CellPress
OPEN ACCESS

Trends in
Plant Science

Review

Emerging trends in nitrogen and phosphorus signalling in photosynthetic eukaryotes

Katherine E. Helliwell [1,2,@,*]

Phosphorus (P) and nitrogen (N) are the major nutrients that constrain plant and algal growth in nature. Recent advances in understanding nutrient signalling mechanisms of these organisms have revealed molecular attributes to optimise N and P acquisition. This has illuminated the importance of interplay between N and P regulatory networks, highlighting a need to study synergistic interactions rather than single-nutrient effects. Emerging insights of nutrient signalling in polyphyletic model plants and algae hint that, although core P-starvation signalling components are conserved, distinct mechanisms for P (and N) sensing have arisen. Here, the N and P signalling mechanisms of diverse photosynthetic eukaryotes are examined, drawing parallels and differences between taxa. Future directions to understand their molecular basis, evolution, and ecology are proposed.

Highlights

The expansion of sequencing resources and the development of advanced plant and algal model systems provide a new framework to examine the distribution and evolution of N and P signalling mechanisms in photosynthetic eukaryotes.

This has revealed that, although core P-starvation signalling components are conserved, distinct mechanisms for P (and N) sensing may have arisen.

Ca^{2+} signalling is emerging as an important player in N and P sensing in photosynthetic eukaryotes, albeit that distinct roles have evolved in different lineages.

Sophisticated mechanisms to integrate N and P signalling networks and maximise nutrient acquisition are employed by diverse photosynthetic eukaryotes.

Integration of plant and algal research will expedite advances in our understanding of N and P signalling, and inform strategies to engineer improved N and P use efficiency in crops.

A new age of understanding N and P signalling in photosynthetic eukaryotes

Photosynthetic eukaryotes (plants and **algae**; see Glossary) comprise widely divergent taxa that have complex evolutionary histories involving several **endosymbiotic** events (Figure 1A) [1,2]. The substantial genetic mixing caused by such endosymbioses probably accounts for the success of photosynthetic eukaryotes on land and in water where they sustain the majority of heterotrophic life. Common to both terrestrial and aquatic ecosystems is the fluctuating availability of the crucial macronutrients N and P [3–5]. Limitation by these nutrients on arable land has driven routine use of fertilisers to boost crop yields. However, P- and N-based fertilisers can be lost through surface run-off, triggering **algal blooms** and **eutrophication** in surrounding aquatic ecosystems [6]. Moreover, because global phosphate reserves will probably become exhausted within 100 years, it is vital to improve P use efficiency [7]. A priority research area is thus to better understand how plants and algae sense, acquire, and optimise P and N usage. Although the importance of maintaining nutritional balance and **elemental stoichiometry** of N and P for optimal plant [8] and algal [9] growth has long been recognised, the molecular mechanisms mediating such processes have remained poorly characterised. The expansion of sequencing resources, as well as the development of genetically tractable model systems for divergent photosynthetic eukaryotes, coupled with novel technologies for imaging cellular processes in real-time, have enabled significant advances in our knowledge of **nutrient signalling** networks [10–14]. This review highlights how these advances now provide a framework to draw a more comprehensive picture of N and P signalling mechanisms across photosynthetic eukaryotes, and aims to identify emerging trends, and stimulate future research questions.

Beyond nutrients: the important roles of phosphate and nitrate as signalling molecules

P-starvation signalling mechanisms

To thrive in dynamic P regimes, it is crucial to coordinate metabolic adaptations to cope with P deprivation. Phosphate starvation response 1 (PSR1) is a transcriptional regulator of P-starvation responses that was first identified in the green alga *Chlamydomonas reinhardtii* (CrPSR1) [15],

[1]Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QD, UK
[2]Marine Biological Association, Citadel Hill, Plymouth PL1 2PB, UK

*Correspondence:
k.helliwell@exeter.ac.uk
katherine.helliwell@mba.ac.uk
(K.E. Helliwell).
@Twitter: @KEHelliwell

and subsequently in *Arabidopsis thaliana* (arabidopsis; AtPHR1) [16] (Figure 1B). '*PSR1-like*' genes belong to the GARP (GOLDEN2/ARR-B/Psr1) transcription factor (TF) family [17]. CrPSR1 and AtPHR1 share a conserved SHLQ(K/M)(Y/F) motif, suggesting that they are closely related [16,17]. Moreover, characterisation of *CrPSR1* and *AtPHR1* demonstrated that they control the expression of P starvation-responsive genes, including phosphate transporters and P scavenging enzymes [15,16]. In arabidopsis, a signalling branch downstream of *AtPHR1* has also been defined in which the expression of miRNA miR399 [18,19] and of the non-protein coding gene *INDUCED BY PHOSPHATE STARVATION 1* (*IPS1*) [20] is induced by P starvation in a *PHR1*-dependent manner. Repression of *PHO2* (encoding an E2 ubiquitin conjugase) by miR399 enhances cellular phosphate content by inhibiting PHO2-dependent degradation of the phosphate transporters PHT1 and PHO1 [21,22]. Although miRNAs have also been detected in *C. reinhardtii* under P deprivation, their regulatory role is less clear because their targets did not show reciprocal changes in transcript abundance according to P availability, suggesting that they are not subject to miRNA-mediated RNA degradation [23].

Beyond arabidopsis and *C. reinhardtii*, *PSR1-like* genes are found across polyphyletic photosynthetic eukaryotes (Figure 1B), including representatives of the trebouxiophyte and prasinophyte lineages [24], as well as in red algae, haptophytes, **diatoms**, brown seaweeds (*Ectocarpus*) [25], and dinoflagellates [24]. The predicted proteins of these genes contain the characteristic SHLQ(K/M)(Y/F) motif in addition to a putative coiled-coil (CC) motif, similar to CrPSR1 and AtPHR1 [24,25] (Figure 1B). Functional characterisation of *PtPSR1* from the model diatom *Phaeodactylum tricornutum* confirmed its role in P-starvation signalling [25]. Moreover, phylogenetic analysis indicated that PtPSR1 is related to other SHLQ(K/M)(Y/F) GARP TFs, including AtPHR1 and CrPSR1 [25]. Mechanistically, PtPSR1 binds to a conserved regulatory element within the promoter regions of the 84 genes that are most strongly upregulated during P starvation. Notably, this motif closely resembles the recognition motifs of arabidopsis GARP TFs [25]. Together, these findings suggest that *PSR1-like* genes represent a conserved mechanism for regulating P-starvation responses in plants and algae. The presence of multiple *PHR1-like* genes in arabidopsis, including *AtPHL1–4* that act redundantly with *AtPHR1* [26–28] and a second *PSR1-like* gene that is upregulated by P starvation in *P. tricornutum* [25] (although functional characterisation is necessary), suggests that this gene family may have expanded. Indeed, only partial impairment of P-limitation responses was detected by *AtPHR1/PtPSR1* loss-of-function mutation studies in these species [16,25]. Similarly, at least four *PHR* genes (*OsPHR1–4*) are involved in P-starvation signalling in rice (*Oryza sativa*) [29–31]. Notably, in addition to *PSR1-like* genes, in an intriguing parallel to arabidopsis, *P. tricornutum* exhibits upregulation of miRNAs (miR5471 and miR5472) and long intergenic non-protein-coding RNAs under P deprivation, suggesting that they may also have regulatory roles in P-starvation responses in diatoms [32]. However, functional characterisation is now necessary to deduce their exact role and determine whether their expression is controlled by PtPSR1.

Although the role of PSR1-like TFs in governing P-starvation responses appears to be conserved across polyphyletic photosynthetic eukaryotes, distinct differences in the features and regulation of these genes are apparent. These include the extent of responsiveness to P starvation. Transcriptional expression of *CrPSR1* and *PtPSR1* is induced 13- and tenfold during P starvation, respectively [15,25]. By contrast, *AtPHR1* transcripts are detected independently of P status and only increase weakly (twofold) under P-limiting conditions [16]. Furthermore, the phosphate content of *AtPHR1* mutants was lower than that of the wild-type (WT) even under P-sufficient conditions, suggesting that AtPHR1 is involved in P homeostasis and regulation even when cells are P-replete [16]. In contrast to *AtPHR1* [16], *PtPSR1* contains multiple *PSR1*-binding motifs in its promoter region [25] that enhance its own expression during P starvation via a positive feedback loop. Differences

Glossary

Algae: an informal term for a polyphyletic 'group' of photosynthetic eukaryotes that are not land plants.
Algal blooms: the rapid proliferation of algal populations in aquatic ecosystems, in response to increases in nutrient supply and other environmental stimuli.
Archaeplastida: a eukaryotic supergroup comprising green algae and land plants (Viridiplantae), red algae (rhodophytes), and glaucophyte algae. Photosynthesis arose in this lineage via a primary endosymbiosis event.
Diatoms: a major group of single-celled algae that live in marine, freshwater, and brackish habitats, and which contribute ~20% of annual global carbon fixation.
Endosymbiosis: the engulfment of a photosynthetic cyanobacterium by a heterotrophic eukaryotic host that gave rise to the first plastids of eukaryotes is referred to as primary endosymbiosis. Subsequent acquisitions of alga-derived plastids are referred to as secondary (or tertiary/quaternary) endosymbioses.
Elemental stoichiometry: the relative proportions of elements (e.g., C, N, P, K, Ca, S, Cu, Zn, Mg, Mn, and Fe) within an organism, controlled by the intracellular macromolecular composition and/or environmental nutrient availability.
Eutrophication: the enrichment of nutrients in a water body that can cause algal bloom formation and other structural changes to the ecosystem.
Nutrient signalling: mechanisms enabling an organism to sense and coordinate adaptations to nutrient supply.

