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Development of symbiosis-specific genes as biomarkers for the early detection of cnidarian–algal symbiosis breakdown

Carys L. Mitchelmore*, Jodi A. Schwarz, Virginia M. Weis

Oregon State University, Department of Zoology, 3029 Cordley Hall, Corvallis, OR 97331, USA

Abstract

Coral bleaching, i.e. the loss of dinoflagellate symbionts from cnidarian hosts, is occurring globally at increasing rates, scales, and severity. The significance of these bleaching events to the health of coral reef ecosystems is extreme, as bleached corals exhibit high mortality, reduced fecundity and productivity and increased susceptibility to disease. This decreased coral fitness leads to reef degradation and ultimately to the breakdown of the coral reef ecosystem. To date there has been little work describing the application of biomarkers to assess coral health. The most commonly applied biomarker is, in fact, the bleaching event itself. We are interested in developing early warning biomarkers that can detect coral stress before bleaching occurs. Recently, several genes that are likely to function in regulating interactions between cnidarians and their symbionts have been characterized, using the temperate sea anemone Anthopleura elegantissima as a model species. One "symbiosis gene" identified from the host genome, sym32, is expressed as a function of anemone symbiotic-state, where sym32 expression is higher in symbiotic cf. aposymbiotic (symbiont-free) anemones. Real-time quantitative RT-PCR suggested that the level of sym32 expression was correlated with the abundance of algae in the host. Furthermore, laboratory exposures of anemones to low levels of cadmium (0, 20, 100 μg^{-1} $CdCl_2$; 14 days), which caused no change in algal cell numbers, resulted in a down-regulation of sym32 compared to controls, indicating that sym32 expression may serve as a new sensitive early warning biomarker of cnidarian–algal symbiosis breakdown.

Keywords: Anemone; Cadmium; Cnidarian; Biomarker; Symbiosis

Reef building corals are a mutualistic association between members of the phylum Cnidaria and dinoflagellate algae in the genus *Symbiodinium*. This partnership forms the trophic and structural foundation of the entire coral reef ecosystem. Coral

* Corresponding author.
E-mail address: mitchelmore@cbl.umces.edu (C.L. Mitchelmore).

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bleaching, defined as the loss of color in corals, is due most often to the breakdown of the symbiosis, with loss of algae from the host coral. Incidents of bleaching are increasing in frequency and severity worldwide (Glynn, 1993; Wilkinson, 1999). Bleaching leads to high mortality and is considered a serious threat to the health of reef ecosystems. To date a common biomarker described for symbiosis breakdown is the bleaching event itself (Brown, 2000; Meehan & Ostrander, 1997). To assess stress in this symbiotic system before bleaching occurs, it is necessary to develop early warning biomarkers, which precede symbiosis breakdown. Recently, cnidarian host genes that are expressed as a function of the symbiotic state have been characterized in the temperate sea anemone *Anthopleura elegantissima*, a model species for studying cnidarian–algal symbiosis (Reynolds, Schwarz, & Weis, 2000; Weis & Reynolds, 1999). This study addresses the possibility that the expression levels of symbiosis-specific genes could be utilized as novel early warning biomarkers for the detection of symbiosis breakdown. Exposure to cadmium was chosen as the environmental stressor as heavy metals are common pollutants, are accumulated by cnidarians and cause bleaching (Harland, Bryan, & Brown, 1990; Harland & Nganro, 1990)

