



# Green algal molecular responses to temperature stress

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## Abstract

Global warming is a critical issue and has great impact on all living organisms, including algae. Generally, algae play significant roles in aquatic ecosystems and employ diverse strategies to survive under abiotic stress. For example, heat stress affects membrane fluidity, and algae, in response, can modify their membrane fatty acid composition to maintain homeostasis. Moreover, they protect their proteins and enzymes using molecular chaperones or degrade denatured proteins in processes involving ubiquitin. In addition, algae regulate their carbohydrate concentrations and structures to utilise the energy of endogenous carbon sources efficiently and protect other molecules via accumulation of compatible solutes. Algae regulate the photosynthetic machinery to acclimatise to stress conditions. In fact, algae have a range of acclimation and repair strategies; and in the case where these strategies fail, programmed cell death (PCD) will be activated. Among algae, green algae have been massively studied due to their broad-range applications such as pharmaceutical, biofuel production and wastewater management, and being a suitable model to study plant and photosynthesis. Enhanced knowledge about the genes and proteins involved in the acclimation of green algae would enlighten our understanding of their acclimation pathways, and enable the genetic improvement of stress-tolerant strains. Thus, the mechanisms and pathways associated with green algal acclimation and repair strategies with an emphasis on temperature-related stress are highlighted in this review.

**Keywords** Global warming · Green algae · Protein · Gene expression

## Introduction

Massive consumption of fossil fuel and the release of greenhouse gases into the air contribute to global warming (Judkins et al. 1993; Solomon et al. 2009; McGlade and Ekins 2015). Global warming is a deleterious factor affecting

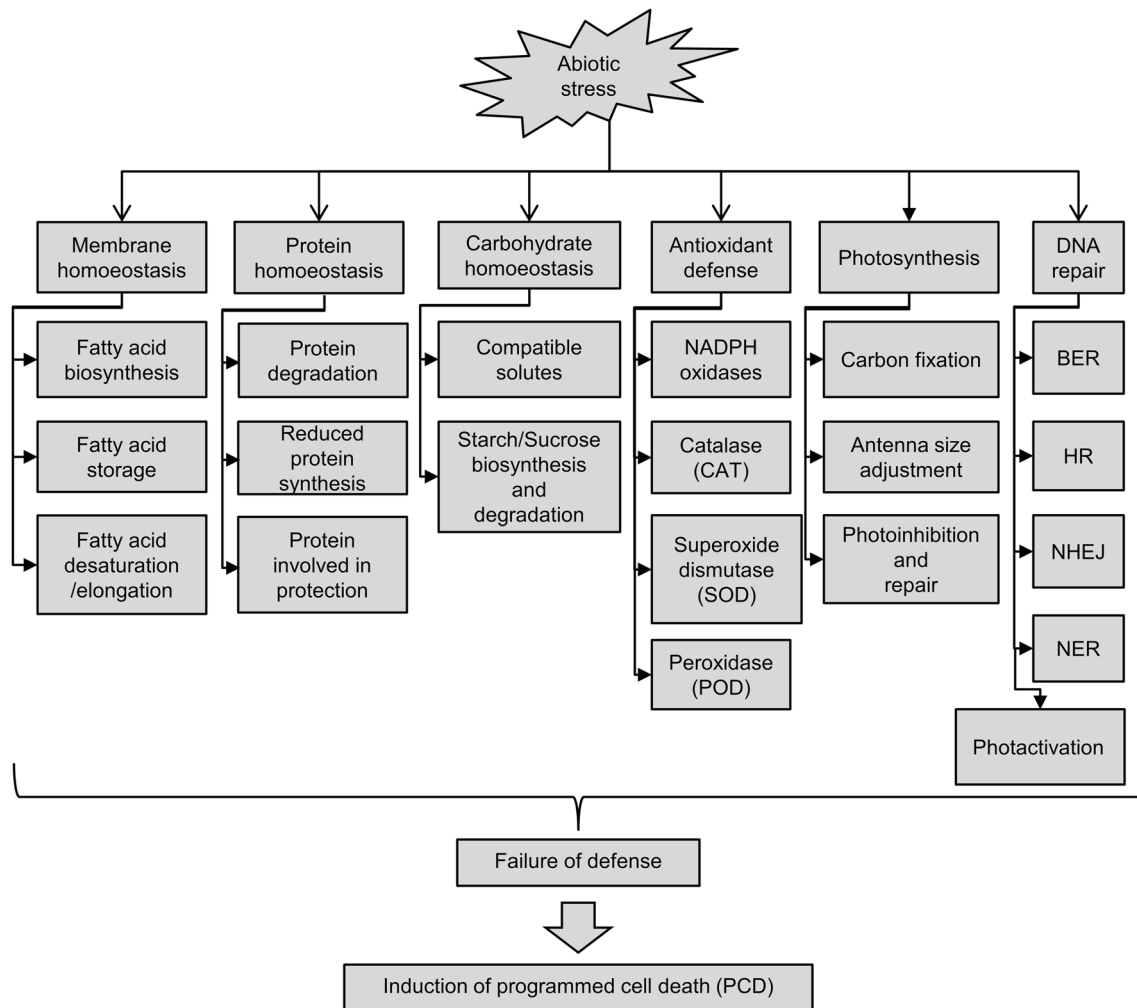
aquatic ecosystems, and sequentially leads to significant shifts in aquatic biogeochemical cycles, dynamics, biodiversity and aquatic food web structure (Harley et al. 2006; Wrona et al. 2006). In aquatic ecosystems, algae play essential roles in carbon sequestration and contribute to food webs as primary producers (Sayre 2010; Tsai et al. 2015). Their roles and distribution are influenced by the shift in environmental variables due to global warming (Beardall and Raven 2004; Bopp et al. 2005; Paul 2008; Olsenz 2011). Acute changes in temperature, irradiance, salinity, or pH will influence their cellular integrity and bio-molecular composition, thereby disrupting cellular homeostasis (Jauzein and Erdner 2013; Van Creveld et al. 2015). Environmental stress could lead to severe damage and initiate responses that result in either acclimation or programmed cell death, depending on the sensitivity of the algae (Zuppini et al. 2007; Sulmon et al. 2015). Commonly, algae utilise several stress management and repair strategies (Fig. 1), such as adjusting membrane fluidity via changing the fatty acid saturation level (Murata and Los 1997; Los et al. 2013; Maksimov et al. 2017), reducing protein synthesis to avoid an upsurge of mis-folded proteins (Schroda et al. 2015), accumulating

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**Fig. 1** Molecular events involved in DNA damage repair and stress adaptation. Algae have developed a range of adaptation and repair strategies. However, these strategies might fail leading to the activation of programmed cell death

compatible solutes to maintain cell osmolality (Klahn and Hagemann 2011), and regulating photosynthesis to balance energy production and consumption (Ras et al. 2013). In this review, green algal acclimation mechanisms and pathways responding to temperature stress are emphasised.

