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Redox regulation of phototactic migration in the green alga *Chlamydomonas reinhardtii* and its possible application

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Most flagellated green algae swim toward or away from the light source, i.e., display positive or negative phototaxis. Usually, the sign of phototaxis, positive or negative, is variable depending on various factors, but the mechanism that controls it has been unclear. Using *Chlamydomonas reinhardtii,* an excellent model organism for studying phototaxis, we have recently shown that cellular redox poise plays a key role; cells show positive phototaxis when treated with reactive oxygen species (ROS), whereas they show negative phototaxis when treated with ROS quenching reagents. Here we discuss the possible mechanism of the redox regulation of phototactic sign, questions to be clarified in the future and its possible application.

Keywords: Chlamydomonas, phototaxis, cilia, flagella, redox, green algae

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Phototaxis is a behavior of organisms to move toward or away from the incident light (called positive or negative phototaxis, respectively). Phototrophic microorganisms switch between positive and negative phototaxis to inhabit under proper light conditions for photosynthesis, like the chloroplasts in higher plant leaves move to optimize their photosynthesis. A unicellular green alga *Chlamydomonas reinhardtii* is an excellent model organism to study phototaxis because cells quickly show phototactic turning upon light perception, and a number of mutants defective in phototaxis have been isolated. Molecular mechanisms of photoreception at the eyespot and the regulation of flagellar beating for phototactic turning have been extensively studied in Chlamydomonas.[1] The switching mechanism of the phototactic sign has also been studied. Factors that have been shown to affect it include light intensity, circadian rhythms and ionic strength of the extracellular solution.[2-4] However, the intrinsic factor that determines the phototactic sign has been an open question. In our recent study, we focused on the cytoplasmic redox state as a candidate determinant of the phototactic sign.[5]

The cytoplasm is kept in a moderately reduced redox state by several mechanisms that include glutathione and thioredoxin (called redox homeostasis).[6] However, it can transiently change to relatively oxidized or overly reduced states upon alterations in activities of photosynthesis or respiration, or upon exposure to external factors. Cellular reactions against such changes in the redox state are called redox regulation. In this decade, the redox regulation has become very important in the cell biology field, as many kinds of cellular activities have been shown to be regulated by redox poise: for example, the regulation of intracellular Ca^{2+} concentration and chemoattraction of leukocytes for wound detection.[7,8]

Previous studies showed that (1) photosynthetic activity affects the phototactic sign; (2) photosynthetic activity affects the cellular redox poise; and (3) redox poise modulates flagellar motility.[9-11] Hence, we surmised that the redox poise could be a good candidate for the determinant of the phototactic sign. After treatments with reactive oxygen species (ROS) reagents to oxidize the cytoplasm, Chlamydomonas cells showed positive phototaxis, while they showed negative phototaxis after treatments with ROS-quenching reagents (**Fig. 1**). Use of a chlorophyll-deficient mutant assured that the effects of those

ROS and ROS-quenching reagents (called "redox reagents") were not through any changes in the photosynthetic activity.[12] This was further confirmed by our direct measurements of photosynthetic activity in Chlamydomonas cells in the presence and absence of the redox agents (data not shown). These data suggest that the cytoplasmic redox poise determines the phototactic sign: when it becomes oxidized, the cells show positive phototaxis, and vice versa.

Redox reagents clearly switched the phototactic sign of Chlamydomonas cells. However, several questions remain to be answered. First, how much do those reagents affect the cytoplasmic redox potential? Since some ROS-quenching reagents not only quench ROS but also produce different types of ROS during itsvquenching reaction, it is necessary to monitor the change in the cellular redox potential after treatment with redox reagents to elucidate the true effects of the redox reagents.[13] Several techniques have been developed to quantify the cellular redox potential in vivo, such as "redox-sensitive GFP" (roGFP, a GFP variant which functions as a ratiometric dual emission redox potential sensor) and "Redoxflour" (tandemly linked CFP and YFP, which show fluorescence resonance energy transfer in a redox-dependent manner).[14-16] Though it may be difficult to directly utilize these fluorescence-based techniques in phototrophic organisms that have strong chloroplast auto-fluorescence, quantification of the cytoplasmic redox potential is clearly the next challenge. Second, how does the redox regulation of phototactic sign contribute to cell viability in nature? One possibility is that it helps cells to maintain the redox homeostasis of the cytoplasm: when the cytoplasm is oxidized, cells migrate toward light and increase photosynthesis activity, and thus the cytoplasm becomes reduced because of the reducing equivalents brought from chloroplast. However, in the chloroplast, stronger light may produce greater amounts of ROS, which may make the cellular redox state more oxidized if diffused into the cytoplasm. To clarify the overall effect resulting from the two apparently opposing effects, further understanding of the mechanisms of redox homeostasis and the relationship between cytoplasmic and chloroplast redox states is necessary.

