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Review

sAC from aquatic organisms as a model to study the evolution of acid/base sensing ☆

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ABSTRACT

Soluble adenylyl cyclase (sAC) is poised to play multiple physiological roles as an acid/base (A/B) sensor in aquatic organisms. Many of these roles are probably similar to those in mammals; a striking example is the evolutionary conservation of a mechanism involving sAC, carbonic anhydrase and vacuolar H^+-ATPase that acts as a sensor system and regulator of extracellular A/B in shark gills and mammalian epididymis and kidney. Additionally, the aquatic environment presents unique A/B and physiological challenges; therefore, sACs from aquatic organisms have likely evolved distinct kinetic properties as well as distinct physiological roles. sACs from aquatic organisms offer an excellent opportunity for studying the evolution of A/B sensing at both the molecular and whole organism levels. Moreover, this information could help understand and predict organismal responses to environmental stress based on mechanistic models.This article is part of a Special Issue entitled "The Role of Soluble Adenylyl Cyclase in Health and Disease," guest edited by J. Buck and L. R. Levin.

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Most aquatic organisms are regularly exposed to fluctuating levels of CO_2, pH and [HCO_3^-], both in the environment and in physiological fluids, as a result of various conditions such as the balance between photosynthesis and respiration on a day/night cycle, feeding, calcification, upwelling and, in the case of aquatic mammals and birds, diving. Moreover, anthropogenic disturbances such as ocean acidification and pollution may induce further acid/base (A/B) disturbances. Because water-breathing animals characteristically have a lower buffering capacity in their internal fluids compared to air-breathing vertebrates, environmental and metabolic disturbances may induce large variations in A/B homeostasis that must be constantly sensed and regulated.

Soluble adenylyl cyclase (sAC), which has been identified in aquatic species from multiple phyla (see [1] for a recent compendium), is a good candidate as a molecular CO_2/pH/HCO_3^- sensor of A/B stress in aquatic organisms. sAC produces the ubiquitous second messenger cAMP in response to elevated CO_2/pH/HCO_3^- and can potentially mediate multiple physiological responses *via* PKA-dependent phosphorylation, ion channel gating and exchange protein activated by cAMP (EPAC) signaling (reviewed in [1–5]). A recent paper has reviewed the established functions of sAC in aquatic animals and identified some potential additional physiological roles [1]. The current paper further discusses environmental and metabolic conditions that may be related to sAC function in aquatic organisms. It will also discuss the apparent evolutionarily conservation of sAC-dependent mechanisms in analogous A/B regulatory organs from diverse animals and extend the debate on potential functions of sAC to phytoplankton.

1. "Aquatic" sAC as a model to study the molecular basis of A/B sensing

From an experimental and methodological perspective, aquatic animals may serve as good model systems to study A/B sensing for two main reasons. First, they experience large A/B disturbances as part of their normal physiology. This is a great experimental advantage as it is possible to expose cells and organisms to a wide suite of A/B conditions ranging from normal ([HCO_3^-] = ~5 mM; pH~ 7.8) to alkaline ([HCO_3^-] = ~20 mM; pH ~8.2) and to acidic (HCO_3^--free; pH ~7.5). The extreme differences between conditions maximize the magnitude of the responses and readouts, thus facilitating detection and quantification. Most importantly, these extreme conditions are physiologically relevant (compared, for example, to mammalian cells, which are often exposed to non-physiological HCO_3^--free conditions to maximize responses). Because A/B stress is universal and sAC is evolutionarily conserved, many of these results may also be applicable to other organisms, including humans.

The second reason why sACs from aquatic organisms are interesting models is for comparative studies on structure and function. For sAC to be an evolutionarily conserved A/B sensor as proposed [6,7], its kinetic

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characteristics should vary according to the distinct A/B challenges that each organism has regularly experienced throughout their evolutionarily history. Because the kinetic differences should be due to key amino acid residues, mutagenesis studies based on amino acids that differ from species to species could inform about the molecular basis for sAC's differential sensitivity to HCO_3^- and other enzyme kinetic parameters. For example, the EC_{50} of dogfish sAC for HCO_3^- is ~5 mM [7], which is much lower than the ~20 mM reported for mammalian sAC [6,8,9]. In human sAC, HCO_3^- binds between lysine 95 and arginine 176, and mutagenesis of the lysine for an alanine completely abolished the stimulatory effect of HCO_3^- [10]. Alternatively, the sAC amino acid sequences from coelacanth, gar, salmon, trout, chimera and dogfish shark posses an asparagine in a position equivalent to human sAC's lysine 95 (Fig. 1). Would mutating lysine 95 for asparagine increase the affinity of human sAC for HCO_3^-? Conversely, would mutating the asparagine to lysine reduce the affinity of shark sAC for HCO_3^-?

Similarly, a comparison between sAC sequences from diverse organisms inform us about potential regulatory mechanisms and interactions with other proteins. The linker region between catalytic domains 1 and 2 in human sAC is 69 amino acids long, has a high proline content and hydrophobicity and has been identified as a potential region for protein–protein interactions (including other sAC domains) [10]. The length of the linker region is essentially the same in sAC from all other vertebrates. However, the equivalent region is 148 amino acids long in sea urchin sAC and 153 amino acids long in oyster sAC. Do sACs from these two invertebrates have additional regulatory properties or interacting partners? This hypothesis is preliminarily supported by studies on sea urchin sAC showing co-immunoprecipitation with at least 10 other proteins [11]. It should also be noted that sea urchin sAC has several PKA phosphorylation sites [12] whereas human sAC has none.

2. Variability of CO_2, pH and HCO_3^- in aquatic environments

Studies to elucidate potential physiological roles of sAC in aquatic organisms require first considering the biology of each organism in relation to its environment. Although the open ocean is chemically stable, some aquatic environments experience pronounced daily or seasonal fluctuations in A/B conditions as a result of biological activity, upwelling, tides or CO_2 vents [13]. For example, in coral reefs and kelp forests, the balance between photosynthesis and respiration can result in daily shifts of ~0.3 pH units [13,14]. In California kelp forests, even larger variations can occur due to sporadic upwelling events of CO_2- and nutrient-rich waters that can last for several days [14]. Similar conditions may be found in other environments with intense biological activity such as mangroves, algal blooms or oxygen minimum zones. Since in most cases the changes in pH are due to CO_2 production, these are associated with equivalent changes in CO_2 and HCO_3^- following the equilibrium $CO_2 \Leftrightarrow H^+ + HCO_3^-$. Hypercapnia can affect the A/B status in the internal fluids of aquatic organisms by inducing an accumulation of CO_2 in internal fluids due to the reduced gradient for metabolic CO_2 excretion (reviewed in [15]), and, in extreme acidic cases, by directly impairing H^+ secretion by Na^+/H^+ exchangers.

