Mechanisms of caesium uptake by plants

PHILIP J. WHITE* AND MARTIN R. BROADLEY

1Department of Plant Genetics and Biotechnology, Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK
2Department of Soil and Environment Sciences, Horticulture Research International, Wellesbourne, Warwick CV35 9EF, UK

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SUMMARY

Caesium (Cs) is a Group I alkali metal with chemical properties similar to potassium (K). It is present in solution as the monovalent cation Cs⁺. Concentrations of the stable caesium isotope ¹³¹Cs in soils occur up to 25 μg g⁻¹ dry soil. This corresponds to low micromolar Cs⁺ concentrations in soil solutions. There is no known role for Cs in plant nutrition, but excessive Cs can be toxic to plants. Studies of the mechanism of Cs uptake by plant roots have been described. The inward-rectifying K⁺ transporters (tentatively ascribed here to KUP genes) also mediate the bulk of Cs uptake. Cation influx through KIR channels is likely to be blocked by extracellular Cs⁺ under typical ionic conditions in the soil. Further simulations suggest that the combined Cs⁺ influx to root cells is mediated by the same molecular mechanism(s). The inward-rectifying K⁺ (KIR) and outward-rectifying K⁺ (KOR) channels are all permeable to Cs⁺. By modelling cation fluxes through these transporters into a stereotypical root cell, it can be predicted that VIC channels mediate most (30–90%) of the Cs⁺ influx to root cells. Further simulations suggest that KOR channels, long half-lives, emissions of Cs from nuclear reactors and thermonuclear explosions. Two radioisotopes of Cs (¹³¹Cs and ¹³³Cs) are of environmental concern owing to their relatively long half-lives, emissions of β and γ radiation during decay and rapid incorporation into biological systems. The soil concentrations of these radioisotopes are six orders of magnitude lower than those of ¹³¹Cs. Early physiological studies demonstrated that K⁺ and Cs⁺ competed for influx to excised roots, suggesting that the influx of these cations to root cells is mediated by the same molecular mechanism(s). The molecular identity and/or electrophysiological signature of many K⁺ transporters expressed in the plasma membrane of root cells have been described. The inward-rectifying K⁺ (KIR), outward-rectifying K⁺ (KOR) and voltage-insensitive cation (VIC) channels are all permeable to Cs⁺ and, by analogy with their bacterial counterparts, it is likely that ‘high-affinity’ K⁺/H⁺ symporters (tentatively ascribed here to KUP genes) also transport Cs⁺. By modelling cation fluxes through these transporters into a stereotypical root cell, it can be predicted that VIC channels mediate most (30–90%) of the Cs⁺ influx under physiological conditions and that the KUP transporters mediate the bulk of the remainder. Cation influx through KIR channels is likely to be blocked by extracellular Cs⁺ under typical ionic conditions in the soil. Further simulations suggest that the combined Cs⁺ influx on

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*Author for correspondence (tel +44 1789 470382; fax +44 1789 470552; e-mail philip-j.white@hri.ac.uk).

Abbreviations: [Ca²⁺]ₜot, cytoplasmic Ca²⁺ concentration; [Ca²⁺]ₜot, extracellular Ca²⁺ concentration; CNG, cyclic-nucleotide-gated; [Cs⁺]ₜot, extracellular Cs⁺ concentration; Eᵦ, Nernst (equilibrium) potential for K⁺; Eᵦ, current-reversal potential; GHK, Goldman-Hodgkin-Katz; [K⁺]ₜot, extracellular K⁺ concentration; Kᵦ, inhibition constant; KIR channel, inward-rectifying K⁺ channel; KOR channel, outward-rectifying K⁺ channel; KIRC, K⁺-selective outward-rectifying channel; KUP, high-affinity K⁺ transporter; NORC, nonspecific outward-rectifying channel; Pᵦ/Pᵦ, relative permeabilities of Cs⁺ and K⁺; Pᵦ, probability of an ion channel being open; TEA⁺, tetraethylammonium; VIC channel, voltage-insensitive cation channel.
influxes through VIC channels and KUP transporters can produce the characteristic ‘dual isotherm’ relationship between Cs⁺ influx to excised roots and external Cs⁺ concentrations below 200 μM. Thus, molecular targets for modulating Cs⁺ influx to root cells have been identified. This information can be used to direct future genetic modification of plants, allowing them to accumulate more, or less, Cs and thereby to remediate contaminated sites.

Key words: caesium (Cs), cation channel, phytoremediation, plasma membrane, potassium (K), root.

I. INTRODUCTION: CAESIUM IN THE ENVIRONMENT

Caesium (Cs) is the second heaviest element of the Group I alkali metals and displays a high degree of chemical similarity with the other alkali metals and with K in particular (Bowen, 1979). In solution, Cs exists predominantly as a monovalent cation. The Cs⁺ cation is a weak Lewis acid with a low charge:radius ratio that interacts only weakly with ligands (Avery, 1995). There are at least 21 isotopes of Cs, mostly in the range of atomic masses 123–144 (Davis, 1963). The only natural isotope of Cs is the stable isotope ¹³²Cs, whose principal source is pollucite, an aluminosilicate mineral. Mean concentrations of ¹³¹Cs have been reported as 1 μg g⁻¹ in granites, 4 μg g⁻¹ in sedimentary rocks, 13.4 μg g⁻¹ in coal, and 10⁻³ μg g⁻¹ in seawater (reviewed in Davis, 1963; Bowen, 1979; Coughtrey & Thorne, 1983).

Seven reactive isotopes of Cs, produced during the fission of various uranium, plutonium and thorium isotopes or by neutron bombardment of ¹³³Cs or ¹³⁶Ba, are considered radiologically important (Nichols & Hunt, 1998). Of these, the two Cs isotopes of environmental concern, owing to their rapid incorporation into biological systems, their relatively long half-lives and emissions of β and γ radiation during decay, are ¹³¹Cs (t₁/₂ = 2.06 yr) and ¹³⁷Cs (t₁/₂ = 30.17 yr). Both these radioisotopes are present in nuclear reactor fuel elements irradiated for long periods and are released into the environment during intentional and unintentional discharges from nuclear installations. Radioisotopes of Cs are also present in the stratosphere as a result of the above-ground explosions of thermonuclear weapons between 1952 and 1963 and have subsequently been deposited as fallout (Davis, 1963; Bell et al., 1988).

After the accident at the Chernobyl nuclear power plant in 1986, soil activities of Cs radioisotopes at the most heavily contaminated site in the UK were 0.12 Bq of ¹³¹Cs cm⁻² and 0.26 Bq of ¹³⁷Cs cm⁻² (Cambray et al., 1987); 1 Bq of ¹³¹Cs weighs 2.1 × 10⁻¹⁴ g and 1 Bq of ¹³⁷Cs weighs 3.1 × 10⁻¹⁵ g (Livens & Rimmer, 1988). These values correspond to soil concentrations of between 2.52 × 10⁻⁹ and 8.06 × 10⁻⁸ μg of radioactive Cs g⁻¹ of soil respectively (assuming a soil bulk density of 1 g cm⁻³). Soil activities of ¹³⁷Cs in excess of 30 Bq cm⁻² (6.2 × 10⁻⁷ μg of ¹³⁷Cs g⁻¹ of soil) persist over large areas in Russia and the Ukraine (Fessenko & Lissiansky, 1997) and concentrations of ¹³⁴/¹³⁷Cs of at least this order have also arisen from the testing and production of nuclear weapons, for example in the Marshall Islands and the Mayak nuclear complex (Graham & Simon, 1996; Aarkrog et al., 1997), and from accidents at other nuclear power stations, such as Three-Mile Island and Windscale. Soil concentrations of stable ¹³³Cs are at least six orders of magnitude higher than those of ¹³⁴/¹³⁷Cs, ranging from 0.3 to 25.7 μg of ¹³³Cs g⁻¹ of soil (reviewed by Davis, 1963; Coughtrey & Thorne, 1983). This corresponds to low micromolar Cs⁺ concentrations in soil solutions. Owing to high affinities of adsorption to clay and organic fractions of the soil, radioisotopes of Cs tend to persist in the upper soil layers (Cawse, 1983; Smith & Elder, 1999).

