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The Symbiotic Anthozoan: A Physiological Chimera between Alga and Animal[1]

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SYNOPSIS. The symbiotic life style involves mutual ecological, physiological, structural, and molecular adaptations between the partners. In the symbiotic association between anthozoans and photosynthetic dinoflagellates (*Symbiodinium* spp., also called zooxanthellae), the presence of the endosymbiont in the animal cells has constrained the host in several ways. It adopts behaviors that optimize photosynthesis of the zooxanthellae. The animal partner has had to evolve the ability to absorb and concentrate dissolved inorganic carbon from seawater in order to supply the symbiont's photosynthesis. Exposing itself to sunlight to illuminate its symbionts sufficiently also subjects the host to damaging solar ultraviolet radiation. Protection against this is provided by biochemical sunscreens, including mycosporine-like amino acids, themselves produced by the symbiont and translocated to the host. Moreover, to protect itself against oxygen produced during algal photosynthesis, the cnidarian host has developed certain antioxidant defenses that are unique among animals. Finally, living in nutrient-poor waters, the animal partner has developed several mechanisms for nitrogen assimilation and conservation such as the ability to absorb inorganic nitrogen, highly unusual for a metazoan. These facts suggest a parallel evolution of symbiotic cnidarians and plants, in which the animal host has adopted characteristics usually associated with phototrophic organisms.

INTRODUCTION

When Wotton in 1552 gave to anthozoan corals and gorgonians, and hydrozoans the name Zoophyte (plant–animal; Daudain, 1926), it was because of their morphological similarity to higher plants. The discovery at the end of the 19th century by Brandt (see Perru, 2003, for a review) of photosynthetic algae (also called zooxanthellae) inside the tissues of these animals confirmed their vegetal nature, although the term zoophyte was abandoned. These algae, most abundant in the highly expandable tentacles and oral disc of the host cnidarian, are still commonly called zooxanthellae, and now this term applies specifically to photosynthetic dinoflagellate Protista (genus *Symbiodinium*) that fix carbon dioxide and produce oxygen *in hospite* (inside the host) (Trench, 1987). In this way, the symbiotic association between cnidarians and their symbiotic dinoflagellates behaves like a plant. Although a large literature documents the mutual benefits of these interactions (Trench, 1987; Goodson *et al.*, 2001), less is known of the restrictions borne by the host for living in symbiosis.

As in plants, the symbiotic life of cnidarians involves major constraints. First, the photosynthetic requirements of zooxanthellae limit symbiotic cnidarians to the euphotic zone of the ocean, and anthozoans in particular adjust their tentacle expansion to irradiance and water movement to capture photons as well as zooplankton and other particulate prey. Second, owing to the intracellular location of the endosymbionts, the host also has to supply the zooxanthellae with CO_2 for their photosynthesis and with inorganic nitrogen and phosphate for biosynthesis. Zooxanthellae represent the main site of assimilation of inorganic nitrogen, phosphate, and carbon transforming it into organic compounds. Third, O_2 produced in photosynthesis by the symbiont leads to hyperoxia in the host's tissues, so the host must have efficient mechanisms of defense against it. Moreover, the location of these symbioses in very shallow marine environments requires defenses against damaging solar radiation, which may exacerbate the effects of hyperoxia. The purpose of the present paper is to review the chimeric nature of this mutualistic association and how the animal partner has adapted to this unusual situation by evolving characteristics usually associated with phototrophic organisms.

THE ANTHOZOAN/DINOFLAGELLATE ASSOCIATION

Cnidarian/dinoflagellate symbioses are widespread in the marine environment and their important ecological role is widely documented (Dubinsky, 1990). The interactions, especially, between anthozoan cnidarians (*i.e.*, corals, sea anemones, zoanthids, and gorgonians) and dinoflagellates of the genus *Symbiodinium* are above all responsible for the formation of coral reefs, which contain a quarter of global marine biodiversity (Reaka-Kudla *et al.*, 1997).

In anthozoans, this symbiosis is a mutualistic intracellular association with advantages for the two partners centering on the photosynthetic activity of the dinoflagellate endosymbiont. The anthozoans are diploblastic animals developing from two epithelial germ cell layers: the ectoderm, facing the seawater, and the endoderm, facing the gastrovascular cavity or coelen-

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teron. The symbionts typically are located in the endodermal cells of the host, and a perisymbiotic membrane of animal origin separates them from the animal cytoplasm. The most important benefits shared by the association are nutritional, for despite their location, the dinoflagellates maintain photosynthetic capacity and transfer to the host most of the organic carbon produced by the photosynthesis, contributing substantially to the host's carbon and energy needs (Muscatine, 1990). Conversely, the host contributes to the endosymbiont's metabolism, translocating essential nutrients (such as nitrogen, phosphorus, and sulfur compounds) produced by its catabolism of macromolecules derived from particulate food or absorbed from the medium (Cook and D'Elia, 1987).

