Mapping the diatom redox-sensitive proteome provides insight into response to nitrogen stress in the marine environment

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Diatoms are ubiquitous marine photosynthetic eukaryotes responsible for approximately 20% of global photosynthesis. Little is known about the redox-based mechanisms that mediate diatom sensing and acclimation to environmental stress. Here we used a quantitative mass spectrometry-based approach to elucidate the redox-sensitive signaling network (redoxome) mediating the response of diatoms to oxidative stress. We quantified the degree of oxidation of 3,845 cysteines in the *Phaeodactylum tricornutum* proteome and identified approximately 300 redox-sensitive proteins. Intriguingly, we found redox-sensitive thiols in numerous enzymes composing the nitrogen assimilation pathway and the recently discovered diatom urea cycle. In agreement with this finding, the flux from nitrate into glutamine and glutamate, measured by the incorporation of ¹⁵N, was strongly inhibited under oxidative stress conditions. Furthermore, by targeting the redox-sensitive GFP sensor to various subcellular localizations, we mapped organelle-specific oxidation patterns in response to variations in nitrogen quota and quality. We propose that redox regulation of nitrogen metabolism allows rapid metabolic plasticity to ensure cellular homeostasis, and thus is essential for the ecological success of diatoms in the marine ecosystem.

phytoplankton | redox proteomics | roGFP | marine diatoms

Aerobic organisms produce reactive oxygen species (ROS) as a byproduct of oxygen-based metabolic pathways, such as photosynthesis, photorespiration, and oxidative phosphorylation (1). Perturbations in oxygenic metabolism under various stress conditions can induce oxidative stress from overproduction of ROS (2, 3). Because ROS are highly reactive forms of oxygenic metabolites, critical mechanisms for ROS detoxification have evolved consisting of ROS-scavenging enzymes and small molecules, including glutathione (GSH) (4). As the most abundant low molecular weight thiol antioxidant, GSH has critical roles in maintaining a proper cellular thiol–disulfide balance and in detoxifying H₂O₂ via the ascorbate–GSH cycle (5).

Although classically ROS were considered toxic metabolic byproducts that ultimately lead to cell death, it is now recognized that ROS act as central secondary messengers involved in compartmentalized signaling networks (1, 6–8). Modulation of various cell processes by ROS signaling is mediated largely by posttranslational thiol oxidation, whereby their physical structure and biochemical activity are modified upon oxidation (9). Thus, the redox states of these proteins possess crucial information needed for cell acclimation to stress conditions (10, 11). The emergence of advanced redox proteomic approaches, such as the OxICAT method (12), has created new opportunities to identify redox-sensitive proteins (e.g., redoxome) on the system level and to quantify their precise level of oxidation on exposure to environmental stress conditions.

Marine photosynthetic microorganisms (phytoplankton) are the basis of marine food webs. Despite the fact that their biomass represents only approximately 0.2% of the photosynthetic biomass

on earth, they are responsible for nearly 50% of the annual global carbon-based photosynthesis and greatly influence the global biogeochemical carbon cycle (13). This high ratio of productivity to biomass, reflected in high turnover rates, makes phytoplankton highly responsive to climate change. Phytoplankton can grow rapidly and form massive blooms that stretch over hundreds of kilometers in the oceans and are regulated by such environmental factors as nutrient availability and biotic interactions with grazers and viruses.

Diatoms are a highly diverse clade of phytoplankton, responsible for roughly 20% of global primary productivity (14). Consequently, diatoms play a central role in the biogeochemical cycling of important nutrients, including carbon, nitrogen, and silica, which constitute part of their ornate cell wall. As members of the eukaryotic group known as stramenopiles (or heterokonts), diatoms are derived from a secondary endosymbiotic event involving red and green algae engulfed within an ancestral protest (15).

