independent in terms of experimental design and interpretation.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

As we planned in the original SOW, we will finish the residual work to evaluate Scribble palmitoylation, localization in more cancer cell lines. In addition, we will validate additional ZDHHC7 antibodies to test the expression and knockout efficiency. We have also planned to use ZDHHC7 wildtype or the catalytic inactive mutant in cell lines to validate. As planned in Subtask 4, we will evaluate the expression junctional proteins, and carry out experiment to test Scribble palmitoylation-defecient mutant in prostate cancer cells. Furthermore, it was not originally proposed in the SOW, we have observed that MTDH, a critical regulator of metastasis is a potential substrate of ZDHHC7, and could bind to Scribble. Therefore, we will explore whether MTDH could also contribute to prostate cancer progression, in conjunction with Scribble mislocalization.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

We have demonstrated that a chemical approach using palmitoylation reporters to detect Scribble palmitoylation in prostate cancer cell lines, and correlated with its mislocalization. In addition, we found that loss of junctional structures are characteristic in prostate cancer cells, which could have significant impact in drug discovery and cancer research.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Actual or anticipated problems or delays and actions or plans to resolve them

As we stated in the Accomplishment section, the commercially available antibody of ZDHHC7 is problematic, and cannot sensitively detect endogenous ZDHHC7 levels in cell lysates. We will use qRT-PCR methods to evaluate the expression and knockout efficiency.

Changes that had a significant impact on expenditures

No change on the expenditure.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

No change.

Significant changes in use or care of human subjects

No change.

Significant changes in use or care of vertebrate animals

No change.

Significant changes in use of biohazards and/or select agents
No change.

6. PRODUCTS:

Journal publications:

Baoen Chen, Yang Sun, Jixiao Niu, Gopala K. Jarugumilli and Xu Wu* "Protein lipidation in cell signaling and diseases: function, regulation and therapeutic opportunities" *Cell Chem. Biol.* **2018**, 25 (7), 817-831. doi: 10.1016/j.chembiol.2018.05.003.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Xu Wu
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-1624-0143
Nearest person month worked:	3
Contribution to project:	Dr. Wu has supervised the research, designed the experiments and interpreted the results
Funding support:	MGH Institutional fund Melanoma Research Alliance NCI NIDDK

Name:	Carla Guarino
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	12
Contribution to project:	Dr. Guarino has carried out the studies of Scribble in prostate cancers. She has developed the biochemical methods to detect Scribble palmitoylation in prostate cancers.
Funding support:	N/A

Name:	Jindan Yu
Project Role:	PI
Researcher Identifier (e.g.	jindany

ORCID ID):	
Nearest person month worked:	1.80
Contribution to project:	Dr. Yu has supervised the research, designed the experiments and interpreted the results
Funding support:	NCI PCF DoD

Name:	Jian Jun Wei
Project Role:	Co-Investigator
Researcher Identifier (e.g. ORCID ID):	Weij03
Nearest person month worked:	0.30
Contribution to project:	Dr. Wei contributed to data analysis of Scribble expression in prostate cancer cells
Funding support:	NCI

Name:	Song Tan
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	5.0
Contribution to project:	Dr. Tan has carried out the analysis of Scribble expression in prostate cancers.
Funding support:	N/A

Name:	Gang Zhen
Project Role:	Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	0.48
Contribution to project:	Dr. Zhen has carried out the analysis of Scribble expression in prostate cancers.
Funding support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?
Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
A duplicated report will be submitted by the collaborative award.

9. APPENDICES:

Yes. PDF copy of the publication is attached.

Cell Chemical Biology
Review

Protein Lipidation in Cell Signaling and Diseases: Function, Regulation, and Therapeutic Opportunities

Baoen Chen,[1] Yang Sun,[1] Jixiao Niu,[1] Gopala K. Jarugumilli,[1] and Xu Wu[1,*]
[1]Cutaneous Biology Research Center, Massachusetts General Hospital, Harvard Medical School, 149, 13th St., Charlestown, MA 02129, USA
*Correspondence: xwu@cbrc2.mgh.harvard.edu
https://doi.org/10.1016/j.chembiol.2018.05.003

Protein lipidation is an important co- or posttranslational modification in which lipid moieties are covalently attached to proteins. Lipidation markedly increases the hydrophobicity of proteins, resulting in changes to their conformation, stability, membrane association, localization, trafficking, and binding affinity to their co-factors. Various lipids and lipid metabolites serve as protein lipidation moieties. The intracellular concentrations of these lipids and their derivatives are tightly regulated by cellular metabolism. Therefore, protein lipidation links the output of cellular metabolism to the regulation of protein function. Importantly, deregulation of protein lipidation has been linked to various diseases, including neurological disorders, metabolic diseases, and cancers. In this review, we highlight recent progress in our understanding of protein lipidation, in particular, S-palmitoylation and lysine fatty acylation, and we describe the importance of these modifications for protein regulation, cell signaling, and diseases. We further highlight opportunities and new strategies for targeting protein lipidation for therapeutic applications.

Introduction

Protein lipidation is a unique co- or posttranslational modification that plays a critical role in cell signaling, and dynamically regulates protein functions in response to extrinsic and intrinsic cues. Lipidation modulates the function of targeted proteins by increasing their binding affinity to biological membranes, rapidly switching their subcellular localizations, affecting folding and stability, and modulating association with other proteins. Proteins can be covalently modified by at least six types of lipids, including fatty acids, isoprenoids, sterols, phospholipids, glycosylphosphatidylinositol (GPI) anchors, and lipid-derived electrophiles (LDEs) (Figure 1). Fatty acids, such as 16-carbon palmitate, are building blocks for the biosynthesis of other complex lipid molecules in cells, and their intracellular concentrations are tightly regulated. Palmitate is biosynthesized by condensation reactions between acetyl-coenzyme A (CoA) and malonyl-CoA catalyzed by the fatty acid synthase (FASN) complex. Acetyl-CoA is generated from pyruvate and citrate, through pyruvate dehydrogenase and ATP citrate lyase, respectively. Malonyl-CoA is synthesized by acetyl-CoA carboxylase (ACC). Both acetyl-CoA and malonyl-CoA are byproducts of glucose metabolism and Krebs cycle (Figure 1). ACC is directly regulated by cellular metabolic regulator and energy sensor, AMP-activated protein kinase. Therefore, lipid biosynthesis is directly involved in cellular energy homeostasis. Deregulation of lipid metabolism plays a prominent role in human disease. However, the detailed mechanism linking deregulation of lipid metabolism to cell signaling and disease remains elusive. It is possible that alterations of lipid metabolism could affect the availability of lipid donors, thus affecting the global protein lipidation levels. Protein lipidation may partially contribute to the pathological consequences of the misregulated lipid metabolism, such as in cancers and metabolic diseases. It is important to understand the detailed regulatory mechanisms, the functions, and the pathological relevance of protein lipidation, which will ultimately lead to new therapeutics. In the following sections, we will first summarize the diversity of protein lipidation, and then focus our discussions primarily on protein fatty acylation.

Diversity of Protein Lipidation

Saturated and unsaturated fatty acids can attach to the cysteine, serine, or lysine residues of proteins, in a process known as fatty acylation. The 14-carbon myristate can be attached to the N-terminal glycine, catalyzed by N-myristoyl transferases (NMTs) as a stable and irreversible co-translational modification. Recent chemical proteomic studies suggest that more than 100 proteins are myristoylated in human cells (Thinon et al., 2014). Typically, myristoylation enhances protein-membrane association that is required for target protein proper localization and biological function. S-Palmitoylation (or S-acylation) is another major form of fatty acylation, in which the 16-carbon palmitate (or other fatty acids, for example, the 18-carbon stearic acid) are attached to cysteine residues. Due to the labile nature of the thioester bond, S-palmitoylation is reversible, and S-palmitoylated proteins can undergo cycles of acylation and deacylation in response to upstream signals (Rocks et al., 2010). In addition, fatty acyl groups can be attached to serine residues (O-palmitoylation) or the N terminus (so-called N-palmitoylation). To date, only a few proteins are known to be O- or N-palmitoylated. For example, Wnt proteins are modified on a conserved serine residue by a monounsaturated fatty acid, *cis*-palmitoleic acid (C16:1Δ9), mediated by a membrane-bound O-acyltransferase called Porcupine (Clevers and Nusse, 2012). Histone H4 can be palmitoylated at Ser45 by lysophosphatidylcholine acyltransferase 1 (LPCAT1) (Zou et al., 2011). The 8-carbon octanoyl group can modify the serine residue of the signaling peptide

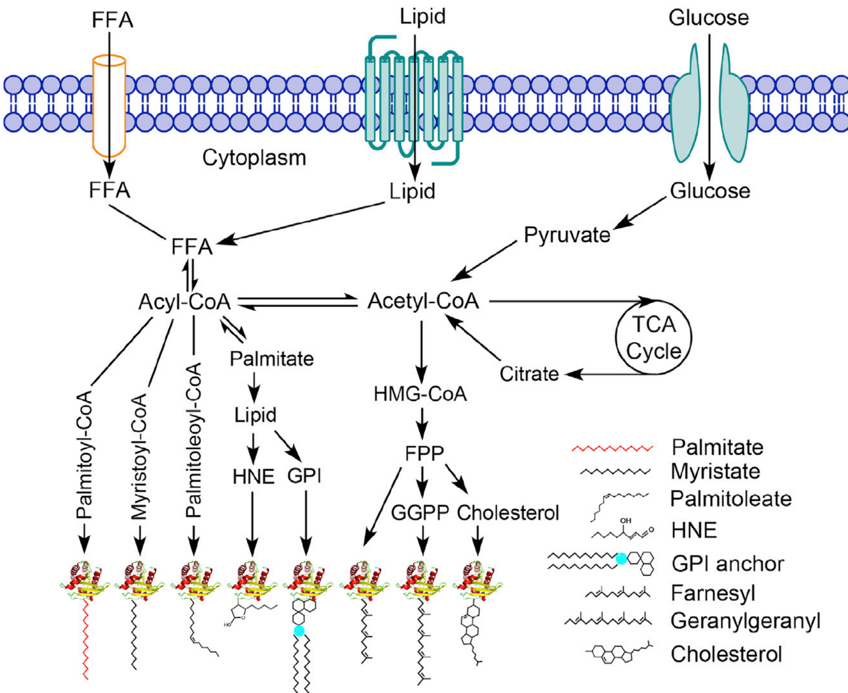

Figure 1. Protein Lipidation Links Lipid Metabolism to the Regulation of Protein Functions

Proteins can be modified by at least six types of lipid, including saturated and unsaturated fatty acids (palmitate, myristate, and palmitoleate, etc.), isoprenoids (farnesyl and geranylgeranyl), GPI anchors, cholesterols, phospholipids (not shown here), and lipid-derived electrophiles (HNE, etc.). Cellular lipid metabolism affects the availability of fatty acyl-CoA and other lipid derivatives, which are used as substrates for protein lipidation. The 16-carbon fatty acid, palmitate, is a critical intermediate for the biosynthesis of other lipids in the cells.

FFA, free fatty acid; HNE, 4-hydroxynonenal; GPI, glycosylphosphatidylinositol; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; TCA cycle, tricarboxylic acid cycle or Krebs cycle.

ghrelin by ghrelin O-acyltransferase (Gutierrez et al., 2008). Hedgehog (Hh) proteins are examples of N-palmitoylated proteins, where the N terminus of the signaling peptide is palmitoylated, catalyzed by Hedgehog acyltransferase (HHAT) (Buglino and Resh, 2008).

Modification of proteins by isoprenoids is known as prenylation. Most prenylated proteins contain a CAAX motif at their C terminus, in which the consensus Cys residue is modified by farnesyl (15-carbon) or geranylgeranyl (20-carbon) groups by farnesyltransferase or geranylgeranyltransferase I, respectively. The Ras-family of proteins, including K-, H-, and N-Ras, are well-studied prenylated proteins (Wang and Casey, 2016). Prenylation is generally considered as an irreversible modification, which alters the membrane affinity of proteins.

Covalent modification of proteins by cholesterols is uncommon. To date, the only well characterized cholesterol-modified proteins are Hedgehog proteins with which cholesterol forms an ester bond at the C-terminal glycine of the cleaved signaling peptide. Recently, chemical probes have been developed to identify other cholesterol-modified proteins (Heal et al., 2011). Smoothened, the co-receptor of Hh pathway, is cholesterol modified on the Asp95 (Xiao et al., 2017).