There is considerable interest in remediating sites contaminated by Cs radioisotopes and, because of the lack of alternative technologies that are practicable, effective and affordable, the use of plants to extract and concentrate Cs is one positive option (Entry et al., 1996; Lasat et al., 1997). In addition, because the produce from contaminated areas enters the food chain, one might beneficially introduce ‘safe’ crops that do not accumulate Cs to these areas. If one discounts the contribution of foliar deposition, the principal route of entry of ¹³⁴Cs and ¹³⁷Cs into the food chain is through the soil–plant pathway (Bell et al., 1988). Thus, the Cs uptake characteristics of a plant determine its potential for phytoextraction or cultivation on contaminated land.

There is no known role for Cs⁺ in plant nutrition (Bowen, 1979; Marschner, 1995). Equally, Cs⁺ is nontoxic to plants at external Cs⁺ concentrations ([Cs⁺]ext) below approx. 200 μM, although this limit depends critically on the concentrations of other ions in the substrate. The toxicity symptoms induced by unnaturally high [Cs⁺]ext include necrosis of shoot and root tissues (Cline & Hungate, 1960; Kordan, 1987). The transport of monovalent cations across the root to the xylem occurs mainly through the root symplast (Marschner, 1995), and Cs⁺ must cross the plasma membrane of root cells at least twice before it can be transported to the shoot. The purpose of this review is to examine the molecular mechanisms contributing to Cs⁺ uptake across the plasma membranes of root cells under different soil ionic conditions. Because there is no convincing evidence of discrimination by biological systems between ¹³³Cs, ¹³⁴Cs and ¹³⁷Cs (Avery, 1995), all Cs isotopes are referred to generically here. Evidence that multiple mechanisms contribute to Cs⁺ uptake by roots under physiological conditions is discussed, on
the basis of studies of Cs\(^+\) uptake from solutions containing femtomolar (often referred to as ‘carrier-free’ Cs in the literature) to millimolar Cs\(^+\) concentrations. The molecular identities of putative Cs\(^+\) transport mechanisms are inferred from recent molecular-biological and electrophysiological studies; these transport mechanisms are incorporated into a theoretical model to predict the contribution of each to Cs\(^+\) influx in a variety of soil ionic conditions. These predictions are compared with kinetic and pharmacological studies of Cs\(^+\) uptake by roots, and used to identify genetic strategies for manipulating Cs\(^+\) accumulation by crops.

II. UPTAKE OF CAESIUM BY PLANT ROOTS

There is a wealth of information, published over the past 50 yr, on the uptake and accumulation of Cs\(^+\) by plants. However, owing to the many confounding effects of soil and plant variables on the uptake of Cs\(^+\) (reviewed by Bell et al., 1988; Broadley et al., 1999a), only those studies investigating Cs\(^+\) uptake from nutrient solutions are reviewed here. Further, for the purposes of resolving Cs\(^+\) uptake mechanisms, solution-culture studies reporting solely the effects of plant growth on Cs\(^+\) uptake (e.g. Weaver et al., 1981; Smolders & Shaw, 1995; Broadley et al., 1999b) have also been excluded.

1. Evidence for multiple mechanisms of Cs\(^+\) uptake by plant roots

Collander (1941) apparently published the first report that plants accumulated Cs\(^+\) from nutrient solutions and also proposed that K\(^+\), rubidium (Rb\(^+\)) and Cs\(^+\) entered the plant through the same uptake mechanism. Collander (1941) observed that the uptake of Cs\(^+\) was almost as rapid as that of K\(^+\) or Rb\(^+\) when these cations were supplied at 0.1 mM in a complete nutrient medium. However, Menzel & Heald (1955) observed that plants discriminated against the accumulation of Cs\(^+\) when both Cs\(^+\) and K\(^+\) were supplied together in the nutrient medium; Middleton et al. (1960) arrived at a similar conclusion when plants were grown in solutions containing a range of K\(^+\) and Cs\(^+\) concentrations.

The pioneering work of Epstein and co-workers (Epstein, 1972) revealed that the concentration dependence of monovalent cation uptake into excised roots could be described by the sum of two Michaelis–Menten hyperbolas. They believed this to represent two distinct transport mechanisms with contrasting affinities for monovalent cations and further suggested that, because Cs\(^+\) and K\(^+\) both inhibited Rb\(^+\) uptake competitively (Epstein & Hagen, 1952), these three cations shared the same uptake mechanisms. Contemporaneously, Bange & Overstreet (1960) studied the concentration dependence of Cs\(^+\) uptake into excised barley (Hordeum vulgare) roots directly and confirmed that this relationship could be described by the sum of two Michaelis–Menten hyperbolae. They proposed that this represented two distinct Cs\(^+\) uptake mechanisms: a high-affinity transport mechanism that followed Michaelis–Menten kinetics with a \(K_m\) of 8 \(\mu\)M and a \(V_{\text{max}}\) of 2 \(\mu\)mol g\(^{-1}\) of fresh root h\(^{-1}\), and a low-affinity transport mechanism that was not saturated even at 75 mM [Cs\(^+\)]\(_{\text{ext}}\). They also observed that low temperatures and decreased oxygen availability had a proportionally greater effect on Cs\(^+\) uptake at low [Cs\(^+\)]\(_{\text{ext}}\). Similarly, Shaw & Bell (1989) undertook a detailed examination of the concentration dependence of Cs\(^+\) influx to excised wheat (Triticum aestivum) roots and concurred with the view that multiple mechanisms contributed to Cs\(^+\) uptake. However, Shaw & Bell (1989) proposed the existence of two distinct Cs\(^+\) influx isotherms operating below a [Cs\(^+\)]\(_{\text{ext}}\) of 200 \(\mu\)M, each with a different affinity for Cs\(^+\) (Fig. 1). At [Cs\(^+\)]\(_{\text{ext}}\) in excess of 200 \(\mu\)M, Shaw & Bell (1989) observed that Cs\(^+\) influx to excised roots increased linearly with increasing [Cs\(^+\)]\(_{\text{ext}}\) and suggested that this repre-
sent a further lower-affinity uptake system that corresponded to the low-affinity transport mechanism invoked by Bange & Overstreet (1960). However, it is a general weakness of flux studies using intact tissues that molecular mechanisms of ion transport cannot be inferred from kinetic parameters alone.

2. Caesium uptake is affected by the presence of other cations

The accumulation of Cs+ in plants grown in solutions containing from trace amounts to 200 μM Cs+ is decreased by increasing the extracellular K+ concentration ([K+]ext) (Cline & Hungate, 1960; Nishita et al., 1962; Smolders et al., 1996). This is consistent with the decrease in Cs+ uptake upon the addition of monovalent cations to nutrient solutions (Sutcliffe, 1957; Bange & Overstreet, 1960; Handley & Overstreet, 1961; Zhu et al., 1999). The effectiveness by which equimolar monovalent cations decreased Cs+ uptake from solutions containing 100 μM [Cs+]ext followed the general sequence Li+ < Na+ < NH4+ < Rb+ < K+ (Bange & Overstreet, 1960; Handley & Overstreet, 1961). Similarly, the results of Shaw & Bell (1991), characterizing the interactions between K+, NH4+, and Cs+ with the use of kinetic competition analyses, demonstrated that Cs+ influx was decreased more effectively by K+ than NH4+ in the external solution (Fig. 2).