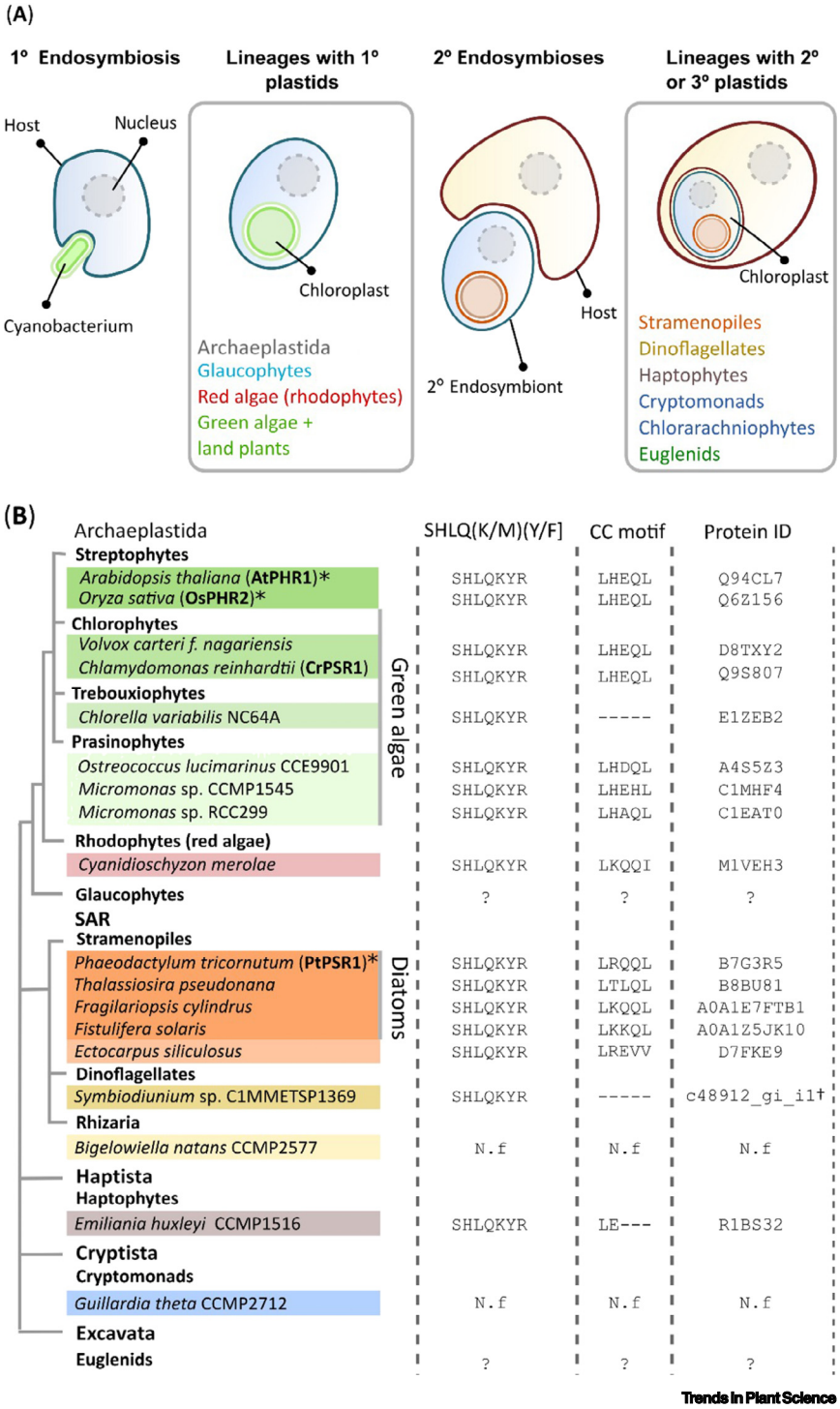

Figure 1. The evolution of photosynthetic eukaryotes and the distribution of *PSR1-like* genes across diverse plant and algal taxa. (A) A simplified schematic diagram of the role of endosymbiosis in driving the evolution of diverse photosynthetic eukaryotes. The primary (1°) endosymbiosis of a photosynthetic cyanobacterium by a heterotrophic eukaryote host gave rise to plants and algae (Archaeplastida) containing primary plastids [1]. Subsequent secondary (2°) and tertiary (3°) endosymbiotic events gave rise to algal taxa with complex plastids, including stramenopiles (e.g., diatoms, pelagophytes, and brown seaweeds), dinoflagellates, haptophytes (e.g., coccolithophores), cryptophytes, chlorarachniophytes, and euglenids [114,115]. (B) The distribution of SHLQ(K/M)(Y/F) 'PSR1-like' GARP TFs in representative taxa of the major lineages of the eukaryote tree of life (including only those containing photosynthetic representatives) [116], as reported previously by Wykoff *et al.* [15], Rubio *et al.* [16], Fiore *et al.* [24], and Sharma *et al.* [25]. Functionally characterised *CrPSR1* (*Chlamydomonas reinhardtii*) [15], *AtPHR1* (*Arabidopsis thaliana*; arabidopsis) [16], *OsPHR2* (*Oryza sativa*) [31], and *PtPSR1* (*Phaeodactylum tricornutum*) [25] are highlighted in bold. Predicted amino acid sequences of *PSR1-like* genes in the region of the GARP 'SHLQ(K/M)(Y/F)' and 'LHEQL' coiled-coil (CC) motif are given, as well as the UniProt protein identifiers for each sequence [†except for *Symbiodinium* sp. C1 MMETSP1369 where the Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP) [110] gene identifier is provided instead [24]]. 'N.f' indicates not found. *In several taxa, more than one *PSR1-like* gene has been identified. These include four *PHR-like* genes (*AtPHL1–4*) in *arabidopsis* that act redundantly with *AtPHR1* [26–28]. Similarly, at least four *PHR* genes (*OsPHR1–4*) are involved in phosphate starvation signalling in rice (*O. sativa*) [29–31]. Moreover, a second *PSR1-like* gene upregulated by P starvation was also identified in *P. tricornutum* [25] (although functional characterisation is necessary).

in the subcellular localisation of PSR proteins have also been observed. For instance, both CrPSR1 and AtPHR1 are localised in the nucleus independently of cellular P status [15,16]. By contrast, OsPHR2 moves from the cytosol to the nucleus during P starvation. This cytosolic-to-nuclear

shuttling is impaired by the OsPHR2 repressor protein, OsSPX4 [33]. In land plants, proteins exclusively containing an SPX domain are known as SPX (SYG1/Pho81/XPR1) proteins [34]. *O. sativa* encodes six *SPX* genes whereas arabidopsis has four. OsSPX4 stability is phosphate-dependent being degraded during P starvation. However, under P-sufficient conditions, OsSPX4 directly binds to OsPHR2, blocking its movement into the nucleus and precluding the activation of P starvation-induced genes [33]. Several other SPX proteins (OsSPX1, OsSPX2, OsSPX4, and OsSPX6) also repress the activity of OsPHR2 in the nucleus by preventing OsPHR2 from binding the promoters of P starvation-induced genes [33–35]. The distribution and role of SPX proteins in algae is not well established. SPX domains have been reported in algal phosphate transporters and polyphosphate (poly-P) biosynthesis proteins [32,36–38]. Moreover, a true *SPX* gene (exclusively harbouring an SPX domain) in *P. tricornutum* contains numerous PtPSR1-binding motifs in its promoter [25]. Gene knockout mutants show elevated expression of the phosphate acquisition machinery, indicating that this gene probably negatively regulates P-starvation responses [38], as in land plants.

Notably, although the PHR–SPX module is crucial for regulating systemic adaptations to phosphate availability in plants [16], PHR-independent mechanisms reportedly govern local responses such as modifications in root architecture observed during P starvation [14,39,40]. In particular, inhibition of primary root growth by P starvation in arabidopsis is controlled by the C_2H_2 zinc-finger TF, SENSITIVE TO PROTON RHIZOTOXICITY 1 (STOP1), which directly induces *ALUMINUM-ACTIVATED MALATE TRANSPORTER 1* (*ALMT1*) to facilitate malate efflux [39,40]. This causes an accumulation of iron and reactive oxygen species that inhibits cell elongation and division at the root tip [39,40]. Intriguingly, the presence of *STOP1–ALMT1* homologues in charophyte (but not chlorophyte) algal genomes indicates that this PHR-independent mechanism was a key innovation for local phosphate sensing in the streptophytes [41].

Plant and algal phosphate-sensing mechanisms

Mechanisms coordinating P-starvation signalling and metabolism are clearly vital for plant and algal survival in low-phosphate environments. These systems rely on the ability to sense phosphate availability and the depletion of intracellular phosphate stores. SPX proteins not only control master regulators of P signalling in plants (i.e., PSR1-like proteins) but they are also crucial for sensing intracellular P availability [42]. SPX domains harbour a highly basic binding surface with strong affinity for inositol polyphosphate (InsP), an important store of intracellular inorganic phosphate [43]. SPX domains exhibit the greatest affinity for highly phosphorylated InsP (PP-InsP) forms [42]. In particular, $InsP_7$ and $InsP_8$ are the most potent in stimulating SPX domain activity [44]. Hence, PP-InsPs act as important P signalling molecules. Their production during P-sufficient conditions activates SPX proteins and alters their interactions with PHR proteins [43]. In rice in P-replete conditions, OsSPX4 stabilises OsSPX4–PHR2 complexes and confines OsPHR2 to the cytosol, thus preventing the activation of nuclear P starvation-induced gene expression [33]. OsSPX4 is thus a crucial phosphate sensor that bridges cellular phosphate status with downstream mediators of P signalling. In *C. reinhardtii*, PP-InsPs also regulate the target of rapamycin (TOR) complex, a central coordinator of nutrient C, N, and P signalling pathways [43,45,46]. However, how PP-InsPs are able to integrate diverse signalling pathways, and their role in algal P signalling more broadly, is poorly understood.