Phospholipids play critical roles in membrane formation and signal transduction. However, protein phospholipid modification is rare. The only known example is the autophagy-related protein Atg8/LC3, which is modified by phosphatidylethanolamine. This modification is mediated by multi-step conjugation processes, and is essential for the double-membrane formation of the autophagosome (Nakatogawa et al., 2007).

GPI anchor is a complex glycolipid that can be covalently attached to the C terminus of proteins as a posttranslational modification. GPIs are synthesized in the ER by sequentially adding monosaccharides, acyl groups, and phosphoethanolamine residues to phosphatidylinositol. The attachment of GPI to the protein mediated by GPI transamidase complex (Yu et al., 2013). About 1% of the eukaryotic proteins are modified by GPI anchors (Orlean and Menon, 2007). In addition to its role in targeting protein to the membrane, GPI anchor has many other biological functions, such as mediating immune response and inflammation, depending on the GPI structural composition (Tsai et al., 2012).

LDEs are reactive lipid metabolites produced by lipid peroxidation or other metabolic pathways via non-enzymatic and enzymatic mechanisms. Known LDEs include 4-hydroxy-2-nonenal, 15-deoxy-Δ12, 14-prostaglandin J2, and 2-*trans*-hexadecenal, etc. LDEs are able to form covalent adducts with nucleophilic residues of proteins, such as cysteine, lysine, and histidine via Michael addition (Chen et al., 2016b). Misregulation of lipid metabolism often leads to the accumulation of LDEs, which are involved in various pathological conditions, such as inflammation, genotoxicity, and tissue degeneration.

Therefore, protein lipidation can regulate many key biological functions of proteins through diverse types of lipids and lipid metabolites. However, it has been challenging to investigate the specific function and regulation of protein lipidation under physiological and pathological conditions due to limited tools and methods. Several recent reviews have discussed the functions of protein prenylation, myristoylation, and GPI modifications (Jiang et al., 2018; Wang and Casey, 2016; Zurzolo and Simons, 2016). Due to the limitation of space, rather than the importance, here we will briefly discuss different methods for studying protein lipidation, and then mainly focus on the function, regulation, and potential therapeutic targets of protein fatty acylation, especially S-palmitoylation of proteins, for which exciting new progress to understand its functions has been made in recent years.

Chemical and Biochemical Methods to Study Protein Lipidation

Traditionally, radioactive isotope-labeled lipids were used to analyze protein lipidation. Although still in practice, such

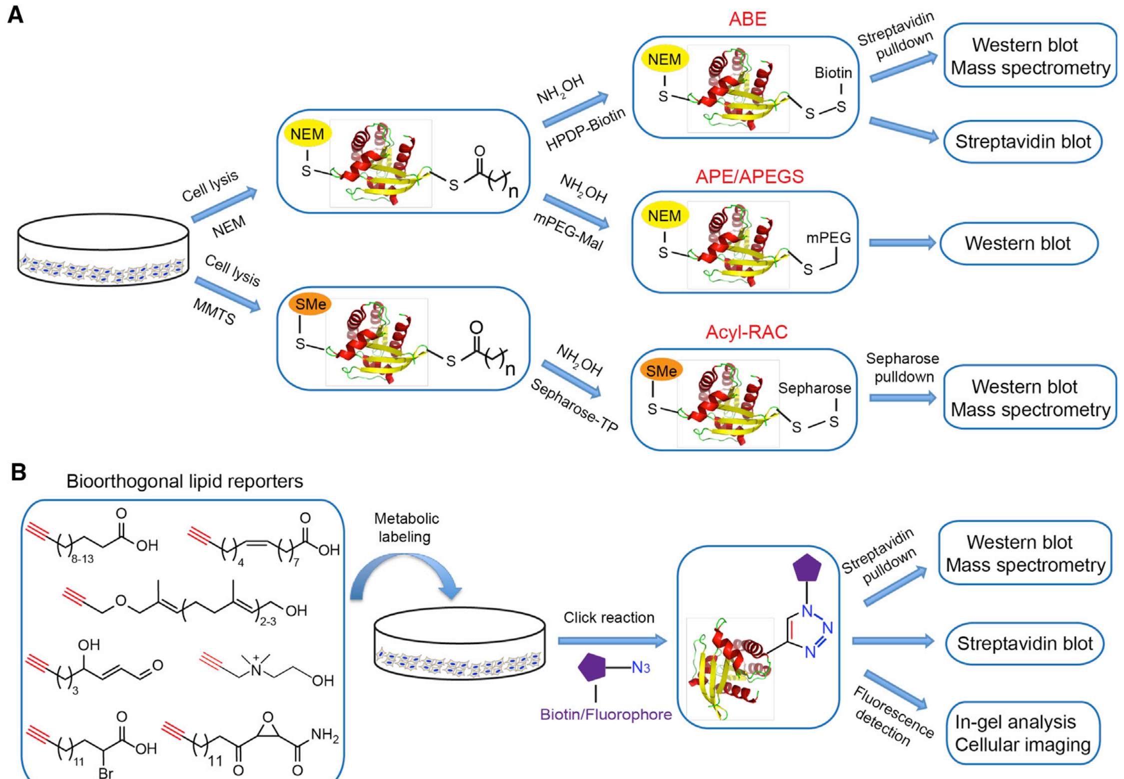

Figure 2. Chemical and Biochemical Methods to Detect Protein Lipidation
(A) Detection of S-acylated proteins by acyl exchange methods.
(B) Detection of protein lipidation using bioorthogonal chemical reporters.

methods are generally not preferred, due to sensitivity and safety issues. Although a recombinant antibody that specifically recognizes the conformation of palmitoylated PSD-95 was reported (Fukata et al., 2013), development of specific antibodies recognizing other lipid modifications has not been successful. S-Fatty acylation is particularly difficult for direct detection by chromatography or mass spectrometry methods, due to the high hydrophobicity of the lipid moiety and the unstable thioester linkage. For these reasons, several alternative methods have been developed to detect the modified cysteines or the tagged lipid moieties. Below we emphasize recent advances of acyl exchange methods and bioorthogonal chemical reporters.

Acyl-biotin exchange (ABE) was one of the first methods developed to detect S-acylation of cysteines, which converts the acyl modification to a stable biotin adduct. It was based on the high reactivity of the thioester bond, which can be readily removed by weak bases, such as hydroxylamine (Figure 2A). ABE has been widely used in proteomic and signaling studies. As S-acylation does not alter the protein mass significantly, and electric charge remains the same, S-acylated species usually do not migrate differently from the non-acylated proteins on protein gel electrophoresis, making it difficult to directly detect the levels and the stoichiometry of acylation of a protein of interest. Acyl-PEG exchange (APE) or acyl-PEGyl exchange gel shift is a mass-tag-labeling method to evaluate the levels of endogenous fatty-acylated proteins (Percher et al., 2016). Similar to ABE, upon liberating the acylated cysteines by hydroxylamine, the resulting free thiol is capped with PEG-N-ethylmaleimide to introduce a mass shift with respect to the non-acylated protein (Figure 2A). It allows easy detection of the mass shift by western blot, and does not require affinity enrichment. In addition, one can easily quantify the ratio of unmodified versus S-acylated proteins, or multiple sites of S-acylation. It has been shown that there are three S-acylated cysteine residues of interferon-induced transmembrane protein 3 (IFITM3) regulating its clustering in membrane compartments, which are critical for IFITM3 antiviral activity (Percher et al., 2016).

Although palmitoylation is often the most common type of S-acylation, other saturated and unsaturated acyl groups could be used. Since the original acyl groups are removed during the "exchange" process, it would be impossible to distinguish the different acyl groups originally attached to the proteins using ABE or APE alone. Therefore, to analyze the preference of lipid chains, lipid-based methods have to be used, such as bioorthogonal chemical reporters, which are usually terminal alkyne or azido derivatives of lipids (fatty acids, sterols, or isoprenoids). Exogenous chemical reporters can be taken up, converted to acyl-CoAs or other intermediates, and used as lipid donors in cells. Therefore, live cells are required for metabolic labeling of lipidation of proteins (Figure 2B). The chemical reporters can be detected by using copper-catalyzed azide-alkyne cycloaddition (click reaction) with biotin or fluorescent tags. ω-Alkynyl fatty acid probes are generally more efficient with higher sensitivity for detection compared with azido-fatty acids (Hannoush and Sun,

2010). ω-Alkynyl fatty acids with various chain lengths can be used to evaluate different type of lipidation, such as myristoylation, palmitoylation, stearoylation, and prenylation (Hannoush and Sun, 2010). In addition, chemical reporters also allow the detection of monounsaturated fatty acylation, prenylation, and N- or O-acylation, which could not be detected by acyl exchange methods (Hang and Linder, 2011). Furthermore, the reported click chemistry-proximity ligation imaging method can be used to detect subcellular localization of fatty-acylated proteins (Gao and Hannoush, 2014). As adding the alkynyl group extended the fatty chain for two extra carbons, one has to be careful when picking the appropriate reporters. We recommend using the total carbon chain, including the two carbons in the alkynyl group, to represent the acyl chain length. In addition, since the metabolic labeling requires high concentrations of reporters (50–100 μM), it would be difficult to evaluate the lipidation levels under physiological concentrations of endogenous acyl donors. Combination of the metabolic labeling of chemical reporters, as well as the acyl exchange methods enables clearer analysis of protein lipidation.

S-Palmitoylation as a Reversible Regulation in Cell Signaling

Palmitoyl-CoA is one of the most abundant fatty acyl-CoAs in cells, and a common intermediate for the biosynthesis of other complex lipid molecules. Proteomic analyses have identified over 1,000 S-palmitoylated proteins. The palmitoylated proteome could be even bigger, and the bioinformatics tool SwissPalm has predicted over 5,000 possible S-palmitoylated proteins (Blanc et al., 2015). Therefore, understanding the functions of S-palmitoylation is critical for cell signaling and therapeutics. It has been widely studied that S-palmitoylation plays an important role in regulating synaptic transmission. At the presynaptic side, many S-palmitoylated proteins, including GAD65, SNAP25, and CSP, are involved in neurotransmitter synthesis and release (Fukata and Fukata, 2010). Heterotrimeric G proteins, consisting of α, β, and γ subunits, are signal-transducers of G protein-coupled receptors (GPCRs). A recent study showed that S-palmitoylation of Gαi1 regulates its interaction with lipid rafts and affects its membrane microdomain localization (Alvarez et al., 2015).

S-Palmitoylation is one of the best-studied lipid modifications, which impact diverse biological processes. S-Palmitoylation can be dynamically regulated by zinc-finger DHHC (Asp-His-His-Cys)-containing palmitoyl acyltransferases (ZDHHC family of PATs) and acyl protein thioesterases (APT1 and APT2). In addition, some proteins can bind to palmitoyl-CoA directly and undergo PAT-independent autopalmitoylation. Below, we will focus on recent progresses of studying the novel functions and regulations of S-palmitoylation.

S-Palmitoylation in Regulation of Immune Receptor Functions

S-Palmitoylation of immune receptors plays an important role in the innate immune response (Chesarino et al., 2014; Mukai et al., 2016), linking lipid metabolism to host defense and immunity. The cyclic GMP-AMP synthase-stimulator of interferon genes (STING) pathway is critical in intracellular signaling of the innate immunity. STING is S-palmitoylated, and this modification promotes its clustering at the *trans*-Golgi membrane, where it recruits and activates TBK1 to phosphorylate IRF3 and induce the type I interferon response (Mukai et al., 2016). Pharmacological inhibition of STING S-palmitoylation, or mutation of its S-palmitoylation sites, blocks the type I interferon response. In addition, Golgi-localized ZDHHC proteins (ZDHHC3, 7, and 15) are potential candidate enzymes responsible for STING S-palmitoylation. Interestingly, ER-localized ZDHHC1 is also required for STING activation and recruitment of downstream effectors TBK1 and IRF3 upon DNA-virus infection (Zhou et al., 2014), implying the novel function of ZDHHC proteins in immune signaling. Further studies are needed to understand whether the ZDHHC3, 7, and 15, or ZDHHC1 play regulatory roles in STING-dependent immune responses.

