Letter

Ussing's conundrum and the search for transport mechanisms in plants

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Plant transport physiologists have developed a range of models describing the movement of ions across cell membranes. However, while substantial progress has been made towards providing precise descriptions of the mechanisms underlying these fluxes, important instances remain in which the prevailing models cannot account for repeated observations, particularly in terms of energy transformations. As we shall show, disagreements with experimental findings may entail a revision of the proposed models, similarly to what has been required in animal transport studies (Ussing, 1994; see below). We present, as a key example, the futile cycling of sodium under toxic conditions (see Britto & Kronzucker, 2006, and discussed later), and show that unidirectional flux magnitudes measured by several groups, including our own, cannot be explained energetically by current models. We attempt to explain these observations by proposing alternative mechanisms of Na^+ transport across the root-cell plasma membrane.

The influx/efflux cycle of Na^+ in plants has been attributed to the sophisticated activity of distinct transport proteins in the plasma membrane (see Fig. 1 in Malagoli *et al.*, 2008). According to this widely accepted view, the majority of Na^+ influx into the plant cell occurs via nonselective cation channels (NSCCs; other possible Na^+ influx transporters include partially specific potassium channels and HKT transporters), while Na^+ efflux is thought to be mediated by a Na^+/H^+ exchanger, possibly SOS1 (Amtmann & Sanders, 1999; Munns & Tester, 2008).

The consensus view of the thermodynamic conditions occuring during salinity stress is that: (1) root cytosolic Na^+ activity (a_{Na^+}) is substantially lower than external a_{Na^+} (in one of the few studies that measured cytosolic a_{Na^+}directly, it was found to be as high as 28 mM, against an external a_{Na^+} of 150 mM (Carden *et al.*, 2003)); and (2) the chemical potential difference for Na^+ across the plasma membrane is amplified greatly by the electrical potential difference (approx. −80 mV in Carden *et al.*, 2003), as per the Nernst equation. A steep electrochemical potential gradient results, favoring passive influx of Na^+ into the cell, and an active efflux, powered by the electrochemical H^+ gradient generated by plasma-membrane proton ATPases. To estimate the energy required to drive the Na^+ fluxing proposed in this model, it is useful to consider that, in many cases, it appears to be almost purely cyclical; that is, relatively little Na^+ accumulates in the plant compared with the large amount that initially enters, and ultimately exits, the system. Evidence for cyclic Na^+ transport includes the loss, within 1 h, of 90% of $^{22}Na^+$ absorbed in 10 min by corn roots (Cheeseman, 1982); the precipitous decline in apparent $^{24}Na^+$ influx within 5 min of labeling intact *Suaeda maritima* roots (Lazof & Cheeseman, 1986; also see Britto & Kronzucker, 2006); the rapid (< 5 min) saturation of $^{22}Na^+$ in roots of *Arabidopsis* (Essah *et al.*, 2003); and efflux : influx ratios of 0.86–0.90 in rice (Malagoli *et al.*, 2008), 0.92–0.95 in *Puccinellia tenuiflora* and wheat (Wang *et al.*, 2009), and 0.95 in barley (Kronzucker *et al.*, 2006).

Thus, the active (efflux) component of this cycle is nearly equal to the passive (influx) component, and therefore influx measurements, which are more commonly found in the literature and more straightforward to interpret, can be used to approximate efflux, with only a slight overestimate (in fact, the influx component may also entail an energy cost, despite being in the thermodynamically passive direction, because it involves an electrogenic uniport that must be counteracted by proton pumping; Na^+ efflux cannot accomplish this, if it is indeed electroneutral, because of the exchange with H^+). The required energy can then be predicted, in terms of O_2 consumed per Na^+ transported, on the basis of the following stoichiometric series that draws upon models of transport energization: a 1:1 stoichiometry for Na^+/H^+ antiport (Shi *et al.*, 2000; Pardo *et al.*, 2006; Yeo, 2007); a second 1:1 stoichiometry between H^+ pumping and ATP hydrolysis (Briskin & Reynolds-Niesman, 1991; Palmgren, 2001); and a third stoichiometry, of 5:1, between ATP synthesized and O_2 consumed in respiration (i.e. 'phosphorylation efficiency' or P : O_2 ratio; Poorter *et al.*, 1991; Scheurwater, 1999; Kurimoto *et al.*, 2004). To summarize, for every O_2 consumed in respiration, five Na^+ ions are extruded from the cell via Na^+/H^+ exchange. Energy demands will be even greater as a result of simultaneous Cl^- transport and other transport steps within the plant, such as across the tonoplast, or from root to shoot (see the analysis by Yeo, 1983).

The problem with this analysis becomes evident upon examining actual Na^+ flux magnitudes measured in plants and comparing them with O_2 normally consumed in respiration. Table 1 predicts the O_2-consumption rates theoretically associated with Na^+ fluxes from a number of studies, based on the preceding analysis; in every case, these values are extremely high and almost certainly exceed the root's respiratory budget. For example, maximal root respiration was only approx. 30 μmol of O_2 g (root FW)$^{-1}$ h^{-1} (assuming, conservatively, a fresh root weight : dry root weight ratio of 10) in a study of 24 wild plant species (Poorter *et al.*, 1991), and was even less in a study of six crop species (Rao & Ito, 1998). These values fall far

Table 1 Na^+ fluxes from four studies, with predicted respiratory requirement

Species	Na^+ flux (μmol g (root FW)$^{-1}$ h^{-1})	Respiratory requirement (O_2 consumption, μmol g (root FW)$^{-1}$ h^{-1})	Reference
Spergularia maritima	600	120	Lazof & Cheeseman (1986)
Arabidopsis thaliana	300*	60	Essah *et al*. (2003)
Oryza sativa (japonica rice)	240*	48	Horie *et al*. (2007)
Oryza sativa (indica rice)	107	21	Malagoli *et al*. (2008)

The asterisk (*) indicates influx measurements that are slightly higher than efflux; respiratory requirements are also therefore slightly higher than active efflux would require.

short of the predicted value in three of the four cases presented in Table 1. In the fourth case (Malagoli *et al*., 2008), measured respiration rates of only 11 μmol g (root FW)$^{-1}$ h^{-1} were compared directly with Na^+ efflux; again, they were only half of what would be required to account for the flux.