LIVING IN THE LIGHT: PROTECTION AGAINST DAMAGING SOLAR RADIATION

To reap the benefits of associating with photosynthetic partners, cnidarian hosts must expose themselves to sunlight, which includes not only photosynthetically available radiation (PAR, 400–700 nm) but also damaging ultraviolet radiation (UVR, 280–400 nm), conditions that non-symbiotic epifauna normally avoid (Jokiel, 1980). Some sea anemones attach reflective shell and gravel to their ectoderm to serve as a sunshade, and anemones and corals change the degree of expansion of their oral disk and tentacles in conjunction with changing irradiance and water movement, apparently optimizing the capture of both prey and PAR while reducing exposure to UVR (reviewed by Shick, 1991; Levy *et al.*, 2001). Intense PAR is also photochemically damaging to the coral host and the symbionts, and is reflected by the skeleton when the tissue is retracted (Brown *et al.*, 1994) or dissipated by fluorescent pigments in the host (Salih *et al.*, 2000) and xanthophyll cycling in the zooxanthellae (Brown *et al.*, 1999).

Zooxanthellate reef corals were among the first organisms reported to contain mycosporine-like amino acids (MAAs), initially detected as ''S-320'' compounds absorbing UVR at about 320 nm (Shibata, 1969). These molecules were subsequently identified in corals as a suite of structurally similar compounds in which a cyclohexenone or cyclohexenimine core is substituted by various amino acids and amino alcohols (Dunlap and Chalker, 1986). MAAs (and the related fungal mycosporines) are also natural products formed in diverse micro- and macroalgae and fungi (reviewed by Bandaranayake, 1998; Dunlap and Shick, 1998; Karentz, 2001; Shick and Dunlap, 2002; Banaszak, 2003). Largely transparent to PAR, MAAs efficiently absorb UVR and dissipate UV energy as heat without forming potentially toxic, reactive intermediates such as free radicals (Conde *et al.*, 2000; Shick *et al.*, 2000). As such they are well suited as intracellular sunscreens, particularly in oxygenic phototrophic symbioses.

MAAs are synthesized via a branch of the shikimic acid pathway (Favre-Bonvin *et al.*, 1987; Shick *et al.*, 1999), which is apparently lacking in metazoans (Herrmann and Weaver, 1999). Therefore, the MAAs found in many anthozoan/dinoflagellate symbioses are widely assumed to originate in the endosymbionts (Shick and Dunlap, 2002), even though they actually may be more concentrated in the tissues of the anthozoan host than in the zooxanthellae (Shick *et al.*, 1995; J. M. Shick, D. Allemand, and C. Ferrier-Pàges, unpublished results). It is unknown whether this is simply a consequence of a wholesale translocation of algal photosynthate to the host (Muscatine, 1990) or whether MAAs are preferentially transferred to and sequestered in the host tissues, as occurs in the accumulation of dietary MAAs by heterotrophic consumers (Shick and Dunlap, 2002), including aposymbiotic sea anemones (*i.e.*, those that temporarily lack the normal population of algal symbionts) (Shick *et al.*, 2002). The localization of MAAs in host tissue, particularly in the ectoderm, protects both the zooxanthellae (*e.g.*, maintains photosynthesis under high UV irradiance: Shick, 1993; Shick *et al.*, 1995) and the host by virtue of the longer optical path over which UVR is attenuated by MAAs, whereas a higher molar concentration would be required within the smaller zooxanthellae to protect them alone (Garcia-Pichel, 1994).

Genotypically varied zooxanthellae maintained in culture consistently have a less diverse complement of MAAs than do the symbioses from which they were isolated (reviewed by Shick and Dunlap, 2002). Moreover, in some cultured strains, zooxanthellae did not produce MAAs, which suggests a cladal specificity of MAA production (Banaszak, 2003). Does living *in hospite* stimulate the zooxanthellae to produce novel MAAs, or does the host modify MAAs that are provided by the algae? For example, the larger array of MAAs in the zooxanthellate coral *Stylophora pistillata* appears to result from the host's secondary modification of a smaller suite of primary, ''*Symbiodinium*-MAAs'' (Shick, 2004) in a shared biosynthetic pathway. In this model, MAAs or other precursors from the algae are converted by the host to the rest of the MAAs characteristic of the symbiotic association. However, it is unknown whether the bacterial microcosm associated with the coral (*e.g.*, Rohwer *et al.*, 2002) participates in these bioconversions, nor is it known definitively whether zooxanthellae *in hospite* receive some signal or precursor from the host and thereby produce a wider array of MAAs than they do in culture. In *S. pistillata*, the four primary MAAs that collectively are the only MAAs reported in cultures of *Symbiodinium* spp. of diverse origin (Banaszak *et al.*, 2000; Shick, 2004) are the first to be synthesized in response to acute exposure of this coral to UVR (Fig. 1A), after which six secondary MAAs accumulate in the longer term, with stoichiometric decreases in the primary (precursor) MAAs (Fig. 1B). That the host provides the first line of defense against UVR by using products of the alga's metabolism, perhaps modifying some of them in its own tissues, seems emblematic of a mutualistic phototrophic symbiosis. The details of