Chemical proteomic studies showed that S-palmitoylation of several Toll-like receptors (TLRs) is important for their functions (Chesarino et al., 2014). For example, S-palmitoylation of TLR2 is required for its proper cell surface localization and nuclear factor κB (NF-κB)-dependent gene expression and cytokine production in response to PAM3CSK4. A palmitoylation-deficient mutant of TLR2 partially lost its ability to activate NF-κB-dependent gene expression. Moreover, it has been shown that S-palmitoylation of TLR2 is mediated by multiple ZDHHC-PATs, including ZDHHC2, 3, 6, 7, and 15.

S-Palmitoylation-Mediated Regulation of Cellular Junction Proteins, Receptors, and Kinases

Proper establishment of cellular junctions is critical for cell identity, and often disrupted in cancers. Several junctional proteins are S-palmitoylated. For example, claudin family members contain membrane-proximal cysteines, which are S-palmitoylated (Gunzel and Yu, 2013). The palmitoylation-deficient mutant localized less to tight junctions, and was localized in lysosomes instead, suggesting that palmitoylation regulates its trafficking. The junctional adhesion molecule C (JAM-C) is involved in cell migration, angiogenesis, cell adhesion and polarity. JAM-C is palmitoylated by ZDHHC7, thus promoting junctional localization and inhibiting cancer cell migration (Aramsangtienchai et al., 2017). The cell polarity protein Scribble (SCRIB) localizes to cell-cell junctions and plays a regulatory role in determining epithelial cell polarity. We recently found that S-palmitoylation of SCRIB at two conserved membrane-proximal cysteine residues is required for its proper membrane localization (Chen et al., 2016a). ZDHHC7 is the primary acyltransferase, and APT2 is a major depalmitoylating enzyme of SCRIB (Hernandez et al., 2017). Therefore, palmitoylation might be a conserved and common regulatory mechanism for regulating many junctional proteins and cell polarity.

S-Palmitoylation plays important regulatory roles in GPCR protein signaling. Agonist-induced activation of the human canonical GPCR protein β2AR led to enhanced S-palmitoylation. The cycle of β2AR palmitoylation and depalmitoylation is mediated by the ZDHHC9, 14, or 18, and APT1, respectively (Adachi et al., 2016). Rhodopsin forms dimers and higher oligomers upon activation by light, and can bind to lipid rafts (raftophilicity) (Seno and Hayashi, 2017). Palmitoylation is required for dimerization-dependent raftophilicity of rhodopsin, playing an important role in the supramolecular organization and response to dim light (Seno and Hayashi, 2017). The melanocortin-1 receptor (MC1R) is melanocyte-specific GPCR, and plays an important role in pigmentation. MC1R is palmitoylated at Cys315, critical

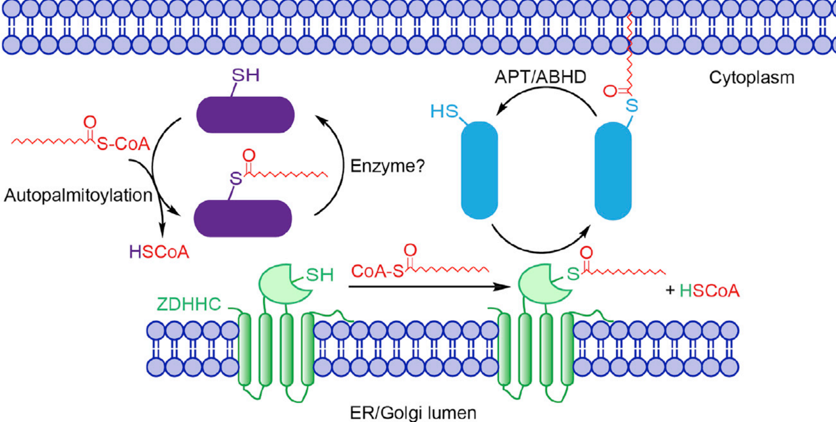

Figure 3. The Dynamic Regulation of Protein S-Palmitoylation
Palmitoyl acyltransferases (ZDHHC family enzymes, LPCAT) or autopalmitoylation are involved in adding palmitate to the Cys residue of proteins. The thioesterases or lipases (APTs and ABHDs) could remove the lipid chain from the proteins.

for MC1R activation and protecting against melanomagenesis (Chen et al., 2017a).

Tyrosine kinases, including receptor (receptor tyrosine kinases [RTKs]) and non-RTKs (nRTKs), function in a wide range of key signal transduction pathways. Both RTKs and nRTKs can be modified by S-palmitoylation. The Src family of nRTKs, including Src, Yes, Fyn, Fgr, Lyn, Hck, Lck, Blk, and Frk, are palmitoylated. S-Palmitoylation of Lyn is required for its protective role against chromosome missegregation during the cell cycle (Honda et al., 2016). The epidermal growth factor receptor (EGFR) is an important RTK associated with tumorigenesis. S-Palmitoylation of EGFR at Cys797 is required for its dimerization and activation, which is dependent on FASN (Bollu et al., 2015). Interestingly, EGFR is also S-palmitoylated at C-terminal Cys1025 and Cys1122, mediated by ZDHHC20 (Runkle et al., 2016). Both studies showed that S-palmitoylation plays a negative role in EGFR activation and that inhibition of EGFR S-palmitoylation may synergize with EGFR inhibitor-induced cell death.

S-Palmitoylation in Regulation of Transcription Factors

Estrogen receptor α (ERα) localizes to both the nucleus and the plasma membrane. S-Palmitoylation at Cys451 is critical for ERα translocation, plasma membrane localization, and interaction with the membrane protein caveolin-1. A mouse model with palmitoylation-deficient ERα (C451A) was generated to obtain membrane-specific loss of function (Adlanmerini et al., 2014). Membrane-bound ERα receptor is critical in ovarian function, including fertility and vascular physiology. In contrast, nuclear ERα is required to mediate the uterine response to estrogen ligands. Therefore, S-palmitoylation of ERα has distinct tissue-specific functions *in vivo*. Similarly, the androgen receptor (AR) is S-palmitoylated, which promotes AR membrane targeting. Several AR splicing variants are highly upregulated in castration-resistant prostate cancer (CRPC) cells. The AR8 variant was primarily localized on the plasma membrane, possibly through palmitoylation. The palmitoylation-deficient mutant of AR8 lost the membrane localization, and its ability to recruit Src and EGFR (Yang et al., 2011b). Therefore, palmitoylation-dependent membrane localization of AR variants may play a role in the development of CRPC.

The TEA domain transcription factors (TEAD1-4) are S-palmitoylated at conserved cysteine residues through a non-enzymatic autopalmitoylation mechanism (Chan et al., 2016). When incubated with physiological concentration of palmitoyl-CoA, TEAD2 undergoes efficient autopalmitoylation with apparent K_m of about 1 μM. We and others found that the palmitoyl chain is deeply buried into the conserved hydrophobic pocket (Chan et al., 2016; Noland et al., 2016). S-Palmitoylation is required for TEAD binding to the transcription co-activator YAP/TAZ and mediates the transcriptional output of the Hippo pathway (Chan et al., 2016). Interestingly, S-palmitoylation does not alter TEAD1 nuclear localization, and might function as a structural motif to rigidify the confirmation of TEADs. It remains unclear whether TEAD palmitoylation level is regulated through upstream signals, intracellular palmitoyl-CoA concentrations, and the depalmitoylation process.

Dynamic Regulation of S-Palmitoylation

S-Palmitoylation of proteins can be mediated by the ZDHHC family of palmitoylating enzymes (Lemonidis et al., 2015). These enzymes catalyze the addition of palmitate to the substrate proteins with a two-step process: autoacylation and acyl-enzyme intermediate formation, followed by transferring the acyl chain to a specific cysteine residue in the substrate protein (Figure 3) (Jennings and Linder, 2012). In mammals, ZDHHC proteins are encoded by more than 20 distinct genes. ZDHHC-PATs are commonly localized in the ER, Golgi, or the plasma membrane (Hentschel et al., 2016). Although some substrates of different ZDHHC proteins have been identified, the substrate specificity of these enzymes are not well understood (Table 1). One possibility is that the regions outside the DHHC domain determine the specificity of the substrate (Korycka et al., 2012). A recent structural study suggests that two conserved residues within the ankyrin repeat domain of ZDHHC17 play a critical role in binding to its substrate SNAP25b (Verardi et al., 2017). In addition, the intracellular localization of ZDHHCs might also specify the interactions with substrates. Genetic alterations of *ZDHHC* genes are observed in various diseases, including cancer and neurological disease (Lemonidis et al., 2015). As summarized in Table 2, several members of the family have been implicated in human physiological and pathological process. ZDHHC2 displays activities toward a broad range of substrates, and has been reported as a potential tumor suppressor in multiple human cancers (Jiang et al., 2015). In contrast, ZDHHC5 catalyzes EZH2 palmitoylation, and might function as an oncoprotein, contributing to the progression of p53-mutated glioma and non-small-cell lung cancer (Chen et al., 2017b; Tian et al., 2015). ZDHHC9 mediates palmitoylation of N-Ras and H-Ras, and alterations of ZDHHC9 are associated with X-linked mental retardation and colorectal cancer (Mansilla et al., 2007; Raymond et al., 2007). Loss of ZDHHC13 or ZDHHC21 leads to hair defects, but these two

Table 1. List of ZDHHC Palmitoyl Acyltransferases

Name	Localization	Known Targets	Tissue Distribution	Reference
ZDHHC1 (ANF377)	early ED	neurochondrin, EGFR	B, L, O	(Bollu et al., 2015)
ZDHHC2 (Ream, ZNF372)	recycling ED/PM	Lck, CKAP4/p63, CD9, CD151, SNAP25/23, PSD95, TLR2, EGFR	B, K, P, T, L, E	(Bollu et al., 2015)
ZDHHC3 (Godz, Gramp1)	Golgi	GABA, PSD-95, eNOS, STING, TLR2, Gα, STREX	LV, S, L, B, PS, C, PL, E	(Mukai et al., 2016; Tian et al., 2010)
ZDHHC4 (ZNF374)	Golgi	BACE1	O, T, K, SK	(Vetrivel et al., 2009)
ZDHHC5 (ZNF375)	PM	flotillin-2, STREX, EZH2	O, T, K, H, SK, LN, BL	(Chen et al., 2017b; Li et al., 2012)
ZDHHC6 (ZFN376)	ER	SSTR5, FLOT2, TLR2	K, LN	(Chesarino et al., 2014; Yang et al., 2010)
ZDHHC7 (ZNF370)	Golgi/PM	Glut4, Fas, CSP, GABA, PSD-95, eNOS, NA, SNAP25, PGR, AR, GAP43, STING, ERα, TLR2, Gα, STREX, Scribble, JAM-C	L, C, B, LV, PS, SK, K	(Aramsangtienchai et al., 2017; Chen et al., 2016a; Chesarino et al., 2014; Mukai et al., 2016)
ZDHHC8	Golgi/PM	ABCA1, PICK1, GRIP1, eNOS	B, L, O, E, P, K	(Thomas et al., 2012)
ZDHHC9 (ZNF380, CXorf11)	ER/Golgi	HRAS, NA, β2AR, STREX	B, PS, L, K, TL	(Adachi et al., 2016; Tian et al., 2010)
ZDHHC11 (ZNF399)	ER	unkown	B, O, T, L	
ZDHHC12 (ZNF400)	ER/Golgi	unkown	ST, ascites, SK, L, PS, B	
ZDHHC13	ER	HTT, SNAP25, MCAT, CTNND1, MC1R	U, B, ST, PL, T, C	(Chen et al., 2017a; Shen et al., 2017)
ZDHHC14	ER	β2AR	B, LN	(Adachi et al., 2016)
ZDHHC15	Golgi	CSP, PSD-95, Sortilin, CI-MPR, GAP43, STING, TLR2	B, O, K	(Chesarino et al., 2014; Mukai et al., 2016)
ZDHHC16	ER	c-ABL, JAB1	K, LV, SK, LN	(Zhang et al., 2006)
ZDHHC17 (HIP14, HIP3, HYPH)	Golgi/CV/SV	SNAP25, PSD-95, GAD65, synaptotagmin I, Huntingtin, CSP, STREX	B, U, E, L, TL	(Tian et al., 2010)
ZDHHC18	Golgi	LCK, β2AR	T, LN, BL	(Adachi et al., 2016; Greaves and Chamberlain, 2011)
ZDHHC19	ER	R-RAS	T	(Baumgart et al., 2010)
ZDHHC20	PM	EGFR	B, O, T, L, C, LN, BL	(Runkle et al., 2016)
ZDHHC21	Golgi/PM	FYN kinase, ERα, EGFR	B, T, U, E, LV	(Bollu et al., 2015)
ZDHHC22	ER/Golgi	KCNMA1	B	(Tian et al., 2012)
ZDHHC23		NOS1, KCNMA1	K	(Saitoh et al., 2004)

ED, endosome; ER, endoplasmic reticulum; PM, plasma membrane; CV, cytosolic vesicle; SV, synaptic vesicle; B, brain; L, lung; O, ovary; K, kidney; P, pancreas; T, testis; E, eye; LV, liver; S, spleen; PS, prostate; C, colon; PL, placenta; SK, skeletal muscle; LN, lymph node; BL, blood; U, uterus; ST, stomach; SK, skin; H, heart; TL, thalamus.
The data of subcellular localization and tissue distribution of ZDHHCs is also collected from (Ohno et al., 2006).

