博 士 論 文 の 内 容 の 要 旨

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論文題目	Molecular mechanism underlying phosphorus transfer in arbuscular mycorrhizal symbiosis （アーバスキュラー菌根共生におけるリン輸送の分子メカニズム）

（博士論文の内容の要旨）

Phosphorus (P) is an essential element for plant growth and development. However, the concentration of available P in soil is low. Thus, plants have evolved several strategies for P acquisition in P-limiting environments. One of these strategies is the formation of symbiotic associations with arbuscular mycorrhizal (AM) fungi belonging to the subphylum Glomeromycotina. AM fungi provide soil P to host plants via hyphae that interconnect the roots and surrounding soil, thereby improving plant P nutrition and growth. Investigating the P delivery process of AM fungi at the molecular level is important to comprehensively understand this symbiotic association. This study investigates the metabolism and distribution of polyphosphate (polyP), a linear polymer of phosphate (Pi), in AM fungi and symbiotic interfaces to elucidate the P transfer mechanism between AM fungi and the host.

AM fungi accumulate a large amount of polyP in their mycelia, which plays a role in P storage and translocation. The vacuolar transporter chaperone 4 (VTC4) localized in the tonoplast is responsible for polyP synthesis in budding yeast and protozoan parasites. In Chapter II, the biochemical properties of the VTC4 protein of the AM fungus *Rhizophagus irregularis* were investigated. The *R. irregularis* VTC4 protein could catalyze polyP synthesis using ATP as a substrate. Notably, the VTC4 protein also catalyzed the reverse reaction (polyP-depolymerizing reaction), in which ATP was generated from polyP in the presence of high ADP concentration. The direction of the reaction was switched at ATP:ADP ratios of 2:1–5:1. These results indicate that AM fungal VTC4 not only synthesizes polyP but also regenerates ATP from polyP, which may be involved in the regulation of polyP and ATP levels in AM fungal cells.

Arbuscules, highly branched fungal structures, are the main site for P exchange. Arbuscules are surrounded by a host-derived periarbuscular membrane with localized symbiotic Pi transporters and H^+-ATPase HA1. The mutation of *HA1* impairs P acquisition through the mycorrhizal pathway. In Chapter III, the subcellular localization of polyP in mature arbuscules colonizing the roots of a *Lotus japonicus ha1-1* mutant was investigated to understand P transfer at the arbuscular interface. PolyP accumulated in the cell walls of trunk hyphae of the wild-type and *ha1-1* mutant, but most fine branches of arbuscules lacked polyP. Double staining of polyP and acid phosphatase (ACP) activity revealed their contrasting distribution patterns in arbuscules, i.e., ACPs were active around fine branches. Notably, polyP was observed in the cell wall of some fine branches formed in the *ha1-1* mutant, indicating that P was released from fungal cells to apoplastic regions. These observations indicate that polyP in fungal cell walls and apoplastic ACPs may play an important role in P transfer at the symbiotic interface of arbuscules.

Based on the findings, the model of P transfer from AM fungi to the host plant was proposed. The polyP accumulated in vacuoles was translocated to arbuscules, depolymerized by VTC4 to short-chain

polyP, and released into the cell wall of fine branches. The secreted polyP was hydrolyzed to Pi by ACP located on the apoplastic region between the AM fungus and the host. The liberated Pi was delivered to host cells by symbiotic Pi transporters driven by the H^+ gradient generated across the periarbuscular membrane of the HA1 H^+-ATPase. The data presented herein indicate that VTC4 and ACP participate in the synthesis and hydrolysis of polyP metabolism during AM symbiosis, which may affect the growth and P nutrition of AM plants. The present work will promote the production of P-efficient crops and reduce the application of P fertilizer in sustainable agriculture by developing molecular diagnostic tools such as polyP, VTC4, and ACP for the evaluation of AM functions.