RGC Ref.: N_CUHK406/12 NSFC Ref. : 31261160490
(please insert ref. above)

The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Mechanistic Analysis of Palmitate Transferase DHHC11 that Regulates Multiple Developmental and Cellular Processes
棕榈酰转移酶 DHHC11 调控植物生长发育的分子及细胞机理研究

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Professor JIANG Liwen	Professor ZHANG Yan
Post	Professor	Professor
Unit / Department / Institution	School of Life Sciences, CUHK	College of Life Sciences, Shandong Agricultural Univ. State Key Lab of Crop Biology
Contact Information	ljiang@cuhk.edu.hk	yzhang@sdau.edu.cn
Co-investigator(s) *(with title and institution)*		Researcher, Sha Li Researcher, Liang-Zi Zhou Researcher, Xin-Ying Zhao Researcher, Fu-Rong Ge (Shandong Agricultural University)

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	1-1-2013		
Project Completion date	31-12-2016		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	31-12-2017		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Characterizing DHHC11-dependent developmental processes and abiotic stress responses.
2. Analyzing subcellular localization of DHHC11 and identifying critical residues or motifs determining its subcellular localization.
3. Identifying DHHC11-regulated vacuolar trafficking routes for tonoplast proteins.
4. Identifying interacting proteins of DHHC11 and building an interacting network centred on DHHC11.

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

Post-translational modifications, such as phosphorylation and lipidation, play important roles in the subcellular localization, interaction, and function of substrate proteins. Protein S-acylation, or palmitoylation is an evolutionarily conserved post-translation modification that catalyzes the reversible addition of palmitate group to substrate cysteine residues. A large number of proteins in eukaryotes have been demonstrated to be palmitoylation substrates. However, functional analysis on the enzymes catalyzing this process, i.e. protein S-acyltransferases (PATs), was scarce. There was only one plant PAT gene with demonstrated function. The purpose of this project is to understand the function of plant PATs in growth, development, as well as responses to environmental cues. Detailed research findings are listed as follows.

1. We have reported functional analysis of *PAT10* (*DHHC11*) in Arabidopsis. *PAT10* is constitutively expressed. Functional loss of *PAT10* resulted in pleiotropic developmental defects, including reduced vegetative and reproductive growth, sporophytic and gametophytic male defects, and compromised ability of pistils to support pollen tube growth. Both cell expansion and cell division were reduced in *pat10*. Using stable transgenic plants for fluorescent colabeling, we showed that PAT10 localizes at vacuolar membrane but not at Golgi or any post-Golgi vesicles. In addition to the developmental defects, mutants of *PAT10* were hypersensitive to salt stresses. We further identified a subfamily of calcineurin B–like proteins (CBLs) as putative substrates of PAT10. Localization of CBL2, CBL3, and CBL6 at the tonoplast depended on functional PAT10. Our results demonstrate that PAT10-mediated protein palmitoylation at the tonoplast is critical for development and salt tolerance in Arabidopsis. Results were published on the Plant Cell (2013).

Related research:
With the support of this grant to graduate students, we have also performed other related researches. We thus consider these publications as the research output of this grant. Selected related research findings are listed as follows.

2. PAT14 plays an evolutionarily conserved role in SA-mediated leaf senescence. We reported the characterization of PAT14, a Golgi-localized PAT. Functional loss of *PAT14* resulted in precocious leaf senescence after floral transition. Transcriptomic data indicated that PAT14 mediates SA biosynthesis and signaling. Indeed, genetic interference of the NPR1-mediated SA signaling abolished the precocious leaf senescence induced by *PAT14* loss-of-function. Interestingly, we have isolated the maize and wheat homologs of Arabidopsis PAT14 and found out that they have conserved function. Results were published on Scientific Reports (2016).

3. Prenylation, the post-translational attachment of prenyl groups to substrate proteins, can affect their distribution and interactomes. Prenylation of ROPs by PLP mediates their membrane association and stability during root hair growth. Prenylation is a post-translational modification that anchors soluble proteins to membranes. Small GTPase ROPs are substrates of prenylation. We reported that PLP-mediated prenylation, together with GDI and palmitoylation, controls ROP signaling in root hairs. Results were published on Plant Journal (2016).