PATs show different substrate preferences (Mill et al., 2009; Saleem et al., 2010). In addition, ZDHHC13 plays an important role in mitochondrial function and metabolism in liver, and is related to skin carcinogenesis and Huntington's disease (Perez et al., 2015; Shen et al., 2017). ZDHHC21 deficiency results in endothelial inflammation and systemic inflammatory response syndrome (Beard et al., 2016). These biochemical and genetic data suggested that PATs are involved in diverse cellular functions, and that substrate specificity and functional redundancy of different ZDHHC-PATs needs careful and detailed studies. Specific inhibitors may need to be developed for different PATs, which will require significant structural, biochemical, and medicinal chemistry efforts.

As there are only 23 DHHC-family PATs (Fukata et al., 2004; Ohno et al., 2006), it is unlikely that they are responsible for all the palmitoylation activities in cells (more than 1,000 protein substrates are S-palmitoylated). Therefore, it is possible that many S-palmitoylated proteins are modified through ZDHHC-PAT-independent processes, such as autopalmitoylation. Previously, autopalmitoylation was considered to be a non-specific reaction that occurred when surface cysteine residues encountered a high concentration of palmitoyl-CoA. However, our studies of

Table 2. Physiological and Pathological Functions of ZDHHCs

Name	Physiological and Pathological Functions	Substrates	Reference
ZDHHC2	colorectal cancer (H) nasopharyngeal carcinoma neurophysiology	CKAP4/p63 (D) CD9, CD151 (H) Mir-155, PSD-95	(Fukata et al., 2013; Jiang et al., 2015)
ZDHHC3	neurophysiology	D2 dopamine receptor	(Ebersole et al., 2015)
ZDHHC4	neurophysiology	D2 dopamine receptor	(Ebersole et al., 2015)
ZDHHC5	NSCLC, glioma	EZH2	(Chen et al., 2017a; Tian et al., 2015)
ZDHHC8	schizophrenia (H, M) neurophysiology	D2 dopamine receptor	(Ebersole et al., 2015)
ZDHHC9	X-linked mental retardation (H) colorectal cancer (H)	NA and HRAS unknown	(Mansilla et al., 2007)
ZDHHC13	skin carcinogenesis (M)	unknown	(Perez et al., 2015)
ZDHHC17	Huntington's disease (M) synaptic transmission (D)	Huntingtin (M) CSP, SNAP25 (D)	(Ohyama et al., 2007)
ZDHHC20	lung cancer	EGFR	(Runkle et al., 2016)
ZDHHC21	endothelial inflammation	PLC-β1	(Beard et al., 2016)

H, human; M, mouse; D, *Drosophila*.

TEAD proteins, together with the studies of yeast Bet3 protein and myelin P0 glycoprotein, have shown that autopalmitoylation can happen under physiological conditions, with specific cysteine residues being modified (Bharadwaj and Bizzozero, 1995; Chan et al., 2016; Turnbull et al., 2005) (Figure 4). It has been noted that ZDHHC proteins also undergo "autopalmitoylation" when bound to palmitoyl-CoA, and form acyl-enzyme adduct (Mitchell et al., 2006; Roth et al., 2006). It is possible that many proteins have such intrinsic enzyme-like activities, and could form acyl-protein adducts. As shown in both the TEAD and Bet3 structures, the modified cysteine residue is located close to the palmitate-binding pocket, similar to the lipid-bound ZDHHC20 protein structure (Rana et al., 2018). It is possible that such an acylation process can be facilitated by proximity-mediated thioester exchange.

If autopalmitoylation can happen readily in the presence of acyl-CoA, how is this process regulated? Two possible mechanisms have been suggested: thiolate formation and the availability of acyl-CoA (Dietrich and Ungermann, 2004). At physiological pH (7.2–7.4), the spontaneous formation of thiolate to the cysteine thiol group (pKa ~8.5) is unlikely. However, in a proper local environment of folded proteins, the nucleophilicity of a cysteine can be potentially modulated by polarization of other side chains. The cellular acyl-CoA levels could also play a role. The conserved acyl-CoA binding protein (ACBP) binds free palmitoyl-CoA and maintains the intracellular concentration (Faergeman and Knudsen, 1997), which might buffer the excessive autopalmitoylation. The "autopalmitoylated" proteins and PATs might compete with ACBP for palmitoyl-CoA binding. Thus, only specific proteins with strong acyl-CoA binding could be autopalmitoylated. Furthermore, upstream regulators of lipid biosynthesis, such as FASN, may indirectly regulate autopalmitoylation through regulating fatty acid biosynthesis (Figure 4).

Palmitoyl protein thioesterases 1 and 2 (PPT1 and PPT2) remove long-chain fatty acids (usually palmitate) from S-fatty-acylated proteins during lysosome degradation (Segal-Salto et al., 2016). The function of PPTs is confined to the lysosomal degradation of S-fatty-acylated proteins. APT1 and APT2 depalmitoylate membrane-anchored proteins and play critical roles in palmitoylation turnover. For a long time, APTs were thought to be the only cytosolic thioesterases, responsible for almost all of the depalmitoylation processes. Pharmacological inhibition of APT1/2 activities block Ras trafficking and inhibit Ras oncogenic activities (Dekker et al., 2010). Recently, specific APT1 and APT2 inhibitors were developed, providing important chemical tools for dissecting the function of APTs (Adibekian et al., 2012; Won et al., 2016). Fluorescence-based depalmitoylation probes have been reported to visualize the enzymatic activity of APTs in cells and explore new mechanism of depalmitoylation (Kathayat et al., 2017).

However, enzymes responsible for depalmitoylation are more diverse than previously thought. α/β Hydrolase domain (ABHD) proteins have been suggested as potential depalmitoylases (Martin et al., 2011). Palmostatin B (Pal B), the inhibitor of APT1/2, also inhibits FASN, PNPLA6, and ABHD proteins (Lin and Conibear, 2015). ABHD17 removes palmitate from N-Ras efficiently (Lin and Conibear, 2015). Almost at the same time, by screening 38 serine hydrolases containing all ABHD proteins, ABHD17 proteins have been identified as physiological PSD-95 depalmitoylating enzymes and regulate local PSD-95 palmitoylation cycles in neurons (Yokoi et al., 2016). The mammalian ABHD family of proteins consists of at least 19 members. The substrates and the physiological functions of most of the ABHD proteins are unknown (Lord et al., 2013). Therefore, studies of ABHD activities might reveal additional depalmitoylases involved in signaling and diseases (Table 3).

Dynamic palmitoylation might also play a critical role in response to the extrinsic signaling stimulations. Stimulation of some receptors can lead to increased palmitoylation turnover or changed palmitoylation levels. Palmitoylation turnover on G proteins is accelerated upon ligand binding, and stimulation of their related GPCRs, thus regulating the downstream signaling activities (Barclay et al., 2005). Stimulation of the fibroblast growth factor receptor by FGF2 results in palmitoylation of neural cell adhesion molecule proteins (Ponimaskin et al., 2008). EGF stimulation can transiently inhibit depalmitoylation activity of

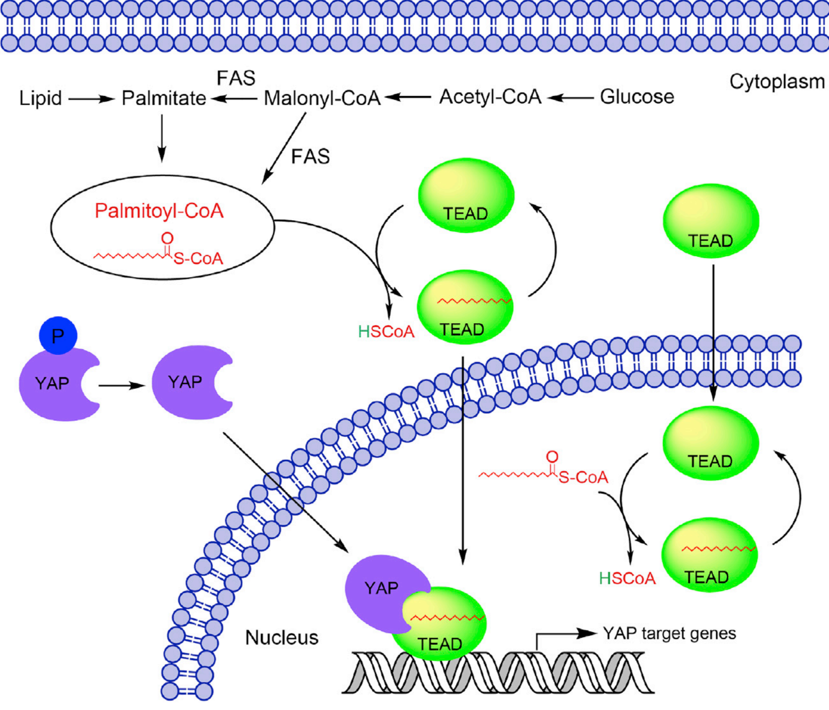

Figure 4. Function and Regulation of TEAD Autopalmitoylation

The Hippo pathway transcription factor TEAD is autopalmitoylated. Autopalmitoylation of TEAD may be regulated by intracellular concentration of palmitoyl-CoA, which is controlled by fatty acid metabolism. Palmitoylation of TEAD is required for its association with YAP and the regulation of transcriptional output of Hippo signaling.

APTs (Kathayat et al., 2017). Palmitoylation of GPCR protein MC1R is stimulated by α-melanocyte-stimulating hormone (α-MSH), and removal of α-MSH from the culture medium diminishes MC1R palmitoylation (Chen et al., 2017a).

Deacylases of Fatty Acylation of Lysine

Besides S-fatty acylation on the cysteine residues, N-fatty acylation on the lysine residues is emerging as an important modification. Lysine residues can be modified by various acyl groups, such as acetyl, butyryl, myristoyl and palmitoyl group. Sirtuin 6 (SIRT6) displays much lower deacetylase activity *in vitro* compared with other sirtuin family of deacetylases. However, free fatty acids, including myristic, oleic, and linoleic acids, at physiological concentrations, induce up to a 35-fold increase of SIRT6 catalytic efficiency (Feldman et al., 2013). Moreover, SIRT6's active site can accommodate longer acyl chains, such as myristoyl, and can remove long-chain fatty acyl groups from lysine residues, raising the possibility that SIRT6 could be a deacylase for N-fatty acylation (Jiang et al., 2013). SIRT6 promotes the secretion of tumor necrosis factor alpha (TNF-α) by removing the fatty acylation of K19 and K20 (Jiang et al., 2013). Recently, it has been reported that SIRT6 regulates the lysine de-fatty acylation of R-Ras2 (Zhang et al., 2017). Sirt6-null mouse embryonic fibroblast cells exhibit elevated R-Ras2 lysine fatty acylation. The histone deacetylase, HDAC8 can remove a variety of acyl groups (C2-C16 acyl chains) from Lys9 of the histone H3 peptide (H3K9) (Aramsangtienchai et al., 2016), and SIRT7 can remove long-chain fatty acyl groups more efficiently than acetyl group (Tong et al., 2017). Taken together, it is possible that N-fatty acylation of lysine residues could be a novel and dynamic regulation, and SIRT6, SIRT7, and HDAC8 could be the potential de-fatty acylases. It remains unclear how many proteins are N-fatty acylated on lysine residues under physiological conditions, and the function of lysine acylation is elusive. The fatty acyltransferase catalyzing the lysine N-fatty acylation remains unknown.

Opportunities in Drug Discovery

As protein lipidation plays a critical role in regulating protein function, and its deregulation is involved in many human diseases, targeting protein lipidation could be an important therapeutic strategy. Proof-of-concept studies have shown that several lipidation-related enzymes are attractive drug targets, and many inhibitors and chemical probes have been developed over the past years (Table 4).

