CRF 8G (Revised Sep 15)

RGC Reference HKBU1/CRF/10
please insert ref. above

The Research Grants Council of Hong Kong
Collaborative Research Fund Group Research Projects
Completion Report

(*for completed projects only*)

Part A: The Project and Investigator(s)

1. Project Title

Identification of Redox-sensitive Proteins and Characterization of Their Functions in Regulating the Oxidative Stress Response in Arabidopsis

2. Investigator(s) and Academic Department/Units Involved *(please highlight approved changes in the composition of the project team and quote the date when RGC granted approval of such changes)*

Research Team	Name/Post	Unit/Department/Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator	XIA Yiji/Prof	Biology/HKBU	9
Co-investigator(s)	ZHANG Jianhua/Prof LI Ning/Prof JIANG Liwen/Prof LAM Hon-ming/Prof GUO Dianjing/Assoc Prof	Life Sciences/CUHK Life Sciences/HKUST Life Sciences/CUHK Life Sciences/CUHK Life Sciences/CUHK	2 3 2 2 2
Collaborators/ Others	Liu Pei, Li Yimin, Jiang Tiantian, Ching Chan, Wang Juan, Wang Yejun, Qin Hu, Weifen Xu, Liguo Jiang, Sun Mingan (graduate students/RAs)	HKBU, CUHK, HKUST	

3. Project Duration

	Original	Revised	Date of RGC Approval *(must be quoted*)
Project Start Date	1 May, 2011		
Project Completion Date	30 April, 2014	Extended to 31 October, 2014	12 April, 2014
Duration (*in month*)	36	42	
Deadline for Submission of Completion Report	30 April, 2015	31 October, 2015	

CRF 8G (Revised Sep 15)

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. Identification of redox-sensitive proteins in Arabidopsis. Redox proteomics approaches will be established and employed in the identification of proteins that undergo in vivo oxidative modifications in Arabidopsis cells that are subjected to redox-perturbing stresses including pathogen infection and the treatment with abscisic acid (ABA), a stress hormone that mediates multiple abiotic stress responses.
2. Characterization of individual redox-sensitive proteins and determination of their redox states under various oxidative stress conditions. The relationship between their redox states and subcellular localizations will be determined.
3. Functional characterization of the selected redox-sensitive proteins. Molecular and mutational analyses will be carried out to understand the roles of the redox-sensitive proteins in oxidative stress signaling and plant stress responses.

5.2 Revised objectives

Date of approval from the RGC: 17 Feb, 2011

Reasons for the change: 1) smaller budget than originally proposed; 2) the reviewers and the CRF committee suggested focusing on fewer proteins for detailed characterization

The three objectives were remained the same but with the following modifications in the experimental plan:

1. We will focus on using OxICAT for identification of redox-sensitive proteins. The DIGE method will be employed only if the OxICAT method does not yield a satisfactory result.
2. We proposed to reduce the number of redox-sensitive proteins that will be subjected to detailed molecular characterization to 7-10 as opposed to 10-20 proteins in the original proposal.
3. The number of proteins subjected to functional characterization will be reduced to 5-8 as opposed to 10-15 proteins in the original proposal.

6. Research Outcome

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

1. Develop redox proteomics approaches for the identification of Arabidopsis proteins that undergo in vivo oxidative modifications under redox-perturbing stresses

In our initial studies, we used gel-based approaches for identifying redox-sensitive proteins. However, the gel-based methods had low sensitivity and post a relatively high risk of false positives. We developed a gel-free redox proteomics method based on iTRAQ (Isobaric Tags for Relative and Absolute Quantitation) and we termed the method oxiTRAQ. The method was also used to identify Arabidopsis proteins that underwent oxidative modifications in response to hydrogen peroxide, salicylate (SA) and flagellin (flag22) treatments. Over 150 proteins have been identified as redox-senstive proteins using the method. In addition to the oxiTRAQ method, we have also developed a $^{14}N/^{15}N$- stable isotope labeling (SILIA)-based method (OxNSIL) for identification of redox sensitive proteins in Arabidopsis seedlings subjected to oxidative stress treatment and pathogen treatment. Over, 150 redox-sensitive proteins have been identified using these methods.