While motile animals have the option of moving, they may prefer to stay for food, protection, mating or socializing purposes. Sessile animals and plants, on the other hand, have no option but to cope with the A/B stressing conditions. Organisms that do not move must either regulate the A/B status of their internal fluids or adjust their physiology to fluctuating A/B conditions. Thus, the ability to sense A/B must be an adaptive and widespread trait that undoubtedly regulates multiple physiological functions in aquatic organisms.

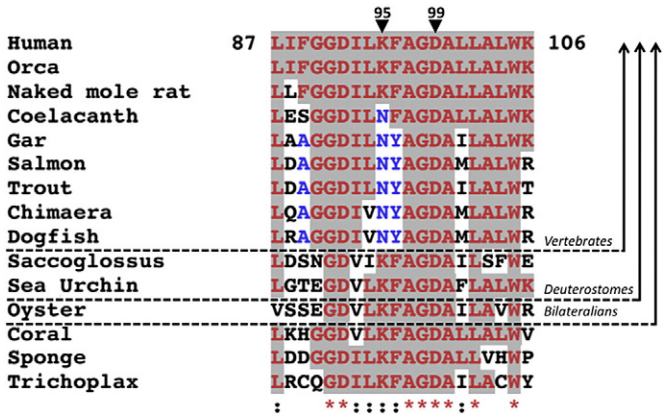

Fig. 1. Alignment of sAC protein sequences from diverse animals in the region corresponding to amino acids 87–106 of human sAC. Residues in red are conserved across all sequences, residues in blue are typical of sACs from fish. Arrowheads indicate two key amino of mammalian sAC: lysine 95 (K) directly binds HCO_3^- and aspartate 99 (D) coordinates Mg^{2+} [10]. While D99 is conserved in all sACs, K95 is an asparagine in sACs from all fish species. This substitution may help explain the lower EC_{50} of shark sAC for HCO_3^- (~5 mM [7]) compared to mammalian sAC (~20 mM [6,8,9]).

3. Regulation of A/B in extracellular fluids in aquatic animals

An important distinction between the A/B physiologies of aerobic water- and air-breathing large animals is that the former typically have lower pCO_2 and $[HCO_3^-]$ in their internal fluids. This is due to the high ventilatory requirement for O_2 uptake and the high capacitance of water for CO_2 relative to O_2 [16]. Since the lower CO_2/HCO_3^- in internal fluids is associated with a lower buffering capacity, water-breathing animals experience more pronounced A/B disturbances compared to air-breathers. Furthermore, the changes are proportionally even larger due the lower CO_2, $[H^+]$ and $[HCO_3^-]$ baseline levels. An additional characteristic of aquatic animals with high metabolic demands is that they must ventilate their respiratory surfaces at a high rate even during resting conditions to be able to extract sufficient oxygen from the surrounding water. As a result, aquatic animals cannot regulate blood A/B status by adjusting the ventilation rate (hyper- or hypo-ventilation) like air-breathing animals do. Instead, they rely heavily on metabolic compensation, typically by the gills, which act as an analogous organ to mammalian kidneys (reviewed in [17,18]).

As an example, the post-feeding period is associated with a metabolic alkalosis both in mammals [19] and sharks [20] as a result of increased H^+ secretion into the stomach and HCO_3^- absorption into the blood. However, while in mammals the alkalosis is mild, localized and transient, in sharks it can more than double resting plasma $[HCO_3^-]$ (from ~ 4 mM to >10 mM, depending on the meal size), elevate pH by ~0.3 pH units, and last ~24 h [20–22]. Another example is exhaustive exercising, which in sharks induces severe metabolic acidosis that can almost completely deplete plasma $[HCO_3^-]$, reduce blood pH by ~0.4 pH units and take 4 h to be compensated [23]. A third and final example is the compensatory A/B response to environmental hypercapnia, which involves the accumulation of HCO_3^- in plasma to levels that can triple or quadruple baseline levels [15,24,25]. Shark sAC's EC_{50} for HCO_3^- is ~5 mM, and its V_{max} is ~15 mM [7], viz. they respectively match normal and upper plasma $[HCO_3^-]$ values found during biologically relevant conditions. Thus, sAC is well suited to be a physiologically relevant AB sensor in sharks ([7], reviewed in [1].

Of the conditions listed above, sAC has to date only been studied in relation to sensing and counteracting blood alkalosis in shark, to which it seems essential [7]. Additionally, sAC has been shown to regulate NaCl and water absorption across the intestine of marine bony fish [26,27]. Promising avenues of future research include regulation of cardiac physiology, vasodilation and vasoconstriction, regulation and coordination of aerobic and anaerobic metabolism and regulation of gene expression. Furthermore, sAC is present in shark red blood cells [1],

where it may additionally regulate Cl^-/HCO_3^- and Na^+/H^+ transport in relation to gas exchange.

4. sAC as a regulator of transepithelial transport

Specialized epithelia such as kidneys and choroid plexus in vertebrates, and gills in fish and many invertebrates, maintain A/B homeostasis in their extra-cellular fluids by secreting and absorbing H^+ and HCO_3^-: The first report of sAC acting as an epithelial A/B sensor and regulator of H^+ transport was in rat epididymis [28] (Fig. 2). The luminal fluid in the distal region of the epididymis is acidic (pH ~6.8) and has low [HCO_3^-] (~5–7 mM) [29], which keep the stored sperm quiescent until ejaculation. In response to elevations in luminal pH and [HCO_3^-], sAC in "clear" cells becomes stimulated to produce cAMP, leading to the insertion of more V-type H^+-ATPase (VHA) into the apical membrane of clear cells, upregulation of H^+ secretion and restoration of an acidic luminal fluid [28]. This mechanism also depends on functional carbonic anhydrase [28]. Subsequent research established the involvement of the actin cytoskeleton [30,31], Ca^{2+} [30] and PKA [32] in VHA insertion into the apical membrane, and that the VHA A subunit becomes phosphorylated [33]. VHA insertion into the apical membrane is negatively modulated by AMPK [33] (reviewed in [34,35]).

