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REVIEW

Chara braunii genome: a new resource for plant electrophysiology

M. J. Beilby[1]

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Abstract

The large-celled green alga *Chara* provided early electrophysiological data, but this model organism lost popularity once the smaller cells of higher plants became accessible to electrophysiology and genetic manipulation. However, with the sequencing of the *Chara braunii* genome (Nishiyama et al. Cell 174: 448–464, 2018), the molecular identity of the underlaying ion transporters in Characeae can be found and placed in evolutionary context. As Characeae are close to ancestors of land plants, the wealth of electrophysiological data will provide insights into important aspects of plant physiology, such as salt tolerance and sensitivity, carbon concentrating mechanisms, pH banding and the action potential generation.

Keywords *Chara* genome · Electrophysiology · Salt tolerance · OH^- channel · pH banding · Action potential mechanism · Hypotonic regulation

Rise and fall of characean electrophysiology

When I started my Ph. D. in early 1970s, talks on *Chara* electrophysiology formed an important part of any conference on plant physiology. Characeae are green algae that inhabit rivers and lakes on all continents except Antarctica. The large size of characean cells (up to 1 mm in diameter and centimeters in length) inspired early scientists. Corti (1774) observed cytoplasmic streaming, Jost (1927) and Osterhout and Harris (1928) inserted microelectrodes to measure potential difference (PD). Blinks et al. (1929) observed action potentials. The early experimental results were inaccurate due to cell damage by large-tipped glass microelectrodes and lack of high input impedance electrometers. The detailed history of early measurements in giant cells can be found in Hope and Walker (1975). It also became obvious that Characeae electrophysiology is very different from that of the marine algae, such as *Valonia* (now *Ventricaria*) with small positive transmembrane PDs (Osterhout et al. 1927). Walker (1955) measured the first reliable negative resting PDs in Characeae *Nitella* and Findlay and Hope (1964) refined the electrode insertion technique into cytoplasm or vacuole to obtain separate plasma membrane and tonoplast PDs in *Chara*. Further, the internal compartments of characean cells could be manipulated by vacuolar or cytoplasmic perfusion (Tazawa 1964; Tazawa et al. 1976; Williamson 1975), plasma membrane permeabilization (Shimmen and Tazawa 1982), or creation of single membrane fragments (Hirono and Mitsui 1981). The development of electronics, computer-controlled experiments, and data-logging has produced a wide range of experimental approaches in Characeae electrophysiology (see Beilby and Casanova 2014 for review).

With improvement in glass microelectrode technology, it became possible to impale the smaller cells of higher plant tissues. Ultimately, the Characeae electrophysiology dominance ended with the invention of patch clamp (Sakmann and Neher 1984) and the development of gene sequencing technologies (The *Arabidopsis* Genome Initiative 2000). Early in the new millennium, Characeae electrophysiology was sidelined as unusual and unrepresentative due to the large cell structure. However, at the same time, the prominent role of Characeae and related charophytes in evolution of land plants was emerging (McCourt et al. 2004). The recent sequencing of *Chara braunii* genome (Nishiyama et al. 2018) demonstrates the close relationship of Characeae and land plants. It will now be possible to identify the molecular makeup of the ion transporters underlying the multitude of Characeae electrophysiological data and compare the sequences to land plants and chlorophyte algae. Perhaps the importance of the characean electrophysiology is on the rise again.

✉ M. J. Beilby
m.j.beilby@unsw.edu.au

[1] School of Physics, University of NSW, Kensington, NSW 2052, Australia

Insight into salt tolerance at cellular level

Most extant Characeae live in freshwater, some survive in brackish conditions, but only a few species are fully salt-tolerant turgor regulators. Some *Tolypellas* (Winter et al. 1996), *Chara longifolia* (Hoffmann and Bisson 1986), and all *Lamprothamnium* species (Bisson and Kirst 1980, Okazaki et al. 1984, Beilby et al. 1999, Casanova 2013) respond to osmolarity/salinity changes by adjusting vacuolar concentrations of K^+, Cl^-, and sometimes Na^+ or sucrose. *Lamprothamnium* exhibits amazing salt tolerance. It can survive in freshwater (where it is not entirely happy), and up to twice (!!) seawater salinity (Burne et al. 1980, Williams 1998). *Chara australis* or *Nitella flexilis*, on the other hand, appear to regulate their internal pressure and do not tolerate brackish media (Gutknecht et al. 1978, Sanders 1981, Bisson and Bartholomew 1984).

