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The ins and outs of intracellular ion homeostasis: NHX-type cation/H^+ transporters

Elias Bassil and Eduardo Blumwald

The biochemical characterization of cation/H^+ exchange has been known since 1985 [1], yet only recently have we begun to understand the contribution of individual exchangers to ion homeostasis in plants. One particularly important class of exchangers is the NHX-type that is associated with Na^+ transport and therefore salinity tolerance. New evidence suggests that under normal growth conditions NHXs are critical regulators of K^+ and pH homeostasis and have important roles, depending on their cellular localization, in the generation of turgor as well as in vesicular trafficking. Recent advances highlight novel and exciting functions of intracellular NHXs in growth and development, stress adaptation and osmotic adjustment. Here, we elaborate on new and emerging cellular and physiological functions of this group of H^+-coupled cation exchangers.

Addresses
Department of Plant Sciences, University of California, Davis, CA 95616, United States

Corresponding author: Blumwald, Eduardo (eblumwald@ucdavis.edu)

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Introduction

Developmental cues and the need to respond to changes in their environment, require plants to constantly adjust their cellular pH and ion contents. Ion transport plays a defining role in the provision of energy, uptake and sequestration of ions and organic metabolites, as well as cell expansion. Cell expansion, and therefore growth, depends on turgor pressure that is generated by the coordinated regulation of vacuolar ion and water uptake, and the augmentation of membrane area and cell wall components that are provided by trafficking vesicles. In plants, H^+ is the motive ion and H^+ electrochemical gradients are generated by the H^+ translocating enzymes, the H^+-ATPase at the plasma membrane or the V-ATPase and PPase in intracellular compartments, to energize the secondary active transport of ions and metabolites. Cation/H^+ exchangers use the H^+ gradient to couple the passive transport of H^+ to the movement of cations against their gradient [2]. The coupled exchange of K^+ or Na^+ for H^+ occurs in all organisms and cellular compartments [3,4,5•] and is mediated in part by a family of transporters known as Na^+/H^+ antiporters (NHXs) in plants or Na^+/H^+ exchangers (NHEs) in animals. Much work has traditionally focused on use of NHXs in salt tolerance but more recent evidence suggests basic cellular roles that go beyond Na^+ transport into vacuoles.

Diversity of plant NHX-type Cation/H^+ antiporters

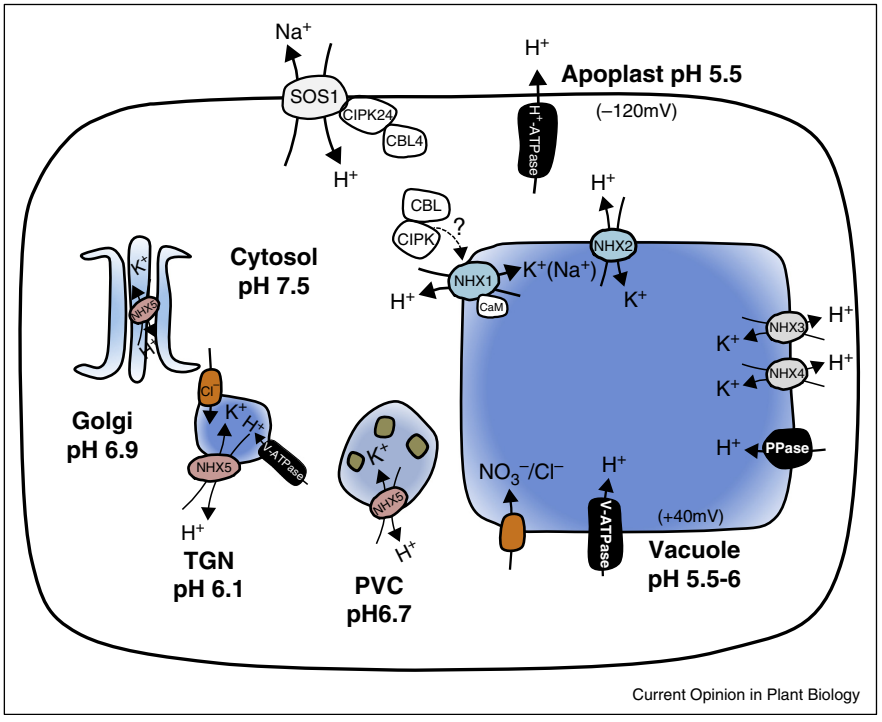
Plant NHXs belong to the large CPA family of monovalent cation/H^+ transporters (CPA1), together with closely related members that include the CHX and KEA (CPA2) exchangers [5•]. Phylogenetic and sequence analysis of available plant genomes (i.e. phytozome.net) indicate that NHXs are ubiquitous to all eukaryotic organisms. Arabidopsis contains eight isoforms belonging to three classes; two divergent members located at the plasma membrane (SOS1/AtNHX7 and AtNHX8); and six intracellular isoforms that are either vacuolar (AtNHX1 to AtNHX4) or in vesicles (AtNHX5, AtNHX6) [6] (Fig. 1). Interestingly, highly similar orthologues to members of each Arabidopsis class are found in genomes ranging from Chlamydomonas to tomato (Table 1). The fact that these NHX classes are represented even in algae, suggests that vacuolar, vesicular and plasma membrane NHXs have unique cellular functions that have been conserved early in evolution [5•,6].