The mammalian epididymis is an excellent model to study renal acid secretion because it is easier to isolate and manipulate compared to kidney nephrons, and because the epididymal "clear" cells resemble renal A-intercalated cells (A-IC) both embryologically [36] and mechanistically [34,37]. Indeed, all the sAC-dependent events described above for epididymis also apply to renal A-IC. Both sAC and VHA are abundantly expressed in A-ICs [28,38]; VHA insertion into the apical membrane is CA-, sAC-, cAMP- and PKA-dependent [39], and it is negatively modulated by AMPK [39,40]. Studies on kidney have additionally revealed a physical interaction between sAC and VHA (based on co-immunoprecipitation and co-immunolocalization) [38], and that the A-subunit becomes phosphorylated at Ser-175 by PKA [41] and at Ser-384 by AMPK [40]. sAC-, cAMP- and PKA-dependent VHA insertion to the apical membrane also takes place in a human salivary gland cell (HSG) [42]. While cAMP and PKA have the same effect in proximal tubule cells [43], it is yet not known if sAC is involved.

sAC is also present in renal B-ICs, where it exhibits a bipolar distribution: it colocalizes with VHA in the apical and basolateral region and with the anion exchanger pendrin in the apical membrane [38]. Although this localization suggests a role in regulating HCO_3^- secretion and H^+ absorption, performing these types of studies is complicated by the low abundance of B-ICs in relation to A-ICs and principal cells. Alternatively, shark gills contain numerous base-secreting cells that are analogous to renal B-ICs from mammals as they co-express VHA, pendrin [44–46], carbonic anhydrase [47] and sAC [7] and are involved in HCO_3^- secretion and H^+ absorption to compensate blood alkalosis [7, 47–49]. Furthermore, acid-secreting cells in shark gills do not express noticeable amounts of VHA (see references above), and sharks regularly experience a pronounced post-feeding alkalosis that is compensated over several hours ("alkaline tide") [20]. Furthermore, since sharks only use H^+ and HCO_3^- movement to regulate blood pH (they cannot efficiently use ventilatory adjustments, see Regulation of A/B in Extracellular Fluids in Aquatic Animals section), experiments with live animals are more easily performed and the results more clearly interpreted. These characteristics make shark an excellent model for studies on the molecular and cellular mechanisms of blood A/B sensing and regulation, especially those related to HCO_3^- secretion and H^+ absorption.

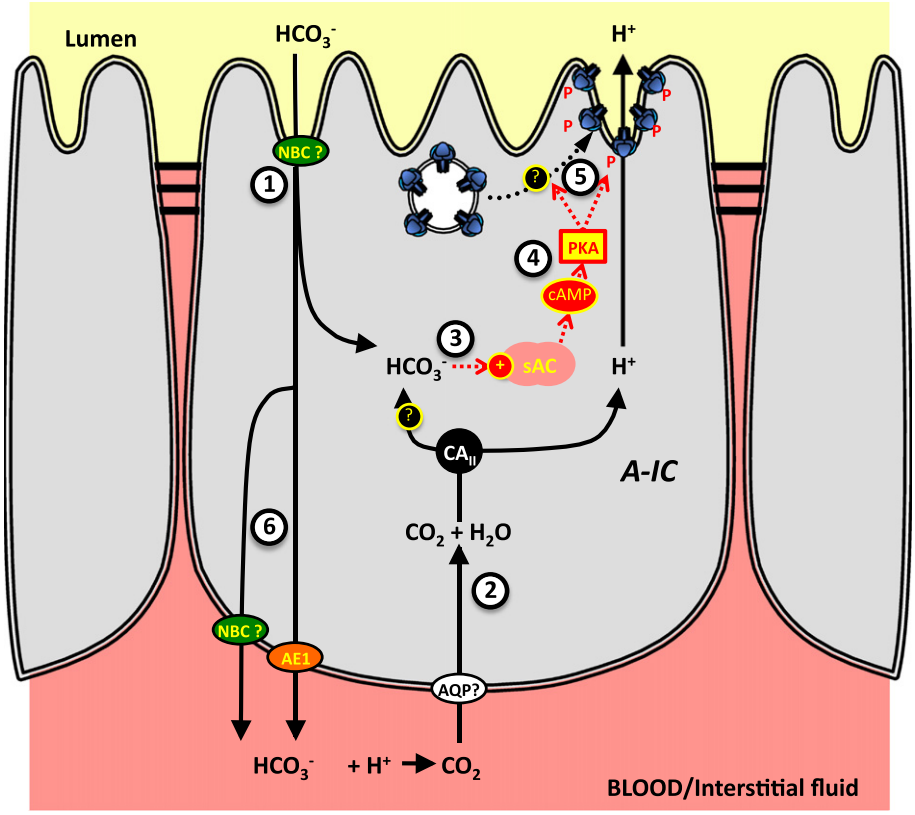

Fig. 2. Model for acid/base sensing and H^+ secretion in mammalian epididymal clear cells and renal A-intercalated cells (A-ICs). (1) Luminal HCO_3^- entering *via* apical Na^+/HCO_3^- cotransporters (NBC) and/or (2) blood CO_2 entering via basolateral aquaporins (AQP) stimulate sAC (3), in the latter case after cytoplasmic carbonic anhydrase II (CA_{II}) hydrates CO_2 into H^+ and HCO_3^-. (4) sAC produces cAMP that activates protein kinase A (PKA), which promotes the insertion of vesicles containing V-type ATPases (VHA, blue icon) into the apical membrane for upregulation of acid secretion (5). Potential PKA targets include Ser-175 in the VHA A subunit, and proteins of the actin cytoskeleton. (6) These acid-secreting cells also absorb HCO_3^- into the blood *via* basolateral NBCs and anion exchanger 1 (AE1).

The elucidation of the role of sAC in sensing and compensation for blood alkalosis involved experiments with live sharks made alkalotic by intravenous infusion of $NaHCO_3$ combined with pharmacological inhibitors of CA, microtubule polymerization, and sAC [7,47–49], as well as sharks in their naturally occurring post feeding period [46,47]. At different times after the onset of alkalosis, gill samples were taken and the intracellular localization of VHA determined by immunohistochemistry and, in some cases, by Western blotting. The resulting cellular model for A/B sensing and regulation (Fig. 3) is mostly a mirror image of mammalian A-ICs (Fig. 2). Interestingly, post-feeding blood alkalosis results in sAC-dependent translocation of VHA from cytoplasmic vesicles to basolateral membranes [7], but also in the translocation of the anion exchanger pendrin to the apical membrane [46]. However, in the latter case, it is not yet known if sAC is involved. To characterize this model in more detail (for example, the potential roles of PKA, EPAC and tmACs), it is essential to first develop primary cultures of shark gill cells enriched for base-secreting cells.

