Minireview

Gianluca Dell'Aquila and Uwe G. Maier*

Specific acclimations to phosphorus limitation in the marine diatom *Phaeodactylum tricornutum*

https://doi.org/10.1515/hsz-2020-0197
Received May 26, 2020; accepted August 19, 2020; published online September 14, 2020

Abstract: Phosphorus (P) is a crucial element and diatoms, unicellular phototrophic organisms, evolved efficient strategies to handle limiting phosphorus concentrations in the oceans. In the last decade, several groups investigated the model diatom *Phaeodactylum tricornutum* concerning phosphate homeostasis mechanisms. Here, we summarize the actual status of knowledge by linking the available data sets, thereby indicating experimental limits but also future research directions.

Keywords: 5′nucleotidase; *Phaeodactylum tricornutum*; phosphate homeostasis; phosphorus limitation.

Introduction

Phosphorus (P) is an essential element for all forms of life (Paytan and McLaughlin 2007). Due to its biochemical properties (Westheimer 1987), P is part of many biological molecules such as nucleic acids, or many lipids and has a fundamental role in important cellular processes such as regulated posttranslational modifications (Hunter 2012). Oceans are highly variable in respect to phosphorus bioavailability (Benitez-Nelson 2000; Paytan and McLaughlin 2007), which strongly influences the ecology and physiology of marine phytoplankton (Lin et al. 2016).

Diatoms, important members of marine phytoplankton, are very efficient primary producers. As being responsible for one-fifth of global primary production, the hereby fixed carbon is an enormous part of the marine food chain. A major interest is, thus, to determine how diatoms fine-regulate their physiology to the changing marine environment. Amongst diatoms, *Phaeodactylum tricornutum* is a widespread used model organism to understand and interpret diatoms biology and ecology. Due to its ease to lab growth and the availability of several genetic tools it has been largely used to study physiological adaptations and responses to biotic/abiotic stimuli (Falciatore et al. 2000), likewise cellular responses to P-limitation in the last decade (Alipanah et al. 2018; Cruz de Carvalho et al. 2016; Feng et al. 2015; Yang et al. 2014). Here we review multidisciplinary data sets aiming to highlight cellular adaptations to phosphorus limitations in *P. tricornutum*.

"Omics" data and what they tell us

"Omics", that quantify changes in protein and transcript abundance at defined conditions, are perfect starting points to study whole-cell responses to environmental stimuli. That is the case of *P. tricornutum*, a model organism for studying cellular responses related to environmental conditions and especially to P-limitations. By using its genome sequence (Bowler et al. 2008), several independent studies indicated specific transcriptomic and proteomic responses of P-starved cells (Alipanah et al. 2018; Cruz de Carvalho et al. 2016; Feng et al. 2015; Yang et al. 2014). Although the "omics" experiments at P limiting conditions differ from each other in respect to used strains, cell concentrations, experimental settings, and methods the overview of the generated data sets is consistent: they all showed a significant impact on the global metabolism of the diatom, underlying molecular strategies that can be classified both as P-limitation-specific or as a stress response. Even though the generated data are perfect for explaining P-correlated responses in different organelles such as the mitochondria, the "omics" results cannot be fully integrated into a metabolic/physiological description of responses to P-limitations when the localizations and functions of the inspected proteins are unknown. Here, we review and discuss P-starvation specific issues with respect to alkaline phosphatases, cellular P uptake, lipid

***Corresponding author: Uwe G. Maier**, Cell Biology Laboratory, Philipps Universität Marburg, Karl-von-Frisch Strasse 8, D-35043 Marburg, Germany; and SYNMIKRO Research Center, Hans-Meerwein-Str. 6, D-35032 Marburg, Germany, e-mail: maier@biologie.uni-marburg.de. https://orcid.org/0000-0002-7106-0516
Gianluca Dell'Aquila, Cell Biology Laboratory, Philipps Universität Marburg, Karl-von-Frisch Strasse 8, D-35043 Marburg, Germany. https://orcid.org/0000-0002-8911-9344

dynamics, P storage, and regulatory networks, hence indicating different levels of cellular responses to limiting environmental conditions.