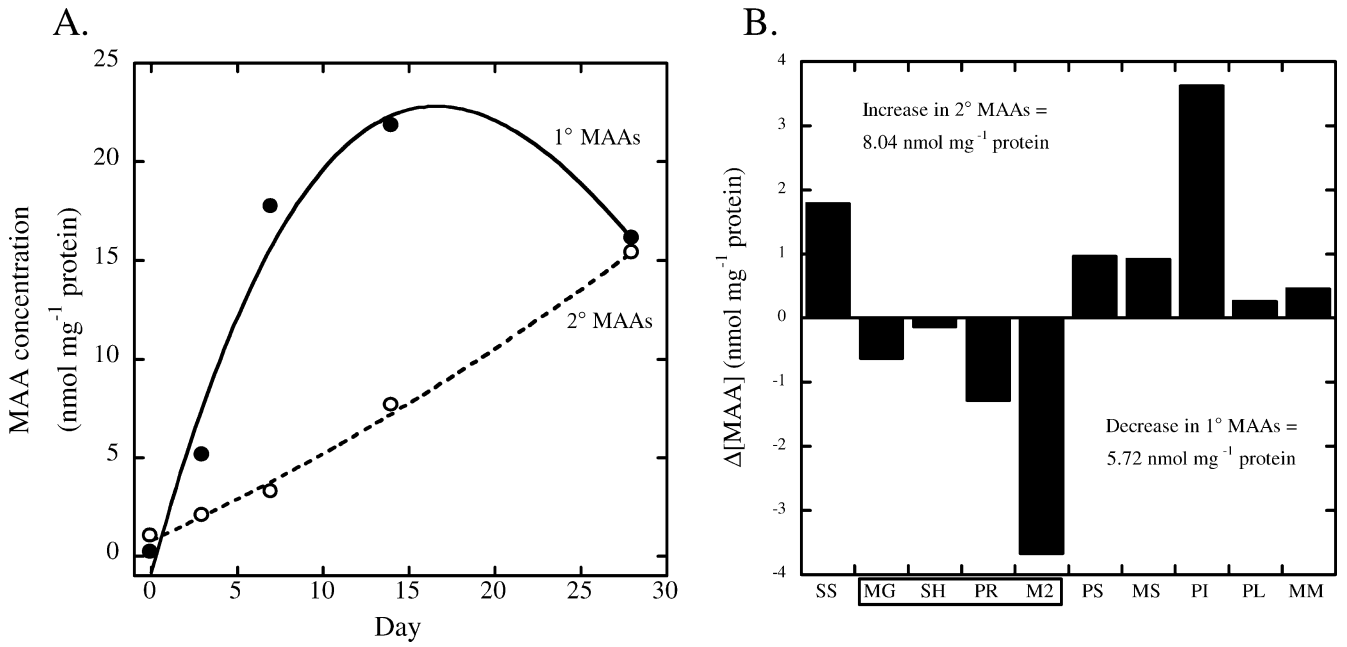

FIG. 1. A. Primary and secondary MAAs show different kinetics of accumulation in colonies of *Stylophora pistillata* during 28 days of acute exposure to UVR. Total MAA concentration is stable by day 14 and there is no net change for the next 14 days. B. Changes in concentrations of individual MAAs occur during days 14–28 of exposure of *S. pistillata* to UVR, when total MAA concentration does not change significantly. The primary, *Symbiodinium*-MAAs are mycosporine-glycine (MG), shinorine (SH), porphyra-334 (PR), and mycosporine-2 glycine (M2); the secondary MAAs are palythine-serine sulfate (SS), palythine-serine (PS), mycosporine-NMA:serine (MS), palythine (PI), palythinol (PL), and mycosporine-NMA:threonine (MM). Net decreases in primary MAAs are stoichiometrically balanced by increases in secondary MAAs, suggesting a precursor-product relationship. Reproduced from Shick (2004) with permission of *Limnology & Oceanography*.

the primary biosynthesis of MAAs, their translocation to the host, and their secondary modification remain to be elucidated.

SOURCE AND SUPPLY OF CO_2 FOR SYMBIONT PHOTOSYNTHESIS

Although the total concentration of dissolved inorganic carbon (DIC) in seawater is about 2.4 mM, dissolved CO_2 represents only about 0.5% of this (*i.e.*, 12 μM), far too low to allow optimal carbon fixation by Rubisco (Ribulose-1,5-biphosphate carboxylase-oxygenase). Consequently, numerous marine phototrophs, including macro- and microalgae, have developed carbon-concentrating mechanisms (CCM) (Raven, 1990). Dinoflagellates contain form II Rubisco (Rowan *et al.*, 1996), which discriminates poorly between CO_2 and O_2. Consequently, dinoflagellates require a higher concentration of CO_2 close to the pyrenoid where the Rubisco is located than do other microalgae (Leggat *et al.*, 2002). We do not know how the genetic diversity of zooxanthellae (Rowan, 1998) affects the concentration of CO_2 that they require, or their discrimination between CO_2 and O_2, nor do we know if there are differences among metazoan hosts in their CO_2-concentrating ability. Therefore, DIC (and DIN: see below) requirements and metabolism in genotypically diverse hosts and zooxanthellae may be another axis of variability (in addition to PAR requirements, UV resistance, and temperature tolerance: see reviews by Baker, 2003; Knowlton and Rohwer, 2003; Lesser, 2004) affecting the stability of the symbiotic relationship and should be taken into account in future investigations.

