Current Biology 20, 1557–1561, September 14, 2010 ©2010 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2010.07.032

Report

Enslavement in the Water Body by Toxic *Aphanizomenon ovalisporum*, Inducing Alkaline Phosphatase in Phytoplanktons

Yehonathan Bar-Yosef,[1] Assaf Sukenik,[2] Ora Hadas,[2] Yehudit Viner-Mozzini,[2] and Aaron Kaplan[1,*]

[1]Department of Plant and Environmental Sciences, Edmond J. Safra Campus, Givat Ram, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

[2]The Yigal Allon Kinneret Limnological Laboratory, Israel Oceanographic and Limnological Research, P.O. Box 447, Migdal 14950, Israel

Summary

The hepatotoxin cylindrospermopsin (CYN) produced by certain cyanobacteria, including *Aphanizomenon ovalisporum* (hereafter *Aphanizomenon*) [1], seriously affects lake water quality [2], but its biological role is not known. Strong correlation between *Aphanizomenon* abundance in Lake Kinneret, Israel, and alkaline phosphatase (APase) activity suggests that inorganic phosphate (Pi) limitation induces the PHO regulon and APase secretion [3]. Staining lake samples with DAPI [4] revealed a high level of polyphosphate bodies (PPB) in *Aphanizomenon*. Application of enzyme-labeled fluorescence (ELF-APase) [5] showed APase in various organisms, but not in *Aphanizomenon*. ELF-APase signals and extracellular APase activity in *Aphanizomenon* were detected only after exploiting PPB under prolonged Pi deprivation in cultures or toward the end of its autumn bloom. Pi deprivation of *Aphanizomenon* induces CYN production, high-affinity Pi uptake, and an internal, not external, APase. Addition of *Aphanizomenon* spent media or CYN to various phytoplanktons, including *Chlamydomonas reinhardtii*, induced genes typically upregulated under Pi limitation and a rise in extracellular APase activity, despite ample surrounding Pi. Coculturing *Aphanizomenon* with *Chlamydomonas* or with *Debarya* sp. showed positive ELF-APase signals, but not in *Aphanizomenon*. CYN producers promote Pi supply by inducing APase secretion by other phytoplanktons, possibly explaining their increased abundance despite reduced Pi supply from watersheds.

Results and Discussion

Correlation between the Abundance of *Aphanizomenon ovalisporum* and Alkaline Phosphatase in Lake Kinneret

Our study was initiated following the observation that the annual summer blooms of *Aphanizomenon ovalisporum* in Lake Kinneret, Israel, are accompanied by high alkaline phosphatase (APase) activity in the water body (Figure 1A). Statistical analysis indicated a strong correlation between *Aphanizomenon* abundances and APase activity (two-way analysis of variance, $Pv < 1e^{-4}$, $R^2 = 0.52$), suggesting that inorganic phosphate (Pi)-limited *Aphanizomenon* secreted APase [3]. However, closer inspection showed significant APase activity at the beginning of the *Aphanizomenon* bloom when the filament abundance was low, and ELF-APase staining of lake samples showed positive signals in various phytoplankton species, but not in *Aphanizomenon* (Figure 1B). DAPI staining (Figure 1C) showed a significant amount of polyphosphate bodies (PPB) in *Aphanizomenon* during the beginning of the bloom, in agreement with the relatively high Pi quota detected in filaments collected from the lake [3]. It became questionable whether secretion from *Aphanizomenon* is indeed the source of the lake's APase activity, as proposed [3]. Only later in the season, after mid-September, did *Aphanizomenon* filaments consume most of the internal Pi (PPB) and show ELF-APase staining (see Figure S1 available online).

Sensing of Pi Limitation by *Aphanizomenon*

Transfer of *Aphanizomenon* cultures to Pi-deprived BG11 medium resulted in a large rise in the extracellular APase activity, but only after an extended lag of 5–7 days (Figure 2A). Because the growth rate of *Aphanizomenon* in the first 3 days was barely affected by Pi removal (data not shown), it is plausible that *Aphanizomenon* used internal Pi resources. DAPI staining showed a clear decline in PPB abundance with the duration of Pi deprivation (Figure 2B), and the ELF-APase assay exhibited positive signals only after 1 week under Pi conditions (Figure 2B). Extracellular APase activity was only observed when the abundance of the PPB declined below our detection level. In this respect, *Aphanizomenon* responds significantly differently than most other microorganisms examined so far, including bacteria [6], cyanobacteria [7] (but see [8]), green algae [9], and yeast [10], where the PHO regulon and APase secretion are induced shortly after depletion of external Pi, even in the presence of ample internal PPB.

