Red/far-red light signals regulate the activity of the carbon-concentrating mechanism in cyanobacteria

Nadav Oren1*, Stefan Timm1, Marcus Frank2,3, Oliver Mantovani1, Omer Murik4, Martin Hagemann1,3

Desiccation-tolerant cyanobacteria can survive frequent hydration/dehydration cycles likely affecting inorganic carbon (Ci) levels. It was recently shown that red/far-red light serves as signal-preparing cells toward dehydration. Here, the effects of desiccation on Ci assimilation by Leptolyngbya ohadii isolated from Israel’s Negev desert were investigated. Metabolomic investigations indicated a decline in ribulose-1,5-bisphosphate carboxylase/oxygenase carboxylation activity, and this was accelerated by far-red light. Far-red light negatively affected the Ci affinity of L. ohadii during desiccation and in liquid cultures. Similar effects were evident in the non–desiccation-tolerant cyanobacterium Synechocystis. The Synechocystis ΔCph1 mutant lacking the major phytochrome exhibited reduced photosynthetic Ci affinity when exposed to far-red light, whereas the mutant ΔSbtB lacking a Ci uptake inhibitory protein lost the far-red light inhibition. Collectively, these results suggest that red/far-red light perception likely via phytochromes regulates Ci uptake by cyanobacteria and that this mechanism contributes to desiccation tolerance in strains such as L. ohadii.

INTRODUCTION

Biological soil crusts (BSCs) are cryptogamic communities on soil surfaces that cover large areas in arid and semiarid regions of Earth. They fulfill numerous biological functions such as carbon and nitrogen enrichment, water retention, and prevention of desertification and contribute substantially to annual CO2 fixation (1–4). It has been predicted that climate change and other human activities will affect the biological composition and function of BSCs in the near future (1).

Filamentous cyanobacteria are regarded as pioneer organisms in BSCs. In addition to their important roles as primary producers by way of carbon and nitrogen fixation, it has been reported that their extracellular polysaccharides form and stabilize the crust structures in topsoil (5). Organisms in BSCs in particularly arid areas live in one of the harshest environments on Earth because they are exposed to extremely low and irregular water availability and large temperature fluctuations. For example, cyanobacteria in BSCs in the Negev desert in Israel are subjected to daily hydration/dehydration cycles (6, 7) because the crust usually receives small amounts of water in the form of early morning dew, followed by dehydration with rising temperature and a rapid decline in humidity during the first hours of the day. Hence, cyanobacteria inhabiting BSCs must be adapted to these conditions (3, 4). Previous studies have mainly focused on microbial community structure and cyanobacterial activity in various BSCs (4, 8–10), whereas the mechanisms that facilitate the maintenance of life under these extreme conditions are less well understood. Osmotic adjustment in BSC-inhabiting cyanobacteria during hydration/dehydration and the consequent changes in salinity probably play an important part in acclimation to desiccation. An extracellular polysaccharide sheath and accumulation of high amounts of compatible solutes trehalose and sucrose are crucial mechanisms for osmotic acclimation in cyanobacteria and other microbes (11–13).

To investigate molecular mechanisms of cyanobacterial desiccation tolerance, detailed studies on strains isolated from BSCs need to be conducted (4, 14, 15). Isolates of the cyanobacterium Leptolyngbya ohadii obtained from BSCs in Israel’s Negev desert exhibiting remarkable desiccation tolerance have been used in these studies (7, 16). Their photosynthetic activity recovers after desiccation, which permits active photosynthetic carbon fixation every morning when a small amount of water provided mostly by dew becomes available to the BSC. A specific acclimation program including changes in gene expression sequence is initiated in desiccation-tolerant cyanobacteria during dehydration, which activates many genes for abiotic stress tolerance (17–19). In L. ohadii, it has been shown that dawn illumination and rising temperature serve as signals that initiate preparation for the inevitable forthcoming dehydration (20). Perception of the light signal is probably mediated via phytochromes, because red/far-red (FR) light cycles reportedly have notable effects on survival rates and transcriptional responses to desiccation (18). Phytochromes are light-sensing proteins that can absorb red and FR light via a bound chromatophore, and red and FR light switch the sensor protein between an active and an inactive conformation, respectively. They regulate numerous cellular processes in various organisms ranging from bacteria to plants, including transcriptional reprogramming (21–23). In plants, sensing light via phytochromes plays an important role in the measurement of the length of daily light periods, which has numerous effects on plant development and many other processes. The function of phytochromes in prokaryotes is far less understood. In cyanobacteria, it has been shown that phytochromes are involved in mediating the circadian clock and in phototactic movement (24, 25).

Hydration/dehydration cycles likely affect inorganic carbon (Ci) levels in BSCs because availability of water allows active photosynthesis that is changing pH and thereby carbonate chemistry in the topsoil (26). Cyanobacteria are able to acclimate to fluctuating Ci
levels via their Ci-concentrating mechanism (CCM). Different systems for bicarbonate and CO₂ uptake contribute to the accumulation of high levels of internal bicarbonate, which then enters the carboxysome. There, carbonic anhydrase converts bicarbonate into CO₂, thereby raising the CO₂ level at the carboxylating site of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and suppressing its oxygenase activity and photorespiration (27–29).

