Chloride in Soils and its Uptake and Movement within the Plant: A Review

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INTRODUCTION: CHLORINE IN THE ENVIRONMENT

Context

Chlorine (Cl) is an essential micronutrient for higher plants, and a minimal requirement for crop growth of 1 g kg⁻¹ dry weight has been suggested (Marschner, 1995). This quantity can generally be supplied by rainfall, and Cl-deficient plants are rarely observed in agriculture or nature. However, high tissue Cl concentrations can be toxic to crop plants, and may restrict the agriculture of saline regions (Xu et al., 2000). Part of the current interest in studying Cl uptake and accumulation in plants relates to the pressures to develop more salt-tolerant crop varieties for use in saline environments. A second reason for studying Cl uptake and accumulation in plants relates to a radioisotope of Cl, ³⁶Cl, which has been identified as an environmental contaminant. Since there is concern that ³⁶Cl may have a detrimental effect on human health (Sheppard et al., 1996), factors affecting the accumulation of ³⁶Cl within the food chain need to be identified. This radioisotope occurs primarily in materials from the operations of the nuclear industry, and an understanding of the movement of ³⁶Cl through soils and into plants is important for the planning of deep repositories for nuclear waste (Norris et al., 1997; Koch-Steindl and Prohl, 2001). The aim of this review is to highlight pertinent aspects of the biodynamics and availabilities of Cl in the soil, the uptake and distribution of Cl within the plant, and the mechanisms of Cl transport across membranes and between tissues, the mechanisms of Cl transport across membranes and the electrical characteristics and molecular biology of Cl-channels.

Key words: Review, Arabidopsis thaliana, channel, chloride (Cl⁻), influx, phloem, plasma membrane, radiochlorine (³⁶Cl), soil, tonoplast, transport, uptake, xylem.

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denote the element, irrespective of chemical form, and ‘chloride’ or $\text{Cl}^-$ is used to denote the ionic form of the element. Since there is no evidence of discrimination in biological processes between isotopes of Cl, no attempt has been made to distinguish between them, except with reference to the radiobiology of $^{36}\text{Cl}$. The radioisotope $^{36}\text{Cl}$ is a low-energy beta emitter. Since $^{36}\text{Cl}$ has a half-life of $3.01 \times 10^5$ years, there is concern that it may have a significant long-term effect on human health through prolonged exposure (Sheppard et al., 1996).

**Inputs of chlorine to the soil**

Chlorine inputs to soils occur mainly as a result of depositions of Cl$^-$ from rainwater, fertilizer applications (KCl), irrigation waters, sea spray, dust and air pollution. The Cl$^-$ content of rainwater depends upon proximity to salt water and varies greatly. Values between 3000 and 0.4 ppm (8.5 mm to 11 $\mu$m) have been recorded (Hewitt and Smith, 1974; Xu et al., 2000). Thus, Cl$^-$ deposition to soils near to the sea or to salt lakes may be extremely high. Öberg (1998) cites Cl deposition rates ranging between 1 and 100 kg ha$^{-1}$ year$^{-1}$ depending on location. Human practices such as irrigation and fertilization also influence the amounts of Cl$^-$ deposited to the soil. The Cl$^-$ concentrations in saline irrigation waters range from 2 to 30 mm, and even irrigation with water of low salinity can easily deposit 1000 kg Cl$^-$ ha$^{-1}$ year$^{-1}$ (Xu et al., 2000). In some circumstances, this may exceed all other forms of Cl$^-$ deposition. Fertilizer inputs also increase soil Cl$^-$ substantially. For example, in one study the Cl$^-$ concentration in the upper 30 cm of a soil was 0.25 mm for untreated plots and 0.73 mm for those receiving 169 kg Cl$^-$ ha$^{-1}$ fertilizer (Parker et al., 1983).

Natural organochlorine compounds are produced in vast quantities, in both marine environments and in the soil, and about 2000 naturally produced organochlorine compounds have been identified to date (Fleming, 1995). Deposition rates of organic-Cl are not as well documented as those for Cl$^-$. In Scandinavia, upper estimates suggest that precipitation may contain 15 $\mu$g l$^{-1}$ organic-Cl (Öberg, 1998). This represents an annual deposition rate of 0.1 kg ha$^{-1}$. Total organic-Cl deposition to soils may be greater than this, due to incorporation of organic-Cl as rain percolates through plant canopies. Based on estimates of plant organic-Cl content (0.01–0.1 mg g$^{-1}$ d. wt), it has been estimated that soils in a Swedish forest ecosystem will receive total organic-Cl deposition rates of between 0.04–0.4 kg ha$^{-1}$ year$^{-1}$ (Öberg, 1998). Anthropogenic sources of organic-Cl will also contribute to Cl deposition rates to soils. These arise from the chlorination of soil (as a disinfectant), the disposal of household waste and the dechlorination of organic compounds. Anthropogenic organochlorines include herbicides (xenobiotics), pesticides, medical pharmaceuticals and artificial sweeteners.

**Inputs of $^{36}\text{Cl}$ to the soil**

$^{36}$Cl occurs naturally in the environment due to (1) cosmic-ray spallation of $^{40}$Ar in the atmosphere, (2) interactions between cosmic radiation and Cl, Ca and K in near-surface rocks and soils, and (3) activation of stable $^{35}$Cl in the subsurface by naturally produced thermal neutrons (see reviews by Bentley et al., 1986; Phillips et al., 1990; Davis et al., 1998). Approximately two-thirds of natural $^{36}$Cl production occurs in the stratosphere. The deposition of this component is determined largely by the latitudinal dependence of stratosphere-troposphere mixing (Keywood et al., 1998). Thus, it is thought that $^{36}$Cl deposition peaks between the mid-latitudes 30°–60°, where the deposition in precipitation may approach 30 atoms m$^{-2}$ s$^{-1}$ (Bird et al., 1991; Keywood et al., 1998). Production of $^{36}$Cl at the Earth’s surface due to cosmic radiation is a function of exposure time, latitude, altitude, and the quantity of target elements in a sample. For example, production rates of $^{36}$Cl from spallation of $^{39}$K and $^{40}$Ca in terrestrial rocks has been calculated at 4160 and 3050 atoms $^{36}$Cl year$^{-1}$ mol$^{-1}$ respectively (Zreda et al., 1991). At a latitude of 30°, $^{36}$Cl production rates have been calculated to range between 0.3 and 1.7 atoms m$^{-3}$ s$^{-1}$ in surface rocks, and between 4.1 $\times$ 10$^{-6}$ and 1.9 $\times$ 10$^{-5}$ atoms m$^{-3}$ s$^{-1}$ in deep rocks, depending on rock type (Bird et al., 1991). Spontaneous fission of $^{238}$U will also induce alpha-particle irradiation of elements such as O, Na, Mg, Al, Si and neutron release, which can lead to $^{36}$Cl production (Bird et al., 1991). However, in areas of elevated natural U deposits $^{36}$Cl in groundwater can be mainly attributed to cosmicogenic radiation (Jiang et al., 1994).

Anthropogenic inputs of $^{36}$Cl to soils arise from (1) nuclear explosions, (2) the operation of nuclear industrial processes and (3) the use of neutron sources in environmental studies. Although the first two sources are likely to represent the dominant anthropogenic inputs, the production of $^{36}$Cl from neutron sources may be significant, particularly if sources are used in proximity to saline waters (Bird et al., 1991). In nuclear explosions, neutrons are released that react with natural solid, liquid and gaseous particles containing $^{35}$Cl, $^{39}$K or $^{36}$Ar (Bentley et al., 1986). For example, following nuclear weapons testing between 1954 and 1960, deposition rates of $^{36}$Cl reached 4000 atoms m$^{-2}$ s$^{-1}$, as measured in a Greenland ice-core. By 1985, deposition rates had declined to pre-testing levels of 10 atoms m$^{-2}$ s$^{-1}$ (Bird et al., 1991). Similarly, seven southern Australian soil profiles showed single peaks corresponding to high nuclide fallout from the 1950s and 1960s. Total fallout represented between 1.2 and 2.4 $\times$ 10$^{12}$ atoms m$^{-2}$, matching that found in similar latitudes in the northern hemisphere (Cook et al., 1994).

During the operation of nuclear industrial processes, and particularly in nuclear power reactors, $^{36}$Cl is produced by neutron activation of $^{35}$Cl in materials such as stainless steels, Magnox alloys, concrete shielding and (possibly) light water reactor UO$_2$ fuel pellets (Sheppard et al., 1996; Parry et al., 1997). Thus, $^{36}$Cl will be present in waste fuel and construction material originating from nuclear installations. An indirect input of $^{36}$Cl into the environment can occur via the combustion of biomass, contaminated as a result of releases from nuclear industries. For example, biomass fires reaching temperatures of 400°C will release up to 40 % of biomass $^{36}$Cl content to the atmosphere.
that consist of straw materials, or that reach temperatures of 1000°C, will release virtually all biomass $^{36}$Cl to the atmosphere (Amiro et al., 1996).

**Availability of chlorine in soils**

Chlorine occurs predominantly as Cl$^-$ in the soil. The Cl$^-$ anion does not form complexes readily and, since exchange sites on layer silicates in soil clays are predominantly negatively charged, Cl$^-$ tends to be repelled from mineral surfaces contained in many soil water particles (Bohn et al., 1979). Thus, the concentration of Cl$^-$ in the bulk solution is greater than in the diffuse layers surrounding soil particles. In addition, these repulsive forces also lead to regions within the soil that are incapable of contributing to Cl$^-$ movement, and the actual volume contributing to Cl$^-$ movement is generally less than that estimated from water-filled porosity measurements. Nevertheless, since Cl$^-$ shows little adsorption to soil components and, unlike other major soil anions such as NO$_3^-$ and SO$_4^{2-}$, is not chemically altered by soil organisms, it is often used as a tracer for soil water movement.

