Competition for Space Among Sessile Marine Invertebrates: Changes in HSP70 Expression in Two Pacific Cnidarians

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Abstract. The role of stress proteins—either constitutive (HSC) or inducible (HSP)—of the HSP70 family in intra- and interspecific competition for space was examined in two sessile Pacific cnidarians, Anthopleura elegantissima, an intertidal anemone, and Corynactis californica, a subtidal corallimorpharian, express HSP70 in the absence of apparent physical stress. HSP70 protein expression is concentrated in the tentacles of A. elegantissima when the animal is exposed to contact with other benthic organisms. Under the same conditions, however, HSP concentrations are similar in the body and tentacles of C. californica. When two different clones of A. elegantissima interact in the field, the outside polyps (warriors) express more HSP70 than the inside ones (2.4 versus 0.6 ng HSP70/µg Protein). When different C. californica clones interact, HSP70 expression in the outside and inside polyps is similar (1.5 versus 1.8 ng HSP70/µg P) and is fairly constant in the corallimorpharian in the different interspecific encounters. HSP70 expression is related to the different kinds of aggression encountered by both cnidarians. HSP70 expression may be involved in the recovery of tissues damaged by the allelochemical, cytotoxic, or corrosive substances produced by different enemies. C. californica clones appear prepared for war, as evidenced by the high constant expression of HSP70 in the polyps. A. elegantissima exhibits differential HSP70 expression depending on the identity of each neighboring intra- or interspecific sessile competitor. We propose that stress proteins can be used to quantify space competition or aggression among sessile marine invertebrates.

Introduction

Space on which to live is often the most limiting resource in marine hard-substratum environments, and patchiness has evolved under the influence of intense competition for living space (Connell, 1961; Pequegnat, 1964; Paine, 1971; Dayton, 1971; Jackson, 1977). Once established, organisms can show aggressive behavior (Chadwick, 1987) that may be especially intense in cryptic environments where free space is almost nonexistent.

In benthic environments, sponges, ectoprocts, cnidarians, and ascidians can produce biologically active substances that may be destructive to enemies during space competition (Whittaker and Feeny, 1973; Uriz et al., 1991). These organisms aggregate in patches that can dominate hard-bottom substrates (Sutherland, 1978; Chornesky, 1983; Chadwick, 1987, 1991; Chadwick and Adams, 1991; Langmead and Chadwick, 1999a, b, among others). Growth is often slow in such organisms, and interactions between competitors are often nonevident. It is difficult to quantify competitive interactions in situ, and the manipulation of organisms is frequently essential to demonstrate the potential effects of space competition (Schoener, 1983). For example, investigators have rarely observed agonistic interactions in wild anemones (A. xanthogrammica), although these organisms frequently exhibit such behavior in forced situations (Sebens, 1984). The quantification of damage from encounters between such organisms and the identification of potential mechanisms used to counter the effect of such aggression have proved difficult. Most studies have dealt with the organismal responses to the attack and the
consequent aggressive behavior displayed by individuals. Few workers have focused on the capacities, and implied mechanisms, for tissue recovery following aggressive interactions. We hypothesize that components of the stress response such as HSPs may provide evidence of the intensity of competitive interactions and are one of the mechanisms by which cnidarians recover from or prepare their tissues for the effects of competitive or aggressive interactions.

HSPs enhance cell survival by reducing the accumulation of damaged or abnormal polypeptides within cells (Feder and Hofmann, 1999). However, whether all wild organisms routinely, occasionally, or seldom express inducible HSPs is unknown. For marine invertebrates, most investigators have examined the effects of thermal variations on constitutive (HSC70) and inducible (HSP70) responses (Feder and Hofmann, 1999). Competitive interactions between sessile organisms can elicit HSP responses due to protein damage following the excretion of harmful substances by one or both competitors (Uriz et al., 1991; Turon et al., 1996; Wiens et al., 1998). One index of tolerance to aggressive sessile organisms could be the presence and abundance of mechanisms (such as HSPs) that would resist or ameliorate the damage inflicted on cellular components by the potential space competitor. Furthermore, once HSP can be related to space competition, no manipulation will be necessary to test such hypotheses. HSP expression could then be a quantitative tool to examine competitive interactions in the field without human interference.

