Sodium transport and salt tolerance in plants

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The ability of plant cells to maintain low cytosolic sodium concentrations is an essential process associated with the ability of plants to grow in high salt concentrations. Recent results have identified pathways for Na^+ entry, and the cloning of vacuolar Na^+/H^+ antiporters has demonstrated the role of intracellular Na^+ compartmentation in plant salt tolerance.

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Abbreviations
KORCs K^+ outward rectifying channels
VIC voltage-independent cation channels

Introduction

Animal cells have adapted to live with relatively high extracellular salt concentrations. The ubiquitous plasma membrane Na^+/K^+-ATPase mediates the efflux of $3Na^+$ and the influx of $2K^+$, which is coupled to the hydrolysis of ATP. This electrogenic Na^+/K^+ exchange establishes a Na^+ gradient across the plasma membrane that is used by the cell for the regulation of nutrient uptake, volume and pH. In contrast to animal cells, Na^+ is not essential for plants. These organisms lack a plasma membrane Na^+/K^+ ATPase. Instead, they possess H^+-ATPase, which generates the H^+ electrochemical gradient that drives the transport of ions and nutrients. Although Na^+ is required in some plants, particularly halophytes [1], a high NaCl concentration is a limiting factor for plant growth in large terrestrial areas of the world. Salinity imposes two stresses on plant tissues: one, a water deficit resulting from the relatively high solute concentrations of the soil; and two, ion-specific stresses resulting from altered K^+/Na^+ ratios and Na^+ and Cl^- ion concentrations that are inimical to plants.

Ion ratios in plants are altered by the influx of Na^+ through K^+ pathways. The similarity of the hydrated ionic radii of Na^+ and K^+ makes it difficult to discriminate between them and this is the basis of Na^+ toxicity. *In vitro* protein synthesis requires physiological K^+ concentrations (100–150 mM) and is inhibited by Na^+ concentrations above 100 mM [2] through competition by Na^+ for K^+-binding sites. Moreover, halophyte cytosolic enzymes are not adapted to high salt levels and display the same sensitivity to salt as enzymes from glycophytes [3]. Thus, plants respond to elevated Na^+ concentrations by maintaining low cytosolic Na^+ concentrations and a high cytosolic K^+/Na^+ ratio.

The strategies for the maintenance of a high K^+/Na^+ ratio in the cytosol include Na^+ extrusion and/or the intracellular compartmentalisation of Na^+ (mainly in the plant vacuole). These are two essential processes for the cytosolic Na^+ detoxification and cellular osmotic adjustment needed to tolerate salt stress. The first part of this review focuses on recent work on ion channels and carriers mediating the influx of Na^+ ions in plant cells. The second part discusses progress towards the identification of the mechanisms controlling cytosolic Na^+ concentrations.

Sodium uptake

Under typical physiological conditions, plants maintain a high cytosolic K^+/Na^+ ratio. Given the negative membrane potential difference at the plasma membrane (–140 mV) [4], a rise in extracellular Na^+ concentration will establish a large electrochemical gradient favoring the passive transport of Na^+ into the cells. As discussed earlier, Na^+ ions can be transported into the cell through K^+ transporters. Plants use low- and high-affinity transporters to take up K^+ from the extracellular medium.

Three classes of low affinity K^+ channels have been identified. Inward rectifying channels (KIRC), such as AKT1 [5], activate K^+ influx upon plasma-membrane hyperpolarization and they display a high K^+/Na^+ selectivity ratio at physiological K^+ and Na^+ external concentrations. A knockout mutant of *AKT1* in *Arabidopsis* (akt1-1) displayed similar sensitivity to salt as the wild type, suggesting that this channel does not play a role in Na^+ uptake [6••]. Moreover, this mutant was able to grow at K^+ concentrations lower than 100 μM in the presence of NH_4, suggesting that AKT1 is involved in the uptake of K^+ in the micromolar concentration range [6••].

K^+ outward rectifying channels (KORCs) could play a role in mediating the influx of Na^+ into plant cells. KORC channels from barley roots showed a high selectivity for K^+ over Na^+ [7]. Nevertheless, in *Arabidopsis* root cells, a lower K^+/Na^+ selectivity ratio was reported [8]. These channels, which open during the depolarization of the plasma membrane (i.e. upon a shift in the electrical potential difference to more positive values), could mediate the efflux of K^+ and the influx of Na^+ [9]. KORC in stelar cells of maize are permeable to Ca^{2+} [10], suggesting that the opening of these channels could result in an increase of cytosolic Ca^{2+} with the concomitant activation of other ion channels. One of these channels, known as NORC (nonselective outward rectifying conductance) does not discriminate between cations and is activated by increased cytosolic Ca^{2+} concentrations [11].

