Review

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Phytohormones in microalgae: a new opportunity for microalgal biotechnology?

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Phytohormones, including auxin, abscisic acid (ABA), cytokinin (CK), ethylene (ET), and gibberellins (GAs), have been found in a broad spectrum of microalgal lineages. Although the functional role of microalgal endogenous phytohormones remains elusive, molecular evidence from the oleaginous microalga *Nannochloropsis oceanica* suggests that endogenous ABA and CK are functional and that their physiological effects are similar to those in higher plants. In this Opinion article, proceeding from genome-based metabolic reconstruction, we suggest that modern higher plant phytohormone biosynthesis pathways originate from ancient microalgae even though some of the microalgal phytohormone signaling pathways remain unknown. Dissection and manipulation of microalgal phytohormone systems could offer a new view of phytohormone evolution in plants and present new opportunities in developing microalgal feedstock for biofuels.

Profile and function of phytohormones in microalgae

Phytohormones (see Glossary) are a class of small molecules that serve as chemical messengers to coordinate cellular activities in higher plants [1]. Phytohormone systems generally involve biosynthesis pathways that produce phytohormones and signal transduction pathways that mediate the effects of phytohormones. It has been suggested that higher plant hormone systems evolved from a pre-existing primary metabolic system in microalgae [2]; however, evidence for the existence and function of phytohormones in microalgae remains fragmentary. A recent surge in the number of microalgal genomic studies being performed owing to the potential use of microalgae as a biofuel feedstock has enabled the footprints of the biosynthetic and signaling processes of phytohormones to be traced in the major microalgal lineages. In this Opinion article we present a survey of the presence of phytohormone molecules in microalgae, including Stramenopiles (diatoms, brown algae, and eustigmatophytes), Archaeplastida (or Plantae, which includes red algae, glaucophytes, and green algae; the first land plants are descendants of ancient green algae, collectively referred to as the streptophyte algae [3]), and cyanobacteria, which share a common ancestor with the endosymbiont that evolved into higher plant chloroplasts [4] (Table S1 in the supplementary material online). Although the physiological roles remain largely unknown, the essential and bioactive forms of the five classical phytohormones, auxin, ABA, CKs, GAs, and ET, have been detected in a wide range of algal lineages [5–32] (Table 1). Moreover, the phytohormone profiles of microalgae can resemble those of higher plants, although they may feature different dominant species and biosynthetic intermediates [10].

The functional roles of most phytohormones in microalgae have been controversial because nearly all the 'evidence' has been derived or inferred from the physiological effect on microalgal cells of exogenous higher plant phytohormones or from correlations between environmental stimuli and the endogenous phytohormone content (whereas functional studies of endogenous microalgal phytohormones have been rare [10,33]; Table 2). Nevertheless, molecular evidence for phytohormone function in microalgae is beginning to accumulate.

Glossary

Autocrine signaling: a signaling mode in which a cell secretes a hormone or chemical messenger that binds to receptors on the same cell, leading to a response.
Endocrine system: a signaling system in which hormones are synthesized and released into a circulatory system and that have actions within the organism itself.
Exocrine system: a signaling system in which hormones are synthesized and released from an organism as liquid or gas and then exert a function on a second organism.
Horizontal gene transfer: the transmission of genetic material (i.e., DNA) between genomes of different species or between the organelles of eukaryotes (i.e., the nucleus, the mitochondrion, and the chloroplast). Horizontal gene transfer is distinguished from vertical gene transfer which is known as gene flow from parents to offspring. The term is also known as lateral gene transfer.
Pheromone: a secreted or excreted chemical factor capable of acting outside of the body (or cell) of the secreting individual to impact on the behavior of the receiving individual.
Phytohormone: a class of signal molecules that are produced in extremely low concentrations and regulate a variety of cellular processes in plants. Phytohormones are produced not only by higher plants but also by algae and by microorganisms such as fungi and bacteria.
Quorum-sensing: a phenomenon in which bacteria, in response to fluctuations in cell population density, secrete and release chemical signal molecules to coordinate activities among different cells in the population.

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Keywords: phytohormone system; microalgae; plant evolution; genetic engineering.

1360-1385/
 http://dx.doi.org/10.1016/j.tplants.2015.01.006

Table 1. Discovery of phytohormones in cyanobacteria and algae

Phytohormone	Cyanobacteria	Diatoms	Eustigmatophytes	Brown algae (multicellular)	Red algae (multicellular)	Green algae
Auxin	*Synechocystis* sp., *Chroococcidiopsis* sp., *Anabaena* sp., *Phormidium* sp., *Oscillatoria* sp., *Nostoc* sp. [16–18]	N/A	N/A	*Ectocarpus siliculosus* [38]	*Prionitis lanceolata*, *Porphyra* sp., *Gelidium* sp., *Gracilaria* sp., *Gracilariopsis* sp., *Chondracanthus* sp., *Hypnea* sp. [9,19]	*Scenedesmus armatus*, *Chlorella pyrenoidosa*, *Chlorella minutissima* [11,20]
ET	*Synechococcus* sp., *Anabaena* sp., *Nostoc* sp., *Calothrix* sp., *Scytonema* sp., *Cylindrospermum* sp. [15]	N/A	N/A	*Padina arborescens*, *Ecklonia maxima* [21]	*Porphyra tenera* [21]	*Chlorella pyrenoidosa* [22]
ABA	*Synechococcus leopoliensis*, *Nostoc muscorum*, *Trichormus variabilis*, *Anabaena variabilis* [23,49,70]	*Coscinodiscus granii* [14]	*Nannochloropsis oceanica* [10]	*Ascophyllum nodosum* [24]	*Porphyra sp.*, *Gelidium sp.*, *Gracilaria sp.*, *Gracilariopsis sp.*, *Chondracanthus sp.*, *Hypnea sp.* [9]	*Chlamydomonas reinhardtii*, *Dunaliella sp.*, *Draparnaldia mutabilis*, *Chlorella minutissima* [11,25]
CK	*Synechocystis* sp., *Chroococcidiopsis* sp., *Anabaena* sp., *Phormidium* sp., *Oscillatoria* sp., *Calothrix* sp., *Chlorogloeopsis* sp., *Rhodospirillum* sp. [17,71]	*Ecklonia* sp. [26]	*Nannochloropsis oceanica* [10]	*Ecklonia maxima*, *Laminaria pallida* [27,28]	*Porphyra* sp., *Gelidium* sp., *Gracilaria* sp., *Gracilariopsis* sp., *Chondracanthus* sp., *Hypnea* sp., *Gigartina clathrata*, *Hypnea* sp. [26,28]	*Chlorella minutissima* [11]
GA	*Anabaenopsis* sp., *Cylindrospermum* sp., *Phormidium foveolarum* [29,71]	N/A	*Nannochloropsis oceanica* (Y. Lu *et al.*, unpublished)	*Ecklonia radiata* [30]	*Hypnea musciformis* [31]	*Chlorella* sp., *Chlamydomonas reinhardtii* [11,32]

Abbreviation: N/A, no reports available.