The uptake of Cs+ into roots is also inhibited by divalent cations (Bange & Overstreet, 1960; Jacobson et al., 1960; Handley & Overstreet, 1961; Resnik et al., 1969; Sze & Hodges, 1977). When [Cs+]ext was <100 μM, the effectiveness with which divalent cations inhibited Cs+ uptake followed the sequence Ca2+ < Mg2+ < Ba2+ (Bange & Overstreet, 1960; Handley & Overstreet, 1961). The data of Smolders et al. (1997) suggest that external Ca2+ ([Ca2+]ext) does not completely inhibit Cs+ accumulation and that the apparent inhibition constant (Ki) for the sensitive portion is approx. 500 μM (Fig. 3). Similarly, some Cs+ uptake by excised barley roots remains even in the presence of 50 mM divalent cation (Ca2+, Mg2+ or Ba2+), although the Ki for the portion of Cs+ uptake sensitive to divalent cations is submillimolar (Handley & Overstreet, 1961). Sze & Hodges (1977) reported that increasing [Ca2+]ext from 0 to 1 mM halved Cs+ influx to excised oat root segments at [Cs+]ext up to 50 mM.

3. Caesium inhibits the uptake of other cations

Epstein & Hagen (1952) showed that [Cs+]ext of 10 and 25 mM inhibited Rb+ uptake in a competitive manner and Sutcliffe (1957) demonstrated that a [Cs+]ext of 10 mM inhibited the uptake of Na+, K+, Rb+, and Li+ into beet discs. More recently, Maathuis & Sanders (1996) reported that Cs+ inhibited Rb+ uptake and that this inhibition was more pronounced at higher [Rb+]ext. Observations such as these led, of course, to the conclusion that Cs+, K+, and Rb+ shared the same transport mechanisms. However, other workers have failed to demonstrate an inhibition by Cs+ of the uptake of other monovalent cations. For example, Bange & Overstreet (1960) reported that a [Cs+]ext of <100 μM inhibited Na+ uptake but had no effect on Rb+ uptake, and Marschner (1986) observed that a [Cs+]ext of 1 mM had no effect on K+ influx from a solution containing 1 mM external K+. These contrasting observations might be a consequence of the use by different workers of plants of different nutrient statuses, employing excised roots or roots of intact plants, or assaying cation uptake (over a period of hours) or influx (over a period of minutes). Nevertheless, on
the basis of the available physiological evidence, it seems likely that Cs\(^+\) enters root cells through a ‘K\(^+\) transporter’.

### III. Molecular mechanisms catalysing caesium uptake

Root cells possess many mechanisms capable of transporting K\(^+\) across the plasma membrane (Table 1). In this section, the evidence for Cs\(^+\) transport into root cells through these various mechanisms is reviewed.

1. ‘High-affinity’ transport mechanisms

Several years ago, direct electrophysiological evidence for a K\(^+\)/H\(^+\) symporter with a stoichiometry of 1K\(^+\)::1H\(^+\) was obtained using protoplasts from Arabidopsis root cells (Maathuis & Sanders, 1994). It was argued that this mechanism could mediate most of the K\(^+\) influx to root cells at submillimolar [K\(^+\)]\(_{ext}\) (Maathuis & Sanders, 1997). However, this electrical activity was observed in <10\(^\circ\) of root cells and the selectivity of the putative K\(^+\)/H\(^+\) symporter was not determined. Since then, other mechanisms mediating high-affinity K\(^+\) transport have been identified genetically and characterized in heterologous systems.

Genes for high-affinity K\(^+\) transporters are expressed in roots of Arabidopsis thaliana (AtKUP1, AtKUP2, AtKUP3 and AtKUP4) and barley (HvHAK1). They are members of large gene families. All these genes confer either high-affinity or dual-affinity K\(^+\) and/or Rb\(^+\) uptake to either yeast (Saccharomyces cerevisiae strain CY162) or Escherichia coli (TK2463) mutants that lack endogenous high-affinity K\(^+\) uptake mechanisms and allow them to grow at low K\(^+\) concentrations (Santa-Maria et al., 1997; Fu & Luan, 1998; Kim et al., 1998). In addition, it has been demonstrated that overexpression of AtKUP1 increases the rate of Rb\(^+\) uptake by Arabidopsis cells in suspension (Kim et al., 1998). On the basis of similarities in the inhibition of \(^{86}\)Rb influx and growth of Arabidopsis on the one hand, and of AtKUP-mediated K\(^+\) fluxes into yeast on the other, it has been suggested that the AtKUP transporters contribute significantly to the K\(^+\) influx required for plant nutrition (Spalding et al., 1999).

The AtKUP1 and HvHAK1 transporters are expressed primarily in roots (Santa-Maria et al., 1997; Fu & Luan, 1998), where it is assumed that they are present on the plasma membrane. The expression of the barley HvHAK1 (Santa-Maria et al., 1997) and the Arabidopsis AtKUP3 (Kim et al., 1998) increase markedly in roots upon K\(^+\) starvation, indicating a possible contribution of these transporters to inducible high-affinity K\(^+\) uptake.

Neither the selectivity nor the transport mechanism(s) of the AtKUP or HvHAK transporters are known for certain. However, AtKUP1-mediated K\(^+\) uptake into yeast CY162 was partly inhibited by 10 mM Cs\(^+\) (Fu & Luan, 1998) and the growth of E. coli TK2463 expressing AtKUP1 or AtKUP2 was completely arrested by 10 mM Cs\(^+\) (Kim et al., 1998), which might indicate competition between K\(^+\) and Cs\(^+\) for transport. In addition, although the AtKUP transporters have only c. 30\% identity (but 60–80\% similarity) to bacterial KUP and fungal HAK transporters at the amino acid level (Kim et al., 1998; Haro et al., 1999), the structure of the transmembrane segments, and that of the fragments connecting these segments, of these proteins is well conserved. Thus, it is likely that their transport mechanism is also conserved. Both the bacterial KUP and fungal HAK transporters function as K\(^+\)/H\(^+\) symporters (Haro et al., 1999; Trchounian & Kobayashi, 1999) and the KUP transporter of E. coli mediates Cs\(^+\) uptake (Bossemeyer et al., 1989).

The genes HKT1 and LCT1, which are expressed in wheat roots, also mediate K\(^+\) uptake and allow S. cerevisiae strain CY162 to grow at low [K\(^+\)]\(_{ext}\) (reviewed by Chrispeels et al., 1999). The locations of HKT1 and LCT1 in a plant cell have not been determined, but they are assumed to reside on the plasma membrane. When expressed in Xenopus oocytes, HKT1 behaves as a Na\(^+\)/K\(^+\) cotransporter (Gassmann et al., 1996). Although HKT1 is expressed in the cortex of wheat roots (Schachtman & Schroeder, 1994), and a homologue of HKT1 is expressed in the cortex and exodermis of rice roots (Golldack et al., 1997), HKT1 does not contribute to Cs\(^+\) uptake because Cs\(^+\) cannot compete for transport at either the Na\(^+\)-binding site or the K\(^+\)-binding site (Gassmann et al., 1996). When expressed in yeast, LCT1 mediates the low-affinity uptake of monovalent (K\(^+\), Rb\(^+\) and Na\(^+\)) and divalent (Ca\(^{2+}\) and Cd\(^{2+}\)) cations (Schachtman et al., 1997; Clemens et al., 1998). It is therefore possible that LCT1 also transports Cs\(^+\), but this is yet to be demonstrated.

Another cDNA (KEA1) has been cloned from Arabidopsis that has 22\% identity with bacterial K\(^+\)/H\(^+\) and Na\(^+\)/H\(^+\) antiporters and mediates a K\(^+\)-dependent inward current in oocytes (Yao et al., 1997). It is not known whether KEA1 transports Cs\(^+\).