Because of the presence of the zooxanthellae in the metabolically active host cytoplasm, one putative source of inorganic carbon for the symbiotic dinoflagellates is the CO_2 produced by the host and symbiont. However, the *net* photosynthesis measured in most symbiotic cnidarians indicates the presence of an external source of inorganic carbon, which implies the transport of exogenous inorganic carbon through the animal tissue (Allemand *et al.*, 1998). Thus, although the metabolic activities of all animal cells involve elimination of inorganic carbon, the coral host must constantly absorb inorganic carbon to supply its symbionts with enough CO_2 for fixation by Rubisco to support the high level of net primary productivity.

The absorption of DIC from seawater therefore implies a transport across the ectodermal cell layer, toward the symbiotic dinoflagellates. Experiments using perfused tentacles, and pieces of tentacles inserted into Ussing chambers, showed that while a passive diffusional, paracellular pathway may exist across ectodermal cells, the major part of DIC (85%) enters via an active transcellular pathway (Allemand *et al.*, 1998). Therefore, the host cells must play a major active role in the uptake of DIC for symbiont photosynthesis.

Both CO_2 and HCO_3^- are potential sources of DIC. While the first is freely diffusible into lipid bilayer membranes, the second is a charged ion, unable to diffuse through this barrier and needing a carrier pro-

