Received: 21 June 2022 | Revised: 18 October 2022 | Accepted: 23 October 2022
DOI: 10.1111/ppl.13811

MINIREVIEW

Physiologia Plantarum

Temperature stress in psychrophilic green microalgae: Minireview

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Funding information
Natural Sciences and Engineering Research Council of Canada

Edited by D. Takahashi

Abstract

Photosynthetic algae are the main primary producers in polar regions, form the basis of polar food webs, and are responsible for a significant portion of global carbon fixation. Many cold-water algae are psychrophiles that thrive in the cold but cannot grow at moderate temperatures (≥20°C). Polar regions are at risk of rapid warming caused by climate change, and the sensitivity of psychrophilic algae to rising temperatures makes them, and the ecosystems they inhabit, particularly vulnerable. Recent research on the Antarctic psychrophile *Chlamydomonas priscuii*, an emerging algal model, has revealed unique adaptations to life in the permanent cold. Additionally, genome sequencing of *C. priscuii* and its relative *Chlamydomonas* sp. ICE-L has given rise to a plethora of computational tools that can help elucidate the genetic basis of psychrophily. This minireview summarizes new advances in characterizing the heat stress responses in psychrophilic algae and examines their extraordinary sensitivity to temperature increases. Further research in this field will help determine the impact of climate change on psychrophiles from threatened polar environments.

1 | INTRODUCTION

Polar regions constitute ~25% of the Earth's surface, are the largest ecosystem on the planet, and are responsible for nearly half of global CO_2 sequestering through microbial photosynthesis (Bax et al., 2021). The Arctic and Antarctic are home to a large biodiversity of aquatic photosynthetic microbes, with representation from most major eukaryotic algal lineages (Cvetkovska et al., 2017). Many algae from these perpetually cold environments are obligate cold extremophiles (psychrophiles) that thrive in low temperature (0–5°C) but cannot survive at more moderate (mesophilic) ones (≥20°C) (Morita, 1975). It should be noted that many terrestrial plants, algae, and cyanobacteria can survive, acclimate, and grow at low temperatures, but exhibit maximal growth rates at ≥20°C (Hüner et al., 2022) and thus are not psychrophilic. The unique feature of psychrophiles is, therefore, not their exceptional ability to thrive in the cold but, rather, their inability to survive at moderate temperatures.

Temperature sensitivity makes psychrophiles particularly threatened by climate change. The surface air temperature of the polar regions has increased at more than double the global average over the past two decades, with some areas in the Arctic experiencing anomalies of +6°C (IPCC, 2019). Increasing air temperatures cause the rapid reduction of ice thickness and "freshening" of saline waters due to the influx of glacial meltwater (Bronselaer et al., 2018; Silvano et al., 2018). Ice thinning also allows for greater penetration of solar radiation within the water column, contributing to further ice melt at the ice/water interface from below (Obryk et al., 2019). This positive feedback mechanism can cause runaway ice cover disintegration, which has already been observed in perennially ice-covered Arctic lakes (Lehnherr et al., 2018; Surdu et al., 2014), areas of the Antarctic Peninsula (Cárdenas et al., 2018) and in the Southern Ocean (Bers et al., 2013). These changes are often abrupt rather than gradual (Mueller et al., 2009; Paquette et al., 2015) and can expose psychrophiles to a myriad of environmental changes: increased temperatures and light availability, decreased salinity, and loss of ice habitats.

The long-term response of polar food webs to climate change is poorly understood, but it is postulated that decreases in marine primary productivity due to warming will have a lasting effect on higher trophic-level species (Steiner et al., 2019). The status and biodiversity of psychrophilic species are excellent biological markers for assessing the impacts of global warming associated with climate change (Gooseff et al., 2017). We are only beginning to elucidate the molecular and cellular basis underlying the sensitivity of psychrophiles to increasing temperatures and accompanying environmental changes. In this work, we summarize and discuss novel insights on the adaptive strategies and stress responses of psychrophilic green microalgae in the context of temperature stress.

2 | GREEN ALGAE FROM THE ORDER CHLAMYDOMONADALES: MODEL PHOTOPSYCHROPHILES

Photosynthetic psychrophiles (photopsychrophiles) are found in diverse cold environments, including ice-covered lakes, sea ice, snowfields, and ephemeral streams (reviewed in Morgan-Kiss et al., 2006). Regardless of their habitat, many psychrophiles employ common strategies for coping with permanently low temperatures, including high levels of polyunsaturated fatty acids (PUFAs) in their lipid membranes, cold-active enzymes, and the accumulation of cryoprotectants (Lyon & Mock, 2014). Unicellular and multicellular photopsychrophiles can be found across all major eukaryotic algal groups, including chlorophytes, rhodophytes, stramenopiles, diatoms and dinoflagellates. This suggests that psychrophily is not an ancestral trait, rather that it has evolved multiple times independently throughout algal evolution. The order Chlamydomonadales (Chlorophyta) harbors more than a third of all confirmed algal psychrophiles (Cvetkovska et al., 2017), including several emerging models for photosynthetic adaptation to the permanent cold.

