Cell Biology of Reef-Building Corals: Ion Transport, Acid/Base Regulation, and Energy Metabolism

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7.1 Summary

Coral reefs are built by colonial cnidarians that establish a symbiotic relationship with dinoflagellate algae of the genus *Symbiodinium*. The processes of photosynthesis, calcification, and general metabolism require the transport of diverse ions across several cellular membranes and generate waste products that induce acid/base and oxidative stress. This chapter reviews the current knowledge on coral cell biology with a focus on ion transport and acid/base regulation while also discussing related aspects of coral energy metabolism.

7.2 Introduction

Reef-building corals, also known as hermatypic or stony corals, are marine invertebrates from the phylum Cnidaria (Class Anthozoa, Subclass Hexacorallia, Order Scleractinia) (Bourne 1900) that build external calcium carbonate skeletons beneath their tissues. The reefs they build provide homes for a variety of other organisms, resulting in one of the most biodiverse ecosystems in the world, that provide ecosystem and economic services for hundreds of millions of people in tropical and subtropical areas (Burke et al. 2011).

Reef-building corals are colonial and establish an endosymbiotic relationship with dinoflagellates of the genus *Symbiodinium*. Reef-building corals fall into one of two clades, robust and complex, which diverged from each other between ~300 and ~400 million years ago (Romano and Cairns 2000; Romano and Palumbi 1996; Stolarski et al. 2011). Species belonging to the robust clade (e.g., *Stylophora*, *Pocillopora*, *Orbicella*) tend to have heavier calcified skeletons, while those from the complex clade (e.g., *Acropora*, *Porites*, *Siderastrea*) tend to have a more porous and less calcified skeleton. Another difference is the type of asexual growth mode used. Robust corals use intratentacular budding, when a parent corallite splits in the middle of the existing corallite into two daughter corallites. However, complex corals use extratentacular budding, when a parent corallite produces a daughter corallite external to the corallite wall (Wijsman-Best 1975). These differences in colony growth modes likely account for some of the skeletal differences between these two major clades of corals.

As the divergence between robust and complex clades occurred before corals developed skeletons, calcification likely evolved multiple independent times and therefore likely resulted in clade-specific or even species-specific mechanisms. This concept may well apply to other physiological processes, having important implications for coral biology and coral's responses to environmental stress, as not all coral species may respond similarly to the same stresses. Whenever possible, this book chapter will mention species- or clade-specific mechanisms. We advise the reader to consult the primary literature for specific details and to keep in mind that many of the mechanisms discussed may vary depending on species or clade, life history, geographic location, and local environmental conditions.

7.3 Coral Anatomy, Histology, and Cytology

A coral colony is comprised of multiple anemone-like polyps that are interconnected by a tissue layer overlying the skeletal plates that join the individual corallites, called the coenosarc, and internally by their gastrovascular cavity, called the coelenteron (◘ Fig. 7.1). Although the paradigm is that all polyps from a given coral colony are genetically identical clones, recent research has found that up to 50 % of coral colonies may be mosaics of different genotypes (Schweinsberg et al. 2015). Like all cnidarians, corals are diploblastic, meaning they have two germ layers, the ectoderm and the endoderm. During embryonic development, the blastula undergoes gastrulation and turns into a larva known as planulae, which result in the subdivision of both the ectoderm and endoderm into oral and aboral tissue layers (Babcock and Heyward 1986). This tissue organization is maintained after larval settlement and growth into juvenile and adult forms, with the oral ectoderm being in contact with seawater, the oral and aboral endoderm "sandwiched" in between and separated from each other by the coelenteron, and the aboral ectoderm (calicoblastic epithelium) sitting on top of the subcalicoblastic medium (SCM) and the skeleton (◘ Fig. 7.1b, c). The oral ectoderm and gastroderm, as well as their aboral counterparts, are interconnected by the mesoglea, an extracellular matrix comprised of connective protein fibers (mostly collagen), water, and some wandering amebocytes (Phillips 1963). ◘ Figure 7.1 shows a simplified diagram of coral anatomy. An example of some of the complexity that is not shown is that the polyp gastrodermal layers form mesenterial filaments with cells specialized for food digestion and reproduction (gonads), and the oral layers surrounding the polyp's mouth are modified into tentacles with abundant nematocysts for predatory food capture and defense (Galloway et al. 2006; Veron 1993). Additionally, areas of rapid growth and calcification may only have the oral and aboral ectodermal layers, with no gastrodermal tissue in between (Jokiel 2011a).

Each of the coral's tissue layers has several cell subtypes; however, the specific physiological function(s) of each cell subtype is not completely characterized. A "generic" coral oral ectoderm contains at least six cell subtypes: ciliated support cells with abundant microvilli, nematocysts, mucocytes, pigment cells, neurons, and epitheliomuscular cells (Goldberg 2002a) (◘ Fig. 7.1c). Ciliated cells are in direct contact with seawater and interact with the diverse microbiota present in the mucus and boundary layer immediately on top of the coral, with which they may exchange diverse substances including nitrogenous compounds, amino acids, and organic acids such as sulfur (Chimetto et al. 2008; Krediet et al. 2013; Raina et al. 2010). Corals may also have endosymbiotic cyanobacteria throughout the oral ectoderm and endoderm, some of which presumably aid in nitrogen fixation (Lesser et al. 2014). It has been argued that this interaction between coral and microbes is as essential as the symbiosis between coral and *Symbiodinium*, and the term "coral holobiont" has been coined to refer to the assemblage of diverse organisms living in close association with a coral colony (the cnidarian animal, *Symbiodinium* endosymbionts, microbes and viruses, endolithic algae, as well as fungi that might be present in between and underneath the coral tissues) (Rohwer et al. 2002). Since these microorganisms are fast evolving, they may provide at least some phenotypic plasticity for the coral holobiont to adapt to rapid environmental change (Rosenberg et al. 2007). However, like most other aspects of coral cell biology, the mechanisms behind these complex processes are largely unknown.

The most prominent cells in the oral gastroderm are the *Symbiodinium* algae and the cnidarian cells that host them; here we will use the term "symbiocyte" to refer to this host cell–symbiont cellular complex. The host cell tightly surrounds the endosymbiont, so its cytoplasm and organelles are only visible using high-magnification microscopy techniques. While most symbiocytes host a single *Symbiodinium*, they may occasionally host two or even

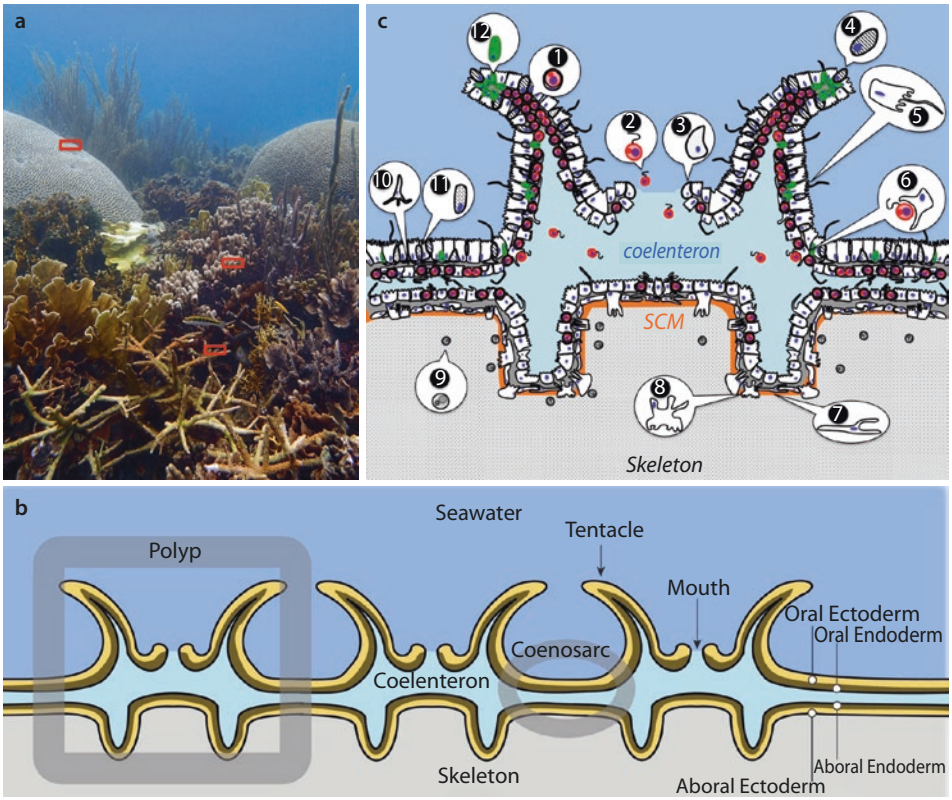

Fig. 7.1 Coral anatomy, histology, and cytology. (**a**) General view of a coral reef in Bocas del Toro, Panama. The areas enclosed in the *red boxes* are shown magnified as cartoons in b and c. (**b**) Diagram showing the different parts of a coral colony as well as the various coral tissue layers (aboral ectoderm = calicoblastic epithelium). (**c**) Coral cytology. (*1*) Symbiotic *Symbiodinium* in the symbiosome of a gastrodermal cell; (*2*) free-living *Symbiodinium*, with flagellum; (*3*) epitheliomuscular cells; (*4*) cnidocyte; (*5*) ciliated support cells; (*6*) gastrodermal cell in the process of phagocytize Symbiodinium; (*7*) calicoblastic cell; (*8*) desmocyte; (*9*) dead *Symbiodinium* encrusted in old skeleton; (*10*) neuron; (*11*) mucocyte; (*12*) pigment cell. *SCM* subcalicoblastic medium. Note: these are artistic renditions that do not necessarily reflect real cell sizes, morphology, relative proportion of the different cell types, or coral species-specific differences (Based on Allemand et al. (1998, 2011); Barott et al. (2015a), Goldberg (2001a, b, 2002a, b), Johnston (1980), Veron (1993))

three, which becomes more readily apparent in cell isolates (Barott et al. 2015b; Venn et al. 2009). Symbiocytes are morphologically and physiologically complex. *Symbiodinium* reside inside within the symbiosome, an intracellular space delimited by the host-derived symbiosome membrane that originates when algae are phagocytized from the gastrovascular cavity, which is later modified to sustain the various metabolic exchanges that make the symbiosis possible (Davy et al. 2012) (discussed in ▶ Sects. 7.6 and 7.7). Of particular importance, the symbiosome membrane must mediate the fluxes of dissolved inorganic carbon (DIC), nitrogenous compounds, and PO_4^{3-} from host cells to *Symbiodinium* and of photosynthates (e.g., organic compounds such as glycerol and glucose) in the opposite direction.

A given coral colony can host different strains or clades of *Symbiodinium*, and the specific strains and their relative abundances can dynamically change over time, potentially resulting in altered physiology and susceptibility or resistance to stress (Cunning et al. 2015a, b; Little et al. 2004). *Symbiodinium* give corals their typical brown/greenish

color, but some corals are also biofluorescent under appropriate illumination conditions (Catala-Stucki 1959) due to fluorescent proteins (FPs). These FPs are produced in pigment cells in the oral layers and stored in granules, and they may also be transported to other cell types. FPs are present throughout the coral colony, including polyp tentacles, polyp wall, and in the coenosarc (FPs are discussed in ► Sect. 7.13).