Phosphatases

P-limitation can trigger the upregulation of transcripts encoding alkaline phosphatases. This class of enzymes hydrolyzes, when acting in the medium, phosphate groups from dissolved organic phosphate (DOP) (Lin et al. 2016), which includes molecules released by cell lysis and grazing activities (Paytan and McLaughlin 2007). One alkaline phosphatase was detected by P-starvation proteome profiling (Phatrdraft_45757), a predicted PhoD metallophosphatase (Feng et al. 2015). In a transcriptome analysis of an analogous experiment by Yang and coworkers (2014), Phatrdraft_49678 (PhoA alkaline phosphatase) and Phatrdraft_45959 (PhoD) were identified as significantly upregulated (Yang et al. 2014). The reason for the discrepancy between both studies might be the limitations of proteins accessible to proteomic analyses.

The P regulated transcriptome was additionally investigated in a microarray approach, after 48 and 72 h of P-starvation (Alipanah et al. 2018). For both time points, additional upregulated putative alkaline phosphatases were identified: Phatrdraft_39432, 48679 and 47612 encoding for PhoD, Aty-PhoA and Phytase respectively. Taken together, the three mentioned analyses cover a range of 72 h of P-starvation. However, the P-dependent regulation of the analyzed genes/proteins is not identical, especially when two transcriptome profiles are compared. This discrepancy, already discussed in Alipanah et al. 2018, might be caused by different experimental designs. In respect to the latter, two different *P. tricornutum* strains were investigated and the sampling was differing in cell densities and growth phases. For example, in the analyses of Yang et al. (2014) the cells were incubated under P-starvation in the early stationary phase, Alipanah and coworkers (2018) studied P starved cells in the exponential phase. In any case, beside some differences found in the above-mentioned datasets, they all show, beside P-specific effects, general stress responses such as a decrease in photosynthetic efficiency under P-stress, which multiplies in the time frame of cultivation. In another study, the transcriptome was studied after four and eight days of P-starvation in addition to P re-supplementation experiments for four days (Cruz de Carvalho et al. 2016). Again, alkaline phosphatases induction was shown under P-starvation (Phatrdraft_39432, 49678, 47869, 45959), but also downregulation was observed when P-starved cells were transferred into fresh f/2 in case of Phatrdraft_39432, 49678, 47869. In another report, the expression patterns of some P-responsive genes have been shown to be strictly related to the external P concentration (Dell'Aquila et al. 2020). Here the regulatory regions (upstream/downstream to the coding region) of several putative alkaline phosphatase genes were used to express an eGFP reporter. In our approach, we showed that the expression of the genes encoding for Phatrdraft_49678, 47612, and 45757 is repressed when the cells were cultivated under P-replete conditions suggesting a strong transcriptional control according to the external P-concentration. However, an additional posttranslational control of these proteins was not excluded (Dell'Aquila et al. 2020).

Several organic phosphorus compounds of the environment can be utilized by diatoms (Benitez-Nelson 2000; Paytan and McLaughlin 2007). For example, *P. tricornutum* can be grown in media having either adenine-monophosphate (AMP) or α-gylcerol-phosphate (αGP), phytin (myoinositol hexaphosphate) and glucose-6-phosphate (G6P) (Cembella et al. 1982) as the only molecules with a phosphate group in the medium. It is likely the case that at least some of these compounds are not imported into the diatom cell, but instead hydrolyzed by extracellular alkaline phosphatases to generate P_i in direct surrounding. Localization studies with putative P-responsive alkaline phosphatases support this concept and for Phatrdraft_49678 and 47612 secretions of the enzymes were described (Buhmann et al. 2016; Erdene-Ochir et al. 2019; Lin et al. 2013). In addition, Flynn and coworkers measured alkaline phosphatase activity in diverse cell fractions and found that it might be associated with the cell surface, extracellular medium, and plasma membrane, respectively (Flynn et al. 1986). In agreement with that, two putative alkaline phosphatases (Phatrdraft_45959, 48789) localized at the cell surface, when expressed as eGFP fusion proteins (Dell'Aquila et al. 2020). A fourth P-regulated putative alkaline phosphatase (Phatrdraft_45757) was shown to be an enzyme of the endoplasmic reticulum (ER) and a membrane surrounding the plastid of diatoms (chloroplast-ER, cER). The latter finding might indicate P scavenging activities in internal compartments as well, especially in the phospholipid (PL) degradation pathway under P-starvation, as proposed by Dell'Aquila et al. (2020).

