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Update on Salt Stress

# Ion Homeostasis in NaCl Stress Environments[1]

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Homeostasis can be defined as the tendency of a cell or an organism to maintain internal steady state, even in response to any environmental perturbation or stimulus tending to disturb normality, because of the coordinate responses of its constituent components. Typically, ions constantly flux in and out of cells in a controlled fashion with net flux adjusted to accommodate cellular requirements, thus creating an ionic homeostasis. When plant cells are exposed to salinity, mediated by high NaCl concentrations, kinetic steady states of ion transport for $Na^+$ and $Cl^-$ and other ions, such as $K^+$ and $Ca^{2+}$, are disturbed (Binzel et al., 1988). High apoplastic levels of $Na^+$ and $Cl^-$ alter aqueous and ionic thermodynamic equilibria, resulting in hyperosmotic stress, ionic imbalance, and toxicity. Thus, it is vital for the plant to re-establish cellular ion homeostasis for metabolic functioning and growth, that is, to adapt to the saline environment.

Comparisons of what have been interpreted to be adaptive responses among various species lead to the conclusion that some salt-tolerant plants have evolved specialized complex mechanisms that allow adaptation to saline stress conditions. In fact, these unique mechanisms, such as salt glands, exist in few plant species and cannot be presumed to be ubiquitously functional for salt adaptation of all plants. However, intrinsically cellular-based mechanisms appear to be common to all genotypes and are a requisite for salt tolerance. Of paramount importance are those mechanisms that function to regulate ion homeostasis while mediating osmotic adjustment through the accumulation and intracellular compartmentation of ions that are predominant in the external environment. In this update we will focus principally on $Na^+$ homeostasis in sodic environments; however, we also include discussions of $H^+$, $K^+$, $Ca^{2+}$, and $Cl^-$ because of the interrelationship of these ions with $Na^+$ homeostasis. Ion transport processes across the plasma membrane and the tonoplast will be emphasized because these are presumed to be most essential for the control of intracellular $Na^+$ uptake and vacuolar compartmentation.

## ION TRANSPORT ACROSS PLANT CELL MEMBRANES

Since ions are hydrated in solution and do not readily traverse the hydrophobic lipid bilayer of membranes, flux across the plasma membrane and tonoplast occurs via transport proteins. Ion flux across membranes is dependent on the thermodynamic gradient ($\Delta\mu$). $\Delta\mu$ consists of two components, the electrical gradient or membrane potential and the chemical gradient (Nobel, 1991). Transport of ions down the $\Delta\mu$ is passive, whereas transport against the gradient is active. The transport proteins that mediate ion flux can be generally categorized as pumps, carriers, and channels (Sussman and Harper, 1989). Pumps directly utilize metabolic energy for vectorial transport, whereas carriers couple uphill transport of one solute to the downhill movement of another, either in the same (symporter) or opposite (antiporter) direction. Channels mediate passive transport, i.e. movement down a free energy gradient, although movement may be electrophoretic flux resulting from an energy-dependent process. In plants many of these transport proteins have been identified based on physiological evidence and biochemical characterization, and in some instances, the genes encoding these proteins have been cloned (summarized in Table I).

Currently, it is presumed that energy-dependent flux of most ions in plants is mediated by the $\Delta\mu_H{}^+$ that generates the pH gradient ($\Delta$pH) and is principally responsible for the $\Delta\Psi$ (Sze, 1985). The proton motive force, which results from the conversion of chemical energy ($\Delta\mu_H{}^+$) to mV, is $\Delta p = \Delta\mu_H{}^+/F = \Delta\Psi - 59\Delta pH$ (at 25°C), where F is the Faraday constant (Sze, 1985). Thus, carrier-mediated ion transport in plants is coupled to the downhill flux of $H^+$. $Ca^{2+}$-ATPases in the plasma membrane and endomembranes are notable exceptions to this generalization about $\Delta\mu_H{}^+$-mediated ion transport, as we discuss below. $H^+$-ATPases in the plasma membrane and tonoplast and the tonoplast $H^+$-pyrophosphatase are primary pumps that couple the free energy of hydrolysis of ATP or PPi, respectively, to vectorial $H^+$ transport and generation of $\Delta\mu_H{}^+$

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Abbreviations: $\Delta\mu$, electrochemical potential; $\Delta\mu_H$, $H^+$ electrochemical gradient; $\Delta\Psi$, membrane potential.

