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# Improvement of Photosynthetic $CO_2$ Fixation at High Light Intensity Through Reduction of Chlorophyll Antenna Size

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## Abstract

At elevated light intensities (greater than ~200 μE/[$m^2$·s]), the kinetic imbalance between the rate of photon excitation and thermally activated electron transport results in saturation of the rate of photosynthesis. Since maximum terrestrial solar radiation can reach 200 μE/($m^2$·s), a significant opportunity exists to improve photosynthetic efficiency at elevated light intensities by achieving a kinetic balance between photon excitation and electron transport, especially in designed large-scale photosynthetic reactors in which a low-cost and efficient biomass production system is desired. One such strategy is a reduction in chlorophyll (chl) antenna size in relation to the reaction center that it serves. In this article, we report recent progress in this area of research. Light-saturation studies for $CO_2$ fixation were performed on an antenna-deficient mutant of *Chlamydomonas* (DS521) and the wild type (DES15) with 700 ppm of $CO_2$ in air. The light-saturated rate for $CO_2$ assimilation in the mutant DS521 was about two times higher (187 μmol/[h·mg of chl]) than that of the wild type, DES15 (95 μmol/[h·mg of chl]). Significantly, a partial linearization of the light-saturation curve was also observed. These results confirmed that DS521 has a smaller relative chl antenna size and demonstrated that reduction of relative antenna size can improve the overall efficiency of photon utilization at higher light intensities. The antenna-deficient mutant DS521 can provide significant resistance to photoinhibition, in addition to improvement in the overall efficiency of $CO_2$ fixation at high light. The experimental data reported herein support the



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idea that reduction in chl antenna size could have significant implications for both fundamental understanding of photosynthesis and potential application to improve photosynthetic $CO_2$ fixation efficiency.

**Index Entries:** Photosynthesis; photosynthetic efficiency; chlorophyll antenna site; $CO_2$ fixation; photoinhibition resistance; *Chlamydomonas* mutant.

## Introduction

Photosynthesis is the fundamental biologic process that converts the electromagnetic energy of sunlight into chemical energy that supports essentially all life on Earth. It is also the foundation for algal biomass, biohydrogen, and agricultural crop production. To develop a viable biohydrogen production process as a potential future energy source, improvement in overall photosynthetic efficiency in a mass algal culture must be achieved. We have previously presented a graphic illustration of a strategy to improve photosynthetic productivity by reduction of chlorophyll (chl) antenna size in photosynthetic reaction centers *(1)*.

The underlying concept is that overall photosynthetic efficiency of an algal population can be improved by reducing the size of the light-harvesting chl antennae. In full sunlight (2000 μE/[$m^2$·s]), a kinetic imbalance exists between the rate of photon excitation of photosynthetic reaction centers and the ability of the thermally activated electron transport chains to process photogenerated electrons (Fig. 1A). Typically, a functional photosystem I (PSI) or photosystem II (PSII) has about 200 chl molecules per reaction center. Since the photo capture properties of a single chl molecule are such that it is hit about 10 times per second in full sunlight, the reaction centers can receive photon excitation at a rate of about 2000/s. However, operation of the electron transport chain is, at most, only about 200/s because of the relatively slow diffusive motion of electron carriers that shuttle between PSII and PSI and/or move through the Calvin cycle (Fig. 1A). Therefore, normal photosynthesis saturates at much less than full sunlight, typically about 10%. Reduction of the antenna size can effectively reduce the rate of photon absorption per reaction center and allow actinic photons to penetrate more deeply into the algal culture, thus improving the overall photosynthetic efficiency of the culture. The rationale underlying this strategy is that a proper reduction in the number of antenna pigments per photosynthetic reaction center could reduce the saturation phenomenon and allow sunlight to penetrate deeper into a dense algal culture (or a thick plant leaf or canopy) so that more photons can be captured and productively utilized by more cells in a unit volume under the same solar illumination area.