P. tricornutum might use further P scavenging strategies. A hint to that is a 5′-nucleotidase (Phatrdraft_43694), transcriptionally upregulated after 72 h of P starvation and proposed to act extracellularly according to topology predictions (Alipanah et al. 2018). It might be of highest interest to investigate this nucleotidase in more detail.

Phosphate transport

Induction of alkaline phosphatases and 5′-nucleotidases acting extracellularly reflects a common strategy to scavenge phosphorus in the surrounding environment. The most readily bioaccessible form of P is inorganic phosphate (PO_4^{3-}, P_i) that can be easily taken up by the cell. For that and for the intracellular transport of phosphate into organelles, P_i-transporters are essential and several genes encoding putative sodium-dependent P_i transporters were identified to be significantly transcriptionally upregulated under P-limitations: Phatrdraft_40433, 47667, 49842, 47239 (Alipanah et al. 2018; Yang et al. 2014) as well as 47666, 47239 detected in Alipanah et al. 2018 only. Another identified upregulated transcript in both datasets was Phatrdraft_23830, predicted to encode for a putative high-affinity P_i-transporter belonging to the PHO4 superfamily. The above-mentioned genes, as well as additional ones (Phatrdraft_39515, 22315, 33266), were shown to be significantly transcriptionally upregulated after four- and eight-days under P starvation as well (Cruz de Carvalho et al. 2016).

To cope with P limitations, cells increase the number of P_i-transporters at the plasma membrane to maximize uptake efficiency. As shown for other organisms, these P_i-induced transporters have high-affinity for P_i and, at the same time, low-affinity Pi-transporter activity is commonly repressed (Lin et al. 2016). So far, no studies where these transporters are characterized in relation their functionality and P_i-binding affinity has been published for *P. tricornutum*. However, a recent publication deals with the dynamics of the response of the P_i uptake machinery in different temporal P_i gradients by combining nutrient-uptake bioassays, transcriptomic and mathematical modeling (Cáceres et al. 2019). They showed that the transcriptional upregulation of three putative transporters (Phatrdraft_47666, 47667, 39515) is coupled with an increase of maximum nutrient-uptake (V_{max}) when the cells are P-starved and P-resupplied with low P amount (3 µM). Both V_{max} and gene expression decreased using a stronger P pulse supplementation (15 µM).

Similarly, a consistent downregulation of putative sodium-phosphate and P_i-transporters was observed after four days in P-replete conditions after cultivation under P-starvation (Cruz de Carvalho et al. 2016), confirming that a high and low-affinity P_i uptake machinery could work in an alternate manner according to external P concentration. However, studies on the regulation of eGFP expression driven by the upstream/downstream regions of putative P_i-transporter genes showed that two of them (Phatrdraft_40433, 47667) still retain a basal level of expression when cultivating the cells in several P-replete conditions, whereas the gene expression of Phatrdraft_23830 was repressed (Dell'Aquila et al. 2020). In any case, transcriptional regulation is not the only mechanism to control the expression of a protein. Protein degradation can occur post translationally and this strategy might lead to a finer control of P uptake as described for other organisms (Hürlimann et al. 2009).

Subcellular localization of the P_i-transporters supports the tendency to increase the number of transporters at cell borders, something which was predicted and observed in the work of Caceres and coworkers (Cáceres et al. 2019). Remarkably, two consistently P-induced sodium-phosphate transporters (Phatrdraft_40433 and 47667) localize indeed at the plasma membrane (Dell'Aquila et al. 2020), probably to increase P uptake during P-stress providing an increase of V_{max}. In addition, it was shown that the putative repressible high-affinity transporter Phatrdraft_23830 instead localizes intracellularly in the endomembrane system (ER/cER), most likely providing influx/efflux of P from/to this compartment. The ER is the compartment in which some steps of PL degradation might take place. The strongly P-regulated putative transporter located there could play a role in phosphate transfer into or out of this organelle. In every case, other transporters, strictly transporting phosphate into organelles, have to be identified and characterized in future.