To clarify whether *Aphanizomenon* is able to sense and respond to external Pi level, we measured the transcript abundances of PHO regulon, including *pstS*, a component of the high-affinity Pi uptake system; *phoD* and *phoX*, which encode intracellular and extracellular alkaline phosphatases, respectively; and *aoaA* and *aoaC*, located on the reverse strands of the *aoa* cluster involved in cylindrospermopsin (CYN) formation [11]. The latter genes were examined because earlier studies [12] and preliminary data indicated a large rise in their transcription in Pi-limited cells. Significant induction of PHO components (Figure 2C) and of *aoa* genes (Figure 2D) was observed shortly after removal of external Pi. Moreover, a large rise in the level of CYN (Figure 2E) and of internal APase (PhoD, data not shown) activity was detected following Pi deprivation well before the appearance of external APase activity (Figure 2A). Finally, our *Aphanizomenon* sequencing project (unpublished data) identified a response regulator with high similarity to that implicated in the *Synechocystis* sp. PCC 6803 response to external Pi deprivation [7]. Induction of the PHO regulon and of *aoa* genes shortly after the removal of Pi, when ample PPB are still present, clearly indicated that *Aphanizomenon* does sense and respond to ambient Pi level. The mechanism involved in inhibition of PhoX translation, as long as PPB is available, is being examined.

Induction of PHO in *Chlamydomonas reinhardtii* by Spent *Aphanizomenon* Media and Cylindrospermopsin

Two lines of evidence suggested that APase activity observed at the beginning of the *Aphanizomenon* bloom did not emerge

*Correspondence: aaronka@vms.huji.ac.il

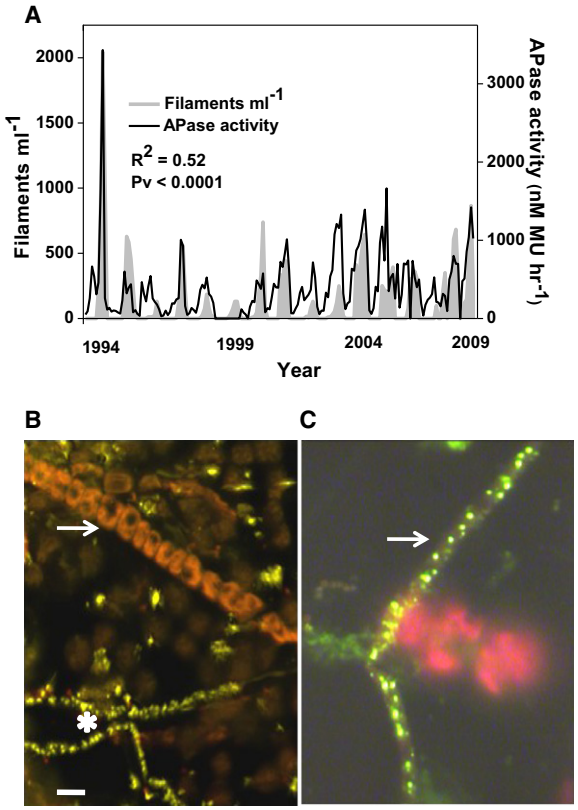

Figure 1. Multiannual Abundance of *Aphanizomenon* Filaments and Alkaline Phosphatase Activities in Lake Kinneret

(A) Gray filled line shows filament counts per ml from its first appearance in 1994 up to 2009, showing blooms each autumn, albeit with reduced intensity than that of 1994. Black line shows alkaline phosphatase (APase) activity measured as nM of MUP hydrolyzed to MU/hr, normalized to biomass g/m^2.

(B) Lake samples (July 2009) showing ELF-Apase staining in *Debarya* sp. (indicated by a white asterisk) and other organisms, but not in *Aphanizomenon* (white arrow).

(C) DAPI staining indicating that the *Aphanizomenon* filament (white arrow) contains many polyphosphate bodies (PPB). Scale bar indicates 10 μm. See also Figure S1.