This theoretical consideration was clearly stated by Clayton *(2)* in 1977, although the idea predates Clayton's publication (Clayton, R. K., personal communication). However, very few experimental studies have been conducted to test this theory. In a developmental study of photosynthesis, Herron and Mauzerall *(3)* first observed a nearly linear light-saturation

curve in a dark-grown greening mutant culture of *Chlorella*, which is remarkably consistent with that predicted by the theory. Using high-light treatment, which physiologically reduces the chl antenna size in *Dunaliella salina* (Chlorophyta), Melis et al. *(4)* recently demonstrated that the high-light-grown algal culture exhibited a threefold higher $P_{max}$ (light-saturated rate of photosynthesis) than the normally pigmented low-light-grown culture, suggesting that algal strains with small antenna size could exhibit higher productivity than that currently achieved with normally pigmented cells. However, the smaller antenna sizes in both the dark-grown greening mutant and the high-light-grown *Dunaliella* used in the previous studies *(3,4)* cannot be sustained because they readily revert to that of the normally pigmented cells on returning to normal light intensity. We report here the first results of an experimental study with an antenna-deficient mutant of *Chlamydomonas*, DS521 *(5)*, and demonstrate the validity of the theory.

## Materials and Methods

### *Algal Strains*

The mutant DS521 was created by chemical mutagenesis of wild-type DES15 strain using fluorodeoxyuridine and ethylmethanesulfate *(5)*. Briefly, colonies grown from mutagenized cells were subjected to selection in the presence of metronidazole in the light. This procedure kills cells capable of full rates of photosynthetic electron transport but allows survival of strains that have lower photosynthetic rates under the conditions used. DS521 is a nuclear gene mutant characterized by a high ratio of chl a to chl b, though it is not completely deficient in chl b. It was estimated that DS521 contains only 5–10% of wild-type levels of the mRNA for chl a/b-binding (cab) proteins *(6)*. As a result, a portion of the antenna chl molecules in DS521 (Fig. 1B) are removed by the mutation, which is known to cause a 90% reduction in light-harvesting complex (LHC) II content and also a significant reduction in PSI antenna size *(7)*. Therefore, DS521 has fewer chl molecules per reaction center than wild-type DES15 (Fig. 1A).

### *Culture and Cell Harvesting*

The algal strains were grown under a light intensity of about 20 μE/($m^2$·s) in minimal-plus-acetate liquid medium. The composition of the culture medium was essentially the same as that of Sueoka medium *(8)*, except that the concentrations of $CH_3COONa$, $NH_4Cl$, $CaCl_2$, and $MgSO_4$ were 14.7, 7.5, 0.35, and 0.41 m*M*, respectively. The rationale for growing these stains photoheterotrophically is that the selection pressure for algal cells to make antennae would be minimal under such a growth condition. When the cultures grew to a density of about $10^6$ cells/mL, the algal cells were harvested by gentle centrifugation (1663*g*) with a JAL-10.500 rotor using a Beckman Avanti J-25I centrifuge. They were then washed and resuspended in fresh minimal medium for photosynthetic $CO_2$ fixation assays.

