MYB-like transcription factor NoPSR1 is crucial for membrane lipid remodeling under phosphate starvation in the oleaginous microalga Nannochloropsis oceanica

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Phosphate (Pi) is an essential nutrient for all living organisms. Pi availability in natural environments is thus tightly associated with their growth and reproduction potential. To cope with Pi-limiting conditions, plants and algae have evolved a variety of physiological responses, which are coordinated by a systematic Pi-sensing system and downstream signal transduction pathways [1,2]. One of the representative responses induced under Pi starvation is a compositional change in cellular membrane lipids: The bulk of constituent membrane glycerophospholipids is reduced, whereas nonphosphorus lipid species, for example, glycolipids and betaine lipids, are increased compensatorily [3–5]. It is considered that the head group of the membrane-derived phospholipids is then metabolized and used as a source of free phosphorus. Within the plastid membranes, a portion of phosphatidylglycerol (PG), which is a major phospholipid inside the plastid that typically accounts for ~10% of the total thylakoid lipid, is replaced by the sulfolipid sulfoquinovosyldiacylglycerol (SQDG) under Pi-starved conditions. Although the adaptive role of SQDG upon Pi starvation is highly conserved from cyanobacteria to higher plants [6,7], the mode of membrane lipid remodeling at extraplastidic compartments appears to have changed over the course of plant evolution. In the case of seed plants, the galactolipid digalactosyldiacylglycerol (DGDG) replaces the major phospholipid phosphatidylcholine (PC), whereas in most microalgae, betaine lipids take part in this event instead [4,5,8]. Characterization of

Abbreviations
BTA1L, betaine lipid synthase 1L; DGTS, diacylglyceryl-N,N,N-trimethylhomoserine; PHR1, phosphate starvation response regulator 1; Pi, inorganic phosphate; PSR1, phosphorus starvation response 1; SQD2, sulfoquinovosyldiacylglycerol 2; SQDG, sulfoquinovosyldiacylglycerol.
lipid-metabolizing enzymes, such as galactolipid/beta-taine lipid synthases and phospholipases, and transcriptome data upon Pi starvation has indicated which metabolic pathways are responsible for the dramatic shift in membrane lipids under Pi starvation [9–14]. There has also been some progress in understanding the regulatory mechanisms involved in the membrane lipid remodeling during Pi starvation. In *Arabidopsis thaliana*, the MYB transcription factor PHOSPHORUS STARVATION RESPONSE REGULATOR 1 (AtPHR1) and its closely related homolog PHR1-Like 1 have been identified as global regulators of the Pi starvation response [15,16]. Functional homologs of AtPHR1 have been found in several species of land plants, including *Oryza sativa*, *Phaseolus vulgaris*, *Brassica napus*, *Triticum aestivum*, and *Glycine max* [17–21]. By characterizing Arabidopsis *phr1* mutants, induction of a series of galactolipid and sulfolipid synthase genes upon Pi limitation was shown to be partially dependent on AtPHR1 [9,22]. In addition, auxin signaling mediated by IAA14 and ARF7/19 is required for full induction of the membrane lipid remodeling in Pi-starved Arabidopsis [23,24]. In the case of the green alga *Chlamydomonas reinhardtii*, PHOSPHORUS STARVATION RESPONSE 1 (CrPSR1), a MYB transcription factor homologous to AtPHR1, is a central regulator of the Pi starvation response [25–28]. Whereas Pi starvation-induced lipid remodeling at the extraplasmatic membrane is likely to be absent in *C. reinhardtii* because it lacks the biosynthetic pathway for PC [29], the genes for plastidic membrane remodeling are controlled by CrPSR1 based on transcriptomic data from *psr1* [28].

Previously, we investigated the membrane lipid composition of Pi-starved *Nannochloropsis oceanica* [14,30]. *N. oceanica* is a marine microalga that has a high potential to accumulate oil and thus is considered to be a promising strain for biofuel production [31,32]. Because oil production is typically induced under conditions of abiotic stress, including nitrogen and Pi starvation, recent work has focused on the responses to these stresses to reveal how *Nannochloropsis* produces a large amount of lipid and adapts to these adverse conditions [13,30,33–36]. We previously observed a substantial increase in the amount of the sulfolipid SQDG and the betaine lipid diacylglycerol-N,N,N-trimethylhomoserine (DGTS) and a corresponding decrease in the amount of phospholipids in Pi-starved *N. oceanica* [14,30]. Consistent with the accumulation of SQDG and DGTS, the expression of genes encoding SQDG and DGTS synthases was also stimulated under Pi starvation, suggesting that the membrane lipid remodeling process is likely to be controlled at the level of transcription. Here, we identified a MYB-like transcription factor responsible for regulating membrane lipid homeostasis during Pi-starved conditions in *N. oceanica*. Our results provide insight into the regulatory mechanism of the Pi starvation response in *N. oceanica*.