### Membrane homeostasis and lipid content

The capability of green algae to survive against various ecological conditions is strongly reflected in the unusual pattern of their cellular lipids and their capacities for changing lipid metabolism (Thompson 1996; Liu et al. 2016). The physical properties of the membrane rely on its fatty acid profile and the degree of saturation which regulates the fluidity of membranes (Singh et al. 2002). The fluidity of membranes is affected by temperature fluctuations. Low temperature reduces membrane fluidity, which is

ameliorated via membrane lipid desaturation by means of fatty acid desaturases (FADs) (Sakamoto and Murata 2002). Indeed, at low temperature, algal cells increase the content of membrane unsaturated fatty acids (UFAs) to achieve a looser packing of lipids, thus, decrease the solidification of membrane lipids (Lyon and Mock 2014). Furthermore, it was noted that by increasing the percentage of unsaturated fatty acids in the membrane, the photosynthetic machinery of algae could also be stabilised under chilling (Wada et al. 1994) and salinity stress conditions (Allakhverdiev et al. 2001). Conversely, heat increases membrane fluidity, which is ameliorated by the integration of de novo-synthesised saturated fatty acids (SFAs) into membrane lipids, and the presence of membrane-stabilizing proteins (Los et al. 2013). Fundamentally, two main family groups of enzymes control fatty acid structures; desaturases and elongases (Khozin-Goldberg and Cohen 2011). During stress conditions, cells show an increase in fatty acid synthesis. The mechanisms

involved in lipid homeostasis can be monitored by analysing the expression of the central regulatory genes, such as genes encoding subunits of acetyl-CoA carboxylase (ACCase), ketoacyl-ACP synthase (KAS), desaturase, and elongases. ACCase is known as one of the enzymes that control fatty acid biosynthesis rate and present in almost all algae (Podkowinski and Tworak 2011; Singh et al. 2016). It comprises four subunits, namely accA, accD, accC and accB. In certain stress conditions, such as metal stress (e.g. iron), up-regulation of ACCase subunits was observed along with elevated ACCase activity and fatty acid synthesis in *Chlorella sorokiniana* (Wan et al. 2014). ACCase activity yields malonyl-ACP, where the malonyl entity participates in a sequence of condensation reactions, resulting in the lengthening of the precursor fatty acid in which KAS is the rate-limiting enzyme (Rismani-Yazdi et al. 2011; Lei et al. 2012). In *Dunaliella salina*, high levels of KAS were found when cells were exposed to increased salinity levels (Azachi et al. 2002).

It is essential to consider that the SFAs formed might undergo elongation and desaturation steps to make longer chains of unsaturated fatty acids, or polyunsaturated fatty acids (PUFAs) (Pereira et al. 2003). PUFAs are fatty acids containing 18 carbons or beyond with the presence of at least two double bonds (Huang et al. 2004). They affect membrane fluidity owing to their very low melting points. The solidification of the membrane lipids can, therefore, be reduced by increasing the ratio of PUFAs in the membrane (Brett and Müller-Navarra 1997). Amidst the enzymes participating in lipid metabolism,  $\Delta 9$  fatty acid desaturases (FADs) catalyse the first committed step in desaturation and initiate the conversion of SFAs to mono-unsaturated fatty acids (MUFAs), which are vital for the generation of PUFAs (Xue et al. 2016). These desaturases introduce the first double bond to palmitic acid (C16:0) and stearic acid (C18:0) and convert them to palmitoleic acid (C16:1) and oleic acid (C18:1), individually (Sakuradani 1999; Xue et al. 2016). Up-regulation of several fatty acid desaturases ( $\Delta 9$ ACPCiFAD,  $\Delta 12$ CiFAD,  $\omega 3$ CiFAD2 and  $\Delta 6$ CiFAD) was observed in the Antarctic ice alga *Chlamydomonas* sp. ICE-L when it was exposed to salinity stress (An et al. 2013a). Under low temperature (0 °C),  $\Delta 9$ CiFAD,  $\omega 3$ CiFAD2 and  $\omega 3$ CiFAD1 were up-regulated in this microalga while  $\Delta 6$ CiFAD amplified with rising temperature. However, the temperature did not cause deregulation of  $\Delta 12$ CiFAD except at a specific temperature (15 °C). These observations suggested that  $\omega 3$ CiFADs might have important roles in cold adaptation while  $\Delta 6$ CiFAD enhanced survival under high temperature (An et al. 2013b). On the other hand, in response to cold and salt stress, Antarctic *C. vulgaris* up-regulated the  $\Delta 12$ FAD enzyme which catalysed desaturation at the  $\Delta 12$  position (Lu et al. 2010a, b). Stearoyl-acyl carrier protein desaturase (SAD) is another enzyme involved in oleic acid

(18:1) synthesis via the insertion of the first double bond into stearic acid (18:0) (Shanklin and Somerville 1991; Shanklin and Cahoon 1998). Stress conditions such as high light and nitrogen deficiency (N-) drastically up-regulated the transcripts of SAD in *C. zofingiensis* and resulted in the accumulation of whole fatty acids, comprising oleic acid (Liu et al. 2012).

In eukaryotes, energy can be stored as triacylglycerols (TAGs), which act as an essential carbon source (Merchant et al. 2012), contributing to a vital part of survival in environmentally harsh conditions (Singh et al. 2002; Fan et al. 2014). Most microalgae accumulate TAG under nitrogen- or phosphorus-limiting conditions (Fan et al. 2014), high salinity (Bartley et al. 2013) and temperature stress (Fakhry and El Maghraby 2015; Elsayed et al. 2017). Two pathways to form TAG are emphasised, namely the acyl-CoA-dependent (Kennedy pathway) and the acyl-CoA-independent pathways (Lenka et al. 2016). In algae, the Kennedy is the main pathway where TAG is generated via consecutive acylation of glycerol-3-phosphate (G3P) using acyl-CoA. Briefly, glycerol-3-phosphate acyltransferase (GPAT) begins TAG biosynthesis via catalysing the transfer of FA from the acyl-CoA pool on to G3P forming lysophosphatidic acid (Wendel et al. 2009), followed by the production of phosphatidic acid (PA) catalysed by lysophosphatidic acid acyltransferase (LPAAT). Overexpression of GPAT and LPAAT led to an increase of lipid percentage in *Chlamydomonas reinhardtii* (Iskandarov et al. 2016; Yamaoka et al. 2016). Meanwhile, diacylglycerol acyltransferase (DGAT) catalyses the last stage of TAG assembly, and is proposed as a key regulator of TAG biosynthesis (Lenka et al. 2016). Indeed, the transformation of the gene encoding DGAT was used as a strategy to obtain higher lipid content in *C. reinhardtii* (Ahmad et al. 2015). Alternatively, TAG can also be produced by three enzymatic reactions in the acyl-CoA-independent pathway, involving phospholipid diacylglycerol acyltransferase (PDAT), DGAT, and diacylglycerol transacylase (DGTA) (Chen and Smith 2012). PDAT was reported as the main enzyme for TAG biosynthesis in *C. reinhardtii* (Yoon et al. 2012) and increased levels of PDAT were observed when the microalga was subjected to nutrient limitations, such as iron (Urzica et al. 2013) and nitrogen (Boyle et al. 2012).