The unique multilineage content of diatom genomes reveals a melting pot of biochemical characteristics that resemble bacterial, plant, and animal traits, including the integration of a complete urea cycle, fatty acid oxidation in the mitochondria, and plant C₄-like related pathways (16, 17). During bloom succession, phytoplankton cells are subjected to diverse environmental

Significance

Phytoplankton form massive blooms in the oceans that are controlled by nutrients, light availability, and biotic interactions with grazers and viruses. Although phytoplankton were traditionally considered passive drifters with currents here we demonstrate how diatom cells sense and respond to oxidative stress through a redox-sensitive protein network. We further demonstrate the redox sensitivity of nitrogen assimilation, which is essential for diatom blooms in the ocean, and provide compelling evidence for organelle-specific oxidation patterns under nitrogen stress conditions using a genetically encoded redox sensor. We propose that redox regulation of metabolic rates in the response to stress provides a mechanism of acclimation to rapid fluctuations in the chemophysical gradients in the marine environment.

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stress conditions that lead to ROS production, such as allelopathic interactions (18), CO₂ availability (19, 20), UV exposure (21), iron limitation (22), and viral infection (23). Recently reported evidence suggests that diatoms possess a surveillance system based on the induction of ROS that have been implicated in response to various environmental stresses (22, 24). Nevertheless, very little is known about cell signaling processes in marine phytoplankton and their potential role in acclimation to rapid fluctuations in the chemophysical gradients in the marine environment (25).

Using a mass spectrometry-based approach, we examined the diatom redoxome and quantified its degree of oxidation under oxidative stress conditions. The wealth of recently identified redox-sensitive proteins participating in various cellular functions suggests a fundamental role of redox regulation in diatom biology. We mapped the redox-sensitive enzymes into a metabolic network and evaluated their role in the adjustment of metabolic flux under variable environmental conditions. We further explored the redox sensitivity of the primary nitrogen-assimilating pathway and demonstrated the role of compartmentalized redox regulation in cells under nitrogen stress conditions using a redox-sensitive GFP sensor targeted to specific subcellular localizations.

Results

We used the recently developed OxICAT method (12) to achieve a global system-level detection of reactive thiols and to quantify the level of oxidation under steady-state and oxidative stress conditions. The OxICAT method allows the identification of thiol groups that undergo stable and reversible oxidative modification based on differential labeling of reduced and oxidized cysteines with isotopically light and heavy forms of the isotope coded affinity tag (ICAT) reagent, coupled to LC-MS/MS proteomics analysis (12). This method enables in vivo assessment of the ROS-sensitive proteome.

We applied this redox proteomic approach to unravel the thiol proteins involved in sensing of the early phase of oxidative stress, which is implicated in diverse environmental stress conditions. We used the diatom *Phaeodactylum tricornutum* as a tractable model phytoplankton and compared exponential growing cells (steady state) and H₂O₂-treated cells (150 μM for 20 min). Significant double labeling with both the heavy and the light ICAT reagent was recognized for 3,845 peptides, representing 1,744 distinct proteins in the diatom proteome.

We calculated the degree of oxidation for each cysteine based on the ratio of the mass intensity of the light and heavy labeled peptides. The average degree of oxidation of cysteines under steady-state conditions was 18.6% (median, 12.6%), demonstrating that the majority of the cysteines were maintained under highly reduced conditions (Fig. 1A). Predications of subcellular protein localization were made using the heterokont subcellular localisation targeting method (HECTAR) tool (26), which is designed to predict subcellular targeting in heterokonts.

We further analyzed the distribution of the identified proteins in various subcellular compartments (*SI Appendix, Fig. S1*). Only minor variations in the average degree of oxidation in the different subcellular compartments were found under steady-state conditions, ranging from $14 \pm 0.65\%$ in the mitochondria to $18 \pm 0.96\%$ in the chloroplast. Despite the highly reduced cellular environment, 219 thiol-containing peptides exhibited 60% oxidation under steady-state conditions (*SI Appendix, Fig. S1*). This group of highly oxidized proteins was enriched with proteins predicted to target the endoplasmic reticulum lumen [Gene Ontology (GO) accession, GO:0005788; $P = 0.0001$, hypergeometric test], such as protein disulfide-isomerase, and with proteins that have aspartic-type endopeptidase activity (GO:0004190; $P = 0.001$, hypergeometric test), such as thermopsin. Many chloroplast-targeted proteins also exhibited a large number of highly oxidized cysteines, including light-harvesting complex II chlorophyll a/b-binding protein and photosystem II oxygen-evolving enhancer protein 1.