How do these responses to saline stress reflect in characean electrophysiology? Thousands of current-voltage (I/V) profiles have been recorded from many characean species and over a huge diversity of conditions (for review see Beilby 2015). The results are consistent for saline stress. *Lamprothamnium* sp. and *Chara longifolia* cells detect decrease in turgor and speed up the proton pumping (see Beilby 2015 review) to maintain a negative membrane potential and proton electrochemical gradient to power Na^+/H^+ exchanger and import K^+ through inward K^+ rectifying channels and Cl^- through $2H^+/Cl^-$ symporter. In salt-sensitive *Chara australis*, the energizing proton pumping diminishes upon exposure to saline (50–100 mM NaCl). The membrane PD becomes more positive and prolonged spontaneous action potentials (APs) cause more K^+ and Cl^- depletion of the cell cytoplasm (Shepherd et al. 2008). *Chara* membrane PD displays a typical noise (Al Khazaaly et al. 2009). The noise was correlated to transient pH changes around the salt-stressed cell (Absolonova et al. 2018) and is thought to be caused by transient opening of groups of OH^- channels (see next section). With further depolarization of membrane PD, the OH^- channels, which remain open, become the dominant membrane conductance and further erode the proton electrochemical gradient leading to cell death (Beilby and Al Khazaaly 2009). None of these changes occur in media of equivalent osmolarity (90–180 mM sorbitol).

The above questions suggest the following tasks for molecular biology/genetics/protein modeling: (1) Compare the structures of the plasma membrane proton pumps from *Lamprothamnium*, *Chara longifolia*, and *Chara australis*. How are they different? (2) Are there similarities to proton pumps from *Arabidodpsis* or *Thellungiella*? And (3), How does the proton pump in *Chara longifolia* and *Lamprothamnium* detect change in turgor pressure or how does it communicate with the turgor pressure sensor?

Insight into carbon concentrating mechanisms and pH banding

Most Characeae live in freshwater ponds with alkaline media of pH 8 and higher, which shifts the equilibrium of dissolved inorganic carbon from CO_2 to HCO_3^-. To get more CO_2, which moves readily across the plasma membrane, characean cells generate acid bands (for proteome of *Chara* acid zones see Pertl-Obermeyer et al. 2018). While the mechanisms of getting carbon into the chloroplasts and into the Calvin cycle are still under dispute (see Beilby and Bisson 2012 for review), the process generates OH^-, which needs to be disposed of without disturbing the acid regions. A pH banding pattern appears in the light and can be visualized by pH indicators added to the medium (Spear et al. 1969). The proton pump on the plasma membrane produces the acid bands, while the alkaline bands are generated by activation of OH^- channels (for review see Beilby et al. 2018). The existence of H^+/OH^- channels in plants is controversial, despite good electrophysiological evidence from Characeae. The availability of the *Chara* genome made it possible to look for the molecular identity of H^+/OH^- channels. Mark Parker's group in the State University of New York at Buffalo (USA) works on Slc4a11 transporter in mice, which performs as a highly selective OH^- channel, when tested in the *Xenopus* oocyte system (Myers et al. 2016). Marion Hoepflinger from Ilse Foissner's group at University of Salzburg (Austria) found 26% homology of *Chara australis* CL5060.2 to Slc4a11. *Xenopus* expression vector pGH19-CL5060.2 was produced as a template to generate cRNA that was injected into *Xenopus* oocytes. Oocytes with CL5060.2 construct show Slc4a11-like characteristics of pH-dependent conductance increase, although the expression in the oocytes still needs optimization (paper in preparation).

Mary Bisson's group at State University of New York at Buffalo (USA) compared CL5060.2 sequence from *Chara australis* to other *Chara* species, most importantly to salt-tolerant *Chara longifolia,* finding only small differences. *Chara longifolia* continues to band in saline media, but there is no membrane PD noise or OH^- channel-mediated drastic depolarization. *Lamprothamnium* bands in freshwater but not at higher salinities (Foissner, paper in preparation). Thus, the salt tolerant Characeae exert control over their OH^- channels in saline media. What are the mechanisms?

A spatial separation of active proton pumps and putative H^+/OH^- channels resulting in zones of differing pH can be found in higher plants. The roots show sub-apical acidification and apical alkalinization with circulating currents to facilitate molybdenum, phosphorus, and iron acquisition, and to reduce aluminum toxicity (Raven 2000, 1991). The wheat root protoplasts seem to switch from “pump state” to “OH^- state” (Tyerman et al. 2001). Pollen tubes display apical acid zone oscillations that affect growth (Feijo et al. 1999; Michard et al. 2008). The aquatic angiosperms (Prins et al. 1980; Lara et al.

