Journal of the Marine Biological Association of the United Kingdom

cambridge.org/mbi

Energizing the plasmalemma of marine photosynthetic organisms: the role of primary active transport

John A. Raven[1,2,3] and John Beardall[4,5]

[1]Division of Plant Science, University of Dundee at the James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK; [2]Climate Change Cluster, University of Technology Sydney, Ultimo, NSW 2007, Australia; [3]School of Biological Sciences, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia; [4]School of Biological Sciences, Monash University, Clayton, VIC 3800, Australia and [5]State Key Laboratory of Marine Environmental Science & College of Ocean and Earth Sciences, Xiamen University, Xiamen 361005, China

Review

Cite this article: Raven JA, Beardall J (2020). Energizing the plasmalemma of marine photosynthetic organisms: the role of primary active transport. *Journal of the Marine Biological Association of the United Kingdom* **100**, 333–346. https://doi.org/10.1017/S0025315420000211

Received: 14 September 2019
Revised: 19 January 2020
Accepted: 26 February 2020
First published online: 13 April 2020

Key words:
Bacteriochlorophyll; Cl^-; F-type ATPases; H^+; ion-pumping rhodopsins; Na^+; P-type ATPases

Author for correspondence:
John A. Raven, E-mail: j.a.raven@dundee.ac.uk

Abstract

Generation of ion electrochemical potential differences by primary active transport can involve energy inputs from light, from exergonic redox reactions and from exergonic ATP hydrolysis. These electrochemical potential differences are important for homoeostasis, for signalling, and for energizing nutrient influx. The three main ions involved are H^+, Na^+ (efflux) and Cl^- (influx). In prokaryotes, fluxes of all three of these ions are energized by ion-pumping rhodopsins, with one archaeal rhodopsin pumping H^+ *into* the cells; among eukaryotes there is also an H^+ influx rhodopsin in *Acetabularia* and (probably) H^+ efflux in diatoms. Bacteriochlorophyll-based photoreactions export H^+ from the cytosol in some anoxygenic photosynthetic bacteria, but chlorophyll-based photoreactions in marine cyanobacteria do not lead to export of H^+. Exergonic redox reactions export H^+ and Na^+ in photosynthetic bacteria, and possibly H^+ in eukaryotic algae. P-type H^+- and/or Na^+-ATPases occur in almost all of the photosynthetic marine organisms examined. P-type H^+-efflux ATPases occur in charophycean marine algae and flowering plants whereas P-type Na^+-ATPases predominate in other marine green algae and non-green algae, possibly with H^+-ATPases in some cases. An F-type Cl^--ATPase is known to occur in *Acetabularia*. Some assignments, on the basis of genomic evidence, of P-type ATPases to H^+ or Na^+ as the pumped ion are inconclusive.

Introduction

Transport of solutes and water across the plasmalemma is vital for homoeostasis and growth of all organisms. For many of these solutes, and perhaps water in some cases, the movement of the ions and molecules is in the direction opposite to that dictated by the free energy difference across the plasmalemma for that ion or compound: this is the strict definition of active transport. For neutral solutes and water, the free energy difference is the difference in chemical activity (concentration times activity coefficient). For ions there is the additional component of electrical potential difference in the overall free energy difference. Active transport necessitates an input of free energy per molecule or ion transported in excess of the free energy difference per molecule or ion. We follow here the distinction made by Mitchell (1979) between primary and secondary active transport (see also Saier, 2000; Saier *et al.*, 2015). In primary active transport the energy input to the transporter is an exergonic scalar chemical reaction (e.g. oxidation of NAD(P)H by O_2; hydrolysis of ATP to produce ADP and Pi) or photons absorbed by a chromophore component of the transporter (e.g. the retinal component of ion-pumping rhodopsins). In secondary active transport the energy input to the transporter is from the energetically downhill transmembrane flux of a driving ion or ions, with re-energization by primary active transport of those driving ions.

The three major ions involved in primary active transport at the plasmalemma of Archaea, Bacteria and Eukarya are H^+, Na^+ and Cl^-. Table 1 shows what is known of the distribution of these pumps among the three higher taxa and the immediate energy sources. In marine photosynthetic (including photoheterotrophic) organisms there are examples of all of the pumps in Table 1 located in the plasmalemma. It is these ions that are the focus of the subsequent discussion in this paper. Other important ions subject to primary active transport are Ca^{2+} and Mg^{2+}, transported out of cells by P-type ATPases, that also pump, in various organisms, Na^+ out:K^+ in, K^+ in, Na^+ out, H^+ out and H^+ out:K^+ in (Kuhlbrandt, 2004; Bublitz *et al.*, 2011; Chan *et al.*, 2011, 2012; Palmgren & Nissen, 2011; Søndergaard & Pedersen, 2015; Palmgren *et al.*, 2020). As well as variants pumping H^+, Na^+, K^+, Mg^{2+} and Ca^{2+} (topological type II: Thever & Saier, 2009, in prokaryotes and eukaryotes), there are also P-type ATPases (topological type I, Thever & Saier, 2009, not considered further here) that pump out metal cations such as Cu(I), Ag(I), Zn(II), Cd(II) and Pb(II) (Kuhlbrandt, 2004; Thever & Saier, 2009; Bublitz *et al.*, 2011; Palmgren & Nissen, 2011; Søndergaard & Pedersen, 2015). There is also an F-type Cl^- influx ATPase in the plasmalemma of some marine algae, as well as P-type Cl^--ATPases as found in metazoans (Gerencser & Zhang, 2003; Raven, 2017). There

Table 1. Energy sources for primary active transporters of H^+, Na^+ and Cl^- in the Archaea, Bacteria and Eukarya

Energy source	Archaea	Bacteria	Eukarya	Reference
Light Rhodopsin	H^+, Cl^-	H^+, Na^+, Cl^-	H^+	Larkum *et al.* (2018), Gleason *et al.* (2019)
Redox	H^+	H^+, Na^+	H^+	Larkum *et al.* (2018), Gleason *et al.* (2019)
ATP	H^+	H^+, Na^+	H^+, Na^+, Cl^-	Larkum *et al.* (2018), Gleason *et al.* (2019)

See also Kuhlbrandt (2004), Bublitz *et al.* (2011), Palmgren & Nissen (2011), Søndergaard & Pedersen (2015).

are also ATP (adenosine triphosphate) binding cassette proteins (sometimes referred to as ABC transporters) involved in active solute influx at the plasmalemma, although there is little evidence of these in marine photosynthetic organisms (Chan *et al.*, 2011, 2012; but see Badger & Price, 2003 for HCO_3^- accumulation in freshwater and marine cyanobacteria).

Possible original functions of primary active H^+, Na^+ and Cl^- pumps at the plasmalemma

An argument for the early origin and functions of primary active H^+ pumps (ATP-driven; light-driven via rhodopsins) is intracellular acid-base regulation in early cells related, for example, to fermentation of neutral substrates (e.g. sugars) to organic acids (e.g. lactic acid) (Raven & Smith, 1981, 1982) in anoxic environments. Occurrence of the redox-driven or light-driven H^+ pumps and ATP-driven H^+ pumps in the plasmalemma of the same cell permits the use of redox or light energy to phosphorylate ADP to ATP, granted appropriate free energy differences and stoichiometries (Raven & Smith, 1981, 1982). Early chemolithotrophy could generate the H^+ free energy difference across the plasmalemma, and thence phosphorylate ADP (Russell & Hall, 1997; Martin & Russell, 2007; Mulkidjanian *et al.*, 2008; Duchuzeau *et al.*, 2014; but see Jackson, 2016). In seawater at the present pH, and with intracellular (cytosolic) pH about 0.5 units lower than seawater (Raven & Smith, 1981, 1982), the electrical potential difference across the membrane must be more negative than is found in most marine eukaryotes to definitely need active H^+ efflux (see below). The requirement for intracellular pH regulation in oxygenic photolithotrophic marine cells involves several metabolic reactions assimilating inorganic nutrient solutes and is shown in Table 2. Volume regulation of wall-less marine cells by active Na^+ efflux would, in the case of primary active H^+ transport, involve H^+:Na^+ antiport (Raven & Smith, 1982; Katz *et al.*, 1991; Gimmler, 2000).

Well characterized in the, mainly freshwater, green algal macrophytes of the Characeae (Walker *et al.*, 1980), localized active efflux of H^+ across the plasmalemma causes a localized acidification in the cell wall and diffusion boundary layer. This shifts the CO_2:HCO_3^- equilibrium in favour of CO_2, increasing the rate of uncatalysed HCO_3^- conversion to CO_2 and thereby improving cellular CO_2 supply (Raven & Beardall, 2016). A similar mechanism is thought to occur in many marine macrophytes as a mechanism of using external HCO_3^- (Raven & Hurd, 2012). However, the evidence for this in marine macroalgae is indirect and based on effects of buffers on HCO_3^- use, inhibition of external carbonic anhydrase using membrane-impermeant inhibitors or a lack of effects of inhibitors of HCO_3^- transport (Raven & Hurd, 2012; Raven & Beardall, 2016). It is considered unlikely that this process occurs in microalgae, however, as their size would mean they have a thinner diffusive boundary layer and greater proton leakage (Flynn *et al.*, 2012; Raven & Beardall, 2016).

For primary active Na^+ efflux at the plasmalemma of wall-less cells, the higher intracellular K^+:Na^+ ratio in the cytosol than in a high-salinity medium such as seawater, driven by the primary active Na^+ efflux, could be involved in cell volume regulation, countering the Donnan effect of negative charge in the cytosol (Raven & Smith, 1982). The high K^+:Na^+ in the cytosol is attributed by Dibrova *et al.* (2015) to the origin of life at inland hot springs with high K^+:Na^+, resulting in a requirement for high (about 100 mol m^{-3}) K^+ for activity of many enzymes. When life invaded the much more widespread freshwater and marine habitats with low K^+:Na^+ ratios, active Na^+ efflux maintained high internal K^+:Na^+ ratios despite a finite Na^+ permeability of the plasmalemma and, in some bacteria, significant energy storage as a Na^+ electrochemical potential gradient (Na^+ motive force) (Skulachev, 1984; Skulachev, 1989; Mulkidjanian *et al.*, 2008; Dibrova *et al.*, 2015). Primary active Na^+ efflux in the marine species of the wall-less chlorophycean *Dunaliella* spp. is involved in osmoregulation with varying external osmolarities (Ehrenfeld & Cousin, 1984; Gimmler, 2000; Popova *et al.*, 2005; Popova & Balnokin, 2013). The Na^+ electrochemical potential gradient is widely used in marine (and some other) photosynthetic organisms to energize the influx of nutrients and osmolytes by Na^+ co-transport (Raven, 1984; Chan *et al.*, 2011, 2012). A further function of the Na^+ electrochemical potential difference is in the action potentials in marine diatoms (Taylor, 2009; Helliwell *et al.*, 2019) and, from genomic evidence, the marine phaeophycean *Ectocarpus* and the marine prasinophyceans *Micromonas* and *Ostreococcus*, as well as freshwater chlorophyceans (Fux *et al.*, 2018). Recovery of the resting state after an action potential requires active Na^+ efflux, either by primary active Na^+ efflux, or by Na^+:H^+ antiport following primary active H^+ efflux.

Primary active transport of Cl^- at the plasmalemma of walled marine ulvophycean algal cells is involved in turgor generation, and in action potentials (Bisson *et al.*, 2006; Raven, 2017). At least the turgor generation role could be performed by Cl^- influx coupled to H^+ or Na^+ influx and primary active H^+ or Na^+ efflux. The roles of Cl^- as an essential micronutrient in photosynthesis (Raven, 2017) can be satisfied by passive Cl^- distribution between seawater and the cytosol even with an inside-negative electrical potential of 150 mV, but not the role established in terrestrial flowering plants as a beneficial nutrient (Raven, 2017).

Not considered in detail here is active Ca^{2+} efflux at the plasmalemma that maintains the very low free Ca^{2+} concentration in the cytosol (100–200 µmol m^{-3}) used in preventing damage to proteins and in signalling (Roberts *et al.*, 1994; Thompson *et al.*, 2007; Wheeler *et al.*, 2019). Ca^{2+}-H^+ antiporters and Ca^{2+} P-ATPases that are involved in Ca^{2+} efflux from the cytosol are known, on genomic evidence, to be very widespread among algae (Emery *et al.*, 2012; Palmgren *et al.*, 2020).

Energetics of primary active transport

An important aspect of analysing primary active ion transport at the plasmalemma is the electrochemical potential difference for ions across that membrane. The electrochemical potential difference is defined by equation (1) (Mitchell, 1979; Raven, 1984; Nobel, 2009; Nichols & Ferguson, 2013).

$$\Delta(\bar{\mu})_{j^{+/-}\mathrm{NP}} = zF\Psi_{\mathrm{NP}} + \mathrm{RT}\ln\frac{[j^{+/-}]_{\mathrm{N}}}{[j^{+/-}]_{\mathrm{P}}} \quad (1)$$

where $\Delta(\bar{\mu})_{j^{+/-}\mathrm{NP}}$ = electrochemical potential of ion $j^{+/-}$ in phase N relative to that in phase P; N = electrically negative phase (cytosol for the plasmalemma); P = electrically positive phase (aqueous medium

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

Table 2. Intracellular H^+ production and consumption in redox reactions, and calcification, in marine phytoplankton

Element	Solute entering cell	Product	Δ H^+	Elemental content, atoms relevant to P	Δ H^+ inside the cell: + = H^+ production, − = H^+ consumption
C	CO_2	1 C in (CH_2O)	0	106	0
C	HCO_3^-	1 C in (CH_2O)	−1	106	−106
N	NH_4^+	1 N in organic matter	+1 to +1.3	16	16–20.8
N	N_2	1 N in organic matter	0 to +0.3	16	0–4.8
N	NO_3-	1 N in organic matter	−0.7 to −1	16	11.2–16
S	SO_4^{2-}	1 S as –SH in organic matter	−2	1	−2
C	HCO_3^-, Ca^{2+}	$CaCO_3$	+1	106	+106

Elemental atomic ratios based on the Redfield ratio, with a upper limit on reduced S from Ksionzek *et al.* (2016) since values are for total S and not all S in the cell is reduced to the −SH level. For intracellular calcification in coccolithophores (Taylor *et al.*, 2017) and some dinoflagellates (Van de Waal *et al.*, 2013), a particulate inorganic C: particulate organic C ratio of 1.0 is assumed. Other references for H^+:N are assimilated from Brewer & Goldman (1976), Smith & Raven (1979) and Raven (2013).

for the plasmalemma); $j^{+/-}$ = ion under consideration, either a cation (j^+) or anion (j^-); z = numerical charge on the ion (+1 for H^+ or Na^+, +2 for Ca^{2+}, −1 for Cl^-); F = Faraday constant (96,485 Joule V^{-1} mol^{-1}); Ψ_{NP} = electrical potential of phase N relative to phase P; R = gas constant (8.314 Joule mol^{-1} °K); T = temperature (°K); ln = natural logarithm; $[j^+]_N$ = concentration of j^+ in phase N (mol m^{-3}); $[j^-]_P$ = concentration of j^- in phase P (mol m^{-3}).