The movement of Cl$^-$ within the soil is determined by water fluxes (Tisdale et al., 1985) and, in particular, the relationship between precipitation and evapotranspiration. For example, in an experiment to determine salt movement in a soil profile, Burns (1974) incorporated CaCl$_2$ to non-irrigated, uncropped plots of sandy loam soil in the UK to a depth of 15 cm. Soil was then sampled between May and October to a depth of 45 cm (Fig. 1). During the early part of the experiment, evaporation exceeded rainfall, and an upward movement of Cl$^-$ was observed. After heavier rainfall, the soil water reached field capacity and a downward movement of Cl$^-$ occurred. In the latter part of the experiment, precipitation and evapotranspiration were equal, and Cl$^-$ redistribution was small.

Although Cl$^-$ redistribution in the soil profile is likely to be dominated by mass flow and convection processes associated with water fluxes, significant diffusive movement may also occur in soils where concentration gradients are high and when the soil is moist. A typical value for the Cl$^-$ diffusion coefficient in moist soil is $2 - 9 \times 10^{-6}$ cm$^2$ s$^{-1}$ (Rowell et al., 1967). Physical irregularities in soil structure, such as the presence of aggregates or cracks, will also affect Cl$^-$ movement. This may be particularly pronounced in soils with high organic or clay components, and can cause deviations from models based solely on precipitation and evapotranspiration (Burns, 1974).

Recent research, reviewed by Öberg (1998), suggests that there may be a significant and previously overlooked organic-Cl pool in soils. This accretes naturally through the chlorination of carbon compounds by micro-organisms, plants and fungi. Evidence of net accumulation of organic-Cl has been obtained from studies in a Swedish spruce forest ecosystem, which might be a significant sink for Cl in areas receiving low Cl$^-$ inputs (Hjelm et al., 1995). Since the dynamics of organic-Cl pools in soils will impact on the atmospheric loading of halogenated hydrocarbons, they will affect environmental pollution.

**Synopsis of chlorine in the environment**

To summarize this section: (1) rates of Cl$^-$ deposition to soils range from 1 to $> 1000$ kg ha$^{-1}$ year$^{-1}$ depending on location and cultural practices; (2) a natural and anthropogenic radiisotope of Cl, $^{36}$Cl, may be of environmental concern due to its long half-life and rapid incorporation into biological systems; (3) chlorine occurs in the soil solution mainly as Cl$^-$, and its movement is largely determined by water flows; and (4) there is evidence of a significant organic-Cl pool in soils.

**Chlorine is a mineral nutrient**

Chlorine is an essential micronutrient for higher plants (Marschner, 1995). It is present mainly as Cl$^-$, although plants do contain compounds with covalently bound Cl (Engvild, 1986). Chloride is a major osmotically active solute in the vacuole and is involved in both turgor- and osmoregulation. In the cytoplasm it regulates the activities of enzymes. Chloride also acts as a counter anion, and Cl$^-$ fluxes are implicated in the stabilization of membrane potential, the regulation of pH gradients and electrical excitability.

**Chlorine deficiency**

The growth of many plants is reduced substantially in Cl$^-$-free media (Broyer et al., 1954; Xu et al., 2000), and environmental factors that enhance growth rate increase susceptibility to Cl deficiency (Ozanne et al., 1957).
Deficiency causes reduced leaf growth and wilting, followed by chlorosis, bronzing and, finally, necrosis. Roots become stunted and the development of laterals is suppressed. Fruits are decreased in numbers and size. The least sensitive plants are (large seeded) beans (Phaseolus spp.), squash (Cucurbita maxima), barley (Hordeum vulgare), maize (Zea mays) and buckwheat (Fagopyrum esculentum). The tissue Cl⁻ concentration at which deficiency symptoms are observed varies between about 0.1 to 5-7 mg g⁻¹ d. wt (Xu et al., 2000). To some extent, Br⁻ can replace Cl⁻ (Ozanne et al., 1957), but this is of no natural significance since the abundance of Br in the Earth's crust is about 100-fold less than that of Cl. Although Cl deficiency symptoms can be induced readily in the laboratory they are rarely observed under field conditions because Cl⁻ concentrations in soils are generally high (see Introduction) and only small amounts of Cl are required by plants. Indeed, assuming a minimal requirement for crop growth of 1 g kg⁻¹ d. wt (Marschner, 1995), only 4 to 8 kg Cl ha⁻¹ is required for the average crop. This can be supplied by rainfall. Nevertheless, a substantial increase in yield has been reported for many crops in response to Cl fertilization (Xu et al., 2000).

Chloride toxicity

The growth responses of plants to high Cl⁻ concentrations in the external medium ([Cl⁻]ₘₑₓ) can be divided into four categories (Fig. 2; Greenway and Munns, 1980). Species can be grouped into (I) halophytes (defined as the native flora of saline soils), which can be subdivided into (IA) species whose growth is stimulated (e.g. Sueda maritima, Atriplex nummularia) or (IB) species whose growth is little affected by 200 mM [Cl⁻]ₘₑₓ (e.g. Atriplex hastata, Spartina spp., sugar beet); (II) halophytes and non-halophytes (glycophytes) whose growth is reduced substantially by 100 mM [Cl⁻]ₘₑₓ, which can be subdivided into tolerant (e.g. Festuca rubris, Puccinella peisonis, cotton, barley), intermediate (e.g. tomatoes) and sensitive (e.g. beans, soybeans) species; and (III) very salt-sensitive non-halophytes (e.g. citrus and woody plant species). Many important cereal, vegetable and fruit crops are susceptible to Cl⁻ toxicity during cultivation. This is a major constraint to horticultural production on irrigated or saline soils (Maas and Hoffman, 1977; Xu et al., 2000). The critical tissue Cl⁻ concentration for toxicity is about 4-7 and 15-50 mg g⁻¹ d. wt for Cl⁻-sensitive and Cl⁻-tolerant plant species, respectively.

Differences between cultivars to withstand Cl⁻ toxicity are frequently related to the ability to restrict Cl⁻ transport to the shoot. This has been observed in soybean (Glycine max; Abel, 1969), wheat (Triticum aestivum; Bernal et al., 1974), barley (Greenway and Munns, 1980), stone fruit trees (Bernstein et al., 1956), grapevine (Vitis spp.; Antcliff et al., 1983) and citrus (reviewed by Maas, 1993; Storey and Walker, 1999). The trait is heritable (Abel, 1969; Sykes, 1992) and, in soybean, it is determined by a single gene (nc1; Abel, 1969). Thus, there is the opportunity for simple breeding and GM approaches to limit Cl⁻ accumulation and to generate plants that withstand Cl⁻ toxicity. Indeed, an interesting practical approach for producing citrus lines tolerant to [Cl⁻]ₘₑₓ is based on the selection of chemically-generated mutants with decreased shoot Cl⁻ concentration ([Cl⁻]ₗₒₒ₅; Garcia-Augustin and Primo-Millo, 1995).

Chloride distribution within the plant

There is considerable variation in the ability of plants to accumulate Cl⁻ (Cram, 1976; Greenway and Munns, 1980). Halophytes generate turgor by accumulating high Cl⁻ concentrations in plant tissues ([Cl⁻]ₜᵋₛᵋᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋₑᵋ_edge). Variable concentrations of Cl⁻ among plant organs (e.g. 340 to 475 mM in halophytes, 10-15 mM in glycophytes) suggest that there are differences in the ability of plants to accumulate Cl⁻.
Synopsis of chloride as a mineral nutrient

Chlorine is an essential plant nutrient, and deficiency symptoms are observed at [Cl]_{tissue} less than about 0.1-5.7 mg g\(^{-1}\) d. wt (approx. 0.03-17 mm). As Cl\(^{-}\), it fills a variety of specific biochemical and biophysical roles. High tissue Cl\(^{-}\) concentrations are toxic, but plant responses to [Cl\(^{-}\)]_{xylem} vary greatly among genera, species and cultivars. Toxicity occurs at [Cl\(^{-}\)]_{tissue} of about 4-7 and 15-50 mg g\(^{-1}\) d. wt in Cl\(^{-}\)-sensitive and Cl\(^{-}\)-tolerant plant species, respectively. Differences between cultivars to withstand Cl\(^{-}\) toxicity are generally inherited, and relate to the ability of the root to restrict Cl\(^{-}\) transport to the shoot. Older leaves tend to accumulate Cl\(^{-}\), whereas seeds and fruit have low [Cl\(^{-}\)]_{tissue}.

CHLORIDE FLUXES WITHIN THE PLANT

Pathways of chloride transport across the root

Plants acquire most of their Cl from the soil solution as the Cl\(^{-}\) anion. To support plant growth, Cl\(^{-}\) is loaded into the xylem and thereby delivered to the shoot. In principle there are two pathways by which anions might reach the xylem and thereby delivered to the shoot. In principle there are two processes by which anions might reach the xylem and thereby delivered to the shoot. In principle there are two processes by which anions might reach the xylem and thereby delivered to the shoot. In principle there are two processes by which anions might reach the xylem and thereby delivered to the shoot.

- Symplastic pathway: The symplastic pathway enters root cells across their plasma membranes, are transferred from cell to cell through plasmodesmatal connections and are exported across the plasma membrane of cells within the stele. This pathway includes at least two processes catalysed by Cl\(^{-}\) transporters in the plasma membrane, which could control the selectivity and magnitude of Cl\(^{-}\) fluxes to the shoot. Alternatively, anions may journey extracellularly through the cell walls and water-filled spaces to reach the stele. This would be a relatively non-selective process governed by the transport of water through ‘solvent drag’. The diffusive penetration of Cl\(^{-}\) in the apoplast (D_{Cl}) approximates 10\(^{-10}\) m\(^2\) s\(^{-1}\) (Pitman, 1982). This figure allows diffusional Cl\(^{-}\) fluxes to be calculated if Cl\(^{-}\) concentration gradients are known. The apoplastic pathway is restricted by the epidermal and endodermal Casparian bands, which span substantial lengths of plant roots (White, 2001). However, Casparian bands are absent at the root apex and may be breached by the formation of secondary roots. It is probable that Cl\(^{-}\) reaches the xylem by both pathways. However, the symplastic pathway is considered to dominate under most circumstances (Pitman, 1982).