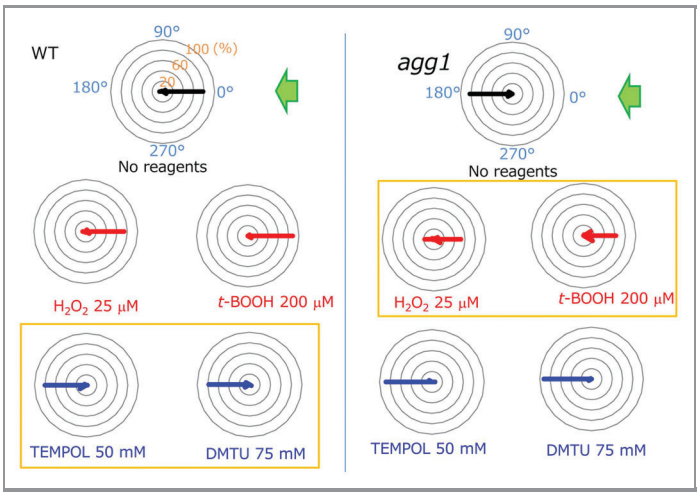

Figure 1. Summary of the effects of ROS or ROS scavengers on the phototactic sign. Polar histograms depict the percentage of cells moving in a particular direction relative to light illuminated from the right, with or without treatment with redox reagents (12 bins of 30°, n = 50 cells per condition). Wild type cells exhibited positive whereas *agg1* mutant cells exhibited negative phototaxis without treatment with any reagents. Note that wild type cells exhibited negative phototaxis after treatment with ROS scavengers (blue), whereas *agg1* cells showed positive phototaxis after treatment with ROS (red) (histograms in yellow boxes). Modified from Figure 2 in Wakabayashi et al.[5]

With our present discovery of redox-dependent phototactic sign, we can now control the swimming direction of Chlamydomonas cells using redox reagents and light. An obvious application of this finding would be to concentrate algae at will. When dimethylthiourea (DMTU), a ROS-quenching reagent, is added to Chlamydomonas wild-type cell culture and green light is applied from above, cells quickly moved to the bottom of the

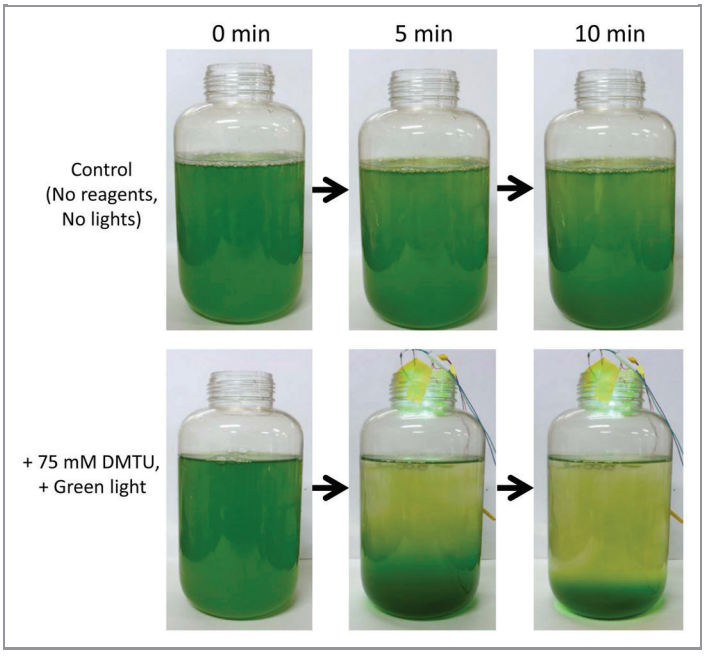

Figure 2. Concentration of Chlamydomonas cells based on the redox regulation of phototaxis. About 1-liter culture of wild type cells were treated with 75 mM dimethylthiourea (DMTU) and illuminated with a green LED from the top.

bottle (**Fig. 2**). By discarding the upper phase, we can easily obtain ~10 fold denser cell culture without using pumps or centrifuges, which is harmful for cells. This method should contribute to industrial use of algae as bioenergy source, as well as to basic science of motile phototrophic algae.

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