Naturally occurring symbiotic and aposymbiotic (symbiont-free) specimens of *A. elegantissima* were collected from Seal Rock or Ecola State Park, Oregon and transferred to the laboratory. Anemones for metal exposures were acclimatized in the laboratory for 2 weeks and then placed in ambient seawater (14 °C) containing $CdCl_2$ (0, 20, 100 μg l^{-1}) in static tanks with water changed every 2 days. Optimal lighting conditions for algal photosynthesis were used (200–250 μmols quanta/m^{-2} s^{-1}, 12/12 h light/dark cycle). Tanks were set up in duplicate for each treatment with both symbiotic and aposymbiotic animals placed in each tank and sampled ($n=4$ for both types) at Day 0 and following 14 days of treatment. A subset of four symbiotic animals from each tank was repeatedly assessed for changes in algal cell numbers using tentacle clips following each water change. Sampled anemones were frozen in liquid nitrogen and stored at −80 °C prior to analysis. Host RNA was extracted using an RNA aqueous kit (Ambion, USA) after removal of algae by centrifugation. Gene expression levels were assessed by real-time quantitative RT-PCR, which is a relatively new, highly sensitive technique to assess differential expression of genes (Heid, Stevens, Livak, & Williams, 1996; Morrison, Weis, & Wittwer, 1998). It employs a fluorochrome that fluoresces as a function of PCR product quantity. Fluorescence is measured at each cycle by a quantitative PCR machine (ABI Prism 7700). Different quantities of template result in differences in the cycle number of threshold fluorescence. For example, samples with abundant template will start to amplify at an earlier cycle number than samples with very little template. Products were amplified using host-specific PCR primers for sym32 and actin (see Fig. 1b). Actin expression was used as a 'housekeeping' or internal standard for the assay as it is expressed equally regardless of host symbiotic state (Reynolds et al., 2000; Weis & Reynolds, 1999). Expression levels were normalized to actin for each sample and expressed in terms of cycle difference (threshold cycle number difference between gene of interest and actin). Statistical analysis for Fig. 2 was carried out using unpaired two-tailed students *t*-test ($\alpha=0.05$) corrected for multiple comparisons using the Bonferroni procedure.

Fig. 1 demonstrates the relationship between algal cell numbers of four field-collected anemones (Fig. 1a) and their corresponding expression levels of sym32 (Fig. 1b). A trend between levels of sym32 and algal abundance in the host is observed; the lowest levels of sym32 (increased cycle difference) occurred in anemones containing lower quantities of algae. The function of this gene in cnidarian-algal symbiosis has not yet been determined although it is a member of the FasI family of cell adhesion molecules (Reynolds et al., 2000). This trend between sym32 expression and algal cell abundance, however, leads to the question of whether this down-regulation can be used to sensitively predict symbiosis-breakdown, before bleaching begins. Fig. 2 details a statistically significant ($P < 0.001$) down-regulation