4. Protein palmitoylation, which is critical for membrane association and subcellular targeting of many signaling proteins, is catalyzed mainly by protein S-acyl transferases. By combining pharmacological and genetic approaches and using root hairs as a model, we show that protein palmitoylation, regulated by protein S-acyl transferases at different endomembrane compartments such as the Golgi and the vacuole, is critical for the polar

growth of root hairs in Arabidopsis. Inhibition of protein palmitoylation by 2-BP disturbed key intracellular activities in root hairs. Although some of these effects are likely indirect, the cytological data reported here will contribute to a deep understanding of protein palmitoylation during tip growth in plants. Results were published on BMC Plant Biology (2015).

5. Characterization of MON1 function in pollen development in Arabidopsis. Programmed cell death (PCD)-triggered degradation of plant tapetum is essential for microspore development and pollen coat formation; however, little is known about the cellular mechanism regulating tapetal PCD. Here, we demonstrate that Rab7-mediated vacuolar transport of tapetum degradation-related cysteine proteases is crucial for tapetal PCD and pollen development in Arabidopsis, with the following evidence: (1) The *monensin sensitivity1* (*mon1*) mutants, which are defective in Rab7 activation, showed impaired male fertility due to a combined defect in both tapetum and male gametophyte development. (2) In anthers, MON1 showed preferential high-level expression in tapetal cell layers and pollen. (3) The *mon1* mutants exhibited delayed tapetum degeneration and tapetal PCD, resulting in abnormal pollen coat formation and decreased male fertility. (4) MON1/CCZ1-mediated Rab7 activation was indispensable for vacuolar trafficking of tapetum degradation-related cysteine proteases, supporting that PCD-triggered tapetum degeneration requires Rab7-mediated vacuolar trafficking of these cysteine proteases. (5) *MON1* mutations also resulted in defective pollen germination and tube growth. Taken together, tapetal PCD and pollen development require successful MON1/CCZ1-mediated vacuolar transport in Arabidopsis. Results were published on the Plant Physiology (2017).

Potential for further development of the research and the proposed course of action *(maximum half a page)*

The research projects have been successfully addressed. No further development of research is proposed.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Palmitoylation regulates enzyme activity, protein stability, subcellular localization, and intracellular sorting. Many plant proteins are palmitoylated. However, little is known about protein S-acyl transferases (PATs), which catalyze palmitoylation. Here, we report that the tonoplast-localized PAT10 (DHHC11) is critical for development and salt tolerance in Arabidopsis thaliana. We have identified several calcineurin B–like proteins as the substrates for PAT10 palmitoylation, by using the cell and molecular biology tools. We propose that PAT10-mediated palmitoylation is critical for vacuolar function by regulating membrane association or the activities of tonoplast proteins.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications	Author(s)	Title and Journal/ Book	Submitted	Attached	Acknowl	Accessi

Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	***bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	*(with the volume, pages and other necessary publishing details specified)*	to RGC *(indicate the year ending of the relevant progress report)*	to this report *(Yes or No)*	edged the support of this Joint Research Scheme *(Yes or No)*	ble from the institutional repository *(Yes or No)*
2014				Gao C, Cai Y, Wang Y, Kang BH, Aniento F, Robinson DG and ***Jiang L**	Retention mechanisms for ER and Golgi membrane proteins. *Trends in Plant Science* 19(8):508-515	2014	Yes	Yes	No
2014				Cui Y, Zhao Q, Gao C, Ding Y, Zeng YL, Ueda T, Nakano A and ***Jiang L**	Activation of the Rab7 GTPase by the MON1-CCZ1 complex is essential for PVC-to-vacuole trafficking and plant growth in *Arabidopsis*. *The Plant Cell.* 26(5): 2080-2097.	2014	Yes	Yes	No
2014				Wang X, Cai Y, Wang H, Zeng Y, Zhuang X, Li B and ***Jiang L**	*Trans*-Golgi network-located AP1 gamma adaptins mediate dileucine motif-directed vacuolar targeting in *Arabidopsis*. *The Plant Cell* 26(10): 4102-18.	2014	Yes	Yes	No
2015				Gao C, Zhuang X, Cui Y, Fu X, He Y, Zhao Q, Zeng Y, Shen J, Luo M and ***Jiang L**	Dual roles of an Arabidopsis ESCRT component FREE1 in regulating vacuolar protein transport and autophagic degradation. *Proceedings of the National Academy of Sciences of the United States of America* 112(6): 1886-91.	No	Yes	Yes	No