2. Inward-rectifying potassium (KIR) channels

KIR channels open upon membrane hyperpolarization and facilitate K\(^+\) influx to root cells (reviewed by Schroeder et al., 1994; White, 1997). They have been characterized in several different cell types from the roots of a number of plant species. Several distinct KIR channels exist, each differing slightly in conductance, gating kinetics and pharmacology. However, KIR channels share several general characteristics. The dominant KIR channels in most root cells are activated at voltages more negative than
Table 1. Potassium ($K^+$) transporters identified in root cells from their gene expression pattern and/or presence in root cell cDNA libraries, or electrophysiologically

<table>
<thead>
<tr>
<th>Identification method</th>
<th>Transporter</th>
<th>Mechanism</th>
<th>Function</th>
<th>Expression</th>
<th>Cs’ permeability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pattern/presence</td>
<td>AKT1</td>
<td>Channel</td>
<td>$K^+$ uptake</td>
<td>Epidermis, cortex, endodermis</td>
<td>Probably?</td>
<td>Gaymard et al. (1996); Lagarde et al. (1996); Berti et al. (1997); Hirsch et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>SKT1</td>
<td>Channel</td>
<td>$K^+$ uptake</td>
<td>Root</td>
<td>Not known</td>
<td>Zimmermann et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>SKT2</td>
<td>Channel</td>
<td>$K^+$ uptake</td>
<td>Vasculature</td>
<td>Not known</td>
<td>Czempinski et al. (1999)</td>
</tr>
<tr>
<td></td>
<td>KAT1</td>
<td>Channel</td>
<td>$K^+$ uptake</td>
<td>Vasculature</td>
<td>Yes</td>
<td>Schachtman et al. (1992); Nakamura et al. (1995)</td>
</tr>
<tr>
<td></td>
<td>AtKC1</td>
<td>Channel</td>
<td>$K^+$ uptake</td>
<td>Root</td>
<td>Not known</td>
<td>Dreyer et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>SKOR1</td>
<td>Channel</td>
<td>$K^+$ efflux to xylem</td>
<td>Stele</td>
<td>$P_{Cs}/P_{K} = 0.15$</td>
<td>Gaymard et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>KCO1</td>
<td>Channel</td>
<td>$K^+$ efflux</td>
<td>Root</td>
<td>Not known</td>
<td>Czempinski et al. (1997, 1999)</td>
</tr>
<tr>
<td></td>
<td>AtKUP1</td>
<td>$K^+/H^+$ symport?</td>
<td>$K^+$ uptake</td>
<td>Root</td>
<td>Probably?</td>
<td>Fu &amp; Luan (1998); Kim et al. (1998)</td>
</tr>
<tr>
<td></td>
<td>AtKUP2</td>
<td>$K^+/H^+$ symport?</td>
<td>$K^+$ uptake</td>
<td>Root</td>
<td>Probably?</td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>AtKUP3</td>
<td>$K^+/H^+$ symport?</td>
<td>$K^+$ uptake</td>
<td>Root</td>
<td>Probably?</td>
<td>&quot; &quot;</td>
</tr>
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<td>AtKUP4</td>
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<td>$K^+$ uptake</td>
<td>Root</td>
<td>Probably?</td>
<td>&quot; &quot;</td>
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<td>HvHAK1</td>
<td>$K^+/H^+$ symport?</td>
<td>$K^+$ uptake</td>
<td>Root</td>
<td>Probably?</td>
<td>Santa-Maria et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>HKT1</td>
<td>$K^+/Na^+$ symport?</td>
<td>$K^+$ uptake</td>
<td>Cortex</td>
<td>No</td>
<td>Schachtman &amp; Schroeder (1994); Gassmann et al. (1996)</td>
</tr>
<tr>
<td>Electrophysiology</td>
<td>LCT1</td>
<td>Not known</td>
<td>Not known</td>
<td>Root</td>
<td>Probably?</td>
<td>Schachtman et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>KIR</td>
<td>Channel</td>
<td>$K^+$ uptake</td>
<td></td>
<td></td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>KORC</td>
<td>Channel</td>
<td>$K^+$ efflux</td>
<td></td>
<td></td>
<td>Wegner &amp; Raschke (1994)</td>
</tr>
<tr>
<td></td>
<td>NORC</td>
<td>Channel</td>
<td>Cation efflux</td>
<td></td>
<td></td>
<td>Maathuis &amp; Sanders (1994)</td>
</tr>
<tr>
<td></td>
<td>VIC</td>
<td>Channel</td>
<td>Cation uptake</td>
<td></td>
<td></td>
<td>&quot; &quot;</td>
</tr>
<tr>
<td></td>
<td>DACC</td>
<td>Channel</td>
<td>$Ca^{2+}$ influx</td>
<td></td>
<td></td>
<td>Roberts &amp; Tester (1997b)</td>
</tr>
<tr>
<td></td>
<td>HACC</td>
<td>Channel</td>
<td>$Ca^{2+}$ influx</td>
<td></td>
<td></td>
<td>&quot; &quot;</td>
</tr>
</tbody>
</table>

A question mark indicates a putative mechanism or function.
Caesium permeates KIR channels. Permeability ratios ($P_K/P_{Ba}$) of 0.07 (Maathuis & Sanders, 1995) and 0.39–0.43 (Wegner & Raschke, 1994) were determined for KIR channels in cortical cells of Arabidopsis roots and xylem parenchyma cells in barley roots, respectively. In addition, extracellular Cs$^+$ inhibits KIR channels in the plasma membrane of root cells at submillimolar concentrations in a voltage-dependent manner (White, 1997) (Fig. 4a). Increasing [Cs$^+$]$_{ext}$ in the presence of constant [K$^+$]$_{ext}$ decreases K$^+$ influx to root cells through KIR channels (Fig. 4b) and the inhibitory effect of [Cs$^+$]$_{ext}$ limits the increase in Cs$^+$ influx with increasing [Cs$^+$]$_{ext}$ (Ichida & Schroeder, 1996). If this is correct, there will be appreciable inhibition of KIR at the K$^+$ and Cs$^+$ concentrations found in soil solutions (Fig. 4a). The KIR channels in the plasma membrane of root cells are also inhibited by millimolar concentrations of tetraethylammonium (TEA$^+$), Ba$^{2+}$, La$^{3+}$ and Ca$^{2+}$ (reviewed by White, 1997). However, the inhibition of KIR channels by Ca$^{2+}$ is unlikely to be physiologically significant at the Ca$^{2+}$ concentrations found in most soils.

The genes for several KIR channels have been cloned. Of these, the Arabidopsis thaliana AKT1 gene and its homologues in Brassica napus (Lagarde et al., 1996), and potato (SKT1) (Zimmermann et al., 1998), AtKCI (Dreyer et al., 1997) and KAT1 (Kochian et al., 1993; Nakamura et al., 1995) are all expressed in plant roots. The AKT1 K$^+$ channel seems to be the dominant K$^+$ channel involved in mineral nutrition (Hirsch et al., 1998; Spalding et al., 1999). The location of AKT1 in the plasma membrane of root cells has been confirmed and, in B. napus, the AKT1 promoter directs β-glucuronidase (GUS) activity to the peripheral cell layers of the mature root (Lagarde et al., 1996). The KAT1 channel is expressed predominantly in guard cells, but also weakly in the root vasculature, where it might serve to scavenge apoplastic K$^+$ (Nakamura et al., 1995).