Targeting Lipidation for Cancer Therapeutics

Ras proteins (K-, H, and N-Ras) are among the best known oncoproteins. H- and N-Ras are prenylated and then palmitoylated. Farnesyl transferase inhibitors have been developed to inhibit Ras prenylation; however, they failed to show significant clinical efficacy (Berndt et al., 2011). Alternatively, inhibitors of PDEδ have been developed. PDEδ binds to farnesyl chain of Ras. The inhibitors occupied the lipid binding pocket and blocked Ras binding (Zimmermann et al., 2013). H- and N-Ras undergo cycles of palmitoylation and depalmitoylation, which are important in the regulation of Ras localization and signaling activity. H- and N-Ras are palmitoylated by ZDHHC9 (Swarthout et al., 2005). Small molecules have been developed to disrupt protein palmitoylation process to inhibit Ras activity (Jennings et al., 2009), but their selectivity is not optimal. Given the functional redundancy of DHHC proteins, it might be a challenge to develop a specific DHHC inhibitor to achieve therapeutic efficacy.

The dual inhibitor of APT1 and APT2, Pal B, has been developed to inhibit depalmitoylation of H-Ras. N-Ras is localized to internal membranes upon Pal B treatment (Dekker et al., 2010). However, the effects of Pal B may be from the inhibition of other enzymes, such as ABHD17 (Lin and Conibear, 2015). In contrast to Pal B, ML348 and ML349 are selective inhibitors of APT1 and APT2 (Adibekian et al., 2012).

Although not discussed at length here, myristoylation also plays a crucial role in protein modification and control, particularly in the regulation of c-Abl kinases (Hantschel et al., 2003). Binding of myristoyl group into the conserved hydrophobic pocket locks the kinase domain in an inactive conformation. The oncoprotein Bcr-Abl lacks N-terminal myristoylation due to

CellPress

Table 3. Localization and Targets of Depalmitoylating Enzymes

Name	Localization	Known Targets	Reference
APT1	mainly localized to the cytosol	SNAP-23, Gsα, eNOS, H-Ras, KCMA1, GAP-43, MCAM, NMNAT2	(Milde and Coleman, 2014)
APT2	mainly localized to the cytosol	H-Ras, GAP-43, NMNAT2, Scribble	(Hernandez et al., 2017; Milde and Coleman, 2014)
PPT1	lysosome	H-Ras, palmitoyl-CoA	(Linder and Deschenes, 2007)
PPT2	lysosome	palmitoyl-CoA	(Linder and Deschenes, 2007)
ABHD17	endosome, plasma membrane	PSD95, NA, MAP6	(Lin and Conibear, 2015; Tortosa et al., 2017; Yokoi et al., 2016)

gene fusion, but the myristoyl-binding pocket is intact in the kinase domain, thus providing opportunities to design allosteric inhibitors. Small-molecule inhibitors (GNF-2 and GNF-5) bind to the myristoyl-binding pocket and alter the structural dynamics of the kinase domain, thus allosterically inhibiting the kinase. GNF-5 can overcome the resistance of kinase inhibitors (imatinib or nilotinib) (Zhang et al., 2010). Another inhibitor, DPH, binds to the myristoyl-binding site and prevents bending of the αI helix via steric hindrance, leading to c-Abl inactivation (Yang et al., 2011a). Other proteins with similar myristoyl-binding sites could be potential candidates for such strategies.

Aberrant Wnt signaling is involved in many types of cancer (Clevers and Nusse, 2012). Wnt proteins are fatty acylated by Porcupine (PORCN). Disruption of Wnt fatty acylation can suppress its secretion, thus inhibiting the growth of Wnt-dependent cancers. Several PORCN inhibitors have been developed. IWP compounds bind to PORCN and block its acyltransferase activity (Chen et al., 2009), and more potent analogs were also developed (You et al., 2016). GNF-1331 is a potent "hit" compound inhibiting Wnt secretion through binding to PORCN. Further optimization of GNF-1331 led to the identification of Wnt-C59 and GNF-6231 (Cheng et al., 2016). Furthermore, LGK974 (also known as WNT974) has been developed as a clinical candidate, with optimal pharmacokinetic and toxicity profiles. LGK974 inhibits Wnt signaling with impressive efficacy and is well tolerated *in vivo* (Liu et al., 2013). LGK974 is currently in clinical trials for treating patients with malignancies dependent on Wnt signaling (clinical trial number: NCT01351103).

Deregulation of the Hedgehog (Hh) pathway is associated with multiple human cancers, including medulloblastoma and basal cell carcinoma. Hh ligands are palmitoylated in the N terminus by the HHAT (Buglino and Resh, 2008), which is critical for the regulation of Hh signaling. A small molecule, RU-SKI 43, inhibits HHAT and blocks both autocrine and paracrine Hh signaling (Petrova et al., 2013). RU-SKI 43 decreases Gli-1 activation, and inhibits Akt/mTOR pathways and pancreatic cancer cell proliferation (Petrova et al., 2015). RU-SKI 43 is a useful chemical tool to explore HHAT functions in cancer. Further optimization and *in vivo* validation are needed before advancing to human clinical trials.

Targeting the deacylating enzymes provides an important approach for disruption of the fatty acylation cycles of S-palmitoylated proteins. Pal B is an inhibitor of APT1/2, which can partially disrupt Ras localization and oncogenic activities (Dekker et al., 2010). However, Pal B inhibits other proteins, including ABHDs, which might be responsible for its Ras depalmitoylation activities. In addition, it has not been shown that Pal B allows *in vivo* studies. Recently, specific inhibitors for APT1 and APT2 have been reported. ML349 and ML348 have good *in vivo* pharmacokinetic properties in mice (Adibekian et al., 2012). ML349 can rescue Snail-induced Scribble mislocalization and tumor suppressor function by selectively inhibiting APT2 activity, but not APT1 (Hernandez et al., 2017).

ABHD family proteins are novel potential deacylases (Lin and Conibear, 2015; Yokoi et al., 2016). Development of potent and selective small-molecule inhibitors of ABHDs will be important. In addition, SIRT6 deacylates TNF-α, thus promoting its secretion (Jiang et al., 2013). SIRT6 might regulate the secretion of many other proteins (Zhang et al., 2016). A specific inhibitor blocking SIRT6 deacylase activity will be helpful for determining its biological function and for drug discovery.

Targeting Lipidation for Infectious Diseases

Plasmodium falciparum, the parasite causing malaria in humans, expresses more than 400 palmitoylated proteins and over 30 myristoylated proteins (Jones et al., 2012; Wright et al., 2014). *P. falciparum* NMT has been validated as an attractive antimalarial drug target. NMT inhibition blocks the formation of essential parasite subcellular structures, leading to cell death (Wright et al., 2014). *Trypanosoma brucei*, the causative agent of human African trypanosomiasis, expresses an NMT that has been identified as a promising therapeutic target for sleeping sickness (Frearson et al., 2010). The inhibitor of the *T. brucei* NMT, DDD85646, inhibits the growth of a bloodstream form *T. brucei* in culture with half maximal effective concentration values between 0.8 and 3 nM (Brand et al., 2012).

Plasmodium PATs are also potential antimalarial drug targets. ZDHHC2 is essential for ookinete morphogenesis and malarial transmission (Santos et al., 2015). Recent studies have identified large families of PATs and palmitoylated proteins in other parasites as well, including *Toxoplasma gondii, T. brucei, Giardia lamblia*, and the fungal pathogen *Cryptococcus neoformans* (Brown et al., 2017). Therefore, inhibiting parasite PATs and thioesterases could be important therapeutic strategies for these devastating diseases.

Similarly, fatty acylation of viral and host proteins plays an important role in virus-host interactions and immune responses. Myristoylation of HIV Gag protein regulates Gag membrane binding and particle budding (Resh, 2004). A recent chemical proteomic study has identified many new acylated viral proteins during infection of the herpes simplex virus (Serwa et al., 2015). Lipidation of some host proteins may also be critical for the host resistance to viral infection. Palmitoylation of IFITM3 is required for its antiviral activity against influenza (Yount et al., 2010).

Table 4. Chemical Modulators of Protein Lipidation

Name	Structure	Mechanism and Biological Activity	Reference
2-Bromopalmitate (2BP)		non-specific, irreversible alkylating palmitoyl acyltransferases	(Resh, 2006)
Cerulenin		irreversible inhibitor of fatty acid synthase and palmitoyl acyltransferases	(Resh, 2006)
Compound V		reversible inhibitor of palmitoylation and myristoylation	(Jennings et al., 2009)
Palmostatin B		inhibitor of APT1/APT2/ABHD17 IC_{50} = 0.67 μM (Ras depalmitoylation)	(Dekker et al., 2010)
ML348		selective reversible inhibitor of APT1, IC_{50} = 0.21 μM	(Adibekian et al., 2012)
ML349		selective reversible inhibitor of APT2, IC_{50} = 0.144 μM	(Hernandez et al., 2017)
IWP-L6		porcupine inhibitor (EC_{50} = 0.5 nM), showing good stability in human plasma	(Wang et al., 2013)
LGK974 (Wnt974)		potent and specific Porcupine inhibitor (IC_{50} = 0.4 nM). In clinical trials	(Liu et al., 2013)
C59		a nanomolar inhibitor of Porcupine, displaying good bioavailability	(Proffitt et al., 2013)
ETC-159		potent and orally available Porcupine inhibitor (IC_{50} = 2.9 nM), in clinical trial	(Madan et al., 2016)
DDD85646		a potent inhibitor of *T. brucei* NMT with IC_{50} of 2 nM	(Brand et al., 2012)
Tipifarnib		a non-peptidomimetic quinolinone inhibitor of farnesyltransferase (FTase), being tested for cancers	(End et al., 2001)
Lonafarnib		a tricyclic derivative of carboxamide inhibitor of FTase, in clinical trial for progeria patients	(Liu et al., 2007)
BMS-214662		an inhibitor of FTase, showing potential antineoplastic activity	(Hunt et al., 2000)
Deltarasin		a potent inhibitor of PDEδ-KRAS interaction by occupying PDEδ farnesyl binding pocket, with a K_d of 0.04 μM	(Zimmermann et al., 2013)

(*Continued on next page*)

Table 4. *Continued*

Name	Structure	Mechanism and Biological Activity	Reference
GNF-5		a selective and allosteric Bcr-Abl inhibitor (IC_{50} = 0.22 μM), occupying the myristate-binding site	(Zhang et al., 2010)
GO-CoA-Tat		a peptide-based bisubstrate antagonist of ghrelin O-acyltransferase, improving glucose tolerance and reducing body weight in mice	(Barnett et al., 2010)

EC_{50}, half maximal effective concentration.

Therefore, targeting lipidated viral or host proteins may lead to new antiviral agents.

Future Outlook

There remain many unanswered questions about the regulation and function of protein lipidation, especially for fatty acylation of proteins. First, are there other classes of palmitoylating enzymes besides ZDHHC proteins? ZDHHC proteins are usually membrane bound and residing in the ER, Golgi, or plasma membrane. However, there are also many non-membrane-bound S-palmitoylated proteins. The discovery of LPCAT1 as a palmitoylating enzyme for histone H4 suggested that indeed other enzymes might have ''moonlight'' functions as fatty-acylating enzymes. Further studies may reveal new enzymatic activities involved in S-palmitoylation, and expand the current spectrum of palmitoylating enzymes. In addition, many S-palmitoylated proteins may undergo ''non-enzymatic'' autopalmitoylation, which will require careful characterization and validation.

Second, are there enzymes catalyzing the N-fatty acylation of Lys residues? It has been shown that many important signaling proteins can be fatty acylated on their Lys residues. Sirtuin family proteins have been shown to regulate de-fatty acylation. Eight types of short-chain fatty acylation of Lys residues in histones have been identified, whereas long-chain fatty acylation of histones has not been well studied (Sabari et al., 2017). Interestingly, histones S- and O-palmitoylation have been reported to regulate gene transcriptional activities (Wilson et al., 2011; Zou et al., 2011). New types of long-chain fatty acylation of histones lysine residues may exist. Therefore, it will be important to reveal the origin of N-palmitoylation on lysine resides, and to determine which enzymes catalyze such modifications.