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Chloride fluxes and accumulation within the root

Plants readily accumulate Cl, and Cl\(^{-}\) fluxes have been estimated for a wide range of plant material using diverse experimental protocols. Before discussing these measurements, several terms need to be defined (Fig. 3). Influx is defined as the unidirectional flux into a cell across the plasma membrane. This is frequently estimated by allowing tissues to accumulate \(^{36}\)Cl\(^{-}\) from solutions containing this radioisotope for a period of 5-10 min and then removing the extracellular \(^{36}\)Cl\(^{-}\) by washing briefly in solutions containing non-radioactive Cl\(^{-}\). Efflux is defined as the unidirectional flux out of a cell across the plasma membrane. This is frequently estimated from the loss of \(^{36}\)Cl\(^{-}\) from tissues preloaded with this radioisotope shortly after transfer to non-radioactive solutions. There may be efflux from a root segment to both the external medium and to the stele/xylem. Uptake is equivalent to the unidirectional flux
from the cytoplasm to the vacuole. It is estimated by allowing tissues to accumulate $^{36}\text{Cl}^-$ from solutions containing this radioisotope for a period of several hours and then removing the extracellular $^{36}\text{Cl}^-$ by washing briefly in solutions containing non-radioactive $\text{Cl}^-$. **Vacuolar efflux** is the unidirectional flux out of the vacuole to the cytoplasm. It is estimated from the loss of $^{36}\text{Cl}^-$ from tissues preloaded with this radioisotope several hours after transfer to non-radioactive solutions. Analysis of the loss of $^{36}\text{Cl}^-$ from tissues preloaded with this radioisotope after transfer to non-radioactive solutions is frequently referred to as 'compartmental analysis' since it enables the experimenter to estimate the $\text{Cl}^-$ concentration in the cytoplasmic and vacuolar compartments (Macklon, 1975; Davies and Higinbotham, 1976; Cram, 1983a; Hajibagheri et al., 1988). **Accumulation** integrates the total $\text{Cl}$ content of tissues, which includes both cellular and extracellular compartments. Both Cram (1975b) and Pitman (1982) have discussed the caveats of these experimental methods.

To investigate $\text{Cl}^-$ dynamics at the cellular level, $\text{Cl}^-$ fluxes into roots of intact plants and excised root segments have been estimated in plants previously grown in solutions containing only $\text{CaSO}_4$, to produce 'low-salt' plants, or in complete nutrient media to produce 'high-salt' plants. The estimated $\text{Cl}^-$ fluxes differ considerably between these treatments. $\text{Cl}^-$ fluxes through the root of intact plants are dominated by $\text{Cl}^-$ translocation to the shoot (Pitman, 1971) and plants deprived of $\text{Cl}$ generally exhibit a greater capacity and affinity for $\text{Cl}^-$ uptake compared to replete plants (Pitman, 1969; Lee, 1982; Cram, 1983b). Since environmental $[\text{Cl}^-]_{\text{ext}}$ is generally replete and constant, $\text{Cl}^-$ fluxes obtained under steady-state conditions in roots of intact plants grown in complete nutrient media are more likely to resemble those prevalent in agricultural, horticultural and natural systems.

The relationships between $\text{Cl}^-$ influx or uptake and $[\text{Cl}^-]_{\text{ext}}$ have been determined for plants grown and assayed under a variety of ionic conditions (Table 1). The relationships obtained depend crucially on the nutritional status of the plant and the ionic composition of the assay medium. Maximal $\text{Cl}^-$ uptake requires a permeable counter cation, particularly at high $[\text{Cl}^-]_{\text{ext}}$ (Latties et al., 1964; Macklon and MacDonald, 1966; Torii and Latties, 1966). When excised roots from low-salt plants are transferred to solutions containing $\text{Cl}^-$, complex dependencies of $\text{Cl}^-$ influx on $[\text{Cl}^-]_{\text{ext}}$ are obtained. These can be fitted (empirically) to the sum of several Michaelis-Menten hyperbolae (Borstlap, 1983) or to the sum of a saturable plus a linear component (Kochian et al., 1985). Such relationships have been taken to imply the existence of multiple mechanisms for $\text{Cl}^-$ transport across the plasma membrane of root cells with contrasting affinities for $\text{Cl}^-$ (Epstein, 1972), but this conclusion must be regarded with caution since individual ion transport proteins can produce equally complex dependencies on $[\text{Cl}^-]_{\text{ext}}$ (Hille, 1992; White et al., 1999). The apparent $K_m$ for high-affinity $\text{Cl}^-$ influx approximates 15 $\mu$M in excised roots from low-salt plants (Epstein, 1972) and data from tall wheatgrass suggest that this does not differ between salt-sensitive (*Atriplex intermedium*) and -insensitive (*Atriplex elongatum*) species (Elzam and Epstein, 1969). However, the $K_m$ for the high-affinity $\text{Cl}^-$ influx to roots of intact barley plants was increased from 24 $\mu$M in low-$\text{Cl}$ plants to 57 $\mu$M in high-$\text{Cl}$ plants (Lee, 1982). Similar values are obtained for high-affinity $\text{Cl}^-$ uptake determined over a period of several hours (Table 1). Contrasting relationships between $\text{Cl}^-$ uptake and $[\text{Cl}^-]_{\text{ext}}$
have been observed in vacuolate and non-vacuolate (tip) tissue and are attributed to the compounding effects of Cl− fluxes across plasma membrane and tonoplast in series (Torii and Laties, 1966). Indeed, Cl− uptake into roots appears to saturate at high Cl− vac because the flux from the cytoplasm to the vacuole (φoc) across the tonoplast saturates, whilst the plasma membrane Cl− influx (φoc) continues to increase. (see Osmond and Laties, 1968; Cram, 1973b, 1975b). The data of both Cram and Laties (1971) and Cram (1973b) suggest that φoc and φev are approximately equal at [Cl−] vac below 5 mM, and at these [Cl−] vac φoc appears to be the rate-limiting step in the influx of Cl− to the vacuole. However, at [Cl−] vac above about 10 mM, φoc continues to increase with increasing [Cl−] vac but φev remains constant. This is consistent with the observation that root Cl− accumulation saturates at higher [Cl−] vac. The maximal rate of Cl− uptake generally declines with increasing Cl− content of tissues (Greenway, 1965; Osmond and Laties, 1968; Pitman et al., 1968; Pitman, 1969; Cram and Laties, 1971; Cram, 1973a, 1983a, b), although there are exceptions (e.g. Jackson and Edwards, 1966). Cram (1973b) suggested that increasing [Cl−] root reduces φev but not φoc. Since cooling reduces the rate of Cl− uptake by roots, but [Cl−] root is unaffected by growth temperature, a homeostatic system for [Cl−] root may operate (Cram, 1983b).

Neither the relationship between Cl− influx and [Cl−] vac, nor the relationship between Cl− uptake and [Cl−] vac determined under non steady-state conditions reflects the eventual accumulation of Cl− by root tissues at contrasting [Cl−] vac (Cram, 1983b). A greater Cl− influx or uptake merely accelerates the attainment of a new steady state. This phenomenon may arise not only because both Cl− influx and Cl− uptake are regulated by feedback through [Cl−] root but also because there is a significant Cl− efflux across the plasma membrane (φev). Indeed, Cl− uptake across the plasma membrane (Jmol) may be only a small fraction of φoc, which may allow better cellular control of Cl− uptake. Efflux across the plasma membrane increases with increasing [Cl−] root (Jackson and Edwards, 1966) and, by analogy with Br−, unidirectional Cl− efflux from the vacuole (φev) is thought to increase linearly with increasing [Cl−] root (Pitman, 1963). In this context it is noteworthy that Cl− uptake is greater in regions of the root where cells are elongating, suggesting that Cl− uptake is modulated by growth demand (Scott et al., 1968). Similarly, the observation that supplying Cl− to a single seminal root can provide all the Cl required by a barley plant (Drew and Saker, 1984) can be explained by demand-driven Cl− uptake.

The presence of Br− in the assay solution inhibits Cl− uptake competitively, with a Ks approximately double the Km for Cl− (Bösörményi and Cseh, 1964; Elzam and Epstein, 1965; Bösörményi, 1966; Läuchli and Epstein, 1983).
Chloride uptake into roots is also inhibited by uncouplers of oxidative phosphorylation (azide, cyanide, arsenate), inhibitors of electron transport (2,4-DNP, CCCP) and anoxia (Elzam and Epstein, 1965; Greenway, 1965; Lüttge and Latties, 1967; Jacoby and Plessner, 1970). These inhibitors are proportionally less effective at higher \([\text{Cl}^-]\) ext. The saturable, high-affinity component of \(\text{Cl}^-\) influx is specifically inhibited by DIDS (Kochian et al., 1985). By contrast, \(\text{Cl}^-\) influx is not greatly sensitive to pH (Elzam and Epstein, 1965; Jackson and Edwards, 1966; Jacoby and Plessner, 1970).

**Long distance transport of chloride in the xylem and the phloem**

During the growth of a plant, \(\text{Cl}^-\) is translocated from the root to the shoot via the xylem and is redistributed between tissues via the phloem. \(\text{Cl}^-\) fluxes through both the xylem and phloem have been determined in plants placed abruptly into solutions of various \([\text{Cl}^-]\) ext as well as under steady-state conditions.