The coral oral and aboral layers are separated by the gastrovascular cavity ("coelenteron"), which internally connects polyps within a colony. The fluid in the coelenteron is circulated throughout the colony by the action of flagella and/or cilia in gastrodermal cells, likely aided by peristaltic muscular contractions (Gladfelter 1983). Although the coelenteron opens to seawater *via* the polyps' mouths, the composition of the coelenteron fluid is quite different from that of seawater. For example, the concentrations of vitamin B_{12} (riboflavin), NO_3^-, NO_2^-, NH_4^+, and PO_4^{3-} can be between 30- and 2000-fold higher in the coelenteron, while bacterial counts can be 100-fold lower (Agostini et al. 2011). Similarly, the fluid in the coelenteron has distinct levels of dissolved oxygen, as well as CO_2, pH [HCO_3^-], and [CO_3^{2-}], which are different from both the surrounding seawater and the coral cells (Agostini et al. 2011; Furla et al. 2000). Furthermore, these parameters change dramatically due to photosynthesis, calcification, and food digestion, with pH fluctuations from 8.5 to 7.5 in *S. pistillata* (Furla et al. 2000) and 7.25 to 6.6 in *Galaxea fascicularis* (Agostini et al. 2011), with the higher values being in the light and the lower values in the dark. Due to the logarithmic nature of the pH scale, these pH values represent fluctuation in [H^+] from ~3 to 30 nM in *S. pistillata* and from ~56 to 250 nM in *G. fascicularis*. The composition and chemistry of the coelenteron fluid is critical when considering calcification mechanisms and other aspects of coral physiology, including the effects of environmental stress such as ocean acidification on coral biology.

The aboral gastroderm is immediately below the coelenteron. This cell layer also contains symbiocytes, which may reach large numbers depending on the position on the coral colony (e.g., base vs. tip of a coral branch), the metabolic status, and the environmental conditions. The cytology and physiology of the aboral gastroderm is the least studied among all coral tissue layers. However, since this tissue layer lies in between the calicoblastic cells and the coelenteron, it likely serves essential functions in the exchange of DIC, Ca^{2+}, and H^+ for calcification. Similarly, the aboral gastroderm must be involved in mechanisms to transport sugars, fatty acids, and other molecules from the symbiocytes to the calicoblastic cells; however, these putative mechanisms remain unknown.

The aboral ectoderm is below the aboral gastroderm and immediately above the skeleton. This tissue layer contains calicoblastic cells and desmocytes. Both cell types seem to be responsible for building and maintaining the skeleton, and desmocytes additionally anchor the coral to the skeleton (described in detail in ► Sects. 7.8 and 7.9). The interface between calicoblastic cells and skeleton forms pockets filled with SCM, a gel-like fluid that is highly alkaline, hypersaturated with respect to aragonite, and contains a variety of proteins secreted by the coral (► see Sect. 7.10). The skeleton lies at the base of the coral colony and is made up of $CaCO_3$. The skeletal crystal structure of adult corals is almost exclusively aragonite (Wainwright 1964), but some of the first-formed elements in larval coral skeletons can be calcite (Vandermeulen and Watabe 1973). Traditionally, skeletal morphology was used for coral identification and classification. However, skeleton morphology is not a reliable indicator of coral evolutionarily history, as it can vary greatly due to environmental conditions. Current models on coral evolution are instead based on molecular phylogenetic analyses, which often do not match skeleton morphology (Fukami et al. 2004; Romano and Cairns 2000; Romano and Palumbi 1996; Stolarski et al. 2011). In addition, coral hybridization, especially during mass spawning events, makes molecular taxonomy challenging (van Oppen et al. 2001; Willis et al. 1997) and

even raises the possibility of reticulate evolution (Diekmann et al. 2001; Odorico and Miller 1997).

Coral skeletons also include organic components, although these are much less abundant compared to aragonite (~0.1 % of the total skeletal dry weight), much more heterogeneous, and show vast species-specific differences (reviewed in Johnston (1980)). Organic components of the skeletal organic matrix (SOM) include proteins, lipids, and polysaccharides. Coral skeletons also have embedded dead host tissue and *Symbiodinium*, as well as micro- and macrosopic endolithic organisms such as cyanobacteria, bacteria, filamentous green algae, fungi, boring mollusks, and sponges. This diversity often complicates determining the various components of coral skeletons and their functions (reviewed in Johnston (1980)).

7.4 Physiological Challenges Associated with Photosynthesis and Calcification

The following chemical equilibrium equations are especially relevant for coral biology and are essential for understanding some of the concepts discussed in this chapter:

i $CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-} + 2H^+$ (DIC equilibria, important for A/B balance, movement of across biological membranes, and OA, among other processes)

ii $Ca^{2+} + 2HCO_3^- \Leftrightarrow CaCO_3 + 2H^+$ (relevant for calcification)

iii $6CO_2 + 12\ H_2O + \text{Light} \Leftrightarrow C_6H_{12}O_6 + 6O_2$ (photosynthesis equation)

The symbiosis between the coral host and *Symbiodinium* endosymbionts drives the productivity of reef ecosystems. Light-driven photosynthesis by *Symbiodinium* can provide the majority of the coral's energy needs (Muscatine 1990), allowing coral reefs to thrive in otherwise oligotrophic waters. The external skeleton is another hallmark of coral reefs, which requires the transport of both Ca^{2+} and DIC to the site of calcification. Photosynthesis and calcification have unique acid/base (A/B) requirements, and meeting these requirements can greatly disturb the A/B status of coral's internal and external microenvironments. However, the A/B requirements of photosynthesis and calcification are at odds, as photosynthesis requires an acidic pH to favor the speciation of DIC into CO_2 (the substrate for carbon fixation for ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo)), while calcification requires an alkaline pH to favor CO_3^{2-} formation (the "building blocks" for the skeleton). Similarly, photosynthesis consumes H^+, but calcification generates H^+, which, respectively, alkalize and acidify the surrounding microenvironment. In fact, diel and seasonal cycles of photosynthesis/respiration and calcification/dissolution of corals and associated reefs organisms can result in large swings in pH in the surrounding seawater, especially on shallow reef flats with long residence times where diel pH ranges can be 0.5–0.6 units and seasonal changes can be 0.7–0.8 pH units (Kline et al. 2015; Shaw et al. 2012; Silverman et al. 2012).

The sources of DIC for photosynthesis and calcification are the surrounding seawater and metabolically produced CO_2. However, once again there is no unifying concept on the relative contribution of each source, as available estimates depend on the coral species, environmental conditions, and experimental techniques utilized. All of the Ca^{2+} necessary for skeleton deposition is of external origin and therefore has to pass through four (if Ca^{2+} is taken up from seawater) or two (if it is taken up from the coelenteron or in the rapidly calcifying areas mentioned above) cell layers. Based on $^{45}Ca^{2+}$ influx and efflux

kinetics, it has been suggested that all skeletal Ca^{2+} passes transcellularly through at least one coral tissue layer, presumably the calicoblastic epithelium (Tambutté et al. 1996). The proposed transcellular mechanisms include Ca^{+} channels and plasma membrane Ca^{+}-ATPases (PMCA), as well as vesicular transport of DIC, Ca^{2+}, and skeletal organic matrix proteins (detailed in ► Sect. 7.9). An essential point to consider is that Ca^{2+} plays several crucial roles in cell homeostasis such as intracellular signaling and serving as a cofactor for various enzymes (e.g., Petersen and Petersen (1994), Friedman and Gasek (1995)). Additionally, free cytoplasmic Ca^{2+} would interact with the intracellular phosphate buffer system. Thus, the transcellular transport of Ca^{2+} likely involves some sort of Ca^{2+} sequestration within coral cells to prevent Ca^{2+} toxicity. Candidate mechanisms include vesicles or Ca^{2+}-binding proteins (cytosolic or intravesicular), which could be directionally trafficked across the cell toward the SCM.

Another physiological challenge for corals is that *Symbiodinium* photosynthesis produces copious amounts of O_2 as an end product. On the contrary, the biota and microbiota associated with corals can drive O_2 levels to hypoxic (or maybe even anoxic) levels at night, depending on density and water flow (Kühl et al. 1995; Ohde and van Woesik 1999; Shashar et al. 1993). These pronounced O_2 fluctuations have important implications for coral physiology in terms of their energy budget (► see Sect. 7.12).

The lack of coral-specific tools is a major disadvantage for studies on coral cellular physiology. For example, many previous studies proposed mechanisms based on the effects of pharmacological inhibitors for proteins from mammals, which might not be specific for the homologous coral proteins. Additionally, these types of pharmacological studies cannot determine the coral cell type or layer where the presumed target proteins are located, because drugs are dissolved in the surrounding seawater and therefore can reach multiple sites within a coral colony. Another hurdle is that drugs can form complexes with the various salts present in seawater, and therefore demonstrate different activity, or require different concentrations, compared to studies using mammalian physiological saline solutions. Recent advances in coral biology are rapidly expanding the experimental toolbox. For example, the sequencing of some coral genomes (Drake et al. 2014; Shinzato et al. 2011) has made it possible to develop coral-specific antibodies and perform shotgun transcriptomic and proteomic studies to identify candidate proteins involved in various responses based on differential regulation following stress. However, there is still the need for coral cell model systems that would allow genetic manipulations such as knocking down, silencing, or knocking in genes of interest to study the functions of encoded proteins. Elucidating the cellular mechanisms behind essential coral physiological processes is of crucial importance in coral research and will be critical for understanding and predicting coral responses to stress.

7.5 Acid/Base (A/B) Regulation

Unlike larger and more active animals such as vertebrate animals, mollusks, and crustaceans, corals do not have specialized organs to regulate the A/B status of their internal fluids. Thus, corals seem to rely on intracellular pH regulation (pHi) to achieve A/B homeostasis. In addition, corals maintain unique acidic and alkaline environments in the symbiosome space (Barott et al. 2015b) and in the SCM (Al-Horani et al. 2003; Venn et al. 2011), which, respectively, promote photosynthesis and calcification.

Tight pHi regulation is vital for all organisms as enzymes and most other cellular components are highly sensitive to pH. Universal challenges to pHi balance include metabolic activity and fluctuations in external pH (pHe). Additionally, photosynthesis and calcification in reef-building corals entail unique A/B conditions in microenvironments that are located only microns apart. In addition to standard "housekeeping" pHi, symbiocytes and calicoblastic cells must have developed specialized mechanisms to maintain extreme pH conditions in the symbiosome space and SCM, respectively, and to compensate for the pHi stress resulting from photosynthesis and calcification.

Like all cells, coral cells regulate pHi by passive buffering and by active transport of A/B relevant ions across cellular membranes. Information about the buffering capacity of cnidarian cells is limited to one study on isolated gastrodermal cells of *Anemonia viridis* (Laurent et al. 2014); their buffering capacity ranged from 20.8 to 43.8 mM/pH unit, similar to cells from other invertebrates. There were few differences between *Symbiodinium*-containing and *Symbiodinium*-free gastrodermal cells, at least in the dark (Laurent et al. 2013). Like all other animal cells (Putnam and Roos 1997; Roos and Boron 1981), active pHi regulation in corals likely involves Na^+/H^+ exchangers (NHEs, SLC9 family) and HCO_3^- transporters (e.g., SLC4 and SLC26 families). Homologous genes are present in the available cnidarian genomes, but they have not been functionally characterized yet. A detailed characterization of these transporters in terms of substrate specificity, kinetics, pharmacology, regulation, and potential cell-specific expression is an essential area of future research.

7.6 DIC Transport Across the Oral Epithelium

Photosynthesis relies heavily on seawater HCO_3^-, which requires ion-transporting proteins to transport it across the apical and basolateral membranes of the oral ectoderm cells and across the symbiocytes' membrane (Allemand et al. 1998). In addition, HCO_3^- could move across the paracellular pathway (i.e., in between cells) from seawater to the symbiocytes or be taken up by the symbiocytes from the coelenteron. In any case, DIC still has to move across the symbiocyte and symbiosome membranes, as well as across various *Symbiodinium* membranes, before finally reaching RuBisCo in the chloroplast stroma and pyrenoid.

