Plant Physiol. (1992) 100, 1269–1276
0032-0889/92/100/1269/08/$01.00/0

Received for publication March 13, 1992
Accepted June 27, 1992

De Novo Synthesis of Plasma Membrane and Tonoplast Polypeptides of Barley Roots during Short-Term K^+ Deprivation[1]

In Search of the High-Affinity K^+ Transport System

Mala Fernando, Jarnail Mehroke, and Anthony D. M. Glass*

Department of Botany, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

ABSTRACT

[^{35}S]Methionine labeling of intact barley roots (*Hordeum vulgare* cv Klondike) after short (6–12 h) and longer (18–24 and 90–96 h) periods of K^+ deprivation revealed that several membrane polypeptides were synthesized in significantly increased amounts following withdrawal of K^+ from nutrient solutions. One of these, a 43-kD polypeptide localized in plasma membrane- and tonoplast-enriched fractions, accounted for a large part of ^{35}S incorporation into membranes when [^{35}S]methionine was administered for 6 h following 6 h of K^+ deprivation. With increasing duration of K^+ deprivation, ^{35}S incorporation into this 43-kD polypeptide decreased. This polypeptide, referred to as KR43, was not synthesized when NO_3^- or inorganic phosphate was removed or when Rb^+ was substituted for K^+. However, it was synthesized when K^+ was removed and replaced by an equivalent concentration of Na^+. The intrinsic nature of this polypeptide and the time course of changes in its expression, which correspond with changes of K^+(^{86}Rb) influx associated with K^+ deprivation, provide evidence that this polypeptide may form part of the high-affinity K^+ transport system in barley roots. A possible role for this polypeptide is discussed in the context of changes in the subcellular distribution of K^+ in barley roots following interruption of K^+ supply. A 45-kD microsomal polypeptide, identified in earlier studies as a response to K^+ deprivation, is suggested to be an extrinsic protein, readily displaced from membranes by exposure to ethylenediaminetetraacetate.

As early as 1906, Brezeale (1) demonstrated that withholding K^+ from hydroponically grown wheat plants caused a >3-fold increase of net K^+ uptake. The same author also examined the effects of withholding other ions (including Ca^{2+}, NO_3^-, and Pi) as well as interactions between Na^+ and K^+. The phenomenon of acclimation of uptake in response to ion deprivation has been reexamined extensively, beginning with Hoagland and Broyer (13), who have generally been assumed to be responsible for its discovery in the context of their "low salt" (high-sugar, high-uptake) barley (*Hordeum vulgare*) roots. Several characteristics of this phenomenon have been investigated, including the identification of influx rather than efflux as responsible for the increased uptake (8, 15, 19), the time courses of responses to K^+ withdrawal or restoration (9, 10, 31), the kinetic rather than thermodynamic nature of the regulation of influx (11), the changes of kinetic constants, V_{max} and K_m, associated with withholding K^+ (2, 8), the nature of the feedback signals responsible for regulating influx (2, 25, 28), and whole plant aspects of regulation (2, 24, 28).

Notwithstanding these studies and the many unsubstantiated hypotheses they have generated concerning the mechanisms responsible for regulating K^+ influx, biochemical details of the high-affinity transport system itself are virtually nonexistent. The paucity of biochemical information regarding the regulation of this system is perhaps not surprising, considering the lack of a consensus concerning the nature of the mechanism of the high-affinity system for K^+ uptake (K^+:H^+ antiport, K^+:H^+ symport, K^+ uniport or channel, or K^+-ATPase).

In *Escherichia coli*, a specific K^+-ATPase known as the Kdp system is responsible for the high-affinity K^+ uptake. This enzyme system, consisting of α, β, and γ subunits, is derepressed under conditions of K^+ deprivation (29). In yeast, also, there is genetic evidence that K^+ uptake is mediated by a high-affinity K^+-ATPase known as the Trk system (6).

A 45-kD microsomal polypeptide (referred to as KR45) was identified in previous studies reported from this laboratory (4, 5) as a response to prolonged (1–6 d) K^+ deprivation in barley roots. Expression of this polypeptide was increased or decreased, respectively, as K^+ was withdrawn or resupplied to hydroponically grown barley plants. KR45 was a specific response to K^+ deprivation; it was not evoked by withholding NO_3^- or Pi. A possible role for this polypeptide as a component of the high-affinity transport system for K^+ influx across the plasma membrane has been suggested (3).

Further investigations of this polypeptide indicated that it was an extrinsic protein whose time course of synthesis, during short-term periods of K^+ deprivation, failed to correspond to the time course of increased K^+ influx. [^{35}S]Methionine labeling of barley root membranes revealed increased expression of several polypeptides, with a 43-kD intrinsic

[1] This research was supported by a National Sciences and Engineering Research Council of Canada Operating Grant and a Potash Phosphate Institute/National Sciences and Engineering Research Council of Canada University-Industry Research Partnership Grant to A.D.M.G. The support of both agencies is gratefully acknowledged.

protein being the most prominent. Synthesis of this polypeptide (referred to as KR43) was most rapid from 6 to 12 h after removal of K^+, corresponding to the period when K^+ influx increases most rapidly.

