Numerous Genes Are Induced during Cold Acclimation

Expression of certain genes and synthesis of specific proteins are common to both heat and cold stress, but some aspects of cold-inducible gene expression differ from that produced by heat stress (Thomashow 2001). Whereas during cold episodes the synthesis of "housekeeping" proteins (proteins made in the absence of stress) is not substantially down-regulated, during heat stress housekeeping-protein synthesis is essentially shut down.

On the other hand, the synthesis of several heat shock proteins that can act as molecular chaperones is up-regulated under cold stress in the same way that it is during heat stress. This suggests that protein destabilization accompanies both heat and cold stress and that mechanisms for stabilizing protein structure during both heat and cold episodes are important for survival.

Another important class of proteins whose expression is up-regulated by cold stress is the **antifreeze proteins**. Antifreeze proteins were first discovered in fishes that live in water under the polar ice caps. As discussed earlier, these proteins have the ability to inhibit ice crystal growth in a noncolligative manner, thus preventing freeze damage at intermediate freezing temperatures. Antifreeze proteins confer to aqueous solutions the property of *thermal hysteresis* (transition from liquid to solid is promoted at a lower temperature than is transition from solid to liquid), and thus they are sometimes referred to as **thermal hysteresis proteins** (**THPs**).

Several types of cold-induced, antifreeze proteins have been discovered in cold-acclimated winter-hardy monocots. When the specific genes coding for these proteins were cloned and sequenced, it was found that all antifreeze proteins belong to a class of proteins such as endochitinases and endoglucanases, which are induced upon infection of different pathogens. These proteins, called **pathogenesis-related** (**PR**) **proteins** are thought to protect plants against pathogens. It thus appears that at least in monocots, the dual role of these proteins as antifreeze and pathogenesis-related proteins might protect plant cells against both cold stress and pathogen attack.

Another group of proteins found to be associated with osmotic stress (see the discussion earlier in this chapter) are also up-regulated during cold stress. This group includes proteins involved in the synthesis of *osmolytes*, proteins for membrane stabilization, and the LEA proteins. Because the formation of extracellular ice crystals generates significant osmotic stresses inside cells, coping with freezing stress also requires the means to cope with osmotic stress.

A Transcription Factor Regulates Cold-Induced Gene Expression

More than 100 genes are up-regulated by cold stress. Because cold stress is clearly related to ABA responses and to osmotic stress, not all the genes up-regulated by cold stress necessarily need to be associated with cold tolerance, but most likely many of them are. Many cold stress–induced genes are activated by transcriptional activators called **C-repeat binding factors** (**CBF1**, **CBF2**, **CBF3**; also called DREB1b, DREB1c, and DREB1a, respectively) (Shinozaki and Yamaguchi-Shinozaki 2000).

CBF/DREB1-type transcription factors bind to **CRT/DRE elements** (C-repeat/dehydration-responsive, ABA-independent sequence elements) in gene promoter sequences, which were discussed earlier in the chapter. CBF/DREB1 is involved in the coordinate transcriptional response of numerous cold and osmotic stress–regulated genes, all of which contain the CRT/DRE elements in their promoters. CBF1/DREB1b is unique in that it is specifically induced by cold stress and not by osmotic or salinity stress, whereas the DRE-binding elements of the DREB2 type (discussed earlier in the section on osmotic stresses) are induced only by osmotic and salinity stresses and not by cold.

The expression of CBF1/DREB1b is controlled by a separate transcription factor, called ICE (*i*nducer of *C*BF *e*xpression). ICE transcription factors do not appear to be induced by cold, and it is presumed that ICE or an associated protein is posttranscriptionally activated, permitting activation of CBF1/DRE1b, but the precise signaling pathway(s) of cold perception, calcium signaling, and the activation of ICE are presently under investigation.

Transgenic plants constitutively expressing CBF1 have more cold–up-regulated gene transcripts than wild-type plants have, suggesting that numerous cold–up-regulated proteins that may be involved in cold acclimation are being produced in the absence of cold in these CBF1 transgenic plants. In addition, CBF1 tansgenic plants are more cold tolerant than control plants.

SALINITY STRESS

Under natural conditions, terrestrial higher plants encounter high concentrations of salts close to the seashore and in estuaries where seawater and freshwater mix or replace each other with the tides. Far inland, natural salt seepage from geologic marine deposits can wash into adjoining areas, rendering them unusable for agriculture. However, a much more extensive problem in agriculture is the accumulation of salts from irrigation water.

