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Review

Stress proteins and auxiliary anti-stress compounds in intertidal macroalgae

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ABSTRACT. Intertidal macroalgae are exposed to strong variation in the physical environment and thus, diverse anti-stress mechanisms are displayed by these organisms. Stress proteins (also called heat shock proteins, HSPs) have been invoked as potential protective mechanism, especially during stressful action of temperature and solar radiation. Therefore, macroalgae have not normally been used as model organisms in studies of these molecules. The present study compiles the existing information from intertidal species in the context of major factors that have been reported to induce them, *e.g.* temperature, enhanced solar radiation, contaminants, etc. Additionally, in order to address the question whether the expression of these proteins operates in intertidal macroalgae complementarily with other protective mechanisms, a case study of induction of HSPs after exposure to UV radiation and high temperature in two upper littoral species, *Ulva* sp. and *Porphyra columbina*, from southern Chile is presented. In parallel, two well-known responses to stress, photoinhibition of photochemical reactions (F_v/F_m) and ROS scavenging were measured. The results indicated that, although stress proteins were detected in a time span between 3 and 24 h, the responses were not correlated with photochemical and antioxidative response. Overall, the study outlines a potential role of stress proteins in ecophysiological responses developed to cope mainly with high temperature and UV radiation. However, other rapid metabolic adjustments (*e.g.* high thermo-tolerance of photosynthesis and efficient ROS scavenging), together with other biomolecules (mycosporines, phenols, polyamines, etc.) and morpho-functional adaptations to the intertidal life (*e.g.* small size, high area/volume ratio) are also important.

Keywords: stress proteins, intertidal macroalgae, photosynthesis, ROS, UV radiation, photoprotection.

Proteínas de estrés y compuestos anti-estrés auxiliares en algas marinas intermareales

RESUMEN. Las macroalgas marinas intermareales están expuestas a extrema variación en las condiciones ambientales y por ello desarrollan una serie de mecanismos anti-estrés. Las proteínas de estrés (HSPs) han sido consideradas como potenciales agentes protectores en respuesta a condiciones estresantes, especialmente durante la acción de elevada temperatura y alta radiación solar. Considerando que las macroalgas marinas no han sido usadas comúnmente como modelos de estudio para analizar estas moléculas, el presente trabajo compila la información existente sobre la inducción de proteínas de estrés en algas intermareales en el contexto de los principales factores hasta ahora reportados como inductores, *e.g.* temperatura, alta radiación solar, contaminantes, etc. Adicionalmente, mediante un estudio de caso usando dos especies del intermareal superior, *Ulva* sp. y *Porphyra columbina* colectadas en el sur de Chile, se examina si la inducción de proteínas de estrés ocurre de forma complementaria con otras respuestas anti-stress. Para ello, dos mecanismos bien conocidos, fotoinhibición de fotosíntesis y actividad antioxidante, fueron medidos en paralelo. Los resultados indicaron que, aunque hubo expresión de proteínas de estrés dentro de un periodo experimental entre 3 y 24 h, estas respuestas no fueron correlacionadas con cambios en fotosíntesis o actividad antioxidante. En general, el estudio perfila un potencial rol de estas moléculas en algunas respuestas ecofisiológicas desarrolladas para contrarrestar los efectos negativos de las altas temperaturas y la radiación solar. Por otro lado, otros ajustes metabólicos de acción rápida (*e.g.* fotoinhibición y actividad antioxidante), distintas biomoléculas (micosporinas, fenoles, poliaminas, etc.), además de las adecuaciones morfo-funcionales a la vida intermareal (*e.g.* pequeño tamaño, alta proporción área/volumen) son importantes para explicar la fisiología de estos organismos.

Palabras clave: proteínas de estrés, macroalgas marinas intermareal, fotosíntesis, radiación UV, ROS, fotoprotección.

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INTRODUCTION

Stress proteins were first discovered in cells of *Drosophila* exposed to thermal shock, which led them to be called "heat shock proteins" (HSPs). However, now it is well known that these proteins are expressed by almost all living organisms in response to a variety of stress factors and thus now they are also generally called stress proteins (see reviews of Lindquist, 1986; Sanders, 1993). Stress proteins have been classified into five major classes based on different molecular weight, similarity of DNA sequence, immunological cross-reactivity and intracellular location (Gusev *et al*., 2002). The stress protein families of different molecular weights (*e.g.* 60, 70 and 90 kD), chaperonins and low molecular stress proteins (LMS) are distributed in different cellular compartments of prokaryotes and eukaryotes (Sanders, 1993). In general, it is now recognized that stress proteins function as molecular chaperones, their encoding genes are highly conserved and not all are inducible in response to environmental stress (Feder & Hofmann, 1999).

