

The demise of the marine cyanobacterium, *Trichodesmium* spp., via an autocatalyzed cell death pathway

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Abstract

We present experimental laboratory evidence and field observations of an autocatalyzed, programmed cell death (PCD) pathway in the nitrogen-fixing cyanobacterium *Trichodesmium* spp., which forms massive blooms in the subtropical and tropical oceans. The PCD pathway was induced in response to phosphorus and iron starvation as well as high irradiance and oxidative stress. Transmission electron microscopy revealed morphological degradation of internal components including thylakoids, carboxysomes, and gas vesicles, whereas the plasma membranes remained intact. Physiologically stressed cells displayed significantly elevated endonuclease activity and terminal d-UTP nick-end labeling. Nucleic acid degradation was concordant with increased immunoreactivity to human caspase-3 polyclonal antisera and enhanced cleavage of a caspase-specific substrate, DEVD. Caspase activity was positively correlated with mortality and was inhibited by the irreversible caspase inhibitor Z-VAD-FMK. A search of the *Trichodesmium erythraeum* genome identified several protein sequences containing a conserved caspase domain structure, including the histidine- and cysteine-containing catalytic diad found in true caspases, paracaspases, and metacaspases. Induction of PCD by caspase-like proteases in a bacterial photoautotroph with an ancient evolutionary history requires a reassessment about the origins and roles of cell death cascades. This process is a previously unappreciated mortality mechanism that can lead to the termination of natural *Trichodesmium* blooms and that can influence the fluxes of organic matter in the ocean.

Planktonic marine cyanobacteria of the genus *Trichodesmium* form extensive blooms in the oligotrophic tropical and subtropical oceans, where they make significant contributions to global nitrogen fixation (Capone et al. 1997). Natural blooms and laboratory cultures of *Trichodesmium* often terminate abruptly, with cell lysis and biomass degradation occurring within 1–2 d (Ohki 1999). The mechanisms controlling the dramatic and abrupt termination of *Trichodesmium* blooms are not well understood, even though this termination drives nutrient flow and biogeochemical cycling of organic and inorganic matter produced by these organisms, including the redistribution of fixed nitrogen in the upper ocean and the flow of organic matter through ecosystem pathways like the grazer food chain, the microbial loop, and vertical sinking flux (Azam 1998).

Grazing by the harpacticoid copepod *Macrosetella gracilis* (O'Neil 1998) and bacteriophage infection (Ohki 1999) are factors that have been implicated in the termination of *Trichodesmium* blooms. At the same time, a variety of microscopic phytoplankton cells also appear to lyse and die via an autocatalytic cell death pathway triggered by nutrient stress (Brussaard et al. 1995; Berges and Falkowski 1998; Vardi et al. 1999; Segovia et al. 2003). This self-destruction is analogous to programmed cell death (PCD) in multicellular organisms and refers to an active, genetically controlled, cellular self-destruction driven by a series of complex biochemical events and specialized cellular machinery—receptors, adapters, signal-kinases, proteases, and nuclear factors (Aravind et al. 1999).

Here we demonstrate that a PCD pathway operates in the cyanobacterium *Trichodesmium* spp. in aging cultures and in response to relevant environmental and physiological stresses, including combined phosphorus and iron depletion, high irradiance, oxidative stress, and cell age. Furthermore, we present evidence that PCD in *Trichodesmium* displays physiological and biochemical characteristics consistent with PCD in metazoans and higher plants. These results, taken together with similar recent findings in yeast (Madeo et al. 2002) and another marine phytoplankter, *Dunaliella tertiolecta* (Segovia et al. 2003), provide mechanistic insight into the control of phytoplankton mortality and intriguing ecological and evolutionary context for PCD.

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Materials and methods

Cultures, natural populations, and growth conditions—*Trichodesmium* IMS101 cultures were grown in YBCII medium at 26°C with a 12:12 L:D cycle and constant aeration. Natural *Trichodesmium* populations, predominantly *Trichodesmium erythraeum*, were collected with 35- μm net tows from surface waters off Noumea (New Caledonia) during the period from November to December 2002. To isolate *Trichodesmium*, tows were size fractionated and zooplankton were separated from *Trichodesmium* by phototaxis.

Trichodesmium was hand-picked, placed in a separating flask in filtered seawater (<0.2 μm), irradiated with $\sim 450 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ and followed with time until the biomass crashed.

Detection of viruses—Presence of viruses was examined via induction of temperate phage with mitomycin C, as described by Ohki (Ohki 1999). Phages were viewed using Epifluorescence microscopy (Zeiss-Axioscope) following staining with SYBR Gold (Molecular Probes), as previously described (Noble and Fuhrman 1998). Cells were also examined for the presence of electron-dense viral particles using transmission electron microscopy (see below).

Measurement of caspase activity—Cells filtered on 5- μm Nucleopore filters were frozen in a buffer containing 100 mmol L^{-1} 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10% sucrose, 500 $\mu\text{mol L}^{-1}$ EDTA (ethylenediaminetetraacetic acid) and 10 mmol L^{-1} dithiothreitol (DTT). After sonication, the extract was incubated with 50 $\mu\text{mol L}^{-1}$ Z-DEVD-AFC (Calbiochem) for 24 h at 26°C. Fluorescence was read in a Spectra Max Gemini XS plate reader (excitation 400 nm, emission 505 nm). Activity was inhibited with the irreversible caspase-3 inhibitor Z-VAD-FMK (Calbiochem).

