

# Application of the Chemiosmotic Hypothesis to Ion Transport Across the Root<sup>1</sup>

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## ABSTRACT

The evidence on how ions accumulated in the root symplasm are released to the xylem vessels is examined. It is suggested that Mitchell's chemiosmotic hypothesis as applied to ion transport might account for the process. A model based on this hypothesis shows the symplasm as an osmotic unit connecting two isolated solutions, but with no significant difference in proton motive force across the unit. If it is assumed that the resistance to transport by plasmalemma uniports and antiports differs in the cortical and stelar ends of the symplasm, the model will provide for an influx of ions to the xylem. The reported properties of isolated steles suggests that the porters (carriers) do have properties in accord with the model.

There is still uncertainty as to how ions actively accumulated by the epidermal and cortical cells of the root are passed to the xylem. The Crafts-Broyer hypothesis (9) proposed cell-to-cell cytoplasmic transport (symplasm transport) through the osmotic barrier of the endodermis into the compact stele, where there is leakage of ions into the xylem due to lower O<sub>2</sub> tension and lower metabolic rate. There is now little doubt that the living cells of the root do form a symplasm through interconnecting plasmodesmata such as Crafts and Broyer visualized (1, 8, 19, 26) but support for the latter part of the hypothesis has not been obtained. Microelectrode determinations of O<sub>2</sub> partial pressure in the stele do not indicate concentrations limiting to respiration (4, cf. 13). Roots accumulate ions into the stele at concentrations comparable to those in the cortex (2, 5, 11, 28), and x-ray microanalysis shows the xylem parenchyma to have high concentrations of K<sup>+</sup> (19). There is substantial evidence that ion transport into the dead xylem vessels shares critical properties with transport into living root cells: *i.e.* transport is energy-linked and produces concentration and electrical potential gradients relative to the external solution (1, 5, 8, 11). Furthermore, there is an electrogenic component to the electrical potential (10, 25).

The uncertainty lies with what these observations signify about release of ions from symplasm to xylem. Läuchli (19) and Pitman (24) summarized the arguments for an active secretion of ions from the xylem parenchyma, which serves as a sink for ions that move across the symplasm from the epidermis-cortex. Briefly, xylem parenchyma cells show characteristics of an active metabolism, including cytoplasm with abundant mitochondria and ER, and plasmalemma ATPase activity; inhibitor studies clearly show that transport to the xylem can be blocked without blocking ion absorption. Oddly enough, certain inhibitors of protein synthesis, cycloheximide or *p*-fluorophenylalanine (which produces ineffec-

tive proteins), are particularly effective, suggesting rapid protein synthesis as essential to ion secretion.

On the other hand there are opinions that the release is passive (2, 5, 18). Bowling (5) concluded "that radial transport is driven by an active step at the outer surface of the root whilst movement from the living cells into the vessels is passive" (*i.e.* down an electrochemical gradient). Baker (2) expressed a similar opinion. Bowling (5) showed that cell potentials and ion activities appear to be stable across the symplasm but drop significantly in the xylem exudate, although the latter still has high ion activities and a negative electrical potential compared with the external solution. An important experiment in demonstrating the intimate relationship between symplast and xylem was that of Dunlop and Bowling (12) showing rapid and parallel depolarization of epidermal cell and xylem exudate electrical potential upon increasing the KCl concentration of the medium.

Baker (2) found that steles in intact corn roots were active in ion accumulation, as reported by Yu and Kramer (28), while freshly isolated steles were not, in agreement with Laties and Budd (18) and Lüttge and Laties (20). Uncouplers prevented ion uptake into the stele by preventing uptake into the epidermis and cortex. Leakage of accumulated ions from isolated steles was more rapid than from the cortex (2, 18) and uncouplers accelerated the loss (2).

With respect to these opposing hypotheses, there is an aspect of Mitchell's chemiosmotic hypothesis (22, 23) which has not been considered; *i.e.* the proton motive force (electrochemical gradient of protons) created by respiratory chain "loops" or by ATP hydrolysis can be utilized to drive a net influx or a net efflux of transportable ions. The concept is simple, and elements of it have been around for some years. In this paper I attempt to show how it might be applied to symplasm transport.