Biochemical functions and regulation

A generally accepted mode of NHX operation, known as alternating access [7], results in the transport of either K^+ or Na^+ into the vacuole or endosome in exchange for H^+ efflux to the cytosol (NHX1–6) and Na^+ efflux out of the cell in exchange for H^+ influx into the cell (plasma membrane-bound NHX7–8) [6]. No available crystallographic structures of plant NHXs are available but biochemical and kinetic studies suggested that NHXs likely contain 9–12 transmembrane (TM) domains [8].

Epitope tagging of heterologously expressed NHX1 revealed that this antiporter has 9 TM domains with an additional 3 'buried' domains that do not entirely span the membrane [8]. The membrane-spanning pores and putative cation binding domains are highly conserved among plants NHXs, yeast Nhx1 and animal NHEs [3,9,10]. NHXs differ most at their C terminus [3,5•].

Figure 1



Schematic diagram of a plant cell indicating the distribution of Arabidopsis NHX antiporters in subcellular compartments. Luminal pH of intracellular compartments is noted below each compartment according to Ref. [49••]. *Trans*-Golgi network (TGN), prevacuolar Compartment (PVC). Note that only NHX5 is shown in the TGN and PVC but that NHX6 is also colocalized in these compartments (Blumwald *et al.*, unpublished results).

Protein-protein interactions, phosphorylation and/or glycosylation [6] are proposed to be a means by which antiporter activity or localization could be differentially regulated. A unique feature of NHX1, that differed from its mammalian NHE orthologues [4], was the localization of the C-terminus in the vacuolar lumen [8,11]. The Arabidopsis NHX1 C-terminus interacted with a calmodulin like protein15 (AtCaM15) within the vacuolar

Table 1

Number and type of NHX genes belonging to each functional class in different plant species. The Phytozome database (http://www.phytozome.net) was blasted with the following *Arabidopsis* sequences; NHX7/SOS1, NHX1, or NHX5 and orthologous sequences in the list of plant species shown were identified. The three *Arabidopsis* genes are members of the plasma membrane, vacuolar (class I) or endosomal/vesicle (Class II) type of NHX genes. Species were selected to represent evolutionarily diverse plants

		NHX Class		
Classification	Species	Vacuolar (Class I)	Endosomal (Class II)	Plasma Membrane
Dicot	*Arabidopsis thaliana*	4	2	2
	Solanum lycopersicum	3	1	1
	Medicago truncatula	7	2	1
	Phaseolus vulgaris	7	2	1
	Glycine max	7	3	1
	Populus trichocarpa	5	1	2
	Manniot esculenta	7	2	1
Monocot	*Sorghum bicolor*	6	2	1
	Zea mays	6	2	1
	Oryza sativa	4	2	1
	Brachypodium distachyon	4	2	2
Lycophyte	*Selaginella moellendorffii*	3	2	2
Bryophyte	*Physcomitrella patens*	5	2	2
Chlorophyte	*Chlamydomonas reinhardtii*	1	3	2