Evaporation and transpiration remove pure water (as vapor) from the soil, and this water loss concentrates solutes in the soil. When irrigation water contains a high concentration of solutes and when there is no opportunity to flush out accumulated salts to a drainage system, salts can quickly reach levels that are injurious to salt-sensitive species. It is estimated that about one-third of the irrigated land on Earth is affected by salt.

In this section we discuss how plant function is affected by water and soil salinity, and we examine the processes that assist plants in avoiding salinity stress.

TABLE 25.6
Properties of seawater and of good quality irrigation water

Property	Seawater	Irrigation water
Concentration of ions (m*M*)		
Na^+	457	<2.0
K^+	9.7	<1.0
Ca^{2+}	10	0.5–2.5
Mg^{2+}	56	0.25–1.0
Cl^-	536	<2.0
SO_4^{2-}	28	0.25–2.5
HCO_3^-	2.3	<1.5
Osmotic potential (MPa)	–2.4	–0.039
Total dissolved salts (mg L^{-1} or ppm)	32,000	500

Salt Accumulation in Soils Impairs Plant Function and Soil Structure

In discussing the effects of salts in the soil, we distinguish between high concentrations of Na^+, referred to as **sodicity**, and high concentrations of total salts, referred to as **salinity**. The two concepts are often related, but in some areas Ca^{2+}, Mg^{2+}, and $SO^2{}_4{}^-$, as well as NaCl, can contribute substantially to salinity. The high Na^+ concentration of a sodic soil can not only injure plants directly but also degrade the soil structure, decreasing porosity and water permeability. A sodic clay soil known as caliche is so hard and impermeable that dynamite is sometimes required to dig through it!

In the field, the salinity of soil water or irrigation water is measured in terms of its electrical conductivity or in terms of osmotic potential. Pure water is a very poor conductor of electric current; the conductivity of a water sample is due to the ions dissolved in it. The higher the salt concentration in water, the greater its electrical conductivity and the lower its osmotic potential (higher osmotic pressure) (Table 25.6).

The quality of irrigation water in semiarid and arid regions is often poor. In the United States the salt content of the headwaters of the Colorado River is only 50 mg L^{-1}, but about 2000 km downstream, in southern California, the salt content of the same river reaches about 900 mg L^{-1}, enough to preclude growth of some salt-sensitive crops, such as maize. Water from some wells used for irrigation in Texas may contain as much as 2000 to 3000 mg salt L^{-1}. An annual application of irrigation water totaling 1 m from such wells would add 20 to 30 tons of salts per hectare (8–12 tons per acre) to the soil. These levels of salts are damaging to all but the most resistant crops.

Salinity Depresses Growth and Photosynthesis in Sensitive Species

Plants can be divided into two broad groups on the basis of their response to high concentrations of salts. **Halophytes** are native to saline soils and complete their life cycles in that environment. **Glycophytes** (literally "sweet plants"), or nonhalophytes, are not able to resist salts to the same degree as halophytes. Usually there is a threshold concentration of salt above which glycophytes begin to show signs of growth inhibition, leaf discoloration, and loss of dry weight.

Among crops, maize, onion, citrus, pecan, lettuce, and bean are highly sensitive to salt; cotton and barley are moderately tolerant; and sugar beet and date palms are highly tolerant (Greenway and Munns 1980). Some species that are highly tolerant of salt, such as *Suaeda maritima* (a salt marsh plant) and *Atriplex nummularia* (a saltbush), show growth stimulation at Cl^- concentrations many times greater than the lethal level for sensitive species (Figure 25.14).

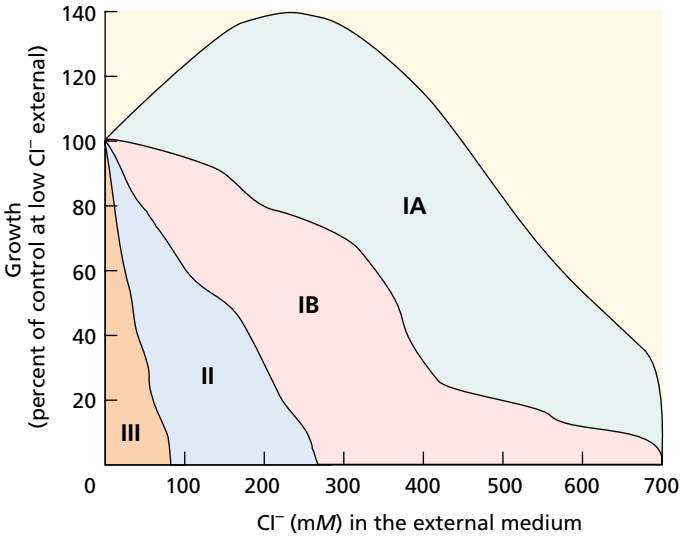
Salt Injury Involves Both Osmotic Effects and Specific Ion Effects