The transport characteristics of AKT1, SKT1 and KAT1 have been studied electrophysiologically...
after their expression in Xenopus oocytes, yeast (S.
cerevisiae strain CY162) and/or baculovirus-infected
insect cells. There is no direct evidence that AKT1
is permeable to Cs\textsuperscript{+} but, by analogy with other KIR
channels, Cs\textsuperscript{+} is likely to permeate this channel. The
K\textsuperscript{+} channel encoded by KAT1 is permeable to Cs\textsuperscript{+}
(Schachtman et al., 1992; Uozumi et al., 1995; Ichida
& Schroeder, 1996), but a P_{Cs}/P_{K} ratio estimated from the current-reversal potential (P_{rev})
under bi-ionic conditions has not been published. Cation influx through AKT1, SKT1 and KAT1 is
inhibited in a voltage-dependent manner by extracellular
Cs\textsuperscript{+}. The K\textsubscript{r} for Cs\textsuperscript{+} inhibition of these
channels ranges from 15 to 500 \mu M when determined
at −130 to −150 mV and at a [K\textsuperscript{+}]\textsubscript{ext} of 30 mM
(Becker et al., 1996; Ichida & Schroeder, 1996;
Dreyer et al., 1997, 1999; Zimmermann et al., 1998).
The sensitivity to inhibition by Cs\textsuperscript{+} increases in the
order KAT1 < SKT1 < AKT1 when determined at the same
[K\textsuperscript{+}]\textsubscript{ext} (Véry et al., 1994; Becker et al.,
1996; Ichida & Schroeder, 1996; Bertl et al., 1997;
Dreyer et al., 1997; Zimmermann et al., 1998).
However, it should be noted that the sensitivity of
KIR to inhibition by Cs\textsuperscript{+} can depend on the
expression system used, the level of expression and
the combination of genes expressed simultaneously
(Véry et al., 1994; Dreyer et al., 1997; Brüggemann
et al., 1999). These channels are also inhibited by TEA\textsuperscript{+} and Ba\textsuperscript{2+}, and weakly by Ca\textsuperscript{2+} (Schachtman
et al., 1992; Véry et al., 1994; Becker et al., 1996;
Ichida & Schroeder, 1996; Bertl et al., 1997; Dreyer
et al., 1998). Their pharmacology therefore corre-
sponds to the KIR channels recorded in root cells.

It is noteworthy that both the sensitivity to
inhibition by Cs\textsuperscript{+} and the cation selectivity of plant
KIR channels seem to be encoded by a region of the
protein termed the P domain, which forms part of
the channel pore. Mutational studies of KAT1 have
identified residues that alter the sensitivity of the
channel to inhibition by Cs\textsuperscript{+} and/or its permeability
to Cs\textsuperscript{+} (Uozumi et al., 1995; Becker et al., 1996;
Ichida & Schroeder, 1996; Hoth et al., 1997; Ichida
et al., 1999). Thus, a molecular-biological approach to
modulating KIR-mediated Cs\textsuperscript{+} influx to root cells
seems feasible. Genetic modifications of root KIR
channels that restrict Cs\textsuperscript{+} permeability might allow
plants to be grown on land contaminated by
128/137Cs\textsuperscript{+}, and modifications decreasing the sen-
tivity of KIR channels to inhibition by [Cs\textsuperscript{+}]\textsubscript{ext}
might increase plant Cs\textsuperscript{+} uptake and the phyto-
 extraction of 138/137Cs\textsuperscript{+}.

3. Outward-rectifying potassium (KOR) channels

KOR channels open at voltages more positive than
the Nernst (equilibrium) potential for K\textsuperscript{+} (E\textsubscript{K}) and
facilitate K\textsuperscript{+} efflux from root cells (White, 1997). In
cells of the xylem parenchyma their role is to load K\textsuperscript{+}
into the xylem for transport to the shoot (Wegner &
De Boer, 1997; Gaymard et al., 1998; Roberts,
1998). In other cells they might function to prevent
excessive plasma membrane depolarization, for ex-
ample during cell signalling or solute uptake.

At least two distinct types of KOR have been
characterized electrophysiologically in root cell pro-
toplasts from several cell types (White, 1997). These
are termed K\textsuperscript{+}-selective outward-rectifying channels
(KORC) and nonspecific outward-rectifying chan-
gles (NORC). They can be distinguished by their
cation selectivity, kinetics, voltage-dependence and
inhibition by TEA\textsuperscript{+}. The NORC select poorly
between monovalent cations (P_{Cs} = P_{K}) (Wegner
& Raschke, 1994), but are activated only at high
positive membrane potentials (Wegner & Raschke,
1994; White, 1997) and elevated cytoplasmic Ca\textsuperscript{2+}
concentration ([Ca\textsuperscript{2+}]\textsubscript{cyt} = 5 \mu M) (Wegner & De
Boer, 1997). These channels are therefore unlikely to
contribute significantly to Cs\textsuperscript{+} influx to root cells,
because they are unlikely to open under physiological
conditions. The KORC activate at physiological
[Ca\textsuperscript{2+}]\textsubscript{cyt} (Wegner & De Boer, 1997; White, 1997).
They show sigmoidal activation kinetics (with be-
tween two and four cooperative gating particles),
open only at voltages more positive than E_{K} and
display a voltage dependence that parallels changes in
E_{K} (Roberts & Tester, 1995; Vogelzang & Prins,
1995; White & Lemtiri-Chlieh, 1995; Wegner & De
Boer, 1997, 1999; White, 1997; Roberts, 1998;
Amtmann et al., 1999). KORC are permeable to
Cs\textsuperscript{+}; estimates of their P_{Cs}/P_{K} ratios, determined
from reversal potential measurements under biionic
conditions, were 0.12 in cortical cells from Arabidop-
sis roots (Maathuis & Sanders, 1995) and 0.31 in
stelar cells from maize roots (Roberts & Tester,
1997b). The KORC are inhibited by extracellular
Ba\textsuperscript{2+} and TEA\textsuperscript{+} at millimolar concentrations, but not
by extracellular Ca\textsuperscript{2+}, Cs\textsuperscript{+} or quinine (Wegner
& Raschke, 1994; Maathuis & Sanders, 1995; Roberts
& Tester, 1995; White & Lemtiri-Chlieh, 1995;
Vogelzang & Prins, 1995; White, 1997). The NORC
are unaffected by extracellular TEA\textsuperscript{+} (Wegner
& Raschke, 1994). The KORC also seem to be
inhibited by cytoplasmic Cs\textsuperscript{+} in a voltage-dependent
manner (Maathuis & Sanders, 1995). Thus, as
cytoplasmic Cs\textsuperscript{+} is likely to increase as the Cs\textsuperscript{+}
concentration in the soil solution increases, this will
restrict K\textsuperscript{+} (and Cs\textsuperscript{+}) efflux from root cells and,
depending on the relative Cs\textsuperscript{+} sensitivities of KIR
and K\textsuperscript{+} uptake) and KOR, might result in increased
K\textsuperscript{+} accumulation by the root. This phenomenon
could therefore reconcile the contrasting reports of the
effects of [Cs\textsuperscript{+}]\textsubscript{ext} on K\textsuperscript{+} uptake by roots (Section
II.3).

A gene (SKORI) encoding a typical KORC has been
identified in Arabidopsis. Its expression is
restricted to the pericycle and xylem parenchyma
within the root stele (Gaymard et al., 1998). Its
expression was downregulated by ABA (Gaymard et
al., 1998), which is consistent with a decrease in the magnitude of KOR currents in stelar cells after pretreatment of maize roots with ABA or water stress (Roberts, 1998). The SKOR1 channel has been characterized after expression in Xenopus oocytes (Gaymard et al., 1998). It displayed sigmoidal activation kinetics and its voltage dependence shifted in parallel with changes in $E_{K}$. It was permeable to Cs$^{+}$; a $P_{Cs}/P_{K}$ ratio of 0.15 was estimated from measurements of $E_{rev}$ under (pseudo)bi-ionic conditions. The SKOR1 channel was inhibited by 10 $\mu$M verapamil, and by Ba$^{2+}$ and TEA$^{+}$ at millimolar concentrations. A physiological role of the SKOR1 channel in xylem K$^{+}$ loading is supported by the observation that Arabidopsis mutants lacking SKOR1 have lower K$^{+}$ concentrations in xylem sap and in shoot tissue than wild-type plants (Gaymard et al., 1998). Whether the down-regulation of stelar KOR channel activities would decrease Cs$^{+}$ movement to the shoot and enable crops to be grown on land contaminated with Cs radioisotopes is unknown, but this could be tested directly with Arabidopsis mutants lacking SKOR1.