lumen in a Ca^{2+}-dependent and pH-dependent manner. Under normal physiological conditions, where the vacuole is acidic (pH 5.5) and Ca^{2+} activity high, AtCaM15 is bound to the AtNHX1 and results in a higher K^+/H^+ than Na^+/H^+ activity. At higher pH (6.0–7.5), AtCaM15 binding to AtNHX1 was reduced and the Na^+/H^+ activity increased relative to the K^+/H^+ activity. Because salinity causes the alkalinization of the vacuole [12,13], the pH dependent change in K^+ for Na^+ selectivity of NHX1 might constitute a mechanism for Na^+ accumulation (at the expense of K^+) into the vacuole. Regulatory effects of phosphorylation are well documented in NHEs [14] and SOS1/NHX7 [15,16] but no direct evidence for any intracellular NHX is available. Under salt stress, the Ca^{2+} sensor protein SOS3/CBL4, activates the protein kinase SOS2/CIPK24 which in turn phosphorylates and activates SOS1/NHX7 to reduce cytoplasmic Na^+ [15–19]. Interestingly the activity of vacuolar NHX activity was reduced in *sos2* and restored with constitutively active SOS2 but no phosphorylation of NHX was found [17]. The possibility that the CBL/CIPK system might regulate intracellular NHX activity should be considered.

Cation homeostasis and salt tolerance

Plant NHXs mediate both Na^+/H^+ and K^+/H^+ exchange [20,22,23] and therefore affect both salinity tolerance and K^+ nutrition. The initial cloning and overexpression of AtNHX1 in Arabidopsis firmly demonstrated the importance of intracellular Na^+ compartmentation for salt tolerance [24]. Many additional studies subsequently confirmed that NHX overexpression lead to improved salt tolerance in diverse species [21,24–29] supporting the idea that maintaining a low Na^+/K^+ cytosolic ratio by removing excess cytosolic Na^+ into the vacuole, in addition to the extrusion of Na^+ into the apoplast by SOS1/NHX7, is critical during salt stress. Enhanced expression of vacuolar NHXs in a salt tolerant tomato variety under salt further confirmed a role of vacuolar NHX in salt tolerance [30]. Salt tolerance of NHX overexpressing transgenics does not seem to depend on the source species or NHX isoform used, but probably affected by the regulation of NHX expression, changes in K^+ homeostasis brought about by high intracellular Na^+, and possible regulation of NHX cation selectivity. For example endosomal/vesicular NHXs may preferentially transport K^+ compared to Na^+ [31]. The precise mechanisms and interactions by which K^+ and Na^+ are regulated remain unclear because NHX overexpression has resulted in contrasting ion accumulation between transgenics and wild type plants and may reflect a primary function of vacuolar NHXs in maintaining osmotic adjustment during both normal growth as well as under salinity. Unexpectedly, the addition of moderate salt (30 mM) to the knockout *nhx1nhx2* lacking the two main Arabidopsis vacuolar antiporters, resulted in improved growth, rather than adversely affecting it, as compared to controls [32••]. Knocking out or silencing endosomal/vesicle NHX isoforms resulted in salt-sensitive plants [26,33••]. Knockouts lacking vacuolar V-ATPase activity had reduced capacity to store NO_3^- or toxic concentrations of Zn^{2+} but did not exhibit sensitivity to high salt [34••], while salt sensitivity was observed instead in knockdowns of the endosomal/*trans*-Golgi network localized V-ATPase. These results point to the importance of the endosomal/vesicle system in ameliorating salt stress as supported by other studies [13,35–37].

Despite the role of NHXs in salt tolerance, NHX cannot simply catalyze Na^+/H^+ exchange in non-salinized plants. In grape NHX1 expression was significantly upregulated at véraison and during cell expansion where berry vacuolar K^+ accumulation and a drop in acidity occur [38]. Genetic studies in *Arabidopsis* firmly demonstrated the importance of NHXs in the regulation of pH and K^+ homeostasis during normal growth and development [10,23,26,32••,39•]. The Arabidopsis knockout *nhx1* had lower antiport activity, smaller cells [20] and displayed an upregulation of high affinity K^+ uptake transporters [40]. Knockout of the closely related isoform *nhx2*, did not display obvious phenotypes [32••] but the double knockout *nhx1nhx2* displayed a dramatic reduction in cell expansion and growth, especially in rapidly elongating tissues as compared to *nhx1*. Interestingly these plants also had reduced seed set that was attributed to unsuccessful pollination due to a lack of anther dehiscence and filament elongation and in which K^+ dependent hydration/dehydration processes have been implicated [41]. Vacuolar K^+ in *nhx1nhx2* plants was one third that of wild type root cells [32••] as well as leaf cells [39•]. K^+/H^+ exchange of tonoplast vesicles was markedly reduced in the same knockout which also displayed impaired osmoregulation, turgor and delayed stomatal closure, resulting in poor maintenance of water status [39•]. Opening of stomata require the accumulation of guard cell vacuolar K^+, a process that relies on NHX1 and NHX2 [42••].