The identity of the HCO_3^--transporter proteins has just begun to be elucidated. A recent paper suggests that Slc4-like transporters present in the apical membrane of oral ectodermal cells mediate HCO_3^- uptake from seawater in *A. yongei* (Barott et al. 2015a). Apically located Na^+/K^+-ATPase (NKA) might energize the uptake of HCO_3^- (and possibly other substances) from seawater (Barott et al. 2015a). However, apical NKA (Barott et al. 2015a) and the SLC4-like transporters present in *A. yongei* (◘ Fig. 7.2) were not detectable in *S. pistillata*. Once again, this highlights potential mechanistic differences between coral species and/or coral clades.

7.7 pHi Regulation in Gastrodermal Cells

Symbiodinium photosynthesis has a strong alkalinizing effect on their gastrodermal host cells. Exposure of isolated *S. pistillata* symbiocytes to light for 20 min induced a significant increase in pHi from ~7.10 to ~7.40 pH units (Venn et al. 2009). The alkalinizing effect is abolished by DCMU (Laurent et al. 2013), a specific blocker of plastoquinone that inhibits

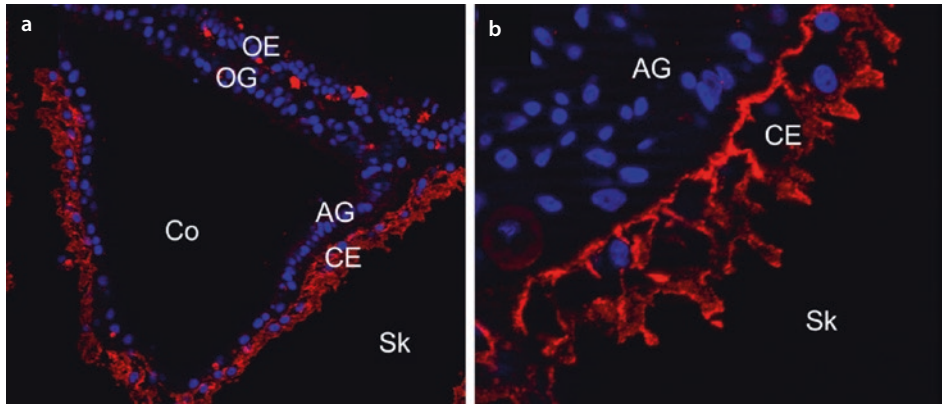

Fig. 7.2 (**a**) 320x magnification; (**b**) m 1000x magnification. Localization of an SLC4-like protein in the coral *Stylophora pistillata* (*red*)**.** Nuclei are stained in *blue*. *OE* oral ectoderm, *OG* oral gastroderm, *Co* coelenteron, *AG* aboral gastroderm, *CE* calicoblastic ectoderm, *Sk* skeleton (Methods followed those described in Barott et al. (2015a))

photosynthesis, confirming that the alkalinization was caused by *Symbiodinium* photosynthesis. Additionally, alkalinization occurred more quickly and reached higher values (maxing out at ~7.46) in cells exposed to irradiance levels of 300 μmol photons $m^{-2}s^{-1}$ or higher (Laurent et al. 2013). Similar effects have been reported in isolated *P. damicornis* cells (Gibbin et al. 2014). Host cytoplasm alkalinization is caused by CO_2 removal by *Symbiodinium* photosynthesis and possibly by OH^- secretion by *Symbiodinium* to the host cytoplasm (Allemand et al. 1998; Venn et al. 2009). Indeed, as photosynthesis draws down CO_2, HCO_3^- and H^+ are converted to CO_2 following the DIC equilibrium reaction, and the decline in $[H^+]$ leads to alkalization. To prevent pHi from rising further, symbiocytes would need to excrete OH^- and/or HCO_3^- or uptake H^+ and/or CO_2. For example, anemone endoderm cells may excrete OH^- into the coelenteron upon light exposure (or uptake H^+) (Furla et al. 1998), and coral symbiocytes potentially transport HCO_3^- into the symbiosome as part of a carbon-concentrating mechanism (CCM) for photosynthesis (Barott et al. 2015b).

7.7.1 pH of the Symbiosome

In *S. pistillata* and *A. yongei*, the host-derived symbiosome membrane abundantly expresses vacuolar H^+-ATPase (VHA), a proton pump whose activity helps acidify the symbiosome to pH ~4 (Barott et al. 2015b). Acidification of the symbiosome space surrounding the zooxanthellae is part of a coral CCM that promotes *Symbiodinium* photosynthesis (Barott et al. 2015b). As *Stylophora* and *Acropora* belong to different coral clades, it is likely that this CCM is universal among coral species. The ultimate goal of the proposed CCM is to increase CO_2 to high-enough levels to sustain photosynthesis, which is required due to the low affinity of dinoflagellate RuBisCo for CO_2 over O_2 (Rowan et al. 1996). However, there are no known cellular mechanisms to actively transport gases such as CO_2, and at a pHi of ~ 7.10 (Venn et al. 2009), the most abundant form of DIC available inside coral gastrodermal cells would be in the form of HCO_3^-. Thus, DIC transport across biological membranes must happen by transporting H^+ and HCO_3^-, which can then

dehydrate into CO_2 (and H_2O) in a reaction reversibly catalyzed by carbonic anhydrase (CA). Experiments using pharmacological drugs support the involvement of CAs, as inhibition of CA by Diamox reduces photosynthetic carbon assimilation by zooxanthellae by up to 85 % (Weis et al. 1989). However, CAs alone are not sufficient to transport DIC against its concentration gradient, which is a requirement for a CCM. This is where VHA becomes relevant by using energy from adenosine triphosphate (ATP) hydrolysis to transport H^+ against its electrochemical gradient ("pumping"), which presumably drives the transport of HCO_3^- into the symbiosome space by yet unidentified transporter proteins. Supporting this model, inhibition of VHA by bafilomycin resulted in an increase of the symbiosome pH by ~0.6 units (a ~70 % change in $[H^+]$) in *S. pistillata* isolated symbiocytes, as well as a reduction in O_2 production by ~30 % in *S. pistillata* colonies, and as high as an 80 % reduction in O_2 production in *A.yongei* colonies (Barott et al. 2015b). A potential additional source of H^+ transport into the symbiosome is the P-type H^+-ATPase, which is expressed by *Symbiodinium* only during symbiosis (Bertucci et al. 2010).

Elucidating the mechanisms of HCO_3^- transport across the symbiosome membrane is an important area of future research. Furthermore, once inside the symbiosome space, CO_2 still has to move across several other *Symbiodinium* membranes before reaching RuBisCo in the chloroplast stroma and pyrenoid; these mechanisms are also largely unknown. Finally, symbiosome acidification could help drive the transport of other molecules between host cells and *Symbiodinium*, as hypothesized in Barott et al. (2015b).

7.8 DIC Transport and pH Regulation in Calcifying Cells

The calicoblastic cells play a vital role in skeleton formation by promoting calcification through elevating pHe in the SCM, transporting DIC and Ca^{2+}, and excreting SOM proteins that promote $CaCO_3$ precipitation (reviewed in Cohen and McConnaughey (2003), Allemand et al. (2004), Johnston (1980)).

The pH of the SCM ranges between 8.1 and 8.4 pH units in the dark and between 8.7 and 9.3 pH units in the light, depending on the coral species and method used (Al-Horani et al. 2003; Venn et al. 2011). At those pH values, the calculated saturation state of aragonite is 20–30-fold hypersaturated in the light and 3–11-fold in the dark. However, the pHi of calicoblastic cells remains stable at ~7.4 in both light and dark conditions (Venn et al. 2011). This indicates that calicoblastic cells are capable of excreting HCO_3^- into the SCM against a steep electrochemical gradient and removing H^+ while also maintaining a stable pHi.

The sources and species of DIC used for calcification are not completely understood but may include CO_2, HCO_3^-, and CO_3^{2-} from seawater or metabolic origin that are ultimately transported and concentrated in the SCM (Allison et al. 2014). Based on immunolocalization data, members of the SLC4 family of HCO_3^- transporters may excrete HCO_3^- into the SCM both in *A. yongei* (Barott et al. 2015a) and *S. pistillata* (Zoccola et al. 2015). However, convincing functional data is not available due to the issues with drug specificity and site of action outlined in ► Sect. 7.2. The driving force for HCO_3^- secretion, and most likely many other transport processes, seems to be provided by NKA that is abundantly expressed in the basolateral membrane of calicoblastic cells from both *A. yongei* and *S. pistillata* (Barott et al. 2015a).

The contribution of metabolic CO_2 to the skeleton's aragonite has been estimated to be as high as 83 % (Erez 1978; Furla et al. 2000; Hughes et al. 2010). Metabolic CO_2 is chiefly produced during respiration in mitochondria, which can be abundantly present through-

out coral tissues including calicoblastic cells (Barott et al. 2015b). This CO_2 could be rapidly hydrated into HCO_3^- by CA in the cell cytosol and exported into the SCM. The resulting H^+ would then be exported across the basolateral membrane into the mesoglea and eventually to the coelenteron. However, other studies have concluded that the respiration rate of corals is too low to significantly contribute DIC to the skeleton (e.g., Falkowski et al. (1984)).

7.9 Ca^{2+} Transport

Despite its central importance in coral biology, the mechanisms for Ca^{2+} transport in corals remain poorly understood. The route of Ca^{2+} transport from seawater to the coelenteron across the oral epithelium has been proposed to be either paracellular (Furla et al. 2000; Tambutté et al. 1996) or transcellular (Clode and Marshall 2002). A contribution of both routes, as well as of species-specific differences, is possible. Ectodermal cells in the oral and calicoblastic layers are connected by septate junctions (Barott et al. 2015a; Clode and Marshall 2002; Ganot et al. 2015; Johnston 1980), which limit the passage of ions and other molecules across the coral epithelium. Electrophysiology of coral microcolonies indicates that the coral epithelium has intermediate resistance, falling on the leaky end of vertebrate tight junctions' continuum but still "tighter" than leaky junctions. However, the relative contribution of the different coral tissue layers to coral epithelial resistance could not be differentiated due to methodological limitations (Tambutté et al. 2011).

There is evidence that all skeletal Ca^{2+} passes through at least one coral tissue layer (Tambutté et al. 1996). An L-type voltage-dependent Ca^{2+} channel was immunolocalized using heterologous antibodies in both the oral and aboral ectoderm of the coral *S. pistillata*, suggesting it functions to take up Ca^{2+} from seawater and export it into the SCM (Zoccola et al. 1999). In addition, pharmacological inhibitors of Ca^{2+} channels reduced calcification rates. However, due to their typically slow inactivation time and short mean open time, L-type Ca^{2+} channels are primarily involved in Ca^{2+} homeostasis and Ca^{2+} signaling in other organisms (Hosey and Lazdunski 1988). Although this does not rule out a role for L-type Ca^{2+} channels in coral calcification, it does highlight the need for studies on the kinetics and specificity of coral Ca^{2+} channels in order to better determine their physiological functions.

