Kuroshio Science **6**-1, 115-119, 2012

Ulva as a Model for the Study of Environmental stress in Intertidal Macroalgae

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Abstract

The macroscopic chlorophyte, *Ulva fasciata* Delile, is abundant in shallow waters and intertidal zones along the Taiwan coast, where light intensity can increase to around 1,800μ mol m^{-2} s^{-1} at noon, particularly in the summer season and salinity can increase to around 60-80‰ in the intertidal pools. A series of experiments were therefore initiated in our laboratory to study how intertidal *U. fasciata* acclimates to high salt stress at the molecular level. We have identified the regulation of ion balance and proline synthesis by Ca and calmodulin via a modification of proline dehydrogenase activity in *U. fasciata,* and the up-regulation of genes encoding protein degradation enzymes and heat shock protein 90 for intertidal green macroalga *Ulva fasciata* against hypersalinity-induced protein oxidation. To cope with hypersalinity, the expression of genes of antioxidant enzymes is up-regulated in *U. fasciata* against oxidative stress. The signals and the following network in sensing the outer salinity changes have now been developed.

Key words: acclimation, hypersalinity, oxidative stress, *Ulva fasciata*

1. Introduction

Intertidal macroalgae frequently suffered from daily fluctuations in light intensity, desiccation, salinity, temperature, and UV exposure (Lobban and Harrison 1997; Kakinuma et al. 2006). The mechanism dealing with the acclimation and adaptation to stressful environments is critical for the survival of intertidal macroalgae. Because macroalgae do not have the advantage of motility like animals, they have developed mechanisms to cope with acute environmental changes, for example, salinity elevation in the intertidal pool. Our preliminary study noted that a marine macroscopic chlorophyte, *Ulva fasciata* Delile, was abundant in shallow waters and intertidal zones along the Taiwan coast, where salinity can increase to around 60-80‰ in the intertidal pools (Fig. 1). A series of experiments were therefore initiated in our laboratory to study how intertidal *U. fasciata* acclimates to high light and high salt stress at the molecular level. Our results in the past decade were summaried as follows.

Fig. 1. *Ulva fasciata* Delile is abundant along the costal shore in Taiwan.

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2. #Regulation of ion balance and proline synthesis in *Ulva fasciata* in response to salinity stress

It is considered that the control of constant cell turgor by regulating osmotic potentials through the adjustment of ion and organic osmolyte contents, is a typical tolerance mechanism observed in most marine algae (Kirst, 1990). When algae are exposed to salinity changes, the movement of water occurs first, followed by ion fluxes for the maintenance of constant cell turgor by regulating osmotic potential; monovalent ions including Na^+, K^+ and Cl^- are the amin ionic osmolytes contributing to the osmotic adjustment (Kirst, 1990). After that, the number of organic osmolytes, such as proline, accumulated in algae in response to salinity, changes. We first examined how *U. fasciata* counteracted the hypersalinity stress through the maintenance of water. These results were published between 10-12 years ago (Lee and, Liu. 1999. Regulation of NaCl-induced proline accumulation by calmodulin via a modification of proline dehydrogenase activity in *Ulva fasciata* (Chlorophyta). Australian Journal of Plant Physiology 26: 595-600; Lee TM, Liu. 1999). Correlation of decreased calcium contents with proline accumulation in the marine green macroalga *Ulva fasciata* exposed to elevated NaCl content in seawater; which is published in Journal of Experimental Botany.50:1855-1859.

Lee and Liu (1999) have found that Na^+, K^+, and Cl^- concentrations can be increased linearly as salinity increases in *U. fasciata* (Fig. 2). It is evident that Na^+, K^+, and Cl^- are used in *U. fasciata* for the control of osmotic potential upon exposure to hypersalinity. It was also found that the increase of salinity by the addition of NaCl led to a decrease in intrace-llular total and soluble Ca^{2+} concentrations but an increase in medium Ca^{2+} concentration, reflecting a efflux of Ca^{2+}. Therefore, hypersaline treatment by concentrated seawater did not influence Ca^{2+} concentrations. Our study showed that calcium effluxes in *U. fasciata* upon exposure to high salinity conditions.