The roles played by ABA and CKs as regulators of physiological processes or of the cellular response to abiotic stresses have been widely reported in higher plants. ABA has been reported to function as a stress molecule in cyanobacteria (salt stress [23]) and in unicellular eukaryotic algae (salt, osmotic, oxidative, drought and nutrient stresses [10,34–37]). In the unicellular oleaginous microalga *Nannochloropsis oceanica*, biosynthetic pathways of ABA and CKs are transcriptionally up- and downregulated, respectively, upon nitrogen deprivation. Moreover, the endogenous ABA and CKs seem to act antagonistically in response to nitrogen depletion. Consistent with this, exogenous CKs were found to stimulate cell cycle progression whereas exogenous ABA acts as both an algal growth repressor and a positive regulator that enhances stress tolerance [10]. These observations suggest that ABA and CKs play a sophisticated regulatory role in the orchestration of cellular homeostasis to cope with variable environmental factors [10]. The auxin indole-3-acetic acid (IAA) regulates growth and development in higher plants; in the multicellular brown alga *Ectocarpus siliculosus*, IAA appears to play a regulatory role in relaying cell–cell positional information and in the induction of a signaling pathway distinct from that known in land plants [38]. ET was recently found to be produced in the filamentous charophyte *Spirogyra pratensis* and regulate cell development, implying that the ET hormone system has emerged before land colonization, and is homologous to that in plants [39]. GAs and ET have been found in *Chlamydomonas* spp. and *Chlorella* spp. and seem to be involved in certain biological activities such as growth and senescence [12,40,41]. Therefore, phytohormone-mediated regulatory mechanisms, at least particular components of them, should be present in a wide range of modern microalgal lineages and, thus, may have been in place in the common unicellular ancestor of microalgae. It has been postulated that the last common unicellular ancestor of the Stramenopiles and Archaeplastida may have already been capable of synthesizing a wide array of phytohormones [42].

Phytohormone biosynthetic pathways in microalgae and higher plants

There is accumulating evidence that, in addition to the various phytohormone metabolites, microalgae possess functional but relatively simple biosynthetic pathways that are equivalent to those in higher plants (Figure 1) [2,5]. However, although microalgal phytohormone biosynthetic pathways and those in higher plants share most key components, there are crucial distinctions.

For instance, both microalgae and higher plants use an 'indirect' pathway for ABA synthesis via the cleavage of a

Table 2. Exploiting phytohormone metabolism for microalgal feedstock development

Phytohormone	Functions in higher plants (examples)	Strategy of perturbation	Physiological impacts in higher plants	Relevant findings in microalgae	Potential implication for microalgal biotechnology
ABA	Development and growth	Overexpression of the zeaxanthin epoxidase (*ZEP*) gene	Increased ABA content and delayed germination [72]	Exogenous ABA decreased growth rate of marine diatom *Coscinodiscus granii* [14] and eustigmatophyte *Nannochloropsis oceanica* [10]	Improvement of stress tolerance
		Silencing *ZEP* gene	Reduced ABA content and rapid seed germination [72]		
	Stress tolerance	Overexpression of the 9-*cis*-epoxycarotenoid dioxygenase gene	Increased ABA level and enhanced drought tolerance [73]	Exogenous ABA improved stress tolerance to dehydration (green alga *Haematococcus pluvialis* [34]), higher salinity (*Dunaliella* sp. [35] and *Chlamydomonas reinhardtii* [37]), nitrogen deprivation (*Nannochloropsis oceanica* [10]), oxidative stress (*Chlamydomonas reinhardtii* [36]), or osmotic stress (*Chlamydomonas reinhardtii* [37])	
CK	Development and growth	Overexpression of the CK oxidase/ dehydrogenase gene (*CKX*) gene	Reduced CK content and increased growth rate [74]	Exogenous CK improved cell cycle progression (*Nannochloropsis oceanica* [10]), growth rate (*Nannochloropsis oceanica* [10] and *Chlamydomonas reinhardtii* [40,75]), and oil content (*Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum*, and *Haematococcus pluvialis* [33]); endogenous CKs are related with light regime (*Chlorella* sp. [7,11]) and cell cycle (*Nannochloropsis oceanica* [10] and *Chlorella* sp. [7]); genetically engineered microalgae with increased oil content (*Chlorella vulgaris* and *Chlamydomonas reinhardtii* overexpressing IPT [33])	Elevation of microalgal growth rate, oil content, and stress tolerance
		Overexpression of the isopentenyltransferase (*IPT*) gene	Elevated CK content, stunted growth and delayed senescence [76]		
	Stress tolerance	Overexpression of the *IPT* gene	Increased CK level and elevated tolerance to water deficit [77]	High temperature led to elevated CK contents (*Ecklonia maxima* and *Macrocystis pyrifera* [78])	
		Overexpression of the *CKX* gene	Enhanced root-specific CK degradation and elevated drought tolerance [79]		
Auxin	Development and growth	Overexpression of the *iaaM* gene	Increased IAA levels and parthenocarpic fruit development [80]	Exogenous IAA improved growth rate (*Chlamydomonas reinhardtii* [40], coccolithophorid alga *Pleurochrysis carterae*, *Chlorella vulgaris*, *Chlorella sorokiniana*, *Haematococcus pluvialis*, diatom *Phaeodactylum tricornutum* and cyanobacterium *Nostoc* sp. [81]) and oil content (*Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum*, and *Haematococcus pluvialis* [33])	Elevation of microalgal growth rate, stress tolerance, oil content, and biomass productivity
	Stress tolerance	Overexpressing PIN3	Improved drought tolerance [80]	Auxin induced tolerance to higher salinity and extreme temperatures (*Chlorella vulgaris*, *Rhizoclonium* sp. and *Pithophora* sp. [81,82])	
GA	Development and growth	Silencing GA 20-oxidase 1, GA20ox2, and GA20ox3 genes	Decreased GAs levels and a semi-dwarf phenotype [83]	Exogenous GA improved growth rate (*Chlamydomonas reinhardtii* [40])	Elevation of microalgal growth rate and biomass productivity
		Overexpression of the GA 20-oxidase gene	Increased GAs levels, elongated hypocotyls and early flowering [83]		
		Overexpressing GA 20-oxidase gene	Increased GA levels, a longer hypocotyl, lighter-green leaves, reduced seed dormancy, and early flowering [84]	Exogenous GA stimulated astaxanthin biosynthesis (*Haematococcus pluvialis* [62])	Target chemical production
ET	Development and senescence	Silencing 1-aminocyclopropane-1-carboxylate (ACC) synthase or ACC oxidase	Reduced ET level and inhibited fruit ripening [85]	ET may be involved in programmed cell death of microalgae (*Chlamydomonas reinhardtii* [12])	Improvement of microalgal growth rate and biomass productivity