Clearly, because there are other demands on respiration (e.g. for growth, maintenance and the fluxes of other ions), these predicted values are in excess of what can be provided to power such large fluxes, according to the proposed mechanisms of transport and energy transduction. We have termed this lack of correspondence 'Ussing's conundrum' because it represents the same problem faced half a century ago by Hans Ussing, a pioneer of tracer-flux research (Ussing, 1947, 1994) and the co-originator of the Ussing–Teorell flux-ratio equation. Ussing had compared theoretically active chloride fluxes and respiration rates in frog epithelia, to find that the available energy was insufficient to account for the observed flux. He thus proposed an alternative transport mechanism, in which the flux of one ionic species in the 'uphill' direction is energetically coupled to the flux of the same (or similar) ion in the 'downhill' direction. This concept of 'exchange diffusion' would later be developed into modern ideas of ionic symport and antiport (Maloney, 1994).

The discrepancy that emerges in Table 1 is scarcely lessened if the Ussing–Teorell equation is applied to sodium fluxes. The energy calculations would then be based only upon the degree to which the total flux in the active (efflux) direction exceeds the maximum free-diffusion flux in that direction, which is a function of cytosolic electrochemical a_{Na^+} (Dainty, 1962; White, 2003). In practice, this diffusive flux can be calculated in relation to influx, and to the external activity, according to the Ussing–Teorell equation:

$$\frac{\phi_{oc}}{\phi_{co}} = \frac{\bar{a}_o}{\bar{a}_c} \qquad \text{Eqn 1}$$

(a simplified version, where ϕ_{oc} and ϕ_{co} represent influx and efflux, and $\bar{a}_c$ and $\bar{a}_o$ represent cytosolic and external electrochemical activities, respectively; Teorell, 1949; Ussing, 1947; also see White, 2003, for critique). Applying values for membrane electrical potential (~ −80 mV) and cytosolic a_{Na^+} (≤ 28 mM), both measured by Carden *et al*. (2003), at an external a_{Na^+} of 150 mM, we can calculate the electrochemical activity ratio (inside : outside) to be *c*. 28:3400 (derived first from the activity ratio 28:150, which is then increased by *c*. 22-fold as a result of the −80 mV electrical potential, according to the Nernst equation; see Nobel, 2005). This indicates that very little (< 1% of the magnitude of influx) of the efflux can be accounted for by passive diffusion. Moreover, this quantity is further reduced by the likelihood that the proposed channel-mediated entry of Na^+ is via a long pore, which exponentially reduces the back flux through the channel (Hodgkin & Keynes, 1955; Hille, 1992).

Thus, like Ussing, we may be required to revise (if not abandon) one or more aspects of the proposed flux-energization model. In Ussing's case, the concept of energetically coupled exchange diffusion was developed to address this concern (Ussing, 1994). Although few examples of ions exchanging with others of the same ionic species exist in the plant literature (Laties, 1959; cf. Horemans *et al*., 1998; Britto & Kronzucker, 2003), it is not impossible that the futile cycling of Na^+ occurs by this means, perhaps via an antiporter, evolved for another function, with low selectivity for monovalent cations. This possibility could be investigated by testing for trans-stimulation. Since the time of Ussing's original conundrum, the broader concept of ionic symport and antiport has encompassed the exchange of similar, as well as dissimilar, ions (Maloney, 1994); in the case of sodium efflux, there may be many possibilities that have been overlooked (e.g. Na^+/Cl^- symport; Colmenero-Flores *et al*., 2007). Indeed, the possibility that sodium exits the cell via processes other than Na^+/H^+ antiport, at least in some species, was suggested in a study of 16 crop plant species (Mennen *et al*., 1990). Interestingly, much of the ground work that established Na^+/H^+ antiport as a leading model for Na^+ efflux was conducted under low-Na^+ conditions (Colombo *et al*., 1979; Jacoby & Teomy, 1988; Mennen *et al*., 1990), where energetic considerations are, by comparison to the present discussion, trivial. In addition, the stoichiometries of ATP hydrolysis and proton pumping may not be uniform (Baunsgaard *et al*., 1996; Low & Rausch, 1996; Morsomme *et al*., 1996).

More radical solutions may also have to be considered. For instance, it may be that Na^+ is not extruded via the plasma membrane by transport proteins, but is secreted via membrane vesicles, as has been suggested for Na^+ transport (Lazof & Cheeseman, 1986; Flowers & Colmer, 2008, and references therein), and more recently shown for photoassimilate (Etxeberria *et al.*, 2007) and Mn^{2+} (Peiter *et al.*, 2007) transport. A still more unconventional proposal is that the majority of apparent ion fluxes exceeding the cell's respiratory capacity are not across the plasma membrane at all, but result from the misinterpretation of tracer accumulating in extracellular spaces, particularly in leaves, as has been demonstrated to some degree in rice plants (Flowers *et al.*, 1991; Gong *et al.*, 2006).

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