学位報告 4

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主　論　文　の　要　旨

論文題目　The family of *Pseudo Response Regulator* genes (*PRR*) in the moss *Physcomitrella patens*.（蘚類ヒメツリガネゴケにおける *Pseudo Response Regulator* 遺伝子族）

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論　文　内　容　の　要　旨

The *PRR* genes play a central role in the flowering plant *A. thaliana* circadian clock. To address the question of the origin of *PRRs*, this gene family was analyzed in the moss *P. patens*, a phylogenetically distant organism.

In *A. thaliana*, *O. sativa* (rice), and all other higher plants five *PRR* homologs exist (*TOC1/PRR1*, *PRR3*, *PRR5*, *PRR7*, *and PRR9*). Four PRR homologs were found in *P. patens*, suggesting that this gene family has ancient origins in the plant kingdom. However, most striking difference observed in the case of *P. patens*, *P. patens* is the first land plants so far studied in that *TOC1* ortholog is absent. Further analysis demonstrated that, all PpPRRs forms separate cluster in phlogenteic tree and they showed close relationship with higher plant PRR7/3 group. Another striking difference found in PpPRRs that, they all retained potential phosphoaccpetor residue similarly as RRs, consistent with this PpPRR2 protein underwent phosphotransfer in an *in-vitro* phosphorlyation assay. An essential feature of *PRR*s function in *A. thalaina* is a circadian controlled rhythm of transcript abundance and they are core clock components in *A. thaliana* circadian clock. The related *PpPRR*s are also found to be under the control of circadian clock, they all showed robust diurnal rhythms in LD (12L: 12D) and endogenous circadian rhythms in DD (continuous dark) conditions. Interestingly, the expression of each *PpPRR* gene only showed significant rhythmicity in constant darkness, not in constant light. This is in contrast with their respective *A. thaliana* homologs, the transcript levels of which showed robust circadian rhythms in continuous light. Moreover, all *PpPRR*s showed acute light responses to all light types, suggesting their role in light signaling.

In *PpCCA1a/1b* double disruptant all *PpPRR*s showed higher expression in light condition, suggesting their expression was suppressed by *PpCCA1a/1b* in presence of light. Similar negative regulation of *PpPRR*s by *PpCCA1a/1b* was already found in their *A. thaliana* counterparts, suggesting the conservation of clock systems in two distantly related species.

The functional similarities of *PpPRR*s and *AtPRR*s were assessed by misexpressing

PpPRR2 gene in *A. thaliana*. *PpPRR2* misexpressiong *A. thaliana* plants exhibited the phenotypes of short period rhythm in continuous dark, early flowering in short day, short hypocotyls in short day and hypersensitivity to red light during early photomorphogenesis, compared with the wild type plants. Similar events were previously demonstrated when the *A. thaliana* clock-associated *PRR* were misexpressed. These similarities provide conclusive evidence that these genes have similar functions as clock components. Therefore, the genetic structures of the circadian oscillators are likely conserved between early land plant and flowering plants; in short the core clock components of circadian systems are likely conserved among land plants.

The results of this study clearly supported the view that plant *PRR*s are indeed the evolutionarily conserved crucial clock components. To further confirm this idea; more direct experiments are requirement, including characterization of multiple loss-of-function mutants of *PpPRRs* in the moss. In a future study, in parallel with gene knock out experiments, it should also be addressed whether PpPRRs are involved in phosphotransfer functions *in planta*. Moreover, a HK(s) and a HPt(s) that are partners to (at least some of) PpPRR proteins should be characterized. It might not be easy to identify a partner HK(s) because the *P. patens* genome contains as many as ~31 HK sequences (Ishida *et al*., 2010), unlike *A. thaliana*, which contains only 8 HK genes (Hwang *et al*., 2002). However, the availability of the entire genome sequence, many full-length cDNA clones and the feasibility of gene functional analysis based on gene targeting techniques will support the identification of such HK and HPt genes in *P. patens*, possibly as novel clock genes.