carotenoid precursor; however, although glucoside ABA has been detected, homologs of known higher plant ABA glucosyltransferases have not been found in microalgae *N. oceanica* [10]. Similarly, diverse glucosyl CKs have been detected in *N. oceanica* despite the lack of land-plant-type glucosyltransferases [10], suggesting the presence of auxiliary or modified ABA and CK biosynthetic pathways in microalgae. By contrast, homologs of known higher plant ABA and CK glucosyltransferases have been identified in several green microalgae [10], although there has been no biochemical evidence to support the presence of glucoside ABA or glucoside CK in these algae. Thus whether the canonical higher plant ABA and CK biosynthetic pathways are present in microalgae remains unknown. Collectively the evidence highlights the diversity and elusiveness of ABA and CK metabolisms in microalgae. The higher plant- and fungal-type GA biosynthesis pathways appear to be absent in cyanobacteria (Figure S1A in the supplementary material online), despite the presence of GAs in miscellaneous cyanobacteria (Table 1), which suggests the presence of as-yet unidentified pathways in cyanobacteria. Our knowledge of the GA biosynthesis pathway in higher plants is still incomplete given that an entirely novel route for GA biosynthesis may exist in *Arabidopsis* (*Arabidopsis thaliana*) [43], suggesting that the presence of algal-type routes in higher plants cannot be ruled out yet. Collectively, the evidence suggests that the phytohormone biosynthetic pathways of microalgae resemble those of higher plants, and that they may also use alternative but as-yet unknown pathways.

Origin of plant hormone systems

Biosynthesis

Microalgae and higher plants produce structurally identical phytohormones using relatively conserved biosynthetic pathways, which raises the question of whether the higher plant hormone systems originated from the hormone systems of microalgae. One possible explanation is that higher plant phytohormone biosynthesis has been inherited from ancient microalgae [44]. Auxin, ABA, CKs, and ET are structurally highly conserved in bacteria, fungi, algae, mosses, ferns, and seed plants. GAs are also largely conserved in chemical structure, even though they have not been found in mosses (Figure S1B). Nevertheless, the moss (i.e., *Physcomitrella patens*) employs GA-type diterpenes as an endogenous regulator of development, suggesting the presence of a rudimentary form of GA-mediated regulatory system in mosses [45]. Some phytohormones (i.e., CKs, GAs, ABA, and IAA) had already evolved to act as signaling molecules to regulate physiology in bacteria [15,44,46–50], including cyanobacteria [23]. Consistent with this, key genes of ABA, CKs, and ET biosynthetic pathways are shared among cyanobacteria, eukaryotic microalgae, and higher plants (Figure 1): for example, the isopentenyltransferases (IPTs), which catalyze the first committed step in CK biosynthesis. Higher plant IPTs form two functional classes: the prokaryote-type transfer RNA (tRNA) IPTs (AtIPT2 and AtIPT9) and the ATP/ADP IPTs (AtIPT1, AtIPT3–AtIPT8). The tRNA IPT homologs have been found in species across a wide range of phylogenetic groups, including bacteria, cyanobacteria, eukaryotic microalgae, and higher plants (Figure 2A). The green microalgae *Micromonas* sp. RCC299 and *Ostreococcus tauri* each harbor two IPT homologs, one of which is more closely related to the ATP/ADP IPTs than to the tRNA IPTs (Figure 2A). The remaining putative algal IPTs exhibit a higher level of similarity to tRNA IPTs than to ATP/ADP IPTs (Figure 2A). Such a gene-tree topology is most readily explained by the scenario that plant tRNA IPTs have been inherited from eukaryotic microalgae and evolved into ATP/ADP IPTs through gene duplication and diversification. Furthermore, it is likely that phytohormone biosynthesis (at least for particular modules) in photosynthetic eukaryotes (both algal lineage and land plants) can be further tracked back to the bacteria that were transformed into the ancient chloroplast [44] (Figure 3).

An alternative explanation is that horizontal gene transfer from bacteria or viruses to the ancestral plants could have also taken place, and this contributed to the genetic reservoir of phytohormone biosynthetic pathways in present-day higher plants and microalgae. For instance, the tryptophan-dependent IAA biosynthesis found in land plants is more likely to have been derived from horizontal gene transfer from bacteria than to have been inherited from microalgae [51]. By contrast, an exhaustive search of the sequenced viral genomes hosted by algae such as unicellular *Chlorella variabilis* NC64A and multicellular *E. siliculosus* revealed the existence of homologs of phytohormone-related genes (although their true functions remain elusive), which suggests that lateral gene transfer via viruses may have also contributed to extending the gene repository of phytohormone metabolism and signaling in microalgae (Table S2 in the supplementary material online). It therefore seems that land plant phytohormone biosynthetic genes might be predominantly derived from either endocytosis of ancient cyanobacteria or by horizontal transfer from microbes (e.g., bacteria and virus).