Dissolved solutes in the rooting zone generate a low (more negative) osmotic potential that lowers the soil water potential. The general water balance of plants is thus affected because leaves need to develop an even lower water potential to maintain a "downhill" gradient of water potential between the soil and the leaves (see Chapter 4). This effect of dissolved solutes is similar to that of a soil water deficit (as discussed earlier in this chapter), and most plants respond to excessive levels of soil salinity in the same way as described earlier for water deficit.

A major difference between the low-water-potential environments caused by salinity versus soil desiccation is the total amount of water available. During soil desiccation a finite amount of water can be obtained from the soil profile by the plant, causing ever decreasing water potentials. In most saline environments a large (essentially unlimited) amount of water at a constant, low water potential is available.

Of particular importance here is the fact that most plants can adjust osmotically when growing in saline soils. Such adjustment prevents loss of turgor (which would slow cell growth; see Figure 25.1) while generating a lower water potential, but these plants often continue to grow more slowly after this adjustment for an unknown reason that curiously is not related to insufficient turgor (Bressan et al. 1990)

In addition to the plant responses to low water potential, specific ion **toxicity effects** also occur when injurious concentrations of ions—particularly Na^+, Cl^-, or SO_4^{2-}—accumulate in cells. Under nonsaline conditions, the cytosol of higher-plant cells contains 100 to 200 m*M* K^+ and 1 to 10 m*M* Na^+, an ionic environment in which many enzymes function optimally. An abnormally high ratio of Na^+ to K^+ and high concentrations of total salts inactivate enzymes and inhibit protein synthesis. At a high concentration, Na^+ can displace Ca^{2+} from the plasma membrane of cotton root hairs, resulting in a change in plasma membrane permeability that can be detected as leakage of K^+ from the cells (Cramer et al. 1985).



Group IA (halophytes) includes sea blite (*Suaeda maritima*) and salt bush (*Atriplex nummularia*). These species show growth stimulation with Cl^- levels below 400 n*M*.

Group IB (halophytes) includes Townsend's cordgrass (*Spartina x townsendii*) and sugar beet (*Beta vulgaris*).These plants tolerate salt, but their growth is retarded.

Group II (halophytes and nonhalophytes) includes salt-tolerant halophytic grasses that lack salt glands, such as *Festuca rubra* subsp. red fescue (*littoralis*) and *Puccinellia peisonis*, and nonhalophytes, such as cotton (*Gossypium* spp.) and barley (*Hordeum vulgare*). All are inhibited by high salt concentrations. Within this group, tomato (*Lycopersicon esculentum*) is intermediate, and common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) are sensitive.

The species in **Group III (very salt-sensitive nonhalophytes)** are severely inhibited or killed by low salt concentrations. Included are many fruit trees, such as citrus, avocado, and stone fruit.

FIGURE 25.14 The growth of different species subjected to salinity relative to that of unsalinized controls. The curves dividing the regions are based on data for different species. Plants were grown for 1 to 6 months. (From Greenway and Munns 1980.)

Photosynthesis is inhibited when high concentrations of Na^+ and/or Cl^- accumulate in chloroplasts. Since photosynthetic electron transport appears relatively insensitive to salts, either carbon metabolism or photophosphorylation may be affected. Enzymes extracted from salt-tolerant species are just as sensitive to the presence of NaCl as enzymes from salt-sensitive glycophytes are. Hence the resistance of halophytes to salts is not a consequence of salt-resistant metabolism. Instead, other mechanisms come into play to avoid salt injury, as discussed in the following section.

Plants Use Different Strategies to Avoid Salt Injury

Plants minimize salt injury by excluding salt from meristems, particularly in the shoot, and from leaves that are actively expanding and photosynthesizing. In plants that are salt sensitive, resistance to moderate levels of salinity in the soil depends in part on the ability of the roots to prevent potentially harmful ions from reaching the shoots.

Recall from Chapter 4 that the Casparian strip imposes a restriction to the movements of ions into the xylem. To bypass the Casparian strips, ions need to move from the apoplast to the symplastic pathway across cell membranes. This transition offers salt-resistant plants a mechanism to partially exclude harmful ions.