The sign of the electrochemical potential difference defines the direction of active transport of the ion, i.e. in the energetically uphill direction, requiring an input of energy from coupling to photons, exergonic redox reactions, or ATP conversion to ADP and phosphate in primary active transport, or coupling to exergonic ion fluxes in secondary active transport. The magnitude of the electrochemical potential difference defines the minimum energy input to primary or secondary active transport. As will be seen in the rest of the paper, Na^+ invariably, and H^+ very widely, are actively transported by marine photosynthetic organisms from the cytosol to the medium. If only one of these ion species, e.g. H^+, is subject to primary active transport, then antiport coupling exergonic H^+ re-entry to secondary active Na^+ efflux must have a H^+:Na^+ stoichiometry consistent with the antiport being overall exergonic.

Hereinafter, the electrical potential of the cytosol (N phase) relative to the outside medium (P phase) is represented as Ψ_{CO}.

Organisms with ion-pumping rhodopsins

Ion-pumping rhodopsins are light energy transducers that bring about active ion transport as the sole product of the photochemical reaction usable in cell metabolism (Oesterhelt & Stoeckenius, 1973). The photoreaction and ion transport occurs using a single retinol-binding opsin protein, and brings about positive charge (H^+ or Na^+) flux from the N to the P side of the membrane, or negative charge (Cl^-) flux from the P to the N side of the membrane, where 'N' and 'P' are as defined by Mitchell (1979). For the plasmalemma, the cytosol is the N side and the outside medium is the P side (see equation (1)).

Some marine bacteria have rhodopsins that pump H^+ or Na^+ out of the cells, or Cl^- into the cells; some marine Archaea have been shown to have H^+ efflux or Cl^- influx rhodopsins, but there are no reports of Na^+ efflux rhodopsins in Archaea (Oesterhelt & Stoeckenius, 1973; Bejá & Lanyi, 2014; Yoshizawa *et al.*, 2014; Larkum *et al.*, 2018). Unexpectedly, there is an *inwardly* directed H^+ pump driven by xenorhodopsin in *Nanosalina* (Shevchenko *et al.*, 2017); the significance of this energized, but energetically downhill, flux is not clear. Some marine Archaea and bacteria have autotrophic CO_2 assimilation pathways, but none of these are energized entirely by ion-pumping rhodopsins despite the possibility of such energization (Raven & Smith, 1981; Raven, 2009*a*, 2009*b*; Berg *et al.*, 2010; Bejá & Lanyi, 2014; Larkum *et al.*, 2018). A marine aerobic anoxygenic photoheterotrophic bacterium, with bacteriochlorophylls, also has an H^+-pumping rhodopsin, presumably in the plasmalemma (Larkum *et al.*, 2018). There seem to be no reports of ion-pumping rhodopsins in marine aerobic or anaerobic anoxygenic photosynthetic bacteria, or in marine photosynthetic cyanobacteria, although a freshwater/terrestrial cyanobacterium (*Gloeobacter*) has a H^+ efflux rhodopsin, with respiratory and photosynthetic H^+ pumps, in the plasmalemma (Larkum *et al.*, 2018). In an analysis of the energetics of phototrophic marine picoplankton, ion-pumping rhodopsins are major energy-transducing pigments in the ocean in organisms that are small enough to pass through a 2 µm filter (Gómez-Consarnau *et al.*, 2019; see also Kirchman & Hanson, 2013).

Ion-pumping rhodopsins also occur in eukaryotes. Among marine photosynthetic eukaryotes, the best-characterized case of ion-pumping rhodopsin in the plasmalemma is in *Acetabularia*, which contains an H^+-pumping rhodopsin. Paradoxically, this rhodopsin moves H^+ *into* the cytosol, rather than the expected direction of acting as a H^+ efflux pump (Raven, 2009*a*; Wada *et al.*, 2011; Bejá & Lanyi, 2014; Tamogami *et al.*, 2017; Larkum *et al.*, 2018), i.e. in the same direction as the *inwardly* directed H^+ pump driven by xenorhodopsin in the bacterium *Nanosalina* (Shevchenko *et al.*, 2017). The Cl^- ATPase in the plasmalemma of *Acetabularia* generates a Ψ_{CO} of −180 mV, inside negative, in the light that, with a probable cytosol pH between 7 and 8, means an inwardly directed H^+ electrochemical difference. This means that the H^+-transporting rhodopsin 'pumps' H^+ downhill. Other cases, where it is thought that the H^+-pumping rhodopsin is in the plasmalemma and functions in H^+ efflux, are found in some diatoms where it is thought to be an Fe-sparing way of energizing the plasmalemma (Raven, 2009*a*; Slamovits *et al.*, 2011; Marchetti *et al.*, 2015; Cohen *et al.*, 2017; Larkum *et al.*, 2018). In at least one case (the dinoflagellate *Noctiluca* which lacks oxygenic photosynthesis, except by symbiosis) the H^+-pumping rhodopsin is found in a digestive vacuole; H^+-pumping rhodopsins also occur in dinoflagellates with oxygenic photosynthesis (Slamovits *et al.*, 2011; Vader *et al.*, 2018).

It is clear that there is a significant variation in the phylogenetic analysis, and the richness of relevant data available among taxa. This is also the case for data considered below for organisms with bacteriochlorophylls and chlorophylls.

Organisms with bacteriochlorophylls

The anoxygenic photosynthetic bacteria function as photolithotrophs with reductants other than water in anaerobic environments

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

or photoheterotrophically by assimilating CO_2 with an organic reductant more oxidizing than H_2 in anaerobic environments. In both of these cases autotrophic CO_2 assimilation pathways are used (Larkum *et al.*, 2018). These photolithotrophic organisms occupy geographically limited benthic marine anoxic illuminated habitats. The other marine bacteria with bacteriochlorophyll are the planktonic aerobic anoxygenic bacteria (Kolber *et al.*, 2001) that lack autotrophic CO_2 assimilation pathways, and so are photoheterotrophs (Larkum *et al.*, 2018; Gómez-Consarnau *et al.*, 2019). Bacteriochlorophylls are less significant in terms of photon absorption than ion-pumping rhodopsins or than chlorophylls as energy-transducing pigments in the ocean in organisms capable of passing through a 2 µm filter (Gómez-Consarnau *et al.*, 2019; see also Kirchman & Hanson, 2013).

Bacteriochlorophylls, like chlorophylls but unlike ion-pumping rhodopsins, are light energy transducers that indirectly bring about active transport of H^+. The primary photochemistry moves an electron from a high potential electron donor on the P side of the membrane (*sensu* Mitchell, 1979) to a low potential acceptor on the N side of the membrane (*sensu* Mitchell, 1979) (equation (1)). H^+ active transport from the P to the N side of the membrane is a result of secondary, thermodynamically downhill, redox reactions.

The freshwater and marine Chlorobi and Chloroflexi and, in inland hot springs, Acidobacteria, are photolithotrophic bacteria with light-harvesting bacteriochlorophyll *c* in chlorosomes. These organisms have no intracellular membranes, and have either Type 1 (Chlorobi, Acidobacteria) or Type 2 (Chloroflexi) reaction centres and associated redox catalysts, and the (photo-) redox H^+ pumps and H^+ electrochemical difference-driven F_OF_1 ATP synthases, are in their plasmalemma (Adams *et al.*, 2013; Larkum *et al.*, 2018). This plasmalemma location of photochemistry is also the case for the firmicute *Heliobacterium* without chlorosomes and with Type 1 reaction centres. H^+ efflux across the plasmalemma is driven by light energy in the photoperiod, and by non-photochemical redox reactions in the scotophase. The H^+ free energy difference across the plasmalemma in the light is large enough to synthesize ATP, granted the H^+:ATP ratio of the bacterial F_OF_1 ATP synthase (Larkum *et al.*, 2018).

The marine photosynthetic Proteobacteria with Type 2 reaction centres are the anaerobic photolithotrophic purple sulphur bacteria, and the anaerobic or aerobic photoheterotrophic purple non-sulphur bacteria. These organisms have their Type 2 reaction centres in plasmalemma invaginations and/or intracellular vesicles or flattened thylakoids (Larkum *et al.*, 2018). These structural features mean that most, or all, of the photochemistry and associated H^+ pumping is in membranes other than the plasmalemma that directly exchanges solutes with the bulk medium. It is, however, unclear what primary ion pumps occur in those parts of the plasmalemma that are not invaginated, and so are involved in nutrient uptake.

The marine anaerobic anoxygenic photosynthetic bacteria of the Chlorobi, Chloroflexi and Proteobacteria make a very small contribution (less than 0.1%) to global marine primary productivity (Johnson *et al.*, 2009; Raven, 2009*b*). Photons absorbed by marine aerobic anoxygenic photoheterotrophic bacteria probably make a larger contribution (0.5–5%) to global marine primary productivity than the anaerobic anoxygenic photolithotrophic bacteria (Kolber *et al.*, 2000, 2001; Goericke, 2002; Johnson *et al.*, 2009; Raven, 2009*b*; Kirchman & Hanson, 2013; Gómez-Consarnau *et al.*, 2019).

Organisms with chlorophylls

Cyanobacteria

Some phylogenetic evidence is consistent with a freshwater/terrestrial origin of cyanobacteria (Blank & Sánchez-Baracaldo, 2010; Blank, 2013*b*; Sánchez-Baracaldo *et al.*, 2014). Data from freshwater cyanobacteria show that respiratory and photosynthetic redox and proton pumping reactions, and the associated CF_OCF_1 ATP synthase, occur in thylakoids. The exception is in *Gloeobacter*, where there are no thylakoids and photosynthesis and respiration, and ion-pumping rhodopsin, as well as nutrient transporters, occur in the plasmalemma (Mullineaux, 2014; Lea-Smith *et al.*, 2016). The plasmalemma of thylakoid-containing freshwater Cyanobacteria has vanadate-sensitive (presumably ATP-driven) H^+ efflux (Scherer & Böger, 1984; Kaplan *et al.*, 1989; Schultze *et al.*, 2009), and can oxidize NAD(P)H and reduce O_2; and contains plastoquinone (PQ) and ATP synthase (Mullineaux, 2014; Lea-Smith *et al.*, 2016), and moves protons out of the cell (Scherer *et al.*, 1984). While this is consistent with primary active H^+ efflux at the plasmalemma in freshwater cyanobacteria, the work of Ritchie (1992) shows that there is an electrogenic Na^+ efflux pump at the plasmalemma of *Synechococcus* R2, and that the H^+ efflux involves a Na^+:H^+ antiporter. This Na^+ active efflux occurs over a wide range of external pH (5–10), K^+ (0.1–300 mol m^{-3}) and Na^+ (0.1–300 mol m^{-3}). The halophilic, alkalophilic cyanobacterium *Aphanothece halophytica* has a Na^+-dependent F_OF_1 ATP synthase which, working as an ATPase in the plasmalemma, could act in active Na^+ efflux (Wiangno *et al.*, 2007; Soontharapirakkul & Incharoensakdi, 2010; Soontharapirakkul *et al.*, 2011; see prediction by Mitchell, 1979). Gabbay-Azaria *et al.* (2000) demonstrated cytochrome oxidase activity in the plasmalemma of the marine cyanobacterium *Spirulina subsalsa* (now *Arthrospira subsalsa*) that could be involved in active H^+ efflux (Gabbay-Azaria *et al.*, 1992). Bergman *et al.* (1993) found cytochrome oxidase in the plasmalemma as well as the thylakoid membranes of the marine diazotrophic cyanobacterium *Trichodesmium thiebaultii*. A P-type Ca^{2+} ATPase has been found in a marine cyanobacterium that can excavate solid $CaCO_3$ (Garcia-Pichel *et al.*, 2010).

Na^+ symport of HCO_3^- is one of the means of concentrating inorganic C in freshwater cyanobacterial cells, although these organisms also have other HCO_3^- transporters, one of which is an ABC transporter (Omata *et al.*, 1999; Badger & Price, 2003). Freshwater cyanobacteria also use ABC transporters for NO_3^- (Maeda & Omata, 1997). However, the HCO_3^- and NO_3^- transporters in marine cyanobacteria are not ABC transporters such as occur in their freshwater counterparts (Wang *et al.*, 2000; Badger & Price, 2003; Maeda *et al.*, 2015).

Eukaryotic algae: relation to the supergroups in the New Tree of Life

Almost all eukaryotic photolithotrophs have a photosynthetic apparatus most of whose core genes were derived from endosymbiosis of a freshwater β-cyanobacterium with a non-photosynthetic unicellular eukaryote to comprise the Archaeplastida (Burki *et al.*, 2020; Palmgren *et al.*, 2020). 'Green' Archaeplastida with chlorophyll *b* produced, by endosymbiosis in an Excavate cell, the secondarily photosynthetic Euglenophyta and, by endosymbiosis in a cell of the Rhizaria in the TSAR (Telonemia, Stramenopila, Alveolata, Rhizaria) supergroup, the secondarily photosynthetic Chlorarachniophyta. In a further set of secondary endosymbioses, 'Red' Archaeplastida with phycobilins produced, in cells of the Cryptista, the phycobilin-containing Cryptophyta and, with the loss of light-harvesting phyobilins, in cells of the Haptista to produce the Haptophyta, and in cells of the TSAR supergroup to produce the chromerids and dinoflagellates in the Alveolata, and the Ochrista in the Stramenopila. A very small minority of photolithotrophic eukaryotes (the genus *Paulinella*) arose by endosymbiosis of an α-cyanobacterium in a rhizarian (TSAR supergroup) (Nowack, 2014).