To compete for P in dynamic nutrient regimes, photosynthetic eukaryotes must also sense transient increases in external phosphate. Diatoms are one of the most ecologically successful algal groups [47]. They thrive in dynamic coastal ecosystems where nutrient supply can vary dramatically because of riverine inputs, turbulent mixing, seasonal fluctuations, and microscale changes [48,49]. Diatoms often dominate algal bloom formation by responding rapidly to

favourable environmental conditions such as increased nutrient supply [50]. Diatoms are thus highly adapted to living in 'feast–famine' nutrient environments. Insight into how diatoms sense and coordinate responses to P replenishment recently came from work employing transgenic lines of *P. tricornutum* expressing a genetically encoded Ca^{2+} biosensor (R-GECO1) [11]. Ca^{2+} is a ubiquitous second messenger employed by eukaryotes to coordinate physiological responses to many different environmental stimuli [11]. Transient elevations in cytosolic [Ca^{2+}] were detected in P-starved *P. tricornutum* R-GECO1 cells within seconds of phosphate resupply (Figure 2A). This response was highly sensitive to external phosphate concentrations (detectable with as little as 0.9 μM phosphate). Moreover, several forms of P other than orthophosphate [e.g., organic P forms such as ATP, D-glucose 6-phosphate (G6P), and inorganic poly-P] triggered Ca^{2+} elevation [11]. However, because cells were not receptive to the non-hydrolysable form of ATP, adenosine-5′-(3-thiotriphosphate) (ATP-γ-S), it is likely that *P. tricornutum* cells rapidly liberate phosphate from organic/poly-P forms (via extracellular phosphatases [51]), which subsequently evokes the Ca^{2+} response.

How the P–Ca^{2+} signalling and P-starvation (PtPSR1) signalling pathways of *P. tricornutum* interact to coordinate responses to fluctuating P availability remains to be elucidated. However, the identification of several Ca^{2+}-related genes in *P. tricornutum* that contain *PtPSR1*-like binding motifs in their promoter regions [25] suggests that the P-starvation signalling and sensing pathways are tightly coordinated. Moreover, whereas P–Ca^{2+} signalling is employed by both pennate (*P. tricornutum*) and centric diatoms (*Thalassiosira pseudonana*) [11] that represent the two major lineages of this highly diverse algal group, the role of Ca^{2+} signalling in P sensing in other algae, and indeed land plants, remains elusive. A role for Ca^{2+} in plant P signalling has long been postulated [52]. Mutants defective in vacuolar Ca^{2+}/H^{+} exchangers show excessive accumulation of phosphate in arabidopsis shoots [53], and exhibit enhanced expression of P starvation-induced genes [52]. Matthus *et al.* [12] directly investigated P nutrition and Ca^{2+} signalling in arabidopsis roots, using genetically encoded Ca^{2+} indicators. They found that P starvation diminishes Ca^{2+} signalling responses to mechanical, salt, osmotic, and oxidative stress compared to fully P-replete plants. However, although plants detect ATP via Ca^{2+}-dependent signalling [54,55], phosphate resupply to P-limited plants did not evoke transient elevations in cytoplasmic calcium ($[Ca^{2+}]_{cyt}$) levels in arabidopsis roots under the P-limitation conditions examined [12]. These findings suggest that distinct mechanisms for phosphate sensing may have arisen in plants and diatoms, which could reflect their different ecologies. In soil, P is heterogeneously spread and highly immobile owing to its propensity to complex with other elements [3]. In aquatic ecosystems, P can also exist in refractory forms [4], but transient increases in phosphate are frequently observed [56] that demand rapid sensory mechanisms.

N signalling mechanisms

N-related signalling pathways also fall into two classes. First, N-starvation responses (NSRs) are activated when the availability of N is limiting, and cause enhanced expression of a suite of N starvation marker genes (namely activation of N uptake and assimilation, and utilisation of organic N sources) [13,57]. Second, primary nitrate responses (PNRs) are triggered within minutes of nitrate replenishment to nitrate-deprived plants/algae [10,58,59]. Arguably, in plants, PNR pathways are better described [13]. In an intriguing parallel with diatom P–Ca^{2+} signalling, nitrate (but not ammonium) provision induces transient Ca^{2+} elevations in arabidopsis cells [10,60] (Figure 2B). The molecular machinery governing the generation and transduction of arabidopsis nitrate-induced Ca^{2+} signals is well described. These Ca^{2+} signatures orchestrate primary responses to nitrate via a signal transduction cascade mediated by Ca^{2+}-dependent protein kinases (CPKs), leading to phosphorylation and cytoplasm-to-nucleus shuttling of the TF NIN-LIKE PROTEIN 7 (NLP7) to control nitrate-responsive gene expression [10]. Remarkably, a cyclic

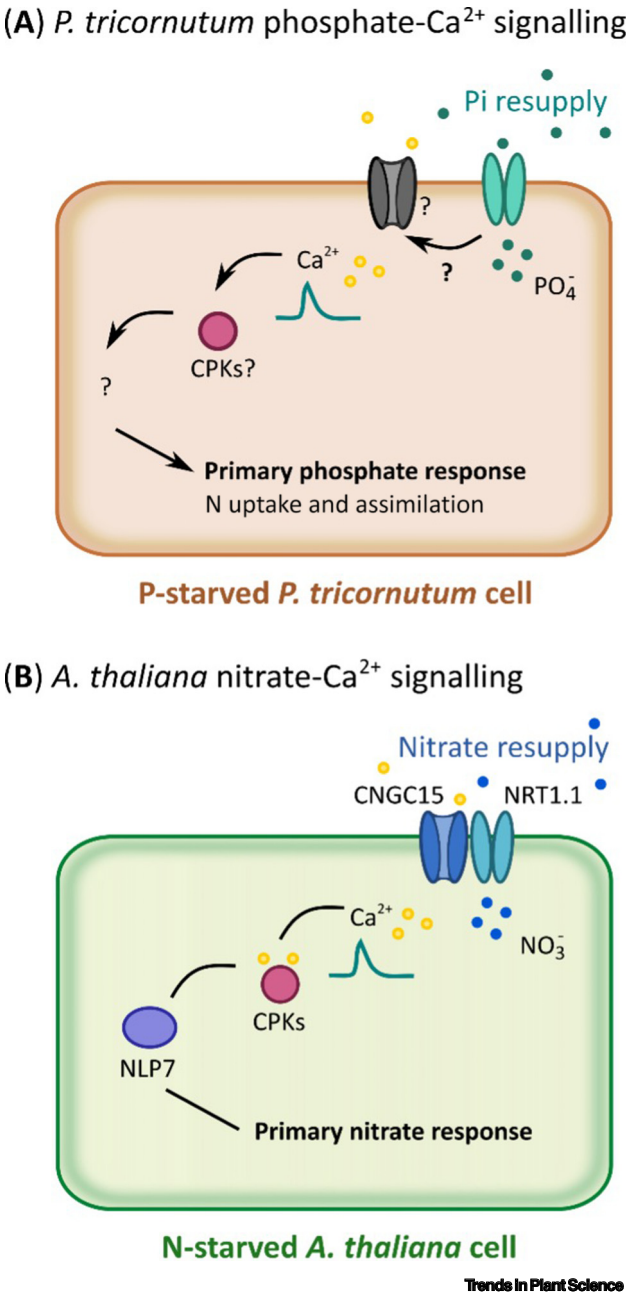

Figure 2. Schematic diagram of Ca^{2+}-dependent P and N signalling pathways described in diatoms (*Phaeodactylum tricornutum*) and plants (*Arabidopsis thaliana*; arabidopsis). (A) Resupply of inorganic phosphate (Pi) to P-limited diatom cells triggers transient elevations in cytosolic Ca^{2+} levels that are necessary for the coordination of primary responses to phosphate replenishment [11]. The molecular components required for the generation and transduction of P–Ca^{2+} signals are currently unknown. (B) A nitrate-coupled Ca^{2+} signalling pathway orchestrates primary responses to nitrate in arabidopsis [10]. A cyclic nucleotide-gated Ca^{2+}-permeable channel (AtCNGC15) and the nitrate transceptor AtNRT1.1 control nitrate-induced Ca^{2+} signals that coordinate adaptations to nitrate supply [62] via a signal transduction cascade mediated by Ca^{2+}-dependent protein kinases (CPKs) and a master regulator of nitrate metabolism, NIN-LIKE PROTEIN 7 (AtNLP7).

nucleotide-gated Ca^{2+}-permeable channel (AtCNGC15) underpins nitrate-induced Ca^{2+} influxes through direct interactions with the nitrate transceptor [nitrate transporter 1 (AtNRT1.1) or AtCHL1] [60–62]. With decreased nitrate, AtNRT1.1 and AtCNGC15 form a transceptor–channel complex which blocks Ca^{2+} channel activity. This complex disassociates under elevated nitrate concentrations to increase Ca^{2+} channel activity and generate nitrate-induced Ca^{2+} signals. Loss-of-function mutants of *AtCNGC15* show impaired cytoplasmic-to-nuclear shuttling of AtNLP7, and PNRs are blocked [62]. AtNRT1.1 serves as the nitrate-sensing element of this complex. In addition to nitrate perception, this protein is a dual-affinity nitrate transporter

(hence 'transceptor'). Phosphorylation of AtNRT1.1 under low-nitrate conditions switches the protein from being a low-affinity to a high-affinity nitrate transporter [61,63]. Thus, AtNRT1.1 enables the sensing and transport of varying extracellular concentrations of nitrate, and couples nitrate perception with downstream nitrate signalling responses through interactions with AtCNGC15 [62] that trigger a Ca^{2+}-dependent signalling cascade [10] (Figure 2B). However, it is important to note that plant nitrate-sensing pathways are not entirely Ca^{2+}-dependent: evidence that the expression of particular nitrate-sensitive genes is not affected by Ca^{2+} inhibitor treatments indicates that Ca^{2+}-independent pathways are also important [60].