The chaperone function of stress proteins is mainly characterized by a capability of these molecules of uniting a wide variety of other polypeptides and proteins, preventing the inactivation of key cellular components and assisting in the refolding of native and structural proteins (Gatenby, 1992; Vinocur & Altman, 2005). These proteins represent often one of various stress-response mechanisms displayed by cells and that act synchronically and synergistically to prevent damage and to re-establish cellular homeostasis. Many stress proteins can be constitutively abundant in the cell or exclusively inducible, depending on the family, during or after stress (Feder & Hofmann, 1999). The activation and induction of a common suite of stress proteins is the molecular basis of both cross-tolerance and stress-hardening that varies depending on the species, cell-type, previous stress history, gene-environment interactions during development, and stress severity (Kültz, 2005).

In aquatic organisms, induction of stress proteins in response to different stress conditions has been demonstrated. The families of 70 and 60 kDa (HSP70 and HSP60) have been used as biomarkers in various algae, invertebrates and fishes (Bond *et al*., 1993; Sanders, 1993; Vayda & Yuan, 1994; Lewis *et al*., 1999, 2001; Ireland *et al*., 2004). In macroalgae (the so-called seaweeds), the expression of stress proteins have been less studied compared to other marine organisms, but results indicate that many thermotolerant species shown a well-developed induction of stress proteins (Ireland *et al*., 2004). Taking into account the intensity and frequency of exposure to the natural stresses, intertidal macroalgae are regarded good models to study the induction of stress proteins because, as result of the tidal variation, they can be suddenly exposed to marked changes in temperature, solar radiation, salinity, etc. (Collen *et al*., 2007; Lago-Lestón *et al*., 2010). If one considers that the exposure to elevated temperature during low tide is closely linked to enhanced solar radiation, a common and synchronised strategy to withstand both light and thermal stress may be advantageous (Jenkins *et al*., 1997). For example, the importance of these proteins in preventing photodamage of photosynthesis has been demonstrated when light stress was applied in combination with a heat shock (Schuster *et al*., 1988). During severe photoinhibition, when the reaction centres of PSII were damaged irreversibly, the HSP70 can reduce the total inactivation of PSII, intensifying its recovery and participating in the assembly of new reaction centres (Schroda *et al*., 1999).

In rocky shores of southern Chile, various sub-Antarctic species adapted to water temperatures <15°C inhabit the upper intertidal fringe and thus they can be exposed to temperature as high as 30°C during summer (Cruces *et al*., 2012). Concomitantly, maximum levels of UV radiation recorded for southern Chile in summer (close to 3 W m^{-2}) can exacerbate the detrimental effects of stress during emersion (Huovinen *et al*., 2006). Various studies carried out in these communities indicate that algae dominating the supra and midlittoral (*e.g. Porphyra columbina* and *Ulva* sp.) exhibit well-developed anti-stress mechanisms such as dynamic photoinhibition (Gómez *et al*., 2004; Gómez & Huovinen, 2011) and synthesis of UV-absorbing substances (*e.g.* mycosporine-like amino-acids) (Huovinen *et al*., 2004). Thus, it is reasonable to argue that induction of stress proteins may be a complementary mechanism in these species to endure thermal and UV stress.

In the present article, the information on the induction of stress proteins in intertidal macroalgae from proteomic, genomic and transcriptomic approaches is compiled in order to delineate their potential role in processes involving stress tolerance to multiples factors such as temperature, enhanced solar radiation and contaminants. Additionally other biomolecules (mycosporine-like amino acids, phenolics, polyamnines and carotenoids) commonly described as anti-stress agents in macroalgae are briefly summarized. Finally, a case study of two upper littoral species from southern Chile, *Porphyra columbina* and *Ulva* sp., is presented in order to exemplify the potential induction of stress proteins simultaneously with other well-known anti-stress responses, such as photoinhibition of maximal quantum yield of fluorescence (F_v/F_m, determined from pulse amplitude modulation fluorometry, PAM) and ROS scavenging activity.

Major factors that induce HSPs in macroalgae

Various stressors have been found to induce the formation of stress proteins in marine macroalgae and the best known are compiled in Table 1.

Temperature

In general, the induction in response to high temperature can be rapid and associated with the duration of the environmental stress. For example, in *Fucus serratus* high induction of HSP70 was observed after 2 h heat shock at 42°C, while in *Chondrus crispus* the highest induction was found after 4 h. Afterwards the concentrations of these proteins decreased (Ireland *et al*., 2004). On the other hand, exposures for 24 h to high temperatures have been found to decrease the levels of stress proteins in *Ulva intestinalis* (Lewis *et al*., 2001).

