Functional polarity of the tentacle of the sea anemone
Anemonia viridis: role in inorganic carbon acquisition

PAOLA FURLA, SYLVIE BÉNAZET-TAMBUTTÉ, JEAN JAUBERT, AND DENIS ALLEMAND
Observatoire Océanologique Européen, Centre Scientifique de Monaco, MC-98000 Monaco, Principality of Monaco

Furla, Paola, Sylvie Bénazet-Tambutté, Jean Jaubert, and Denis Allemand. Functional polarity of the tentacle of the sea anemone Anemonia viridis: role in inorganic carbon acquisition. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R303–R310, 1998.—The oral epithelial layers of anthozoans have a polarized morphology: photosynthetic endosymbionts live within endodermal cells facing the coelenteric cavity and are separated from the external seawater by the ectodermal layer and the mesoglea. To study if this morphology plays a role in the supply of inorganic carbon for symbiotic photosynthesis, we measured the change in pH and the rate of $\text{OH}^-$ ($\text{H}^+$) fluxes induced by each cell layer on a tentacle of the sea anemone Anemonia viridis. Light-induced pH increase of the medium bathing the endodermal layers led to the generation of a transepithelial pH gradient of $\sim 0.8$ pH units across the tentacle, whereas darkness induced acidification of this medium. The light-induced pH change was associated with an increase of total alkalinity. Only the endodermal layer was able to induce a net $\text{OH}^-$ secretion ($\text{H}^+$ absorption). The light-induced $\text{OH}^-$ secretion by the endodermal cell layer was dependent on the presence of $\text{HCO}_3^{-}$ in the compartment facing the ectoderm and was sensitive to several inhibitors of ion transport. [$^{14}$C] $\text{HCO}_3^{-}$ incorporation into photosynthates confirmed the ectodermal supply, the extent of which varied from 25 to $\geq 90\%$, according to $\text{HCO}_3^{-}$ availability. Our results suggest that the light-induced $\text{OH}^-$ secretion by the endodermal cell layer followed the polarized transport of $\text{HCO}_3^{-}$ and its subsequent decarboxylation within the endodermal cell layer. This polarity may play a significant role both in inorganic carbon absorption and in the control of light-enhanced calcification in scleractinian corals.

The objective of the present study was therefore to study $\text{OH}^-$ ($\text{H}^+$) fluxes and transport mechanisms by the two faces of the oral epithelial layers of the sea anemone Anemonia viridis to determine if the tentacles present a functional polarity linked to photosynthesis of dinoflagellate endosymbionts. For this purpose, we used two types of experimental preparations described previously (5, 6): the tentacle bag and the Ussing chamber. Further, to evaluate the use of the tentacle bag as a model, we also carried out measurements under in vivo conditions. Evolution of pH and fluxes of $\text{OH}^-$ ($\text{H}^+$) were measured by titration in media facing both ectodermal and endodermal cells. A functional polarity similar to that previously described in aquatic plants was demon-

MATERIALS AND METHODS

Biological material. Specimens of the Mediterranean sea anemone Anemonia viridis (Forskål) were collected in Villefranche-sur-mer, France, and were maintained in an open-circuit seawater aquarium supplied with Mediterranean sea-
water pumped at a depth of 50 m. Light was provided by a metal halide lamp (HQI-TS 400 W; Philips, Somerset, NJ), with a photosynthetic photon flux density of 125 µmol m⁻² s⁻¹ and a 12:12 h light-dark photoperiod. Unless otherwise indicated, all experiments were carried out under a constant saturating irradiance of 300 µmol photons m⁻² s⁻¹ (6) (HQI-TS 400 W, Philips) and a constant temperature of 16.0 ± 0.5°C maintained with a thermostatic circulator (Lauda RM 20).