In contrast to arabidopsis, nitrate-deprived *P. tricornutum* does not exhibit nitrate-induced Ca^{2+} signals [11], and indeed this alga does not harbour *CNGC* genes [64]. This suggests that an analogous nitrate–Ca^{2+} signalling pathway is not utilised by this diatom. However, nitrate responsiveness of CPKs in the green alga *Ostreococcus tauri* tentatively indicates a role for CPKs and/or Ca^{2+} signalling in nitrate status sensing in this species, which warrants further investigation [65]. *C. reinhardtii* and *Micromonas* sp. RCC299 (but interestingly not *O. tauri* or *Ostreococcus lucimarinus*) also have genes encoding for CNGCs [64]. In addition, *C. reinhardtii* encodes other components resembling the nitrate-sensing pathway of plants. Notably, the master regulator of nitrate assimilation in *C. reinhardtii* (*NIT2*) is structurally related to NLP TFs of plants [66] which have a central regulatory role in plant nitrate signalling [67] and, as discussed above, are phosphorylated via the nitrate-Ca^{2+}–CPK signalling pathway of arabidopsis (Figure 2B) [10]. Further work will be necessary to deduce whether an 'arabidopsis-like' nitrate–Ca^{2+}–CPK–NLP signal transduction cascade is employed by green algae. However, NLPs were not detected in a range of stramenopile genomes (including diatoms), indicating that this family of TFs may be absent from these lineages (or have diverged significantly) [68], which suggests that alternative mechanisms for nitrate sensing may have arisen in photosynthetic eukaryotes outside the **Archaeplastida**.

An important role for nitrate transporters (in this case, the high-affinity class, *NRT2*) in nitrate sensing in *C. reinhardtii* has also emerged [69]. Both phylogenetically unrelated *NRTs* (*NRT1* and *NRT2*) are distributed across photosynthetic eukaryotic taxa (Table 1). However, whereas the (typically low-affinity) nitrate permease *NRT1* genes of the oligopeptide transporter (POT) family [63] have expanded considerably in plants, *NRT2* genes more typically exceed the number of *NRT1* genes in algal genomes. Further functional characterisation of algal nitrate-associated genes to deduce their roles in nitrate transport and signalling is essential. The observed distribution, and apparent patchiness, of known plant nitrate-sensing components across algal taxa raises many questions regarding the evolution of nitrate signalling mechanisms in photosynthetic eukaryotes (see Outstanding questions). It is likely that different innovations have arisen in distinct algal lineages, reflecting their complex evolutionary histories (Figure 1A). Certainly, the observation that diatom *NRT2* genes closely resemble those of oomycetes [58], and probably originated from the heterotrophic stramenopile host of the secondary endosymbiotic event that gave rise to the diatoms (Figure 1A), adds complexity to this important question.

An understanding of the molecular elements modulating plant and algal NSR is also emerging. In arabidopsis, the calcium sensor CBL7 (CALCINEURIN B-LIKE PROTEIN 7) influences expression of nitrate transporters *NRT2.4* and *NRT2.5* during N starvation [70]. miRNAs also have functional roles in coordinating the plant NSR; miR169 controls NFYA (nuclear Factor Y, subunit A) TFs and influences *NRT2.1* and *NRT1.1* expression during N deprivation [71]. In addition, members of the *LATERAL ORGAN BOUNDARY DOMAIN* (*LBD*) TF gene family negatively regulate anthocyanin biosynthesis in arabidopsis when N is scarce [72]. Although understanding of key regulators of algal NSRs is far from complete, several significant advances have been made. An R2R3-type MYB TF, CmMYB1, has been identified as a central regulator of N-assimilation genes during N

Table 1. Distribution of *NRT1* and *NRT2* genes in representative plant and algal genomes

Genus	Species/strain	*NRT1*	*NRT2*	Refs
Arabidopsis	*thaliana*	53	7	[117]
Oryza	*sativa*	93	5	[118]
Volvox	*carteri* f. *nagariensis*	1	3	[119,120]
Chlamydomonas	*reinhardtii*	1	6	[119,120]
Chlorella	*variabilis* NC64A	2	1	[119,120]
Ostreococcus	*lucimarinus* CCE9901	1	1	[119,120]
Micromonas	sp. CCMP1545	NF[a]	1	[119,120]
Micromonas	sp. RCC299	1	3	[119,120]
Cyanidioschyzon	*merolae*	1	1	[120]
Cyanophora	*paradoxa*	NF	2	[120]
Phaeodactylum	*tricornutum*	1	6	[119]
Thalassiosira	*pseudonana*	NF	3	[119]
Aureococcus	*anophagefferens*[b]	1	3	[119]
Emiliania	*huxleyi* CCMP1516[b]	NF	9	[119]

[a]Abbreviation: NF, not found.
[b]Indicates that at the time of the search the genome was not completely assembled and the number of *NRT1/2* genes may be under-represented.

starvation in the thermoacidophilic red alga, *Cyanidioschyzon merolae* [73]. Unlike land plants (and green algae), diatoms encode a complete animal-like urea cycle that helps them to cope with episodic changes in N availability [74]. Regulatory elements identified to coordinate the transcriptional rewiring of diatom N metabolism in response to N starvation include a bZIP TF (bZIP14) [75] that controls the upregulation of the tricarboxylic acid cycle, and a novel family of RING-domain TF that is conserved in stramenopile algae [76].

Crosstalk between N and P signalling networks in plants, algae, and beyond

Our understanding of N and P signalling pathways in photosynthetic eukaryotes has been shaped largely by investigations into single-nutrient effects. An emerging theme from the molecular examination of plant and algal nutrient signalling systems is the importance of regulatory crosstalk between N and P networks [13,14,77]. Plants and algae frequently encounter combined N and P limitations and/or resupply. Likewise, environmental levels of these nutrients can fluctuate independently of one another, necessitating highly coordinated molecular regulation of signalling and uptake pathways. However, although the importance of maintaining N and P stoichiometry has long been recognised [8,9], we are only beginning to understand the molecular mechanisms driving such coordination.

N status-regulated P signalling and acquisition

Early insights of the molecular mediators governing the interplay between N and P nutrition in plants have arisen from the examination of nitrate-dependent regulation of phosphate uptake driven by the *NITRATE LIMITATION ADAPTATION* (*AtNLA*) gene (encoding an E3 ligase). *Atnla* mutants are impaired in their ability to adapt to N starvation, and exhibit early senescence and increased phosphate uptake under low-N conditions compared to the WT [78]. The evidence suggests that AtNLA functions as a negative regulator of phosphate transport machinery [*PHOSPHATE TRANSPORTER TRAFFIC FACILITATOR 1* (*AtPHF1*) and *PHOSPHATE TRANSPORTER 1.1* (*AtPHT1.1*)] by acting in concert with PHO2 during N starvation [78,79]. The fact that overaccumulation of phosphate in *Atnla* and *Atpho2* mutants causes phosphate

toxicity under N-deficient conditions [18,78] emphasises the crucial importance of crosstalk between N and P metabolism for adapting to dynamic nutrient regimes.

Integration of N and P signals in plants also applies to nitrate-inducible responses. Nitrate treatment causes increased phosphate uptake in a range of plants [80–82]. Specific molecular interactions between N and P signalling components can underlie nitrate-mediated enhancement of P acquisition. In rice, direct physical interactions between the primary nitrate sensor OsNRT1.1B and the P-starvation signalling master regulator (OsPHR2) repressor, OsSPX4, govern the enhancement of P uptake by nitrate [81]. Nitrate perception strengthens the OsNRT1.1B–OsSPX4 interaction and promotes the degradation of OsSPX4 via an E3 ubiquitin ligase (called NRT1.1B INTERACTING PROTEIN 1, NBIP1). This allows OsPHR2 to translocate to the nucleus to activate the transcription of phosphate acquisition genes. N-mediated control of P-starvation responses that are dependent on the *NRT1.1* nitrate sensor has also been reported in arabidopsis [83]. In arabidopsis the majority of P-sensitive genes are also significantly regulated by N availability [83]. Analogously to rice, P starvation-response genes (including *AtSPX4*) are also upregulated during P limitation, but only in the presence of nitrate, and this response is also dependent on a functional *AtNRT1.1* gene. Interactions between *SPX* genes and crucial N signalling components extend beyond NRT1.1. *AtNIGT1s* (*NITRATE-INDUCIBLE GARP-TYPE TRANSCRIPTIONAL REPRESSOR 1*) encode GARP TFs closely related to *AtPSR1* that are strongly upregulated by nitrate [84]. AtNIGT1s repress *SPX* gene expression, thus activating phosphate utilisation [82,85]. Indeed, *Atnigt1.1–Atnigt1.4* mutants have impaired nitrate-dependent enhancement of phosphate uptake. Thus, NIGT1–SPX–PHR cascades are crucial in mediating N status-responsive regulation of phosphate uptake and starvation signalling. These pioneering studies provide the first clues into the intricacy of regulatory crosstalk between plant N and P signalling networks, but probably only scratch the surface of the extent of such interactions.