Apparently, the expression of stress proteins in some macroalgae depends strongly on the thermal history of the populations. In the invasive kelp *U. pinnatifida*, local acclimation was associated whith latitudinal differences in temperature, while in the native *Egregia menziesii* latitudinal differences in the expression of stress proteins were detected (Henkel & Hoffmann, 2008b). In small green algae, which generally dominate upper littoral levels, the course of the expression of stress protein induction appears to confirm a common eurythermal characteristic of this group of macroalgae. For example, in *Ulva prolifera* HSP70 transcription measured through a temperature gradient 5-40°C was very low at its growth temperature (25°C), but substantially increased at the extremes of the range (Zhang *et al*., 2012). Similarly, levels of HSP70 in the intertidal *Ulva intestinalis* were considerably higher at temperatures >25°C compared to the prevailing temperatures at the collection site (15°C) (Lewis *et al*., 2001). In *Ulva* sp. and *Porphyra columbina*, two species adapted to cold-temperate conditions in southern Chile, exposure to high temperatures (25-35°C) for various hours resulted in degradation of proteins (in all the treatments only fragments <50kD were detected).

UV radiation

The disruption of the activity of PSII and inhibition of the photosynthetic electron transport are probably the most known effects of the exposure to UV-B radiation (Vass, 1997). In general, stress proteins and chaperones can facilitate the assembly of proteins and their insertion into the membrane (Stapel *et al*., 1993). It is well established that diverse types of stress proteins prevent the inactivation of PSII components caused by excess solar radiation (included UV radiation). For example, stress proteins have been associated with the repair processes and stabilization of thylakoid membranes during stress (Vigh *et al*., 1998) and also in the D1 cycle repair, with functions such as removal of damaged and reassembly of new D1 subunits during exposure to UV-B radiation (Mattoo & Edelman, 1987). It is believed that the expression of HSP70 would be a major determinant of the ability of the cell to resist photodamage (*e.g.* after injury by UV radiation) by reducing the inactivation of PSII and intensifying the recovery. The role of the stress proteins in the repair of the photodamaged PSII would be the stabilization of the core of PSII reaction centres (Yokthongwattana *et al*., 2001). In macroalgae, exposure to UV radiation in the laboratory has proved to be a factor inducing HSP70, which depends on the UV dose and species. In *Ulva* sp., HSP70 induction has been detected after time spans of 2.5 (Zhang *et al*., 2012) and 5 h (Ahamad, 2010). However, stress proteins can be detected several hours after the stress, as was observed in the present study for the Rhodophyta *Porphyra columbina*, where stress proteins of the HSP70 family were detected after 24 h (see below). In species of *Ulva rotundata* from locations strongly exposed to solar UV radiation in southern Spain, stress proteins (chaperonin 60) can be induced in time scales of days (Bischof *et al*., 2002) (Table 1). In general, the induction of stress proteins in response to high light is exacerbated under high temperatures. The over expression of gene products in response to combined action of high light and high temperature stress was observed in *Chondrus crispus* (Collén *et al*., 2007). The relationship between light

Table 1. Stress protein responses in intertidal macroalgae upon exposure to temperature, high irradiation, contaminants and combined factors.
Tabla 1. Respuestas de proteínas de estrés en macroalgas expuestas a elevada temperatura, alta irradiación, contaminantes y factores combinados.

Factors / species	Site	HSP	Stressor and exposure conditions	Observed response	Reference
Temperature					
Ulva lactuca Linnaeus *Chondrus crispus* Stackhouse *Fucus serratus* Linnaeus	Penmon Point, Anglesey, UK	HSP70	42°C (*Fucus* up to 24 h; *Ulva* and *Chondrus* 4 h)	Elevated HSP70 in *Fucus* after a 2 h exposure (not after longer exposure). Increase in HSP70 in *Chondrus* after 4 h exposure, but not in *Ulva*.	Ireland *et al*. (2004)
Undaria pinnatífida (Harvey) Suringar (gametophytes)	Culture from four sites in California	Gene expression (HSP70)	Incubations for 1 h between 12 and 36°C (under low PAR <5 µmol m^{-2} s^{-1}).	Extremely low expression and no upregulation of the HSP70 genes. Yield decreased with increasing temperature from 17 to 31°C. Gametophytes broadly thermotolerant.	Henkel & Hofmann (2008a)
Ulva sp. Linnaeus *Porphyra columbina* Montagne	Valdivia, Southern Chile	HSP70	Exposure to 20, 25, 30 and 35°C for 3, 6 and 24 h.	Low induction of HSP70. At temperatures >25°C marked degradation of proteins (fragments between 20-40 kD detected).	Present study
Ulva intestinalis Linnaeus	Wembury Beach, Devon, UK	HSP70	Exposure to 15, 20, 25, 30 and 35°C for 24 h (prior to exposure at 15°C)	HSP70 increased at 25 and 30°C compared to 15°C. Levels reduced at 35°C.	Lewis *et al*. (2001)
Ulva prolifera O.F. Müller	Qindao, Yelow Sea	HSP70 transcripts	Gradient between 5 and 40°C for 1 h.	HSP70 transcription was increased at low (5-14°C) and high (>30°C). At growth temperature (25°C), HSP70 levels were at lowest.	Zhang *et al*. (2012)
Undaria pinnatífida (Harvey) Suringar *Egregia menziesii* (Turner) Areschoug	West coast of California and Baja California	Gene expression (HSP70)	Incubation for 1 h at 12, 17, 22, 26, 31, 33 and 36°C (previously acclimated at 10°C).	Gene expression of HSP70 in *Egregia* showed latitudinal responses to heat stress. The invasive *Undaria* exhibited differences in the thermal according to habitat depth.	Henkel & Hofmann (2008b)
UV radiation					
Ulva sp. Linnaeus *Porphyra columbina* Montagne	Valdivia, Southern Chile	HSP70	Exposure to UV radiation at 10°C for 3, 6 and 24 h.	In *Ulva* sp. HSP70 induction by UV after 3 and 6 h exposure, in *P. columbina* after 24 h.	Present study

