Chloroplast Photoorientation in *Adiantum* gametophytes

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Ⅰ. Abstract

Fern gametophytes are a good model system to study plant morphogenesis, because of their simple organization and various photocontrolled responses. We studied fern photomorphogenesis including chloroplast photoorientation using *Adiantum* gametophytes to analyze signal transduction pathways of plant photomorphogenesis. Chloroplast photoorientation will be shown in detail and molecular structure of fern phytochromes and blue light absorbing pigments will also be discussed.

Ⅱ. Introduction

Fern gametophytes are haplophase stage of fern life cycle and are autotrophically growing, simple independent organisms and are not surrounded by other tissue, meaning that direct observation under a microscope is very easy. Although their form is simple, gametophytes possess almost all physiological phenomena found in higher plants, such as germination, cell elongation, cell division, two-dimensional differentiation, Chloroplast photoorientation and development of reproductive organs. These physiological phenomena are controlled by red and/or blue light. Given these characteristics, fern gametophytes are a good model system to study plant photomorphogenesis

(Wada and Sugai, 1994)

Among various environmental factors which control plant development, light is an excellent tool for analytical studies, because wavelength, fluence rate, direction of electrical vector, and direction of incident ray can be specified. Moreover, light irradiation can be controlled spatially and temporally by the irradiation with microbeams or short pulses aimed at specific photoreceptors.

We studied various phenomena found in fern gametophytes from the photobiological stand point as shown in Table 1. Among these responses, chloroplast photoorientation is a good model system to analyze signal transduction of photomorphogenesis, although it is not a developmental step, not only because the same photoreceptors, phytochrome and blue light absorbing pigment used in photomorphogenesis are involved in this physiological phenomenon, but also because chloroplast photoorientation can be observed within a short period

Table 1 Photoresponses in *Adiantum* gametophytes

germination	prom	R	Sugai, Furuya	1985
(spore)	inhib	B	Sugai, Furuya	1985
growth	prom	R	Wada, Furuya	1970
(protonema)	inhib	B	Kadota et al	1970
	resumption	R	Kadota, Furuya	1981
phototropism	prom	R	Kadota et al	1982
	prom	B	Hayami et al	1986
polarotropism	prom	R	Wada et al	1981
	prom	B	Hayami et al	1992
swelling	prom	B	Wada et al	1978
branching	prom	R	Wada	1995
cell division	G0 arrest	R	Wada et al	1984
	G1 short	B	Miyata et al	1979
	G2 length	FR	Miyata et al	1979
	plane	W	Wada, Furuya	1971
2-dimension	orientation	W	Kadota, Wada	1986
Chloroplast	low fl resp	R & B	Yatsuhashi et al	1985
movement	high fl resp	R & B	Yatsuhashi, Wada	1990

Fern effect Color authors year

Phenomena

after light treatment comparing with other developmental phenomena (Wada et al, 1993). In this symposium, chloroplast photoorientation of fern *Adiantum* gametophytes will be summarized.

Ⅲ. General aspect of chloroplast photoorientation

Chloroplast photoorientation is induced in almost all plants by blue light but not by red light with some exceptions such as *Adiantum* gametophytes, *Mougeotia, Mesotaenium* and *Vallisneria* (Fig.1) In these four organisms, the interaction of red and blue light on phytochrome-mediated chloroplast movement is quite different, namely both phytochrome and blue light absorbing pigment simply mediate Adiantum chloroplast photoorientation, but in *Mougeotia* and *Mesotaenium* blue light works to modify or potentiate the phytochrome-mediated chloroplast movement. In *Vallisneria* epidermal cells, no interaction could be found, but blue light mediates only high fluence rate response (Izutani et al, 1990).

The direction of chloroplast photoorientation is dependent on the fluence rate of light irradiated. In *Adiantum* at low fluence rate, both in red and blue light, chloroplast move towards the light source, but at the high fluence rate, 10 Wm^{-2} in blue light and 500 Wm^{-2} in red light, they tend to escape from the irradiated area. The same photoreceptors, phytochrome and blue light receptor are thought to involve in high as well as low fluence rate responses.

Ⅳ. Dichroic orientation of photoreceptors

Dichroic effect of these pigments in *Adiantum* chloroplast movement indicates that the transition moments of the photoreceptors are well arranged

arranged in cells. It is shown that Pr is parallel and Pfr is perpendicular to the plasma membrane as shown in Mougeotia (Fig.2). Blue light receptor is also shown to be parallel to the plasma membrane.