Plasma membrane Ca^{2+}-ATPases (PMCA) have been proposed to export Ca^{2+} across the apical membrane of calicoblastic cells in exchange for H^+ (Zoccola et al. 2004, Allemand et al. 2011, Davy et al. 2012, Cohen et al. 2009). Thus, PMCA could both deliver the Ca^{2+} needed for calcification and remove protons produced during $CaCO_3$ precipitation to maintain the alkaline pH of the SCM. However, this model is largely based on results from pharmacological inhibition, in this case with ruthenium red (Al-Horani et al. 2003; Ip et al. 1991; Marshall 1996). Although PMCA messenger RNA (mRNA) is present in calicoblastic cells, it is also abundantly expressed throughout other coral cell layers (Zoccola et al. 2004). Moreover, a recent immunolocalization study found PMCA protein in the cytoplasm and not the membrane of calicoblastic cells in *A. yongei* and *S. pistillata* (Barott et al. 2015a), further questioning the role of PMCA in Ca^{2+} excretion and H^+ removal. In *A. yongei*, PMCA protein was present throughout all coral tissue layers, with more intense localization in the cytoplasm of calicoblastic cells followed by the apical pole of cells in the oral ectoderm. PMCA protein was similarly present throughout all coral tissue layers of *S. pistillata*; however, it was most abundant in gastrodermal cells, more specifically in the cytoplasmic area facing the coelenteron.

Abundant vesicles have been observed in the calicoblastic cells of multiple species of corals, in some cases pinching in and out at the basolateral and apical membranes of calicoblastic cells (Barott et al. 2015a; Isa 1986; Johnston 1980). Furthermore, inhibitors of actin and tubulin polymerization reduced Ca^{2+} incorporation into the skeleton, suggesting trafficking of intracellular vesicles is required for calcification (Tambutté et al. 1996). However, these results could also be explained by effects on the trafficking of HCO_3^- or H^+ transporters (see (Tresguerres et al. 2006) for an example of transepithelial HCO_3^- secretion in shark gills).

7.10 Skeletal Organic Matrix (SOM)

In addition to $CaCO_3$ in the form of aragonite, coral skeletons have numerous proteins, lipids, and polysaccharides that are collectively referred as the skeletal organic matrix (SOM). A majority of the SOM components seem to be synthesized and secreted by calicoblastic cells (Puverel et al. 2004), although some precursors of the SOM may be synthesized by *Symbiodinium* or other coral tissues. Not surprisingly, significant differences in SOM composition exist between coral species (Johnston 1980; Puverel et al. 2005).

The SOM has been proposed to be especially important in the early mineralization phase, as "substrate, catalyst, or controlling agent in the chemical reactions that culminate in the deposition of new skeletal material" (Johnston 1980). Organic components present in deeper parts of the skeleton could be remnants from the early calcification process, or they could play additional structural roles such as modifying skeletal mechanical properties.

The SOM confers the skeleton with chemical properties that are different from pure $CaCO_3$; for example, some coral acid-rich proteins (CARPs) recently identified in the SOM of *S. pistillata* are able to spontaneously catalyze $CaCO_3$ precipitation in vitro, even at a seawater pH of 7.6 (Mass et al. 2013). Immunolabeling confirmed that at least four CARPs are present in calicoblastic cells and embedded in skeletal aragonite crystals but also in noncalcifying coral tissues suggesting roles other than calcification (Mass et al. 2014).

Another interesting protein found in the SOM is a secreted form of CA, hypothesized to play roles in calcification and A/B regulation in calicoblastic cells and the SCM (Moya et al. 2008). The SCM, which is the microenvironment where calcification occurs, clearly is vastly different from bulk seawater due to significant biological control by the coral tissues.

7.11 Light-Enhanced Calcification

The stimulatory effect of light on coral calcification was noted as early as the 1930s (Kawaguti 1937), subsequently demonstrated chemically by measuring the disappearance of Ca^{2+} from seawater (Kawaguti and Sakumoto 1948), and later corroborated by radioisotope studies showing incorporation of $^{45}Ca^{2+}$ from seawater into the coral skeleton (Goreau 1959; Goreau and Goreau 1959). This phenomenon, which was termed light-enhanced calcification (Chalker and Taylor 1975), was subsequently confirmed across multiple coral species (reviewed in Allemand et al. (1998), Allemand et al. (2011), Cohen and Holcomb (2009), Gattuso (1999), Johnston (1980)). Despite a few exceptions (e.g., Wijgerde et al. (2012, 2014)) (which might be explained by husbandry and/or illumination conditions), light-enhanced calcification seems to be a universal phenomenon among corals.

The physiological mechanisms supporting light-enhanced calcification are not completely understood, but all hypotheses proposed over the last 80 years are based on *Symbiodinium* photosynthetic activity facilitating calcification in one or multiple ways. Thus, the term "photosynthetically enhanced calcification" (Chalker and Taylor 1975) seems more appropriate. Some possible mechanisms for light-enhanced calcification include photosynthesis providing (a) carbohydrates as fuel to support the energetic cost of transporting DIC to the SCM and maintaining its alkaline pH, (b) O_2 to support aerobic metabolism, (c) a supply of SOM precursors, and (d) for the removal of calcification waste products such as CO_2, H^+, PO_4^{3-}, and NH_4^+ (reviewed in Allemand et al. (2011), Chalker and Taylor (1975), Johnston (1980)). Clearly, the ability to study any of these hypotheses requires the elucidation of the basic mechanisms for calcification, energy metabolism, and metabolic communication between coral host cells and *Symbiodinium*.

7.12 Coral Energy Metabolism

Symbiodinium translocate the majority of net-fixed carbon to the host, in some cases >95 % (Davies 1984; Falkowski et al. 1984; Muscatine et al. 1984). Under high light conditions, autotrophy from *Symbiodinium* photosynthesis can supply over 100 % of a coral's daily energy requirements (Falkowski et al. 1984). Most of the translocated carbon is immediately respired by the host, but a considerable amount (potentially as high as 50 %) is lost in coral mucus secretions (Crossland et al. 1980; Muscatine et al. 1984). The role of mucus is unknown but could be related to aiding in feeding; cleaning; staving off epiphytic, epizoic, and bacterial growth (Ducklow and Mitchell 1979; Muscatine 1973); protecting against damage (Benson et al. 1978); and getting rid of excess translocated carbon (Davies 1984). In any case, coral mucus release likely represents a significant source-dissolved organic carbon for reef microbial communities (Muscatine et al. 1984; Wild et al. 2004).

Originally, glycerol was believed to be the main organic compound supplied by *Symbiodinium* (Muscatine and Cernichiari 1969); however, more recent results indicate it is in fact glucose (Burriesci et al. 2012). Either way, both glycerol and glucose are nitrogen deficient and can sustain metabolic respiration but cannot be used to build new tissue (Davies 1984; Falkowski et al. 1984). Thus, corals must obtain nitrogenous compounds from heterotrophic feeding on zooplankton, bacteria, and sessile organisms, which they do using cnidocysts, mesenterial filament eversion and extrusion, and mucus trapping (Goreau et al. (1971) and references therein). Corals can also absorb PO_4^{3-}, amino acids, and other micronutrients directly from seawater (Goreau et al. 1971). The relative contribution of autotrophy and heterotrophy likely varies according to species and dynamic changes in environmental conditions such as light, *Symbiodinium* abundance and type, and nutrient availability.

Corals experience large daily variations in O_2 levels due to *Symbiodinium* photosynthesis during the day (which can increase O_2 concentrations in coral tissues and their diffusive boundary layer to over 4x higher than the surrounding seawater) and to respiration of reef organisms and microorganisms during the night that can result in very low O_2 levels (hypoxia) or even lack thereof (anoxia) (Kühl et al. 1995; Ohde and van Woesik 1999; Shashar et al. 1993). This implies that corals are able to dynamically switch between aerobic and anaerobic respiration; however, virtually nothing is known about coral anaerobic metabolic pathways and their relative contribution to the coral energy budget.

Because hypoxia and anoxia likely also occur during stressful conditions that reduce *Symbiodinium* abundance, the capacity to generate energy using anaerobic metabolic pathways might be especially important in determining a coral species' ability to survive mass-bleaching events.

7.13 Coral Fluorescence

Cnidarians, including corals, display biofluorescence under appropriate illumination conditions (Catala-Stucki 1959). Originally, coral fluorescence was proposed to play roles in UV protection and optimization of algal productivity (Ben-Zvi et al. 2015; Dawson 2007; Kawaguti 1969; Schlichter et al. 1994). As part of these studies, fluorescent mycosporine-like amino acids were discovered; however, these are found in the secreted mucus, and their fluorescence is relatively weak and labile. Subsequent research demonstrated that coral biofluorescence is largely due to endogenous fluorescent proteins (FPs).

The green fluorescence protein (GFP) was originally found associated with the luminous jellyfish *Aequorea victoria* (Shinomura et al. 1962) and was proposed to play a role in mimicry by changing the bioluminescence color of luciferase from blue (best for color dispersion in deeper waters) to green (best in shallower water) *via* Förster Resonance Energy Transfer (Ohmiya and Hirano 1996; Haddock et al. 2009). More recently, FPs were found in corals and sea anemones that do not produce bioluminescence (Matz et al. 1999; Wiedenmann et al. 2004). Although FPs are predominantly green, other colors have also evolved ranging from blue to red (Labas et al. 2002; Sabine et al. 2004; Alieva et al. 2008), and FPs of up to four colors have been found in a single coral (Kelmanson and Matz 2003). In addition, some proteins in this family, called chromoproteins, absorb but do not emit light (Dove et al. 1995, 2001). Altogether, FPs are one of the main pigments in cnidarians and give them a diversity of fluorescent colors (Oswald et al. 2007).

Adult corals express FPs throughout the colony, including in polyp tentacles, body wall, and coenosarc. The fluorescence intensity is usually stronger in the growing tips of corals, probably because the reduced *Symbiodinium* abundance in the coral tips results in less shading of FP fluorescence. FPs are also expressed in the eggs, larvae, and juveniles of many coral species (Kenkel et al. 2011; Roth et al. 2013); however, the specific fluorescence color may change throughout the coral life cycle.

The precise cellular localization of FPs remains a challenge (Leal et al. 2015). In many cnidarians, FPs occur largely in granules of coral pigments; however, FPs could also be transported across tissues in corals, as described in other organisms (Hanson and Kohler 2001; Schonknecht et al. 2008; McLean and Cooley 2013). FPs can represent up to 14 % of the soluble protein content in anthozoans and have slow decay lives with half-lives of about 20 days (Leutenegger et al. 2007). Furthermore, FPs can be accumulated at various stages of protein maturity, possibly building up as a nonfluorescent premature stage before maturing and becoming fluorescent following oxidation of the chromophore (Leutenegger et al. 2007). Thus, large amounts of FPs could be produced and stored in pigment cells but be readily available readily to other coral tissues where they might play different physiological functions.

Despite abundant information about FPs' spectral and biochemical properties in vitro, FP in vivo functions remain unclear. Since FPs can absorb shorter, potentially damaging high-energy wavelengths of light and transform them into longer, lower energy wavelengths that are beneficial for photosynthesis, two of the most likely FP functions are pho-

toprotection (Roth et al. 2010; Salih et al. 2000; Smith et al. 2013) and photoenhancement of *Symbiodinium* photosynthesis (Dove et al. 2008; Kawaguti 1969; Roth 2014). Additionally, some FPs display antioxidant activity in vitro (Bou-Abdallah et al. 2006; Bomati et al. 2009), suggesting that they protect corals from reactive oxygen species generated as by-products of *Symbiodinium* photosynthesis in vivo and other sources of oxidative stress.