Continued

Ulva aff. *rotundata* Blinding	Cádiz, Spain	Chaperonin 60 (RubisCO binding protein)	A 3-d outdoor exposure to UV radiation and PAR	Increase in the concentration of CPN60 under PAR UV-A + UV-B, and PAR UV-A. Possible effect of elevated temperature. No induction under unfiltered solar radiation.	Bischof *et al*. (2002)
Ulva lactuca L. *Palmaria palmata* (L.) Kuntze *Solieria chordalis* (Ag.) J. Agardh *Dictyocha dichotoma* (Hudson) Lamour.	North Sea, Germany	HSP60, HSP70	UV radiation and PAR: exposure for 5 h and 18 h recovery period.	Induction of HSP60 and HSP70 in all the algae. Variation between species and light treatments. Highest induction in *Ulva lactuca*.	Ahamad (2010)
Ulva prolifera O.F. Müller	Qindao, Yelow Sea	HSP70 transcripts	UV radiation exposures for 1 to 4 h	Induction of HSP70 transcripts was maximal after 2.5 h exposure.	Zhang *et al*. (2012)
Heavy metals					
Ulva intestinalis Linnaeus	Wembury Beach, Devon, UK	HSP70	Exposure to copper (5 d) and antifouling Irgarol 1051 (92 h)	HSP70 was induced by copper but not changes in HSP70 induction were observed after exposure to Irgarol 1051.	Lewis *et al*. (2001)
Ulva lactuca L. *Chondrus crispus* (Stackh.) *Fucus serratus* L.	Penmon Point, Anglesey, UK	HSP70	Exposure for 4 h to a gradient in Cd^{2+} concentrations	High induction at 25 mM Cd^{2+}, but at higher concentrations (50-100 mM), stress proteins levels decreased.	Ireland *et al*. (2004)
Multiple factors					
Fucus vesiculosus Linnaeus (embryos) *Fucus spiralis* L. (embryos)	Schoodic Point, Maine	HSP60	Temperature and salinity: 3 h exposure to heat, with and without acclimation to a sub-lethal temperature and hyper-saline media.	Higher levels of HSP60 in embryos exposed to 29-33°C than to 14°C; lower levels of HSP60 in embryos exposed to hypersaline conditions than in normal sea-water.	Li & Brawley (2004)
Fucus vesiculosus L. *Fucus radicans* L. Bergström & L. Kautsky	Skagerrak (North Sea) and Central Baltic Sea	Gene expression (HSP90, HSP70, sHSP_3, sHSP_5, 14-3-3, LEA-like)	Temperature and light: 30 min to 250 µmol m^{-2} s^{-1} (control 50 µmol m^{-2} s^{-1}) and/or to 25°C (control 15°C).	Sympatric populations of *Fucus radicans* displayed divergent heat shock responses, while from allopatric *F. vesiculosus* populations did not. *F. radicans* was more sensitive to heat shock at 25°C under high irradiance and desiccation than *F. vesiculosus*.	Lago-Lestón *et al*. (2010)
Chondrus crispus (Stackh.) (gametophytes)	Roscoff, Brittany, France	cDNA micro-arrays (containing 1920 different cDNAs representing 1295 unique genes).	Temperature, light, osmotic and natural stress: incubation for 4 h at 16°C (PAR 100 µmol photons m^{-2} s^{-1}; control), at 32°C (in darkness) and to high PAR of 1800-2000 µmol photons m^{-2} s^{-1} at 15-18°C.	High-temperature and high-light stress increased the expression of HSPs. Expression of stress protein genes was induced in response to different stresses. High light stress was associated with synthesis of antioxidative proteins.	Collén *et al*. (2007)

and thermal stress varies between populations of related species with different distributional patterns (*e.g. Fucus vesiculosus versus Fucus radicans*) (Lago-Leston *et al*., 2010).