In the present study, the effects of different Ci preacclimatization states and FR light on Ci assimilation and desiccation tolerance were investigated in the cyanobacterium L. ohadii under simulated natural conditions. Moreover, experiments with specific mutants of the non–desiccation-tolerant cyanobacterium Synechocystis sp. PCC 6803 provided mechanistic evidence on how the FR light is sensed and to which target protein the signal is transduced. Collectively, our results allowed to conclude that red/FR light–sensing photoreceptors such as phytochromes regulate the activity of the cyanobacterial CCM and thereby affect desiccation tolerance in cyanobacteria.

RESULTS

Effects of Ci amount and FR light on desiccation tolerance in L. ohadii

The inability of L. ohadii to recover from dehydration when exposed to FR light prompted the current investigation of the underlying mechanisms in cyanobacteria acclimated to different amounts of Ci. With regard to the effects of CO₂ levels on acclimation to desiccation and the ability to recover thereafter, FR light almost completely abolished the desiccation tolerance of L. ohadii grown under low Ci (LC) conditions (ambient air with 0.04% CO₂), whereas in the absence of FR light, approximately 70% recovery was observed (Fig. 1). The FR light–mediated decrease in desiccation tolerance could be reversed to a large extent, when the FR light was switched off after 1 hour, and the further drought acclimation continued with growth light (Fig. 1). When the dehydration experiment was performed with L. ohadii preacclimated to high Ci (HC) conditions (5% CO₂), recovery was significantly lower, and photosynthesis dropped to approximately 15% of the initial value. The extent of recovery in the HC-preacclimated L. ohadii was similar when they were exposed to FR light treatment (Fig. 1). These results suggest that Ci-mediated and phytochrome-mediated signals play an important role in desiccation tolerance in L. ohadii.

Effects of Ci amount and FR light on the metabolome of L. ohadii under dehydration

To investigate the effects of Ci preacclimatization and FR light on responses to dehydration, we performed metabolic profiling of L. ohadii under various hydration conditions. Relative levels of primary metabolites during dehydration were compared in L. ohadii preacclimated to LC or HC conditions. Primary metabolite levels exhibited multiple changes during natural dehydration (Fig. 2 and fig. S2). The amounts of 3-phosphoglycerate (3PGA), the stable carboxylation product of Rubisco-mediated CO₂ fixation, increased approximately twofold during the first 60 to 160 min of dehydration treatment in LC-acclimated and HC-acclimated L. ohadii. During ongoing dehydration, the 3PGA levels decreased three- to fourfold compared to levels before desiccation (Fig. 2). When LC-acclimated L. ohadii were exposed to additional FR light during dehydration, 3PGA levels rose during the first 60 min as in non-FR–treated L. ohadii but remained elevated throughout the dehydration process.

In addition to increased amounts of the Rubisco carboxylation reaction product 3PGA, an increased steady-state level of the Rubisco oxygenation reaction product 2-phosphoglycolate (2PG) was observed in LC-acclimated L. ohadii, and it was even more pronounced in HC-acclimated L. ohadii after light exposure (Fig. 2). The most substantial 2PG accumulation (an almost 10-fold increase) was evident in LC-acclimated L. ohadii exposed to FR light (Fig. 2), which exhibited significantly higher and more stable 2PG accumulation than LC-acclimated L. ohadii. The increase in the steady level of 2PG likely indicates enhanced photorespiratory activity after FR light in L. ohadii, suggesting a reduced CO₂/O₂ ratio during dehydration. The concept of enhanced photorespiration is supported by the finding that the photorespiratory intermediates glycine and serine also increased upon dehydration, particularly in HC-acclimated L. ohadii, in which there was a consistent increase to 8- to 10-fold higher levels (Fig. 2).

Additional evidence for strong effects of dehydration and FR light on carbon metabolism was reflected by glycogen levels in L. ohadii. The glycogen pool is rapidly mobilized during dehydration in HC-acclimated L. ohadii. Glycogen breakdown probably replenishes the Calvin cycle and other metabolic routes to compensate for reduced carbon fixation, as has been previously reported in the model cyanobacterium Synechocystis sp. PCC 6803 (30). Similarly, in the current study, the lower glycogen pool in LC-acclimated L. ohadii was also mobilized during the first 160 min of dehydration, whereas FR-treated LC-acclimated L. ohadii exhibited almost no glycogen decrease; i.e., FR light treatment kept the carbon storage at constant levels (Fig. 2). The strong changes in carbon fixation and storage
compounds observed also had effects on many other metabolites. Tricarboxylic acid cycle intermediates such as malate and citrate accumulate during dehydration (fig. S2). The steady-state amount of 2-oxoglutarate also increased, which could be interpreted as an indication that dehydration also slows ammonia assimilation (31). This assumption is supported by reduced amounts of some soluble amino acids such as arginine (fig. S2). The observed metabolic responses by *L. ohadii* to dehydration indicated that water shortage had strong effects on primary metabolism and that their adjustment to new conditions is influenced by FR light signals.

