Plant Signaling & Behavior 5:2, 132-133; February 2010; © 2010 Landes Bioscience

The ever expanding role of aquaglyceroporins

Confirmation of protein-facilitated boron transport

Kate L. Fitzpatrick* and Robert J. Reid
School of Earth and Environmental Sciences; The University of Adelaide; Adelaide, Australia

The exact mechanism of transport of boron (B) entering the plant cell as boric acid $B(OH)_3$, has become hotly debated with evidence for both passive and protein facilitated transport. Here we put the controversy to rest by confirming that boron influx into plants can be partially controlled by opening and closing of channel-like transport proteins. Using treatments that were likely to inhibit membrane transporters capable of facilitating B transport, we confirmed that at least 50% of B transport could be contributed by a transporter of some type in barley roots. Based on the physiochemical similarities between $B(OH)_3$ and other solutes that were known to be transported via aquaglyceroporins, we hypothesised that aquaglyceroporins would be likely candidates to facilitate $B(OH)_3$ transport into the cytoplasm. We demonstrated using functional yeast complementation that two barley root aquaglyceroporins, HvPIP1;3 and HvPIP1;4, were both capable of facilitating B transport. This finding has demonstrated yet another function of aquaglyceroporins.

Key words: aquaporin, aquaglyceroporin, boron, transport, PIPs

Submitted: 10/11/09

Accepted: 10/12/09

Previously published online: www.landesbioscience.com/journals/psb/article/10305

*Correspondence to: Kate L. Fitzpatrick; Email: kate.fitzpatrick@adelaide.edu.au

Addendum to: Fitzpatrick KL, Reid RJ. The involvement of aquaglyceroporins in transport of boron in barley roots. Plant Cell Environ 2009; 32:1357–65; PMID: 19552667; DOI: 10.1111/j.1365-3040.2009.02003.x.

The major intrinsic protein (MIP) superfamily contains aquaporins and the related 'aquaglyceroporins' (AQGP), whose numbers and functionality are rapidly expanding.[1] These transport proteins are responsible for not only the bidirectional transport of water and glycerol, but also for the transport of other small neutral uncharged solutes. Based on their size, net charge and volume compared to the diameter of the aquaglyceroporin pore, it was predicted that a range of other molecules such as arsenite (AsIII) and silicic acid $Si(OH)_4$ would also permeate aquaglyceroporins, and this has been confirmed.[2-5] It has long been argued that because of the strong similarity with H_2O, it could reasonably be assumed that H_2S would cross membranes via aquaporins. However, it has very recently been demonstrated that membrane fluxes of H_2S were insensitive to treatments that inhibited influx of H_2O, leading to the conclusion that H_2S simply passed through the phospholipid bilayer and not through a protein transporter.[6]

Boron (B), available to plants as boric acid, $B(OH)_3$, can be classed as a small neutral uncharged molecule based on physiochemical similarities to glycerol and arsenite.[7] Like H_2S, the research surrounding B transport across biological membranes has been highly debated and the literature contains conjecture about the exact mode of transport with evidence for both passive and active transport. Several studies have demonstrated substantial passive B movement through both lipid bilayers and plant membranes, consistent with measurements indicating that B has high lipid solubility which would favor permeation through such membranes.[8-12] These data suggested that protein-mediated transport into cells would be redundant and would be short-circuited by the passive leak pathway. However, other reports have indicated that B transport may have an active transport component when plants were grown under B deficient conditions.[12,13] The presence of protein-assisted passive transport has proved hard to establish.