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

Eukaryotic algae: Archaeplastida (Glaucophyta, Rhodophyta, Chlorophyta, algal members of the Streptophyta)

The primary endosymbiosis of a β-cyanobacterium in an aerobic eukaryote that gave rise to chloroplasts of the Archaeplastida involved a cyanobacterium whose closest living relative is the freshwater *Gloeomargarita lithophora* (Brasier, 2013; Blank, 2013*a*; Lewis, 2017; Ponce-Toledo *et al*., 2017; Sánchez-Baracaldo *et al*., 2017). The basal extant Archaeplastida are the freshwater Glaucophyta; the basal Rhodophyta, the Cyanidiophyceae, are non-marine, but their habitat of acid hot springs cannot be described as freshwater (Dittami *et al*., 2017). There have been numerous freshwater–marine transitions in the Rhodophyta and, especially, in the Chlorophyta, but less so in the predominantly freshwater algal Streptophyta (Dittami *et al*., 2017). These habitat variations make it difficult to predict which primary active ion transporters occur in the plasmalemma of these organisms.

In the Rhodophyta, the non-marine (acid hot spring) *Cyanidium caldarium*, *Cyanidioschyzon merolae* and *Galdieria sulphuraria* (Cyanidiophyceae) have P-type H^+-ATPases but not Na^+-ATPases (Ohta *et al*., 1997; Lee *et al*., 2017). Two Na^+-ATPases (PyKPA1 and PyKPA2) have been characterized in the marine bangiophycean *Porphyra yezoensis* (now *Pyropia yezoensis*) (Barrero-Gil *et al*., 2005; Uji *et al*., 2012*a*, 2012*b*; see also Chan *et al*., 2012; Kishimoto *et al*., 2013). This organism also has two plasmalemma Na^+/H^+ antiporters (Uji *et al*., 2012*b*). An Na^+:H^+ antiporter has also been described in *Pyropia haitanensis* by Chen *et al*. (2019), who assume, on the basis of vanadate inhibition, that there is a H^+-ATPase at the plasmalemma of this alga. However, vanadate inhibition is the case for all P and F ATPases (Müller *et al*., 1999; Araki & González, 1998; Kuhlbrandt, 2004; Hong & Pedersen, 2008; Pedersen *et al*., 2012), but to a much smaller extent for V ATPases (Müller *et al*., 1996; Araki & González, 1998). Accordingly, vanadate is a general inhibitor of H^+, Na^+ and Ca^{2+} P-ATPases, and is not specific for H^+ ATPases. Reed *et al*. (1981) and Reed & Collins (1981) examined the energetics of ion transport in *Porphyra purpurea* over a wide range of hypo- and hyper-saline (relative to seawater) media. Under all salinities (1/16 seawater to 3× seawater) there is active Na^+ efflux (Reed *et al*., 1981). It should be noted that the measurement of Ψ_{CO} in the essentially non-vacuolate *Porphyra purpurea* cells by Reed and co-workers was determined using the distribution of the lipophilic cation $TPMP^+$; there are reservations about the use of this method of measuring transplasmalemma electrical potential differences in eukaryotes (Ritchie, 1982, 1984). The florideophycean *Chondrus crispus* has a P-type Na^+-ATPase but no H^+-ATPase (Lee *et al*., 2017). There seem to be no relevant data for freshwater red algae.

In the Chlorophyta, the microalgal Prasinophyceae occur in marine and also freshwater habitats (Tragin & Vaulot, 2018; Del Cortona *et al*., 2020). There is functional evidence for the presence in the plasmalemma of the marine *Tetraselmis* (=*Platymonas*) *viridis* of an ATP-driven Na^+ pump (Balnokin & Popova, 1994; Balnokin *et al*., 1997, 1999, 2004; Gimmler, 2000; Pagis *et al*., 2001, 2003; Popova & Balnokin, 2013) and an ATP-driven H^+ pump (Popova & Balnokin, 1992; Gimmler, 2000; Pagis *et al*., 2003). There is genomic evidence of a P-type Na^+-ATPase in the marine *Ostreococcus tauri* (Rodríguez-Navarro & Benito, 2010). Gimmler (2000) provides an energetic background to infer the presence of active H^+ and Na^+ efflux in *Tetraselmis viridis*, although there are no data from that organism on the electrical potential difference across the plasmalemma, or Na^+ and H^+ concentrations in the cytoplasm.

The Chlorophyta: Chlorophyceae are mainly freshwater but with some marine representatives (Tragin & Vaulot, 2018; Del Cortona *et al*., 2020), the P-type ATPases of the genus *Dunaliella* has been the subject of considerable investigation (Wolf *et al*., 1995; Weiss & Pick, 1996; Popova *et al*., 2018). Sequences encoding P-type H^+-ATPases have been found in the genomes of the halophilic *Dunaliella bioculata* (Smahel *et al*., 1990; Wolf *et al*., 1995), *Dunaliella salina* (Weiss & Pick, 1996; Katz *et al*., 2007), *Dunaliella bioculata* (Bertucci *et al*., 2010) and *Dunaliella tertiolecta* (Popova *et al*., 2018), as well as the acidophilic *Dunaliella acidophila* (Weiss & Pick, 1996; Bertucci *et al*., 2010). While Popova *et al*. (2018) could not find a P-type Na^+-ATPase in the genome of *Dunaliella tertiolecta*, Popova *et al*. (2005; see also Shumkova *et al*., 2000) functionally identified an electrogenic Na^+-translocating ATPase in *Dunaliella maritima* using inside-out plasmalemma vesicles (see also Popova & Balnokin, 2013). The possibility of a primary H^+ pump with H^+:Na^+ antiport was ruled out by the observation of stimulation, rather than inhibition, of Na^+ transport by the uncoupler CCCP (m-chlorophenyl carbonyl cyanide phenylhydrazone). Popova *et al*. (2005) did not attempt to identify whether the catalyst of Na^+ transport was a P-type ATPase. Further investigation is needed into the possibility of direct redox energization of Na^+ efflux from *Dunaliella salina* (Katz & Pick, 2001). The freshwater *Chlamydomonas reinhardtii* has a P-type Na^+-ATPase (Barrero-Gil *et al*., 2005; Rodríguez-Navarro & Benito, 2010), as well as a P-type H^+-ATPase (Campbell *et al*., 2001; Barrero-Gil *et al*., 2005; Bertucci *et al*., 2010).

An important aspect of the functioning of primary active transport of H^+ and Na^+ in driving symport, antiport and uniport of other solutes by *Dunaliella* is the free energy difference across the plasmalemma. There are several reports of intracellular Na^+ concentrations (Katz & Avron, 1985; Bental *et al*., 1986; Pick *et al*., 1986; Wegmann, 1986; Gimmler, 2000), cytoplasmic pH (Burns & Beardall, 1987; Gimmler *et al*., 1988; Ginzburg *et al*., 1988; Gimmler, 2000) and Ψ_{CO} (Gimmler & Greenway, 1983; Oren-Shamir *et al*., 1990; see the critique by Ritchie, 1982, 1984) in a variety of halophilic *Dunaliella* species. Ψ_{CO} of *Dunaliella acidophila* has been measured using the preferable microelectrodes method (Remis *et al*., 1992), but not so far for halophilic *Dunaliella* species. The thermodynamic analysis by Gimmler (2000) dealt with *Dunaliella salina* and suggested active efflux of both H^+ and Na^+, and pre-dates the discovery of the electrogenic Na^+ ATPase in *Dunaliella maritima* (Popova *et al*., 2005; see also Shumkova *et al*., 2000). Khramov *et al*. (2019) showed increased transcript abundance of a putative H^+ P-ATPase under hypersaline conditions in *Dunaliella maritima*.

As with the Chlorophyceae, the Chlorophyta: Trebouxiophyceae are mainly freshwater but with some marine members (Tragin & Vaulot, 2018; Del Cortona *et al*., 2020). There is genomic evidence of both Na^+-ATPase and H^+-ATPases in the halotolerant *Picochlorum* sp. (Foflonker *et al*., 2014). The only contribution to a thermodynamic analysis of the need for active H^+ and/or Na^+ efflux is the work of Bock *et al*. (1996) who, using ^{31}P NMR, found a cytoplasmic pH of 7.8 in the high intertidal–supralittoral macroalga *Prasiola crispa*. For a freshwater *Chlorella* sp. there is genomic evidence of a Na^+ -ATPase (Uji *et al*., 2012*a*), and functional evidence consistent with a H^+-ATPase (Komor *et al*., 1989).

The earliest Chlorophyta: Ulvophyceae fossils are from marine sediments, and most extant ulvophyceans are marine, exceptions being the freshwater *Dichotomosiphon* and some species of *Cladophora* and the terrestrial Trentepohliales (Del Cortona *et al*., 2020). Blount & Levedahl (1960) found active electrogenic Na^+ efflux and Cl^- influx in the marine gametophyte *Halicystis ovalis* phase of the marine ulvophycean *Derbesia marina*. There is genomic evidence for a P-type Na^+ ATPase of the marine ulvophycean *Flabellia petiolata* (formerly *Udotea petiolata*) (Rodríguez-Navarro & Benito, 2010), and for a number of

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

P-type ATPases in *Ulva* (Zhang *et al.*, 2012; De Clerck *et al.*, 2018).

Blinks (1940, 1949) showed that *Halicystis ovalis* (the giant-celled coenocytic gametophyte phase of *Derbesia marina*) had a potential difference between the vacuole and seawater medium (Ψ_{VO}) of −75 to −80 mV; for *Halicystis osterhoutii* (the gametophyte phase of *Derbesia osterhoutii*) the value is −65 to −70 mV. Replacing external Cl^- with NO_3^- (or SO_4^{2-}, or a number of organic anions) caused the potential difference to become negligible, or positive. This is consistent with the short-circuit current experiments of Blount & Levedahl (1960) showing the occurrence of an electrogenic Cl^- influx pump in *Halicystis ovalis*. Graves & Gutknecht (1976, 1977*a*, 1977*b*) examined ion concentrations and fluxes, and the effects of decreased external Cl^- and low temperatures and of clamped Ψ_{VO}, in the gametophyte *Halicystis parvula* phase of *Derbesia tenuissima*, and also concluded that there is an electrogenic Cl^- influx pump. Graves & Gutknecht (1976, 1977*a*, 1977*b*) infer that the Cl^- pump is at the plasmalemma, as is the case for *Acetabularia*, discussed below.

Saddler (1970*a*, 1970*b*) measured ion (K^+, Na^+, Cl^-) fluxes between uninucleate giant-celled marine *Acetabularia* and the seawater medium, and electrical properties of the cell, concluding that there was an electrogenic Cl^- influx pump. The occurrence of active electrogenic Cl^- influx was confirmed by Mummert & Gradmann (1976) and by Gradmann *et al.* (1982). The Ψ_{CO} in the light is −170 mV (Saddler, 1970*a*) to −180 mV (Amtmann & Gradmann, 1994). Saddler (1970*a*, 1970*b*) suggested that cytoplasmic Cl^- in *Acetabularia* is the same (500 mol m^{-3}) as that in seawater, although this is not consistent with observed inhibitory effects of Cl^- on metabolism (Raven, 2017). Using the value of 500 mol m^{-3} for cytoplasmic Cl^-, the electrochemical potential gradient for Cl^- is 17–18 kJ mol^{-1}, driving Cl^- from the cytoplasm to the medium (Saddler, 1970*a*, 1970*b*). Mummert & Gradmann (1991*a*) showed the role of Cl^- in the action potential of *Acetabularia*, and also (Mummert & Gradmann, 1991*a*, 1991*b*) discovered the role of vesicular transport of ions across the cytosol to the vacuole. The Cl^- influx is driven by a Cl^--ATPase (Gradmann *et al.*, 1982; Goldfarb & Gradmann, 1983; Ohhashi *et al.*, 1992) inhibited by vanadate (Smahel *et al.*, 1992; see discussion of vanadate inhibition of primary active ion-pumping ATPase under 'Rhodophyta' above). Goldfarb *et al.* (1984) reported that the Cl^- pump can be reversed, coupled to net ATP synthesis (as in the prediction of Mitchell, 1979). Ikeda *et al.* (1990*a*, 1990*b*) showed that the Cl^--ATPase was not identical with the (C)F ATPase of the F_OF_1/CF_OCF_1 ATP synthase, but Ikeda *et al.* (1997) demonstrated the interchangeability of the b subunit of the *Acetabularia* Cl^--ATPase and the β-subunit of the *Escherichia coli* F-ATPase. Finally, Moritani *et al.* (1997) determined the primary structure of the b subunit of the *Acetabularia* Cl^--ATPase. There seems to have been no further work on this Cl^--ATPase other than studies on action potentials (Raven, 2017).

For the major ions commonly subject to primary active transport, Na^+ and H^+, the cytoplasmic Na^+ concentration in *Acetabularia* is 60 mol m^{-3} (Amtmann & Gradmann, 1994), and the cytoplasmic pH is estimated at 8.0–8.4 using pH indicators, and pH 7.6–7.7 from the pH at which isolated intact chloroplasts exhibit their highest rate of photosynthesis (Dodd & Bidwell, 1971). If external Na^+ is 450 mol m^{-3}, the internal:external Na^+ concentration difference is 0.133, the electrochemical potential difference for Na^+ is 22 kJ mol^{-1} and will drive Na^+ into the cell. For H^+, the indicator dye-measured cytoplasmic pH of 8.0–8.2 (Dodd & Bidwell, 1971) is essentially identical to that of seawater, so the electrochemical potential difference for H^+ is 17 kJ mol^{-1}, driving H^+ into the cell. No evidence is available for the occurrence of primary active transport processes at the plasmalemma of *Acetabularia* other than the inward Cl^- pump and the inward H^+ pump. If secondary active efflux of Na^+ is driven by Cl^- symport, granted the electrochemical potentials for the two ions calculated above, the Cl^-:Na^+ ratio must be not less than 2. Active H^+ efflux cannot be driven by the inwardly directed H^+-pumping rhodopsin. Since the calculated electrochemical potential differences for H^+ and Cl^- are equal and opposite, net H^+ efflux driven by Cl^- efflux requires a ratio of Cl^-:H^+ > 1.0.