Samples were filtered on a black 0.22 μm polycarbonate filter (GE Water and Process Technologies), placed on slides, and visualized by Axioplan2 Imaging fluorescent microscope (Carl Zeiss) at an excitation of 365/12 nm and emission of 500–540 nm. Photos were taken by an ORCA R^2 digital camera (Hamamatsu). Images were analyzed by MicroManager 1.3 software (Vale laboratory, University of California, San Francisco) and by AutoQuant X2 software (Media Cybernetics).

from this organism: (1) ELF-APase was observed in various phytoplankton species, but not in *Aphanizomenon* (Figure 1B), and (2) the Pi quota in lake filaments was rather high [3], and a large abundance of PPB was observed (Figure 1C), whereas *Aphanizomenon* secreted APase only when the amount of PPB declined significantly (Figures 2A and 2B). Induction of the *aoa* cluster and CYN production shortly after Pi deprivation (Figures 2D and 2E) suggested involvement in Pi acquisition and prompted us to examine whether secondary metabolites such as CYN could activate APase in other organisms.

The ELF-APase assays on natural populations (Figure 1B) suggested that various organisms respond to the presence of *Aphanizomenon*. We selected *Chlamydomonas reinhardtii* (hereafter *Chlamydomonas*) for further experiments because basic genetic information and tools are available [9]. Exposure of *Chlamydomonas* to *Aphanizomenon* spent media resulted in a fast and significant rise of APase activity, although the cells were not Pi limited (Figure 3A). APase activity increased also in control, untreated *Chlamydomonas* cells, but only after several days, during which they depleted the ambient Pi. Exposure of *Chlamydomonas* to CYN purified from *Aphanizomenon* (provided by S. Carmeli, Tel Aviv University [1]) also induced a large rise in APase activity (Figure 3B), albeit slower and less intensive than with the spent *Aphanizomenon* medium. A similar but significantly smaller effect (40% rise in 96 hr) was observed following exposure of a *Chlorella* sp. strain isolated from Lake Kinneret to spent *Aphanizomenon* media (data not shown).

The response of target organisms to *Aphanizomenon* spent medium was greater than that observed following application of purified CYN (50 μg/l, Figures 3A and 3B), although its concentration in the used media (7–8 μg/l) was considerably smaller. This suggests a synergistic involvement of other secondary metabolites in the used media, the nature of which is being clarified. This resembles the stronger induction of *mcy* genes (encoding microcystin synthetase) in *Microcystis* sp. by its own spent medium than by isolated microcystin [13]. This is due to the synergistic effect of the protease inhibitors micropeptin and microginin, produced by *Microcystis* and released to the medium by lysing cells. These secondary metabolites serve as infochemicals, i.e., their release to the medium by lysing *Microcystis* cells "reports" to the rest of the cells that the population is under stress [13].

Most of our experiments were conducted with *Chlamydomonas*, a convenient model organism that, for an unknown reason, responded more strongly than did organisms growing natively in the lake, such as the *Chlorella* sp. This might be related to adaptations that developed during long interactions in the water body, possibly leading to some desensitization. In addition to suggesting a biological role for CYN, these findings indicated that other components secreted by *Aphanizomenon* function synergistically with CYN to induce APase secretion by *Chlamydomonas*.

The spent *Aphanizomenon* medium induced large changes in APase activity (Figure 3A) and in the abundances of *Chlamydomonas pta1* and *ptb2* transcripts encoding low- and high-affinity Pi transporters, respectively, and *phoX* encoding APase (Figure 3C). This reflects a typical *Chlamydomonas* response to Pi deprivation [9], despite the presence of sufficient Pi. The mechanism whereby the spent *Aphanizomenon* media and CYN affect *Chlamydomonas* and affect what is being sensed is not clear. We did not detect significant alterations in the rate of Pi uptake in the treated *Chlamydomonas* cells. Possibly the expected rise in Pi uptake following activation of *ptb2* is compensated for by the declines in *pta1* (Figure 3C) and in ambient Pi concentration because of continuous removal from the medium.

Further confirmation that *Chlamydomonas* and *Debarya* sp. respond to allelochemicals produced by *Aphanizomenon* came from experiments in which we cocultured each of them with *Aphanizomenon* in the same flasks. Despite the presence of sufficient Pi (20 μM), positive ELF-APase stainings were obtained in *Chlamydomonas* and *Debarya* sp. cells within 24 hr of coculturing, but not in *Aphanizomenon* (Figure 4).