Importantly, as shown in Fig. 14, only a minor change in average oxidation was observed under the steady-state (18.6%) and oxidative stress (22.8%) conditions. This finding suggests that most of the cysteines do not contain thiols that readily react with H_2O_2 . This finding is supported by the relatively low constant rate of thiol oxidation by H_2O_2 (9). Alternatively, oxidized essential thiols can be reduced rapidly by the cell antioxidant/redox buffer capacity.

We found 338 cysteine-containing peptides, representing 278 proteins that exhibited a significantly increased degree of oxidation (ΔOX) under oxidative stress conditions (Fig. 1B). These proteins, which can be directly oxidized by H_2O_2 or indirectly by redoxins, were considered redox-sensitive proteins (i.e., the redoxome). The complete list of identified redox-sensitive cysteines, their subcellular localization, and their degree of oxidation under steady-state and oxidative stress conditions is provided in

Fig. 1. Differential cysteine oxidation of *P. tricornutum* proteome under oxidative stress conditions. (A) Distribution of the redox state of 3,845 peptidyl cysteine residues as measured by OxICAT redox proteomics in steady state (blue line) and in response to 150 μ M H_2O_2 for 20 min (red line). (B) Degree of oxidation of identified cysteines was plotted according to their difference in oxidation level in H_2O_2 -treated cells versus steady-state conditions. The main plot shows cysteines with at least 10% difference in their redox state between the oxidized and steady-state conditions. (Inset) Distribution of all identified cysteines. The degree of oxidation of selected redox-sensitive proteins is shown. (C and D) Mass spectra for iCAT-labeled Cys¹⁰⁰ of the peroxiredoxin (Prx) active site under steady-state (C) and oxidative stress (D) conditions.

SI Appendix, Table S1. For example, a significant change in the degree of oxidation was found for the active site of peroxiredoxin (Cys¹⁰⁰; $\Delta\text{OX} = 35\%$) (Fig. 1 *C* and *D*), who oxidation state has been shown to play a role in mediating cell survival signal transduction pathways (27) and serves as a universal marker for circadian rhythms (28). The stable oxidation of these proteins and their variation in degree of oxidation may enable the transmission of specific ROS signals (29, 30). Analysis of the redoxome distribution among various subcellular compartments showed that chloroplast-targeted proteins were overrepresented (hypergeometric test $P = 0.01$) (Fig. 2*A*) and oxidized at greater degrees compared with other cell compartments (Fig. 2*B*). These findings underscore the prevalent role of redox regulation in chloroplast reactions.

Eukaryotic Orthologous Group (KOG) and GO assignment of redox-sensitive proteins revealed their representation in numerous cell function categories, including signaling, primary and secondary metabolism, translation, transcription, and trafficking (Fig. 2*C*). Interestingly, overrepresentation of assigned metabolic pathways was found in the redoxome (Fig. 2*C* and **SI Appendix, Table S2**); thus, we mapped the redoxome to metabolic networks using the DiatomCyc database (31). We discovered redox sensitivity of key metabolic enzymes involved in numerous essential pathways, including photosynthesis, glycolysis, antioxidant activity, amino acid biosynthesis, and nitrogen and sulfur metabolism (Fig. 3 and **SI Appendix, Fig. S2**). Posttranslational modifications of reactive cysteines in these biosynthetic pathways potentially can lead to alterations in the metabolic fluxes through these pathways (10, 32–34).

We found significant enrichment in reactive cysteines in nitrogen metabolism proteins (glutamine metabolism, GO:0006541; $P = 0.008$) (**SI Appendix, Table S2**). Detailed inspection identified the presence of redox-sensitive cysteines in key enzymes

participating in the assimilation of nitrate to glutamate (Fig. 3 and **SI Appendix, Table S1**), including Cys¹⁹⁵ and Cys⁴⁶² ($\Delta\text{OX} = 16\%$ and 21% , respectively) as redox-sensitive cysteines in NADPH nitrite reductase. In addition, Cys³⁵³ in the chloroplastic glutamine synthase (GS) ($\Delta\text{OX} = 11\%$) and three cysteines in the chloroplastic glutamate synthase (GOGAT; Cys¹²⁰, Cys¹⁵⁶, and Cys⁷⁰⁴; $\Delta\text{OX} = 15\%$, 16% , and 25% , respectively) were found to be redox-sensitive, suggesting the redox regulation of ammonia sequestration.