Given the importance of K^+ as an enzyme cofactor, in charge balance and an osmoticum, cytosolic K^+ concentrations must be tightly maintained [43]. At the typical electrochemical potentials of the plasma membrane and tonoplast, transport of K^+ into the cytosol is passive but would require energy to accumulate above ~20 mM in the vacuole [44–46]. To maintain constant cytosolic K^+, both uptake of K^+ from the apoplast and exchange with the vacuole are essential [44]. The sensitivity of *nhx1nhx2* plants to added K^+, their reduced vacuolar K^+ content [32••] and accumulation of cytosolic K^+ [39•] highlights the importance of vacuolar NHX in intracellular K^+ homeostasis. The technical limitation to measurement of K^+ in vesicles, due to the lack of targeted K^+ specific probes, limit our understanding of the role of endosomal/vesicular NHX

in Na^+/K^+ homeostasis and their possible functions in trafficking and salt responses.

pH homeostasis

Luminal pH is not uniform throughout the cell, but rather maintained within specific values depending on the intracellular compartment and becomes progressively more acidic with maturity along the secretory pathway [47]. The specific luminal pH of plant cellular compartments was only recently measured using targeted genetically encoded pH sensors [48•,49••]. *In vivo* pH measurements revealed that a gradual acidification of pH, ranging from pH 7.1 in the ER to ~5.5 in the vacuole, except that the trans-Golgi network (TGN) was more acidic than prevacuolar compartments (PVC) [49••]. Vesicles that colocalized with NHX5 were significantly more alkaline than those colocalizing with the endosomal V-ATPase, while the application of V-ATPase or NHX inhibitors, caused either a respective alkalinization or acidification of vesicles. Such data support the idea that vesicle pH homeostasis requires H^+-pumps to establish the initial acidity, and alkalinizing mechanisms (NHX), in order to 'fine-tune' the luminal pH, as has been suggested in animals cells [4].

The localization of NHX5 and NHX6 to the Golgi, TGN [33••], and PVC (Blumwald *et al.*, unpublished results), the tomato orthologue NHX2 to vesicles [26], as well as the phenotypes of *nhx5nhx6*, suggest that endosomal/vesicular NHXs like AtNHX5 control vesicle pH and trafficking. Vacuolar NHXs have been associated with pH homeostasis. For example, morning glory petal requires NHX activity for coloration [50]. In Arabidopsis *nhx1nhx2* roots, vacuolar pH was significantly more acidic especially in cortical cells of the elongation and maturation zones [32••]. Nevertheless, the precise role of vacuolar NHXs in pH regulation is difficult to discern from their roles in vacuolar K^+ accumulation especially since many of the knockout phenotypes can be attributed to altered K^+ homoeostasis.

Essential for vesicular trafficking

Firm demonstration of pH regulation and protein trafficking by NHX-like antiporters was provided in yeast *Nhx1Δ* where cytosolic and vacuolar pH were altered and protein trafficking out of the Golgi was blocked [51,52]. In Arabidopsis, a role of vesicular/endosomal NHXs in endomembrane trafficking was initially provided by the *nhx5nhx6* double knockout [33••]. These plants missorted vacuolar destined cargo to the apoplast and displayed a notable delay in labeling of the vacuole with the tracer, FM4-64 [6]. A functional link between the TGN localized v-ATPase complex and NHX5 and NHX6 was proposed based on high colocalization between the TGN localized V-ATPase and NHX5 and NHX6 [33••]. More recent data indicated that the Golgi and *trans*-Golgi network in *nhx5nhx6* were significantly more acidic than wild type (Blumwald, unpublished results). These data suggest that endosomal/vesicular NHXs have important roles in vesicle pH homeostasis that is essential to trafficking.