**Materials and methods**

**N. oceanica strains and culture conditions**

*Nannochloropsis oceanica* strain NIES-2145 obtained from the National Institute for Environmental Studies was routinely cultured in a 50 mL volume at 25 °C with continuous bubbling of 2% CO₂ and constant light (40 µmol photons·m⁻²·s⁻¹) in F2N medium [37]. Before each experiment, cells were precultured for 3 days from an initial density of 10⁵ cells·mL⁻¹. Cell density was measured by an optical microscope with a bacteria counter. To induce nitrogen or Pi starvation, cells were washed twice with nitrogen- or phosphorus-free F2N medium, respectively, as described [30].

**Identification of R1-type MYB genes in N. oceanica strain NIES-2145**

Three R1-type MYB-like genes were identified from transcript data of *N. oceanica* CCMP1779 v1.0 [38] by tblastn with AtPHR1 and CrPSR1 as queries. We remodeled these gene structures (transcript ID: 7158, 4932, and 6410) based on genome mapping and de novo assembly of previous RNA-Seq data from *N. oceanica* NIES-2145 [34]. The RNA-Seq data were mapped to assembled scaffolds of *N. oceanica* CCMP1779 v1.0 with SAT2 [39]. De novo assembly was performed with Trinity [40]. We named these R1-type MYB-like genes NoPSR1, NoPSL1, and NoPSL2 (DDBJ accession number, LC532100 for NoPSR1, LC532101 for NoPSL1, and LC532102 for NoPSL2).

**Reverse transcription–quantitative PCR**

Total RNA was extracted from each 1- to 1.5-mL culture using TRI Reagent (Sigma-Aldrich, St. Louis, MO, USA). First-strand cDNA was synthesized from the obtained total RNA (~ 500 ng) using Superscript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA). For quantitative PCR, cDNA was amplified using SYBR Premix Ex Taq II (Takara, Kusatsu, Japan) with each gene-specific primer set (Table S1), and the signal was detected by the Thermal Cycler Dice Real Time System (Takara). The gene encoding NADH dehydrogenase subunit 11 was used for normalization.

**Plasmid construction and nuclear transformation**

General strategies for producing gene knockout or overexpression lines are described in Ref. [14,34]. Briefly, to make
gene knockout constructs, an ~1-kb region consisting of the 5' and 3' flanking genomic regions of the relevant gene was amplified by PCR using *N. oceanica* genomic DNA as a template and was cloned into the *PstI* and *KpnI* sites of the vector pUC18/NT7. To make the gene overexpression line, the promoter region of *VCP1* (~1 kb) with a *NotI* site was cloned between *Sh ble* and *terVCP1* of pUC18/NT7 by inverse PCR. The *NoPSR1* coding sequence (1563 bp) was amplified by PCR using a cDNA template. Then, the coding sequence was cloned into the *NotI* site (ProVCP2:sh ble:ProVCP1:NoPSR1:terVCP1). Information about primers used for plasmid construction is provided in Table S1. After genetic transformation, experiments were carried out as described [34].

**Lipid analyses**

Membrane lipid composition was determined as described [14]. Briefly, total cellular lipids were extracted as described [41] and then developed by two-dimensional thin-layer chromatography (TLC). For the first dimension, chloroform/methanol/28% ammonia water (120 : 80 : 8, v/v/v) was used as the solvent system, whereas chloroform/methanol/acetic acid/water (170 : 30 : 15 : 3, v/v/v/v) was used as the solvent system for the second dimension. To quantify the amount of triacylglycerol (TAG), total lipids were separated by hexane/diethyl ether/acetic acid (160 : 40 : 4, v/v/v). Each lipid class was isolated and quantified by gas chromatography (GC) with a flame ionization detector (GC-2030; Shimadzu, Kyoto, Japan).