A number of studies have reported voltage-independent cation channels (VIC) in plant plasma membranes [12–15]. These channels have a relatively high Na^+/K^+ selectivity and in contrast to the voltage-dependent channels (KIRC

and KORC), they are not gated by voltage. In a recent review on the mechanisms of Na^+ uptake by plant cells, Amtmann and Sanders [16] proposed a simple model for the different cation channels and concluded that voltage-independent channels constitute the main pathway for Na^+ uptake in high salt conditions. The permeation of Na^+ via VICs is inhibited by an increase in extracellular Ca^{2+} concentration [17,18••]. Tyerman and Skerrett [14•] found that the Ca^{2+}-dose-response curves of the Na^+ currents in root protoplasts from wheat [19] and maize [13] and the dose-response curves for short term $^{22}Na^+$ influx in whole roots [20] and root segments [21] at similar external Na^+ concentrations were in good agreement. These findings support the role of VICs in a pathway for Na^+ influx in plant cells.

Two families of high-affinity transporters play a role in K^+ transport. They may also determine the K^+/Na^+ ratio in plant cells. The KUP-HAK (K^+ uptake transporter-high affinity K^+ transporters) high-affinity K^+ transporters have been identified in *Arabidopsis* [22–24] and in barley [25]. They are widely distributed in bacteria, fungi and higher plants, and at least six genes in *Arabidopsis* [26] and two genes in barley [25] have been identified. HAK transporters couple K^+ movement to the H^+ gradient and are very selective for K^+. Na^+ competitively blocks them in the mM range of concentrations [23,24], and HAK1 from barley allows Na^+ permeation [25].

The notion that a high-affinity K^+ transporter can be also a low-affinity Na^+ transporter is a shared property of HKT1, the second family of high-affinity K^+ transporters [27,28]. Heterologous expression of HKT1 in *Xenopus* oocytes [29] showed that HKT1 is a Na^+/K^+ symporter. HKT1 contains two binding sites, one that is specific for Na^+ and the other that can bind to both Na^+ and K^+ and induce their transport [30]. Although its purported function as a high affinity K^+ transporter remains controversial [18••], HKT1 could mediate Na^+ uptake, particularly at high NaCl concentrations, when its binding sites are saturated with Na^+.

Regulation of cytosolic Na^+ concentrations

Sodium efflux

In plants, the main mechanism for Na^+ extrusion is mediated by the plasma membrane H^+-ATPase [31]. The H^+-ATPase uses the energy of ATP hydrolysis to pump H^+ out of the cell, generating an electrochemical H^+ gradient. This protonmotive force generated by the H^+-ATPase operates the Na^+/H^+ antiporters, which couple the movement of H^+ into the cell along its electrochemical gradient to the extrusion of Na^+ against its electrochemical gradient. Biochemical evidence consistent with the operation of plasma membrane Na^+/H^+ antiporters has been obtained in different plant species [32], and a putative Na^+/H^+ antiporter with significant sequence similarity to plasma membrane Na^+/H^+ antiporters from bacteria and fungi has been identified (sse update).

Vacuolar sodium compartmentation

The cytosolic Na^+ concentration is maintained at non-toxic levels by both halophytes and glycophytes in spite of the considerable influx of Na^+ into the cytosol. The compartmentalisation of Na^+ into vacuoles averts the deleterious effects of Na^+ in the cytosol. Moreover, the compartmentalisation of Na^+ (and Cl^-) into the vacuole allows the plants to use NaCl as an osmoticum, maintaining an osmotic potential that drives water into the cells. The relatively detailed genetics of yeast, easy transformation protocols, and the homology between numerous plant and yeast genes have been used to identify and characterize of a number of plant transporters. Recent evidence suggests that Na^+ detoxification in yeast may be similar to plants. This is particularly true for the role of Ca^{2+}-dependent signal transduction in response to salinity stress [33••,34,35•]. Putative Na^+/H^+ antiporters from both organisms are also similar [36–38,39••,40].