Seawater preparation. Filtered seawater (FSW) was obtained by filtering Mediterranean seawater through a 0.45-µm Millipore membrane. HCO₃⁻-free seawater (0-HCO₃ SW) was prepared according to Bénazet-Tambutté et al. (6). The pH was adjusted to 6.6 or 8.2 with CO₂-free NaOH (6). pH was titrated in 100 ml of FSW-0.37 M MgCl₂ (1:1), after which a bag (5–6 cm pedal disk diameter) was first anesthetized with 0.01 N NaOH. After dissection, the tentacles were added to the endodermal compartment. The medium was either normal FSW (pH 8.2) or FSW adjusted to pH 9.0; the external medium was FSW. 

Measurement of photosynthetic rates. The photosynthetic rate of tentacle bags was measured as the rate of net O₂ evolution simultaneously in 5 ml of FSW in an oxygen chamber (see below). Inorganic carbon incorporation into photosynthates was measured in a Ussing chamber and in tentacle bags. The internal medium was either normal FSW (pH 8.2) or FSW adjusted to pH 9.0; the external medium was FSW. 

Presentation of results. Results are reported, results are given as means ± SD. Results were taken every 15 min after a 10-min equilibration period. The changes in H⁺ concentration permitted the calculation of net OH⁻ (H⁺) fluxes. In both cases, H⁺ concentration was measured by titration using an automatic titrator (Taucussel TTP-2). Titration was performed with a 5 mM HCl solution. The pH end point was the initial pH of the samples (30). To obtain reproducible measurements, the sample was thoroughly mixed during titration. The volume of HCl added allowed the calculation of the acid (base) net fluxes during the incubation period. In this paper, we refer to the pH increase of the medium as OH⁻ efflux and the pH decrease as H⁺ efflux. It should be noted that this might equally have been caused by H⁺ (OH⁻) influx.

Pharmacology. Pharmacological experiments were carried out in a Ussing chamber. The anion carrier inhibitor, 4,4'–disothiocyanostilbene-2,2'-disulfonic acid (DIDS) and the carbonic anhydrase inhibitors acetazolamide (N-[5-sulfamoyl-1,3,4-thiadiazol-2-yl]acetamide) (AZ, Diamox) and ethoxzolamide (EZ) were dissolved in dimethyl sulfoxide (DMSO) and buffered with 1 M Tris to pH 8.2. The H⁺-pump inhibitor diethylstilbestrol (DES) was dissolved in absolute ethanol and buffered with 1 M Tris to pH 8.2. Aminolactate, a potent and relatively specific inhibitor of the Na+/H⁺ antiporter, was dissolved in DMSO. All chemicals were obtained from Sigma (St. Louis, MO). All chemicals were obtained from Sigma (St. Louis, MO). All chemicals were obtained from Sigma (St. Louis, MO). All chemicals were obtained from Sigma (St. Louis, MO).
were standardized by the protein content of tentacle. Protein determination was carried out according to the method of Lowry, with bovine serum albumin as a standard using an autoanalyzer (Alliance Instruments). Results are presented as OH\(_2\)(H\(_1\)) flux expressed in nanoequivalents per minute per milligrams of protein or as percentage of control flux.

**RESULTS**

Effect of light on rate of O\(_2\) evolution and pH change of external and internal media. The light dependence of the rate of O\(_2\) evolution and pH change of both the internal and external media of a tentacle bag of the sea anemone *Anemonia viridis* is presented in Fig. 1. Increase of internal pH occurred only in the light, whereas acidification was observed in the dark. Although the rate of O\(_2\) production was constant during the 45-min light period, the internal pH increased rapidly from pH 7.4 to around 8.9 \(\pm\) 0.2 within 20–30 min and then ceased. In contrast, the external pH changed slightly (<0.1 pH units). The gradient of pH between the external seawater and the internal medium was –0.8 pH units. To verify if the bag could be considered representative of in vivo physiological conditions, internal pH was monitored on a tentacle of a whole sea anemone. Figure 2 shows a representative pH evolution. As in the tentacle bags, we observed a light-induced pH increase of the internal medium, with a similar plateau around pH 9.0, and acidification was observed in darkness. To determine the specific role of the ectodermal and endodermal layers, a piece of tentacle was mounted in the Ussing chamber. In this case, both tissue layers were bathed with the same volume of seawater. Figure 3 shows that although pH variations were <0.1 pH units in the ectodermal compartment, an increase of 1.6 pH units (from 7 to 8.6) was measured in the endodermal compartment. Again, the evolution of pH reached a plateau after 20 min.