Other epithelia also secrete H^+ or HCO_3^- but for physiological functions other than systemic A/B regulation. As examples, the stomach secretes H^+ for food digestion, the pancreas secretes HCO_3^- that activates digestive enzymes and neutralizes the acid chyme entering the duodenum, the ciliary body secretes HCO_3^- that drives water transport into the aqueous humor in the eye and the coral calicoblastic epithelium and the mollusk mantle secrete base to promote calcification. Furthermore, CO_2/pH/HCO_3^- are known to modulate the transport of other molecules such as NaCl and water across many epithelia. In many cases, the effects of CO_2/pH/HCO_3^- on transepithelial transport may be explained by the substrate-law of mass action, by allosteric modulation of ion transporting proteins or by inducing changes in protein structure. However, a detailed examination of the literature also reveals a recurrent link between epithelial ion transport and CO_2/pH/HCO_3^-, carbonic anhydrase and cAMP, three variables intrinsic to sAC activity. Examples of mammalian epithelia where this link has been described include intestine [50], colon [51], jejenum [52], choroid plexus [53] and cornea [54], all of which express sAC [6,55–58]. Regulation of epithelial ion transport by CO_2/pH/HCO_3^- also occurs in aquatic animals, for example in crab gills [59], frog stomach [60] and fish intestine [27,61,62] (in the latter example, sAC has already been shown to be involved [26,27]). Put together, it is likely that sAC is a widespread mediator of the effects of CO_2/pH/HCO_3^- on epithelial ion transport by sensing A/B parameters and regulating and coordinating activities of various transporter proteins via cAMP-dependent post-translational modifications. Moreover, apical and basolateral epithelial membranes may display differential permeabilities to CO_2, H^+ and HCO_3^-, depending on lipid composition and expression of aquaporins, ion channels and transporters [63–65]. This implies that sAC can respond to (and sense) the A/B status from one epithelial side and not from the other (as shown in isolated dogfish gills in which dfsAC-dependent VHA translocation occurs in response to alkalosis in the basolateral side but not in the apical side [7]). Finally, sAC may also regulate cell physiology in response to A/B disturbances induced by cellular respiration in the cytoplasm (e.g., [27,66] and inside mitochondria [67, 68].

5. sAC in photosynthesizing aquatic organisms

Marine phytoplankton is composed of many species of diverse photosynthesizing microorganisms that generate half our planet's oxygen [69] and are essential to marine foodwebs and the global carbon cycle [70]. Some of these microorganisms additionally establish symbiotic associations with cnidarians, mollusks and other marine animals. Prominent examples are the scleractinian corals, a symbiosis between cnidarians and dinoflagellates that forms coral reefs, one of the most biodiverse ecosystems in the world.

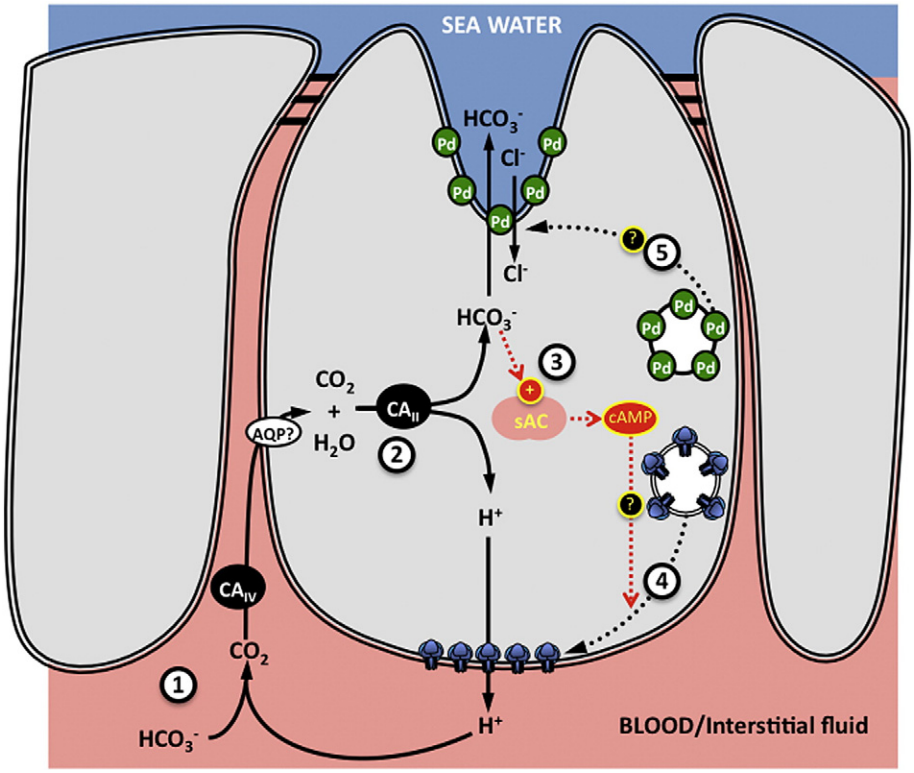

Fig. 3. Model for acid/base sensing and HCO_3^- secretion in shark gill base secreting cells. (1) Blood HCO_3^- is dehydrated by extracellular carbonic anhydrase IV (CA_{IV}), and CO_2 enters the cell possibly *via* aquaporins (AQP). (2) Cytoplasmic carbonic anhydrase II (CA_{II}) hydrates CO_2 into H^+ and HCO_3^-. The elevated intracellular HCO_3^- stimulates sAC to generate cAMP, which triggers the microtubule-dependent translocation of VHA (blue icon) containing cytoplasmic vesicles to the basolateral membrane (4). The targets of cAMP action are unknown. Basolateral VHA reabsorbs H^+ into the blood to counteract the original alkalosis. (5) The anion exhanger pendrin (Pd) translocates to the apical membrane to secrete the excess HCO_3^- to seawater. sAC is presumed to trigger the translocation of pendrin; however, this has not been experimentally confirmed.

For photosynthetic organisms, CO_2 (along with light and water) represents their food. Photosynthetic activity involves the transport of CO_2 and HCO_3^- and its accumulation in intracellular compartments via carbon concentrating mechanisms (CCM). This is essential for photosynthesis because the affinity of ribulose-1,5-bisphosphate carboxylase/oxygenase (commonly known as RuBisCo) for CO_2 is generally much lower than environmental CO_2 levels [71]. Photosynthetic activity is also associated with substantial changes in pH in the cytoplasm of aquatic cyanobacteria (~7.2 in the light and ~6.7 in the dark [72]), as well as in the cytoplasm of coral gastrodermal cells hosting symbiotic algae (~pH 7.4 in the light and ~7.1 in the dark in [73]). Thus, CO_2/pH/HCO_3^- sensors potentially have several essential roles in coordinating cell physiology in photosynthesizing organisms.

BLAST searches of genomic and transcriptomic databases reveal genes encoding for putative sAC-like proteins in representatives of the major marine phytoplanktonic groups: the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* [74], cyanobacteria of the genus *Synechococcus*, the foraminifer *Emiliania huxleyi* and the dinoflagellate *Karenia brevis*, as well as in corals [75]. The physiological roles of sAC in phytoplankton remain unknown; however, experiments with the diatoms *P. tricornutum* suggest sAC is involved in regulating gene expression as cAMP represses the expression of certain genes under elevated environmental CO_2 conditions [76]. Furthermore, *P. tricornutum* has three adenylyl cyclases that show reasonable conservation of amino acids that are key for HCO_3^- stimulation in mammalian sAC (e.g., K95), and *P. tricornutum* lysates demonstrate stimulation of cAMP by HCO_3^- [74]. Similarly, lysates of the diatom *T. pseudonana* show HCO_3^--stimulated cAMP production that is sensitive to the sAC inhibitor KH7 (Fig. 4).