Heavy metals

Environmental pollutants, especially heavy metals, can induce stress proteins in marine organisms and thus these proteins have been considered as environmental biomarkers (Ireland *et al*., 2004; Torres *et al*., 2008). Although the induction is generally slower than under heat shock (Sanders, 1993), some studies using Cu and Cd confirm that increases in the levels of stress proteins are concentration dependent with levels decreasing at high metal concentrations (Lewis *et al*., 2001; Ireland *et al*., 2004). Similar to temperature and UV radiation, metals are redox active and participate in many reactions generating reactive oxygen species (ROS) (Sakihama *et al*., 2002). Increased ROS can lead to irreversible photooxidative damage, affecting lipid membranes, D1 protein synthesis and thylakoid proteins (Anderson *et al*., 1997; Niyogi, 1999; Takahashi & Murata, 2008). For example, macroalgae in copper impacted coastal areas of northern Chile have been shown to develop oxidative stress, which can partially be counteracted by rapid and reversible antioxidant activity (Ratkevicius *et al*., 2003; Contreras *et al*., 2005). There is evidence that stress proteins (and other biomolecules, see below) located in the cell protect proteins through mechanisms removing ROS, *e.g.* through the formation of methionine residues, thus protecting other proteins sensitive to oxidation (Levine *et al*., 1996). An important issue in the role of stress proteins as biomarkers under metal pollution is that the effects of metals are normally exacerbated by other environmental factors, such as high light, temperature and nutrients (Ireland *et al*., 2004; Huovinen *et al*., 2010). Additionally, high levels of metals (*e.g.* copper) can cause the breakdown of protein metabolism, thus decreasing the synthesis of stress proteins (Lewis *et al*., 2001).

Interactive effects of multiple stress factors

In nature, living organisms are never exposed to a single environmental factor and thus the action (synergistic or antagonistic) of different stressors in the intertidal zone is a common scenario to which macroalgae adapt. For example, during low tide, high solar radiation and temperature are accompanied by desiccation stress and extreme changes in salinity and nutrient availability (Davison & Pearson, 1996). However, the detection of metabolic mediated stress responses of macroalgae under these conditions is normally a very difficult task and few studies are available. It is known that intertidal macroalgae display rapid metabolic adjustments based on the up regulation of stress genes (including various transcripts of stress proteins) (Collén *et al.,* 2009). In the case of combined action of temperature and salinity, apparently high salinity can modify the capacity of algae to induce stress proteins in response to heat shock (Li & Brawley, 2004) (Table 1). However, the impact of various factors simultaneously cannot be additive. For example, in *Fucus* from the Baltic Sea stress proteins were up-regulated after thermal stress, which was not modified by the simultaneous exposure to high light or desiccation (Lago-Leston *et al*., 2010). Overall, recent studies appear to indicate that under multiple stress conditions, the same suite of stress protein genes can be up-regulated in response to different stressors as has been postulated for some species of *Fucus* (Pearson *et al*., 2010).

Other bio-molecules with putative anti-stress properties

Mycosporine-like amino acids

Mycosporine-like amino acids (MAAs) are probably the most common and best known group of photoprotective compounds in macroalgae. These substances belong to a family of chemically related, colourless, water-soluble amino acid derivatives commonly found in red algae. These compounds have been determined in polar and cold-temperate species, and their function as intracellular screening agents has been inferred from a decrease in concentration with increasing depth (Karentz *et al.*, 1991; Hoyer *et al.*, 2001; Huovinen *et al.*, 2004). Supra- and eulittoral species can be exposed to high solar stress, and consequently accumulate very high MAA contents, which are positively correlated with the natural UV doses (Karsten *et al.*, 1998). Although an antioxidant activity has been reported for various groups of MAAs from red algae (Tao *et al*., 2008; Coba *et al*., 2009), is not clear if the action mechanisms of these molecules during severe UV stress are directly related with the induction of stress proteins. Apparently, the sunscreen function of MAAs represents a primary shielding barrier against UV radiation, while stress proteins could act after damage (*e.g*., at the thylakoid membranes).

Phenolic compounds

Various phenolic compounds of green and brown algae have also been associated with photoprotection against excess solar radiation and other environmental stresses. Tri-hydroxi-coumarins found in some green

