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The chloroplast signal recognition particle (CpSRP) pathway as a tool to minimize chlorophyll antenna size and maximize photosynthetic productivity

Henning Kirst, Anastasios Melis *

Plant and Microbial Biology, University of California, Berkeley, CA 94720–3102, USA

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ABSTRACT

The concept of the Truncated Light-harvesting chlorophyll Antenna (TLA) size, as a tool by which to maximize sunlight utilization and photosynthetic productivity in microalgal mass cultures or high-density plant canopies, is discussed. TLA technology is known to improve sunlight-to-product energy conversion efficiencies and is hereby exemplified by photosynthetic productivity estimates of wild type and a TLA strain under simulated mass culture conditions. Recent advances in the generation of TLA-type mutants by targeting genes of the chloroplast signal-recognition particle (CpSRP) pathway, affecting the thylakoid membrane assembly of light-harvesting proteins, are also summarized. Two distinct CpSRP assembly pathways are recognized, one entailing post-translational, the other a co-translational mechanism. Differences between the post-translational and co-translational integration mechanisms are outlined, as these pertain to the CpSRP-mediated assembly of thylakoid membrane protein complexes in higher plants and green microalgae. The applicability of the CpSRP pathway genes in efforts to generate TLA-type strains with enhanced solar energy conversion efficiency in photosynthesis is evaluated.

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1. Introduction

Photosynthesis relies on the absorption and utilization of sunlight by the photosystems, embedded in the chloroplast thylakoids. Higher plants and green microalgae have developed an extensive light-harvesting system, composed of highly coordinated chlorophylls and carotenoids that absorb and funnel the energy of sunlight towards the photochemical reaction centers. Green algae and plants have developed rather large arrays of such light-harvesting complexes associated with the reaction centers of photosystem-I (PSI) and photosystem-II (PSII). These light-harvesting holocomplexes are assembled and become associated with PSI and PSII in the developing thylakoid membrane of chloroplasts. The *Lhca* gene subfamily encodes for proteins of the light-harvesting complex I (LHCI) associated with PSI, while the *Lhcb* gene subfamily encodes for proteins of the light-harvesting complex II (LHCII), associated with PSII (Jansson et al., 1992). The large light-harvesting antenna size found in wild type plants and algae is thought to confer survival advantages for the cell in the wild: it enables chloroplasts in these organisms to operate the photochemical reaction centers at maximum capacity even under low sunlight intensities. Thus, cells can grow under a wide range of light conditions, which are encountered in the natural environment, from the early morning hours right after sunrise, when the light intensity is low, over midday where the light intensity reaches a maximum, to late evening before sunset. Limiting light conditions for microalgae could arise from growing further down in the water column, where the light intensity is low even at midday or when plants grow in

Abbreviations: CpSRP, chloroplast signal recognition particle; TLA, truncated light-harvesting antenna; D1, the psbA-encoded 32 kD PSII reaction center protein; GTP, guanosine triphosphate; LHC, light-harvesting complex; NPQ, non-photochemical quenching; Chl, chlorophyll; PS, photosystem.

* Corresponding author at: Plant and Microbial Biology, 111 Koshland Hall, MC-3102, University of California, Berkeley, Berkeley, California 94720–3102, USA. Tel.: +1 510 642 8166; fax: +1 510 642 4995.
E-mail address: melis@berkeley.edu (A. Melis).

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the lower canopy of trees and forests. In both cases, the intensity of sunlight is attenuated often to below saturation for the process of photosynthesis. Independently, attenuation of sunlight intensity occurs due to cloud cover in the atmosphere (Kirk, 1994). It has been hypothesized that possessing a large light-harvesting chlorophyll antenna size in its chloroplasts affords the organism a competitive growth advantage under these light-limiting conditions. The other advantage of a large light-harvesting antenna size is indirect; it can be plausibly argued that by virtue of shading competing organisms in the same ecotype, enables an organism to grow more efficiently, while slowing down growth of competitors.

Having a large light-harvesting Chl antenna size results in over-absorption of direct sunlight, not all of which can be used for photosynthesis, as this is limited by the rate of the carbon reactions and the associated biochemical steps. Excess absorbed irradiance from direct sunlight is then dissipated by the process of non-photochemical quenching (NPQ), a mechanism designed to prevent photodamage of the photosystems in the chloroplast (reviewed by Müller et al., 2001). In summary, evolution has applied selective pressure for the assembly and function of large light-harvesting Chl antenna complexes in all photosynthetic systems and, at the same time, efficient non-photochemical quenching processes, that are both characteristics of the photosynthetic apparatus in wild type plants and algae.

When plants and algae are grown under direct sunlight in high-density unicultures for product generation, e.g. fuel and chemicals, efficient conversion of sunlight energy to product by the culture as a whole is highly desirable. This requirement is compromised by the over absorption and wasteful dissipation of sunlight by cells at the surface of an alga culture, or upper canopy of plants, thereby compromising productivity (Melis, 2009). In such production systems, it is desirable to prevent the over-absorption of sunlight, to alleviate unnecessary shading, and to enable better sunlight penetration and more efficient utilization, thus enabling cells deeper in a culture or canopy to perform photosynthetically. Engineering strains that do not over-absorb and wastefully dissipate sunlight, but photosynthetically utilize all of it, could theoretically afford substantial productivity benefits to mass cultures. This was recognized long ago (Kok, 1953, 1960; Myers, 1957), but a solution could not be found. The concept of the truncated light-harvesting antenna (TLA) in photosynthetic organisms has recently attracted attention, as a way by which to optimize growth and productivity in mass cultures under bright sunlight conditions. This review discusses the conditions upon which a strain with a truncated light-harvesting antenna (TLA) would be more productive in mass-culture compared to a corresponding wild type. The work also summarizes recent progress in the elucidation of the CpSRP assembly pathway for the integration of the light-harvesting proteins into developing thylakoid membranes and examines how this process can be exploited in biotechnology to generate strains with a truncated light-harvesting chlorophyll antenna size.