Third, how is cellular lipid metabolism regulating protein lipidation? Lipid peroxidation products can covalently modify proteins and participate in the regulation of multiple biological processes (Chen et al., 2016b). Although many targets have been identified through chemoproteomic studies, detailed follow-up studies are required to validate these findings. In addition, FASN, which is often overexpressed in cancers, has been identified as a potential therapeutic target. These finding suggest that cancer cells might become ''addicted'' to elevated lipid levels. It will be important to validate and characterize the ''lipid addiction'' phenotype of cancers, and to reveal whether high lipid levels are linked to deregulated protein lipidation. Such work may provide insights into the role of lipidation in complex diseases, and bring novel therapeutic targets for drug development.

ACKNOWLEDGMENTS

We acknowledge the support from NIH R01CA181537 and R01DK107651, Melanoma Research Alliance (Samuel Fisher Memorial-Established Investigator Award), and Department of Defense (W81XWH-17-1-0361). We thank Drs. J. Hersch, M. Arkin, and C. Cosma for their comments of the manuscript.

AUTHOR CONTRIBUTIONS

B.C. and X.W. contributed to the concepts, writing, and editing. Y.S., J.N., and G.K.J contributed to the writing and organizing the references.

REFERENCES

Adachi, N., Hess, D.T., McLaughlin, P., and Stamler, J.S. (2016). S-Palmitoylation of a novel site in the beta2-adrenergic receptor associated with a novel intracellular itinerary. J. Biol. Chem. *291*, 20232–20246.

Adibekian, A., Martin, B.R., Chang, J.W., Hsu, K.L., Tsuboi, K., Bachovchin, D.A., Speers, A.E., Brown, S.J., Spicer, T., Fernandez-Vega, V., et al. (2012). Confirming target engagement for reversible inhibitors in vivo by kinetically tuned activity-based probes. J. Am. Chem. Soc. *134*, 10345–10348.

Adlanmerini, M., Solinhac, R., Abot, A., Fabre, A., Raymond-Letron, I., Guihot, A.L., Boudou, F., Sautier, L., Vessieres, E., Kim, S.H., et al. (2014). Mutation of the palmitoylation site of estrogen receptor alpha in vivo reveals tissue-specific roles for membrane versus nuclear actions. Proc. Natl. Acad. Sci. USA *111*, E283–E290.

Alvarez, R., Lopez, D.J., Casas, J., Llado, V., Higuera, M., Nagy, T., Barcelo, M., Busquets, X., and Escriba, P.V. (2015). G protein-membrane interactions I: galphai1 myristoyl and palmitoyl modifications in protein-lipid interactions and its implications in membrane microdomain localization. Biochim. Biophys. Acta *1851*, 1511–1520.

Aramsangtienchai, P., Spiegelman, N.A., He, B., Miller, S.P., Dai, L., Zhao, Y., and Lin, H. (2016). HDAC8 catalyzes the hydrolysis of long chain fatty acyl lysine. ACS Chem. Biol. *11*, 2685–2692.

Aramsangtienchai, P., Spiegelman, N.A., Cao, J., and Lin, H. (2017). S-Palmitoylation of junctional adhesion molecule c regulates its tight junction localization and cell migration. J. Biol. Chem. *292*, 5325–5334.

Barclay, E., O'Reilly, M., and Milligan, G. (2005). Activation of an alpha2A-adrenoceptor-Galphao1 fusion protein dynamically regulates the palmitoylation status of the G protein but not of the receptor. Biochem. J. *385*, 197–206.

Barnett, B.P., Hwang, Y., Taylor, M.S., Kirchner, H., Pfluger, P.T., Bernard, V., Lin, Y.Y., Bowers, E.M., Mukherjee, C., Song, W.J., et al. (2010). Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor. Science *330*, 1689–1692.

Baumgart, F., Corral-Escariz, M., Perez-Gil, J., and Rodriguez-Crespo, I. (2010). Palmitoylation of R-Ras by human DHHC19, a palmitoyl transferase with a CaaX box. Biochim. Biophys. Acta *1798*, 592–604.

Beard, R.S., Jr., Yang, X., Meegan, J.E., Overstreet, J.W., Yang, C.G., Elliott, J.A., Reynolds, J.J., Cha, B.J., Pivetti, C.D., Mitchell, D.A., et al. (2016). Palmitoyl acyltransferase DHHC21 mediates endothelial dysfunction in systemic inflammatory response syndrome. Nat. Commun. *7*, 12823.

Berndt, N., Hamilton, A.D., and Sebti, S.M. (2011). Targeting protein prenylation for cancer therapy. Nat. Rev. Cancer *11*, 775–791.

Bharadwaj, M., and Bizzozero, O.A. (1995). Myelin P0 glycoprotein and a synthetic peptide containing the palmitoylation site are both autoacylated. J. Neurochem. *65*, 1805–1815.

Blanc, M., David, F., Abrami, L., Migliozzi, D., Armand, F., Burgi, J., and van der Goot, F.G. (2015). SwissPalm: protein palmitoylation database. F1000Res. *4*, 261.

Bollu, L.R., Katreddy, R.R., Blessing, A.M., Pham, N., Zheng, B., Wu, X., and Weihua, Z. (2015). Intracellular activation of EGFR by fatty acid synthase dependent palmitoylation. Oncotarget *6*, 34992–35003.

Brand, S., Cleghorn, L.A., McElroy, S.P., Robinson, D.A., Smith, V.C., Hallyburton, I., Harrison, J.R., Norcross, N.R., Spinks, D., Bayliss, T., et al. (2012). Discovery of a novel class of orally active trypanocidal N-myristoyltransferase inhibitors. J. Med. Chem. *55*, 140–152.

Brown, R.W., Sharma, A.I., and Engman, D.M. (2017). Dynamic protein S-palmitoylation mediates parasite life cycle progression and diverse mechanisms of virulence. Crit. Rev. Biochem. Mol. Biol. *52*, 145–162.

Buglino, J.A., and Resh, M.D. (2008). Hhat is a palmitoylacyltransferase with specificity for N-palmitoylation of Sonic Hedgehog. J. Biol. Chem. *283*, 22076–22088.

Chan, P., Han, X., Zheng, B., DeRan, M., Yu, J., Jarugumilli, G.K., Deng, H., Pan, D., Luo, X., and Wu, X. (2016). Autopalmitoylation of TEAD proteins regulates transcriptional output of the Hippo pathway. Nat. Chem. Biol. *12*, 282–289.

Chen, B., Dodge, M.E., Tang, W., Lu, J., Ma, Z., Fan, C.W., Wei, S., Hao, W., Kilgore, J., Williams, N.S., et al. (2009). Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat. Chem. Biol. *5*, 100–107.

Chen, B., Zheng, B., DeRan, M., Jarugumilli, G.K., Fu, J., Brooks, Y.S., and Wu, X. (2016a). ZDHHC7-mediated S-palmitoylation of Scribble regulates cell polarity. Nat. Chem. Biol. *12*, 686–693.

Chen, Y., Qin, W., and Wang, C. (2016b). Chemoproteomic profiling of protein modifications by lipid-derived electrophiles. Curr. Opin. Chem. Biol. *30*, 37–45.

Chen, S., Zhu, B., Yin, C., Liu, W., Han, C., Chen, B., Liu, T., Li, X., Chen, X., Li, C., et al. (2017a). Palmitoylation-dependent activation of MC1R prevents melanomagenesis. Nature *549*, 399–403.

Chen, X., Ma, H., Wang, Z., Zhang, S., Yang, H., and Fang, Z. (2017b). EZH2 palmitoylation mediated by ZDHHC5 in p53 mutant glioma drives malignant development and progression. Cancer Res. *77*, 4998–5010.

Cheng, D., Liu, J., Han, D., Zhang, G., Gao, W., Hsieh, M.H., Ng, N., Kasibhatla, S., Tompkins, C., Li, J., et al. (2016). Discovery of pyridinyl acetamide derivatives as potent, selective, and orally bioavailable porcupine inhibitors. ACS Med. Chem. Lett. *7*, 676–680.

Chesarino, N.M., Hach, J.C., Chen, J.L., Zaro, B.W., Rajaram, M.V., Turner, J., Schlesinger, L.S., Pratt, M.R., Hang, H.C., and Yount, J.S. (2014). Chemoproteomics reveals Toll-like receptor fatty acylation. BMC Biol. *12*, 91.

Clevers, H., and Nusse, R. (2012). Wnt/beta-catenin signaling and disease. Cell *149*, 1192–1205.

Dekker, F.J., Rocks, O., Vartak, N., Menninger, S., Hedberg, C., Balamurugan, R., Wetzel, S., Renner, S., Gerauer, M., Scholermann, B., et al. (2010). Small-molecule inhibition of APT1 affects Ras localization and signaling. Nat. Chem. Biol. *6*, 449–456.

Dietrich, L.E., and Ungermann, C. (2004). On the mechanism of protein palmitoylation. EMBO Rep. *5*, 1053–1057.

Ebersole, B., Petko, J., Woll, M., Murakami, S., Sokolina, K., Wong, V., Stagljar, I., Luscher, B., and Levenson, R. (2015). Effect of C-terminal S-palmitoylation on D2 dopamine receptor trafficking and stability. PLoS One *10*, e0140661.

End, D.W., Smets, G., Todd, A.V., Applegate, T.L., Fuery, C.J., Angibaud, P., Venet, M., Sanz, G., Poignet, H., Skrzat, S., et al. (2001). Characterization of the antitumor effects of the selective farnesyl protein transferase inhibitor R115777 in vivo and in vitro. Cancer Res. *61*, 131–137.

Faergeman, N.J., and Knudsen, J. (1997). Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling. Biochem. J. *323* (Pt 1), 1–12.

Feldman, J.L., Baeza, J., and Denu, J.M. (2013). Activation of the protein deacetylase SIRT6 by long-chain fatty acids and widespread deacylation by mammalian sirtuins. J. Biol. Chem. *288*, 31350–31356.

Frearson, J.A., Brand, S., McElroy, S.P., Cleghorn, L.A., Smid, O., Stojanovski, L., Price, H.P., Guther, M.L., Torrie, L.S., Robinson, D.A., et al. (2010). N-Myristoyltransferase inhibitors as new leads to treat sleeping sickness. Nature *464*, 728–732.

Fukata, Y., and Fukata, M. (2010). Protein palmitoylation in neuronal development and synaptic plasticity. Nat. Rev. Neurosci. *11*, 161–175.

Fukata, M., Fukata, Y., Adesnik, H., Nicoll, R.A., and Bredt, D.S. (2004). Identification of PSD-95 palmitoylating enzymes. Neuron *44*, 987–996.

Fukata, Y., Dimitrov, A., Boncompain, G., Vielemeyer, O., Perez, F., and Fukata, M. (2013). Local palmitoylation cycles define activity-regulated postsynaptic subdomains. J. Cell Biol. *202*, 145–161.

Gao, X., and Hannoush, R.N. (2014). Single-cell in situ imaging of palmitoylation in fatty-acylated proteins. Nat. Protoc. *9*, 2607–2623.

Greaves, J., and Chamberlain, L.H. (2011). DHHC palmitoyl transferases: substrate interactions and (patho)physiology. Trends Biochem. Sci. *36*, 245–253.

Gunzel, D., and Yu, A.S. (2013). Claudins and the modulation of tight junction permeability. Physiol. Rev. *93*, 525–569.

Gutierrez, J.A., Solenberg, P.J., Perkins, D.R., Willency, J.A., Knierman, M.D., Jin, Z., Witcher, D.R., Luo, S., Onyia, J.E., and Hale, J.E. (2008). Ghrelin octanoylation mediated by an orphan lipid transferase. Proc. Natl. Acad. Sci. USA *105*, 6320–6325.

Hang, H.C., and Linder, M.E. (2011). Exploring protein lipidation with chemical biology. Chem. Rev. *111*, 6341–6358.

Hannoush, R.N., and Sun, J. (2010). The chemical toolbox for monitoring protein fatty acylation and prenylation. Nat. Chem. Biol. *6*, 498–506.

Hantschel, O., Nagar, B., Guettler, S., Kretzschmar, J., Dorey, K., Kuriyan, J., and Superti-Furga, G. (2003). A myristoyl/phosphotyrosine switch regulates c-Abl. Cell *112*, 845–857.

Heal, W.P., Jovanovic, B., Bessin, S., Wright, M.H., Magee, A.I., and Tate, E.W. (2011). Bioorthogonal chemical tagging of protein cholesterylation in living cells. Chem. Commun. (Camb.) *47*, 4081–4083.

