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Review

Chloroplast movement

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ABSTRACT

Chloroplast movement is important for plant survival under high light and for efficient photosynthesis under low light. This review introduces recent knowledge on chloroplast movement and shows how to analyze the responses and the moving mechanisms, potentially inspiring research in this field. Avoidance from the strong light is mediated by blue light receptor phototropin 2 (phot2) plausibly localized on the chloroplast envelop and accumulation at the week light-irradiated area is mediated by phot1 and phot2 localized on the plasma membrane. Chloroplasts move by chloroplast actin (cp-actin) filaments that must be polymerized by Chloroplast Unusual Positioning1 (CHUP1) at the front side of moving chloroplast. To understand the signal transduction pathways and the mechanism of chloroplast movement, that is, from light capture to motive force-generating mechanism, various methods should be employed based on the various aspects. Observation of chloroplast distribution pattern under different light condition by fixed cell sectioning is somewhat an old-fashioned technique but the most basic and important way. However, most importantly, precise chloroplast behavior during and just after the induction of chloroplast movement by partial cell irradiation using an irradiator with either low light or strong light microbeam should be recorded by time lapse photographs under infrared light and analyzed. Recently various factors involved in chloroplast movement, such as cp-actin filaments and CHUP1, could be traced in Arabidopsis transgenic lines with fluorescent protein tags under a confocal laser scanning microscope (CLSM) and/or a total internal reflection fluorescence microscope (TIRFM). These methods are listed and their advantages and disadvantages are evaluated.

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1. Introduction

Chloroplasts move toward an area irradiated with weak light (accumulation response) in a manner that absorbs more light allowing efficient photosynthesis but move away from strong light when irradiated directly (avoidance response), avoiding the damage caused by the absorption of excess light [1,2] (Fig. 1). Senn initiated experiments that explored chloroplast movement in 1900, studying basic chloroplast distribution patterns under various light conditions in various tissues of many plant species, including algae, mosses, ferns, herbaceous plants, and trees. His results and the knowledge known at that time were published in 1908 [3]. However, Senn was not the first to study such responses. A light-dependent chloroplast distribution pattern in a Crassulaceae plant

Abbreviations: cp-actin filaments, chloroplast-actin filaments; TIRFM, total internal reflection fluorescence microscope.

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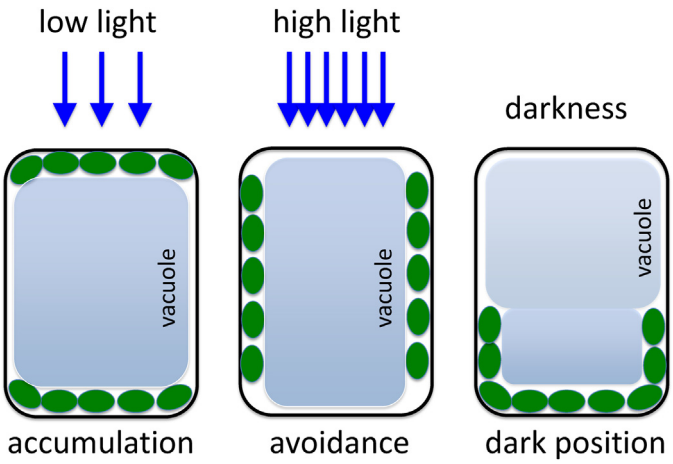

Fig. 1. Schematic chloroplast distribution patterns in *Arabidopsis* mesophyll cells. Chloroplasts are embedded in a thin layer of cytoplasm between the plasma membrane and the tonoplast of a large vacuole.
Left. Accumulation response. Chloroplasts re-distribute to the upper and lower sides of palisade cell under low light conditions, achieving maximal light absorption.
Center. Avoidance response. Chloroplasts re-distribute on the side walls of palisade cell, minimizing the absorption of strong light.
Right. Dark positioning. Chloroplasts distribute on the bottom of the cell in the dark, although the physiological function of this distribution is not yet known.

was first reported in 1856 [4]. The induction of chloroplast accumulation movement by light was found in 1871 [5]. Although Senn's book was published more than hundred years ago, it includes a large amount of detailed and extensive knowledge regarding chloroplast movement and is considered the fundamental resource for researchers studying chloroplast movements.