## HYPOTHESIS

The phenomenon of influx and efflux salt pumping has been extensively studied in mitochondria, and the evidence from animal (6) and plant (14) mitochondria has been reviewed. Brierley and colleagues (7, 16) have recently shown a dynamic energy-linked influx and efflux of K<sup>+</sup> in heart mitochondria at osmotic steady-state. In corn mitochondria a similar dynamic osmotic steady-state has been deduced from the rapid osmotic swelling or shrinkage resulting from inhibition or acceleration of carrier activity (15).

Figure 1 is a chemiosmotic model of influx and efflux transport. In general terms the model shows an equivalent of salt influx or efflux driven by an equivalent of electrogenic H<sup>+</sup> efflux.<sup>3</sup> The

<sup>3</sup> This equivalency is correct for the model, but it is not established as fact. It is conceivable, for example, that an equivalent of anion influx might be by symport (or co-transport) with two or more equivalents of H<sup>+</sup> thus utilizing part of the energy available in the electrical gradient as well as that in the pH gradient. However, this possibility does not affect the basic question explored here, and the hypothesis is presented in its simplest form.

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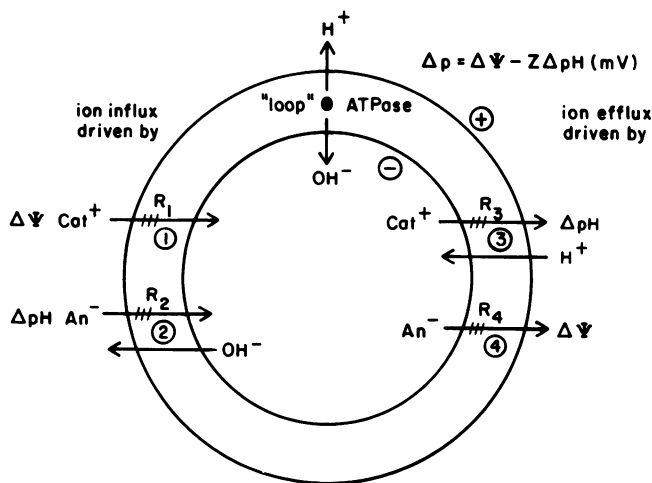


FIG. 1. Chemiosmotic hypothesis applied to ion transport. The model shows an equivalent of salt influx or salt efflux driven at the expense of an equivalent of electrogenic proton efflux. Numbers are used to designate the two uniports (1 and 4) and the two antiports (2 and 3). R stands for corresponding resistances to transport. See text for discussion.

proton motive force ( $\Delta p = \Delta\psi - Z\Delta pH$  where  $Z = 2.3 RT/F$ ) created by the proton pump is collapsed in the transport of the cation and the anion. For salt influx, the anion<sup>-</sup>/OH<sup>-</sup> antiport is driven by  $-Z\Delta pH$  (an anion<sup>-</sup>/H<sup>+</sup> symport would serve as well), while the cation<sup>+</sup> enters via a uniport down the electrical gradient ( $\Delta\psi$ ). For salt efflux, the utilization of the components of  $\Delta p$  is reversed:  $-Z\Delta pH$  drives cation<sup>+</sup>/H<sup>+</sup> exchange, and the anion<sup>-</sup> effluxes down  $\Delta\psi$  via a uniport. Uniports and antiports are generic terms for avenues of transport through otherwise impermeable membranes, presumably via proteinaceous carriers or channels which have some capacity for ion discrimination. Outside of Donnan effects the hypothesis assumes that the ion content of the osmotic unit is a function of  $\Delta p$ . (In mitochondria this is readily demonstrated by the rapid efflux of salt when the energy supply is blocked or uncoupled. Efflux in this instance is driven by the electrochemical gradients of transportable ions, which had been balanced in steady-state by  $\Delta p$ . Passive salt efflux might be accommodated by either cation plus anion uniports or by cation plus anion antiports; the evidence on passive efflux of phosphate salts [where the Pi/OH antiporter can be reversibly inhibited] indicates that the antiports are the principal avenues of passive exit [15].)