2. Analysis of redox states of individual redox-sensitive proteins under different stresses and determination of their subcellular localizations under oxidative stress

We have chosen 9 oxidant-sensitive proteins for more detailed analysis of their redox states in seedlings under stress conditions. These proteins include AtAnamorson 1, protein tyrosine

phosphatase 1 (AtPTP1), eukaryotic elongation factor 1a (eEF1a), nucleosome assembly protein 1;1 (AtNAP1), protein disulfide isomerase like 1-1 (AtPDIL 1-1), 14-3-3λ., bZIP68, bZIP16, and GBF1. We have generated transgenic Arabidopsis lines which express each of these proteins tagged with an epitope (a double tag consisting of FLAG and StrepII) for analysis using two methods: one is based on protein electrophoretic mobility shift (PEMSA) and the other one is based on differential pull-down of oxidized and reduced proteins through affinity purification with the anti-FLAG antibody-conjugated resin. Five of them were confirmed to undergo oxidation in the Arabidopsis seedlings subjected to the hydrogen peroxide treatment. AtANA1 was found to also undergo oxidative modifications under the treatments of SA and flg22, two inducers of the defense response, suggesting that AtANA1 could be involved in the innate immune response. Two of them (bZIP16 and bZIP68) have been found to be localized in nuclei under normal growth conditions; however, under oxidative stress, they underwent nucleocytoplasmic shuttling.

3. Functional characterization of the selected redox-sensitive proteins

Mutational analysis has been carried out to understand biological functions of the redox-sensitive proteins. We have obtained putative mutants (T-DNA insertion lines) for over 50 of the genes encoding the redox-sensitive proteins. Those mutants were screened for any discernible phenotype under various stress treatments.

Loss-of-function mutations of AtANA1 were found to lead to embryonic lethality and affect development of both male and female gametophytes. It was reported recently by another group that AtANA1 functions in epigenetics and parental effects. We are now working on determining whether AtANA1 plays a role in epigenetic control of oxidative stress responses.

bZIP16 and bZIP68 have been found to act as transcriptional suppressors of stress responsive genes under normal conditions. Under oxidative stress, they became oxidized and translocate to the cytosol, leading to activation of stress responsive genes. The mutation of bZIP68 causes hyper-responsiveness to oxidative stress and other stresses (such as cold stress).

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

One of the main goals of the project is to develop redox proteomics methods to identify redox-sensitive proteins. The results from this study generated very useful information and resources for the plant science community to understand oxidative stress responses and redox signaling pathways. The redox proteomics method we developed can be used in other experimental systems to identify and characterize redox-sensitive proteins, and many of those proteins can be further characterized to understand their roles in redox sensing and signaling mechanism. We are recently focusing on defining the role of bZIP16, bZIP68, and GBF1 in coordinating responses to different stresses. These proteins were found to undergo oxidative modifications using the OxiTRAQ method. Both bZIP16 and bZIP68 apparently function as transcriptional suppressors of stress-responsive genes and undergo nucleocytoplasmic shuttling under oxidative stress to de-repress those genes. They might have the same and distinct sets of genes they regulate and respond differently to different stresses to ensure appropriate responses. We are also working on two other redox-sensitive proteins that are likely involved in epigenetic control to connect oxidative stress responses with epigenetic mechanisms.

6.3 Research collaboration achieved *(please give details on the achievement and its relevant impact)*

In addition to the PC/PI (Xia), the team contributed their expertise in proteomics (Li), cell biology (Jiang), bioinformatics (Guo), and stress physiology (Zhang and Lam) to achieve the project goal. The metabolic labeling approach for redox-sensitive proteome study was developed mainly by Prof. Li group in collaboration with Prof. Xia group. Prof. Guo group, with collaboration of Prof. Xia group, conducted bioinformatics analysis of redox-sensitive proteins including setting us a

database of known redox-sensitive proteins and analysis of sequence patterns associated with redox-sensitive cysteines in proteins. Prof. Xia's and Prof. Jiang's groups collaborated in studying nucleocytoplasmic shuttling of redox-sensitive proteins. Prof. Zhang's and Prof. Lam's group did phenotypic screening of some mutants in the genes encoding for the redox-sensitive proteins under abiotic stresses.

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)

Various stress conditions can lead to over-accumulation of reactive oxygen species (ROS). ROS can cause oxidative damage of cellular molecules, leading to oxidative stress. Organisms evolved mechanism to recognize accumulation of ROS as a signal of stress, leading to activation of stress response pathways to cope with the stress. Some of redox-sensitive proteins are involved in sensing presence of ROS to initiate the stress response. Identification of redox-sensitive proteins is an important step toward understanding the stress response pathway.

Through this CRF project, we developed new methods for identifying redox-sensitive proteins using the model plant Arabidopsis. Using the methods, we identified over 150 redox-sensitive proteins that underwent oxidative modifications in response to various oxidative stress treatments. Some of those proteins were further cauterized to reveal their roles in stress response pathways. Among them, two transcriptional regulators, bZIP16 and bZIP68, were found to act as suppressors of stress responsive genes under normal conditions to prevent unnecessary activation of the stress response. Upon oxidative stress, they become oxidized and apparently could no longer suppress the stress response genes, leading to activation of stress responsive pathways.