To determine whether HSP expression patterns could be related to competitive interactions in marine hard-bottom sessile invertebrates, two Pacific cnidarians were chosen for study: the intertidal anemone Anthopleura elegantissima and the subtidal corallimorpharian Corynactis californica. A. elegantissima forms contiguous aggregations composed of individuals of a single clone, the products of asexual reproduction (Francis, 1973b; Sebens, 1982a, b). Free zones are created where competition between clones occurs through the outside polyps of the aggregation (called “warriors,” Francis, 1973a). Compared with polyps in the center of the clone, the warriors have larger and more abundant acrorhagi (specialized nonfeeding tentacles) and lack mature gonads (Francis, 1973b, 1976). The aggressive response is not directly involved in either defense against predators or capture of prey (Francis, 1973b), but functions in the competition for space. We hypothesize that A. elegantissima warriors may exhibit higher HSP levels than interior clonemates because they interact more frequently with competitors.

In the subtidally distributed C. californica, the polyps have no distinctive roles within each clone (Chadwick, 1987). Although the physiology of this group is not as well understood as that of anemones, several studies have described the competition for space and the specific responses to aggression in corallimorpharians (Chadwick, 1987, 1991; Chadwick and Adams, 1991; Langmead and Chadwick, 1999a, b). Space competition experiments demonstrate that C. californica influences the abundance and population structure of other cnidarians by means of its aggressive behavior (Chadwick, 1987, 1991; Chadwick and Adams, 1991). We sought to determine whether the high aggression in this species is related to elevated HSP levels as preparation for possible damage resulting from such interspecies encounters.

We tested two main hypotheses in this work: first, that stress produced by space competition can induce HSP expression to counter the effects of aggressive neighbors; second, that HSP expression can provide a quantitative assay for space competition in sessile invertebrates.

Materials and Methods

Animals and treatments

Anthopleura elegantissima and Corynactis californica were collected from the Bodega Bay area and held in the running seawater system of the Bodega Marine Laboratory. All animals were held in ambient seawater (13–15 °C) and fed adult brine shrimp or frozen seafood. The seawater from the Bodega Bay area is considered clean, and the animals used in these experiments are considered to have had minimal contact with anthropogenic chemicals that are known to induce HSP expression (McCain et al., 1988). All experiments (aquarium and field) were done in September–October 1998 and 1999 to avoid seasonal differences in cnidarian behavior. Each experiment, whether forced interactions in an aquarium or in situ interaction, was designed to assess the effects of neighboring competition for space on HSP70 expression.

Forced aquarium experiments

The first experiment examined HSP70 protein expression in A. elegantissima and C. californica in a forced situation. Six isolated polyps of each species (attached to stones, no physical stress induced) were moved into contact with each other (i.e., one polyp of A. elegantissima against one polyp of C. californica). After 24 h, tentacle samples from three individuals of each species were removed and frozen in liquid nitrogen. To quantify the differences between tentacles and body, the other three polyps of each species were sampled 48 h later, frozen in liquid nitrogen, and then assayed for HSP70 level by methods detailed below. As controls, isolated polyp tentacles (n = 5–6, no interacting species) of A. elegantissima and C. californica were likewise sampled in the aquarium.

In situ intraspecific competition

We assessed HSP70 expression related to competition for space in a natural environmental situation (i.e., in natural
clones in the field). Because collection and transport of animals to artificial holding conditions can stimulate a stress response (Sharp et al., 1994; Roberts et al., 1997), clones of *A. elegantissima* and *C. californica* were located and sampled from the Bodega Bay Jetty from a minimum 2 m below the tide level (permanently submerged). This avoided desiccation, changes in temperature, fluctuations in salinity and pH, and other effects that are typical of the environment for the intertidal *A. elegantissima* but not for the subtidal *C. californica*.