Other large-celled ulvophyceans include the Siphonocladales and Cladophorales, mainly marine and comprising one or more coenocytic cells. In the cases examined, the vacuole-positive electrical potential difference across the tonoplast is much greater than in other vacuolated organisms, in some cases making the inside-positive tonoplast–medium electrical potential difference greater than the inside-negative cytosol–medium electrical potential difference (Hope & Walker, 1975). There are data for the giant-celled marine *Chaetomorpha darwinii* (now *Chaetomorpha coliforme*) on the Ψ_{CO} of −72 mV, cytoplasmic Na^+ concentration (25 mol m^{-3}) and cytoplasmic pH (pH 8.0–8.3 in the light; pH 7.5–7.8 in the dark), with external Na^+ 500 mol m^{-3} and external pH 8.0. The free energy difference across the plasmalemma, cytosol relative to medium, for H^+ is −7.6 kJ mol^{-1}, driving H^+ into the cell, and −12.6 kJ mol^{-1} for Na^+, also driving Na^+ into the cell (Dodd *et al.*, 1966; Findlay *et al.*, 1971; Raven and Smith, 1980), in the light. No data are available for electrical potentials, or intracellular ion concentrations, in the dark. Less information is available for the closely related large-celled alga, i.e. species of *Valonia*, *Valoniopsis* and *Ventricaria* (Bisson *et al.*, 2006), but the available information is consistent with a situation similar to that in *Chaetomorpha darwinii*.

Finally among the Ulvophyceae, there are important data on *Ulva* spp., now incorporating the genera *Ulva* and *Enteromorpha* (Hayden *et al.*, 2003). *Ulva* is multicellular, with small cells of which half or less of the volume is taken up by a vacuole. Ritchie (1985) examined the energetics of ion transport in *Enteromorpha* (=*Ulva*) *intestinalis*. The cytoplasmic pH was not measured in that study so Ritchie (1985) used a value of pH 7.3 from a wide range of studies on (mostly non-marine) cyanobacteria, eukaryotic algae and plants, with an external pH of 8.0. With the measured Ψ_{CO} of −54 ± 5 mV in seawater in the light, and −30 ± 5 mV in the dark, the proton electrochemical potential difference across the plasmalemma is not significantly different from zero (Ritchie, 1985). For Na^+, the ion most likely to be subject to primary active efflux in *Ulva*, the electrochemical potential difference (cytosol relative to medium) across the plasmalemma is −15.5 kJ mol^{-1} in the light and −13.1 kJ mol^{-1} in the dark (Ritchie, 1985).

There are also data for the closely related *Ulva lactuca*. Reed & Collins (1981) used the distribution of the lipid-soluble cation $TPMP^+$ to measure Ψ_{CO} for *Ulva lactuca* cells in seawater; they found values of −54 ± 1.8 mV in the light and −44 ± 3.5 mV in the dark. Ritchie (1988), using microelectrodes, showed that Ψ_{CO} of *U. lactuca* in seawater was −39 ± 1.1 mV in the light and −25 ± 1.9 mV in the dark. In view of the comments by Ritchie (1982, 1984) on problems with the use of lipid-soluble cations to estimate the electrochemical potential difference between cells and the medium in eukaryotes, the following calculations use the electrical potential difference values of Ritchie (1988). Thus, using intracellular and extracellular Na^+ concentrations, we calculate the electrochemical potential difference for Na^+ (cytosol relative to medium) across the plasmalemma to be −13.8 kJ mol^{-1} in the light and −12.2 kJ mol^{-1} in the dark. Ritchie (1988) does not give values for the electrochemical potential differences for H^+ across the plasmalemma, but with the assumption made by Ritchie (1985) for cytoplasmic pH (7.3), the H^+

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

electrochemical potential difference (cytosol relative to medium) across the plasmalemma is +0.30 kJ mol^{-1} in the light and +1.65 kJ mol^{-1} in the dark. The choice of using the cytoplasmic pH value of Ritchie (1988) is supported by Lundberg *et al.* (1989) who, using ^{31}P NMR, found a cytoplasmic pH of 7.2 in *U. lactuca*.

The algal members of the Streptophyta are the Charophyceae *sensu lato* (Del Cortona *et al.*, 2020). The most morphologically complex of the Charophyceae are the Charales with most of the thallus volume occupied by giant cells (Beilby, 2015; Nishiyama *et al.*, 2018). Most of the Charales are freshwater, although some species occur in brackish waters (e.g. in the Baltic) and *Lamprothamnium* spp. grows in coastal lagoons with very large changes in salinity, with the highest values twice that of seawater (Beilby, 2015). Energization of transport at the plasmalemma of *Lamprothamnium*, like that of freshwater Charales, involves a P-type H^+ efflux ATPase, with active Na^+ efflux driven by H^+ antiport and active Cl^- influx driven by H^+ symport (Beilby, 2015). The electrochemical potential difference across the plasmalemma for H^+, with external pH of 8 and cytoplasmic pH of 7.7 and a Ψ_{CO} of −160 mV (Kirst & Bisson, 1982) at 25° C, is −13.7 kJ mol^{-1}. For the major ions the electrochemical potential differences, cytosol relative to medium, are −24.2 kJ mol^{-1} (Na^+), −3.14 kJ mol^{-1} (K^+) and +9.27 kJ mol^{-1} (Cl^-) (Kirst & Bisson, 1982). The primary active efflux of H^+ energizes, directly or indirectly, the active efflux of Na^+ and K^+ and active influx of Cl^-.

Eukaryotic algae: organisms with secondary and tertiary chloroplast endosymbiosis

The earliest fossils of the diatoms (Bacillariophyceae *sensu lato*; Ochrophyta) are from marine habitats, with later invasion of fresh waters (Falkowski *et al.*, 2004; Siver *et al.*, 2018). There is functional evidence of a plasmalemma Na^+-ATPase in the colourless marine diatom *Nitzschia alba* (Bhattacharya & Volcani, 1980). The work of Flynn *et al.* (1987) on plasmalemma vesicles of *Phaeodactylum tricornutum* is also consistent with the occurrence of an Na^+-ATPase. The available evidence on cytoplasmic pH is for pH 7.6 in a non-vacuolate marine pennate diatom *Phaeodactylum tricornutum* (Burns & Beardall, 1987), and pH 7.3 in the centric marine diatom *Thalassiosira weissflogii* at an external pH of 8 (Hervé *et al.*, 2002). The Ψ_{CO} is −60 to −90 mV for the marine centric diatom *Coscinodiscus wailesii* (Gradmann & Boyd, 1995, 1999*a*, 1999*b*) and −84 mV for the marine centric diatom *Odontella sinensis* (Taylor *et al.*, 2017). These values for a range of marine diatoms are consistent with the occurrence of active H^+ efflux. For Na^+ the only intracellular concentration values are for whole cells of the marine *Coscinodiscus granii* (46 mol m^{-3}) and *Coscinodiscus wailesii* (125 mol m^{-3}) (Kesseler, 1974). Boyd & Gradmann (1999) assume that these mean intracellular concentrations, dominated by the concentration in the largest cell compartment, the vacuole, also apply to the cytosol. With this assumption, and Ψ_{CO} of −75 mV across the plasmalemma in the marine *C. wailesii* (Gradmann & Boyd, 1999*a*, 1999*b*), the Na^+ free energy difference across the plasmalemma is 12 kJ mol^{-1}, inside negative. Jones & Morel (1988) suggested that redox reactions in the plasmalemma of *Thalassiosira weissflogii* can act as an H^+ efflux pump. Bertucci *et al.* (2010) cite genomic evidence for a P-type H^+-ATPase in the diatom *Phaeodactylum tricornutum*. Diatoms also have vacuolar H^+ pyrophosphatases and V-type H^+-ATPases (Bussard & Lopez, 2014). While there is evidence from metazoans for the expression of V-type H^+-ATPases in the plasmalemma (Beyenbach & Wieczorek, 2006), there is no evidence as to whether this occurs in diatoms (Wieczorek *et al.*, 1999; Bussard & Lopez, 2014).

Almost all of the extant Ochrophyta: Phaeophyceae are marine. For the marine phaeophycean *Ectocarpus siliculosus* there is genomic evidence for a P Na^+-ATPase (Uji *et al.*, 2012*a*, 2012*b*). The only estimates of the electrochemical potential differences across the plasmalemma of the Phaeophyceae are for the development of the just-fertilized eggs of the fucoid *Pelvetia fastigiata* (Allen *et al.*, 1972; Gibbon & Kropf, 1993) and the unfertilized eggs of the fucoid *Fucus serratus* (Taylor & Brownlee, 1993). Gibbon & Kropf (1993) used microelectrodes to measure the Ψ_{CO} of −60 mv, inside negative), and the pH difference (0.5 units, inside low) between the cytosol and seawater medium. Gibbon & Kropf (1993) calculated the proton motive force across the plasmalemma, equivalent to an electrochemical potential difference, cytosol relative to medium for H^+ of −2.5 kJ mol^{-1}, i.e. close to electrochemical equilibrium. The data for ion content of Allen *et al.* (1972) are for the whole zygote, rather than the cytosol, so calculations of electrochemical potential difference across the plasmalemma for K^+, Na^+ and Cl^- are less accurate than the value for H^+ (Gibbon & Kropf, 1993). The calculations of Gibbon & Kropf (1993) show that while K^+ and Cl^- are near electrochemical equilibrium, the electrochemical potential difference, cytosol relative to medium, for Na^+ is −14 kJ mol^{-1}. Gibbon & Kropf (1993) therefore suggest that the primary active transport at the plasmalemma is for Na^+, with Na^+:H^+ antiport generating the much smaller H^+ electrochemical potential difference. Taylor & Brownlee (1993) examined the electrical properties of the plasmalemma, and the ion content, of unfertilized eggs of *Fucus serratus* in seawater. The Ψ_{CO} using microelectrodes is −40 to −65 mV, with a mean of −50 mV. With the same cautions as for *Pelvetia fastigiata* zygotes, the driving force for H^+ is 1 kJ mol^{-1} directed inwards, for Na^+ 12 kJ mol^{-1}, directed inwards, and for Cl^-, zero kJ mol^{-1}, i.e. equilibrium. These values are consistent with the occurrence of a Na^+ efflux, with very weak evidence of a H^+ efflux pump, and for Cl^- at equilibrium. However, replacement of external Cl^- by isethionate$^-$ makes the plasmalemma electrical potential difference 20 mV less negative, consistent with active electrogenic Cl^- influx, and a lower Cl^- concentration in the cytosol than in some other intracellular compartments. Further experiments are needed to examine this possibility.

Klenell *et al.* (2002, 2004) suggested that *Laminaria digitata* and *Laminaria saccharina* (now *Saccharina latissima*) have a P-type H^+-ATPase that is involved in acidifying part of the thallus surface; however, the evidence (inhibition by vanadate) does not distinguish a P-type H^+ ATPase from a P-type Na^+-ATPase in parallel with a H^+:Na^+ antiporter (see discussion above for Rhodophyta). Klenell *et al.* (2004) also used erythrosin B as an inhibitor of the plasmalemma P-type H^+-ATPase; however, erythrosin B also inhibits a range of other processes (Gimmler, 1988).

In the Ochrophyta: Raphidophyceae, inverted plasmalemma vesicles of the marine *Heterosigma akashiwo* show ATP-dependent Na^+ accumulation. The Na^+ accumulation was inhibited by vanadate, and was shown not to be an accumulation down an inside-negative electrical potential, or a result of Na^+:H^+ exchange following H^+ accumulation by an H^+-ATPase (Shono *et al.*, 1995, 1996). These findings are supported by genomic evidence of a Na^+-ATPase (Shono *et al.*, 2001; Jo *et al.*, 2010; Uji *et al.*, 2012*a*).

For the Haptophyta the earliest known fossils of the calcified coccoliths of coccolithophores in the Class Prymnesiophyceae are from marine strata, and there is no evidence that the coccolithophores ever invaded fresh waters (Falkowski *et al.*, 2004). The coccolithophore *Emiliania huxleyi* (Haptophyta: Prymnesiophyceae) has a putative P-type H^+-ATPase in the plasmalemma (Lohbeck *et al.*, 2014). The Ψ_{CO} of a calcifying strain of *Emiliania huxleyi* (Sikes & Wilbur, 1982) is −81 mV

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

using K^+-valinomycin and −145 mV using a fluorescent dye (cf. Ritchie, 1982, 1984). However, using the preferable microelectrode technique, Taylor *et al.* (2011) found a Ψ_{CO} of −46 mV. There are conflicting reports on the intracellular pH of *Emiliania huxleyi* (Dixon *et al.*, 1989; Nimer *et al.*, 1994; see also Suffrian *et al.*, 2011). Using the fluorescent dye method the internal pH was determined as 7.28 (Dixon *et al.*, 1989), 7.03 (Nimer *et al.*, 1994) and 7.0 (Gibbin *et al.*, 2014), at an external pH of 8 in the presence of 2 mol m^{-3} inorganic carbon. Lower values for internal pH are found with the DMO method and/or in the absence of inorganic C (Dixon *et al.*, 1989; Nimer *et al.*, 1994). Another coccolithophore, *Pleurochrysis* sp., has a P-type Ca^{2+}-ATPase (Araki & González, 1998).

The earliest known fossil Dinophyta (Alveolata) are from marine sediments with subsequent invasion of fresh waters (Lenz *et al.*, 2002; Falkowski *et al.*, 2004). Symbiotic dinoflagellates of the Symbiodiniaceae may have a Na^+-ATPase in the plasmalemma (Goiran *et al.*, 1997), although a H^+-ATPase and H^+-Na^+ antiport has not been ruled out as the mechanism of Na^+ efflux. Bertucci *et al.* (2010) and Mies *et al.* (2017*a*, 2017*b*) showed that the gene for a plasmalemma-located P-type H^+-ATPase in the Symbiodiniaceae shows increased expression during symbiosis with corals, possibly related to acidification of the perisymbiotic space (Bertucci *et al.*, 2010). It is not clear which ion is pumped by the P-ATPase in the calcifying dinoflagellate *Thoracosphaera helmii*, but it is probably Ca^{2+} (Van de Waal *et al.*, 2013).

Submerged marine flowering plants: seagrasses

Fernández *et al.* (1999), Garcia-Sánchez *et al.* (2000) and Rubio *et al.* (2011) provided electrophysiological evidence of a H^+-ATPase of the leaf and root cell plasmalemmas of *Zostera marina*. The Ψ_{CO} is −150 to −160 mV, inside negative, and is hyperpolarized by fusicoccin, a compound known to stimulate non-halophytic flowering plant plasmalemma H^+-ATPases; the cytosolic pH is 7.3 (Fernández *et al.*, 1999; Garcia-Sánchez *et al.*, 2000). With an external seawater pH of 8.0 and the cytosol −160 mV negative relative to the seawater, the H^+ electrochemical potential difference, cytosol relative to medium, across the plasmalemma is thus −13.7 kJ mol^{-1} kJ per mol, favouring H^+ entry. Rubio *et al.* (2005) showed that the cytosol Na^+ concentration (measured with Na^+-selective microelectrodes) of 10.7 ± 3.3 mol m^{-3} in cells of *Zostera marina* in seawater has a Ψ_{CO} of −150 mV, so the electrochemical potential difference across the plasmalemma is 25 kJ mol^{-3}, driving Na^+ into the cytosol. Following Pak *et al.* (1995), Fukuhara *et al.* (1996) and Muramoto *et al.* (2002) characterized a salt-tolerant P-type H^+-ATPase in the plasmalemma of *Zostera marina*. Generation of the −25 kJ mol^{-1} gradient (cytosol relative to medium) for Na^+ using a Na^+:H^+ antiporter and a −13.7 kJ mol^{-1} gradient for H^+ requires a Na^+:H^+ ratio of 0.5 or lower. Rubio *et al.* (2017, 2018) showed that the cytosol pH and the electrical potential difference across the plasmalemma of the seagrass *Posidonia oceanica* are very similar to those of *Zostera marina*.

