RGC Ref. No.:
UGC/FDS25/M09/19
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR
THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines:
1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Molecular Characterisation of Lipid-associated Protein Trafficking Pathway in Plant Cells

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

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr. Angus Ho-yin LAW / Assistant Professor	School of General Education & Languages, Technological and Higher Education Institute of Hong Kong
Co-Investigator(s)	NIL	NIL
Others	NIL	NIL

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	1 Jan 2020	1 Nov 2019	22 Oct 2019
Project Completion Date	31 Dec 2022	30 Apr 2023	19 Nov 2022
Duration *(in month)*	36 months	42 months	19 Nov 2022
Deadline for Submission of Completion Report	31 Dec 2023	30 Apr 2024	19 Nov 2022

4.4 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.

NIL

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To study the subcellular localization of selected Arabidopsis GPI-APs in plants

2. To characterize the molecular mechanism of trafficking of Arabidopsis GPI-APs
3. To probe the clathrin-independent endocytosis with the use of Arabidopsis GPI-Aps
4. To study the function of GPI glycan and lipid remodeling enzymes in Arabidopsis

5.2 Revised objectives

Date of approval from the RGC:	N/A
Reasons for the change:	N/A

5.3 Realisation of the objectives
(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

Objective 1: To study the subcellular localization of selected GPI-APs in plant cells. (100% achieved)

Selected Glycosylphosphatidylinositols (GPI)-anchored proteins (GPI-APs), and the remodeling enzymes of the lipid-associated protein trafficking pathway in Arabidopsis were cloned. To study their localization, they are further sub-cloned into signal peptide (SP)-fluorescent protein-containing vector for transient and stable expression in Arabidopsis seedlings. Transient co-expression of fluorescent protein-tagged GPI-AP and marker proteins of organelles were carried out to understand its subcellular location in Arabidopsis PSBD protoplasts. Arabidopsis seedlings stably expressing fluorescent protein-tagged GPI-APs were generated. Pure lines of fluorescent protein-tagged GPI-APs have been crossed with those transgenic lines expressing fluorescent protein-tagged organelle markers.

Objective 2: To characterize the molecular mechanism of secretory trafficking of Arabidopsis GPI-APs (75% achieved)

Pharmacological drugs inhibiting sphingolipid and ceramide biosynthesis in Arabidopsis system were applied on the transgenic lines generated under objective 1 to observe their inhibitory effects on trafficking of GPI-APs. Mutants with defective trafficking machinery and lipid biosynthesis were used to test the molecular requirements of trafficking of selected GPI-APs, by crossing with stably-expressed transgenic lines of fluorescent protein-tagged GPI-AP and by electroporation of fluorescent protein-tagged GPI-AP in the protoplasts of mutants.

Objective 3: To probe the clathrin-independent endocytosis with the use of Arabidopsis GPI-APs (75 % achieved)

Pharmacological tools were used to abrogate clathrin-mediated endocytosis. FM4-64 dye were used to study the uptake of membrane by clathrin-independent manner, under the expression of stably-expressing fluorescent protein-tagged GPI-APs generated under objective 1. The process was further characterized with the involvement of actin and microtubule.

Objective 4: To study the function of GPI glycan and lipid remodeling enzymes in Arabidopsis (75% achieved)

Loss-of-function mutants of biosynthetic and remodeling enzymes for the pathway were generated and characterized. Double transgenic lines were generated, to look for clue of valuable information on their participation in the lipid-associated protein trafficking pathway.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. To study the subcellular localization of selected Arabidopsis GPI-APs in plants	✓	100%
2. To characterize the molecular mechanism of trafficking of Arabidopsis GPI- APs	✓	75%
3. To probe the clathrin-independent endocytosis with the use of Arabidopsis GPI-APs	✓	75%
4. To study the function of GPI glycan and lipid remodeling enzymes in Arabidopsis	✓	75%

6. Research Outcome

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

Major findings

In this research, we have provided functional evidences to demonstrate the trafficking machinery required for the lipid-associated protein trafficking in plant cell, which is essential for various elaborated process in plants, such as pollen tube guidance and reception, cuticle formation and cellulose deposition in cell wall.

With the use of confocal live cell imaging on transgenic lines, we have demonstrated the trafficking trajectories of signal peptide-fluorescent protein-tagged GPI-sensing sequence of COBRA and LTPG1, that go through conventional biosynthetic/secretory pathway from ER to Golgi and *trans*-Golgi network (TGN) and destined at plasma membrane by pulse chase experiment. Interestingly, they also partially colocalised with marker of recycling compartment, AtEHD1, hinted that portion of the plasma membrane-localised GPI-APs might be internalized. To investigate further into the molecular machinery and lipid requirement for GPI-APs export along the biosynthetic/secretory pathway, Arabidopsis mutants and pharmacological tools were used.

