

# MYCOSPORINE-LIKE AMINO ACIDS AND RELATED GADUSOLS: Biosynthesis, Accumulation, and UV-Protective Functions in Aquatic Organisms

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■ **Abstract** Organisms living in clear, shallow water are exposed to the damaging wavelengths of solar ultraviolet radiation (UVR) coincident with the longer wavelengths of photosynthetically available radiation (PAR) also necessary for vision. With the general exception of bacteria, taxonomically diverse marine and freshwater organisms have evolved the capacity to synthesize or accumulate UV-absorbing mycosporine-like amino acids (MAAs), presumably for protection against environmental UVR. This review highlights the evidence for this UV-protective role while also considering other attributed functions, including reproductive and osmotic regulation and vision. Probing the regulation and biosynthesis of MAAs provides insight to the physiological evolution and utility of UV protection and of biochemically associated antioxidant defenses.

## INTRODUCTION: BIOCHEMICAL DEFENSES AGAINST UV RADIATION

Solar radiation reaching the Earth consists of infrared (>800 nm), visible or photosynthetically available (PAR, 400–750 nm), ultraviolet-A (UVA, 320–400 nm), and the more energetic ultraviolet-B (UVB, 280–320 nm) wavelengths. Highly energetic ultraviolet-C radiation (UVC, 200–280 nm) does not reach the Earth because it is absorbed by atmospheric ozone and O<sub>2</sub>, in the latter case initiating reactions forming the ozone that itself absorbs most solar UVB. Intertidal and epipelagic marine organisms are exposed to the highest levels of ultraviolet radiation (UVR), and even planktonic and benthic organisms may experience harmful levels to depths >20 m (1).

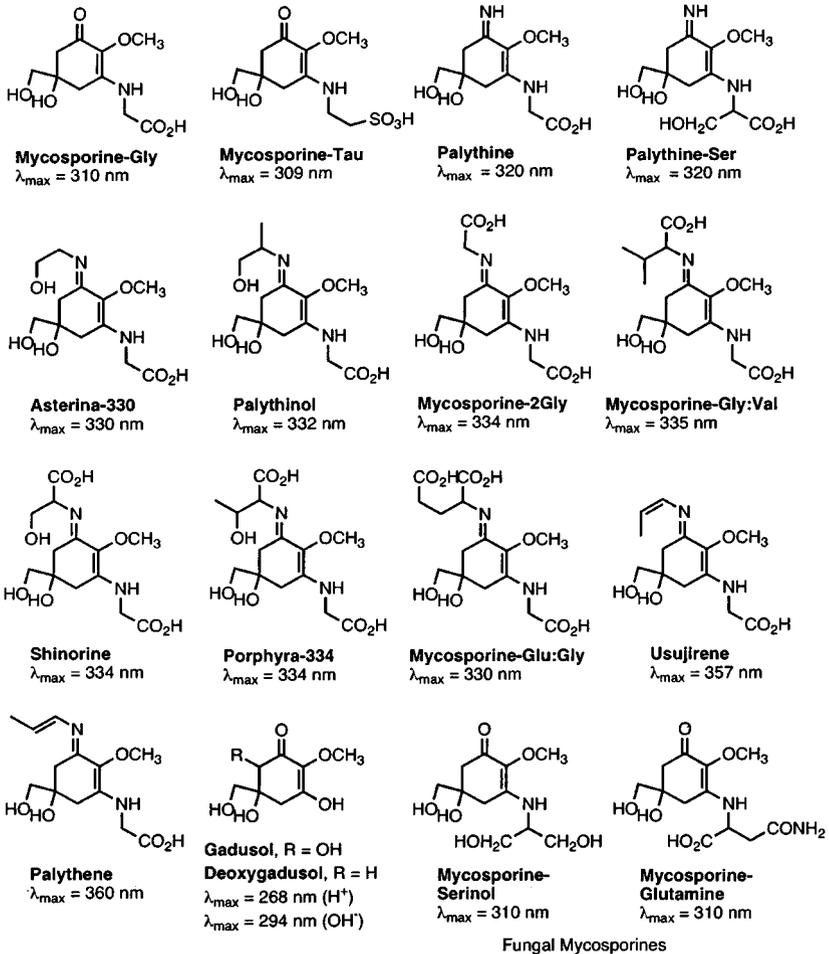
Environmental UVB and the short wavelengths of UVA can be detrimental to marine life (2, 3), and enhancement of UVB (via ozone loss) can disrupt trophic interactions (3, 4). Cellular damage from UV exposure can occur by direct photochemical reaction, e.g., thymine dimerization in DNA (5), or via the photodynamic production of reactive oxygen species (ROS) such as singlet oxygen ( $^1\text{O}_2$ ) and superoxide radical ( $\text{O}_2^{\bullet-}$ ) (6). Accordingly, shallow-dwelling organisms exposed to high levels of solar UVR have evolved biochemical defenses against such damage, protection that includes the elaboration of natural UV-absorbing sunscreens, the expression and regulation of antioxidant enzymes, the accumulation and cycling of small-molecule antioxidants, and molecular repair. This review focuses on the physiological aspects of UV-absorbing mycosporine-like amino acids (MAAs) in aquatic organisms; antioxidant (2, 7–9) and molecular repair (10, 11) functions are reviewed elsewhere.

Nearly ubiquitous among marine organisms is the ability to synthesize or otherwise acquire MAAs absorbing maximally in the range 310–360 nm (Figure 1). MAAs are transparent to visible light (i.e., they are not pigments) and have high molar absorptivity ( $\epsilon = 28100\text{--}50000 \text{ M}^{-1} \text{ cm}^{-1}$ ) for UVA and UVB. MAAs are particularly common in coral-reef algae and animals but also occur in organisms from most shallow-water environments, from tropical coral reefs, to high alpine lakes, to polar seas (8, 12). A protective function for MAAs, inferred from their efficient UV absorption and correlation between their concentrations and ambient levels of UVR such as occur over a bathymetric range, has been verified experimentally. The origin of MAAs as products of photosynthetic organisms necessitates more coverage of algal and cyanobacterial physiology than is usual in this series but is justified because the compounds are important in the photophysiology of invertebrate-microbial symbioses and in metazoans that obtain MAAs from their diet.

## HISTORICAL CONTEXT

### Discovery of MAAs in Marine Organisms

The abundance of life in marine surface waters is evidence of effective UV protection. Wittenburg (13) was first to report a strong UV-absorbing agent ( $\lambda_{\text{max}} = 305 \text{ nm}$ ) in the gas gland of the epipelagic Portuguese man-of-war, but the isolated substance ( $\lambda_{\text{max}}$  corrected to 310 nm) was never fully characterized (14). Soon thereafter, UV-absorbing materials were found to be characteristic of the Rhodophyta (red macroalgae) (15). Similarly, Shibata (16) observed strong UV absorption by the aqueous extracts of several zooxanthellate scleractinian corals and a cyanobacterium from the Great Barrier Reef. This substance (named S-320) in coral reef samples showed broad UV absorption centered at 320 nm, but variations in the exact  $\lambda_{\text{max}}$  (315–323 nm) suggested that S-320 is made up of a group of spectrally similar compounds. The first evidence that S-320 is photoprotective was provided by Maragos (17) on finding that S-320 absorbance in colonies of



**Figure 1** Molecular structures and wavelengths of maximum absorption ( $\lambda_{\max}$ ) of two fungal mycosporines, and of mycosporine-like amino acids and related gadusols in marine organisms.

*Porites lobata* varied inversely with depth, presumably compensating for ambient levels of UVR. A decade later, Jokiel & York (18) showed experimentally that S-320 decreased in tissues of *Pocillopora damicornis* on long-term exclusion of UVR, providing the first indication that S-320 was produced in response to UV exposure rather than some other depth-related factor. Contemporaneous reports indicate that UV-absorbing material is almost ubiquitous among marine algae (19, 20).

After the early biological observations of S-320 in coral reef invertebrates, natural products chemists at Nagoya (21), searching for palytoxin, observed a

water-soluble metabolite from a tropical zoanthid, *Palythoa tuberculosa*, having a sharp absorption maximum at 310 nm. This metabolite proved to be mycosporine-glycine (Figure 1), having a basic structure identical to a family of metabolites previously described in terrestrial fungi. Following this discovery, Hirata's group isolated a number of imino-mycosporine derivatives (to be grouped later as mycosporine-like amino acids, MAAs), including palythine, palythanol, and palythene, also from *P. tuberculosa* (22); asterina-330 was later characterized from the sea star, *Asterina pectinifera* (23). Rhodophytes yielded several MAAs, including porphyra-334 from *Porphyra tenera* (24), shinorine from *Chondrus yendoi* (25), and usujirene from *Palmaria palmata* (26). There are now 19 known MAAs (see 12 for a complete listing with their taxonomic distribution). Thus S-320 has been identified as a suite of MAAs in corals and other marine organisms (27, 28). Interest in the physiological role of MAAs following the early years of chemical discoveries has intensified, particularly because of environmental concern about global stratospheric ozone depletion (29, 30).

## Mycosporines in Terrestrial Fungi

Fungal metabolites strongly absorbing UVB ( $\lambda_{\max} = 310$  nm), first described by Leach (31) and notionally designated P-310, were present in the mycelia of several genera when sporulation had been induced with near-UVR, but were absent from non-sporulating colonies grown in darkness. The first P-310 substance was isolated from *Stereum hirsutum*, and its structure was elucidated (32) as 2-methoxy-3-bis(hydroxymethyl)methylamino-5-hydroxy-5-hydroxymethyl-2-cyclohexene-1-one (i.e., mycosporine-serinol; Figure 1). An early review (33) indicated that mycosporines are widespread among fungi, with the exception of Agaricales, but their presumed sporogenic activity was questioned on finding mycosporines only in conidiospores within the mycelium during sexual development. These UV-absorbing metabolites were later postulated to provide protection to fungal spores exposed to solar radiation during atmospheric dispersal (34). Although this contention is arguable (35), an evolutionary divide appears between the parent class of mycosporines expressed by fungi exclusively as oxo-carbonyl chromophores (absorbing UVB) and MAAs in aquatic organisms and terrestrial cyanobacteria containing mainly imino-carbonyl constituents (absorbing UVB and principally the short wavelengths of UVA) (Figure 1). Interestingly, only oxo-carbonyl mycosporines occur in the fungal-cyanobacterial symbiosis of terrestrial lichens (36).