MATERIALS AND METHODS

Growth of Plants

Barley (*Hordeum vulgare* L. cv Klondike), oat (*Avena sativa* L. cv Jasper), and corn (*Zea mays* L. cv G-4066) plants were germinated in sand and grown for 3 to 5 d in hydroponic solutions containing one-fifth strength Johnson's modified inorganic medium containing 1.2 mM K^+ (27). Plants were then transferred to solutions lacking K^+ (−K treatment) or allowed to continue in complete solutions (+K treatment) for additional periods of up to 96 h.

Labeling with [^{35}S]Methionine

Roots of intact plants were exposed to [^{35}S]methionine for 6 h in the period from 6 to 12, 18 to 24, or 90 to 96 h after removal of K^+. In each case, the appropriate nutrient solutions were supplemented with 37 MBq of ^{35}S-labeled methionine (carrier free). In some experiments designed to evaluate the specificity of the response to K^+ deprivation, NO_3^- or Pi was removed from the nutrient solutions for 12 h and [^{35}S]methionine labeling undertaken (as in the −K treatments) during the period from 6 to 12 h after withdrawal of the particular ion. In another variation of this method, −K conditions were established, but either Na^+ or Rb^+ replaced K^+ to determine whether either of these ions was capable of suppressing the K^+ response.

Membrane Isolation and Purification of KR43

Microsomal membrane preparations and plasma membrane- and tonoplast-enriched fractions were isolated using standard methods (3). Following the labeling period, root tissue (40 g) was briefly washed in ice-cold distilled water, finely cut with scissors, and ground in ice-cold homogenizing buffer (80 mL). This consisted of 0.25 M sucrose, 5 mM EGTA, 0.2 mM PMSF, 2 mM SHAM, 1 mM DTT, 1.5% soluble PVP, and 25 mM Tris-Mes, pH 7.6. All isolation procedures were conducted at 4°C. Homogenates were strained through cheesecloth and centrifuged at an average 100*g* for 20 min in a GSA rotor. The resulting supernatant was centrifuged at an average 10,000*g* for 20 min in a Sorvall SS 34 rotor to remove mitochondria and nuclei, before a final centrifugation at an average 80,000*g* for 45 min in a Beckman SW 27 rotor to obtain the microsomal fraction.

The microsomal preparation (1 or 2 mL) was layered on a sucrose gradient consisting of 15, 30, 38, and 45% sucrose in 1 mM Tris-Mes (pH 7.3), 1 mM EDTA, 2 mM PMSF, 1 mM DTT, and 10 mM KCl, and the gradient was centrifuged at an average 80,000*g* in a Beckman SW 27 rotor for 2 h. The plasma membrane- and tonoplast-enriched fractions were recovered from the 34 to 45% and the 15 to 30% interphases, respectively (3). These fractions were tested for e.r.[2] contamination using NADH Cyt *c* reductase activity (22).

Electrophoresis and Autoradiography

SDS-PAGE was performed using 10% gels. Equal amounts of protein or equal counts of ^{35}S were solubilized at 70°C for 20 min in 50 μL of sample buffer. Stacking and separation of polypeptides were achieved at 12 and 25 mA, respectively, for 6 to 10 h. Polypeptides were stained with Coomassie blue, and radioactivity was revealed by exposure to Kodak X-OMAT 2R film after exposure of the gel to "AMPLIFY" (Amersham) for 15 min.

Triton X-114 Fractionation of Intrinsic Polypeptides

Membrane polypeptides were partitioned between two phases of a two-phase (aqueous/detergent) system for 15 min at 37°C. Intrinsic proteins, by virtue of their hydrophobicity, partition into the detergent phase, whereas extrinsic proteins enter the aqueous phase.

RESULTS AND DISCUSSION

Withholding K^+ from growth media for 1 to 6 d caused increased expression of a 45-kD polypeptide, referred to as KR45 (Fig. 1) as described in previous communications (4, 5). This polypeptide was displaced from its association with microsomal membranes following resuspension of microsomal vesicles in resuspension buffer or during sucrose gradient centrifugation to obtain tonoplast- and plasma membrane-enriched membrane fractions. We rejected the possibility that KR45 was a soluble protein contaminant of microsomal membranes because KR45 was not evident among proteins of the soluble fraction. A careful investigation of the components of the resuspension buffer that might be responsible for this loss revealed that EDTA was the causative agent.

Figure 2 shows typical one-dimensional gels of microsomal proteins from low-K^+ roots, stained with Coomassie blue, after washing in the presence or absence of 1 mM EDTA. Lane 2 (+EDTA) is significantly depleted of KR45. Thus, KR45 is probably loosely associated with microsomal membranes and, possibly, is a peripheral protein. Binding proteins (siderophores for micronutrients such as iron) are known to be produced in response to a shortage in micronutrient supply; KR45 may be such a protein. In the present case, Mg^{2+} or Ca^{2+} appears to be critical for the membrane association of this polypeptide. When released from its membrane association, KR45 appeared to be sensitive to proteolysis because it was not possible to locate this polypeptide in the supernatant after treating microsomal vesicles in EDTA-containing buffers.

When microsomal membrane preparations were subjected to further purification by sucrose gradient centrifugation in buffers lacking EDTA, yields of KR45 in plasma membrane-

[2] Abbreviations: e.r., enriched fraction; $\Delta\psi$, transmembrane electrical potential difference; pmf, proton motive force; $\Delta\tilde{\mu}_{K^+}$, electrochemical potential difference for K^+ between apoplasm and cytoplasm (co), vacuole and cyroplasm (cv), and cytoplasm and xylem (cx).