Senn described the fundamental phenomena of chloroplast movement, that is, the chloroplast distribution pattern and chloroplast behavior. He described that the effective wavelength for chloroplast movement was, in general, blue light, and he also described that red light was effective with the alga *Mougeotia*. He hypothesized why chloroplasts adopted distribution patterns based on the aspects of light paths through individual cells and leaf tissues. However, the mechanisms and signal transduction pathways of chloroplast movement were unknown at the time. Indeed, the signaling factors involved in signal transduction pathways, including photoreceptors [6,7], protein factors [8–11], and the actin structure that underlies the motive force of the movement [12,13], were uncovered a hundred years after Senn's first experiment not only in Arabidopsis but also in fern and moss. Weak light, which induces the accumulation response, is perceived by the blue light receptors phototropin 1 (phot1) and phot2 [7] at the plasma membrane, whereas phot2 that mediates the strong light-induced avoidance response [6] is likely localized on the chloroplast envelope [14].

The "signal" that is transmitted from the photoreceptor to the chloroplasts, the most interesting and important subject is not yet known despite the rapid advances during the last 13 years. Phosphoinositides and Ca^{2+}, however, have been proposed as candidates [15].

The number of groups working on chloroplast movements and/or researchers joining this field is increasing, and various attempts have been made to study the mechanism and/or physiological relevance of chloroplast movements. Due to the advances in molecular biological techniques, many genes involved in chloroplast movements have been identified through mutant analyses [6,8–11]. However, to fully understand them, chloroplast movements should be analyzed from a different perspective. Precise observation of chloroplast behavior and functional analyses of the protein factors involved using various techniques are still needed. As each method has its advantages and disadvantages, the preferable method is dependent on the researcher's experimental purposes and the available facilities.

The main method currently employed for the detection of chloroplast movement is the measurement of red light transmittance through a leaf [16]. Transmittance decreases when the chloroplasts gather at the palisade cell surface, whereas the transmittance increases when the chloroplasts move from that position. Therefore, chloroplast movement can be recorded automatically by placing leaves in a photometer [16], and the resulting chart facilitates the data analysis.

The merits and shortcomings of each method used to detect chloroplast movement (Table 1), i.e., observation of chloroplast distribution pattern using fixed and sectioned leaves, measurement of red light transmittance through leaf tissue, and direct observation of chloroplast movement under microscopy, is discussed below.

2. Fixed-cell sectioning

Senn [3] studied chloroplast movement in various tissues of many plant species mainly by sectioning plant materials that were most likely fixed with chemicals (Fig. 2A). When we observe chloroplast movement using a whole leaf without sectioning, it is difficult to detect the precise distribution pattern of all chloroplasts (the number and their position) in a long, thin palisade cell (even if the uppermost cell) of a thick leaf from the leaf surface through an epidermal cell layer, even when using a confocal microscope changing its focal plane from the top to the bottom of the cell (Fig. 2B). The best way is fixed cell observation of leaf cross sections, as Senn did (Fig. 2A). Although the chloroplast distribution pattern can be observed with a thin section (1 μm or less), the whole cell should be included in the section if the number of chloroplasts in a cell is to be enumerated; in this case the section should be more than 50 μm in thickness. The sectioning of a leaf (with or without fixation) embedded in an agar block with a vibrating blade microtome, such as a Leica VT1200S, is commonly used (Fig. 2C).

Table 1
The merits and demerits of the chloroplast movement analysis methods.

Methods	Best for	Useless for, (disadvantage)
Fixed-cell-sectioning	Chloroplast-distribution pattern, chloroplast number-counting	Time course study
Band assay	Rapid analysis of many samples, mutant screening	Time course study, weak response
Light transmittance	Time course, comparison between mutants	Direct observation of chloroplasts, (influenced by other factors)
Microbeam assay	Single chloroplast behavior, control of chloroplast movement	Rapid analysis of many samples, (need special equipment)
Movie analysis	Time course, chloroplast behavior, speed of movement	(need special equipment)
CLSF microscopy	Observation of cp-actin filaments, time course of cp-actin filament behavior	(need transgenic line), (need costy equipment)
TIRF microscopy	Single cp-actin filament behavior	(need transgenic line), (need costy equipment)
Cryoelectron microscopy	Structure of cp-actin filaments	(need costy equipment), (time consuming)

The most appropriate method "Best for" or less appropriate method "Useless for" and (Disadvantage) for chloroplast movement analyses were listed with comments. CLSF microscopy: confocal laser scanning fluorescent microscopy. TIRF microscopy: total internal reflection fluorescence microscope.