Two Chlamydomonas species are particularly well studied: *Chlamydomonas priscuii* and *Chlamydomonas* sp. ICE-L. The former, recently renamed from *Chlamydomonas* sp. UWO241 (Stahl-Rommel et al., 2022), was isolated 17 meters below the ice-covered surface of the Antarctic Lake Bonney (Neale & Priscu, 1995) and represents a unique lineage within the Moewusinia clade of the Chlamydomonadales (Possmayer et al., 2016). This polyextremophile thrives in perpetually low temperatures (~5°C), high salinity (~40 ppt), extreme shading (5–15 μmol m^{-2} s^{-1}), high oxygen levels (200% air saturation levels), low phosphorus (N:P ~ 1000), and extreme photoperiod. The perennial ice cover of Lake Bonney promotes a stratified environment where the salinity, nutrient, and temperature profiles remain extraordinarily constant year-round due to the lack of wind-driven mixing (Morgan-Kiss et al., 2006). *Chlamydomonas* sp. ICE-L, which belongs to the Monadinia clade, was isolated from Antarctic sea ice (Liu et al., 2006). This habitat comprises a system of brine channels characterized by low temperatures (ca. −2 to −20°C), high salinity (35–200 ppt), high pH (up to 11), low irradiance (<1 μmol m^{-2} s^{-1}), and highly oxic conditions. Unlike the extraordinarily stable conditions in Lake Bonney, sea ice is a very dynamic environment that undergoes rapid annual changes. During warmer months, algae are found both within the ice and in the open water of surface melt ponds, where they cope with greatly increased light and UV exposure (Morgan-Kiss et al., 2006). Thus, sea ice photopsychrophiles must have cellular mechanisms that enable survival in an extreme and dynamic environment. Closely related species have been detected in other Antarctic locations, including floating ice (ICE-W; Liu et al., 2006), Lake Bonney (ICE-MDV; Li et al., 2016), and within cyanobacterial mats (Antarctic 2E9; Jungblut et al., 2012), suggesting a wide distribution in Antarctic ecosystems. Life in these extreme environments has resulted in many unique photosynthetic features that have been extensively studied (reviewed in Hüner et al., 2022).

A breakthrough in the study of green algal psychrophiles was the sequencing of the nuclear genomes of *C. priscuii* (Zhang et al., 2021) and ICE-L (Zhang et al., 2020). Comparative genomics between the psychrophiles and their mesophilic relatives, including the well-studied model *Chlamydomonas reinhardtii*, revealed several features that may be signatures of life at the extremes. Both psychrophiles have very large genomes (at least twice the size of that of *C. reinhardtii*), which are rich in noncoding and repetitive DNA. Psychrophiles also harbor ~4-fold higher number of highly similar duplicate genes (≥90% pairwise identity) when compared to related mesophiles (Zhang et al., 2021). Gene duplicates encode proteins involved in key cellular processes, including photosynthesis, carbohydrate synthesis, lipid metabolism, and progression of the cell cycle. Thus, it is possible that the fixation of duplicate genes is driven by positive selection for enhanced gene dosage (i.e., higher protein amount) in key pathways as an adaptive mechanism for survival in extreme environments (Kondrashov, 2012). Comparative genomics, backed by experimental data, has opened the door to systemic research on psychrophilic characteristics that enable life in the cold, and in those that underlie the inherent sensitivity to temperature increases.

3 | KEY DIFFERENCES IN THE TEMPERATURE RESPONSES BETWEEN PSYCHROPHILIC AND MESOPHILIC ALGAE

Mesophilic algae often experience temperature fluctuations in their natural environment and are well-equipped to cope with both transient and prolonged heat by mounting a multifaceted heat stress response (HSR) that limits cellular damage and enables recovery. Heat-increased membrane fluidity can lead to alterations in cellular function, which is compensated by rapid membrane remodeling, synthesis of saturated fatty acids, and membrane rigidification (Los et al., 2013). Heat can also damage the photosynthetic and respiratory apparatus, cause an increase in reactive oxygen species, and lead to protein misfolding and denaturation (Schroda et al., 2015). In *C. reinhardtii*, the accumulation of unfolded proteins during heat stress activates the heat shock transcription factor HSF1, which regulates the accumulation of stress-related genes, including heat shock proteins (HSPs) (Schulz-Raffelt et al., 2007). The amplitude of the HSR depends on the magnitude and duration of the heat stress. Exposure to an acute high temperature (42°C) results in rapid cell cycle arrest and a shift in energy usage from cell cycle operation to energy storage in *C. reinhardtii* (Ivanov et al., 2021), accompanied by rapid changes in lipid metabolism, increased fatty acid saturation, and accumulation of HSPs (Hemme et al., 2014; Légeret et al., 2016). Sustained exposure to 35°C, a moderately high but not lethal temperature, transiently inhibits cell cycle progression in *C. reinhardtii*, followed by acclimation and resumption of rapid growth. Cellular and molecular responses triggered by exposure to 35°C are transient, while those accompanying severe heat at ≥40°C are sustained and dramatic (Zhang et al., 2022).

