Studies on regulation of the plasma membrane H+-ATPase activity with respect to blue light-dependent stomatal opening

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論 文 名　：Studies on regulation of the plasma membrane H^+-ATPase activity with respect to blue light-dependent stomatal opening（青色光依存の気孔開口を指標とした細胞膜 H^+-ATPase の活性制御に関する研究）

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論　文　内　容　の　要　旨

The plasma membrane H^+-ATPase acts as a primary transporter in fungi and plants, and drives a large number of secondary transporters by enhancing the negative membrane potential and pH gradient across the plasma membrane. The H^+-ATPase is responsible for various physiological responses, including cell elongation, phloem loading, and stomatal opening. Stomatal opening is one of the suitable responses to investigate the activity of H^+-ATPase. Functions of H^+-ATPase have been analyzed mainly by biochemical technique, but genetic evidence for the functions are scarcely available because all 11 H^+-ATPase isoforms in *Arabidopsis* are expressed in stomatal guard cells. The functional redundancy of the H^+-ATPase makes it difficult to identify the specific role of the H^+-ATPase in plants.

In this study, I performed genetic screening by thermography and isolated *blus2* (*blue light signaling2*), in which opening responses of stomata were reduced. Whole genome re-sequencing demonstrated that the responsible gene of *blus2* is *AHA1* (*Arabidopsis* H^+-*ATPase1*) that encodes one of the isoform of H^+-ATPase. I thus renamed *blus2* as *aha1-10*. By contrast, the mutation in *AHA2* (*aha2*) and *AHA5* (*aha5*) did not affect stomatal opening in response to blue light, although the transcripts of two isoforms were highly expressed in guard cells. I showed that the amount of AHA1 protein was larger than those of AHA2 and AHA5 in guard cells. From these results, I conclude that AHA1 plays a major role in blue light-dependent stomatal opening.

Phosphorylation of the penultimate threonine in the C-terminus and subsequent 14-3-3 protein binding are essential for the activation of H^+-ATPase. However, no genetic evidence for this was obtained. Using *aha1-9* mutant that lacks AHA1, I determined the activity of H^+-ATPase with respect to stomatal response. To do this, I transformed *aha1-9* with non-phosphorylatable form of the penultimate threonine in AHA1 (Threonine 948 to Ala; T948A). The transformant having T948A did not recover the stomatal phenotype of *aha1-9*. 14-3-3 protein were not bound to the H^+-ATPase in response to blue light in the transformant. The results provide genetic evidence that phosphorylation of the penultimate threonine is essential for H^+-ATPase activation.