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High-light avoidance response of chloroplasts and reorganization of actin filaments are induced only in the exposed area to blue light in the epidermal cell of *Vallisneria gigantea*

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In many plant cells, the positions of chloroplasts change in response to changes in light conditions. In the epidermal cells of the aquatic angiosperm *Vallisneria gigantea*, the avoidance response of chloroplasts is induced specifically by irradiation with blue light of high intensity. Possible roles of actin cytoskeleton in the blue-light-induced avoidance response of chloroplasts were investigated by partial irradiation and phalloidin staining. We showed that the blue-light-dependent redistribution of chloroplasts was induced only in the limited area, where exposed to blue light, even in individual cells. In addition, in the exposed area, the configuration of actin filaments strikingly changed compared with that before the irradiation. Short and thick bundles of actin filaments surrounding the chloroplasts changed to much longer and thinner bundles with a more stretched array. In contrast, in the unexposed area, neither the distribution of chloroplasts nor the configuration of actin filaments exhibited any changes. Cytochalasin D and latrunculin B inhibited the avoidance response of chloroplasts concomitantly with the fragmentation of actin filaments. These results indicate that the reorganization of actin filaments plays a crucial role in the induction of avoidance response of chloroplasts.

Key words : actin cytoskeleton, avoidance response of chloroplasts, blue light, partial irradiation, *Vallisneria gigantea*

INTRODUCTION

In a variety of plant species, light-dependent intracellular rearrangements of chloroplasts are observed [1]. Basically, under weak light, chloroplasts accumulate on the outer periclinal layer of the cytoplasm (P side) to optimize photosynthesis, whereas under strong light, chloroplasts move into the anticlinal layer of the cytoplasm (A side) to avoid photodamage. Both responses are controlled by blue light [2]. Recently, in *Arabidopsis thaliana*, phot1/phot2 were identified as blue light photoreceptors functioning in the light-induced movements of chloroplasts [3,4,5], though their intracellular localization has not been determined. In several plants, it was reported that the high-light avoidance response of chloroplasts was induced only in the exposed area. Actin cytoskeleton is generally involved in intracellular movements of organelles [6]. In the algae [7,8] and fern [9], the actin filaments exhibited dynamic reorganization, concomitantly with light-induced movements of chloroplasts

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In epidermal cells of the aquatic angiosperm *Vallisneria gigantea*, weak red light most effectively induces the accumulation of chloroplasts on the P side, and strong blue light specifically induces the high-light avoidance response of chloroplasts into the A sides [10]. Concomitantly with a loss of motility of chloroplasts on the P sides induced by weak red light, the configuration of actin filaments changes from a network to a honeycomb array [11]. Moreover, the chloroplasts surrounded by the actin filaments with a honeycomb array become anchored on the P sides in a cytochalasin-sensitive manner [12]. In mesophyll cells of *Arabidopsis thaliana*, latrunculin B destroyed actin filaments that surround the chloroplasts and brought about a disordered arrangement of chloroplasts [13].

In this study, we examined possible roles for actin cytoskeleton in the high-light avoidance response of chloroplasts in partially irradiated epidermal cells of *V. gigantea* with strong light.

MATERIALS AND METHODS

Vallisneria gigantea Graeber was grown as described [10].

We constantly used the mature parts of the leaves of nearly the same age. The leaf was cut into smaller pieces of about 4 mm in length. Each leaf piece was cut open in the middle of mesophyll layers and the adaxial half piece was exclusively used for experiments after preparation as described [10]. The dark-adapted specimen was irradiated on the stage of a light microscope from below the mesophyll layers. The monochromatic light of various wavelengths (450 nm, 550 nm, 650 nm, 750 nm) was produced by combination of an interference filter and an appropriate cut-off filter. Each monochromatic light was applied as a spot of 60 μm in diameter at a constant fluence rate of 60 μmol/m^2/sec. Before and after actinic irradiation, photographs of the P sides of epidermal cells were taken. The movement of chloroplasts was recorded with a time-lapse video recorder.

Actin filaments on the P sides of epidermal cells were visualized by staining with fluorescent phalloidin (Alexa Fluor™ 488 phalloidin) as described by Dong et al. [12] with slight modifications. After incubation in the staining solution for 20 min at 25 °C in darkness, stained cells were examined with an epifluorescence microscope within 1 h. Cytochalasin D and latrunculin B dissolved in dimethylsulfoxide were diluted up to 100 μM and 0.1 μM at use, respectively, with artificial pond water (APW). The inhibitor containing APW was applied to a specimen by gentle irrigation of the space between the coverslip and the glass slide.

RESULTS AND DISCUSSION

Effects of partial irradiation on movement of the chloroplast.