Sodium ions enter roots passively (by moving down an electrochemical-potential gradient; see Chapter 6), so root cells must use energy to extrude Na^+ actively back to the outside solution. By contrast, Cl^- is excluded by negative electric potential across the cell membrane, and the low permeability of root plasma membranes to this ion. Movement of Na^+ into leaves is further minimized by absorption of Na^+ from the transpiration stream (xylem sap) during its movement from roots to shoots and leaves.

Some salt-resistant plants, such as salt cedar (*Tamarix* sp.) and salt bush (*Atriplex* sp.), do not exclude ions at the root, but instead have salt glands at the surface of the leaves. The ions are transported to these glands, where the salt crystallizes and is no longer harmful. In general, halophytes have a greater capacity than glycophytes for ion accumulation in shoot cells.

Although some plants, such as mangroves, grow in saline environments with abundant water supplies, the ability to acquire that water requires that they make osmotic adjustments to obtain water from the low-water-potential external environment. As discussed earlier in relation to water deficit, plant cells can adjust their water potential (Ψ_w) in response to osmotic stress by lowering their solute potential (Ψ_s). Two intracellular processes contribute to the decrease in Ψ_s: the accumulation of ions in the vacuole and the synthesis of compatible solutes in the cytosol.

As mentioned earlier in the chapter, compatible solutes include glycine betaine, proline, sorbitol, mannitol, pinitol, and sucrose. Specific plant families tend to use one or two of these compounds in preference to others. The amount of carbon used for the synthesis of these organic solutes can be rather large (about 10% of the plant weight). In natural vegetation this diversion of carbon to adjust water potential does not affect survival, but in agricultural crops it can reduce growth and therefore total biomass and harvestable yields.

Many halophytes exhibit a growth optimum at moderate levels of salinity, and this optimum is correlated with the capacity to accumulate ions in the vacuole, where they can contribute to the cell osmotic potential without damaging the salt-sensitive enzymes. To a lesser extent, this process also occurs in more salt-sensitive glycophytes, but the adjustment may be slower.

Besides making adjustments in water potential, plants adjusting to salinity stress undergo the other osmotic stress–related acclimations described earlier for water deficit. For example, plants subjected to salt stress can reduce leaf area and or drop leaves via leaf abscission just as during episodes of osmotic stress. In addition, changes in gene expression associated with osmotic stress are similarly associated with salinity stress. Keep in mind, however, that in addition to acclimation to a low-water-potential environment, plants experiencing salinity stress must cope with the toxicity of high ion concentrations associated with salinity stress.

Ion Exclusion Is Critical for Acclimation and Adaptation to Salinity Stress

In terms of metabolic energy, use of ions to balance tissue water potential in a saline environment clearly has a lower energy cost for the plant than use of carbohydrates or amino acids, the production of which has a significantly higher energy cost. On the other hand, high ion concentrations are toxic to many cytosolic enzymes, so ions must be accumulated in the vacuole to minimize toxic concentrations in the cytosol.

Because NaCl is the most abundant salt encountered by plants under salinity stress, transport systems that facilitate compartmentation of Na^+ into the vacuole are critical (Binzel et al. 1988). Both Ca^{2+} and K^+ affect intracellular Na^+ concentrations (Zhong and Läuchli 1994). At high concentrations of Na^+, K^+ uptake through a high-affinity K^+–Na^+ transporter, HKT1, is inhibited, and the transporter operates as an Na^+ uptake system (Figure 25.15). Calcium, on the other hand, enhances K^+/Na^+ selectivity and in so doing increases salt tolerance (Liu and Zhu 1997).

Sodium Is Transported across the Plasma Membrane and the Tonoplast

As discussed in Chapter 6, H^+ pumps in the plasma membrane and tonoplast provide the driving force (H^+ electro-