Emergent marine flowering plants: tidal (=salt) marsh plants (herbaceous) and mangroves (trees)

Brügemann & Janiesch (1989) compared the plasma membrane ATPase from control specimens and those grown with added NaCl of the tidal marsh plant *Plantago maritima*. Wu & Seliskar (1998) investigated salinity adaptations in the H^+-ATPase of the tidal marsh plant *Spartina patens*. The H^+-ATPase also energizes NaCl secretion by salt glands in recretohalophytic tidal marsh plants and mangroves (Yuan *et al.*, 2016; Dassanayake & Larkin, 2017). The suggestion that the *Limonium* salt gland has primary active transport involving a Cl^--ATPase has not been substantiated (Raven, 2017).

Table 3. Phylogenetic distribution of plasmalemma ion transport ATPases in marine photosynthetic eukaryotes

Phylum, Class	H^+ efflux	Na^+ efflux	Cl^- influx
Rhodophyta	–	P-ATPase	
Chlorophyta Prasinophyceae	(P-ATPase?)	P-ATPase	
Chlorophyta Chlorophyceae	P-ATPase	(P-ATPase?)	
Chlorophyta Trebouxiophyceae	(P-ATPase?)	(P-ATPase)	
Chlorophyta Ulvophyceae		(P-ATPase)	F-ATPase
Streptohyta Charophyceae	P-ATPase	–	
Streptophyta flowering plants	P-ATPase	–	
Ochrophyta Bacillariophyceae	(P-ATPase?)	(P-ATPase?)	
Ochrophyta Phaeophyceae		P-ATPase	
Ochrophyta Raphidophyceae		(P-ATPase?)	
Haptophyta Prymnesiophyceae	P-ATPase		
Alveolata Dinophyceae	(P-ATPase?)		

For references see text.

Evolutionary aspects of primary active ion pumps in marine photosynthetic organisms

The occurrence of primary active H^+, Na^+ and Cl^- at the plasmalemma of marine photosynthetic organisms is summarized in Table 3, encapsulating the outcomes of the analysis in the previous section. Among the Archaeplastida, the brackish Characeae and brackish and marine flowering plants only have P(II)-type H^+-ATPases. However, the Streptophyta are not basal among the Archaeplastida, so it cannot be concluded that P(II)-type H^+-ATPase is the ancestral energizer of the plasmalemma. No data seem to be available for Glaucophyta; among Rhodophyta, marine representatives have P(II)-type Na^+-ATPases and acidophilic 'freshwater' Cyanidiophyceae have P(II)-type H^+-ATPases. Marine Chlorophyta have both P(II)-type H^+-ATPases and P(II)-type Na^+-ATPases. In these two phyla of Archaeplastida it is likely that horizontal gene transfer has been involved, e.g. via virus-encoded P-ATPases: an example is a Ca^{2+}-ATPase in a freshwater *Chlorella* virus (Bonza *et al.*, 2010). A range of transporters are encoded by other viruses (Greiner *et al.*, 2018) and horizontal gene transfer has been shown for ATPases in prokaryotes (Hilario & Gogarten, 1993) and other transporters in eukaryotic algae (Chan *et al.*, 2011, 2012). Similar horizontal gene transfer is required to account for the distribution of P(II)-type H^+-ATPases and P(II)-type Na^+-ATPases among marine algae with plastids derived from secondary or tertiary endosymbiosis (Chan *et al.*, 2011, 2012; Burki *et al.*, 2020). The origin of the F-ATPase that pumps Cl^- in some ulvophycean Chlorophyta (Table 3) is unclear; metazoan Cl^--ATPases seem to be P-ATPases (Gerencser & Zhang, 2003).

Conclusions

In Archaea there are ion-pumping rhodopsins that actively transport H^+ out of, and Cl^- into, the cells; in one archaean there is an *inward* H^+-pumping rhodopsin. In Bacteria all three of the ions H^+, Na^+ and Cl^- are moved (H^+ and Na^+ out, Cl^- in) by ion-

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

pumping rhodopsins. There is an H^+ *influx* rhodopsin in *Acetabularia*, and (probably) H^+ efflux rhodopsins in diatoms. Photochemistry based on bacteriochlorophyll exports H^+ from the cytosol in some marine anoxygenic photosynthetic bacteria, but chlorophyll-based redox reactions do not export H^+ from cells of marine cyanobacteria. Exergonic redox reactions export H^+ and Na^+ in photosynthetic bacteria, H^+ in cyanobacteria and possibly H^+ in eukaryotic algae. H^+-and/or Na^+-ATPases occur in the plasmalemma of all photosynthetic marine organisms tested. P-type H^+ efflux ATPases occur in the marine Streptophyta, i.e. marine charophycean algae and seagrasses and emergent marine flowering plants. P-type Na^+-ATPases are the main primary active ion pumps in the plasmalemma of other marine green algae and non-green algae. However, there may be P-type H^+-ATPases in some cases, and a F-type Cl^--ATPase occurs in the ulvophycean *Acetabularia*. Some assignments of P-type ATPases as H^+ or as Na^+ pumps using genomics are not conclusive. Despite the insights that genomics can provide, it seems that there are still large gaps in the availability of electrophysiological data from many of the ecologically (and economically) important marine (and freshwater) phototrophs. There is thus a need for more such data to improve our understanding of the functioning of primary active transport in these organisms.

Acknowledgements. The University of Dundee is a registered Scottish charity, No. 015096. Discussions over the years with Jim Barber, Mary Beilby, Mary Bisson, Jack Dainty, Geoff Findlay, Mario Giordano, Alex Hope, Tony Larkum, Enid MacRobbie, Ray Ritchie; Hugh Saddler, Dale Sanders, Vladimir Skulachev, F Andrew Smith, Roger Spanswick, N Alan Walker and Philip White have been very helpful.

References

Adams PG, Cadby AJ, Robinson B, Tsukatani Y, Tank M, Wen J, Blankenship RE, Bryant DA and Hunter CN (2013) Comparison of physical characteristics of chlorosomes from three different phyla of green phototrophic bacteria. *Biochimica Biophysica Acta* **1827**, 1235–1244.

Allen RD, Jacobsen L, Joaquen J and Jaffe LF (1972) Ion concentrations in developing *Pelvetia* eggs. *Developmental Biology* **27**, 538–545.

Amtmann A and Gradmann D (1994) Na^+ transport in *Acetabularia* bypasses conductance of plasmalemma. *Journal of Membrane Biology* **139**, 117–125.

Araki Y and González EL (1998) V- and P-type Ca^{2+}-stimulated ATPase in a calcifying strain of *Pleurochrysis* sp. (Haptophyceae). *Journal of Phycology* **34**, 79–88.

Badger MR and Price GD (2003) CO_2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. *Journal of Experimental Botany* **54**, 609–622.

Balnokin Y and Popova L (1994) The ATP-driven Na^+ pump in the plasma membrane of the marine unicellular alga, *Platymonas viridis*. *FEBS Letters* **343**, 61–64.

Balnokin Y, Popova L and Gimmler H (1997) Further evidence for an ATP-driven sodium pump in the marine alga *Tetraselmis* (*Platymonas*) *viridis*. *Journal of Plant Physiology* **150**, 264–270.

Balnokin YV, Popova LG and Andreev IM (1999) Electrogenicity of the Na^+-ATPase from the marine microalga *Tetraselmis* (*Platymonas*) *viridis* and associated H^+ countertransport. *FEBS Letters* **462**, 402–406.

Balnokin YV, Popova LG, Pagis LY and Andreev IM (2004) The Na^+-translocating ATPase in the plasma membrane of the marine microalga *Tetraselmis viridis* catalyses Na^+/H^+ exchange. *Planta* **219**, 332–337.

Barrero-Gil J, Garciadeblás B and Benito B (2005) Sodium, potassium-ATPases in algae and oomycetes. *Journal of Bioenergetics and Biomembranes* **37**, 269–278.

Beilby MJ (2015) Salt tolerance at single cell level in giant-celled Characeae. *Frontiers in Plant Science* **6**, art. 226.

Bejá O and Lanyi JK (2014) Nature's toolkit for microbial rhodopsin. *Proceedings of the National Academy of Sciences USA* **111**, 6538–6539.

Bental M, Degani H and Avron M (1986) ^{23}Na-NMR studies of the intracellular sodium ion concentration in the halotolerant alga *Dunaliella*. *Plant Physiology* **87**, 813–817.

Berg IA, Kockelhorn D, Ramos-Vera WH, Say RF, Zarzycki J, Hügler M, Alber BE and Fuchs G (2010) Autotrophic carbon fixation in Archaea. *Nature Reviews Microbiology* **8**, 447–460.

Bergman B, Siddiqui PJA, Carpenter EJ and Pescher GA (1993) Cytochrome oxidase: subcellular distribution and relationship to nitrogenase expression in the nonheterocystous marine cyanobacterium *Trichodesmium thiebaultii*. *Applied and Environmental Microbiology* **59**, 3239–3244.

Bertucci A, Tambutté R, Tambutté S, Allemand D and Zoccola D (2010) Symbiosis-dependent gene expression in coral dinoflagellate association: cloning and characterization of a P-type H^+-ATPase gene. *Proceedings of the Royal Society of London B* **277**, 87–95.

Beyenbach KW and Wieczorek H (2006) The V-type H^+-ATPase: molecular structure and function, physiological roles and regulation. *Journal of Experimental Biology* **209**, 577–589.

Bhattacharya P and Volcani BE (1980) Sodium-dependent silicate transport in the apochlorotic marine diatom *Nitzschia alba* (Na^+ gradient/Na^+, K^+-ATPase/membrane vesicles). *Proceedings of the National Academy of Sciences USA* **77**, 6386–6390.

Bisson MA, Beilby MJ and Shepherd VA (2006) Electrophysiology of turgor regulation in marine siphonous green algae. *Journal of Membrane Biology* **211**, 1–15.

Blank CE (2013*a*) Origin and early evolution of photosynthetic eukaryotes; reinterpreting Proterozoic palaeobiology and biogeochemical processes in light of trait evolution. *Journal of Phycology* **49**, 1040–1056.

Blank CE (2013*b*) Phylogenetic distribution of compatible solute synthesis genes support a freshwater origin for cyanobacteria. *Journal of Phycology* **49**, 880–895.

Blank CE and Sánchez-Baracaldo P (2010) Timing of morphological and ecological innovations in the cyanobacteria – a key to understanding the rise in atmospheric oxygen. *Geobiology* **8**, 1–23.

Blinks LR (1940) The relations of bioelectric phenomena to ionic permeability and to metabolism in large plant cells. *Cold Spring Harbor Symposium* **8**, 204–215.

Blinks LR (1949) The source of the bioelectric potentials in large plant cells. *Proceedings of the National Academy of Sciences USA* **35**, 566–575.

Blount RW and Levedahl BH (1960) Active sodium and chloride transport in the single celled marine alga *Halicystis ovalis*. *Acta Physiologia* **49**, 1–9.

Bock C, Jacob A, Kirst GO, Leibfritz D and Mayer A (1996) Metabolic changes of the Antarctic green alga *Prasiola crispa* subjected to water stress investigated by *in vivo* ^{31}P NMR. *Journal of Experimental Botany* **47**, 241–249.

Bonza MC, Martin H, Kang M, Lewis G, Greiner T, Giacometti S, Van Etten JL, De Michelis MI, Thiel G and Moroni A (2010) A functional calcium-transporting ATPase encoded by chlorella viruses. *Journal of General Virology* **91**, 2620–2629.

Boyd CM and Gradmann D (1999) Electrophysiology of the marine diatom *Coscinodiscus wailesii*. III. Uptake of nitrate and ammonium. *Journal of Experimental Botany* **50**, 461–467.

Brasier MD (2013) Green algae (Chlorophyta) and the question of freshwater symbiogenesis in the early Proterozoic. *Journal of Phycology* **49**, 1036–1039.

Brewer PG and Goldman JC (1976) Alkalinity changes generated by phytoplankton growth. *Limnology and Oceanography* **21**, 103–117.

Brügemann W and Janiesch P (1989) Comparison of plasma membrane ATPase from salt-treated and salt-free grown *Plantago maritima*. *Journal of Plant Physiology* **134**, 20–25.

Bublitz M, Morth JP and Nissen P (2011) P-type ATPases at a glance. *Journal of Cell Science* **124**, 2525–2519.

Burki F, Roger AJ, Brown MW and Simpson AGB (2020) The new tree of eukaryotes. *Trends in Ecology and Evolution* **35**, 43–55.

Burns BD and Beardall J (1987) Utilization of inorganic carbon by marine microalgae. *Journal of Marine Biology and Ecology* **107**, 75–86.

Bussard A and Lopez PJ (2014) Evolution of vacuolar pyrophosphatases and vacuolar H^+ ATPases in diatoms. *Journal of Marine Science and Technology* **22**, 50–59.

Campbell AM, Coble AJ, Cohen LD, Ch'ng TH, Russo KM, Long EM and Armbrust EV (2001) Identification and DNA sequence of a new H^+-ATPase in the unicellular green alga *Chlamydomonas reinhardtii* (Chlorophyceae). *Journal of Phycology* **37**, 536–542.

Chan CX, Reyes-Prieto A and Bhattacharya D (2011) Red and green algal origin of diatom membrane transporters: insights into environmental adaptation and cell evolution. *PLoS ONE* **6**, art.e29138.

Chan CX, Zäuner A, Wheeler G, Grossman AR, Prochnik SE, Blouin NA, Zhuang Y, Benning C, Berg GM, Yarish C, Ekiksen RL, Klein AS, Lin S,

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

Levine I, Brawley SH and Bhattachaya D (2012) Analysis of *Porphyra* membrane transporters demonstrates gene transfer among photosynthetic eukaryotes and numerous sodium-coupled transport systems. *Plant Physiology* **158**, 2001–2012.