Our recent work has focused on putting this controversy to rest by attempting to modify B uptake using treatments

that should not affect B transfer through the lipid phase of the membrane.[7] Firstly, we hypothesized that aquaglyceroporins may be involved in the transport of B and examined influx, efflux and concentration-dependence of B uptake in barley roots using inhibitors known to cause the closure of aquaporins though cytoplasmic acidification (butyric acid) or metabolic inhibition (sodium azide). Results from these experiments demonstrated that a significant component of both B influx and efflux was responsive to these treatments. Metabolic inhibition by sodium azide reduced influx and efflux by 40–50%, while cytoplasmic acidification with butyric acid reduced influx to a lesser but still significant degree.[7]

Secondly, in order to elucidate which transport proteins may be involved, we hypothesised more specifically that the PIP1 subgroup may be able to facilitate the movement of $B(OH)_3$ based on the location of such proteins on the plasma membrane. This had previously been suggested by Dordas et al.[14] who showed that a maize aquaporin ZmPIP1 when expressed in *Zenopus oocytes* could account for at least 25% of B uptake. We selected two aquaglyceroporins isoforms previously characterised from barley roots,[15-17] HvPIP1;3 and HvPIP1;4, and functionally expressed these in a *Saccharomyces cerevisiae* mutant containing a deletion of the yeast native aquaglyceroporin, FPS1. Expression of these PIP1 constructs caused the yeast to become sensitive to B toxicity. Influx measurement revealed that both HvPIP1;3 and HvPIP1;4 were capable of transporting B as indicated by increases of up to 40% in the rate of B uptake. Activation in yeast of some plant Nod 26-like intrinsic proteins (NIPs) that also function as aquaglyceroporins, requires truncation of the N-terminal sequence, presumably because this region contains a control domain. In our yeast experiments, a truncated version of HvPIP1;3 (HvPIP1;3t) was engineered to determine the effect of the removal of the first 44 amino acids from the N-terminal tail on the expression and subsequent B transport capacity. Surprisingly truncation of *HvPIP1;3* had little effect on either the expression or transport capacity of HvPIP1;3.

As a result of this study it has been firmly established that boron entry into plants can be partially controlled by opening and closing of channel-like transport proteins. Specifically, we have demonstrated that B can be transported via two aquaglyceroporins, HvPIP1;3 and HvPIP1;4. However, we suspect that most of the HvPIP1 subgroup, which contains another 3 members, may all have some capacity to transport B based on high sequence homology amongst the PIP1 subgroup.

The confirmation of the ability of PIP1s to transport B contributes greatly to the overall understanding of B transport in the plant system. Recently other aquaglyceroporins NIP5;1 and NIP6;1 have also been shown to be involved in B influx[18-20] while a separate class of non-aquaglyceroporins, that are structurally related to anion exchangers, are involved in the active efflux of B under toxicity conditions[21,22] or xylem loading under deficiency conditions.[23,24]

Aquaglyceroporins may have evolved to facilitate transport of beneficial and essential nutrients such as $Si(OH)_4$,[2] $B(OH)_3$, urea and ammonia[25] but other toxic molecules with similar physiochemical characteristics such as AsIII and $Sb(OH)_3$ may have 'piggy backed' on the process allowing these toxins to also enter the plant system. An understanding of selectivity mechanism that allows both essential and toxic elements to pass through the aquaglyceroporin pore and into the cytoplasm may have important implications for research into the potential bioremediation of toxic substances. It seems highly probable that other small molecules will be shown to be transported by aquaglyceroporins. There is still much to be learnt about the roles of other classes of MIPs, in particular NIPs, small basic intrinsic proteins (SIPs)[26] and tonoplast intrinsic proteins (TIPs) in the movement of these molecules into and within cells. No doubt the roles and functions of aquaglyceroporins within the plant system will continue to grow.

