

# Intracellular NHX-Type Cation/H<sup>+</sup> Antiporters in Plants

Cells depend on the homeostatic maintenance of pH within specific cellular compartments to ensure optimal conditions for metabolic and enzymatic processes as well as protein structure and function. In the animal secretory pathway, cells maintain distinct luminal pHs within various compartments (Paroutis et al., 2004). Among the many molecular players that contribute to pH and ion homeostasis in plants, Na<sup>+</sup>(K<sup>+</sup>)/H<sup>+</sup> exchangers (also known as NHX-type cation/H<sup>+</sup> antiporters) appear to be particularly important for the regulation of a wide variety of physiological processes, including cell expansion, cell volume regulation, osmotic adjustment, pH regulation, membrane trafficking, protein processing, and cellular stress responses (Pardo et al., 2006; Rodriguez-Rosales et al., 2009; Bassil et al., 2012). In plants, NHX antiporters appeared early in evolution and are ubiquitously encoded members of the CPA1 cation/H<sup>+</sup> antiporters subgroup that belongs to the large family of monovalent cation/H<sup>+</sup> transporters CPA (Brett et al., 2005). NHX antiporters are found, thus far, in all sequenced plant genomes (Bassil et al., 2012; Chanroj et al., 2012). In *Arabidopsis*, the NHX family consists of eight isoforms, six of which are intracellular (AtNHX1–AtNHX6), located either to the vacuole (AtNHX1 to AtNHX4) or endosomes (AtNHX5 and AtNHX6) and an additional two more divergent members (AtNHX7/SOS1 and AtNHX8) at the plasma membrane (Bassil et al., 2012). Orthologous sequences in each of the three classes (plasma membrane, vacuolar, or endosomal) appear in all sequenced genomes, suggesting that distinct functional NHX classes appeared early in evolution and may have conserved roles that are compartment-specific (Bassil et al., 2012). Emerging new evidence highlights the importance of particular intracellular NHX antiporters in the regulation of vesicular and vacuolar pH and K<sup>+</sup> homeostasis. Vacuolar NHXs are needed to maintain K<sup>+</sup> homeostasis between the vacuole and cytosol, without which cell expansion is compromised (Bassil et al., 2011b). Other NHX isoforms (endosomal) are required for membrane trafficking and raise interesting new questions about the role of pH and ion homeostasis in protein processing and trafficking in the endomembrane system (Bassil et al., 2011a). In this update, we aim to highlight recent new evidence on intracellular NHX antiporters and emphasize possible novel and important cellular processes regulated by this particularly interesting group of transporters.

## Na<sup>+</sup>/H<sup>+</sup> ANTIPORTERS ARE REGULATORS OF pH AND ION HOMEOSTASIS

The importance of vacuolar H<sup>+</sup> pumps such as vacuolar (V-) ATPases and the vacuolar H(+)-translocating pyrophosphatase (V-PPase) in the generation of intracellular pH gradients and in energizing secondary active transport is now well established (Schumacher and Krebs, 2010). Nevertheless, the activity of V-ATPase alone is not enough to either set up the initial pH or to regulate it homeostatically, especially if indeed intracellular pH differs across the endomembrane system as is known to occur in animal cells (Paroutis et al., 2004; Casey et al., 2010; Ohgaki et al., 2011). For example, a significant electrical potential difference, positive inside, would limit further acidification unless an anionic conductance (mainly Cl<sup>-</sup>) ensues to dissipate this membrane potential. In addition, evidence from studies in yeast, plants, and animals strongly suggests that Na<sup>+</sup>/H<sup>+</sup>-type antiporters may act as proton leaks to fine-tune the luminal pH of specific intracellular compartments to a particular pH (Casey et al., 2010). Intracellular NHX-dependent pH regulation was initially shown in morning glory (*Ipomea*) flower petals (Yoshida et al., 2005) which suggested that a coordination between NHX and the vacuolar pumps might occur during flower petal development. A direct interaction between the V-ATPase and the NHX antiporters has not been demonstrated but the possibility of an indirect coordination through the sharing of regulatory partners has been postulated (Bassil et al., 2012; Pittman, 2012). Recently, direct measurements of vacuolar pH in NHX knockouts strongly suggested that vacuolar NHX antiporters are critical in vacuolar pH regulation (Bassil et al., 2011b; Barragán et al., 2012). In addition, Bassil et al. (2011a) demonstrated that AtNHX5 and VHAA1 co-localized to the *trans*-Golgi network, supporting the notion that H<sup>+</sup> pumps and cation/H<sup>+</sup> antiporters may act jointly to fine-tune endosomal pH during vesicular trafficking. The precise effect(s) of altered endosomal/vacuolar pH remains unclear given the concomitant alteration of K<sup>+</sup> changes that are also observed in these plants.