Fluorescence is dynamic in corals, being up- or downregulated as a function of life stage (Kenkel et al. 2011), presence of trematode parasites (Palmer et al. 2009), and various environmental conditions. For example, corals exposed to different intensities of light showed associated changes in FPs, with exposure to higher light levels resulting in larger FP abundance and greater fluorescence intensity (Roth et al. 2010). Similarly, a temperature shock (either cold or warm) induced a rapid decrease in fluorescence, which was detectable earlier than a RGB color change (Roth and Deheyn 2013; Roth et al. 2012). Fluorescence intensity returned to normal levels after corals adjusted to the cold shock or became more intense after continued exposure to warm water that induced bleaching; this was due to a loss of light shading by *Symbiodinium*. Similar declines in fluorescence level were observed under other stressors such as anoxia (Haas et al. 2014) and light intensity (Roth et al. 2010, 2013). Most likely, the decrease in fluorescence is related to the biochemical antioxidant property of GFP. Indeed, GFP otherwise is a very stable protein with a pKa between 5 and 6 that is sheltered from changes in the surrounding biochemical environment, except for its susceptibility to free radicals. These studies suggest that changes in fluorescence can be used as a proxy for physiological adjustments associated with oxidative stress in corals.

7.14 Potential Role of cAMP in Regulating Coral Physiology

The cyclic adenosine 3'5' monophosphate (cAMP) pathway can modulate virtually every aspect of cell biology by regulating the activity of target proteins *via* protein kinase A-dependent phosphorylation, exchange proteins activated by cAMP, and cyclic nucleotide-gated channels (Wong & Scott, 2004). The cAMP pathway seems to have a special significance in corals, as homogenates of *A. yongei* and *P. damicornis* have the highest cAMP production rates ever recorded for any organism: 17,000 pmol mg^{-1} min^{-1} and 30,000 pmol mg^{-1} min^{-1}, respectively (Barott et al. 2013). Furthermore, cAMP levels in *P. damicornis* fluctuate with the light/dark cycle, reaching their highest values during the day and the lowest at night (Barott et al. 2013). However, the physiological roles of cAMP in corals are virtually unknown.

Given the importance of A/B status on coral photosynthesis and calcification and the dynamic changes in A/B status corals experience during the diel cycle, corals must be able to sense A/B status to adjust and coordinate their physiology accordingly. A potential A/B sensor in corals is soluble adenylyl cyclase (sAC), an evolutionarily conserved HCO_3^--sensitive enzyme that produces cAMP (Buck et al., 1999; Chen et al., 2000). In vivo, sAC can potentially sense intra- and extracellular CO_2, pH, and HCO_3^- levels and trigger a variety of physiological responses *via* the cAMP pathway (reviewed in Tresguerres (2014), Tresguerres et al. (2010a, 2011, 2014)). Evidence for sAC presence in corals is available in genomic and transcriptomic databases from *A. digitifera* (Shinzato et al. 2011) and *S. pistillata* (Karako-Lampert et al. 2014); however, these putative sAC sequences have not been validated, and the activity of the encoded proteins has not been characterized.

This task, which is essential before characterizing sAC physiological roles in corals, is greatly complicated by the presence of multiple isoforms and splice variants, as previously described for mammals (Farrell et al. 2008; Geng et al. 2005). There is, however, strong biochemical evidence for sAC in corals: cAMP production in homogenates is stimulated by HCO_3^- in a dose–response manner, and the HCO_3^--stimulated cAMP activity is inhibited by KH7 (Barott et al. 2013), a pharmacological inhibitor of sAC in mammals (Hess et al. 2005) and sharks (Tresguerres et al. 2010b). Given the A/B physiology of corals, sAC is a potential regulator of coral calcification, photosynthesis, pHi and pHe, and metabolism, among other functions. Other potential A/B sensors found in other organisms include H^+-sensing G-protein-coupled receptors, transient receptor potential channels, and acid-sensing ion channels (reviewed in Tresguerres et al. (2010a)).

7.15 Effects of Ocean Acidification (OA) on Coral Biology

The oceans have absorbed over 25 % of the CO_2 emitted to the atmosphere since the beginning of the industrial revolution (Bindoff et al. 2007; Millero 2007; Sabine et al. 2004). Dissolved CO_2 reacts with water to create H_2CO_3, which in turn dissociates into H^+ (and HCO_3^-), which has resulted in a decline in surface open ocean pH of approximately 0.1 pH units. In addition to elevated CO_2 and reduced pH, OA reduces $[CO_3^{2-}]$ in seawater when H^+ combines with CO_3^{2-} to form HCO_3^-. This phenomenon has been termed ocean acidification (OA) (Caldeira and Wickett 2003; Feely et al. 2004).

The current atmospheric CO_2 concentration of 400 parts per million is the highest observed in the last 800,000 years (Lüthi et al. 2008) and possibly since the middle Pliocene (~3MYA). This rate of increase is likely 2–3 orders of magnitude faster than most natural CO_2 excursions known via geohistory (Beerling and Royer 2011; Pagani et al. 2010). The rate of OA will match the increase in atmospheric CO_2, raising concerns on whether marine organisms can cope. Calcifying organisms such as corals are believed to be particularly vulnerable to OA because reduced seawater $[CO_3^{2-}]$ might mean fewer "building blocks" available to build calcium carbonate skeletons (Kleypas and Langdon 2006) and reduced pH increases the dissolution of dead skeleton, reef framework, and sediments (Andersson and Gledhill 2013; Silverman et al. 2009).

The biological mechanisms described in previous sections of this book chapter indicate that OA impacts on corals are likely much more complex. Five characteristics of coral biology are particularly important when predicting the potential effects of OA on corals: (1) most of the coral skeleton is not directly exposed to seawater, but instead it is underneath four tissue layers and the coelenteron (► Sect. 7.2); (2) the DIC source for coral skeletons is predominantly CO_2 and HCO_3^- from seawater and metabolic origin, rather than CO_3^{2-} from seawater (► Sects. 7.5 and 7.8); (3) the actual site of coral calcification (the SCM) is highly alkaline, has elevated Ω_{ARG}, and SOM proteins that make it extremely different chemically from the surrounding seawater (► Sects. 7.8 and 7.10); (4) as a result of photosynthesis, respiration, calcification, and dissolution (► Sect. 7.3), corals induce daily changes in the pH and DIC status of their immediate environment that are much more pronounced than those predicted due to OA in the surface open ocean; and (5) elevated CO_2 levels associated with OA could actually promote *Symbiodinium* photosynthesis, which might mean more energy transferred to the corals (► Sects. 7.7 and 7.12).

The "proton flux" hypothesis proposes that the negative effect of OA on coral calcification is caused by the reduced H^+ gradient between coral tissues and seawater, which

requires corals to spend more energy to secrete the excess H^+ generated during calcification (Jokiel 2011a, b, 2013; Ries 2011). This hypothesis could explain reduced calcification rates under OA from an energetic point of view. In addition, other studies (e.g., Castillo et al. (2014)) have suggested that increased *Symbiodinium* photosynthesis as a result of elevated CO_2 and subsequent translocation of carbon compounds to the host could offset the increased energetic demand of calcification.

It is also important to consider the experimental challenges and limitations associated with OA research on corals. To date, the majority of studies on the effects of OA on coral calcification have been manipulative experimental studies in aquarium or mesocosms in which the corals were removed from their natural ecosystem and placed under artificial light, seawater, nutrition, and flow conditions. Extrapolating these results to the ecosystem scale and different future scenarios becomes difficult because many variables impact corals synergistically with elevated CO_2 conditions, which often lead to nonlinear effects. For example, several studies suggest that a coral's response to OA is highly dependent on food supply (Cohen and Holcomb 2009; Langdon and Atkinson 2005), temperature (Anthony et al. 2008), and to natural diel and seasonal variability of the carbonate chemistry (Bates et al. 2010; Dufault et al. 2012; Kline et al. 2015; Price et al. 2012). Another limitation of aquarium and mesocosm studies is their duration, which typically ranges from days to only a few months and therefore cannot investigate long-term impacts. These points help explain why OA studies have reported negative, neutral, and even "positive" effects on corals depending on the species and experimental setup (reviewed in Kroeker et al. (2010)).

In situ studies are a critical complement to aquarium and mesocosm findings, as they can include much more natural conditions such as light, nutrition, and environmental variability. The two main types of in situ experimental methods that are commonly used to assess OA impacts are observational studies of natural systems with high-CO_2 levels such as vent/seep sites and upwelling areas, and semi-enclosed controlled CO_2 manipulative experiments. Studies in natural vent/seep and upwelling regions are beginning to provide data about the long-term impacts of OA on organisms, communities, and ecosystems (Fabricius et al. 2011; Hall-Spencer et al. 2008; Kroeker et al. 2013) and are one of the few approaches that can assess multiyear-long and potentially evolutionary responses to OA. However, there is no control over the carbonate chemistry, and there may be other unmeasured variables that influence the results (Andersson et al. 2015). Fully enclosed in situ studies have been previously used to measure reef metabolism (Yates and Halley 2003), and results across different coral reef zones suggest that reef flats would shift to net dissolution at pCO_2 levels between 470 and 1000 ppm (Yates and Halley 2006). However, the duration of this approach is limited because the enclosed community has major biogeochemical feedbacks to the seawater chemistry and waste products' buildup after 1–2 days. An emerging and highly promising approach is to perform controlled in situ manipulative experiments with semi-enclosed replicate Free Ocean Carbon Enrichment (FOCE) flumes (Gattuso et al. 2014; Kline et al. 2012; Marker et al. 2010). FOCE style experiments allow for controlled CO_2 manipulations that can be performed as a controlled offset in pH from ambient, with natural food, diel and seasonal carbonate chemistry variability and natural environmental conditions. Additionally FOCE style experiments make it possible to address critical questions that are unanswerable in aquariums or mesocosms such as the study of ecological interactions such as herbivory or competition and OA impacts on feeding and overall energy budgets.

In addition to impairing calcification, OA has been suggested to have negative effects on many other aspects of coral biology, including sperm flagellar motility (Morita et al. 2010), fertilization, larvae settlement (Albright et al. 2010) and metamorphosis (Nakamura et al. 2011), and energy metabolism (Kaniewska et al. 2012; Vidal-Dupiol et al. 2013). The mechanistic bases behind these effects are unknown but likely are related to A/B disturbances in the coral intracellular environment. However, when analyzing the relevance of this type of studies, one must carefully analyze the conditions used as commonly the experimental CO_2/pH levels are too extreme, are administered too rapidly, are applied as a constant level even if the corals are from a dynamic environment, or are applied over very short periods of time, which do not match realistic OA conditions. Similarly, conclusions based on transcriptomics studies must be taken with caution due to potential mismatches between mRNA abundance and protein abundance and enzymatic activity (Rocca et al. 2015), posttranslational modifications not detectable by transcriptomics, and lack of information about the specific cell types where mRNA changes take place.

7.16 Conclusions

Despite their relatively simple body plan and their basal position within the phylogenetic tree, corals are complex at both the cellular and molecular level. Corals possess several specialized cell types that achieve unique and essential physiological functions, for example, symbiotic metabolic exchanges with *Symbiodinium* or deposition of massive skeletons that provide the foundation for coral reefs. The cellular and molecular mechanisms behind these functions are mostly unknown; however, recent technological advances are making it possible to study coral cellular and molecular biology at unprecedented levels of detail. In addition to being a fascinating area of research in its own right, mechanistic information about coral cell biology is essential for better understanding and predicting coral-general and species-specific responses to ocean warming, acidification, pollution, and disease.