**Results**

**Identification of three *Nannochloropsis* R1-type MYB-like transcription factors that are weakly similar to *AtPHR1* and *CrPSR1***

We hypothesized that a homolog (or homologs) of *AtPHR1* and/or *CrPSR1* in *N. oceanica* strain NIES-2145 may be involved in regulation of lipid compositional changes under Pi starvation. To isolate such a protein, we first searched for transcript sequences that show similarity to *AtPHR1* or *CrPSR1* among published nuclear genome data from *N. oceanica* CCMP1779 v1.0 [38]. Three transcript sequences (transcript ID: 7158, 4932, and 6410) were obtained by tblastn search no matter which coding sequence was used as the query, either *AtPHR1* or *CrPSR1*. We then deduced these gene structures based on our previously reported RNA-Seq data from *N. oceanica* NIES-2145 [34]. As a result, we obtained three R1-type MYB-like protein sequences (Fig. 1A), which we named NoPSR1 (DDBJ accession number, LC532100), NoPSR1-like 1 (NoPSL1; LC532101), and NoPSL2 (LC532102), based on the results from the following experiments. Although NoPSR1 and NoPSL1 and NoPSL2 showed only 12–25% similarity to *AtPHR1* or *CrPSR1* orPtPSR (from *Phaeodactylum tricornutum*) across their amino acid sequences, they shared a highly conserved motif, SHLQKYR, in their MYB-like domain (Fig. 1B,C). The SH-A/L-QKY-R/F motif (NCBI accession, TIGR01557) is found in several plant transcription factors, such as circadian clock components AtLHY and AtCCA1 and a regulator of floral meristem homeostasis, AtUFI1 [42,43]. The coiled-coil (CC) domain is also found in downstream of the MYB domain in *AtPHR1* and *CrPSR1* and PtPSR, which identifies them as members of the MYB-CC family [44] (Fig. 1A). Similar CC domain was predicted in NoPSR1 and NoPSL1, albeit with relatively weak expected values (Fig. S1). PtPSR and its homologs in *P. tricornutum* are known to have other conserved domains (CDs). We also investigated whether these CDs are present in the *Nannochloropsis* proteins, and found that they had weakly related regions (Fig. S2).

**Transcriptional changes in NoPSR1, NoPSL1, and NoPSL2 and membrane lipid synthases involved in the membrane lipid remodeling upon early Pi-starved conditions**

Upon Pi starvation, transcription of *AtPHR1* is only moderately induced [15], whereas that of *CrPSR1* is rapidly and strongly induced and culminates at 8 h after the cells are transferred to Pi-starved conditions [26]. To examine the transcriptional response of *NoPSR1*, *NoPSL1*, and *NoPSL2* themselves to Pi starvation, we cultured wild-type (WT) *Nannochloropsis* cells in Pi-replete or Pi-depleted medium and harvested them at 0, 3, 6, 12, and 24 h after inoculation. The mRNA levels of *NoPSR1*, *NoPSL1*, and *NoPSL2* as measured by reverse transcription–quantitative PCR (RT-qPCR) were not increased under Pi-starved conditions relative to the onset of culture (0 h) but were rather stable under both Pi-controlled conditions (Fig. 2A–C). We also assessed expression of *betaine lipid synthase 1L* (*BTACL*) and *sulfoquinovosyldiacylglycerol 2* (*SQD2*), both of which are known to be induced by Pi deprivation in *N. oceanica* [14,30]. *BTACL* is an enzyme responsible for the two catalytic steps of DGTS biosynthesis [14], whereas *SQD2* is the enzyme that mediates the final step of SQDG biosynthesis [30]. In contrast to *NoPSR1*, expression of *BTACL* and *SQD2* increased up to ~2- and 4-fold, respectively, after 12 h of Pi deficiency as compared with the value at time 0 h, although their mRNA
levels did not change significantly under Pi-sufficient conditions (Fig. 2D,E). Therefore, although NoPSR1 and NoPSL1 and NoPSL2 are not subjected to transcriptional regulation during early Pi-sufficient or Pi-deficient conditions, lipid compositional remodeling at extraplastidic and plastidic compartments is triggered by 12 h after the onset of Pi deficiency.