Hentschel, A., Zahedi, R.P., and Ahrends, R. (2016). Protein lipid modifications – more than just a greasy ballast. Proteomics *16*, 759–782.

Hernandez, J.L., Davda, D., Cheung See Kit, M., Majmudar, J.D., Won, S.J., Gang, M., Pasupuleti, S.C., Choi, A.I., Bartkowiak, C.M., and Martin, B.R. (2017). APT2 inhibition restores scribble localization and S-palmitoylation in snail-transformed cells. Cell Chem. Biol. *24*, 87–97.

Honda, T., Soeda, S., Tsuda, K., Yamaguchi, C., Aoyama, K., Morinaga, T., Yuki, R., Nakayama, Y., Yamaguchi, N., and Yamaguchi, N. (2016). Protective role for lipid modifications of Src-family kinases against chromosome missegregation. Sci. Rep. *6*, 38751.

Hunt, J.T., Ding, C.Z., Batorsky, R., Bednarz, M., Bhide, R., Cho, Y., Chong, S., Chao, S., Gullo-Brown, J., Guo, P., et al. (2000). Discovery of (R)-7-cyano-2,3,4,5-tetrahydro-1-(1H-imidazol-4-ylmethyl)-3- (phenylmethyl)-4-(2-thienylsulfonyl)-1H-1,4-benzodiazepine (BMS-214662), a farnesyltransferase inhibitor with potent preclinical antitumor activity. J. Med. Chem. *43*, 3587–3595.

Jennings, B.C., and Linder, M.E. (2012). DHHC protein S-acyltransferases use similar ping-pong kinetic mechanisms but display different acyl-CoA specificities. J. Biol. Chem. *287*, 7236–7245.

Jennings, B.C., Nadolski, M.J., Ling, Y., Baker, M.B., Harrison, M.L., Deschenes, R.J., and Linder, M.E. (2009). 2-Bromopalmitate and 2-(2-hydroxy-5-nitro-benzylidene)-benzo[b]thiophen-3-one inhibit DHHC-mediated palmitoylation in vitro. J. Lipid Res. *50*, 233–242.

CellPress

Jiang, H., Khan, S., Wang, Y., Charron, G., He, B., Sebastian, C., Du, J., Kim, R., Ge, E., Mostoslavsky, R., et al. (2013). SIRT6 regulates TNF-alpha secretion through hydrolysis of long-chain fatty acyl lysine. Nature *496*, 110–113.

Jiang, Y.X., Du, Z.M., Jiao, L., Shao, Q., Fu, S., Shao, J.Y., Zhu, X.F., Ernberg, I., and Li, Y.H. (2015). Inhibition of MiR-155 suppresses cell migration in nasopharyngeal carcinoma through targeting ZDHHC2. Int. J. Clin. Exp. Med. *8*, 8472–8484.

Jiang, H., Zhang, X., Chen, X., Aramsangtienchai, P., Tong, Z., and Lin, H. (2018). Protein lipidation: occurrence, mechanisms, biological functions, and enabling technologies. Chem. Rev. *118*, 919–988.

Jones, M.L., Collins, M.O., Goulding, D., Choudhary, J.S., and Rayner, J.C. (2012). Analysis of protein palmitoylation reveals a pervasive role in *Plasmodium* development and pathogenesis. Cell Host Microbe *12*, 246–258.

Kathayat, R.S., Elvira, P.D., and Dickinson, B.C. (2017). A fluorescent probe for cysteine depalmitoylation reveals dynamic APT signaling. Nat. Chem. Biol. *13*, 150–152.

Korycka, J., Lach, A., Heger, E., Boguslawska, D.M., Wolny, M., Toporkiewicz, M., Augoff, K., Korzeniewski, J., and Sikorski, A.F. (2012). Human DHHC proteins: a spotlight on the hidden player of palmitoylation. Eur. J. Cell Biol. *91*, 107–117.

Lemonidis, K., Werno, M.W., Greaves, J., Diez-Ardanuy, C., Sanchez-Perez, M.C., Salaun, C., Thomson, D.M., and Chamberlain, L.H. (2015). The zDHHC family of S-acyltransferases. Biochem. Soc. Trans. *43*, 217–221.

Li, Y., Martin, B.R., Cravatt, B.F., and Hofmann, S.L. (2012). DHHC5 protein palmitoylates flotillin-2 and is rapidly degraded on induction of neuronal differentiation in cultured cells. J. Biol. Chem. *287*, 523–530.

Lin, D.T., and Conibear, E. (2015). ABHD17 proteins are novel protein depalmitoylases that regulate N-Ras palmitate turnover and subcellular localization. Elife *4*, e11306.

Linder, M.E., and Deschenes, R.J. (2007). Palmitoylation: policing protein stability and traffic. Nat. Rev. Mol. Cell. Biol. *8*, 74–84.

Liu, J., Pan, S., Hsieh, M.H., Ng, N., Sun, F., Wang, T., Kasibhatla, S., Schuller, A.G., Li, A.G., Cheng, D., et al. (2013). Targeting Wnt-driven cancer through the inhibition of porcupine by LGK974. Proc. Natl. Acad. Sci. USA *110*, 20224–20229.

Liu, G., Marrinan, C.H., Taylor, S.A., Black, S., Basso, A.D., Kirschmeier, P., Robert Bishop, W., Liu, M., and Long, B.J. (2007). Enhancement of the antitumor activity of tamoxifen and anastrozole by the farnesyltransferase inhibitor lonafarnib (SCH66336). Anticancer Drugs *18*, 923–931.

Lord, C.C., Thomas, G., and Brown, J.M. (2013). Mammalian alpha beta hydrolase domain (ABHD) proteins: lipid metabolizing enzymes at the interface of cell signaling and energy metabolism. Biochim. Biophys. Acta *1831*, 792–802.

Madan, B., Ke, Z., Harmston, N., Ho, S.Y., Frois, A.O., Alam, J., Jeyaraj, D.A., Pendharkar, V., Ghosh, K., Virshup, I.H., et al. (2016). Wnt addiction of genetically defined cancers reversed by PORCN inhibition. Oncogene *35*, 2197–2207.

Mansilla, F., Birkenkamp-Demtroder, K., Kruhoffer, M., Sorensen, F.B., Andersen, C.L., Laiho, P., Aaltonen, L.A., Verspaget, H.W., and Orntoft, T.F. (2007). Differential expression of DHHC9 in microsatellite stable and instable human colorectal cancer subgroups. Br. J. Cancer *96*, 1896–1903.

Martin, B.R., Wang, C., Adibekian, A., Tully, S.E., and Cravatt, B.F. (2011). Global profiling of dynamic protein palmitoylation. Nat. Methods *9*, 84–89.

Milde, S., and Coleman, M.P. (2014). Identification of palmitoyltransferase and thioesterase enzymes that control the subcellular localization of axon survival factor nicotinamide mononucleotide adenylyltransferase 2 (NMNAT2). J. Biol. Chem. *289*, 32858–32870.

Mill, P., Lee, A.W., Fukata, Y., Tsutsumi, R., Fukata, M., Keighren, M., Porter, R.M., McKie, L., Smyth, I., and Jackson, I.J. (2009). Palmitoylation regulates epidermal homeostasis and hair follicle differentiation. PLoS Genet. *5*, e1000748.

Mitchell, D.A., Vasudevan, A., Linder, M.E., and Deschenes, R.J. (2006). Protein palmitoylation by a family of DHHC protein S-acyltransferases. J. Lipid Res. *47*, 1118–1127.

Mukai, K., Konno, H., Akiba, T., Uemura, T., Waguri, S., Kobayashi, T., Barber, G.N., Arai, H., and Taguchi, T. (2016). Activation of STING requires palmitoylation at the Golgi. Nat. Commun. *7*, 11932.

Nakatogawa, H., Ichimura, Y., and Ohsumi, Y. (2007). Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. Cell *130*, 165–178.

Noland, C.L., Gierke, S., Schnier, P.D., Murray, J., Sandoval, W.N., Sagolla, M., Dey, A., Hannoush, R.N., Fairbrother, W.J., and Cunningham, C.N. (2016). Palmitoylation of TEAD transcription factors is required for their stability and function in hippo pathway signaling. Structure *24*, 179–186.

Ohno, Y., Kihara, A., Sano, T., and Igarashi, Y. (2006). Intracellular localization and tissue-specific distribution of human and yeast DHHC cysteine-rich domain-containing proteins. Biochim. Biophys. Acta *1761*, 474–483.

Ohyama, T., Verstreken, P., Ly, C.V., Rosenmund, T., Rajan, A., Tien, A.C., Haueter, C., Schulze, K.L., and Bellen, H.J. (2007). Huntingtin-interacting protein 14, a palmitoyl transferase required for exocytosis and targeting of CSP to synaptic vesicles. J. Cell Biol. *179*, 1481–1496.

Orlean, P., and Menon, A.K. (2007). Thematic review series: lipid posttranslational modifications. GPI anchoring of protein in yeast and mammalian cells, or: how we learned to stop worrying and love glycophospholipids. J. Lipid Res. *48*, 993–1011.

Percher, A., Ramakrishnan, S., Thinon, E., Yuan, X., Yount, J.S., and Hang, H.C. (2016). Mass-tag labeling reveals site-specific and endogenous levels of protein S-fatty acylation. Proc. Natl. Acad. Sci. USA *113*, 4302–4307.

Perez, C.J., Mecklenburg, L., Jaubert, J., Martinez-Santamaria, L., Iritani, B.M., Espejo, A., Napoli, E., Song, G., del Rio, M., DiGiovanni, J., et al. (2015). Increased susceptibility to skin carcinogenesis associated with a spontaneous mouse mutation in the palmitoyl transferase Zdhhc13 gene. J. Invest. Dermatol. *135*, 3133–3143.

Petrova, E., Rios-Esteves, J., Ouerfelli, O., Glickman, J.F., and Resh, M.D. (2013). Inhibitors of hedgehog acyltransferase block Sonic Hedgehog signaling. Nat. Chem. Biol. *9*, 247–249.

Petrova, E., Matevossian, A., and Resh, M.D. (2015). Hedgehog acyltransferase as a target in pancreatic ductal adenocarcinoma. Oncogene *34*, 263–268.

Ponimaskin, E., Dityateva, G., Ruonala, M.O., Fukata, M., Fukata, Y., Kobe, F., Wouters, F.S., Delling, M., Bredt, D.S., Schachner, M., et al. (2008). Fibroblast growth factor-regulated palmitoylation of the neural cell adhesion molecule determines neuronal morphogenesis. J. Neurosci. *28*, 8897–8907.

Proffitt, K.D., Madan, B., Ke, Z., Pendharkar, V., Ding, L., Lee, M.A., Hannoush, R.N., and Virshup, D.M. (2013). Pharmacological inhibition of the Wnt acyltransferase PORCN prevents growth of WNT-driven mammary cancer. Cancer Res. *73*, 502–507.

Rana, M.S., Kumar, P., Lee, C.J., Verardi, R., Rajashankar, K.R., and Banerjee, A. (2018). Fatty acyl recognition and transfer by an integral membrane S-acyltransferase. Science *359*, https://doi.org/10.1126/science.aao6326.

Raymond, F.L., Tarpey, P.S., Edkins, S., Tofts, C., O'Meara, S., Teague, J., Butler, A., Stevens, C., Barthorpe, S., Buck, G., et al. (2007). Mutations in ZDHHC9, which encodes a palmitoyltransferase of NRAS and HRAS, cause X-linked mental retardation associated with a Marfanoid habitus. Am. J. Hum. Genet. *80*, 982–987.

Resh, M.D. (2004). A myristoyl switch regulates membrane binding of HIV-1 Gag. Proc. Natl. Acad. Sci. USA *101*, 417–418.

Resh, M.D. (2006). Use of analogs and inhibitors to study the functional significance of protein palmitoylation. Methods *40*, 191–197.

Rocks, O., Gerauer, M., Vartak, N., Koch, S., Huang, Z.P., Pechlivanis, M., Kuhlmann, J., Brunsveld, L., Chandra, A., Ellinger, B., et al. (2010). The palmitoylation machinery is a spatially organizing system for peripheral membrane proteins. Cell *141*, 458–471.

Roth, A.F., Wan, J., Bailey, A.O., Sun, B., Kuchar, J.A., Green, W.N., Phinney, B.S., Yates, J.R., 3rd, and Davis, N.G. (2006). Global analysis of protein palmitoylation in yeast. Cell *125*, 1003–1013.