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References

Agostini S, Suzuki Y, Higuchi T, Casareto BE, Yoshinaga K, Nakano Y, Fujimura H (2011) Biological and chemical characteristics of the coral gastric cavity. Coral Reefs 31:147–156

Al-Horani FA, Al-Moghrabi AM, De Beer D (2003) Microsensor study of photosynthesis and calcification in the scleractinian coral, *Galaxea fascicularis*: active internal carbon cycle. J Exp Mar Biol Ecol 288:1–15

Albright R, Mason B, Miller M, Langdon C (2010) Ocean acidification compromises recruitment success of the threatened Caribbean coral *Acropora palmata*. Proc Natl Acad Sci U S A 107:20400–20404

Alieva NO, Konzen KA, Field SF, Meleshkevitch EA, Hunt ME, Beltran-Ramirez V, Miller DJ, Wiedenmann J, Salih A, Matz MV (2008) Diversity and evolution of coral fluorescent proteins. PLoS One 3(7):e2680. doi:10.1371/journal.pone.0002680

Allemand D, Furla P, Bénazet-Tambutté S (1998) Mechanisms of carbon acquisition for endosymbiont photosynthesis in Anthozoa. Can J Bot 76:925–941

Allemand D, Ferrier-Pagès C, Furla P, Houlbrèque F, Puverel S, Reynaud S, Tambutté E, Tambutté S, Zoccola D (2004) Biomineralisation in reef-building corals: from molecular mechanisms to environmental control. Comptes Rendus Palevol 3:453–467

Allemand D, Tambutté E, Zoccola D, Tambutté S (2011) Coral calcification, cells to reefs. In: Dubinsky Z, Stambler N (eds) Coral reefs an ecosystem in transition. Springer, New York, pp 119–150
Allison N, Cohen I, Finch AA, Erez J, Tudhope AW, Edinburgh Ion Microprobe Facility (2014) Corals concentrate dissolved inorganic carbon to facilitate calcification. Nat Commun 5:5741
Andersson AJ, Gledhill D (2013) Ocean acidification and coral reefs: effects on breakdown, dissolution, and net ecosystem calcification. Ann Rev Mar Sci 5:321–348
Andersson AJ, Kline DI, Edmunds PJ, Archer SD, Bednaršek N, Carpenter RC, Chadsey M, Goldstein P, Grottoli AG, Hurst TP et al (2015) Understanding ocean acidification impacts on organismal to ecological scales. Oceanography 28:16–27
Anthony KR, Kline DI, Diaz-Pulido G, Dove S, Hoegh-Guldberg O (2008) Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc Natl Acad Sci U S A 105:17442–17446
Babcock RC, Heyward AJ (1986) Larval development of certain gamete-spawning scleractinian corals. Coral Reefs 5:111–116
Barott KL, Helman Y, Haramaty L, Barron ME, Hess KC, Buck J, Levin LR, Tresguerres M (2013) High adenylyl cyclase activity and in vivo cAMP fluctuations in corals suggest central physiological role. Sci Rep 3:1–7
Barott KL, Perez SO, Linsmayer LB, Tresguerres M (2015a) Differential localization of ion transporters suggests distinct cellular mechanisms for calcification and photosynthesis between two coral species. Am J Physiol Regul Integr Comp Physiol 309:R235–R246
Barott KL, Venn AA, Perez SO, Tambutté S, Tresguerres M (2015b) Coral host cells acidify symbiotic algal microenvironment to promote photosynthesis. Proc Natl Acad Sci U S A 112:607–612
Bates NR, Amat A, Andersson A (2010) Feedbacks and responses of coral calcification on the Bermuda reef system to seasonal changes in biological processes and ocean acidification. Biogeosciences 7: 2509–2530
Beerling DJ, Royer DL (2011) Convergent Cenozoic CO_2 history. Nat Geosci 4:418–420
Ben-Zvi O, Eyal G, Loya Y (2015) Light-dependent fluorescence in the coral *Galaxea fascicularis*. Hydrobiologia 759:15–26
Benson AA, Patton JS, Abraham S (1978) Energy exchange in coral reef ecosystems. Atoll Res Bull 220:33–54
Bertucci A, Tambutté E, Tambutté S, Allemand D, Zoccola D (2010) Symbiosis-dependent gene expression in coral-dinoflagellate association: cloning and characterization of a P-type H^+–ATPase gene. Proc Biol Sci 277:87–95
Bindoff NL, Willebrand J, Artale V, Cazenave A, Gregory J, Gulev S (2007) *Observations: oceanic climate change and sea level*. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt K, Tignor M (eds) Climate change 2007: the physical science basis contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, New York
Bomati EK, Manning G, Deheyn DD (2009) Amphioxus encodes the largest known family of green fluorescent proteins, which have diversified into distinct functional classes. BMC Evol Biol 9:1–11
Bou-Abdallah F, Chasteen ND, Lesser MP (2006) Quenching of superoxide radicals by green fluorescent protein. Biochim Biophys Acta Gen Subj 1760(11):1690–1695
Bourne GC (1900) The anthozoa. In: Lankester ER (ed) A treatise on zoology. Part II. The porifera and coelenterata. Adam & Charles Black, London, pp 1–84
Buck J, Sinclair ML, Schapal L, Cann MJ, Levin LR (1999) Cytosolic adenylyl cyclase defines a unique signaling molecule in mammals. Proc Natl Acad Sci 96:79–84
Burke L, Reytar K, Spalding M, Perry A (2011) Reefs at risk revisited. World Resources Institute, Washington, DC
Burriesci MS, Raab TK, Pringle JR (2012) Evidence that glucose is the major transferred metabolite in dinoflagellate-cnidarian symbiosis. J Exp Biol 215:3467–3477
Caldeira K, Wickett ME (2003) Oceanography: anthropogenic carbon and ocean pH. Nature 425:365
Castillo KD, Ries JB, Bruno JF, Westfield IT (2014) The reef-building coral *Siderastrea siderea* exhibits parabolic responses to ocean acidification and warming. Proc R Soc B Biol Sci 281:20141856
Catala-Stucki R (1959) Fluorescent effects from corals irradiated with ultra-violet rays. Nature 183:949
Chalker BE, Taylor DL (1975) Light-enhanced calcification, and the role of oxidative phosphorylation in calcification of the coral *Acropora cervicornis*. Proc R Soc B Biol Sci 190:323–331
Chen Y, Cann MJ, Litvin TN, Iourgenko V, Sinclair ML, Levin LR, Buck J (2000) Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. Science 289:625–628
Chimetto LA, Brocchi M, Thompson CC, Martins RC, Ramos HR, Thompson FL (2008) Vibrios dominate as culturable nitrogen-fixing bacteria of the Brazilian coral *Mussismilia hispida*. Syst Appl Microbiol 31:312–319

Clode PL, Marshall AT (2002) Low temperature FESEM of the calcifying interface of a scleractinian coral. Tissue Cell 34:187–198
Cohen A, Holcomb M (2009) Why corals care about ocean acidification. Oceanography 22:118–127
Cohen AL, McConnaughey TA (2003) Geochemical perspectives on coral mineralization. Rev Mineral Geochem 54:151–187
Crossland CJ, Barnes DJ, Borowitzka MA (1980) Diurnal lipid and mucus production in the staghorn coral *Acropora acuminata*. Mar Biol 60:81–90
Cunning R, Silverstein RN, Baker AC (2015a) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. Proc Biol Sci 282:20141725
Cunning R, Vaughan N, Gillette P, Capo TR, Matté JL, Baker AC (2015b) Dynamic regulation of partner abundance mediates response of reef coral symbioses to environmental change. Ecology 96: 1411–1420
Davies PS (1984) The role of zooxanthellae in the nutritional energy requirements of *Pocillopora eydouxi*. Coral Reefs 2:181–186
Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinoflagellate symbiosis. Microbiol Mol Biol Rev 76:229–261
Dawson TL (2007) Light-harvesting and light-protecting pigments in simple life forms. Color Technol 123:129–142
Diekmann O, Bak R, Stam W, Olsen J (2001) Molecular genetic evidence for probable reticulate speciation in the coral genus Madracis from a Caribbean fringing reef slope. Mar Biol 139:221–233
Dove SG, Takabayashi M, Hoegh-Guldberg O (1995) Isolation and partial characterization of the pink and blue pigments of Pocilloporid and Acroporid corals. Biol Bull 189(3):288–297
Dove SG, Hoegh-Guldberg O, Ranganathan S (2001) Major colour patterns of reef-building corals are due to a family of GFP-like proteins. Coral Reefs 19(3):197–204
Dove SG, Lovell C, Fine M, Deckenback J, Hoegh-Guldberg O, Iglesias-Prieto R, Anthony KR (2008) Host pigments: potential facilitators of photosynthesis in coral symbioses. Plant Cell Environ 3:1523–1533
Drake JL, Mass T, Haramaty L, Zelzion E, Bhattacharya D, Falkowski PG (2014) Proteomic analysis of skeletal organic matrix from the stony coral *Stylophora pistillata*. Proc Natl Acad Sci 111:12728–12733
Ducklow HW, Mitchell R (1979) Composition of mucus released by coral reef coelenterates. Limnol Oceanogr 24:706–714
Dufault AM, Cumbo VR, Fan T-Y, Edmunds PJ (2012) Effects of diurnally oscillating pCO_2 on the calcification and survival of coral recruits. Proc Biol Sci 279:2951–2958
Erez J (1978) Vital effect on stable-isotope composition seen in foraminifera and coral skeletons. Nature 273:199–202
Fabricius KE, Langdon C, Uthike S, Humphrey C, Noonan S, Death G, Okazaki R, Muehllehner N, Glas MS, Lough JM (2011) Losers and winners in coral reefs acclimated to elevated carbon dioxide concentrations. Nat Clim Chang 1:165–169
Falkowski PG, Dubinsky Z, Muscatine L, Porter JW (1984) Light and bioenergetics of a symbiotic coral. BioScience 34:705–709
Farrell J, Ramos L, Tresguerres M, Kamenetsky M, Levin LR, Buck J (2008) Somatic 'soluble' Adenylyl Cyclase isoforms are unaffected in $Sacy^{tm1Lex}$/$Sacy^{tm1Lex}$ 'knockout' mice. PLoS One 3:e3251
Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305:362–366
Friedman PA, Gesek FA (1995) Cellular calcium transport in renal epithelia: measurement, mechanisms, and regulation. Physiol Rev 75:429–471
Fukami H, Budd AF, Paulay G, Solé-Cava A, Allen Chen C, Iwao K, Knowlton N (2004) Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. Nature 427:832–835
Furla P, Bénazet-Tambutté S, Jaubert J, Allemand D (1998) Functional polarity of the tentacle of the sea anemone *Anemonia viridis*: role in inorganic carbon acquisition. AJP: Regul Integr Comp Physiol 274:303–310
Furla P, Galgani I, Durand I, Allemand D (2000) Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis. J Exp Biol 203:3445–3457
Galloway SB, Work TM, Bochsler VS, Harley RA, Kramarsky-Winters E, McLaughlin SM, Meteyer CU, Morado JF, Nicholson JH, Parnell PG et al (2006) Coral disease and health workshop: coral histopathology II. National Oceanic and Atmospheric Administration (NOAA), Silver Spring
Ganot P, Zoccola D, Tambutté E, Voolstra CR, Aranda M, Allemand D, Tambutté S (2015) Structural molecular components of septate junctions in cnidarians point to the origin of epithelial junctions in eukaryotes. Mol Biol Evol 32:44–62

