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Chapter 21

Sensing and signalling in diatom responses to abiotic cues

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Abbreviations

UV: ultra-violet; CPF: Cryptochrome/Photolyase family; FAD : Flavin adenine dinucleotide; MTHF: 5,10-methenyltetrahydrofolic acid; FMN: flavin-mononucleotide; PHR: Photolyase-related domain; PL: photolyase; DNA: deoxyribonucleic acid; CPD: cyclobutane pyrimidine dimer; CRY: Cryptochrome; AUREO: Aureochrome; TF: Transcription factor; Ser/Thr: Serine/threonine ; R: red light; FR: far-red light; PCM: photosensory core module; BV: biliverdin; PHY: phytochrome; DPH: diatom phytochrome; DCMU : 3-(3', 4'-dichlorophenyl)-1, 1-dimethylurea; cAMP : cyclic adenosine monophosphate; CCREs : CO₂/cAMP-responsive elements ; CA :carbonic anhydrase ; ROS : reactive oxygen species; bHLH: basic helix–loop–helix domain; PAS: Per-Arnt-Sim domain (from “period”, “aryl hydrocarbon receptor nuclear transporter” and “single-minded” proteins); NPF : nitrate transporter 1/peptide transporter family; TOR: target of rapamycin; ATP: adenosine triphosphate; NAGK : N-acetyl-L-glutamate kinase; ncRNA : non coding RNA; snRNA derived sRNAs; sRNA : small RNA; lincRNA : long intergenic non-protein coding RNA; NAT : natural antisense transcript ; TE : transposable element.

Abstract

Diatoms are prominent microalgae that proliferate in a wide range of aquatic environments. Still, fundamental questions regarding their biology, such as how diatoms sense and respond to environmental variations, remain largely unanswered. In recent years, advances in the molecular and cell biology of diatoms and the increasing availability of genomic data have made it possible to explore sensing and signalling pathways in these algae. Pivotal studies of photosensory perception have highlighted the great capacity of diatoms to accurately detect environmental variations by sensing differential light signals and adjust their physiology accordingly. The characterization of photoreceptors and light-dependent processes described in this review, such as plastid signalling and diel regulation, is unveiling sensing systems which are unique to these algae, reflecting their complex evolutionary history and adaptation to aquatic life. Here, we also describe putative sensing components involved in the responses to nutrient, osmotic changes, and fluid motions. Continued elucidation of the molecular systems processing endogenous and environmental cues and their interactions with other biotic and abiotic stress signalling pathways is expected to greatly increase our understanding of the mechanisms controlling the abundance and distribution of the highly diverse diatom communities in marine ecosystems.

I. Introduction

The life history of diatoms in aquatic environments is strongly influenced by a multitude of abiotic factors including light, nutrients, salinity, and temperature (Mann and Lazier 2006). Physical factors such as circulation and turbulent mixing of water, sea level changes and proximity to coastal regions can cause drastic changes in the characteristics of abiotic resources which, if limiting or in excess, can quickly become a source of stress and adversely affect diatom growth and productivity (Seckbach and Gordon 2019).

Before the advent of the genomic era, several pioneering studies offered the first indications that diatoms are able to sense their environment and respond to a variety of signals. Observations of migrating diatom mats between deep nutrient pools and the ocean surface (Villareal et al. 1999) or of photoperiodic control of diatom spore growth (Eilertsen et al. 1995) implied that active responses are controlling their physiology and life strategies. This observation also challenged historical paradigms that considered diatom growth and distribution only influenced by the availability of the resources (Sverdup et al. 1942).

When diatoms became accessible to genetic transformation, the analysis of calcium changes in transgenic *Phaeodactylum tricornutum* cells expressing an aequorin reporter revealed the existence of calcium-dependent signal transduction mechanisms involved in responses to fluid motion, osmotic stress, and iron limitation (Falciatore et al. 2000). It was also shown that calcium mediates desensitization to prolonged exposure to stimuli such as turbulence or infochemicals (Falciatore et al. 2000; Vardi et al. 2006), suggesting that these physiological responses are controlled by “sense-process-respond” chains with specific receptors and feedback mechanisms, similar to those known in terrestrial plants or animals. However, compared to the latter systems, our understanding of diatom signal perception mechanisms is still very fragmentary, and mostly limited to light sensing. Molecular aspects of environmental perception remain largely uncharacterized in diatoms, although the increasing availability of data from cells exposed to different cues clearly indicates that cellular responses involve significant changes in gene expression, likely driven by the processing of external and internal signals.













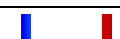



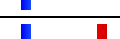














The aim of this chapter is to provide an overview of abiotic signal perception by diatoms, with a particular focus on the regulators involved in the responses to light changes, including light-absorbing photoreceptors, circadian rhythm regulation and plastid-derived signals. We also provide an initial appraisal of nutrient sensing systems and of possible components of the diatom response systems to osmotic changes and fluid motion. Finally, the ecological implication of these processes and future challenges are also discussed.

II. Light sensing and responses in diatoms

Being photosynthetic organisms, diatoms require light as an essential source for growth. Light provides also a significant amount of information from the surrounding environment. From sunrise to sunset, the intensity, orientation, and spectrum of sunlight vary because the sun’s rays hit the atmosphere at different angles, and they are selectively attenuated before reaching the earth’s surface. In addition to such daily light/dark changes, there is periodic variation in day length with the changing seasons across latitudes each year. The intensity and relative ratio of different wavebands can also be affected when radiation is filtered by a layer of light-absorbing elements. For example, photosynthetic organisms absorb blue and red light, so the light reflected by them is enriched in green and far-red bands. In plants, perception of such low red/far-red ratios allows them to detect surrounding phototrophs competing for light and trigger an appropriate shade avoidance response (Kendrick 1994). In aquatic environments, variations in intensity and spectral composition occur because of the strong and selective absorption of light (Kirk 2011). Light is absorbed by water itself, mostly in the red part of the spectrum, so the blue and green bands penetrate the water column more deeply. Suspended particles and dissolved chromophoric organic matter in water scatter light and/or differentially absorb wavelengths. Therefore, the light field in aquatic habitats is structured in depth and it can vary significantly with the diverse concentrations of constituents occurring in open ocean or coastal regions, estuaries, lakes or intertidal zones (Kirk 2011).

Sensing of light relies on pigment-bound proteins known as photoreceptors that absorb light of specific wavelengths and activate specific signalling cascades (Duanmu et al. 2017). However, in the cells of phototrophs, light is also absorbed by various photosynthetic pigments collectively absorbing a larger fraction of the incident light than the photoreceptors. As will be described in this chapter, photosynthetic activities in plastids also regulate the responses to light via retrograde pathways from plastids to the nucleus. Therefore, it is often difficult to disentangle information on the relative influence of individual sensors and signalling pathways involved in the responses to light. Experimental

Table 21.1. Effective wavebands triggering physiological responses in diatoms.

	Responses	Photosensing system	Diatom species	References
	cell cycle progression	Aureo1a	<i>P. tricornutum</i>	(Huysman et al. 2013)
	cell cycle progression		<i>Navicula sp.</i>	(Cao et al. 2013)
	growth rate	Phytochrome?	<i>D. brightwellii</i>	(Lipps 1973)
	auxospore formation	Phytochrome ?	<i>H. ostreraria</i>	(Mouget et al. 2009)
	sexual reproduction	Phytochrome ?	<i>S. palmeriana</i>	(Ren et al. 2005)
	sexual reproduction	Cryptochrome ? Rhodopsin ?	<i>C. didymus</i>	(Baatz 1941)
	resting spore germination and growth		<i>Chaetoceros sp., S. costatum, T. minima</i>	(Shikata et al. 2009)
	resting spore germination	Plastid ?	<i>L. danicus</i>	(Shikata et al. 2011)
	chloroplast assembly		<i>P. laevis</i>	(Furukawa et al. 1998)
	chloroplast dispersion		<i>P. laevis</i>	(Furukawa et al. 1998)
	upward migration in sediment	Cryptochromes ? Aureochromes ?	Environmental samples, <i>G. fasciola, N. spartinetensis</i>	(Barnett et al. 2020)
	upward migration in sediment		Wadden sea populations	(Wenderoth and Rhiel 2004)
	sinking rate		<i>T. weissflogii</i>	(Fisher et al. 1996)
	photophobotaxis		<i>N. communis</i>	(Nultsch 1971)
	photophobotaxis	Cryptochromes ? Aureochromes ?	<i>N. ostrearia, N. gregaria, N. communis</i>	(Wenderoth 1979)
	photophobotaxis	Plastid ?	<i>N. peregrina, P. viridis, N. sigmaidea</i>	(Wenderoth 1979)
	photophobotaxis		<i>C. cuspidata, Nitzschia cf linearis, P. viridis</i>	(Cohn and Weitzell 1996)
	photophobotaxis		<i>S. phoenicenteron</i>	(Cohn and Weitzell 1996)
	photophobotaxis and phototopotaxis		<i>N. communis</i>	(Nultsch 1971)
	phototopotaxis		<i>N. perminuta</i>	(McLachlan et al. 2009)
	possible repelling		<i>N. linearis, C. cuspidate, P. viridis</i>	(Cohn and Weitzell 1996)
	possible repelling		<i>N. communis</i>	(Nultsch 1971)
	possible repelling		<i>S. phoenicenteron</i>	(Cohn and Weitzell 1996)
	photokinesis		<i>C. Closterium</i>	(McLachlan et al. 2009)
	photokinesis		<i>N. perminuta</i>	(McLachlan et al. 2009)
	photokinesis		<i>N. communis</i>	(Nultsch 1971)
	chromatic acclimation,		<i>P. tricornutum, N. closterium</i>	(Fujita and Ohki 2004; Herbstová et al. 2015)
	high light acclimation	Aureo1a and Aureo1b	<i>P. tricornutum</i>	(Schellenberger Costa et al. 2013a; Mann et al. 2017)
	photoprotection	Aureo1a and CPF family	<i>P. tricornutum</i>	(Coesel et al. 2009; Schellenberger Costa et al. 2013b; Juhas et al. 2014)
	pigment production		<i>H. ostreraria</i>	(Mouget et al. 2005)