Yeast salt-sensitive calcineurin mutants (*cnb1*), which lack the ability to increase expression of the *PMR2A/ENA1* gene and modulate K^+ transport to high affinity, were used in screening for genetic suppressors [36]. Interestingly, loss-of-function mutations in the plasma membrane H^+-ATPase (*PMA1*) confer Na^+ tolerance. The mutant cells (*pma1-α4*) had a number of phenotypes, which included: reduced Na^+ influx, increased Na^+ tolerance in response to low extracellular pH, and increased intracellular Na^+ levels. These results suggested that in addition to the limited Na^+ influx, a mechanism for intracellular sequestration of Na^+ may exist.

Nass, Cunningham and Rao [36] identified a novel gene for a Na^+/H^+ exchanger (*NHX1*) by *in silico* analysis of the yeast genome and examined its possible intracellular role. The strong phenotype of *Δnhx1* cells, observed in response to Na^+-stress, favors prevacuolar/vacuolar compartmentalization of Na^+ via Nhx1 in yeast [37]. This model is supported by the requirement for Nhx1 and the yeast Cl^- channel Gef1 in Na^+ sequestration. Both were recently colocalized to the prevacuolar compartment [38]. The proposed prevacuolar distribution of Nhx1 raises the possibility of a role in the regulation of vesicle volume and pH, which may, in turn, be important for vacuole biogenesis. However, the effects of *Δnhx1* on the prevacuolar compartment and/or vacuole biogenesis are yet to be examined. Recently, Nass and Rao [41•] showed that yeast vacuolar shrinkage and recovery was altered in a *Δnhx1* mutant after hyperosmotic stress. This supports the role of Nnhx1 in vacuolar volume regulation and its contribution to osmotolerance.

In the current model for intracellular Na^+ sequestration, yeast cells rely on an endosomal H^+-ATPase to establish a H^+ gradient that can drive Na^+ and Cl^- influx via Na^+/H^+ antiporters and chloride channels, respectively [38]. According to this model, increased H^+ influx into an endosomal compartment will enhance cation sequestration via the Nhx1 Na^+/H^+ antiporter. To test this model, Gaxiola *et al.* [38] overexpressed the *Arabidopsis* vacuolar H^+-pyrophosphatase (*AVP1*) in salt-sensitive *ena1* yeast mutants. These cells do not express plasma membrane Na^+-ATPase, the primary Na^+ extrusion mechanism and, therefore, have a significantly increased level of cytosolic Na^+. Under these conditions,

cytosolic detoxification is only achieved by the compartmentalization of Na^+ into an endosomal organelle. Yeast cells overexpressing *AVP1-D*, a mutant H^+-pyrophosphatase with an enhanced H^+-pumping ability, suppress the salt-sensitive phenotype of the *ena1* mutant. Although the restoration of the salt-tolerant phenotype seems to confirm their hypothesis, measurements of intracellular Na^+ in these cells contradicted the original rationale behind the experiment. The intracellular Na^+ content in the *ena1* mutant is eight-fold higher than in the wild type, whereas in the salt-resistant strain — *ena1 AVP-D* — it is only four-fold higher than the wild type (in other words, two-fold less than the *ena1* cells) [38]. This does not support the targeting of the *AVP-D* gene product to the yeast vacuole: enhanced Na^+ sequestration through a vacuolar pyrophosphatase would be expected to result in an equivalent, if not an increased, intracellular Na^+ content to that observed in the *ena1* strain. However, the decreased intracellular Na^+ content would suggest an exclusion or extrusion of Na^+ at the plasma membrane upon expression of the *AVP-D* product. Unfortunately, Gaxiola *et al.* [38] have neither attempted to localize the *AVP-D* product nor have they addressed their puzzling observations.

The *A. thaliana* genome-sequencing project has identified genes with significant similarity to the *Saccharomyces cerevisiae NHX1* gene product [38,39••]. Homologues have also been identified in rice [40]. These genes may well be the first plant Na^+/H^+ antiporters cloned. As mentioned before, there is overwhelming biochemical evidence for the role of plant Na^+/H^+ antiporters in salt tolerance [32]. Nevertheless, the lack of progress in the characterization of these antiporters has hindered our understanding of the cellular and molecular bases of salt tolerance. The availability of plant genes coding for Na^+/H^+ antiporters, especially those from *A. thaliana*, a model plant amenable to genetic analysis, allows for the direct probing of their function. Gaxiola *et al.* [38] have described the cloning of *AtNHX1* and its expression in *S. cerevisiae*. The predicted *AtNHX1* gene product is a protein of 538 amino acids with a putative amiloride-binding domain. It has a high degree of similarity to Na^+/H^+ antiporters from *Caenorhabditis elegans*, human (mitochondrial, NHE6) and yeast (ScNhx1), except for the amino- and carboxy-terminal regions [38].