6. Conclusions and perspectives

The most fascinating aspects of sAC are that it provides a mechanism to sense and respond to a fundamental variable in biology such as A/B conditions, that sAC and sAC-related enzymes are evolutionarily conserved from cyanobacteria to mammals and that sAC produces the ubiquitous signaling molecule cAMP, which can regulate virtually every aspect of cell biology and physiology. However, for obvious funding reasons, most of our understanding on sAC comes from biomedical studies, which, although certainly important, usually lack an evolutionary focus. Comparative studies on marine organisms have the potential to provide insights about the evolution of A/B sensing while also being relevant for understanding and predicting responses to environmental stress. Furthermore, some marine organisms may provide useful models to biomedical problems related to A/B sensing and regulation (e.g., shark base-secreting cells).

Although sAC ortholog genes are present in a large variety of aquatic animals [1], it is still for the most part unknown in which cell types sAC is expressed, if sAC protein is present inside organelles like shown for a few mammalian cell lines and tissues and what physiological roles sAC plays in addition to blood A/B regulation in shark gills [7], NaCl and water absorption in fish intestines [26,27], and flagellar movement and the acrosome reaction in sea urchin sperm [12,77]. The dual link of sAC with A/B and cAMP makes the possibilities for additional functions endless; however, characterizing sAC function in marine organisms entails multiple challenges. To begin with, there is a lack of experimental models suitable for reverse genetics, so experiments rely on pharmacological inhibitors such as KH7 and derivatives of catechol estrogens (dCE). Most of our current knowledge on physiology and cell biology has been obtained using this approach, so it is certainly very useful. However, this approach complicates studies on live animals due to the potential inhibition of sAC in multiple parts of the body. Additionally, the effectiveness of KH7 and dCEs on sAC from each organism, as well as their specificity for sAC over tmACs, should be confirmed.

This illuminates a second challenge, which is to discern the cellular functions regulated by sAC from those regulated by tmACs. The cAMP signaling microdomain model proposes that cAMP is produced by sAC and tmACs in discreet intracellular areas and that cAMP diffusion is restricted by phosphodiesterases that degrade cAMP produced in each microdomain, thus preventing (or regulating?) cross-talk and allowing for signal specificity (reviewed in [2,3]). There is evidence for cAMP-micordomains in marine fish intestine, as sAC stimulates NaCl and water absorption but tmAC decreases it (and maybe stimulates NaCl and water secretion) [26,27]. Furthermore, experiments using forskolin to stimulate tmACs and study their function should be interpreted with caution because forskolin stimulation of tmACs is so high that is non-physiologically relevant and almost certainly disrupts cAMP microdomains. The bottom line is that elucidating the roles of sAC requires multiple experimental approaches, and that previous results about the roles of cAMP may require a fresh interpretation that considers the cellular microdomain model.

While there is solid genetic and functional evidence for sAC in elasmobranchs, an open question in fish physiology is if sAC is present in any other fishes. Although studies on toadfish [27] and sea bream [26] intestine suggest sAC is present, those studies relied on Western blotting with heterologous antibodies against shark sAC, and on pharmacological inhibition with KH7 and dCE. Thus, they cannot be considered definitive evidence. The lack of sAC genes in the available genomic and transcriptomic databases from zebrafish,

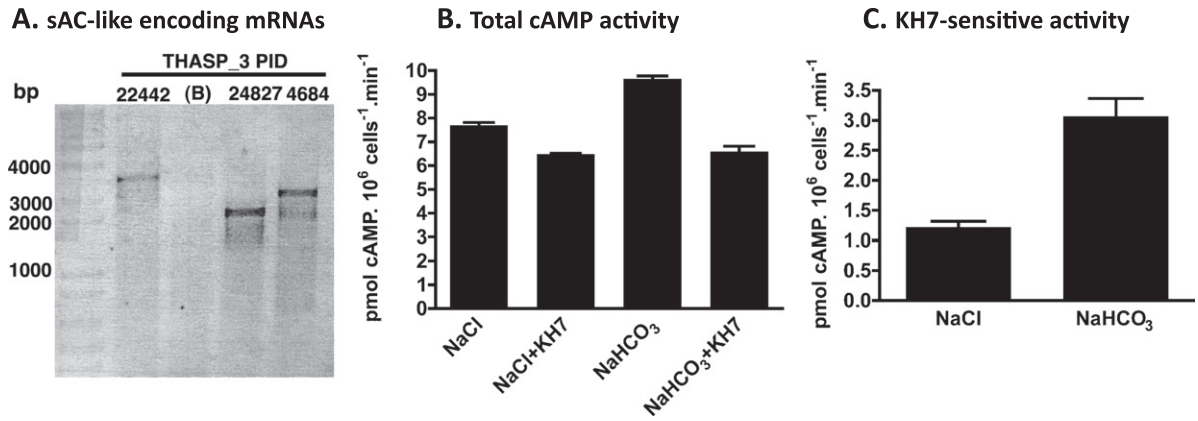

Fig. 4. Evidence for sAC-like enzymes in the diatom *Thalassiosira pseudonana.* (A) RT-PCR of the region encoding the catalytic domains of sACs. PID = protein id according to the THASP_3 database annotation. B = blank. (B, C) Stimulation of cAMP production in *T. pseudonana* extracts by HCO_3^-, and sensitivity to KH7, an inhibitor of eukaryote sACs. Diatoms supernatant aliquots were incubated in 100 mM Tris pH 7.5, 5 mM $MnCl_2$, 2.5 mM ATP, 0.5 mM IBMX, 20 mM creatine phosphate and 100 $U.ml^{-1}$ creatine phoshpkinase (30 min, 25°C). NaCl, $NaHCO_3$ = 40 mM. KH7 = 100 μM (control = DMSO) **(N = 3).**

fugu, stickleback, pufferfish, killifish and Atlantic cod would seem to suggest that sAC genes have been lost in some fish lineages. However, sAC orthologs are definitely present in trout, salmon, gar and coelacanth [1], and there is at least one sAC-like gene in catfish. This raises technical questions of potential problems in the annotation of some of the fish genomes, but also scientific questions about the evolution of A/B sensing. Why would sAC be present in some fish and in most land vertebrates, but absent in another subset of fish species? If this is indeed the case, what genes (if any) have taken over sAC's regulatory roles?

Another question is whether sAC genes from organisms other than mammals undergo alterative splicing, and if, unlike human, the genomes of some species may contain more than one sAC gene. For example, there is evidence for more than one sAC gene in corals [75], which could have implications for specialization of function of the potential sAC isoforms based on differential kinetic properties, localization within the cell and interaction with other proteins.