**Table I.** *Plant genes encoding transport proteins that are presumed to function in establishment and maintenance of ion homeostasis for salt adaptation*

In instances where yeast genes are listed, the transport process mediated is assumed to be so fundamental that a functional higher plant homolog must exist. Only one isogene is listed per species.

| Category | Name | Location | Gene | Characteristics | Species | Reference |
|---|---|---|---|---|---|---|
| Pumps | $H^+$-ATPase | Plasma membrane | *AHA3* | 12 isogenes (?) | *Arabidopsis thaliana* | Pardo and Serrano (1989) |
| | | | *PMA2* | 4 isogenes | *Nicotiana plumbaginifolia* | Boutry et al. (1989) |
| | | | *LHA1* | 2 isogenes | *Lycopersicon esculentum* | Ewing et al. (1990) |
| | | | *OSA1* | | *Oryza sativa* | Wada et al. (1992) |
| | | Tonoplast | *Unnamed* | Subunit A (69 kD) | *Daucus carota* | Zimniak et al. (1988) |
| | | | *CVA69.24* | Subunit A (69 kD) | *Gossypium hirsutum* | Wilkins (1993) |
| | | | *At57* | Subunit B (57 kD) | *A. thaliana* | Manolson et al. (1988) |
| | | | *HTB1* | Subunit B, 2 isogenes | *Hordeum vulgare* | Berkelman et al. (1993) |
| | | | *Unnamed* | Subunit B (60 kD) | *G. hirsutum* | Wan and Wilkins (1994) |
| | | | *VATP-P1* | Subunit c (16 kD) | *Avena sativa* | Lai et al. (1991) |
| | $H^+$-PPase | Tonoplast | *AVP* | 3 isogenes | *A. thaliana* | Sarafian et al. (1992) |
| | $Ca^{2+}$-ATPase | ER | *LCA1* | | *L. esculentum* | Wimmers et al. (1992) |
| | $Na^+$-ATPase | Plasma membrane | *ENA1* | 2 isogenes | *Saccharomyces cerevisiae* | Haro et al. (1991) |
| Carriers | $Na^+/H^+$ antiporter | Plasma membrane | *SOD2* | | *Schizosaccharomyces pombe* | Jia et al. (1992) |
| | | | *Unnamed* | | *Zygosaccharomyces rouxii* | Watanabe et al. (1991)[a] |
| | $K^+$-$H^+$ symporter | Plasma membrane | *HKT1* | | *Triticum aestivum* | Schachtman and Schroeder (1994) |
| Channels | $K^+$ inward | Plasma membrane | *KAT1* | | *A. thaliana* | Anderson et al. (1992) |
| | | | *AKT1* | | *A. thaliana* | Sentenac et al. (1992) |
| | | | *KST1* | | *Solanum tuberosum* | Müller-Röber et al. (1995) |

[a] GenBank accession No. D43629.

(Maathuis and Sanders, 1992). The $H^+$ pump in the plasma membrane is a P-type ATPase (Michelet and Boutry, 1995) that establishes a ΔpH of about 1.5 to 2 units (pH 5.5 to 5.0 in the apoplast) and is principally responsible for the inside negative membrane potential of −120 to −200 mV across this membrane under physiologically steady-state conditions (Sze, 1985). From the Nernst equation, $\Delta\Psi$ values in this range would establish free-energy gradients that would result in the concentration of monovalent cations ($K^+$ or $Na^+$) 100- to 1000-fold or greater in the cytosol (Nobel, 1991). Conversely, these free-energy gradients would make intracellular uptake of anions thermodynamically untenable. The two $H^+$ pumps present in the tonoplast, vacuolar-type $H^+$-ATPase (Sze, 1985) and $H^+$-pyrophosphatase (Rea and Sanders, 1987), establish a $\Delta\mu_{H}{}^{+}$ across this membrane. This $\Delta\mu_{H}{}^{+}$ is predominantly the result of ΔpH (about 2 units between the cytosol and inside the vacuole), since a $\Delta\Psi$ of 0 to +20 mV (inside positive) normally occurs across the tonoplast.

Under typical physiological conditions, homeostatic concentrations of ions in the cytosol are 100 to 200 mM $K^+$, 1 to 10 mM $Na^+$ and $Cl^-$, and 100 to 200 nM $Ca^{2+}$ (Binzel et al., 1988; Bush, 1995). The $\Delta\mu_{K}{}^{+}$ across the plasma membrane is near equilibrium, and $K^+$ homeostasis is achieved by the gating of inward- and outward-rectifying $K^+$ channels and by the influx activity of a high-affinity $K^+$-$H^+$ symporter (Schachtman and Schroeder, 1994; Schroeder et al., 1994). Uptake of $Na^+$ and $Ca^{2+}$ occurs passively across the plasma membrane, and efflux is presumably due to the activities of a $Na^+/H^+$ antiporter and a $Ca^{2+}$-ATPase, respectively. $Cl^-$ uptake is assumed to be coupled to a $H^+$ symporter because of the large inside negative $\Delta\Psi$ (Poole, 1988). Extrusion of $Cl^-$ is by electrophoretic flux.