## OCCURRENCE AND DISTRIBUTION OF MAAs

Their near-ubiquity among marine taxa suggests not only an early origin of MAAs but also a functional importance that has been retained during subsequent evolution. Extensive sampling within some taxa in environments ranging from tropical to polar has discerned relationships between concentrations of MAAs in their

tissues and environmental fluences of UVR that the organisms experience on local (bathymetric) and latitudinal scales. The predominantly superficial localization of MAAs in multicellular organisms, and their apparently homogeneous cytoplasmic distribution within single cells, are consistent with optical considerations for a sunscreensing role.

## MAAs in Marine Micro- and Macroalgae

MAAs occur in all microalgal taxa examined (reviewed in 12), as well as in natural assemblages of phytoplankton (37). When cultured under PAR alone, bloom-forming dinoflagellates tend to have the greatest capacity to accumulate MAAs (38), and when stimulated by changing irradiance, may alter their MAA complements and hence their UV-absorption spectra on the order of hours (39).

Marine, freshwater, and terrestrial cyanobacteria contain multiple MAAs, many being the same as in phylogenetically diverse eukaryotes, but most remaining unidentified (40). As in eukaryotic microalgae, the ability of small (typically  $<10\ \mu\text{m}$  diameter), seemingly vulnerable, cyanobacteria to inhabit intensely bright environments has prompted study of their UV defenses.

Small cell size itself limits the efficacy of molecular sunscreens because the absorption of UVR is a function both of the concentration of the chromophore and of cell size (= optical path length), with decreases in either reducing the sunscreen factor [ $S$ , the fraction of radiation of a given wavelength incident on a cell that will be absorbed by the sunscreen and thus not impinge on other cellular constituents (41)]. In no case examined has  $S$  achieved 1.0, i.e., some UVR always reaches other cellular targets and may exert biological effects (42–44), so the term sunscreen rather than sunblock is apt.

Although sometimes listed as sources of MAAs (12, 45), marine bacteria have not been systematically examined for their ability to synthesize these compounds. Only one bacterium, *Micrococcus* sp., is reported to contain an MAA, shinorine (46), although several species in the genera *Pseudoalteromonas* and *Vibrio* can convert the algal MAAs shinorine and porphyra-334 in the medium to mycosporine-glycine or to the related 4-deoxygadusol (8, 47).

The bio-optical model for UV absorption by single cells assumes a homogeneous cytoplasmic distribution of the sunscreen, a condition apparently met by MAAs in most cyanobacteria, where the water-soluble compounds occur free in the cytoplasm and not in cell walls or photosynthetic membranes (48); MAAs covalently linked to oligosaccharides uniquely occur in the extracellular glycan coat of *Nostoc commune* (40, 49), where they yield an  $S$  of  $\sim 0.7$ . Cytosolic homogeneity has not been checked in eukaryotic microalgae, where localization of MAAs around UV-sensitive organelles might increase their efficacy (50), although the small size of organelles presumably would require both very high local concentrations of MAAs for them to be effective and a more complicated optical model taking into account the cytological density and distribution of the organelles to assess this.

MAAs were early discovered in the Rhodophyta, and it is here among the algae that they achieve their highest concentrations and greatest diversity (12, 51, 52) and where we know most about macroalgal UV photoacclimatization. The extensive surveys by Karsten and colleagues enable the generalizations that Phaeophyta (brown algae such as kelps) either lack MAAs or have only trace amounts of them, presumably relying on other UV absorbers such as phlorotannins (53), and that Chlorophyta (green algae) have a higher proportion of genera containing MAAs, but, again, in minimal concentrations (54). Geographic and bathymetric trends are also evident.

## MAAs in Algal-Invertebrate Symbioses

As we have emphasized (2, 8, 9), the UV-intense environment of many coral reefs is rich in MAA-containing symbioses between diverse invertebrates and unicellular phototrophs (including cyanobacteria; Prochlorales, *Prochloron* sp.; and dinoflagellates, especially *Symbiodinium* spp., known as zooxanthellae). Such symbioses present a heterogeneous array of MAAs, the concentration of which may vary according to habitat depth, so the symbioses have been studied for environmental determinants of their levels of MAAs.

Reef-building corals (Scleractinia) are particularly well studied and collectively contain at least 13 MAAs, the number in a given zooxanthellate species ranging from 2 (55) to 10 (56). Corals and Indo-Pacific “giant clams” (57) overlap in their MAA complements, seemingly because they form symbioses with the same phylotypes of *Symbiodinium* (58).

MAAs usually are more concentrated in the hosts’ tissues than in symbionts freshly isolated from them [(57, 59–61); but for an exception, see (62)], a distribution suggesting that the host’s tissues, by virtue of their high concentrations of MAAs and relatively long optical path, afford the first line of defense against UVR. As befit UV sunscreens, MAAs are more concentrated in superficial tissues of tridacnid clams (57) and the ascidian *Lissoclinum patella* (61) than in underlying layers of the tissues where the algae reside. Likewise, in the sea anemone *Anemonia viridis*, MAAs are more concentrated in the tentacular ectoderm than in the endoderm, where the zooxanthellae are located (D. Allemand & J. M. Shick, unpublished data). The higher concentration of MAAs in the upper surface than in the sides or base of hemispherical colonies of the coral *Montastraea annularis* (63), and in branch tips than in tissues closer to the center of arborescent colonies of *Pocillopora damicornis* (64), probably is a photoadaptive response affording greater protection in the most exposed tissues. Likewise, MAAs are more concentrated in peripheral, actively growing apical tissues than in older, self-shaded parts of macroalgal thalli (65, 66; K. Wong, N. L. Adams & J. M. Shick, unpublished data).

Unlike the glycosylated MAAs (and scytonemin, a unique cyanobacterial sunscreen) (40, 49) in cyanobacterial sheaths, an extracellular location of MAAs in marine symbioses has been reported only in the mucus of corals (67, 68), wherein

they seem to be passively released rather than actively regulated, particularly as the relatively low concentration of MAAs in the mucus ( $\sim 1 \mu\text{M}$ ) would intercept only 7% of the incident solar UVR over the 1 mm thickness of the mucous layer (68).

MAAs in algal-invertebrate symbioses presumably originate in the phototrophic partner via the shikimate pathway. Four MAAs have been found among *Symbiodinium* species in culture: mycosporine-glycine, shinorine, and porphyra-334 (58) and mycosporine-2 glycine (J. M. Shick & C. Ferrier-Pagès, unpublished data), although a given species or phylotype contains only from zero to three of these MAAs. The suites of MAAs in the tridacnid clams and cnidarians from which the algal cultures were derived are similar to those of the algae, but the intact symbioses typically have a greater number of compounds. Differences in their kinetics of biosynthesis suggest that certain algal-type MAAs, such as shinorine and mycosporine-glycine that are synthesized quickly in response to UV exposure or other precursors, may subsequently be converted to different MAAs, perhaps in the host's tissues (56). In extreme cases, the host inhabited by zooxanthellae that produce no MAAs in vitro may have up to seven MAAs (56, 69), so the compounds seemingly must originate other than in the algae.

The finding that zooxanthellae in hospite (within the host) have different patterns of protein expression from the algae in vitro (70) is in keeping with the long-standing notion that the host can alter the biochemistry of its endosymbionts, e.g., there are qualitative differences in photosynthate produced by zooxanthellae in hospite and those in vitro (71). Thus it is possible that similar differences in the MAAs synthesized by cultured algae and by those in hospite explain the foregoing discrepancies. This remains to be tested.

Zooxanthellae generally produce a more restricted suite of MAAs than do free-living species of dinoflagellates in culture, the latter having from four to eight identified MAAs, plus additional, unidentified, presumed MAAs (38, 43, 50, 72–74). Might this reflect phylogenetic differences among dinoflagellates in the complexity of their biosynthetic machinery, and might the animal host harboring zooxanthellae have assumed the function of bioconversion among MAAs (concomitantly broadening the band of UV absorption) during evolution of the symbiosis?

Less is known of other symbioses. For example, a coral-reef sponge, *Dysidea herbacea*, contains four MAAs, one of them the novel mycosporine-glutamate: glycine, as well as the common mycosporine-glycine, and the isomers palythene and usujirene (75), the latter two being relatively rare among marine invertebrates. The unusual MAA complement in this sponge may be related to its symbiosis with *Oscillatoria spongelliae*, a cyanobacterium, a group for which most MAAs remain undescribed (40).

## MAAs in Asymbiotic Invertebrates and Vertebrates

MAAs occur in a plethora of asymbiotic animals (see tables in Reference 12), especially in the epidermis (76, 77), as expected for UV sunscreens. An intriguing

example is the presence of MAAs in the ocular tissues of fishes. Two of the MAAs found in the eyes of fishes (palythanol and palythene), plus shinorine, also occur in the cornea and lens of the cuttlefish *Sepia officinalis*, a cephalopod mollusc, where the absorption spectra of the ocular MAAs ( $\lambda_{\max}$  from 332 to 360 nm) and the single visual pigment ( $\lambda_{\max} \sim 490$  nm) are distinct; thus the MAAs in *Sepia* probably do not affect its visual sensitivity (78).

There is a clear sexual dichotomy in the occurrence of MAAs: Whereas ovaries and eggs often have the highest concentration of MAAs among the tissues tested, testes and sperm have, at most, trace amounts (55, 76, 79–84). In asymbiotic animals, ovarian MAAs originate in the adult's food (42, 44, 83, 85, 86), and because the sexual difference persists when the dietary availability of MAAs is controlled, the dichotomy probably has an adaptive physiological basis. There are also implications for the specificity of transport mechanisms by which dietary MAAs are sequestered.

As in algal unicells (41), size may determine the occurrence of MAAs in gametes: Relatively large eggs (150  $\mu\text{m}$  in diameter or greater in various species of sea urchins and tunicates, for example) have an optical pathlength sufficiently large so that MAAs in the observed concentrations could have biologically relevant sun-screening effectiveness. As in cyanobacteria, MAAs in eggs of sea urchins appear to occur free in the cytosol and not associated with any specific subcellular fractions (N. L. Adams, A. K. Carroll & J. M. Shick, unpublished data). Conversely, sperm averaging 2–3  $\mu\text{m}$  in diameter would have to accumulate MAAs amounting to  $\sim 25\%$  of their dry mass to achieve similar sun-screening factors (42, 69), a physiologically infeasible concentration because of osmotic constraints. The seeming UV-vulnerability of sperm shed to the environment may thus explain why so many marine animals, especially on coral reefs (81), spawn at night. Even the sperm of species spawning in daylight must remain undamaged for only minutes to hours before fertilizing an egg, so their cumulative UV dose is far less than in eggs and in the larvae developing from them (42, 44). The developmental manifestations of UV exposure of embryos having different concentrations of MAAs are considered below.