We examined the evolutionary conservation of these cysteines and found remarkable conservation of two GOGAT reactive cysteines, Cys¹²⁰ and Cys⁷⁰⁴, across photosynthetic organisms from cyanobacteria to higher plants (**SI Appendix, Fig. S3**). We also found redox-sensitive cysteines in two key enzymes of the urea cycle: mitochondrial carbamoyl-phosphate synthase (CPSIII; Cys⁹⁸⁰ and Cys¹³⁵⁸; $\Delta\text{OX} = 16.5\%$ and 14.4% , respectively), which facilitates the formation of carbamoyl phosphate from bicarbonate and ammonium or glutamine, and arginase (Cys²¹²; $\Delta\text{OX} = 40\%$), the final enzyme of the urea cycle, which catalyzes the hydrolysis of arginine to form ornithine (Fig. 3). This pathway, which is similar to that of metazoans but is absent from green algae and plants, was recently identified in diatoms and shown to facilitate rapid recovery from prolonged nitrogen limitation (16).

Importantly, we identified a high abundance of these redox-sensitive enzymes participating in nitrogen metabolism in EST libraries (35) derived from diatom cells with variable nitrogen sources and availability (**SI Appendix, Fig. S4**). To further examine the redox sensitivity of the nitrogen assimilation pathway in diatoms, we measured the transfer of isotope-labeled ¹⁵N from nitrate into glutamine (Gln) and glutamate (Glu) by LC-MS. We detected rapid and efficient incorporation of ¹⁵N into Gln and Glu in cells under normal growth conditions, reaching ~80% Glu and Gln labeling (labeled by ¹⁵N in both amide and amino groups) after 140 min (Fig. 4*A* and *B*) and ~50% of Gln labeling in either the amide or the amino group (**SI Appendix, Fig. S5**). In contrast, we found hardly any incorporation of ¹⁵N into Gln and Glu in H₂O₂-treated cells (Fig. 4*A* and *B* and **SI Appendix, Fig. S5**), demonstrating the redox sensitivity of nitrogen assimilation pathways in diatoms (Fig. 4*C*).

Our finding of the redox sensitivity of nitrogen metabolism pathways prompted us to examine whether intracellular redox homeostasis is affected by nitrogen availability. We detected a decrease in GSH content under nitrogen starvation conditions as measured by fluorescent staining with monochlorobimane (**SI Appendix, Fig. S8**). However, because the redox state of reactive thiols relies mainly on the compartmentalized GSH redox potential (E_{GSH}) (8, 36), it was essential to map the spatial resolution of in vivo E_{GSH} at the subcellular level. To this end, we generated *P. tricornutum* transformants expressing the redox-sensitive green fluorescent protein (roGFP) in specific organelles, including the chloroplast (chl-roGFP), mitochondria (mit-roGFP), and nucleus (nuc-roGFP) (Fig. 5 and **SI Appendix, Fig. S6**). Emission ratios measured after excitation with 405 nm and 488 nm represent the in vivo oxidation level of the roGFP probe that reports E_{GSH} (36). Complete oxidation and reduction was achieved by application of H₂O₂ and DTT, respectively (Fig. 5*D–I*). Under steady-state conditions, roGFP was highly reduced (~97%) in all of the examined compartments, corroborating the highly reduced state of the redoxome (Fig. 5*A–C*). Application of 150 μM H₂O₂, which was used for redoxome quantification, led to complete oxidation of roGFP in all organelles (Fig. 5*M–O*). Distinct roGFP oxidation patterns were detected in various subcellular compartments in response to a range of H₂O₂ concentrations (Fig. 5*M–O*). These data indicate compartmentalized redox microenvironments and high antioxidant capacity in the chloroplast compared with the mitochondria and nucleus.