But what constitutes heat stress in psychrophiles? Can polar algae, which never experience high temperatures in their environment, mount a protective HSR? The upper temperature limit for survival is the most common approach for defining temperature stress in psychrophilic algae, but this limit is species-specific. For instance, the upper growth limit for *C. priscuii* is 18°C (Possmayer et al., 2011) but many polar algal species are stenotherms and can only grow within a much narrower temperature range (e.g., 0–5°C). Temperature stress also depends on the exposure time, developmental and reproductive stages, growth media (particularly its salinity) and complex interactions with other organisms (discussed in more detail in Cvetkovska et al., 2017).

Previous work has shown that algal psychrophiles have a primary metabolism shifted towards constitutive accumulation of compounds with known stress-related functions, presumably due to life in the permanent cold. *C. priscuii* accumulates high levels of soluble carbohydrates, antioxidants, and polyamines when cultured at 4°C (Cvetkovska et al., 2022; Kalra et al., 2020; Stahl-Rommel et al., 2022). ICE-L grown at 5°C has a high PUFA levels in its lipid membranes, which counteracts membrane rigidification in the cold (An et al., 2013). The Arctic marine psychrophile *Chlamydomonas malina*, which thrives at 4°C, also accumulates PUFAs and soluble carbohydrates (Morales-Sánchez et al., 2020). Complex Arctic and Antarctic snow algal communities are characterized by high levels of antioxidants and carotenoids, including tocopherols and astaxanthin, high PUFA levels, and accumulation of glycerol, sugar alcohols, and soluble carbohydrates (Davey et al., 2019; Lutz et al., 2015; Remias et al., 2010). These stress-associated metabolic signatures, however, appear to be insensitive to moderate temperature increases and do not confer temperature resilience in psychrophiles. When *C. priscuii* is grown at 10 and 15°C, nonlethal temperatures that are close to its upper growth limit of 18°C (Possmayer et al., 2011), its steady-state metabolome and transcriptome remain largely unchanged relative to growth at 4°C (Cvetkovska et al., 2022). Growth at 8 and 15°C stimulated soluble carbohydrate and lipid accumulation in *C. malina*, but this did not translate to temperature resilience and this alga had severely stunted growth at 15°C (Morales-Sánchez et al., 2020). Thus, psychrophiles have a limited ability to acclimate to moderate temperature increases.

Laboratory cultures of *C. priscuii* exist as heterogenous populations of single cells and multicellular palmelloid colonies (Pocock et al., 2004). Direct comparison between the two phenotypes has shown that palmelloids have a 2-fold higher Chl *a*/*b* ratios, decreased levels of light-harvesting complex (LHCB) proteins of Photosystem II, and increased capacity for nonphotochemical quenching (Szyszka-Mroz et al., 2022). These modifications are opposite of what may be expected if the palmelloid phenotype was leading to intercellular shading (Anderson et al., 1995). This suggests that the phenotypic transformation to a palmelloid may provide protection through a reduction in light absorption coupled with enhanced nonregulated energy dissipation of excess absorbed light. Palmelloid formation increases by ~50% when *C. priscuii* is grown at 16°C (Szyszka-Mroz et al., 2022), and it was proposed that this species respond to temperature stress by inducing a developmental change from motile, single cells to colonial palmelloids to protect the photosynthetic apparatus from temperature-induced photoinhibition. We suggest that palmelloid formation in *C. priscuii* may be part of its general response to heat stress, geared towards protecting the photosynthetic apparatus (and likely other cellular structures as well).

Acute stress at lethal temperatures has also been examined in psychrophilic algae. Incubation of *C. priscuii* at 24°C results in alterations in global metabolomic and transcriptomic responses at 6 h (Cvetkovska et al., 2022), followed by a rapid drop in the rate of photosynthesis and respiration after 12 h (Possmayer et al., 2011). Notably, algae acclimated to higher temperatures (10 and 15°C) before heat stress, did not exhibit increased heat resilience and had an attenuated HSR compared to those acclimated to 4°C (Cvetkovska et al., 2022). In the Antarctic green algal isolate AnF0048, exposure to a lethal 15°C for 24 h induced the activities of several antioxidant enzymes (Choi & Lee, 2012). An early transcriptomic response was observed in the Antarctic *Chaetoceros neogracile* when the culture was shifted from 4 to 10°C, but this was not sufficient to allow the alga to acclimate to the temperature increase (Hwang et al., 2008). Although limited, these data suggest that psychrophiles do retain a limited ability to respond to temperature increases but this HSR is not sufficient to overcome their inherent temperature sensitivity.

4 | HEAT SHOCK PROTEINS IN PSYCHROPHILES: NECESSITY OR LUXURY?

A hallmark of heat stress in mesophilic green algae is the rapid accumulation of HSPs (Hemme et al., 2014; Légeret et al., 2016; Zhang et al., 2022). HSPs are molecular chaperones that repair thermally denatured proteins, prevent aggregation or target them for degradation, thus limiting the consequences of heat stress. HSPs are highly conserved among evolutionarily distant clades and are considered molecular markers for environmental, physical, and chemical stresses in algae and plants (Schroda et al., 2015).