There is also evidence that N availability drives P acquisition, metabolism, and storage in algae. N enrichment enhanced phosphate uptake in the intertidal multicellular brown seaweed *Fucus vesiculosus* [86]. Moreover, phosphate uptake rates by the freshwater green alga *Scenedesmus* sp. were reduced eightfold under N-limited compared to N-sufficient conditions [87]. However, N starvation enhanced phosphate uptake in *Chlorella vulgaris* [88]. This is likely to sustain increases in the synthesis of poly-P observed in *C. vulgaris* under N starvation [89], which has also been reported for *C. reinhardtii* [90] and the diatom *T. pseudonana* [91]. Intriguingly, provision of organic N led to enhanced alkaline phosphatase activity in representative dinoflagellate, diatom, and haptophyte species [92]. However, unlike in plants, the cellular mechanisms governing N/P crosstalk are completely unknown.

P status-regulated N signalling and acquisition

Notably, N acquisition can also be driven by P status, and reductions of N uptake during P limitation have been reported in several plant species [93–95]. Insights into the complex molecular mechanisms underlying P-driven adaptations to N uptake and assimilation were gained through further study of *AtNIGT1.1*. Maeda *et al.* demonstrated that AtNIGT1.1 is a direct negative regulator of the high-affinity nitrate transporter *AtNRT2.1* [96], and competes with AtNLPs that positively regulate *AtNRT2.1* immediately following nitrate provision [96]. Together these interactions fine-tune the transient increase in *AtNRT2.1* expression observed in response to nitrate. However, *AtNIGT1.1* is not only under tight control by nitrate availability [84], but expression of this gene is also promoted by phosphate starvation in an *AtPHR1*-dependent manner [96] (Figure 3A). AtPHR1 binds to the promoter region of the *AtNIGT1* gene to activate its expression, thus downregulating *AtNRT2.1* expression (and nitrate uptake) during P starvation [96]. In a further level of complexity, *AtNIGT1* clade genes also negatively regulate their own expression by directly competing with AtPHR1-

dependent activation of *AtNIGT1* [96]. That *AtNIGT1* is also promoted by *AtNLP7* (as well as by *AtPHR1*) [96] suggests that *AtNIGT1* is a major hub between N and P starvation signalling pathways in plants. Notably, the PHR-independent regulator of local responses to P starvation AtSTOP1 also intersects with N uptake by directly binding to the promoter region of *AtNRT1.1* to control its expression under acidic conditions and modulate soil pH [97]. Together, these studies highlight the reciprocity between plant N and P regulatory networks. Albeit, intriguingly, Medici *et al.* [83] reported an apparent prioritisation towards N signalling in arabidopsis. They found that, whereas the majority (85%) of 'P starvation response' genes were regulated by N status, fewer 'NSR' genes (45%) were controlled by P provision. The authors postulated that this could be due to the immobile nature of P in the soil, leading plants to invest in growth and P retrieval only if N is available [83]. However, at the level of root system architecture, multifactorial experiments with different combinations of nutrients (N, P, K, and S) showed that P is the most important factor regulating main root parameters, whereas N had a greater influence on lateral root parameters [98].

A strong influence of P availability on N uptake has also been reported in diverse algae [11,37,99,100], including the reduction of N acquisition machinery during P limitation [11,101,102] (Figure 3B, left). Remarkably, following P replenishment to P-starved *P. tricornutum* cells, one of the earliest detectable metabolic adaptations was a rapid enhancement of nitrate uptake (8 h after P resupply) [11]. This response was accompanied by substantially increased abundance of proteins associated with N uptake and assimilation (Figure 3B, right). Because this response was blocked by the Ca^{2+} channel inhibitor ruthenium red (RuR) that abolishes P–Ca^{2+} signals [11], it is likely that the rapid modulation of nitrate acquisition by phosphate is controlled by P–Ca^{2+} signalling. Reductions in N-assimilation proteins during P limitation, tightly coupled to their enhancement following P resupply, have also been reported in the bloom-forming pelagophyte *Aureococcus anophagefferens* [101] and green alga *Micromonas commoda* [102]. This demonstrates that coordination between P signalling and N acquisition is probably fundamentally important for diverse algae. The question is, given the imperative role of NIGT1 in balancing N and P acquisition in plants [82,84,96], do algae encode *NIGT1* homologues? Both NIGT1 and PSR1 belong to the GARP family of TFs that harbour a characteristic SHLQ(K/M)(Y/F) motif [17]. This family is greatly expanded in plant genomes, and 56 GARP TFs (including four *NIGT1*-clade genes [96]) have been identified in arabidopsis [17] compared to five in *C. reinhardtii*, two in *P. tricornutum*, and four in the red alga *Porphyridium purpureum* [103]. Given the diverse functions of GARPs in plants, from regulating nutrient sensing to coordinating circadian clock oscillation [17], gene characterisation is imperative to determine whether any such algal GARPs are probable NIGT1 functional homologues. The answer will have an important bearing on understanding the evolution of GARP TFs, and of N/P sensing and crosstalk, in photosynthetic eukaryotes.

P signalling and N metabolism are clearly closely intertwined in diverse photosynthetic eukaryotes. Notably, the evidence suggests that coordination of P and N signalling is fundamentally important more broadly. Certainly, direct phosphate control over N signalling and metabolism extends beyond eukaryotes [104,105]. In the bacterium *Streptomyces*, regulation of P-responsive metabolism is exerted by the two-component system, PhoR–PhoP [106]. PhoR is a membrane sensor protein kinase that detects phosphate scarcity, whereas PhoP is a response regulator that binds to the 'PHO boxes' of P-responsive genes to control their expression during P starvation. PhoP binds to the promoters of the major N regulator (*glnR*), as well as to the *amtB–glnK–glnD* operon that encodes an ammonium transporter and two N-sensing proteins (Figure 3C). This interaction exerts a negative impact on the expression of these genes during P starvation [104,105]. Negative regulation of N-related genes by P starvation has also been described in *Sinorhizobium meliloti* [107] and *Escherischia coli* [108]. Thus, direct interactions between P