For the *A. elegantissima* intraspecific competition experiments, clones were located by scuba and photographed (Nikonos V camera, 35-mm lens with macro 1:1 or close-up lens). Polyps of each clone were sampled (n = 3, tentacles) from the outside (touching the competitor) and the inside (touching only the same clone, 10–20 cm from the outside polyps). Samples were dissected, kept in 13°C seawater for no longer than 30 min before freezing in liquid nitrogen, and stored at −70°C. As a control to assess whether HSP70 levels were affected by the extra 30-min tissue incubation in ambient seawater before freezing, the following experiment was performed. Individual tentacle samples were obtained from three individuals of two clones exposed to elevated temperatures in the intertidal zone (elevated HSP70 is found in these conditions, Snyder and Rossi, unpubl. obs.). Each sample was divided into three parts, of which two were immediately frozen in liquid nitrogen and the third was submerged in ambient seawater for 40 min prior to freezing as above.

For the *C. californica* intraspecific competition experiments, six clones were located and sampled as above. Color varies greatly between different clonal aggregations, which is useful in distinguishing clones that show potential intraspecific competition. Outside and inside polyps (tentacle crowns) of each clone were sampled to compare interacting (<2.5 mm apart) and non-interacting individuals (5–10 cm apart from the outside ones).

**Interspecific competition**

To examine the effects that different space competitors in the benthic substrata have on HSP70 protein levels, we chose two genera of algae that compete for space with *A. elegantissima* and *C. californica* and two intertidal and two subtidal invertebrates for *A. elegantissima* and *C. californica*, respectively. The sampled and photographed anemone clones were always submerged (as described before).

Four clones of *A. elegantissima* and three of *C. californica* that were interacting with a calcareous red alga (*Lithothamnium* sp.) were dissected (outside and inside clone tentacles). Another alga interacting with both cnidarians was a fleshy green alga (*Ulva* sp.), and six clones of each cnidarian were sampled as above.

In the high subtidal, common space competitors of *A. elegantissima* are the anemone *A. xanthogrammica* and the cirrhiped *Balanus amphitrite*. Five *A. elegantissima* clones interacting with *A. xanthogrammica* were sampled in the outside and inside parts of the clones. For *B. amphitrite*, three clones competing for space were likewise sampled. For *C. californica*, the subtidal organisms chosen (sponge *Haliclona permollis*; ascidian *Synoicum parfustis*) were considered potentially more aggressive than the fleshy algae. Six *C. californica* clones were chosen for their clear interactions with *H. permollis*, and polyps of the outside and inside part of each clone were dissected. For *S. parfustis*, the interaction of the clones was observed in four populations in the dive area, and outside and inside polyps were sampled.

**HSP70 measurements**

The western immunoblotting for HSP70 expression was done as follows. Frozen tentacle samples (stored at −70°C) were individually homogenized in 0.2 ml of buffer K containing 5 mM NaHPO₄, 40 mM HEPES (pH 7.4), 5 mM MgCl₂, 70 mM potassium gluconate, 150 mM sorbitol, and 1% SDS. Homogenates were centrifuged 10 min at 10,000 × g, and the supernatants were combined with equal volumes of SDS sample buffer (Laemmli, 1970) and boiled for 5 min. Supernatant protein levels were determined by BioRad DC assay, and 20 µg of tentacle protein was loaded in each gel lane. For each blot, 50 ng of standard HSP70 protein (human, StressGen) was included. Discontinuous SDS gels (1 mm) were 6.2% for the stacking gel and 12% for the resolving gel. After running for 2 h at 150 V, SDS gels were electroblotted onto PDVF membranes (for 1 h at 100 V). The protein bands in each western blot were visualized by staining with Ponceau S. HSP70 protein was detected with mouse monoclonal anti-HSP70 (SPA-822, StressGen, Victoria, BC); the secondary antibody was goat-anti-mouse IgG, conjugated to peroxidase (Sigma), and was detected with ECL reagents (Amersham) and exposure of blots to X-ray film.

Blot band intensities were compared by scanning the X-ray films and analyzing the scans with the NIH Image software package. For each blot, the scanned intensity of the HSP was normalized against the intensities of the HSP70 protein standard from that blot; that is, the NIH Image datum point was divided by the intensity of the HSP70 standard.