Light-sensitive OH\(_2\) flux. Figure 4 shows that when the measurement of light-sensitive OH\(_2\) flux was performed in the external medium of a bag, a lag phase of 24 \(\pm\) 5 min was observed before the onset of titration. On an inside-out tentacle bag, this lag phase decreased to 2.7 \(\pm\) 1.5 min, suggesting that the primary site of OH\(_2\) excretion is the endodermal layer. To confirm the cellular origin of OH\(_2\), titration experiments were conducted in the Ussing chamber. A 15-min duration was chosen for the titration experiments to avoid any diffusion of OH\(_2\) from the endodermal toward the ectodermal compartment. In darkness, an H\(_+\) efflux was measured in both compartments, with higher excretion observed in the endodermal compartment (0.74 \(\pm\) 0.02 and 1.14 \(\pm\) 0.35 neq·min\(^{-1}\)·mg protein\(^{-1}\) in ectodermal and endodermal compartments, respectively). This higher rate of H\(_+\) efflux may be due to the
higher respiratory rate related to the presence of zooxanthellae. Under saturating light irradiance, no net OH⁻ excretion was measured in the ectodermal compartment, whereas a net OH⁻ excretion (3.55 ± 1.48 neq·min⁻¹·mg protein⁻¹) was measured in the endodermal compartment, showing that OH⁻ came primarily from the endodermal cell layer. If we assume that the rate of H⁺ efflux was constant in the light, then an OH⁻ excretion into the ectodermal compartment (0.74 neq·min⁻¹·mg protein⁻¹) was induced by light, whereas the total rate of OH⁻ excretion into the endodermal compartment was 4.69 neq·min⁻¹·mg protein⁻¹. In subsequent experiments, we only measured the net rate of OH⁻ excretion in the Ussing chamber.

Pharmacology of light-sensitive OH⁻ flux. The ion transport properties of the oral epithelial layers were characterized by exploring the effect of putative inhibitors of specific ion transport on OH⁻ excretion by both ectodermal and endodermal cell layers. OH⁻ movement may result either from carrier-mediated anion exchange, carbonic anhydrase activity, or H⁻–pump activity. Consequently, we tested inhibitors known to interfere with each of these pathways: DIDS (400 µM) (1), Diamox (AZ) and EZ (600 µM) (1), and DES (100 µM) (1). For all inhibitors tested, no effect was measured on OH⁻ (H⁻) fluxes by the ectodermal cell layer (results not shown). Consequently, Fig. 5 shows inhibitor effects on OH⁻ excretion by the endodermal cell layer only. DIDS added to the ectodermal or endodermal compartment decreased OH⁻ excretion by 35%. When added to both compartments, the inhibition was greater, although the difference does not appear to be significant. Diamox and EZ added to the ectodermal compartment inhibited OH⁻ excretion by ~50 and 20%, respectively. A 50% inhibition was also observed when Diamox was added to the endodermal compartment or in both compartments, showing that the effect of Diamox was not additive. When added to the endodermal compartment (or in both compartments) EZ almost totally inhibited OH⁻ excretion. The proton-pump inhibitor DES decreased OH⁻ secretion when added to the ectodermal compartment (~35% inhibition). The percentage of inhibition was 50% when DES was added to the endodermal compartment. Its effect was not additive when added to both compartments.