algae (*e.g.* the siphonal *Dasycladus vermicularis*) (Menzel *et al*., 1983) have several functions in the cell including UV photoprotection, antioxidant activity and anti-herbivory (Pérez-Rodríguez *et al*., 1998, 2001; Gómez *et al*., 1998). Apparently, the UV screening capacity of the coumarins remains even after massive excretion indicating that algae allocate considerably energy to these compounds (Pérez-Rodríguez *et al*., 2001). Phlorotannins (polymers of phloroglucinol; 1,3,5-trihydroxybenzen; Ragan & Glombitza, 1986), are phenolic compounds found exclusively in brown algae and play a series of roles as secondary metabolites, mainly as anti-herbivory defense (Targett & Arnold, 1998; Jormalainen & Honkanen, 2008) and antifouling activity (Wikström & Pavia, 2004). Phlorotannins form up to 25% of dry weight (Ragan & Glombitza, 1986) and are present as soluble and cell wall-bound fractions. Due to their UV absorbing properties and peripheral localization in cells and tissues, phlorotannins have been related with an increased tolerance to UV radiation, which has been demonstrated in kelp species such as *Ascophyllum nodosum* (Pavia *et al*., 1997), *Macrocystis integrifolia* (Swanson & Druehl, 2002), and in *Saccharina latissima* and *Nereocystis luetkeana* in relation to a combination of high UV and high CO_2 (Swanson & Fox, 2007). UV mediated increases in phlorotannins can minimize photodamage of key physiological process and cellular components such as photosynthesis and DNA in the intertidal kelp *Lessonia nigrescens* (Gómez & Huovinen, 2010). High levels of phlorotannins have been correlated with enhanced ROS scavenging activity in intertidal kelps exposed to high UV doses and metals suggesting that these compounds represent primary metabolic anti-stress agents (Huovinen *et al*., 2010; Cruces *et al*., 2012). In the kelp *Laminaria digitata*, high tolerance of sporogenesis and low DNA damage within the sorii were related with high concentrations of phenols in the paraphysis (Gruber *et al*., 2011). In *Saccharina latissima*, this higher allocation of phenolics in soral tissues was correlated with an enhanced antioxidant capacity compared to vegetative regions (Holzinger *et al*., 2011).

Polyamines

A less known group of molecules called polyamines (PAs), which can be free in the cell or bound to thylakoid membranes of plants and algae, have been associated with repair or photoprotective functions during severe high light and UV stress (Sfichi-Duke *et al*., 2004), resembling functions of some stress proteins. The three major polyamines, putrescine (PUT), spermine (SPM) and spermidine (SPD) have been reported to have roles in many biological processes such as cell division, growth and senescence (Igarashi & Kashiwagi, 2000). Their involvement in acclimation processes of PSII such as the down regulation of antenna size, increases in non-photochemical quenching and ROS scavenging during light stress suggest important photoprotective roles (Groppa & Benavides, 2008; Ioannidis *et al*., 2011). For macroalgae, the involvement of polyamines in UV stress responses has been examined in very few studies indicating that UV-B exposure can increase the amounts of polyamines (mainly PUT) up to 200% as reported for the red alga *Porphyra cinnamomea* (Schweikert *et al*., 2011). The action mechanism of polyamines in macroalgae remains unclear, but some evidence suggests that these molecules bind to other compounds (*e.g.* polysaccharides or phenols) thus stabilizing membranes or the cell wall.

Carotenoids

Carotenoids represent a large family of compounds, which, apart of their well-known functions as accessory pigments, have been associated with different photoprotective mechanisms. In general, it has been reported that carotenoids, due to their chemical structure and localization in the cell, can serve as screens against UV radiation and also as efficient antioxidant compounds, *e.g.* scavenging free radicals produced in the peroxidation reactions in the thylakoids (Götz *et al*., 1999; Hupel *et al*., 2011). However, the most known role of carotenoids in the so-called xanthophyll cycle, a trans-membrane interconversion of zeaxanthin to violaxanthin observed in plants to dissipate excess energy (Demmig-Adams & Adams, 1996). This cycle has been recognized to operate in macroalgae exposed to high PAR (Schofield *et al*., 1998; Bischof *et al*., 2002), but there is some evidence that UV radiation can affect xanthophyll cycle by inhibiting the de-epoxidation of violaxanthin to zeaxanthin (Pfündel *et al*., 1992). Additionally, increases in lutein have also been associated with a possible energy dissipation pathway in algae (Schäfer *et al*., 1994; Bischof *et al*., 2002).

Overall, the link between these different biomolecules and their photoprotective mechanisms in macroalgae has not been studied so far, however, it is reasonable to argue that many of them are complementary or are induced by different factors or combinations of factors. In fact, studies examining multiple cDNA microarrays from intertidal algae exposed to different stressors outlined the up regulation of specific genes for different stresses and additionally, emphasized the importance of ROS in the gene expression during environmental stress

(Collén *et al*., 2007). Thus, the putative antioxidant capacity of several of the described biomolecules could be a key connecting element that allows cells, by different ways, to protect finally the integrity and functionality of the photosynthetic machinery (Kreslavski *et al*., 2007).

Stress tolerance of upper intertidal macroalgae: a case study from southern Chile species

Stress proteins of the HSP70 family were detected in *Ulva* sp. from the coast of Valdivia (39º48'S, 73º14'W) exposed for 3 and 6 h to UV radiation in the laboratory, resembling summer conditions (Fig. 1). In