## Bathymetric Distribution of MAAs

The concentration of MAAs in corals is greater in shallow than in deep water (2, 8, 9, 86a). This is apparently an adaptive response to the exponential increase in the fluence of UVR with decreasing depth, and although other depth-dependent variables such as PAR and water movement may contribute (64, 87), the net effect is that corals contain MAAs in positive relation with their need for UV-sunscreen protection. The capacities of some corals to accumulate MAAs are correlated with their depth ranges (88, 89).

Palythine ( $\lambda_{\max} = 320$  nm) and mycosporine-glycine ( $\lambda_{\max} = 310$  nm) are the most prevalent MAAs in corals (reviewed in 2, 12), perhaps owing to the transparency of tropical waters to shorter, more damaging wavelengths. Mycosporine-glycine usually shows the greatest increase in shallow water, a correlate that may

be related not only to its UVB-absorbing but also to its antioxidant properties (90), for phototrophic corals necessarily experience conditions conducive to oxidative stress (reviewed in 2, 8, 9).

Bathymetric differences in the concentration of MAAs also occur in boreal and polar red macroalgae (51, 66, 86, 91–94). Transplanting deep-growing algae into shallow water often eliminates or reverses such differences, an effect that depends on both PAR and UVR (66, 92, 93), which strengthens the case for bathymetric photoacclimatization of UV defenses. High-intertidal species tend to have higher concentrations of MAAs than do low-shore or subtidal species. Some deep-water species of Rhodophyta seemingly lack the capacity to produce MAAs, whereas some littoral species have constitutively high concentrations (94).

Attempts to correlate the abundance in corals (89) and macroalgae (66) of MAAs having particular absorption maxima with the underwater spectrum or wavelengths stimulating their synthesis have been less convincing. This is partly because of the relatively great breadth of the absorption spectra of some MAAs (44, 88) and because of the apparent multiplicity of interacting signals stimulating the biosynthesis of MAAs, as well as differences in the kinetics of their accumulation, which result in temporal differences in the complement of MAAs and the combined UV-absorption spectra they present.

Data on asymbiotic animals are too few to generalize about bathymetric patterns of their MAA concentrations. Based on very small sample sizes in a broad survey, Shick et al. (76) postulated intergeneric differences among coral reef sea cucumbers (holothuroid echinoderms) associated with their depths of occurrence. Karentz et al. (84) found significantly higher MAA concentrations in the ovaries of the Antarctic sea urchin *Sterechinus neumayeri* collected intertidally and at 8 m than in those from 15 and 24 m depth. Conversely, no depth-related differences in ovarian MAA concentrations occur in the boreal sea urchin *Strongylocentrotus droebachiensis* over a range of 0.5 to 10 m, perhaps because the turbid water at the collection sites itself attenuates UVR (86). Because MAAs in asymbiotic herbivores are derived from their food, including cyanobacterial mats in the case of tropical holothuroids and macroalgae in the case of sea urchins, any depth-related differences in MAAs in the consumers' tissues arise from digesting differentially photoacclimatized phototrophs because UV exposure of the consumers themselves does not affect their accumulation of MAAs (86, 95). The lack of bathymetric trends in the consumers' MAAs may also arise from their ingesting "drift algae" and from their own mobility, which enables them to graze at different depths (76, 86) so that MAA concentrations in consumers' tissues spatially and temporally integrate the UV exposure of their food.

## Geographic and Seasonal Occurrence of MAAs

MAAs are ubiquitous, occurring in biomes ranging from polar to tropical (summarized in 12). MAAs occur most frequently and reach their greatest concentrations among tropical species, which may stem from the exposure of tropical organisms

to higher levels of UVR owing both to the smaller solar zenith angle and thinner ozone layer there (96). The increase in fluences of UVR with decreasing latitude (97) may help to explain a similar geographic trend in the concentration of MAAs in red algae (51, 52). However, a latitudinal gradient in temperature paralleling that in UVR has not been considered, nor can local variation in temperature be ruled out as a contributor to the variation in MAAs in primary producers, particularly in tropical species under unusually warm conditions associated with the El Niño–Southern Oscillation (98). The primacy of the role of UVR compared with PAR or temperature may be indicated by the higher concentration of MAAs in Antarctic red macrophytes (which rivals those in warm-temperate species) (51, 94), than in antipodal Arctic species, where the UV transparency of seawater is less, owing to higher concentrations of dissolved organic material (99).

Seasonal changes in concentrations of MAAs in a tropical symbiotic sponge were positively related to seawater temperature and PAR (75) and thus presumably to UVR. MAA levels in a scleractinian coral tracked seasonal changes in solar UVB (with a one-week lag) but not temperature, so that UV acclimatization by the symbiotic algae is implicated (100). In comparison, concentrations of MAAs in the soft corals *Lobophytum compactum* and *Sinularia flexibilis* were significantly correlated with annual cycles of both solar irradiation and seawater temperature (101). Conversely, ovarian MAA levels were negatively related to seasonal temperature (and UVR) in a boreal sea urchin (86). In both the tropical sponge and the boreal sea urchin, MAA concentrations are maximal at the time of the annual spawning, which occurs at seasonally high and low fluences of UVR, respectively, so that accumulating UV-protective MAAs in eggs released to the environment is more the determinant than are direct thermal or UV effects on their accumulation.

Despite the bathymetric differences in ovarian concentrations of MAAs in the Antarctic sea urchin *S. neumayeri*, there were no differences attributable to seasonal changes in daylength, or in fluences of PAR or of UVB, even during springtime ozone depletion (84). This is perhaps because MAAs accumulate in ovaries more gradually, in synchrony with gametogenesis and maturation (86) (spanning a wide seasonal range of conditions) and, moreover, depend on diet (42, 44, 83).

In summary, geographic and seasonal trends—probably determined more by differences in solar UVR than by PAR or temperature—in concentrations of MAAs in tissues are more clearly seen in the photosynthetic organisms (notably marine red algae) that produce MAAs than in species that consume them. The lack of clear environmental correlates of MAA accumulation by animals seems related to the tissue-specificity of MAAs sampled and particularly to the tendency for them to be sequestered in ovaries and eggs, which may be released at different seasons by various species in diverse habitats. The strong dietary dependence of MAAs in consumers and the limited knowledge of what they may eat in the field for months before being sampled complicate the matter.

## MAAs in Freshwater and Terrestrial Organisms

MAAs are found in cyanobacteria (40) and green microalgae (Chlorophyta) (102) from freshwater, hot spring, and terrestrial habitats. Mycosporine-glycine and several unidentified MAAs absorbing at 309–310 nm occur in terrestrial cyanobacterial lichens, but it is unknown whether the MAAs are produced by the cyanobacterial or fungal partner (36). MAAs occur in phytoplankton in high-alpine lakes and in the copepods that consume them (103, 103a). The MAA concentrations in the copepods are positively correlated with the altitude and the clarity of the water in the lakes, factors that increase the conditions of UV exposure (103a). Benthic cyanobacteria in the same lake have a more diverse suite of MAAs and in higher concentration than do phytoplankton; the unique occurrence of mycosporine-glycine in epilithic cyanobacteria on the lake shore may be related to their greater exposure to UVB (103). Like their marine and brackish-water relatives, freshwater fishes contain MAAs, both in their lenses and skin (104; N. L. Adams & J. M. Shick, unpublished data).

The sampling of freshwater and terrestrial species has not been extensive enough to enable other than the broadest generalizations. MAAs are unreported in higher plants, where the principal protection from UVR is by the multifunctional flavonoids (105, 106). Not only have MAAs not been found in the higher vertebrates (their sunscreens functions assumed by melanins), but unlike diverse invertebrates and fishes, mammals apparently cannot absorb MAAs from their food (77).

## PROTECTIVE FUNCTIONS OF MAAs AND RELATED GADUSOLS

The photo-physiochemical properties of a natural “sunscreening” agent are vital to its UV-protective effectiveness. A sunscreen must not only be efficient at absorbing appropriate wavelengths of UVR, but also at dissipating the absorbed energy without transferring it to sensitive biomolecules, or causing the photodynamic production of  $^1\text{O}_2$  and  $\text{O}_2^{\bullet-}$  to impose oxidative stress. Sunscreening and antioxidant functions for UV protection are thus closely entwined (107).

### Photophysics of UV Sunscreening and Related Antioxidant Functions

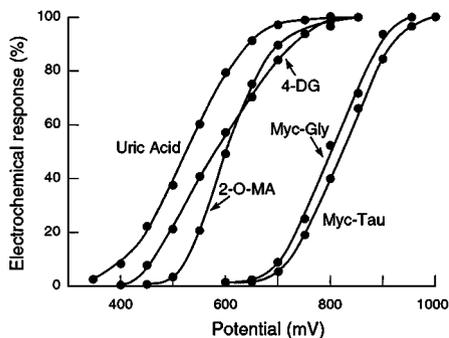
There are only two reports on the photophysical characteristics of UV dissipation by MAAs, and both examine the excited-state properties and photostability of macroalgal imino-mycosporines in vitro. Shick et al. (69) reported that shinorine (17  $\mu\text{M}$ ), despite its high absorptivity for UVA ( $\epsilon = 44,670 \text{ M}^{-1} \text{ cm}^{-1}$ ), showed no detectable fluorescence when excited across its half-maximal absorption waveband (312–348 nm). Furthermore, electron paramagnetic resonance (EPR) spectroscopy

revealed that purified shinorine (50  $\mu\text{M}$ ) did not produce detectable radicals when irradiated from 305 to 700 nm in the EPR cavity together with the spin-traps 5,5-dimethylpyrroline-*N*-oxide or  $\alpha$ -(4-pyridyl-1-oxide)-*N*-tert-butyl nitron. The absence of free radical formation by UV irradiation and a lack of fluorescence are consistent with a high efficiency of thermally dissipating absorbed UV energy.