Polar green algae encode a higher number of HSP genes compared to their mesophilic relatives, despite never experiencing increased temperatures in their natural habitat. The genomes of *C. priscuii* and ICE-L contain >50 HSP genes, which is significantly higher than mesophilic *Chlamydomonas* species, which typically encode ~40 HSP genes (Cvetkovska et al., 2022). This is consistent with recent reports on the widespread gene duplication in the nuclear genomes of polar species (Zhang et al., 2021). Transcriptomic examination in *C. priscuii* revealed that most HSP genes are actively

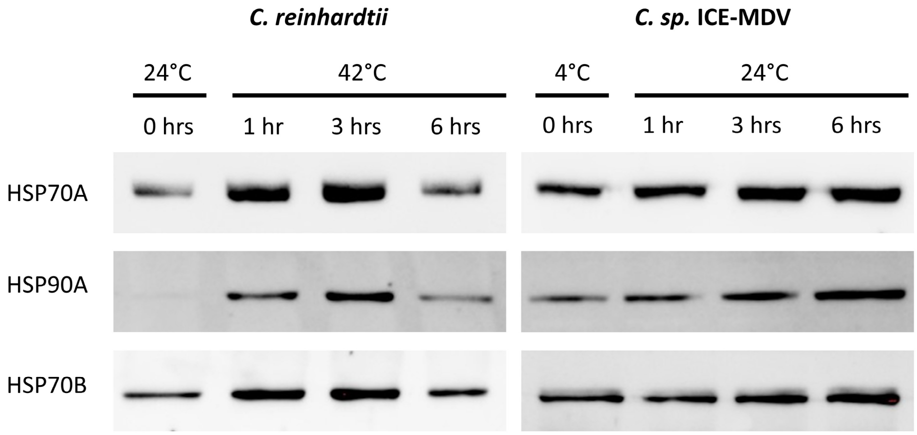

FIGURE 1 Accumulation of heat shock proteins (HSPs) during acute thermal shock in mesophilic and psychrophilic green algae. The mesophile *Chlamydomonas reinhardtii* rapidly accumulates HSPs after only 1 h of heat shock at 42°C. The Antarctic psychrophile *Chlamydomonas* sp. ICE-MDV accumulates HSPs after acute heat shock, but the response is attenuated, with a moderate change in HSP accumulation, after 6 h at 24°C. In all cases, extracted proteins were loaded on an equal protein basis, and membranes were probed with primary antibodies raised against *C. reinhardtii* and specific for HSP70A (1:3000), HSP90A (1:1500) and HSP70B (1:10,000) predicted to bind specifically to their closest homolog in ICE-MDV (Agrisera, Sweden; personal communication). All other experimental methods are as described in Cvetkovska et al. (2022). The immunoblots shown here reflect typical results of three biological experiments (Vakulenko and Cvetkovska, unpublished)

transcribed at 4°C, but there was no evidence of HSP upregulation in algae acclimated to elevated steady-state temperatures (10 and 15°C). When exposed to short-term heat stress at 24°C, *C. priscuii* responded by upregulating genes involved in protein processing pathways, including increased HSP transcription, but lacked the corresponding increase in HSP protein amounts (Cvetkovska et al., 2022).

In contrast to *C. priscuii*, the sea ice alga ICE-L increases HSP70 transcription when exposed to both low (0°C) and moderate (12°C) temperature stress (Liu et al. 2010) and its close relative ICE-MDV exhibits a moderate increase in the protein levels of several HSPs when exposed to 24°C for 6 h (Figure 1). Despite the psychrophilic nature of these two species, their responses to temperature stress appear to be similar to mesophiles, where HSP accumulation is one of the first responses to increased temperatures (Schroda et al., 2015). It is tempting to speculate that the lack of HSP accumulation during temperature stress in *C. priscuii* is the exception rather than the rule, and likely the result of life in a highly stable environment. It should be noted, however, that a systematic examination of the HSR in polar psychrophilic algae is lacking and most studies to date have only examined very few HSPs.

5 | THE ROLES OF HSPS IN THE PERPETUAL COLD

Examination of HSP protein levels in *C. priscuii* suggested that constitutively high and sustained HSP accumulation may be a result of life at low temperatures rather than a hallmark of acute heat stress. Immunoblot analysis of several major HSPs (HSP60A, HSP70A, HSP90A, HSP90C) demonstrated that *C. priscuii* constitutively accumulates high chaperone levels at a range of permissible steady-state temperatures (4–17°C) in the absence of heat stress. In comparison, HSP levels were low in *C. reinhardtii* in the absence of acute stress, regardless of the steady-state growth temperature (22–37°C) (Cvetkovska et al., 2022). When *C. reinhardtii* cultures were acclimated to 10°C (a temperature that supports robust albeit slow growth), HSPs accumulated at significantly higher amounts than what was observed during growth at 28°C (Cvetkovska et al., 2022). Thus, the accumulation of high levels of HSPs could be a result of low-temperature growth in green algae broadly. But what is the role of HSPs in the cold?