Finally, it is still unknown whether sAC is present inside organelles such as mitochondria and nucleus of any organism other than humans and rodents. Unpublished results suggests that sAC is present in the nucleus of cells from coral (Barott and Tresguerres) and shark (Roa and Tresguerres), which could provide a mechanistic model for the regulation of gene expression in response to environmental and metabolic A/B stress based on sAC-dependent phosphorylation of transcription x

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References

[1] M. Tresguerres, K.L. Barott, M.E. Barron, J.N. Roa, Potential and established physiological roles of soluble adenylyl cyclase (sAC) in aquatic animals, J. Exp. Biol. 217 (2014) 663–672.
[2] M. Tresguerres, L.R. Levin, J. Buck, Intracellular cAMP signaling by soluble adenylyl cyclase, Kidney Int. 79 (2011) 1277–1288.
[3] M. Tresguerres, J. Buck, L. Levin, Physiological carbon dioxide, bicarbonate, and pH sensing, Pflügers Arch. Eur. J. Physiol. 460 (2010) 953–964.
[4] N. Rahman, J. Buck, L.R. Levin, pH sensing via bicarbonate-regulated "soluble" adenylyl cyclase (sAC), Front. Physiol. 4 (2013) 343.
[5] J.-C. Chang, R. Oude Elferink, Role of the bicarbonate-responsive soluble adenylyl cyclase in pH sensing and metabolic regulation, In addition to maintenance of pH homeostasis, sAC is also involved in metabolic regulation such as coupling of Krebs cycle to oxidative phosphorylation via bicarbonate/CO2 sensing. Additionally, sAC also regulates CFTR channel and plays an important role in regulation of barrier function and apoptosis. These observations suggest that sAC, via bicarbonate-sensing, plays an important role in maintaining homeostatic status of cells against fluctuations in their microenvironment, 2014.
[6] Y. Chen, M.J. Cann, T.N. Litvin, V. Iourgenko, M.L. Sinclair, L.R. Levin, J. Buck, Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor, Science 289 (2000) 625–628.
[7] M. Tresguerres, S.K. Parks, E. Salazar, L.R. Levin, G.G. Goss, J. Buck, Bicarbonate-sensing soluble adenylyl cyclase is an essential sensor for acid/base homeostasis, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 442–447.
[8] J.A. Chaloupka, S.A. Bullock, V. Iourgenko, L.R. Levin, J. Buck, Autoinhibitory regulation of soluble adenylyl cyclase, Mol. Reprod. Dev. 73 (2006) 361–368.
[9] T.N. Litvin, M. Kamenetsky, A. Zarifyan, J. Buck, L.R. Levin, Kinetic properties of "soluble" adenylyl cyclase. Synergism between calcium and bicarbonate, J. Biol. Chem. 278 (2003) 15922–15926.
[10] S. Kleinboelting, A. Diaz, S. Moniot, J. van den Heuvel, M. Weyand, L.R. Levin, J. Buck, C. Steegborn, Crystal structures of human soluble adenylyl cyclase reveal mechanisms of catalysis and of its activation through bicarbonate, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 3727–3732.
[11] M. Nomura, V.D. Vacquier, Proteins associated with soluble adenylyl cyclase in sea urchin sperm flagella, Cell Motil. Cytoskeleton 63 (2006) 582–590.
[12] M. Nomura, C. Beltran, A. Darszon, V.D. Vacquier, A soluble adenylyl cyclase from sea urchin spermatozoa, Gene 353 (2005) 231–238.
[13] G.E. Hofmann, J.E. Smith, K.S. Johnson, U. Send, L.A. Levin, F. Micheli, A. Paytan, N.N. Price, B. Peterson, Y. Takeshita, P.G. Matson, E.D. Crook, K.J. Kroeker, M.C. Gambi, E.B. Rivest, C.A. Frieder, P.C. Yu, T.R. Martz, High-frequency dynamics of ocean ph: a multi-ecosystem comparison, PLoS ONE 6 (2011) e28983.
[14] C.A. Frieder, S.H. Nam, T.R. Martz, L.A. Levin, High temporal and spatial variability of dissolved oxygen and pH in a nearshore California kelp forest, Biogeosciences 9 (2012) 3917–3930.
[15] F. Melzner, M.A. Gutowska, M. Langenbuch, S. Dupont, M. Lucassen, M.C. Thorndyke, M. Bleich, H.O. Portner, Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6 (2009) 2313–2331.
[16] P. Dejours, Carbon dioxide in water- and air-breathers, Respir. Physiol. 33 (1978) 121–128.
[17] D.H. Evans, P.M. Piermarini, K.P. Choe, The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste, Physiol. Rev. 85 (2005) 97–177.
[18] S.F. Perry, K.M. Gilmour, Acid-base balance and CO_2 excretion in fish: unanswered questions and emerging models, Respir. Physiol. Neurobiol. 154 (2006) 199–215.
[19] Y. Niv, G.M. Fraser, The alkaline tide phenomenon, J. Clin. Gastroenterol. 35 (2002) 5–8.
[20] C.M. Wood, M. Kajimura, T.P. Mommsen, P.J. Walsh, Alkaline tide and nitrogen conservation after feeding in an elasmobranch (*Squalus acanthias*), J. Exp. Biol. 208 (2005) 2693–2705.
[21] C.M. Wood, M. Kajimura, C. Bucking, P.J. Walsh, Osmoregulation, ionoregulation and acid-base regulation by the gastrointestinal tract after feeding in the elasmobranch (*Squalus acanthias*), J. Exp. Biol. 210 (2007) 1335–1349.