The photophysical properties of porphyra-334 in aqueous solution were studied in detail (108). Irradiation of porphyra-334 (6  $\mu\text{M}$ ) at its absorption maximum produced a weak fluorescence (emission maximum = 395 nm) of extremely low quantum yield ( $\Phi_{\text{F}} = 1.6 \times 10^{-3}$ ). The short lifetime of the excited singlet-state indicated its rapid internal conversion to ground state. Direct excitation of 6  $\mu\text{M}$  porphyra-334 by laser flash photolysis (355 nm) revealed no triplet-state absorption transient in the microsecond range, consistent with a lack of triplet-state reactivities. The excited triplet state, however, could be measured by sensitization with benzophenone, and showed a low quantum efficiency ( $\Phi_{\text{T}} < 0.05$ ) and a strongly exothermic triplet energy level ( $E_{\text{T}} \leq 250 \text{ kJ mol}^{-1}$ ). Conde et al. (108) conclude, "...the very low quantum yields of fluorescence, intersystem crossing, and photolysis, are in agreement with a photoprotective role of porphyra-334 in living systems. In particular, the very low triplet quantum yield will preclude the action of [this] MAA as a photodynamic agent via singlet oxygen generation." The same holds for the lack of fluorescence and radical production observed for shinorine (69).

The foregoing properties of shinorine and porphyra-334, together with their high degree of photostability *in vitro* (42, 108) and *in vivo* (42, 44), are expected attributes of an efficient UV-screening agent elaborated by evolution. Moreover, the MAAs (principally shinorine) in the microalga *Phaeocystis antarctica* do not transfer (directly or by fluorescence) the UV energy that they absorb to chlorophyll *a* and thus do not participate in photosynthesis (109).

As part of their defense against photooxidative stress, marine invertebrates and fishes often contain high levels of gadusols (110, 111), which are related structurally (Figure 1) and biosynthetically to MAAs. The sunscreens and antioxidant roles of these cyclohexenone molecules are not always distinct. Examining the relationship between sunscreens and possible antioxidant functions of MAAs revealed that imino-MAAs are oxidatively robust, whereas the oxo-carbonyl mycosporine-glycine (and mycosporine-aurine, unique to sea anemones of the genus *Anthopleura*) (59) has moderate, concentration-dependent antioxidant activity (90). This may explain why corals growing in shallow water generally have disproportionately greater quantities of mycosporine-glycine than deeper-water conspecifics. Moreover, 4-deoxygadusol (4-DG), presumed to be the immediate precursor of MAAs, has strong antioxidant properties (8), as demonstrated by a comparison of electrochemical properties of water-soluble antioxidants found in marine organisms (Figure 2). 4-DG has been prepared by a bacterial "retrobiosynthetic" pathway (47), yet the biosynthetic relationship between the antioxidant function of 4-DG (and oxo-MAAs) and the sunscreens function of oxo- and imino-MAAs has not been fully explored. No doubt this area of investigation will



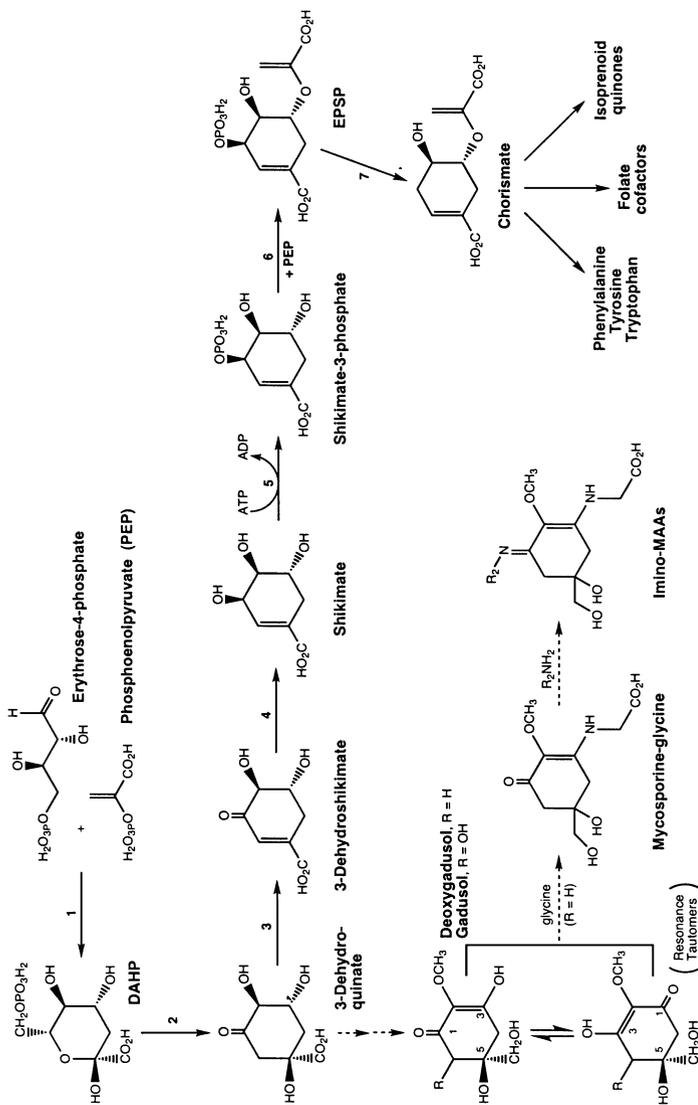
**Figure 2** Comparison of voltamperograms for water-soluble antioxidants commonly found in marine organisms: 4-DG, 4-deoxygadusol; 2-O-MA, 2-*O*-methylascorbate, a stable, methylated form of ascorbate (W. C. Dunlap, unpublished data); Myc-Gly, mycosporine-glycine; Myc-Tau, mycosporine-aurine. Antioxidant activities generally increase with decreasing half-wave potential (50% electrochemical response).

flourish as implications of thermal and photooxidative stress in coral bleaching (2, 112–116) gain recognition.

## Evolution of Structure and Function of MAAs and Gadusols

The requirement for UV protection in the evolution of phototrophic life on the early Earth has been renewed in scientific discourse (117, 118). The first life developed in the absence of atmospheric O<sub>2</sub>, so the early biosphere lacked this defense against the highly energetic wavelengths of solar UVC, and little evidence remains of how early photosynthesizers withstood it. The simpler structure of 4-DG and its intermediate position between MAAs and the shikimate pathway (Figure 3) suggest that gadusols evolved prior to MAAs and may have served originally as a UVB/C screen ( $\lambda_{\text{max}} = 294 \text{ nm}$  at physiological pH), albeit with lower absorptivity than MAAs (117). The strong antioxidant properties of gadusols concomitantly would have protected early cyanobacteria against oxidative damage at the intracellular sites of oxygenic photosynthesis. More speculatively, gadusols likewise might have detoxified sulfur- and oxygen-centered free radicals in microoxic, sulfidic interfacial microhabitats (119). Biochemical evolution involving amine condensation with 4-DG provided MAAs with strong UVB- and UVA-absorbing characteristics but at the expense of moderating or eliminating the antioxidant activity of 4-DG. Oxo-MAAs (absorbing UVB maximally at  $\sim 310 \text{ nm}$ ) might have predominated in cyanobacteria in early evolution, whereas imino-MAAs developed later as rising atmospheric oxygen levels increased the need for protection from UVA and photooxidative stress (117).

The absence of imino-mycosporines from the fungi suggests that these compounds in eukaryotic algae were inherited from the cyanobacterial progenitors



**Figure 3** The shikimate pathway, showing intermediates and enzyme-catalyzed steps (*numbered*). DAHP, 3-deoxy-D-arabinoheptulosin-7-phosphate; EPSP, 5-enolpyruvylshikimate-3-phosphate. Enzymes: 1, DAHP synthase; 2, DHQ synthase; 3, DHQ synthase; 4, shikimate dehydratase; 5, shikimate kinase; 6, EPSP synthase; 7, chorismate synthase. Broken arrows represent the putative biosynthetic relationship between 3-dehydroquinate (DHQ), gadusols, and MAAs. R<sub>2</sub>, amino acids and amino alcohols characterizing individual MAAs. Compiled from various sources.

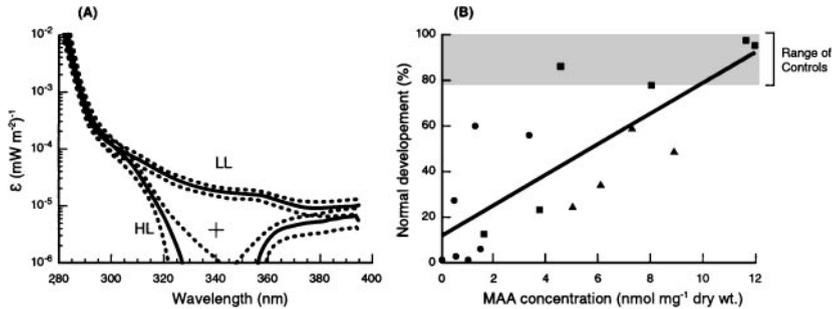
of their plastids, rather than having a cytosolic, eukaryotic provenance (117). Whether the fungal oxo-mycosporines are redox-active (i.e., have antioxidant activity) apparently has not been examined, but for most this is probable, based on chemical considerations. Their role in sporulation (35) indicates that these mycosporines have assumed additional functions, roles which may be paralleled by mycosporine-glycine in algae and invertebrates and which may involve redox signaling—all intriguing possibilities for future research.

## A Critical Look at the Protective Functions of MAAs

Organismal studies have documented MAA concentration-dependent protection of embryonic and larval development (42, 44, 55) and of growth and photosynthesis in free-living algae (43, 50, 92, 93). Also, protection of photosynthesis in symbiotic microalgae in hospite probably is owing to the higher concentration of MAAs (and other UV-absorbing materials) in the hosts' cells (which offer a longer optical path over which UVR is attenuated) because UVR does inhibit photosynthesis in the freshly isolated endosymbionts (60–62, 107, 120). Collectively, these studies also indicate that the protection by MAAs is incomplete, so that MAAs are appropriately seen as part of a suite of defenses against the manifold effects of UVR. Nor is it clear that all of the protection attributed to MAAs indeed derived from them, because MAA levels in some of the test organisms resulted from differential prior exposure to UVR, which might have enhanced other defenses such as antioxidants (69).

Protection against acute effects of UVR is unambiguous in the larvae of a sea urchin (42, 44) and in a free-living dinoflagellate (50), where the concentrations of MAAs were experimentally altered without prior exposure to UVR. In the latter case, protection was evaluated using the biological weighting function (BWF)—a polychromatic action spectrum (121, 122)—showing the wavelength-dependent inhibition of photosynthesis (Figure 4A). In that case, the BWFs for cells grown under high PAR (but no UVR) and rich in MAAs diverged from MAA-depauperate, low-PAR cells in the range of 320 to 360 nm, where cells having higher concentrations of MAAs showed the greatest enhancement of UV absorbance and resistance to acute UV exposure. Moreover, because lowering of the UV effect (biological weight) at 340 nm in MAA-rich cells compared with MAA-depauperate cells exceeded the value of 80% predicted by Garcia-Pichel's (41) optical model for regionally homogeneous intracellular sunscreens, Neale et al. (50) suggested that MAAs may not be uniformly distributed within algal cells; as already noted, there are virtually no data on any such subcellular localization.