Information on the effective wavebands has been extracted from the specific conditions used in the reported experiments. Note that the illumination conditions (light sources, irradiance) are not comparable between each study. Phototopotaxis, movement towards or away from the light source; photophobotaxis, reversing the direction of movement caused by a sudden decrease or increase in light intensity; photokinesis, acceleration of movement by light; repulsion, migration away from light.

evidence is needed to characterize the dependency of these responses on light quality and quantity. When a response is only induced by a particular waveband, we can deduce that a photoreceptor that specifically perceives, say, blue, green or red light must be involved. Alternatively, when a response is triggered by both blue and red light, it can be supposed that it is regulated by photosynthesis or/and the action of different photoreceptors. Photoreceptor-mediated responses can be activated by very low light fluences and are independent of photosynthesis, and plastid signalling is abolished in conditions that perturb plastid function (Kendrick 1994). Photoreceptor activity can also be identified in acute light response experiments using pulses of light after a prolonged dark treatment that abolishes both photosynthesis and circadian clock regulation (Kendrick 1994).

Many facets of diatom life are known to be tightly linked to light conditions as summarized in Table 21.1 and described in more detail in many chapters of this book. For instance, cell cycle progression (Chapter 9), germination of resting spores and sexual reproduction (Chapter 8) have been observed to occur at certain light intensities and photoperiods. Movement in several diatom species is also affected by the light intensity applied (Chapter 14). The strong impact of light on diatom gene expression has been also evidenced by independent genome-wide expression analyses in different diatom species exposed to different light regimes (e.g. Nymark et al. 2009 ; Chauton et al. 2013 ; Nymark et al. 2013 ; Ashworth et al. 2013 ; Valle et al. 2014 ; Smith et al. 2014 ; Mann et al. 2020 ; Bilcke et al. 2021). A wide palette of light colours from violet to far-red has been observed to trigger different responses, with blue and red light being the most effective bands. Light properties triggering each of these responses vary among diatom species, which could reflect the species-specific optimization of spectral light quality for growth and the involvement of multiple signalling pathways regulating acclimation to particular environments.

In the next session, we review the different light sensors controlling responses to these light changes in diatoms discovered through genomic, genetic and functional characterization in model species.

II.A . Diatom photoreceptors for sensing the light environment

A variety of photoreceptors have been found by exploring the protein sequences encoded in diatom genomes (Jaubert et al. 2017) (Table 21.2). Some of these proteins are similar to photoreceptors found in bacteria, plants or animals, while others have distinct combinations of protein domains (Figure 21.1). This evidence supports the hypothesis that diatoms evolved their light-sensing abilities from different ancestors via complex routes to fully exploit light cues in their environment. The spectral and functional properties are known for several members of the blue light cryptochrome/photolyase and aureochrome photoreceptor families, and for the red/far-red light phytochromes (Figure 21.1B). It should be noted that UV-light sensing photoreceptors have not been described in diatoms. While some gene products have been found that show similarities to plant UVR8 (Duanmu et al. 2017), they lack the specific tryptophan residues needed to act as a chromophore, so it is unlikely that they may function as photoreceptors.

The Cryptochrome/Photolyase Family. The cryptochrome/photolyase family (CPF) are widespread blue light absorbing flavoproteins with similar primary sequences but very diverse functions. Most CPFs non-covalently bind a flavin adenine dinucleotide (FAD) cofactor as a specific prosthetic

chromophore. Other chromophores such as 5,10-methenyltetrahydrofolic acid (MTHF), 8-hydroxy-5-deazaflavin, or flavin-mononucleotide (FMN) may also be associated with some CPFs as light antennae (Essen et al. 2017). CPF members have a characteristic conserved photolyase-related (PHR) domain but amino and carboxy terminal extensions are highly variable in both length and primary sequence. Photolyases (PL) are blue light-activated enzymes repairing UV-induced DNA lesions, such as cyclobutane pyrimidine dimer (CPD PL) or (6-4) pyrimidine-pyrimidone photoproducts. The cryptochromes (CRY) of plants and some insects do not have any DNA repair activity but are either blue light photoreceptors, or light-independent components of the central circadian oscillator (Chaves et al. 2011). The activation of light-dependent CRY has been well studied. The photoperception process starts with a very fast photoinduced reduction of FAD. This redox- and light-dependent change at the core of the protein leads to conformational changes allowing specific interactions with other protein partners. Globally, both light-dependent and -independent CRY, are involved in transcriptional regulation, respectively by inhibiting transcriptional repressors or by inactivating transcription.

Phylogenetic relationships in the CPF family helped to identify five major super-classes (sc) which do not necessarily converge functionally. For instance, sc1 includes (6-4) PLs but also light-dependent animal CRYs and light-independent CRYs involved in the transcription/translation feedback loop of the circadian clock. Class I and III CPD photolyases group also together phylogenetically with proteins with different functions such as light dependent plant photoreceptor CRY and plant-like photoreceptor CRY in sc3 but are separate from class II CPD photolyases which are all found in sc4. The last two super-classes (sc2 and sc5) include all CRY-DASH (named after *Drosophila*, *Arabidopsis*, *Synechocystis*, Human) and the proteobacterial PL/CRYs (Fortunato et al. 2015; Ozturk 2017). A surprising result from genome sequence analyses was that diatoms do not possess canonical plant CRY photoreceptors, even though blue light is preponderant in the ocean and CRY regulate so many physiological processes in plants. However, further genomic and functional investigations in diatoms revealed novel CPF variants, including the animal-like CPF1 and plant-like CRYs (CryP) (see below) and several DASH CRYs (Table 21.2). The biological function of Cry DASH is not yet clearly defined in diatoms or indeed in other organisms, but several studies suggest that they might have a double stranded DNA CPD PL activity, a signalling role, or be involved in the regulation of metabolism, consistent with their organellar localization (Kleine et al. 2003; Froehlich et al. 2010).

Only two diatom CPFs have been characterized in detail to date: CPF1 and CryP of *P. tricornutum* (Figure 21.1B). Initially discovered in diatoms, these proteins are extensively represented in metatranscriptomic data derived from marine environments (Coesel et al. 2021). CPF1 belongs to sc1, is localised in the nucleus, and has (6-4) PL DNA repair activity both *in vitro* (Coesel et al. 2009) and *in vivo* (De Riso et al. 2009). However, like plant CRY, CPF1 regulates the transcription of several genes acting in pathways modulating photoprotection, cell division, nutrient assimilation, etc, under blue light (Coesel et al. 2009), so is likely to be also a photoreceptor. CPF1 is also able to bind mammalian CLOCK protein in heterologous cells where it partially represses the CLOCK/BMAL1 heterodimer (Coesel et al. 2009) in the positive loop of the circadian clock (Kume et al. 1999).