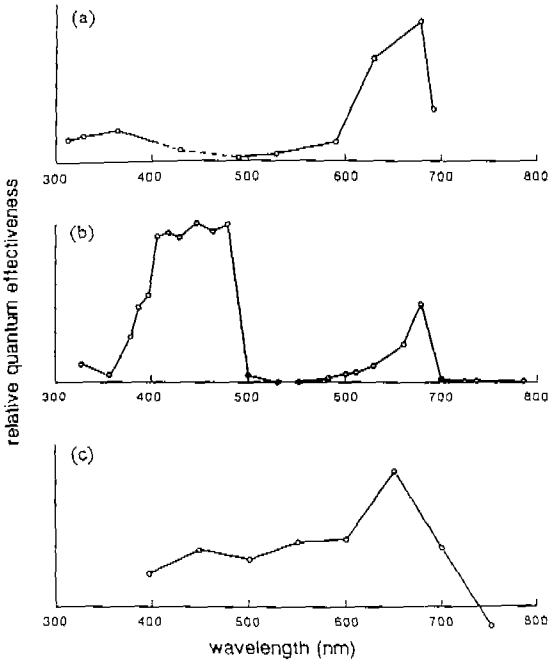


Fig. 1 Action spectra of chloroplast photo-orientation in (a) *Mougeotia scalaris*, (b)protonemal cell of *Adiantum*, and (c) epidermal cell of *Vallisneria gigantea*. Red light is effective in all three species.(from Wada et al. 1993)

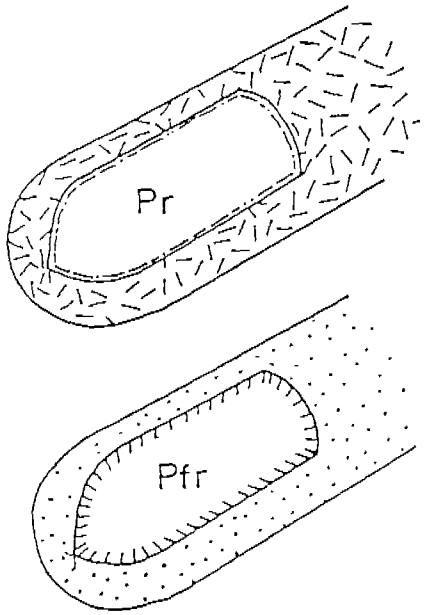


Fig. 2 Schematic drawing of three dimensional orientation of phytochrome molecules at the apical part of a protonema of *Adiantum capillus-veneris*. The dashed showed the absorption vector
(from Wada and Kadota 1987)

V. Partial irradiation of dark adapted prothallial cells

When prothalli were kept in the dark for 2 days, chloroplast moved from the periclinal walls to the anticlinal walls. In these dark adapted cells, partial irradiation with blue or red microbeam(10 um in diameter) at an area without chloroplasts for 60 sec induces the chloroplast movement towards the irradiated area during the subsequent dark condition. meaning that a signal is transferred

from the irradiated area to the place where chloroplasts exist (Fig. 3). The higher the fluence of the light, the grater the distance from which chloroplasts could be induced. Chloroplasts started to move anytime up to 20 min after the red stimulus. The velocity of chloroplast movement was independent of light-fluence in both red and blue light(Fig.4).

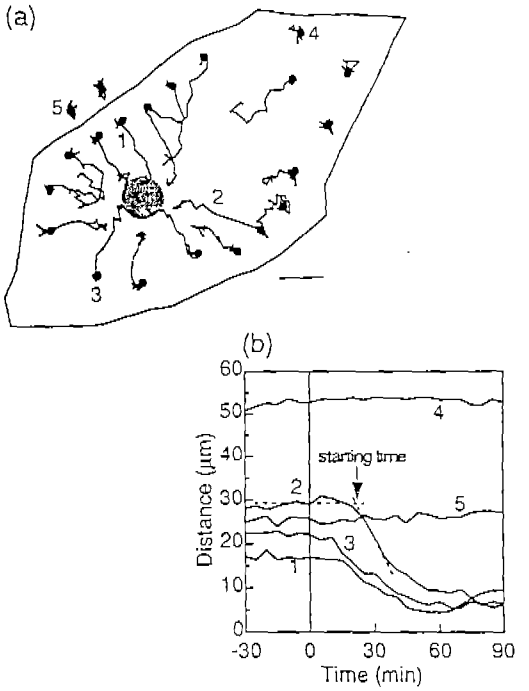


Fig. 3 chloroplast movement induced in a dark-adapted prothallial cell of *Adiantum* by irradiation for 60sec at 30 Wm^{-2} with a red microbeam. A Chloroplast movement was traced for 30 min before irradiation to 90 min after irradiation The hatched circle shows the position of the irradiated locus in the cell. The bar; 10㎛. b. Changes in distances between the center of the irradiated locus and the chloroplasts with time. The line numbers in b correspond to the chloroplast numbers shown in a.(from Kagawa and Wada 1996)