Chen T, Wang W, Xu K, Xu Y, Ji D, Chen C and Xie C (2019) K^+ and Na^+ transport contribute to K^+/Na^+ homeostasis in *Pyropia haitanensis* under hypersaline stress. *Algal Research* **40**, art. 101526.

Cohen NR, Ellis KA, Lampe RH, McNair H, Twining BS, Maldonado MT, Brzezinski MA, Kizminov FI, Thamatrakoln K, Till CP, Bruland KW, Sunda WG, Bangu S and Marchetti A (2017) Diatom transcriptional and physiological responses to changes in iron bioavailability across ocean provinces. *Frontiers in Marine Science* **4**, art. 360.

Dassanayake M and Larkin JC (2017) Making plants break a sweat: the structure, function, and evolution of plant salt glands. *Frontiers in Plant Science* **8**, art. 406.

De Clerck O and 35 co-authors (2018) Insights into the evolution of multicellularity from the sea lettuce genome. *Current Biology* **28**, 2921–2933.

Del Cortona A, Jackson CJ, Bucchini F, Van Bel M, D'hondt S, Škaloud P, Delwiche CF, Knoll AH, Raven JA, Verbruggen H, Vandepoel K, De Clerck O and Leliaert F (2020) Neoproterozoic origin and multiple transitions to macroscopic growth in green seaweeds. *Proceedings of the National Academy of Sciences USA* **117**, 2551–2559. doi: 10.1073/pnas.19.1006017.

Dibrova DV, Galperin MY, Koonin EV and Mulkidjanian AY (2015) Ancient systems of sodium/potassium homeostasis as predecessors of membrane bioenergetics. *Biochemistry (Moscow)* **80**, 495–516.

Dittami SM, Heesch S, Olsen JL and Collén J (2017) Transition between marine and freshwater environments provide new clues about the origins of multicellular plants and algae. *Journal of Phycology* **53**, 731–745.

Dixon GK, Brownlee C and Merrett MJ (1989) Measurement of internal pH in the coccolithophore *Emiliania huxleyi* using 1′,7′-bis'(2-carboxyethyl)-5 (and −6)carboxy-fluorescein acetoxymethylester and digital imaging microscopy. *Planta* **178**, 443–449.

Dodd WA and Bidwell RGS (1971) The effect of pH on the products of photosynthesis in $^{14}CO_2$ by chloroplast preparations from *Acetabularia mediterranea*. *Plant Physiology* **47**, 779–783.

Dodd WA, Pitman MG and West KR (1966) Sodium and potassium transport in the marine alga *Chaetomorpha darwinii*. *Australian Journal of Biological Sciences* **19**, 341–354.

Duchuzeau A-L, Schoepp-Cothenet B, Baymann F, Russell MJ and Nitscke W (2014) Free energy conversion in the LUCA: quo vadis? *Biochimica et Biophysica Acta* **1837**, 882–888.

Ehrenfeld J and Cousin J-L (1984) Ionic regulation of the unicellular green alga *Dunaiella tertiolecta*: response to hypotonic shock. *Journal of Membrane Biology* **77**, 45–55.

Emery L, Whelan S, Hirschi KD and Pittman JK (2012) Protein phylogenetic analysis of Ca^{2+}/cation antiporters and insights into their evolution in plants. *Frontiers in Plant Science* **3**, art. 1.

Falkowski PG, Katz ME, Knoll AH, Quigg A, Raven JA, Schofield O and Taylor FJ (2004) The evolution of modern eukaryotic phytoplankton. *Science (New York, N.Y.)* **305**, 354–360.

Fernández JA, Garcia-Sánchez MJ and Felle HH (1999) Physiological evidence for a proton pump and sodium exclusion mechanisms at the plasma membrane of the marine angiosperm *Zostera marina* L. *Journal of Experimental Botany* **50**, 1763–1768.

Findlay GP, Hope AB, Pitman MG, Smith FA and Walker NA (1971) Ionic relations of marine algae III *Chaetomorpha* membrane electrical properties and chloride fluxes. *Australian Journal of Biological Sciences* **24**, 731–746.

Flynn KJ, Blackford JC, Baird ME, Raven JA, Clark DR, Beardall J, Brownlee C, Fabian H and Wheeler GL (2012) Changes in pH at the exterior surface of plankton with ocean acidification. *Nature Climate Change* **2**, 510–513.

Flynn KJ, Öpik H and Syrett PJ (1987) The isolation of plasma membrane from the diatom *Phaeodactylum tricornutum* using an aqueous two-polymer phase system. *Journal of General Microbiology* **133**, 93–101.

Foflonker F, Price DC, Qiu H, Palenik B, Wang S and Bhattacharya D (2014) Genome of the halotolerant green alga *Picochlorum* sp. reveals strategies for thriving under fluctuating environmental conditions. *Environmental Microbiology* **17**, 412–426.

Fukuhara T, Pak JY, Ohwaki H, Tsujimura H and Nitta T (1996) Tissue-specific expression of the gene for a putative plasma membrane H^+-ATPase in a seagrass. *Plant Physiology* **110**, 35–42.

Fux JE, Mehta A, Moffat J and Spafford JD (2018) Eukaryotic voltage-gated sodium channels: on their origins, asymmetries, losses, diversification and adaptations. *Frontiers in Physiology* **9**, art. 1406.

Gabbay-Azaria R, Schonfeld M, Tel-Or S, Messinger R and Tel-Or E (1992) Respiratory activity in the marine cyanobacterium *Spirulina subsalsa* and its role in salt tolerance. *Archives of Microbiology* **57**, 183–190.

Garcia-Pichel F, Ramirez-Reinat E and Guo Q (2010) Microbial excavation of solid carbonates powered by a P-type ATPase-mediated transcellular Ca^{2+} transport. *Proceedings of the National Academy of Sciences USA* **107**, 21749–21754.

Garcia-Sánchez MJ, Jaime MP, Ramos A, Sanders D and Fernández JA (2000) Sodium-dependent nitrate transport at the plasma membrane of leaf cells of the marine higher plant *Zostera marina*. *Plant Physiology* **122**, 979–885.

Gerencser GA and Zhang J (2003) Existence and nature of the chloride pump. *Biochimica et Biophysica Acta* **1618**, 133–139.

Gibbin EM, Putnam HM, Davy SK and Gates RD (2014) Intracellular pH and its response to CO_2-driven seawater acidification in symbiotic versus non-symbiotic coral cells. *Journal of Experimental Biology* **217**, 1963–1969.

Gibbon BC and Kropf DL (1993) Intracellular pH and its regulation in *Pelvetia zygotes*. *Developmental Biology* **157**, 259–268.

Gimmler H (1988) Erythrosine B – a specific inhibitor of plasmalemma ATPase in intact microalgae. *Journal of Plant Physiology* **132**, 545–551.

Gimmler H (2000) Primary sodium plasma membrane ATPases in salt-tolerant algae: facts and fictions. *Journal of Experimental Botany* **51**, 1171–1178.

Gimmler H and Greenway H (1983) Tetraphenylphosphonium (TPP^+) is not suitable for assessment of electrical potentials in *Chlorella emersonii*. *Plant Cell and Environment* **6**, 739–744.

Gimmler H, Kugel H, Leibfritz D and Mayer A (1988) Cytoplasmic pH of *Dunaliella parva* and *Dunaliella parva* as monitored by in vivo ^{31}P-NMR spectroscopy and the DMO method. *Physiologia Plantarum* **74**, 521–555.

Ginzburg M, Ratcliffe RG and Southon TE (1988) Phosphorus metabolism and intracellular pH in the halotolerant alga *Dunaliella parva* studied by ^{31}P NMR. *Biochimica et Biophysica Acta: Molecular Cell Research* **969**, 225–235.

Gleason FH, Larkum AWD, Raven JA, Manohar CS and Lilje O (2019) Ecological implication of recently discovered and poorly studied sources of energy for true fungi, especially in extreme environments. *Fungal Ecology* **39**, 380–387.

Goericke R (2002) Bacteriochlorophyll *a* in the ocean: is anoxygenic bacterial photosynthesis important? *Limnology and Oceanography* **47**, 290–295.

Goiran C, Allemand D and Galgani I (1997) Transient Na^+ stress in symbiotic dinoflagellates after isolation from coral-host cells and subsequent immersions in seawater. *Marine Biology* **129**, 581–589.

Goldfarb V and Gradmann D (1983) ATPase activity in partially purified membranes of *Acetabularia*. *Plant Cell Reports* **2**, 152–155.

Goldfarb V, Sanders D and Gradmann D (1984) Reversal of electrogenic Cl^- pump in *Acetabularia* increases level and ^{32}labelling of ATP. *Journal of Experimental Botany* **35**, 645–658.

Gómez-Consarnau L, Raven JA, Levin NM, Cutter L, Wang D, Seegers B, Arístegui J, Fuhrman J A, Gasel JM and Sañudo-Wilhelmy SA (2019) Microbial rhodopsins are major contributors to the solar energy captured in the sea. *Science Advances* **5**, eaaw8855.

Gradmann D and Boyd CM (1995) Membrane voltage of marine phytoplankton, measured in the diatom *Coscinodiscus radiatus*. *Marine Biology* **124**, 645–650.

Gradmann D and Boyd CM (1999a) Electrophysiology of the marine diatom *Coscinodiscus wailesii* I. Endogenous changes of membrane voltage and resistance. *Journal of Experimental Botany* **50**, 445–452.

Gradmann D and Boyd CM (1999b) Electrophysiology of the marine diatom *Coscinodiscus wailesii* II. Potassium currents. *Journal of Experimental Botany* **50**, 453–459.

Gradmann D, Tittor J and Goldfarb V (1982) Electrogenic Cl^- pump in *Acetabularia*. *Philosophical Transactions of the Royal Society B* **299**, 447–457.

Graves JS and Gutknecht J (1976) Ion transport studies and determination of the cell wall elastic modulus in the marine alga *Halicystis parvula*. *Journal of General Physiology* **67**, 579–592.

Graves JS and Gutknecht J (1977a) Chloride transport and membrane potential in the marine alga *Halicystis parvula*. *Journal of Membrane Biology* **36**, 65–81.

Graves JS and Gutknecht J (1977b) Current-voltage relationship and voltage sensitivity in the Cl^- pump in *Halicystis parvula*. *Journal of Membrane Biology* **36**, 83–91.

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

Greiner T, Moroni A, Van Etten JI and Thiel G (2018) Genes for membrane transport proteins: not so rare in viruses. *Viruses* **10**, art. 456.

Hayden HS, Blomster J, Maggs CA, Silva PC, Stanhope MJ and Walland JR (2003) Linnaeus was right all along: *Ulva* and *Enteromorpha* are not distinct genera. *European Journal of Phycology* **38**, 277–294.

Helliwell KE, Chrachri A, Koester JA, Wharam S, Verret F, Taylor AR, Wheeler GL and Brownlee C (2019) Alternative mechanisms for fast Na^+/Ca^{2+} signalling in eukaryotes via a novel class of single-domain voltage-gated channels. *Current Biology* **29**, 1503–1511.

Hervé V, Derr J, Douady S, Quinet M, Moisan L and Lopez JL (2002) Multiparametric analyses reveal the pH-dependence of silicon mineralisation in diatoms. *PLoS ONE* **7**, art. e46722.

Hilario E and Gogarten JP (1993) Horizontal transfer of ATPase genes – the tree of life becomes a net of life. *Biosystems* **31**, 111–119.

Hong S and Pedersen PL (2008) ATP synthase and the actions of inhibitors utilized to study its roles in human health, disease, and other scientific areas. *Microbiology and Molecular Biology* **72**, 590–641.

Hope AB and Walker NA (1975) *The Physiology of Giant Algal Cells*. Cambridge: Cambridge University Press.

Ikeda M, Schmid R and Oesterhelt D (1990*a*) A chloride-translocating adenosine triphosphatase in *Acetabularia acetabulum*. 1. Purification and characterization of a novel type of adenosine triphosphatase that differs from chloroplast F1 adenosine triphosphatase. *Biochemistry* **29**, 2057–2065.

Ikeda M, Schmid R and Oesterhelt D (1990*b*) A chloride-translocating adenosine triphosphatase in *Acetabularia acetabulum*. 2. Reconstitution of the enzyme into liposomes and effect of net charge of liposomes on chloride permeability and reconstitution. *Biochemistry* **29**, 2065–2070.

Ikeda M, Kadowaki H, Ikeda H, Moritani C and Kanazawa H (1997) Exchangeability of the b subunit of the Cl^--translocating ATPase of *Acetabularia acetabulum* with the β-subunit of *E. coli* F1-ATPase: construction of the chimeric β-subunits and complementation studies. *Biochimica Biophysica Acta* **1322**, 33–40.

Jackson BZ (2016) Natural pH gradients in hydrothermal alkali vents were unlikely to have played a role in the origin of life. *Journal of Molecular Evolution* **83**, 1–11.

Jo T, Shono M, Wada M, Ito S, Nomoto J and Hara Y (2010) Homology modelling of an algal membrane protein, *Heterosigma akashiwo*, Na^+-ATPase. *Membrane* **35**, 80–85.

Johnson DT, Wolfe-Simon F, Pearson A and Knoll AH (2009) Anoxygenic photosynthesis modulated Proterozoic oxygen and sustained Earth's middle age. *Proceedings of the National Academy of Sciences USA* **106**, 16925–16929.

Jones GJ and Morel FM (1988) Plasmalemma redox activity in the diatom *Thalassiosira*: a possible role for nitrate reductase. *Plant Physiology* **87**, 143–147.

Kaplan A, Scherer S and Lerner M (1989) Nature of the light-induced H^+ efflux and Na^+ uptake in Cyanobacteria. *Plant Physiology* **89**, 1220–1225.

Katz A and Avron M (1985) Determination of intracellular osmotic volume and sodium concentrations in *Dunaliella*. *Plant Physiology* **78**, 817–820.

Katz A and Pick U (2001) Plasma membrane electron transport coupled to Na^+ extrusion in the halotolerant alga *Dunaliella*. *Biochimica et Biophysica Acta* **1504**, 423–431.

Katz A, Bental M, Degani H and Avron M (1991) *In vivo* pH regulation by a Na^+/H^+ antiporter in the halotolerant alga *Dunaliella salina*. *Plant Physiology* **96**, 110–115.