Transmission electron microscopy (TEM)—Cells were filtered onto 5- μm polycarbonate filters under low vacuum, blocked in 2% agar (in seawater), and preserved in Thrump's electron microscopy (EM) fixative (4% formaldehyde and 1% glutaraldehyde in phosphate buffer, pH 7.2). The cells were rinsed three times (15 min each time) in Millonig's phosphate buffer (pH 7.3), postfixed for 2 h in 1% buffered OsO_4 , washed three times, and dehydrated through a graded series of EtOH. After replacement of ethanol with propylene oxide, cells were embedded in Epon-Araldite cocktail. Sections were cut using an LKB 2088 ultramicrotome, collected on 200-mesh copper grids, and stained with uranyl acetate and lead citrate. The stained sections were photographed with a JEM-100CXII electron microscope.

Terminal d-UTP nick-end labeling (TUNEL)—Cells were labeled using the APO-BrdU TUNEL Assay Kit (Molecular Probes). Fixation of *Trichodesmium* cells prior to labeling was done by filtration of cells onto 5- μm polycarbonate filters and resuspension in ice-cold 100% ethanol for at least 18 h before labeling with APO-BrdU TUNEL; this process was examined using an epifluorescent Zeiss Axioscope microscope.

Western analysis—Samples for immunochemical analysis were loaded on an equal protein basis, separated on 12% polyacrylamide gels, and transferred onto polyvinylidene fluoride (PVDF) membranes. Membranes were probed with polyclonal antibodies for recombinant human caspase-3 (Stressgen) and detected using a horseradish peroxidase chemiluminescence system (SuperSignal, Pierce).

Nuclease activity assay—*Trichodesmium* IMS101 cells grown in complete YBCII media were gravity filtered and resuspended in either complete YBCII or YBCII medium minus phosphorus and iron. At designated times, 100–150 ml of each culture type was harvested onto 5- μm -pore size membrane filters. Filters were immediately frozen in liquid nitrogen and stored at -80°C until processing. Cells were resuspended in 300 μl of nuclease activity buffer (10 mmol L^{-1} Tris-Cl, pH 7.5; 2.5 mmol L^{-1} MgCl_2 ; 0.1 mmol L^{-1} CaCl_2) and probe sonicated (Misonix Microson ultrasonic cell disruptor; Power 2) for 1 min on ice at 20-s intervals. Cellular debris was pelleted by centrifugation (16,000 \times g; room temperature; 1 min). Cell lysates were adjusted to equal protein concentrations in nuclease activity buffer ($\sim 200 \mu\text{g ml}^{-1}$). One microgram of linear plasmid DNA (pBK-CMV) was added to cell lysates and incubated at 26°C for 1–3 h. The reaction was stopped by addition of 1 μl of 0.5 mol L^{-1} EDTA and stored at -20°C . The entire reaction was loaded onto 0.8% agarose gel run in $1\times$ tris-acetate EDTA (TAE) buffer and visualized with ethidium bromide (0.5 $\mu\text{g ml}^{-1}$). Control reaction consisted of linear plasmid DNA digested with 1 U DNase I in nuclease reaction buffer for 1 h.

Photosynthetic efficiency—Fast repetition rate fluorometer measurements of fluorescence kinetics were used to derive the maximum photochemical quantum yield of photosystem II (F_v/F_m) (Kolber et al. 1998).

Results and discussion

We observed a catastrophic decrease in biomass (>45% in 24 h) in stationary-phase laboratory cultures of *Trichodesmium* IMS101 (Fig. 1A, days 30–31), independent of viral infection. Neither epifluorescence microscopy nor TEM revealed evidence of an inducible, temperate phage in response to mitomycin C treatment (Ohki 1999) or in physiologically stressed cells. We considered whether PCD was responsible for this abrupt mortality by searching for distinct morphological changes (Kerr et al. 1972). Based on TEM images, cell death was characterized by degradation of thylakoids, carboxysomes, gas vesicles, and cyanophycin granules (Janson et al. 1995), but there was no evidence of plasma membrane rupture (Fig. 1B, right panels). The cells had large areas of electron-transparent, intracellular vacuolar spaces, an increase in the number of electron-dense particles that are likely lipid droplets, and increased gaps between cells along a trichome (Fig. 1B, right panels) that are indicative of cellular shrinkage. Epifluorescence microscopy of cultures stained with SYTOX green verified membrane integrity during the early stages of cell death. In contrast, cells with compromised membrane integrity (e.g., due to boiling)