In addition to their well-documented involvement in stress, many HSPs have housekeeping functions in the folding of nascent proteins, translocation across membranes, and complex assembly (Zhao & Liu, 2018). Protein synthesis and folding are temperature-sensitive processes and slow down at lower temperatures. The physiological consequence of low protein folding rates is the slowing down of the cell cycle, which could be the reason behind the inability of many mesophiles to grow rapidly in the cold. The maintenance of a properly folded proteome has been deemed crucial for survival of psychrophiles (Feller, 2013). In support of this hypothesis, molecular chaperone gene silencing frequently leads to cold-sensitive phenotypes in mesophilic yeast (Verghese et al., 2012) and *E. coli* (Choi & Hwang, 2018).

Several HSPs with known homeostatic functions are encoded by expanded gene families in Antarctic green algae and constitutively accumulate at high levels during low-temperature growth. The HSP70 (DnaK-like) family of chaperones is ubiquitously found in both prokaryotes and eukaryotes. In *C. reinhardtii*, HSP70A is the only cytosolic DnaK-like chaperone with many key functions, including flagellar assembly, cell division and microtubule stability (Schroda & Vallon, 2009). The genome of *C. priscuii* encodes for six HSP70A genes, while ICE-L encodes for three HSP70A isoforms (Figure 2A). The chloroplast chaperonins CPN60s (GroEL/GroES-like) play a

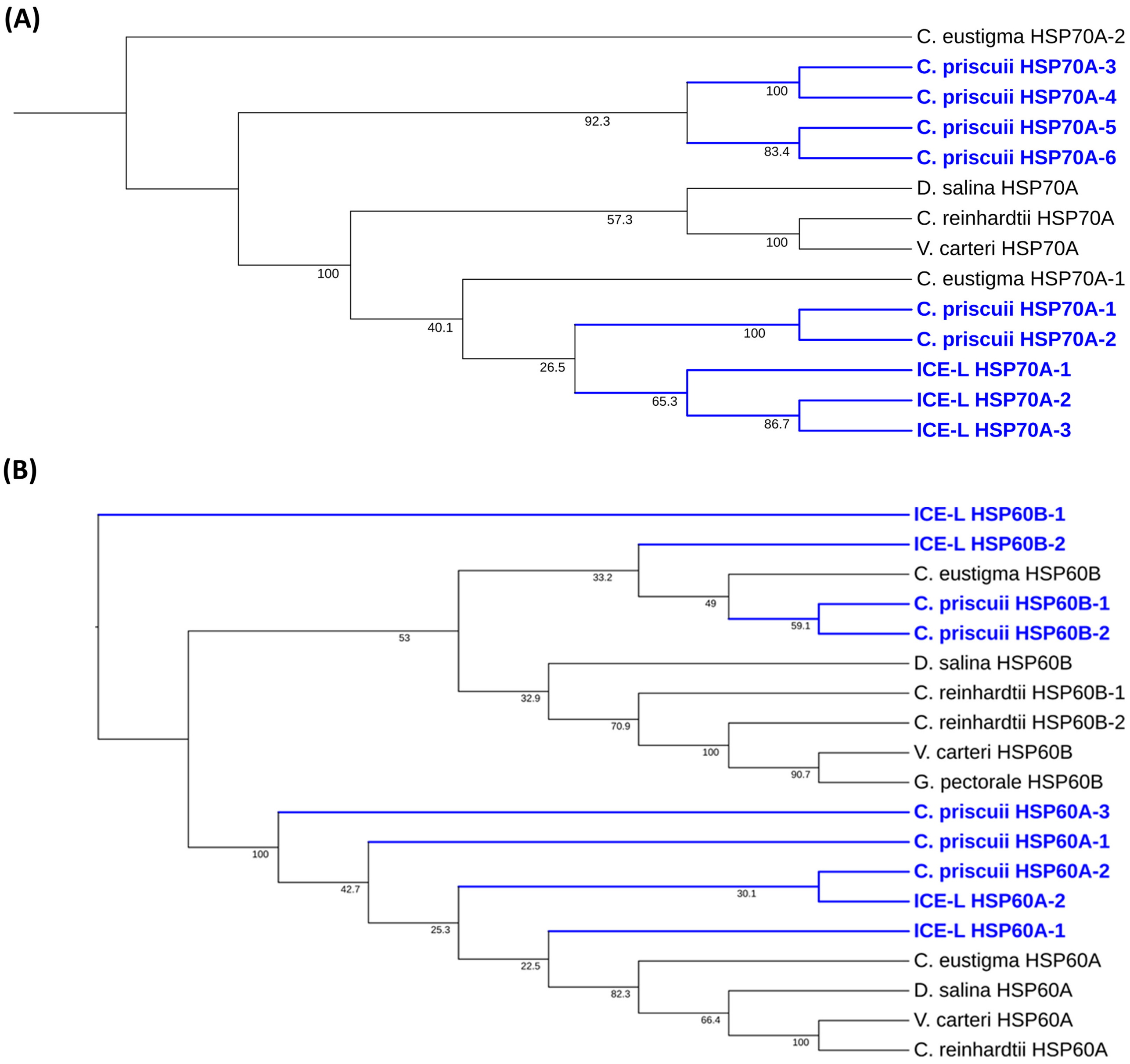

FIGURE 2 Phylogenetic tree of the (A) cytosolic HSP70A family and the (B) chloroplast HSP60 family in Chlamydomonadalean algae with sequenced genomes, illustrating the expansion of HSP gene families with important homeostatic functions in the genomes of psychrophilic algae. The trees were constructed using the neighbor-joining method based on the predicted amino acid sequences of the HSP70 and HSP60 members from *C. priscuii*, ICE-L, *C. reinhardtii*, *Chlamydomonas eustigma*, *Dunaliella salina*, *Gonium pectorale*, and *Volvox carteri*. The HSP genes in psychrophilic algal genomes are highlighted in blue. Numbers at each branch indicate the percentage support for the node among 1000 bootstrap replicates (modified from Cvetkovska et al., 2022)