**Effects of Ci and FR light on the CCM in *L. ohadii* under dehydration**

Changes in 3PGA and 2PG levels suggested that dehydration had strong effects on the activity of the CCM in *L. ohadii*. To investigate the mechanisms involved, we examined carboxysome structures in...
L. ohadii during dehydration via electron microscopy. Specifically, the numbers and integrity of carboxysomes during dehydration treatments were assessed. There were no significant differences between HC-acclimated and LC-acclimated L. ohadii exposed to water loss for different durations (fig. S3).

Photosynthetic Ci affinity measurements were conducted in L. ohadii at different time points during the dehydration process to test whether Ci uptake was affected by desiccation. Because photosynthetic dependence measurements over a broad range of Ci concentrations require more than 1 hour (fig. S4A), which is not compatible with defined dehydration times, photosynthetic oxygen evolution was compared under Ci-limiting conditions (75 μM NaHCO₃) and Ci-saturating conditions (1 or 50 mM NaHCO₃). In these experiments, there was increased photosynthetic activity in LC-acclimated L. ohadii during the first 60 min under a limiting bicarbonate concentration, due to light activation of the CCM, followed by gradual decline thereafter (Fig. 3A). Similar trends were evident at saturating bicarbonate concentrations in LC-acclimated L. ohadii (Fig. 3B), whereas HC-acclimated L. ohadii exhibited lower photosynthetic activity at limiting bicarbonate concentrations, corresponding to lower induction of the CCM in HC-grown L. ohadii as has been previously reported (32). In contrast, a steady decline in photosynthesis was evident at saturating bicarbonate levels during the dehydration treatment. When LC-acclimated L. ohadii were exposed to FR light, however, their photosynthetic activity declined significantly. In the presence of limited or saturating Ci amounts, it reached lower levels, similar to those of L. ohadii preacclimated to HC (Fig. 3, A and B). The reduced photosynthetic activity in FR-treated L. ohadii could be restored by supplying very high amounts of bicarbonate (50 mM) (Fig. 3C), suggesting that the observed decline in photosynthetic activity was caused by dysfunction of the Ci uptake machinery.

Effects of Ci and FR light on the CCM of L. ohadii in liquid culture

The previously unknown effects of FR light on Ci affinity may be specific for the preparation of L. ohadii to desiccation, or they may not be. To differentiate effects of FR light from effects of the dehydration process itself, we exposed L. ohadii cells to FR light in liquid cultures and then measured Ci-dependent photosynthesis. Again, there was a clear difference in photosynthetic activity at limiting or saturating Ci concentrations, i.e., FR light-exposed L. ohadii always exhibited lower photosynthesis than non–FR light–exposed LC-acclimated L. ohadii. Photosynthetic activity dropped to <20% after FR light exposure in the presence of limiting or saturating bicarbonate concentrations compared with control LC-grown L. ohadii (Fig. 4A). The negative impact of FR light on the photosynthetic activity was found to be reversible. The photosynthetic activities returned to control levels when the FR light was switched off, and the cells were incubated for additional 1 hour in the presence of red light (Fig. 5A) or of white light (fig. S5A). Furthermore, it could almost be restored to control levels in the presence of an oversaturating Ci concentration (Fig. 4A). Similar effects were observed when the photosynthetic activity of L. ohadii was measured in an artificial short light/dark/light cycle. Incubation of LC-acclimated cells for 1 hour in darkness decreased the photosynthetic activity at 75 and 500 μM bicarbonate to the same extent as FR light exposure, and it could be reactivated to the initial levels with white light as before with red light (fig. S6A). These results indicate that Ci-dependent photosynthetic activity changes with light availability and that FR light might have an effect similar to darkness on this process. No significant differences in photosynthetic activity were observed in HC-acclimated L. ohadii under a limiting Ci concentration (75 μM), because of lower photosynthetic activity in the control (fig. S7A), which was consistent with the lower CCM capacity under HC growth conditions. However, the FR light signal had an inhibitory effect under higher Ci conditions (500 μM) in HC-grown L. ohadii, similar to LC-acclimated L. ohadii. Again, the decline in photosynthetic bicarbonate affinity could be recovered with saturating (1 mM) and oversaturating (50 mM) bicarbonate concentrations (fig. S7A). Thus, FR light had a direct, dehydration-independent, negative effect on photosynthetic activity in L. ohadii, which could be reversed by either red light or high bicarbonate concentrations.

Impact of Ci amount and FR light on the CCM of Synechocystis

The effects of FR light on L. ohadii in both the presence and absence of dehydration stress prompted us to investigate whether this previously

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**Fig. 3. Changes in the photosynthesis in L. ohadii during dehydration.** The photosynthetic activity in L. ohadii that have been preacclimated to LC or HC conditions with and without the addition of FR light was measured in an oxygen electrode in the presence of different NaHCO₃ concentrations (A, limited amount of 75 μM; B, saturated amounts of 1 mM; C, excess amounts of 50 mM) after different times of dehydration. Samples for photosynthetic measurements were withdrawn during dehydration at the indicated time points (see fig. S1; DESI, after complete desiccation). All values indicate mean photosynthetic activities ± SD of three independent biological replicates.
unknown effect of FR light on cyanobacterial Ci affinity may be a specific adaptation of desiccation-tolerant cyanobacteria such as *L. ohadii* or whether similar effects could be observed in non–desiccation-tolerant cyanobacteria. The above-described experiments were repeated using the non–desiccation-tolerant strain *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*). Negative effects of FR light on photosynthetic activity were clearly evident in both LC-acclimated *Synechocystis* (Fig. 4B) and HC-acclimated *Synechocystis* (Fig. S7B).