When plants are placed abruptly in solutions containing \(\text{Cl}^-\), the anion is taken up at a constant rate for several hours (Böszörményi and Cseh, 1961; Helder, 1964; Greenway, 1965; Pitman, 1971; Storey and Walker, 1987). Initially, the root retains a large fraction and swiftly reaches a constant concentration. Gradually, \(\text{Cl}^-\) movement to the xylem increases until a constant translocation rate is reached within a few hours. Although the efficiency of \(\text{Cl}^-\) transport to the xylem decreases from the root apex to more basal zones (Hodges and Vaadia, 1964a; Pitman, 1971), there is considerable \(\text{Cl}^-\) movement to the xylem even in regions where the Casparian band is fully suberized. This suggests that \(\text{Cl}^-\) moves symplastically to the xylem, which is consistent with the observation that the lateral transport of \(\text{Cl}^-\) is competitively inhibited by Br\(^-\) (Böszörményi and Cseh, 1961; Lüttge and Latties, 1966; Läuchli and Epstein, 1971). Measurements of the fluxes of \(^{36}\text{Cl}^-\) to the xylem in excised roots of \(\text{Cl}^-\)-replete plants suggested to Läuchli and Epstein (1971) that \(\text{Cl}^-\) release from the root vacuole did not influence \(^{36}\text{Cl}^-\) flux to the xylem, indicating that the unidirectional rate constant \(\phi_\text{xc}\) was much greater than \(\phi_\text{cx}\). Similarly, Hodges and Vaadia (1964b) observed that higher \([\text{Cl}^-]_{\text{root}}\) resulted in lower accumulation rates in root tissues and a lower rate of delivery to the xylem. This is consistent with the regulation of both \(\phi_\text{xc}\) and \(\phi_\text{cx}\) by \([\text{Cl}^-]_{\text{root}}\) through \(\text{Cl}^-\) uptake across the plasma membrane. However, it is noteworthy that the addition of abscisic acid (ABA) to solutions containing 10 mM \([\text{Cl}^-]_{\text{ext}}\) bathing barley or maize roots resulted in continued \(\text{Cl}^-\) uptake, but the retention of \(\text{Cl}^-\) by the root and a reduced xylem loading and transfer of \(\text{Cl}^-\) to the shoot (Cram and Pitman, 1972; Cram, 1983b). This implies that \(\phi_\text{xc}\) and \(\phi_\text{cx}\) can be regulated independently by a mechanism that does not act through \([\text{Cl}^-]_{\text{root}}\).

Although \([\text{Cl}^-]_{\text{root}}\) saturates with increasing \([\text{Cl}^-]_{\text{ext}}\), \([\text{Cl}^-]_{\text{root}}\) increases proportionally with \([\text{Cl}^-]_{\text{ext}}\) (Storey and Wyn Jones, 1978; Jeschke et al., 1986; Jeschke and Wolf, 1988; Storey, 1995). In addition to the rootstock, which has a major influence on \([\text{Cl}^-]_{\text{root}}\) (see section on Chloride Toxicity), \([\text{Cl}^-]_{\text{root}}\) is affected by both transpiration and shoot growth rate (Greenway, 1965; Pitman, 1982; Storey, 1995; Moya et al., 1999). For example, the halophyte *Atriplex spongiosa* had 8 mM Cl in its leaves when grown in high humidity (95–100 %), but 93 mM when grown at 40–45 % humidity (Pitman, 1982). A model for predicting \(\text{Cl}^-\) fluxes and \([\text{Cl}^-]_{\text{root}}\) in citrus has been proposed based solely upon the rate of \(\text{Cl}^-\) transport from root to leaf and the relative growth rate (RGR) of root and shoot (Storey, 1995). The \([\text{Cl}^-]_{\text{xylem}}\) has been estimated either as the \([\text{Cl}^-]\) of sap exuding from excised roots or roots of intact plants under pressure, or by mathematical modelling techniques. It has been observed (1) that \([\text{Cl}^-]_{\text{xylem}}\) decreases with increasing applied pressure (Munns, 1985; Schurr and Schulze, 1995) and with increasing transpiration (Greenway, 1965; Walker et al., 1990; Storey, 1995) and (2) that the \(\text{Cl}^-\) fluxes to the xylem calculated for intact transpiring plants may be an order of magnitude lower than those measured under applied pressure yielding the same water flow (Pitman, 1982). This raises some concern about the equivalence of measurements with intact plants and excised roots.

\([\text{Cl}^-]_{\text{xylem}}\) generally increases with increasing \([\text{Cl}^-]_{\text{ext}}\), but the magnitude of this increase differs greatly and probably depends upon the exact experimental conditions. In barley, there was little increase in \([\text{Cl}^-]_{\text{xylem}}\) between 0-1 and 200 mM \([\text{Cl}^-]_{\text{ext}}\) when determined in sap exuding under root pressure (Dunlop and Bowling, 1971a; Munns, 1985), but \([\text{Cl}^-]_{\text{xylem}}\) increased from 1 to 6.8 mM between these \([\text{Cl}^-]_{\text{ext}}\) when pressure was applied to excised roots (Munns, 1985). By contrast, \([\text{Cl}^-]_{\text{xylem}}\) determined in sap exuding from roots of lupin (*Lupinus albus*) under pressure increased from 1.8 to 33.2 between 1 and 50 mM \([\text{Cl}^-]_{\text{ext}}\) (Jeschke et al., 1986). \([\text{Cl}^-]_{\text{xylem}}\) of sap exuding under root pressure from roots of *Leptochloa fusca* increased from 0.8 to 54 mM between 0 and 100 mM \([\text{Cl}^-]_{\text{ext}}\) (Jeschke et al., 1995a) and \([\text{Cl}^-]_{\text{xylem}}\) determined in sap exuding from the xylem of intact maize plants whose roots were subject to applied pressure increased from 10 to 54 mM between 0-1 and 100 mM \([\text{Cl}^-]_{\text{ext}}\) (Lohaus et al., 2000). \([\text{Cl}^-]_{\text{xylem}}\) is also influenced by nitrogen nutrition and increases when plants are fed NH\(_4^+\) rather than NO\(_3^-\) (Siebrecht and Tischner, 1999). This probably reflects a biophysical role of \(\text{Cl}^-\). When plants are supplied with NO\(_3^-\), NO\(_3^-\) is the dominant anion in the xylem sap. However, when plants are supplied with NH\(_4^+\), N is transported to the shoot as organic N to prevent chronic pH perturbations occurring within the shoot through N assimilation. Under these conditions, the concentrations of other anions such as \(\text{Cl}^-\) in the xylem sap are increased to maintain charge balance.

Chloride is also relatively mobile within the phloem (Lessani and Marschner, 1978) and the recirculation of \(\text{Cl}^-\) (defined as the ratio of phloem/xylem nutrient fluxes)
Fig. 4. Schematic representation of Cl$^-$ fluxes between the shoot and root at low and high [Cl$^-$]$_{\text{ext}}$ (salinity). At low salinity, Cl$^-$ is accumulated equally by shoot and root tissues and the Cl$^-$ fluxes are related to plant shoot/root ratio. The ratio of Cl$^-$ fluxes in xylem and phloem approximates 5:1. At high salinity, the Cl$^-$ accumulated by root cells remains constant, whereas the Cl$^-$ flux to the shoot increases greatly. Again, the ratio of Cl$^-$ fluxes in xylem and phloem approximates 5:1.

approximately 0.2 (20%) in castor bean (Ricinus communis; Jeschke and Pate, 1991; Peuke and Jeschke, 1993; Peuke et al., 1994), lupin (Jeschke et al., 1992), maize (Lohaus et al., 2000) and the salt-tolerant grass Leptochloa fusca (Jeschke et al., 1995b), across a wide variety of saline growth conditions (Fig. 4). Indeed, when plants with high [Cl$^-$]$_{\text{shoot}}$ are transferred abruptly to solutions of low [Cl$^-$]$_{\text{ext}}$ there may be a net Cl$^-$ redistribution from the shoot to the root. Analysis of phloem sap indicates that the phloem Cl$^-$ concentration ([Cl$^-$]$_{\text{phloem}}$) varies with the salinity of the solution in which plants are grown. For example, the [Cl$^-$]$_{\text{phloem}}$ in lupin increased linearly from 6.2 to 47 mM as [Cl$^-$]$_{\text{ext}}$ was increased from 1 to 40 mM (Jeschke et al., 1986) and the [Cl$^-$]$_{\text{phloem}}$ in maize increased from 8.7 to 31.6 mM as [Cl$^-$]$_{\text{ext}}$ was increased from 0.1 to 100 mM (Lohaus et al., 2000).

Modelling chloride fluxes in cereals

A mechanistic model for Cl$^-$ fluxes in excised, low-salt barley roots was developed by Pitman (1969). This model incorporated known and suspected relationships of Cl$^-$ fluxes at the plasmalemma and tonoplast of low-salt barley roots, together with estimates of passive permeabilities of Cl$^-$ at the plasmalemma and tonoplast of low-salt barley roots, and the Goldman-Hodgkin-Katz (GHK) equation (Hille, 1992). The estimated permeability ratio ($P_{\text{Cl}}/P_K$) varies greatly. Estimates of 0.03 for roots of barley (Pitman, 1969), 0.002 for roots of Vicia faba (Scott et al., 1968), 0.023 for maize roots (Davis and Higinbotham, 1976) and 0.15 for onion roots (Macklon, 1975) are available. From such values absolute permeabilities to Cl$^-$ between 0.04 and 6.9 x 10$^{-8}$ mol cm$^{-1}$ s$^{-1}$ at [Cl$^-$]$_{\text{ext}}$ between 1 and 80 mM have been estimated (Pitman, 1969; Pierce and Higinbotham, 1970; Cram and Lattes, 1971; Pitman et al., 1971; Cram, 1973b).