Lipid dynamic

Environmental stress can cause lipid accumulation in many phytoplanktonic species. This is also true for *P. tricornutum* where P-limitation triggers an increase of the lipid content (Abida et al. 2015; Alipanah et al. 2018; Cruz de Carvalho et al. 2016; Gong et al. 2013; Huang et al. 2019; Yang et al. 2014). Transcriptomic data of *P. tricornutum* cells under P stress provided information on the dynamics of this process. According to Yang et al. (2014), triacylglycerol (TAG) accumulation was proposed to be partially attributed to *de novo* synthesis under P stress. This might be observed in a slight upregulation of the transcription of two genes encoding for ACCases (Acetyl-CoA carboxylase) (putative cytosolic Phatrdraft_55209 and for a putative chloroplast Phatrdraft_54926) that are responsible for the synthesis of malonyl-CoA, a key intermediate in fatty acid biosynthesis. Moreover, the upregulation of the transcription of the genes encoding pyruvate-dehydrogenase precursor (Phatrdraft_55035, 20183 alpha, and beta subunits, respectively) and ketoacyl-CoA

synthase (Phatrdraft_26714), might increase the enzymatic capacities to promote the reservoir for fatty acids and further steps of fatty acid biosynthesis (Yang et al. 2014).

Again, the experimental settings have a huge impact as it was observed in exponential-phase P-starved *P. tricornutum* cells, in which the above-mentioned genes encoding enzymes that promote *de novo* fatty acid biosynthesis, were not significantly differentially expressed and in some cases even downregulated (Alipanah et al. 2018).

In any case, it is not clear whether the P-induced lipid accumulation is totally supported by a *de novo* process. This might be seen in the lipid metabolism under P_i- deprivation that can be channeled in an alternative way. A hint into that is the decrease of the PL content under P-deprivation and the parallel increase of betaine class lipids (Abida et al. 2015; Cañavate et al. 2017a,b; Gong et al. 2013; Huang et al. 2019) such as diaglyceryl-hydroxymethyl-*N,N,N*-trimethyl-β-alanine (DGTA), and digalactosyldilacyl-glycerol (DGDG) (Abida et al. 2015; Huang et al. 2019). The substitution of phospho-lipids to betaine class lipids under P-stress is accompanied by the transcriptional upregulation of phospholipase type-C and D (PLC and PLD respectively) (Alipanah et al. 2018; Cruz de Carvalho et al. 2016; Yang et al. 2014), and therefore coherent with the PL recycling process. PLs recycling might directly feed the TAG metabolic pathway and the gene encoding the enzyme phospholipid-diaglycerol-acyltransferase (PDAT), converting diaglycerol (DAG) to TAG by a transesterification reaction, is transcriptionally upregulated under P-starvation (Alipanah et al. 2018; Cruz de Carvalho et al. 2016). PDAT can utilize acyl groups coming from phospholipase-catalyzed PLs to generate TAG, suggesting that the accumulation is directly connected to the membrane PLs recycling process TAG accumulation correlates with the presence of lipid bodies that were consistently observed during P-stress (Abida et al. 2015; Alipanah et al. 2018; Cruz de Carvalho et al. 2016; Yang et al. 2014).

Phosphate storage

Several organisms store phosphate in the form of polyphosphate (polyP) in their vacuole (Lin et al. 2016). However, no direct evidence for a P_i-storage function of the vacuole have been found for *P. tricornutum*, but a predicted vacuolar phosphate transporter (Phatrdraft_19586) might point to that (Alipanah et al. 2018). For storing P_i in vacuoles, other organisms such as yeast, express a set of proteins named vacuolar transporter chaperones (VTCs) and *P. tricornutum* encodes homologs to the subunits Vtc1 to Vtc4 (Phatrdraft_48811, 35739, 48537, 50019). Moreover, Vtc2 (Phatrdraft_35739) was shown to be a tonoplast located protein in *P. tricornutum* (Schreiber et al. 2017). Three of the VTC-encoding genes are differentially expressed after two days under P-starvation in the early stationary phase (Phatrdraft_48811 slightly upregulated and Phatrdraft_35738 together with Phatrdraft_50019 slightly downregulated). Downregulation, especially for the transcript of Phatrdraft_50019, which is supposed to encode for the polymerization core of the putative VTC complex, is in line with the hypothesis that the VTC-complex might be involved in polyP accumulation (Yang et al. 2014). A slight downregulation of the transcription of the same gene was found after two days under P-deprivation in the exponential phase as well (Alipanah et al. 2018). However, after four and eight days under P-stress, transcription of Phatrdraft_50019 was strongly upregulated followed by downregulation after P-resupplementation (Cruz de Carvalho et al. 2016), a pattern also identified in *Thalassiosira pseudonana* cultivated under P-starvation (Dyhrman et al. 2012). This expression pattern, especially the upregulation under P-limiting conditions, does, however, not support the hypothesis of a possible polyP accumulation catalyzed by the VTC complex. Thus, further studies are necessary to determine the VTC complex and especially the localization of the subunits. In any case, the available data are not consistent with the hypothesis that the vacuole of *P. tricornutum* acts as a P_i storage compartment.