The elevation of salinity by the addition of NaCl induced proline accumulation in *Ulva fasciata* via increased synthesis and decreased degradation (Fig. 3). Evidence suggests that

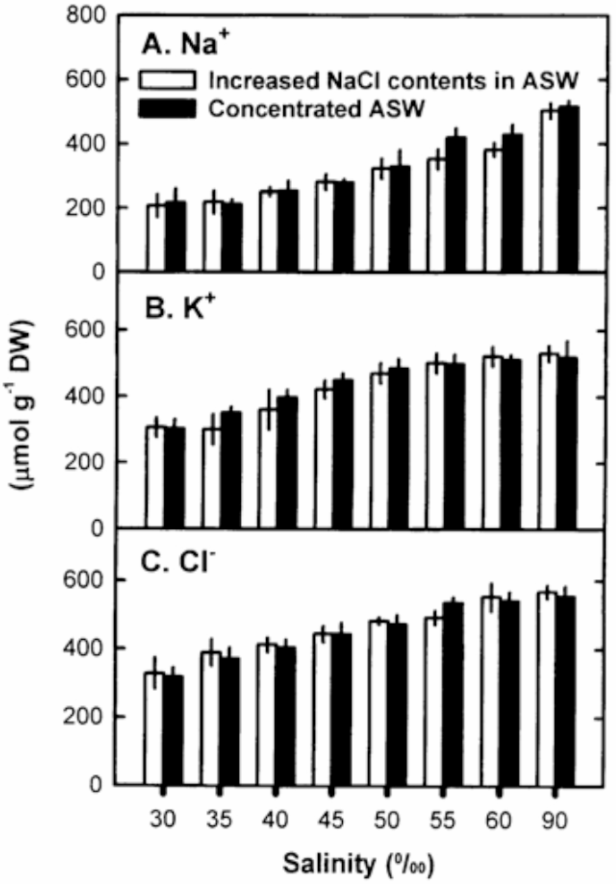

Fig. 2. Changes in Na^+ (A), K^+ (B), and Cl^- (C) in *Ulva fasciata* in response to elevated ASW salinity by increasing NaCl concentration in ASW (open bar) or concentrating ASW (solid bar). Vertical bar indicates the standard error (n = 3) (Lee and Liu, 1999)

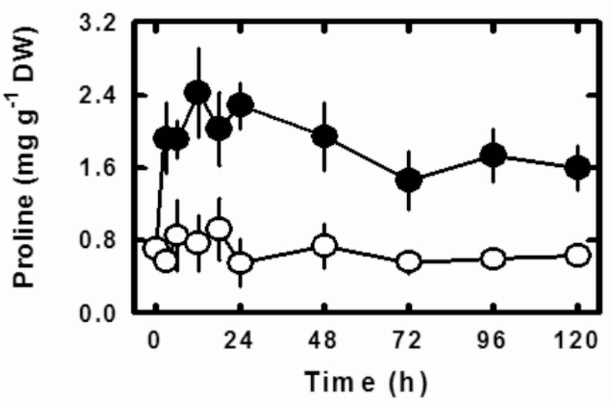

Fig. 3. Changes in proline in *Ulva fasciata* in response to elevated ASW salinity by increasing NaCl concentration in ASW. Solid circle: 55 ‰, Open circle: 30 ‰. Vertical bar indicates the standard error (n = 3) (Lee and Liu, 1999).

calcium is the factor in the regulation for proline synthesis for *U. fasciata* against hypersalinity stress. The activity and Vmax value of P5C reductase were increased by hypersalinity, while proline dehydrogenase (PDH) and P5C dehydrogenase (P5CDH) activities were significantly decreased, accompanied by an increase in Km value. Both a decrease in Ca^{2+} availability and a block of calmodulin action cause a decrease in PDH activity and and an increase in proline concentration. These suggest that when exposed to hypersalinity (NaCl), a loss of Ca^{2+} from *Ulva fasciata* cells alters the calmodulin-mediated pathway in the modulation of proline synthesis and degradation, and in turn, proline accumulates.