Pitman (1969) assumed that Cl$^-$ influx across the plasma membrane of root cortical cells was the sum of two transport components: an active transport with a Michaelian dependence on [Cl$^-$]$_{\text{ext}}$, that contributed most to total Cl$^-$ influx, and a diffusive flux calculated from $P_{\text{Cl}}$ [Cl$^-$]$_{\text{ext}}$ and [Cl$^-$] and membrane potential according to the GHK equation (cf. Table 1). He considered that Cl$^-$ efflux across the plasma membrane was a diffusional flux. He also suggested that Cl$^-$ influx to the vacuole could be calculated as the sum of active (following a Michaelian dependence on [Cl$^-$]$_{\text{ext}}$) and diffusive transport (following the GHK equation) components, and that Cl$^-$ efflux from the vacuole was solely diffusional. The simulations of Pitman (1969) accurately described the timecourse of Cl$^-$ uptake and its dependence on both [Cl$^-$]$_{\text{ext}}$ and [Cl$^-$]$_{\text{root}}$. However, to account for the observation that steady-state [Cl$^-$]$_{\text{vac}}$ saturated with increasing [Cl$^-$]$_{\text{ext}}$, he concluded that some feedback from [Cl$^-$]$_{\text{vac}}$ to limit the rate of Cl$^-$ influx to the vacuole was required.

To address more realistic situations, Cram (1983b) developed the hypothesis that the root is homeostatic for...
[Cl\textsuperscript{−}]\textsubscript{root}, and that Cl\textsuperscript{−} influx to excised root tissues is controlled by an error-actuated negative-feedback system responding to the difference between [Cl\textsuperscript{−}]\textsubscript{root} and a reference [Cl\textsuperscript{−}]\textsubscript{root}, which approximates 170 pmol g\textsuperscript{−1} f. wt. This feedback system ensures that steady-state [Cl\textsuperscript{−}]\textsubscript{root} becomes virtually independent of the immediate unidirectional flux rates, membrane permeability or Michaelis constants. Thus, Cram (1983b) suggested that attempts to control [Cl\textsuperscript{−}]\textsubscript{root} by changing these parameters would not be effective, and that taxa differing in root Cl\textsuperscript{−} accumulation did so because of differences in their ‘set point’ [Cl\textsuperscript{−}]\textsubscript{root}. Thus, cannot appear to have a higher set point than beet. He noted also that although anions may interact for uptake into root cells, this does not necessarily impact on steady-state [Cl\textsuperscript{−}]\textsubscript{root}.

The effect of growth on [Cl\textsuperscript{−}]\textsubscript{shoot} was modelled by Greenway and Thomas (1965) assuming that the shoot initially contained no Cl\textsuperscript{−}, that the total Cl\textsuperscript{−} content of the shoot depended solely on a constant rate of Cl\textsuperscript{−} translocation from the root (i, expressed in mol g\textsuperscript{−1} f. wt root d\textsuperscript{−1}) and that plant shoot f. wt / root f. wt ratio was constant (W\textsubscript{p}/W\textsubscript{r} = k). The latter assumptions allowed them to base their calculations of translocation on root weight. They assumed an exponentially growing plant (W\textsubscript{p} = W\textsubscript{p0}e\textsuperscript{rt}) and, thereby, calculated the temporal change in [Cl\textsuperscript{−}]\textsubscript{root} as d[Cl\textsuperscript{−}]\textsubscript{root} / dt = (i/k) (1−e\textsuperscript{−rt}). Thus, they surmised that [Cl\textsuperscript{−}]\textsubscript{root} was sensitive to the balance between root uptake and plant relative growth rate. This has important implications for ion balance, osmotic adjustment and salt tolerance.

**MOLECULAR MECHANISMS OF CHLORIDE TRANSPORT ACROSS MEMBRANES**

Chloride requires a transport protein to catalyse its movement across membranes. The mechanism by which Cl\textsuperscript{−} is transported across a particular membrane is determined by thermodynamic criteria. The principal driving forces for Cl\textsuperscript{−} movement are the voltage and concentration gradients across a membrane. Together, these make up the Cl\textsuperscript{−} electrochemical gradient. Transport is defined as ‘passive’ when Cl\textsuperscript{−} moves in the direction of its electrochemical gradient. Under these conditions, Cl\textsuperscript{−} transport can be mediated by either ‘channel’ or ‘carrier’ mechanisms. This is also known as ‘facilitated diffusion’. The flux of Cl\textsuperscript{−} through a channel may be several orders of magnitude greater than that through a carrier. Transport is defined as ‘active’ when Cl\textsuperscript{−} is accumulated against its electrochemical gradient. This is effected either by chemically coupling Cl\textsuperscript{−} transport to ATP hydrolysis (primary active transport) or by harnessing Cl\textsuperscript{−} transport to the movement of a second ion (generally H\textsuperscript{+} in plants) down its electrochemical gradient. The latter may occur in the same (symport) or opposite (antiport) direction as Cl\textsuperscript{−} movement.

Classical evidence for the mechanism of Cl\textsuperscript{−} transport across membranes was obtained by comparing measurements of membrane potential and Cl\textsuperscript{−} activities with the predictions of the Nernst equation or by comparing estimates of kinetic parameters for Cl\textsuperscript{−} transport with the predictions of the Goldman-Hodgkin-Katz (GHK) or Teorell-Ussing equations (Hille, 1992). Unfortunately, these comparisons often relied heavily on assumed membrane potentials, indirect measurements of [Cl\textsuperscript{−}]\textsubscript{cyt} or [Cl\textsuperscript{−}]\textsubscript{apc} and/or equivocal flux measurements. Therefore, it is probably necessary to reappraise such studies using modern electrophysiological techniques employing multibarrelled electrodes measuring Cl\textsuperscript{−} activity, pH and membrane-potential simultaneously. Nevertheless, the evidence suggests that under non-saline conditions: (1) there is active Cl\textsuperscript{−} influx across the plasma membrane of root cells though a H\textsuperscript{+}/Cl\textsuperscript{−} symport; (2) Cl\textsuperscript{−} efflux across the plasma membrane is mediated by anion channels; (3) Cl\textsuperscript{−} fluxes across the tonoplast are passive and mediated by anion channels; and (4) Cl\textsuperscript{−} is loaded into the xylem down its electrochemical gradient. Under saline conditions most Cl\textsuperscript{−} influx across the plasma membrane becomes passive.

Various experimental approaches have suggested that active Cl\textsuperscript{−} transport across the plasma membrane dominates Cl\textsuperscript{−} influx to root cells at low [Cl\textsuperscript{−}]\textsubscript{ext}, and that passive Cl\textsuperscript{−} influx to root cells occurs under more saline conditions. Measurements of Cl\textsuperscript{−} influx and membrane potential (outside-vacuole) initially led to the suggestion that when Cl\textsuperscript{−} was supplied as KCl, Cl\textsuperscript{−} influx to root cells was passive at [Cl\textsuperscript{−}]\textsubscript{ext} between 1 and 40 mM. This conclusion was based on a consistency between the observed Cl\textsuperscript{−} fluxes and those predicted by the GHK current equations (Laties et al., 1964; Macklon and MacDonald, 1966). However, this consistency was not apparent when Cl\textsuperscript{−} was supplied as CaCl\textsubscript{2}, and other factors in addition to electrochemical ones were involved. From similar observations, Cram and Laties (1971) suggested that the constant P\textsubscript{Cl} observed at [Cl\textsuperscript{−}]\textsubscript{ext} greater than 10 mM implied passive transport, but the greater P\textsubscript{Cl} observed at lower [Cl\textsuperscript{−}]\textsubscript{ext} indicated the contribution of an active transport mechanism. Cram (1975a) subsequently observed that replacing 10 mM Cl\textsuperscript{−} with SO\textsubscript{4}\textsuperscript{2−} caused the membrane potential to become more negative, which is inconsistent with passive Cl\textsuperscript{−} transport across the plasma membrane. This conclusion was supported by the studies of Pierce and Higinbotham (1970) who argued that Cl\textsuperscript{−} was actually accumulated across the plasma membrane of oat coleoptile cells bathed in a medium containing [Cl\textsuperscript{−}]\textsubscript{ext} of 10 mM based on comparisons with the Teorell-Ussing equation.

Approaches based on thermodynamic principles, which compare the Cl\textsuperscript{−} electrochemical gradient across the plasma membrane with the predictions of the Nernst equation (Cram, 1968, 1983a; Gerson and Poole, 1972; Macklon, 1975; Davis and Higinbotham, 1976; Binzel et al., 1988; Hajibagheri et al., 1988, 1989; Felle, 1994; Zhang and Greenway, 1995), also support these general conclusions and imply that active Cl\textsuperscript{−} transport dominates Cl\textsuperscript{−} influx to root cells at [Cl\textsuperscript{−}]\textsubscript{ext} up to 10−60 mM and that passive Cl\textsuperscript{−} influx dominates under more saline conditions. Laterly, studies using ion-selective electrodes have provided direct evidence for the presence of an electrogenic Cl\textsuperscript{−}/2H\textsuperscript{+} symporter in the plasma membrane of root-hair cells (Felle, 1994). This transporter generates a voltage-independent current whose magnitude is determined primarily by the pH gradient across the plasma membrane, and can produce a rapid increase in [Cl\textsuperscript{−}]\textsubscript{ext} after an abrupt increase in [Cl\textsuperscript{−}]\textsubscript{ext} (Felle, 1994). Electrophysiological studies also...
Chloride channels in the plasma membrane

Chloride channels are integral membrane proteins that form aqueous pores, allowing permeant anions to cross a membrane rapidly. They have been observed in all plant membranes studied to date including the plasma membrane, tonoplast, endoplasmic reticulum, mitochondrial and chloroplast membranes of plant cells (Hedrich, 1994; Schroeder, 1995; Allen and Sanders, 1997; Barbier-Brygoo et al., 1999, 2000; Tyerman and Skerrett, 1999; Krol and Trebacz, 2000). They have been characterized from their electrical properties using a variety of voltage-clamp techniques.