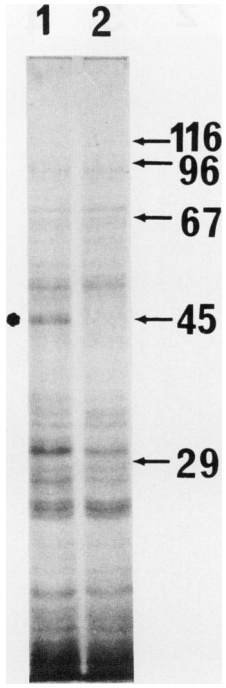

Figure 1. Microsomal proteins from barley roots separated by SDS-PAGE and stained with Coomassie blue. Lane 1, Low-K^+ roots (6 d without K^+); lane 2, high-K^+ roots (K^+ supplied continuously at 1.2 mM). Arrows, Molecular mass in kD.

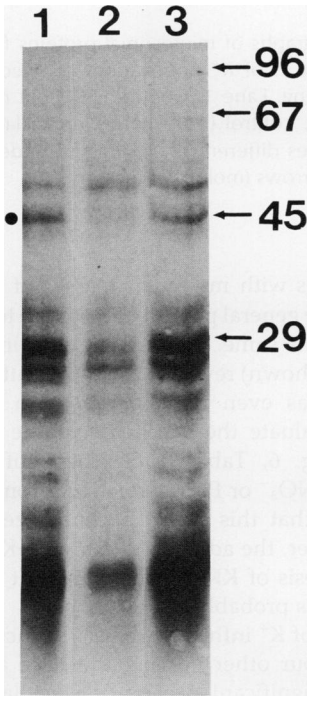

Figure 2. SDS-PAGE of microsomal proteins from roots of low-K^+ plants stained with Coomassie blue. Lane 1, Unwashed microsomes; lane 2, microsomes washed in buffer containing 1 mM EDTA; lane 3, microsomes washed in buffer lacking EDTA. Arrows, Molecular mass in kD.

and tonoplast-enriched fractions were too low to detect with confidence. As a consequence, [^{35}S]methionine labeling of KR45 was attempted to increase the sensitivity of detection.

Autoradiographs of microsomal proteins separated by one-dimensional SDS-PAGE were prepared from roots of plants labeled with ^{35}S for 6 h following various periods of K^+ deprivation. These ranged from 0 h (controls) to 6, 18, and 90 h. In control experiments, K^+ concentrations were maintained at 1.2 mM throughout the ^{35}S-labeling period, whereas in all other experiments ($-K^+$ for 6, 18, and 90 h), roots remained in $-K^+$ solutions for the labeling period. Autoradiographs (Fig. 3) showed no evidence of ^{35}S labeling of KR45. We conclude that the rate of synthesis of this polypeptide is relatively low compared with several other membrane polypeptides.

Of the several microsomal polypeptides that became differentially labeled in response to K^+ deprivation, a 43-kD polypeptide (designated KR43) was most prominent. This is evident in Figure 3 showing autoradiographs obtained from SDS-PAGE gels of the various +K and −K treatments. The period of most rapid synthesis of KR43 corresponded with the period of greatest increase in K^+ influx following removal of K^+ from growth media (3, 7). Incorporation of ^{35}S label into this polypeptide when [^{35}S]methionine was applied after longer periods of K^+ deprivation revealed that by 24 and 90 h, respectively, the synthesis of KR43 had declined sig-

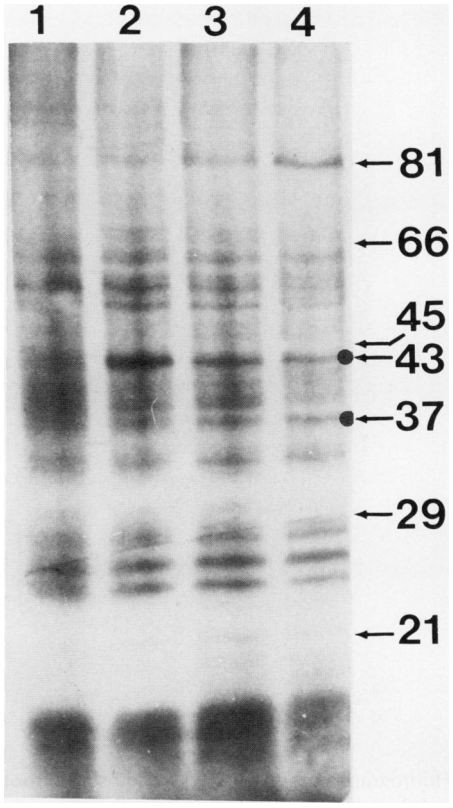

Figure 3. Fluorographs of microsomal proteins from barley roots labeled with [^{35}S]methionine for a total of 6 h following 0 (control, lane 1), 6 (lane 2), 18 (lane 3), or 90 h (lane 4) of K^+ deprivation. Arrows, Molecular mass in kD.

nificantly. This corresponds to the leveling off of K^+ influx between 24 and 36 h after removing K^+ from nutrient media (3).