3. Molecular acclimation of *Ulva fasciata* to hypersalinity

In attempts to identify the whole picture of *U. fasciata* to hypersalinity stress, the transcripteomics analysis has been carried out using suppression subtractive hybridization (SSH) (Sung et al., 2010. Implications of the up-regulation of genes encoding protein degradation enzymes and heat shock protein 90 for intertidal green macroalga *Ulva fasciata* against hypersalinity-induced protein oxidation. Marine Biotechnology 13: 684-694.). SSH has been used to extract differentially expressed genes in algae in response to hypersalinity. In this study, mRNAs from 30‰ (control) and 90‰ (hypersalinity) treatments (24 h) were extracted for the construction of the SSH library to elucidate the whole picture of metabolism responses to hypersalinity in *U. fasciata* at the molecular level. We found that the SSH cDNAs for protein synthesis and destination were abundant. From these, several genes were selected for cloning of the full-length cDNAs and the examination of time course changes in mRNA expression levels in relation to protein degradation. In attempts to explore the role of these genes in the hypersalinity survival of *U. fasciata* in the removal of oxidatively modified proteins, ROS scavengers were treated to 90‰ grown thalli to scavenge overproduced ROS, and the recovery growth ability as the growth rate after recovery to 30‰ condition, H_2O_2 and protein carbonyl group contents, and mRNA levels of these genes were examined.

A forward cDNA library was constructed via the suppressive subtractive hybridization between 30‰ and 90‰ (24 h) and by the time-course dynamics of several abundantly expressed genes.

Among the genes with known sequences, the expressed sequence tags (ESTs) are abundant in the function of protein synthesis (ribosomal protein) and destination (Fig. 4). The cDNAs of ATP-dependent Clp protease, (*UfClpC*), 20S proteasome B subunit type 1 domain (*UfPbf1*), ubiquitin-conjugating enzyme E2 I (*UfUbc9*), and heat shock protein 90A (*UfHsp90A*) were cloned. *UfClpC* transcript increased 3 h after 90‰ treatment, followed by a decrease, while *UfPbf1* and *UfUbc9* transcripts increased after 12 h and decreased at 48 h. The transcripts of *UfHsp90A* increased 1 h after 90‰ treatment, followed by a drop and to the control level at 48 h. Protease activity increased 3 h after 90‰ treatment and decreased to the control level at 48 h (Fig. 5). H_2O_2 contents increased 1 h after 90‰ treatment and then remained unchanged but protein carbonyl group contents increased after 48 h. The treatments of reactive oxygen species scavengers partially alleviated 90‰ damage (partial growth rescue) and suppressed the increases in H_2O_2 content, protein carbonyl group content, protease activity, and *UfClpC, UfPbf1, UfUbc9*, and *UfHsp90A* transcripts by 90‰. The induction of specific chaperones and proteases at the molecular level for protein quality control can be considered as one of molecular mechanisms of hypersalinity acclimation in *U. fasciata.*

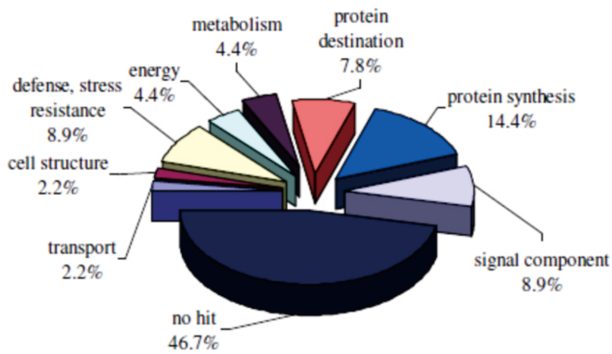

Fig. 4. The functional grouping of forward cDNA library via the suppressive subtractive hybridization between 30‰ and 90‰ (24 h) (Sung et al. 2010).