**Results**

*Anthopleura elegantissima* and *Corynactis californica* express a single HSC70 or HSP70 protein (Fig. 1). In other eukaryotes, the HSP70-DnaK protein family comprises multiple proteins, more than one of which may be detected by the antibody. For the sake of convenience, we will collectively term these as “HSP70.” The inclusion of protease inhibitors did not affect HSP70 levels (Fig. 1A,
Anthopleura 1 and 2, a versus b; therefore they were omitted from our studies during the homogenization steps. The 30-min ambient seawater submersion of subtidal tentacle samples prior to freezing had no effect compared with immediate freezing (Fig. 1A, Anthopleura 1 and 2, c versus a and b). In comparing tentacles of the same polyp 24 h after the first forced interaction between the two cnidarian species in the laboratory, no differences were observed (F(3, 8) = 2.0, P < 0.1929) (Fig. 2). Two days later, HSP70 levels in Anthopleura elegansissima tentacle were 4 times greater than before (4.0 ± 0.5 ng HSP70/µg P in the tentacles; 0.0 ± 0.1 ng HSP70/µg P in the body, power of test = 0.87), but no differences were detected in Corynactis californica tentacles (1.7 ± 0.9 ng HSP70/µg P in the tentacles; 0.8 ± 0.9 ng HSP70/µg P in the body) (Fig. 2). Differences between tentacles and body were found in Anthopleura elegansissima but not in Corynactis californica (Fig. 1; F(3, 8) = 18.55, P < 0.0006, power of test = 0.98). Algal symbionts are at the highest concentration in Anthopleura elegansissima oral disk (Fitt et al., 1982; Weis and Levine, 1996); these data imply that we are measuring HSP70 responses in animal tissue. No such differences were found in the corallimorpharian, which lacks algal symbionts.

HSP70 levels in isolated polyps were also examined under the same conditions (no contact with any other invertebrate). Anthopleura elegansissima tentacles had very low expression (0.2 ± 0.3 ng HSP70/µg P) compared with the previous contact experiments. Corynactis californica had high expression (2.1 ± 1.3 ng HSP70/µg P) even when there was no direct (contact) aggression present. Comparing this analysis with the anemone-corallimorpharian experiments, no differences were found between HSP70 expressions in Corynactis californica. There were differences in the HSP70 expression of polyps between the two cnidarians when they were compared together (F(1, 9) = 10.81, P < 0.0094).

The mean distance between competitors in field studies as determined from the photographs was 2.4 ± 0.9 mm (n = 17). This distance is clearly within the range that Anthopleura elegansissima tentacle crowns sway during seawater movements (Francis, 1973a). The results of intraspecific competition in selected patches of both cnidarians are shown in Figure 3. There were clear differences in Anthopleura elegansissima HSP70 expression between the outside warrior polyps and the inside ones (in contact, 2.4 ± 0.5 ng HSP70/µg P; no contact, 0.6 ± 0.7 ng HSP70/µg P; F(3, 20) = 3.93, P < 0.0234, power of test = 0.82) when two clones of the same species interacted. Interestingly, Corynactis californica had similar HSP70 amounts in polyps of different clones (outside 1.5 ± 1.1 ng HSP70/µg P; inside 1.8 ± 1.3 ng HSP70/µg P).

The regular cnidarian HSP70 expression in both outside and inside polyps of the clone in different competition-for-space situations is illustrated in Figure 4. Anthopleura elegansissima
had more HSP70 in the warriors than in the inside clone polyps in general, depending on the competing species (Fig. 4). In Figure 5A, B we show HSP70 levels when both cnidarians interacted with the same competitors in the field: crustose red (Lithothamnium sp.) and fleshy green (Ulva sp.) algae. Contact with *A. xanthogrammica* and *Corynactis californica* tentacles from inside not interacting (i) and outside interacting (o) analyzed with competitors in the field. *C. californica* competitors were Ulva sp. and *H. permollis*. *A. elegantissima* competitors were *A. xanthogrammica* and Ulva sp.

Figure 4. Western blot of HSP70 levels in *Anthopleura elegantissima* and *Corynactis californica* tentacles from inside not interacting (i) and outside interacting (o) analyzed with competitors in the field. *C. californica* competitors were Ulva sp. and *H. permollis*. *A. elegantissima* competitors were *A. xanthogrammica* and Ulva sp.