KR43 was not displaced from microsomal membranes by exposure to EDTA, and it was possible to obtain both plasma membrane- and tonoplast-enriched fractions by sucrose gradient centrifugation in which KR43 was evident (Fig. 4). Based on NADH Cyt *c* reductase assays, these fractions were found to be virtually devoid of contamination by e.r. Partitioning of ^{35}S-labeled microsomal preparations in a two-phase (aqueous/detergent) system resulted in the distribution of KR43 to the detergent phase. Thus, KR43 has the characteristics anticipated for an intrinsic polypeptide.

Four other microsomal polypeptides were specifically labeled by [^{35}S]methionine in response to K^+ deprivation in the 6- to 12-h period. These included polypeptides with molecular masses of 23, 35, 71, and 81 kD. These are particularly evident in Figure 5, although the major change in labeling pattern was in KR43. Lanes 1 and 2 also include data from parallel studies in which oat roots were used. In general, the pattern of labeling in response to 12 h of K^+ deprivation was very similar. In particular, KR43 was strongly labeled, as were

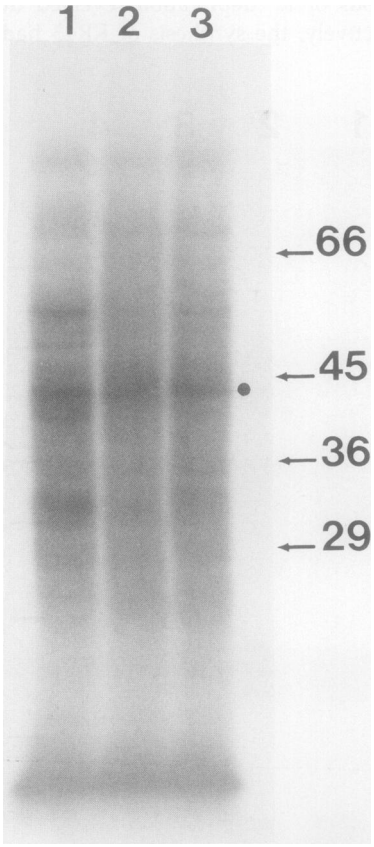

Figure 4. Fluorographs of microsomal proteins from roots deprived of K^+ from 6 h (labeled with [^{35}S]methionine from 6–12 h) and fractionated by sucrose gradient centrifugation. Lane 1, Tonoplast-enriched fraction (15–30% interphase); lane 2, plasma membrane-enriched fraction (34–45% interphase); lane 3, e.r. Arrows, Molecular mass in kD.

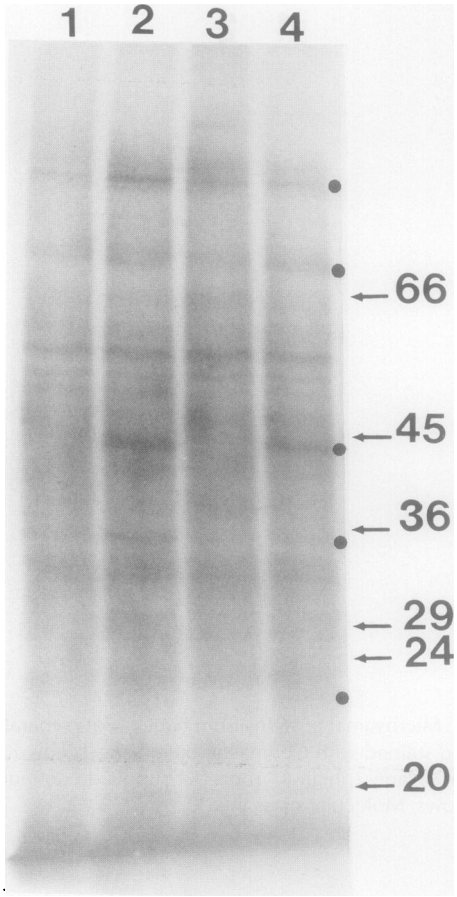

Figure 5. Fluorographs of microsomal proteins from roots of oats and barley after 6 h of K^+ deprivation followed by 6 h of [^{35}S]-methionine labeling. Lane 1, Control ($+K^+$) oat roots; lane 2, $-K^+$ oat roots; lane 3, control ($+K^+$) barley roots; lane 4, $-K^+$ barley roots. Polypeptides differentially expressed under $-K^+$ conditions are shown with arrows (molecular mass in kD).

the polypeptides with molecular masses of 81, 71, 35, and 23 kD. Thus, the general pattern of response to K^+ deprivation appears to be the same. Subsequent experiments with corn roots (data not shown) revealed the same pattern of response. This pattern was even more apparent in the experiment designed to evaluate the specificity of the response to K^+ withdrawal (Fig. 6, Table I). Synthesis of KR43 did not increase when NO_3^- or Pi was removed from growth media, demonstrating that this is not a generalized ion-stress response. Moreover, the addition of Na^+ to $-K$ plants failed to suppress synthesis of KR43, indicating that the response to K^+ deprivation is probably not osmotic.