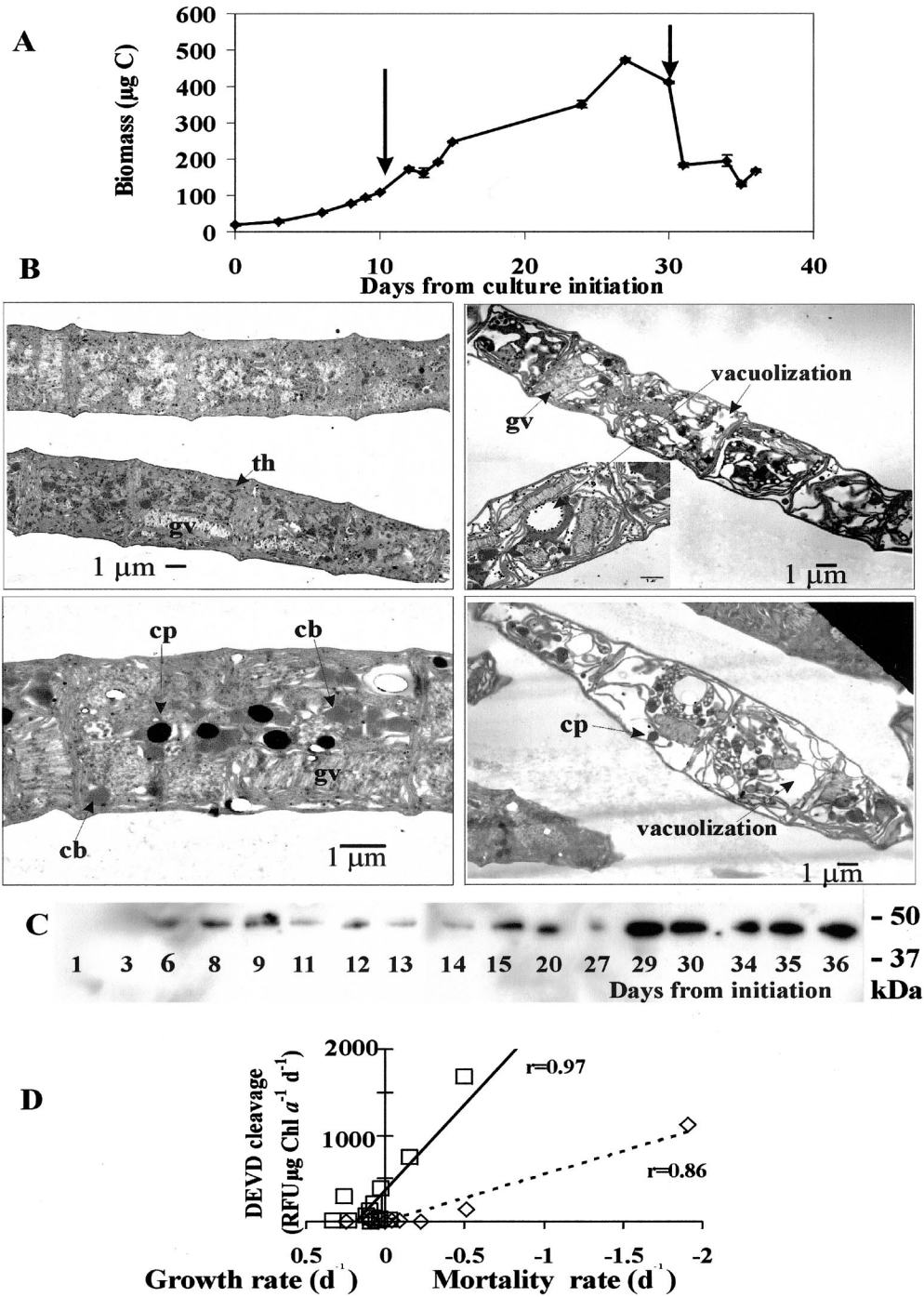


Fig. 1. A) Growth and crash of *Trichodesmium* IMS101 during a simulated bloom. Arrows indicate phase from which cultures were sampled for TEM. B) TEM micrographs showing changes in cellular morphology of the aging culture. Left panels are of cells during the early stages of the bloom, when growth rates were positive. Right panels are of cells sampled during biomass decline. Abbreviations: th, thylakoids; gv, gas vesicles; cp, cyanophycin granules; cb, carboxysomes. C–D) Caspases in *Trichodesmium*. C) Western blot illustrating an increase in caspase-3 immunoreactivity during *Trichodesmium* IMS101 bloom progression. Whole-cell protein extracts were challenged with polyclonal antibodies to recombinant human caspase-3. Samples were loaded with equal protein and detected using a horseradish peroxidase chemiluminescence system. Lane numbers identify age of culture in days. Days 1–13 and days 14–36 were run on separate gels. Gels were run, blotted, probed, and exposed to film at the same time under identical conditions. D) Dependence of DEVD cleavage on physiological status of *Trichodesmium* IMS101 (mortality rate, expressed as changes in particulate organic carbon). Different symbols represent two separate bloom simulations. Diamonds represent the samples presented in Western analysis. Correlation coefficients are indicated for linear regression.