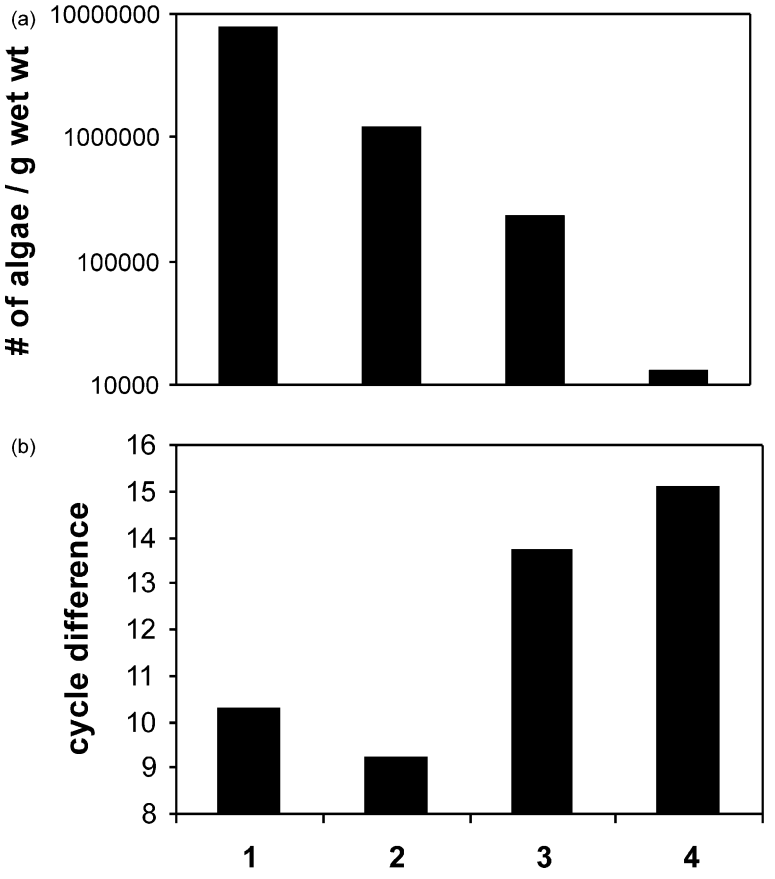

Fig. 1. Algal cell number (a) is correlated with the level of sym32 gene expression (b) in four field collected symbiotic anemones. Algal cell numbers are presented as # of algae/g^{-1} wet weight of anemone. Differences in gene expression are expressed in terms of threshold cycle number difference between sym32 and actin (internal control). A higher cycle difference represents a lower amount of sym32 template hence decreased expression or down-regulation of sym32. Host specific primers (forward and reverse respectively) for actin; 5′-CTGATGGACAGGTCATCACCAT-3′, 5′-CTCGTGGATACCAGCAGATTCC-3′; for sym32, 5′-TGCCCAAAAAGGACATACGA-3′, 5′-GATTTCTTTCAGCTTTTCGGCA-3′.

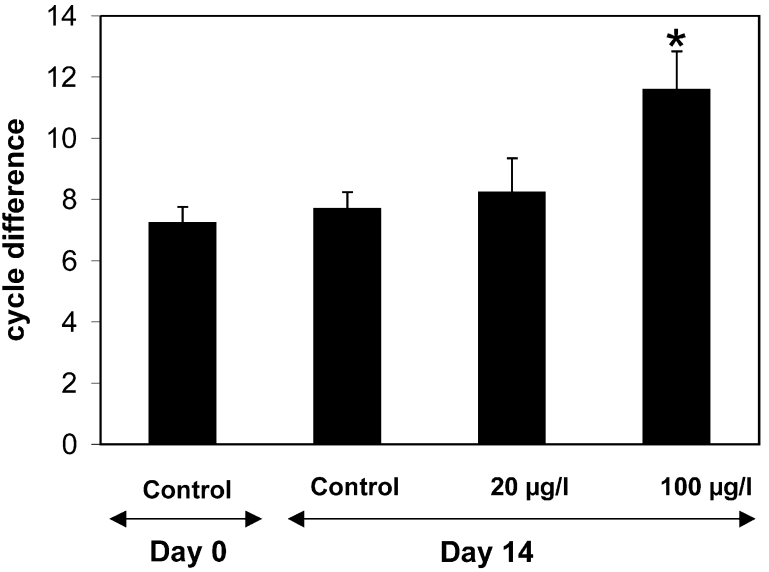

Fig. 2. Decreased gene expression of sym32 in symbiotic anemones following 14 day laboratory exposure to cadmium. Data expressed as mean (±S.D., $n=3$–6) threshold cycle number differences between sym32 and actin (internal control). *, Significant differences from controls (Day 0 and Day 14) and 100 $\mu g/1^{-1}$ cadmium (Day 14) were observed ($P<0.001$) using unpaired two-tailed Students t-tests. Host specific primers used are the same as in Fig. 1 except for sym32, 5′-GAATGGTGGGATCATGACTC-3′.

(increased cycle difference) of sym32 in symbiotic anemones exposed for 14 days to 100 $\mu g/1^{-1}$ $CdCl_2$ cf. controls at Day 0 and 14 ($n=3$–6). No evidence of algal cell loss was observed in any of the anemones repeatedly sampled from each tank (average 1.7×10^8 algal cells/g^{-1} wet weight anemone tissue ± 0.32 S.D.) suggesting that indeed down-regulation of sym32 may represent stress in this symbiosis before loss of algae occurs.

Our results provide the first description of the use of applying cnidarian "symbiosis genes" as sensitive early warning indicators of stress and presents evidence that these responses may serve as useful biomarkers as part of a multi-disciplinary biomonitoring regime investigating cnidarian–algal symbiosis health. Further studies are underway investigating other symbiosis-enhanced genes in response to various heavy metal stressors in long-term exposure and recovery studies. These responses will be compared with other early warning biomarkers that have been developed in our laboratory to investigate stress in cnidarians and their algal symbionts.

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