To test if the effect of the inhibitors added to the ectodermal compartment on OH⁻ secretion by the endodermal layer was due to a direct action on the ectodermal cells or to diffusion of inhibitors through the tentacle, we tested the permeability of inhibitors through the oral tissue in a Ussing chamber. Two kinds of experiments were carried out. The permeability was first determined spectrophotometrically. Inhibitors, at appropriate concentrations, were added to the ectodermal compartment. After 15 min (duration of a titration experiment), the endodermal medium was sampled and an absorption spectrum was carried out. None of the inhibitors tested was detected in this medium, suggesting that their permeability through the epithelial cell layers was low. To confirm the nonpermeation of inhibitors, we tested the effect of DIDS added to the ectodermal compartment on light-induced pH increase mediated by FIZ added in the endodermal compartment. In this case, DIDS did not affect pH increase in the opposite compartment, suggesting that its effect on intact tissue was specific.

Effect of pH and HCO₃⁻ availability. The effects of pH and HCO₃⁻ SW were tested on OH⁻ excretion. No effect of any protocol tested was observed on OH⁻ excretion by the ectodermal cell layer, whereas OH⁻ secretion by the endodermal cell layer was modulated by action on the opposite compartment. Figure 6 shows that elimination of HCO₃⁻ from the ectodermal compartment at pH 8.2 reduced by ~30% OH⁻ excretion by the endodermal cell layer, whereas a reduction of 90% was observed when HCO₃⁻ was eliminated from the endodermal compartment. A similar pattern of inhibition of OH⁻ excretion was observed when the endodermal layer of the tentacle was immersed in FSW ad-
justed to pH 9.0. Elimination of HCO$_3^-$ from both endodermal and ectodermal compartments at pH 6.6 decreased OH$^-$ excretion within the endodermal compartment by $\sim$75%. Elimination of HCO$_3^-$ from endodermal or ectodermal compartments at pH 6.6 led to a smaller inhibition of OH$^-$ excretion compared with that observed at pH 8.2 (25 instead of 90% and 10 instead of 28%, respectively).

Dependence on external Na$^+$ and amiloride-sensitive H$^+$ excretion. Na$^+$-dependent H$^+$ excretion is well known in vertebrate and invertebrate cells (2). To study the Na$^+$ dependence of OH$^-$ excretion, we tested the effect of 0-Na$^+$ ASW. Figure 7 shows that incubation of the ectodermal cell layer in 0-Na$^+$ ASW triggered a net OH$^-$ excretion by this cell layer. This stimulation was totally cut by 500 µM amiloride, suggesting that the OH$^-$ excretion observed in 0-Na$^+$ ASW was due to the reversal of the Na$^+$/H$^+$ exchange (leading to H$^+$ uptake) after modification of the Na$^+$ electrochemical gradient, which energizes this antiport. The same effect was observed when the endodermal cell layer was incubated in 0-Na$^+$ ASW. In this case, an OH$^-$ excretion triggered by 0-Na$^+$ ASW was superimposed on the light-dependent OH$^-$ excretion, leading to a twofold increase in this parameter. This OH$^-$ excretion was sensitive to amiloride. When 0-Na$^+$ ASW bathed the two compartments, an amiloride-sensitive OH$^-$ excretion by the two layers of cells was triggered. This set of experiments demonstrated the presence of a Na$^+$/H$^+$ exchange on both layers of cells. This exchange did not apparently play a major role in the light-dependent OH$^-$ excretion.

Inorganic carbon incorporation into photosynthates. To confirm the external supply of inorganic carbon, we carried out measurements of DIC incorporation into photosynthates. Experiments were carried out in both Ussing chambers and tentacle bags. Figure 8 shows that in the Ussing chamber, when the pH of the endodermal compartment was that of natural seawater, i.e., 8.2, $\sim$75% of inorganic carbon came from the endodermal compartment. When the pH of the endodermal compartment increased to 9.2, only 25% of inorganic carbon was supplied from this compartment. In the tentacle bag, a similar pattern was observed with a more pronounced effect: the inorganic carbon supply from the endodermal compartment decreased from $\sim$40% for an initial pH of 8.2 to 6% for a pH of 9.2.