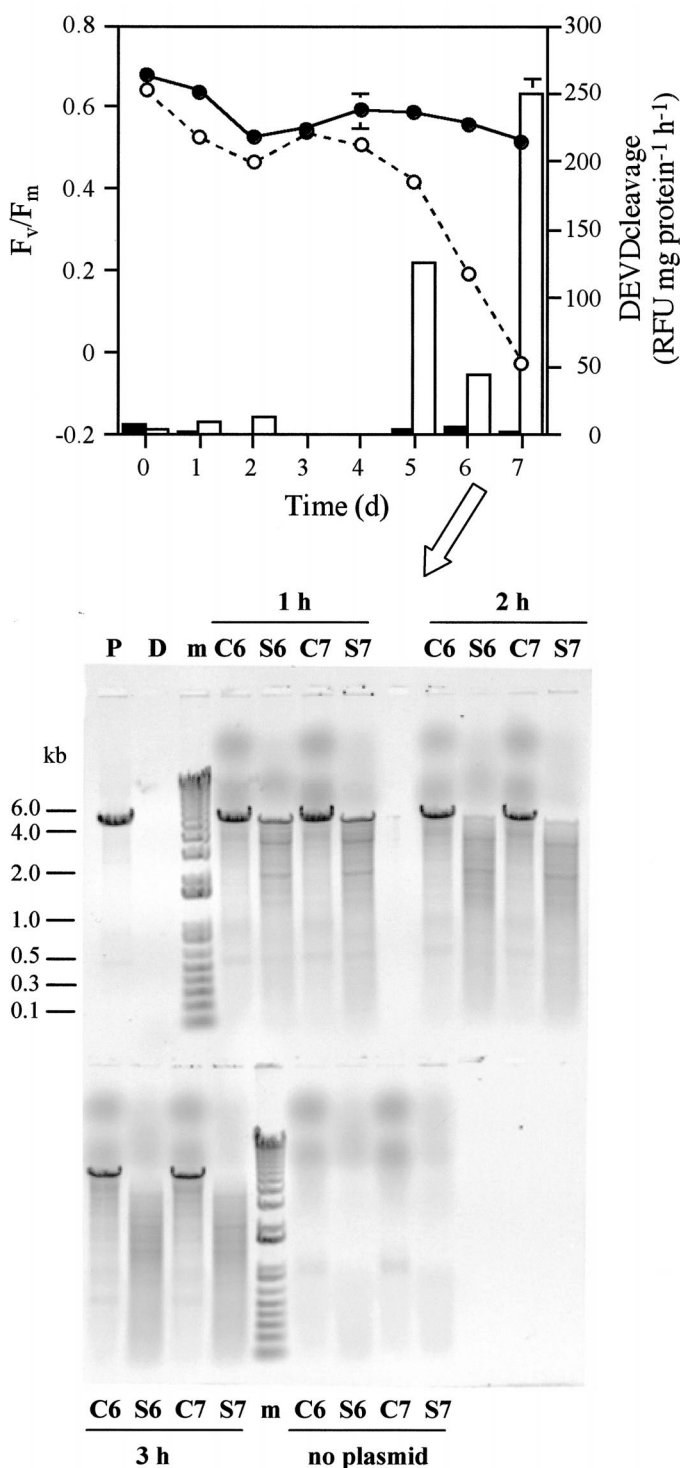


Fig. 2. Phosphorus (P) and iron (Fe) limitation leads to reduction in photosynthetic efficiency, activation of DEVD cleavage, and activation of nuclease activity. Upper panel: Time series of the maximum quantum yield of photosynthesis (F_v/F_m ; circles) and DEVD cleavage (bars) for *Trichodesmium* IMS101 incubating in either complete YBCII growth medium (filled symbols/bars) or YBCII lacking in P and Fe (open symbols/bars). DEVD cleavage was not determined on days 3–4. Error bars are included and indicate standard deviation from triplicate measurements. Lower panel: 0.8% Agarose gel showing elevated nuclease activity in cell lysates of

were all positively labeled with SYTOX green (data not shown).

In metazoans, PCD is both initiated and executed by caspases, a unique family of cysteine aspartate-specific proteases (Thornberry and Lazebnik 1998) that subsequently activate other degradative enzymes, including endonucleases. Although “true” caspases have only been identified in metazoans (ranging from *Hydra* to humans), proteases with similar catalytic properties have been reported in higher plants during hypersensitive response (Del Pozo and Lam 1998; Korthout et al. 2000), in aging yeast cells (Madeo et al. 2002), and in physiologically stressed eukaryotic algae (Segovia et al. 2003). For example, the unicellular chlorophyte alga *Dunaliella tertiolecta* activates a caspase-like protease when kept in darkness for several days (Segovia et al. 2003). Studies on autocatalyzed cell death in other photoautotrophs also hint at similar biochemical control, but these studies lack sufficient detail to ascribe death to a similar pathway. The freshwater cyanobacterium *Anabaena* activates PCD and increases general protease activity after exposure to univalent-cation salts (Ning et al. 2002). The diatom *Thalassiosira weissflogii* induces a specific, unidentified protease under nitrogen stress (Berges and Falkowski 1998). The dinoflagellate *Peridinium gatunense* activates PCD in response to reactive oxygen stress (ROS) that involves cysteine proteases (treatment with E-64, an inhibitor of cysteine proteases, suppressed autolysis) (Vardi et al. 1999).