Some VTC proteins (Phatrdraft_48811, 35739, 50019) show a non-vacuolar localization when expressed as eGFP proteins, being integrated most likely in the endomembrane system or in not specified compartments in case of Phatrdraft_48538 (Dell'Aquila et al. 2020). Despite this, independently to the result described in Alipanah et al. 2018, we identified the afore-mentioned vacuolar transporter Phatrdraft_19586, and localized it in the tonoplast (Dell'Aquila et al. 2020). In addition, Phatrdraft_19586, proposed to have a high similarity with VPT1 that mediates P_i transport into the vacuole in *A. thaliana* (Liu et al. 2016), was shown to be upregulated under P_i limitations (Alipanah et al. 2018; Dell'Aquila et al. 2020).

Regulatory network

In *P. tricornutum*, P-limitation induces cellular responses that are found to be present when cells are growing under limiting conditions in general, in addition to P-specific ones. This is documented by a comparison of the transcriptional responses with respect to P and N starvation (Alipanah et al. 2018). Under both conditions, central carbon and amino

acid metabolism are similarly affected thereby indicating general responses to these limiting conditions. The specific P-starvation response, such as upregulation of phosphatases or phosphate transporters, must be tightly controlled and generally, eukaryotes regulate transcription factors (TFs) essential for the expression of genes encoding factors involved in the phosphate starvation response (PSR) (Rubio et al. 2001). This is true for *P. tricornutum* as well. The first hint into that are the results of Cruz de Carvalho and coworkers (Cruz de Carvalho et al. 2016) that demonstrated that 62.5% of the annotated TFs were differentially expressed under P limiting conditions, and 32% of the upregulated ones are members of the heat shock factor family (HSF). Interestingly, after P-supplementation, the abundance of HSFs in correlation to another group of TFs, the Myeloblastosis (MYB) family, changes in a way that the MYB TFs are more abundant. Two putative HSF TFs were predicted to interact with upstream/downstream regions of several protein-coding genes, including such for signaling/sensing and TFs functions. In addition, an MYB TF (Phatrdratf_47256), identified in the most 50 upregulated genes after 48 and 72 h of P-starvation (see S1 dataset, supplementary material from Alipanah et al. 2018), is upregulated in early and late P starvation and slightly also in P-supplemented conditions (see Table S6, supplementary material from Cruz de Carvalho et al. 2016). The function of this TF was studied by analyzing knockout lines (Sharma et al. 2019). In this work it was demonstrated that the *ptPsr* knockout lines showed reduced induction of P-stress genes, reduced PL degradation and almost total reduction of alkaline phosphatase activity. Furthermore, the authors identified the binding site of this factor (5′-YGAATCTH-3′), present in the promoter regions of at least 84 P-responsive genes in different quantities and orientations.

Other non-protein players might be involved in the P stress response in *P. tricornutum* and the transcriptome analysis by Cruz de Carvalho et al. (2016) suggested a possible role of micro-RNAs (miRNAs) and long intergenic non-coding RNAs (lincRNAs). First, two miRNAs precursors were significantly upregulated in P-stress (Table S3, supplementary material Cruz de Carvalho et al. 2016) together with 202 putative lincRNAs (Table S4, supplementary material Cruz de Carvalho et al. 2016) that resulted additionally in downregulation after P-supplementation. Subsequently, several lincRNAs were predicted to interact with two HSFs which were P-stress-specific expressed and only two of them were detected in an N-starvation study (Levitan et al. 2015). If these lincRNAs are involved in the regulation of up- or downstream located genes needs to be investigated. Taken together, obtained data on the regulation mechanisms already indicated the sophisticated complex regulatory network that controls the P-stress response. However, future studies are necessary to reveal further aspects of the P-related regulatory network.