Signaling

For most phytohormones, the biosynthetic pathways are highly conserved between higher plants and eukaryotic microalgae; however, this is not the case for the signaling pathways (Figure 1). Orthologs encoding the ET receptor complexes, including ETHYLENE RESPONSE 1/ETHYLENE RESPONSE SENSOR/ETHYLENE INSENSITIVE 4 (ETR1/ERS/EIN4), are widely present in microalgal lineages (Figure 1); moreover, ET binding sites have been validated in a cyanobacterial protein [52]. By contrast, the remaining signaling components [e.g., ETHYLENE-INSENSITIVE 3 (EIN3) and EIN3-Like proteins] have only been found in land plants (Figure 3).

To date, the higher plant ABA receptors PYR (PYRABACTIN RESISTANT)/PYL (PYRABACTIN RESISTANT-LIKE)/RCAR (REGULATORY COMPONENT OF ABA RECEPTOR) have not been identified in microalgae, even though the downstream phosphatases from the ABA signaling pathway (e.g., SNF1-RELATED PROTEIN KINASE 2) are conserved in sequence in species ranging from microalgae to higher plants [48]. These findings suggest that the higher plant ET and ABA signaling components mentioned, but not the entire signaling pathways, may have been derived from microalgae.

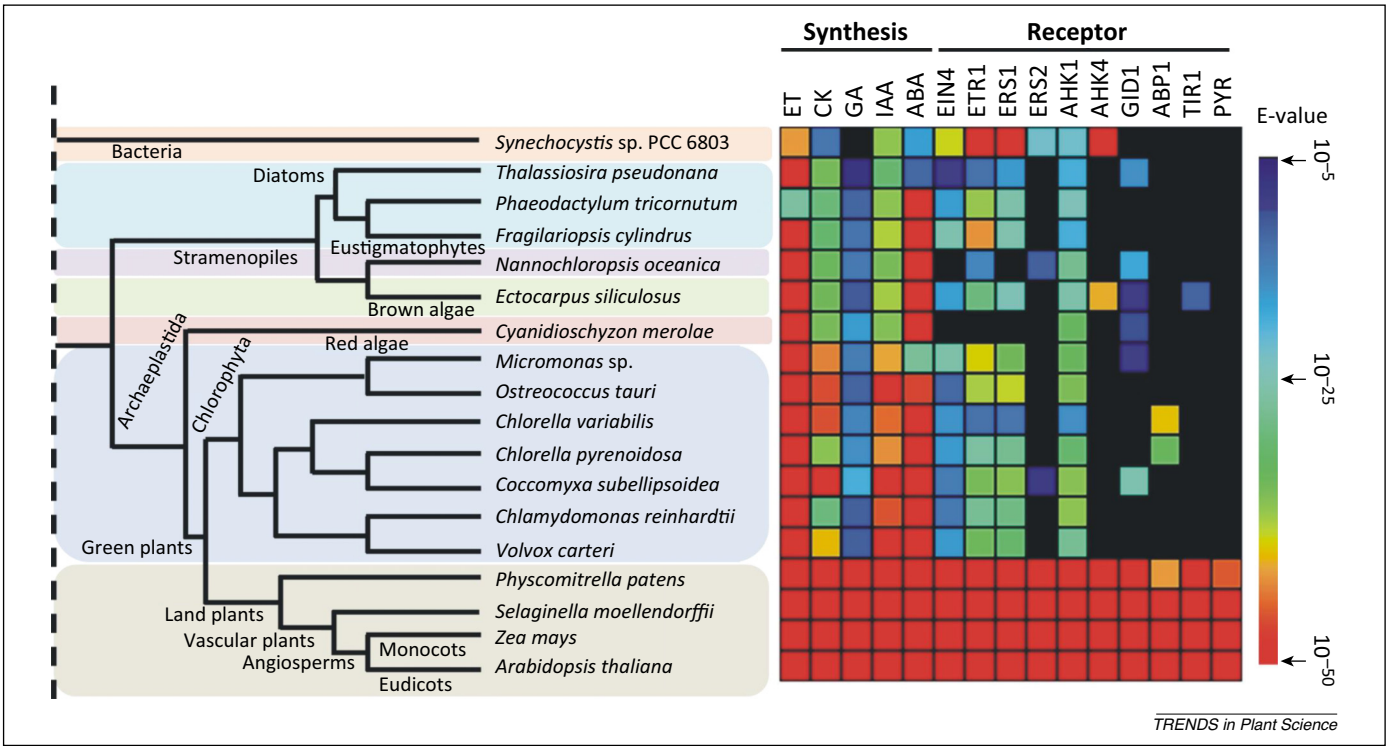

Figure 1. Distribution of phytohormone biosynthetic pathways and phytohormone receptors in microalgae. The phylogenetic tree was built based on ribosomal 18S RNA genes of microalgae using MEGA 6.0, followed by manual modification (according to Baldauf [86]). The color key indicates the similarity of a gene to its closest match, and ranges from low similarity (black) to high similarity (red). Black areas indicate that no BLASTp hits below the e-value threshold ($1e^{-5}$) were found. Red areas indicate orthologs with BLASTp e-values below $1e^{-50}$. For genes with multiple isoforms, homologs with the lowest BLASTp e-values were selected. The similarity of each of the phytohormone biosynthesis pathways between algae and *Arabidopsis* is indicated by the average e-value of all the selected genes. Owing to space limitations, this figure does not fully reflect the complexity of the biosynthetic pathways. For detailed diagrams of the biosynthetic pathways, please refer to the excellent reviews for auxin [38], abscisic acid (ABA) [10], cytokinins (CKs) [10], ethylene (ET) [87], and gibberellins (GAs) [88]. Green algae include *Micromonas* sp. RCC299, *Ostreococcus tauri*, *Chlorella variabilis* NC64A, *Chlorella pyrenoidosa*, *Coccomyxa subellipsoidea* C-169, and *Chlamydomonas reinhardtii*. The red algae are represented by the simple cellular architecture of *Cyanidioschyzon merolae*. Diatoms include *Fragilariopsis cylindrus*, *Phaeodactylum tricornutum*, and *Thalassiosira pseudonana*. Eustigmatophyte algae are represented by *Nannochloropsis oceanica*. The green alga *Volvox carteri* and the brown alga *Ectocarpus siliculosus* are included as representatives to probe the roles of phytohormones in the evolution of multicellularity and differentiation. The bryophyte *Physcomitrella patens* and the lycophyte *Selaginella moellendorffii* were selected to represent phylogenetically basal land plants, and the monocot maize (*Zea mays*) and the dicot *A. thaliana* were included as representatives of seed plants.