ELF-APase Staining in Late Summer

As indicated, samples withdrawn from the lake in the early phase of the *Aphanizomenon* bloom showed ELF-APase

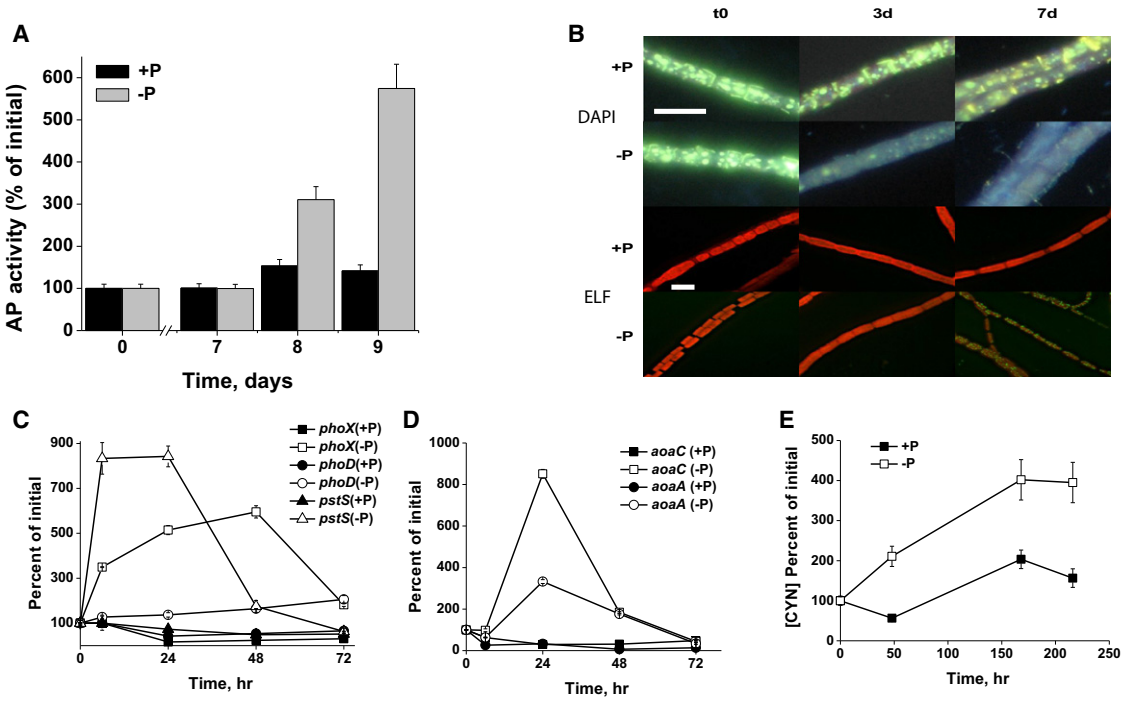

Figure 2. Response of *Aphanizomenon* to Inorganic Phosphate Deprivation

(A) External APase activity in control and inorganic phosphate (Pi)-deprived *Aphanizomenon* cells, presented as percentage of the initial APase activity in the culture.

(B) Dynamics of the PPB status and the in situ APase activity (ELF-APase) in control and Pi-deprived *Aphanizomenon*. Scale bars indicate 10 μm.

(C) qPCRs showing the transcript abundances of certain PHO regulon genes: *phoD*, internal APase; *phoX*, external APase; *pstS*, a subunit of the high-affinity Pi transporter; *aoaA* and *aoaC*, involved in CYN biosynthesis (D); and CYN (E), affected by the duration of Pi deprivation. 100% corresponds to 7–8 μg/l (CYN) or 50 nM MUP hydrolyzed μg^{-1} Chl hr^{-1} in different experiments. For CYN analyses, 50 ml samples were lyophilized and resuspended in 5% formic acid, lysed by sonication, and harvested. Measurements of the CYN levels were performed as described in [21]. Additional analyses were held with the CYN-ELISA kit (Abraxis). Data were normalized to chlorophyll a concentrations to compensate for culture growth. Error bars indicate standard deviation (SD), n = 3. Average and standard error are presented throughout.

staining in various phytoplankton species, but not in *Aphanizomenon*, which possessed ample Pi supply, as indicated by the amount of PPB (Figures 1B and 1C). On the other hand, DAPI staining of lake samples withdrawn in mid-September barely detected any PPB (Figure S1). Consequently, and as expected (see Figure 2), a large ELF-APase signal was observed in the *Aphanizomenon* filaments in these samples (Figure S1). It is important to note that the Pi levels in Lake Kinneret are rather low throughout the summer, 1–3 μg/l, which is close to the analytical detection level. Hence, we are unable to conclude whether the difference in the PPB and ELF-APase signals in *Aphanizomenon* between July and September reflects a decline in the lake's Pi level. Apparently the biological system is more sensitive than the analytical one.