We examined whether caspase-like proteases were potentially involved in executing cell death in *Trichodesmium* IMS101 by challenging whole-cell protein extracts with polyclonal antibodies to human caspase-3, the main executioner caspase that is activated by initiator caspases 8 or 9 (Thornberry and Lazebnik 1998). Caspase-specific immunoreactivity, expressed as a fraction of total cellular protein, increased markedly (Fig. 1A,C) as cultures entered death phase (>29 d). The temporal pattern of caspase-like immunoreactivity was further supported by direct measurements of enzymatic cleavage of the fluorogenically labeled, caspase-specific substrate DEVD. DEVD cleavage was extremely low in exponentially growing cells, but greatly increased as mortality increased (Fig. 1D; $r > 0.86$ in duplicate experiments). Elevated DEVD cleavage was not accompanied by associated increases in general protease activity (data not shown). Moreover, addition of the broad-spectrum caspase-specific inhibitor z-VAD-FMK to *Tricho-*

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P- and Fe-stressed cells (S) compared to control cells incubating in complete YBCII (C). Data are for cells harvested on days 6 and 7, as indicated by arrow and lane number. One microgram of linear plasmid DNA (pBK-CMV; ~6 kb) was added to cell lysates and incubated at 26°C for 1–3 h. Cell lysates were adjusted to equal protein concentrations (200 $\mu\text{g ml}^{-1}$) in nuclease activity buffer. Nuclease activity is represented by degradation of linear plasmid DNA. Reactions with linear plasmid DNA incubating for 1 h in nuclease buffer with (D) or without (P) DNase I (Ambion) are included. Cell lysates were also incubated in nuclease buffer without addition of plasmid DNA in order to depict background DNA (“no plasmid”). Abbreviation: m, molecular weight markers.