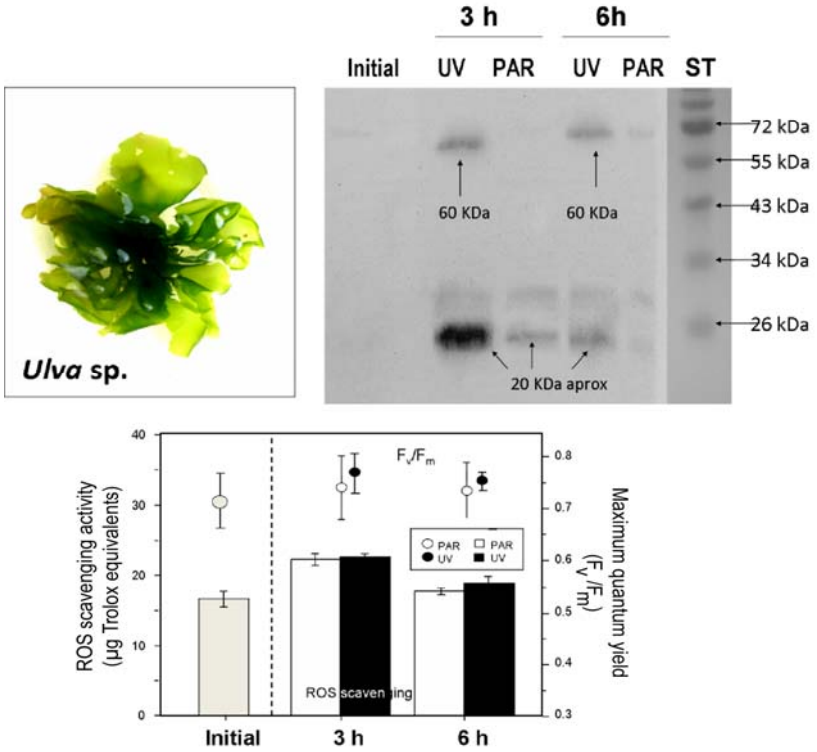

Figure 1. Western immunoblots to detect stress proteins in *Ulva* sp. from the coast of Valdivia, exposed for 3 and 6 h to UV and PAR (photosynthetically active radiation) treatments at 10°C. Algae were incubated under a combination of lamps (UV-B 2.3 W m^{-2}; UV-A 8.4 W m^{-2}); Q-Panel-313 and 340, Co., Cleveland, OH) and PAR (85 µmol m^{-2} s^{-1}). Samples were covered with Ultraphan 295 and 395 cut off foils (Digefra, Munich, Germany). The protein isolation, SDS-PAGE and immunoblotting followed the procedure described by Huovinen (2000). The membranes were probed with the monoclonal anti-Hsp-70 (MA3-008) primary antibody (Thermo Scientific) with a dilution of 1:2000. The secondary antibody conjugated to horseradish peroxidase (goat anti-IgG mouse) (Thermo Scientific, USA) with dilution of 1:1000 was used. Protein bands were imaged using chemiluminiscence (ECL, Thermo Scientific) in an Ultralum system (UltraLum Inc., USA). Results of quantum yield of chlorophyll fluorescence of photosystem II (F_v/F_m) and antioxidant activity (2.2-diphenyl-1-picrylhydrasyl; DPPH radical scavenging) are also shown (means ± SD, n = 6-10) and were measured according to the methodology described by Cruces *et al.* (2012).

Figura 1. Western immunoblots para la detección de proteínas de estrés en *Ulva* sp. colectada en la costa de Valdivia, expuesta por 3 y 6 h a tratamientos de radiación UV y PAR (radiación fotosintéticamente activa) a una temperatura de 10°C. Las algas fueron incubadas bajo una combinación de lámparas UV (UV-B 2.3 W m^{-2}; UV-A 8,4 W m^{-2}) (Q-Panel 313 y 340, Q-Panel Co., Cleveland, OH) and PAR (85 µmol m^{-2} s^{-1}). Las muestras fueron cubiertas con filtros Ultraphan 295 y 395 cut off foils (Digefra, Munich, Germany). La aislación, SDS-PAGE e inmuno-detección de proteínas fue realizada de acuerdo a la metodología descrita por Huovinen (2000). Las membranas se probaron con el anticuerpo primario específico para Hsp-70 (MA3-008 (Thermo Scientific) con una dilución de 1:2000. Se utilizó anticuerpo secundario conjugado a peroxidasa de rábano (cabra anti-IgG ratón) (Thermo Scientific, USA) con dilución de 1:1000. Las bandas de proteínas fueron detectadas por medio de quimioluminiscencia (ECL, Thermo Scientific) en un sistema de imagen Ultralum (UltraLum Inc., USA). Se muestran también los resultados de rendimiento cuántico máximo de fluorescencia de clorofilas del fotosistema II (F_v/F_m) y actividad antioxidante (2,2-diphenyl-1-picrylhydrasyl; DPPH radical scavenging) (promedios ± DS, n = 6-10). Estas respuestas fisiológicas fueron medidas de acuerdo a la metodología descrita por Cruces *et al*. (2012).