A second gene (KCO1) encoding a KOR channel has also been identified in roots of Arabidopsis (Czempinski et al., 1999). The membrane location of KCO1 is not known. The KCO1 K$^{+}$ channel has been characterized after heterologous expression in baculovirus-infected insect cells (Czempinski et al., 1999). The channel was activated at voltages more positive than $E_{K}$. Its activity was critically dependent on $[Ca^{2+}]_{cyt}$, and it was activated maximally at $[Ca^{2+}]_{cyt}>300$ nM. The channel was blocked by 5 mM Ba$^{2+}$. It is not known whether KCO1 is permeable to Cs$^{+}$.

4. Voltage-insensitive cation (VIC) channels

Caesium-permeable VIC channels in the plasma membrane of cereal root cells have been characterized electrically (White, 1997; Amtmann & Sanders, 1999; Tyerman & Skerrett, 1999). These channels have been observed ubiquitously in all root cell types studied. When incorporated into planar lipid bilayers, a $P_{Ca}/P_{K}$ of 0.85 was estimated from measurements of $E_{rev}$ under bi-ionic conditions for the VIC channel from rye roots (White & Tester, 1992). In general, VIC channels have a probability of being open ($P_{o}$) of 0.65–0.80 at voltages more positive than −120 mV, but opening might decline at extreme negative voltages (White, 1997, 1999; Davenport & Tester, 2000). Cation influx through VIC channels is insensitive to TEA$^{+}$ and Cs$^{+}$, but is partly inhibited by Ca$^{2+}$, Ba$^{2+}$, Gd$^{2+}$, La$^{3+}$ and, in some species, quinine (Roberts & Tester, 1997a; Tyerman et al., 1997; White, 1997, 1999; Davenport & Tester, 2000). When plants are grown under natural conditions, cation permeation through VIC channels will be partly inhibited by Ca$^{2+}$ in the soil solution (White, 1999). However, blockade by extracellular Ca$^{2+}$ is incomplete and a high fraction (30–50%) of the potential cation influx through VIC channels can be observed even at an extracellular Ca$^{2+}$ activity of 3 mM (White, 1999).

The nonselective cation permeability, voltage-independence and pharmacology of VIC channels resemble cyclic-nucleotide-gated (CNG) channels in animals (Zagotta & Siegelbaum, 1996). Genes homologous with those for animal CNG channel have been identified in plants. These include HvCBT1, which is expressed in the plasma membrane of barley aleurone cells (Schuurink et al., 1998), NtCBP4 and NtCBP7, which are expressed on the plasma membrane of tobacco cells (Arazi et al., 1999), and a family of genes (AtCNGC1–6) present in Arabidopsis (Köhler et al., 1999; Leng et al., 1999). These genes encode proteins containing six putative transmembrane domains and binding sites for cyclic nucleotide monophosphates and calmodulin. The characteristic pore structure of K$^{+}$-selective channels (GYGD (one-letter amino acid codes)) is absent from these channels. It is replaced by the motif GQNL in HvCBT1, NtCBP4, AtCNGC1 and AtCNGC3, by GQGL in NtCBP7, by ANDL in AtCNGC2 and by GN–L in AtCNGC4. Both AtCNGC1 and AtCNGC2 complement the S. cerevisiae mutant (strain CY162) that lacks endogenous high-affinity K$^{+}$ transporters (Köhler et al., 1999) and AtCNG2 has been shown to function as a nonselective cation channel activated by lipophilic cyclic nucleotides (Leng et al., 1999). Curiously, Maathuis & Sanders (1999) observed that the activity of VIC channels in Arabidopsis root cells was decreased by increasing the cytoplasmic concentration of cyclic AMP; they nevertheless speculated that genes encoding homologues of CNG channels encode isoforms of the VIC channel.

5. Ca$^{2+}$-permeable channels

The depolarization-activated Ca$^{2+}$ channels in the plasma membrane of root cells are also permeable to Cs$^{+}$ (White, 1997, 1998). However, at Ca$^{2+}$ concentrations approaching 1 mM, Cs$^{+}$ is likely to be excluded from the pore of these channels in a manner analogous to the exclusion of Na$^{+}$ (White, 2000). These channels are therefore unlikely to contribute to Cs$^{+}$ uptake under typical ionic conditions in soil.

IV. MODELLING CAESIUM INFUX TO ROOT CELLS

Contributory mechanisms to Cs$^{+}$ influx to root cells seem to be KIR channels, KOR channels, VIC channels and high-affinity K$^{+}$ transporters such as KUP. This section describes a modelling approach aimed at elucidating the dominant mechanism(s) mediating Cs$^{+}$ influx under physiological ionic conditions. The modelling presents a paradigm that
should be revisited as more is learnt about the diversity, expression and regulation of plant uptake mechanisms. It is assumed that a root cell has a cytoplasmic K⁺ activity of 70 mM and that, before the rate of Cs⁺ influx is determined, the cytoplasm contains no Cs⁺. Influx of Cs⁺ has been calculated from solutions having K⁺ activities of either 10 μM or 1 mM and Cs⁺ activities of either 0.1 or 10 μM, which approximate the concentrations found in soil solutions.

1. Predicted Cs⁺ influx through high-affinity mechanisms

The putative, idealized high-affinity Cs⁺ transporter described in this section is based on certain assumptions about the mechanism of cation transport by KUP. Evidence suggests that KUP can facilitate K⁺/Rb⁺ uptake against its electrochemical gradient and therefore must couple K⁺/Rb⁺ transport either directly to ATP hydrolysis or to the electrochemical gradient of another ion (for example Na⁺ or H⁺). The homologous bacterial KUP and fungal HAK transporters seem to function as H⁺/K⁺ symports (Haro et al., 1999; Trchounian & Kobayashi, 1999); this is consistent with electrophysiological evidence (Maathuis & Sanders, 1994) and physiological evidence (Spalding et al., 1999) for a H⁺/K⁺ symport in plant roots. The E. coli KUP transporter apparently obeys Michaelis–Menten kinetics and has a Kₘ of 5 mM for Cs⁺ transport, a Kₘ of 0.37 mM for K⁺ transport and a Vₘₐₓ for Cs⁺ transport two-thirds that for K⁺ transport (Bossemeyer et al., 1989). If the idealized high-affinity transporter of plants exhibited a similar competition between Cs⁺ and K⁺ for transport, this would mean that increasing Cs⁺ from 0.1 to 10 μM would have little effect on the K⁺ currents mediated by the high-affinity transport mechanism, but that increasing K⁺ from 0.01 to 1 mM would decrease the Cs⁺ flux by >73%. Most of the inward current would be carried by K⁺ under all these ionic conditions. The current through the H⁺/K⁺ symport studied in Arabidopsis is approximately linearly related to voltage between 0 and −200 mV (Maathuis & Sanders, 1994). The conductance into a cortical protoplast at negative voltages and saturating [K⁺]ₜₑｘ is approx. 1200 pS (Maathuis & Sanders, 1994). Half of this is contributed by K⁺ influx. Assuming similar competition between Cs⁺ and K⁺ for transport to that observed for the KUP transporter of E. coli, the relationship between Cs⁺ influx and voltage can be calculated under various ionic conditions for a putative high-affinity Cs⁺ transporter (Fig. 5a,b). Both higher [Cs⁺]ₜₑₓ and higher [Cs⁺]ₜₑₓ/[K⁺]ₜₑₓ ratios favour Cs⁺ influx through this mechanism.