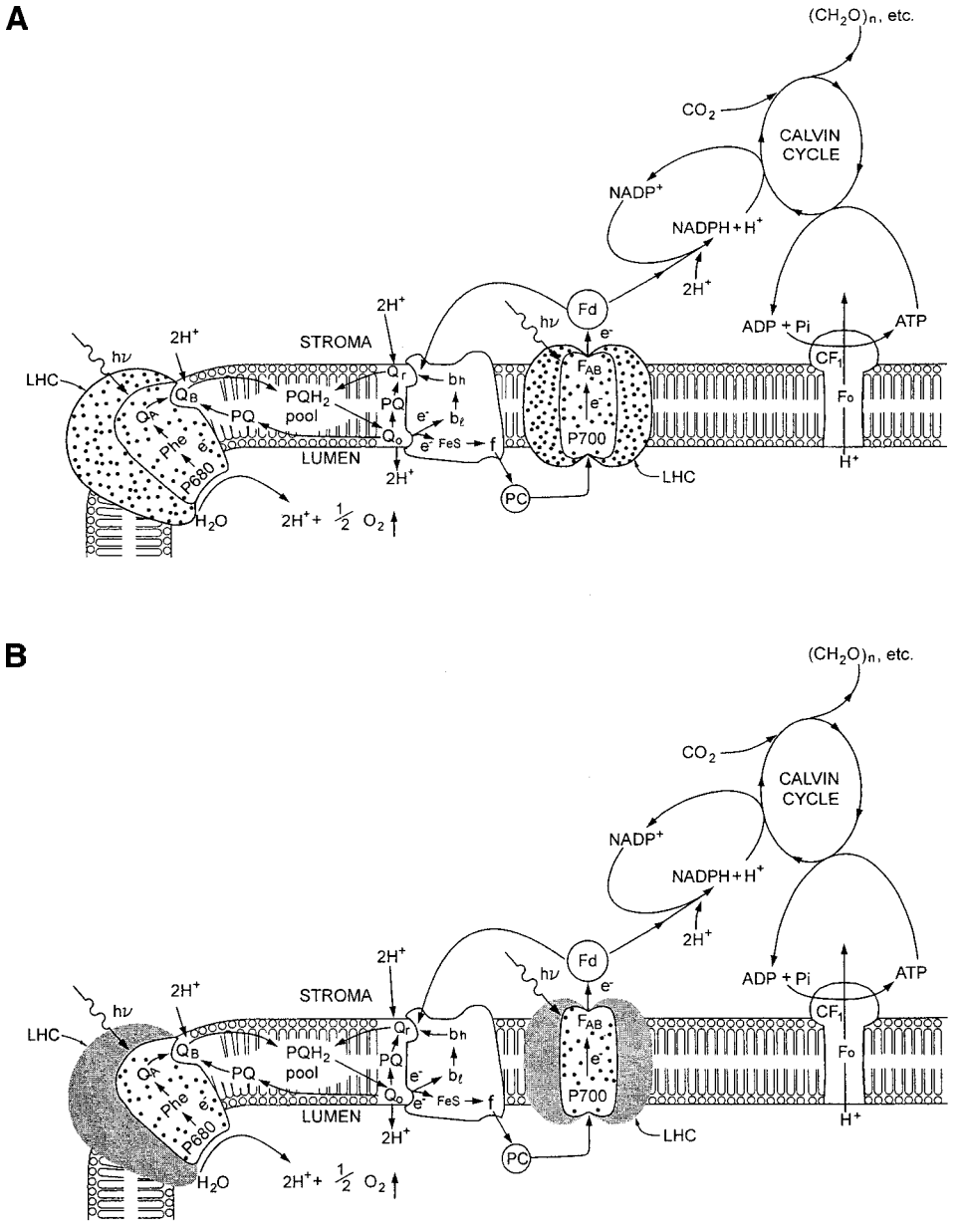


Fig. 1. (A) Conventional photosynthetic antenna size (about 200 chlorophylls per P680 or P700) and pathway as in wild-type DES15; (B) genetic removal of a significant fraction of light-harvesting chlorophylls from both photosystems I and II in antenna-deficient mutant DS521.

### *Photosynthetic Assays*

The $CO_2$ fixation assays were performed with 700 ppm of $CO_2$ in helium or air using our unique dual reactor flow detection system (Fig. 2). For each assay, algal samples of DS521 and DES15 (3 µg of chl/mL, 35 mL each) were placed and sealed in the two reactors, which were water jacketed and held at 20°C with a temperature-controlled water bath (Lauda RM6; Brinkmann, Germany). The algal samples were then purged by 50 mL of gas/min of air (containing 700 ppm of $CO_2$) through the liquid reaction medium. This carrier gas flow serves two purposes: (1) to remove $O_2$ from the algal sample to establish and maintain a constant aerobic (air) condition, and (2) to carry the remaining $CO_2$ in the gas-flow streams into the $CO_2$ analyzers. The actinic illumination was provided by a red (peak wavelength at 670 nm) light-emitting diode (LED) light source that was controlled by a computerized stepper motor whose drive shaft was connected to the intensity dial. Because of the automatic control of the LED light source, precise and reproducible step functions of actinic intensity were generated. The actinic intensity was monitored by a light meter (IL1700; International Light) and recorded by a computer simultaneously with the rates of $CO_2$ fixation. Any systematic error of the dual reactor flow detection system was minimized by interchanging two algal samples between the two reactors for each replication of assays. Chl concentration was determined spectrophotometrically with methanol extracts.