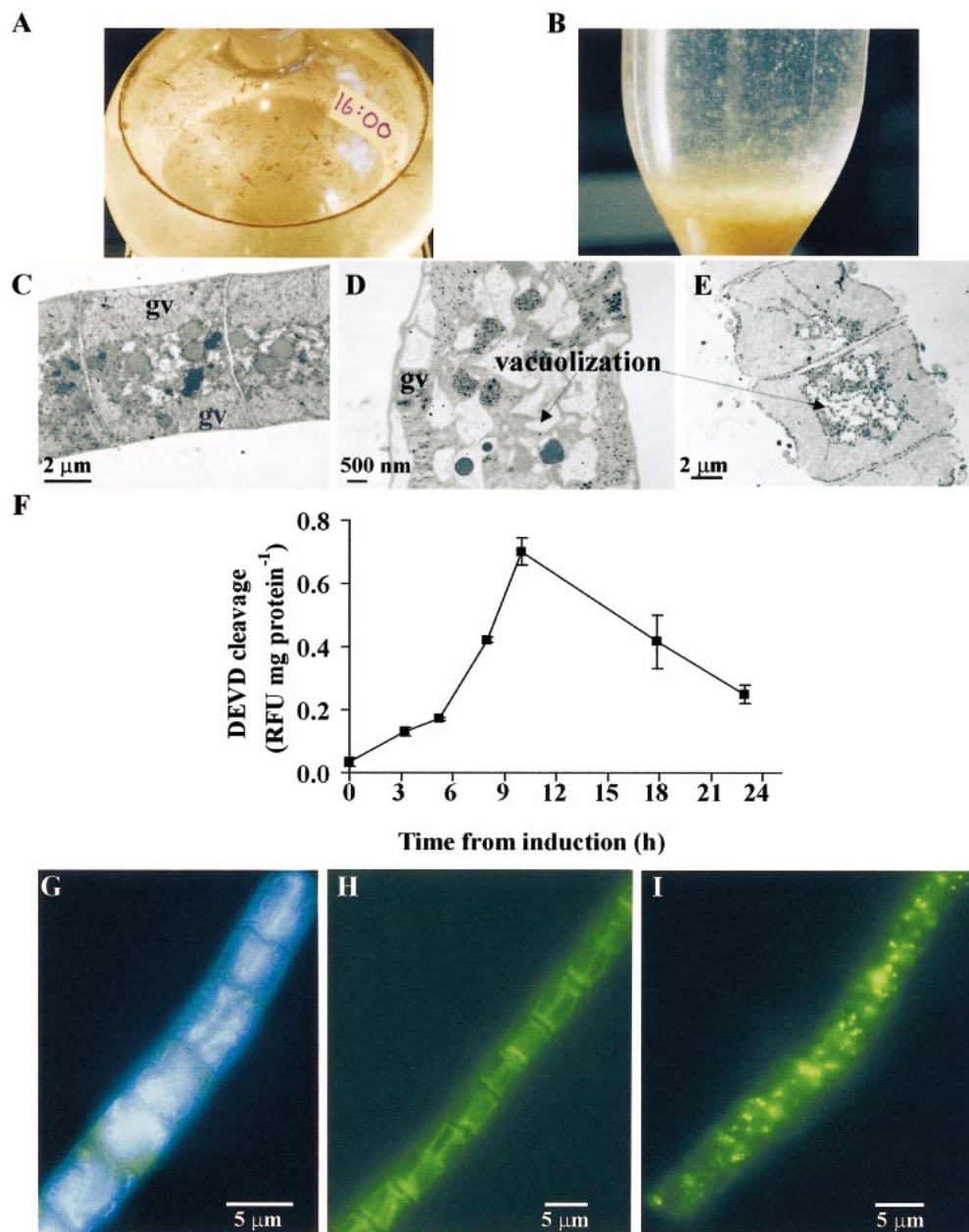


Fig. 3. Oxidative stress induces cell death and elevated caspase-like activity in natural *Trichodesmium* populations collected in the subtropical western Pacific Ocean (off the coast of New Caledonia). Populations were collected from surface waters, cleaned and separated from the majority of other phytoplankton and zooplankton populations, and resuspended in enclosed spheres under high irradiance ($\sim 450 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$). A) After 3 h, a majority of colonies and single trichomes are suspended throughout the sphere and are positively buoyant. B) At >7 h, exposure led to massive sinking of *Trichodesmium* populations. C–E) TEM micrographs of trichomes. C) After 3 h gas vesicles (gv) occupied a high percentage of the cells, which are also packed with other cellular components, such as thylakoids, carboxysomes, and cyanophycin granules; D) after 8 h, vacuolization (v) increased and gas vesicle volume decreased; E) sinking trichomes after 24 h, at which point cellular interior is highly vacuolated and external membranes have begun to lyse. F) A time course of caspase-like activity (DEVD cleavage) in the suspended population during exposure to high irradiance. G–I) Visualization of *Trichodesmium* DNA exposed to ROS (G) DNA of healthy, uninduced (no peroxide added) *Trichodesmium* IMS101 with a general DNA stain, Hoechst. Being a prokaryotic organism, DNA is distributed throughout the cells and trichomes rather than packaged in a nucleus. H) Low-level, diffuse TUNEL staining of uninduced “healthy” cells. We observed a similar low-level background staining from healthy human lymphoma cells supplied with the TUNEL kit. I) Cells under enhanced ROS (3 h after induction with $10 \mu\text{mol L}^{-1} \text{H}_2\text{O}_2$) displayed intense, localized incorporation of the TUNEL fluorescent label indicative of DNA fragmentation and a large population of 3'-hydroxyl ends.

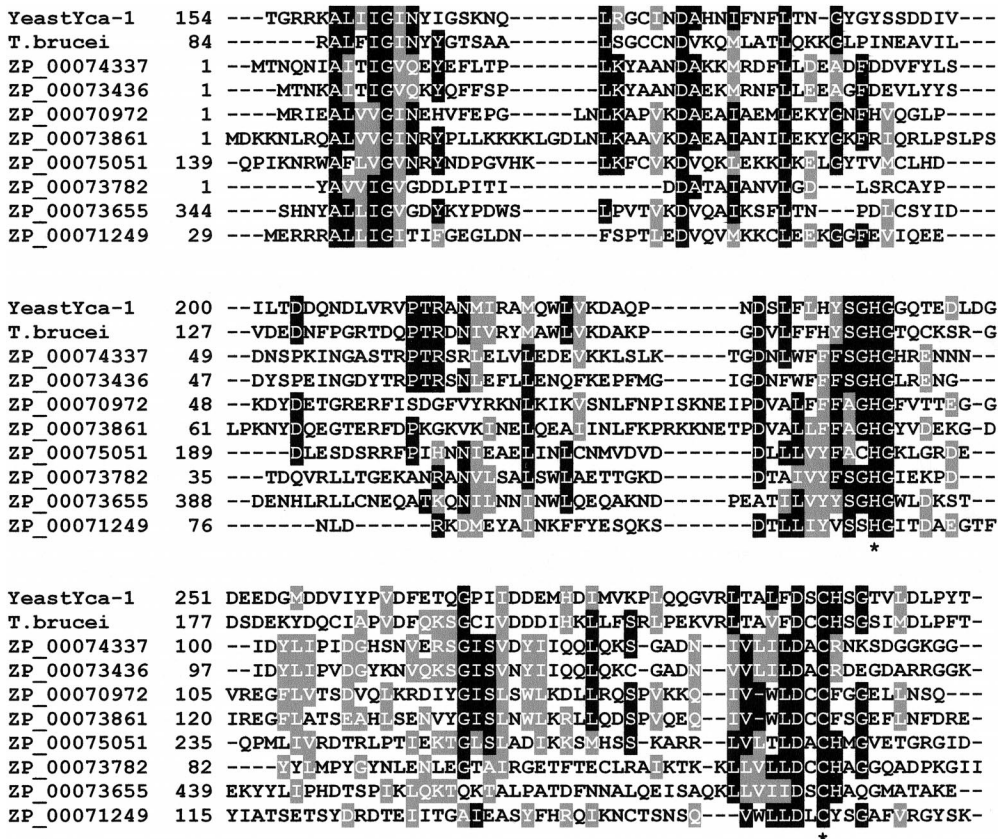


Fig. 4. Alignment of partial protein sequences from *Trichodesmium erythraeum* (accession numbers are given) and metacaspases from *Saccharomyces cerevisiae* (Yca-1; NP_014840) and *Trypanosoma brucei* (CAD24803). The region containing the conserved caspase domain (COG4249) was chosen for alignment. Homologous sequences (black denotes identity and gray denotes similarity) highlight similarities in key functional regions, including the histidine-cysteine catalytic diad (indicated in white and with an asterisk) shared by caspases, paracaspases, and metacaspases. Alignments were done using ClustalW 1.8 (<http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html>). Amino acid numbering within the full-length gene is provided on each line.

desmium cell lysates inhibited DEVD cleavage by 87% at a 50 $\mu\text{mol L}^{-1}$ final inhibitor concentration.