Recently, Apse *et al.* [39••] demonstrated that *AtNHX1* codes for a vacuolar Na^+/H^+ antiporter. To assess the Na^+/H^+ exchange function, Na^+-dependent H^+ movement was measured in intact vacuoles isolated from both wild-type plants and plants overexpressing AtNHX1. Although Na^+/H^+ exchange rates were very low in vacuoles from wild-type plants, much higher rates were observed in vacuoles from transgenic plants [39••]. The higher vacuolar Na^+/H^+ antiporter activity in the transgenic plants correlated with an increase in AtNHX1 protein. Moreover, transgenic plants overexpressing AtNHX1 grew in the presence of 200 mM NaCl, supporting the role of the vacuolar Na^+/H^+ antiporter in salt tolerance [39••]. As *Arabidopsis* is a glycophytic plant with a sensitivity to salt, similar to most crop plants, these results suggest the feasibility of genetic engineering of crop plants with improved salt tolerance.

Conclusions

The identification of proteins mediating sodium transport and their biochemical characterization in plants is advancing rapidly. There is no doubt that the use of yeast as a model system for sodium transport in plants has facilitated the molecular identification of plant ion transporters. The cloning of the *Arabidopsis* Na^+/H^+ antiporters was certainly expedited by the identification of its yeast homologue. Nevertheless, the situation in plants is inherently more complex and at least four *Arabidopsis* antiporters similar to the yeast Nhx1 have been identified (E Blumwald, unpublished data). The number of putative Na^+/H^+ antiporters can be accounted for, *a priori*, by different tissue specificity, development and the response to a specific stress condition. However, the yeast model may point to a Na^+/H^+ antiporter function other than Na^+ detoxification. For example, volume and osmotic regulation in organellar biogenesis and ion homeostasis may be roles fulfilled by Na^+/H^+ antiporters. These roles have yet to be investigated in plants. The progress of the different genomic sequence projects, together with the development of knockout mutants, will greatly facilitate this task.

Update

Recent work has identified the *SOS1* (salt overly sensitive 1) locus in *Arabidopsis thaliana* [42••]. *SOS1* encodes a putative Na^+/H^+ antiporter that has significant sequence similarity to plasma membrane Na^+/H^+ antiporters from bacteria and fungi.

Acknowledgements

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References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Glenn E, Brown JJ, Blumwald E: **Salt-tolerant mechanisms and crop potential of halophytes.** *Crit Rev Plant Sci* 1999, **18**:227-255.

2. Wyn Jones G, Pollard A: **Proteins, enzymes and inorganic ions.** In *Encyclopedia of Plant Physiology, New Series,* Edited by Lauchli A, Person A. New York: Springer; 1983:528-562.

3. Flowers T, Troke PF, Yeo AR: **The mechanisms of salt tolerance in halophytes.** *Annu Rev Plant Physiol* 1977, **28**:89-121.

4. Higinbotham N: **Electropotentials of plant cells.** *Annu Rev Plant Physiol* 1973, **B24**:25-46.

5. Sentenac H, Bonneaud N, Minet M, Lacroute F, Salmon JM, Gaymard F, Grignon C: **Cloning and expression in yeast of a plant potassium ion transport system.** *Science* 1992, **256**:663-665.

6. •• Spalding EP, Hirsch RE, Lewis DR, Zhi Q, Sussman MR, Lewis BD: **Potassium uptake supporting plant growth in the absence of AKT1 channel activity. Inhibition by ammonium and stimulation by sodium.** *J Gen Physiol* 1999, **113**:909-918.

The authors used a reverse genetic screen to identify an *Arabidopsis thaliana* mutant in which AKT1 gene was disrupted. The mutants showed reduced $^{86}Rb^+$ uptake and reduced growth on media containing 100 micromolar K^+ or less. This paper is of particular interest because it suggests that the AKT1 channel is able to mediate K^+ uptake in plants growing in solutions containing as little as 10 micromolar potassium.

7. Wegner LH, Raschke K: **Ion channels in the xylem parenchyma of barley roots.** *Plant Physiol* 1994, **105**:799-813.

8. Maathuis FJM, Sanders D: **Contrasting roles in ion transport of two K^+-channel types in root cells of *Arabidopsis thaliana*.** *Planta* 1995, **197**:456-464.

9. Maathuis FJM, Sanders D: **Regulation of K absorption in plant root cells by external K: interplay of different plasma membrane K transporters.** *J Exp Bot* 1997, **48**:451-458.