## Results and Discussion

Antenna-deficient mutant DS521 and wild-type DES15 were comparatively assayed for photoassimilation of $CO_2$ under various actinic intensities. Analysis of experimental data showed that the maximal rate of $CO_2$ fixation in DS521 with 700 ppm of $CO_2$ in air was about two times higher (187 µmol/[h·mg of chl]) than that of the wild type, DES15 (95 µmol/[h·mg of chl]). Figure 3 presents a comparative illustration of the light-saturation curves in DS521 and DES15. Photoassimilation of $CO_2$ in DS521 saturated at about twice the actinic intensity of that in DES15. The light intensities that give half-saturation of the photosynthetic rate were 276 and 152 µE/($m^2$·s) in DS521 and DES15, respectively. This result confirmed that DS521 has a smaller relative chl antenna size and demonstrated that the reduction of antenna size can indeed improve the overall efficiency of photon utilization and reduce photoinhibition.

DS521 also had significantly more resistance to photoinhibition than the wild type. As illustrated in Fig. 4, the simultaneous monitoring of $CO_2$ photoassimilation and actinic intensity showed that the photosynthetic activity in the wild type was photoinhibited almost completely when the LED actinic light reached its full intensity (red [670 nm] photons: 2000 µE/[$m^2$·s]), while DS521 retained about 25% of its maximal photosynthetic activity. The photoinhibited wild-type cells released more $CO_2$ by respiration than the amount of $CO_2$ fixation, while DS521 was still capable of