## IMPACT OF NaCl STRESS ON ION HOMEOSTASIS AND ADAPTIVE RESPONSES

When plants are exposed to NaCl, ions reduce the apoplastic water potential and accumulate excessively in the cytosol (Binzel et al., 1988). Plant cells adjust to the water relations imbalance through osmotic adjustment by synthesizing compatible organic solutes and accumulating ions from the external environment. Osmotic adjustment must be achieved without undue concentration, in the cytosol, of ions from the external environment, and the ion activities of those that do accumulate must be attenuated by osmoprotectants. Presumably, genotypes that are most adapted to salt tightly regulate ion uptake across the plasma membrane at a rate that is compatible with the capacity for vacuolar compartmentation (Binzel et al., 1988). Thus, transport processes at the plasma membrane and tonoplast that regulate ion influx and efflux, particularly those involved in the control of $Na^+$ uptake and vacuolar compartmentation, are of crucial importance to salinity adaptation.

### Sodium Influx, Association with Potassium Uptake

The $Na^+$ electrochemical gradient dictates that $Na^+$ influx across the plasma membrane is passive and efflux is

active at physiological $\Delta\Psi$ values. When the NaCl concentration increases in the surrounding environment, the high extracellular level of $Na^+$ (relative to the cytosol) and inside-negative $\Delta\Psi$ establish a steep thermodynamic gradient for $Na^+$ influx. Vacuolar compartmentation of $Na^+$, which reduces cytosolic levels of this ion, further facilitates the energetically downhill influx across the plasma membrane.

The mechanism of $Na^+$ influx across the plasma membrane is unknown. $Na^+$ acts as a competitor of $K^+$ uptake (Watad et al., 1991; Schroeder et al., 1994), suggesting that the uptake mechanisms for both cations are similar. Plant roots utilize two systems for $K^+$ acquisition. System 1 has a high affinity for $K^+$ ($K_m$ of 10–30 $\mu$m) to allow uptake at low $K^+$ concentrations; it is not inhibited by $Na^+$ (Rains and Epstein, 1967). System 2 mediates uptake at higher external $K^+$ concentrations (mM) and has a less pronounced $K^+/Na^+$ selectivity. $Na^+$ influx into plants likely occurs via the low-affinity rather than the high-affinity $K^+$ uptake system (Rains and Epstein, 1967). Presumably, system 1 is an active transporter (perhaps a $K^+$-$H^+$ symporter; Schachtman and Schroeder, 1994) because of thermodynamic constraints to passive influx at very low external $K^+$ concentrations, whereas inward-rectifying $K^+$ channels may mediate $K^+$ uptake by system 2 at external concentrations above 0.3 mM (Maathuis and Sanders, 1993; Schroeder et al., 1994). However, available data indicate that plasma membrane inward-rectifying $K^+$ channels have extremely high $K^+/Na^+$ selectivity, whereas the $K^+$-$H^+$ symporter is less selective (Anderson et al., 1992; Schachtman and Schroeder, 1994).

Schachtman et al. (1991) have suggested that $Na^+$ uptake may occur through outward-rectifying cation channels. Plasma membrane depolarization and exposure to high external NaCl increases the open probability of outward-rectifying cation channels in wheat root and tobacco cells, thereby allowing $Na^+$ influx to occur down its steep electrochemical gradient. Any regulatory process that decreases the open probability of these outward-rectifying cation channels would reduce both the entry of $Na^+$ into the cell and the leakage of $K^+$ out of the cell, thus hypothetically representing a mechanism of adaptation to NaCl stress.