2002) also produce acid and alkaline sides of leaves as a biophysical carbon concentrating mechanism. Marion Hoepflinger found 22–51% homologies to CL5060.2 in *Arabidopsis thaliana*, *Glycine soja*, *Selaginella moellendorffii*, *Physcomitrella patens*, and *Marchantia polymorpha*. Are the acid/alkaline zones in the roots disrupted by salinity in salt sensitive, but not in salt tolerant land plants?

Insight into action potential in plants

In a recent paper Brunet and Arendt (2016) suggest that the action potential (AP) evolved from ancient damage response. Calcium is low in the cytoplasm, as it would interfere with the phosphate-based energetic metabolism. Upon rupture of cell membrane, Ca^{2+}, which is at much higher concentration in external media, will flood in due to negative membrane PD and low internal concentration. Characean AP increases internal Ca^{2+} concentration by using "tame" calcium from internal stores and the vacuole, which can be re-sequestered by the calcium pumps. The high cytoplasmic Ca^{2+} activates Cl^- channels and outflow of Cl^- from cytoplasm and vacuole depolarizes the membrane PD to close to zero. If the membrane is damaged, then repair happens quickly and usually involves calcium. High cytoplasmic Ca^{2+} also stops the cytoplasmic streaming to minimize loss of cell contents. While the APs will certainly be observed upon cell damage, the cells also "anticipate trouble" and APs are excited upon threshold depolarization to around − 100 mV by passing current across the membrane, by various depolarizing chemical agents or by mechanical deformation (for review see Beilby 2007, Beilby and Casanova 2014). In higher plants, APs propagate along some of the tissues and are now implicated in affecting many important functions, such as stomatal movements, production of phytohormones, or even gene expression. APs can inform plants of various types of biotic and abiotic stress (for review see Sukhov and Vodeneev 2009, Sukhov et al. 2011). Beilby and Al Khazaaly (2016, 2017) modeled *Chara* AP, based on work by the Thiel group (Wacke et al. 2003), who utilized an animal model of excitation with second messenger inositol 1,4,5-triphosphate (IP_3) (Othmer 1997). While the role of IP_3 is uncertain in plants, there have to be second messengers to connect threshold membrane depolarization to opening of Ca^{2+} channels on the internal stores. The mechanism is very likely to be the same in higher plants and it will be interesting to compare Ca^{2+}-activated Cl^- channels on the plasma membrane, Ca^{2+} channels and Ca^{2+} pumps on internal stores in Characeae and in higher plants. The Ca^{2+} channels might also be similar to those in animal cells.

The turgor-regulating Characeae, *Lamprothamnium* and *Chara longifolia*, employ the AP mechanism at the time of hypotonic stress. The salinity of a shallow pond may decrease drastically in a sudden downpour. The turgor increase triggers Ca^{2+} rise in the cytoplasm, opening of Cl^- channels and high conductance K^+ channels dumping K^+ and Cl^- to adjust the cell turgor to set point (Beilby and Shepherd 1996). A similar mechanism is likely to function in the roots of salt-tolerant land plants and aquatic higher plants. *Lamprothamnium* cells have another protection from sudden salinity decreases (and turgor increases): they form extracellular sulphated mucilage similar to that of chlorophyte sea algae, which absorbs some of the osmotic shock (Beilby et al. 1999, Shepherd and Beilby 1999). Aquino et al. (2011) found that mangroves, sea grasses, and salt-tolerant fern *Acrostichum aureum* produce sulphated mucilage in roots and shoots. On the other hand, glycophytes (*Zea mays* L., *Oryza sativa* L., and *Phaseolus vulgaris* L.) were unable to synthesize such mucilage, even when challenged by increased salinity. What genes are involved in this mucilage production?

Conclusion

Other examples of characean electrophysiology can be found in Hope and Walker (1975) and Beilby and Casanova (2014). The wealth of Characeae electrophysiological data can now be utilized to find the underlaying molecular identities of ion transporters, which in turn can inform on similar mechanisms in higher plants and elucidate evolutionary pathways. It is the author's hope that this short Commentary describing some of the recent breakthroughs in the field of Characeae electrophysiology, will stimulate scientists possessing the necessary biophysical related skill sets (such as molecular biology, electrophysiology, and protein modeling) to engage in research on this topic. Indeed, release of the *Chara* genome makes this area, once again, an excellent model system for modern biophysical research.

Compliance with ethical standards

Conflict of interest M. J. Beilby declares that she has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

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