Conclusion

Recent advances highlight novel NHX cellular and physiological roles that go beyond their importance in salt tolerance. Genetic studies provided compelling evidence to support earlier biochemical data and indicate that NHXs regulate a multitude of cellular and physiological processes including cell expansion, cation homeostasis, turgor and osmotic adjustment, pH regulation, vesicle trafficking, stomatal function and plant water status as well as flowering. The development of multiple NHX knockout lines, together with the generation of genetically-encoded ion sensors will facilitate further understanding of how ion and pH homeostasis regulate mechanisms that control vesicular trafficking and protein processing.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Blumwald E, Poole RJ: **Na^+/H^+ antiport in isolated tonoplast vesicles from storage tissue of *Beta vulgaris***. *Plant Physiol* 1985, **78**:163-167.

2. Blumwald E: **Tonoplast vescicles as a tool in the study of ion-transport at the plant vacuole**. *Physiol Plant* 1987, **69**:731-734.

3. Brett CL, Donowitz M, Rao R: **Evolutionary origins of eukaryotic sodium/proton exchangers**. *Am J Physiol Cell Physiol* 2005, **288**:C223-C239.

4. Orlowski J, Grinstein S: **Na^+/H^+ exchangers**. *Compr Physiol* 2011, **1**:2083-2100.

5. • Chanroj S, Wang G, Venema K, Zhang MW, Delwiche CF, Sze H: **Conserved and diversified gene families of monovalent cation/H^+ antiporters from algae to flowering plants**. *Front Plant Sci* 2012, **3**:1-18.
In this review, a phylogenetic analysis of many plant genomes indicated that the three distinct clades of the Na^+/H^+ exchangers, plasmamembrane, vacuolar and endosomal, have been conserved from single-celled algae to higher plants.

6. Bassil E, Coku A, Blumwald E: **Cellular ion homeostasis: emerging roles of intracellular NHX Na^+/H^+ antiporters in plant growth and development**. *J Exp Bot* 2012, **63**:5727-5740.

7. Post MA, Dawson DC: **Basolateral Na^+–H^+ antiporter: mechanisms of electroneutral and conductive ion transport**. *J Gen Physiol* 1994, **103**:895-916.

8. Yamaguchi T, Apse MP, Shi HZ, Blumwald E: **Topological analysis of a plant vacuolar Na^+/H^+ antiporter reveals a luminal C terminus that regulates antiporter cation selectivity**. *Proc Natl Acad Sci U S A* 2003, **100**:12510-12515.

9. Yokoi S, Quintero FJ, Cubero B, Ruiz MT, Bressan RA, Hasegawa PM, Pardo JM: **Differential expression and function**

of *Arabidopsis thaliana* NHX Na^+/H^+ antiporters in the salt stress response. *Plant J* 2002, **30**:529-539.

10. Aharon GS, Apse MP, Duan S, Hua X, Blumwald E: **Characterization of a family of vacuolar Na^+/H^+ antiporters in *Arabidopsis thaliana***. *Plant Soil* 2003, **253**:245-256.

11. Yamaguchi T, Aharon GS, Sottosanto JB, Blumwald E: **Vacuolar Na^+/H^+ antiporter cation selectivity is regulated by calmodulin from within the vacuole in a Ca^{2+}- and pH-dependent manner**. *Proc Natl Acad Sci U S A* 2005, **102**:16107-16112.

12. Okazaki Y, Kikuyama M, Hiramoto Y, Iwasaki N: **Short-term regulation of cytosolic Ca^{2+}, cytosolic pH and vacuolar pH under NaCl stress in the charophyte alga *Nitellopsis obtusa***. *Plant Cell Environ* 1996, **19**:569-576.

13. Leshem Y, Melamed-Book N, Cagnac O, Ronen G, Nishri Y, Solomon M, Cohen G, Levine A: **Suppression of Arabidopsis vesicle-SNARE expression inhibited fusion of H2O2 containing vesicles with tonoplast and increased salt tolerance**. *Proc Natl Acad Sci U S A* 2006, **103**:18008-18013.

14. Khaled AR, Moor AN, Li A, Kim K, Ferris DK, Muegge K, Fisher RJ, Fliegel L, Durum SK: **Trophic factor withdrawal: p38 mitogen-activated protein kinase activates NHE1, which induces intracellular alkalinization**. *Mol Cell Biol* 2001, **21**:7545-7557.