Conclusions

Our experiments clarified the biological role and function of CYN. It serves in an allelopathic interaction, novel of its kind, whereby one organism (*Aphanizomenon* and possibly other filamentous cyanobacteria, see below) induces others to excrete large amounts of APase to the water body. As shown (Supplemental Experimental Procedures), in terms of N allocation, CYN production is cheaper for the cells than APase secretion. Naturally, we do not know the exact sequence of events that takes place in the water body, but a likely scenario may develop as follows. When Pi becomes limiting, *Aphanizomenon* activates CYN production and its high-affinity Pi uptake, PstS, an efficient Pi scrubbing system [3]. *Aphanizomenon* can then compete for the Pi-released consequence of APase activity from other organisms. Apparently *Aphanizomenon* does it quite successfully, grows rapidly, and dominates in the seasonal bloom.

We do not know how specific the *Aphanizomenon* effect is on the neighboring cells, whether other filamentous cyanobacteria activate a similar response, or what the ecological implications are on the population dynamics. Naturally, we expect other CYN producers such as *C. rachiborskii* [14] to induce APase in other organisms. Lack of ELF-APase staining in lake samples of *Anabaena* sp., *Planktothrix* sp., and *Planktolyngbya* sp. [5], and delayed APase secretion in Pi-deprived *Trichodesmium* sp. [8], may indicate similar capabilities in other cyanobacterial lineages that are not known to produce CYN.

Finally, there are numerous reports over the last decade showing a significant rise in the abundance of toxic filamentous N_2 fixing cyanobacteria in fresh water bodies [15, 16]. The reason is not known, particularly because it occurs simultaneously with intensive efforts of lake management authorities to reduce the nutrient inflow from the watershed. The rise in cyanobacterial abundance has been attributed to global warming [17, 18], but, in view of the results presented here, we raise an alternative possibility that superior competitiveness for Pi enables them to flourish in such lakes.

Experimental Procedures

All of the experiments presented here were performed at least three times, each one on three independent replicates.

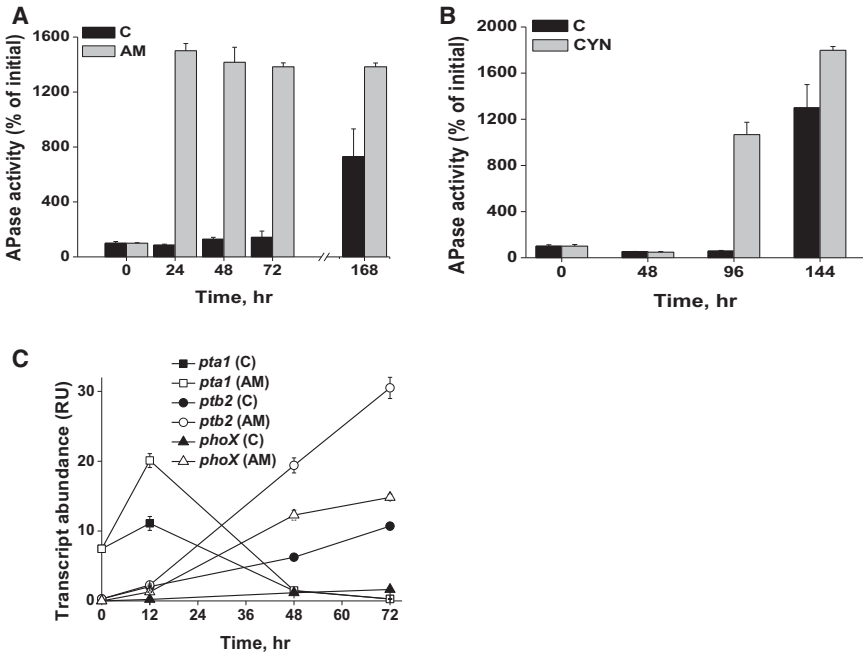

Figure 3. Induction of PHO Genes and Extracellular APase Activity in *Chlamydomonas reinhardtii* by Spent *Aphanizomenon* Medium and CYN

(A) Effect of spent medium from *Aphanizomenon* on APase activity in *Chlamydomonas*. APase activity is represented as a percentage of the initial value.