A Ca^{2+}-dependent signaling pathway might play an essential role in P-homeostasis as well. This pathway was

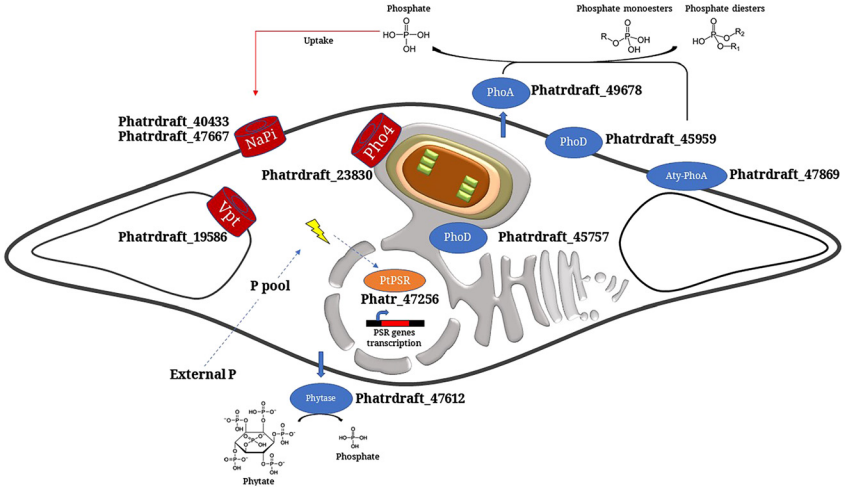

Figure 1: A model on the cellular strategy of *P. tricornutum* to overcome P-limitation. The P concentration in the medium is sensed and in case of limitations an unknown signal activates, through an uncharacterized cascade (blue dotted line), the transcription factor *PtPSR* which leads to the PtPSR-catalyzed transcriptional regulation of several phosphate starvation (PSR) genes (Sharma et al. 2019). According to expression data (Alipanah et al. 2018; Cruz de Carvalho et al. 2016; Yang et al. 2014) and localization studies (Dell'Aquila et al. 2020), we depict a possible model that summarizes P-limitation adaptations: alkaline phosphatases (blue circles, Phatrdraft_49678, 45959, 47869) act extracellularly (Buhmann et al. 2016; Erdene-Ochir et al. 2019; Lin et al. 2013) (blue arrows indicate secretion to the extracellular environment) and possibly hydrolyze phosphate mono and -diesters. Phatrdraft_47612 (blue box) might attack phytate molecules. Extracellular phosphorus mobilization generates phosphates readily accessible to plasma membrane-localized NaP_i cotransporters (Dell'Aquila et al. 2020). Phatrdraft_45757 (blue circles) has been localized in the ER and cER. Its activity might be correlated with P-starvation lipid recycling as well as a putative phosphate transporter found in the cER (Phatrdraft_23830). The putative vacuolar P_i-transporter (VPT, Phatrdraft_19586) might mediate phosphate storage/reallocation possibly *via* an SPX domain. Notice that the respective protein roles and functions for each reaction are not experimentally demonstrated, likewise the functionality of P_i-transporters.

shown to be active when the cells where resupplied with 36 µM P, after four days in P-limitation (1.8 µM), indicating a possible sensing of external P concentrations (Helliwell et al. 2020).

Conclusion

We have summarized major findings related to the understanding of the phosphorus stress response in the diatom *P. tricornutum*. By combining expression data, knowledge on the gene regulation of major players could be gained, being combined in parallel with recently published localization data into a model (Figure 1).

The studies conducted in the last decade have consequently opened up many directions for future research. There are still many aspects, which should be addressed in further studies. Examples for this are functional studies concerning to factors involved in P-scavenging, where different phosphatases might have diverse specificity for various substrates or in regulating the P-metabolism, as well as the molecular search for a possible P_i-storage compartment in *P. tricornutum*.

Acknowledgments: We would like to thank Dr. Daniel Moog (Philipps University Marburg) and Mariano Santoro (Leibniz Institute for Baltic Sea Research, Warnemünde) for useful comments on the manuscript. Funding was provided by the Deutsche Forschungsgemeinschaft (DFG) through the framework of the DFG Collaborative Research Centre SFB 987.

Author contribution: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: This study was supported by Deutsche Forschungsgemeinschaft (DFG).

Conflict of interest statement: The authors declare no conflicts of interest regarding this article.