Considerable evidence points to a role of NHX-type antiporters in the regulation of cellular K<sup>+</sup> and Na<sup>+</sup> homeostasis.

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Although much work focused on the overexpression of different NHX isoforms for the purpose of conferring salt tolerance, as initially demonstrated by Apse et al. (1999), the endogenous function of NHX antiporters cannot be solely to accumulate Na<sup>+</sup> into vacuoles. This is firmly demonstrated by recent genetic studies in which mutants lacking particular vacuolar NHX antiporters display deficient cellular K<sup>+</sup> regulation. The *Arabidopsis* double knockout *nhx1/nhx2* exhibited smaller cells and reduced growth, especially of rapidly growing tissues such as filaments and hypocotyls, and significantly lower turgor and tissue water contents. In addition, *nhx1/nhx2* knockouts not only had 70% less vacuolar K<sup>+</sup>, but were also highly sensitive to the addition of external K<sup>+</sup>, suggesting that these vacuolar NHX antiporters are the main mediators of cytosolic K<sup>+</sup> uptake into the vacuole (Bassil et al., 2011b; Barragán et al., 2012). The sensitivity of *nhx1/nhx2* to external K<sup>+</sup> raises interesting questions about the 'compatibility' of cytosolic K<sup>+</sup>. These studies complemented previous work by Walker et al. (1998) that showed that variations of K<sup>+</sup> supply, which would otherwise result in a fluctuation of cytosolic K<sup>+</sup>, is essentially buffered by K<sup>+</sup>/H<sup>+</sup> exchange with the vacuole. The analysis of *nhx1/nhx2* double mutant plants provides a unique opportunity to further decipher the mechanisms that regulate cytosolic K<sup>+</sup> and the effects derived from changes in cytosolic K<sup>+</sup> on cell function. Interestingly, the growth of *nhx1/nhx2* knockouts was improved under moderate amounts of external Na<sup>+</sup> supply, suggesting that Na<sup>+</sup> uptake into the vacuole may occur via transporters other than NHX1 and NHX2.

## EMERGING FUNCTIONS OF Na<sup>+</sup>/H<sup>+</sup> ANTIPORTERS

Perhaps the most exciting new finding in NHX research has been the discovery of key roles played by the endosomal-localized isoforms NHX5 and NHX6 of *Arabidopsis* (Bassil et al., 2011a). Similarly to studies done in yeast and animals, this work highlighted novel roles for plant intracellular Na<sup>+</sup>/H<sup>+</sup> antiporters (NHXs), outside of the vacuole, and, collectively, the data suggested that endosomal NHXs control vesicular trafficking, probably via the regulation of endosomal ion and pH homeostasis. pH is not the only determinant for the maintenance of endosomal enzyme activity and protein stability, but also for vesicle identity, the regulation of receptor and cargo interactions, and ultimately endomembrane trafficking (Paroutis et al., 2004). Thus, luminal pH of intracellular compartments should be maintained within a narrow range to ensure the function of intracellular compartments and endomembrane trafficking. The first evidence of the requirement of NHX antiporters in vesicular trafficking was reported in yeast (Bowers et al., 2000). Using the knockout *Scnhx1Δ*, Bowers et al. (2000) demonstrated the requirement of the yeast *Nhx1*, that is orthologous to AtNHX5 and AtNHX6 and is localized to the prevacuolar compartment. Similarly, mounting evidence supported the role of mammalian endosomal Na<sup>+</sup>/H<sup>+</sup> exchangers

(NHE) in the control of endosomal pH and the regulation of vesicular trafficking (Casey et al., 2010; Ohgaki et al., 2011). In plants, the most direct evidence demonstrating the role of NHX-type Na<sup>+</sup>/H<sup>+</sup> antiporters in vesicular trafficking was generated using knockouts lacking both endosomal NHX5 and NHX6 antiporters (Bassil et al., 2011a). *nhx5/nhx6* double knockouts exhibited severely reduced cell expansion and growth, differential gene expression associated with vesicular trafficking, and defects in trafficking to the vacuole. Although the specific mechanisms remain unclear, the notion of the control of vesicular pH by AtNHX5 and AtNHX6 in regulating vesicular trafficking is firmly established. *In vivo* measurements of vesicular luminal pH of endomembrane compartments and their contribution to the regulation of protein maturation will aid in establishing the biochemical basis of these processes.

## CONCLUSIONS/FUTURE DIRECTIONS

Novel functions of the NHX-type Na<sup>+</sup>/H<sup>+</sup> antiporters are beginning to emerge in plants, including roles in cell expansion, flower development, stomatal conductance, salt stress response, and vesicular trafficking. The development of molecular tools enabling *in vivo* measurements of pH and ion concentrations in different intracellular compartments and the identification of the protein partners regulating the transporters' activities are essential requirements in order to better understand the mechanisms underlying NHX functions.

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