Katz A, Waridel P, Shevchenko A and Pick U (2007) Salt-induced changes in the plasma membrane proteome of the halotolerant alga *Dunaliella salina* as revealed by blue native gel electrophoresis and nano-LC-MS/MS analysis. *Molecular and Cellular Proteomics* **6/9**, 1459–1472.

Kesseler H (1974) Die anorganische-chemische Zussamensetzung dor Zellsaftes von *Coscinodiscus granii* (Bacillariophyceae, Centrales). *Helgoländer Wissenschaftliche Meeresuntersuchungen* **26**, 481–489.

Khramov DE, Matalin DA, Karpichev IV, Balnokin YV and Popova LG (2019) Expression of P-type ATPases of the marine green microalga *Dunaliella maritima* under hyperosmotic salt shock. *Doklady Biochemistry and Biophysics* **488**, 327–333.

Kirchman DL and Hanson TE (2013) Bioenergetics of photo-heterotrophic bacteria in the oceans. *Environmental Microbiology* **5**, 188–199.

Kirst GO and Bisson MA (1982) Vacuolar and cytoplasmic pH, ion composition, and turgor pressure in *Lamprothamnium* as a function of external pH. *Planta* **155**, 287–295.

Kishimoto M, Shimajiri Y, Oshima A, Hase A, Mikami K and Akama K (2013) Functional expression of an animal-type-Na^+-ATPase gene from a marine red seaweed *Porphyra yezoensis* increases salinity tolerance in rice plants. *Plant Biotechnology* **30**, 417–422.

Klenell M, Snoeijs P and Pedersén M (2002) The involvement of a plasma membrane H^+-ATPase in the blue-light enhancement of photosynthesis in *Laminaria digitata* (Phaeophyta). *Journal of Phycology* **38**, 1143–1149.

Klenell M, Snoeijs P and Pedersén M (2004) Active carbon uptake in *Laminaria digitata* and *L. saccharina* (Phaeophyta) is driven by a proton pump in the plasma membrane. *Hydrobiologia* **514**, 41–53.

Kolber ZS, VanDover CL, Niederman RA and Falkowski PG (2000) Bacterial photosynthesis in surface waters of the open ocean. *Nature* **407**, 177–179.

Kolber ZS, Gerald F, Lang AS, Beatty JT, Blankenship RE, VanDover CL, Vetriani M, Ratheber C and Falkowski PG (2001) Contribution of aerobic photoheterotrophic bacteria to the carbon cycle in the ocean. *Science (New York, N.Y.)* **292**, 2492–2495.

Komor E, Cho B-H, Schricker S and Schobert C (1989) Charge and acidity compensation during proton-sugar symport in *Chlorella*: the H^+-ATPase does not fully compensate for the sugar-coupled proton influx. *Planta* **177**, 9–17.

Ksionzek KB, Lechtenfeld OJ, McAllister SL, Schmitt-Kopplin P, Geuer JK, Geibert W and Koch BP (2016) Dissolved organic sulfur in the ocean: biogeochemistry of a petagram inventory. *Science (New York, N.Y.)* **354**, 456–459.

Kuhlbrandt W (2004) Biology, structure and function of P-type ATPases. *Nature Reviews Microbiology Molecular Cell Biology* **3**, 282–295.

Larkum AWD, Ritchie RJ and Raven JA (2018) Living of the sun: chlorophylls, bacteriochlorophylls and rhodopsins. *Photosynthetica* **56**, 11–43.

Lea-Smith DJ, Bombelli P, Vasudevan R and Howe CJ (2016) Photosynthetic, respiratory and extracellular electron transport pathways in cyanobacteria. *Biochimica et Biophysica Acta* **1857**, 247–255.

Lee J, Ghosh S and Saier MH Jr (2017) Comparative genomic analysis of transport proteins encoded within the red algae *Chondrus crispus*, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*. *Journal of Phycology* **53**, 503–521.

Lenz OK, Wilde V and Riegel W (2002) Distribution and palaeoecologic significance of the freshwater dinoflagellate cyst *Messelodinium thielpfeifferae* gen. et sp. nov. from the middle Eocene of Lake Messel, Germany. *Palynology* **31**, 117–134.

Lewis LA (2017) Hold the salt: freshwater origin of primary plastids. *Proceedings of the National Academy of Sciences USA* **114**, 9759–9760.

Lohbeck KT, Riebesell U and Reusch TBH (2014) Gene expression changes in the coccolithophore *Emiliania huxleyi* after 500 generations of selection to ocean acidification. *Proceedings of the Royal Society of London B* **281**, 2014.0003.

Lundberg P, Welch RG, Jensén P and Vogel HJ (1989) Phosphorus-31 and nitrogen-14 NMR studies of the uptake of phosphorus and nitrogen compounds in the marine macroalga *Ulva lactuca*. *Plant Physiology* **89**, 1380–1387.

Maeda S-I and Omata T (1997) Substrate-binding lipoprotein of the cyanobacterium *Synechococcus* sp. strain PCCC 7942 involved in the transport of nitrate and nitrite. *Journal of Biological Chemistry* **272**, 3016–3041.

Maeda S-i, Murakami A, Ito H, Tanaka A and Omata T (2015) Functional characterization of the FNT family nitrite transporter of marine picocyanobacteria. *Life (Chicago, ILL)* **5**, 432–446.

Marchetti A, Corlett D, Hoplinson BM, Ellis K and Cassar N (2015) Marine diatom proteorhodopsins and their role in coping with low iron availability. *ISME Journal* **9**, 2745–2748.

Martin W and Russell MJ (2007) The origin of biochemistry at an alkaline hydrothermal vent. *Philosophical Transactions of the Royal Society of London B* **367**, 1887–1925.

Mies M, Van Sluys MA, Metcalfe CJ and Sumida PYG (2017*a*) Molecular evidence of symbiotic activity between *Symbiodinium* and *Tridacna maxima* larvae. *Symbiosis* **72**, 13–22.

Mies M, Voolstra CR, Castro TB, Pires DO, Calderon EN and Sumida PYG (2017*b*) Expression of a symbiosis-specific gene in *Symbiodinium* Type A1 associated with coral, nudibranch and giant clam larvae. *Royal Society Open Science* **4**, 170253.

Mitchell P (1979) Compartmentation and communication in living systems. Ligand conduction: a general catalytic principle in chemical, osmotic and chemiosmotic reaction system (The Ninth Sir Hans Krebs Lecture). *European Journal of Biochemistry* **95**, 1–20.

Moritani C, Ohnashi T, Kadowaki H, Tagaya M, Lottspeich F, Oesterhelt D and Ikeda M (1997) The primary structure of Cl^--translocating ATPase,

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

beta subunit of *Acetabularia acetabulum*, which belongs to the F-type ATPase family. *Archives of Biochemistry and Biophysics* **359**, 115–124.

Mulkidjanian AY, Dibrov P and Galperin MY (2008) The past and present of the sodium energetics: may the sodium-motive force be with you. *Biochimica et Biophysica Acta* **1777**, 985–992.

Müller ML, Irkens-Kiesecker U, Rubinstein B and Taiz L (1996) On the mechanism of hyperacidification in lemon: comparison of the vacuolar H^+-ATPase in the fruits and epicotyls. *Journal of Biological Chemistry* **271**, 1918–1924.

Müller ML, Jensen M and Taiz L (1999) The vacuolar H^+-ATPase of lemon fruits is regulated by variable H^+/ATP coupling and slip. *Journal of Biological Chemistry* **274**, 10706–10716.

Mullineaux CW (2014) Co-existence of photosynthetic and respiratory activities in cyanobacterial thylakoid membranes. *Biochimica et Biophysica Acta* **1837**, 503–511.

Mummert H and Gradmann D (1976) Voltage dependent potassium fluxes and the significance of action potentials in *Acetabularia*. *Biochimica et Biophysica Acta* **443**, 4443–4450.

Mummert H and Gradmann D (1991*a*) Ion fluxes in *Acetabularia*: vesicular shuttle. *Journal of Membrane Biology* **124**, 255–263.

Mummert H and Gradmann D (1991*b*) Action potentials in *Acetabularia*: measurements and simulation of voltage-gated fluxes. *Journal of Membrane Biology* **124**, 265–273.

Muramoto Y, Harada A, Ohkaki Y, Takagi S and Fukuhara T (2002) Salt-tolerant ATPase activity in the plasma membrane of the marine angiosperm *Zostera marina* L. *Plant Cell Physiology* **43**, 1137–1143.

Nichols DG and Ferguson SJ (2013) *Bioenergetics*, 4th Edn. New York, NY: Academic Press/Elsevier.

Nimer NA, Brownlee C and Merrett MJ (1994) Carbon dioxide availability, intracellular pH and growth rate of the coccolithophore *Emiliania huxleyi*. *Marine Ecology Progress Series* **100**, 257–262.

Nishiyama T *et al.* (2018) The *Chara* Genome: secondary complexity and implications for plant terrestrialisation. *Cell* **174**, 448–464.

Nobel PS (2009) *Physicochemical and Environmental Plant Physiology*, 4th Edn. Amsterdam: Elsevier.

Nowack ECM (2014) *Paulinella chromaphora* – rethinking the transition from endosymbiont to organelles. *Acta Societas Botanicorum Poloniae* **83**, 387–397.

Oesterhelt D and Stoeckenius W (1973) Functions of a new photoreceptor membrane. *Proceedings of the National Academy of Sciences USA* **70**, 2853–2857.

Ohhashi T, Katsu T and Ikeda M (1992) Improvement of reconstitution of the Cl^--translocating ATPase isolated from *Acetabularia acetabulum* into liposomes and several anion pump characteristics. *Biochimica et Biophysica Acta* **1106**, 165–170.

Ohta H, Shirakawa H, Uchida K, Yoshida M, Matuo Y and Ehami I (1997) Cloning and sequencing of the gene encoding the plasma membrane H^+-ATPase from an acidic red alga, *Cyanidium caldarium*. *Biochimica et Biophysica Acta Bioenergetics* **1319**, 9–13.

Omata T, Price GD, Badger MR, Okamura M, Gohta S and Ogawa T (1999) Identification of the ATP-binding cassette transporter involved in bicarbonate uptake in the cyanobacterium *Synechococcus* sp. strain PCC 7942. *Proceedings of the National Academy of Sciences USA* **96**, 13571–13376.

Oren-Shamir M, Pick U and Avron M (1990) Plasma membrane potential of the alga *Dunaliella*, and its relation to osmoregulation. *Plant Physiology* **93**, 403–408.

Pagis LY, Popova LG, Andreev IM and Balnokin YV (2001) Ion specificity of Na^+-transporting systems in the plasma membrane of the halotolerant alga *Tetraselmis* (*Platymonas*) *viridis*. *Russian Journal of Plant Physiology* **48**, 281–286.

Pagis LY, Popova LG, Andreev IM and Balnokin YV (2003) Comparative characterization of two primary pumps, H^+-ATPase and Na^+-ATPase, in the plasma membrane of the marine alga *Tetraselmis viridis*. *Physiologia Plantarum* **118**, 514–522.

Pak J-Y, Fukuhara T and Nitta T (1995) Discrete subcellular localization of membrane-bound ATPase activity in marine angiosperms and marine algae. *Planta* **196**, 15–22.

Palmgren M and Nissen P (2011) P-type ATPases. *Annual Review of Biophysics* **40**, 243–266.

Palmgren MG, Sorenson DM, Hallström BM, Säll T and Broberg K (2020) Evolution of P2A and P5A ATPases: ancient gene duplications and the red algal connection to green plants revisited. *Physiologia Plantarum* **168**, 630–647. doi: 10.111/ppl.13008.

Pedersen CS, Axelsen KB, Harper JF and Palmgren MG (2012) Evolution of plant P-type ATPases. *Frontiers in Plant Science* **3**, art. 31.

Pick U, Kanni L and Avron M (1986) Determination of ion concentration and ion fluxes in the halotolerant alga *Dunaliella salina*. *Plant Physiology* **81**, 92–96.

Ponce-Toledo RI, Deschamps P, Lôpez-García P, Zivanovic Y, Benzenara K and Moreira D (2017) An early-branching freshwater cyanobacterium at the origin of plastids. *Current Biology* **27**, 386–391.

Popova LG and Balnokin YV (1992) H^+-translocating ATPase and Na^+/H^+ antiport activities in the plasma membrane of the marine alga *Platymonas viridis*. *FEBS Letters* **3**, 333–336.

Popova L and Balnokin YV (2013) Na^+-ATPases of halotolerant algae. *Russian Journal of Plant Physiology* **68**, 472–482.

Popova LG, Shumkova GA, Andreev IM and Balnokin YV (2005) Functional identification of electrogenic Na^+-translocating ATPase in the plasma membrane of the halotolerant microalga *Dunaliella maritima*. *FEBS Letters* **579**, 5002–5006.

Popova LG, Belyaev DW, Shuvalov AV, Yurchenko AA, Matalin DA, Khramov DE, Orlova YV and Balnokin YV (2018) In silico analyses of transcriptomes of the marine green microalga *Dunaliella tertiolecta*: identification of sequences encoding P-type ATPases. *Molecular Biology* **52**, 520–531.

Raven JA (1984) *Energetics and Transport in Aquatic Plants*. New York, NY: AR Liss.

Raven JA (2009*a*) Functional evolution of photochemical energy transformations in oxygen-producing organisms. *Functional Plant Biology* **36**, 505–515.

Raven JA (2009*b*) Contribution of anoxygenic and oxygenic phototrophy and chemolithotrophy to carbon and oxygen fluxes in aquatic environments. *Aquatic Microbial Ecology* **56**, 35–52.

Raven JA (2013) Half a century of pursuing the pervasive proton. *Progress in Botany* **74**, 3–34.

Raven JA (2017) Chloride: essential micronutrient and multifunctional beneficial ion. *Journal of Experimental Botany* **68**, 359–367.

Raven JA and Beardall J (2016) The ins and outs of CO_2. *Journal of Experimental Botany* **67**, 1–13.

Raven JA and Hurd CJ (2012) Ecophysiology of photosynthesis in macroalgae. *Photosynthesis Research* **113**, 105–125.

Raven JA and Smith FA (1980) Intracellular pH regulation in the giant-celled marine alga *Chaetomorpha darwinii*. *Journal of Experimental Botany* **31**, 1357–1369.

Raven JA and Smith FA (1981) H^+ transport in the evolution of photosynthesis. *Biosystems* **14**, 95–111.

Raven JA and Smith FA (1982) Solute transport at the plasmalemma and the early evolution of cells. *Biosystems* **15**, 13–26.