As shown for *L. ohadii*, the negative impact of FR light on the photosynthetic activity could be also reversed in *Synechocystis* when the FR light was switched off, and the cells were incubated for additional 1 hour in the presence of red light (Fig. 5B) or of white light (fig. S5B). Moreover, the incubation of *Synechocystis* wild-type cells in darkness inhibited photosynthesis, while light reactivated it as observed before with *L. ohadii* (fig. S6B). Hence, the general photosynthetic responses of *Synechocystis* were highly similar to those of *L. ohadii*, i.e., more pronounced FR light inhibition in LC-acclimated *Synechocystis* under limiting bicarbonate conditions, followed by recovery at red light or at oversaturating Ci concentrations.

The prime candidates with respect to a connecting link between the effects of red/FR light signals and cellular carbon responses are phytochromes, specifically red/FR light–sensitive photoreceptors. Cph1 was the first plant-type phytochrome to be identified in *Synechocystis* that behaves like a plant phytochrome (22, 23). Hence, we used a *Synechocystis* ∆cph1 mutant, in which the gene encoding the major phytochrome Cph1 is impaired, and tested the involvement of this phytochrome in FR light-mediated inhibition of photosynthetic Ci affinity. The LC-acclimated *Synechocystis* ∆cph1 mutant exhibited much lower affinity to Ci than wild-type *Synechocystis* under the same conditions (Fig. 4, B and C). Measurements of the photosynthetic activity with LC-acclimated cells of mutant ∆cph1 under a wide range of Ci concentrations revealed that the mutant

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**Fig. 4. Changes in the photosynthesis in *L. ohadii* and *Synechocystis* sp. PCC 6803 from liquid cultures.** The photosynthetic activity of *L. ohadii* wild type (A; WT), *Synechocystis* sp. PCC 6803 wild type (B, WT), and *Synechocystis* sp. PCC 6803 mutants ∆cph1 (C) and ∆sbtB (D) measured in an oxygen electrode in the presence of different NaHCO₃ concentrations. Cells were cultivated in liquid BG11 medium under LC conditions. After a dark period, they were exposed to white light of 50 μmol photons m⁻² s⁻¹ with or without the addition of FR light of 0.3 μmol photons m⁻² s⁻¹ for 4 hours and then used for photosynthetic activity measurements. All values indicate mean photosynthetic activities ± SD of three independent biological replicates.
has a lowered Ci affinity than wild type, while the \( V_{\text{max}} \) at saturating bicarbonate amounts was not changed (fig. S4B). The Ci affinity of photosynthesis in the \textit{Synechocystis} \( \Delta \text{cph1} \) mutant remained similarly low with or without exposure to FR light (Fig. 4C), and it could not be reactivated in the presence of red light (fig. S8A). The photosynthesis was at a comparable level to that of LC-acclimated wild-type \textit{Synechocystis} exposed to FR light. However, higher photosynthetic activity of the \textit{Synechocystis} \( \Delta \text{cph1} \) mutant was measured when dark-acclimated cells are exposed to red light (fig. S8A) compared to white light exposure at the same light intensity (Fig. 4C). This observation suggests that additional red/FR light–sensitive photoreceptors such as Cph2 are also capable to initially sense the red light signal then transducing it to Cph1. These results suggest that red/FR light–induced modulation of Ci uptake can be mediated by several photoreceptors, likely phytochromes. In the case of \textit{Synechocystis} particularly, Cph1 is involved in the reactivation of bicarbonate uptake after FR light signals.

Two bicarbonate transporters, namely, SbtA (sodium bicarbonate transporter A) and BCT1 (bicarbonate transporter 1), are induced in cyanobacteria under LC conditions (27–29). Recently, it has been shown that the previously unknown Ci acclimation regulator SbtB can directly interact with SbtA (32) and has a negative impact on the SbtA bicarbonate transport activity (33). Hence, we used a \textit{Synechocystis} \( \Delta \text{sbtB} \) mutant, in which the gene encoding this regulatory protein is impaired, and tested the involvement of SbtB in FR light–mediated inhibition of photosynthetic Ci affinity. The LC-acclimated \textit{Synechocystis} \( \Delta \text{sbtB} \) mutant exhibited similar affinity to Ci than wild-type \textit{Synechocystis} under the same conditions (Fig. 4D). The Ci affinity of photosynthesis in the mutant \( \Delta \text{sbtB} \) remained similarly high in white or red light with or without exposure to FR light, and it was always at a comparable level to that of LC-acclimated wild-type \textit{Synechocystis} not exposed to FR light (Fig. 4B and fig. S8B). These results suggest that the FR light–induced reduction of carbon metabolism is indeed mediated by a negative impact on Ci uptake because the FR light effect was not further observed in the absence of the negative regulator SbtB known to inhibit SbtA bicarbonate transport activity.