References

Abida, H., Dolch, L.-J., Meï, C., Villanova, V., Conte, M., Block, M.A., Finazzi, G., Bastien, O., Tirichine, L., and Bowler, C. (2015). Membrane glycerolipid remodeling triggered by nitrogen and phosphorus starvation in *Phaeodactylum tricornutum*. Plant Physiol. 167: 118–136.

Alipanah, L., Winge, P., Rohloff, J., Najafi, J., Brembu, T., and Bones, A.M. (2018). Molecular adaptations to phosphorus deprivation and comparison with nitrogen deprivation responses in the diatom *Phaeodactylum tricornutum*. PloS One 13: e0193335.

Benitez-Nelson, C.R. (2000). The biogeochemical cycling of phosphorus in marine systems. Earth Sci. Rev. 51: 109–135.

Bowler, C., Allen, A.E., Badger, J.H., Grimwood, J., Jabbari, K., Kuo, A., Maheswari, U., Martens, C., Maumus, F., and Otillar, R.P. (2008). The Phaeodactylum genome reveals the evolutionary history of diatom genomes. Nature 456: 239.

Buhmann, M.T., Schulze, B., Förderer, A., Schleheck, D., and Kroth, P.G. (2016). Bacteria may induce the secretion of mucin-like proteins by the diatom *Phaeodactylum tricornutum*. J. Phycol. 52: 463–474.

Cáceres, C., Spatharis, S., Kaiserli, E., Smeti, E., Flowers, H., and Bonachela, J.A. (2019). Temporal phosphate gradients reveal diverse acclimation responses in phytoplankton phosphate uptake. ISME J. 13: 2834–2845.

Cañavate, J.P., Armada, I., and Hachero-Cruzado, I. (2017a). Aspects of phosphorus physiology associated with phosphate-induced polar lipid remodelling in marine microalgae. J. Plant Physiol. 214: 28–38.

Cañavate, J.P., Armada, I., and Hachero-Cruzado, I. (2017b). Interspecific variability in phosphorus-induced lipid remodelling among marine eukaryotic phytoplankton. New Phytol. 213: 700–713.

Cembella, A.D., Antia, N.J., and Harrison, P.J. (1982). The utilization of inorganic and organic phosphorous compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: Part I. CRC Crit. Rev. Microbiol. 10: 317–391.

Cruz de Carvalho, M.H., Sun, H.-X., Bowler, C., and Chua, N.-H. (2016). Noncoding and coding transcriptome responses of a marine diatom to phosphate fluctuations. New Phytol. 210: 497–510.

Dell'Aquila, G., Zauner, S., Heimerl, T., Kahnt, J., Samel-Gondesen, V., Runge, S., Hempel, F., and Maier, U.G. (2020). Mobilization and cellular distribution of phosphate in the diatom *Phaeodactylum tricornutum*. Front. Plant Sci. 11: 579.

Dyhrman, S.T., Jenkins, B.D., Rynearson, T.A., Saito, M.A., Mercier, M.L., Alexander, H., Whitney, L.P., Drzewianowski, A., Bulygin, V.V., and Bertrand, E.M. (2012). The transcriptome and proteome of the diatom *Thalassiosira pseudonana* reveal a diverse phosphorus stress response. PloS One 7: e33768.

Erdene-Ochir, E., Shin, B.-K., Kwon, B., Jung, C., and Pan, C.-H. (2019). Identification and characterisation of the novel endogenous promoter HASP1 and its signal peptide from *Phaeodactylum tricornutum*. Sci. Rep. 9: 9941.

Falciatore, A., d'Alcalà, M.R., Croot, P., and Bowler, C. (2000). Perception of environmental signals by a marine diatom. Science 288: 2363–2366.

Feng, T.-Y., Yang, Z.-K., Zheng, J.-W., Xie, Y., Li, D.-W., Murugan, S.B., Yang, W.-D., Liu, J.-S., and Li, H.-Y. (2015). Examination of metabolic responses to phosphorus limitation via proteomic analyses in the marine diatom *Phaeodactylum tricornutum*. Sci. Rep. 5: 10373.

Flynn, K.J., Öpik, H., and Syrett, P.J. (1986). Localization of the alkaline phosphatase and 5′-nucleotidase activities of the diatom *Phaeodactylum tricornutum*. Microbiology 132: 289–298.