[22] C.M. Wood, A.G. Schultz, R.S. Munger, P.J. Walsh, Using omeprazole to link the components of the post-prandial alkaline tide in the spiny dogfish, *Squalus acanthias*, J. Exp. Biol. 212 (2009) 684–692.
[23] J.G. Richards, G.J.F. Heigenhauser, C.M. Wood, Exercise and recovery metabolism in the pacific spiny dogfish (*Squalus acanthias*), J. Comp. Physiol. B. 173 (2003) 463–474.
[24] N. Heisler, Acid-base regulation in fishes, in: W.S. Hoar, D.J. Randall (Eds.), Fish Physiology, 10, Academic Press, 1984, pp. 315–401, (Part 1).
[25] N. Heisler, Acid-Base regulation, in: T.J. Shuttleworth (Ed.), Physiology of Elasmobranch Fishes, Springer, Verlag, Berlin, 1988, pp. 215–252.
[26] E.S. Carvalho, S.F. Gregorio, D.M. Power, A.V. Canario, J. Fuentes, Water absorption and bicarbonate secretion in the intestine of the sea bream are regulated by transmembrane and soluble adenylyl cyclase stimulation, J. Comp. Physiol. B. 182 (8) (2012) 1069–1080.
[27] M. Tresguerres, L.R. Levin, J. Buck, M. Grosell, Modulation of NaCl absorption by $[HCO_3^-]$ in the marine teleost intestine is mediated by soluble adenylyl cyclase, Am. J. Physiol. Regul. Integr. Comp. Physiol. 299 (2010) 62–71.
[28] N. Pastor-Soler, V. Beaulieu, T.N. Litvin, N. Da Silva, Y. Chen, D. Brown, J. Buck, L.R. Levin, S. Breton, Bicarbonate-regulated adenylyl cyclase (sAC) is a sensor that regulates pH-dependent V-ATPase recycling, J. Biol. Chem. 278 (2003) 49523–49529.
[29] N. Levine, D.J. Marsh, Micropuncture studies of electrochemical aspects of fluid and electrolyte transport in individual seminiferous tubules, epididymis and vas deferens in rats, J. Physiol. 213 (1971) 557–570.
[30] V. Beaulieu, N. Da Silva, N. Pastor-Soler, C.R. Brown, P.J.S. Smith, D. Brown, S. Breton, Modulation of the actin cytoskeleton via gelsolin regulates vacuolar H^+-ATPase recycling, J. Biol. Chem. 280 (2005) 8452–8463.
[31] W.W.C. Shum, C. Belleannee, D. Brown, S. Breton, Regulation of V-ATPase recycling via a RhoA- and ROCKII-dependent pathway in epididymal clear cells, Am. J. Physiol. Cell Physiol. 301 (2010) C31–C43.
[32] N.M. Pastor-Soler, K.R. Hallows, C. Smolak, F. Gong, D. Brown, S. Breton, Alkaline pH- and cAMP-induced V-ATPase membrane accumulation is mediated by protein kinase A in epididymal clear cells, Am. J. Physiol. Cell Physiol. 294 (2008) C488–C494.
[33] K.R. Hallows, R. Alzamora, H. Li, F. Gong, C. Smolak, D. Neumann, N.M. Pastor-Soler, AMP-activated protein kinase inhibits alkaline pH- and PKA-induced apical vacuolar H + -ATPase accumulation in epididymal clear cells, Am. J. Physiol. Cell Physiol. 296 (2009) C672–C681.
[34] S. Breton, D. Brown, Regulation of luminal acidification by the V-ATPase, Physiology (Bethesda) 28 (2013) 318–329.
[35] N. Pastor-Soler, C. Pietrement, S. Breton, Role of acid/base transporters in the male reproductive tract and potential consequences of their malfunction, Physiology (Bethesda) 20 (2005) 417–428.
[36] B. Hinton, T. Turner, Is the epididymis a kidney analogue? News Physiol. Sci. 3 (1988) 28–31.
[37] D. Brown, R. Bouley, T.G. Paunescu, S. Breton, H.A.J. Lu, New insights into the dynamic regulation of water and acid-base balance by renal epithelial cells, 2012.
[38] T.G. Paunescu, N. Da Silva, L.M. Russo, M. McKee, H.A. Lu, S. Breton, D. Brown, Association of soluble adenylyl cyclase with the V-ATPase in renal epithelial cells, Am. J. Physiol. Renal Physiol. 294 (2008) F130–F138.
[39] F. Gong, R. Alzamora, C. Smolak, H. Li, S. Naveed, D. Neumann, K.R. Hallows, N.M. Pastor-Soler, Vacuolar H + -ATPase apical accumulation in kidney intercalated cells is regulated by PKA and AMP-activated protein kinase, Am. J. Physiol. Renal Physiol. 298 (2010) F1162–F1169.
[40] R. Alzamora, M.M. Al-Bataineh, W. Liu, F. Gong, H. Li, R.F. Thali, Y. Joho-Auchli, R.A. Brunisholz, L.M. Satlin, D. Neumann, K.R. Hallows, N.M. Pastor-Soler, AMP-activated protein kinase regulates the vacuolar H + -ATPase via direct phosphorylation of the A subunit (ATP6V1A) in the kidney, Am. J. Physiol. Renal Physiol. 305 (2013) F943–F956.
[41] R. Alzamora, R.F. Thali, F. Gong, C. Smolak, H. Li, C.J. Baty, C.A. Bertrand, Y. Auchli, R.A. Brunisholz, D. Neumann, K.R. Hallows, N.r.M. Pastor-Soler, PKA regulates vacuolar H^+-ATPase localization and activity via direct phosphorylation of the a subunit in kidney cells, J. Biol. Chem. 285 (2010) 24676–24685.
[42] O. Oehlke, C. Schlosshardt, M. Feuerstein, E. Roussa, Acidosis-induced V-ATPase trafficking in salivary ducts is initiated by cAMP/PKA/CREB pathway via regulation of Rab11b expression, Int. J. Biochem. Cell Biol. 44 (2012) 1254–1265.