Adams & Shick (42) fed omnivorous sea urchins macroalgae having different concentrations of MAAs that were transferred to the ovaries and eggs during gametogenesis and maturation in the absence of UVR. Fertilized eggs acutely exposed to simulated solar UVR showed a delay in cleavage that was inversely and logarithmically related to their concentration of MAAs, a result in keeping with the continuous absorption of UVR along the intracellular optical path, where the highest levels



**Figure 4** (A) Biological weight ( $\epsilon$ , reciprocal of  $\text{mW m}^{-2}$ ) for the inhibition of photosynthesis by acute exposure to UVR in the dinoflagellate *Gymnodinium sanguineum* grown under high PAR (HL) or low PAR (LL) in the absence of UVR. HL cells contained 44.4 and LL cells contained 2.1  $\text{nmol total MAAs nmol}^{-1}$  chlorophyll *a*. The broken lines represent the 95% confidence belts for the HL and LL mean curves. The cross indicates the weight predicted by applying a sunscreen factor (*S*) of 0.8 to the biological weight at 340 nm [reproduced from (50) with permission of *J. Phycol.*]. (B) When acutely exposed to PAR + UVR, the percentage of embryos of *Strongylocentrotus droebachiensis* developing normally is correlated with the total MAA-concentration in the eggs ( $r^2 = 0.614$ ,  $P = 0.001$ ). The data are from separate experiments on embryos from laboratory-maintained adults fed *Laminaria saccharina* (●), which lacks MAAs, or a combination diet of *L. saccharina* plus *Chondrus crispus* rich in MAAs (■), and field-fresh sea urchins eating a natural diet (▲). The shaded area indicates the range of normal development in control embryos (irradiated with PAR only) from the same batches of eggs at the same time of development (day 4) [reproduced from (44) with permission of *Mar. Biol.*].

of shinorine and porphyra-334 in the eggs gave a calculated sunscreen factor of 0.86. A protective effect extended to later stages when the incidence of normal development during UV irradiation depended on the concentration of MAAs in the eggs and larvae (Figure 4B). The nominal UVA- (334 nm) absorbing MAAs protected against UVB- (<320 nm) induced damage because their broad absorption properties have high molar extinction coefficients extending into the UVB.

When endogenous ascorbate (having the lowest electrochemical potential among biological antioxidants and thus easily oxidized to form the ascorbate free radical) was used as a spin-trap for in vivo electron paramagnetic resonance analysis, sea urchin ovaries irradiated with broad-spectrum UVR showed an inverse relationship between their concentration of MAAs and this measure of oxidative stress (69). Thus, although the shinorine and porphyra-334 in the eggs are not themselves antioxidants (90), their suncreening role includes intercepting and harmlessly dissipating UVR before it reaches more photoreactive biomolecules.

Despite their demonstrated role in protecting developing embryos in laboratory cultures, the ecological importance of UV protection by MAAs in early development (known to be the most sensitive phase of the life cycle) remains largely unexplored. The reported effects of UVR and MAA protection of reproduction in tropical corals are scant and scattered. Early evidence offers that MAA-containing planulae released by shallow-water colonies of *Agaricia agaricites* may be near their limits of tolerance to environmental UVR (55). By extension, this poses the question of UV protection in broadcast spawners: Do deep-water corals provide the same level of UV protection for their buoyant eggs as in those from shallow-water corals acclimatized to UV exposure? The zooxanthellate eggs of *Montipora verrucosa* and the azooxanthellate eggs of *Fungia scutaria* have strikingly different MAA compositions and concentrations, the concentrations being more than eight times greater in the positively buoyant eggs of *M. verrucosa* than in the negatively buoyant eggs of *F. scutaria* (82). A systematic inter- and intraspecific comparison of the UV tolerance and MAA composition of coral eggs and embryos is necessary to establish meaningful correlations.

Although a protective role is clearly demonstrated in dinoflagellates and sea urchin embryos, exactly what targets MAAs protect has not been determined. Such targets are known in principle from studies of UV-induced damage to biological molecules, but demonstration of their protection in vivo is scarce. Damage to DNA, RNA, and proteins is especially well known (5, 123), but in marine organisms, the repair of DNA damage is better documented than its prevention, and there are no published studies showing specifically whether high concentrations of MAAs can reduce UV-related damage to these molecules or impairment of enzyme function. The UV-induced accumulation of MAAs in a dinoflagellate did not prevent a concurrent decrease in its activity of Rubisco (43), the primary CO<sub>2</sub>-fixing enzyme.

The pyridine nucleotides NAD(P)H, which absorb maximally at 340 nm, are potential targets of UVR. The metabolic ubiquity of the NAD(P)/NAD(P)H redox couple indicates its early origin (124). The transfer of electrons from NADH to O<sub>2</sub> in the electron transport system is generally familiar to animal physiologists, but NAD(P)/NAD(P)H also maintains redox balance in anaerobic fermentations such as glycolysis and alternate schemes where pyruvate can have fates other than conversion to lactate (125), and in anoxygenic photosynthesis (126). Another crucial role of NAD(P)H is the reduction of coenzyme Q, important in preventing lipid peroxidation in the plasma membrane (127). NADPH is also central to photosynthetic electron flow and important in the oxidative defenses in chloroplasts (128). Thus there might be widespread metabolic consequences of the UV irradiation of NAD(P)H, especially in the presence of O<sub>2</sub>, where the potential for the photosensitized production of O<sub>2</sub><sup>•-</sup> (129) and H<sub>2</sub>O<sub>2</sub>, and thence HO<sup>•</sup>, would multiply the secondary effects of its irradiation (6).

NAD(P)H has a molar extinction coefficient of 6230 M<sup>-1</sup> cm<sup>-1</sup> at 340 nm, compared with the values on the order of 40,000 M<sup>-1</sup> cm<sup>-1</sup> for various UVA-absorbing MAAs (12). Given this within-order-of-magnitude similarity of the molar absorptivities of NAD(P)H and UVA-absorbing MAAs, the latter would have to be

present in much greater concentration in order to be protective, and this seems to be the case: MAA concentrations in various invertebrates, including eggs of sea urchins where they demonstrably protect cleavage from UVR, are about 500–2,000 nmol/g of fresh tissue, whereas the total concentration of NAD(P)H is ~50 nmol/ml (1 ml ~1 g) of unfertilized eggs of sea urchins and 135 nmol/ml of fertilized eggs (130, 131). A high molar ratio of MAAs to flavin nucleotides, which are other UVA-targets in cells (6), may also minimize in vivo any O<sub>2</sub>-dependent, flavin-mediated photolysis of redox-active oxo-MAAs such as occurs at approximately equimolar concentrations of FAD and fungal mycosporines in vitro (132).

## MAAs in Coral Bleaching

The role of MAAs as UV photoprotectants in the synergy of photic and thermal stresses causing coral bleaching has gained prominent attention. Early papers by Lesser et al. (112) and Glynn et al. (133) inferred that MAAs or their biosynthesis is thermally labile, so high temperatures would diminish UV protection, thus providing a molecular link between high UV irradiance and temperature to explain this synergistic stress in coral bleaching. Until recently, there has been no systematic examination of MAA levels in corals during a bleaching event to test this hypothesis.

MAAs in the mucus of the solitary coral *Fungia repanda* (1 m depth), monitored over 18 months, were positively correlated with solar UVR with a lag time of 1 week (100), consistent with the kinetics of UV-stimulated MAA-biosynthesis in corals (56). This significant correlation did not extend to seawater temperature or to the volume of mucus secreted. Although the corals observed during two bleaching events that occurred in this study were pale or partially bleached, the authors did not observe any shift of MAA concentrations or modification of composition in the mucus.

Similarly, over a two-year period, Michalek-Wagner (101) found positive correlations between MAA concentrations in reef-flat colonies of the soft corals *Lobophytum compactum* and *Sinularia flexibilis* and annual cycles in solar radiation and seawater temperature. MAAs in these soft corals during the 1998 mass bleaching event on the Great Barrier Reef clearly were not degraded at bleaching temperatures (134). On the contrary, MAAs were up-regulated under thermal stress, and concentrations were further enhanced during simultaneous exposure to UVR (98). Thermally acclimatized colonies having high MAA levels were not fully protected against solar UV-induced bleaching (loss of zooxanthellae) and, as may be expected, MAAs provided no discernible protection against thermal stress alone.

Another question regards the effects of bleaching and subsequent recovery on UV protection in coral reproduction. Experimental bleaching of the soft coral *Lobophytum compactum* reduced fecundity, fertilization success, and offspring viability in the subsequent breeding season (135). This negative impact on reproduction was associated with lowered levels of protein, lipid, MAA, and carotenoid in bleached adults (136). In contrast, MAAs were not as greatly depleted as were

other constituents in the eggs of bleached soft corals (136), but the importance of this conservation of MAA content in UV protection for larval recruitment remains untested. These results provide evidence for Gleason's (86a) hypothesis that the biosynthesis of MAAs is costly and may necessitate trade-offs with other metabolic demands during multiple abiotic stresses.

## Other Roles Attributed to MAAs

Because of their UV absorbance, MAAs have been studied primarily in photobiological contexts (sunscreens, vision). Owing to their seasonal changes in concentration that parallel ovarian maturation and their high aqueous solubility and high concentrations in vivo, MAAs have also been considered in reproductive and osmotic contexts.

**REPRODUCTIVE REGULATION** Largely by analogy with the case in the fungi, where mycosporines are involved with morphogenesis and sporulation (see review in 35), Bandaranayake proposed that MAAs in marine invertebrates have an undefined but intrinsic involvement with reproduction (75). There is scant empirical evidence to support this hypothesis, which stems mainly from the case of fungal mycosporines and from the observation that the concentrations of individual MAAs, especially in the ovaries, by various marine invertebrates is not necessarily correlated with the seasonal maximum of solar irradiation (which might be expected if the primary role of MAAs is as a UV sunscreen), but in many cases may reach their peak at the time of ovarian reproductive maturity.