(B) Effect of isolated cylindrospermopsin (CYN) on APase activity in *Chlamydomonas*. 100% in (A) and (B) corresponds to 5 nM MUP hydrolyzed $\mu g\ Chl^{-1}\ hr^{-1}$. C denotes control, AM denotes *Aphanizomenon* medium. Error bars indicate SD, n = 3.

(C) Abundance (qPCR) of PHO genes in *Chlamydomonas* exposed to spent medium from *Aphanizomenon*. *pta1* and *ptb2* (encoding the low- and high-affinity Pi transporters, respectively), as well as *phoX* (encoding extracellular APase), are presented. qPCR data were normalized to CBLP (Rack1 [9]). Error bars indicate SD, n = 3.

Chlamydomonas cultures in exponential growth phase were washed with Pi-depleted standard culture medium (SCM) and resuspended into three different flasks, each containing 20 μM Pi, 50% SCM, and 50% *Aphanizomenon* medium (from Pi-depleted culture) or 100% SCM with or without (control) 50 $\mu g\ l^{-1}$ of CYN. Samples were taken for APase activity and RNA extraction. Results were normalized to Chl a concentrations, measured by a Spectronic 20 Genesys spectrophotometer.

Growth Conditions

A toxic strain of *Aphanizomenon* was isolated from Lake Kinneret, and an axenic culture was also isolated [11]. The cultures were grown on a shaker (100 rpm) in BG11 medium [19] supplemented with 20 mM TAPS-NaOH at pH 9 and 5 mM $NaHCO_3$ at 28°C. Light intensity was 50 μmol photons $m^{-2}s^{-1}$ provided by a cool fluorescent lamp.

Chlamydomonas wild-type strain CC-125 was grown in standard culture medium [20] at 22°C on a shaker (100 rpm), and then 5 mM $NaHCO_3$ was added, as well as 10 mM of TRIS buffer at pH 7.5. Light intensity was 50 μmol photons $m^{-2}s^{-1}$ provided by a cool fluorescent lamp.

For Pi deprivation, *Aphanizomenon* filaments were washed twice in BG11 medium in which Pi-containing compounds were substituted with KCl. The cultures were then divided into two equal volumes. One lot was resuspended in Pi-depleted BG11, whereas the controls were resuspended in regular BG11. Cell densities corresponded to ~0.5 μg Chl a ml^{-1}. Samples were withdrawn as indicated for further analyses.

APase Activity

We mixed 50 μl with 350 μl of 0.1 M TRIS buffer (pH 8.3), 4 μl of 0.1 M $MgCl_2$, and 50 μl of 0.1 mM 4-Methylumbelliferyl phosphate (4-MUP, Sigma-Aldrich) and incubated it for 30 min at 37°C. Fluorescence was measured in a Fluoroskan Ascent plate reader (Thermo), excitation 360 nm, emission 450 nm. Results were normalized to Chl a concentrations and measured in duplicates after extraction with 80% acetone.

DAPI and ELF-APase Staining

One milliliter of cultures was prefixed with 5% formaldehyde, and then 50 μl was withdrawn and then mixed in 5 ml of double distilled water and 4 μg of

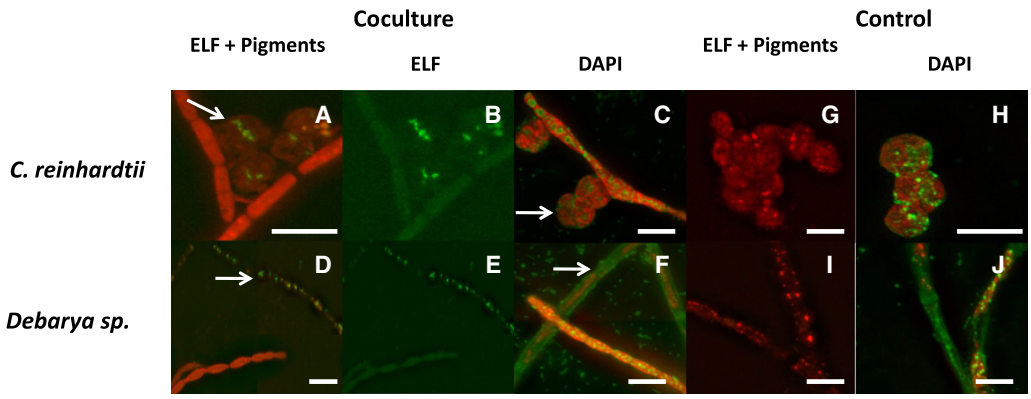

Figure 4. Induction of APase in Various Green Algae by Coculturing with *Aphanizomenon*