2. Predicted Cs⁺ influx through cation channels

Cation permeation through KIR and KOR channels in the plasma membrane of root cells has been approximated here by the Goldman–Hodgkin–Katz (GHK) current equation (Hille, 1992). This equation describes the independent diffusion of cations in a constant electric field and follows the precedents of Maathuis & Sanders (1997), Amtmann & Sanders (1999) and White (1999). Although cation permeation through these channels might eventually be described better by using energy-barrier or reaction-kinetic models (White et al., 1999), such models are not available at present. However, at low cation concentrations, cation permeation obeys the rules of independence (i.e. cations do not interact within the pore or compete for a transport binding-site) and permeation can be described qualitatively with the GHK current equation.

For these simulations it was assumed that the opening of KIR channels was unaffected by [K⁺]ₜₑₓ (White, 1997). The predicted Cs⁺ influx through KIR was greatest when both [Cs⁺]ₜₑₓ and [K⁺]ₜₑₓ were high (Fig. 5c). Under these conditions Cs⁺ influx is promoted not only by raising the concentration of the permeant cation but also by high [K⁺]ₜₑₓ decreasing the magnitude of the inhibition of the KIR channels by [Cs⁺]ₜₑₓ. At low (micromolar) [K⁺]ₜₑₓ, [Cs⁺]ₜₑₓ effectively inhibits the KIR channels (Fig. 5c,d).

It was assumed that KOR channels would open at voltages substantially more positive than Eᵣ, and at all [K⁺]ₜₑₓ, their opening would be regulated to parallel changes in Eᵣ (White, 1997). On the basis of these assumptions, an appreciable Cs⁺ influx through the KOR channel was predicted to occur only at extremely low [K⁺]ₜₑₓ and high [Cs⁺]ₜₑₓ (Fig. 5e). However, these simulations overestimate Cs⁺ influx through KOR channels if [K⁺]ₜₑₓ regulates the opening of KOR by an additional allosteric interaction, which decreases Pᵣ substantially at low [K⁺]ₜₑₓ, in the manner proposed by Gaymard et al. (1998).

Cation permeation through the VIC channel was modelled by using an energy-barrier model derived for the VIC channel in the plasma membrane of rye roots (White, 1997, 1999), supplemented with the free-energy profiles for Cs⁺ permeation deduced from data presented by White & Tester (1992). This model assumes single-file permeation through the pore of VIC channels, and places two cation-binding sites within the pore. These sites can be occupied simultaneously and cations can interact within the pore. One consequence of these interactions is that Cs⁺ influx through VIC channels is inhibited by increasing [K⁺]ₜₑₓ (Fig. 5f,g). Thus, the greatest Cs⁺ influx through VIC channels is predicted to occur when [K⁺]ₜₑₓ is low and [Cs⁺]ₜₑₓ is high (Fig. 5f).

Simulations of Cs⁺ influx to a stereotypical root cell from solutions with K⁺ activities of either 10 μM or 1 mM and Cs⁺ activities of either 0.1 or 10 μM indicated that most of the Cs⁺ influx would be mediated by VIC channels, supplemented by high-
Fig. 5. (a, b) The relationships between Cs⁺ influx into a stereotypical root cell through idealized H⁺/K⁺ transporters and the cell membrane potential. The Cs⁺ influx was calculated on the assumption of competition between Cs⁺ ($K_r = 5$ mM) and K⁺ ($K_m = 0.37$) for transport, and maximum conductances of 400 pS for Cs⁺ and 600 pS for K⁺. Extracellular solutions contained Cs⁺ activities of either 10 μM (a) or 0.01 μM (b) and K⁺ activities of either 1 mM (broken line) or 10 μM (solid line). Note the different scales on the y-axis. (c, d) The relationships between Cs⁺ influx into a stereotypical root cell through idealized KOR channels and the cell membrane potential. Extracellular solutions contained Cs⁺ activities of either 10 μM (c) or 0.01 μM (d) and K⁺ activities of either 1 mM (broken line) or 10 μM (solid line). The Cs⁺ currents were calculated by multiplying together the fraction of uninhibited channels (determined as described for KAT1 in Fig. 4), the probability of finding KOR open (P_sl = 1/(1 + exp(V/Vₗ₉₅))/S), where Vₗ₉₅ = −140 mV and S = 18.66) and the maximum Cs⁺ currents calculated from the GHK current equation assuming an absolute K⁺ permeability (Pₖ) of $2.67 \times 10^{-17}$ m⁵ s⁻¹ (equivalent to c. 400 channels) and a $P_{Cs}/P_{K}$ ratio of 0.4. (e) The relationships between Cs⁺ influx into a stereotypical root cell through idealized KIR channels and the cell membrane potential. Extracellular solutions contained Cs⁺ activities of 10 μM and K⁺ activities of either 1 mM (broken line) or 10 μM (solid line). The Cs⁺ currents were calculated by multiplying the probability of finding KIR open (P_sl = 1/(1 + exp((Vₗ₉₅ − V)/S)))², where Vₗ₉₅ = 100 + Eₗ, V = cell membrane potential and S = 28) (White, 1997) by the maximum cation currents calculated from the GHK current equation assuming an absolute K⁺ permeability (Pₖ) of $9.60 \times 10^{-18}$ m⁵ s⁻¹ (equivalent to c. 150 channels) and a $P_{Cs}/P_{K}$ ratio of 0.15. (f, g) The relationships between Cs⁺ influx into a stereotypical root cell through idealized VIC channels and the cell membrane potential. Extracellular solutions contained Cs⁺ activities of either 10 μM (f) or 0.01 μM (g) and K⁺ activities of either 1 mM (broken line) or 10 μM (solid line). Permeation through a single VIC channel was modelled by using the computer program AJUSTE (Alvarez et al., 1992) with the parameters for pore structure and free-energy profile for K⁺ permeation given by White (1997). This was supplemented by a free-energy profile for Cs⁺ permeation ($G_i = 2.41, G_o = −4.99, G_s = 4.51, U_1 = −10.66, U_2 = −8.93 \ RT$, reference state 55.5 M) deduced from data presented by White & Tester (1992). Cellular Cs⁺ currents were calculated by assuming that a cell contained 50 VIC channels, each with a voltage-insensitive $P_o$ of 0.8.
affinity transporters (Fig. 6). The KIR channels were predicted to contribute between 8% and 14% of the total Cs\(^+\) influx only at voltages more negative than \(-140\) mV and in the presence of high [K\(^+\)]\(_{ext}\) (Fig. 6a,b). A tiny absolute Cs\(^+\) influx (but between 8% and 26% of the total influx) was predicted through KOR in the presence of low [K\(^+\)]\(_{ext}\) and high [Cs\(^+\)]\(_{ext}\) at voltages more positive than \(-20\) mV (Fig. 6d). However, this might not be manifested if KOR are modulated allosterically by [K\(^+\)]\(_{ext}\) in the manner described by Gaymard et al. (1998). If the high-affinity transporters are only present in 10% of root cells (Maathuis & Sanders, 1994), the relative contributions of the VIC and KIR channels to the total Cs\(^+\) influx are, of course, greater.