Indeed, the seasonal accumulation of MAAs in ovaries of *Strongylocentrotus droebachiensis* occurs in inverse relation to solar irradiation: MAAs increase throughout the autumn and early winter, and peak in late winter just prior to spawning (83). Conversely, MAAs are most concentrated in ovaries and eggs of the crown-of-thorns sea star at its time of spawning in austral summer (35). Likewise, MAAs in the coral reef sponge *Dysidea herbacea* peak in summer, the time of spawning (75). It is a fair generalization that most MAAs reach their maximum concentration in eggs at about the time of spawning, which in the foregoing cases may be near the seasonal minima and maxima, respectively, of solar irradiation.

The synchronous accumulation of MAAs and the development of ovaries does not necessarily mean that MAAs are regulating or controlling such development, and their ovarian sequestration may be among the anabolic processes (e.g., vitellogenesis) that must be coordinated in conjunction with oogenesis and maturation. Thus MAA concentration may be an indicator of ovarian ripeness, not a regulator of it. Sea urchins eating only kelp (lacking MAAs), and in whose ovaries concentrations of previously assimilated MAAs remained constant or declined during gametogenesis, had gonadal indices (percentage of body mass allocated to gonads) as high or higher than those eating MAA-rich diets (83, 86). Importantly, the ovaries in adults on these disparate diets did not differ in their percentage

of nutritive or gametic cells in various stages of development despite their very different concentrations of MAAs (86).

For MAAs to exert their demonstrated effectiveness as UV sunscreens in spawned eggs in nature, they would ideally reach their maximum intracellular levels at the time of the eggs' release to the environment, regardless of whether UVR is at its seasonal high or low, which indeed is what occurs, but only if adults have eaten diets containing MAAs. That UVR does not enhance the accumulation by adults of dietary MAAs into their ovaries (86) suggests that the preferential concentration of MAAs in the ovaries is under a more general physiological control. It remains to be seen whether the environmental [e.g., photoperiod: (137)] and physiological [e.g., neurochemical and locally secreted chemical messengers (138)] factors that regulate ovarian development also control the sequestration and metabolism of MAAs; however, it does seem clear that MAAs themselves are not controlling oogenesis or maturation in sea urchins. Thus whether MAAs that are so prevalent among marine and freshwater organisms were evolutionarily co-opted to serve as chemical regulators of physiological processes such as reproduction remains speculative, and like so many gaps in our knowledge, testing this experimentally is hindered by the lack of commercial sources of MAAs.

**OSMOTIC REGULATION** Perhaps because of their "extremophily," cyanobacteria thriving in harsh environments have been investigated for other functions of MAAs. This includes a postulated role as osmolytes (139), and indeed the ~100 mM intracellular concentration of MAAs in halophilic cyanobacteria approaches that of free amino acids (FAAs) in diverse marine invertebrates, where FAAs account for about 25–75% of the total intracellular osmotic concentration. Such a high concentration of MAAs in cells suggests that they are compatible solutes with respect to their effect on macromolecular function (125), and their zwitterionic or acidic nature is consistent with this hypothesis. Similar concentrations of MAAs (~60 mM) occur in the ocular lenses of some tropical fishes (W. C. Dunlap & M. Inoue, unpublished data).

As would be expected if they have a role in cellular volume regulation, MAAs are released from cyanobacteria under hypo-osmotic stress (139, 140), although they are not absorbed from a more concentrated medium (139). As is the case for FAAs in many taxa, the steady-state concentration of MAAs is positively related to environmental salinity in *Chlorogloeopsis*, where the osmotically induced biosynthesis of mycosporine-glycine is synergistically enhanced by UVB, whereas shinorine accumulation is more under the control of UVB (140). Nevertheless, in this cyanobacterium, which tolerates salt concentrations only up to 70% of normal seawater, MAAs represent less than 5% of total intracellular osmolytes, so their physiological role here is not in osmotic regulation. It may be fortuitous that osmotic shock induces the biosynthesis of UV-absorbing MAAs where they do not serve as osmolytes; such a dual control by UV and osmotic stress is reminiscent of the activation of the c-Jun amino-terminal protein kinase (JNK) cascade in mammalian cells by these separate stressors (141).

In marine invertebrates, MAAs are far less important than FAAs as organic osmolytes. For example, in sea anemones living in normal seawater of  $\sim 1000$  milliosmoles, concentrations of FAAs are about  $100 \text{ mol g}^{-1}$  wet weight of tissue (142); assuming that tissues are 80% water by weight and that 50% of tissue water is intracellular, this gives an intracellular concentration of FAAs of 250 mM. By comparison, concentrations of MAAs in sea anemones (59, 143; J. M. Shick, unpublished data) are about  $15 \mu\text{mol g}^{-1}$  dry weight, or  $3 \mu\text{mol g}^{-1}$  wet weight; this gives an intracellular concentration of MAAs of about 7.5 mM, or only 3% of the FAA concentration in sea anemones and only 7.5% of the MAA concentration in halophilic cyanobacteria.

Intracellular concentrations of MAAs in sea urchin eggs, which are notably stenohaline, are lower, about 2 mM. Concentrations of MAAs in other marine invertebrates are similar to those in sea anemones, whereas FAA concentrations are generally higher, which suggests that MAAs are not major contributors to the intracellular pool of osmolytes in these cases. The extraordinarily high concentrations of MAAs (from 2200 up to 8800  $\text{nmol mg}^{-1}$  tissue protein, equivalent to  $\sim 220\text{--}880 \mu\text{mol g}^{-1}$  tissue wet weight) reported in some corals (54, 64, 144) would accordingly represent intracellular osmotic concentrations of  $\sim 0.55$  to 2.2 M, or from about half to double the total osmotic concentration (including organic plus inorganic solutes) of cells in osmotic equilibrium with seawater. Such concentrations are physiologically implausible, unless MAAs in these corals have domains other than free cytosolic dispersal.

## MAAs IN MARINE VISION

UVR, even the longer wavelengths of UVA, can damage ocular tissues, and photooxidative damage is the usual consequence of light exposure of the retina (145). Thus the corneal and lenticular MAAs accumulated by many fish may protect their retinas from damage by environmental UVR. Some species having near-UV-sensitive vision, however, have UVA-transparent ocular tissues to allow stimulation of retinal opsin absorbing in the range 360–380 nm. Assuming that UV-sensitive vision imposes a metabolic cost, it follows that vision in the near-UV range is functionally important (146).

### Presence of MAAs in Ocular Tissues of Fishes

Kennedy & Milkman (147) and Bon et al. (148) early postulated the existence in the ocular lenses of fishes, amphibians, and cephalopods of UV-absorbing substances having a characteristic absorbance at 320–360 nm. These UV-absorbing pigments (149) were unlike the kynurenine derivatives found in terrestrial animals, including humans (150). The biochemical properties reported by Zigman et al. (149) and Zigman (150) suggested they belong to the mycosporine family of metabolites, later confirmed by analysis of ocular tissues from a wide diversity of

fishes from the Great Barrier Reef (151). The specific group of MAAs present in the lenses of tropical fishes includes palythine, asterina-330, palythinol, and palythene. Notably absent are the UVB-absorbing mycosporine-glycine ( $\lambda_{\max} = 310$  nm) and the imino-MAAs shinorine and porphyra-334, common to many marine algae and corals. The interspecific comparison of MAAs in fish lenses revealed no clear behavioral or taxonomic trends: e.g., MAA concentrations in the lenses of two diurnal surface-feeders were more than three orders of magnitude lower than in common reef species feeding in deeper water. In the Sciaridae (parrotfish), concentrations of palythene ( $\lambda_{\max} = 360$  nm) were so great that the lenses were noticeably yellow, the only instance of a pigmented MAA. The presence of MAAs in particular tissues of fishes is attributed to accumulation from the diet and selective sequestration (77); such dietary accumulation of MAAs is treated separately.

### Role of MAAs in Vision

Protecting ocular tissues from damaging UVR and preventing the transmission (and focusing) of this energy to the retina would seem adaptive for fishes inhabiting the shallow photic zone. Accordingly, many species have highly absorbing substances (including MAAs) in their ocular tissues that effectively block transmission of wavelengths  $<400$  nm (104). The general presence of UVA-absorbing chromophores in ocular tissues is often attributed to improving visual acuity by reducing chromatic aberration caused by the scattering of short-wavelength radiation (152). However, this principle does not appear relevant to all fishes, as many species have tetrachromatic vision (UV opsin  $\lambda_{\max} = 360$ – $380$  nm) with functional near-UV perception (reviewed in 153), as do many insects, reptiles, birds, and some mammals (154). Siebeck & Marshall (155) compare light transmittance by the ocular media of 211 species of coral reef fishes, where 50.2% of them strongly absorb light of wavelengths below 400 nm, which is generally consistent with known lenticular MAA composition (151), particularly for palythene ( $\lambda_{\max} = 360$  nm). The remaining 49.8% have eyes that transmit wavelengths sufficient to allow UV-sensitive vision. Of the Labridae (wrasses), one of the largest and most diverse families of coral reef fishes, only 5 of 36 species have UV-capable vision (156), a variability again consistent with available data on MAAs (151).

While contemporary research focuses on the ocular transmission of UV wavelengths to evaluate the constraints of UV vision, visual perception of an object also depends on its reflection or absorption of the relevant wavelengths. Given that MAAs are generally localized in the epidermis of fishes and other marine organisms (8), the occurrence of these UV chromophores in dermal tissues may have relevance in the perception of UV coloration by marine animals having UV-sensitive vision. Notwithstanding evolutionary constraints necessary to achieve UV-sensitive vision, the role of MAAs is unlikely restricted to UV photoprotection, and potential involvement in visual UV perception may implicate MAAs as contributors to the sensory physiology, behavior, and ecology of marine animals.

## BIOSYNTHESIS OF MAAs BY ALGAL PRODUCERS

### The Shikimate Pathway

Details of the biosynthesis of MAAs in marine algae and phototrophic symbioses remain to be demonstrated, but their origin via the shikimate pathway has been a persistent assumption. Favre-Bonvin et al. (157) showed that the shikimate pathway-intermediate, 3-dehydroquinate (DHQ), is the precursor for the six-membered carbon ring common to fungal mycosporines (Figure 1). Synthesis of fungal mycosporines and of MAAs presumably proceeds from DHQ via gadusols (cyclohexenones) (Figure 3) (see references in 35, 69). Based on this knowledge, mycosporine-glycine and fungal mycosporine-serinol (Figure 1) were prepared starting with natural D-(-)-quinic acid (158).