Orthologs of the GA higher plant receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1) have been identified in microalgae (Figure 1). Functional motif analysis has revealed that GID homologs in microalgae and in the moss *P. patens* have the catalytic triad (S, D, and H) of the hormone-sensitive lipase (HSL) family; however, the GA-binding amino acid residues have not been found (Figure 2B). By contrast, the lycophyte *Selaginella moellendorffii* GID1 homolog has lost the H of the catalytic triad (but still has D and S) (Figure 2B). The *P. patens* GID1 orthologs are unable to complement rice (*Oryza sativa*) GA receptor mutants, whereas the *S. moellendorffii* GID1s show a high binding affinity for GA (supporting its role as a functional GA receptor [53]). Therefore, GID1 appears to have originated from HSL and has been further modified during vascular plant evolution to exhibit stricter selectivity for bioactive GAs [54]. This further supports the proposal that inheritance from microalgae may be a vital source for elements of higher plant hormone systems. However, the DELLA-domain proteins and the F-box protein SLEEPY1, which are involved in mediating GA signaling, have not been identified in microalgae and have been found only in land plants [55], suggesting that the GID–DELLA growth-regulatory mechanism arose after the emergence of land plants (Figure 3).

The CK and auxin signaling pathways are more complex than the ET, ABA, and GA signaling pathways given that at least two cascades have been documented in higher plants for each hormone. Homologs of the first CK receptor to be discovered, ARABIDOPSIS HISTIDINE KINASE 1 (AHK1), are common in algal genomes [10] (Figure 1). Moreover, homologs of the essential components of the downstream CK signaling cascade (type B ARABIDOPSIS RESPONSE REGULATORS and HISTIDINE-CONTAINING PHOSPHOTRANSMITTER 1) have been found in plants, including green microalgae [10]. By contrast, homologs of the CK receptor AHK4, which harbor the CK-binding cyclases/histidine kinases associated sensory extracellular (CHASE) domain, are present only in *Synechocystis* sp. PCC 6803 and *E. siliculosus* [10] (Figure 1). Thus although CK signaling components have evolved in microalgae, parallel evolution occurred among different algal lineages (Figure 3).

Proteins with sequence similarity to the *Arabidopsis* auxin receptor TRANSPORT INHIBITOR RESPONSE 1 (TIR1) were not found in the sampled algal genomes (*E. siliculosus* has a TIR1 homolog but it lacks the auxin binding residues; Figure S2). By contrast, *C. variabilis* NC64A and *Chlorella pyrenoidosa* genomes harbor auxin receptor AUXIN-BINDING-PROTEIN1 (ABP1) homologs