**Mutation of NoPSR1 impairs Pi starvation-induced membrane lipid remodeling**

To determine the molecular function of these proteins, gene knockout mutants of NoPSR1 and NoPSL1 were separately isolated by homologous recombination (Fig. 3A,B). We isolated two individual knockout lines for NoPSR1 and NoPSL1 (#1, #2) but could not obtain a mutant for NoPSL2 at this time. These mutants (nopsl1, nopsl2) were subjected to Pi-replete and Pi-depleted culture conditions to examine whether the impairment of either of their function has relevance to membrane lipid homeostasis. Whole cellular membrane lipids extracted from cells cultured for 3 days were analyzed using two-dimensional TLC followed by GC. Under Pi-replete conditions, these mutants showed no significant differences as compared with WT for any major membrane lipid species (Fig. 3C). Under Pi-depleted conditions, WT and nopsl1 mutants showed a substantial increase in DGTs and SQDG of ~184% and 69%, respectively, and a concomitant decrease in phospholipids [PC, PG, and phosphatidylethanolamine (PE)] as compared with Pi-sufficient conditions. In contrast, nopsl1 mutants did
not show such a dynamic alteration (Fig. 3C): The proportion of DGTS and SQDG was almost equivalent to the value under Pi-sufficient conditions, and the reduction in PC and PG by Pi starvation was less obvious. It should be noted that the relative amount of PE was similarly reduced by Pi starvation in noprsl as in WT and noprsl (Fig. 3C). Thus, NoPSR1 plays a crucial role in inducing dynamic membrane lipid remodeling during Pi-starved conditions, but not in membrane lipid homeostasis under Pi-sufficient conditions. Because no obvious influence was observed in membrane lipid composition of noprsl, this mutant was not analyzed further at this time.

Next, we performed RT-qPCR analysis in the mutants cultured under Pi-replete or Pi-depleted conditions to reveal whether the lack of the membrane lipid alteration in noprsl was accompanied by misinduction of respective lipid synthase genes. The expression of BTA1L and SQD2 increased ~2- and 4-fold, respectively, by Pi starvation in WT as indicated in Fig. 2; however, these increases were totally abolished in noprsl (Fig. 3D,E). We also checked transcriptional changes in the putative homolog of PHT1 family (PHT1a, DDBJ accession number, LC532103) as a reference gene. PHT1 family encodes high-affinity Pi transporters whose expression level was generally induced under Pi-starved growth in A. thaliana and the moss Physcomitrella patens [45,46]. PHT1a was also significantly upregulated under Pi-starved conditions in WT, but not in noprsl (Fig. 3F). These results suggested that NoPSR1 is required for inducing membrane lipid synthase genes (BTA1L and SQD2) and the consequent membrane lipid remodeling under Pi starvation, and also for other aspects of the Pi starvation response.

To further characterize the influence of NoPSR1 disruption, a growth assay and storage lipid analysis were carried out for noprsl cultured under nutrient-sufficient (+Pi/+N), nitrogen-starved (+Pi/~N), or Pi-starved (~Pi/+N) conditions. noprsl cells proliferated similarly as compared with the empty vector control strain (EV) under these conditions (Fig. S3A–C). Also, there were almost no major differences in the accumulation of the storage lipid triacylglycerol (TAG) between noprsl and EV after 2 and 4 days in culture under the same conditions, except for cells cultured for 4 days under +Pi/+N, for which noprsl showed a lower level than EV (Fig. S3D,E). We further compared the acyl composition of TAG in 2-day cultures of EV and noprsl and observed no large differences between them other than a slight increase in the proportion of 16 : 1 (number of carbons:number of double bonds) in Pi-starved noprsl (Fig. S3F). Thus, NoPSR1 does not play a major role in the regulation of oil synthesis under N starvation or Pi starvation, and the absence of membrane lipid remodeling in noprsl has no apparent effect on cell proliferation, at least during the early stage (~ 4 days) of Pi starvation.

![Regulation of lipid remodeling under P starvation](image-url)
Regulation of lipid remodeling under P starvation

Fig. 3. Generation of knockout mutants of NoPSR1 and NoPSL1 and their membrane lipid composition and gene expression under Pi-sufficient and Pi-deficient conditions. (A) Schematic diagram of gene knockout by homologous recombination method. Coding region (exons were indicated as black boxes) was replaced by Zeocin resistance cassette (for NoPSR1) or hygromycin resistance cassette (for NoPSL1). (B) Confirmation for the gene knockout by genomic PCR and RT-PCR. Two independent lines (#1, #2) were tested. Alphabets indicated the right side of each panel was primers used for the genomic PCR whose location was described in (A). For RT-PCR, the gene encoding NADH dehydrogenase subunit 11 was used as a control. gDNA, genomic DNA. (C) Membrane lipid composition of WT, nopsr1, and nopsl1 cultivated for 3 days under Pi-sufficient (+Pi) or Pi-deficient (–Pi) medium is indicated as the mole percentage of the eight major polar lipids. Two independently isolated lines (#1, #2) for nopsr1 and nopsl1 were analyzed. (D–F) Expression of BTA1L (D), SQD2 (E), and PHT1a (F) mRNA in the same cultures of WT and nopsr1 as in (C). Data represent the mean ± SD from three biologically independent samples. Statistical significance was determined with a one-tailed Student’s t-test by comparing the values for +Pi and −Pi culture for each strain. **P < 0.01. MGDG, monogalactosyldiacylglycerol; PI, phosphatidylinositol.
Overexpressing NoPSR1 during Pi-replete conditions is not enough to induce the membrane lipid remodeling