## Protein homeostasis

Several stress conditions, particularly heat, disrupt weak interactions in protein structures and affect the stability of protein conformation (Daniel et al. 1996). Heat stress could induce the aggregation and denaturation of proteins, resulting in membrane injury and alterations to metabolic fluxes (Fu et al. 2008). Consequently, accumulation of unfolded proteins under stress conditions triggers the expression of

molecular chaperones, namely heat-shock proteins (HSPs), that act to re-establish protein homeostasis (Fulda et al. 2010). Expression of HSPs induced by high-temperature is commonly observed in various living organisms (Parsell and Lindquist 1993; Gupta et al. 2010). According to their molecular masses, several HSPs have been identified including HSP90 and HSP70 (Waters et al. 1996). HSP90s are known to have a noticeable function in conserving protein homeostasis by stabilising and maintaining the conformation of unstable proteins close to their native forms. In addition, HSP90s are also involved in various signalling and cellular pathways (Richter and Buchner 2001; Young et al. 2001). Meanwhile, HSP70 chaperones are involved in other aspects of protein processing, specifically protein translocation and protein folding (Bukau and Horwich 1998). Chloroplast stromal HSP70B in *C. reinhardtii* and *Dunaliella* were reported to contribute to the molecular protection and repair of photosystem II (PSII) (Schroda et al. 1999; Yokthongwattana et al. 2001). An increase of 5–10 °C above normal temperature triggered the expression of the *HSP70B* gene in the Antarctic alga (Vayda and Yuan 1994). Chankova et al. (2013) recommended that HSP70B could be a marker for temperature stress in *Chlorella* from different environments and with different temperature tolerance. They observed that under similar temperature stress, about 30% higher levels of HSP70B were recorded for Antarctic *C. vulgaris* when compared with thermophilic *C. vulgaris* and mesophilic *C. kesslery*. Interestingly, no reduction of HSP70B levels was observed in Antarctic *C. vulgaris* at low temperature indicating the role of HSP70B for cell survival under Antarctic extreme conditions. Similarly, levels of HSPs increased in *C. acidophila* under temperature stress and HSPs might also contribute to the survival of acidophilic algae in harsh conditions (Gerloff-Elias et al. 2006). Moreover, the expression of several small HSPs was up-regulated at species-specific threshold temperature indicating the association of temperature sensing systems in algae (Kobayashi et al. 2014).

In addition to HSPs, several other proteins are involved in protecting cells against stress-induced damages. Late-embryogenesis abundant (LEA) proteins reported in green algae (Honjoh et al. 1995, 2000) increased freezing tolerance in *Chlorella* (Liu et al. 2011). They are thought to stabilise membranes and other proteins during cellular dehydration via a mechanism involving hydrogen-bond stabilisation (Shih et al. 2008). During stress, proteins that are irreversibly denatured or mis-folded are labelled by ubiquitins for degradation (Hershko and Ciechanover 1998). The ubiquitinated proteins can then be acted upon by proteolytic enzymes known as proteasomes. Ubiquitination can be induced by different stresses and it regulates various biological processes in eukaryotes, namely receptor endocytosis, cell-cycle control, intracellular signalling, transcription, gene silencing, DNA repair, and stress responses (Kwapisz

et al. 2005; Kaliszewski and Zoladek 2008). Enhanced ubiquitin levels are associated with high levels of protein degradation and turnover (Hawkins 1991; Shahsavarani et al. 2012). Up-regulation of ubiquitin has been observed in *C. reinhardtii* in response to heat and light stress (Wettern et al. 1990; Von Kampen et al. 1995). It was described that the ubiquitin–proteasome pathway (UPP) in *C. reinhardtii* could be impaired by severe or prolonged stress exposure due to the accumulation of reactive oxygen species (ROS) (Valentine et al. 2014). In addition to protein degradation, under unfavourable conditions such as heat stress, protein synthesis might be reduced as a strategy to limit the risk of generating mis-folded proteins (Allakhverdiev and Murata 2004).

## Carbohydrate homeostasis

According to energy allocation models, there is an increase in cell maintenance costs under harsh conditions due to the higher energy demand to maintain homeostasis (Calow 1991). Hence, carbohydrate metabolism is affected by abiotic stress leading to a decrease of carbon storage and an upsurge in levels of dissolved sugars (Arbona et al. 2013). González-Fernandez and Ballesteros (2012) reviewed the impacts of various environmental parameters on the carbohydrate content of algae. Cultivation parameters such as limiting nutrients or starvation, inorganic carbon supply, sodium chloride concentration, irradiance and temperature can all affect the activity of enzymes associated with carbohydrate accumulation (Vinocur and Altman 2005). In contrast, in a study on tropical microalgae from Australia, no consistent pattern was found in carbohydrate content in response to temperature treatment experiments (Renaud et al. 2002). Among carbohydrates, sucrose is the major outcome of photosynthesis and has crucial parts in plant growth and development, storage reserve, cell signalling and stress acclimation (Jiang et al. 2015). Sucrose formation is heavily temperature dependent and is enhanced by an increase in temperature, which may be due to the involvement of a thermophilic enzyme system in sucrose synthesis (Müller and Wegmann 1978). However, algae respond differently in altering sucrose levels when subjected to temperature changes; for example, in *Dunaliella*, sucrose was accumulated at elevated temperatures (Müller and Wegmann 1978), while in *Chlorella* (Guy 1990) and *Klebsormidium flaccidum* (Nagao and Uemura 2012) sucrose accumulation was instead reported at low temperatures. In *C. vulgaris* at temperatures above its ambient, the sucrose level greatly increased due to degradation of starch (Nakamura and Miyachi 1982).

The enzyme sucrose phosphate phosphatase (SPP), an essential component of the sucrose synthesis pathway, is involved in hydrolysing sucrose 6-phosphate (S6P) into sucrose and inorganic phosphate (Nagao and Uemura 2012).

The SPP family exists ubiquitously in algae (Jiang et al. 2015). In *C. reinhardtii*, the enhanced expression of the SPP gene in response to cold stress resulted in a consistent rise in sucrose levels (Valledor et al. 2013). Algae also respond to environmental stress, especially nutrient deficiency, by accumulating starch as the primary carbon and energy storage (Geider and La Roche 2002; Zhu et al. 2014b). The biosynthesis of starch is regulated by the rate-limiting enzyme ADP-glucose pyrophosphorylase (AGPase) whose activity is affected by temperature and redox mechanisms (Ball and Morell 2003; Thitisaksakul et al. 2012). For example, in *C. zofingiensis* starch abundance increased significantly at an early stage of nitrogen starvation, with a significant reduction of starch synthesis rate at a later stage, followed by an increase in starch degradation and lipid production (Zhu et al. 2014a). Similarly, AGPase was highly expressed in *C. vulgaris* in the first few hours of response to N-starved conditions, while its expression was reduced after 120 h of incubation (Ikarán et al. 2015). Vitova et al. (2015) conducted a review of the approaches to enhance the bioaccumulation of starch and lipids, namely the use of inhibitors to prevent DNA synthesis or cell division as well as by nutrient starvation involving the macro-elements such as nitrogen, phosphorus and sulphur. Starch is known as the most common storage form of carbohydrate and as a compact and osmotically inert form of energy storage (Pfister and Zee-man 2016). In phototrophically grown algae, starch which is accumulated prior to cellular division, is utilised as a carbon and energy reserve to ensure completion of cell division processes is independent of external supplies of carbon and energy either during photosynthesis or in the dark (Vitova et al. 2015). Hence, starch degradation is an essential process in the cell, especially in photosynthetic organisms during the dark (Smith et al. 2005). Starch phosphorylase plays an essential role in starch degradation (Rathore et al. 2009) and its expression is significantly induced in green algae by nitrogen starvation (Li et al. 2012).