**DISCUSSION**

It has been shown that light signals activate a specific cellular acclimation program including a defined gene expression sequence preparing desert BSC-inhabiting cyanobacteria for the impending dehydration (18,20). The acclimation program to achieve high desiccation tolerance is disturbed by FR light, thus strongly implicating the involvement of phytochromes. The \textit{L. ohadii} genome harbors three genes encoding classical Cph1-type phytochromes (WP_088894538, WP_088888826, and WP_088899285); two of them showed decreased expression during desiccation (18). In addition, there is one phytochrome-like gene (WP_088899917), which is slightly up-regulated after dehydration. FR light prevented the down-regulation of the gene WP_088894538,
which could indicate that this phytochrome might be the most likely candidate to be involved in preparing *L. ohadii* toward desiccation (18). FR light and darkness usually switch phytochromes to an inactive state, i.e., histidine protein kinase domains that play key roles in the regulation of physiological and developmental activities in plants are inactivated, inhibiting the positive effects of light (34–36). In cyanobacteria, it has previously been reported that phytochromes are involved in the timing of circadian rhythms and in phototactic movement (24, 25), but their direct involvement in metabolic and/or CCM activity control has not been confirmed or characterized.

In the present study, we provide evidence that acclimating *L. ohadii* to HC conditions or exposing it to FR light, which negatively affects the activity of red/FR light–sensitive photoreceptors such as phytochromes, significantly reduced the cells ability to withstand desiccation (Fig. 1). These results suggest that Ci-mediated and phytochromes-mediated changes in carbon metabolism play an important role in desiccation tolerance. The cells carbon metabolism was strongly altered through dehydration (Fig. 2). The observed 3PGA dynamics, equating to a short period of carbon fixation with sunrise followed by rapid decline early in the dehydration process when the water level is still high, may reflect metabolic acclimation to the forthcoming desiccation. Disruption of this pattern by FR light indicates a central role for red/FR light sensing in the process of metabolic acclimation (Fig. 2). The higher relative increase in 2PG and other photorespiratory intermediates in HC-acclimated *L. ohadii* is consistent with their lower CCM activity, which less effectively reduces the oxygenase activity of RubisCO (27). The enhanced accumulation of 2PG in LC-acclimated cells exposed to FR light indicates that light signals triggered changes in the cellular CO₂/O₂ ratio affecting RubisCO activity.

The effect of the Ci preacclimation state and FR light on RubisCO activity was also reflected in *L. ohadii* performance in photosynthetic Ci affinity measurements during dehydration (Fig. 3), with no notable effect on the carboxysome structure. These results indicate that reduction of photosynthetic activity in *L. ohadii* due to reduced uptake of Ci, affected by FR light, is part of the desiccation acclimation program, because it reduced the cell survival rates. During dehydration, light intensity is rising considerably above that saturates the rate of photosynthesis. The excess energy may cause oxidative stress and photodamage. Previous studies showed that Ci cycling associated with the CCM activity, i.e., part of the transported HCO₃⁻ is subsequently lost from the cell after conversion into CO₂, may serve as a means to dissipate excess light energy (37, 38), thereby contributing to desiccation tolerance. Similar FR light effects on the gene expression profiles of canonical Ci uptake systems such as the bicarbonate transporters SbtA and BCT1 (the latter encoded by the *cmp* operon) as well as carbonic anhydrase were previously reported (18), which may be involved in the FR light–triggered reduction of Ci uptake in *L. ohadii*.

Dysfunction of the Ci uptake machinery when exposed to FR light was observed not only in the desiccation-tolerant *L. ohadii* but also similarly in the desiccation-sensitive cyanobacterium *Synechocystis*. This finding clearly indicates that FR light had a direct, dehydration-independent, negative effect on cyanobacterial photosynthetic activity. In both strains, the negative effect of FR light could be reversed by red light (Fig. 5), supporting the hypothesis that red/FR light photoreceptors mediated this switch. The similar reactions to FR and red light with regard to the CCM activity in terrestrial and aquatic cyanobacteria suggest that the red/FR light–dependent CCM regulation may have appeared early in the evolution of cyanobacteria. In future studies, the FR/red light effects on cyanobacterial CCM activity should be tested in a broader spectrum of strains to further support the hypothesis that this mechanism occurs in the entire cyanobacterial phyllum. Light is differentially attenuated in terrestrial and aquatic ecosystems. It is known that light of specific wavelength is absorbed to a different degree in the water column, especially FR light penetration that decreased much stronger compared to red light (39). Hence, it can be hypothesized that light and particularly red light is able to activate the cyanobacterial CCM via various red/FR light photoreceptors during the day in aquatic strains as in terrestrial ones. FR/red light signaling via phytochromes has been demonstrated in diatoms occurring in the marine water column (40).

