Summary of Doctoral Dissertation

Degree requested Doctor of Life Science　　Applicant's name　Yongming Luo

Title of Doctoral Dissertation

Studies on ubiquitin ligases and deubiquitinating enzymes involved in nutrient and phytohormone signaling in *Arabidopsis*
(シロイヌナズナの栄養および植物ホルモンシグナル伝達に関与するユビキチンリガーゼおよび脱ユビキチン化酵素の研究)

Protein ubiquitination is a dynamic and reversible post-translational modification that controls diverse cellular processes in eukaryotes. The 76-amino acid protein ubiquitin is covalently conjugated to a lysine residue on the substrate through an enzymatic cascade, comprising a ubiquitin-activating enzyme (E1), a ubiquitin-conjugation enzyme (E2) and a ubiquitin ligase (E3). However, the conjugation machinery is antagonized by the reverse process — deubiquitination, catalyzed by the deubiquitinating enzymes (DUBs). Seven lysine residues and the N-terminal methionine of ubiquitin allow the formation of polyubiquitin chains with different topologies, which are associated with distinct cellular outputs. The two most abundant forms are K48- and K63-linked polyubiquitin chains, which mainly regulate proteasomal degradation and intracellular trafficking, respectively. Protein ubiquitination status, including the abundance of ubiquitination and ubiquitination topology, is determined by the balance between ubiquitination and deubiquitination, therefore, ubiquitin ligases and DUBs play important roles in regulating the cellular fates of target proteins.

Ubiquitination substrates are involved in a wide spectrum of physiological processes, in my study I focus on how the ubiquitin signals regulate plant nutrient and hormone responses, these are important processes for plant growth and development. Understanding the role of ubiquitin in such regulatory mechanisms will add substantially to our knowledge of this modification and provide possible entry points to engineer sustainable crops under suboptimal growth conditions.

In chapter I, I identified a ubiquitin ligase ATL8, involves in sugar starvation response. The expression of *ATL8* was significantly increased under sugar starvation conditions but was repressed by an exogenous sugar supply. The ATL8 protein was found to possess ubiquitin ligase activity *in vitro* and located to the membranes in plant

cells. In addition, Starch Synthase 4 was identified as a putative interactor of ATL8, suggesting that ATL8 may be involved in the early steps of starch granule formation. These findings suggest that ATL8 functions as a membrane-localized ubiquitin ligase likely to be involved in the adaptation to sugar starvation stress in *Arabidopsis*.

In chapter II, I discovered two DUBs UBP12 and UBP13 regulate protein degradation of the brassinosteroids receptor BRI1. Brassinosteroids (BRs) are a group of plant steroid hormones essential for growth and development. BRs are perceived at the apoplast by the plasma membrane (PM)-localized receptor BR INSENSITIVE 1 (BRI1). The BRI1 protein abundance is decreased in the *ubp12i/ubp13* double mutant, which displayed severe growth defects and reduced sensitivity to BRs. Additionally, UBP13 directly interacted with and effectively removed the K63-linked polyubiquitin chains from BRI1, thereby negatively modulating its vacuolar targeting and degradation. My study reveals that UBP12 and UBP13 play crucial roles in governing BRI1 protein abundance and BR signaling activity to regulate plant growth. In addition, I provided evidence the plant DUBs play opposite functions in intracellular trafficking: contrary to the demonstrated roles for the ASSOCIATED MOLECULE WITH THE SH3 DOMAIN OF STAM3 (AMSH3) protein, which is the DUB that positively regulates ubiquitin-mediated vacuolar degradation of membrane proteins, UBP12 and UBP13 antagonize this process by removing K63-linked polyubiquitin chains from their substrate BRI1.

Finally, these studies emphasized the importance of ubiquitination in plant nutrient response and hormone signaling. The identification of ubiquitin ligase and DUBs involved in those processes revealed many layers of complexity of ubiquitination *per se*, owing to this, future studies on ubiquitin-mediated physiological processes would be both challenging and fascinating.