The high-affinity $K^+$ uptake system in barley roots can be induced by low ($\mu$M) and inhibited by elevated (mM) extracellular $K^+$ concentrations (Fernando et al., 1992, and refs. therein). Whether the functions of root $K^+$ uptake systems 1 and 2 are also regulated by $Na^+$ stress, thereby altering $Na^+$ influx into the root, remains to be determined. Suggested by analogy with yeast (discussed below), the prediction is that high external $Na^+$ would trigger, either by excessive $Na^+$ influx or reduced $K^+$ uptake, the activation of system 1 and the inhibition of system 2. Consistent with this hypothesis, tobacco cells adapted to NaCl exhibited enhanced capacity for $K^+$ uptake relative to wild-type cells, presumably due to a higher degree of coupling between $H^+$ efflux and $K^+$ influx across the plasma membrane (Watad et al., 1991). Further, no inhibition of $K^+$ uptake by NaCl was observed in salt-adapted cells, indicating that these cells had a higher $K^+/Na^+$ selectivity at the plasma membrane (Watad et al., 1991). Therefore, increased $K^+/Na^+$ selectivity of the $K^+$ uptake system might represent a significant adaptation to high concentrations of NaCl. At the whole plant level, it is generally accepted that increased $K^+/Na^+$ selectivity during uptake and reduced $Na^+$ translocation from the root to the shoot contribute to the overall salt tolerance of glycophytes.

### Sodium Efflux and Vacuolar Compartmentation

Active efflux of $Na^+$ from the cytosol across the plasma membrane and the tonoplast is essential for the regulation of net intracellular uptake and vacuolar compartmentation. Physiological and biochemical data support the likelihood that $Na^+/H^+$ antiporters in the plasma membrane and tonoplast mediate these fluxes (DuPont, 1992, and refs. therein). Mechanistically, the $Na^+/H^+$ antiporters in the plasma membrane and tonoplast are coupled to the $\Delta$pHs generated by the $H^+$ pumps located in these membranes.

Induction of plasma membrane $Na^+/H^+$ antiport activities by NaCl occurred in the halotolerant alga *Dunaliella salina* (Katz et al., 1992) and the halophyte *Atriplex nummularia* (Hassidim et al., 1990). The $H^+$-translocating activities of the plasma membrane ATPase increased in response to NaCl treatment in tobacco cells and roots of the halophyte *A. nummularia* (Braun et al., 1986; Watad et al., 1991). This up-regulation may be mediated, at least in part, by increased gene expression (Niu et al., 1993a, 1993b; Perez-Prat et al., 1994). The levels of the plasma membrane pump mRNA were substantially higher in the elongation and differentiation zones after NaCl treatment (Niu et al., 1993a). The gene expression and physiological responsiveness of the plasma membrane $H^+$-ATPase to NaCl is positively correlated with salt tolerance, since halotolerant cells and plants exhibited higher transcript levels and/or pump activities than intolerant counterparts (Braun et al., 1986; Niu et al., 1993a, 1993b; Perez-Prat et al., 1994).

Plasma membrane $H^+$-ATPase mRNA accumulation is induced only during NaCl adaptation, since lower and similar mRNA levels were detected in samples obtained from cells that were either growing in the absence of salt or had been adapted to salt for several hundred generations (Niu et al., 1993b; Perez-Prat et al., 1994). These results indicate that salt-induced plasma membrane $H^+$-ATPase gene expression, which is assumed to be a basis for at least part of higher $H^+$ transport activity, occurs during stress adaptation but not after the new adaptive state has been achieved. Presumably, after adaptation (when cells are growing actively in salt), the pump re-establishes a $H^+$ transport steady state that is the same as for cells growing in a nonsaline environment. Thus, during the period of stress adaptation, active plasma membrane transport mechanisms (e.g. $Na^+/H^+$ antiporter) are induced or activated, requiring higher pump activity, whereas after adaptation and when ion gradients have been established across the membrane, altered membrane permeability (e.g. reduced passive transport restricting $Na^+$ influx) may be the primary basis for ion homeostasis (Binzel et al., 1988; Watad et al., 1991).

Vacuolar compartmentation of $Na^+$ and $Cl^-$ is an essential mechanism for salt tolerance, since it results in lower cytosolic ion levels and facilitates osmotic adjustment required for cell expansion and maintenance of turgor. Tobacco cells adapted to and growing in 428 mM NaCl accumulated 780 mM $Na^+$ and 624 mM $Cl^-$ in the vacuole, whereas cytoplasmic levels of both ions were below 100 mM (Binzel et al., 1988). $Na^+/H^+$ antiport activities of tonoplast vesicles from beet cells and barley roots (DuPont, 1992) were induced by NaCl. Antibodies that reacted to a 170-kD polypeptide and that accumulated in tonoplast vesicles after salt treatment inhibited $Na^+/H^+$ activity of these membrane vesicles. Similarly, $H^+$ transport by the vacuolar $H^+$-ATPase increased during adaptation to NaCl in several plants (DuPont, 1992, and refs. therein). In barley roots, increased $H^+$ transport activity did not peak until 3 d after the onset of NaCl treatment, and this induction was inhibited by protein synthesis inhibitors, indicating that de novo synthesis was required. Accordingly, 70-kD subunit (subunit B) mRNA of the vacuolar $H^+$-ATPase accumulated in response to NaCl treatment (Narasimhan et al., 1991). These data imply that salt-induced $H^+$ transport activity of the tonoplast ATPase is mediated through increased gene expression. The induction of tonoplast $H^+$-ATPase activity by NaCl apparently occurs only during stress adaptation but not after adaptation, since both vesicle $H^+$ pumping activities and 70-kD subunit mRNA levels were similar in wild-type and salt-adapted cells (Narasimhan et al., 1991). The tonoplast $H^+$-ATPase of salt-adapted cells exhibited altered biochemical characteristics consistent with a higher specific $H^+$ pumping activity. Enhanced activity of the tonoplast pyrophosphatase in NaCl-stressed carrot cells has also been reported (Colombo and Cerana, 1993).