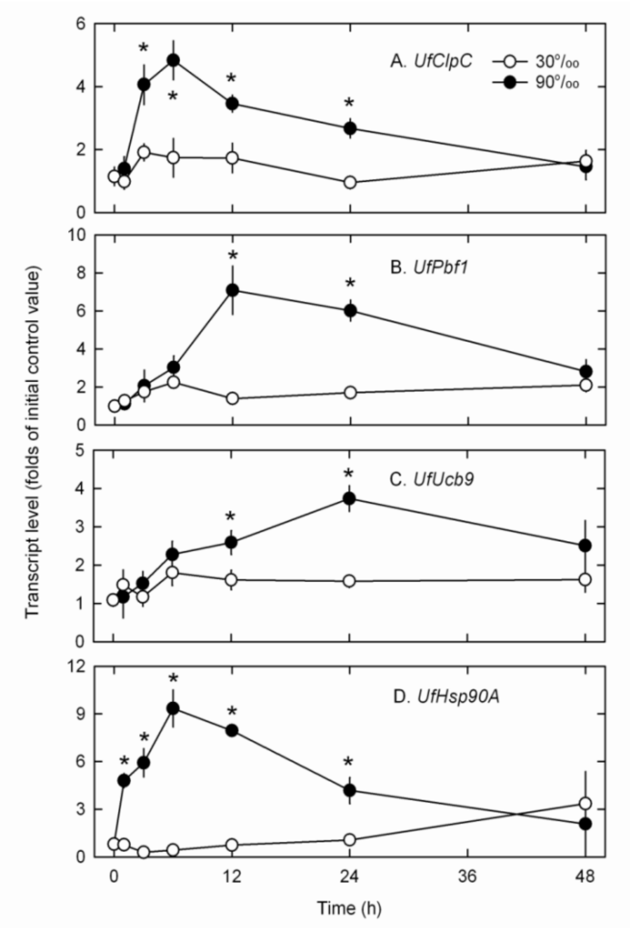

Fig. 5. Time course changes in the transcripts of UfClpC (a), UfPbf1 (b), UfUbc9 (c), and UfHsp90A (d) (means ± SD, n=3) of *U. fasciata* exposed to 30‰ or 90‰ . The relative transcript abundances were assayed by the detection of SYBR Green fluorescence in quantitative real-time PCR and presented as the fold change in mRNA abundance, normalized to an endogenous reference gene (α -tubulin; GenBank no. EU701065), relative to the RNA sample of 30‰-grown *U. fasciata* at 0 h. Asterisk indicates significant difference by t-test (Sung et al. 2010)

4. Induction of antioxidant defense system in U. faciata against oxidative stress in hypersalinity stress.

Because one of EST genes, ascorbate peroxidase, as the antioxidant enzyme, has been found in *U. fasciata* after 24 h of exposure to hypersalinity, we proposed that antioxidant enzymes may be involved in *U. fasciata* in the defense of oxidative stress. In addition, our previous study, (Lu IF, Sung MS, Lee TM. (2006) Salinity stress and hydrogen peroxide regulation of antioxidant defense system in *Ulva fasciata.* Marine Biology 150: 1-15.) noted that hypersalinity stress increased antioxidant defense enzyme activity. Therefore, we have determined the expression of genes of these antioxidant enzymes in *U. fasciata* in response to hyper-salinity stress (Sung et al., (2009) Hypersalinity and hydrogen peroxide up-regulation of gene expression of antioxidant enzymes in *Ulva fasciata* against oxidative stress. Marine Biotechnology 11: 199-209).

We have identified that hypersalinity (60‰ , 90‰) as well as hyposalinity (15‰) caused lipid peroxidation and H_2O_2 accumulation, and that a H_2O_2 scavenger, dimethylthiourea, effectively inhibited H_2O_2 increase and oxidative stress. Antioxidants and the enzyme activity and mRNA expression levels of several antioxidative enzymes, including superoxide dismutase (SOD) (Fig. 6), ascorbate peroxidase (APX), glutathione reductase (GR) (Fig. 7), and catalase were differentially up-regulated against salinity stress.