Alkalinity of the internal medium of tentacle bags. TA of the internal medium of tentacle bags exposed to light for 1 h increased from 2.626 ± 0.003 meq/l (TA of FSW pH 8.2) to 3.353 ± 0.591 meq/l. We could not measure any significant change of TA in the external medium due to its high volume related to the internal medium.

**DISCUSSION**

Our results demonstrate that under light conditions, the tentacle of the sea anemone Anemonia viridis generates a pH gradient of $\sim$0.8 units across its epithelial layers, with the endodermal face being alkaline. Measurements in a bag or in the Ussing chamber were corroborated by the direct measurement of internal pH within a whole sea anemone. Our results showed that in vivo, the endodermal cell layer was subjected to cyclic variations of pH, from acidic in darkness to alkaline in light conditions. Furthermore, this paper confirms previous results (6, 9) showing that inorganic carbon is absorbed from the seawater pool by the ectodermal cells and then transferred to endodermal cells where a net OH$^-$ secretion (or H$^+$ uptake) occurs. One of the functions of the epithelial layers of the tentacle of the sea anemone is then to transport HCO$_3^-$ across the epithelial barrier as renal tubular or gastric epithelia.

OH$^-$ secretion occurred originally in the internal medium of the tentacle, as demonstrated by the titration experiment carried out in the Ussing chamber. This net secretion of OH$^-$ was further confirmed by the light-dependent increase of TA in the internal medium. Indeed, if both CO$_2$ absorption and H$^+$/OH$^-$ fluxes affect pH, only H$^+$/OH$^-$ (or HCO$_3^-$, CO$_3^{2-}$) fluxes affect...
alkalinity (27). As the pH gradient across the tentacle became maximal (i.e., after 20 min of saturating light), a slight diffusion of OH\textsuperscript{-} took place from the internal toward the external compartment, as shown by the lag phase of 24 ± 5 min before the onset of titration in this medium. Although the increase of pH in the internal medium plateaued at ~9 after 20 min, the rate of O\textsubscript{2} evolution remained linear. An inhibition of photosynthesis when extracellular pH becomes alkaline has been recorded in various photosynthetic organisms, including marine microalgae (26), cyanobacteria (14), and in the symbiotic association between corals and dinoflagellates (12). Such inhibition has been attributed to low levels of HCO\textsubscript{3}\textsuperscript{-} at this pH. In contrast to these results, only one side of the photosynthetic cell (i.e., the apical membrane of the endodermal cell) of the sea anemone tentacle was bathed in alkaline pH. The ectodermal side of the tentacle remained around pH 8.2, thus allowing the normal supply of DIC. Such functional polarization was found to an even larger extent in various macroalgae and angiosperms. In Potamogeton, the pH at the upper side increased to a value of ~11, whereas at the lower side the pH decreased to a value as low as 4.0 (21). Although the function of polarity in these organisms is still unclear, it has been suggested that a ΔpH or proton motive force is needed to drive HCO\textsubscript{3}\textsuperscript{-} uptake or HCO\textsubscript{3}\textsuperscript{-}-to-CO\textsubscript{2} conversion (22). In the giant hydrothermal vent tubeworm Riftia pachyptila, symbiotic with intracellular carbon-fixing sulfide-oxidizing bacteria, it has been recently demonstrated that the maintenance of an alkaline pH within the extracellular fluids of the tubeworms acts to concentrate inorganic carbon (11). However, the rate of transepithelial HCO\textsubscript{3}\textsuperscript{-} diffusion which usually supplies ~16% of symbiont photosynthesis (9) did not present any significant difference when the tentacle was perfused with FSW at pH 8.2 or 9.0 (unpublished data). The generation of a transepithelial pH gradient in sea anemones is therefore probably a part of the carbon-concentrating mechanism (6) rather than being the carbon-concentrating mechanism itself.