Recent studies of influx and efflux pumping of KH<sub>2</sub>PO<sub>4</sub> and K<sub>2</sub>SO<sub>4</sub> in corn mitochondria have shown that the direction of salt transport is governed by the relative resistance to transport (17). "Resistance" is here used as a generalized parameter which integrates all rate-limiting factors in transport, including the kinetic parameters of carrier transport. It is recognized that this comprehensive definition of "resistance" glosses over biophysical and biochemical aspects of transport driven by  $\Delta p$  (see later discussion). However, I desire here to make a simple distinction between the driving force provided by  $\Delta p$  on one hand, and everything else on the other. In Figure 1 each of the two uniports and antiports is assigned a corresponding resistance, and ion fluxes are expressed by the simple relationship: flux = potential/resistance.

For any one organelle or cell there will be a single  $\Delta p$ , but the contributions of  $\Delta\psi$  and  $\Delta pH$  to  $\Delta p$  can vary with the resistance. For example, consider a net influx of salt where  $R_1 > R_2$ :

$$\phi_{Cat}^{in} = \frac{\Delta\psi}{R_1} = \phi_{An}^{in} = \frac{-Z\Delta pH}{R_2} \quad (1)$$

and since  $R_1 > R_2$ ,  $\Delta\psi > -Z\Delta pH$ .

Obviously, the converse will be true for  $R_1 < R_2$ , and the general expression is  $R_1/R_2 = \Delta\psi/-Z\Delta pH$ . For net efflux the relationship is  $R_3/R_4 = -Z\Delta pH/\Delta\psi$ .

At osmotic steady-state the contributions of  $\Delta\psi$  and  $-Z\Delta pH$  to  $\Delta p$  will be determined by the relative values of all four resistances. It is not possible at present to predict quantitatively the steady-state osmotic balance, but observations have been made on osmotic swelling and shrinking of corn mitochondria in 5 mM K-phosphate or sulfate which give a qualitative picture (17). Briefly,  $R_2^{SO_4} > R_1^K > R_2^{PO_4}$  (Fig. 1), and hence  $\Delta\psi < -Z\Delta pH$  during respiration-driven swelling in sulfate, while  $\Delta\psi > -Z\Delta pH$  for swelling in phosphate. At osmotic steady-state it appears that for sulfate  $E_K > \Delta\psi$ , and for phosphate  $E_K < \Delta\psi$  ( $E_K$  = equilibrium potential for K<sup>+</sup>). Addition of valinomycin at steady-state reduces both  $R_1$  and  $R_3$  (Fig. 1), resulting in rapid net salt influx in sulfate and net salt influx in phosphate, and producing a new steady-state. With sulfate, the decrease in  $R_1^K$  is of little consequence since  $R_2^{SO_4}$  remains high and rate-limiting to salt influx. However, the decrease in  $R_3^K$  causes K<sup>+</sup>/H<sup>+</sup> exchange to become the path of least resistance to H<sup>+</sup> entry, and with the efflux of K<sup>+</sup> at the expense of  $\Delta pH$  (giving a proportionate rise in  $\Delta\psi$ ) there is an accompanying exit of sulfate via the uniport. The converse argument is applied to valinomycin-induced K-phosphate influx;  $R_2^{PO_4}$  is low and lowering  $R_1^K$  permits additional salt influx. Although in both cases the salt fluxes are transients, the point is made that changing resistances can change ion fluxes and produce net salt transport.

It is not yet demonstrated that plasmalemmas of root cells possess these uniports, antiports, and proton pump, but their existence is increasingly postulated to explain experimental observations (see the recent symposium on cell membrane activities in plants, ref. 21). If one assumes that they are present and that the "resistances" of the porters in the cortex and stele are different, a new picture of transport to the xylem emerges.

