Evolutionary appearance of the plasma membrane H\(^+\)-ATPase containing a penultimate threonine in the bryophyte

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The plasma membrane H\(^+\)-ATPase provides the driving force for solute transport via an electrochemical gradient of H\(^+\) across the plasma membrane, and regulates pH homeostasis and membrane potential in plant cells. However, the plasma membrane H\(^+\)-ATPase in non-vascular plant bryophyte is largely unknown. Here, we show that the moss Physcomitrella patens, which is known as a model bryophyte, expresses both the penultimate Thr-containing H\(^+\)-ATPase (pT H\(^+\)-ATPase) and non-pT H\(^+\)-ATPase as in the green algae, and that pT H\(^+\)-ATPase is regulated by phosphorylation of its penultimate Thr. A search in the P. patens genome database revealed seven H\(^+\)-ATPase genes, designated PpHA (Physcomitrella patens H\(^+\)-ATPase). Six isoforms are the pT H\(^+\)-ATPase; a remaining isoform is non-pT H\(^+\)-ATPase. An apparent 95-kD protein was recognized by anti-H\(^+\)-ATPase antibodies against an isoform of Arabidopsis thaliana and was phosphorylated on the penultimate Thr in response to a fungal toxin fusicoccin and light in protonemata, indicating that the 95-kD protein contains pT H\(^+\)-ATPase. Furthermore, we could not detect the pT H\(^+\)-ATPase in the charophyte alga Chara braunii, which is the closest relative of the land plants, by immunological methods. These results strongly suggest the pT H\(^+\)-ATPase most likely appeared for the first time in bryophyte.

The Plasma Membrane H\(^+\)-ATPase in the Moss Physcomitrella patens

The plasma membrane H\(^+\)-ATPase actively transports H\(^+\) out of the cell and creates an electrochemical gradient of H\(^+\) across the plasma membrane for energizing substance transport coupled with many secondary transporters, the maintenance of membrane potential and pH homeostasis.\(^1\)-\(^4\) The structure of the H\(^+\)-ATPase is highly conserved, apart from the C-terminal region. In vascular plants, the H\(^+\)-ATPase contains the C-terminal region consisting around 100 amino acids, which is known as an autoinhibitory domain and contains a penultimate threonine (Thr).\(^2\) It has been demonstrated that phosphorylation of the penultimate Thr and subsequent binding of the 14–3-3 protein to the phosphorylated penultimate Thr in response to physiological signals has shown to be a major common regulatory mechanism of H\(^+\)-ATPase in vascular plants.\(^5\)-\(^10\) On the other hand, the H\(^+\)-ATPases in yeasts and green algae, including Chlamydomonas reinhardtii, Chlorella variabilis NC64A and Volvox carteri, lack such a C-terminus,\(^1\)-\(^14\) suggesting that the H\(^+\)-ATPase containing a penultimate Thr might have appeared after green algae during evolution.

A recent our study has revealed that plasma membrane H\(^+\)-ATPase in the liverwort Marchantia polymorpha as a non-vascular plant bryophyte, which represents the most basal lineage of extant land plants, expresses both the penultimate Thr-containing H\(^+\)-ATPase (pT H\(^+\)-ATPase) and non-penultimate Thr-containing H\(^+\)-ATPase (non-pT H\(^+\)-ATPase). We further provided the evidence that the pT H\(^+\)-ATPase in M. polymorpha is regulated by phosphorylation of its penultimate Thr in response to physiological signals, such as light, sucrose and osmotic shock, and that light-induced phosphorylation of the pT H\(^+\)-ATPase depends on photosynthesis.\(^15\)