CryP belongs to Plant-like photoreceptor CRY (sc3). Recombinant *P. tricornutum* CryP produced in *E. coli* binds FAD and MTHF chromophores (Juhas et al. 2014). Moreover, FAD photoreduction, a common mechanism in CPF that bind FAD, is also active *in vitro* suggesting that activation of CryP is light-dependent. Originally, CryP was identified as a blue light regulator of light harvesting proteins directly involved in photoprotection (LHCX) (Juhas et al. 2014). However, subsequent analyses of transcriptional responses to illumination by blue light after prolonged dark, revealed that gene expression was already deregulated in the dark in *CryP* knockdown lines compared

Table 21.2. Photoreceptors in diatom genome sequence resources.

Species	ID prefix	Photolyse 6-4 /PtCPF1	PlantCRY-like	CryDASH	Aureo1a	Aureo1b	Aureo1c	Proteo-rhodopsin	Helio-rhodopsin	PHY	Source
<i>Tp</i> centric	Thaps3	262946	7368	23500 268988 35005	33340	20065	37198			22848	JGI
<i>To</i> centric	EJK	58421.1	58052.1 62994.1	51584.1 57652.1 70255.1* 57807.1	65981.1	47540.1	75561.1				GenBank
<i>Cc</i> centric		g1243.t1		g11904.t1 g3306.t1	g18270.t1	g20747.t1	g23579.t1			g17859.t1	http://genomes.mcdb.ucla.edu/Cyclotella/download.html
<i>Pt</i> pennate	Phatr3_	J27429.p1	J54342.p1	J55091.p1 J34592.p1	8113.p1	J15977.p1	J51933.p1		J43903.p1	J54330.p1	Ensembl Genomes
<i>Fc</i> pennate	OEU	16032.1	23746.1	18916.1 15165.1 21140.1	18451.1	21654.1	06878.1, 17811.1*	10445.1 23745.1*			GenBank
<i>Sr</i> pennate	Sro	82_g043740.1	580_g170080.1	147_g068020.1 2726_g335660.1*	5_g004540.1 624_g177380.1	1194_g251270.1	971_g226430.1		149_g068360.1 149_g068370.1	219_g090570.1 537_g162340.1 537_g162360.1 537_g162370.1	https://bioinformatics.psb.ugent.be/orcae/overview/Semro
<i>Pme</i> pennate	Psemu1	133684	107549 107549	285541 190760	172818	294561	202247				JGI
<i>Pmu</i> pennate	VEU	36634.1	42420.1 44083.1	40361.1 41071.1	?	34876.1 34877.1*	?				GenBank
<i>Fs</i> pennate	GAX	14982.1, 23745.1*		14744.1 19840.1* 23334.1 28242.1*	11767.1 20834.1*	09413.1 20262.1*	16809.1, 13918.1*				GenBank
<i>Sa</i> pennate	Sac	19225	1435, 23010	6021	24026 25403*	24510	?		16855 16858*	18038 1907 1973 22009 24689	http://www.lin.irk.ru/sacus/index.php?r=site/page&view=downloads

Identification numbers are given for photoreceptor sequences found in the indicated source. Asterisks indicate uncertainty whether the sequence is another gene or an allelic variant of the same gene. Species abbreviations: *Thalassiosira pseudonana*, *Tp*; *Thalassiosira oceanica*, *To*; *Cyclotella cryptica*, *Cc*; *Phaeodactylum triconutum*, *Pt*; *Fragilariopsis cylindrus*, *Fc*; *Seminavis robusta*, *Sr*; *Pseudo-nitzschia multiseriata*, *Pme*; *Pseudo-nitzschia multistriata*, *Pmu*; *Fistulifera solaris*, *Fs*; *Synedra acus*, *Sa*. (Armbrust et al. 2004; Bowler et al. 2008; Lommer et al. 2012; Galachyants et al. 2015; Tanaka et al. 2015; Traller et al. 2016; Mock et al. 2017; Basu et al. 2017; Osuna-Cruz et al. 2020).

to wild type (König et al. 2017). Therefore, despite the presence of chromophores, CryP may be not a major blue light photoreceptor but rather a component involved in the global modulation of transcription, requiring other blue light photoreceptors to signal the light responses.

The Aureochromes. The aureochromes (AUREOs) are unique blue light photoreceptors that possess both an FMN-binding light-oxygen-and-voltage (LOV) domain (Crosson et al. 2003), and a bZIP domain typical for bZIP transcription factors (TFs) (Dröge-Laser et al. 2018) (Figure 21.1). The blue light phototropin photoreceptors of green algae and plants also possess two LOV domains, but utilize a Serine/threonine (Ser/Thr) kinase domain for signal transduction instead (Christie 2007). AUREOs were originally discovered in the xanthophyte alga *Vaucheria frigida* in 2007 (Takahashi et al. 2007). Their name refers to “aurum” (Latin for gold), because of the golden-brownish color of most stramenopiles. Takahashi et al. (2007) identified two orthologs in *V. frigida*, VfAUREO1 and VfAUREO2. Using an RNA interference approach to silence these two genes individually, they demonstrated that both AUREOs are involved in the regulation of photomorphogenic responses. Meanwhile, AUREOs have been identified in other stramenopiles (Ishikawa et al. 2009; Jungandreas et al. 2014) and in a raphidophyte (Ji et al. 2017), but not in non-photosynthetic oomycetes. AUREOs are not present in red algae, which are considered to represent the endosymbiotic ancestors of stramenopile plastids (Archibald 2015). This indicates that the ancestral *AUREO* gene with its unique combination of LOV and bZIP domains may have been provided either by the putative host cell of the secondary endosymbiosis event, or that it evolved very early within the stramenopiles, possibly via domain shuffling (Di Roberto and Peisajovich 2014).

Stramenopile AUREOs differ in their structures. While AUREO1 proteins possess the typical LOV domain, AUREO2 proteins have a mutation within the LOV domain, which prevents non-covalent binding of flavin needed for light absorbance in the blue range. The reason is a steric hindrance from a methionine residue within the binding cavity (Banerjee et al. 2016a). AUREO2 therefore is not a real photoreceptor, but could still be involved in light regulation, e.g. by forming a dimer with a light sensing AUREO1 protein. In reciprocal experiments, genetic modification of AUREO2 from *P. tricornutum* restored flavin binding (Serif 2017), while introducing a point mutation at the same site in PtAUREO1a led to loss of flavin binding (Banerjee et al. 2016a). Based on this distinction and on phylogenetic analyses, aureochromes in diatoms and other organisms have been classified as either Aureo1-type (a/b/c etc.) or as Aureo2 (Schellenberger Costa et al. 2013b). The algae studied so far all possess one Aureo2 protein and one or more Aureo1 isoforms (Table 21.2).

Blue light absorption, both in aureochromes and phototropins, causes the formation of an adduct between the flavin and a nearby cysteine within a few microseconds, starting the signalling cascade (Toyooka et al. 2011; Kerruth et al. 2014). The domain topology of AUREOs is inverted as compared to most other characterized LOV proteins, because the sensory domain of AUREOs is at the C-terminus of the receptor. The J α helix of the AUREO-LOV domain, allosterically regulates the fold of the N-terminally flanking A' α helix (Herman and Kottke 2015). Subsequent unfolding of the A' α helix exposes a high affinity dimerization site and enables the formation of the light state dimer of LOV (Herman et al. 2013; Herman and Kottke 2015; Heintz and Schlichting 2016). Indeed, in full-length AUREO, the J α helix plays a crucial role in the formation of the light state of the receptor (Banerjee et al. 2016b). The activation of the LOV domain results in a loss of the helical secondary structure of the bZIP domain (Banerjee et al. 2016b), indicating that there is a direct communication between the two domains. If a DNA binding site is available, the helical fold of the bZIP domain is increased by light (Banerjee et al. 2016b), resulting in rigidification of the domain (Heintz and Schlichting 2016). In a

complementary approach, Tian and colleagues showed the importance of a previously overlooked α helix in promoting the conformational protein changes (Tian et al. 2020). bZIP domains have a general tendency to dimerize, and are only capable of binding DNA as dimers (Tateyama et al. 2018). For some AUREOs, dimerization and DNA binding have been shown to be induced by blue light (Hisatomi and Furuya 2015; Banerjee et al. 2016b; Nakatani and Hisatomi 2018; Nakajima et al. 2021). PtAUREO1a occurs in the dark as a dimer/higher oligomer (Banerjee et al. 2016b) or as a monomer in equilibrium with a dimer (Heintz and Schlichting 2016). Light induces the dimerization of LOV domains and the association of the monomers (Kobayashi et al. 2020; Goett-Zink et al. 2020), which is the rate-limiting step in the process of DNA binding (Akiyama et al. 2016). Based on these properties, AUREOs could potentially be used as an optogenetic tool, for instance, to increase protein stability under blue light conditions (Hepp et al. 2020) or directly as a light-driven gene switch.