(A–J) ELF (APase) and DAPI (PPB) staining of mixed culture of *Aphanizomenon* with *C. reinhardtii* (A–C) or *Debarya* sp. (D–F). Control *Chlamydomonas* and *Debarya* sp. without *Aphanizomenon* are presented in (G) and (H) and (I) and (J), respectively. (B) and (E) show the ELF staining without the autofluorescence of photosynthetic pigments.

Chlamydomonas or *Debarya sp.* suspensions corresponding to 20 μg Chl a ml^{-1} at stationary phase, as well as *Aphanizomenon* of 1 μg Chl a ml^{-1} at exponential growth phase, were washed twice and resuspended in BG11 containing 20 μM of Pi. ELF-APase and DAPI staining were taken at 0 and 24 hr. Scale bars indicate 10 μm. Arrows show unicellular *Chlamydomonas* or filamentous *Debarya* sp.

DAPI (4′,6-diamidino-2-phenylindole, Sigma-Aldrich). Samples were incubated for 5 min in room temperature in darkness before inspection using a fluorescence microscope.

For ELF-APase, 1 ml (concentrated from 5 ml of culture or 500 ml of lake samples) was centrifuged for 5 min at 6000 × g and fixed with 75% EtOH for 30 min. EtOH was removed by centrifugation for 3 min at 6000 × g. Pellets were resuspended in 1:20 dilution of ELF 97 phosphatase substrate in the detection buffer (components A and B, respectively, ELF 97 Endogenous Phosphatase Detection Kit E6601, Molecular Probes) in the dark for exactly 30 min. Reactions were stopped by centrifugation for 5 min at 6000 × g and went through four stages of washing with a phosphate-buffered saline buffer, 25 mM EDTA, at pH 8. This procedure mostly stained the extracellular APase (see Supplemental Experimental Procedures).

qPCR Analyses

RNA was extracted using TRI-Reagent (Sigma-Aldrich) according to the manufacturers' instructions and was resuspended in 50 μl DEPC water. Two micrograms of RNA was treated with a Turbo-DNA-free kit (Ambion), and 120 ng of DNA-free (after DNase treatment, absence of DNA verified by a reverse transcriptase [RT] control) RNA was taken for RT reaction using the random primers of ImProm-II Reverse Transcriptase kit (Promega), according to the manufacturers' instructions. Analyses of *Aphanizomenon* and *Chlamydomonas* transcripts were each performed in triplicate using Rotor-Gene 6000 real-time PCR analyzer (Corbett). cDNA was diluted to 1:5 and then to 1:10 in the reaction tube, which contained 200 ng of specific primers and Absolute QPCR SYBR green mix (Thermo). The same RNA was used for the analysis of transcript abundances of the various genes examined. Primers used in the qPCR reactions are shown in Table S1. Results were normalized to 16SrDNA and Cblp genes in *Aphanizomenon* and *Chlamydomonas* [9], respectively. See further discussion on the normalization in the Supplemental Experimental Procedures.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, one figure, and one table and can be found with this article online at doi:10.1016/j.cub.2010.07.032.

Acknowledgments

We thank the Israel Ministry of Science, the Israel Science Foundation, the Deutsche Forschungsgemeinschaft, and the Israel Water Authority for supporting this study.

Received: May 18, 2010
Revised: June 27, 2010
Accepted: July 12, 2010
Published online: August 12, 2010