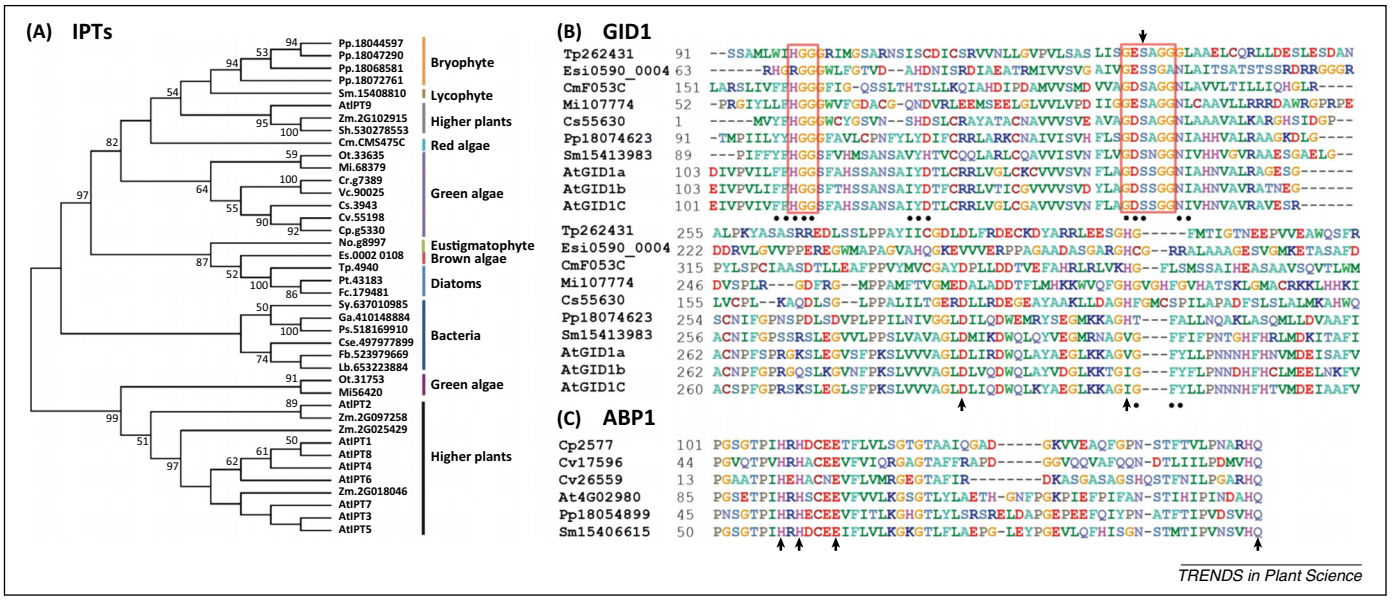

Figure 2. Evolution of isopentenyltransferases (IPTs), GID1, and ABP1. **(A)** Phylogenetic tree of IPTs. IPT orthologs are phylogenetically divided into two major groups: clade I members are from flowering plants (AtIPT1–AtIPT8 and two maize IPTs) with the exception of particular genes from *O. tauri* and *Micromonas* sp. RCC299; clade II harbors AtIPT9, the IPT-like proteins from bacteria, cyanobacteria, bryophytes, and lycophytes, and the remaining algal IPT-like proteins. **(B)** Multiple protein-sequence alignments of GID1. Essential residues for gibberellic acid (GA) binding are indicated by a black dot. Three amino acid residues corresponding to the catalytic triad of the hormone-sensitive lipase (HSL) are represented as arrows. The conserved motif of the HSL family (the HGG and GXSXG motifs, which are also important for GA binding of GID1) are outlined in red. **(C)** Multiple protein-sequence alignments of ABP1. The auxin binding residues of ABP1 that interact with a zinc ion are indicated by an arrow. Abbreviations: At, *Arabidopsis thaliana*; Cm, *Cyanidioschyzon merolae*; Cp, *Chlorella pyrenoidosa*; Cr, *Chlamydomonas reinhardtii*; Cs, *Coccomyxa subellipsoidea* C169; Cse, *Clostridium senegalense*; Cv, *Chlorella variabilis* NC64A; Es, *Ectocarpus siliculosus*; Fb, *Firmicutes bacterium*; Fc, *Fragilariopsis cylindrus*; Ga, *Glaciecola arctica*; Lb, *Lachnospiraceae bacterium*; Mi, *Micromonas* sp. RCC299; No, *Nannochloropsis oceanica*; Ot, *Ostreococcus tauri*; Pp, *Physcomitrella patens*; Ps, *Pseudomonas stutzeri*; Pt, *Phaeodactylum tricornutum*; Sh, *Saccharum hybrid*; Sm, *Selaginella moellendorffii*; Sy, *Synechocystis* sp. PCC 6803; Tp, *Thalassiosira pseudonana*; Vc, *Volvox carteri*; Zm, *Zea mays*.

(Figure 2C), which have also been found in *Chlamydomonas reinhardtii* [56]. The algal ABP1-like protein and their land-plant counterparts share the conserved motif that forms the auxin-binding pocket (Figure 2C). These findings point to an early emergence of primitive forms of auxin receptor in microalgae (Figure 3).

Together, the above observations suggest that some components (albeit likely not the full set) of higher plant canonical phytohormone signaling systems emerged in microalgae, where the recruitment of a molecule that was previously constrained for a different role into a new functional complex likely plays a crucial role. This has been proposed as one of the mechanisms underlying the emergence of 'new genes' in higher plants [57]. Whether and to what degree this mechanism plays a role in the functional evolution of higher plant phytohormone signaling systems from microalgae deserve additional investigation.

However, independent origins followed by parallel evolution may also underlie the evolution of signaling cascades in higher plants and in microalgae, resulting in other presently unknown mechanisms for transmitting the phytohormone signals in present-day microalgae [15]. For instance, for ABA and CKs, although a functional flowering plant-like phytohormone regulatory mechanism appears to be present in *Nannochloropsis* spp., a complete set of components from the canonical ABA and CK signaling pathways has not been found [10]. For auxin, the active physiological roles of IAA and the absence of an auxin canonical signaling cascade in *E. siliculosus* suggest the likely presence of alternative signaling pathways [38]. Despite the controversy surrounding the role of ET and GAs in microalgal cells, to date the corresponding canonical higher plant signaling circuitries have not been identified in microalgae. The possibility of such potentially novel signaling mechanisms points to specific regulatory roles of phytohormones in microalgae that are distinct from those in higher plants.

Therefore, higher plant phytohormone systems might be derived from pre-existing systems that already operated in ancient microalgae, with biosynthetic pathways being relatively conserved and signaling pathways that have been greatly altered. It is thus possible that the current plant signaling models might represent an oversimplified or incomplete view of the diversity of cellular adaptation strategies in aquatic environments.

Implication of phytohormone manipulation for developing microalgal feedstock for biofuels

In higher plants, the manipulation of phytohormone systems has been one pillar of the 'Green Revolution', which has involved the introduction or enhancement of agriculturally useful traits to achieve ever-higher productivity of food crop cultivars. Both genetic engineering of endogenous phytohormone systems and the exogenous application of phytohormones (alone or in conjunction with other plant growth regulators) have been widely used to improve crop tolerance to a broad range of abiotic stresses (e.g., high levels of light, salinity, temperature extremes, drought, flooding, and lack of nutrients) (Table 2) [58].

As part of endeavors to tackle the energy crisis and global warming, oleaginous microalgae have been considered as a potential feedstock for biofuels [59]. However, few