2. Minimizing the light-harvesting chlorophyll antennae to maximize photosynthetic productivity

There is current interest and on-going efforts to renewably generate fuel and chemicals for human industrial and domestic consumption, through the process of microalgal and plant photosynthesis. Such bioproducts include H_2 and other suitable hydrocarbon and alcohol fuel molecules (Greenwell et al., 2010; Hankamer et al., 2007; Hu et al., 2008; Mata et al., 2010; Melis, 2007, 2012), and antigens (Dauvillée et al., 2010; Mayfield et al., 2007; Michelet et al., 2011). To aid the economic aspects of such effort, sunlight energy conversion efficiency in photosynthesis must occur with the maximum possible, as this would help to make renewable fuel and chemical processes economically feasible. In plants and algae, the solar energy conversion efficiency of photosynthesis is thus a most critical factor for the economic viability of renewable biomass, fuel and chemical production (Melis, 2009).

At low sunlight intensities, i.e., below those required for the saturation of photosynthesis, all absorbed photons are utilized efficiently to drive electrons in the electron-transport chain. Under these conditions, photosynthetic productivity increases linearly with the level or irradiance (Fig. 1, 0–500 μmol photons m^{-2} s^{-1} range). As the level of irradiance increases further, photosynthesis becomes saturated and reaches a plateau due to the fact that the carbon reactions cannot keep up with the linear increase in light absorption (Fig. 1, linear). Strains with a wild type light-harvesting antenna system (Fig. 1, WT) reach this light intensity for saturation, I_s, at lower levels of irradiance than their TLA counterparts (Fig. 1, *tla3*). The light-saturation of photosynthesis signifies that the sunlight harvested by the chlorophyll antenna exceeds the maximal operational capacity of the electron-transport chain and of the carbon reactions of photosynthesis, rendering the excess absorbed photons useless. Under bright sunlight conditions (2500 μmol photons m^{-2} s^{-1}) wild type strains with their fully developed light-harvesting antenna utilize photons inefficiently; only about 20% of the incoming sunlight energy is converted into useful photosynthesis (Fig. 1), excess absorbed energy is dissipated by the NPQ process. This inefficiency can theoretically be alleviated upon minimizing the light-harvesting chlorophyll antenna size (Kok, 1953, 1960; Myers, 1957) to limit sunlight absorption. It has been shown that cultures populated with TLA strains are more productive, when grown under high cell densities and sunlight intensities (Formighieri et al., 2012; Melis, 2009; Melis et al., 1999; Nakajima and Ueda, 1997, 1999; Polle et al., 2003). Greater culture productivity results by diminishing over-absorption of sunlight at the surface of the culture and thus minimizing wasteful dissipation of energy, while at the same time allowing for a far greater transmittance of sunlight deeper into the culture by eliminating unwanted shading. Ideally, no saturation of photosynthesis should occur within the range of the natural daylight intensities (0–2500 μmol photons m^{-2} s^{-1} range, Fig. 1), such that every single absorbed photon is utilized in photosynthesis. Such corrective action could improve productivity of mass cultures by over 500% under bright sunlight conditions, as evident by comparing the measured productivity of a wild type strain at 2500 μmol photons m^{-2} s^{-1}, which is about 100 mmol O_2 per mol Chl per s for the wild type (Fig. 1, WT) to over 500 mmol O_2 per mol Chl per s, as the case would be when photosynthesis could increase linearly with light intensity in the entire spectral range (Fig. 1, linear). Practically, the theoretical max efficiency is not achieved under ambient sunlight, as photosynthetic productivity is

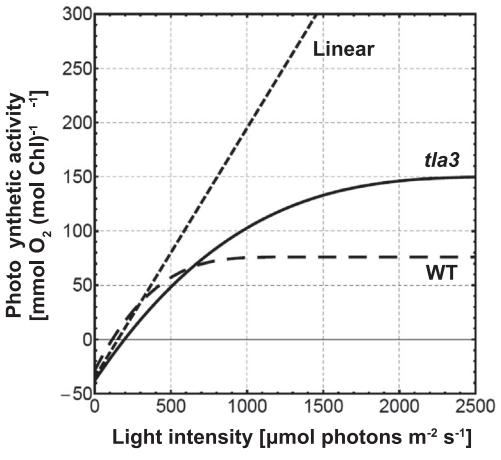

Fig. 1. The light saturation curves of photosynthesis. Activity was measured in O_2-evolution as a function of light intensity. Dotted line: theoretical maximal photosynthetic efficiency, which is linear with light intensity; dashed line: WT, wild type strains showed saturation of photosynthesis at about 500 μmol photons m^{-2} s^{-1}; solid line: *tla3* strain (Kirst et al., 2012b) shown here as an example for a TLA-type strain, showed saturation of photosynthesis at about 1000 μmol photons m^{-2} s^{-1}. A light intensity of 2500 μmol photons m^{-2} s^{-1} corresponds to that of bright sunlight.

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