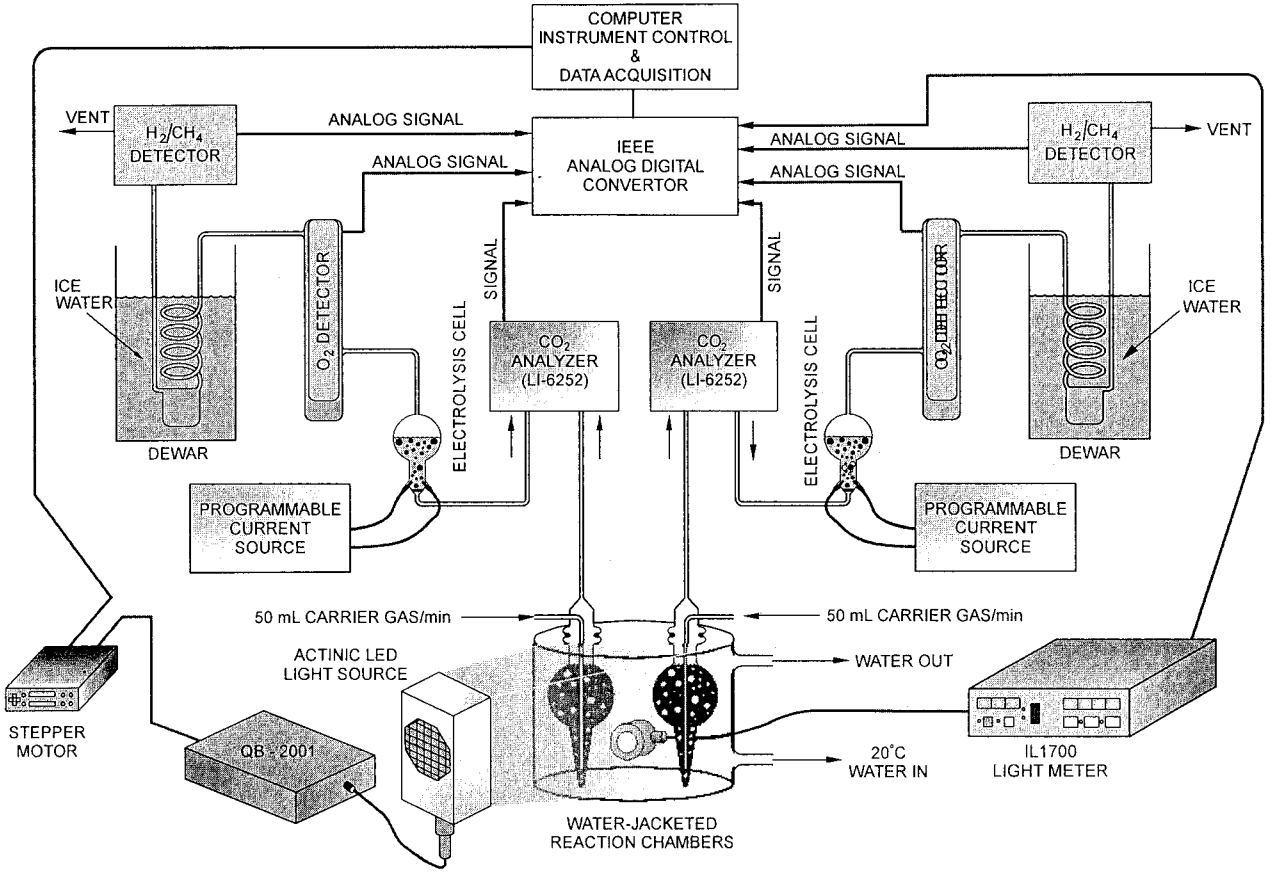


Fig. 2. Schematic of a dual reactor flow detection system for simultaneous detection of $CO_2$, $H_2$, $O_2$, and $CH_4$. This assay system allowed us to perform reliable characterization for the effect of chl antenna size on photosynthetic $CO_2$ fixation in the two algal strains under well-controlled experimental conditions. Any systematic error of the dual reactor flow detection system was minimized by interchanging two algal samples between the two reactors for each replication of assays.