prominent role in the folding of the Rubisco large subunit in photosynthetic organisms (Zhao & Liu, 2018). High levels of Rubisco have been detected in *C. priscuii* (Dolhi et al., 2013), suggesting efficient protein folding. The genomes of both *C. priscuii* and ICE-L encode for six CPN60 isoforms (Cvetkovska et al., 2022), while their mesophilic relatives typically encode 3–4 CPN60 genes (Figure 2B). *C. priscuii* has a smaller free amino acid pool compared to *C. reinhardtii* when grown at low temperatures (Cvetkovska et al., 2022). Amino acid accumulation during prolonged cold stress has been interpreted as a consequence of decreased protein synthesis in *C. reinhardtii* (Valledor et al., 2013) and the lack of free amino acids in *C. priscuii* could be due to effective protein synthesis machinery that is not negatively affected by the cold (Cvetkovska et al., 2022). Thus, unlike their protective functions during heat stress in mesophiles, HSPs in psychrophiles could play a more prominent homeostatic role during low-temperature growth.

Insights on the involvement of HSPs at low temperatures can also be also gained through experimental work on mesophilic algae exposed to cold stress. When *C. reinhardtii* is exposed to a sudden cold shock (7°C), there is an increase in transcript and protein levels of three HSP70 isoforms and the HSF1 transcription factor (Maikova et al., 2016). The trebouxiophyte green alga *Auxenochlorella protothecoides* induced the expression of HSFA1d and several HSP genes during prolonged cold stress (Xing et al., 2022). Heat shock elements that regulate HSP expression during cold stress (10°C) were detected in the HSP70 promoter region of the green macroalga *Ulva prolifera* (Wu et al., 2019). Cold stress (5°C) induced rapid upregulation of pathways related to protein processing and transcript accumulation of several small HSPs in the rhodophytes *Pyropia yezoensis* and *Gracilariopsis lemaneiformis* (Qin et al., 2021; Uji et al., 2019). HSPs are also prominent players in cold and freezing stress in land plants (Timperio et al., 2008), suggesting a broad role in low-temperature resilience.

6 | THE HSR IN OTHER PSYCHROPHILES: BEYOND MICROALGAE

HSP expression has been examined in a handful of other polar photosynthetic species, all of which originate from dynamic polar habitats. The Antarctic benthic rhodophyte *Plocamium cartilagineum* induces HSP70 expression when exposed to the elevated but nonlethal temperature of 10°C for 1 h, but undergoes rapid death and reduced HSP70 expression when incubated at 20°C (Vayda & Yuan, 1994). Expression of small HSPs, HSP70 and HSP90, was significantly increased in the ochrophyte *Fucus distichus* from Arctic intertidal zones when exposed to 24°C for 24 h (Smolina et al., 2016). The Antarctic moss *Pohlia nutans*, shifted from 4 to 10°C for 24 h and exhibited a modest upregulation of a mitochondrial HSP70 isoform (Liu et al., 2014). The Antarctic grass *Deschampsia antarctica* accumulated HSP70 in leaf tissue when exposed to temperatures >25°C (Reyes et al., 2003).

Antarctic marine heterotrophs have been recognized as some of the most stenothermal species on the planet. Many marine fish and invertebrates suffer extensive temperature-induced cellular damage with temperature rises of just 2–3°C, and examining their temperature stress responses has been of interest (Clark & Peck, 2009). Yusof et al. (2022) recently reviewed HSP expression and accumulation in Antarctic organisms and provide a comprehensive list of heat stress responses in psychrophilic animals. Although this review proposes that HSP70 is an excellent marker for environmental stress in polar biota, careful examination of published data suggests a much more complex scenario. For instance, there are many examples of Antarctic species that lack the ability to induce HSP expression under temperature stress (Buckley et al. 2004; Bilyk et al. 2018; Collins et al. 2021; Nieva et al. 2021; Saravia et al. 2021). Some species induce a robust HSR, but only during moderate stress or with slower kinetics than related mesophiles (Clark et al., 2008; Toullec et al., 2020). Recent large-scale genomic investigations have revealed multiple gene duplications in the HSP70 and HSP40 families of Antarctic fish and krill (Cascella et al., 2015; Tercero & Place, 2020) accompanied by constitutively high housekeeping HSP expression during steady-state low-temperature growth (Bilyk et al., 2021; Toullec et al., 2020). While it may seem that gene duplications accompanied by constitutive HSP expression is a common feature of psychrophily, more systematic studies on psychrophiles from different taxa are needed to conclusively determine this.

7 | ADAPTATIONS TO THE COLD AND SENSITIVITY TO HEAT: TWO SIDES OF THE SAME COIN?