Reed RD and Collins JC (1981) Membrane potential measurements of marine macroalgae: *Porphyra purpurea* and *Ulva lactuca*. *Plant Cell and Environment* **4**, 257–260.

Reed RD, Collins JC and Russell G (1981) The effects of salinity upon ion content and ion transport of the marine red alga *Porphyra purpurea* (Roth) C. Ag. *Journal of Experimental Botany* **32**, 347–367.

Remis D, Simonis W and Gimmler H (1992) Measurements of the transmembrane electrical potential of *Dunaliella acidophila* by microelectrodes. *Archives of Microbiology* **158**, 350–355.

Ritchie RJ (1982) Comparison of a lipophilic cation and microelectrodes to measure membrane potentials of the giant-celled algae *Chara australis* (Chlorophyta) and *Griffithsia monilis* (Rhodophyta). *Journal of Membrane Biology* **69**, 57–63.

Ritchie RJ (1984) A critical assessment of the use of lipophilic cations as membrane potential probes. *Progress in Biophysics and Molecular Biology* **43**, 1–32.

Ritchie RJ (1985) Energetic considerations of ion transport in *Enteromorpha intestinalis* (L) Link. *New Phytologist* **100**, 5–24.

Ritchie RJ (1988) The ionic relations of *Ulva lactuca*. *Journal of Plant Physiology* **133**, 183–192.

Ritchie RJ (1992) Sodium transport and the origin of the membrane potential in the cyanobacterium *Synechococcus* R-2 (*Anacystis nidulans*) PCC 7943. *Journal of Plant Physiology* **139**, 320–330.

Roberts DSE, Illot I and Brownlee C (1994) Cytoplasmic calcium and *Fucus* egg activation. *Development (Cambridge, England)* **120**, 155–163.

Rodríguez-Navarro A and Benito B (2010) Sodium or potassium efflux ATPase. A fungal, bryophyte and protozoal enzyme. *Biochimica et Biophysica Acta* **1799**, 1841–1853.

Rubio L, Linares-Rueda A, Garcia-Sánchez MJ and Fernández JA (2005) Physiological evidence for a sodium dependent high affinity phosphate and nitrate transport at the plasma membrane of leaf and root cells of *Zostera marina* L. *Journal of Experimental Botany* **56**, 613–622.

Rubio L, Belver A, Venene K, Garcia-Sánchez MJ and Fernández JA (2011) Evidence for a sodium efflux mechanism in the leaf cells of the seagrass *Zostera marina* L. *Journal of Experimental Marine Biology and Ecology* **402**, 56–64.

Rubio L, Garcia D, Garcia-Sánchez MJ, Niell FX, Felle HH and Fernández JA (2017) Direct uptake of HCO_3^- in the marine angiosperm *Posidonia oceanica* (L.) Delile driven by a plasma membrane H^+ economy. *Plant Cell and Environment* **40**, 2820–2830.

Rubio L, Garcia-Pérez D, Garcia-Sánchez MJ and Fernández JA (2018) Na^+-dependent high-affinity nitrate, phosphate and amino acids transport in leaf cells of the seagrass *Posidonia oceanica* (L.) Delile. *International Journal of Molecular Sciences* **12**, art. 1570.

Russell MJ and Hall AJ (1997) The emergence of life from iron monosulphide bubbles at a submarine hydrothermal vent. *Journal of the Geological Society of London* **154**, 377–402.

Saddler HD (1970a) The membrane potential of *Acetabularia mediterranea*. *Journal of General Physiology* **55**, 802–821.

Saddler HDW (1970b) The ionic relations of *Acetabularia mediterranea*. *Journal of Experimental Botany* **21**, 345–359.

Saier MH Jr (2000) Vectorial metabolism and evolution of transport systems. *Journal of Bacteriology* **182**, 5029–5035.

Saier MH Jr, Reddy VS, Tsu BV, Ahmed MS, Li C and Moreno-Hagelsieb G (2015) The Transporter Classification Database (TCDB): recent advances. *Nucleic Acid Research* **44**, D372–D379.

Sánchez-Baracaldo P, Ridgwell A and Raven JA (2014) A Neoproterozoic transition in the marine nitrogen cycle. *Current Biology* **24**, 652–657.

Sánchez-Baracaldo P, Raven JA, Pisani D and Knoll AH (2017) Early photosynthetic eukaryotes inhabited low-salinity habitats. *Proceedings of the National Academy of Sciences USA* **114**, E7737–E7745.

Scherer S and Böger P (1984) Vanadate-sensitive proton efflux by filamentous cysnobacteria. *FEMS Microbiology Letters* **22**, 190–192.

Scherer S, Stürzl E and Böger P (1984) Oxygen-dependent proton efflux in Cyanobacteria (blue-green algae). *Journal of Bacteriology* **158**, 609–614.

Schultze M, Forberich B, Rexroth S, Dyczmons NG, Roegner M and Appel J (2009) Localization of cytochrome b_6f complexes implies an incomplete respiratory chain in the cytoplasmic membranes of the cyanobacterium *Synechocystis* sp. PCC 6803. *Biochimica et Biophysica Acta* **1787**, 1479–1485.

Shevchenko V, Mager T, Kovalev K, Polovinkin V, Alekseev A, Juettner J, Chizhov I, Bamann C, Vavourakis C, Ghai R, Gushchin I, Borshchevskiy V, Rogachev A, Melnikov I, Popov A, Balandin T, Rodriguez-Valera F, Manstein DJ, Bueldt G, Bamberg E and Gordelly V (2017) Inward H^+ pump xenorhodopsin: mechanism and alternative optogenetic approach. *Science Advances* **3**, e1603187.

Shono M, Wada M and Fujii T (1995) Partial purification of a Na^+-ATPase from the plasma membrane of the marine alga *Heterosigma akashiwo*. *Plant Physiology* **108**, 1615–1621.

Shono M, Hara Y, Wada M and Fujii T (1996) A sodium pump in the plasma membrane of the marine alga *Heterosigma akashiwo*. *Plant and Cell Physiology* **37**, 385–388.

Shono M, Wada M, Hara Y and Fuji T (2001) Molecular cloning of Na^+-ATPase cDNA from a marine alga, *Heterosigma akashiwo*. *Biochimica et Biophysica Acta* **1511**, 193–199.

Shumkova GA, Popova LG and Balnokin YV (2000) Export of Na^+ from cells of the halotolerant microalga *Dunaliella maritima*: Na^+:H^+ antiporter or primary Na^+ pump? *Biochemistry (Moscow)* **65**, 917–923.

Sikes CS and Wilbur KH (1982) Functions of coccolithophore formation. *Limnology and Oceanography* **27**, 18–26.

Siver PA, Velez M, Clivetti M and Binda P (2018) Early freshwater diatoms from the Cretaceous Battle formation in Western Canada. *Palaios* **33**, 525–534.

Skulachev VP (1984) Sodium bioenergetics. *Trends in Biochemical Science* **9**, 483–485.

Skulachev VP (1989) The sodium cycle: a novel type of bacterial energetics. *Journal of Bioenegetics and Biomembranes* **21**, 635–647.

Slamovits CH, Okamoto N, Burri L, James ER and Keeling PJ (2011) A bacterial proteorhodopsin proton pump in marine eukaryotes. *Nature Communications* **2**, art. 183.

Smahel M, Hamann A and Gradmann D (1990) The prime plasmalemma ATPase of the halophilic alga *Dunaliella bioculata*: purification and characterisation. *Planta* **181**, 496–504.

Smahel H, Klieber H-G and Gradmann D (1992) Vanadate-sensitive ATPase in the plasmalemma of *Acetabularia*: biochemical and kinetic characteristics. *Planta* **188**, 62–64.

Smith FA and Raven JA (1979) Intracellular pH and its regulation. *Annual Review of Plant Physiology* **30**, 289–311.

Søndergaard D and Pedersen CNS (2015) PATBox: a toolbox for classification and analysis of P-type ATPases. *PLoS ONE* **10**, art. e0139571.

Soontharapirakkul K and Incharoensakdi A (2010) Na^+-stimulated ATPase of alkalophilic halotolerant cyanobacterium *Aphanothece halophytica* translocates Na^+ into proteoliposomes via Na^+ uniport mechanism. *BMC Biochemistry* **11**, art. 30.

Soontharapirakkul K, Promden W, Yamada N, Kageyama H, Incharoensakdi A, Iwamoto-Kihara A and Takabe T (2011) Halotolerant cyanobacterium *Aphanothece halophytica* contains an Na^+-dependent F_1F_O-ATP synthase with a potential role in salt-stress tolerance. *Journal of Biological Chemistry* **286**, 10169–10176.

Suffrian K, Schulz KG, Gurowska MA, Riebesell U and Bleich M (2011) Cellular pH differences in *Emiliania huxleyi* reveal pronounced membrane proton permeability. *New Phytologist* **190**, 595–608.

Tamogami J, Kikuyawa T, Wada T, Demura M, Kimura-Someya T, Shirouzo M, Yokayama S, Miauchi S, Simono K and Kamo N (2017) Evidence of two O-like intermediates in the photocycle of *Acetabularia* rhodopsin II, a light-driven proton pump from a marine alga. *Biophysics Physicobiology* **14**, 49–55.

Taylor AR (2009) A fast Na^+/Ca^{2+}-based action potential in a marine diatom. *PLoS ONE* **4**, art. e4966.

Taylor AR and Brownlee C (1993) Calcium and potassium currents in the *Fucus* egg. *Planta* **189**, 109–119.

Taylor AR, Chrachri A, Wheeler C, Goddard H and Brownlee C (2011) A voltage-gated H^+ channel underlies pH homeostasis in calcifying coccolithophores. *PLoS Biology* **9**, art. e1001085.

Taylor AR, Brownlee C and Wheeler G (2017) Coccolithophore cell biology: chalking up progress. *Annual Review of Marine Science* **9**, 283–310.

Thever MD and Saier MH Jr (2009) Bioinformatic characterization of P-type ATPases encoded within the fully sequenced genomes of 26 eukaryotes. *Journal of Membrane Biology* **229**, 115–130.

Thompson SEM, Callow JA, Callow ME, Wheeler GC, Taylor AH and Brownlee C (2007) Membrane recycling and calcium dynamics during settlement and adhesion of zoospores on the green alga *Ulva linza*. *Plant Cell and Environment* **30**, 723–744.

Tragin M and Vaulot D (2018) Green microalgae in marine coastal waters: the Ocean Sampling Day (OSD) dataset. *Scientific Reports* **8**, art. 14020.

Uji T, Hirata R, Mikami K, Mizuto H and Saga N (2012*a*) Molecular characteristics and expression analysis of sodium pump genes in the marine red alga *Porphyra yezoensis*. *Molecular Biology Reports* **39**, 7973–7980.

Uji T, Monma R, Mizuta H and Saga N (2012*b*) Molecular characterization and expression of two Na^+/H^+ antiporter genes in the marine red alga *Porphyra yezoensis*. *Fisheries Science* **78**, 985–991.

Vader A, Laughinghouse IV HD, Griffiths C, Jakobsen KS and Gabrielsen TM (2018) Proton-pumping rhodopsins are abundantly expressed by microbial eukaryotes in a high-Arctic fjord. *Environmental Microbiology* **20**, 890–902.

Van de Waal DB, John U, Ziveri G-J, Hoins M, Sluija A and Röst B (2013) Ocean acidification in a marine dinoflagellate. *PLoS ONE* **8**, e65987.

Wada T, Shimono K, Kikukawa T, Hato M, Shinya N, Ki SY, Kimura-Somega T, Shirouza M, Taogami J, Miyachi S, Juing KH, Kamo N and Yokoyama S (2011) Crystal structure of the eukaryotic light-driven proton-pumping rhodopsin, *Acetabularia* rhodopsin II, from marine algae. *Journal of Molecular Biology* **411**, 986–998.

Walker NA, Smith FA and Cathers IR (1980) Bicarbonate assimilation by freshwater charophytes and higher plants. I. Membrane transport of bicarbonate is not proven. *Journal of Membrane Biology* **12**, 241–256.

Wang Q, Li H and Post AF (2000) Nitrate assimilation genes of the marine diazotrophic, filamentous cyanobacterium *Trichodesmium* sp. Strain WH9601. *Journal of Bacteriology* **182**, 1764–1767.

Wegmann K (1986) Osmoregulation in eukaryotic algae. *FEMS Microbiology Reviews* **39**, 37–43.

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press

Weiss M and Pick U (1996) Primary structure and effect of pH on the expression of the plasma membrane H^+-ATPase from *Dunaliella acidophila* and *Dunaliella salina*. *Plant Physiology* **112**, 1693–1702.

Wheeler G, Helliwell K and Brownlee G (2019) Calcium signalling in algae. *Perspectives in Phycology* **6**, 1–10.

Wiangno K, Raksajit W and Incharoensakdi A (2007) Presence of a Na^+-type ATPase in the plasma membrane of the alkaliphilic halotolerant cyanobacterium *Aphanothece halophytica*. *FEMS Microbiology Letters* **270**, 139–145.

Wieczorek H, Brown D, Grinstein S, Ehrenfeld J and Harvey WR (1999) Animal plasma membrane energization by protein-motive V-ATPases. *Bioessays* **21**, 637–648.

Wolf AH, Slayman C and Gradmann D (1995) Primary structure of the plasma membrane H^+-ATPase from the halotolerant alga *Dunaliella biosculata*. *Plant Molecular Biology* **26**, 657–666.

Wu J and Seliskar DM (1998) Salinity adaptation of plasma membrane H^+-ATPase in the salt marsh plant *Spartina patens*: ATP hydrolysis and enzyme kinetics. *Journal of Experimental Botany* **49**, 1005–1013.

Yoshizawa S, Kumuga Y, Kim H, Ogawa Y, Hayashi T, Iwasaki W, DeLong EF and Kogure K (2014) Functional characterization of flavobacteria rhodopsin reveals a unique class of light-driven chloride pumps in bacteria. *Proceedings of the National Academy of Sciences USA* **111**, 6732–6737.

Yuan F, Leng B and Wang B (2016) Progress in studying salt secretion from the salt glands in recretohalophytes: how do plants secrete salt? *Frontiers in Plant Science* **7**, art. 977.

Zhang X, Ye N, Liang C, Mou S, Fan Z, Xu J, Xu D and Zhuang Z (2012) *De novo* sequencing and analysis of the *Ulva linza* transcriptome to discover putative mechanisms associated with its successful colonization of coastal ecosystems. *BMC Genomics* **13**, art. 565.

https://doi.org/10.1017/S0025315420000211 Published online by Cambridge University Press