The variable kinetics of increase among individual MAAs (when their synthesis is stimulated) in dinoflagellates, where the MAA complement may change on the order of hours (39, 72), and in red macroalgae, where the suite of MAAs varies over several days (51, 92), may also indicate interconversions among MAAs following the initial synthesis of a smaller number of primary compounds, as seems to be the case in the coral *Stylophora pistillata* (56). As in *S. pistillata*, shinorine is among the first MAAs to be synthesized in the red macrophyte *Chondrus crispus* (92), and likewise a compound absorbing at 334 nm (the  $\lambda_{\max}$  of shinorine) is the first to increase in the free-living dinoflagellate *Alexandrium excavatum* (72). Reciprocal changes in shinorine and palythine concentrations occur in *C. crispus* as they do in *S. pistillata*, suggesting a precursor-product relationship.

Blockage of the synthesis of MAAs in *S. pistillata* by *N*-phosphonomethylglycine (glyphosate), a specific inhibitor of the shikimate pathway, provides the only direct evidence that MAAs in marine organisms are indeed formed via this route (56). DHQ is formed from 3-deoxy-D-arabinoheptulosinate 7-phosphate (DAHP) at the second step in the shikimate pathway by the enzyme 3-dehydroquinase synthase (DHQ synthase). Both this enzyme and one isozyme of DAHP synthase (which catalyzes the first step in the pathway, the condensation of phosphoenolpyruvate and erythrose 4-phosphate) require  $\text{Co}^{2+}$ , and chelation of this metal by glyphosate may be the basis for its inhibition (at near-millimolar concentrations) of these enzymes (159, 160). Glyphosate (at 1  $\mu\text{M}$ ) is a competitive inhibitor for PEP of 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, the sixth enzyme in the pathway. Therefore, the use of 1 mM glyphosate on *S. pistillata* would have blocked the first two steps, as well as the penultimate step, in the shikimate pathway. This is important because, if the synthesis of MAAs indeed proceeds via a branchpoint at DHQ, inhibition of EPSP synthase alone would not block accumulation of MAAs (Figure 3).

The biosynthesis of MAAs via the shikimate pathway in a zooxanthellate coral presumably occurs in the algal partner, because animals purportedly lack this pathway, which is thought to be restricted to bacteria, algae, plants, and fungi. However, the oft-repeated dogma that animals lack this pathway apparently stems from the inability of vertebrates (variously given as animals, vertebrates, fish,

mammals, and humans in literature accounts) to synthesize essential aromatic amino acids, which they must obtain from their diets (159–162), and is not based on empirical evidence such as failures to detect activities of enzymes of the shikimate pathway or DNA sequences encoding these enzymes.

Nevertheless, which amino acids are essential for most invertebrates is incompletely known, and some these animals apparently can synthesize amino acids that vertebrates cannot (163, 163a). The report that azooxanthellate corals can produce small amounts of tyrosine and phenylalanine (164) seemingly points to the existence of the shikimate pathway and post-chorismate aromatic biosynthesis in these metazoans (although the authors could not rule out the production of the essential amino acids by bacteria associated with the corals). The shikimate pathway does occur exceptionally in a protist, the malaria parasite *Plasmodium falciparum* (165), perhaps in an evolutionarily enigmatic cytosolic form, as in the fungi (166).

Zooxanthellae freshly isolated from their hosts frequently have the same or similar MAA complement as the holobiont (intact symbiosis) or host tissues (60, 62, 143), reinforcing the notion that the algae produce the MAAs. However, in some cases, the freshly isolated zooxanthellae from MAA-containing holobionts lack MAAs (57, 143), which, together with the inability of these and some other zooxanthellae to synthesize MAAs in culture, suggests that the MAAs have another provenance in these cases. If dietary acquisition of MAAs can be excluded, the occurrence in corals and other symbioses of MAAs not synthesized by their endosymbionts demands explanation. Might cnidarians have the enzymes of the early shikimate pathway and the DHQ branchpoint to MAAs, or even the rest of the pre- and post-chorismate pathway, and thus be capable of synthesizing compounds assumed to be essential? We pose this question based on limited but suggestive data and mindful of the different protist ancestry for Cnidaria than for other Metazoa, from which stem the Cnidaria diverged very early (167).

It is noteworthy that cyanobacteria, phototrophic eukaryotes, and the symbioses in which they occur contain imino-MAAs that are lacking from terrestrial fungi, which have only oxo-mycosporines (35). Thus the imino-MAAs probably arose in the plastid line (117), where eukaryotic algae produce a suite of MAAs that both overlaps with and is broader than that in extant cyanobacteria, indicating evolutionary diversification in MAA-biosynthetic pathways in photosynthetic eukaryotes. The occurrence in phototrophic symbioses of MAAs lacking from their endosymbionts in culture and from cyanobacteria may again indicate either bioconversion of primary MAAs or, more speculatively, a de novo synthesis by the hosts themselves.

The metabolic cost of MAAs as a determinant of their concentration in organisms under different photic conditions or in different tissues has been debated (18, 60, 86a, 87, 88, 92, 168, 169). None of the foregoing sources, however, includes a reckoning of the actual costs of synthesizing MAAs, which may or may not affect growth and reproduction, and deposition of protein and lipids in UV-exposed corals and algae (37, 43, 86a, 136, 170–174).

Based on stoichiometry and ATP-coupling coefficients, Haslam (161) quotes a cost of 60 ATP-equivalents for synthesizing one mole of chorismate. Presumably the cost of synthesizing one mole of MAA would be similar, if it proceeds from the DHQ branchpoint prior to investing the final ATP and PEP in synthesizing shikimate-3-phosphate and EPSP in the pathway to chorismate (Figure 3), but with a corresponding ATP-cost of condensing amino acid(s) to the cyclohexenone base structure. This is about double the cost for the more direct synthesis of most non-aromatic amino acids from glycolytic or citrate cycle intermediates. Raven (175) estimates the cost as 300 moles of photons captured in photosynthesis per mole of MAA synthesized, which is about the same as the cost of synthesizing chlorophyll and one-tenth that of producing the light-harvesting complex of chlorophyll, proteins, and accessory pigments (176). Because MAAs are about 5 to 10 times more concentrated than chlorophyll in microalgal (50, 175) and cyanobacterial (177) cells, the cost of MAA synthesis is considerable—perhaps 19% of the total cost of cell production (175). Therefore, although MAA-biosynthesis in phototrophs is coupled to photosynthesis, the frequently observed necessity of a UV stimulus may further check the operation of a costly anabolic process that might compete with other energetic and material demands. In particular, the biosynthesis of MAAs *de novo* requires nitrogen (scarce in oligotrophic waters) and may divert this limiting nutrient from the competing needs of growth and reproduction (86a).

## Stimulation and Regulation of Biosynthesis of MAAs

The concentrations of MAAs in photosynthetic organisms and in symbioses containing them are positively related to the total irradiance they experience in nature, but because solar PAR and UVR co-vary, it is not always clear what wavelengths determine this (reviewed in 2, 8, 12, 69). Experiments using filtered sunlight and sources of artificial light on diverse cyanobacteria, micro- and macroalgae, and corals reveal stimulating effects of UVB, UVA, white light lacking UVR, and blue light, but no effect of red or green light, indicating the presence of specific photoreceptors.

Chemically inhibiting photosynthesis arrests the synthesis of MAAs in cultured dinoflagellates (72), but bright white light stimulates the biosynthesis of MAAs disproportionately more than it enhances photosynthesis (50), so that MAAs do not simply follow photosynthetic carbon, and the upregulation of their biosynthesis in microalgae is part of a suite of responses to high irradiance (50, 109). Non-photonic factors such as water flow that enhance photosynthesis also increase the accumulation of MAAs in the coral *Pocillopora damicornis*, but the effect is small compared with that of UVR (64) and is transient in *Porites compressa* (87). The effect of PAR on the levels of MAAs in *Montastraea faveolata* is only 30% of that of UVR when these wavelengths co-vary over a depth range of 3 to 30 m (144).

Studies using controlled spectral irradiance are too few to allow many generalizations about the specific stimuli for biosynthesis of MAAs. UVB but not UVA

wavelengths stimulate the synthesis of intracellular and sheath MAAs in diverse cyanobacteria (reviewed in 40, 49, 177a), whereas UVA and blue light are positive effectors in free-living dinoflagellates (72) and in the red macrophyte *Chondrus crispus* (177b). Under the same fluence of PAR, MAAs are synthesized in response to combined UVB + UVA radiation, but not to UVA alone, in the coral *Stylophora pistillata* (56). A differential response occurs for MAA biosynthesis during exposure of Antarctic diatoms to UVB, UVA, and PAR (178), a response also seen in a dinoflagellate (178a). Like cyanobacteria, zooxanthellae isolated from diverse hosts may contain constitutive levels of MAAs in the absence of UVR in culture and may enhance these under UVR (57, 58, 143, 173). Zooxanthellae originally isolated from *S. pistillata* and maintained under the same light source as this species of coral for two weeks scarcely increase their MAAs beyond constitutive levels (J. M. Shick & C. Ferrier-Pagès, unpublished data), although the coral does. The limited complement of MAAs in zooxanthellae may change with the age of the culture (143).

It has not been established whether the different kinetics of increase for various MAAs in *S. pistillata* indicate different UV sensitivities of de novo biosynthesis of these MAAs or whether there is a conversion of some MAAs synthesized early to those accumulating later; moreover, some interconversions may occur in the host's tissues and not in the algae (56, 69). Similar temporal changes in the complement of MAAs occur in red macroalgae under controlled spectral irradiance, where, e.g., only shinorine is synthesized in *C. crispus* under UVR alone but full-spectrum PAR + UVR elicits the additional synthesis of palythine (92, 179), which is also synthesized under blue light alone (177b). The former indicates not only a wavelength-specificity of stimulation, but also that the biosynthesis of shinorine does not directly depend on photosynthesis (at least in experiments of 7 days duration), as no PAR was present.

Little is known of the photoreceptors determining the spectral specificity of MAA biosynthesis. The pronounced effect of blue light and UVA on such biosynthesis in the red-tide dinoflagellate *Alexandrium excavatum* (72) and *C. crispus* (177b) indicates the presence of blue-light/UVA receptors, perhaps the flavoprotein cryptochromes (180, 181), but this has not been ascertained. The biosynthesis of MAAs in response particularly to UVB in cyanobacteria, microalgae, rhodophytes, and zooxanthellate corals suggests the presence of the corresponding receptor, although no receptor specific to UVB has been identified in any organism. Intriguingly, there is a selective induction by UVB of mRNA transcripts for DAHP synthase (the first enzyme in the shikimate pathway) (Figure 3) and for enzymes involved in the biosynthesis of UV-screening flavonoids in higher plants (182), but the signaling mechanism has not been demonstrated.