In the contemporary open ocean, phosphorus and iron are often drawn down to extremely low concentrations during late-phase phytoplankton blooms, leading to physiological limitation of metabolic functions. Incubation of *Trichodesmium* IMS101 in phosphorus- and iron-free media resulted in elevated DEVD cleavage after 5 d, concomitant with physiological stress, as indicated by the dramatic decline in the maximum quantum yield of photosynthetic energy conversion efficiency (F_v/F_m) (Kolber et al. 1998) (Fig. 2A). DEVD cleavage remained very low under nutrient-replete conditions (Fig. 2A). Significant reductions in F_v/F_m also coincided with a dramatic increase in nuclease activity in stressed cultures compared to healthy control cells. One hallmark of PCD is a commitment to degradation of the dying cells' genome by the activation of endonucleases. Cell lysates (containing in situ endonucleases) from nutrient-stressed cells collected after 6 d demonstrated enhanced degradation of linear pBK-CMV plasmid DNA. Little to no degradation was detected in nutrient-stressed cells in earlier

time points or in control cells over the complete time course of the experiment (Fig. 2B).

We also examined whether natural populations of *Trichodesmium* activated caspase-like proteases in response to environmental stress. A time course of DEVD cleavage was measured in natural populations of *Trichodesmium*, which were collected in the subtropical western Pacific Ocean (off the coast of New Caledonia) and exposed to the high irradiance (HL) ($\sim 450 \mu\text{mol quanta m}^{-2} \text{ s}^{-1}$) commonly experienced by surface blooms of *Trichodesmium*. HL enhances the production of ROS in oxygenic photoautotrophs (Berman-Frank et al. 2001), which can induce PCD (Chandra et al. 2000). During early exposure to HL (<3 h), a large proportion of *Trichodesmium* cells, which were positively buoyant by virtue of intracellular gas vesicles (Walsby 1978) (indicative of healthy cells), displayed low DEVD cleavage (Fig. 3A,C). Increased exposure (>7 h) to HL resulted in increased DEVD cleavage, enhanced sinking, increased vacuolization (Fig. 3B,D-E), and the selective removal of cells with high caspase activity (Fig. 3F). Sinking trichomes had elevated (by a factor of 24 to 400) caspase-like specific ac-

Table 1. Conserved PCD domains that were detected in *Trichodesmium erythraeum* IMS101 protein sequences.

Domain description	Domain identifier*	Domain function	Contig	Gene	Length (a.a.)	Accession No.
DegQ trypsin-like serine protease, HtrA-like protease	COG0265	Posttranslational modification, protein turnover, chaperones	Contig102 Contig116 Contig106	186 2,881 665	415 405 407	ZP_00072870 ZP_000739790 ZP_00071776
Predicted NTPase (NACHT family)	COG5635	Signal-transduction mechanism	Contig103 Contig102 Contig118 Contig122	328 186 3,871 6,284	570 1,244 447 599	ZP_00073901 ZP_00073386 ZP_00071486 ZP_00072978
AP-ATPase, NB-ARC domain	pfam00931	Novel signaling motif shared by plant resistance gene products and regulators of cell death in animals				
WD-40 domain	cd0020 COG2319	A wide variety of functions, including adaptor/regulatory modules in signal transduction, pre-mRNA processing	Contig120 Contig105 Contig102 Contig117	4,806 552 266 3,499	1,041 1,287 1,243 1,858	ZP_00070972 ZP_00073861
APAF-1	KO†02084	Apoptotic protease activating factor	Contig120 Contig121 Contig122	4,764 5,723 7,167	1,789 1,304 1,510	
TPR, tetratricopeptide repeat	cd00189	Protein-protein interactions, chaperones, cell-cycle, transcription, protein transport	Contig121 Contig119 Contig115 Contig122 Contig111 Contig117	5,773 4,347 2,564 6,284 1,474 3,248	455 531 866 599 731 317	ZP_00071792 ZP_00071396 ZP_00073301 ZP_00072978 ZP_00072841 ZP_00072090

* <http://www.ncbi.nlm.nih.gov/>.

† KEGG; <http://www.genome.ad.jp.>

tivity ($17.1 \text{ RFU } \mu\text{g protein}^{-1}$) compared to buoyant trichomes ($0.30 \pm 0.22 \text{ RFU } \mu\text{g protein}^{-1}$; range 0.04 to 0.70) and lysed after 24 h (Fig. 3E). Direct exposure of *Trichodesmium* to ROS ($10 \mu\text{mol L}^{-1} \text{H}_2\text{O}_2$) induced an ordered fragmentation of DNA, as revealed by positive TUNEL, characteristic of PCD (Gavrieli et al. 1992) (Fig. 3I).