The increase of K^+ influx as well as the increased synthesis of KR43 and four other polypeptides (see above) occurred well before a significant decline of vacuolar [K^+] could be detected (Table I). Rb^+ was able to repress synthesis of KR43 as well as the increase of K^+ influx, indicating that Rb^+ may be equivalent to K^+ not only at the influx step (10) but also at the level of repressing protein synthesis. In all the short-term labeling experiments, KR43 was always evident as a

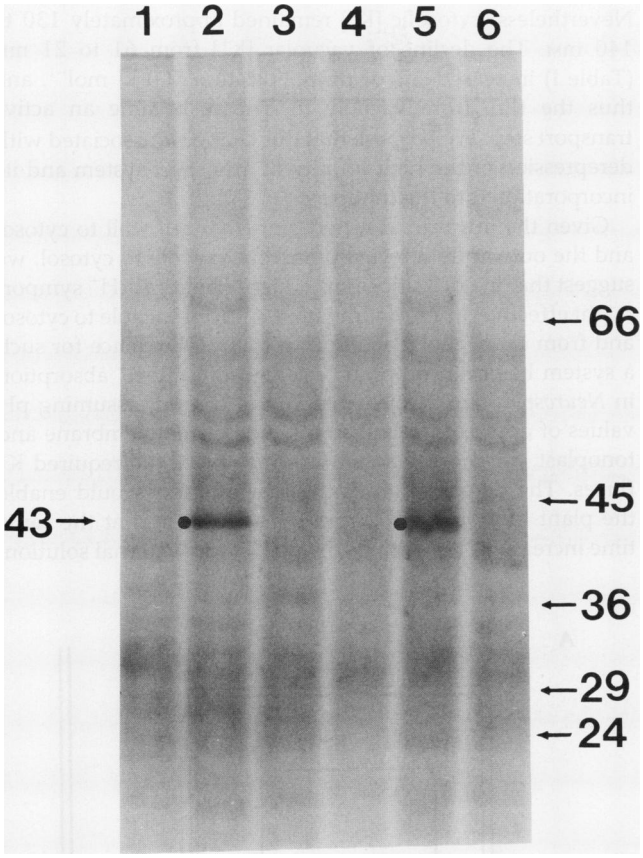

Figure 6. Effects of withdrawing or supplementing the external medium with various ions for the 6 h before and during the 6 h ^{35}S-labeling period. Lane 1, Control (+K^+); lane 2, −K^+; lane 3, −NO_3^-; lane 4, −Pi; lane 5, −K^+ + 1.2 mM Na^+; lane 6, −K^+ + 1.2 mM Rb+. Arrows, Molecular mass in kD.

prominently labeled polypeptide, although not as conspicuously as in Figure 6.

Thermodynamic Considerations

It is evident from our earlier studies of the subcellular distribution of K^+ in roots of barley (19) that cytoplasmic [K^+] tends to be held at values close to 150 mM at the expense of vacuolar [K^+], as proposed by Leigh and Wyn Jones (18). It has, therefore, been argued (30) that changes of vacuolar [K^+] trigger subsequent changes of K^+ influx. Nevertheless, an interruption of K^+ supply must inevitably lead to short-term perturbations of cytoplasmic [K^+], primarily because of the continued supply of K^+ to the stele (14). These perturbations may play some part in triggering the series of events leading to the acclimation of influx across the plasma membrane and efflux across the tonoplast.

Indeed, although the negative correlations between tissue [ion] (i.e. vacuolar [ion]) and plasmalemma influx have been used to argue that the vacuole (rather than cytoplasm) is the source of the regulatory signal(s) responsible for acclimation, it is evident from short-term studies of K^+ influx following changes in external [K^+] (5, 10, 11, 31) that changes of K^+ influx appear to occur too rapidly to be associated with large changes in vacuolar [ion]. Moreover, it is difficult to envisage the long-term maintenance of cytoplasmic [K^+] at some prescribed value without feedback from cytoplasmic [K^+] itself. If vacuolar [K^+] is exclusively responsible for regulating K^+ fluxes, then changes evoked by K^+ deprivation must be extremely sensitive to vacuolar [K^+]. This appears to be the case; after 12 h of K^+ deprivation, synthesis of KR43 was significantly elevated, K^+ influx had increased 2.75-fold, and tissue (vacuolar) [K^+] had declined by only 14% (Table I). In view of these arguments, it is probable that both cytoplasmic and vacuolar compartments participate in the regulation of K^+ influx.

We considered the distributions of K^+ in subcompartments of the root and electrical potential differences across major membranes to calculate approximate values of the thermodynamic driving forces for the major K^+ fluxes between subcompartments before and after removal of exogenous K^+ (Table II). Concentration terms and typical values for $\Delta\psi$ are taken from the literature and thus should be viewed as indicators of the directions of the passive driving forces rather than absolute values. Consider roots of barley plants grown in one-fifth strength Johnson's medium containing 1.2 mM K^+. Table II indicates that under these conditions $\Delta\tilde{\mu}_{K^+co}$ (−0.5 kJ mol^{-1}) is in the direction to permit passive entry of K^+ across the plasma membrane into the cytosol. Kinetic evidence from flux measurements in corn roots (17) and electrophysiological evidence from patch-clamp studies of *Vicia faba* stomata and corn roots have demonstrated the existence of plasma membrane K^+ channels (12, 16). Kinetic studies have demonstrated that when barley is grown in 1 mM K^+, the high-affinity K^+ transport system, which operates at low external K^+ concentrations and is considered to be an active transport system (Table II), is strongly repressed (2, 8, 10, 28). For example, K^+ influx, measured at 100 μM [K^+], is reduced >20-fold by this level of K^+ pretreatment. Likewise, in *E. coli*, growth at high external [K^+] causes repression of

Table I. *Effects of Various Pretreatments on K^+ Influx, Root [K^+], and Synthesis of KR43*

Plants were grown for 3 d in complete nutrient solution containing 1.2 mM K^+. They were then transferred to solutions supplemented or depleted of various ions, as shown, for a total period of 12 h. Six hours after the transfer, the same solutions were supplemented with 37 MBq of ^{35}S-labeled methionine (carrier free). Twelve hours after transfer, ^{86}Rb (K^+) influx was measured from complete nutrient medium containing 100 μM [K^+], and root [K^+] was measured by flame photometry.