15. Quintero FJ, Ohta M, Shi H, Zhu JK, Pardo JM: **Reconstitution in yeast of the Arabidopsis SOS signaling pathway for Na^+ homeostasis**. *Proc Natl Acad Sci U S A* 2002, **99**:9061-9066.

16. Halfter U, Ishitani M, Zhu J-K: **The Arabidopsis SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3**. *Proc Natl Acad Sci* 2000, **97**:3735-3740.

17. Qiu QS, Guo Y, Quintero FJ, Pardo JM, Schumaker KS, Zhu JK: **Regulation of vacuolar Na^+/H^+ exchange in *Arabidopsis thaliana* by the salt-overly-sensitive (SOS) pathway**. *J Biol Chem* 2004, **279**:207-215.

18. Liu J, Ishitani M, Halfter U, Kim C-S, Zhu J-K: **The *Arabidopsis thaliana* SOS2 gene encodes a protein kinase that is required for salt tolerance**. *Proc Natl Acad Sci* 2000, **97**:3730-3734.

19. Liu J, Zhu J-K: **A calcium sensor homolog required for plant salt tolerance**. *Science* 1998, **280**:1943-1945.

20. Apse MP, Sottosanto JB, Blumwald E: **Vacuolar cation/H^+ exchange, ion homeostasis, and leaf development are altered in a T-DNA insertional mutant of AtNHX1, the Arabidopsis vacuolar Na^+/H^+ antiporter**. *Plant J* 2003, **36**:229-239.

21. Zhang HX, Blumwald E: **Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit**. *Nat Biotechnol* 2001, **19**:765-768.

22. Venema K, Quintero FJ, Pardo JM, Donaire JP: **The Arabidopsis Na^+/H^+ exchanger AtNHX1 catalyzes low affinity Na^+ and K^+ transport in reconstituted liposomes**. *J Biol Chem* 2002, **277**:2413-2418.

23. Leidi EO, Barragan V, Rubio L, El-Hamdaoui A, Ruiz MT, Cubero B, Fernandez JA, Bressan RA, Hasegawa PM, Quintero FJ *et al.*: **The AtNHX1 exchanger mediates potassium compartmentation in vacuoles of transgenic tomato**. *Plant J* 2010, **61**:495-506.

24. Apse MP, Aharon GS, Snedden WA, Blumwald E: **Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in Arabidopsis**. *Science* 1999, **285**:1256-1258.

25. Zhang HX, Hodson JN, Williams JP, Blumwald E: **Engineering salt-tolerant Brassica plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation**. *Proc Natl Acad Sci U S A* 2001, **98**:12832-12836.

26. Rodriguez-Rosales MP, Jiang XY, Galvez FJ, Aranda MN, Cubero B, Venema K: **Overexpression of the tomato K^+/H^+ antiporter LeNHX2 confers salt tolerance by improving potassium compartmentalization**. *New Phytol* 2008, **179**:366-377.

27. Liu H, Wang Q, Yu M, Zhang Y, Wu Y, Zhang H: **Transgenic salt-tolerant sugar beet *Beta vulgaris* constitutively expressing an *Arabidopsis thaliana* vacuolar Na^+/H^+ antiporter gene, AtNHX3, accumulates more soluble sugar but less salt in storage roots**. *Plant Cell Environ* 2008, **31**:1325-1334.

28. Ohta M, Hayashi Y, Nakashima A, Hamada A, Tanaka A, Nakamura T, Hayakawa T: **Introduction of a Na^+/H^+ antiporter gene from *Atriplex gmelini* confers salt tolerance to rice**. *FEBS Lett* 2002, **532**:279-282.

29. Brini F, Hanin M, Mezghani I, Berkowitz GA, Masmoudi K: **Overexpression of wheat Na^+/H^+ antiporter TNHX1 and H^+-pyrophosphatase TVP1 improve salt- and drought-stress tolerance in *Arabidopsis thaliana* plants**. *J Exp Bot* 2007, **58**:301-308.

30. Galvez FJ, Baghour M, Hao GP, Cagnac O, Rodriguez-Rosales MP, Venema K: **Expression of LeNHX isoforms in response to salt stress in salt sensitive and salt tolerant tomato species**. *Plant Physiol Biochem* 2012, **51**:109-115.