Gattuso JP (1999) Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. Am Zool 39:160–183
Gattuso JP, Hendriks IE, Brewer PG (2014) Free-ocean CO_2 enrichment (FOCE) systems: present status and future developments. Biogeosciences 11:4057–4075
Geng W, Wang Z, Zhang J, Reed BY, Pak CY, Moe OW (2005) Cloning and characterization of the human soluble adenylyl cyclase. AJP: Cell Physiol 288:C1305–C1316
Gibbin EM, Putnam HM, Davy SK, Gates RD (2014) Intracellular pH and its response to CO2-driven seawater acidification in symbiotic versus non-symbiotic coral cells. J Exp Biol 217:1963–1969
Gladfelter EH (1983) Circulation of fluids in the gastrovascular system of the reef coral *Acropora cervicornis*. Biol Bull 165:619–636
Goldberg WM (2001a) Acid polysaccharides in the skeletal matrix and calicoblastic epithelium of the stony coral *Mycetophyllia reesi*. Tissue Cell 33:376–387
Goldberg WM (2001b) Desmocytes in the calicoblastic epithelium of the stony coral *Mycetophyllia reesi* and their attachment to the skeleton. Tissue Cell 33:388–394
Goldberg WM (2002a) Feeding behavior, epidermal structure and mucus cytochemistry of the scleractinian *Mycetophyllia reesi*, a coral without tentacles. Tissue Cell 34:232–245
Goldberg WM (2002b) Gastrodermal structure and feeding responses in the scleractinian *Mycetophyllia reesi*, a coral with novel digestive filaments. Tissue Cell 34:246–261
Goreau TF (1959) The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. Biol Bull 116:59–75
Goreau TF, Goreau NI (1959) The physiology of skeleton formation in corals. II. Calcium deposition by hermatypic corals under various conditions in the reef. Biol Bull 117:239–250
Goreau TF, Goreau NI, Yonge CM (1971) Reef corals: autotrophs or heterotrophs? Biol Bull 141:247–260
Haas AF, Smith JE, Thompson M, Deheyn DD (2014) Effects of reduced dissolved oxygen concentrations on physiology and fluorescence of hermatypic corals and benthic algae. Peer J 2:e235. doi:10.7717/peerj.235
Haddock SHD, Moline MA, Case JF (2009) Bioluminescence in the sea. 2:443–493. http://dx.doi.org/10.1146/annurev-marine-120308-081028
Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E, Fine M, Turner SM, Rowley SJ, Tedesco D, Buia M-C (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454:96–99
Hanson MR, Kohler RH (2001) GFP imaging: methodology and application to investigate cellular compartmentation in plants. J Exp Bot 52(356):529–539
Hess KC, Jones BH, Marquez B, Chen Y, Ord TS, Kamenetsky M, Miyamoto C, Zippin JH, Kopf GS, Suarez SS et al (2005) The "soluble" adenylyl cyclase in sperm mediates multiple signaling events required for fertilization. Dev Cell 9:249–259
Hosey MM, Lazdunski M (1988) Calcium channels: molecular pharmacology, structure and regulation. J Membr Biol 104:81–105
Hughes AD, Grottoli AG, Pease TK, Matsui Y (2010) Acquisition and assimilation of carbon in non-bleached and bleached corals. Mar Ecol Prog Ser 420:91–101
Ip YK, Lim ALL, Lim RW (1991) Some properties of calcium activated adenosine triphosphatase from the hermatypic coral *Galaxea fascicularis*. Mar Biol 111:191–197
Isa Y (1986) An electron microscope study on the mineralization of the skeleton of the staghorn coral *Acropora hebes*. Mar Biol 93:91–101
Johnston IS (1980) The ultrastructure of skeletogenesis in hermatypic corals. Int Rev Cytol 67:171–214
Jokiel P (2011a) The reef coral two compartment proton flux model: a new approach relating tissue-level physiological processes to gross corallum morphology. J Exp Mar Biol Ecol 409:1–12
Jokiel PL (2011b) Ocean acidification and control of reef coral calcification by boundary layer limitation of proton flux. Bull Mar Sci 87:639–657
Jokiel PL (2013) Coral reef calcification: carbonate, bicarbonate and proton flux under conditions of increasing ocean acidification. Proc Biol Sci 280:20130031
Kaniewska P, Campbell PR, Kline DI, Rodriguez-Lanetty M, Miller DJ, Dove S, Hoegh-Guldberg O (2012) Major cellular and physiological impacts of ocean acidification on a reef building coral. PLoS One 7:e34659
Karako-Lampert S, Zoccola D, Salmon-Divon M, Katzenellenbogen M, Tambutté S, Bertucci A, Hoegh-Guldberg O, Deleury E, Allemand D, Levy O (2014) Transcriptome analysis of the scleractinian coral *Stylophora pistillata*. PLoS One 9:e88615
Kawaguti S (1937) On the physiology of reef corals. II. The effect of light on color and form of reefs. Palao Trop Biol Stat Stud 2:199–208

Kawaguti S (1969) The effect of green fluorescent pigment on the productivity of the reef corals. Micronesica 5:313
Kawaguti S, Sakumoto D (1948) The effect of light on the calcium deposition of corals. Bul Oceanogr Inst Taiwan 4:65–70
Kelmanson IV, Matz MV (2003) Molecular basis and evolutionary origins of color diversity in great star coral *Montastraea cavernosa* (Scleractinia: Faviida). Mol Biol Evol 20(7):1125–1133
Kenkel CD, Traylor MR, Wiedenmann J, Salih A, Matz MV (2011) Fluorescence of coral larvae predicts their settlement response to crustose coralline algae and reflects stress. Proc Biol Sci 278:2691–2697
Kleypas J, Langdon C (2006) Coral reefs and changing seawater carbonate chemistry. In: Phinney JT, Hoegh-Guldberg O, Kleypas J, Skirving W, Strong A (eds) *Coral reefs and climate change science and management*. American Geophysical Union, Washington, DC
Kline DI, Teneva L, Hauri C, Schneider K, Miard T, Chai A, Marker M, Dunbar R, Caldeira K, Lazar B et al (2015) Six month in situ high-resolution carbonate chemistry and temperature study on a coral reef flat reveals asynchronous pH and temperature anomalies. PLoS One 10:e0127648
Kline DI, Teneva L, Schneider K, Miard T, Chai A, Marker M, Headley K, Opdyke B, Nash M, Valetich M et al (2012) A short-term in situ CO_2 enrichment experiment on Heron Island (GBR). Sci Rep 2:413
Krediet CJ, Ritchie KB, Paul VJ, Teplitski M (2013) Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. Proc Biol Sci 280:20122328
Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol Lett 13:1419–1434
Kroeker KJ, Micheli F, Gambi MC (2013) Ocean acidification causes ecosystem shifts via altered competitive interactions. Nat Clim Chang 3:156–159
Kühl M, Cohen Y, Dalsgaard T, Jorgensen BB, Revbech NP (1995) Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O_2, pH and light. Mar Ecol Prog Ser 117:159–172
Labas YA, Gurskaya NG, Yanushevich YG, Fradkov AF, Lukyanov KA, Lukyanov SA, Matz MV (2002) Diversity and evolution of the green fluorescent protein family. Proc Natl Acad Sci U S A 99(7):4256–4261
Langdon C, Atkinson MJ (2005) Effect of elevated pCO_2 on photosynthesis and calcification on corals and interactions with seasonal change in temperature/irradiance and nutrient enrichment. J Geophys Res 110:C09S07
Laurent J, Tambutté S, Tambutté E, Allemand D, Venn A (2013) The influence of photosynthesis on host intracellular pH in scleractinian corals. J Exp Biol 216:1398–1404
Laurent J, Venn A, Tambutté E, Ganot P, Allemand D, Tambutté S (2014) Regulation of intracellular pH in cnidarians: response to acidosis in *Anemonia viridis*. FEBS J 281:683–695
Leal MC, Jesus B, Ezequiel J, Calado R, Rocha RJM, Cartaxana P, Serodio J (2015) Concurrent imaging of chlorophyll fluorescence, Chlorophyll a content and green fluorescent proteins-like proteins of symbiotic cnidarians. Mar Ecol Evol Perspect 36(3):572–584
Lesser MP, Mazel CH, Gorbunov MY, Falkowski PG (2014) Discovery of symbiotic nitrogen-fixing cyanobacteria in corals. Science 305:1–5
Leutenegger A, D'Angelo C, Matz MV, Denzel A, Oswald F, Salih A, Nienhaus GU, Wiedenmann J (2007) It's cheap to be colorful. Anthozoans show a slow turnover of GFP-like proteins. FEBS J 274: 2496–2505
Little AF, van Oppen MJ, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. Science 304:1492–1494
Lüthi D, Le Floch M, Bereiter B, Blunier T, Barnola J-M, Siegenthaler U, Raynaud D, Jouzel J, Fischer H, Kawamura K et al (2008) High-resolution carbon dioxide concentration record 650,000–800,000 years before present. Nature 453:379–382
Marker M, Kline DI, Kirkwood WJ, Headley K, Brewer PG, Peltzer ET, Miard T, Chai A, James M, Schneider K et al (2010) The coral proto free ocean carbon enrichment system (CP-FOCE): engineering and development, Proceedings of OCEANS IEEE 1–10
Matz MV, Fradkov AF, Labas YA, Savitsky AP, Zaraisky AG, Markelov ML, Lukyanov SA (1999) Fluorescent proteins from nonbioluminescent Anthozoa species. Nat Biotechnol 17:969–973
Marshall AT (1996) Calcification in hermatypic and ahermatypic corals. Science 271:637–639
Mass T, Drake JL, Haramaty L, Kim JD, Zelzion E, Bhattacharya D, Falkowski PG (2013) Cloning and characterization of four novel coral acid-rich proteins that precipitate carbonates *in vitro*. Curr Biol 23: 1126–1131
Mass T, Drake JL, Peters EC, Jiang W, Falkowski PG (2014) Immunolocalization of skeletal matrix proteins in tissue and mineral of the coral *Stylophora pistillata*. Proc Natl Acad Sci U S A 111:12728–12733