Figure 5. Interspecific competition I. HSP70 expression between tentacles of the inside and outside polyps in *Anthopleura elegantissima* and *Corynactis californica* in contact with calcareous red (Lithothamnium sp.) (A) and fleshy green (Ulva sp.) (B) algae. The bars are +1 standard deviation of 4–6 clones. Asterisks indicate significant differences between groups (*P* ≤ 0.05); ns indicates a lack of significant differences between groups.

Figure 6. Interspecific competition II. HSP70 expression between tentacles of the inside and outside polyps in *Anthopleura elegantissima* and *Corynactis californica* with different competitors. (A) *A. elegantissima* against *A. xanthogrammica* and Balanus; (B) *C. californica* against *Haliclona permollis* and *Synoicum parfustis*. The bars are +1 standard deviation of 3–5 clones. Asterisks indicate significant differences between groups (*P* ≤ 0.05); ns indicates a lack of significant differences between groups.

0.8 ng HSP70/μg P). *C. californica* HSP70 expression was always the same in the outside and inside polyps (1–1.8 ng HSP70/μg P) in encounters with either *A. elegantissima*, other *C. californica* clones, or either algal species.

For *A. elegantissima*, two intertidal competitors were tested in submersed conditions: *A. xanthogrammica* and Balanus amphitrite (Fig. 6A). Encounters with *A. xanthogrammica* resulted in higher HSP70 in *A. elegantissima* outside polyps (0.6 ± 0.2 ng HSP70/μg P; inside ones 0.1 ± 0.1 ng HSP70/μg P, *F*(3, 12) = 2.88, *P* < 0.048, power of test = 0.99). However, HSP70 levels were low compared with other situations (interactions with calcareous algae or other *A. elegantissima* clones). No differences in HSP70 level were found with the *B. amphitrite* interactions (outside 0.5 ± 0.6 ng HSP70/μg P; inside 0.4 ± 0.4 ng HSP70/μg P).

Differences in *C. californica* HSP70 levels occurred when potential encounters and fights for space were against the sponge *Haliclona permollis* or the ascidian *Synoicum parfustis* (Fig. 6B). HSP70 expression was the same in the outside and inside polyps, but was slightly higher than with other competitors. Both sponge and ascidian appear to activate higher HSP70 expression (*H. permollis* outside 3.1 ± 0.5 ng HSP70/μg P; inside 2.5 ± 0.5 ng HSP70/μg P; *S. parfustis* outside 2.4 ± 1.0 ng HSP70/μg P; inside 1.8 ± 0.6 ng HSP70/μg P). Again, no significant differences were found between inside and outside polyps. When comparing the response of this cnidarian against the sponge and the ascidian with all the other encounters, significant HSP70 differences were found (*F*(5, 79) = 18.58, *P* < 0.00001). HSP70 expression in the sponge and ascidian...
encounters was 2.2 ± 0.7 ng HSP70/μg P, and in all the other encounters (A. elegantissima and C. californica, calcareous and fleshy algae) the HSP70 level was 1.3 ± 0.6 ng HSP70/μg P.

Discussion

Anthopleura elegantissima and Corynactis californica express HSP70 without physical stress (e.g., from temperature, desiccation, changes in pH) or pollution stress (e.g., due to heavy metals, organochlorines). There are few examples of cnidian HSP expression patterns, and all are directly (Bosch et al., 1988; Bosch and Praetzel, 1991; Sharp et al., 1994) or indirectly (Hayes and King, 1995; Sharp et al., 1997; coral bleaching) related to temperature stress. This is the first set of observations relating aquatic invertebrate HSP levels to biological stress and relating cnidian HSP expression to parameters other than temperature.

There were significant differences in HSP70 levels between the two cnidarians, and these depended on the particular competing species. Perhaps the aggressive behavior of C. californica (Chadwick, 1987, 1991; Chadwick and Adams, 1991) causes cellular damage, thereby increasing HSP70 expression levels in A. elegantissima tentacles (Fig. 2) in the first aquarium experiments. C. californica extrudes mesentarial filaments upon contact with nonfood species, suggesting that this behavior is used in interspecies aggressive encounters (Chadwick, 1987; Chadwick and Adams, 1991). Prolonged contact with C. californica mesentarial filaments kills the competitor. In this forced situation, no stresses other than contact between polyps appear to affect the tentacles of both cnidarians. In comparison with isolated (non-interacting) A. elegantissima polyps (Fig. 2), the expression of HSP70 is nearly 20 times greater after 48 h of interspecific interactions. The differences shown between tentacle crown and whole body in A. elegantissima were not found in C. californica.