First, we examined effects of partial irradiation with monochromatic light of various wavelengths with high intensity on the distribution of chloroplasts on the P sides of the epidermal cells. As we used a spot of 60 μm in diameter, we could irradiate a part of an epidermal cell, which was a few tens to a hundred of μm in its size. The avoidance response of chloroplasts was specifically induced by continuous irradiation with blue light. Monochromatic light of other wavelengths exhibited little effects. In addition, a partial irradiation with blue light induced the avoidance response of chloroplasts only in the exposed area (Fig. 1). In contrast, the positions of chloroplasts in the unexposed area did not seem to change at all.

Within a few minutes of irradiation with blue light, the chloroplasts in the exposed area started to sway in random directions. In the meanwhile, the chloroplasts began to move more directionally and some of them exited from the exposed area to the unexposed areas. Consequently, the number of chloroplasts in the exposed area with blue light rapidly decreased. After 60 min of the irradiation, the number of chloroplasts resided in the exposed area decreased to less than 10% compared with that before the irradiation. The chloroplasts exited from the exposed area moved very vigorously within the cell. These chloroplasts came into and exited from the exposed area but never stayed in the exposed area on the P side. On the other hand, in the unexposed areas, the chloroplasts did not move at all.

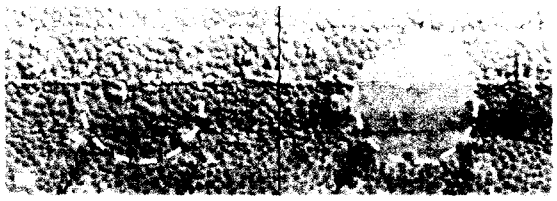

Fig. 1 The effects of partial irradiation with blue light on the distribution of chloroplasts in the epidermal cells of *V. gigantea*. Dark-adapted epidermal cells were irradiated with blue light (450nm) of a spot of 60 μm in diameter at a constant fluence rate of 60 μmol/m^2/sec. The P sides of epidermal cells under DIC optics were photographed immediately before (left) and 60 min after (right) partial irradiation with blue light. Each circle of broken line indicates the irradiated area. Bar = 10 μm.

Effects of partial irradiation on the configuration of actin filaments.

Next, we visualized the configuration of actin filaments of the epidermal cells before and after the partial irradiation. On the P sides of the dark-adapted epidermal cells, we observed two types of configuration of actin filaments; several short and thick bundles near the surface of each chloroplast and longer and thinner bundles constructing a loose network over the whole P side. In the epidermal cells irradiated with blue light, the configuration of actin filaments changed from the short and thick bundles into much longer and stretched bundles. Even more strikingly, the blue-light-induced reorganization of actin filaments occurred only in the exposed cytoplasmic region even in the partially irradiated single epidermal cell (Fig. 2). In contrast, in the unexposed areas, the configuration of actin filaments remained unchanged. Partial irradiation at other wavelengths had no effect on the configuration of actin filaments.

We suppose that a plausible blue-light photoreceptor functioning in the avoidance response of chloroplasts is distributed uniformly on the whole P side of the epidermal cell. Because the localized redistribution of chloroplasts and reorganization of actin filaments were induced in any

place on the P side we selected to be irradiated. Furthermore, signals that mediate between the photoreceptor and the avoidance response of chloroplasts do not diffuse from the exposed area into the neighboring unexposed areas.

Fig. 2 Configuration of actin filaments after partial irradiation with blue light of an epidermal cell of *V. gigantea*. After partial irradiation with blue light (450 nm, 60 μmol/m^2/sec) for 60 min, actin filaments in the epidermal cell was visualized by staining with Alexa 488-phalloidin.
Bar = 10 μm

Effects of actin inhibitors.

We examined effects of actin inhibitors on the blue-light-induced avoidance response of chloroplasts. After treatment of the dark-adapted epidermal cells with 100 μM cytochalasin D for 120 min or 0.1 μM latrunculin B for 60 min, the epidermal cells were irradiated with blue light in the presence of the reagents. During 60 min of continuous irradiation, all the chloroplasts kept the same positions and did not move from the exposed area. Consequently, the distribution of chloroplasts on the P sides exhibited little change before and after the partial irradiation. Concomitantly, cytochalasin D and latrunculin B caused a substantial fragmentation of actin filaments on the P sides. After each reagent was removed, the blue-light-induced changes in the distribution of chloroplasts on the P side were observed as in untreated cell.

A proposed model.

Our results show that blue-light-dependent reorganization of actin cytoskeleton may be one of the indispensable steps to induce the avoidance response of chloroplasts. When chloroplasts accumulate on the P side, the short and thick actin bundles close to the surface of chloroplasts function to anchor those chloroplasts. Upon irradiation with blue light, the short and thick bundles change to the much longer bundles with a stretched network array. The chloroplasts in the exposed area move along the longer and straight bundles to the unexposed area.

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