AUREO function has been extensively characterized in *P. tricornutum*, especially for PtAUREO1a that acts as a key regulator of the diatom cell cycle (Table 21.1; Chapter 9). Evidence from silenced and knockout lines of *P. tricornutum* further indicates that PtAureo1a and PtAureo1b might be involved in regulation of photoacclimation (Table 21.1; Schellenberger Costa et al. 2013b; Serif et al. 2017; Mann et al. 2017; Madhuri et al. 2019). PtAureo1c might be a high light sensor *in vivo* because it recovers faster and is much less sensitive to light than PtAUREO1a (Bannister et al. 2019). Recent studies indicate that aureochromes may have a large impact on the cells. Changes in transcriptomes in response to a shift from red to blue light were analysed in wild-type *P. tricornutum* cells, in PtAureo1a knockout and in PtAureo1a complemented lines (Mann et al. 2020). Wild-type cells react within minutes by up- or down-regulating 75% of the genes, while this massive change in gene expression is mostly inhibited in *Ptaureo1a* knockout strains (Mann et al. 2020). PtAureo1a therefore has a specific function in cellular regulation that cannot be complemented by other AUREOs. This raises the question of how a single photoreceptor can affect such a large number of genes. Possibly, a cascade of TF transduces the initial response of PtAureo1a to blue light. This is supported by findings that the transcript abundance of a large number of diatom TFs and photoreceptors (Rayko et al. 2010) is strongly and rapidly affected by blue light in wild-type cells, but not in PtAureo1a knockout mutants (Mann et al. 2020). When common gene expression patterns are analysed, the aureochromes are placed in different clusters (Ait-Mohamed et al. 2020), supporting the idea that Aureo1 isoforms may have different roles. There also is some evidence that aureochromes might be involved in regulation of the diel cycle and the expression of some aureochromes follows a different diurnal pattern (Banerjee et al. 2016b). The diurnal rhythmic expression of PtAureo1a and 1c can still be detected when the cells are kept in the dark, while PtAureo1b expression appears to be light activated, and PtAureo2 oscillates only weakly throughout the day. The recently discovered diatom clock component RITMO1/PtbHLH1a (Annunziata et al. 2019) is strongly induced by blue light in wild-type cells, but not in the *Ptaureo1a* mutant, indicating that PtAureo1a might be involved in triggering the diatom clock in response to blue light.

Beside the aureochromes, *P. tricornutum* was previously reported to possess putative LOV-Histidine kinase photoreceptors (Bowler et al. 2008). However, closer examination of the corresponding genes showed that although they encode a recognizable PAS- Histidine kinase structure, the PAS signal sensor domain lacks the critical residues required for LOV photosensing, putting into question its function as a photoreceptor.

The Diatom Phytochromes (DPHs). Originally discovered in terrestrial plants as red/far-red light (R/FR) sensors, phytochromes (PHY) form a family of modular proteins that have a conserved N-terminal

photosensory core module (PCM) for light perception and a variable C-terminal output module involved in signal transduction (Rockwell and Lagarias 2020). The PCM encompasses a conserved architecture of PAS, GAF and PHY domains. The photosensitivity of phytochromes is conferred by a linear tetrapyrrole, covalently bound to a cysteine residue in the PAS or GAF domains, and derived from the cleavage of heme to biliverdin (BV), which can be further reduced by specific bilin reductases (Rockwell and Lagarias 2020). Phytochromes have been found in cyanobacteria, photosynthetic and non-photosynthetic bacteria, fungi, and diverse algae (Rockwell and Lagarias 2020). Canonical phytochromes exhibit the unique capacity to switch between R and FR absorbing forms (Pr and Pfr respectively) in a photoreversible way, with bacterial and fungal BV-binding PHY absorbing at longer R and FR wavelengths compared to the more reduced chromophore-binding PHY from cyanobacteria, plant or algae.

Among marine algae, phytochromes have been found in Glaucophyta, Prasinophyta, Streptophyta, Cryptophyta and Ochrophyta, but not in Haptophyta, Rhodophyta and Chlorophyta (Rockwell and Lagarias 2020). Recombinant PHY from some Glaucophyta and Prasinophyta microalgae and the multicellular *Ectocarpus siliculosus*, exhibit a large spectral diversity with some of them being able to sense orange, green or blue light (Rockwell et al. 2014). Possibly molecular evolution of PHY absorption properties has been driven by the particular spectral composition in aquatic environments (Rockwell et al. 2014).

DPHs predicted from genomic sequences (Table 21.2) all exhibit the typical output domain architecture of bacterial phytochromes (Figure 21.1B). Probably complex processes led to DPH evolution. Based on the phylogeny of the PCM, diatoms and other heterokont phytochromes are a sister clade of fungal phytochromes, separate from other algal phytochromes, and more similar to bacterial phytochromes, suggesting a different origin (Duanmu et al. 2014; Li et al. 2015; Fortunato et al. 2016). Pennate phytochromes seem to be closer to those from Phaeophyta than to those from centric diatoms. Moreover, predicted PHYs found in some brown algal viruses are also phylogenetically close to DPHs, suggesting that horizontal gene transfer events may have contributed to the evolution and diversification of this photoreceptor class in marine diatoms.

Recombinant DPHs from the centric diatom *T. pseudonana* and from the pennate *P. tricornutum* can both bind BV as chromophore and exhibit a genuine R/FR bacteriophytochrome absorption spectrum, shifted towards longer wavebands compared to the PHY found in plants or the other microalgae mentioned (Fortunato et al. 2016). This feature is indeed surprising given the rapid absorption of the R and FR wavelengths in the water column. Another particularity is the poor sequence similarity of the N-terminal part of DPH of pennate diatoms with known PAS domains. An additional cysteine in the GAF domain, at a position conserved with archaoplastidal phytochromes, has been found in some diatom DPH such as those from the pennate *Amphora coffeaformis* (Fortunato et al. 2016). Some phytochromes from the Phaeophyceae *E. siliculosus* also have these two conserved cysteines. One of these phytochromes has been spectrally characterized and has a green/FR photocycle in the presence of phytochromobilin (Rockwell et al. 2014). Therefore, we cannot exclude the possibility that some DPH might also bind phytochromobilin or other BV-derived chromophores and display different light sensing abilities.

In recent years, functional studies in *P. tricornutum* have provided initial information on DPH activities. Comparing gene expression in wild-type and *dph* knockout mutants in dark adapted cells exposed to either low R or FR treatments revealed a specific deregulation of FR-induced genes in knockout mutants, while responses to R were unaltered. This study demonstrated the activity of DPH as a photoreceptor triggering signal propagation in diatoms (Fortunato et al. 2016). The discovery that

FR sensing occurs in the ocean is significant although the function of this photoreceptor in diatom life is still unexplained. In *P. tricornutum*, DPH-regulated genes include some involved in transcription and signalling, but the majority are of unknown function. No physiological defects have been identified in the *P. tricornutum* knockout lines so far. In other diatoms, R- and FR-induced phenomena have been described, for example, the induction of sexual reproduction in *Stephanopyxis palmeriana* (Table 21.1). In the absence of genomic data in most of these species, it is still not possible to confidently propose that DPH are involved in the regulation of these processes.

Phytochrome signalling mechanisms usually involve two-component systems in bacteria (Auldridge and Forest 2011), and phytochrome interacting factors (PIF) TFs in land plants (Chen and Chory 2011). In the model fungi *Aspergillus nidulans*, two different pathways for phytochrome signalling have been investigated. One signalling cascade involves the SakA pathway, which starts with a two-component like phosphorelay, followed by a mitogen-activated protein kinase cascade and activation of the TF (Yu et al. 2016). In another pathway, fungal PHY interacts with chromatin regulation complexes, affecting gene expression via histone modification (Hedtke et al. 2015). In *P. tricornutum*, the phytochrome has been shown to auto-phosphorylate in response to FR, suggesting a phosphorelay mechanism, but the rest of the signalling cascade activating the observed gene expression changes is still unknown (Fortunato et al. 2016). No homologs of PIFs have been identified in diatoms.

