Photoorientation of Chloroplasts in Protonemal Cells of the Fern Adiantum as Analyzed by Use of a Video-tracking System

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Photoorientation of chloroplasts mediated by phytochrome and blue light-absorbing pigment in protonemal cells of the fern Adiantum was studied by use of inhibitors of the cytoskeleton and was analyzed with a video-tracking system. The photoorientation responses were inhibited by cytochalasin B and by N-ethylmaleimide (NEM) but not by colchicine, suggesting that the photomovement depends on the actomyosin system. In the dark, chloroplasts moved randomly, being independent of one another. After induction of photoorientation by polarized red light, most chloroplasts that had been located at the margin of cells moved almost perpendicularly to the cell axis toward the site of photoorientation. This type of movement was hardly ever observed in the dark. Under polarized blue light, such specific movements were less evident but were still observed in the case of a few chloroplasts. After photoorientation was complete, chloroplasts still moved in random directions but their mobility was lower than that in the dark, indicating the presence of some anchoring mechanism.

When EGTA was applied, photoorientation was inhibited but this inhibition was overcome by the addition of CaCl₂. Video-tracking of chloroplasts in the dark revealed that the mobility of chloroplasts was higher in medium with EGTA than in medium with EGTA plus CaCl₂ and that many of the chloroplasts moved jerkily in the medium with EGTA. This change in the nature of movements was also seen under polarized light, resulting in the disturbance of photoorientation. These results indicate that the inhibition of photoorientation at low concentrations of Ca²⁺ ions may be due to changes in the nature of chloroplast movement.

Key words: Blue light-absorbing pigment (cryptochrome) — Chloroplast movement — Fern protonema — Photomovement (chloroplast) — Phytochrome

In plant cells, chloroplasts respond to unilateral light by intracellular orientation, perhaps in order to maximize photosynthetic activity in the case of weak irradiation and to avoid damage to the photosynthetic apparatus in the case of strong irradiation. The photoorientation responses have been investigated in some detail from the photobiological point of view (Haupt 1982, 1987, Haupt and Scheuerlein 1990, Schönbohm 1980, Zurzycki 1980). In most cases, the responses are induced by blue light,

Abbreviations: DMSO, dimethylsulfoxide; EGTA, ethyleneglycol-bis-(β-aminoethylether)-N, N', N'-tetraacetic acid; MES, 2-(N-morpholino)-ethanesulfonic acid; NEM, N-ethylmaleimide

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and the blue light-absorbing pigment (cryptochrome) has been assigned as the photoreceptor. Occasionally, phytochrome also functions as the receptor pigment (Haupt 1959, Haupt and Thiele 1961, Izutani et al., 1990, Yatsuhashi et al., 1985). However, the mechanism of chloroplast translocation and its regulation during the orientation response are poorly understood even though the response has potential as a model system for the study of the movement of intracellular organelles in plant cells. By contrast, our understanding of cytoplasmic streaming is much more extensive (Kamiya 1986, Shimmen 1988). One of the difficulties encountered in attempts to analyze chloroplast movement stems from the fact that the speed of movement is much lower than that of cytoplasmic streaming. It is not easy to trace the path of a chloroplast or to measure the speed of movement under a microscope. To solve these problems, we have developed a video-tracking system that automatically tracks the chloroplasts in a cell and allows both reconstruction of the path of movement and measurement of the speed of movement. Chloroplast photoorientation mediated by phytochrome and blue light-absorbing pigment in protonemal cells of the fern *Adiantum* was analyzed by use of this tracking system.

**Materials and Methods**

*Plant material and aseptic culture*

Spores of *Adiantum capillus-veneris* L. were collected in the summer of 1982 in a greenhouse at the Botanical Gardens, University of Tokyo, Koishikawa, Tokyo, and were stored in the dark at about 5°C until use.

Spores were sterilized with 0.1-strength “Purelox” (4-6% solution of sodium hypochlorite; Oyalox Co., Tokyo) and sown in a line on a membrane placed on a coverslip. The membrane was prepared from a mixture of 0.75% agar and 0.3% gelatin (Murata and Wada 1989). Spores were covered with another agar-gelatin membrane and were placed at the bottom of a Petri dish that contained ten-fold diluted modified Murashige and Skoog’s mineral salt solution (Kadota and Furuya 1977). After imbibition for 1 day in the dark, spores were cultured for 9 days at 25°C under continuous red light at 0.5 Wm⁻² which was applied horizontally. Protonemata cultured in this way were irradiated for 6 h with continuous white light at 3.6 Wm⁻² and then kept in the dark for 2 days (Yatsuhashi et al., 1987). This treatment resulted in two-celled protonemata of more than 1 mm in length. The photoorientation response of chloroplasts was monitored in the basal cell of these non-growing two-celled protonemata (Yatsuhashi et al., 1987).

*Induction of chloroplast photoorientation*

Intracellular photoorientation of chloroplasts was induced by continuous irradiation with linearly polarized red or blue light vibrating horizontally, which was given from the tip of the protonema (Fig. 1). Red and blue light were obtained by passing light from a fluorescent lamp (FL20SD; Toshiba Corp., Tokyo) through a red plastic plate (Shinkolite A, #102; Mitsubishi Rayon Co., Ltd., Tokyo) and a blue plastic film