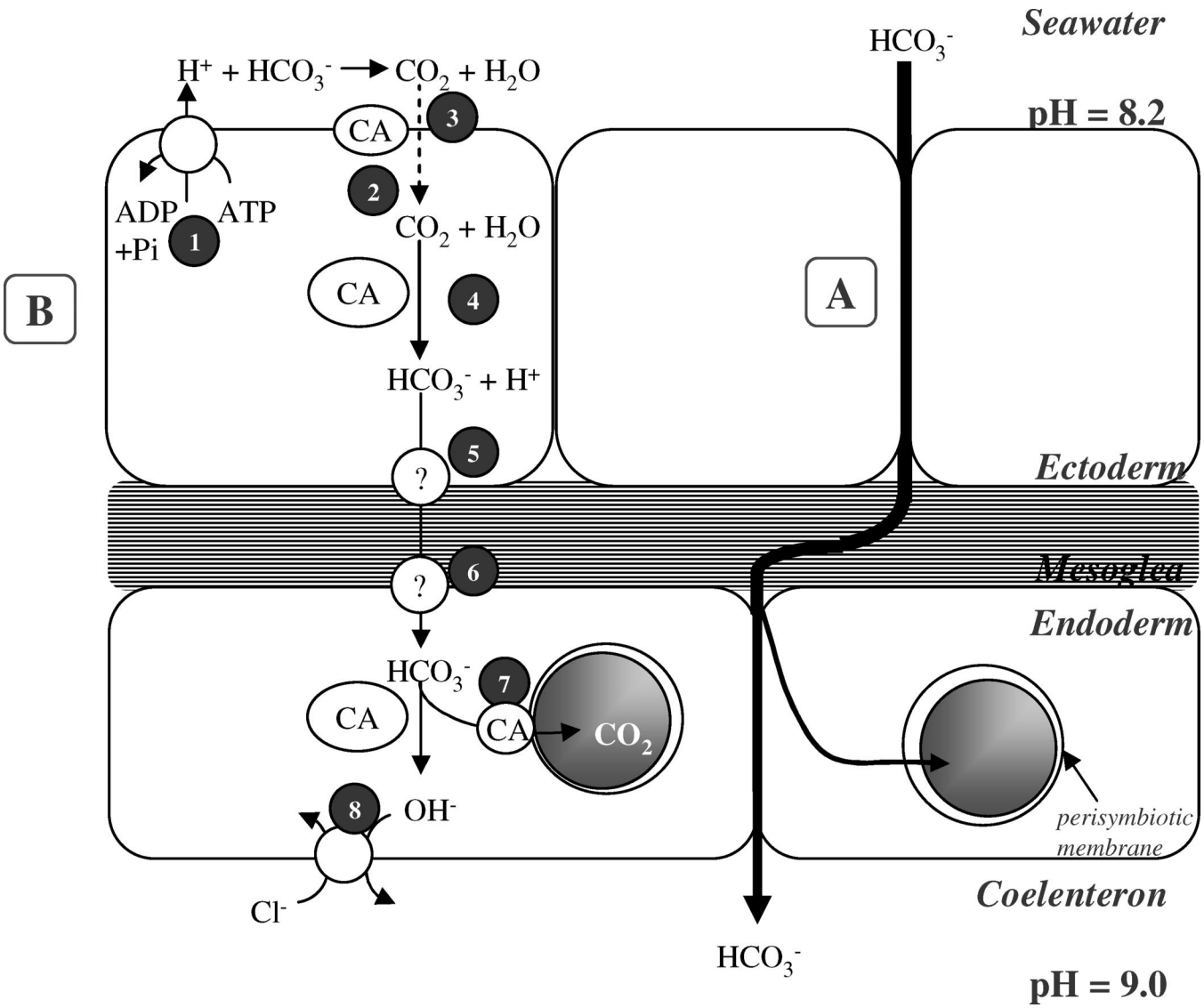

FIG. 2. Mechanism of inorganic carbon absorption in a symbiotic sea anemone. A: Paracellular pathway representing 15% of the DIC absorption. B: Transcellular pathway representing 85% of the DIC absorption. 1: Expulsion of protons into the external medium by a P-type proton ATPase. 2: Conversion of HCO_3^- to CO_2 favored by a carbonic anhydrase activity in the apical plasma membrane of the ectodermal cells. 3: Diffusion of CO_2 into the cytoplasm of the ectodermal cell. 4: Conversion of CO_2 to HCO_3^- in the cytoplasm of ectodermal cell accelerated by cytoplasmic carbonic anhydrase activity. 5, 6: Extrusion of bicarbonate and entry into the endodermal cell by an unknown mechanism. 7: Final conversion of HCO_3^- to CO_2 near (or in) the zooxanthella (Zoox). 8: Expulsion of hydroxyl ion into the coelenteron by a chloride-dependent mechanism, which increases coelenteron pH.

tein. To determine the cellular mechanisms involved in the inorganic carbon absorption by the ectodermal cell, membrane vesicles of the apical plasma membrane of ectodermal cells were produced (Furla *et al.*, 2000*a*). It was shown that the uptake of DIC depends on the secretion of H^+ by an H^+-ATPase (Fig. 2), leading to local acidification of the boundary layer, and resulting in the protonation of HCO_3^- to carbonic acid, following the reaction:

$$HCO_3^- + H^+ \rightarrow H_2CO_3$$

A membrane-bound carbonic anhydrase (CA) next dehydrates carbonic acid into CO_2:

$$H_2CO_3 \rightarrow CO_2 + H_2O$$

The uncharged CO_2 molecule then diffuses into the ectodermal cell following the concentration gradient created by the extrusion of H^+ in the external medium. Once in the animal cytoplasm, CO_2 is equilibrated with HCO_3^- according to the intracellular pH by another CA isoform, which prevents back-diffusion of CO_2. The presence of symbiosis-inducible CA isoforms in the host has been highlighted by Weis (1991) and by Weis and Reynolds (1999) in the sea anemones *Aiptasia pulchella* and *Anthopleura elegantissima*, underlining the pivotal role of this enzyme in the absorption

of inorganic carbon. In those models, the activity, and amounts of enzyme and transcript, were greatly enhanced in symbiotic animals compared with aposymbiotic animals, demonstrating that the presence of symbionts affects the expression of a host gene (Weis and Reynolds, 1999).

This system therefore acts like a plant CCM. Indeed, it has been observed that that the DIC pool in the tissues of the coral *Stylophora pistillata* increases 39-fold upon illumination (Furla *et al.*, 2000*b*), leading to a ratio DIC_{int}/DIC_{ext} of about 61, a value close to that reported for various micro- and macroalgae (Aizawa and Miyachi, 1986; Bowes and Salvucci, 1989).

Therefore, the anthozoan/dinoflagellate association possesses a CCM, including inducible host CA in proportion to the density of zooxanthellae. This allows the intracellular symbionts to fix inorganic carbon actively, paralleling the example of micro- and macroalgae. Although DIC is also essential for calcification in scleractinian corals (see Furla *et al.*, 2000*b*), no data are available on a putative role of CCM in the carbonate precipitation, and this is an important topic for future work.

PROTECTION AGAINST PHOTOSYNTHESIS-INDUCED HYPEROXIA

Symbiont photosynthesis leads not only to the fixation of CO_2 but also to the release of O_2. Before O_2 diffuses to the external seawater, it causes local hyperoxia (2–3 fold above atmospheric normoxia) in both symbiont and host (D'Aoust *et al.*, 1976; Dykens and Shick, 1982; Shashar *et al.*, 1993; Harland and Davies, 1995; Kühl *et al.*, 1995; Richier *et al.*, 2003). Although this situation is usual for algae and plants, the hyperoxic state is exceptional for metazoans, which possess physiological mechanisms poised to ensure an adequate supply of O_2 to their comparatively hypoxic cells.

Hyperoxia enhances the photodynamic generation of reactive oxygen species (ROS) (Valenzeno and Pooler, 1987), among them free radicals, which if unchecked produce oxidative stress involving oxidation of membrane lipids, DNA, or proteins, and thereafter cellular aging and death (Halliwell and Gutteridge, 1999). ROS have been implicated in the dysfunction of zooxanthellae *in hospite* under unusually high temperature and solar irradiance leading to ''coral bleaching'' (Lesser, 1997; Jones *et al.*, 1998), perhaps involving effects of ROS in the host's cells as well (Dykens *et al.*, 1992). Photosynthetic cnidarians and other organisms have evolved anti-oxidative metabolism, including enzymic mechanisms involving superoxide dismutases (SODs), catalases, and peroxidases, that detoxify ROS.