Exploring the diversity of diatoms has yielded some interesting observations on DPHs. First, not all diatoms have a DPH. Several species such as *Thalassiosira oceanica*, *Fragilariopsis cylindrus*, *Pseudo-nitzschia multistrata* and *multiseries* do not possess the DPH gene, indicating that this photoreceptor is not essential for diatom biology. Moreover, differences have been also found between pennate and centric species. In addition to forming phylogenetically distinct clades, DPH genes in centric diatoms are always found as a single gene copy, while gene multiplication has occurred in several pennate species which may possess up to 5 phytochrome genes (Table 21.2). Data are yet too scarce to determine when multiplication occurred. However, the expansion of the phytochrome family observed in some biofilm-forming motile diatoms (Osuna-Cruz et al. 2020) suggests that DPH duplication might be the result of adaptation to specific environments and life strategies. Extensive mining of genomic and metagenomic data, coupled with careful functional analyses of DPHs selected from various diatom species, will hopefully provide enough evidence to resolve how phytochromes arose in heterokont microalgae and explain the cryptic functions in species that possess DPHs.

The Rhodopsins. These light sensing integral membrane proteins found in Archaea, bacteria, and eukaryotes share a topology of seven transmembrane alpha helices within which a retinal chromophore is covalently bound (Ernst et al. 2014). Rhodopsins exhibit a wide variety of spectral tuning in the blue-green part of the spectrum depending on the nature of a few influential amino acids interacting with the retinal (Man 2003; Ernst et al. 2014). Rhodopsins function as light-driven ion pumps, ion channels, or light sensors (Grote et al. 2014). The discovery that H⁺-pump rhodopsin converts light into ATP has challenged the assumed monopoly of photosynthesis as a phototrophy-enabling mechanism (Béjà et al. 2001; Finkel et al. 2013). Recently, a distinct group of microbial rhodopsins, the heliorhodopsins, has been identified after analysing environmental genomic samples (Pushkarev et al. 2018). Heliorhodopsins do not have the capacity for light-triggered ion transport but they do have a long photocycle, suggesting that they could act as signalling photoreceptors (Pushkarev et al. 2018). H⁺-pump rhodopsins and heliorhodopsins are present in bacteria, Archaea and algae and are highly represented in environmental genomic data (Pushkarev et al. 2018; Coesel et al. 2021).

Diatom rhodopsin-like sequences falling into the H⁺-pump group, based on conservation of key amino acids and phylogeny, were first identified in the transcriptome of *Pseudo-nitzschia granii* (Marchetti et al. 2012), then in those of other species (Marchetti et al. 2015) and in the genome of *F. cylindrus* (Mock et al. 2017) (Table 21.2). Because *P. granii* rhodopsin-like transcripts are highly abundant in low iron conditions, it has been hypothesized that this proteorhodopsin-like protein could be involved in energy production under conditions of iron deficiency that affect photosynthesis (Marchetti et al. 2012). It is noteworthy that a gene homologous to heliorhodopsin has been identified in the genomes of *P. tricornutum* and other pennate diatoms, but not in centric ones (Pushkarev et al. 2018) (Table 21.2). G-protein-coupled receptor rhodopsin-like genes, homologs of receptors that transduce a wide range of stimuli including light, hormones, volatile molecules, glycoproteins, nucleotides, and chemokines in eukaryotes (Costanzi et al. 2009), have also been identified in various diatom genomes (Port et al. 2013), with evidence of expansion of this gene family in some species (Osuna-Cruz et al. 2020). However, information about the spectral and functional properties of these and other diatom rhodopsins is still lacking, so their function as light sensors remains to be established.

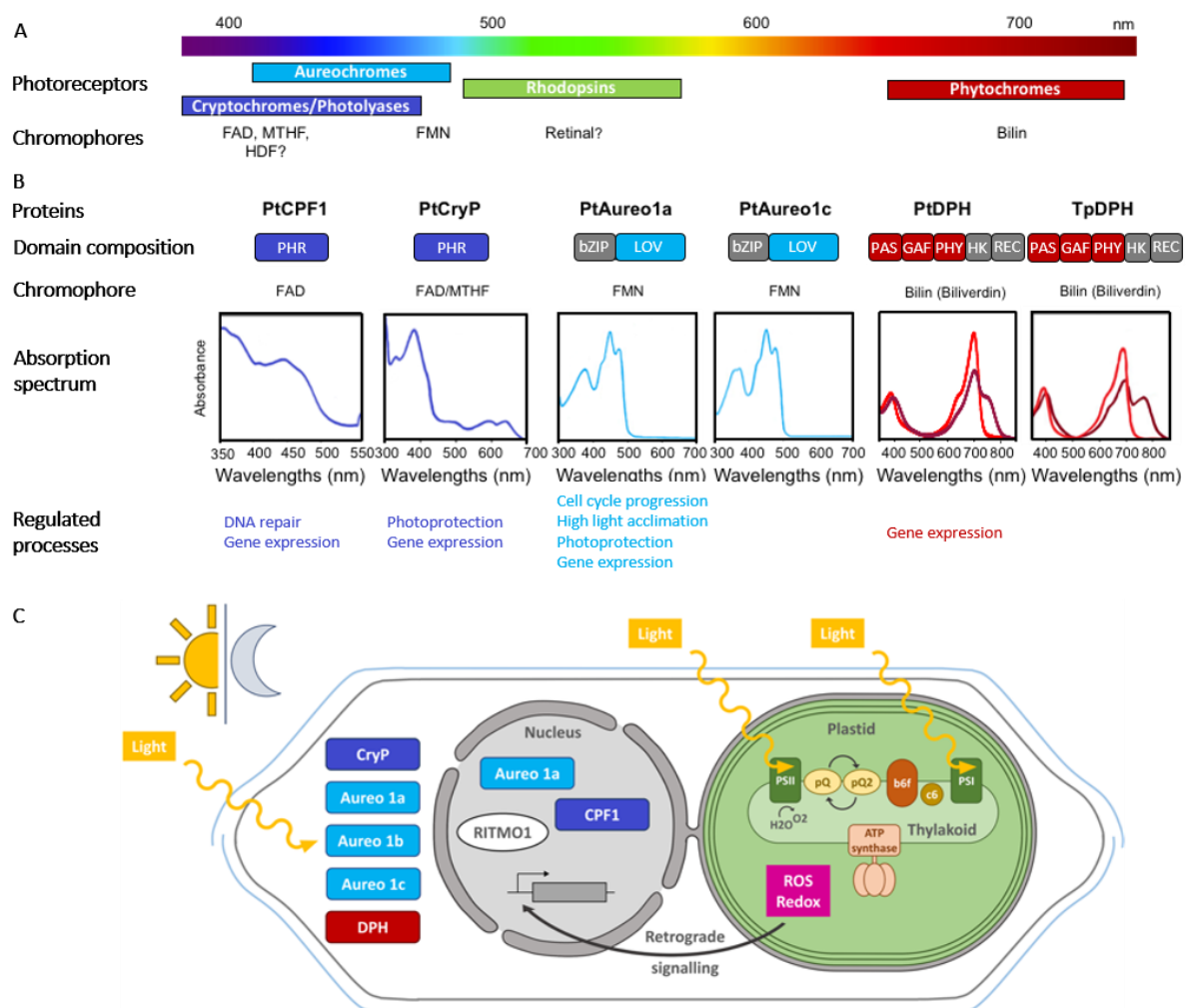


Figure 21.1. Sensing of abiotic cues in diatoms. A. Photoreceptor classes identified in diatom genomes. Cryptochrome-Photolyase, Aureochrome, Rhodopsin and Phytochrome families are positioned schematically according to the range of wavelengths they usually absorb. The nature of the chromophore is indicated below the respective photoreceptor boxes, with question marks indicating putative chromophore. B. Summary of characterized photoreceptors from *P. tricornutum* (Pt) and *T. pseudonana* (Tp). Domain architecture,

chromophore, absorption spectrum, and regulated processes are indicated for: cryptochrome photolyase 1 (PtCPF1)(Coesel et al. 2009); plant-like Cry (PtCryP)(Juhás et al. 2014); aureochromes PtAureo1a (Heintz and Schlichting 2016) and PtAureo1c (Bannister et al. 2019) and diatom phytochromes (PtDPH and TpDPH)(Fortunato et al. 2016). Domains involved in the light sensing are indicated in blue or red. C. Schematic representation of the characterized *P. tricornutum* regulators processing external and internal cues and controlling nuclear gene expression in response to light changes. They include photoreceptors, the regulator of cellular rhythmicity RITMO1, and plastid to nucleus retrograde signaling pathways related to the photosynthetic processes (ROS and redox signals). In the nucleus are shown the factors whose cellular localization or DNA binding has been experimentally validated.