Pretreatment	86Rb (K^+) Influx	Root [K^+]	KR43 Synthesis
	$\mu mol\ g^{-1}\ h^{-1}$	$\mu mol\ g^{-1}$	
+K^+ (control)	0.57[a]	108[a]	−
−K^+	1.57	93	+
+K^+−NO_3^-	0.1	113	−
+K^+−Pi	0.48	99	−
−K^++Na^+	1.26	89	+
−K^++Rb^+	0.33	90	−

[a] Standard errors were all within 5% of the mean.

Table II. *Estimates of the $\Delta\tilde{\mu}_{K^+}$ between Root Apoplasm and Cytoplasm ($\Delta\tilde{\mu}_{K^+co}$), between Vacuole and Cytoplasm ($\Delta\tilde{\mu}_{K^+cv}$), and between Cytoplasm and Xylem Sap ($\Delta\tilde{\mu}_{K^+cx}$)*

Positive values for $\Delta\tilde{\mu}_{K^+co}$, $\Delta\tilde{\mu}_{K^+cv}$, and $\Delta\tilde{\mu}_{K^+cx}$ are indicative of a requirement for active transport for net transfer of K^+ from apoplasm to cytoplasm, vacuole to cytoplasm, and cytoplasm to xylem, respectively. Sources of literature values selected for parameters shown are indicated by the references in parentheses.

Cell Parameter	External [K^+] of Growth Medium in mmol L^{-1}		
	1.2	0.1	0.01
[K^+] in cytoplasm in mmol L^{-1} (19)	144	140	131
[K^+] in vacuole in mmol L^{-1} (19)	85	61	21
[K^+] in xylem sap in mmol L^{-1} (20)	6	6	
$\Delta\psi_{co}$ in mV (21, 12)	−126	−138	−150
$\Delta\psi_{cv}$ in mV (21)	+15	+15	+15
$\Delta\tilde{\mu}_{K^+co}$ in kJ mol^{-1}	−0.5	+4.3	+8.6
$\Delta\tilde{\mu}_{K^+cv}$ in kJ mol^{-1}	−0.2	+0.5	+3.0
$\Delta\tilde{\mu}_{K^+cx}$ in kJ mol^{-1}	+2.2	+2.2	

the high-affinity Kdp system for K^+ absorption (29). Thus, at high external [K^+], when the high-affinity transport system is repressed, the entry of K^+ into barley roots might readily occur down the electrochemical gradient via K^+ channels.

K^+ distribution between cytoplasm and vacuole under these conditions is close to equilibrium ($\Delta\tilde{\mu}_{K^+cv}$ = −0.2 kJ mol^{-1}). Hence, redistribution of K^+ from vacuole to cytoplasm could be passive initially, perhaps via the nonspecific tonoplast (F.V. type) channels (12) when the external supply of K^+ is removed. Hooymans (14) demonstrated that following removal of external K^+ there was continued transport of K^+ to the shoots of intact barley until root K^+ was reduced to extremely low levels approximately 32 h after withdrawal of K^+. Transport of K^+ from cytosol to stele in the absence of incoming K^+ external solution would be anticipated to generate a transient reduction of cytoplasmic [K^+] (see above) and a favorable gradient for a passive flux of K^+ from vacuole to cytosol. This flux would tend to maintain cytoplasmic [K^+] at the expense of vacuolar [K^+], while sustaining net transport to the shoot.

The reduction of external [K^+] from 1.2 mM to 100 μM or less thus causes significant changes in the gradients of K^+ between apoplasm and cytoplasm and between vacuole and cytoplasm. At 10 or 100 μM [K^+] $\Delta\tilde{\mu}_{K^+co}$ is +8.6 or +4.3 kJ mol^{-1}, respectively, and K^+ entry into the cytosol from external solution is thermodynamically uphill. We propose that these treatments lead to derepression of the high-affinity K^+ transport system and its incorporation into the plasma membrane.

Despite the increased capacity for plasma membrane K^+ influx resulting from derepression of the high-affinity system, net absorption of K^+ from a 10-μM solution may be insufficient to satisfy plant demand for K^+, and vacuolar [K^+] is reduced. For example, when external [K^+] was lowered to 10 μM (19), vacuolar [K^+] declined from 61 to 21 mM in barley. Nevertheless, cytosolic [K^+] remained approximately 130 to 140 mM. The decline of vacuolar [K^+] from 61 to 21 mM (Table I) increased $\Delta\tilde{\mu}_{K^+cv}$ from −0.5 to + 3.0 kJ mol^{-1}, and thus the flux from vacuole to cytosol became an active transport step. We propose that this change is associated with derepression of the high-affinity K^+ transport system and its incorporation into the tonoplast.