The cellular mechanisms that lead to cold adaptation in psychrophiles have received considerable attention. Cold temperatures place severe physiochemical constraints on cellular functions by negatively influencing water viscosity, solute diffusion rates, membrane fluidity, enzyme kinetics and macromolecule interactions (Lyon & Mock, 2014). The ability of psychrophiles to overcome these constraints is dependent on a number of adaptive strategies to maintain vital cellular functions, including a rerouted metabolism, the presence of cold-active enzymes and more fluid membranes with altered fatty acid content (Lyon & Mock, 2014). Additionally, photopsychrophiles must maintain photostasis: the balance between energy input as light and energy utilization through metabolism and growth. The biophysical processes of light absorption are rapid (10^{-12} to 10^{-6} s) and temperature-insensitive, in contrast to enzyme-driven carbon fixation that occur slowly (10^{-3} s to hours) with a rate greatly decreasing at low temperatures. Such extreme disparities in temperature sensitivities and reactions rate pose a formidable challenge. Photopsychrophiles, including green algae, have many characteristics that protect them from light-induced damage, including varying pigment composition, cold-active photosynthetic proteins and increased capacity for nonphotochemical quenching (reviewed in detail by Hüner et al., 2022). But many of these features, which are beneficial in the cold, may be a detriment when the temperature rises.

C. priscuii and its polar relatives constitutively accumulate stress-related compounds (e.g., soluble sugars, antioxidants) and proteins (e.g., HSPs), likely as a strategy for survival at low temperatures. *C. priscuii* exhibits a metabolome-wide response to heat stress (24°C); however, many metabolites associated with stress functions are already present at high levels in nonstressed cells and do not accumulate further in response to heat. What *C. priscuii* is lacking is the ability to fine-tune the regulation of this network upon exposure to temperature stress (Cvetkovska et al., 2022). In contrast, the mesophile *C. reinhardtii* can respond strongly to heat stress and rapidly increase the amounts of protective compounds and proteins (Légeret et al., 2016; Zhang et al., 2022) Thus, an organism's ability to tightly regulate its central defense network when exposed to stress could play a more prominent role in thermotolerance rather than the absolute amounts of protective compounds.

Enzymes from psychrophiles are uniquely adapted to maintain high catalytic rates at low temperatures that significantly slow down the reaction rates of mesophilic proteins. The high activity in the cold arises from increased flexibility within the protein structure, but the consequence is weak stability and loss of activity at increased temperatures (Feller, 2013). Several cold-active algal proteins have been