The superoxide anion (O_2^-) is the first free radical normally produced in aerobic cells. By removing superoxide, SODs constitute the first line of defense against oxidative stress. They work together with catalases and peroxidases, which eliminate the hydrogen peroxide (H_2O_2) produced in O_2^- dismutation. Different SOD isoforms exist within organisms, distributed in different cellular locations (Halliwell and Gutteridge, 1999):

- an intracellular copper-zinc-containing SOD (CuZnSOD), generally located in the cytoplasm and present in virtually all eukaryotic cells and in some prokaryotes;
- an extracellular copper-zinc-containing SOD (ECSOD);
- a manganese-containing SOD (MnSOD), present in prokaryotes and in mitochondria and peroxisomes of plants and animals;
- an iron-containing SOD (FeSOD), present in prokaryotes, in chloroplasts, and in peroxisomes.

Whereas phototrophs generally harbor a large number of isoforms, animals exhibit only two or three isoforms (Halliwell and Gutteridge, 1999). Dykens and Shick (1982) showed that symbiotic *Anthopleura elegantissima* exhibits high SOD activity. Such activity is proportional to the potential for photooxidative damage in this and taxonomically diverse symbioses (Dykens and Shick, 1984; Shick and Dykens, 1985; Dykens *et al.*, 1992; Lesser and Shick, 1989), because it decreases as depth increases (Shick and Dykens, 1985; Shick *et al.*, 1995). More recently, by using native gel electrophoresis, Richier *et al.* (2003) demonstrated the presence in the animal host of the temperate symbiotic sea anemone, *Anemonia viridis*, of at least seven different active isoforms. Some isoforms were specific to the animal tissues, others to the symbiont, and some were common to both partners. Although the hypothesis of cross-contamination between the two partners has been excluded (Richier *et al.*, 2003), the origin of these similarities is still unknown. Moreover, a detailed analysis of the distribution of these isoforms within the different host compartments suggests a specific role for each:

- a CuZnSOD specific to the animal host and located in both endoderm and ectoderm;
- a MnSOD, located in the mitochondria of the symbiont and the host cells, where it is present in both endoderm and ectoderm (MnSODI);
- three MnSODs present in the symbiont and within the endodermal cell layer of the host (MnSODII, III and IV); these isoforms are highly unusual since they are non-mitochondrial (eukaryotic MnSOD is typically located within mitochondria) and dimeric (mitochondrial SODs are usually tetrameric);
- two FeSODs present in the symbiont and within the endodermal cell layer of the host (FeSODI and II).

In addition to this already unusally high number of SOD isoforms, Plantivaux *et al.* (2004) found, by a molecular approach, an extracellular SOD within the animal host that is expressed in both epithelial cell layers.

We extended these observations to other symbiotic cnidarians; all displayed a similarly high number of SOD isoforms (Fig. 3). Within the Actiniidae, whereas