3. Predicted dependence of Cs\(^+\) influx on [Cs\(^+\)]\(_{ext}\)

Caesium uptake into plant roots has frequently been determined from solutions containing only CsCl (plus 0.5 mM Ca\(^{2+}\)). Under these conditions the presence of Cs\(^+\) induces marked membrane depolarizations in plant roots (Rubio et al., 1996; Sacchi et al., 1997). To determine a theoretical relationship between Cs\(^+\) influx and [Cs\(^+\)]\(_{ext}\), the projected Cs\(^+\) influx through the various Cs\(^+\) transporters was calculated at appropriate membrane potentials. By analogy with K\(^+\)-induced depolarizations (Maathuis & Sanders, 1994), it was assumed that Cs\(^+\)-induced membrane depolarizations would have a Michaelis–Menten dependence on [Cs\(^+\)]\(_{ext}\) at concentrations <500 \(\mu\)M (Fig. 7a). A maximum depolarization (\(V_{\text{max}}\)) of 26.4 mV and a \(K_m\) of 9.8 \(\mu\)M were calculated from the data obtained by Sacchi et al. (1997) under appropriate external ionic conditions. The projected Cs\(^+\) influx into a stereotypical root cell containing H\(^+\)/K\(^+\) transporters with a maximum Cs\(^+\) conductance of 400 pS, 400 KIR, 150 KOR and 50 VIC channels exhibited a sublinear, non-Michaelis–Menten dependence on [Cs\(^+\)]\(_{ext}\) (Fig. 7b,c). This resembles the observed relationship between Cs\(^+\) influx into excised roots and [Cs\(^+\)]\(_{ext}\) (Shaw & Bell, 1989) (Fig. 1a,b). The projected Cs\(^+\) influx was dominated at all [Cs\(^+\)]\(_{ext}\) by the contribution of VIC channels. The remainder was contributed by the ‘high-affinity’ H\(^+\)/K\(^+\) transporters. No significant contribution to the projected Cs\(^+\) influx was made by either KIR or KOR. The contribution of VIC channels to the projected Cs\(^+\) influx would be even greater if the ‘high-affinity’ transporters were restricted to <10% of root cells (Maathuis & Sanders, 1994). Ironically, the relative contribution of the VIC channels was greatest at the lowest [Cs\(^+\)]\(_{ext}\) and that of the ‘high-affinity’ transporters at higher [Cs\(^+\)]\(_{ext}\). The reasons for this are (1) that channel-mediated Cs\(^+\) influx can occur at all [Cs\(^+\)]\(_{ext}\) because there is no [Cs\(^+\)]\(_{cyt}\), and (2) that the \(K_m\) of the ‘high-affinity’ transporters towards [Cs\(^+\)]\(_{ext}\) was assumed to be 5 mM. These calculations support the conclusion that the VIC channels are a major mechanism mediating Cs\(^+\) influx to root cells.

The conclusion that the VIC channels are a major mechanism mediating Cs\(^+\) influx to root cells is corroborated by the similar pharmacologies of the VIC channel, Cs\(^+\) influx and uptake into excised roots, and plant accumulation of Cs\(^+\). Both VIC channels (White, 1997, 1999) and Cs\(^+\) transport processes (Handley & Overstreet, 1961; Sze & Hodges, 1977; Smolders et al., 1997) are incompletely inhibited by both Ca\(^{2+}\) and Ba\(^{2+}\). The \(K_i\) values for the decrease in the Ca\(^{2+}\)-sensitive portions

![Fig. 6. The relationship between the total Cs\(^+\) current into a stereotypical root cell and the cell membrane potential. Extracellular solutions contained activities of (a) 1 mM K\(^+\) and 0.1 \(\mu\)M Cs\(^+\), (b) 1 mM K\(^+\) and 10 \(\mu\)M Cs\(^+\), (c) 10 \(\mu\)M K\(^+\) and 0.1 \(\mu\)M Cs\(^+\), (d) 10 \(\mu\)M K\(^+\) and 10 \(\mu\)M Cs\(^+\). The total Cs\(^+\) current (solid line) was calculated by summing the individual Cs\(^+\) currents through the idealized H\(^+\)/K\(^+\) transporters, KIR, KOR and VIC channels presented in Fig. 5. The contributions of the VIC channels (stippled) and the H\(^+\)/K\(^+\) transporters (hatched) to the total Cs\(^+\) current are shown. Note the different scales on the y-axis.](image-url)
... into a stereotypical root cell and the extracellular Cs

+ potential and the extracellular Cs

+ concentration. Currents were calculated by assuming the

+ concentration shown in (b). The line is plotted to a

+ relationship with a maximum depolarization (V

+ mV, a K

+ of 9.8 μM towards [Cs

+ ]_ext and a membrane potential of −144 mV in the absence of

+ Cs. (b) The relationship between the total Cs

+ current (solid line) or the current through VIC channels (broken

+ line) into a stereotypical root cell and the extracellular Cs

+ concentration. Currents were calculated by assuming the

+ presence of H

+ /K

+ transporters (with a maximal Cs

+ conductance of 400 pS), 400 KIR, 150 KOR and 50 VIC channels. (c) Hofstee transformation of the relationship

+ between the total Cs

+ current into a stereotypical root cell and the extracellular Cs

+ concentration shown in (b).

**V. perspective**

Stable 133Cs is present in soils at concentrations up
to 25 μg g

−1 of dry soil. This corresponds to low

micromolar Cs

+ concentrations in soil solutions. Although there is no known role for Cs in plant

nutrition, two radioactive Cs isotopes (134Cs and

137Cs) are of environmental concern owing to their rapid incorporation into biological systems, relatively long half-lives and emissions of β and γ radiation during decay. Soil activities of these isotopes can exceed 30 Bq cm

−2 (6.2 × 10

−7 μg of

137Cs g

−1 of soil) in contaminated areas. There is therefore considerable interest in (1) preventing radioactive Cs isotopes from entering the food chain through the development of crops that do not accumulate Cs

+, and (2) remediation of contaminated areas by using plants that accumulate large amounts of Cs. Because Cs enters plants through the root symplasm, it is argued that Cs

+ transport across the plasma membrane of root cells determines the potential of a plant for the cultivation or phyto-

remediation of contaminated land.

The molecular identities of putative Cs

+ transport mechanisms in the plasma membrane of root cells have been inferred from recent molecular-biological and electrophysiological studies (Fig. 8). Both the pharmacology of Cs

+ influx to roots and predictions from theoretical models of Cs

+ fluxes through cation transporters in the plasma membrane suggest that...
VIC channels mediate most of the Cs\textsuperscript{+} influx to root cells under realistic soil conditions. Thus, the downregulation of VIC channel activities could decrease Cs uptake by plants, and genes for these channels provide molecular targets to enable crops to be cultivated on land contaminated with radioactive Cs isotopes. Additional Cs\textsuperscript{+} influx might be mediated by Cs\textsuperscript{+}/H\textsuperscript{+} symporters, tentatively ascribed here to the KUP/HAK gene family. The KOR channels in the xylem parenchyma facilitate Cs\textsuperscript{+} efflux to the xylem; it is possible that the downregulation of stelar KOR channel activities would reduce Cs\textsuperscript{+} movement to the shoot and also enable crops to be grown on \(^{134,137}\)Cs-contaminated soil. This hypothesis can be tested directly by using Arabidopsis mutants lacking SKOR1 (Gaymard et al., 1998). Modelling studies indicate that negligible Cs\textsuperscript{+} uptake occurs through the dominant KIR channel in the root plasma membrane (AKT1), which is inhibited by [Cs\textsuperscript{+}]\textsubscript{ext}. However, genetic modifications of the P domain of KIR channels have been identified that decrease their sensitivity to inhibition by [Cs\textsuperscript{+}]\textsubscript{ext} and increase their permeability to Cs\textsuperscript{+}. Modified KIR channels could enhance the uptake of Cs\textsuperscript{+} by plants and enable the phytoextraction of radioactive Cs isotopes.

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Mechanisms of caesium uptake