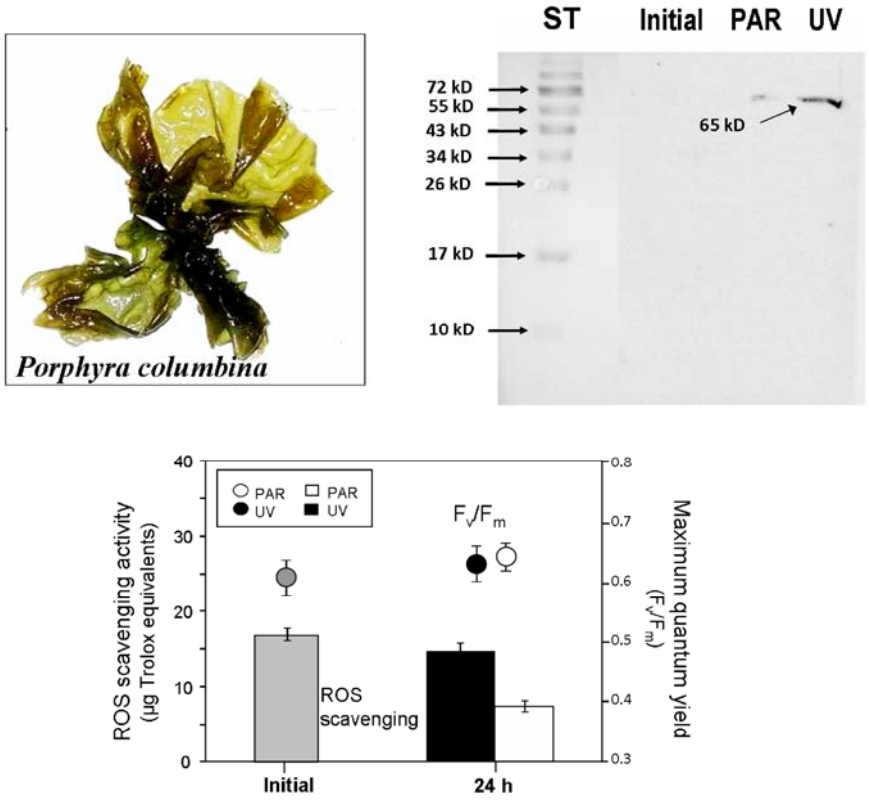

Figure 2. Western immunoblots to detect stress proteins in *Porphyra columbina* exposed for 24 h to UV and PAR (photosynthetically active radiation) treatments at 10°C. Results of quantum yield of chlorophyll fluorescence (F_v/F_m) and antioxidant activity measured in parallel are also shown. Means ± SD, n = 6-10. Conditions and procedures as described in Figure 1.

Figura 2. Western immunoblots para la detección de proteínas de estrés en *Porphyra columbina* expuesta por 24 h a tratamientos de radiación UV y PAR (radiación fotosintéticamente activa) a una temperatura de 10ºC. Se muestran también los resultados de rendimiento cuántico máximo de fluorescencia de clorofilas del fotosistema II (Fv/Fm) y actividad antioxidante medidos en paralelo. Promedios ± DS, n = 6-10).Las condiciones y procedimientos son los descritos en Figura 1.

Porphyra columbina, stress proteins were detected after 24 h exposure to UV radiation (Fig. 2). Confirming the well-recognized light tolerance of these species, which allow them to cope with high solar radiation (Gómez *et al.*, 2004), the induction of stress proteins did not correlate with changes in photochemical reactions (F_v/F_m) and antioxidant activity, which remained constant along time and were not affected by UV treatments. However, after 24 h exposure, antioxidant activity of UV exposed samples was higher than those under PAR treatment. These findings support the idea that the expression of stress proteins is not necessarily regarded as the most relevant anti-stress mechanism in upper littoral species subjected to intense environmental variation. On the other hand, the marked decrease in photosynthesis, suggesting photoinhibition, was correlated with low expression of proteins and after 6 and 24 h to temperatures >35°C, the presence of degradation products with molecular weights <40kD was detected (data not shown). This suggests that physiology of these algae, adapted normally to sub-Antarctic conditions, is strongly impaired at these extreme conditions. It is well known that synthesis and accumulation of stress proteins imply energy costs at expenses of other important processes. Thus, this type of algae display other metabolic mechanisms (*e.g.* photoinhibition, ROS scavenging) as primary strategy, at least at short-term, to cope with environmental factors or alternatively, have developed constitutive stress tolerance such as thermo-tolerance of photosynthesis. For example, in gametophytes of *Undaria pinnatifida*, an invasive and highly plastic species, the low or null expression of HSP70 transcripts together to a remarkable thermotolerance of photosynthesis was associated with an intrinsic physiological strategy

for example, to colonize different types of habitats (Henkel & Hoffman, 2008a). In the genera *Ulva* and *Porphyra*, opportunistic strategies associated with small size (*e.g.* high area/volume ratio), an annual phenology and rapid photosynthetic adjustments to cope with high solar radiation (Huovinen *et al*., 2006, Gómez & Huovinen, 2011) have probably not favoured the up regulation and synthesis of energy expensive stress proteins or, alternatively, these species maintain normally high levels of stress proteins (Henkel & Hoffmann, 2008b).

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