[43] M.M. Al-bataineh, F. Gong, A.L. Marciszyn, M.M. Myerburg, N. Pastor-Soler, Regulation of proximal tubule vacuolar H^+-ATPase by PKA and AMP-activated protein kinase, 2014.
[44] P.M. Piermarini, J.W. Verlander, I.E. Royaux, D.H. Evans, Pendrin immunoreactivity in the gill epithelium of a euryhaline elasmobranch, Am. J. Physiol. Regul. Integr. Comp. Physiol. 283 (2002) R983–R992.
[45] B.D. Reilly, R.L. Cramp, J.M. Wilson, H.A. Campbell, C.E. Franklin, Branchial osmoregulation in the euryhaline bull shark, *Carcharhinus leucas*: a molecular analysis of ion transporters, J. Exp. Biol. 214 (2011) 2883–2895.
[46] J.N. Roa, C.L. Munévar, M. Tresguerres, Feeding induces translocation of vacuolar proton ATPase and pendrin to the membrane of leopard shark (*Triakis semifasciata*) mitochondrion rich gill cells, Comp. Biochem. Physiol. A 174 (2014).
[47] M. Tresguerres, S.K. Parks, C.M. Wood, G.G. Goss, V-H^+-ATPase translocation during blood alkalosis in dogfish gills: interaction with carbonic anhydrase and involvement in the postfeeding alkaline tide, Am. J. Physiol. Regul. Integr. Comp. Physiol. 292 (2007) R2012–R2019.
[48] M. Tresguerres, F. Katoh, H. Fenton, E. Jasinska, G.G. Goss, Regulation of branchial V-H^+-ATPase Na^+/K^+-ATPase and NHE2 in response to acid and base infusions in the Pacific spiny dogfish (*Squalus acanthias*), J. Exp. Biol. 208 (2005) 345–354.
[49] M. Tresguerres, S.K. Parks, F. Katoh, G.G. Goss, 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 (2006) 599–609.
[50] D.D.K. Rolston, M.J. Kelly, M.M. Borodo, A.M. Dawson, M.J.G. Farthing, Effect of bicarbonate, acetate, and citrate on water and sodium movement in normal and cholera toxin-treated rat small intestine, Scand. J. Gastroenterol. 24 (2009) 1–8.
[51] P.C. Dagher, R.W. Egnor, A.N. Charney, Effect of intracellular acidification on colonic NaCl absorption, Am. J. Physiol. Gastrointest. Liver Physiol. 264 (1993) G569–G575.
[52] G.E. Sladen, A.M. Dawson, Effect of bicarbonate on sodium absorption by the human jejunum, Nature 218 (1968) 267–268.
[53] A. Ames, K. Higashi, F.B. Nesbett, Effects of PCO_2, acetazolamide and ouabain on volume and composition of choroid-plexus fluid, J. Physiol. 181 (1965) 516–524.
[54] K.Y. Kuang, M. Xu, J.P. Koniarek, J. Fischbarg, Effects of ambient bicarbonate, phosphate and carbonic anhydrase inhibitors on fluid transport across rabbit corneal endothelium, Exp. Eye Res. 50 (1990) 487–493.
[55] J. Farrell, L. Ramos, M. Tresguerres, M. Kamenetsky, L.R. Levin, J. Buck, Somatic "soluble" adenylyl cyclase isoforms are unaffected in Sacy™1Lex /Sacy™1Lex "knock-out" mice, PLoS ONE 3 (2008) e3251.
[56] W. Geng, Z. Wang, J. Zhang, B.Y. Reed, C.Y. Pak, O.W. Moe, Cloning and characterization of the human soluble adenylyl cyclase, Am. J. Physiol. Cell Physiol. 288 (2005) C1305–C1316.
[57] S.T. Halm, J. Zhang, D.R. Halm, β-Adrenergic activation of electrogenic K^+ and Cl^- secretion in guinea pig distal colonic epithelium proceeds via separate cAMP signaling pathways, Am. J. Physiol. Gastrointest. Liver Physiol. 299 (2010) 81–95.
[58] M. Strazzabosco, R. Fiorotto, S. Melero, S. Glaser, H. Francis, C. Spirli, G. Alpini, Diferentially expressed adenylyl cyclase isoforms mediate secretory functions in cholangiocyte subpopulation, Hepatology 50 (2009) 244–252.
[59] M. Tresguerres, S.K. Parks, S.E. Sabatini, G.G. Goss, C.M. Luquet, Regulation of ion transport by pH and [HCO_3^-] in isolated gills of the crab *Neohelice* (*Chasmagnathus*) *granulata*, Am. J. Physiol. Regul. Integr. Comp. Physiol. 294 (2008) R1033–R1043.
[60] E.C. Manning, T.E. Machen, Effects of bicarbonate and pH on chloride transport by gastric mucosa, Am. J. Physiol. Gastrointest. Liver Physiol. 243 (1982) G60–G68.
[61] M. Field, K.J. Karnaky, P.L. Smith, J.E. Bolton, W.B. Kinter, Ion transport across the isolated intestinal mucosa of the winter flounder, *Pseudopleuronectes americanus*, J. Membr. Biol. 41 (1978) 265–293.
[62] M. Field, P.L. Smith, J.E. Bolton, Ion transport across the isolated intestinal mucosa of the winter flounder, *Pseudopleuronectes americanus*: II. Effects of cyclic AMP, J. Membr. Biol. 55 (1980) 157–163.
[63] W.F. Boron, Acid-base transport by the renal proximal tubule, J. Am. Soc. Nephrol. 17 (2006) 2368–2382.
[64] J.S. Hub, B.L. de Groot, Does CO_2 permeate through aquaporin-1? Biophys. J. 91 (2006) 842–848.
[65] V. Endeward, G. Gros, Low carbon dioxide permeability of the apical epithelial membrane of guinea-pig colon, J. Physiol. 567 (2005) 253–265.
[66] L.S. Ramos, J.H. Zippin, M. Kamenetsky, J. Buck, L.R. Levin, Glucose and GLP-1 stimulate cAMP production via distinct adenylyl cyclases in INS-1E insulinoma cells, J. Gen. Physiol. 132 (2008) 329–338.
[67] R. Acin-Perez, B. Hoyos, F. Zhao, V. Vinogradov, D.A. Fischman, R.A. Harris, M. Leitges, N. Wongsiriroj, W.S. Blaner, G. Manfredi, U. Hammerling, Control of oxidative phosphorylation by vitamin A illuminates a fundamental role in mitochondrial energy homoeostasis, FASEB J. 24 (2010) 627–636.
[68] R. Acin-Perez, E. Salazar, M. Kamenetsky, J. Buck, L.R. Levin, G. Manfredi, Cyclic AMP produced inside mitochondria regulates oxidative phosphorylation, Cell Metab. 9 (2009) 265–276.
[69] P.G. Falkowski, M.E. Katz, A.H. Knoll, A. Quigg, J.A. Raven, O. Schofield, F.J.R. Taylor, The evolution of modern eukaryotic phytoplankton, Science 305 (2004) 354–360.
[70] P. Falkowski, R.J. Scholes, E. Boyle, J. Canadell, D. Canfield, J. Elser, N. Gruber, K. Hibbard, P. Hogberg, S. Linder, F.T. Mackenzie, B. Moore III, T. Pedersen, Y. Rosenthal, S. Seitzinger, V. Smetacek, W. Steffen, The global carbon cycle: a test of our knowledge of earth as a system, Science 290 (2000) 291–296.
[71] J.R. Reinfelder, Carbon concentrating mechanisms in eukaryotic marine phytoplankton, Ann. Rev. Mar. Sci. 3 (2011) 291–315.
[72] S. Belkin, R.J. Mehlhorn, L. Packer, Proton gradients in intact cyanobacteria, Plant Physiol. 84 (1987) 25–30.
[73] A.A. Venn, E. Tambuttee, S. Lotto, D. Zoccola, D. Allemand, S. Tambuttee, Imaging intracellular pH in a reef coral and symbiotic anemone, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 16574–16579.
[74] Y. Matsuda, K. Nakajima, M. Tachibana, Recent progresses on the genetic basis of the regulation of CO_2 acquisition systems in response to CO2 concentration, Photosynth. Res. (2011) 1–13.
[75] K.L. Barott, Y. Helman, L. Haramaty, M.E. Barron, K.C. Hess, J. Buck, L.R. Levin, M. Tresguerres, High adenylyl cyclase activity and *in vivo* cAMP fluctuations in corals suggests central physiological role, Sci. Rep. 3 (2013) 1379.
[76] H. Harada, K. Nakajima, K. Sakaue, Y. Matsuda, CO_2 sensing at ocean surface mediated by cAMP in a marine diatom, Plant Physiol. 142 (2006) 1318–1328.
[77] C. Beltran, V.D. Vacquier, G. Moy, Y. Chen, J. Buck, L.R. Levin, A. Darszon, Particulate and soluble adenylyl cyclases participate in the sperm acrosome reaction, Biochem. Biophys. Res. Commun. 358 (2007) 1128–1135.