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Quantitative Biochemcial Characterization of *Chlamydomonas reinhardtii* Mutants with Altered Antenna Size by Hyperspectral Confocal Fluorescence Microscopy

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Algae and plants have ability to adapt in different light conditions and have evolved complex and dynamic responses to variable light intensities. They balance energy absorption and utilization by changing the absorption cross-section of the antenna system through state-transitions which involves redistribution of light harvesting complex II between the two photosystems. Under high-light conditions, additional photoprotective mechanisms are employed to avoid photodamage such as non-photochemical quenching of the excited state of chlorophyll as well a regulation of the antenna system at the genetic lever. For example, a class of well studied blue-light receptors known as phototropins is involved in regulation of chlorophyll and carotenoid biosynthesis genes and can moderate the amounts of these pigments *in vivo*. We present hyperspectral confocal fluorescence microscopy (HCFM) of novel mutants of *Chlamydomonas reinhardtii* with altered antenna size and pigment stoichiometries to probe the global distribution and concentration of their photosynthetic complexes in the membrane. We examined an array of mutants including two phototropin knockouts, Chlorophyll *a* oxygenase knockdown lines having intermediate antenna size (CAO RNAi) and other mutant cell lines deficient in chlorophyll *b* (small antenna size) or *psb*A. HCFM records the entire emission spectrum (500-850 nm) at each spatial pixel of an image and when paired with multivariate analysis, unique fluorescence components were mathematically isolated and quantified for LCHII, PSII, and carotenoid emission features. Our results indicate that Chl *b* knockdown mutants display decreased emission attributed to LHCII and carotenoid confirming a decrease in the amount of LHCII size. The overall chloroplast morphology appeared less organized compared to wild-type cells and this result was confirmed with transmission electron microscopy. Consistent with these results relative distribution of LHCII and PSII in the membrane was also altered in CAO RNAi lines and chl b less mutant compared to wild-type cells. Mutants deficient in *psb*A and that do not assemble functional PSII reaction centers, had similar chloroplast morphology compared to wild-type cells, however the distribution of LHCII in the thylakoid membrane was significantly different. These results are complemented by bulk pigment quantification and photosynthetic efficiency studies and are presented on an accompany abstract submitted to this meeting.

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