Given that diatom blooms in the ocean are highly dependent on episodic nitrate availability, we measured intracellular E_{GSH}

Fig. 2. Redoxome distribution among subcellular compartments and biological pathways. (A) Subcellular distribution of redox-sensitive proteins were assigned using the HECTAR tool (26). The percentage of proteins in each compartment from the total redoxome is depicted; the number in brackets represents the percentage of each group from the entire identified protein population irrespective of redox sensitivity. (B) Averaged difference in the thiol-oxidized state between steady state and exposure to H₂O₂ treatment in different subcellular compartments. P values were determined by the Student unpaired two-tailed t test. Chl, chloroplast; Mit, mitochondria; Nuc, nucleus; SP, signal peptide; SA, signal anchor; NT, not targeted. (C) KOG assignment of *P. tricornutum* redox-sensitive proteome. KOG categories were assigned using the server at <http://weizhong-lab.ucsd.edu/metagenomic-analysis/server/kog/>.

Fig. 3. Integrated diagram of redox-sensitive proteins in key metabolic pathways in diatoms. Identified redox-sensitive proteins are in red, and quantification of the difference in their degree (Δ OX) of oxidation in response to oxidative stress is shown. Redox-sensitive reactions participating in nitrogen metabolism are highlighted in bold. A complete list of abbreviations is provided in *SI Appendix, Table S3*.

in response to nitrogen starvation. We found differential changes in the redox state of subcellular compartments in nitrate-starved cells (Fig. 5 *J–L* and *P–R*). A profound oxidation of 60% was observed in chl-roGFP on nitrogen depletion (Fig. 5 *J* and *P*), which is equivalent to the increase in chloroplastic E_{GSH} from -345 mV to -284 mV (Fig. 5*M*). Concomitantly, an increase in oxidation was also observed in the mit-roGFP probe (Fig. 5*Q*); however, the level of oxidation was lower compared with that in chl-roGFP, equivalent to the increase in mitochondrial E_{GSH} from -348 mV to -318 mV (Fig. 5*N*). In contrast, no changes were observed in nuc-roGFP (Fig. 5 *L* and *R*), highlighting the specificity of subcellular localization of the redox microenvironment. Importantly, roGFP oxidation preceded the reduction in growth rate due to nitrogen limitation, implying regulation of the cellular acclimation by redox signaling (*SI Appendix, Fig. S7*).

Diatom blooms are dominant in high NO_3^- upwelling environments and continental margins, which are characterized by seasonal pulses of nutrients and a well-mixed water column (37). Under such fluctuating nitrate availability, diatoms can out-compete other dominant phytoplankton groups, such as coccolithophores (38). To mimic these natural conditions and examine the role of redox regulation in recovery from nitrogen starvation, we monitored subcellular alterations in redox potential. We found a differential response in chl-roGFP and mit-roGFP in response to the addition of nitrate, ammonia, or urea to nitrogen-starved cells (Fig. 5 *S–U*). Whereas the addition of nitrate induced specific oxidation of chl-roGFP above the level observed in nitrate-starved cells, the addition of ammonia or urea had no further effect on chloroplast redox potential (Fig. 5*S*). In contrast, the addition of ammonia or urea to starved cells led to specific oxidation of mit-roGFP (Fig. 5*T*). No change in the degree of oxidation in nuc-roGFP was noted in response to the addition of any nitrogen source (Fig. 5*U*). Taken together, our findings demonstrate an organelle-specific oxidation pattern in diatom response to nitrogen status and quality.