## Compatible solutes

Under several stress conditions, typically osmotic stress, green algae accumulate organic solutes, such as the amino acid proline and sugar alcohols. These ‘compatible solutes’ do not hinder enzymatic reactions even at high concentration, but protect the membranes and enzymes against the lethal effects of destabilising ions (Yancey et al. 1982; Rathinasabapathi 2000). These compatible solutes comprised quaternary amino acid derivatives (Ahmad and Hellebust 1988; Parvaiz and Satyawati 2008); monosaccharides, disaccharides, and polysaccharides (Peters et al. 2007; Nagao et al. 2008); sugar alcohols (Borowitzka and Brown 1974; Wegmann 1986; Bohnert et al. 1995; Hagemann 2016) and

sulphonium compounds (Rhodes and Hanson 1993; Scholz and Liebezeit 2012). Proline accumulation is known to occur under various extreme conditions such as UV radiation and low temperature (Hayat et al. 2012). Amplified levels of proline also protect enzymes against inactivation by heat (Liang et al. 2013). The biosynthesis of proline through the glutamate pathway is catalysed via  $\Delta^1$ -pyrroline-5-carboxylate synthetase (P5CS) and  $\Delta^1$ -pyrroline-5-carboxylate reductase (P5CR), where the latter limits the rate of flux through the pathway (Hu et al. 1992). In various plant studies, the expression pattern of P5CS was examined as an indicator of proline production (Zhang et al. 2008; Amini et al. 2015). In green algae, an increased level of P5CS gene expression was found in *C. reinhardtii* exposed to copper (Zhang et al. 2008) and mercury (Elbaz et al. 2010). Proline exhibited protection against metal-induced oxidative stress via quenching oxygen singlets and scavenging radicals such as hydroxyl radicals (Siripornadulsil et al. 2002). However, report about the effect of temperature on proline level in green microalgae was not found in the literature.

Another compatible solute, trehalose or a non-reducing disaccharide stabilizes biological structures under abiotic stress, possibly by its capacity to absorb water, thus, protecting biomolecules from dehydration (Garg et al. 2002; Vinocur and Altman 2005; Lunn et al. 2014). It was proposed that trehalose works as a ‘chemical chaperone’ due to its ability to stabilise proteins and other biological structures against damage (Crowe 2007). In addition, it is very stable and can tolerate high temperature and acidic conditions (Richards et al. 2002). It was stated that increase levels of trehalose resulted in enhanced photosynthetic rates and reduced photo-oxidative damage induced by stress. The most common trehalose biosynthesis pathway involves two enzymes: trehalose-6-phosphate synthase (TPS) and T6P-phosphatase (TPP) (Avonce et al. 2010). The expression of these genes (TPS and TPP) improved plant resistance to abiotic stresses (Jang et al. 2003; Miranda et al. 2007). Accumulation of trehalose was reported in *Chlorella* sp. during osmotic stress (Bremauntz et al. 2011). However, unexpected repression of the gene involved in the synthesis of trehalose was observed in *Chlorella* strain subjected to UV stress (Poong et al. 2018).

## Oxylipins and polyamines

In addition, two other groups of molecules known as oxylipins and polyamines are involved in defence systems. Oxylipins are lipid metabolites generated from unsaturated fatty acids either through chemical auto-oxidation or enzymatically. Oxylipins resulting from the oxidative metabolism of PUFAs are recognized as performing in response to environmental changes and developmentally regulated processes



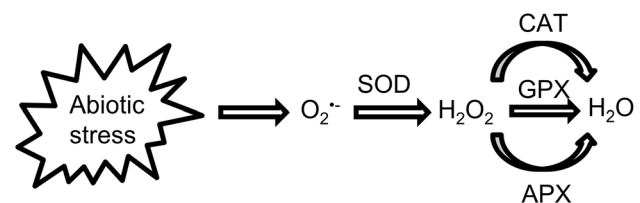
in both terrestrial animals and plants (Andreou et al. 2009). Among eukaryotic algae, oxylipins have been found to be abundant as a defence mechanism against both biotic and abiotic stresses (Bouarab et al. 2004). Algae undergo octadecanoid and/or eicosanoid oxylipin biosynthetic pathways where several enzymes such as lipoxygenases (LOXs) and cyclooxygenase (COX) are involved in the production of a variety of oxylipins. LOXs are the initial and fundamental enzymes in the oxylipin pathway and are omnipresent in plants, prokaryotes, and animals (Andreou and Feussner 2009). The presence of LOXs was reported in *Chlorella pyrenoidosa* (Zimmerman and Vick 1973; Nuñez et al. 2002). Much of the research about oxylipins focused on the jasmonate family of molecules, which comprises jasmonic acid (JA) and methyl jasmonate (MeJA) (Bouarab et al. 2004; Cosse et al. 2007; Potin et al. 2002). Jasmonic acid (JA) plays diverse functions including defence and stress response activity (Wasternack and Hause 2013). In several microalgae, JA affects growth, levels of the pigments (chlorophyll, carotenoids and astaxanthin) and protein profiles (Czerpak et al. 2006; Lu et al. 2010a, b; Raman and Ravi 2011; Piotrowska-Niczyporuk et al. 2012). In addition, Fujii et al. (1997) suggested the existence of JA in axenic cultures of *Dunaliella*. However, further research about the role of this molecule in microalgae is needed.

On the other hand, polyamines (PAs) are aliphatic amines which can appear in free form or as conjugated molecules. Their roles as stress alleviators have only recently been recognized (Kumar et al. 2010, 2012; Li et al. 2013). It was proposed that PAs could exert protection to cells through the formation of complexes with anions which led to reduced reactive oxygen species (ROS) production (García-Jiménez and Robaina 2012). Numerous studies have stated the involvement of PAs in response to environmental stress (Kumar et al. 2011, 2012, 2014).

## Antioxidant defence

Under optimal growth conditions, ROS or free radicals such as singlet oxygen ( $^1\text{O}_2$ ) and superoxide ( $\text{O}_2^{\cdot-}$ ) are primarily produced as a part of the cellular growth at low levels in various organelles including chloroplasts and mitochondria (Ahmad et al. 2014). However, the ROS homeostasis could be affected by stress, leading to high ROS levels and eventually oxidative stress (Malan et al. 1990; Apel and Hirt 2004; Murik et al. 2014). In chloroplasts, the main reason for ROS production is the suppression of  $\text{CO}_2$  fixation, linked to the over-reduction of the electron transport chain in thylakoid (Davidson and Schiestl 2001). High ROS levels in chloroplasts may occur via the Mehler reaction where the formation of superoxide occurs owing to the transfer of electrons from photosystem I (PSI) to  $\text{O}_2$  (Dietz et al. 2016).