Overview of chloride channels in the plasma membrane

Chloride channels in the plasma membrane catalyse anion fluxes both into and out of plant cells (Fig. 5). They are generally more permeable to NO₃⁻ than to Cl⁻. They serve a variety of functions. In certain cells they facilitate anion uptake (Skerrett and Tyerman, 1994; Dieudonné et al., 1997). However, in the majority of cells, and under non-saline conditions, they catalyse the rapid release of anions and have been implicated in both turgor- and osmoregulation. They have also been implicated in the stabilization of membrane potential and/or electrical excitability. Calcium activated Cl⁻ channels are thought to develop membrane depolarization through Cl⁻ efflux during the slow action potentials observed in plant cells, which are important in both intracellular signalling and signal propagation.

Chloride channels in the plasma membrane can be classified on the basis of their voltage-dependence into depolarization-activated and hyperpolarization-activated channels (Table 2). These classes can then be subdivided on the basis of kinetics, pharmacology and stretch-activation. The Cl⁻ channels in the plasma membrane of stomatal guard cells have been characterized extensively, and frequently serve as paradigms. However, it must be remembered that Cl⁻ channels in guard cells have specific physiological roles: they must amplify membrane depolarization and mediate the osmotically significant anion efflux required for the closure of stomata. Chloride channels have also been characterized in leaf mesophyll cells, hypocotyl/cotyledon cells, root cells and suspension-cultured cells. Although Cl⁻ channels may contribute to turgor regulation in these cells, their primary roles are in Cl⁻ uptake, the stabilization of membrane potential and the amplification of the slow action potential (and cell signalling).

Chloride channels in the plasma membrane of guard cells

At least three types of Cl⁻-permeable channels occur in the plasma membrane of guard cells. There are rapidly activating anion channels (R-type or GCAC1), slowly activating anion channels (S-type) and stretch-activated anion channels (Cosgrove and Hedrich, 1991; Hedrich, 1994; Schroeder, 1995; Barbier-Brygoo et al., 2000). Both the R-type and S-type channels are highly selective for anions and activate at voltages more negative than the Nernst (equilibrium) potential for the dominant permeant anion (Eₐ,anion) with a steep voltage-dependence to allow anion efflux upon membrane depolarization (Fig. 6). It is possible that R-type and S-type channels represent contrasting kinetic activities of the same channel protein (Dietrich and Hedrich, 1994), but their properties will be described independently here.

The R-type anion channels in guard cells (GCAC1) exhibit complex kinetics. Although they activate rapidly upon membrane depolarization, they inactivate following prolonged depolarization (Keller et al., 1989; Hedrich et al., 1990; Schroeder and Keller, 1992; Kolb et al., 1995). Thus, they will facilitate transient anion efflux and membrane depolarization, which is characteristic of the slow action potentials observed in plants. They are permeable to a wide variety of anions, following the permeability sequence SCN⁻ > NO₃⁻ > Br⁻ > F⁻ > I⁻ > Cl⁻ > malate⁽⁻²⁾, but are impermeable to glutamate and gluconate (Keller et al., 1989; Hedrich et al., 1990; Dietrich and Hedrich, 1994, 1998). The unitary conductance of GCAC1 was approx. 30 pS with 154 mM [Cl⁻]ₑₓ and 40 mM [Cl⁻]ₑₓ (Keller et al., 1989; Marten et al., 1991). However, increasing [Cl⁻]ₑₓ increases unitary conductance from 0 to 60 pS under similar [Cl⁻]ₑₓ (Kₑₓ = 3 to 16 mM; Hedrich and Marten, 1993; Dietrich et al., 1994; Lohse and Hedrich, 1995; Dietrich and Hedrich, 1998). This 'anion feedback' will promote Cl⁻ efflux even though the anion gradient is being dissipated.
GCAC1 is activated by increasing [Ca^{2+}]_{cyt} or [Ba^{2+}]_{cyt} (Keller et al., 1989; Hedrich et al., 1990; Marten et al., 1992), by direct interactions with cytoplasmic nucleotides (Hedrich et al., 1990; Schultz-Lessdorf et al., 1996) and by cytoplasmic (pK 6-9) or apoplastic acidification (Schultz-Lessdorf et al., 1996). It is also modulated directly by extracellular anions such as auxin (optimum at 10^{-5} M; Marten et al., 1991), isophthalate, malate (K_{m} = 0.4 mM; Hedrich and Marten, 1993; Hedrich et al., 1994), acetate and propionate (Dietrich and Hedrich, 1998), which shift its current vs. voltage (I/V) relationship to more negative membrane potentials (Fig. 6B). These interactions are antagonized by increasing [Cl^{-}]_{ext} (Hedrich et al., 1994; Lohse and Hedrich, 1995). Models for anion permeation and gating kinetics of the GCAC1 have been described (Kolb et al., 1995; Schulz-Lessdorf et al., 1996; Dietrich and Hedrich, 1998), but the physiological significance of these phenomena for the control of the stomatal aperture is unclear (see Schroeder, 1995). GCAC1 is reversibly inhibited by extracellular ethacrinic acid (100 μM), A9C (anthracene-9-carboxylic acid; K_{i} = 100 μM), niflumic acid (K_{i} = 20 μM), IAA-94 (((6,7-dichloro-2-cyclopentyl-2,3-dihydro-2-methyl-1-oxo-1H-inden-5-y1)oxy) acetic acid; K_{i} = 7 μM) and NPPB (5-nitro-2-(3-phenylpropylamino)benzoic acid; K_{i} = 4 μM). These compounds reduce the magnitude of GCAC1-mediated currents and shift the I/V relationship to more negative voltages (Marten et al., 1992). The sensitivity of GCAC1 to stilbene derivatives such as...
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<th>Channel</th>
<th>Cell type</th>
<th>Unitary conductance (pS)</th>
<th>Selectivity</th>
<th>Physiological regulators</th>
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<td>DIDS &gt; DNDS &gt; SITS NPPB &gt; IAA-94 &gt; NiF &gt; A9C &gt; EA</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>'High conductance'</td>
<td>Cotyledon A. tricolor</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'High conductance'</td>
<td>Root</td>
<td>148</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-IRAC</td>
<td>Root xylem parenchyma</td>
<td>71</td>
<td>NO₃⁻, Cl⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stretch-activated</td>
<td>Carrot cell suspension</td>
<td>100</td>
<td>F⁻ &gt; NO₃⁻ &gt; Cl⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Voltage-independent'</td>
<td>Cofix cell suspension</td>
<td>100</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Channels are classified on the basis of their voltage dependence and are subdivided on the basis of their activation kinetics or mechanism of activation. Note that hyperpolarization-activated Cl⁻ channels may, under certain assay conditions, appear 'voltage-independent'. A9C, Anthracene-9-carboxylic acid; DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; DNDS, 4,4'-dinitrostilbene-2,2'-disulfonic acid; DPAC, diphenylamine-2-carboxylic acid; EA, ethacrinic acid; Gli, glibenclamide; IAA-94, [(6,7-dichloro-2-cyclopentyl-2,3-dihydro-2-methyl-1-oxo-1H-inden-5-yl)oxy] acetic acid; NiF, niflumate; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoic acid; SITS, 4-acetamido-4-isothiocyanostilbene-2,2'-disulfonic acid.
SITS ($K_0 = 4 \mu M$), DNDS ($K_0 = 0.5 \mu M$) and DIDS ($K_0 = 0.2 \mu M$; Marten et al., 1993) differentiates it from the S-type Cl$^-$ channel in guard cells (Schroeder et al., 1993; Dietrich and Hedrich, 1994; Schwartz et al., 1995).

Neither channel is affected by the 'K$^+$-channel blockers' Ba$^{2+}$, Cs$^+$ or TEA$^+$, or by extracellular Zn$^{2+}$ (Marten et al., 1993; Schroeder et al., 1993).

The S-type anion channels in the plasma membrane of guard cells are active at voltages substantially more positive than the R-type anion channels under the same assay conditions (Fig. 6A; Schroeder and Keller, 1992). The S-type anion channels activate and deactivate slowly, and do not inactivate (Linder and Raschke, 1992; Schroeder and Keller, 1992). Thus, S-type anion channels can facilitate the prolonged Cl$^-$ efflux and membrane depolarization required for stomatal closure. Single S-type channels have long open times (Linder and Raschke, 1992; Schroeder et al., 1993; Dietrich and Hedrich, 1994; Schmidt and Schroeder, 1994) and exhibit multiple, apparently cooperative, subconductance states with a dominant unitary conductance of about 33 pS with 154 mM [Cl$^-$] cyt (Linder et al., 1999).

The S-type anion channels are also inhibited by diphenylamine-2-carboxylic acid (DPC) and glibenclamide ($K_0 = 3-3 \mu M$). This inhibition is reversed upon the addition of cromakalim or ABA (Leonhardt et al., 1999).

The phytohormone abscisic acid (ABA) causes stomatal pores to close in response to drought stress. This closure is effected by the loss of solutes, and turgor, from the two guard cells that form the stomatal pore. It is thought that ABA elicits anion efflux from guard cells through its effects on the S-type anion channel. However, the mechanisms by which ABA exerts its effects appear to be plant species-specific (Schroeder et al., 1998; Barbier-Brygoo et al., 1999). In arabidopsis, ABA induces channel activation through phosphorylation events, in Nicotiana benthamiana it increases the current amplitude and slows down the deactivation kinetics of the S-type anion channel, and in Vicia faba and Commelina communis ABA maintains channel activity by downregulating protein dephosphorylation.

Chloride channels in the plasma membrane of hypocotyl and coleoptile cells

At least three distinct Cl$^-$-permeable channels are present in the plasma membrane of hypocotyl and coleoptile cells (Table 2). These channels resemble the R-type (Thomine et al., 1995, 1997) and S-type (Barbier-Brygoo et al., 1999; Frachisse et al., 2000) anion channels described for guard cells, together with a blue-light activated channel (Spalding, 2000). By analogy with guard cells, it is thought that R-type channels are involved in electrical signalling, whilst S-type channels mediate sustained anion efflux.