II.B . Metabolic signalling

As for all photosynthetic eukaryotes, diatom genes encoding plastid and mitochondrial proteins are located in the nuclear and in the organellar genomes. Accordingly, organelle biogenesis and function require strong coordination of gene expression in different compartments. In plants and green algae, it is well established that nuclear-encoded proteins participate in the regulation of organellar gene expression and functions (anterograde signalling) and that mitochondrial and plastid factors control the expression of nuclear encoded genes (retrograde signalling) (Hernández-Verdeja and Strand 2018). The coordination of these different signalling pathways is essential to adjust cellular metabolism to environmental changes. The plastid itself is considered as an internal sensor of environmental signals because most of the metabolic reactions occurring in this organelle are extremely sensitive to external changes. Different intermediates of plastid processes such as tetrapyrroles or carotenoids, and products of the photosynthetic process can act as indicators of the plastid metabolism and nutrient and redox states and modify the expression of nuclear genes involved in stress responses (Hernández-Verdeja and Strand 2018).

In organisms with a secondary plastid such as diatoms, the information about retrograde signalling is still very limited. However, studies of the photosynthetic processes (Chapter 15 and 16) are providing initial evidence that different metabolites are involved in the relay of information from plastid to nucleus. In particular, it has been shown that light-induced expression of several nuclear genes involved in photoprotection, such as LHCX1 and 2, can be altered by artificially modulating the redox state of plastoquinone with drugs such as DCMU (3-(3', 4'-dichlorophenyl)-1, 1-dimethylurea) and DBMIB (2, 5-dibromo-3-methyl-6-isopropyl-p-benzoquinone) that mimic low light and high light phenotypes, respectively, as originally shown in green algae (Escoubas et al. 1995). The effect of these inhibitors has been observed for only some of the LHCX genes and only under specific light conditions, supporting the idea that multiple signalling pathways control their expression (Lepetit et al. 2013; Taddei et al. 2016). Plastid-to-nucleus signals have also been identified by genome-wide expression analyses performed in *P. tricornutum* exposed to equal doses of photosynthetic usable radiation of blue, green and red light (Valle et al. 2014). This study identified light-quality independent responses for many photosynthetic-associated nuclear genes, which are altered by inhibiting photosynthetic electron transport with DCMU. This study also showed blue light dependent control of some genes implicated to be involved in photoprotection and PSII repair. Functional studies of the CPF1 and aureochrome photoreceptors, support the involvement of blue light receptors in the regulation of photoacclimation processes. On the contrary, FR induced nuclear genes regulated by DPH are not

affected by the inhibition of photosynthetic electron transport (Fortunato et al. 2016), as expected for wavebands at the extreme end of the visible light spectrum that have a minimal input to photosynthesis.

Nutrient stress can alter plastid metabolism and in turn nuclear gene expression (Chapters 19 and 20). Genes implicated in carbon fixation are tightly controlled by both light and nutrient signalling. It has been proposed that the CO₂ concentration itself elicits the physiological modifications observed upon changes of the external CO₂ levels, by activating a signalling cascade involving the TF PtbZIP11 and cAMP (cyclic adenosine monophosphate) as a second messenger (Harada et al. 2006; Ohno et al. 2012). TF PtbZIP11 binds to CO₂/cAMP-responsive elements (CCREs) in CO₂-regulated genes such as the *P. tricornutum* carbonic anhydrase genes (*PtCAs*) (Harada et al. 2005; Ohno et al. 2012). These genes are also regulated by light activated pathways that crosstalk with CO₂/cAMP signals by acting on the same CCREs in promoters (Kikutani et al. 2012; Tanaka et al. 2016). It has been shown that different photosynthetically active monochromatic-lights confer similar transcriptional activation of *PtCAs* genes when CO₂ is limiting, while an artificial electron acceptor from the reduction side of PSI efficiently inhibits their expression. These data support the idea that there is redox control within CO₂/light crosstalk (Tanaka et al. 2016). At the same time, the direct involvement of photoreceptors in *PtCAs* activation has not been ruled out.

Recent evidence also indicates that reactive oxygen species (ROS) which accumulate under exposure to excess light can also play a signalling role in diatoms, acting as secondary messengers, as is known to occur in other organisms (Mittler et al. 2011). As described in detail in Chapter 22 a compartmentalized redox-sensitive signalling network has been characterized in *P. tricornutum* by analysing the redox proteome as well as redox-sensitive biosensors (e.g., reduction-oxidation sensitive green fluorescent protein) localized in different cellular compartments (Rosenwasser et al. 2014; Graff van Creveld et al. 2015). Single-cell analysis of responses to environmental stresses that generate ROS revealed that changes in the chloroplast redox state play a critical role in controlling cell fate decisions toward acclimation or cell death (Mizrachi et al. 2019). It is still unclear which molecules act as plastid redox signal(s) or how signals are transmitted to the nucleus in diatoms, plants or green algae.

II.C . Regulation of diatom biological rhythms: integrating external and internal signals

Like most other organisms, diatoms experience periodic variation in light/dark cycles because of the Earth's rotation. Numerous laboratory and field-based investigations have clearly indicated synchrony of algal life processes with these cycles. As in many microalgae, cell cycle progression is strongly linked to light and dark alternations (Vaulot et al. 1986) and progression through various cell cycle phases is tightly controlled by both light and nutrient availability (Chapter 9). Temporal variations in key metabolites have also been described. Synthesis of photosynthetic pigments (Ragni and d'Alcalà 2007), fixed carbons (β -glucans), or lipids (Chauton et al. 2013; Ashworth et al. 2013) normally increase during the light to sustain other energy-demanding cellular activities at night. Diatoms also vary in their sensitivity to oxidative stress over a diurnal cycle, due to the light-dependent production of antioxidants (e.g. glutathione GSH cellular pool) which decrease during the dark period (Volpert et al. 2018). Rhythmic movements of benthic diatoms dependent on diel cycles and on tides have been well documented over the years (Round and Haphey 1965). It has been shown more recently that this migration strategy relates to the decoupling of cell division, which occurs in darkness deep in the sediment, and photosynthesis, which occurs at the surface of the sediment during light periods (Saburova and Polikarpov 2003; Barnett et al. 2020).

At a molecular level, diverse diurnal transcript profiling experiments performed in the diatom *T. pseudonana* (Ashworth et al. 2013), *P. tricornutum* (Chauton et al. 2013; Smith et al. 2016; Annunziata et al. 2019) and *Seminavis robusta* (Bilcke et al. 2021) revealed a strict temporal separation of diatom transcriptional gene networks with day/night cycles. A 24-h periodicity in transcriptional responses have also been reported in diatoms *in situ* from the metatranscriptome analysis of environmental populations over multiple diurnal cycles (Kolody et al. 2019; Coesel et al. 2021). The data show robust diel transcriptional patterns and the temporal partitioning of gene expression implicated in key biological processes such as photosynthesis, cell-cycle and light perception. Moreover, semidiurnal (12-h) gene expression periodicity has been described in the diatom *S. robusta* (Bilcke et al. 2021), which may reflect adaptations to the occurrence of tides in coastal benthic ecosystems or might be linked to the twice daily vertical migration of diatoms into the sediment previously described.

It is still largely unknown how diatoms orchestrate their life according to light/dark cycles. Processes occurring during the light phase could be directly activated by photoreceptor-mediated signalling pathways. Other light-driven processes such as photosynthesis may also contribute to temporal segregation of cellular processes and metabolism, as in other phototrophs (Haydon et al. 2013; Dodd et al. 2015). Several studies indicate that diatom cellular rhythmicity is also regulated by an endogenous circadian clock. This has been demonstrated by monitoring rhythms of cellular fluorescence (Annunziata et al. 2019) or photosynthesis and migration behaviours (Round and Haphey 1965; Harding et al. 1981) over light/dark cycles compared to constant light. The persistence of these rhythms for several days in the absence of periodic cues clearly support the presence of an autonomous timekeeper regulating circadian rhythms in diatoms, as in most organisms (Dunlap 1999). Nevertheless, the diatom circadian clock system remains largely uncharacterized because no orthologs of bacterial, animal, or plant circadian clock components have yet been found in the diatom genomes, except for CRYs and casein kinases. The first regulator of diatom biological rhythms named RITMO1 has been identified recently in *P. tricornutum* by searching within all diatom TFs for those showing robust rhythmic expression profiles in light/dark cycles and in the dark (Annunziata et al. 2019). RITMO1 is a nuclear localized protein containing bHLH and PAS domains, and alteration of its expression levels and timing by ectopic overexpression resulted in lines with deregulated diurnal gene expression profiles compared with wild-type cells (Annunziata et al. 2019). Gene expression fluctuations were also reduced in these lines in continuous darkness, showing that the regulation of rhythmicity by RITMO1 is independent of light input. Also, cell fluorescence rhythms were perturbed by deregulation of RITMO1 expression in light/dark cycles and, even more so in constant light, strongly supporting its role in circadian regulation.