Discussion

A common feature of cells exposed to environmental stress conditions is generation of ROS, which can vary in terms of chemical species and subcellular localization. ROS levels can be accurately sensed and activate specific biological pathways, mainly by thiol

oxidation in redox-sensitive proteins. Using a redox proteomics approach, we identified the diatom redoxome and demonstrated its involvement in various cellular functions (*SI Appendix, Table S1*). More specifically, we identified a high proportion of redox-sensitive enzymes that regulate key metabolic pathways in diatom biology, including photosynthesis, photorespiration, lipid biosynthesis, and nitrogen metabolism (Fig. 3 and *SI Appendix, Fig. S2*). These redox-sensitive enzymes can serve as a network to transmit information derived from the cell redox state into metabolic pathways. Whereas long-term adaption to environmental stress

Fig. 4. Redox regulation of primary nitrate assimilation in diatoms. (A and B) *P. tricornutum* cells under steady-state or oxidative stress conditions were supplied with ^{15}N -nitrate and the enrichment of ^{15}N in glutamine (labeled in amide and amino groups) (A) and glutamate (B) was detected by LC-MS. Data are presented as mean $\pm$ SD; $n = 3$. (C) Proposed model for redox regulation of nitrogen assimilation in diatoms. Nitrogen assimilation enzymes use the reducing power (Fd; NADPH) generated in the chloroplast to assimilate nitrate to glutamate. This pathway can serve as a sink for excess electrons produced under highly reducing conditions. The activity of these enzymes is redox-regulated by ROS level modifying reactive thiols and Grx/Trx activities. The redox regulation of nitrogen assimilation enzymes can act as a feedback mechanism in which the chloroplast redox state is constantly monitored by nitrogen assimilation enzymes, thereby ensuring proper adjustment of the assimilation process and redox metabolism.

Fig. 5. In vivo quantification of compartmentalized redox state in *P. tricornutum* cells under nitrogen starvation recovery cycle. (A–L) Micrographs of fluorescence images of *P. tricornutum* cells expressing roGFP in the chloroplast, mitochondria, and nucleus under steady-state (control cells, A–C) conditions, following treatments with an oxidant (200 μ M H_2O_2 ; D–F) and a reductant (1 mM DTT; G–I) and of nitrate-starved cells (J–L). For each *P. tricornutum* transformant ratiometric images (excitation, 405/488 nm; emission, 525 nm) under steady-state, oxidized, and reduced conditions are shown in pseudocolor. The dashed line represents the cell outline and is based on bright field images. (Scale bar: 10 μ m.) (M–U) In vivo determination of roGFP oxidation in the chloroplast (M, P, and S), mitochondria (N, Q, and T), and nucleus (O, R, and U) on application of a range of H_2O_2 concentrations (M–O), under nitrogen-starvation conditions (P–R), and during recovery by the addition of nitrate, ammonia, or urea (S–U). roGFP oxidation was calculated based on averaged fluorescence measurements of at least 6,000 cells by flow cytometry. Data on recovery from nitrogen starvation are presented as the difference in roGFP oxidation between starved cells and those supplemented by different nitrogen sources. Representative data from at least five experiments are shown in M–U and presented as mean $\pm$ SD; $n = 3$.

conditions is generally mediated by transcriptome reprogramming, alterations in protein activity by redox-dependent post-translational modifications may serve as a mechanism to enable rapid responses to variable conditions.

Nitrogen availability is one of the key factors limiting phytoplankton growth and bloom formation in the marine environment. Our redox proteomic analysis revealed numerous redox-sensitive cysteines in the proteins participating in nitrogen metabolism, suggesting that this pathway is redox-regulated (Fig. 3). By targeting the roGFP probe to various subcellular localizations, we also showed a organelle-specific E_{GSH} oxidation pattern under nitrogen stress conditions (Fig. 5). In addition, we demonstrated reduced nitrogen assimilation activity under oxidative stress conditions (Fig. 4). The latter analysis cannot distinguish between redox sensitivity caused by the redox regulation of the nitrogen assimilation proteins and redox sensitivity resulting from lower availability of electron equivalents for nitrogen reduction under oxidative stress conditions, and further biochemical and functional studies on each of the identified reactive cysteines are needed to validate their role in the diatom redox-signaling cascade. Taken together, our findings point to a central role of redox regulation in nitrogen stress conditions.