### $Cl^-$

Little is known about intracellular uptake and vacuolar compartmentation of $Cl^-$ (Binzel et al., 1988). The extreme inside negative membrane potential across the plasma membrane that occurs when ion homeostasis is established and maintained is a substantial thermodynamic barrier to $Cl^-$ influx, even at relatively high external concentrations. However, active uptake could be mediated by a $Cl^-$-$H^+$ symporter (Poole, 1988). If $Na^+$ influx depolarizes the $\Delta\Psi$ across the plasma membrane, the $Cl^-$ can be taken up passively through an anion channel (Skerrett and Tyerman, 1994). Vacuolar compartmentation of $Cl^-$ is an essential adaptation for NaCl tolerance. $Cl^-$ movement from the cytosol to the vacuole may be achieved through channels with transport driven by the electrophoretic flux generated by $H^+$ pumps across the tonoplast, i.e. under saline conditions, $Cl^-$ may be the counterion to $H^+$, since the $\Delta\mu_H{}^+$ across this membrane is primarily due to $\Delta$pH. Slow-vacuolar-type channels can account for all ion conductances at elevated cytosolic (>1 $\mu$M) $Ca^{2+}$ levels (Plant et al., 1994). An alternative possibility is a $H^+$/anion antiporter that would actively transport $Cl^-$ across the tonoplast (Rea and Sanders, 1987).

### $Ca^{2+}$

The role of $Ca^{2+}$ in NaCl stress adaptation is complex and not well defined, although this cation and its homeostasis are thought to be essential. Externally supplied $Ca^{2+}$ ameliorates NaCl stress through an unknown function that preserves $K^+/Na^+$ selectivity (Zhong and Läuchli, 1994). Moreover, $Ca^{2+}$ inhibits inward-rectifying $K^+$ channels, that may reduce the $Na^+$ influx mediated by the low-affinity component of $K^+$ uptake (Schroeder et al., 1994). Cytosolic $Ca^{2+}$ levels are usually maintained at 100 to 200 nM by active transport, and small increases in concentration typically initiate very specific signal transduction cascades (Bush, 1995). NaCl causes a rapid increase in cytosolic $Ca^{2+}$ that probably acts as a general stress signal (Lynch et al., 1989). Although it is not clear if this increase is an effector of salt tolerance, increases in cytosolic $Ca^{2+}$ that mediate salt adaptation must be transitory, as is the case for all signals. Current information indicates that reestablishment of cytosolic $Ca^{2+}$ homeostasis is a requisite for adaptation.

The rise in cytosolic $Ca^{2+}$ may occur via influx from the apoplast through stretch-activated $Ca^{2+}$ channels similar to those in guard cells that respond to tension produced by osmotic stress (Cosgrove and Hedrich, 1991). Additionally, inositol (1,4,5)-triphosphate-regulated channels in the tonoplast and other endomembranes may release $Ca^{2+}$ from internal pools (Alexandre et al., 1990), perhaps in response to G-protein activation and inositol (1,4,5)-triphosphate formation. Several transport mechanisms facilitate $Ca^{2+}$ efflux from the cytosol, including $Ca^{2+}$-ATPases in the plasma membrane and endomembranes (Bush, 1995) and a tonoplast $Ca^{2+}/H^+$ antiporter. Plant $Ca^{2+}$-ATPase cDNAs have been isolated that encode putative ER pumps. Transcripts detected by these cDNAs accumulated in response to NaCl treatment, and $Ca^{2+}$-ATPase mRNA levels remained elevated after adaptation (Perez-Prat et al., 1992). These results suggest that the increase in cytosolic $Ca^{2+}$ that follows exposure to NaCl may be lowered by increased activity of the $Ca^{2+}$-ATPase.