During oxidative stress, superoxide radicals act as oxidants and facilitate the production of short-lived hydroxyl radicals (Fridovich 1995) which are highly reactive and can induce DNA damage via denaturation (Lesser 2006). Furthermore, ROS hinder the mending of PSII (Nishiyama et al. 2004). It is suggested that both the singlet oxygen and superoxide radical are strong oxidants and both oxidise elongation factor G (EF-G), which participates in the production of the D1 protein (Nishiyama and Murata 2014). Initially, ROS were thought to be toxic by-products generated from aerobic metabolism; however, studies revealed that ROS played important role in signal transduction (Mittler et al. 2004). Thus, a basal level of ROS is vital for the functions of living cells (Mittler 2017). Principally, ROS elevation induces the synthesis of ROS scavenging enzymes, namely ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD) and thioredoxins (TRXs) as well as enzymes involved in the biosynthesis of glutathione (Lemaire et al. 2007; Gill and Tuteja 2010). As the first line of defence, superoxide dismutases (SODs) rapidly convert  $\text{O}_2^{\cdot-}$  to  $\text{O}_2$  and  $\text{H}_2\text{O}_2$  and the generated  $\text{H}_2\text{O}_2$  can be converted into  $\text{H}_2\text{O}$  by peroxidases, such as APX and GPX found in vacuoles, cell walls and the cytosol (Alscher et al. 2002; Elbaz et al. 2010; Caverzan et al. 2012) (Fig. 2). Induced activities of SODs, APX and GPX under diverse abiotic stress conditions have been reported in microalgae (Fischer et al. 2006; Park et al. 2006; Yildiztugay et al. 2014; Chen et al. 2015; Yanguéz et al. 2015). High levels of SOD were reported in *Chlorella ellipsoidea* in response to temperature stress (Clare et al. 1984). The high APX activity seen in *Chlamydomonas* under stress conditions indicated that APX potentially contributed to this alga's tolerance to oxidative stress (Tanaka et al. 2011). In addition, catalase (CAT) is recognised as a dominant enzyme that catalyses the exchange of  $\text{H}_2\text{O}_2$  to  $\text{H}_2\text{O}$  or other non-toxic molecules (Mittler et al. 2004; Luis et al. 2006). In *C. reinhardtii*, catalase (CAT) scavenges  $\text{H}_2\text{O}_2$  from photorespiration and oxidation of fatty acids (Kato et al. 1997; Vega et al. 2005). Interestingly, CAT activity was correlated with the chlorophyll content during photoacclimation, and exhibited protection against photodynamic damage induced by ROS (Dykens and



**Fig. 2** ROS and antioxidant defence mechanism. The generated  $\text{O}_2^{\cdot-}$  due to abiotic stress is converted to  $\text{H}_2\text{O}_2$  by SOD activity, then three primary enzymes, catalase (CAT), glutathione peroxidase (GPX) and ascorbate peroxidase (APX) catalyse, the conversion of  $\text{H}_2\text{O}_2$  to  $\text{H}_2\text{O}$

Shick 1984). Manganese superoxide dismutase (MnSOD) and CAT are the predominant enzymes responsible for mitochondrion protection in *C. reinhardtii* under metal stress, while SOD and CAT are associated with the recovery of photosynthesis (Aksmann et al. 2014). In addition, CAT activity also contributed to the antioxidant defence and acclimation of the green alga *Scenedesmus vacuolatus* when exposed to copper (Sabatini et al. 2009). The other reported antioxidant compounds in relation to photosynthesis are carotenoids and  $\alpha$ -tocopherols. Carotenoids are essential components found in photosynthetic algae. They are mainly involved in the light-harvesting process during photosynthesis while protecting the photosynthetic system against photo-induced oxidative stress (Vidhyavathi et al. 2008). As antioxidants, carotenoids are capable of quenching  $^1\text{O}_2$  and excitation energy from chlorophyll, hence reducing the production and accumulation of  $^1\text{O}_2$  (Demmig-Adams 1990; Young 1991). It was also noted that ROS triggers the parallel accumulation of carotenoids in *Dunaliella* (Ye et al. 2008) and amplified expression of carotenoid biosynthesis-related genes was observed in *Haematococcus pluvialis* under several stress conditions (Vidhyavathi et al. 2008). Furthermore, tocopherols (Toc) also have vital roles in maintaining redox homeostasis and their biosynthesis is found to increase during stress. Toc functions by deactivating ROS (mainly  $^1\text{O}_2$  and OH $^\cdot$ ) generated in thylakoid membranes during photosynthesis and by inhibiting lipid peroxidation via removal of lipid peroxy radicals (LOO $^\cdot$ ) (Maeda et al. 2005). Although ROS are produced in both normal and stress conditions, cell defence mechanisms counter radical damage by reducing ROS production and facilitating ROS removal.

## Photosynthetic efficiency

Alterations in the environmental factors could cause an inequity between energy consumption and supply in photosynthetic algae, leading to alterations to the photosynthetic apparatus. In algae and higher plants, the reaction centre of PSII is bounded by the central antenna (CP47 and CP43) and the outer antenna, which comprises the less-abundant minor light-harvesting complex I (LHC) and the major light-harvesting complex II (LHCII) (Elrad et al. 2002). These LHCS comprise proteins that bind chlorophylls (Chl *a* and Chl *b*). The *LHCb* gene family encodes both the minor LHC proteins and the LHCII. In the thylakoid membrane, LHCII is the most abundant pigment–protein complex and about half of all chlorophylls are associated with it. The main role of LHCII is to receive and pass on the energy to the reaction centre of PSII and participate in controlling the supply of excitation energy among PSII and PSI. Transcriptional control of the genes encoding LHCII plays an essential role in antenna size adjustment (Elrad et al. 2002; Teramoto et al.

2001). Algae regulate the expression of nuclear-encoded chlorophyll *a–b* light-harvesting complex genes, (*LHCA*, *LHCb*), thereby controlling the antenna size. For example, when *C. reinhardtii* was grown under light-limiting conditions, the up-regulation of *LHC* gene expression resulted in a larger antenna size (Elrad et al. 2002). Moreover, environmental changes can influence the subunit arrangement of reaction centres (PSI and PSII). For example, the *psbC* gene encodes a PSII chlorophyll-binding protein, functioning as an oxygen-evolving enzyme and participate in water-splitting of photosynthesis (Qian et al. 2009a), and is influenced by severe circumstances like temperature stress (Chong et al. 2011). The gene *psaB*, encoding one of the subunits of PSI (Qian et al. 2009a), was down-regulated in *Chlorella vulgaris* when this alga was in contact to toxic chemicals (Qian et al. 2011). Although other stresses, such as salinity, might have an influence on the expression of these genes, high light illumination was more commonly associated with their regulation (Shapira et al. 1997; Nama et al. 2015). It is noteworthy to consider that gene expression is controlled at several steps starting with transcription of a gene, splicing, editing steps, and finally mature mRNA. The expression of chloroplast genes (*psaB*, *psbC* and *rbcL*) was initially thought to be regulated at the post-transcriptional level, associated with mRNA stability (Pfannschmidt 2003). While it is likely that photosynthetic gene expression is controlled at all levels of expression (Qian et al. 2008), studying their expression at the transcriptomic level can provide a suitable resolution of their synthesis.

Photosynthetic fixation of  $\text{CO}_2$  is vital for the development and growth of algae, supplying the carbohydrates needed for metabolism, as well as for structural constituents and supplying cellular structural units. Also, photosynthesis acts as a global sensor of environmental stress that causes an imbalance in cellular energy which leads to the distinct changes in redox chemistry linked to thylakoid membranes and adjustment of cellular sugar grade (Biswal et al. 2011). Photosystems and key enzymes associated with carbon dioxide fixation, such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) play critical roles in photosynthesis. The expression levels of genes involved in photosystem structure and RubisCO subunits were significantly influenced by various stresses (Qian et al. 2009a, b, 2012; Luo et al. 2015). For example, *rbcL*, which encodes the large subunit of RubisCO (Spreitzer and Salvucci 2002) was up-regulated upon a temperature increase to 20 °C, but with a further temperature rise, its expression was down-regulated (Deng et al. 2014b). Also, expression of *rbcL* was inhibited under desiccation, low and high salinity plus at temperatures under and beyond the normal ambient temperature (Xu et al. 2013).