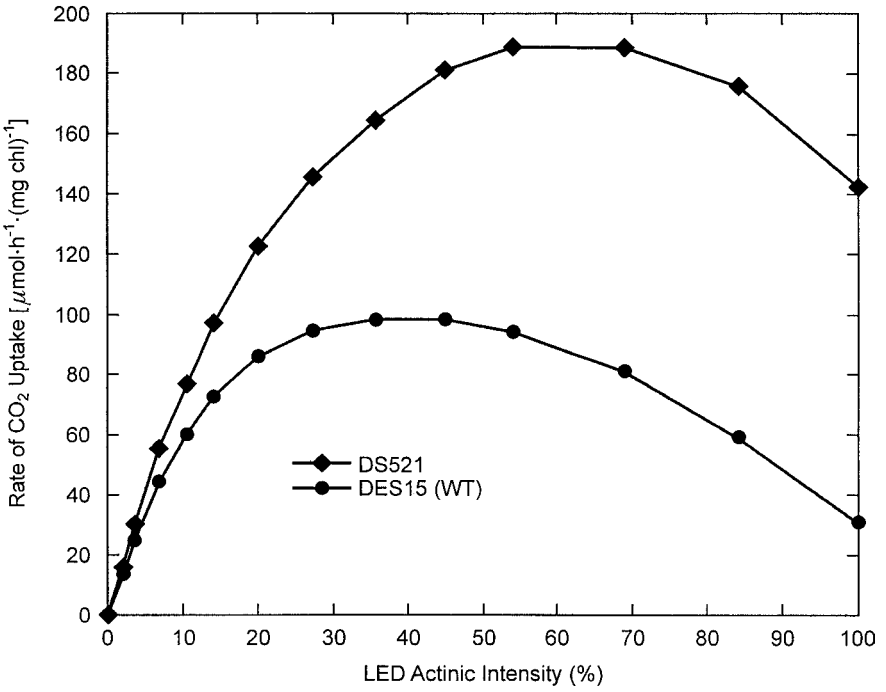


Fig. 3. Light-saturation curve as measured with photosynthetic fixation of $CO_2$ by DS521 and DES15 with 700 ppm of $CO_2$ in air. The full (100%) LED actinic intensity corresponds to 2000 μE/(m$^2$·s) of red (670-nm) photons. In this experiment, the observed difference in the maximal rate of $CO_2$ fixation between the mutant DS521 and the wild-type DES15 reflects the two effects of a reduced antenna size: (1) improving photosynthetic productivity and (2) reducing the probability or degree of photoinhibition.

photosynthesizing. These results demonstrate that reduction of photosynthetic antenna size in green alga can provide partial resistance to photoinhibition, in addition to improving the light utilization efficiency in $CO_2$ fixation.

Previous studies with chl fluorescence lifetime measurements have shown that the DS521 mutation significantly reduces chl antenna size in photosynthetic systems *(7,9)*. The effect of the DS521 mutation was first demonstrated in *Chlamydomonas reinhardtii* mutant strains A4d and 4D1c, which also carry the DS521 mutation that causes deficiency in the cab proteins. The strain 4D1c contains the DS521 mutation but retains normal PSI and PSII activities, in a manner similar to that of DS521, which was used in our study. The mutant A4d is a PSII-minus strain that was constructed by crossing B1 (a PSII-minus mutant) with 4D1c, a strain carrying the DS521 mutation. Therefore, in addition to the psbA (PSII) deletion, strain A4d also contains the DS521 nuclear mutation, which causes a 90% reduction in LHCII content as well as a significant reduction in PSI antenna size *(7)*. The A4d cells have a reduced PSI core antenna size (60–65 chl a/P700) relative to that of wild-type cells (120 chl a/P700) and a slightly altered composition

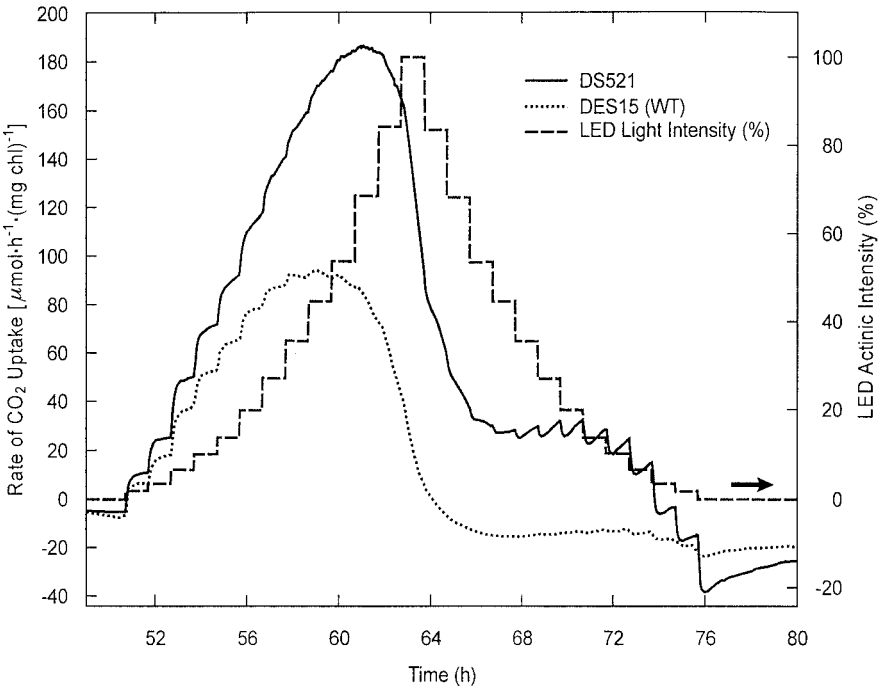


Fig. 4. Simultaneously recorded data of actinic intensity and $CO_2$ photoassimilation by DS521 and DES15 with 700 ppm of $CO_2$ in air. The vertical axis on the left is the rate of photosynthetic $CO_2$ fixation by the algal samples (3 µg of chl/mL, 35 mL for each strain). The axis on the right is the LED actinic intensity. The full (100%) LED light intensity corresponds to 2000 µE/($m^2$·s) of red (670-nm) photons. In comparison with the wild-type DES15, significant resistance to photoinhibition was visible in the mutant DS521.