Figure 2 is a schematic model of the cortex-stele symplast of the root, with the endodermis providing an osmotic barrier which separates the apoplasts of cortex and stele. The model differs from Figure 1 in having different solutions on the two sides of the osmotic unit, and different membrane properties on these two sides. Ions enter via the cortical plasmalemma and are transported to the stele with very little gradient in ion concentration, ion activity, and cell electrical potential (5, 11). There is a gradient in vacuolar pH (3), but this may not be reflected in cytoplasmic pH. Transport through the symplast is largely by diffusion (27). Efflux through the plasmalemma of the stelar parenchyma is driven by  $\Delta p$ . Steady-state transport across the symplast is secured by influx pumping exceeding efflux pumping at the cortical plasmalemma, with the converse true of the stelar plasmalemma. During transport the following relationship will hold (number

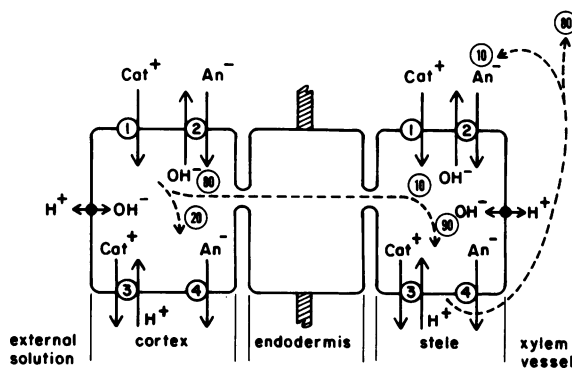


FIG. 2. Chemiosmotic hypothesis applied to ion transport across the symplast of the root. The endodermis separates the apoplastic solutions of cortex and stele, and the symplast becomes an osmotic unit bridging these solutions. Net ion flux across the symplast is secured by differential resistance to influx and efflux transport in cortex and stele (see text). Uniports and antiports are numbered as in Figure 1, with corresponding resistances (not shown; see Fig. 1). Dotted line and circled numbers are given to illustrate a hypothetical example discussed in the text.

refers to transport carriers identified in Fig. 2, c to cortex, s to stele):

$$\phi_1^c - \phi_3^c = \phi_3^s - \phi_1^s \text{ for cations} \quad (2)$$

$$\phi_2^c - \phi_4^c = \phi_4^s - \phi_2^s \text{ for anions} \quad (3)$$

For simplicity of illustration one can visualize transport of a salt where the steady-state fluxes of cation and anion into the xylem are equal. In this case the terms above for cation and anion transport in and out of the symplast will be equal, and the following can be written:

$$\begin{aligned} \left( \frac{\Delta\psi}{R_1^c} - \frac{-Z\Delta pH}{R_3^c} \right) &= \left( \frac{-Z\Delta pH}{R_3^s} - \frac{\Delta\psi}{R_1^s} \right) \\ &= \left( \frac{-Z\Delta pH}{R_2^c} \frac{\Delta\psi}{R_4^c} \right) = \left( \frac{\Delta\psi}{R_4^s} - \frac{-Z\Delta pH}{R_2^s} \right) \end{aligned} \quad (4)$$

For this relationship to hold during net transport of a salt, there must be differences between cortex and stele in corresponding resistances and/or in  $\Delta\psi$  and  $\Delta pH$ . Illustration of this can be made by assuming that  $\Delta p$ ,  $\Delta\psi$  and  $\Delta pH$  are constant across the symplast, and inserting some arbitrary figures to give flux units for the above terms. If  $\Delta p$  is set at 200,  $\Delta\psi$  and  $-Z\Delta pH$  at 100,  $R_1^c$  and  $R_2^c$  at 1,  $R_3^c$  and  $R_4^c$  at 5, 80 units/unit time will cross the symplast to the xylem (Fig. 2).

$$\left( \frac{100}{1} - \frac{100}{5} \right) = \left( \frac{100}{1.1} - \frac{100}{10} \right) = \left( \frac{100}{1} - \frac{100}{5} \right) = \left( \frac{100}{1.1} - \frac{100}{10} \right) \quad (5)$$

In this simplest of cases, transport is secured by increasing the resistance of  $R_1^s$  and  $R_2^s$  10-fold over that of  $R_1^c$  and  $R_2^c$ , while decreasing that of  $R_3^s$  and  $R_4^s$  about 4.5-fold compared with  $R_3^c$  and  $R_4^c$ . Of primary importance are the ratios  $R_1:R_3$  and  $R_2:R_4$  within the cortex and the stele, as these will govern the direction and rate of net transport in each end of the system. In the example above,  $R_1:R_3$  is 1:5 for cortex and 10:1.1 for stele;  $R_2:R_4$  give the same ratios.