CRF 8G (Revised Sep 15)

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 corresponding 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)						
2012				Wang H, Wang S, Lu Y, Alvarez S, Hicks L, Ge X, Xia Y*	Proteomic Analysis of Early-Responsive Redox-Sensitive Proteins in *Arabidopsis*. **Journal of Proteome Research,** 11:412-424.	Yes, 2012	no	yes	no
2012				Xu W, Jia L, Baluska F, Ding G, Shi W, Ye N, Zhang J*	PIN2 is required for the adaptation of *Arabidopsis* roots to alkaline stress by modulating proton secretion. J Exp Bot, doi:10.1093/jxb/ers259	Yes, 2012	no	yes	no
2012				Cheng H, Zhang Q, and Guo D*	Genes that respond to H2O2 are also evoked under light in Arabidopsis. Molecular Plant. 2012. 10.1093/mp/sss108	Yes, 2012	no	yes	no
2012				M Sun, Y Wang, H Cheng,Q Zhang,W Ge and D Guo*	RedoxDB – a curated database for experimentally verified protein oxidative modification. Bioinformatics. 28(19): 2551-2552.	Yes, 2012	no	yes	no
2012				Y Li, Y Shu, C Peng, L Zhu, G Guo, N Li*	Absolute quantitation of isoforms of post-translationally modified proteins in transgenic organisms. Molecular& Cellular Proteomics 11:10.1074/mcp.M111.016568		yes	yes	no
2013				Liu P, Zhang H, Wang H, Xia Y*	Identification of redox- sensitive cysteines in the Arabidopsis proteome using OxiTRAQ, a quantitative redox proteomics method. Proteomics, DOI: 10.1002/pmic.201300307	Yes, 2014	no	yes	no

CRF 8G (Revised Sep 15)

2013				L Zhu, D Liu, Y Li, N Li*	Functional Phosphoproteomic Analysis Reveals That a Serine-62-Phosphorylated Isoform of Ethylene Response Factor110 Is Involved in Arabidopsis Bolting. Plant Physiology, 2013, 161:1-14		yes	yes	no
2013				Z Yang, G Guo, M Zhang, CY Liu, Q Hu, H Lam, H Cheng, Y Xue, J Li, N Li*	Stable Isotope Metabolic Labeling-based Quantitative Phosphoproteomic Analysis of Arabidopsis Mutants Reveals Ethyleneregulated Time-dependent Phosphoproteins and Putative Substrates of Constitutive Triple Response 1 Kinase. Molecular&Cellular Proteomics, 12:10.1074/mcp.M113.031633, 1-24		yes	yes	no
2013				Jia L, Xu W, Li W, Ye N, Liu R, Shi L, Bin Rahman AN, Fan M, Zhang J*	Class III peroxidases are activated in proanthocyanidin-deficient Arabidopsis thaliana seeds. Ann Bot, 111(5):839-47		yes	yes	no
2013				Yu Xing, Qingqin Cao, Qing Zhang, Ling Qin, Wensuo Jia and Jianhua Zhang*	MKK5 Regulates High Light-Induced Gene Expression of Cu/Zn Superoxide Dismutase 1 and 2 in Arabidopsis. Plant Cell Physiology, 54(7):1217-1227		yes	yes	no
2014				Chan C, Lam H*	A putative Lambda class Glutathione-S-transferase enhances plant survival under salinity stress. Plant Cell Physiology, 55(3):570-9		yes	yes	no
2014				Qin Hu, Guangyu Guo, Zhu Yang, Yaojun Li, Yiji Xia and Ning Li*	Stable Isotope Metabolic Labeling-Based Quantitative Thiol Redox Proteomic Analysis of Hydrogen Peroxide-treated Arabidopsis plant. J Proteomics Bioinform, 7:121-133		yes	yes	no
2015				P Liu, H Zhang, B Yu, L Xiong, Y Xia*	Proteomic identification of early salicylate-and flg22-responsive redox-sensitive proteins in Arabidopsis. Scientific Reports,		yes	yes	no

					5:doi:10.1038/srep08625.				

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)*
July, 2013	OxiTRAQ: a redox proteomic method for identification and quantification of redox-sensitive proteins in Arabidopsis (poster)	Plant Biology 2013, organized by ASPB		yes	no	no
June, 2015	Redox Signaling Mediated by a Redox-Sensitive bZIP Transcription Factor in *Arabidopsis* (oral presentation)	Gordon Conference on Posttranslational Modifications		yes	yes	no
July, 2015	Understanding Redox Signaling in Plants Through Identification and Functional Characterization of Redox-sensitive Proteins (poster)	Plant Biology 2015, organized by ASPB		yes	no	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
Pei Liu	PhD	September, 2011	November, 2014/November, 2015
Yimin Li	PhD	September, 2011	August, 2015/not graduated yet
Qin Hu	PhD	September, 2010	March, 2014/May, 2014
Mingan SUN	PhD	August, 2010	June, 2012/December, 2013

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

Project Coordinator

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