If a net OH\textsuperscript{-} secretion seemed to occur only in the endodermal compartment, our results show unambiguously that this light-sensitive excretion may be modulated by inhibitors (Fig. 5) or by use of HCO\textsubscript{3}\textsuperscript{-}-free SW (Fig. 6) within the ectodermal compartment. This inhibition resulted specifically from an effect on endodermal cells and not from diffusion of inhibitors through the tentacle. The requirement for external HCO\textsubscript{3}\textsuperscript{-} was further supported by the measurement of dissolved inorganic \textsuperscript{14}C incorporation into photosynthates. Whatever the biological preparation, our results demonstrate that at least a part of the carbon incorporated into photosynthates was supplied by endodermal cells. The extent of this supply changed according to the preparation and the initial internal pH conditions (Fig. 8). For an initial pH of 8.2, the difference observed between the Ussing chamber and tentacle bag was likely the consequence of DIC availability (6). In the Ussing chamber, the availability of DIC between the two compartments being the same, the uptake of DIC was performed mostly by endodermal cells. In tentacle bags, which are supposed to be closest to in vivo conditions, the internal volume was 25-fold lower than the external one, and the internal DIC availability was not high enough to supply symbiotic photosynthesis, leading to a predominant uptake from the external compartment. The extent of ectodermal supply was a function of endodermal pH (which governs carbon speciation). The endodermal supply decreased to nearly zero as light-dependent pH increase occurred, suggesting that the transport capacity of the endodermal layer was stimulated to compensate for any decrease of direct endodermal supply. These results demonstrate that the epithelial layers of the tentacle displayed a great plasticity according to DIC availability.

Bénazet-Tambutté et al. (6) demonstrated that part of the DIC used for symbiotic photosynthesis was supplied by a DIDS-sensitive DIC uptake site located in the membrane of ectodermal cells. Similarly, the present results, using either the anion inhibitor DIDS or HCO\textsubscript{3}\textsuperscript{-}-free SW, demonstrated a dependence of OH\textsuperscript{-} secretion by endodermal cells on HCO\textsubscript{3}\textsuperscript{-} availability in the ectodermal compartment. These results demonstrate that a HCO\textsubscript{3}\textsuperscript{-} carrier is present in the membrane of ectodermal cells. The involvement of a secondary active transport of HCO\textsubscript{3}\textsuperscript{-} in sea anemone tentacles is consistent with the fact that such transports are generally involved in transepithelial acid transport in leaky epithelia (7) such as the tentacle of the sea anemone (5).

HCO\textsubscript{3}\textsuperscript{-} uptake may also be involved in membrane-bound carbonic anhydrase-catalyzed dehydration of HCO\textsubscript{3}\textsuperscript{-} followed by CO\textsubscript{2} uptake, leading to extracellular pH increase (4). A Diamox and EZ-sensitive process was found in ectodermal cells; however, EZ, which is thought to be more membrane permeable than Diamox (1), led to a lower inhibition of OH\textsuperscript{-} excretion by endodermal cells. A possible explanation for this paradoxical result is a nonspecific effect of Diamox. Diamox is known to inhibit both HCO\textsubscript{3}\textsuperscript{-}-ATPase in the blue crab gallblader (28), and chemical conversion between HCO\textsubscript{3}\textsuperscript{-} and CO\textsubscript{2} may be mediated by H\textsuperscript{+} excretion. A luminal Na\textsuperscript{+}/H\textsuperscript{+} exchanger was demonstrated to mediate transcellular HCO\textsubscript{3}\textsuperscript{-} flux in the absorptive direction by intraluminal CO\textsubscript{2} generation in various absorptive epithelia, such as renal proximal tubule (2), Necturus gallbladder (28), and
rabit intestine (16). We demonstrated the presence of an amidolide-sensitive Na+/H+ exchanger in the tentacle of the sea anemone that seems to play no role in HCO$_3^-$ transport. H+ excretion may also be mediated by an H+–ATPase. Experiments carried out with DES suggest that such an enzyme was involved in HCO$_3^-$ transport. We observed a similar inhibition (~50%) when DES was added to the internal medium or both the internal and external media, suggesting the involvement of two H+–ATPases in series within each epithelial cell layer. It is possible that these H+–pumps facilitate the diffusion of DIC between the two cell layers by acidifying the mesoglea.