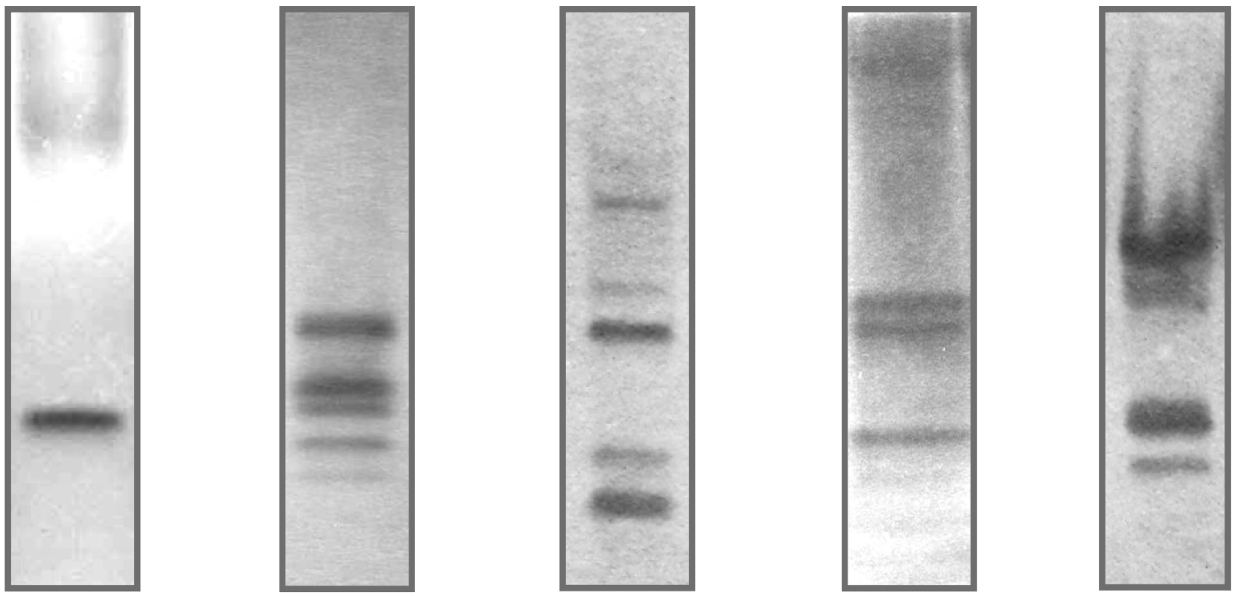

FIG. 3. Superoxide dismutase diversity in symbiotic and non-symbiotic Cnidaria. SOD activities of the whole symbiotic association are revealed by native PAGE 8% and NBT staining. Each lane was loaded with 150 μg of protein. The analyzed Cnidaria were a non-symbiotic sea anemone (*Actinia schmidti*), two symbiotic sea anemones (*Anemonia viridis* and *Entacmaea quadricolor*), a symbiotic scleractinian coral (*Stylophora pistillata*), and a symbiotic soft coral (*Plerogyra sinuosa*).

the temperate symbiotic sea anemone *Anemonia viridis* expresses at least eight SOD isoforms, the temperate non-symbiotic species *Actinia schmidti* expresses only three isoforms, as in most animals. We suggest that this increase of SOD isoforms in symbiotic cnidarians follows from selection pressure driven by photosynthesis-induced hyperoxia and ROS production, as occurs in plants.

UPTAKE OF INORGANIC NITROGEN

Most symbiotic anthozoans live in nutrient-poor tropical waters. The sources of inorganic nitrogen in these reef waters are sediments, nitrogen fixation by cyanobacteria, runoff near the continent, and upwelling. However, the amounts of inorganic nitrogen are usually very low in such oligotrophic environments (Szmant, 1997). The acquisition and retention of essential nutrients therefore presents a formidable problem (Muscatine and Porter, 1977) and symbiotic cnidarians have developed adaptations for assimilating and conserving nitrogen. Animals in general, and non-symbiotic cnidarians in particular, are not able to take up external inorganic nitrogen (Muscatine *et al.*, 1979) and generally excrete ammonium and urea into the surrounding water (Rahav *et al.*, 1989). In contrast, symbiotic cnidarians, owing to the presence of algae in their tissue, are able to take up, retain, and incorporate inorganic nitrogen from seawater at micromolar or even nanomolar concentrations (Muscatine and D'Elia, 1978; Grover *et al.*, 2002).

In symbiotic cnidarians, nitrogen is assimilated and conserved in several ways. First, animals acquire nitrogen by eating and subsequently digesting zooplankton (Erez, 1990). Ammonium resulting from animal metabolism and digestion of prey by the host is not excreted into the surrounding water but is immediately re-assimilated by the algae and recycled (Muscatine *et al.*, 1989). This minimizes the loss of nitrogen via excretion. Second, symbiotic cnidarians are able to absorb diverse forms of inorganic nitrogen such as ammonium and nitrate.

In the zooxanthellae, the main route of ammonium assimilation is into glutamate, via the NADPH-linked glutamate dehydrogenase (GDH) enzyme (Yellowlees *et al.*, 1994). The role of the animal tissue in the direct acquisition of ammonium is not yet well described and understood. Some studies have suggested a depletion-diffusion model for ammonium uptake (D'Elia *et al.*, 1983), in which zooxanthellae deplete the animal tissue of NH_4^+, creating a concentration gradient through which additional nutrient diffuses into the animal tissue from seawater. Other workers (Rees, 1987) using metabolic inhibitors hypothesized that the host, and not the algae, was responsible for active uptake of NH_4^+. Indeed, both zooxanthellae and also all animal cells contain the GDH enzyme (Wilkerson and Muscatine, 1984; Yellowlees *et al.*, 1994), which promotes glutamate synthesis from ammonium. Furthermore, ammonium could be assimilated to produce glutamine by host cell glutamine synthase (GS), which has been identified in several species (Yellowlees *et al.*, 1994). Finally, additional evidence of animal assimilation of ammonium comes from the work of Lipschultz and Cook (2002), who studied the incorporation of $^{15}NH_4^+$

in two species of sea anemones and demonstrated a simultaneous appearance of ^{15}N in host and zooxanthellae. Uptake of ^{15}N also occurred in aposymbiotic anemones, suggesting a persistent adaptation of these animals to symbiosis.

Like ammonium, nitrate is generally not assimilated in animals but can be converted to ammonium in algae through the combined action of nitrate and nitrite reductases (Miller and Yellowlees, 1989). However, some symbiotic corals have also developed mechanisms to take up nitrate (Wilkerson and Trench, 1986; Marubini and Davies, 1996; Ferrier-Pagès *et al.*, 2001), even at very low concentrations (Bythell, 1990; Grover *et al.*, 2003). As for phytoplankton, the uptake of nitrate should be via a carrier transport system located in the host tissue. Such system represents an additional adaptive trait, but it has not yet been investigated in corals.