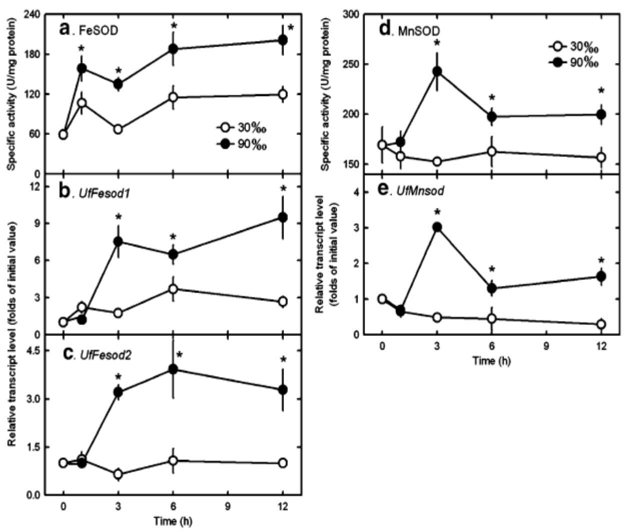

Fig. 6. Time-course changes in the activity and transcript of FeSOD (a-c) and MnSOD (d, e) of *U. fasciata* in response to 30‰(open circle) or 90‰(closed circle). Data are means ± SD (n=3) and asterisk indicates significant difference by t-test (Sung et al. 2009).

Ulva fasciata underwent oxidative stress in response to hypersaline condition (Lu et al. 2006; Sung et al. 2009). To counteract oxidative stress, antioxidant enzymes including manganese superoxide dismutase (MnSOD), FeSOD, APX, GR, and catalase were up-regulated by short-term (Sung et al. 2009) or long-term (Lu et al. 2006) hypersalinity (90‰) treatments. However, the capacity for scavenging ROS was not sufficient for inhibiting ROS over-production, as reflected by a significant H_2O_2 accumulation after exposure to 90‰ . As a result, macromolecules including membrane lipids, proteins, and nucleic acids are potentially oxidized. Therefore, in addition to the activation of antioxidant defense system, the removal and repairing of oxidative macromolecules, such as oxidatively damaged proteins, and the modification of other metabolic changes have become critical for the adaptation of *U. fasciata* to hypersalinity-induced oxidative stress.

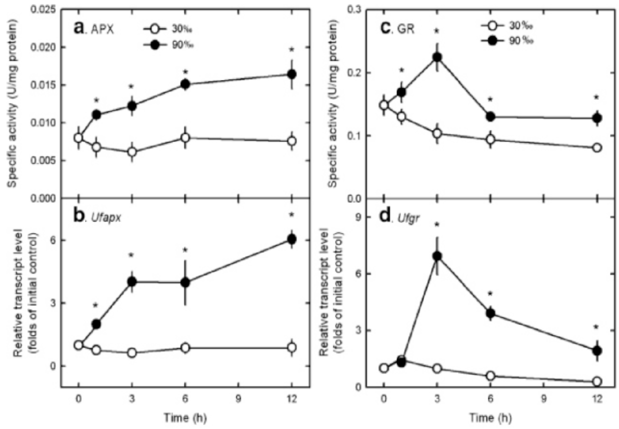

Fig. 7. Time-course changes in the activity and transcript of APX (a,b) and GR (c,d) of *U. fasciata* in response to 30‰ (open circle) or 90‰ (closed circle). Data are means ± SD (n=3) and asterisk indicates significant difference by t-test (Sung et al. 2009).

5. Conclusion

Ulva fasciata modulates gene expression, including protein destination and synthesis, the antioxidative defense system, and the repairing system of oxidized proteins, for the acclimation to high salinity conditions. The molecular acclimation of intertidal green macroalga *Ulva fasciata* Delile to high salinity stress was examined by the construction of a forward cDNA library via the suppressive subtractive hybridization between 30‰ and 90‰ (24 h) and by the time course dynamics of several abundantly expressed genes. Among the genes with known sequences, the expressed sequence tags are abundant in the function of protein s ynthesis (ribosomal protein) and destination. The signals and the following network in sensing the outer salinity changes are now developed.

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