The *Synechocystis* genome harbors six genes encoding different light-sensing proteins. Among them, the two phytochromes Cph1 and Cph2 that specifically respond to red/FR light signals have been characterized (41). Insusceptibility of the *Synechocystis* mutant Δcph1 to FR when exposed to white light and the irreversibility of the FR signal when exposed to subsequent red light signal provided evidence that the phytochrome Cph1 is crucial for reactivating the CCM activity in FR light–treated *Synechocystis* cells. In contrast to white light (Fig. 5), pure red light was able to activate the photosynthesis in dark-acclimated cells of mutant Δcph1 (fig. S8). From these experiments, we conclude that not only the phytochrome Cph1 but also other red/FR light photoreceptors can participate in the reversible increase/decrease of Ci uptake in *Synechocystis*. It has been shown that different red/FR light ratios had marked impact on gene expression patterns in *Synechocystis* (34). Probably, changes in the red/FR light ratio can also explain the observed differences in the activation of photosynthesis in dark-acclimated cells of the Δcph1. The incubation with 50 μmol m⁻² s⁻¹ of white light that contains a relatively low proportion of red light was not sufficient to activate photosynthesis in Δcph1 to wild-type levels at 75 μM bicarbonate, whereas pure red light of 50 μmol m⁻² s⁻¹ had a clear stimulatory effect in this mutant. This observation indicates that in the presence of higher red light fluence rates, other photoreceptors such as Cph2 are also capable to activate photosynthetic Ci affinity and to receive FR light inhibiting it.

Collectively, our results suggest that red/FR light–mediated signals reversibly switch on and off the cyanobacterial CCM activity with respect to light availability (Fig. 6). The enriched FR signal during sunset might already initiate cell preparation to upcoming darkness, a condition under which CCM activity and Ci uptake should be switched off. The FR or red light signal is somehow transduced from phytochromes onto the regulatory protein SbtB because the FR light had no effect on photosynthetic Ci affinity in ΔsbtB mutant. It has been shown that SbtB can directly inhibit the SbtA bicarbonate transport activity (33). These findings support the conclusion that FR light is switching off bicarbonate transport via SbtA. This is consistent with the more pronounced FR light effects in LC-grown cyanobacteria, which are characterized by high sbtAB operon expression (32). The mechanism underlying the red/FR light– and particularly the Cph1-mediated regulation of the SbtA activity via SbtB in *Synechocystis* is still unknown and requires further investigations. Transcriptional regulation via Cph1 seems to be unlikely because the expression of sbtAB and other CCM-related genes did not change in the *Synechocystis* Δcph1 mutant (34). The *cph1* (sir0473) mRNA increased about twofold when *Synechocystis* wild-type cells were shifted from HC to LC...
conditions for 24 hours (42); however, the protein level of Cph1 remained unchanged under different Ci conditions (43). Postranslational protein modifications such as SbtB phosphorylation (44) or second messenger signals [Ci-dependent SbtB interaction with adenosine 3′,5′-monophosphate was shown (32)] are likely candidates possibly involved in the signal transduction between Cph1 and SbtB.

Hence, we conclude that the previously unknown red/FR light–mediated Ci uptake regulation in *Synechocystis* is triggering the activation/inactivation of the cyanobacterial CCM activity (Fig. 6). This red/FR light–regulated CCM activity control was clearly adopted by desiccation-tolerant cyanobacteria such as *L. ohadii*, in which it is a central part of the light-initiated acclimation program conceivably to prepare the organism’s entire metabolic system for impending dehydration. Last, preacclimation to high-CO₂ conditions diminished the desiccation tolerance of *L. ohadii*. This observation suggests the possibility that elevated anthropogenic atmospheric CO₂ levels will imperil BSC-inhabiting cyanobacteria, as proper functioning of their Ci uptake machinery is essential for their survival under desiccation.

**MATERIALS AND METHODS**

**Culture growth and Ci preacclimation**

Axenic *L. ohadii* and *Synechocystis* sp. PCC 6803 cultures, respectively, were grown on BG11-agar plates or in liquid BG11 medium (45) at 30°C under light-emitting diode light (warm white, 3000 K, 40 μmol photons m⁻² s⁻¹). For Ci preacclimation, the cells were continuously grown either with ambient air bubbling for low-Ci acclimation (LC) or with air enriched by 5% CO₂ for high-carbon acclimation (HC) in liquid cultures. All experiments presented here were performed with samples from at least three independent cultures.

**Dehydration, desiccation, FR treatment, and characterization of revival**

These experiments were performed essentially as described previously (7, 18, 20) using the typical autumn conditions (as monitored on 14 October 2009), when the early morning dew in the relevant BSC region is substantial. Autumn conditions were presented in (18, 20) and are provided here for comprehensiveness in fig. S1. Samples corresponding to 0.25 mg of chlorophyll were transferred onto glass fiber filters, placed on 1.5% agar plates (supplemented with 10 mM NaHCO₃ for the HC-preacclimated cells) overnight, at 14°C (the autumn early morning temperature) in the dark. The filters were then placed at ambient air in an environmental chamber to initiate the dehydration protocol described in (7), simulating the natural BSC conditions. Following dehydration, filters were exposed to autumn conditions, ground temperatures of 60°C, air temperature of 45°C, light intensity of 2000 μmol photons m⁻² s⁻¹, and a relative humidity of 3 to 5% for 4 hours, simulating noon conditions in the daily cycle (hereafter desiccation). To assess the impact of light sensing, we supplemented the filters bearing the cells with FR light (730 nm) as in (18, 20) during the first hour or continuously throughout the