The ratios need not be equal for cation and anion, and will not be for cases where  $\Delta\psi$  and  $\Delta pH$  are not the same for cortex and stele. For example, if in the above illustration all parameters for the cortex remain unchanged, still providing 80 units of net influx/unit time, but reducing  $-Z\Delta pH$  for the stele to 20 ( $\Delta\psi$  remaining at 100), the following ratios are obtained:

$$R_1^c:R_3^c = R_2^c:R_4^c = 1:5 \quad (6)$$

$$R_1^s:R_3^s = 10:0.22 \text{ and } R_2^s:R_4^s = 2:1.1 \quad (7)$$

As summarized above, however, there is no reason to believe that  $\Delta p$  or its components differ very much across the symplast, and during steady-state transport ion activities can be considered stabilized.

These illustrations serve to show that net flux of salt across the symplast could be secured by varying the resistance of the carriers. Or perhaps it would be more exact to say that if transport is chemiosmotic, there must be differences in resistance to transport between the two osmotic phases of the system. However, it is not required that there be differences in metabolic rate between these phases, or that gradients of chemical or electrical potential exist (other than for internal diffusion of salt). Note that this is not to say such gradients are excluded; it is simply that the hypothesis for net salt flux across the system is not based upon them. It is based on a membrane-bound osmotic unit which faces two different external solutions, and with which it conducts separate energy-linked ion traffic. The carrier systems for this traffic have the same type of energy linkage, but differ in unknown properties which are pooled here under "resistance." It is the differential functioning of these two sets of carriers that produces net energy-linked ion transport. Each end of the symplast attempts to come to steady-state with its external solution, but as these steady-states are different, net transport results.

A profitless debate can be conducted as to whether the net transport is active or passive. It is active in that net efflux into the xylem is energy-linked; it is passive in that the electrochemical potential of mobile ions drops between stelar parenchyma and xylem exudate (5, 11). A purely passive efflux of salt down an electrochemical gradient via carriers can be demonstrated in mitochondria (see Fig. 2 of ref. 15), but without evidence for lack of proton motive force in the stelar parenchyma it is necessary to assume that ion efflux is energy-linked. The carriers respond to  $\Delta p$  as well as to  $\Delta\bar{\mu}_{ion}$ .

Since "resistance" must be broadly defined to include all of the rate-limiting parameters of transport, we are concerned with at least the following: (a) specific ions being transported; (b) ion activities within and without the symplast; (c) access of ions to carrier-binding sites; (d) affinity of ions for binding sites (this can include any energy barrier in removing water of hydration); (e) turnover of carriers; (f) number of carriers.

There is little to indicate which of these parameters would be implicated in the relative carrier resistance of cortex and stele. Freshly isolated steles have little capacity for influx transport (2, 18, 20), which translates as a high resistance in  $R_1^s$  and  $R_2^s$  of Figure 2. However, washing or aging the steles induces influx transport (18, 20), signifying a decline in  $R_1^s$  and  $R_2^s$ . The decline might be due to biosynthesis of more carrier, activation of existing carrier, change in membrane lipids, etc. In a similar vein, the rapid leakage of ions from freshly excised steles (2, 18) might only be an expression of low resistance in  $R_3^s$  and  $R_4^s$ , especially when the steles are placed in a much more dilute solution than that with which their carrier resistances are designed to equilibrate. The accelerating effect of dinitrophenol on ion efflux from fresh steles (2) is what would be expected if part of the resistance of the  $H^+$ / $K^+$  antiport (*i.e.*  $R_3^s$ ) lies with mobilizing  $H^+$ . Hensley and Hanson (15) have demonstrated this response to uncoupler in corn mitochondria.

In summary, the chemiosmotic hypothesis as applied to salt transport permits efflux as well as influx pumping of ions at the expense of a proton motive force. If an osmotic unit such as the symplast of a root (or salt gland or the phloem) connects two isolated solutions, and if the properties of the carriers on the two sides differ in "resistance" a net flow of ions through the unit can be established. No alteration of proton motive force across the unit is required (nor is it excluded). The known properties of the stele in ion uptake and loss are such as to suggest that resistance does differ from that of the cortex. In view of the increasing support which the chemiosmotic hypothesis is receiving, it seems reasonable that symplast transport across the root be studied with chemiosmotic mechanisms in mind.

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