Runkle, K.B., Kharbanda, A., Stypulkowski, E., Cao, X.J., Wang, W., Garcia, B.A., and Witze, E.S. (2016). Inhibition of DHHC20-mediated EGFR palmitoylation creates a dependence on EGFR signaling. Mol. Cell *62*, 385–396.

Sabari, B.R., Zhang, D., Allis, C.D., and Zhao, Y. (2017). Metabolic regulation of gene expression through histone acylations. Nat. Rev. Mol. Cell Biol. *18*, 90–101.

Saitoh, F., Tian, Q.B., Okano, A., Sakagami, H., Kondo, H., and Suzuki, T. (2004). NIDD, a novel DHHC-containing protein, targets neuronal nitric-oxide synthase (nNOS) to the synaptic membrane through a PDZ-dependent interaction and regulates nNOS activity. J. Biol. Chem. *279*, 29461–29468.

Saleem, A.N., Chen, Y.H., Baek, H.J., Hsiao, Y.W., Huang, H.W., Kao, H.J., Liu, K.M., Shen, L.F., Song, I.W., Tu, C.P., et al. (2010). Mice with alopecia, osteoporosis, and systemic amyloidosis due to mutation in Zdhhc13, a gene coding for palmitoyl acyltransferase. PLoS Genet. *6*, e1000985.

Santos, J.M., Kehrer, J., Franke-Fayard, B., Frischknecht, F., Janse, C.J., and Mair, G.R. (2015). The *Plasmodium* palmitoyl-S-acyl-transferase DHHC2 is essential for ookinete morphogenesis and malaria transmission. Sci. Rep. *5*, 16034.

Segal-Salto, M., Sapir, T., and Reiner, O. (2016). Reversible cysteine acylation regulates the activity of human palmitoyl-protein thioesterase 1 (PPT1). PLoS One *11*, e0146466.

Seno, K., and Hayashi, F. (2017). Palmitoylation is a prerequisite for dimerization-dependent raftophilicity of rhodopsin. J. Biol. Chem. *292*, 15321–15328.

Serwa, R.A., Abaitua, F., Krause, E., Tate, E.W., and O'Hare, P. (2015). Systems analysis of protein fatty acylation in herpes simplex virus-infected cells using chemical proteomics. Chem. Biol. *22*, 1008–1017.

Shen, L.F., Chen, Y.J., Liu, K.M., Haddad, A.N.S., Song, I.W., Roan, H.Y., Chen, L.Y., Yen, J.J.Y., Chen, Y.J., Wu, J.Y., et al. (2017). Role of S-palmitoylation by ZDHHC13 in mitochondrial function and metabolism in liver. Sci. Rep. *7*, 2182.

Swarthout, J.T., Lobo, S., Farh, L., Croke, M.R., Greentree, W.K., Deschenes, R.J., and Linder, M.E. (2005). DHHC9 and GCP16 constitute a human protein fatty acyltransferase with specificity for H- and N-Ras. J. Biol. Chem. *280*, 31141–31148.

Thinon, E., Serwa, R.A., Broncel, M., Brannigan, J.A., Brassat, U., Wright, M.H., Heal, W.P., Wilkinson, A.J., Mann, D.J., and Tate, E.W. (2014). Global profiling of co- and post-translationally N-myristoylated proteomes in human cells. Nat. Commun. *5*, 4919.

Thomas, G.M., Hayashi, T., Chiu, S.L., Chen, C.M., and Huganir, R.L. (2012). Palmitoylation by DHHC5/8 targets GRIP1 to dendritic endosomes to regulate AMPA-R trafficking. Neuron *73*, 482–496.

Tian, H., Lu, J.Y., Shao, C., Huffman, K.E., Carstens, R.M., Larsen, J.E., Girard, L., Liu, H., Rodriguez-Canales, J., Frenkel, E.P., et al. (2015). Systematic siRNA screen unmasks NSCLC growth dependence by palmitoyltransferase DHHC5. Mol. Cancer Res. *13*, 784–794.

Tian, L., McClafferty, H., Jeffries, O., and Shipston, M.J. (2010). Multiple palmitoyltransferases are required for palmitoylation-dependent regulation of large conductance calcium- and voltage-activated potassium channels. J. Biol. Chem. *285*, 23954–23962.

Tian, L., McClafferty, H., Knaus, H.G., Ruth, P., and Shipston, M.J. (2012). Distinct acyl protein transferases and thioesterases control surface expression of calcium-activated potassium channels. J. Biol. Chem. *287*, 14718–14725.

Tong, Z., Wang, M., Wang, Y., Kim, D.D., Grenier, J.K., Cao, J., Sadhukhan, S., Hao, Q., and Lin, H. (2017). SIRT7 is an RNA-activated protein lysine deacylase. ACS Chem. Biol. *12*, 300–310.

Tortosa, E., Adolfs, Y., Fukata, M., Pasterkamp, R.J., Kapitein, L.C., and Hoogenraad, C.C. (2017). Dynamic palmitoylation targets MAP6 to the axon to promote microtubule stabilization during neuronal polarization. Neuron *94*, 809–825.e7.

Tsai, Y.H., Liu, X., and Seeberger, P.H. (2012). Chemical biology of glycosylphosphatidylinositol anchors. Angew. Chem. Int. Ed. *51*, 11438–11456.

Turnbull, A.P., Kummel, D., Prinz, B., Holz, C., Schultchen, J., Lang, C., Niesen, F.H., Hofmann, K.P., Delbruck, H., Behlke, J., et al. (2005). Structure of palmitoylated BET3: insights into TRAPP complex assembly and membrane localization. EMBO J. *24*, 875–884.

Verardi, R., Kim, J.S., Ghirlando, R., and Banerjee, A. (2017). Structural basis for substrate recognition by the ankyrin repeat domain of human DHHC17 palmitoyltransferase. Structure *25*, 1337–1347.e6.

Vetrivel, K.S., Meckler, X., Chen, Y., Nguyen, P.D., Seidah, N.G., Vassar, R., Wong, P.C., Fukata, M., Kounnas, M.Z., and Thinakaran, G. (2009). Alzheimer disease Abeta production in the absence of S-palmitoylation-dependent targeting of BACE1 to lipid rafts. J. Biol. Chem. *284*, 3793–3803.

Wang, M., and Casey, P.J. (2016). Protein prenylation: unique fats make their mark on biology. Nat. Rev. Mol. Cell Biol. *17*, 110–122.

Wang, X., Moon, J., Dodge, M.E., Pan, X., Zhang, L., Hanson, J.M., Tuladhar, R., Ma, Z., Shi, H., Williams, N.S., et al. (2013). The development of highly potent inhibitors for porcupine. J. Med. Chem. *56*, 2700–2704.

Wilson, J.P., Raghavan, A.S., Yang, Y.Y., Charron, G., and Hang, H.C. (2011). Proteomic analysis of fatty-acylated proteins in mammalian cells with chemical reporters reveals S-acylation of histone H3 variants. Mol. Cell Proteomics *10*, M110.001198.

Won, S.J., Davda, D., Labby, K.J., Hwang, S.Y., Pricer, R., Majmudar, J.D., Armacost, K.A., Rodriguez, L.A., Rodriguez, C.L., Chong, F.S., et al. (2016). Molecular mechanism for isoform-selective inhibition of acyl protein thioesterases 1 and 2 (APT1 and APT2). ACS Chem. Biol. *11*, 3374–3382.

Wright, M.H., Clough, B., Rackham, M.D., Rangachari, K., Brannigan, J.A., Grainger, M., Moss, D.K., Bottrill, A.R., Heal, W.P., Broncel, M., et al. (2014). Validation of N-myristoyltransferase as an antimalarial drug target using an integrated chemical biology approach. Nat. Chem. *6*, 112–121.

Xiao, X., Tang, J.J., Peng, C., Wang, Y., Fu, L., Qiu, Z.P., Xiong, Y., Yang, L.F., Cui, H.W., He, X.L., et al. (2017). Cholesterol modification of smoothened is required for hedgehog signaling. Mol. Cell *66*, 154–162.e10.

Yang, J., Campobasso, N., Biju, M.P., Fisher, K., Pan, X.Q., Cottom, J., Galbraith, S., Ho, T., Zhang, H., Hong, X., et al. (2011a). Discovery and characterization of a cell-permeable, small-molecule c-Abl kinase activator that binds to the myristoyl binding site. Chem. Biol. *18*, 177–186.

Yang, X., Guo, Z., Sun, F., Li, W., Alfano, A., Shimelis, H., Chen, M., Brodie, A.M., Chen, H., Xiao, Z., et al. (2011b). Novel membrane-associated androgen receptor splice variant potentiates proliferative and survival responses in prostate cancer cells. J. Biol. Chem. *286*, 36152–36160.

Yang, W., Di Vizio, D., Kirchner, M., Steen, H., and Freeman, M.R. (2010). Proteome scale characterization of human S-acylated proteins in lipid raft-enriched and non-raft membranes. Mol. Cell. Proteomics *9*, 54–70.

Yokoi, N., Fukata, Y., Sekiya, A., Murakami, T., Kobayashi, K., and Fukata, M. (2016). Identification of PSD-95 depalmitoylating enzymes. J. Neurosci. *36*, 6431–6444.

You, L., Zhang, C., Yarravarapu, N., Morlock, L., Wang, X., Zhang, L., Williams, N.S., Lum, L., and Chen, C. (2016). Development of a triazole class of highly potent Porcn inhibitors. Bioorg. Med. Chem. Lett. *26*, 5891–5895.

Yount, J.S., Moltedo, B., Yang, Y.Y., Charron, G., Moran, T.M., Lopez, C.B., and Hang, H.C. (2010). Palmitoylome profiling reveals S-palmitoylation-dependent antiviral activity of IFITM3. Nat. Chem. Biol. *6*, 610–614.

Yu, S., Guo, Z., Johnson, C., Gu, G., and Wu, Q. (2013). Recent progress in synthetic and biological studies of GPI anchors and GPI-anchored proteins. Curr. Opin. Chem. Biol. *17*, 1006–1013.

Zhang, J., Adrian, F.J., Jahnke, W., Cowan-Jacob, S.W., Li, A.G., Iacob, R.E., Sim, T., Powers, J., Dierks, C., Sun, F., et al. (2010). Targeting Bcr-Abl by combining allosteric with ATP-binding-site inhibitors. Nature *463*, 501–506.

Zhang, X., Khan, S., Jiang, H., Antonyak, M.A., Chen, X., Spiegelman, N.A., Shrimp, J.H., Cerione, R.A., and Lin, H. (2016). Identifying the functional contribution of the defatty-acylase activity of SIRT6. Nat. Chem. Biol. *12*, 614–620.

Zhang, X., Spiegelman, N.A., Nelson, O.D., Jing, H., and Lin, H. (2017). SIRT6 regulates Ras-related protein R-Ras2 by lysine defatty-acylation. Elife *6*, https://doi.org/10.7554/eLife.25158.

Zhang, F., Di, Y., Li, J., Shi, Y., Zhang, L., Wang, C., He, X., Liu, Y., Wan, D., Huo, K., et al. (2006). Molecular cloning and characterization of human Aph2 gene, involved in AP-1 regulation by interaction with JAB1. Biochim. Biophys. Acta *1759*, 514–525.

CellPress

Zhou, Q., Lin, H., Wang, S., Wang, S., Ran, Y., Liu, Y., Ye, W., Xiong, X., Zhong, B., Shu, H.B., et al. (2014). The ER-associated protein ZDHHC1 is a positive regulator of DNA virus-triggered, MITA/STING-dependent innate immune signaling. Cell Host Microbe *16*, 450–461.

Zimmermann, G., Papke, B., Ismail, S., Vartak, N., Chandra, A., Hoffmann, M., Hahn, S.A., Triola, G., Wittinghofer, A., Bastiaens, P.I., et al. (2013). Small molecule inhibition of the KRAS-PDEdelta interaction impairs oncogenic KRAS signalling. Nature *497*, 638–642.

Zou, C., Ellis, B.M., Smith, R.M., Chen, B.B., Zhao, Y., and Mallampalli, R.K. (2011). Acyl-CoA:lysophosphatidylcholine acyltransferase I (Lpcat1) catalyzes histone protein O-palmitoylation to regulate mRNA synthesis. J. Biol. Chem. *286*, 28019–28025.

Zurzolo, C., and Simons, K. (2016). Glycosylphosphatidylinositol-anchored proteins: membrane organization and transport. Biochim. Biophys. Acta *1858*, 632–639.