## THE FUNGAL MODEL

Ion transport in plants and fungi shares many common features, and it has been suggested by Haro et al. (1993) that this organism can be a model for the characterization of salt-tolerant mechanisms in plant cells because of similarities with higher plants and the available powerful molecular genetic tools. Genetic and molecular studies of yeast have shown that restricted $Na^+$ uptake, rapid $Na^+$ efflux, and efficient $Na^+$ compartmentation into the vacuole are important salt-tolerant determinants similar to those in plant cells. As has been established for plant roots, fungi exhibit a dual $K^+$ uptake system, with high or low affinity of $K^+$, that adjusts in response to environmental stimuli. In NaCl-stressed *Saccharomyces cerevisiae* cells, the $K^+$ uptake system changes to a state with increased affinity for $K^+$, whereas the affinity for $Na^+$ is relatively unaltered, thereby effectively reducing $Na^+$ influx (Ramos et al., 1985). The high-affinity $K^+$ uptake system depends on

*TRK1*, a gene encoding a membrane protein presumed to be the $K^+$ transporter, although direct biochemical evidence for such a function is not yet available (Gaber et al., 1988). A *trk1* mutant, deficient in the high-affinity $K^+$ transport mode, is sensitive to $Na^+$ and $Li^+$ (Haro et al., 1993), consistent with the presumption that enhanced $K^+/Na^+$ selectively is required for NaCl tolerance.

Two different $Na^+$ efflux systems have been characterized in yeast. The *SOD2* gene of *Schizosaccharomyces pombe* encodes a plasma membrane $Na^+/H^+$ antiporter (Jia et al., 1992). In *S. cerevisiae*, $Na^+$ efflux is mediated by a novel plasma membrane P-type $Na^+$-ATPase, the ENA protein (Haro et al., 1991). Apparently, SOD2 and ENA proteins are the only $Na^+$ efflux mechanisms present in *S. pombe* and *S. cerevisiae*, respectively, because null mutations in the corresponding genes completely abolished $Na^+$ efflux and rendered cells highly sensitive to NaCl. Gene amplification of *SOD2* enhanced $Na^+$ efflux and increased tolerance to NaCl, indicating that $Na^+$ efflux capacity delineates the upper limit for $Na^+$ tolerance in yeast (Jia et al., 1992). Theoretically, overexpression of the ENA ATPase might increase $Na^+$ tolerance even further because the efflux of $Na^+$ would be less dependent on the transmembrane $Na^+$ and $H^+$ gradients.

In *S. cerevisiae*, the tonoplast has a functional role in salt and osmotic stress tolerances. Some *vpt* and all *ssv* mutants, with altered tonoplast function or morphology, were sensitive to high concentrations of salts and polyols, indicating that correct vacuolar function is required for osmoregulation (Banta et al., 1988; Latterich and Watson, 1991). Besides this osmoregulatory role, ion compartmentation in the large vacuolar space may also prevent the buildup of toxic levels of ions in the cytoplasm. A *vatC/vma3* mutant defective in vacuolar $H^+$-ATPase function, and therefore having disabled energization of the tonoplast, shows a reduced capacity for $Na^+$ accumulation. Furthermore, it was sensitive to low levels of $Na^+$ and $Li^+$ that do not represent a significant osmotic stress (Haro et al., 1993).

The genetic analysis of NaCl tolerance in the yeast *S. cerevisiae* has produced important insights into the regulatory mechanisms controlling adaptation to a saline environment. A screening for genes that enhance tolerance to NaCl led to the isolation of *HAL1* (Gaxiola et al., 1992). The overaccumulation of the HAL1 protein resulted in increased intracellular $K^+$ levels in salinized media. Preliminary data suggested that plants might also contain a *HAL1* homolog whose expression is induced by NaCl and ABA (Gaxiola et al., 1992). Additionally, the isolation of yeast mutants unable to adapt to mild NaCl stress led to the identification of the $Ca^{2+}$/calmodulin-dependent protein phosphatase, calcineurin, as a key component of the signaling pathway controlling adaptation to NaCl stress (Mendoza et al., 1994). A calcineurin-deficient cell was unable to fully induce *ENA1* gene expression and convert the $K^+$ uptake system to the high-affinity state. Hence, calcineurin seems to be a common intermediate in signaling pathways leading to the control of both $Na^+$ efflux and influx that regulate net uptake of this ion across the plasma membrane.