Several lines of evidence indicate a partial conservation of the regulatory program generating rhythmicity in animals and diatoms. RITMO1 contains bHLH and PAS protein domains that are also present in the CLOCK and BMAL proteins, key components of the mammalian central oscillator (Dunlap 1999), as already mentioned. This raises novel questions on the evolution of circadian clock in diatoms and in many other phytoplankters, in which RITMO-like proteins have been found (Annunziata et al. 2019). bHLH-PAS RITMO-like proteins might have independently acquired a function in rhythm regulation by convergent evolution. However, it has also been hypothesised that this function might be present in the ancient heterotrophic host that subsequently acquired plastids via endosymbiosis. Remarkably, an expansion of bHLH-PAS TFs has been found in the genome of the benthic diatom *S. robusta*, with seven out of ten genes showing rhythmic expression either at dawn or broadly during the day (Bilcke et al. 2021). It has been proposed that the expansion and expression divergence of

these putative circadian genes represent an adaptation of diatoms to the highly rhythmic benthic environment. Thus, these factors currently represent ideal entry points to further characterize the mechanisms generating cellular rhythmicity in diatoms and address their relevance for life in the marine ecosystem that is governed by a multitude of rhythms of different periods, including daily, tidal, lunar, or seasonal cycles.

III. Nutrient Sensing Pathways

The availability of macronutrients such as nitrogen, silicon, phosphorus, and carbon, or micronutrients such as iron are essential for diatom growth. Whether freely living in the water column or attached to substrates, diatoms experience frequent variations in nutrient concentrations due to the movement of currents, water mixing, or tides. It is well known that these microalgae have a strong capacity to adjust their physiology and metabolism to the nutrients available, due to genomic and metabolic features that have been described in detail in chapters 19 and 20. However, compared to other established model systems (Chantranupong et al. 2015), we still lack information on how diatoms sense and respond to nutrient changes in their environment.

Diatoms have evolved an array of nutrient transporters, including diverse families of channels, carriers and pump proteins, that are modulated in accordance with nutrient demand and availability (Chapter 19 and 20). A comprehensive description of these transporters is beyond the scope of this review. However, it is noteworthy that in plants it has been demonstrated that some of these proteins, known as transceptors, play a signalling role in addition to their transport function (Gojon et al. 2011). In particular, the plant low affinity nitrate transporters (NPF) can bind and transport different substrates, such as phytohormones controlling organogenesis according to nitrate availability (Fan et al. 2017). NPF genes have been found in the genomes of several diatom species (Rogato et al. 2015). Considering the low concentration of nitrate in the oceans, it has been hypothesized that the diatom NPFs, predicted to be located in the plasma membrane, could bind alternative substrates such as oligopeptides or hormones and be involved in the control of different processes such as interactions between diatoms and bacteria (Santin et al. 2021). A similar function in nutrient sensing has been proposed for the silicic acid transporter (SIT) of *T. pseudonana* (Shrestha and Hildebrand 2015). By generating and analysing knock-down mutants of *sit1* and *sit2* genes, it was demonstrated that in growth conditions with sufficient silicic acid, the transport role of the SITs is relatively minor. It has been proposed that under these conditions, their primary role is to sense silicic acid levels to evaluate whether the cell can proceed with its cell wall formation and division processes.

A recent study integrating transcriptomic and phosphoproteomic analyses in *P. tricornutum* highlighted that protein phosphorylation is an important part of regulation in systemic nutrient sensing (Tan et al. 2020). Proteins that are conditionally phosphorylated as a function of diel cycling and iron and nitrogen availability have been identified, as well as a number of stress related kinases (e.g. calcium dependent protein kinase, calcium calmodulin dependent protein kinase, inositol hexakisphosphate/diphosphoinositol-pentakisphosphate kinase and Ser/Thr kinase) that might be responsible for phosphorylating them. TOR (target of rapamycin) protein kinases have also been found in diatoms (Prioretti et al. 2017). As the name indicates, TOR Ser/Thr kinases are inactivated by selective ATP-competitive inhibitors such as rapamycin. These widespread proteins play a central regulatory role in cell homeostasis because they connect information about the quantity and quality of different environmental cues (including nutrients) to metabolic processes (Dobrenel et al. 2016). A current working model for TOR signalling proposes that these kinases relay a permissive signal to

downstream targets only in the presence of sufficient nutrients to fuel protein synthesis. Compared to mammalian TOR, the TOR gene described in *P. tricornutum* (Prioretti et al. 2017) possesses highly conserved motifs and amino acids of the ATP-binding pocket in the TOR kinase domain. In *P. tricornutum*, the inhibition of TOR with inhibitors developed for mammalian TOR, resulted in a nutrient starvation response (Prioretti et al. 2017) as in other organisms (Ingargiola et al. 2020). It has been shown that the inhibition of the TOR activity reduced but did not halt cell proliferation unlike growth under N starvation and also improved triacylglycerol (TAG) productivity, strongly suggesting that this pathway is somehow involved in nutrient sensing and metabolic remodelling.

The PII proteins are signalling components widely distributed in all domains of life that help to coordinate carbon and N assimilation but they have not been found in diatoms (Rogato et al. 2015). However, in the genome of *T. pseudonana* and *P. tricornutum* the gene *argB* encodes the enzyme N-acetyl-L-glutamate kinase (NAGK) (Chellamuthu et al. 2013), which in plants and cyanobacteria has been demonstrated to form a complex with PII in response to N starvation. NAGK is a member of the amino acid kinase family that control a step in arginine synthesis. Arginine is needed to make proteins and, together with its precursor ornithine, to make polyamines and urea. Therefore, it is reasonable to hypothesise that diatom NAGKs are involved in the response to urea, which is indeed part of N metabolism, and that a diatom specific mechanism of N signalling might have evolved in these microalgae (Chellamuthu et al. 2013; Rogato et al. 2015). Phylogenetically, diatom NAGK cluster with alphaproteobacterial NAGK (Chellamuthu et al. 2013). It is thus possible that during the evolution of Stramenopiles, the *argB* gene was acquired by horizontal gene transfer, while the original cyanobacterial/plant NAGK and PII genes were lost.

The recent discovery of a novel Ca^{2+} signalling pathway involved in environmental phosphorus (P) sensing and acclimation has provided new insights on the peculiar nutrient sensory system of diatoms (Helliwell et al. 2021a). By using the fluorescent Ca^{2+} biosensor R-GECO1, it has been shown that centric and pennate diatoms grown under P-limited conditions exhibited rapid and transient raise of cytosolic Ca^{2+} within seconds following resupply of environmentally relevant concentrations of a range of P forms. These responses were not detected in P-replete cells. Remarkably, this study also highlights the existence of rapid cross-talk between P and N metabolism based on P- Ca^{2+} signalling. In particular, primary diatom responses to P limitations involves substantial increase in the assimilation of N, a major constituent of proteins, nucleic acids and chlorophyll, thus exploiting another vital nutrient for cellular recovery and restoration of growth. This coordinated regulation of P and N metabolism could enhance diatom ability to compete for resources in dynamic nutrient environments.

Although the molecular machinery underpinning nutrient sensing and signalling remains largely uncharacterized, it is well established that diatom responses to nutrient changes require massive changes in gene expression that ultimately controls nutrient transport, mobilization, or storage. How the environment impacts genome expression is still largely unknown, but it is likely that this requires the integrated action of signalling components and genetic and epigenetic factors that we are just starting to discover in these microalgae (Chapter 7). TFs that are differentially expressed under different nutrient conditions have been identified in many transcriptomic studies. However, only in few cases the involvement of TFs in the regulation of nutrient metabolism has been demonstrated. Examples are the novel N starvation-inducible RING-domain protein called RING-GAF-Gln-containing protein (RGQ1), which regulates the response of *P. tricornutum* to N starvation (Matthijs et al. 2016), and the leucine zipper bZIP14 which has been proposed to regulate remodelling of the TCA cycle and N metabolism (Matthijs et al. 2017). Massive changes in the expression of different TF gene families have also been described in response to phosphate (P) fluctuation (Cruz de Carvalho et al. 2016). A

regulator of P limitation in the Myb TF family called PtPSR has recently been characterized in *P. tricornutum* (Kumar Sharma et al. 2020). *ptpsr* knockout mutants show reduced cell growth under P limitation and reduced induction of the P-stress response genes containing PtPSR recognition motifs. PtPSR therefore has a pivotal role in *P. tricornutum* adaptation to P limitation (Kumar Sharma et al. 2020).

