Integration of Carbon Assimilation Modes with Photosynthetic Light Capture in the Green Alga *Chlamydomonas reinhardtii*

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ABSTRACT **The unicellular green alga *Chlamydomonas reinhardtii* is capable of using organic and inorganic carbon sources simultaneously, which requires the adjustment of photosynthetic activity to the prevailing mode of carbon assimilation. We obtained novel insights into the regulation of light-harvesting at photosystem II (PSII) following altered carbon source availability. In *C. reinhardtii*, synthesis of PSII-associated light-harvesting proteins (LHCBMs) is controlled by the cytosolic RNA-binding protein NAB1, which represses translation of particular LHCBM isoform transcripts. This mechanism is fine-tuned via regulation of the nuclear *NAB1* promoter, which is activated when linear photosynthetic electron flow is restricted by CO_2-limitation in a photoheterotrophic context. In the wild-type, accumulation of NAB1 reduces the functional PSII antenna size, thus preventing a harmful overexcited state of PSII, as observed in a NAB1-less mutant. We further demonstrate that translation control as a newly identified long-term response to prolonged CO_2-limitation replaces LHCII state transitions as a fast response to PSII over-excitation. Intriguingly, activation of the long-term response is perturbed in state transition mutant *stt7*, suggesting a regulatory link between the long- and short-term response. We depict a regulatory circuit operating on distinct timescales and in different cellular compartments to fine-tune light-harvesting in photoheterotrophic eukaryotes.**

Key words: light-harvesting antenna; translation control; state transitions; NAB1; carbon metabolism; *Chlamydomonas reinhardtii*.

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INTRODUCTION

Photosynthetic acclimation ensures unaffected photosynthetic performance in a constantly changing environment. Light-harvesting is one of its prime targets, being modulated on multiple levels with implicated mechanisms operating on different timescales. A sudden rise in light intensity or a drop in CO_2 availability increases excitation pressure at PSII, which has deleterious effects, if not immediately relieved by short-term acclimation mechanisms. Seconds to minutes after the onset of high excitation pressure, non-photochemical quenching (NPQ) mechanisms are activated (Allorent et al., 2013). The fast, energy-dependent part of NPQ relies on a reversible switch of light-harvesting complexes from a harvesting into a photoprotective state that is required to dissipate excess excitation energy as heat. This process is regarded as the major photoprotective mechanism in high light (Iwai et al., 2007; Ruban et al., 2007), whereas state transitions represent the predominant fast mechanism that reduces PSII excitation pressure under low light conditions (Rintamäki et al., 1997). An over-reduced plastoquinone pool triggers STT7/STN7-dependent LHCII phosphorylation (Lemeille and Rochaix, 2010; Lemeille et al., 2010) and the subsequent migration of extra or loosely bound trimers (Wientjes et al., 2013; Drop et al., 2014) to PSI. Since an enhanced photon absorption capacity at PSI following the state I–state II transition increases cyclic electron flow, this process not only relieves PSII excitation pressure (Bonaventura and Myers, 1969; Murata, 1969), but also adjusts the ATP/NADPH ratio to meet the demands of the Calvin cycle (Bulté et al., 1990;

