学 位 論 文 要 旨

Physiological and molecular studies of the chloroplast movements in the liverwort *Apopellia endiviifolia*

苔類ホソバミズゼニゴケにおける葉緑体運動の生理学的・分子生物学的研究

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Chloroplast movements refer to the subcellular localisation of chloroplasts in plant cells that respond to ambient light and temperature. The relocation of chloroplasts optimises photosynthetic activity under different environmental conditions and supports plant growth and development to survive. These behavioural chloroplast movements under room temperature conditions have induced chloroplast to migrate to the anticlinal wall bordering with neighbouring cells in dark (dark-positioning response), accumulate towards weak light (accumulation response), and avoid strong light (avoidance response). While at low-temperature conditions, chloroplast moves away from the weak light (cold-avoidance response). In the present study, I identified a liverwort *Apopellia endiviifolia* with simple thalloid thalli comprising uniformly developed chloroplasts without any air chamber. From the thallus, clear views of the four chloroplast responses were observed. Moreover, the light-dependent chloroplast responses including accumulation, avoidance and cold-avoidance in *A. endiviifolia* were only reacted to blue light irradiation, indicating mediation from the blue light photoreceptor phototropin. A single copy phototropin gene of *A. endiviifolia* (*AePHOT*) was identified through the next-generation RNA sequencing and Southern blot analysis, similar to the *MpPHOT* gene in the closely related liverwort *Marchantia polymorpha* that perform a simple signalling pathway. These results showed a beneficial combination of the simple thalloid thallus and single copy *AePHOT* gene in *A. endiviifolia*, which can be useful for the following study of chloroplast movements.

To further understand the functional role of the *AePHOT* gene in regulating the chloroplast movements, I performed a comparative analysis on the transient expression of *AePHOT* and *MpPHOT* in *A. endiviifolia* and *M. polymorpha*. I have cross-introduced the plasmids of AePHOT or MpPHOT into the thalli of *A. endiviifolia* and

gemmalings of *M. polymorpha* by particle bombardment. Avoidance response from the transient overexpression of the *AePHOT* gene was observed in the AePHOT-transformed cells of *A. endiviifolia* under weak blue light (BL) at 22°C. This result has confirmed the functions of the *AePHOT* gene in *A. endiviifolia*. However, the avoidance response was not induced in AePHOT-transformed cells of *M. polymorpha*, indicating incompatibility of *AePHOT* to *M. polymorpha*. This observation showed the species specificity of *AePHOT* that only functions in *A. endiviifolia*. On the other hand, the transient overexpression of *MpPHOT* has induced avoidance responses in both MpPHOT-transformed cells of *A. endiviifolia* and *M. polymorpha* under the weak BL conditions, showing the compatible function of *MpPHOT* in *A. endiviifolia*.

As *AePHOT* signalling in the chloroplast movements of *A. endiviifolia* has been demonstrated, cytoskeleton components employed in the downstream pathway that facilitate the chloroplast movements were investigated. To date, two cytoskeleton components, microtubules and actin filaments have been reported to move chloroplasts in plant cells. Through the respective fluorescent localisations, I observed the microtubules and actin filaments in the thallus cells of *A. endiviifolia*. The visualisation of microtubules and actin filaments was specifically disrupted by the microtubule and actin filament polymerisation inhibitors oryzalin and latrunculin-A (lat-A), respectively. To find the responsible cytoskeleton components of the chloroplast movements in *A. endiviifolia*, the chloroplast positionings in the inhibitor-treated thalli with disrupted microtubules or actin filaments have been examined. In the oryzalin-treated thalli, light-dependent chloroplast responses including accumulation, avoidance and cold-avoidance were induced unaffectedly despite the disrupted microtubules. Whereas, these chloroplast responses were inhibited in the lat-A-treated thalli, as the disrupted actin filaments have restricted the chloroplast mobility in the formation of chloroplast responses. These results indicate that the light-dependent chloroplast responses are regulated by actin filaments only, which is consistent with the photosynthetic organs reported in previous data. Additionally, a rapid dark-positioning response of *A. endiviifolia* that can be achieved after 3 h of dark incubation was demonstrated in the present study. This response was considered a faster reaction than in any reported plant species. Under the dark conditions, the dark-positioning response of *A. endiviifolia* was not inhibited by the disrupted microtubules, but by the disrupted actin filaments. This result proves the actin filament regulation in the dark-positioning response, and it is the first to be reported here.

In conclusion, my study demonstrated the beneficial characteristics of clear chloroplast observation and simple *AePHOT* signalling in *A. endiviifolia*. These characteristics thus facilitated the subsequent analysis to determine the mechanism of chloroplast movements in *A. endiviifolia*, where *AePHOT* signalling in mediating the chloroplast movements through the actin filament motility mechanism was shown. Moreover, the rapid dark-positioning response of *A. endiviifolia* was highlighted in the present study, as it shows the potential uses of *A. endiviifolia* as a model plant to elucidate the mechanism of dark-positioning response.