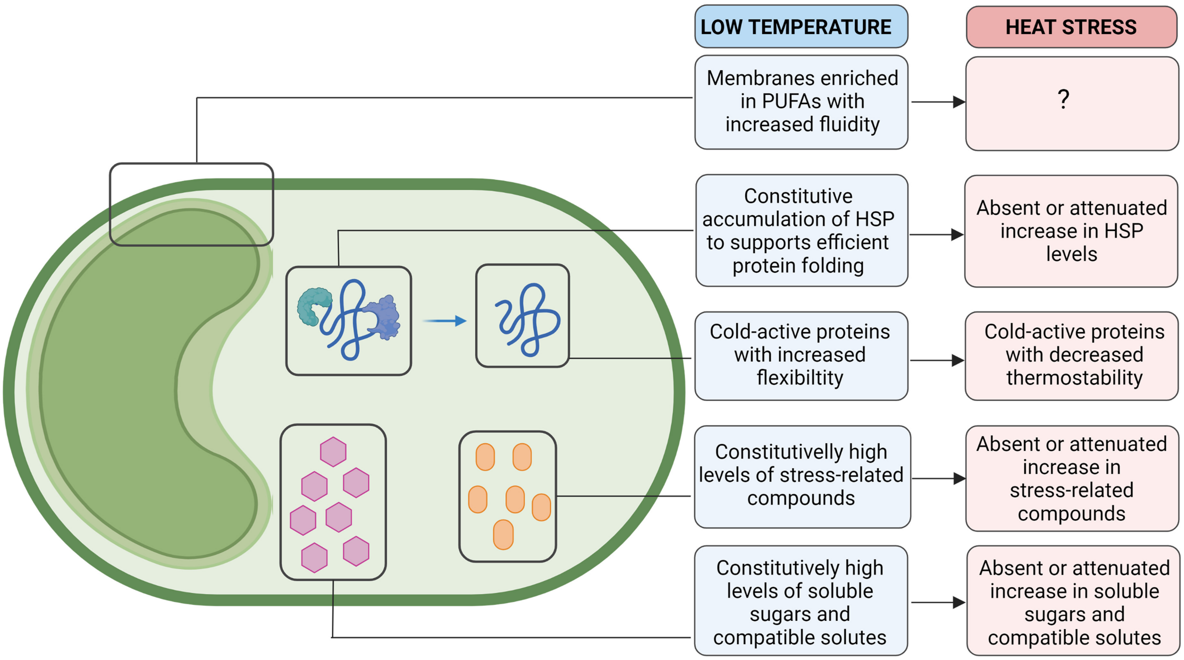

FIGURE 3 A model of the cellular and molecular characteristics in psychrophilic green algae under perennially low temperatures and our current knowledge on how these cellular features are affected by increased temperatures. Psychrophiles have membranes enriched in PUFAs that lead to increased fluidity in the cold, but it is not known how psychrophilic lipid metabolism responds to heat stress. Constitutive accumulation of soluble sugars, compounds with known stress-related functions and high levels of molecular chaperones (HSPs) has been demonstrated in psychrophilic algae, but these species lack the ability to rapidly regulate their central defense network when exposed to sudden temperature stress. Finally, psychrophilic proteins have increased flexibility when compared to their mesophilic counterparts, but at the expense of decreased thermostability at higher temperatures. Combined, these characteristics could be the underlying reason behind both the exceptional ability of psychrophilic algae to thrive in permanent cold as well as their extreme sensitivity to increased temperature

characterized in algae: ferredoxin and the chloroplast kinase STT7 in *C. priscuii* (Cvetkovska et al., 2018; Szyszka-Mroz et al., 2019), nitrate reductase and argininosuccinate lyase in *Chloromonas* sp. ANT1 (Loppes et al., 1996), glycose-6-phosphate dehydrogenase and nitrate reductase in *Koliella antarctica* (Ferrara et al., 2013; Vona et al., 2004). Compared to their mesophilic homologs, these psychrophilic proteins have: (1) higher activities at low temperatures and (2) increased structural sensitivity and loss of activity at moderate and high temperatures. This suggests that the sensitivity of psychrophilic algae to increased temperatures might be, at least in part, due to the sensitivity of cold-active proteins to heat.

The examples above illustrate that what could be perceived as an advantage to life in the permanent cold could also be a detriment during temperature increases (Figure 3). This could be the underlying reason behind both the exceptional ability of psychrophilic algae to thrive inpermanent cold and their extreme sensitivity to increased temperature. Psychrophily is a complex trait that requires systemic adaptations, so it could be similarly surmised that heat sensitivity in these organisms is also a systemic failure of different cellular mechanisms at increased temperatures. Recent advances in -omics techniques, accompanied by the sequencing of the genomes of polar algae such as *C. priscuii* and ICE-L, will enable large-scale investigations of the biology of cold adaptation and heat sensitivity in photopsychrophiles.

8 | FUTURE PERSPECTIVES

Polar regions are the most rapidly warming locations on Earth and are predicted to experience a myriad of environmental changes. Given these trends, understanding the stress responses in psychrophilic primary producers has been pinpointed as a primary objective of polar research (IPCC, 2019). But do psychrophilic algae, such as *C. priscuii* and its relatives, have the necessary mechanisms to survive the warming trends observed in polar ecosystems? The questions below are specific avenues of research that have not been addressed.

8.1 | The regulation of HSP accumulation

HSF1 is a master regulator of HSP expression during stress and cytotoxicity in *C. reinhardtii* (Schroda et al., 2015). HSF1 activity is triggered by the accumulation of unfolded proteins and is attenuated by de novo synthesized and folded proteins (Schulz-Raffelt et al., 2007). An HSF1 homolog was detected in *C. priscuii* and ICE-L (Cvetkovska et al., 2022), but whether it plays the same role in psychrophiles is not known. Characterizing HSF using genetic approaches could lead to important insights into the role of HSPs during low-temperature growth and temperature stress responses in psychrophiles.

8.2 | The role of stress-related metabolites and proteins in the cold

The constitutively high accumulation of HSPs and stress-related metabolites in psychrophilic algae suggests that these strategies are beneficial for life in the cold. The genomic sequencing of several psychrophiles has opened the door for genome editing. Developing CRISPR-Cas9 methodologies in psychrophiles and modifying genes involved in stress-metabolite synthesis would provide direct experimental evidence to support this hypothesis.

8.3 | Combining lab- and field-based studies

Wet-lab experimental studies give a high-resolution insight in the cellular processes that operate in psychrophiles, but true understanding of psychrophilic stress biology can only come from combining wet-lab work with ecological studies. Climate change is a complex phenomenon and rising temperatures often translate to significant changes in many environmental factors in polar aquatic environments (e.g., nutrients, salinity, and light availability) (Obryk et al., 2019). Furthermore, psychrophilic algae exist in complex communities that involve protists, bacteria and viruses (Morgan-Kiss et al., 2006). The added effects of multiple abiotic and biotic factors on psychrophilic biology have not been examined but are crucial in gaining a true understanding of psychrophiles and their vulnerabilities in the face of a changing climate.

AUTHOR CONTRIBUTIONS

M. Cvetkovska and N.P.A. Hüner conceptualized the manuscript and had a primary role in manuscript writing. G. Vakulenko contributed data for the figures and wrote sections of the manuscript. X. Zhang and D. R. Smith had a significant contribution towards the manuscript design. All authors edited the manuscript and approved of the final version.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Cvetkovska, M., Vakulenko, G., Smith, D.R., Zhang, X. & Hüner, N.P.A. (2022) Temperature stress in psychrophilic green microalgae: Minireview. *Physiologia Plantarum*, 174(6), e13811. Available from: https://doi.org/10.1111/ppl.13811