Next, we tested whether NoPSR1 overexpression under Pi-sufficient conditions can induce the membrane lipid remodeling. The promoter of violaxanthin/chlorophyll a-binding protein 1 (VCP1) was used to enhance NoPSR1 expression, as this promoter strongly induces the expression of its downstream gene, especially during optimal culture conditions [14,37]. We isolated two overexpression strains (NoPSR1-OX #1 and NoPSR1-OX #2), which had ~27- and ~16-fold higher expression, respectively, of NoPSR1 than WT (Fig. 4A). These NoPSR1-OX lines were cultured in Pi-sufficient medium for 3 days and were then subjected to RT-qPCR and membrane lipid analysis. The NoPSR1-OX lines showed no statistically apparent changes in BTA1L expression, although they had a slight increase in SQD2 expression (Fig. 4B,C). Moreover, membrane lipid analysis revealed that despite the absence of BTA1L upregulation, NoPSR1-OX #1, which expresses higher levels of NoPSR1, exhibited a proportion of DGTS that was slightly but significantly higher than that of WT (Fig. 4D). NoPSR1-OX #1 also showed compositional changes reminiscent of the membrane lipid remodeling under Pi starvation (an increase in SQDG and a decrease in PC and PG), but these changes were not significant based on Dunnett’s test (Fig. 4D). In contrast, NoPSR1-OX #2, which overexpresses NoPSR1 at a lower level than NoPSR1-OX #1, showed no significant change in the proportion of each lipid class, except for a 12% decrease in MGDG (Fig. 4D). Altogether, enhancing NoPSR1 expression up to 27-fold under Pi-replete conditions was not sufficient to evoke the drastic membrane lipid remodeling process as seen under Pi-starved conditions, suggesting another layer of regulation during this event.

Discussion

In N. oceanica, we found MYB-like transcription factors related to AtPHR1 and CrPSR1, which are well-characterized global regulators of the Pi starvation response in the plant and green alga, respectively. Although NoPSR1 shared few similarities with AtPHR1 and CrPSR1 at the amino acid level except for the highly conserved motif in the MYB domain (Fig. 1B,C), phenotypic analyses in NoPSR1 knockout mutants demonstrated that NoPSR1 was necessary for inducing the dynamic membrane lipid remodeling that occurs during Pi starvation (Fig. 3C–F). At this time, whether NoPSR1 acts as a master regulator in the Pi starvation response, as do AtPHR1 and CrPSR1, has not been clarified. As upregulation of the Pi transporter gene PHT1a was abolished in Pi-starved nopsr1,
NoPSR1 is likely to regulate an aspect of the Pi starvation response other than the membrane lipid remodeling (Fig. 3F). We note, however, that as disruption in NoPSR1 did not largely affect cell growth or TAG accumulation, at least during the early stage of Pi starvation (Fig. S3C–E), unlike the effects in Arabidopsis

**et al**. Phaeodactylum psr1 [15,28]. NoPSR1 may be involved in some aspects of the Pi starvation response. It is conceivable that NoPSL2 controls other aspects of the response, as we failed to isolate a knockout of its gene. It is also possible that NoPSL1 is also involved in the Pi starvation response through an unknown function. However, as for regulation of membrane lipid metabolism under Pi starvation, NoPSR1 apparently plays a dominant role, as the main parts of the membrane lipid alteration process, especially the increase in DGTS and SQDG and the upregulation of BTA1L and SQD2, were almost totally repressed in Pi-starved nopsr1 (Fig. 3C).