The acquisition of both ammonium and nitrate in symbiotic corals is a light-dependent process (Grover *et al.*, 2002, 2003). The light-stimulated uptake of ammonium, for instance, indicates the involvement of the enzyme glutamate synthase, which, in many other photosynthetic systems, is driven by photochemically derived reduced ferredoxin (Lea and Mifflin, 1979). Zooxanthellae are therefore thought to be the main site of assimilation of this inorganic nitrogen (Grover *et al.*, 2002, 2003), transforming it into organic compounds and translocating at least a portion of these compounds to the host (Swanson and Hoegh-Guldberg, 1998; Wang and Douglas, 1999). The animal host can thus be considered to have gained access to a complex metabolic capacity generally absent from the animal kingdom

CONCLUSION

Symbiosis with unicellular phototrophs has existed in anthozoans at least since the Triassic (225 million years ago) (Rosen, 2000) and likely constitutes a source of selective pressures that have deeply modified the host's physiology. The host shows convergent mechanisms with algae and plants, inventing or acquiring new metabolic capacities such as efficient transport and concentration of inorganic carbon, antioxidant abilities, accumulation of molecular sunscreens, and uptake of inorganic nitrogen. It is important to note that these traits are not peculiar to symbiotic anthozoans but have also been identified in other phototrophic endosymbioses as mollusc/dinoflagellate (Leggat *et al.*, 2002), sponge/cyanobacteria (Regoli *et al.*, 2000), and annelid/thiotrophic bacteria (Goffredi *et al.*, 1997). Moreover, these traits are unique to the symbiotic animal partner and none of the novel characteristics described are usually present in a non-symbiotic metazoan. Richier *et al.* (2005) have demonstrated that the diversity (in number and classes) of antioxidant superoxide dismutases is lower in non-symbiotic and aposymbiotic sea anemones. Muscatine *et al.* (1979) demonstrated that uptake of DIN is not measurable in aposymbiotic sea anemones. Finally, the animal hosts with particular zooxanthellae have more diverse MAAs than do those algae in culture (Shick and Dunlap, 2002). No data are available for CCM in non-symbiotic Anthozoa, but it would be most unlikely for the animal to accumulate inorganic carbon without the presence of CO_2 users.

One could speculate that new metabolic properties, necessitated by the constraints of symbiosis, originated from the evolutionary selection of key genes that are essential for stability of the symbiosis, or from lateral gene transfers between the two partners. As an example of such key genes, Reynolds *et al.* (2000) have identified a symbiosis-specific gene (symb32) that could be implicated in the formation and maintenance of the symbiosis. While analyses of bacterial and archaeal genomes have made it clear that lateral gene transfer is an important force in prokaryotic evolution (Doolittle *et al.*, 2003; Ochman *et al.*, 2000), the impact of such transfer on eukaryote genomes is less clear-cut (Ochman *et al.*, 2000; Raymond and Blankenship, 2003). It has nevertheless been suggested that eukaryotes possess the same capacity and similar mechanisms for effective lateral gene transfer as do prokaryotes (De la Cruz and Davies, 2000), and lateral eukaryote/eukaryote gene transfer has recently been suggested between a non-photosynthetic protist and a cnidarian (Steele *et al.*, 2004). Similarly, the authors of the last two studies offer two explanations: a gene ancestrally present in all eukaryotes was secondarily lost from most taxa or, more likely, a lateral gene transfer occurred.

A consequence of the symbiotic life style is the presence of major molecular and physiological differences between symbiotic and non-symbiotic cnidarians, some of them resulting from the induction of gene activity in one partner by the other. The signals mediating such differences are not known with certainty (taurine is a recent candidate for a ''host factor'' that causes zooxanthellae *in hospite* selectively to release their photosynthate: Wang and Douglas, 1997), but might be sought among the signalling systems shared by plants and animals (Schultz, 2002). It remains to be seen whether gene transfers between the endosymbionts and their cnidarian hosts have also contributed to these differences, which should imply different sensitivity and resistance to environmental changes.

Edmunds and Gates (2003) have suggested that the increasing frequency of geographically widespread coral bleaching, as well as practical experimental considerations, have led to a focus on the zooxanthellae as the environmentally-sensitive weak link, with less attention being paid to the host's sensitivity and its effects on the integrity of the symbiosis. We have argued that more than the alga's, it is the host's metabolism that has had to adjust to the conditions of phototrophic endosymbiosis. This includes actively increasing the supply of CO_2 to the zooxanthellae (which, beyond its effect on productivity, might help to avoid the proposed initiation of coral bleaching by ROS linked to inorganic carbon limitation: Jones *et*

al., 1998), and enhancing its own antioxidant defenses (likewise an important consideration for the well-being of the symbiosis: Dykens *et al.*, 1992). Therefore, genotypic diversity within each partner, and their interplay, likely contribute to a spectrum of environmental sensitivities in each and in the holobiont, and to the persistence of these symbioses in changing environments, a topic of growing interest.

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