References

1. Benga G. Water channel proteins (later called aquaporins) and relatives: past, present and future. IUBMB Life 2009; 61:112-33.
2. Chiba Y, Mitani N, Yamaji N, Ma JF. HvLsi1 is a silicon influx transporter in barley. Plant J 2009; 57:810-8.
3. Isayenkov S, Maathuis F. The *Arabidopsis thaliana* aquaglyceroporin AtNIP7;1 is a pathway for arsenite uptake. FEBS Lett 2008; 582:1625-8.
4. Kamiya T, Tanaka M, Mitani N, Ma JF, Maeshima M, Fujiwara T. NIP1;1, an Aquaporin Homolog, Determines the Arsenite Sensitivity of *Arabidopsis thaliana*. J Biol Chem 2009; 284:2114-20.
5. Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M, et al. A silicon transporter in rice. Nature 2006; 440:688-91.
6. Mathai JC, Missner A, Kugler P, Saparov SM, Zeidel ML, Lee JK, et al. No facilitator required for membrane transport of hydrogen sulfide. Proc Natl Acad Sci USA 2009; 106:16633-8.
7. Fitzpatrick KL, Reid RJ. The involvement of aquaglyceroporins in transport of boron in barley roots. Plant Cell and Environ 2009; 32:1357-65.
8. Dordas C, Brown PH. Permeability of boric acid across lipid bilayers and factors affecting it. J Membrane Biol 2000; 175:95-105.
9. Dordas C, Brown PH. Evidence for channel mediated transport of boric acid in squash (*Cucurbita pepo*). Plant Soil 2001; 235:95-103.
10. Hayes JE, Reid RJ. Boron tolerance in barley is mediated by efflux of boron from the roots. Plant Physiol 2004; 136:3376-82.
11. Raven JA. Short and long distance transport of boric acid in plants. New Phytol 1980; 84:231-49.
12. Stangoulis JCR, Reid RJ, Brown PH, Graham RD. Kinetic analysis of boron transport in Chara. Planta 2001; 213:142-6.
13. Dannel F, Pfeffer H, Romheld V. Characterization of root boron pools, boron uptake and boron translocation in sunflower using the stable isotopes ^{10}B and ^{11}B. Aust J Plant Physiol 2000; 27:397-405.
14. Dordas C, Chrispeels MJ, Brown PH. Permeability and channel-mediated transport of boric acid across membrane vesicles isolated from squash roots. Plant Physiol 2000; 124:1349-61.
15. Katsuhara M. Molecular mechanisms of water uptake and transport in plant roots: research progress with water channel aquaporins. Plant Root 2007; 1:22-6.
16. Katsuhara M, Akiyama Y, Koshio K, Shibasaka M, Kasamo K. Functional analysis of water channels in barley roots. Plant Cell Physiol 2002; 43:885-93.
17. Katsuhara M, Hanba YT. Barley plasma membrane intrinsic proteins (PIP aquaporins) as water and CO_2 transporters. Pflüg Arch Eur J Phy 2008; 456:687-91.
18. Kato Y, Miwa K, Takano J, Wada M, Fujiwara T. Highly Boron Deficiency-Tolerant Plants Generated by Enhanced Expression of NIP5;1, a Boric Acid Channel. Plant Cell Physiol 2009; 50:58-66.
19. Takano J, Wada M, Ludewig U, Schaaf G, von Wiren N, Fujiwara T. The Arabidopsis major intrinsic protein NIP1;5 is essential for efficient boron uptake and plant development under boron limitation. Plant Cell 2006; 18:1498-509.
20. Tanaka M, Wallace IS, Takano J, Roberts DM, Fujiwara T. NIP6;1 Is a boric acid channel for preferential transport of boron to growing shoot tissues in Arabidopsis. Plant Cell 2008; 20:2860-75.
21. Reid R. Identification of boron transporter genes likely to be responsible for tolerance to boron toxicity in wheat and barley. Plant Cell Physiol 2007; 48:1673-8.
22. Sutton T, Baumann U, Hayes JE, Collins NC, Shi B-J, Schnurbusch T, et al. Boron-toxicity tolerance in barley arising from efflux transporter amplification. Science 2007; 318:1446-9.
23. Miwa K, Takano J, Fijiwara T, eds. Roles of *BOR1* paralogs in boron transport *Arabidopsis thaliana* Beijing, China: Tsinghua University Press 2005.
24. Takano J, Noguchi K, Yasumori M, Kobayashi M, Gajdos Z, Miwa K, et al. Arabidopsis boron transporter for xylem loading. Nature 2002; 420:337-40.
25. Dynowski M, Mayer M, Moran O, Ludewig U. Molecular determinants of ammonia and urea conductance in plant aquaporin homologs. FEBS Lett 2008; 582:2458-62.
26. Maurel C, Verdoucq L, Luu DT, Santoni V. Plant aquaporins: membrane channels with multiple integrated function. Annu Rev Plant Biol 2008; 59:595-624.