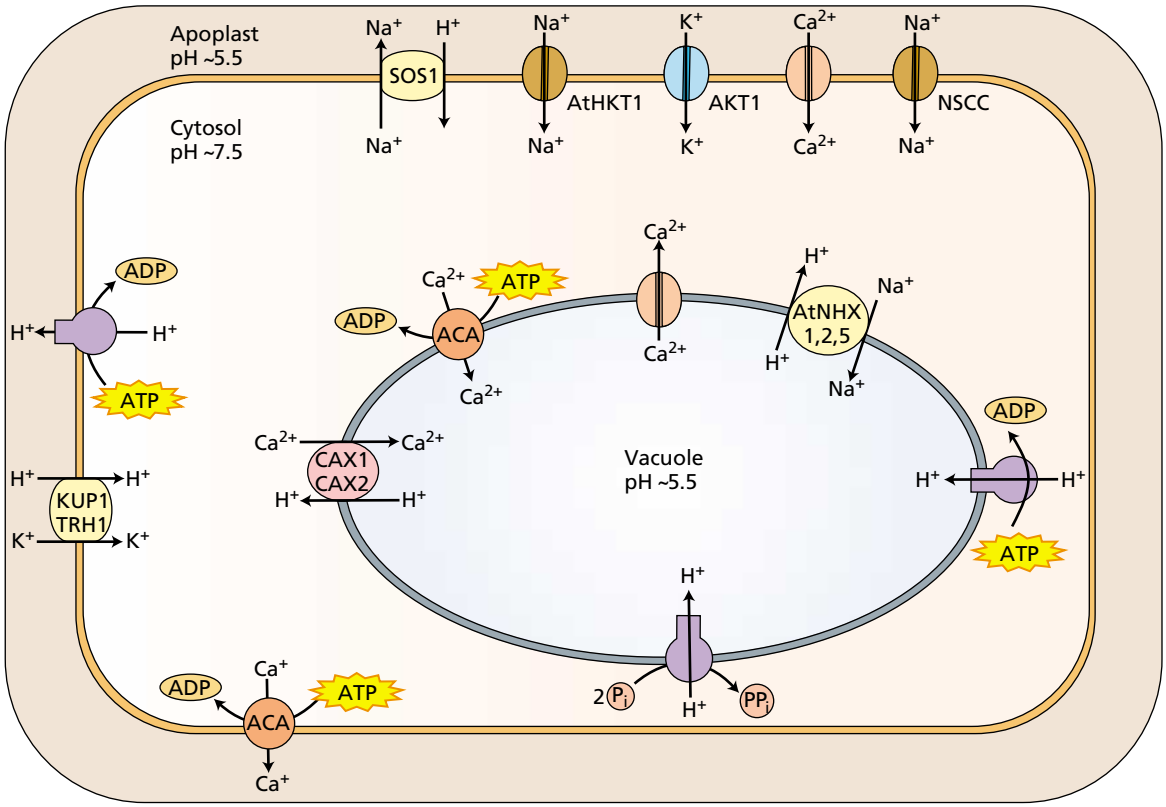


FIGURE 25.15 Membrane transport proteins mediating sodium, potassium, and calcium transport during salinity stress. SOS1, plasma membrane Na^+/H^+ antiporter; ACA, plasma/tonoplast membrane Ca^{2+}-ATPase; KUP1/TRH1, high-affinity K^+-H^+ co-transporter; atHKT1, sodium influx transporter; AKT1, K^+_{in} channel; NSCC, non selective cation channel; CAX1 or 2, Ca^{2+}/H^+ antiporter; atNHX1, 2 or 5, endomembrane Na^+/H^+ antiporter. Also indicated in the figure are proteins that have been implicated in ion homeostasis, but whose molecular identity is either not presently known or cofirmed in plants. These include plasma membrane and tonoplast calcium channel proteins, and vacuolar proton-pumping ATPases and pyrophosphatases. The membrane potential difference across the plasma membrane is typically 120 to 200 mV, negative inside; across the tonoplast 0 to 20 mV; positive inside.

chemical potential) for secondary transport of ions (see Figure 25.15). An ATPase is primarily responsible for the large ΔpH and membrane potential gradient found across the plasma membrane. A vacuolar H^+-ATPase generates a ΔpH and membrane potential across the tonoplast (Hasegawa et al. 2000).

Activity of these pumps is required for the secondary transport of excess ions associated with plant responses to salinity stress. This is indicated by findings showing that the activity of these H^+ pumps is increased by salinity, and induced gene expression may account for some of this up-regulation.

Energy-dependent transport (efflux) of Na^+ from the cytosol of plant cells across the plasma membrane is mediated by the gene product of the *SOS1* (*s*alt *o*verly *s*ensitive *1*) gene that function as a Na^+–H^+ antiporter (Figure 25.16). The SOS1 antiporter is regulated by the gene products of at least two other genes, referred to as *SOS2* and *SOS3* (Shi et al. 2000). SOS2 is a serine/threonine kinase that is apparently activated by calcium through the function of SOS3, a calcium-regulated protein phosphatase (see **Web Topic 25.4** for details on Ca^{2+} signaling and the *SOS* gene family).