Genes involved in nutrient stress responses are likely to be regulated also by mechanisms involving non coding RNAs (ncRNAs). High-throughput sequencing of small RNAs (sRNAs) extracted from *P. tricornutum* cells grown under different conditions of light and iron starvation showed the unexpected complexity of sRNA populations in these algae (Rogato et al. 2014). Some sRNAs were identified and experimentally validated as being specifically expressed under iron limitation, including U2 snRNA derived sRNAs and tRNA-derived sRNAs. A similar analysis in *T. pseudonana* and *F. cylindrus* (Lopez-Gomollon et al. 2014) revealed that a significant number of tRNA-derived sRNAs were up-regulated under specific conditions of Si and Fe limitation. Moreover, both studies reported a huge subset of sRNAs associated to repetitive transposable elements (TEs) marked by DNA methylation. Interestingly, some *P. tricornutum*-specific Ty1/copia-like retrotransposons have been shown to be induced under N deficiency (Maumus et al. 2009), and cytosine methylation is thought to control the mobility of these TEs, as they are hypomethylated when expressed (Veluchamy et al. 2015). Altogether, these data suggest that complex small RNA-dependent DNA methylation processes may contribute to controlling the expression of TEs in diatoms and regulating gene expression and alterations in genome structure when certain nutrients are limiting. Long non-coding RNAs of the long intergenic non-protein coding RNA (lincRNA) and natural antisense transcript (NAT) classes have been described in *P. tricornutum* (Cruz de Carvalho et al. 2016; Cruz de Carvalho and Bowler 2020). Remarkably, the P-regulated noncoding transcriptome (NATs and lincRNAs) is almost equivalent in number to the coding transcriptome (messenger RNA), providing compelling evidence of the relevance of ncRNAs in the regulation of environmental stress responses in diatoms (Cruz de Carvalho and Bowler 2020).

IV. Diatom sensing of other abiotic stimuli

Besides the light and nutrient sensing pathways previously described, some information is also emerging on the mechanisms controlling diatom responses to other abiotic cues. It is well established that rapid shifts in osmolarity in coastal and estuarine regions, as well as in sea-ice environments, can trigger specific metabolic and morphological responses in diatoms (Kirst 1990). Several studies in *P. tricornutum* revealed a key role of intracellular Ca^{2+} signalling in diatom osmoregulation (see also Chapter 22). Elevation of cytosolic Ca^{2+} detected with the bioluminescence aequorin reporter were described in response to hypo-, but not hyper-osmotic shock (Falciatore et al. 2000). More recent studies employing novel single-cell imaging techniques revealed intracellular spatiotemporal patterns of osmotic-induced Ca^{2+} elevations, regulating K^{+} efflux. These processes enable the fine-tuned cell volume regulation across the whole cell, preventing cell bursting (Helliwell et al. 2021b). Additionally, Ca^{2+} independent pathways controlling organic osmolytes efflux also contribute to the diatom osmotic stress tolerance (Kirst 1990; Helliwell et al. 2021b).

There are also evidences in diatoms of sophisticated sensing systems for detecting and responding to fluid motion (shear stress), which also involve transient changes in cytosolic Ca^{2+} (Falciatore et al. 2000). In *P. tricornutum*, the response to fluid motion is rapid (seconds) and highly controlled: the cells lose their ability to respond with their original sensitivity, when a second stimulus

is applied shortly after the first. With this short-term adaptation or desensitization process, diatoms can reversibly adjust their sensitivity to the level of the stimulus (Falciatore et al. 2000). Differential gene expression analyses and microscopy investigations also revealed morphological and transcriptional changes of the chain-forming diatom *Chaetoceros decipiens* in response to measured levels of turbulence in nutrient replete conditions (Amato et al. 2017). These active responses support the hypothesis that turbulence not only favour nutrient uptake, but it can also act as signal, that diatoms exploit to reorganise their physiology in particular environmental conditions.

V. Future directions

Experimental and *in silico* studies on diatom signalling pathways described in this review is revealing intriguing facts about the environmental sensing abilities of these microalgae. Biophysical and functional characterization of photoreceptors such as AUREO, CPF and DPH have provided novel information on the role and the function of these sensors in aquatic environments, as well as their involvement in the control of key processes in diatoms. There is still insufficient information to demonstrate the importance of the CRY-DASHs, which are highly represented in the genome of all organisms, including diatoms, and possibly have conserved functions. Similarly, several hypotheses exist on how rhodopsin could be involved in the regulation of diatom metabolism in response to nutritional stress, but more evidence is needed to understand the pertinence of known rhodopsin variants. Many novel protein domains potentially involved in light sensing have been predicted from diatom genomes, but they are linked to other protein domains in combinations which have not been described before (Bowler et al. 2008). It is important to mention that possible novel light sensors have also been recently identified in planktonic organisms after extensive exploration of metagenomic data (Coesel et al. 2021), which could now be characterised in diatom model species (Falciatore et al. 2020).

Significant work remains to characterize the signalling pathways downstream of the receptors. Intriguingly, equivalents of the components of light signalling pathways of plants (Chen and Chory 2011) have not been found to be encoded in diatom genomes, suggesting distinct differences in the regulatory cascades between these organisms. It is interesting to note that diatom genomes do have several genes predicted to encode bacterial-like proteins with features of two-component phospho-relay systems. Therefore, it is likely that previously unreported combinations of bacterial and eukaryotic signalling systems will be identified by studying receptor-interacting factors and downstream targets with biochemical and genetic approaches.

Much remains to be done to identify sensing and signalling components involved in the responses to other abiotic cues of relevance for diatoms. Diatoms are particularly abundant at high latitudes and show specific temperature acclimation and adaptation responses (Mock et al. 2017; Liang et al. 2019). It is still unclear if these responses are regulated by a true temperature sensor or by the modification of the physical properties of enzymes, proteins or membranes in the cell caused by temperature changes. There is also the possibility that the signalling pathways transducing temperature information in marine environments overlap with those of other cues (e.g., light), as shown in plants (Lamers et al. 2020). Therefore, the exploration of how diatoms sense and respond to temperature changes will be particularly informative, also to assess the impact of global warming on their growth and distribution. Moreover, though circadian and seasonal rhythms have been described in diatoms, the genetic basis regulating physiological adaptation to varying latitudinal and seasonal photoperiods remain completely unknown. In terrestrial organisms, the endogenous circadian clock has a key role in regulation of photoperiodism (Millar 2016) coordinating physiology not only to

photoperiodic cues through specific photoreceptors, but also to temperature and other abiotic stresses (Greenham et al. 2017). Uncoupling temperature and photoperiod signals can have a dramatic consequence on the reproductive success of species adapted to specific habitat by altering physiology or predator–prey mismatch. Therefore, as the most advanced molecular phytoplankton model species, diatoms represent the ideal system for the identification and characterization of novel signalling components processing external and internal signals to anticipate and respond to environmental variations, and also to address the relevance of these pathways in the adaptation of phytoplankton to specific latitudinal and temporal niches.

Another challenging questions for the future will be to address the ecological relevance of the sensing systems in the real environment, where diatoms are exposed to a multitude of different signals and stresses. A strong crosstalk between different pathways, like the light-activated processes described here, would be expected to optimize diatom physiology and metabolism in changing environments. We also expect there to be significant interactions between regulatory pathways implicated in the perception of abiotic and biotic cues and ensuing responses. Master regulators of multiple signalling pathway are likely to be discovered by comparing responses to individual and combined signals and stresses. Omics techniques applied to selected mutants could help in identifying common players in environmental perception and responses. An important contribution to these studies could derive from targeted exploration of the vast amount of environmental data that are now available for marine plankton. Analysing the expression of a specific receptor in a particular environment or the identification of its genetic functional variant correlating with specific abiotic or biotic parameters could help to pinpoint novel regulators of diatom life in complex ecosystems. The remarkable diversity of diatoms could be exploited as an additional tool to gain insight into the function of environmental sensors. As noted in this chapter, DPHs and rhodopsins have only been found in some diatom species. By further investigating the diversity and distribution of diatom species possessing a particular receptor or modified receptor-like properties, it will be possible to identify novel regulators of diatom phenotypic plasticity and to assess how they equip these algae to colonize highly diverse marine environments.

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