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Fig. 6. Hypothetical model of the red/FR light effects on Ci accumulation in *Synechocystis* sp. PCC 6803 and desiccation tolerance in *L. ohadii*. (A) The red light signal is sensed by red/FR photosensors, likely phytochromes, that then transmit it via a yet unknown signal transduction chain to the negative regulator SbtB. This signaling removes the negative impact of SbtB on SbtA-mediated bicarbonate uptake, leading to active CCM supporting photosynthesis when light is available. (B) In darkness or because of the FR light signal, the phytochrome-mediated positive signal is blocked; hence, SbtB can act as negative regulator of SbtA activity, shutting down CCM activity under nonphotosynthetic conditions. In the drought-resistant strain *L. ohadii*, this red/FR light signaling is adopted to ensure metabolic balance resulting in high desiccation tolerance as depicted below the figure.
dehydration protocol. Following the various treatments, the cells were incubated overnight under growth conditions in BG11 medium with vigorous ambient air bubbling, and photosynthetic net oxygen evolution measurements were taken as a proxy for cell performance to assess recovery after the treatments. The rate of O₂ exchange of 20-µg chlorophyll cell concentration (using a Clark-type O₂ electrode, Chlorolab 2 System, Hansatech, Norfolk, UK) was measured at 30°C.

**Ci-dependent photosynthetic affinity measurements during dehydration**

Simulated natural dehydration with or without FR light was performed as described above, using LC- and HC-preacclimated *L. ohadii* cultures. Samples were withdrawn during dehydration at time points indicated at fig. S1, resuspended in 10 mM Hepes-NaOH buffer (pH 7.8), and placed in an O₂ electrode chamber, at given NaHCO₃ concentrations. Measurements were performed with 20-µg chlorophyll cell concentration.

**Ci-dependent photosynthetic affinity measurements under liquid conditions**

LC- and HC-preacclimated *L. ohadii* and *Synechocystis* sp. PCC 6803 cultures were incubated in BG11 medium, supplemented with 10 mM NaHCO₃, for the HC-preacclimated cells, at 30°C in the dark overnight. Then, cells were exposed to 50 µmol photons m⁻² s⁻¹ of white or red light, with or without exposure to FR light for 4 hours (as natural dehydration protocol duration). To investigate whether the FR effect is reversible, FR light was switched off, and the cells were exposed to red light or white light for an additional 1 hour continuously stirred in open beaker. To investigate whether darkness has effects similar to those of FR light, cells were incubated overnight in the dark as described above, followed by incubation in white light (50 µmol photons m⁻² s⁻¹) for 1 hour. Then, the cells were incubated in darkness for 1 hour and subsequently again exposed to white light for 1 hour. Samples were withdrawn, resuspended in 10 mM Hepes-NaOH buffer (pH 7.8), and placed in the O₂ electrode chamber, 20-µg chlorophyll cell concentration, at given NaHCO₃ concentrations. The *Synechocystis* sp. PCC 6803 mutant ∆sbtB (sbr473, Hik35) strain used in the current study was described before (46), while the mutant ∆sbtB2 (sbr1513) was generated and characterized in (32). Spectra of the white light (fig. S9), red light, and FR light (fig. S10) sources can be found in the Supplementary Materials.

**Metabolite analysis**

Samples for liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis were withdrawn during dehydration at the indicated time points (fig. S1). Extraction of soluble primary intermediates was carried out using LC-MS grade chemicals as described in (47) with some modifications. Briefly, at the selected time points, a 100-µg chlorophyll pie slice was placed in a 2-ml Eppendorf tube and frozen in liquid nitrogen until all samples were collected and stored in −80°C until metabolite extraction. Frozen *L. ohadii* samples were resuspended in 1 ml of subfrozen LC-MS/MS buffer [600 µl of methanol (−80°C), 400 µl of chloroform (−20°C), and 1 µl of carnitine as internal standard (1 mg ml⁻¹)] and was vigorously vortexed. To break the cells completely, cells were exposed to 5 cycles of thaw-freeze in liquid nitrogen and ice, then disrupted by bead beating in the presence of 0.3 g of mixed glass beads (150 to 212 µm; Sigma-Aldrich) in 4°C for 3 min, and then incubated in an ice-cold ultrasonic bath (Bandelin Sonorex RK 255 H) for 10 min. Ice-cold water (800 µl) was added, and the sample was vigorously vortexed. The aqueous fraction was collected after centrifugation (5 min, 14,000 rpm, 4°C). Each sample was divided to two 2-ml Eppendorf tubes, and 300 µl was added to each tube. Samples were freeze-dried overnight (Alpha 3-4 LSCbasic; Christ). Dry extracts were dissolved and unified in 250 µl of ice-cold water and filtrated through 0.2-µm filters (Omnifit-F, Braun, Germany). The cleared supernatants were analyzed using a high-performance liquid chromatograph mass spectrometer LCMS-8050 system (Shimadzu) and the incorporated LC-MS/MS method package for primary metabolites (version 2, Shimadzu). Briefly, 4 µl of each extract was separated on a pentfluorophenylpropyl column (Supelco Discovery HS FS; 3 µm, 150 x 2.1 mm) with a mobile phase containing 0.1% (v/v) formic acid. The compounds were eluted at 0.25 ml min⁻¹ using the following gradient: 1-min 0.1% (v/v) formic acid [95% A. dest. (distilled water), 5% acetonitrile], within 15-min linear gradient to 0.1% (v/v) formic acid (5% A. dest., 95% acetonitrile) and 10-min 0.1% (v/v) formic acid (5% A. dest., 95% acetonitrile). Aliquots were continuously injected in the MS/MS part and ionized via electrospray ionization. The compounds were identified and quantified using the multiple reaction monitoring values given in the LC-MS/MS method package and the LabSolutions software package (Shimadzu). Authentic standard substances (Merck) at varying concentrations were used for calibration and peak areas normalized to signals of the internal standard.