The process involving HCO$_3^-$ transport at the membrane level implies that the dehydration reaction of HCO$_3^-$ into CO$_2$ is intracellular. Numerous transport systems have been suggested to account for OH$^-$ secretion: an electrogenic HCO$_3^-/OH^-$ antiport, H+–HCO$_3^-$ cotransport system (15, 18), or Cl-/OH$^-$ antiport (25). The fact that OH$^-$ secretion was inhibited by 90% when HCO$_3^-$ was lacking from the endodermal compartment whereas O$_2$ evolution was only inhibited by 40% (6) suggests a possible coupling between OH$^-$ and HCO$_3^-$. Such an antiport would be neutral (18). When the internal medium becomes alkaline, the OH$^-$ concentration gradient inhibits the OH$^-$ efflux, whereas at acidic pH the opposite is observed. At pH 6.6 the OH$^-$ concentration gradient will favor OH$^-$ efflux (or H+ influx). Surprisingly, even when HCO$_3^-$-free SW was present in both compartments, a light-induced OH$^-$ efflux was still recorded, whereas no change was recorded in the ectodermal compartment. This demonstrates that this OH$^-$ efflux was specific to the endodermal cell layer, suggesting a higher OH$^-$ (H+) cell permeability of endodermal cells than ectodermal cells. Such a different permeability may play a role in intracellular pH regulation during photosynthesis.

Our results demonstrate that the epithelial layers of the sea anemone tentacle carried out transepithelial HCO$_3^-$ transport and OH$^-$ secretion like numerous other epithelia. However, mechanisms underlying this process appeared to be different from other animals, thus providing a new model for studying HCO$_3^-$ transport. A pH gradient across the tentacle, resulting from the activity of a carbon-concentrating mechanism, was generated by light. Such a functional polarity has been previously shown to play a role in HCO$_3^-$ utilization in macrophytes (22). This role can now be extended to symbiotic anthozoans. It is likely that this phenomenon also occurs in scleractinian corals, in which the oral epithelial layers are similar to those of sea anemones. If this is the case, an alkaline coelenteric space may facilitate diffusion of H+ produced by the calcium carbonate precipitation following the reaction

$$\text{HCO}_3^- + \text{Ca}^{2+} \rightarrow \text{CaCO}_3 + \text{H}^+$$

which consequently results in enhancement of calcification. A role of H+ in coupling photosynthesis and calcification has been previously proposed (20). However, in contrast to this earlier study, our model does not allow calcification to stimulate photosynthesis. Thus the polarized oral epithelial layers of anthozoans may play some role in light-enhanced calcification by coupling OH$^-$ production by endodermal cells and H$^+$ production by calcification.

**Perspectives**

Anthozoans provide an interesting model of HCO$_3^-$ transport involving functional polarity of the oral tissue associated with a morphological polarity (the presence of symbiotic algae only in endodermal cells). The originality of this model is the inferred presence of carbon-concentrating mechanisms in the animal cells, whereas such mechanisms have presently been described in vegetal cells only. Moreover, the light-dependent increase of coelenteric pH could play a significant role in the process of light-enhanced calcification. The epithelial approach enables us to increase significantly the knowledge of the physiology of symbiotic Anthozoans, but a lot of work is required to determine more precisely the transepithelial transport of HCO$_3^-$ and to characterize the carriers at cellular and molecular levels.

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Address for reprint requests: D. Allemand, Observatoire Oceanologique Europeen, Centre Scientifique de Monaco, Ave. Saint Martin, MC-98000 Monaco, Principality of Monaco.

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