The more striking result is the lack of differences between the solitary and interacting C. californica polyps in the aquarium experiences (in Fig. 2, compare 24 and 48 h). The expression of HSP70 is high and very constant in the three interspecific encounters (1.3–2.1 ng HSP70/μg P). One explanation could be that the aggressive behavior of some corallimorpharians requires cellular protection to counter the effect of the competing species’ response (Chadwick, 1987; Langmead and Chadwick, 1999a, b). After a period of contact with C. californica, A. elegantissima moved away via pedal locomotion, suggesting that the specialized aggressive structures of the anemone were ineffective against the corallimorpharian (Francis, 1973a, b; Chadwick, 1987).

Strong intraspecific competition has been clearly demonstrated between clones of A. elegantissima (Francis, 1973a, b; Ayre and Grossberg, 1995, 1996). Contact between genetically different individuals of this species initiates elaborate behaviors involving aerorhagial contact (leaving patches of tissue containing high numbers of nematocysts) and results in damage to one or both competitors. In addition, anemones of the genus Anthopleura, including A. xanthogrammica (discussed below), produce cytolytic and sodium-channel toxins that presumably damage cellular constituents such as proteins following contact (Bernheimer and Lai, 1985; Cline and Wolowyk, 1997; Kelso and Blumenthal, 1998). These toxic mechanisms could explain the high HSP70 levels found in the examined clones (Fig. 3). The outside warrior polyps bordering neighboring clones have more HSP70 than the inside ones. Sessile organisms discontinuously fight for space, depending on growth and reproductive cycles, the age of competitors, or the nature of the enemies (Connell, 1961; Jackson, 1977; Chadwick, 1991). Perhaps when warrior polyps encounter a “known” competitor (i.e., in this case a different clone of the same species), they become “prepared for war,” producing HSP70 levels high enough to avoid serious cellular damage when real interactions begin. Alternatively, some interactions have already caused some tissue damage, resulting in higher HSP70.

No differences in HSP70 expression were expected in interactions between A. elegantissima and a fleshy green alga (Ulva sp., Fig. 5B). This algal type escapes from direct competition for space by growing as rapidly as nutrients and light levels permit (Lewis, 1964; Paine, 1971). No direct interactions were evident, and the low HSP70 levels found in the outside interacting polyps of these clones seem to confirm their absence, although algae in this genus are capable of producing harmful secondary compounds (Paine, 1990; Whitfield et al., 1999). In the case of Lithothamnium sp. (Fig. 5A), it is known that coralline algae grow slowly (Steneck, 1986; Garrabou and Ballesteros, 2000) and can synthesize allelochemicals (as do some other red algae) to compete for space (Whitfield et al., 1999). Perhaps the anemone better detects or is more affected by these Lithothamnium chemicals than by those produced by Ulva.

A. xanthogrammica is a common intertidal competitor with A. elegantissima for space (Francis, 1973b; Sebens, 1984). This solitary anemone elicits aggression in A. elegantissima (Francis, 1973b) but does not display the same mechanisms of defense. Observations made by Sebens (1984) support the idea that aggression is common between these two species, which explains the higher levels of HSP70 in the outside A. elegantissima polyps in these interactions (Fig. 6A). Balanus amphitrite, another common space competitor, seems to have no effect on HSP70 expression (Fig. 6A). It is possible that the lack of effect was due to exposure to small individual cirripeds, and it would be interesting to examine A. elegantissima clones that are in competition for space with larger clumps of barnacles.
In C. californica, HSP70 levels are similar in outside and inside clone polyps. Therefore the corallimorpharian does not distinguish between the exposed (outside polyps) and nonexposed (inside polyps) areas of the clone. More importantly, even without apparent interactions (Fig. 2), C. californica expresses HSP70 at constant levels (1–2 ng HSP70/μg P). In this species, intraspecific competition results in HSP70 levels that are within the “normal” range (Fig. 3), and there is no aggressive behavior in intraspecific contacts (Chadwick, 1987). Perhaps the key to interpreting HSP70 expression as a mechanism of competence in C. californica is the finding that the highest HSP70 levels were found in polyps interacting with Haliclona or Synoicium (Fig. 6B). Also of importance is that these differences between interacting and non-interacting polyps are significant. It is known that sponges and ascidians use chemical substances to defend themselves or attack potential foes (Fig. 6B). Also of importance is that these differences found when the encounter involves ascidians or sponges may reflect the aggressive toxic substances used by these enemies (Uriz et al., 1991).