of LHC, which contains 60–70 chl a + b per reaction center *(7,10)*. Although the exact molecular nature of the DS521 mutation is yet to be determined, these early studies clearly demonstrated that this mutation causes a large reduction in three LHCII polypeptides (mol wt = 30.5, 25, and 24 kDa) and a significant reduction in PSI antenna size with altered LHC composition *(7)*. With the previously reported characterizations of the DS521 mutation, the theory for improvement of photosynthetic efficiency through reduction of antenna size can explain perfectly the observed photosynthetic $CO_2$ fixation light-saturation curves in DS521 and DES15 (Figs. 3 and 4).

Our study therefore demonstrated a novel method for improving photosynthetic productivity by reducing the number of the light-harvesting antenna chls of the photosystems through molecular genetics. Direct experimental evidence showed that in comparison with the wild type (DES15), a genetically truncated light-harvesting antenna size in green algae *C. reinhardtii* (DS521) can result in greater yield of photosynthetic $CO_2$ fixation and improved resistance to photoinhibition in liquid culture. This conclusion is also consistent with a recent report *(11)* on a phycocya-

nin-deficient mutant (PD-1) of *Synechocystis* PCC 6714, which also demonstrated that photosynthetic productivity can be improved by reducing the light-harvesting pigment content in high-cell-density cultures at high light intensities.

The benefit of a proper reduction of chl antenna size is twofold: it improves photosynthetic productivity, and it reduces the probability or degree of photoinhibition. As shown in Figs. 3 and 4, the difference between the wild-type and mutant data comprises the two parts: the antenna effect of improved photosynthetic productivity on a per-chl basis and the antenna effect on the reduction of photoinhibition in the presence of atmospheric oxygen.

Genetic reduction in antenna size is probably a better approach than physiologic reduction in antenna size by high-light adaptation. Under high-light growth conditions, it is known that wild-type organisms can develop some physiological resistance to photoinhibition through alterations in the pigment and photosystem content of the cells—such as the changes in chl a/b ratio and optical properties of the culture; an increase in damaged and photochemically nonproductive PSII component ($PSII_b$); and formation of more carotenoids (particularly xanthophyll), which are capable of dissipating excess photon energy through a phenomenon known as the nonphotochemical energy dissipation *(12)*. Because of the nonphotochemical energy dissipation, high-light-grown algal cultures usually have a lower quantum yield than that of low-light-grown organisms. The relative quantum yield on a chl basis in high-light-grown algal culture was demonstrated to be only 37% of that in low-light-grown cells *(4)*. Therefore, physiologic reduction of antenna size by high-light adaptation has the disadvantage of loss of photosynthetic quantum efficiency, in addition to its sustainability problem. (The antenna size readily reverts to that of the normally pigmented cells on returning to normal light intensity.) However, genetic reduction in antenna size does not have these problems. The initial slope of the rate of photosynthesis (on a chl basis) vs irradiance is a measure of the relative quantum yield. The experimental data (Fig. 3) show that in the linear region of photosynthesis, the antenna-deficient mutant DS521 had a slope (relative quantum yield) equal to or somewhat better than that of the wild-type DES15. This result is consistent with the observation noted in 1966 by Schmid et al. *(13)*. The slight improvement in the relative quantum yield that we observed in the mutant DS521 is probably owing to the fact that it does not suffer yield loss by quenching of excitation energy in the antenna. In any event, our results indicate that the photosynthetic productivity of DS521 mass culture should be significantly higher than that of DES15 under high light intensities and that its productivity at low light intensities should be at least equal to, if not better than, that of the wild type.