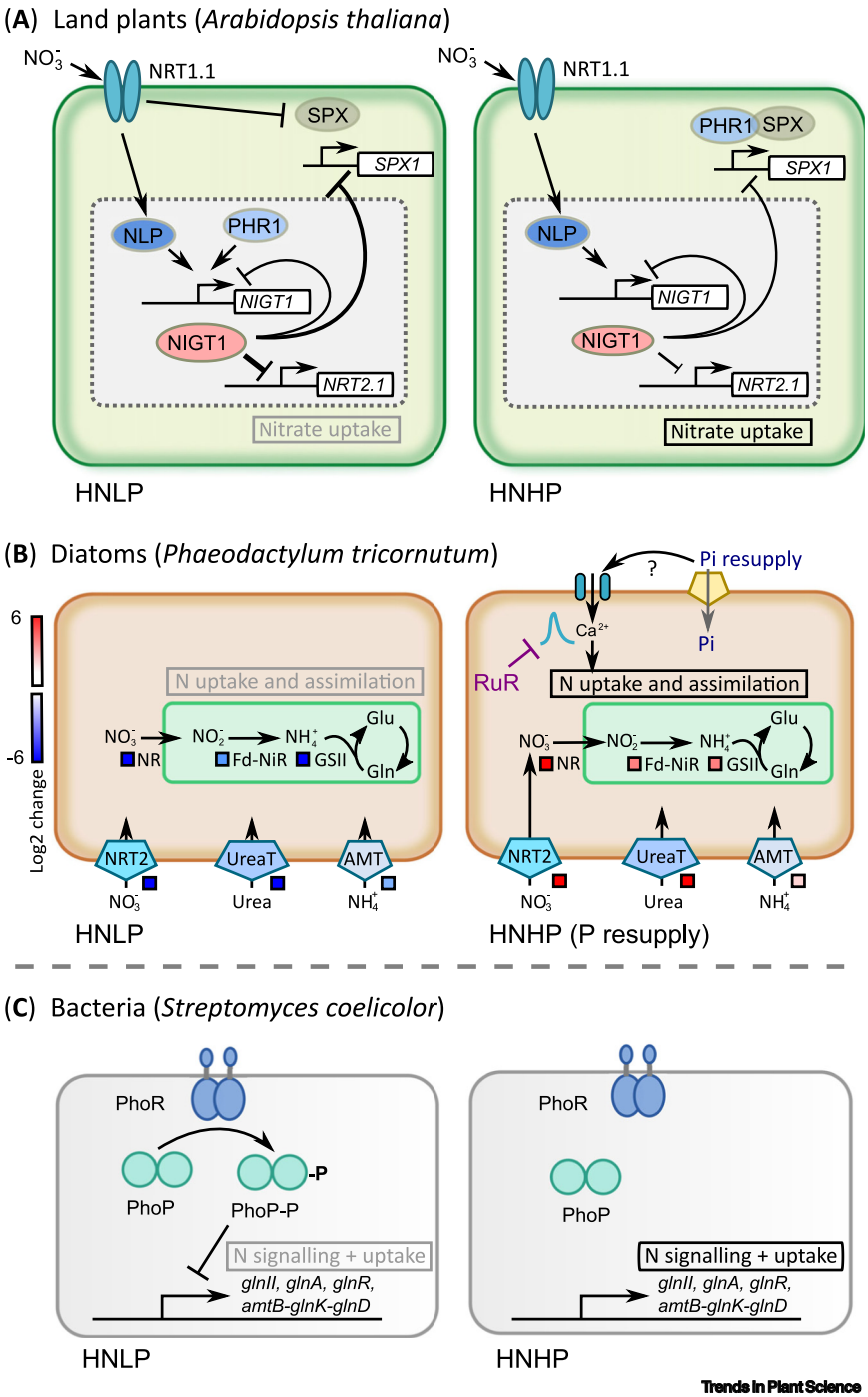

Figure 3. P-driven control of N uptake and assimilation in plants, diatoms, and bacteria. (A) Schematic diagram of a model for the role of NIGT1s in balancing N and P responses in plants, based on [82,85,96]. The nitrate–AtNRT1.1–AtNLP cascade in *Arabidopsis thaliana* (At) activates the expression of the transcription factor AtNIGT1 that is a transcriptional repressor of the high-affinity nitrate transporter gene *AtNRT2.1* [85,96]. Under P starvation, AtPHR1 enhances the expression of *AtNIGT1*, leading to further reductions in *AtNRT2.1* expression and nitrate uptake [96]. NIGT1 proteins also directly repress the expression of *SPX* genes (that encode repressors of PHR1), thus enhancing phosphate starvation responses in the presence of nitrate [82]. *NIGT1* clade genes also negatively regulate their own expression by directly competing with PHR1-dependent activation of *NIGT1* [87]. In rice (*Oryza sativa*), OsNRT1.1B (homologue of AtNRT1.1) physically interacts with and promotes the degradation of OsSPX4, causing cytoplasmic–nuclear shuttling of OsPHR2 (homologue of *AtPHR1*) to activate P uptake [81]. (B) In *Phaeodactylum tricornutum*, N uptake and assimilation proteins are less abundant under P limitation (as compared to P-replete conditions) [11]. However, their abundance rapidly increases (within 4 h) following P resupply (compared to P-limited cells), leading to increases in N uptake rates [11]. P resupply-triggered upregulation of nitrate uptake is abolished when cells are pretreated with the P–Ca^{2+} signalling inhibitor ruthenium red (RuR) before phosphate resupply. (C) Phosphate status-mediated control of N signalling and acquisition in *Streptomyces coelicolor* is governed by the two-component P starvation signalling system, PhoR–PhoB [104,105]. PhoR binds to the promoter sequences of N-related genes, including *glnII* and *glnA* (encoding glutamine synthetases), *glnR* (a major N regulator), and genes encoding the ammonium transporter (*amtB*) and two putative N-sensing/regulatory proteins (*glnK* and *glnD*) [104]. This interaction exerts a negative effect on the expression of these genes during P starvation. Abbreviations: AMT, ammonium transporter; Fd-NiR, ferredoxin-dependent nitrite reductase; GSII, glutamine synthetase II; HNHP, high-nitrogen high-phosphorus conditions; HNLP, high-nitrogen low-phosphorus conditions; NR, nitrate reductase; NRT2, nitrate transporter 2; Pi, inorganic phosphate; UreaT, urea transporter.

signalling and N metabolism that optimise the acquisition and usage of these nutrients under dynamic nutrient regimes are likely to be universally important because they have arisen independently in prokaryotes and eukaryotes.

Concluding remarks

The development of sophisticated and genetically tractable model systems has significantly advanced our understanding of N and P signalling in photosynthetic eukaryotes. These advances provide a new framework to compare and contrast taxa of diverse evolutionary origins and distinct ecological lifestyles. This has revealed the importance of conserved P-starvation signalling machinery, namely PSR1-like proteins, which are distributed across photosynthetic eukaryotes [15,16,24,25]. The importance of Ca^{2+} signalling in nutrient sensing is also emerging. Nitrate–Ca^{2+} signalling is crucial for N sensing in arabidopsis [10,60], but is not employed by the diatom *P. tricornutum* [11]. Even so, P-limited diatoms do use Ca^{2+} signalling for phosphate sensing, which has not been observed in land plants (arabidopsis) [12]. Advancing insights into the molecular machinery underpinning such processes will enable us to better understand their distribution and evolution through mining expanding 'omic' resources [109,110]. Meanwhile, the acceleration of genetic tool development for diverse photosynthetic eukaryotes [111] will support essential functional studies and allow evolutionary hypotheses to be tested. Coupled with new techniques such as phosphoproteomics, this will present opportunities to identify regulatory targets [112,113]. Crucially, it is vital to recognise how one system can advance insights into another. The early identification of *CrPSR1* in *C. reinhardtii* [15] helped to inform our understanding of plant P-starvation mechanisms [16]. Similarly, the discovery of nitrate–Ca^{2+} signalling in arabidopsis [10,60] stimulated investigations into Ca^{2+}-dependent nutrient-sensing mechanisms in diatoms [11]. Future breakthroughs will undoubtedly inform new directions in algal research, and vice versa. This will be vital not only for expediting knowledge of the evolution of N and P signalling in photosynthetic eukaryotes but could also help in engineering strategies to optimise N and P acquisition in crops.

Acknowledgments

This work was supported by Natural Environment Research Council (NERC) Independent Research Fellowship NE/R015449/2 and Biotechnology and Biological Sciences Research Council (BBSRC) New Investigator Grant BB/W006286/1. Thanks also go to two anonymous reviewers for their careful evaluation and insightful comments that greatly improved this manuscript. This paper did not generate any new data.

Declaration of interests

No interests are declared.

References

1. Archibald, J.M. (2009) The puzzle of plastid evolution. *Curr. Biol.* 19, R81–R88
2. Burki, F. *et al.* (2020) The new tree of eukaryotes. *Trends Ecol. Evol.* 35, 43–55
3. Raghothama, K.G. and Karthikeyan, A.S. (2005) Phosphate acquisition. *Plant Soil* 274, 37–49
4. Duhamel, S. *et al.* (2021) Phosphorus as an integral component of global marine biogeochemistry. *Nat. Geosci.* 14, 359–368
5. Elser, J.J. *et al.* (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10, 1135–1142
6. Malone, T.C. and Newton, A. (2020) The globalization of cultural eutrophication in the coastal ocean: causes and consequences. *Front. Mar. Sci.* 7, 670
7. Baker, A. *et al.* (2015) Replace, reuse, recycle: improving the sustainable use of phosphorus by plants. *J. Exp. Bot.* 66, 3523–3540
8. Güsewell, S. (2004) N:P ratios in terrestrial plants: variation and functional significance. *New Phytol.* 164, 243–266
9. Geider, R.J. and La Roche, J. (2002) Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* 37, 1–17
10. Liu, K. *et al.* (2017) Discovery of nitrate–CPK–NLP signalling in central nutrient–growth networks. *Nature* 545, 311–316
11. Helliwell, K.E. *et al.* (2021) A novel Ca^{2+} signaling pathway coordinates environmental phosphorus sensing and nitrogen metabolism in marine diatoms. *Curr. Biol.* 31, 978–989
12. Matthus, E. *et al.* (2019) Phosphate starvation alters abiotic-stress-induced cytosolic free calcium increases in roots. *Plant Physiol.* 179, 1754–1767
13. Krouk, G. and Kiba, T. (2020) Nitrogen and phosphorus interactions in plants: from agronomic to physiological and molecular insights. *Curr. Opin. Plant Biol.* 57, 104–109
14. Ueda, Y. and Yanagisawa, S. (2019) Perception, transduction, and integration of nitrogen and phosphorus nutritional signals in the transcriptional regulatory network in plants. *J. Exp. Bot.* 70, 3709–3717
15. Wykoff, D.D. *et al.* (1999) Psr1, a nuclear localized protein that regulates phosphorus metabolism in *Chlamydomonas*. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15336–15341
16. Rubio, V. *et al.* (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes Dev.* 15, 2122–2133
17. Safi, A. *et al.* (2017) The world according to GARP transcription factors. *Curr. Opin. Plant Biol.* 39, 159–167
18. Aung, K. *et al.* (2006) pho2, a phosphate overaccumulator, is caused by a nonsense mutation in a microRNA399 target gene. *Plant Physiol.* 141, 1000–1011

Outstanding questions

Molecular mechanisms

Are the nitrate signalling mechanisms of land plants employed by different algal taxa? If so, are N signalling components integrated with P-starvation signalling master regulators in a similar manner?

How do N and P signalling networks interact with the regulatory networks of other nutrients?

What specific genes and proteins are involved in the generation and transduction of diatom P–Ca^{2+} signals?

Evolutionary questions

Is P–Ca^{2+} signalling employed by other photosynthetic eukaryotes?

If P–Ca^{2+} signalling is confined to diatoms, have photosynthetic eukaryotes evolved alternative mechanisms to sense P replenishment? If so, what are the selective and/or functional benefits of one mechanism over another?

How has the process of endosymbiosis (primary vs secondary/tertiary) shaped the origin and distribution of nutrient signalling pathways in photosynthetic eukaryotes?

Ecological questions

Do the distinct mechanisms for N and P sensing/signalling in photosynthetic eukaryotes reflect the adaptation of plants and algae to different physical environments – for example, terrestrial versus freshwater and marine (coastal and open ocean ecosystems)?

Is the influence of N status on P-responsive gene expression greater than that of P status on N-responsive gene expression in aquatic algae, as has been observed in arabidopsis? If not, does this reflect differences in phosphate accessibility in terrestrial versus aquatic ecosystems?

Applied research question

Can learning from the diversity and efficiency of plant and algal nutrient signalling systems help to better engineer optimised N and P acquisition and storage in important crop species?