The observed oxidation in chloroplastic E_{GSH} under nitrogen starvation (Fig. 5 J and P) may be a result of impaired photosynthetic reaction, leading to overproduction of ROS owing to uncontrolled electron leakage and, consequently, reduced antioxidant buffer capacity. Alternatively, the observed oxidation of chloroplastic E_{GSH} may be a result of the decreased GSH content as synthesis of amino acids becomes limiting during nitrogen depletion (SI Appendix, Fig. S8). This oxidation may affect chloroplastic metabolic processes through oxidative modifications of chloroplast-targeted redox-sensitive proteins (Fig. 3 and SI Appendix, Table S1). Because primary nitrogen assimilation is an important sink for reducing power (i.e., through reduced ferredoxin and NADPH) (39), the addition of nitrate to nitrogen-starved cells may divert the distribution of electrons among different chloroplastic metabolic pathways, including redox metabolism (e.g., GSH, NADPH). Indeed, when nitrate was added to nitrogen-starved cells, oxidation of chloroplastic E_{GSH} increased,

whereas that of mitochondrial and nuclear E_{GSH} did not change (Fig. 5S).

As proposed in Fig. 4C, nitrogen assimilation enzymes use the reducing power generated by the photosynthetic apparatus to reduce nitrate to ammonia. These enzymes are also redox-regulated by ROS levels and by the flux of electrons via thioredoxins (Trx) and glutaredoxin (Grx) activity, which is required to reduce essential oxidized thiols for optimal enzymatic activities. This may enable feedback mechanisms in which the chloroplast redox state is continuously monitored by the nitrogen assimilation enzymes to ensure precise adjustments of the assimilation process to maintain cellular homeostasis. Interestingly, in alga, the expression of the nitrate reductase gene, which encodes the first enzyme in nitrate assimilation pathway, was shown to be redox-regulated via the redox state of the plastoquinone pool (40). In addition, GS and GOGAT have been identified as targets of Grx in *Arabidopsis* plants (41), and GS also has been identified as a target of Trx (42, 43). Coordination of primary nitrogen assimilation, localized in the chloroplast, and mitochondrial nitrogen metabolism and respiration processes to maintain cellular homeostasis is crucial. Specific oxidation of the mitochondrial E_{GSH} was observed when nitrate-starved cells were fed with ammonia or urea (Fig. 5T).

Supplementation of starved cells with ammonia as a sole nitrogen source will stimulate activation of the mitochondrial urea cycle to achieve the essential distribution and storage of nitrogen (16). Furthermore, it will increase the demand for carbon skeletons in the form of the tricarboxylic acid (TCA) cycle intermediate α -ketoglutarate to produce glutamine. These modulations of mitochondrial metabolism may affect the supply of reducing equivalents generated by the TCA cycle and, as such, the flow of electrons in the respiratory chain and, subsequently, ATP production. Therefore, the substantial variations in nitrogen supply and light intensities during diatom blooms require a mechanism for cross-talk between the chloroplast and mitochondria. The redox sensitivity of nitrogen metabolism enzymes, along with specific modulation of the redox microenvironments in the chloroplast and mitochondria, may enable this interorganelle signaling.

To conclude, we have provided a comprehensive systematic analysis of the redox network of diatoms and its function in the response of diatoms to environmental stresses, such as nitrogen

availability. The data presented here can serve as a fundamental resource for future studies aimed at elucidating the functional role of these components in sensing and acclimation to the ever-changing marine environment. We propose that the diatom redoxome evolved as an essential mechanism for sensing oxidative stress derived from oxygen-based metabolism since the great oxygen event 2.8 billion years ago (44, 45). This redoxome plays a pivotal role in regulating cellular redox metabolism and, consequently, algal bloom dynamics.

Materials and Methods

For ^{15}N labeling of glutamate and glutamine, amino acids were extracted from *P. tricornutum* cells under steady-state or oxidative stress conditions as

described previously (46) and analyzed by ultra-performance liquid chromatography (ACQUITY ultra performance liquid chromatography system; Waters) coupled online to a triple-quadrupole mass spectrometer (Xevo TQ-S; Waters). ^{15}N enrichment was calculated as described previously (47). Complete descriptions of the methods used in these experiments are provided in *SI Appendix, Materials and Methods*.

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