## CONCLUSIONS AND PERSPECTIVES

Figure 1 illustrates a typical scenario of the consequences of NaCl stress to plant cells and the transport processes that are involved in the establishment of ion homeostasis. NaCl stress environments impose water deficit and ion imbalance on plants, and both must be alleviated for survival and growth. Maximizing utilization of the predominant ions in the stress environment for osmotic adjustment and controlling cytosolic levels of these ions (i.e. ion activities) to minimize metabolic toxicity are principal salt-adaptation mechanisms. During the initial period of NaCl stress adaptation, a primary necessity is to evacuate $Na^+$ and $Cl^-$ from the cytosol by efflux to the apoplast and the vacuole. Activation of the $H^+$ pumps in the plasma membrane and tonoplast generates $\Delta\mu_H{}^+$ required for active transport of $Na^+$ across these membranes and compartmentation of $Cl^-$ in the vacuole. Concurrent with $H^+$ pump activation must be adaptations that more effectively regulate passive transport of $Na^+$ across the plasma membrane and, perhaps, passive efflux of $Na^+$ and $Cl^-$ from the vacuole. The control of passive transport processes is required to maintain $Na^+$ and $Cl^-$ gradients that result from active transport and electrophoretic flux mediated by the re-establishment of the plasma membrane $\Delta\Psi$. These new gradients can be maintained by an ion flux steady state that does not require the high level of active transport necessary for their establishment. Consequently, the energy costs are not substantially greater than before adaptation (Schnapp et al., 1991), presumably due in large part to the down-regulation of the $H^+$ pumps from the levels induced by salt exposure (Niu et al., 1993a). Therefore, membrane transport proteins and their regulators are critical salt-tolerant determinants.

The immediate research challenge is to identify and characterize the genes controlling ion homeostasis in saline environments. Plant genes encoding putative $K^+$ transport systems in the plasma membrane (Table I) (Schroeder et al., 1994) can now be used for molecular genetic and biochemical experimentation to determine if these mediate $Na^+$ uptake. Perhaps molecular modifications can be made to these proteins that would increase $K^+/Na^+$ selectivity. Current evidence indicates that $Na^+$ efflux across the plasma membrane in plants occurs via a $Na^+/H^+$ antiporter. It is not known whether a $Na^+$ pump analogous to yeast ENA exists in higher plants, but a $Na^+$-activated plasma membrane P-type ATPase has been described in the marine alga *Heterosigma akashiwo* (Wada et al., 1989), and a primary $Na^+$ pump has been implicated in the halophilic alga *D. salina* (Katz et al., 1991). Theoretically, overexpression of a plasma membrane $Na^+$ efflux transport protein from a heterologous system could establish the significance of active $Na^+$ efflux across the plasma membrane in salt adaptation. The identification of a 170-kD polypeptide as a probable constituent of the tonoplast $Na^+/H^+$ antiporter (Barkla and Blumwald, 1991) should lead eventually to the isolation of a gene critical to vacuolar compartmentation of $Na^+$.

A key factor in the genetic capacity for salt adaptation must involve an appropriately responsive and coordinated