19. Bari, R. *et al.* (2006) PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. *Plant Physiol.* 141, 988–999
20. Franco-Zorrilla, J.M. *et al.* (2007) Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat. Genet.* 39, 1033–1037
21. Liu, T.Y. *et al.* (2012) PHO2-dependent degradation of PHO1 modulates phosphate homeostasis in *Arabidopsis*. *Plant Cell* 24, 2168–2183
22. Huang, T.K. *et al.* (2013) Identification of downstream components of ubiquitin-conjugating enzyme PHOSPHATE2 by quantitative membrane proteomics in *Arabidopsis* roots. *Plant Cell* 25, 4044–4060
23. Voshall, A. *et al.* (2017) miRNAs in the alga *Chlamydomonas reinhardtii* are not phylogenetically conserved and play a limited role in responses to nutrient deprivation. *Sci. Rep.* 7, 5462
24. Fiore, C. *et al.* (2021) A phosphate starvation response gene (PSR1-like) is present and expressed in *Micromonas pusilla* and other marine algae. *Aquat. Microb. Ecol.* 86, 29–46
25. Kumar Sharma, A. *et al.* (2020) The Myb-like transcription factor phosphorus starvation response (PtPSR) controls conditional P acquisition and remodelling in marine microalgae. *New Phytol.* 225, 2380–2395
26. Bustos, R. *et al.* (2010) A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in *Arabidopsis*. *PLoS Genet.* 6, e1001102
27. Sun, L. *et al.* (2015) *Arabidopsis* PHL2 and PHR1 act redundantly as the key components of the central regulatory system controlling transcriptional responses to phosphate starvation. *Plant Physiol.* 170, 499–514
28. Wang, Z. *et al.* (2018) Functional characterization of *Arabidopsis* PHL4 in plant response to phosphate starvation. *Front. Plant Sci.* 9, 1432
29. Ruan, W. *et al.* (2017) Phosphate starvation induced OsPHR4 mediates Pi-signaling and homeostasis in rice. *Plant Mol. Biol.* 93, 327–340
30. Guo, M. *et al.* (2015) Integrative comparison of the role of the PHOSPHATE RESPONSE 1 subfamily in phosphate signaling and homeostasis in rice. *Plant Physiol.* 168, 1762–1776
31. Zhou, J. *et al.* (2008) OsPHR2 is involved in phosphate-starvation signaling and excessive phosphate accumulation in shoots of plants. *Plant Physiol.* 146, 1673–1686
32. Cruz de Carvalho, M.H. *et al.* (2016) Noncoding and coding transcriptome responses of a marine diatom to phosphate fluctuations. *New Phytol.* 210, 497–510
33. Lv, Q. *et al.* (2014) SPX4 negatively regulates phosphate signaling and homeostasis through its interaction with PHR2 in rice. *Plant Cell* 26, 1586–1597
34. Zhong, Y. *et al.* (2018) Rice SPX6 negatively regulates the phosphate starvation response through suppression of the transcription factor PHR2. *New Phytol.* 219, 135–148
35. Wang, Z. *et al.* (2014) Rice SPX1 and SPX2 inhibit phosphate starvation responses through interacting with PHR2 in a phosphate-dependent manner. *Proc. Natl. Acad. Sci. U. S. A.* 111, 14953–14958
36. Sanz-Luque, E. *et al.* (2020) Polyphosphate: a multifunctional metabolite in cyanobacteria and algae. *Front. Plant Sci.* 11, 938
37. Dyhrman, S.T. *et al.* (2012) The transcriptome and proteome of the diatom *Thalassiosira pseudonana* reveal a diverse phosphorus stress response. *PLoS One* 7, e33768
38. Zhang, K. *et al.* (2021) SPX-related genes regulate phosphorus homeostasis in the marine phytoplankton, *Phaeodactylum tricornutum*. *Commun. Biol.* 4, 797
39. Balzergue, C. *et al.* (2017) Low phosphate activates STOP1–ALMT1 to rapidly inhibit root cell elongation. *Nat. Commun.* 8, 15300
40. Mora-Macías, J. *et al.* (2017) Malate-dependent Fe accumulation is a critical checkpoint in the root developmental response to low phosphate. *Proc. Natl. Acad. Sci. U. S. A.* 114, E3563–E3572
41. Rico-Reséndiz, F. *et al.* (2020) Transcriptional and morpho-physiological responses of *Marchantia polymorpha* upon phosphate starvation. *Int. J. Mol. Sci. 2020, Vol. 21, Page 8354* 21, 8354
42. Wild, R. *et al.* (2016) Control of eukaryotic phosphate homeostasis by inositol polyphosphate sensor domains. *Science* 352, 986–990
43. Lorenzo-Orts, L. *et al.* (2020) Identity and functions of inorganic and inositol polyphosphates in plants. *New Phytol.* 225, 637–652
44. Gerasimaite, R. *et al.* (2017) Inositol pyrophosphate specificity of the SPX-dependent polyphosphate polymerase VTC. *ACS Chem. Biol.* 12, 648–653
45. Couso, I. *et al.* (2016) Synergism between inositol polyphosphates and TOR kinase signaling in nutrient sensing, growth control, and lipid metabolism in *Chlamydomonas*. *Plant Cell* 28, 2026–2042
46. Couso, I. *et al.* (2020) Phosphorus availability regulates TORC1 signaling via LST8 in *Chlamydomonas*. *Plant Cell* 32, 69–80
47. Malviya, S. *et al.* (2016) Insights into global diatom distribution and diversity in the world's ocean. *Proc. Natl. Acad. Sci. U. S. A.* 113, E1516–E1525
48. Litchman, E. (2007) Resource competition and the ecological success of phytoplankton. In *Evolution of Primary Producers in the Sea* (Falkowski, P. and Knoll, A., eds), pp. 351–375, Elsevier
49. Downes-Tettmar, N. *et al.* (2013) Seasonal variation in *Pseudo-nitzschia* spp. and domoic acid in the Western English Channel. *Cont. Shelf Res.* 53, 40–49
50. Armbrust, E.V. (2009) The life of diatoms in the world's oceans. *Nature* 459, 185–192
51. Lin, H.Y. *et al.* (2013) Identification and characterization of an extracellular alkaline phosphatase in the marine diatom *Phaeodactylum tricornutum*. *Mar. Biotechnol.* 15, 425–436
52. Chiou, T.J. and Lin, S.I. (2011) Signaling network in sensing phosphate availability in plants. *Annu. Rev. Plant Biol.* 62, 185–206
53. Cheng, N.H. *et al.* (2005) Functional association of *Arabidopsis* CAX1 and CAX3 is required for normal growth and ion homeostasis. *Plant Physiol.* 138, 2048–2060
54. Tanaka, K. *et al.* (2014) Extracellular ATP acts as a damage-associated molecular pattern (DAMP) signal in plants. *Front. Plant Sci.* 5, 446
55. Choi, J. *et al.* (2014) Identification of a plant receptor for extracellular ATP. *Science* 343, 290–294
56. Jordan, M.B. and Joint, I. (1998) Seasonal variation in nitrate: phosphate ratios in the English Channel 1923–1987. *Estuar. Coast. Shelf Sci.* 46, 157–164
57. Alipanah, L. *et al.* (2015) Whole-cell response to nitrogen deprivation in the diatom *Phaeodactylum tricornutum*. *J. Exp. Bot.* 66, 6281–6296
58. Smith, S.R. *et al.* (2019) Evolution and regulation of nitrogen flux through compartmentalized metabolic networks in a marine diatom. *Nat. Commun.* 10, 4552
59. Engelsberger, W.R. and Schulze, W.X. (2012) Nitrate and ammonium lead to distinct global dynamic phosphorylation patterns when resupplied to nitrogen-starved *Arabidopsis* seedlings. *Plant J.* 69, 978–995
60. Riveras, E. *et al.* (2015) The calcium ion is a second messenger in the nitrate signaling pathway of *Arabidopsis*. *Plant Physiol.* 169, 1397–1404
61. Ho, C.-H. *et al.* (2009) CHL1 functions as a nitrate sensor in plants. *Cell* 138, 1184–1194
62. Wang, X. *et al.* (2021) A transceptor–channel complex couples nitrate sensing to calcium signaling in *Arabidopsis*. *Mol. Plant* 14, 774–786
63. Liu, K. and Tsay, Y. (2003) Switching between the two action modes of the dual-affinity nitrate transporter CHL1 by phosphorylation. *EMBO J.* 22, 1005–1013
64. Verret, F. *et al.* (2010) Calcium channels in photosynthetic eukaryotes: implications for evolution of calcium-based signalling. *New Phytol.* 187, 23–43
65. Caló, G. *et al.* (2017) Ancient signal for nitrogen status sensing in the green lineage: functional evidence of CDPK repertoire in *Ostreococcus tauri*. *Plant Physiol. Biochem.* 118, 377–384
66. Camargo, A. *et al.* (2007) Nitrate signaling by the regulatory gene NIT2 in *Chlamydomonas*. *Plant Cell* 19, 3491–3503
67. Konishi, M. and Yanagisawa, S. (2013) *Arabidopsis* NIN-like transcription factors have a central role in nitrate signalling. *Nat. Commun.* 4, 1617
68. Rayko, E. *et al.* (2010) Transcription factor families inferred from genome sequences of photosynthetic stramenopiles. *New Phytol.* 188, 52–66
69. Llamas, A. *et al.* (2002) Nitrate signalling on the nitrate reductase gene promoter depends directly on the activity of the nitrate transport systems in *Chlamydomonas*. *Plant J.* 30, 261–271