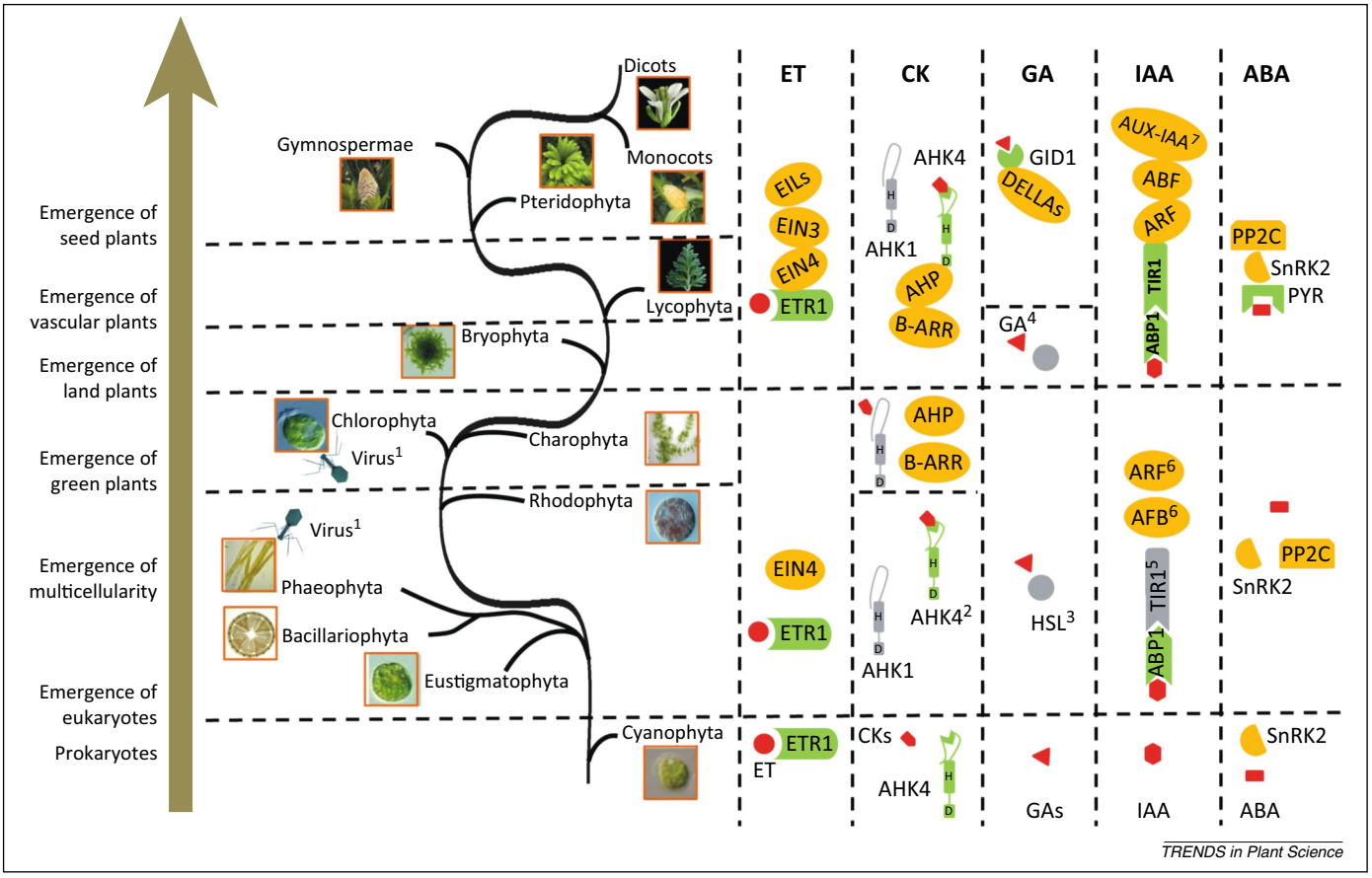

Figure 3. Proposed model for the evolution of phytohormone pathways: ethylene (ET), cytokinin (CK), gibberellin (GA), indole-3-acetic acid (IAA), and abscisic acid (ABA). Biosynthesis of these plant hormones may be largely inherited from cyanobacteria via endosymbiosis, whereas the signaling components may have acquired their current functions through stepwise evolution. Phytohormones, receptor precursors, receptors, and transcriptional factors are shown as red, grey, green, and yellow symbols, respectively. The superscript numbers denote: 1, lateral gene transfer of phytohormone-related elements may occur between viruses and microalgae (i.e., *Chlorella variabilis* NC64A and *Ectocarpus siliculosus*); 2, homologs of AHK4 are only found in cyanobacteria *Synechocystis* sp. PCC 6803 and the brown alga *Ectocarpus siliculosus*; 3, the ancient hormone-sensitive lipase (HSL) is the precursor of plant GID1, and the first functioning GID1 may have evolved in ancient lycophytes; 4, although GAs have not been found in moss, the moss (i.e., *P. patens*) produces and utilizes GA-type diterpenes as an endogenous regulator in development; 5, homologs of auxin receptor ABP1 are found in green algae *Chlorella variabilis* NC64A and *Chlorella pyrenoidosa*, whereas a homolog of auxin receptor TIR1 is found only in brown alga *Ectocarpus siliculosus* but lacks the auxin-binding motifs; 6, homologs of the auxin signaling components ARFs have been found in the green algae *Chlamydomonas reinhardtii*, *Coccomyxa subellipsoidea*, and *Volvox carteri*, and in the brown alga *Ectocarpus siliculosus*, while AFBs have been found in the green algae *Chlorella pyrenoidosa*, *Coccomyxa subellipsoidea*, and in the diatom *Thalassiosira pseudonana*; 7, Brophyta harbor an intermediate form of AUX-IAA that is unlikely to be an active factor in the early auxin response. Note that Bryophyta, Lycophyta and gymnosperms as represented here comprise several lineages and might not be monophyletic. The signaling components in microalgae indicated here have not been experimentally studied, thus whether they are *bona fide* signaling components remains to be validated. Moreover, the presence of homologs of the signaling pathways does not necessarily indicate the occurrence of classical phytohormone responses.

microalgal strains found in nature have a sufficient level of biomass productivity, oil content, and environmental tolerance to sustain cost-competitive production. Given the important regulatory roles played by phytohormones in higher plants, for example, in germination, seed development, growth, vegetative development, biomass production, dormancy, senescence, and in response to environmental factors, the discovery and dissection of phytohormone systems in microalgae could help researchers to devise rational strategies to select or genetically engineer algal species for beneficial industrial traits, such as increased biomass productivity and elevated tolerance to adverse environmental conditions (Figure 4 and Table 2). Such hormone-related components, whose functions might or might not be similar to their counterparts in higher plants, may serve as potential targets for microalgal feedstock development (Table 2).

Manipulation of phytohormone metabolism in algae is still in its infancy, and past experiments have largely focused on exogenous application of phytohormones. For example, algal extracts in which multiple phytohormones are present have been applied as biostimulants to enhance the growth and reduce abiotic and biotic stresses of higher plants [15]. However, several reports have demonstrated the effects of exogenous phytohormones, such as auxin, GAs, ABA, or CKs, on algal growth [40,41,60] or on the stress response of microalgal cells [36,37,61,62] (Table 2). Whether the active ingredients are microalgal phytohormones remains unclear; however, these results suggest a degree of functional conservation between the microalgal and higher plant phytohormones.