Thomine et al. (1995, 1997) described a typical depolarization-activated R-type anion channel in arabidopsis hypocotyl epidermal cells that facilitated Cl$^-$ efflux. This channel was selective for NO$_3^-$ and SO$_4^{2-}$ relative to Cl$^-$ (Frachisse et al., 1999). It appeared to have two modes, which were characterized by different voltage dependencies and kinetic behaviours. In the presence of ATP (fast mode), the channel activated at depolarized potentials and deactivated increasingly rapidly at more positive voltages. In absence of ATP there was incomplete deactivation and the I/V relationship shifted to more negative potentials. The effect of ATP did not require nucleotide hydrolysis (Thomine et al., 1997) and it has no

![Fig. 6. A, Schematic representations of the steady-state current vs. voltage relationships for the R-type and S-type anion channels in the plasma membrane of guard cells. B, Schematic representation of the effects of extracellular organic anions on the outward (negative) Cl$^-$ current through the R-type anion channel in the plasma membrane of guard cells (GCACl). Here the addition of extracellular auxin (100 µM) shifts the steady-state current vs. voltage relationship to more negative voltages.](http://aob.oxfordjournals.org/)

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![Graph](http://aob.oxfordjournals.org/)
been proposed that the channel is blocked by the voltage-dependent entry of nucleotides into the channel pore at hyperpolarized potentials. It has been proposed that the regulation of the channel by nucleotides may couple the electrical properties with the metabolic status of the cell and/or plasma membrane H+-ATPase activity (Thomine et al., 1997). The channel exhibited rundown in the presence of extracellular NO$_3^-$ and Cl$^-$, but not in the presence of SO$_4^{2-}$ (Frachisse et al., 1999). This suggests that SO$_4^{2-}$ may play a regulatory role in channel activity. The channel was inhibited by niflumic acid ($K_c = 83 \mu$m) and NPPB ($K_c = 100 \mu$m) but was relatively insensitive to IAA-94 (Thomine et al., 1997). Furthermore, the channel was insensitive to A9C and DIDS (unlike its counterpart in guard cells). The R-type channel in arabidopsis hypocotyl cells had unitary conductances of 11, 21 and 20 pS in the presence of 10, 50, 100 mM [CaCl$_2$]$_{ext}$ respectively.

The S-type anion channel in arabidopsis hypocotyl cells was also activated by membrane depolarization, but exhibited slow activation and deactivation kinetics (Frachisse et al., 2000). This channel was highly permeable to NO$_3^-$ but totally impermeable to SO$_4^{2-}$. Activation of the channel required cytosolic ATP, which acted both directly and through protein phosphorylation. The slow channel was inhibited by DIDS ($K_c = 26 \mu$m) and, with lower potencies, SITS, NPPB and niflumic acid. Neither IAA-94 nor A9C were effective. These properties can be contrasted with the S-type channel of arabidopsis guard cells whose activation requires both cytoplasmatic ATP and ABA (Pei et al., 1997) and is insensitive to DIDS but inhibited by A9C (Forestier et al., 1998). This may reflect molecular differences between these channel proteins. The S-type anion channel in the hypocotyl plasma membrane has been implicated in the regulation (inhibition) of hypocotyl growth by auxin (Barbier-Brygoo et al., 2000).

Blue light inhibits hypocotyl elongation. In arabidopsis hypocotyls, blue light activates an anion channel with unitary conductance of 23 pS in 137 mM [Cl$^-$] (Ryan et al., 1997; Kollmeier et al., 2001) and their pharmacological profile resembles that observed in protoplasts from Asclepias tuberosa (100 pS in 100 mM KCl; Schaaf and Wilson, 1987). The channel from A. tricolor was activated by membrane depolarization, but not in the presence of SO$_4^{2-}$ (Ryan et al., 1997; Kollmeier et al., 2001). A channel in Samanea saman (Kollmeier et al., 2000) and Asclepias tuberosa was also activated by membrane depolarization, but not in the presence of SO$_4^{2-}$ (Frachisse et al., 1999).

**Chloride channels in the plasma membrane of root cells**

Three types of depolarization-activated anion channels have been observed in the plasma membrane of root cells (Table 2; Fig. 5). The first two types of depolarization-activated anion channels in root cells appear to be similar to the S-type and R-type anion channels recorded in guard cells. These have been observed in the xylem parenchyma (X-SLAC and X-QUAC; Köhler and Raschke, 2000) and apical cells of the root (A1AAC; Ryan et al., 1997; Kollmeier et al., 2001; Piñeros and Kochian, 2001; Zhang et al., 2001). They mediate anion efflux and are thought to be involved in loading the xylem and in organic acid secretion, respectively. The kinetics of X-SLAC and A1AAC resemble S-type anion channels and the kinetics of X-QUAC resemble R-type anion channels. X-QUAC in the xylem parenchyma of barley roots are permeable to Cl$^-$, NO$_3^-$ and malate, and their activity is reduced by increasing [Ca$^{2+}$]$_{cyt}$ (Köhler and Raschke, 2000). The X-QUAC appear to dominate Cl$^-$ delivery to the xylem under physiological conditions and the Cl$^-$ fluxes potentially catalysed by X-QUAC (up to 11 μmol h$^{-1}$ g$^{-1}$ f. wt root) greatly exceed measured values (Pitman, 1971; 1982). The A1AAC are activated by Al$^{3+}$ (but not La$^{3+}$) in cells from the apex (but not the elongation or basal zones) of wheat and maize roots (Ryan et al., 1997; Kollmeier et al., 2001). Individual A1AAC exhibit unitary conductances from 27 to 144 pS with approx. 100 mM [Cl$^-$]$_{cyt}$ (Ryan et al., 1997; Kollmeier et al., 2001; Piñeros and Kochian, 2001). The A1AAC do not inactivate and will facilitate sustained anion efflux inhibitable by niflumate, DPC and DIDS. They are permeable to Cl$^-$, malate and citrate (Kollmeier et al., 2001; Zhang et al., 2001) and their pharmacological profile resembles that observed for organic acid efflux from cereal roots, which is thought to reduce Al$^{3+}$ stress by chelating this cation. In addition to A1AAC, constitutively active Cl$^-$
channels with a smaller conductance (9 pS with 115 mM \([\text{Cl}^-]_{\text{ext}}\)) have occasionally been observed in cells from the apex of maize roots (Piñeros and Kochian, 2001). These also resemble S-type channels in their kinetics, and have been termed small conductance anion channels (SCAC).

A third type of depolarization-activated anion channel has been observed in (cortical) cells of wheat roots (Skerrett and Tyerman, 1994). This channel appears to be gated by voltage and \([\text{Cl}^-]_{\text{ext}}\), such that it allows only anion influx to root cells and is termed an ‘outward rectifying’ (OR) anion channel. The OR anion channel in the plasma membrane of wheat roots is permeable to \(\text{NO}_3^-\), \(\text{Cl}^-\) and \(\text{I}^-\). It is insensitive to external pH and \([\text{Ca}^{2+}]_{\text{ext}}\) but its activity is increased by increasing \([\text{Ca}^{2+}]_{\text{cyt}}\). The channel has a low unitary conductance (4 pS in 300 mM KCl) and activates rapidly (Skerrett and Tyerman, 1994). It is inhibited by DIDS (at 200 μM), NPPB (\(K_i = 150 \mu M\)) and niflumic acid (\(K_i = 100 \mu M\)), but neither Zn\(^{2+}\) nor phenylglyoxal have any effect (Skerrett and Tyerman, 1994; Garrill et al., 1996). It is noteworthy that DIDS also inhibits \(\text{Cl}^-\) uptake into protoplasts and segments from maize roots (Lin, 1981; Kochian et al., 1985). However, the usefulness of any of these compounds as diagnostics is limited because of their lack of specificity (Garrill et al., 1996).

Two types of hyperpolarization-activated anion channels have been observed in the plasma membrane of root cells. Following the incorporation of plasma membrane fractions from rye roots into PLB, a putative hyperpolarization-activated \(\text{Cl}^-\) channel was characterized (White, 1995) that appears to be similar to X-IRAC observed in xylem parenchyma cells (Wegner and Raschke, 1994; Köhler and Raschke, 2000). X-IRAC are permeable to \(\text{Cl}^-\) and \(\text{NO}_3^-\) and do not inactivate (Köhler and Raschke, 2000). Single channels exhibited multiple conductance states (up to 71 pS in 600 mM KCl) and activated rapidly (Skerrett and Tyerman, 1994). It is inhibited by DIDS (200 μM), NPPB (\(K_i = 150 \mu M\)) and niflumic acid (\(K_i = 100 \mu M\)), but neither Zn\(^{2+}\) nor phenylglyoxal have any effect (Skerrett and Tyerman, 1994; Garrill et al., 1996). It is noteworthy that DIDS also inhibits \(\text{Cl}^-\) uptake into protoplasts and segments from maize roots (Lin, 1981; Kochian et al., 1985). However, the usefulness of any of these compounds as diagnostics is limited because of their lack of specificity (Garrill et al., 1996).

Chloride channels in the plasma membrane of suspension-cultured cells

Zimmermann et al. (1994, 1998) studied anion channels (TSAC) in protoplasts from tobacco suspension-cultured cells. The single channel conductance of the TSAC was 15 pS in pipette:bath 154:110 mM KCl. The TSAC exhibited the hallmarks of R-type anion channels. They activated rapidly upon membrane depolarization and inactivated with prolonged depolarization. They were regulated by phos-
propelling membrane voltage from a steady state (Buschmann and Gradmann, 1997), which establishes the importance of this transport protein in electrical perturbations, such as the slow action potential. The opening of anion channels in the plasma membrane effects anion efflux and plasma membrane depolarization. This depolarization may activate depolarization-activated channels, such as Ca\(^{2+}\) channels, or serve as an electrical shunt for the H\(^+\)-ATPase. Thus, anion channels may contribute to short-term electrical and/or [Ca\(^{2+}\)]\(_{cyt}\) signals (Barbier-Brygoo et al., 1999; Spalding, 2000), to the regulation of membrane potential and pH gradients across the membrane (Schroeder, 1995), to turgor- and osmoregulation (Xu et al., 2000), and to net salt loss (Hedrich, 1994; Schroeder, 1995; Barbier-Brygoo et al., 2000).