McLean PF, Cooley L (2013) Protein equilibration through somatic ring canals in Drosophila. Science 340(6139):1445–1447
Millero FJ (2007) The marine inorganic carbon cycle. Chem Rev 107:308–341
Morita M, Suwa R, Iguchi A, Nakamura M, Shimada K, Sakai K, Suzuki A (2010) Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates. Zygote 18:103–107
Moya A, Tambutté S, Bertucci A, Tambutté E, Lotto S, Vullo D, Supuran CT, Allemand D, Zoccola D (2008) Carbonic anhydrase in the scleractinian coral *Stylophora pistillata*: characterization, localization, and role in biomineralization. J Biol Chem 283:25475–25484
Muscatine L (1973) Nutrition of corals. In: Jones OA, Endean R (eds) Biology and geology of coral reefs, vol II, Biology I. Academic, New York, pp 77–115
Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z (ed) Ecosystems of the world. Elsevier, Amsterdam, pp 75–87
Muscatine L, Cernichiari E (1969) Assimilation of photosynthetic products of zooxanthellae by a reef coral. Biol Bull 137:506–523
Muscatine L, Falkowski PG, Porter JW, Dubinsky Z (1984) Fate of the photosynthetic fixed carbon in light- and shade-adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc R Soc B Biol Sci 222:181–202
Nakamura M, Ohki S, Suzuki A, Sakai K (2011) Coral larvae under ocean acidification: survival, metabolism, and metamorphosis. PLoS One 6:e14521–e14527
Odorico DM, Miller DJ (1997) Variation in the ribosomal internal transcribed spacers and 5.8S rDNA among five species of *Acropora* (Cnidaria; Scleractinia): patterns of variation consistent with reticulate evolution. Mol Biol Evol 14:465–473
Ohde S, van Woesik R (1999) Carbon dioxide flux and metabolic processes of a coral reef, Okinawa. Bull Mar Sci 65:559–576
Ohmiya Y, Hirano T (1996) Shining the light: the mechanism of the bioluminescence reaction of calcium-binding photoproteins. Chem Biol 3:337–347
Oswald F, Schmitt F, Leutenegger A, Ivanchenko S, D'Angelo C, Salih A, Maslakova S, Bulina M, Schirmbeck R, Nienhaus GU, Matz MV, Wiedenmann J (2007) Contributions of host and symbiont pigments to the coloration of reef corals. FEBS J 274(4):1102–1109
Pagani M, Liu ZH, LaRiviere J, Ravelo AC (2010) High earth-system climate sensitivity determined from Pliocene carbon dioxide concentrations. Nat Geosci 3:27–30
Palmer CV, Roth MS, Gates RD (2009) Red fluorescent protein responsible for pigmentation in trematode-infected *Porites compressa* tissues. Biol Bull 216:68–74
Petersen OH, Petersen C (1994) Calcium and hormone action. Annu Rev Physiol 56:297–319
Phillips JH (1963) Immune mechanisms in the phylum Coelenterata. In: Dougherty EC (ed) The lower metazoa. University of California Press, Berkeley, pp 425–431
Price NN, Martz TR, Brainard RE, Smith JE (2012) Diel variability in seawater pH relates to calcification and benthic community structure on coral reefs. PLoS One 7:e43843
Putnam RW, Roos A (1997) Intracellular pH. In: Hoffman JF, Jamieson DJ (eds) Handbook of physiology. Oxford University Press, New York, pp 389–440
Puverel S, Tambutt E, Zoccola D, Domart-Coulon I, Bouchot A, Lotto SV, Allemand D, Tambutt S (2004) Antibodies against the organic matrix in scleractinians: a new tool to study coral biomineralization. Coral Reefs 24:149–156
Puverel S, Tambutté E, Pereira-Mouriès L, Zoccola D, Allemand D, Tambutté S (2005) Soluble organic matrix of two Scleractinian corals: partial and comparative analysis. Comp Biochem Physiol B Biochem Mol Biol 141:480–487
Raina J-B, Dinsdale EA, Willis BL, Bourne DG (2010) Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? Trends Microbiol 18:101–108
Ries JB (2011) A physicochemical framework for interpreting the biological calcification response to CO_2-induced ocean acidification. Geochim Cosmochim Acta 75:4053–4064
Rocca JD, Hall EK, Lennon JT, Evans SE, Waldrop MP, Cotner JB, Nemergut DR, Graham EB, Wallenstein MD (2015) Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. ISME J 9:1693–1699
Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1–10
Romano SL, Cairns SD (2000) Molecular phylogenetic hypotheses for the evolution of scleractinian corals. Bull Mar Sci 67:1043–1068

Romano SL, Palumbi SR (1996) Evolution of scleractinian corals inferred from molecular systematics. Science 271:640–642
Roos A, Boron WF (1981) Intracellular pH. Physiol Rev 61:296–434
Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355–362
Roth MS (2014) The engine of the reef: photobiology of the coral-algal symbiosis. Front Microbiol 5:422
Roth MS, Deheyn DD (2013) Effects of cold stress and heat stress on coral fluorescence in reef-building corals. Sci Rep 3:1421
Roth MS, Fan T-Y, Deheyn DD (2013) Life history changes in coral fluorescence and the effects of light intensity on larval physiology and settlement in Seriatopora hystrix. PLoS One 8:e59476
Roth MS, Goericke R, Deheyn DD (2012) Cold induces acute stress but heat is ultimately more deleterious for the reef-building coral *Acropora yongei*. Sci Rep 2:240
Roth MS, Latz MI, Goericke R, Deheyn DD (2010) Green fluorescent protein regulation in the coral *Acropora yongei* during photoacclimation. J Exp Biol 213:3644–3655
Rowan R, Whitney SM, Fowler A, Yellowlees D (1996) Rubisco in marine symbiotic dinoflagellates: form II enzymes in eukaryotic oxygenic phototrophs encoded by a nuclear multigene family. Plant Cell 8:539–553
Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DW, Tilbrook B et al (2004) The oceanic sink for anthropogenic CO2. Science 305:367–371
Salih A, Larkum A, Cox G, Kühl M, Hoegh-Guldberg O (2000) Fluorescent pigments in corals are photoprotective. Nature 408:850–853
Schlichter D, Meier U, Fricke HW (1994) Improvement of photosynthesis in zooxanthellate corals by autofluorescent chromatophores. Oecologia 99:124–131
Schonknecht G, Brown JE, Verchot-Lubicz J (2008) Plasmodesmata transport of GFP alone or fused to potato virus X TGBp1 is diffusion driven. Protoplasma 232(3–4):143–152
Schweinsberg M, Weiss LC, Striewski S, Tollrian R, Lampert KP (2015) More than one genotype: how common is intracolonial genetic variability in scleractinian corals? Mol Ecol 24:2673–2685
Shashar N, Cohen Y, Loya Y (1993) Extreme diel fluctuations of oxygen in diffusive boundary layers surrounding stony corals. Biol Bull 185:455–461
Shaw EC, Mcneil BI, Tibrook B (2012) Impacts of ocean acidification in naturally variable coral reef flat ecosystems. J Geophys Res Oceans 117:C03038
Shinomura O, Johnson FH, Saiga Y (1962) Extraction, purification and properties of aequorin, a bioluminescent protein from the luminous hydromedusan, Aequorea. J Cell Comp Physiol 59:223–239
Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M, Fujiwara M, Koyanagi R, Ikuta T et al (2011) Using the *Acropora digitifera* genome to understand coral responses to environmental change. Nature 476:320–323
Silverman J, Kline DI, Johnson L, Rivlin T, Schneider K, Erez J, Lazar B, Caldeira K (2012) Carbon turnover rates in the one tree Island reef: a 40-year perspective. Geophys Res Biogeosci 1127:G03023
Silverman J, Lazar B, Cao L, Caldeira K, Erez J (2009) Coral reefs may start dissolving when atmospheric CO_2 doubles. Geophys Res Lett 36:L05606
Smith EG, Angelo D, Salih C, Wiedenmann J (2013) Screening by coral green fluorescent protein (GFP)-like chromoproteins supports a role in photoprotection of zooxanthellae. Coral Reefs 32:463–474
Stolarski J, Kitahara MV, Miller DJ, Cairns SD, Mazur M, Meibom A (2011) The ancient evolutionary origins of Scleractinia revealed by azooxanthellate corals. BMC Evol Biol 11:316
Tambutté E, Allemand D, Mueller E, Jaubert J (1996) A compartmental approach to the mechanism of calcification in hermatypic corals. J Exp Biol 199:1029–1041
Tambutté E, Tambutté S, Segonds N, Zoccola D, Venn A, Erez J, Allemand D (2011) Calcein labelling and electrophysiology: insights on coral tissue permeability and calcification. Proc R Soc B BiolSci 279: 19–27
Tresguerres M (2014) sAC from aquatic organisms as a model to study the evolution of acid/base sensing. BBA Mol Basis Dis 1842:2629–2635
Tresguerres M, Barott KL, Barron ME, Roa JN (2014) Established and potential physiological roles of bicarbonate-sensing soluble adenylyl cyclase (sAC) in aquatic animals. J Exp Biol 217:663–672
Tresguerres M, Buck J, Levin LR (2010a) Physiological carbon dioxide, bicarbonate, and pH sensing. Pflugers Arch Eur J Physiol 460:953–964
Tresguerres M, Levin LR, Buck J (2011) Intracellular cAMP signaling by soluble adenylyl cyclase. Kidney Int 79:1277–1288

Tresguerres M, Parks SK, Katoh F, Goss GG (2006) Microtubule-dependent relocation of branchial V-H^+–ATPase to the basolateral membrane in the Pacific spiny dogfish (*Squalus acanthias*): a role in base secretion. J Exp Biol 209:599–609

Tresguerres M, Parks SK, Salazar E, Levin LR, Goss GG, Buck J (2010b) Bicarbonate-sensing soluble adenylyl cyclase is an essential sensor for acid/base homeostasis. Proc Natl Acad Sci U S A 107:442–447

van Oppen MJ, McDonald BJ, Willis B, Miller DJ (2001) The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence? Mol Biol Evol 18:1315–1329

Vandermeulen JH, Watabe N (1973) Studies on reef corals. I. Skeleton formation by newly settled planula larva of *Pocillopora damicornis*. Mar Biol 23:47–57

Venn AA, Tambutté E, Lotto S, Zoccola D, Allemand D, Tambutté S (2009) Imaging intracellular pH in a reef coral and symbiotic anemone. Proc Natl Acad Sci U S A 106:16574–16579

Venn A, Tambutté E, Holcomb M, Allemand D, Tambutté S (2011) Live tissue imaging shows reef corals elevate pH under their calcifying tissue relative to seawater. PLoS One 6:e20013–e20019

Veron JE (1993) Corals of Australia and the Indo-Pacific, 2nd edn. University of Hawaii Press, Honolulu

Vidal-Dupiol J, Zoccola D, Tambutté E, Grunau C, Cosseau C, Smith KM, Freitag M, Dheilly NM, Allemand D, Tambutté S (2013) Genes related to Ion-transport and energy production are upregulated in response to CO_2-driven pH decrease in corals: new insights from transcriptome analysis. PLoS One 8: e58652

Wainwright SA (1964) Studies of the mineral phase of coral skeleton. Exp Cell Res 34:213–230

Weis VM, Smith GJ, Muscatine L (1989) A "CO_2 supply" mechanism in zooxanthellate cnidarians: role of carbonic anhydrase. Mar Biol 100:195–202

Wiedenmann J, Ivanchenko S, Oswald F, Nienhaus GU (2004) Identification of GFP-like proteins in nonbioluminescent, azooxanthellate anthozoa opens new perspectives for bioprospecting. Marine Biotechnol 6(3):270–277

Wijgerde T, Jurriaans S, Hoofd M, Verreth JA, Osinga R (2012) Oxygen and heterotrophy affect calcification of the scleractinian coral *Galaxea fascicularis*. PLoS One 7:e52702

Wijgerde T, Silva CIF, Scherders V, van Bleijswijk J, Osinga R (2014) Coral calcification under daily oxygen saturation and pH dynamics reveals the important role of oxygen. Biol Open 3:489–493

Wijsman-Best M (1975) Intra-and extratentacular budding in hermatypic reef corals. pp. 471–475

Wild C, Huettel M, Klueter A, Kremb SG, Rasheed MY, Jørgensen BB (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature 428:66–70

Willis BL, Babcock RC, Harrison PL, Wallace CC (1997) Experimental hybridization and breeding incompatibilities with the mating systems of mass spawning reef corals. Coral Reefs 16:S53–S65

Wong W, Scott JD (2004) AKAP signalling complexes: focal points in space and time. Nat Rev Mol Cell Biol 5:959–970

Yates KK, Halley RB (2003) Measuring coral reef community metabolism using new benthic chamber technology. Coral Reefs 22:247–255

Yates KK, Halley RB (2006) Diurnal variation in rates of calcification and carbonate sediment dissolution in Florida Bay. Estuar Coast 29:24–39

Zoccola D, Ganot P, Bertucci A, Caminiti-Segonds N, Techer N, Voolstra CR, Aranda M, Tambutté E, Allemand D, Casey JR et al (2015) Bicarbonate transporters in corals point towards a key step in the evolution of cnidarian calcification. Sci Rep 5:9983

Zoccola D, Tambutté E, Kulhanek E, Puverel S, Scimeca J-C, Allemand D, Tambutté S (2004) Molecular cloning and localization of a PMCA P-type calcium ATPase from the coral *Stylophora pistillata*. Biochim Biophys Acta 1663:117–126

Zoccola D, Tambutté E, Sénégas-Balas F, Michelis J-F, Failla J-P, Jaubert J, Allemand D (1999) Cloning of a calcium channel a1 subunit from the reef-building coral, *Stylophora pistillata*. Gene 227:157–167