Photoinhibition associated with impairment to the D1 protein of PSII can occur whenever algal cells are receiving

light, and the impairment is constantly fixed by de novo synthesis of D1, after the activation of the reaction centre to achieve a balance among the photodamage of PSII and its re-production (Takahashi and Murata 2008). Three key steps in PSII repair are conspicuously sensitive to abiotic stress, namely the breakdown of the D1 protein in photo-damaged PSII, generation of pre-D1 and its conversion to the mature D1 protein (Nishiyama and Murata 2014). It was suggested that adverse stresses might not increase photodamage directly but rather affect the PSII repair process by hindering the (re)synthesis of D1 (Takahashi and Murata 2008). In chloroplasts of *C. reinhardtii*, the expression of *psbA* was highly controlled by mRNA processing, especially during the initiation of translation (Mulo et al. 2012). Besides the regulation of D1 protein synthesis during photoinhibition, amino acid changes in the D1 protein were reported in response to temperature changes. Together with the physicochemical properties of the thylakoid membrane, these changes might contribute to the structural flexibility required for electron transfer in the PSII system (Giardi et al. 1997; Lukes et al. 2014). Table 1 shows a list of genes related to acclimation and repair strategies of algae under different stress conditions.

## DNA damage and repair mechanisms

Among cellular biomolecules only DNA is regularly repaired and preserved, while the others such as proteins and lipids are broken down when damaged, followed by de novo biosynthesis. Cells have developed several checkpoints during DNA replication, where the cell cycle is arrested to avoid error by providing additional time for DNA repair prior to mitosis (Ševčovičová et al. 2008). In the process, DNA damage is detected and repaired to ensure the integrity and proper functioning of the genome and the organism (Holzinger and Karsten 2013). Discontinuities that occur in one strand of the DNA double helix can also be referred to as single-strand breaks (SSBs). SSBs can happen directly via the disintegration of the oxidised sugar moiety or indirectly throughout the DNA base-excision repair (BER). In addition, functional errors of enzymes such as DNA topoisomerase 1 (TOP1) may result in SSBs (Caldecott 2008). Both ROS and ultraviolet radiation (UVR) are known as the primary causes of SSBs in DNA (Bray and West 2005). Alternatively, if discontinuities take place in double-stranded DNA, these are called DNA double-strand breaks (DSBs) and these can be made by endogenous and exogenous factors including mistakes throughout the DNA replication process, ionising radiation, or particular chemicals (Dudas and Chovanec 2004). A DSB is considered to be the most relevant signal-activating recombination process in the cell (Barlow et al.

2008). Living cells have developed a variety of mechanisms to mitigate the effects of DNA damage. BER is the first type of repair and is used to repair SSB damage by the processes in which DNA glycosylases, namely methyladenine glycosylase and uracil glycosylase, play key roles (Seeberg et al. 1995). BER is also used for dealing with lesions resulting from strong alkylating agents and ionising radiation (Seeberg et al. 1995). Though little knowledge about BER in algae, its presence has been stated in *C. reinhardtii* (Vlcek et al. 2008). On the other hand, DSBs are usually fixed by non-homologous end joining (NHEJ) or homologous recombination (HR). HR is the favourite manner of repair in numerous unicellular organisms, whereas NHEJ is the common pathway in plants and animals (Nagaria et al. 2013; Plecenikova et al. 2014). It was suggested that *C. reinhardtii* principally carried out DNA repair by involving NHR, rather than HR (Vlcek et al. 2008; Plecenikova et al. 2013). The contribution of NHEJ in the repair of DSBs in *C. reinhardtii* was observed under heat shock and gamma radiation exposure (Boreham and Mitchel 1993). The NHEJ pathway is facilitated by the DNA-dependent protein kinase catalytic subunit (DNA-PKcs), the Ku70–Ku80 heterodimer (Ku complex), and the DNA ligase IV–Xrcc4 complex (Ninomiya et al. 2004). Formation of cyclobutane pyrimidine dimers (CPDs) is another type of DNA damage and its occurrence as a result of UVR exposure was reported in green algae (Lud et al. 2001). CPDs can be fixed by a light-dependent procedure named photoreactivation or by dark repair mechanisms, namely nucleotide excision repair (NER) (Hercegoва et al. 2008). During photoreactivation, the enzyme photolyase is triggered by blue light and UVA emission. It splits the covalent bond of the pyrimidine dimer (Sancar 1990; Britt 2004). The enzyme photolyase is perhaps one of the simplest and oldest DNA repair systems found ubiquitously in all organisms. Photolyase from *Ostreococcus tauri* (a green alga) has been characterised (Usman et al. 2009). A gene called *PHR2* showing DNA photolyase activity in chloroplast and the nucleus was identified in *C. reinhardtii* (Petersen et al. 1999) and *Dunaliella salina* (Cheng et al. 2007). On the other hand, NER recognises lesions that distort the DNA helix. It is the most flexible and adaptable DNA repair pathway of organisms, as it works with a wide variety of structurally distinct DNA lesions (Costa et al. 2003). NER is well characterised in yeast and mammalian cells, and has been presented to be composed of approximately 30 gene products (Sinha and Hader 2002). NER has been observed to also function in the unicellular green alga *C. reinhardtii* (Hsu et al. 2000). Five proteins associated with NER were identified in *C. reinhardtii*, including the excision repair protein ERCC1. The DNA damage and associated repair mechanisms are summarised in Fig. 3.



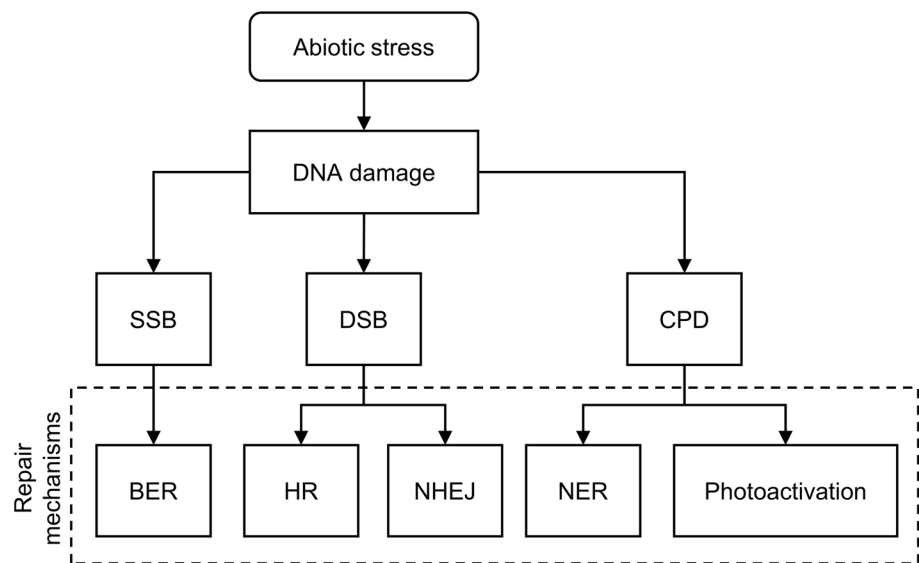
**Table 1** Genes involved in acclimation and repair strategies of algae under various stress conditions