Vacuolar compartmentation of Na^+ results in part from the activity of a family of Na^+–H^+ antiporters such as *Arabidopsis* AtNHX1 (see Figure 25.15). Transgenic *Arabidopsis* and tomato plants overexpressing the gene that encodes AtNHX1 exhibit enhanced salt tolerance (Apse et al. 1999; Quintero et al. 2000). (See **Web Topic 25.5** for details on molecular studies of Na^+ compartmentation.) These molecular findings are another example of the wealth of new information emerging from studies on transgenic plants, gene sequencing, and protein characterization (see **Web Topic 25.6** for details on work with transgenic plants for stress studies).

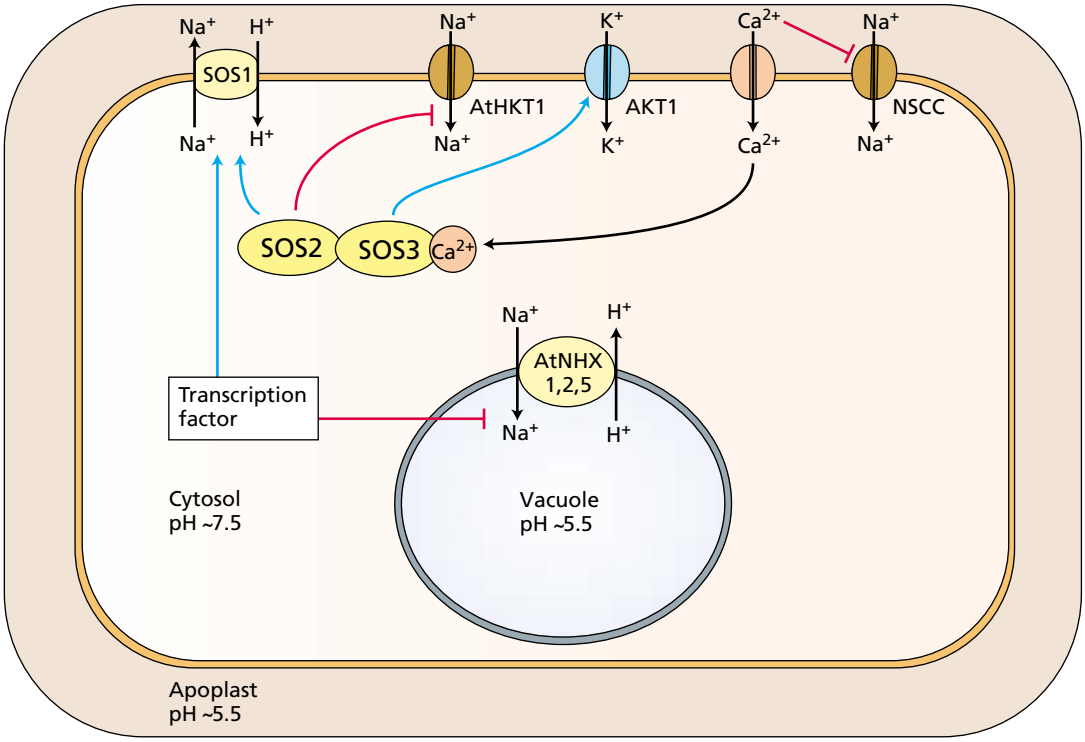


FIGURE 25.16 The regulation of ion homeostasis by the SOS signal transduction pathway, salinity stress, and calcium levels. Red arrows indicate positive regulation of the effected transport protein while blue arrows indicate negative regulation. Proteins shown in yellow are activated by salinity stress. SOS1, plasma membrane Na^+/H^+ antiporter; SOS2, serine/threonine kinase; SOS3, Ca^{2+} binding protein; HKT1, sodium influx transporter; AKT1, K^+_{in} channel; NSCC, non selective cation channel; NHX1, 2 or 5, endomembrane Na^+/H^+ antiporter; shown in orange is an undertermined calcium channel protein. Salinity stress activates a calcium channel leading to an increase in cytosolic calcium that activates the SOS cascade through SOS3. The SOS cascade must negatively regulate HKT1 which in turn secondarily regulates AKT1. At the same time, the SOS cascade increases the activity of SOS1 and AKT1. Working through an as yet undefined transcription factor the SOS cascade increases transcription of SOS1 while decreasing transcription of NHX gene(s). At low calcium NSCC can also function as an alternative sodium influx system, but this transporter is inhibited at high calcium levels. The membrane potential difference across the plasma membrane is typically 120 to 200 mV, negative inside, that of the tonoplast is 0 to 20 mV, positive inside.