Recently, a counterpart of CrPSR1 in the diatom *P. tricornutum* (PtPSR) was shown to be a key transcription factor regulating Pi scavenging, phospholipid degradation, and cell growth under Pi stress conditions [47]. Previously, Canavate *et al* [48] investigated the effects of P deprivation on cell growth, P-cell content, and lipid composition in a taxonomically diversified group of nine marine microalgae, including *Phaeodactylum* and *Navochloropsis*. According to their study, *Navochloropsis* had higher P storage capacity than *Phaeodactylum*. In addition, growth decline by P depletion was more intense in *Phaeodactylum* compared to other species. These data suggested that *Phaeodactylum* may have a high sensitivity against P starvation than *Navochloropsis*, which could explain the difference in P starvation growth of *ptpsr* and *nopsr1* mutants. Although PtPSR is obviously responsible for eliciting the various PI starvation responses, it is still not known whether the biosynthesis of TAG and nonphosphorus membrane lipids is controlled by PtPSR [47]. In Arabidopsis, a defect in AtPHR1 results in the repressed transcriptional induction of genes encoding glycolipid synthases and phospholipases during Pi starvation, but the effect on lipid content was only partial [9,22]. Thus, we have identified a type of AtPHR1- and CrPSR1-related transcription factor that is deeply involved in membrane lipid remodeling during Pi-starved conditions. It should be noted that the decrease in PE and the increase in DGDG upon Pi limitation were not impaired in *nopsr1* (Fig. 3C), suggesting that these events may not be under the control of NoPSR1.

As Pi starvation-dependent upregulation of *BTA1L* and *SQD2* was blocked by disrupting NoPSR1 function, these events are considered to be regulated by NoPSR1 (Fig. 3D–F). The time course measurement of gene expression revealed that *BTA1L* and *SQD2* were upregulated by 12 h after the onset of Pi starvation, whereas NoPSR1 itself was not subjected to transcriptional regulation at least during early (~ 24 h) Pi-sufficient or Pi-deficient conditions (Fig. 2). These results suggested that the function of NoPSR1 was controlled post-transcriptionally in accordance with Pi availability. The fact that NoPSR1 overexpression under Pi-replete conditions alone did not fully induce membrane lipid remodeling is consistent with this idea (Fig. 4D). In *A. thaliana*, the function of AtPHR1 is repressed under Pi-sufficient conditions by SPX (Syg1/Pho81/XPR1) domain-containing proteins [49]. When plants suffer from Pi starvation, AtPHR1 is released from suppression by the SPX protein and functions to elicit a wide array of responses [49].

We previously succeeded in activating a diacylglycerol acyltransferase gene using the *SQD2* promoter of *C. reinhardtii* (pCrSQD2-CrDGTT4) within Pi-starved *N. oceanica* [30]. This result implies that there is a common regulatory system for activating *SQD2* under Pi-starved conditions among algal species. Moreover, recently Hidayati *et al* [50] reported that the R2R3 MYB transcription factor LIPID REMODELING REGULATOR 1 (LRL1) in *C. reinhardtii* is involved in different mechanisms than CrPSR1, particularly during the later stage of the response to Pi depletion, including plastidic membrane lipid remodeling. According to a transactivation assay using tobacco leaves, those authors demonstrated that LRL1 and CrPSR1 directly activate transcription from the *SQD2*-2 promoter in the presence of their appropriate partners. This implies that counterparts of LRL1 and CrPSR1 are highly conserved in algal lineages as responsible factors for regulation of plastidic membrane lipid remodeling upon Pi starvation. In *N. oceanica*, we have now identified and characterized NoPSR1 as an indispensable player for inducing membrane lipid alteration under Pi starvation. Although disruption of NoPSR1 resulted in a lack of *SQD2* induction upon Pi-starved conditions (Fig. 3E) and, conversely, NoPSR1 overexpression resulted in upregulation of *SQD2* (Fig. 4C), it is still unclear whether...
NoPSR1 directly controls SQD2 transcription. Our present results may facilitate future work to elucidate the core regulatory system of the Pi starvation-induced membrane lipid remodeling, which is prevalent among a wide range of algae and plants.

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Author contributions

HM and HO designed this work. HM and NK performed the experiments. HM, NK, KH, and HO analyzed the data. HM wrote the manuscript with contributions from all authors.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Coiled-coil prediction of NoPSR1, NoPSL1, NoPSL2, PtPSR, CrPSR1 and AtPHR1.

Fig. S2. NoPSR1, NoPSL1, NoPSL2, PtPSR, and related MYB genes of diatoms were aligned using MAFFT (v7.471) with DASH Integration and E-INS-I iterative refinement methods and visualized with the BioEdit v7.2.5.

Fig. S3. Comparison of cell growth and storage lipid amounts and composition in nopsr1 and control cells during nutrient-sufficient (+Pi/+N), nitrogen-deficient (+Pi/−N) and Pi-deficient (−Pi/+N) conditions.

Table S1. Sequence of primers used in this work.