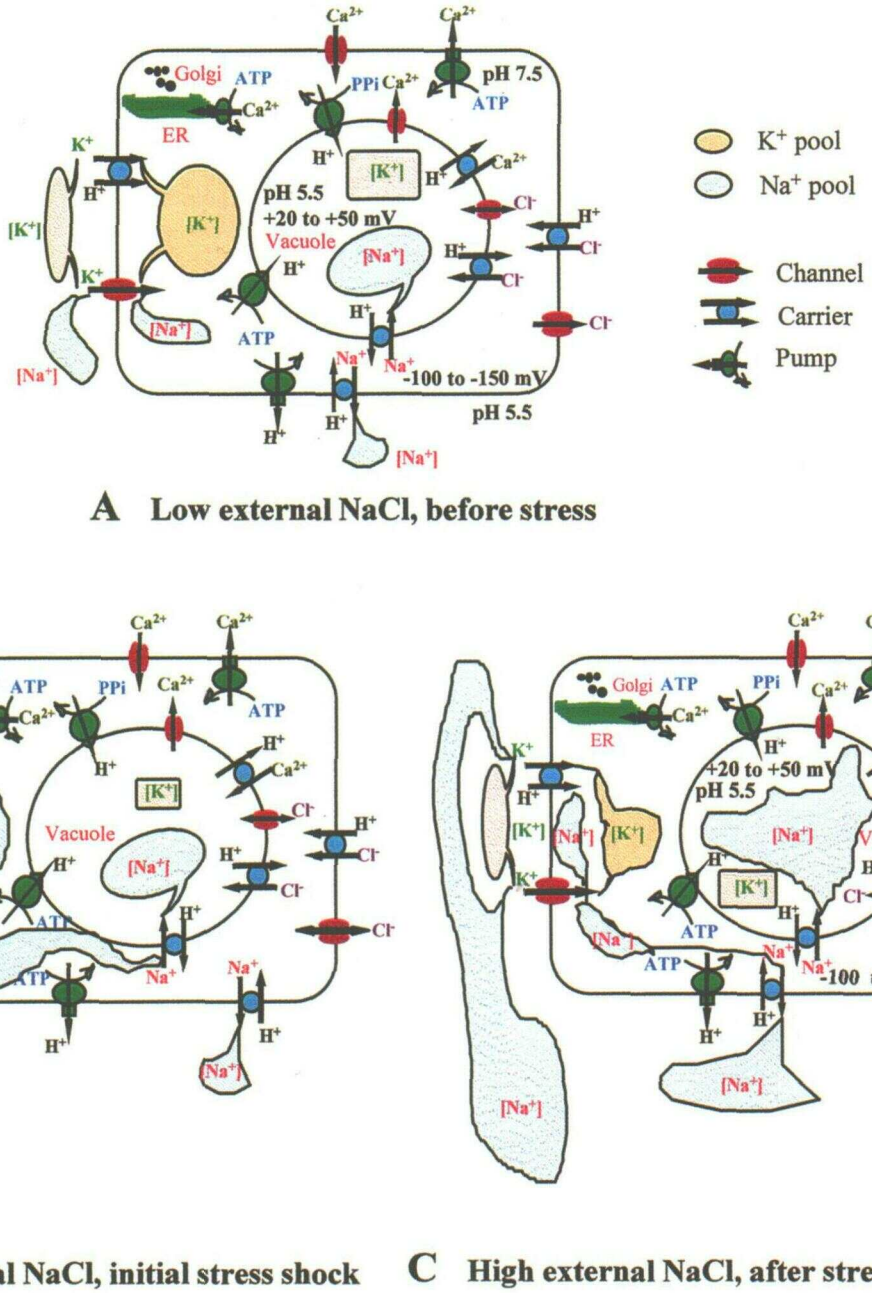


**Figure 1.** Ionic status of plant cells and proposed transport processes that control cytosolic $Na^+$ levels in low NaCl (A) and high NaCl during (B) and after (C) adaptation. Simplified diagrams illustrated plasma membrane and tonoplast pumps, carriers, and channels presumed to be involved in the regulation of $Na^+$, $Cl^-$, $K^+$, $Ca^{2+}$, and $H^+$ transport required to establish and maintain ion homeostasis in a saline environment. *Tonoplast $\Delta\Psi$ is relative to the cytosol. Illustrated are* comparisons of relative $Na^+$ and $K^+$ pools before NaCl stress, immediately after stress imposition, and after stress adaptation.

signal transduction system. As we have indicated, signaling molecules in cascades that directly regulate proteins involved in ion flux or regulate the expression of the genes that encode transport proteins are principal components of salt adaptation and probably limit the efficiency of salt adaptation in many important crop plants. Physiological evidence implicates $Ca^{2+}$ as a secondary messenger in the signal transduction of salt-stress perception to the mechanisms that control ion homeostasis in plants. In yeast, the $Ca^{2+}$/calmodulin-dependent phosphatase calcineurin is a key intermediate in the regulation of $Na^+$ influx and efflux systems (Mendoza et al., 1994) that are responsible for salt adaptation. Perhaps a functional calcineurin homolog facilitates the effect of $Ca^{2+}$ on transport processes that regulate $K^+/Na^+$ selectivity in plants. There is still little known about the genes involved in such signal transduction pathways. However, overcoming the limitations placed on ion homeostasis adaptability by a poorly respon-

sive or inadequately coordinated signal transduction system remains a major objective in understanding and achieving salt-stress tolerance in plants.

An important consideration for future research will be the possible impact of manipulating the $K^+/Na^+$ selectivity of transporters on $K^+$ nutrition. Higher $K^+/Na^+$ selectivity may reduce the ability to transport $K^+$ rapidly and thereby affect $K^+$ uptake to the extent that growth rates are compromised. An interesting speculation is that there is a relationship between $K^+/Na^+$ selectivity, $K^+$ uptake capacity, and the well-noted slow growth rates of highly $Na^+$-tolerant halophytic species. In other words, have highly $Na^+$-tolerant species sacrificed $K^+$ uptake capacity for reduced $Na^+$ uptake, thereby obtaining $Na^+$ tolerance but losing rapid growth capability? Only further research will answer these important questions.

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