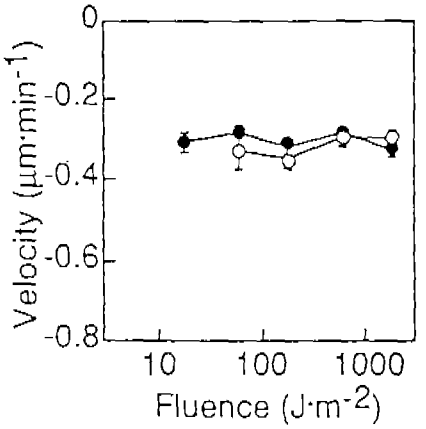
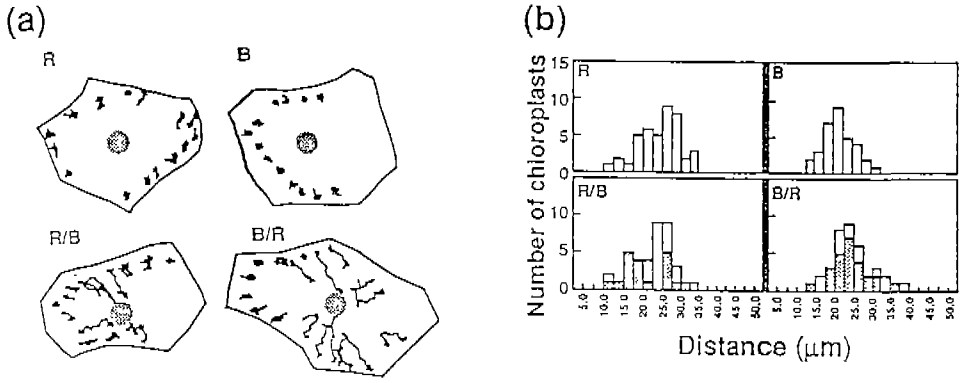


Fig. 4 Velocities of chloroplast movement induced by red(closed circles) and blue light(open circles) of different fluences. Dark-adapted prothallial cells were irradiated with a microbeam of various fluences and chloroplast movements were traced.(from Kagawa and Wada 1996).

VI. Signal transduction in phytochrome and blue light absorbing pigment

Sequential treatment with red and blue microbeams, whose fluences were less than the threshold values when applied separately, resulted in an additive effect and induced chloroplast movement (Fig.5). suggesting that signals from



g 5. Additive effects of red and blue light on chloroplast movement. a. A dark-adapted prothallial cell was adiated with a microbeam of red ($5Wm^{-2}$, 2 sec), blue ($30Wm^{-2}$, 2 sec). red followed by blue. or blue followed red at the same locus. b. Each histogram was obtained from three independent experiments.(from Kagawa d Wada 1996).

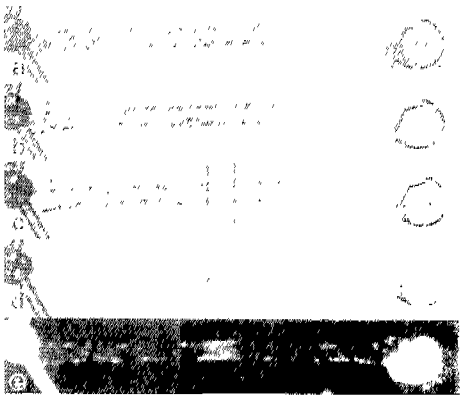


Fig. 6 Red-light-induced chloroplast photoorientatio n in enucleated protonemata. Polarized red light an d then microbeam (30㎛ in width shown by black l ines) of red light were irradiatede, DAPI fluorescen ce microscopy. Note that no nucleus is included(fro m Wada 1988)

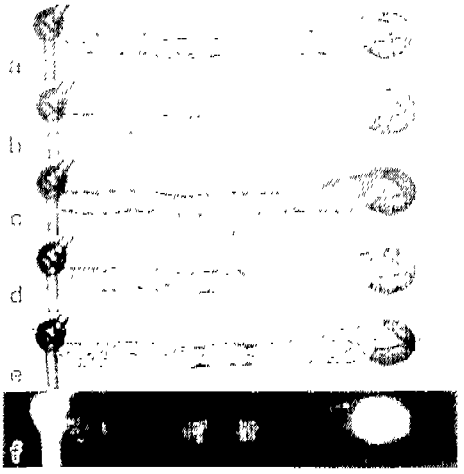


Fig. 7 Blue-light-induced chloroplast photoorientatio n in enucleated protonemata. Polarzed blue light an d then a microbeam of blue light of low and then high fluence rate were irradiated. f. DAPI fluoresce nce microscopy.(from Wada 1988)

phytochrome and blue light absorbing pigment could interact at some point before the induction of chloroplast movement (Kagawa and Wada, 1996). Gene expression is not involved in this phenomenon, because enucleated protonemata show chloroplast photoorientation (Figs. 6 & 7, Wada 1988).

Ⅶ. Photoreceptors of *Adiantum*

Genomic clones of the photoreceptors are now cloned and under sequencing. *Adiantum* has at least 4 phytochrome genes and several genes of blue light absorbing pigments. One of the phytochrome genes has a novel structure which has photoreceptive domain at N-terminus and protein kinase domain ao C-terminus. Similar phytochrome has been reported in moss *Ceratodon* (Thümmler et al, 1992). The detail of the gene structure will be shown.

Reference

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