To date, little is known about the functional role of phytohormones in microalgae, and further investigations will be necessary to determine the opportunities for exploiting phytohormones for biotechnological purposes. The genome-wide metabolic and regulatory networks of the emerging systems-biology research models of industrial oleaginous microalgae, such as *Nannochloropsis* spp. and others [10,63,64], should provide an excellent foundation

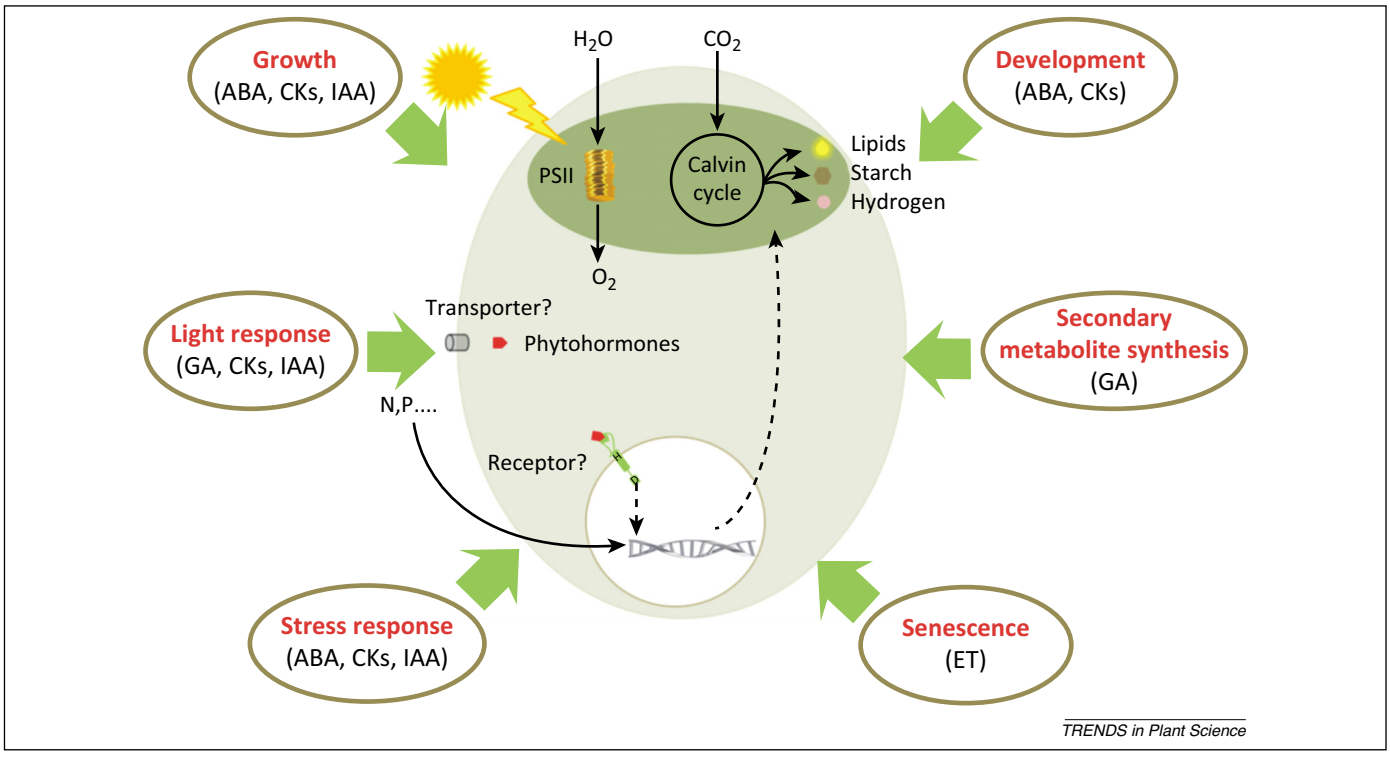

Figure 4. Potential strategies for manipulating phytohormone metabolism to improve economically important traits in microalgae. The effects of endogenous phytohormones on microalgae were deduced from the results of applying exogenous phytohormones to microalgal cultures as well as via experiments manipulating phytohormone pathways in higher plants. The *in vivo* functions of endogenous phytohormones in microalgae remain to be elucidated. Abbreviation: PSII, photosystem II.

for understanding and engineering phytohormone systems for enhanced biofuel production. Chemical or genetic manipulation of enzymes involved in phytohormone metabolism can alter the levels of the active hormones, and could thus yield engineered microalgae of industrial interest (Figure 4). These endeavors should also generate new research tools for probing the molecular mechanisms of phytohormone biosynthesis and signaling, which in turn should open new doors for genetically or chemically engineering microalgal production processes [33]. Furthermore, microalgal phytohormone-related genes could serve as an expanded gene bank for improving agronomic traits in crops (i.e., similar to the introduction of bacteria phytohormone genes in higher plants; e.g., [65]). Moreover, microalgae can serve as a valuable system for screening genes of value for crop breeding given their phylogenetic closeness to higher plants, the amenity to genomic and genetic approaches, the usually shorter generation time, the typically more streamlined genomes, and presumably more tractable metabolic and regulatory networks.

Concluding remarks and future perspectives

Unraveling the functional modes of hormonal systems in eukaryotic microalgae, which are mostly unicellular, might also provide insights into the diversity and evolution of intercellular signaling mechanisms in eukaryotes. Comparison of hormone signaling pathways between unicellular microalgae and multicellular microalgae (i.e., green algae *Volvox carteri*) or macroalgae (i.e., brown alga *E. siliculosus*) revealed that the transition from unicellular to multicellular organisms appears to involve far fewer new hormone-related genes than the transition from aquatic to terrestrial life. Multicellular organisms have an endocrine system in which one cell type typically produces the signal molecule, which is perceived by other cells. In unicellular eukaryotes it is unclear how the communications are mediated, despite reports supporting such intercellular signaling roles for the excreted hormones in several protozoa and microalgae [66–68]. In microalgae, are the physiological effects of phytohormones limited to the hormone-producing cells (known as autocrine signaling) [69]? Can these hormones function as intercellular signals (exocrine system, e.g., pheromones) [66]? Does phytohormone-based communication among aquatic microalgal cells demonstrate a parallel evolution with the quorum-sensing by which bacteria use secreted signal molecules to regulate cell population density? Can microalgal phytohormone metabolism be exploited to regulate population traits of microalgae?

In conclusion, the recently ignited enthusiasm for microalgal biofuels might lead to exciting new knowledge about the metabolic and regulatory networks of phytohormones in the vast and phylogenetically divergent microalgal world, and offer fresh views of phytohormone evolution in plants and copious new opportunities for microalgal biotechnology.

Acknowledgments

We are indebted to Steven M. Smith (University of Western Australia) for his valuable input. This work was supported by the Natural Science Foundation of China (31200063 and 31401705), the Ministry of Science and Technology of China (2012CB721101), and the Basic Research in Science and Technology Plan Projects of Qingdao (13-1-4-229-jch).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tplants.2015.01.006.

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