Cultures of *A. excavatum* kept in logarithmic growth phase increase their MAA content and change its composition within 3 to 6 h of exposure to high irradiance (39). Such rapid UV photoacclimation may be necessary in growing phytoplankton that experience radically different light fields on a daily basis (39). Slower rates of accumulation requiring days occur in batch cultures (presumably in stationary phase) of Antarctic diatoms (45, 178) and natural assemblages of Antarctic

phytoplankton (37). Zooxanthellae in stationary-phase culture show smaller changes in MAA content, and increases that do occur require two weeks or more (143, J. M. Shick & C. Ferrier-Pagès, unpublished data). Time-courses of several days to weeks for MAAs to increase are also evident in corals (reviewed in 2, 8), where the steady-state population of zooxanthellae in hospite may have low metabolic rates like those in stationary-phase cultures. Kinetics similar to those in corals are seen in red macrophytes (93).

## BIOACCUMULATION OF MAAs BY CONSUMER ORGANISMS

No metazoan has been shown to synthesize MAAs *de novo*, and the long-standing assumption that consumers acquire them from their diet (39, 76, 183) has been confirmed experimentally (77, 83, 85, 95). Sea cucumbers (Figure 5), sea urchins (83), and fishes (77) remove MAAs from their digesta, whereas hairless mice (which lack MAAs in their tissues) do not absorb or degrade MAAs present in a formulated diet (77).

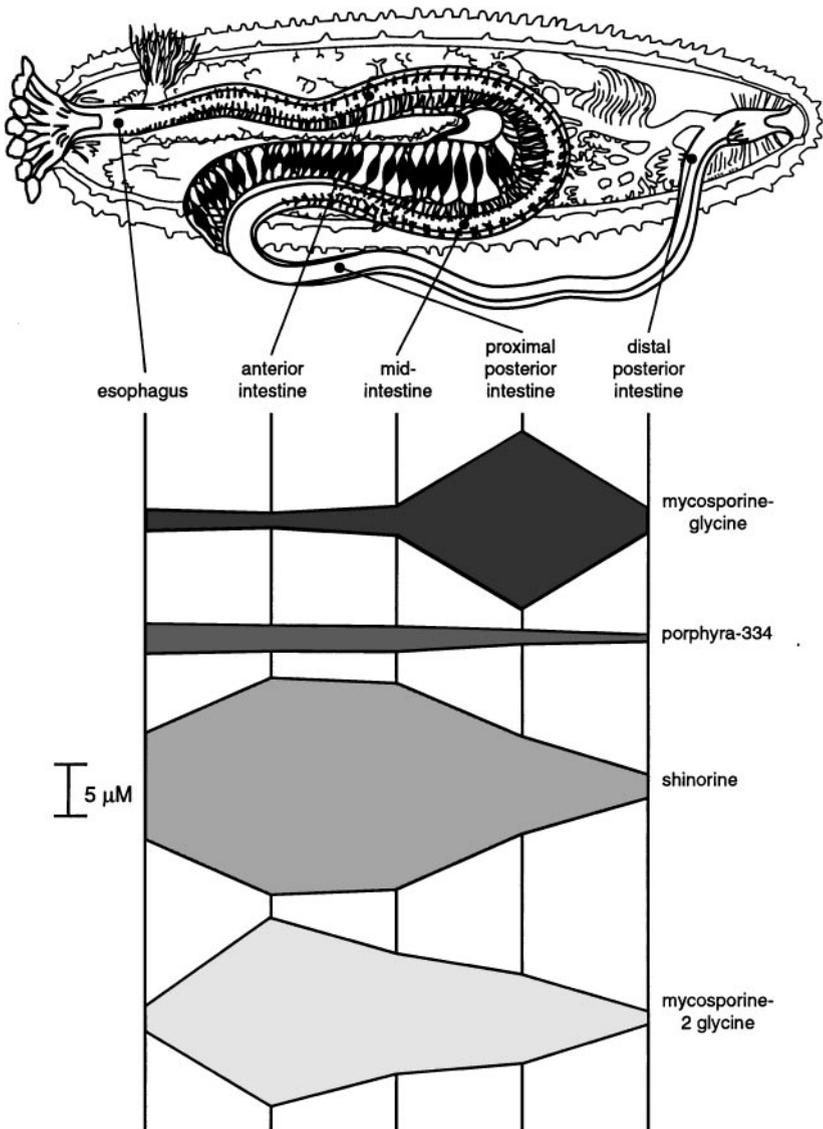
### Uptake of MAAs

The selective uptake of MAAs from food suggests there are specific transporters for them in the gut. Sea urchins accumulate principally shinorine (an acidic MAA) from *Chondrus crispus*, although this red macrophyte contains not only this MAA but also higher concentrations of the uncharged molecules palythine, asterina-330, and usujirene (86). Conversely, the medaka fish *Oryzias latipes* absorbs and accumulates neutral palythine and asterina-330 but not the acidic shinorine from *Mastocarpus stellatus* (77). These results suggest the presence of a transporter for acidic MAAs in sea urchins and one for neutral MAAs in fishes (69). *Sepia officinalis*, a cephalopod mollusc, can assimilate neutral and acidic MAAs, as its eyes contain both classes of MAAs (78), as do eggs and follicle/test cells of the tunicate *Ascidia ceratodes* (184).

The inhibition of the translocation of shinorine across the holothuroid gut by the presence of an equimolar concentration of the structurally similar porphyra-334 (J. M. Shick & W. C. Dunlap, unpublished data) suggests that the passage of MAAs occurs via carrier-mediated mechanisms rather than by paracellular diffusion, as likewise seems the case in the medaka (77). There are also regional differences in the echinoderm gut's capacity to transport MAAs (83; J. M. Shick & W. C. Dunlap, unpublished data). Although the mammalian (murine) gut cannot absorb it, shinorine is taken up in a concentration-dependent fashion by human skin cells in culture (77).

### Accumulation of MAAs

The accumulation of MAAs in cultured microalgae, macroalgae, and zooxanthellate corals may occur within hours to days of the appropriate photic stimulation,



**Figure 5** MAAs in the enteric fluid of the holothuroid *Thelenota ananas* feeding on cyanobacterial mats and associated microalgae (W. C. Dunlap & J. M. Shick, unpublished data).

whereas measurable accumulation of dietary MAAs in the consumers' tissues requires weeks to months (77, 83, 86, 95). Although echinoderm larvae can accumulate dissolved FAAs from seawater (163), they do not take up dissolved shinorine (44). The differential occurrence of particular MAAs among organs of metazoan consumers implies a specificity of transport systems, and perhaps tissue-specific interconversions among MAAs, to produce this distribution (8, 76).

### Interconversion of Dietary MAAs

The composition of MAAs accumulated by non-symbiotic consumers, notably echinoderms, differs from that in their diets (76, 86). MAAs in sea cucumbers are predominantly localized in their epidermal tissues and gonads, where asterina-330 is usually a major component, yet this MAA is absent from their diet of benthic microalgae and filamentous cyanobacteria, which contain mostly shinorine, mycosporine-2 glycine, and porphyra-334. This conundrum was partially solved by detailed examination of a coral reef sea cucumber, *Thelenota ananas*. The MAA complement in the foregut corresponds with that in dietary microflora, with the lumen of the digestive tract showing decreasing concentrations of algal MAAs and increasing concentrations of mycosporine-glycine (absent from the sediment algae) distally until enteric concentrations of all MAAs decline as they are translocated to body fluids and tissues (Figure 5). Asterina-330 is rarely present in the gut contents but reaches high concentrations in the intestinal tissue and especially in the epidermis. Further examination showed that strains of the ubiquitous marine bacterium *Vibrio harveyi*, isolated from enteric fluids, selectively hydrolyze the hydroxyamino acid substituents of shinorine and porphyra-334, yielding mycosporine-glycine (see 8 for the biochemical scheme), which may explain the complementary changes in these MAAs in the gut fluid (Figure 5). Whereas mycosporine-glycine is the postulated intermediate in the conversion to asterina-330, the pathway to reaminate mycosporine-glycine with endogenous ethanolamine has not been elucidated. Interestingly, this conversion appears not to operate in the medaka fish, which absorbs the small amount of asterina-330 available in a formulated diet but does not absorb or convert the far greater amount of shinorine (77).

### CONCLUSION: OUTLOOK AND FUTURE DIRECTIONS

The occurrence and physiological importance of MAAs are becoming standard inclusions in studies of the effects of UVR on aquatic organisms. Most involve environmental correlations between levels of UVR and MAAs, particularly in light of stratospheric ozone depletion, and more intensive and extensive sampling may discern a match between the UV absorption maxima of diverse MAAs and the UV spectral irradiance in the habitats where they occur. Possible localization of MAAs in chloroplasts or other UV-sensitive intracellular sites will be studied.

Cellular targets protected from UVR by MAAs will be elucidated, particularly as measuring damage to DNA becomes more feasible in taxonomically diverse organisms and as techniques of proteomics are applied. Molecular studies of UV signal-transduction eliciting biosynthesis of MAAs can be expected, particularly as researchers on cyanobacteria, algae, and corals extend their collaborations to include workers on higher plants, where UV-signaling is of intense interest. The possibility that MAAs themselves serve as chemical messengers or otherwise regulate physiological function will be examined further. Cooperation between physiologists and photobiochemists has already proved productive, and details of the cellular machinery for synthesizing and interconverting MAAs may yet emerge from interactions between these groups and metabolic biochemists and molecular biologists. Structures, UV-absorbing, and redox properties of unidentified MAAs will be elucidated, particularly in cyanobacteria, perhaps in the context of their protecting biological nitrogen fixation from UVR. The study of symbiotic associations will continue to broaden the physiological horizons of algal, bacterial, fungal, plant, and animal physiologists alike. Comparative genomics might test for the wider occurrence of the shikimate pathway in protists (which are under-represented in studies of the presence of MAAs) and in Cnidaria, where in some instances the presence of MAAs is otherwise enigmatic. Finally, it may be possible to reintroduce the biosynthetic pathway for MAAs into a higher plant (such as a flavonoid-deficient mutant of *Arabidopsis*) to examine the biomolecular evolution of tolerance to UVR.

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