31. Venema K, Belver A, Marin-Manzano MC, Rodriguez-Rosales MP, Donaire JP: **A novel intracellular K^+/H^+ antiporter related to Na^+/H^+ antiporters is important for K^+ ion homeostasis in plants**. *J Biol Chem* 2003, **278**:22453-22459.

32. •• Bassil E, Tajima H, Liang Y-C, Ohto M-A, Ushijima K, Nakano R, Esumi T, Coku A, Belmonte M, Blumwald E: **The Arabidopsis Na^+/H^+ antiporters NHX1 and NHX2 control vacuolar pH and K^+ homeostasis to regulate growth, flower development, and reproduction**. *Plant Cell* 2011, **23**:3482-3497.

In this study, the Arabidopsis double knockout mutant nhx1nhx2 was shown to have reduced growth and severe flower phenotypes. Knockouts had a lower concentration of vacuolar K^+ and more acidic vacuoles, compared to wild type. Knockouts also exhibited high sensitivity to external K^+ concentrations but not to high Na^+, suggesting that the role of these transporters is to regulate vacuolar pH and the uptake of vacuolar K^+.

33. •• Bassil E, Ohto MA, Esumi T, Tajima H, Zhu Z, Cagnac O, Belmonte M, Peleg Z, Yamaguchi T, Blumwald E: **The Arabidopsis intracellular Na^+/H^+ antiporters NHX5 and NHX6 are endosome associated and necessary for plant growth and development**. *Plant Cell* 2011, **23**:224-239.

This work demonstrates the importance of the two Arabidopsis endosomal NHXs, NHX5 and NHX6 to cell expansion and growth as well as salt stress. NHX5 and NHX6 were located to the Golgi and trans-Golgi network. In the double knockout inhx5nhx6 trafficking to the vacuole is compromised. Results suggested that NHX5 and NHX6 activity might regulate vesicular pH, K^+ and Na^+ homeostasis. The paper highlights the importance of vesicle ion homeostasis and trafficking in higher plants.

34. •• Krebs M, Beyhl D, Gorlich E, Al-Rasheid KAS, Marten I, Stierhof YD, Hedrich R, Schumacher K: **Arabidopsis V-ATPase activity at the tonoplast is required for efficient nutrient storage but not for sodium accumulation**. *Proc Natl Acad Sci U S A* 2010, **107**:3251-3256.

The study shows that a mutant lacking tonoplast V-ATPase activity is less critical for growth and salt stress responses than is the V-ATPase in the trans-Golgi network/early endosome and provides interesting insights into the importance of the V-ATPase in vesicle function including salt tolerance.

35. Mazel A, Leshem Y, Tiwari BS, Levine A: **Induction of salt and osmotic stress tolerance by overexpression of an intracellular vesicle trafficking protein AtRab7 (AtRabG3e)**. *Plant Physiol* 2004, **134**:118-128.

36. Hamaji K, Nagira M, Yoshida K, Ohnishi M, Oda Y, Uemura T, Goh T, Sato MH, Morita MT, Tasaka M *et al.*: **Dynamic aspects of ion accumulation by vesicle traffic under salt stress in Arabidopsis**. *Plant Cell Physiol* 2009, **50**:2023-2033.

37. Hernandez A, Jiang XY, Cubero B, Nieto PM, Bressan RA, Hasegawa PM, Pardo JM: **Mutants of the *Arabidopsis thaliana* cation/H^+ antiporter AtNHX1 conferring increased salt tolerance in yeast. The endosome/prevacuolar compartment is a target for salt toxicity**. *J Biol Chem* 2009, **284**:14276-14285.

38. Hanana M, Cagnac O, Yamaguchi T, Hamdi S, Ghorbel A, Blumwald E: **A grape berry (*Vitis vinifera* L.) cation/proton antiporter is associated with berry ripening**. *Plant Cell Physiol* 2007, **48**:804-811.

39. • Barragán V, Leidi EO, Andrés Z, Rubio L, De Luca A, Fernández JA, Cubero B, Pardo JM: **Ion exchangers NHX1 and NHX2 mediate active potassium uptake into vacuoles to regulate cell turgor**

and stomatal function in Arabidopsis. *Plant Cell* 2012, **24**:1127-1142.
Similar to results in Ref. [32••], this paper shows that nhx1nhx2 knockouts have aberrantly high cytosolic K^+ impaired osmoregulation and defective stomatal functions. The study also reports similar exchange activites of between NHX1 and NHX2. Results further support that NHX1 and NHX2 are important for vacuolar accumulation of K^+.