C. californica appears to be always “prepared for war” by its aggressive behavior (Chadwick, 1991). Another organism that exhibits this strategic use of stress proteins (by maintaining a basal level of HSP expression) is the desert-dwelling ant Cataglyphis. This ant presynthesizes HSPs at relatively low nest temperatures to limit damage from heat shock on the desert floor. Coupled with continued HSP production at higher temperatures, this protects the ant from the high temperatures it experiences when foraging in daytime (Gehring and Wehner, 1995). Perhaps the presynthesis of HSP70 in C. californica provides protection from neighbors that intermittently excrete harmful substances. Alternatively, the constant HSP70 levels might protect the corallimorpharian against its own aggressive substances, which it uses to catch prey and to fight for space (Chadwick, 1987). The aggressive behavior of C. californica includes the extrusion of mesenteric filaments containing gland cells that secrete strong proteolytic enzymes and nematocysts that may inject cytolytic toxins into prey or enemies (Van-Praet, 1985).

Because of the high cost of the HSP expression and its occasional harmful effect if constantly highly expressed (Feder et al., 1992; Krebs and Feder, 1997), we suggest that expression varies depending on the kind of neighboring competitor or enemy. Furthermore, A. elegantissima also expresses high levels of HSP70 in response to physical factors, especially temperature (Rossi and Snyder, unpubl. obs.). The anemone has to “share” HSP70 expression between biological (e.g., competition for space) and physical (e.g., temperature) factors.

It is also possible that other stress proteins contribute to the responses against biological phenomena such as competitive interactions for space in the benthic environment. For example, unexpected low-molecular-weight HSP70 homologs have been found in other cnidarians (Sharp et al., 1994). HSP60 has known roles in thermal acclimation of the cnidarians Hydra vulgaris and Acropora grandis (Bosch et al., 1988; Fang et al., 1997). The use of SPA-822 HSP70 antiserum can possibly underestimate the number of HSP70 isoforms, and consequently may explain the finding of single HSP70 proteins by our methods. However, we have successfully used the same antiserum and measured two and three to four different HSP70 isoforms in larval lobsters, (Homarus americanus), and juvenile abalone (Haliotis rufescens) and adult mussels (Mytilus galloprovincialis) respectively (Snyder and Mulder, 2001; Snyder et al., 2001). Many questions remain unanswered, such as the identity of the harmful substances or aggressive behaviors that activate HSP70 expression in competitive interactions among sessile marine invertebrates. Among the likely candidates for cellular damaging allelochemicals are cnidarian sodium-channel toxins (Kelso and Blumenthal, 1998), cytotoxic and cytolytic factors (Bernheimer and Lai, 1985; Cline and Wohowik, 1997), and an array of toxic alkaloids found in cnidarians and sponges (e.g., Dura and Faulkner, 1980; Koh and Sweatman, 2000). Such chemicals can diffuse and act at some distance from the source or can be deposited on neighboring organisms by direct contact (e.g., Schmitt et al., 1995; Slattery et al., 1997). Further studies of HSP proteins may provide important information about the consequent distribution and hierarchy of species in the rocky benthos.

With this work we propose HSP70 expression as a tool for evaluating space competition among sessile marine invertebrates, without manipulative experiments. From our results, it is clear that the expression of the stress proteins depends on both the particular competing species and the interacting life stages of each competitor. The energy required to repair tissue damage cannot be used for other processes such as reproduction and growth. It will be interesting to measure how the amount of energy an organism devotes to growth and reproduction varies with the level of HSP produced during prolonged competition for space.

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