Given the inwardly directed pmf from cell wall to cytosol and the outwardly directed pmf from vacuole to cytosol, we suggest that in energetic terms a high-affinity K^+:H^+ symport might effectively serve to transport K^+ from vacuole to cytosol and from extracellular solution to cytosol. Evidence for such a system has been proposed for high-affinity K^+ absorption in *Neurospora* (26). Calculated values of pmf, assuming pH values of approximately 2 across the plasma membrane and tonoplast, are more than sufficient to drive the required K^+ fluxes. The derepression of these symporters would enable the plant to mobilize K^+ from the vacuole and at the same time increase its capacity to absorb K^+ from external solution.

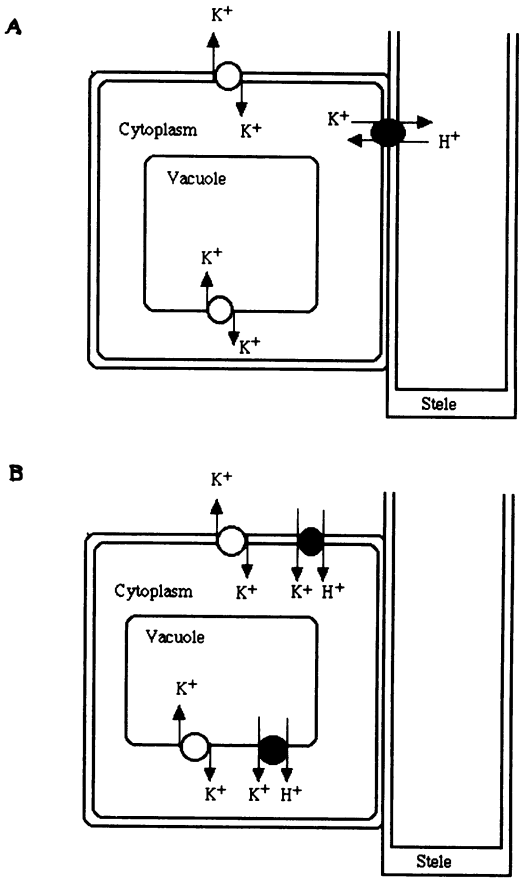

Figure 7. Model of the regulation of K^+ fluxes between various subcompartments of the root. A, Under conditions of high external [K^+] the putative high-affinity (K^+/H^+ symport) transport system is repressed. B, Transfer to conditions of low external [K^+] results in the insertion of this system into plasma membrane and tonoplast membranes. Under extreme conditions of K^+ deprivation (several days without K^+), the putative K^+:H^+ antiport responsible for loading K^+ into the stele is repressed. Proton:K^+ porters are represented by black circles; K^+ channels are represented by open circles.

Measurements of xylem [K^+] in barley are in the range of 6 mM (20), hence the transport step from cytosol to xylem apoplasm is uphill, requiring approximately 2.2 kJ mol^{-1} because of the negative membrane potential of the plasma membrane at the root cytosol/xylem interface. Provided that cytoplasmic [K^+] is maintained at approximately 140 mM, the redistribution of K^+ from vacuole to stele, via the cytosol, will not alter the energetic requirement for the xylem loading step.

When vacuolar [K^+] is reduced to approximately 20 mM, the transfer of K^+ to the stele is terminated in barley (10, 14). Resupply of K^+ to these plants caused root [K^+] to increase approximately 4-fold in the following 6 to 8 h without significant increase of shoot [K^+] (10). After 6 to 8 h, when root [K^+] began to plateau, the translocation of K^+ to the shoot increased substantially. We propose that these changes correspond to the activation or de novo synthesis of a K^+ transport system that is distinct from the plasma membrane and tonoplast carriers. On the basis of differential sensitivity to inhibitors, Pitman (23) proposed that transport of K^+ to the shoot involved a second active transport step, distinct from that responsible for uptake from external solution. Given that the proton gradient between cytosol and stele is in the direction of the cytosol, the transport of K^+ from cytosol to xylem vessels could occur via a K^+:H^+ antiporter. Thus, conditions leading to derepression of the putative high-affinity K^+:H^+ symporters may ultimately cause repression of the K^+:H^+ antiporter responsible for the translocation step. It is reasonable to assume that regulation of this (K^+:H^+ antiporter) transport step should be distinct from that involved in absorption of K^+ or mobilization of vacuolar K^+. The major components of this model are summarized in Figure 7.

How do the predictions of this model relate to the present biochemical observations concerning the synthesis of ^{35}S-labeled polypeptides under conditions of K^+ deprivation? The observation that KR43 was present in both plasma membrane- and tonoplast-enriched fractions is consistent with the model's prediction of the synthesis and incorporation in plasma membrane and tonoplast of a K^+:H^+ symporter. The slower rate of turnover of the KR45 polypeptide identified in our previous report and its apparently extrinsic character is not consistent with a role in the high-affinity transport of K^+. Perhaps KR45 may participate as a K^+-binding protein analogous to the iron-binding proteins (siderophores) produced in response to iron deprivation.

Work is presently underway to purify sufficient quantities of KR43 to allow amino acid sequencing and preparation of antibody.