2015				Zhuang X, Cui Y, Gao C and ***Jiang L**	Endocytic and autophagic pathways crosstalk in plants. *Current Opinion in Plant Biology* 28:39-47.	No	Yes	Yes	No
2015				Zeng Y, Chung KP, Li B, Lai CM, Lam SK, Wang X, Cui Y, Gao C, Luo M, Wong KB, Schekman R, and ***Jiang L**	Unique COPII component AtSar1a/AtSec23a pair is required for the distinct function of protein ER export in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America* 112(46):14360-5.	No	Yes	Yes	No
2015				Zhang, Li, Feng, Zhao, Ge, Zhang, Li	Protein palmitoylation is critical for the polar growth of root hairs in Arabidopsis. *BMC Plant Biology* 15;50	No	Yes	Yes (NSFC: 31261160490)	No
2016				Cui Y, Shen J, Gao C, Zhuang X, Wang J and ***Jiang L**	Biogenesis of Plant Prevacuolar Multivesicular Bodies. *Molecular Plant* 9(6):774-86.	No	Yes	Yes	No
2016				Cui Y, Gao C, Zhao Q and ***Jiang L**	Using Fluorescent Protein Fusions to Study Protein Subcellular Localization and Dynamics in Plant Cells. *Methods in Molecular Biology* 1474: 113-123	No	Yes	Yes	No

2016				Zhao, Wang, Song, Wang, Kang, Zhang, Li	Precocious leaf senescence by functional loss of PROTEIN S-ACYLTRANSFERASE14 involves the NPR1-dependent salicylic acid signaling. *Scientific Report* 6, 20309	No	Yes	Yes (NSFC: 31261160490)	No
2016				Chai, Ge, Feng, Li, Zhang	PLURIPETALA mediates ROP2 localization and stability in parallel to SCN1 but synergistically with TIP1 in root hairs. *The Plant Journal* 86, 413-426	No	Yes	Yes (NSFC: 31261160490)	No
2017				Cui Y, Zhao Q, Xie HT, Wong WS, Wang X, Gao C, Ding Y, Tan Y, Ueda T, **Zhang Y** and ***Jiang L**	MONENSIN SENSITIVITY 1 (MON1)/CALCIUM CAFFEINE ZINC SENSITIVITY 1 (CCZ1)-mediated Rab7 activation regulates tapetal programmed cell death and pollen development. P*lant Physiology* 173(1):206-218.	No	Yes	Yes	No

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*

June 2013 Suzhou, China	Biogenesis and function of COPII Vesicles in Plants.	Cold Spring Harbor Asia - Plant Cell and Developmental Biology	2014	Yes	Yes	No
June 2013 Suzhou, China	Functional Study of Endomembrane Proteins (EMPs) in Plant	Cold Spring Harbor Asia - Plant Cell and Developmental Biology	2014	Yes	Yes	No
June 2013 Suzhou, China	Golgi Retention Mechanisms of Endomembrane Membrane Proteins (EMPs) in Eukaryotes	Cold Spring Harbor Asia - Plant Cell and Developmental Biology	2014	Yes	Yes	No
Apr 2015 Shenzhen, China	Functional Roles of MON1-CCZ1 Complex in Vacuolar Trafficking	CSCB The Biennial Conference and the 15th Congress of the Chinese Society for Cell Biology	No	Yes	Yes	No
Apr 2015 Shenzhen, China	Mon1 Repressor Mutant Screening for new Components involved in membrane fusion, vacuolar trafficking and vacuole biogenesis	CSCB The Biennial Conference and the 15th Congress of the Chinese Society for Cell Biology	No	Yes	Yes	No
Aug 2016 France	European Network for Plant Endomembrane Research 2016	Prevacuolar compartments contribute to the vacuole biogenesis in Arabidopsis root cortex and epidermis	No	Yes	Yes	No

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation

Zhuang Xiaohong	PhD	1/8/2008	1/8/2013
Shen Jinbo	PhD	1/8/2009	1/8/2013
Cui Yong	PhD	1/8/2011	1/9/2014
Lin Youshun	MPhil	1/8/2012	31/12/2014
Joanne WOO	MPhil	1/8/2012	1/6/2015
Lai Ching Man	MPhil	1/8/2012	1/3/2015
Wang Xiangfeng	PhD	3/8/2009	1/6/2015
Wang Juan	PhD	1/8/2010	1/12/2015
Zeng Yonglun	PhD	12/7/2010	1/1/2016

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*