10. Roberts SK, Tester M: **Permeation of Ca^{2+} and monovalent cations through an outwardly rectifying channel in maize root stelar cells.** *J Exp Bot* 1997, **48**:839-846.

11. de Boer AH, Wegner LH: **Regulatory mechanisms of ion channels in xylem parenchyma cells.** *J Exp Bot* 1997, **48**:441-449.

12. Amtmann A, Laurie S, Leigh R, Sanders D: **Multiple inward currents provide flexibility in Na^+/K^+ discrimination at the plasma membrane of barley suspension culture cells.** *J Exp Bot* 1997, **48**:481-497.

13. Roberts SK, Tester J: **A patch clamp study of Na transport in maize roots.** *J Exp Bot* 1997, **48**:431-440.

14. • Tyerman SD, Skerrett IM: **Root ion channels and salinity.** *Sci Hort* 1999, **78**:175-235.
An extensive review that focuses on the function of cation and anion channels under saline conditions with emphasis on the root cells.

15. White PJ: **The molecular mechanism of sodium influx to root cells.** *Trends Plant Sci* 1999, **4**:245-246.

16. Amtmann A, Sanders D: **Mechanisms of Na^+ uptake by plant cells.** *Adv Bot Res* 1998, **29**:76-112.

17. White PJ, Lemtiri-Chlieh F: **Potassium currents across the plasma membrane of protoplasts derived from rye roots: a patch clamp study.** *J Exp Bot* 1995, **46**:497-511.

18. •• Maathuis FJM, Amtmann A: **K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios.** *Ann Bot* 1999, **84**:123-133.
This review deals with the different plasma membrane K^+ and Na^+ transporters operating in plant cells in general and on the soil/root interface in particular. This paper is of particular interest because the authors correlate the current data on the presence and regulation of the individual transporters and their impact on the cytosolic K^+/Na^+ ratio.

19. Tyerman SD, Skerrett M, Garrill A, Findlay GP, Leigh RA: **Pathways for the permeation of Na and Cl into protoplasts derived from the cortex of wheat roots.** *J Exp Bot* 1997, **48**:459-480.

20. Davenport RJ, Reid RJ, Smith FA: **Sodium-calcium interactions in two wheat species differing in salinity tolerance.** *Physiol Plant* 1997, **99**:323-327.

21. Zidan I, Jacoby B, Ravina I, Neumann PM: **Sodium does not compete with calcium in saturating plasma membrane sites regulating $^{22}Na^+$ influx in salinized maize roots.** *Plant Physiol* 1991, **96**:331-334.

22. Quintero FJ, Blatt MR: **A new family of K^+ transporters from *Arabidopsis* that are conserved across phyla.** *FEBS Lett* 1997, **415**:206-211.

23. Kim EJ, Kwak JM, Uozumi N, Schroeder JL: **AtKUP1: An *Arabidopsis* gene encoding high-affinity potassium transport activity.** *Plant Cell* 1998, **10**:51-62.

24. Fu H, Luan S: **A dual affinity K^+ transporter from *Arabidopsis*.** *Plant Cell* 1998, **10**:63-73.

25. Santa-Maria G, Rubio F, Dubcovsky J, Rodriguez-Navarro A: **The HAK1 gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter.** *Plant Cell* 1997, **9**:2281-2289.

26. Schachtman D, Liu W: **Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants.** *Trends Plant Sci* 1999, **4**:281-286.

27. Schachtman D, Schroeder JI: **Structure and transport mechanism of a high-affinity potassium uptake transporter from higher plants.** *Nature 1994,* **370**:655-658.

28. Wang TB, Gassmann W, Rubio F, Schroeder JI, Glass ADM: **Rapid upregulation of *HKT1,* a high-affinity potassium gene, in root of barley and wheat following withdrawal of potassium.** *Plant Physiol* 1998, **B118**:651-659.

29. Rubio F, Gassmann W, Schroeder JI: **Sodium-driven potassium uptake by the plant potasium transporter HKT1 and mutations conferring salt tolerance.** *Science* 1995, **270**:1660-1663.

30. Gassmann W, Rubio F, Schroeder JI: **Alkali cation selectivity of the wheat root high-affinity potassium transporter HKT1.** *Plant J* 1996, **10**:869-882.

31. Sussman MR: **Molecular analysis of protein in the plant plasma membrane.** *Annu Rev Plant Physiol Plant Mol Biol* 1994, **45**:211-234.