LITERATURE CITED

1. **Brezeale JF** (1906) The relation of sodium to potassium in soil and solution cultures. J Am Chem Soc **28:** 1013–1025
2. **Drew MC, Saker LR** (1984) Uptake and long-distance transport of phosphate, potassium and chloride in relation to internal ion concentration in barley: evidence for non-allosteric regulation. Planta **160:** 490–499
3. **Dupont FM, Tanaka CK, Hurkman WJ** (1988) Separation and immunological characterization of membrane fractions from barley roots. Plant Physiol **86:** 717–724
4. **Fernando M, Kulpa J, Siddiqi MY, Glass ADM** (1990) Potassium-dependent changes in the expression of membrane-associated proteins in barley roots. 1. Correlations with K^+ (86Rb) influx and root K^+ concentration. Plant Physiol **92:** 1128–1132
5. **Fernando M, Siddiqi MY, Glass ADM** (1989) Changes in membrane-associated polypeptides in relation to K^+ supply (abstract No. 904). Plant Physiol **89:** S-151
6. **Gaber RF, Styles CA, Fink GR** (1988) TRK1 encodes a plasma membrane protein required for high affinity K^+ transport in *Saccharomyces cerevisiae*. Mol Cell Biol **8:** 2848–2859
7. **Glass ADM** (1975) The regulation of potassium absorption in barley roots. Plant Physiol **56:** 377–380
8. **Glass ADM** (1976) The regulation of potassium absorption in barley roots: an allosteric model. Plant Physiol **58:** 33–37
9. **Glass ADM** (1977) The regulation of K^+ influx in barley roots: evidence for direct control by internal K^+. Aust J Plant Physiol **4:** 313–318
10. **Glass ADM** (1978) The regulation of potassium influx into intact roots of barley by internal potassium levels. Can J Bot **56:** 1759–1764
11. **Glass ADM, Dunlop J** (1979) The regulation of K^+ influx in excised barley roots: relationships between K^+ influx and electrochemical potential differences. Planta **145:** 395–397
12. **Hedrich R, Schroeder J** (1989) The physiology of ion channels and electrogenic pumps in higher plants. Annu Rev Plant Physiol **40:** 539–569
13. **Hoagland DR, Broyer TC** (1936) General nature of the process of salt accumulation by roots with description of experimental methods. Plant Physiol **11:** 471–507
14. **Hooymans JJM** (1974) Role of cell components in the redistribution of K and Na ions absorbed by the roots of intact barley plants. Z Pflanzenphysiol **73:** 234–242
15. **Johansen CD, Edwards DG, Loneragan JF** (1970) Potassium fluxes during potassium absorption by intact barley roots of increasing potassium content. Plant Physiol **45:** 601–603
16. **Ketchum K, Shrier A, Poole RJ** (1989) Characterization of potassium-dependent currents in corn suspension cells. Plant Physiol **89:** 1184–1192
17. **Kochian LV, Lucas W** (1982) Potassium transport in corn roots. I. Resolution of kinetics into a saturable and linear component. Plant Physiol **70:** 1723–1731
18. **Leigh RA, Wyn Jones RG** (1984) An hypothesis relating critical potassium concentration for growth to the distribution of this ion in plant cells. New Phytol **97:** 1–13
19. **Memon AR, Saccomani M, Glass ADM** (1985) Efficiency of K^+ utilization by barley varieties: the role of subcellular compartmentation. J Exp Bot 1860–1876
20. **Mertz SM** (1973) Electrical potentials and kinetics of potassium sodium and chloride uptake in barley roots. Doctoral thesis. Washington State University, Pullman, WA
21. **Munns R** (1985) Na^+, K^+ and Cl^- in xylem sap flowing to shoots of NaCl-treated barley. J Exp Bot **36:** 1032–1042
22. **Omura T, Siekevitz P, Palade GE** (1967) Turnover of constituents of the endoplasmic reticulum membranes of rat hepatocytes. J Biol Chem **242:** 2389–2393
23. **Pitman MG** (1972) Uptake and transport of ions in barley seedlings. II. Evidence for two active stages in transport to the shoot. Aust J Biol Sci **25:** 243–257
24. **Pitman MG, Cram WJ** (1977) Regulation of ion content in whole plants. *In* DH Jennings, ed, Integration of Activity in the Higher Plant, Society for Experimental Biology Symposium 31. Cambridge University Press, Cambridge, UK, pp 391–424
25. **Pitman MG, Mowat J, Nair H** (1971) Interaction of processes for accumulation of salt and sugar in barley plants. Aust J Biol Sci **24:** 619–631
26. **Rodriguez-Navarro A, Blatt MR, Slayman CL** (1986) A potassium-proton symport in *Neurospora Crassa*. J Gen Physiol **87:** 649–674

27. **Siddiqi MY, Glass ADM** (1983) Studies of the growth and mineral nutrition of barley varieties. II. Effects of potassium supply on the uptake of potassium and growth. Can J Bot **61:** 671–678
28. **Siddiqi MY, Glass ADM** (1987) Regulation of K^+ influx in barley: evidence for a direct control of influx by K^+ concentration of root cells. J Exp Bot **38:** 935–947
29. **Siebers A, Altendorf K** (1988) The K^+-translocating Kdp-ATPase from *Escherichia coli*: purification, enzymatic properties and production of complex- and subunit-specific antisera. Eur J Biochem **178:** 131–140
30. **Tomos AD, Wyn Jones RG** (1988) Some transport properties of cells within tissues. *In* D Baker, JL Hall, eds, Solute Transport in Plant Cells and Tissues. Longman, Bath, UK, pp 220–250
31. **Young M, Sims AP** (1972) The potassium relations of *Lemna minor*. I. Potassium uptake and plant growth. J Exp Bot **23:** 958–969