

(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	BAO HAONAN
Thesis Title	Functional analysis of the histidine kinase CKI1 in female gametogenesis of the liverwort <i>Marchantia polymorpha</i>		
(Thesis Summary)			
<p>Flowering plants produce the central cell, a specialized type of female gamete, alongside the egg cell in the embryo sac for double fertilization, which generates the endosperm and embryo within a seed. The central cell specification in <i>Arabidopsis thaliana</i> is controlled by the histidine kinase CYTOKININ-INDEPENDENT 1 (CKI1), which activates two-component signaling (TCS) via downstream regulatory components shared with the cytokinin signaling pathway. However, the evolutionary history and the biological function of CKI1 in non-flowering plants remained largely unknown.</p> <p>The author identified and analyzed MpCKI1, the single CKI1 ortholog in the model liverwort <i>Marchantia polymorpha</i>. Phylogenetic analysis suggested that CKI1 orthologs are widely present in land plant lineages, including hornworts, liverworts, basal mosses, ferns, and seed plants. Mp<i>ckil</i> mutants showed abnormal thallus morphology, which was partially rescued by artificial activation of TCS signaling. Moreover, inducible overexpression of MpCKI1 suppressed thallus growth and regeneration, which was not observed in Mp<i>hpt</i> or Mp<i>rrb</i> mutant background. These results suggested that MpCKI1 mediated TCS via evolutionarily conserved downstream components. MpCKI1 was essential for egg cell formation and archegonium development. In <i>M. polymorpha</i>, the initial cell of the archegonium undergoes a series of asymmetric cell divisions and three-dimensional growth to form an egg-bearing archegonium. This process is under the regulation of MpBONOBO (MpBNB), a key factor for germline specification. The accumulation of MpBNB was restricted to female germline cells in asymmetric cell divisions during early archegonium development, while in Mp<i>ckil</i> mutants, both asymmetric cell division and MpBNB accumulation pattern were disrupted, resulting in failure of proper female gametogenesis. These observations demonstrated that MpCKI1 regulates female gametogenesis by specifying the female germline during archegonium development. The observation on the archegonia of Mp<i>hpt</i> and Mp<i>rrb</i> mutants and complementation test by MpRRB activation for Mp<i>ckil</i> mutants supported that MpCKI1-mediated TCS was also critical for female gametogenesis. In summary, this study demonstrated that CKI1-mediated TCS is evolutionarily conserved and plays an important role in female gametogenesis in highly divergent land plant lineages.</p>			

(Form 2)

(Thesis Evaluation Summary)

In land plants, gametes develop during the gametophyte generation. In angiosperms, one central cell and one egg cell are differentiated in the embryo sac as female gametes for double fertilization, while in non-flowering plants, only one egg cell is generated in the female sexual organ, called the archegonium. The central cell specification of *A. thaliana* is controlled by the histidine kinase CYTOKININ-INDEPENDENT 1 (CKI1). The previous genome analysis suggested that *M. polymorpha* possesses a single ortholog of *CKI1* (MpCKI1). However, the role of CKI1 in non-flowering plants remains unclear. This thesis applied molecular genetic approaches to understand the developmental function of CKI1 in *M. polymorpha*.

The Ph.D. candidate demonstrated that CKI1 orthologs are widely present in land plants by phylogenetic analysis. Moreover, the candidate showed that MpCKI1 functions for proper development via TCS pathway in *M. polymorpha* by various methods, including RNA-seq and imaging techniques with the loss-of-function and gain-of-function mutants. Furthermore, the candidate found that MpCKI1 regulates the female germline specification for egg cell development in the archegonium. The candidate visualized female germline specification by capturing the distribution pattern of MpBNB, a master regulator for germline initiation, and differentiation in discrete stages of early archegonium development. The candidate found that MpCKI1 is required for the proper MpBNB accumulation in the female germline and is critical for the asymmetric cell divisions that specify the female germline cells. These results suggest that CKI1-mediated TCS originated during early land plant evolution and participates in female germ cell specification in diverged plant lineages.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of plant molecular genetics and developmental biology, and presents new discoveries that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On February 5th, 2024, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary (Form 1), and thesis evaluation summary (Form 2) will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date below that the thesis can be published.

(Please note, however, based on Article 8 of the Degree Regulations, that the thesis must be published within three months of the date that the degree is awarded.)

Publication date of the thesis summary (Form 1) and thesis evaluation summary (Form 2) : mm dd , yyyy