Strategies	Class of genes	Functions	Associated stress	Species	Ref.
Protein homeostasis	<i>HSP90</i>	Keep inherently unstable proteins in a close-to-native conformation	Temperature and salinity	<i>Chlorella sp</i>	Liu et al. (2014)
	<i>HSP70</i>	PSII repair and protection throughout and after photoinhibition	UV irradiation, heat and salinities	<i>Ulva prolifera</i>	Zhang et al. (2012)
	<i>HIC6, HIC12</i>	Encoding LEA, hydrogen-bond stabilising effects on enzymes	Low temperature	<i>C. vulgaris</i>	Machida et al. (2008)
	<i>UBI</i>	Encoding ubiquitin, protein degradation	Temperature	<i>Chlamydomonas sp</i>	Von Kampen et al. (1995)
Fatty acid synthesis	<i>accA, accD</i>	Encoding subunit of ACCase regulate fatty acid synthesis rate	Nutrition limitation	<i>C. pyrenoidosa</i>	Fan et al. (2014)
	<i>KAS</i>	Encoding 3-ketoacyl-ACP-synthase, regulate fatty acid synthesis rate	Temperature and nitrogen starvation	<i>Haematococcus pluvialis</i>	Lei et al. (2012)
	<i><math>\Delta 9ACPCiFAD</math></i>	Stearoyl ACP desaturase, desaturate fatty acids	Salt	<i>Chlamydomonas sp</i>	An et al. (2013b)
	<i><math>\Delta 12CiFAD</math></i>	Fatty acid desaturase, balance the proportion between MUFAs and PUFAs	Temperature	<i>Chlamydomonas sp</i>	An et al. (2013a)
	<i>CiFAD3</i>	Desaturate fatty acids	Temperature and salinity	<i>Chlamydomonas sp</i>	Zhang et al. (2011b)
	<i>PDAT1</i> <i>PDAT2</i>	Phospholipid:diacylglycerol acyltransferase, TAG biosynthesis	Nitrogen starvation	<i>Chlamydomonas sp</i>	Boyle et al. (2012)
	Carbohydrate homeostasis	<i>P5CS</i>	Pyrroline-5-carboxylate synthase, proline (Pro) accumulation	Metal toxicity	<i>Chlamydomonas sp</i>
<i>T6P</i>		Trehalose 6-phosphate synthase, trehalose accumulation	High light	<i>Parachlorella kessleri</i>	Deng et al. (2014a, b)
<i>AGPase</i>		ADP-glucose pyrophosphorylase, starch synthesis	Nitrogen starvation	<i>C. vulgaris</i>	Ikaran et al. (2015)
<i>SP</i>		Starch phosphorylase, starch degradation	Nitrogen starvation	<i>C. vulgaris</i>	Ikaran et al. (2015)
ROS scavenging	<i>codA</i>	Glycine betaine (GB) accumulation	Temperature, UV and salinity	<i>Chlamydomonas sp</i>	Hema et al. (2007)
	<i>GAD</i>	Glutamate decarboxylase, $\gamma$ -aminobutyric acid (GABA) accumulation	Cold stress	<i>Klebsormidium flaccidum</i>	Dittami et al. (2011)
	<i>SOD</i>	Superoxide dismutases (convert $O_2^{\cdot -}$ to $O_2$ and $H_2O_2$ )	Heat stress	<i>H. pluvialis</i>	Okamoto et al. (2001); Eom et al. (2005)
	<i>APX</i>	Ascorbate peroxidase synthesis (converts $H_2O_2$ to $H_2O$ )	High light	<i>D. salina</i>	Park et al. (2006)
	<i>GPX</i>	Glutathione peroxidase synthesis (converts $H_2O_2$ to $H_2O$ )	Toxic chemical	<i>Chlamydomonas sp</i>	Wang et al. (2008)
	<i>CAT</i>	Catalase synthesis (converts $H_2O_2$ to $H_2O$ )	Toxic chemical	<i>Chlamydomonas sp</i>	Wang et al. (2008)

**Table 1** (continued)

Strategies	Class of genes	Functions	Associated stress	Species	Ref.
Photosynthesis efficiency	<i>LhcII</i>	Antenna size adjustment	Metal	<i>Chlamydomonas sp</i>	Jamers et al. (2006)
	<i>LHCSR</i>	Non-photochemical quenching	Light and temperature	<i>U. linza</i>	Dong et al. (2012)
	<i>rbcL</i>	Large subunit of RubisCO	Metal toxicity	<i>C. sorokiniana</i>	Wan et al. (2014)
	<i>psbC</i>	Encodes a PSII chlorophyll (Chl)-binding protein	Salinity	<i>C. vulgaris</i>	Kebeish et al. (2014)
	<i>psaB</i>	Reaction centre subunits of photosystem I	Herbicide	<i>C. vulgaris</i>	Qian et al. (2009a, b)
	<i>psbA</i>	D1 synthesis	UV radiation	<i>C. vulgaris</i>	Garcia-Gomez et al. (2016)
Programmed cell death	<i>Metacaspase</i>	PCD inducer	Oxidative stress	<i>C. reinhardtii</i>	Murik et al. (2014)

**Fig. 3** DNA damage and repair mechanisms. Single-strand breaks (SSB), double-strand breaks (DSB) and cyclobutane pyrimidine dimers (CPD) are the common DNA lesions which trigger a range of specific repair mechanisms in algae to protect genomic integrity



## Programmed cell death (PCD)

Organisms' tolerance or sensitivity to abiotic stress may also be linked to the stimulation or suppression of death in the cells (Darehshouri et al. 2008). Breakdowns of DNA, chromatin condensation, the release of cytochrome *c*, the contribution of caspase-like enzymes, a reduction in photosynthetic activity, vacuolization of cytoplasm, ATP depletion, and disruption of membrane integrity have all been observed to convoy cell death following various kinds of inductions (Franklin and Berges 2004; Geitmann et al. 2004; Andronis and Roubelakis-Angelakis 2010). The two terms apoptosis and PCD are often confused. PCD results from changes in the gene expression of dying cells and is mediated by the activity of a series of enzymes resulting in a distinctive morphology of the dead cells. On the other hand, apoptosis refers to a series of morphological features found in dead/dying metazoan cells throughout/

after the initiation of PCD (Franklin et al. 2006). PCD is a cellular suicide process controlled genetically that removes unwanted or damaged cells (Vaux and Korsmeyer 1999). The presence of a PCD process in single-cell organisms has also been recognised (Zuo et al. 2012). It is hypothesised that the essential factor in the cell death pathway was available in the genome of primary eukaryotes via ancient viral infection, prior to the development of multicellular organisms (Segovia et al. 2003). Stress might cause severe damage to DNA via the introduction of alterations to its structure and conformation and, in spite of DNA repair mechanisms, cells might then initiate mechanisms leading to programmed cell death. Table 2 shows some well-studied examples of PCD in microalgae. The hypotheses concerning the advantages of PCD in unicellular organisms include the fact that PCD can eliminate impaired and/or ageing cells and result in the release of nutrients to supplement the nutritional necessities of other non-PCD algae as well as to maximise the biological fitness of the