To understand if clathrin was involved in the endocytosis of GPI-APs, a range of clathrin-mediated endocytosis (CME)-ablating drugs, e.g. such TyrA23, NAA and IKA were used at an optimal and non-toxic concentrations to study the expression of GPI-APs and the endocytosed FM 4-64 dye. It's found that GPI-APs could be internalized in such environment, and further investigations will be needed to confirm the morphology of such organelle with immunogold labeling of thin sections cut from high-pressure frozen/freeze-substituted samples, which might shed light on the elusive clathrin-independent endocytosis, and the possibility of existence of an analogous structure to the "GPI-AP-enriched early endosomal compartments" in mammalian cells.

To test the hypothesis that glycan and lipid remodeling on the GPI moiety can regulation the trafficking and functions of GPI-APs and exhibits its physiological and developmental functions in plants, previously uncharacterized homogenous Arabidopsis GPI biosynthesis and lipid remodeling enzymes mutants were generated and studied. Further research will have to carry out to induce complete abrogation of expressions and observation of phenotypes in defects in plant development.

Research outcome

The research has yielded two manuscripts for submission (Part C; currently under revision, Law et al.2024a, Law et al 2024b) and one presentation in the 19th International Workshop on Plant Membrane Biology 2023 in Taipei Taiwan.

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

With our understanding on the molecular machinery required for delivery and endocytosis of GPI-APs, further research could be directed on engineering of GPI "tag" that could be used for potential cellular engineering applications in biopharmaceutical production of useful biomolecules, which upon tagging, could be efficiently exported to extracellular space. Further research could be done to apply knowledge of protein and lipid trafficking into biotechnological applications.

7. Layman's Summary

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

Plants, as sessile organism, have developed a complex but flexible developmental program to sense and adjust to persistently changing environment. One of such programs is membrane trafficking mechanism, which selectively transport membranes from endomembrane compartments to plasma membrane and extracellular space to maintain cellular homeostasis. Over the decade, much has been known about protein trafficking, but the role of lipid in regulating such mechanism were less characterized.

In this project, we have utilised a number of glycosylphosphatidylinositols (GPI)-anchored proteins (GPI-APs) as molecular marker to track the lipid-associated protein trafficking pathway. With a combination of the state-of-the-art cellular, molecular, biochemical and cell imaging techniques, we have revealed that AtLTPG1 localized on the biosynthetic/secretory pathways, interestingly, partially colocalised with the putative recycling compartments marked by AtEHD1. Genetic approach has been used to knockdown expression of glycan and lipid remodeling enzymes, which has to be further studied to look for developmental processes participated in these mutants.

The project has shed light on the possibility of GPI-APs-specific secretory and recycling pathways. Further research on the pathway will further reveal the molecular machinery in building the recycling vesicles.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project
(Please attach a copy of the 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)** *(denote the correspond-ing author with an asterisk*)*	**Title and Journal / Book** *(with the volume, pages and other necessary publishing details specified)*	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Year of Publication	**Year of Acceptance** *(For paper accepted but not yet published)*	**Under Review**	**Under Preparation** *(optional)*						
			✓	**Angus Ho Yin Law*, Wing Hang Wu, Liwen Jiang***	**"Molecular machinery requirement at ER, Golgi, TGN for export and endocytosis of GPI-APs"** **Research paper prepared for submission to The Plant Journal**	**No**	**No**	**Yes**	**No**
			✓	**Angus Ho Yin Law* & Liwen Jiang***	**"Ticket to the ride: Potentials for efficient export with GPI"** **Review paper prepared for submission to Protoplasma**	**No**	**No**	**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 conference abstract)

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 RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Mar / 2023 / Taipei, Taiwan	Characterising Glycosylphosphatidylinositol (GPI)-Anchored Proteins: A Trafficking Perspective	19th International Workshop on Plant Membrane Biology 2023	No	Yes	Yes	Yes

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning
(Please elaborate)

Research experience of the PI and new knowledge generated on protein trafficking pathway in plant cells, for example, the contribution on the lipid-associated pathway to the development of cell wall and plasma membrane, have been incorporated into the topics of "protein trafficking in plant cell", "plant physiology & development" of a General Education elective module titled "Plants & Human Civilisations", which the PI acted as the module convenor.

Students were further engaged into the topic of protein trafficking with the laboratory session on observing different types of plant cells and organelles under light microscope. With these inputs provided, students from a diverse background have a better understanding on the impacts of protein trafficking in plant development and subsequently onto issues which would affect mankind (e.g. crop yield, resistance to environmental stress and food production).

11. 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**
NIL	NIL	NIL	NIL

12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

The research project is a collaboration between Technological and Higher Education Institute of Hong Kong and The Chinese University of Hong Kong.

The work on protein trafficking has enhanced the teaching of a General Education elective module titled "Plants & Human Civilisations" (please refer to Q10).

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	**Conference Papers**	**Scholarly Books, Monographs and Chapters**	**Patents Awarded**	**Other Research Outputs (please specify)**	
No. of outputs arising directly from this research project	2 (under revision)	1	0	0	Type	No.
					NIL	NIL

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	**Reasons**
NIL	NIL