70. Ma, Q. *et al.* (2015) The calcium sensor CBL7 modulates plant responses to low nitrate in *Arabidopsis*. *Biochem. Biophys. Res. Commun.* 468, 59–65
71. Zhao, M. *et al.* (2011) Involvement of miR169 in the nitrogen-starvation responses in *Arabidopsis*. *New Phytol.* 190, 906–915
72. Rubin, G. *et al.* (2009) Members of the LBD family of transcription factors Repress anthocyanin synthesis and affect additional nitrogen responses in *Arabidopsis*. *Plant Cell* 21, 3567–3584
73. Imamura, S. *et al.* (2009) R2R3-type MYB transcription factor, CmMYB1, is a central nitrogen assimilation regulator in *Cyanidioschyzon merolae*. *Proc. Natl. Acad. Sci. U. S. A.* 106, 12548–12553
74. Allen, A.E. *et al.* (2011) Evolution and metabolic significance of the urea cycle in photosynthetic diatoms. *Nature* 473, 203–207
75. Matthijs, M. *et al.* (2017) The transcription factor bZIP14 regulates the TCA cycle in the diatom *Phaeodactylum tricornutum*. *EMBO J.* 36, 1559–1576
76. Matthijs, M. *et al.* (2016) Profiling of the early nitrogen stress response in the diatom *Phaeodactylum tricornutum* reveals a novel family of RING-domain transcription factors. *Plant Physiol.* 170, 489–498
77. Hu, B. and Chu, C. (2020) Nitrogen–phosphorus interplay: old story with molecular tale. *New Phytol.* 225, 1455–1460
78. Kant, S. *et al.* (2011) Genetic regulation by NLA and microRNA827 for maintaining nitrate-dependent phosphate homeostasis in *Arabidopsis*. *PLoS Genet.* 7, e1002021
79. Lin, W.Y. *et al.* (2013) NITROGEN LIMITATION ADAPTATION, a target of microRNA827, mediates degradation of plasma membrane–localized phosphate transporters to maintain phosphate homeostasis in *Arabidopsis*. *Plant Cell* 25, 4061
80. Smith, F.W. and Jackson, W.A. (1987) Nitrogen enhancement of phosphate transport in roots of *Zea mays* L. *Plant Physiol.* 84, 1314–1318
81. Hu, B. *et al.* (2019) Nitrate–NRT1.1B–SPX4 cascade integrates nitrogen and phosphorus signalling networks in plants. *Nat. Plants* 5, 401–413
82. Ueda, Y. *et al.* (2020) Nitrate-inducible NIGT1 proteins modulate phosphate uptake and starvation signalling via transcriptional regulation of SPX genes. *Plant J.* 102, 448–466
83. Medici, A. *et al.* (2019) Identification of molecular integrators shows that nitrogen actively controls the phosphate starvation response in plants. *Plant Cell* 31, 1171–1184
84. Medici, A. *et al.* (2015) AtNIGT1/HRS1 integrates nitrate and phosphate signals at the *Arabidopsis* root tip. *Nat. Commun.* 6, 6274
85. Kiba, T. *et al.* (2018) Repression of nitrogen starvation responses by members of the *Arabidopsis* GARP-type transcription factor NIGT1/HRS1 subfamily. *Plant Cell* 30, 925–945
86. Perini, V. and Bracken, M.E.S. (2014) Nitrogen availability limits phosphorus uptake in an intertidal macroalga. *Oecologia* 175, 667–676
87. Rhee, G.-Y. (1974) Phosphate uptake under nitrate limitation by *Scenedesmus* sp. and its ecological implications. *J. Phycol.* 10, 470–475
88. Chu, F.F. *et al.* (2013) Phosphorus plays an important role in enhancing biodiesel productivity of *Chlorella vulgaris* under nitrogen deficiency. *Bioresour. Technol.* 134, 341–346
89. Chu, F.F. *et al.* (2015) Polyphosphate during the regreening of *Chlorella vulgaris* under nitrogen deficiency. *Int. J. Mol. Sci.* 16, 23355–23368
90. Sanz-Luque, E. *et al.* (2020) Metabolic control of acclimation to nutrient deprivation dependent on polyphosphate synthesis. *Sci. Adv.* 6, eabb5351
91. Perry, M.J. (1976) Phosphate utilization by an oceanic diatom in phosphorus-limited chemostat culture and in the oligotrophic waters of the central North Pacific. *Limnol. Oceanogr.* 21, 88–107
92. Fitzsimons, M.F. *et al.* (2020) Dissolved organic phosphorus uptake by marine phytoplankton is enhanced by the presence of dissolved organic nitrogen. *J. Exp. Mar. Bio. Ecol.* 530–531, 151434
93. Rufty, T.W. *et al.* (1990) Phosphorus stress effects on assimilation of nitrate. *Plant Physiol.* 94, 328–333
94. Lee, R.B. (1982) Selectivity and kinetics of ion uptake by barley plants following nutrient deficiency. *Ann. Bot.* 50, 429–449
95. De Magalhães, J.V. *et al.* (1998) Nitrate uptake by corn under increasing periods of phosphorus starvation. *J. Plant Nutr.* 21, 1753–1763
96. Maeda, Y. *et al.* (2018) A NIGT1-centred transcriptional cascade regulates nitrate signalling and incorporates phosphorus starvation signals in *Arabidopsis*. *Nat. Commun.* 9, 1376
97. Ye, J.Y. *et al.* (2021) STOP1 activates NRT1.1-mediated nitrate uptake to create a favorable rhizospheric pH for plant adaptation to acidity. *Plant Cell* 33, 3658–3674
98. Kellermeier, F. *et al.* (2014) Analysis of the root system architecture of *Arabidopsis* provides a quantitative readout of crosstalk between nutritional signals. *Plant Cell* 26, 1480–1496
99. Wurch, L.L. *et al.* (2019) Transcriptional shifts highlight the role of nutrients in harmful brown tide dynamics. *Front. Microbiol.* 10, 136
100. Rokitta, S.D. *et al.* (2016) P- and N-depletion trigger similar cellular responses to promote senescence in eukaryotic phytoplankton. *Front. Mar. Sci.* 3, 109
101. Wurch, L.L. *et al.* (2011) Proteome changes driven by phosphorus deficiency and recovery in the brown tide-forming alga *Aureococcus anophagefferens*. *PLoS One* 6, e28949
102. Guo, J. *et al.* (2018) Specialized proteomic responses and an ancient photoprotection mechanism sustain marine green algal growth during phosphate limitation. *Nat. Microbiol.* 3, 781–790
103. Thiriet-Rupert, S. *et al.* (2016) Transcription factors in microalgae: genome-wide prediction and comparative analysis. *BMC Genomics* 17, 282
104. Rodríguez-García, A. *et al.* (2009) Phosphate control over nitrogen metabolism in *Streptomyces coelicolor*: direct and indirect negative control of glnR, glnA, glnII and amtB expression by the response regulator PhoP. *Nucleic Acids Res.* 37, 3230–3242
105. Rodríguez-García, A. *et al.* (2007) Genome-wide transcriptomic and proteomic analysis of the primary response to phosphate limitation in *Streptomyces coelicolor* M145 and in a ΔphoP mutant. *Proteomics* 7, 2410–2429
106. Sola-Landa, A. *et al.* (2003) The two-component PhoR–PhoP system controls both primary metabolism and secondary metabolite biosynthesis in *Streptomyces lividans*. *Proc. Natl. Acad. Sci. U. S. A.* 100, 6133–6138
107. Krol, E. and Becker, A. (2004) Global transcriptional analysis of the phosphate starvation response in *Sinorhizobium meliloti* strains 1021 and 2011. *Mol. Gen. Genomics.* 272, 1–17
108. VanBogelen, R.A. *et al.* (1996) Global analysis of proteins synthesized during phosphorus restriction in *Escherichia coli*. *J. Bacteriol.* 178, 4344–4366
109. Leebens-Mack, J.H. *et al.* (2019) One thousand plant transcriptomes and the phylogenomics of green plants. *Nature* 574, 679–685
110. Keeling, P.J. *et al.* (2014) The marine microbial eukaryote transcriptome sequencing project (MMETSP): illuminating the functional diversity of eukaryotic life in the oceans through transcriptome sequencing. *PLoS Biol.* 12, e1001889
111. Faktorová, D. *et al.* (2020) Genetic tool development in marine protists: emerging model organisms for experimental cell biology. *Nat. Methods* 17, 481–494
112. Liu, K.-H. *et al.* (2020) Primary nitrate responses mediated by calcium signalling and diverse protein phosphorylation. *J. Exp. Bot.* 71, 4428–4441
113. Tan, M.H. *et al.* (2020) The importance of protein phosphorylation for signaling and metabolism in response to diel light cycling and nutrient availability in a marine diatom. *Biology (Basel)* 9, 155
114. Stiller, J.W. *et al.* (2014) The evolution of photosynthesis in chromist algae through serial endosymbioses. *Nat. Commun.* 5, 5764
115. Dorrell, R.G. *et al.* (2017) Chimeric origins of ochrophytes and haptophytes revealed through an ancient plastid proteome. *eLife* 6, e23717

116. Keeling, P.J. and Burki, F. (2019) Progress towards the tree of eukaryotes. *Curr. Biol.* 29, R808–R817
117. Fan, X. *et al.* (2017) Plant nitrate transporters: from gene function to application. *J. Exp. Bot.* 68, 2463–2475
118. Feng, H. *et al.* (2011) Spatial expression and regulation of rice high-affinity nitrate transporters by nitrogen and carbon status. *J. Exp. Bot.* 62, 2319–2332
119. McDonald, S.M. *et al.* (2010) The mixed lineage nature of nitrogen transport and assimilation in marine eukaryotic phytoplankton: a case study of *Micromonas*. *Mol. Biol. Evol.* 27, 2268–2283
120. Sanz-Luque, E. *et al.* (2015) Understanding nitrate assimilation and its regulation in microalgae. *Front. Plant Sci.* 6, 899