Glycogen was quantified as described in (48) with some modifications. Briefly, the cell pellets were washed twice with 1 ml of double-distilled H₂O. Afterward, the pellet was resuspended in 400 µl of KOH (30%, w/v) and incubated for 2 hours at 95°C. For the subsequent glycogen precipitation, 1200 µl of ice-cold ethanol (final concentration of 70%) was added. The mixture was incubated at −20°C overnight. Next, the solution was centrifuged at 4°C for 10 min at 10,000g. The pellet was washed twice with 70 and 98% ethanol and dried in a SpeedVac for 20 min at 60°C. Then, the pellet was resuspended in 1 ml of 100 mM sodium acetate (pH 4.5), and 8 µl of an amyloglucosidase solution (4.4 U µl⁻¹) was added. For the enzymatic digest, the cells were incubated at 60°C for 2 hours. For the spectrophotometric glycogen determination, 200 µl of the digested mixture was used and added to 1 ml of O-toluidine reagent (6% O-toluidine in 100% acetic acid). The tubes were incubated for 10 min at 100°C. The samples were cooled down on ice for 3 min, before the optical density at 635 nm (OD₆₃₅) was measured. The final result was normalized to the sample fresh mass. A glucose standard curve was used to calculate the glucose contents in the sample from their OD₆₃₅. Data shown represent mean log₂ fold change of the normalized steady-state metabolites levels compared to time 0. Where indicated, significant differences were estimated using Student’s t test (P values of ≤0.05) with values from at least three biological replicates. Error bars represent SD.

**Transmission electron microscopy**

Samples for transmission electron microscopy (TEM) analysis were withdrawn during dehydration at the indicated time points (fig. S1) and were fixed in 1 ml of fixation buffer [1% paraformaldehyde, 2% glutaraldehyde, and 0.1 M phosphate buffer (pH 7.3)]. Samples were stored at 4°C in the dark. After washing with phosphate buffer, cells were collected by centrifugation and were embedded in low-melting agarose for further processing. This included postfixation in 1% osmium tetroxide, washes in distilled water, and subsequent stepwise dehydration with an ascending acetone series. Resin infiltration was
started with a 1:1 mixture of acetone and epon resin overnight, followed by two steps of pure epon resin (Epon 812, Serva, Heidelberg, Germany). The samples were transferred to rubber molds and were cured at 60°C in an oven for 2 days before trimming and ultramicrotomy (Ultra-cut S, Reichert, Vienna, Austria) using a diamond knife (Diatome, Biel, Switzerland). Thin sections (approximately 70 to 90 nm) were cut and, after contrasting with lead citrate and uranyl acetate, were analyzed in a Zeiss EM902 transmission electron microscope operated at 80 kV. Digital images were acquired with a side-mounted 1 × 2 k frame transfer charge-coupled device camera (TRS Proscan, Scheuring, Germany) using TEM camera control and imaging software (Olympus Soft Imaging Solutions, Münster, Germany).

SUPPLEMENTARY MATERIALS
Supplemental material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/34/eabg0435/DC1

View/request a protocol for this paper from Bio-protocol.

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Acknowledgments: We thank K. Michl for technical assistance in strain cultivation and sample preparation, as well as U. Schulz and K. Schulz for technical assistance with sample preparation for electron microscopy. The help of A. Schoor (Department of Ecology, University of Rostock) in the measurements of light spectra is acknowledged. Funding: The postdoctoral stay of N.O. at the University of Rostock is funded by the Minerva Foundation and the Alexander von Humboldt Foundation. The project was also funded by grants from the German Research Foundation (DFG) to M.H. (HA 2002/23-1 and HA2002/24-1). The LC-MS/MS equipment at University of Rostock was financed through the HBFG program (GZ: INST 264/125-1 FUGG). Author contributions: N.O. and M.H. designed the study. N.O. performed experiments, analyzed and evaluated data, and drafted figures. S.T. performed and evaluated metabolome experiments. M.F. performed and evaluated the electron microscopy. O.Ma. analyzed the sbtB mutant. O.Mu. evaluated data and performed statistical analysis. N.O. and M.H. wrote manuscript with the input from all authors. Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Submitted 7 December 2020
Accepted 28 June 2021
Published 18 August 2021
10.1126/sciadv.abg0435

Citation: N. Oren, S. Timm, M. Frank, O. Mantovani, O. Murik, M. Hagemann, Red/far-red light signals regulate the activity of the carbon-concentrating mechanism in cyanobacteria. Sci. Adv. 7, eabg0435 (2021).