For commercial mass algal production, conditions that maximize photosynthetic productivity and minimize photodamage are important factors *(14–16)*. So far, most of the research and development on increasing

the productivity of algal mass production has focused on rapid mixing of the cultures to achieve a "flashing-light effect" *(17–21)* and on vertical reactors, prisms, or fiberoptics to better diffuse light into the cultures *(22–30)*. These options are cumbersome and prohibitively expensive for large-scale mass cultures in which a low-cost production system is desired *(31)*. We have demonstrated here that a mutant green alga with a smaller antenna size can indeed exhibit both higher maximum rates of photosynthesis and resistance to photoinhibition at high light intensities—the photobiologic characteristics desired in commercial mass cultures. Therefore, mutant algae with a truncated chl antenna size may find a variety of commercial applications, including mitigation of $CO_2$, and production of biomass, fine biochemicals, and hydrogen.

Although application of an antenna size–reduced mutant for a dense cell culture could provide a collectively more efficient solar energy conversion technology, it should be noted that—to an individual cell—genetic reduction of antenna size would not necessarily confer any survival advantages in the natural environment. An antenna size–reduced mutant would have fewer chls per cell, although it may contain the same number of photosynthetic reaction centers and carbon assimilation enzymes as that of its wild type. Consequently, an individual cell of the mutant would capture fewer photons and would not necessarily assimilate any more $CO_2$ on a per-cell basis than its wild-type counterpart. Therefore, such a mutant cell may not have any advantage in competing with its wild-type cells in a natural environment. This probably explains why no such mutant forms of green algae have been found in nature and why this type of mutant has to be created for the benefit of human application. For two mass cultures with an equal chl concentration (or equal chl optical density) that is sufficient to absorb most of the sunlight, the culture of an antenna-deficient mutant would have more cells (thus more photosynthetic reaction centers and $CO_2$ fixation enzymes) per culture volume than the culture of the wild type. Since each cell of the mutant absorbs fewer photons because of its smaller antenna size, thereby minimizing saturation and photoinhibition, actinic photons should penetrate deeper and reach a larger number of cells in the mutant culture than in the wild-type culture—the actinic photons would thus be utilized more productively by more cells in the culture of the mutant than in the culture of the wild type. This, in essence, explains why application of an antenna size–reduced mutant for a mass culture could provide a collectively more efficient solar energy conversion technology. DS521 is the first chl antenna–deficient mutant of a green alga that showed promise in improving the collective photon utilization efficiency of a liquid culture.

Furthermore, the concept demonstrated in our study may also find application for improvement in crop productivity. The Green Revolution of the 1960s and 1970s (the use of chemical fertilizers, irrigation, and crop breeding) dramatically improved crop yield per land area *(32)*. The success of crop breeding is mainly the result of the development of varieties

that are less susceptible to pests and diseases or that can tolerate hostile environmental conditions such as drought or salty soils. However, the annual increases in crop yield have recently slowed and showed signs of reaching an "immovable ceiling" because all of these early techniques exploit the existing yield potential but do not focus on improvement of the molecular photosynthetic apparatus *per se (32)*. For example, the yields of wheat and rice were significantly increased by creating shortened "dwarf" varieties with strong stalks that could hold more grain. This boosted the "harvest index"—the percentage of the plant's mass that is grain—to the present value of about 50%, almost double the previous figure *(33)*. However, the present harvest index value has almost reached a physical limit. Breeding still-shorter plants ultimately produces such a low, uneven canopy of leaves that it is difficult to obtain uniform interception of sunlight, which interferes with photosynthesis; breeding for thinner, lighter stalks makes plants more likely to collapse under the weight of their own grains. Therefore, many agricultural scientists now believe that further improvement in yield potential has to come from reengineering of photosynthesis *per se (32)*. The improvement in productivity by reduction in chl antenna size demonstrated in our study could provide such an opportunity. With the advance of molecular genetic engineering, the DS521 mutation may find application in crops such as rice and wheat through genetic transformation. It may also be possible to create mutations similar to that of DS521 in higher plants for further improvement in agricultural crop productivity.

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