Gong, Y., Guo, X., Wan, X., Liang, Z., and Jiang, M. (2013). Triacylglycerol accumulation and change in fatty acid content of four marine oleaginous microalgae under nutrient limitation and at different culture ages. J. Basic Microbiol. 53: 29–36.

Helliwell, K.E., Harrison, E., Christie-Oleza, J., Rees, A., Downe, J., Aguilo-Ferretjans, M., Al-Moosawi, L., Brownlee, C., and Wheeler, G. (2020). A novel Ca^{2+} signalling pathway co-ordinates environmental phosphorus sensing and nitrogen metabolism in marine diatoms. bioRxiv.

Huang, B., Marchand, J., Blanckaert, V., Lukomska, E., Ulmann, L., Wielgosz-Collin, G., Rabesaotra, V., Moreau, B., Bougaran, G., and Mimouni, V. (2019). Nitrogen and phosphorus limitations induce carbon partitioning and membrane lipid remodelling in the marine diatom *Phaeodactylum tricornutum*. Eur. J. Phycol. 54: 342–358.

Hunter, T. (2012). Why nature chose phosphate to modify proteins. Phil. Trans. Biol. Sci. 367: 2513–2516.

Hürlimann, H.C., Pinson, B., Stadler-Waibel, M., Zeeman, S.C., and Freimoser, F.M. (2009). The SPX domain of the yeast low-affinity phosphate transporter Pho90 regulates transport activity. EMBO Rep. 10: 1003–1008.

Levitan, O., Dinamarca, J., Zelzion, E., Lun, D.S., Guerra, L.T., Kim, M.K., Kim, J., van Mooy, B.A.S., Bhattacharya, D., and Falkowski, P.G. (2015). Remodeling of intermediate metabolism in the diatom *Phaeodactylum tricornutum* under nitrogen stress. Proc. Natl. Acad. Sci. U.S.A. 112: 412–417.

Lin, H.-Y., Shih, C.-Y., Liu, H.-C., Chang, J., Chen, Y.-L., Chen, Y.-R., Lin, H.-T., Chang, Y.-Y., Hsu, C.-H., and Lin, H.-J. (2013). Identification and characterization of an extracellular alkaline phosphatase in the marine diatom *Phaeodactylum tricornutum*. Mar. Biotechnol. 15: 425–436.

Lin, S., Litaker, R.W., and Sunda, W.G. (2016). Phosphorus physiological ecology and molecular mechanisms in marine phytoplankton. J. Phycol. 52: 10–36.

Liu, T.-Y., Huang, T.-K., Yang, S.-Y., Hong, Y.-T., Huang, S.-M., Wang, F.-N., Chiang, S.-F., Tsai, S.-Y., Lu, W.-C., and Chiou, T.-J. (2016). Identification of plant vacuolar transporters mediating phosphate storage. Nat. Commun. 7: 11095.

Paytan, A., and McLaughlin, K. (2007). The oceanic phosphorus cycle. Chem. Rev. 107: 563–576.

Rubio, V., Linhares, F., Solano, R., Martín, A.C., Iglesias, J., Leyva, A., and Paz-Ares, J. (2001). A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Gene Dev. 15: 2122–2133.

Schreiber, V., Dersch, J., Puzik, K., Bäcker, O., Liu, X., Stork, S., Schulz, J., Heimerl, T., Klingl, A., and Zauner, S. (2017). The central vacuole of the diatom *Phaeodactylum tricornutum*: identification of new vacuolar membrane proteins and of a functional di-leucine-based targeting motif. Protist 168: 271–282.

Sharma, A.K., Mühlroth, A., Jouhet, J., Maréchal, E., Alipanah, L., Kissen, R., Brembu, T., Bones, A.M., and Winge, P. (2019). The Myb-like transcription factor Phosphorus Starvation Response (PtPSR) controls conditional P acquisition and remodeling in marine microalgae. New Phytol 225: 2380–2395.

Westheimer, F. H. (1987). Why nature chose phosphates. Science. 235: 1173–1178.

Yang, Z.-K., Zheng, J.-W., Niu, Y.-F., Yang, W.-D., Liu, J.-S., and Li, H.-Y. (2014). Systems-level analysis of the metabolic responses of the diatom *Phaeodactylum tricornutum* to phosphorus stress. Environ. Microbiol. 16: 1793–1807.