32. Blumwald E, Aharon GS, Apse MP: **Sodium transport in plant cells.** *Biochim Biophys Acta* 2000, **1465**:140-151.

33. •• Halfter U, Ishitani M, Zhu, JK: **The *Arabidopsis* SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3.** *Proc Natl Acad Sci USA* 2000, **97**:3735-3740.
This report shows that SOS3 physically interacts with and activates SOS2 protein kinase. SOS3 is an EF hand-type calcium-binding protein with sequence similarity to animal and yeast calcium sensors. SOS2 is a serine/threonine protein kinase from the SNF1/AMpK family. Both *SOS2* and *SOS3 Arabidopsis thaliana* genes are required for intracellular Na^+ and K^+ homeostasis. This paper is of special interest because it defines a novel regulatory pathway for the control of intracellular ion homeostasis in plants.

34. Pardo JM, Reddy MP, Yang S, Maggio A, Huh GH, Matsumoto T, Coca MA, Paino-D'Urzo M, Koiwa H, Yun DY *et al.*: **Stress signaling through Ca^{2+}/calmodulin-dependent protein phosphatase calcineurin mediates salt adaptation in plants.** *Proc Natl Acad Sci USA* 1998, **95**:9681-9686.

35. • Kudla J, Xu Q, Harter K, Guissem W, Luan S: **Genes for calcineurin B-like proteins in *Arabidopsis* are differentially regulated by stress signals.** *Proc Natl Acad Sci USA* 1999, **96**:4718-4723.
The *Arabidopsis* calcineurin B-type like protein (AtCBL) was characterized. This paper is of particular interest because it shows that AtCBL proteins are encoded by a family of at least six genes in *Arabidopsis* and that the expression of some of these genes was regulated by specific stress signals.

36. Nass R, Cunningham KW, Rao R: **Intracellular sequestration of sodium by a novel Na^+/H^+ exchanger in yeast is enhanced by mutations in the plasma membrane H^+-ATPase.** *J Biol Chem* 1997, **272**:26145-26152.

37. Nass R, Rao R: **Novel localization of a Na^+/H^+ exchanger in a late endosomal compartment of yeast. Implications for vacuole biogenesis.** *J Biol Chem* 1988, **273**:21054-21060.

38. Gaxiola RA, Rao R, Sherman A, Grisafi P, Alper SL, Fink GR: **The *Arabidopsis thaliana* proton transporters AtNhx1 and Avp1 can function in cation detoxification in yeast.** *Proc Natl Acad Sci USA* 1999, **96**:1480-1485.

39. •• Apse MP, Aharon GS, Blumwald E: **Salt tolerance conferred by overexpression of a vacuolar Na^+/H^+ antiport in *Arabidopsis.*** *Science* 1999, **285**:1256-1258.
This paper demonstrated that the overexpression of a vacuolar Na^+/H^+ antiporter allowed transgenic *Arabidopsis thaliana* plants to grow in solutions containing 200 mM NaCl. The salinity tolerance of the transgenic plants was correlated with higher than normal levels of *AtNHX1* transcripts, protein and vacuolar Na^+/H^+ antiport activity. This paper is of particular interest because it clearly demonstrates the role of vacuolar Na^+ compartmentalization in plant salt tolerance.

40. Fukuda A, Nakamura A, Tanaka Y: **Molecular cloning and expression of the Na^+/H^+ exchanger gene in Oryza sativa.** *Biochim Biophys Acta* 1999, **1446**:149-155.

41. • Nass R, Rao R: **The yeast endosomal Na^+/H^+ exchanger, Nhx1, confers osmotolerance following acute hypertonic shock.** *Microbiol* 1999, **145**:3221-3228.
This paper shows that the yeast endosomal Na^+/H^+ exchanger Nhx1 contributes to osmotolerance following the sudden exposure of yeast to a hyperosmotic stress. This paper is of special interest because it provides evidence for the role of vacuolar volume regulation in the initial response of the yeast cells to osmotically-induced cell shrinkage.

42. •• Shi H, Ishitani M, Kim C, Zhu J-K: **The *Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na^+/H^+ exchanger.** *Proc Natl Acad Sci* USA 2000, **97**:6896-6901.
This paper suggests that a plasma-membrane-type Na^+/H^+ antiporter could play a significant role in plant salt tolerance. This paper is of special interest because it provides evidence for the regulation of *SOS1* gene expression by the SOS2/SOS3 kinase complex, a regulatory pathway that controls Na^+ and K^+ homeostasis in plants.