40. Sottosanto JB, Gelli A, Blumwald E: **DNA array analyses of *Arabidopsis thaliana* lacking a vacuolar Na^+/H^+ antiporter: impact of AtNHX1 on gene expression**. *Plant J* 2004, **40**:752-771.

41. Heslop-Harrison Y, Heslop-Harrison JS: **Lodicule function and filament extension in the grasses: potassium ion movement and tissue specialization**. *Ann Bot* 1996, **77**:573-582.

42. •• Andrés Z, Pérez-Hormaeche J, Leidi EO, Schlücking K, Steinhorst L, McLachlan DH, Schumacher K, Hetherington AM, Kudla J, Cubero B *et al.*: **Control of vacuolar dynamics and regulation of stomatal aperture by tonoplast potassium uptake**. *Proc Natl Acad Sci* 2014.
In this work the authors demonstrate convincingly the role of NHX1 and NHX2 in stomatal opening and closing by regulating guard cell vacuolar dynamics. Vacuolar K^+/H^+ exchange, mediated by NHX1 and NHX2 is necessary to accumulate K^+ and for normal stomatal function.

43. Leigh RA: **Potassium homeostasis and membrane transport**. *J Plant Nutr Soil Sci* 2001, **164**:193-198.

44. Walker DJ, Leigh RA, Miller AJ: **Potassium homeostasis in vacuolate plant cells**. *Proc Natl Acad Sci U S A* 1996, **93**:10510-10514.

45. Carden DE, Walker DJ, Flowers TJ, Miller AJ: **Single-cell measurements of the contributions of cytosolic Na^+ and K^+ to salt tolerance**. *Plant Physiol* 2003, **131**:676-683.

46. Walker DJ, Black CR, Miller AJ: **The role of cytosolic potassium and pH in the growth of barley roots**. *Plant Physiol* 1998, **118**:957-964.

47. Paroutis P, Touret N, Grinstein S: **The pH of the secretory pathway: measurement, determinants, and regulation**. *Physiology* 2004, **19**:207-215.

48. • Shen J, Zeng Y, Zhuang X, Sun L, Yao X, Pimpl P, Jiang L: **Organelle pH in the Arabidopsis endomembrane system**. *Mol Plant* 2013, **6**:1419-1437.
Using targeted pH sensitive GFP this work reports the luminal pH of the main organelles of plant cells. pH values indicate that the cytosol, nucleus, peroxisomes, the mitochondrial matrix and plastid stroma are alkaline while the pH of the secretory pathway are gradually more acidic.

49. •• Martinière A, Bassil E, Jublanc E, Alcon C, Reguera M, Sentenac H, Blumwald E, Paris N: **In vivo intracellular pH measurements in tobacco and Arabidopsis reveal an unexpected pH gradient in the endomembrane system**. *Plant Cell* 2013, **25**:4028-4043.
In this work, the use of genetically encoded pH sensors, targeted to specific cellular compartments, together with colocalization experiments were used to provide the measurements of luminal pH in all main cellular compartments. Interestingly, and different from animal cells, the pH of prevacuolar compartments was more alkaline than the trans-Golgi network. The work also suggests that endosomal pH requires both V-ATPase for acidifcation and NHX5 for alkalnization.

50. Yamaguchi T, Fukada-Tanaka S, Inagaki Y, Saito N, Yonekura-Sakakibara K, Tanaka Y, Kusumi T, Iida S: **Genes encoding the vacuolar Na^+/H^+ exchanger and flower coloration**. *Plant Cell Physiol* 2001, **42**:451-461.

51. Bowers K, Levi BP, Patel FI, Stevens TH: **The sodium/proton exchanger Nhx1p is required for endosomal protein trafficking in the yeast *Saccharomyces cerevisiae***. *Mol Biol Cell* 2000, **11**:4277-4294.

52. Brett CL, Tukaye DN, Mukherjee S, Rao RJ: **The yeast endosomal $Na^+(K^+)/H^+$ exchanger Nhx1 regulates cellular pH to control vesicle trafficking**. *Mol Biol Cell* 2005, **16**:1396-1405.