In this study, we examined the plasma membrane H\(^+\)-ATPase in the moss Physcomitrella patens, which is known as a model bryophyte, and the genome has been sequenced.\(^16\),\(^17\) We searched H\(^+\)-ATPase genes with similarity to the typical plasma membrane H\(^+\)-ATPase in Arabidopsis thaliana, AHA2 in the P. patens genome database (www.cosmoss.org) and found seven H\(^+\)-ATPase homologs, designated PpHA1–PpHA7. Of these, six isoforms (PpHA1–PpHA6) possess a penultimate Thr in the C-terminal region. In contrast, the remaining isoform, PpHA7, lacks such a penultimate Thr in the C-terminus. Phylogenetic analysis using full-length amino acid sequences indicated that PpHA1–PpHA5 are clustered with Arabidopsis H\(^+\)-ATPase, and that PpHA7 is close to the non-pT H\(^+\)-ATPase of Chlamydomonas reinhardtii (Crlpump), which has no penultimate Thr (Fig. 1A). Note that PpHA6 is clustered with the non-pT H\(^+\)-ATPases, although this isoform possesses the penultimate Thr. According to classification of gene families in the

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The Charophyte Green Alga \textit{Chara braunii} is Unlikely to Express the \( pT \) H\(^+\)-ATPase

Next, we investigated whether the charophyte alga \textit{Chara braunii}, which is the closest relative of the land plants, \(^1\) expresses the \( pT \) H\(^+\)-ATPase using immunoblot and protein blot analyses. The results showed that there are no proteins in \textit{C. braunii} that are recognized by anti-H\(^+\)-ATPase against Arabidopsis H\(^+\)-ATPase; furthermore, the signal did not appear in immunoblots using anti-pThr and protein blots using 14-3-3 protein as a probe when characean cells were treated with FC (Fig. 2A), although endogenous 14-3-3 proteins (31.6 kD, 32.5 kD and 32.9 kD) were recognized by anti-14-3-3 against Arabidopsis GF14phi (Fig. 2B). In contrast, Arabidopsis H\(^+\)-ATPase having \( pT \) H\(^+\)-ATPase, PpHA1–PpHA5 localize between subfamilies I and IV.\(^1\)\(^8\) These results suggest that \textit{P. patens} genome encodes both \( pT \) H\(^+\)-ATPase and non-\( pT \) H\(^+\)-ATPase genes (Fig. 1A). In addition, we identified 11 typical 14-3-3 protein genes in \textit{P. patens} (Fig. 1B).

We then examined a fungal toxin fusicoccin (FC)- and light-induced phosphorylation of the penultimate Thr in H\(^+\)-ATPase in protonemata of \textit{P. patens}.\(^1\)\(^9\) An apparent 95-kD protein as the H\(^+\)-ATPase was phosphorylated in response to FC and light (50 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) for 30 min) as well as in thalli of \textit{M. polymorpha} (Fig. 1C and D), suggesting that the 95-kD protein contains the \( pT \) H\(^+\)-ATPase in \textit{P. patens}, and that light also acts as a physiological signal regulating phosphorylation status of the \( pT \) H\(^+\)-ATPase in protonemata of \textit{P. patens}.

\textbf{The Charophyte Green Alga \textit{Chara braunii} is Unlikely to Express the \( pT \) H\(^+\)-ATPase}

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an apparent mass of 95 kD from the etiolated seedlings was recognized by anti-H+-ATPase and was phosphorylated and bound to 14-3-3 protein in response to FC. In addition, we could not find the H+-ATPase containing the penultimate Thr of charophyte algae in the available database such as National Center for Biotechnology Information. Characean cells, however, have plasma membrane H+-ATPase activity, suggesting that characean cells are unlikely to express pT H+-ATPase, which binds with 14-3-3 protein on phosphorylation of the penultimate Thr, and that they express only the non-pT H+-ATPase.

From these results, we conclude that the pT H+-ATPase most likely appeared for the first time in the bryophyte; in other words, during the transition of plants from water to the terrestrial land. To verify the evolutionary appearance of the pT H+-ATPase in plants, elucidation of whole genome sequence in charophyte algae is required.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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