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Lemeille and Rochaix, 2010). State transitions are of particular relevance during the acclimation to varying inorganic and organic carbon supply (Bulté et al., 1990; Johnson and Alric, 2012, 2013; Lucker and Kramer, 2013). Microalgae like *Chlamydomonas reinhardtii* grow photoautotrophically using CO_2 as a carbon and light as an energy source, but in addition reduced carbon sources can be assimilated (Harris, 2009). Being ATP-demanding processes, acetate assimilation and the induction of carbon concentrating mechanisms triggered by inorganic carbon limitation were both shown to induce a transition to state II, thereby enhancing ATP-generating cyclic electron flow (Iwai et al., 2007; Lucker and Kramer, 2013). CO_2-limitation reduces the consumption of ATP and NADPH formed within the photosynthetic light reaction leading to an over-reduced photosynthetic electron transport (PET) chain. Previous studies attributed the initial fast reduction in excitation pressure immediately after the onset of CO_2-limitation to state transitions as the underlying mechanism (Palmqvist et al., 1990; Falk and Palmqvist, 1992; Iwai et al., 2007). Reversal of the state II transition during prolonged low CO_2 supply (Iwai et al., 2007), however, indicated that excitation pressure relief based on state transitions is replaced by alternative mechanisms operating on longer timescales. In accordance with this notion, the functional antenna size was shown to be reduced as part of the long-term response to CO_2-limitation in *C. reinhardtii* (Spalding et al., 1984). Photosynthetic long-term acclimation processes are based on stoichiometric adjustments within the photosynthetic machinery, which require a modulated expression of genes encoding individual subunits. Previous studies analyzing transcriptome changes following CO_2-limitation under low light conditions revealed that the abundance of LHCII (*LHCBM*) transcripts did not change significantly (Yamano et al., 2008). Under such conditions, expression of antenna proteins might therefore be regulated posttranscriptionally involving translation control (Wobbe et al., 2008) and this type of control was found in evolutionarily diverse photosynthetic organisms (Durnford et al., 2003; Floris et al., 2013; Gutu et al., 2013). In *C. reinhardtii*, the cytosolic RNA-binding protein NAB1 represents a key factor controlling the translation of light-harvesting protein encoding transcripts (Mussgnug et al., 2005), that selectively binds to the mRNA of particular *LHCBM* isoforms with a preference for the *LHCBM6* transcript. By sequestrating *LHCBM* mRNA in sub-polysomal ribonucleoprotein complexes, it represses its translation, thereby adjusting the synthesis of LHCII proteins. Given that the demand for light-harvesting proteins in the thylakoid membrane constantly changes in response to environmental cues, cytosolic LHCII translation repression has to be fine-tuned. Two distinct molecular switches in NAB1 were shown to determine its activity and include redox-based modification of cysteine residues (Wobbe et al., 2009) besides arginine methylation (Blifernez et al., 2011). Considering that NAB1 represents a key element of the regulatory circuit fine-tuning the PSII light capture, a multi-layer regulation of NAB1-mediated translation control seems reasonable. We investigated the complex regulation of light-harvesting in the photoheterotroph *C. reinhardtii* that follows a switch between carbon assimilation modes and which implicates processes in the nucleus, chloroplast and cytosol. As a key finding, NAB1 was identified as a regulatory hub connecting short- and long-term photoacclimatory responses that control PSII excitation pressure.

RESULTS

The Cellular NAB1 Level Is Determined by the Prevailing Carbon Assimilation Mode

Green alga like *C. reinhardtii* are photoheterotrophs assimilating organic in addition to inorganic carbon (Harris, 2009). Acetate supply was previously shown to have a repressive effect on photosynthesis including LHCBM expression (Kindle, 1987; Heifetz et al., 2000; Kovács et al., 2000; Humby et al., 2009). The light-harvesting antenna is a prime target of photosynthetic acclimation (Kindle, 1987; Teramoto et al., 2002; Durnford et al., 2003; Rochaix, 2013) and, in *C. reinhardtii*, translation control of LHCII mRNAs requires the cytosolic translation repressor NAB1 (Mussgnug et al., 2005). Besides its repressor activity, the cellular NAB1 amount is modulated in response to changes in the carbon acquisition mode (Blifernez et al., 2011). Immunoblot studies (Figure 1A) showed that photoheterotrophic cultivation using air levels (≈0.04% (v/v)) of CO_2 ($-CO_2$ throughout the manuscript) and acetate (+Ac) was accompanied by an increased cellular NAB1 amount as compared to photoautotrophic growth with CO_2-enriched air (3% (v/v); $+CO_2$ throughout the manuscript). *C. reinhardtii* was recently shown to utilize cellulose as a carbon source (Blifernez-Klassen et al.,

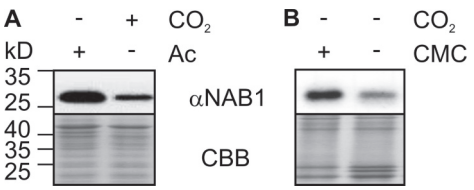

Figure 1 The Availability and Type of Carbon Source Triggers NAB1 Accumulation.

(A) Immunodetection of NAB1 in whole cell protein extracts derived from cultures grown in the presence (+) or absence (–) of acetate (Ac). Cultures were either bubbled with air ($-CO_2$) or CO_2-enriched (3% (v/v)) air ($+CO_2$).

(B) Immunodetection of NAB1 after growth in the presence (+) or absence (–) of carboxymethyl cellulose (CMC) and air levels of CO_2 ($-CO_2$).

Lower panels (A, B): Coomassie-Brilliant Blue-stain (CBB) serving as a loading control.