Chloride channels in the tonoplast

Plant cell vacuoles can accumulate up to 500 mM Cl\(^-\) (reviewed by Cram, 1976) and Cl\(^-\) channels in the tonoplast have important roles in maintaining [Cl\(^-\)]\(_{cyt}\) homeostasis and in turgor and osmoregulation. Chloride channels may be involved in acidification of intracellular compartments by effecting charge balance.

Anion channel activities in the tonoplast of plant cells have been studied indirectly, using Cl\(^-\)-sensitive dyes (Wissing and Smith, 2000) or through the effects of anions on the generation of a trans-tonoplast pH gradient by the vacuolar H\(^+\)-ATPase (White and Smith, 1989), and directly, using electrophysiological techniques (Allen and Sanders, 1997; Krol and Trehbach, 2000). Both approaches have described two classes of anion channel (V\(_{Cl}\) and V\(_{mal}\)), but more detail is forthcoming from electrophysiological studies. Klughammer et al. (1992) reported numerous Cl\(^-\)-permeable channels from barley leaf vacuoles reconstituted into PLB. The most selective channel had an unitary conductance of 24 pS in 100 mM KCl. Two classes of hyperpolarization-activated anion channels (V\(_{Cl}\) and V\(_{mal}\)) have been recorded in the tonoplast using patch-clamp techniques. The V\(_{Cl}\) are responsible for carrying Cl\(^-\) into the vacuole. The V\(_{Cl}\) channel from arabidopsis vacuoles had a unitary conductance of 110 pS with 100 mM Cl\(^-\) as the charge carrier (Ping et al., 1992). A V\(_{Cl}\) was also identified in the tonoplast of Vicia faba guard cells and in red beet (Pei et al., 1996). This channel was permeable to Cl\(^-\) and possibly malate, but not to glutamate. It was activated by a Ca\(^{2+}\)-dependent serine/threonine protein kinase (calmodulin domain protein kinase, CDPK) in the presence of ATP. It had a unitary conductance of 34 pS with 89 mM Cl\(^-\) as the charge carrier, and was inhibited by niflumic acid, but not by DIDS. The V\(_{mal}\) channel has been identified in plants performing CAM (Iwasaki et al., 1992; Cheffings et al., 1997). However, the V\(_{mal}\) channel in the vacuole of sugar beet was inhibited by 2-5 mM Zn\(^{2+}\) on the cytoplasmic side (Pantoja et al., 1992). The V\(_{mal}\) channel displayed a unitary conductance of 8-6 pS with 50 mM [malate]\(_{cyt}\) in sugar beet vacuoles (Plant et al., 1994) and 120 pS with 10 mM [malate]\(_{cyt}\) in Gropopenatum vacuoles (Iwasaki et al., 1992). It should be noted that 'slow-vacuolar' (SV) channels are not anion channels (White, 2000).

The molecular biology of plant chloride channels

The plant’s genome contains genes related to at least two families of Cl\(^-\) channels in animals. These are the ATP-binding cassette (CFTR) family and the Cl\(^-\) channel (CLC) family (Barbier-Brygoo et al., 2000). The presence of CFTR homologues in plant cells is indicated by the pharmacological studies of Leonhardt et al. (1999), who observed that the slow-anion channels in guard cells were susceptible to CFTR-specific inhibitors. Both gene sequence similarities and functional analyses indicate the presence of CLC Cl\(^-\) channels in plants.

Genes for CLC channels have been cloned from Arabidopsis thaliana (AtCLCa-d; Hechenberger et al., 1996) and tobacco (CLC-Nt1 or NiCLC-c; Lurin et al., 1996). The CLC channels have ten-12 predicted transmembrane domains, and N-terminal and C-terminal domains localized in the cytoplasm. They are thought to function as dimers, containing two pores. Several transmembrane segments contribute to each pore. The conserved sequence motifs (GxGxPE, GKxGPxxH, PxxGxLF) are located in the cytoplasmic loops between transmembrane domains D2-D3 and D5-D6, and are thought to contribute to anion selectivity (Barbier-Brygoo et al., 2000).

In arabidopsis, CLC genes are expressed in all tissues. The AtCLC-a mRNA is induced by the addition of NO\(_3^-\) to N-starved plants (Geelen et al., 2000), which may suggest an involvement in adaptation to excess NO\(_3^-\) and location on an intracellular membrane. Indeed, to date there is no direct evidence that CLC are located in the plasma membrane of plant cells and Hechenberger et al. (1996) have suggested that the hyperpolarization-activated currents induced by heterologous expression of NiCLC-c in oocytes (Lurin et al., 1996) are similar to endogenous oocyte Cl\(^-\) currents. Both AtCLC-c and AtCLC-d complemented a yeast mutant with a Golgi CLC gene (gef1) disruption (Hechenberger et al., 1996; Gaxiola et al., 1998), and the fusion protein AtCLCd-GFP demonstrated an intracellular, non-vacuolar location. Antibodies to the CLC-Nt1 protein have indicated a mitochondrial location for this channel, and Lurin et al. (2000) speculate that CLC-Nt1 may correspond to the inner membrane anion channel in this organelle. Knockout (T-DNA) arabidopsis mutants have been selected by reverse genetic approaches for AtCLC-a (Geelen et al., 2000). The capacity to accumulate NO\(_3^-\) in both root and shoot under conditions of NO\(_3^-\) excess was reduced in these mutants. However, the levels of chloride and other halogens were similar to those of the wild-type.
Synopsis of chloride channels in the plasma membrane and tonoplast

Chloride channels are present in all membranes of plant cells, and have been characterized using a variety of electrophysiological, voltage-clamp techniques. They are generally permeable to other physiological anions including nitrate, sulfate and malate. Chloride channels in the plasma membrane can be divided into several classes based on their voltage-dependence (depolarization-activated or hyperpolarization-activated) and then subdivided on the basis of their kinetics, pharmacology and stretch-activation (Table 2). Depolarization-activated Cl⁻ channels include stretch-activated and light-activated Cl⁻ channels as well as the R-type and S-type Cl⁻ channels that are thought to be involved in electrical excitability and sustained Cl⁻ efflux, respectively. They also include several OR Cl⁻ channels that mediate Cl⁻ influx. Hyperpolarization-activated Cl⁻ channels generally have higher conductances than depolarization-activated Cl⁻ channels. They are present in many cell types and facilitate Cl⁻ efflux. Two classes of Cl⁻ channel have been demonstrated in the tonoplast (VCl and Vma). These channels mediate Cl⁻ fluxes in both directions across the tonoplast.

PERSPECTIVE

Studies of the biodynamics and availability of Cl⁻ in the soil, and its uptake and accumulation by plants, are of topical interest for two reasons. First, high tissue Cl⁻ concentrations are toxic to many crop plants, and restrict the agriculture of saline areas. Second, radioactive ³⁶Cl⁻ present in materials produced by the nuclear industry could be detrimental to human health if it enters the food chain. It is recognized that an understanding of the mechanisms of Cl⁻ uptake and accumulation in plants can be used to inform conventional breeding and genetic modification strategies for the development of more salt-tolerant crop varieties, and that a knowledge of the movement of radioactive ³⁶Cl⁻ through soils and into plants is important for the planning of deep repositories for nuclear waste.

Root cells take up Cl⁻ from the soil solution through H⁺/Cl⁻ symporters at low [Cl⁻]ext, and also through anion channels under saline conditions (Fig. 5). To reach the xylem, and then the shoot, Cl⁻ traverses the root by a symplastic pathway and is released from cells within the stele through specific anion channels. Since this route includes at least two processes catalysed by Cl⁻ transporters in the plasma membrane of root cells, the selectivity and magnitude of Cl⁻ fluxes to the shoot can be controlled. Differences between cultivars in their ability to transport Cl⁻ are frequently related to the ability to restrict Cl⁻ transport to the shoot. In some species, such as soybean (Abel, 1969), this is determined by a single gene. This implies that a simple screening and selection strategy utilizing chemically generated mutants, such as that performed by Garcia-Augustin and Primo-Millo (1995) for citrus, could be successful in identifying lines with restricted Cl⁻ transport to the shoot and, thereby, greater tolerance of Cl⁻ salinity. In addition, since the genes encoding Cl⁻ channels and H⁺-coupled transporters are being identified (Barbier-Brygoo et al., 2000), a targeted, transgenic approach to manipulate the selectivity, activity or expression of genes encoding Cl⁻ transporters in the plasma membrane of root cells could be considered.

To predict the transfer of ³⁶Cl⁻ from a source of contamination into the food chain, ³⁶Cl⁻ movement within the soil, and ³⁶Cl⁻ transfers between the soil solution and edible plants, must be modelled. Although modelling of Cl⁻ movements within the soil is advanced (Norris et al., 1997), a complimentary synthesis of the Cl⁻ fluxes between soils and plants, and the movement of Cl⁻ within the plant to edible portions, is lacking. However, it is known that plants readily accumulate Cl⁻, and this review presents data on Cl⁻ uptake, accumulation and fluxes within the plant determined on a variety of plant materials under a wide range of environmental conditions. These data can be used for the initial parameterization of compartmental models to describe Cl⁻ transfers between the soil solution and plants, and the movement of ³⁶Cl⁻ within the plant into edible portions.

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LITERATURE CITED

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