Using the Position-Specific Iterative BLAST (PSI-BLAST) program, we identified several protein sequences in the *T. erythraeum* genome (<http://www.jgi.doe.gov>) that are homologous to the family of caspase-like proteases (“metacaspases”). Metacaspase sequences have been identified in plants, fungi, unicellular protozoa, and multiple bacterial species, including *Streptomyces*, *Rhizobium*, *Anabaena*, *Bordetella*, *Geosulfurococcus*, *Rhodospira*, *Dehalococcoides*, *Xylella*, and *Synechocystis* (Uren et al. 2000). Several deduced *T. erythraeum* protein sequences contained a caspase domain structure (Domain identifier COG4249) (NCBI Conserved Domain Database; <http://www.ncbi.nlm.nih.gov>) and display sequence similarities to metacaspases in the unicellular protists *Saccharomyces cerevisiae* (Yca-1) and *Trypanosoma brucei* (Fig. 4), including the histidine- and cysteine-containing catalytic diad found in true caspases (p20 subunit), paracaspases, and metacaspases (Uren et al. 2000). Yeast Yca-1 has been shown to mediate PCD in aging yeast cells and in response to H_2O_2 stress (Madeo et al. 2002), and overexpression of the *T. brucei* metacaspase causes loss of respiration competence and clonal death in yeast (Szallies et al. 2002). Some of the metacaspase-like *T. erythraeum* sequences also contained WD-40 propeller domains (Table 1), which are regulatory nodules for signal transduction. Orthologs were also identified for other key enzymes of the eukaryotic PCD machinery, including apoptosis activating factor (APAF-1), apoptotic ATPases (AP-ATPases), NACHT family NTPases, and mitochondrial HtrA-like proteases (Table 1).

The demise of *Trichodesmium* via PCD with caspase-like components and its retention in modern eukaryotic algae (i.e., *D. tertiolecta*) indicates that it is an integral mortality pathway in aquatic photoautotrophs with early origins. It also provides another dimension to the current debate on the evolutionary origins and the role of PCD in unicellular and prokaryotic organisms (Aravind et al. 1999; Ameisen 2002). As these organisms have very different evolutionary histories (Delwich 2000), PCD components either evolved independently in host cells prior to the appropriation of a plastid or were derived from plastid inheritance of a cyanobacterium early in the endosymbiotic association. *Trichodesmium* displays biochemical similarities to components of metazoan-like PCD execution, but it evolved well before the first identifiable metazoans appear in the fossil record (Lipps 1993). The origin of cyanobacteria is traced to ca. 3 Ga (Holland and Rye 1997; Summons et al. 1999; Kasting and Siefert 2002), with clades closely related to *Trichodesmium* branching early in the diazotroph phylogenetic NifH tree (Zehr et al. 1997). Furthermore, execution of PCD in *Trichodesmium* appears to be distinct from controls of PCD in other bacteria involving “addiction modules” (Ameisen 2002), providing intriguing evolutionary context on the involvement of caspase-like proteases in PCD. Indeed, caspase-like cysteine proteases may represent the initial, ancestral core of execu-

tioners that allowed the emergence of PCD and essential effectors of the cell death machinery (Ameisen 2002).

In other bacteria, PCD is known to play an important role in the developmental and differentiation processes as part of an adaptation to environmental stress. Examples include sporulation in *Bacillus* and *Streptomyces*, swarmer cell formation and differentiation in *Caulobacter cereus*, the formation of multicellular fruiting bodies in *Myxobacteria*, heterocyst formation in *Anabaena*, the development of bacteroids in *Rhizobium*, and transition to the viable but non-culturable state in various Gram-negative bacteria (Hochman 1997; Lewis 2000). It is noteworthy that in some of these bacteria, PCD pathways are independent of metacaspases.

An additional function of the PCD pathway in diazotrophic cyanobacteria may be the formation of hormogonia, small groups of cells that are released from a parent filament after death and serve as dispersal and infective units in plant-cyanobacterial symbioses. Hormogonia occur in several diazotrophs (e.g., *Nostoc*) under unfavorable growth conditions. In *Trichodesmium* populations, hormogonia are observed, typically deep in the euphotic zone around or below the 1% light level after a large fraction of the trichomes has decomposed (E. Carpenter pers. comm.). Thus, it is tempting to speculate that PCD pathways in *Trichodesmium* may regulate differentiation of selected cells into hormogonia, providing an inocula for a new bloom upon improved environmental conditions. In the dinoflagellate *P. gatunense*, PCD leads to spore formation under limited CO_2 availability and oxidative stress (Vardi et al. 1999).

Induction of PCD in response to nutrient, light, salt, or oxidative stress (Berges and Falkowski 1998; Vardi et al. 1999; Ning et al. 2002; Segovia et al. 2003) further indicates that this mortality pathway operates in the modern ocean and may facilitate biogeochemical cycling through transfer of organic and inorganic matter to heterotrophic microbial communities. Although retention of a PCD pathway would appear to exert negative selection pressure, there must be a selective advantage based on phenotypic expression. *Trichodesmium* sp. IMS101 (this study), *D. tertiolecta* (Segovia et al. 2003), and *Emiliania huxleyi* (Bidle unpubl. data) display immunoreactivity to caspase-3 antisera in both healthy and physiologically stressed cells, but immunoreactivity increases under elevated physiological stress. This pattern of expression indicates that these proteins may have additional cellular roles, such as housekeeping or regulatory functions. Under physiological stress, however, overexpression and posttranslational processing may initiate catastrophic cell death that appears to be maladaptive. Examination of the activation pathways and ensuing processes in cyanobacteria may provide critical insight into the evolution of PCD in eukaryotic organisms.

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