References

1. Banker, R., Carmeli, S., Hadas, O., Teltsch, B., Porat, R., and Sukenik, A. (1997). Identification of cylindrospermopsin in *Aphanizomenon ovalisporum* (Cyanophyceae) isolated from Lake Kinneret, Israel. J. Phycol. *33*, 613–616.
2. Anderson, D., Glibert, P., and Burkholder, J. (2002). Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries *25*, 704–726.
3. Hadas, O., Pinkas, R., Malinsky-Rushansky, N., Shalev-Alon, G., Delphine, E., Bernert, T., Sukenik, A., and Kaplan, A. (2002). Physiological variables determined under laboratory conditions may explain the bloom of *Aphanizomenon ovalisporum* in Lake Kinneret. Eur. J. Phycol. *37*, 259–267.
4. Aschar-Sobbi, R., Abramov, A., Diao, C., Kargacin, M.E., Kargacin, G.J., French, R.J., and Pavlov, E. (2008). High sensitivity, quantitative measurements of polyphosphate using a new DAPI-based approach. J. Fluoresc. *18*, 859–866.
5. Rengefors, K., Ruttenberg, K.C., Haupert, C.L., Taylor, C., Howes, B.L., and Anderson, D.M. (2003). Experimental investigation of taxon-specific response of alkaline phosphatase activity in natural freshwater phytoplankton. Limnol. Oceanogr. *48*, 1167–1175.
6. Vershinina, O.A., and Znamenskaya, L.V. (2002). The Pho regulons of bacteria. Microbiology *71*, 497–511.
7. Hirani, T.A., Suzuki, I., Murata, N., Hayashi, H., and Eaton-Rye, J.J. (2001). Characterization of a two-component signal transduction system involved in the induction of alkaline phosphatase under phosphate-limiting conditions in *Synechocystis* sp. PCC 6803. Plant Mol. Biol. *45*, 133–144.
8. Orchard, E.D., Webb, E.A., and Dyhrman, S.T. (2009). Molecular analysis of the phosphorus starvation response in *Trichodesmium* spp. Environ. Microbiol. *11*, 2400–2411.
9. Moseley, J.L., Chang, C.W., and Grossman, A.R. (2006). Genome-based approaches to understanding phosphorus deprivation responses and PSR1 control in *Chlamydomonas reinhardtii*. Eukaryot. Cell *5*, 26–44.
10. Nishizawa, M., Komai, T., Katou, Y., Shirahige, K., Ito, T., and Toh-E, A. (2008). Nutrient-regulated antisense and intragenic RNAs modulate a signal transduction pathway in yeast. PLoS Biol. *6*, 2817–2830.
11. Shalev-Malul, G., Lieman-Hurwitz, J., Viner-Mozzini, Y., Sukenik, A., Gaathon, A., Lebendiker, M., and Kaplan, A. (2008). An AbrB-like protein might be involved in the regulation of cylindrospermopsin production by *Aphanizomenon ovalisporum*. Environ. Microbiol. *10*, 988–999.
12. Bácsi, I., Vasas, G., Surányi, G., M-Hamvas, M., Máthé, C., Tóth, E., Grigorszky, I., Gáspár, A., Tóth, S., and Borbely, G. (2006). Alteration of cylindrospermopsin production in sulfate- or phosphate-starved cyanobacterium *Aphanizomenon ovalisporum*. FEMS Microbiol. Lett. *259*, 303–310.
13. Schatz, D., Keren, Y., Vardi, A., Sukenik, A., Carmeli, S., Börner, T., Dittmann, E., and Kaplan, A. (2007). Towards clarification of the biological role of microcystins, a family of cyanobacterial toxins. Environ. Microbiol. *9*, 965–970.
14. Pearson, L.A., Moffitt, M.C., Ginn, H.P., and B A, N. (2008). The molecular genetics and regulation of cyanobacterial peptide hepatotoxin biosynthesis. Crit. Rev. Toxicol. *38*, 847–856.
15. Dokulil, M.T., and Teubner, K. (2000). Cyanobacterial dominance in lakes. Hydrobiol *438*, 1–12.
16. Hudnell, H.K. (2010). The state of U.S. freshwater harmful algal blooms assessments, policy and legislation. Toxicon *55*, 1024–1034.
17. Paerl, H.W., and Huisman, J. (2008). Climate: Blooms like it hot. Science *320*, 57–58.
18. Wagner, C., and Adrian, R. (2009). Cyanobacteria dominance: Quantifying the effects of climate change. Limnol. Oceanogr. *54*, 2460–2468.
19. Stanier, R.Y., Kunisawa, R., Mandel, M., and Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. *35*, 171–205.
20. Moss, B. (1972). The influence of environmental factors on the distribution of freshwater algae: An experimental study. I. Introduction and the influence of calcium concentration. J. Ecol. *60*, 917–932.
21. Törökné, A., Asztalos, M., Bánkiné, M., Bickel, H., Borbély, G., Carmeli, S., Codd, G.A., Fastner, J., Huang, Q., Humpage, A., et al. (2004). Interlaboratory comparison trial on cylindrospermopsin measurement. Anal. Biochem. *332*, 280–284.