**Table 2** Well-studied examples of PCD in microalgae

Stress	Organism	References
Light deprivation	<i>Dunaliella tertiolecta</i>	Segovia et al. (2003)
H <sub>2</sub> O <sub>2</sub> induction	<i>Micrasterias denticulata</i>	Darehshouri et al. (2008)
UV radiation	<i>C. reinhardtii</i>	Moharikar et al. (2006)
Heat stress	<i>Chlorella saccharophila</i>	Zuppini et al. (2007)
Salt stress	<i>C. reinhardtii</i>	Vaviala et al. (2016)

community (Huang et al. 2016). *Caspase* or *metacaspase* gene activity can be used to determine PCD occurrence in the cell. In animals, caspase proteins are cysteine proteases that are vital regulators of PCD. However, structural homologs of caspase genes are not found in plants. As an alternative, they contain metacaspases, which are also found in organisms such as protozoa, fungi, bacteria and algae (Saheb et al. 2014; Fagundes et al. 2015). Increased level of caspase-like protein or *metacaspase* was reported in green algal cells (*Dunaliella viridis*) experiencing PCD under different stressors (Jiménez et al. 2009). Moreover, it was suggested that metacaspases might contribute in other metabolic procedures including signal transduction cascades, stress acclimation and regulation of protein aggregations, rather than entirely being accountable for PCD initiation (Bidle and Falkowski 2004; Bidle et al. 2007; Richie et al. 2007; Lee et al. 2010; Huang et al. 2016). Generally, a greater understanding of processes involved in phytoplankton cell death will possibly deliver knowledge about intercellular signalling, population regulation and bloom development.

## Omics applications and future directions

Knowledge about algae biological basics and regulatory networks can assist in enlightening their successful distribution, proliferation, and survival. Furthermore, understanding their responses to stress conditions is required if successful outcomes are needed for the potential role of algae in dealing with increased demand for biomass, feed and food (Moreno-Risueno et al. 2010). A large body of literature has been devoted to genomic studies to define the functions and roles of genes related to stress. This knowledge could be translated into practical industrial applications, for example, the production of lipids and antioxidants induced by stress. Lipid content is extremely important to biofuel production, whereas antioxidants such as astaxanthin are recognised to be the main biomolecules in the pharmaceutical industry (Hu et al. 2008; Ambati et al. 2014). Although the desired product can be obtained by introducing the associated stress, if the growth is compromised by

the stress, the overall productivity is also reduced. Lately, advances in ‘omics’ sciences including genomics, proteomics, transcriptomics, and metabolomics have provided deeper understanding about algae (Nouri et al. 2015). For instance, genomics studies contributed to the complete sequence of *C. reinhardtii* (Merchant et al. 2007). Grossman et al. (2007) have compiled genomic information of four algae including *C. reinhardtii* for a better understanding of many metabolic pathways. In the area of transcriptomics, the development of a microarray for *C. reinhardtii* containing 10,000 oligonucleotide sequences which cover nearly its full genome (<http://www.chlamy.org>) may further contribute to algal research. Similarly, *C. reinhardtii* proteomics would provide valuable information about the functional groups of proteins and cellular subunits (Stauber and Hippler 2004). Furthermore, metabolomics, a comprehensive and quantitative analysis of metabolites in a living cell at a particular moment could reveal the factual combination of protein expression and gene regulation as well as the influence of the environment (Fiehn 2002). Current advances in bioinformatics and analytical tools have improved our ability to analyse a large number of metabolites, assess metabolic alterations in response to exterior stimuli and explain metabolic pathways (Kumar et al. 2016). A series of analytical systems have been established such as nuclear magnetic resonance (NMR) and mass spectrometry (MS). Combination of chromatographic separation with different mass spectra is presented in systems such as gas chromatography (GC–MS) and liquid chromatography (LC–MS). Owing to a high level of structural variety, polarity and molecular weight differences among metabolites, no single analytical tool is able to measure and detect all conceivable metabolites in one run (Kueger et al. 2012). Bölling and Fiehn (2005) demonstrated metabolite profiles generated using GC–MS system could distinguish *C. reinhardtii* cell growth under different nutrient deficiency conditions. Finally, ‘omics’ analyses are crucial in understanding the complete processes of molecular networks in response to abiotic stress. It is essential to define the roles of newly identified stress-responsive molecules to explain the complex abiotic stress responses of algae.

Interestingly, the genomic information not directly encoded in the DNA sequence can also be transferred among generations. For example, non-genetic information can be transferred when DNA or its related proteins are altered, such as through DNA methylation and histone modifications (Chinnusamy and Zhu 2009; Cortijo et al. 2014). Together, these alterations are called epigenetic modifications. It has now been established that epigenetic alterations can be passed on not only via mitotic cell division but also from parent to offspring (Johannes et al. 2008; Cortijo et al. 2014; Audergon et al. 2015). Mutation accumulation experiments have suggested that spontaneous epigenetic modifications happen much like

genetic mutations. Nevertheless, epigenetic mutations happen at a faster rate, although they are less stable than genetic mutations (Kronholm et al. 2017). Kornholm et al. (2017) summarized that epigenetic variation can contribute to adaptation, although environmental selection plays an important role in controlling its extent.

Furthermore, various gene manipulation or gene editing approaches such as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9 (CRISPR-associated gene 9) can be applied to generate stress-tolerant algae (Gan and Mags 2017). CRISPR/Cas9 technology has been adapted to the model microalga *C. reinhardtii* to efficiently produce targeted gene editing while reducing Cas9-associated toxicity (Jiang et al. 2014; Shin et al. 2016; Greiner et al. 2017). CRISPR/Cas9 can directly edit the genome precisely at the region of interest by either NHEJ or HDR mutations, which leads to a defined DNA replacement, deletion and insertion (Xu et al. 2014). With this technology, further accomplishments can also be achieved in the functional study of algal proteins and genes associated with abiotic stress, understanding of their biology, and description of their metabolic pathways (Lander et al. 2016; Gan and Mags 2017). There is an extensive relation among constituents of several regulatory, signalling and metabolic pathways, which results in adaptation or responses (Nakashima et al. 2009; Garg et al. 2014; Mickelbart et al. 2015). In many instances, it is challenging to find the role of the genes using knockout of a single gene since this may not produce any desired phenotype. However, using the CRISPR–Cas9 system several genes can be knocked out at the same time, which can beat the issue due to genetic redundancy (Jain 2015). Although CRISPR technology is still in its infancy in algal studies, it is hoped to be of more use in the near future where it can contribute to the improvement of our understanding on algal metabolism and responses to environment, and also be used in producing more stress-tolerant algae to be used in industry.

**Author contribution statement** All authors contributed to this work and commented on the manuscript at all stages and revised it accordingly. BB, SYG, P-EL and SMP contributed in the conception of the content of the manuscript. BB wrote the manuscript, S-YG substantially helped in improvement of the manuscript by her comments and revisions, P-EL supervised the work and revised the manuscript, figures and tables. JB and S-MP substantially helped in both English and scientific revision.

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